Comparative Analysis of Gut Microbiome between Captive and Wild Red Deer 1 (Cervus elaphus) in Inner Mongolia

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Comparative analysis of Gut Microbiome between captive and wild red deer (*Cervus elaphus*) in Inner Mongolia

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Abstract: The digestive tract of ruminants is the home of the gut microbiome ecosystem, which plays a huge role in the diagnosis of various health conditions and the analysis of physiological conditions in wild animals. Red deer is a second-class protected animal in China. In this study, we used microsatellite and high-throughput sequencing of the 16S rRNA gene in fecal samples of red deer to investigate differences in the gut bacterial microbiota were analyzed between wild and captive in winter. Our results revealed that proportions of bacterial taxa, alpha-and beta-diversities, and relative abundances of amplicon sequence variants in the gut bacterial microbiota of the two groups differed. Firmicutes (79.46%), Bacteroidetes (16%) and Tenericutes (1.25%) were the most predominant phyla in wild red deer. While in captive red deer, Firmicutes (62.5%) was the dominant phylum, followed by Bacteroidetes (29.1%) and Tenericutes.(3.21%). The specific function and mechanism of Tenericutes in red deer need further study. The wild red deer had higher fecal bacterial diversity than the captive in farm. These differences were attributed to the enrichment of bacterial taxa involved in the digestion of the supplementary food and to different natural diets consumed in the forest. Also the dominant and differential microflora of intestinal microflora in various populations were mined and their related metabolic pathways. In terms of functional data, most of the genes annotated are related to metabolism. The second most commented gene is related to genetic information processing. The comparative study of the intestinal flora of the two populations can not only assess the health status of the two populations, but also provide important suggestions for the breeding of captive red

47 deer and the protection of wild populations.

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49 **Kywords:** Cervus elaphus; gut microbiome; Molecular scatology; 16SrRNA

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1. Background

Red deer (*Cervus elaphus*), a second-class protected animal with relatively high ecological and economic values, is widely distributed in Asia, Europe, and North America [1]. Among 23 subspecies of red deer, eight were found in China [2]. Dongbei red deer (*Cervus elaphus xanthopygus*) is found in The Greater Hinggan and Changbai mountain ranges in the northeast of China [3].

In recent years, with the advancement of techniques in molecular biology and the development of high-throughput sequencing technology, it has been discovered gradually that gut microbiome not only plays a role in digestion and absorption but also participates in a variety of physiological activities and plays a regulatory role [4]. Using 16S rRNA gene amplification sequencing or whole genome sequencing (WGS) and other advanced technologies to study the intestinal microbiota of red deer is the focus of animal conservation research in microbial ecology.

Intestinal microbiome plays a crucial role in its digestion, driving multiple aspects of the ecosystem of wild animals, such as nutrient acquisition, antimicrobial production, protection of the gut against pathogen invasion, and systemic changes in immune capacity. However, the structure of the intestinal microbiota depends on their habitat, environmental conditions, and diet [5,6].Nontargeted analysis of intestinal metabolites using fecal samples has become a convenient and reliable method for biomarker discovery [7].Many researchers have studied the gut microbiota of the deer family, such as Firmicutes (68%), where Bacteroidetes (14%) and Proteobacteria (10%) dominate the composition of gut bacteria in healthy horses [8].A large body of

91 evidence supports the need for the intestinal microbiome to maintain the balance of
92 the intestinal environment in herbivores. For example, compared with healthy musk
93 deer, Clostridium Escherichia coli is the main pathogen in the intestinal flora of
94 diarrhea musk deer [9].Costa et al. found that actinomycetes and spirochetes were the
95 main groups of intestinal flora of healthy horses, while the abundance of
96 Fusobacterium in colitis horses was significantly higher. If the homeostasis of the
97 intestinal microbiota is disrupted, the host may become ill [10].Rumen microbiome of
98 red deer in captivity and the effect of winter captivity on intestinal microbiome of red
99 deer have been studied [11,12].In the metagenomic analysis of the intestinal
100 microbiome of healthy and bacterial pneumonia forest deer, Zhao Wei et al. concluded
101 that the intestinal microbiome of bacterial pneumonia group had significantly changed
102 [13].In general, the study of intestinal microbiome interactions by measuring feces has
103 great potential to uncover diagnostic and physiological analyses of various health
104 conditions in wild animals. May help improve the health of captive red deer and be
105 helpful for captive management and future reintroduction programs.
106
107 **2. Description of study area**
108 The Gaogestai Reserve in Chifeng, Inner Mongolia, with an east longitude of
109 119°03′30″ to 119°39′08″, and north latitude of 44°41′03″ to 45°08′44″ is the location
110 for this study. The altitude of the area is between 900 and 1500 m, with a total area of
111 106284 hm2. It belongs to a semi-arid continental monsoon climate, with an average
112 temperature of 3.8°C, a frost-free period of 115 days, 437.3 mm of annual

precipitation, and 1958.1 mm of annual evaporation. This area, a double transition zone from leaf forest to coniferous forest, grassland to forest, has a clear diversity of grassland, forest, and shrub, and retains the original characteristics of vegetation, providing a favorable living environment for wild animals and plants. Fourteen rivers have been originated from the reserve, flowing eastward into the West Liao River and westward into the Xilin Gol Prairie. Hence, it is a typical and important forest for the conservation of water in Northeast of China.

3. Method

3.1. Field data sampling

From December 2018 to March 2020, 38 footprint chains were tracked in the red deer concentrated area according to the conditions of each gully section and the distribution of red deer in the Gaogestai Reserve in Chifeng, Inner Mongolia. A total of 117 stool samples were collected. Fecal samples were collected along the transect with PE gloves, and 30 grains of each pile of faeces were taken and put into a sealed bag. Forty-three samples of feces were individually identified in the laboratory. According to the GPS coordinate points of the collection site and the freshness of the samples, 22 individual samples were screened for 16S rRNA amplification and sequencing. The feces of ten different individuals red deer were collected in a captive farm in Chifeng, Inner Mongolia, and the males and females were recorded. In December of the winter of 2019, 10 samples of captive red deer feces were collected from a farm in Chifeng, Inner Mongolia, from different red deer individuals, and the

male and female conditions were recorded.

3.2. ***Individual recognition and gender identification***

Primers were selected according to the research results of our research group and the 10 pairs of microsatellite primers (C143, T507, DM42, DM45, T123, BM203, ETH225, T530, BM1225, N) with good polymorphism obtained in the literature. Eight microsatellite loci with a high polymorphism were selected for the individual identification of wild red deer in the genetic diversity analysis. Genotyping was performed by capillary electrophoresis. Cervus 3.0 was used to compare the genotype to determine whether the samples were taken from the same individual.

Gender identification was based on the unique SRY gene fragment of the Y chromosome. Specific primers SRY (F:5 '-3' TGAACGCTTTCATTGTGTGGTC; R: 5 '- 3' GCCAGTAGTCTCTGTGCCTCCT). Specific primers have been designed to amplify the DNA sequence, while after electrophoresis, a specific band appeared as male. Else, it was female. However, due to the improper experimental operation, there were often no specific bands. Hence, SRY12 and BMC1009 were used to amplify SRY gene fragments of the Y chromosome and autosomal microsatellite sites, respectively, in the case of gender misjudgment caused by experimental failures. However, SRY primers were used to amplify all samples separately to improve the accuracy of gender identification in the context of competition and interference in multiplex amplification. Each sample was amplified three times, and gender identification was based on a comparison of multiplex amplification and single amplification.

157 *3.3 Analysis of gut microbial community*

158 Microbial DNA was extracted from 22 samples using the E.Z.N.A.® Soil DNA
159 Kit (Omega Bio-tek, Norcross, GA, U.S.) according to manufacturer's protocols.
160 The universal primer 338F_806R of bacterial 16 S TDNA was used to amplify the
161 V3-V4 region. The total reaction system was 20 L, in which the DNA template was
162 10 ng, 5X fast pfu buffer was 4 pL, and 2.5 mM DNTP was 2 μL were used. 0.8 μL
163 of forward primer (5 UM), 0.8 wL of reverse primer (5 WM), 0.4 pL of fastpfu DNA
164 polymerase, 0.2 μL of BSA, and DI water was added into the volume. The
165 amplification process was divided into three steps, and the first step was to melt at
166 95℃ for 3 min, the second step was to melt at 95°C for 30 s, annealing at 52°C for
167 30 s, and then to extend at 72°C for 45 s with a cycle of 30 times. The third step had
168 to continue at 72°C for 10 min. Gel electrophoresis has been used for detection. The
169 enzyme-digested product was purified by an enzymatic purification kit purchased
170 from Sigma to remove residual enzymes and salts to clear peaks, which may appear
171 in the subsequent spectrum analysis. The proper linker sequence of Illumina was
172 added to the outside of the target area by PCR, while the Truseqtm DNA gel
173 recovery kit was able to recover the PCR product. Tris-HC buffer was used to elute,
174 2% agarose electrophoresis was used to detect, and finally, NaOH was used to
175 denature single-stranded DNA fragments for library construction and quality-tested
176 by Qubit 2.0 fluorometer and Agilent 2100 biochip analysis system. Subsequently,
177 the library was sequenced with Umina Hiseq2500.

178 *3.4.* ***Analysis of gut microbial community***

The original sequencing data was obtained by sequencing, in which there was a certain proportion of incorrect data. After the splicing and filtering of the original data the results of analysis information can be more accurate and reliable and get the Valid data. Then, the available data were used for clustering and species classification analysis of OTUs. According to OTUs clustering results, species annotation was carried out for each sequence of OTUs to calculate species richness. At the same time, the abundance and Alpha diversity of OTUs were calculated to obtain the information of species richness and evenness within the sample, as well as the common and unique OTUs among different samples or groups. On the other hand, multi-sequence alignment of OTUs can be performed and phylogenetic trees can be constructed to find the differences in community structure among different samples or groups. To further analyze the differences of sample community structure between groups, LEFSE statistical analysis method was used to test the significance of the differences in species composition and community structure of samples between groups. PicRust2 software was used to predict the function of microbial communities in ecological samples.

4. Results

4.1. Identification of individuals and sex

The sample DNA solution was melted on ice, thoroughly mixed and centrifuged, and 5ul solution was taken for 1% agarose gel electrophoresis detection. The main band was clear and single, without primer dimer, DMA degradation or impurity

contamination. The concentration and purity of the sample DNA were detected by ultraviolet spectrophotometer, and the test results were shown in Table 1, suggesting that the concentration and purity of the sample DNA were in line with the subsequent sequencing standards.

Table 1. The purity and concentration of DNA

Number	number of samples	concentration （ng/ul）	OD260/280	OD260/230
1	z1c	47.45	1.79	0.93
2	z16c	22.33	1.69	0.55
3	z23c	23.25	1.76	0.56
4	z31c	15.36	1.63	0.36
5	z48c	71.03	1.79	1.10
6	w3c	14.00	1.70	0.38
7	w15c	13.01	1.50	0.33
8	w31c	15.32	1.74	0.33
9	w36c	17.56	1.93	0.45
10	w41c	9.94	1.83	0.28
11	z11x	60.71	1.79	0.65
12	z12x	35.74	1.76	0.79
13	z15x	18.64	1.63	0.44
14	z19x	36.43	1.74	0.47
15	z53x	23.25	1.76	0.56
16	z55x	20.65	1.70	0.45
17	w9x	57.71	1.63	0.72
18	w11x	28.63	1.75	0.38
19	w12x	15.89	1.65	0.28
20	w13x	19.07	1.65	0.42
21	w16x	32.62	1.75	0.65
22	w33x	17.30	1.72	0.45

209 **Table 2.** Diversity index table for each sample

alpha_name	chao1	observed_species	PD_whole_tree	shannon	simpson	goods_coverage
w13x	2460.180	2015.000	115.229	9.200	0.992	0.979
JY12	2681.368	1903.000	119.146	8.825	0.992	0.973
w11x	2288.812	1857.000	106.696	8.894	0.994	0.979
JY14	2423.527	1734.000	112.251	8.507	0.991	0.976
JY15	2737.426	2214.000	146.515	9.139	0.994	0.973
w41c	2434.148	1918.000	109.421	8.591	0.986	0.977
w3c	2326.778	1885.000	109.823	8.999	0.993	0.979
JY13	2252.893	1689.000	102.032	8.576	0.990	0.978
w9x	2245.573	1850.000	105.257	9.127	0.996	0.980
z11x	2212.910	1753.000	105.438	8.667	0.992	0.979
w36c	2350.132	1942.000	111.958	9.112	0.994	0.979
w15c	2251.218	1857.000	111.926	9.200	0.996	0.981
z1c	2115.380	1780.000	109.076	9.227	0.996	0.983
z15x	2050.621	1732.000	109.849	8.911	0.994	0.984
z48c	2673.453	2106.000	113.786	9.261	0.995	0.976
w16x	2417.795	1837.000	104.647	8.920	0.994	0.977
z19x	1977.243	1659.000	100.080	8.868	0.994	0.983
z23c	2341.474	1947.000	109.356	9.241	0.995	0.980
z31c	2386.997	1914.000	112.495	8.801	0.992	0.978
w12x	2585.148	2108.000	117.892	9.517	0.997	0.978
z53x	2139.243	1708.000	104.918	9.008	0.995	0.982
JY6	2396.423	1797.000	115.879	8.746	0.993	0.976
z55x	2216.000	1737.000	104.937	8.905	0.994	0.980
JY4	2200.415	1665.000	102.227	8.639	0.992	0.978
JY5	2222.648	1615.000	106.449	8.345	0.991	0.977
JY2	2218.333	1642.000	102.984	8.588	0.992	0.978
JY3	2122.012	1637.000	98.371	8.592	0.992	0.980
z16c	2648.386	2057.000	111.204	8.950	0.993	0.975
JY1	2303.047	1720.000	103.377	8.643	0.992	0.977
w33x	2263.121	1786.000	107.190	8.933	0.994	0.980
z12x	2172.088	1705.000	98.541	8.653	0.991	0.980
w31c	2327.086	1828.000	107.238	9.057	0.995	0.979

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212 *4.2 PCR amplification*

213 For PCR amplification, 2% agarose gel electrophoresis was used to detect the
214 PCR products, and 3ul samples were taken for testing. The results were shown in
215 Figure 31. The fragment size in the amplified region V3-V4 was about 750bp, and the
216 size and concentration of the bands in the figure were in line with subsequent
217 sequencing operations.

218 *4.3 Overview of the sequencing data*

219 A total of 68572 effective sequences were obtained from 32 fresh winter feces of
220 wild and captive red deer. The diversity index of each sample includes:
221 observed_species, Shannon, Simpson, Chao1, ACE and good coverage. See Table 2
222 for details.The rarefaction curve refers to the calculation of the number of species or
223 diversity index it represents.With the increase of sequencing depth, when the curve
224 flattens, the sequencing data volume at this time is reasonable. The Rank-Abundance
225 curve explains two aspects of sample diversity(Fig. 2b), the abundance and uniformity
226 of the species contained in the sample. The wider the curve, the richer the species. The
227 shape of the curve represents the uniformity of species composition.

228 *4.4 Bacteria composition and relative abundance*

229 In the analysis of the components of the bacterial community, the relative
230 abundance of bacteria at the level of phylum and genus was mainly compared. As
231 shown in figure 3.At phylum level of red deer, Firmicutes was the predominant
232 phylum. According to the annotated results, the four phyla with the highest content in
233 the intestinal flora of red deer were established, accounting for more than 98% of the

annotated species. In the YS and JY groups, the relative abundance of Firmicutes (79.46%, 62.5%), Bacteroidete (16%, 29.1%), Tenericutes (1.25%, 3.21%), and Actinobacteria (0.7%, 0.37%) were identified. At genus level of red deer, The genus with the highest average abundance in both groups is Ruminococcaceae UCG-005, and the average abundance is 22.23% in wild group and 12.02% in captive group. The wild group is Christensenellaceae R-7 group with an average abundance of 7.97%, and the average abundance of this genus in captive group is 6.81%. The captive group is Ruminococcus UCG-010 with an average abundance of 7.38%, while the wild group has an abundance of 5.18%.

Figure 4 is a heatmap at the genus level. Wild red deer (W3-W33x) were grouped together while captive red deer (J15–JY2) were grouped in the other one. Horizontal represents the clustering situation of a sample in TOP20 species, which is the same as vertical clustering. The shorter the branch length, the more similar the species composition between the samples.The darker the color, the higher the relative abundance. The wild group Ruminococcaceae UCG-005 is more abundant than the captive group. With the weighted Unifrac and unweighted Unifrac distance matrix, we made the unweighted pair-group method with arithmetic mean (UPGMA) clustering analysis to study the similarity between samples in Fig. 5.

4.5 Species analysis of differences between groups

The T test of Alpha (observed species and Shannon) and Beta-diversity (using Unweighted and Weighted Unifrac distance matrix) between wild and captive groups were shown in Fig. 6 ($P = 0.028$, 0.005, 0.022, $2.096e^{-13}$). The larger the shannon

value, the higher the community diversity, and the difference in bacterial community diversity among individuals in the wild group(YS) is greater than that in the captive group(JY). The number of species annotated for each sample in the wild group is more than that of the captive group. The heatmap of Beta-diversity index calculated by Bray Curtis、weighted and unweighted Unifrac distance was plotted in Fig. 7 to suggest the discrepancy of species diversity between samples. The similarity coefficient between the wild group and the captive group is significantly greater than the value within the group, indicating that the difference in species diversity between the two groups is smaller. The similarity coefficient between the field group and the captive group was significantly greater than the value within the group, indicating that the difference of species diversity within the two groups was smaller. We also demonstrated the non-metric multi-dimensional scaling (NMDS) plot and the principle co-ordinates analysis (PCoA) plots in Fig.8. NMDS (Nonmetric Multidimensional Scaling) is often used to compare differences between sample groups. Principal Co-ordinates Analysis (PCoA) is a kind of dimensionality reduction sorting method similar to PCA.Each dot represents a sample, and the dots of the same color come from the same group. The closer the distance between the two points, the smaller the difference in community composition between them. The NMDS and PCoA analyses using different methods were also showing there was obvious separation between wild and captive red deer samples. It was found that the flora of faeces of wild and captive red deer were obviously separated, The results indicated that the similarity of intestinal flora composition between the two groups was lower

than that within the group.And the community diversity of intestinal flora of the two groups was significantly different. The artificial feeding process decreased the diversity of intestinal flora of red deer.

T test and LDA effect size (LEfSe) analyses were used to calculate the significant difference between samples of the groups.The bar chart of LDA value distribution shows the species with LDA Score greater than the set value (the default setting is 3) .As shown in Figure 9,the species with different abundance were: In the wild male group, the top 4 species with different abundance were Ruminococcaceaeucg_014, Ruminococcaceaeucg_013, Roseburia and Prevotellaeucg_004, respectively.In the wild female group, the top 4 species with different abundance were Clostridiales, Firmicutes, Clostridia, and Ruminococcaceae. In the captive male group, the top 4 species with different abundance were Eubacterium_coprostanoligenesgroup, Clostridium, Tenericutes, Mollicutes, and Ruminococcaceaeucg_002.The top 4 species with different abundance in the captive female group were Bacteroidetes, Bacteroidia, Bacteroidales, and Rikenellaceae. This cladogram shows core strains that vary significantly at each level in Fig. 7b.

4.6 Predicted Metabolic Functions

Picrust software was used to predict the composition of the functional genes composed of the intestinal flora obtained by 16S sequencing in the samples in this study. In the KEGGPAYHWAY database, the KEGG database is divided into five levels. We also found several predicted metabolic functions (KEGG pathway level 3) were enriched in wild and captive red deer based on PICRUSt2 results (Figure 10). In

the Picrust2 pathway level 1 annotation results，top three of the genes annotated are related to metabolism、 genetic information processing、environmental information processing. In the Picrust2 pathway level 2 annotation results，top five of the genes annotated are related to Carbohydrate metabolism、Amino acid metabolism、Energy metabolism、Nucleotide metabolism、Translation. In the Picrust2 pathway level 3 annotation results，top five of the genes annotated are related to ko03010(Ribosome)、ko00230(Purine metabolism)、ko02010(ABC transporters)、ko00240(Pyrimidine metabolism)、ko02020(Two-component system).

5. Discussion

Due to the limited availability of high-throughput sequencing data on the gut microbiota of wild red deer to date, it is particularly useful to analyze the differences between wild and captive populations of red deer. The 16S rRNA Illumina MiSeq high-throughput sequencing technology was used for the first time in this study to compare the gut microbiota of captive and wild red deer.

Overall, the results of our study are basically consistent with the characteristics of the gut of previous herbivores, like the red deer in the Bavarian Forest National Park[14], musk deer[15], horses [8]and North America white-tailed deer[16], cattle[17]. Our results showed different abundance of microbiotic communities at phylum level between wild and captive red deer species. We found that the abundance of Firmicutes in the intestine of wild red deer was significantly higher than that of captive red deer. But the abundance of Bacteroidetes of captive red deer was

significantly higher than in wild. Firmicutes are the main cellulolytic bacteria, and they can degrade cellulose into volatile fatty acids for the host to use[18]. The main function of Bacteroides is to help the host degrade carbohydrates, proteins, and other substances to increase the nutrient-utilization rate of the host[19]. Bacteroides can also maintain intestinal microbial ecological balance[20,21]. Wild red deer mainly consumed the branches and leaves of wild high-fiber plants. While captive red deer was mainly fed on grain, elm leaves, grass. Therefore, differences in the microflora between captive and wild red deer may be closely related to dietary differences. At the genus level, the two species had similar core flora, and the relative abundance of Ruminococcus was higher, and the abundance of Ruminococcus contained in wild was higher than that in captive. In addition, the core flora species at the level of captive and wild red deer are similar. The core flora of red deer gut contains 25 OTUs. Among them, 24 OUT belonged to Firmicutes and the remaining one belonged to Bacteroidetes. For ruminants, rumen coccus is vital for digesting dietary fiber [22].Ruminococcaceae_UCG-005($P<0.001$) Ruminococcaceae_UCG-010($P<0.001$) and Christensenellaceae are dominant and abundant genera of rumen coccaceae in wild and captive red deer. In this study, Christensenellaceae are the also belongs to the dominant genus, belonging to Firmicutes, which is widely found in human and animal intestines and mucosa and is very important for host health. This result is consistent with the difference in musk deer [15,23].

The diversity of α and β of gut microbiota of captive and wild red deer was significantly different. This result is consistent with research on red deer in the

Bavarian Forest National Park This was consistent with the results of red deer in the Bavarian Forest National Park, Red deer in this park are kept in enclosures and free-range free-ranging[14]. Food is critical to the formation of bacterial communities in the gut of ruminants that ferment starch and sugars from highly fibrotic plants [24]. In addition, age, sex and host heredity are also non-negligible influencing factors of mammalian intestinal microbiota [25].

In this study based on KEGG database of red deer of gut flora metabolism enrichment is forecasted, We speculated that the functional differences of intestinal flora between wild and captive red deer were due to the host maintaining the homeostasis of intestinal microenvironment by changing the enrichment degree of metabolic functional pathways of intestinal flora, Our study of functional data, most of the genes annotated are related to metabolism, and the second gene is related to genetic information processing. In the functional studies of Amur tiger, the most metabolomes were annotated, and carbohydrate metabolism, amino acid metabolism, nucleotide metabolism, cofactor and vitamin metabolism, and energy metabolism were relatively high[26].

In the later stage, the correlation between intestinal flora and feeding habits, as well as the corresponding biochemical indicators in the intestine will be determined, and the correlation between intestinal flora and related physical and chemical indicators will be explored, so as to provide a theoretical basis for better protection of wild red deer in terms of feeding habits and metabolism.

6. Conclusion

In this study, 16S high-throughput sequencing technology combined with microsatellite molecular individual identification technology was used to determine the basic composition and structure of gut Microbiome in red deer faeces. The experimental results show that there are significant differences between the wild red deer and captive deer at different levels. The reason for this difference may be related to food. Wild red deer mainly eat branches and leaves with high fibrosis in winter. Compared with captive red deer, food richness is higher. By predicting the function of the microflora, it was concluded that most of the gene annotations were related to metabolism. Further studies are needed to study the intestinal flora of red deer, such as using metagenomic research methods to analyze the related metabolic pathways of unknown bacteria in the intestinal flora.

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Availability of data and materials : All data generated or analysed during this study are included in this published article and its supplementary information files.

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Figure legends

Figure 1. Results of gene fragment amplification of Northeast red deer samples

Figure 2. (a) Rarefaction curves : The horizontal axis represents the number of clean reads randomly selected from a sample, and the vertical axis represents the alpha

diversity index corresponding to the number of clean reads. (b) rank abundance curves: Horizontal axis: The relative abundance of OTU was ranked in descending order. Vertical axis: relative abundance ratio.

Figure 3. Histogram of species profiling at phylum and genus level

Figure 4. The heatmap of clustering for species abundance.

Figure 5. Based on unweighted Unifrac distance(a) and weighted Unifrac distance(b).

Figure 6. The comparisons for Alpha-diversity (a.Shannon index and b.observed species)and beta-diversity (with c.weighted and d.unweighted Unifrac distance matrix) between wild and captive red deer.

Figure 7. Heatmap of beta-diversity. The numbers in grids are the dissimilarity coefficient between samples. The smaller the coefficient of divergence was, the smaller the difference of species diversity was. In the same grid, the upper, middle and lower values represent Bray Curtis, Weighted Unifrac and Unweighted Unifrac distances, respectively.

Figure 8. PCoA Unweighted(a) and weighted Unifrac distance(b)、NMDS weighted Unifrac distance(c) and unweighted Unifrac distance(d)

Figure 9. The results of LEfSe (LDA Effect Size) analysis (a). In the cladogram (b), The above figure is a clustering tree. Different colors indicate different groups, and nodes of different colors indicate the microbial groups that play an important role in the group represented by the color. Yellow nodes indicate groups of microorganisms that have not played an important role in different groups. The name of the species represented by the English letters in the figure shows the species of the family

520 classification level in the legend on the right.

521 **Figure 10.** Annotation results of pathway level 1 and pathway level 2 in Picrust2

Figures

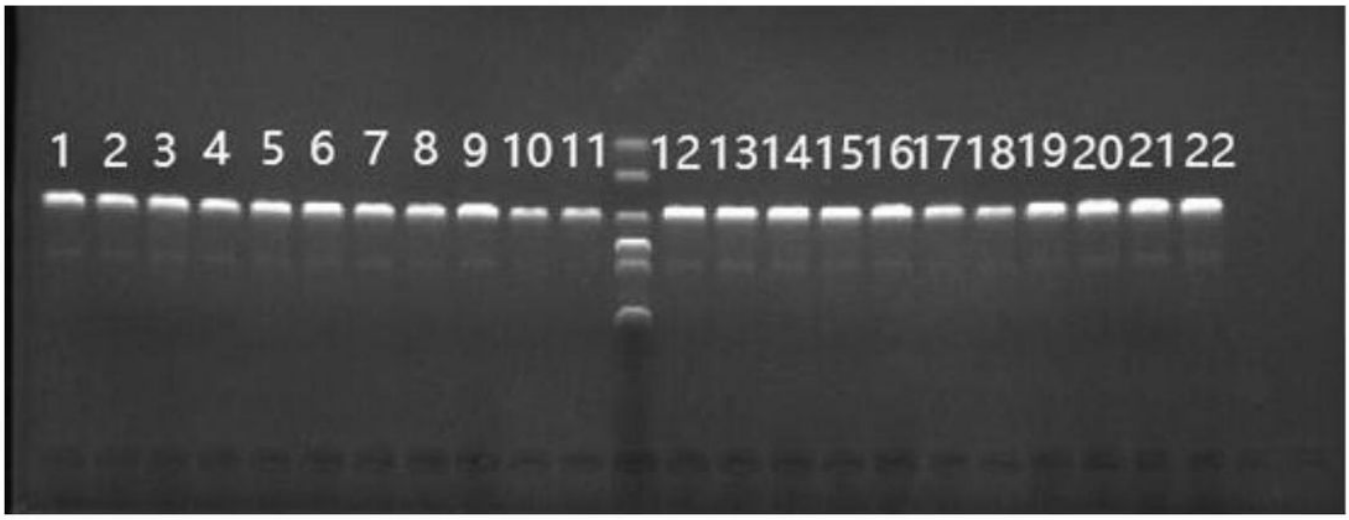

Figure 1

Figure 1

Results of gene fragment amplification of Northeast red deer samples

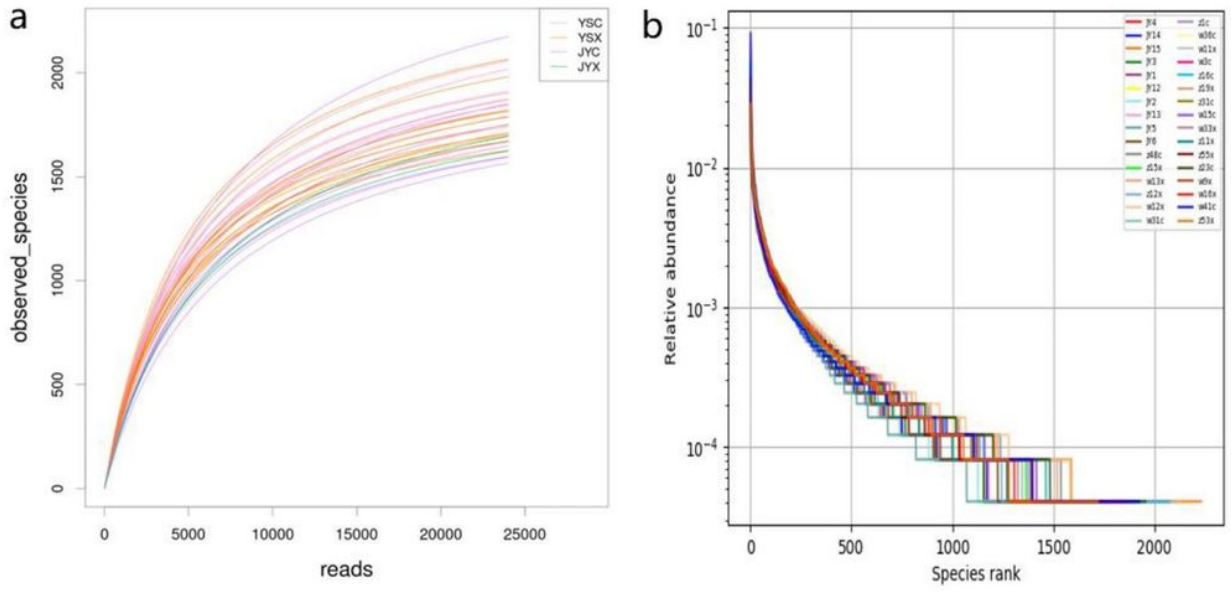

Figure 2

Figure 2

(a) Rarefaction curves : The horizontal axis represents the number of clean reads randomly selected from a sample, and the vertical axis represents the alpha diversity index corresponding to the number of clean reads. (b) rank abundance curves: Horizontal axis: The relative abundance of OTU was ranked in descending order. Vertical axis: relative abundance ratio.

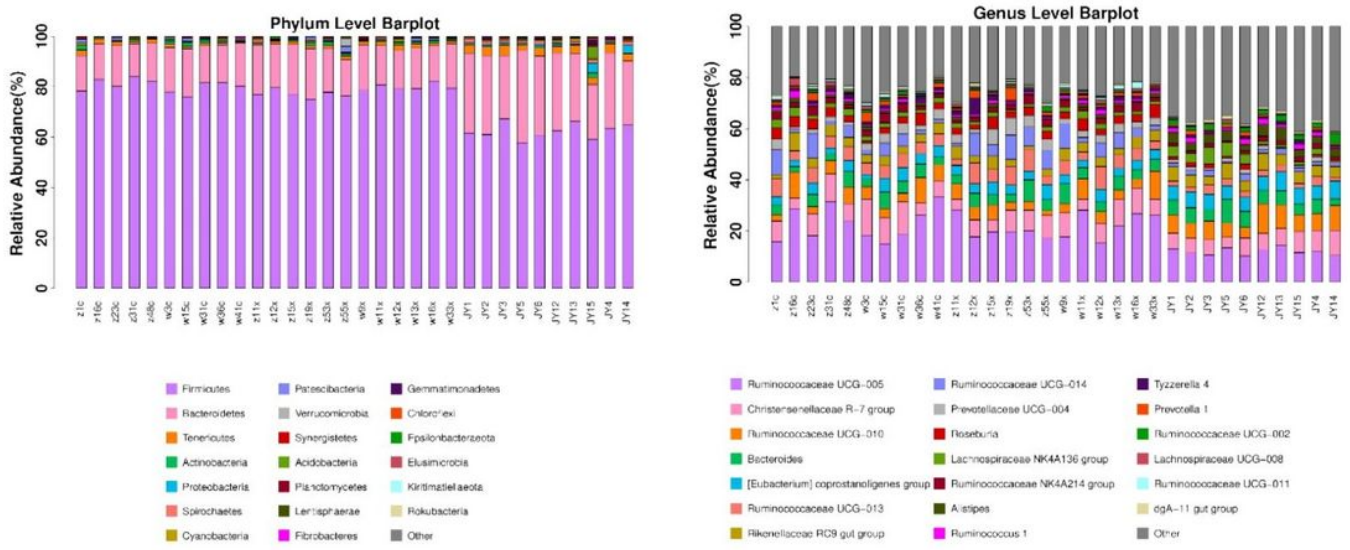

Figure 3

Figure 3

Histogram of species profiling at phylum and genus level

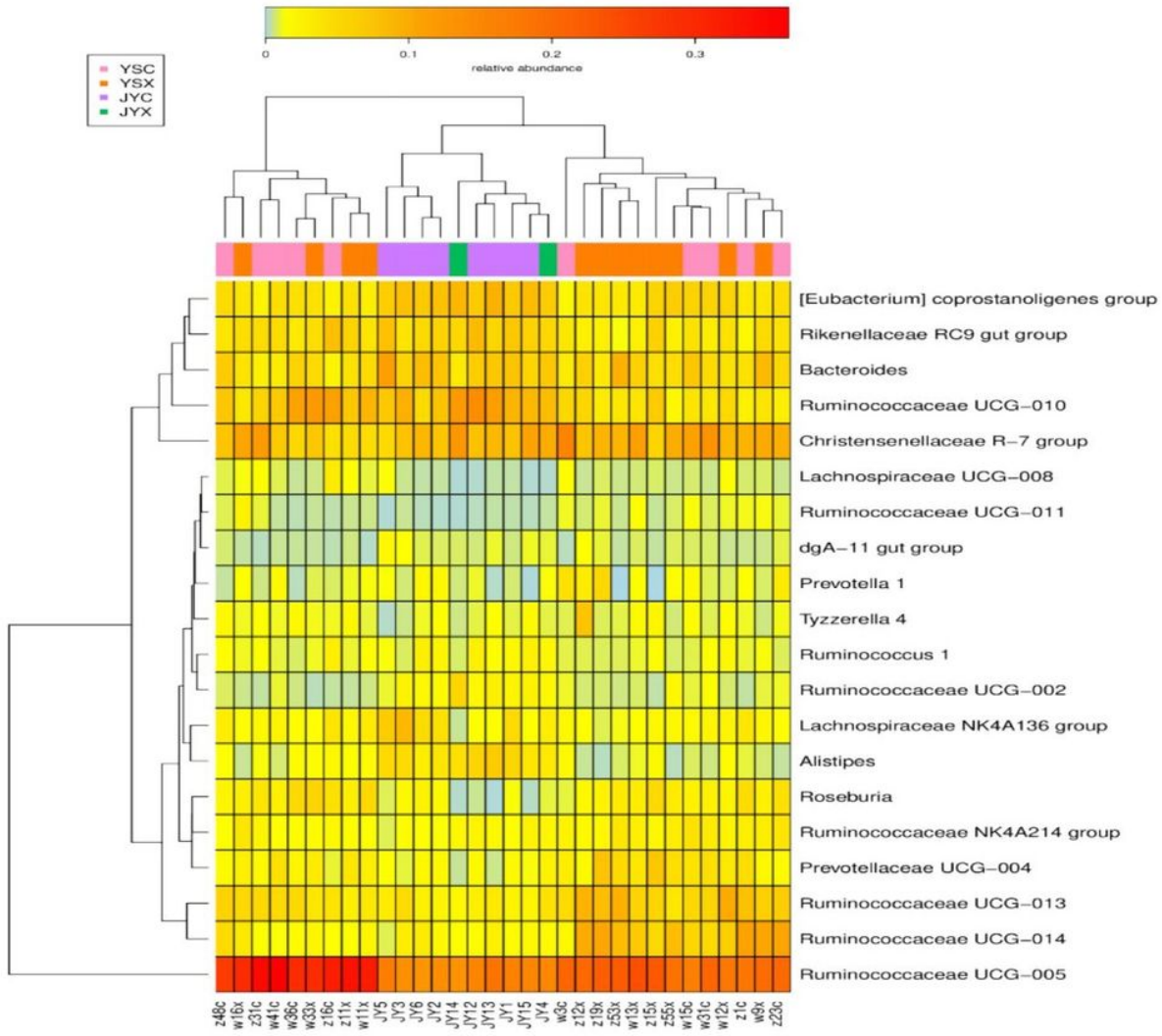

Figure 4

Figure 4

The heatmap of clustering for species abundance.

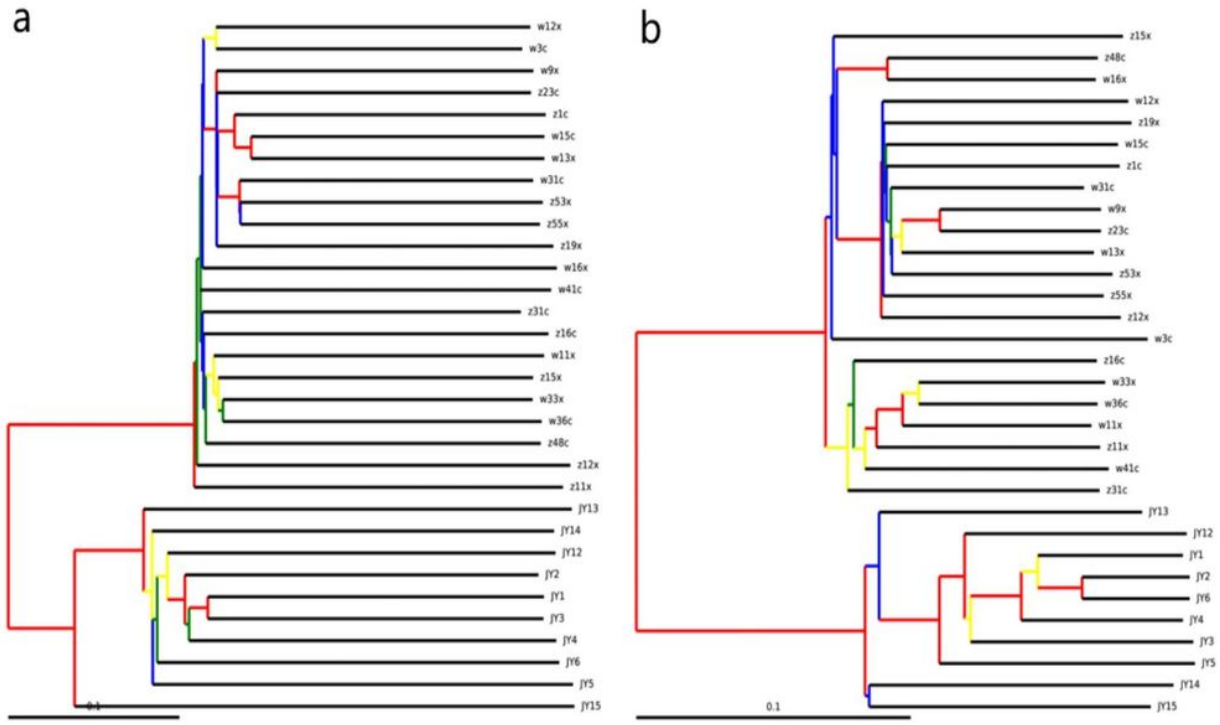

Figure 5

Figure 5

Based on unweighted Unifrac distance(a) and weighted Unifrac distance(b).

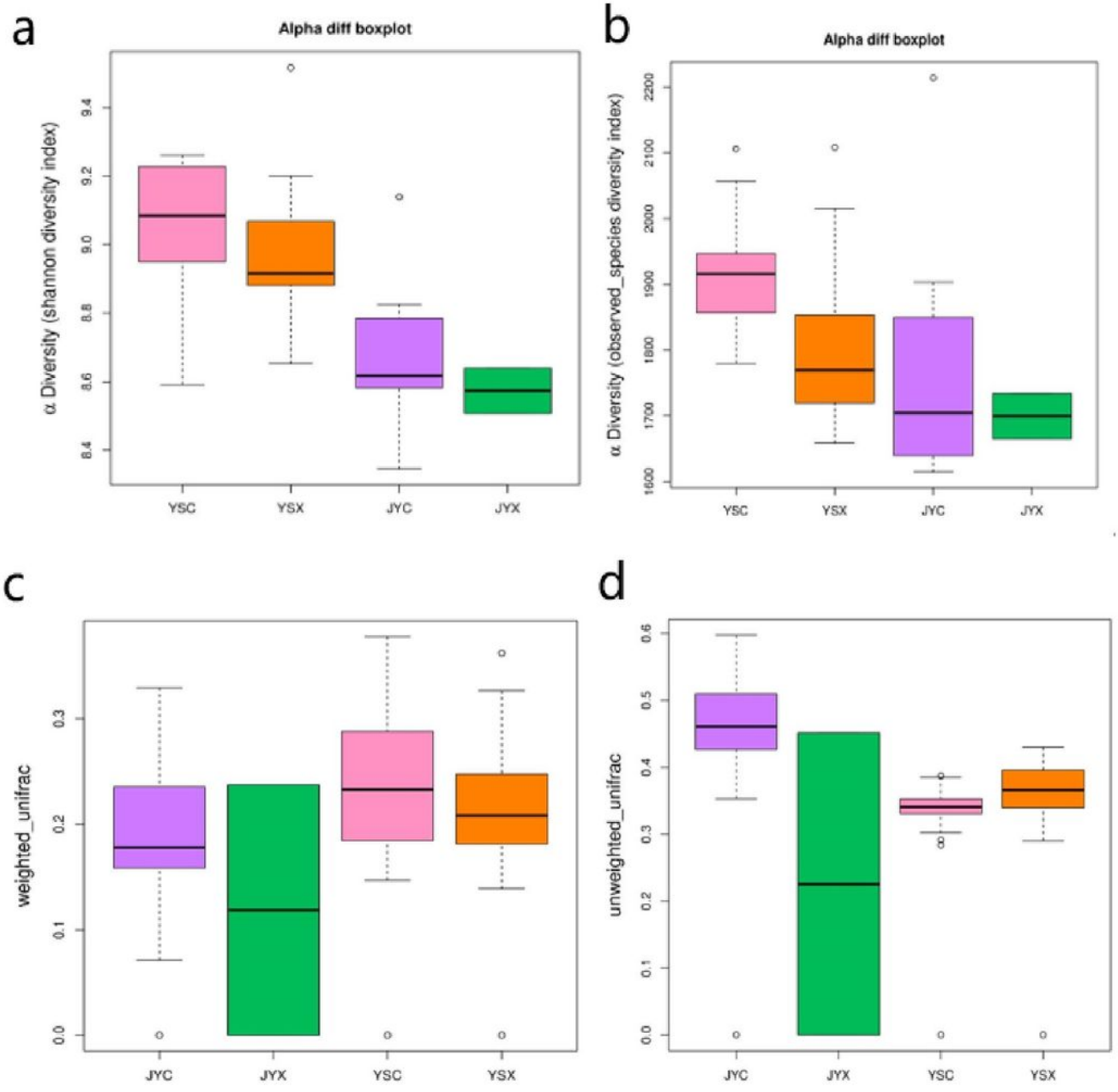

Figure 6

Figure 6

The comparisons for Alpha-diversity (a.Shannon index and b.observed species)and beta-diversity (with c.weighted and d.unweighted Unifrac distance matrix) between wild and captive red deer.

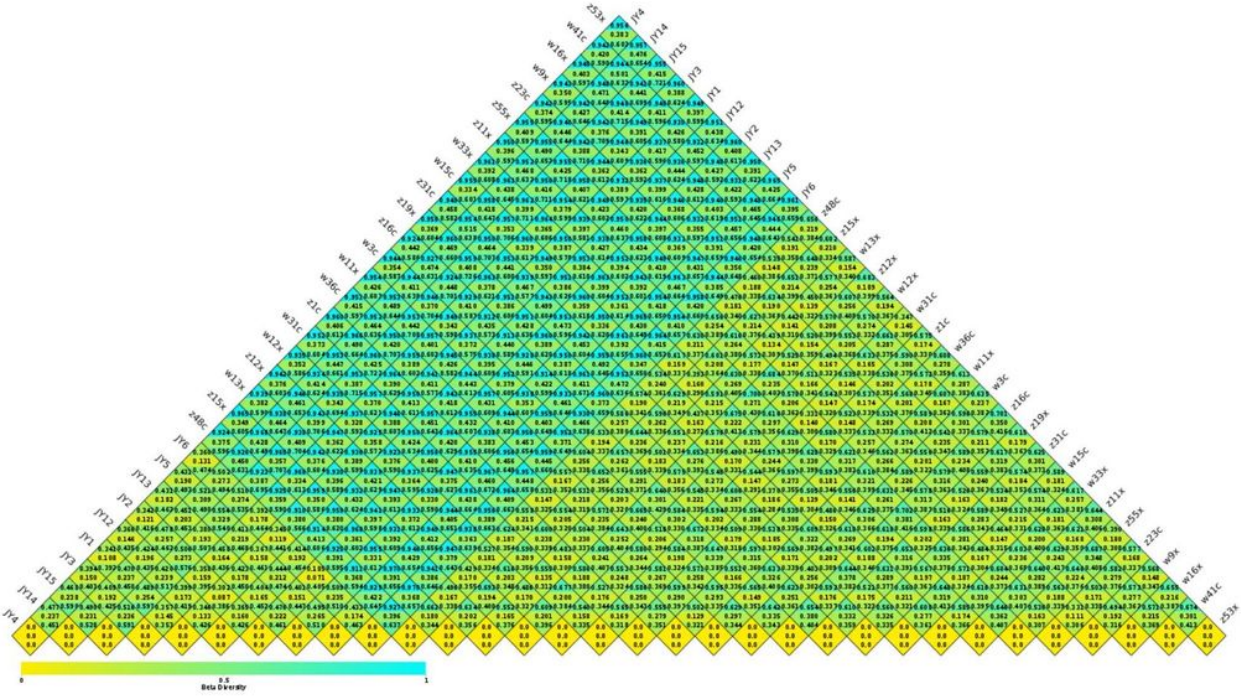

Figure 7

Figure 7

Heatmap of beta-diversity. The numbers in grids are the dissimilarity coefficient between samples. The smaller the coefficient of divergence was, the smaller the difference of species diversity was. In the same grid, the upper, middle and lower values represent Bray Curtis, Weighted Unifrac and Unweighted Unifrac distances, respectively

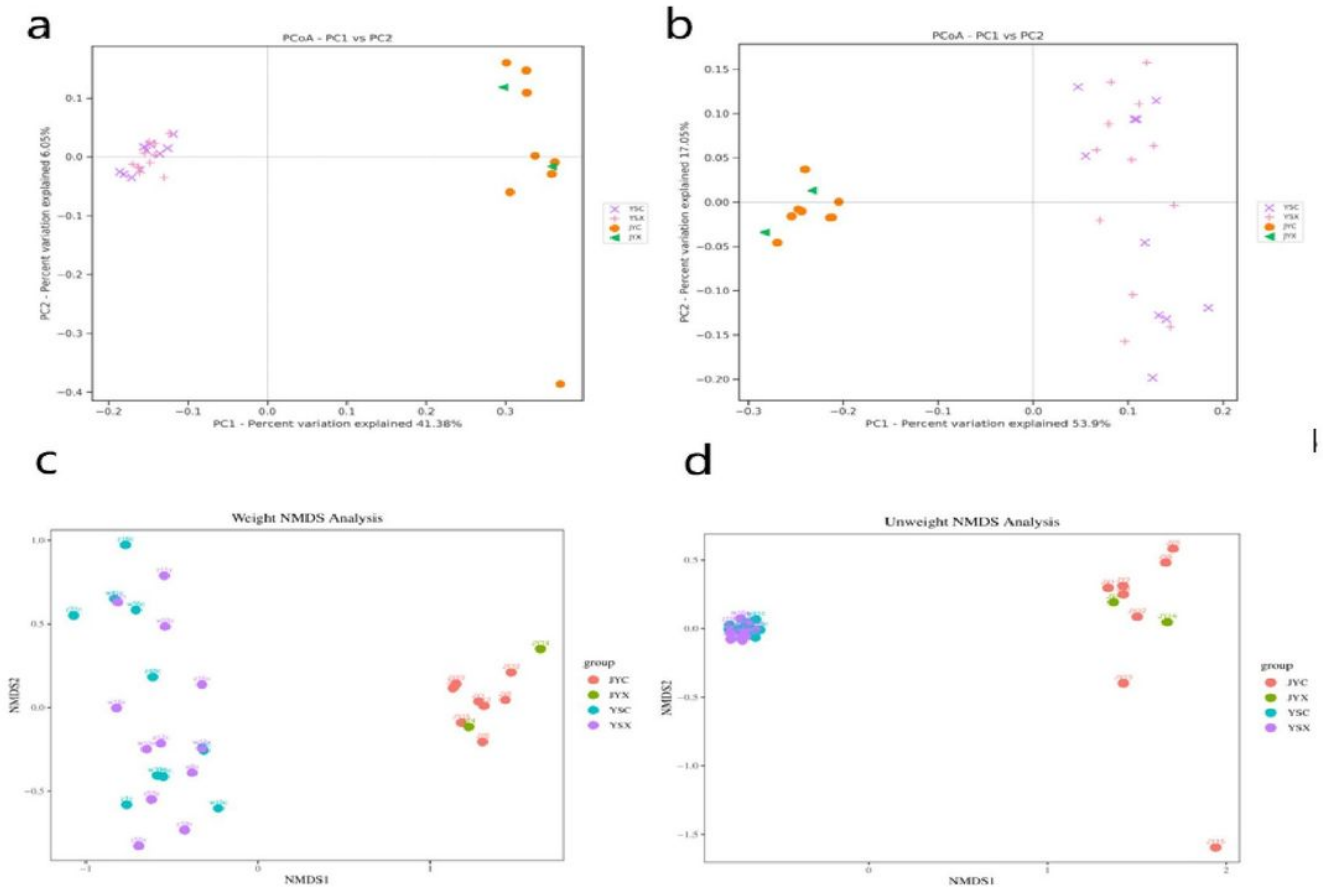

Figure 8

Figure 8

PCoA Unweighted(a) and weighted Unifrac distance(b)NMDS weighted Unifrac distance(c) and unweighted Unifrac distance(d)

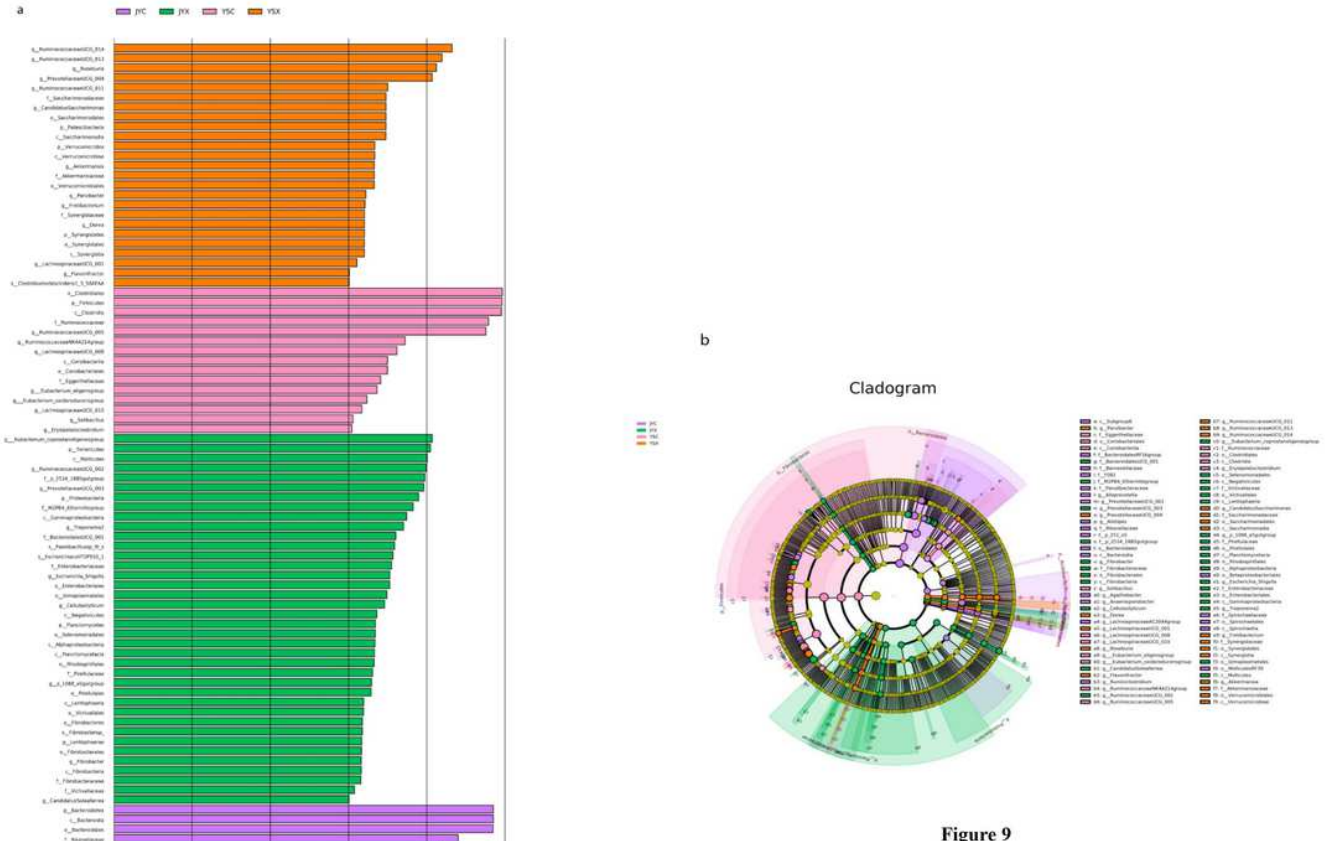

Figure 9

Figure 9

The results of LEfSe (LDA Effect Size) analysis (a). In the cladogram (b), The above figure is a clustering tree. Different colors indicate different groups, and nodes of different colors indicate the microbial groups that play an important role in the group represented by the color. Yellow nodes indicate groups of microorganisms that have not played an important role in different groups. The name of the species represented by the English letters in the figure shows the species of the family classification level in the legend on the right.

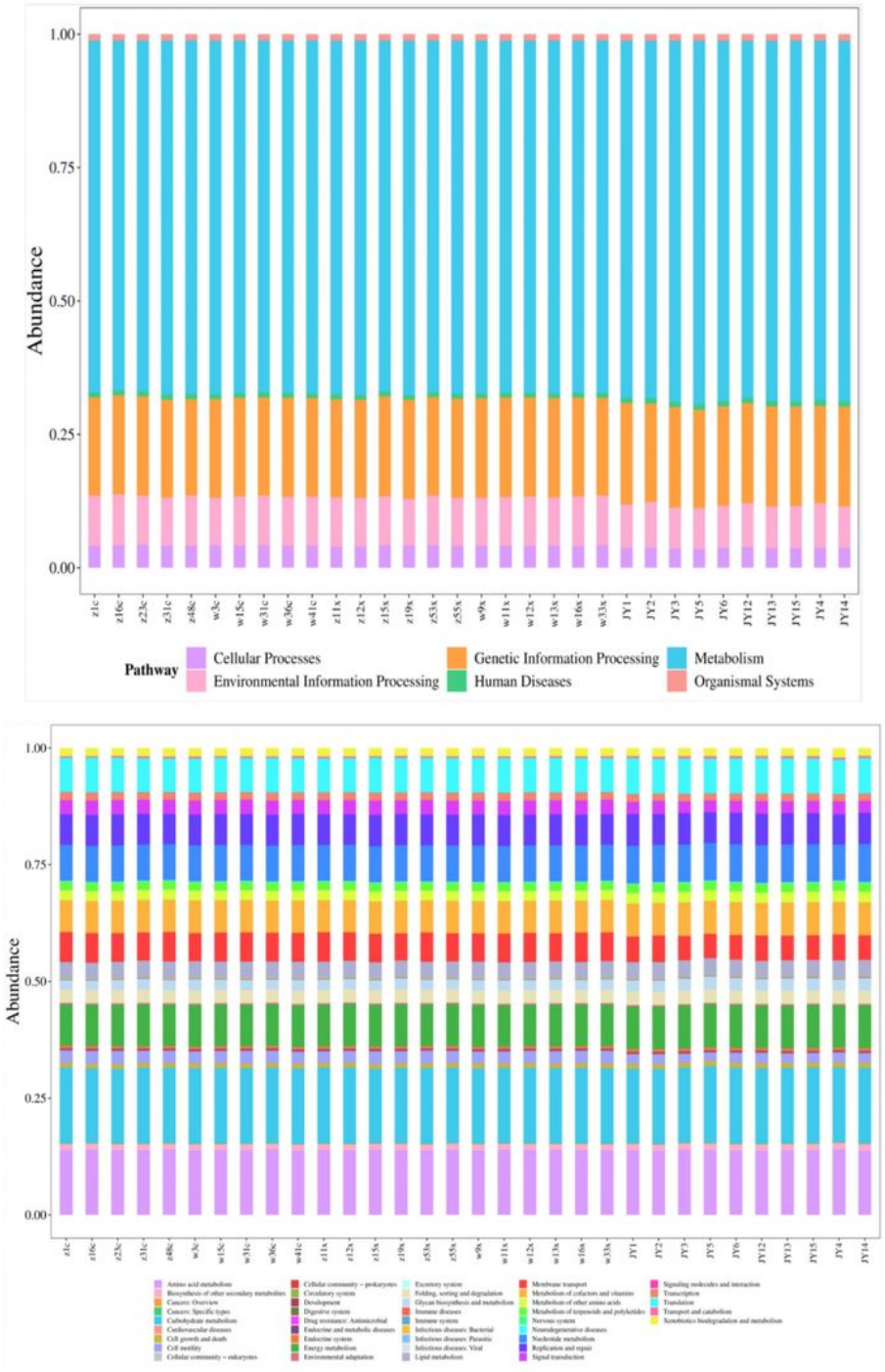

Figure 10

Figure 10

Annotation results of pathway level 1 and pathway level 2 in Picrust2