ORIGINAL ARTICLE

MicrobiologyOpen WILEY

Gut microbial diversity and stabilizing functions enhance the plateau adaptability of Tibetan wild ass (*Equus kiang*)

Hongmei Gao[1,2] | Xiangwen Chi[1,2] | Guangying Li[3] | Wen Qin[1,2] | Pengfei Song[1,2] | Feng Jiang[1,2] | Daoxin Liu[1,2] | Jingjie Zhang[1,2] | Xiaowen Zhou[4] | Shengqing Li[5] | Tongzuo Zhang[1,6]

[1]Key Laboratory of Adaptation and Evolution of Plateau Biota, Northwest Institute of Plateau Biology Chinese Academy of Sciences, Xining, China

[2]University of Chinese Academy of Sciences, Beijing, China

[3]Qinghai Provincial Environmental Protection Department, Xining, China

[4]Lanzhou Zoo, Lanzhou, China

[5]Qinghai Academy of Animal Science and Veterinary Medicine, Qinghai University, Xining, China

[6]Qinghai Provincial Key Laboratory of Animal Ecological Genomics, Xining, China

Correspondence
Tongzuo Zhang, Key Laboratory of Adaptation and Evolution of Plateau Biota, Northwest Institute of Plateau Biology Chinese Academy of Sciences, No. 23 Xinning Road, Chengxi District, Xining 810001, Qinghai Province, China.
Email: zhangtz@nwipb.cas.cn

Abstract

Interactions between gut microbiota not only regulate physical health, but also form a vital bridge between the environment and the host, thus helping the host to better adapt to the environment. The improvement of modern molecular sequencing techniques enables in-depth investigations of the gut microbiota of vertebrate herbivores without harming them. By sequencing the 16S rRNA V4-V5 region of the gut microbiota of both the captive and wild kiang in winter and summer, the diversity and function of the microbiota could be compared. The reasons for observed differences were discussed. The results showed that the dominant phyla of the kiang were Bacteroidetes and Firmicutes, and the structure and abundance of the gut microbiota differed significantly between seasons and environments. However, the relatively stable function of the gut microbiota supplies the host with increased adaptability to the environment. The diversity of the intestinal flora of the kiang is relatively low in captivity, which increases their risk to catch diseases to some extent. Therefore, importance should be attached to the impact of captivity on wildlife.

KEYWORDS
adaption, gut microbiota, Qinghai–Tibet Plateau, Tax4Fun, Tibetan wild ass (*Equus kiang*)

1 | INTRODUCTION

Animal guts have a large number of bacterial communities, which play important roles in host immunity (Yun et al., 2017), health and disease (Pulikkan et al., 2018), nutrient metabolism (Kau, Ahern, Griffin, Goodman, & Gordon, 2011), and energy acquisition (Shepherd, DeLoache, Pruss, Whitaker, & Sonnenburg, 2018). The symbiotic relationship between microbes and host is critical for their mutual survival. Herbivores utilize gut symbionts to convert plant biomass into fermentable sugars, volatile fatty acids, and microbial
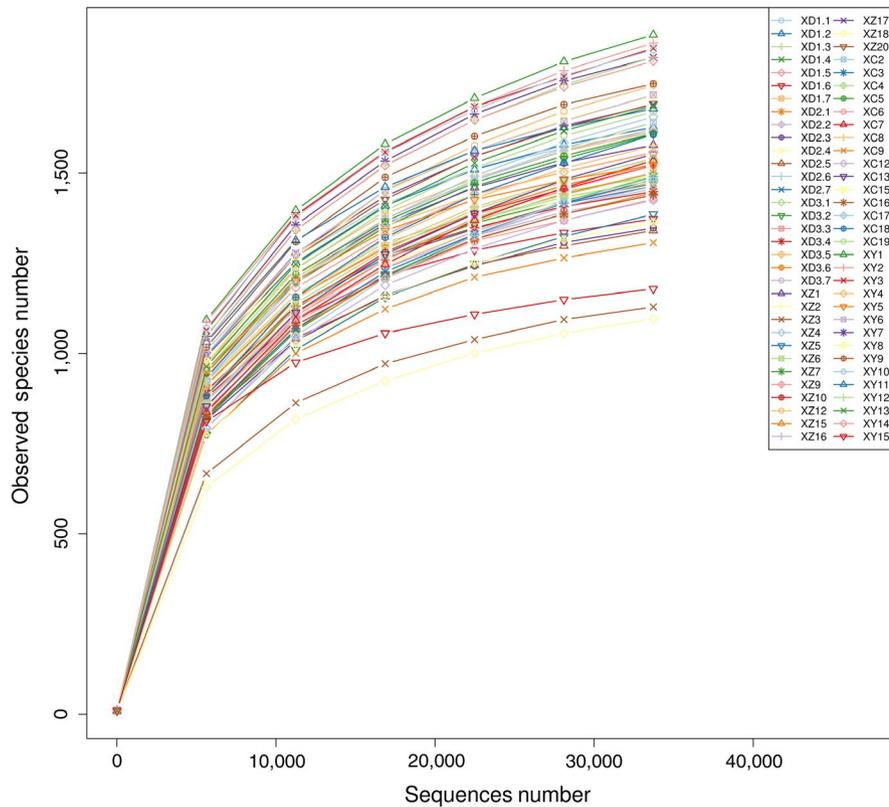
FIGURE 1 Rarefaction curves of all fecal samples of the kiang in summer

TABLE 1 Composition of the food of the captive kiang

Food composition	Fresh alfalfa	Fodder	Semidry oat grass	Carrots
Moisture (%)	72.00	13.20	10.10	92.70
Ash (%)	1.70	6.60	4.50	42.42
Crude fiber (%)	13.83	8.49	35.31	0.95
Crude protein (%)	3.46	17.50	6.28	0.89
Crude fat (%)	0.28	0.20	0.06	0.06
Total sugar (%)	5.15	5.00	2.48	2.60
Carbohydrate (%)	8.75	54.00	43.80	4.55
Energy (KJ/kg)	2,170	12,230	8,530	950
Na (g/kg)	0.02	2.11	0.86	0.32
P (g/kg)	0.27	2.09	0.67	0.22
Ca (g/kg)	1.94	5.01	1.50	0.31

proteins (Xiao et al., 2019). The composition of these intestinal microorganisms is not stable and is easily affected by host diet (David et al., 2014), age, sex, genetics (Ren et al., 2017), and environmental fluctuations (Amato et al., 2015).

Food is the main source of energy for the host (Leftwich, Hutchings, & Chapman, 2018), and the gut microbiome forms a key symbiotic relationship with the host. Prior studies identified diet as the main factor for gut microbial variations between mammalian species (David et al., 2014; Zhang, Ju, & Zuo, 2018). Temporal variations of the relative abundance of individual bacterial groups are closely related to changes in the host diet (Amato et al., 2015). In recent years, the relationship between host gut microbiota and their nutritional metabolism has been increasingly studied (Jeong, Jang, & Kim, 2019; Zhao et al., 2018). However, these studies still suffer from a number

of shortcomings, such as lack of detailed dietary information for wild populations or only focusing on captive animals (Mariat et al., 2009).

The Tibetan wild ass (*Equus kiang*) is the only wild equid on the Tibetan Plateau (St-Louis & Côté, 2009). Due to the harshness of the environment of the Qinghai–Tibet Plateau, wild herbivores need more efficient nutrient metabolism mechanism (Gibson et al., 2019). However, the diversity and function of the gut microbiota in the wild kiang have not been investigated exhaustively (Gao et al., 2019). This study examined the stability of the captive and wild kiang gut microbiomes during both summer and winter of one year. The unique living environment of the kiang provides a rare opportunity to study the gut microbiome and to assess how the kiang and its gut microbiota adapt to the Qinghai–Tibet Plateau. Analyzing the changes in their intestinal microbiota can help to understand

FIGURE 2 Stacked histograms of the relative abundance of gut microbiota phyla in the wild and captive kiang in summer. SC, summer captive group; SW, summer wild group
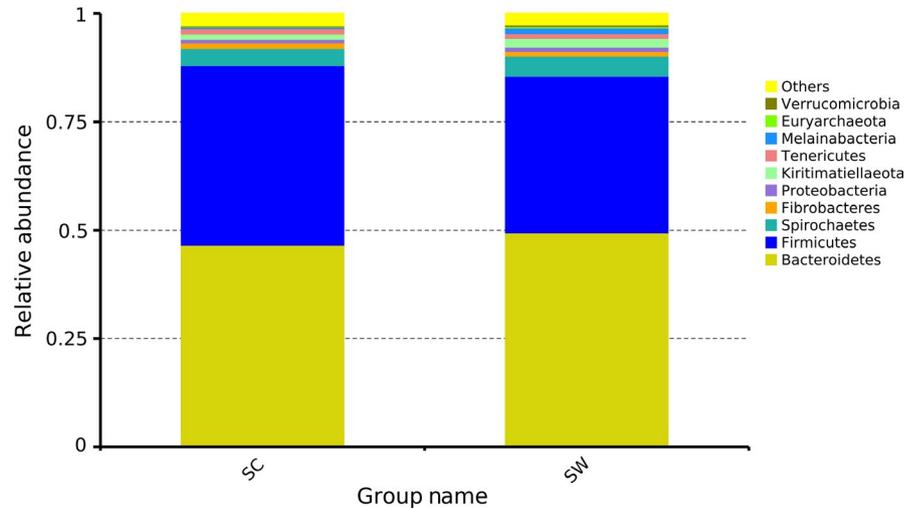

TABLE 2 Comparison of alpha diversity indices of gut microbiota from different groups

alpha diversity	WC	WW	SC	SW
Observed_species	1,745.1 ± 203.2	1,909.1 ± 123.9	1,490 ± 83.4	1,579.3 ± 186.1
Shannon	8.4 ± 0.64	8.8 ± 0.25	8.6 ± 0.21	8.4 ± 0.57
Simpson	0.984 ± 0.020	0.992 ± 0.002	0.993 ± 0.002	0.987 ± 0.022
Chao1	2,154.5 ± 207.5	2,337.2 ± 169.3	1,684.3 ± 130.4	1,837.2 ± 248.5
ACE	2,145.1 ± 206.44	2,329.7 ± 163.2	1,682.1 ± 119.9	1,833.0 ± 237.9

Abbreviations: SC, summer captive group; SW, summer wild group; WC, winter captive group; WW, winter wild group.

the relationship between nutrients, health, and adaptive evolution. The results provide promising targeted methods for the conservation of the kiang.

2 | MATERIALS AND METHODS

2.1 | Collection of fecal samples

Feces samples from the wild kiang were collected from Maduo county, Qinghai province, and samples of animals in captivity were collected from the Qinghai–Tibet Plateau Wildlife Park. Winter samples were collected in January 2018 and summer samples were collected in July. Both in the wild and in the Qinghai–Tibet Plateau Wildlife Park, the kiang was observed. After defecating, fresh feces were quickly collected in a clean zipper bag (using PE gloves) and samples were stored in dry ice. Since this happened in their natural habitat, the kiang is very alert; therefore, they were observed for a long time from the distance, and after they had left an area, fresh feces were collected. After sampling, samples were stored at −80°C. All fecal samples were collected in their natural state.

Twenty-one fecal samples were collected from the captive kiang in summer (SC), and 21 samples were collected in winter (WC). Moreover, 45 fecal samples were collected from the wild kiang in summer (SW) and 60 samples were collected in winter (WW).

2.2 | DNA extraction, PCR amplification, and purification

Total genomic DNA from samples was extracted using the CTAB method (Miaomiao, Guangcheng, Xiaohong, Huailei, & Weizhen, 2008). 1% agarose gels were used to detect both DNA concentration and purity. The DNA was diluted to 1 ng/μl with sterile water, depending on the concentration. The V4-V5 region of 16S rRNA gene was amplified using the following primers: 515F: 5′-GTGCCAGCMGCCGCGGTAA-3′ and 907R: 5′-CCGTCAATTCCTTTGAGTTT-3′ (Gao et al., 2019).

The PCR products were mixed with the same volume of 1× loading buffer (containing SYB green) and were detected by electrophoresis on a 2% agarose gel. The PCR products were mixed at equal density ratio. Then, mixed PCR products were purified using the GeneJET™ Gel Extraction Kit (Thermo Scientific). A sequencing library was generated using the Ion Plus Fragment Library Kit 48 rxns (Thermo Scientific) according to the manufacturer's recommendations. The library quality was assessed using a Qubit@ 2.0 Fluorometer (Thermo Scientific). The library was sequenced on an Ion S5™ XL platform (Thermo Scientific), and 400-bp single-end reads were generated.

2.3 | Sequence analysis

The raw reads were filtered under specific conditions to obtain high-quality clean reads according to the Cutadapt quality

controlled process (Martin, 2011). Then, the clean reads of all samples were clustered using Uparse software (Uparse v7.0.1001; Edgar, 2013), and the sequences were clustered into operational taxonomic units (OTUs) with 97% identity (Identity). According to the results of OTU clustering, the Silva database (Version 132; Quast et al., 2012) was used based on the Mothur algorithm to annotate taxonomic information and to statistically calculate the community composition of each sample at each classification level.

Food was collected from the captive kiang and the composition and nutritional content of the food were analyzed at the Center for Analysis and Testing of the Northwest Plateau Institute of Biology, Chinese Academy of Sciences (Qinghai, China).
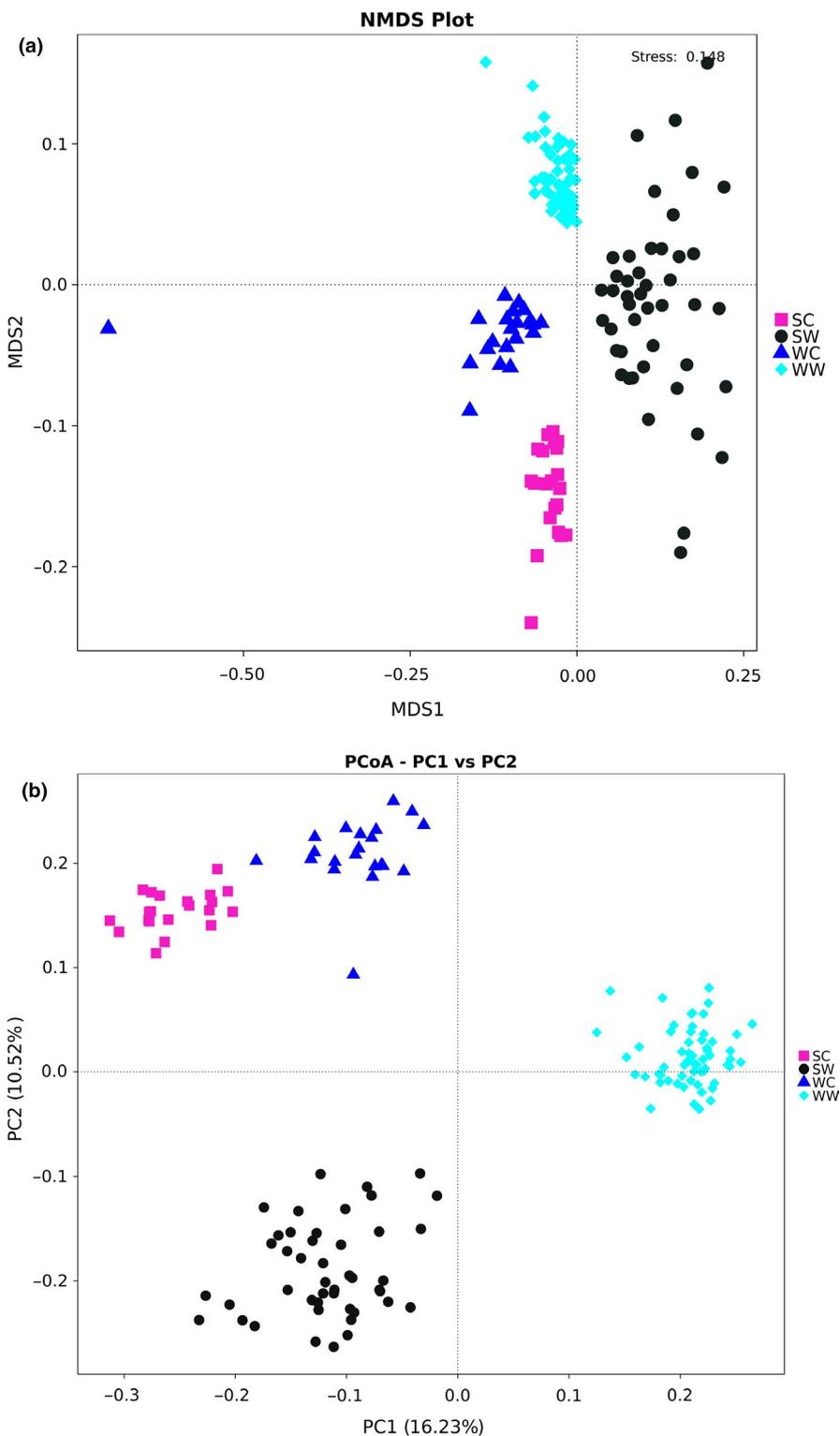

FIGURE 3 Cluster analysis result for both winter group and summer group according to NMDS (a) and Principal coordinate analysis (PCoA) (b). PCoA was performed using Bray-Curtis. SC, summer captive group; SW, summer wild group; WC, winter captive group; WW, winter wild group

TABLE 3 Major differences of gut microbiota at the phylum level for different groups

Phylum/group	WC-vs.-SC	WW-vs.-SW	SW-vs.-SC	WC-vs.-WW
Acidobacteria	—	**	—	—
Spirochaetes	—	**	—	**
Planctomycetes	—	**	—	**
Verrucomicrobia	**	—	*	—
Deferribacteres	**	**	**	—
Thermus	—	**	**	—
Actinobacteria	**	**	—	*
Armatimonadetes	*	**	—	—
Thaumarchaeota	—	*	—	—
Chloroflexi	**	—	**	—
Firmicutes	**	—	*	**
Melainabacteria	—	—	**	**
Fusobacteria	**	—	—	—
Cyanobacteria	—	**	**	—
Synergistetes	—	**	**	**
Bacteroidetes	**	**	—	**
Tenericutes	**	**	—	**
Kiritimatiellaeota	**	**	**	—
Gemmatimonadetes	**	—	—	**
Fibrobacteres	**	**	—	**
Elusimicrobia	**	*	—	**
Lentisphaerae	**	**	*	**
Chlamydiae	**	—	—	—
Gracilibacteria	**	**	—	—
Proteobacteria	—	—	—	**

Abbreviations: SC, summer captive group; SW, summer wild group; WC, winter captive group; WW, winter wild group.

$*p < .01$.

$**p < .001$.

2.4 | Statistical analysis

QIIME (version 1.9.1) was used to calculate all diversity indexes in these samples, and R software (version 2.15.3) was used for visual analysis. LEfSe software (LEfSe 1.0) was used for linear discriminant analysis (LDA; Eckburg et al., 2005). Principal coordinate analysis (PCoA) was performed using Bray–Curtis. The functions of gut microbiota were predicted for the winter and summer groups and for both captive and wild groups using Tax4Fun in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Asshauer, Wemheuer, Daniel, & Meinicke, 2015).

3 | RESULTS

3.1 | 16S rDNA sequence diversity of the wild and captive kiang in summer

Via data quality control, 1,611,185 high-quality reads were obtained in the summer captive group (SC), which were divided into 3,300 OTUs. In the summer wild group (SW), 3,402,962 high-quality reads were obtained, which were divided into 3,995 OTUs. Rarefaction curves (Figure 1) can directly reflect the rationality of data and indirectly reflect the richness of samples. Each curve in the figure represents a different sample and is displayed using a different color. With increasing number of samples, the curve tended to flatten, which proved that the sequenced amount of these samples was reasonable.

3.2 | Dietary composition of the food provided for the captive kiang

In the Qinghai–Tibet Plateau Wildlife Park, the captive kiang mainly eats fresh alfalfa and fodder in summer, and mainly semidry oat grass, carrots, and fodder in winter. The nutritional composition of the food of the captive kiang is listed in Table 1. Their fodder contained more crude protein, carbohydrate, and energy, which provides rich nutrients for the captive kiang. Fresh alfalfa contains high crude fat in summer, and semidry oat grass has high levels of crude fiber in winter.

3.3 | Bacterial composition and their relative abundances in summer

In the summer wild group, 25 phyla, 27 classes, 47 orders, 74 families, 127 genera, and 77 species were detected. In the summer captive group, 22 phyla, 25 classes, 40 orders, 72 families, 109 genera, and 57 species were detected. We have published the winter data in a previous article (Gao et al., 2019).

The gut bacterial community composition and structure were analyzed at different taxonomical levels and seasons. In the summer captive group (SC), Bacteroidetes (46.55%) and Firmicutes (41.51%) were the predominant phyla and Phascolarctobacterium (1.87%) was the predominant genus. In the summer wild group (SW), Bacteroidetes (49.43%) and Firmicutes (36.11%) were the predominant phyla and Phascolarctobacterium (1.23%) was the predominant genus. To visually assess the relative abundance of bacterial communities, the top 10 of each group were selected and the cumulative histogram percentage of their relative abundance at the phylum level is shown in Figure 2.

3.4 | Analysis of between-group discrepancies

The alpha diversity (observed species, Shannon, Simpson, Chao1, and ACE) of the captive and wild kiang was calculated in summer and winter (Table 2). The Shannon index and observed species index were higher in the wild winter group than in the summer group ($P_{Shannon}$ = 0.0094, $P_{observed\ species}$ = 0.0174), and the wild winter group had a higher Shannon index than the captive winter group ($P_{Shannon}$ = 0.0067). In addition, the ACE and Chao1 indexes of the summer group were significantly lower than in the winter group (P_{ACE} = 0.0014, P_{Chao1} = 0.0094) and they were higher in the wild group than in the captive group (P_{ACE} = 0.0001, P_{Chao1} = 0.0001) in all samples. Consequently, the diversity of gut bacteria in the winter group was higher than in the summer group, and their diversity in the wild group was higher than in the captive group.

Multi-response permutation procedure (MRPP) testing between the summer and winter groups and the captive and wild groups yielded A > 0. The intergroup differences were larger than

TABLE 4 Major differences of gut microbiota at the genus level for different groups

Name	WC-vs.-SC	WW-vs.-SW	SW-vs.-SC	WC-vs.-WW
Phascolarctobacterium	**	**	**	—
Fibrobacter	**	**	—	**
Alloprevotella	**	**	*	**
Anaerovibrio	—	**	—	**
Saccharofermentans	—	*	**	**
Papillibacter	—	*	—	—
Sphaerochaeta	**	**	—	*
Akkermansia	**	—	*	—
Methanocorpusculum	**	**	**	**
Agathobacter	—	**	*	**
Flexilinea	*	—	**	—
Oribacterium	*	—	**	**
Pseudobutyrivibrio	—	**	**	**
Mycoplasma	**	—	—	—
Candidatus_Soleaferrea	—	**	—	—
Bacteroides	**	**	*	—
Marvinbryantia	**	—	—	**
Erysipelatoclostridium	—	**	*	—
Parabacteroides	**	—	*	—
Streptococcus	**	**	**	**
Gillisia	**	**	**	**
Fusobacterium	*	—	—	—
Lachnoclostridium	**	*	—	—

Abbreviations: SC, summer captive group; SW, summer wild group; WC, winter captive group; WW, winter wild group.

*p < .01.

**p < .001.

FIGURE 4 Results of LEfSe analysis. The histogram shows species whose LAD Score exceeded the default value of 4. The length of the histogram represents the impact of the different species. The species without significant difference are marked in yellow, and different species have the same color as the group in the evolutionary branch diagram. SC, summer captive group; SW, summer wild group; WC, winter captive group; WW, winter wild group
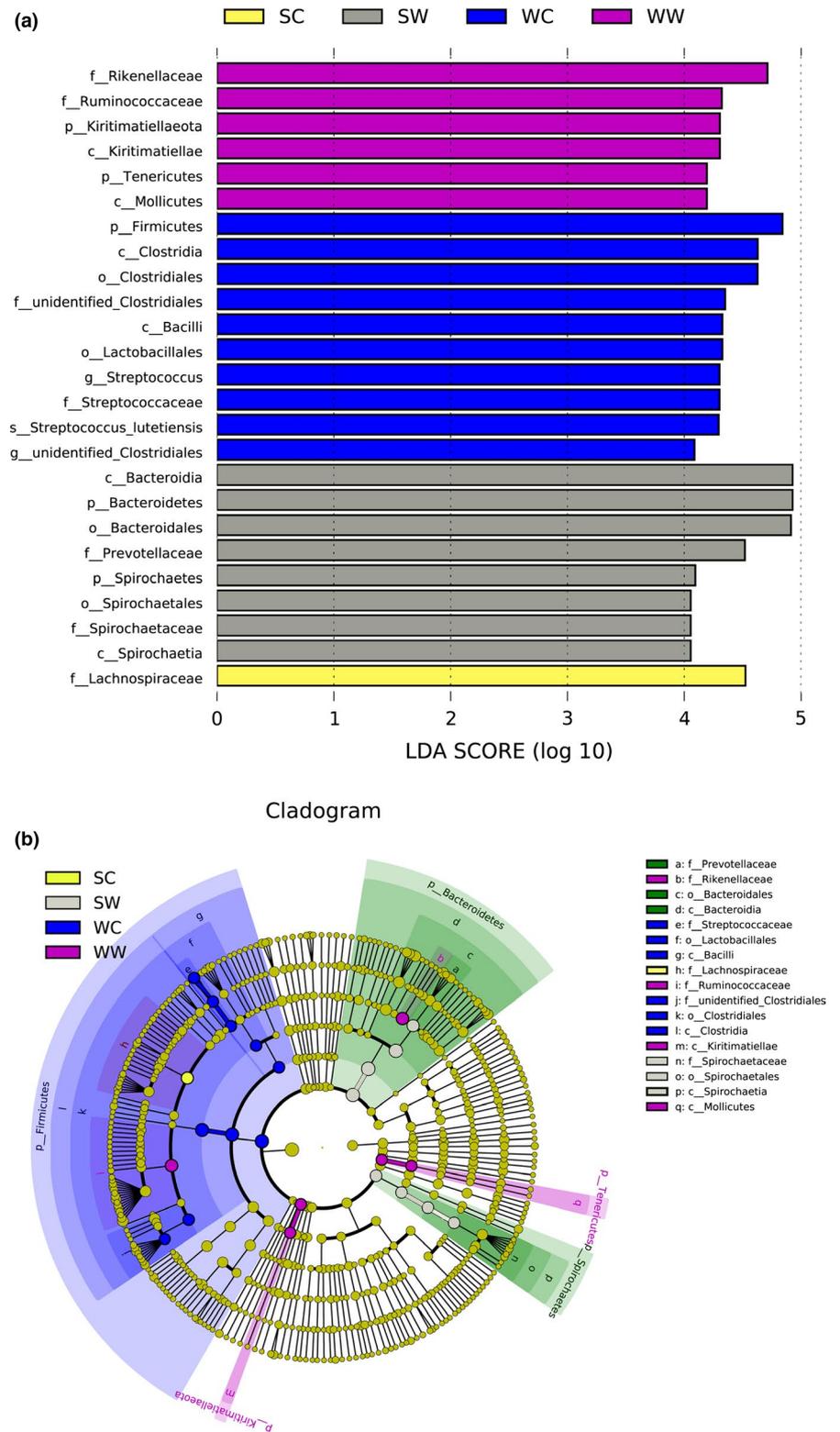
the intragroup differences (O'Reilly & Mielke, 1980; Sullivan, Skeffington, Gormally, & Finn, 2010), which further indicate that the grouping of this study was reasonable. Combined with the nonmetric multi-dimensional scaling (NMDS) plot (Figure 3a) and PCoA plots (Figure 3b), it was shown that the summer captive group, summer wild group, winter captive group, and winter wild group were significantly different.

3.5 | Analysis of different species among groups

Significant differences were found between the diversity and abundance of intestinal microbiota for the different seasons. To investigate the intestinal bacteria in different seasons and environments, a difference analysis of two aspects was conducted, using Metastat analysis. As shown in Table 3, major differences were found for intestinal

TABLE 5 Relative abundance of predicted function of the gut microbiota of the kiang

KO hierarchy	WC (%)	WW (%)	SC (%)	SW (%)
Level 1				
Metabolism	44.66	45.47	44.90	44.39
Genetic information processing	23.17	22.74	23.13	23.52
Environmental information processing	13.48	13.18	13.36	13.32
Cellular processes	8.43	8.20	8.34	8.55
Human diseases	2.77	2.77	2.73	2.74
Organismal systems	2.00	2.01	1.98	2.02
Level 2				
Carbohydrate metabolism	11.28	11.53	11.35	11.12
Membrane transport	10.10	9.80	9.97	9.97
Replication and repair	9.61	9.38	9.64	9.79
Translation	9.26	9.11	9.22	9.40
Amino acid metabolism	9.09	9.43	9.18	9.02
Energy metabolism	4.38	4.54	4.40	4.42
Nucleotide metabolism	4.24	4.16	4.23	4.30
Signal transduction	3.26	3.26	3.28	3.24
Metabolism of cofactors and vitamins	3.13	3.21	3.17	3.17
Glycan biosynthesis and metabolism	3.10	3.08	3.09	3.06

Abbreviations: SC, summer captive group; SW, summer wild group; WC, winter captive group; WW, winter wild group.

microbiota at the phylum level between different seasons and environments. The results listed in Table 4 describe the major differences of intestinal microbiota at the genus level for different seasons and environments.

The results of the LDA Effect Size analysis show species with significantly different abundance among groups (Eckburg et al., 2005). The results have two parts: LDA value distribution histogram (Figure 4a) and evolutionary branch diagram (phylogenetic distribution; Figure 4b). Lachnospiraceae is the most important family in the summer captive group (SC). Bacteroidetes and Spirochaetes are more important phyla and Bacteroidia and Spirochaetia are more important classes in the summer wild group (SW). In the winter wild group (WW), Kiritimatiellaeota, Tenericutes, and Kiritimatiellaeota are more important. In the

winter captive group (WC), Firmicutes is the most important phylum.

3.6 | Analysis of gut microbiome function

According to the results of the predicted function, the main functional information of maximum abundance was selected for each sample of the KEGG classification level (level 1 and level 2, where level 1 showed all six categories of functional information), and a relative functional abundance column diagram was generated (Table 5). At level 1 (Figure 5a), the main functions were metabolism, genetic information processing, and environmental information processing. The function of human diseases in the captive summer group was significantly 1ower than that in the captive winter group ($P_{\text{human diseases}}$ = 0.0460), while genetic information processing was significantly higher ($P_{\text{genetic information processing}}$ = 0.0479) in the wild summer group compared with the wild winter group. In winter, metabolism was higher ($P_{\text{metabolism}}$ = 0.0005) in the wild group; however, genetic information processing, environmental information processing, and cellar processes were higher ($P_{\text{genetic information processing}}$ = 0.0336, $P_{\text{environmental information processing}}$ = 0.0149, and $P_{\text{cellar processes}}$ = 0.0324) in the captive group.

At level 2 (Figure 5b), carbohydrate metabolism, membrane transport, and replication and repair were the main functions of the gut microbiome of the captive kiang. The function of metabolism of cofactors and vitamins in the captive summer group was higher than in the captive winter group ($P_{\text{metabolism of cofactors and vitamins}}$ = 0.0068). Carbohydrate metabolism was higher ($P_{\text{Carbohydrate metabolism}}$ = 0.0460) in the wild winter group than in the wild summer group. In winter, carbohydrate metabolism, amino acid metabolism, energy metabolism and cofactors, and vitamins were higher ($P_{\text{carbohydrate metabolism}}$ = 0.0224, $P_{\text{amino acid metabolism}}$ = 0.0104, $P_{\text{Energy metabolism}}$ = 0.0064, $P_{\text{cofactors and vitamins}}$ = 0.0151) in the wild group. Membrane transport and replication and repair were higher ($P_{\text{Membrane transport}}$ = 0.0071, $P_{\text{replication and repair}}$ = 0.0140) in the captive group. In summer, carbohydrate metabolism was higher ($P_{\text{carbohydrate metabolism}}$ = 0.0466) in the captive group than that in the wild group.

4 | DISCUSSION

Bacteroidetes and Firmicutes were identified as the most dominant phyla in all samples. In herbivores, these organisms are vital for the digestion and fermentation of fibrous forage (Flint & Bayer, 2008). They can derive energy and carbon from the degradation of complex carbohydrates (Williams et al., 2013). The phyla Bacteroidetes and Firmicutes are major components of all fecal microbial communities, and their presence is vitally important for host animals (Brice et al., 2019; Gao et al., 2019).

The relative abundance of the phylum Firmicutes was significantly higher ($P_{\text{SC-SW}}$ = 0.002, $P_{\text{WC-WW}}$ = 0.001) in the captive group (WC group was 49.74%) than in the wild group (WW group was 37.6%; Gao et al., 2019). The abundance of Bacteroidetes in the wild winter group (42.59%) was higher ($P_{\text{WC-WW}}$ = 0.001) than

FIGURE 5 Relative abundance column diagram of microbiota functions based on the KEGG database. (a) Microbiota functions are shown on the first level; (b) top-ten microbiota functions are shown on the second level. SC, summer captive group; SW, summer wild group; WC, winter captive group; WW, winter wild group
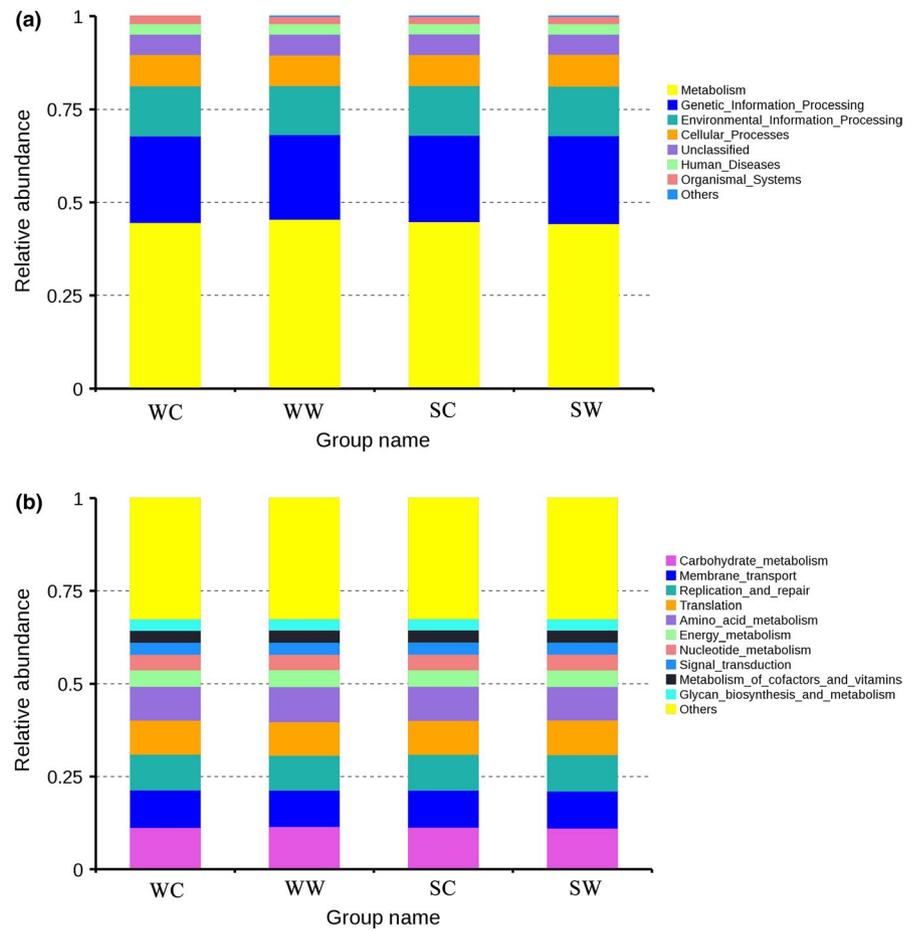
that of the captive winter group (32.88%). The main food of the wild kiang is *Poaceae* and *Cyperaceae* (Yin, Huai, Zhang, Le, & Wei, 2007). Research has shown that in summer, the average content of *Poaceae* crude protein accounts for 9.16%, crude fat accounts for 2.17%, crude fiber accounts for 38.41% (Li, Wang, Kong, Jiang, & Wang, 2018), the average crude protein of *Cyperaceae* accounts for 12.58%, crude fat accounts for 2.56%, and crude fiber accounts for 35.72% (Song, 2002). Numerous bacteria of Bacteroidetes have a degrading function of organics with high molecular weight (Abdallah Ismail et al., 2011), such as fat, plant cell walls, and complex carbohydrates (Coelho et al., 2018). Scientific evidence suggests that the proportion of Firmicutes to Bacteroidetes is related to fat accumulation; however, the effect of Firmicutes is more apparent (Abdallah Ismail et al., 2011).

In any environment, a higher ($P_{SC-WC} = 0.001$, $P_{SW-WW} = 0.001$) abundance of Bacteroidetes was observed in the summer group in comparison with the winter group (the relative abundance of Bacteroidetes of the WC group was 32.88% and that of the WW group was 42.59%; Gao et al., 2019). These results showed that Firmicutes play an important role in the degradation of fiber and cellulose (Ben David et al., 2015), which is consistent with the result that the food of the winter group contains more fiber than that in summer group, and an increased proportion of Firmicutes enhances

the kiang to obtain the nutrition and energy from their food in winter (Boutton, Tieszen, & Imbamba, 1988). Dietary fiber fermentation typically happens through butyrogenic bacteria, most of which belong to Firmicutes (Klement & Pazienza, 2019). The increase in the relative abundance of this phylum during summer is consistent with the observation that fresh alfalfa has a lower carbohydrate and energy but higher fat contents. In addition, the other bacterial flora showed significant differences between both groups. The nutritional interaction between the gut microbiome and the host is complex and requires further research (Abenavoli et al., 2019).

The alpha diversity of gut microbiomes in the wild group was significantly higher than that in the captive group, and in the winter group, this was significantly higher than in the summer group. Interestingly, a decrease in diversity has been reported to be associated with a number of diseases (Kau et al., 2011). However, the content of pathogenic bacteria increased in the captive group, such as Fusobacteria (Roggenbuck et al., 2014) and Tenericutes (Ludwig, Euzéby, & Whitman, 2010), which increases their risk to catch diseases to some extent. Industrialized food affects the diversity and function of the gut microbiome (Sonnenburg & Sonnenburg, 2019). This suggests that the kiang in the wild winter group had the best health, followed by the wild summer group, while the captive summer group had the worst health. This result is consistent with recent research (Chi et al., 2019). Therefore, in the captive

environment, breeders should focus on disease prevention and control of wild animals.

In the captive environment, significant differences in composition and abundance of intestinal flora were found; however, their functions are basically identical, indicating that the taxonomic composition of microbial communities may differ, while being similar in function (Phillips et al., 2017). Different combinations of microbial lineages may achieve comparable community functions (Xiao et al., 2019). These results suggest that diet only alters the intestinal flora. To adapt to a new environment, its function remains unchanged. However, between the wild winter group and the captive winter group, significant differences in function were found. This suggests that the wild environment is more complex and offers less food. Consequently, the wild kiang requires more energy to survive; therefore, the function of metabolism, energy metabolism, and carbohydrate was enhanced. In contrast, the captive kiang has a higher fat and protein diet as well as contact with many humans; consequently, the function of environment information and amino acid metabolism is stronger.

In summary, the composition and abundance of the gut microbiome of the host can rapidly change their metabolic activity in response to changes in the environment. This has enhanced the flexibility of the gut microbiome, helped to maintain normal physiological needs, and improved the adaptability of the host. This study provides a preliminary examination of the seasonal and environmental changes of intestinal flora in the kiang. It not only emphasizes the commonalities of mammals, but also provides a basis for the interaction between intestinal flora and host.

5 | CONCLUSION

In summer and winter, the composition and abundance of the intestinal flora of both the captive and wild kiang differed significantly. Environment was identified as the main reason for the observed differences. However, under the same habitat conditions, the function of the gut flora did not change significantly, which plays an important role in the adaptability of the kiang. During the same season, the diversity of the intestinal flora in the captive environment decreased and the content of pathogenic bacteria increased. This suggests that in an environment affected by industrial food, the health level of the captive kiang was lower than that of the wild population and great importance should be attached to the impact of captivity on wildlife.

AUTHOR CONTRIBUTION

Hongmei Gao: conceptualization; data curation; formal analysis; methodology; visualization; writing-original draft; writing-review & editing. Xiangwen Chi: data curation; formal analysis; writing-original draft. Guanying Li: conceptualization; visualization; writing-original draft. Wen Qin: conceptualization; data curation; writing-original draft. Pengfei Song: data curation; formal analysis; methodology. Feng Jiang: data curation; formal analysis; visualization. Daoxin Liu: data curation; methodology; visualization. Jingjie Zhang: data curation; methodology; visualization. Xiaowen Zhou: conceptualization; methodology; writing-original draft; Writing-review and editing. Shengqing Li: conceptualization; formal analysis; methodology. Tongzuo Zhang: conceptualization; writing-original draft; writing-review and editing.

ORCID

Hongmei Gao https://orcid.org/0000-0003-4401-2743
Wen Qin https://orcid.org/0000-0002-8584-1663
Tongzuo Zhang https://orcid.org/0000-0001-5524-4262

REFERENCES

Abdallah Ismail, N., Ragab, S. H., Abd Elbaky, A., Shoeib, A. R., Alhosary, Y., & Fekry, D. (2011). Frequency of Firmicutes and Bacteroidetes in gut microbiota in obese and normal weight Egyptian children and adults. *Archives of Medical Science*, 7(3), 501–507. https://doi.org/10.5114/aoms.2011.23418

Abenavoli, L., Scarpellini, E., Colica, C., Boccuto, L., Salehi, B., Sharifi-Rad, J., … Capasso, R. (2019). Gut microbiota and obesity: A role for probiotics. *Nutrients*, 11(11), 2690. https://doi.org/10.3390/nu11112690

Amato, K. R., Leigh, S. R., Kent, A., Mackie, R. I., Yeoman, C. J., Stumpf, R. M., … Garber, P. A. (2015). The gut microbiota appears to compensate for seasonal diet variation in the wild black howler monkey (*Alouatta pigra*). *Microbial Ecology*, 69(2), 434–443. https://doi.org/10.1007/s00248-014-0554-7

Asshauer, K. P., Wemheuer, B., Daniel, R., & Meinicke, P. (2015). Tax4Fun: Predicting functional profiles from metagenomic 16S rRNA data. *Bioinformatics*, 31(17), 2882–2884. https://doi.org/10.1093/bioinformatics/btv287

Ben David, Y., Dassa, B., Borovok, I., Lamed, R., Koropatkin, N. M., Martens, E. C., … Moraïs, S. (2015). Ruminococcal cellulosome systems from rumen to human. *Environmental Microbiology*, 17(9), 3407–3426. https://doi.org/10.1111/1462-2920.12868

Boutton, T. W., Tieszen, L. L., & Imbamba, S. K. (1988). Seasonal changes in the nutrient content of East African grassland

vegetation. *African Journal of Ecology*, *26*(2), 103–115. https://doi.org/10.1111/j.1365-2028.1988.tb00961.x

Brice, K. L., Trivedi, P., Jeffries, T. C., Blyton, M. D. J., Mitchell, C., Singh, B. K., & Moore, B. D. (2019). The Koala (*Phascolarctos cinereus*) faecal microbiome differs with diet in a wild population. *PeerJ*, *7*, e6534. https://doi.org/10.7717/peerj.6534

Chi, X., Gao, H., Wu, G., Qin, W., Song, P., Wang, L., … Zhang, T. (2019). Comparison of gut microbiota diversity between wild and captive bharals (*Pseudois nayaur*). *BMC Veterinary Research*, *15*(1), 243. https://doi.org/10.1186/s12917-019-1993-7

Coelho, L. P., Kultima, J. R., Costea, P. I., Fournier, C., Pan, Y., Czarnecki-Maulden, G., … Bork, P. (2018). Similarity of the dog and human gut microbiomes in gene content and response to diet. *Microbiome*, *6*(1), 72. https://doi.org/10.1186/s40168-018-0450-3

David, L. A., Maurice, C. F., Carmody, R. N., Gootenberg, D. B., Button, J. E., Wolfe, B. E., … Turnbaugh, P. J. (2014). Diet rapidly and reproducibly alters the human gut microbiome. *Nature*, *505*(7484), 559–563. https://doi.org/10.1038/nature12820

Eckburg, P. B., Bik, E. M., Bernstein, C. N., Purdom, E., Dethlefsen, L., Sargent, M., … Relman, D. A. (2005). Diversity of the human intestinal microbial flora. *Science*, *308*(5728), 1635–1638. https://doi.org/10.1126/science.1110591

Edgar, R. C. (2013). UPARSE: Highly accurate OTU sequences from microbial amplicon reads. *Nature Methods*, *10*(10), 996–998. https://doi.org/10.1038/nmeth.2604

Flint, H. J., & Bayer, E. A. (2008). Plant cell wall breakdown by anaerobic microorganisms from the Mammalian digestive tract. *Annals of the New York Academy of Sciences*, *1125*, 280–288. https://doi.org/10.1196/annals.1419.022

Gao, H., Chi, X., Qin, W., Wang, L., Song, P., Cai, Z., … Zhang, T. (2019). Comparison of the gut microbiota composition between the wild and captive Tibetan wild ass (*Equus kiang*). *Journal of Applied Microbiology*, *126*, 1869–1878. https://doi.org/10.1111/jam.14240

Gibson, K. M., Nguyen, B. N., Neumann, L. M., Miller, M., Buss, P., Daniels, S., … Pukazhenthi, B. (2019). Gut microbiome differences between wild and captive black rhinoceros - implications for rhino health. *Scientific Reports*, *9*(1), 7570. https://doi.org/10.1038/s41598-019-43875-3

Jeong, M. Y., Jang, H. M., & Kim, D. H. (2019). High-fat diet causes psychiatric disorders in mice by increasing Proteobacteria population. *Neuroscience Letters*, *698*, 51–57. https://doi.org/10.1016/j.neulet.2019.01.006

Kau, A. L., Ahern, P. P., Griffin, N. W., Goodman, A. L., & Gordon, J. I. (2011). Human nutrition, the gut microbiome and the immune system. *Nature*, *474*(7351), 327–336. https://doi.org/10.1038/nature10213

Klement, R. J., & Pazienza, V. (2019). Impact of different types of diet on gut microbiota profiles and cancer prevention and treatment. *Medicina*, *55*(4), 84. https://doi.org/10.3390/medicina55040084

Leftwich, P. T., Hutchings, M. I., & Chapman, T. (2018). Diet, gut microbes and host mate choice: Understanding the significance of microbiome effects on host mate choice requires a case by case evaluation. *BioEssays*, *40*(12), 1800053. https://doi.org/10.1002/bies.201800053

Li, L. I., Wang, Y., Kong, L., Jiang, W. U., & Wang, S. (2018). Study on nutritional components of 35 common gramineous forage plants in Guizhou Province. *Journal of Grassland and Forage Science*, *3*, 13–18. https://doi.org/10.3969/j.issn.2096-3971.2018.03.004

Ludwig, W., Euzéby, J., & Whitman, W. B. (2010). Taxonomic outlines of the phyla Bacteroidetes, Spirochaetes, Tenericutes (Mollicutes), Acidobacteria, Fibrobacteres, Fusobacteria, Dictyoglomi, Gemmatimonadetes, Lentisphaerae, Verrucomicrobia, Chlamydiae, and Planctomycetes. In W. B. Whitman (Ed.), *Bergey's manual® of systematic bacteriology* (pp. 21–24). New York, NY: Springer.

Mariat, D., Firmesse, O., Levenez, F., Guimarães, V. D., Sokol, H., Doré, J., … Furet, J.-P. (2009). The Firmicutes/Bacteroidetes ratio of the human microbiota changes with age. *BMC Microbiology*, *9*, 123. https://doi.org/10.1186/1471-2180-9-123

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.Journal*, *17*(1), 10–12. https://doi.org/10.14806/ej.17.1.200

Miaomiao, Y., Guangcheng, W., Xiaohong, P., Huailei, M., & Weizhen, L. (2008). A method suitable for extracting genomic DNA from animal and plant-modified CTAB method. *Agricultural Science and Technology*, *9*(2), 39–41. https://doi.org/10.1007/s11442-008-0073-x

O'Reilly, F. J., & Mielke, P. W., Jr. (1980). Asymptotic normality of MRPP statistics from invariance principles of U-statistics. *Communications in Statistics-Theory and Methods*, *9*(6), 629–637. https://doi.org/10.1080/03610928008827907

Phillips, C. D., Hanson, J., Wilkinson, J. E., Koenig, L., Rees, E., Webala, P., & Kingston, T. (2017). Microbiome structural and functional interactions across host dietary niche space. *Integrative and Comparative Biology*, *57*(4), 743–755. https://doi.org/10.1093/icb/icx011

Pulikkan, J., Maji, A., Dhakan, D. B., Saxena, R., Mohan, B., Anto, M. M., … Sharma, V. K. (2018). Gut microbial dysbiosis in indian children with autism spectrum disorders. *Microbial Ecology*, *76*(4), 1102–1114. https://doi.org/10.1007/s00248-018-1176-2

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., … Glöckner, F. O. (2012). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research*, *41*(D1), D590–D596. https://doi.org/10.1093/nar/gks1219

Ren, T., Boutin, S., Humphries, M. M., Dantzer, B., Gorrell, J. C., Coltman, D. W., … Wu, M. (2017). Seasonal, spatial, and maternal effects on gut microbiome in wild red squirrels. *Microbiome*, *5*(1), 163. https://doi.org/10.1186/s40168-017-0382-3

Roggenbuck, M., Bærholm Schnell, I., Blom, N., Bælum, J., Bertelsen, M. F., Sicheritz-Pontén, T., … Hansen, L. H. (2014). The microbiome of New World vultures. *Nature Communications*, *5*, 5498. https://doi.org/10.1038/ncomms6498

Shepherd, E. S., DeLoache, W. C., Pruss, K. M., Whitaker, W. R., & Sonnenburg, J. L. (2018). An exclusive metabolic niche enables strain engraftment in the gut microbiota. *Nature*, *557*(7705), 434–438. https://doi.org/10.1038/s41586-018-0092-4

Song, Q. (2002). Quality analysis of several forage grasses in Cyperaceae in Qinghai. *Journal of Sichuan Grassland*, *4*, 37–39. https://doi.org/10.3969/j.issn.1673-8403.2002.04.007

Sonnenburg, J. L., & Sonnenburg, E. D. (2019). Vulnerability of the industrialized microbiota. *Science*, *366*(6464), eaaw9255. https://doi.org/10.1126/science.aaw9255

St-Louis, A., & Côté, S. D. (2009). Equus kiang (Perissodactyla: Equidae). *Mammalian Species*, *835*, 1–11. https://doi.org/10.1644/835.1

Sullivan, C. A., Skeffington, M. S., Gormally, M. J., & Finn, J. A. (2010). The ecological status of grasslands on lowland farmlands in western Ireland and implications for grassland classification and nature value assessment. *Biological Conservation*, *143*(6), 1529–1539. https://doi.org/10.1016/j.biocon.2010.03.035

Williams, C. L., Willard, S., Kouba, A., Sparks, D., Holmes, W., Falcone, J., … Brown, A. (2013). Dietary shifts affect the gastrointestinal microflora of the giant panda (Ailuropoda melanoleuca). *Journal of Animal Physiology and Animal Nutrition*, *97*(3), 577–585. https://doi.org/10.1111/j.1439-0396.2012.01299.x

Xiao, Y., Xiao, G., Liu, H., Zhao, X., Sun, C., Tan, X., … Feng, J. (2019). Captivity causes taxonomic and functional convergence of gut microbial communities in bats. *PeerJ*, *7*, e6844. https://doi.org/10.7717/peerj.6844

Yin, B., Huai, H., Zhang, Y., Le, Z., & Wei, W. (2007). Trophic niches of *Pantholops hodgsoni*, *Procapra picticaudata* and *Equus kiang* in Kekexili region. *Ying yong sheng tai xue bao=. The Journal of Applied Ecology*, *18*(4), 766–770.

Yun, D., Qi, W., Yibo, H., Xiao, W., Yonggang, N., Xiaoping, W., & Fuwen, W. (2017). Advance and prospects of gut microbiome in

wild mammals. *Acta Theriologica Sinica*, *37*(4), 399–406. https://doi.org/10.16829/j.slxb.201704010

Zhang, N., Ju, Z., & Zuo, T. (2018). Time for food: The impact of diet on gut microbiota and human health. *Nutrition*, *51–52*, 80–85. https://doi.org/10.1016/j.nut.2017.12.005

Zhao, L., Zhang, F., Ding, X., Wu, G., Lam, Y. Y., Wang, X., … Zhang, C. (2018). Gut bacteria selectively promoted by dietary fibers alleviate type 2 diabetes. *Science*, *359*(6380), 1151–1156. https://doi.org/10.1126/science.aao5774