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Gut Microbiota Composition of Wild Birds in Different Habitats in Suai Niah, Miri, Sarawak

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ABSTRACT

Birds host diverse communities of micro-organisms in their guts that play a crucial role in providing nutrition and protection from pathogens. Various studies have reported on the influence of habitat factors that shape the gut microbiome of wild birds. However, there have been limited studies conducted in Sarawak. In this study, we collected the gut composition of wild birds from different habitats at Suai Niah, Miri. Fifteen faeces samples from various bird species were gathered from three distinct habitats: a secondary forest, an oil palm plantation, and a village. The faeces samples were analysed using the V3-V4 region of the 16S rRNA molecule as a microbial fingerprint, and metagenomic analyses were performed with microbial ecology packages in QIIME to characterise the gut microbiota in wild birds. Metagenomic analysis identified four primary bacterial phyla: Firmicutes (59.23%), Bacteroidetes (17.98%), Proteobacteria (14.06%), and Actinobacteria (3.71%), collectively accounting for the majority of the total relative abundance across all samples. This study highlights habitat as a key factor shaping the gut microbiome of wild birds. Gut microbes influence host nutrition, immunity, and adaptation, while birds may act as pathogen reservoirs affecting other species. Microbiome studies in wild birds are therefore important for understanding health, disease risk, and the ecological impacts of environmental changes, with broader implications for conservation and biodiversity. The information gathered from this study can inform future research and enhance our understanding of the gut microbiome of birds in Borneo.

Keywords: Faeces, gut microbiota, habitat, wild birds

INTRODUCTION

Birds are regarded as the ideal taxa for investigating the relationship between microorganisms and host animals, representing a diverse and evolutionarily successful lineage with over 11,200 species currently existing worldwide (Gill *et al.*, 2025). Furthermore, they serve as important indicators of forest ecosystem health due to their high mobility and unique capacity for flight, facilitated by their feathers and wings (Moreno *et al.*, 2007). Beyond their ecological importance, wild birds can also transmit diseases, acting as zoonotic vectors. For instance, species like wild birds can carry human enteric pathogens such as *Salmonella*, Avian Influenza Virus (AIV), *Escherichia coli*, Campylobacter, and *Yersinia enterocolitica*, posing a potential risk to nearby communities (Mancini *et al.*, 2020; Malekian *et al.*, 2021).

Several factors affect the composition of gut microbiota in birds. These include environmental factors such as diet, which reflects food type and availability, and habitat (Keenan *et al.*, 2013; Phillips *et al.*, 2018; Youngblut *et al.*, 2019). For example, studies on wild and captive white-tailed eagles utilizing next-generation sequencing (NGS) of 16S rRNA gene reveal variations in the avian microbiome linked to their dietary patterns (Ogasawara *et al.*, 2023). Other contributing factors involve climate change (Schmiedova *et al.*, 2023) and levels of pollution (Fackelmann *et al.*, 2023; Li *et al.*, 2023), which also shape birds' gut microbiota profiles. Additionally, host-related aspects such as age, sex, and genetics significantly influence a bird's gut microbiota (Liu *et al.*, 2020; Gongora *et al.*, 2021; Li *et al.*, 2021). For instance, research into the gut microbiome of domestic chickens indicates significant differences in bacterial communities between their ilea and caeca as they age (Mohd

Shaufi *et al.*, 2015). However, there is likely a complex interaction between microbial communities, environmental influences, and host traits, leading to a dynamic and adaptable microbiome critical for avian health (Grond *et al.*, 2018).

Many studies on wild bird's gut microbiota have been conducted in other parts of the world over the past few decades (Grond *et al.*, 2018). However, in Malaysia, there is still a lack of data on the microbiota in wild birds because most of the studies have been conducted on domestic and livestock birds, which only focus on certain bacteria that are potential pathogens (Matheen *et al.*, 2022; Mohamed *et al.*, 2022; Rasmussen & Chua, 2023). Hence, it is a good start to conduct the study on gut microbiota from wild bird species in Malaysia Borneo, specifically Sarawak. This study focuses on whether the environmental factor is the main factor that influences the gut composition of Bornean wild birds. Hence, we specifically examine different habitats as a parameter of the main environmental factor. This effort will advance our understanding of microbial diversity associated with wildlife as well as its potential implications for their health and conservation and how it relates to the role of zoonotic pathogens in wild birds.

MATERIALS ANS METHODS

Sample Collection

The sampling was conducted at Wilmar oil palm plantation, Suai Niah Miri (N03°33′36.″, E113°48′27.99″). The oil palm plantation belongs to Wilmar International Ltd. The company also has a mill, Saremas Sdn Bhd, located in the same area. The sampling sites primarily encompassed three distinct habitats: a secondary forest, an oil palm plantation, and a village area that includes the human settlement of Rantau's Longhouse and its farms. Two weeks of sampling were needed to cover all three habitats (see Table 1 and Figure 1). There are 15 mist nets at each site, set up along the potential birds' flyways. The mist nets were opened from 0600 to 1770 hours, and regular checkups were conducted for 1 or 2 hours. This was to avoid casualties among captured birds and to minimise stress induced by mist netting. Each captured bird was placed in cloth bags, weighed, banded, and identified. Then, faeces samples were collected from the captured wild birds.

Table 1. The sampling site with coordinated locations. The sampling sites, each different habitat, cover two (2) sites

Site	Habitat	Coordinate
Wilmar Oil Palm Plantation, Suai Niah, Miri	Secondary Forest	N03°27′24.7″, E113°48′24.9″
		N03°27′28.0″, E113°39′44.6″
	Oil Palm Plantation	N03°28′31.7″, E113°47′04.5″
		N03°31′14.7″, E113°45′08.3″
	Village	N03°30′31.9″, E119°46′15.1″
		N03°30′41.1″, E113°46′01.5″

The protocol developed by Knutie and Gotanda (2018) for collecting faeces samples from live wild birds was used and proved particularly suitable for field conditions. The materials used to construct the collection bag included paper lunch bags with a flat bottom, hardware cloth fencing, plastic weigh boats, scissors, and clothespins. The weigh boats and hardware cloth fencing were fitted inside the paper bag. The hardware cloth fencing was used to prevent birds from stepping in the faeces, thereby reducing potential contamination of feral samples. The collection materials were sterilised between uses to minimise the risk of cross-contamination. A 10% bleach solution was stored in a spray bottle and used to sterilise the tray and grate. The collection materials were ensured to be dry before reuse to prevent bleach from degrading the DNA in the samples. To facilitate field collection, the collection bag was placed inside a cloth bag, with a hole cut in the paper bag for air. Each paper bag was stored in a separate cloth bag. During collection, gloves were worn to prevent transfer of human-associated microbes into the bag and samples. The faeces were initially placed in a cold box with ice packs, then transferred to a -80 °C freezer for long-term storage pending DNA extraction.

From the 32 individual birds that were caught, only 15 wild birds were selected for metagenomic analysis (Table 4). In this study, birds were randomly captured at each site, reflecting the natural diversity of species present in the habitat. Then, individuals were selected based on their diets. Although diet was not the primary focus of the study, it allowed us to assess whether observed differences in gut microbiota were driven more by habitat or by host dietary factors. This approach strengthens the reliability of our findings by helping to separate habitat influence from host-specific traits.

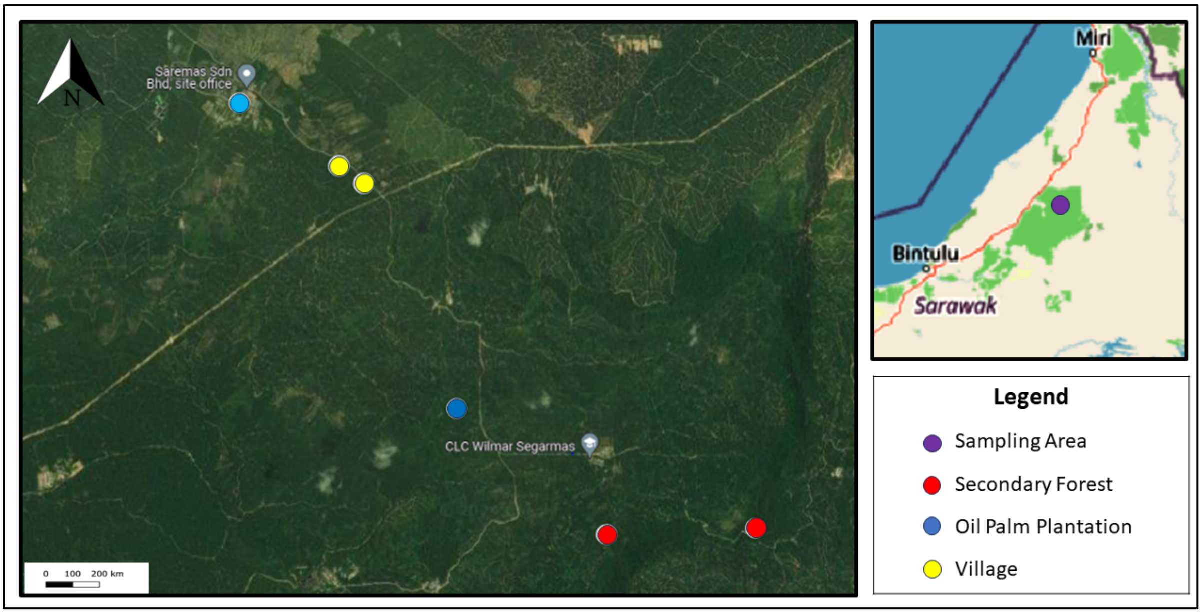

Figure 1. Map showing the location of the sampling site in the Suai Niah, Miri. The sampling site is about 48 km from Niah town. Saremas Sdn. Bhd belongs to Wilmar International Ltd

DNA Extraction and Polymerase Chain Reaction (PCR)

Genomic DNA (gDNA) extractions from faeces samples were performed following the NucleoSpin DNA Stool Kit (Macherey-Nagel, Germany) at the Molecular Ecology Laboratory of the University of Malaysia Sarawak (UNIMAS). However, the methods in the protocol (Nagel, 2023) are not entirely suited to faeces samples from wild birds; hence, the protocol has been adjusted (Gawin *et al*., 2025) to better align with the sample requirements for metagenomic analysis. The methodology incorporated combinatorial primers and extensive multiplexing of PCR amplicons, as per the approach elucidated by Jalali *et al.* (2017), in preparation for high-throughput sequencing on an Illumina Hi-Seq.

The quality of the purified gDNA was assessed using 1.7% TAE agarose gel electrophoresis. Its concentration was measured with a spectrophotometer (Implen Nanophotometer® N60/N50) and fluorometrically quantified using the iQuant™ Broad Range dsDNA Quantification Kit. Table 4 presents the gDNA concentrations for 15 bird samples, which ranged from 3.25 to 42.20 ng/µl. Despite some samples having lower quality, all were suitable for 16S rRNA gene amplicon library construction, although with some risk. Several samples with concentrations as low as 5 ng/µl successfully passed visual quality checks and amplified during PCR. No noticeable decline in microbial diversity or sequencing output was observed in samples with lower gDNA concentrations, indicating that DNA quality did not significantly impact the analysis of gut microbiota diversity and composition.

The 16S rRNA V3-V4 hypervariable region was amplified using primers 341F and 805R (Table 2), as described by Klindworth *et al.* (2013). The amplification of the 15 avian gDNA templates was carried out using REDiant II X

PCR Master Mix (1st Base, Singapore), ensuring high-fidelity profiling of the bird gut microbiota. PCR products were visualised on a 1.7% TAE agarose gel (100V, 56 minutes), producing discrete bands of approximately 500 bp across all samples.

Table 4. Order, family, common name and scientific name, and sampling habitat of each selected individual. All 15 individuals are from three (3) orders and 11 families and 14 species

Order	Family	Common Name	Scientific Name	Habitat	DNA Concentration (ng/µl)
Columbiformes	Columbidae	Zebra Dove	*Geopelia striata*	3	42.20
		Spotted Dove	*Streptopelia chinensis*	2	3.90
Coraciiformes	Alcedinidae	Rufous-backed Kingfisher	*Ceyx rufidorsa*	1	6.45
Gruiformes	Rallidae	White-breasted Waterhen	*Amaurornis phoenicurus*	3	3.80
Passeriformes	Acrocephalidae	Oriental reed Warbler	*Acrocephalus orientalis*	2	4.35
	Artamidae	White-breasted Woodswallow	*Artamus leucorynchus*	2	20.80
	Dicruridae	Bronzed Drongo	*Dicrurus aeneus*	1	3.85
	Dicruridae	Crow-billed Drongo	*Dicrurus annectens*	1	12.25
	Muscicapidae	Oriental Magpie-Robin	*Copsychus saularis_1*	2	7.30
	Muscicapidae	Oriental Magpie-Robin	*Copsychus saularis_2*	3	7.30
	Nectarinidae	Red-throated Sunbird	*Anthreptes rhodolaemus*	3	10.05
	Nectarinidae	Little Spiderhunter	*Arachnothera longirostra*	1	7.20
	Passeridae	Eurasin Tree Sparrow	*Passer montanus*	3	4.65
	Pityriaseidae	Long-tailed Shrike	*Lanius schach*	2	3.25
	Phylloscopidae	Gray-headed Babbler	*Stachyris poliocephala*	1	4.975

Secondary forest = 1; Oil palm plantation = 2; Village = 3

NGS Library Preparation and Sequencing

The amplicon library was constructed using a two-step PCR protocol. In the first stage of PCR amplification, the V3-V4 region of the 16S rRNA gene was amplified using the gene-specific primers 341F and 805R (Table 2). Subsequently, a second round of PCR was performed using the same primers modified with Illumina overhang adapter sequences (Table 3). This step was performed using KOD-Multi & Epi-® polymerase (Toyobo, Japan). Dual indices and Illumina sequencing adapters were then added using the Nextera XT Index Kit v2 (Illumina, USA) following the manufacturer's protocol.

The quality and size distribution of the final libraries were assessed using the Agilent 2100 Bioanalyzer System (Agilent Technologies, USA) with the Agilent DNA 1000 Kit. Library concentrations were quantified using Helixyte Green™ Quantifying Reagent (AAT Bioquest, USA). The libraries were normalised, pooled and sequenced on the Illumina MiSeq platform using 300 bp paired-end reads (2 × 300 PE). This enables high-resolution analysis of the microbial communities across the faeces samples.

Table 2. Primers used for encoding the V3-V4 region of 16S rDNA

Direction	Primer
Forward (341F)	5'-CCTACGGGNGGCWGCAG-3'
Reverse (805R)	5'-GACTACHVILLAGEGGGTATCTAATCC-3'

Table 3. Primers modified with Illumina overhang adapter sequences used for the second round of PCR

Direction	Primer
Forward	5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG–[341F] 3'
Reverse	5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG–[805R]–3'

Bioinformatics Analysis

QIIME (Qiime2 v_Amplicon 2023.9) was used in this project because it provides a comprehensive suite of tools for analysing microbial communities (Caporaso *et al.*, 2010; Bolyen *et al.*, 2019). Fifteen FASTQ files of the 15 birds' samples were analysed in the virtual computer hosting the QIIME2 pipeline. Cut-adapt has been used for demultiplexing and primer trimming of the raw paired-end reads to remove adapters and low-quality sequences. To denoise Amplicon Sequence Variants (ASVs), the sequence reads were processed using the q2-dada2 plugin in the QIIME 2 platform, which integrates the core functionalities of the DADA2 algorithm (Callahan *et al.*, 2016) to ensure accurate error correction and high-resolution unique sequence identification. Then, the taxonomic assignment of the ASV was performed using the q2-feature-classifier (Bokulich *et al.*, 2018) trained on the SILVA 16S rRNA database (132) (Quast *et al.*, 2013; Henderson *et al.*, 2019). The ASV and taxonomic classification tables were exported from QIIME2 using QIIME2 tools in tab-separated values (TSV) format.

Additionally, an appropriate sampling depth was determined, followed by conducting alpha rarefaction analysis using the q2-diversity plugin to generate rarefaction curves and assess the sufficiency of the sequencing depth. Finally, alpha diversity metrics (e.g., Shannon, Faith's Phylogenetic Diversity, Evenness, and Observed Features (richness) were calculated using the chosen sampling depth to evaluate the within-sample microbial diversity. To compare alpha diversity across habitats, the Kruskal–Wallis test was used, as it is a nonparametric test suitable for detecting differences among multiple groups without assuming normality. Where relevant, pairwise Kruskal–Wallis tests were conducted to examine differences among individual habitat types. The q2-diversity plugin was also used to assess beta diversity in microbial communities between habitats, utilising metrics like UniFrac (Unweighted or Weighted) and statistical tests such as PERMANOVA (Permutational Multivariate Analysis of Variance) (Anderson, 2001).

RESULTS

Wild Bird Gut Microbiota Composition

There are 2,366,453 read pairs remaining with no errors in the primer sequence. After filtering, the same number of sequences remained, averaging 157,763 sequences per individual. In total, 5584 (Table 6) were obtained, with 9 phyla, 14 classes, 20 orders, 29 families, 39 genera, and 34 species. Several ASVs remained unclassified at each level, with 1 phylum, 3 at class, 6 at order, 11 at family, 14 at genus, and 18 at the species level. Most ASVs were habitat-specific, with the highest number of unique ASVs found in secondary forest (n = 44), followed by village (n = 29) and oil palm plantation (n = 28). While the majority of ASVs were unique to individual habitats, only one ASV was shared between two habitats, and none were shared across all three habitats

Table 6. The summary of unique and shared ASVs across habitat

Habitat	ASVs Count
Oil_Palm_Plantation Unique	28
Secondary_Forest Unique	44
Village Unique	29
Shared across 2 habitats	1
Shared across 3 habitats	0

The most abundant phylum was Firmicutes (59.23%), followed by Bacteroidetes (17.98%), Proteobacteria (14.06%), and Actinobacteria (3.71%). Minor proportions were also observed for Cyanobacteria (3.21%), Fusobacteria (0.79%), Patescibacteria (0.48%), Epsilonbacteraeota (0.14%), and Unclassified taxa (0.43%). No sequences were assigned to Chloroflexi (Figure 2; Table S1A). Six other phyla- Cyanobacteria, Fusobacteria, Patescibacteria, Epsilonbacteraeota, and Chloroflexi - were also discovered (Figure 2; Table S1A). However, only one phylum remained unclassified (Table S1A(d)).

The results showed a very different microbial composition among bird species, with Firmicutes appearing to be the most abundant phylum in some species (Figure 2). For example, Crow-billed Drongo (*D. annectens*) and Long-tailed Shrike (*L. schach*) consisted entirely of the bacterial phylum Firmicutes. The other wild bird species also contained Firmicutes, except for the Little Spiderhunter (*A. longirostra*), whose faeces matter predominantly contained Proteobacteria (Figure 2). Meanwhile, Fusobacteria was found in the Spotted dove (*S. chinensis*), while Patescibacteria was detected in the Red-throated Sunbird (*A. rhodolaemus*) at low relative frequency. Specifically, fourteen bacterial classes were identified across the six bacterial phyla (Table S2A). There are Bacilli, Clostridia, Bacteroidia, Gammaproteobacteria, Negativicutes, Actinobacteria, Alphaproteobacteria, Oxyphotobacteria, Fusobacteria, Saccharimonadia, Deltaproteobacteria, Coriobacteria, Campylobacteria, Ktedonobacteria. In addition, two bacterial classes remained unknown.

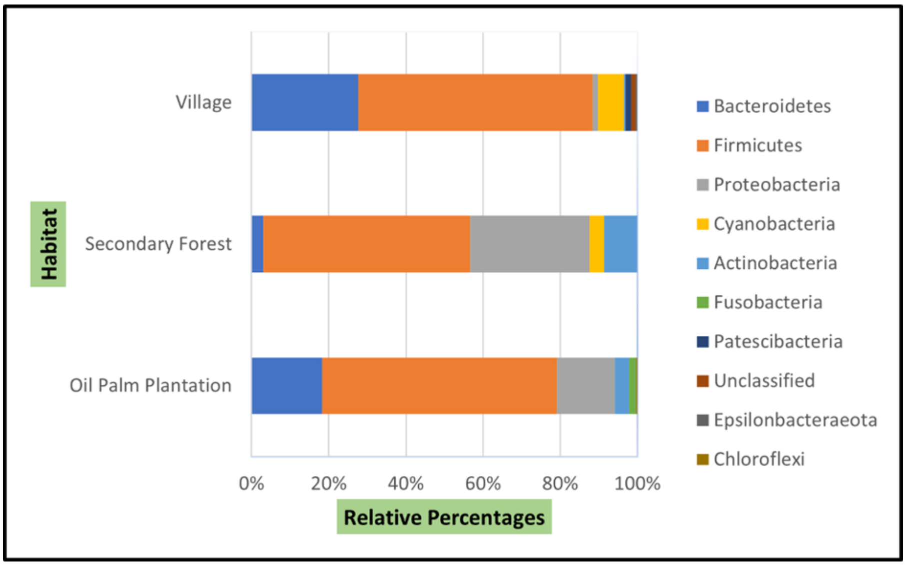

Figure 2. Taxonomy bar plots of bacteria at the phylum level for 5 bird species were selected in three habitats at the Wilmar Plantation and its adjacent areas. Refer to Table S2A(a) through S2A(e) for details on bacteria types in each phylum

Influence of Habitat on Gut Microbiota Diversity

Analyzing the microbiota of all 15 faeces samples from the three different habitats revealed distinct patterns in microbial community composition and diversity (Figure 3). In the secondary forest, Firmicutes are commonly found in the gut habitats of the Bronzed Drongo (*D. aeneus*), Grey-headed Babbler (*S. poliocephala*), Crow-billed Drongo, and Rufous-backed Kingfisher (*C. rufidorsa*), but not in the Little Spiderhunter. These four bird species harbour a combined 53.69% abundance of Firmicutes. Besides Firmicutes, other phyla in the secondary forest were Proteobacteria with a combined abundance at 30.96%, followed by Actinobacteria at 8.61%, and Cyanobacteria at 3.73 %.

In contrast, the oil palm plantation showed a decrease in microbial diversity, with a dominance of Firmicutes constituting 60.50%, followed by Bacteroidetes at 18.29%. However, in the village area, seven bacterial phyla were identified: Firmicutes (60.75%), Bacteroidetes (27.69%), Cyanobacteria (6.70%), Proteobacteria (1.34%), Patescibacteria (1.39%), Epsilonbacteraeota (0.41%), and one more unclassified phylum (1.23%). The village habitat also has a high abundance of Firmicutes at 60.75%, followed by Bacteroidetes at 27.90% and Cyanobacteria at 6.07%.

In addition, the Venn diagram (Figure 4) revealed an interesting distribution of bacterial phyla across the three different habitats. The phyla Fusobacteria and Chloroflexi were found exclusively, making up 22.20% of the total microbiota in the five faeces samples from the oil palm plantation. Conversely, the village habitat identified Patescibacteria, and one unclassified phylum, together comprising 22.20% of the total microbiota (Figures 3 and 4). Meanwhile, no phyla are exclusive to the secondary forest (Figure 4). Besides, four common phyla- Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria, comprised 44.40% of the total microbiota, shared across all three habitats (Figure 4). No common phyla were shared between the oil palm plantation and the village, nor between the oil palm plantation and the secondary forest (Figures 3 and 4). However, Cyanobacteria were shared between the secondary forest and the village (Figures 3 and 4). Furthermore, based on a comparative analysis of bacterial phyla (Figure 3), birds in oil palm plantations have the highest abundance of Firmicutes compared to those in the secondary forest and the village.

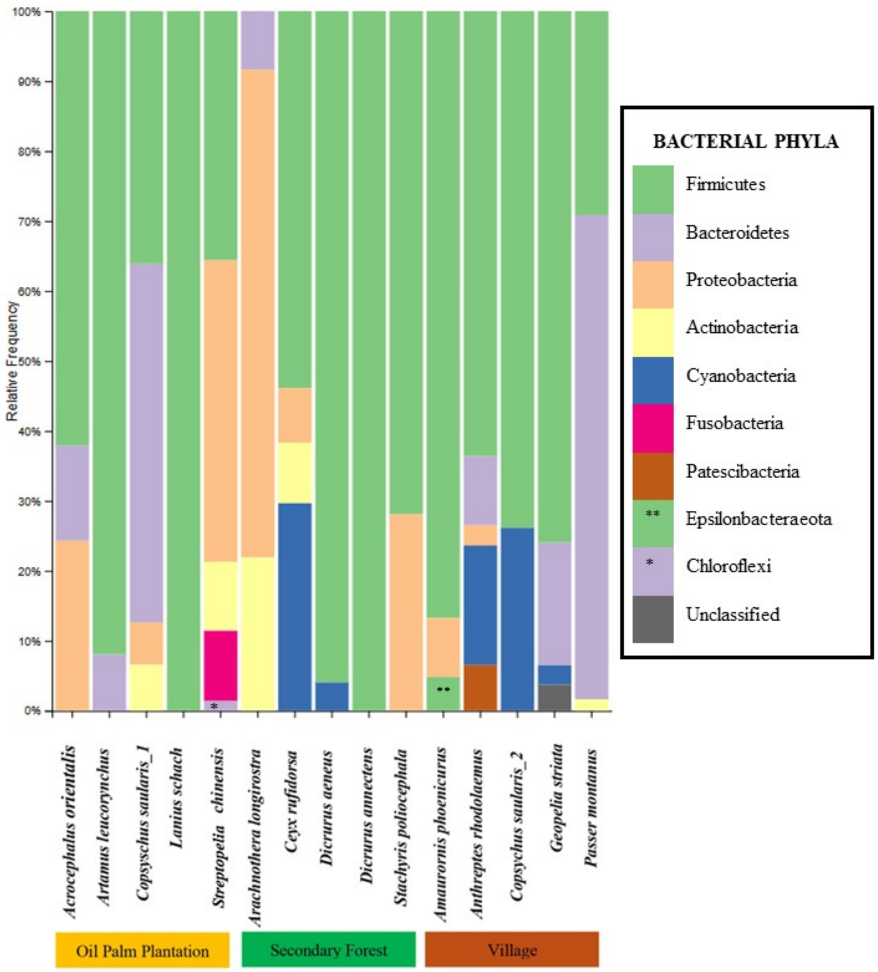

Figure 3. The bar plot of bacteria at the phylum level in different habitats, in which 10 phyla were identified from 15 faeces samples. The number of phyla in the village habitat is greater than in the secondary forest and the oil palm plantation

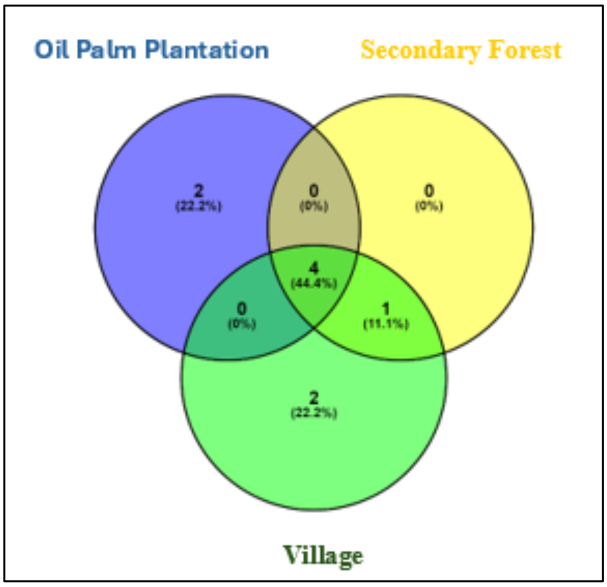

Figure 4. The Venn diagram of bacterial phyla common to three distinct habitats, with five bird species selected from each habitat

The analyses of microbial diversity across habitats revealed no significant differences in Faith's Phylogenetic Diversity (PD) (H = 2.40, p = 0.30), Shannon diversity (H = 2.96, p = 0.23), or observed features (richness) (H = 2.60, p = 0.27). The Kruskal-Wallis test for each alpha-diversity matrix indicated that differences in microbiota diversity among the three habitats were not statistically significant. Similarly, the beta diversity for the Unweighted UniFrac distance matrix showed a *p-value* of 0.236 from the PERMANOVA test (Table 5). This indicates that there was no statistically significant difference between the habitats at the chosen significance level, suggesting that any observed variation among habitats may be due to chance. Hence, various factors can contribute to the lack of significant differences in the gut microbiota among the wild birds. While habitat did not appear to strongly influence gut microbiota composition, host identity may still play an important role. Future analyses at the host species or family level, including alpha- and beta-diversity comparisons and statistical testing, could help determine whether host species and associated dietary habits exert a stronger influence on microbial community structure than habitat alone.

Table 5. Pairwise PERMANOVA results based on unweighted UniFrac habitat significance

Number of groups	Sample size	Permutations	Test statistic	*p-value*
3	15	999	1.13621	0.236

DISCUSSION

Microbiome analysis in wild birds provides valuable insights into the diversity and composition of microbial communities inhabiting bird hosts across different habitats. The results of this study revealed that the gut microbiota of the selected Bornean wild birds was abundant in Firmicutes but lacked other common phyla, such as Bacteroidetes, Proteobacteria, and Actinobacteria, in secondary forests, oil palm plantations, and villages. This finding aligns with the works of Waite and Taylor (2014) and Jiang (2023), who also mentioned that Firmicutes was the dominant phylum in most birds' gut microbiota. This contrasts with the study by Hird *et al.* (2015), which found that Proteobacteria were the most abundant, followed by Firmicutes, Bacteroidetes, and Actinobacteria across the bird species studied.

This predominance of Firmicutes in the gut microbiota of Bornean wild birds raises questions about the ecological factors driving this pattern, which may be related to their adaptive advantages in the gut environment. Firmicutes are often the most abundant bacterial phylum in vertebrate gut primarily due to their superior adaptability to the anaerobic conditions prevalent in the gut environment (Sun *et al.,* 2022), and their efficiency in fermenting complex carbohydrates into short-chain fatty acids (SCFAs) which are absorbed by the host and used as an energy source (den Besten *et al.*, 2013; Fujisaka *et al.*, 2023). A high abundance of Firmicutes in the vertebrate gut also benefits hosts by contributing to mass gain and improving immune function (Zhang *et al.*, 2015). Additionally, Firmicutes produce bacteriocins that inhibit the growth of competing bacteria, including some Proteobacteria and Actinobacteria, thereby furthering their competitive edge in the gut environment (Drissi *et al.*, 2015). The lower relative abundance of phyla Proteobacteria, Bacteroidetes, and Actinobacteria in comparison to Firmicutes in this study might be attributed to the more homogenous or disturbed environments resulting from agricultural practices that often lead to a reduction in microbial diversity both in the environment and within the gut microbiota of resident species (Sheng *et al.*, 2013; Santosa *et al.*, 2018; Salamat *et al.*, 2021).

However, Proteobacteria was approximately 21.00% higher than Firmicutes in the gut of the Spotted Dove, with relative abundances of 43.15% and 35.62%, respectively. This inconsistency might be due to an underlying methodological bias (Hird *et al.*, 2015). The detection of Fusobacteria and Chloroflexi in the gut of the Spotted Dove also suggests environmental interactions that may have exposed this bird species to specific bacteria from the two phyla, similar to findings in Shang *et al.* (2020). Besides, it is quite interesting to detect the presence of Cyanobacteria in the secondary forest and the village, as they are typically associated with aquatic ecosystems rather than terrestrial or vertebrate gut environments (Lu *et al.*, 2021). The Cyanobacteria in the gut microbiome of both habitats are likely non-photosynthetic strains, such as those from the class 'Melainabacteria' (Hu & Rzymski, 2022). The next identified phylum, Epsilonbacteraeota, is frequently present in the intestines of birds inhabiting human settlements, likely due to human activity affecting their environment. This phylum is prevalent in areas with significant human presence, where birds may be exposed to human waste or other contaminants (Boukerb *et al.*, 2021). For instance, Epsilonbacteraeota were detected in the gut of White-breasted Waterhen (*A. phoenicurus*), a finding consistent with the Boukerb *et al.* (2021) study, which also identified these bacteria in wild waterbirds commonly found in human-impacted areas. Meanwhile, Patescibacteria in the Red-throated Sunbird might connect to the birds' interaction with human activities. These bacteria are frequently located in wastewater treatment facilities (Hu *et al.*, 2023), suggesting sunbirds could have been exposed to them. The unclassified phyla detected in Zebra Dove (*G. striata*) could be because of a few factors, such as many limitations in current taxonomic databases in the vertebrate gut (Werner *et al.*, 2012) and the discovery of potentially novel bacteria taxa such as in the study of Hoatzin (Godoy-Vitorino *et al.*, 2008).

While changes in habitat can impact the composition of microbiota in certain bird species (San Juan *et al.*, 2020), previous studies have consistently shown that diet plays the most significant role in shaping bird gut microbiomes (Bodawatta *et al.*, 2018; Aimeric, 2020; Matheen *et al.*, 2022) with phylogeny, the nest environment, and seasonal variation factors playing secondary roles (Matheen *et al.*, 2022). Interestingly, the phyla Firmicutes were the most dominant among all three habitats, particularly in insectivorous and omnivorous diets. For example, insectivorous birds in both oil palm plantations and secondary forests consistently maintained Firmicutes as the most abundant phylum, suggesting a possible link between insect-based diets and gut communities dominated by Firmicutes, as observed by Waite and Taylor (2015). By comparing the feeding guilds among the wild bird species, the finding of this study is similar to those of Wang *et al.* (2022), which showed that omnivorous birds exhibited greater bacteria than herbivorous and carnivorous. For example, in the village setting, the Red-throated Sunbird (omnivorous) displayed higher bacterial diversity than the Eurasian tree sparrow (*P. montanus*) (herbivorous) and the Oriental Magpie Robin (*C. saularisI*) (carnivorous).

However, when comparing different individuals of the same species, bacterial diversity might be influenced more by foraging behaviour than by feeding guilds. In this study, an Oriental Magpie-robin in the oil palm plantation lacked Cyanobacteria in its gut, unlike another individual in the village, which did harbour the bacteria. Moreover, individuals from oil plantations were dominated by Bacteroidetes, whereas those from the village were dominated by Firmicutes. This difference could be due to the village bird consuming unnatural food items introduced by humans, such as human waste or food for domesticated animals. These findings are consistent with the study by Knutie and Gotanda (2018) on the Galapagos Islands, which found that the gut microbiota of finch species in urban areas differed from that in non-urban sites. Hence, it is unsurprising that the Zebra Dove and Red-throated Sunbird in the village were also exposed to the same bacteria due to human presence. Additionally, the Rufous-backed dwarf kingfisher (*C. rufidorsa*) probably acquired Cyanobacteria from its primary diet of fish, which are known to harbour Cyanobacteria (Drobac *et al.*, 2016). When the kingfisher consumes the fish, the bacteria are indirectly transferred to its gut habitat.

It is quite interesting to find that the abundance of Bacteroidetes was higher than that of Firmicutes in the gut of the Oriental Magpie Robin found in the oil palm plantation. Although the presence of this phylum in the bird's gut is not fully explained, the bird likely foraged for food in nearby villages before moving to the oil palm plantation. Similarly, the presence of Epsilonbacteraeota in the gut of White-breasted Waterhen in the village could also be linked to its diets, likely due to ingestion of contaminated food and water from human and domesticated animal sources (Knutie & Gotanda, 2019; Boukerb *et al.*, 2021). In addition, the higher abundance of Proteobacteria in the gut of the Little Spiderhunter might be attributed to the consumption of nectar containing high densities of this phylum, which is consistent with the finding of Lee *et al.* (2019). This observation supports the idea that diet influences gut microbiota through natural foraging behaviour and environmental context. However, due to the lack of direct dietary analysis, this interpretation remains speculative and should be further explored in future studies.

Although pairwise comparisons for each metric also showed no significant differences, indicating broadly comparable microbial diversity among oil palm plantations, secondary forests, and villages. Nevertheless, boxplots showed that secondary forests had slightly higher microbial diversity, while oil palm plantations had the lowest across all metrics. The reduced diversity observed in oil palm plantations may be attributed to external factors such as habitat disturbance, lower dietary diversity, or limited environmental microbial exposure in monoculture settings.

CONCLUSION

Overall, the findings of this study indicate that habitat factors are secondary influences on gut bacterial diversity. Diet has been shown to influence gut bacterial diversity, but foraging behaviours also shape the composition of the bacterial microbiome, especially among individuals of the same species across different habitats. Generally, all three factors can influence the gut microbiota composition of wild birds. This study contributes to expanding the field of avian microbiome research by providing one of the first characterisations of gut microbiota in wild birds from Borneo across multiple habitat types. Further analysis is required by adding at least two individuals per species per habitat, as well as soil and water samples, to determine whether habitat is the primary factor influencing the gut microbiota composition of the wild birds. Additionally, detailed observation of host foraging behaviour in each habitat is recommended to better understand its role, which can help with the conservation of birds, especially in Sarawak.

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