**Gut microecology of four sympatric desert rodents varies by diet**

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**Abstract**

The gut microbiome can be one pathway by which a host rapidly acclimates and adapts to its ecological environment. Exploring how the microbiome has evolved to differ between hosts with different diets provides insights into the profound interactions between hosts and microbes within these systems. In this study, we used DNA metabarcoding techniques and macrogenomic prediction techniques to study the gut microbes of four desert rodent species with different feeding strategies in the same habitat. One species is herbivorous (*Spermophilus alashanicus*), one is seed-eating (*Phodopus roborovskii*), another is omnivorous (*Dipus sagitta*), and the last (*Orientallactaga sibirica*) has a diet with a relatively high proportion of meat. Diets rich in plants and insects can be challenging to digest due to the abundance of indigestible fiber and stable chitin, respectively*.* Out of the species studied, the herbivorous *Spermophilus alashanicus* has the highest density of *UCG-005* genes and the highest predicted abundance of genes related to digestive complexity. The composition of *Phodopus roborovskii*'s microbiome has the highest variation between individuals, yet *Phodopus roborovskii* has the highest predicted abundance of genes associated with simple sugars—reflecting this species’ potential adaptability to the fiber within plant seeds and its constraints brought about by its smaller body size. The most insectivorous species,  *Orientallactaga sibirica*, exhibits the highest predicted abundance of genes related to chitin degradation. This study has enhanced our understanding of the gut microbiota in the intestines of rodents as they adapt to various dietary strategies.

**Keywords:** Sympatric coexistence,Rodents, Dietary strategy, gut microbiome

**Introduction**

The mammalian gut is one of the major sources of microbial biodiversity on Earth [1], and the mammalian gut microbiome is a particularly dense and diverse community that co-encodes 150 times more genes than its host [2]. The composition of gut microbial communities is shaped by a complex set of host and environmental factors, including host genotype, species and individual development, diet, habitat, geographic location, and anthropogenic disturbance [3]. While geography, sex, reproductive status, and social structure have all been associated with mammalian gut microbiome diversity, the main factors that consistently drive it appear to be host evolutionary history and diet [4-5-6].

The gut microbiome, heralded as an "applied genotype" for animals, affects the host in a wide variety of ways. Gut microbes carry out various physiological functions for the host, including the digestion and absorption of nutrients, energy metabolism, immune responses, signal conveyance, and the breakdown of toxins [7-8-9]. Therefore, the gut microbiome has been proposed as one pathway through which the host organism can rapidly adapt to its ecological environment [10]. These microbial communities are vital for degrading complex biological polymers or toxins that are indigestible by animals, such as the lignocellulose found within plant cells walls and substances like cellulose, lignin, and guaiac acid[11]. Therefore, many species of herbivorous mammals depend on their gut microbiota for their metabolic processes, subsequently absorbing the meta-products of this metabolic activity. Most studies indicate that herbivorous mammals possess a higher abundance of genes related to cellulose degradation compared to species with other diets [12]. These studies are conducted across various taxonomic groups by comparing related species that live in similar ecological habitats but which have different diets. The analysis of the differences between their microbiomes provides insights into the symbiotic relationships between hosts and their microorganisms.

Chitin is a polysaccharide compound primarily found within the exoskeletons of arthropods. The polymer is composed of N-acetylglucosamine and D-glucose. Chitin is a linear chain of polymers with resilience and biodegradability, and it is so common in nature that it is second only to cellulose globally in material abundance, so it is common in the diet of numerous vertebrates [13-14]. A study has revealed that animals that ingest chitin-rich foods may have a symbiotic relationship with chitin-decomposing bacteria, which can facilitate their digestion of prey. The presence of convergently evolved chitinases in various species, such as the *Pipistrellus nathusii*, *Callithrix pygmaea*, and baleen whales, signifies a convergence of their gut microbiomes [15]. However, the functions of these gut microbial communities and their potential role in the digestion of chitin exoskeletons remain unexplored [16]. Additionally, much remains unknown about differences in the composition of the gut microbiome between insectivorous and non-insectivorous species of the same taxa.

Rodents are particularly suited to addressing these pivotal questions about host-microbe interactions. They are a particularly diverse order of mammals, with a rich array of ecological strategies. These animals have adapted to a multitude of different habitat types and exhibit a wide range of dietary strategies, including grazing, seed consumption, omnivory, and arthropod consumption [17-18]. Therefore, rodents have emerged as a ubiquitous model in evolutionary ecology, particularly in the investigation of the host-gut microbiome interactions and how these related to environmental factors [19]. Although previous studies have investigated the microbiological adaptations of herbivorous rodents, there has been no investigation into how the gut microbiome varies between rodent species that live in the same region but have distinct diets.

Of the numerous techniques available for studying microbial communities, the most common approach involves sequencing the genomes of microbial DNA, namely by amplifying and decoding the 16S rRNA genes of bacteria and then generating a catalog of microbes present [22-23]. This method is known as "metagenomic sequencing," and it is a technique dedicated to the investigation of the microbial DNA sequences within an entire ecological community. The sequencing of genomic DNA, specifically that focusing on the 16S rRNA gene, is limited to specific genetic regions. In contrast, metagenomic sequencing assesses the entire genome, encompassing genes and their functions from all microbial species; however, such sequencing is exorbitantly costly, leaving researchers often constrained by financial limitations [24]. Therefore, researchers have developed tools like Tax4FUN, Piphillin, PICRUSt, and PICRUSt2 (an upgraded version of PICRUSt that augments the precision and scope of function predictions) to generate a predicted functional database (the predicted metagenome) based on 16S rRNA databases. These technologies are suitable for the preliminary analysis of the predicted abundance of functional categories across various samples, yet they bear limitations due to the potential for uncharacterized microbial taxa within sequencing results derived from 16S rRNA [25].

In this experiment, we employed DNA metabarcoding techniques and predictive metagenomic techniques to investigate the gut microbiota of four rodent species with distinct diets. These four murine species coexist in the same bioregion but have distinct feeding ecologies (Table 1). We hypothesized that due to their different diets, these four species of rodents would possess unique microbial communities [26]. Specifically, we predicted that grazing rodents would possess the highest microbial diversity, akin to what is observed in other grazing mammals [27]. Finally, we hypothesized that the microbial taxa or functions present in a particular species’gut would correspond to the nutritional components of that host’s diet. For instance, herbivores would have microbes that break down cellulose, and insectivores would have microbes that degrade chitin. We anticipated that the predicted abundance of genes associated with cellulose degradation would be highest within the grazing *Spermophilus alashanicus*, and that the genes related to chitin degradation would be most plentiful among the most insectivorous host, *Orientallactaga sibirica*  (predicted based on the PICRUSt2 ).

**Table 1.**

**Methods**

**Rodent collection**

In 2021, four rodent species were collected from the desert area located in the southern part of Alashan Left Banner, Alashan League, Inner Mongolia Autonomous Region, China (37.887497°N, 105.381715°E). Among them were 7 SA, 10 PR, 10 DS, and 10 OS (Table1). In the sampling area, we used live-trap cages to capture rodents, using fresh peanut rice as bait. Traps were deployed early in the morning and checked in the evening and then again early the subsequent morning. It has been previously shown that consumption of bait in live-trap cages does not significantly affect the gut microbiome of rodents over a short period of time [28].

Rodents captured in live-trap cages were humanely euthanized, and rodent tissue samples were collected immediately afterwards. Cecal contents were stored in 2 mL sterile cryostat tubes, and samples were snap-frozen in liquid nitrogen immediately after sampling and then stored at -80 °C.

Although several rodents were collected from slightly different locations in the desert zone, differences in gut microbial communities between rodent species are much greater than geographic differences [29-30], and our sampling areas were not far from each other. Furthermore, changes in microbial composition caused by differences in collection or storage procedures are even smaller than individual differences [31-32], so these factors do not strongly influence our results.

This study was conducted in accordance with the guidelines issued by the Ethics Committee for Animal Care and Treatment of Inner Mongolia Agricultural University. The committee requires all researchers and students associated with wildlife and laboratory animals to be certified in accordance with the requirements of the Ethics Committee of Inner Mongolia Agricultural University (NND2017012 and NND2022093).

**DNA extraction**

DNA was extracted from the cecum contents using the FastDNA®SpinKitforSoilDNA extraction kit following the manufacturer’s instructions. We chose the cecum contents because the cecum has the highest microbial density and activity [33-34]. The extracted DNA was stored on dry ice and transported to Shanghai Meiji Biomedical Technology Co. The V3-V4 region of the bacterial 16SrRNA gene was amplified by PCR using upstream primer 338F (ACTCCTACGGGGAGGCAGCAG) and downstream primer 806R (GGACTACHVGGGGTWTCTAAT) [35-36], and the resulting amplicon products were combined and purified. Libraries were constructed using NEXTFLEXRapidDNA-SeqKit and sequenced to generate 2 × 300 base-paired end reads. Detailed methods for amplification, library preparation, and sequencing are described in the Supplemental Material. Sequence reads have been deposited in the NCBI database at (([PRJNA1083208](https://www.ncbi.nlm.nih.gov/portal/utils/pageresolver.fcgi?recordid=667440ae30bd3e09ebd96089)).

Quality control of the raw sequences was performed using fastp (https://github.com/OpenGene/fastp, version0.20.0) software [37]. Splicing was performed using FALSH (http://www.cbcb.umd.edu/software/flash, version1.2.7) software [38]. We filtered the bases with a quality value below 20 at the end of the reads, set a window of 50 bp, and truncated the back-end bases from the beginning of the window if the average quality value within the window was below 20. We filtered the reads below 50 bp after quality control and removed the reads containing N bases. According to the overlap relationship between PEreads, we spliced pairs of reads into one sequence. The minimum overlap length was 10 bp, and the maximum mismatch ratio allowed in the overlap region of the spliced sequences was 0.2. We screened the non-compliant sequences based on the barcodes at the beginning and end of the sequence, with a mismatch ratio of 0.2. The barcode and primers at the beginning and end of the sequence distinguished the samples and adjusted the sequence orientation. The allowed mismatch number of the barcode was 0, and the maximum primer mismatch number was 2. Using UPARSE software (http://drive5.com/uparse/, version 7.1), sequences were OTU-clustered based on 97% similarity, and any reads identified as archaea, chloroplasts, or mitochondria were removed from further analyses [39-40]. Species classification was annotated for each sequence using the RDPclassifier (http://rdp.cme.msu.edu/, version2.2), comparing it to the Silva16SrRNA database (v138) with a comparison threshold set at 70% [41]. In this experiment, we also followed the recommended protocol of extracting samples in mixed batches, so that any contaminants should be evenly distributed in the samples and therefore not lead to contamination being the cause of any observed species differences [42].

**Statistical analysis**

We utilized QIIME2 software to generate abundance tables for different taxonomic levels. We employed the Uparse software for OTU clustering and used it to conduct statistical analysis on OTUs to discern both shared and unique OTUs among the different rodent species. To compare the diversity of gut microbial communities across various species, we used the Mothur software to compute several metrics alpha diversity for the gut microbiomes. Subsequently, we applied the Kruskal-Wallis test and post-hoc inquiries to test for differences in these indices among the different rodent species. Subsequently, we employed Bray-Curtis [43] and the UniFrac metrics[44] (both with and without weighting) to compute the difference values between all sample pairs, thereby comparing the community composition of the gut microbiomes across these rodent species. The NMDS diagrammatically represents the principal component analysis, demonstrating the interconnectedness of the gut microbiota. We test for microbiome differences using two methods: the PERMDISP test within QIIME2 (999 arrangements) and the pairwise Bray-Curtis distances calculated for inbreeding samples, with the Kruskal-Wallis test and post-hoc analyses used for conducting more detailed comparisons. Ultimately, we employed PERMANOVA within QIIME2 to conduct a statistical comparison of the microbial community composition between the rodent species.

To discern the ways in which the bacterial communities of rodent species differ based on their diets, we employed the ANCOM difference test within QIIME2, comparing the abundances of specific phyla and genera. Before the detection of diversity abundance, we filtered out all bacterial taxonomic groups with read counts of less than 20, thereby eliminating the rare categories [45].

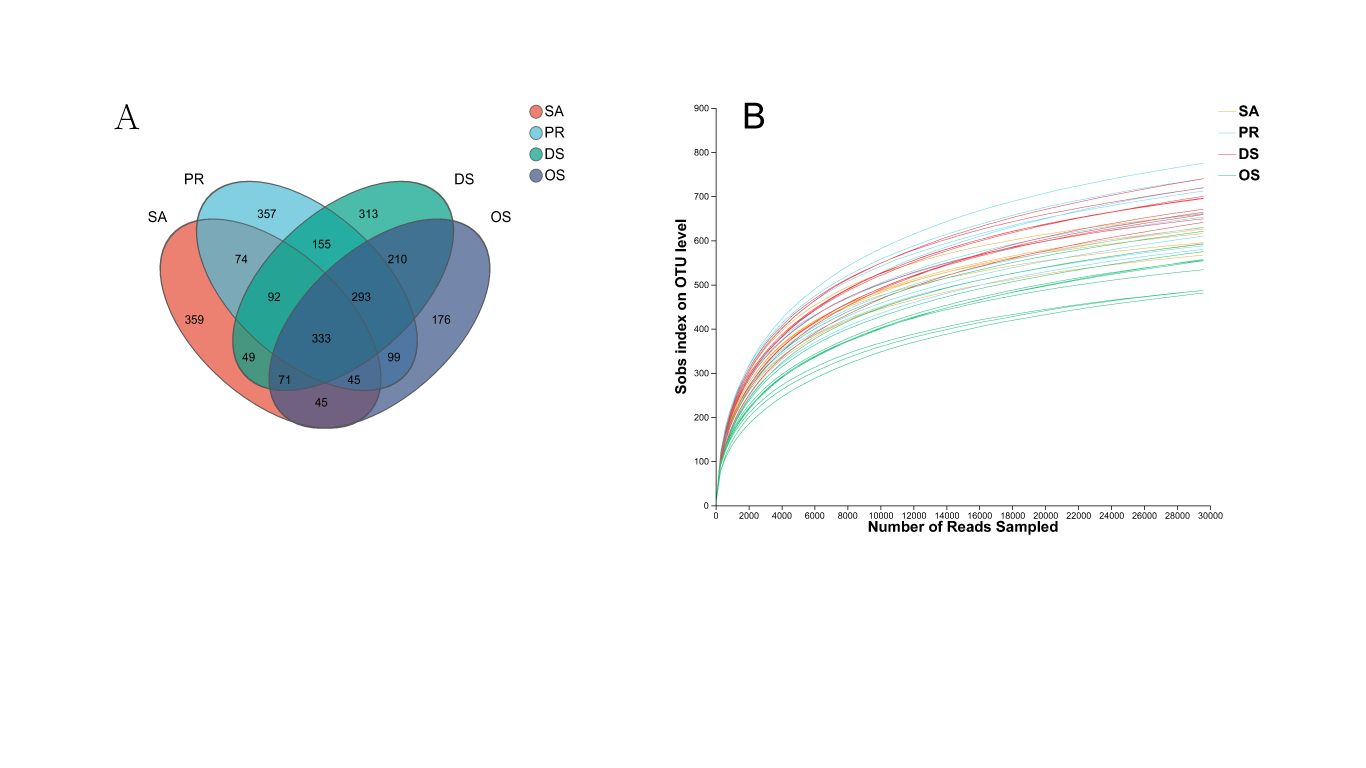
We used the QIIME2 plugin PICRUSt2[46] to generate a predicted functional list from our 16S rRNA database (predicted metagenome). We excluded any OTUs whose NSTI values exceeded 2.0 during this process. Considerations regarding the predictive power of metagenomic modeling requires a focus on prior knowledge, particularly with regard to the comparison of functional categories associated with biopolymer degradation.

By pulling the glycoside hydrolase subset from the carbohydrate activity enzyme database [47] with the KEGG entity database, we searched for pathways that are associated with our predicted results. We selected and analyzed the genes associated with simple sugars: α-glucosidase (K01187), oligosaccharide-1,6-glucosidase (K01182),α-amylase (K07405), maltose-6’-phosphate glucosidase (K01232), and α-amylase (K01176). To investigate the metabolic response of microbes to complex fibers, we also screened the predicted abundance of KEGG entities associated with the degradation of composite fibers: β-glucosidase (K05349), en-doglucanase (K01179), endo-1,4-β-xylanase (K01181), β-glucosidase (K05350), and xylan-1,4-β-xylosidase (K01198).

We compared the relative abundance of several KEGG entities associated with chitin degradation [48]. The following KEGG entities are related to chitin: chitinase (K01183), chitosanases (K01233), phospho-chitobiase (K01222), and chitin disaccharide deacetylase (K03478). We also anticipated the existence of two enzymes associated with the metabolism of chitin carbon-nitrogen bonds: β-hexosaminidase (K01207) and α-N-acetylgalactosaminidase (K01143). Kruskal-Wallis tests and post-hoc analyses were used to compare the predicted abundance between rodent species.

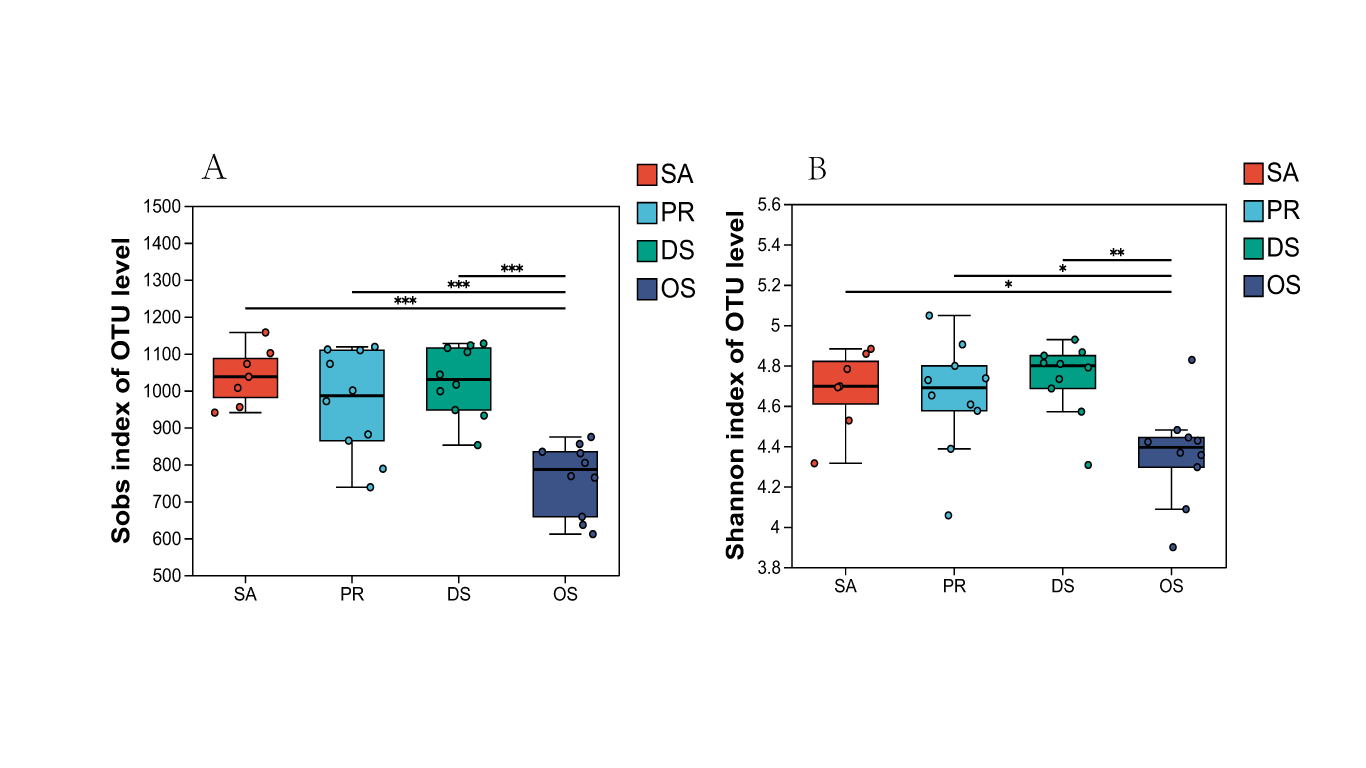
**Results**

This study analyzed the microbial composition of fecal samples from four rodent species coexisting in the same desert habitat. The sequence range of the 37 samples was 28759-29013, with an average of 28146 valid sequences per sample. A total of 1412 core OTUs were identified based on 97% sequence similarity. The DS species had an average of 483 OTUs per sample, OS had an average of 398 OTUs per sample, PR had an average of 515 OTUs per sample, and SA had an average of 450 OTUs per sample. The four species shared 333 OTUs. OS had the fewest unique OTUs, with 176, while SA had the most unique OTUs, with up to 359 (Fig. 1A). This indicates that the gut microbiome has microbes that are widely distributed across these four host species as well as ones that are unique to each host’s particular microbiome. Based on the rarefaction curve, the estimate of species richness is stable and unbiased, and the sequencing depth has covered almost all bacteria in the samples, making it suitable for subsequent data analysis (Fig. 1B).



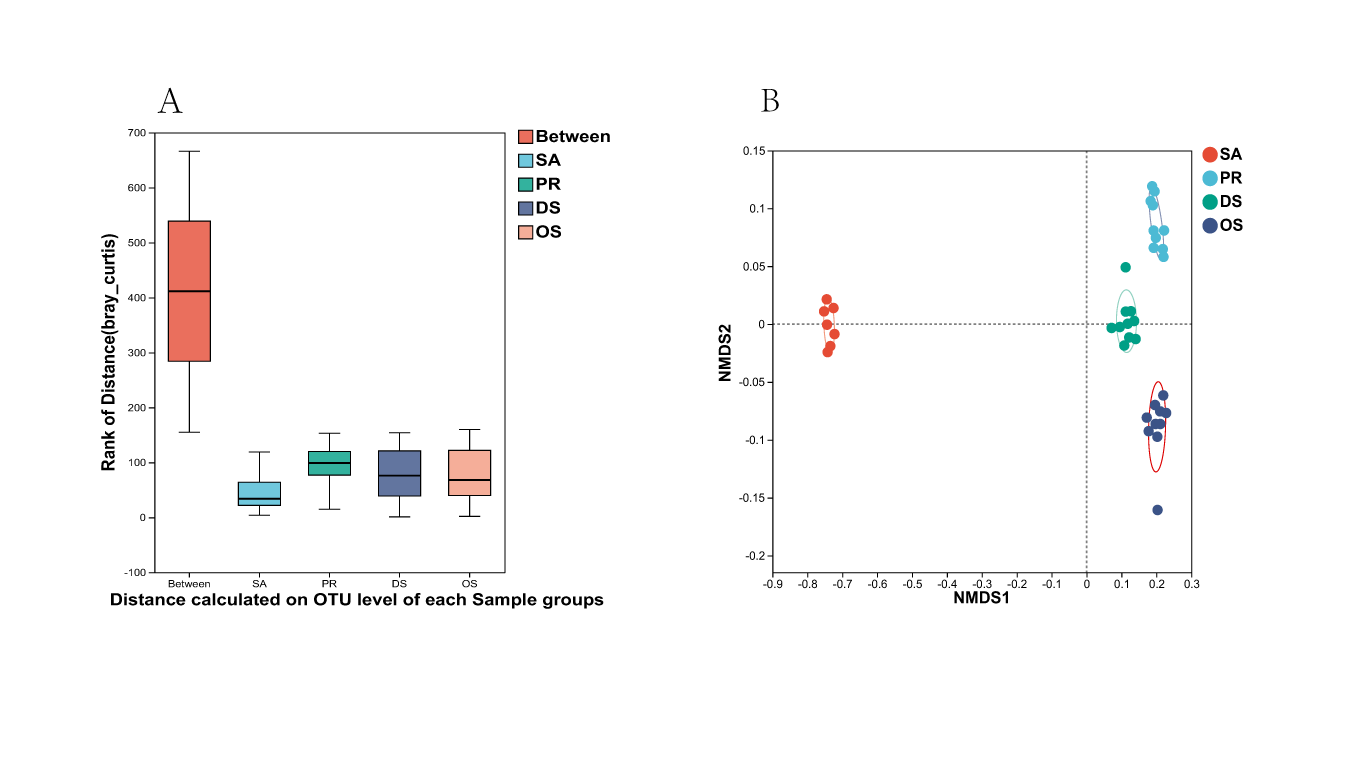
**Fig. 1**

We calculated two different alpha diversity indices, the Sobs index and Shannon index, for the gut microbiota of the four desert rodent species. As predicted, the alpha diversity was highest in the herbivorous SA. The most insectivorous species, OS, had the lowest alpha diversity and was significantly different from the other three species for both indices (Fig. 2A. B).



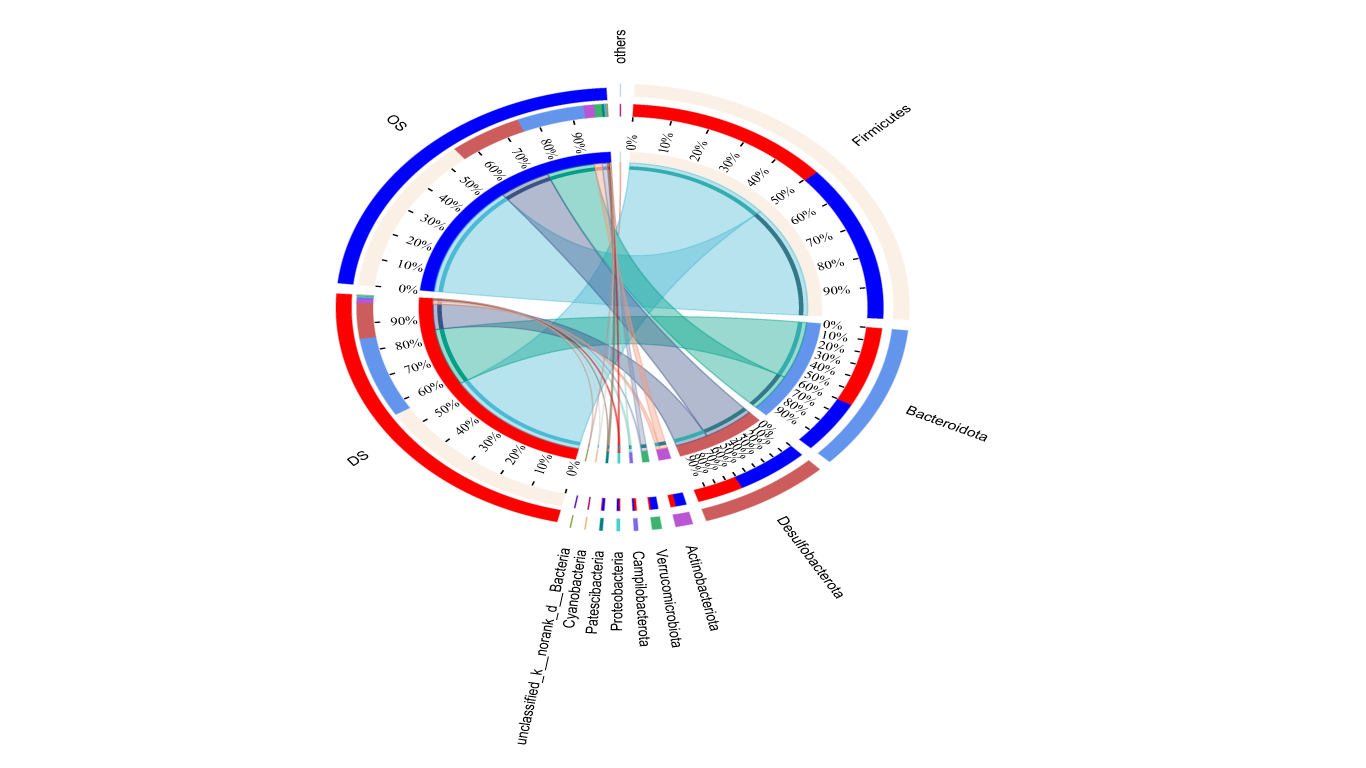
**Fig. 2**

Microbial communities also vary between individual hosts, so we used an ANOSIM analysis to assess how much the gut microbiomes varied within and between species. This analysis showed that the seed-feeding PR had greater inter-individual variation than the other rodent species. We also found significant inter-species differences that were much greater than intra-species differences (Fig. 3A, r=0.999, p=0.001), a result that was also confirmed in the NMDS analysis, where the different species showed strong clustering. This included a clear separation of the seed-feeding PR microbiome from the microbiomes of the other species. (Fig. 3B, stress=0.093, p=0.001). These differences were statistically supported by the significant effect of host species on microbiome structure (PERMANOVA: pseudo-F=9.28, p=0.001), indicating that these rodents possessed different microbial communities.



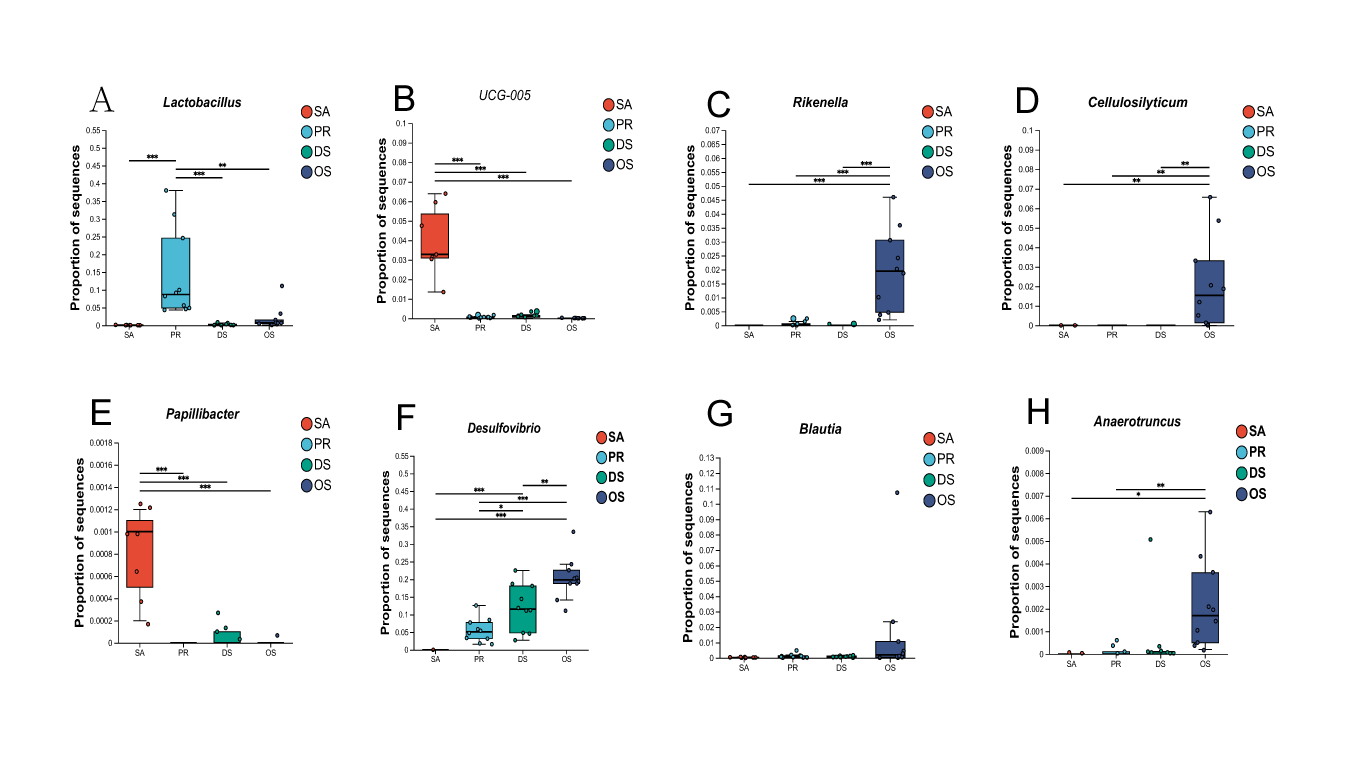
**Fig. 3**

We determined the distinctive patterns of bacterial abundance at the phylum level for the gut microbiomes of the different rodent species. Firmicutes and Bacteroidota were the main bacterial phyla in several rodents, and both of these phyla were less abundant in the OS gut compared to the other three rodent species. Desulfobacterota, Patescibacteria and Actinobacteriota were most abundant in the gut of OS, which has the highest proportion of insects in its diet. Verrucomicrobiota was most abundant in the SA gut, but least abundant in the gut of seed-eating PR (Fig. 4).



**Fig. 4**

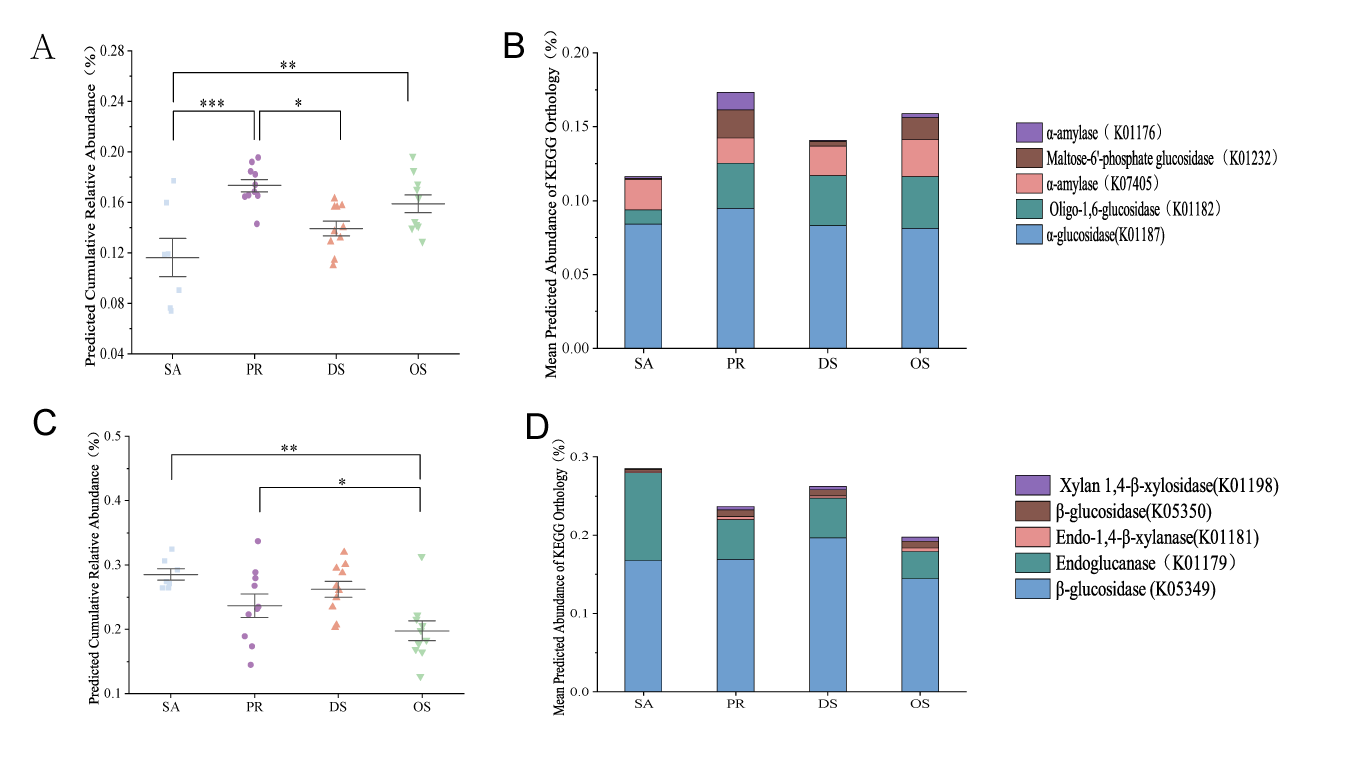
At the genus level, we were able to identify genera that were uniquely present or absent in each rodent species studied. Lactobacillus (a bacterium with lactic acid fermentation capacity that converts carbohydrates into lactic acid, Fig. 5A) [49] was only common in the intestine of PR. Papillibacter (a bacterium involved in the breakdown of dietary fiber in food, Fig. 5E) [50] was found only in the intestinal tracts of the herbivorous SA and the omnivorous DS. In addition, we were able to identify missing taxa in certain species. For instance, the microbial genus UCG-005 (Fig. 5B), a bacterium associated with the degradation of fibers in herbivorous hosts, was completely absent in seed-eating PR and omnivorous DS and OS. Desulfovibrio (Fig. 5F) showed a conspicuous absence in the SA gut. We also identified more bacterial genera that were significantly more abundant in the gut of the insect-eating OS, such as Rikenella (Fig. 5C), Cellulosilyticum (Fig. 5D), Blautia (Fig. 5G), and Anaerotruncus (Fig. 5H).



**Fig. 5**

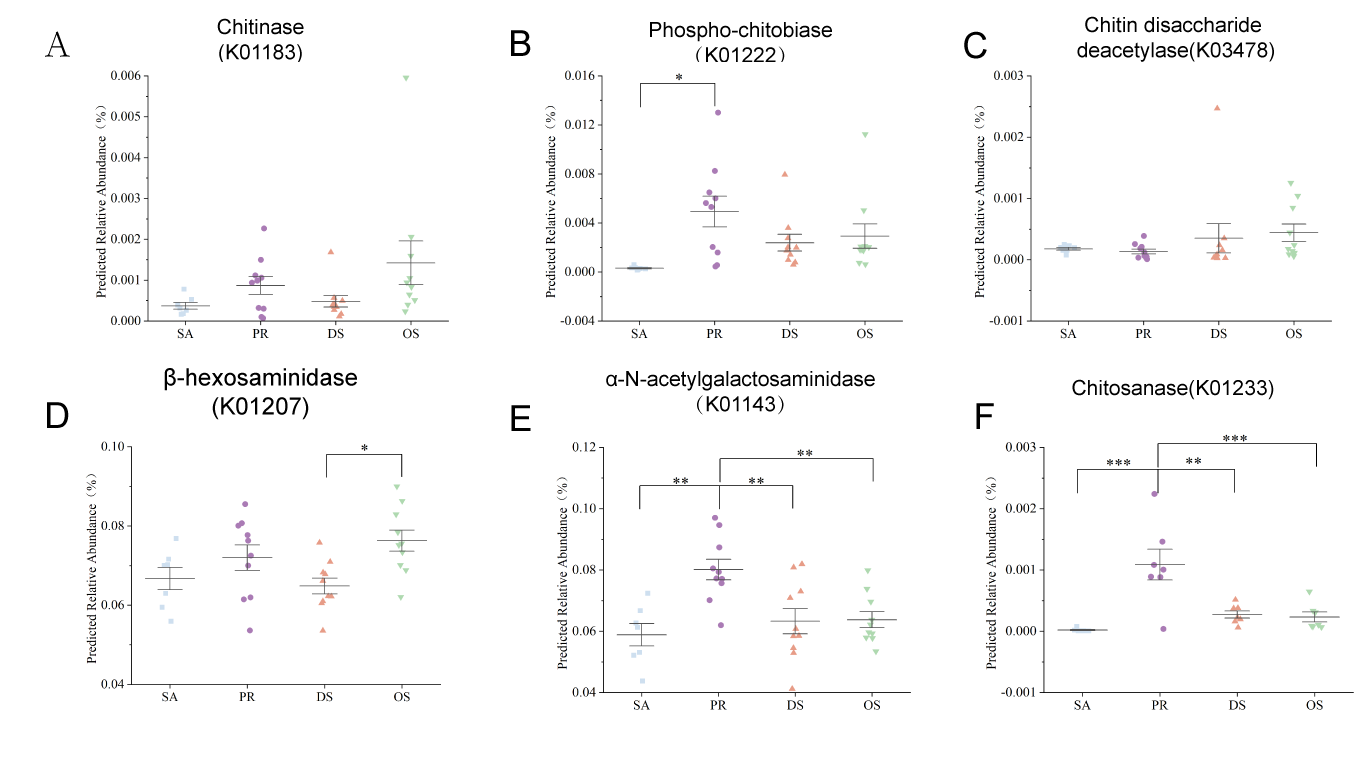
In comparing the microbial OTUs detected with in dataset to those present in the PICRUSt2 database, we found that all 2901 OTUs' NSTI values (the most recent sequencing classification index) were less than 2.0, thus ensuring that no OTU was excluded from the PICRUSt2 analysis. All OTUs' NSTI values are comparably modest (average ± standard deviation: 0.321 ± 0.194; median: 0.279). The average weighted NSTI values differed significantly by rodent species (Kruskal-Wallis: H = 22.182, p < 0.001), with OS boasting the highest weighted NSTI value (average: 0.261), and PR exhibiting the lowest average (average: 0.194). These values are comparable to those obtained in human research; hence one should be able to predict the content of the microbiome based on the 16S rRNA gene library.

The microbiome of the herbivorous SA boasts the highest predicted abundance of fiber-decomposing genes; conversely, the microbiome of the more insectivorous OS exhibits the lowest abundance of such genes. The seed-eating PR had the highest predicted abundance of microbial genes for metabolizing carbohydrates. When examining the genetic elements associated with the digestion of simple sugars (Fig. 6A and B; Kruskal-Wallis: H = 15.859; p < 0.001) or fibers (Fig.6C and D; Kruskal-Wallis: H = 12.317; p < 0.01), the outcomes remain in line with our predictions. The PR has the most genes associated with the digestion of simple sugars. The herbivorous SA has the most genes related to the degradation of complex fibers, whereas the more insectivorous OS has the fewest such genes. Across rodent species, the most plentiful gene related to the digestion of simple sugars is predicted to be alpha-glucosidase, KEGG Ontology (K01187), and the most abundant gene associated with the complex fiber metabolism is expected to be β-glucosidase, KEGG Ontology (K05349).



**Fig. 6**

Genes associated with chitin digestion are most abundant within the microbiome of the most insectivorous species, OS (Fig. 7). The predicted chitinase and chitinases-based disaccharide deacetylase abundance was higher for OS than for any of the other rodent species, at approximately two to four times higher abundance (Fig. 7A and C; Kruskal-Wallis: H = 17.115; all genes p < 0.001). It is worth noting that neither the phosphoryl chitinases nor the chitinase domain containing the acetyl group deacetylase genes are predicted to exist within the herbivorous SA , yet they are predicted to be present within the omnivorous DS (Fig. 7B and C). The genes associated with the metabolic pathways of chitinase and the carbon-nitrogen bonds within chitin varied significantly across rodent host species; however, the most insectivorous species, OS, exhibits lower predicted abundance for these categories (Fig. 7E and F; Chitinase K01233, α-N-acetyl neuraminidase K01143).



**Fig. 7**

**Discussion**

In this study, we employed DNA metabarcoding techniques to investigate intestinal microbial ecology of four sympatric desert rodent species. Our focus was on the structural and functional relationships between gut microbiome composition of these species and their dietary strategies. We have demonstrated that these four desert rodent species possess distinct gut microbiome communities. This outcome mirrors the findings of other studies on wild rodents [51-52], with the structure and composition of gut microbiome significantly influenced by the host's evolutionary history and diet [53-54]. In terms of their evolutionary history, the two most closely related rodent species in this study were the two jerboa species (DS and OS); these two hosts had the most similar gut microbiomes, but due to their distinct diets, their microbial communities were also markedly different. We also observed differences in the predicted abundance of genes with specific metabolic functions among these rodent species with different diets. We will now discuss two main findings regarding the gut microbiomes of these rodents: (i) the gut microbiota of herbivorous/seed-based rodents is correlated with complex carbohydrate digestion, and (ii) the gut microbiota of predominantly insectivorous rodents is associated with chitinase. Finally, we will delve into the merits and limitations of the DNA metabarcoding techniques in the study of microbiota and the pursuit of scientific advances.

**Feeding habits of herbivorous/seed-eating rodents and the degradation of carbohydrates**

We found that SA, the herbivore, has the most diverse gut microbiome out of the rodents studied. Prior research has found that, in mammals, the gut microbiomes of herbivores are far more diverse than those of mammals with other diets [55-56]. It is believed that this higher diversity is the result of the presence of fibers in plant tissue. These fibers are difficult for animals to directly digest and absorb [57-58], so the diversity of gut microbiota among grazing mammals like SA may aid in their food digestion. Gut microbial diversity is also correlated with the host’s body size, with larger animals typically containing greater gut microbial diversity [59-60]. Interestingly, SA is also the largest of our study species (Table 1), which could be an additional contributing factor for the higher microbial diversity in this species.

We also discovered that, in line with our predictions, the grazing herbivore SA has the highest abundance of cellulose-decomposing genera—UCG-005, and the highest predicted abundance of genes associated with complex fiber digestion. However, the predicted abundance of genes associated with simple sugar digestion is highest within the intestinal tract of PR. The predicted abundance of genes related to complex fiber digestion was elevated in PR, defying our anticipated pattern. We expect that genes associated with simple sugars are also most plentiful within the grazing SA , due to the need for animal bodies to break down cellulose into simpler sugars for their absorption and utilization [61-62]. This might be linked to the cellulose content within plant leaves and seed kernels, which also have indigestible fibers [63-64]. Therefore, the seed-eating PR may also rely on its microbiome in order to digest and absorb the fibers found in seeds. Research on mammal digestive processes has contrasted the strategies of maximum yield and maximum velocity. Animals that exhibit maximum yield generally show longer intestinal passage times, whereas those that maximize velocity are characterized by rapid digestive tract movement[65-66]. Maximum yield is typically associated with a larger body size, which increases the amount of time that food is located within the intestine [67]. The small body size of PR implies that it is expected to maximize velocity rather than yield, which would require its microbiome to be capable of utilizing simple sugars. Of course, this hypothesis requires additional investigation and validation.

**Feeding habits of insectivorous rodents and the decomposition of chitin**

OS, the most insectivorous species studied, possesses a unique gut microbiome distinct from that of the other rodents studied, with the lowest species diversity in this study. This is more common among mammals that consume higher nutrient levels, so perhaps this is due to its relatively narrow diet with a high proportion of insects [68-69]. The diets of insectivorous animals are generally high in proteins and chitin [70-71], and the relative scarcity of carbohydrates might restrict the survival and reproduction of certain microorganisms. The stomachs of insectivores are typically more acidic [72-73], which could also be detrimental to microbes, thus possibly contributing to why OS's intestinal microbiome has relatively low diversity.

Upon further examination of the microbial community of OS, several distinctive patterns emerge. For instance, the genus Anaerotruncus was detected exclusively within the guts of OS. Members of this genus may possess anti-inflammatory properties, which promotes the health of intestinal mucosa and mitigates chronic inflammation [74-75]. Some studies have indicated that insectivorous mammals are prone to intestinal inflammation [76]. Therefore, the presence of Anaerotruncus may help the host resist inflammation and thus maintain its physiological health. Furthermore, the genera Rikenella and Blautia are also abundant within the intestinal microbiota of insectivores. These microbial communities are ubiquitously present within the intestinal tracts of animals, yet their specific role within the host's intestinal ecosystem remains unclear. They have been found to exert a profound influence on the host's physiological processes, including energy metabolism, communication of signals, and immune responses [77-78].

Interestingly, we discovered that the genes associated with chitin digestion were predicted in the intestinal tracts of all four rodent species, including the grazing SA, which may be due to the propensity of rodents to opportunistically feed on insects[79]. However, the species that eats the most insects, OS, possesses three genes with higher predicted abundance related to chitin degradation. Chitinase (a hydrolyzing enzyme, utilized for the degradation of long-chain chitin), chitin disaccharide deacetylase (an enzyme catalyzing the removal of acetyl groups in chitin degradation), and β-hexosaminidase (a gene category related to the metabolism of carbon–nitrogen bonds in chitin) are intimately tied to the degradation of chitin [80]. Therefore, their increased abundance may help the host’s digestion. However, the abundance of microbial functions does not necessarily result in increased functional capacity. Microbes may be digesting chitin for their own benefit without providing any benefits for the host; this hypothesis requires further investigation and validation. In addition, we have observed the predicted abundances of chitosanases and another gene related to the metabolism of carbon-nitrogen bonds in chitin were not enriched in the gut of OS(Fig. 7). Therefore, chitin metabolism within animal hosts requires increased attention.

**Constraints, Limitations, and Scientific Validity**

Our study on the gut microbiomes of four rodent species is primarily descriptive due to the constraints of our sample size—the limitations of our samples are determined by species, population, and individual differences. Rodents’ diets may vary over time and space [81-82], yet these fluctuations are not measured in our sample. Moreover, as the dietary diversity within our sample varying by species only rather than within species, this limits our ability to discern the difference between the effects of the host’s diet from other effects that vary by species. However, our research was a naturalistic study and also employed advanced sequencing technologies, thus ensuring the natural validity of our measures.

DNA metabarcoding techniques is a commonly employed molecular biology method for investigating microbial community structure. Despite its significant advantages in characterizing microbial diversity, it does possess certain limitations. Firstly, the sequence of DNA metabarcoding provides only relatively low-resolution data, so in some cases the precise microbial strains cannot be distinguished. Secondly, due to the varying copy numbers of DNA metabarcoding genes, there exists a bias towards taxa with a higher copy number, which may lead to an underestimation or overestimation of microbial abundance [83-84]. We used the PICRUSt method to infer the functional characteristics of microbial communities through the anticipation of functional genomes. Although PICRUSt offers valuable insights into function prediction, its predictions are still subject to several possible biases. Firstly, this method relies on the accuracy of the DNA metabarcoding and the integrity of the database. Subsequently, because of any possible inaccuracies in microbial genomes, the predicted functions of PICRUSt may not thoroughly reflect the reality [85-86]. Despite their inherent constraints, these methods continue to be essential tools in the field of microbial community structure and function. These limitations must be kept in mind, but we believe that despite them, these methods allow for the discovery of reasonably accurate information. Advanced sequencing techniques and methodologies do indeed furnish a more plentiful and precise dataset, yet they can never completely escape such limitations.

The benefits of DNA metabarcoding techniques lie in its ability to resolve a multitude of issues by leveraging a single dataset. Despite the constraints imposed on the depth of our sequencing, this paper's data contributes to our understanding of how the gut microbiome and its potential functions vary across hosts. Therefore, the sequencing technology of DNA metabarcoding is a useful tool that can lead to new discoveries in various topics. For instance, we have discovered the potential adaptations of the intestinal microbiota of herbivorous rodents and rodents with higher proportions of insects in their diet. Furthermore, we have investigated individual variation, enhancing our understanding of the gut microecology of rodents with different dietary strategies, thereby opening up new avenues for research into the microbial ecology of these wild rodents.

**Conclusion**

We observed significant differences in the gut microbial composition of four species of rodents living in the same habitat but each consuming different diets. These differences in gut microbiota may arise as adaptations to their distinct diets and digestive needs, thereby enhancing their ability to live within the same ecosystem.

**Supplementary information**

Additional file :Sample amplification, library preparation, and sequencing. NSTI value of sample OTUs. Prediction\_KO.

**Authors’ contributions**

SY and HPF designed and coordinated the study. XDW acquired the funding. LLL, YLJ, HTZ and HWY examined the animals and collected samples. LN and ZHNS conducted flea identification and data summarization. DYCand SY analyzed the data and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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**Conflict of interest statament**

The authors declare that they have no competing interests.

**Date Availability statement**

All sequence reads from this study have been deposited in the NCBI database ([PRJNA1083208](https://www.ncbi.nlm.nih.gov/portal/utils/pageresolver.fcgi?recordid=667440ae30bd3e09ebd96089)),（https://www.ncbi.nlm.nih.gov/biosample?LinkName=bioproject\_biosample&from\_uid=1083208）.The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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**Table 1**: The following species abbreviations will be used throughout. SA: *Spermophilus alashanicus* ; PR: *Phodopus roborovskii* ; DS: *Dipus sagitta* ; OS: *Orientallactaga sibirica*.

**Figure. 1:** Sequencing quality and OTU analysis. A: Venn diagram comparing the number of OTUs shared among species. The four ellipses in different colors represent four species, with numbers indicating the number of unique or shared OTUs for each species. B: Dilution curve of gut microbiome sequences.

**Figure. 2:** Differences in lpha diversity of the gut microbiome among different host species. A: Sobs diversity index B: Shannon diversity index, with horizontal lines indicating the results of pairwise Kruskal-Wallis tests. Adjusted p-values are represented by the following symbols: (\*) for p < 0.05; (\*\*) for p < 0.01; (\*\*\*) for p < 0.001.

**Figure. 3:** Beta diversity of the gut microbiome in rodents. A: Boxplot of ANOSIM analysis. The x-axis represents the values between species as well as within each species. The y-axis represents the magnitude of the distance values. R values typically range from 0 to 1; higher R values indicate greater between-group differences compared to within-group differences. B: NMDS analysis of gut microbiota in four rodent species.

**Figure. 4:** Circos plot showing the distribution of microbial taxa at the phylum level in four rodent species. The left semicircle represents the composition of species in the samples, where the outer colored bands represent the rodent host species, the inner colored bands represent the microbial taxa, and the lengths represent the relative abundance of the species in the corresponding samples. The right semicircle illustrates the distribution of different microbes across hosts at the taxonomic level, where the outer bands represent microbial taxa, the inner colored bands represent rodent host species, and the lengths represent the proportion of hosts that the taxon is distributed among.

**Figure. 5:** Relative abundance of selected microbial genera with large differences in abundance among rodent species. Points represent data from individual rodent species, and lines represent the median. Lines with asterisks indicate the results of pairwise Kruskal-Wallis tests. Adjusted p-values are denoted by the following symbols: (\*) for p < 0.05; (\*\*) for p < 0.01; (\*\*\*) for p < 0.001.

**Figure. 6:** Predicted abundance of genes related to sugar metabolism. A: Predicted abundance of genes related to monosaccharide degradation. B: Stacked bar chart depicting the relative contributions of the top five most abundant KEGG categories related to simple sugar digestion. C: Predicted abundance of genes related to complex fiber degradation. D: Stacked bar chart depicting the relative contributions of the top five most abundant KEGG categories related to fiber digestion. For A and C, points represent data from individual rodent species, and lines with bars represent the mean ± standard error. Brackets with asterisks indicate the results of pairwise Kruskal-Wallis tests. Adjusted p-values are denoted by the following symbols: (\*) for p < 0.05; (\*\*) for p < 0.01; (\*\*\*) for p < 0.001.

**Figure. 7**: Predicted abundance of genes related to chitin digestion. Points represent data from individual rodent species, and lines with bars represent the mean ± standard error. Brackets with asterisks indicate the results of pairwise Kruskal-Wallis tests. Adjusted p-values are denoted by the following symbols: (\*) for p < 0.05; (\*\*) for p < 0.01; (\*\*\*) for p < 0.001.

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