Exploring gut microbiota diversity in Catharsius molossus: influence of dietary conditions on ecosystem functionality

Yue Mao[1], Xingjian Yang[2], Hui Yuan[3], and Tao Xiong[1]

[1]Yangtze University College of Life Science
[2]Grinnell College
[3]Yangtze University

September 16, 2023

Abstract

Dung beetle serve as valuable indicators for studying environmental changes and as model systems for exploring ecosystem functionality. By analyzing the diversity and composition of gut microbiota in Catharsius molossus under starvation and refeeding conditions, this study investigates the effects of dietary states on the gut microbiota of these insects. Artificial rearing methods, along with 16S rRNA high-throughput sequencing and bioinformatics, were used to analyze Catharsius molossus gut microbiota under varying dietary conditions. The results indicate that at the phylum and genus levels, the gut microbiota of Catharsius molossus under refeeding conditions is more diverse than that under starvation conditions, with seven phyla and twenty-two genera showing significant differences ($P < 0.05$). In terms of functional prediction, the predicted functional genes of the gut microbiota were annotated to the KEGG database, revealing significant differences in thirty-two metabolic pathways at the third level ($P < 0.05$). Furthermore, it provides functional prediction information related to specific microbial taxa. Additionally, Dysgonomonas is speculated to participate in nitrogen fixation, and the gut microbiota of Catharsius molossus may potentially serve as a source of antimicrobial agents like anshanmycin. These findings provide novel insights into Coleoptera ecosystem microbial interactions and offer theoretical support for future applications.

Article title

Exploring gut microbiota diversity in *Catharsius molossus* : influence of dietary conditions on ecosystem functionality

2. Names of all authors

Yue Mao[1], Xingjian Yang[2], Hui Yuan[1], Tao Xiong[1*]

3.Affiliation of all authors

[1]Department of Life Science, Yangtze University, Jingzhou 434000, Hubei Province, China;

[2]Department of Biology, Grinnell College, Grinnell 50112, IA, USA

4. Postal and E-mail address of the corresponding author

Corresponding author: Tao Xiong. E-mail: xiongtao@hotmail.com

Exploring gut microbiota diversity in *Catharsius molossus* : influence of dietary conditions on ecosystem functionality

Yue Mao[1], Xingjian Yang[2], Hui Yuan[1], Tao Xiong[1*]

[1]Department of Life Science, Yangtze University, Jingzhou 434000, Hubei Province, China;

[2]Department of Biology, Grinnell College, Grinnell 50112, IA, USA

*Corresponding author:

Tao Xiong,

Department of Life Science, Yangtze University, Jingzhou 434000, Hubei Province;

E-mail address: xiongtao@hotmail.com

Abstract: Dung beetle serve as valuable indicators for studying environmental changes and as model systems for exploring ecosystem functionality. By analyzing the diversity and composition of gut microbiota in *Catharsius molossus* under starvation and refeeding conditions, this study investigates the effects of dietary states on the gut microbiota of these insects. Artificial rearing methods, along with 16S rRNA high-throughput sequencing and bioinformatics, were used to analyze *Catharsius molossus* gut microbiota under varying dietary conditions. The results indicate that at the phylum and genus levels, the gut microbiota of *Catharsius molossus* under refeeding conditions is more diverse than that under starvation conditions, with seven phyla and twenty-two genera showing significant differences ($P < 0.05$). In terms of functional prediction, the predicted functional genes of the gut microbiota were annotated to the KEGG database, revealing significant differences in thirty-two metabolic pathways at the third level ($P < 0.05$). Furthermore, it provides functional prediction information related to specific microbial taxa. Additionally, *Dysgonomonas* is speculated to participate in nitrogen fixation, and the gut microbiota of *Catharsius molossus*may potentially serve as a source of antimicrobial agents like anshanmycin. These findings provide novel insights into Coleoptera ecosystem microbial interactions and offer theoretical support for future applications.

Keywords : *Catharsius molossus* ; gut microbiota; 16S rRNA high-throughput sequencing; functional prediction; nitrogen fixation

1. INTRODUCTION

There are over twenty thousand species of beetles worldwide, distributed across all continents except Antarctica. Notably, beetles play a crucial role in ecosystems. They have the ability to disrupt the breeding habitats of pests and parasites harmful to livestock(deCastro-Arrazola et al., 2023; Gregory et al., 2015), thereby facilitating the spread of human pathogens(Nichols & Gómez, 2014), Additionally, they accelerate the return of nutrients from feces to soil(Sitters et al., 2014), contributing to improved soil aeration, water retention, and fertility(Kirsch, 2011). Moreover, beetles promote plant growth(deCastro-Arrazola et al., 2020), assist in seed dispersal(Pedersen & Blüthgen, 2022), and even provide essential pollination services(Pokhrel et al., 2021; Sakai & Inoue, 1999).

The microbial community within the digestive tract of insects, also known as gut microbiota, comprises protozoa, fungi, archaea, and bacteria(Rozadilla et al., 2020). This diverse consortium plays a crucial role in providing essential nutrients to the host, aiding in efficient food digestion, enhancing host defense mechanisms, and enabling detoxification processes(Kaltenpoth, 2020). Moreover, it exerts a significant influence on various aspects of the host insect's life, including its lifespan, developmental cycle, mating behaviors, and reproductive capacities(Dong et al., 2021). Despite its importance, research into the gut microbiota of beetles is currently in its early exploratory stages. However, several studies have already unveiled noteworthy findings. For instance, Franzini et al. conducted a comparison of the gut microbiota among two distinct small desert beetle species, revealing intriguing intra-species variations(Franzini et al., 2016). In a separate investigation by Suarez-Moo et al., greater similarity in gut microbiota was observed between the larvae of*Copris incertus Say* and the female adults, suggesting a potential mode of vertical transmission that shapes the composition and diversity of offspring gut microbiota(Suárez-Moo et al., 2020). Furthermore, a novel yeast species named *Trichosporon heliocopridis* , extracted from the gut of *Heliocopris bucephalus Fabricius* , has exhibited the remarkable ability to assimilate various carbon sources while withstanding environmental stressors such as high temperature, salt, sugar, and ethanol concentrations(Nwaefuna et al., 2021). Additionally, the gut of

Thorectes lusitanicus harbors several species belonging to the Actinobacteria phylum, known to produce an array of secondary metabolites with antibiotic properties, thus contributing to host protection(Kaltenpoth, 2009). It's worth noting that symbiotic bacteria within insects also serve as a significant source for discovering new bioactive small molecules, presenting valuable opportunities for the identification and utilization of beneficial natural products.

Dung beetles, exemplified by *Catharsius molossus* , are a distinctive group of coprophagous insects that confront specific nutritional challenges within their daily dietary regime. Being reliant on fecal matter as their primary food source, they encounter limitations in accessing adequate nitrogen nutrition, as feces typically harbor low nitrogen content(Madzivhe et al., 2020). Moreover, fecal matter is laden with diverse parasites and pathogens that could potentially impact the health of dung beetles(Woo et al., 2023). In this study, we utilized advanced 16S rRNA high-throughput sequencing technology to extensively scrutinize the diversity and differential composition of the gut microbiota of*Catharsius molossus* under conditions of both starvation and refeeding. Our endeavor is centered on unraveling the consequences of periods of food deprivation followed by subsequent refeeding on the structural dynamics of the gut microbiota in *Catharsius molossus* . This research holds substantial promise in enhancing our comprehension of the intricate ecological functionalities of the gut microbiota in dung beetles, with particular emphasis on the roles assumed by nitrogen-fixing bacteria and the prospective development and application of antimicrobial agents. The outcomes anticipated from this study are poised to contribute significantly to the foundational knowledge of dung beetle ecosystems and the broader domain of physiological ecology, thereby ushering in novel insights and perspectives to this realm of scientific inquiry.

2. MATERIALS AND METHODS

2.1 Animal selection

Wild *Catharsius molossus* dung beetles were captured in their natural habitat in Xichuan County, Nanyang City, Henan Province, China, from April 2021 to June 2021. The captured beetles were transported to the laboratory facilities at the College of Life Sciences, Yangtze University, for further study. The laboratory was maintained at a constant temperature of 21°C with a soil moisture content of 26%. Adequate ventilation was ensured in the laboratory, and the beetles were provided with a daily diet of fresh cow dung.

2.2 Experimental procedures

2.2.1 Sample collection and grouping

The gut samples from dung beetles under starvation and refeeding conditions were collected on August 15th and August 22nd, respectively. Six samples were collected for each condition. Before collection, the dung beetles were artificially reared for one month to stabilize their physiological conditions. For the starvation treatment, the beetles were observed for defecation daily until their defecation frequency significantly decreased. Once the beetles exhibited minimal defecation, their gut samples were collected for the starvation condition group. Subsequently, the beetles were re-fed for seven days and their gut samples were collected for the refeeding condition group. The collected gut samples were placed in sterile tubes, sequentially numbered, and stored at -80°C for further analysis (Table 1).

2.2.2 DNA extraction and Illumina NovaSeq 6000 sequencing

DNA extraction of the entire gut microbial community genome was performed using the NovaSeq 6,000 SP Reagent Kit (Illumina, USA). For bacterial 16S rRNA gene V3-V4 hypervariable region amplification, genomic DNA served as the template, and the fusion primers 341F/805R were utilized(Tsou et al., 2020). The PCR reaction mixture consisted of 1 μL 10× Toptaq Buffer, dNTPs (2.5 mM), 0.2 μL Primer F/R (10 μM), 0.2 μL Toptaq DNA Polymerase, 1˜3 μL Template DNA, with the final volume adjusted to 10 μL with

supplementary ddH_2O. The PCR cycling program comprised an initial denaturation step at 94 °C for 2 min, followed by 26 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min. Following amplification, the products were purified as per the Agencourt AMPure XP Nucleic Acid Purification Kit instructions. Subsequently, Illumina NovaSeq 6000 sequencing was performed by Shanghai Tianhao Biotechnology Co., Ltd. (Shanghai, China).

2.2.3 Analysis of 16S rRNA gene sequences and bioinformatics

We initially subjected the raw sequencing data to preprocessing steps to ensure the accuracy of subsequent analyses. The raw sequencing data may contain artificial additives such as adapter sequences and primers. To eliminate potential noise from these sources, the cutadapt plugin of QIIME2 software (v0.5.0) was employed to remove possible adapter sequences and primers. Subsequently, the raw data subjected to filtering were quality assessed to guarantee high data quality(Lima et al., 2021). For obtaining high-quality sequencing data and enhancing the accuracy of subsequent bioinformatics analyses, the DADA2 plugin of QIIME2 software was utilized for quality filtering, denoising, merging, and chimera removal, thereby generating Amplicon Sequence Variants (ASVs) to assess sample diversity(Liu et al., 2023). To evaluate the adequacy of sequencing depth for each sample, dilution curves were constructed using QIIME2 and the ggplot2 package (v3.3.0) in R, assessing sequencing saturation and determining the need for further sequencing. Furthermore, Mothur software (v1.41.1) was employed for taxonomic annotation, species composition was assessed, and Alpha and Beta diversity analyses were conducted using R software(Langbo et al., 2017). Leveraging amplicon sequencing data, PICRUSt2 analysis was employed for predictive and analytical functional profiling of microbial communities(C. Yang et al., 2023). Employing Wilcoxon rank-sum tests and Metastats analysis, a significance threshold of p-value <0.05 was used for differential significance screening, with the Bonferroni correction applied to the p-values to minimize the potential for false positives(Feng et al., 2023; Neuhäuser, 2015). This methodology was adopted to assess whether significant interspecies diversity exists among groups.

3. RESULTS

3.1 Sequencing quality and ASV analysis

In this study, the composition of gut microbiota in *Catharsius molossus* under starvation and refeeding conditions was analyzed. A total of 12 samples were sequenced, yielding a range of 49,222 to 69,752 sequences after removing chimeric sequences. The average number of valid sequences per sample was 66,620. The hungry group exhibited 1,289 ASVs, while the refed group displayed 2,847 ASVs. There were 419 common ASVs between the two groups, with 870 ASVs exclusive to the hungry group and 2,428 ASVs exclusive to the refed group (Fig. 1A). This indicates shared bacteria within the two gut microbiota groups while also highlighting their distinctive microbial communities. The sparse curve analysis showed a plateauing trend with increasing extracted sequence counts, indicating that the sequencing data volume and depth were appropriately set for the samples (Fig. 1B).

3.2 Composition and differential analysis of two gut microbiota

Through ASV annotation, a total of 26 phyla, 45 classes, 68 orders, 177 families, and 399 genera were identified across the two gut microbiota. At the phylum level (abundance $> 1\%$), the predominant phyla in *Catharsius molossus* under starvation conditions were Proteobacteria (51.93%), Firmicutes (33.11%), Actinobacteria (5.65%), Bacteroidetes (5.63%), Others (2.66%), and Chloroflexi (1.05%), with the highest abundance observed in Proteobacteria (Fig. 2A). In contrast, the predominant phyla in *Catharsius molossus* under refed conditions were Firmicutes (44.88%), Proteobacteria (19.73%), Bacteroidetes (15.16%), Actinobacteria (4.78%), Synergistetes (4.15%), Candidatus_Saccharibacteria (3.04%), Planctomycetes (2.31%), Others (2.18%), No_Rank (1.49%), Acidobacteria (1.21%), and Chloroflexi (1.08%), with the highest abun-

dance observed in Firmicutes. This reveals distinct predominant phyla between the two conditions, with the gut microbiota of refed beetles displaying higher phylum-level diversity (Fig. 2B).

At the genus level, the predominant genera in *Catharsius molossus*under starvation conditions were Unassigned (51.02%), Others (15.42%),*Vagococcus* (15.11%), No_Rank (8.93%), *Dysgonomonas*(2.66%), *Sphingobacterium* (1.71%), *Gordonia* (1.68%),*Acinetobacter* (1.26%), and *Paracoccus* (1.13%), with the highest classified and abundant genus being *Vagococcus* . In contrast, the predominant genera in *Catharsius molossus* under refed conditions were Others (25.96%), *Romboutsia* (13.81%), No_Rank (12.24%), Unassigned (10.09%), *Clostridium_XI*(7.67%), *Proteiniphilum* (6.33%), *Cloacibacillus*(3.83%), *Clostridium_sensu_stricto* (3.75%), Saccharibacteria_genera_incertae_sedis (3.04%), Turicibacter (2.69%), Lysinibacillus (1.9%), *Corynebacterium* (1.62%),*Luteimonas* (1.38%), *Hydrogenophaga* (1.22%),*Ercella* (1.22%), *Dysgonomonas* (1.08%), *Serpens*(1%), *Anaerovorax* (1%), with *Romboutsia* being the highest classified and abundant genus. This indicates higher genus-level diversity in the gut microbiota of refed beetles, with different dominant genera observed between the two conditions (Fig. 2C).

Based on Metastats analysis, comparisons of phylum-level classifications between the starvation and refed gut microbiota of *Catharsius molossus* revealed significant differences ($P < 0.05$) in Hydrogenedentes, Proteobacteria, Synergistetes, Planctomycetes, Verrucomicrobia, Fusobacteria, and Bacteroidetes (Table 2). At the genus level, 22 genera exhibited significant differences ($P < 0.05$) (Table 3).

3.3 Alpha diversity and differential analysis of two gut microbiota

Alpha diversity analysis was conducted based on the Wilcoxon rank-sum test to compare the richness and diversity indices of the gut microbiota between the starvation and refed states of *Catharsius molossus* . The Chao1 and ACE indices, representing species richness(M. Yang et al., 2022), were found to be significantly higher in the refed group than in the starvation group ($P < 0.05$), indicating a greater species diversity in the refed microbiota. Additionally, the Shannon index, reflecting microbial diversity(Zhang et al., 2022), was also higher in the refed group, while the Simpson index, indicating lower microbial diversity, was lower in the refed group ($P < 0.05$). These results collectively suggest that the gut microbiota of the refed group exhibited both higher richness and diversity compared to the starvation group, underscoring significant differences between the two feeding conditions (Table 4).

3.4 Beta diversity and differential analysis of the two gut microbiota

Principal Coordinate Analysis (PCoA) was employed to evaluate the similarity in the structure of gut microbiota between the starved and refed groups(Song et al., 2021). The PCoA plot visualized the primary sources of dissimilarity among the samples along the horizontal (Axis 1) and vertical (Axis 2) axes, which accounted for the most significant variations. Distinct colors on the PCoA plot represented different groups, with shorter distances indicating greater similarity and reduced dissimilarity in microbial structures between paired samples. Employing unweighted UniFrac distances, the PCoA analysis revealed that Axis 1 contributed to 27.89% of the variance, while Axis 2 contributed to 15.47% (Fig. 3). Importantly, the gut microbiota of *Catharsius molossus* exhibited clustering patterns corresponding to their feeding conditions. Notably, the starvation group displayed more scattered microbial compositions among samples, whereas the refed group demonstrated a higher degree of intra-group clustering. These findings imply that under conditions of starvation, *Catharsius molossus*may employ diverse strategies in response to environmental changes, leading to differences in their gut microbiota configurations.

3.5 Functional prediction using PICRUSt

Functional gene annotations predicted by PICRUSt provided a comprehensive insight into the metabolic potential of the gut microbiota in Catharsius molossus. These annotations were mapped to primary, secondary, and tertiary pathways within the KEGG database, encompassing various hierarchical levels of metabolic

classification. The primary pathways fell under five fundamental metabolic categories: metabolism, genetic information processing, environmental information processing, cellular processes, and organismal systems. Within these primary pathways, a total of 27 secondary-level pathways and 175 tertiary pathways were identified. Remarkably, metabolic pathways were dominant within the primary metabolic category, accounting for approximately 80% of the total annotations. Among the secondary pathways, amino acid metabolism exhibited the highest gene abundance, constituting around 15%. Notably, utilizing the Wilcoxon rank-sum test, 32 tertiary metabolic pathways displayed significant differences ($P< 0.05$), highlighting potential alterations in the metabolic landscape between groups at the granularity of these specific pathway levels (Fig. 4).

4. DISCUSSION

4.1 Analysis of gut microbiota composition in different states of *Catharsius molossus*

Gut microbiota, as the "inner ecosystem" of the gut, plays a crucial role in insects' digestive systems that rely on limited nutrients or hard-to-digest food sources(Tafesh-Edwards & Eleftherianos, 2023; Wang et al., 2020). We observed stable and enduring microbial community structures in the host, referred to as "dominant microbial taxa." The presence of such microbial communities is vital for maintaining ecological balance and host health(Chen et al., 2021). Studies have demonstrated that gut microbiota in insects like*Pachysoma MacLeay* from arid coastal regions of southwestern Africa and flightless *Thorectes lusitanicus* contain dominant taxa such as Bacteroidetes, Firmicutes, Proteobacteria, and Actinobacteria(Franzini et al., 2016; Hernández et al., 2015). In our study, the predominant phyla in the gut of *Catharsius molossus* in both hungry and refeeding states were Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes. Notably, Bacteroidetes, known as obligate or strict anaerobes, are challenging to culture in vitro, suggesting potential similarities in the dominant microbial taxa among different beetle species and their significant roles in host growth and development. After refeeding, the proportion of Firmicutes and Bacteroidetes increased, while Proteobacteria decreased. The consistent proportion of Actinobacteria may relate to the fiber-rich diet of*Catharsius molossus* , as Firmicutes possesses genes involved in fermenting dietary fiber and interacts with intestinal mucosa, contributing to maintaining host equilibrium (Sun et al., 2022). Bacteroidetes, known for polysaccharide degradation and aiding dietary fiber energy release(Pereira et al., 2021), exhibited significant differences between hungry and refeeding states, indicating its potential role in polysaccharide metabolism that warrants further experimental validation (Vera-Ponce de León et al., 2020). Regarding the variation in Proteobacteria, previous studies have suggested that an increase in its proportion could lead to an elevated risk of diseases, serving as an indicator of microbial community disruption, and potentially even as a diagnostic marker for diseases (Rizzatti et al., 2017; Shin et al., 2015). Importantly, within the context of our study, we have observed a relatively higher abundance of Proteobacteria within the gut microbiota of *Catharsius molossus* during periods of dietary deprivation compared to the refeeding state. This observation provides suggestive insights that starvation could potentially induce an unfavorable physiological state, potentially contributing to the instability of the host's gut microbiota ecology. Additionally, the relatively small proportion of *Dysgonomonas* in our study differs from *Vagococcus* and *Romboutsia* , which were dominant genera according to Suarez-Moo et al. (Suárez-Moo et al., 2020).*Dysgonomonas* has been associated with nitrogen fixation capabilities (Bar-Shmuel et al., 2020; Skrzypczak & Przybylski, 2022), and its higher abundance during starvation suggests a potential role in nitrogen fixation to sustain host survival. However, more research is needed to explore whether *Dysgonomonas* assimilates nitrogen into the beetle or its direct environment. In summary, hunger and refeeding affect gut microbiota diversity and variability in *Catharsius molossus* . Yet, the impact of changes in gut microbial abundance on host physiological functions and their mechanisms remains to be investigated. Our study provides valuable insights into the adaptability of gut microbiota in beetles under unique environments and their ecological functions.

4.2 Predictive analysis of *Catharsius molossus* gut microbiota functions

Utilizing PICRUSt, we conducted functional predictions, revealing a higher abundance of genes related to primary metabolic pathways and genetic information processing at the primary pathway level. At the tertiary pathway level, the gut microbiota of hungry *Catharsius molossus* showed higher gene abundances in pathways related to ansamycin biosynthesis, vancomycin group biosynthesis, and D-glutamine and D-glutamate metabolism, while refeeding state displayed elevated gene abundances in pathways related to ansamycin biosynthesis, valine, leucine, and isoleucine biosynthesis, and D-glutamine and D-glutamate metabolism. Ansaamycins are essential antibiotics, particularly against Mycobacterium tuberculosis(Skrzypczak & Przybylski, 2022). This suggests that *Catharsius molossus* may utilize its gut microbiota's metabolic functions to interact with the environment, assisting in defense against pathogen and parasite infections, which may contribute to its unique adaptation to a diet of feces and living in such an environment. However, it's important to note that while our functional predictions offer valuable clues, they are based on predictions and don't fully represent the actual functions of gut microbiota. To delve into the precise roles of these gene functions in*Catharsius molossus'* environmental adaptability, integration of multi-omics data is essential. Such comprehensive research would enhance our understanding of the physiological functions, interdependencies, and coordination mechanisms of gut microbiota, uncovering their complex functions and potential applications. This approach could facilitate further resource development and utilization in the context of gut microbiota.

5. CONCLUSIONS

In conclusion, this study elucidates the influence of starvation and refeeding conditions on the diversity and differential composition of gut microbiota in *Catharsius molossus* . Additionally, it provides insights into functional predictions associated with specific microbial taxa. Furthermore, *Dysgonomonas* is postulated to engage in nitrogen fixation within the host and potentially contribute to the host's defense against pathogens and parasites through gut microbiota metabolic functions. The gut microbiota of *Catharsius molossus*also presents a potential reservoir of antimicrobial agents akin to anshanmycin. These findings not only introduce a fresh perspective on microbial interactions within the Coleoptera ecosystem but also offer theoretical underpinnings for future applications. Overall, this research contributes to a deeper understanding of the intricate interplay between gut microbiota and the host, paving the way for further investigations into the potential therapeutic applications of gut microbiota modulation.

AUTHOR CONTRIBUTIONS

Yue Mao : Conceptualization (supporting); Data curation (equal); Formal analysis (lead); Investigation (equal); Methodology (equal); Software (lead); Visualization (equal); Writing – original draft (lead); Writing – review & editing (supporting). **Xingjian Yang** : Conceptualization (supporting); Formal analysis (supporting); Investigation (equal); Methodology (supporting); Software (supporting).**Hui Yuan** : Conceptualization (supporting); Formal analysis (supporting); Investigation (equal); Methodology (supporting); Software (supporting). **Tao Xiong** : Conceptualization (equal); Data curation (equal); Funding acquisition (lead); Formal analysis (supporting); Investigation (equal); Methodology (equal); Project administration (equal); Resources (equal); Supervision (lead); Visualization (equal); Writing – review & editing (lead).

DECLARATION OF COMPETING INTEREST

None.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge financial support from the State Veterinary Biotechnology State Key Laboratory Open Topics of China (Youth Fund SKLVBF202002) and Northern University Agricultural Fund (20220013).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Figshare at doi: 10.6084/m9.figshare.24143640.

References

Bar-Shmuel, N., Behar, A., & Segoli, M. (2020). What do we know about biological nitrogen fixation in insects? Evidence and implications for the insect and the ecosystem. *Insect Science, 27* (3), 392-403.

Chen, Q. L., Hu, H. W., Yan, Z. Z., Li, C. Y., Nguyen, B. T., Zheng, Y., Zhu, Y. G., & He, J. Z. (2021). Termite mounds reduce soil microbial diversity by filtering rare microbial taxa. *Environmental Microbiology, 23* (5), 2659-2668.

deCastro-Arrazola, I., Andrew, N. R., Berg, M. P., Curtsdotter, A., Lumaret, J. P., Menéndez, R., Moretti, M., Nervo, B., Nichols, E. S., Sánchez-Piñero, F., Santos, A. M. C., Sheldon, K. S., Slade, E. M., & Hortal, J. (2023). A trait-based framework for dung beetle functional ecology. *Journal of Animal Ecology, 92* (1), 44-65.

deCastro-Arrazola, I., Hortal, J., Noriega, J. A., & Sánchez-Piñero, F. (2020). Assessing the functional relationship between dung beetle traits and dung removal, burial, and seedling emergence. *Ecology, 101* (10), e03138.

Dong, Z. X., Chen, Y. F., Li, H. Y., Tang, Q. H., & Guo, J. (2021). The Succession of the Gut Microbiota in Insects: A Dynamic Alteration of the Gut Microbiota During the Whole Life Cycle of Honey Bees (Apis cerana).*Frontiers in Microbiology, 12* , 513962.

Feng, X., Hua, R., Zhang, W., Liu, Y., Luo, C., Li, T., Chen, X., Zhu, H., Wang, Y., & Lu, Y. (2023). Comparison of the gut microbiome and resistome in captive African and Asian elephants on the same diet.*Frontiers in Veterinary Science, 10* , 986382.

Franzini, P. Z., Ramond, J. B., Scholtz, C. H., Sole, C. L., Ronca, S., & Cowan, D. A. (2016). The Gut Microbiomes of Two Pachysoma MacLeay Desert Dung Beetle Species (Coleoptera: Scarabaeidae: Scarabaeinae) Feeding on Different Diets. *PLoS One, 11* (8), e0161118.

Gregory, N., Gómez, A., Oliveira, T. M., & Nichols, E. (2015). Big dung beetles dig deeper: trait-based consequences for faecal parasite transmission. *International Journal for Parasitology, 45* (2-3), 101-105.

Hernández, N., Escudero, J. A., San Millán, Á., González-Zorn, B., Lobo, J. M., Verdú, J. R., & Suárez, M. (2015). Culturable aerobic and facultative bacteria from the gut of the polyphagic dung beetle Thorectes lusitanicus. *Insect Science, 22* (2), 178-190.

Kaltenpoth, M. (2009). Actinobacteria as mutualists: general healthcare for insects? *Trends in Microbiology, 17* (12), 529-535.

Kaltenpoth, M. (2020). An endosymbiont's journey through metamorphosis of its insect host. *Proceedings of the National Academy of Sciences of the United States of America, 117* (35), 20994-20996.

Kirsch, I. (2011). Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences. Preface. *Philosophical Transactions of the Royal Society B: Biological Sciences, 366* (1572), 1781-1782.

Langbo, Yi, Guirong, Su, Guang, Hu, Qingzhong, & Peng. (2017). Diversity study of microbial community in bacon using metagenomic analysis. *Journal of Food Safety, 37* (3), e12334.

Lima, J., Manning, T., Rutherford, K. M., Baima, E. T., Dewhurst, R. J., Walsh, P., & Roehe, R. (2021). Taxonomic annotation of 16S rRNA sequences of pig intestinal samples using MG-RAST and QIIME2 generated different microbiota compositions. *Journal of Microbiological Methods, 186* , 106235.

Liu, G., Li, T., Zhu, X., Zhang, X., & Wang, J. (2023). An independent evaluation in a CRC patient cohort of microbiome 16S rRNA sequence analysis methods: OTU clustering, DADA2, and Deblur. *Frontiers in Microbiology, 14* , 1178744.

Madzivhe, F. M., Woolley, C., & Byrne, M. (2020). Getting the most out of waste: how dung beetles boost the nitrogen content in their food.*Physiological Entomology* .

Neuhäuser, M. (2015). Combining the t test and Wilcoxon's rank-sum test.*Journal of Applied Statistics, 42* (12), 2769-2775.

Nichols, E., & Gómez, A. (2014). Dung beetles and fecal helminth transmission: patterns, mechanisms and questions. *Parasitology, 141* (5), 614-623.

Nwaefuna, A. E., Rumbold, K., Boekhout, T., & Zhou, N. (2021). Bioethanolic yeasts from dung beetles: tapping the potential of extremophilic yeasts for improvement of lignocellulolytic feedstock fermentation. *Biotechnology for Biofuels, 14* (1), 86.

Pedersen, K. M., & Blüthgen, N. (2022). Seed size and pubescence facilitate secondary dispersal by dung beetles. *Biotropica, 54* (1), 215-225.

Pereira, G. V., Abdel-Hamid, A. M., Dutta, S., D'Alessandro-Gabazza, C. N., Wefers, D., Farris, J. A., Bajaj, S., Wawrzak, Z., Atomi, H., Mackie, R. I., Gabazza, E. C., Shukla, D., Koropatkin, N. M., & Cann, I. (2021). Degradation of complex arabinoxylans by human colonic Bacteroidetes. *Nature Communications, 12* (1), 459.

Pokhrel, M. R., Cairns, S. C., Hemmings, Z., Floate, K. D., & Andrew, N. R. (2021). A Review of Dung Beetle Introductions in the Antipodes and North America: Status, Opportunities, and Challenges.*Environmental Entomology, 50* (4), 762-780.

Rizzatti, G., Lopetuso, L. R., Gibiino, G., Binda, C., & Gasbarrini, A. (2017). Proteobacteria: A Common Factor in Human Diseases. *BioMed Research International, 2017* , 9351507.

Rozadilla, G., Cabrera, N. A., Virla, E. G., Greco, N. M., & Mccarthy, C. B. (2020). Gut microbiota of Spodoptera frugiperda (J.E. Smith) larvae as revealed by metatranscriptomic analysis. *Journal of Applied Entomology, 144* (5), 351-363.

Sakai, S., & Inoue, T. (1999). A new pollination system: dung-beetle pollination discovered in Orchidantha inouei (Lowiaceae, Zingiberales) in Sarawak, Malaysia. *American Journal of Botany, 86* (1), 56-61.

Shin, N. R., Whon, T. W., & Bae, J. W. (2015). Proteobacteria: microbial signature of dysbiosis in gut microbiota. *Trends in Biotechnology* , 496-503.

Sitters, J., Maechler, M. J., Edwards, P. J., Suter, W., Olde Venterink, H., & Kay, A. (2014). Interactions between C:N:P stoichiometry and soil macrofauna control dung decomposition of savanna herbivores.*Functional Ecology, 28* (3), 776-786.

Skrzypczak, N., & Przybylski, P. (2022). Modifications, biological origin and antibacterial activity of naphthalenoid ansamycins.*Natural Product Reports, 39* (9), 1653-1677.

Song, X., Feng, Z., Tan, J., Wang, Z., & Zhu, W. (2021). Dietary administration of Pleurotus ostreatus polysaccharides (POPS) modulates the non-specific immune response and gut microbiota diversity of Apostichopus japonicus - ScienceDirect. *Aquaculture Reports, 19* (2021), 100578.

Suárez-Moo, P., Cruz-Rosales, M., Ibarra-Laclette, E., Desgarennes, D., Huerta, C., & Lamelas, A. (2020). Diversity and Composition of the Gut Microbiota in the Developmental Stages of the Dung Beetle Copris incertus Say (Coleoptera, Scarabaeidae). *Frontiers in Microbiology, 11* , 1698.

Sun, Y., Zhang, S., Nie, Q., He, H., Tan, H., Geng, F., Ji, H., Hu, J., & Nie, S. (2022). Gut firmicutes: Relationship with dietary fiber and role in host homeostasis. *Critical Reviews in Food Science and Nutrition* , 1-16.

Tafesh-Edwards, G., & Eleftherianos, I. (2023). The role of Drosophila microbiota in gut homeostasis and immunity. *Gut Microbes, 15* (1), 2208503.

Tsou, A. M., Olesen, S. W., Alm, E. J., & Snapper, S. B. (2020). 16S rRNA sequencing analysis: the devil is in the details. *Gut Microbes, 11* (5), 1139-1142.

Vera-Ponce de León, A., Jahnes, B. C., Duan, J., Camuy-Vélez, L. A., & Sabree, Z. L. (2020). Cultivable, Host-Specific Bacteroidetes Symbionts Exhibit Diverse Polysaccharolytic Strategies. *Applied and Environmental Microbiology, 86* (8).

Wang, S., Wang, L., Fan, X., Yu, C., Feng, L., & Yi, L. (2020). An Insight into Diversity and Functionalities of Gut Microbiota in Insects.*Current Microbiology* , 1-11.

Woo, C., Bhuiyan, M. I. U., Eo, K. Y., Lee, W. S., Kimura, J., & Yamamoto, N. (2023). Diversity of fecal parasitomes of wild carnivores inhabiting Korea, including zoonotic parasites and parasites of their prey animals, as revealed by 18S rRNA gene sequencing.*International Journal for Parasitology: Parasites and Wildlife, 21* , 179-184.

Yang, C., Mai, J., Cao, X., Burberry, A., Cominelli, F., & Zhang, L. (2023). ggpicrust2: an R package for PICRUSt2 predicted functional profile analysis and visualization. *Bioinformatics, 39* (8).

Yang, M., Qi, Y., Liu, J., Wu, Z., Gao, P., Chen, Z., Huang, F., & Yu, L. (2022). Dynamic changes in the endophytic bacterial community during maturation of Amorphophallus muelleri seeds. *Frontiers in Microbiology, 13* , 996854.

Zhang, M., Chen, S., Ding, S., Yao, X., Wang, Z., & Sang, L. (2022). Effects of 7 Years of Warming and Straw Application on Soil Bacterial, Fungal, and Archaeal Community Compositions and Diversities in a Crop Field. *Journal of Soil Science and Plant Nutrition, 22* (2), 2266-2281.

Hosted file

`figure.docx` available at https://authorea.com/users/665512/articles/666644-exploring-gut-microbiota-diversity-in-catharsius-molossus-influence-of-dietary-conditions-on-ecosystem-functionality

Hosted file

`table.docx` available at https://authorea.com/users/665512/articles/666644-exploring-gut-microbiota-diversity-in-catharsius-molossus-influence-of-dietary-conditions-on-ecosystem-functionality