ARTICLE

Mapping the bacterial diversity and functional landscape of the Philippine bat gut microbiome using a 16S-based metataxonomics approach

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ABSTRACT

Understanding the gut microbiome of bats is essential for elucidating its role in host health, metabolism, and the transmission of potentially zoonotic pathogens. Despite this, the gut microbiomes of Philippine bats remain largely uncharacterized. In this study, we present a comprehensive examination of the gut microbiome profiles of 112 bats captured from multiple cave sites in the CALABARZON region of Luzon, Philippines. Using 16S ribosomal RNA (rRNA) gene amplicon sequencing, we compared the microbiomes of various insectivorous (n=60) and frugivorous (n=52) bat species, accounting for dietary niches, host identity, and location. In terms of microbial diversity, results reveal that insectivorous bats show significantly lower Shannon diversity (H') and Inverse Simpson metrics than frugivorous bats (K-W; $p<0.05$). Beta diversity analysis indicates a strong influence of cave location on microbial community composition, while diet had weaker effects—suggesting overlap and similarity of gut communities across dietary guilds. Stratified permutational analysis also highlights notable convergence among bats within the same dietary guild or species roosting at different caves, particularly among frugivorous bats such as *Ptenochirus jagori*. Compositional analysis shows a predominance of Pseudomonadota and Bacillota across samples, with several frugivorous bats harboring potentially pathogenic families (Neisseriaceae, Campylobacteraceae, Mycoplasmoidaceae, Helicobacteraceae).

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KEYWORDS

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Functional predictions using PiCRUSt2 suggest that insectivores, despite their lower taxonomic diversity, possess higher functional diversity, notably in amino acid metabolism pathways. This enrichment may reflect specific metabolic adaptations or dietary requirements. These interesting findings elucidate the role of ecological factors in shaping the gut microbiome of cave bats, particularly in the South Luzon region.

INTRODUCTION

The gut microbiome is considered as a "forgotten" auxiliary organ in mammals and is known to play an active role in the metabolism, internal signalling, and immunity of its hosts (Wu et al., 2018). It is known to be shaped by a variety of factors, including the host diet, phylogeny, and other externalities in the host's environment. Among these, diet is considered as one of the primary drivers as it directly influences the availability of nutrients and substrates for the colonization of the gut. In particular, herbivorous mammals tend to feature cellulose-degrading bacteria while carnivorous mammals exhibit microbiomes adapted towards protein and fat metabolism (Muegge et al., 2011).

Bats (order Chiroptera) are among the world's most diverse mammalian orders, with over 1400 extant species spread over two distinct lineages—the Yinchiropterans and the Yangochiropterans. Bats are known to feature a number of distinct adaptations, such as true flight, that enabled them to colonize a large variety of habitats around the world, including forests and subterranean habitats (Tanalgo & Hughes, 2018). Bats feature a wide variety of dietary adaptations, ranging from insectivory, nectarivory and hematophagy (Mena Canata et al., 2024). Recent studies into the bat gut have shed light on how diet influences microbial composition and adaptations among bats of different diets and bat species (Carrillo-Araujo et al., 2015; Fleischer et al., 2024; Mena Canata et al., 2024; Wu et al., 2018). Previous studies have established the predominance of the Proteobacteria, Actinobacteria and Firmicutes phyla among sampled bat gut and fecal matter (Corduneanu et al., 2023; Guo et al., 2023; Mena Canata et al., 2024).

Interestingly, the gut microflora of bats are known to exhibit profiles more similar to avians rather than to other mammalians, posing further questions as to how their compositions are shaped, their roles in their respective hosts and whether such composition is brought by convergent evolution (Carrillo-Araujo et al., 2015; Jones et al., 2022). Further studies corroborated the role of host identity and ecology in shaping the gut microbiomes of bats, implying a strong location-based factor influencing the structure of the bat microbiome – a trait shared even among other similarly cosmopolitan mammals such as rodents (Wang et al., 2022; Dai et al., 2024; Mena Canata et al., 2024). Gastrointestinal microbiota modulate host immunity and govern pathogen carriage; they constitute a mechanistic bridge between bat ecology and the likelihood of cross-species transmission events (Carrillo-Araujo et al., 2015; Round & Mazmanian, 2009). Understanding the diet- and site-specific drivers of these communities offers insight not only into spill-over risk but also into the development of conservation measures that safeguard cave ecosystems.

Studies on bat gut microbiome focused mainly on inferences based on samples collected from feces, oral swabs, or rectal swabs. However, indirect approaches such as these are limited such that it cannot capture the complete picture of the microbial landscape of the gut (Ingala et al., 2018; Wu et al., 2018; Ahn et al., 2023). At present, there is a paucity of literature with a large-scale analysis of bat gut microbiomes particularly in biodiversity hotspots such as Southeast Asia. The Philippines is known to harbor over 70 extant bat species, around 30 species are known to roost in caves and subterranean habitats (Tanalgo & Hughes, 2018).

With Philippines offering a critical setting for bat microbiome research, this study aims to characterize and map the taxonomic and functional profiles of bats from caves across the CALABARZON region of Luzon, Philippines, that include the provinces of Laguna, Batangas, Rizal and Quezon. Characterization of the bat gut microbiome using systematic microbial ecology approaches that considers the location, host identities, diets and functional profiles using prediction tools allows a robust understanding of the ecological processes and factors shaping the bat gut microbiome and insights on public health implications.

MATERIALS AND METHODS

Sampling Sites

Bats were sampled from five distinct cave systems from the 4 provinces of the CALABARZON region of Luzon, Philippines. These caves are Cavinti Underground River and Cave Complex (Cavinti, Laguna), Kamantigue Cave (Lobo, Batangas), Bat Cave and Lobog Cave, Pamitinan Protected Landscape (Pamitinan, Rizal) and Palale and Sungwan Caves (Tayabas, Quezon) **(Table 1 and Figure 1)**. The caves sampled were mainly Class II and III based on the Philippine Department of Environment and Natural Resources (DENR) Cave Classification Scheme (DENR-PAWB, 2008). Samples reported in the paper of Datul et al. (2024) were incorporated into the present analysis.

Table 1: A summary table of all sampled sites and locations.

Geographical Location	Caves	Coordinates	Number of Bat Individuals	Philippine Wildlife Gratuitous Permit
Philippines: Cavinti, Laguna	CURCC	14.2818 N 121.636017 E	12	R4A-WGP-2021-LAG-004
Philippines: Lobo, Batangas	Kamantigue Cave	13.646481 N 121.347342 E	18	R4A-WGP-2021-BAT-006
Philippines: Pamitinan Protected Landscape, Rizal	Bat Cave	14.73105 N 121.189783 E	10	R4A-WGP-2023-RIZ-003
	Lobog Cave	14.746467 N 121.19515 E	14	

Philippines: Tayabas, Quezon	Palale Cave	14.031431 N 121.685736 E, 14.037767 N 121.678233 E	28	R4A-WGP-2023-QUE-004
	Sungwan Cave	14.02475 N 121.665333 E	30	R4A-WGP-2021-QUE-005

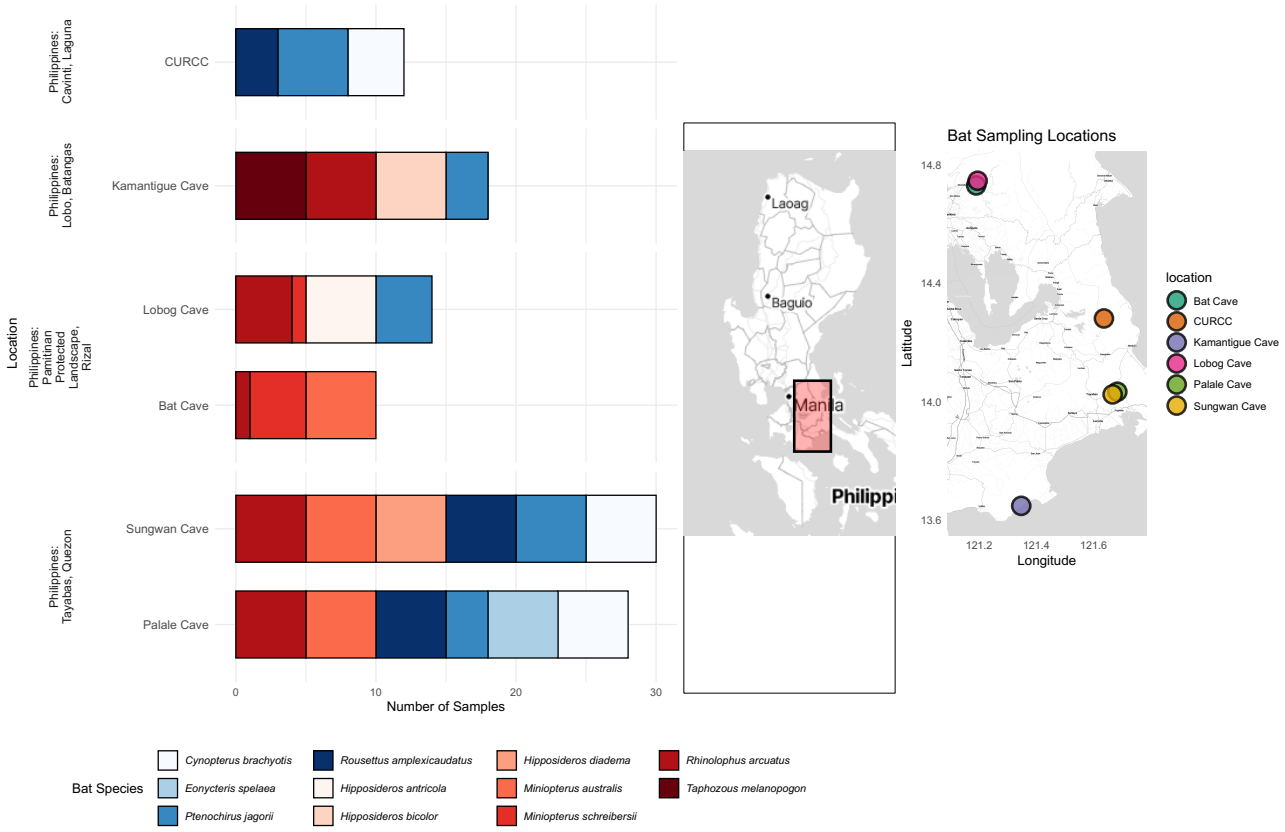

Figure 1: Summary of all bats sampled for this study (left) as well as the sampling locations (right). Frugivorous bats are represented in the shades of blue while insectivorous bats are represented in shades of red.

Sample Collection

A total of 112 bat individuals from 11 unique bat species, 5 distinct bat families and spanning 3 distinct dietary guilds (Frugivore, Insectivore and Nectarivore) were collected from multiple cave sites in the CALABARZON region. Table 2 summarizes the bat populations sampled and their respective locations. Among these bats are *Hipposideros antricola* Peters and *Ptenochirus jagori* Peters, two of whom are known to be endemic to the Philippines.

Table 2: A summary table of all sampled bat species.

Bat Species	Suborder	Family	Diet	Locations sampled	Number of Individuals
Cynopterus brachyotis (Müller, 1838)	Yinpterochiroptera	Pteropodidae	Frugivore	CURCC, Sungwan Cave, Palale Cave	14
Eonycteris spelaea (Dobson, 1871)	Yinpterochiroptera	Pteropodidae	Nectarivore	Palale Cave	5
Hipposideros antricola (Peters, 1861)	Yinpterochiroptera	Hipposideridae	Insectivore	Lobog Cave	5
Hipposideros bicolor (Temminck, 1834)	Yinpterochiroptera	Hipposideridae	Insectivore	Kamantigue Cave	5
Hipposideros diadema (É. Geoffroy, 1813)	Yinpterochiroptera	Hipposideridae	Insectivore	Sungwan Cave	5
Miniopterus australis (Tomes, 1858)	Yangochiroptera	Miniopteridae	Insectivore	Sungwan Cave, Bat Cave, Palale Cave	15
Miniopterus schreibersii (Kuhl, 1817)	Yangochiroptera	Miniopteridae	Insectivore	Bat Cave, Lobog Cave	5
Ptenochirus jagori (Peters, 1861)	Yinpterochiroptera	Pteropodidae	Frugivore	CURCC, Sungwan Cave, Kamantigue Cave, Lobog Cave, Palale Cave	20
Rhinolophus arcuatus (Peters, 1871)	Yinpterochiroptera	Rhinolophidae	Insectivore	Sungwan Cave, Kamantigue Cave, Bat Cave, Lobog Cave, Palale Cave	20
Rousettus amplexicaudatus (É. Geoffroy, 1810)	Yinpterochiroptera	Pteropodidae	Frugivore, Nectarivore	CURCC, Sungwan Cave, Palale Cave	13

Bats were captured via mist netting and were characterized based on diet and species. Species identification was conducted by mammalian and bat experts based on the *Key to the Bats of the Philippine Islands* (Ingle & Heaney, 1992). Bats were euthanized using inhalant anesthetic (isoflurane) following approved UPLB-IACUC protocols (Approval No. UPLB-2021-027). Individual bats were restrained in roosting pouches and placed in sealed plastic containers with cotton balls saturated with isoflurane, with death confirmed within 2 hours of exposure. Immediately after euthanization, the whole intestine was excised and placed in 95 percent ethanol prior to storage in liquid nitrogen and transport to the laboratory for further processing. All field identifications were later verified at the UPLB Museum of Natural History, where voucher specimens were also later deposited.

In the laboratory, the intestinal tissues were opened and scraped to collect the mucosal lining and gut contents based on (Nordgård et al., 2005; Phillips et al., 2012). The DNA content of each sample was extracted using the Microbiome DNA Isolation kit (Norgen Biotek, ON, Canada), with modifications to the lysis step reported by Datul et al. (2024) to enhance sample yield. All sample processing was conducted under sterile conditions using dedicated equipment and sterile techniques.

Targeted 16S Amplicon Metagenomics Sequencing

The extracted DNA was then sent to Macrogen Inc. for the subsequent library preparation and sequencing. Sequencing libraries were prepared using the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA) following the 3rd party service provider's specifications. The V3-V4 regions were amplified using the 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3') primers with Illumina adapters. Sequencing was performed using the Illumina MiSeq (300bp x 2) platform with a set sequencing depth of up to 100,000 reads.

Processing of Reads using QIIME2 and R

Processing of raw reads was performed within the QIIME2 Amplicon sequencing (2024.2) pipeline (Callahan et al., 2016). Trimming parameters for the denoising step was inferred with the use of the FIGARO (0.1) package (Sasada et al., 2020). Trimming and truncation parameters were set globally to minimize variation during the taxonomic mapping step. The samples were subsequently denoised and filtered for chimeras with the use of the q2-DADA2 (1.26.0) package (Callahan et al., 2016) on a per-sequencing batch basis using the global truncation and error model parameters. In particular, truncation parameters (-p-trunc-f and -p-trunc-r) were set to [297, 226]

while trimming parameters (--p-trim-left-f and –p-trim-left-r) are set to [17, 21]. Lastly, global error thresholds were set to [2, 2] for forward and reverse reads, respectively.

The denoised reads were subsequently merged within the Qiime2 environment. Taxonomic mapping was done with the use of the Greengenes v2024.5 (McDonald et al., 2024) database using the *classify-sklearn* (1.4.2; Qiime2-2024.5) function. Contaminant reads (Chloroplast and mitochondrial rRNA) were subsequently removed using *qiime taxa filter-table* function. The resulting artifacts were exported as a *phyloseq* object and subsequent post-processing of the dataset was done in R environment (4.2.2) (McMurdie & Holmes, 2013). Downstream analysis and visualization is then handled with the use of bound *Phyloseq* (1.48.0) and *ggplot* (3.5.1) methods.

Alpha Diversity Analysis

Computation of the Shannon and Inverse Simpson metrics were done using the *estimate_richness()* function of the phyloseq R package and compared using the Kruskal-Wallis statistic. Pairwise Post-hoc tests were performed after global K-W analysis to assess which sample groupings (based on diet, bat identity, and sample location) were significantly different from one another. Adjustment of P-values were made with the use of the Holm correction method.

Beta Diversity Analysis

Beta diversity analysis was performed using the *vegan* package (v2.6-6.1) (Oksanen, 2010). Pairwise sample distances were computed using the Bray-Curtis method on raw feature counts. Prior to statistical testing, homogeneity of multivariate dispersions were assessed using the Permutational Analysis of Dispersion (PERMDISP; *betadisper()* function; 999 permutations). Permutational Multivariate Analysis of Variance (PERMANOVA; *adonis2*() function, 999 permutations) was conducted to test for significant differences in community composition across diet, bat identity, sampling site, and host phylogeny (suborder level).

The partial omega squared (ω^2) values were calculated using the *adonis_omegaSq*() function (MicEco R package - 0.9.19) (Russel, 2021) to estimate effect size of each parameter while accounting for sample size and variance, using the formula,

$$\omega^2 = (df \times (MS\_effect - MS\_residual)) / (df \times MS\_effect + (N - df) \times MS\_residual)$$

where df = degrees of freedom
MS = mean squares
N = total sample size

Two analytical approaches were employed in this paper. First, Global PERMANOVA model was used to assess the total explained variance of confounding variables that included both the main effects (diet, location, bat identity, and suborder) and biologically relevant interaction terms (diet*location, bat identity*location, suborder*location).

Second, stratified PERMANOVA analyses were conducted to test specific hypotheses by grouping samples in two ways: (1) samples were grouped based on the synthetic term suborder*diet to assess the host evolutionary lineage influences on the diet-associated microbiome patterns, and (2) samples were grouped according to location-bat source combinations to assess habitat and species effects on microbiome composition. Only subgroups with samples more than 3 were considered for statistical validity. Stratified PERMANOVA was performed using the *Ecole* R package (0.9-2021; *Phytomosaic/Ecole*, n.d.) to assess community similarities in a pairwise manner while adjusting for global significance thresholds. P-values were adjusted for global results using FDR-based correction.

Differential Abundance Analysis

To identify bacterial genera with significant abundance differences across bat species, we utilized ANCOM-BC2 R package (2.6.0), which is designed to address the data compositionality issues with microbiome data while controlling for false discovery rates (Lin & Peddada, 2024). Groups were defined according to bat species, dietary guild, as well as geographic location were agglomerated at the level of Phylum and Class. We considered bacterial genera differentially abundant if they had W-statistics >0.9 and adjusted p-values <0.05. We further filtered results using the passed_ss parameter to identify taxa that passed sensitivity analysis after CLR transformation. The results in turn were clustered using the ward method and visualized using the *MicroViz* R package (0.12.4) (Barnett et al., 2021).

Functional Prediction Analysis

To further assess the implications of the microbiome, PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) (2.5.2) was used to predict the presence of certain metabolic pathways associated with each observed representative taxa associated with the sampled microbial communities (Douglas et al., 2020). The output of the default standalone pipeline using *predict_metagenomes.py* was used for this analysis, particularly the KO metagenome and MetaCyc. The outputs specific for KO entries were preprocessed into KEGG pathways using *ggpicrust2* R package (1.7.4) (Yang et al., 2023), annotated for BRITE hierarchies using the KEGGREST API (Tenenbaum et al., 2019) and afterwards packaged as a *phyloseq* object for ease of downstream processing. Prior to analysis, validation was made with the use of the Nearest Sequence Taxon Index (NSTI) to assess the level of functional mapping of the 16S rRNA database to the reference genomes of PICRUSt2. Output counts were center-log transformed (CLR) across samples to facilitate comparisons and to minimize the effect of large count ranges across the predicted functional types. Visualization of the predicted functional loadings was done via both Principal Component (PCA) and Principal Coordinate Analysis (PCoA) to visualize which particular KEGG pathways influence the Principal Component loadings the most, and to visualize the pairwise distances of each particular sample, respectively.

Further comparisons have been made between the Spearman correlation alpha diversity metrics (Shannon and Inverse Simpson) of Observed features in both taxonomic and functional data sets to assess the concordance between sample-wise taxonomic diversity and functional diversity, respectively. Lastly, differential abundance analysis using ANCOM-BC2 was performed to assess which particular pathways were enriched across sample diet types and bat species, using default settings and an alpha value set at 0.05. To reduce False Discovery Rates, a filter was implemented (based on default alpha = 0.05) such that only features that have passed the center-log ratio sensitivity test are considered for downstream analysis.

RESULTS AND DISCUSSION

Gut Bacterial Microbiome Sequence Analyses

In this study, we comprehensively examined the gut bacterial microbiomes of 112 individual bat samples including 9 samples reported by Datul et al. (2024). Samples were collected from multiple cave sites across the CALABARZON region of the Philippines spanning three distinct dietary guilds (insectivores, frugivores, and nectarivores). By integrating 16S rRNA gene

amplicon metagenomics, hierarchical clustering, and functional prediction, our objective was to provide a detailed overview of how host identity, diet, and geographic location interact to shape the bacterial communities within the bat gut.

A total of 8,621,053 individual reads were considered in this analysis, with an average sequencing depth of 76,973.69 ± 41,182.09 after denoising and chimera removal. Samples were not rarefied to an equal sequencing depth after preliminary analysis and it showed a minimal effect in terms of alpha and beta diversity (Supplementary Figure 1). This was also not done to retain rare taxa that might be removed by subsampling.

Key design considerations were implemented to enhance the robustness and interpretability of our findings—namely, (1) utilization of the Amplicon Sequence Variant (ASV)-based clustering over traditional Operational Taxonomic Unit (OTU)-based methods. ASV-based approaches employ error-modeling frameworks to correct for inherent sequencing errors in short-read amplicon sequencing pipelines, yielding nucleotide-resolved variants (Callahan et al., 2016). This method circumvents the arbitrary similarity thresholds that characterize OTU-based clustering, thereby recovering more accurate community profiles and reducing spurious taxa, as demonstrated by recent benchmarks (Fasolo et al., 2024).

Furthermore, ASVs facilitate direct cross-study comparisons due to consistent sequence labels across datasets, establishing a more robust and replicable reference for future bat-microbiome survey; and targeting the V3–V4 hypervariable region of the 16S rRNA gene for sequencing for optimal balance between taxonomic resolution, phylogenetic coverage, and sequencing costs (Castelino et al., 2017; Zhang Han et al., 2024). Its widespread adoption in microbial ecology studies is attributed to its compatibility with short-read sequencing platforms and its ability to capture a broad spectrum of bacterial lineages (Klindworth et al., 2013; Wasimuddin et al., 2020).

Certain caveats are acknowledged with regards to this approach: these include potential primer biases, challenges with inconsistent taxonomic mapping, and inherent resolution limits in specific bacterial lineages. Notably, current limitations of this methodology include the underrepresentation of archaeal clades (Fadeev et al., 2021; Wasimuddin et al., 2020) and limited taxonomic resolution at the genus and species levels for certain groups, such as members of the Enterobacteriaceae family (Greay et al., 2019; Popov et al., 2025). Nevertheless, this methodology provides a comprehensive and cost-effective map of the bacterial landscape of bat gut microbiomes, maximizing sampling breadth and prioritizing taxonomic coverage and comparability for future research.

Alpha Diversity Analysis Reflects Dietary Differences and Species-level Variation

Alpha diversity analysis showed differences in gut bacterial communities between dietary groups, bat species, and sampling locations. In this approach, we have used two indices—the Shannon diversity index (*H'*) and the Inverse Simpson index (1/D)—to obtain a thorough picture of the evenness and richness of each sample type (Figure 2).

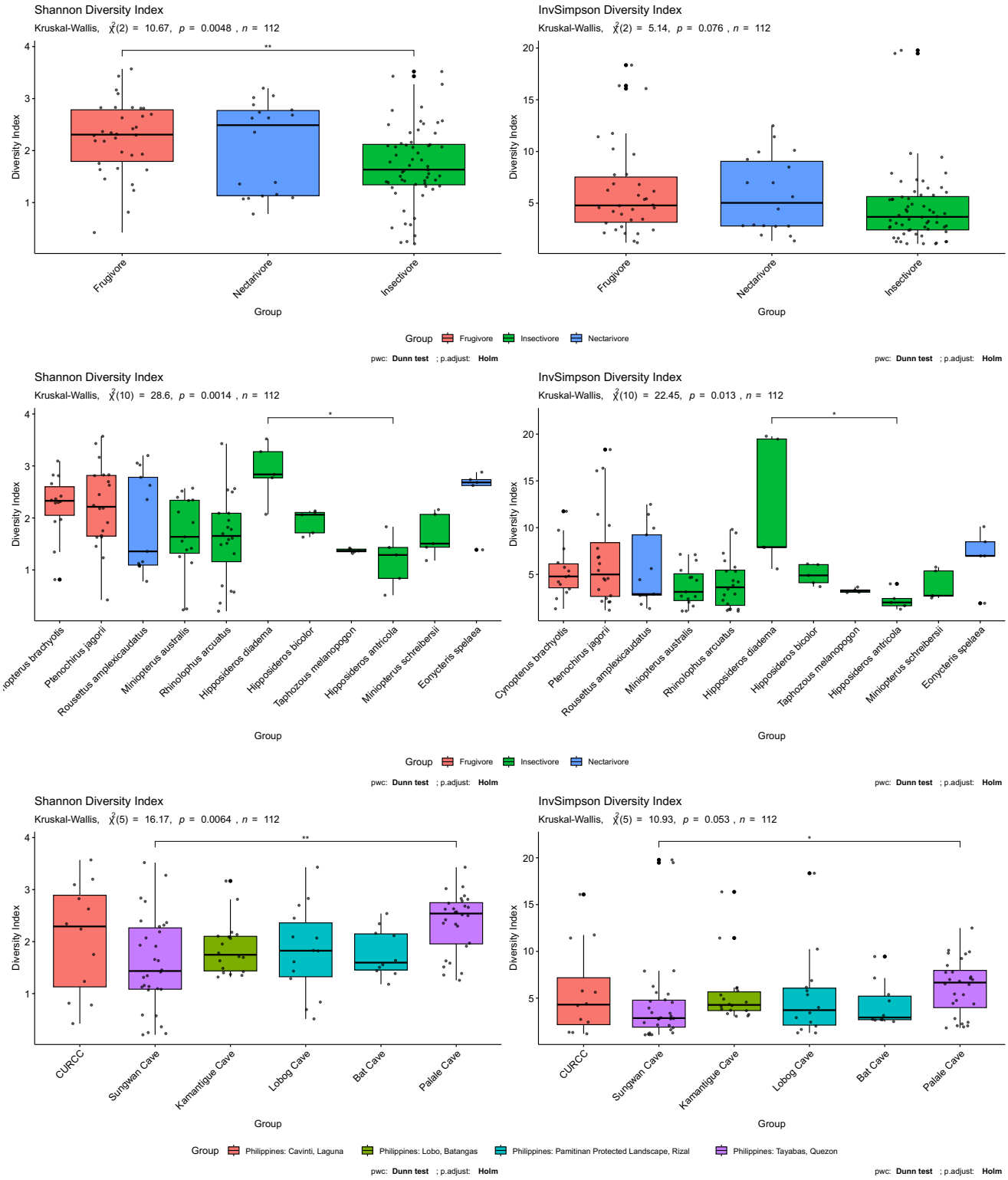

Figure 2: *Comparison of Alpha Diversity Indices (Shannon and Inverse Simpson Indices) Across Bat Dietary Guilds.* Boxplots illustrate the distribution of alpha diversity metrics, aggregated by diet (top), bat species (middle), and location (bottom). Global significance was assessed using the Kruskal-Wallis test, while pairwise significance was determined using Dunn's test. Significance levels are indicated as follows: $p \leq 0.05$ (*); $0.05 \leq p \leq 0.01$ (**).

Kruskal-Wallis tests showed that insectivorous bats had significantly lower Shannon diversity than frugivorous bats ($p<0.001$). However, their Inverse Simpson indices were not significantly different ($p>0.05$). Further comparisons between the two dietary guilds among the frugivores (Nectarivores and Typical Frugivores) show similar alpha diversity profiles for both Shannon and Inverse Simpson indices, indicating that both dietary guilds feature similarly diverse bacterial profiles. The difference between these two diversity measures suggests that diet may not strongly influence which bacterial species dominate the gut microbiome.

In terms of the bacterial diversity per bat species, we observed that one particular bat species (*H. diadema*) has both a significantly higher *H'* and 1/D diversity indices compared to the insectivorous sample with the lowest indices (*H. antricola*), implying that despite having a lower sample-wise diversity as a dietary guild, specific insectivores may show high bacterial

diversity.

This observation aligns with previous studies noting that the gut microflora of herbivores often harbors more specialized microbes, potentially leading to more enriched but less evenly distributed community structures (Phillips et al., 2012; Carrillo-Araujo et al., 2015). In contrast, other studies imply that insectivorous bats have more diverse microbial communities (Mena Canata et al., 2024). In this present study, frugivorous bats had higher Shannon diversity, meaning they had more bacterial species. This could have resulted from eating diverse plant foods and encountering different environmental bacteria while foraging across various ecosystems in the CALABARZON region. In contrast, the more consistent and protein-rich diet of insectivores might have been selected for a less diverse microbial community. The lack of significant difference in the Inverse Simpson index, which is more sensitive to dominant taxa, supports that, while richness differs, the most abundant microbial groups might be similarly represented across these dietary guilds.

Global Beta Diversity Analysis Demonstrates Bat Species as a Key Driver of Microbiome Composition

Beta diversity assessments were performed based on four factors—host diet, sampling location, host identity, and host phylogeny (at the suborder level). Global and stratified PERMANOVA analyses of the sequences revealed high similarities of bacterial communities across sample distributions.

A global approach was first done to assess the relative contributions of various ecological and host-related factors to gut microbiome variation (**Table 3**). All tested factors significantly influenced composition ($p > 0.001$) with varying effect sizes and dispersion patterns. Collectively, all factors described explained 57.4% of the total variance in microbial community composition, with 42.7% residual variance unaccounted for.

Table 3: Results of Global Permutational Analysis of Variance (PERMANOVA) and PERMDISP using bat.source, location, diet, and suborder as model terms (including interaction terms, indicated by *)

Term	Df	F	R^2	ω^2 (partial)	P-value (PERMANOVA)	P-value (PERMDISP)
Bat species	8	4.954	0.197	0.22	0.001***	0.001***
Dietary Guild	1	7.375	0.037	0.054	0.001***	0.376
Location	5	5.991	0.149	0.182	0.001***	0.072
Host suborder	1	4.503	0.022	0.03	0.001***	0.005**
Bat species*location	4	2.826	0.056	0.061	0.001***	
Diet*location	3	4.661	0.069	0.089	0.001***	
Suborder*Location	3	2.925	0.044	0.049	0.001***	
Total Variance			**0.574**			
Residual			**0.427**			

Bat species identity showed the strongest effect on microbiome composition ($R^2 = 0.197$, $p = 0.001$), indicating that host-specific traits are the primary drivers of microbial community structure. Significant PERMDISP results ($p=0.001$) indicate heterogeneous dispersion when comparing bat species, likely due to locational effects, as highlighted in **Figure 4B**.

Sampling location is the second most influential factor ($R^2 = 0.149$, $p = 0.001$). PERMDISP analysis shows insignificant dispersion differences between the sampled locations ($p = 0.072$) indicating that the variability of microbial community compositions remains consistent among sites and may represent actual differences rather than variance-related artifacts.

Surprisingly, compared to other factors, dietary guild (Insectivorous vs. Frugivorous) has a minor effect on microbiome composition of the bat individuals compared to the other tested factors ($R^2 = 0.037$, $p = 0.001$) with a homogenous dispersion across the two main groups ($p = 0.376$). This, in turn, suggests a substantial overlap in microbial communities despite differences in nutritional outputs, which is highlighted in **Figure 4A.**

Among the factors examined, host phylogeny at the suborder level exhibited the weakest influence on microbiome composition ($R^2 = 0.022$, $p = 0.001$), accompanied by significant differences in dispersion (PERMDISP $p = 0.005$). This dispersion heterogeneity suggests that one chiropteran suborder may possess significantly larger microbiome variance than the other, likely attributable to uneven sample distributions between the two suborders.

Our analysis revealed that, while interaction effects were statistically significant, their effect sizes were relatively low

compared to the main factors influencing microbiome composition. Specifically, the bat species × location interaction (*ω2= 0.061, PERMDISP p = 0.001*), diet × location interaction (*ω2= 0.089, PERMDISP p = 0.175*), and suborder × location interaction (*ω2= 0.049, PERMDISP p = 0.001*) all contributed to the observed community variation.

We noted that the R^2 and effect sizes obtained for the factors bat identity and geographic effects are consistent with previous literature (Carrillo-Araujo et al., 2015; Dai et al., 2024). However, the low effect size associated with diet was unexpected and reported and contrasts with earlier findings where diet was considered a significant driver of microbiome variation (Carrillo-Araujo et al., 2015; Dai et al., 2024). A substantial portion of variance remains unaccounted for (R^2 =0.47) but is comparable to the residual levels reported in previous studies (Carrillo-Araujo et al., 2015). Previous studies noted factors such as sex, seasonal variations, roosting habits, as well as captivity status as possible factors that may affect the composition of the gut microbiome (Carrillo-Araujo et al., 2015; Xiao et al., 2019; Lebeuf-Taylor et al., 2025) and may be considered for future characterization.

Given the significant interaction effects and PERMDISP results, we conducted stratified analyses to separate true compositional differences from variance artifacts and to isolate specific factor effects, highlighted in the following paragraphs.

Stratified Permutational Analysis Reveals Conservation and Divergence of Microbiomes across Locations and Bat Phylogeny

To further examine the phylogenetic influences at suborder-level and the interactions with diet, we performed a pairwise PERMANOVA testing using a synthetic *diet * suborder* interaction term (i.e. Yangochiroptera-Insectivore, Yinpterochiroptera-Frugivore) with community structure visualized using Non-Metric Dimensional Scaling (NMDS) ordination (Shown in **Figure 3A)**. This approach allowed us to disambiguate between specialized diet types, further refining our analysis. We identified several notable distribution patterns based on these two factors. Interestingly, samples from the two distinct lineages of insectivorous bats exhibited similar bacterial community profiles, while also partially segregating from samples associated with other dietary guilds.

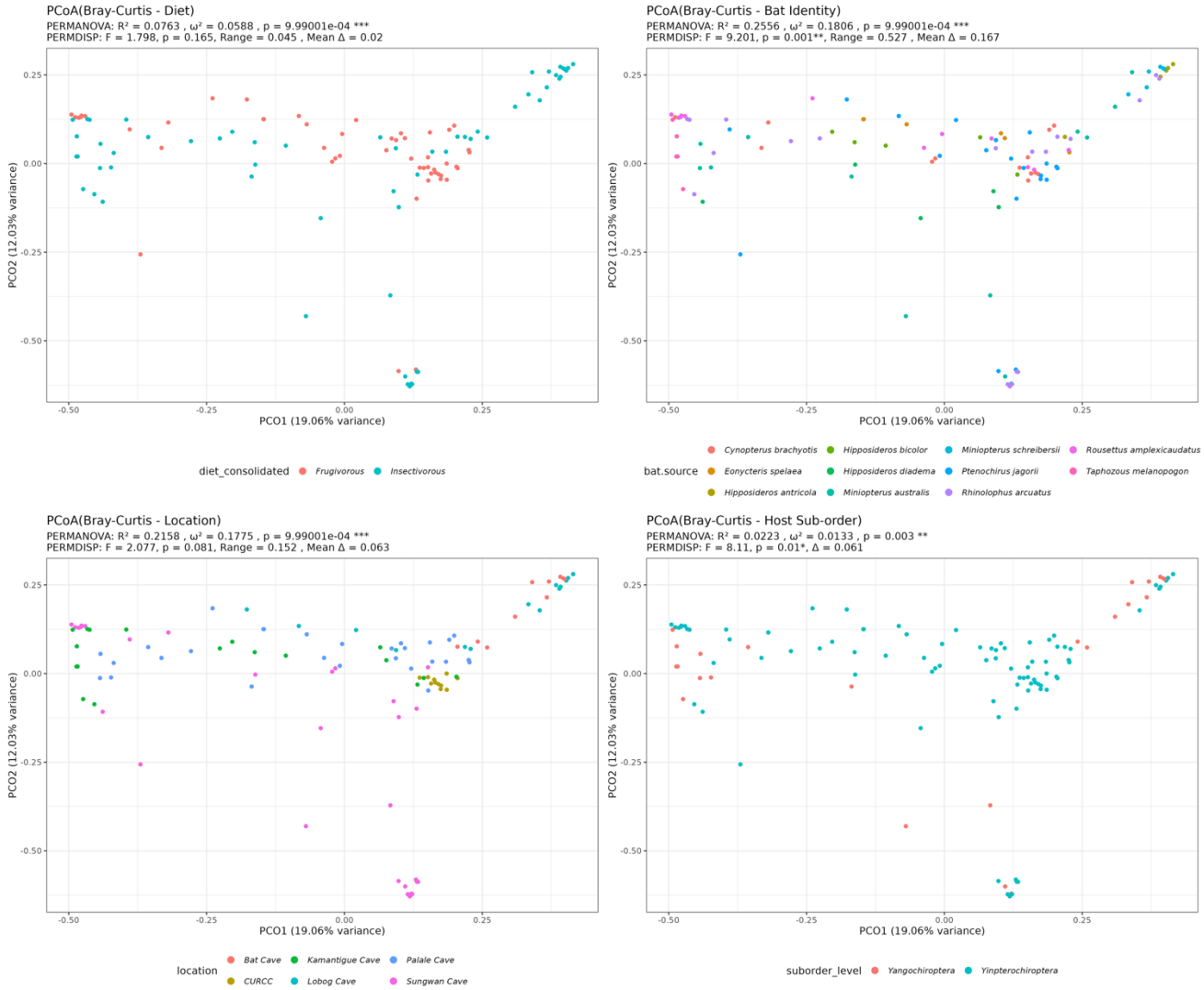

Figure 3: Principal Coordinate Analysis (PCoA) using the Bray-Curtis dissimilarity metric, showing ordinations annotated based on diet (**Fig. 4A**), species (**Fig. 4B**), bat suborder (**Fig 4C**), and location (**Fig 4D**) (clockwise from top left). Plots include the result for single-factor PERMANOVA (R^2, Effect Size (Omega^2) and p-values), PERMDISP (F value, P value, Range and Mean Centroid difference) using each indicated parameter as the model term (*dist ~ parameter*).

Pairwise PERMANOVA results, detailed in Table 4, strongly support a phylogenetic convergence pattern in the microbial communities. Notably, comparisons between Yangochiroptera-Insectivore and Yinpterochiroptera-Insectivore showed no

significant difference (R^2 = 0.025, p-adjusted = 0.762). This suggests a lack of a strong phylogenetic signal influencing the microbiome composition in these insectivorous bats, indicating that similar diets may lead to similar gut microbiomes regardless of host evolutionary lineage.

Table 4: Pairwise PERMANOVA Analysis using Suborder*diet as model term.

Comparison	R^2	p-value	P.adj (PERMANOVA)	PERMANOVA	PERMDISP (p-value)
Yinpterochiroptera-Frugivore vs Yinpterochiroptera-Nectarivore	0.066	0.001	0.006**	**	0.224 (ns.)
Yinpterochiroptera-Frugivore vs Yangochiroptera-Insectivore	0.075	0.001	0.006**	**	0.885 (ns.)
Yinpterochiroptera-Frugivore vs Yinpterochiroptera-Insectivore	0.059	0.001	0.006**	**	0.716 (ns.)
Yinpterochiroptera-Nectarivore vs Yangochiroptera-Insectivore	0.075	0.01	0.06 (n.s.)	n.s.	0.932 (ns.)
Yinpterochiroptera-Nectarivore vs Yinpterochiroptera-Insectivore	0.068	0.001	0.006**	**	0.127 (ns.)
Yangochiroptera-Insectivore vs Yinpterochiroptera-Insectivore	**0.025**	**0.127**	0.762 (n.s)	n.s.	0.917 (ns.)

Figure 4A (Left) and Figure 4B (Right). Beta Diversity Analysis showing mapping of community distributions using Non-Metric Dimensional Scaling (NMDS), showing the distributions of each suborder*diet pair. The right plot shows groupings according to Location. Both plots are annotated based on Bat Species identification as shapes.

Similarly, the comparison between Yinpterochiroptera-Nectarivore and Yangochiroptera-Insectivore was not statistically significant (R^2 = 0.075, p-adjusted = 0.06). While this might initially suggest further convergence, it is important to consider that uneven sample distributions could be a confounding factor, potentially masking subtle differences.

Further analysis, however, revealed clear dietary specialization within the Yinpterochiroptera suborder. Yinpterochiroptera-Frugivore communities were significantly different from all other dietary groups, including Yinpterochiroptera-Nectarivore (R^2 = 0.066, p-adjusted = 0.006). This highlights that even within the same phylogenetic lineage, dietary shifts can drive significant divergences in microbiome composition.

Finally, PERMDISP analysis consistently confirmed

homogeneous dispersions across all comparisons *(p 0.05)*. This is a crucial finding, as it validates the reliability of our PERMANOVA results, ensuring that observed differences are truly due to variations in community centroids rather than differences in within-group variability.

To assess whether the community compositions of each particular bat species converge at the location level or whether each composition is conserved regardless of location, we performed pairwise PERMANOVA using a synthetic interaction term *diet* * location. Our investigation was guided by two primary hypotheses regarding factors shaping bat gut microbiome composition. First, bats of the same species will exhibit similar gut microbiomes even across different geographic locations, implying that host physiology is a predominant factor in shaping microbiome structure. Second, bats co-roosting at the same location will possess similar gut microbiomes, suggesting that habitat, shared resources, or similar microbial exposures are key drivers of community composition.

To ensure robustness in our comparisons, only bat samples with a minimum of three individuals per site were included in this analysis. Following initial pairwise PERMANOVA analyses, we proceeded to assess which specific communities exhibited significantly different compositions from one another. A schematic diagram illustrating the comparisons is shown in **Figure 5. Figure 6** summarizes the results of comparison, showing the counts of non-significant vs. significant communities across species-level and location-level comparisons. The raw results of the pairwise permutational analysis are included in **Supplementary table 2.**

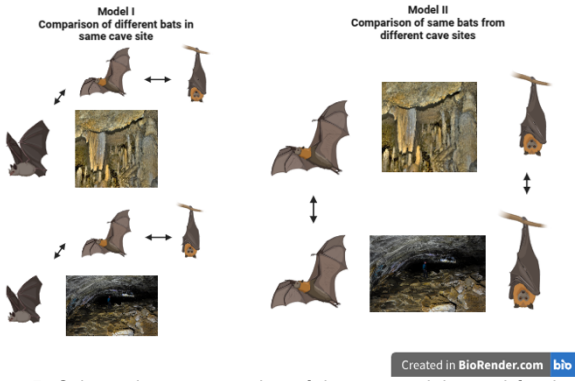

Figure 5: Schematic representation of the two models used for the Stratified PERMANOVA approach comparing bats within the same sampled site (Model I) and same bats from different cave sites (Model II). This schematic was created using Biorender using images from (*Harrison's Cave*, n.d.; *Lava River Cave*, n.d.)

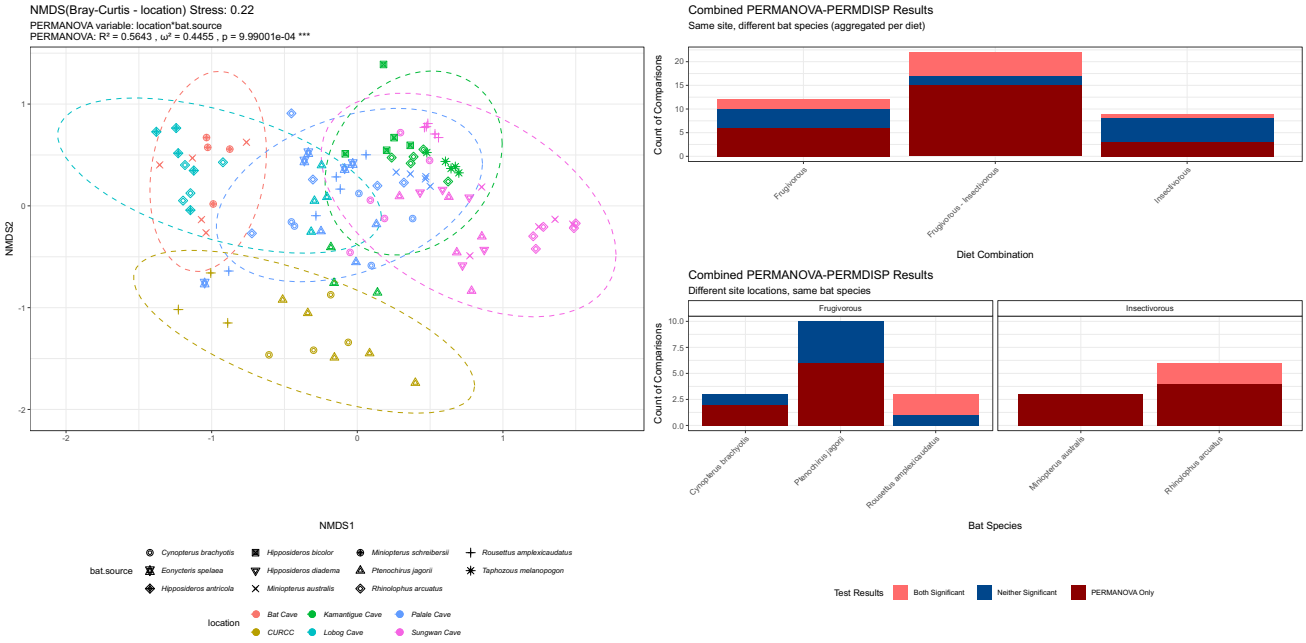

Figure 6: Summary of the Pairwise comparisons of Gut Bacterial Communities across bat species and locations. The plot on the left shows an Ordination Plot (NMDS) (**Figure 6A**), annotated based on the identity of bats and the locations where they were obtained. The panel on the right shows in particular the number of community-species pairs found to be significantly different per same site (Top right; **Figure 6B**) and per same bat species, controlling for different sampling locations (Bottom right; **Figure 6C**). Significance threshold based on adjusted (FDR-controlled) p-value of 0.05. Results are further annotated based on whether PERMDISP also returns a significant result (based also on *p.adj < 0.05*).

Stratified PERMANOVA analysis for the location*bat species term yielded several key insights. First, we observed a global R^2 of 0.577 for the location:bat.source interaction term, indicating that this combined term explains a substantial portion of the variance in the overall community distribution. This highlights the significant interplay between where a bat is found and the specific bat source in determining its microbiome.

Furthermore, we also identified distinct trends when examining comparisons across and within dietary guilds. On comparisons across different dietary guilds (e.g., Frugivore vs. Insectivore), we found a large fraction of significant differences. This suggests that dietary habits are a strong determinant of microbiome composition when comparing broadly diverse groups. Conversely, when comparisons were made within the same dietary guilds, at least 50% of the pairwise PERMANOVA comparisons showed insignificant differences (*p.adj > 0.05*). This implies that within specific dietary niches, the microbial communities tend to be more similar, particularly among Insectivorous niches.

Our analysis revealed patterns concerning location and host species. When comparing microbial communities across different sites while controlling for the same bat species, we observed that *P. jagori* had a significant of non-significant comparisons across sites (p.adj > 0.05). Suggesting that the *P. jagori* may harbor a more stable, host-driven community when compared to other bats. In contrast, we also observed a

significant location-driven variance across sites when counting the number of significant hits among members of the same species. This indicates that for many bat species, the environment of a particular location does indeed play a crucial role in shaping their gut microbiome. We noted however that several pairwise comparisons—such as with *R. amplexicaudatus* and *R. arctuatus*—show significant PERMDISP results ($p.adj < 0.05$), implying a possibility that the significant PERMANOVA results may arise due to differences in variances rather than "true" differences in microbial composition.

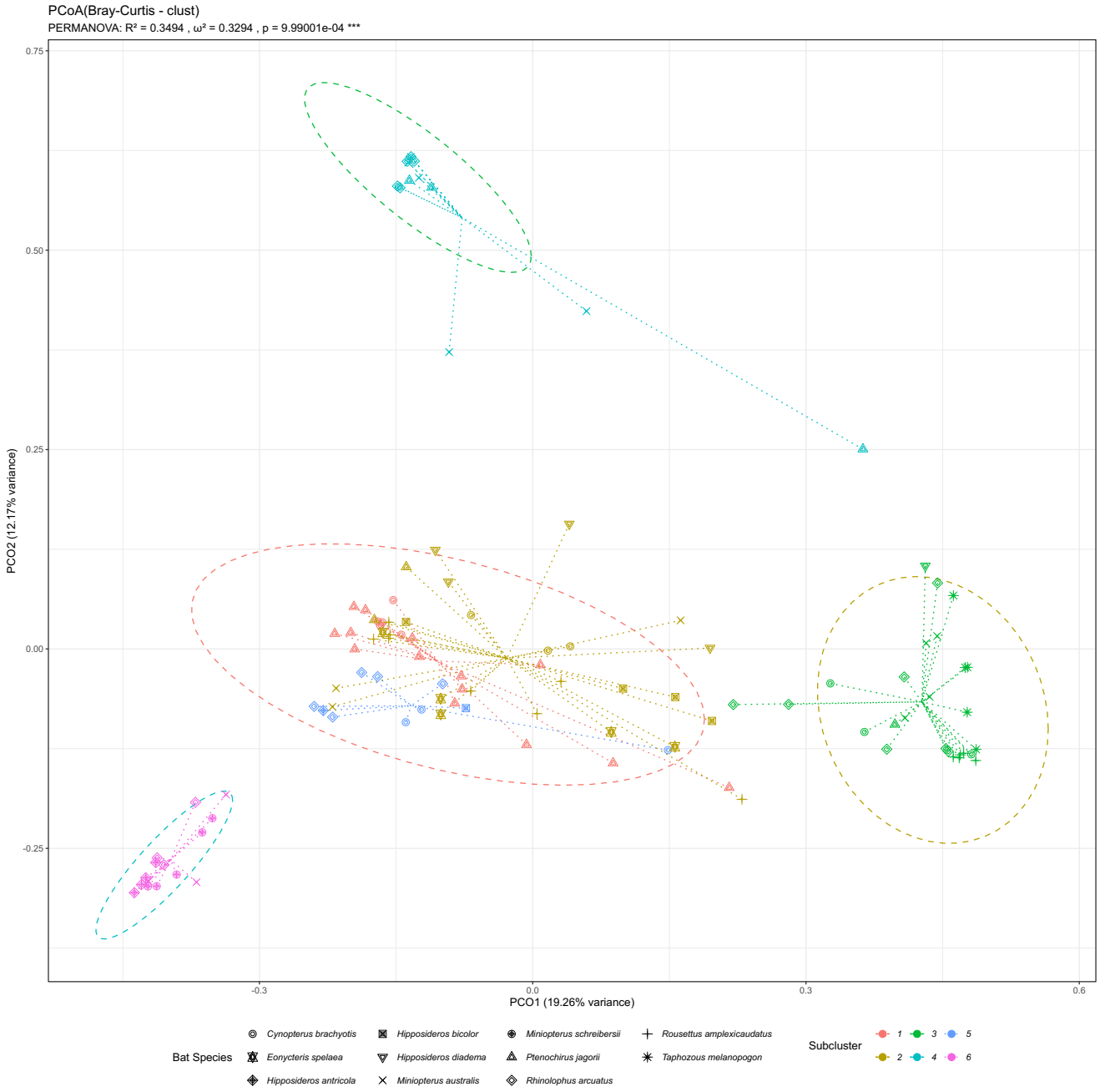

Figure 7: Shows an Ordination (Principal Coordinate Analysis) plot showing the results of hierarchical clustering analysis. The main clusters are highlighted as ellipses (95% confidence interval) while subclusters are indicated via spider graphs and colorations pointing to each sub-cluster centroid. Points are annotated based on bat species composition

Hierarchical Clustering Analysis Highlights Compositional Differences among Sampled Bats

The variation in microbial composition is brought by a confluence of multiple factors, including location, bat identity, and diet. A top-down approach was employed to better understand the composition of the microbial communities per sample. Classes Alphaproteobacteria and Gammaproteobacteria predominate alongside Campylobacteria and Bacilli **(Highlighted in Fig. 8A)**. One primary cluster consisting of multiple subclusters is shown to have lesser predominance of *Proteobacteria* while other clusters are predominated with *Bacilli* and *Campylobacteria*. At the Family level, we noted the significant presence of *Leptospirae* in both subclusters 1 and 3. Differentiation is highlighted in **Figure 8B,** where the two main clusters were primarily differentiated by the dominance of Gammaproteobacteria, while differentiation at lower clustering levels is apparent at the Family level with the difference in subclusters 4, 5, and 6 are defined by the families Enterobacteriaceae, Burkholderiaceae, and Xanthomonadaceae, respectively.

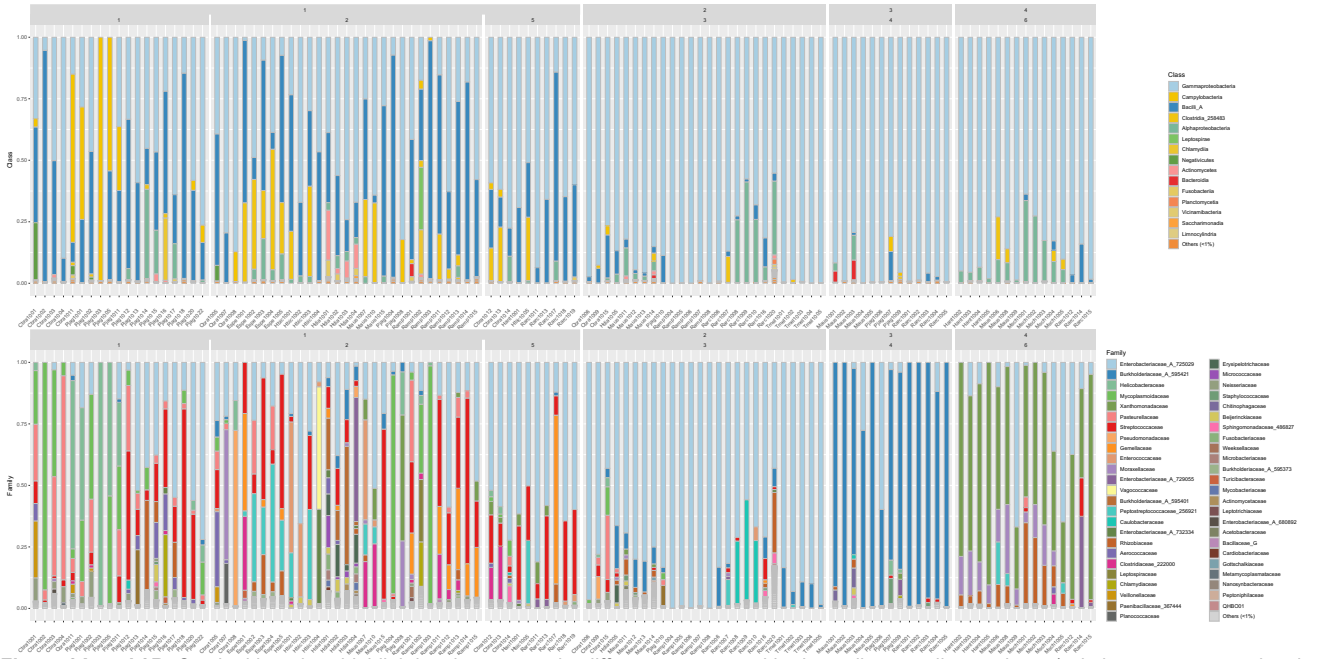

Figure 8A and 8B: Stacked bar chart highlighting the community differences arranged horizontally according to cluster/subcluster, aggregated at the Class (**Figure 8A**) and Family (**Figure 8B**) levels, respectively.

For the rest of the peripheral clusters 2, 3 and 4, it is shown to be predominantly composed of Gammaproteobacteria, but differ specifically in their respective compositions. Cluster 5, for instance, is seen to have a predominance of Helicobacteraceae (Gammaproteobacteria) while cluster 6 is seen to be composed of members of Xanthomonadaceae and Pasteurellaceae (**Figures 8B and 9**).

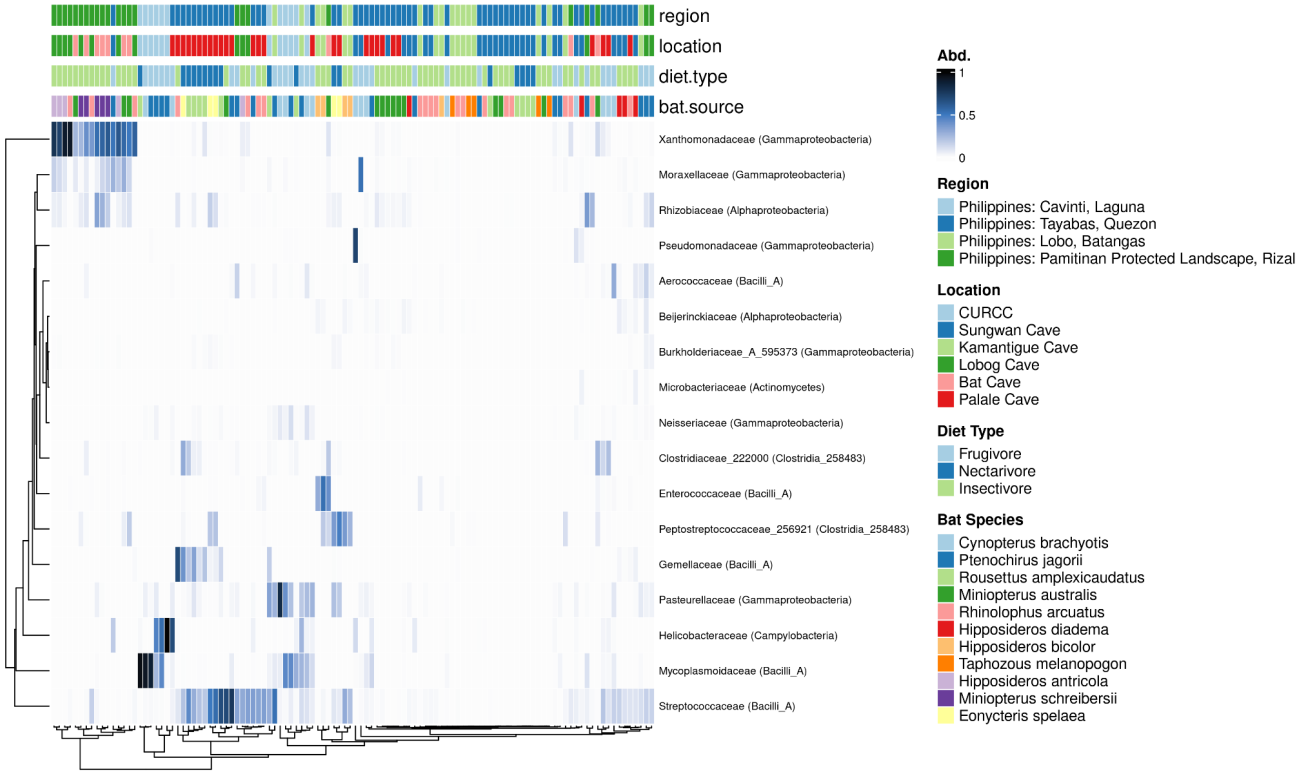

Figure 9: Heatmap indicating differentially abundant ASVs across all sampled bat species, merged at the Family Level. Differential abundance results were filtered based on the adjusted p-value threshold of was assessed using global ANCOMBC2, selecting only ASVs that have passed sensitivity analysis. Both Samples and taxa are ordered based on hierarchical clustering (Ward method). Samples (columns) were transformed to Relative Abundances prior to mapping and were annotated based on sampling region, location, diet type and bat.source.

Further analysis of hierarchical clustering among differentially abundant ASVs implies the following: first, while the Class Gammaproteobacteria was generally predominant across multiple samples, it was notably not identified as differentially abundant. Instead, the primary drivers of variation across all sites were the Classes Bacilli, Alphaproteobacteria, and Clostridia. Additionally, we observed specific associations between certain bacterial classes and bat dietary guilds. The Class Campylobacteria was predominantly found in frugivorous bats and showed a strong association with the frugivorous

species *P. jagori*; and Clostridia in insectivores and the nectarivore *E. spelaea*.

By analyzing more bacterial families were revealed that distinguished samples across species and locations. In particular, the family Burkholderiaceae (Class Gammaproteobacteria) was observed to be both primarily predominant among Insectivorous bats across multiple caves and bat species. In contrast, members of the Helicobacteraceae (Class Campylobacteria) and the Mycoplasmoidaceae (Class Bacilli) were observed occurring in frugivorous bats. Lastly, Streptococcacaeae (Class Bacilli) exists mainly among samples from Palale cave, Quezon regardless of the identity and dietary guild of bats.

We found several bacterial families that occurred together. Xanthomonadaceae appeared with other Gammaproteobacteria families like Moraxellaceae and Rhizobiaceae. Streptococcaceae occurred with Gemellaceae (both from class Bacilli). Another cluster, mainly composed of frugivorous bats from the Cavinti Cave site, is noted to have a cluster of pathogens, namely *Mycoplasmoidaceae*, *Helicobacteriaceae*, and *Neisseriaceae*. This, in turn, may indicate horizontal transmission of putative pathogens, and may raise further questions of the pathogenic potentials of such microbes among bat populations and may warrant further study to determine their actual pathogenicity and any other associated impacts.

In the wild, bats are also known to roost together in heterospecific groups, even between species of varying sizes and dietary guilds. Some of the noted benefits for co-roosting include protection from predation and thermoregulation, while in turn causing resource competition and the propagation of diseases and parasites. Such roosting behavior was observed to occur more between species of different diets, implying that members of differing dietary niches tend to roost together to minimize dietary competition, and as such may influence each other's microbiome profiles through proximity (Kelm et al., 2021).

Previous studies noted the convergence of microbiomes in captive bat populations both in the functional and taxonomic sense, owing primarily to both proximity and the influence of similarly given diets to the captive bats (Xiao et al., 2019). These factors, in turn, may explain the horizontal transmission of gut-associated microbes, where close physical contact or shared roost substrates may facilitate the exchange of bacteria.

Functional Analysis Reveals Concordance and Enrichment of Key Predicted Pathways in the Bat Gut Microbiome

In order to have an initial assessment of the functional landscape of the bat gut microbiome, we conducted functional prediction analysis using PICRUSt2 (Douglas et al., 2020). Our approach examined three particular aspects: first, we assessed the reliability of the functional predictions using the Nearest Sequence Taxon Index (NSTI) metric; second, we attempted to map the relationship between taxonomic and functional diversity by comparing the alpha diversities of taxonomic and functional loadings, and third, we attempted to identify dietary-guild specific functional enrichments using microbial ecology methods (alpha, beta diversity and differential abundance analysis). A summary map of all predicted functional loadings is represented in **Figure 12C.**

The validity of our functional predictions is further corroborated by assessing the weighted NSTI metric, as highlighted in **Figure 10**. Our assessment of NSTI scores revealed that most samples fell within the recommended threshold of 0.2, with no sample exceeding the maximum acceptable threshold of 2.0. While the typical weighted NSTI threshold in human microbiome studies averages around 0.03, indicating very high mapping to reference genomes, our results for bat microbiomes are still well within the range considered acceptable for reliable functional imputation (Castaño-Rodríguez et al., 2018; Parras-Moltó & Aguirre de Cárcer, 2020).

Afterwards, we assessed the relationship between bat gut microbial taxonomic diversity and predicted functional diversity by computing alpha diversity metrics on the PICRUSt2 output and obtaining the Spearman's ρ correlation across all sample types. We found a surprising negative correlation between bacterial species diversity and functional diversity using the KEGG database (Shannon $\rho = -0.12$; Inverse Simpson $\rho = -0.07$), highlighted in **Figure 11A**. This finding suggests a potential uncoupling between the diversity of bacterial communities and the diversity of their predicted functions when using the KO framework. Specifically, higher taxonomic diversity did not necessarily translate to a greater number of distinct metabolic functions. Comparisons with MetaCyc pathway outputs (**Figure 11B)** showed more concordant results, yielding positive Spearman correlations of $\rho = 0.55$ for the Shannon index and $\rho = 0.56$ for the Inverse Simpson index. This contrast between KO and MetaCyc results is notable.

These divergent correlations collectively lead us to infer that the diversity of bacterial communities may be, to some extent, uncoupled from the diversity of predicted functions. The negative correlation with KO pathways, particularly, suggests that a richer taxonomic community might not automatically correspond to a broader range of core metabolic capabilities (Louca et al., 2018). Instead, this could imply that a significant portion of functional diversity, especially for rare functions, may be contributed by a smaller number of rare bacterial taxa. It is also possible that the resolution and hierarchical structure of the KO database, versus MetaCyc, influence these observations.

Alpha diversity analysis of functional features using the Shannon diversity metric (H') indicates that insectivores have significantly higher (H') in terms of imputed KEGG features compared to MetaCyc **(Figure 12A and Figure 12B)**. In both cases, comparisons between the Nectarivores and Frugivores resulted in no significant differences (Wilcoxon test; $p>0.05$; Figures 12A-1 and 12B-1). This discrepancy reflects possible differences in pathway granularity between the two databases, where KEGG may capture a wider and broader range of functional pathways. Further analysis of ordinations shows high overlapping of features across dietary guilds.

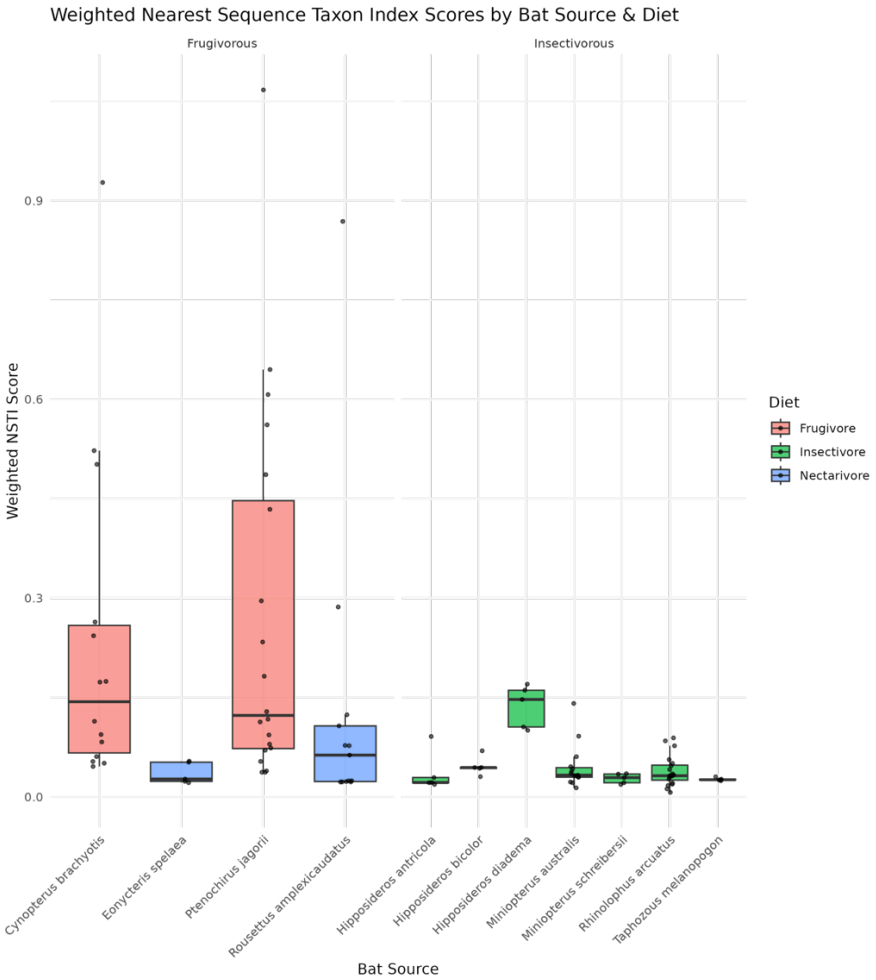

Figure 10: Nearest Sequence Taxon Index (NSTI) metric of each predicted bacterial sample, aggregated according to bat species.

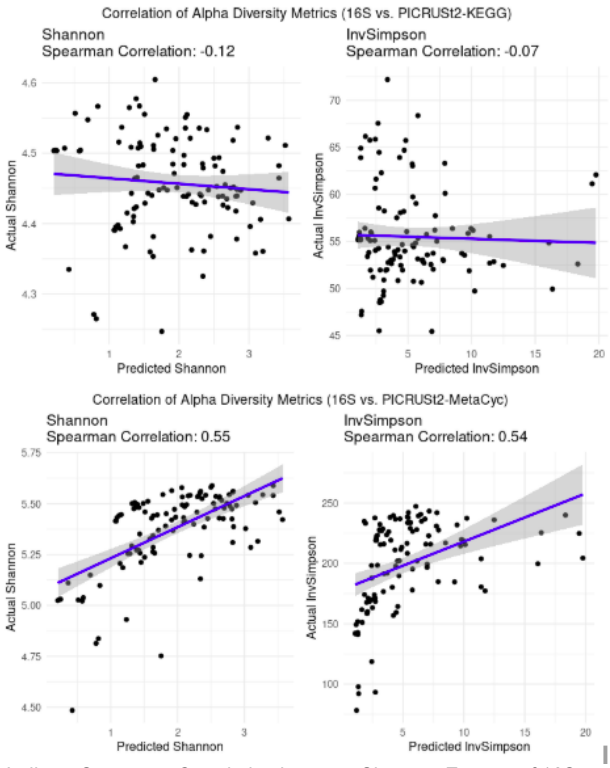

Figure 11: Correlation of Alpha Diversity Indices. Spearman Correlation between Shannon Entropy of 16S and Predicted Functional diversity of KEGG pathways (left) and Spearman Correlation between Inverse Simpson metric of 16S and Predicted Functional diversity of KEGG pathways (right) **Fig. 11A (Top row).** Spearman Correlation between Shannon Entropy of 16S and Predicted Functional diversity of MetaCyc annotations (left) and Spearman Correlation between Inverse Simpson metric of 16S and Predicted Functional diversity of MetaCyc annotations (right) **Fig. 11B (Bottom row).**

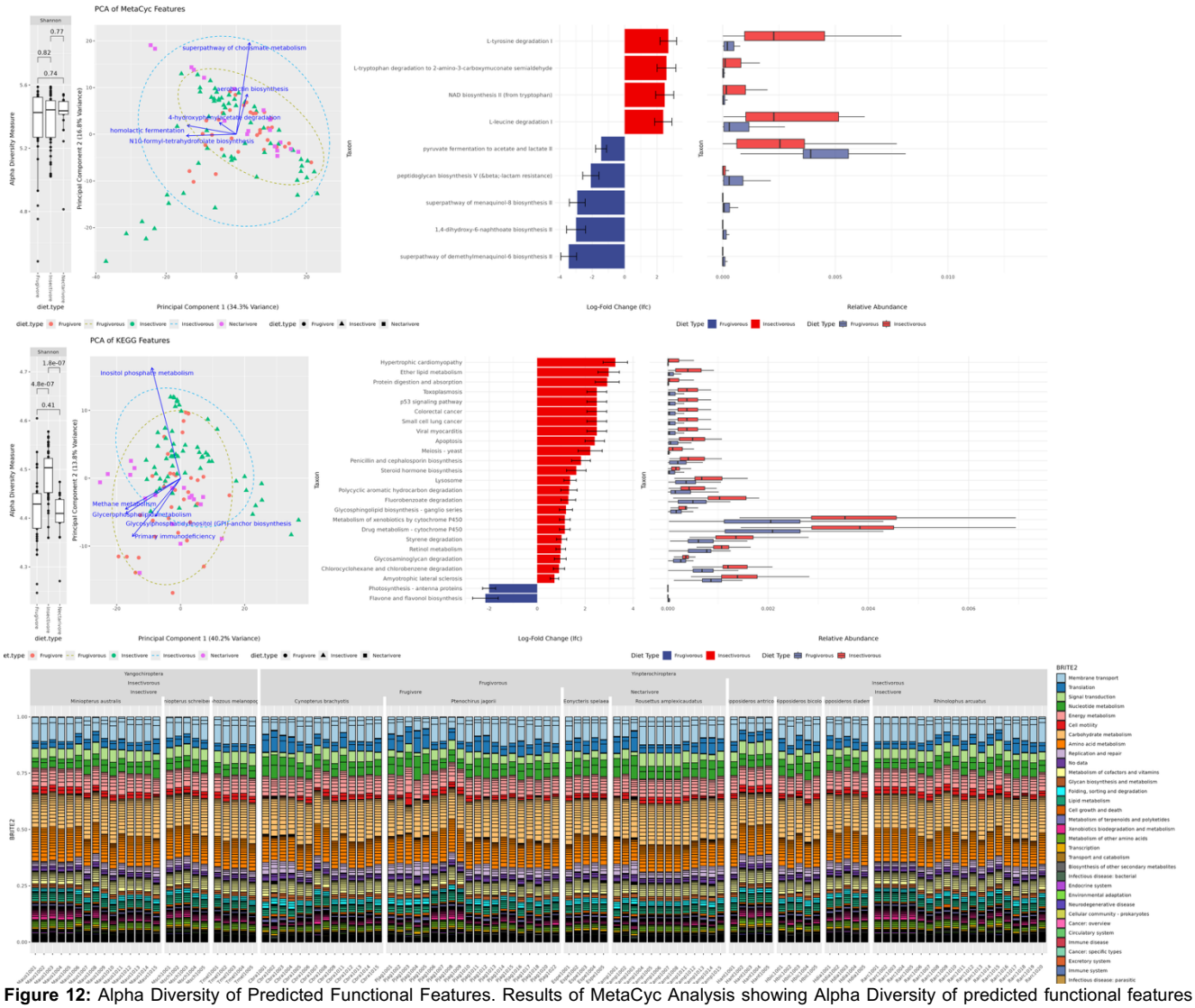

Figure 12: Alpha Diversity of Predicted Functional Features. Results of MetaCyc Analysis showing Alpha Diversity of predicted functional features (left; 12A-1), Beta diversity (Middle; 12A-2) and Differential abundance Analysis (right; 12A-3) **Fig 12A (Top row).** Results of KEGG Analysis showing Alpha Diversity of predicted functional features (left; 12B-1), Beta diversity (Middle; 12B-2) and Differential abundance Analysis (right; 12B-3) **Fig 12B (Middle row).** Relative abundances of each imputed KEGG functional profiles aggregate using each respective BRITE category **Fig 12C (Bottom row).**

Beta diversity analysis revealed a high degree of functional overlap across the bacterial communities, with only a few key differentially abundant features distinguishing them. This suggests that microbial communities, despite their taxonomic diversity, maintain a stable set of core functional traits largely independent of host physiology or dietary guilds.

A number of Metacyc and KEGG pathways have been detected to be differentially abundant across samples. Most notably, a number of pathways relating to amino acid catabolism such as L-tyrosine degradation I, L-leucine degradation, L-tryptophan degradation and metabolism is noted to be higher in guts of insectivorous bat, while a number of pathways relating to menaquinol metabolism were revealed in frugivorous bats. Most notably, the biosynthesis pathway of peptidoglycan (contributing to beta-lactam resistance) was predicted to be enriched in frugivorous bats. *Ptenochirus jagori* primarily contributed to this enrichment compared to other frugivorous samples. Lastly, most enriched features in frugivores, with the exceptions of peptidoglycan biosynthesis and pyruvate fermentation, represented only a small fraction of the total imputed features, suggesting their marginal presence in the frugivore gut microbiome (**Figure 12B-3).**

We also attempted to correlate the alpha diversity metrics of both taxonomic and functional profiles and observed a divergence in terms of their coefficients of determination (R^2), despite expecting a modest concordance between taxonomic and functional diversity. This may indicate that rare taxa may contribute disproportionately towards certain metabolic capabilities, or that multiple lineages of the same family converge towards similar functional profiles, consistent with (Phillips et al., 2012).

Functional imputation using PICRUSt2 indicates that, despite taxonomic variability, many of the core pathways show conservation across bat gut communities. During our analysis, we noted only a number of differentially abundant pathways and were primarily enriched across insectivores in terms of relative pathway quantity. In particular, Insectivorous bats show enrichment in pathways related towards amino acid catabolism while frugivores show higher menaquinol metabolism and peptidoglycan biosynthesis, the latter of which may potentially confer resistance against beta-lactamases and hinting at the potential for antibiotic resistance elements to persist among frugivorous bat microbiome populations.

Present Limitations and Future Directions

While our study provides valuable insights into the bat gut microbiome, it is important to acknowledge several inherent limitations that warrant consideration when interpreting our findings and for guiding future research.

First, the nature of our analysis is constrained by the 16S rRNA gene amplicon sequencing framework. Although sufficient for providing baseline information on microbial distribution and diversity, this methodology offers limited resolution for species and strain-level characterization. Consequently, finer distinctions within bacterial lineages, particularly relevant for understanding host-microbe interactions or pathogen dynamics, could not be fully resolved.

Additionally, despite employing stringent sterile techniques throughout sample collection and processing, the absence of negative extraction and PCR controls represents a methodological limitation. This could potentially affect the confident interpretation of low-abundance taxa, as it precludes a definitive assessment of background contamination, especially for rare community members. This study addresses this in part by focusing primarily on highly abundant taxa and filtering low abundance ASVs prior to downstream analysis.

Our study utilized functional imputation techniques to infer the metabolic potential of the bat gut microbiome. While highly appropriate given the context of the mammalian gut microbiome and supported by relatively high mapping to reference genomes as indicated by the Nearest Sequence Taxon Index (NSTI), these predictions may not fully reflect the actual metabolic activities or functional potential *in vivo*. Future studies employing shotgun metagenomics would be crucial for validating these predicted pathways and providing a more refined understanding of the precise functional capabilities of the bat gut microbiome.

Furthermore, limitations in sampling sizes, constrained to a maximum of five individuals per location due to regulatory permit requirements, may have limited the statistical power of subsample-level comparisons. To overcome this, we strongly recommend conducting longitudinal sampling across multiple seasons or diverse roost types in future investigations. This would allow for a more robust assessment of temporal dynamics and fine-scale environmental influences.

Despite successfully identifying several major determinants of community variation, a sizable portion of the total microbial community variance remains unexplained. This suggests the influence of other factors unaccounted for in the current analysis. Future research should consider incorporating additional environmental data, such as cave microclimate, roosting density, or specific dietary item analyses, to capture these unaddressed variables.

Lastly, our imputation of antibiotic resistance traits and the indication of certain bacterial families containing potential pathogens, particularly within frugivorous bats, highlight an important area for targeted research. Isolation and comprehensive genomic characterization of these specific bacterial strains are recommended to elucidate their exact characteristics, including their pathogenic potential and co-occurrence patterns with other microbial members.

CONCLUSION

Our study provides a detailed account of the microbial communities of the bat gut specimens across multiple cave sites from the CALABARZON region of Luzon, Philippines. By integrating 16S rRNA amplicon sequencing, microbial ecology and functional prediction, we demonstrated a number of key insights. Insectivorous bats generally exhibit lower Shannon diversity than frugivorous bat microbiomes. Taxonomic compositions of the bat microbiome indicate two distinct clusters, with the first showing the predominance of Gammaproteobacteria while the second cluster having a more diverse microbiome, composed of various classes, including Bacilli, Campylobacteria and Clostridia. Furthermore, we observed a strong location-specific influence on community composition, which may reflect local environmental conditions and roosting tendencies. Most bat species inhabiting the same cave with similar diets share similar gut microbiota compositions, implying the possible effect of co-roosting in shaping the gut microbial composition. We also performed stratified PERMANOVA analysis for each cave and bat species. This showed clear differences between dietary groups, but fewer differences within the same dietary group. Lastly, functional analysis implies that, although insectivorous bats feature a lower taxonomic diversity, they feature more diverse functional features as opposed to frugivorous bats, with the former featuring enriched pathways specific for amino acid metabolism while frugivorous bats mainly show enrichment in terms of peptidoglycan biosynthesis and pyruvate fermentation.

This is the first comprehensive report of the Philippine bat gut microbiome that compares bats across multiple cave sites and population parameters. The authors recommend additional research into other potential ecological drivers of microbiome diversity and to further study the role of behavioral factors such as co-roosting and social interactions in the horizontal transmission of gut flora among different bat species.

ETHICS STATEMENT

All protocols used for handling all live specimens were approved by the University of the Philippines Los Baños Institutional Animal Care and Use Committee (IACUC) with Approval Reference No. UPLB-2021-027. Furthermore, all sample collections pertinent to each site were approved by the Department of Environment and Natural Resources (DENR) Region IV Office (Calamba, Laguna) with the following Wildlife Gratuitous Permits (No. R4A-WGP-2021-LAG-004; R4A-WGP-2021-QUE-005; R4A-WGP-2021-BAT-006; R4A-WGP-2023-QUE-004; R4A-WGP-2021-RIZ-010).

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DATA AVAILABILITY

All raw 16S rRNA sequences used for this analysis are available via the Bioproject Accession no. PRJNA1096155. All figures and supplementary data indicated in this manuscript can be found in 10.6084/m9.figshare.29533766

CONFLICT OF INTEREST

The authors declare no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

R.T.S. was involved in the methodology, software, formal analysis, data curation, writing original draft, review and editing; R.J.D. F. in the conceptualization, methodology, writing original draft, review and editing; A.D.M. in the conceptualization, methodology, formal analysis, data curation, writing original draft, review and editing, and M.P.L. in the conceptualization, methodology, resources, writing original draft, review and editing, project administration and funding acquisition.

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SUPPLEMENTARY FILES*

Supplementary File 1: Results of Rarefaction validation analysis and correlation analysis between Alpha and Beta diversity metrics

Supplementary File 2: Tabular Results of the Pairwise Permutational Analysis of Variance (PERMANOVA) across all sites

Supplementary File 3: Raw Phyloseq File used for the Analysis of results above

*These files may be retrieved from https://shorturl.at/XoyVC