Seasonal variations in the composition and diversity of gut microbiota in white-lipped deer (*Cervus albirostris*)

Zhangqiang You, Jing Deng, Jialin Liu, Junhua Fu, Huan Xiong, Wei Luo and Jianli Xiong

Ecological Security and Protection Key Laboratory of Sichuan Province, Mianyang Normal University, Mianyang, Sichuan Province, China

ABSTRACT

The gut microbiota has key physiological functions in host adaptation, although little is known about the seasonal changes in the composition and diversity of the gut microbiota in deer. In this study, seasonal variations (grassy and withering season) in the gut microbiota of white-lipped deer (*Cervus albirostris*), which lives in alpine environments, were explored through 16S rRNA high-throughput sequencing based on sixteen fecal samples collected from Gansu Qilian Mountain National Nature Reserve in China. At the phylum level, Firmicutes, Bacteroidota, and Actinobacteriota dominated the grassy season, while Firmicutes, Proteobacteria, and Actinobacteriota dominated the withering season. At the genus level, *Carnobacterium* dominated the grassy season, while *Arthrobacter* and *Acinetobacter* dominated the withering season. Alpha diversity results (Shannon: $P = 0.01$, ACE: $P = 0.00$, Chao1: $P = 0.00$) indicated that there was a difference in the diversity and richness of the gut microbiota between the two seasons, with higher diversity in the grassy season than in the withering season. Beta diversity results further indicated that there was a significant difference in the community structure between the two seasons ($P = 0.001$). In summary, the composition, diversity, and community structure of the gut microbiota showed significant seasonal variations, which could be explained by variations in the seasonal food availability, composition, diversity, and nutrition due to phenological alternations. The results of this study indicate that the gut microbiota can adapt to changes in the environment and provide the scientific basis for health assessment of white-lipped deer.

INTRODUCTION

The gut microbiota, which refers to all microorganisms present in the gastrointestinal tract (*Kim et al., 2020*), has important roles in the digestion (*Rowland et al., 2018*), metabolism (*Nicholson et al., 2012*), immunity (*Round & Mazmanian, 2009*; *Thaiss et al., 2016*), and entro-endocrine function (*Rastelli, Cani & Knauf, 2019*) of the host. However, in a state of dysbiosis, the gut microbiota can cause many diseases, such as diarrhea (*Wang et al., 2018*; *Xi et al., 2021*), follicular cysts (*Feng et al., 2021*), and bacterial pneumonia (*Zhao et al., 2021*). Thus, the gut microbiota can affect the health of the host either positively or negatively. The composition and diversity of the gut microbiota are affected by biotic

and abiotic factors, such as genetic background, diet, season, region, habitat, and other environmental factors (*e.g.*, *Xin et al., 2019*; *Chang et al., 2020*; *Fan et al., 2020*; *Aricha et al., 2021*; *Guo et al., 2021*; *Ilina et al., 2021*; *Jiang et al., 2022*), but diet is considered one of the predominant factors influencing the diversity and composition of the gut microbial community (*Kim et al., 2014*; *Khafipour et al., 2016*; *Guo et al., 2021*; *Wei et al., 2021*).

In recent years, variations of the gut microbiota of herbivores have been widely studied. For example, different seasons can influence the composition and diversity of the gut microbiota of American bison (*Bison bison*) (*Bergmann et al., 2015*), Musk deer (*Moschus berezovskii* and *M. chrysogaster*) (*Hu et al., 2018*; *Jiang et al., 2021*), Blue sheep (*Pseudois nayaur*) (*Wu, 2020*), yaks (*Bos grunniens*) and Tibetan sheep (*Ovis aries*) (*Wei et al., 2021*). Captivity can shift the diversity of fecal bacteria of Sika deer (*Cervus Nippon hortulorum*) (*Guan et al., 2017*) and Père David's deer (*Elaphurus davidianus*) (*Wang et al., 2019*). Different regions can also influence the composition and diversity of the gut microbiota of *E. davidianus* (*Zhang et al., 2018a*; *Zhang et al., 2018b*). Winter enclosures alter the microbial communities of red deer (*C. elaphus*) (*Menke et al., 2019*), while diet (crop-raiding versus noncrop-raiding) and habitat (forest versus savanna) influence the microbial communities of African savanna elephant (*Loxodonta africana*) (*Budd et al., 2020*). Provenance and sex can also influence the microbial communities of white-tailed deer (*Odocoileus virginianus*) (*Minich et al., 2021*). Thus, the variations in the composition and diversity of the gut microbiota in these herbivores can be associated with shifts in dietary nutrition.

White-lipped deer (*C. albirostris*), a Cervidae species endemic to China, are mainly distributed throughout Gansu, Qinghai, Tibet, Sichuan, and Yunnan Provinces in China, and they live in alpine meadows and alpine shrubs at elevations ranging from 3,500 to 5,100 m (*Cai, 1988*). As the historic over-exploitation, grazing competition with livestock and habitat degradation (*Harris, 2015*), white-lipped deer has been defined as an endangered species, and it is on the Red List of China's Vertebrates (*Jiang et al., 2016*) and the Category I key National Protected Wild Animal Species List in China. The microbial composition of the gut (*Li et al., 2017*), rumen, reticulum, omasum, and abomasum (*Wang, 2020*) of white-lipped deer has been reported. Furthermore, captivity can significantly influence the composition of the gut microbiota of white-lipped deer (*Li et al., 2022*). However, seasonal changes of the gut microbiota in white-lipped deer have not been thoroughly investigated. In recent years, two seasonal fecal samples of white-lipped deer have been collected from the Gansu Qilian Mountain National Nature Reserve, which provides an opportunity to study the seasonal variations of the gut microbiota. Due to the unique climatic conditions of the Gansu Qilian Mountain National Nature Reserve, the division of the four seasons is not obvious; thus, one year was divided into the grassy season (May to October) and the withering season (November to the next April) based on the growth of vegetation. In the grassy season, white-lipped deer live in alpine meadows and feed on herbs, whereas in the withering season, they migrate to lower altitudes and live at the boundary of alpine meadows and alpine shrubs, where they not only feed on withered herbs but also graze on tree barks and shrubs (pers. obs. of Zhangqiang, You, 2018–2019). During these two seasons, the shapes of the fecal samples were significantly different, and showed significant

environment and diets effects. The shape of fecal samples was granular in the withering season, similar to goat fecal samples, while it was pile-like in the grassy season, similar to cattle fecal samples. Thus, we wonder how the gut microbiota of white-lipped deer can adapt to the ever-changing environments and diets. Here, we explore the seasonal variations of the gut microbiota in white-lipped deer through 16S rRNA high-throughput sequencing based on fecal samples. We hypothesize that the composition and diversity of the gut microbiota differ significantly between the seasons. This study provides new insights into the evolutionary adaptation of the gut microbiota to changes in environments and diets, and provides a scientific basis for the health assessment of white-lipped deer.

MATERIALS AND METHODS

Ethics statement

Fecal samples were collected after foraging to ensure that the white-lipped deer were devoid of human disturbance. Permission for the collection of fecal samples was obtained from A Cheng of Gansu Qilian Mountain National Nature Reserve.

Sample collection and preservation

Fecal samples were collected from white-lipped deer in the Gansu Qilian Mountain National Nature Reserve (97°25′–103°46′E, 36°43′–39°36′N). Eight fecal samples were collected for each season (grassy season: October 1 to 10, 2018; withering season: May 1 to 10, 2019).

Fecal samples were collected from the daily patrol route of the nature reserve. To ensure that the samples came from different individuals, only one fecal sample was collected from the same fecal pile, and the distance of each fecal sample was greater than 2.0 m. The surface of each fecal sample was removed, and only the middle portion was used to ensure the freshness of the samples and to avoid air and soil contaminants. Fecal samples were initially stored in a cooler for no more than one day, and then stored at ∼196 °C in liquid nitrogen until further study. Samples did not consider the sex and age of the individuals because we did not witness the process of excretion.

DNA extraction, 16S rRNA amplification, and sequencing

Genomic DNA was extracted from each sample using the Soil FastDNA® SPIN Kit (MP Biomedicals, Santa Ana, CA, USA) following the protocol of the manufacturer. The quality and concentration of the DNA were quantified using the ND-1000 NanoDrop® spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) and 1% agarose gels for electrophoresis. The universal primers 338F and 806R (5′-ACTCCTACGGGAGGCAGCAG-3′; 5′-GGACTACHVGGGTWTCTAAT-3′) were used to amplify the V3-V4 hypervariable region of the bacterial 16S ribosomal RNA gene in fecal samples by the polymerase chain reaction (*Zhou et al., 2016*). PCR amplifications were performed in a 20 μL volume of the reaction mixture containing 19 Phanta® Max Buffer, 200 μM dNTPs, 0.5 U Phanta® Max Super-Fidelity DNA Polymerase (Vazyme Biotech Co., Ltd, Beijing, China), 0.2 μM forward primer, 0.2 μM reverse primer, and 10 ng of the DNA template (*Li et al., 2017*). Thermal cycling consisted of an initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 15 s, annealing at

50 °C for 15 s, and elongation at 72 °C for 15 s. A final extension at 72 °C for 5 min was included at the end of the thermal cycling protocol. PCR products were extracted from 1.5% agarose gels and purified using the EZNA® Gel Extraction Kit (Omega Bio-Tek, Inc., USA). Purified products were sequenced on the Illumina MiSeq 2500 System (Illumina Inc., USA) according to a standard protocol, and 2×300 bp paired-end reads were generated. Sequencing procedures were delegated to a commercial company: Shanghai Majorbio Bio-Pharm Technology Co., Ltd (https://cloud.majorbio.com/).

Data analysis

The raw data were deposited into GenBank (Accession number: PRJNA792628). All sequences were trimmed and denoised with Mothur Software (version1.30.2) using the following criteria: reads that were shorter than 200 bp, contained any ambiguous bases, and exhibited homopolymers that were longer than 8 bp were discarded (*Schloss et al., 2009*). Sequences were clustered into operational taxonomic units (OTUs) using a 97% identity threshold. Taxonomic analysis of the OTUs was performed by aligning each 16S rRNA gene sequence to the SILVA ribosomal RNA gene database (release 138, https://www.arb-silva.de/) using a confidence threshold of 80% (*Quast et al., 2013*).

Alpha diversity indices (Shannon, Simpson, abundance-based coverage (ACE), and Chao1) were calculated with Mothur Software, and the significant differences between two seasons were determined by the Wilcoxon rank-sum test. Beta diversity was assessed using principal coordinates analysis (PCoA), which was performed using QIIME (Quantitative Insights Into Microbial Ecology) Software (version 1.9.1) (*Caporaso et al., 2010*) based on weighted and unweighted unifrac distance matrices. Analysis of similarities (ANOSIM) was used to determine the significance of the difference in two seasons. The Wilcoxon rank-sum test was used to verify significant differences between two seasons at phylum and genus levels. Linear discriminant analysis effect size (LEfSe) (*Segata et al., 2011*) was applied to analyze the potential biomarkers with statistical differences between the two seasons. All statistical analyses were performed on the services platform of Majorbio Bio-Pharm Technology Co., Ltd. (Majorbio Bio-Pharm Technology Co., Ltd., Shanghai China: https://cloud.majorbio.com/). Values are presented as mean $\pm$ standard error, and the significance level used in all tests was $P < 0.05$.

RESULTS

Gut microbiota profiles

After quality filtering, a total of 906,913 high-quality sequences were obtained, with a mean of 56,682.06 sequences per sample. The sequences yielded 1,510 OTUs at a 97% similarity level with an average length of 425.78 bp per sequence. Among these OTUs, 957 OTUs were shared between seasons, and 467 and 86 were unique in the grassy and withering seasons, respectively (Fig. 1). Sobs curves, Shannon index curves, and rank abundance curves (Fig. S1) for all samples suggested that there were sufficient sequences for further analyses. The Good's coverage (>99.78%) indicated that most gut bacterial communities of diverse species were retrieved from the samples.
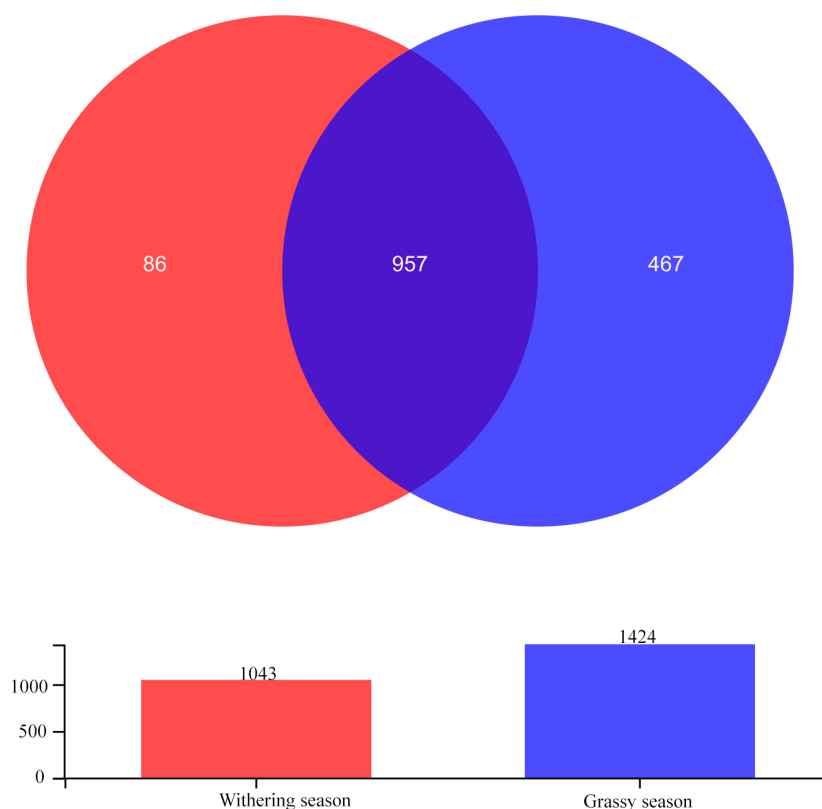
Figure 1 Venn diagram showing the unique and shared gut bacterial operational taxonomic units (OTUs) between grassy and withering season.

Full-size 🖼 DOI: 10.7717/peerj.13753/fig-1

Variations in the gut microbiota diversity between grassy and withering seasons

Alpha diversity indices, including Shannon, Simpson, ACE, and Chao1, were calculated for each season to examine whether there were diversity differences in the gut microbial community between the two seasons. The Simpson index was not significantly different ($P = 0.44$) between the two seasons (Fig. 2B), whereas Shannon ($P = 0.01$), ACE ($P = 0.00$), and Chao1 ($P = 0.00$) indices of the grassy season were significantly higher than those of the withering season (Figs. 2A, 2C, and 2D).

With regard to the beta diversity, PCoA analysis based on weighted (Fig. 3A) and unweighted (Fig. 3B) unifrac distances was carried out to determine the differences between the two seasons. The PCoA plot showed that the samples of the grassy and withering seasons clustered separately (ANOSIM tests, weighted $R = 0.523$, $P = 0.001$; unweighted $R = 0.843$, $P = 0.001$), suggesting that there were differences in the structure of the gut microbiota in the two seasons.
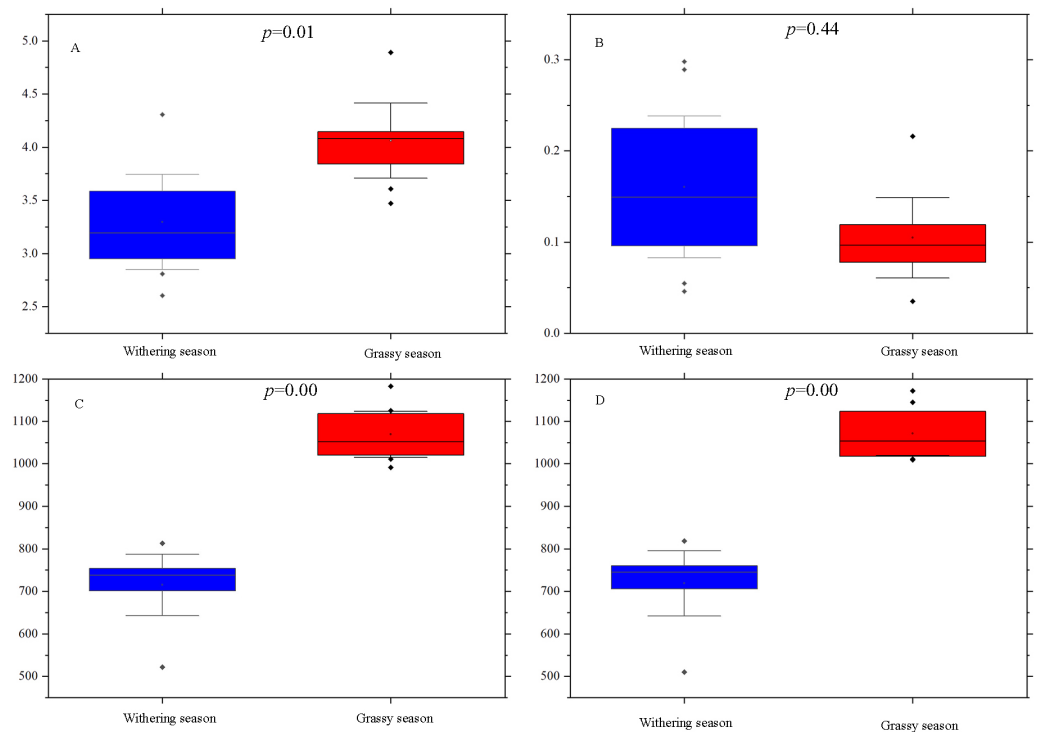
Figure 2 **Alpha diversity indices difference analysis between grassy and withering season.** (A) Shannon index, (B) Simpson index, (C) ACE, (D) Chao1. Error bars indicate standard deviation.

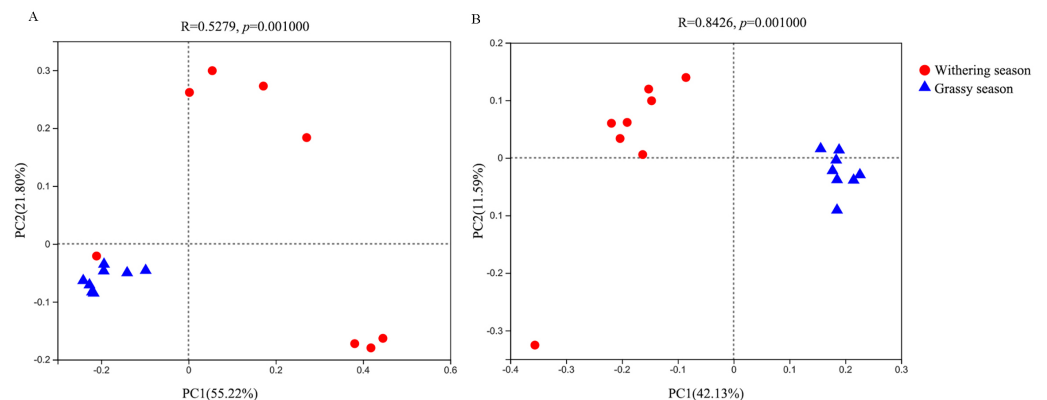Full-size 🖼 DOI: 10.7717/peerj.13753/fig-2

Figure 3 **Beta diversity difference in gut microbiota between grassy and withering season.** (A) Weighted-unifrac, (B) unweighted-unifrac.

Full-size 🖼 DOI: 10.7717/peerj.13753/fig-3

Variations in the gut microbiota composition between grassy and withering seasons

Representatives of 574 species, 362 genera, 187 families, 106 orders, 42 classes, and 20 phyla were detected based on taxonomic assignments at a sequencing identity level of 97%. At the phylum level, the gut microbiota was mainly comprised of Firmicutes (55.18%
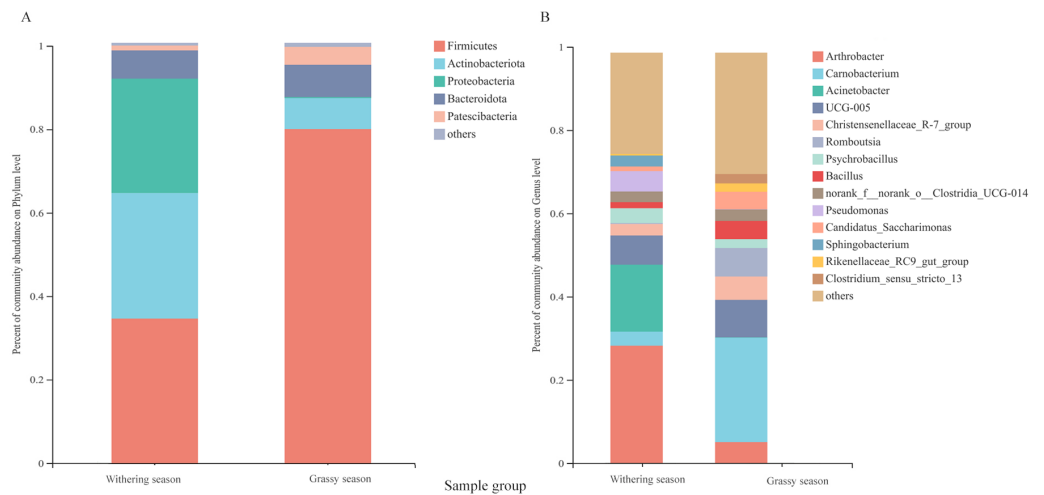
Figure 4 Gut microbiota composition at the phylum and genus level between grassy and withering season. (A) Phylum level, (B) genus level. Only phyla and genera with relative abundance greater than 1% are shown in the bar chart and the other taxons (<0.01%) are combined.

± 0.02%), Actinobacteria (19.52% ± 0.12%), Proteobacteria (14.78% ± 0.06%), and Bacteroidota (7.16% ± 0.00%), and these phyla accounted for approximately 96.64% of the gut microbial community (Fig. S2A). At the genus level, the gut microbiota was mainly comprised of (relative abundance, >5%) *Arthrobacter* (17.75% ± 3.94%), *Carnobacterium* (13.60% ± 0.00%), *Acinetobacter* (8.81% ± 0.79%), and Ruminococcaceae *UCG-005* (8.04% ± 0.24%) (Fig. S2B). However, the relative abundance of the predominant phyla and genera between the two seasons were different. The predominant phyla were Firmicutes (79.51% ± 0.01%), Bacteroidota (7.68% ± 0.01%), Actinobacteria (7.39% ± 0.18%), and Patescibacteria (4.25% ± 0.01%) in the grassy season, and they shifted to Firmicutes (34.41% ± 0.03%), Actinobacteria (29.87% ± 0.04%), Proteobacteria (27.22% ± 0.00%), and Bacteroidota (6.71% ± 0.00%) in the withering season (Fig. 4A). The predominant genera were *Carnobacterium* (25.49% ±0.00%) in the grassy season, and changed to *Arthrobacter* (28.57% ± 1.26%), and *Acinetobacter* (16.32% ± 0.00%) in the withering season (Fig. 4B). The top five phyla and ten genera with significant different between two seasons were compared based on the Wilcoxon rank-sum test (Fig. 5). In the grassy season, the relative abundances of Firmicutes, Patescibacteria, and Cyanobacteria were significantly higher than those in the withering season. By contrast, the relative abundances of Actinobacteriota and Proteobacteria in the grassy season were significantly lower than those in the withering season (Fig. 5A). At the genus level, the relative abundances of *Carnobacterium*, *Christensenellaccea_R-7_group*, *Romboutsia*, *Candidatus_Saccharimonas*, and *Rikenellaceae_RC9_gut_group* in the grassy season were significantly higher than those in the withering season. By contrast, the withering season had significantly higher relative abundances of *Arthrobacter*, *Acinetobacter*, *Psychrobacillus*, *Pseudomonas*, and *Sphingobacterium* compared to the grassy season (Fig. 5B).
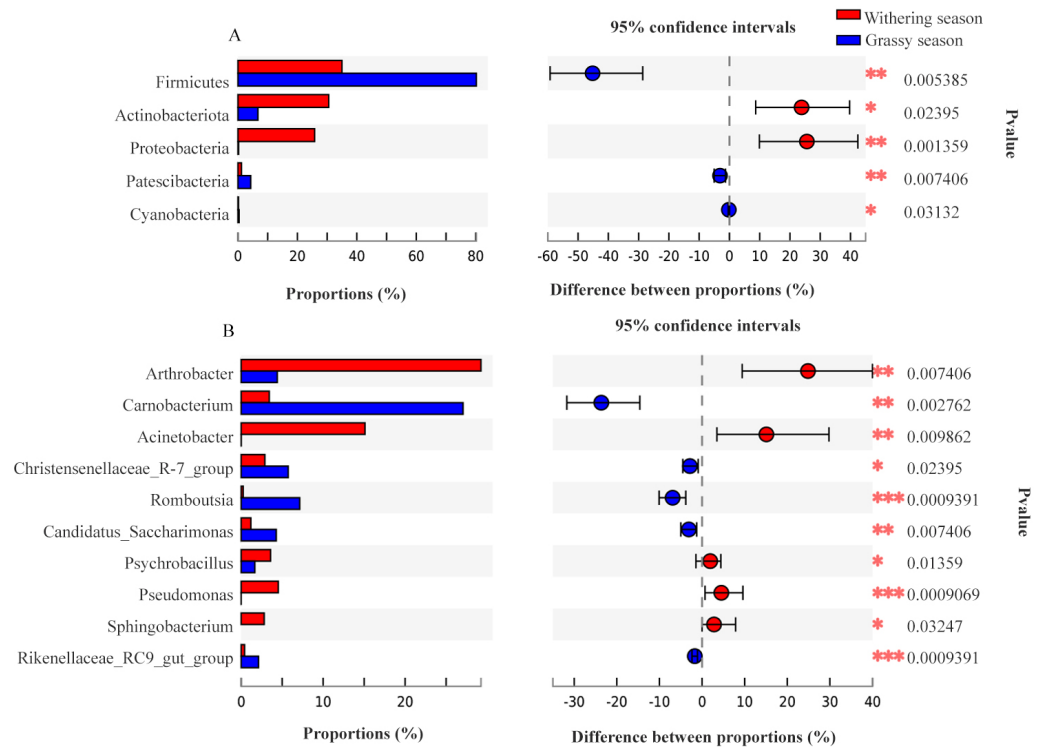
Figure 5 The top five phyla and ten genera with significant different between grassy and withering season. (A) Phylum level, (B) genus level.

To identify the specific microbial communities that existed in each group, LEfSe analysis was conducted. Thirty-nine biomarkers were significantly different (LDA > 4.0, $p < 0.05$, Fig. 6), and fifteen and twenty-four biomarkers were presented in grassy and withering seasons, respectively. At the phyla level, Actinobacteria and Proteobacteria were significantly enriched in the withering season, whereas Firmicutes, Bacteroidota, and Patescibacteria were significantly enriched in the grassy season (Fig. 6A). At the genus level, *Arthrobacter*, *Acinetobacter*, *Pseudomonas*, and *Psychrobacillus* were significantly enriched in the withering season, whereas *Carnobacterium*, *Romboutsia*, *Candidatus_Saccharimonas*, *Christensenellaccea_R-7_group*, and *Clostridium_sensu_stricto_13* were significantly enriched in the grassy season (Fig. 6A). The core bacterial species with remarkable differences ($P < 0.05$) at all levels are shown in Fig. 6B.

DISCUSSION

In recent years, several studies have been published on the gut (*Li et al., 2017*; *Li et al., 2022*; *Wang, 2020*) microbiota of white-lipped deer, but the influence of seasons on the gut microbiota has not been investigated. This is the first study to explore the seasonal variations of the gut microbiota in white-lipped deer based on 16S rRNA gene sequencing of fecal samples. The results demonstrated that the composition and diversity of the gut microbiota, as expected, were significantly influenced by season.
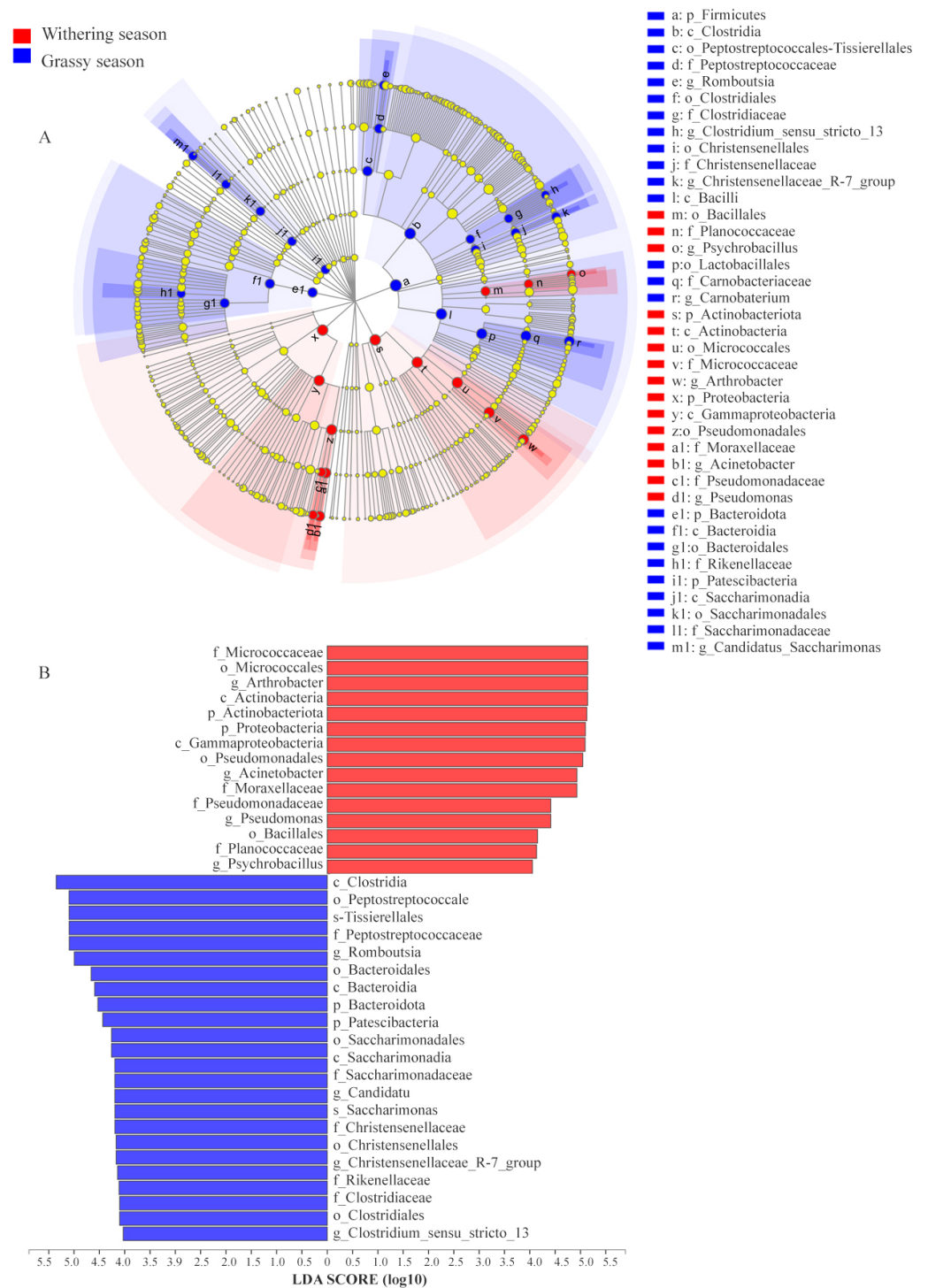
A

Withering season
Grassy season

a: p_Firmicutes
b: c_Clostridia
c: o_Peptostreptococcales-Tissierellales
d: f_Peptostreptococcaceae
e: g_Romboutsia
f: o_Clostridiales
g: f_Clostridiaceae
h: g_Clostridium_sensu_stricto_13
i: o_Christensenellales
j: f_Christensenellaceae
k: g_Christensenellaceae_R-7_group
l: c_Bacilli
m: o_Bacillales
n: f_Planococcaceae
o: g_Psychrobacillus
p:o_Lactobacillales
q: f_Carnobacteriaceae
r: g_Carnobaterium
s: p_Actinobacteriota
t: c_Actinobacteria
u: o_Micrococcales
v: f_Micrococcaceae
w: g_Arthrobacter
x: p_Proteobacteria
y: c_Gammaproteobacteria
z:o_Pseudomonadales
a1: f_Moraxellaceae
b1: g_Acinetobacter
c1: f_Pseudomonadaceae
d1: g_Pseudomonas
e1: p_Bacteroidota
f1: c_Bacteroidia
g1:o_Bacteroidales
h1: f_Rikenellaceae
i1: p_Patescibacteria
j1: c_Saccharimonadia
k1: o_Saccharimonadales
l1: f_Saccharimonadaceae
m1: g_Candidatus_Saccharimonas

B

Figure 6 Linear discriminant analysis effect size (LEfSe) analysis of gut microbiota composition between grassy and withering season (LDA ≥ 4.0, $P < 0.05$). (A) Taxonomic representation of statistically and biologically consistent differences between grassy and withering season.

Among the alpha diversity indices, Chao1 and ACE indices were used to estimate species
richness, and Shannon and Simpson indices revealed species diversity (*Zhang et al., 2018a*;
Zhang et al., 2018b). Higher Chao1 and ACE indices indicate higher richness, while a
higher Shannon index and a lower Simpson index indicate higher diversity. In this study,
the diversity and richness of the gut microbiota of white-lipped deer in the grassy season
was higher than those of the withering season. Differences in food resources are the most
direct and important factor affecting the diversity and abundance of the gut microbiota
(*Yun et al., 2014*). Food resources can also change over temporal scales. In the grassy
season, all vegetation thrives, while it withers in the withering season. For white-lipped
deer, food resources and choices were plentiful in the grassy season, but relatively poor
in the withering season. Furthermore, nutrient levels (*e.g.*, crude fat, crude protein, and
crude fiber) in the grassy season were higher than those in the withering season (*Zhang
et al., 2018a*; *Zhang et al., 2018b*; *Buxton, 1996*), with higher nutrient levels being more
conducive to microbial fermentation (*Yang et al., 2012*). Although the nutritional content
of the diet of white-lipped deer was not investigated, those of other species of Cervidae
were examined, and the findings demonstrated that the nutritional content is influenced
by different seasons. For example, the averaged winter-spring diets of *Rangifer tarandus*
contained a significantly higher amount of crude fiber and lower amounts of crude protein
and crude fat compared to summer-autumn diets (*Yildirim et al., 2021*). Thus, the diversity,
richness, and high nutrient levels of food in the grassy season led to greater species richness
and diversity of the gut microbiota.

At the phylum level, white-lipped deer had higher abundances of Firmicutes,
Bacteroidota, and Patescibacteria in the grassy season, and higher abundances of
Actinobacteria and Proteobacteria in the withering season (Figs. 4 and 5). Firmicutes
can degrade various substances, which helps the host to digest and absorb certain nutrients
(*Kaakoush, 2015*). The proportion of Firmicutes in the gut microbiota was positively
correlated with the level of nutrition (*De Filippo et al., 2010*). Bacteroidetes is responsible
for degrading carbohydrates and proteins (*Fernando et al., 2010*; *Jami, White & Mizrahi,
2014*), and it can improve the host's nutritional outlook (*Colston & Jackson, 2016*). The
diversity, resources, and nutrient levels of food of white-lipped deer in the grassy season
were relatively rich, and the higher abundances of Firmicutes and Bacteroidetes could
improve the breakdown of food (*Xu et al., 2003*; *Backhed et al., 2005*; *Kaakoush, 2015*),
thereby generating more energy. Patescibacteria (also known as candidate phyla radiation)
is ubiquitous and abundant in groundwater (*Rinke et al., 2013*; *He et al., 2021*). Drinking
water is a source of Patescibacteria in the human oral cavity (*Pinto et al., 2014*; *Bautista-de
Los Santos et al., 2016*). Furthermore, Patescibacteria abundance was positively correlated
with ambient temperature (*Qiu et al., 2020*). The ambient temperature in the grassy

season is higher than that of the withering season, and groundwater flows freely in the grassy season, but it freezes in the withering season. Thus, the ambient temperature and availability of groundwater for white-lipped deer may have contributed to the differences in the abundance of Patescibacteria. Furthermore, Patescibacteria abundance was positively correlated with plasma total protein, revealing that Patescibacteria may accelerate the absorption of total protein (*Qiu et al., 2020*). Thus, a high Patescibacteria abundance in the grassy season contributes to diets with high protein levels. Proteobacteria can promote cellulose activity, degrade a variety of aromatic compounds (*Reid et al., 2011*), and flexibly adjust metabolic processes to tolerate low-nutrition foods (*Berg et al., 2016*). The cellulolytic enzymes of Actinobacteria can promote the degradation of cellulose (*Berlemont & Martiny, 2013*). In the withering season, most vegetables are withered, indicating that food diversity and food resources are low. To survive, white-lipped deer must acquire food with a lower nutrient level, which has a higher proportion of crude fiber. Furthermore, white-lipped deer migrate to lower altitudes in the withering season, and the habitat shifts from alpine meadows to the boundary at alpine meadows and alpine shrubs. White-lipped deer not only feed on withered herbs but also graze on tree barks and shrubs. Thus, the food of white-lipped deer in the withering season has a higher proportion of crude fiber. A higher abundance of Proteobacteria and Actinobacteria can help to degrade crude fiber, such as lignin, in food sources (*Fang et al., 2012*). However, some members of the phylum Proteobacteria are common opportunistic pathogenic bacteria, which can cause a variety of diseases (*Xi et al., 2021*). A higher abundance of Proteobacteria in the withering season indicates that white-lipped deer are susceptible to diseases. Actinobacteria can produce many bioactive compounds (*Raissa, Waturangi & Wahjuningrum, 2020*), with actinomycetes producing many antibiotics that play important roles in the immunity of the host (*Matsui et al., 2012*). Thus, a high abundance of Actinobacteria can resist the onset of disease, while the combined actions of Proteobacteria and Actinobacteria can promote animal health. Variations in the components of the gut microbiota at the phylum level are indicative of the adaptation of the gut microbiota in relation to the changed environments and diets.

In addition, significantly different gut microbial species between two seasons were found at the genus level. For example, samples from the withering season were enriched in *Arthrobacter*, *Acinetobacter*, *Pseudomonas*, and *Psychrobacillus*, whereas those from the grassy season were enriched in *Carnobacterium*, *Romboutsia*, *Candidatus_Saccharimonas*, *Christensenellaccea_R-7_group*, and *Clostridium_sensu_stricto_13* (Figs. 5 and 6). In the grassy season temperatures were relatively high, foods were relatively rich, and nutritional levels of food were relatively high, but the opposite was true in the withering season, which lead to starvation and malnutrition of white-lipped deer in the withering season. *Arthrobacter* not only has nutritional versatility but also plays important roles in desiccation resistance, long-term starvation, and environmental stress (*Niewerth et al., 2012*), and enriched *Arthrobacter* can ensure white-lipped deer are not subject to these restrictions. *Acinetobacter* and *Pseudomonas*, two conditional pathogens, can cause a variety of diseases (*Gauthier, 2015*). In addition, some species of *Pseudomonas* play an important role in protein degradation (*Liu et al., 2017*). Higher abundances of *Acinetobacter* and

Pseudomonas indicate that white-lipped deer had a higher risk of disease in the withering season, with a higher abundance of Pseudomonas compensating for the decrease of other protein-degrading microbes. *Carnobacterium* responds to thermal stress, resulting in more efficient feed assimilation (*Nguyen et al., 2020*). *Romboutsia* is involved in the fermentation of carbohydrates and the utilization of single amino acids (*Gerritsen, 2015*). *Candidatus_Saccharimonas* is associated with inflammation and the host's immunological response (*Li et al., 2021a*; *Li et al., 2021b*). *Clostridium* can digest simple carbohydrates and complex polysaccharides (*Bäckhed et al., 2004*; *Ramos et al., 2015*; *Aristilde, 2017*), which may lead to increased carbohydrate metabolism (*Dong et al., 2019*). *Christensenellaccea_R-7_group* is involved in amino acid and lipid metabolism (*Waters & Ley, 2019*) and plays crucial roles in increasing blood sugar levels and promoting obesity in the host (*Tavella et al., 2021*). Enriched *Carnobacterium*, *Romboutsia Candidatus_Saccharimonas*, *Christensenellaccea_R-7_group*, and *Clostridium_sensu_stricto_13* can increase immunity and promote digestion in white-lipped deer.

CONCLUSION

To the best of our knowledge, this is the first report on the seasonal variations of the gut microbiota in white-lipped deer. The composition and diversity of the gut microbiota in white-lipped deer showed significant seasonal variations. The seasonal variations of the composition and diversity of the gut microbiota in white-lipped deer can be explained by variations in seasonal food availability, composition, diversity, and nutrition. This study indicates that the adaptive evolution of the gut microbiota changes according to the environment and provides the scientific basis for the health assessment of white-lipped deer.

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ADDITIONAL INFORMATION AND DECLARATIONS

Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Zhangqiang You conceived and designed the experiments, performed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Jing Deng performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Jialin Liu performed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Junhua Fu performed the experiments, prepared figures and/or tables, and approved the final draft.
- Huan Xiong performed the experiments, analyzed the data, prepared figures and/or tables, and approved the final draft.
- Wei Luo performed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Jianli Xiong conceived and designed the experiments, performed the experiments, authored or reviewed drafts of the article, and approved the final draft.

Field Study Permissions

The following information was supplied relating to field study approvals (i.e., approving body and any reference numbers):

Gansu Qilian Mountain National Nature Reserve approved the study. Permission for the collection of fecal samples was oral authorized by A Cheng of Gansu Qilian Mountain National Nature Reserve.

Data Availability

The following information was supplied regarding data availability:

The gut microbiota sequences are available at GenBank: PRJNA792628.

Supplemental Information

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REFERENCES

Aricha H, Simujide H, Wang CJ, Zhang J, Lv WT, Jimisi X, Liu B, Chen H, Zhang C, He L, Cui YX, Gao RJ, Aorigele C. 2021. Comparative analysis of fecal microbiota of grazing Mongolian cattle from different regions in Inner Mongolia, China. *Animals* **11**:1938 DOI 10.3390/ani11071938.

Aristilde L. 2017. Metabolite labelling reveals hierarchies in Clostridium acetobutylicum that selectively channel carbons from sugar mixtures towards biofuel precursors. *Microbial Biotechnology* **10**:162–174 DOI 10.1111/1751-7915.12459.

Bäckhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, Semenkovich CF, Gordon JI. 2004. The gut microbiota as an environmental factor that regulates fat storage. *Proceedings of the National Academy of Sciences of the United States of America* **101**:15718–15723 DOI 10.1073/pnas.0407076101.

Backhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. 2005. Host-bacterial mutualism in the human intestine. *Science* **307**:1915–1920 DOI 10.1126/science.1104816.

Bautista-de Los Santos QM, Schroeder JL, Sevillano-Rivera MC, Sungthong R, Ijaz UZ, Sloan WT, Pinto AJ. 2016. Emerging investigators series: microbial communities in full-scale drinking water distribution systems –a meta-analysis. *Environmental Science Water Research & Technology* **2**:631–644 DOI 10.1039/C6EW00030D.

Berg M, Stenuit B, Ho J, Wang A, Parke C, Knight M, Alvarez-Cohen L, Shapira M. 2016. Assembly of the Caenorhabditis elegans gut microbiota from diverse soil microbial environments. *The ISME Journal* **10**:1998–2009 DOI 10.1038/ismej.2015.253.

Bergmann GT, Craine JM, Robeson NMS, Fierer N, Maldonado JE. 2015. Seasonal shifts in diet and gut microbiota of the American Bison (*Bison bison*). *PLOS ONE* **10**:e142409 DOI 10.1371/journal.pone.0142409.

Berlemont R, Martiny AC. 2013. Phylogenetic distribution of potential cellulases in bacteria. *Applied and Environmental Microbiology* **79**:1545–1554 DOI 10.1128/AEM.03305-12.

Budd K, Gunn JC, Finch T, Klymus K, Sitati N, Eggert LS. 2020. Effects of diet, habitat, and phylogeny on the fecal microbiome of wild African savanna (*Loxodonta africana*) and forest elephants (*L. cyclotis*). *Ecology and Evolution* **10**:5637–5650 DOI 10.1002/ece3.6305.

Buxton DR. 1996. Quality-related characteristics of forages as influenced by plant environment and agronomic factors. *Animal Feed Science and Technology* **59**:37–49 DOI 10.1016/0377-8401(95)00885-3.

Cai GQ. 1988. Notes on white-lipped deer (*Cervus albirostris*) in China. *Act Prarataculturae Sinica* **8**:7–12.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R. 2010. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* **7**:335–336 DOI 10.1038/nmeth.f.303.

Chang J, Yao X, Zuo C, Qi Y, Chen D, Ma W. 2020. The gut bacterial diversity of sheep associated with different breeds in Qinghai province. *BMC Veterinary Research* **16**:254 DOI 10.1186/s12917-020-02477-2.

Colston TJ, Jackson CR. 2016. Microbiome evolution along divergent branches of the vertebrate tree of life: what is known and unknown. *Molecular Ecology* **25**:3776–3800 DOI 10.1111/mec.13730.

De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, Collini S, Pieraccini G, Lionetti P. 2010. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proceedings of*

the National Academy of Sciences of the United States of America **107**:14691–14696
DOI 10.1073/pnas.1005963107.

Dong Y, Xiang X, Zhao G, Song Y, Zhou L. 2019. Variations in gut bacterial communities of hooded crane (*Grus monacha*) over spatial–temporal scales. *PeerJ* **7**:e7045
DOI 10.7717/peerj.7045.

Fan QS, Wanapat M, Yan TH, Hou FJ. 2020. Altitude influences microbial diversity and herbage fermentation in the rumen of yaks. *BMC Microbiology* **20**:370
DOI 10.1186/s12866-020-02054-5.

Fang W, Fang Z, Zhou P, Chang F, Hong Y, Zhang X, Peng H, Xiao Y, Neufeld J. 2012.
Evidence for lignin oxidation by the giant panda fecal microbiome. *PLOS ONE*
7:e50312 DOI 10.1371/journal.pone.0050312.

Feng T, Ding HX, Wang J, Xu W, Liu Y, Á Kenéz. 2021. Alterations of serum metabolites and fecal microbiota involved in ewe follicular cyst. *Frontiers in Microbiology*
12:675480 DOI 10.3389/fmicb.2021.675480.

**Fernando SC, Purvis HT, Najar FZ, Sukharnikov LO, Krehbiel CR, Nagaraja TG, Roe
BA, Desilva U. 2010.** Rumen microbial population dynamics during adaptation to a high-grain diet. *Applied and Environmental Microbiology* **76**:7482–7490
DOI 10.1128/AEM.00388-10.

Gauthier DT. 2015. Bacterial zoonoses of fishes: a review and appraisal of evidence for linkages between fish and human infections. *The Veterinary Journal* **203**:27–35
DOI 10.1016/j.tvjl.2014.10.028.

Gerritsen J. 2015. The genus *Romboutsia*: genomic and functional characterization of novel bacteria dedicated to life in the intestinal tract. PhD thesis, Wageningen University, Wageningen, NL.

Guan Y, Yang H, Han S, Feng L, Wang T, Ge J. 2017. Comparison of the gut microbiota composition between wild and captive sika deer (*Cervus nippon hortulorum*) from feces by high-throughput sequencing. *AMB Express* **7**:1–13
DOI 10.1186/s13568-016-0313-x.

**Guo N, Wu QF, Shi FY, Niu JH, Zhang T, Degen AA, Fang QG, Ding LM, Shang ZH,
Zhang ZG, Long RJ. 2021.** Seasonal dynamics of diet-gut microbiota interaction in adaptation of yaks to life at high altitude. *NPJ Biofilms and Microbiomes* **7**:38
DOI 10.1038/s41522-021-00207-6.

Harris RB. 2015. Cervus albirostris. The IUCN Red List of Threatened Species 2015:
e.T4256A61976756. *Available at https://dx.doi.org/10.2305/IUCN.UK.2015-2.RLTS.
T4256A61976756.en* (accessed on 23 March 2022).

He C, Keren R, Whittaker ML, Farag IF, Doudna JA, Cate J, Banfield JF. 2021. Genome-resolved metagenomics reveals site-specific diversity of episymbiotic CPR bacteria and DPANN archaea in groundwater ecosystems. *Nature Microbiology* **6**:354–365
DOI 10.1038/s41564-020-00840-5.

Hu X, Liu G, Li Y, Wei Y, Lin S, Liu S, Zheng Y, Hu D. 2018. High-throughput analysis reveals seasonal variation of the gut microbiota composition within forest Musk Deer (*Moschus berezovskii*). *Frontiers in Microbiology* **9**:1674
DOI 10.3389/fmicb.2018.01674.

Ilina LA, Filippova VA, Brazhnik EA, Dubrovin AV, Yildirim EA, Dunyashev TP, Laptev GY, Novikova NI, Sobolev DV, Yuzhakov AA, Laishev KA. 2021. The comparative analysis of the ruminal bacterial population in Reindeer (*Rangifer tarandus L.*) from the Russian Arctic Zone: regional and seasonal effects. *Animals* **11**:911 DOI 10.3390/ani11030911.

Jami E, White BA, Mizrahi I. 2014. Potential role of the bovine rumen microbiome in modulating milk composition and feed efficiency. *PLOS ONE* **9**:e85423 DOI 10.1371/journal.pone.0085423.

Jiang F, Gao H, Qin W, Song P, Wang H, Zhang J, Liu D, Wang D, Zhang T. 2021. Marked seasonal variation in structure and function of gut microbiota in forest and Alpine Musk Deer. *Frontiers in Microbiology* **12**:699797 DOI 10.3389/fmicb.2021.699797.

Jiang ZG, Jiang JP, Wang YZ, Zhang E, Zhang YY, Li LL, Xie F, Cai B, Cao L, Zheng GM, Dong L, Zhang ZW, Ding P, Luo ZH, Ding CQ, Ma ZJ, Tang SH, Cao WX, Li CW, Hu HJ, Ma Y, Wu Y, Wang YX, Zhou KY, Liu SY, Chen YY, Li JT, Feng ZJ, Wang Y, Wang B, Li C, Song XL, Cai L, Zang CX, Zeng Y, Meng ZB, Fang HX, Ping XG. 2016. Red list of China's vertebrates. *Sheng Wu Duo Yang Xing* **24**:500–551 DOI 10.17520/biods.2016076.

Jiang F, Song P, Wang HJ, Zhang JJ, Liu DX, Cai ZY, Gao HM, Chi XW, Zhang TZ. 2022. Comparative analysis of gut microbial composition and potential functions in captive forest and alpine musk deer. *Applied Microbiology and Biotechnology* **106**:1325–1339 DOI 10.1007/s00253-022-11775-8.

Kaakoush NO. 2015. Insights into the role of erysipelotrichaceae in the human host. *Frontiers in Cellular and Infection Microbiology* **5**:84 DOI 10.3389/fcimb.2015.00084.

Khafipour E, Li S, Tun HM, Derakhshani H, Moossavi S, Plaizier JC. 2016. Effects of grain feeding on microbiota in the digestive tract of cattle. *Animal Frontiers* **6**:13–19 DOI 10.2527/af.2016-0018.

Kim M, Kim J, Kuehn LA, Bono JL, Berry ED, Kalchayanand N, Freetly HC, Benson AK, Wells JE. 2014. Investigation of bacterial diversity in the feces of cattle fed different diets. *Journal of Animal Science* **92**:683–694 DOI 10.2527/jas.2013-6841.

Kim YS, Tatsuya U, Kim B, Park M. 2020. Sex differences in gut microbiota. *The World Journal of Men's Health* **38**:48–60 DOI 10.5534/wjmh.190009.

Li Y, Cai Y, Huang Q, Tan W, Li B, Zhou H, Wang Z, Zou J, Ding C, Jiang B, Yoshida S, Zhou Y. 2021b. Altered fecal microbiome and metabolome in a mouse model of choroidal neovascularization. *Frontiers in Microbiology* **12**:738796 DOI 10.3389/fmicb.2021.738796.

Li Q, Chen C, Liu C, Sun W, Liu X, Ci Y, Song Y. 2021a. The effects of cellulose on AOM/DSS-Treated C57BL/6 colorectal cancer mice by changing intestinal flora composition and inflammatory factors. *Nutrition and Cancer-an International Journal* **73**:502–513 DOI 10.1080/01635581.2020.1756355.

Li B, Gao H, Song P, Liang C, Jiang F, Xu B, Liu D, Zhang T. 2022. Captivity shifts gut microbiota communities in white-lipped deer (*Cervus albirostris*). *Animals* **12**:431 DOI 10.3390/ani12040431.

Li JG, Wang CD, Tang ZH, Guo YQ, Zheng TC, Li YZ, You ZQ. 2017. The gut bacterial community composition of wild *Cervus albirostris* (white-lipped deer) detected by the 16S ribosomal RNA fene sequencing. *Current Microbiology* **74**:1100–1107 DOI 10.1007/s00284-017-1288-9.

Liu KZ, Xu Q, Wang LZ, Wang JW, Guo W, Zhou ML. 2017. The impact of diet on the composition and relative abundance of rumen microbes in goat, Asian-Australasian. *Journal of Animal Sciences* **30**:531–537 DOI 10.5713/ajas.16.0353.

Matsui T, Tanaka J, Namihira T, Shinzato N. 2012. Antibiotics production by an actinomycete isolated from the termite gut. *Journal of Basic Microbiology* **52(6)**:731–735 DOI 10.1002/jobm.v52.6.

Menke S, Heurich M, Henrich M, Wilhelm K, Sommer S. 2019. Impact of winter enclosures on the gut bacterial microbiota of red deer in the Bavarian Forest National Park. *Wildlife Biology* **2019**:1–10 DOI 10.2981/wlb.00503.

Minich D, Madden C, Evans MV, Ballash GA, Barr DJ, Poulsen KP, Dennis PM, Hale VL. 2021. Alterations in gut microbiota linked to provenance, sex, and chronic wasting disease in white-tailed deer (*Odocoileus virginianus*). *Scientific Reports* **11**:13218 DOI 10.1038/s41598-021-89896-9.

Nguyen C, Amoroso G, Ventura T, Elizur A. 2020. Assessing the pyloric caeca and distal gut microbiota correlation with flesh color in Atlantic Salmon (*Salmo salar L.*, 1758). *Microorganisms* **8**:1244 DOI 10.3390/microorganisms8081244.

Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, Pettersson S. 2012. Host-gut microbiota metabolic interactions. *Science* **336**:1262–1267 DOI 10.1126/science.1223813.

Niewerth H, Schuldes J, Parschat K, Kiefer P, Vorholt JA, Daniel R, Fetzner S. 2012. Complete genome sequence and metabolic potential of the quinaldine-degrading bacterium *Arthrobacter* sp. Rue61a. *BMC Genomics* **13**:534 DOI 10.1186/1471-2164-13-534.

Pinto AJ, Schroeder J, Lunn M, Sloan W, Raskin L. 2014. Spatial–temporal survey and occupancy-abundance modeling to predict bacterial community dynamics in the drinking water microbiome. *mBio* **5**:e1114–e1135 DOI 10.1128/mBio.01135-14.

Qiu QH, Gao CY, Azizur Rahman M, Cao BH, Su HW. 2020. Digestive ability, physiological characteristics, and rumen bacterial community of Holstein finishing steers in response to three nutrient density diets as fattening phases. *Advanced Microorganisms* **8**:335 DOI 10.3390/microorganisms8030335.

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research* **41**:D590–D596 DOI 10.1093/nar/gks1219.

Raissa G, Waturangi DE, Wahjuningrum D. 2020. Screening of antibiofilm and anti-quorum sensing activty of *Actinomycetes* isolates extracts against aquaculture pathogenic bacteria. *BMC Microbiology* **20**:343 DOI 10.1186/s12866-020-02022-z.

Ramos JL, Sol CM, Molina-Santiago C, Segura A, Duque E, Gomez-Garcia MR, Udaondo Z, Roca A. 2015. Mechanisms of solvent resistance mediated by interplay

of cellular factors in Pseudomonas putida. *Fems Microbiology Reviews* **39**:555–566 DOI 10.1093/femsre/fuv006.

Rastelli M, Cani PD, Knauf C. 2019. The gut microbiome influences host endocrine functions. *Endocrine Reviews* **40**:1271–1284 DOI 10.1210/er.2018-00280.

Reid NM, Addison SL, Macdonald LJ, Lloyd-Jones G. 2011. Biodiversity of active and inactive bacteria in the gut flora of wood-feeding Huhu beetle larvae (*Prionoplus reticularis*). *Applied and Environmental Microbiology* **77**:7000–7006 DOI 10.1128/AEM.05609-11.

Rinke C, Schwientek P, Sczyrba A, Ivanova NN, Anderson IJ, Cheng JF, Darling A, Malfatti S, Swan BK, Gies EA, Dodsworth JA, Hedlund BP, Tsiamis G, Sievert SM, Liu WT, Eisen JA, Hallam SJ, Kyrpides NC, Stepanauskas R, Rubin EM, Hugenholtz P, Woyke T. 2013. Insights into the phylogeny and coding potential of microbial dark matter. *Nature* **499**:431–437 DOI 10.1038/nature12352.

Round JL, Mazmanian SK. 2009. The gut microbiota shapes intestinal immune responses during health and disease. *Nature Reviews Immunology* **9**:313–323 DOI 10.1038/nri2515.

Rowland I, Gibson G, Heinken A, Scott K, Swann J, Thiele I, Tuohy K. 2018. Gut microbiota functions: metabolism of nutrients and other food components. *European Journal of Nutrition* **57**:1–24 DOI 10.1007/s00394-017-1445-8.

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF. 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology* **75**:7537–7541 DOI 10.1128/AEM.01541-09.

Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C. 2011. Metagenomic biomarker discovery and explanation. *Genome Biology* **12**:R60 DOI 10.1186/gb-2011-12-6-r60.

Tavella T, Rampelli S, Guidarelli G, Bazzocchi A, Gasperini C, Pujos-Guillot E, Comte B, Barone M, Biagi E, Candela M, Nicoletti C, Kadi F, Battista G, Salvioli S, O'Toole PW, Franceschi C, Brigidi P, Turroni S, Santoro A. 2021. Elevated gut microbiome abundance of *Christensenellaceae*, Porphyromonadaceae and *Rikenellaceae* is associated with reduced visceral adipose tissue and healthier metabolic profile in Italian elderly. *Gut Microbes* **13**:1–19 DOI 10.1080/19490976.2021.1880221.

Thaiss CA, Zmora N, Levy M, Elinav E. 2016. The microbiome and innate immunity. *Nature* **535**:65–74 DOI 10.1038/nature18847.

Wang S. 2020. *Analysis of microbiome structure and function in stomach of Cervidae.* Northeast Forestry University.

Wang L, Ding J, Yang Z, Chen H, Yao R, Dai Q, Ding Y, Zhu L. 2019. Père David's deer gut microbiome changes across captive and translocated populations: implications for conservation. *Evolutionary Applications* **12**:622–635 DOI 10.1111/eva.12743.

Wang YJ, Zhang H, Zhu L, Xu YL, Liu N, Sun XM, Hu LP, Huang H, Wei K, Zhu RL. 2018. Dynamic distribution of gut microbiota in goats at different ages and health states. *Frontiers in Microbiology* **9**:2509 DOI 10.3389/fmicb.2018.02509.

Waters JL, Ley RE. 2019. The human gut bacteria Christensenellaceae are widespread, heritable, and associated with health. *BMC Biology* **17**:83 DOI 10.1186/s12915-019-0699-4.

Wei X, Dong Z, Cheng F, Shi H, Zhou X, Li B, Wang L, Wang W, Zhang J. 2021. Seasonal diets supersede host species in shaping the distal gut microbiota of Yaks and Tibetan sheep. *Scientific Reports* **11**:22626 DOI 10.1038/s41598-021-99351-4.

Wu HL. 2020. Analysis of intestinal microbial diversity of Blue sheep in different seasons. *Chinese Journal of Animal Infectious Diseases* **28**:71–78.

Xi L, Song YM, Qin XN, Han JC, Chang YF. 2021. Microbiome analysis reveals the dynamic alternations in gut microbiota of diarrheal *Giraffa camelopardalis*. *Frontiers in Veterinary Science* **8**:649372 DOI 10.3389/fvets.2021.649372.

Xin J, Chai Z, Zhang C, Zhang Q, Zhu Y, Cao H, Zhong J, Ji Q. 2019. Comparing the microbial community in four stomach of dairy cattle, yellow cattle and three yak herds in Qinghai-Tibetan Plateau. *Frontiers in Microbiolology* **10**:1547 DOI 10.3389/fmicb.2019.01547.

Xu J, Bjursell MK, Himrod J, Deng SU, Carmichael LK, Chiang HC, Hooper LV, Gordon JI. 2003. A genomic view of the human: bacteroides thetaiotaomicron symbiosis. *Science* **299**:2074–2076 DOI 10.1126/science.1080029.

Yang DZ, Yan DH, Chen C, Yi Q, Chen GY. 2012. Effects of microbial fermentation on the nutritional quality of rape straw. *Guizhou Journal of Animal Husbandry & Veterinary Medicine* **35**:14–16.

Yildirim E, Ilina L, Laptev G, Filippova V, Brazhnik E, Dunyashev T, Dubrovin A, Novikova N, Tiurina D, Tarlavin N, Laishev K. 2021. The structure and functional profile of ruminal microbiota in young and adult reindeers (*Rangifer tarandus*) consuming natural winter-spring and summer-autumn seasonal diets. *PeerJ* **9**:e12389 DOI 10.7717/peerj.12389.

Yun J, Roh SW, Whon TW, Jung M, Kim M, Park D, Yoon C, Nam Y, Kim Y, Choi J, Kim J, Shin N, Kim S, Lee W, Bae J, Drake HL. 2014. Insect gut bacterial diversity determined by environmental habitat, diet, developmental stage, and phylogeny of host. *Applied and Environmental Microbiology* **80**:5254–5264 DOI 10.1128/AEM.01226-14.

Zhang M, Shi M, Fan M, Xu S, Li Y, Zhang T, Cha M, Liu Y, Guo X, Chen Q, Li Y, Zhang S, Hu D, Liu S. 2018a. Comparative analysis of gut microbiota changes in Père David's deer populations in Beijing Milu Park and Shishou, Hubei Province in China. *Frontiers in Microbiology* **9**:1258 DOI 10.3389/fmicb.2018.01258.

Zhang PP, Xue SY, Jin H, Li CQ, Cui C, Zhao YH. 2018b. Effects of different phenophas on rumen fermentation of grazing sheep. *China Animal Husbandry & Veterinary Medicine* **45**:698–704.

Zhao W, Ren ZW, Luo Y, Cheng JG, Wang J, Wang Y, Yang ZX, Yao XP, Zhong ZJ, Yang W, Wu X. 2021. Metagenomics analysis of the gut microbiome in

healthy and bacterial pneumonia forest musk deer. *Genes Genomics* **43(1)**:43–53
DOI 10.1007/s13258-020-01029-0.

Zhou S, Xu J, Zhu H, Wu J, Xu J, Yan R, Li X, Liu H, Duan S, Wang Z, Chen H, Shen H, Li S. 2016. Gut microbiota-involved mechanisms in enhancing systemic exposure of ginsenosides by coexisting polysaccharides in ginseng decoction. *Scientific Reports* **6**:22474 DOI 10.1038/srep22474.