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RESEARCH ARTICLE

Characteristics and Differences of Gut Bacterial and Fungal Communities in Chickens with Different Breeds

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ABSTRACT

Tibetan chickens (TBC) and Snowland white chickens (SWC) are distinctive poultry breeds primarily found on the Qinghai-Tibet Plateau, known for their strong adaptability to the plateau environment. The gut microbiota has been recognized to play a crucial role in host health and intestinal function, and its composition is closely associated with the environment. However, there is currently a lack of research on the gut microbiota of TBC and SWC native to Tibet, China. Therefore, this study aimed to investigate the differences in the gut bacterial and fungal compositions and structures in TBC and SWC. Results revealed that 26,627 bacterial OTUs and 1,937 fungal OTUs were identified in SWC and TBC. Moreover, both groups had no distinct differences in the gut bacterial and fungal diversities. Bacterial taxonomic analysis demonstrated a significant increase in the relative richness of 1 phylum and 187 genera in SWC compared to TBC, while the relative richness of 3 phyla and 197 genera exhibited a significant decrease. Fungal taxonomic analysis also showed a substantial increase in the relative richness of 5 phyla and 105 genera in SWC compared to TBC, while the relative richness of 2 phyla and 82 genera exhibited a significant decrease. In summary, this study highlights the notable variations in the gut bacterial and fungal communities between SWC and TBC. The findings are novel and intriguing and contribute to the development of the chicken industry in high-altitude regions and the exploration of Tibetan specialty poultry products.

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INTRODUCTION

Due to the increase in per capita income and population, the livestock industry has experienced significant growth in the past few decades, effectively addressing the shortage of meat products. Among poultry, chicken is the most commonly raised by humans and serves as an important source of animal protein through its meat and eggs (Chen *et al*., 2024; Wu *et al*., 2024). Statistics indicate that China's annual chicken production in 2022 reached 16.608 million tons, accounting for 14.32% of the global chicken production and ranking second worldwide. Furthermore, China possesses a rich variety of chicken breeds, with approximately 100 different local breeds. TBC, as an Indigenous breed of the Qinghai-Tibet Plateau, offers various advantages such as tolerance to rough feeding, strong disease resistance, delicious meat, and highly nutritious eggs (Liu *et al*., 2020). However, the number of TBC is still very limited because of the low reproductive rate and slow growth. SWC is a new breed characterized by rapid growth, strong stress resistance, high feed conversion efficiency, and low feeding cost. TBC and SWC are crucial for the development of the chicken industry in the Qinghai-Tibet Plateau, serving as valuable resources to produce high-quality and distinctive poultry products in Tibet.

The gut microbiota is a highly complex microecological system consisting of 100 trillion microorganisms (Tong *et al*., 2023). Previous research has indicated that the gut microbiota plays a crucial role as the metabolic and immune organ in the host, exerting its influence throughout life (Michaudel and Sokol, 2020). By coexisting with the host, the gut microbiota offers numerous health benefits, such as producing beneficial metabolites and influencing intestinal function (Wang and Zhao, 2018). Previous studies have demonstrated the vital role of the gut microbiota in maintaining intestinal barrier function, nutrient intake, and preventing colonization by pathogenic bacteria (Wang *et al*., 2022a). Furthermore, recent research has highlighted the significant contribution of the gut microbiota in skeletal development, energy supply, and intestinal epithelial differentiation (Zhao *et al*., 2023b). However, the gut microbial composition can be affected by both the external environment and host genetics. Compared with host genetics, the external environment has a greater impact on shaping the gut microbiota. Factors such as antibiotics, exposure to environmental pollutants (heavy metals, pesticides, microplastics), dietary structure, and geographic location have all been shown to significantly alter the gut microbiota and potentially lead to gut microbial dysbiosis (Chen *et al*., 2023; Li *et al*., 2023b). Several gastrointestinal diseases, including inflammatory bowel disease, diarrhea, and bowel cancer, have been associated with gut microbial dysbiosis (Li *et al*., 2019). Furthermore, conditions such as arthritis, ventilation, diabetes, obesity, and mastitis have also been frequently observed alongside gut microbial dysbiosis (Zhao *et al*., 2023a; Chu *et al*., 2025). Notably, the influence of the gut microbiota extends beyond intestinal function, as it also affects the physiology and function of the brain, liver, and kidneys through interactions with the host (Wang *et al*., 2021a).

Metagenomics and bioinformatics tools have been successfully employed to analyze the gut microbial composition and structure in the gastrointestinal tract (Yang *et al*., 2021). By conducting a thorough analysis of the gut microbiota, we can gain insights into the health status of the host and its correlation with host traits. Moreover, recent studies have focused on exploring the variations in gut microbiota among different breeds. For instance, Liu *et al*. (2022a) examined Shouguang chickens and Luqin chickens and observed obvious differences in the composition and structure of their gut microbiota. Similarly, a study comparing Weining chickens and Jinlinghua chickens found notable disparities in the abundance of beneficial bacteria between the two breeds (Yan *et al*., 2021). However, there is currently a lack of research investigating the relationship between the gut microbiota of TBC and SWC. Therefore, our objective was to conduct a comparative analysis of the gut microbial composition between TBC and SWC.

MATERIALS AND METHODS

Sample acquisition: Samples were collected from a standardized breeding farm in Lhasa, China, located approximately 3500 meters above sea level. Fecal samples were obtained from 10 healthy TBC and 10 healthy SWC. Before sampling, all selected subjects had not been administered any antibiotics or other medications. Furthermore, the chickens' health status was assessed to minimize the influence of other diseases on the gut microbiota. The chickens were housed in the same environment and provided with a sufficient diet and water supply. Before each sample, the equipment was disinfected with 75% ethanol. Additionally, individual sampling of each selected chicken was conducted to prevent cross-contamination. The samples were in a -80°C ultra-low temperature refrigerator until DNA extraction.

Analysis of gut microbiota: The specific steps of amplicon sequencing, bioinformatics, and statistical analysis were referred to previous studies (Ning *et al*., 2020; Liu *et al*., 2021).

RESULTS

Sequence data and OTUs analysis: Results indicated that the TBC and SWC generated 1,552,520 (TBC=770,093, SWC=782,427) original bacterial sequences, in the range of 79,726 to 80,339 sequences per sample (Table 1). Moreover, we also collected 1,599,304 (TBC=799,250, SWC=800,054) raw fungal sequences from 20 samples in TBC and SWC, varying from 69,850 to 80,278 sequences per sample (Table 2). After sequence filtering, a total of 1,216,066 (TBC=637,473, SWC=578,593) valid bacterial sequences and 1,205,700 (TBC=606,482, SWC=599,218) valid fungal sequences were achieved, resulting in an effective rate of approximately 78.32% and 75.39%, respectively. Based on 97% sequence similarity, the valid sequences obtained from TBC and SWC were clustered into 26,627 (TBC=11,396, SWC=16,521, ranging from 266 to 4,984 OTUs per sample) bacterial OTUs and 1,937 (TBC=996, SWC=1,024, ranging from 68 to 271 OTUs per sample) fungal OTUs (Fig. 1A, B, C, G, H, I). Among these OTUs, the number of individual bacterial OTUs was 10,106 and 15,231, while the individual fungal OTUs were 913 and 941 in the TBC and SWC, respectively. Additionally, there were 1,290 bacterial OTUs and 83 fungal OTUs shared between the TBC and SWC, making up approximately 4.84% and 4.28% of the total OTUs, respectively. We also observed that the rarefaction curves gradually approach saturation when the number of effective sequences is over 20,000 (Fig. 1D, E, J, K). In this microbiome investigation, the quantity of effective bacterial sequences and fungal sequences for each sample exceeded 40,000, indicating that the sequencing depth was sufficient. Moreover, the results of the rank abundance curves showed that the compositions of gut bacterial and fungal communities were relatively homogeneous (Fig. 1F, L).

Comparison of gut bacterial and fungal diversities between the TBC and SWC: The bacterial Chao1 and ACE indices in TBC were 1245.20 and 1249.47, while these indices in SWC were 1806.51 and 1812.46, respectively (Fig. 2A, B). Moreover, the average gut bacterial Shannon index in the TBC and SWC was 5.73 and 7.26, whereas the gut bacterial Simpson index was 0.86 and 0.95, respectively (Fig. 2C, D). Inter-group analysis of the above-mentioned four indices intuitively showed that there was no difference in the gut bacterial diversity and abundance between TBC and SWC. Similarly, there were no noticeable differences in the Chao1 (159.50±30.31 versus 112.7±11.53, P=0.18), ACE (159.73±30.31 versus 112.83±11.55, P=0.17), Simpson

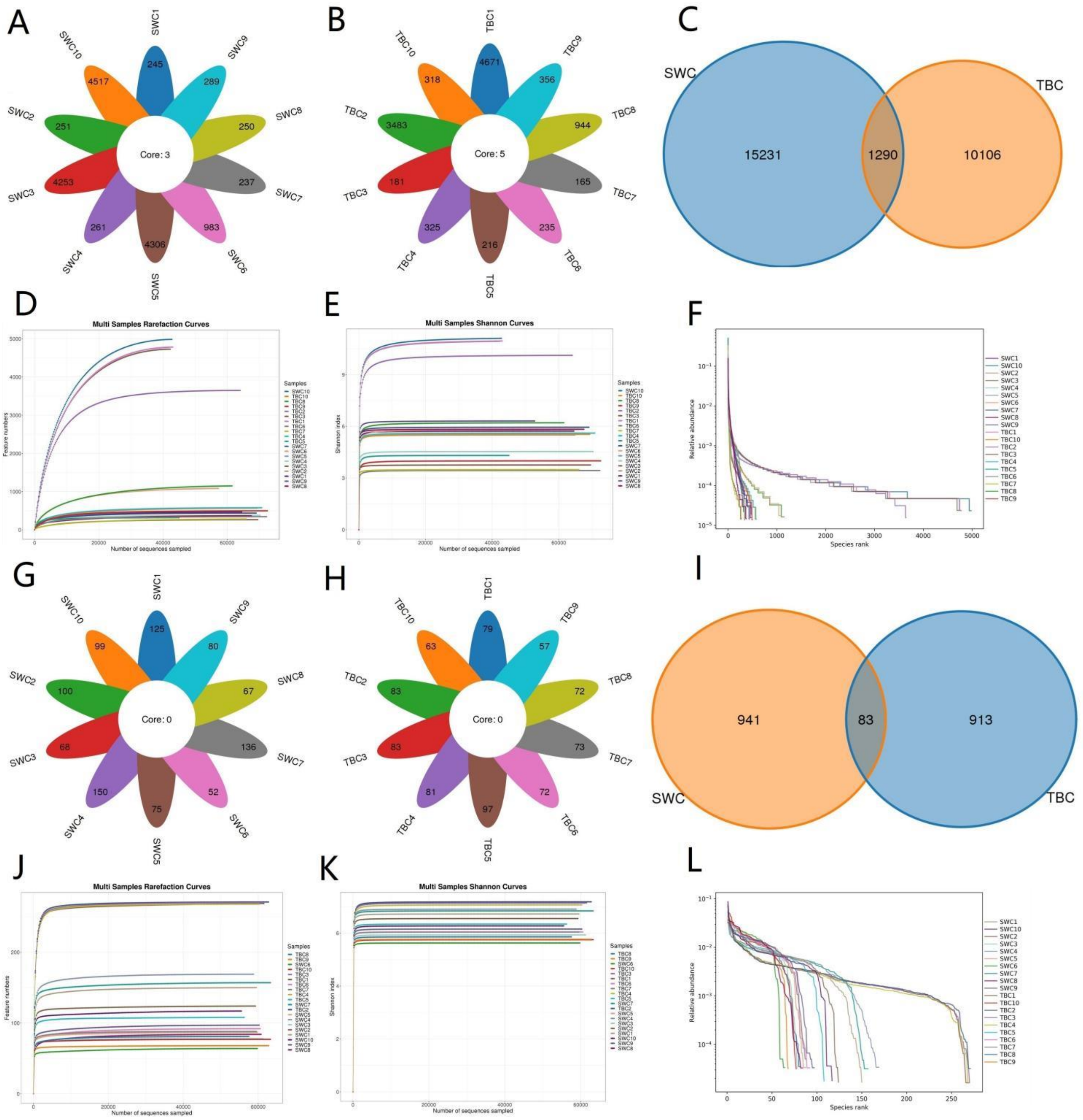

Fig. 1: Analysis of OTUs quantity and sequencing depth. The Venn diagram shows the quantity of bacterial (A, B, C) or fungal (G, H, I) OTUs in the TBC and SWC. The depth and evenness of sequencing are represented by rarefaction curves (D, E: bacterial rarefaction curves; J, K: fungal rarefaction curves) and rank abundance curves (F: bacterial rank abundance curves, L: fungal rank abundance curves). Each curve or polyline in the rarefaction curve and rank abundance curve represents a sample.

(0.98±0.001 versus 0.98±0.0014, P=0.47) and Shannon (6.43±0.19 versus 6.28±0.14, P=0.54) indices for fungi between TBC and SWC, indicating no significant differences in gut fungal diversity and abundance between both groups (Fig. 2G, H, I, J). PCoA analysis results showed that the points representing gut bacterial and fungal communities clustered together, suggesting that there were no significant differences in the gut microbial structures between the TBC and SWC (Fig. 2E, F, K, L).

Comparison of gut bacterial community composition between the TBC and SWC: There were 51 phyla and 1708 genera identified from TBC and SWC, ranging from 19 to 47 phyla and 129 to 894 genera per sample (Table 3). Among them, the Firmicutes (69.40%, 53.07%), Bacteroidota (7.65%, 18.35%), Proteobacteria (11.87%, 12.15%) and Actinobacteriota (2.42%, 3.99%) were the most prevalent bacterial phyla in the TBC and SWC, accounting for approximately 90.00% of the total taxonomic groups identified (Fig. 3A). However, the proportions of unclassified_Bacteria (1.22%,1.33%), Chloroflexi (0.88%, 0.94%), Acidobacteriota (0.87%, 0.80%), Patescibacteria (0.53%, 1.00%) and Desulfobacterota (0.37%, 0.84%) were lower in the gut bacterial community of TBC and SWC. The preponderant bacterial genera observed in the TBC were *Romboutsia* (22.72%), *Lactobacillus* (17.25%) and *Ligilactobacillus* (10.73%), whereas *Lactobacillus* (15.05%), *Bacteroides*

Metastats analysis was used to determine the differential taxa between the TBC and SWC (Fig. 4A, B). At the phylum level, the abundances of Euryarchaeota, Thermoplasmatota and Dadabacteria in TBC were distinctly predominant than SWC, whereas Bacteroidota was lower. Additionally, a comparison of the gut bacterial community of TBC and SWC showed significant decrease in the abundances of 187 genus (*Abiotrophia*, *Actibacter*, *Costertonia*, *Crocinitomix*, *Defluviicoccus*, *Delftia*, *Elusimicrobium*, *Erysipelotrichaceae_UCG_009*, *Fontimonas*, *Gemmatirosa*, *Halothiobacillus*, *Ideonella*, *Lachnobacterium*, *Lachnospiraceae_FE2018_group*, *Lachnospiraceae_NC2004_group*,*Lachnospiraceae_UCG_010*, *Lawsonia*, *Micropepsis*, *Nannocystis*, *Nitratireductor*, *Pantalinema*, *Pelagibius*, *Polymorphobacter*, *Tepidimicrobium*, *Terrabacter*, *Thiogranum*, *Xanthobacter*, *Aurantimicrobium*, *Crenobacter*, *uncultured_Lactococcus*, *Thiorhodococcus*, *Enterorhabdus*, etc.) as well as an obvious increase in the richness of 197 genus (*Chryseomicrobium*, *Citrifermentans*, *Citrobacter*, *Clostridium_sensu_stricto_11*, *Cohnella*, *Gelidibacter*, *Georgenia*, *Globicatella*, *Gluconobacter*, *Hathewaya*, *Herminiimonas*, *Hymenobacter*, *Idiomarina*, *Isosphaera*, *Lachnospiraceae_UCG_007*, *Lacibacter*, *Lactivibrio*, *Leucobacter*, *Longispora*, *Lysinibacillus*, *Mariniradius*, *Methanobrevibacter*, *Methylobacter*, *Methylocaldum*, *Rhodococcus*, *Salinispora*, *Sinomonas*, *Sporosarcina*,

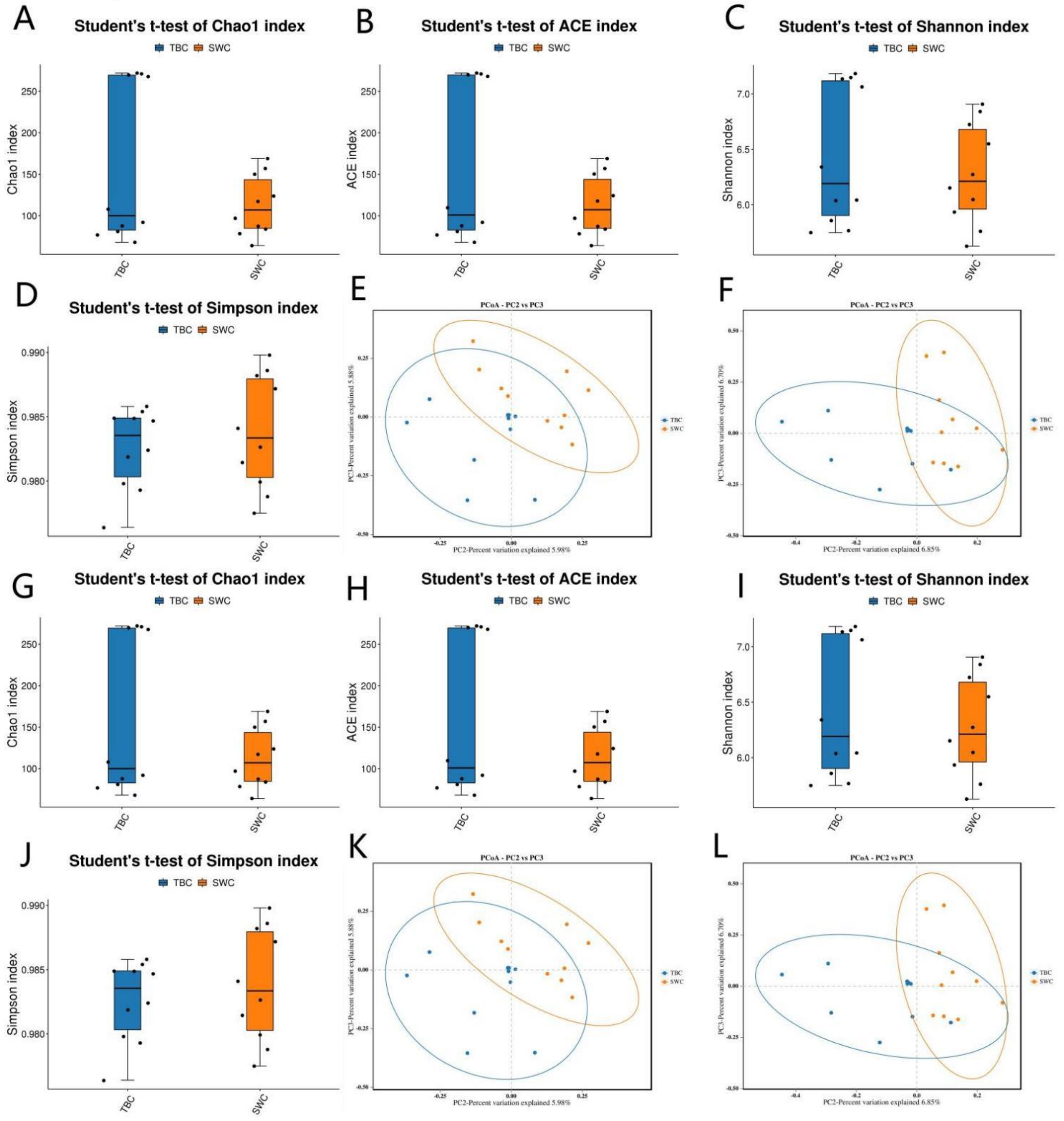

Fig. 2: Difference analysis of gut bacterial and fungal diversities between TBC and SWC. Chao1, ACE, Simpson, and Shannon were used to assess differences in alpha diversity of gut bacterial (A, B, C, D) and fungal (G, H, I, J) communities. PCoA scatter plots were employed to analyze the variations in gut bacterial (E, F) and fungal (K, L) structures between the TBC and SWC.

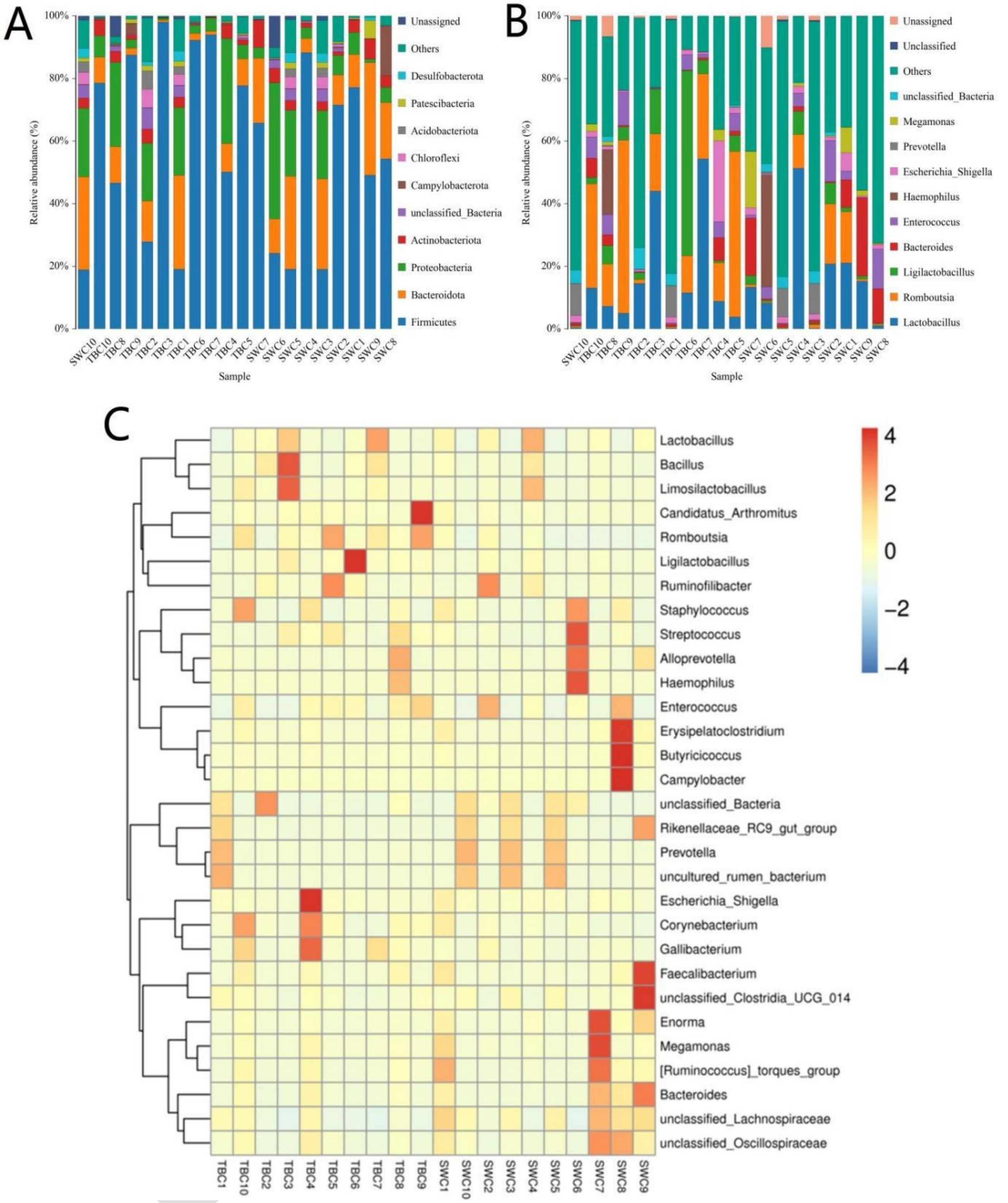

Fig. 3: Compositional analysis of gut bacterial community in TBC and SWC. Types and relative proportions of the 10 bacterial phyla (A) and genera (B) with the highest abundance of gut microbiota. Clustering heat map of bacterial (C) genera in different samples. Each row represents a bacterial or fungal genus, and the rectangular areas of different colors indicate its relative abundance. Darker colors indicate greater abundance.

Verticiella, *Alishewanella*, *Azovibrio*, *Clostridium_sensu_stricto_7*, *Ercella*, *Pseudochrobactrum*, *Robinsoniella*, *Saccharopolyspora*, etc.). To further identify differential taxa between TBC and SWC, we also performed LEfSe analysis. We also observed that *Halomonas*, *Proteiniclasticum* (7.27%), and *Romboutsia* (5.70%) were enriched in the SWC (Fig. 3B). Moreover, the clustering heatmaps further revealed the changes in bacterial types and abundance in each sample of the TBC and SWC (Fig. 3C).

Proteiniphilum and *Romboutsia* in the TBC were significantly preponderant than SWC, whereas the *unclassified_Lachnospiraceae*,*unclassified_Ruminococcaceae*, *unclassified_Muribaculaceae*, and *unclassified_Eubacterium_coprostanoligenes_group* were lower (Fig. 5A, B).

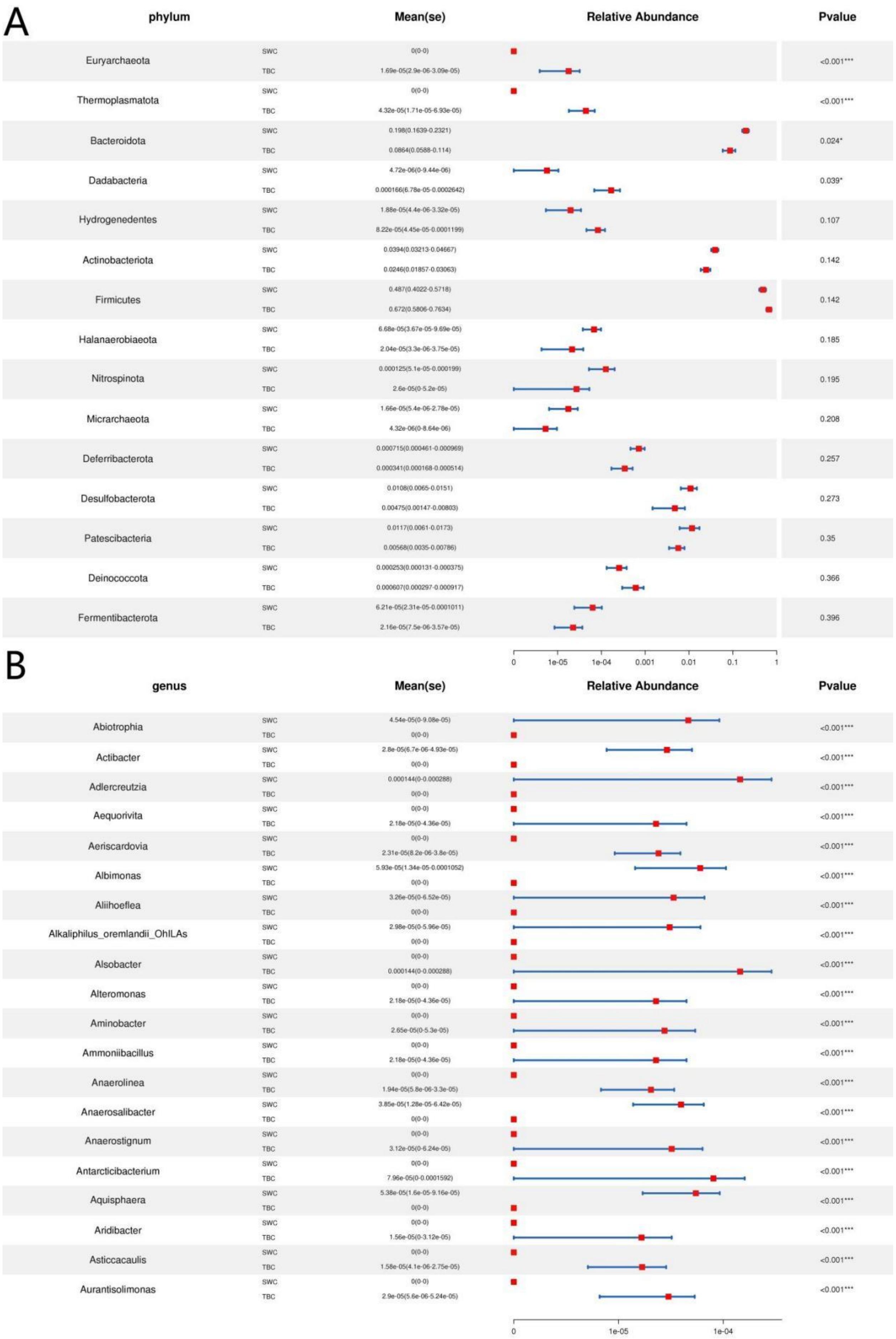

Fig. 4: Taxonomic composition and relative variations of gut bacterial community between TBC and SWC. Data were not fully shown.

Table 1: Basic bacterial information statistics of amplicon sequencing.

Sample	Raw Reads	Clean Reads	Denoised Reads	Merged Reads	Effective Reads	Effective (%)
TBC1	80004	74450	73131	56049	44523	55.65
TBC2	80038	74327	73805	69223	64394	80.45
TBC3	79712	73473	73415	73034	69571	87.27
TBC4	80063	74351	74222	73364	70835	88.47
TBC5	49993	46201	46157	45974	45264	90.54
TBC6	80024	73530	73493	73255	72555	90.66
TBC7	80200	73803	73759	73205	66265	82.62
TBC8	79897	73240	72947	67899	61987	77.58
TBC9	79954	74003	73930	73449	72556	90.74
TBC10	80208	74631	74415	72857	69523	86.67
SWC1	80143	74476	74324	71828	64802	80.85
SWC2	72810	66763	66682	66460	64813	89.01
SWC3	79981	74339	72902	54808	43837	54.80
SWC4	79290	73219	73196	72914	70486	88.89
SWC5	80112	75272	73913	55526	43590	54.41
SWC6	80058	73680	73473	66493	57542	71.87
SWC7	80134	74225	74146	73148	69143	86.28
SWC8	80278	74527	74275	71861	67624	84.23
SWC9	79771	73922	73669	67385	52979	66.41
SWC10	69850	64317	63256	52459	43777	62.67

Table 2: Basic fungal information statistics of amplicon sequencing.

Sample	Raw Reads	Clean Reads	Denoised Reads	Merged Reads	Effective Reads	Effective (%)
TBC1	79847	65538	65389	64755	61704	77.27
TBC2	79968	66719	66668	66236	62754	78.47
TBC3	80050	65990	65940	65334	61188	76.43
TBC4	79781	66214	66128	65520	60496	75.82
TBC5	79974	62928	62926	62749	56278	70.37
TBC6	79892	65837	65831	65513	60549	75.78
TBC7	79726	63206	63185	63138	59557	74.70
TBC8	80161	64988	64979	64708	57738	72.02
TBC9	79978	67097	67077	67006	62814	78.53
TBC10	79873	67200	67200	67192	63404	79.38
SWC1	80175	66223	66210	65724	59666	74.41
SWC2	80067	64074	64057	63567	59483	74.29
SWC3	79855	64072	64071	63794	61376	76.85
SWC4	80074	64081	64047	63828	58892	73.54
SWC5	80002	65442	65401	65310	59803	74.75
SWC6	79986	65445	65444	65382	59979	74.98
SWC7	79828	67885	67882	67565	63305	79.30
SWC8	79902	67197	67192	66532	60887	76.20
SWC9	80339	64455	64448	64291	60305	75.06
SWC10	79826	64754	64750	64347	55522	69.55

Table 3: Statistical table of bacterial species at different taxonomic levels in TBC and SWC.

Sample	Phylum	Class	Order	Family	Genus
TBC1	40	95	255	456	824
TBC2	47	111	283	519	894
TBC3	22	36	75	116	158
TBC4	28	42	92	162	245
TBC5	26	45	87	142	187
TBC6	29	45	99	153	196
TBC7	25	39	83	121	168
TBC8	28	50	101	170	255
TBC9	23	41	90	162	249
TBC10	31	46	93	162	262
SWC1	19	30	63	116	182
SWC2	25	45	95	146	204
SWC3	42	95	243	467	849
SWC4	25	47	99	148	204
SWC5	45	104	264	472	849
SWC6	28	52	111	183	268
SWC7	26	41	84	139	206
SWC8	26	40	71	116	172
SWC9	16	22	44	76	129
SWC10	41	101	263	472	855
Total	51	133	376	790	1708

Comparative analysis of gut fungal community composition between the TBC and SWC: We also comparatively analyzed the composition of the gut fungal community at different taxonomic levels and observed significant changes between the TBC and SWC. At the phylum level, 51 fungal phyla were identified from the TBC and SWC, ranging from 16 to 47 phyla per sample (Table 4). Among identified fungal phyla, Ascomycota (60.55%, 60.68%), Basidiomycota (22.66%, 17.28%), unclassified_Fungi (6.72%, 11.07%), Mortierellomycota (3.44%, 5.33%) and Chytridiomycota (5.21%, 3.01%) were abundantly present in the fungal community of TBC and SWC, accounting for over 1% of total sequences on average (Fig. 6A). However, other fungal phyla such as Glomeromycota (0.72%, 1.09%), Rozellomycota (0.39%, 0.87%), Olpidiomycota (0.28%, 0.12%), Kickxellomycota (0.00%, 0.32%) and Mucoromycota (0.00%, 0.11%) in both groups were detected in low abundances. In addition, there were 325 fungal genera identified from 20 samples in TBC and SWC, ranging from 39 to 125 fungal genera per sample. Specifically, the preponderant fungal genera found in TBC were *Fusarium* (4.49%), *Mortierella* (3.41%), *unclassified_Sordariomycetes* (3.33%) and *Aspergillus* (3.00%) in descending order (Fig. 6B). Moreover, *unclassified_Ascomycota* (6.41%) was the most preponderant fungal genera in the SWC, followed by *Mortierella* (5.29%), *Fusarium* (5.12%) and *unclassified_Basidiomycota* (4.38%). Moreover, changes in the distribution and abundance of more fungal genera between the TBC and SWC can also be visualized via cluster heatmaps (Fig. 6C).

Table 4: Statistical table of fungal species at different taxonomic levels in TBC and SWC.

Sample	Phylum	Class	Order	Family	Genus
TBC1	7	21	48	95	125
TBC2	7	21	49	95	125
TBC3	7	21	48	94	125
TBC4	7	21	49	94	124
TBC5	7	20	31	47	57
TBC6	6	14	26	39	55
TBC7	5	16	29	44	56
TBC8	5	16	31	45	54
TBC9	6	20	28	38	39
TBC10	6	16	28	38	52
SWC1	8	24	42	56	69
SWC2	8	23	37	52	64
SWC3	6	17	26	36	43
SWC4	9	19	37	61	78
SWC5	7	15	29	36	46
SWC6	8	17	29	36	38
SWC7	9	24	38	58	77
SWC8	7	17	27	36	46
SWC9	7	14	26	34	42
SWC10	7	20	32	47	57
Total	12	41	87	178	325

Metastats analysis revealed differential taxa at different taxonomic levels between TBC and SWC (Fig. 7A, B). At the phyla level, TBC enriched for *Chytridiomycota* and *Basidiomycota*, whereas the SWC showed dramatically higher abundances of *Calcarisporiellomycota*, *Kickxellomycota*, *Mucoromycota*, *Neocallimastigomycota*, and *unclassified_Fungi*. Moreover, we also observed that 187 genera exhibited significant differences between the TBC and SWC. Compared with the TBC, the relative abundances of 105 genera (*Epicoccum*, *Epicoleosporium*, *Fellomyces*, *Funneliformis*, *Geoglossum*, *Geosmithia*, *Goffeauzyma*, *Hymenoscyphus*, *Kernia*, *Knufia*, *Kockovaella*, *Kodamaea*, *Kotlabaea*, *Laetisaria*, *Lasionectria*, *Lectera*, *Lepiota*, *Madurella*, *Septoglomus*, *Sodiomyces*, *Sporidiobolus*, *Talaromyces*, *Thelebolus*, *Thermoascus*, *Thyrostroma*, *Tomentellopsis*, *Trichothecium*, *Uwebraunia*, *Verrucocladosporium*, *Wardomyces*, *Xenomyrothecium*, *Zygosaccharomyces*, etc.) observably increased, while the relative abundances of 82 genera (*Acephala*, *Achaetomium*, *Agaricus*, *Aleurodiscus*, *Amphinema*, *Aporospora*, *Arachnomyces*, *Arnium*, *Arthrocladium*, *Athelia*, *Atractospora*, *Ceratobasidium*, *Cetraspora*, *Chaetomidium*, *Chaetosphaeria*, *Clavaria*, *Clavispora*, *Clonostachys*, *Comoclathris*, *Cordyceps*, *Corynascella*, *Cryptomarasmius*, *Cuphophyllus*, *Cutaneotrichosporon*, *Erysiphe*, *Gamsia*, *Hansfordia*, *Hasegawazyma*, *Myxozyma*, etc.) significantly decreased in SWC. LEfSe analysis was used to further identify differential fungi between TBC and SWC. Results indicated that *Cercospora*, *Sterigmatomyces*, *Alternaria*, *unclassified_Sordariomycetes*, and *Rhodotorula* in the TBC were dramatically preponderant than SWC, while the *unclassified_Ascomycota*, *Mortierella*, and *Podospora* were lower (Fig. 5C, D).

Correlation network analysis: *unclassified_Lachnospiraceae* was positively associated with *Bacteroides* (0.78) **(Fig. 8A)**. *Sterigmatomyces* was positively associated with *Rhodotorula* (0.99), *Pseudallescheria* (0.89), *Acrophialophora* (0.78), *unclassified_Xylariales* (0.69), *unclassified_Hypocreales* (0.63), *Botryotrichum* (0.62), *Wallemia* (0.59), *Russula* (0.49), *Truncatella* (0.48) and *Saitozyma* (0.46), but negatively correlated to *Malassezia* (0.53). *Rhodotorula* was positively related to *Botryotrichum* (0.62). *Alternaria* was negatively associated with *unclassified_Fungi* (0.57) **(Fig. 8B)**.

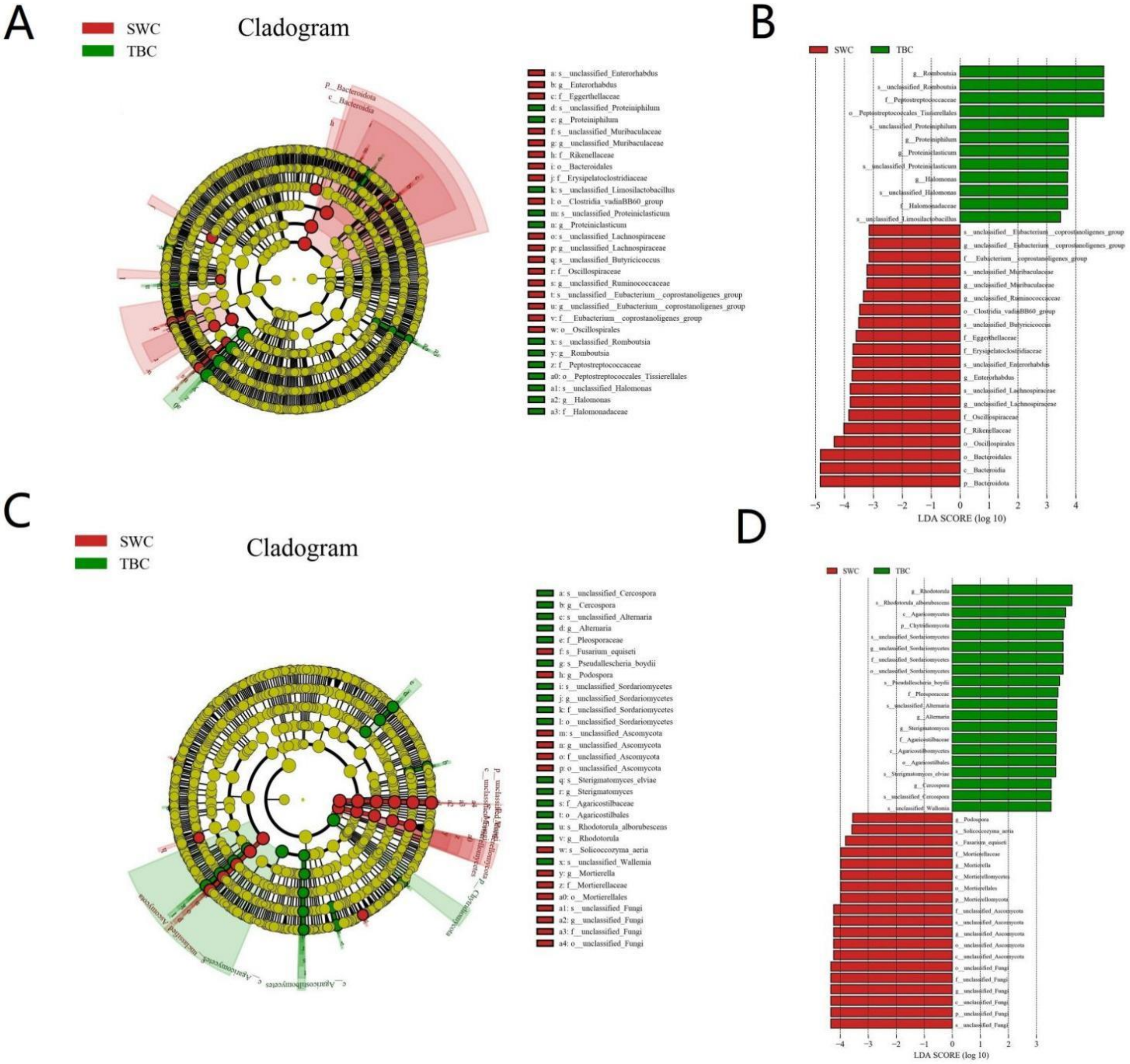

Fig. 5: Gut bacterial (A) and fungal (C) taxa that differed at different taxonomic levels in the TBC and SWC were visualized using cladograms. Significantly abundant bacteria (B) or fungi (D) in the SWC and TBC were represented by negative LDA scores and positive LDA scores, respectively. Only taxa with LDA>3 can be displayed.

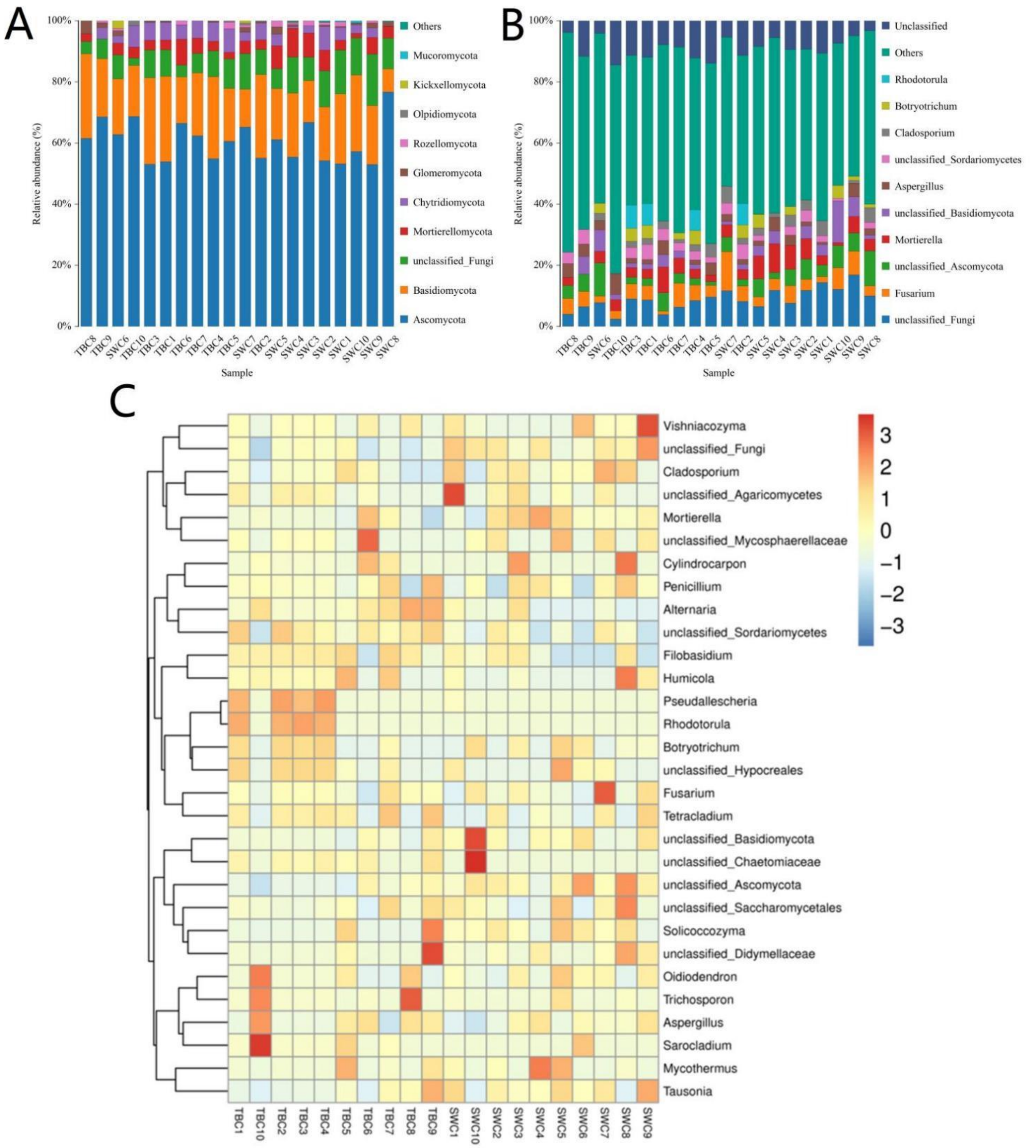

Fig. 6: Compositional analysis of gut fungal community in TBC and SWC. Types and relative proportions of the 10 fungal phyla (A) and genera (B) with the highest abundance of gut microbiota. Clustering heat map of fungal (C) genera in different samples. Each row represents a bacterial or fungal genus, and the rectangular areas of different colors indicate its relative abundance. Darker colors indicate greater abundance.

DISCUSSION

China possesses a rich variety of chicken breeds, with local breeds being widely distributed across the country. Two typical local breeds, TBC and SWC, are predominantly found in Tibet, China (Zhang *et al*., 2017). The gut microbiota has been demonstrated to have multiple beneficial functions and plays a crucial role in maintaining the host's health and traits (Guo *et al*., 2022). Growing evidence suggests that the gut microbiota is specific to each species and closely linked to the host's genetics. For instance, Ma *et al*. (2022) discovered significant differences in the gut microbial composition and structure among Duroc, Landrace, and Yorkshire pigs. Similarly, Li *et al*. (2022b) examined two breeds of cats and observed that the diversity and richness of the gut microbiota in Felinae Cats were significantly higher compared to Ragdoll Cats. These findings underscore the substantial influence of genetic background on the gut microbiota. However, to date, no relevant studies have explored the differences in gut microbiota between Chinese native TBC and SWC. Therefore, this study aimed to address this gap by collecting fecal samples from TBC and SWC for amplicon sequencing. The results

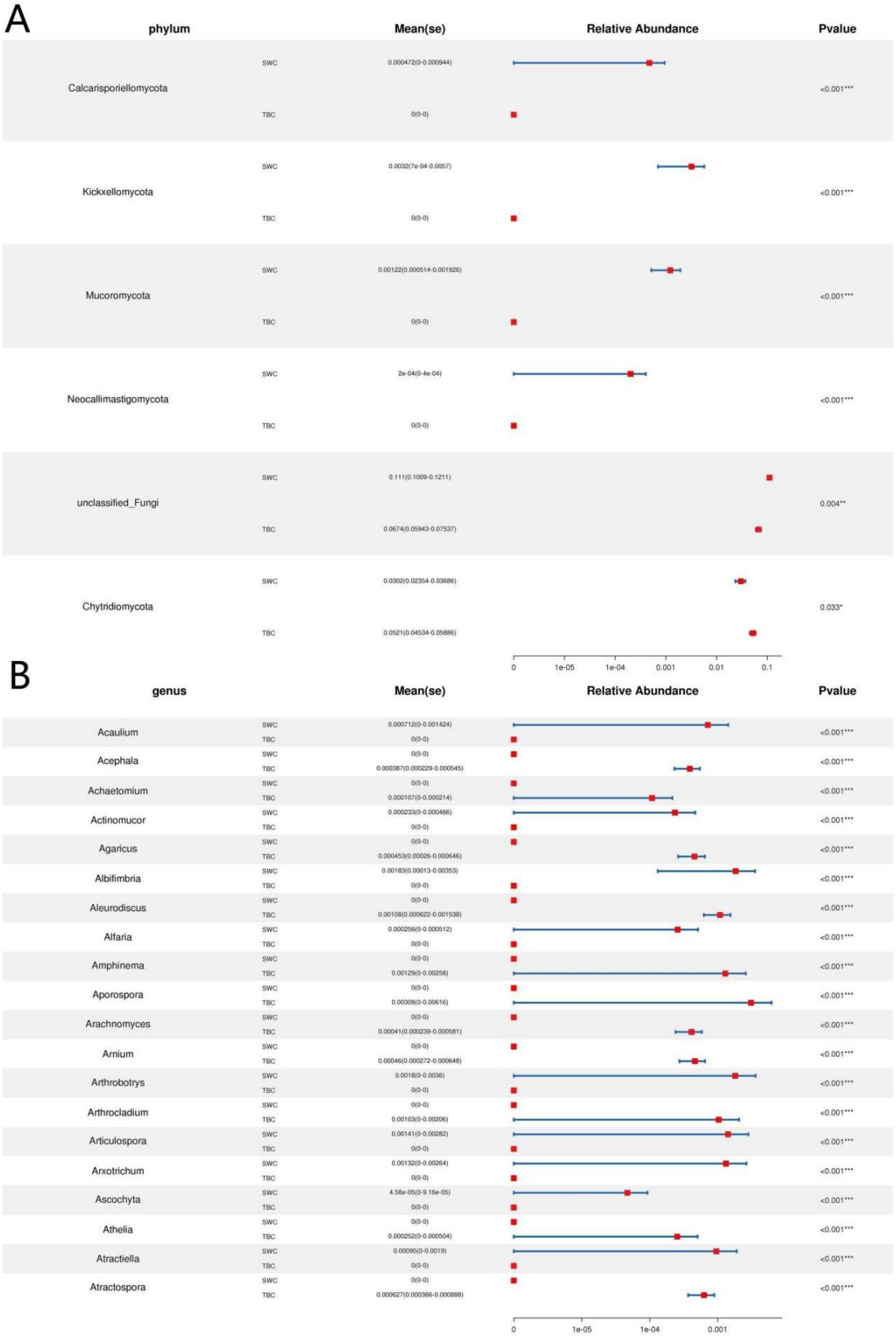

Fig. 7: Taxonomic composition and relative variations of gut fungal community between TBC and SWC. Data were not fully shown.

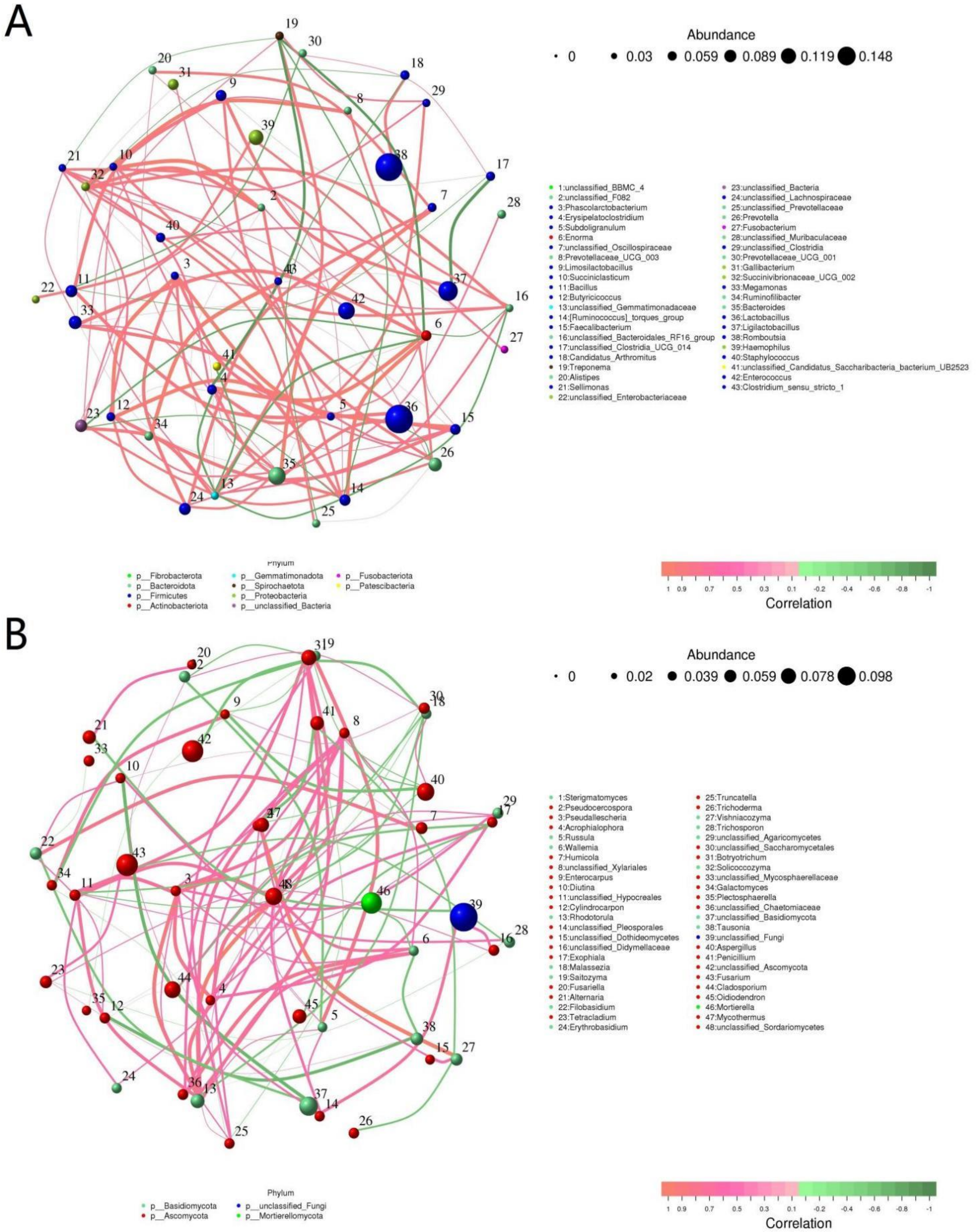

Fig. 8: Co-occurrence networks constructed to reveal correlations among gut bacterial (A) or fungal (B) communities. The color of the node indicates the gut bacterial or fungal genus. Edge color represents positive (red) or negative (green) correlations.

revealed significant disparities in the composition of the gut bacterial and fungal communities between the two chicken breeds.

In this study, we found that the core bacterial phyla and fungal phyla were the same in TBC and SWC, suggesting that the genetic background did not have a significant impact on the core microbial communities. These core bacterial and fungal phyla have also been observed in previous studies on cattle, pigs, and sheep, highlighting their importance in the animal intestinal ecosystem (Wang *et al*., 2022b; Zhao *et al*., 2023c). Firmicutes, which is the most prevalent bacterial phylum in the gut bacterial community, plays a crucial role in breaking down carbohydrates and facilitating nutrient absorption (Gavande *et al.,* 2021). Bacteroidetes can break down complex polysaccharides and maintain intestinal homeostasis (McKee *et al.,* 2021). Both major bacterial phyla are closely involved in the breakdown and metabolism of fibrous and non-fibrous components of the host's diet. Additionally, we also found that Proteobacteria constituted a significant proportion of the gut bacterial community in TBC and SWC. Research has shown that members of Proteobacteria are key bacteria in digesting soluble carbohydrates and are also involved in host biofilm formation and gastric content fermentation (Zhou *et al.,* 2020). The higher abundances of Firmicutes and Proteobacteria indicate that TBC and SWC possess stronger capabilities for food decomposition and digestion.

Notably, we also observed significant differences in bacterial and fungal communities between TBC and SWC. Additionally, some of these differential taxa, such as *Halomonas*, *Lachnospiraceae_FE2018_group*, *Lachnospiraceae_NC2004_groupLachnospiraceae_UCG_007*, *Lachnospiraceae_UCG_010*, *uncultured_Lactococcus*, *Rhodococcus*, *Romboutsia*, *Enterorhabdus*, *Pseudochrobactrum*, *unclassified_Ruminococcaceae*, and *Lachnobacterium* are considered potential beneficial bacteria. Previous research has indicated that significant changes in specific bacteria or fungi can indicate potential connections between gut microbiota and host phenotype (Wang *et al*., 2018; Liu *et al*., 2022b). Therefore, these differential taxa may play important roles in the intestinal ecosystems and their function. Previous studies have demonstrated that *Halomonas* is capable of secreting α-amylase and exhibits significant potential in the degradation of organic compounds such as phenol and catechol (Lin *et al*., 2021). *Lachnospiraceae* have been identified as potential producers of short-chain fatty acids and are inversely related to intestinal inflammation (Konturek *et al*., 2020). *Lactococcus* has long been recognized as a beneficial intestinal bacterium due to its positive influence on host growth performance, immune system, and antioxidant capacity (Li *et al*., 2023a). Moreover, *Lactococcus* has been found to maintain gut microbial homeostasis and improve the intestinal environment by inhibiting the growth of pathogenic bacteria and producing antimicrobial peptides (Chen *et al*., 2021). In addition to these advantageous properties, *Lactococcus* also demonstrates promising potential in enhancing intestinal barrier function and alleviating symptoms of diarrhea and inflammation (Wang *et al*., 2019). As a recognized beneficial intestinal bacterium, *Ruminococcaceae* has demonstrated the ability to break down cellulose and starch (Opdahl *et al*., 2018). Furthermore, *Ruminococcaceae* has been found to positively regulate the host intestinal environment and immune system. Recent research on *Ruminococcaceae* has shown a reverse correlation between its abundance and increased intestinal permeability, cirrhosis, and non-alcoholic fatty liver disease (Milton-Laskibar *et al*., 2022). *Lachnobacterium*, an obligate anaerobic bacterium, can ferment glucose to produce acetic acid, butyric acid, and lactic acid (Wang *et al*., 2021b). Additionally, *Lachnobacterium* is inversely related to age, and its reduced content can have negative effects on host health. Interestingly, studies involving *Lachnobacterium* have also revealed a significant reduction in its abundance among patients with asthma and atopic dermatitis (Galazzo *et al*., 2020). Previous studies have demonstrated an inverse correlation between *Enterorhabdus* and pro-inflammatory cytokine levels (Pang *et al*., 2021). Moreover, there is compelling evidence indicating a significant reduction in *Enterorhabdus* levels during colitis (Cheng *et al*., 2023). *Rhodococcus* possesses properties that allow it to degrade organic matter and may be beneficial in mitigating the toxic effects of metal exposure (Nazari *et al*., 2022). *Pseudochrobactrum* has been demonstrated to reduce Cr (VI) and contribute to the bioremediation of chromium pollution (Li *et al*., 2022a). *Romboutsia* has been reported to play a role in carbohydrate and amino acid metabolism. Considering the significant attributes of these microorganisms, we hypothesized that they play a crucial role in maintaining gut microbial balance and ensuring proper intestinal function. Although there is a wide variety of differential microbial taxa found in TBC and SWC, these differential microorganisms all contribute to achieving gut microbial homeostasis and enhancing the diversity of intestinal functions. Notably, we also observed the presence of certain bacteria or fungi exclusively in TBC or SWC. We speculate that these shared bacterial or fungal communities serve common intestinal functions, while the unique bacterial or fungal communities are responsible for specific functions.

Conclusions: In summary, this study compared the gut bacterial and fungal compositions and structures of TBC and SWC. Results revealed significant differences in the gut bacterial and fungal compositions between TBC and SWC, indicating that genetic background plays a key role in changing the gut microbiota. These results are vital for understanding the gut microbial characteristics in different native chicken species in China. Future research should consider integrating other omics technologies, such as metagenomics and metabolomics, to further investigate the influence of genetic background on the gut microbiota and host functions of TBC and SWC. However, this research has several limitations, including a relatively small sample size and uncontrollable environmental factors.

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