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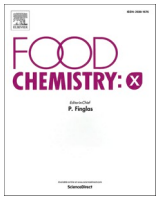

Initial abiotic factors as key drivers in core microbe assembly: Regulatory effects on flavor profiles in light-flavor *Baijiu*

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ABSTRACT

Instability in initial abiotic factors of open solid-state fermentation systems can significantly alter *Baijiu*'s flavor profile, but the mechanisms governing microbial interactions and flavor formation remain unclear. This study comprehensively monitored changes in abiotic factors, microbial communities, and flavor profiles across two distinct fermentation processes in a *Baijiu* distillery, which differed significantly in their management of initial abiotic factors. Our results revealed significant differences in abiotic factors between the two groups, including moisture, ethanol, acidity, glucose, and organic acid levels. The assembly of microbial communities in fermented grains was primarily driven by deterministic processes. The moisture content in the fermented grains positively affected the growth and metabolism of core microbiota. The rapid proliferation and metabolism of core microbes led to a rapid increase in the acidity of the fermented grains, alongside a significant accumulation of ethyl lactate. This study provides technical support and theoretical guidance for *Baijiu* production.

1. Introduction

Baijiu is a traditional Chinese distilled spirit that utilizes *Daqu* as a fermentation starter culture and sorghum as primary raw material, undergoing solid-state fermentation to shape its distinctive flavor profile (Zheng & Han, 2016). During the fermentation process of *Baijiu*, microorganisms sourced from *Daqu*, environment, and raw materials engage in complex interactions (Kang et al., 2024; Wu, Zhu, Fang, Wijffels, & Xu, 2021). These microbial interactions are essential in shaping the structure of microbial communities and regulating metabolic functions, which subsequently influences the flavor and quality of *Baijiu* (Huang et al., 2020). The multiple interactions between environmental factors and microorganisms in the open solid-state fermentation system of *Baijiu* complicate efforts to establish clear and stable interaction relationships for evaluating the metabolic potential of microbial communities. While interactions among microorganisms significantly influence the succession of these communities, the intricate interplay between various biotic and abiotic factors also plays a crucial regulatory role in community function (Kang et al., 2022). Numerous studies have demonstrated a complex relationship between microbial community succession and flavor metabolism during *Baijiu* fermentation, influenced by environmental factors (Du, Ji, Xing, Wang, & Xu, 2021; Tan, Zhong, Zhao, Du, & Xu, 2019; Wu et al., 2021). However, significant challenges remain in identifying and managing the key drivers that influence microbial interactions and community assembly during fermentation, as well as clarifying their cascading effects on the formation of flavor profiles. Addressing these challenges is crucial for manipulating microbial communities and regulating their metabolism.

Initial abiotic factors, including temperature and moisture, as well as biotic factors represented by the initial microbiota and careful control of fermentation parameters, collectively influence microbial succession and interactions (Ji et al., 2023; Kang et al., 2024; Wang et al., 2023; Zhang et al., 2021). The effective regulation of initial temperature and moisture is a critical operational element in the pretreatment of raw materials following the cooking process. Given that *Baijiu* production largely relies on traditional manual operations rooted in experiential knowledge, inadequate control during the initial fermentation phase can result in abnormal change in environmental factors and microbial succession (Tu et al., 2022; Zhao et al., 2020). For example, improper management of the initial abiotic factors in fermented grains can cause a

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rapid increase in acidity, leading to an excessive accumulation of ethyl lactate and an imbalance between ethyl acetate and ethyl lactate ratios (Wang et al., 2023). This issue frequently arises in the production of light-flavor *Baijiu* (Pang et al., 2018; Zhao et al., 2020; Zheng & Han, 2016). Excessive ethyl lactate content leads to an unpleasant, stuffy, astringent, and slightly bitter flavor profile (Zhao et al., 2020). Although high-alcohol distillation can lower ethyl lactate levels, it may also result in the loss of other critical flavor compounds and decrease the yield of base liquor (Li, 2022). Effective control of the production and accumulation of key flavor compounds can be indirectly achieved by regulating the growth of core microbiota through adjustments in initial environmental conditions.

Thus, in this study, two independent fermentation batches were selected to perform experiments in a famous distillery with a long-term producing light-flavor *Baijiu*. The two batches used the same raw materials (sorghum), *Daqu* and the same fermentation environment, while exhibited significant differences in the control of initial moisture and temperature in fermented grain. This study aims to elucidate the intricate interplay between abiotic factors and microbial communities, which shape the fermentation process and the final product quality of light-flavor *Baijiu*, highlighting the cascade regulation of core microbial assembly mediated by abiotic factors in the formation of flavor compounds of *Baijiu*. To this end, we compared differences in microbial community succession, core microbial interactions, microbial community assembly modes, and the formation and accumulation of flavor profiles, all under the influence of initial abiotic factors. Through multi-omics data analysis, we explored the intricate relationships between environmental factors, core microbes, and key metabolites. Understanding these interactions is vital for elucidating how environmental drivers shape the metabolic activity of core microorganisms during *Baijiu* fermentation. This knowledge provides a foundation for achieving standardized and stable fermentation management, advancing both the quality and consistency of the brewing process.

2. Materials and methods

2.1. Experimental design and sample collection

Daqu and fermented grain samples were collected from a well-known light-flavor *Baijiu* distillery in Shanxi province, China, in 2020, following the traditional light-flavor *Baijiu* fermentation process (Kang et al., 2022). Two independent fermentation batches were selected for sampling and monitoring based on the differing control of initial moisture and temperature: a normal group, in which the initial moisture and temperature were within empirical ranges, and a defect group, characterized by abnormal moisture and temperature conditions. Sampling points for the fermented grains were at 0, 7, 14, 21, 28, 35, 42, 49, and 56 d. Samples were collected using a customized sampler at five locations within the ground jar according to the method of Kang et al. (2022). After each sampling, the five samples collected from the same ground jar were mixed evenly and labeled as one sample. Portions of the collected *Daqu* and fermented grain samples were used for microbial counts and physicochemical parameter monitoring, with the remainder stored at −80 °C for further analysis.

2.2. Microbiological and physicochemical analysis during fermentation

Viable cell counts in fermented grains, including mesophilic bacteria, yeast, lactic acid bacteria, and molds, were determined using corresponding selective media (Huang et al., 2020). Changes in the physicochemical parameters of fermented grains, including core temperature, moisture, pH, and titratable acidity, were measured as described by Pang et al. (2018). Starch content in fermented grains was assessed using the DNS method: 5 g of fermented grain sample was hydrolyzed with 50 mL of 20 % (*v*/v) HCl for 30 min. The pH was then adjusted to 7.0, and the DNS method was used to detect the reducing sugar content (Miller, 1959). Glucose, ethanol, glycerol, and organic acids, including acetic acid, lactic acid, tartaric acid, and succinic acid, were all determined using an Agilent HPLC system (Agilent Technologies Inc., Santa Clara, CA, USA) with an Aminex HPX-87H column (300 × 7.8 mm; Bio-Rad Laboratories, Hercules, CA, USA) (Kang et al., 2022).

2.3. Microbial community composition and succession by amplicon sequencing

The extraction of metagenomic DNA from *Daqu* and fermented grain samples was performed using our previously described CTAB-based method (Huang et al., 2020). Briefly, microbial cells were pelleted by centrifugation in phosphate-buffered saline (120 mM, pH 8.0). The microbial pellet of each sample was then resuspended in 1 mL of CTAB lysis buffer (0.1 M Tris-HCl, 0.1 M EDTA, 0.1 M Na_3PO_4, 1.5 M NaCl, 1 % *w/v* CTAB (pH = 8), 5 μL of proteinase K (20 mg/mL), 10 μL of lysozyme (50 mg/mL), 10 μL of lyticase (10 u/mL), and 5 μL of RNase A (10 mg/mL)). After mechanical grinding and SDS precipitation, the DNA was extracted using the chloroform-isoamyl alcohol method, precipitated with isopropanol, and washed with 70 % ethanol. For bacterial community analysis, the primer pair 338F (5'-ACTCCTACGGGAGGCAGCA-3′) and 860R (5'-GGACTACHVGGGTWTCTAAT-3′) was used to amplify the V3-V4 region of the 16S rRNA gene. For fungal community analysis, the internal transcribed spacer (ITS) region was amplified with the primer pair ITS5F (5'-GGAAGTAAAAGTCGTAACAAGG-3′) and ITS1R (5'-GCTGCGTTCTTCATCGATGC-3′) (Kang et al., 2022).

The PCR products were purified and paired-end sequenced on an Illumina NovaSeq 6000 PE250 platform (Illumina, San Diego, CA, USA). Data analysis was performed using the unoise3 denoising algorithm in USEARCH 11 to cluster sequencing fragments into ASVs (Amplicon Sequence Variants). The representative sequences of bacterial ASVs were compared against the SILVA 138.1 and EzBioCloud (www.ezbiocloud.net) databases, while the representative sequences of fungal ASVs were compared against the UNITE 8.2 database and the Central Bureau of Fungal Cultures database (CBS-KNAW, www.wi.knaw.nl).

2.4. Volatile compounds profile during fermentation

Volatile compounds were analyzed using a HS-SPME/GC–MS system (Agilent 6890 GC and 5975B series MS detector, Agilent Technologies Inc.) equipped with an Agilent 19091F-105 HP-FFAP column (50 m × 200 μm × 0.33 μm). A 50:30 mm DVB-CAR-PDMS SPME fiber (Supelco Co., Bellefonte, PA, USA) was used for headspace solid-phase microextraction (SPME). Specifically, 3 g of fermented grain sample was added to 20 mL of deionized water. After treatment for 30 min, the mixture was centrifuged at 6000 ×*g* for 10 min at 4 °C, and the supernatant was collected. Then, 3 mL of the collected supernatant was transferred to a 15 mL vial, to which 10 μL of 4-methyl-2-pentanol (125 mg/L) was added as an internal standard, along with 3 g of NaCl. The vial was sealed with a PTFE silicon septum, and volatile compounds were collected at 60 °C for 30 min, followed by GC–MS analysis. The injection port temperature was set to 270 °C with a thermal desorption time of 10 min. The flow rate of the helium carrier gas was 1 mL/min. The temperature program started at 50 °C (held for 2 min), increased at 2 °C/min to 85 °C and was held at 85 °C for 0.1 min, increased at 5 °C/min to 230 °C, and was held at 230 °C for 2 min. Mass spectrometry analysis was performed using a 70 eV ionization source (EI). The mass spectrometer operated in full scan mode (20–350 *m/z*). The retention index (RI) was calculated using a series of standard alkanes C8-C40 as an external reference under the same conditions. The volatile compounds were qualitatively identified by comparing the mass spectra and RI values in the NIST17 standard library, with the matching index≥70 %. Semi-quantification of volatile compounds was calculated from peak areas according to the internal standard.

2.5. Statistical analysis

All experiments were performed in triplicate, and the results are presented as mean ± standard deviation. Alpha and beta diversity indices were calculated using the "core diversity" plugin in QIIME2 (Bolyen et al., 2019). The Wilcoxon signed-rank test was used to determine statistically significant differences among different fermented grain samples, with significance levels indicated as follows: *: $P < 0.05$, **: $P < 0.01$, and ***: $P < 0.001$. Differences in microbial community structure were analyzed using the Bray-Curtis distance algorithm, incorporating Principal Coordinates Analysis (PCoA), Analysis of Similarities (ANOSIM), Multi-Response Permutation Procedure (MRPP) group difference analysis, and Adonis multi-factor variance analysis, all conducted through the Vegan package in R. Linear Discriminant Analysis (LDA) Effect Size (LEfSe) analysis was used to identify significant variations among different fermented grain samples (LDA > 2, $P < 0.05$) using the online platform available at https://www.microbiomeanalyst.ca. PICRUSt2 (Phylogenetic investigation of communities by reconstruction of unobserved states) were used to predict the potential metabolic functions of microbial community. The thresholds for the co-occurrence network analysis were established as follows: for bacterial networks, the criteria were set at |Spearman's rho| > 0.8 and $P < 0.01$, while for fungal networks, the criteria were |Spearman's rho| > 0.7 and $P < 0.01$. Within-module connectivity (*Zi*) and among-module connectivity (*Pi*) were determined to identify keystone species in the network. The bacterial and fungal co-occurrence networks were performed using Gephi 0.9.2. Random Forest analysis (Number of trees = 500) was employed to identify key volatile compounds contributing to the variation of fermented grains during the fermentation using R software (version 4.3.1). Correlation network among abiotic factors, key volatile metabolites, and core microbes in the fermented grains were constructed with |Spearman's rho| > 0.6 and $P < 0.05$ and visualized via Cytoscape (version 3.8.0).

3. Results

3.1. Dynamics of physicochemical parameters in fermented grains during fermentation

The fermentation process of fermented grains typically followed a two-phase pattern characterized by a rapid change phase followed by a stable phase in both the normal and defect groups (Fig. 1). The initial temperature and moisture content were higher in the defect group compared to the normal group. The temperature in both groups peaked on the 7th day of fermentation and subsequently decreased (Fig. 1A). Throughout the fermentation period, the defect group maintained higher moisture content (Fig. 1B). Ethanol, primarily produced through yeast metabolism during *Baijiu* fermentation, may also be generated by lactic acid bacteria through heterolactic fermentation (Wang et al., 2021). Ethanol and glycerol production were slower in the normal group during the early stages of fermentation and remained lower than in the defect group later (Fig. 1C and D). As fermentation progressed, the starch content in the fermented grains of both groups dropped sharply (Fig. 1E). The glucose content initially increased and then decreased (Fig. 1F). Changes in pH, acidity, and organic acid content in the fermented grains reflect the metabolic activities of the microbial community, particularly lactic acid bacteria, which are crucial physicochemical indicators (Kang et al., 2022). As fermentation proceeded, the acidity of the fermented grains increased, and pH decreased (Fig. 1G and H). In the defect group, lactic acid production from day 0 to day 7 of fermentation was slightly higher than in the normal group, continuing to increase and reaching 6.09 g/100 g by the end of fermentation. In contrast, the lactic acid content in the normal group reached 1.70 g/100 g on the 7th day and remained stable throughout the fermentation process (Fig. 1I). Additionally, the contents of acetic acid, succinic acid, malic acid, and tartaric acid in the defect group were higher than those in the normal group (Figs. 1J–1M). The differences in initial abiotic factors -temperature and moisture- could significantly affect a group of fermentation physicochemical parameters between the two groups, with values being higher in the defect group compared to the normal group (Fig. 1N).

3.2. Microbial composition and structural succession of fermented grains during fermentation

First, the viable count of culturable microbes in the fermented grains during fermentation was monitored. The trends in the changes of total bacteria and lactic acid bacteria (LAB) in the normal group and the defect group differed more than those of yeast and molds (Fig. S1). Next, the microbial composition and structure were analyzed using amplicon sequencing. The alpha diversity, including both the Chao1 and Faith's_pd indices, revealed that the overall richness and diversity of the bacterial community in the normal group were significantly higher than those in the defect group; however, they were lower in the normal group than in the defect group at the beginning of fermentation (Fig. 2A and B). For the fungal community, the diversity was lower in the normal group compared to the defect group at the beginning of fermentation. However, Wilcoxon rank-sum test analysis showed no significant overall difference in fungal alpha diversity between the two groups (Fig. 2C and D). A PCoA analysis based on the Bray-Curtis distance matrix of ASVs was conducted to compare the structural differences in microbial communities. There was a distinct separation in the overall structure of bacterial and fungal communities in the fermented grains of the normal and defect groups (Fig. S2 A and B). Additionally, ANOSIM, MRPP, and Adonis confirmed that there were significant differences in the microbial structures between the normal and defect groups (Table 1).

The dominant microbial genus succession in the normal and defect groups is shown in Fig. 2E. Despite using the same *Daqu* for fermentation, the microbial communities in the fermented grains exhibited different compositions. At the beginning of fermentation, the dominant bacterial genera in both groups *were Leuconostoc, Weissella, Pediococcus*, and *Pantoea*. Starting from the 14th day, *Lactobacillus* rapidly succeeded and became the most abundant genus in the defect group, maintaining its dominance until the end of fermentation. In the normal group, by the 7th day of fermentation, the dominant bacteria had evolved to *Lactiplantibacillus* and *Leuconostoc*, maintaining their dominance until the 21st day. From the 28th day onward, the relative abundance of *Lactobacillus* continuously increased, dominating until the end of fermentation. Regarding the fungal communities, *Saccharomycopsis* and *Pichia* were the dominant fungi throughout fermentation in both groups. Additionally, *Thermoascus* rapidly increased from the 14th day of fermentation, becoming a dominant fungal genus in the defect group.

3.3. Comparison of microbial community composition and potential metabolic function between normal and defect groups

Differences in microbial composition between bacterial and fungal communities of the normal and defect groups were analyzed using LEfSe analysis. There were more bacteria and less fungi enriched in the normal group compared to the defect group. In terms of bacterial communities, there were 20 bacteria enriched in the normal group, predominantly lactic acid bacteria such as *Lactiplantibacillus*, *Leuconostoc*, *Lacticaseibacillus*, *Paucilactobacillus*, and *Lactococcus*. In contrast, there were only 7 bacteria enriched in the defect group, with *Lactobacillus* being the most prominent (Fig. 3A). Regarding fungal communities, there were 8 fungi enriched in the normal group, such as *Saccharomyces*, *Kazachstania*, and *Saccharomycopsis*. In contrast, the defect group exhibited 18 genus-level biomarkers, prominently featuring *Thermoascus*, *Pichia*, and *Aspergillus* (Fig. 3B).

Furthermore, PICRUSt2 was used to predict the genes encoding enzymes related to starch and sucrose metabolism, glycolysis/gluconeogenesis, and pyruvate metabolism, which mainly related to flavor formation of *Baijiu* (Kang et al., 2021). The abundance of these enzyme-

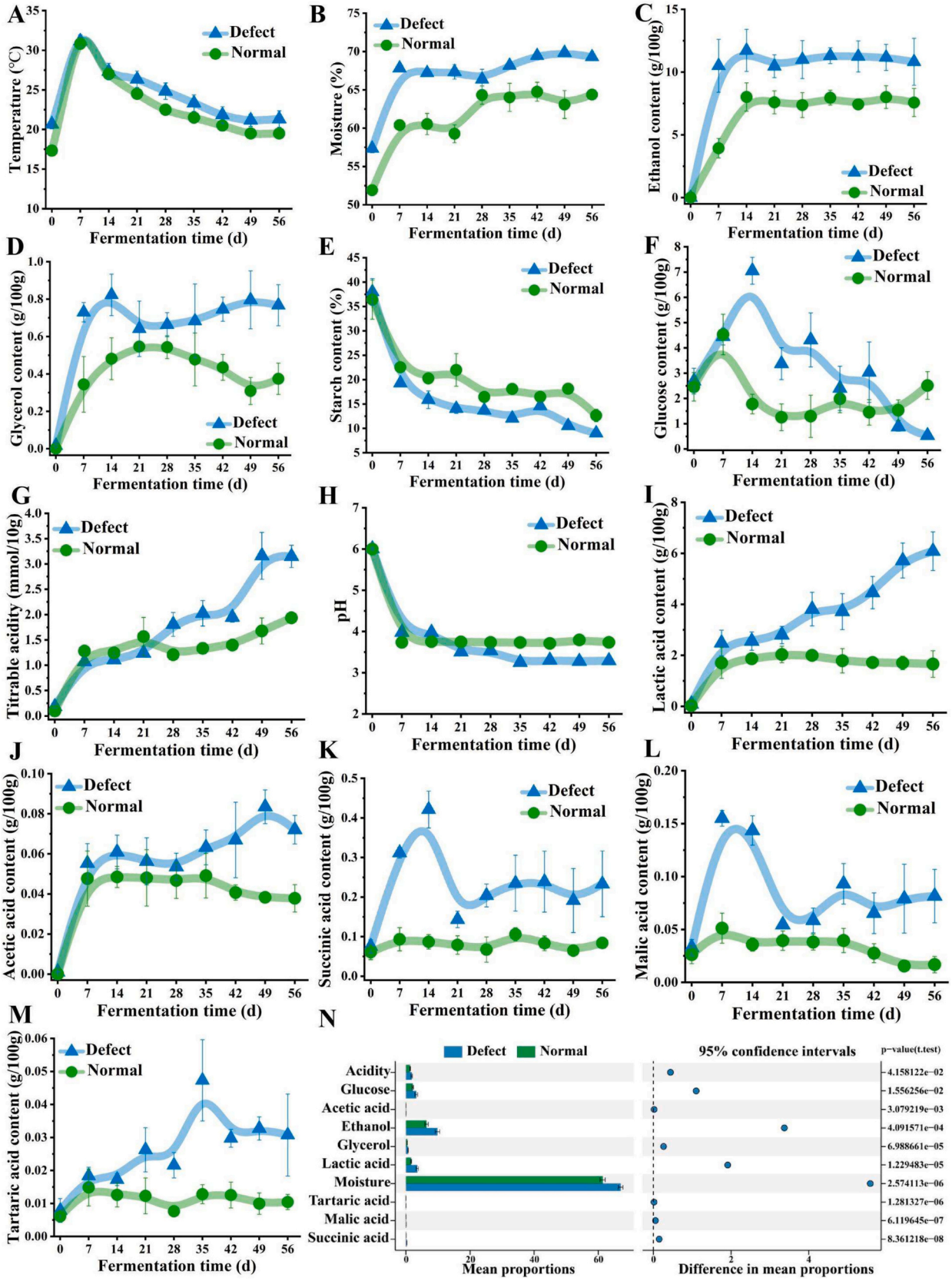

Fig. 1. The evolution of physicochemical properties of fermented grains during the fermentation.
A: Temperature, B: Moisture, C: Ethanol, D: Glycerol, E: Starch, F: Glucose, G: Titratable acid, H: pH, I: Lactic acid, J: Acetic acid, K: Succinic acid, L: Malic acid, M: Tartaric acid, N: Comparison of the differences in physicochemical factors between defect and normal groups.

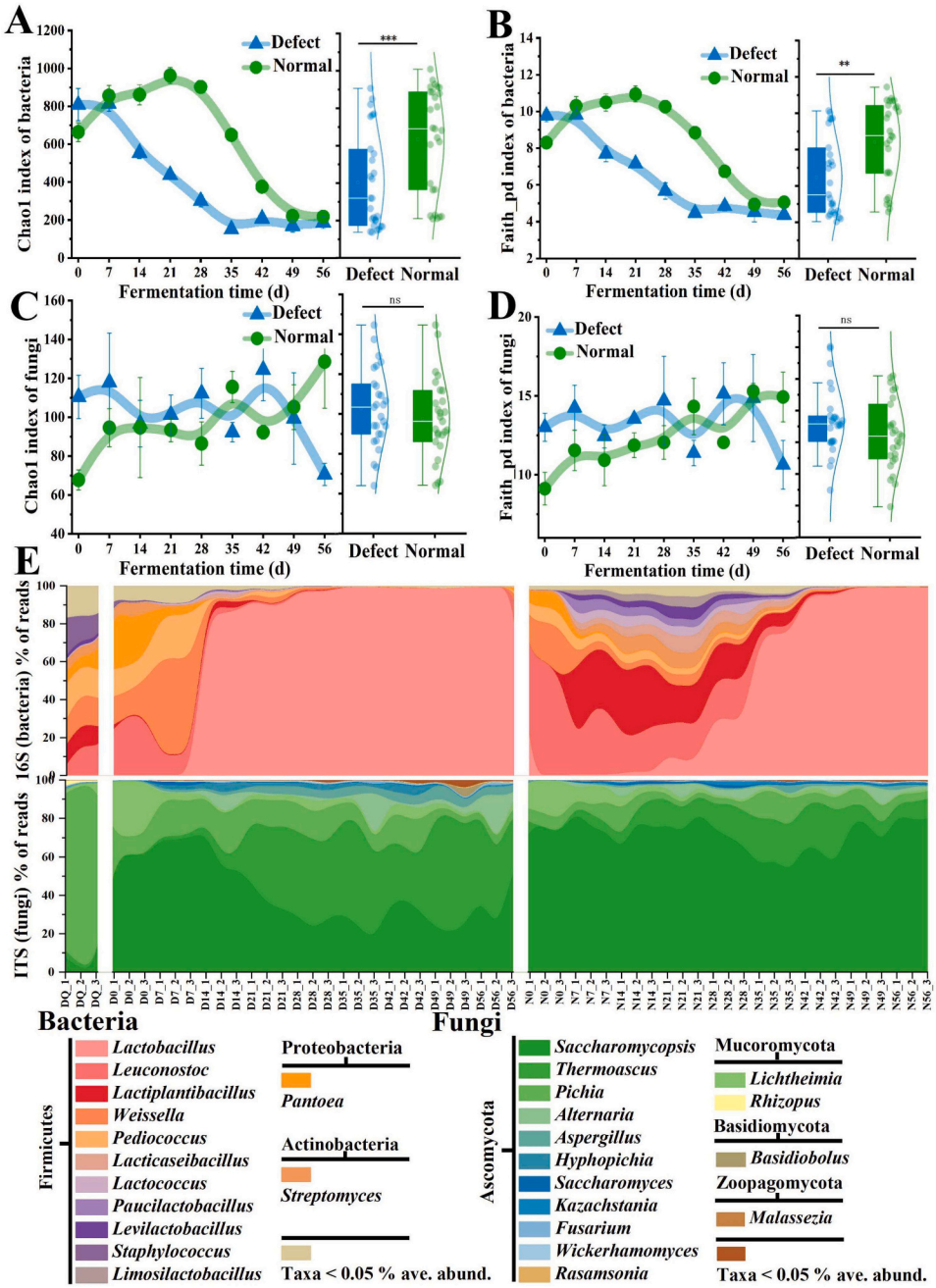

Fig. 2. Microbial community dynamics of fermented grains during the fermentation.
Changes in the α-diversity of the microbial community during the fermentation, including A: Bacterial richness; B: Bacterial diversity; C: Fungal richness; D: Fungal diversity. E: Dynamics in the microbial community at the genus level with a relative abundance of >0.05 %.

Table 1
Dissimilarity tests of the microbial communities between normal and defect groups.

Group	MRPP		ANOSIM		Adonis	
	A	*P*	*r*	*P*	*F*	*P*
Bacteria	0.10	0.004*	0.13	0.002*	7.91	0.001*
Fungi	0.24	0.001*	0.48	0.001*	24.18	0.001*

Notes: A significant difference is indicated by * for $P < 0.05$.

encoding genes was compared between the normal and defect groups in the bacterial and fungal communities, respectively (Fig. S3). In the bacterial community, all the enzymes showed higher abundance in the normal group compared to the defect group. Specifically, nine enzymes involved in pyruvate metabolism, which are closely related to the production of lactic acid and acetic acid, were identified. Among these, L-lactate dehydrogenase (EC:1.1.1.27) is essential for microorganisms to produce lactic acid and acetic acid (Huang et al., 2020). The bacterial contribution to this enzyme is much higher than that of fungi. Both the normal and defect groups maintained a high abundance of L-lactate dehydrogenase, with the normal group exhibiting a higher abundance. Due to the greater contribution of the fungal community to starch degradation, the abundance of fungal genes encoding α-amylase (EC:3.2.1.1) and oligo-1,6-glucosidase (EC:3.2.1.10) was significantly higher in the defect group compared to the normal group. This result may explain why the high moisture content in the defect group derived the rapid growth and metabolism of filamentous fungi, leading to the

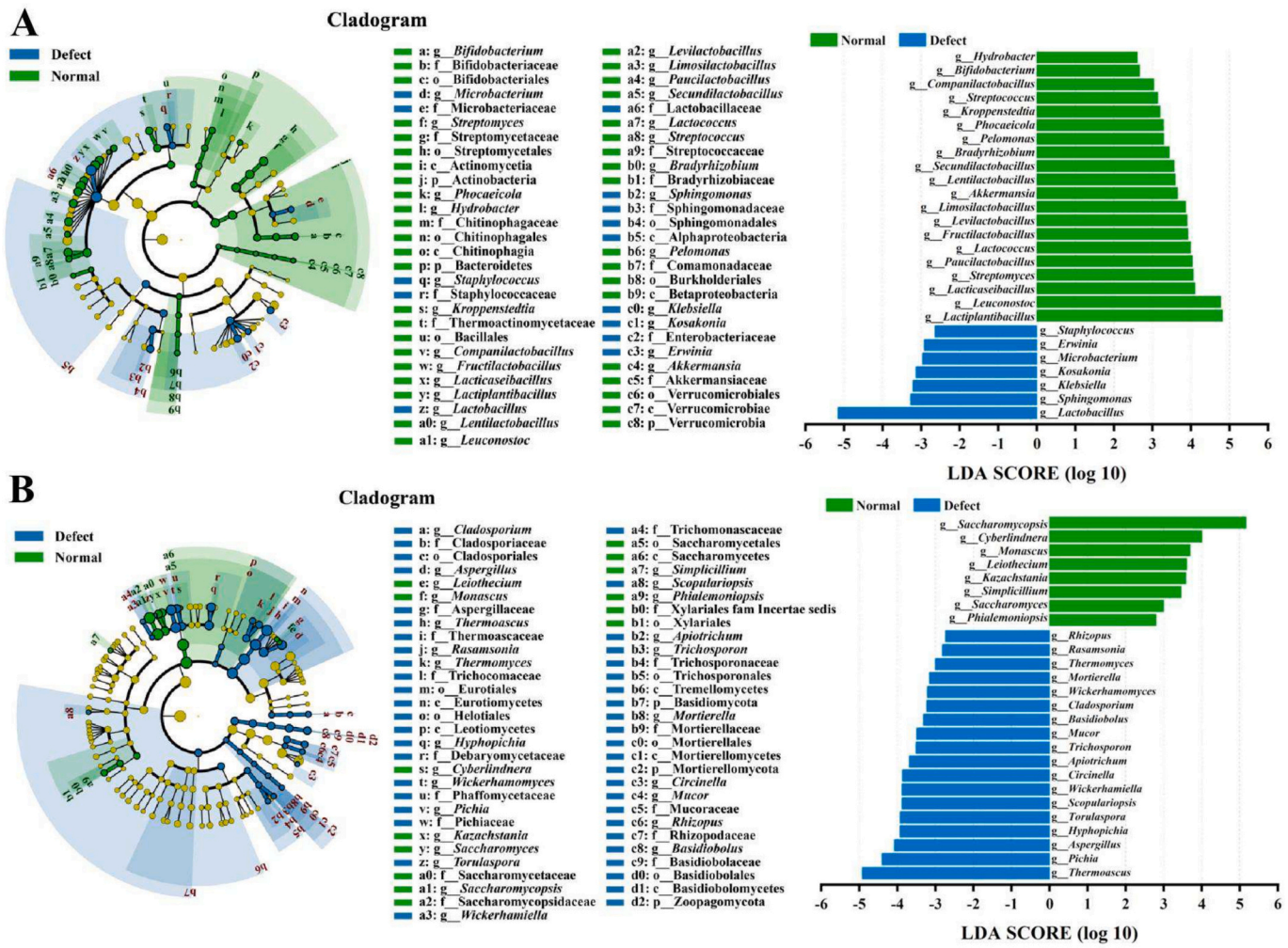

Fig. 3. Comparison of microbial composition of the microbial community during fermentation between normal and defect groups. LEfSe analysis of bacterial (A) and fungal (B) communities, the threshold on the logarithmic LDA score for discriminative features was set to 2.0.

swift accumulation of ethanol and lactic acid. In contrast, the normal group achieved a balance through a slow and steady fermentation process involving the metabolism of filamentous fungi, yeasts, and lactic acid bacteria. The high abundance of functional metabolic enzymes in the bacterial community would also play an important role in the accumulation of flavor compounds.

3.4. Microbial assembly process of fermented grains in normal and defect groups

The dominance and abundance of microbes varied significantly between the normal and defect groups. To investigate the assembly mechanisms of microbial communities in fermented grains during fermentation in both groups, we employed the neutral community model (NCM) and the modified stochasticity ratio (MST). These statistical approaches have been widely used to analyze assembly processes in various microbial communities, including those involved in *Baijiu* fermentation (Ren et al., 2024; Yuan et al., 2023). Traditional ecological theory suggests that deterministic processes, driven by biotic factors like species interactions and abiotic factors such as environmental conditions, predominantly shape microbial community assembly. In contrast, neutral theory posits that stochastic processes-such as birth, death, migration, and dispersal- play a significant role in determining community structure (Sloan et al., 2006).

The relationship between the occurrence frequency and relative abundance of microbial ASVs in the normal and defect groups was predicted using the NCM. For the bacterial communities, the normal group exhibited a higher R^2 value compared to the defect group, indicating a better fit with the neutral model (Fig. 4A and B). This suggests that the microbial community in the normal group was more influenced by stochastic processes than in the defect group. Additionally, the normal group showed higher Nm values, indicating lower dispersal limitations and higher dispersal rates during fermentation. Conversely, for fungal communities, the R^2 and Nm values were similar in both the normal and defect groups (Fig. 4D and E). The MST index indicated that bacterial communities in both groups were primarily governed by deterministic processes (MST < 0.5). However, compared to the defect group, the bacterial community in the normal group's fermented grains exhibited significantly higher stochasticity (Fig.4C). In contrast, the fungal communities in the normal group were predominantly influenced by deterministic processes (MST < 0.5), whereas the defect group showed a notable increase in stochastic processes (MST > 0.5) (Fig. 4F). The microbial community assembly in the normal group was primarily governed by deterministic processes, highlighting the dominance of microbial interactions and abiotic factors during fermentation. The difference of fungi assembly process in two groups would lead to the distinction in flavor formation.

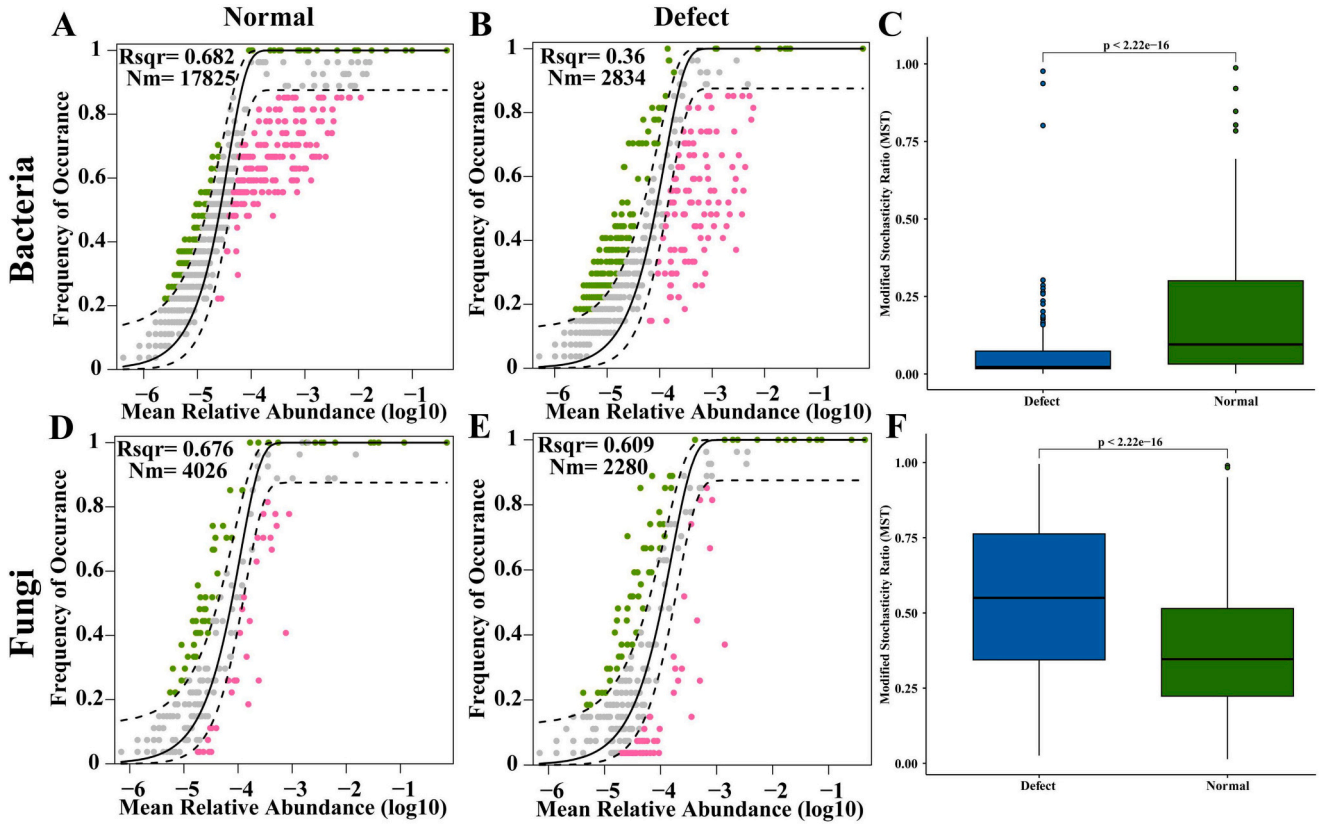

Fig. 4. Neutral community model (NCM) and modified stochasticity ratio (MST) of bacterial and fungal communities during the fermentation of fermented grains. The fit of the NCM for bacterial community assembly processes in normal group (A) and defect group (B). The fit of the NCM for fungal community assembly processes in normal group (D) and defect group (E). ASV occurring more frequently than predicted by the model are depicted in green, while those occurring less frequently are shown in pink. The solid line describes the fit of the neutral model, with the upper and lower dotted lines representing the 95 % confidence interval of the model prediction. R^2 values indicate the goodness of fit of the neutral model, ranging from 0 to 1, while m values indicate the estimated migration rate. Nm is the product of metacommunity size (N) and migration rate (m), quantifying dispersal potential among communities. C: MST analysis for bacterial community in normal group and defect group. F: MST analysis for fungal community in normal group and defect group. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.5. Microbial co-occurrence network of fermented grains in normal and defect groups

Since microbial interactions and abiotic factors have a significant effect on light-flavor *Baijiu* fermentation, a co-occurrence network was conducted to gain deeper insights into the intra-community associations within bacterial and fungal communities of fermented grains in the normal and defect groups. As shown in Table 2, the bacterial network connectivity in both groups followed a power-law distribution, indicating that the network exhibited scale-free properties, with a small number of microorganisms having many connections while the majority have few connections (Barberán, Bates, Casamayor, & Fierer, 2012; Deng et al., 2016). Additionally, average clustering coefficient and modularity were much lower in the random networks compared to the empirical ones, indicating that the clustering and modularity observed in the empirical networks were biologically significant.

For the bacterial communities in the normal group, the co-occurrence network contained 422 nodes and 7164 edges (Fig. 5A), whereas the defect group's bacterial network contained 145 nodes and 757 edges (Fig. 5B). The average degree (AD value) of the bacterial network in the normal group was significantly higher than that in the defect group, indicating that the scale and complexity of the bacterial network in the normal group were greater than those in the defect group. The network density of the bacterial community in the normal group was also higher, implying greater cohesion (Table 2). In the normal group, 71.11 % of the associations were positive (Fig. 5A), compared to 69.62 % in the defect group (Fig. 5B). The proportion of positive associations in both groups exceeded half, indicating that

Table 2
Topological properties of the empirical bacterial and fungal co-occurrence networks in fermented grains and their associated random networks.

Topological Parameters		Bacteria		Fungi	
		Normal	Defect	Normal	Defect
Empirical Network	R^2 of power law	0.719	0.867	0.790	0.917
	Average degree (AD)	33.953	10.299	3.907	3.929
	Average clustering coefficient(ACC)	0.459 ^{b}	0.464 ^{a}	0.271 ^{a}	0.211 ^{b}
	Average path length (APL)	2.495 ^{b}	3.044 ^{a}	2.633 ^{b}	3.011 ^{a}
	Density	0.081	0.071	0.093	0.071
	Modularity	0.344	0.426	0.483	0.512
Random Network	ACC	0.196 ± 0.008	0.077 ± 0.013	0.025 ± 0.021	0.032 ± 0.02
	APL	2.133 ± 0.006	2.488 ± 0.025	2.766 ± 0.085	2.931 ± 0.095
	Modularity	0.113 ± 0.005	0.222 ± 0.005	0.385 ± 0.019	0.395 ± 0.015

Notes:Randomized networks were performed by rewiring all the nodes and links corresponding to empirical networks 100 time; Significant difference in the ACC, APL, and modularity between normal and defect group was detected based on *t*-test ($P < 0.01$).

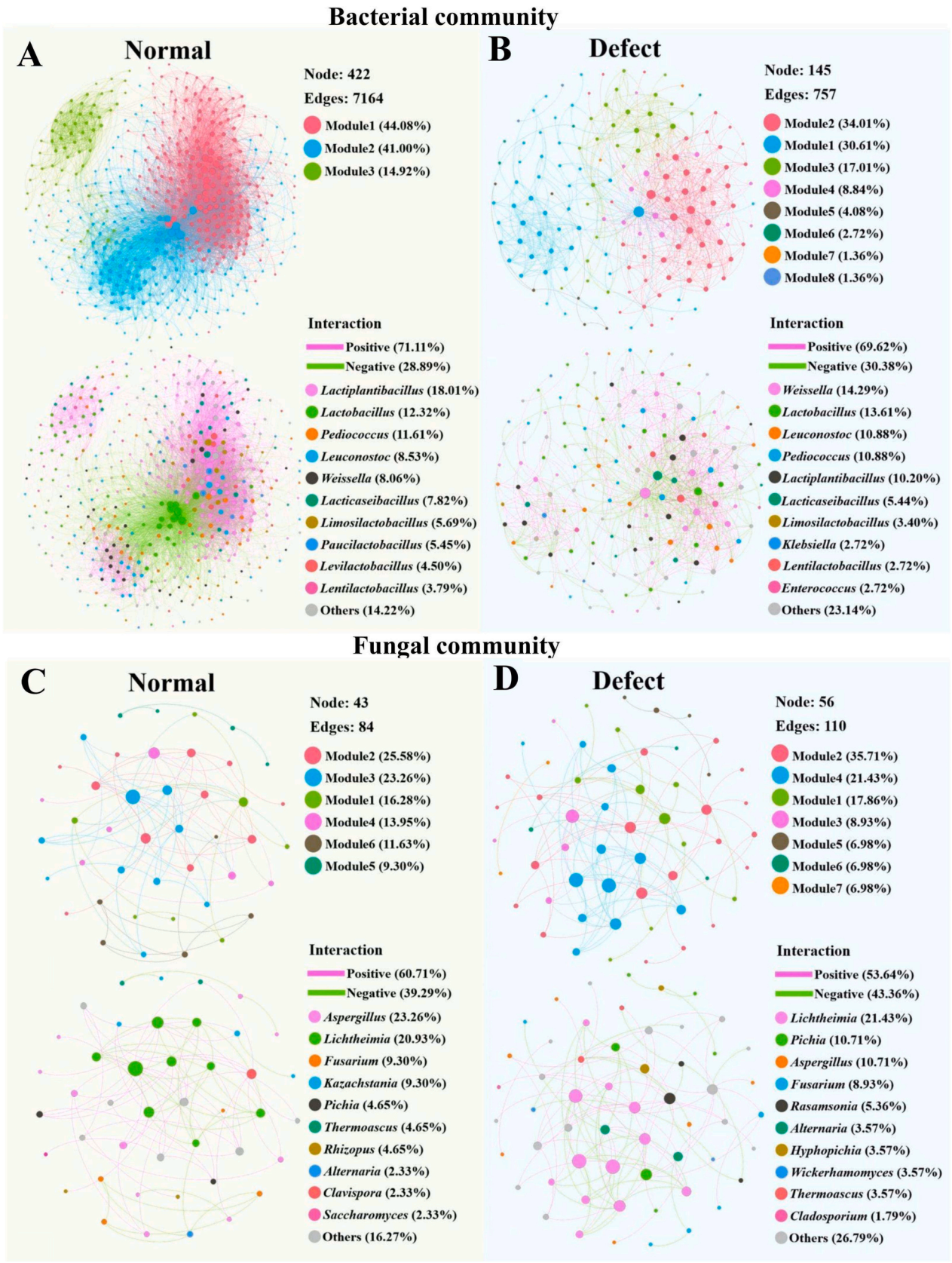

Fig. 5. Co-occurrence network of microbial communities in normal and defect groups. A-B: Based on the bacterial communities in normal and defect groups. Network analysis displaying the associations among different network module (up) and bacterial genera (down) in normal group (A) and defect group (B). The nodes are colored by the network module or bacterial genera. The size of a node represents the degree of each node. C-D: Based on the fungal communities in normal and defect groups. Network analysis displaying the associations among different network module (up) and fungal genera (down) in normal group (C) and defect group (D).

bacteria predominantly coexisted in cooperative or symbiotic relationships during the fermentation process (Tang et al., 2023; Yuan et al., 2021), with the relationship being slightly stronger in the normal group compared to the defect group. Furthermore, key species in bacterial networks were identified. In the normal group's bacterial network, 13 key species were identified, including one connector hub and 12 module hubs. The connector hub was annotated as *Lactobacillus*. Among the 12 module hubs, six were *Lactobacillus*, and the others included *Lacticaseibacillus*, *Lactiplantibacillus*, and *Lactococcus* (Fig.S4A). In the defect group, nine key bacteria were identified, including two *Lactobacillus* species as well as *Furfurilactobacillus*, and *Weissella* (Fig. S4B). Overall, the bacterial community interactions in the normal group were more complex compared to the defect group, involving a greater number of key species, most of which were lactic acid bacteria. This suggests that the diversity of lactic acid bacteria mediated bacterial network relationships, playing a crucial role in the fermentation process. The fungal network in the normal group contained 43 nodes and 84 edges (Fig. 5C), while the defect group's fungal network contained 56 nodes and 110 edges (Fig. 5D). Although the defect group's network was slightly larger, the fungal networks in both groups were relatively similar in size and complexity. However, the cohesion of the fungal network was higher in the normal group (Table 2). Additionally, in both the normal and defect groups, more than 50 % of the fungal network associations were positive, with a higher proportion in the normal group. This indicates that cooperative and symbiotic relationships were more prevalent among fungi in the normal group compared to the defect group. Key species identification in the fungal communities revealed that the normal group's fungal network contained three key species, all of which were connector hubs. Two of them belonged to *Lichtheimia*, and one to *Clavispora* (Fig. S4C). In the defect group, two connector hubs were identified, belonging to *Lichtheimia* and *Leucosporidium* (Fig. S4D). The fungal network in both groups was significantly smaller than the bacterial network, with *Lichtheimia* playing a significant role during the fermentation process. This genus contributed to starch degradation and saccharification in the early stages of fermented grain fermentation. All in all, the microbial community succession in the normal and defect groups resulted in distinct microbial structures and networks, which may lead to differences in community metabolism. The complex microbial interaction during *Baijiu* fermentation would contribute to the characteristic flavor compounds formation. Therefore, we further examined the composition and content of volatile metabolites during the fermentation process in both groups.

3.6. Flavor profile of fermented grains during fermentation

A total of 51 volatile compounds were identified during the fermentation process of the fermented grains using HS-SPME-GC–MS, including 24 esters, 9 alcohols, 6 phenols, 5 acids, 4 aldehydes and ketones, and 3 other compounds (Fig. S5). The content of the majority of volatile compounds rapidly decreased or increased in the early stages, likely due to the intense metabolic activity of microorganisms at this stage. This observation aligns with previous findings that the most substantial changes in metabolites occurred during the first seven days of *Baijiu* fermentation (Huang et al., 2020). Overall, the volatile compounds in the fermented grains exhibited a clear temporal continuity, with esters and alcohols being the predominant volatile compounds in the mid to late stages of fermentation. Specifically, the content of ethyl lactate was higher in the defect group during the mid to late stages of fermentation, while the content of ethyl acetate was higher in the normal group from the 7th day onwards compared to the same period in the defect group. Ethyl acetate was identified as the most typical flavor compound in light-flavor *Baijiu*.

To further compare the structural characteristics of volatile metabolites during the fermentation process between the normal and defect groups, we conducted a PCoA analysis based on the Bray-Curtis distance matrix. The results indicated a clear separation of volatile metabolite profiles between the two groups (Fig. 6A). The ANOSIM test further confirmed significant differences in the structural characteristics of volatile metabolites between the normal and defect groups (Fig. 6B). Subsequently, a random forest model was used to identify the top 20 key volatile compounds that may contribute to the significant differences in volatile metabolite profiles between the normal and defect groups (Fig. 6C). In the normal group, volatile metabolites with higher content included 3-hydroxy-2,2,4-trimethylpentyl isobutyrate, veratrole, styrene, 4-ethylphenol, acetic acid, ethyl acetate, acetophenone, and 3-methyl-1-butanol. In contrast, the defect group had higher levels of diethyl glutarate, ethyl isopentyl succinate, diethyl succinate, ethyl caproate, ethyl 9-hexadecenoate, 2,4-Di-tert-butylphenol, 4-ethyl-2-methoxyphenol, ethyl caprylate, ethyl palmitate, phenol, 1-hexanol, and ethyl lactate. This group had a greater variety of long-chain fatty acid esters compared to the normal group. Long-chain esters are generally less volatile and contribute less to flavor compared to short-chain esters. Based on these findings, it is speculated that the formation and accumulation of characteristic flavor compounds in the fermented grains were related to the unique microbial community structures and succession patterns in each group.

3.7. Correlation network analysis of microbial communities and characteristic volatile metabolites mediated by abiotic factors

A correlation network analysis was conducted to explore the relationships among biotic factors, microbial communities, and volatile metabolites using the Spearman rank correlation coefficient (|Spearman's rho| > 0.6, $P < 0.05$) (Fig. 6D). The analysis identified seven positive and six negative correlations between moisture content and microbial communities. Moisture content showed positive correlations with genera such as *Aspergillus*, *Hyphopichia*, *Thermoascus*, *Wickerhamomyces*, *Lactobacillus*, and *Rhizopus*, while negative correlations were observed with *Lactiplantibacillus*, *Staphylococcus*, *Saccharomycopsis*, and *Leuconostoc*. Additionally, nine correlations were observed between acidity and microbial communities. Among these, three were positive: acidity with *Lactobacillus*, *Thermoascus*, and *Rasamsonia*. Notably, acidity demonstrated a strong positive correlation with *Lactobacillus* ($r = 0.86$, $P < 0.01$). The six negative correlations included acidity with *Staphylococcus* and *Leuconostoc*. Ethanol and most organic acids exhibited positive correlations with fungi, such as *Aspergillus* and *Wickerhamomyces*, but negative correlations with bacteria. Among volatile metabolites, ethyl lactate had the most associations with the microbial community. *Lactobacillus* showed the strongest positive correlation with ethyl lactate ($r = 0.89$, $P < 0.01$), followed by *Thermoascus* ($r = 0.80$, $P < 0.01$). *Lactobacillus* also positively correlated with diethyl succinate and 1-hexanol. In contrast, bacteria such as *Lactococcus*, *Paucilactobacillus*, *Streptomyces*, *Staphylococcus*, and *Leuconostoc* showed negative correlations with ethyl lactate. *Aspergillus* exhibited the highest correlation with metabolites, including eight positive correlations and one negative correlation, such as positive correlations with diethyl succinate, ethyl 9-hexadecenoate, ethyl lactate, and ethyl palmitate.

4. Discussion

Microbial interaction and assembly process significantly affect the function and stability of microbial community (de Vries et al., 2018; Gao et al., 2022; Liu et al., 2020; Liu & Salles, 2024). In this study, we investigated microbial succession, interactions, and assembly processes, as well as the driving factors in two different fermentation groups with different initial abiotic factors, including moisture and temperature. Our findings indicated that bacterial community assembly in both groups was primarily governed by deterministic processes, although stochastic processes had a greater influence in the normal group compared to the defect group. This difference may be attributed to the higher microbial diversity in the normal group, which may confer greater resilience to

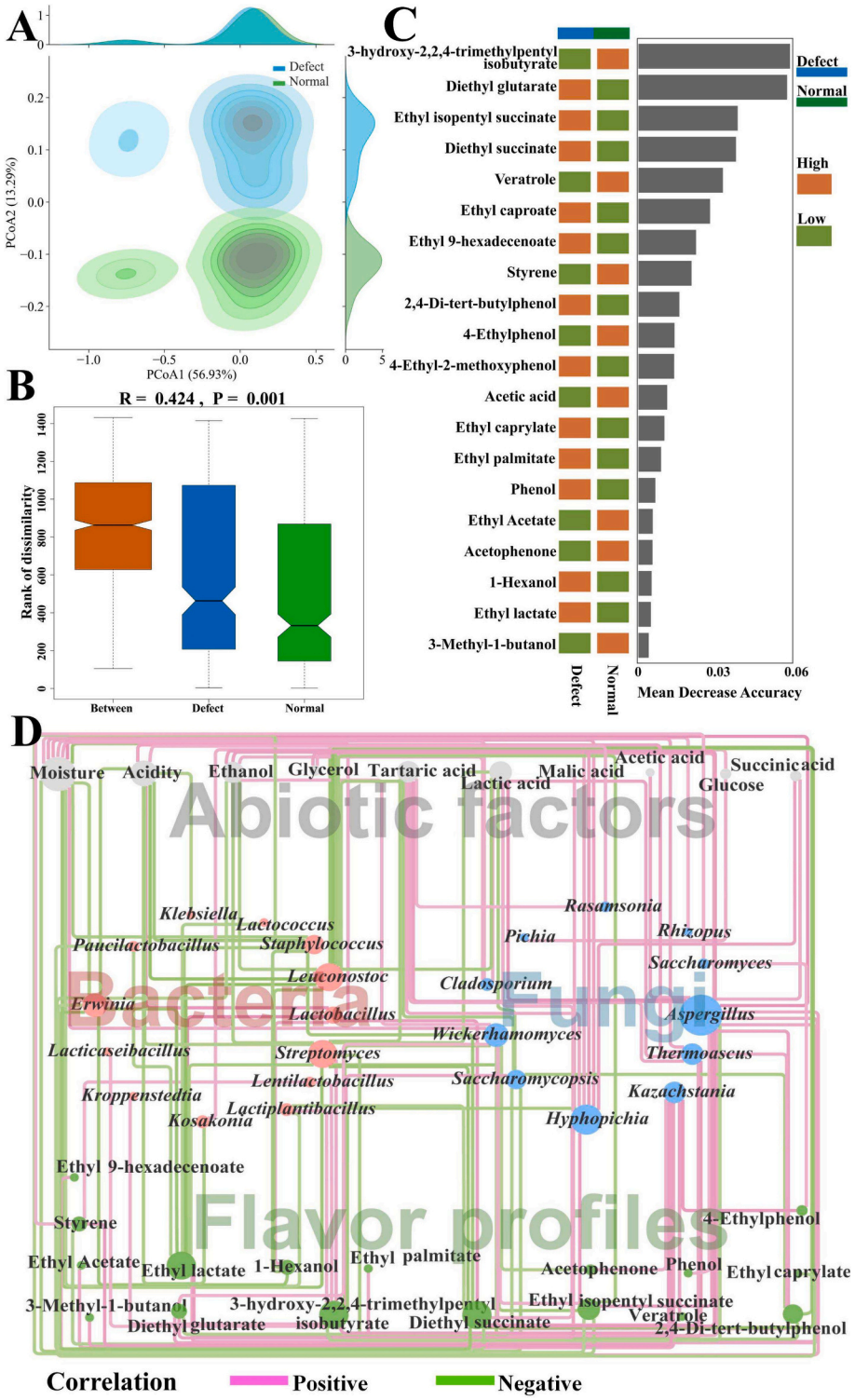

Fig. 6. Volatile profile of fermented grains of normal and defect groups during fermentation. A-C: Differences analysis in the volatile compounds between normal and defect group using PCoA plot analysis (A); ANOSIM analysis (B); random forest analysis (C). D: Correlation network analysis of core microbiota and important volatile metabolites mediated by abiotic factors. The size of a network node indicates the number of relationships it has with others.

environmental changes such as pH reduction or ethanol accumulation during *Baijiu* fermentation, thereby reducing vulnerability to both biotic and abiotic selection pressures (Chen & Wen, 2021). This observation aligned with previous studies on strong-flavor *Baijiu* fermentation, which suggested that while deterministic processes dominated community assembly, stochastic processes also played a positive role (Yuan et al., 2023). Selection processes preserve adaptable microorganisms, enhancing specific community functions, while neutral processes promote functional diversity and contribute to maintaining ecosystem stability (Liu et al., 2021). Thus, both deterministic and stochastic processes influenced the dynamics observed during fermentation (Wei et al., 2023; Yuan et al., 2023). For fungal communities, deterministic processes predominantly influenced the normal group, whereas the defect group exhibited a notable increase in stochastic processes.

Communities assembled through stochastic processes were more susceptible to invader survival (Liu & Salles, 2024). Given the openness of the *Baijiu* fermentation environment, microbial invasion due to changes in physicochemical factors poses a risk, which may explain why the fungal community in the defect group was dominated by stochastic processes.

Abiotic factors, such as moisture and temperature, are critical initial parameters in the fermentation process of light-flavor *Baijiu*. Initial temperature is influenced by the raw material pre-treatment before fermentation, while moisture is regulated through manual water addition. Improper control of these factors can lead to abnormal fermentation. Moisture, temperature and acidity play critical roles in shaping microbial community structures and their metabolic activities, particularly during the initial stages of fermentation (Ji et al., 2023; Pan, Qiu, Lv, & Li, 2023; Zhang et al., 2021). Our study found that differences in initial moisture and temperature led to distinct microbial and metabolic profiles during light-flavor *Baijiu* fermentation. For sauce-flavor *Baijiu* fermentation, variations in initial temperature influenced microbial succession rates, volatile compound production, and acetic acid concentration (Zhang et al., 2021), which was consistent with our results. Additionally, Ji et al. (2023) found that acidity was the main factor impacting volatile compounds during sesame-flavor *Baijiu* fermentation. The initial moisture content significantly affected microbiota structure and flavor formation during *Xiaoqu* fermentation. With increasing temperature, the total volatile compound content and acetic acid concentration also rose (Wang et al., 2023), aligning with our results. Lactic acid, the primary organic acid produced during *Baijiu* fermentation, inhibited the growth of undesirable microbes and guided the fermentation process. Additionally, acidic conditions enhanced fermentation capacity by providing an optimal environment for the enzyme systems essential for *Baijiu* production (Fan, Chen, Du, & Fang, 2024). However, excessive lactic acid accumulation in the fermented grains can adversely affect the growth of functional fermentation microorganisms such as yeast, disrupt the microbial community structure in subsequent fermentations, and unbalance flavor compounds in the final product (Fan et al., 2024; Wang et al., 2022).

Over-acidification during fermentation is the primary cause of abnormal Chinese *Baijiu* fermentation and a common issue in the industry. This is influenced by multiple factors. For instance, high starch content in raw materials may promotelactic acid bacteria to produce more lactic acid, leading to ethyl lactate accumulation; *Daqu* with a high abundance of lactic acid bacteria can further increase lactic acid production; high environmental temperatures and humidity also promote the growth of these bacteria, resulting in more lactic acid formation (Yang, Hua, & Zhou, 2024). Our results indicated that moisture content in fermented grains positively regulated the growth and metabolism of filamentous fungi, thereby promoting the growth of *Lactobacillus* and yeasts, as observed in sesame-flavor *Baijiu* fermentation (Ji et al., 2023). Enzymes derived from filamentous fungi, such as *Aspergillus*, *Rhizopus*, and *Mucor*, including α-amylase and glucoamylase, were positively correlated with starch hydrolysis and ethanol production during *Baijiu* fermentation (Wang, Wu, Xu, & Sun, 2020). *Lactobacillus* was the primary contributor to acid production during *Baijiu* fermentation (Kang et al., 2024). The rapid proliferation and metabolic activity of *Lactobacillus* led to increased acidity and a significant accumulation of ethyl lactate in the fermented grains. These findings were consistent with previous research indicating that inoculation with indigenous *Lactobacillus* significantly promoted the formation of ethyl lactate during *Baijiu* fermentation (Pang et al., 2021). Moisture in the fermented grains not only ensured the growth and metabolism of microorganisms but also acted as an effective solvent for metabolic products, diluted acidity, and regulated the fermentation temperature (Wang et al., 2023). Maintaining an equilibrium of both acidity and moisture content during *Baijiu* fermentation is crucial. This balance ensures an optimal fermentation speed for the microbial community, leading to the best production of flavor compounds. Future studies will focus on developing predictive models using machine learning algorithms or structural equation modeling combined with experimental data to examine the impact of initial abiotic factors on the evolution of important abiotic factors and core microbiotas during the fermentation process, as well as the formation and accumulation of key flavor profiles. It is reasonable to assert that effectively managing initial environmental factors will be crucial for optimizing fermentation processes and advancing the intelligent development of the *Baijiu* brewing industry.

5. Conclusion

In conclusion, variations in the initial abiotic factors of light-flavor *Baijiu* fermentation—such as moisture and temperature—led to significant differences in the physicochemical properties of fermented grains, including ethanol, acidity, glucose, and lactic acid concentration. These variations subsequently influenced the microbial composition, succession, assembly, interactions, and metabolic functions during fermentation. The normal fermentation group was enriched with lactic acid bacteria, including *Lactiplantibacillus*, *Leuconostoc*, *Lacticaseibacillus*, *Paucilactobacillus*, and *Lactococcus*, as well as fungi primarily from the genera *Saccharomyces*, *Kazachstania*, and *Saccharomycopsis*. In contrast, *Lactobacillus* and *Thermoascus* were primarily enriched in the defect group. Moisture content positively regulated the growth and metabolism of filamentous fungi, which in turn promoted the succession of other microbes, particularly *Lactobacillus*. During the acid production phase, the rapid proliferation of *Lactobacillus* significantly increased the acidity of the fermented grains and led to a substantial accumulation of ethyl lactate. This study highlights the intricate interplay between abiotic factors and microbial communities, which shape the fermentation process and the final product quality of light-flavor *Baijiu*. Understanding these dynamics is essential for optimizing fermentation conditions and enhancing product consistency and quality.

CRediT authorship contribution statement

Xiaoning Huang: Writing – original draft, Visualization, Methodology, Formal analysis. **Jiamu Kang:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Yuandi Zhang:** Methodology, Investigation, Data curation. **Xiaoxue Chen:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Beizhong Han:** Writing – review & editing, Project administration, Conceptualization.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101982.

Data availability

All raw sequence data generated in this research has been deposited in the NCBI Sequence Read Archive under accession number PRJNA933937.

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