



RESEARCH ARTICLE

Fungal Microbiota Comparison Analysis of Different Breeds of Plateau Sheep

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ABSTRACT

Tibetan sheep are economically important and adapted to high altitude animals of the Qinghai Xizang Plateau, characterized by hypoxia, low temperature, and limited forage quality. Despite their importance, the fecal fungal microbiota of different plateau sheep breeds and their potential role in altitude adaptation remain largely unexplored. Here, we conducted ITS1 amplicon sequencing to comprehensively compare the cecal fungal communities among three representative breeds: Pengbo semi-fine-wool sheep (P), Elephant male semi-fine-wool sheep (XX, and Holba sheep (HRB) (n = 6 males per breed, 4 years old). From 18 cecal samples, we generated 1 347 654 raw and 1 227 756 high quality filtered sequences. In addition to sufficient sequencing depth confirmed by rarefaction curves, alpha diversity indices, including Pielou (P<0.01), Shannon (P<0.01), and Simpson (P<0.0001), significantly declined with increasing altitudes. Comparatively, beta-diversity analyses (PCoA, UPGMA, and ANOSIM) revealed clear, statistically significant separation among the three breeds (P<0.01). LEfSe and t-tests identified three phyla (*Ascomycota*, *Basidiomycota*, *Mucoromycota*) and 21 genera as robust biomarkers, including genera of *Caecomyces*, *Myceliophthora*, *Naganishia*, *Leptobacillum*, *Ustilago*, *Filobasidium*, *Mucor*, *Wallemia*, etc. Our results may give novel insights for a better understanding of the high-altitude adaptability of plateau animals.

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INTRODUCTION

Tibetan sheep (*Ovis aries*) are primitive ruminant breeds that live on the Qinghai-Xizang Plateau at altitudes exceeding 3000m. Additionally, these animals exhibit exceptional physiological adaptations to the hostile plateau environment, including chronic hypoxia, low ambient temperatures, intense ultraviolet radiation, and insufficient forage (Li *et al.*, 2022; Wu *et al.*, 2024). In addition to their remarkable resilience, Tibetan sheep play an important socio-economic role for local communities, supplying meat, milk, cashmere, leather, and others while numbering over 50 million across this plateau (Ren *et al.*, 2023; Issa *et al.*, 2024). Comparatively, the common Tibetan sheep breeds include Black Tibetan sheep, Pengbo semi-fine-wool sheep (Pengbo sheep), Chaka semi-fine wool sheep (Chaka sheep), Elephant male semi-fine wool sheep (Elephant sheep), Holba sheep, Oula

sheep, and others (Peng *et al.*, 2024; Xu *et al.*, 2024). Pengbo sheep are primarily found in Lhünzhub county (northeast of Lhasa; 90°51'–91°28' E, 29°45'–30°08' N), at an average altitude of approximately 3860 m and an annual temperature of 7.5°C. Similarly, Elephant sheep living in the Zanda County in the southwest of Ngari Prefecture (76°12'–80°54' E, 30°24'–32°42' N), with an average altitude of ~4 500m and a temperature of -1.5°C. Holba sheep are usually inhabited in Zhongba county, which is located in the west of Xigaze with east longitude 82°00'~84°76', northern latitude 29°15'~31°80'. The average altitude and temperature in Zhongba County are 5000 m and 5°C, respectively. In contrast to lower-altitude sheep breeds, these plateau populations face progressively harsher conditions with increasing elevation, making them valuable models for studying environmental adaptation.

In the host intestine, there are trillions of various microorganisms, including fungi, bacteria, and protozoa,

which is commonly named gut microbiota or “the hidden organ” (El-Sayed *et al.*, 2021). Additionally, this complex community influences host physiology through nutrient extraction, metabolism, and immunity of the host (Bouskra *et al.*, 2008; Hou *et al.*, 2022; Xu *et al.*, 2025). For decades, research on gut microbiota in ruminants has predominantly focused on bacteria rather than the fungal mycobiome (Pe Rez and Johnson, 2013). However, recently, some studies demonstrated that intestinal fungi play a significant role in important physiological processes such as the induction of T helper 17 cells and immunoregulation (Pérez, 2021), and interact with gut bacteria (van Tilburg Bernardes *et al.*, 2020). Similarly, previous studies have documented altitude-driven shifts in gut microbiota among plateau animals, including Tibetan chicks, rats, and yaks (Du *et al.*, 2022; Pan *et al.*, 2022; Zhu *et al.*, 2024). Recent multi-omics investigations further indicate that high-altitude Tibetan sheep exhibit enhanced forage fermentation capacity, with enrichment of fibrolytic taxa and alterations in short-chain fatty acid production that support energy harvesting under nutritional stress. Comparatively, fungal communities also respond to altitude gradients, with shifts in Ascomycota, Basidiomycota, and Neocallimastigomycota potentially aiding cellulose degradation and stress resilience. However, the extensive bacterial-focused literature, information on the cecal fungal microbiota of different Tibetan sheep breeds remains scarce. Therefore, we conducted this study to compare the cecal fungal communities among Pengbo semi-fine-wool sheep, Elephant male semi-fine-wool sheep, and Holba sheep originating from the Qinghai–Xizang Plateau, China, using ITS1 amplicon sequencing. Additionally, by identifying altitude-associated biomarkers at phylum and genus levels, our findings aim to illuminate the potential contribution of the mycobiome to high-altitude adaptability in plateau ruminants.

MATERIALS AND METHODS

Sheep samples collection: Male sheep aged four years were used in this study. Cecal content samples were collected from a local slaughterhouse in Lhasa, Xizang, in 2025. Additionally, the animals comprised six Pengbo semi-fine-wool sheep (P1-P6), six Elephant male semi-fine wool sheep (XX1-XX6), and six Holba sheep (HRB1-HRB6). The animals were carefully selected to minimize biological variability, including similar age, sex, management, and environmental conditions, which helped improve the reliability of comparisons among groups. All procedures were performed in accordance with the guidelines approved by the Ethics Committee of the College of Animal Science and Technology, Henan Agricultural University (approval No. 11-00850).

Sheep microbiota sequencing: Genomic DNA was extracted from approximately 0.25gm of each cecal sample using the Soil and Fecal Genomic DNA Extraction Kit (Tiangen Biotech, Beijing, China) following the manufacturer’s instructions. In addition to quantity and quality assessment via NanoDrop spectrophotometer (Thermo Fisher Scientific, USA) and 1.5% agarose gel electrophoresis, only high-quality DNA extracts (A260/A280 ratio: 1.8–2.0, concentration: 20 ng/ μ L) were

used for downstream analysis (Chen *et al.*, 2023). The qualified extracts from plateau sheep were used for ITS gene (V1 region) amplification targeting universal primer (F:5’-CTTGGTCATTTAGAGGAAGTAA-3’; R: 5’-GCTGCGTTCTTCATCGATGC-3’) (Li *et al.*, 2024). The PCR reactions were performed under standardized conditions, and amplicons were purified and quantified for further processing. The libraries were constructed and subjected to 2 \times 250 bp paired-end sequencing on the Illumina NovaSeq platform at Bioyi Biotechnology Co., Ltd, China (Xu *et al.*, 2025).

Bioinformatics analysis of different breeds of sheep: Microbiome bioinformatics analyses of the three breed plateau sheep were carried out using QIIME2 (2024) according to its instructions (Bolyen *et al.*, 2019). Briefly, all raw sequences of plateau sheep were first re-demultiplexed, primers removed, and the data were quality filtered, denoised, and merged via the DADA2 plugin (Callahan *et al.*, 2016). This pipeline was subsequently used to generate non-singleton amplicon sequence variants (ASVs) with sequence alignment performed using MAFFT (Katoh *et al.*, 2019). Secondly, the taxonomy of ASVs from different sheep breeds was assigned using the classify-sklearn naïve Bayes classifier against the UNITE database (Abarenkov *et al.*, 2024). Thirdly, alpha-diversity metrics (Chao1, Observed species, Shannon, Simpson, etc.) for sheep in different groups were evaluated through QIIME2 (Rust *et al.*, 2025). Fourthly, beta-diversity metrics (unweighted UniFrac and Jaccard distance) were analyzed and visualized using principal coordinate analysis (Xu *et al.*, 2025), nonmetric multidimensional scaling (Vanhatalo *et al.*, 2025), unweighted pair-group method with arithmetic means hierarchical clustering (Liang *et al.*, 2025), and principal component analysis to depict the structural variation among different sheep groups (Wu *et al.*, 2025). Fifthly, co-existing ASVs among different sheep groups were identified by using a Venn diagram through the R package, and biomarkers among different sheep breeds were detected employing Linear discriminant analysis effect size (LEfSe) and t-tests (Pan *et al.*, 2024; Xu *et al.*, 2025).

Statistical analysis: Group differences among different sheep groups were examined by statistical analyses performed with SPSS (27.0). All results are depicted as means \pm standard deviation, and the significance threshold ($P < 0.05$), to improve clarity and reproducibility.

RESULTS

Sequencing information and alpha diversity analysis of sheep in different groups. A total of 461,434 (P), 431,939 (XX), and 454,281 (HRB) raw sequences were obtained, of which 421,767 (P), 392,296 (XX), and 413,693 (HRB) high-quality sequences remained after filtering (Table 1). Rarefaction curves plateaued rapidly and approached saturation, indicating that sequencing depth was sufficient to capture the microbial diversity of the samples (Fig. 1a). Rank abundance curves were smooth and gradually declining, suggesting high richness and evenness in the sheep microbiota (Fig. 1b). Alpha diversity indices, including Pielou’s evenness ($P < 0.01$),

Shannon ($P < 0.01$), and Simpson ($P < 0.0001$), differed significantly among the three groups, with lower diversity observed in sheep from higher altitudes (Table 2, Fig. 1c).

Table 1: Sequencing information of plateau sheep

Samples	Input	Filtered	Denosed	Merged	Non-chimeric	Non-singleton
P1	79945	73317	73193	71650	71645	71644
P2	80136	72435	72345	71989	71960	71959
P3	79896	72818	72705	71673	71671	71671
P4	61544	56731	56671	56331	56329	56329
P5	79981	73989	73875	72797	72796	72796
P6	79932	72477	72325	70634	70340	70340
XX1	79955	70698	70552	69759	69754	69754
XX2	51425	46629	46561	39374	39371	39371
XX3	79745	72808	72716	71986	71983	71982
XX4	79927	72629	72377	70189	70186	70186
XX5	60885	55406	55365	54516	54516	54516
XX6	80002	74126	74077	73457	73457	73457
HRB1	63840	58326	58266	56916	56916	56916
HRB2	80017	72111	72037	69698	69698	69698
HRB3	79963	72902	72871	70430	70422	70422
HRB4	79926	73037	72910	71075	71075	71075
HRB5	79958	72400	72318	70347	70347	70347
HRB6	70577	64917	64870	63373	63373	63373

Microbiota comparison of sheep in different taxa: A total of 1,531 ASVs were identified across all plateau sheep samples, including 628 in group P, 583 in group XX, and 482 in group HRB, with 34 ASVs shared among all groups (Fig. 2a). At the phylum level, *Ascomycota* (P: 80.85%, XX: 85.19%, HRB: 92.54%), *Basidiomycota* (P: 16.10%, XX: 6.02%, HRB: 3.05%) and *Neocallimastigomycota* (P: 1.68%, XX: 7.67%, HRB: 3.93%) were dominant across all groups (Fig. 2b). At the class level, *Sordariomycetes* (51.44%), *Eurotiomycetes* (23.36%), and *Tremellomycetes* (14.14%) predominated in group P; *Sordariomycetes* (79.64%), *Neocallimastigomycetes* (7.68%), and *Tremellomycetes* (3.76%) in group XX; and *Sordariomycetes* (86.50%), *Neocallimastigomycetes* (3.93%) and *Leotiomycetes* (2.43%) in group HRB (Fig. 2c). At the order level, *Hypocreales*, *Eurotiales*, and *Filobasidiales* were dominant in group P; *Hypocreales*, *Neocallimastigales*, and *Filobasidiales* in group XX; and *Hypocreales*, *Neocallimastigales*, and *Thelebolales* in group HRB (Fig. 2d).

Table 2: Alpha diversity analysis of different breed sheep groups

Sample	Chao1	ACE	Goods coverage	Observed species	Pielou e	Shannon	Simpson
P1	162	162	1	162	0.539138953504544	3.95719904943554	0.834823791802347
P2	103.5	103.358880046584	0.999946526923694	103	0.404729130571144	2.70622154493036	0.707089910006794
P3	91	91.1986873008648	0.999973263461847	91	0.346132611386676	2.25255995318019	0.587181521701812
P4	112.111111111111	112.470820247187	0.999946526923694	112	0.305641361042603	2.08060922347775	0.498969740435065
P5	123	122.677192378329	0.999919790385541	122	0.410225646587756	2.84316620563164	0.704207194791694
P6	181.5	181.241799502636	0.999893053847388	180	0.539900139651116	4.04485253295404	0.847293834148271
XX1	133	133.19607812028	0.999973263461847	133	0.327179009228773	2.30834031707644	0.499637206007773
XX2	83	83	1	83	0.372831210334568	2.37681366711967	0.532747402597716
XX3	141.5	142.150972594669	0.999839580771082	140	0.203415487368308	1.45020657947846	0.289699067646118
XX4	181.5	180.75352461701	0.999919790385541	180	0.457579116658374	3.42811552195284	0.618180268065412
XX5	49	49	1	49	0.131917421925407	0.740678047494885	0.154353034236514
XX6	103.142857142857	103.547399124939	0.999946526923694	103	0.222655319691715	1.4887849124988	0.312817406320416
HRB1	94	94.2543480664979	0.999973263461847	94	0.169311561909439	1.10976767615174	0.210431930096321
HRB2	124	124.236154556274	0.999973263461847	124	0.264661065230205	1.84050500332695	0.35563290478346
HRB3	105	105.301388888889	0.999973263461847	105	0.297434019530115	1.99704503243149	0.419847614399536
HRB4	101	101.217766596023	0.999973263461847	101	0.207914887179244	1.38434128925189	0.30879475611921
HRB5	88.5	88.9336734693878	0.999946526923694	88	0.169092799285482	1.09224337418853	0.198069021191737
HRB6	74	74.1954868148148	0.999973263461847	74	0.153683193745094	0.954288624641077	0.183385563489205

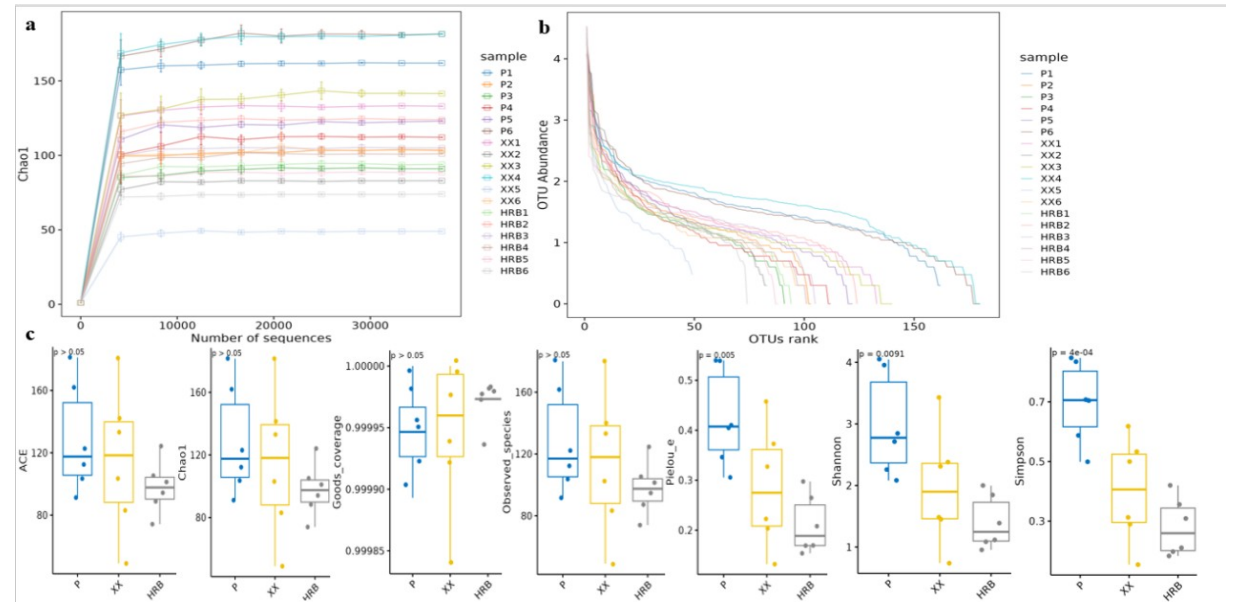


Fig. 1: Alpha diversity analysis of plateau sheep in different groups. (a) Rarefaction curve, (b) Rank abundance curve, (c) Diversity indexes. Data were shown as the mean \pm SEM (n=6), significance is depicted as * $P < 0.05$ and *** $P < 0.0001$.

Additionally, at the family level, Cordycipitaceae, Aspergillaceae, and Filobasidiaceae were dominant in group P; Cordycipitaceae, Neocallimastigaceae, and Filobasidiaceae in group XX; and Cordycipitaceae, Neocallimastigaceae, and Thelebolaceae in group HRB (Fig. 2e). While at the genus level, Leptobacillium, Naganishia and Thermomyces were dominant in group P; Leptobacillium, Naganishia and Caecomyces in group XX; and Leptobacillium, Caecomyces and Naganishia in group HRB (Fig. 2f). Moreover, heatmap analysis showed that

Basidiomycota and Mucoromycota were more abundant in group P, Neocallimastigomycota, Blastocladiomycota and Olpidiomycota were enriched in group XX and Ascomycota and Kickxellomycota were more abundant in group HRB (Fig. 3a). Finally, the genus level, Alfaria, Cladosporium, Neoascochyta, Buckleyzyma, Debaryomyces and Glomeraceae gen. incertae sedis were enriched in group P; Khoyollomyces, Agaricus, Gelatinospora, and Russula in group XX; and Meyerozyma, Preussia and Wallemia in group HRB (Fig. 3b).

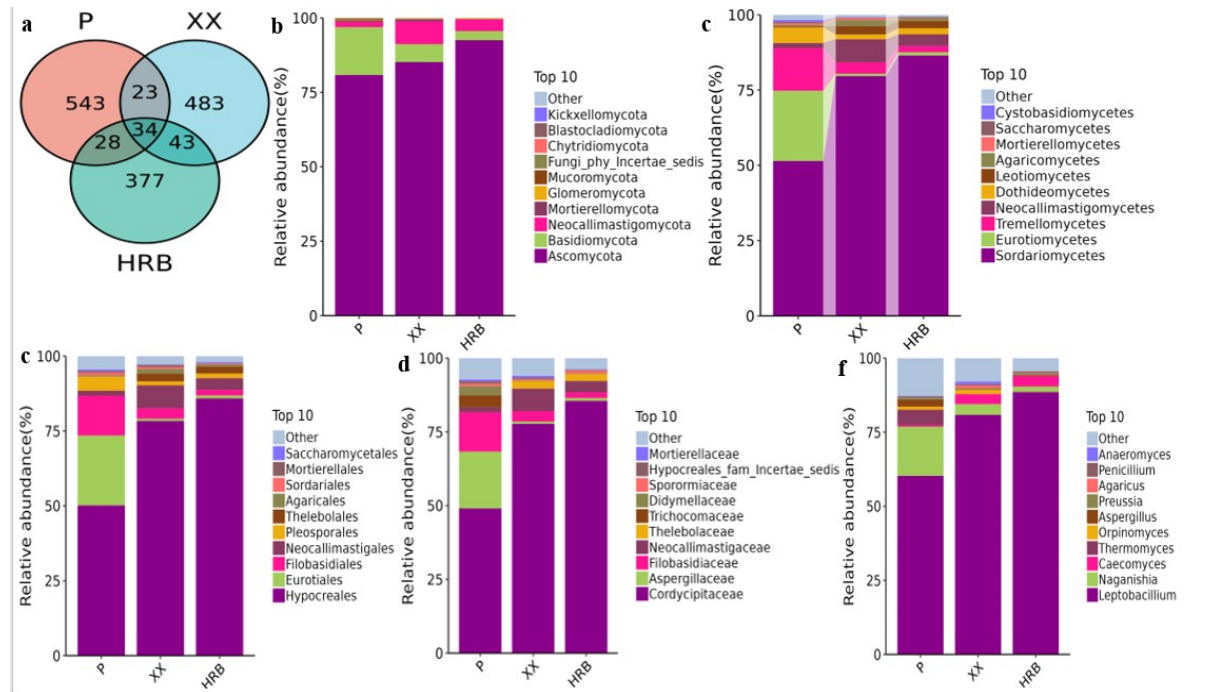


Fig. 2: Venn map and microbiota comparison analysis of plateau sheep in different taxa. (a) Venn map, (b) Phylum, (c) Class, (d) Order, (e) Family, (f) Genera.

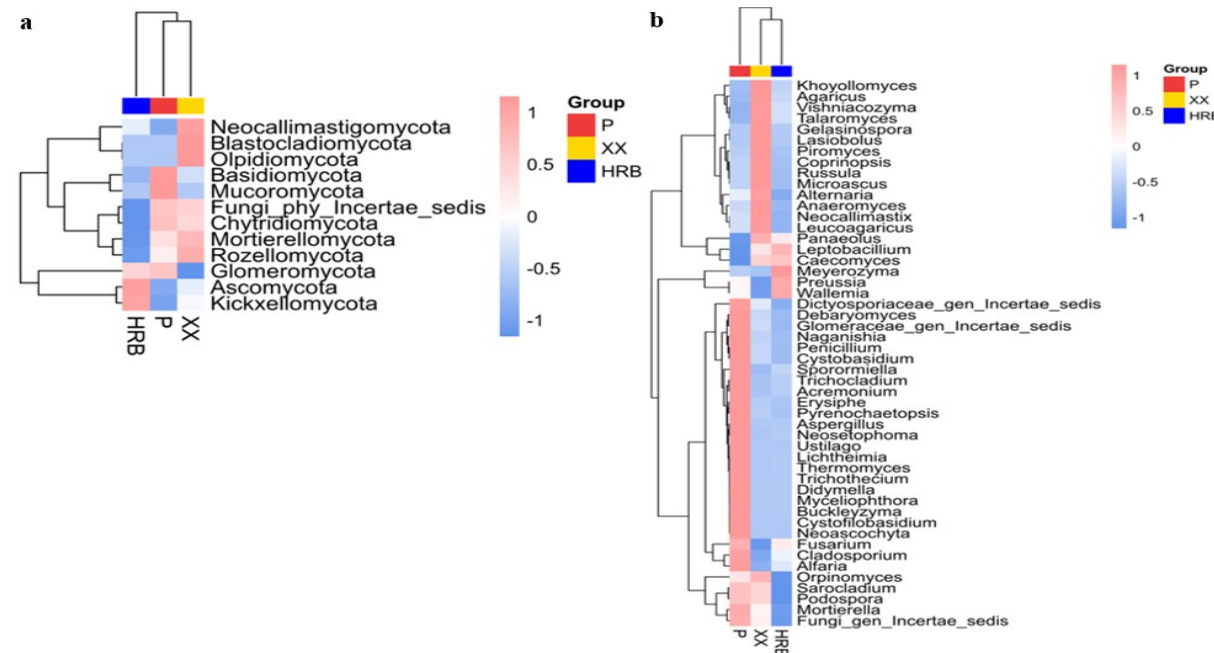


Fig. 3: Microbiota comparison of sheep in different groups via heatmap. (a) Phylum, (b) Genera.

Biomarkers of the microbiota of sheep in different groups: Microbiota beta diversity analysis of sheep in different groups showed obvious group separation via PCoA ($P < 0.01$) (Fig. 4a), UPGMA (Fig. 4d) and ANOSIM analysis ($P < 0.01$) (Fig. 4e). However, no significant difference was revealed by NMDS (Fig. 4b) and PCA (Fig. 4c). LEfSe showed that *Naganishia* ($P < 0.01$), *Thermomyces* ($P < 0.01$), *Cladosporium* ($P < 0.05$), *Aspergillus* ($P < 0.01$), *Myceliophthora* ($P < 0.01$), *Debaryomyces* ($P < 0.01$) and *Trichothecium* ($P < 0.001$) were significantly higher in group P, whereas *Lasiobolus* ($P < 0.05$), *Coprinopsis* ($P < 0.05$), *Debaryomyces* ($P < 0.01$), and *Anaeromyces* ($P < 0.05$) were markedly higher in group XX and *Caecomyces* ($P < 0.01$), *Leptobacillum*

($P < 0.01$) and *Preussia* ($P < 0.05$) were significantly higher in group HRB (Fig. 5).

T-tests showed that *Ascomycota* in group HRB was significantly higher than that in group P ($P < 0.01$). In contrast, *Basidiomycota* in group P was significantly higher than that in group XX ($P < 0.05$) and HRB ($P < 0.001$), respectively. *Mucoromycota* in group P was significantly higher than that in group XX ($P < 0.01$) and HRB ($P < 0.01$), respectively (Fig. 6a). *Leptobacillum* ($P < 0.05$; $P < 0.01$), *Caecomyces* ($P < 0.05$, $P < 0.05$), and *Myceliophthora* ($P < 0.01$; $P < 0.01$) in group P were significantly lower than those in groups XX and HRB, respectively.

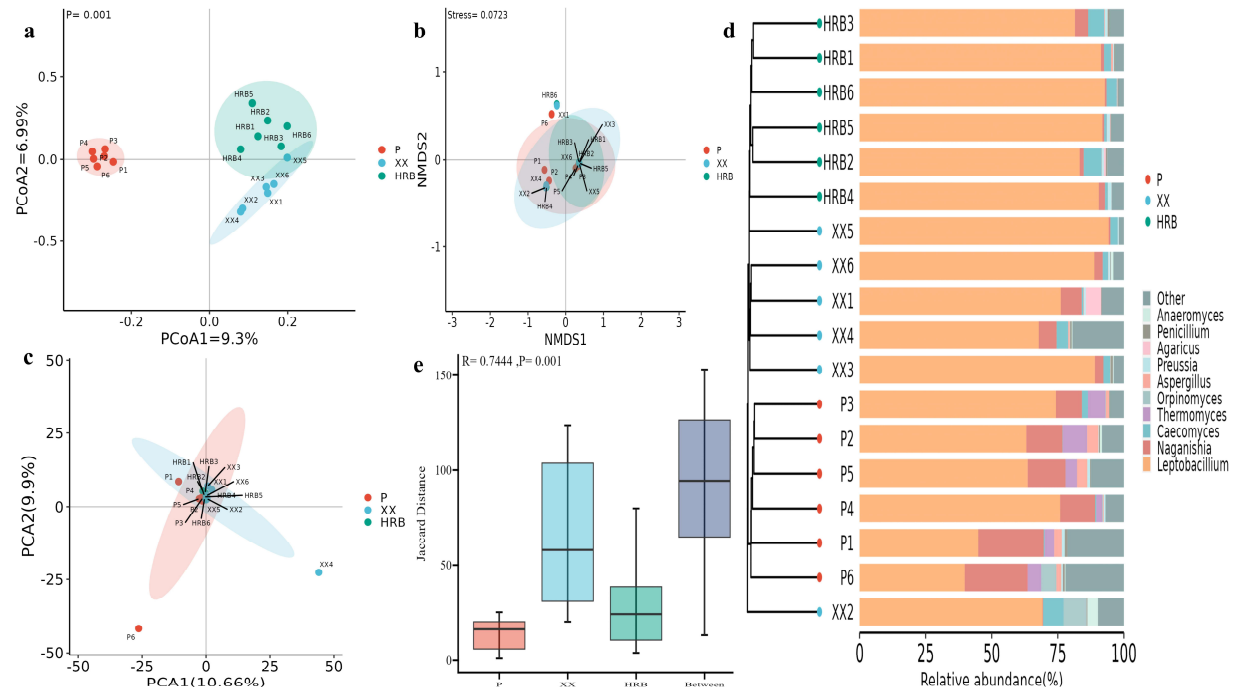


Fig. 4: Beta diversity analysis of sheep in different groups. (a) PCoA, (b) NMDS, (c) PCA, (d) UPGMA, (e) ANOSIM.

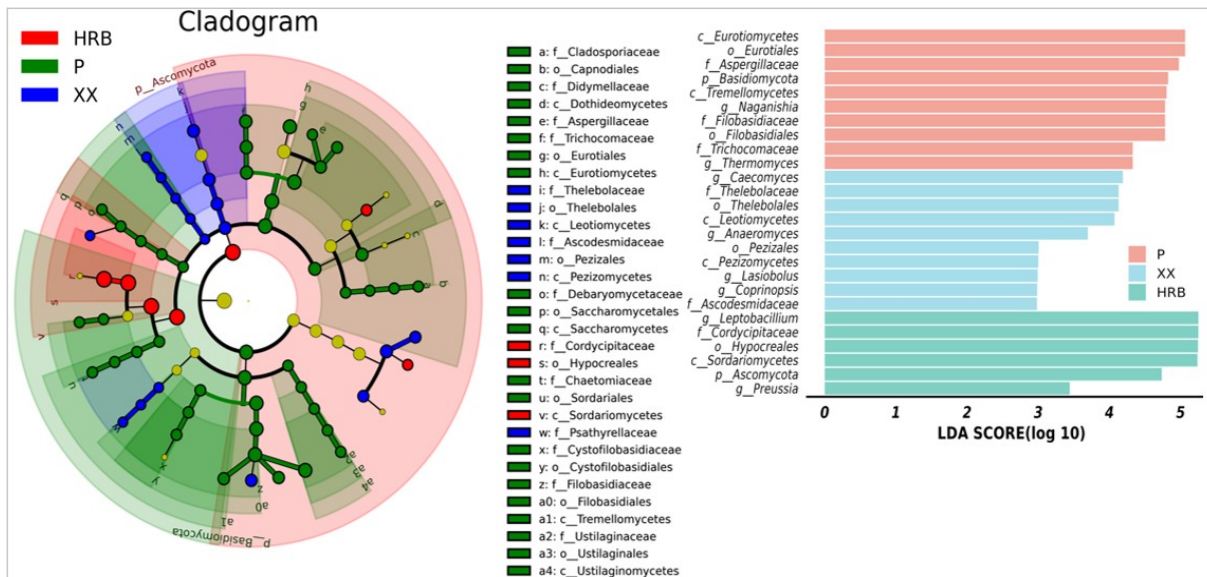


Fig. 5: Differential fungal taxa among sheep groups revealed by LEfSe analysis.

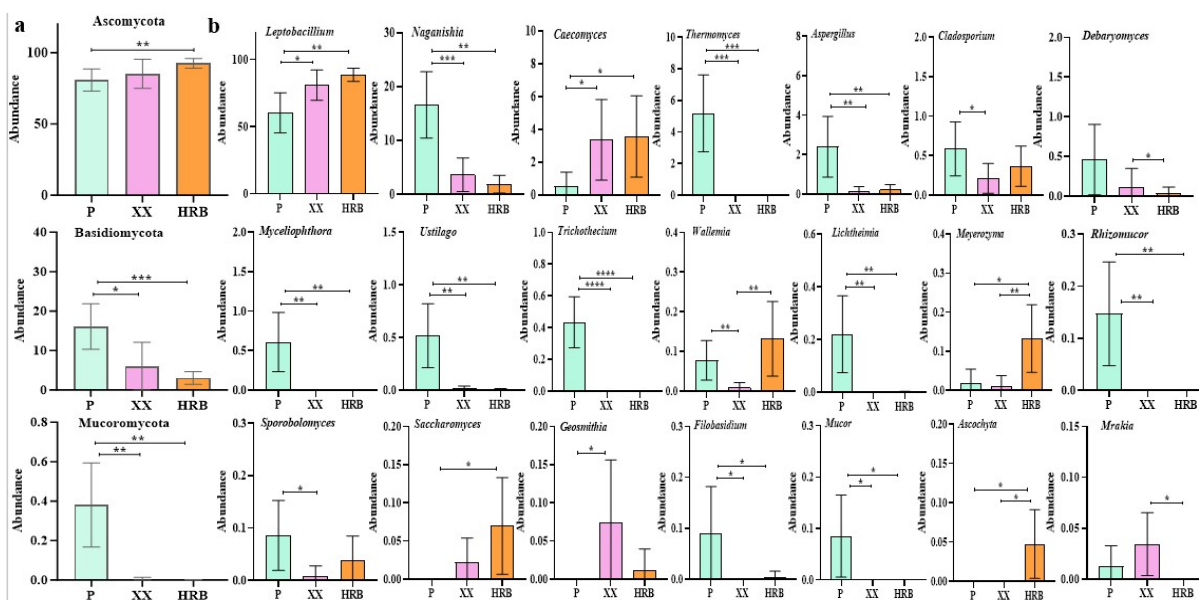


Fig. 6: Differential fungal taxa among sheep groups identified using t-tests. (a) Phylum, (b) Genera. Significance is presented as * $P<0.05$, ** $P<0.01$ and *** $P<0.001$; data are presented as the mean \pm SEM ($n=6$).

In contrast, *Naganishia* ($P<0.001$; $P<0.01$), *Thermomyces* ($P<0.001$; $P<0.001$), *Aspergillus* ($P<0.01$; $P<0.01$), *Ustilago* ($P<0.01$; $P<0.01$), *Trichothecium* ($P<0.0001$; $P<0.0001$), *Lichtheimia* ($P<0.01$; $P<0.01$), *Rhizomucor* ($P<0.01$; $P<0.01$), *Filobasidium* ($P<0.05$; $P<0.05$) and *Mucor* ($P<0.05$; $P<0.05$) in group P were significantly higher than those in groups XX and HRB, respectively. *Cladosporium* ($P<0.05$) and *Sporobolomycetes* ($P<0.05$) were significantly higher in group P compared with group XX, while *Geosmithia* was significantly higher in group XX ($P<0.05$). *Debaryomyces* ($P<0.05$) and *Mrakia* ($P<0.05$) were significantly higher in group XX compared with group HRB.

Wallemia in group XX was significantly lower than that in group P ($P<0.01$) and HRB ($P<0.01$), respectively. *Meyeromyza* ($P<0.05$; $P<0.01$) and *Ascochyta* in group HRB were significantly higher than those in group P and XX, respectively. *Saccharomyces* in group HRB were markedly higher compared with group P ($P<0.05$) (Fig. 6b).

DISCUSSION

Sheep are important food animals, especially in the cold, high-altitude regions of China, where they must cope with chronic hypoxia, low temperatures, and nutrient-limited forage. Additionally, exploring cecal fungal microbiota (mycobiome) provides a valuable window into microbial contributions to plateau adaptability. In this study, we performed ITS1 amplicon sequencing on cecal samples from three representative Tibetan sheep breeds, such as Pengbo sheep, Elephant sheep, and Holba sheep (HRB) to characterize altitude-associated shifts in fungal community structure and function. Microbiome studies are potentially influenced by technical factors such as contamination, batch effects, and sequencing bias, which may affect microbial community composition and diversity estimates. To minimize these effects, all samples in the present study

were collected, processed, and sequenced under standardized laboratory conditions using the same protocols and sequencing platform. Similar approaches have been recommended in previous microbiome studies to reduce technical variability and improve reproducibility (Salter *et al.*, 2014; Kim *et al.*, 2017). Nevertheless, low-biomass contamination, PCR amplification bias, and sequencing-related variation cannot be completely excluded in high-throughput microbiome analyses (Pollock *et al.*, 2018).

A total of 1,347,654 raw and 1,227,756 high-quality filtered sequences were obtained (Table 1). In addition to confirming sufficient sequencing depth via rarefaction curves, alpha-diversity indices (Pielou's evenness, Shannon, and Simpson as $P<0.01$; $P<0.01$ and $P<0.0001$, respectively) declined significantly with increasing altitude (Fig. 1). Comparatively, these findings align with previous observations in high-altitude yaks and Tibetan chicks, where microbial diversity and evenness decreased under harsher plateau conditions (Du *et al.*, 2022; Li *et al.*, 2024; Zhu *et al.*, 2024). Similarly, studies on plateau pikas and other ruminants have reported reduced fungal α -diversity with elevation, potentially reflecting simplified ecological networks and selective pressure favoring resilient taxa in hypoxic, low-temperature environments (Tang *et al.*, 2023; Zhang *et al.*, 2023). In contrast to some bacterial-focused investigations that show stable or increased diversity in certain high-altitude ruminants (e.g., enhanced fibrolytic capacity in Tibetan sheep), our results suggest that the mycobiome may respond more sensitively to altitude gradients, with reduced evenness possibly limiting functional redundancy while enriching specialized fungi.

Beta-diversity analyses further demonstrated clear separation among breeds via PCoA, UPGMA, and ANOSIM ($P<0.01$, Fig. 4), consistent with altitude-driven structural differences reported in Tibetan chicks, yaks, and pikas (Du *et al.*, 2022; Pan *et al.*, 2022). Additionally, taxonomic comparisons at multiple levels (Fig. 2 and Fig.

3) and LEfSe analysis (Fig. 5) confirmed pronounced shifts, particularly at the genus level. The t-test analysis identified three phyla (*Ascomycota*, *Basidiomycota*, *Mucoromycota*) and 21 genera as statistically robust biomarkers (Fig. 6). Comparatively, *Ascomycota* dominated across groups but increased in higher-altitude HRB sheep, mirroring patterns in rumen fungi of Tibetan sheep and yaks, where *Ascomycota* enrichment supports organic matter degradation under nutritional stress (Guo *et al.*, 2025; Wang *et al.*, 2025). In contrast, *Basidiomycota* and *Mucoromycota* were more abundant in lower-altitude P sheep, potentially linked to broader environmental exposure or pasture differences. At the genus level, several biomarkers warrant functional interpretation. Similarly, to prior reports, *Caecomyces* (a *Neocallimastigomycota* member) showed higher abundance in higher-altitude groups; as an anaerobic gut fungus specialized in lignocellulose breakdown, particularly hemicellulose and cellulose fractions, its enrichment may enhance nitrogen metabolism and fiber fermentation efficiency in nutrient-scarce plateau conditions (Brown *et al.*, 2021; Wang *et al.*, 2024).

In addition to this, *Myceliophthora* (plant-beneficial) and *Naganishia* (extreme-environment-tolerant, oleaginous yeast associated with dietary fiber digestion) were differentially abundant. Comparatively, elevated *Naganishia* in certain high-altitude contexts has been noted in Tibetan sheep rumen and wild animals, suggesting adaptation to hypoxia and UV stress (Tang *et al.*, 2023; Wang *et al.*, 2025). Thermophilic cellulose-degraders such as *Thermomyces* were more prominent at lower altitudes, while opportunistic pathogens (*Aspergillus*, *Ustilago*, *Filobasidium*) decreased at higher elevations. Similarly, reduced pathogen loads in harsher altitudes may reflect stronger host-microbe filtering or inhibitory effects of hypoxia on fungal colonization (Last *et al.*, 2021; Cheng *et al.*, 2026). Genera like *Leptobacillium* and *Meyerozyma* appeared largely environmental and less directly tied to altitude adaptation. Differences in *Trichothecium* and *Mucor* (linked to cellulose decomposition) likely stem from variations in available pasture quality across altitudes (Guo *et al.*, 2025; Zhang *et al.*, 2025).

In contrast to most prior studies that emphasize bacterial contributions to energy harvesting in Tibetan livestock, e.g., volatile fatty acid pathways in yaks and sheep (Ren *et al.*, 2023; Wu *et al.*, 2024), this work highlights the understudied mycobiome. Fungal communities, though lower in biomass, excel at initial plant cell wall degradation and cross-kingdom interactions that modulate bacterial activity and host immunity (van Tilburg Bernardes *et al.*, 2020; Pérez, 2021). The observed altitude-associated mycobiome restructuring decreased diversity, coupled with enrichment of fiber- and nitrogen-related taxa, may complement bacterial adaptations, collectively support efficient nutrient extraction, and reduce pathogenic pressure in extreme environments. Limitations include the focus on cecum (vs. rumen) and lack of metagenomic/functional validation; future multi-omics and culture-based studies could validate the biological significance and adaptive roles of identified microbial communities. Future studies with

larger sample sizes and multiple geographic locations are recommended.

Conclusions: In conclusion, this study reveals significant differences in the cecal fungal microbiota among Pengbo semi-fine-wool sheep, Elephant male semi-fine-wool sheep, and Holba sheep inhabiting distinct altitudes on the Qinghai–Xizang Plateau. Additionally, three phyla (*Ascomycota*, *Basidiomycota*, *Mucoromycota*) and 21 genera, including *Caecomyces*, *Myceliophthora*, *Naganishia*, *Leptobacillium*, *Ustilago*, *Filobasidium*, *Mucor*, and *Walleimia*, emerged as robust biomarkers. Comparatively, the decline in alpha diversity and shifts toward specialized degraders at higher altitudes suggest that the mycobiome contributes to high-altitude adaptability by modulating nutrient metabolism and pathogen resistance. Similarly, these findings extend beyond bacterial-centric views and provide novel insights into microbial mechanisms underlying the resilience of plateau ruminants. Our results provide a basis for further investigations into mycobiome-host interactions and potential applications in improving livestock performance under extreme conditions.

Data available statement: All of the raw data from animals were deposited in the NCBI Sequence Read Archive under accession number: PRJNA1447109.

Ethics statement: All the experiment procedures were guided by the instructions and approval of the ethics committee of the College of Animal Science and Technology, Henan Agricultural University (No. 11-00850).

Authors contribution: DZ: research idea and methodology; DZ, LZ, and XC: reagents, materials, and analysis tools; DZ: writing – original draft and preparation; DZ, RA, JW, and MHA: writing – review and editing; DZ: visualization and supervision; All authors approved the final manuscript.

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