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Original Research

High-Throughput Sequencing as a Tool for Detecting Microbial Communities in Lake Ecosystem and Its Implications in Fish Farming in Lake Buluan, Mindanao, Philippines

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Abstract

Lake Buluan, the third-largest lake in the Philippines, is renowned for its diverse ecosystem, including various endemic fish species and fish pens dedicated to aquaculture. This study focused on analyzing the microbial community diversity in the lake using metabarcoding of the V3-V4 16S rRNA gene amplicon sequencing on llumina MiSeq platform. A total of six amplicon libraries were created, representing sites inside and outside fishing pens. After quality control and processing 917,192 amplicon sequence variants (ASVs) were obtained. The most abundant ASVs belonged to genera such as *Cylindrospermopsis, Sphaerospermopsis, Stenotrophomonas, Vibrio, Anabaena, Microcystis, Serratia, Elizabethkingia* and some unidentified genera that belong to phyla Verrucomicrobia, Planctomycetes, Chloroflexi, Firmicutes, Actinobacteria, Bacteroidetes and Acidobacteria. As assessed by the Shannon index, the microbial community's alpha diversity was high inside fish pens. In addition, the pH level significantly influences the composition of bacterial communities (BCC), leading to the notable presence of bacteria from the phylum Firmicutes, certain Proteobacteria, and Cyanobacteria. The analysis revealed an increased abundance of bacteria associated with toxin production, antibiotic

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resistance, and opportunistic pathogens. This poses risks to human health, water quality, and ecological systems. This information may impact fish farming practices in the area. Moreover, the predicted functions of the bacterial communities, analyzed using the PICRUSt algorithm within the Parallel-Meta Suite (PMS), indicated their involvement in metabolism and environmental information processing. Notably, the presence of bacteria involved in xenobiotic biodegradation suggests potential applications in bioremediation, including the breakdown of pollutants, hydrocarbons, and heavy metals.

Keywords: lake, xenobiotic, microbes, diversity, metabarcoding

Introduction

The lake ecosystem has gained significant attention, and within its inconspicuous inhabitants, prokaryotes play a critical role in the decomposition of organic matter and mineralization of nutrients in freshwater sediments [1, 2]. However, human activity and global climate change have significantly impacted aquatic ecosystems' functional activity in terms of biogeochemical cycling in recent years [3]. The ability of an aquatic ecosystem to handle, reduce, and recover from the impacts of pollutants and hydro-physical changes relies heavily on the diversity and composition of its microbiota and its functional traits. These traits include photosynthesis and the breakdown, oxidation or fermentation of both inorganic and organic substances [4-6]. Understanding the evolution of bacterial community composition (BCC) in lake ecosystems is crucial for comprehending microbial biodiversity and assessing the impact of specific habitats and climate change on BCC distribution and diversity [7]. Freshwater BCC could be an effective alternative and monitoring tool for the condition of big lakes [8].

Prokaryotes' remarkable adaptability to diverse environments highlights their evolutionary success. In lake ecosystems, BCC plays a significant role in decomposing organic materials into nutrients, which are then utilized by other organisms, influencing water quality [9]. Bacterial activities profoundly impact ecosystem function and metabolism, as they dominate a significant portion of planktonic biomass. The active bacterial community involves substrate uptake, growth, reproduction, and metabolic processes [10]. Prokaryotes are responsible for crucial geological, biological, and chemical processes in aquatic environments [11]. Microorganisms' activities are vital for all organisms within an ecosystem, and researchers are exploring the extraction of useful chemicals from bacterial by-products.

The BCC not only varies along the larger gradient within and between the aquatic habitats [12, 13]. A particular study reported the use of 16S rRNA gene sequences from some 11 freshwater lakes and resulted in the collection of 689 bacterial 16S rRNA gene sequences. This enabled the identification of 10 freshwater phyla and 34 supposed bacterial freshwater clusters, defined as monophyletic branches of a phylogenetic tree containing at least two sequences with ≥95% gene identity from more than one freshwater environment [14]. Many identified clusters were common in freshwater, representing the unique bacterial taxa found only in freshwater ecosystems. Lake water and sediments of the different habitats with unique intrinsic environmental conditions result in their unique bacterial community composition [15] and such differences account for different microbial biogeographies in lake environments [16, 17]. However, over the past many years, the frequent use of molecular techniques has helped to fill the gap in the literature about freshwater bacterial communities to a more significant extent by retrieving innumerable bacterial groups from these freshwater ecosystems.

Moreover, the 16S rRNA gene has become a standard for prokaryotic taxonomy. The 16S rRNA gene is approximately 1600 base pairs long and includes nine hypervariable regions of varying conservation (V1-V9). The conserved regions are useful for determining the higher-ranking taxa, whereas more quickly evolving ones can help identify genera or species. Metabarcoding using 16S rRNA markers is widespread in the studies of various microbial communities with the introduction of next-generation sequencing (NGS) techniques. This has led to novel applications of metabarcoding methods. Increased read counts have allowed for quantitative estimates of the microbial community composition. Another advantage of NGS-based metabarcoding is that quantitative analysis has become available for communities of uncultured microbes. Utilizing the 16S ribosomal gene as a phylogenetic marker is a cost-effective and efficient microbiome analysis method, including predicting functional content based on taxon abundances [18, 19].

Lake Buluan, located in the southern part of Mindanao, spans the provinces of Maguindanao and Sultan Kudarat and is the third-largest lake in the Philippines. The lake is renowned for its rich biodiversity, particularly its abundance of endemic fish species and its significant fish pen aquaculture industry. Over the past decades, the water quality of Lake Buluan has been significantly impacted by various anthropogenic activities. These include bathing, washing, open defecation, religious practices, tourism, surface drainage, irrigation runoff, industrial discharge, and domestic wastewater. Such activities can cause rapid changes in the sensitive ecosystem of the lake and its watershed [20].

In this context, the presence and activities of autotrophic and heterotrophic microorganisms play a crucial role in assessing and predicting aquatic environments' ecological and water-quality conditions. By identifying and understanding the composition of the microbial community (BCC) in Lake Buluan, researchers can gain insights into the functioning of these microorganisms in maintaining the ecosystem's health. This knowledge becomes instrumental in recognizing and predicting the responses of the lake's ecosystem to changes in water quality or other environmental disturbances.

The primary objective of this study is to detect and enhance our knowledge regarding the diversity of bacterial community composition (BCC) and gain insights into the functional role of BCC within a freshwater lake setting. Notably, this study represents the initial investigation of BCC in lake communities in the Philippines utilizing high-throughput sequencing techniques, establishing itself as one of the pioneering studies to implement an innovative pipeline.

Implementing the Parallel-Meta Suite (PMS), an online web service with a user-friendly graph-based interface (GUI), presents a significant advancement in usability, eliminating the complexity associated with command-based operations while incorporating highly versatile toolkits. PMS offers an interactive, efficient, and comprehensive approach to microbiome analysis, building upon established marker-gene-based analysis protocols and workflows.

Examining the composition of bacterial communities in freshwater ecosystems holds considerable significance due to the influence of bacterial groups on the regulation of dissolved organic matter and their contributions to various vital processes, such as matter and energy cycling. Understanding BCC dynamics and microorganisms' ecological roles in Lake Buluan, Philippines is vital.

Materials and Methods

Study Site and Sample Collection

Lake Buluan, situated in the island of Mindanao in the Philippines, is the third largest lake in the region, following Lake Lanao and Lake Mainit. It covers an estimated surface area of 61.34 square kilometres, an average elevation of 4.5 meters, mean depth of 4.5 m and maximum depth of 6 m.

Located between the provinces of Maguindanao and Sultan Kudarat, the lake (6°38′26″N-124°49′30″E/ 6.64056°N-124.82500°E) falls within the administrative boundaries of the municipalities of Buluan and Mangudadatu in Maguindanao, as well as President Quirino and Lutayan in Sultan Kudarat (Fig. 1). The lake is formed by the confluence of several marshy basins, including those of the Pulangi, Maanoy, Buluan, and Alah rivers, all of which are tributaries of the Mindanao River [20].

Prior informed consent was obtained from the barangays for the study. Two sampling sites were established: inside fish pens (F) and outside fish pens (OF). Physico-chemical parameters such as average water temperature, pH and dissolved oxygen (DO) were measured and noted. All water containers were bleached prior to use. At each site, 30 liters (L) of water were collected, with approximately 10 L per subsample, and

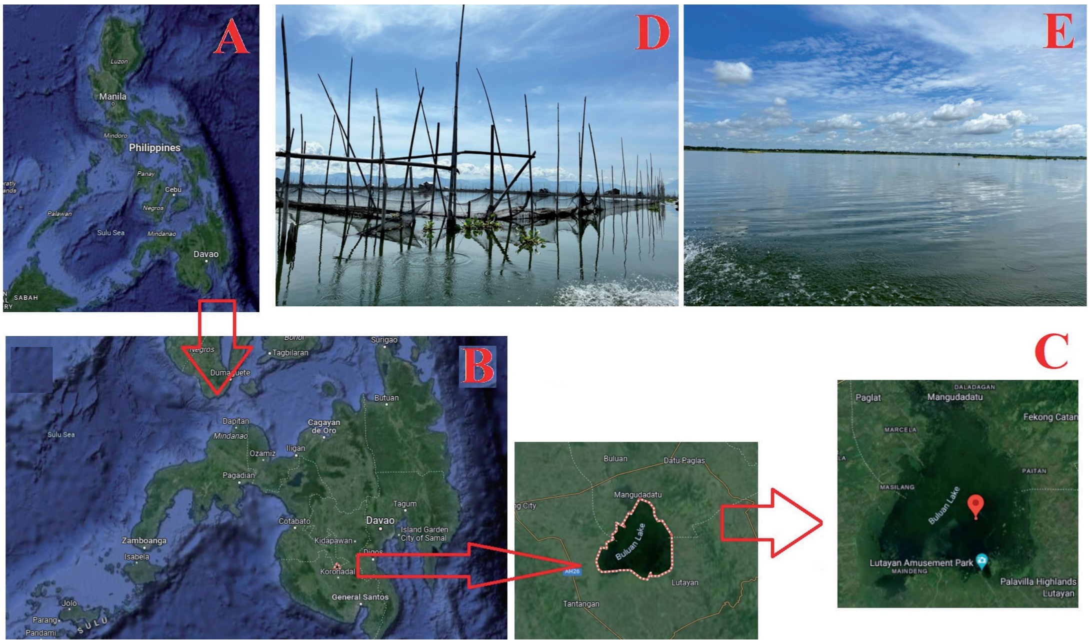

Fig. 1. Lake Buluan, Maguindanao: a) Map of the Philippines; b) Mindanao showing location of Lake Buluan; c) Aerial view of Lake Buluan; d) Inside fish pens; e) Outside fish pens

subsampling was randomly taken at 10-meter intervals. All the collected samples were promptly transported to the laboratory and subjected to immediate processing. Water samples were filtered using sterile 60 mm buchner funnel with 50 mm Polyethersulfone (PES) membrane (0.22 μm pore size). The membranes were then placed in capped sterile containers for DNA extraction.

DNA Extraction, High-Throughput Sequencing, and Microbiome Analysis

Total genomic DNA was extracted from water samples using HiPurA™ DNA Extraction Kit (Vadhani Industrial Estate, Mumbai, India) according to the manufacturer's instructions. Purified DNA were sent to Macrogen, located in South Korea, for high-throughput sequencing using the Illumina MiSeq platform. Microbial communities were analyzed using high-throughput amplicon sequencing. There were six amplicon libraries corresponding to the sites inside fish pens (F1, F2, F3) and outside fish pens (OF1, OF2, OF3). The amplification of the 16S rRNA gene was performed using universal bacterial primers designed to target the V3-V4 regions Bakt_341F:CCTACGGGNGGCWGCAG and Bakt_805R: GACTACHVGGGTATCTAATCC.

Quality check of sequences was done using FastQC. This provides a convenient way to execute a quality control check on raw sequence data from high-throughput sequencing pipelines. FLASH (Fast Length Adjustment of Short reads) was utilized to merge the paired-end reads, a rapid and precise tool for genome assembly and analysis. The resulting sequences were uploaded to the Metagenomics RAST (MG-RAST) server for analysis and made publicly available under the accession code numbers 4984173.3-4984178.3. MG-RAST offers an automated pipeline for processing environmental DNA sequences. It is a comprehensive system with an R-interface that allows users to upload raw sequence data, which is then normalized, processed, and summarized for metagenomic analysis [21]. The Parallel-Meta Suite (PMS) was employed for efficient and accurate microbiome analysis. PMS processed the amplicon sequences as the primary input, performing denoising and de-chimera of gene markers to generate amplicon sequence variants (ASVs). These sequences were aligned against reference databases (such as SILVA) using the built-in vsearch tool for profiling and taxonomy annotation. The relative abundance of taxa was determined, and gene families were inferred using the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) algorithm to assign KEGG Orthology. KEGG (Kyoto Encyclopedia of Genes and Genomes) systematically analyze gene functions, linking genomic information with higher-order functional information. The metabolic pathways were annotated using the KEGG BRITE hierarchy. The prediction accuracy of functional annotations was assessed using the Nearest Sequenced Taxonomy Index, which measures the phylogenetic similarity between operational taxonomic units and their closest individually sequenced relatives. Alpha diversity metrics such as Shannon, Simpson, and Chao1 indexes were also calculated. The results were visualized into boxplots with Wilcoxon rank-sum test. Beta-diversity pattern was demonstrated via principal component analysis diagram and visualized as a heatmap. Beta diversity refers to the variation in species composition between different samples or locations. Heatmaps are particularly useful for representing the dissimilarity or similarity matrix between samples based on their species composition. Each cell in the heatmap represents the degree of dissimilarity or similarity between two samples, with different colors or intensity levels indicating different levels of dissimilarity or similarity. Meta-storms distance analysis was used to show the relationship of bacterial taxa beta-diversity distribution to physico-chemical parameters. For biomarker analysis, PMS utilizes statistical tests such as the Wilcoxon or Kruskal rank-sum test to identify organisms or gene units with significant differences among different groups based on discrete metadata. These identified candidates are then ranked according to their importance using the Random Forest algorithm [22].

Results and Discussion

Lake Buluan, known as the third largest lake in the Philippines, boasts a rich diversity of fish species including climbing gourami (*Anabas testudineus*), snakehead murrel (*Channa striata*), milkfish (*Chanos chanos*), walking catfish (*Clarias batrachus*), common carp (*Cyprinus carpio carpio*), sundari bele (*Glossogobius giuris*), Mozambique tilapia (*Oreochromis mossambicus*), common barb (*Puntius binotatus*), and snakeskin gourami (*Trichopodus pectoralis*). Within lake ecosystems, the bacterial community plays a crucial role in decomposing organic matter into essential nutrients that serve as food for other organisms while exerting control over water quality [23].

In this context, autotrophic and heterotrophic microorganisms serve as highly sensitive indicators of aquatic environments' ecological and water quality conditions. Identifying the bacterial community composition (BCC) and understanding the functional roles of microorganisms in healthy aquatic ecosystems are essential for recognizing and predicting responses to changes in water quality and other environmental disturbances.

Identification of Bacterial Community Composition (BCC)

High-throughput sequencing based on the 16S rRNA gene explored bacterial communities. Bacterial datasets were separated and analyzed based on the two sampling sites (inside and outside fish pens). After post-quality control, there were a total of 917,192 amplicon sequence variants (ASVs) obtained from nine amplicon libraries (inside fish pens-F1-163,507; F2-139,192; F3-165,452; outside fish pens-OF1-145,100; OF2-152,435; OF3-151,506) belonging to 486 families and 662 genera inside the fishing pens and 505 families and 621 genera outside fishing pens. Results showed that the most abundant ASVs at the genus level were affiliated with *Cylindrospermopsis, Sphaerospermopsis, Stenotrophomonas, Anabaena, Microcystis, Serratia, Elizabethkingia*, CL500 from the family Phycisphaeraceae and some unidentified bacteria at the genus level (Fig. 2). Most unidentified genera belong to phyla Verrucomicrobia, Planctomycetes, Chloroflexi, Firmicutes, Actinobacteria, Bacteroidetes and Acidobacteria. For phylum Planctomycetes most bacteria come from families Rubinisphaeraceae, Phycisphaeraceae, and Pirellulaceae. As for phylum Chloroflexi the common families are Caldilineaceae and Roseiflexaceae while family Solibacteraceae is the most common family under phylum Acidobacteria. *Candidatus megaira* (Rickettsiales), known as symbiotic microbes and are common associates of algae and ciliates were abundant [24].

Alpha diversity indices (Shannon, Simpson, and Chao1) were computed at the genus level to assess the diversity measures. The Shannon index yielded the highest bacterial diversity value of 2.3618 inside fishing pens and 2.0898 outside fishing pens. Simpson index was 0.7817 inside fishing pens and 0.7590 outside fishing pens. Simpson's index emphasized relative abundance while the Shannon index focused on the richness of the community's species. At the same time, Chao1 is a nonparametric method for estimating the number of species in a community. More species were identified inside fishing pens. To evaluate beta diversity, pairwise distance matrices were generated using Hierarchical Meta-Storms and visualized as a heatmap showing high abundance of species inside fish pens. Additionally, Principal Component Analysis (PCA) was conducted to explore the clustering of amplicon libraries based on beta diversity. The PCA plot separated the amplicon libraries collected inside and outside the fish pens, reducing the multidimensional data to distinct clusters. Moreover, specific phyla (Chloroflexi, Planctomycetes, Firmicutes, Verrucomicrobia, Actinobacteria, Bacteroidetes, Acidobacteria) were identified as biomarkers that distinguished the two sampling sites. The importance score, represented on the x-axis, was obtained from the Random Forest Model, which evaluated the significance of each group in distinguishing between the different sites. This analysis provided insights into the key phyla that contributed to the differentiation of the sampling locations Fig. 3(a-d).

Fig. 4 shows the amplicon sequence variants (ASVs) count comparison between outside and inside fish pens based on the most abundant and common genera. *Cylindrospermopsis* predominate inside fish pens. The genus belongs to the family Cylindrospermopsidaceae of photosynthetic bacteria commonly found in aquatic environments such as lakes, reservoirs, and slow-flowing rivers. Some strains are known to produce toxins, particularly cylindrospermopsin. It is a potent hepatotoxin that can harm humans and animals if exposed to contaminated water. Certain species can form dense blooms, especially nutrient-rich waters. These blooms can have ecological impacts, including altering

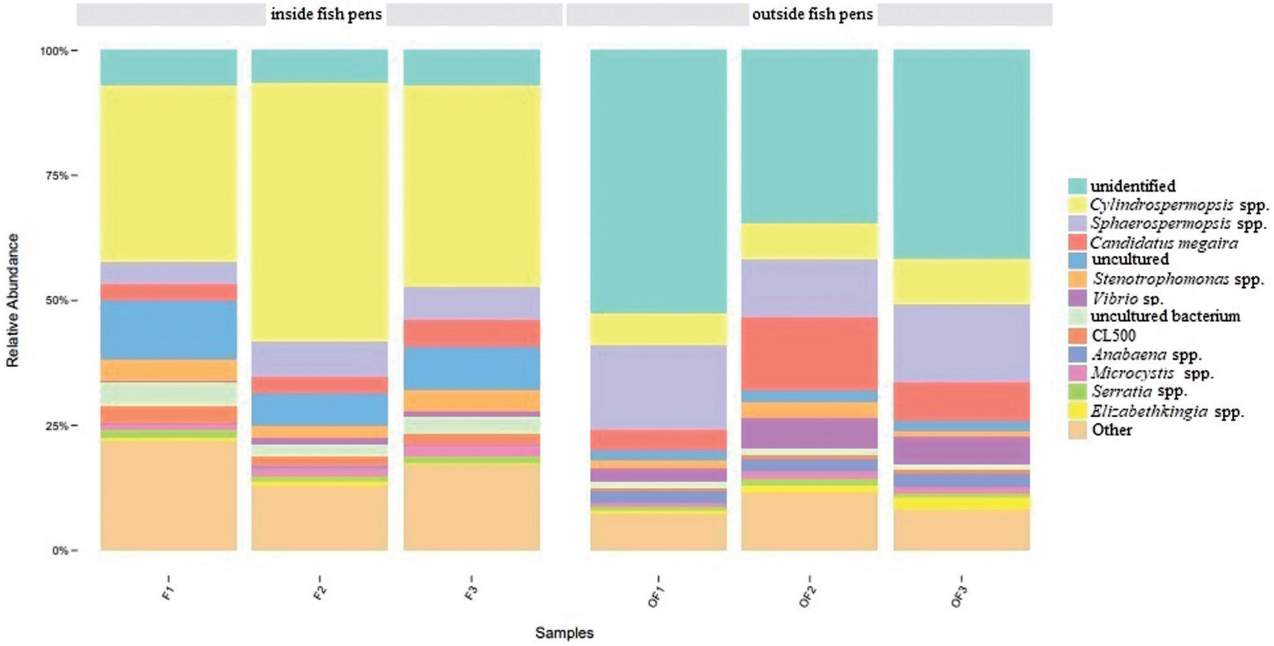

Fig. 2. Comparison in terms of Relative Abundance of bacterial community between sites (inside fish pens- F1, F2, F3; outside fish pens – OF1, OF2, OF3).

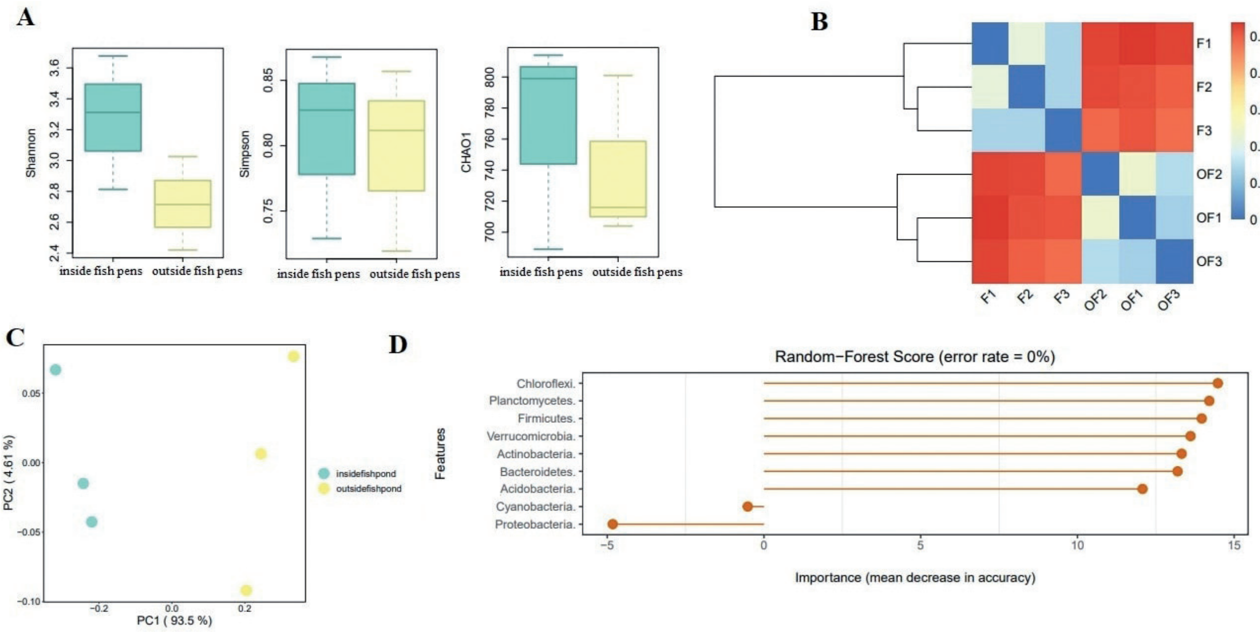

Fig. 3. a) Alpha diversity indices comparing both sites (outside and inside fish pens); b) Heatmap showing the beta diversity pairwise distance matrices between sites (inside fish pens- F1, F2, F3; outside fish pens – OF1, OF2, OF3); c) Principal Component Analysis show amplicon library clustering in reduced dimensions based on beta diversity analysis; d) Selected phyla as biomarkers distinguishing the two sampling site; the x-axis is the importance score (mean decrease in accuracy) produced by Random Forest Model evaluating the importance of each group in distinguishing the different site.

water quality, oxygen levels, affecting the balance of aquatic ecosystems. Its toxin-producing strain received attention due to its potential impacts on water quality, human health, and ecological systems. This also become the focus of much research [25-27]. The dynamics and toxicity of *Cylindrospermopsis* in freshwater bodies are particularly important because freshwater is a scarce resource, and toxic cyanobacterial development could restrict its use [28].

The family Methylacidiphilaceae is a group of bacteria under phylum Verrucomicrobia known for their unique metabolic capabilities and ability to thrive in extreme environments. They are found in geothermal or volcanic environments such as hot springs, volcanic soils and acidic environments adapted to habitats with low pH and high temperatures. They can utilize methane as their primary carbon and energy source (methanotrophs). This unique ability sets them apart from other bacteria. They are of scientific interest due to their unique metabolic capabilities and potential in biotechnology such as methane mitigation and bioremediation [29]. This group constitutes many bacteria but is classified as unidentified at the genus level in Fig. 4.

Among the abundant ASVs detected belong to the genus *Serratia* which comprises of bacteria that belong to the Family Enterobacteriaceae from phylum Proteobacteria. These gram-negative, facultative anaerobic bacteria are widely distributed in various environments, including soil, water, plants, and the gastrointestinal tract of animals. Additionally, they are commonly found in clinical settings as opportunistic pathogens, causing infections in humans such as urinary tract infections, respiratory tract infections, wound infections, and bloodstream infections [30]. *Serratia* bacteria are recognized for their diverse enzymatic activities, which enable them to produce various extracellular enzymes, including proteases, lipases, and DNAses. These enzymatic capabilities contribute to their pathogenic potential and ecological adaptability. Furthermore, many *Serratia* species have developed innate or acquired resistance to multiple antibiotics, complicating treatment options and posing challenges for the future [31]. Despite their opportunistic pathogenicity, certain strains of Serratia have beneficial applications in biotechnology. They are utilized for enzyme production, biodegradation of pollutants, and plant growth hormones. However, it is worth noting that the increased usage of antibiotics has led to their dominance within fish pens, which raises concerns about the development of antibiotic resistance in these environments [32].

The genus *Stenotrophomonas*, classified within the family Xanthomonadaceae, of phylum Proteobacteria demonstrates adaptability to diverse environments, including soil, water, plants, and hospital settings. They are recognized as potential opportunistic pathogens which may pose a threat, especially inside fish pens. These bacteria exhibit metabolic versatility, utilizing various carbon sources, such as sugars, organic acids, and aromatic compounds. It is important to note their intrinsic resistance to multiple antibiotics, which poses a challenge in clinical settings. *Stenotrophomonas*

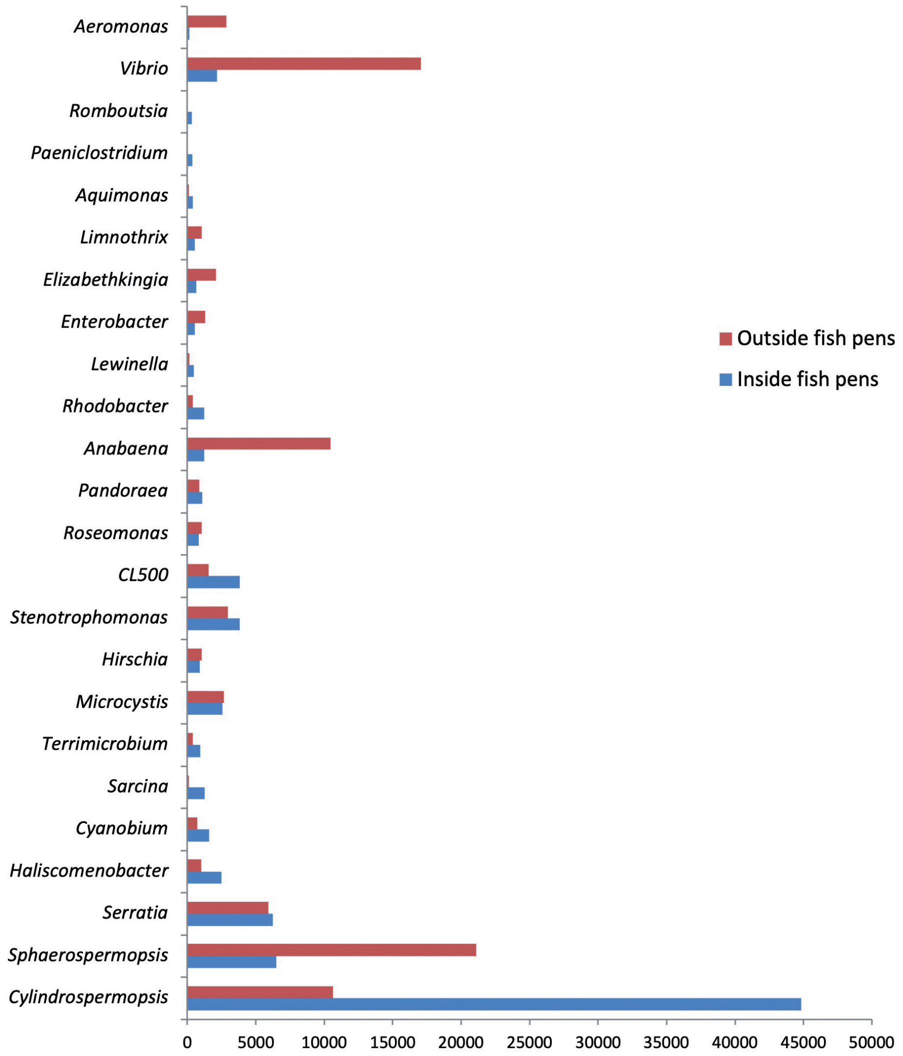

Fig. 4. Amplicon sequence variants (ASVs) count comparison between outside and inside fish pens based on most abundant and common genera.

species are generally considered opportunistic pathogens, capable of causing infections in individuals with underlying conditions. These infections can manifest in various areas, including the respiratory tract, urinary tract, bloodstream, or wounds. However, certain strains of *Stenotrophomonas* have shown promise in biotechnological applications, specifically in bioremediation processes. They can degrade pollutants like hydrocarbons and heavy metals, making them potentially useful in environmental cleanup efforts [33, 34].

Haliscomenobacter bacteria from phylum Bacteroidetes are characterized as obligate aerobic heterotrophs. Their cellular growth occurs in the form of distinctive 'needle-like' filaments, which can serve as a structural framework for forming floccular biomass. These bacteria have been observed in activated sludge and influent wastewater that also flows to fish pens [35, 36].

The genus *Sarcina* consists of bacteria classified within the phylum Firmicutes. They can be found in soil and the digestive tracts of ruminant animals, including cows and goats. They predominate in the lake. While most species are generally considered non-pathogenic and are part of the normal gastrointestinal flora in ruminants, there have been rare human infections, particularly among individuals with compromised immune systems. These infections are infrequent but may occur, underscoring the importance of vigilance, particularly in immunocompromised individuals [37, 38].

The genus *Terrimicrobium* is classified within the phylum Actinobacteria. Its occurrence in fish pens is noteworthy since these bacteria are predominantly found in soil environments. They are characterized by their aerobic nature, meaning they require oxygen for their metabolic processes. *Terrimicrobium* organisms derive energy by breaking down organic compounds present in their surroundings. Their presence and metabolic activities contribute to the complex ecosystem dynamics and nutrient cycling within soil environments [39].

The genus Cyanobium encompasses a diverse group of photosynthetic bacteria inside fish pens. They are frequently encountered in many freshwater environments, such as lakes, rivers, ponds, and reservoirs. Additionally, they can be found in soil and other moist terrestrial habitats. These bacteria are vital in cycling nutrients and energy within freshwater ecosystems, contributing to these environments' overall ecological balance and functioning [40, 41].

CL500 belongs to the family Phycisphaeraceae within the phylum Planctomycetes. This phylum is commonly found in aquatic environments. Its presence is also noteworthy in the lake. The organisms in this group possess diverse metabolic capabilities, with some species functioning as chemoorganotrophs and others as photoheterotrophs. These metabolic characteristics enable them to utilize a variety of organic compounds as energy sources [42].

The genus *Pandoraea*, classified within the Burkholderiaceae family of the Betaproteobacteria class, exhibits a wide distribution across various environments, including soil, water, and clinical settings. These bacteria in fish pens, have been isolated from both natural environments and individuals with respiratory tract infections, cystic fibrosis, and other underlying medical conditions. Certain species within the genus, such as *Pandoraea apista* and *Pandoraea pnomenusa,* have been identified as opportunistic pathogens, causing severe infections primarily in immunocompromised individuals. *Pandoraea* species have demonstrated resistance to multiple antibiotics, including beta-lactams and aminoglycosides. This resistance poses a significant challenge in treating infections caused by these bacteria, necessitating careful consideration when selecting appropriate therapeutic approaches [43].

The genus *Lewinella* belongs to Family Flavobacteriaceae within phylum Bacteroidetes are mainly found in aquatic environments. They are aerobic bacteria and known to possess extracellular enzymes that enable them to degrade complex organic matter which breakdown polymers and various organic substrates. They have potential applications for bioremediation, especially in the breakdown and removal of pollutants in aquatic environments [44].

Outside the fishing pens very interesting groups of bacteria are found. The genus *Sphaerospermopsis* predominates outside the fishing pens and comprises cyanobacteria belonging to the Microcystaceae family. These organisms are commonly found in various aquatic environments, including lakes, ponds, and reservoirs. They exhibit adaptability across various nutrient levels, from eutrophic (nutrient-rich) to oligotrophic (nutrient-poor) waters. It is important to note that certain species within this genus have the potential to produce toxins, which can be detrimental to both humans and animals if they come into contact with contaminated water. Consequently, they pose a risk to the health of aquatic ecosystems. The strain BCCUSP55 has been the subject of numerous research studies focusing on taxonomy, ecology, toxin production, and genetic diversity. *Sphaerospermopsis* species play a vital ecological role and contribute to primary production through photosynthesis. However, their propensity to form dense blooms can profoundly impact the overall dynamics of the ecosystem. It is essential to monitor and manage these blooms to maintain the health and balance of the surrounding environment [45].

The genus *Anabaena* comprises freshwater cyanobacteria commonly found in soil, damp environments, and various aquatic habitats, including lakes, ponds, rivers, and rice fields. They are recognized for their significant contributions to nitrogen fixation and photosynthesis processes. It is important to note that certain species within this genus can produce toxins, such as microcystins. These hepatotoxins threaten humans and animals if they are ingested or exposed to contaminated water. Under conditions of high nutrient levels, warm temperatures, and stagnant water, *Anabaena* species have the potential to form extensive blooms. These blooms can have significant ecological impacts, including oxygen depletion and the production of harmful toxins. The occurrence of such blooms in Lake Buluan requires careful monitoring and management to mitigate their adverse effects on the environment and the organisms inhabiting it [46, 47].

Meanwhile, the genus *Elizabethkingia*, from phylum Bacteroidetes prevalent in water, soil, and plants, includes opportunistic pathogens, notably *Elizabethkingia anopheles* and *Elizabethkingia meningoseptica*. These bacteria can potentially cause infections, especially in immunocompromised patients and the elderly. Infections can occur in various sites within the body, including the bloodstream, respiratory tract, central nervous system, and other locations. It is worth noting that *Elizabethkingia* species have exhibited resistance to multiple antibiotics, including beta-lactams, aminoglycosides, and fluoroquinolones. This antibiotic resistance poses challenges in treating infections caused by these bacteria, necessitating careful consideration when selecting appropriate therapeutic strategies [48, 49]. It is among the most abundant ASVs detected in the lake.

Another prominent group is *Vibrio* from phylum Proteobacteria consisting of diverse species that play various ecological roles and possess potential implications for human and animal health. These bacteria are commonly found in aquatic environments and exhibit associations with aquatic animals, including shellfish and fish. While they can be harmless and beneficial, certain *Vibrio* species can cause pathogenic infections in both humans and animals. Notably, *Vibrio cholerae* is a well-known causative agent of cholera, a severe diarrheal disease. Other pathogenic species within this group, such as *Vibrio parahaemolyticus* and *Vibrio vulnificus*, can give rise to foodborne illnesses often linked to seafood consumption. The presence of *Vibrio* species in Lake Buluan necessitates careful monitoring and implementation of appropriate measures to prevent and manage the associated health risks [50].

Of significance is the genus *Microcystis,* which comprises colonial cyanobacteria predominantly found in freshwater environments. They thrive in eutrophic waters with elevated nutrient concentrations, particularly phosphorus and nitrogen. Notably, many species within this genus can produce toxins known as microcystins. These hepatotoxins pose a risk to humans, animals, and other aquatic organisms if consumed through contaminated water, potentially resulting in various health issues, including liver damage. Under favorable conditions such as calm and warm water temperatures, appropriate pH levels, sufficient light intensity, and high nutrient availability, *Microcystis* can form blooms. These blooms can potentially reduce oxygen levels, leading to fish kills and disrupting the ecological balance within the ecosystem. Human activities, such as agricultural runoff in the lake contribute to the high nutrient loads that promote the formation of harmful algal blooms. Monitoring and mitigating these blooms is crucial to safeguard human and aquatic ecosystems' health [51, 52].

Among the important species detected are from the genus *Roseomonas* from phylum Proteobacteria. These bacteria exhibit versatility in their metabolic capabilities and can utilize diverse carbon sources. While they are typically considered opportunistic pathogens, causing infections primarily in immunocompromised individuals, certain species such as *Roseomonas mucosa* and *Roseomonas gilardii* have been associated with opportunistic infections such as bacteremia, endocarditis, respiratory tract infections, and urinary tract infections. In addition to their pathogenic potential, *Roseomonas* species have also garnered attention for their biotechnological applications. They have been extensively studied for their capacity to degrade environmental pollutants and produce enzymes with industrial applications. These characteristics make them of particular interest in environmental remediation and enzyme production for various industrial processes [53, 54].

The *Limnothrix* species are frequently found in aquatic environments and play a crucial role in converting nitrogen gas into a form usable by living organisms, thereby contributing to nutrient cycling in the lake. However, it is important to note that they can also produce toxins known as microcystins. These hepatotoxins can harm humans and animals if ingested in water contaminated with these toxins. The formation of *Limnothrix* blooms can result in discoloration of the water, reduced oxygen levels, and the production of harmful toxins. Monitoring and managing these blooms in the lake is essential to mitigate their potential adverse effects on the environment and the health of organisms that inhabit the affected water bodies [55, 56].

Aeromonas, a genus of Gram-negative bacteria from phylum Proteobacteria is widely distributed in aquatic environments such as freshwater, marine, and brackish water habitats. These bacteria exhibit a facultative anaerobic nature, enabling them to thrive and reproduce in aerobic and anaerobic conditions. *Aeromonas* species are characterized by their diverse metabolic capabilities, allowing them to utilize a broad spectrum of carbon sources. They can cause infections as opportunistic pathogens in both humans and animals. In humans, *Aeromonas* infections can give rise to various clinical manifestations, including gastroenteritis, wound infections, septicemia, and respiratory tract infections. It is worth noting that certain strains of *Aeromonas* have been linked to antibiotic resistance, presenting challenges in their treatment. Consequently, these bacteria have attracted significant attention in public health and research. Gaining insights into the ecology, virulence factors, and mechanisms of antibiotic resistance exhibited by *Aeromonas* is crucial for effectively managing and preventing infections associated with these bacteria as its presence in the lake poses a great challenge [57, 58].

Physico-Chemical Parameters in Relation to BCC

Moreover, water pH, temperature and dissolved oxygen (DO) are essential environmental factors that significantly impact the structure and composition of bacterial communities in lake ecosystems. Temperature plays a crucial role in bacterial growth rates, with higher temperatures generally promoting faster growth and influencing overall abundance and activity. On the other hand, different pH levels can favor specific bacterial taxa growth, leading to community composition variations [59].

Based on the analysis, although the average water temperature was 25ºC, it was not found to be significant in relation to the distribution of bacterial taxa diversity (beta-diversity). However, Fig. 5, which illustrates the distribution of bacterial taxa beta-diversity in relation to pH, shows significant results from the meta-storms distance analysis ($p = 0.0055$). This indicates that pH substantially influences the composition of bacterial communities (BCC). The pH levels impact nutrient and organic matter availability, influencing bacterial diversity.

In this case, the pH range observed was 7.3-7.9, leaning towards an alkaline condition. Alkaline environments with high pH levels promote the prevalence of alkaliphilic bacteria. These bacteria include members of the phylum Firmicutes, some Proteobacteria, and Cyanobacteria. As a result, the notable high presence of *Cylindrospermopsis, Sphaerospermopsis, Stenotrophomonas, Anabaena, Serratia, Vibrio, Microcystis*, and *Sarcina* inside and outside fishing pens can be explained. Different bacteria have specific pH optima for growth, with some thriving in neutral pH conditions while others prefer extremely acidic or alkaline conditions.

The observed dissolved oxygen (DO) levels inside and outside of the fishing pens ranged from 1.59 mg/L to

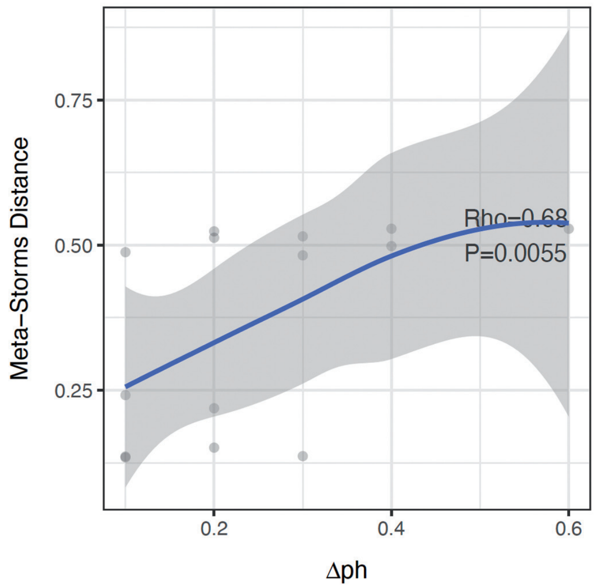

Fig. 5. Meta-storms distance analysis illustrating the distribution of bacterial taxa beta-diversity in relation to pH (p = 0.0055)

2.19 mg/L, which is below the acceptable standard level. A hypothesis suggests a correlation between increasing pH levels and decreasing DO levels. In more polluted waters, oxygen availability decreases [60]. Interestingly, it was observed that as DO levels decrease, the pH increases, resulting in a more alkaline environment. This alkaline condition may explain the notable presence of photosynthetic bacteria (e.g. *Cylindrospermopsis*) that are known to thrive in alkaline conditions.

Prediction of Microbiome Functional Composition

Moreover, it was possible to predict the microbiome's functional composition at the KEGG pathway level by utilizing the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt). The outcomes unveiled a significant abundance of bacterial assemblages engaged in diverse metabolic pathways, including amino acid metabolism, carbohydrate metabolism, lipid metabolism, cofactors and vitamins metabolism, energy metabolism, and association with protein families (Fig. 6). This suggests the potential of the microbiome as a valuable source of cellulolytic enzymes, which could be utilized in various applications such as biofuel production, biotechnology, and bioremediation processes.

Another intriguing finding is the presence of bacteria capable of xenobiotic biodegradation [61]. Microorganisms possess the unique ability to metabolize xenobiotic substances partially or completely in various ecosystems. A study highlighted the abundance of putative genes and taxa associated with xenobiotic biodegradation [62]. Another study also reported that xenobiotic compounds alter the homeostasis in fishes and lead to oxidative stress due to large numbers of reactive oxygen species and suppressing the antioxidant system [63]. Interestingly, this study identified five genera linked to xenobiotic biodegradation: *Serratia, Haliscomenobacter, Stenotrophomonas, Pandoraea,* and *Enterobacter.*

It is worth noting that certain species of *Serratia* exhibit diverse metabolic capabilities and can degrade a

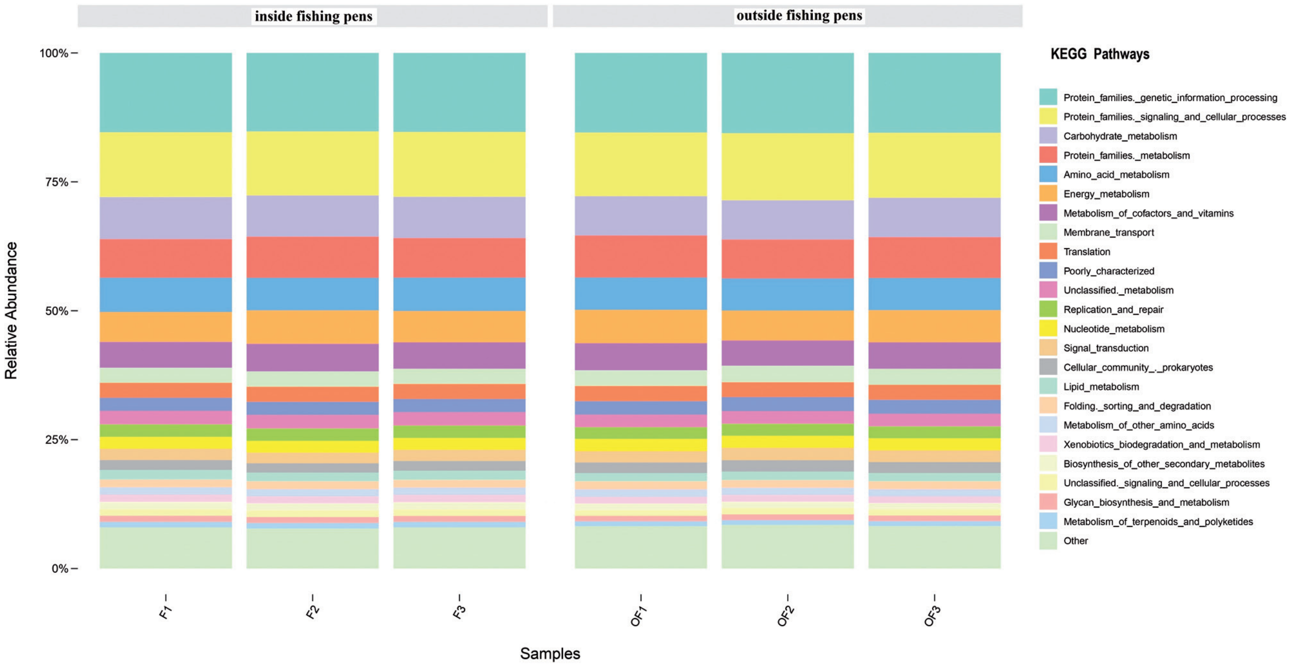

Fig. 6. PICRUSt predictions of the functional composition of the respective microbiome (inside fish pens – F1, F2, F3; outside fish pens – OF1, OF2, OF3) as represented at KEGG pathway

wide range of organic compounds, including xenobiotics. *Haliscomenobacter* has been reported to be able to degrade complex organic compounds, particularly associated with hydrocarbon degradation [64]. Some species of *Stenotrophomonas* have demonstrated the capacity to degrade various xenobiotic compounds, including aromatic hydrocarbons and pesticides [65]. Species belonging to the genus *Pandoraea,* is known to possess xenobiotic degradation capabilities and have been isolated from contaminated environments [66]. Additionally, certain strains of *Enterobacter* have been recognized for their ability to degrade various xenobiotic compounds, including aromatic hydrocarbons and industrial pollutants. However, further studies are required to determine the extent of xenobiotic degradation, particularly focusing on specific strains within these genera. Biotransformation is recognized as the most effective metabolic process, enables microorganisms to degrade xenobiotic compounds and survive in toxic environments by utilizing available carbon, phosphorus, sulfur, and nitrogen sources [67].

Implications in Fish Farming in the Lake

The analysis identified a significant increase in the abundance of bacteria associated with toxin production, antibiotic resistance, and opportunistic pathogens. This raises potential risks to human health, water quality, and ecological systems. This information may impact fish farming practices in the area. It is crucial to note that the excessive use of antibiotics has contributed to the prevalence of antibiotic-resistant bacteria within and outside fish pens.

Certain bacteria, including *Microcystis, Cylindrospermopsis, Sphaerospermopsis, Anabaena*, and *Limnothrix,* can form harmful algal blooms. These blooms have the potential to deplete oxygen levels, leading to fish mortality and disrupting the ecological balance of the affected ecosystem. Human activities, such as agricultural runoff, contribute to the high nutrient loads that promote the growth of these harmful algal blooms. Monitoring and mitigating these blooms is essential to safeguard both human health and the well-being of aquatic ecosystems [68].

Alkaline environments with high pH levels promote the prevalence of alkaliphilic bacteria. These bacteria include members of the phylum Firmicutes, some Proteobacteria, and Cyanobacteria. As a result, there is a notable high presence of *Cylindrospermopsis, Sphaerospermopsis, Stenotrophomonas, Anabaena, Serratia, Vibrio, Microcystis*, and *Sarcina* inside and outside fishing pens. *Cylindrospermopsis* was found to be the most prevalent bacteria among the identified species. Understanding the dynamics and toxicity of *Cylindrospermopsis* in freshwater bodies is particularly important, considering that fresh water is a valuable resource, and the development of toxic cyanobacteria can limit its utilization [69].

Based on the results, this calls for ecosystem governance. The Philippine Environmental Governance Project (EcoGov) is actively addressing key environmental threats in collaboration with the United States Agency for International Development (USAID). The Philippine government is committed to enhancing the decision-making process and taking appropriate actions against threats such as illegal fishing, overfishing, and improper solid waste disposal that could impact aquatic environments such as rivers, lakes and marine ecosystems. The project aims to protect and restore species and habitats while addressing human activities such as overexploitation, overfishing, habitat destruction, pollution, and related issues that negatively impact aquatic life [70]. However, policy recommendations in the local government unit (LGU) backed with scientific data are very important steps towards conservation. This study serves as a baseline data for future policy recommendations.

Conclusion

In lake ecosystems, it is crucial to identify the composition of the bacterial community (BCC) and understand the functional roles of microorganisms in maintaining the health of aquatic ecosystems. This knowledge is essential for recognizing and predicting responses to changes in water quality and other environmental disturbances. The bacterial community plays a vital role in decomposing organic matter, converting it into essential nutrients that serve as food for other organisms while influencing water quality control. High-throughput sequencing based on the 16S rRNA gene explored the bacterial communities. The analysis revealed a notable increase in the abundance of bacteria associated with toxin production, antibiotic resistance, and opportunistic pathogens. This raises concerns about potential risks to human health, water quality, and ecological systems. This information may impact fish farming practices in the area. Moreover, the presence of bacteria involved in xenobiotic biodegradation indicates their potential application in bioremediation, including the breakdown of pollutants, hydrocarbons, and heavy metals. This study will serve as baseline data for further studies and ecosystem governance, especially aquatic resources.

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Conflict of Interest

The authors declare no conflict of interest.

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