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Metabarcoding insights into microbial drivers of flavor development and quality stability in traditional Chinese red pepper sauce: impacts of varietal selection and solar/shade fermentation

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Abstract

Background Red pepper sauce is a traditional Chinese condiment, which is rich in nutrients and popular worldwide. However, the changes in the microbial community of red pepper sauce during fermentation and the effects of such changes on quality stability have been under studied. In this study, we systematically analyzed the relationship between the microbial community composition of multiple red pepper sauces and the biochemical indexes. Moreover, we also explored the dynamics of changes in the microbial community composition using metabarcoding sequencing.

Results Our analysis revealed significant differences in amino acids (AA), lactate, pectin, reducing sugar, flavonoids, phenolics, pigments, and alcohol dehydrogenase (ADH) activity among the six red pepper sauces. Moreover, the relative abundance of bacteria and fungi showed significant differences among multiple red pepper sauces. Among these biochemical indexes, water content, pigment, and capsaicin showed a significant negative correlation with the abundance of multiple bacterial genera. ADH activity showed a significant positive correlation with the abundance of multiple bacterial genera. The content of AA, flavonoid, pectin, and gamma-aminobutyric acid (GABA) was significantly correlated with the relative abundance of multiple fungi such as *Rhodotorula*, *Dipodascus*, *Leucosporidium*, *Hannaella*, and *Coniochaeta*.

Conclusions These results provide a basis for revealing the biological basis of the quality stability and flavor characteristics of red pepper sauce, which are of great significance for further investigation of the fermentation mechanism and control of the product quality of red pepper sauce.

Keywords Red pepper sauce, Fermentation, Microbial community function, Metabarcoding sequencing, Flavor characteristics, Capsicum annuum

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Background

Red pepper sauce, which is also known as doubanjiang or "Pixian douban", as a traditional condiment in China, is not only important to the Chinese food culture, but also favored by consumers for its special flavor and nutritional value [1, 2]. The traditional production of red pepper sauce relies on natural fermentation, made from red peppers (*Capsicum annuum* L.), broad beans (*Vicia faba* L.), wheat flour (*Triticum aestivum* L.), and salt. This process involves the combined action of various microorganisms [3]. The flavor characteristics of red pepper sauce are determined by its complex chemical composition, which includes amino acids, organic acids, esters, and aldehydes, which together constitute the special aroma and flavor of red pepper sauce [4–6]. Despite the cultural significance of artisanal doubanjiang, industrial standardization remains challenging: studies report > 30% batch-to-batch variation in amino acid content [2] and inconsistent microbial succession patterns under fluctuating solar exposure [7]. These quality instabilities stem from two underexplored factors: (i) fungal community dynamics governing secondary metabolite synthesis, and (ii) cultivar-specific substrate-microbe interactions. By optimizing fermentation conditions, including temperature, humidity, and salinity, we can control the fermentation process and enhance the production of various compounds, thereby improving the overall flavor of red pepper sauce [3, 8].

The microorganisms in red pepper sauce include bacteria, yeasts, and molds, which play an indispensable role in fermentation. Li et al. investigated the bacterial community evolution and metabolite changes during the fermentation of red pepper sauce by high-throughput sequencing of the 16S rRNA gene and found that *Pseudomonas* and *Streptococcus* were remarkably correlated with nitrogenous and carbonic metabolites [9]. Li et al. also found that different salt levels can affect the microbial community and the expression of antimicrobial genes in red pepper sauce [7]. By identifying the microbial species in red pepper sauce and understanding their roles during fermentation, a theoretical basis is provided to improve the stability of the fermentation quality and the flavor of the red pepper sauce [7, 9].

Although Zhang et al. investigated physicochemical factors and microbial dynamics during red pepper fermentation, existing research predominantly emphasizes bacterial composition in red pepper sauce [2]. However, fungal community structure and its impact on fermentation quality remain poorly characterized. Current research efforts remain constrained to homogeneity analyses of standardized products generated through fixed combinations of raw ingredients and fermentation regimes, neglecting variability assessment across diversified production systems. Different red pepper varieties and fermentation methods, including sun and shade treatment, were used in producing red pepper sauce, which have a certain impact on the quality of red pepper sauce.

In this study, three red pepper varieties were used as raw materials, and six types of red pepper sauce were prepared. The composition and abundance of bacteria and fungi in the six red pepper sauce were identified and analyzed, and 15 biochemical indicators were used to analyze the relationship with different bacterial and fungal compositions. The results will provide a reference for the optimization of the production method and flavor characteristics of red pepper sauce, as well as the identification of microbial compositions. Furthermore, the results of this study will provide a scientific basis for improving the quality and safety of red pepper sauce.

Materials and methods

Red pepper sauce preparation

In this study, three red pepper varieties were used to produce red pepper sauce, including "Chuan Teng No.6 (CT6)", "Hong Guan No.4 (HG4)", and "Hong Guan No.5 (HG5)". Once cleaned, the red peppers were manually chopped into pieces approximately 0.1–0.2 cm in length. All samples were prepared using the same method. The broad beans (*Vicia faba* L.) were harvested and removed from the pod. After sun drying, the broad beans were softened under warm water (45 °C). Next, the shells were removed before mixing the beans (40 kg) with wheat flour (8 kg, *Triticum aestivum* L.) and fermented "naturally" at room temperature for 8 months. Then, the mixtures were further fermented at room temperature for 4 months (which was known as shade treatment) or transferred to drying cylinders for 4 months sun-drying fermentation (which was known as sun treatment). Subsequently, the mixtures were added with cutting red peppers, koji (*Aspergillus oryzae,* the pure strain was purchased from QuFu biological technology company (Chengdu, China)), 10–12% sodium chloride (w/v), and 20% water (w/v) and fermented in the sun for 2 years. All samples were divided into two groups; one group was fermented in the sun (S) and the other in shade (Y) at 22–25 °C. These samples were labeled as follows: CT6_S, CT6_Y, HG3_S, HG3_Y, HG4_S, and HG4_Y. The experiment for each treatment was performed at least in triplicate.

DNA extraction and metabarcoding of bacterial and fungal communities

Each red pepper sauce sample (5 g) was mixed with sterile water (25 mL), filtered through three layers of coarse sterile gauze to remove large particles, and centrifuged at 18,500 g for 10 min at 4 °C. The pellets were used for

genomic DNA extraction in accordance with the Zymo Research BIOMICS DNA Microprep Kit instruction (Cat# D4301). The concentration of red pepper sauce DNA samples was detected by using Tecan F200 (Tecan, Switzerland).

The 16S rRNA primers 515F (5′- GTGYCAGCMGCC GCGGTAA -3′) and 806R (5′- GGACTACHVGGG TWTCTAAT -3′) [10] and internal transcribed spacer (ITS) rRNA primers ITS1 (5′-GGTCATTTAGAGGAA GTAA-3′) and ITS4 (5′-AGCCTSCSCTTANTDATA TGC-3′) [11] were used for amplification by Applied Biosystems PCR System 9700 (ABI, USA). The H_2O and the Microbial Community DNA Standard (Zymo Research #D6305, USA) were used as negative and positive control. The PCR amplification program included an initial denaturation at 98 °C for 30 s, followed by 30 cycles of 98 °C for 5 s, 54 °C for 15 s, and 72 °C for 45 s, and a final extension at 72 °C for 2 min. PCR products were analyzed using 1% (w/v) agarose gel electrophoresis. Bands with a correct size were purified using the Zymoclean Gel Recovery Kit (D4008) and quantified using the Tecan F200. The library was constructed in accordance with Illumina (NEB#E7645L) library preparation protocols and then sequenced by NovaSeq 6000 at Rhonin Biosciences Co. (Chengdu, China). All the raw metabarcoding datasets in this study are publicly available in the NCBI BioProject with the accession PRJNA1127001.

Data analysis

Sequence data analysis was carried out using QIIME2 [12]. Raw sequences were assembled and demultiplexed based on barcodes using FLASH [13] and Sabre, respectively. Sequences failing to meet quality thresholds (average quality < 29 and maximum ambiguous > 1) were discarded by the quality-filtering module. Denoising and chimera checking were performed using DADA2 [14]. The Deblur algorithm was employed in QIIME2 to generating an Amplicon Sequence Variant (ASV) feature list and sequence. Microbial community structure refers to the taxonomic composition and organization of microbial populations within the ecosystem, characterized by phylogenetic diversity, alpha diversity, and beta diversity. It was analyzed as follows: The representative sequences from denoised 16S rRNA and ITS data were taxonomically classified using a Naïve Bayes classifier, employing the SILVA 138.1 database (release date: December 2020) for prokaryotic sequences and UNITE 9.0 for fungal identification (Note: The SILVA 138.1 database retains the historical nomenclature for *Lactobacillus* prior to its reclassification into multiple genera (e.g., *Lacticaseibacillus*, *Limosilactobacillus*) proposed by Zheng et al. [15]). Phylogenetic trees were constructed using muscle and FastTree [16, 17]. Faith's phylogenetic diversity was measured by using the Picante package [18]. All samples were homogenized and resampling was carried out according to the least amount of data. Chao1, Simpson, and Shannon–Wiener indices were estimated by using the vegan package. Unifrac distances were computed using the GuniFrac package [19]. Bray–Curtis and Jaccard distances were calculated using the vegan package. Principal co-ordinate analyses (pCoA) were performed using the ape package [20]. The Principal PCoA and non-metric multi-dimensional scaling analysis were performed using the vegan package. The ANOSIM (Analysis of Similarity) and PerMANOVA (Permutational Multivariate Analysis of Variance) were computed using the vegan package in R (https://github.com/vegandevs/vegan/). Differential species analysis was performed using Linear Discriminant Analysis Effect Size (LEfSe) tool [21] and Metastats based on machine learning [22]. Functional prediction was conducted using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) [23], and Fungi Functional Guild (FUNGuild) [24]. The coefficient of variation was used to measure the degree of variation in species abundance.

Changes in chemical composition of different red pepper sauces

Red pepper sauce samples (three biological replicates) were collected. pH was measured using a digital pH meter (PHS-3C pH Meters, Shanghai Kang Yi Instrument Co., LTD., China) in accordance with the National Standard of China (GB 5009.237-2016). Similarly, water content was measured in accordance with the National Standard of China (GB 5009.3-2016). Total soluble solid content (SSC) was measured in accordance with the Agricultural Industry Standard of China (NY/T2637-2014). Pigment content was measured in accordance with the National Standard of China (GB 1886.34-2015). Capsaicin content was measured in accordance with the National Standard of China (GBT21266-2007). Amino acid, lactic acid, pectin, reducing sugar (RS), flavonoid, phenolics, alcohol dehydrogenase (ADH), gamma-aminobutyric acid (GABA), nitrite, and carotenoid content were measured in accordance with the kit instructions (Ruixin Bio, Quanzhou, China).

Results

Chemical characteristics of different red pepper sauces

Fifteen biochemical indices were examined in six types of red pepper sauces, and eight biochemical indices were found to be remarkably different in part of red pepper pastes, including AA, lactate, pectin, reducing sugar, flavonoid, phenolics, pigment, and ADH (Fig. 1A–O).

Analysis of 15 biochemical indices revealed significant interrelationships: Water content positively correlated

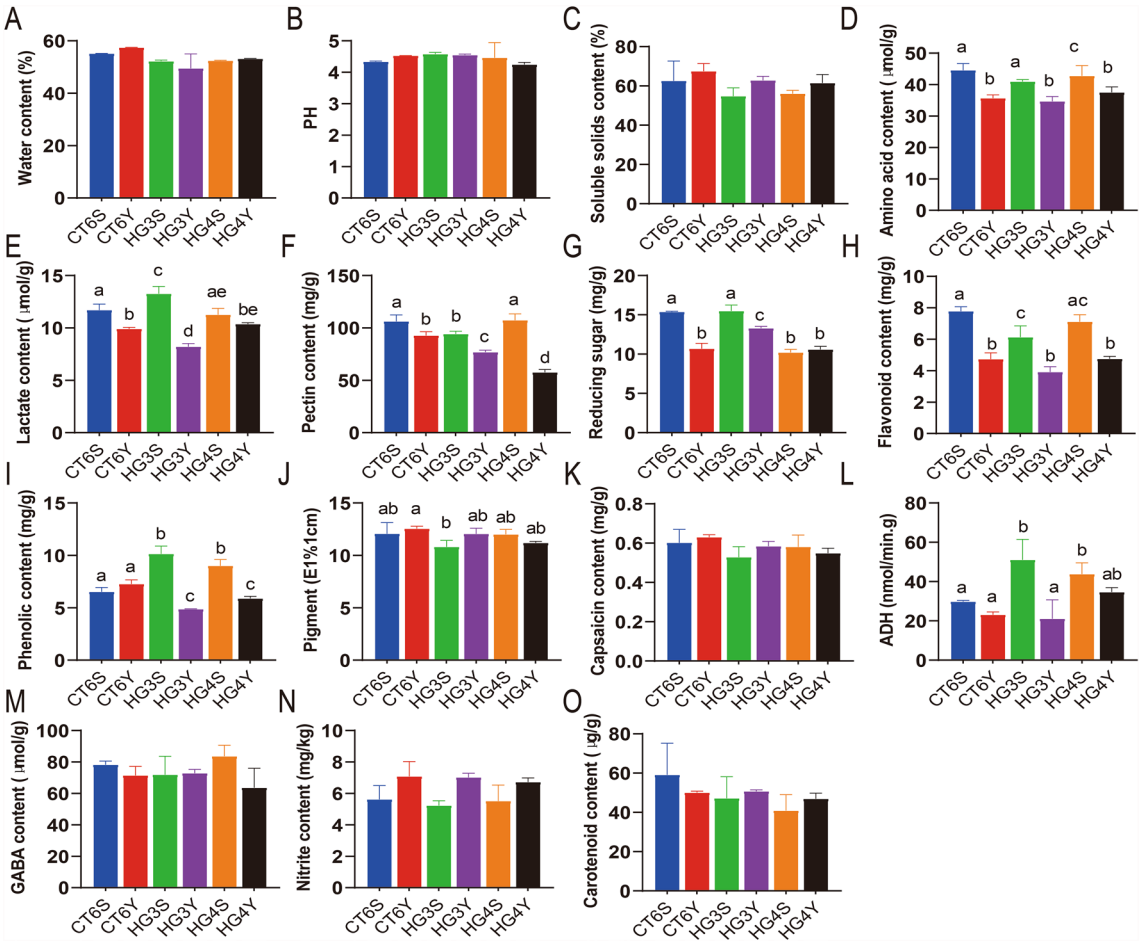

Fig. 1 Chemical composition detected in different red pepper sauce. **A** Water content. **B** PH value. **C** Soluble solids content. **D** Amino acid content. **E** Lactate content. **F** Pectin content. **G** Reducing sugar. **H** Flavonoid content. **I** Phenolics content. **J** Pigment. **K** Capsaicin content. **L** ADH activity. **M** GABA content. **N** Nitrite content. **O** Carotenoid content. Roman letters indicate significant differences among different samples ($p < 0.05$). Values are means ± SD (n = three biological replicates). S, fermented in the sun; Y, fermented in shade

with pigment levels, while negatively correlated with ADH activity. Notably, AA content exhibited strong positive associations with lactate, pectin, and flavonoid concentrations, but inversely correlated with nitrite levels. Furthermore, nitrite content showed negative correlations with lactate, pectin, flavonoid, phenolics, and ADH activity (Additional File 1: Fig. S1).

Bacterial community structure in different red pepper sauces

In this study, 6 red pepper sauces were sequenced, and after removing low-quality reads, a total of 608,447 high-quality reads were obtained, with an average of about 33,800 reads per sample. The bacterial community composition of the six red pepper sauces varied considerably at the genus level, with CT6 (CT6_S and CT6_Y) having a relative abundance of less than 34%. In addition, HG3 and HG4 had relative abundances higher than 42% (Fig. 2A). Overall, *Pseudomonas* was the most dominant species in the six red pepper sauces, followed by the genera *Bradyrhizobium*, *Janthinobacterium*, and *Ralstonia*, and the relative abundance of these genera was higher than the relative abundance of CT6 in HG3 and HG4 (Fig. 2A; Additional File 2: Table S1). Alpha diversity indices showed no significant differences in species richness between the two treatments for CT6 and HG3, but significant differences in species richness were found between HG4_S and HG4_Y. CT6_S and CT6_Y were also significantly different from HG4_Y (Fig. 2B). Comparing the species richness among red pepper sauces of different varieties, no significant difference was observed (Additional File 3: Fig. S2A). However, a significant difference in species abundance was found between sun-fermented and shade-fermented red pepper sauces (Fig. 2C).

The results of PCoA showed that the bacterial compositions of all red pepper sauces were mainly separated into

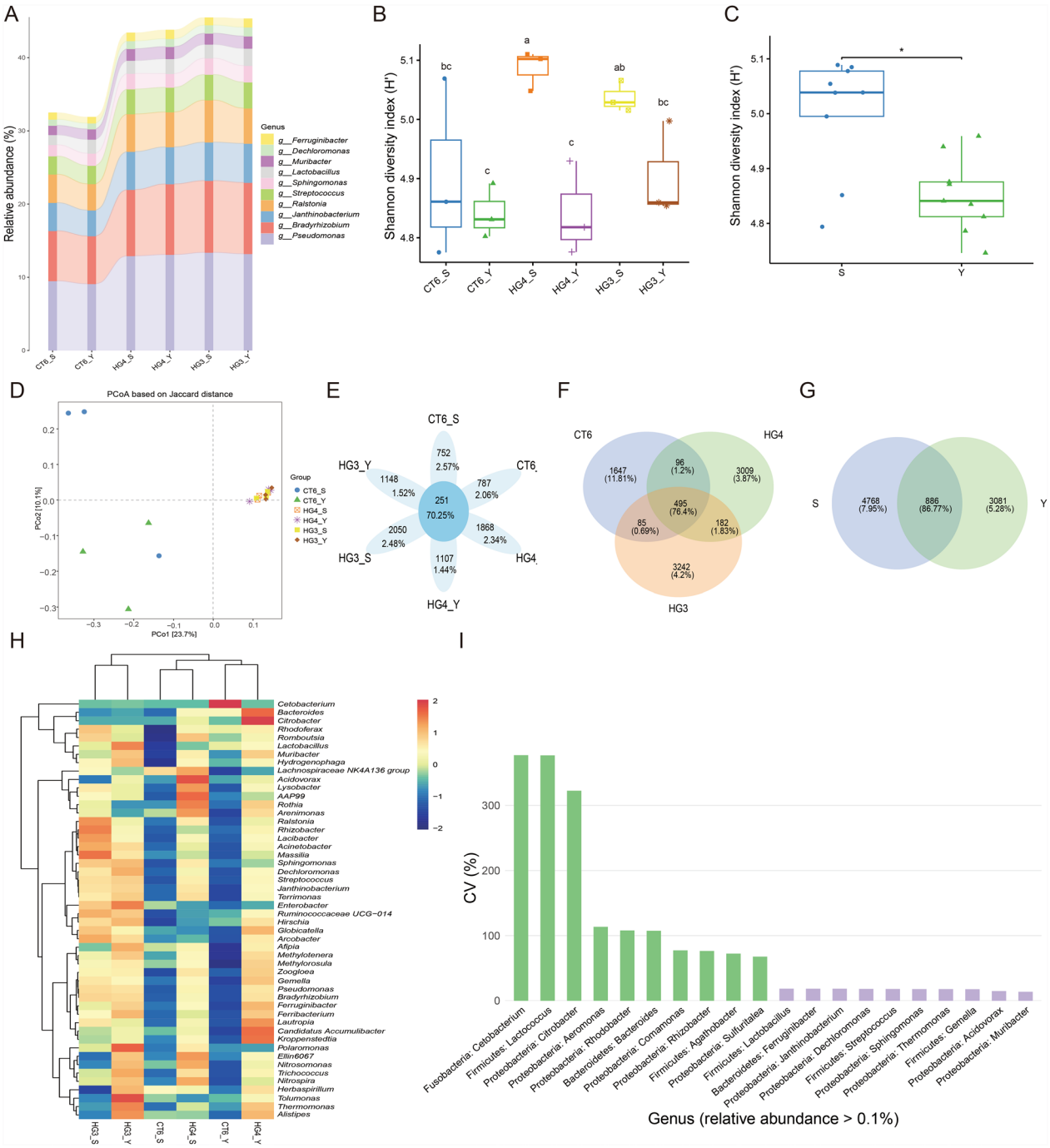

Fig. 2 The bacterial abundance analysis of red pepper sauces. **A** Sankey plot of high abundance genus (sorted by total mean abundance). **B** alpha diversity of six red pepper sauces. **C** Alpha diversity of shade and sun treatment red pepper sauces. **D** PCoA based on Jaccard distance. **E** Venn plot of six red pepper sauces. **F** Venn plot of red pepper sauces made by three red pepper varieties. **G** Venn plot of shade and sun treatment red pepper sauces. **H** Heatmap at genus level. **I** Coefficient of variation at genus level. S, fermented in the sun; Y, fermented in shade

two groups, where HG3_S, HG3_Y, HG4_S, and HG4_Y clustered into one group, and CT6_S and CT6_Y were relatively far away, with a large variation between samples. Using unweighted Jaccard, the first two axes (PCoA1 and PCoA2) accounted for 23.7% and 10.1% of the total variance, respectively (Fig. 2D). Using unweighted UniFrac, PCoA1 and PCoA2 accounted for 13.6% and 7.7% of the total variance, respectively (Additional File 3: Fig.

S2B). This result indicates that changes in bacterial community may be due to changes in the relative abundance of OTUs.

For the bacterial composition of the six red pepper sauces, 251 common OTUs were found, and the specific strains to each red pepper sauce accounted for about 1.44–2.57% of the total number of strains (Fig. 2E). For the different pepper varieties, 495 common OTUs were identified. CT6 varieties had about 11.81% specific OTUs, which was more than the specific OTUs in HG3 and HG4 (Fig. 2F). Analyzing the strains of sun-fermented and shade-fermented red pepper sauces, 886 common OTUs were found (86.77%, Fig. 2G).

Analyzing the changes in bacterial abundance at the genus level, the red pepper sauces made by HG3 and HG4 varieties had higher relative abundance compared with most genera in CT6, such as Cluster II (including *Ralstonia* etc.). In addition, sun-fermented and shade-fermented red pepper sauces differ between HG3 and HG4, and the difference was primarily found in Cluster III, including *Polaromonas* etc. In CT6, the differences were mainly concentrated in Cluster I, such as *Cetobacterium* (Fig. 2H). The genera with large fluctuations in abundance mainly include *Cetobacterium* (*Fusobacteria*), *Lactococcus* (Firmicutes), and *Citrobacter* (Proteobacteria, Fig. 2I).

Fungal community structure

The ITS gene was used to evaluate fungal abundance. Using the abovementioned methods, 563,364 high-quality reads were obtained, with an average of about 31,300 reads per sample. The fungal community composition of the six red pepper sauces varied considerably at the genus level. The relative abundance of *Starmerella* in CT6_S is high (~30% of the total), whereas that of *Pichia* spp. is low (~5% of the total). In addition, the relative abundance of *Starmerella* in CT6_Y decreases sharply (less than 5% of the total), whereas that of *Pichia* spp. increases sharply (~60% of the total). HG4_S had the lowest total relative abundance of genera (about 25%), and the relative abundance of *Debaryomyces*, *Starmerella*, and *Candida* in HG4_Y were all massively increased. The genera with large differences in relative abundance between HG3_S and HG3_Y included *Debaryomyces*, *Pichia*, *Starmerella*, *Candida*, etc. (Fig. 3A). Alpha diversity indices showed significant differences between CT6_S and CT6_Y, as well as between HG4_S and HG4_Y (Fig. 3B). Significant differences were also found between HG3 and HG4 varieties (Fig. 3C). In addition, a significant difference in species abundance was observed between sun-fermented and shade-fermented red pepper sauces (Fig. 3D).

The PCoA results indicated that the fungal compositions of all red pepper sauces greatly varied among samples. Using unweighted Jaccard, the first two axes (PCoA1 and PCoA2) accounted for 25.1% and 16.9% of the total variance, respectively (Fig. 3E). For the fungal composition of the six red pepper sauces, 34 common OTUs were identified, and the specific strains to each red pepper sauce were less than 0.5% of the total number of strains (Fig. 3F). For pepper sauces prepared with different pepper varieties, 84 common OTUs were found. CT6 varieties have 20 specific OTUs, whereas HG3 and HG4 have 41 and 108 specific OTUs, respectively (Fig. 3G). Analyzing the strains of sun-fermented and shade-fermented red pepper sauces, 99 common OTUs were found (86.77%, Fig. 3H).

After analyzing the changes in fungal abundance at the genus level, sun-fermented and shade-fermented red pepper sauces were found to be clustered together. *Saccharomycetes* and *Leotiomycetes* showed high relative abundance in shade-fermented red pepper sauces, whereas *Agaricomycetes* and *Sordariomycetes* showed high relative abundance in sun-fermented red pepper sauces (Fig. 3I). The genera with large fluctuations in abundance mainly include *Diutina* (*Ascomycota*), *Cystofilobasidium* (*Basidiomycota*), *Toxicocladosporium* (*Ascomycota*), etc. (Fig. 3J).

Different bacterial community structures and functions

In identifying important species with significant differences among the groups, random forest method analysis was performed, and the results showed 24 significantly different species ($p < 0.05$), including *Lactobacillus* (now reclassified into *Lacticaseibacillus* and other genera [15]), *Nitrospira*, *Bacillus*, *Methylotenera*, *Thermomonas*, *Ralstonia*, and *Lacibacter* (Fig. 4A). Further analyzing of the metabolic activities or ecological functions of different genera between red pepper sauces by using the random forest method, the results indicated: different genera between CT6 and HG3/4 are related with aromatic hydrocarbon degradation, non-photosynthetic cyanobacteria, aromatic compound degradation, ureolysis, methanol oxidation, aerobic chemoheterotrophy, hydrocarbon degradation, nitrate denitrification and so on (Fig. 4B).

Different fungal community structures and functions

The six red pepper sauces were further analyzed using the FUNGuild (Fungi Functional Guild) tool, and the results showed significant differences among *Pichia*, *Starmerella*, *Hannaella*, *Dipodascus*, and *Didymella* (Fig. 5A). The fungal function prediction showed that the predominant guild contained two types, namely, "animal endosymbiont–animal pathogen–plant pathogen–undefined saprotroph" and "undefined saprotroph" (Fig. 5B). The trait type is soft rot in six red pepper

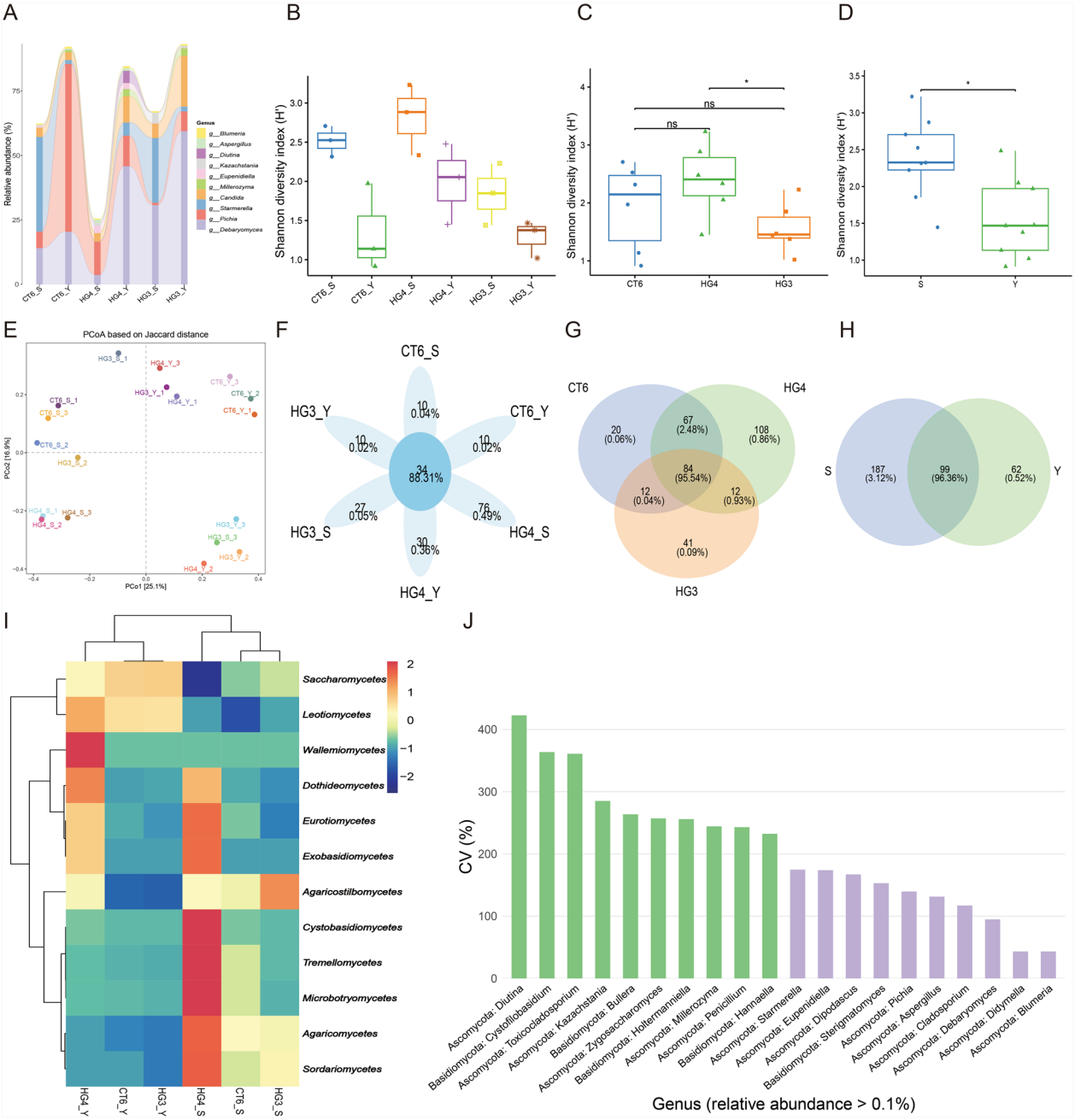

Fig. 3 The fungal abundance analysis of red pepper sauces. **A** Sankey plot of high abundance genus (sorted by total mean abundance). **B** Alpha diversity of six red pepper sauces. **C** Alpha diversity of red pepper sauces made by three red pepper varieties. **D** Alpha diversity of shade and sun treatment red pepper sauces. **E** PCoA based on Jaccard distance. **F** Venn plot of six red pepper sauces. **G** Venn plot of red pepper sauces made by three red pepper varieties. **H** Venn plot of shade and sun treatment red pepper sauces. **I** Heatmap at genus level. **J** Coefficient of variation at genus level. S, fermented in the sun; Y, fermented in shade

sauces, and white rot accounts for a high percentage in HG4_S (Fig. 5C). Growth form analysis showed that yeast is the most dominant component of the six red pepper sauces (Fig. 5D).

Different microbial community effects of the chemical composition of red pepper sauce

Different microorganisms affected the biochemical indices of red pepper sauces. The bacterial analysis results

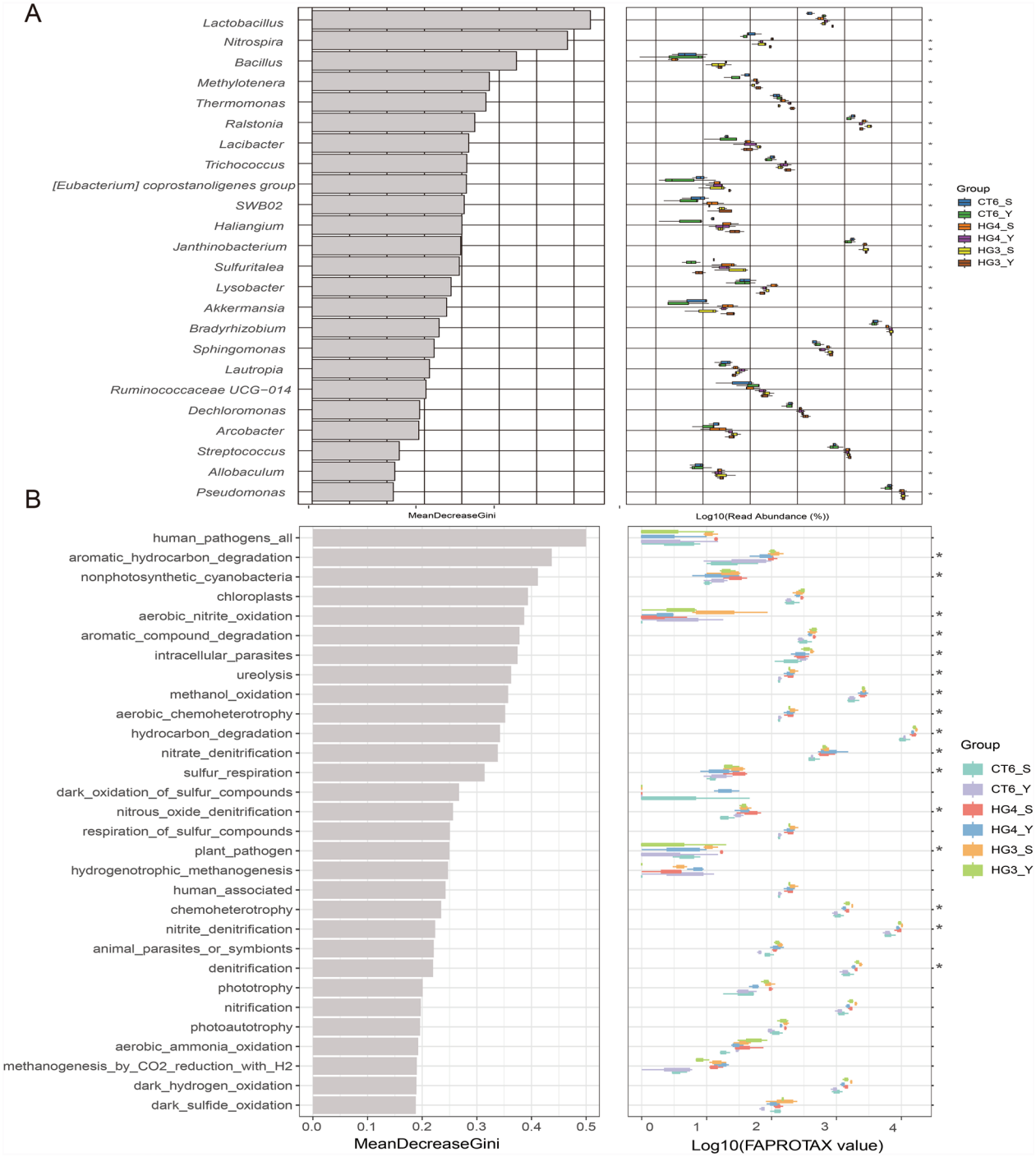

Fig. 4 Different bacterial community structure and function. **A** Random Forest analysis of different bacterial. **B** Random Forest analysis of bacterial function by FAPROTAX. The horizontal coordinate of the left figure is the average reduction value of Gini index, the vertical coordinate is the classification information of the genus, and the right figure is the box chart of the abundance of different groups. The * sign on the right represents the significance of the difference between groups (Kruskal–Wallis rank sum test) (***$p < 0.001$; **$p < 0.01$; *$p < 0.05$). S, fermented in the sun; Y, fermented in shade

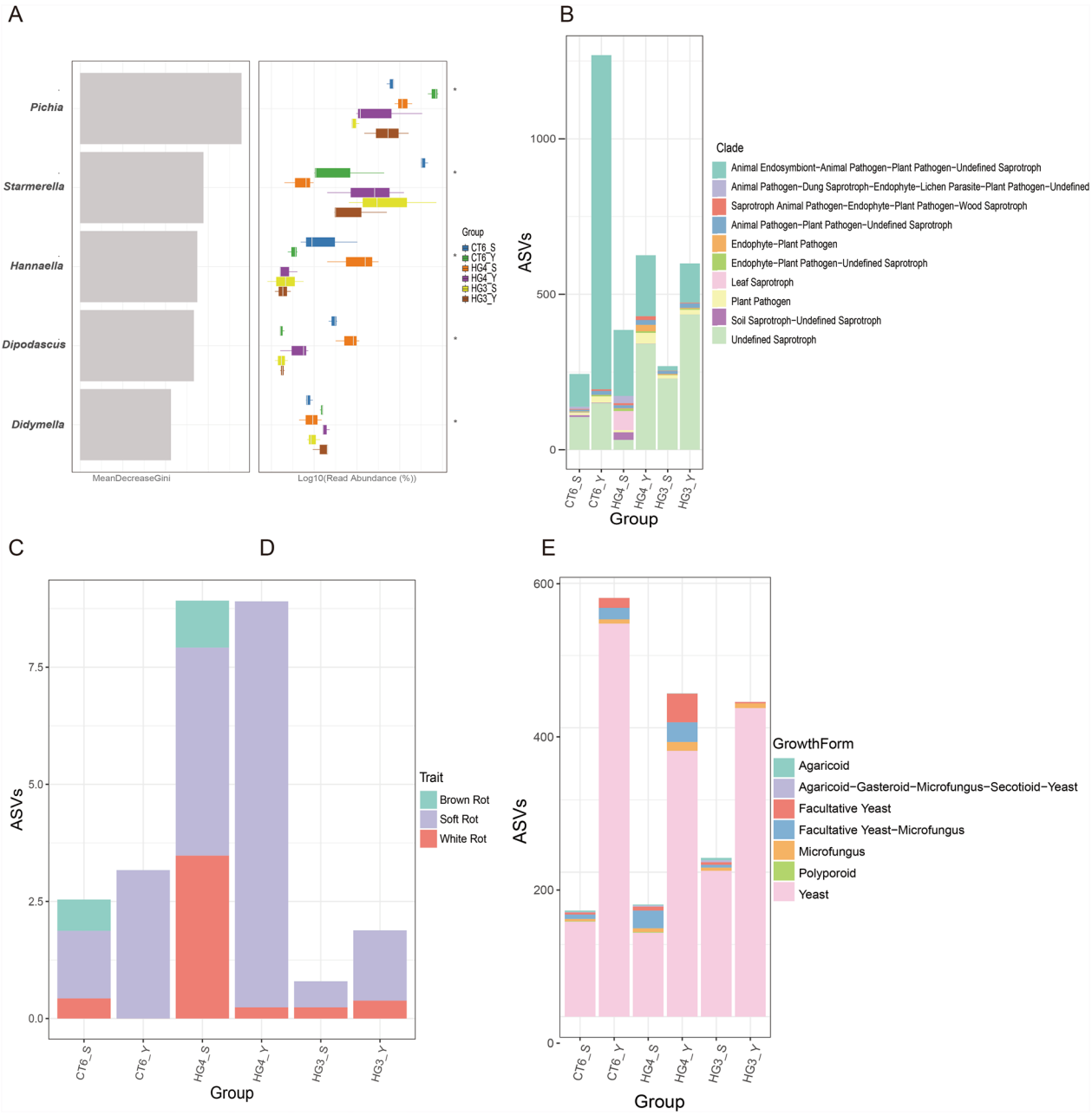

Fig. 5 Different fungal community structure and function. **A** Random Forest analysis of different fungal. **B** the fungi functional Guild statistics. **C** The trait of fungal. **D** The Growth Form of fungal. S, fermented in the sun; Y, fermented in shade

indicated that the content of water, pigment, and capsaicin was significantly and negatively correlated with the abundance of the genera *Ferruginibacter*, *Methylotenera*, *Arcobacter*, *Acinetobacter*, *Methylorosula*, *Gemella*, *Dechloromonas*, *Sphingomonas*, *Romboutsia*, *Lacibacter*, and *Bradyrhizobium*. The content of lactate, phenolics, pectin, AA, and flavonoid was significantly and negatively correlated with *Thermomonas*. Similarly, the level of lactate, pectin, AA, and flavonoid was also negatively correlated with the abundance of *Lactobacillus*. ADH levels were significantly and positively correlated with the abundance of various bacteria, including *Romboutsia*, *Lacibacter*, *Rhizobacter*, *Rothia*, *Pseudomonas*, *Bradyrhizobium*, *Lysobacter*, *Ralstonia*, and *Lautropia*. Moreover, the content of SSC was significantly and positively correlated with the abundance *Herbaspirillum*.

Similarly, nitrite content was significantly and positively correlated with the abundance of *Hirschia*, *Cetobacterium*, *Tolumonas*, *Lactobacillus*, and *Bacteroides*. The pH was significantly and positively correlated with *Cetobacterium* and *Sphingomonas*. RS was significantly and negatively correlated with *Acidovorax* and *Bacteroides*. Furthermore, the carotenoid content showed a significantly negative correlation with *Arenimonas* and *Lysobacter* (Fig. 6A).

The results of correlation analysis between fungal abundance and biochemistry indexes indicated that AA, flavonoid, pectin, and GABA content were significantly and negatively correlated with *Didymella*, *Juncaceicola*, *Ogataea*, *Blumeria*, *Diutina*, *Toxicocladosporium*, and *Debaryomyces* but positively correlated with *Rhodotorula*, *Dipodascus*, *Leucosporidium*, *Hannaella*, and *Coniochaeta*. The other biochemistry indexes were significantly correlated with 1–4 genera (Fig. 6B).

Discussion

Although the Chinese national standard (GB/T 20560-2006) of red pepper sauce has been formulated, the fermentation of red pepper sauce was greatly affected by the environment and human factors, and obtaining a stable quality for skilled workers is difficult. Thus, in this study, the relationship between the structure of microbial communities and the contents of different chemical components and flavor substances in red pepper sauce produced by different raw materials (red pepper varieties) and different forms of fermentation was investigated to analyze the main factors affecting the quality of red pepper sauce during their preparation.

In this study, AA, flavonoid, and pectin content first showed significant differences between sun-fermented and shade-fermented red pepper sauce (Fig. 1D, F, and H), and a significantly positive correlation was found among these three biochemical indicators (Fig. 1P). A significant difference in biodiversity was also observed between sun-fermented and shade-fermented red pepper sauce (Figs. 2C, 3D), and the content of AA, flavonoid, and pectin was negatively correlated with *Thermomonas* and *Lactobacillus* (now reclassified into *Lacticaseibacillus* and other genera [15]) (Fig. 6A). *Lactobacillus* and *Thermomonas* are prominent bacteria with differences in abundance among six red pepper sauces, which play important roles in aromatic hydrocarbon degradation, aerobic nitrite oxidation, aromatic compound degradation, methanol oxidation, hydrocarbon degradation, nitrate denitrification, and regulation of AA, flavonoid, and pectin content (Fig. 4A, B). In addition, the observed correlations between the content of AA, flavonoid, pectin, GABA and the relative abundance of fungi (e.g., *Didymella*, *Rhodotorula*, *Dioszegia*) may reflect synergistic interactions within the microbial community, where these taxa potentially contribute to metabolic pathways collectively rather than independently (Fig. 6B). The content of AA, flavonoid, GABA, and pectin may be relatively unaffected by bacteria but mainly related to the abundance of various fungi.

The content of AA was significantly higher in all three types of sun-treated red pepper sauces than in shade-treated red pepper sauce, and its content had a greater effect on the flavor of red pepper sauce. Zhang et al. (2020) found that Glu, Pro, Asp, Asn, and Arg were the major free amino acids found in red pepper sauce, which might contribute to the umami, sweet, and bitter tastes [25, 26]. Zofia et al. found that proteolysis can enhance the amino acid compounds in fermented broad bean products [27]. Sun treatment can increase the temperature of red pepper sauce fermentation, which is favorable to the growth of some microorganisms. *Debaryomyces* can play a role in improving the flavor of bacon sausages and influence the microbial community in post-harvest fruits to reduce spoilage [28, 29].

Flavonoids exhibit significant antioxidant, anti-aging, and anti-inflammatory properties [30]. The different abundance of microorganisms found in sun- and shade-treated red pepper sauces indicates that these significantly correlated microorganisms can convert the flavonoids bound in the raw materials into free flavonoids, and sun treatment may promote the activity of microorganisms to increase flavonoid concentration. Although flavonoids can affect the color of red pepper sauces, only a significant difference in the pigment of HG3S red pepper sauces was observed, indicating that the method of fermentation and the raw materials used in the preparation of the red pepper sauces had a relatively small effect on the color of the red pepper sauces. Our results indicate that multiple bacteria whose differences in abundance were significantly and negatively correlated with pigmentation showed significantly negative correlations with WATER and capsaicin content, indicating that higher water content may primarily affect the optimal environment for the growth of these bacteria, with concomitant diluting effects on pigmentation and capsaicin content.

In addition, lactate, phenolics, pectin, and ADH were correlated with at least one of the factors, either in fermentation method or chili variety. Lactate was significantly and negatively correlated with two bacteria (*Thermomonas* and *Lactobacillus*) and four fungi (*Didymella*, *Juncaceicola*, *Ogataea*, and *Blumeria*) but significantly and positively correlated with three fungi (*Plectosphaerella*, *Tausonia*, and *Dioszegia*). Although no significant difference was found between HG4S and HG4Y, the significant difference in the other four bean

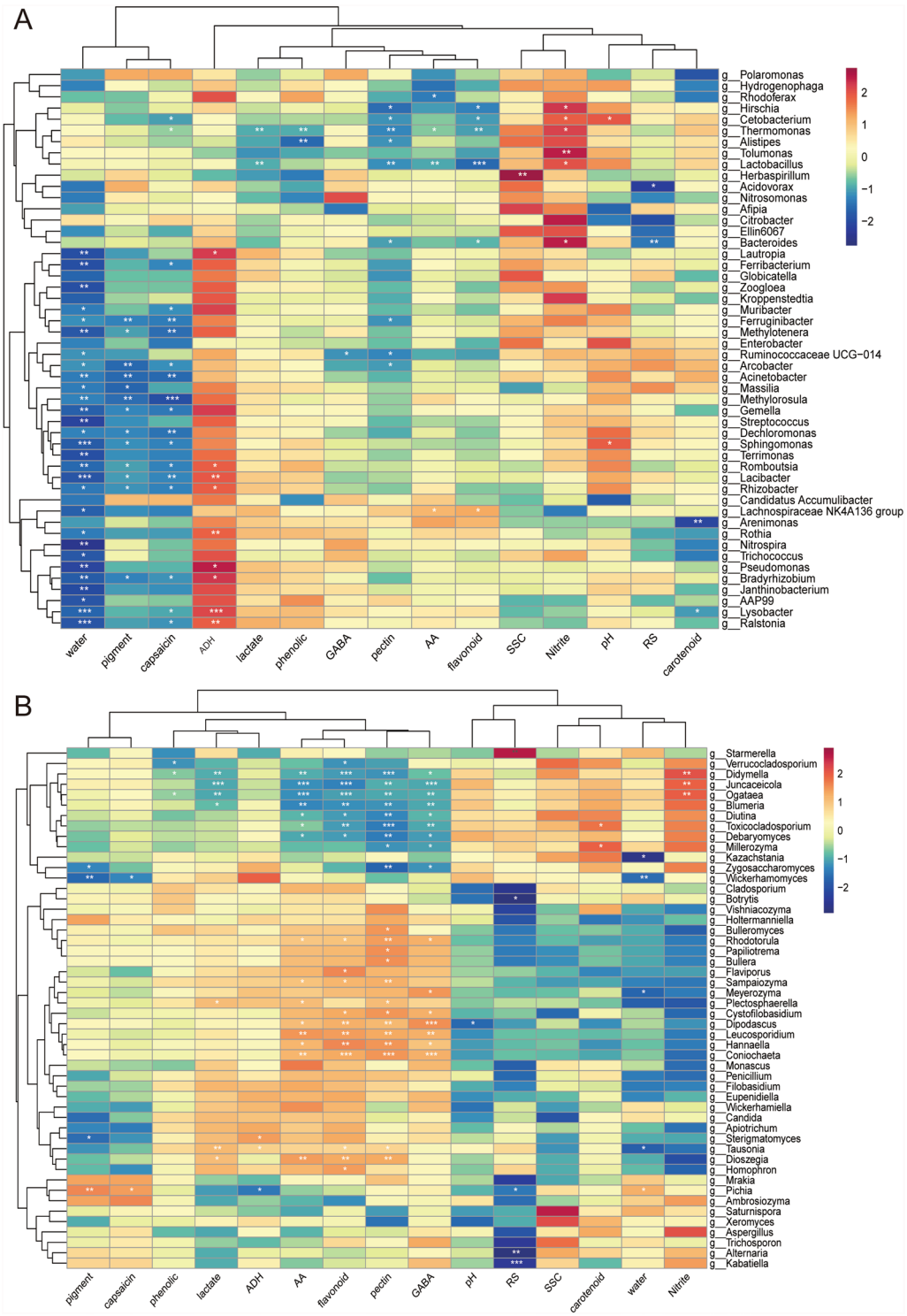

Fig. 6 Correlation analysis between high abundance genus and biochemistry indexes. **A** The correlation of bacterial and biochemistry indexes. **B** The correlation of fungal and biochemistry indexes. The horizontal coordinate is the name of the biochemical index, the vertical showed the genus. The 50 genera with the highest abundance were selected for calculation. The blue color means negative correlation, the red color showed positive correlation. The asterisk represents significance (***$p < 0.001$; **$p < 0.01$; *$p < 0.05$). Horizontal and vertical coordinates are arranged using hierarchical clustering. Correlation analysis indicates statistical associations between microbial taxa and biochemical indices, which may reflect community-wide metabolic interactions rather than direct causation by individual taxa

pastes could be used for screening relevant strains for subsequent use. Li et al. also found a significant correlation between *Lactobacillus* and lactate [30]. Artilha-Mesquita et al. found that applying ultrasonication and thermosonication can improve the phenolics and flavonoid content in jalapeno pepper [31]. This finding also provides novel insights into improving the content of the corresponding components in the preparation and fermentation of bean paste. Pectin content was strongly influenced by the fermentation method, and it was significantly higher in sun-dried dal than in shade-treated red pepper sauces, which indicated that sun-treated red pepper sauces may have promoted the accumulation of pectin by increasing the fermentation temperature.

Interestingly, ADH activity was higher in HG3 and HG4 sun-treated red pepper sauces, suggesting that elevated temperatures may favor the growth of various bacteria significantly associated with ADH, thereby increasing ADH activity, promoting ethanol conversion during the fermentation of red pepper sauces, and improving the flavor of red pepper sauces.

Although FUNGuild predicted potential pathogenic functions (e.g., 'animal pathogen'), these classifications are based on phylogenetic inference rather than direct evidence of pathogenicity. The detected genera (e.g., *Pichia*, *Debaryomyces*) are commonly found in fermented foods and are generally recognized as safe. No known human pathogens were identified in our metagenomic data, suggesting negligible health risks under standard fermentation conditions.

Conclusion

In this study, metabarcoding genomic sequencing and biochemical indicators were used to investigate six red pepper sauces produced by sun and shade treatment, as well as different red pepper varieties. The results showed that different fermentation methods had significant effects on indicators such as AA, flavonoid, pectin, and GABA, which were closely related to the changes in the abundance of bacteria and fungi. Water may affect the content of pigment and capsaicin; these indicators primarily affect the abundance of various bacteria. The results of this study provide comprehensive understanding of the quality stability of red pepper sauces and an important reference for improving the nutrition, quality, and flavor of red pepper sauces.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40793-025-00717-2.

Additional file 1: Fig. S1. Correlation analysis conducted by 15 types chemical composition in 6 red pepper sauces. The horizontal and vertical coordinates indicate the range of corresponding chemical composition in 6 red pepper sauces. Inside each plot of the diagonal is a density curve fitted. Each graph in the upper right triangle section is the correlation value and significance between the two indicators in the row and column. The text size is proportional to the absolute value of the numeric size. The asterisk represents significance (***$p < 0.001$; **: $p < 0.01$; *$p < 0.05$).

Additional file 2: Table S1. Relative abundance at genus level

Additional file 3: Fig. S2. The abundance analysis of red pepper sauces. (A) Alpha diversity of sauces by three red pepper varieties. (B) PCoA based on Unweighted unifrac distance.

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Author contributions

ZS conceived and design the study. XG and YX was involved in data interpretation. SH, HL and XC organized and performed the experiments. XG, YX and ZS wrote the manuscript. All authors read and approved the final version of the manuscript.

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Data availability

All the raw metabarcoding datasets in this study are publicly available in the NCBI BioProject with the accession PRJNA1127001.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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