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Linking Environmental Microbiomes to Baijiu Brewing: Microbial Assembly in the Upper Yangtze River Basin Shapes the Functional Potential for Fermentation

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ABSTRACT: The unique quality of Baijiu, China's traditional distilled spirit, is intimately linked to its regional micro-ecology. However, the origins and assembly processes of these fermentation-relevant microbes in natural environments within major Baijiu production basins, like the upper Yangtze River, remain largely unexplored. This study comprehensively profiled the microbial communities (bacteria and fungi) across four habitats (water, soil, air, and plant surfaces) from 15 locations in the upper Yangtze River basin, a critical region for Baijiu production. Results indicated that habitat type was the primary factor shaping microbial structures. Notably, a core set of 30 microbial genera (14 bacterial and 16 fungal) with known functions in Baijiu fermentation was identified. These key taxa were predominantly enriched in the air and exhibited significant regional variation. Co-occurrence network analysis revealed higher structural complexity in river and plant soil communities, suggesting these as potential stability hubs for microbial resources. Community assembly was mainly governed by homogeneous selection, indicating deterministic environmental filtering. Our findings directly bridge environmental microbiology with food fermentation practices by mapping the distribution of functional brewing microbes. This work provides a crucial theoretical foundation for harnessing natural microbial resources to enhance Baijiu quality control, develop starter cultures, and ultimately support the sustainable development of the Baijiu industry.

Keywords: Chinese Baijiu fermentation; Environmental microbiome; Microbial resource; Co-occurrence network; Habitat variation

1. Introduction

As a typical traditional Chinese fermented food, Baijiu has distinctive flavor diversity and regional characteristics[1-3]. The upper reaches of the Yangtze River, especially the Chishui River basin, are among the core production areas of Chinese baijiu. Baijiu is mostly produced using an open fermentation system, allowing microorganisms from the environment to actively or passively migrate into the fermentation

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system[4-7], with local microbial communities considered to play a key role in promoting spontaneous fermentation and enhancing flavor complexity[8]. Unique regional microbial communities contribute to shaping the sensory characteristics and metabolite profiles of Baijiu from different areas[3]. Du et al. (2025) pointed out that the completely different ecological environments of Shennongjia and Maotai Town have given rise to their respective unique microbial compositions, resulting in significant differences in metabolite profiles and aroma styles between the sauce-flavor Baijiu of the two regions[9]. Research by Zhou et al. (2024) pointed out that in the ecosystem of Maotai Town distilleries, Actinobacteria, Firmicutes, Proteobacteria, Ascomycota, and Basidiomycota are the dominant phyla and make important contributions to flavor formation and quality[10].

However, current research on the fermentation environment of Baijiu mainly focused on the microbial traceability of fermented grains and Daqu, or was limited to the microbial composition analysis of workshop environments[11-13]. For example, it has been found that *Streptomyces*, *Lactobacillus*, *Aspergillus, Thermomyces*, and *Thermoascus* are dominant genera within brewing workshop environments[11], while *Aspergillus*, *Streptomyces*, *Saccharopolyspora,* and so on were widely present in the Daqu production room[13]. Studies have shown that 50% to 75% of the viable bacteria in fermented grains can be traced to various environmental media, such as air, tool surface, and plant soil[14]. Nevertheless, there is a lack of in-depth exploration regarding the overall characteristics of microbial communities in production areas and their association with functional brewing microorganisms. In particular, the community structure, migration pathways, and relationships of potential microbial sources such as water, soil, air, and plant surfaces with the fermentation microbiome remain to be systematically analyzed.

This study covered the upper reaches of the Yangtze River to the Chishui River basin, encompassing several provinces and 15 different locations, and systematically collected samples from four habitats: soil, water, air, and plant surfaces. The community structure, diversity, and migration of microorganisms in different habitats were analyzed by high-throughput sequencing and bioinformatics analysis. The impact of environmental microbial movement on the biodiversity and function of Baijiu fermentation-associated microbiota was evaluated through bioinformatics analysis. This study aims to clarify the ecological process and resource distribution characteristics of microorganisms in the producing area, and to provide a scientific basis for the utilization of microbial resources, process optimization, and sustainable development of Baijiu production.

2. Materials and Methods

2.1 Sample collection

A total of 15 distinct sites were selected in the upper Yangtze River (the area above Yichang) and its tributary, the Chishui River Basin, spanning six cities. Samples from four habitats, including water, soil, air, and plant surface, were taken in each site (Figure 1, Supplementary Table S1). The sampling points were categorized into two groups along the river flow direction: those located in the upper Yangtze River basin were labeled as L (L1 to L6), and those located near the Chishui River basin were marked as S (S1 to S9).

Sampling was conducted from July 6 to July 23, 2023, to minimize temporal variation. Extreme weather was avoided during sampling.

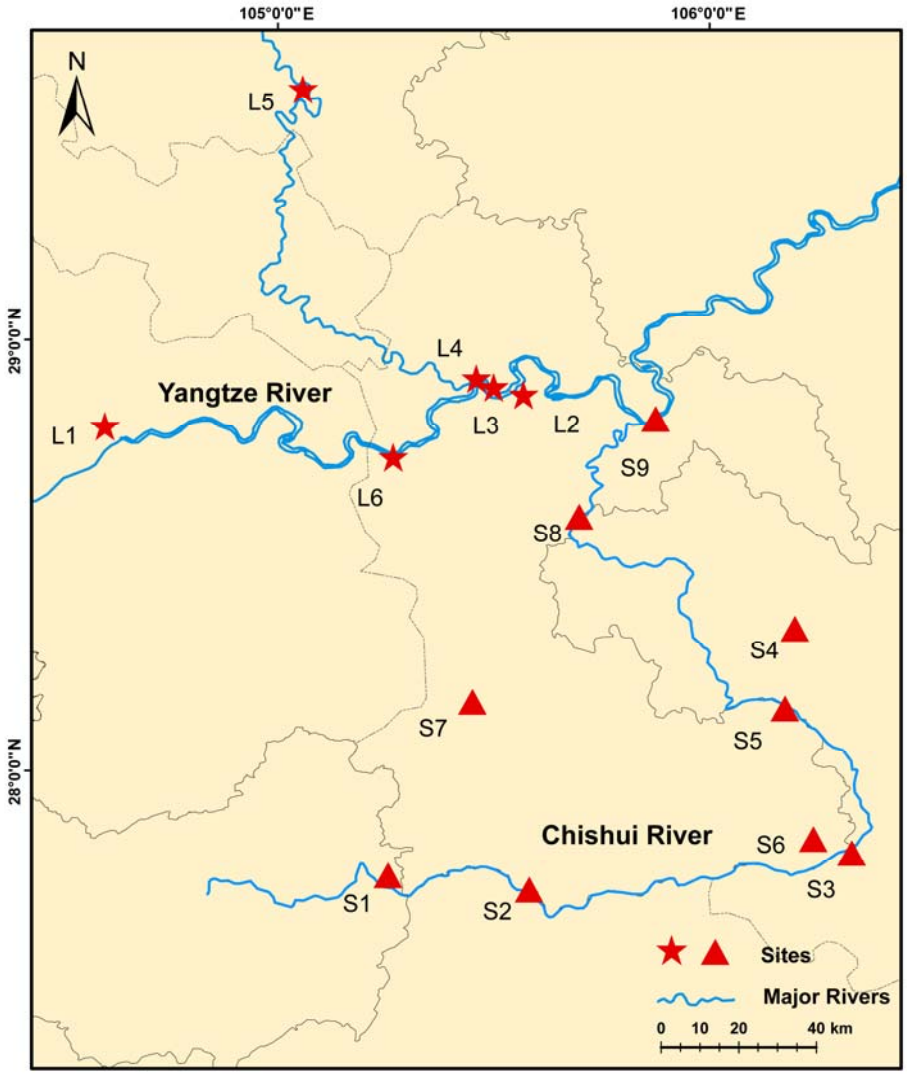

Figure 1 Study area location and the distribution of sampling points.

Six liters of surface water were collected from each sampling site in triplicate and subsequently filtered using 0.22-μm pore-size membranes (Millipore, Billerica, MA, USA) according to Walden, Carbonero, and Zhang[15]. The membranes were then kept in sterile sampling bags. Microbial samples from the plant surface were obtained in triplicate from each site by wiping plant leaves and branches with sterile cotton wool soaked in pre-sterilized 0.1 M phosphate buffer solution (pH = 7.4; GIBCO) in 50 mL centrifuge tubes[16]. For soil samples, at least five separate areas were selected at each sampling site, following the method by Pepper, Gerba, and Maier[17]. Samples were dug to a depth of 0-10 cm, removed from visible rocks, roots, and plant materials, mixed thoroughly, and then placed in sterile sampling bags. Airborne microbial samples were collected using a high-volume air collector (Laoying 2031 TSP/PM10/PM2.5, Laoshan Institute of Applied Technology, Qingdao, China) with a glass fiber membrane (200 mm × 250 mm). The sampler operated at a flow rate of 1.05 m^3/min, with each sample collected for at least 10 h. The filter membranes in

the atmospheric sampler were collected in triplicate and stored in sterile sampling bags. All samples collected from the four habitats across 15 sites were stored at -80°C in the laboratory until further analysis.

2.2 DNA extraction and high-throughput sequencing analysis

The genomic DNA in the samples was extracted using the VAMNE Stool/Soil DNA Extraction Kit (DM401-C3-P2, Vazyme Biotech Co., Ltd, Nanjing, China), according to the manufacturer's instructions. Full-length 16S rRNA and ITS genes were amplified with the full-length primer sequences. The PCR products were detected by electrophoresis with 1.8% agarose gel to detect their integrity and were purified using a Monarch DNA gel recovery kit. Sequencing was then performed using the PacBio Sequel II platform (PacBio in CA, USA, and BMKCloud Biotechnology Co., Ltd in Qingdao, China), a tri-generation sequencing technology. Sequences were assembled into contigs and subjected to quality control. Subsequently, sequences were clustered to operational taxonomic units (OTUs) at a 97% similarity threshold using nearest-neighbor (single linkage) joins, ensuring a conservative assignment of sequences to OTUs.

2.3 Identification of Baijiu fermentation-associated microbiota

All bacterial and fungal genera were screened according to reported articles[18-20]. The following steps were conducted to identify the key microbiota[21]: (i) Bacterial and fungal genera with an average relative abundance of ≥0.05% were selected to form a keyword list, resulting in the identification of 295 bacterial genera and 192 fungal genera across all samples. (ii) Two sets of literature searches were conducted using the "Core Collection" of Web of Science (https://webofscience.clarivate.cn). The algorithm for bacteria was as follows: TITLE (baijiu OR pit mud OR fermented grain OR Qu) and (TITLE (bacteria∗) OR ABSTRACT (bacteria∗)), on September 28, 2024. The search yielded 206 articles, which were narrowed down to 124 after further verification. Building on the well-established taxonomy of fungi from prior research, the algorithm for fungi was outlined as follows: TITLE (baijiu OR pit mud OR fermented grains OR Qu) and (TITLE (fung*) OR ABSTRACT (fung*)), on September 27, 2024. The search yielded 167 articles, which were narrowed down to 115 after further verification. The time span was set to all years. (iii) the Lost Pearls (https://rookiethx.shinyapps.io/LostPearls)[21] was adopted to select the Baijiu fermentation-associated (BF-associated) genera. The tool requires two input files: a target word list and PDF documents for searching. It processes each word from the list by conducting individual searches across all documents. In the event of a match, the tool outputs the identified word, the sentence in which it appears, and the document source. The list obtained in step (i) was used as the input wordlist, leading to 71 bacterial genera in 117 papers and 60 fungal genera in 112 papers (Supplementary Tables S2 and S3). (iv) Based on further literature analysis, we identified a common core microbiome that frequently appeared in more than 10 papers, totaling 14 bacterial and 16 fungal genera. Meanwhile, these genera were defined as BF genera.

2.4 Statistical analysis

All samples were merged at the supergroup level, and hierarchical clustering was performed using the "ward. D2" method based on Bray-Curtis dissimilarity. Permutational multivariate analysis of variance

(PERMANOVA) at the supergroup level was conducted to explore the relative contribution of the geographical region, site, and habitat to the difference at the community level. Non-metric multidimensional scaling (NMDS) ordination and PERMANOVA were used to investigate differences in community compositions among different groups (four habitats and fifteen sites) for bacterial and fungal datasets. The cross-kingdom bacteria-fungi co-occurrence network constructions were constructed using the R (version 4.1.3) package "psych". To construct a microbial community symbiotic network, the top 50 genera with the highest average relative abundance were selected from those detectable in at least 80% of the samples. Symbiotic interactions among taxa were deemed statistically significant when the correlation coefficient exceeded an absolute value of 0.6 ($|r| > 0.6$) and $P < 0.01$ (Spearman's correlation coefficient). The network was visualized using Cytoscape (v.3.10.2). SourceTracker uses a Bayesian framework to quantify the relative contributions of designated "sources" to a "sink" microbiota[22]. Here, the four habitats in pairs from fifteen sites were selected as unknown sinks and potential sources, respectively. There were 45 replicates for each habitat (3 replicates for each habitat in one site × 15 sites); hence, there were 45 replicates for each sink-sources matrix. The input file was prepared under the guidance of the source tracker (v1.0.1).

3. Results and Discussion

3.1 Diversity of bacterial and fungal communities

In the study, microorganisms from 15 sites across four habitats (soil, air, river, and plant surfaces) were evaluated. After amplification and sequencing of microbial 16S rRNA genes and ITS genes, quality testing and sequence screening yielded 47,698 high-quality OTUs. Both hierarchical clustering and permutational multivariate analysis of variance revealed that habitat types were the predominant factor driving the difference in microbial community structure, with site-specific differences contributing to a lesser extent (Figure 2A and Supplementary Table S4). This finding is consistent with the view of Jiraska et al. (2023) that physical habitat is the primary factor driving the construction of microbial communities[23]. The Shannon and Simpson diversity indices showed significant differences among different habitats ($P < 0.05$, Figure 2B and Figure S1), but no significant changes were observed among the 15 sites ($P > 0.05$, Figure S2, S3). Moreover, there were no significant differences in the species richness or diversity index between different sites[24]. At the environmental ecosystem level, microbes may maintain the stability of biodiversity through their inherent spatial distribution and diffusion mechanisms[4, 25]; whereas at the regional baijiu production level, current brewing activities have not exerted significant directional selection pressure on the diversity of local environmental microbial communities[26]. Together, these results indicate that microorganisms originating from Baijiu fermentation did not have a significant directional effect on the diversity of the local environmental microbial communities[26]. In soil and river water samples, the taxonomic and phylogenetic diversity was the highest, and the plant surface was the lowest (Figure 2C). It is worth noting that bacteria are more diverse in classification and phylogeny than fungi. Bacterial communities in water samples exhibited the widest niche breadth, whereas fungal communities in soil showed the narrowest (Figure 2D). This may be the result of the differential evolutionary strategies of fungi and bacteria,

combined with habitat-specific selection: the higher taxonomic diversity of bacteria gives them a wider potential ecological niche, while the more disturbed river water further selects for bacterial taxa with broad niche breadth[27]; the relatively stable microhabitat of the soil environment is more conducive to fungal communities with highly specialized niches. This habitat-specific variation in microbial niches suggests distinct ecological adaptations.

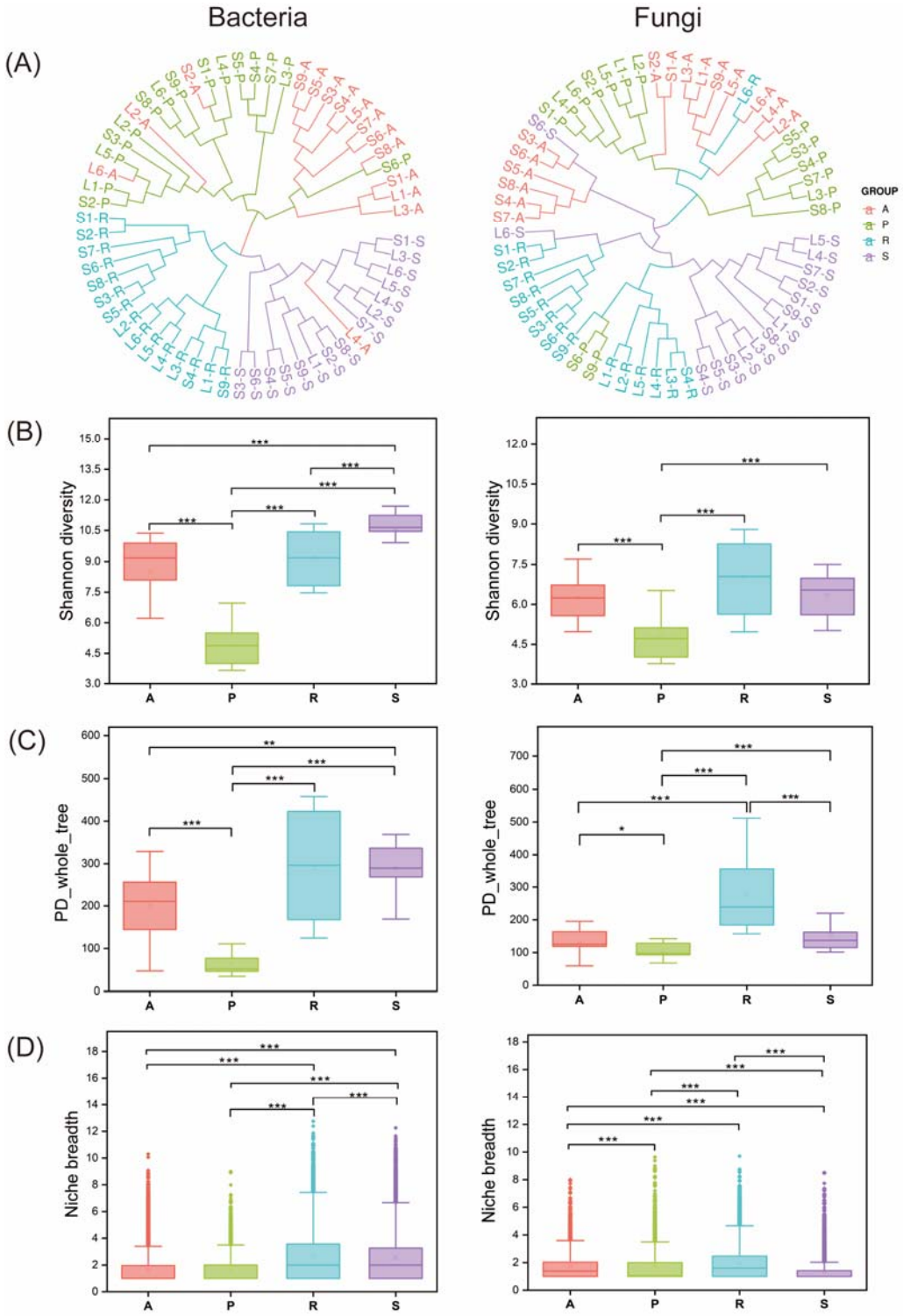

Figure 2 Microbial community α-diversity. (A) Hierarchical cluster of 180 samples containing fifteen sites, four habitats, and three replicates. (B–D) α-Diversity (Shannon richness, PD_whole_tree, and niche breadth) of bacterial and fungal communities in four habitats, respectively. Alpha (α) diversity was analyzed by the Wilcoxon test, and * represents $P < 0.05$, ** represents $P < 0.01$, *** represents $P < 0.001$.

3.2 Microbial Community Composition

The NMDS plots and permutational multivariate analysis of variance (PERMANOVA) based on Bray-Curtis distance showed the same results. Both bacterial (R^2 = 0.302, P = 0.001) and fungal (R^2 = 0.184, P = 0.001) community compositions differed significantly among habitats (Figure 3A), instead of the sampling sites (Figure S4). Forty-four bacterial and 18 fungal phyla were identified across all samples, which showed that bacteria were more abundant than fungi. In all samples, bacterial communities were largely dominated by the phyla of Proteobacteria, Bacteroidota, and Actinobacteriota, with average relative abundance of 55.97%, 11.74%, and 6.10%, respectively (Figure S5A, C). Globally, Proteobacteria consistently dominated airborne bacterial communities, though the relative abundance rankings of other major phyla differed regionally[28]. Proteobacteria had the highest percentage composition (83.14%) on the plant surface. Additionally, Proteobacteria, Actinobacteria, Acidobacteria, and Firmicutes have been frequently documented in the mid-lower Yangtze River basin[29].

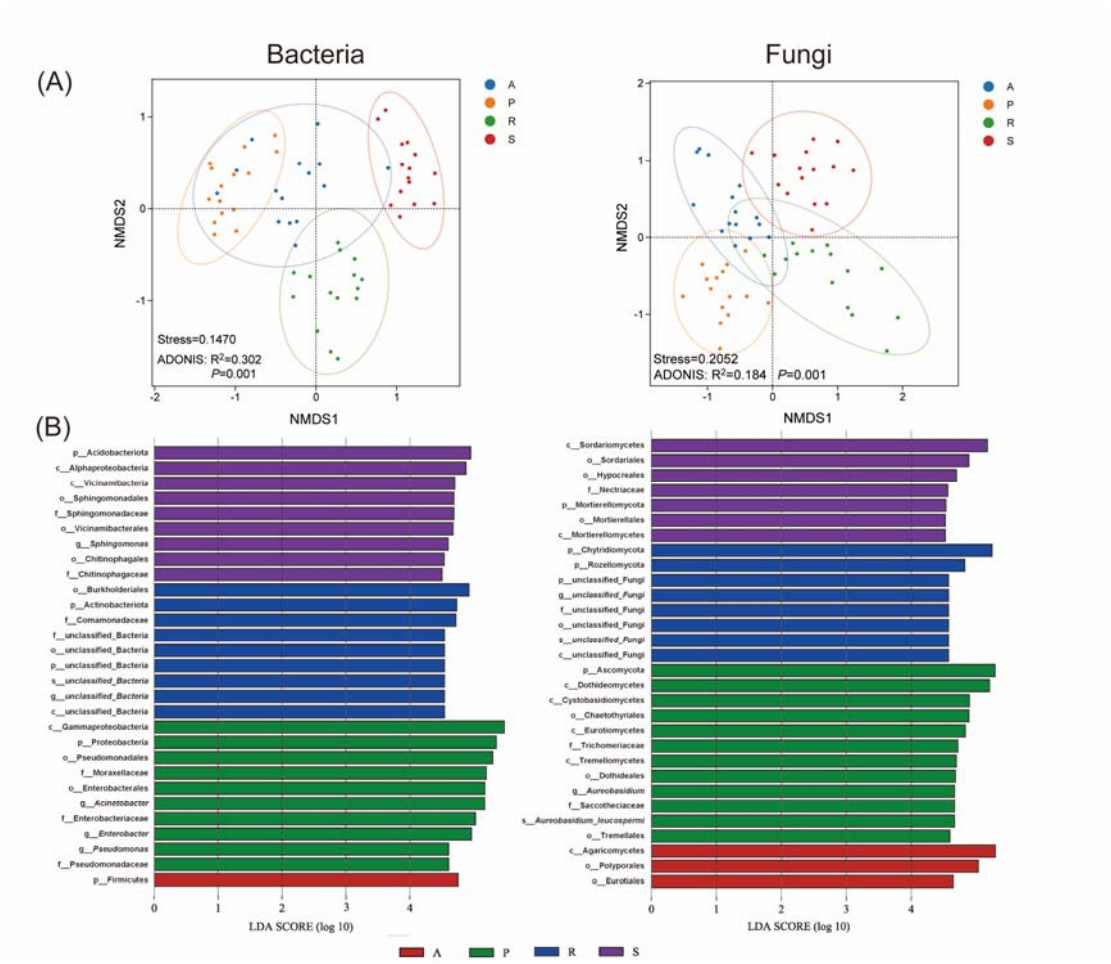

Figure 3 Analysis of the difference in microbial communities in four habitats. (A) Nonmetric multidimensional scaling (NMDS) ordination of microbial communities based on the Bray–Curtis dissimilarity for the bacteria and fungi, respectively. (B) LEfSe analysis identified biomarkers in samples from different habitats. LDA scores (LDA > 4.5, P < 0.05) were computed for taxa differentially abundant between different samples.

Ascomycota (48.23%), Basidiomycota (27.98%), and Chytridiomycota (12.71%) constituted the predominant fungal phyla, with Ascomycota and Basidiomycota dominance in airborne communities aligning with prior studies (Figures S5B, D)[30-31]. Additionally, the highest percentage of Ascomycota was

found on the plant surface, at 62.35%. In river samples, Chytridiomycota (36.71%) dominated fungal communities, followed by Ascomycota (24.94%). Among the major bacterial genera, the relative abundances of *Acinetobacter* and *Enterobacter* were higher on airborne and plant surfaces than in rivers and soil (Figures S6A, B). Fungal communities were dominated by *Cladosporium* (9.97-10.48%) on plant surface and airborne, and the most abundant genus was *Fusarium* (7.41%) in soil (Figures S6C, D). It is worth noting that these dominant taxa (Proteobacteria, Actinobacteria, Ascomycota, and Basidiomycota, etc.) are also widely reported in the fermented grains of Baijiu[10, 18], which indicates that the spontaneous fermentation system of Baijiu shares the microbial community with the local natural environment.

According to the LEfSe pipeline, 5 phyla, 8 classes, 9 orders, 4 families, 2 genera, and 2 species showed significant differences in abundance across the four habitats when an LDA threshold of 4.5 was used (P<0.05, Figure 3B). Twenty-nine bacterial clades showed significant differences between different habitats. Bacterial communities on plant surfaces were characterized by more characteristic clades, predominantly belonging to the order Sphingomonadales (Sphingomonadaceae, *Sphingomonas*) and Chitinophagales (Chitinophagaceae). More bacterial characteristic clades of soil were mainly from classes Gammaproteobacteria, including order Pseudomonadales (Moraxellaceae, Acinetobacter; Pseudomonadaceae, *Pseudomonas*), and Enterobacterales (Enterobacteriaceae, *Enterobacter*). Fungal orders Sordariales, Hypocreales (Nectriaceae), and Mortierellales were enriched in soil communities, while the plant surface supported significantly higher abundance of orders Chaetothyriales (Trichomeriaceae), Dothideales (Saccotheciaceae, *Aureobasidium*, *Aureobasidium_leucospermi*), and Tremellales. Variation in microbial community composition across geographic sites was primarily reflected in dominant clades (Figure S7). Overall, variations in bacterial and fungal compositions across different locations were primarily driven by species-level differences (Figure S7). For instance, within bacteria, *Lachnospiraceae bacterium_DW22* was particularly abundant in L4; *Proteobacterium BF_4* showed higher relative abundance in S6; and *Aeromonas sanarellii* and *Aeromonas taiwanensis* were more prevalent in S7. In fungi, *Periconia epilithographicola* dominated in L3, while *Aspergillus carbonarius* exhibited higher relative abundance in L5.

Microbial community assembly across spatial scales reflected dual drivers: niche selection (habitat) and geographical distance (sites) (Figure 2). The stochastic ratio of phylogenetic normalization (pNST) was quantified using a null model approach. Zero-modeling analyses indicated that the relative distributions of deterministic processes ($|\beta NTI| \geq 2$) and stochastic processes ($|\beta NTI| < 2$) in the assembly of environmental microbial communities were influenced by habitat type and microbial type, especially for airborne and soil samples (Figure 4). Microbial communities dominated by deterministic processes can respond predictably to changes in the environment, whereas stochastically assembled communities may effectively adapt to the changing conditions[32-33]. Specifically, airborne fungal communities exhibited greater deterministic assembly (88.5%) than bacterial communities (47.6%). In soil samples, the effect of deterministic processes on bacterial community assembly was higher (about 67.6%), and the effect of stochastic processes

(Ecological drift, referred to as “undominated” processes) on fungal community assembly was higher (74%). According to Zhang et al. (2023), homogeneous selection primarily structured soil bacterial communities across growing seasons, while stochastic processes dominated fungal community assembly[32]. In river water, stochastic processes dominated both bacterial and fungal communities' assembly, consistent with a previous study on the Chishui River, which showed high stochastic dominance (78.87-82.2%) across abundant bacterial and protist, and rare bacterial communities[34]. The differences in these assembly mechanisms are closely related to environmental conditions: The high hydrological connectivity of river water may reduce the spatial heterogeneity of environmental factors, which is more conducive to the dominance of stochastic processes in microbial community assembly[35]. Moreover, the contribution of deterministic processes generally increases with elevation[36] and exhibits seasonal fluctuations (for example, homogeneous selection can rise to 60.8% in winter[37]). Suitable temperatures and abundant nutrients favor the growth of diverse microorganisms (especially rare species), which not only promote stochastic ecological processes but also significantly enhance microbial diversity and the complexity of ecological networks[37]. Climate change may affect the input pathways and community structure of brewing environment microorganisms by altering regional wind fields, precipitation, and other conditions, thereby further regulating the balance between deterministic and stochastic processes in microbial assembly.

Overall, the assembly of bacterial and fungal communities was mainly driven by ecological drift (undominated) and homogeneous selection. Airborne fungal communities exhibited a high degree of deterministic assembly, reflecting strong habitat filtering effects; this facilitates the stable colonization of specific functional microbes, which could potentially enhance the predictability and stability of core microbial communities in fermentation systems. On the contrary, the randomness of fungal assembly in habitats such as soil and water is high, suggesting that these environments could serve as potential microbial sources for rare species related to brewing. However, this also means that there is greater variability in microbial input during fermentation, which requires appropriate regulation through environmental management in actual production.

3.3 Co-occurrence patterns of microbial communities

Microbial co-occurrence networks were constructed for each habitat using random matrix theory to infer bacterial-fungal interactions. Upon analysis, it indicated that co-occurrence patterns differed across habitats, each with distinctive network topological features, detailed in Table S5. Each modular cluster in the co-occurrence network embodies polymicrobial consortia characterized by cross-kingdom interactions, including bacterial-fungal cross-feeding, co-aggregation, co-colonization, and mutualistic symbiosis[38-39]. The connections of all networks were dominated by positive correlations (67.29-84.30%), indicating that mutualism may be the main form of interaction in these environmental systems[40]. Regardless of habitat type, the dominant taxa in the network mainly come from Basidiomycota, Ascomycota, Bacteroidota, Acidobacteriota, Proteobacteria, and Firmicutes.

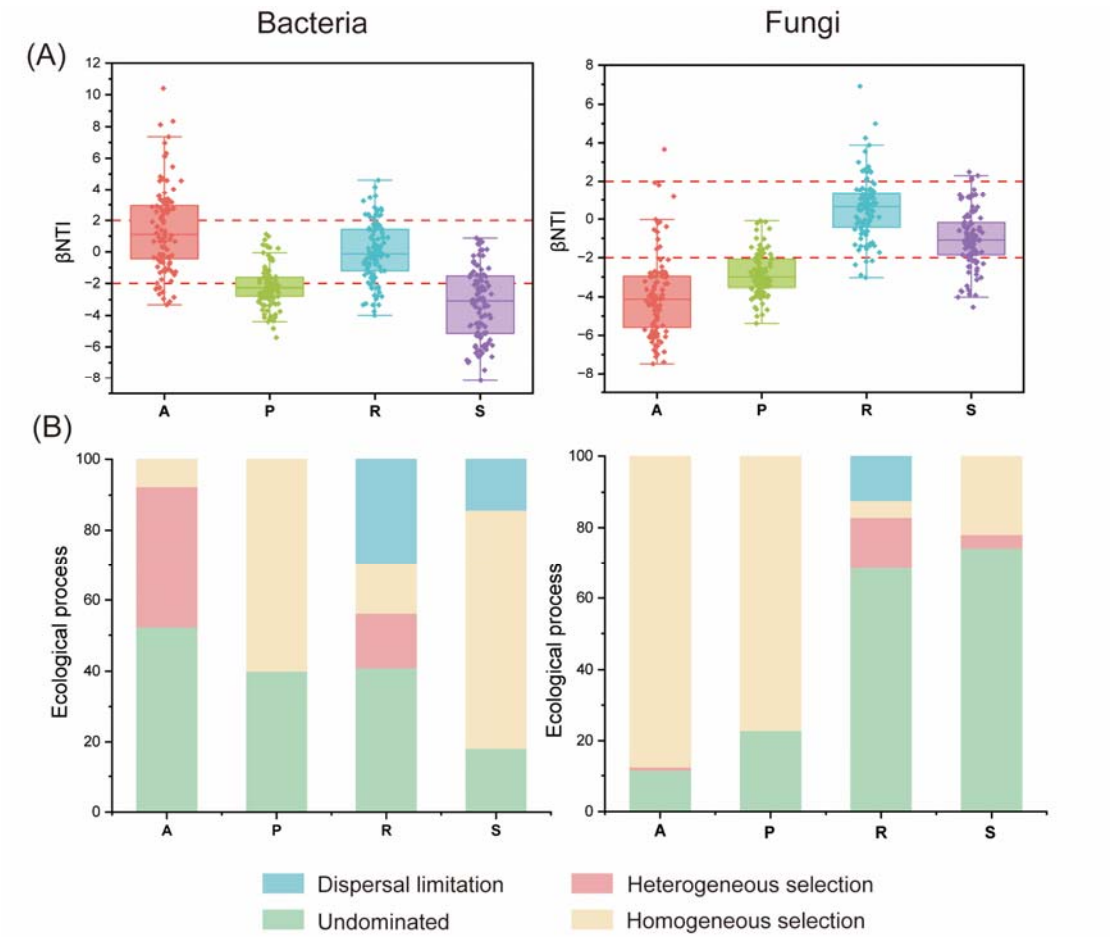

Figure 4 Microbial assembly processes of four habitats. (A) Distribution box plots of βNTI of bacteria and fungi in four habitats. The horizontal red dashed lines indicate the upper and lower significance limits at βNTI = +2 and −2, respectively. (B) Ecological assembly processes for bacteria and fungi were inferred by the phylogenetic information and Bray-Curtis matrices with null models.

The *co*-occurrence network of airborne was clear, consisting of 482 edges and divided into 8 modules, with 82.57% positive (significant positive correlations) (Figure 5A). Its relatively high average clustering coefficient and modularity indices reflect obvious niche differentiation and modular organization[41]. The soil network was relatively simple, with a total of 321 edges, and 67.29% of the connections were positive correlations (Figure 5D). The river water network was the most complex, with 839 edges and 81.88% positive correlations, among which module I accounted for 22% of the entire network (Figure 5C). This network also exhibited a high average degree (16.78) and network density (0.152), as well as a short path length (2.404), indicating that microbial interactions in the aquatic environment were close-knit and efficient. However, higher complexity and diversity may reduce community stability[37]. Therefore, implementing long-term microbial monitoring in Baijiu production areas, especially tracking the dynamic changes of networks at key interfaces such as air, may help alert to the invasion of exogenous pathogens or imbalance of functional microbial communities, thereby providing an ecological basis for healthy management of the brewing environment. In addition, compared with other environments, the bacterial diversity indices (Shannon, Simpson, PD_whole_tree) in river water were significantly higher (Figure 2 and S1, $P < 0.05$), supporting the hypothesis that enhanced niche availability promotes metabolic versatility in

these communities. Cross-habitat comparisons also found that the modularity index of bacterial communities was higher than that of fungi, suggesting that bacteria may have stronger collaborative potential and functional resilience in response to environmental changes.

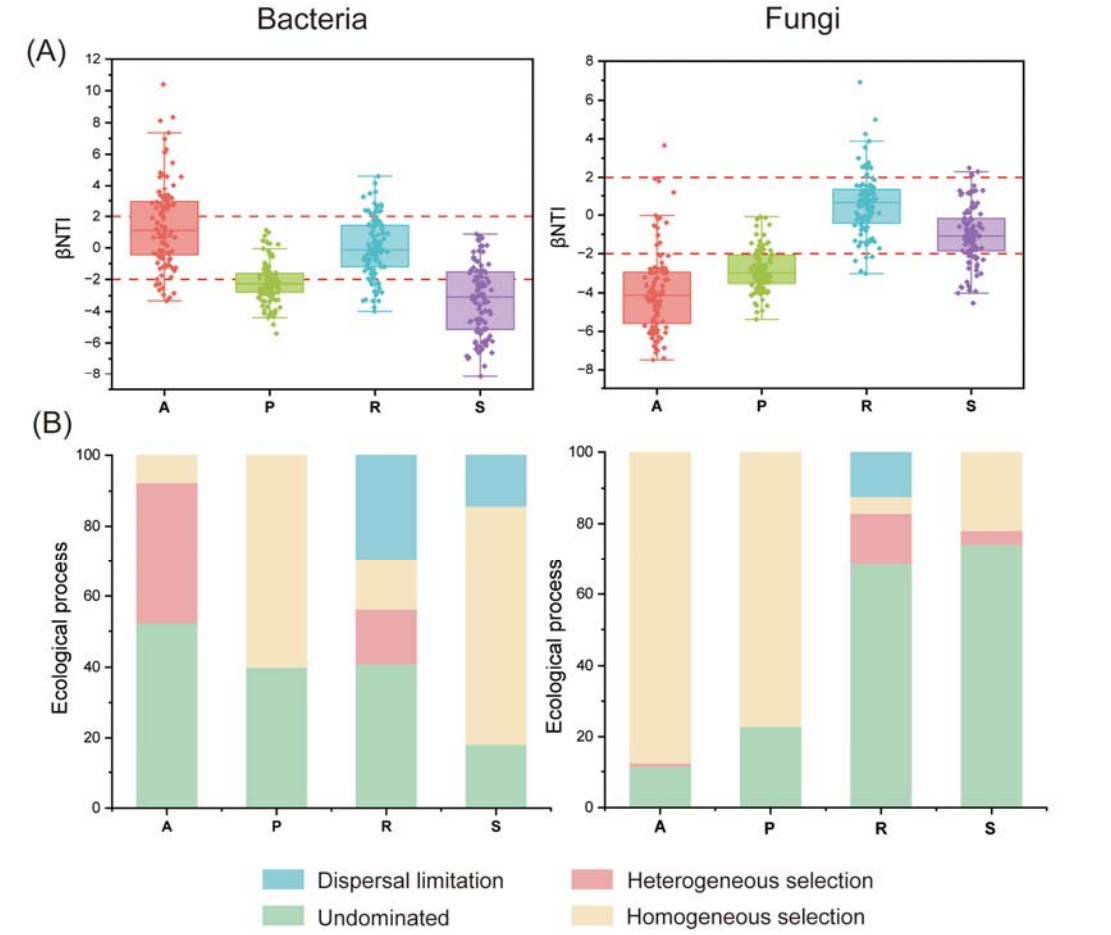

Figure 5 Co-occurrence networks of bacteria and fungi in air (A), plant surface (B), river (C), and soil (D).

3.4 Microorganism movement across habitats

Microbial source tracking analysis indicated that air played a dominant role in cross-habitat microbial transmission, contributing the most to other habitats (bacteria: 93.33%; fungi: 72.71%) (Figure 6). Specifically, in bacterial communities (Figure 6A), the air is the main source of microorganisms for river water and plant surfaces, contributing 52.06% and 93.33%, respectively. River water was also an important source of microorganisms on the plant surface (63.30%), while soil contributes to the microorganisms in air (29.98%) and river water (8.70%) to some extent. In fungal communities (Figure 6B), plant surface microorganisms were mainly sourced from the air (72.71%) and river water (69.88%), and the exchange rate of microorganisms between river water and air was also relatively high. Additionally, there was about 20% moderate connectivity between aquatic (river water) and terrestrial (soil) habitats, indicating that various components of the ecosystem can serve as potential microbial sources. This bidirectional source-sink dynamics can help maintain biodiversity by facilitating microbial exchange during environmental fluctuations[42]. Overall, in most habitats, the proportion of clearly identifiable sources in fungal communities was significantly higher ($P < 0.05$) than that in bacterial communities (Figure S8), a

trend consistent with the study by Li et al. (2023)[43]. As a key transmission medium, air plays a role closely related to the wind-driven long-distance[44-47] transport of particles and microbes, which often leads to increased microbial concentrations and diversity in downwind areas[6, 48]. However, some community members remain untraceable to specific sources, which may reflect unique species in each habitat or the detection of species only at the molecular level[49]. In addition, microorganisms can actively or passively spread through various routes such as human activities, wildlife, winds, rains, or other unknown disturbances[50]. In general, air, as a key medium for the spread of microorganisms across habitats, can affect the microbial inoculation sources of starter (Jiuqu) and fermented grains in the open fermentation system. The highly consistent microbial composition between the plant surface and the air further suggests that environmental microorganisms may participate in the fermentation process through direct sedimentation.

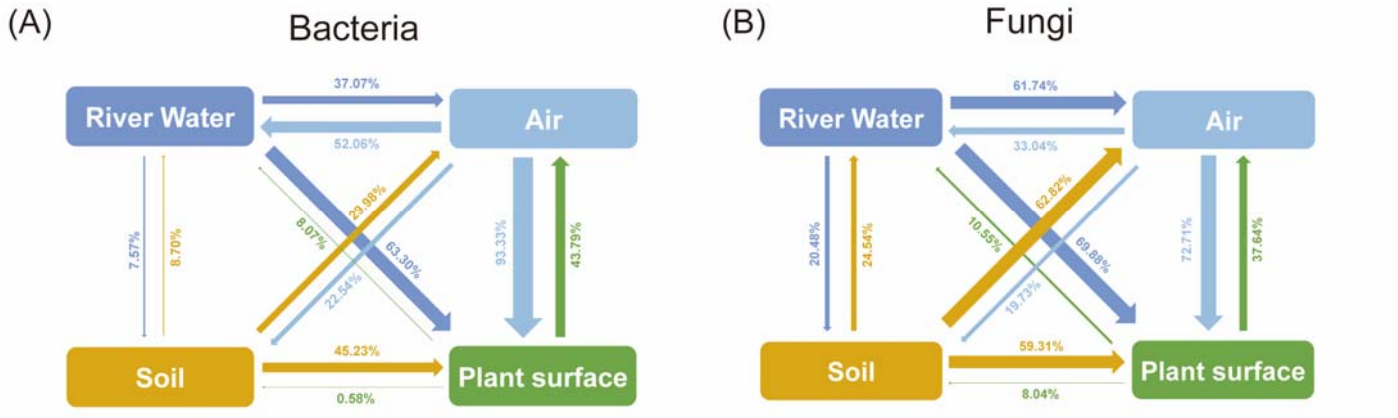

Figure 6 Connectivity of bacteria (A) and fungi (B) across different habitats. The direction of the arrows represents the source-sink relationships, and percentages represent the contribution that each source provides.

3.5 Identification of Baijiu fermentation-associated microbiota

As the core driving factor in the formation of flavors in traditional fermented foods, environmental microorganisms constitute an important microbial resource pool[18]. This principle is reflected in various traditional fermented products, such as Zha-chili[51] and cheese[52], where region-specific microbial communities are key to shaping unique flavor characteristics. In the process of Baijiu brewing, environmental microorganisms not only participate in the formation of initial fermentation flora but also directly or indirectly influence the synthesis of flavor compounds[19]. In this study, 30 microbial genera (14 genera of bacteria and 16 genera of fungi) related to Baijiu fermentation were screened in the upstream environment of the Yangtze River. The distribution of these functional microorganisms showed obvious habitat preference and geographical differentiation (Figure 7).

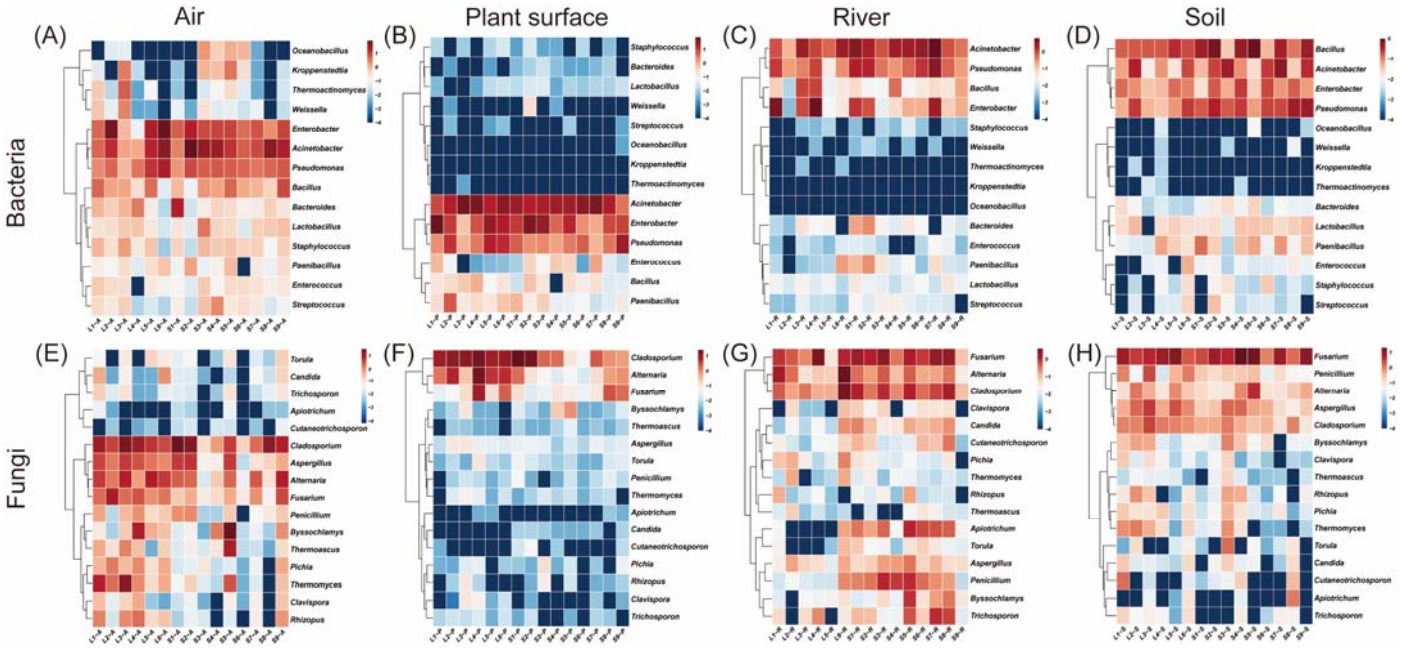

Figure 7 Relative abundance of the Chinese Baijiu fermented-associated microbe at the genus level in four habitats. (A), (B), (C), and (D), 14 bacterial abundances in air, plant surface, river water, and soil sample; (E), (F), (G), and (H), 16 fungal abundances in air, plant surface, river water, and soil sample.

For example, *Oceanobacillus*, *Kroppenstedtia*, *Thermoactinomyces*, and *Weissella* had low relative abundances in most habitats, yet they were enriched in air samples from certain localized areas of Baijiu factories (such as S5, S3, and L3), confirming that air is an important source of brewing microorganisms[10]. On the other hand, long-term brewing activities may also promote the enrichment of specific microorganisms, leading to differences in the composition of fermentation microorganisms in different regions. In contrast, *Acinetobacter*, *Bacillus*, *Enterobacter*, and *Pseudomonas* maintained high relative abundance at all sampling sites, demonstrating broad ecological adaptability and a wide ecological niche. As reported, in the brewing of Baijiu, *Pseudomonas sp.* and *Bacillus oleronius* primarily originate from air, while *Weissella cibaria* and *Bacillus sonorensis* mainly come from soil[53]. Some taxa, such as *Enterobacter* and *Oceanobacillus,* are also present in Daqu and fermented grains, forming the core microbiota of the fermentation system[54]. These microorganisms have specific roles in the formation of Baijiu flavor: *Kroppenstedtia* can synthesize amino acids, lactic acid, terpene, and isoprene flavor substances[1]. *Bacillus* can secrete hydrolytic enzymes (e.g., proteases, amylases), promoting metabolic processes and synthesizing characteristic flavor components like 4-methylpyrazine[18]. Additionally, *Weissella* and *Lactobacillus* are the main producers of organic acids (such as lactic acid and acetic acid), providing the basis for flavor composition[54]. Fungal communities showed stronger regional specificity. *Cladosporium* (0.057%-28.185%), *Alternaria* (0.020%-20.521%), *Fusarium* (0.027%-20.383%), and *Aspergillus* (0.006%-5.604%) maintained high relative abundance across all sampling sites, with fluctuations.

Overall, the distribution of BF-associated microbial genera is regulated by geographical and habitat factors, and long-term brewing activities also domesticate and enrich the microbial composition in the environment, forming a benign mutual feedback between the “environment-fermentation” system[10, 20].

Furthermore, the core microbial taxa identified in this study, which show clear habitat preferences and are closely associated with the brewing environment (such as *Kroppenstedtia*, *Thermoactinomyces*, *Weissella*, and related fungi), can be considered as a highly promising reservoir of environment-derived brewing microbes. Based on the habitat distribution map provided in this study (Figure 7), future efforts could focus on selectively isolating and purifying these core strains from key habitats, especially air (particularly around the factory area), factory soil, and plant surfaces, and systematically conducting fermentation phenotype validation. This strategy may not only assess the fermentation performance of each strain but also help reveal the evolutionary trajectories and ecological shaping mechanisms of brewing microbes, optimizing microbial resources and enhancing the stability and controllability of the brewing process.

3.6 Predicted functional potential of microbial community

Changes in the composition and diversity of microbial communities usually indicate functional differences. In this study, PICRUSt2 was used to predict the functional potential of bacterial communities in four habitats (Figure S9A). KEGG L2 metabolic pathway prediction analyses identified 30 significantly different functional pathways, which can be classified into 6 main functional groups: cellular processes (4 pathways), genetic information processing (4 pathways), human disease (6 pathways), and metabolic and other (16 pathways) (Figure 8A). Among them, the relative abundance of the "global and overview map" pathway was the highest (41.14%–42.35%), followed by carbohydrate metabolism (8.42%–8.79%). This functional feature was highly correlated with the hydrolysis and transformation requirements of carbohydrates during starter (Jiuqu) making and Baijiu fermentation, suggesting that environmental microorganisms may be used as potential resources for fermentation. In addition, the soil samples were enriched with various key functions, including cell growth and death, folding, sorting, and degradation, replication and repair, transcription, translation, and the biosynthesis of secondary metabolites. These processes may enhance the metabolic activity and structural diversity of microbial communities[55]. Characterization of fungal functional guilds using the FUNGuild database showed that saprotrophs (45.59-69.37%) dominated in all habitats, followed by pathotrophs (21.29-39.90%) and symbiotrophs (9.34-25.58%) (Figure S9B). The composition of fungal guilds differed significantly between habitats (Figure 8B), with the relative abundance of endophyte, epiphyte, wood_Saprotroph, dung_Saprotroph, and leaf_Saprotroph being significantly influenced by habitat types ($P < 0.05$). Specifically, wood_Saprotroph was significantly enriched in the air, and endophyte and epiphyte showed higher relative abundance on the plant surface sample.

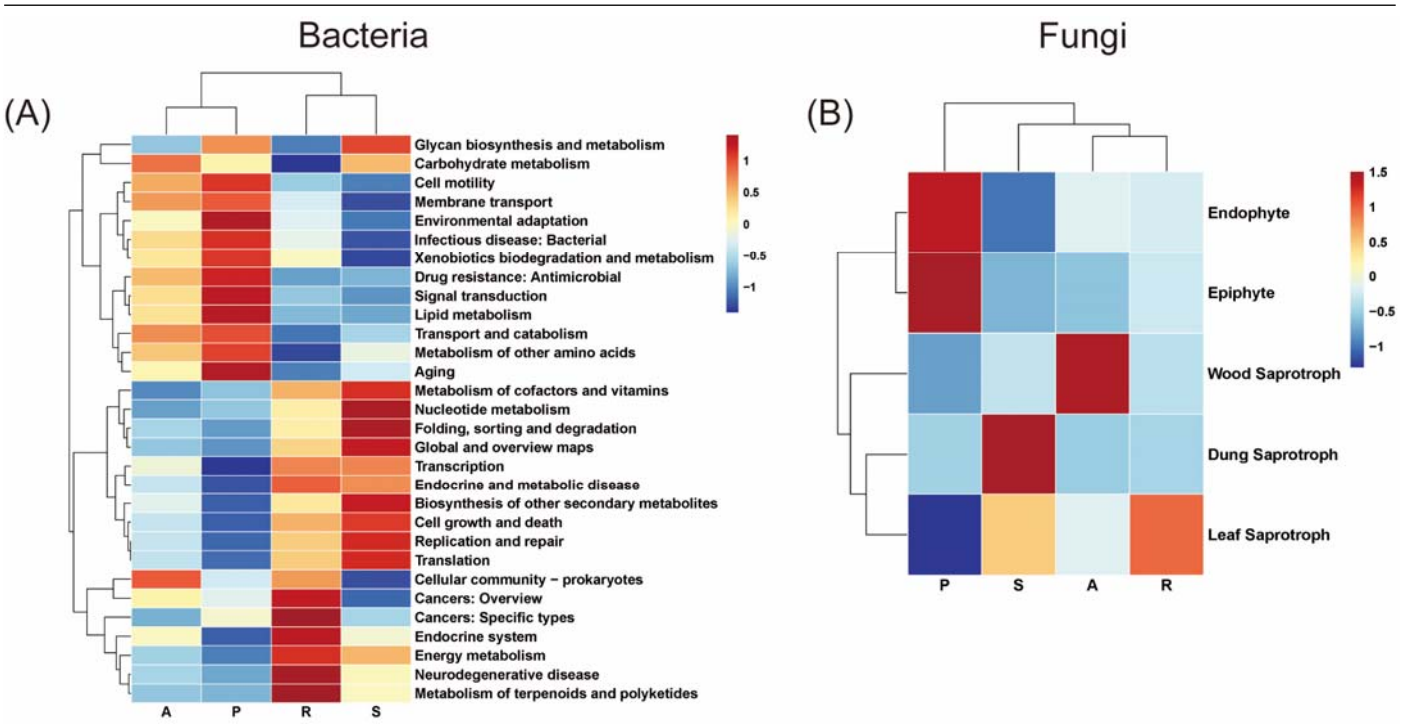

Figure 8 Differential functional analysis among four habitats. (A), Significantly different functions of bacterial communities in four habitats were predicted based on the KEGG pathway. (B), Significantly different function guilds of fungal communities in four habitats were predicted based on the FUNGuild database.

Functional analysis of BF-associated microbes was conducted by further using the OmicStudio tools (https://www.omicstudio.cn/tool) [56]. We focused on amino acid metabolism and carbohydrate metabolism pathways closely related to Baijiu fermentation, and analyzed the predicted abundance of the corresponding enzyme-encoding genes (EC numbers) (Figure 9). These results showed that in bacteria, except for point L4, the abundance of metabolic genes is generally higher in air and plant surface samples, and lowest in soil. Fungi showed a different trend, with lower abundances at S3, S4, S6, and S7, and higher abundance at L2, L3, and S5. It is worth noting that the metabolic potential in air and plant surface samples was higher than that of soil and river water samples, indicating that carbohydrate and amino acid metabolic genes carried by brewing-related functional microorganisms in these habitats were more abundant, which may affect the fermentation efficiency and the formation of flavor metabolites. A suitable micro-ecological environment is beneficial for maintaining the stability of brewing microecology. Our results further revealed the potential impact of airborne microbiomes on local ecological functions, and can support the regional baijiu industry by regulating environmental microbes and ecological factors. However, although PICRUSt2 and FUNGuild are widely used and highly flexible, their functional predictions based on amplicon sequencing have limitations: they cannot provide the resolution to distinguish strain-specific functions and are constrained by reference genomes[57], making it difficult to comprehensively and accurately reveal the true functions of microbial communities[58]. To systematically clarify the specific pathways and impact of these microbes and their functions in baijiu fermentation, further studies combining metagenomics, metabolomics, and other technologies are needed in the future.

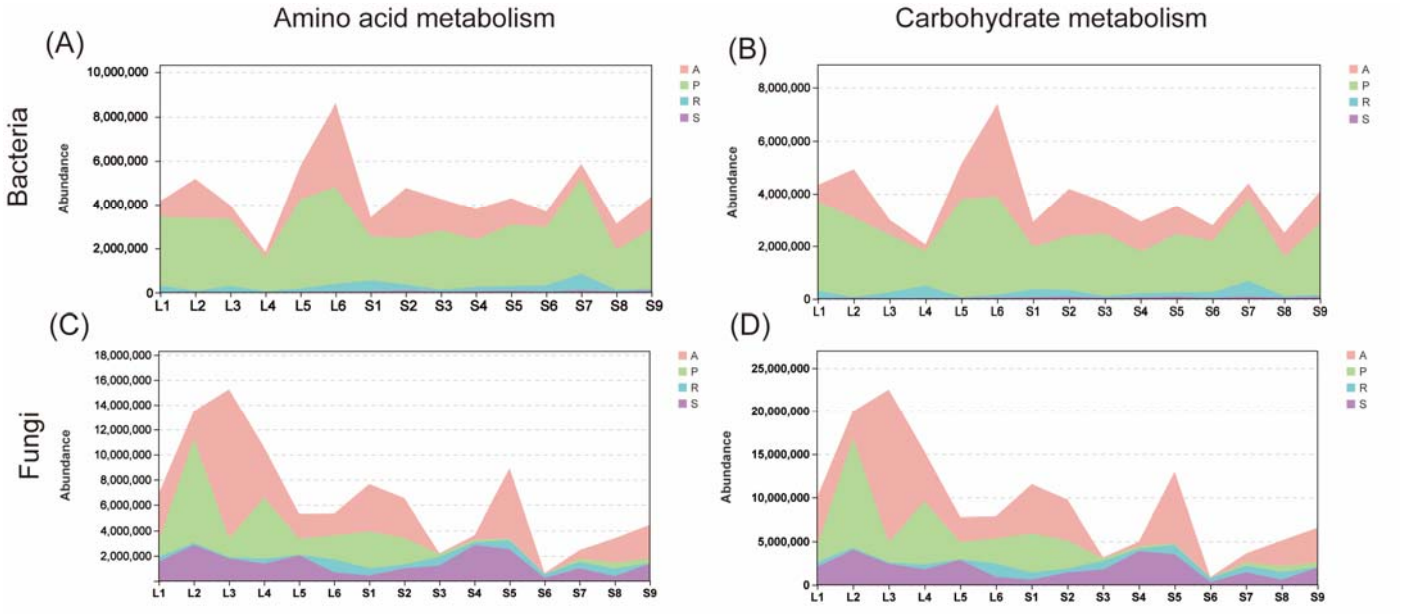

Figure 9 Abundance variation of the functional genes encoding key enzymes involved in amino acid metabolism and carbohydrate metabolism in microorganisms associated with Chinese Baijiu fermentation at fifteen sites by PICRUSt2 analysis.

4. Conclusion

This study systematically analyzed the distribution patterns, assembly mechanisms, and functional potential of environmental microorganisms in the core baijiu-producing regions of the upper Yangtze River. The results show that habitat type was the primary factor regulating microbial community construction, and air served as a key medium for microbial dispersal across environments. Community assembly was mainly driven by ecological drift and homogeneous selection, with the importance of each varying depending on microbial groups (bacteria/fungi) and habitats. In addition, we identified a core microbial resource set containing 30 genera, which were associated with Baijiu fermentation and have clear metabolic functions (carbohydrate conversion and flavor compound synthesis). In addition, the distribution of related microbial genera and their functions was distinctly influenced by geographical and habitat-specific factors. These findings not only deepen the understanding of the microbial biogeography in traditional fermentation ecosystems but also transform environmental microbiomes into actively controllable 'brewing resources', providing a theoretical basis for discovering region-specific brewing microbes and achieving targeted regulation of fermentation processes. In the future, it will be necessary to further reveal the functions of environmental microbes and their interaction mechanisms with fermentation systems through the integration of time-series dynamic monitoring, key strain cultivation, and multi-omics technologies, thereby promoting the deep integration of microbial ecology theory with brewing practice.

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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Data availability

The raw data reported in this paper have been deposited in the Genome Sequence Archive in the National Genomics Data Center, China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences, with BioProject number PRJCA041281 (GSA: CRA026601 for fungi, and CRA026602 for bacteria).

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