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Comparative Analysis of Microbial Community Structure and Composition across Tropical Freshwater Ecosystems Using 16S rRNA Metagenomics

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Abstract

Understanding the microbial diversity of freshwater bodies provides insight into their trophic status, habitat type, and degree of contamination. This study represents the first attempt to evaluate the taxonomic composition and microbial diversity of three freshwater bodies (Iyiube Lake, Abala Lake, and Iyioku River) located in Enugu East, Nigeria. Genomic DNA was isolated from the collected water samples, and the compositions of the microbial communities were characterized using the 16S rRNA sequencing method. The results of metagenomics analysis identified a total of 31 phyla, 81 classes, 139 orders, 177 families, and 209 genera. The two dominant bacterial phyla identified in the present study are Proteobacteria and Acidobacteria. Additionally, other prevalent phyla identified are Chloroflexi, Bacteroidota, Verrucomicrobiota, Myxococcota, and Nitrospirota. Among the bacterial phyla present in the freshwater ecosystems studied, Fusobacteria was the least prevalent phylum, only detected in Abala Lake. Additional phyla unique to single freshwater bodies are Sumerlaeota, DTB120, Deferrisomatota, Hydrogenedentes, Elusimicrobiota, and NB1-j, suggesting niche differentiation. Thus, when compared to other freshwater bodies, Iyiube Lake exhibits the largest abundance of Acidobacteria, which may indicate anthropogenic influence because of high sewage deposit. This study provides the first baseline inventory of bacterial assemblages in these tropical freshwater systems, underscoring their ecological uniqueness and the potential of specific taxa as bioindicators for environmental monitoring and freshwater ecosystem restoration in the tropical regions.

Keywords

16S rRNA, Diversity, Freshwater Bodies, Microbial Structures, Metagenomics

1. Introduction

A sustainable environment relies on the hydrosphere, which covers approximately 75% of the Earth's crust, yet only a limited amount of it is available as freshwater [1]. While a significant portion of the Earth's surface is covered by water, merely 3% of that is freshwater, and the majority of terrestrial organisms cannot directly use the salty water that constitutes the rest [2]. The availability of freshwater is crucial for the survival of terrestrial forms as well as other forms of life. Lakes, rivers, streams, and wetlands are examples of freshwater ecosystems that provide humans with valuable ecosystem services, including drinking water, fishing, and enjoyment. Water bodies serve as natural barriers between people and different kinds of organisms, but they can be contaminated by a variety of sources, which can change the water's quality and condition [3]. Therefore, protecting aquatic ecosystems and priceless water resources is crucial [1]. In freshwater habitats, the activities of autotrophic and heterotrophic microbes regulate the natural cycles of oxygen, carbon, and other nutrients [4].

Freshwater ecosystems harbor vast microbial communities, playing critical roles in nutrient cycling, ecosystem stability, and overall ecological balance [5]. According to Fierer *et al.* [6], these communities comprise bacteria, archaea, fungi, viruses, and other micro-eukaryotes, collectively forming a complex microbial consortium that drives essential biogeochemical processes. Studying the composition, structure, and functional potential of these communities is crucial for understanding the dynamics of freshwater ecosystems and their responses to environmental perturbations [7] [8]. The state of freshwater resources has deteriorated considerably in recent times, and the impact of water pollution events on the safety of water quality and the well-being of local populations is now being recognized on a global scale [1]. Presently, no studies investigating the microbial community structure of the freshwater bodies in Enugu State, Nigeria are available. Even though data regarding the composition of microbial communities plays a crucial role in freshwater ecosystems, such studies subjected to other water bodies of different regions of the world are available in the literature. Traditionally, the study of microbial communities relied on culture-based approaches, which were limited in capturing the true extent of diversity, mostly due to the nonculturable nature of most microorganisms [9]. Thus, the advent of high-throughput sequencing technologies, particularly metagenomics, has revolutionized the ability to explore the genetic and functional diversity of microorganisms, including the VBNC (viable but nonculturable), within their natural habitats [10] [11].

Notably, the cumulative effects of anthropogenic stress on freshwater ecosystems are becoming more noticeable and concerning. In addition to natural stressors, anthropogenic stressors like increased water use, overuse of hydrobiological resources, nutrient inputs, hydrological stress, and the disposal of industrial, urban, and agricultural waste are damaging freshwater ecosystems [12] [13]. The accelerated changes in our ecosystem due to climate change and anthropogenic activities have created an urgent need for monitoring, empirical studies, and conservation of our biodiversity [14]. Thus, one of the key steps towards ensuring healthy conditions of a freshwater ecosystem is to have a good understanding of its microbial community structure [15]. According to Brar *et al.* [1], there is a growing demand for routine aquatic ecosystem monitoring and assessment in order to take further actions to maintain the sustainability of these resources.

In recent years, metagenomic studies have shed light on the vast diversity of freshwater microbial communities, revealing a huge reservoir of untapped genetic resources [16]. Furthermore, this technique has enabled the identification of key functional attributes, such as nutrient cycling, secondary metabolite production, and stress response mechanisms, which are crucial for understanding the ecological roles of these microorganisms [17]-[19]. Quince *et al.* [20] emphasized that by directly sequencing DNA extracted from environmental samples, metagenomics offers a culture-independent alternative that allows for the comprehensive characterization of microbial communities in their entirety. Therefore, this study seeks to address this gap by conducting a comparative analysis of freshwater bodies' microbial communities from Enugu State, Nigeria using metagenomic approaches. The complete microbial profile of the three freshwater bodies studied is analyzed for the first time to the best of our knowledge. Thus, by characterizing their taxonomic composition and metabolic diversity, we aim to gain deeper insights into the factors shaping the structure and ecological functions of these microorganisms.

2. Materials and Methods

2.1. Study Site and Sample Collection

Three freshwater ecosystems in Ugwuomu-Nike were selected for this study. Ugwuomu-Nike is a remote community located in the Enugu East local government area of Enugu State (Southeastern Nigeria). It lies at latitude 6˚32'N and longitude 7˚32'E with an area of about 383 km^2 [21]. The area is characterized by its lush vegetation and numerous freshwater bodies. Sample collection was carried out on the morning of August 21, 2022, using 1000 ml of sterile polyethylene bottles from the three sampling sites, namely Abala lake, Iyiube lake, and Iyioko river. The water samples were collected using a Van Dorn sampler from the lowest layer (1 m above the sediment), the middle section of the water column, and the superficial layer at a depth of 1 m. Furthermore, samples were taken in triplicate from each site to ensure data reproducibility and were carried to the laboratory at 4˚C and stored at −20˚C till further analysis. The samples were then passed over a membrane with a pore size of 1.2 µm to remove debris and coarse particles. To concentrate microbial cells, the samples were filtered through 0.22 μm pore size polycarbonate filters (Whatman, UK) using a peristaltic pump. Filters were then aseptically transferred to sterile microcentrifuge tubes and stored at −80˚C pending DNA extraction.

2.2. DNA Extraction and PCR Amplification

Genomic DNA was extracted from each sample using the Qiagen DNeasy PowerWater Kit following the manufacturer's instructions while strictly adhering to all laboratory procedures. The DNA extracts were then stored at −20˚C until PCR. The 16S rRNA regions were amplified by PCR using cytiva PuReTaqTM Ready-To-Go PCR Beads (Global Life Sciences Solutions, UK). Each individual bead contains 2.5 units of Taq DNA polymerase, 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM of each dNTP, and 10 mM Tris-HCl (pH 9.0). The primer/loading dye mix contains 640 μL of molecular grade water, 460 μL of Cresol Red Loading Dye, 20 μL of 15 pmol/μL 5' primer, and 20 μL of 15 pmol/μL 3' primer. Amplification was carried out using the Bio-Rad's T100™ Thermal Cycler. Reactions of 25 μL were prepared by adding 2 μL of template DNA to 23 μL of the primer/loading dye mix. The optimized PCR conditions involved an initial denaturation at 94˚C for one minute, 30 cycles of denaturation at 94˚C for 30 seconds, annealing at 54˚C for 15 seconds, initial extension at 72˚C for 30 seconds, and a final elongation step at 72˚C for 5 minutes. A 2% agarose gel electrophoresis was performed at 100 volts for 30 minutes to visualize the PCR products and confirm the successful amplification of the 16S rRNA gene. The gels were incorporated with ethidium bromide (0.1 μg/ml). Visualization was conducted using the Accuris UV–Transilluminator E-3000-E. A 100-bp DNA ladder (New England BioLabs Inc) served as molecular weight markers. The PCR products were then sent to GeneWiz (Azenta Life Sciences, South Plainfield, NJ, USA) for sequencing using Novaseq 6000 Illumina to generate raw data in the form of fastq reads.

2.3. Bioinformatics and Statistical Analysis

The Purple Line, a new feature of DNA Subway that allows for metagenomics investigation of microbiomes, was used to examine the resulting fastq read data [22]. Thus, the Purple Line implements a simplified version of the QIIME 2 workflow that analyzed the Illumina sequencing reads. Briefly, the raw fastq data generated by sequencing were checked for noise and processed by computational tools. This was done by removing reads with more than 10% of nucleotides (N) and reads with more than 50% of bases having Q quality scores less than 20, while removing reads containing adapters. Therefore, high-quality reads were utilized for downstream analysis after the data were pre-processed and their quality was enhanced through trimming and filtering. The quality-filtered reads were assembled into contigs, and open reading frames (ORFs) were predicted. Taxonomic classification of the sequences and functional annotation were then conducted. Furthermore, statistical analysis for the taxonomic abundance, phylogeny, and diversity indices (alpha diversity and beta diversity) was calculated from the DNA Subway interface to assess the microbial community composition and diversity within the freshwater ecosystems.

3. Results

3.1. Sequence Analysis

High molecular weight genomic DNA was extracted from each sample across the freshwater bodies, and it yielded the required sequence reads. In total, across the three freshwater bodies, 323,286 reads were generated with a mean value of 5213.48 for all the studied samples (Supplementary **Table 1**). The eDNA extracted from all 62 samples was successfully used to create libraries containing 16S rRNA amplicons. After Illumina sequencing, resulting FASTQ sequences were demultiplexed; a minimum of 559 reads was seen in both forward and reverse, while a maximum of 13,838 reads was recorded for each. Rarefaction curves, which were employed as a qualitative technique to quantify species richness at different taxonomic levels as a function of sequencing depth, began to plateau for all taxonomy levels, indicating that sequencing saturation had been reached (**Figure 1**). The sequence length of the dataset was distributed within the range of 200 to 2000 bp.

Table 1. Distribution of the bacteria identified based on the phylum, class, order, family, and genus for the three freshwater bodies.

Parameters	Abala-Lake	Iyioku-River	Iyiube-Lake
Phylum	24	28	28
Class	41	61	56
Order	65	108	99
Family	74	129	116
Genus	87	147	131

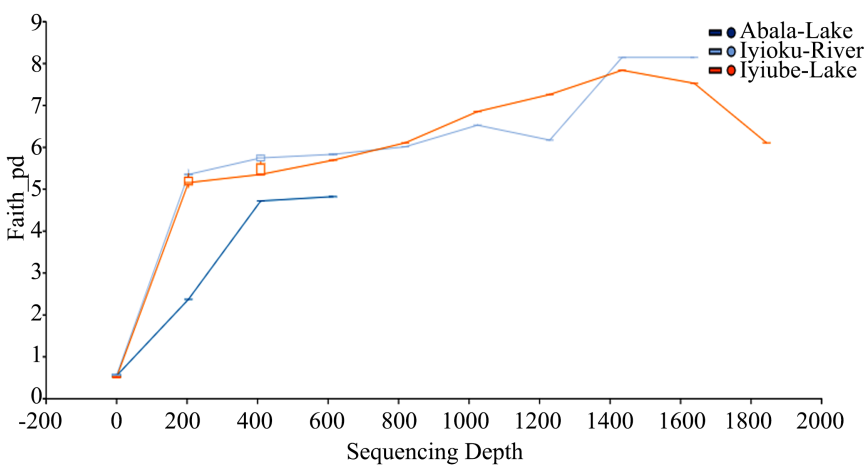


Figure 1. Refraction curves for the studied freshwater bodies showing the sequencing depth of the 16S rRNA.

3.2. Comparative Taxonomic Analysis

In the present study, the comprehensive description of the data has been provided as a comparative report at the phyla, classes, orders, families, and genera levels (Table 1). The results of the metagenomics analysis revealed the identification of 31 phyla, 81 classes, 139 orders, 177 families, and 209 genera. Furthermore, the analysis of the taxonomic community showed that the freshwater bodies were dominated by Bacteria (92%) and Archaea (8%). Also, the analysis of the three freshwater bodies' sequences revealed that at the phylum level, the bacterial community was strongly dominated by Proteobacteria and Acidobacteriota (Figure 2). Other phyla identified with high levels of bacterial community abundance are Chloroflexi, Bacteroidota, and Verrucomicrobiota. Members of the phyla Fosobacteriota and Sumerlaeota were only detected at the Abala-Lake sites, while Deferrisomatota, Hydrogenedentes, and DTB120 phyla were only identified at the Iyioku-River. However, Elusimicrobiota and NB1-j phyla were identified only in the Iyiube lake. Also, the dominant classes found in the three freshwater bodies are Acidobacteriae, Gammaproteobacteria, Bacteroidia, Verrucomicrobiae, and Myxococcia.

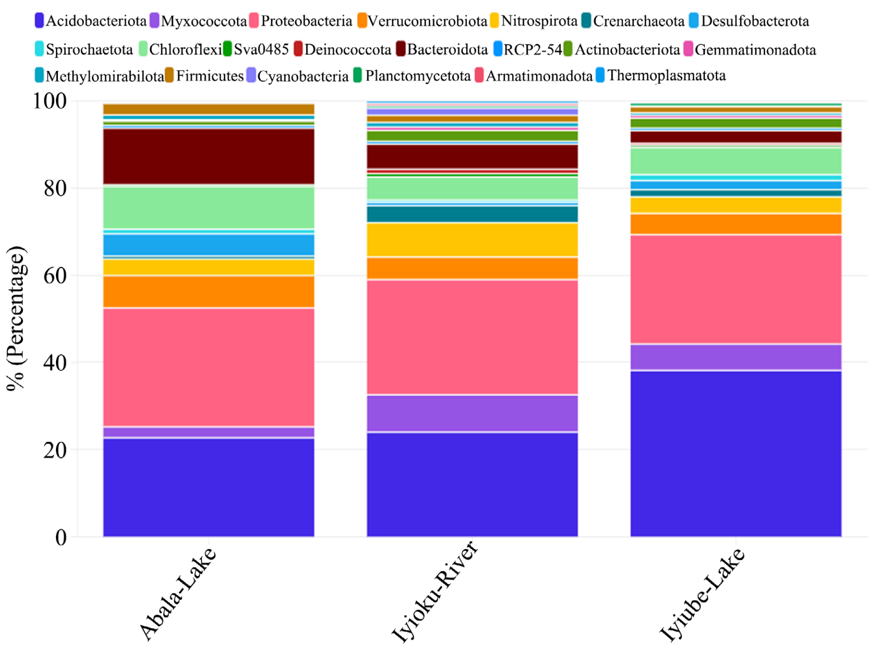


Figure 2. Taxonomic composition of the three freshwater bodies studied at the phylum level.

3.3. Abala-Lake Bacterial Composition and Diversity

The bacterial composition identified at the Abala-lake site consists of 24 phyla, 41 classes, 65 orders, 74 families, and 87 genera (Table 1). The top six most abundant phyla at Abala-lake are Proteobacteria (27%), Acidobacteriota (22%), Bacteroidota (13%), Chloroflexi (10%), Verrucomicrobiota (7%), and Desulfobacterota (5%), while the classes are Gammaproteobacteria (23%), Acidobacteriae (13%), Bacteroidia (11%), Subgroup_18 (7%), and Anaerolineae (Table 2). Additionally, the top six orders are as follows: Burkholderiales (19%), Bacteroidales (8%), Subgroup_18 (7%), Pedosphaerales (6%), Acidobacteriales (6%), and Rhizobiales (4%).

Table 2. Relative abundance of the top 6 bacterial species identified from Abala Lake.

Phylum	Class	Order	Genus
Proteobacteria (27%)	Gammaproteobacteria (23%)	Burkholderiales (19%)	Comamonadaceae (15%)
Acidobacteriota (22%)	Acidobacteriae (13%)	Bacteroidales (8%)	Subgroup_18 (7%)
Bacteroidota (13%)	Bacteroidia (11%)	Subgroup_18 (7%)	Pedosphaeraceae (6%)
Chloroflexi (10%)	Subgroup_18 (7%)	Pedosphaerales (6%)	uncultured (6%)
Verrucomicrobiota (7%)	Verrucomicrobiae (7%)	Acidobacteriales (6%)	Bacteroidetes_vadinHA17 (4%)
Desulfobacterota (5%)	Anaerolineae (7%)	Rhizobiales (4%)	Xanthobacteraceae (4%)
Others (16%)	Others (32%)	Others (50%)	Others (58%)

3.4. The Iyioku-River Bacterial Composition and Diversity

The results of the bacterial composition and diversity of the Iyioku River revealed 28 phyla, 61 classes, 108 orders, 129 families, and 147 genera (Table 1). At the phylum level, the order of dominance in decreasing order for the top six is Proteobacteria (26%), Acidobacteriota (22%), Myxococcota (9%), Nitrospirota (8%), Bacteroidota (6%), and Chloroflexi (Table 3). Also, the top six most abundant classes in this sample site are Gammaproteobacteria (23%), Acidobacteriae (19%), Myxococcia (8%), Nitrospiria (6%), Verrucomicrobiae (5%), and Bacteroidia (4%), while the Burkholderiales (17%), Acidobacteriales (11%), Myxococcales (8%), Nitrospirales (6%), Subgroup_2 (4%), and Pedosphaerales (3%) are for the orders.

Table 3. Relative abundance of the top 6 bacterial species identified from Iyioku River.

Phylum	Class	Order	Genus
Proteobacteria (26%)	Gammaproteobacteria (23%)	Burkholderiales (17%)	uncultured (10%)
Acidobacteriota (22%)	Acidobacteriae (19%)	Acidobacteriales (11%)	Anaeromyxobacteraceae (8%)
Myxococcota (9%)	Myxococcia (8%)	Myxococcales (8%)	Nitrospiraceae (6%)

Continued

Nitrospirota (8%)	Nitrospiria (6%)	Nitrospirales (6%)	Comamonadaceae (4%)
Bacteroidota (6%)	Verrucomicrobiae (5%)	Subgroup_2 (4%)	Subgroup_2 (4%)
Chloroflexi (5%)	Bacteroidia (4%)	Pedosphaerales (3%)	Burkholderiales (4%)
Other (22%)	Others (33%)	Others (50%)	Others (63%)

3.5. The Composition and Diversity of Iyiube-Lake

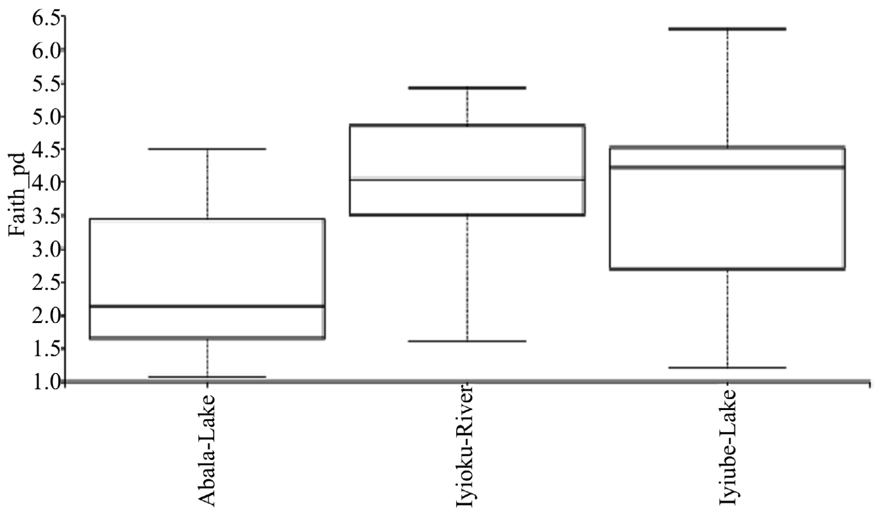
Metagenomics analysis of the Iyiube Lake revealed the identification of 28 phyla, 56 classes, 99 orders, 116 families, and 121 genera (Table 1). The top six phyla that dominate the Iyiube Lake are Acidobacteriota (38%), Proteobacteria (25%), Chloroflexi (6%), Myxococcota (6%), Verrucomicrobiota (5%), and Nitrospirota (4%), while the classes are Acidobacteriae (33%), Gammaproteobacteria (17%), Alphaproteobacteria (8%), Verrucomicrobiae (5%), Myxococcia (3%), and Anaerolineae (Table 4). Furthermore, the top six most abundant orders are Acidobacteriales (22%), Burkholderiales (11%), Subgroup_2 (7%), Rhizobiales (6%), Myxococcales (3%), and Pedosphaerales (3%).

Table 4. Relative abundance of the top 6 bacterial species identified from Iyiube Lake.

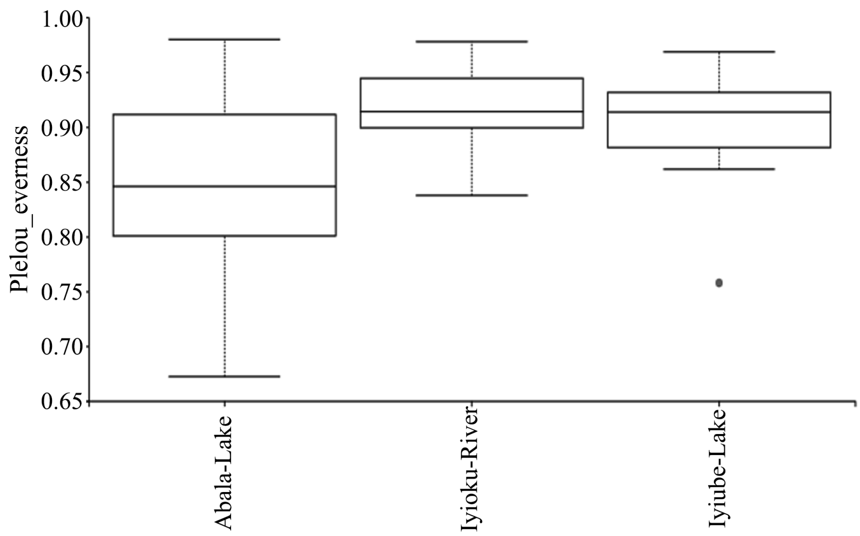
Phylum	Class	Order	Genus
Acidobacteriota (38%)	Acidobacteriae (33%)	Acidobacteriales (22%)	uncultured (21%)
Proteobacteria (25%)	Gammaproteobacteria (17%)	Burkholderiales (11%)	Subgroup_2 (7%)
Chloroflexi (6%)	Alphaproteobacteria (8%)	Subgroup_2 (7%)	Xanthobacteraceae (5%)
Myxococcota (6%)	Verrucomicrobiae (5%)	Rhizobiales (6%)	Burkholderiales (3%)
Verrucomicrobiota (5%)	Myxococcia (3%)	Myxococcales (3%)	Anaeromyxobacteraceae (3%)
Nitrospirota (4%)	Anaerolineae (3%)	Pedosphaerales (3%)	Pedosphaeraceae (3%)
Others (16%)	Others (30%)	Others (48%)	Others (57%)

3.6. Differences in Microbial Communities of the Three Freshwater Bodies

We analyzed the differences in the freshwater bodies from three sampling sites and the microbiota community diversity. A significant difference ($p < 0.05$) was observed in various alpha-diversity (Pielou's Evenness and Faith's Phylogenetic Diversity) indices between Abala-lake/Iyioku-river and Abala-lake/Iyiube-lake (**Figure 3(a)** and **Figure 3(b)**). Furthermore, the Bray-Curtis dissimilarities of these bacterial communities, which were the basis for the principal coordinate (PCoA) analysis, showed that the various freshwater bodies differed significantly from one another (**Figure 4**). Notably, the distribution in the Iyioku River exhibited a more dispersed pattern compared to the Abala and Iyiube lakes. The distributions within the Iyioku River were found to be more closely related to those observed in the Abala Lake than with the patterns observed in the Iyiube Lake. Notably, the Abala Lake presented the least number of clusters among the observed sites.



(a)



(b)

Figure 3. (a) Boxplots depicting alpha diversity based on Faith's phylogenetic diversity measure among the freshwater bodies studied; (b) Boxplots depicting alpha diversity based on Pielou's Evenness measure among the freshwater bodies studied.

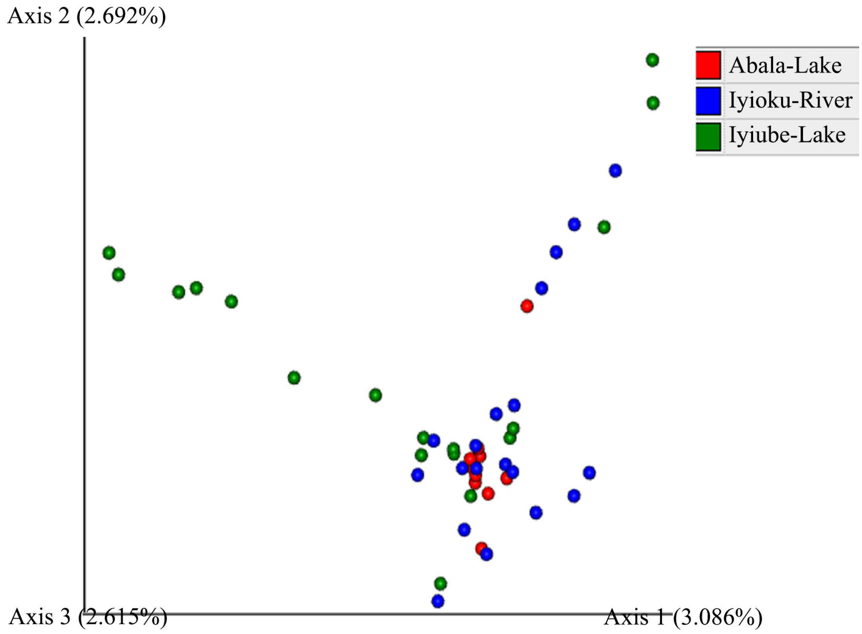


Figure 4. PCoA analysis based on the Bray-Curtis distance matrix for the studied freshwater bodies.

4. Discussion

Our research offers the initial descriptive examination of the microbial community profiles in the freshwater bodies of Enugu East, which is an essential step in understanding the microbial ecology of reservoirs. Microbial structures or communities, primarily bacteria, of the water system contribute significantly to the aquatic ecosystem due to their role in biogeochemical processes [23]. In recent years, with the development of industrialization, the lake environment has deteriorated to varying degrees, and lake health has become a hot research topic [24] [25]. The result of the analysis of the taxonomic community showed that the freshwater bodies were dominated by prokaryotic organisms (92% bacteria and 8% archaea). This distribution highlights prokaryotes' importance in maintaining ecological balance and their potential roles in biogeochemical processes within freshwater ecosystems. Prokaryotes are considered the main drivers of transformation and the cycling of most biologically active elements in aquatic ecosystems via the degradation and mineralization of organic compounds to their inorganic constituents. The abundance of bacteria in the present findings agrees with other reports. Bacteria are the most common organisms on Earth; their makeup and abundance significantly influence ecosystem functions and stability, whether they exist in host-associated communities, soil, grasslands, or oceans [1].

The two most dominant phyla in the present study are Proteobacteria and Acidobacteria. The abundance of the two phyla may be attributed to human interference and the discharge of human wastes in freshwater bodies. The dominance of these groups is in agreement with other freshwater studies [26]-[28]. Therefore, utilizing the studied freshwater bodies (Iyiube lake, Abala lake, and Iyioku river) for drinking water presents significant health hazards for residents of those regions, as human activities contribute to environmental contamination. Proteobacteria, which primarily consist of gram-negative bacteria, have previously been identified as the dominant taxa in surface water bodies defined by anthropogenic pollution and waste disposal [29]. Additionally, it has been observed that the phylum Proteobacteria is predominantly found in environments with low salinity and minimal electrical conductivity [3]. Proteobacteria mainly consist of rapidly growing copiotrophs that flourish in nutrient-rich environments, and they are believed to play an essential role in nitrogen cycling, connecting iron-carbon biogeochemistry, carbon sequestration, nutrient flux, and various biochemical processes [1] [30].

Among the five classes of Proteobacteria (alpha, beta, gamma, delta, and Epsilon), the most abundant across all the freshwater bodies in this study is gamma-proteobacteria. The abundance of gamma-proteobacteria in freshwater bodies has been previously reported by Betiku *et al.* [31]. Gamma-proteobacteria comprise families of N-fixing bacteria [32] and favor high pH [33]. Gamma-proteobacteria are transient members of the bacterioplankton, brought in by surrounding environments, and the members of the group are naturally occurring in freshwater environments that are probably copiotrophs, which could be one of the main reasons for their disproportionate distribution among the studied sites [34]. Phylum Acidobacteriota has been reported in previous studies in freshwater lakes to thrive in settings with acidic systems [34], limited nutritional availability, slower growth rates, high tolerance to toxic compounds [35], and sulfidic conditions [36]. A significant prevalence of Acidobacteria has been documented in a water stream in Japan [37].

Additionally, other prevalent phyla identified in all three freshwater ecosystems examined included Chloroflexi, Bacteroidota, Verrucomicrobiota, Myxococcota, and Nitrospirota, which align with observations from various other studies on freshwater bodies [3] [31] [38]. Similar trends in the prevalence of the primary bacterial groups have been observed globally across various freshwater ecosystems, even with differences in hydrology and water chemistry across the locations examined [39] [40]. Verrucomicrobia identified in examined freshwater ecosystems have been shown to possess a strong ability to degrade various forms of carbon, such as xylan, cellulose, pectin, sugars, and methane, and they play a beneficial role in nitrogen fixation in soils and polysaccharide breakdown in termites [31] [41].

The identification of the phyla Fusobacteriota and Sumerlaeota for Abala Lake and DTB120, Deferrisomatota, and Hydrogenedentes for Iyioku River that are specific to a single location suggests that the freshwater bodies differ in terms of pollution, nutrient levels, pH, oxygen availability, and human influence, all of which affect the composition of the microbial communities. Fusobacteriota are rod-shaped, anaerobic, Gram-negative bacteria that are well-known for their capacity to flourish in low oxygen conditions [42]. While Sumerlaeota have been confirmed to exist in diverse natural and artificial environments, including freshwater and marine sediments, they are still not well understood because there are not enough cultured specimens [43]. The phylum Deferrisomatota is enriched in anoxic and highly salinized environments and is involved in anaerobic respiration and metal cycling, particularly the reduction of iron and sulfur. The discovery of the candidate phylum DTB120, which is mainly known from metagenomic data and lacks cultured representatives, emphasizes the hidden microbial diversity and the possibility of new metabolic capacities that have not yet been identified. McAllister *et al.* [44] noted DTB120 is still uncharacterized and was identified using metagenomics analysis.

Although there were some anticipated variations among the three freshwater sources from different locations, the findings revealed overlap in the composition of the microbial communities across the analyzed freshwater bodies. These findings align with other research that indicates freshwater bodies harbor similar microbial communities [29] [31]. Our study found that the alpha diversity at Abala Lake was lower than that of the other freshwater bodies examined and indicated a significantly lower level in comparison to the Iyioku River. The results highlight the abundance of microbial diversity in the Iyioku River. The Iyioku River represents a typical river-fed ecosystem, and the higher diversity here is probably due to the influx of foreign bacterial populations from other smaller freshwater sources that feed into the river.

Furthermore, a significant difference was also observed in the beta diversity between the Iyioku river and the other studied freshwater bodies. These data all demonstrated that the bacterial community structure of the Iyioku river was quite different from that of the Abala lake. Iyiube lakes show the highest abundance of Acidobacteria when compared to other sites, revealing it to be the most polluted site because of human interference and the discharge of human wastes near the lake bank. These observed human activities could have an impact on the river's aquatic microbiome and fish health, based on a study that showed host-habitat as a primary determinant of gut microbiome in fish [45]. Adedire *et al.* [29] reported that the abundance of Acidobacteriota at the Idah river in Kogi State, Nigeria is as a result of industrial pollutants in the surrounding area. Future research on the studied freshwater bodies (Abala lake, Iyiube lake, and Iyioku river) should integrate metatranscriptomics to uncover microbial functions and pathways, while culture-based approaches remain essential for characterizing the ecological roles of uncultured or poorly defined taxa. Thus, the study was restricted to 16S rRNA-based taxonomic profiling of prokaryotic communities and did not capture other microbial groups such as fungi and protists, highlighting an important avenue for future investigation.

5. Conclusion

The initial metagenomic analysis of three freshwater ecosystems (Iyiube Lake, Abala Lake, and Iyioku River) in Enugu East, Nigeria, showed that Proteobacteria and Acidobacteria were the predominant phyla across all sampling sites, suggesting pollution from community waste, human sewage, and other anthropogenic sources. The identification of Acidobacteria in Iyiube Lake indicates that it is the most contaminated among the tested freshwater bodies, with a significant amount of sewage disposal. The three studied freshwater bodies (Iyiube Lake, Abala Lake, and Iyioku River) should not be used for drinking water due to environmental contamination, as consuming them poses substantial health risks for locals. Given that the water is used for agricultural purposes in growing vegetables, it is important that it be purified of pollutants through natural methods (slow sand and cloth filtration). The results of this study could serve as a reference or case study for future research on freshwater microbiomes and provide insights into the microbial communities of these three freshwater bodies.

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

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