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Distance decay pattern of fermented-related microorganisms in the sauce-flavor *Baijiu* producing region

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ABSTRACT

Microbial terroir is essential to the development of regional fermented food characteristics. Environmental microbiota participates in the fermentation process and fermentation process also domesticates the microorganisms in the environments, leading to an interaction between the fermentation process and the fermentation environment. Here, we reported microbiota from different environments (fermentation process, fermentation environment, and ecological environment) of two distilleries. We used amplicon full-length sequencing to study the regional microbial characteristics and the domestication of the fermentation process on environment. Fungi in the *Chishui* River producing region contributed more to the brewing, which contained many functional fungi, such as *Pichia*, *Wickerhamomyces anomalus*, and *Debaryomyces*. The fermentation process had a strong domestication effect on microbiota in the fermentation environment. Fermented grains and *Daqu* were the primary microbiota sources in the fermentation environment. Microorganisms in the producing area exhibited a distance decay relationship. With increasing distance from the distillery, the dissimilarity between microbial communities increased and the microbial community showed an obvious deterministic-stochastic pattern. The ecological environment 50m away from the distillery was less domesticated by the fermentation process. Abiotic factors (pH, acidity, and water content) and microbial interactions synergistically led to microbial differences across environments. These results first determined that a regional microbial model had been established in sauce-flavor *Baijiu* and comprehensively clarified the interaction between the fermentation process and the environment. It laid the foundation for further elucidation of the influence of microorganisms on the yield and flavor quality of sauce-flavor *Baijiu*.

1. Introduction

Most fermented foods are fermented in an open process, so the fermentation process is highly dependent on the microbiota in the fermentation environment and the ecological environment of the producing area (Wang, 2022). Researchers (Knights et al., 2011) demonstrated that the fermentation environment contributed significantly to the flavor and quality of fermented foods by using SourceTracker, such as *Daqu* (Xiao et al., 2021), *Baijiu* (Li et al., 2022; Wang et al., 2018; H. Zhang et al., 2021), cheese (Bokulich & Mills, 2013), beer (Bokulich et al., 2015), and fermented meats (Zwirzitz et al., 2020).

The relationship between microbial biogeography and fermented foods terroir appears to be a developing public issue (R. Li, Tian, et al., 2021; Tan et al., 2022). The study of wine "terroir" demonstrated that regional, site-specific, and grape-variety factors shaped the microbial consortia inhabiting wine-grape surfaces (Bokulich et al., 2014).

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Geographical delineations among *Saccharomyces cerevisiae* populations in New Zealand vineyards have been recorded, indicating that grape yeasts dispersed regionally (Gayevskiy & Goddard, 2012). By fermenting grapes with distinct yeasts, Knight (Knight et al., 2015) proved that the geographical peculiarities of wine were tied to specific microorganisms. Microbial biogeography implies that "microorganism is everywhere, the environment selects" (Bokulich et al., 2014). The environmental factors (such as pH, humidity, and acidity) (H. Zhang et al., 2021), geographic distance (Jiao et al., 2017), and fermentation process parameters (Xiao et al., 2021) played a crucial role in the biogeographical distribution of microorganisms. Therefore, revealing the microbial characteristics of the producing areas and analyzing the interactions between the environment and the brewing process can promote the development of high-quality fermented foods (Gao et al., 2019, 2020, 2021).

The profit of *Baijiu* ranked among the top in the world. It was classified into three primary types according to the flavor: light-flavor *Baijiu*, strong-flavor *Baijiu*, and sauce-flavor *Baijiu*. Current research has revealed the microbial geography of Chinese major *Baijiu* producing regions and the contribution of latitude-dependent fungi to the flavor of *Baijiu* (Tan et al., 2022). More research was required to determine whether a regional microbial model had been established in *Baijiu*. The sauce-flavor *Baijiu* is fermented in two stages: heap fermentation and pit fermentation (H. X. Zhang et al., 2021). Its raw materials (such as sorghum, rice, glutinous rice, and corn) are fermented into spirit containing thousands of trace components. *Baijiu* is produced using bilateral solid-state fermentation method (Jin et al., 2017), with *Jiuqu* (*Daqu*, *Xiaoqu*, or *Fuqu*) as a saccharifying and fermenting starter (Liu & Sun, 2018). Sauce-flavor *Baijiu* had a strong dependence on water, soil, air, climate, microorganisms, and raw materials (Tan et al., 2022). Environmental changes affected not only the quality of raw materials (Bokulich et al., 2014; J. Chen, Feng, et al., 2022; Zhang et al., 2022) but also the composition of environmental microbiota (Wang et al., 2018). Environmental microorganisms contributed to the brewing process (Li et al., 2022), and there was a certain connection between the fermentation process and environmental microbiota. Therefore, it is necessary to study the effect of the brewing process on the fermentation environment and ecological environment.

In this study, high-throughput sequencing and bioinformatics statistical analysis were used to study the microbiota of different environments (fermentation process, fermentation environment and ecological environment) in two breweries and elucidate the interactions between the brewing process and ecological environment. The similarities and differences of microorganisms in different environments based on Bray-Curtis distances were investigated. Besides, abiotic (pH, acidity, water content) and biotic factors influencing the construction of microbial communities in different environments were identified. The results advanced our understanding of the role of environmental microbiota during fermentation.

2. Materials and methods

2.1. Sampling collection and environmental factors detection

Samples were collected from two sauce-flavor *Baijiu* distilleries (A and B) located in the core producing area of sauce-flavor *Baijiu* in Luzhou, Sichuan Province. A is a ten-year distillery (27°53′N, 106°22′E), while B is a twenty-year distillery (27°52′N, 106°22′E). Raw materials (rice husk and sorghum), water, *Daqu*, heap fermented grains, and cellar fermented grains were collected in the fermentation process (FP). Pits, brewing sites, tools, and indoor air samples were collected in the fermentation environment (FE). Outdoor soil, 50 m away from distillery environmental soil, and 1 km away from distillery environment soil were collected in the environment outside the brewing workshop (BW). Ecological environmental soil (ND) was collected in the newly developed area (Table S1). A mixture of steamed sorghum and high-temperature *Daqu* initiated spontaneous heap fermentation in an open environment. The fermented grain was transferred to a cellar (anaerobic tank) after 3–7 days of heap fermentation and fermented for another 30 days (cellar fermentation). Fig. 1 shows the sampling strategy. Table S1 summarizes the detailed information and abbreviations.

For samples of FP, uncooked rice husk, sorghum, and *Daqu* powder, were chosen at random and blended into sterile sampling bags. The precipitate of water in *Chishui* river was collected using 0.22 μm membrane filtration. Fermented grains on the last (3 rd or 4th) day of heap fermentation and on the last (30th) day of cellar fermentation were gathered. They were gathered from each distillery's four distinct cellars. Environmental samples were taken from the pits, brewing site, tools, air, and soil. When excavating soil samples, at least five separate areas were sampled, and samples weighing more than 10 g from one spot were dug and put in a sterile sampling bag. All surface samples were wiped and collected with the sterile absorbent cotton that has been pre-soaked in sterile phosphate-buffered saline (PBS, 0.1 M). Air samples were selected with the membrane filtration air extraction equipment (JCH-120F) at a flow rate of 100 L/min until the filter membrane turned black. Five-point sampling method were used for each sample. All of them were collected in four parallel samples, for a total of 124 samples, put into a −80 °C refrigerator for freezing, and then transferred back to the laboratory by the cold chain.

We measured the pH of the sample suspension (10 g/100 mL) by using the pH meter, and determined the titratable acidity using NaOH (0.1 mol/L). The moisture content was determined by drying the sample at 112 °C to constant weight. Acetic acid, lactic acid and ethanol were determined by HPLC and GC-MS (S. Li, Tian, et al., 2021; Lou et al., 2022).

2.2. DNA extraction and PCR amplification

Total genome DNA from samples were extracted with the Fast DNA Spin Kit for Soil (MP Biomedicals, Santa Ana, CA, USA). DNA concentration was determined through a Thermo Scientific NanoDrop 8000 UV–Vis Spectrophotometer (NanoDrop Technologies, Wilmington, DE). 16S rRNA was amplified in bacteria using the universal primers with the barcode (Adedire et al., 2022). The internal transcribed spacer (ITS) region of fungi was amplified by the barcoded universal primer ITS4_ITS9 (Bengtsson Palme et al., 2013). All PCR reactions were carried out with DNA Polymerase (TransGen Biotech). The same volume of 1x loading buffer was mixed with PCR products and detected by electrophoresis on a 2% agarose gel.

2.3. PacBio sequencing

We detected the number of library fragments after constructing the SMRT Bell library as required using the Agilent 2100 Bioanalyzer (Agilent Technologies, USA) and purified it with AM Pure PB beads, and sequenced on PacBio Sequel II platform. The PacBio SMRT portal was used to process raw sequences at first (Mosher et al., 2013; Singer et al., 2016). Sequences were filtered for a minimum of three passes and a minimum predicted accuracy of 90% (min full pass = 3, min Predicted Accuracy = 0.9). The PacBio platform files were then utilized for amplicon size trimming to remove sequences that were larger than the expected amplicon size (for 16S rRNA reads, min Length 1340 bp, max Length 1640 bp; for ITS reads, min Length 600 bp) (Yan et al., 2019). The reads were assigned to samples using their unique barcode and then truncated by cutting off the barcode and primer sequence.

2.4. Feature sequence analyses

Sequence analyses were performed by Qiime 2 (qiime2-2021.2, https://qiime2.org/). For bacteria, UCLUST was used to cluster high-quality sequences into several Operational Taxonomic Units (OTUs) according to 97% similarity. For fungi, DADA2 was used to cluster high-

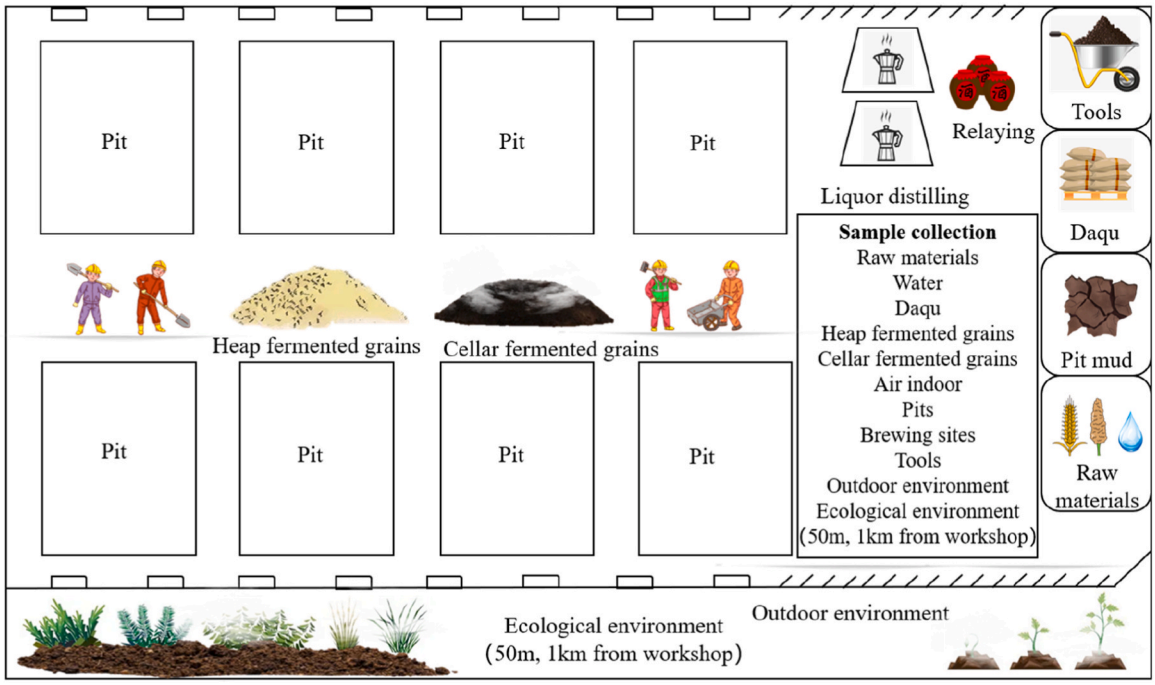

Fig. 1. Schematic of the brewing microecology and sampling procedures of traditional sauce-flavor *Baijiu* distillery.

quality sequences into several Amplicon Sequence Varian (ASVs) (Callahan et al., 2016). Each clustered OTU/ASV utilized a single representative sequence to align to the SILVA bacterial database and the UNITE fungal database (Kõljalg et al., 2013).

2.5. Diversity and similarity analysis

The Shannon diversity index was calculated by Qiime 2. *p* value determined by ANOVA with Turkey's test. The cluster analysis was conducted with the unweighted-pair group method using average linkages (UPGMA) based on the Bray-Curtis distance in R (Pavlopoulos et al., 2010). Principal coordinates analysis (PCoA) provides visualization of the microbial community composition. ANOSIM with 999 permutations was used to test significant differences between samples (Clarke, 1993). SourceTracker is a tool for quantitative analysis of microbial sources based on Bayesian technique. Set the fermentation environment (P, Bs, T, A) and the ecological environment outside the brewing workshop (OE, 50m-EE, 1 km-EE) to "sink" respectively. And set other samples to "source". Calculate the modeled data using the R package of "sourcetracker" (Knights et al., 2011). The shared OTUs proportion index between microbial communities in different environments and samples in FP was used to indicate community similarity.

2.6. Identification of differential microorganisms

Galaxy Web application (https://huttenhower.sph.harvard.edu/galaxy/) was used to calculate the linear discriminant analysis (LDA) effect size. Kruskal–Wallis test was used to determine the difference between the categories, with a significance level of $p < 0.05$, and a threshold LDA score of 4.0 (Segata et al., 2011). The number of marker taxa were identified with a 10-fold cross-validation implemented with the rfcv function in the R package "randomForest" with five repeats (Zhang et al., 2018). In order to elucidate the causes of differences in community assembly in different environments, we further examined the correlation between Euclidean distance and Bray-Curtis distance and used the Pearson correlation to express the strength of the association (Feng et al., 2019; Liu et al., 2020).

2.7. Correlation analysis between microorganisms and environmental factors and construction of microbial networks

RDA multiple linear regression analysis was used to reveal the relationship between microorganisms and environmental factors, and R was used to realize visualization. Based on Spearman correlation among microorganisms, microbial network was built in R "Hmisc" package, selected the correlation of $R \geq 0.7$ and $p < 0.05$, visualized it through Gephi (v0.9.2), and calculated the average degree of each node and modularity of each network.

2.8. Null community model analysis

The beta nearest taxon index (βNTI) and Raup Crick index of Bray-Curtis dissimilarity (RC_{bray}) were calculated using the Picante package in R software (Zhao et al., 2021) to evaluate the assembly process of the microbial community in different environment. $\beta NTI < -2$ indicates lower than expected phylogenetic turnover, representing homogeneous selection. $\beta NTI > 2$ indicates greater than expected phylogenetic turnover, implying variable selection. $|\beta NTI| < 2$ shows that the community in microecology is affected by the stochastic process (Chase et al., 2011; Stegen et al., 2012). $RC_{bray} > 0.95$ indicates that turnover in microbial community composition is mainly limited by dispersal limitation consistent with drift or mean diffusion, while $RC_{bray} < -0.95$ indicates that homogenizing dispersal is the dominant process. $|RC_{bray}| < 0.05$ is considered to be an uncertain process, indicating that the community assembly is highly random and dispersed (Kembel et al., 2010; Stegen et al., 2013).

3. Results and discussion

3.1. Microbial alpha diversity and environmental factors in different environments

According to specific substrates and the distance from processing area, the macroecology of *Baijiu* production were divided into three parts: fermentation process, fermentation environment, and ecology outside the brewing workshop. High-throughput sequencing technology

was used to assess the microbial community structure in three environments. 867,633 high-quality reads from the full-length of bacterial 16S rRNA gene sequences, and 1,235,248 high-quality reads from the fungal ITS rRNA gene sequences were detected from all samples. For bacteria, there was an average of 7171 reads per sample, with a range from 2255 to 16206 reads. For fungi, there was an average of 10380 reads per sample, with a range from 2115 to 40122 reads. The rarefaction curves of microbes were close to saturation, indicating that the depth of sequencing was representative for subsequent analysis (Fig. S1).

Alpha diversity was used to characterize the species diversity (Simpson, 1949). The Shannon index was calculated to assess the species diversity in the fermentation environments. Microbial α-diversity in the three environments showed similar trends between the two distilleries. The Shannon index in the fermentation environment was close to that of the fermented grains. The fungal diversity in the fermentation environment was lower than that of the bacteria, with a bacterial Shannon index of 6 and the fungal Shannon index of 4 (Fig. 2A and B). The microbial diversity in the ecological environment outside the distillery increased with distance. Microbial α-diversity in the ecological environment 1 km away from the distillery was the highest, with a bacterial Shannon index of up to 8 and a fungal Shannon index of up to 7 (Fig. 2). It has been confirmed that increased species diversity in a given environment may reduce fluctuations in species abundance and improve community stability (Feng et al., 2017). Increased microbial diversity implied high community stability of the ecosystem. Compared with the fermentation environment, the microbial community structure in the ecological environment was more stable. In addition, there was no significant difference in the microbial diversity of the ecological environment among the newly developed area and which has been developed for 10 and 20 years (Fig. 2).

Dataset S2 (Mendeley Data: https://data.mendeley.com/datasets/82g57ypbrf/1) showed the pH, acidity, acetic acid, lactic acid, water, and ethanol content of different samples. These environmental factors had significant differences ($p < 0.01$) in different environments. In general, pH increased with the increase of geographical distance (3.82 ± 0.01 to 6.47 ± 0.10), and acidity (17.2 ± 0.16 to 8.43 ± 0.58 g/kg), acetic acid (1.72 ± 0.01 to 0 g/kg), lactic acid (19.55 ± 0.09 to 0 g/kg), water (46.89 ± 0.77 to 10.51 ± 2.17%), and ethanol content (123.35 ± 27.99 to 0 mg/kg) decreased (Fig. 2C, D, and 2E).

3.2. Domestication of fermentation environment by fermentation process

The fermented grains of both distilleries were inoculated with high-temperature *Daqu* for fermentation, and similar microorganisms were present on the surfaces of the equipments (Fig. 3, Dataset S3, S4, S5, and S6, Mendeley Data: https://data.mendeley.com/datasets/82g57ypbrf/1). *Pseudomonas* sp., *Sphingomonas* sp., *Thermomyces lanuginosus*, and *Mucor* sp. were common in raw materials and water, which were not associated with fermentation. *Bacillus* (mainly *Bacillus thermolactis* and *Bacillus licheniformis*), *Thermoactinomyces sanguinis*, *Aspergillus* and *Saccharomycopsis fibuligera* were rich in *Daqu*. *Acetilactobacillus jinshanensis*, *Lactobacillus*, *Pichia*, and *S. fibuligera* became dominant microorganisms in fermented grains. They were the core functional microorganisms reported for sauce-flavor *Baijiu* (H. Zhang et al., 2021).

The prevalent microorganisms in the fermentation environment were similar to those found in fermented grains and *Daqu* (Fig. 3, Dataset S3, S4, S5, and S6, Mendeley Data: https://data.mendeley.com/datasets/82g57ypbrf/1). *Lactobacillus*, *A. jinshanensis*, *Bacillus ruris*, *Pichia kudriavzevii*, *Saccharomyces cerevisiae*, *S. fibuligera*, and several filamentous fungi were abundant in the pits, brewing sites, and tools. *Ochrobactrum haematophilum*, *T. sanguinis*, *Bacillus*, *Thermomyces ibadanensis*, and *S. fibuligera* were the common microorganisms in the air. The results revealed that fermentation-related microbiota occupied the majority of the equipment surfaces. Some functional microorganisms, such as *Pichia*, *Saccharomyces*, and *Saccharomycosis* in the fermentation environment were more abundant than in the starter (*Daqu*), which played a complementary role of *Daqu*. The dominant microorganisms in BW were primarily *Sphingomonas*, *Sediminibacterium* sp., *Glutamicibacter protophormiae*, *Cladosporium*, *Mucor*, and *Trichosporon*. Besides, *Pichia*, *Wickerhamomyces anomalus*, *Debaryomyces*, and *Thermomyces*

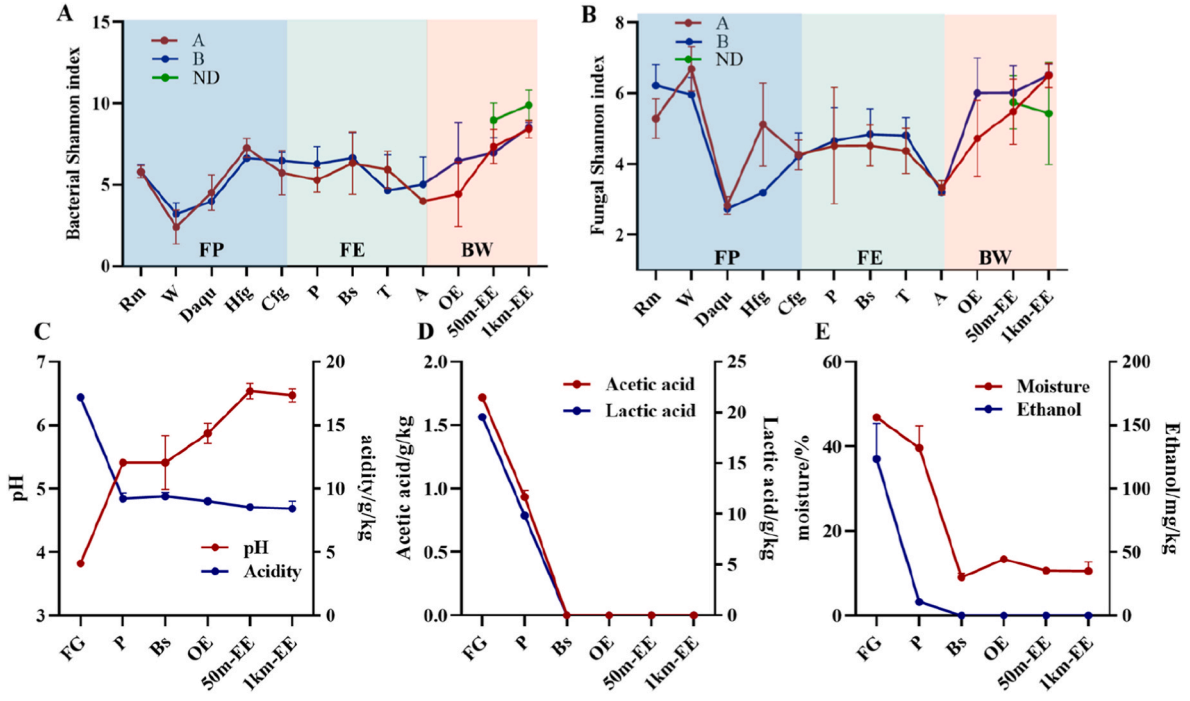

Fig. 2. Microbial diversity and physicochemical factors changes in different environments. Shannon index for bacteria (A) and fungi (B) among samples in fermentation process, fermentation environment and environment outside brewing workshop. Changes of pH, Acidity (C), Acetic acid, Lactic acid (D), Moisture and Ethanol (E) in different environments. FG: heap fermented grains and cellar fermented grains. ND: newly developed area.

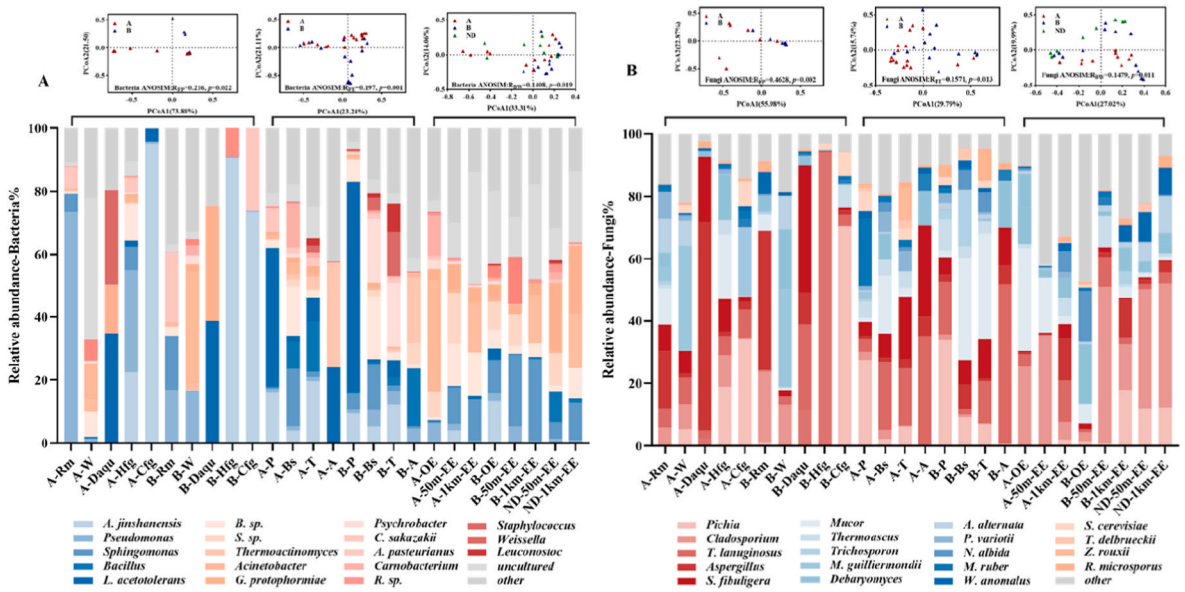

Fig. 3. The compositions of the microbial community in the brewing microecology. Bacterial community composition (A). Fungal community composition (B). The PCoA results above represent the differences between distillery A and B in the same environment.

lanuginosus were abundant in this environment (Fig. 3, Dataset S3, S4, S5, and S6, Mendeley Data: https://data.mendeley.com/datasets/82g57ypbrf/1), but no fermentation-related bacteria. The ecological environment of the newly developed area had similar bacterial communities. There were still many unidentified bacteria and fungi in the environment, indicating that more research into the ecological environment of the sauce-flavor *Baijiu* core production area was required. Microorganisms in the fermentation environment between A and B had significant differences, especially for bacteria (Fig. 3, $p_{\text{bacteria}} = 0.001$, $p_{\text{fungi}} = 0.013$). Distillery A was mainly dominated by *P. sp.*, *Burkholderia stabilis*, *Mucor circinelloides*, and a few fermented-related microorganisms. While distillery B was mainly dominated by fermentation-related microorganisms, such as *Weissella*, *Leuconostc*, *Lactiplantibacillus plantarum*, *S. fibuligera*, *Torulaspora delbrueckii*, and *Thermoascus*, it indicated that the domestication of the FP on microorganisms in the FE increased with time. However, the difference of microorganisms in the BW between A and B was lower than that of the FE (Fig. 3, $p_{\text{bacteria}} = 0.019$, $p_{\text{fungi}} = 0.011$). It indicated that the brewing process domesticated the fermentation environment in the workshop more than the ecological environment.

Microorganisms on the indoor ground, worker's skin, and equipment surfaces played a role in microbial transfer and made up for the deficiency of the starter, which was consistent with the previous study (H. Zhang et al., 2021). It was advantageous to direct the continuous fermentation process (Bokulich & Mills, 2013). The workshop was akin to a natural screening medium, which explained why fermented-related microorganisms dominated the fermentation environment (Wang, 2022). Only microorganisms that acclimated to the fermentation environment had a chance of survival. Furthermore, the fluidity of the air in the workshop was low and the temperature changed modest, promoting the growth and reproduction of microorganisms. The air outside the distillery was quite brisk, and the nutrients available for microbial growth in the air and ecological environment were limited. Besides, the number of microorganisms especially fungi in the ecological environment was smaller than that in the workshop due to the UV radiation emitted by sunlight.

3.3. Fermented grains and Daqu were the main sources of fermentation environment microorganisms

PCoA based on the Bray-Curtis distance was used to determine the distribution patterns of bacteria and fungi in different environment (Fig. 4). The first two principal axes of bacteria and fungi accounted for 33.82%, 25.35% (A) and 52.66%, 27.07% (B) of the total variance. Fungi in the same environment clustered well, but established distinct fungal communities in different environments. However, bacteria in different environments partially overlapped.

Microorganisms in the fermentation environment were similar to those found in the fermented grains, which may be attributed to the presence of *Lactobacillus*, *A. jinshanensis*, *P. kudriavzevii*, *Thermoascus*, *T. lanuginosus*, *S. cerevisiae*, and *Monascus ruber*. The fungal community structure of indoor air was similar to that of *Daqu*, and they were located in the third quadrant, indicating the considerable impact of *S. fibuligera* and *T. ibadanensis*. The environment outside the brewing workshop was clustered with raw materials and water, which was related to the high abundance of *C. haloolerans*, *Trichomerium*, and *M. racemosus*. PERMANOVA indicated substantial differences in microbial community structure among different environments ($p = 0.001$). However, the differences of bacteria in different environments were smaller than that of fungi (A: $R_{\text{bacteria}} = 0.5038$, $R_{\text{fungi}} = 0.7011$; B: $R_{\text{bacteria}} = 0.5332$, $R_{\text{fungi}} = 0.7076$).

Changes in the makeup of microbial communities in different environments revealed different assembly processes. The variance of pairs β diversity was used to further explain this assembly process. The proportion of OTUs shared by bacteria and fungi followed the same downward trend (Fig. 4E and F). The proportion of shared OTUs between FP and FE (bacteria: 71.07%–77.31%, fungi: 39.39%–44.67%) was higher than the shared OTUs between FP and BW (bacteria: 44.34%–65.22%, fungi: 31.02%–42.68%), which proved the domestication effect of fermentation process on fermentation environment microorganisms. Whether in the fermentation environment or outdoor environment, bacteria shared a much higher proportion of OTUs with fermented grains and *Daqu* than fungi, which indicated that the FP had a stronger domestication effect on the bacteria of the FE than on the fungi.

SourceTracker was used to quantify the potential microbial sources in the fermentation environment and environmental ecology (Fig. S2). Raw substrates (raw materials, water, *Daqu*, and fermented grains) and extraneous sources (pits, brewing site, tools, air, and soil) were tested as primary microbial sources (Bokulich et al., 2015; H. X. Zhang et al., 2021). Results revealed similar patterns of microbial sources across distinct distilleries. Cellar fermented grains were predicted as the major bacterial (11.44%) and fungal (42.29%) contributor to pits, which

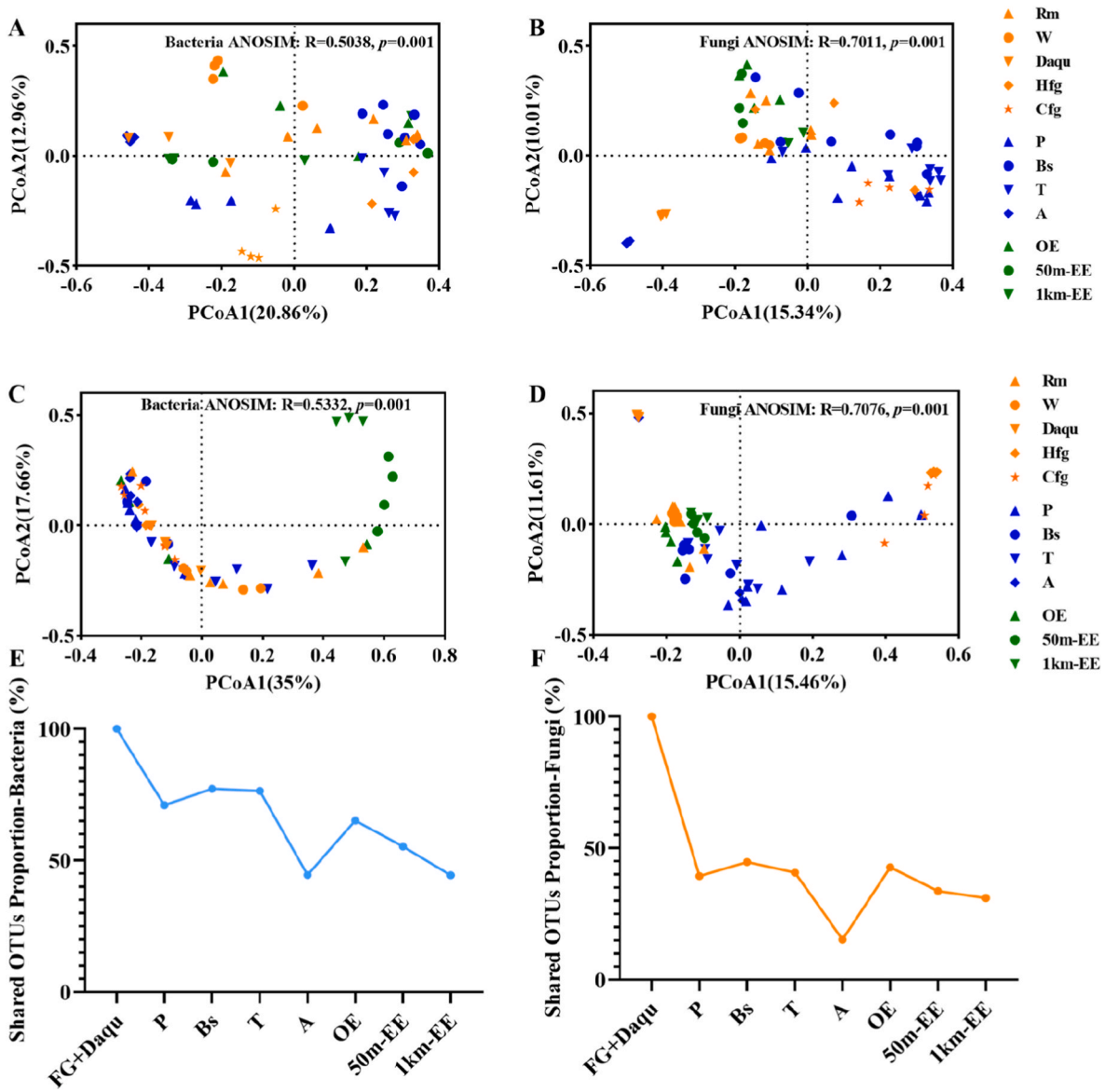

Fig. 4. Similarity of three different environmental microecology. Principal coordinate analysis (PCoA) of the bacterial and fungal communities based on Bray-Curtis distances in distillery A (A, B). Principal coordinate analysis (PCoA) of the bacterial and fungal communities based on Bray-Curtis distances in distillery B (C, D). Proportion of OTUs shared by microbial communities in various environments (E, F).

mainly provided 58.61% *A. jinshanensis*, 6.95% *L. acetotolerans*, 36.64% *S. cerevisiae*, 34.31% *Pichia*, and 33.46% *Thermoascus*. Raw substrates (19.00%) were the largest bacterial contributors to brewing sites and tools surfaces, which mainly provided 62.03% *Pseudomonas*, 13.37% *Burkholderia* sp., and 10.36% *Sphingomonas*. Fermented grains (13.43%–21.01%) were the largest fungal contributors to brewing sites and tools surfaces, mainly provided *S. cerevisiae*, *Pichia*, and *Thermoascus*. Fungi on the brewing sites and tool surfaces passed through and affected each other. The reason could be that the environment was not cleaned up quickly enough following the heap fermentation, resulting in microbial colonization in the indoor ground or the equipment surfaces. After pit fermentation, the fermented grains transferred the microorganisms in the cellar into the fermentation environment through tools, resulting in microbial domestication and reproduction in the fermentation environment. *Daqu* was the most common source of fungi in the air (2.63%), mainly providing 18.04% *S. fibuligera*. While there were many unknown sources of bacteria (47.07%–89.21%) and fungi (29.67%–94.96%) in the fermentation environment. The source of microorganisms in the environmental ecology was mostly unknown. It was because the ecological environmental microorganisms were complex and the community structure had reached a stable state. These results showed that fermented grains, especially cellar fermented grains, were the primary sources of environmental fungi. The sources of bacteria in the workshop were more diverse, including fermented grains, raw materials, *Daqu*, and the ecological environment.

These predicted relationship could suggest that both the source and sink came from another untested source, such as flies, workers, and other vectors that could transfer microbes among these surfaces (Bokulich et al., 2015). These predictions highlighted potential sources of feedback domestication effect on the fermentation environment, or at least shared microbial transmission patterns between raw materials and surfaces in the distillery (Bokulich et al., 2015). It was reported that microorganisms in the environment contributed significantly to the fermented grains and the flavor of *Baijiu* (H. X. Zhang et al., 2021). So, the fermentation environment is an essential carrier for the bidirectional transfer of microorganisms in the fermentation process.

3.4. Distance decay relationship highlighted the spatial dispersal pattern of microbiota in producing region

LEfSe was used to further examine the microbial distribution patterns. Microorganisms with LDA values greater than 4 were regarded statistically significant differential marker microorganisms. Firmicute and Bacilli were the representative bacteria in the FP, which was consistent with previous coverage that Firmicute and Bacillus were the major bacteria in the sauce-flavor *Baijiu* fermentation process (Wang et al., 2018). *Burkholderia*, Firmicute, Bacilli, *Acetobacter*, and *Rhodospirillales* were the representative bacteria in brewing sites and tools (Figs. S4A and S4B). *Pantoea*, *Sphingomonas*, and *Staphylococcus* were representative bacteria in the outdoor soil (Fig. 5 and S4A). Bacteria were not statistically different between FP and FE ($p > 0.05$), but there were significant differential bacteria between FP, FE, and BW ($p < 0.01$, Figs. S4A and S4B). For fungi, *Pichia* (mainly *P. kudriavzevii* and *Pichia manshurica*), *Thermoascus* (especially *Thermoascus aurantiacus*), *Aspergillus penicillioide*, and *Rasamsonia* were represented in FP. Brewing sites, pits and tools were represented by *Monascus* and *Rhizopus microsporus*. In the BW, the standard differential fungi were *Cladosporium*, *Alternaria* and *Mucor* (Fig. 5, S4C, and S4E). The number of differential fungi in FP and FE was significantly lower than that in FP, FE, and BW (Figs. S4C, S4D, and S4E). The results revealed distinct bacterial and fungal aggregation patterns in the different environments.

The random forest classification algorithm depicted the relative abundance of some essential microbes in three different environments (Fig. S3). The abundance of fermented-related microorganisms differed in the three environments, implying that microorganisms in the FP, FE, and BW had distinct aggregation patterns.

We further examined the correlation of microbial dissimilarity with geographical distance to demonstrate the assembly of environmental microbiota in different environments. Pearson correlation was employed to express correlation strength. As distance increased, the dissimilarity between microbial communities increased (bacteria $R^2 = 0.1882$, $p < 0.05$; fungi $R^2 = 0.5991$, $p < 0.0001$) (Fig. 5C and D). It was worth noting that the microorganisms in ecological environment 50m away from the distillery were significantly different from those in the environment inside the workshop (Fig. 5C and D). These results illustrated the spatial dispersal pattern of microbiota in the sauce-flavor *Baijiu* producing area. Aerosols and direct contact often occurred in *Baijiu* distilleries, increasing the chance of microorganisms spreading from one surface to another (Bokulich et al., 2015). Human and insect activities also improved the speed of transfer among these surfaces (for example, from pit to indoor ground). However, some physical obstacles (such as geographical distance and walls), as well as physical and chemical conditions (such as humidity, temperature, and precipitation), could limit the spread of some microorganisms. This will decrease the possibility of ecological environment contamination produced by food processing to some extent.

Distance decay relationship proved that only a few conspecific species can be detected in different environments, and most species disappeared in the migration due to environmental pressure (W. Chen, Feng, et al., 2022). Dominant fungi in the fermentation process, such as *Pichia, W. anomalus, Debaryomyces, and T. lanuginosus* occurred frequently in the three environments. One possible reason was that compared with rare species, rich species could occupy a wider niche, making use of larger living spaces and resources to survive in more diverse environments.

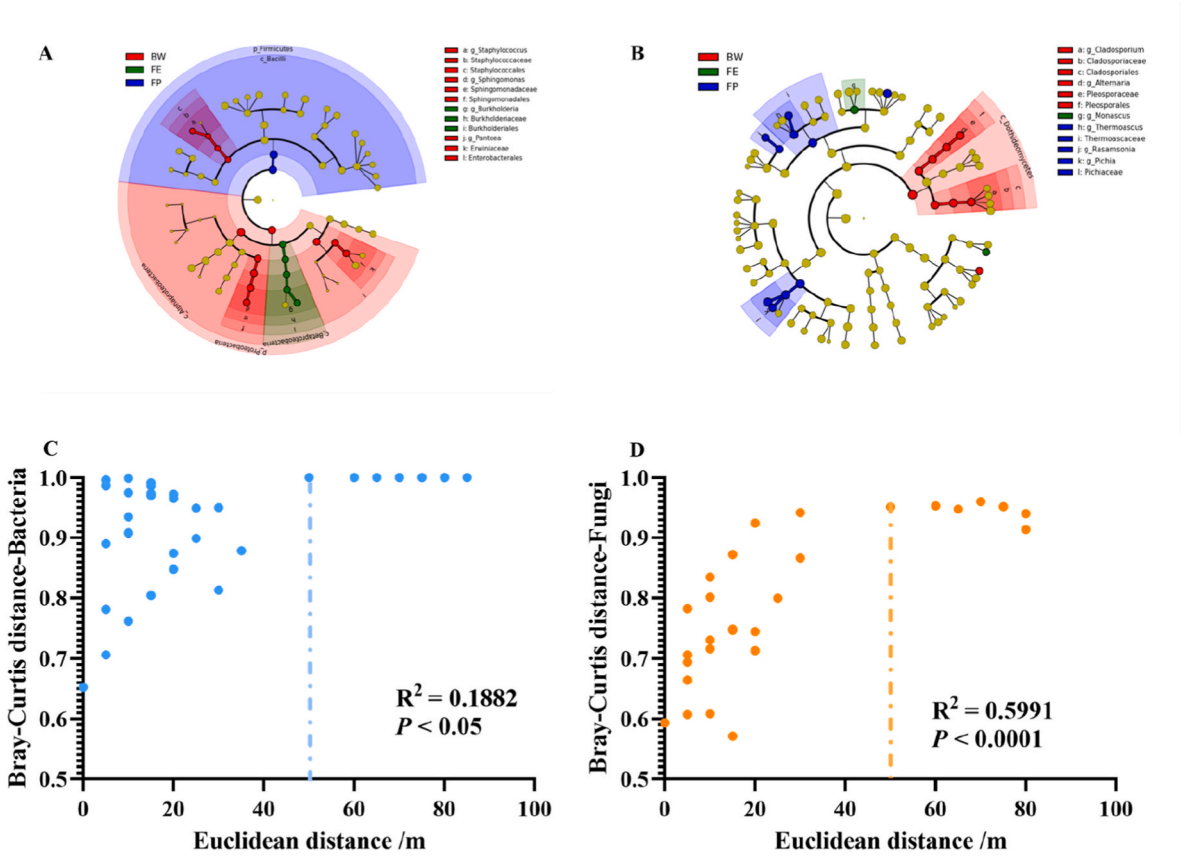

Fig. 5. Difference among three different environmental microecology. Linear discriminant effect size (LEfSe) analysis of the bacterial (A) and fungal (B) composition in distinct environments. Variation of bacterial (C) and fungal community (D) with geographical distance.

3.5. Abiotic factors and microbial interactions synergistically led to microbial differences in different environments

We used the relative abundance and occurrence frequency to select the core microorganisms in the environment. LEfSe analysis showed that the core microorganisms had environmental specificity. Correlation analysis and microbial network relationship were used to explain the correlation of environmental factors (abiotic factors) and microorganisms (biological factors) with microbial community construction.

By calculating the Spearman correlation between environmental factors and core microorganisms, we found that pH, acidity, and water content were significantly related to bacteria and fungi in different environments (Fig. 6). Environmental factors explained 65.17% of the changes in the bacterial community and 58.48% of fungi. It showed that the microbial environmental specificity was related to environmental factors, included pH, acidity, and water content of the soil. There were 2 pairs of positive correlation and 11 pairs of negative correlation between core bacteria and acidity, 5 pairs of positive correlation and 6 pairs of negative correlation between core fungi and acidity, among which *A jinshanensis*, *L. acetotolerans*, *Pichia*, and *S. cerevisiae* was positively correlated with acidity, acetic acid, lactic acid, and ethanol, while *Bacillus*, *Brevundimonas*, *Mucor* and *Cladosporium* were negatively correlated with acidity. It indicated that low pH, high ethanol, high water content, high temperature, and other extreme environments in the fermentation process and fermentation environment had screening effects on microorganisms (L. Chen, Feng, et al., 2022; Lin Chen, Feng, et al., 2022; Zhao et al., 2022; Zhao et al., 2019), resulting in low microbial diversity and high content of functional microorganisms related to fermentation.

In order to test the community stability in different environments, the molecular ecological network of three environment was analyzed (Fig. 7, Dataset S9, Mendeley Data: https://data.mendeley.com/datasets/82g57ypbrf/1). In the network of FP, 129 nodes and 1398 edges were obtained. In the network of FE, 61 nodes and 101 edges were obtained. In the network of BW, 76 nodes and 99 edges were obtained. The degree of modularity of the three environmental networks showed an increasing trend, which was 0.341, 0.657 and 0.798, respectively. Microbial connectivity increased in the fermentation environment (3.31) compared with the ecological environment (2.61). The above results indicated that the microbial community structure in the fermentation environment was affected by the fermentation process.

We used βNTI comparative analysis (Fig. 8) to evaluate the influence of the deterministic and stochastic processes on the assembly of environmental microbiota in different environments. With the increase in the distance from the distillery, the microbial community construction pattern showed an obvious deterministic-stochastic pattern. Homogeneous selection dominated the construction of bacteria (91.67%) and fungi (83.34%) in the FP, showing that the fermentation process was mainly affected by the deterministic process, which was caused by the environmental selection pressure. Both deterministic (27.67%) and stochastic processes (72.33%) dominated the construction of bacteria and fungi in the FE. Stochastic processes had a greater impact on the fermentation environment, and the cause of this stochastic process might be microorganisms in the fermentation process. The construction of bacteria in the BW was mainly affected by stochastic processes, while fungi were also affected by a small part of the variable selection (27.76%). Due to the weak change of ecological environment factors, the succession of bacteria and fungi was more affected by the random

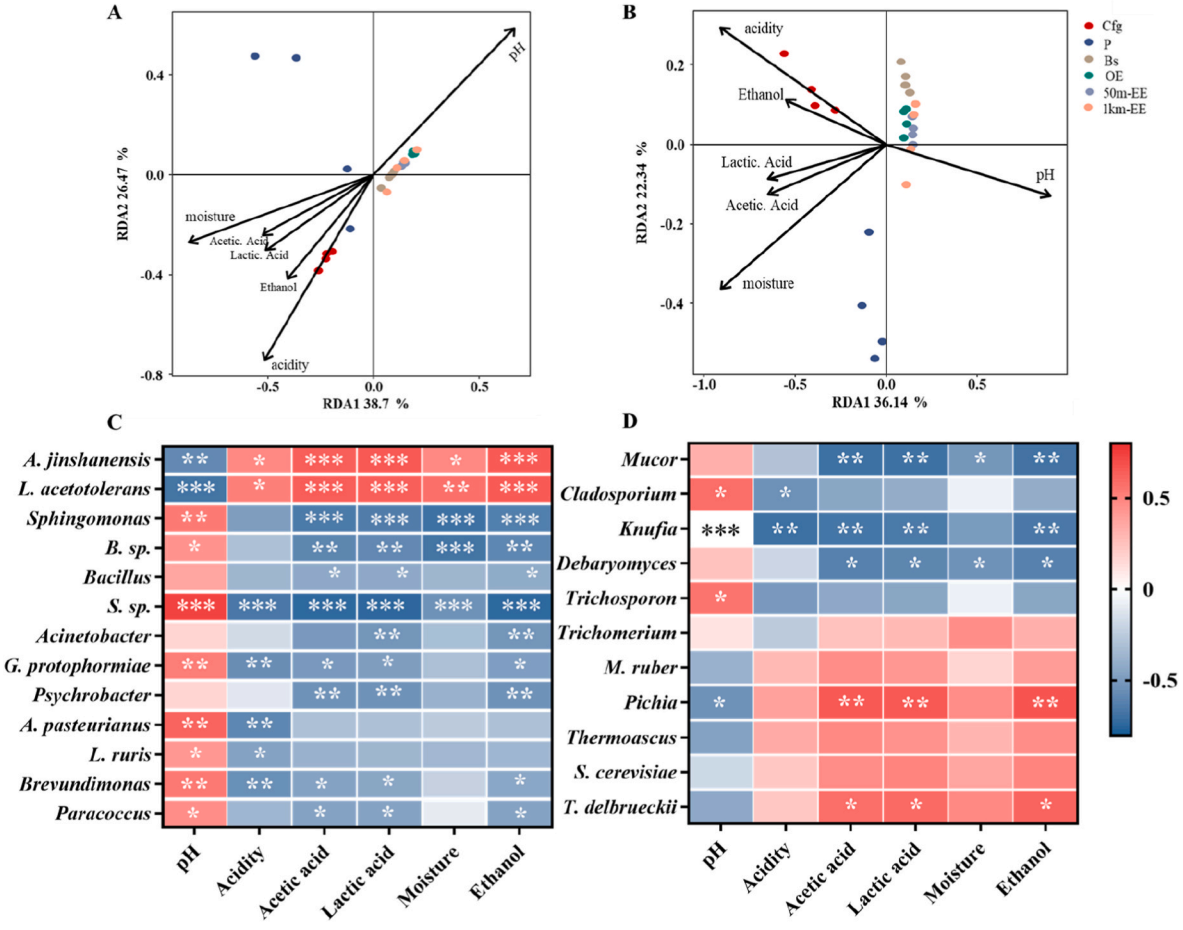

Fig. 6. Correlation analysis of microbial communities with environmental factors. RDA analysis between bacteria (A), fungi (B) and environmental factors in different environments. Heatmap of the correlation between dominant bacteria (C), fungi (D) and environmental factors.

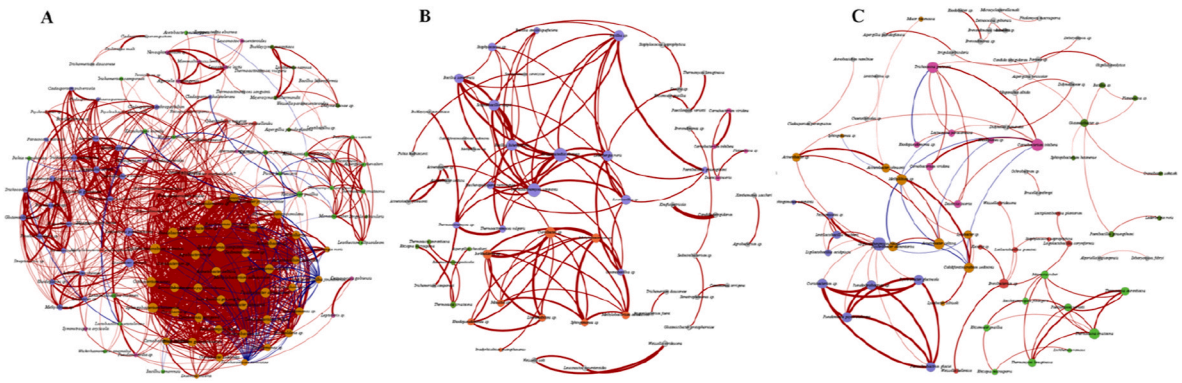

Fig. 7. Microbial network diagram in different environments. Microbial network in fermentation process (A). Microbial network in fermentation environment (B). Microbial network in environment outside brewing workshop (C). The red line represents positive correlation and the blue line represents negative correlation. Different colors of OTUs represent distribution in different modules. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

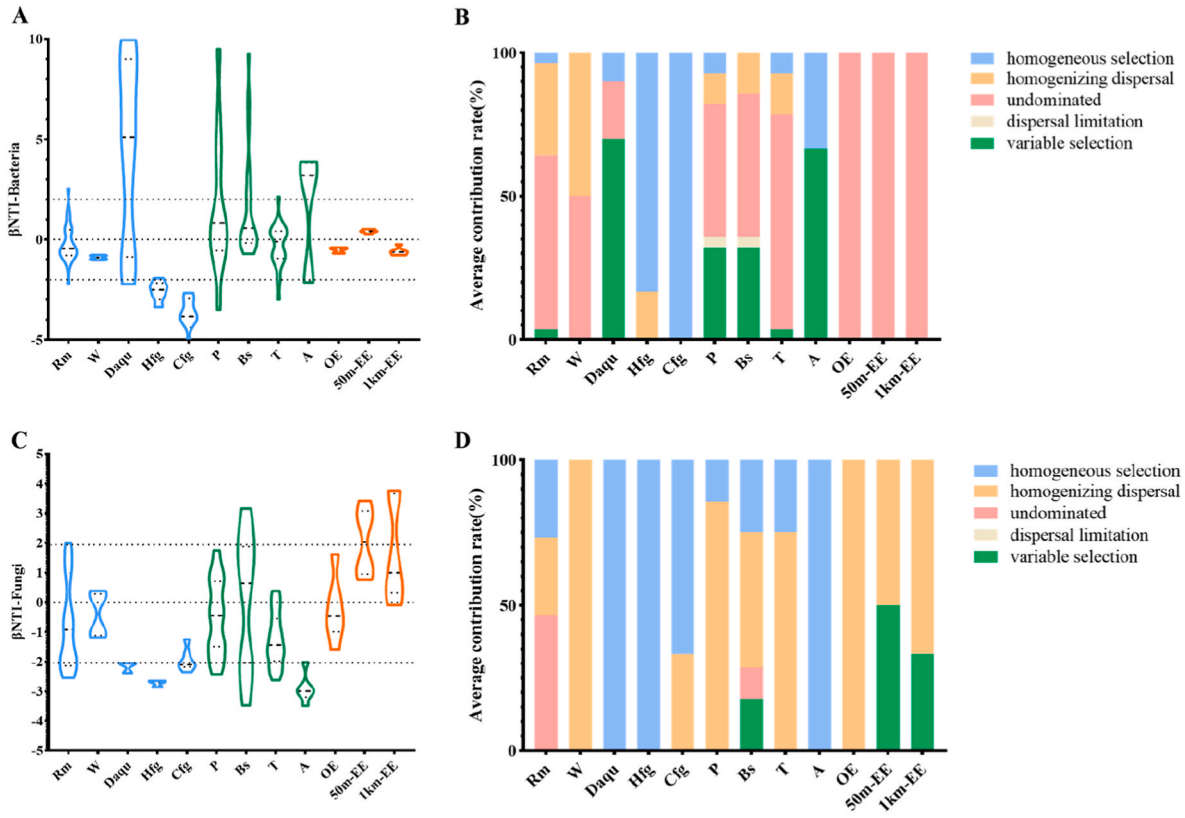

Fig. 8. Environmental bacteria (A, B) and fungi (C, D) community construction in various environments. The βNTI value is represented by a violin diagram, the wider the violin, the more sample points it represents.

birth, death, and reproduction of microorganisms.

4. Conclusion

Taking sauce-flavor *Baijiu* as an example, we clarified the regional microbial characteristics in the *Chishui* River producing area and the domestication effect of the fermentation process on environment. The ecological environment of the producing area showed high stability and the microbial community structure was not easily changed. Fungi in the core producing area contributed more to the *Baijiu* brewing process. Fermented grains and *Daqu* were the main sources of microbiota for indoor ground and tools. Microbiota in the air mainly came from *Daqu*. In addition, we found the distance decay pattern of fermented-related microorganisms in the sauce-flavor *Baijiu* producing region. The ecological environment 50m away from the distillery was less domesticated by the fermentation process, which demonstrated that the physical barrier, as well as some physical and chemical conditions, could reduce the risk of ecological environment pollution caused by *Baijiu* brewing to a certain extent. pH, acidity, and water content were the main environmental factors that caused microbial changes in different environments. Extreme environments in the fermentation process and fermentation environment had screening effects on microbial colonization. Abiotic factors and microbial interactions synergistically led to microbial differences across environments. This study provided the first comprehensive account of the interactions between the fermentation process and the environment. Besides, it highlighted the role of

geographic distance in the biogeographical distribution of microorganisms. These findings expanded our knowledge regarding sauce-flavor *Baijiu*, and provided a theoretical basis for using microbial terroir to improve the yield and flavor of regional *Baijiu*.

CRediT authorship contribution statement

Shuangping Liu: Funding acquisition, Project administration, Writing – review & editing. **Zhengfei Jiang:** Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Writing – original draft. **Dongna Ma:** Supervision, Writing – review & editing. **Xiaogang Liu:** Sample collection, Data curation. **Yilun Li:** Visualization, Supervision, Writing – review & editing. **Dongliang Ren:** Supervision, Writing – review & editing. **Ying Zhu:** Supervision, Writing – review & editing. **Hongyuan Zhao:** Method provision, Data curation. **Hui Qin:** Data curation. **Mengyang Huang:** Data curation. **Suyi Zhang:** Data curation, Writing – review & editing. **Jian Mao:** Funding acquisition, Supervision, Writing – review & editing.

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

Data will be made available on request.

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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.2022.102305.

References

Adedire, D. E., Jimoh, A. O., Kashim-Bello, Z., Shuaibu, B., Popoola, O. A., Pate, K. I., Uzor, O. S., Etingwa, E., Joda, J. F., & Opaleye, O. O. (2022). Microbiome diversity analysis of the bacterial community in Idah river, Kogi state, Nigeria. *Advances in Microbiology, 12*, 343–362. https://doi.org/10.4236/aim.2022.125025

Bengtsson Palme, J., Ryberg, M., Hartmann, M., Branco, S., Wang, Z., Godhe, A., De Wit, P., Sánchez-García, M., Ebersberger, I., de Sousa, F., Amend, A., Jumpponen, A., Unterseher, M., Kristiansson, E., Abarenkov, K., Bertrand, Y. J. K., Sanli, K., Eriksson, K. M., Vik, U., Veldre, V., & Nilsson, R. H. (2013). Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. *Methods in Ecology and Evolution, 4*(10), 914–919. https://doi.org/10.1111/2041-210X.12073

Bokulich, N. A., Bergsveinson, J., Ziola, B., & Mills, D. A. (2015). Mapping microbial ecosystems and spoilage-gene flow in breweries highlights patterns of contamination and resistance. *Elife, 4*, Article e04634. https://doi.org/10.7554/eLife.04634

Bokulich, N. A., & Mills, D. A. (2013). Facility-specific "house" microbiome drives microbial landscapes of artisan cheesemaking plants. *Applied and Environmental Microbiology, 79*(17), 5214–5223. https://doi.org/10.1128/aem.00934-13

Bokulich, N. A., Thorngate, J. H., Richardson, P. M., & Mills, D. A. (2014). Microbial biogeography of wine grapes is conditioned by cultivar, vintage, and climate. *Proceedings of the National Academy of Sciences of the United States of America, 111*(1), E139–E148. https://doi.org/10.1073/pnas.1317377110

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J., & Holmes, S. P. (2016). DADA2: High-Resolution sample inference from illumina amplicon data. *Nature Methods, 13*(7), 581–583. https://doi.org/10.1038/nmeth.3869

Chase, J. M., Kraft, N. J. B., Smith, K. G., Vellend, M., & Inouye, B. D. (2011). Using null models to disentangle variation in community dissimilarity from variation in α-diversity. *Ecosphere, 2*(2), art24. https://doi.org/10.1890/ES10-00117.1

Chen, J., Feng, T., Wang, B., He, R., Xu, Y., Gao, P., Zhang, Z. H., Zhang, L., Fu, J., Liu, Z., & Gao, X. (2022a). Enhancing organic selenium content and antioxidant activities of soy sauce using nano-selenium during soybean soaking. *Frontiers in Nutrition, 9*, Article 970206. https://doi.org/10.3389/fnut.2022.970206

Chen, L., Li, X., Lou, X., Shu, W., Hai, Y., Wen, X., & Yang, H. (2022b). NMR-Based metabolomics reveals the antibacterial effect of electrolysed water combined with citric acid on *Aeromonas spp.* in barramundi (*Lates calcarifer*) fillets. *Food Research International, 162*(Pt A), Article 112046. https://doi.org/10.1016/j.foodres.2022.112046

Chen, L., Liu, Q., Zhao, X., Zhang, H., Pang, X., & Yang, H. (2022c). Inactivation efficacies of lactic acid and mild heat treatments against *Escherichia coli* strains in organic broccoli sprouts. *Food Control, 133*, Article 108577. https://doi.org/10.1016/j.foodcont.2021.108577

Chen, W., Wei, J., Su, Z., Wu, L., Liu, M., Huang, X., Yao, P., & Wen, D. (2022d). Deterministic mechanisms drive bacterial communities assembly in industrial wastewater treatment system. *Environment International, 168*, Article 107486. https://doi.org/10.1016/j.envint.2022.107486

Clarke, K. R. (1993). Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology, 18*(1), 117–143. https://doi.org/10.1111/j.1442-9993.1993.tb00438.x

Feng, M., Tripathi, B. M., Shi, Y., Adams, J. M., Zhu, Y. G., & Chu, H. (2019). Interpreting distance-decay pattern of soil bacteria via quantifying the assembly processes at multiple spatial scales. *Microbiologyopen, 8*(9), e851. https://doi.org/10.1002/mbo3.851

Feng, K., Zhang, Z., Cai, W., Liu, W., Xu, M., Yin, H., Wang, A., He, Z., & Deng, Y. (2017). Biodiversity and species competition regulate the resilience of microbial biofilm community. *Molecular Ecology, 26*(21), 6170–6182. https://doi.org/10.1111/mec.14356

Gao, X., Feng, T., Liu, E., Shan, P., Zhang, Z., Liao, L., & Ma, H. (2021). Ougan juice debittering using ultrasound-aided enzymatic hydrolysis: Impacts on aroma and taste. *Food Chemistry, 345*, Article 128767. https://doi.org/10.1016/j.foodchem.2020.128767

Gao, X., Liu, E., Zhang, J., Yang, L., Huang, Q., Chen, S., Ma, H., Ho, C. T., & Liao, L. (2020). Accelerating aroma formation of raw soy sauce using low intensity sonication. *Food Chemistry, 329*, Article 127118. https://doi.org/10.1016/j.foodchem.2020.127118

Gao, X., Zhang, J., Liu, E., Yang, M., Chen, S., Hu, F., Ma, H., Liu, Z., & Yu, X. (2019). Enhancing the taste of raw soy sauce using low intensity ultrasound treatment during moromi fermentation. *Food Chemistry, 298*, Article 124928. https://doi.org/10.1016/j.foodchem.2019.05.202

Gayevskiy, V., & Goddard, M. R. (2012). Geographic delineations of yeast communities and populations associated with vines and wines in New Zealand. *The ISME Journal, 6*(7), 1281–1290. https://doi.org/10.1038/ismej.2011.195

Jiao, S., Chen, W., & Wei, G. (2017). Biogeography and ecological diversity patterns of rare and abundant bacteria in oil-contaminated soils. *Molecular Ecology, 26*(19), 5305–5317. https://doi.org/10.1111/mec.14218

Jin, G., Zhu, Y., & Xu, Y. (2017). Mystery behind Chinese liquor fermentation. *Trends in Food Science & Technology, 63*, 18–28. https://doi.org/10.1016/j.tifs.2017.02.016

Kembel, S. W., Cowan, P. D., Helmus, M. R., Cornwell, W. K., Morlon, H., Ackerly, D. D., Blomberg, S. P., & Webb, C. O. (2010). Picante: R tools for integrating phylogenies and ecology. *Bioinformatics, 26*(11), 1463–1464. https://doi.org/10.1093/bioinformatics/btq166

Knight, S., Klaere, S., Fedrizzi, B., & Goddard, M. R. (2015). Regional microbial signatures positively correlate with differential wine phenotypes: Evidence for a microbial aspect to terroir. *Scientific Reports, 5*, Article 14233. https://doi.org/10.1038/srep14233

Knights, D., Kuczynski, J., Charlson, E. S., Zaneveld, J., Mozer, M. C., Collman, R. G., Bushman, F. D., Knight, R., & Kelley, S. T. (2011). Bayesian community-wide culture-independent microbial source tracking. *Nature Methods, 8*(9), 761–763. https://doi.org/10.1038/nmeth.1650

Kõljalg, U., Nilsson, R. H., Abarenkov, K., Tedersoo, L., Taylor, A. F., Bahram, M., Bates, S. T., Bruns, T. D., Bengtsson-Palme, J., Callaghan, T. M., Douglas, B., Drenkhan, T., Eberhardt, U., Dueñas, M., Grebenc, T., Griffith, G. W., Hartmann, M., Kirk, P. M., Kohout, P., … Larsson, K. H. (2013). Towards a unified paradigm for sequence-based identification of fungi. *Molecular Ecology, 22*(21), 5271–5277. https://doi.org/10.1111/mec.12481

Li, R., Lin, M., Guo, S., Yang, S., Han, X., Ren, M., Song, Y., Du, L., You, Y., Zhan, J., & Huang, W. (2021a). A fundamental landscape of fungal biogeographical patterns across the main Chinese wine-producing regions and the dominating shaping factors. *Food Research International, 150*(Pt A), Article 110736. https://doi.org/10.1016/j.foodres.2021.110736

Li, Y., Liu, S., Zhang, S., Liu, T., Qin, H., Shen, C., Liu, H., Yang, F., Yang, C., Yin, Q., & Mao, J. (2022). Spatiotemporal distribution of environmental microbiota in spontaneous fermentation workshop: The case of Chinese Baijiu. *Food Research International, 156*, Article 111126. https://doi.org/10.1016/j.foodres.2022.111126

Li, S., Tian, Y., Jiang, P., Lin, Y., Liu, X., & Yang, H. (2021b). Recent advances in the application of metabolomics for food safety control and food quality analyses. *Critical Reviews in Food Science and Nutrition, 61*(9), 1448–1469. https://doi.org/10.1080/10408398.2020.1761287

Liu, H., & Sun, B. (2018). Effect of fermentation processing on the flavor of Baijiu. *Journal of Agricultural and Food Chemistry, 66*(22), 5425–5432. https://doi.org/10.1021/acs.jafc.8b00692

Liu, J., Zhu, S., Liu, X., Yao, P., Ge, T., & Zhang, X. H. (2020). Spatiotemporal dynamics of the archaeal community in coastal sediments: Assembly process and Co-occurrence relationship. *The ISME Journal, 14*(6), 1463–1478. https://doi.org/10.1038/s41396-020-0621-7

Lou, X., Wen, X., Chen, L., Shu, W., Wang, Y., Hoang, T. T., & Yang, H. (2022). Changes in texture, rheology and volatile compounds of golden pomfret sticks inoculated with *Shewanella baltica* during spoilage. *Food Chemistry, 404*(Pt A), Article 134616. https://doi.org/10.1016/j.foodchem.2022.134616

Mosher, J. J., Bernberg, E. L., Shevchenko, O., Kan, J., & Kaplan, L. A. (2013). Efficacy of a 3rd generation high-throughput sequencing platform for analyses of 16S rRNA genes from environmental samples. *Journal of Microbiological Methods, 95*(2), 175–181. https://doi.org/10.1016/j.mimet.2013.08.009

Pavlopoulos, G. A., Soldatos, T. G., Barbosa-Silva, A., & Schneider, R. (2010). A reference guide for tree analysis and visualization. *BioData Mining, 3*(1), 1. https://doi.org/10.1186/1756-0381-3-1

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., & Huttenhower, C. (2011). Metagenomic biomarker discovery and explanation. *Genome Biology, 12*(6), R60. https://doi.org/10.1186/gb-2011-12-6-r60

Simpson, E. H. (1949). Measurement of diversity. *Nature, 163*(4148), 688. https://doi.org/10.1038/163688a0

Singer, E., Bushnell, B., Coleman-Derr, D., Bowman, B., Bowers, R. M., Levy, A., Gies, E. A., Cheng, J. F., Copeland, A., Klenk, H. P., Hallam, S. J., Hugenholtz, P., Tringe, S. G., & Woyke, T. (2016). High-resolution phylogenetic microbial community profiling. *The ISME Journal, 10*(8), 2020–2032. https://doi.org/10.1038/ismej.2015.249

Stegen, J. C., Lin, X., Fredrickson, J. K., Chen, X., Kennedy, D. W., Murray, C. J., Rockhold, M. L., & Konopka, A. (2013). Quantifying community assembly processes and identifying features that impose them. *The ISME Journal, 7*(11), 2069–2079. https://doi.org/10.1038/ismej.2013.93

Stegen, J. C., Lin, X., Konopka, A. E., & Fredrickson, J. K. (2012). Stochastic and deterministic assembly processes in subsurface microbial communities. *The ISME Journal, 6*(9), 1653–1664. https://doi.org/10.1038/ismej.2012.22

Tan, Y., Du, H., Zhang, H., Fang, C., Jin, G., Chen, S., Wu, Q., Zhang, Y., Zhang, M., & Xu, Y. (2022). Geographically associated fungus-bacterium interactions contribute to the formation of geography-dependent flavor during high-complexity spontaneous fermentation. *Microbiology Spectrum, 10*(5), Article e0184422. https://doi.org/10.1128/spectrum.01844-22

Wang, L. (2022). Research trends in jiang-flavor Baijiu fermentation: From fermentation microecology to environmental ecology. *Journal of Food Science, 87*(4), 1362–1374. https://doi.org/10.1111/1750-3841.16092

Wang, X., Du, H., Zhang, Y., & Xu, Y. (2018). Environmental microbiota drives microbial succession and metabolic profiles during Chinese liquor fermentation. *Applied and Environmental Microbiology, 84*(4), Article e02369. https://doi.org/10.1128/aem.02369-17, 02317.

Xiao, C., Yang, Y., Lu, Z. M., Chai, L. J., Zhang, X. J., Wang, S. T., Shen, C. H., Shi, J. S., & Xu, Z. H. (2021). Daqu microbiota exhibits species-specific and periodic succession features in Chinese Baijiu fermentation process. *Food Microbiology, 98*, Article 103766. https://doi.org/10.1016/j.fm.2021.103766

Yan, Y., Li, X., Guan, H., Huang, L., Ma, X., Peng, Y., Li, Z., Nie, G., Zhou, J., Yang, W., Cai, Y., & Zhang, X. (2019). Microbial community and fermentation characteristic of Italian ryegrass silage prepared with corn stover and lactic acid bacteria. *Bioresource Technology, 279*, 166–173. https://doi.org/10.1016/j.biortech.2019.01.107

Zhang, H., Wang, L., Tan, Y., Wang, H., Yang, F., Chen, L., Hao, F., Lv, X., Du, H., & Xu, Y. (2021a). Effect of *Pichia* on shaping the fermentation microbial community of sauce-flavor *Baijiu*. *International Journal of Food Microbiology, 336*, Article 108898. https://doi.org/10.1016/j.ijfoodmicro.2020.108898

Zhang, H. X., Wang, L., Tan, Y. W., Wang, H. Y., Yang, F., Chen, L. Q., Hao, F., Lv, X. B., Du, H., & Xu, Y. (2021b). Effect of *Pichia* on shaping the fermentation microbial community of sauce-flavor Baijiu. *International Journal of Food Microbiology, 336*, Article 108898. https://doi.org/10.1016/j.ijfoodmicro.2020.108898

Zhang, Y., Zhang, Z. H., He, R., Xu, R., Zhang, L., & Gao, X. (2022). Improving soy sauce aroma using high hydrostatic pressure and the preliminary mechanism. *Foods, 11*(15). https://doi.org/10.3390/foods11152190

Zhang, J., Zhang, N., Liu, Y. X., Zhang, X., Hu, B., Qin, Y., Xu, H., Wang, H., Guo, X., Qian, J., Wang, W., Zhang, P., Jin, T., Chu, C., & Bai, Y. (2018). Root microbiota shift in rice correlates with resident time in the field and developmental stage. *Science China Life Sciences, 61*(6), 613–621. https://doi.org/10.1007/s11427-018-9284-4

Zhao, L., Li, H., Wang, K., Li, X., Guo, C., & Yang, H. (2022). Effects of electrolysed water and levulinic acid combination on microbial safety and polysaccharide nanostructure of organic strawberry. *Food Chemistry, 394*, Article 133533. https://doi.org/10.1016/j.foodchem.2022.133533

Zhao, W., Wang, X. B., Shi, L. Y., Zhu, W. Y., Ma, L., Wang, J. J., Xu, S. Y. R., Zhang, X. X., Han, Y. F., & Huang, Z. Y. (2021). Calculation method for stochastic and deterministic assembly processes of prokaryotic communities. *Bio-protocol.* , Article e2003400. https://doi.org/10.21769/BioProtoc.2003400

Zhao, L., Zhao, M. Y., Phey, C. P., & Yang, H. (2019). Efficacy of low concentration acidic electrolysed water and levulinic acid combination on fresh organic lettuce (*Lactuca sativa var. Crispa L.*) and its antimicrobial mechanism. *Food Control, 101*, 241–250. https://doi.org/10.1016/j.foodcont.2019.02.039

Zwirzitz, B., Wetzels, S. U., Dixon, E. D., Stessl, B., Zaiser, A., Rabanser, I., Thalguter, S., Pinior, B., Roch, F.-F., Strachan, C., Zanghellini, J., Dzieciol, M., Wagner, M., & Selberherr, E. (2020). The sources and transmission routes of microbial populations throughout a meat processing facility. *Npj Biofilms and Microbiomes, 6*(1), 26. https://doi.org/10.1038/s41522-020-0136-z