Seasonal Breeding Leads to Changes for Gut Microbiota Diversity in the Wild Ground Squirrel (*Spermophilus Dauricus*)

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Abstract

Background: Seasonal breeding is a normal phenomenon that animals adapt to natural selection and reproduce only in specific seasons. With the gradual popularization of Next-generation sequencing (NGS), large studies have shown that seasonal breeding has been affected by gut microbiota. Consequently, the purpose of this study is to explore the effect of seasonal breeding on the gut microbiota of wild ground squirrel (*Spermophilus dauricus*). We used 16S rRNA gene sequencing technology to sequence the gut microbiota of the wild ground squirrel in the breeding season and non-breeding season. We also predicted the function of gut microbiota by bioinformatic software.

Results: The results showed that the main components of gut microbiota in all samples consisted of *Firmicutes* (61.8%), *Bacteroidetes* (32.4%), and *Proteobacteria* (3.7%). Microbial community composition analyses revealed significant differences between these two groups. At the genus level, *Alistipes*, *Mycoplasma*, *Anaerotruncus,* and *Odoribacter* were up-regulated in the non-breeding season, while *Blautia* and *Streptococcus spp.* were up-regulated in the breeding season. The result of function prediction suggested that the relative abundance of functional categories related to lipid metabolism, carbohydrate metabolism, and nucleotide metabolism was higher in the breeding season. The expression of transcription, energy metabolism, and signal transduction was enriched in the non-breeding season.

Conclusions: Overall, the results of this study emphasized the significant effects of seasonal breeding on gut microbiota community composition of the wild ground squirrel and laid a foundation for further study of gut microbiota on seasonal breeding in the future.

1. Background

The gut microbiota of animals is composed of a large number of microbes. The micropopulation stays in the host and interacts with each other, forming a balanced, complex, and diverse gut microbiota system [1]. Gut microbiota is closely related to the physiological activities and growth of the body, and participate in many physiological processes, including metabolic, reproduction [2, 3].

With the gradual popularization of Next-generation sequencing (NGS), which has low cost and a large amount of data, research on gut microbiota has gradually increased. Many studies have shown that gut microbiota has essential effects on host growth [4], bone mineral density [5], energy metabolism [2] and immune regulation [6]. Furthermore, it has been found that gut microbiota is closely linked with a variety of diseases, including metabolic diseases such as obesity [7, 8] and diabetes [9], neurological diseases such as Alzheimer's disease [10, 11] and Parkinson's disease [12], cardiovascular diseases such as hypertension [13] and atherosclerosis [14], cancer [15] and liver cirrhosis [16], etc…The effect of gut microbiota on reproduction has also received considerable attention. For example, studies on zebrafish (*Danio rerio*) illustrated that probiotic *Lactobacillus* rhamnosus could activate leptin, which regulates the hypothalamus-pituitary-gonadal(HPG) axis to affect reproduction, thereby promoting the maturation of follicles and improving the reproductive ability of animals [3]. Besides, in the study of human beings,

significant changes in gut microbiota diversity occur during pregnancy, thus adjusting their metabolism to adapt to the growing energy needs [17]. Gut microbiota also plays an important role in the synthesis, metabolism, and recycling of nitrogenous compounds such as amino acids, It has a significant influence on the host in terms of fecundity [18]. Therefore, the gut microbiota is crucial for the health and physiological activities of the host, including maintaining the health and ensuring the reproductive capacity of the host.

The seasonal breeding strategy is a survival strategy that animals can reproduction in the most favorable environment. Survival and reproduction are the primary tasks of each individual, which depend on their ability to adapt to seasonal variation, to meet their own needs under the conditions of changes in food distribution, supply, and abundance [19]. The reproductive function of mammals is mainly regulated by the HPG axis. The hypothalamus secretes gonadotropin releasing hormone (GnRH), which stimulates the anterior pituitary to secrete gonadotropins, such as luteinizing hormone (LH) and follicle stimulating hormone (FSH). Therefore, the HPG axis was activated only during the breeding season [20]. Studies have shown that the reproductive system of seasonal breeding animals is regulated by melatonin [21], thyroid hormone (TH) [22], kisspeptin [23], gonadotropin inhibitory hormone (GnIH) [24, 25],leptin [26], and other hormones. In recent years, the gut microbiota has been shown to interact with estrogens [27]. Estrobolome gene exists in gut microbiota, which is defined as the gene pool that can metabolize estrogen [28]. The gut microbiota can affect the estrogen level by the secretion of β-glucuronidase, which can bind to estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ) and affect downstream physiological effects [27]. However, the mechanism of how gut microbiota affects seasonal breeding is still unclear.

The wild ground squirrel (*Spermophilus dauricus*) belongs to *Mammalia*, *Rodentia*, *Sciuridae*, and *Spermophilus*, which is a typical seasonal breeding small mammal. During the year, the wild ground squirrel doesn't breed in any month except April to May. Therefore, the wild ground squirrel includes breeding season (April to May) and non-breeding season (June to March of the following year). The female wild ground squirrel is most likely to become pregnant in May [29–31]. At the same time, the wild ground squirrel is a very suitable animal model for the study of seasonal breeding. Previous studies have showed that android receptor (AR), estrogen receptors α and β (ER α, ER β), and aromatase cytochrome P450 (P450arom) are seasonally expressed in the hypothalamus, hippocampus, uterus, testis, and epididymis [32–36]. Luteinizing hormone receptor (LHR), Follicle stimulating hormone (FSHR) also showed seasonal expression in testis and epididymis [37, 38]. In addition, ghrelin, obestatin, insulin, and other gut-derived hormones have direct or indirect effects on the reproductive axis and play their roles in regulating energy balance and reproductive function [39]. In summary, the important role of gut microbiota in regulating various physiological activities of the host prompted us to research on seasonal breeding and gut microbiota. Thus, the study aimed to explore the effect of seasonal breeding on the gut microbiota of the wild ground squirrels, and to explore the differences of gut microbiota between breeding season and non-breeding season, further analyzed the main functional differences.

2. Methods

2.1 Sample collection

All wild ground squirrels were collected by mousetraps in the wild in Hebei Province, China. Six individuals were captured in the breeding season (B; April to May; n = 6) and non-breeding season (NB; June to September; n = 6) respectively. The gut contents were collected as soon as possible after the animals were captured (within 24hours). The animals were killed by decapitation after anesthesia. The surface of animals was disinfected with alcohol. The contents of the gut tract were collected quickly by opening the abdominal cavity. After collection, the intestinal contents were frozen in liquid nitrogen immediately and stored at − 80℃ before DNA extraction.

All animal experiments were approved by the Policy on the Care and Use of Animals by the Ethics Committee of Beijing Forestry University and Hebei Provincial Department of Agriculture (jnzf11 / 2007).

2.2 DNA extraction and sequencing

Total genomic DNA in each gut sample was extracted according to the instructions of the TIANamp DNA Kit (TIANGEN BIOTECH Inc., Beijing, China). The quality and integrity of DNA were evaluated by the A260/280 ratio and agarose gel electrophoresis. After extracted the DNA from gut samples,V3–V4 region of the bacteria 16S rRNA gene was amplified by two-step PCR, which was used forward primer 340F (5'-ACTCCTACGGGAGGCAGCAG-3') and reverse primer 806R (5'- GGACTACHVGGGTWTCTAAT-3'). All PCR reactions were carried in 25 ul total volume consisting of 12.5 ul KAPA HiFi HotStartReadyMix (2×), 0.25umol L-1 of each primer, and 10 ng of DNA template. KAPA HiFi Hotstart PCR Kit (KAPA Biosystems, United States) was used for the first round of PCR amplification. First, pre denaturation at 95℃ for 3 min, then denaturation at 95℃, annealing at 55℃, and extension at 72℃ for 30 s respectively. These three steps are subjected to 25 cycles, and finally extended at 72℃ for 5 min. The PCR products were purified with 1 x AMPure XP Beads, and then perform the second round PCR amplification was performed. Except for 8 cycles, the other cycle steps were the same as the first round PCR amplification. The PCR products of each sample were mixed to prepare the PCR amplicon libraries. Amplicons were extracted from 2% agarose gels and recycled using the QIAquick Gel Extraction Kit (QIAGEN, CA, United States), and then quantified using the KAPA Library Quantification Kit (KAPA Biosystems, United States) as the standard of Libraries mixing. After the quality assessment and quantification, the same amount of amplified products were collected and sequenced by pair-end 2 × 300-bp in the Illumina Miseq platform at ORI-GENE Technology Co., Ltd. (Beijing, China).

2.3 Bioinformatic analyses

High throughput sequencing results were received as the FASTQ file. Paired-end reads were merged using VSEARCH (v2.14.1) [40] to form consensus sequences and truncated at both primers, then reads with the error threshold above 0.01 were discarded. After removed low abundance noise with miniquesize 8, sequences were denoised to obtain single-base precision amplicon sequence variants (ASVs) using the UNOISE3 in USEARCH (v10.0.240) [41, 42], during which part of chimeric sequences was also removed.

To improve accuracy, the Ribosomal Database Project (RDP) (v1.6) was used as a reference sequence to remove chimeras again. Further, a feature table was created using USEARCH (v10.0.240) [43]. Subsequently, the taxonomic origin of each ASV was determined in VSEARCH (v2.14.1) with the confidence value of 0.60, based on the RDP (v1.6), and plastid and non-Bacteria were removed.

The 16S rRNA gene sequences were analyzed mainly using USEARCH (v10.0.240) and R (v3.6.2). After normalizing by subsample, the community richness index and community diversity index were calculated using the Vegan package [44] to determine the alpha diversity within the groups of the wild ground squirrels. Then, the number of ASVs in 1% – 100% sequence without replacement was taken to calculate the richness change of the dilution process. Next, β-diversity was calculated using the Bray-Curtis distance and visualized with Principal Coordinate Analysis (PCoA) analyses to find the difference of microbiota structure among groups. Taxonomies can annotate species information from phylum to genus level, and Linear Discriminant Analysis Effect Size (LEFSe) was used to analyze the difference at each level [45]. Microbial functions were predicted by Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt), based on high-quality sequences [46]. STAMP (v2.1.1.0) [47] was used to explore the functional pathways with significant differences. The R script is used to generate the corresponding figures.

3. Results

3.1 Data summary

A total of 1812298 16S rRNA gene reads were obtained from the raw offline data, with an average of 151025 reads per sample. The raw data were merged at two ends, and the clean sequences were obtained after quality control. The number of ASV in each sample was obtained by denoising based on the clean sequences, eliminating the wrong sequence, and selecting the credible sequence with higher abundance as the representative sequence. A total of 1638 ASVs were obtained from 12 samples. The average number of ASV in the breeding season was 847 and that in the non-breeding season was 224 (Table S1).

To evaluate whether the sequencing depth was enough to make a stable estimation of the species richness we used the rarefaction curve, which tends to be gentle indicated that sequencing is saturated and there is no need to increase the sample size. The sequencing depth has basically covered all species in the sample, and the marginal contribution of more data to the discovery of new ASV is very small (Fig.S1).

3.2 Microbial Diversity

We analyzed chao1, ACE, Shannon, and Simpson as four common alpha diversity indexes of gut microbiota (Table 1). The alpha diversity index showed that there was no significant difference in both the diversity index and the abundance index of gut microbiota between breeding season and non-breeding season.

Table 1
Number of community richness index (ACE and Chao1), community diversity index (Shannon and Simpson) for each sample.

SampleID	Chao1	ACE	Shannon	Simpson
B1	1189.168	1161.4	5.188146	0.980939
B2	960.5446	952.2633	4.300073	0.944246
B3	1067.103	1022.224	5.406034	0.989723
B4	962.8922	948.7459	3.569155	0.841802
B5	883.2471	863.3297	4.720447	0.971907
B6	393.6512	397.6003	2.217882	0.750912
NB1	1025.778	1027.65	4.867893	0.967579
NB2	1022.585	989.4314	5.301287	0.98375
NB3	905.1235	870.5195	4.879087	0.970492
NB4	957.8264	957.1833	4.542708	0.956739
NB5	1029.827	1018.301	5.195961	0.986655
NB6	930.1932	895.0828	4.755879	0.976622
B, breeding season; NB, non-breeding season				

In the statistics of β diversity, the PCoA analysis was used to determine the differences between these two groups. PCoA plots and sample correlation heatmap based on the Bray-Curtis distance matrices showed that the samples of the breeding season and non-breeding season could cluster basically. The first principal axis can explain 25.24% of the total sample difference, and the second principal axis can explain 12.46% of the sample difference, indicating a possible effect of seasonal breeding strategy (Fig. 1).

3.3 Taxonomic Composition of Microbiotas

At the phylum level, three major bacterial were found in the wild ground squirrel gut microbiota, most of which were classified as *Firmicutes* (61.8%), followed by *Bacteroidetes* (32.4%), and some *Proteobacteria* (3.7%) (Fig. 2 and Table S2). The other phyla were *Verrucomicrobia*, *Elusimicrobia*, *Actinobacteria*, and *Tenericutes*, but not abundant (< 1%). The rest of the sequences of the wild ground squirrel gut microbiota were not annotated, called unassigned group.

At the genus level, the unassigned group also means that the sequences cannot be compared to any known genus. The proportion of this unassigned group at the genus level is 46.2% in all samples. The top

13 genera were listed in Fig. 2d and the top four genera in all samples are *Lactobacillus* (7.9%), *Barnesiella* (7.4%), *Streptococcus* (6.3%), *Ruminococcus* (5.7%) (Table S3).

3.4 Analysis of the microbiota difference between groups

To identify specific microbial communities that exist in the breeding season and non-breeding season, we used the LEFSe analysis to explore the microbial community in these two different periods (Fig. 3). At the genus level, the wild ground squirrel in the non-breeding season had a significantly higher relative abundance of *Alistipes*, *Mycoplasma*, *Anaerotruncus* and *Odoribacter*. In contrast, the wild ground squirrel in the breeding season had significantly more *Blautia* and *Streptococcus spp*. At the order level, the number of *Rhodospirillales* in the non-breeding season was significantly more than that in the breeding season, and *Lactobacillales* are more in the breeding season. At the class level, *Alphaproteobacteria* were more in the non-breeding season, and *Bacilli* were more in the breeding season.

We also draw the Manhattan plot at the phylum level with the negative logarithm of the P-value, which showed that *Proteobacteria*, *Tenericutes*, *Verrucomicrobiais* were significantly down-regulated in the breeding season compared with the non-breeding season, and the abundance of *Elusimicrobia* was significantly higher in the breeding season (Fig. 4). These results suggest that there are abundant microbial communities in the gut of the wild ground squirrel, and their composition and relative abundance may affect the physiological activities of the host.

3.5 Analysis of the functional difference of gut microbiota between groups

We used PICRUSt to predict the function of gut microbiota. Based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database, analysis of functional categories of the KEGG pathway indicated that the predicted functional categories changed significantly between the breeding season and the non-breeding season, including cell motility, carbohydrate metabolism, nucleotide metabolism, transcription, energy metabolism, signal transduction, lipid metabolism and cancers (Fig. 5 and Table S4). The relative abundance of functional categories related to carbohydrate metabolism, nucleotide metabolism, and lipid metabolism was higher in the breeding season. Besides, the expression of transcription, energy metabolism, and signal transduction was higher in the non-breeding season. These results illustrated that the metabolic function of gut microbiota in the seasonal breeding wild ground squirrel is altered by the changes in season.

4. Discussion

The seasonal breeding of animals is a phenomenon that only occurs in the specific season. In this study, for the first time, we used the NGS to explore the composition of gut microbiota in the wild ground squirrel, and we demonstrated the effect of seasonal breeding on gut microbiota. Diet, phylogeny, age, genotype, and other factors can affect the composition of host gut microbiota [48–51]. Among them, diet is considered to be the most important factor [52]. Our study found that there was no significant

difference in the diversity and richness of gut microbiota between the breeding season and the non-breeding season of the wild ground squirrel. It may be due to the foraging environment, which relatively free in the wild and their feeding preference is not easy to change. The PCoA based on the Bray-Curtis distance matrices showed that the community structure of the wild ground squirrel gut microbiota clustered basically by breeding seasons, with differences in community structure between samples from the breeding season and non-breeding season.

The analysis for the taxonomic of gut microbiota showed that the main bacteria of both breeding season and non-breeding season wild ground squirrel belonged to *Firmicutes* and *Bacteroidetes*, which are the same as the dominant microbiota species of mammalian gut [53]. In addition, these results are consistent with studies on the gut microbiome of other Spermophilus mammals, such as arctic ground squirrels (*Urocitellus parryii*) [54] and 13-lined ground squirrels (*Ictidomys tridecemlineatus*) [55]. *Firmicutes* can degrade polysaccharides through amyloplast and cellulosome multi-enzyme complexes [56], and it has a positive effect on nutrient and energy absorption from feed ingredients [57]. *Bacteroides* can not only degrade polysaccharides, carbohydrates, and proteins improve in the gut but also can assemble polysaccharides to assist the host to get nutrients from the diet [56, 58]. Besides, it can also improve the gut environment and make it more friendly to itself and other microorganisms [56]. At the same time, there is a mutual promoting symbiosis between *Firmicum* and *Bacteroides*. The high abundance of *Firmicum* and *Bacteroides* have the potential to promote the host to absorb or store energy and the high proportion of *Firmicum* / *Bacteroides* are also related to the prevalence of some diseases [59, 60].At the genus level, *Lactobacillus* has the largest proportion, which is similar to other rodent mammals like wild wood mice (*Apodemus sylvaticus*) [61] and Siberian hamsters (*Phodopus sungorus*) [62]. These results also indicate that in the evolution of vertebrate gut microbiota, the species of bacteria that can settle in the gut are limited [63].

The wild ground squirrel of the breeding season and non-breeding season had four representative different communities at the phyla level, including *Proteobacteria*, *Tenericutes*, *Verrucomicrobiais*, and *Elusimicrobia*. *Proteobacteria* have versatile physiology and greatly variable morphology, which are the most unstable phylum compared with the other three major phyla (*Firmicutes*, *Bacteroidetes*, and *Actinobacteria*) [64]. The phylum can be divided into six classes according to the rRNA sequence, including Alpha-, Beta-, Gamma-, Delta-, Epsilon-, and Zetaproteobacteria. It can adjust metabolism flexibly and tolerate low nutritional food [65], but at the same time, the growth of *Proteobacteria* can cause biological diseases, which can be used as a diagnostic marker of potential disease risk [66]. Our study showed that the phylum of *Proteobacteria*, the class of *Alphaproteobacteria* and the order of *Rhodospirillales* increased in the gut microbiota of the wild ground squirrel during the non-breeding season, we hypothesize that *Proteobacteria* which is high abundance in the non-breeding season helps to improve the digestion and absorption efficiency of the wild ground squirrel and adjust its metabolism to balance its consumption during the breeding period. *Tenericutes* are a special class of bacteria that famous for lack of cell walls, consisting of the sole class *Mollicutes* [67]. The most significant genus in the phylum *Tenericutes* is Mycoplasma (*Mycoplasmataceae, Mycoplasmatales*) [68]. In humans, Mycoplasma (*Mycoplasmataceae, Mycoplasmatales*, Tenericutes) infection can cause some

reproductive disorder diseases [69, 70]. Our study shows that the relative abundance of Mycoplasma in the non-breeding season is significantly increased, which indicates that Mycoplasma has a certain negative impact on reproduction. During the breeding period, the abundance of order *Lactobacillales* belongs to the class *Bacilli* increased significantly. Though *Lactobacillales*, one of the diverse and phylogenetically heterogeneous orders of lactic acid producing bacteria, are not dominant microbiota in the gut, they play an important role in animal reproduction. They can use carbohydrates through fermentation to produce lactic acid and have a bearing on vitamin B9 (folate) biosynthesis in humans [71, 72]. Folate is mainly involved in the synthesis of genetic material, which is essential for cell growth and reproduction [73]. Besides, *Lactobacillales* can also indirectly regulate the HPG axis through the activation of leptin [3]. Therefore, the increase of *Lactobacillales* in the wild ground squirrel during the breeding season is conducive to the improvement of reproductive performance.

In this study, we also used PICRUSt to predict the potential function of the gut microbiota, and the functional pathways with significant differences in expression between the breeding season and non-breeding season were identified. The results showed that three KEGG pathways (carbohydrate metabolism, nucleotide metabolism, and lipid metabolism) were significantly expressed in the breeding season, and five KEGG pathways (cell motility, transcription, energy metabolism, signal transduction, and cancers) were increased in the non-breeding season. It has been reported that gut microbiota plays an important role in a variety of metabolic processes in the host, including glucose metabolism, lipid metabolism, and energy homeostasis [74]. In terms of lipid metabolism, gut microbiota may affect host lipid metabolism through metabolites and lipopolysaccharides produced by gut microbiota [75]. Lipid metabolism affects fetal growth and late pregnancy outcome. Studies in women and rats have found that fat pools accelerate breakdown during the last third of pregnancy [76]. Our results indicated that the increase of lipid metabolism pathway expression in the wild ground squirrel during the breeding season may be due to the increased expression of gut microbiota related to lipid metabolism. It avoids the negative effect of fat accumulation on the body in the breeding season. The interaction between carbohydrate and lipid metabolism is mutual [77], so it is understandable that the carbohydrate metabolism pathway is up-regulated during the breeding season as a lipid metabolism pathway. We speculate that the reason for the up-regulation of the energy metabolism pathway in the non-breeding period may be the host needs to metabolize the energy accumulated during the breeding season to maintain its health. These pathways may contribute to the reproduction and homeostasis of the wild ground squirrel.

5. Conclusion

In summary, we sequenced the gut microbiota of the wild ground squirrel in the breeding season and non-breeding season by 16S rRNA gene sequencing technology to learn the effects of seasonal breeding on gut microbiota. The difference analysis of LEFSe showed that there were different expression groups at phylum to genus level in the breeding season and non-breeding season. The functional prediction results of PICRUSt showed that the gut microbiota with significant differences mainly existed in metabolic pathways under different reproductive strategies. These results suggest that the seasonal breeding of

animals has a certain impact on gut microbiota. The results of this study expand our knowledge of the symbiosis and co-evolution of seasonal breeding animals and their gut microbiota, and provide a prerequisite for future studies on the special effects of gut microbiota on seasonal breeding through metagenomic analysis.

Abbreviations

NGS
Next-generation sequencing; HPG axis:hypothalamus-pituitary-gonadal axis; GnRH:gonadotropin releasing hormone; LH:luteinizing hormone; FSH:follicle stimulating hormone; TH:hyroid hormone; GnIH:gonadotropin inhibitory hormone; ERα:estrogen receptor alpha; ERβ:estrogen receptor beta; AR:android receptor; P450arom:aromatase cytochrome P450; LHR:Luteinizing hormone receptor; FSHR:Follicle stimulating hormone; ASVs:amplicon sequence variants; RDP:Ribosomal Database Project; LEFSe:Linear Discriminant Analysis Effect Size; PICRUSt:Phylogenetic Investigation of Communities by Reconstruction of Unobserved States; KEGG:Kyoto Encyclopedia of Genes and Genomes

Declarations

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Authors' contributions

XY Y: Conceptualization, Resources, Writing – original draft, Writing - review & editing. YC Y: Resources, Investigation, Writing - review & editing. XY Z, JH Z: Resources. FL G, HL Z, YY H, Q W: Conceptualization, Writing - review & editing. ZR Y: Conceptualization, Writing - review & editing, Supervision. All authors read and approved the final version of this manuscript.

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Availability of data and materials

All data generated or analyzed during this study can be made available by the corresponding author upon reasonable request.

Ethics approval and consent to participate

All animal experiments were approved by the Policy on the Care and Use of Animals by the Ethics Committee of Beijing Forestry University and Hebei Provincial Department of Agriculture (jnzf11 / 2007).

Consent for publication

Not applicable.

Competing Interests

The authors declare no conflicts of interest.

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References

1. Savage DC. Microbial ecology of the gastrointestinal tract. *Annual review of microbiology.* 1977;31:107-133.
2. Cani PD, Delzenne NM. Gut microflora as a target for energy and metabolic homeostasis. *Curr Opin Clin Nutr Metab Care.* 2007;10(6):729-734.
3. Gioacchini G, Maradonna F, Lombardo F, Bizzaro D, Olivotto I, Carnevali O. Increase of fecundity by probiotic administration in zebrafish (Danio rerio). *Reproduction.* 2010;140(6):953-959.
4. McFall-Ngai M, Hadfield MG, Bosch TCG, et al. Animals in a bacterial world, a new imperative for the life sciences. *Proceedings of the National Academy of Sciences.* 2013;110(9):3229-3236.
5. Quach D, Britton RA. Gut Microbiota and Bone Health. *Adv Exp Med Biol.* 2017;1033:47-58.
6. Pickard JM, Zeng MY, Caruso R, Nunez G. Gut microbiota: Role in pathogen colonization, immune responses, and inflammatory disease. *Immunol Rev.* 2017;279(1):70-89.
7. Ridaura VK, Faith JJ, Rey FE, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science.* 2013;341(6150):1241214.
8. Patterson E, Ryan PM, Cryan JF, et al. Gut microbiota, obesity and diabetes. *Postgrad Med J.* 2016;92(1087):286-300.
9. Pedersen HK, Gudmundsdottir V, Nielsen HB, et al. Human gut microbes impact host serum metabolome and insulin sensitivity. *Nature.* 2016;535(7612):376-381.
10. Jiang C, Li G, Huang P, Liu Z, Zhao B. The Gut Microbiota and Alzheimer's Disease. *J Alzheimers Dis.* 2017;58(1):1-15.
11. Hu X, Wang T, Jin F. Alzheimer's disease and gut microbiota. *Sci China Life Sci.* 2016;59(10):1006-1023.
12. Quigley EMM. Microbiota-Brain-Gut Axis and Neurodegenerative Diseases. *Curr Neurol Neurosci Rep.* 2017;17(12):94.
13. Ma J, Li H. The Role of Gut Microbiota in Atherosclerosis and Hypertension. *Front Pharmacol.* 2018;9:1082.
14. Zeng C, Tan H. Gut Microbiota and Heart, Vascular Injury. *Advances in Experimental Medicine and Biology.* 2020;1238:107-141.

15. Viaud S, Saccheri F, Mignot G, et al. The Intestinal Microbiota Modulates the Anticancer Immune Effects of Cyclophosphamide. *Science.* 2013;342(6161):971-976.
16. Philips CA, Augustine P, Yerol PK, et al. Modulating the Intestinal Microbiota: Therapeutic Opportunities in Liver Disease. *J Clin Transl Hepatol.* 2020;8(1):87-99.
17. Koren O, Goodrich JK, Cullender TC, et al. Host remodeling of the gut microbiome and metabolic changes during pregnancy. *Cell.* 2012;150(3):470-480.
18. Dai Z, Wu Z, Hang S, Zhu W, Wu G. Amino acid metabolism in intestinal bacteria and its potential implications for mammalian reproduction. *Mol Hum Reprod.* 2015;21(5):389-409.
19. Sun B, Wang X, Bernstein S, et al. Marked variation between winter and spring gut microbiota in free-ranging Tibetan Macaques (Macaca thibetana). *Sci Rep.* 2016;6:26035.
20. Nishiwaki-Ohkawa T, Yoshimura T. Molecular basis for regulating seasonal reproduction in vertebrates. *J Endocrinol.* 2016;229(3):R117-R127.
21. Sirotkin AV, Schaeffer HJ. Direct regulation of mammalian reproductive organs by serotonin and melatonin. *The Journal of endocrinology.* 1997;154(1):1-5.
22. Yoshimura T. Thyroid hormone and seasonal regulation of reproduction. *Front Neuroendocrinol.* 2013;34(3):157-166.
23. Smith JT. The role of kisspeptin and gonadotropin inhibitory hormone in the seasonal regulation of reproduction in sheep. *Domest Anim Endocrinol.* 2012;43(2):75-84.
24. Smith JT, Coolen LM, Kriegsfeld LJ, et al. Variation in kisspeptin and RFamide-related peptide (RFRP) expression and terminal connections to gonadotropin-releasing hormone neurons in the brain: a novel medium for seasonal breeding in the sheep. *Endocrinology.* 2008;149(11):5770-5782.
25. Dardente H, Birnie M, Lincoln GA, Hazlerigg DG. RFamide-related peptide and its cognate receptor in the sheep: cDNA cloning, mRNA distribution in the hypothalamus and the effect of photoperiod. *J Neuroendocrinol.* 2008;20(11):1252-1259.
26. Smith GD, L M Jackson DLF. Leptin regulation of reproductive function and fertility. *Theriogenology.* 2002;57(1):73-86.
27. Baker JM, Al-Nakkash L, Herbst-Kralovetz MM. Estrogen-gut microbiome axis: Physiological and clinical implications. *Maturitas.* 2017;103:45-53.
28. Plottel CS, Blaser MJ. Microbiome and malignancy. *Cell Host Microbe.* 2011;10(4):324-335.
29. Yuan Z, Wang Y, Yu W, et al. Seasonal expressions of oxytocin and oxytocin receptor in the epididymides in the wild ground squirrels (Citellus Dauricus Brandt). *Gen Comp Endocrinol.* 2020;289:113391.
30. Yu W, Zhang Z, Liu P, et al. Seasonal expressions of SPAG11A and androgen receptor in the epididymis of the wild ground squirrels (Citellus dauricus Brandt). *Eur J Histochem.* 2020;64(2):120-128.
31. Wang Y, Yao Y, Zhang C, et al. Seasonal expressions of COX-1, COX-2 and EP4 in the uteri of the wild Daurian ground squirrels (Spermophilus dauricus). *Prostaglandins Other Lipid Mediat.*

2019;143:106343.

32. Zhang F, Wang Y, Han W, et al. Seasonal changes of androgen receptor, estrogen receptors and aromatase expression in the hippocampus of the wild male ground squirrels (Citellus dauricus Brandt). *Gen Comp Endocrinol.* 2017;249:93-100.
33. Zhang F, Wang J, Jiao Y, et al. Seasonal changes of androgen receptor, estrogen receptors and aromatase expression in the medial preoptic area of the wild male ground squirrels (Citellus dauricus Brandt). *Eur J Histochem.* 2016;60(2):2621.
34. Li Q, Zhang F, Zhang S, et al. Seasonal expression of androgen receptor, aromatase, and estrogen receptor alpha and beta in the testis of the wild ground squirrel (Citellus dauricus Brandt). *Eur J Histochem.* 2015;59(1):2456.
35. Wang J, Liu Q, Qi H, et al. Seasonal expressions of androgen receptor, P450arom and estrogen receptors in the epididymis of the wild ground squirrel (Citellus dauricus Brandt). *Gen Comp Endocrinol.* 2019;270:131-138.
36. Wang Y, Wang Z, Yu W, et al. Seasonal expressions of androgen receptor, estrogen receptors and cytochrome P450 aromatase in the uteri of the wild Daurian ground squirrels (Spermophilus dauricus). *Eur J Histochem.* 2018;62(1):2889.
37. Wang J, Wang Y, Zhu M, et al. Seasonal expression of luteinizing hormone receptor and follicle stimulating hormone receptor in testes of the wild ground squirrels (Citellus dauricus Brandt). *Acta Histochem.* 2017;119(7):727-732.
38. Wang J, Liu Q, Wang Z, et al. Seasonal expressions of luteinising hormone receptor, follicle-stimulating hormone receptor and prolactin receptor in the epididymis of the male wild ground squirrel (Spermophilus dauricus). *Reprod Fertil Dev.* 2019;31(4):735-742.
39. Comninos AN, Jayasena CN, Dhillo WS. The relationship between gut and adipose hormones, and reproduction. *Hum Reprod Update.* 2014;20(2):153-174.
40. Rognes T, Flouri T, Nichols B, Quince C, Mahé F. VSEARCH: a versatile open source tool for metagenomics. *PeerJ.* 2016;4.
41. Edgar RC, Flyvbjerg H. Error filtering, pair assembly and error correction for next-generation sequencing reads. *Bioinformatics.* 2015;1(31):3476-3482.
42. Liu YX, Qin Y, Chen T, et al. A practical guide to amplicon and metagenomic analysis of microbiome data. *Protein Cell.* 2020.
43. Edgar RC. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics.* 2010;26(19):2460-2461.
44. Zhang J, Liu YX, Zhang N, et al. NRT1.1B is associated with root microbiota composition and nitrogen use in field-grown rice. *Nat Biotechnol.* 2019;37(6):676-684.
45. Segata N, Izard J, Waldron L, et al. Metagenomic biomarker discovery and explanation. *Genome Biol.* 2011;12(6):R60.

46. Langille MG, Zaneveld J, Caporaso JG, et al. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nat Biotechnol.* 2013;31(9):814-821.
47. Parks DH, Tyson GW, Hugenholtz P, Beiko RG. STAMP: statistical analysis of taxonomic and functional profiles. *Bioinformatics.* 2014;30(21):3123-3124.
48. David LA, Maurice CF, Carmody RN, et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature.* 2014;505(7484):559-563.
49. Goodrich JK, Waters JL, Poole AC, et al. Human genetics shape the gut microbiome. *Cell.* 2014;159(4):789-799.
50. Amato KR, Martinez-Mota R, Righini N, et al. Phylogenetic and ecological factors impact the gut microbiota of two Neotropical primate species. *Oecologia.* 2016;180(3):717-733.
51. Yatsunenko T, Rey FE, Manary MJ, et al. Human gut microbiome viewed across age and geography. *Nature.* 2012;486(7402):222-227.
52. Wu GD, Chen J, Hoffmann C, et al. Linking long-term dietary patterns with gut microbial enterotypes. *Science.* 2011;334(6052):105-108.
53. Leser TD, Molbak L. Better living through microbial action: the benefits of the mammalian gastrointestinal microbiota on the host. *Environ Microbiol.* 2009;11(9):2194-2206.
54. Stevenson TJ, Duddleston KN, Buck CL. Effects of season and host physiological state on the diversity, density, and activity of the arctic ground squirrel cecal microbiota. *Appl Environ Microbiol.* 2014;80(18):5611-5622.
55. Carey HV, Walters WA, Knight R. Seasonal restructuring of the ground squirrel gut microbiota over the annual hibernation cycle. *Am J Physiol Regul Integr Comp Physiol.* 2013;304(1):R33-42.
56. Wexler AG, Goodman AL. An insider's perspective: Bacteroides as a window into the microbiome. *Nat Microbiol.* 2017;2:17026.
57. Turnbaugh PJ, Backhed F, Fulton L, Gordon JI. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host Microbe.* 2008;3(4):213-223.
58. Tremaroli V, Backhed F. Functional interactions between the gut microbiota and host metabolism. *Nature.* 2012;489(7415):242-249.
59. Murphy EF, Cotter PD, Healy S, et al. Composition and energy harvesting capacity of the gut microbiota: relationship to diet, obesity and time in mouse models. *Gut.* 2010;59(12):1635-1642.
60. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology□human gut microbes associated with obesity. *Nature.* 2006;444(7122):1022-1023.
61. Maurice CF, Knowles SC, Ladau J, et al. Marked seasonal variation in the wild mouse gut microbiota. *ISME J.* 2015;9(11):2423-2434.
62. Sylvia KE, Jewell CP, Rendon NM, St John EA, Demas GE. Sex-specific modulation of the gut microbiome and behavior in Siberian hamsters. *Brain Behav Immun.* 2017;60:51-62.
63. Ley RE, Lozupone CA, Hamady M, Knight R, Gordon JI. Worlds within worlds□evolution of the vertebrate gut microbiota. *Nature Reviews Microbiology.* 2008;6:776–788.

64. Faith JJ, Guruge JL, Charbonneau M, et al. The long-term stability of the human gut microbiota. *Science.* 2013;341(6141):1237439.
65. Tong Q, Liu XN, Hu ZF, et al. Effects of Captivity and Season on the Gut Microbiota of the Brown Frog (Rana dybowskii). *Front Microbiol.* 2019;10:1912.
66. Shin NR, Whon TW, Bae JW. Proteobacteria: microbial signature of dysbiosis in gut microbiota. *Trends Biotechnol.* 2015;33(9):496-503.
67. Gupta RS, Sawnani S, Adeolu M, Alnajar S, Oren A. Phylogenetic framework for the phylum Tenericutes based on genome sequence data: proposal for the creation of a new order Mycoplasmoidales ord. nov., containing two new families Mycoplasmoidaceae fam. nov. and Metamycoplasmataceae fam. nov. harbouring Eperythrozoon, Ureaplasma and five novel genera. *Antonie Van Leeuwenhoek.* 2018;111(9):1583-1630.
68. Lock BA, Wellehan J. Ophidia (Snakes). In: *Fowler's Zoo and Wild Animal Medicine, Volume 8.*2015:60-74.
69. Huang C, Zhu HL, Xu KR, Wang SY, Fan LQ, Zhu WB. Mycoplasma and ureaplasma infection and male infertility: a systematic review and meta-analysis. *Andrology.* 2015;3(5):809-816.
70. Lis R, Rowhani-Rahbar A, Manhart LE. Mycoplasma genitalium Infection and Female Reproductive Tract Disease: A Meta-Analysis *Clinical Infectious Diseases.* 2015;61(3):418-426.
71. Goldstein EJ, Tyrrell KL, Citron DM. Lactobacillus species: taxonomic complexity and controversial susceptibilities. *Clin Infect Dis.* 2015;60 Suppl 2:S98-107.
72. Wang W, Cao J, Li JR, Yang F, Li Z, Li LX. Comparative analysis of the gastrointestinal microbial communities of bar-headed goose (Anser indicus) in different breeding patterns by high-throughput sequencing. *Microbiol Res.* 2016;182:59-67.
73. Lamers Y. Folate recommendations for pregnancy, lactation, and infancy. *Ann Nutr Metab.* 2011;59(1):32-37.
74. Sonnenburg JL, Backhed F. Diet-microbiota interactions as moderators of human metabolism. *Nature.* 2016;535(7610):56-64.
75. Schoeler M, Caesar R. Dietary lipids, gut microbiota and lipid metabolism. *Rev Endocr Metab Disord.* 2019;20(4):461-472.
76. Herrera E. Lipid metabolism in pregnancy and its consequences in the fetus and newborn. *Endocrine.* 2002;19(1):43-55.
77. Tsimihodimos V, Elisaf M. Effects of evolving lipid-lowering drugs on carbohydrate metabolism. *Diabetes Res Clin Pract.* 2018;137:1-9.

Figures

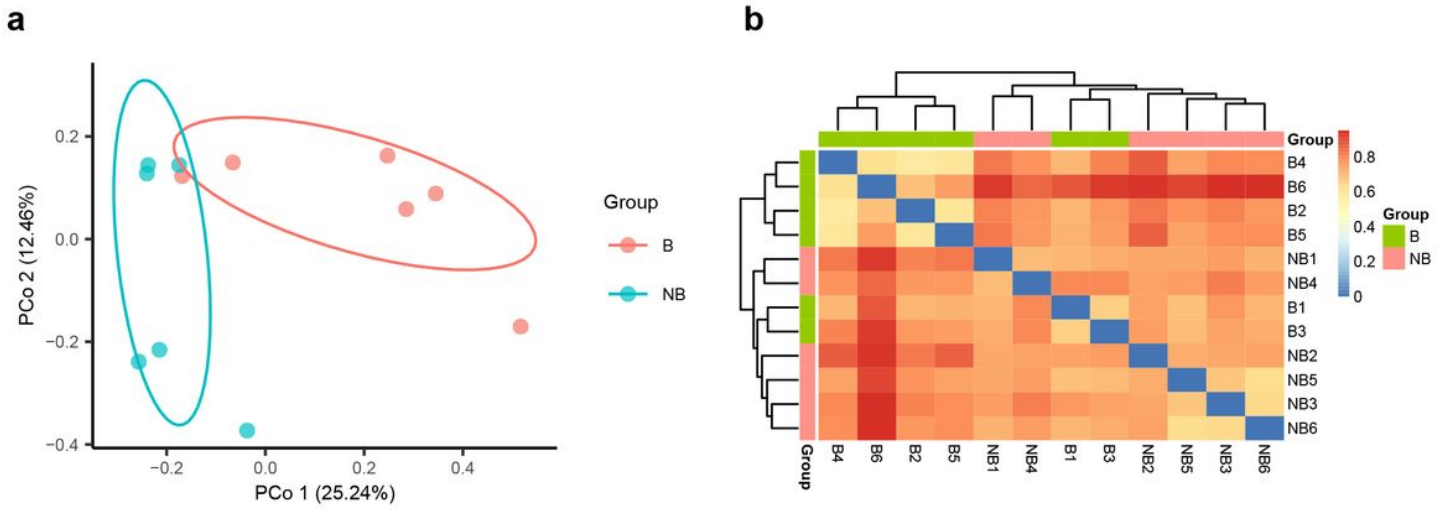

Figure 1

Beta diversity of the gut microbiota in the wild ground squirrel. B, breeding season; NB, non-breeding season. (a) Principal coordinate analysis (PCoA) plot of gut microbiota structure using the Bray-curtis distance metric. (b) Sample correlation heatmap based on the Bray-curtis distance matrices.

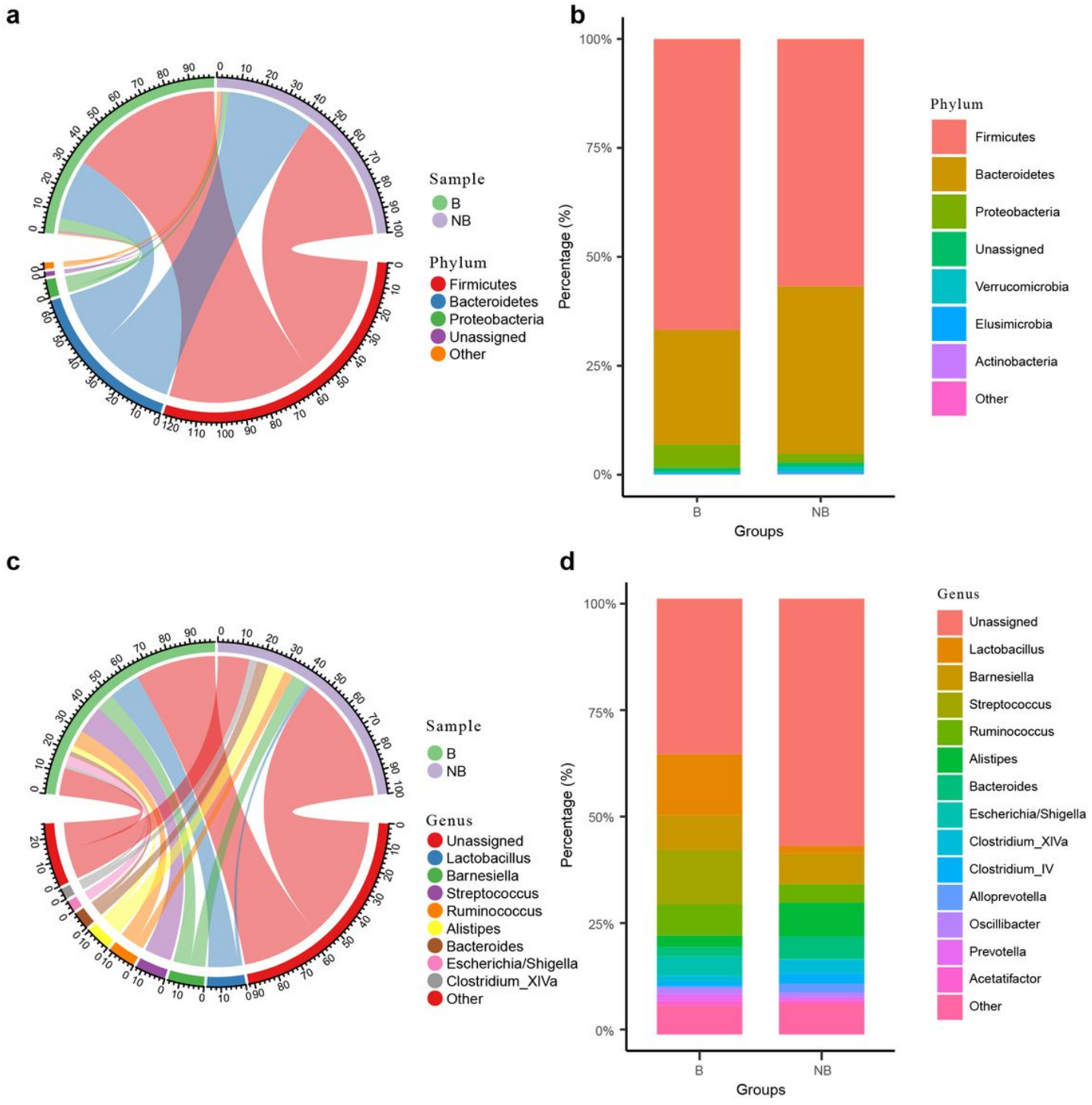

Figure 2

Taxonomic analysis of the gut microbial community composition of each group. Sequences that could not be assigned at phylum level and genus level were marked as“Unassigned”. B, breeding season; NB, no-breeding season. (a) Chord diagram at phylum level. (b) The bar chart at phylum level. (c) Chord diagram at genus level. (d) The bar chart at genus level.

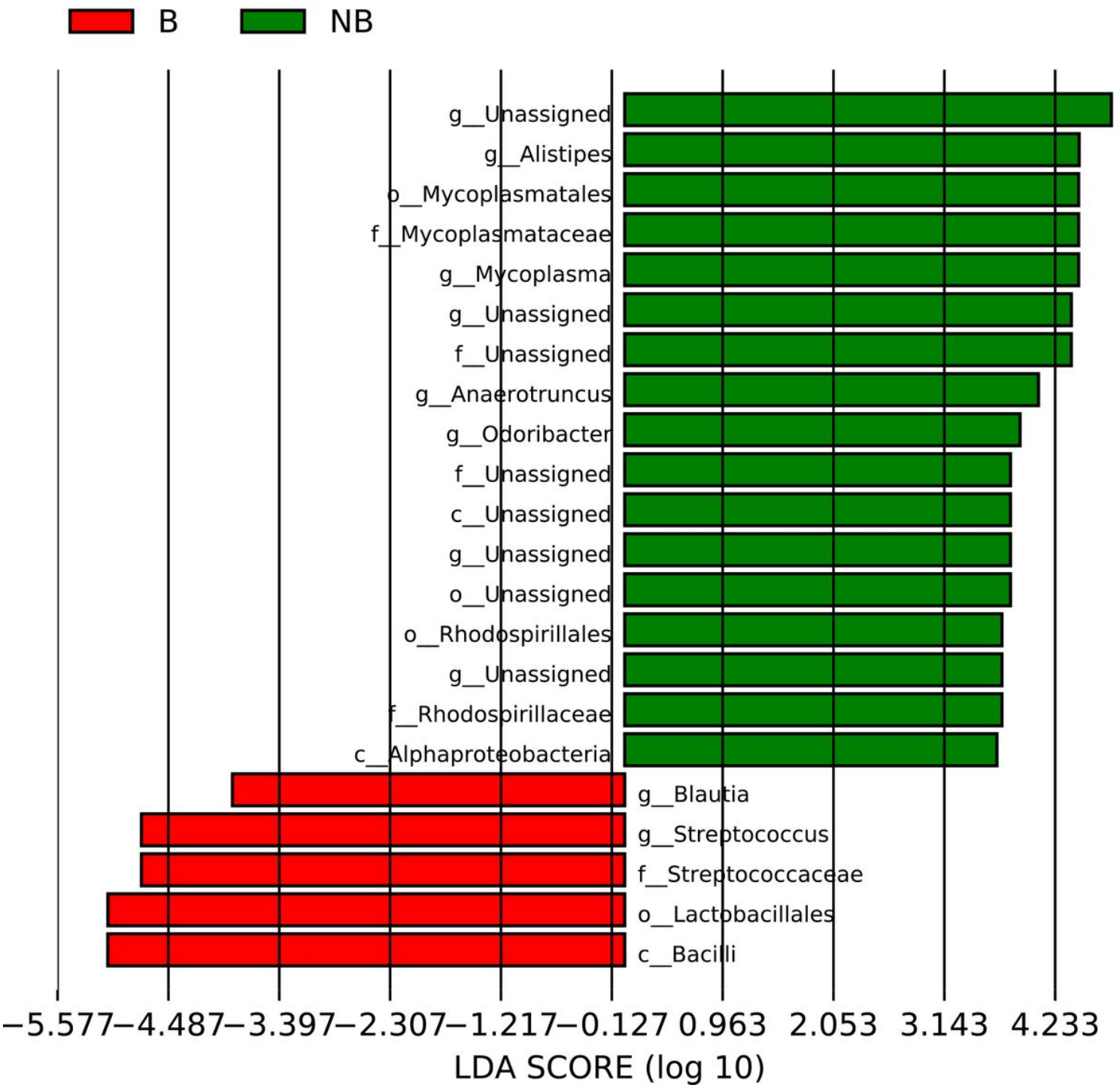

Figure 3

Representative differential microbiota among two groups identified by linear discriminant analysis coupled with effect size (LEfSe). Linear discriminant analysis (LDA) is the influence degree of species with significant differences between two groups. B, breeding season; NB, no-breeding season.

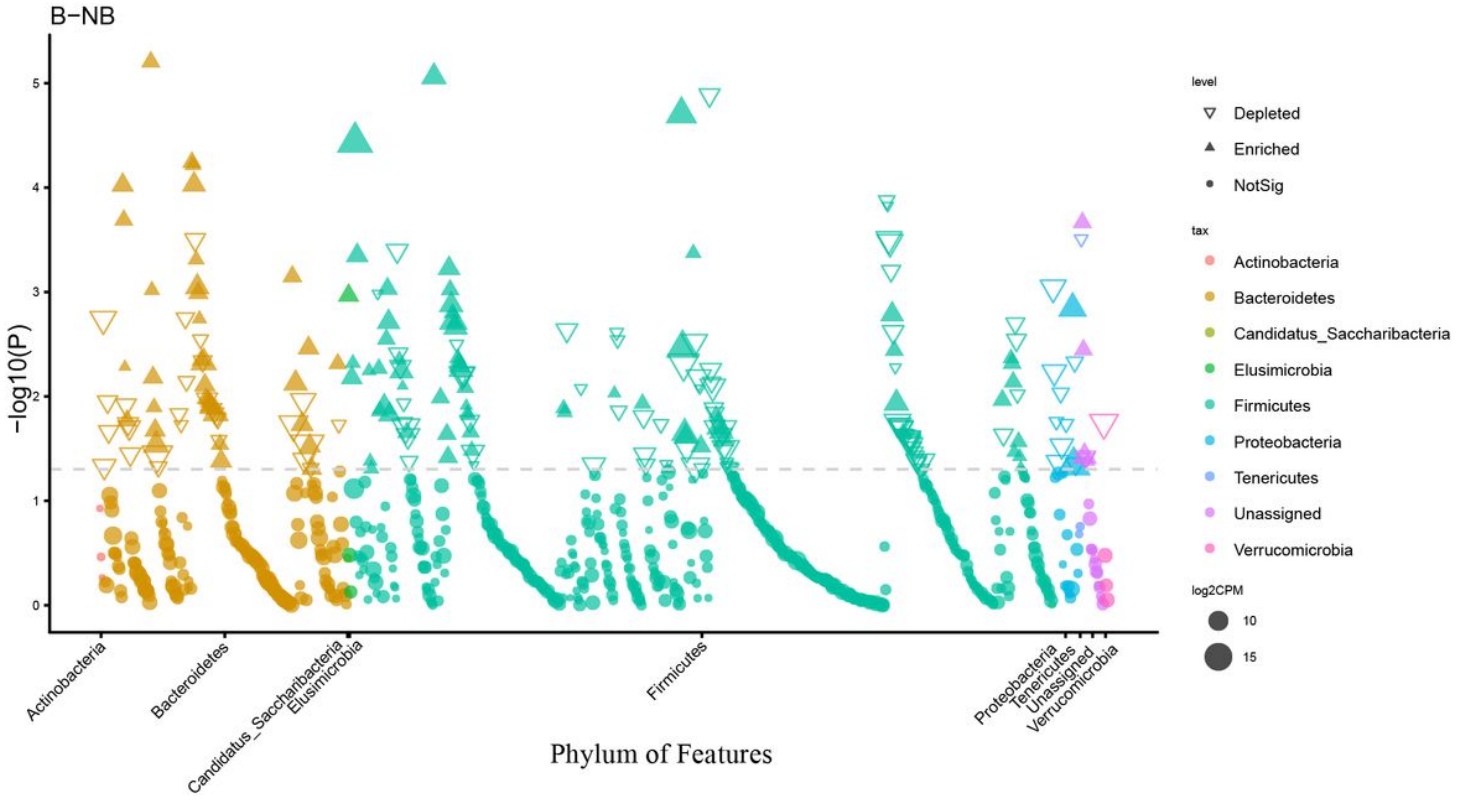

Figure 4

Manhattan plot of different microbiota at phylum level. The group of non-breeding season (NB) was used as the control, and the group of breeding season (B) was compared with it. The color of each dot represents the different taxonomic of the ASVs at the phylum level, and the size of the dot represents the relative abundance of the ASV, taken log2CPM. CPM, count per million.The shape of the dot indicates the type of the change.

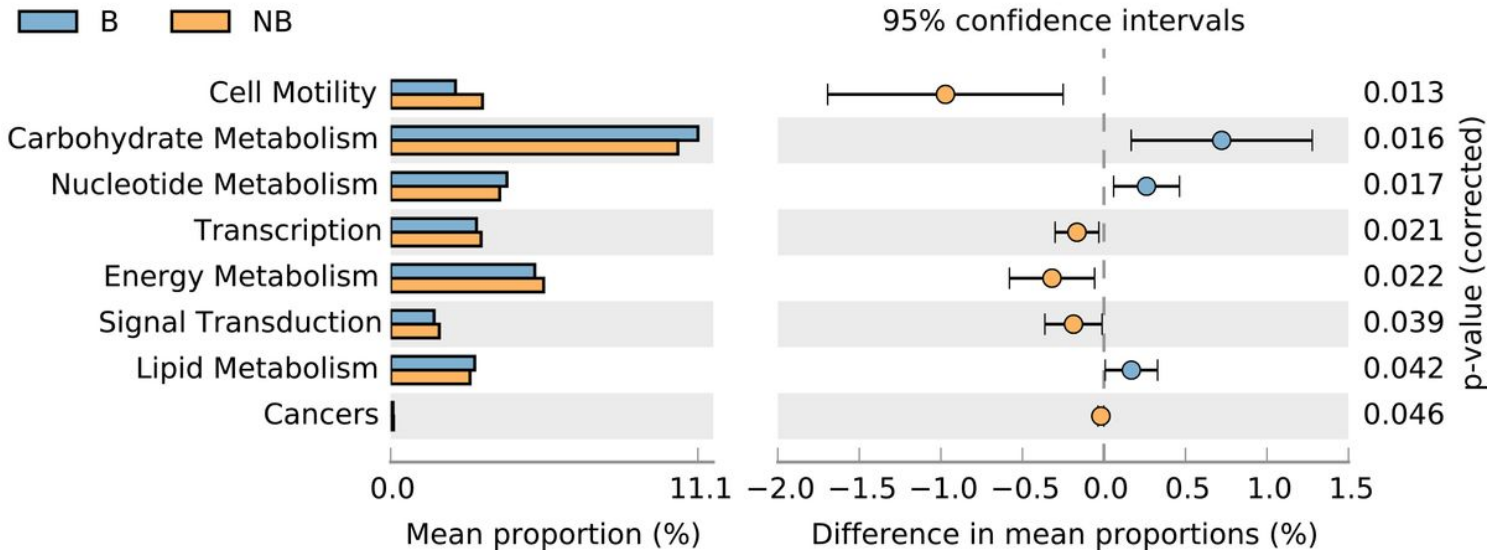

Figure 5

Extended error bar for significant differences in the second level of functional categories of KEGG pathway. B, breeding season; NB, no-breeding season.

Supplementary Files

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