Characterizing the correlation between species/strain-specific starter with community assembly and metabolic regulation in Xiaoqu Pei

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ABSTRACT

Studying the correlation between microbiome metabolism and flavor of fermented foods has garnered significant attention recently. Understanding the contribution of metabolic regulation and environmental stress to microecosystems is essential for exploring the mechanisms of action of traditional fermented foods. Here, the interaction between microbial communities was investigated using a Xiaoqu fermentation system, processed as “simulative microecosystems,” in which starters were composed of Rhizopus-specific species/strains, Meyerozyma guilliermondii, and Bacillus licheniformis. The differences between community succession and metabolites were also explored. The results indicated that Rhizopus species/strain specificity affected starch hydrolyzation, resulting in a remarkable difference in the type and content of organic acids. This further suggested that the differences in nutrient abundance and organic acids influenced the colonization of microorganisms in the fermentation system, thereby influencing the succession of their communities. The fungi in the community predominantly originated from starters, whereas the bacteria were derived from both the environment and starter. Environmentally colonized microbes were the major contributors to the co-occurrence network and were strongly correlated with network. Regional characteristics of fermented foods were closely related to environmental microbes. These results contribute to the understanding of microbial assembly and flavor metabolism in fermented foods and provide strategies for quality regulation.

1. Introduction

Fermented foods, especially traditional fermented foods, have attracted wide attention for improving the functional and nutritional value of the raw material in recent decades. Fermentation processes, which are operated in open or semi-open environments (Kabak and Dobson, 2011; Chaudhary et al., 2021). Therefore, the spatiotemporal characteristics of these techniques have always been considered irreproducible and unregulated. The quality and distinctive flavor of these products are closely correlated with microbial community assembly and metabolic regulation stressed by the microenvironment, in addition to the diversity of raw materials. Thus far, the mechanism underpinning microbial community assembly remains unknown, leading to low controllability and inconsistency in the fermentation process. Difficulties in understanding the directed evolution and regulation of communities have also hindered the transition from individual empirical techniques to scientific fermentation ones (Wu et al., 2021). Scholars inferred that assembled and applied experimentally controllable fermentation model ecosystems to help understand community formation and metabolic mechanisms (Wolfe et al., 2014; Wolfe and Dutton, 2015; Lawson et al., 2019; Garcia-Jimenez et al., 2021; Wu et al., 2021). This assumption was partially confirmed by exploring the interaction and metabolic regulation in a modeled biosystem comprising Escherichia coli and Saccharomyces cerevisiae (Barber et al., 2021).

Traditional Chinese food fermentation techniques involve several process parameters that are regulated periodically under extreme conditions, such as high acidity and high ethanol content (Wolfe and Dutton, 2015; Sakandar et al., 2020; Wu et al., 2021). Therefore, they are potential model microecosystems in which unique communities are formed by enriching microbiota originating from niches, such as raw materials, production facilities, the environment, and directed evolution (Wu et al., 2021). Communities in relatively isolated systems are

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regulated and measurable, thereby providing opportunities to explore the mechanisms of assembly and succession (Wolfe et al., 2014; Wolfe and Dutton, 2015). Furthermore, these communities can also be reconstructed based on known substrates and by screening functional microbes in the laboratory.

Xiaoqu Jiu fermentation is similar to Daqu Jiu’s—both are solid-state fermentation processes. A common feature between them is multilateral fermentation through synergistic intercommunity action (Sakandar et al., 2020). The fermenting starter for Xiaoqu consists of pure cultures of *Rhizopus* and *Saccharomycoses cerevisiae*, wherein only 0.2%–0.5% (w/w) of *Xiaoqu* is used, and the fermentation periods are short (Sakandar et al., 2020). Conversely, the microbial community of *Daqu* is more complex, originating from the niches, and the proportion of *Daqu* used is 15%–50% (w/w) (Wei et al., 2020), resulting in a weakly controllable community in fermented grains; the fermentation periods are also relatively longer. The microbrial structure and population size of *Xiaoqu*-fermented grains are more moderate and easier to regulate and replicate than those of *Daqu*-fermented grains (Shen et al., 2018). Thus, the microecosystem of *Xiaoqu Jiu* fermentation is a more suitable model system for exploring the community evolution and metabolic regulation of traditional fermented foods.

We used the fermentation process of *Xiaoqu Jiu* as a model. The community characteristics of the different types of starters and their metabolites in *Xiaoqu Pei* (*Xiaoqu*-fermented grains) were analyzed using high-throughput sequencing, gas chromatography–mass spectrometry (GC–MS), and high-performance liquid chromatography (HPLC). These starter types consist of *Rhizopus* species/strain-specificity, *Meyerozyma guilliermondii*, and *Bacillus licheniformis*. The correlations between fermentation metabolites, initial microbiota, and environmental microorganisms were explored using bioinformatics. This study aimed to use *Xiaoqu* as a model to lay the foundation for a follow-up study on community succession and quality control of fermented foods.

2. Materials and methods

2.1. Construction of the Xiaoqu fermentation microecosystem

The starter PZS was provided by a *Xiaoqu* factory (Chengdu, China), and the fungi mainly consisted of *Rhizopus oryzae*. The other starters, namely, SYS, N4–9, No3–12, and No4–21, were composed of strains selected by us in starters from various major production areas of *Xiaoqu Jiu* in China (Supplementary Table S1). The fungi in No3–12 and No4–21 consisted of purely cultured *R. oryzae* and *Rhizopus aszygosporus* mixed in a 1:1 (w/w) ratio. The two starters differed only at the strain level.

Except for PZS, the remaining starters contained the same proportions of purely cultured *M. guilliermondii* and *B. licheniformis*. *Xiaoqu* fermentation was performed as previously described (Tang et al., 2022). Briefly, each of the five different starters was inoculated at 0.45% (w/w) in 10 kg of steamed sorghum and saccharified at 30 ± 1 °C in an open environment for 1 day. The saccharified grains were placed in sealed plastic boxes and fermented at 30 ± 1 °C for 11 days to produce *Xiaoqu Pei*. *Xiaoqu Pei* were stored at -20 °C and -80 °C for determination of physicochemical properties and high-throughput sequencing analysis, respectively. Experiments were performed in triplicate, with samples 1, 2, and 3 representing the biological replicates.

2.2. High-throughput sequencing and analysis

Total genomic DNA was extracted directly from these samples using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA). DNA was extracted and purified as previously described (Wang et al., 2008). The concentration and quality of the purified DNA were determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and PCR amplification was performed subsequently. Universal primers were used to amplify the V3–V4 high variant region of the 16S rRNA gene (F: 5′- ACTCCTACGGGAGGCAGCA-3′; R: 5′- GGACTACHVGGGTWTCTAAT-3′). The internal transcribed spacer (ITS) region of the fungal gene was targeted using ITS1 universal primers (F: 5′-GGAAGTAAAGCTGAACACAGG-3′; R: 5′-GTTCGCGTCTTCTCATGATGC-3′). The amplification procedures for bacteria and fungi were the same as those described in our previous study (Tang et al., 2019). Amplicon sequence variants were analyzed using QIIME2 (version 2019.4), which included removal, quality control, denoising, splicing, and chimera removal. Each de-weighted sequence resulting from quality control using DADA2 was called amplicon sequence variant. The calculation and drawing of the sparse curve, Chao1 index, Shannon index, taxonomic tree, non-metric multi-dimensional scaling (NMDS), hierarchical clustering, and redundancy analysis (RDA) were performed using QIIME2 (version 2019.4) and R (version 3.2.0). Linear discriminant analysis effect size (LEfSe) analysis was conducted on the Galaxy website (http://huttenhower.sph.harvard.edu/galaxy/root) to analyze differences in metabolite content among samples (Ter Braak and Smilauer, 2002; Jiang et al., 2015). The source of microorganisms in the fermentation system was analyzed using Source Tracker (Knights et al., 2011).

2.3. Determination of physicochemical properties of starters and fermentation microecosystems

The physicochemical properties of the starter and fermentation microecosystems were determined as previously described (Yan et al., 2015; Tang et al., 2019). These properties included moisture, fermentation activity, saccharification activity, liquefaction activity, esterification activity, acidity, starch, reducing sugar, alcohol, and total ester content. Correlations between the physicochemical properties of the starters and fermentation microecosystems were calculated using the Spearman rank correlation method (*P* < 0.05).

The determination of organic acids (OAs) was based on Cocchi et al.’s study with some modifications (Cocchi et al., 2006). Briefly, the samples were extracted with 9 mM H$_2$SO$_4$, treated with a C18 SPE column (Swell scientific instruments Co., Ltd. Chengdu, China), and filtered through a 0.22 μm filter (Micron Separation Inc., Westborough, MA, USA). The filtrate was injected into an Agilent 1260 HPLC system (Agilent Technologies Inc., Wilmington, Delaware, USA) equipped with an Alltech OA-1000 OA column (300 mm × 7.8 mm) and maintained at 75 °C. Degassed H$_2$SO$_4$ (9 mM) was used as the mobile phase, and the extracts were detected using an ultraviolet detector (215 nm).

Volatiles were analyzed using GC–MS/MS (Gas Chromatography Coupled System TSQ 9000 Mass Spectrometer, Thermo trace 1300, Waltham, MA, USA) equipped with an HPINNOWAX capillary column (30.0 m × 0.25 mm × 0.25 mm, Agilent Technologies Inc., Electron Corporation, Waltham, MA, USA). The detection protocol and data acquisition procedure were the same as those described previously (Tang et al., 2019).

2.4. Data availability

All sequencing data are available at the NCBI Bio-project database (accession number: PRJNA722838).

3. Results

3.1. Microbial community characteristics of the fermentation microecosystem

The sequencing depth represented the composition of microorganisms in the samples, as evidenced by the ratio of high-quality sequences of microorganisms (Supplementary Table S2) and sparse curves (Supplementary Fig. S1). Across all fermentative communities, differences in within-habitat diversity were expressed using Chao1, Shannon, and Pielou’s evenness indices (Fig. 1). For the fungal communities, the order
of Chao1 was PZS > SYS > N4–9 > No3–12 > No4–21, whereas the order of both Shannon and Pielou’s evenness was N4–9 < SYS < PZS < No4–21 < No3–12 (Fig. 1a). In addition, the Chao1 index of bacterial communities varied considerably between microecosystems, decreasing notably from PZS (644.74) to No3–12 (434.43). The Shannon and Pielou’s evenness indices among PZS, SYS, and N4–9 were similar and higher than those of No3–12 and No4–21 (Fig. 1b). These results indicated differences in species richness and evenness of bacterial and fungal communities in the five fermentation microecosystems.

The distance in NMDS suggested that microbial communities were structurally similar in biological replicate samples but differed markedly across microecosystems (Fig. 2a, Fig. 2b). The fungal composition was
highly similar for No3–12 and No4–21, whereas SYS, N4–9, and No4–21 had a high degree of similarity in bacterial composition. Cluster analysis revealed community similarity based on the hierarchical tree and dominant microbial composition (Fig. 2c, Fig. 2d). Hierarchical clustering of fungi showed that the sum of the abundances of Rhizopus, Meyerozyma, and Pichia exceeded 90%. Moreover, the proportion of the three microbes in the microecosystems affected the similarity in the fungal community structure. The evolutionary similarity was higher for No4–21 and No3–12, but evolutionary distances differed slightly among the other three microecosystems (Fig. 2c). Hierarchical clustering of bacteria showed that Bacillus, Cupriavidus, Acinetobacter, Enterobacter, and Klebsiella were dominant, wherein the sum of their abundances was greater than 60%. The bacterial composition of N4–9 was similar to that of SYS but different from that of No4–21. The other three microecosystems had notably different structural bacterial microbiota, with distribution trends similar to those of the NMDS (Fig. 2c, Fig. 2d).

Microbial composition of the microecosystems was determined using taxonomic trees and LEfSe (LDA > 2, P < 0.05). Rhizopus, Meyerozyma, and Pichia were the dominant fungi in the microecosystems. In addition, No3–12 and No4–21 had similar proportions of dominant fungi but differed markedly from the other microecosystems (Fig. 3a). Some of the microbial abundances in PZS and SYS were much higher than those in the other microecosystems because they involved more R. oryzae than the other starters. Their strong substrate hydrolysis provided more opportunities for microbial enrichment (Fig. 3c, Table 1). Compared with the circular map of fungi, the composition of bacteria was more diverse and complex. The latter was mainly composed of Gammaproteobacteria, Bacilli, and Alphaproteobacteria, of which Bacillus, Cupriavidus, Enterobacter, Acinetobacter, and Sphingomonas were the dominant genera (Fig. 3b). Moreover, notable differences were observed in the number of bacteria present in the samples. The abundance of Bacillus spp. differed during fermentation community succession, with much higher levels in the No3–12 microecosystem than in the other fermentation ones (Fig. 3d). These results suggested that the composition of fungal microorganisms in the microecosystems was consistent with the composition of the starter microbiota, while the bacterial compositions were diverse. This indicates that the degree of co-evolution of fungi and bacteria via environmental influences during fermentation differs (Wolfe et al., 2014; Wang et al., 2018).

Furthermore, we explored the sources of microbes in the fermented microecosystem using Source Tracker (Fig. 4). Fungal microbes in the three microecosystems were the primary source of the starter, with the proportions of PZS, SYS, and No3–12 being 71.55%–76.47%, 99.29%–99.60%, and 99.96%–99.99%, respectively. In contrast, the bacterial microbes in the PZS and SYS communities were presumed to be predominantly of environmental origin, as the proportion of starters in the bacterial communities was only PZS (19.32%–20.44%) and SYS (21.15%–35.04%). The source of bacteria in No3–12 was different from that in PZS and SYS, and the ratio of starters in its bacterial community was 70.71%–81.46%, which suggested that the bacterial microbiota in the No3–12 starter was well propagated and enriched during the fermentation process. This is consistent with the results presented in Figs. 2 and 3. A high similarity existed between the No4–21 and No3–12 microecosystems in terms of starter and fermentation communities. The origin of the No4–21 fermentation microbial community was also presumably highly similar to that of No3–12 fermentation microbial community.

3.2. Metabolic capacity of starters and metabolites features in the corresponding microecosystems

The physicochemical properties of the five starters were markedly different (Table 1). The saccharifying and liquefying activities of PZS and SYS were almost the same and were significantly higher than those of the other three starters. The fermenting, liquefying, saccharifying, and esterifying activities of No3–12 were slightly higher than those of

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Fig. 3. Microbial composition of fermentation ecosystems. Taxonomic tree of microecosystems: (a) fungi and (b) bacteria. The largest circle represents the phylum level, and the gradually shrinking circle represents class, order, family, and genus according to the gradient. LEfSe analysis of (c) fungal and (d) bacterial microecosystems (LDA > 2, P < 0.05).
The fermentability of N4–9 was only 0.57 g/0.5 g ⋅ 72 h, which was lower than that of other starters. Ethanol production is one of the primary functions of the starter as the starting microbiota of a unique microecosystem (Sakandar et al., 2020). The difference in the ability to produce ethanol resulted in not only the divergence of ethanol content in the microecosystem but also the contents of accompanying products, such as OAs, amino acids, and volatiles, during the subsequent fermentation process (Table 2). For example, malic acid was not detected, while the contents of the other six OAs were similar in microecosystems No3–12 and No4–21, as the composition of the dominant functional strains in the starters was almost the same. However, the tartaric acid was the dominant OA in the microecosystem N4–9, and lactic acid and ace acid were not detected. These results support the species- and strain-specificity of Rhizopus. The properties of OA-producing microbes are species- or strain-specific (Abe et al., 2007), although Rhizopus has an excellent ability to produce OAs and is often used in the industrial production of OAs (Goldberg et al., 2006).

The effect of starters on the microecosystem was investigated using the Spearman’s correlation heat map (Fig. 5). The differences in the

Table 1
Physicochemical properties of five starters.

| Samples | Moisture (%) | Fermenting activity (g/0.5 g ⋅ 72 h) | Liquefying activity (g/g ⋅ h) | Saccharifying activity (mg/g ⋅ h) | Esterifying activity (mg/50 g ⋅ 7 d) |
|---------|--------------|-------------------------------------|-----------------------------|-------------------------------|-----------------------------------|
| PZS     | 6.63±0.00    | 0.86±0.20                           | 4.35±0.00                   | 1688.43±8.26                 | 253.80±28.37                      |
| SYS     | 6.84±0.01    | 1.41±0.03                           | 6.68±0.00                   | 1197.42±4.09                 | 448.47±8.58                       |
| N4–9    | 8.92±0.00    | 0.57±0.02                           | 1.01±0.00                   | 371.54±19.00                 | 85.73±18.77                       |
| No3–12  | 9.01±0.01    | 1.40±0.02                           | 1.65±0.00                   | 699.30±13.96                 | 709.82±2.84                       |
| No4–21  | 8.45±0.01    | 1.20±0.30                           | 1.54±0.00                   | 550.52±4.54                  | 673.66±36.48                      |

Fig. 4. Patterns of microbes originating from the starter and the environment. (genus level). (a), (c), and (e) represent the source composition of fungi in each microecosystem. (b), (d), and (f) represent the source composition of bacteria in each microecosystem.

Table 2
Organic acid content in five fermentation microecosystems.

| Organic acid (mg/kg) | PZS     | SYS     | N4–9    | No3–12  | No4–21  |
|----------------------|---------|---------|---------|---------|---------|
| Oxalic acid          | 184.42  | 183.68  | 51.89   | 79.74   | 79.82   |
| Citric acid          | 598.95  | 580.95  | 1090.39 | #N/A    | #N/A    |
| Tartaric acid        | 1167.75 | 1262.78 | 2560.43 | 182.54  | 163.43  |
| Malic acid           | 598.95  | 580.95  | 1090.39 | #N/A    | #N/A    |
| Succinic acid        | 3762.49 | 2949.33 | 2261.18 | 2677.89 | 2653.03 |
| Lactic acid          | 2331.33 | 2339.52 | #N/A    | 2662.24 | 2644.09 |
| Acetic acid          | 839.02  | 609.82  | #N/A    | 505.16  | 510.02  |

Fig. 5. Correlation heat map of the physicochemical properties between starters and microecosystems.
fermentation microecosystems were closely related to various parameters of the starters, including fermenting, liquefying, saccharifying, and esterifying activities. These parameters regulate the levels of reducing sugars, total esters, and lactic acid, thereby influencing microbiota succession through differences in the nutrient network. The acidity of the fermentation microecosystems was positively related to that of the starter, especially in case of tartaric acid and malic acid. NMDS analysis showed that some metabolites clustered near the central location, with parallel samples from each organism clustered together (Fig. 6). Only N4–9 was distant from the other microecosystems.

Understanding microbial interactions is essential for understanding community functions and metabolite regulation (Faust and Raes, 2012; Mac Aogain et al., 2021). Together, 4 genera of fungi and 92 genera of bacteria formed a network of 242 pairs of relationships (Fig. 7). All the fungal microorganisms originated from starters, such as Meyerozyma and Pichia, and formed fewer interrelationships. Eight genera in the network that displayed strong positive correlations ($r > 0.93$) accounted for 0.93%–18.70% in the bacterial community of fermented grains. Of them, five genera were not detected in the corresponding starters, exceeding 86% of the eight genera (Fig. 7b). It was inferred that the five genera might have originated from the environment and were the most dominant components of the network in the fermented grains. In contrast, Bacillus and other bacteria had a weaker correlation, even though its abundance was 12.72%–60.66%. Regarding microbe–metabolite relationships, a positive correlation existed between the multiple microorganisms that could form a strong correlation and the metabolites (Fig. 7c, Fig. 7d), which may explain the diversity of microbial composition and the central clustering of metabolism in the community. Although the content of strongly related microorganisms is low, they may play an essential role in metabolism (Banerjee et al., 2018). Bacillus was positively correlated with six metabolites: isoamyl acetate, lactic acid, ethyl myristate, ethyl palmitate, 2,3-butanediol, and ethyl 9-hexadecenoate (Fig. 7c, Fig. 7d). Bacillus spp. can efficiently produce lactic acid at the industrial level (Meng et al., 2012). Our results support that these strongly related microorganisms, including Gluconobacter, Ochrobactrum, Bradyrhizobiurn, and Sphingomonas, are closely associated with the production of OAs; this finding is consistent with the results of previous studies (Jensen et al., 1995; Deppenmeier et al., 2002; Chen et al., 2014; Yang et al., 2021).

4. Discussion

Xiaoqu is a crude enzyme and a microbial source in the Xiaoqu Jiu fermentation process (Li et al., 2016; Zheng and Han, 2016; Wu et al., 2017), and this fermentation process involves various species and genera of microbes (Zheng et al., 2015). Therefore, it is difficult to determine whether the observed microbial composition can be a factor that endows the microecosystem’s features (David et al., 2014), or is it only one of the phenotypes of a complex community evolution. The general statistical characteristics of microecosystems appear to vary markedly in taxonomic diversity, even when species turnover and community ecological function remain stable in simple environments (David et al., 2014; Louca et al., 2017). The taxonomic levels of abundant species are strongly influenced by their concentrations and nutrient interaction relationships (Ratzke et al., 2020). A tightly connected network of trophic interactions can stabilize competition among communities of highly related species that compete for the carbon source. Trophic interaction networks drive communities to interact together rather than in pairs. Higher order interactions are strongly associated with the stability of complex community structures (Baier et al., 2016; Levine et al., 2017). In our present study, we explored the knowledge gap in community formation and succession and its function by constructing communities based on simulated microecosystems. The system is experimentally tractable and also allows for the regulation of differences in community diversity between microecosystems through a starter consisting of species/strains specific of Rhizopus (Tang et al., 2022).

Upon comparing the microbiota constituents in both microecosystems, it was found that the fungal microbes of the system originated mainly from the starter (Fig. 4). For example, the similarity of the fungal composition in SYS and No3–12 was more than 99% with the corresponding starters, and both Rhizopus and Meyerozyma were dominant. Conversely, bacterial communities were more complex in origin and composition than fungal communities were, and their succession processes were likely to be more complex (Fig. 3). After inoculating the starter into cooked sorghum, the fermented grains completed the mounding saccharification phase within 24 h in an open environment permeated by numerous fungi and bacteria, followed by anaerobic fermentation for 11 days (Zheng and Han, 2016; Tang et al., 2022). Bacteria spread quickly and have relatively shorter generation times, and compared with fungi, they are smaller in size and more abundant in nature (Qian et al., 2012; Cao et al., 2014). The high chance of bacterial colonization of saccharified sorghum contributes to an enrichment advantage prior to anaerobic fermentation. These coupled starter metabolic properties resulted in markedly different community structures in the five microecosystems (Fig. 3). Community formation results from dense multiplication of different species in small-scale spaces (Ley et al., 2006; Fuhrman, 2009; Faust and Raes, 2012). Exposure to airborne saccharification and increasing nutrients provides opportunities for the colonization and enrichment of exogenous microbiota (Zomorrodi and Segre, 2017). The type and concentration of nutrients affect the abundance and homogeneity of species in the community (Goldford et al., 2018; Ratzke et al., 2020), as well as the directed domestication, booming, and ecological suicide of the community (Ratzke et al., 2018, 2020).

The fungi in this process were mainly derived from the starter (Fig. 4, Fig. 5). A similar result was reported in a survey of cheeses originating worldwide (Wolle et al., 2014). In the present study, notable differences were observed in the physiochemical properties of the five microecosystems constructed by inoculating with different types of starter (Table 1). These starters included identification strains of M. griseus and B. licheniformis, and their ratio was also the same; the process was regulated by the identification pattern. Similar results were reported in a study of Chinese Daqu fermentation (Wang et al., 2018). These studies demonstrate that the fungal communities involved in the traditional fermentation process could assemble regardless of the region and substrate but were firmly associated with the starter fungal microbiota. These results unravel an opportunity to understand and regulate microbial community formation.

During sorghum microecosystem development, the starch in
The fermentation cheese and Daqu, the structure of fungal communities in the constructed microecosystem was closely related to that of the starter, whereas the bacterial community succession was notably different at the initial stage, resulting in different colonization intensities by environmental microbes. In particular, the negative interactions are usually inhibited by the production of OAs, which can change the pH of bacterial cells to induce antibacterial activity (Eswaranandam et al., 2004; Elsheshtawy et al., 2017) and inhibit the growth of some microorganisms. OAs with lower molecular weights have higher antimicrobial activity (Eswaranandam et al., 2004). Although the microecosystem N4–9 contained abundant tartaric acid, the molecular weight of tartaric acid is higher than that of lactic acid and acetic acid. In addition, the microecosystem of the N4–9-fermented grains lacked lactic acid and acetic acid accumulation (Table 2). These factors resulted in N4–9 having higher bacterial diversity in community succession when the degree of nutrient richness was lower than that of the other systems. The contribution of the initiating microbiota to microbial community formation indicated that their metabolic activities also affected the interaction between the microecosystem and environmental microbes. Similar to the results of the fermented cheese and Daqu, the structure of fungal communities in the constructed microecosystem was closely related to that of the starter, whereas the bacterial community succession was diversified. This result indicates the universality of the Xiaqu microbial community and that the similarity of microbial communities.
Microbial diversity varied with the fermentation process. However, the metabolic functions of the community, except for N4–9, and the profiles of volatiles in the four other fermentation systems exhibited a central aggregation trend (Fig. 6). These results suggested that a few microorganisms affect community function, as illustrated by the relationship between microorganisms and metabolites (Fig. 7). Externally colonized microorganisms had a strong interrelationship with less content and were positively correlated with the production of various dominant metabolites. This may explain the regional characteristics of fermented foods. However, the volatile profile of N4–9 was far from that of the other microecosystems (Fig. 6). This may be related to the no amount of lactic acid that accumulated in the microecosystem N4–9. Lactic acid is involved in signal communication in the microbial community (Garcia et al., 2016; Tuite, 2016) and also affects the completion of the ecological function in the microbial community, resulting in its flavor being far more conspicuous than that of other microecosystems.

There were significant differences in the microbial composition of Xiaogu fermentation communities reconstructed by fermentation and that of the corresponding starters. The fungal composition of the reconstructed microecosystem was similar to that of the starter; however, the bacterial composition was significantly different. Bacterial community have different ecological functions, although evolutionary processes are regulated by the same biotic and abiotic contexts. Our findings indicate that the assembly and function of bacterial microbiota are driven by trophic interactions, and the nutrient intensity was closely related to community assembly. In contrast, the assembly and function of fungal microbiota were driven by OAs. Although the abundances of some microbial genera and species are relatively low in the environment, they play an essential role in completing ecological functions. The fungal composition of the community (Garcia et al., 2016; Tuite, 2016) and also affects the completion of the ecological function in the microbial community, resulting in its flavor being far more conspicuous than that of other microecosystems.

Declaration of Competing Interest

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

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ccmrc.2022.100170.

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