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## Research Article

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**Posted Date:** October 13th, 2026

**DOI:** <https://doi.org/10.21203/rs.3.rs-10768139/v1>

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**Additional Declarations:** No competing interests reported.

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# Seasonal dynamics of bacterial community structure in a mixed land-use Tropical Borneo River

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## Abstract

Waterborne diseases pose significant risks to communities in the Batang Layar river catchment that depend on the river for recreation, consumption, agriculture, aquaculture, and transportation. Despite the widespread use of Next-Generation Sequencing (NGS) globally, its application to biodiverse yet understudied tropical river systems such as those in Sarawak, Malaysia, remains limited. Sarawak's rivers traverse both pristine rainforests and urban areas and likely host distinct microbial communities influenced by both natural and anthropogenic activities. This study presents the first NGS-based characterisation of microbial communities in this understudied river, providing a foundational dataset for ecological and public health monitoring. Surface water samples were collected from five sites during wet and dry seasons. Illumina 16S rRNA sequencing revealed 62 phyla, 340 families, and 700 genera, with Pseudomonadota (69-82%) dominating across all samples. Seasonal variation exerted stronger influence on microbial community diversity than land-use effects, with significantly greater richness and evenness in the wet season. The core microbiome contracted from 43 genera in the wet season to six in the dry season, with *Pseudomonas* (70.9%) dominating the dry core. Putative faecal-associated genera shifted from low-abundance classical indicators (*Escherichia-Shigella*, *Enterococcus*, *Bacteroides*) in the wet season to site-specific dominance of opportunistic genera (*Pseudomonas*, *Stenotrophomonas*) in the dry season. Redundancy analysis (RDA) identified temperature (75.95%) and biochemical oxygen demand (BOD) (24.05%) as significant drivers of the distribution of faecal-associated genera. These findings demonstrate the value of seasonally stratified molecular monitoring for water quality assessment in rural tropical catchments, providing new insights into the river microbiome of Borneo.

Keywords: tropical basins, riverine microbiome, seasonal dynamics, faecal-associated bacteria, water safety

## Key Points

- Seasonal variation exerts a stronger influence on microbial diversity compared to land-use effects.
- Temperature and BOD are significant environmental drivers of faecal-associated genera distribution.
- Application of NGS-based characterisation of Batang Layar River demonstrates value in classical water quality assessment in rural tropical catchments.

## Introduction

Lakes and rivers represent complex aquatic environments that harbour diverse microbial ecosystems, which also serve as the medium of drinking water sources (Garner et al. 2021). River systems face heightened vulnerability to contamination from land use pressures and seasonal fluctuations, acting as reservoirs for potentially pathogenic bacteria, thereby exposing vulnerable populations to waterborne diseases (Bae et al. 2019; Kiama et al. 2021; Starevich et al. 2024). This vulnerability is especially pronounced in rural regions such as Batang Layar, where this catchment sustains domestic and livelihood activities. In such settings, even subtle shifts in microbial community composition carry direct public health implications (Ho et al. 2022; Justin et al. 2026). Water is deemed potable only when pathogenic microorganisms are absent (Dhengesu et al, 2022). Nevertheless, microbial contamination can persist in treated and distributed systems, raising particular concern for understudied catchments frequently utilised by rural populations (Church et al. 2020). Anthropogenic activities, including urban and industrial discharges, can alter microbial community structure and ecosystem functions (Starevich et al. 2024).

Traditional waterborne pathogen detection relies on culture-based enumeration of faecal indicator bacteria (FIB), such as *E. coli* and coliforms. However, this approach cannot capture the overall microbial diversity in the water, particularly viable but non-culturable (VBNC) or stress-resistant organisms, thereby providing an incomplete water safety assessment (Acharya et al. 2020; Church et al. 2020; Garner et al. 2021; Kiama et al. 2022). The emergence of NGS has transformed the potential to address complex microbiological challenges in the water industry, although its application remains constrained by cost and technical expertise (Garner et al. 2021). Among NGS approaches, 16S rRNA amplicon sequencing is widely used to characterise overarching shifts in microbial communities, encompassing responses to environmental disturbances, successional patterns, and temporal trends (Garner et al. 2021). This technique can serve as a routine monitoring tool alongside physicochemical parameters, enabling comprehensive and integrated assessment of drinking water resources (Zhang et al. 2017). High-throughput 16S rRNA amplicon sequencing thereby facilitates broad bacterial profiling and the detection of taxa associated with faecal contamination and potential public-health relevance, enabling more representative microbial risk assessment compared to predetermined indicator-based approaches (Bae et al. 2019; Church et al. 2020).

In tropical mixed-land-use catchments, 16S rRNA amplicon sequencing offers deeper insights into microbial dynamics across diverse land-use types and hydrological conditions. This approach effectively establishes links between microbial community composition and environmental drivers alongside anthropogenic impacts in aquatic environments (Garlapati et al. 2021; Kiama et al. 2021; Ting et al. 2021). Understanding environmental drivers of microbial communities is essential for monitoring and designing sustainable remediation strategies (Starevich et al. 2024). Although the V3-V4 hypervariable region combination is widely employed in riverine microbiome studies (Kiama et al. 2021; Ting et al. 2021; Zhang et al. 2017), targeting a single hypervariable V3 region can provide a cost-effective approach for broad characterisation of bacterial community composition, making it appropriate for pilot studies in resource-limited settings (Ahmad et al. 2019; Mittal et al. 2019; García-López et al. 2020). It has been proven effective for characterising bacterial communities across diverse aquatic environments (Ahmad et al. 2019; García-López et al. 2020; Mittal et al. 2019). Such an approach is particularly valuable in tropical watersheds where conventional monitoring inadequately captures the complete spectrum of microbial contaminants.

Despite increasing interest in molecular approaches to water-quality assessment, community-level bacterial profiling of Sarawak's river systems remains limited, particularly across contrasting land-use and seasonal conditions. Applying NGS based approach enables assessment of shifts in microbial composition across land use types and seasonal variations, while identifying environmental variables associated with microbial community variation. Critically, it transcends single-indicator frameworks to deliver an integrated understanding of how seasonality, land use, and water quality gradients collectively shape microbial assemblages (Garlapati et al. 2021; Ting et al. 2021). The findings will reveal microbial community structure, including faecal-associated taxa, and contribute to a deeper understanding of riverine microbial ecology and its implications for water safety in this understudied catchment (Bae et al. 2019). This study had successfully characterised the bacterial community composition and diversity in Batang Layar river water, assessed the influence of seasonal variation and land-use on microbial community structure, and identified the environmental drivers of faecal-associated genera distribution across seasons and sites.

## **Materials and Methods**

### **Study area and sampling sites**

The study was conducted in the Batang Layar watershed in the Betong region of Sarawak, Malaysian Borneo. The sampling was conducted at five sites that represented a gradient of land-use types (Fig. 1): Lubok Paoh (LS1), Nanga Tiga (LS2), Nanga Linsum (LS3), Nanga Spak (LS4), and Jelau (LS5). These sites ranged from forested upstream areas to downstream regions influenced by agricultural activities, schools, and settlements. The area of interest (AOI) for each sampling site was generated using a geographic information system (GIS), with land-use information derived from field observations and GIS-based mapping. Table 1 describes sites with sampling points A, B, and C within each site, providing replication to capture micro-spatial variability, resulting in five sites X three points X two seasons (total n = 30).

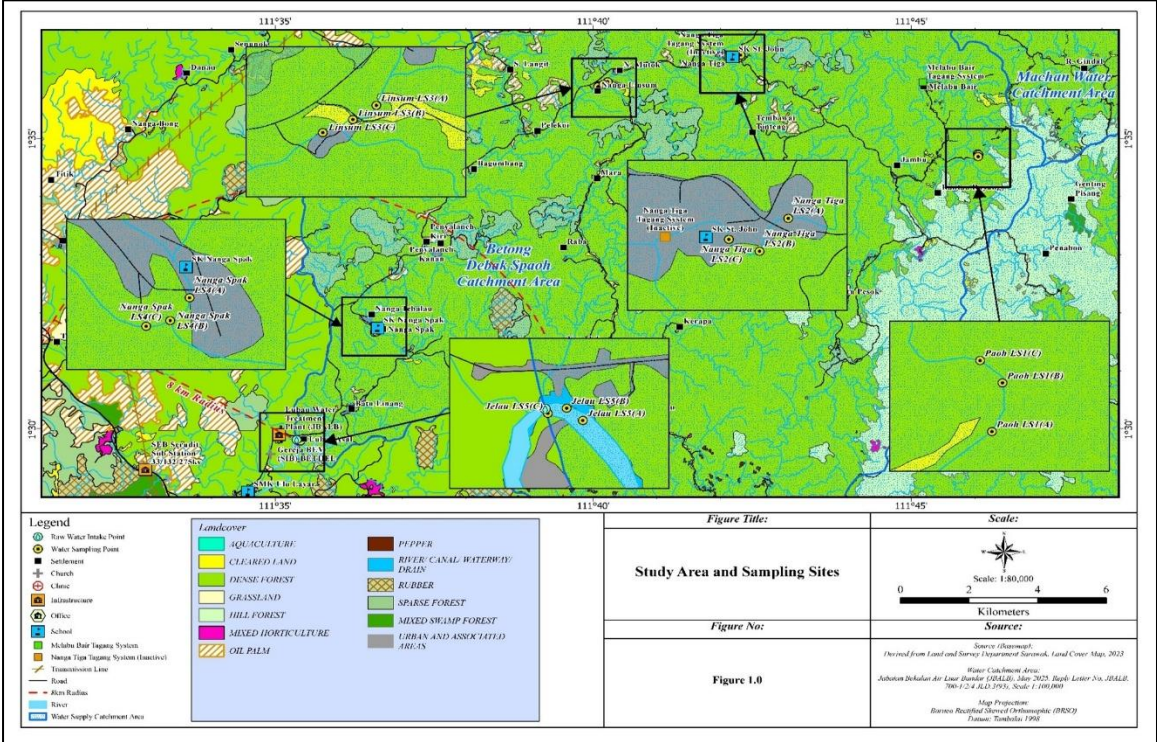


**Fig 1.** Sampling area along the Batang Layan river with distinct land-use types. Inset map highlighted in box shows the sampling points within each site (Adapted from Justin et al. 2026).

**Table 1.** Description of sampling sites along Batang Layan watershed, Sarawak, including the geographic coordinates and land use characteristics for of each locality. Adapted from Justin et al. (2026)

| Site             | GPS coordinates                                                                                    | Land use description                                                                                                                                                                                         |
|------------------|----------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Paoh (LS1)       | A: N 01.57791°<br>E 111.75753°<br>B: N 01.57859°<br>E 111.76764°<br>C: N 01.57906°<br>E 111.76776° | Upstream, away from built-up areas; dense forest (98.74%; 24.68 Ha) and minimal cleared land (1.23%; 0.31 Ha).                                                                                               |
| Nanga Tiga (LS2) | A: N 01.60737°<br>E 111.70507°<br>B: N 01.60663°<br>E 111.70447°<br>C: N 01.60721°<br>E 111.70407° | Midstream, school and settlements, with an inactive <i>tagang*</i> system; dense forest (64.30%; 16.07 Ha), urban/associated areas (35.67%; 8.92 Ha), including small oil palm and mixed agricultural farms. |
| Linsum (LS3)     | A: N 01.59943°<br>E 111.67016°<br>B: N 01.59916°<br>E 111.66927°<br>C: N 01.59833°<br>E 111.66789° | Midstream, settlement area with mixed agricultural farms; dense forest (77.7%; 19.4 Ha), cleared land (12.5%; 3.1 Ha), and urban zones (9.7%; 2.4 Ha).                                                       |
| Nanga Spak (LS4) | A: N 01.52844°<br>E 111.61006°<br>B: N 01.52801°<br>E 111.60981°<br>C: N 01.52801°<br>E 111.60950° | Midstream, school and small poultry farm; dense forest (74.6%; 18.6 Ha), and urban/associated areas (25.4%; 6.4 Ha).                                                                                         |

|             |                                                                                                    |                                                                                                                                                                   |
|-------------|----------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Jelau (LS5) | A: N 01.49652°<br>E 111.58996°<br>B: N 01.49688°<br>E 111.58953°<br>C: N 01.49667°<br>E 111.58882° | Downstream, settlements and mixed agricultural farms; dense forest (73.1%; 18.3 Ha), urban/associated areas (18.3%; 4.6 Ha), and waterways/drains (8.5%; 2.1 Ha). |
|-------------|----------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------|

Note: *\*tagang* system, a traditional community practice in Sarawak for aquatic conservation. Land cover percentages (%) and total area (Ha).

### Water sample collection

Sampling was conducted in December 2023 (wet season) and June 2024 (dry season). A total of 30 water samples were collected throughout this study (15 samples/season/site). Sampling was performed at depths of 20-40 cm below the water surface, avoiding disturbed sediment and bankside vegetation. Each sample was collected in a pre-cleaned, acid-washed 1 L sterile polypropylene bottle to minimise contamination. Samples were stored in an icebox and transported to the laboratory on the same day to preserve integrity (Justin et al. 2026).

### DNA Extraction, PCR Amplification and Sequencing

The total genomic DNA for each water sample was extracted using the Nucleospin® eDNA Water Kit (Macherey-Nagel, Germany). DNA purity was assessed using a spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific) and DNA concentration was quantified using a Quantus™ Fluorometer (Promega, USA) (Azaroual et al. 2022; Wu et al. 2024). The extracted DNA that met the minimum quality thresholds (A260/280 within an acceptable range and sufficient DNA yield) was then used for library preparation and sequencing (Romantseva et al. 2022). The bacterial 16S rRNA gene V3 hypervariable region was amplified using primers 341F (CCTACGGGNGGCWGCAG) and 534R (ATTACCGCGGCTGCTGG) (Ahmad et al. 2019; Mittal et al. 2019; García-López et al. 2020). PCR was performed using Rediant II PCR Mastermix (Apical Scientific, Malaysia) under the following conditions: initial denaturation at 95 °C for 2 min; 30 cycles of denaturation at 95 °C for 15 s, annealing at 50 °C for 30 s, and extension at 72 °C for 30 s. Amplicons were purified using 0.8× volume of solid-phase reversible immobilisation (SPRI) beads. Purified amplicons were used as templates for 8 cycles of index PCR to incorporate full Illumina adapters and dual-index barcodes. Libraries were size-selected, pooled, and sequenced on an Illumina NovaSeq6000 platform (Illumina, San Diego) using the 2×150 bp paired-end sequencing configuration.

### Bioinformatic processing

Raw FASTQ data were processed in R (Version 4.4.1) using the DADA2 pipeline to infer amplicon sequence variants (ASVs) (Callahan et al. 2016; Fares et al. 2025; Kwon et al. 2025). Taxonomy was assigned against the SILVA SSU Ref NR99 v138.2 reference database. Non-bacterial sequences (eukaryotes, archaea, chloroplasts, and mitochondria) were removed. The resulting ASV table, metadata and taxonomy were combined into a phyloseq object for downstream analyses. Samples were rarefied to 25,000 reads per sample, with good coverage values of 99% for all samples, indicating adequate sequencing depth (Bardenhorst et al. 2022; Daly et al. 2024; He et al. 2023; Morni et al., 2025).

### Statistical analysis

All statistical analyses were performed in R (Version 4.4.1) using the phyloseq and vegan packages (Jiang et al. 2023; Daly et al. 2024). Relative abundance was determined at phylum, family and genus levels using ggplot2 (Ogola et al. 2025; Wang et al. 2026). The rarefied dataset was used for all diversity analyses (Schloss 2024). Alpha-diversity (observed ASVs and Shannon index) was compared between seasons using the Wilcoxon rank sum test and among sites using the Kruskal-Wallis rank sum test (Bardenhorst et al. 2022; Cavaco et al. 2019; Jensen et al. 2022; Ogola et al. 2025; Snyder et al. 2025). Beta diversity was assessed using Bray-Curtis dissimilarity, visualised via principal coordinates analysis (PCoA), with permutational multivariate analysis of variance (PERMANOVA) and permutational analyses of multivariate dispersions (PERMDISP) applied to test for composition differences and dispersion homogeneity, respectively (Jeunen et al. 2022; Nasuelli et al., 2023; Jiang et al. 2023; Ogola et al. 2025). Core microbiome analysis was performed using the microbiome package,

with a detection threshold of 0.02% relative abundance and 80% prevalence (Custer et al. 2023; Hallett and Comte 2025; Mayol et al. 2026). Putative faecal-associated genera were identified by screening ASV profiles against published lists of enteric taxa (Glaeser et al. 2022; Stefaniak et al. 2026; Yang et al. 2025; Zeng et al. 2025). Redundancy Analysis (RDA) was performed using the *rda* function in the *vegan* package (Akita et al. 2021; Dimpor et al. 2025; Wu et al. 2024), with environmental parameters (BOD, COD, pH, etc.) measured at the same sites and sampling events (Justin et al. 2026) as explanatory variables. Forward selection identified significant drivers, with significance tested by a Monte Carlo permutation test ( $\alpha = 0.05$ , 999 permutations) (Akita et al. 2021; Xie et al. 2022).

## Results

### Analysis of 16S rRNA gene sequencing results

A total of 2,403,239 high-quality 16S rRNA sequences were generated. The bacterial microbiome was classified into 62 phyla, 121 classes, 270 orders, 340 families, and 700 genera. Figure 2 below shows the rarefaction curves of ASVs based on dry and wet seasons.

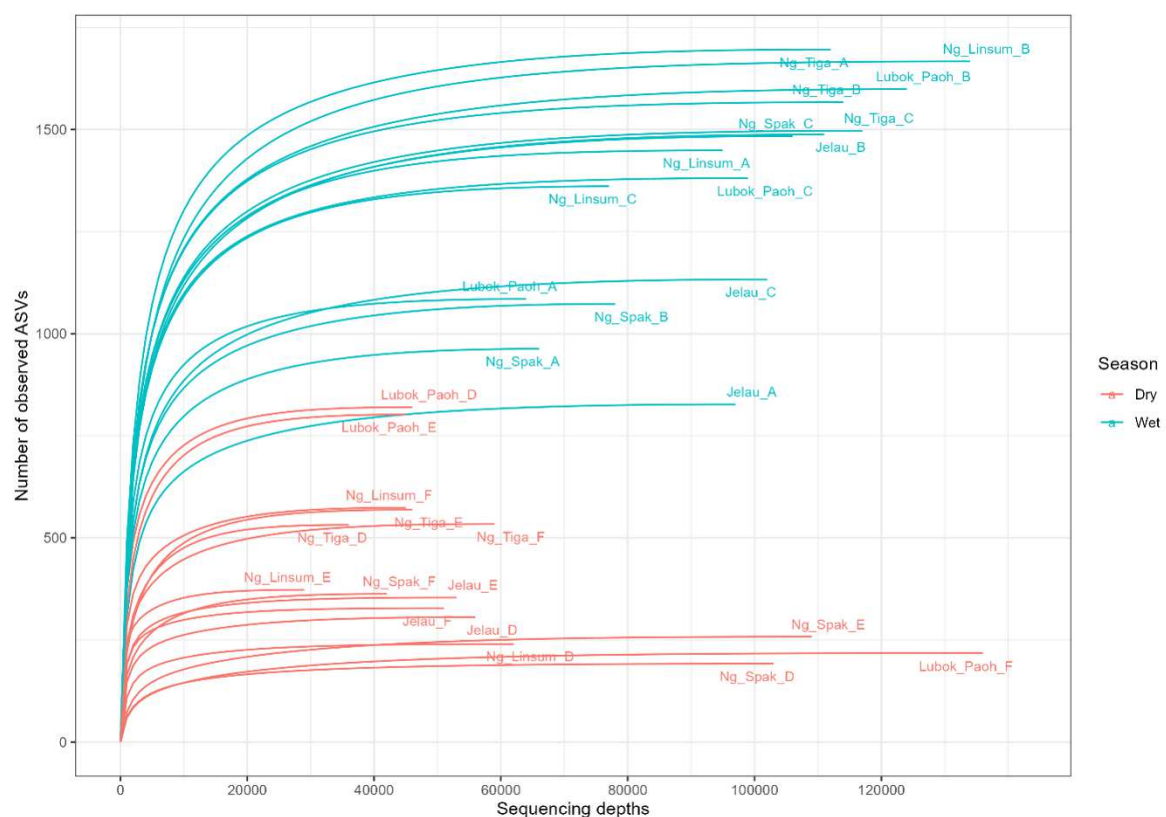


**Figure 2.** The rarefaction curves of ASVs. The x-axis shows the number of valid sequences per sample, and the y-axis shows the observed amplicon sequence variants (ASVs). Each line in the graph represents one sample. The seasonal grouping is shown through two different colours (wet=blue, dry=red). The abbreviation for Ng is for *Nanga* (river mouth in Iban dialect).

### Bacterial diversity in river water

Alpha-diversity indices, namely Observed ASVs and Shannon index, were calculated for sites and seasons (Table 2). Both Observed ASVs and the Shannon index (Fig 3) were significantly higher in the wet season compared to the dry season (Wilcoxon rank-sum test,  $p < 0.05$ ). No significant differences were detected among sites within either season (Kruskal-Wallis rank sum test,  $p > 0.05$ ), indicating that season was the primary driver of alpha diversity compared to land-use effect.

**Table 2.** Table of alpha diversity indices across sites and seasons.

| Sites | Season | Observed ASVs | Shannon, $H'$ |
|-------|--------|---------------|---------------|
|-------|--------|---------------|---------------|

|                    |     |                  |             |
|--------------------|-----|------------------|-------------|
| Lubok Paoh (LS1)   | Wet | 1249 ± 191.54    | 6.13 ± 0.09 |
|                    | Dry | 592 ± 357.73     | 4.57 ± 1.94 |
| Nanga Tiga (LS2)   | Wet | 1428.67 ± 91.51  | 6.13 ± 0.17 |
|                    | Dry | 531.33 ± 23.59   | 4.88 ± 0.31 |
| Nanga Linsum (LS3) | Wet | 1352.33 ± 99.57  | 6.11 ± 0.03 |
|                    | Dry | 386.33 ± 160.42  | 4.68 ± 0.95 |
| Nanga Spak (LS4)   | Wet | 1079.67 ± 217.92 | 5.98 ± 0.28 |
|                    | Dry | 248.67 ± 96.26   | 3.11 ± 1.02 |
| Jelau (LS5)        | Wet | 1042.33 ± 299.52 | 5.80 ± 0.42 |
|                    | Dry | 322 ± 27.06      | 4.33 ± 0.42 |

Note: Results are expressed as mean value ± standard deviation

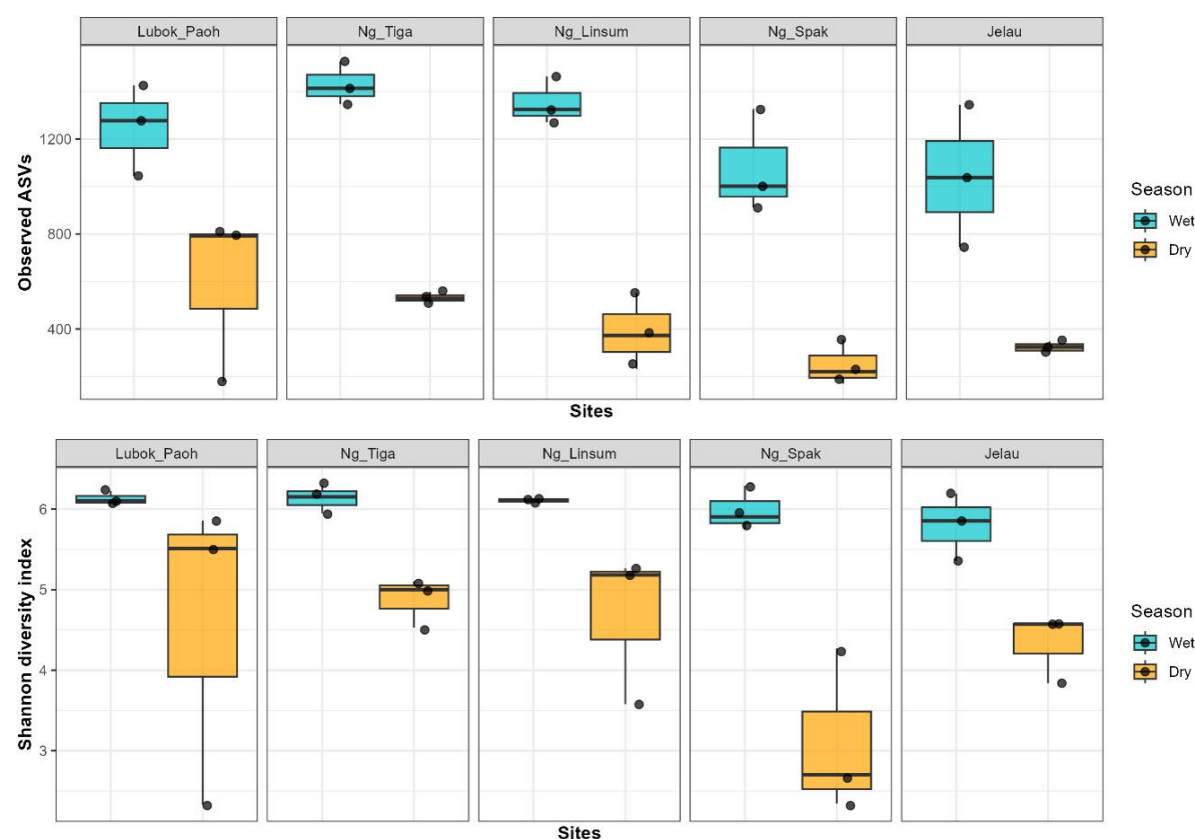


**Fig 3.** Alpha diversity indices (Observed ASVs and Shannon diversity index) of the riverine microbial community across sites and seasons. The seasonal grouping is shown through two different colours (wet=blue, dry=yellow). The abbreviation for Ng is Nanga.

Principal coordinates analysis (PCoA) based on Bray-Curtis dissimilarity revealed distinct clustering of microbial communities by season along PCoA1, explaining 43.5% of the total variation (Fig 4). PERMANOVA confirmed that season explained a larger proportion of variance ( $R^2 = 0.262$ ,  $F = 9.93$ ,  $p = 0.001$ ) than site ( $R^2 = 0.200$ ,  $F = 1.57$ ,  $p = 0.089$ ). A significant Season  $\times$  Site interaction was observed ( $R^2 = 0.195$ ,  $F = 2.85$ ,  $p = 0.004$ ), indicating that seasonal shifts differed among sites. PERMDISP revealed that wet-season communities were more homogeneous (mean distances: 0.20–0.26) than dry-season communities (mean distances: 0.40–0.64;  $F = 34.48$ ,  $p < 0.001$ ), while no significant differences were observed among sites ( $F = 0.28$ ,  $p = 0.890$ ).

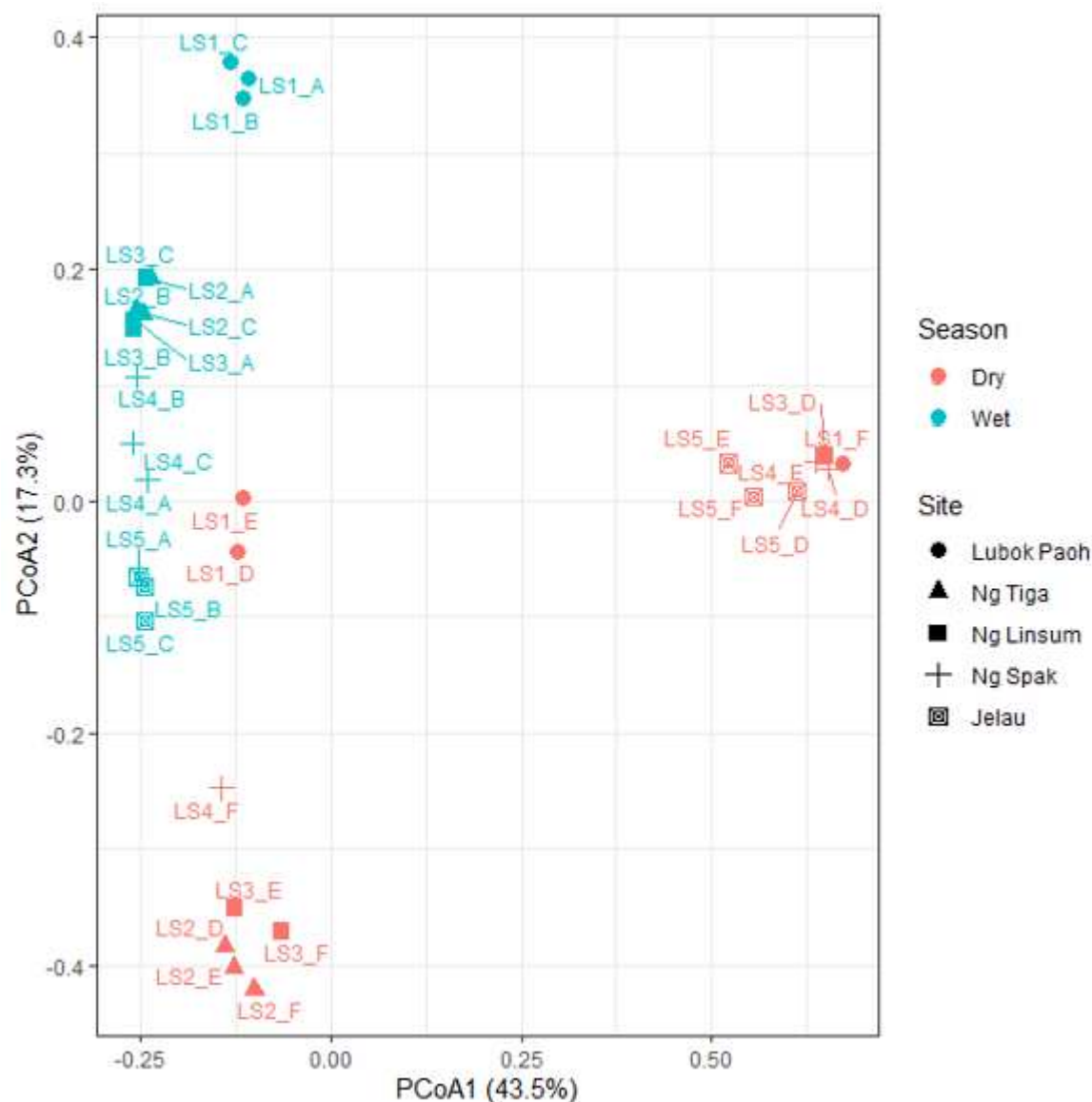


**Fig 4.** Genus-level PCoA (Bray-Curtis) of riverine bacterial communities across sites and seasons. Abbreviations of LS1 to LS5 follow Table 1, and Ng mean Nanga. A, B, C= wet season samples; D, E, F= dry season samples.

#### Relative abundance of riverine microbiome

To streamline the data presentation, the riverine microbiome are grouped into top 10 phyla, top 10 families and the top 20 genera, ranked by relative abundance. Across all sites and seasons, Pseudomonadota is the dominant phylum (69–82% in wet season; >95% at selected dry-season sites), followed by subdominant phyla including Actinomycetota, Bacteroidota, Patescibacteria, and Verrucomicrobiota, each contributing <25% of relative abundance (Fig 5). Bacteroidota and Actinomycetota showed higher relative abundances in dry-season samples (5–40%) compared to wet-season samples. Verrucomicrobiota occasionally reached higher levels in certain dry-season samples, increasing from 3–4% (wet) to 18–37% at Nanga Tiga and Nanga Linsum.

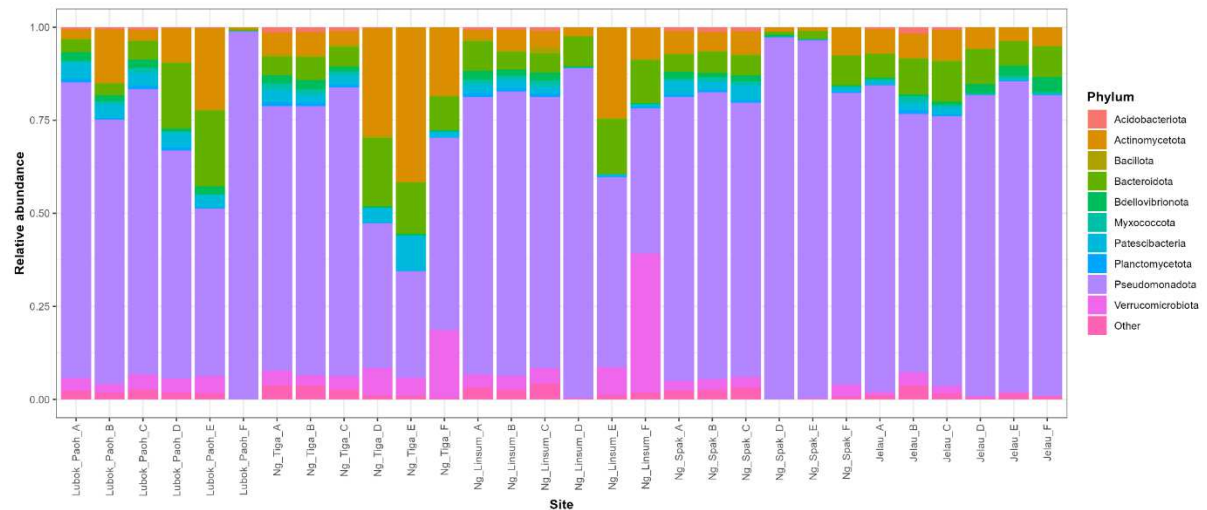


**Fig 5.** Histogram of relative abundance of the top 10 phyla. The x-axis represents sampling sites, and the y-axis represents relative abundance presented as a decimal percentage. A, B, C= wet season samples; D, E, F= dry season samples.

Analysing the family relative abundance, a large percentage of Comamonadaceae (49-71%) across all sites in wet-season communities (Fig 6A), reaching its highest proportion at Jelau A (71%). In contrast, dry-season communities (Fig 6B) exhibited greater heterogeneity with site-specific dominance patterns. Microbacteriaceae reached high proportions at Nanga Tiga (16–42%), Oxalobacteraceae was enriched at Nanga Linsum (20%) and Jelau (26–35%), and Pseudomonadaceae showed strong enrichment at Lubok Paoh F, Nanga Spak D and E (>85%).

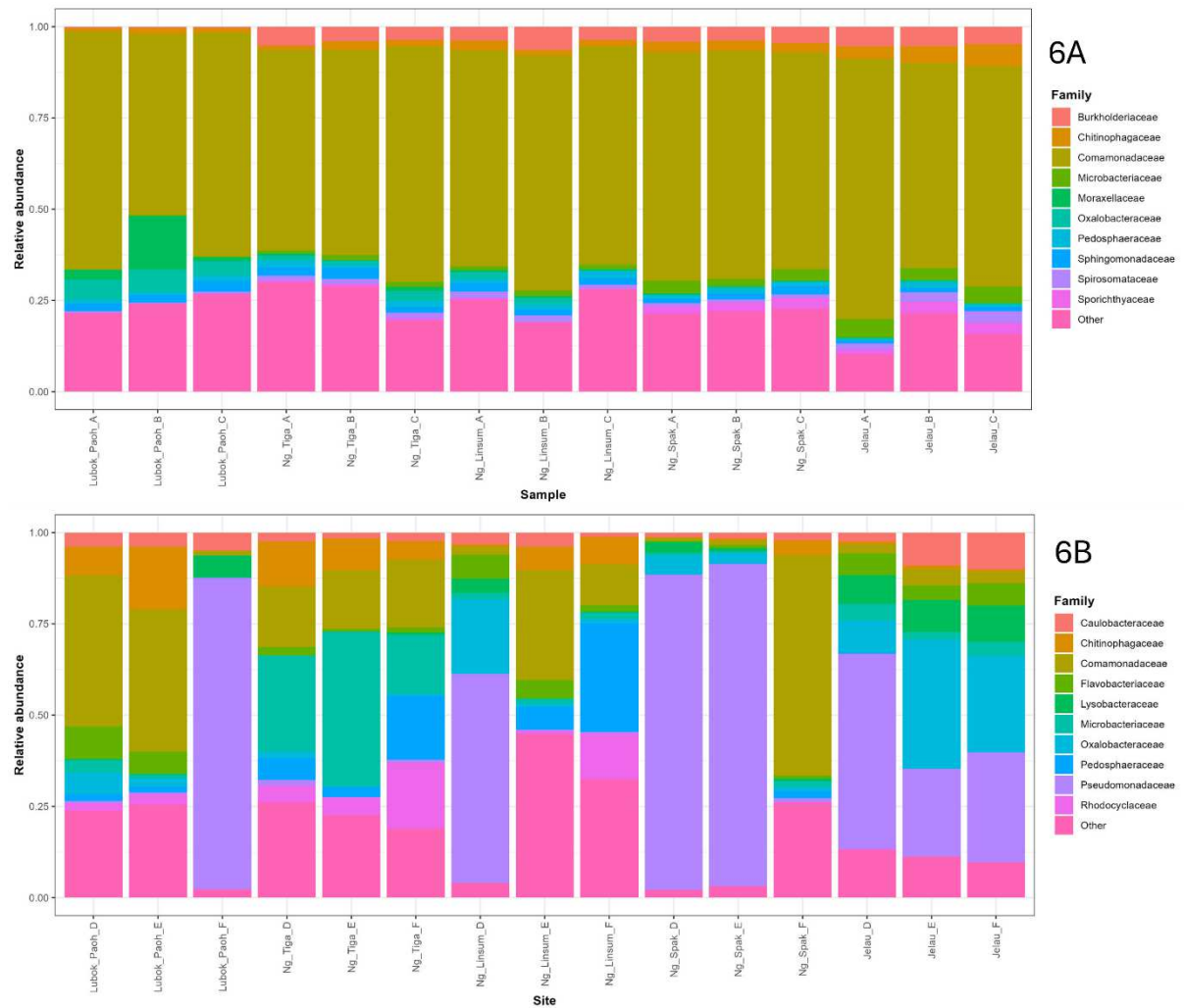


**Fig 6.** Histogram of relative abundance of the top 10 families, wet season (6A) and dry season (6B). The x-axis represents sampling sites, and the y-axis represents relative abundance presented as a decimal percentage. A, B, C= wet season samples; D, E, F= dry season samples.

In terms of genus-level composition, wet-season samples displayed broad diversity with no single dominant genus (Fig 7A). *Rhodoferrax* was abundant at Lubok Paoh (34–40%), while *Acinetobacter* dominated Lubok Paoh B (29%). *Polynucleobacter* (10–17%), *Curvibacter* (3–20%), *Limnohabitans* (4–12%), and *Candidatus Flaviluna* (3–10%) are consistently detected across sites. Eight genera, including *Acinetobacter* (<29%), *Aquabacterium* (<9%), *Brachymonas* (<4%), *Candidatus Omnitrophus* (<3%), *Cellvibrio* (<2%), *Inhella* (<8%), *Kinneretia* (<3%), and *Ramlibacter* (<5%), were exclusive to the wet-season top 20. In contrast, dry-season samples exhibited stronger site-specific dominance (Fig 7B). *Pseudomonas* dominated at Lubok Paoh F, Nanga Spak D and E (>90%), while *Candidatus Flaviluna* reached 21–53% at Nanga Tiga. Eight genera were exclusive to the dry-season top 20, including *Asticcacaulis* (<3%), *Flavipsychrobacter* (<3%; Jelau, 0.04%), *Luteolibacter* (<3%; 0.06 at Jelau, 0.06%), *Phenylobacterium* (<8%; absent at Jelau), *Rugomonas* (Jelau, 8–26%), *Rugosibacter* (Nanga Spak, 23%; Nanga Linsum, 17%), *SH3-11* (Nanga Tiga F, 18%; Nanga Linsum F, 40%), and *Stenotrophomonas* (Jelau, 8–10%).

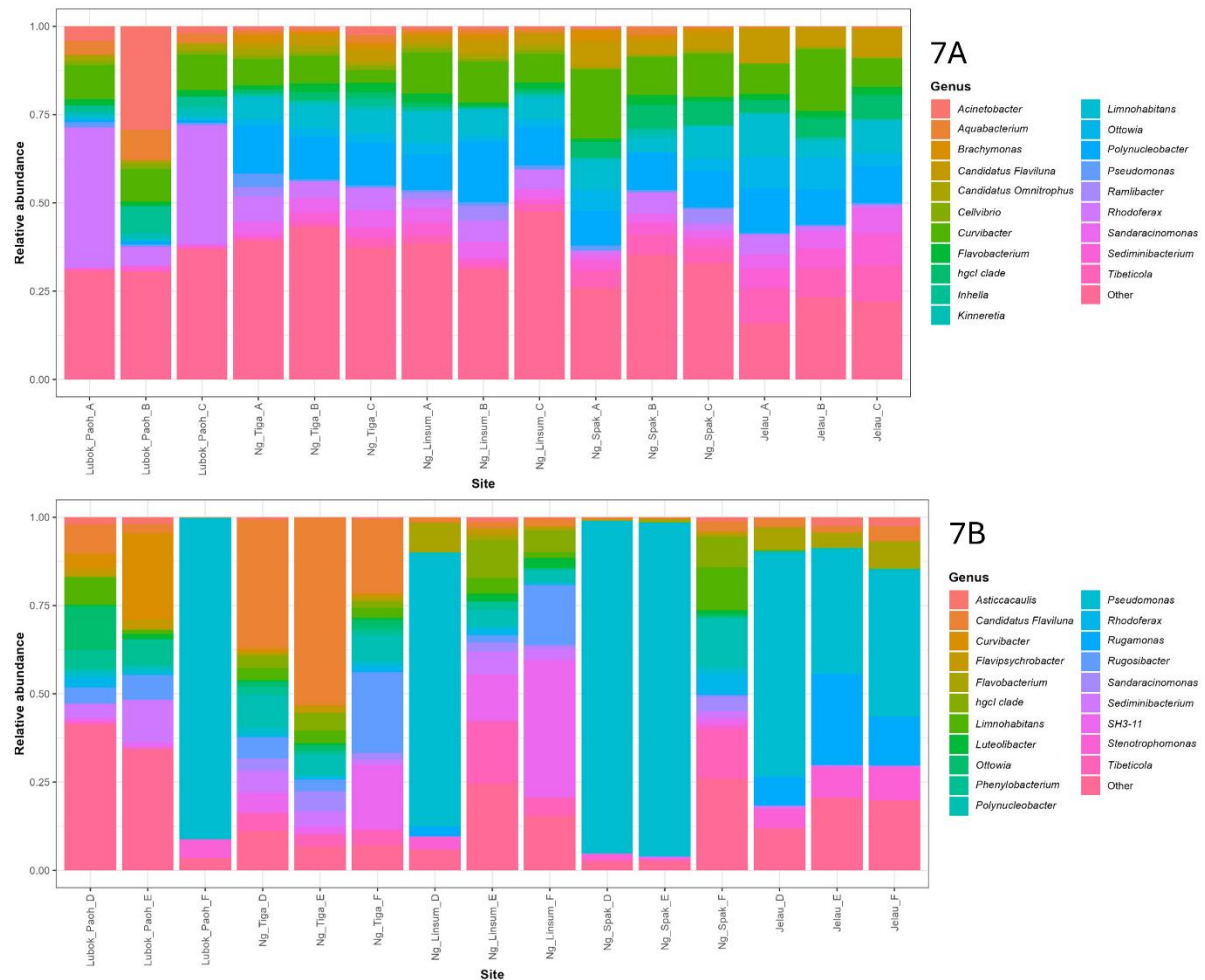


**Fig 7.** Histogram of relative abundance of the top 20 genera, wet season (7A) and dry season (7B). The x-axis represents sampling sites, and the y-axis represents relative abundance presented as a decimal percentage. A, B, C= wet season samples; D, E, F= dry season samples.

#### Core microbiome and putative faecal-associated genera

Analysis of the core microbiome identified 43 core genera in wet-season samples and 6 in dry-season samples, with only 5 bacterial genera shared across both seasons: *Pseudomonas* (64.8%), followed by *Candidatus Flaviiluna* (16.8%), *Polynucleobacter* (11.5%), *Sediminibacterium* (5.5%) and *Asticcacaulis* (1.4%) (Fig 8). Putative faecal-associated genera detected across sites included *Acinetobacter*, *Aeromonas*, *Arcobacter*, *Bacteroides*, *Enterococcus*, *Escherichia-Shigella*, *Klebsiella*, *Pseudomonas*, *Serratia*, and *Stenotrophomonas* (Fig 9). Seasonal stratification was evident. In the wet season, most genera exhibited low mean relative abundances (<2%) across all sites, except *Acinetobacter* (11.88% at Lubok Paoh). Classical indicators such as *Bacteroides* (0.02–0.18%), *Enterococcus* (0.01%), and *Escherichia-Shigella* (0.01–0.12%) were detected at low abundances. In contrast, the dry season showed reduced richness but clear dominance of specific genera. *Pseudomonas* was highly abundant at Lubok Paoh (31.28%), Nanga Linsum (26.36%), Nanga Spak (63.34%), and Jelau (46.33%), while *Stenotrophomonas* increased across all sites (0.30–7.91%), with the highest abundance at Jelau (7.91%). *Acinetobacter* (<0.04%) and *Bacteroides* (<0.09%) were undetected at Nanga Spak and Jelau, while *Serratia* occurred sporadically at trace levels (<0.03%) at Lubok Paoh and Nanga Linsum.

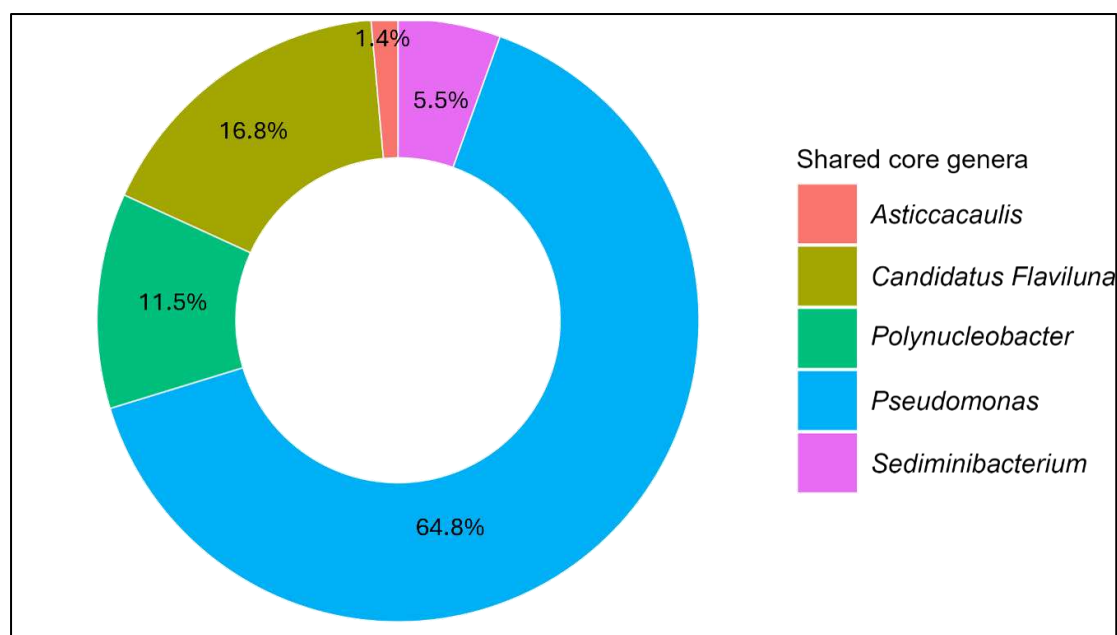


**Fig 8.** The doughnut shape of shared core genera across seasons and sites in abundance percentage.

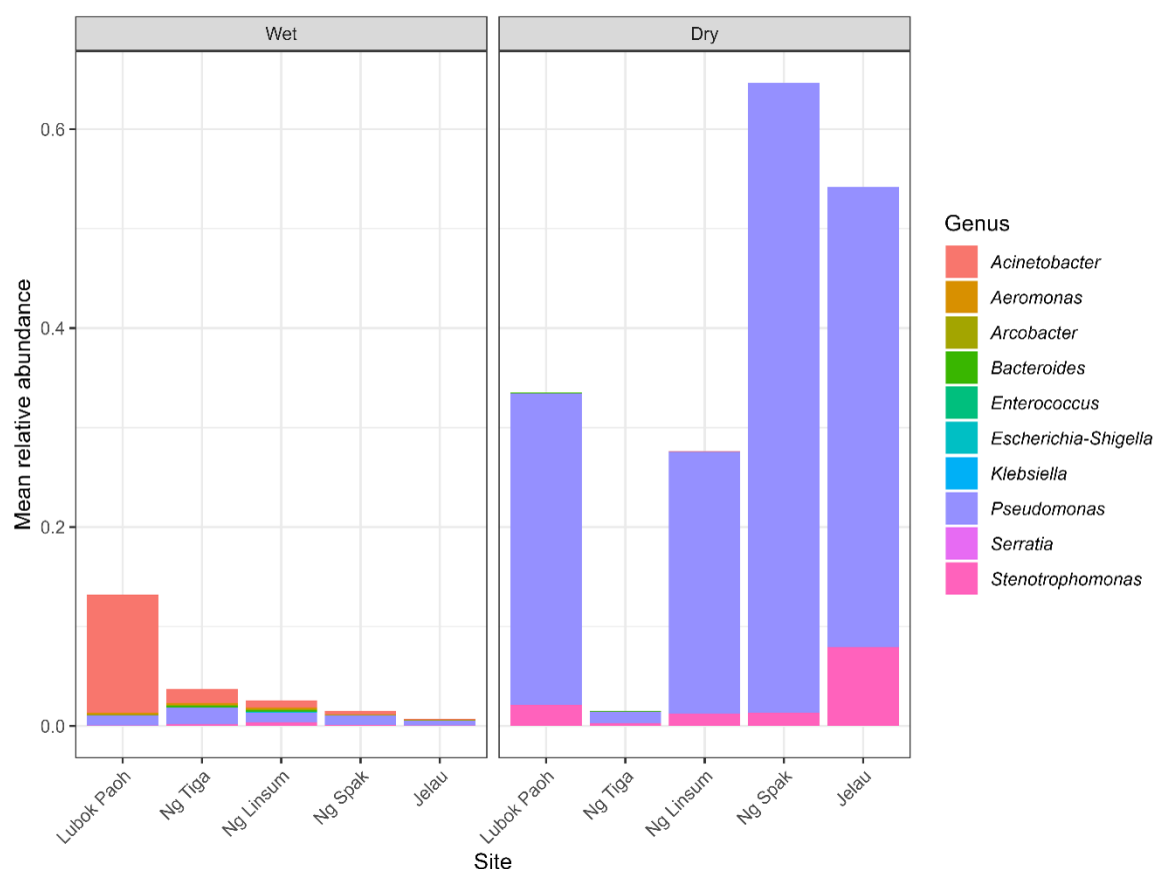


**Fig 9.** Histogram of mean relative abundance of putative faecal-associated genera across sites and seasons.

#### Environmental drivers of faecal-associated genera

The redundancy analysis (RDA) revealed that temperature (75.95%) and BOD (24.05%) were the most significant environmental variables ( $p < 0.05$ ) that influenced the distribution of faecal-associated genera in the Batang Layar watershed (Table 3). The overall RDA model was significant ( $F = 9.88$ ,  $p = 0.002$ ), with RDA1 explaining 95.5% and RDA2 4.5% of the constrained variance. and the community composition of the faecal-associated genera

across sites and seasons. The overall RDA model was significant ( $F = 9.88$ ,  $p = 0.002$ ), with RDA1 explaining 95.5% and RDA2 4.5% of the constrained variance. The RDA biplot (Fig. 10) clearly separated wet-season samples (negative RDA1 scores) from dry-season samples (positive RDA1 scores). Temperature was positively correlated with RDA1 (score = 0.886), while BOD was negatively correlated with RDA1 (score = -0.734), indicating that higher temperatures and lower BOD levels characterised dry-season conditions. High-BOD sites (mainly wet-season samples) clustered together, whereas high-temperature sites (mainly dry-season samples) formed a separate cluster. BOD was associated with the abundance of *Acinetobacter*, *Aeromonas*, *Arcobacter*, and *Bacteroides*, while temperature promoted an increase in *Pseudomonas*, consistent with its dominance during the dry season. The Lubok Paoh wet-season sample was separated from other wet-season samples along RDA2, suggesting distinct local conditions at this upstream forested site.

Table 3. Summary of RDA and forward selection of environmental variables influencing faecal-associated genera distribution in Batang Layar

| Analysis “constrained”                                                                                      |              |                  |       |       |        |
|-------------------------------------------------------------------------------------------------------------|--------------|------------------|-------|-------|--------|
| Method: RDA                                                                                                 |              |                  |       |       |        |
| Total variation is 0.209; explanatory variables account for 73.85% (Adjusted explained variation is 66.38%) |              |                  |       |       |        |
| Summary table                                                                                               |              |                  |       |       |        |
| Statistics                                                                                                  | Axis 1       | Axis 2           |       |       |        |
| Eigenvalues                                                                                                 | 0.147        | 0.007            |       |       |        |
| Pseudo-canonical correlation                                                                                | 0.977        | 0.212            |       |       |        |
| Explained fitted variation (cumulative)                                                                     | 95.50        | 100              |       |       |        |
| Forward selection results                                                                                   |              |                  |       |       |        |
| Environmental variables                                                                                     | Explains (%) | Contribution (%) | F     | P     | P(adj) |
| Temperature                                                                                                 | 75.95        | 75.95            | 15.01 | 0.001 | 0.002  |
| BOD                                                                                                         | 24.05        | 24.05            | 4.75  | 0.036 | 0.036  |

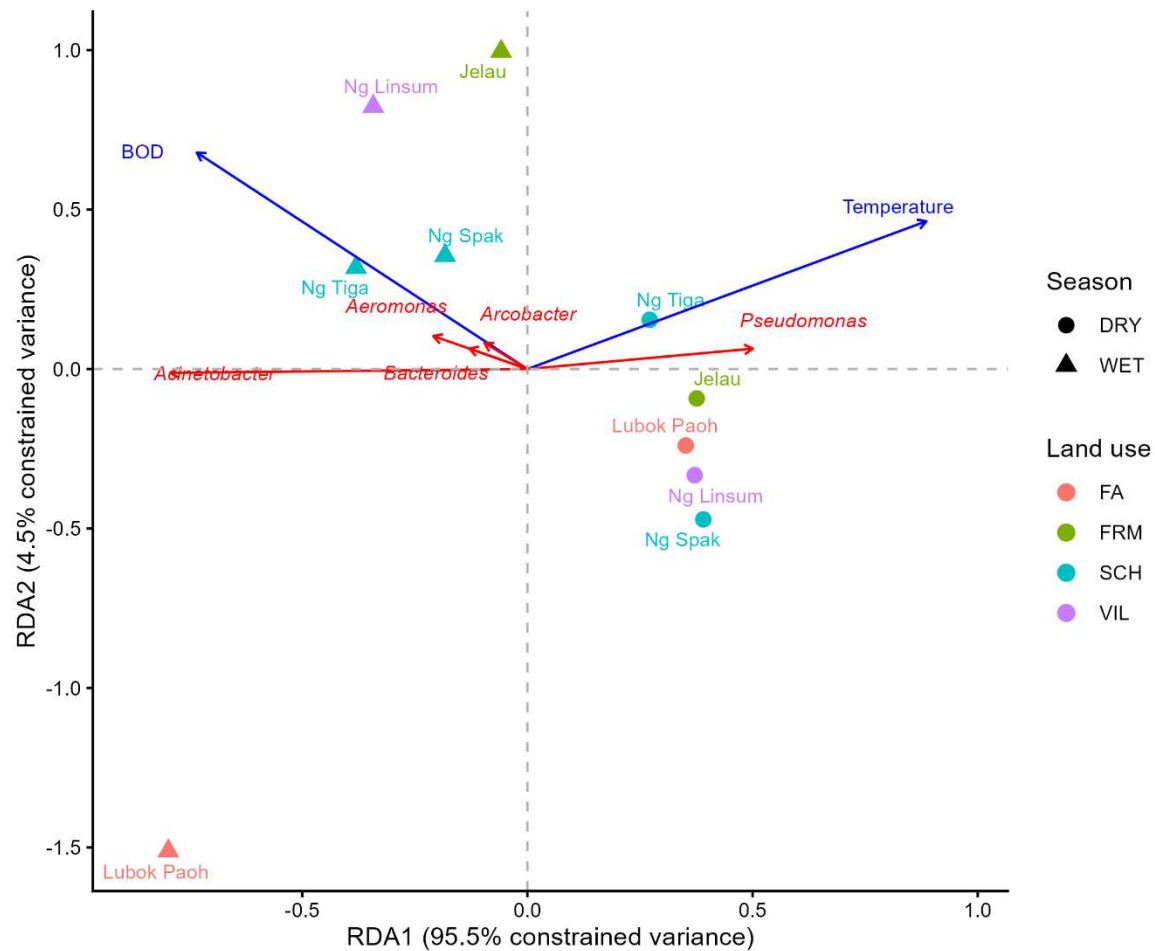


**Fig 10.** RDA biplot of environmental variables influencing faecal-associated genera (Top 5) in Batang Layar.

## Discussions

This study established a comprehensive baseline characterisation of the riverine bacterial community in Batang Layar, Sarawak, using high-throughput 16S rRNA amplicon sequencing across five sites and contrasting hydrological seasons (wet and dry seasons). The river harboured a taxonomically diverse bacterial community comprising 62 phyla, 340 families, and 700 genera, which has greater richness than some reported Malaysian rivers such as Perak River (Yang et al. 2025). Dominance of Pseudomonadota is consistent with observations from other tropical freshwater systems, reflects the phylum's broad metabolic capabilities, and tolerance to diverse environmental conditions (Boeraş et al. 2021; Li et al. 2023; de Oliveira et al. 2024; Yang et al. 2025). Subdominant phyla included Actinomycetota, Bacteroidota, Patescibacteria, and Verrucomicrobiota, which together formed a structural core community, aligning with reports from tropical rivers in China, Brazil, and Malaysia, as these groups are closely linked to organic-nutrient cycling (Boeraş et al. 2021; Lv et al. 2021; Li et al. 2023; de Oliveira et al. 2024; Yang et al. 2025).

Seasonal hydrology emerged as the strongest tested factor associated with bacterial community structure, outweighing the effects of spatial variation among sites. Alpha diversity was significantly higher in the wet season, with richer and more evenly distributed communities, while dry-season communities exhibited reduced richness and greater site-specific heterogeneity. These findings are consistent with studies from the Yellow River and other river systems where wet-season conditions enhanced richness and diversity (Aguilar et al. 2025; Stefaniak et al. 2026; Thorpe et al. 2026; Wang et al. 2026). The absence of significant alpha diversity differences among sites in Batang Layar parallels observations from the Nandu River (Li et al. 2023) and other systems (Kodera et al. 2023; Jia et al. 2025) where hydrological connectivity acts as a homogenising force that constrains site-specific divergence. In contrast, studies from anthropogenically pressured systems such as the Perak River and Bandar Sunway River reported significant site-specific differences driven by land-use pressure, suggesting that

anthropogenic filters can override seasonality as the dominant driver (Ting et al. 2021; Yang et al. 2025). The comparative analysis suggests a gradient from systems where seasonal filtering dominates (Batang Laya) to systems where anthropogenic filtering dominates (Perak River and Bandar Sunway River), demonstrating that strong site effects emerge where external human impacts impose distinct ecological filters.

Beta diversity analyses confirmed clear wet-dry separation, with PERMANOVA attributing 26.2% of community variation to season compared to non-significant site effects. The significant Season  $\times$  Site interaction indicated that seasonal restructuring varied among sites, reflecting modulation by local environmental conditions. In addition, this study's diversity pattern mirrored She et al. (2024), with microbial communities in intensive agricultural watersheds clustering in the wet season but dispersing in the dry. PERMDISP further revealed that wet-season communities were more homogeneous, while dry-season communities exhibited greater heterogeneity, consistent with greater hydrological mixing during the wet season and stronger local selection under dry, low-flow conditions (Jiang et al. 2023; Chen et al. 2025; Ogola et al. 2025). These findings indicate that seasonal variability was strongly associated with the riverine microbiome composition than spatial heterogeneity, while land use modulates site-specific responses to seasonal variation (Garlapati et al. 2021; Ting et al. 2021).

Core microbiome analysis revealed a dynamic seasonal pattern, from 43 genera in the wet season contracting to six in the dry season, with only five genera persisting across both seasons. This expansion-contraction pattern reflects resilience expressed through multi-taxon coexistence under high connectivity, with dominance contraction under dry-season stress, yet a small set of persistent taxa anchors both. The shared core comprising *Pseudomonas*, *Candidatus Flaviluna*, *Polynucleobacter*, *Sediminibacterium* and *Asticcacaulis* represents a persistent component of the Batang Laya bacterial community, underscoring their role as ecological sentinels across disturbance regimes (Custer et al. 2023; Mayol et al. 2026).

Putative faecal-associated genera exhibited a distinct seasonal shift from a diverse assemblage of low-abundance classical indicators (*Escherichia-Shigella*, *Enterococcus*, *Bacteroides*) in the wet season to site-specific dominance of environmental opportunists (*Pseudomonas* and *Stenotrophomonas*) in the dry season. This pattern is consistent with episodic runoff-driven inputs during high flow and concentration effects under low-flow conditions (Mushi et al. 2021; Alkhadher et al. 2025). Classical indicators such as *Bacteroides* and *Enterococcus* align with their recognition as promising microbial source tracking markers due to their prevalence in faecal matter and anaerobic growth (Khodaparast et al. 2024). The detection of both *Bacteroides* and *Enterococcus* in this study warrants further investigation of their potential relevance to public health significance in this rural tropical river. The elevated abundance of *Pseudomonas* during the dry season aligns with observations from the Yamuna River, where opportunistic pathogens persist under environmental stress (Raghav et al., 2024). Similarly, increased *Stenotrophomonas* abundance in the dry season parallels reports from rural Cameroonian household water, where it was identified as an opportunistic contaminant with public health relevance (Bae et al. 2019). In contrast, *Acinetobacter* exhibited peak abundance during the wet season at the upstream forested site (Lubok Paoh), plausibly linked to natural inputs including wildlife faecal matter (Dahiru and Enabulele 2015; Wilharm et al. 2026), as *Acinetobacter* occupies multiple ecological niches across environments, animals, and humans (Kisková et al. 2023; Benoit et al. 2024). This contrast underscores that faecal-associated genera in rivers may arise from both human and wildlife inputs, complicating source attribution in rural catchments.

Redundancy analysis (RDA) identified temperature and biochemical oxygen demand as the environmental variables most strongly associated with the distribution of faecal-associated genera. These findings revealed two distinct seasonal pathways: an organic-enriched wet season favouring *Aeromonas*, *Acinetobacter*, *Arcobacter*, and *Bacteroides* through rainfall-induced runoff and terrestrial inputs, and a temperature-driven dry season promoting *Pseudomonas* dominance under warmer, low-flow conditions. Similar responses have been reported for other faecal-associated genera under high-BOD conditions (Marie and Lin, 2018; Dimpot et al., 2025). At the basin scale, land use further modulates these seasonal dynamics; agricultural and urban expansion amplify runoff-driven contamination, whereas forested areas may reduce these impacts (Pakoksung et al. 2025). This pattern is

evident in Batang Layar, where wet-season effects are more pronounced in impacted areas, while the upstream forested site (Lubok Paoh) showed distinct separation along RDA2, suggesting that forested areas may buffer contamination and support distinct faecal-associated assemblages despite similar rainfall inputs. The RDA thereby provide quantitative evidence that seasonal hydrology, operating through thermal regime (dry season) and organic loading (wet season), governs the distribution of faecal-associated genera in the Batang Layar watershed. These advances understanding beyond presence-absence characterisation by providing evidence for associations between environmental conditions and seasonal bacterial assemblages.

The findings demonstrate that 16S rRNA amplicon sequencing, when integrated into seasonally stratified monitoring frameworks, provides valuable insights beyond conventional faecal indicator bacteria. This approach agrees with other studies (Bae et al. 2019; Mittal et al. 2019; Acharya et al. 2020; Garner et al. 2021; Yang et al. 2025) that applying NGS in river monitoring extends pathogen risk assessment, in which the current study captures community-level ecological dynamics, identifies persistent core taxa and seasonal opportunists, and elucidates environmental drivers of faecal-associated genera distribution. Such integrated molecular surveillance is particularly relevant for rural tropical catchments like Batang Layar, where conventional monitoring fails to capture the full spectrum of microbial contaminants and their seasonal variability. However, the application of NGS in water practice presents challenges, including cost, the requirement for specialised expertise and equipment, complex data analysis and interpretation, and the current lack of standardised protocols, all of which must be addressed before proper integration into national water quality frameworks (Garner et al. 2021). Complementing amplicon sequencing with targeted PCR assays can further provide community-level ecological insights while maintaining the capacity for specific pathogen detection (Bae et al. 2019; Acharya et al. 2020). The identification of persistent core genera and seasonally responsive taxa provides candidates for developing molecular indicators or ecological metrics tailored to Sarawak's riverine systems, supporting the long-term goal of embedding microbial ecology within One Health surveillance frameworks for sustainable water resource management in developing tropical regions.

## Conclusions

The Batang Layar riverine bacterial community profiles revealed a diverse bacterial community with Pseudomonadota dominant throughout this study. Seasonal variation was the strongest factor associated with bacterial community structure, outweighing land-use effects. Alpha diversity was significantly greater in richness and evenness during the wet season compared to the dry season, while beta diversity confirmed clear wet-dry separation, as the season explained 26.2% of the community variation. The core microbiome contracted from 43 (wet) to 6 (dry) genera, with only 5 genera persisting across seasons. Putative faecal-associated genera shifted from classical indicators in the wet season to opportunistic genera (*Pseudomonas*, *Stenotrophomonas*) in the dry season. Temperature (75.95%) and BOD (24.05%) were identified as significant environmental drivers of the distribution of faecal-associated genera. These findings demonstrate the value of seasonally stratified molecular monitoring for water quality assessment in rural tropical catchments, providing critical baseline data on Borneo's riverine microbiome and supporting the integration of NGS into routine water surveillance frameworks. Future research should expand sampling across multiple rural tropical rivers for robust cross-system comparisons, incorporate metagenomic approaches to investigate functional gene profiles and antimicrobial resistance determinants, and integrate molecular data with quantitative microbial risk assessment frameworks to better inform public health interventions.

## Declarations

*Ethical approval: Not applicable*

*Data availability:* The R scripts used for the data analyses in this study are available from the first and second authors upon reasonable request.

*Conflict of interest*

The authors declare no conflict of interest

## Funding

This research was funded by Universiti Malaysia Sarawak, Malaysia (High Impact Research Grant no. UNI/I01/VC-HIRG/85485/P03-02/2022).

## Author contributions statement

Conceptualisation, SJ, SL, AO and MAM; Formal analysis, SJ and AO; Funding acquisition, SL; Investigation, SJ; Writing - original draft, SJ; Writing – review & editing, SJ, SL, AO and MAM.

## Acknowledgments

We are highly thankful to the Universiti of Malaysia Sarawak for funding this research. This manuscript was corrected for language issues using Grammarly.

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