

Original Research

# Microbial and AMR Profiles of Mandulog River, Iligan City, Philippines: Insights into Ecosystem Health and Long-Term Flood Legacy

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## Abstract

The Mandulog River, in Iligan City, Philippines, sustains local livelihoods, fisheries, and biodiversity, but is subjected to perpetual pollution and the aftereffects of Typhoon Sendong in 2011. We utilized 16S rRNA high-throughput sequencing and Chan Zuckerberg ID analysis to screen for bacterial community profiles in the upstream, midstream, and downstream areas, and to detect antimicrobial resistance (AMR) genes. The midstream region (M1, M2, M3) was revealed as the most diverse area. The microbiomes harbored pollutant-degrading genera (*Pseudomonas*, *Sphingomonas*, *Bacillus*, *Methylobacterium*), mediating nutrient cycling and bioremediation. Yet, these co-occurred with AMR determinants – tetA(60) and aminoglycoside acetyltransferases (AAC(6′)-Ia, AAC(6′)-Iaj) – linked to wastewater, agriculture, and aquaculture. Their persistence suggests that sediments and biofilms act as long-term reservoirs of resistance, mobilized during high-flow conditions. Results validate our proposed "Dual-Score Hypothesis", which conceptualizes microbial communities following disturbance as a symphony of harmonious (biodegradation, nutrient cycling) and discordant notes (AMR, pathogens), the balance between which defines ecosystem health and public risk, thereby highlighting a dual function as providers of ecosystem services and as sentinels of ecological stress. Policy interventions should include

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microbial and AMR monitoring in surveillance programs, decrease pollutant loads, replenish riparian buffers, and enhance antibiotic stewardship to uphold ecosystem integrity and public security in tropical urban rivers.

**Keywords:** bacterial community composition, urban river, antimicrobial resistance, flood legacy

## Introduction

Urban river systems are active interfaces between land activities and aquatic habitats, but they are highly prone to impairment by wastewater discharges, agricultural runoff, and other anthropogenic impacts. These ecosystems' capacity to counteract, recover from, and adapt to environmental stress, including pollution and hydrophysical alterations, predominantly depends on the structure and richness of their microbiota. Bacterial community structure (BCC) is gaining prominence as a sensitive bioindicator of such effects since changes in microbial assemblages record changes in nutrient loading, organic contamination, and habitat quality. For example, floodwaters commonly bring many types of pollutants into a river and can radically change the river's microbial community, affecting water quality, nutrient cycling, and ecosystem resilience in general [1-4]. Breakthroughs in high-throughput sequencing, especially next-generation sequencing (NGS), now permit high-resolution description of abundant as well as rare taxa, including those missed by culture-based approaches. NGS in aquatic monitoring simultaneously offers insights into taxonomic diversity, functional potential, and the presence of pathogenic or antimicrobial-resistant microorganisms. Its responsiveness allows for the detection of ecological changes before their appearance in traditional water-quality parameters and, as such, represents a strong tool for anticipatory management of dynamic and high-hazard systems like urban rivers [5, 6].

The Mandulog River in Iligan City, southern Philippines, is a vital watercourse that supports fisheries, agriculture, and domestic uses before it discharges into Iligan Bay. In December 2011, Typhoon Sendong (Washi) devastated the watershed with catastrophic flooding, geomorphological disturbance, widespread sedimentation, and damage to infrastructure. While devastating floods are known to trigger short-term microbial changes, their longer-term ecological legacies are poorly defined, especially in tropical river systems where data are limited [7].

No studies have yet described the BCC of the Mandulog River or assessed whether Sendong left microbial signatures a decade after the event. Since floods can reshape sediments, nutrient fluxes, and hydrology for years, they may leave persistent imprints on microbial assemblages [8]. Knowledge of such dynamics is necessary since bacteria underpin essential ecosystem processes like photosynthesis, the breakdown of organic matter, and nutrient cycling, and are also sentinels of ecological stress and reservoirs of AMR.

Their metabolic plasticity and resilience underscore both their environmental value and their likely role in post-disturbance recovery [9, 10].

The research fills two gaps: the lack of baseline microbial information from the Mandulog River and the scarcity of evidence of flood legacies in tropical city rivers. We used 16S rRNA amplicon sequencing to profile microbial diversity along the upstream, midstream, and downstream sections, and assessed correlations with physicochemical gradients and human pressures. Special focus was placed on the potential long-term legacy of Typhoon Sendong, as seen through microbial community signatures.

We proposed the "Dual-Score Hypothesis", in a biological sense, that models microbial communities following perturbation as a symphony with both harmonious (functions beneficial to ecosystem services, such as biodegradation and nutrient cycling) and discordant notes (functions associated with antimicrobial resistance and pathogenicity). The harmony between these functions, we hypothesize, defines ecosystem well-being and public risk. Notably, we predicted that (i) midstream or downstream areas would be more diverse but enriched with pollutant-tolerant and AMR-related taxa, which would indicate both continuing urban discharges and residual impacts from Sendong sediments, and (ii) predicted microbial functions would exhibit a dual purpose in maintaining ecosystem processes while indicating ecological stress.

Through the integration of microbial taxonomic and functional composition with physicochemical parameters, this study provides the first molecular baseline for one of the most socially and economically valuable rivers in Mindanao. It also demonstrates how historical mega-flooding, combined with current pressures of urbanization, can shape microbiomes in a manner that impacts both ecosystem resilience and public health. More generally, results underscore the need to integrate microbial and AMR surveillance into river monitoring and post-disaster management programs in tropical developing countries. This work aligns with the United Nations Sustainable Development Goals (SDGs), specifically SDG 6 (clean water and sanitation), SDG 11 (sustainable communities), and SDG 14 (life below water).

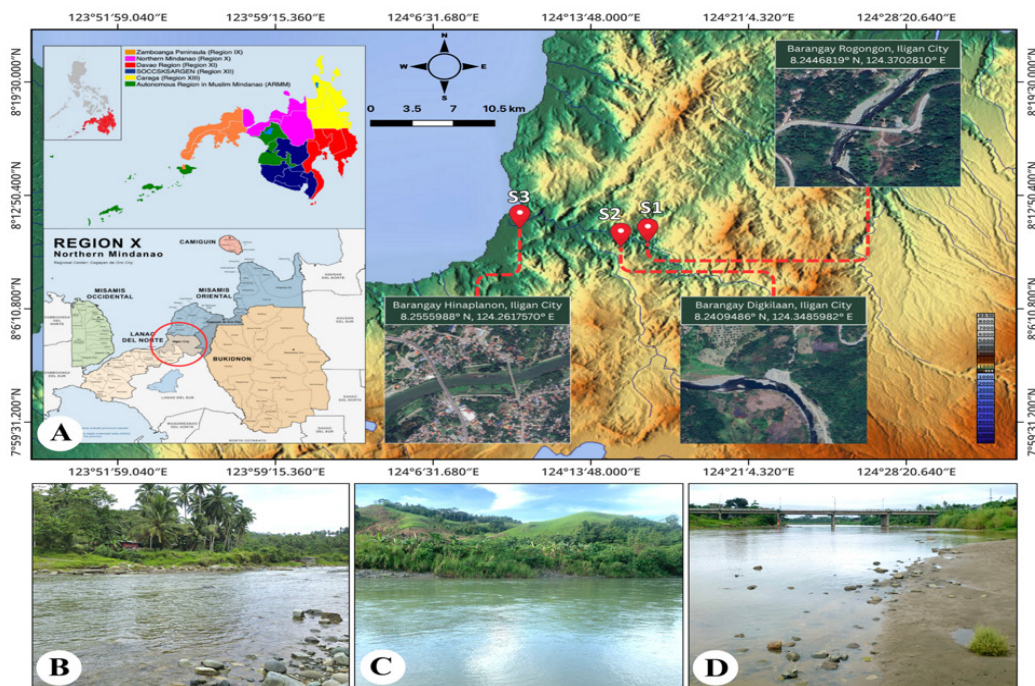


Fig. 1. Map of the study area – a) Sampling sites; b) photo of upstream portion in Barangay Rogongon, Iligan City – Site 1; c) photo of midstream portion in Barangay Digkilaan, Iligan City – Site 2; and d) photo of downstream portion in Barangay Hinaplanon, Iligan City – Site 3.

## Materials and Methods

### Study Area and Sampling Locations

Three sampling locations were designated along the Mandulog River in Iligan City, Lanao del Norte, Philippines (Fig. 1). Prior informed consent and courtesy call were conducted before sampling proper. The Mandulog River is a central river system that stretches about 50 km, emanating from upland watersheds of Lanao del Sur and flowing northward, passing through Iligan City before emptying into Iligan Bay. The river is an essential factor in the hydrology and ecology of the area and is a source of domestic water, irrigation, and fisheries resources for the surrounding communities [11]. It crosses varied landforms such as forested uplands, hilly areas, and highly populated lowland settlements. Yet, the river has been suffering from mounting environmental stressors like sedimentation, illegal quarrying, and spontaneous settlements, all of which have impacted water quality

and flow regime [12]. The three sample stations are critical river reaches: Site 1 (Upstream) is at Barangay Rogongon, which has a predominantly rocky and sandy landscape with little human settlement. Vegetation cover was mainly composed of grasses and riverbank trees. Site 2 (Midstream) is in Barangay Digkilaan, along a moderately sloping upland covered with grass, where the river has slightly more transparent water and moderate turbidity due to the presence of small-scale quarrying in the area. Site 3 (Downstream) is located close to the Mandulog Bridge in Barangay Hinaplanon, which has a lowland location with higher turbidity, finer sediments, and proximity to residential structures and major roads (Table 1).

### Sample Collection

Physico-chemical characteristics, such as average surface water temperature, pH, and dissolved oxygen (DO), salinity, and total dissolved solids (TDS) were recorded *in situ*. All containers were pre-treated with

Table 1. GPS location of sampling sites with a description of each site.

Sites	Coordinates	Descriptions	Human Influence
Site 1: Upstream	8.2446819°N, 124.3702810°E	Mountainous terrain; rocky and sandy riverbed; dense vegetation	Minimal; few nearby households; agricultural practices
Site 2: Midstream	8.2409486°N, 124.3485982°E	Slightly grassy riparian slope; sand, gravel, and silt substrate; moderately clear	Moderate; quarrying, livestock presence; agricultural practices
Site 3: Downstream	8.2555988°N, 124.2617570°E	Lowland urban area; turbid water; finer sediments; dense grass cover	High; nearby highway, settlements, runoff

bleach before use. In each location, 30 L of water were gathered, divided into 10 L per subsample. The samples were immediately taken to the laboratory for processing. Water was filtered through a sterile 60 mm Büchner funnel equipped with 50 mm polyethersulfone (PES) membranes (0.22 µm pore size). Membranes were stored in sterile, capped containers and transported to the Molecular Systematics and Conservation Genomics Laboratory, Premier Research Institute of Science and Mathematics (PRISM), Mindanao State University–Iligan Institute of Technology, for DNA extraction.

### DNA Extraction and Sequencing

Total genomic DNA was extracted using the manufacturer's protocol with the HiPurA™ DNA Extraction Kit from Vadhani Industrial Estate, Mumbai, India. The purified DNA was outsourced for high-throughput sequencing on the Illumina MiSeq platform to Macrogen, Seoul, South Korea. Nine amplicon libraries were prepared corresponding to the downstream (D1, D2, D3), midstream (M1, M2, M3), and upstream (U1, U2, U3) sites. The 16S rRNA gene was amplified with universal bacterial primers for the V3–V4 regions: Bakt\_341F (CCTACGGGNGGCWGCAG) and Bakt\_805R (GACTACHVGGGTATCTAATCC).

### Microbial Community Analysis

Raw sequence quality was evaluated with FastQC. Paired-end reads were joined together using FLASH (Fast Length Adjustment of Short Reads), and the resulting sequences were deposited in NCBI under accession numbers: SRR34935872–SRR34935880. Taxonomic profiling and functional prediction were carried out by Parallel-Meta Suite (PMS). PMS performed denoising, removal of chimeras, and amplicon sequence variant (ASV) generation, with taxonomic classification against the SILVA database using the in-built VSEARCH tool. KEGG Orthology was assigned, and pathways were annotated using the KEGG BRITE hierarchy to infer predicted functional profiles using the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt). KEGG (Kyoto Encyclopedia of Genes and Genomes) systematically examines gene functions, integrating genomic data with higher-order functional information. The accuracy of prediction was assessed by the Nearest Sequenced Taxonomy Index (NSTI). Alpha diversity metrics (Shannon, Simpson, Chao1) were calculated and represented through boxplots with Wilcoxon rank-sum tests; beta diversity was investigated by principal component analysis (PCA) and heatmaps [13, 14].

### Metagenomic Screening through CZ ID Pipeline

Further processing was performed with the Chan Zuckerberg ID (CZ ID). It is an open-source cloud pipeline for objective pathogen discovery and

microbial community characterization [15]. It takes in raw NGS data, removes host reads and filters quality, and then assembles and aligns the sequences and assigns taxonomic identities. Data are visualized through an interactive web interface where known and potentially novel taxa can be detected. The pipeline also incorporates detection of antimicrobial resistance (AMR) genes through the Comprehensive Antibiotic Resistance Database (CARD), coupled with the Resistance Gene Identifier tool [16].

## Results and Discussion

### Identification of Bacterial Community Composition (BCC)

In the river ecosystem, bacteria play a central role in organic matter decomposition, nutrient cycling, and water quality management [17]. This case study applied an eDNA metabarcoding strategy (16S rRNA gene, V3–V4 region) to characterize bacterial community composition (BCC) in the Mandulog River across downstream (D1, D2, D3), midstream (M1, M2, M3), and upstream (U1, U2, U3) regions, which are represented by nine amplicon libraries. After quality control, a total of 462,280 amplicon sequence variants (ASVs): (D1–8,134; D2–8,728; D3–21,952; M1–20,762; M2–100,502; M3–88,130; U1–108,209; U2–18,594; U3–87,269), were affiliated with 281 families and 594 genera. Fig. 2 shows the relative abundance of each group of bacteria with respect to the sampling sites. The dominant genera were *Sphingomonas*, *Pseudomonas*, *Bosea*, *Patulibacter*, *Brevundimonas*, *Bacillus*, *Methylobacterium*, *Flavobacterium*, and *Flectobacillus*.

Autotrophic and heterotrophic microbes are sensitive bioindicators of aquatic ecosystem health. Determining bacterial community composition (BCC) and functional functioning is instrumental in understanding ecosystem integrity and predicting ecological responses to water quality changes and environmental perturbations. Fig. 3 shows an amplicon sequence variant (ASV) count comparison for the different sites (downstream: D1, D2, D3; midstream: M1, M2, M3; upstream: U1, U2, U3), showing the abundant and important genera linked to ecosystem valuation and public health relevance.

*Sphingomonas* predominates in both the downstream and midstream areas, comprising 110 species. It is notable for its ecological adaptability and biotechnological significance. It is an excellent bacterial genus to investigate habitat preferences to facilitate applications for bioremediation. Many *Sphingomonas* species have been recovered from environments with toxic substance contamination, where they exhibit the capacity to degrade a wide range of xenobiotics [18–20] and recalcitrant pollutants such as polycyclic aromatic hydrocarbons, pesticides, and industrial waste products [21, 22]. This metabolic activity suggests that their abundance in the river can be attributed

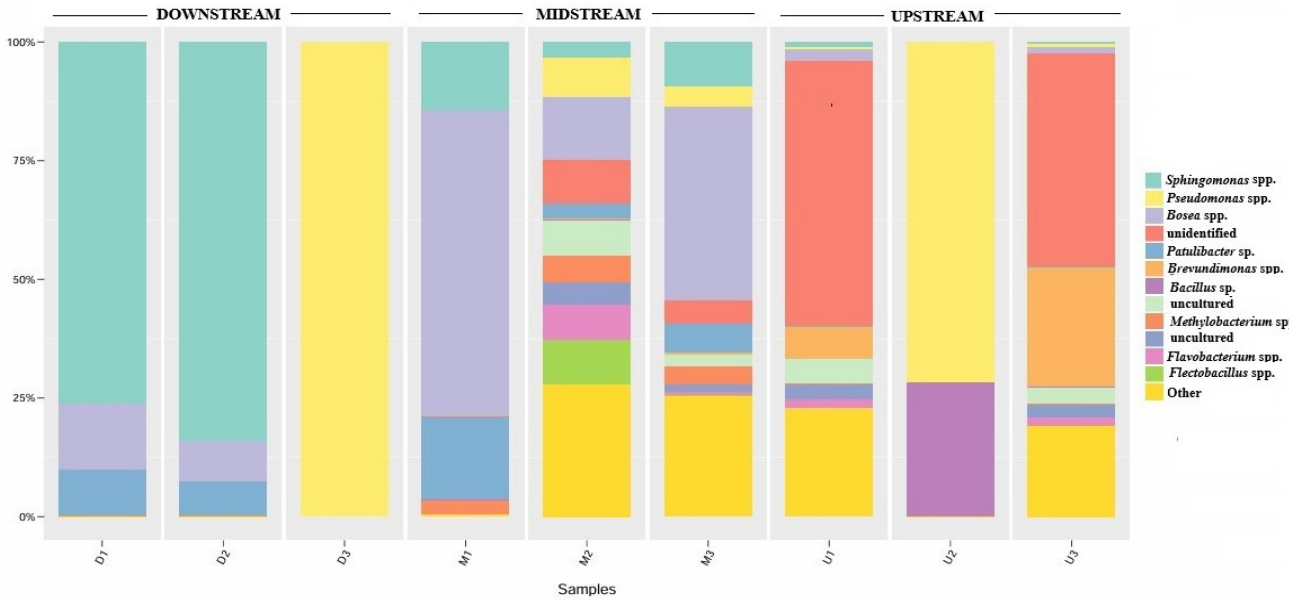


Fig. 2. Relative abundance of bacterial community composition (BCC) between sites (downstream-D1, D2, D3; midstream-M1, M2, M3; upstream-U1, U2, U3) in the Mandulog River, Iligan City, Philippines.

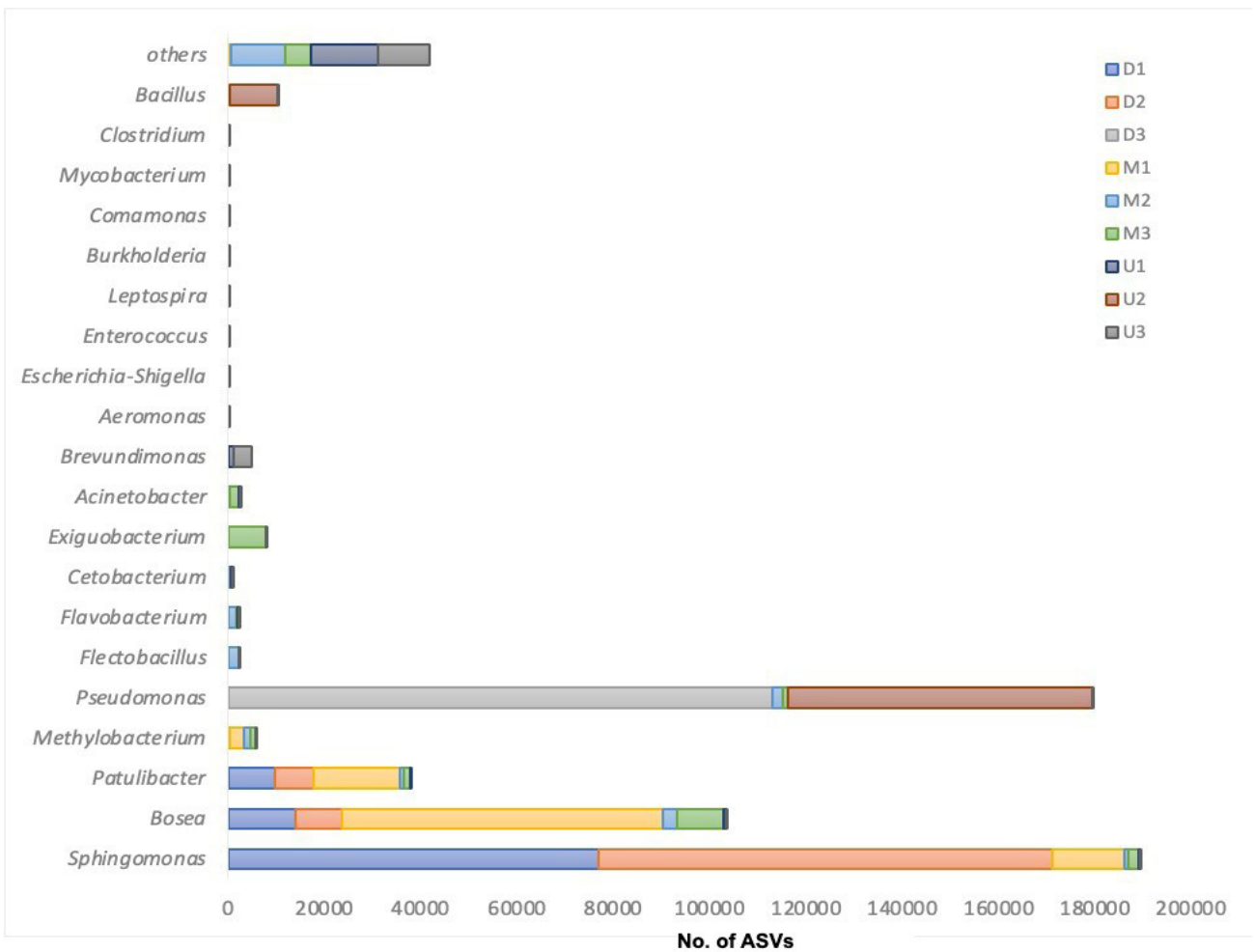


Fig. 3. Amplicon sequence variant (ASV) count comparison between sites (downstream-D1, D2, D3; midstream-M1, M2, M3; upstream-U1, U2, U3) in the Mandulog River, Iligan City, Philippines, based on the most abundant and important genera linked to ecosystem health and public health relevance.

to anthropogenic sources, such as urban runoff and agricultural effluents, which may have implications for the natural bioremediation of contaminants [23]. Their abundance in the Mandulog River, particularly at midstream and downstream stations, can be attributed to anthropogenic sources such as urban runoff and agricultural effluents [23, 24], with extractive activities like quarrying or mining [25] potentially adding further sediment and chemical inputs. Alternatively, some species, most notably *S. paucimobilis*, are known opportunistic pathogens, causing mostly nosocomial infections that are usually not life-threatening and can be cured with routine antibiotics [26]. This bifunctional nature is highlighted by the significance of *Sphingomonas* monitoring both as a biomarker of environmental stress and as a reservoir of opportunistic pathogens. In addition, *Sphingomonas* are characterized by their biosynthetic ability to produce extracellular polysaccharides like sphingans (e.g., gellan, welan, rhamsan), which find extensive use in food, pharmaceutical, and industrial applications [27, 28]. Evidence of practical application of *Sphingomonas* when added to the drinking water of broilers can improve the histological structure of gut microbiota of their intestines, resulting in body weight gain and an increase in survival rate [29]. Hence, finding *Sphingomonas* in the Mandulog River presents both ecological risks and opportunities, as they are potential agents of pollutant degradation and ecosystem resilience. Still, they deserve attention when viewed through the lens of public health and water safety.

Another ubiquitous genus found in the Mandulog River was the *Pseudomonas* (77 species detected), a metabolically heterogeneous group renowned for its ecological versatility and biotechnological value. *Pseudomonas* species are dominant in diverse aquatic and terrestrial habitats and are used as ecological indicators of water quality and level of pollution. This group of bacteria is often linked to contaminated or nutrient-rich environments, because of their capacity to metabolize a broad array of organic pollutants like hydrocarbons, pesticides, and heavy metals [30, 31]. This ability places *Pseudomonas* as an essential participant in natural bioremediation mechanisms within affected freshwater ecosystems. Additionally, their ability to form biofilms and secrete extracellular enzymes improves their ecological success in fluctuating environments like rivers that are subject to periodic flooding and sediment resuspension. From a public health point of view, several members of this genus, particularly *P. aeruginosa*, are opportunistic pathogens that can infect immunocompromised persons. It is a multidrug-resistant pathogen. Some examples are urinary tract infections, pneumonia, gastrointestinal infections, meningitis, and ear infections (otitis externa) that can be acquired by contact with recreational waters containing *P. aeruginosa* [32]. Other known pathogens are *P. putida* and *P. fluorescens* [33]. Co-detection of antimicrobial resistance genes in the Mandulog River

further highlights the possible risk of *Pseudomonas* as reservoirs and vectors of resistance in water ecosystems. Although the abundance of *P. aeruginosa*, *P. putida*, and *P. fluorescens* may indicate a eutrophic water, which is a paramount concern and could be due to nutrient sources, these species play a vital role in bioremediation by breaking down organic pollutants [34]. Concurrently, *Pseudomonas* species have been used in biotechnology, their applications extending from agricultural biocontrol to industrial enzyme manufacture, biosurfactant production, and the treatment of wastewater [35]. Therefore, as with *Sphingomonas*, the two-way ecological role of *Pseudomonas* indicates both potential for ecosystem services (nutrient cycling, pollutant degradation) and hazards associated with human health and antimicrobial resistance. Their abundance in the Mandulog River indicates that monitoring microbial communities can serve as an early warning for achieving a balance between conservation, ecosystem function, and public health.

Concurrently, other interesting taxa encountered were *Bosea* (57 species), *Patulibacter* (2 species), *Flavobacterium* (67 species), and *Flectobacillus* (12 species). Eutrophication may indicate blooms of *Flavobacterium*, the population of which is highest upstream, likely due to shallow waters and wind-driven suspension of particles [36]. Ecologically, these are significant players in the process of nutrient cycling and organic matter decomposition. *Bosea* and *Patulibacter* have been shown to have metabolic versatility with reported degradative abilities of intricate organic matter and survival in soils and sediments that have been affected by human activity. They were found across the three regions (upstream, midstream, downstream). Among the important species is *P. medicamentivorans*, which is a Gram-positive, ibuprofen-degrading, non-spore-forming, aerobic, and non-motile bacterium [37]. Meanwhile, *Flavobacterium* is mainly found in abundance in aquatic environments, where it is pivotal to the breakdown of polysaccharides, proteins, and other biopolymers, supporting carbon flow and microbial food webs. Likewise, *Flectobacillus* has been associated with the degradation of dissolved organic matter and can be used as a freshwater bacterial community marker [38, 39]. Both are abundant in the upstream and midstream regions only.

From the biotechnological point of view, particular *Flavobacterium* species form enzymes of economic value, whereas the *Bosea* genus has been explored for bioremediation potential. *Flavobacterium*, though, contains fish pathogens (e.g., *Flavobacterium columnare*), which indicates risks for aquaculture operations. The co-occurrence of these ecologically valuable yet at times pathogenic taxa reflects the dual nature of riverine microbiomes – as sources of ecosystem services and sites for upcoming health and management issues [40, 41].

The genus *Bacillus* (23 species) was also adequately covered in the Mandulog River samples (midstream and

upstream). The members of this genus are endospore-forming bacteria that exhibit excellent resistance to environmental stress, enabling them to survive long after flood disturbance through survival within sediments or biofilms. Their ecological roles comprised complex organic matter breakdown, nitrogen and phosphorus cycling, and extracellular enzyme production that help regulate water quality [42]. *Bacillus* species are extensively used in bioremediation techniques, such as the elimination of hydrocarbons, dyes, and heavy metals from polluted waters [43]. In aquaculture operations, some *Bacillus* strains have been employed as probiotics because of their capacity to suppress pathogens, enhance nutrient utilization, and promote overall fish well-being [44]. These include pathogens such as viruses, fungi, protozoa, bacteria, and helminths. However, opportunistic pathogenic strains like *Bacillus cereus* pose problems because they are linked to food poisoning and opportunistic diseases [45]. The dual ecological function of *Bacillus* highlights its significance as both an environmental engineer and a potential public-health risk factor.

Another bacterium of interest predominantly in the midstream region is *Methylobacterium* (8 species), which is also known as "pink-pigmented facultative methylotrophs". These species are considered common residents of aquatic and terrestrial ecosystems. They grow on single-carbon (C1) compounds, such as methanol and formaldehyde, and play crucial roles in carbon cycling and organic matter turnover [46]. In river systems, they contribute significantly to pollutant attenuation by degrading methanol and other volatile organic compounds. Certain strains are found to induce plant growth via the production of phytohormones and the fixation of nitrogen, and hence associate with farm and riparian ecosystem well-being [47]. Opportunistic pathogenic strains have been described, especially in immunocompromised hosts, where they may induce bloodstream and device-related infections [48]. The concurrent presence of *Methylobacterium* in the Mandulog River indicates its ecological flexibility – sustaining ecosystem services through pollutant degradation and with potential human health implications.

Concurrently, *Brevundimonas* is recognized as globally distributed with common affiliation with soil, water, and biofilm-enriched habitats. This genus is dominant in the upstream region with 84 species. The species show excellent metabolic adaptability, with the ability to degrade hydrocarbons, aromatic molecules, and other xenobiotics [49]. In riverine aquatic environments, they are involved in nutrient turnover and natural water filtering activities. Its life strategy of forming biofilms and desiccation resistance allows survival in fluctuating riverine environments, such as post-flood scenarios. Often traditionally ubiquitous in the environment, some species (e.g., *B. diminuta*) are linked to opportunistic infections, particularly within the hospital environment, and pose questions about pathogenic reservoirs [50].

Its presence implies both bioremediation potential and caution regarding water use and public health.

Moreover, some clinically and environmentally significant genera were found as well, such as *Clostridium*, *Mycobacterium*, *Comamonas*, *Burkholderia*, *Leptospira*, *Enterococcus*, *Escherichia-Shigella*, *Aeromonas*, *Acinetobacter*, *Exiguobacterium*, and *Cetobacterium*. They comprise possible pathogens and antimicrobial-resistant taxa, indicating threats to human and animal health. Water-borne pathogens such as *Clostridium perfringens* may act as an alternative fecal indicator in surface waters, which suggests the need for water quality monitoring for public health [51].

Alpha diversity analyses revealed the highest Shannon (2.679) and Simpson (0.911) values at midstream site M2, reflecting richness as well as evenness. Simpson's Index highlighted relative abundance, whereas the Shannon index weighed the richness of species in the community. Meanwhile, Chao1 is a nonparametric estimator for assessing the number of species in a community. Chao1 estimates were also highest midstream, implying higher species richness compared with upstream and downstream sites (Fig. 4). Patterns in beta diversity, as represented by a heatmap and PCA, showed strong clustering by river zone, where midstream samples clustered in the center and overlap was detected between D3 and U1. These spatial trends indicate midstream sites contain the most diverse bacterial communities, possibly fueled by urban inputs, nutrient gradients, and hydrodynamic conditions (Fig. 5).

Following a large-scale catastrophic flood, certain bacterial genera tend to colonize or remain long-term in river systems, particularly when floods embed nutrient-rich sediments, mobilize contaminants, and change hydrology. Most taxa are robust, opportunistic, and tolerant of environmental variation (Table 2) [52].

Some of the genera that have been identified in this study – like *Sphingomonas*, *Pseudomonas*, *Bacillus*, *Flavobacterium*, *Methylobacterium*, *Acinetobacter*, and *Aeromonas* – are recognized to survive in river systems many years following destructive flood events, owing to characteristics such as spore development, biofilm production, and metabolic adaptability (Table 2). Through these characteristics, they can survive post-flood environmental changes and retain ecological processes, while in other instances presenting public health hazards.

### Influence of Physico-Chemical Parameters on BCC

The Mandulog River, which was rich in biodiversity – freshwater goby populations in particular – has been targeted by water quality monitoring and analyses of anthropogenic effects. Typhoon Sendong (Washi) produced catastrophic flooding in 2011, bringing about pollutants, changing hydrology, and possibly restructuring microbial populations. Table 3 consolidates parameters that were measured at

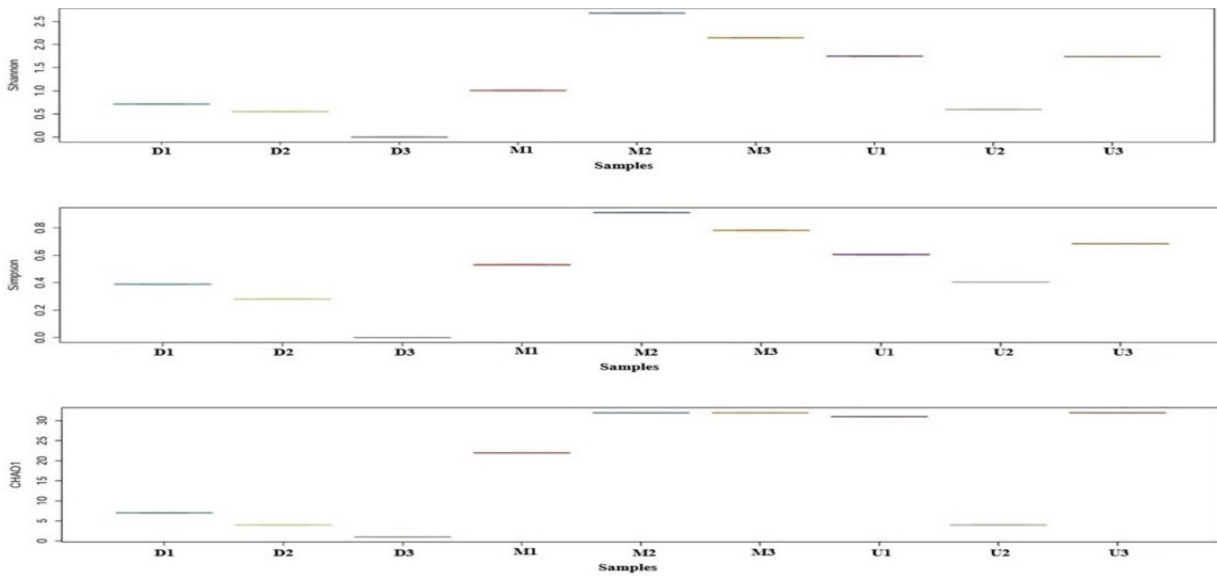


Fig. 4. Alpha diversity indices comparing the three sites (downstream-D1, D2, D3; midstream-M1, M2, M3; upstream-U1, U2, U3) in the Mandulog River, Iligan City, Philippines.

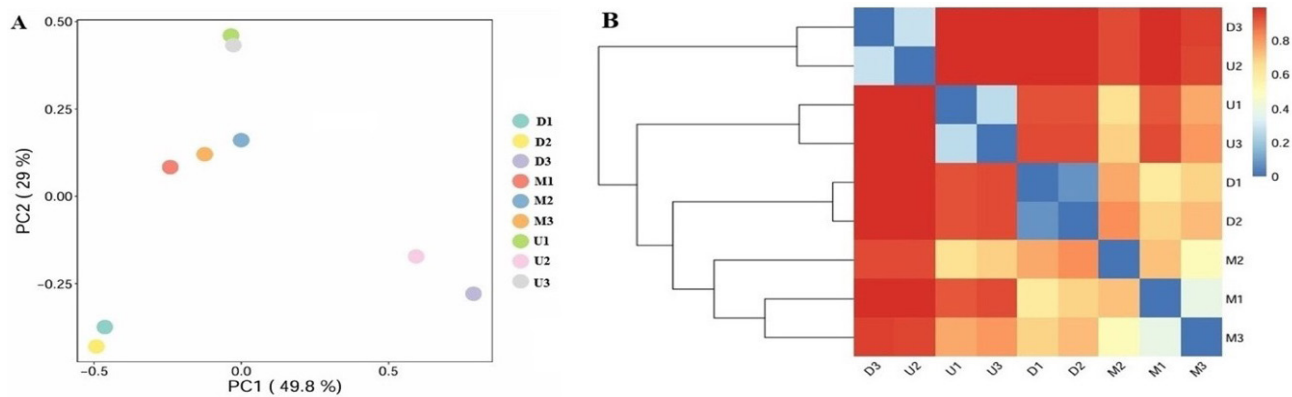


Fig. 5. Principal Component Analysis (PCA) shows amplicon library clustering in reduced dimensions based on beta diversity analysis; Heatmap shows the beta diversity pairwise distance matrices (downstream-D1, D2, D3; midstream-M1, M2, M3; upstream-U1, U2, U3) in the Mandulog River, Iligan City, Philippines.

upstream, midstream, and downstream sections. Physico-chemical parameter variations of the river determined the structuring pattern of the observed bacterial community composition (BCC). Temperature gradients between 23.4°C and 30.8°C seemed to affect microbial activity and relative abundance, as mesophilic genera *Pseudomonas* and *Bacillus* dominated warmer downstream waters. Dissolved oxygen (DO) content, which was above the DENR guideline range of 6.5-7.4 mg/L, favored a diverse assemblage of aerobic bacteria such as *Sphingomonas* and *Methylobacterium*, both of which have important roles in degrading organic matter. Salinity levels (<0.5 ppt) verified that the river was freshwater and were consistent with the dominance of freshwater-related taxa such as *Exiguobacterium* and *Brevundimonas*. Generally, water quality parameters are still within acceptable limits, aside from slightly raised pH.

The slightly increased pH recorded for the Mandulog River seems to have shaped the bacterial community composition by preferring alkalitolerant genera. The pH is a vital factor for BCC [53]. These taxa, including *Bacillus*, *Pseudomonas*, *Sphingomonas*, *Methylobacterium*, *Exiguobacterium*, *Flavobacterium*, *Acinetobacter*, and *Comamonas*, are known to be tolerant of environments in the range of neutral to alkaline, and several have (e.g., *Bacillus* and *Exiguobacterium*) alkaliphilic strains obtained from environments with a pH greater than 9. Their stability and relative abundance downstream (pH = 9.1) indicate that these taxa are key players in sustaining ecosystem processes under slight alkaline stress. Conversely, genera like *Clostridium*, *Leptospira*, *Enterococcus*, *Escherichia-Shigella*, *Aeromonas*, and *Cetobacterium* are presumably less alkalitolerant and can potentially be less competitive at high pH. The predominance of alkalitolerant taxa indicates ecological flexibility of the

Table 2. Bacterial genera commonly persisting in river systems long after major catastrophic floods, their ecological roles, persistence traits, and public health relevance.

Genus	Ecological Role	Persistence Traits	Public Health Relevance
<i>Pseudomonas</i>	Degrades diverse organic compounds; biofilm formation; nutrient cycling	Metabolically versatile; survives in low-nutrient and polluted environments	Opportunistic pathogens ( <i>P. aeruginosa</i> ); antibiotic resistance reservoirs
<i>Flectobacillus</i>	Degradation of complex polysaccharides; key member of freshwater consortia	Adapted to oligotrophic and cold environments; stable in aquatic ecosystems	Not pathogenic; ecological importance as a degrader
<i>Patulibacter</i>	Slow-growing but resilient; tolerates variable pH and low-nutrient conditions	Slow-growing but resilient; tolerates variable pH and low-nutrient conditions	Slow-growing but resilient; tolerates variable pH and low-nutrient conditions
<i>Bosea</i>	Nitrogen cycling; degradation of organic matter	Oligotrophic lifestyle; persistence in sediments and water	Rarely opportunistic; isolated from clinical cases but primarily environmental
<i>Brevundimonas</i>	Organic matter degradation, nutrient cycling	Survives in oligotrophic waters; biofilm-associated persistence	Emerging opportunistic pathogen, isolated from hospital and water systems
<i>Acinetobacter</i>	Organic matter degradation; nitrogen cycling	Resistant to desiccation, heavy metals, and disinfectants	Opportunistic infections; multidrug-resistant strains
<i>Aeromonas</i>	Aquatic animal pathogen; organic matter decomposer	Thrives in nutrient-rich waters; biofilm-former	Gastroenteritis, wound infections; zoonotic potential
<i>Enterococcus</i>	Fecal contamination indicator	Survives in sediments and re-suspends during floods	Nosocomial and community-acquired infections
<i>Escherichia/Shigella</i>	Fecal indicator; nitrogen cycling	Sediment persistence; regrowth under favorable conditions	Diarrheal diseases; AMR gene reservoirs
<i>Bacillus</i>	Organic matter decomposition; nutrient regeneration	Spore-forming; survives decades in sediments	Some pathogenic species (e.g., <i>B. cereus</i> )
<i>Clostridium</i>	Anaerobic decomposition; fermentation	Spore-forming; tolerates anoxia	Tetanus, botulism, and other toxin-related diseases
<i>Sphingomonas</i>	Biodegradation of complex pollutants	Adapted to oligotrophic and disturbed waters	Rare opportunistic infections
<i>Methylobacterium</i>	Carbon cycling; plant-microbe interactions	Survives in biofilms, low-nutrient niches	Rare opportunist; potential AMR gene carrier
<i>Leptospira</i>	Pathogenic and saprophytic nutrient cycling	Survives in moist sediments and floodwaters	Leptospirosis (flood-associated outbreaks)
<i>Mycobacterium</i>	Organic matter degradation	Resistant to disinfectants; slow-growing but persistent	Pulmonary and skin infections
<i>Flavobacterium</i>	Degrades high-molecular-weight organics	Thrives in nutrient pulses; biofilm-associated	Fish pathogens; rare human infections
<i>Comamonas</i>	Nitrogen and carbon cycling	Adaptable to diverse substrates	Rare opportunistic pathogen
<i>Burkholderia</i>	Pollutant degradation; plant interactions	Resistant to antibiotics; survives in varied habitats	Opportunistic infections ( <i>B. cepacia</i> complex)

Mandulog River microbiome. This shows that shifts in pH levels, whether caused by natural processes, algal blooms, or anthropogenic additions, can serve as significant drivers of microbial community structure and resilience. The stability of the ecosystem hinges on resistance, resilience, and redundancy, as expressed through the community composition or ecosystem function [54].

### Functional Prediction of Microbiome Composition

Prediction of the microbiome functional profile at the KEGG pathway level, based on Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt), showed a high relative proportion of bacterial assemblages associated with protein families, cellular processes and signaling, miscellaneous metabolic pathways (such as energy, lipid, carbohydrate, amino acid, cofactor, vitamin, and nucleotide metabolism), membrane transport,

Table 3. Recorded physico-chemical parameters from three sampling sites in the Mandulog River.

Parameters	Quality Range (DENR, 2016)	Upstream	Midstream	Downstream
Temperature (°C)	25-31°C	26.0	23.4	30.8
Salinity (ppt)	< 0.5 ppt	0.067	0.067	0.044
Dissolved Oxygen (mg/L)	≥5 mg/L	7.3	7.4	6.5
Total Dissolved Solids (ppm)	< 500 ppm	105.2	104.9	69.0
pH	6.5-8.5 pH	8.8	8.6	9.1

Note: Recorded numbers are average values.

and xenobiotic degradation. These functional abilities underscore the promise of the riverine microbiome as a prolific source of cellulolytic enzymes with high utility in the production of biofuels, biotechnology, and bioremediation (Fig. 6).

#### Xenobiotic Degradation Potential of Mandulog River Microbiome

PICRUSt-based functional inference revealed substantial enrichment of KEGG pathways related to xenobiotic degradation and metabolism. Several dominant genera identified in the Mandulog River – *Pseudomonas*, *Sphingomonas*, *Bacillus*, *Methylobacterium*, and *Brevundimonas* – are extensively reported for their ability to degrade a wide range of pollutants. *Pseudomonas* species contain heterogeneous oxygenases and hydrolases that can break down hydrocarbons, synthetic dyes, pesticides, and solvents [55, 56]. *Sphingomonas* is known for its degradation of polycyclic aromatic hydrocarbons (PAHs), phenols, and resistant aromatic compounds [57]. Metabolic versatility is shown by *Bacillus* spp. in the degradation of petroleum hydrocarbons, azo dyes, and organophosphate pesticides [42, 58]. *Methylobacterium* can degrade methanol, formaldehyde, and some aromatic compounds as a source of carbon [59]. In addition, *Brevundimonas* has also been associated with the biotransformation of hydrocarbons, phenols, and industrial solvents [60].

The persistence of these genera in the Mandulog River may be indicative of historical contamination – possibly associated with post-Typhoon Sendong

sediment redistribution and continuous anthropogenic inputs from urban, agricultural, and industrial activities. The BCC's functional potential reflects how the BCC is attuned to environments with intermittent pollutant exposure, which may contribute to natural attenuation processes. This relates to the predicted metabolic processes participating in aromatic compound degradation, xenobiotic biotransformation, and detoxification, reflecting riverine microbiomes' role in ecosystem resilience and contaminant processing.

#### Detection of Antimicrobial Resistance Genes (AMR)

Metagenomic screening identified the occurrence of antimicrobial resistance (AMR) genes in upstream and downstream parts of the Mandulog River, given its status as an environmental reservoir of resistance determinants (Table 4).

In the upstream location, the gene *tetA(60)* was found, which codes for one of the subunits of the *tetAB(60)* ABC transporter. This efflux pump confers resistance to tetracycline and tigecycline through pumping antibiotics out of bacterial cells. ATP-binding cassette (ABC) transporters are prevalent in prokaryotes and eukaryotes and utilize ATP hydrolysis to transport substrates through membranes, indicating the potential for extensive resistance dissemination [61].

In the downstream locations, two aminoglycoside acetyltransferase genes, *AAC(6')-Ia* and *AAC(6')-Iaj*, were detected. These genes encode resistance by enzymatic acetylation of the 6'-amino group of

Table 4. Detected Antimicrobial Resistance Genes in Mandulog River.

Location	Gene	Drug Class	High-level Drug Class	Mechanism	Model
upstream	<i>tetA(60)</i>	tetracycline antibiotic	tetracycline antibiotic	antibiotic efflux	protein homolog
downstream	<i>AAC(6')-Ia</i>	aminoglycoside antibiotic	aminoglycoside antibiotic	antibiotic inactivation	protein homolog
downstream	<i>AAC(6')-Iaj</i>	aminoglycoside antibiotic	aminoglycoside antibiotic	antibiotic inactivation	protein homolog

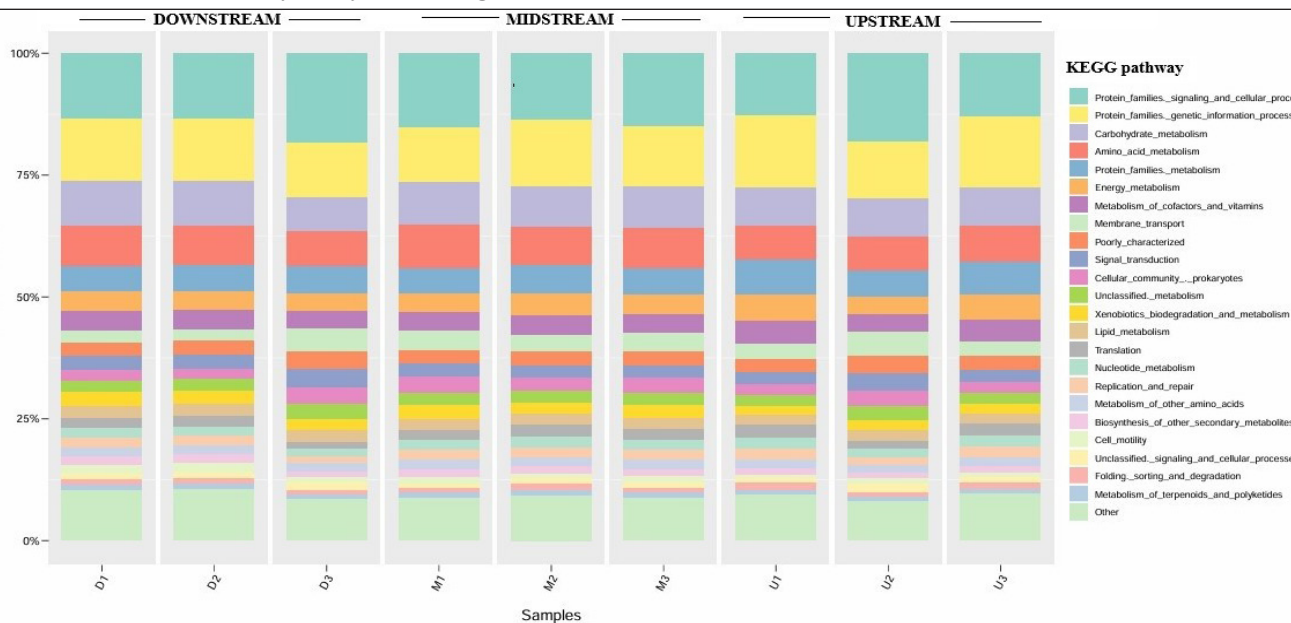


Fig. 6. PICRUSt predictions of the functional composition of the respective microbiome (downstream-D1, D2, D3; midstream-M1, M2, M3; upstream-U1, U2, U3) in the Mandulog River, Iligan City, Philippines, as represented at the KEGG pathway.

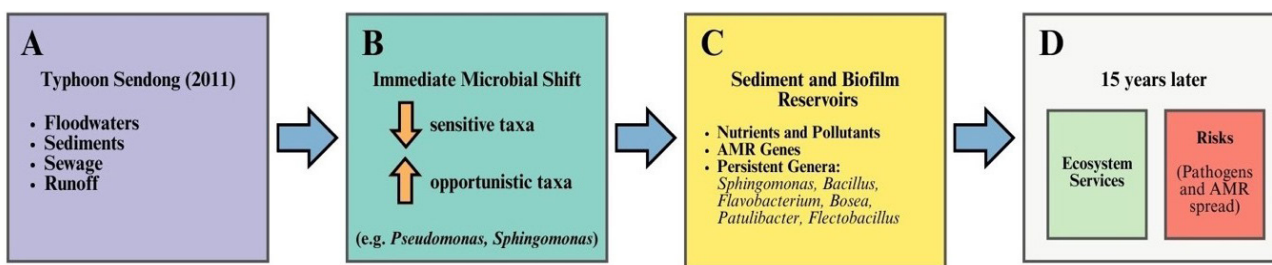


Fig. 7. Legacy effects of Typhoon Sendong on the Mandulog River microbiome.

aminoglycoside antibiotics, inactivating drugs like gentamicin, tobramycin, and amikacin. AAC(6)-Ia is reported to occur in plasmids, transposons, and integrons in pathogenic organisms like *E. coli*, *Shigella sonnei*, and *P. aeruginosa*. AAC(6)-Iaj, likewise, alters aminoglycosides and plays a role in resistance in *P. aeruginosa* [62]. The functional categorization of the genes according to the CARD (Comprehensive Antibiotic Resistance Database) ontology is shown in Table 4.

The results highlight the establishment of AMR genes in a post-disturbance river ecosystem. Upstream tetracycline resistance most likely indicates agricultural or livestock runoff, while aminoglycoside resistance downstream is more typical of domestic wastewater and urban effluents. Overall, these results highlight the risk of horizontal gene transfer to pathogenic bacteria, with ecological and public health implications.

### Legacy Effects of Typhoon Sendong

Severe flood events impose lasting ecological impacts that reach far beyond the initial disturbance. For the Mandulog River, the Typhoon Sendong disaster of 2011

redistributed sediments, organic pollutants, and matter throughout the watershed. These changed substrates, combined with geomorphic modifications in the river channel, likely provided new and long-term niches that continue to shape microbial assemblages. The prevalence of pollutant-resistant and metabolically flexible genera like *Spingomonas*, *Pseudomonas*, *Bacillus*, and *Methylobacterium* indicates a microbial community organized not only by continuous anthropogenic inputs but also by legacy impacts of historic flooding. These types of taxa are well-suited to grow in nutrient-rich and chemically dynamic conditions, indicating the lasting influence of Sendong on riverine microbiomes. This coincides with several studies on microbial responses to flooding, and findings imply that they reshape microbial community structure [63].

Over a decade later, the durability of these functional groups indicates how microbial communities can serve as "ecological memory banks", carrying records of disturbance history in their taxonomic and functional structure. This agrees with overall theories of microbial resilience and legacy effects, whereby communities do not completely restore to pre-disturbance conditions but rather reorganize into new stable combinations.

Identification of these persistent microbial signatures is also key to river management in tropical, disaster-affected areas because they influence ecosystem recovery pathways and the ability to deliver essential functions like nutrient cycling, pollutant decay, and biodiversity maintenance [64, 65].

#### Conceptual model of the legacy effects of Typhoon Sendong (2011) on the Mandulog River Microbiome

Fig. 7 shows a summary of the Legacy Effects of Typhoon Sendong on the Mandulog River Microbiome. Typhoon Sendong triggered catastrophic flooding that introduced sediments, organic matter, sewage, and pollutants into the river system (Panel A). These inputs caused immediate disturbance, altering river geomorphology and favoring opportunistic microbial taxa while reducing sensitive groups (Panel B). Over time, deposited sediments and biofilms acted as long-term reservoirs of nutrients, contaminants, and antimicrobial resistance genes, sustaining pollution-tolerant and metabolically versatile genera such as *Sphingomonas*, *Pseudomonas*, *Bacillus*, *Methylobacterium*, *Flavobacterium*, *Bosea*, *Patulibacter*, and *Flectobacillus* (Panel C). After nearly 15 years, the bacterial community composition reflects this ecological “memory”, balancing ecosystem services (nutrient cycling, pollutant degradation, bioremediation potential) with risks such as pathogen persistence, antimicrobial resistance dissemination, and reduced resilience of native biodiversity (Panel D). This framework highlights how extreme climatic events can produce long-lasting microbial signatures that shape freshwater ecosystem health and inform conservation and management strategies.

A similar study covering the Tandag River, Surigao del Sur, also applied amplicon sequencing to describe bacterial diversity among upstream, midstream, and downstream stations. A rich microbiome with *Bacillus*, *Pseudomonas*, *Ralstonia*, *Alcaligenes*, and *Pseudarcicella* as dominant genera was found, with a decrease in alpha diversity from upstream to downstream as urban effluents became more concentrated. Microbial community organization was influenced mainly by human disturbance and urban wastes, and higher alkalinity benefited alkaliphilic taxa [66].

In contrast, the Mandulog River's microbiome not only exhibited sustained anthropogenic inputs but also a microbial legacy of Typhoon Sendong (2011). The typhoon, however, caused high loads of domestic, agricultural, and industrial contamination, particularly in settlement areas. This devastating flood redirected hydrology, redistributed sediments, and perhaps reconfigured microbial reservoirs. The survival of taxa associated with xenobiotic degradation (e.g., *Pseudomonas*, *Sphingomonas*, *Bacillus*, *Methylobacterium*) and the presence of antimicrobial resistance genes indicate that the river retains ecological signatures of this extreme disturbance, with sediments

and biofilms serving as reservoirs of functional and pathogenic microbes. Whereas the Tandag River is an example of chronic, cumulative modification by urbanization, the Mandulog River shows a post-disturbance recovery pathway, whereby microbial communities still register the aftermath of a single extreme flood superimposed on existing anthropogenic stresses.

This divergence underlines the need to look at both sudden catastrophic events and chronic urban stressors when interpreting microbial signatures in tropical rivers, since both routes condition resilience, ecological function, and human community risk.

In this respect, we propose and coin the “Dual-Score Hypothesis” to describe this phenomenon, where selective pressures screen for microbial persistence characteristics, and disturbances orchestrate the functional niches that species fill within the ecosystem. In this model, the microbial “symphony” post-disturbance consists of harmonious notes, such as biodegradation and nutrient cycling, and discordant notes, including antimicrobial resistance and pathogenicity. The interaction and equilibrium of these opposing functions eventually determine ecosystem health and underpin related public risks.

#### Implications for Ecosystem Health, Services, and Conservation

The Mandulog River BCC is of critical importance to understanding ecosystem resilience, function, and potential vulnerabilities. Results of this study point out pollutant-degrading bacteria like *Sphingomonas*, *Pseudomonas*, *Bacillus*, and *Methylobacterium*, reflecting a resilient microbial community capable of mitigating specific anthropogenic impacts through the natural bioremediation process. These processes are essential for preserving water quality, facilitating nutrient cycling, and maintaining aquatic biodiversity, including commercially valuable species such as freshwater gobies. Though their joint occurrence points to risks for public health, fisheries, and recreational activities, with this bifold function – both ecosystem service provision as well as an indicator of ecological distress – it becomes pertinent to include monitoring bacterial community composition (BCC) in river management plans. Conservation-wise, the incorporation of high-throughput sequencing into routine surveys can facilitate early warnings of trends toward dominance by pollution-resistant taxa, triggering on-time mitigation measures. This molecular monitoring is in line with international efforts at freshwater ecosystem health, especially in climate-vulnerable and disaster-risk-prone tropical areas. Long-term monitoring can also determine if legacy impacts from mega-disasters such as Typhoon Sendong last, providing a unique window to track post-disturbance recovery paths and inform habitat restoration and policy-making.

Action Points for Conservation that need elaborate consideration include: improve local water-quality monitoring programs by incorporating AMR and BCC monitoring into river systems through eDNA and NGS to detect early microbial shifts; adopt more stringent controls on wastewater and agricultural runoff to limit nutrient and pollutant loading; restore riparian zones to enhance shading, temperature control, and habitat complexity; formulate contingency plans for the management of microbial risks during extreme weather events; introduce bioremediation strategies and involve communities in focus group discussions as well as in participatory river stewardship programs that link biodiversity conservation with livelihoods.

### Conclusions

This research presents the first comprehensive picture of bacterial community structure and antimicrobial resistance in the Mandulog River, a watershed still characterized by the impact of Typhoon Sendong. The river contains rich microbial diversity, including pollutant-degrading taxa capable of supporting natural bioremediation, but also possible pathogens and resistance genes (tetA(60), AAC(6')-Ia, AAC(6')-Iaj) that highlight threats to the ecosystem and public health. Slightly higher pH and human-derived inputs were found to be the principal drivers of these microbial changes.

The shared function of the microbiome as both an ecosystem services provider and environmental stress indicator points to the necessity of regular molecular monitoring. Seasonal and post-disturbance dynamics should be followed in future studies. Functional metagenomics should be used to determine resistance gene mobility, and microbial data should be combined with land-use and hydrological surveys. Integrating these tools into local water governance and community-based monitoring will enhance conservation efforts as well as provide biodiversity and human health protection.

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### Conflict of Interest

The authors declare no conflict of interest.

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