

# Amplicon metagenome comparison of Tandag River, Surigao del Sur, Philippines, based on high-throughput parallel DNA sequencing analysis of bacterial communities

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**Abstract.** *Eupeña-Caray RG, Ramos GB, Panduyos JB, Bacquial LS, Tabugo SRM. 2024. Amplicon metagenome comparison of Tandag River, Surigao del Sur, Philippines, based on high-throughput parallel DNA sequencing analysis of bacterial communities. Biodiversitas 25: 3464-3472.* Rivers serve as a vital freshwater reservoir for the environment and human consumption. Biomonitoring based on eDNA is essential for aquatic ecosystems. The present study investigates the bacterial taxonomic diversity of this ecosystem using the V1-V3 16S rRNA regions obtained from paired-end Illumina MiSeq reads. A total of nine amplicon libraries (U1, U2, U3, M1, M2, M3, D1, D2, D3) were constructed corresponding to the sites (U-upstream, M-midstream, D-downstream) of the Tandag River. After post-quality control and processing, there were 608,099 amplicon sequence variants (ASVs), representing 1194 families and 2090 genera. Results showed that the most abundant ASVs were affiliated with genera *Bacillus*, *Pseudomonas*, *Ralstonia*, *Alcaligenes*, and *Pseudarcicella*, along with unclassified bacteria derived from Gammaproteobacteria, Alphaproteobacteria, Verrucomicrobia subdivision, Flavobacteria, and Pseudomonadaceae. The bacterial alpha diversity was ranked upstream>midstream>downstream. An increase in the abundance of *Bacillus* (Firmicutes) and Gammaproteobacteria in an urbanized zone indicated a high level of human activity and an important threat to public health. Noteworthy is that the alkaline environment characterized by increased pH levels favors the occurrence of alkaliphilic bacteria. Microbial community structure was influenced by anthropogenic interference and selective pressure from loads of urban discharge in the area. The functional prediction of bacterial communities by Parallel-Meta Suite (PMS) using the PICRUST algorithm suggests functions related to metabolism and environment information processing. The presence of xenobiotic biodegradation bacteria highlights future possible applications. Findings contribute to understanding microbial communities in response to rapid urbanization. This is the first account of high-throughput sequencing analysis of bacterial communities in an urban river under anthropogenic stress in the Philippines.

**Keywords:** ASV, amplicon sequencing, functional prediction, PMS, river

## INTRODUCTION

Rivers that flow into important cities greatly contribute to the water supply (Medeiros et al. 2016). These complex ecosystems have experienced significant damage or destruction due to overexploitation and increasing population growth. The close relationship between rivers and human society and their role in socio-economic development has contributed to these ecosystems' substantial degradation and transformation. Despite this, rivers continue to provide essential goods and services, such as drinking water, water purification, power generation, organic matter retention, nutrient recycling, recreational opportunities, and habitats for various unique plants and animals, all of which have been adversely affected by human activities. Assessing the health of rivers is a primary tool that water management organizations employ to help prevent further harm (Khoroshi et al. 2016). Urban growth is driving greater demand for freshwater resources; however, it is noteworthy that the water sources of major cities worldwide have never

been evaluated on a global scale. This lack of assessment hinders efforts to understand the distribution and underlying causes of urban water stress (McDonald et al. 2014; Herbert et al. 2015).

Various studies demonstrated the impact of urbanization and anthropogenic stress on freshwater ecosystems; however, many of which focused on physico-chemical parameters such as increased levels of phosphorus, nitrogen, nitrate, ammonia, and potassium as a gauge (Egbueri et al. 2023) and not on biological components such as bacterial composition communities (BCC). In this study, the BCC of aquatic ecosystems is essential for the maintenance and sustainability of these environments since microbes are highly sensitive to anthropogenic stress. They represent a large and diverse species responsible for sustaining metabolic activities, biogeochemical processes, and recycling of nutrients and organic matter. A river adjacent to residential, agricultural, and industrial areas are major wastewater discharge sites that can reshape bacterial structures (Wang et al. 2018; Kupiec et al. 2022). Only a few studies have

analyzed the effect of urbanization on the microbial community. To date, there are limited studies on comprehensive descriptions of the taxonomy and functionality of microbes in urban freshwater ecosystems. Notably, diversity can change at a given time depending on environmental conditions. Thus, amplicon metagenomic comparisons provide a method that involves comparing the genetic material (amplicons) of different microbial communities in relatively disturbed areas of important river systems, which may contribute significantly to a better understanding of real anthropogenic impacts on aquatic environments. Amplicon metagenomic studies are important for understanding microbial ecosystems, given their ability to provide information about the diversity and distribution of the different members of the community and their metabolic potential (Hugerth and Andersson 2017). In this context, the study aims to develop a comprehensive description and taxonomic profiling of the microbial community associated with an urban river by comparing areas under different anthropogenic stress. This was done using high-throughput Illumina amplicon sequencing of the 16S V1-V3 rRNA gene region.

In this study, the Tandag River (Surigao del Sur, Philippines) has been selected to illustrate the spatial distribution and variation of microbial compositions in an urban river. It is hypothesized that microbial communities could be structured and evolved according to the present condition of surroundings and increase adaptability. This study also reveals the dominant microbial assemblage in response to anthropogenic and natural factors.

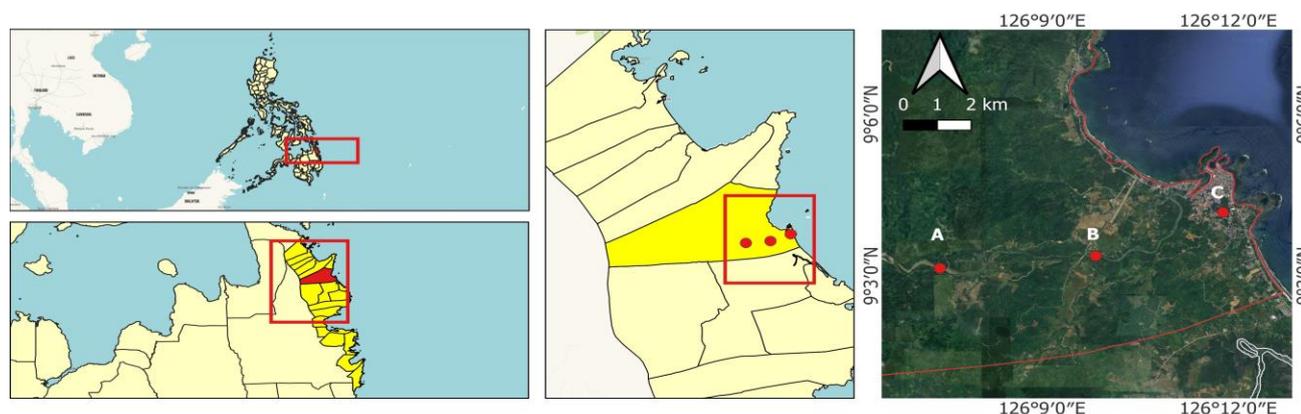
Natural and anthropogenic activities can shape the bacterial community in an urban river. This is the first account of high-throughput sequencing analysis of bacterial communities based on environmental DNA (eDNA) in an urban river under anthropogenic stress in the Philippines. Meanwhile, high-throughput parallel DNA sequencing based on the 16S rRNA gene provided a powerful culture-independent tool to assess the bacterial community composition of river systems, thus helping in biomonitoring purposes. Results may contribute to understanding urban rivers' microbial ecology in response to rapid urbanization. Science-based conservation can be done to support and

recommend policies. Moreover, accounts on the functional profile of microbes may indicate microbial action on methane, nitrogen, and sulfur metabolism in urban areas. This can lead to the dissemination of virulence/defense (antibiotic and metal resistance) and stress response-related genes in an urbanized environment. The microbial community's structure can ultimately be altered by uncontrolled anthropic interference, highlighting the impact of selective pressure imposed by high loads of urban sewage discharged into freshwater environments (Medeiros et al. 2016). Hence, this study provides biological insights to craft policies for sustainability and conservation aspects.

## MATERIALS AND METHODS

### Study site and sample collection

Tandag River is approximately 84 km long and is located in the eastern portion of Mindanao Island, particularly in Tandag City, Philippines (urban population is approximately 300,000). The river is the principal drainage way of the basin originating from Brgy. Pakwan, Lanuza, Surigao del Sur, and passes along the entire basin length in a northeasterly direction. It drains to Brgy. Telaje and Dagocdoc, Tandag city and discharges into the Philippine Sea (Figure 1). The river traverses through agriculture, recreational, and residential areas. Three sampling sites were established. Upstream samples (U1, U2, U3) were collected from waterways traversing Barangay Maitum, midstream samples (M1, M2, M3) were collected traversing Barangay Quezon, and the downstream samples (D1, D2, D3) were collected from Brgy. Telaje, Tandag City. Acquisition of prior informed consent to barangays was done for the study. For each site, 5L water was collected, and three subsamples at each site were obtained randomly at 3m intervals. All water samples were immediately transported to the laboratory, filtered using a sterile 0.22  $\mu\text{m}$  pore-size filter membrane in a Buchner funnel, and processed for eDNA extraction. Additionally, physico-chemical parameters such as surface water temperature and pH were measured.



**Figure 1.** Map of Tandag River (urban river), showing the sampling sites A. Upstream; B. Midstream; C. Downstream Tandag City, Surigao del Sur, Philippines

### DNA extraction and high-throughput sequencing

Bacterial communities were analyzed using high-throughput amplicon sequencing. Total genomic DNA (gDNA) was extracted from water samples using HiPurA™ DNA Extraction Kit (Vadhani Industrial Estate, Mumbai, India) according to the manufacturer's instructions. Universal bacterial primers targeting V1-V3 regions (5'-AGA GTT TGA TCC TGG CTA G-3') (3'-ATT ACC GCG GCT GCT GG-5') were used for amplification of the 16S rRNA gene, whose purified products were sent to Macrogen, South Korea for high-throughput sequencing on Illumina MiSeq platform. Nine amplicon libraries (U1, U2, U3, M1, M2, M3, D1, D2, D3) were constructed corresponding to the sites (U-upstream, M-midstream, D-downstream).

### Microbiome data analysis

FastQC (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) was used to check the quality control of raw sequence data. FLASH (Fast Length Adjustment of Short reads), an accurate and fast tool for genome assembly and analysis, was used to merge paired-end reads. The resulting sequences were then uploaded to the Metagenomics RAST (MG-RAST) server for analysis and made publicly available under the accession code numbers 4984164.3-4984172.3. MG-RAST provides automated processing of environmental DNA sequences via a pipeline. MG-RAST is an automated pipeline with an R-interface that can upload raw sequence data for normalization, processing, and summaries of metagenomic data (Meyer et al. 2008). Moreover, Parallel-Meta Suite (PMS), available at (<https://github.com/qdu-bioinfo/parallel-meta-suite>) was used for fast and reliable microbiome analysis. It is a highly interactive and efficient software encompassing data analysis, statistical tools, and visualization tools.

PMS process amplicon sequences as an original input for amplicon sequence variants (ASVs) denoising and de-chimera for gene markers. The sequences were then aligned against reference databases (SILVA) with built-in VSEARCH for profiling and taxonomy annotation. Relative abundance was determined, and gene families were inferred into the Kyoto Encyclopedia of Genes and Genomes (KEGG) Orthology via Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) algorithm; metabolic pathways were further annotated by the KEGG BRITE hierarchy. Prediction accuracy of functions was done through the Nearest Sequenced Taxonomy Index value as the sum of distances between operational taxonomic units and the nearest individually sequenced relatives in the phylogenetic architecture.

Then, alpha diversity Shannon, Simpson, Chao1 indexes and beta diversity were calculated per sample via pairwise distance matrices unweighted/weighted Meta-Storms algorithm (for taxonomy) and Hierarchical meta-storms (for function) and plotted as a heatmap. In a co-occurrence network, the nodes represent community features (e.g., taxon), and edges are their Spearman correlation values. Meanwhile, the network density, diameter, radius, and

centralization were also computed to quantify the network property (Chen et al. 2021).

## RESULTS AND DISCUSSION

Throughout human history, rivers have always been the lifeline of civilizations, and this trend continues today. However, because of contemporary socio-economic development, this lifeline of civilization has been facing burgeoning threats from several quarters: pollution, habitat deterioration, and anthropogenic stress. Improving the health of river systems is gaining international prominence and becoming a prime mandate of governments all over the globe (Shinde et al. 2018).

Tandag River (in Surigao del Sur) is a major snaking river ecosystem that traverses several barangays from the hinterlands to the urban city. This ecosystem has been used extensively for anthropogenic purposes, namely drinking water for domesticated animals, recreation, and agriculture, and most often, as a catch basin for the city's wastewater distribution. Science-based conservation can be done for this river if data can be obtained to support and recommend policies. Studies can provide biological insights to craft such policies.

### Physico-chemical parameters

The physico-chemical parameters were measured accordingly, revealing an average water temperature of 26.2°C and a water pH level of 7.76. Research indicates that microbial communities are vitally influenced by various factors, such as pH, temperature, salinity, dissolved oxygen, and nutrient levels, which shape their composition. The pH is correlated to soil and water, where fluctuations can be related to pollution (Jordaan and Bezuidenhout 2016). The pH level significantly impacts the composition of bacterial communities (BCC), which led to the notable presence of bacteria from the phylum Firmicutes, Bacteroidetes, certain Gammaproteobacteria, Alphaproteobacteria, Verrucomicrobia subdivision, Flavobacteria, and Pseudomonadaceae (Tabugo et al. 2023).

An average pH of 7.76 leans towards an alkaline condition; it is noteworthy that alkaline environments characterized by high pH levels favor the occurrence of alkaliphilic bacteria (Borkar 2015). Notable representatives of these bacteria are from the phyla Firmicutes, Bacteroidetes, and Proteobacteria (Hahnke et al. 2016). The results indicate a significant presence of species of *Bacillus*, *Pseudomonas*, *Ralstonia*, *Alcaligenes*, and *Pseudarcicella*.

### Identification of bacterial communities

High-throughput sequencing of the 16S rRNA gene was used to explore bacterial communities in an urban river. Bacterial datasets were separated and analyzed based on the three sampling sites (upstream-scenic, recreation, agriculture; midstream-recreation, agriculture; downstream-highly urbanized) (Figure 1). After post-quality control, there were a total of 608,099 amplicon sequence variants (ASVs) obtained from nine amplicon libraries (upstream-273,307; midstream-100,016; downstream-234,776), representing 1,194

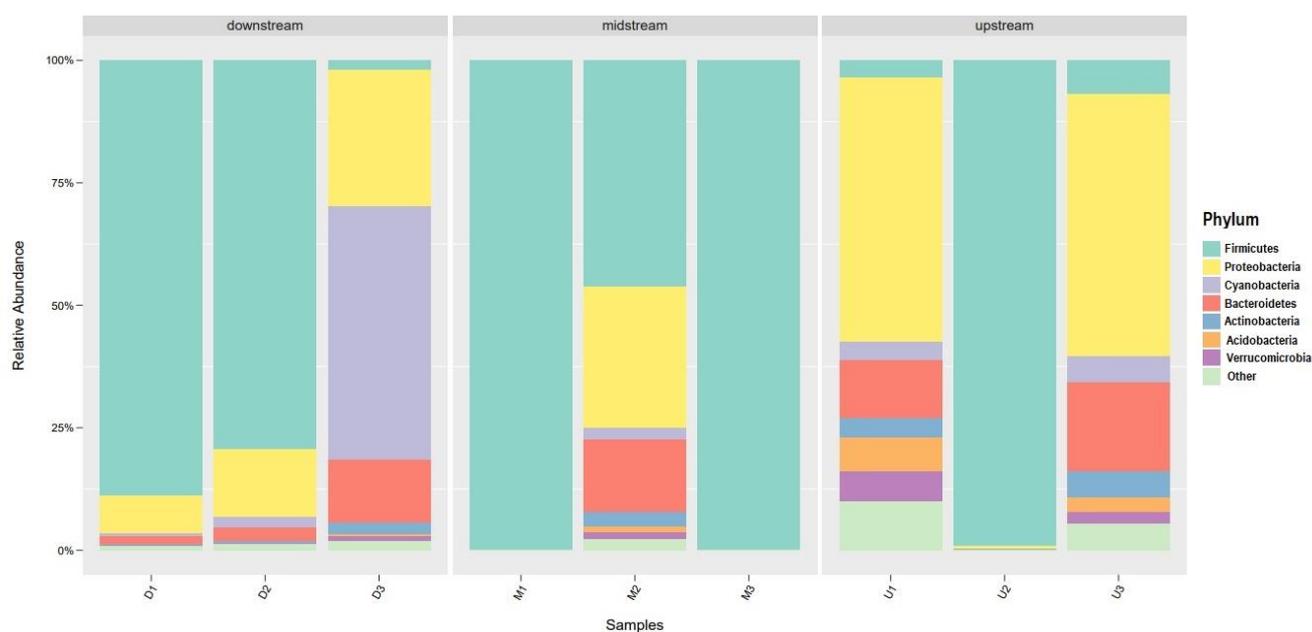
families and 2,090 genera. Results showed that the most abundant ASVs were affiliated with Bacillaceae, unclassified bacteria derived from Gammaproteobacteria, Alphaproteobacteria, Pseudomonadaceae Flavobacteria, and Verrucomicrobia subdivision. The presence of significantly enriched Firmicutes (*Bacillus*) and Gammaproteobacteria in the urbanized zone implies the effects of human activity in the area. Figure 2 compares the taxonomic bacterial diversity of urban river amplicon metagenomes using 16S rRNA high-throughput sequencing at the phylum level. The predominant groups are Firmicutes, Proteobacteria (Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria), Cyanobacteria, Flavobacteria (Bacteroidetes), Actinobacteria, Acidobacteria and Verrucomicrobia. Figure 3 shows the relative abundance of bacterial communities in an urban river, comparing the nine amplicon metagenomes at the species level. The results indicate a significant presence of species of *Bacillus*, *Pseudomonas*, *Ralstonia*, *Alcaligenes*, and *Pseudarcicella*. Measures of alpha diversity indices (Shannon, Simpson, and Chao1) based on genus level identification for all sites ranked bacterial diversity as follows: upstream>midstream>downstream, albeit overlapping genera were observed between sites. The Shannon index values showed the highest bacterial diversity of 3.0697 (Table 1 and Figure 4.A). Shannon's diversity index considers both richness and evenness. The highest diversity was observed in the upstream area (U1, U2, U3). A combination of environmental and anthropogenic disturbances can cause microbial diversity between locations.

For beta diversity, pairwise distance matrices were calculated using a weighted Meta-Storms algorithm (for

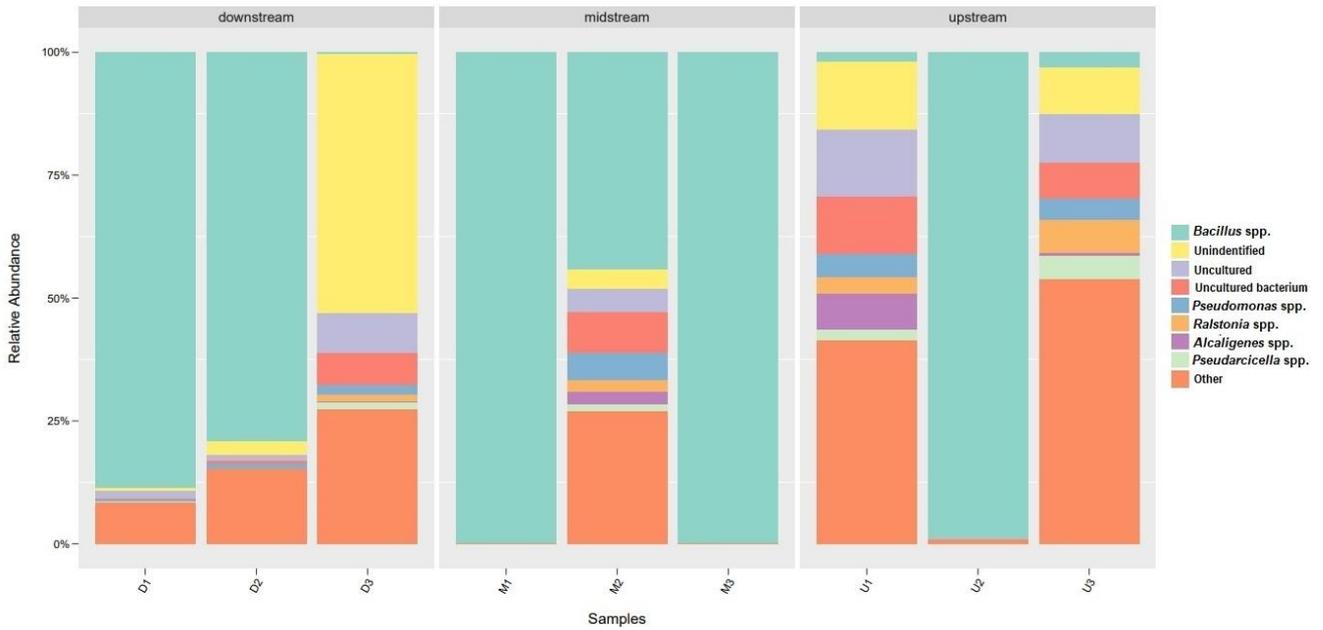
taxonomy) or Hierarchical Meta-Storms and plotted as a heatmap. Still, there were overlapping amplicon metagenomes for the midstream and upstream (Figures 4.B-4.C) because both sites were utilized for recreation and agriculture purposes. Figure 4D also shows the microbial co-occurrence network. Herewith, the presence of *Bacillus* species is highly correlated to Proteobacteria, Actinobacteria, and Bacteroidetes with a Spearman correlation value near 1. Bacteroidetes are anaerobic, indicating high levels of intestinal waste and recent contamination in the aquatic ecosystem. The relative abundance of Betaproteobacteria and Gammaproteobacteria positively correlates to dissolved organic carbon (DOC) concentration (Kuang et al. 2022). This is a key environmental parameter shaping bacterial community composition.

**Table 1.** Measures of alpha diversity based on genus-level identification for each sample type

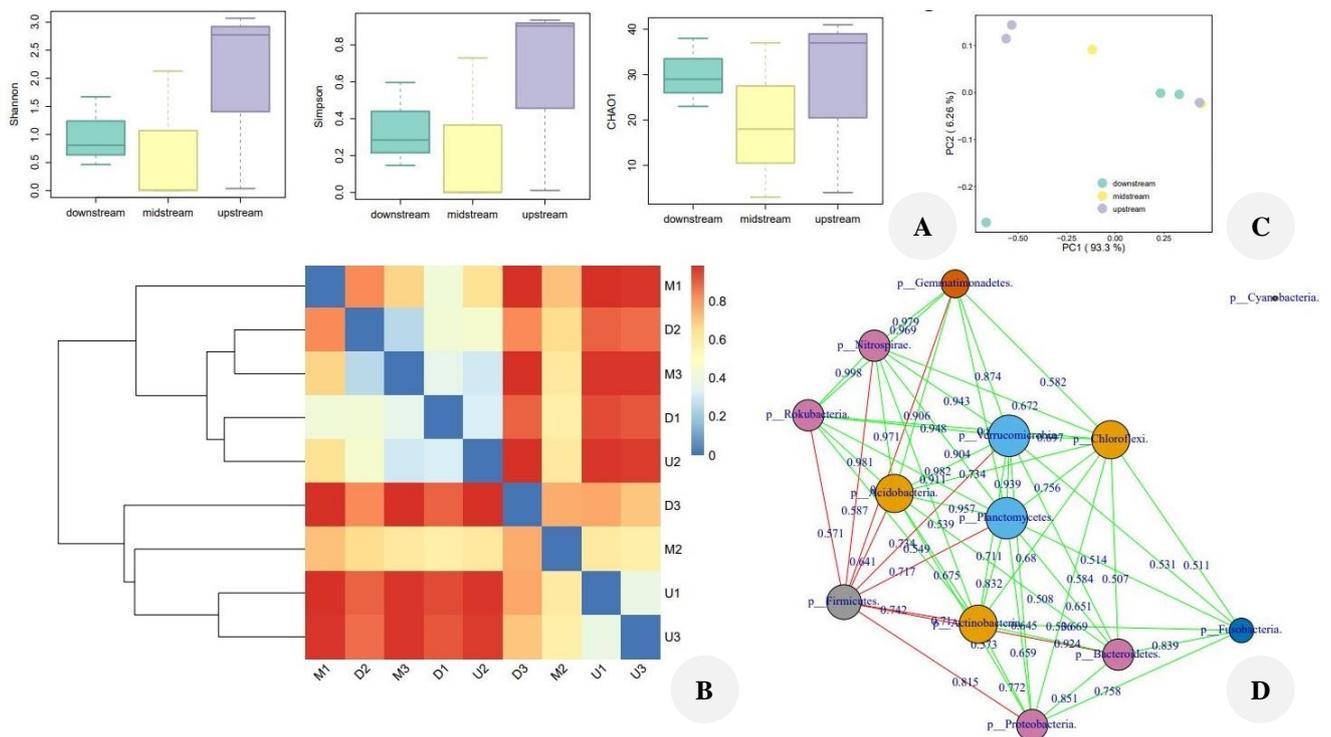
Sample	Shannon	Simpson	Chao1
D1 (downstream)	0.4643	0.1466	23
D2 (downstream)	0.8090	0.2845	29
D3 (downstream)	1.6716	0.5962	38
M1 (midstream)	0.0092	0.0017	18
M2 (midstream)	2.1284	0.7292	37
M3 (midstream)	0.0007	0.0001	3
U1 (upstream)	2.7743	0.9028	37
U2 (upstream)	0.0372	0.0111	4
U3 (upstream)	3.0697	0.9347	41



**Figure 2.** Relative abundance of bacterial communities per phylum between nine amplicon libraries: D1, D2, D3, M1, M2, M3, U1, U2, U3) corresponding to the sites (U-upstream, M-midstream, D-downstream)



**Figure 3.** Relative abundance of bacterial species: nine amplicon libraries (D1, D2, D3, M1, M2, M3, U1, U2, U3) corresponding to the sites (U: upstream, M: midstream, D: downstream)



**Figure 4.** A. Comparison of alpha diversity indices (Shannon, Simpson, Chao) for water samples composed of nine amplicon libraries (D1, D2, D3, M1, M2, M3, U1, U2, U3) corresponding to the sites (U-upstream, M-midstream, D-downstream); B. Heatmap showing the beta diversity pairwise distance matrices between samples; C. Principal Component Analysis shows the clustering of amplicon libraries in reduced dimensions derived from beta diversity analysis; D. Microbial co-occurrence network at the phylum level

Members of Bacilli (Firmicutes), Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, and Flavobacteria (Bacteroidetes) make up most of the human intestinal microbiota and other animals thus, are easily found in stools; hence, this explains their dominance in the three sites. In the highly urbanized zone (downstream), industrial, agricultural, and domestic sewage enters the river. The abundance of *Bacillus* and unidentified/unclassified bacteria derived from Gammaproteobacteria, Alphaproteobacteria, Verrucomicrobia subdivision, and Flavobacteria (Bacteroidetes) predominate the area.

In the upstream (U1, U2, U3) region, there was a significant increase in the percentage of unclassified bacteria derived from Verrucomicrobia subdivision and Gammaproteobacteria, *Elizabethkingia*, *Candidatus solibacter*, and *Pseudomonas*. However, there was one library (U2) where Bacilli predominated owing to the presence of farm animals defecating in the area. Verrucomicrobiae species are identified and isolated from freshwater, marine, and soil environments and human feces. *Elizabethkingia* are commonly found in the environment (particularly soil, river, and water reservoirs). It has been identified as an emerging pathogen in hospital settings (Hem et al. 2022). The upstream area was regarded as scenic but has been utilized for agriculture and recreation purposes. Also, animals were seen in the area along with the human populace using the water source for everyday routine.

Bacilli species dominated the midstream (M1, M2, M3). Numerous Bacilli (Firmicutes) species are associated with human activities. They are widely distributed in construction sites with inorganic and organic pollution. Bacilli (Firmicutes) are indicators of nosocomial infection and human fecal contamination (Köchling et al. 2017; Dechavez et al. 2022). The area had been used for agriculture and recreation purposes. Often, farm animals were seen in the area, and the human populace utilized the water source for everyday chores like washing clothes and dishes and even defecating.

For the downstream (D1, D2, D3) area, the predominant bacteria belong to *Bacillus*, unidentified bacteria (derived from Gammaproteobacteria and Flavobacteriaceae), unclassified bacteria derived from Verrucomicrobia subdivision, genera *Elizabethkingia*, *Lactobacillus*, *Listeria*, and *Pseudomonas*. The midstream area is often subjected to industrial and domestic sewage. External pollution has a significant impact on the bacterial community assembly in rivers and contributes to the formation of unique bacterial co-occurrence patterns (Zhang et al. 2020); hence, it may explain the observed high alpha diversity next to the upstream zone.

An increase in the abundance of human pathogens of Bacilli (Firmicutes) and Gammaproteobacteria in an urbanized zone indicated a high level of human activity and an important threat to public health (Durand et al. 2015; Numberger et al. 2022). The presence and impact of these bacteria in the river deserve further study.

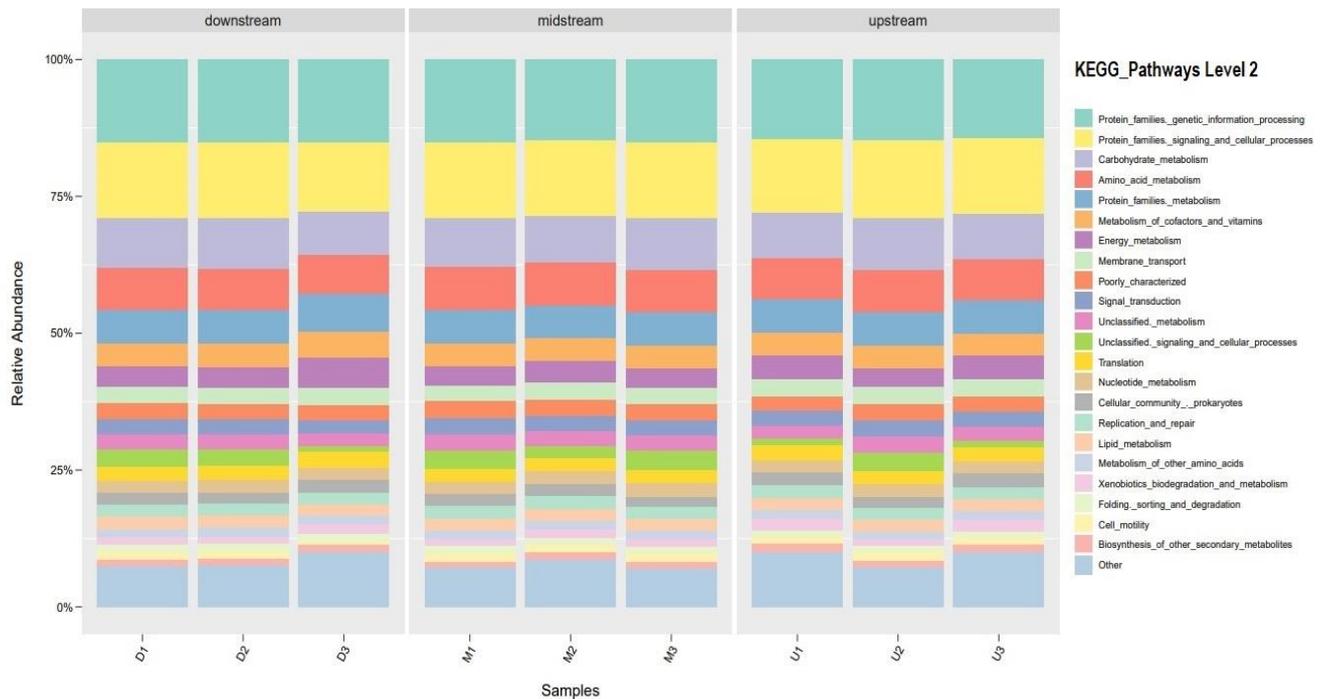
Gammaproteobacteria is a class of several medically, ecologically, and scientifically important groups of bacteria. They are abundant in soil, freshwater lakes, and rivers. Flavobacteriaceae are comprised of environmental bacteria. Species are aerobic, while some are microaerobic to anaerobic. It is noted that the health of an ecosystem is an interplay of the combination of physico-chemical (e.g. pH, temperature) and biological (e.g. bacteria community composition) plus the balance of nutrients (Erlacher et al. 2014).

In addition, a percentage of Coscinodiscophyceae (a major class of diatoms) was also detected from the metagenomes of the downstream area. Many bacterial 16S rRNA primers have a high affinity for eukaryotic plastid DNA such that non-target sequences from chloroplasts and mitochondria are often co-amplified (Gan et al. 2019), thus detecting diatoms as well. A previous study presented the plausibility of uncovering diatom assemblage data from rRNA sequences. In that study, diatom reads were obtained using a universal 16S primer from freshwater communities (Bonfantine et al. 2021). Diatoms are particular about the quality of water in which they live. A particular algae profile can be linked to the pollution level.

Diatoms are highly sensitive to nitrogen and phosphorus concentrations; hence, they are used for stream monitoring programs. The presence of *Paralia sulcata*, indicates that the river is under pressure from pollution. The species' proliferation can be attributed to high nutrient concentration in the river and had become more enhanced by low flow conditions, high evaporation rate and low water level. Other studies also reported these species as indicators of pollution in reservoirs and lagoons (Edward and Ugwumba 2013; Onyema 2013). Runoff from agricultural lands can carry excess nutrients such as nitrogen and phosphorus into rivers, streams, lakes, and groundwater supplies. The excess nutrients degrade the water quality (Withers et al. 2014). The accumulation of these nutrients happened downstream, which also triggered algal growth.

### Functional prediction of the microbiome

Bacteria are a significant component of microbial communities and perform essential tasks in river nutrient cycles. Moreover, Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) predictions of the functional composition of the respective microbiome as represented at the KEGG pathway at level 2 analysis revealed that there is a relative abundance of bacterial assemblage in the river that is involved in many metabolism pathways such as amino acid metabolism, carbohydrate metabolism, lipid metabolism, cofactors and vitamins metabolism, energy metabolism and association with protein families (Figure 5). There is also promise as a source of cellulolytic enzymes, that can be important and helpful for biofuel production. The presence of bacteria for xenobiotic biodegradation is also of interest (Hu et al. 2018).



**Figure 5.** PICRUSt analysis of predictive functional analysis of bacterial communities as represented by KEGG pathways for water samples composed of nine amplicon libraries (D1, D2, D3, M1, M2, M3, U1, U2, U3) corresponding to the sites (U-upstream, M-midstream, D-downstream)

Xenobiotic contaminants such as pharmaceuticals' active compounds (PhACs), polycyclic aromatic hydrocarbons (PAHs), phenolics, azodyes, polycyclic aromatic hydrocarbons (PAHs), personal care products (PCPs), halogenated compounds, triazines, nitroaromatic compounds, pesticides, and chlorinated compounds adversely impact the environment because of long-term persistence to slow or no biodegradation in the ecosystems. Once xenobiotics are released into the environment, they enter the food chain, leading to harmful impacts at each trophic level and inevitably affecting human and animal health. Hence, removing toxic, undegradable xenobiotics from the environment is important (Godheja et al. 2016). Interestingly, this study identified a high bacterial load for the five genera linked to xenobiotic biodegradation: *Bacillus*, *Pseudomonas*, *Alcaligenes*, *Ralstonia*, and *Pseudarcicella*. These bacteria are said to be cultivable with high xenobiotic degradative potential (Migłani et al. 2022; Zhao et al. 2024).

A study by Pal et al. (2022) highlighted the abundance of putative genes and unidentified taxa, which was possibly associated with xenobiotic biodegradation. Microorganisms have the unique property that allows them to metabolize xenobiotic substances partially or completely in various ecosystems. Biotransformation is the most effective metabolic process for degrading xenobiotic compounds. The ability of microorganisms to detoxify xenobiotic compounds enables them to persist in a toxic environment using available sources of carbon, phosphorus, sulfur, and nitrogen (Migłani et al. 2022). Betaproteobacteria, Firmicutes, and Bacteroidetes are abundant in a freshwater environment (Bai et al. 2014). The Firmicutes (*Bacillus*) are well-known

for having many members that can degrade even very recalcitrant organic compounds (Köchling et al. 2017). Betaproteobacteria are comprised of genera that play a role in denitrification and biological phosphate removal (Ginige et al. 2004). A high abundance of proteobacteria was associated with nutrient availability. The relative abundance of *Pseudarcicella* of phylum Bacteroidetes (Bacteroidia) is worth mentioning, especially in the downstream region. This group is highly diverse and has an important role in the ecosystem. It can efficiently degrade many high molecular-weight compounds such as cellulose, pectin, protein, and chitin. Some species of Bacteroidetes are very good indicators of recent fecal contamination (Zhang et al. 2020). High levels of nitrate, feces, ammonia, and other pollutants from residential wastewater may cause relative abundance in the area. Some species demonstrate the degradation ability, including *Ralstonia eutropha*, *Bacillus subtilis* strain MTCC 441, *Alcaligenes* spp., and *Alcaligenes faecalis*. These microbes potentially remove more than 85% methylene blue (MB) in a study conducted and are confirmed to affect degradation cases with other xenobiotic wastes. *Ralstonia pickettii* is a xenobiotic bacteria that can biodegrade some organic pollutants. Numerous research on various related wastes have verified this phenomenon, resulting from the metabolic complexity alongside the encoding genes for biodegradation enzymes (Purnomo et al. 2021). In addition, in a study by Lang et al. 2010, they examined two strains of gram-negative bacteria from the genus *Pseudomonas*, isolated because of their ability to decompose xenobiotic compounds. It was subjected to a

polyphasic taxonomic study based on 16S rRNA gene sequence analysis.

Furthermore, the findings in this study confirm other studies that show the occurrence of potential pathogens in freshwater environments is influenced by varying levels of urbanization. This provides insights into community assemblage under the influence of human activities. A study by Zhang et al. (2020) stressed that many anthropogenic stressors threaten urban rivers, and bacterial communities in this type of polluted environment quickly respond, thus making them useful for water quality assessment and predictive insight. Ramond et al. (2015) stated that amplicon next-generation sequencing (NGS) and normalization of metagenomic DNA is a promising method in characterizing members of 'microbial dark matter' where keystone species may be numerically rare but are vital to the ecosystem. Environmental factors influence bacterial diversity and abundance (Kusunur et al. 2022). Illumina MiSeq high-throughput sequencing technology effectively analyzes bacterial community structure, diversity, co-occurrence patterns, and functional changes reflected in rivers under different domestic pollution types (Zhang et al. 2020). This study is the first account of high-throughput sequencing analysis of bacterial communities in an urban river under anthropogenic stress in the Philippines.

In conclusion, natural and anthropogenic activities shape the bacterial community in an urban river. The microbial composition in water samples adjacent to urban zones was linked to human activities as evidenced by increased pathogens such as Gammaproteobacteria and *Bacillus*, which could threaten public health. Microbial variation can reflect the environmental changes and quality of aquatic ecosystems and contribute to understanding urban rivers' microbial ecology in response to rapid urbanization. Given the findings, it is crucial to propose recommendations to local government bodies to establish policies that guarantee the safety and sustainability of water supplies in river systems. Meanwhile, eDNA and high-throughput parallel DNA sequencing based on the 16S rRNA gene provided a powerful culture-independent tool to assess bacterial community composition in river systems, thereby aiding in biomonitoring purposes. Investigation concerning seasonal variation can be done for future studies.

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