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The comparison of gut microbiota between wild and captive Asian badgers (*Meles leucurus*) under different seasons

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The gut microbiota plays an important role in the immunology, physiology and growth and development of animals. However, currently, there is a lack of available sequencing data on the gut microbiota of Asian badgers. Studying the gut microbiota of Asian badgers could provide fundamental data for enhancing productivity and immunity of badgers' breeding, as well as for the protection of wild animals. In this study, we first characterized the composition and structure of the gut microbiota in the large intestines of wild and captive Asian badgers during summer and winter by sequencing the V3-V4 region of the 16S ribosomal RNA gene. A total of 9 dominant phyla and 12 genera among the bacterial communities of the large intestines exhibited significant differences. Our results showed that Firmicutes and Proteobacteria were the most predominant in both wild and captive badgers, regardless of the season. *Romboutsia*, *Streptococcus* and *Enterococcus* may represent potential sources of zoonoses, warranting further attention and study. Our findings indicated that the diversity and availability of food resources were the most important influencing factors on the gut microbiota of Asian badgers, providing fundamental data for the protection and conservation of wild animals. Variation in the gut microbiota due to season, age and sex in both wild and captive Asian badgers should be considered in future research directions. Furthermore, combined multi-omics studies could provide more information for wild animal conservation, and enhancing our understanding of the molecular mechanism between the microbiota and host.

Keywords Asian badger (*Meles leucurus*), Gut microbiota, 16S rRNA gene, High-throughput sequencing, Seasonal variation

The Asian badger (*Meles leucurus*) is a species of Mustelidae widely distributed across central Asia, including the southern portion of Russia east of the Urals, Mongolia, Kazakhstan, China and the Korean Peninsula[1]. The taxonomic status of badgers is comparatively controversial due to the collision between traditional taxonomy[2–4] and modern molecular biology[5,6]. Although classified as least concern class by the International Union for the Conservation of Nature (IUCN)[7], the Asian badger is on the list of terrestrial wildlife that is beneficial or of important economic or scientific research value under state protection in China. However, significant declines in the badger population have been observed due to increased human activity, illegal hunting, habitat loss and fragmentation[8,9]. Fortunately, with the establishment of the National Park for the Amur Tiger and Amur Leopard in China, the detection rate of Asian badgers is gradually increasing. The variation in population and distribution range of Asian badgers, considered crucial secondary consumers, has a significant impact on the balance and stability of the entire ecosystem. Moreover, Asian badgers play other important roles in the economy and traditional Chinese medicine[10].

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Studies on European badgers were conducted relatively earlier and in more depth than those on Asian badgers, covering various aspects of their ecology, physiology and behavior[11–14], as well as population structure and dynamics[15,16]. Currently, most previous studies on Asian badgers focus on habitat selection, behavioral ecology, activity rhythms and the breeding industry[17–19]. Although studies of Asian badgers using molecular biology methods, including phylogenetic analysis and genetic diversity assessment, are increasing[20,21], a deep understanding of this species and appropriate protection strategies remain insufficient, especially in China.

In the past decade, the gut microbiota has become a research focus due to its role in nutrient availability, food digestion and host protection from pathogens, as well as its influence on host behavior, development, reproduction, and health[22,23]. The composition and dynamics of the host gut microbiota are determined by external factors, such as the environment, food resources and behavior, as well as internal factors, including diet, age, sex and health status[24,25]. However, gut microbiota data of Asian badgers have not yet been reported.

The primary objective of this study is to characterize the basic bacterial community composition and structure of the large intestine contents from both captive and wild Asian badgers for the first time, and to compare the gut microbiota of badgers in different seasons. While it's true that having data on basic gut microbiome composition may not directly translate into practical conservation or management strategies, it can still serve as a valuable baseline for understanding the health and ecology of the Asian badger population. By establishing this benchmark, researchers can monitor changes in the gut microbiota over time, which may reflect broader shifts in the environment or health status of the badgers. Additionally, understanding the gut microbiota of Asian badgers can provide insights into their dietary habits, disease susceptibility, and overall well-being, all of which are critical factors for effective conservation and management efforts.

Materials and methods

Sample collection

A total of 25 large intestine samples from Asian badgers were collected between September 2018 and December 2019. Sample information and sampling locations are provided in Table S1 and Figure S1 (Oviframe Interactive Map, Version V9.9.8.31753, https://www.ovital.com/product/), respectively. Samples from captive Asian badgers during both summer (CS1-CS7) and winter (CW1-CW8) were collected from Haodong Farm in Cangzhou, Hebei Province, China. The farmed badgers were fed mainly corn, chicken and modified feed for foxes, which ensured a sufficient food supply in both summer and winter. The captive badgers were housed individually in outdoor enclosures and were euthanized by electrocution. The large intestines were collected immediately from deceased Asian badger individuals on the farm. The entire sampling process was conducted by a professional veterinarian. All captive individuals have not received veterinary treatments or antibiotics.

Large intestine samples from wild Asian badgers in summer (WS1-WS6) and winter (WW1-WW4) were obtained from deceased badgers stored at the Forest Public Security Bureau of Hunchun, Jilin Province. Police officers collected the corpses of wild Asian badgers that died of natural causes or road accidents during their daily patrols and temporarily stored them at −20 ℃ within 1–2 h after discovering these bodies. The large intestine sampling process and scientific research on deceased badgers were permitted by the Forest Public Security Bureau of Hunchun. All necropsies were conducted by professionals in a sterile environment within the laboratory.

All the large intestine samples were stored in a customized freezer at − 20 ℃ and then transferred to − 80 ℃ in the laboratory for further experiments.

DNA extraction

All samples were collected from feces in the large intestine of badgers under aseptic conditions. The QIAamp® Stool Mini Kit (QIAGEN, Germany) was used to extract total genomic DNA following the manufacturer's protocols. Subsequently, the extracted DNA was analyzed using 1% agarose gel electrophoresis.

16S rRNA gene PCR and sequencing

The V3-V4 region of the bacterial 16S ribosomal RNA gene was amplified by PCR using the following protocol: initial denaturation at 95 ℃ for 3 min; followed by 25 cycles at 95 ℃ for 30 s, 55 ℃ for 30 s, and 72 ℃ for 30 s; and a final extension at 72 ℃ for 5 min The primers used were338F (5′-barcode-ACTCCTACGGGAGGCAGC AG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). The PCR reaction was performed in a total volume of 50 μL containing the following: 6 μL of the template DNA, 25 μL of 2 × Taq PCR Master Mix, 2 μL of each primer (10 mM) and 15 μL of ddH_2O to complement the reaction system. After detection by 2% agarose gel electrophoresis and recovery using the AxyPrep DNA Gel Extraction Kit (AXYGEN, USA), the PCR products were quantified using the Quantified Fluor™ ST Blue Fluorescence Quantification system (Promega, USA).

Sequencing libraries were generated using the TruSeq™ DNA Sample Prep Kit (Illumina, USA) and sequencing was conducted on an Illumina MiSeq platform following the manufacturer's recommendations.

Sequence processing and data analysis

Raw fastq files were demultiplexed, quality-filtered and assembled using QIIME (version 1.9.1)[26] and FLASH (version 1.2.11). Chimeric sequences were identified and removed using UCHIME[27]. Subsequently, sequences were clustered into the same operational taxonomic units (OTUs) with a 97% sequence identity cutoff value using UPARSE (version 7.0.1 http://drive5.com/uparse/). Taxonomy and annotation of the obtained sequences were performed by RDP Classifier (version 2.11) against the SILVA (SSU132) 16S rRNA database[28].

The sequencing depth was 28,763 for rarefaction. Alpha diversity index values, including the Sobs (observed species richness), Shannon, Simpson, Chao 1, ACE and Good's coverage indices, were generated and analyzed by Mothur (version 1.30.2), along with rarefaction curves. Rank-abundance curves were calculated and displayed using R software (version 3.3.1). Stacked histogram of relative abundance and the heatmap showing clustering

of species abundance were created using R software (version 3.3.1). Hierarchical clustering trees were generated and displayed using QIIME (version 1.9.1) and R software (version 3.3.1) based on the unweighted pair-group method with arithmetic mean (UPGMA). Wilcoxon rank-sum test for alpha diversity indices, principal component analysis (PCA), principal coordinate analysis (PCoA) and nonmetric multidimensional scaling (NMDS) were also performed and demonstrated using R software (version 3.3.1). We used the Euclidean distance matrix for PCA and weighted unifrac distance matrix for both PCoA and NMDS. Permutational multivariate analysis of variance (PERMANOVA) was conducted in QIIME (version 1.9.1) to test the grouping of the samples. To determine the species with significant differences, we first performed a log10 transformation on the abundance data before conducting the relevant tests, then the Kruskal–Wallis H test was performed using R software (version 3.3.1). Finally, linear discriminant analysis effect size (LEfSe) was performed using LEfSe software, with a filter value of the LDA score was set as 4 by default[29].

The data were analyzed using the free online platform of Majorbio Cloud Platform (www.majorbio.com). The dataset from our study is available in the Sequence Read Archive (SRA) of NCBI under the accession number PRJNA751208.

All methods were carried out in accordance with relevant guidelines and regulations. All methods are reported in accordance with ARRIVE guidelines. This study was performed in accordance with the permission from the Ethics and Animal Welfare Committee of Beijing Normal University (approval reference number: CLS-EAW-2019-029).

Results

Overview of the sequencing data

A total of 1,072,316 reads were obtained after quality control and filtration from 25 of large intestine contents, comprising 10 from wild and 15 from captive badgers. Subsequently, the high-quality reads were classified into 1,759 OTUs with a 0.97 identity cutoff.

The alpha diversity indices (Sobs, Shannon, Simpson, ACE, Chao 1 and Good's coverage) are presented in Table 1. The Shannon and Sobs indices of each sample are displayed in Figure S2. Rarefaction curves (Figure S3a) are depicted to assess whether the quantity of OTUs used in the study was sufficient. To analyze the richness and evenness of species in the contents of the large intestines of badgers, rank-abundance curves are shown in Figure S3b.

Bacterial composition and relative abundance

Overall, 33 phyla, 85 classes, 192 orders, 321 families and 572 genera were detected in the microbiota of the large intestine contents from 25 badgers.

At the phylum level (Fig. 1a), for the captive badgers in the summer (CS group), Firmicutes (58.663% ± 27.453%), Proteobacteria (40.367% ± 27.569%) and Actinobacteria (0.671% ± 0.491%) were the predominant phyla, followed by Bacteroidetes (0.160% ± 0.287%) and Fusobacteria (0.045% ± 0.102%). For the captive badgers in the winter (CW group), the top three predominant phyla were also Firmicutes (82.881% ± 9.204%), Proteobacteria (16.435% ± 8.656%) and Actinobacteria (0.279% ± 0.309%). Fusobacteria (0.247% ± 0.567%) and Camplyobacteria (0.079% ± 0.213%) were ranked fourth and fifth, respectively.

For the wild badgers, in the summer group (WS group), the top five phyla were Firmicutes (60.013% ± 41.412%), Proteobacteria (18.128% ± 14.961%), Actinobacteria (13.072% ± 16.595%), Verrucomicrobia (3.064% ± 4.141%) and Chloroflexi (1.638% ± 2.239%). For the wild badgers in the winter (WW group), the predominant phyla were Firmicutes (71.122% ± 26.140%), Proteobacteria (16.003% ± 12.584%), Bacteroidetes (7.372% ± 14.734%), Actinobacteria (3.664% ± 4.281%) and Deltaproteobacteria (0.929% ± 1.853%).

At the genus level (Fig. 1b), *Escherichia-Shigella* (39.007% ± 26.443%), *Streptococcus* (23.429% ± 27.924%) and *Enterococcus* (12.703% ± 11.590%) were the predominant genera in the CS group. For the CW group, *Streptococcus* (26.567% ± 14.787%) was the predominant genus, followed by *Clostridium_sensu_stricito_1* (17.256% ± 7.117%) and *Escherichia-Shigella* (15.985% ± 8.812%).Interestingly, the most predominant genus in the wild badgers was *Romboutsia* (44.196% ± 35.401% WS and 46.256% ± 19.527%WW). The genera *Terrisporobacter* (5.761% ± 13.714%) and *Clostridium_sensu_stricto_1* (4.682% ± 7.382%) were ranked second and third in the WS group, while *Escherichia-Shigella* (10.674% ± 14.815%) and *Terrisporobacter* (9.707% ± 10.975%) were ranked second and third in the WW group.

To analyze the similarities and differences in the microbiota community composition of the badger large intestine content, a cluster heatmap for species abundance at the phylum level is shown in Fig. 2. There were obvious differences in the community structure between the captive and wild groups, especially at the genus level. Hierarchical clustering trees using UPGMA at the phylum and genus levels are shown in Fig. 3a,b respectively, which indicated similar results to Fig. 2. Samples CS2 and CS6 were clustered more closely with the WS group than other samples in the CS group.

Analysis of differences in community composition

The Wilcoxon rank-sum test for alpha diversity indices (ACE, Chao 1, Shannon and Simpson indices at the OTU level) among the four groups is shown in Fig. 4. The p values of the test for ACE and Chao 1 indices between the CS and WS groups were both 0.005. Significant differences in the Shannon and Simpson indices were also detected between the CS and CW groups, respectively (Wilcoxon rank-sum test: Shannon diversity, CS vs. CW, $W = 8$, $p = 0.024$; Simpson diversity, CS vs. CW, $W = 47$, $p = 0.032$). However, there were no significant differences among the other combinations (the CW and WW group, the WS and WW group).

To demonstrate the discrepancies among the four groups more intuitively, the principal component analysis (PCA) plot, the principal coordinates analysis (PCoA) plot and the nonmetric multidimensional scaling (NMDS)

Sample	Sobs	Shannon	Simpson	ACE	Chao 1	Good's coverage
CS1	127.000	2.267	0.157	193.310	158.167	0.999
CS2	116.000	1.146	0.616	203.474	161.882	0.999
CS3	89.000	1.693	0.265	116.480	107.400	0.999
CS4	242.000	2.371	0.166	444.812	348.310	0.998
CS5	124.000	2.064	0.209	294.556	205.667	0.998
CS6	113.000	1.181	0.539	240.906	186.929	0.998
CS7	101.000	0.926	0.638	313.258	238.800	0.999
Mean	130.286	1.664	0.370	258.114	201.022	0.999
CW1	122.000	2.356	0.150	235.476	173.250	0.999
CW2	108.000	2.541	0.116	216.625	152.400	0.999
CW3	101.000	2.336	0.167	403.271	230.375	0.999
CW4	108.000	2.030	0.247	249.123	165.000	0.999
CW5	108.000	2.295	0.165	316.229	186.833	0.999
CW6	102.000	2.474	0.126	273.725	225.000	0.999
CW7	107.000	2.156	0.230	207.916	150.588	0.999
CW8	129.000	2.504	0.140	156.468	147.455	0.999
Mean	110.625	2.337	0.168	257.354	178.863	0.999
WS1	1224.000	5.362	0.018	1307.203	1282.508	0.996
WS2	1144.000	5.075	0.025	1238.293	1222.537	0.996
WS3	768.000	1.575	0.608	1024.499	998.556	0.994
WS4	364.000	1.712	0.413	506.923	501.459	0.997
WS5	170.000	1.518	0.352	436.305	311.778	0.998
WS6	282.000	1.631	0.326	729.233	508.957	0.996
Mean	658.667	2.812	0.290	873.743	804.299	0.996
WW1	60.000	1.600	0.257	102.571	88.500	0.999
WW2	735.000	3.726	0.084	907.443	905.850	0.995
WW3	161.000	2.064	0.234	524.594	410.400	0.998
WW4	564.000	2.068	0.246	835.710	807.100	0.995
Mean	380.000	2.364	0.205	592.579	552.962	0.997

Table 1. Alpha diversity of gut microbiota in large intestine contents from captive and wild Asian badgers.

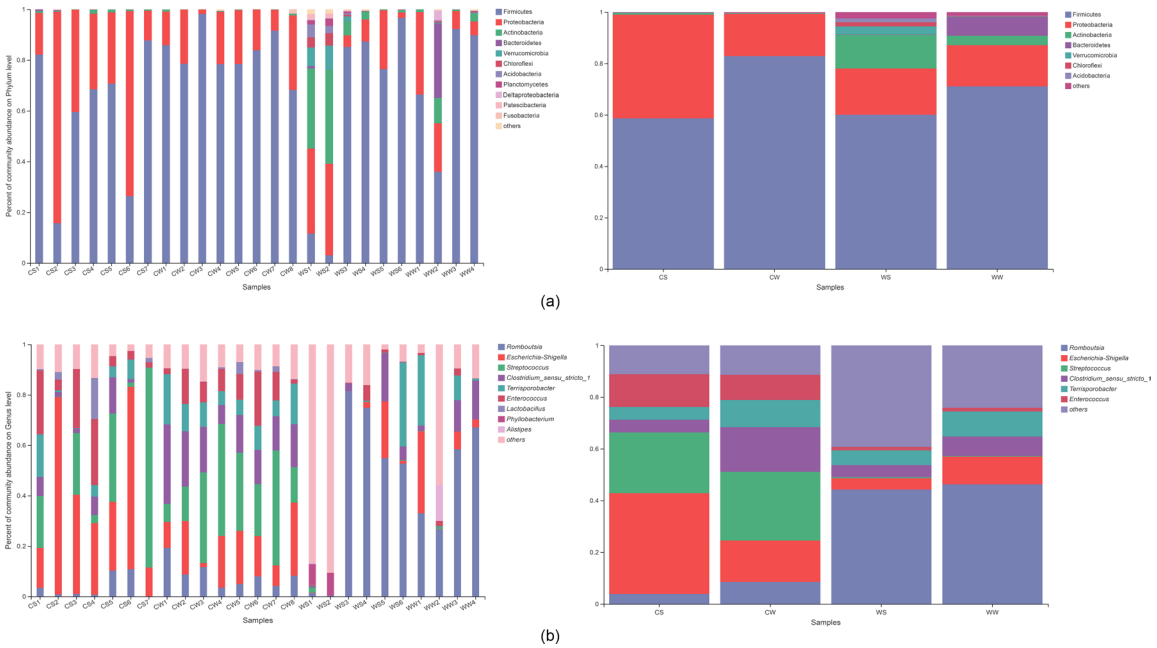

Figure 1. The histogram of relative abundance for species in the gut microbiota of wild and captive Asian badgers at phylum (**a**) and genus (**b**) level.

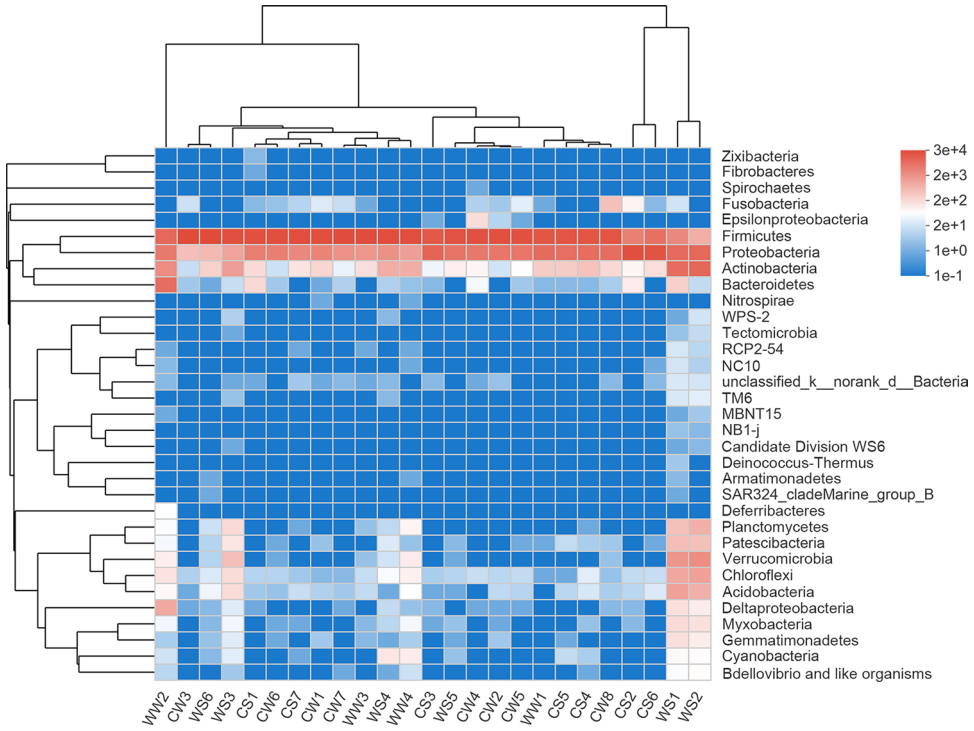

Figure 2. The heatmap of clustering for species abundance. The color gradient of the color block represents the variation of the abundance of different species in the sample. The value represented by the color gradient is on the right of the figure. The software and algorithm used to generate this chart: R language (version 3.3.1) vegan package (https://cran-archive.r-project.org/bin/windows/base/old/3.3.1/).

plot are depicted in Fig. 5. All samples within each group were clustered in the three plots, indicating that the bacterial composition and structure of the gut microbiota were similar. Additionally, the captive (CS and CW) and wild (WS and WW) groups showed distinct clustering patterns, further supporting the differences between captive and wild badgers. We also performed PERMANOVA to conduct inter-group similarity analysis on the grouped samples and test the significance of inter-group differences. The PERMANOVA results of wild/captive ($R = 0.306$, $p = 0.001$) and summer/winter ($R = 0.093$, $p = 0.035$) indicated that the groupings were statistically significant and in line with expectations, consistent with the findings presented in Fig. 4.

Then the Kruskal–Wallis H test (Fig. 6) and linear discriminant analysis effect size (LEfSe) (Fig. 7) were used to calculate and detect species with significant differences at different taxonomic levels. At the phylum level (Fig. 6a), significant differences were observed in the relative abundances of Verrucomicrobia ($3.064\% \pm 4.141\%$, $H = 16.745$, $p < 0.001$), Planctomycetes ($0.880\% \pm 1.173\%$, $H = 13.975$, $p = 0.003$), Patescibacteria ($0.685\% \pm 0.855\%$, $H = 10.465$, $p = 0.015$), Cyanobacteria ($0.158\% \pm 0.161\%$, $H = 12.097$, $p = 0.007$) and Gemmatimonadetes ($0.162\% \pm 0.230\%$, $H = 10.471$, $p = 0.015$) in the WS group. Only the phylum Fusobacteria ($0.247\% \pm 0.567\%$, $H = 14.524$, $p = 0.002$) showed a significant difference in the CW group. Other species that exhibited significant differences at the genus level are shown in Fig. 6b. Different color nodes in the cladogram in Fig. 7a represent microbial groups that were significantly enriched in the corresponding groups and had a notable influence on the differences between groups. The LEfSe bar plot, depicted in Fig. 7b, shows the taxa (CS: 10, CW: 10, WS: 17 and WW: 4) with significant differences among the four groups.

Discussion

Over the past decade, the role of the gut microbiota in animal health has been extensively studied, encompassing developmental, immunological and physiological functions[30–32]. Diet plays a crucial role in modulating the composition of the gut microbiota[33,34]. Additionally, for many wild animals, seasonal dietary shifts influence changes in gut microbiota composition due to fluctuations in food availability and energy intake[28,34,35]. However, studies on the gut microbiota of Asian badgers are currently lacking.

In this study, we first characterized the gut microbiota of both captive and wild Asian badgers and compared it across different seasons. Our findings align with previous studies on Mustelidae species like the sable (*Martes zibellina*), mink (*Mustela vison*), and North American river otter (*Lontra canadensis*)[36–39], showing Firmicutes, Proteobacteria, Actinobacteria, and Bacteroidetes as the predominant phyla, which are commonly found in mammals.

At the phylum level, regardless of the season, Firmicutes and Proteobacteria were consistently the most abundant phyla in both captive and wild Asian badgers. Notably, these two phyla accounted for over 98% of the microbiota in captive Asian badgers, whereas their proportions were lower than 90% in wild individuals. The

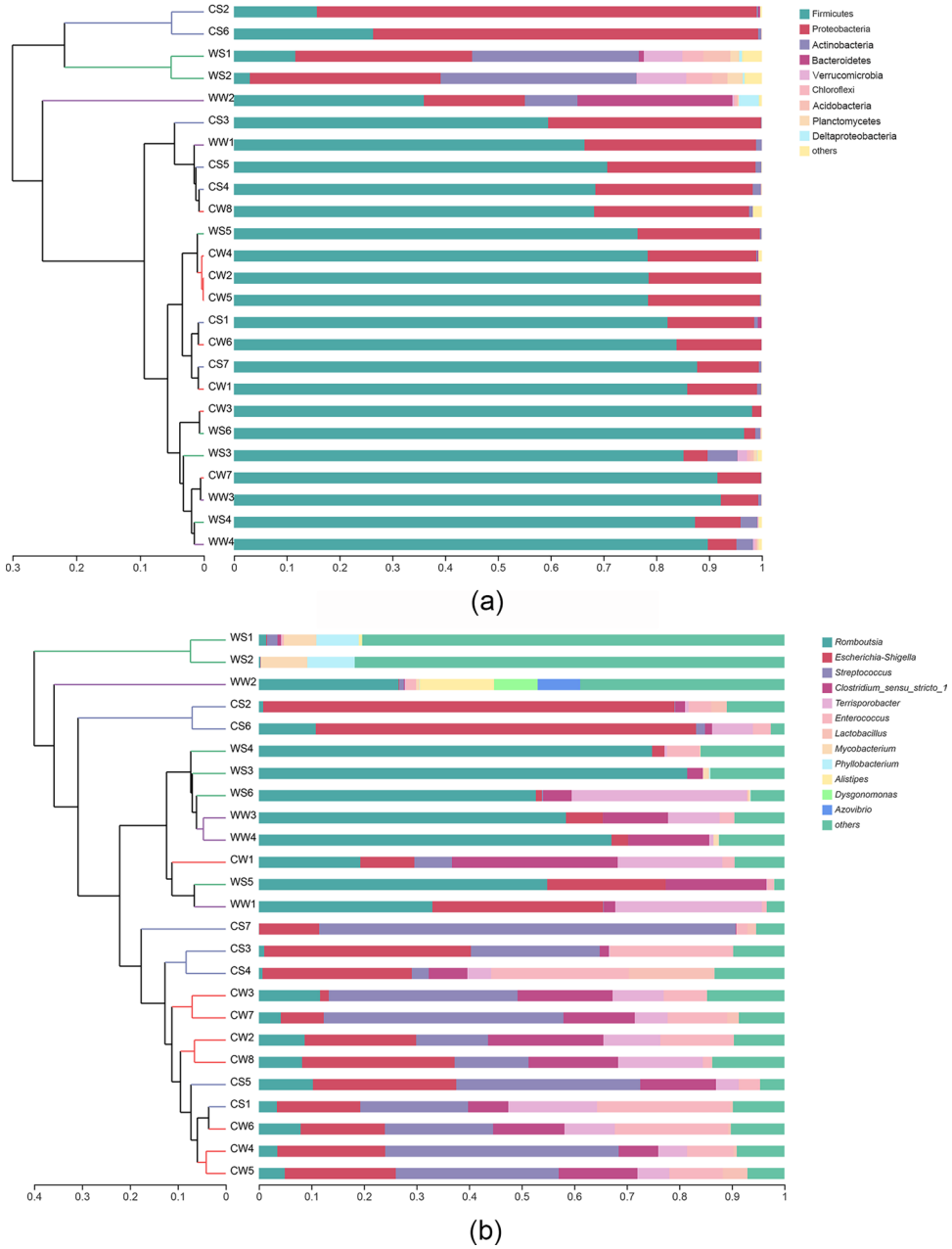

Figure 3. The hierarchical clustering trees. (**a**) and (**b**) were generated based on weighted unifrac distance matrix at phylum and genus level, respectively.

varying relative abundance of the third and subsequent dominant phyla indicated differences in bacterial community richness between captive and wild Asian badgers. The composition of the bacterial microbiota is known to be closely related to long-term dietary habits[40]. The feeding strategy of the Asian badger largely depends on food availability and environmental factors.

The relative abundances of Bacteroidetes, which play an important role in the degradation of high molecular weight substances and carbohydrates secreted by the intestines[41,42], present a remarkable proportion in the WW group, while in the other groups they were almost undetectable. Moreover, it was also shown that an increase of Bacteroidetes was detected when the weight of obese mice decreased[43]. The Firmicutes/Bacteroidetes ratio is closely related to the dietary habits and physiological function of the host[44,45]. First, in the wild, the food resources and availability in winter were limited compared to those in summer. However, the farmed badgers were fed mainly corn, chicken and modified feed for foxes, which ensured a sufficient food supply in winter. Second, studies have shown that hibernation is an adaptive mechanism for badgers to maintain their energy balance when winter climatic conditions lead to food shortages and increased heat loss[14]. Although the lower body temperature could save considerable energy and reduce fat storage requirements during hibernation[46], weight

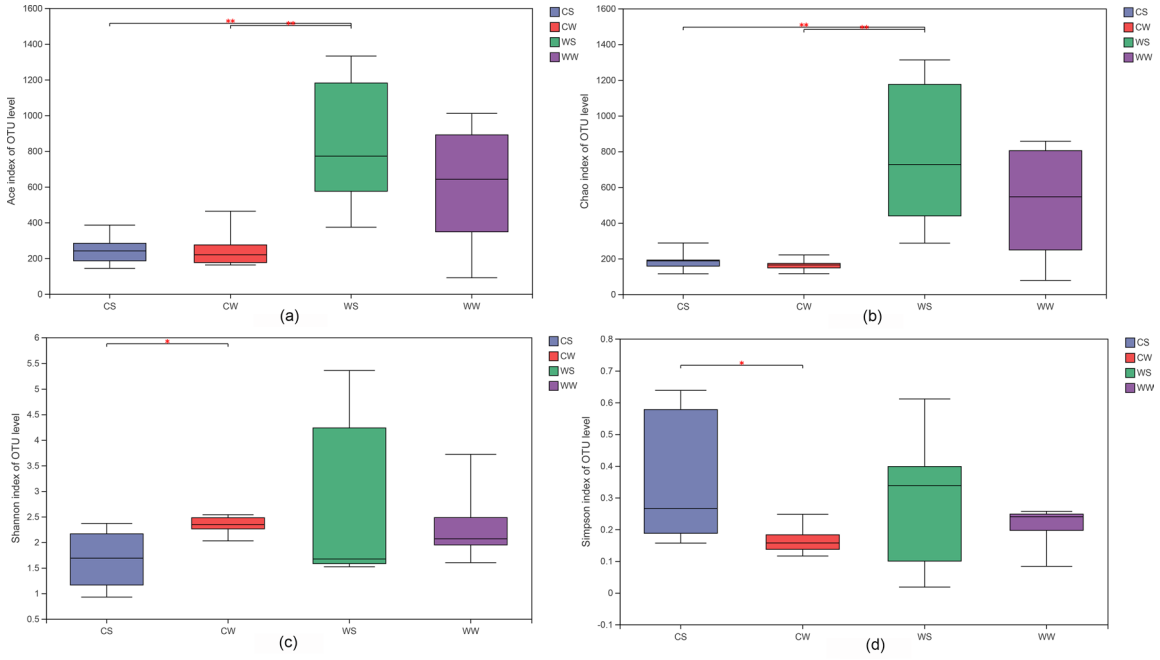

Figure 4. The Wilcoxon rank-sum test for alpha diversity indices of the Asian badger samples, including ACE (**a**), Chao 1 (**b**), Shannon (**c**) and Simpson (**d**) indices.

loss of badgers would occur after long and cold winters at high latitudes. Nevertheless, due to the difficulties of sampling, the body weight data of the Asian badgers in this study were insufficient.

Furthermore, the relative abundance of Verrucomicrobia in the WS group was also significantly higher than that in other groups. Due to the difficulties in isolation and the deficiency of genomic data, the ecological and metabolic roles of Verrucomicrobia are poorly understood. Several studies have suggested that Verrucomicrobia is a potential target for inducing regulatory immunity[47], and some relatively rare taxa of this phylum may be highly effective in polysaccharide degradation[48]. The higher abundance of Verrucomicrobia in the WS group may indicate that more polysaccharides in food need to be degraded. However, at present, due to the lack of fresh samples and the strict protection of wild animals, we know very little about the actual health of wild badgers. Therefore, the potential role of Verrucomicrobia should be studied further with more wild samples.

At the genus level, *Romboutsia* was the only genus that was significantly higher in the WW group. This genus is associated with the host's healthy status and is also regarded as a biomarker of intestinal dysbiosis[49]. In the present study, the relative abundance of *Romboutsia* was low in captive Asian badgers. It is well known that the primary objective of breeding farms is to profit maximization. The Asian badger is a valuable economic animal, prized for its skin, fur and meat, especially in traditional Chinese medicine. Nevertheless, scientific breeding strategies and effective monitoring methods for badger health are still lacking. This result indicates that existing breeding strategies may not be entirely suitable for captive Asian badgers.

Another genus deserving attention and showing a significantly higher abundance in the CW group was *Streptococcus*. Group B *Streptococcus* (GBS) has been identified as the primary cause of mastitis in dairy herbs, directly impacting milk productivity[50,51]. Other species within this genus, such as *Streptococcus iniae*, are commonly found infecting finfish, leading to Streptococcosis[52]. The transmission routes of *Streptococcus agalactiae* from bovines vary and include milk, water contaminated by milk, dairy workers and consumers of dairy products. Therefore, given the hard lessons learned from the spread of COVID-19 worldwide, the prevention and control of zoonotic diseases should be further reinforced, regardless of whether captive or wild animals are involved.

In addition, *Enterococcus* was identified at higher level in captive Asian badgers than in the wild groups. *Enterococcus* typically colonizes various environments and animal intestines and is implicated in many diseases, such as urinary tract infections, hepatobiliary sepsis and surgical wound infection[53]. Some studies have indicated that *Enterococcus* is common nosocomial pathogens and have been detected in the fecal microbiota of birds that underwent surgery[54,55]. Although there were no abnormal conditions in the badger farm, the health conditions of badgers should be evaluated carefully based on the most recent developments in epidemiology and zoology.

In summary, the predominant phyla of the gut microbiota related to the digestion of fat and carbohydrates, as well as the degradation of monosaccharides and polysaccharides, indicated that the diversity and availability of food resources of wild Asian badgers in summer were higher than those for captive badgers. Our results also revealed that wild badgers in summer had a higher richness of the bacterial community, consistent with reports that wild animals harbor a more abundant gut microbiota. Thus, we inferred that dietary diversity is the most important factor influencing the composition and structure of the gut microbiota in Asian badgers. Next, the health of captive badgers must be thoroughly examined using more advanced methods, such as metagenomics and proteomics analyses. Moreover, further analysis of the gut microbiota from more wild samples could help improve the optimal dietary conditions for captive Asian badgers in farming systems.

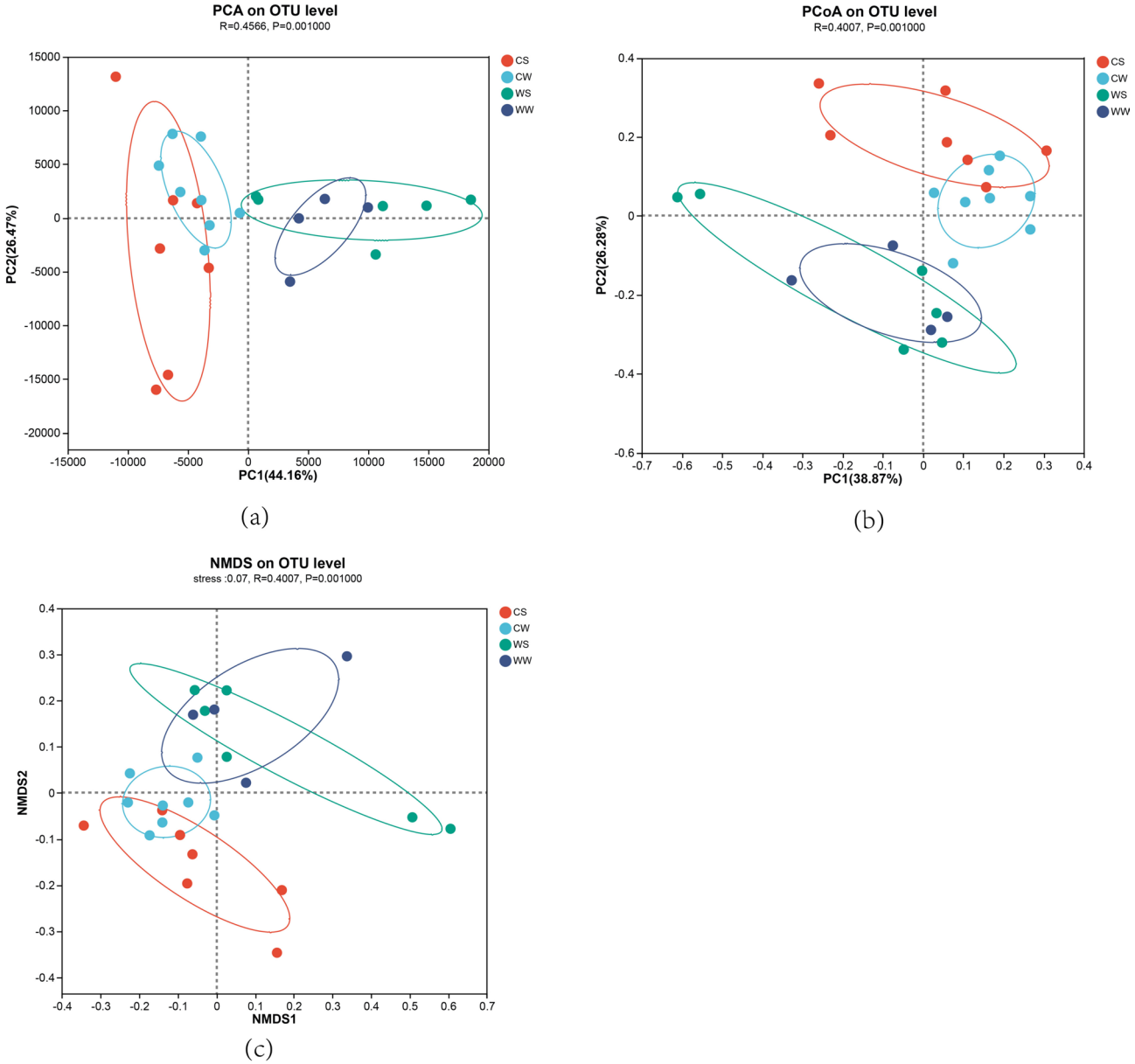

Figure 5. PCA (**a**), PCoA (**b**) and NMDS (**c**) of intestianl bacterial community structures of the wild and captive Asian badgers. The different shape with colors represented all samples of badgers respectively. (**b**) and (**c**) were generated with weighted unifrac distance. The test results in figure are all from the ANOSIM test.

We are aware of and acknowledge that other factors may have influenced the results of this study. Firstly, we cannot ascertain the health status of wild badgers prior to their demise. Therefore, we cannot rule out the possibility that wild badgers may have died due to illness. Additionally, recent research has shown variation in the gut microbiota of other mustelids according to breeding and non-breeding seasons[56]. Secondly, we have not yet considered the potential effects of age or sex on the gut microbiota, both of which could contribute to variation in microbial composition. Additionally, due to the differences in sampling areas, there is significant biogeographical variation between the captive and wild populations. Therefore, in further research and analysis, we will continue to take these factors into account to ensure the accuracy and reliability of our study results.

While the results of our study offer primary data on the gut microbiota of Asian badgers, the scarcity and value of wild samples limited the information we could gather. To enhance our understanding, it is essential to collect a wider variety of samples, including fresh feces and urine, along with detailed information on individual animals, in a more scientific and systematic manner in the future.

Conclusions

In general, we have reported for the first time the bacterial composition and structure of the gut microbiota from large intestine samples of wild Asian badgers using high-throughput sequencing of the V3-V4 region of the 16S rRNA gene. We observed that Firmicutes and Proteobacteria were the most predominant phyla in the

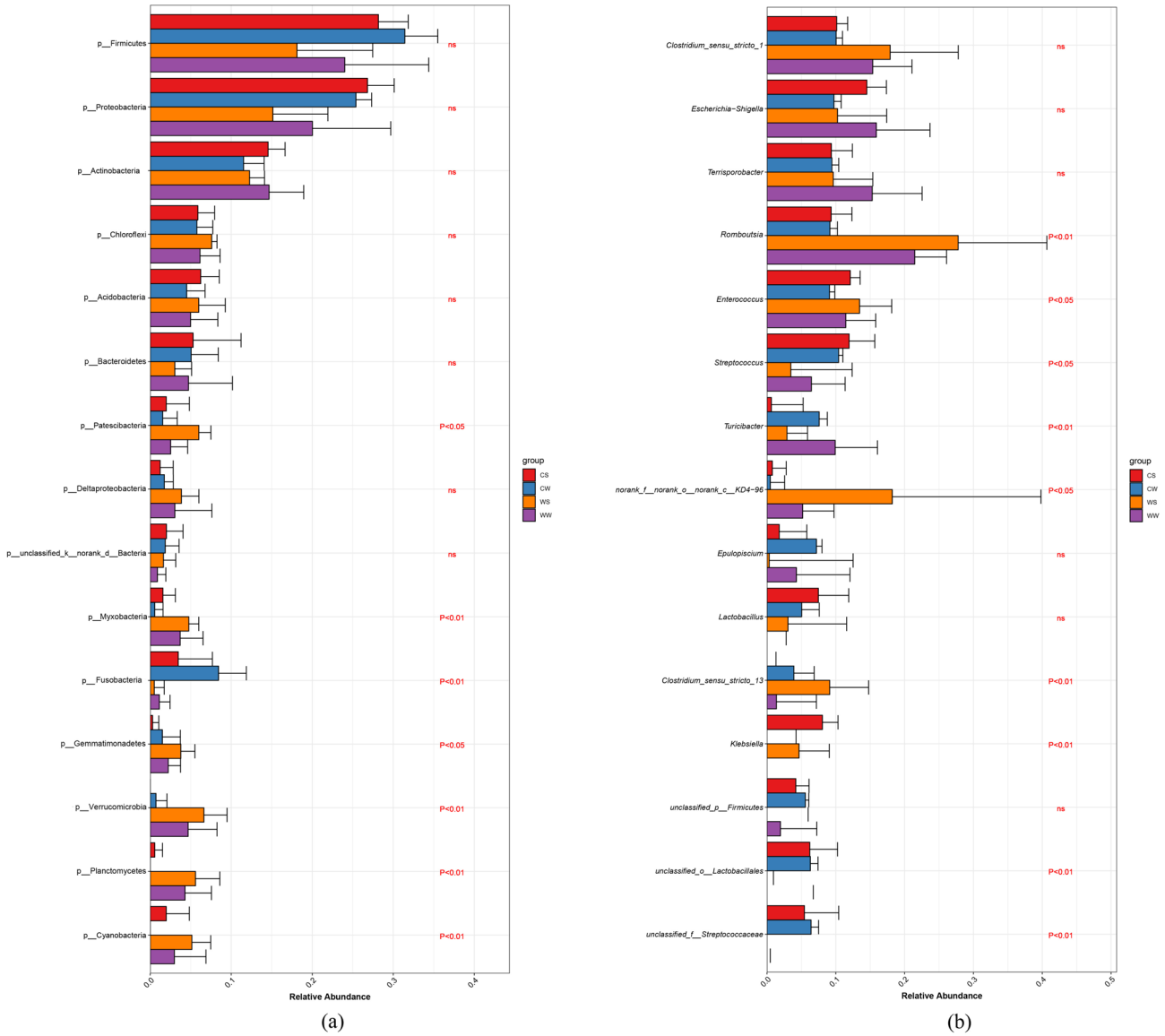

Figure 6. Kruskal-Wallis H test bar at phylum (**a**) and genus (**b**) level. The Y-axis represents the species names at a certain taxonomic level, the X-axis represents the relative abundance of species in different groups.

gut microbiota of both wild and captive Asian badgers, regardless of the season. We speculated that the diversity and availability of food resources are the most important factors influencing the gut microbiota of Asian badgers. Several genera may serve as potential sources of zoonoses, warranting further attention and study. Certainly. This study could significantly contribute to conservation and management efforts in several ways. Firstly, by understanding the gut microbiota of Asian badgers, we can gain insights into their overall health status and ecosystem interactions, which are crucial for effective conservation measures. Secondly, identifying any patterns or changes in the microbiota could help in early detection and prevention of disease outbreaks among badger populations, thus aiding in their conservation. Additionally, this research can inform wildlife management strategies by providing valuable information on the factors influencing badger populations and their habitats. Overall, a better understanding of the gut microbiota of Asian badgers can lead to more informed conservation and management decisions aimed at protecting this species and their ecosystems.

Data availability

The dataset from our study is available in the Sequence Read Archive (SRA) of NCBI under the accession number PRJNA751208.

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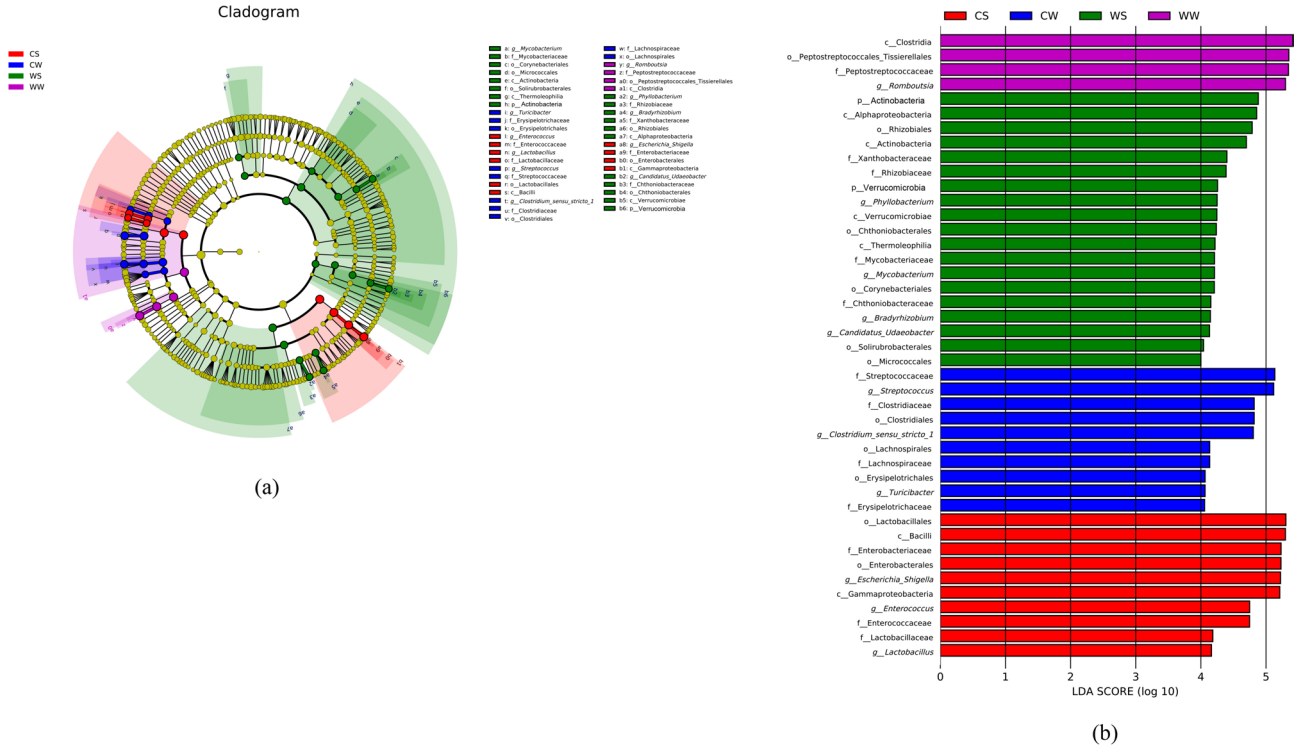

Figure 7. The results of LEfSe analysis. The different color nodes in cladogram (**a**) represent the microbial groups that are significantly enriched in the corresponding groups and have significant influence on the differences between groups. The LEfSe bar chart (**b**) counts the microbial groups with significant effects in multiple groups. The greater the LDA score, the greater the impact of species abundance on the difference effect.

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