Seasonal Dietary Shifts Alter the Gut Microbiota of a Frugivorous Lizard Teratoscincus roborowskii (Squamata, Geckonidae)

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Abstract

Seasonal dietary shifts of animals are important ecological adaptation strategies. An increasing number of studies have shown that seasonal dietary shifts can influence or even determine the composition of gut microbiota. The turpan wonder gecko Teratoscincus roborowskii lives in extreme desert environments, which have flexible dietary shift to fruit-eating in warm seasons. But the impact of such shifts on gut microbiota is poorly understood. Here, 16SrRNA sequencing and LC-MS metabolomics we used to examine the changes of gut microbiota composition and metabolic pattern of T. roborowskii. The results demonstrated that the gut microbes of T. roborowskii had significant seasonal changes, the diversity and abundance of gut microbiota in autumn were higher than those of in spring. Firmicutes, Bacteroidetes and Proteobacteria were the core gut microbes of T. roborowskii. Verrucomicrobia and Proteobacteria exhibit dynamic pattern of ebb and flow between spring and autumn.The composition and structure of gut microbes in different seasons perform specific metabolic functions, and this change may be an important adaptation for T. roborowskii to cope with dietary shifts and improve energy acquisition. Our study will provide a theoretical basis for exploring the adaptive evolution to special frugivorous behavior of the T. roborowskii, which is an important supplement to the study of the gut microbiology of desert lizards.

Seasonal Dietary Shifts Alter the Gut Microbiota of a Frugivorous Lizard *Teratoscincus roborowskii* (Squamata, Geckonidae)

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Abstract

Seasonal dietary shifts of animals are important ecological adaptation strategies. An increasing number of studies have shown that seasonal dietary shifts can influence or even determine the composition of gut microbiota. The turpan wonder gecko *Teratoscincus roborowskii* lives in extreme desert environments, which have flexible dietary shift to fruit-eating in warm seasons. But the impact of such shifts on gut microbiota is poorly understood. Here, 16SrRNA sequencing and LC-MS metabolomics we used to examine the changes of gut microbiota composition and metabolic pattern of *T. roborowskii* . The results demonstrated that the gut microbes of *T. roborowskii* had significant seasonal changes, the diversity and abundance of gut microbiota in autumn were higher than those of in spring. Firmicutes, Bacteroidetes and Proteobacteria were the core gut microbes of *T. roborowskii* . Verrucomicrobia and Proteobacteria exhibit dynamic pattern of ebb and flow between spring and autumn.The composition and structure of gut microbes in different seasons perform specific metabolic functions, and this change may be an important adaptation for *T. roborowskii* to cope with

dietary shifts and improve energy acquisition. Our study will provide a theoretical basis for exploring the adaptive evolution to special frugivorous behavior of the *T. roborowskii* , which is an important supplement to the study of the gut microbes of desert lizards.

Keywords: *Teratoscincus roborowskii*, *s*easonal dietary changes, gut microbiota, metabolomics, ecological adaptation

Introduction

The intestinal structure of animals satisfies the requirements of food digestion and nutrient absorption. It also maintains homeostasis of gut microbes (Sommer & Bäckhed, 2013). Complex interactions of the gut microbial ecosystem occur between the host and its gut microbes (McFall-Ngai et al., 2013). The gut microbiota plays an important role in the intestinal immune system, energy metabolism (Sommer & Bäckhed, 2013), reproductive activity (MacLeod et al., 2022), and longevity (Kim & Benayoun, 2020)of the host.

The composition, structure, and function of gut microbiota are influenced by multiple factors (Suzuki & Worobey, 2014). Seasonal environmental fluctuations can significantly alter the gut microbiota of animals. For example, the gut microbiota of *Pteromys volansorii*varies seasonally on an ecological scale (Liu et al., 2019). In addition, seasonal physiological changes associated with hibernation also alter gut microbiota in greater horseshoe bats (*Rhinolophus ferrumequinum*) and brown bears (*Ursus arctos*) (Sommer et al., 2016; Xiao et al., 2019). Trophic niche expansion is an active adjustment of wildlife to seasonal and environmental changes (Guisan et al., 2014), and dietary shifts can affect or even determine the composition of animal gut microbes that change dynamically with the season (Maurice et al., 2015). Studies on wood mice (*Apodemus sylvaticus*), plateau pikas (*Ochotona curzoniae*), brown frogs (*Fejervarya limnocharis*), black howler monkeys (*Alouatta pigra*), and giant pandas (*Ailuropoda melanoleuca*) have shown that gut microbes undergo dynamic seasonal changes (Maurice et al., 2015; Fan et al., 2022; Huang et al., 2021; Amato et al., 2015; Wu et al., 2017). These results indicate that seasonal dietary shifts can significantly alter animal gut microbiota.

At present, fecal metabolomics is widely considered a key tool for studying the relationship between hosts and their gut microbiota and has attracted extensive attention (Zierer et al., 2018). Metabolomics enables researchers to identify a large proportion of the metabolites present in a sample. By analyzing these products of cellular metabolism, metabolomics can reveal valuable information about an organism's metabolic or physiological state at the time of sampling (Tang et al., 2020). For example, in wildlife research, joint analysis of metabolomics and gut microbiota has revealed differences in the abundance and metabolic phenotypes of gut microbes of *Panthera pardus japonensis* in captive and wild environments (Hua et al., 2020). The co-metabolic patterns of the gut and microbes in mountain gorillas (*Gorilla beringei beringei*) and western lowland gorillas (*Gorilla gorilla*) indicate that dietary restriction is a potential factor driving specific changes in gut microbes (Gomez et al., 2016). Combined analysis of gut microbes and metabolomics has also become an effective approach for identifying relevant biomarkers to reveal complex changes in metabolic and biochemical pathways (Tran et al., 2020).

Significant differences in the composition, structure, and abundance of gut microbiota in different animal groups, such as mammals (Zhao et al., 2018), birds (Grond et al., 2018), and fishes (Egerton et al., 2018), have been extensively studied. Reptiles, especially lizards, have a special evolutionary history and environmental adaptation behaviors (Pianka & Vitt, 2003). however, relatively little research has been conducted on the gut microbes of lizards (Colston & Jackson, 2016). Relevant studies have focused on changes in the patterns of gut microbes of crocodile lizards (*Shinisaurus crocodilurus*), western fence lizards (*Sceloporus occidentalis*), northern grass lizards (*Takydromus septentrionalis*), and mesquite lizards (*Sceloporus grammicus*) under the influence of human activities, temperature, diet, altitude, and other factors (Tang et al., 2020; Moeller et al., 2020; Zhou et al., 2020; Montoya-Ciriaco et al., 2020).

The Turpan wonder gecko (*Teratoscincus roborowskii*) is a walking gecko species endemic to the Turpan Depression in the Xinjiang Uyghur Autonomous Region of China (Shi et al., 2007; Shi et al., 2002). They live in extreme desert environments (Song et al., 2009; Zhou et al., 2019). Research on Turpan wonder geckos

mainly focuses on foraging mode, activity rhythms, and seed dispersal (Song et al., 2017; Werner et al., 1997; Yang et al., 2021). Field observations and dietary analysis have shown that *T. roborowskii* feeds mainly on insects in spring, whereas the proportion of caper fruits in the food can reach 85% in summer and autumn, showing a significant seasonal shift in dietary habits (Liu et al., 2010; Yang et al., 2021). This seasonal dietary shift may be an important factor in *T. roborowskii* adaptation to harsh and arid desert habitats.

The Turpan wonder gecko goes through a long hibernation process every year (Li et al., 2010). Fruit-eating strategies are more likely to be deployed with seasonal variations in food availability to maximize pre-hibernation food intake and satisfy nutritional requirements (Naya et al., 2010; Robbins et al., 2012). Related studies have shown that switching from insects to plant fruits is a common mechanism through which migrating birds accumulate fat for energy (Marshall et al., 2016). Gerbils (*Psammomys obesus*), which also live in a desert environment, can convert a small amount of carbohydrates into endogenous fructose due to the stimulation of a high-salt diet, thereby aggravating the tendency to obesity and effectively adapting to the lack of food (Kaiser et al., 2012). Turpan wonder geckos face abundant caper fruit resources in summer and autumn. From this, we can speculate that fresh caper fruit is not only an excellent source of water and nutrients but also a special way for Turpan wonder geckos to acquire energy and store fat during the pre-hibernation period. This dramatic shift in seasonal dietary habits can lead to alterations in gut microbes to meet the functional needs of dietary transition. Therefore, 16S rRNA sequencing technology and LC-MS metabolomics were used in this study to explore changes in the gut microbiota and metabolic patterns of Turpan wonder geckos during spring and autumn.

Materials and methods

Animal and feces collection

T. roborowskii individuals were captured from the desert in the suburb of Turpan in May 2021 and September 2021. The capture site is located near the Turpan Eremophytes Botanic Garden. In consideration of the search ability of the site, we use the coordinates of the Turpan Eremophytes Botanic Garden to locate it. Which is located in the Turpan Basin in the Xinjiang Uyghur Autonomous Region of China (40°51'N, 89deg11'E). The experiment was divided into two groups: (1) 11 lizards were captured in spring (spring group; SG), and 23 complete fresh fecal pellets were collected during the fasting period; (2) 15 lizards were captured in autumn (autumn group; AG), and 21 complete fresh fecal pellets were collected during the fasting period.

After capture, Turpan wonder geckoes were numbered, and their sex and other basic information were recorded. Subsequently, each *T. roborowskii* was placed in a clean feeding box and fasted during this period. A noninvasive fecal sampling method was used to check for excretion every 2 h to ensure the timely collection of fresh fecal samples. Complete fecal samples were collected into sterile cryovials using fresh, sterilized tweezers. Samples were immediately marked and snap-frozen in liquid nitrogen, and then transferred to a -80 freezer for subsequent experimental operations. Our previous study showed that the transit time of food through the digestive tract of *T. roborowskii* is up to 15 days (Yang et al., 2021), therefore, fecal collection was performed while no food was provided for 15 days. After the experiment, all the animals were in good physiological condition and were treated in strict accordance with the relevant regulations on animal welfare. All of the experiments and handling of the animals were conducted according to the research protocols approved by the Animal Welfare and Ethics Committee of Xinjiang Agricultural University.

DNA extraction and PCR amplification

Fecal samples from different seasons were homogenized to obtain four groups of mixed samples for spring and autumn (Zhou et al., 2020; Xiao et al., 2019; Gong et al., 2021). Extraction of total microbial DNA from fecal samples was performed. The V3-V4 hypervariable regions of the microbial 16S rRNA gene were amplified by PCR using primers 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The PCR conditions were as follows: 98degC for2 min; 30 cycles at 98degC for 15s, 55degC for 30s, and 72degC for 30s; and extension at 72degC for 5 min (Zhou et al.,

2020). The products were purified, quantified, and normalized to generate a sequencing library (SMRT Bell). The constructed library was first subjected to library quality inspection, and the qualified products were sequenced using PacBio Sequel at Biomarker Technologies Co., Ltd. (Beijing, China).

Raw subreads were calibrated to get circular consensus sequencing (CCS) (SMRT Link, version 8.0), then Lima v1.7.0 software was used to identify CCS sequences of different samples through barcode sequences and remove chimeras to obtain Effective-CCS sequences. Operational taxonomic units (OTUs) were clustered with a 97% similarity cutoff using UPARSE (version 10.0) (Edgar, 2013).

Alpha diversity index analysis was performed using QIIME2 software, via the Wilcoxon rank-sum test to compare the community diversity indices (Ace richness estimator and Shannon-Wiener index) (Prehn-Kristensen et al., 2018; Schloss et al., 2011).

Dimensionality reduction of data was based on the Bray-Curtis distance matrix by principal coordinate analysis (PCoA), and nonmetric multidimensional scaling (NMDS) was used to observe the differences in gut microbial community structure (Oksanen et al., 2013). We modeled the binary Jaccard distance dissimilarities based on an operational taxonomic unit (OTU) level table via analysis of similarities (ANOSIM) (Anderson, 2001).

Using SILVA as the reference database, the community species composition analysis of the two groups of experiments was performed at various levels: phylum, order, family, genus, and species. Linear discriminant effect size (LEfSe) analysis was used to screen microorganisms with large differences as potential markers (Segata et al., 2011), the significance of different species was determined using Matestats software, and differential strains were screened according to the criteria of LDA>4, q<0.05. Picrust2 was used to predict changes in KEGG-enriched functional pathways across the groups (Kanehisa, 2019).

LC-MS metabolomics detection

Metabolites of fecal samples were detected in positive and negative ion mode, and metabolites were separated using a Waters Acquity 1-Class PLUS ultraperformance liquid chromatography system (Waters XevoG2-XS QTOF high-resolution mass spectrometer). Primary and secondary mass spectrometry data were collected in MSe mode under the control of acquisition software (MassLynx V4.2, Waters). In each data acquisition cycle, dual-channel data acquisition can be performed at both low and high collision energies simultaneously. The low collision energy is 2 V, the high collision energy range is 10˜40 V, and the scI ion source is as follows: capillary voltage, 2000 V (positive ion mode) or -1500 V (negative ion mode); cone voltage, 30 V; ion source temperature, 150 degC; desolvent gas temperature, 50 degC; backflush gas flow rate, 50 L/h; desolventizing gas flow rate, 800 L/h (Wang et al., 2020).

The raw data collected using MassLynx V4.2 was processed by Progenesis QI software for peak extraction, peak alignment, and other data processing operations, based on the Progenesis QI software online METLIN database and Biomark's self-built library for identification; at the same time, theoretical fragment identification and mass deviation were within 100 ppm.

Quality control and data analysis of fecal metabolite detection

Principal component analysis (PCA) was performed on metabolite data, and the OPLS-DA model was calculated using the R (3.3.2) package "ropls" to visualize the clustering of samples (Thevenot et al., 2015). The differential metabolites were screened by combining the fold difference, P-value of the t -test, and VIP value of the OPLS-DA model. The screening criteria were FC>1, P-value <0.05, and VIP>1. The cluster profiler used the hypergeometric test method to perform enrichment analysis on the annotation results of the differential KEGG metabolites and screened some representative metabolic pathways for impact value and significance as the enrichment criteria.

Results

Composition of gut microbiota

A total of 468 different OTUs were divided into the spring (SG) and autumn (AG) groups (Figure 1A). Among these, there were 373 OTUs in the spring group, 434 OTUs in the autumn group, and 339 OTUs in both groups (Figure 1B).

Analysis of alpha diversity of gut microbiota

The results of alpha diversity analysis based on the phylum level using the Wilcoxon rank sum test showed that the Ace richness estimator in the autumn group (P =0.023<0.05) was significantly higher than that in the spring group (Figure 2A). However, the Shannon–Wiener diversity index in the spring group (P =0.029<0.05) was significantly higher than in the autumn group (Figure 2B). At the family level, the Ace (P =0.029<0.05) and Shannon (P =0.029<0.05) indices in the autumn group were significantly higher than those in the spring group (Figure 2C, D).

Analysis of beta diversity of gut microbiota

PCoA was conducted to observe the differences in the gut microbial community structure. The results show that the contribution rates of the first and second principal components are 50.28% and 20.22%, respectively. The distance between the groups was relatively close, and the distance between the groups was further; that is, the samples of the two treatment groups were well-clustered, and the separation between the different groups was clear. Compared with the spring group, the gut microbial community structure among *T. roborowskii* individuals in the autumn group had higher similarity (Figure 3A).

The results of NMDS and similarity analysis indicated a significant difference in the gut microbiota between the spring and autumn groups (Stress=0.0008; Anosim: R=0.865, P =0.026). The distribution within the group was more discrete in the spring group and more uniform in the autumn group, suggesting that food and environmental influences after hibernation promoted the clustering and stability of the gut microbes of the Turpan wonder gecko (Figure 3B). In conclusion, the gut microbes of Turpan wonder geckos changed significantly between spring and autumn, manifesting as increased microbial abundance and diversity.

Analysis of gut microbiota species composition and differences

The composition of the gut microbiota in the spring and autumn groups of *T. roborowskii* is shown in Figure 4. At the phylum level, the main annotated groups were Firmicutes (32.79%; 36.92%), Bacteroidetes (31.53%; 34.45%), Proteobacteria (7.28%; 18.99%), Verrucomicrobia (19.58% and 4.30%), and Tenericutes (4.62% and 1.93%, respectively) (Figure 4A).

At the family level, the main annotated groups were Bacteroidaceae (15.15%; 16.54%), Lachnospiraceae (10.12%; 20.54%), Akkermansiaceae (19.58%; 4.30%), Erysipelotrichaceae (11.90%; 3.67%), Tannerellaceae (9.06%; 4.99%), Lactococcaceae (6.38%; 7.31%), Pseudomonas (0.00%; 11.15%), and others were highly abundant in fecal samples (Figure 4B).

Bacteroides (15.15%; 16.54%), *Akkermansia* (19.58%; 4.30%), *Parabacteroides* (9.06%; 4.99%), *Roseburia*(0.06%; 11.72%), *Pseudomonas* (0.00%; 11.15%),*Odoribacter* (1.89%; 6.83%), and *Alistipes* (4.52%; 2.68%), among others, were the core bacterial genera of *T. roborowskii* (Figure 4C).

Based on the above analysis of community structure and species composition, the gut microbes of the Turpan wonder geckos changed to a certain extent in different seasons. We further used LEfSe analysis based on the effect pattern of LDA (LDA>4,P <0.05) to screen the microorganisms with significant differences in relative abundance between the two groups. At the phylum level, the relative abundance of Tenericutes (LDA=4.08, P =0.02) in the spring group was significantly higher than in the autumn group; however, the abundance of Proteobacteria was higher in the autumn group (LDA=4.74, P =0.02).

At the family level, Erysipelotrichacea (LDA=4.64, P =0.021), Tannerellaceae (LDA=4.31, P =0.02), and uncultured bacterium Mollicutes RF39 (LDA=4.06, P =0.02) were present at significantly higher levels in the spring group than in the autumn group. In the autumn group, Enterobacteriaceae (LDA=4.22, P =0.018), Marinifilaceae (LDA=4.41, P =0.02), Lachnospira (LDA=4.67,P =0.021), and Pseudomonas (LDA=4.68, P =0.014) were significantly more prevalent than in the spring group.

At the genus level, *Erysipelatoclostridium* (LDA=4.64,P =0.02), *Parabacteroides* (LDA=4.28, P =0.021),*Breznakia* (LDA=4.10, P =0.021), and*uncultured_ bacterium_ o_ Mollicutes_ RF39* (LDA=4.10,P =0.021) were significantly higher in the spring group, while*Odoribacter* (LDA=4.38, P =0.021), *Rothella*(LDA=4.73, P =0.014) and *Pseudomonas* (LDA=4.18,P =0.014) were significantly higher in the autumn group (LDA>4, P <0.05; Figure 5).

Predictive analysis of gut microbial function

The functional pathway abundance of KEGG corresponding to the 16S rRNA sequencing data was predicted using picrust2. The results show that the KEGG metabolic pathways at the first level mainly consisted of the following functional categories: metabolism (78.6%), genetic information processing (8.0%), environmental information processing (6.5%), cellular processes (3.0%), human diseases (2.5%), and organic systems (1.4%).

In the differential analysis of KEGG metabolic pathways at the second level, the carbohydrate metabolism, replication and repair, and drug resistance pathways were significantly enhanced in the spring group (P <0.05). Amino acid metabolism, metabolism of terpenoids and polyketides, xenobiotic biodegradation and metabolism, cell motility, signal transduction, and lipid metabolism were significantly enhanced in the autumn group (P <0.05; Figure 6A).

In the enrichment process of the third-level metabolic pathways, glycolysis/gluconeogenesis, amino sugar and nucleotide sugar metabolism, and metabolic pathways such as fructose and mannose metabolism, galactose metabolism, and purine metabolism were significantly higher in the spring group (P <0.05). The two-component system and glycine, serine, and threonine metabolism were significantly enhanced in the autumn group (P <0.05; Figure 6B).

Fecal metabolic profiling ofT. roborowskii

The fecal metabolic profiles of *T. roborowskii* were acquired by LC-MS, and metabolism was detected with good intra-group reproducibility. Fecal metabolites were significantly separated in the first principal component (PC1: 82.9%) (Figure 7A). In the OPLS-DA model, the metabolic curves were clearly different between the two sample groups (Q2=0.99>0.9 indicates an excellent model), and the results show that there are obvious differences in fecal metabolites between the spring and autumn groups (Figure 7B).

The absolute values of log2FC (Fold Change, FC) were sorted to get the top 10 metabolites in each group. In the spring group, the levels of hexaethylene glycol, leucyl-tyrosine, scillipheosidin 3-[glucosy-(1->2)-rhamnoside], 1-O-caffeoyl-(b-D-glucose 6-O-sulfate), serinyl-gamma-glutamate, bradykinin hydroxyproline, phaseolic acid, TR-saponin B, corepoxylone, and ethylparaben were high. Methyl dihydrojasmonate, dodecanoic acid, trans-2-hexyl-1-cyclopropaneacetic acid, cochliophilin A, "PC(20:5(5Z,8Z,11Z,14Z,17Z)/24:0)," uridine diphosphate glucose, grevillol, benzyl hexanoate, trans-cinnamic acid, etc. were higher in the autumn group (Figure 8).

The differential metabolites were classified as carboxylic acids and derivatives, fatty acids, organooxygen compounds, steroids and steroid derivatives, glycerol glycerophospholipids, prenol lipids, and glycerolipids.

Differential metabolites were screened based on the criteria of$P$ =0.05, VIP=1, and FC=1. Finally, 1264 differential metabolites were detected in the two groups of samples, of which 668 were upregulated (higher in the autumn group), and 596 were downregulated (higher in the spring group). The relative contents of deoxycholic acid glycine conjugate and 26-methyl nigranoate were relatively higher in the spring group. Simulansine, 3-oxocholic acid, and asparaginyl-proline were the three metabolites with the highest relative contents in the autumn group (Figure 9).

KEGG functional classification of metabolites showed that metabolites participate mainly in metabolism, organismal systems, human diseases, environmental information processing, and other functional pathways. Among these, metabolic pathways, biosynthesis of secondary metabolites, and microbial metabolism in different environments are related to a variety of metabolites.

The KEGG enrichment network map of differential metabolites showed that lysine degradation, pantothenate and CoA biosynthesis, steroid hormone biosynthesis, carbon fixation pathways in prokaryotes, and other differential metabolites between different seasons had a close positive or negative relationship (Figure 10).

Fecal metabolic profiling and its correlation with the gut microbiota

Metabolite clusters were obtained by hierarchical cluster analysis (HCA). Pearson correlation analysis of significantly different metabolites and significantly different OTUs (microbes) was performed to determine the relationships between metabolites and microbes (Figure 11). The results showed that Proteobacteria, Verrucomicrobia, Lentisphaerae, Elusimicrobia, Tenericutes, and other phyla had the closest relationship with the metabolites, and the dominant microorganisms showed different positive or negative correlations with different metabolite clusters. This confirms the results of the LEfSe analysis; that is, bacterial phyla with significant seasonal differences correspond to some differential metabolites, suggesting that they are closely related and may be the key factors affecting gut microbes.

Discussion

The strict nocturnal activity rhythm of *T. roborowskii* (Song et al., 2009) and their special burrow construction not only avoid high temperatures in the daytime but also create a relatively constant living temperature. These behaviors are effective mechanisms for adapting to harsh and hot desert environments (Song et al., 2017). It is clear that seasonal dietary shifts are also a way to cope with the drought environment and enrich the food composition of *T. roborowskii* , which may be the main reason for the significantly higher gut microbial abundance and diversity of *T. roborowskii* in autumn relative to the spring. Studies on insectivorous and carnivorous bats have shown that diet variation significantly affects the gut microbial community structure (Gong et al., 2021). Therefore, improvement of gut microbial diversity and changes in community structure in *T. roborowskii* may be the main factors in coping with seasonal dietary shifts. Kohl et al. (2016) fed *Liolaemus ruibali* a diet ratio of 50% insects + 50% plants or 10% insects + 90% plants and found that lizards fed a rich, plant-based diet showed a higher level of gut microbial diversity. This is consistent with our results; that is, the intake of caper fruit significantly increased the diversity of gut microbes, and this change may be beneficial to the achievement of gut function and the stabilization of microbiota in *T. roborowskii* (Soriano et al., 2018).

At the phylum level, the contents of Firmicutes, Bacteroidetes, Proteobacteria, and Verrucobacterium in the gut microbes of *T. roborowskii* in spring and autumn accounted for more than 91% of the core flora. Studies have shown that Firmicutes, Bacteroidetes, and Proteobacteria are important members of the gut microbiota in most vertebrates (Zhao et al., 2018; Hale et al., 2019; Wang et al., 2018; Kohl et al., 2013). As a nocturnal desert gecko, the leopard gecko (*Eublepharis macularus*) possesses a gut microbial composition that is highly similar to that of *T. roborowskii* and remains stable after 28 d of fasting, suggesting that animals living in extreme desert environments have a high degree of control over gut microbes (Kohl et al., 2014). Other lizard species, such as a toad-headed lizard (*Phrynocephalus vlangalii*) (Zhang et al., 2018), *S. crocodilurus* (Jiang et al., 2017), *T. septentrinalis* (Zhou et al., 2020), and *S. occidentalis* (Moeller et al., 2020), are also dominated by Firmicutes and Bacteroidetes, which indicates that different lizard taxa have similar gut microbial composition at higher taxonomic levels.

However, the gut microbial composition of *T. roborowskii* is unique at both the family and genus levels. *S. crocodilurus* lives in warm, moist habitats and feeds mainly on earthworms and loaches. The main bacterial families are Pasteurellaceae, Deinococcaceae, and Comamonadaceae (Jiang et al., 2017). There were significant differences compared with *T. roborowskii* . A study of *T. septentrionali* s from eastern China showed that this insect-eating lizard has a composition similar to that of gut microbes of *T. roborowskii* at the family level, but the abundance differs greatly among families (Zhou et al., 2020). *P. vlangalii* living at high altitudes appears to have a more similar gut microbial composition to that of *T. roborowskii* (Zhang et al., 2018), which may be caused by similar food sources and arid environments. It can be seen that some factors such as diet (Campos et al., 2018) and environmental factors (Kartzinel et al., 2019) can significantly affect the composition of lizards' gut microbes.

Individuals with a high ratio of Firmicutes to Bacteroidetes have a higher ability to obtain energy from food (Clarke et al., 2012). Our results showed that *T. roborowskii* has more than 65% abundance of Firmicutes and Bacteroidetes in different seasons. Firmicutes are responsible for the metabolic transformation of carbohydrates and proteins (Flint et al., 2015), and can produce large amounts of energy-rich short-chain fatty acids that are associated with digestive efficiency (Turnbaugh et al., 2009). Bacteroidetes have the capacity to ferment amino acids and carbohydrates and are involved in bile acid, polysaccharide, and steroid metabolism (Rios-Covian et al., 2017). They work together to promote fat accumulation (Turnbaugh et al., 2006). The relative stability of the core flora may be favorable for maintaining a high level of nutrient absorption capacity during the active season. On one hand, this meets the direct cost of summer breeding input (Speakman, 2008), and on the other hand, it ensures energy accumulation before hibernation.

The special frugivory strategy chosen by *T. roborowskii* in the long-term evolutionary process promotes seasonal changes in its gut microbes. For example, the abundance of Proteobacteria in the autumn group was significantly higher than in the spring group ($P < 0.05$), which seems to be an effective way to cope with this shift. Relevant studies have shown that Proteobacteria are related to a variety of metabolic pathways and can secrete a large number of enzymatic substances related to polysaccharide and protein metabolism, which can effectively decompose polysaccharide and vitamin production (Abdul Rahman et al., 2016; Colston & Jackson, 2016), thus satisfying the combination of *Capparis spinosa* fruit and insect food and providing the possibility for the accumulation of energy materials before hibernation. The relative abundances of Enterobacteriaceae and Pseudomonas in the autumn group were significantly higher than those in the spring group, which may also be the result of adaptation to fruit food with higher sugar content. Related studies have shown that some Enterobacteriaceae species possess sucrose-specific phosphotransferase systems and sugar transporters with different functions and structures (Le Bouguenec & Schouler, 2011). Carbohydrate metabolism is also widely recognized as a nutritional basis for γ-proteobacteria to colonize the gut and maintain strains (Chang et al., 2004).

Lachnospira and Ruminococcaceae were the most abundant Firmicutes species in the *T. roborowskii* gut. Most studies have confirmed that both have abundant genes related to polysaccharide degradation and that their ability to utilize dietary polysaccharides is effective (Vacca et al., 2020). The high abundance of ABC transporters is the basis for the utilization of complex plant materials and the transport of various degradation products (Biddle et al., 2013), which can use lactic acid and acetate to produce short-chain fatty acids such as butyrate through the butyryl-CoA: acetate CoA-transferase pathway (Flint et al., 2015). The significant increase in Lachnospiriaceae (Roseburia) in autumn may be an adaptation to the abundant heteropolysaccharides in caper fruits (Bai et al., 2007), and may improve the efficiency of nutrient metabolism.

Akkermansiaceae are widely present in the gut of hibernating animals (Tang et al., 2019), and are considered probiotics with functions such as promoting intestinal mucosal barrier repair and regulating intestinal flora metabolism (Belzer & De Vos, 2012). The relative abundance of*Akkermansia muciniphila* was significantly higher in the spring group than in the autumn group. We speculate that the period when*T. roborowskii* was collected was still in the transitional period of enterotype transition; therefore, these individuals still had a higher abundance of Verrucobacterium. Akkermansiaceae can produce short-chain fatty acids such as acetate and propionate by degrading intestinal mucin (Feng et al., 2018). The production of fatty acids can maintain the immune state of the intestine and create an anaerobic environment required for the growth of strictly anaerobic symbiotic microorganisms, thus establishing a mutually beneficial relationship with the host (Shealy et al., 2021). Most Enterobacteriaceae and Pseudomonas are facultative anaerobes, and the destruction of the anaerobic environment leads to their rapid proliferation (Rivera-Chávez et al., 2016). Therefore, the high abundance of Verrucomicrobia in spring limited Proteobacteria; however, the abundance of Verrucomicrobia decreased in autumn, and some aerobic Proteobacteria dominated.

Analysis based on PCA and PLS-DA showed significant differences in fecal metabolites among different seasons, which may be caused by differences in seasonal diets (Li et al., 2020). Plant-derived chemicals such as lauric acid and cinnamic acid were significantly enriched in the autumn group because *T. roborowskii* had limited ability to digest caper seeds (Yang et al., 2021), and some plant-derived components were retained.

Deoxycholic acid glycine conjugate and 3-oxocholic acid are secondary bile acids. Bile acids participate in cholesterol metabolism through the enterohepatic circulation. This plays a crucial role in the digestion and absorption of components, regulates carbohydrate metabolism, and effectively regulates metabolic homeostasis (Bao et al., 2016).

From the differential metabolite KEGG enrichment network map, it can be seen that the biosynthetic pathways of pantothenate and CoA are significantly associated with a variety of metabolites. Pantothenate and D-4'-phosphopantothenate are precursors of pantothenate and CoA. CoA is involved in various biochemical reactions, including the tricarboxylic acid cycle, fatty acid synthesis and oxidation, and amino acid metabolism (Choudhary et al., 2014; Ma et al., 2020). The high CoA biosynthesis in autumn suggests that *T. roborowskii* has a vigorous metabolic level, which is consistent with our speculation. L-piperic acid, 5-aminopentanoicacid, and D-1-piperideine-2-carboxylic acid were higher in the spring group, demonstrating that lysine was degraded through the pipecolic acid pathway. Related studies have shown that L-lysine can improve the absorption and utilization of food proteins (Hallen et al., 2013). This appears to explain the adjustment of the Turpan wonder geckos in spring to cope with a protein-rich, insect-based diet.

Correlation analysis between metabolite clusters and gut microbes revealed an interesting phenomenon: Verrucomicrobia and Tenericutes were more abundant in spring, whereas Proteobacteria and Elusimicrobia were more abundant in autumn. These have the same correlation with certain metabolite clusters, which means that the gut microbiota in different seasons performs specific metabolic functions, so the gut microbiota of*T. roborowskii* shows obvious seasonal patterns with changes in the seasonal diet.

Conclusion

In summary, there were seasonal changes in the gut microbes of *T. roborowskii* , with higher diversity and relative abundance in autumn than in spring. Firmicutes and Bacteroidetes had a stable proportion, Proteobacteria and the significant growth of Lachnospiriaceae in autumn may be an adaptive factor to cope with seasonal dietary shifts. Secondary bile acids, such as deoxycholate-glycine conjugates, showed higher levels in different seasons, suggesting their important role in metabolism. Picrust functional predictions showed that metabolism-related pathways varied in different seasons, and the core microbiota in different seasons appeared to perform functions associated with specific metabolic pathways. In the future, captive breeding and artificial manipulation of the food ratio will be an effective way to further understand seasonal dietary shifts and the effects of frugivory strategies on the gut microbiota and metabolic functions of lizards such as *T. roborowskii* .

Authors' contributions

ZG, YY, and LS conceived the ideas and designed methodology, W-ZG and LS do field work. W-ZG and YY collected the data. W-ZG analyzed the data. All authors contributed to the article and approved the submitted version.**Acknowledgments**

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Ethics approval and consent to participate

The authors declare that this article is reported in accordance with the ARRIVE guidelines 2.0. All experimental procedures involving animals were approved (animal protocol number: 2021079) by the Animal Welfare and Ethics Committee of Xinjiang Agricultural University, Urumqi, Xinjiang, China.

Conflict of interest

The authors declare that they have no competing interests.

Availability of data and materials

The data that support the findings of this study are openly available in National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) at

https://www.ncbi.nlm.nih.gov/bioproject/PRJNA887219, reference number [PRJNA887219].

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Figures and legends

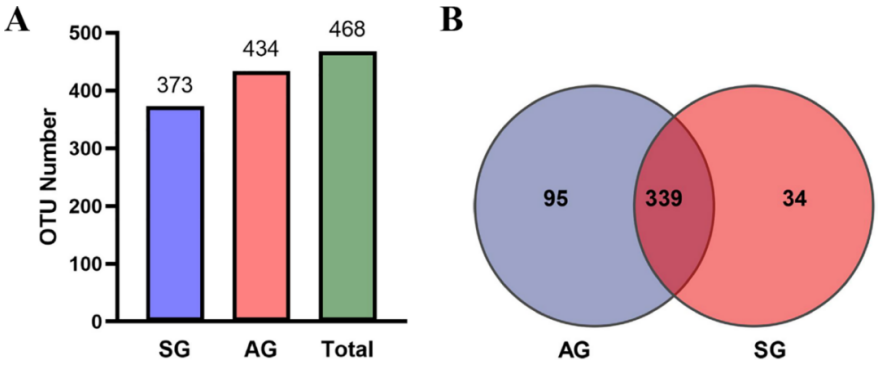


Figure 1 Bar diagram (A) and Venn plot (B) of gut microbiota OTU from *Teratoscincus roborowskii.*

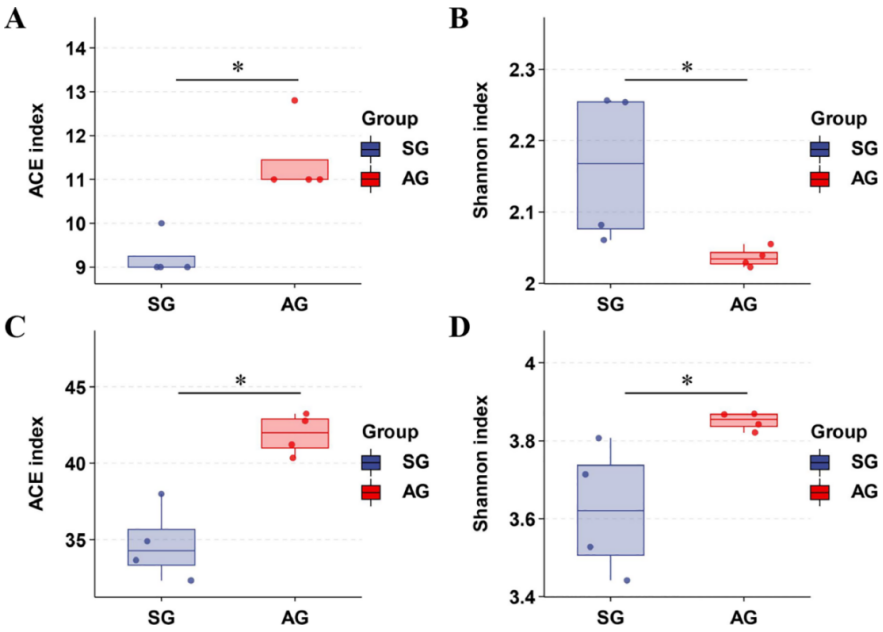


Figure 2 Alpha diversity of gut microbiota from *Teratoscincus roborowskii* . (A) ACE index at Phylum level; (B) Shannon index at Phylum level (C) ACE index at Family level; (D) Shannon index at Family level. Significant differences between AG and SG groups were tested by Wilcoxon rank-sum test (*, P <0.05).

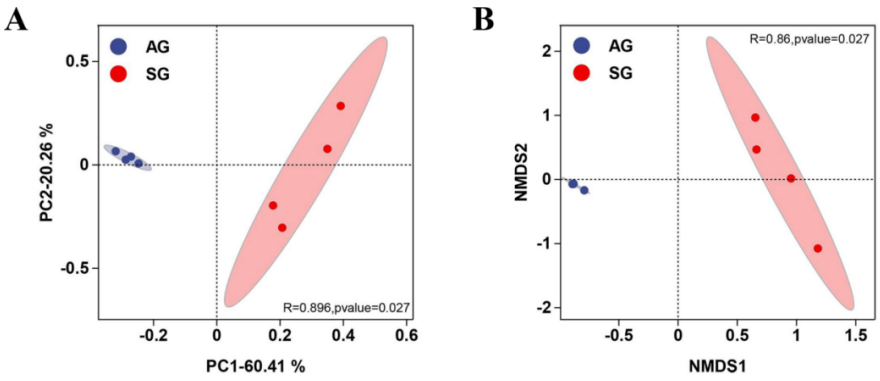


Figure 3 Beta diversity analysis of gut microbiota from *Teratoscincus roborowskii* according to principal coordinates analysis (PCoA) (A) and nonmetric multidimensional scaling (NMDS) (B). (A) Bray-Curtis distance matrix PCoA of the gut microbiota in the SG and AG. The boxplot shows the discrete distribution of samples along the PC1 and PC2 axes. (B) nonmetric multidimensional scaling analysis (NMDS) of the binary Jaccard distance of the two groups. The P -value was based on ANOSIM.

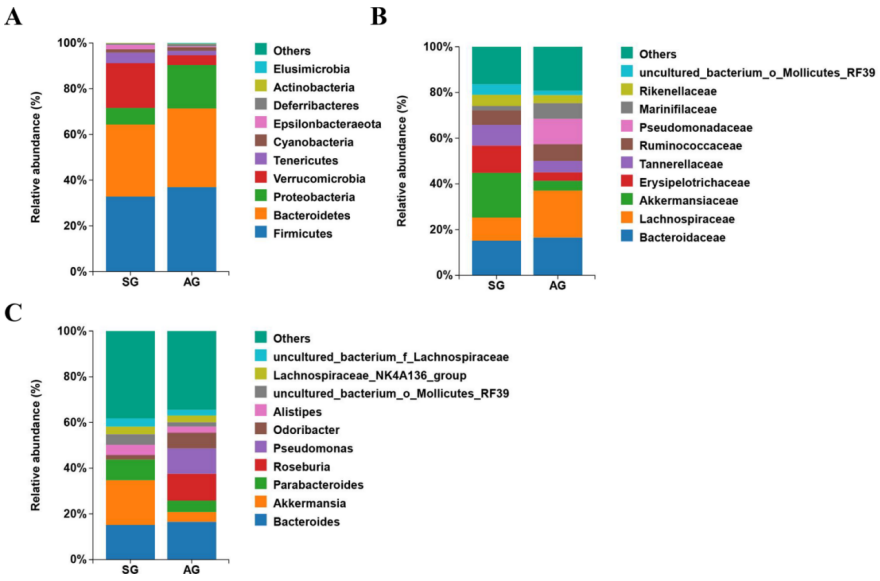


Figure 4 The relative abundance of gut microbiota at the phylum (A), family (B), and genus (C) levels in *Teratoscincus roborowskii*. The histogram shows the top 10 bacterial phyla, families and genera with relative abundance. The vertical coordinates of the figure indicate the relative abundance of bacterial species and the species names indicated by letters in the figure are shown in the legend on the right.

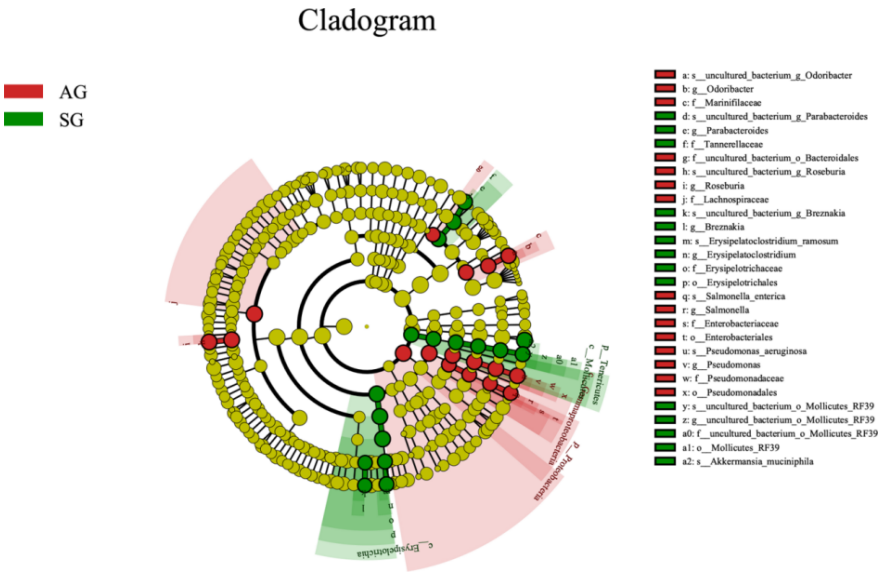


Figure 5 Linear discriminant analysis effect size (LEfSe) analysis of gut microbiota composition between seasons for *Teratoscincus roborowskii* (LDA>4,P <0.05). Red boxes and green boxes represent enrichment in the AG (autumn) and SG (spring) groups. Circles radiating from the inside to the outside represent taxonomic levels from phylum to species. Each circle's diameter is proportional to the taxon's abundance. The letters p, o, f, g, and s represent phylum, order, family, genus, and species, respectively.

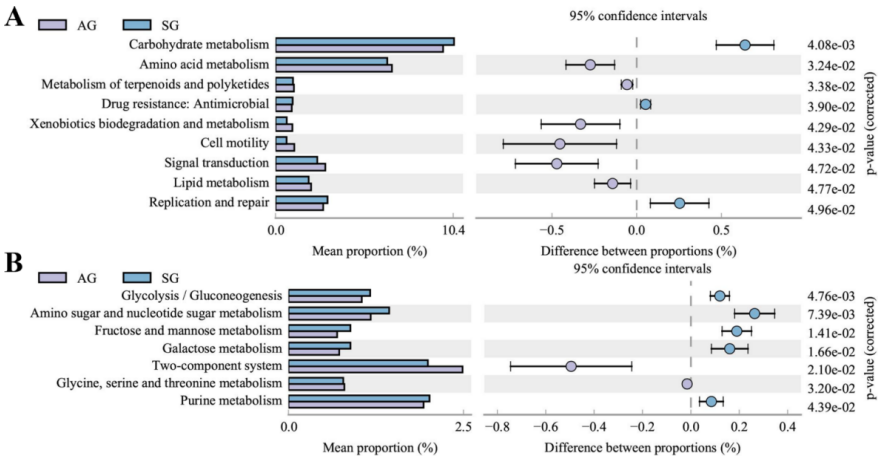


Figure 6 Differential analysis of metabolic functional pathways predicted by KEGG in the AG and SG groups. Significantly changed pathways of the AG and SG at the secondary(A) and tertiary (B) levels (metabolism pathways). The vertical axis represents different pathways, and the horizontal axis represents the proportions of corresponding pathways (P <0.05).

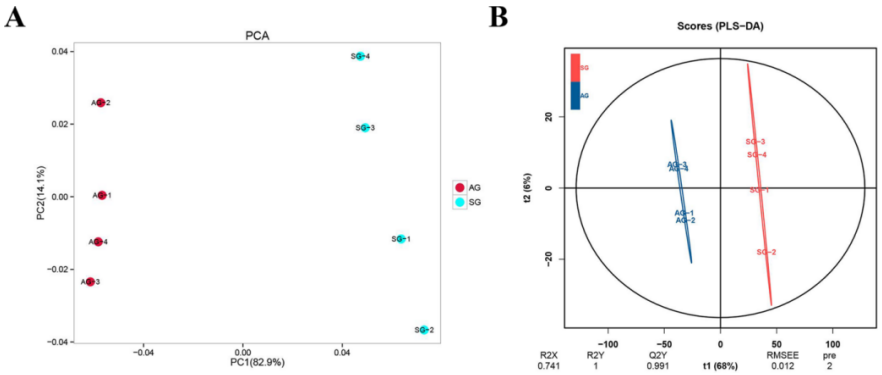


Figure 7 Principal component analysis (PCA)(A) and partial least-squares discrimination analysis (B) of fecal metabolites in *Teratoscincus roborowskii.* AG1-4 are fecal metabolites of *Teratoscincus roborowskii* from autumn group. SG1-4 are fecal metabolites of *Teratoscincus roborowskii* from spring group.

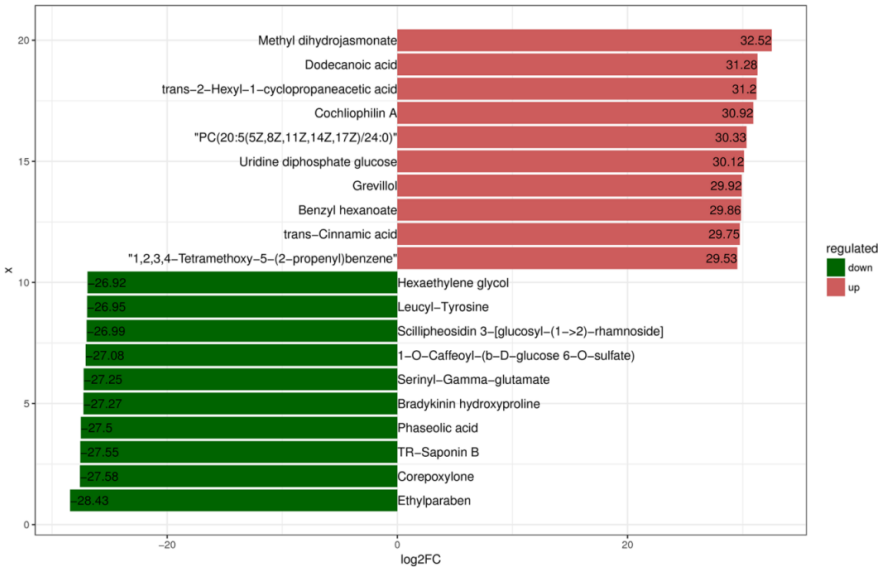


Figure 8 Fold change analysis of fecal metabolite differences based on log2FC size ordering. The figure shows the top 10 metabolites of up and down logFC, and the labels of each column indicate the name of the metabolite.

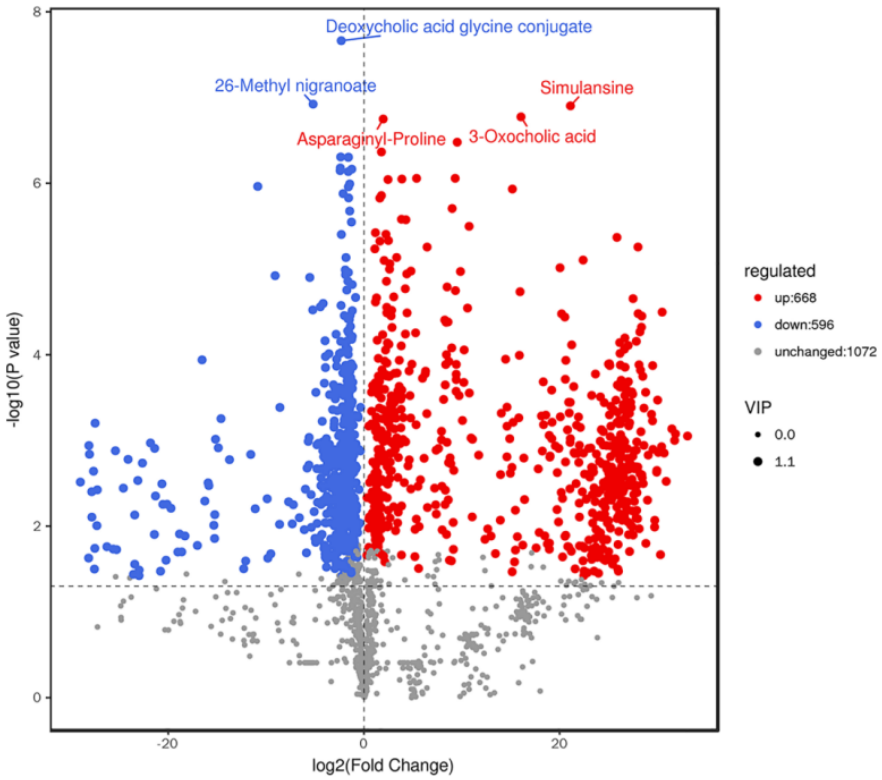


Figure 9 Volcano plot for statistical analysis of fecal differential metabolites. Note: Each point in the volcano plot represents a metabolite, the abscissa represents the multiple change of the group compared with each substance, the ordinate represents the$P$ -value of the t-test , and the size of the scatter represents the VIP value of the OPLS-DA model. The blue dots in the figure represent the down regulated differential expression metabolites, the red dots represent the up regulated differential expression metabolites, and the gray dots represent the detected but not significantly different metabolites. In addition, the top 5 qualitative metabolites are selected and marked in the figure after sorting by P value.

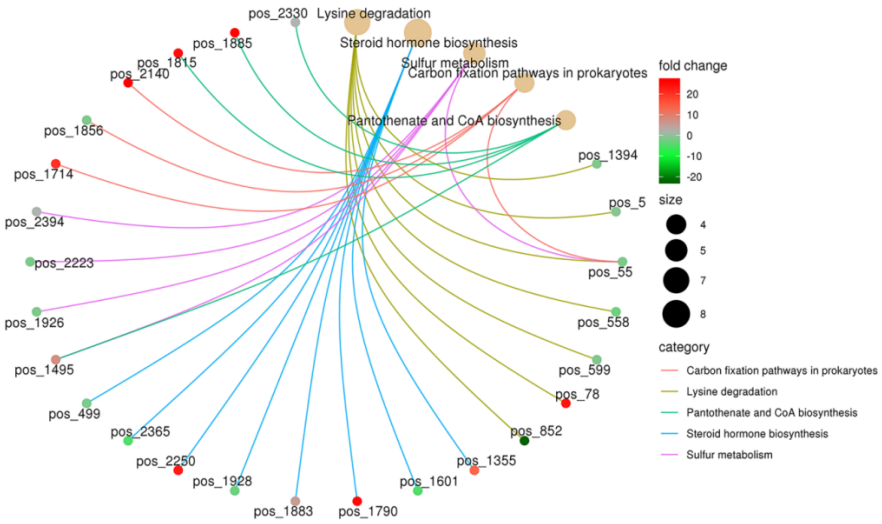


Figure 10 Enrichment of functional pathways corresponding to differential metabolites. Note: The light yellow node in the figure is the pathway, and the small node connected with it is the specific metabolite annotated to the pathway.The value of log2 in the difference multiple determines the color depth.

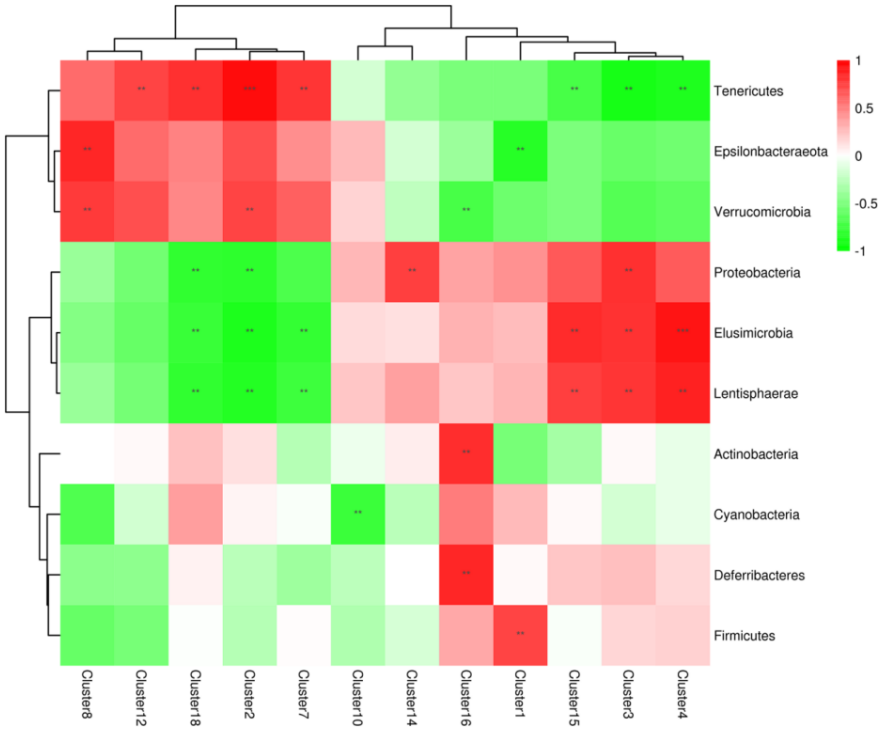
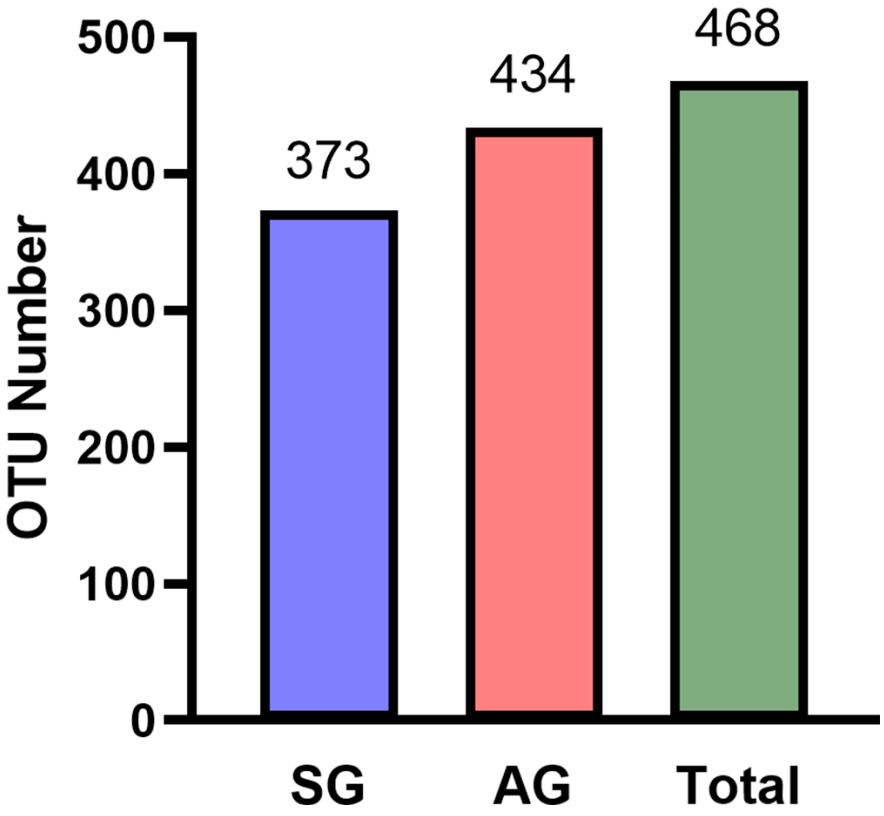
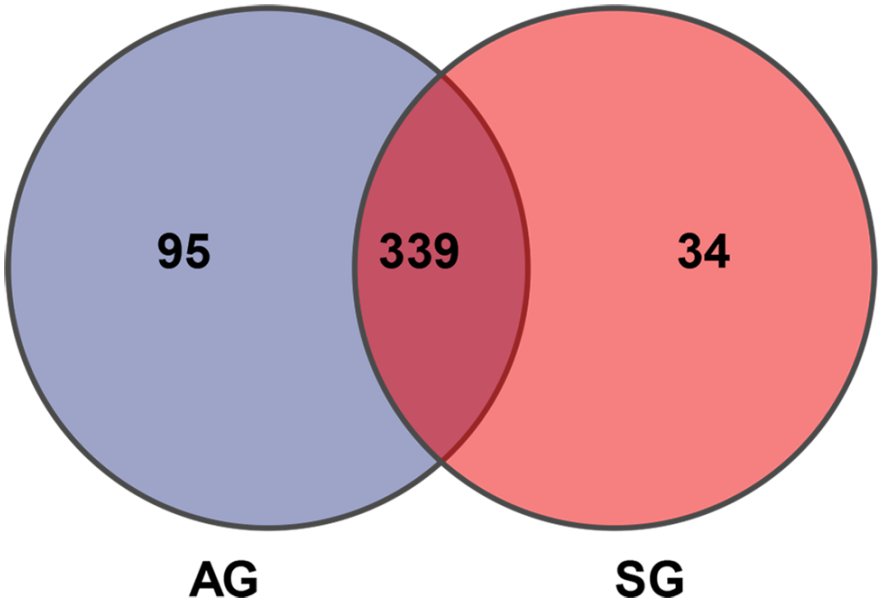
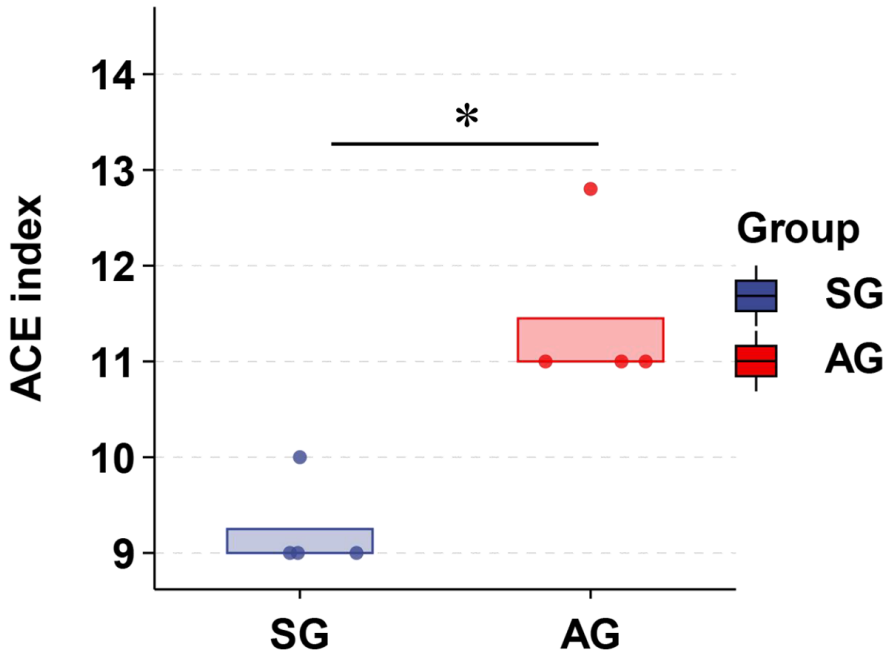
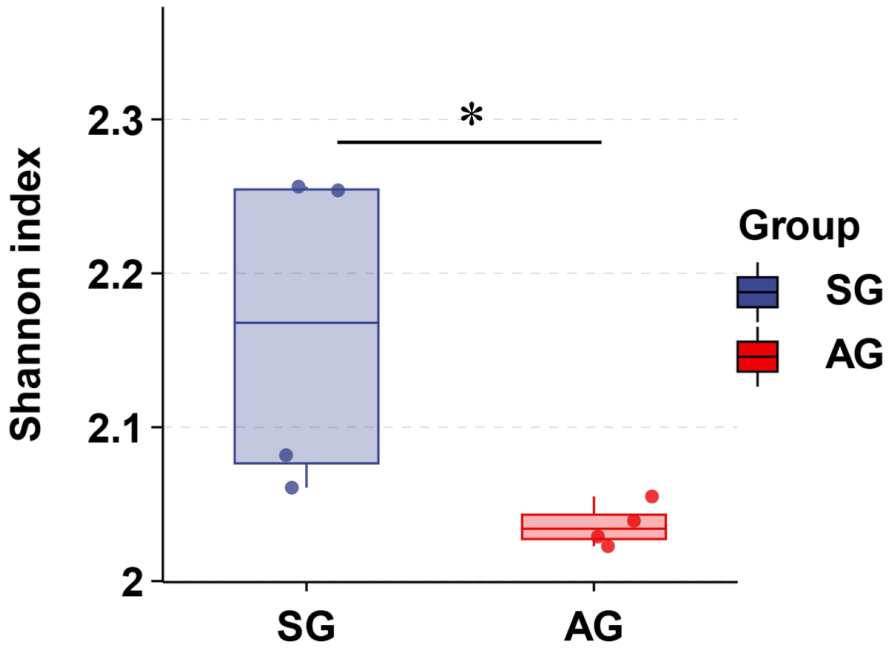


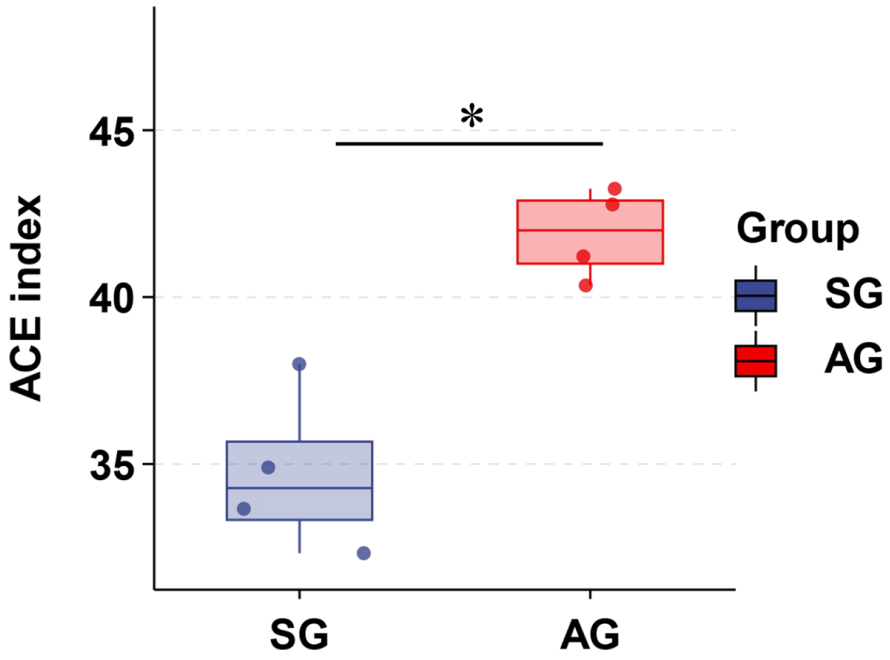
Figure 11 Heat map of the correlation between metabolite clusters and gut microbiota in *Teratoscincus roborowskii.* Note: Different colors represent the size of the Pearson correlation coefficient. Red indicates a positive correlation, whereas green indicates a negative correlation. Asterisks indicate significant correlations between metabolite clusters and microorganisms (P <0.05); *, **, and *** indicate$P$ <0.05, P <0.01, and$P$ <0.001, respectively.

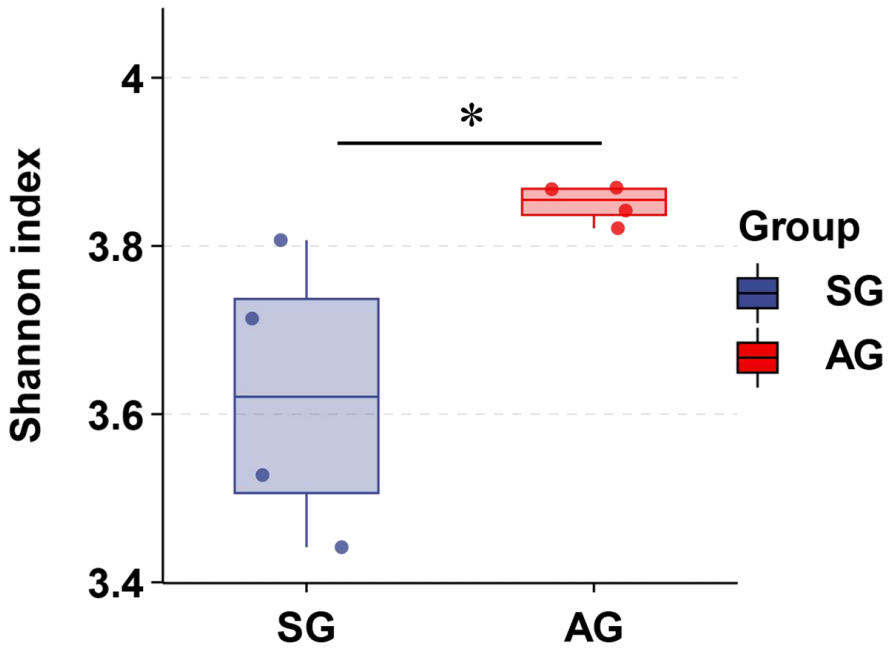


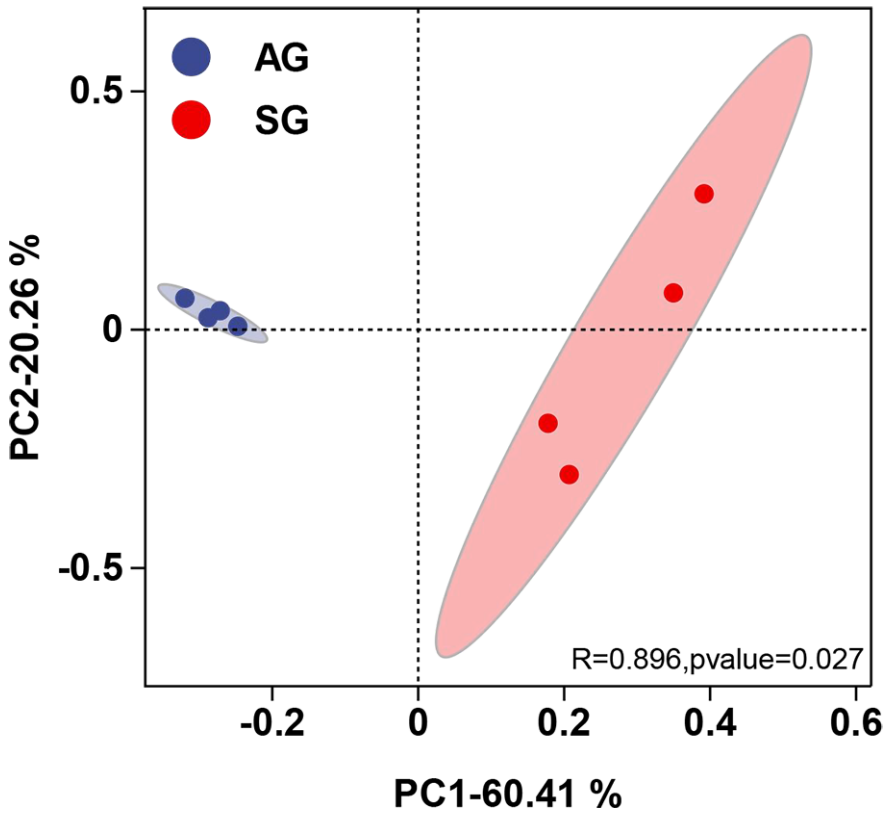


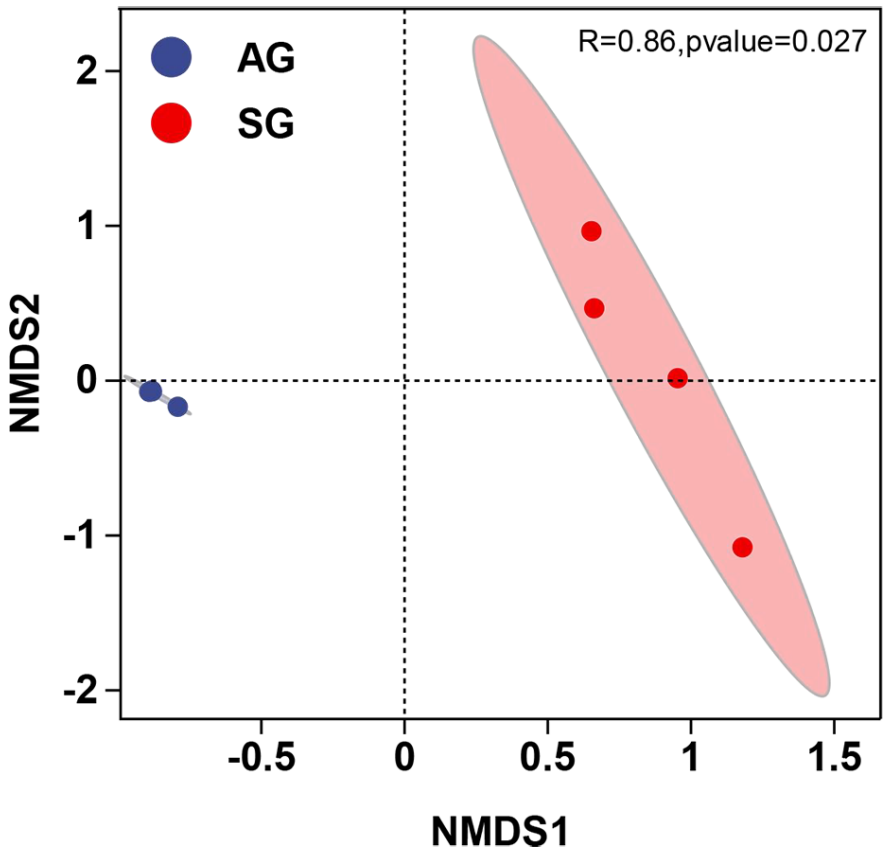


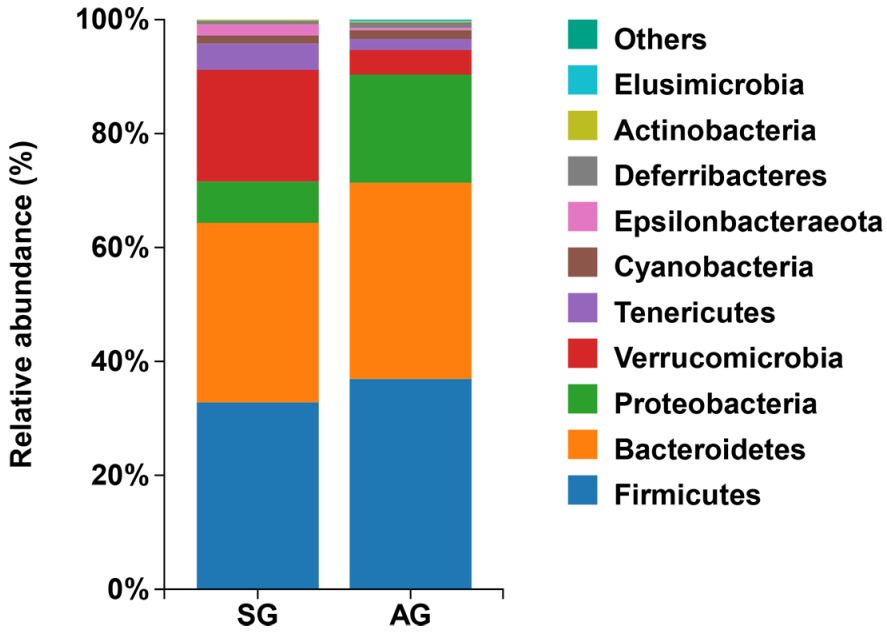


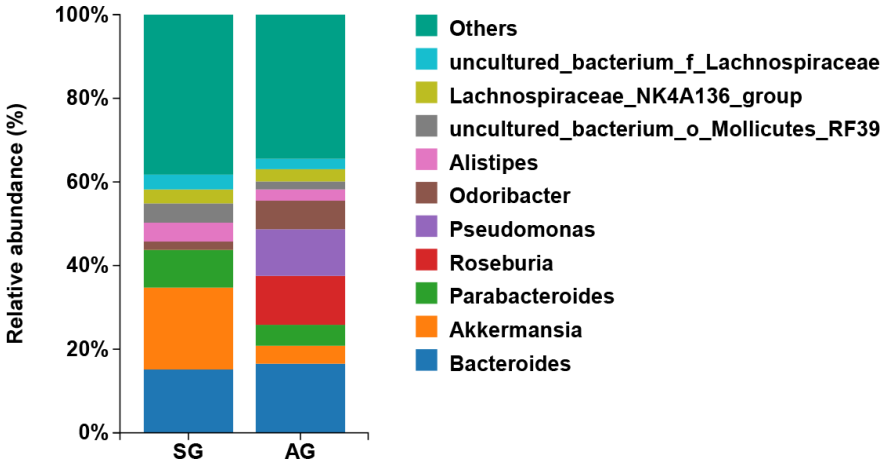


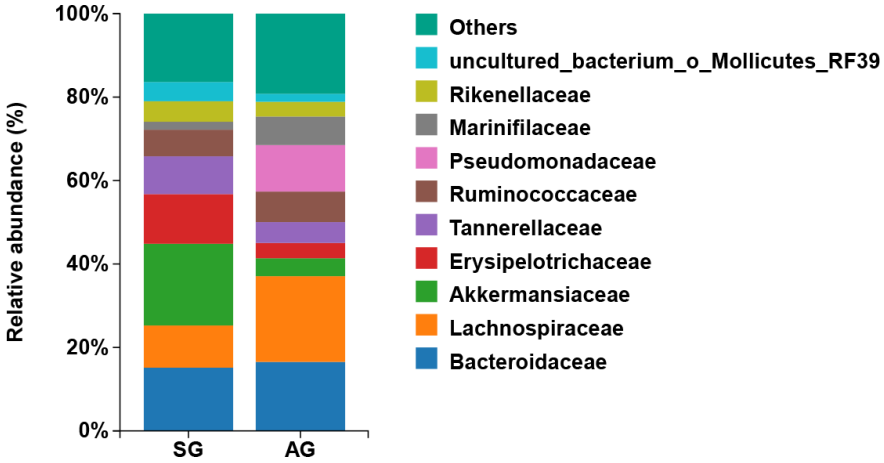












Cladogram