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Dynamic changes of fungal community structure of yak Qula during storage

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Yak Qula is a traditional fermented dairy product from the Tibetan Plateau, but the dynamic changes of its fungal community during storage remain poorly understood. This study employed Illumina MiSeq high-throughput sequencing to investigate the fungal community structure of yak Qula stored for 0 (fresh): 3, and 6 months. Results showed significant succession: at the phylum level, Ascomycota dominance (97.0%–98.2%) was temporarily replaced by increased Basidiomycota (16.2%–28.9%) at 3 months before recovering; at the genus level, fresh Qula dominated by unclassified Dipodascaceae and *Galactomyces* shifted to *Trichosporon* and *Yarrowia* at 3 months, and finally to absolute dominance of Pichia (61.8%–86.2%) at 6 months, with the Shannon index decreasing significantly ($P = 0.026$). LEfSe analysis detected *Aspergillus versicolor* and *Trichosporon inkin*, two species previously associated with mycotoxin production and opportunistic infections, suggesting potential safety concerns that require further study. SparCC network analysis revealed a dynamic succession of interaction patterns from "tight co-occurrence" to "bimodal mutual exclusion" to "polycentric loose" networks. These findings provide a scientific basis for quality evaluation, safety assessment, and storage optimization of Qula.

Keywords: Yak Qula, Fungal community, High-throughput sequencing, Dynamic succession

INTRODUCTION

Yak Qula, also known as milk residue, is a traditional fermented dairy product unique to the Tibetan Plateau. It is produced by skimming yak milk, followed by natural fermentation for casein coagulation and subsequent sun-drying[1]. Compared with fresh yak milk, Qula exhibits higher protein content, enhanced storability, and easier transportability[1-2]. Beyond serving as a crucial strategy for Tibetan herders to preserve dietary protein during long winters, Qula is also utilized as a natural starter culture for yogurt production and as a raw material for casein manufacturing[3]. Owing to its open, non-aseptic processing environment, diverse environmental microorganisms actively participate in the fermentation process, collectively shaping Qula's distinctive flavor and quality attributes[4]. Following production, Qula is typically stored at ambient temperature for extended periods. Notably, the resident microbial community can undergo substantial successional changes during this storage phase, potentially exerting significant impacts on both product quality and safety[5-7].

Methodologically, the analysis of microbial diversity has evolved from traditional culture-dependent approaches to advanced culture-independent molecular techniques. Although straightforward, conventional pure-culture

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methods are limited to isolating only a minor fraction of culturable microorganisms, thereby failing to capture the full spectrum of microbial diversity. Early molecular fingerprinting techniques-including denaturing gradient gel electrophoresis (DGGE)[8], single-strand conformation polymorphism (SSCP)[9], temperature gradient gel electrophoresis (TGGE)[10], and terminal restriction fragment length polymorphism (T-RFLP)[11-13] have been widely applied in dairy microbiology[14-16]. However, these techniques generally provide limited taxonomic resolution and lack quantitative capacity. In contrast, the recently emerged Illumina MiSeq high-throughput sequencing platform enables deep, comprehensive, and accurate characterization of microbial composition and structure. Compared with traditional methods, this technology offers superior sequencing depth, richer data output, and the ability to delineate the relative abundance of both dominant and rare microbial taxa. It has been successfully employed in profiling microbial diversity in raw milk[17] and fermented yogurt[13].

Fungi play a pivotal role in the ripening of traditional fermented dairy products, exerting profound influences on flavor development, textural properties, and bioactive compound formation[13]. Despite this recognized importance, no study to date has systematically investigated the dynamic succession of fungal communities in yak Qula across the entire post-production storage timeline (fresh, 3-month, and 6-month storage). Previous studies on Qula and other fermented dairy products have primarily focused on fermentation-stage microbial composition or bacterial diversity, while the storage-stage fungal succession patterns, interaction network shifts, and associated safety implications remain largely unexplored.

To fill this gap, the present study, for the first time, employs Illumina MiSeq high-throughput sequencing to (1) delineate the phased succession trajectory of fungal communities during Qula storage at both phylum and genus levels; (2) identify potential safety-related fungal biomarkers (e.g., Aspergillus versicolor and Trichosporon inkin) that emerge during storage; and (3) reveal the dynamic reconfiguration of fungal co-occurrence networks, transitioning from a tightly cooperative structure to a bimodal mutually exclusive pattern and finally to a polycentric loose network. These findings provide novel insights into the ecological succession of fungi during dairy product storage and offer a scientific basis for quality control and safety assessment of traditional fermented foods.

Materials and Methods

Sample collection

Qula samples were collected from Kashe Village, Hezuo City, Gannan Tibetan Autonomous Prefecture, Gansu Province. For each storage time point (fresh, 3 months, and 6 months): ten independent samples were obtained from different households. The ten samples from each time point were thoroughly pooled into a composite sample, which was then divided into three subsamples serving as biological replicates (designated M01–M03 for the fresh group M0, M31–M33 for the 3-month group M3, and M61–M63 for the 6-month group M6). All subsamples were placed in self-sealing bags, labelled, and transported to the laboratory in ice boxes for subsequent analysis.

Extraction of total microbial DNA from Qula

A 0.2 g Qula sample was taken, and total DNA was extracted using the E.Z.N.A. Soil DNA Kit D5625-01 according to the manufacturer's instructions. The extracted DNA was then quantified using a Qubit 2.0 spectrophotometer, and the integrity of the extracted DNA was verified by 0.8% agarose gel electrophoresis.

PCR amplification and sequencing

Fungal-specific primers were used to amplify the ITS region of the DNA extracted from Qula samples. The primers for ITS region amplification were ITS5F (GGAAGTAAAAGTCGTAACAAGG) and ITS1R (GCTGCGTTCTTCATCGATGC). The PCR conditions were as follows: initial denaturation at 95°C for 5 min, followed by 25 cycles of 95°C for 30 s, 56°C for 30 s, and 72°C for 30 s, with a final extension at 72°C for 10 min, and then held at 4°C. The PCR products were detected by agarose gel electrophoresis, then recovered using a kit for quality assessment and library construction. Finally, paired-end sequencing was performed on the MiSeq sequencing platform by Shanghai Personal Biotechnology Co., Ltd.

High-throughput sequencing data processing and bioinformatics analysis

Raw paired-end reads were processed using QIIME 2 (version 2023.5)[18] and Mothur (v.1.48.0)[19]. Quality filtering was performed with the following criteria: reads were truncated at any site with an average quality score < Q20 over a 10 bp sliding window; reads shorter than 200 bp after truncation were discarded; and sequences with ambiguous bases (> 2 Ns) or mismatches in primer sequences were removed. High-quality reads were then merged using FLASH (v.1.2.11)[20] with a minimum overlap of 10 bp and a maximum mismatch rate of 0.1. Chimeric sequences were identified and removed using UCHIME (v.4.2.40)[21] in de novo mode against the UNITE reference database.

For taxonomic assignment, clean non-chimeric reads were clustered into operational taxonomic units (OTUs) at 97% sequence similarity using the UCLUST algorithm[20]. OTU representative sequences were taxonomically classified using the Naïve Bayesian classifier[22] (confidence threshold ≥ 0.7) against the UNITE fungal ITS database (version 8.3, released 2023-01-20). To minimize the effect of sequencing depth on diversity comparisons, all samples

were rarefied to the minimum sequence count (14,343 reads per sample) prior to alpha and beta diversity analyses. OTU tables were generated and used for all downstream analyses.

Alpha diversity metrics (Shannon, Simpson, Chao1, and ACE indices) were calculated using Mothur[19], and differences among storage groups were assessed by one-way analysis of variance (ANOVA) followed by Tukey's HSD post-hoc test, with $P < 0.05$ considered statistically significant. Beta diversity was evaluated using principal coordinate analysis (PCoA) based on Weighted Unifrac distances and non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarities; group differences were tested by permutational multivariate analysis of variance (PERMANOVA, adonis function in QIIME 2 with 999 permutations[18]. Linear discriminant analysis effect size (LEfSe) was performed to identify differentially abundant taxa among groups, using a logarithmic LDA score threshold of ≥ 2.0 and an alpha value of 0.05 for the factorial Kruskal-Wallis test. Network analysis was conducted using the SparCC method to infer microbial co-occurrence patterns, retaining only correlations with $|r| \geq 0.6$ and $P < 0.05$. All statistical analyses were performed using R software (version 4.2.0) with the vegan, ggplot2, and Hmisc packages, unless otherwise specified.

Results and discussion

Sequencing results of the samples

The sequencing read counts and OTU profiles of the Qula samples are shown in Table 1. High-throughput sequencing of nine Qula samples yielded a total of 57,926 to 75,670 raw reads per sample. After quality control and filtration, 18,051 to 66,627 valid reads were obtained per sample, with an average quality control retention rate of approximately 67.3%. Following denoising, merging, and chimera removal, 14,343 to 58,647 chimera-free reads were obtained, with a merging efficiency exceeding 96%. The number of OTUs per sample ranged from 31 to 181, with an average of approximately 78. The coverage value for all samples was ≥ 0.9992, indicating that the sequencing depth sufficiently covered the major microbial species in each sample, and the data quality was reliable enough to support subsequent diversity and composition analyses.

Alpha diversity analysis

Alpha diversity analysis (Fig. 1) revealed significant differences in the Shannon index of fungal communities across different storage periods ($P = 0.026$): with a gradual decreasing trend as storage time prolonged, indicating that the community structure tended to simplify. Although the Chao1 and ACE indices also showed a decline in species richness, the differences did not reach statistical significance ($P > 0.05$): which may be attributable to the high heterogeneity of individual samples within groups.

Beta diversity analysis

NMDS analysis based on fungal community structure (Fig. 2A) showed a Stress value of 0.0418, indicating an excellent model fit. The three groups of samples were clearly separated along the NMDS1 axis: the M0 group (fresh Qula) was distributed on the left, the M3 group (3-month storage) on the right, and the M6 group (6-month storage) on the left. The M0 and M3 groups were completely separated, suggesting a significant difference in fungal community structure between fresh Qula and Qula stored for three months, which is consistent with the outbreak of Basidiomycota at the phylum level in the M3 group (1.5% → 28.9%). Partial overlap was observed between the M0 and M6 groups, indicating that after long-term storage, the community structure returned to a state similar to that of fresh Qula, although the dominant genus had shifted from unclassified Dipodascaceae to Pichia. Regarding within-group aggregation, samples in the M3 group were relatively dispersed, reflecting the diversity and complexity of the community structure during the mid-storage period, which is consistent with the bimodal mutually exclusive network observed in the M3 group by network analysis. In contrast, samples in the M6 group were highly aggregated (almost overlapping): indicating a high degree of convergence in community structure after long-term storage, consistent

Table 1. Statistics of sample sequencing data processing results

Sample ID	Raw Reads	Clean reads	Denoised reads	Merged reads	Non-chimeric reads	OTUs	Coverage
M01	59243	50406	50406	50334	46384	95	0.9995
M02	60022	44117	44106	44011	40397	163	0.9992
M03	57926	48685	48681	48556	43197	181	1
M31	62923	18701	18667	18632	14343	41	0.9999
M32	71529	18051	18016	17981	16377	54	0.9998
M33	75670	19745	19735	19728	15417	32	0.9998
M61	71680	66627	66624	66607	58633	49	0.9996
M62	69445	65292	65292	65272	58647	31	0.9996
M63	65599	62658	62623	62397	51695	61	0.9998

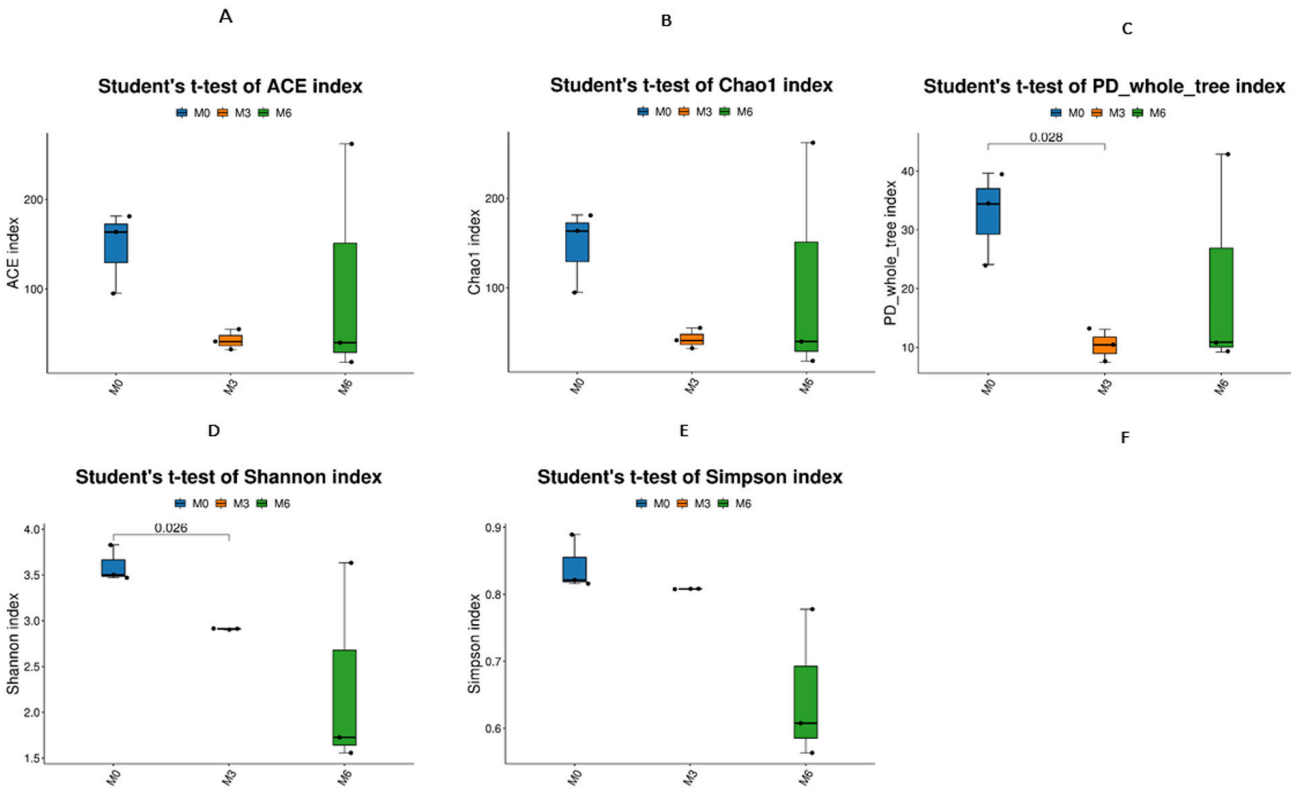

Figure 1. Alpha diversity indices of fungal communities in yak Qula at different storage stages (M0: fresh; M3: 3 months; M6: 6 months). (A) Shannon index; (B) Chao1 index; (C) ACE index. Data are shown as mean ± SD (n = 3)

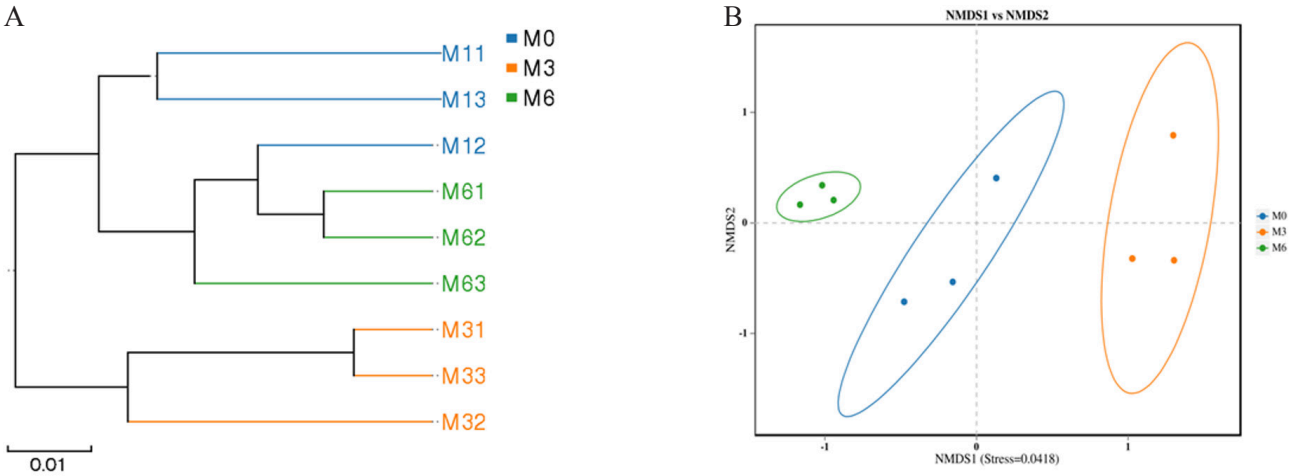

Figure 2. Beta diversity analysis of fungal communities. (A) UPGMA cluster tree based on Weighted Unifrac distance. Each point/leaf represents a sample, colored by storage group (M0, M3, M6); (B) Non-metric multidimensional scaling (NMDS) plot based on Bray-Curtis dissimilarities (Stress = 0.0418)

with the absolute dominance of Pichia at the genus level (86.2%) and the recovery of saprotrophic function in functional prediction (99.9%). These results validate the dynamic succession patterns of fungal communities during Qula storage from the perspective of β-diversity.

PERMANOVA analysis confirmed significant differences in fungal community structure among the three storage groups (R^2 = 0.612, P = 0.001): with pairwise comparisons showing significant separation between all groups (M0 vs. M3: P = 0.003; M3 vs. M6: P = 0.004; M0 vs. M6: P = 0.022). UPGMA cluster tree analysis based on Weighted Unifrac distance (Fig. 2B) revealed clear clustering characteristics of the three Qula groups at all taxonomic levels (phylum, class, order, family, genus, and

species). At the genus level, the three groups formed three main branches: the M0 group (fresh Qula): the M3 group (3-month storage): and the M6 group (6-month storage) each clustered into separate branches. Branch lengths showed greater distances between the M0 and M3 groups and between the M3 and M6 groups, while the distance between the M0 and M6 groups was relatively smaller, indicating higher similarity in community structure between the M0 and M6 groups than with the M3 group. Regarding within-group aggregation, samples in the M6 group were highly clustered with extremely short branch lengths, indicating a highly consistent community structure after six months of storage. Samples in the M3 group were relatively dispersed with longer branch lengths, reflecting the diversity of community structure during the mid-storage period, while the M0 group fell between the two. These results further confirm the significant effect of storage time on the fungal community structure of Qula from a cluster analysis perspective and reveal the relative similarity in community structure between the M0 and M6 groups. It should be noted, however, that the three replicates within each storage group were derived from pooled household samples rather than independent biological replicates, which may reduce the estimated within-group variability and should be considered when interpreting the beta-diversity patterns. Nevertheless, the clear inter-group separation and high sequencing coverage support the robustness of the observed successional trends.

Analysis of fungal community composition

Analysis of fungal community composition at the phylum level (Fig. 3A) revealed significant dynamic succession of fungal communities in Qula samples during storage. In fresh Qula (M0 group): Ascomycota was the absolutely dominant phylum, with a relative abundance of 97.0%–98.2%. After three months of storage (M3 group): the abundance of Ascomycota decreased to 70.8%–83.8%, while Basidiomycota increased markedly from 1.4%–1.5% to 16.2%–28.9%, indicating a restructuring of the phylum-level community structure during the mid-storage period. After six months of storage (M6 group): the abundance of Ascomycota recovered to 93.8%–99.9%, re-establishing itself as the absolutely dominant phylum, while Basidiomycota dropped back to 0.02%–5.9%. The abundances of other phyla (Mortierellomycota, Rozellomycota, Glomeromycota, etc.) were extremely low (totaling <0.1%): with negligible effects on community structure. These results indicate that during Qula storage, the fungal community at the phylum level underwent a dynamic succession of "Ascomycota dominance → Basidiomycota increase → Ascomycota dominance recovery".

Analysis of fungal community composition at the genus level (Fig. 3B) revealed significant dynamic succession of fungal communities in Qula samples during storage. In fresh Qula (M0 group): the dominant genera were unclassified Dipodascaceae (33.8%–53.7%): *Galactomyces* (15.5%–20.8%): and *Pichia* (6.5%–11.0%). After three months of storage (M3 group): the community structure changed dramatically: *Trichosporon* exploded from 0.2%–0.3% to 13.8%–27.0%; *Yarrowia* (2.7%–3.4%) and *Apiotrichum* (1.1%–1.7%) appeared specifically; while *Aspergillus* decreased sharply from 13.9% to 0.3%–1.0%. These observations indicate that the M3 group represents a critical turning point in fungal community succession. After six months of storage (M6 group): Pichia became the absolutely dominant genus (61.8%–86.2%): while the abundances of unclassified Dipodascaceae (3.8%–7.2%) and *Galactomyces* (6.9%–11.5%) decreased substantially, and genera such as *Trichosporon* and *Yarrowia* almost disappeared, indicating a trend toward community simplification. These results quantitatively validate the dynamic succession patterns of fungal communities during Qula storage and are highly consistent with the findings of network analysis and LEfSe analysis.

LEfSe analysis of fungal community biomarkers in Qula during different storage periods

LEfSe analysis (LDA score threshold ≥ 2.0) further identified differential biomarkers of fungal communities in Qula samples at different storage times (Fig. 4). The cladogram displays the distribution of differentially abundant taxa from the phylum to species levels from the inside out, with green nodes indicating fungal taxa enriched in the M3 group and blue nodes indicating those enriched in the M6 group.

The results showed that the characteristic fungi of the M3 group (3-month storage) mainly belonged to Ascomycota, including *Aspergillus versicolor*, *Galactomyces pseudocandidus*, *Yarrowia lipolytica*, and *Pichia fermentans*. Among these, *Yarrowia lipolytica* and *Pichia fermentans* are known for their excellent enzyme-producing and aroma-producing capabilities in fermented dairy products. Studies have shown that *Y. lipolytica* possesses high lipase and protease activities, which can break down milk fat to produce free fatty acids, thereby promoting flavor formation during cheese ripening[23]; *P. fermentans* primarily produces alcohols and esters through metabolism, contributing fruity and floral aromas.

The characteristic fungi of the M6 group (6-month storage) mainly belonged to Basidiomycota, particularly the order Trichosporonales, including *Trichosporon aquatile*, *Trichosporon inkin*, and *Saitozyma*. This succession pattern from Ascomycota to Basidiomycota reflects the restructuring of the fungal community structure during long-term storage. Similar phenomena have been reported in other traditional fermented foods, such as the observed phased succession of fungal communities

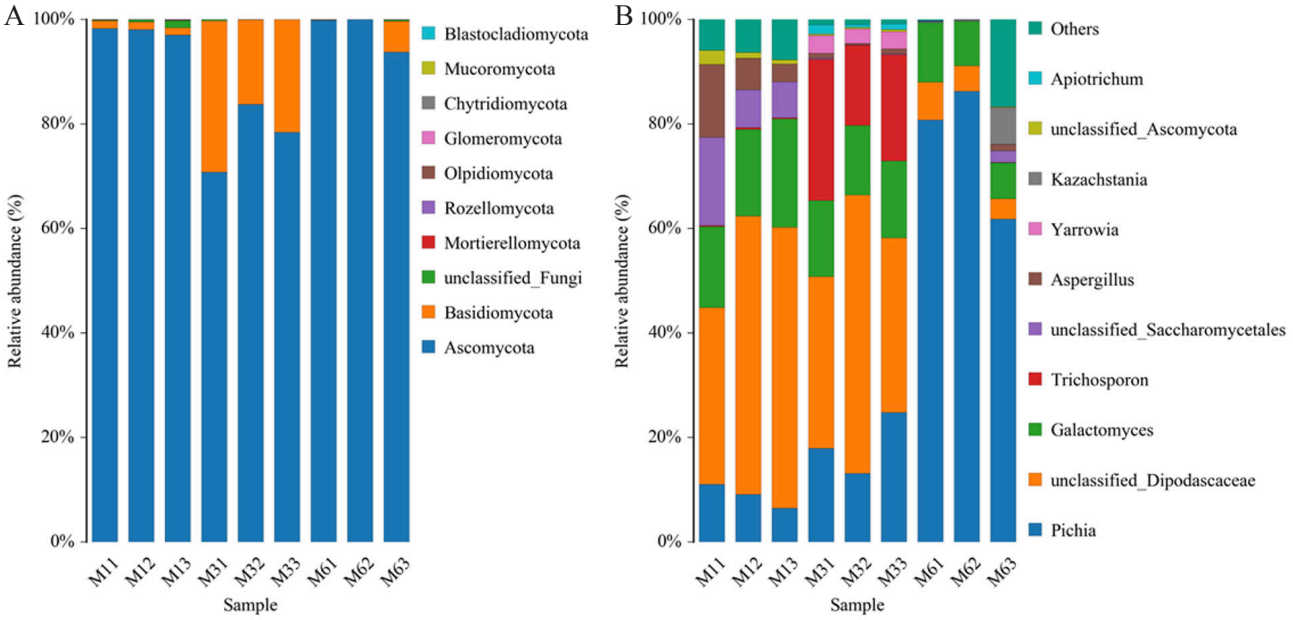

Figure 3. Fungal community composition at the phylum (A) and genus (B) levels across storage stages. Only phyla/genera with relative abundance > 1% are shown; remaining taxa are grouped as "Others". Relative abundance is based on OTU read counts after rarefaction

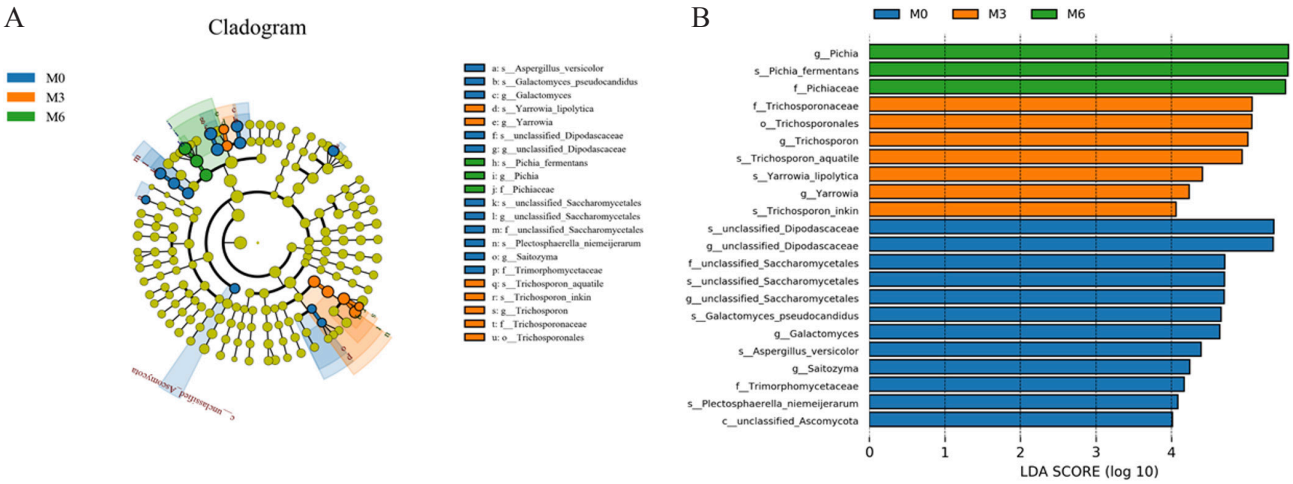

Figure 4. Linear discriminant analysis effect size (LEfSe) identification of differentially abundant fungal taxa among storage groups. (A) Cladogram showing taxonomic representation of enriched taxa from phylum to species level (inner to outer rings); (B) LDA scores (log10) of taxa with significant differential abundance (LDA score ≥ 2.0, $\alpha = 0.05$ for Kruskal-Wallis test). Taxa enriched in the M3 group are shown in orange, and those enriched in the M6 group in green

during the fermentation and storage of Nongxiangxing Daqu[24]. Additionally, the coexistence of Ascomycota (*Eurotium* spp., *Aspergillus* spp.) and Basidiomycota (*Wallemia*, *Cryptococcus*) has been detected during the pile-fermentation process of Fuzhuan brick tea, with the dominant microbial communities changing dynamically over fermentation time[25].

It is noteworthy that *Aspergillus versicolor* was detected in the M3 group. This species has been reported as a potential producer of sterigmatocystin, a mycotoxin with documented carcinogenicity in animals that mainly contaminates grains and grain products [26]. While our sequencing data confirm its presence in Qula at the 3-month storage point, they do not confirm actual toxin production, as this would require targeted metabolomic analyses (e.g., LC-MS/MS). Nevertheless, its detection highlights a potential risk that merits attention during Qula storage. Similarly, *Trichosporon inkin* detected in the M6 group is a species that has been associated with superficial fungal infections (e.g., white piedra) and systemic infections

in immunocompromised patients[27]. However, the mere presence of this fungus does not imply pathogenicity in the context of Qula consumption, as infection risk depends on host susceptibility, viable cell counts, and exposure routes. Thus, our findings should be interpreted as an early warning signal rather than a confirmed safety hazard, and further studies, including viability assessment and pathogenicity testing, are warranted.

These results indicate that the fungal community in Qula underwent a phased succession from Ascomycota to Basidiomycota during storage: the M3 group was characterized by enzyme- and aroma-producing yeasts and potentially toxigenic *Aspergillus*, while the M6 group was dominated by Trichosporonales fungi. This provides an important basis for understanding the ecological succession of fungal roles during Qula ripening and for safety assessment.

Microbial co-occurrence network analysis

Comparative SparCC network analysis ($|r| \geq 0.6$, $P < 0.05$) of fungal communities in the three Qula groups (Fig. 5) revealed significant differences in fungal interaction patterns across different storage periods. The M0 group (fresh Qula) exhibited a highly aggregated star-like network with unclassified Dipodascaceae as the core hub, showing completely positive correlations (corr = +1.00) with approximately 30 genera, including *Galactomyces*, *Pichia*, *Aspergillus*, and *Trichosporon*. This indicates that multiple saprotrophic and fermentative fungi in fresh Qula tend to coexist synergistically, collectively participating in the initial fermentation process, similar to networks reported in other traditional fermented foods[28]. The M3 group (3-month storage) presented a bimodal mutually exclusive network: all genera that highly co-occurred with the core in the M0 group became mutually exclusive with the core (corr = -1.00): while another group of positively correlated taxa (unclassified Ascomycota, *Lecanicillium*, *Fusarium*, *Monascus*, etc.) emerged, with the two modules completely separated. This reflects the nonlinear succession characteristics and niche differentiation of fungal communities during the mid-storage period[29-30], indicating that the M3 group represents a critical turning point in fungal community succession[31]. The M6 group (6-month storage) exhibited a polycentric loose network: the Ascomycota module recovered co-occurrence (unclassified Dipodascaceae ↔ *Galactomyces*, corr = +0.9998), while new independent Basidiomycota co-occurrence modules emerged (*Botryotrichum* ↔ *Trichosporon*, corr = +0.9644; *Leptobacillium* ↔ *Saitozyma*, corr = +0.9625). This suggests that after long-term storage, environmental selection pressures tend to homogenize, and Ascomycota and Basidiomycota form relatively independent metabolic functional modules, reducing direct competition[29]. These results reveal a dynamic succession of fungal interaction patterns from "tight co-occurrence" to "bimodal mutual exclusion" and then to "polycentric loose" networks, with the M3 group representing the stage with the most consistent network structure between fungi and bacteria, providing network-level evidence for understanding the ecological roles of fungi during Qula ripening.

The fungal succession patterns observed in this study are consistent with findings from other traditional fermented dairy products and related systems. For instance, in naturally fermented cow's milk, yeasts initially increase during early fermentation but decline as lactic acid bacteria (LAB) proliferate, accompanied by a pH decrease that selectively suppresses acid-sensitive fungi[32-33]. A similar phased succession from Ascomycota to Basidiomycota has been documented during the fermentation of traditional fermented milks in Inner Mongolia, where environmental selective pressures drive the replacement of initial fermentative taxa by more stress-tolerant species[34]. Notably, the emergence of Trichosporon and Apiotrichum during mid-storage in our Qula samples parallels observations in Tarag (a traditional fermented milk from the same region), where these genera were also detected as part of the post-fermentation fungal community[35].

From an ecological perspective, the observed successional shifts can be explained by changes in key environmental determinants during storage. Fresh Qula (M0) supports a diverse community dominated by Galactomyces and unclassified Dipodascaceae-taxa commonly associated with milk fermentation[36]. These fungi are adapted to the relatively high moisture and nutrient-rich environment of freshly produced Qula, where cooperative interactions (as reflected by the "tight co-occurrence" network) facilitate initial colonization. During the 3-month storage (M3), moisture loss, oxygen availability, and the accumulation of metabolic byproducts (e.g., organic acids) create selective pressures that favor stress-tolerant Basidiomycota (e.g., Trichosporon), while suppressing less tolerant Ascomycota[31]. The bimodal mutually exclusive network observed at this stage reflects intensified competition for limiting resources-a classic ecological response to environmental stress[29]. By 6 months (M6), the community stabilizes under more homogeneous storage conditions, with Pichia emerging as the dominant genus due to its well-known tolerance to low moisture and high osmotic pressure[37-38]. The reversion to a "polycentric loose" network suggests that, after an initial competitive phase, surviving taxa partition into relatively independent metabolic niches, reducing direct competition and achieving a new equilibrium[30]. Similar community stabilization patterns have been reported in stored barley grain and during Daqu storage, where storage fungi gradually replace field fungi as the substrate desiccates and matures[39,40].

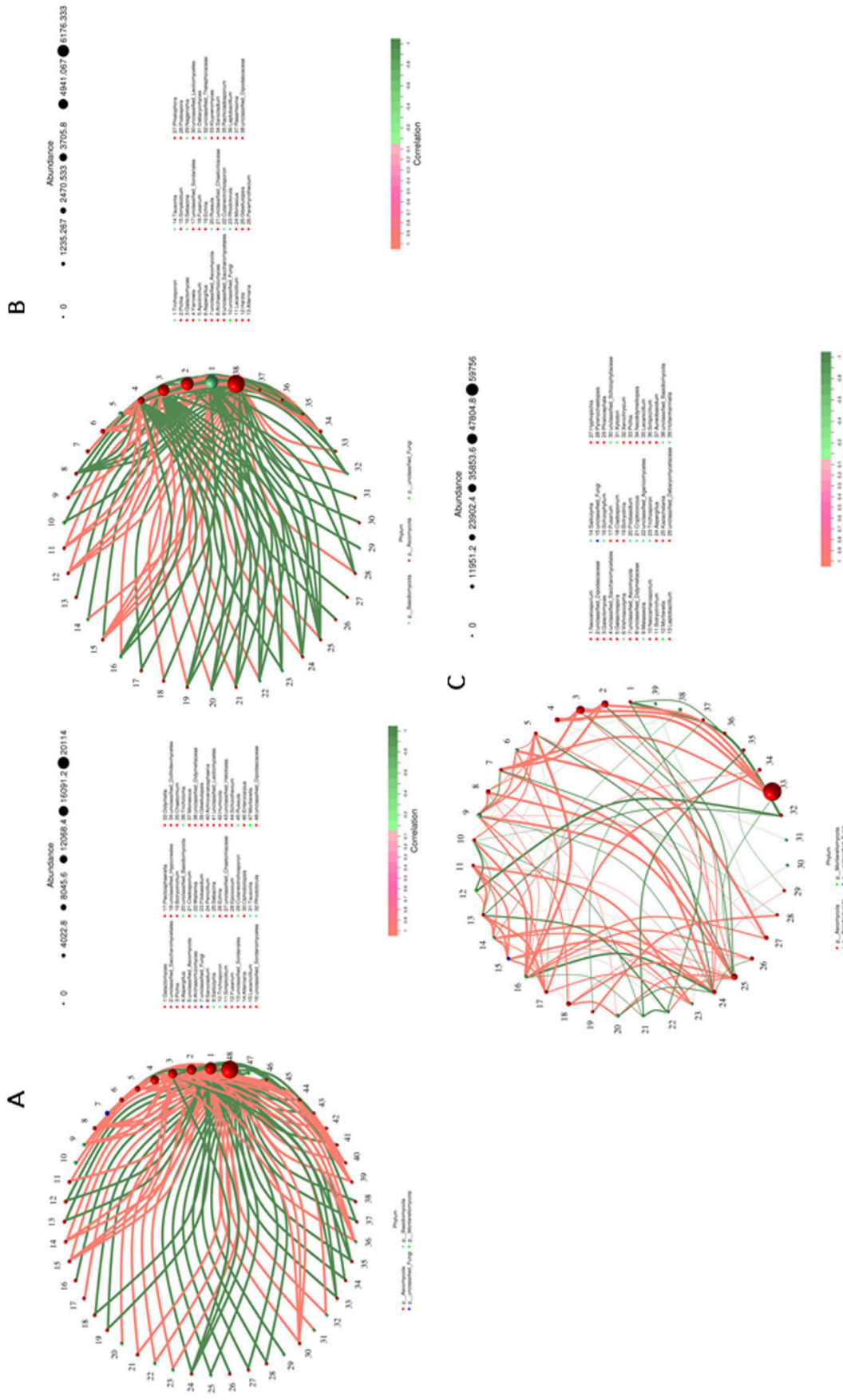

Figure 5. SparCC network analysis of fungal co-occurrence patterns. (A) M0 (fresh); (B) M3 (3 months); (C) M6 (6 months). Each node represents a genus, with node size proportional to its relative abundance. Edges represent significant correlations ($|r| \geq 0.6$, $P < 0.05$): red edges indicate positive correlations, green edges indicate negative correlations

Conclusions

This study reveals a clear fungal succession pattern in yak Qula during storage, with the 3-month time point identified as a critical turning point in community structure and network dynamics. The detection of *Aspergillus versicolor* and *Trichosporon inkin* suggests potential safety risks, although sequencing alone does not confirm toxin production or pathogenicity. Practically, these findings suggest that (i) routine fungal monitoring should be intensified around the 3-month storage mark, and (ii) targeted chemical analyses (e.g., mycotoxin detection) are recommended to verify actual safety hazards. This study establishes a foundation for storage management and quality control of Qula, while highlighting the need for functional validation through metabolomic and viability assays in future research.

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Declaration of competing interest

All authors ensure the absence of known conflicts of interest or personal relationships that could bias the research work presented in this paper.

Data availability

Data are available within the article or its supplementary material.

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