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Fungal community composition and function in different Chinese post-fermented teas

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Chinese post-fermented teas are produced through special fermentation by microorganisms, with fungi significantly contributing to their flavor and sensory characteristics. Here, the fungal community structure and function were investigated using Illumina HiSeq sequencing of the fungal ITS rDNA region across different post-fermented teas, including Fuzhuan, Qingzhuan, Tianjian black, Liupao, and raw and ripened Pu-erh. Additionally, the headspace solid-phase microextraction gas chromatography–mass spectrometry (HS-SPME-GC–MS) technology was used to compare the volatile components of tea samples, and moisture content, pH, total nitrogen, carbon–nitrogen ratio, and total sulfur were measured. All the tea samples were slightly acidic, with pH values of 5.56–6.43, and Ascomycota was the most dominant phylum, representing over 90% of the relative abundance. However, there were significant differences at the genus level in the six typical post-fermented teas. *Aspergillus* was the most dominant genus in Fuzhuan (91.16%), Qingzhuan (54.89%), Tianjian (64.11%), and Liupao (47.43%) teas, whereas *Debaryomyces* and *Blastobotrys* were the most dominant genera in raw (35.67%) and ripened (78.88%) Pu-erh tea, respectively. A functional prediction analysis revealed that most fungal gene functions were involved in metabolism. A total of 26 main volatile components were detected, which differed in composition among six tea samples. This is the first comparative analysis of fungal communities and volatile components in different typical Chinese post-fermented teas, and the results will aid the design of better culturing strategies for the specific dominant fungal species and the influence of fungi on aroma types of post-fermented teas.

Keywords Chinese post-fermented tea, Fungal diversity, Community structure, Illumina sequencing, Volatile components

Post-fermented teas are very popular throughout different provinces in China, including Yunnan, Hunan, Shannxi, Hubei, Guangxi, and Sichuan[1]. Chinese post-fermented teas are made by fixing, rolling, pile-fermenting, and drying the tea leaves. Pile-fermentation is a kind of solid-state fermentation process that is crucial in the production of post-fermented tea[2,3]. In addition, post-fermented teas can be divided into different types, such as Qingzhuan, Tianjian, Liupao, Fuzhuan, and Pu-erh, based on processing methods and appearances[4]. Pu-erh tea can be divided into raw and ripened based on the manufacturing procedures. Post-fermented tea is reportedly beneficial to human health, and this may be attributed to the presence of various bioactive substances, such as tea polyphenols, polysaccharides, and theaflavins[5]. The transformations of some bioactive substances are associated with various functional core microorganisms in post-fermented tea, including fungi and bacteria[6]. Furthermore, the volatile components of post-fermented tea were related to its functional core microorganisms and the main components of flavor compounds in post-fermented tea, such as ketones, aldehydes, esters, and alcohols[7]. The headspace solid phase microextraction (HS-SPME) combined with gas chromatography–mass spectrometry (GC–MS) has become a commonly used method for determining the changes in volatile components of post-fermented tea during the fermentation process[8–10]. The volatile components vary among the different teas, and even teas of the same category may have different volatile components[11].

The core functional microbial genera vary among different types of post-fermented teas. For example, *Eurotium cristatum* and *Aspergillus* spp. are dominant fungi in Fuzhuan tea[12,13], *Aspergillus* spp. is a dominant fungus in Liupao tea[14], and *Penicillium chrysogenum* is a dominant fungus in Pu-erh tea[15]. Therefore, fungi play

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significant roles in the fermentation of post-fermented teas. Additionally, the fungal community structure can be analyzed using high-throughput sequencing and cultivation methods. High-throughput sequencing technique has been the main method to reveal changes in fungal community structure during the post-fermented tea fermentation process[16–18]. Moreover, the high-throughput sequencing method has been used to compare fungal community compositions in post-fermented tea from different years or subjected to de-enzyming methods[19,20].

There are differences in the fungal community composition among different types of post-fermented teas. Li et al. found that fungi in Fuzhuan tea were dominated by the genus *Aspergillus*[21], which was responsible for 90% of the total effective sequences. Long et al. found that *Blastobotrys*, *Aspergillus*, *Debaryomyces*, and *Aureobasidium* were the predominant genera in Liupao tea[22]. Much research has focused on the fungal community structure in single types of post-fermented tea. However, there are few comparative studies on the fungal community structures and compositions of different types of post-fermented teas. In this study, we used high-throughput sequencing methods to compare the differences in the fungal community structures and compositions in six typical post-fermented teas and analyzed the influence of environmental factors. Moreover, HS-SPME-GC–MS was used to compare the volatile components of post-fermented teas.

Materials and methods

Sample collection

Tea samples were collected from different provinces of China and represented all the typical Chinese post-fermented teas, including raw and ripened Pu-erh (RAP and RIP, respectively), Fuzhuan (FZT), Qingzhuan (QZT), Tianjian (TJT), and Liupao (LBT). The morphology and sample sources of these teas are represented in Table 1 and Fig. 1. All the tea samples were transported to the laboratory in plastic containers. Then, samples were stored in a − 80 °C freezer for further use.

Moisture contents, pH values, and carbon-to-nitrogen ratios of samples

The moisture contents of tea samples were determined using the gravimetric method[23]. Specifically, 5-g tea samples were dried at 103 °C for 4 h and then at 80 °C until constant weights were achieved. The pH values of tea samples were determined using the method previously described by Lin et al.[24]. Briefly, 5-g tea samples were independently mixed with 45 mL deionized water overnight at 4 °C. After filtration, the pH of each solution was determined using a pH meter (PHS-25, Shanghai Yidian Analysis Instrument Co., Ltd., Shanghai, China). Total carbon (TC), total nitrogen (TN), total sulfur (TS), and the carbon-to-nitrogen (C/N) ratio of each tea sample were determined using an elemental analyzer (vario EL cube, Elementar, Hanau, Germany).

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from each tea sample using an E.Z.N.A.™ DNA Kit (Omega Bio-tek, Norcross, GA, USA) in accordance with the manufacturer's instructions. The ITS1 and ITS2 regions of the ITS rDNA were amplified using PCR with primers ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2R (5′-GC TGCGTTCTTCATCGATGC-3′) to analyze the fungal communities[25]. The PCR reactions were performed at a volume of 20.0 μL containing 4 μL 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer, 0.4 μL of FastPfu polymerase, 10 ng of template DNA, and ddH_2O to the final volume. The PCR samples were subjected to initial denaturation at 95 °C for 5 min, followed by 25 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s, and amplification at 72 °C for 45 s, with a final extension at 72 °C for 10 min. They were then stored at 10 °C. The PCR products were separated from 2% agarose gels and further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) in accordance with the manufacturer's instructions. The purified PCR products were used to construct a DNA library, which was evaluated using Illumina HiSeq 2500 sequencing. The raw sequencing data were deposited in the NCBI SRA database under the number PRJNA1141190. All of the sequencing was performed by Shanghai Biozeron Biotechnology Co. Ltd (Shanghai, China).

Sequence analysis

The raw reads obtained from the Illumina HiSeq 2500 platform were subjected to quality control using SMRT Link Analysis software (version 9.0) to remove low-quality reads. To obtain clean sequences, sequences were further filtered by removing barcodes, primer sequences, chimeras, and sequences containing 10 consecutive identical bases. Finally, the clean sequences were clustered into operational taxonomic units (OTUs) of 97% similarity using UPARSE (version 7.1), and chimeric sequences were identified and removed using UCHIME. The sequences for each OTU were used for annotation against the Unite database using the RDP Classifier

Number	Tea types	Production	Year	Source
FZT	Fuzhuan tea	Hunan	2015	Anhua Fengjiaoling Tea Industry Co., Ltd
LPT	Liupao tea	Guangxi	2016	Wuzhou Hongchun Dabao Tea Co., Ltd
RIP	Ripened Pu-erh tea	Yunnan	2013	Pu'er Jindian Tea Industry Co., Ltd
RAP	Raw Pu-erh Tea	Yunnan	2013	Pu'er Jindian Tea Industry Co., Ltd
QZT	Qingzhuan tea	Hubei	2012	Hubei Zhaoliqiao Tea Factory Co., Ltd
TJT	Tianjian tea	Hunan	2012	Hunan Hepin Tea Industry Co., Ltd

Table 1. Specific information on samples of Chinese post-fermented teas.

Fig. 1. The morphological characteristics of the Chinese post-fermented dark teas. (**A**) Ripened Pu-erh tea. (**B**) Raw Pu-erh tea. (**C**) Fuzhuan tea. (**D**) Qingzhuan tea. (**E**) Tianjian dark tea. (**F**) Liupao tea.

(Version 2.2), and the community composition of each sample was statistically analyzed at the levels of phylum, class, order, family, genus, and species[26,27].

Analyses of alpha and beta diversity were performed based on normalized data for each sample. The alpha diversity was used to analyze the species diversity of samples, including the Observed, Chao1, ACE, Shannon, and Simpson indices. A principal component analysis (PCA) and principal coordinate analysis (PCoA) were performed to evaluate the beta diversity. Moreover, rarefaction curves were calculated based on the OTUs, and a Venn diagram was used to further compare and analyze the core OTUs shared by all the samples. Finally, the functional predictions for fungal communities were conducted using the PICRUSt2 program based on the Kyoto Encyclopedia of Genes and Genomes database[28].

HS-SPME procedure

The method of Reference[11] was followed with some modifications. Briefly, 1 g of crushed post-fermented tea samples were weighed into a 10 mL head-space vial, brewed by adding 5 mL of boiling distilled water, and immediately closed tightly and incubated at 80 °C. After 10 min of sample conditioning, 50 μm DVB/CAR/PDMS extraction head was exposed to head-space and performed for 60 min, then immediately inserted into the GC–MS inlet at 250 °C for 5 min.

GC–MS analysis

Using an HP-5MS elastic quartz capillary column (30 m × 0.25 mm, 0.25 μm, Agilent J&W Scientific, USA), the flow rate of the GC carrier gas (helium) was 1.0 mL/min, and there was no shunt injection, the temperature of the injection port was 250 °C. The heating program was as follows: The initial temperature was 50 °C and was held for 5 min. The temperature was increased to 210 °C at the rate of 3 °C/min, equilibrated for 3 min, and finally increased to 230 °C at the rate of 15 °C/min. Electron bombardment ion source (EI), an electron energy of 70 eV, an ion source temperature of 230 °C, and a mass spectrum interface temperature of 280 °C were used. The scanning mass number range m/z is 25 to 500, and the mass spectral database is NIST08.

Statistical analyses

Results of physicochemical properties and diversity indices are presented as means ± SEMs and subjected to an analysis of variance. All the analyses were conducted using Microsoft Office Excel 2019.

Sample	pH	moisture contents%	TN	C/N	TS
FZT	6.28 ± 0.03	0.12 ± 0.00	4.41 ± 0.02	14.83 ± 0.29	0.38 ± 0.02
LPT	6.32 ± 0.02	0.13 ± 0.00	3.83 ± 0.00	11.11 ± 0.11	4.40 ± 0.89
RIP	6.43 ± 0.02	0.09 ± 0.00	4.09 ± 0.16	10.73 ± 0.08	5.04 ± 1.51
RAP	5.98 ± 0.02	0.07 ± 0.00	2.60 ± 0.07	12.39 ± 0.03	4.40 ± 0.89
QZT	5.56 ± 0.03	0.09 ± 0.00	4.22 ± 0.03	17.80 ± 0.21	3.68 ± 0.82
TJT	5.61 ± 0.01	0.08 ± 0.00	3.16 ± 0.06	11.48 ± 0.22	0.39 ± 0.02

Table 2. The physicochemical properties of Chinese post-fermented tea. Values are means ± standard error (n = 3). Statistical significance was calculated using Dunn's test.

Sample	Sequences	Bases (bp)	Average Length (bp)
FZT	39,445 ± 2,588	8,668,358 ± 534,078	219.80 ± 0.98
LPT	38,805 ± 2,994	9,100,667 ± 569,846	234.72 ± 4.57
RIP	36,211 ± 5,107	8,128,245 ± 1,192,634	224.34 ± 1.45
RAP	30,123 ± 170	7,613,034 ± 315,669	252.74 ± 10.82
QZT	30,438 ± 90	6,934,855 ± 18,698	227.83 ± 0.11
TJT	31,811 ± 2,712	7,724,646 ± 799,504	242.59 ± 4.25

Table 3. Statistics of the ITS sequencing data of Chinese post-fermented tea.

Results

Analysis of physicochemical properties

The physicochemical properties of the six typical post-fermented teas (FZT, LPT, QZT, RAP, RIP, and TJT) are shown in Table 2. All the tea samples were slightly acidic (pH 5.56–6.43), and their water contents were low (0.07–0.13%). The pH value of RIP was the most acidic at 5.56, whereas QZT showed the highest pH value at 6.43. The TN concentrations and C/N ratios of FZT and QZT were significantly higher than those of LPT, RAP, RIP, and TJT. However, the TN concentrations were the lowest in FZT compared with the other samples. Additionally, the TN concentrations were comparable between LPT and RAP, but lower than RIP.

Statistical analysis of sequencing data

The sequencing of the fungal ITS amplicons from post-fermented teas yielded 620,499 high-quality sequences from 18 samples (Table 3). FZT had the maximum number, with an average of 39,445 sequences. The average sequencing length of each sample varied from 219 to 252 bp.

Rarefaction curves reflect the rationality of the sequencing data size and the abundance of species in each sample. The rarefaction curves tended to plateau with increasing sequencing depth in all the post-fermented tea samples, indicating that the sequencing had covered most fungi in the samples to accurately assess fungal diversity (Fig. 2).

Operational taxonomic unit cluster analysis

The high-quality sequences of all the samples were clustered using the Uparse software into OTUs according to a 97% identity threshold. A total of 966 fungal OTUs were detected (Table S1). Among them, 466 OTUs were shared OTUs in 90% of all the samples and defined as core OTUs. A Venn diagram showed that 179 core OTUs were common among all the post-fermented tea samples, and the highest core OTU unique number (117) appeared in the RAP sample (Fig. 3). Additionally, the numbers of core OTUs unique to FZT, LPT, QZT, RIP, and TJT were 11, 5, 92, 11, and 51, respectively.

Fungal diversity analysis

The alpha diversity was evaluated using Observed, Chao1, ACE, Shannon, and Simpson indices. The higher the Chao1, ACE, and Shannon index values, the greater the community richness and diversity in the sample, whereas the opposite is true for the Simpson index[29]. The mean Chao1 and ACE index values (at 740.10 and 743.65, respectively) of QZT were the highest, whereas RIP had the lowest values at 343.06 and 327.80, respectively (Table 4). The results suggested that the overall fungal species abundance in QZT was the largest, whereas the lowest number of species was observed in RIP. The mean Shannon index values for QZT, TJT, RAP, LPT, RIP, and FZT were 3.54, 3.26, 2.79, 2.78, 1.90, and 1.06, respectively, and the mean Simpson index values were 0.89, 0.90, 0.76, 0.83, 0.54 and 0.30, respectively. Thus, fungal species richness and uniformity were the highest in QZT. In contrast, no significant differences were observed between RAP and LPT, and the lowest abundance and evenness were observed in FZT.

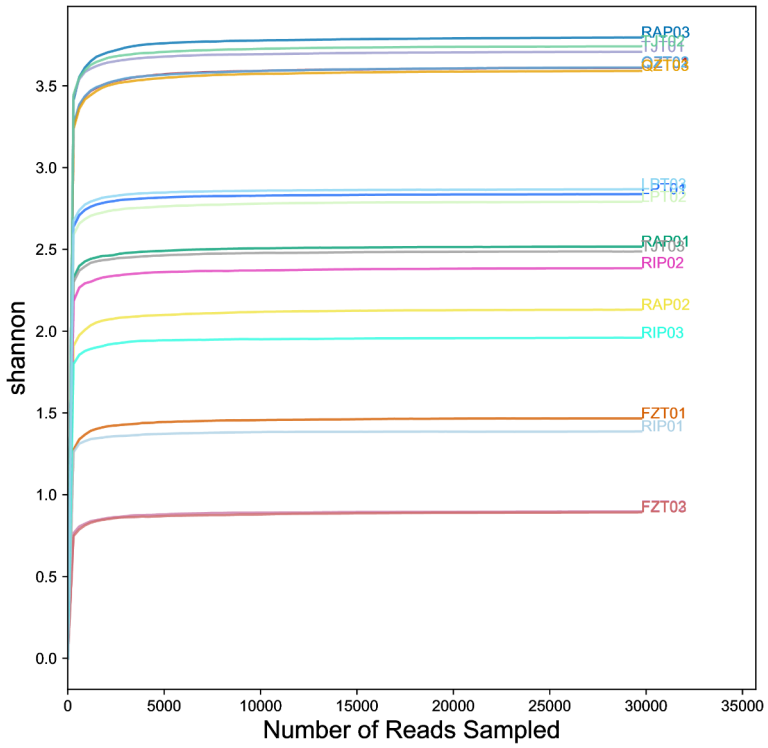

Fig. 2. Rarefaction curves of all the samples of Chinese post-fermented tea.

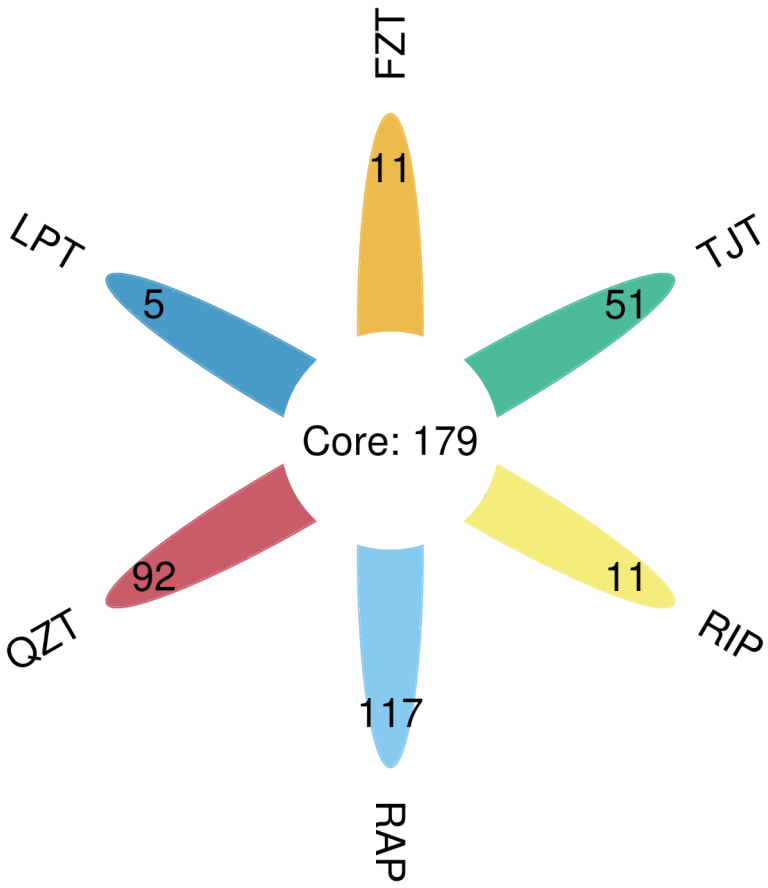

Fig. 3. Venn diagram analysis of core OTU level in Chinese post-fermented tea.

Sample	Observed	Chao1	ACE	Shannon	Simpson
FZT	267.33 ± 19.66	356.76 ± 16.38	362.35 ± 19.34	1.06 ± 0.32	0.30 ± 0.10
LPT	276.67 ± 32.32	373.80 ± 66.37	367.09 ± 63.86	2.78 ± 0.03	0.83 ± 0.03
RIP	249.67 ± 51.79	343.06 ± 99.27	327.80 ± 75.24	1.90 ± 0.49	0.54 ± 0.13
RAP	340.67 ± 94.74	361.71 ± 89.51	368.41 ± 89.52	2.79 ± 0.87	0.76 ± 0.17
QZT	518.00 ± 5.57	740.10 ± 20.83	743.65 ± 2.61	3.54 ± 0.01	0.89 ± 0.00
TJT	333.67 ± 55.61	386.60 ± 26.21	393.87 ± 30.24	3.26 ± 0.73	0.90 ± 0.08

Table 4. Statistical table of fungal community diversity indices in Chinese post-fermented tea.

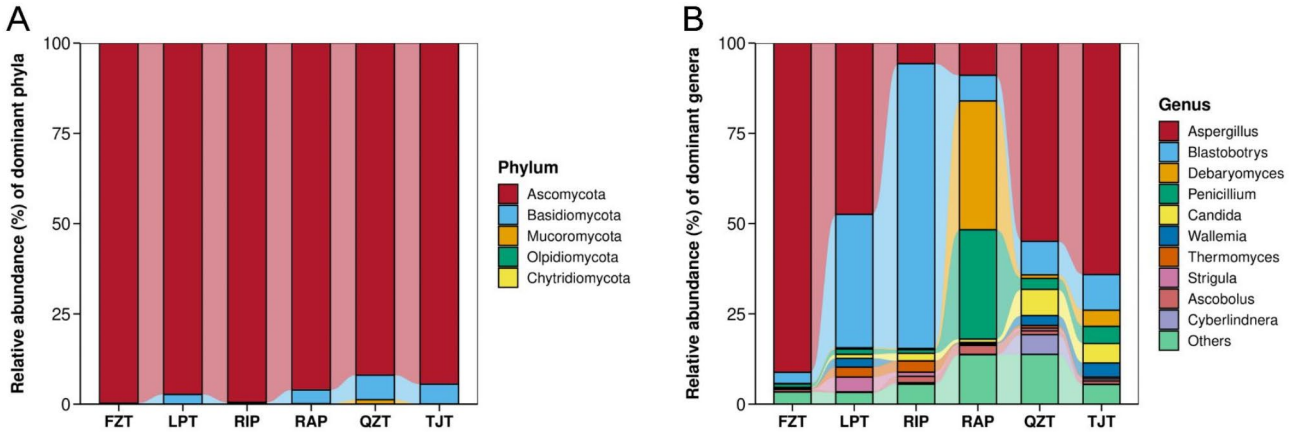

Fig. 4. Distribution of fungi at the phylum (**A**) and genus (**B**) level associated with Chinese post-fermented tea.

Fungal community composition analysis

Fungal community compositions in six different post-fermented teas were analyzed using high-throughput sequencing. The fungal OTUs in all the samples belonged to 6 phyla, 25 classes, 60 orders, 135 families, and 227 genera.

The dominant phylum in all the samples was Ascomycota (Fig. 4A). As the dominant fungi, Ascomycota accounted for 99.69% of the relative abundance in FZT, followed sequentially by RIP, LPT, RAP, TJT, and QZT, accounting for 99.51%, 97.29%, 96.07%, 94.46%, and 91.96%, respectively. The second most common phylum was Basidiomycota (0.24–6.80%), which was most abundant in QZT and least abundant in FZT. In addition, the relative abundance of Mucoromycota in QZT was significantly higher than in other samples.

At the genus level, different post-fermented tea samples had different dominant fungal community compositions (Fig. 4B). *Aspergillus* was the dominant genus in FZT (91.16%), TJT (64.11%), QZT (54.89%), and LPT (47.43%). Moreover, *Blastobotrys* was the second most abundant genus in FZT (3.09%), TJT (9.87%), QZT (9.30%), and LPT (36.96%). In contrast, *Blastobotrys* (78.88%) and *Aspergillus* (5.68%) were the first and second dominant genera in RIP. However, the dominant genera were *Debaryomyces* (35.67%) and *Penicillium* (30.26%) in RAP, followed by *Aspergillus* (8.92%) and *Blastobotrys* (7.10%). Smaller levels of other genera, such as *Candida*, *Wallemia*, *Thermomyces*, *Strigula*, *Ascobolus*, and *Cyberlindnera*, were also detected in all the samples.

Fungal community structure analysis

To evaluate the degree of variation in the fungal communities of six post-fermented teas, a PCA and PCoA were conducted (Fig. 5). According to the PCA, 78.67% of the total variance was contributed by PC1 (54.44%) and PC2 (24.23%) (Fig. 5A). Additionally, PC1 explained 33% of the variation, and PC2 explained 21% of the variation in the PCoA plots (Fig. 5B). Notably, the fungal communities were closely clustered among RAP, TJT, LPT and among QZT, RAP, and TJT, whereas FZT and RIP were separate from the four other samples. Thus, the combined PCA and PCoA results indicated obvious differences in fungal communities from the different samples.

Fungal community functions

In this study, functional predictions for the fungal communities in post-fermented teas were performed using PICRUSt2 based on ITS sequencing data. Functional annotations were based on Kyoto Encyclopedia of Genes and Genomes (level 2) databases, including cellular processes, environmental information processing, genetic information processing, human disease, metabolism, and organismal systems pathways, genetic information processing, environmental information processing, cellular processes, human disease, and organismal systems pathways (Fig. 6). Most of the fungal gene functions were concentrated in metabolism, such as amino acid,

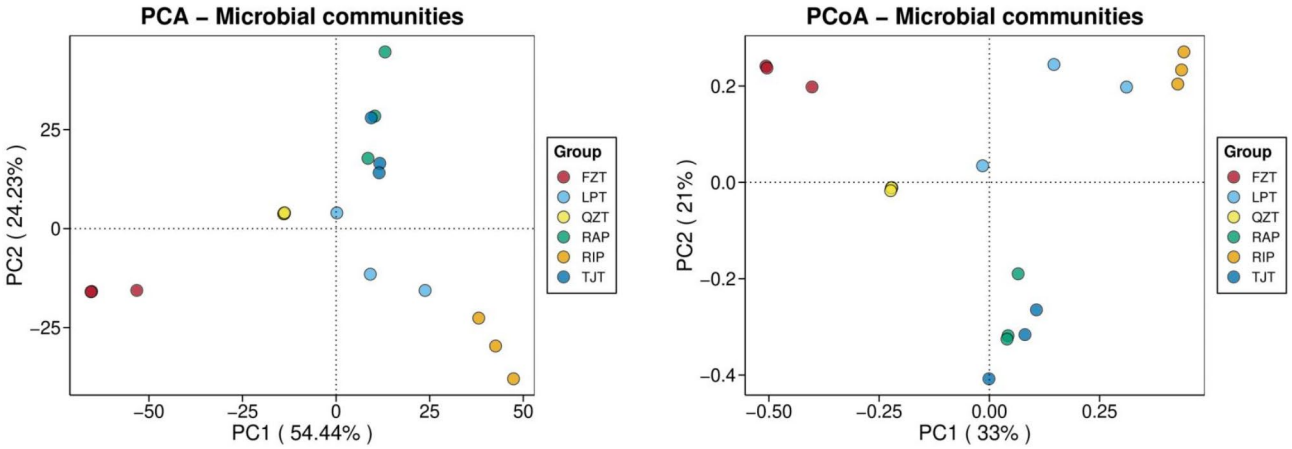

Fig. 5. Multiple sample principal component analysis (PCA) (**A**) and principal coordinate analysis (PCoA) (**B**) of the OTUs.

carbohydrate, energy, cofactor and vitamin, and nucleotide. Also represented were genetic information processing (translation, replication and repair, and folding, sorting and degradation), environmental information processing (signal transduction and membrane transport), and cellular processes (cell motility, and cell growth and death). Although different samples had different fungal community structures, the functional abundance levels of the fungal communities did not differ significantly.

Identification of volatile compounds profiling

The volatile components in different types of post-fermented tea were comparatively analyzed by HS-SPME-GC–MS. The volatile components with relative content greater than 1% and matching degrees greater than 80% were statistically analyzed (Table 5). A total of 26 volatile components were identified, of which 15, 16, 18, 14, 11, and 18 were in FZT, LPT, RIP, RAP, ZZT, and TJT, respectively. Among them, there were six common volatile components, namely (2E)-Oct-2-enal (3E,5E)-3,5-Octadien-2-one,(5E)-6,10-Dimethyl-5,9-undecadien-2-one,4-(2,2,6-Trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-3-buten-2-one,4,4,7a-Trimethyl-5,6,7,7a-tetrahydro-1-benzofuran-2(4H)-one, and 6,10,14-Trimethyl-2-pentadecanone. There were certain differences in the volatile components of six post-fermented teas, among which (1S, 4R, 6R)-4,7,7-Trimethylbicyclico [4.1.0] hept-2-ene, and (5Z) -6,10-Dimethyl-5,9-undecadien-2-one were the unique volatile components in RAP. 4-Isopropylidene-1-methylcyclohexexene, and (9Z,12Z,15Z)-9,12,15-Octadecatrienoic acid were the unique volatile components in RAP. (2Z) -2-decanal was the unique volatile component in QZT. 4-Ethyl-2-methoxyphenol, and 8-Propoxycedrane were the unique volatile components in TJT.

Discussion

Chinese post-fermented teas offer characteristic flavors that are closely related to pile fermentation. Pile fermentation is an important productive process that involves the participation of many microorganisms, including bacteria, yeast, and mold. There are many studies on post-fermented tea that have mainly focused on the dynamic changes in, and roles of, microorganisms during the fermentation process[36–39]. However, there is little research on the differences in microbial community composition among different types of post-fermented teas. This study described the differences in fungal community composition in six different types of post-fermented tea (Qingzhuan, Tianjian, Liupao, Fuzhuan, raw Pu-erh, and ripened Pu-erh) as determined by high-throughput ITS amplicon sequencing analyses. After optimizing the original data, 334,593 high-quality fungal sequences were obtained from 18 samples. In total, 996 fungal OTUs were given taxonomic assignments, and they were divided into 6 phyla, 25 classes, 60 orders, 135 families, and 227 genera.

The species richness, diversity, and evenness of microbial communities were calculated using Chao1, Shannon, and Simpson indices, respectively[40]. In this study, we compared the richness and diversity of the fungal communities among six post-fermented teas. The species richness and diversity in QZT were significantly higher than those in the other samples, which may be related to the pH and C/N of the samples. Similar studies have shown that pH values negatively correlate with the fungal richness and diversity on the surface of grape berries[41]. The richness and diversity of the fungal community in RAP were slightly higher than in RIP, which was consistent with previous reports[42]. In addition, fermentation and storage conditions affected the fungal diversity in post-fermented teas.

The dominant fungal phyla, including Ascomycota and Basidiomycota, were similar in all the samples. Ascomycota was the most dominant phylum, with a relative abundance of over 90%. Similarly, Ascomycota is also the dominant fungal phyla in tea leaves, black tea, Kombucha, and Miang[43–46]. However, the relative abundance of Basidiomycota was varied across the different tea samples, being higher in QZT, TJT, LBT, and RAP, and a lower in FZT and RIP. Li et al.[2] identified Basidiomycota in the early stage of FZT fermentation, but it was almost undetectable after 6 days of fermentation. The relative abundance of Basidiomycota was detected to be 3.78% in RAP, whereas it was only 0.42% in RIP. The results of this study were consistent with previous

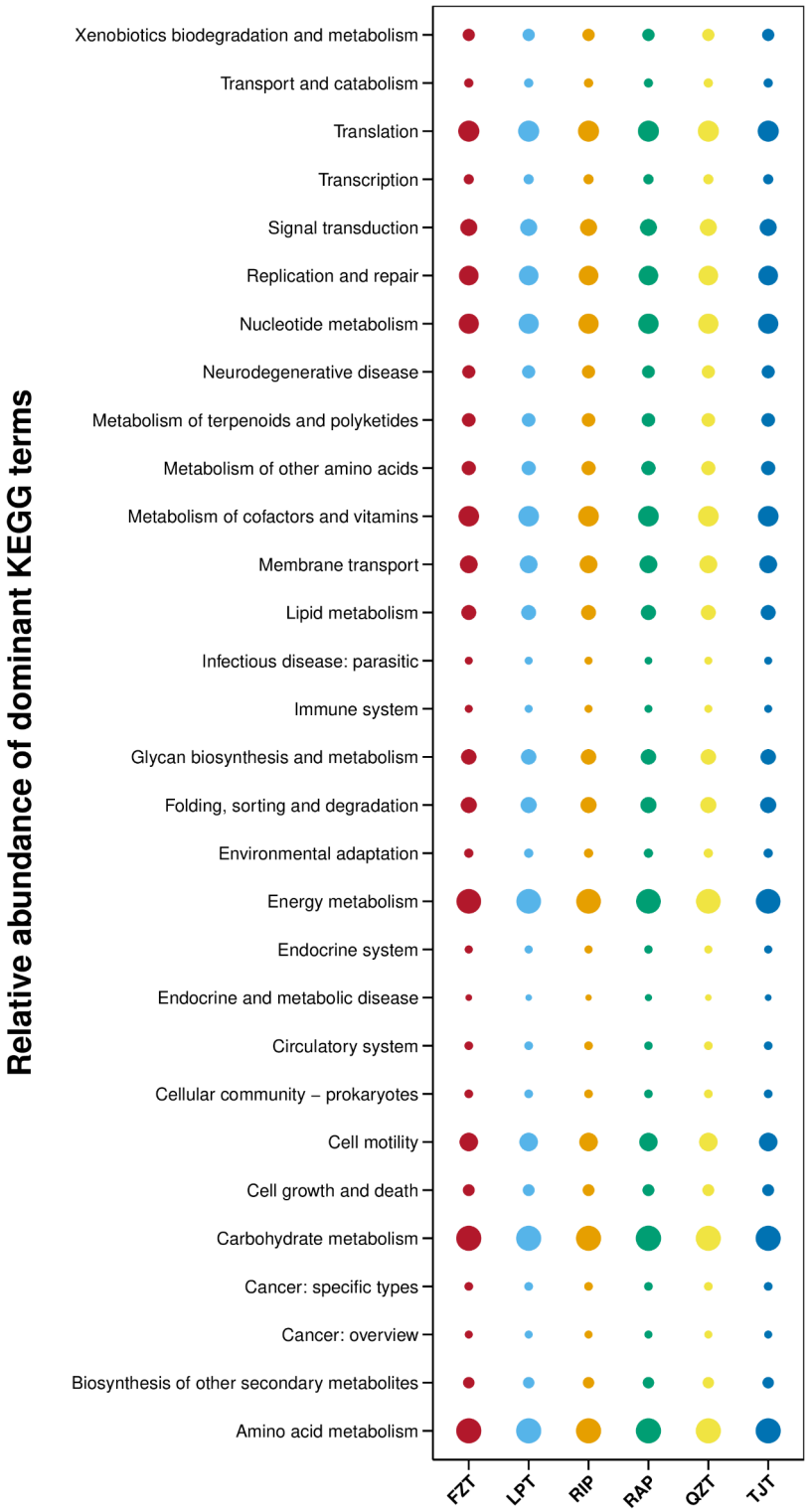

Fig. 6. Functional annotation based on the functional classification of KEGG (level 2). The horizontal axis is the sample name, and the vertical axis represents the Functional annotation and classification. The size of each point corresponds to the relative abundance of each functional annotation.

research, and the difference between RIP and RAP may be due to variations in microbial communities caused by the RIP fermentation process[42]. Notably, the relative 1.5% abundance of Mucoromycota detected in QZT was significantly higher than in the other samples, and the presence of Mucoromycota has also been detected in Black hawk tea[47].

No.	Retention time/min	Name	Formula	CAS	Matching degree	FZT	LPT	RIP	RAP	QZT	TJT	Correlated genera	Literatures
1	12.951	2-pentylfuran	$C_9H_{14}O$	3777–69-3	93	+	+	–	+	+	+	*Eurotium*	[30]
2	13.3111	(2E,4E)-2,4-heptadienal	$C_7H_{10}O$	4313–03-5	94	+	+	+	+	+	–	*Aspergillus*	[31]
3	16.4533	(2E)-oct-2-enal	$C_8H_{14}O$	2548–87-0	95	+	+	+	+	+	+		
4	18.2699	(3E,5E)-3,5-octadien-2-one	$C_8H_{12}O$	30,086–02-3	87	+	+	+	+	+	+		
5	18.7664	(3E)-6-methyl-3,5-heptadien-2-one	$C_8H_{12}O$	1604–28-0	91	+	+	+	–	+	+		
6	23.3814	(1R,6S)-3,7,7-trimethylbicyclo[4.1.0]hept-2-ene	$C_{10}H_{16}$	554–61-0	90	+	–	+	+	–	–	*Aspergillus*	[32]
7	23.3815	(1S,4R,6R)-4,7,7-trimethylbicyclo[4.1.0]hept-2-ene	$C_{10}H_{16}$	13,837–63-3	90	–	–	–	+	–	–		
8	23.3815	4-isopropylidene-1-methylcyclohexene	$C_{10}H_{16}$	586–62-9	87	–	–	+	–	–	–		
9	26.5347	(2Z)-2-decenal	$C_{10}H_{18}O$	2497–25-8	95	–	–	–	–	+	–		
10	26.9547	4-ethyl-2-methoxyphenol	$C_9H_{12}O_2$	220–500-4	94	–	–	–	–	–	+	*Thielavia*	[33]
11	28.575	1,2,3-trimethoxybenzene	$C_9H_{12}O_3$	634–36-6	98	–	–	+	–	–	+	*Blastobotrys*	[34]
12	29.1096	4-ethyl-1,2-dimethoxy benzene	$C_{10}H_{14}O_2$	5888–51-7	95	–	+	+	+	–	+		
13	32.5356	1,2,3-trimethoxy-5-methylbenzene	$C_{10}H_{14}O_3$	6443–69-2	97	+	+	+	–	–	+		
14	33.0593	4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-2-butanone	$C_{13}H_{22}O$	31,499–72-6	91	+	+	–	–	–	+		
15	33.9321	4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-butanone	$C_{13}H_{22}O$	17,283–81-7	98	+	+	+	+	–	+		
16	34.5976	6,10-dimethyl-5,9-undecadien-2-one	$C_{13}H_{22}O$	689–67-8	86	–	–	+	–	–	+		
17	34.6195	(5Z)-6,10-dimethyl-5,9-undecadien-2-one	$C_{13}H_{22}O$	3879–26-3	94	–	–	–	+	–	–		
18	34.7068	(5E)-6,10-dimethyl-5,9-undecadien-2-one	$C_{13}H_{22}O$	3796–70-1	91	+	+	+	+	+	+		
19	35.8524	(3E)-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one	$C_{13}H_{20}O$	14,901–07-6	98	+	+	–	+	–	–		
20	35.9669	4-(2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-3-buten-2-one	$C_{13}H_{20}O_2$	23,267–57-4	92	+	+	+	+	+	+		
21	37.8708	4,4,7a-trimethyl-5,6,7,7a-tetrahydro-1-benzofuran-2(4H)-one	$C_{11}H_{16}O_2$	15,356–74-8	96	+	+	+	+	+	+	*Cryptococcus*	[35]
22	41.0186	8-propoxycedrane	$C_{18}H_{32}O$	19,870–75-8	94	–	–	–	–	–	+		
23	49.507	6,10,14-trimethyl-2-pentadecanone	$C_{18}H_{36}O$	502–69-2	99	+	+	+	+	+	+		
24	50.9089	(7Z)-7-hexadecene	$C_{16}H_{32}$	35,507–09-6	97	–	+	–	–	–	–		
25	53.0966	butyl isobutyl phthalate	$C_{16}H_{22}O_4$	17,851–53-5	96	–	–	+	–	–	+		
26	59.0265	(9Z,12Z,15Z)-9,12,15-octadecatrienoic acid	$C_{18}H_{30}O_2$	463–40-1	99	–	–	+	–	–	–		

Table 5. GC–MS analysis results of volatile compounds in different types of post-fermented teas. "+" means detected. "-" means not detected.

The dominant genera of post-fermented teas have been reported to be *Aspergillus*, *Blastobotrys*, *Debaryomyces*, *Penicillium*, and *Candida*[13–15,48,49]. These fungal genera also play key roles in the unique aromas and active ingredients of post-fermented teas. *Aspergillus* was the most dominant fungal genus in QZT, FZT, LBT, and TJT, and it is crucial for achieving the desired quality and taste of tea[14,36,50]. For example, *Aspergillus fumigatus* M1 was the core strain in QZT responsible for polyphenol conversion, which shortens the pile-fermentation time[51]. *Eurotium cristatum* PW-1 accelerates the biotransformation of phenolic compounds and provides the good taste and desired infusion color of FZT[52]. In addition, some aflatoxins have been detected in post-fermented tea samples in recent years[53–55]. *Blastobotrys* was the most dominant genus in RIP, and the second most dominant genus in LBT. It has been isolated from corresponding tea samples[22,49]. A different fungal community structure was observed in RIP and RAP, with *Blastobotrys* being the most abundant genus in RIP (78.88%) and *Debaryomyces* being the most abundant genus (35.67%), followed by *Penicillium* (30.26%), in RAP. A similar finding was previously described and was likely due to the different production processes of Pu-erh teas[42]. Overall, the relative abundances of dominant fungal genera varied among different post-fermented teas, mainly due to differences in raw materials and production processes.

Fungi are indispensable for the fermentation processes used to produce post-fermented teas. In this study, most of the functional annotations of the fungal communities were related to metabolism, suggesting that the fungi may help improve the quality of post-fermented teas. Other studies have found that *Aspergillus* and *Penicillium* affect the metabolism of substances related to flavor in post-fermented teas[13,20,39,44]. Therefore, most of these fungi play irreplaceable key roles during the fermentation of post-fermented teas.

The dominant microorganisms unique to post-fermented tea lead to the production of different volatile components. This study further used HS-SPME-GC–MS to identify the volatile components in various samples and found that they were mainly ketones, aldehydes, and esters. Some volatile components have specific aroma types, such as 2-pentylfuran with roast aroma attributes[56], (5E)-6,10-dimethyl-5,9-undecadien-2-one with fresh, flowery, and weak sweet[57], (2E,4E)-2,4-heptadienal with fatty, green, oily, vegetable aroma[58], (3E,5E)-3,5-octadien-2-one with stale aroma[30]. Of which 2-pentylfuran with a burnt and sweet odor was detected in the Fuzhuan tea leaves fermented by *E. cristatum*[30]. (E,E)-2,4-heptadienal was one of the key aroma-active compounds in RIP and primarily occurs through the microbial bioconversion of tea components facilitated by *Aspergillus*[31]. Additionally, it was found that the (E,E)-2,4-heptadienal was also abundant in LBT and FZT[59,60]. The volatile component 1,2,3-trimethoxybenzene with stale aroma was also found in RIP and TJT. Moreover, the synthesis of 1,2,3-trimethoxybenzene in tea was correlation with *Blastobotrys*, *Rasamsonia*, and *Thermomyces*[34]. The dominant microorganisms of post-fermented tea influence the formation of key aroma-active compounds, resulting in different aroma types for the post-fermented tea[61].

Conclusions

To the best of our knowledge, this is the first reported use of high-throughput sequencing technology to assess the differences in fungal communities in six typical post-fermented teas. The fungal community structures of different post-fermented teas were similar at the phylum level, but they were quite different at the genus level. Our study provides a comprehensive understanding of the fungal composition and potential functions in post-fermented teas. We further revealed the dominant fungal genera of different post-fermented teas, providing a reference for the selective isolation of dominant fungi. In addition, this study clarified the differences in the composition of volatile components in different types of post-fermented tea. Therefore, future research will focus on the species identification of dominant fungi and their correlations with crucial metabolic pathways and aroma mechanisms. This research also provides a theoretical basis for understanding the formation mechanisms of post-fermented teas from a microfloral perspective.

Data availability

Sequence data that support the findings of this study have been deposited in the NCBI SRA database with the accession number PRJNA1141190.

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Author contributions

The conception and design of the research were designed by Zhuoting Gan. Pu Cui, Jia Li, and Ting Yao performed analysis and interpretation of data and statistical analysis. Pu Cui wrote the first draft of the manuscript. Pu Cui and Zhuoting Gan revised the manuscript. All authors have read and approved the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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