Profiling the surface water bacterial communities of riverine mangroves of Placer, Surigao del Norte, Philippines using 16S rRNA eDNA metabarcoding

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Abstract. Riverine mangroves provide critical ecosystem services yet remain understudied in the Philippines. This study profiled the surface water bacterial communities of the Amoslog and Panhutongan riverine mangroves in Placer, Surigao del Norte, using 16S rRNA eDNA metabarcoding and PICRUSt2 functional prediction. Water samples were collected from six sites (downstream, midstream, upstream) across both rivers, and 12 amplicon libraries were sequenced on the Illumina MiSeqi100 Plus platform. High-throughput sequencing generated 1,009,764 amplicon sequence variants (ASVs) representing 337 families and 758 genera. The most dominant taxa were uncultured bacteria, *HIMB11*, and *Synechococcus CC9902*, whose distribution patterns reflected tidal marine influence and salinity gradients. Beta diversity analysis (PCoA) revealed distinct river-specific clustering driven by contrasting salinity regimes: freshwater in the Amoslog River (0.03-0.43 ppt) versus brackish conditions in the Panhutongan River (1.95-2.69 ppt). PICRUSt2 predictions indicated functional potential for organic matter cycling, xenobiotic biodegradation, and nitrogen metabolism across all sites. However, the detection of *Acinetobacter* sp. and *Flavobacterium* sp. signals potential public health and aquaculture concerns. This study provides the first microbial baseline for these riverine mangroves, offering bacterial indicators that can inform water quality monitoring and conservation strategies in mining- and aquaculture-impacted coastal ecosystems.

Keywords: bacteria, eDNA metabarcoding, mangrove microbiome, physico-chemical parameters, Surigao del Norte.

Introduction. The mangrove habitat is one of the most productive and ecologically important transitional ecosystems on earth, where terrestrial, freshwater, and marine ecosystems are interconnected (Alongi 2008). They are also unique ecosystems that deliver irreplaceable ecosystem services, including coastal protection against storms and erosion, flood control, blue carbon sequestration, water filtration, nutrient cycling, and as vital nursery habitats for fish, crustaceans, and mollusks (Duke et al 2007; Barbier et al 2011). In contrast, the integrity of mangrove functions is tightly coupled to the quality of the surrounding water, especially in riverine-associated mangroves, where freshwater inflow from upstream affects physicochemical and biological conditions.

The riverine mangroves of Placer, Surigao del Norte, particularly along the Amoslog and Panhutongan Rivers, are facing increasing anthropogenic pressures. Placer is a municipality historically known for its mining activities, growing aquaculture ponds, agricultural and livestock runoff, and domestic wastewater discharge. These activities can contribute organic pollutants, heavy metals, and excess nutrients to mangrove waterways, which may affect the microbial community structures (Kathiresan & Bingham 2001). Although these mangroves are known to be vulnerable, no detailed microbiological analysis has been done in this particular locality.

The mangrove water bacterial community is a special case for monitoring environmental changes. They control important biogeochemical reactions such as sulfate reduction, nitrification, denitrification, and the degradation of organic matter (Holguin et al 2001). Bacterial community composition (BCC) can change before any visible changes in macrofauna and/or vegetation occur, indicating pollution stress, eutrophication, or heavy metal contamination. Therefore, profiling of BCC can be a very effective tool for early-warning biomonitoring. Traditional culture-based techniques, however, account for only 1% of the true bacterial diversity, and molecular methods are essential (Amann et al 1995).

To mitigate this limitation, this study uses 16S rRNA eDNA metabarcoding, a molecular-based, high-throughput approach that amplifies and sequences the hypervariable regions of the 16S ribosomal RNA gene from environmental DNA (eDNA). This allows for an in-depth understanding of bacterial communities without the need for cultivation (Taberlet et al 2012). In addition, 16S rRNA data can be used to infer potential ecological functions, for example, xenobiotic degradation, nitrogen cycling, and pathogenicity, using the PICRUSt2 pipeline (Douglas et al 2020).

The application of 16S rRNA sequencing techniques has illuminated the diversity and ecological importance of mangrove microbial communities worldwide. The bacterial diversity in mangrove sediments has been investigated in China, India, and Brazil, and representative taxa involved in nitrogen cycling, hydrocarbon degradation, and pollutant detoxification have been identified (Basak et al 2015). These results emphasize the ecological significance of bacteria in the health and resiliency of mangroves and that these bacteria could be utilized in biotechnological applications, including bioremediation. In the Philippines, however, there is a paucity of studies on the mangrove microbiome. A recent study in South Asia used 16S rRNA sequencing to characterize bacterial communities in surface water samples from the Sundarbans mangrove and identified various bacterial taxa with the potential to metabolize and biodegrade xenobiotics (Ghosh et al 2022). However, the studies conducted are limited, and their coverage is also limited; baseline data on the bacterial community in most mangrove areas in the country are still lacking.

Therefore, to compensate for this, the study seeks to address the total absence of baseline information on the riverine mangroves of Placer, Surigao del Norte. Apart from adjacent areas in the Caraga region, which were studied for mangrove floristic composition or heavy metal content, no studies have been conducted on waterborne bacterial communities in the Amoslog and Panhutongan Rivers using 16S rRNA metabarcoding. In the absence of this baseline data, it will be impossible to identify future microbial changes driven by mining discharges, aquaculture effluents, crop-livestock runoff, or climate change.

This study aims to profile the surface water BCC of the two riverine mangroves (Amoslog River and Panhutongan River) in Placer, Surigao del Norte, using 16S rRNA eDNA metabarcoding, coupled with functional prediction via PICRUSt2. If robust results are found, for example, a prevalence of putative pollution-tolerant taxa, or the lack of sensitive indicator groups, then the results can be used as a science-based guideline for local authorities to enhance water quality monitoring and mangrove conservation measures. Ultimately, this research will provide the first baseline microbial reference for future long-term ecological evaluations and informed decision-making within Placer's riverine-mangrove ecosystems.

Material and Method

Study area. This study was conducted to analyze BCC in two rivers in Placer, Surigao del Norte, Philippines (Figure 1). This municipality has a mangrove-associated riverine system, where the Amoslog and Panhutongan Rivers drain into the coastal area. Six sampling sites were established across both rivers, with three sites per river — downstream, midstream, and upstream: Amoslog River (downstream: ADR1, ADR2; midstream: AMR1, AMR2; upstream: AUR1, AUR2) and Panhutongan River (downstream: PDR1, PDR2; midstream: PMR1, PMR2; upstream: PUR1, PUR2). Prior-informed consent was obtained from officials of Barangay Amoslog and Barangay Panhutongan, as well as from the municipal Local Government Unit (LGU) of Placer.

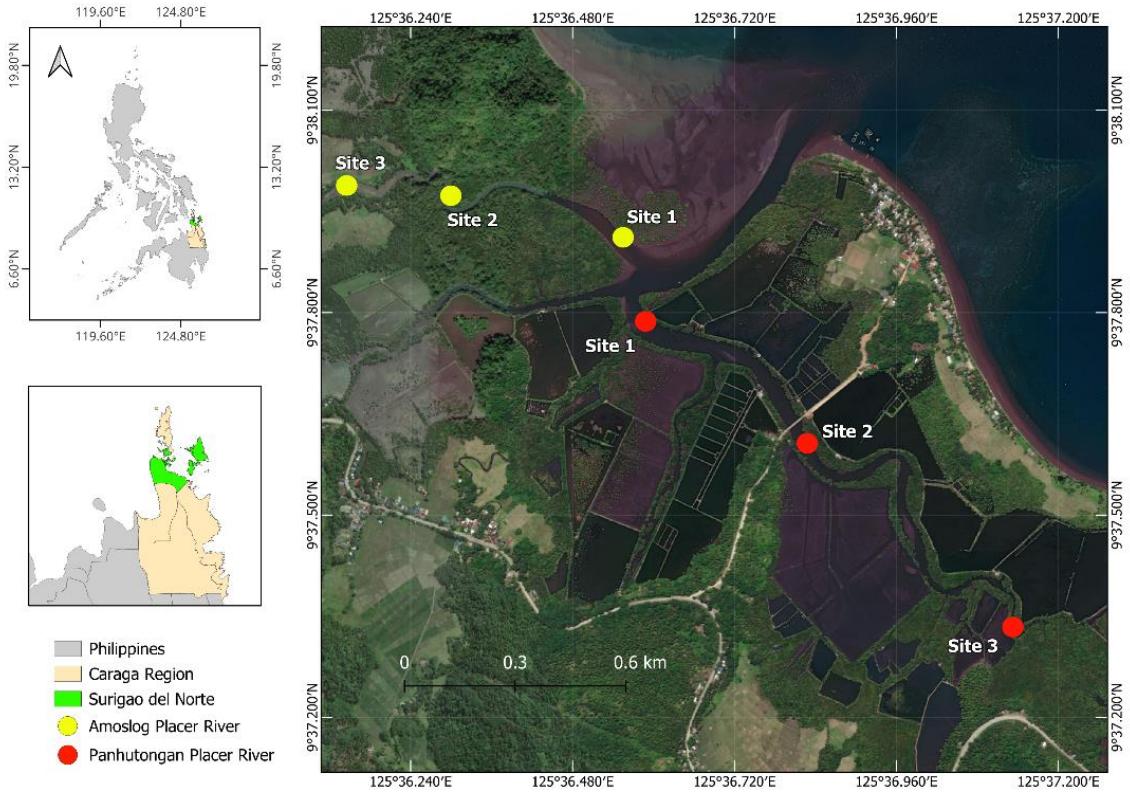

Figure 1. Map of the study area showing the sites in Amoslog Riverine mangroves (Site 1: downstream, Site 2: midstream, Site 3: upstream) and Panhutongan River (Site 1: downstream, Site 2: midstream, Site 3: upstream), Placer, Surigao del Norte, Philippines.

Sample collection. Sampling was done within the one-month (December 2025) period. Water samples were collected and processed in replicates. At each sampling site, 5 liters of water were obtained, and three subsamples were randomly collected at 3-meter intervals and combined to form a composite sample, following the method of Eupeña-Caray et al (2024) with slight modifications. Physicochemical parameters, including water temperature, pH, salinity, and dissolved oxygen (DO), were measured in situ. The collected water samples were filtered on-site using a sterile 60 mm Büchner funnel with a 50 mm × 0.22 µm polyethersulfone (PES) membrane. Filtration was performed aseptically to avoid contamination. The membrane was then placed in sterile capped containers, stored in an icebox, and transported to the Molecular Systematics and Conservation Genomics Laboratory at the Center for Biodiversity Studies and Conservation (CBSC), Premier Research Institute of Science and Mathematics (PRISM), Mindanao State University–Iligan Institute of Technology (MSU-IIT), for eDNA extraction.

DNA extraction, amplification, and MiSeq sequencing. Prior to eDNA extraction, water samples underwent centrifugation at 13,000 rpm for at least 10 minutes to concentrate microbial biomass. eDNA from water samples was extracted using the HiPurA™ DNA Extraction Kit (Vadhani Industrial Estate, Mumbai, India) following the manufacturer’s protocol. Extracted eDNA was evaluated using gel electrophoresis in Certified Molecular Biology Agarose gel (Bio-Rad) in 1× TBE buffer using a Cleaver Scientific electrophoresis system (MSMINIONE), and DNA concentration and purity were assessed using a NanoDrop Spectrophotometer, before samples were sent to Macrogen, South Korea, for high-throughput sequencing on the Illumina MiSeqi100 Plus platform. Universal bacterial primers targeting the V3-V4 region — Bakt_341F: CCTACGGGNGGCWGCAG and Bakt_805R: GACTACHVGGGTATCTAATCC — were used for amplification of the 16S rRNA gene (Tabugo et al 2024). A total of 12 amplicon libraries were generated across the two rivers: Amoslog (ADR1, ADR2, AMR1, AMR2, AUR1, AUR2) and Panhutongan (PDR1, PDR2, PMR1, PMR2, PUR1, PUR2), corresponding to downstream, midstream, and upstream sampling sites.

***Data processing**.* Fast QC was used to check the quality of sequences. Resulting sequences were deposited to NCBI GenBank and made publicly available under the accession nos. SRR38839784-SRR38839795. Raw sequencing data underwent Fast Length Adjustment of Short Reads (FLASH) processing to merge paired-end reads, with a minimum overlap of 15 bp and a maximum overlap of 150 bp, thereby combining the higher-quality base calls from both reads into a single read. Further, processing of reads were done using the Parallel-Meta Suite (PMS) pipeline available at https://github.com/qdu-bioinfo/parallel-meta-suite. Within PMS, the amplicon sequences were denoised and screened for chimeras to generate high-resolution Amplicon Sequence Variants (ASVs), and taxonomic profiling was built by aligning the ASVs against the SILVA database using the built-in VSEARCH for accurate classification.

The functional potential of the bacterial communities was inferred from 16S rRNA data using the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) algorithm, integrated into the PMS pipeline. The predicted genes were annotated against the Kyoto Encyclopedia of Genes and Genomes (KEGG) Orthology (KO) database. Metabolic pathways were further classified according to the KEGG BRITE hierarchy. The prediction accuracy of these metagenomic functions was assessed using the Nearest Sequenced Taxon Index (NSTI), which calculates the sum of phylogenetic distances between each ASV and its nearest sequenced relative in the reference database. Alpha diversity indices — including the Shannon index (species richness and evenness), Simpson's index (dominance), and Chao1 (richness estimator) — and beta diversity, visualized as principal coordinate analysis (PCoA), were calculated per sample using pairwise distance matrices with the unweighted/weighted Meta-Storms algorithm for taxonomy and Hierarchical Meta-Storms for functional profiles; the results were then plotted as a heatmap. A co-occurrence network was constructed to examine the interactions among dominant bacterial taxa. In this network, nodes represented individual taxa (e.g., genera), and edges represented substantial Spearman correlation values. Key network properties, including density, diameter, radius, and centralization, were computed to quantify the overall structure and complexity of the microbial community interactions (Chen et al 2021; Eupeña-Caray et al 2024).

Results and Discussion. High-throughput sequencing of the V3-V4 region of the 16S rRNA gene generated a total of 1,009,764 Amplicon Sequence Variants (ASVs) from 12 amplicon libraries, representing 337 families and 758 genera across the six sampling sites of the Amoslog and Panhutongan riverine mangroves (downstream, midstream, upstream) (Figure 2).

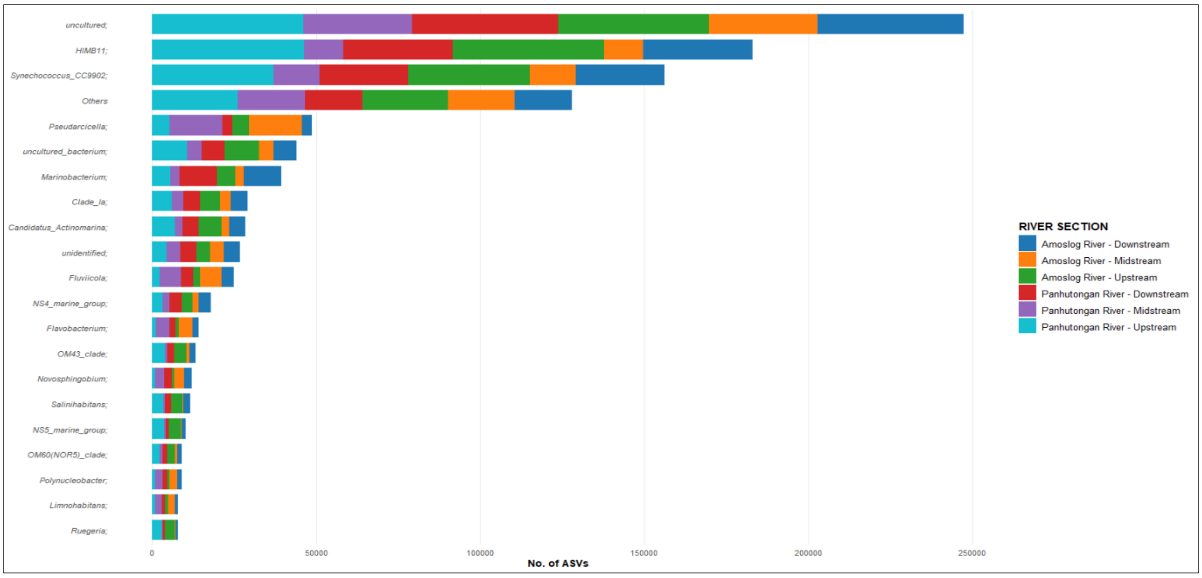

Figure 2. Amplicon sequence variants (ASVs) count comparison between sites (downstream, midstream, upstream) in the Amoslog and Panhutongan riverine mangroves, Placer, Surigao del Norte, Philippines.

Results showed the dominant genera detected across all sampling stations were uncultured, *HIMB11*, *Synechococcus_CC9902*, *Pseudarcicella*, uncultured_bacterium, *Marinobacterium*, *Clade_Ia*, *Candidatus_Actinomarina*, unidentified, *Fluviicola*, NS4_marine_group, *Flavobacterium*, *OM43_clade*, *Novosphingobium*, *Salinihabitans*, NS5_marine_group, OM60(NOR5) _clade, *Polynucleobacter*, *Limnohabitans*, and *Ruegeria*.

The relative abundance of each BCC across sampling stations is shown in Figure 3. Within each river, the dominant genera sorted into three ecological guilds whose distribution patterns mapped onto a consistent downstream-midstream-upstream gradient driven by tidal marine influence, freshwater discharge, and anthropogenic inputs.

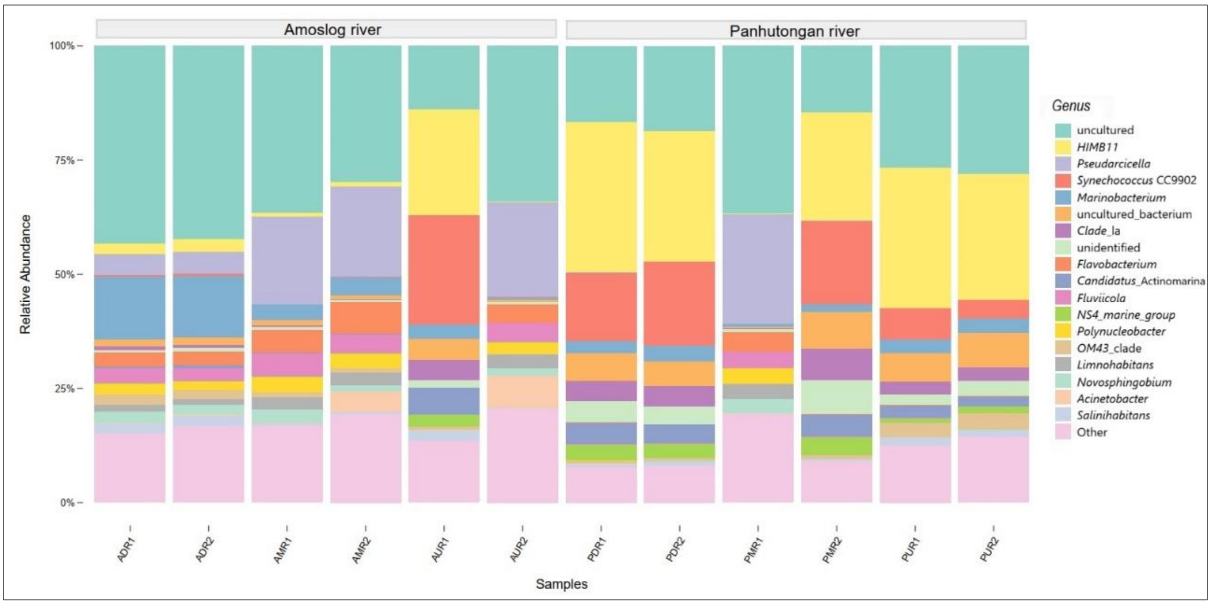

Figure 3. Relative abundance of bacterial community composition (BCC) between sites in Amoslog riverine mangroves (downstream-ADR1, ADR2; midstream-AMR1, AMR2; upstream-AUR1, AUR2) and Panhutongan riverine mangroves (downstream-PDR1, PDR2; midstream-PMR1, PMR2; upstream-PUR1, PUR2), Placer, Surigao del Norte, Philippines.

***Guild 1*: *marine*-*influenced taxa*.** A diverse group of phylogenetically distinct taxa shared a common distribution pattern: highest relative abundance at the upstream and downstream stations, with marked suppression at the midstream zone. This guild included *HIMB11*, *Synechococcus CC9902*, *Clade_Ia*, *Candidatus_Actinomarina*, the NS5 marine group, the OM60(NOR5) clade, *Ruegeria*, and *Salinihabitans*. Their congruent distribution points to a shared environmental control: tidal seawater that intrudes into the upper reaches as a saltwater wedge and simultaneously influences the downstream zone, while the midstream represents a transitional dilution zone where salinity drops to its minimum and marine-adapted lineages are excluded.

HIMB11 is a marine bacterioplankton strain belonging to the ubiquitous and metabolically versatile *Roseobacter* clade (family Rhodobacteraceae, class Alphaproteobacteria). It is an environmental opportunist that persists on a few reduced substrates and alternative energy metabolisms until conditions favor rapid growth, such as during a phytoplankton bloom (Durham et al 2014). Ecologically, the occurrence of *HIMB11* may indicate active cycling of microbial carbon and sulfur. Its genome encodes bacteriochlorophyll-based aerobic anoxygenic phototrophy, degradation of the algal compound dimethylsulfoniopropionate (DMSP) with concomitant production of the climate-relevant gas dimethylsulfide (DMS), and oxidation of the greenhouse gas carbon monoxide (CO) (Durham et al 2014). Beyond its metabolic versatility, *HIMB11* also contributes to vitamin B12 biosynthesis via the aerobic pathway in coastal waters (Beauvais et al 2023). In the present study, *HIMB11* was the second most abundant bacterial genus detected within the riverine-mangrove ecosystem (Figure 2). The strain exhibited its highest relative abundance at the upstream station, followed by the downstream and midstream stations (Figure 3). The elevated *HIMB11* abundance may reflect post-storm conditions during sampling. Low-pressure disturbances during the northeast monsoon, coupled with increased runoff, likely enhanced nutrient input, organic matter transport, and phytoplankton productivity within the ecosystem. Yeo et al (2013) originally reported that the *Roseobacter* clade lineage containing *HIMB11* became highly abundant after storm-induced phytoplankton blooms in Kaneohe Bay, coastal Hawaii. Durham et al (2014) later confirmed *HIMB11* as one of the taxa exhibiting this post-storm enrichment. This disturbance-associated pattern has since been documented following El Niño-driven disruption in the Galapagos Archipelago (Gifford et al 2020), further supporting the interpretation of *HIMB11* as a broadly adapted opportunist that thrives in nutrient-enriched, biologically productive, and disturbed aquatic systems regardless of season.

Another notable, *Synechococcus* CC9902, is a unicellular picocyanobacterium belonging to the phylum Cyanobacteriota, family Synechococcaceae, and is classified within marine clade IV of sub-cluster 5.1 (Scanlan et al 2009). Marine *Synechococcus* species are among the most abundant photosynthetic organisms in the global ocean and contribute substantially to aquatic primary production and carbon fixation (Scanlan et al 2009). Clade IV strains, including CC9902, are typically associated with coastal and estuarine environments and possess efficient nutrient-scavenging adaptations; they allocate a high proportion of their membrane transport resources toward phosphate uptake, enabling persistence under variable nutrient conditions (Teoh et al 2020). In the present study, *Synechococcus* CC9902 was the third most abundant taxon detected. It exhibited its highest relative abundance at the upstream stations, followed by the downstream stations, and its lowest at the midstream stations (Figures 2 and 3). The strain was more abundant in the Panhutongan River, where salinity was higher (1.95-2.69 ppt) than in the Amoslog River (0.03-0.43 ppt), consistent with CC9902's known preference for marine-influenced waters. Kim et al (2018) demonstrated in the Kwangyang Bay estuary that CC9902 was enriched at open-sea sites with salinity exceeding 30 ppt. In contrast, the freshwater-influenced river mouth was dominated by a different *Synechococcus* lineage (RCC307), confirming that salinity gradients are a key factor in partitioning *Synechococcus* populations along river-to-sea continua. The detection of CC9902 in the present riverine-mangrove system at salinities substantially lower than its typical marine range suggests that tidal seawater intrusion transports marine picocyanobacteria into the river reaches, and that the upstream and downstream stations represent zones where tidal influence, light availability, and nutrient conditions favor the persistence of this lineage. Furthermore, *Synechococcus* strains, including clade IV representatives, exhibit enhanced tolerance to copper and oxidative stress through the expression of genomic island genes (Stuart et al 2009; Stuart et al 2013) and possess abundant Type II toxin–antitoxin systems that enable a recoverable persistent state under adverse conditions (Fucich & Chen 2020). These stress-tolerance mechanisms are particularly relevant to the present study area because the municipality of Placer has a history of mining, and the presence of metal-tolerant *Synechococcus* lineages may reflect long-term adaptation of the microbial community to metal-enriched conditions in the riverine-mangrove environment. The observed distribution pattern of CC9902, elevated at both upstream and downstream stations but suppressed at midstream, may therefore reflect not only salinity and tidal gradients but also differential heavy metal tolerance across the three zones. Furthermore, *Synechococcus* distribution patterns have been employed as indicators of trophic status and water quality in tropical and subtropical coastal waters (Rajaneesh et al 2015; Kolda et al 2020), supporting the potential utility of CC9902 relative abundance as a bioindicator of tidal marine influence and environmental conditions in Philippine riverine mangrove ecosystems.

The NS5 marine group, OM60(NOR5) clade, and *Ruegeria* each showed upstream-peaking abundance consistent with their marine character and known salinity preferences (Liu et al 2015; Seo et al 2017; Wang et al 2021; Li et al 2024). *Candidatus Actinomarina*, an ultra-small streamlined photoheterotroph (Ghai et al 2013), and the placeholder lineage Clade_Ia further corroborated the pattern of marine taxa displaced at the midstream freshwater mixing zone. *Salinihabitans*, a halophilic genus (Yoon et al 2009), similarly tracked saline conditions at both river ends. The collective suppression of this entire guild at midstream reinforces the interpretation that the midstream zone functions as a freshwater dilution barrier that filters out obligately marine bacterioplankton.

***Guild 2**: **midstream freshwater-associated taxa**.* A contrasting group of heterotrophic bacteria reached peak relative abundance at the midstream stations and lower abundance at the upstream and downstream extremes. This guild included *Pseudarcicella*, *Fluviicola*, *Novosphingobium*, *Polynucleobacter*, OM43 clade and *Limnohabitans*. Their midstream dominance reflects the transitional nature of this zone, where freshwater discharge and tidal mixing create a dynamic nutrient regime enriched in terrestrially derived and tidally imported organic substrates.

Pseudarcicella was predominant in the midstream station. The genus belongs to the phylum Bacteroidota and the family Spirosomataceae (Kämpfer et al 2012). Members of *Pseudarcicella* are chemoorganotrophic degraders of complex organic matter and play a role in the cycling of carbon and polysaccharides in freshwater systems (Pitt et al 2019). Ecologically, *Pseudarcicella* has been identified as a potential indicator of environmental conditions. Its abundance is sensitive to changes in aquatic systems. Studies from the Songhua River showed that *Pseudarcicella* was significantly more abundant in lightly disturbed areas (Yang et al 2019a), and its abundance increased following aeration treatment as dissolved oxygen rose (Wu et al 2019). The abundance of *Pseudarcicella* at midstream stations may reflect the transitional nature of this zone, where freshwater discharge and tidal mixing create conditions favorable for aerobic heterotrophic bacteria that specialize in degrading terrestrial organic matter. The midstream zone, characterized by intermediate salinity (0.20-2.55 ppt) and elevated dissolved oxygen (6.89-7.30 mg L^{-1}), likely receives pulsed inputs of organic substrates from both upstream freshwater runoff and downstream tidal sources, providing a favorable niche for chemoorganotrophic Bacteroidota lineages such as *Pseudarcicella*.

Fluviicola is a genus of Gram-negative, rod-shaped, aerobic to facultatively anaerobic bacteria belonging to the phylum Bacteroidota and the family Cryomorphaceae (O'Sullivan et al 2005). Notably, *Fluviicola* is the only genus within Cryomorphaceae that is exclusively adapted to freshwater. All other family members are marine origin and require Na+ ions or natural seawater for growth (Woyke et al 2011). One species of the *Fluviicola* genus, *F. taffensis*, has intrinsic resistance to chloramphenicol, streptomycin, and kanamycin (Woyke et al 2011). This resistance indicates a potential to persist under selective pressure from antibiotic contamination in aquatic environments. The mechanisms underlying this resistance may include the presence of specific efflux pumps that actively expel antibiotics from the cell, enzymatic inactivation of antibiotics, or mutations in target sites that reduce drug binding. Such mechanisms are common among Gram-negative bacteria and illustrate how genetic and biochemical traits influence ecological fitness in environments exposed to antibiotics. Ecologically, members of the genus *Fluviicola* are chemoheterotrophic bacteria. They degrade complex organic compounds in freshwater environments and possess genomic potential to break down cellulose, cellobiose, hemicellulose, and aromatic compounds (Woyke et al 2011; Banfield et al 2017). Furthermore, the genus has been detected in many aquatic habitats, including rivers, reservoirs, and lake ecosystems, where its abundance is often linked with organic matter availability (Bashenkhaeva et al 2023; Engloner et al 2023).

The genus *Novosphingobium* consists of Gram-negative, aerobic, rod-shaped, chemoorganotrophic bacteria in the phylum Proteobacteria and family Sphingomonadaceae. These bacteria degrade xenobiotic compounds, including polycyclic aromatic hydrocarbons, pesticides, and chlorophenols (Kertesz & Kawasaki 2010). *Novosphingobium chloroacetimidivorans* degrades chloroacetamide herbicides such as acetochlor, butachlor, and alachlor (Kumar et al 2020). *Novosphingobium indicum* breaks down aromatic compounds like naphthalene, biphenyl, acenaphthene, dibenzofuran, 2-methylnaphthalene, 2,6-dimethylnaphthalene, dibenzothiophene, 4-methyldibenzothiophene, phenanthrene, chrysene, anthracene, and fluoranthene (Kumar et al 2020). *Novosphingobium lentum* degrades 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol, and pentachlorophenol, while *Novosphingobium lindaniclasticum* degrades hexachlorocyclohexane (HCH) isomers (Kumar et al 2020). *Novosphingobium pentaromativorans* breaks down phenanthrene, fluorene, fluoranthene, anthracene, benz[a]anthracene, pyrene, chrysene, benz[b]fluoranthene, and benzo[a]pyrene (Kumar et al 2020). *Novosphingobium rosa*, isolated from plant roots, demonstrates metabolic versatility. Closely related species from landfill leachate can degrade catechol, protocatechuic acid, and phthalic acid (He et al 2022). Ecologically, *Novosphingobium* indicates environments exposed to organic enrichment and anthropogenic disturbance. The genus is found in soil, water, sediments, acidic lakes, deep-sea environments, plant rhizospheres, activated sludge, pesticide-contaminated soil and water, and oil-contaminated sites (Kumar et al 2020). Previous studies have also reported that *Novosphingobium* was abundant in mining sludge-impacted river systems. This suggests its potential role as an indicator of stressed aquatic environments (Reis et al 2020). *Novosphingobium*'s presence may reflect active degradation of organic matter and adaptation to fluctuating riverine conditions, highlighting its relevance for bioremediation.

The genus *Polynucleobacter* belongs *to* the family Burkholderiaceae and the phylum Proteobacteria and is ubiquitous in freshwater ecosystems globally. These bacteria typically constitute an average of 11.6% of total bacterioplankton cells across a range of aquatic habitats (Newton et al 2011). The genus *Polynucleobacter* serves as important indicator of anthropogenic nutrient enrichment and is frequently associated with eutrophic or urban-impacted waters (Ma et al 2016; Hosen et al 2017). Additionally, the *Polynucleobacter* genus possesses specialized metabolic pathways that enable the utilization of photodegraded and terrestrial dissolved organic matter (Hahn et al 2012). As aerobic heterotrophs with streamlined genomes, their elevated abundance in the midstream zones of the Amoslog and Panhutongan riverine mangroves underscores their function as copiotrophic organisms that thrive in nutrient-rich transitional environments (Ma et al 2016). Donchev et al (2024) reported that the genus can be enriched downstream of wastewater treatment plants, along with antibiotic resistance genes (ARGs). Yang et al (2019b) identified *Polynucleobacter* as a dominant genus within potential pathogenic communities in highly polluted river ecosystems, suggesting it may serve as an environmental vector for the dissemination of resistance.

The genus *Limnohabitans* belongs to the family Comamonadaceae and the phylum Proteobacteria. These bacteria are characterized by high rates of substrate uptake and rapid growth and possess larger cell volumes than typical bacterioplankton (Simek et al 2010; Kasalický et al 2013). They are recognized as a prominent and highly active component of freshwater communities, often thriving in nutrient-enriched reaches of anthropogenically influenced rivers (Ma et al 2016). Moreover, they demonstrate significant metabolic versatility, including a widespread capacity for aerobic anoxygenic photosynthesis (Kasalický et al 2017). Their distribution in riverine-mangrove systems is specifically associated with the availability of algal-derived organic carbon, particularly in midstream sections where optimal freshwater conditions and high phosphorus availability support their biomass production (Simek et al 2010). Wang et al (2022) reported that *Limnohabitans* species, particularly *Limnohabitans* sp. *63ED37-2* were identified as "supercarriers" of antibiotic resistance in the large Yangtze River system, contributing disproportionately to the dissemination of antibiotic resistance genes despite their relatively low abundance. The OM43 clade belongs to the family Methylophilaceae within the class Betaproteobacteria. These bacteria are obligate methylotrophs that utilize reduced one-carbon compounds, particularly methanol, as sole carbon and energy sources through the ribulose monophosphate cycle (Giovannoni et al 2008). Members of this lineage are characterized by exceptionally small, streamlined genomes (1.30-1.33 Mbp) and are widely distributed in coastal marine and freshwater ecosystems worldwide (Huggett et al 2012). Ecologically, the OM43 clade and its freshwater sister lineage LD28 (*Candidatus Methylopumilus* sp.) are closely associated with phytoplankton blooms, as the methanol they consume is released during the decomposition of algal cell walls (Morris et al 2006; Ramachandran & Walsh 2015). The freshwater LD28 lineage can constitute up to 4% of total bacterioplankton in lakes and rivers, with pronounced seasonal peaks during spring and autumn blooms (Salcher et al 2019). Fontaine et al (2023) identified *Candidatus Methylopumilus* sp. as one of the top six candidate bioindicator genera for biological status classification along the Danube River, demonstrating that Methylophilaceae abundances reflect water quality gradients in large river systems. The clade exhibited its highest relative abundance at the upstream stations. Given the low-salinity conditions of the upstream stations (0.03-0.20 ppt), the sequences classified as the OM43 clade in this study most likely represent the freshwater LD28 lineage rather than the truly marine OM43. The elevated abundance at upstream sites is consistent with their classification as freshwater bioindicators (Fontaine et al 2023), while the reduced abundance at the midstream stations may reflect the salinity barrier of the mixing zone (0.20-2.55 ppt), which falls between the optima of the strictly freshwater LD28 and the marine OM43 lineages.

***Guild 3**: **downstream-associated taxa**.* A smaller group of genera showed downstream-dominated distributions, reflecting the accumulation of urban runoff and anthropogenic pollutants at the river mouth. The genus *Marinobacterium* belongs to the phylum Pseudomonadota and family Alteromonadaceae (González et al 1997). Ecologically, *Marinobacterium* is recognized as a metabolically versatile heterotrophic genus capable of degrading hydrocarbons, including benzene and polycyclic aromatic hydrocarbons (Dos Santos et al 2011; Bae et al 2018). Dos Santos et al (2011) reported that increased relative abundance of *Marinobacterium* was associated with oil contamination, leading to its proposal as a potential bacterial proxy for oil pollution monitoring in mangrove ecosystems. Similarly, Chen et al (2021) identified *Marinobacterium* as a bioindicator of eutrophication and hypoxia in the Pearl River Estuary, linking its abundance to nutrient-enriched and oxygen-depleted conditions in anthropogenically impacted waters. Furthermore, *Marinobacterium* has been detected across diverse aquatic habitats, including freshwater wetlands, mangrove rivers, and coastal waters, demonstrating its ecological adaptability and potential role in the natural attenuation of organic pollutants (Huo et al 2009; Kim et al 2010). In the present study, the elevated abundance of *Marinobacterium* at downstream stations may reflect the greater anthropogenic influence in these areas, including urban runoff, domestic wastewater discharge, and the accumulation of organic pollutants. This observation is consistent with previous reports describing *Marinobacterium* as a genus commonly associated with hydrocarbon-contaminated and nutrient-enriched environments (Dos Santos et al 2011; Chen et al 2021). Moreover, the downstream zone of the Amoslog River exhibited the highest overall bacterial diversity, suggesting that environmental conditions influenced by tidal mixing and anthropogenic inputs support a diverse microbial assemblage, including hydrocarbon-degrading and pollution-tolerant taxa such as *Marinobacterium*. In contrast, the reduced abundance of this genus at midstream stations may be attributed to the transitional hydrological conditions of the area, where the mixing of freshwater and saline water, together with relatively adequate dissolved oxygen concentrations (6.89-7.30 mg L^{-1}), may favor other heterotrophic bacterial groups over marine-associated Gammaproteobacteria.

The detection of *Acinetobacter* and *Flavobacterium* among the dominant genera warrants attention. *Acinetobacter* includes the clinically significant, multidrug-resistant pathogen *A. baumannii*, while *Flavobacterium* includes fish pathogens such as *F. psychrophilum* and *F. columnare.* Their presence signals potential public health and aquaculture concerns given the proximity of populated areas and aquaculture ponds to these riverine mangroves.

The dominance of uncultured bacterial sequences across all sampling sites, underscores the vast proportion of microbial diversity that remains uncharacterized in these ecosystems. Less than 1% of environmental bacteria are readily culturable under standard conditions (Amann et al 1995; Steen et al 2019), and the dominance of uncultured and uncultured_bacterium sequences reflects the presence of novel lineages adapted to the dynamic physicochemical conditions of riverine mangroves. Both categories showed elevated abundance at the upstream stations and suppression at the midstream mixing zone, suggesting that these uncharacterized taxa may be more sensitive to salinity fluctuations and competitive pressures. In contrast, the unidentified sequences were more uniformly distributed across all zones, suggesting broader environmental tolerance among these phylogenetically divergent lineages. Future metagenomic studies are needed to resolve the taxonomic identity and functional potential of this hidden diversity, which may harbor novel enzymes relevant to bioremediation and ecosystem resilience.

Alpha diversity was assessed using the Shannon, Simpson, and Chao1 indices to evaluate species richness, evenness, and diversity across the six sampling stations (Figure 4).

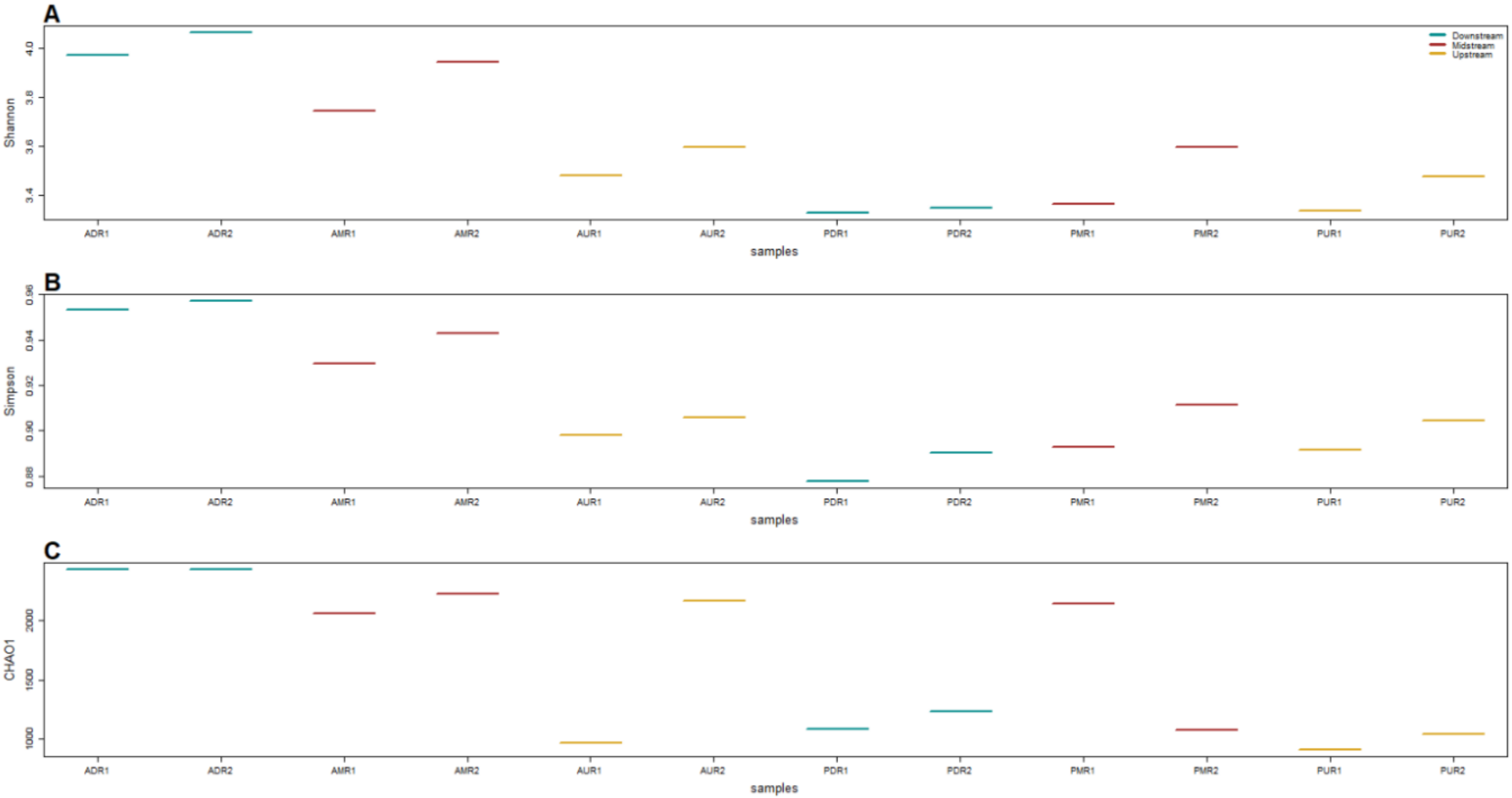

Figure 4. Alpha diversity indices comparing the six sampling stations in the Amoslog and Panhutongan Riverine mangroves in Placer, Surigao del Norte, Philippines (downstream, midstream, upstream).

Across all three indices, the downstream sites of the Amoslog River (ADR1 and ADR2) consistently exhibited the highest values, indicating the most diverse, evenly distributed, and species-rich bacterial community among all sampling stations. Specifically, ADR1 and ADR2 recorded Shannon index values around 4.0, Simpson index values of approximately 0.95, and Chao1 richness estimates exceeding 2,300, markedly higher than all other stations. In contrast, the downstream sites of the Panhutongan River (PDR1 and PDR2) showed the lowest alpha diversity across all three metrics, with Shannon indices near 3.3-3.4, Simpson indices around 0.82, and Chao1 estimates of 1,050-1,200, suggesting a less diverse and more uneven bacterial community. All values were significant with $p < 0.05$. Along the Amoslog River, a clear downstream-to-upstream gradient was observed, with diversity declining progressively from downstream to midstream to upstream.

In contrast, in the Panhutongan River, the midstream and upstream stations showed comparable or slightly higher diversity than the downstream station. These spatial patterns suggest that the downstream reaches of the Amoslog River provide more heterogeneous habitats or more favorable physicochemical conditions that support higher bacterial diversity. Conversely, the reduced diversity at the Panhutongan downstream sites may reflect localized environmental stressors such as higher salinity (1.95-2.69 ppt in Panhutongan versus 0.03-0.43 ppt in Amoslog) or anthropogenic inputs that filter out sensitive taxa and favor a more specialized, less diverse community. Microorganisms are highly sensitive to environmental changes and shifts in microbial community diversity and structure can serve as effective indicators of ecological disturbance and environmental stress (Ma et al 2022). The contrasting diversity patterns between the two rivers highlight the influence of environmental conditions, particularly salinity and anthropogenic impacts, on the structure of bacterial communities in these riverine mangrove ecosystems.

Beta diversity was assessed using principal component analysis (PCA) and a hierarchical clustering heatmap based on pairwise distance matrices to evaluate the differences in bacterial community composition among sampling sites (Figure 5). The PCA plot revealed a clear separation between the bacterial communities of the two river systems, with the Amoslog River samples (ADR1, ADR2, AMR1, AMR2, AUR1, AUR2) clustering distinctly from the Panhutongan River samples (PDR1, PDR2, PMR1, PMR2, PUR1, PUR2). The first principal component (PC1) explained 83.4% of the total variance. In comparison, the second principal component (PC2) accounted for 8%, indicating that the majority of the compositional variation between samples was driven by differences between the two riverine mangrove zones. The heatmap corroborated this pattern, showing lighter colors (higher similarity) within the same river system and darker reds (higher dissimilarity) between samples from different rivers, with hierarchical clustering dendrograms grouping all Amoslog samples separately from all Panhutongan samples. These results demonstrate that the bacterial community composition was strongly structured by the riverine-mangrove zone, with each river harboring a distinct microbial assemblage. This spatial differentiation is likely driven by the contrasting physicochemical conditions between the two rivers, particularly salinity. Ma et al (2016) similarly reported that salinity was a major factor driving spatial differentiation in microbial community structure along an exorheic river, clearly separating riverine from estuarine bacterial assemblages. Kieft et al (2018) also demonstrated that estuarine bacterial communities in Yaquina Bay exhibited habitat-specific taxonomic structures driven by physico-chemical gradients, resulting in spatially distinct microbial assemblages across different zones of the estuary. In the present study, the Amoslog River, with lower salinity (0.03-0.43 ppt) characteristic of a freshwater-dominated system, supported a bacterial community composition distinct from that of the Panhutongan River, where higher salinity (1.95-2.69 ppt) reflects a stronger tidal marine influence. The strong river-specific clustering observed in both the PCA and heatmap underscores the role of environmental conditions, particularly salinity, as primary drivers of beta diversity in these riverine mangrove ecosystems. Herewith, salinity emerged as the driver of the environmental variable structuring the bacterial communities in these riverine mangroves. Beta diversity analysis (PCoA) revealed that the first principal component explained 83.4% of the total variance, clearly separating the freshwater-dominated Amoslog riverine mangroves (0.03-0.43 ppt) from the brackish Panhutongan riverine mangroves (1.95-2.69 ppt) (Figure 5).

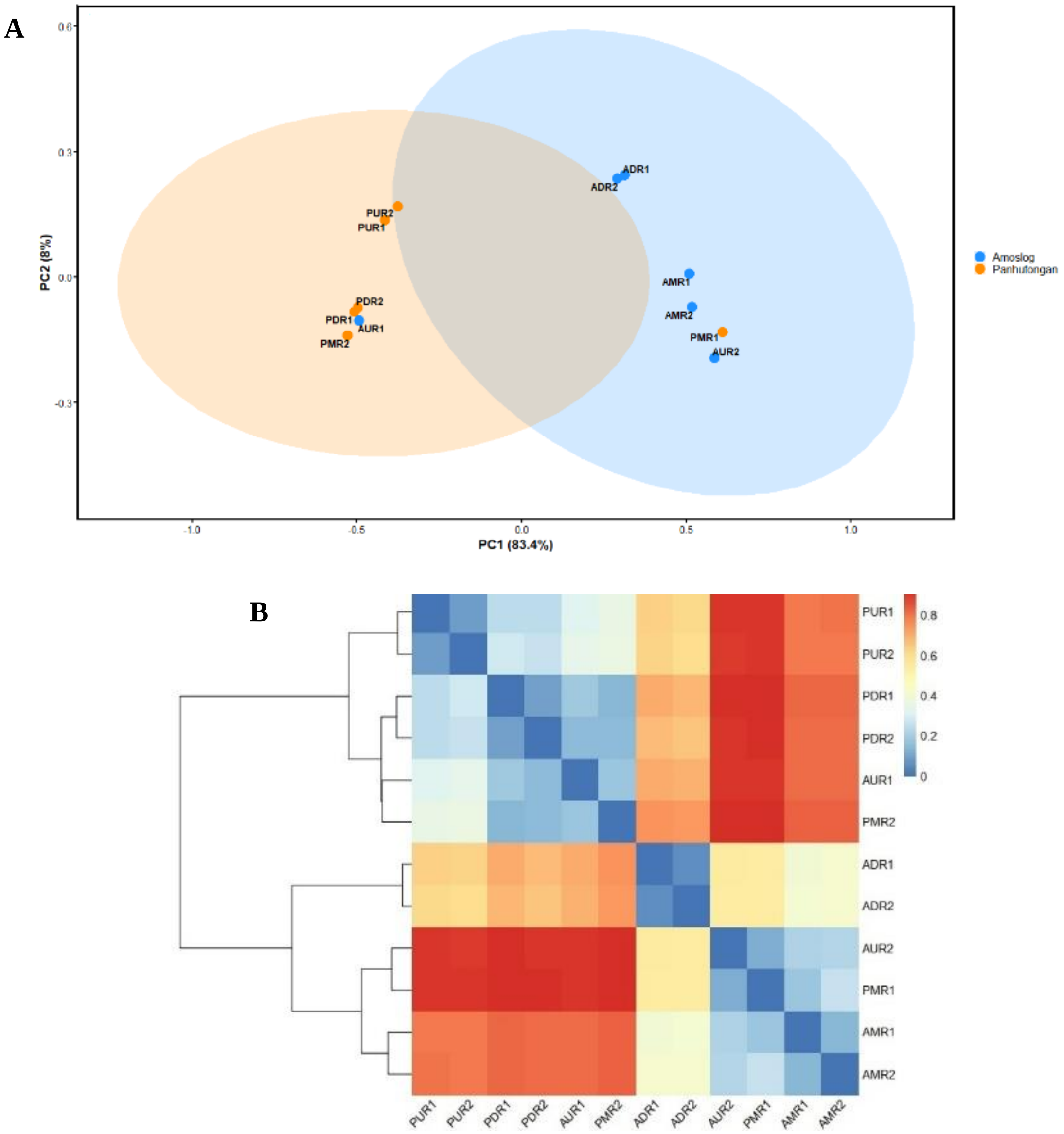

Figure 5. a) Principal component analysis show amplicon library clustering in reduced dimensions based on beta diversity analysis; b) Heatmap showing the beta diversity pairwise distance matrices between sites Amoslog riverine mangroves (downstream-ADR1, ADR2; midstream-AMR1, AMR2; upstream-AUR1, AUR2) and Panhutongan riverine mangroves (downstream-PDR1, PDR2; midstream-PMR1, PMR2; upstream-PUR1, PUR2), Placer, Surigao del Norte, Philippines.

A co-occurrence network was constructed at the genus level to identify potential ecological interactions among the dominant bacterial taxa across the Amoslog and Panhutongan riverine mangrove ecosystems (Figure 6). The network comprised 49 nodes (genera) connected by 795 significant Spearman correlations (|r| > 0.5), with 406 positive and 389 negative edges. The nearly balanced ratio of positive to negative associations suggests that both cooperative (co-occurrence) and competitive (co-exclusion) interactions shape microbial community structure in these riverine mangrove habitats (Cheung et al 2018). The network density of 0.676 indicates a relatively high degree of connectivity among the bacterial genera, implying that the community is characterized by a complex web of interdependencies rather than isolated or loosely connected taxa. The absence of isolated nodes (islands = 1) further confirms that all 49 genera were interconnected within a single network, reflecting a highly integrated microbial community. The network diameter of 1.774 and radius of 1.18 suggest a relatively compact network structure, where most genera are closely connected within a few interaction steps. This type of network topology, often described as a "small world" network, is associated with efficient transfer of resources and information across the community (Wang et al 2021). The centralization value of 0.283 indicates a moderately decentralized network, suggesting that no single genus dominates the interactions; rather, multiple genera contribute to overall network stability. Genera for instance, such as *Acinetobacter*, *Marinobacterium*, *Synechococcus CC9902*, *Pseudomonas*, along with OM43 clade and others emerged as highly connected nodes within the network, suggesting they may function as keystone taxa whose presence or absence could disproportionately affect community structure and ecosystem function. The high proportion of both positive and negative correlations underscores the complex ecological dynamics in these riverine mangrove ecosystems, where bacterial communities are shaped not only by environmental filtering but also by intricate biotic interactions, including niche partitioning, syntrophy, and antagonism.

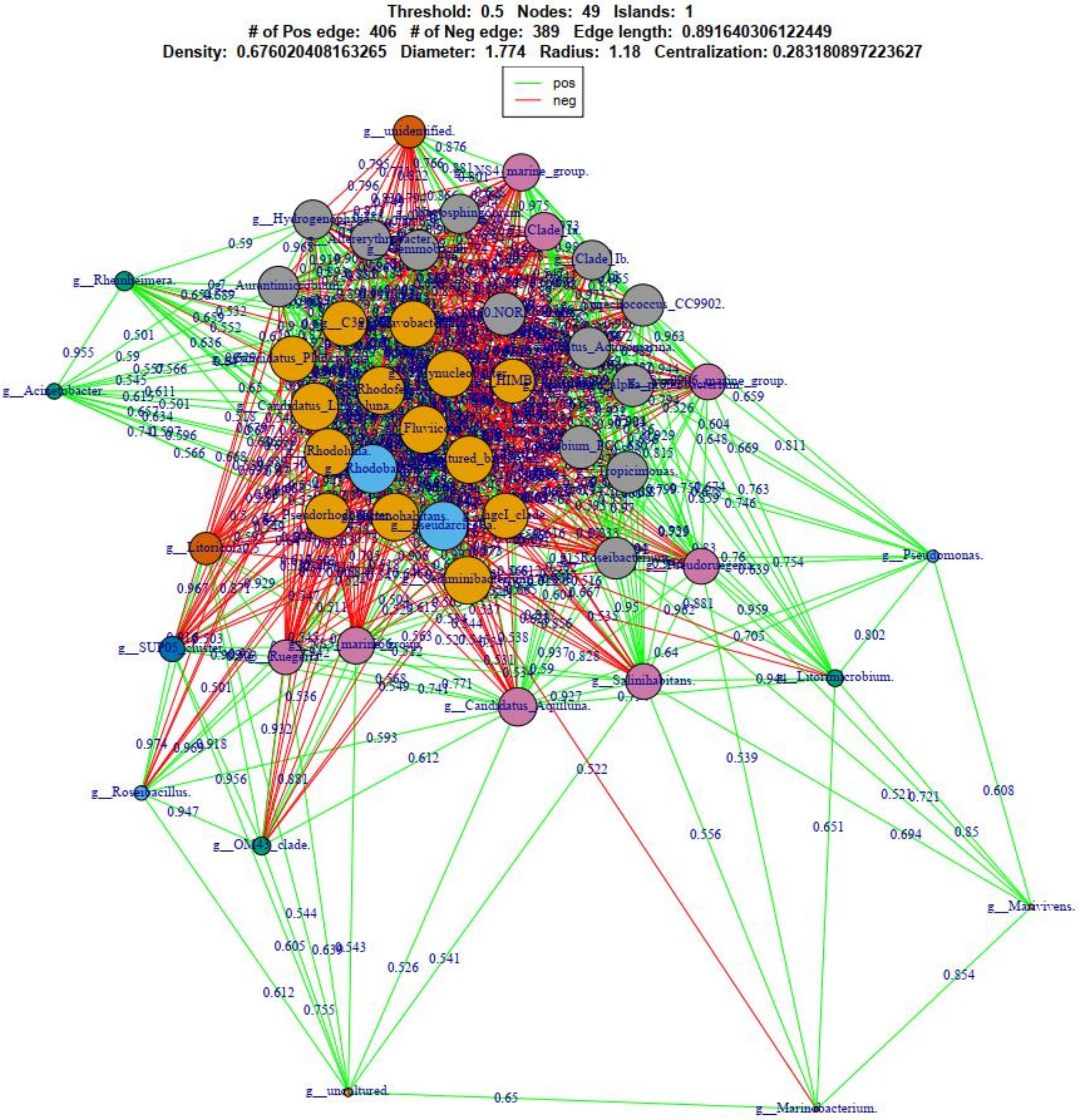

Figure 6. Microbial co-occurrence network at the genus level.

***Physico-chemical parameters**.* The physico-chemical parameters recorded from the six sampling sites (Table 1) revealed distinct environmental conditions between the Amoslog and Panhutongan riverine mangroves, all of which fell within the acceptable quality range set by the Department of Environment and Natural Resources (DENR 2016) for freshwater bodies. Water temperature was consistently lower in the Amoslog River (26.3-26.7°C) than in the Panhutongan River (29.6-29.9°C), likely reflecting differences in shading, water depth, or tidal mixing between the two rivers. The pH values ranged from slightly acidic to neutral in the Amoslog River (7.49-7.55) and from slightly alkaline to neutral in the Panhutongan River (7.68-7.91), both within the DENR standard of 6.5-8.5. All sites recorded dissolved oxygen (DO) concentrations above the 5 mg L^{-1} standard, with the Amoslog River showing an increasing trend from downstream (6.19 mg L^{-1}) to upstream (7.27 mg L^{-1}), while the Panhutongan River exhibited the opposite pattern, with DO decreasing from downstream (7.46 mg L^{-1}) to upstream (5.86 mg L^{-1}). Spietz et al (2015) demonstrated that bacterial community composition in estuarine systems is strongly associated with DO gradients, with significant community shifts occurring at DO concentrations between 5.18 and 7.12 mg L^{-1}, a range that encompasses all values recorded in this study.

Among all measured parameters, salinity exhibited the most pronounced spatial variation between the two rivers and emerged as the primary factor structuring the bacterial community composition. The Amoslog River maintained freshwater conditions across all sites (0.03-0.43 ppt), well within the DENR freshwater standard of < 0.5 ppt. In contrast, the Panhutongan River recorded elevated salinity levels (1.95-2.69 ppt) exceeding the freshwater standard, indicating a strong tidal marine influence. This salinity contrast was directly reflected in the bacterial community composition: genera with marine or brackish-water affinities, such as the NS5 marine group, OM60(NOR5) clade, and *Ruegeria*, were most abundant at the upstream sites where tidal marine intrusion reaches. In contrast, freshwater-adapted genera, including *Polynucleobacter* and *Limnohabitans*, were most abundant at the midstream stations where salinity was lower. Doherty et al (2017), in their study of the Amazon River-ocean continuum, similarly identified salinity as a master variable structuring bacterial communities across freshwater-to-marine gradients. Tee et al (2021) further demonstrated that microbial communities across river-to-sea continua exhibit distinct taxonomic and metabolic profiles along salinity gradients, with non-saline and saline communities adopting divergent osmoregulation strategies and nutrient cycling capacities. The strong river-specific clustering observed in the PCA and heatmap (Figure 5), where PC1 explained 83.4% of the total variance separating the Amoslog and Panhutongan communities, further confirms that the contrasting salinity regimes between the two rivers are the dominant environmental drivers of bacterial community structure in these riverine mangrove ecosystems. Additionally, the higher bacterial diversity observed at the downstream sites of the Amoslog River (Shannon index ~4.0, Chao1 > 2,300) compared to the lower diversity at the downstream sites of the Panhutongan River (Shannon ~3.3-3.4, Chao1 ~1,050-1,200) may reflect the filtering effect of elevated salinity, which typically reduces species richness by favoring only salt-tolerant taxa.

Table 1

Recorded physico-chemical parameters from six sampling sites in the Riverine Mangrove of Amoslog and Panhutongan, Placer, Surigao del Norte, Philippines (downstream, midstream, and upstream)

Parameters	*Quality range (DENR 2016)*	*Amoslog River*			*Panhutongan River*		
		Downstream	*Midstream*	*Upstream*	*Downstream*	*Midstream*	*Upstream*
Temp (ºC)	25-31	26.3	26.5	26.7	29.6	29.7	29.9
pH	6.5-8.5	7.55	7.52	7.49	7.91	7.96	7.68
Salinity (ppt)	< 0.5	0.43	0.20	0.03	2.69	2.55	1.95
DO (mg L^{-1})	> 5	6.19	6.89	7.27	7.46	7.30	5.86

Prediction of microbiome functional composition. Bacteria are a significant component of microbial communities and perform essential tasks in river nutrient cycles. The functional potential of the bacterial communities in the Amoslog and Panhutongan riverine mangroves was inferred using the PICRUSt algorithm integrated within the Parallel-Meta Suite pipeline, with predicted genes annotated against the KEGG Orthology database and classified according to the KEGG BRITE hierarchy (Douglas et al 2020). The prediction accuracy was assessed through the Nearest Sequenced Taxon Index (NSTI). Figure 7 presents the relative abundance of KEGG pathways across all 12 amplicon libraries, revealing the predicted functional composition of the bacterial assemblages at each site (downstream, midstream, upstream). The functional profiles were broadly similar between the Amoslog and Panhutongan rivers, suggesting a core functional resilience in these riverine mangrove microbiomes despite differences in taxonomic composition.

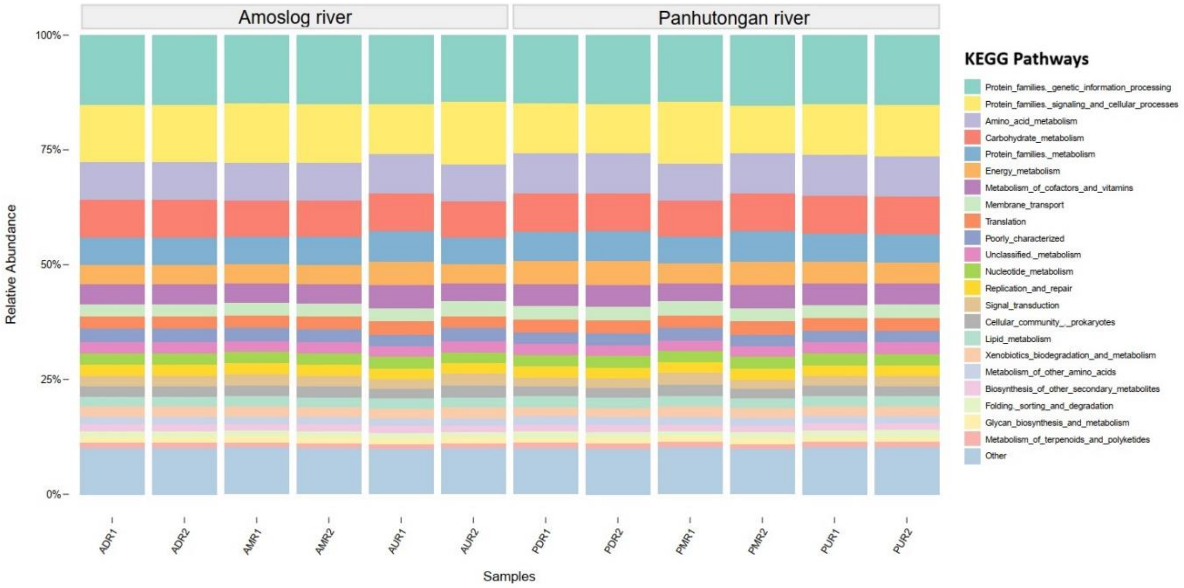

Figure 7. PICRUSt analysis of predictive functional composition of bacterial communities as represented by KEGG pathways for water sample composed of 12 amplicon libraries (ADR1, ADR2, AMR1, AMR2, AUR1, AUR2, PDR1, PDR2, PMR1, PMR2, PUR1, PUR2) corresponding to the sites (downstream, midstream, upstream).

PICRUSt predictions revealed that a high relative abundance of the bacterial assemblage in both rivers is involved in many metabolic pathways, including amino acid, carbohydrate, lipid, cofactor/vitamin, and energy metabolism, as well as associations with protein families (Figure 7). The prominence of amino acid and carbohydrate metabolism reflects the active heterotrophic activity of dominant genera such as *Limnohabitans*, *Polynucleobacter*, *Flavobacterium*, and *Fluviicola*, which are known for their high rates of organic matter utilization (Jezbera et al 2012; Kasalický et al 2013). The consistent representation of energy metabolism pathways is consistent with the presence of phototrophic and photoheterotrophic taxa, including *Synechococcus_CC9902* (cyanobacterial photosynthesis) and the aerobic anoxygenic photoheterotrophs OM60 (NOR5) clade and HIMB11 (Spring et al 2013). There is also promise in the Bacteroidota as a source of cellulolytic enzymes, which can be important and helpful for biotechnological applications, as members of the Bacteroidota (e.g., *Fluviicola*, *Flavobacterium*) are known to efficiently degrade high-molecular-weight compounds such as cellulose and pectin (Eupeña-Caray et al 2024).

The presence of pathways for xenobiotic biodegradation and metabolism across all sampling sites is of particular interest. This indicates a natural bioremediation capacity within the bacterial communities of both rivers, consistent with findings in the Tandag River, where similar functional potentials were attributed to taxa such as *Bacillus*, *Pseudomonas*, and *Pseudarcicella* (Eupeña-Caray et al 2024). In the present study, the detection of *Novosphingobium*, *Acinetobacter*, and *Pseudarcicella* among the dominant genera supports this functional prediction, as these genera are well documented for their ability to degrade aromatic and polycyclic aromatic hydrocarbons and other organic pollutants (Kumar et al 2020; Eupeña-Caray et al 2024).

In addition, an increase in the abundance of certain genera with known pathogenic members signals potential public health concerns. *Acinetobacter*, a genus that includes the clinically significant and multidrug-resistant pathogen *Acinetobacter baumannii*, was detected among the dominant genera across sampling sites. *Flavobacterium* includes species known as fish pathogens (*Flavobacterium psychrophilum*, *Flavobacterium columnare*), which are relevant given the proximity of these riverine mangroves to aquaculture areas (Eupeña-Caray et al 2024). The presence and impact of these bacteria in the river warrant further study, particularly in urbanized or densely populated areas where exposure risk is higher.

Conclusions. This study provides the first comprehensive baseline profiling of surface water bacterial communities in the Amoslog and Panhutongan riverine mangroves of Placer, Surigao del Norte, using 16S rRNA eDNA metabarcoding. The detection of 758 bacterial genera across 12 amplicon libraries highlights the high microbial diversity in these transitional ecosystems. The dominance of uncultured lineages and opportunistic taxa, such as the *HIMB11* strain and *Synechococcus CC9902*, underscores the dynamic nature of these habitats and their adaptation to fluctuating environmental conditions. Salinity emerged as an environmental variable structuring bacterial assemblages, evidenced by the distinct clustering of the two river systems in beta-diversity analyses. This spatial differentiation reflects a stronger tidal marine intrusion in the Panhutongan Riverine mangroves than in the freshwater-dominated Amoslog Riverine mangroves.

Furthermore, the higher alpha diversity observed in the downstream Amoslog sites suggests that these areas may offer more heterogeneous niches or more stable conditions than the salt-stressed downstream reaches of the Panhutongan Riverine mangroves. Functional predictions through PICRUSt2 indicate a core functional resilience within these microbiomes, with significant metabolic potential for organic matter cycling and xenobiotic biodegradation. However, the detection of potential pathogens, such as *Acinetobacter* and *Flavobacterium*, underscores the need for continued monitoring to assess public health and aquaculture risks. Ultimately, these findings establish a critical microbial reference for the riverine mangroves of Brgy. Amoslog and Panhutongan, Placer, Surigao del Norte. The identified taxa and functional profiles can serve as early-warning biomonitoring tools for local authorities to assess the impacts of mining, aquaculture, and urbanization, providing a science-based foundation for enhanced water-quality management and mangrove conservation.

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Conflict of Interest. The authors declare that there is no conflict of interest.

Data Availability. The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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