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Microbiome Diversity Analysis of the Bacterial Community in Idah River, Kogi State, Nigeria

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Abstract

The analysis of bacterial diversity in aquatic systems particularly in rivers, lakes, and streams can provide useful data on the effect of anthropogenic activities on such water bodies to humans and fishes. Idah River, the focal point of this study, is an offshoot of the two major Nigerian rivers characterized by observed human activities and pollution sources. Water samples were collected from four designated sites and assessed for their bacterial assemblages and structure, using PacBio Single-Molecule Real-Time (SMRT) sequencing technology. The full length of the 16S rRNA gene was sequenced, and Amplicon Sequence Variants were generated using the DADA2 workflow optimised for PacBio long-read amplicons in Rstudio. A total of 8751 high-quality reads obtained were taxonomically classified as 24 phyla, 42 classes, 84 orders, 125 families, 156 genera, and 106 species. Taxonomical composition revealed Proteobacteria as the most abundant phyla across all sample sites. At the genera level, *Azospira* (57.03%) was the most dominant ASV in Docking Point A, while *Acinetobacter* (66.67%) was the most abundant ASV in Docking Point B. In Idah Axis Confluence, hgcl clade (65.66%) was the most prevalent ASV, whereas *Holophaga* (42.86%) was the most common ASV in Idah Axis Midstream. Genera analysis also revealed that 12.9% of the total ASVs were discovered across all sample sites. Among these were pathogenic bacteria, reducers, and degraders of domestic and animal wastes. Observed results provide evidence that sampled sites of Idah River are contaminated, most likely through constant human activities and thus, could have an impact on resident fishes as well. This study, therefore, agrees with a previous report from the river, which used standard microbial procedures. However, next-generation se-

quencing techniques employed revealed more bacterial community than the former, including unresolved taxonomic sequences that may be novel.

Keywords

Idah River, Dada2, PacBio Sequencing, Bacterial Diversity, Amplicon Sequence Variants

1. Introduction

In aquatic water bodies, microorganisms are essential components, as they support the provision of a variety of ecosystem services [1]. Bacteria, archaea, microbial eukaryotes and viruses are the aquatic microbial communities that represent practically immeasurable levels of the aquatic biodiversity [2]. However, the activities carried out by these microbial communities within the aquatic environment impact the quality of human life, the survival of animals and plants population, and the productivity level of the habitat directly [3]. Freshwater ecosystems, such as rivers, can differ in terms of salinity, average temperature, depth and nutrient content, yet they all provide good habitats for these life forms.

In the world today, the identification and role of microbes in aquatic biomes have substantially improved due to the metagenomic sequencing of environmental DNA [4]. The entire microbial communities in samples can be profiled easily and faster using the Next-generation sequencing (NGS) technology by researchers. To analyse and characterize microbial communities of different habitats such as soil and water, the 16S rRNA gene and other genetic markers have been used as targets [5] [6] [7]. The information generated by using these advanced molecular genetic approaches enables the ability to understand the microbial composition, their distribution in the environment and the pollution mechanisms [8] [9]. Nigeria has abundant surface water resources, and rivers serve as the major source of fresh water. The Idah River in Kogi State, Nigeria is an offshoot of the two major Nigerian rivers: River Niger (named after the country, Nigeria) and River Benue, after their confluence in Lokoja. It is located between latitude 7˚6'1"N and longitude 6˚42'23"E. A prior study used culture-based approaches to determine the physicochemical properties and microbiological profile of the Idah River in order to acquire insight into the microbial diversity and its impact on the environment and neighbouring communities [10].

Due to the limitations of the culture-based approach which have been well documented to identify less than 1% of microbes in an environment [11] [12], we sought to evaluate the microbiome diversity of the river utilizing 16S rRNA sequencing technology and microbiome pipelines in the present study.

2. Materials and Methods

2.1. Sample Collection

One litre each of water samples were collected from four sampling sites of Idah

River, namely Docking point A (DPA), Docking Point B (DPB), the designated point of the midstream of the river (IAM), and the confluence of the river (IAC). Water samples were collected using sterile bottles which were lowered 20 cm deep into the river. Samples were transported in a cooler box with ice packs to the laboratory for analysis.

2.2. DNA Extraction

Briefly, bacteria from water samples were concentrated on to membrane filters, by vacuum-filtering 1 litre of water samples through a 47 mm diameter membrane filter of 0.2 μm pore-size. Following this, DNA was directly extracted from concentrated bacteria on the membrane by subjecting them to DNA extraction protocol of the DNeasy PowerLyzer PowerSoil kit (Qiagen, Germany).

The integrity of the extracted DNA was assessed on an ethidium bromide-stained 1.0% agarose electrophoresis gel. Electrophoresis was done at 90 V for 1 hour (BioRad Power Pac Basic Electrophoresis systems, USA), and visualisation of DNA in the gel was captured in the gel documentation system (Biorad Gel Documentation System, USA) under UV light. All DNA samples were quantified using NanoDrop Spectrophotometer 2000 (Thermo Scientific, USA) and stored at −20˚C for further experiment.

2.3. Library Preparation, Amplification and Full-Length 16S rRNA Sequencing

The bacterial community present in water samples was analysed using the PacBio Single-Molecule Real-Time platform (Pacific Biosciences, USA).

Primer pairs 27F (AGRGTTYGATYMTGGCTCAG) and 1492R (RGYTACCTTGTTACGACTT) tailed with 16-bp barcode sequences were employed to amplify the full-length 16S ribosomal RNA from DNA samples.

Two rounds of PCR amplification were performed using KAPA HiFi HotStart ReadyMix PCR Kit (KAPA Biosystems, USA). The first round of amplification was performed using 10 μM of universal tagged 16S primers (0.75 μL each for forward and reverse), 2X KAPA HiFi HotStart ReadyMix (12.5 μL), template DNA and PCR-grade water required for 25 μL final volume. PCR conditions used were, initial denaturation at 95˚C for 3 minutes and 20 cycles of denaturation at 95˚C for 30 seconds, annealing at 57˚C for 30 seconds and extension at 72˚C for 60 seconds and a final elongation step at 72˚C for 5 minutes. Following the first round of PCR, DNA samples were inspected by loading them onto an agarose gel and quantified using the Agilent 2100 Bioanalyzer system (Agilent Technologies, USA). The second round of PCR was performed using the same PCR conditions using PCR product from the first round of amplification. Purification of 16S-barcoded amplicons was performed with the AMPure PB beads (Pacific Bioscience, USA). Purified 16S-barcoded amplicons were used to prepare DNA libraries using SMRTbell Express Template Prep Kit 2.0 (Pacific Biosciences, USA). HiFi DNA libraries were then sequenced on a PacBio Sequel Sys-

tem at Inqaba Biotech. (South Africa).

2.4. Sequence Analysis

Raw data were processed to generate HiFi fastq reads using the consensus circular sequence algorithm in the SMRT Link version 8 software. These data were then further quality filtered using the DADA2 package (version 1.20.0), in R software [13], chiefly implementing DADA2 workflow designed for PacBio long-read amplicons [14]. Reads were filtered using the following parameters in DADA2: minQ set to 3, minLen set at 1000, maxLen fixed to 1600 and maxN set to 0. Following filtering, a number of steps such as error-model learning, chimera detection and denoising were performed on all reads, to produce denoised amplicon sequence variants (ASVs). The Multiple Sequence Alignment was done using the Decipher package [15] in R. A phylogenetic tree was constructed using FastTree (version 2.1.10; [16]) and taxonomy assignment was performed against the trained SILVA database (version 138; [17]) for DADA2. Generated taxonomy, phylogenetic tree and sample metadata were merged into a phyloseq object for downstream processing using the phyloseq package (version 1.36.0; [18]). Downstream analysis such as alpha diversity calculation and statistics were computed using 4 metrics: Observed species, PD, Shannon and Simpson's using the package picante (version 1.8.2; [19]) and microbiome package (version 1.14.1; [20]). Taxonomic bar plots were computed for phylum detected across all samples and for top 20 genera in all samples using microbiome and microbiomeutilities (version 10.0.16; [21]) and Venn diagram depicting unique and shared bacterial genera from all sample sites were also constructed using the ggVenn package (version 0.1.9; [22]).

2.5. Statistical Analysis

To further investigate bacterial composition for each sample site, alpha diversity statistics were computed using four metrics namely: Shannon Index, Simpson Index, Phylogentic Diversity index and Observed species metrics. The non-parametric Kruskal-Wallis rank sum test was performed to evaluate their variations in bacterial diversity.

2.6. Metagenomics Data Availability

Raw PacBio HiFi fastq files generated in this study have been deposited into NCBI under Bioproject ID PRJNA779155 with SRA accession numbers SAMN23012752-SAMN23012755.

3. Results

A total of 8751 high quality reads were generated across all samples, with an average read of 2179.75. High quality reads were within the range of 394 - 4158, while the sequence length of the dataset were distributed within the range of 1401 to 1600 bp. Percentage read distribution of each sample site is shown in **Figure 1**.

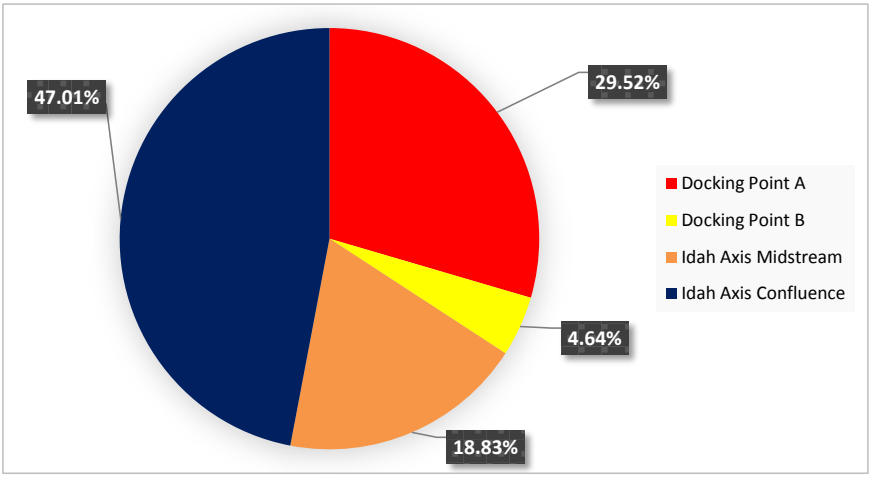

Figure 1. Percentage distribution of reads per sample site.

Bacterial Composition and Diversity of Idah River Samples

Bacterial community in DPA sample was represented by 67 species, derived from 19 phyla, 31 classes, 65 orders, 95 families and 108 genera. Bacterial community in DPB was represented by 14 phyla, 24 classes, 36 orders, 43 families, 40 genera and 15 species, while composition of bacteria captured in IAC were 19 phyla, 32 classes, 55 orders, 75 families, 91 genera and 59 species. Lastly, IAM sample revealed its bacterial composition as 20 phyla, 34 classes, 56 orders, 72 families, 77 genera and 39 species (Table 1). The Shannon index, Simpson diversity index, Phylogenetic Diversity (PD) index, and observed species of each sample were computed to examine the bacterial diversity and species richness of each sample. Shannon statistics varied from 4.634 to 6.813 while the Simpson diversity index varied from 0.987 to 0.997, PD values varied considerably from 10.776 - 82.247, while observed species ranged from 463 - 1228 (Table 1). Nineteen bacterial phyla were identified in all sample sites with Proteobacteria being the most abundant followed by Bacteroidota (Figure 2).

Figure 3 shows the twelve phyla that are common in all the sample sites in Idah River, namely Proteobacteria, Bacteroidota, Firmicutes, Desulfobacterota, Acidobacteriota, Planctomycetes, Actinobacteriota, Cyanobacteria, Verrucomicrobiota, Spirochaetota, Bdellovibrionota and Chloroflexi. Docking Point A had the highest relative abundance of the following phyla: Proteobacteria (28.54%), Bacteroidota (36.07%), Firmicutes (42.94%) and Desulfobacterota (32.41%). There was an indicated higher abundance of *Cyanobacteria* (42.02%) in Docking Point B relative to the other three sample sites. Idah Axis Midstream area had the highest abundance levels of the phyla: Acidobacteriota (52.68%) and Spirochaetota (42.37%). The sample site—Idah Axis Confluence area had the highest number of phyla with most abundance: Planctomycetes (42.38%), Actinobacteriota (34.32%), Verrucomicrobiota (40.76%), Bdellovibrionota (82.63%) and Chloroflexi (46.51%).

Table 2 shows the unique phyla in each sample site and their abundance counts. Docking Point A (DPA) had 3 unique phyla recorded, namely: Depen-

dentiae, Hydrogenedentes and Patescibacteria, albeit with low abundance counts of 6, 4 and 2 respectively. There were two unique phyla in Idah Axis Midstream area: Fibrobacterota (6) and Deferrisomatota (3). There were no unique phyla found in Docking Point B and Idah Axis Confluence region. However, these phyla mentioned above—Dependentiae, Hydrogenedentes, Patescibacteria, Fibrobacterota and Deferrisomatota had unclassified genera and species.

Table 1. Summary of bacterial taxa distribution and alpha diversity statistics of Idah River samples.

	DPA	DPB	IAC	IAM
Total number of reads	2529	394	4158	1670
Number of Phylum	19	14	19	20
Number of Class	31	24	32	34
Number of Order	65	36	55	56
Number of Family	95	43	75	72
Number of Genus	108	40	91	77
Species	67	15	59	39
Shannon's Index	6.152	4.634	6.813	5.856
Simpson's Index	0.994	0.987	0.997	0.992
Observed Species	683.000	120.000	1228.000	463.000
PD	56.055	10.776	82.247	26.557

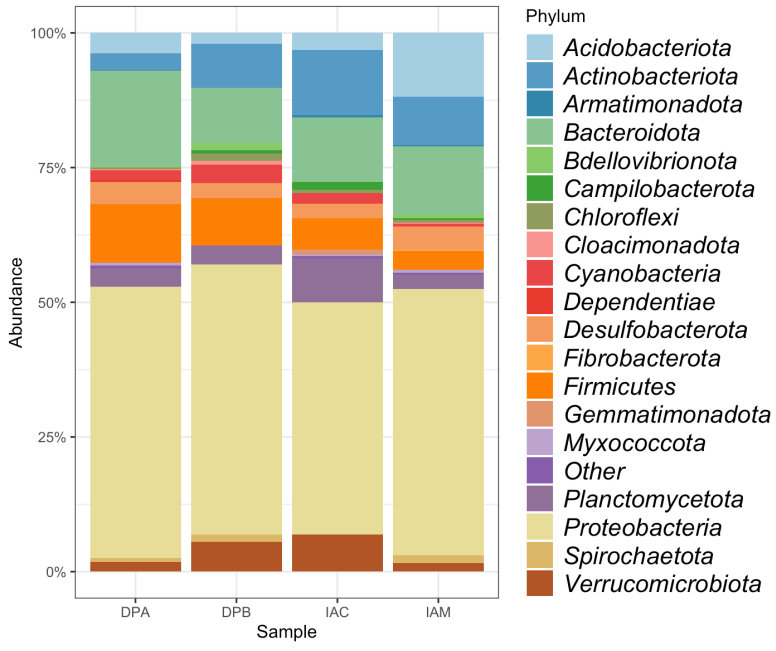

Figure 2. Taxonomic bar plots depicting the relative abundance of top 19 bacterial phyla identified from each sampling sites of Idah River, where DPA is Docking Point A, DPB: Docking Point B, IAC: Idah Axis Confluence and IAM: Idah Axis Midstream.

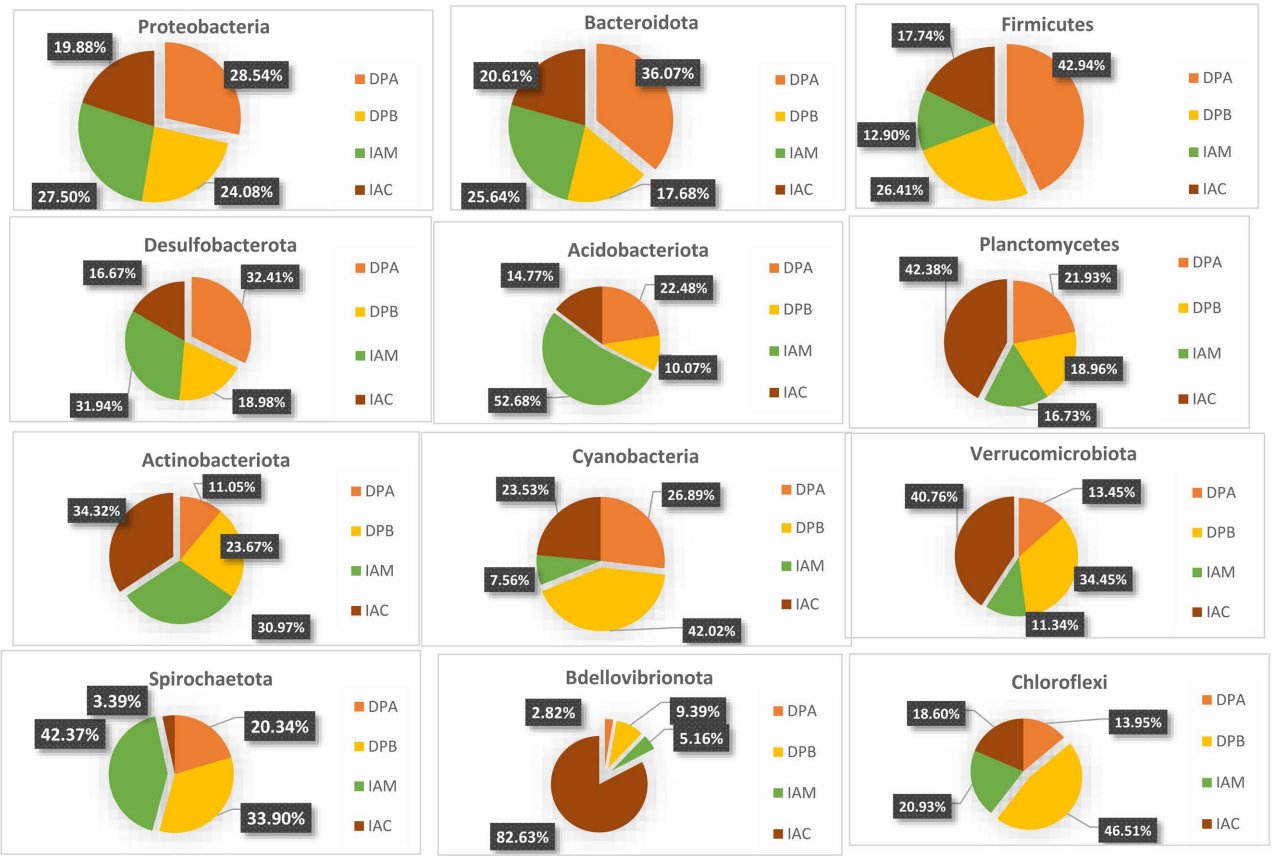

Figure 3. Two-D Pie chart of the twelve phyla common in all sample sites. Key: DPA: Docking Point A, DPB: Docking Point B, IAC: Idah Axis Confluence, IAM: Idah Axis Midstream.

Table 2. Unique phyla distinct to each sample site in Idah river.

	Docking Point A (DPA)	Docking Point B (DPB)	Idah Axis Midstream (IAM)	Idah Axis Confluence (IAC)
Phylum And Abundance Counts	Dependentiae (6) Hydrogenedentes (4) Patescibacteria (2)	NIL	Fibrobacterota (6) Deferrisomatota (3)	NIL

In Figure 4, the Venn diagram shows overlapping areas representing shared genera among sample sites, as well as unique taxa distinct to each site as non-overlapping areas. Figure 4(a) describes DPA and DPB sample sites as having 30 shared genera, resulting in 25.9% of all genera observed from both sites. In addition, DPA site was observed to possess more unique genera than DPA, with DPA occupying 66.4% of the total genera detected across both samples, and DPB occupying 7.8% of total genera as its unique genera. Figure 4(b) describes IAC and IAM sample sites. Likewise, Figure 4(b) shows common genera shared between IAM and IAC as 42.7% of the total genera detected among these sites, with IAC and IAM possessing 34.2% and 23.1% respectively, as their unique bacterial genera. Lastly, Figure 4(c) depicts shared genera observed from all sample sites and the unique taxa of each sample site. DPA, DPB, IAM, and IAC was seen to occupy 20 genera (12.9%) of the total genera identified across all

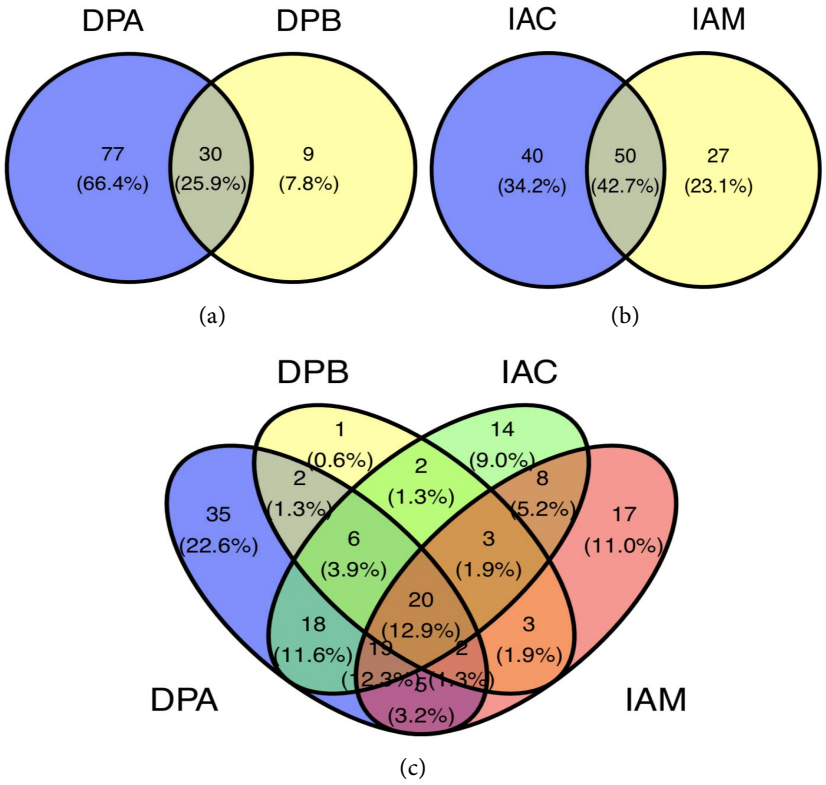

Figure 4. Venn diagram representing common genera as overlapping areas within samples of (a) Docking Point A and Docking Point; (b) Idah Axis Midstream and Idah Axis Confluence; (c) All sample sites (Docking Point A, Docking Point B, Idah Axis Confluence and Idah Axis Midstream).

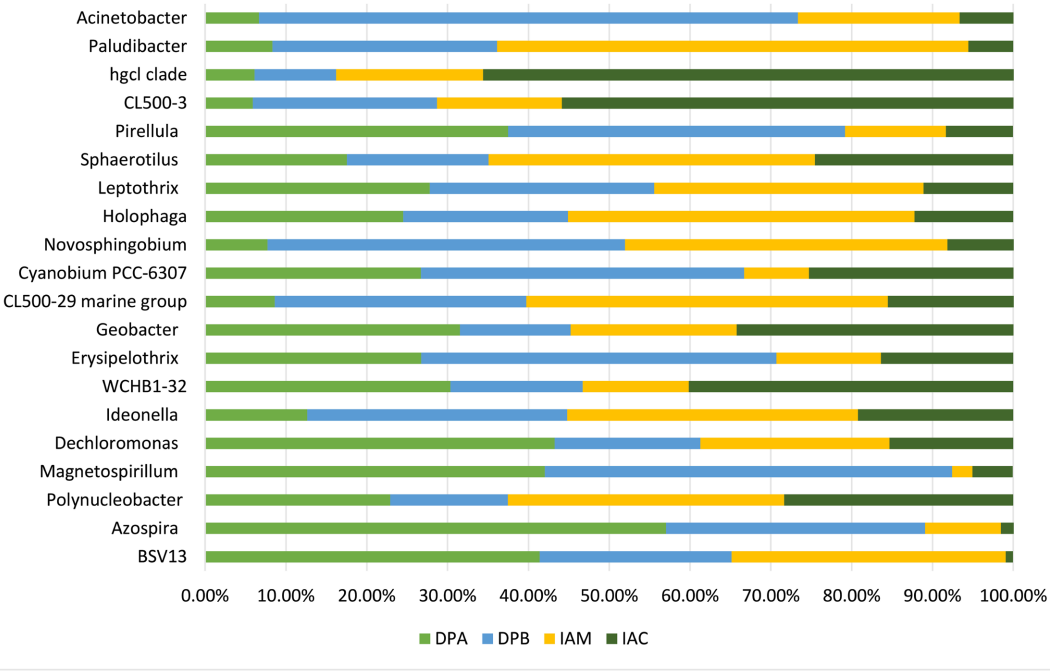

Figure 5. The relative abundances of the 20 taxonomic genera (100% stacked bar chart) common in the four sample sites.

sample sites. Moreover, the site with the most unique genera was seen as DPA, occupying 22.6% of the total genera of all sample site, while the site with the least unique genera was observed as DPB possessing 1 genus (0.6%) of the overall genera detected.

Figure 5 shows the distribution of the common genera with its percentage abundance in the four sites. The highest percentage abundances of genera observed in Docking Point A are BSV13 (41.40%), *Azospira* (57.03%), *Dechloromonas* (43.24%) and *Pirellula* (37.50%) while that of Docking Point B, a nearby site, was recorded to have the following highest abundances: *Acinetobacter* (66.67%), *Magnetospirillum* (50.42%), *Erysipelothrix* (43.97%), *Cyanobium* PCC-6307 (40.00%), *Novosphingobium* (44.26%), *Pirellula* (41.67%). *Polynucleobacter*, *Ideonella*, *Holophaga*, *Leptothrix*, *Paludibacter* had the highest percentage abundances in Idah Axis Midstream with values of 34.18%, 35.96%, 42.86%, 33.33%, and 40.35% respectively. Idah Axis Confluence region was more abundant in the following genera: *Geobacter* (34.25%), WCHB1-32 (40.16%), *Sphaerotilus* (24.56%), CL500-3 (55.88%) and hgcl clade (65.66%).

4. Discussion

This study was conducted in continuation of our initial investigation, which established the physicochemical attributes and bacterial diversity of Idah River using conventional techniques [10]. The use of conventional techniques had been well established and been in existence before next-generation methodologies were birthed. Some of these conventional techniques consider parameters such as morphological evaluation (in terms of shape, sizes, and pattern of growth on plates), biochemical analysis, physiological observation as well as chemical assays to detected proven patterns [23]. Nonetheless, these techniques are not without shortcomings; they are time-consuming, prone to contamination [24], require several culture media to establish growth patterns, and is insensitive to "difficult-to-grow" microbes, hence underestimating viable organisms in samples to be tested [25] [26]. In the report, it was recommended that a more comprehensive overview of the microbial structure of the river, be generated, by employing deep sequencing techniques. Deep sequencing techniques have proven to be more efficient in characterising microbes as it has been noticed to address limitations of conventional methodologies, therefore, allowing for the identification of a wide range of previously unidentified microbes [27]. This study addressed the recommendation and therefore, employed the use of Next Generation Sequencing techniques to unravel the bacterial diversity of the River. Full-length sequencing of the 16S rRNA was adopted using the PacBio Single-Molecule Real-Time (SMRT) sequencing technology, to obtain bacterial reads of all water samples. Previous studies such as [28] [29] and [30] also employed the use of PacBio SMRT platform to analyse bacterial communities from various samples. To our knowledge, this is the first metagenomic study of Idah River, which brings to the fore, a deeper bacterial structure and community of the popular river.

In this study, all sequence reads were taxonomically classified at the phyla and genera levels. Alpha diversity metrics to measure the evenness (Shannon and Simpson), richness (observed species), and phylogenetic diversity (PD) were also computed. Evenness, richness, and phylogenetic diversity of the bacterial community detected in the river were highest in IAC, whereas richness, phylogenetic diversity, and evenness of the bacterial population were lowest in DPB, and also recorded the lowest number of taxa detected in the area (**Table 1**).

In **Figure 3**, the highest percentage abundance (28.54%) of the phylum *P*roteobacteria in Docking Point A may be owing to human interference and discharge of human wastes near the riverbank, and the steady flow of the river is likely to have dispersed the flow of Proteobacteria to the other three sample sites. Proteobacteria, which primarily consists of gram-negative bacteria, have previously been identified as the dominant taxa in surface water bodies defined by anthropogenic pollution and waste disposal. Proteobacteria's ubiquity and vast dispersion over the other three sample sites, with genera such as *Acinetobacter*, *Pseudomonas*, *Zoogloea*, *Asticcacaulis* raises concerns on the implications to human health and the fishes due to their pathogenic nature. In particular, *Acinetobacter* and *Pseudomonas* are known to have a proclivity for antimicrobial resistance, thus posing an environmental hazard [31]. Interestingly, some genera of this phylum found in our study have been linked to the degradation of metals and hydroaromatic compounds, and some of them included *Propionivibrio*, *Aquabacterium*, *Dechloromonas*, *Novosphingobium*. This shows the presence of hydrocarbons in the river, and the source could be fuel discharge from fishing and transit motorboats as observed in the Idah River.

Actinobacteriota, Bacteroidota and Firmicutes were also found in abundance throughout the four sites, and the genera in these phyla have been largely connected to the gut microbiota of humans and animals. It has been proposed as being an indicator of faecal contamination [32] and identified as human biomarkers (**Figure 2**). It is interesting to note that *Bacteroidetes* and Firmicutes, along with Proteobacteria have been observed to account for 90% of fish intestinal microbiota according to [33]. We deduced that the 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 [34]. Desulfobacterota is a group of sulfate-reducing bacteria that is common in marine habitats. It has been observed that it plays a significant role in the biogeochemical cycling of sulphur in paleosediments [35]. The genera detected in this phylum in the microbiome analysis—*Desulfovibrio*, *Geobacter*, *Desulfobulbus*, *Pseudopelobacter* and *Geothermobacter*—indicate that there might be sulfate residues in the river and ongoing degradation is not unlikely. The abundance of Acidobacteriota at the Idah Axis midstream sample site (52.68%) is most likely connected to industrial pollutants in the surrounding area. The phylum's genera found in the river—*Geothrix* and *Holophaga*—were shown to be heavy metal tolerant as seen in previous studies including waterbo-

dies and soil environments [36] [37]. Their presence also confirms the presence of heavy metals in the Idah River, such as arsenic and selenium, which were found in high levels in our previous study [10], and can induce cytotoxic effects on fish, making it a major source of organic arsenic exposure for humans in the Idah community. However, the role *Geothrix* plays in reducing metal ions makes the bacteria environmentally essential. Planctomycetes are ubiquitous in both aquatic and soil ecosystems, and they are mostly chemoorganotrophic microorganisms that break down complex organic compounds [38]. In the river, this phylum contained *Pirellula*, *Blastopirellula*, *Candidatus Annammoximicrobium moscowii*, which was discovered as a novel microorganism involved with anaerobic ammonium oxidation for treatment of filtrate of wastewater fermented residue [39]. *Candidatus Nostocoida limicola*, a filamentous bacterium previously reported to be associated with activated sludge plants [40]. This could mean that the Idah confluence region, which is a junction of the water influx from both the Idah and Agenebode communities, is a low-oxygen habitat for marine organisms. *Cyanobacteria* and *Verrucomicrobiota* are prevalent in extremely contaminated waterbodies [41], and this phylum's distribution in Idah River reflects this. Members of the Spirochaetota in the river samples – *Salinispira* and *Spirochaeta* sp. and *Chloroflexi* are free-living, non-pathogenic microorganisms or commensals and thus, they do not pose harm to the aquatic body. *Bdellovibrionota* are obligate predators preying on gram-negative bacteria. It is worth noting that the Idah Axis Confluence area had the highest percentage distribution of this phylum at 82.63%, however, the genera were unknown. This could mean that they may be novel microorganisms. The influence of these unidentified microorganisms on the environment is not fully understood. The only genera identified from *Bdellovibrionota* was *Bacteriovorax* located at Docking Point A and Docking Point B (**Figure 3**) and they are considered microbial therapeutic agents due to their predatory nature on gram negative bacteria [42]. This indicates they are beneficial to aquatic life as they may be deleterious to potential bacterial pathogens in fishes.

In terms of the distinct phylum discovered at each site, all of them in **Table 2** had unknown genera with extremely low abundance and nearly negligible. There is little known about the biology of the phylum, Dependentiae, in Docking Point A, although studies show that its members are pathogens of aquatic protists [43]. This could imply that the unknown representatives of these genera in Idah River may exhibit such characteristics. Hydrogenedentes phylum is a diverse chemotrophic metabolic group linked to nitrogen, carbon and sulphur pathways [44], however, it has previously been found to be present in the gut microbiota of Mexican children [45]. Given that this was only discovered in Docking Point A, where the majority of human activities occurred, this could be a trigger for the phylum's existence. Patescibacteria, another phylum identified only in Docking Point A, is typically found in groundwater and is primarily associated with seepage from agricultural soils. The presence of this phylum could be related to the

farming activities near the riverside. The unique phyla—Fibrobacterota and Deferrisomatota in the Idah Axis Midstream region, have been previously linked to the cycling of methane, sulfur and nitrogen cycling [46].

About 25.9% of the total bacterial genera were observed as shared taxa among sampled docking points (DPA and DPB) (**Figure 4(a)**). However, the most prevalent among these commonly shared taxa were *BSV13*, *Ideonella*, *Azospira*, *Magnetospirillum*, *Polynucleobacter* and *Pseudopelobacter*. Their dominance is not surprising, as 66.66% of them were among the large and ubiquitous Proteobacteria phylum. Among these prevalent genera, an unclassified genus, BSV 13 is currently unknown among taxonomic classification; however, this group might be an epidemiologically important group of organisms resident in freshwater habitats. *Ideonella* is a member of the broad Proteobacteria phylum and belongs to the class Betaproteobacteria and the family Comamonadaceae. This organism has been affiliated with biofilms in polluted rivers [47], and its affinity for degrading plastics (Polyethylene terephthalate) [48]. Therefore, its presence in both docking points might not be unconnected to the pollution that these sampling sites are being exposed to almost constantly. A good example of human activities that might have contributed to the pollution of both sampling sites is the washing of motor bicycle parts, washing of plastics and fibres, and dumping of bits and pieces of household items by locals in the vicinity. Because of its propensity for degrading plastics, its presence in Idah River could benefit the fishes by eliminating the challenge or threat posed by plastics. Another common bacterium discovered in both sites was *Azospira*. *Azospira* has been phylogenetically connected to Betaproteobacteria, of the family Rhodocyclaceae. Formerly, this genus was mostly associated with root nodules in plants, as they have been described as Nitrogen-fixers [49], however, currently, it is being recovered in freshwater bodies as well [50]. Interestingly, this genus as well as *Dechloromonas* and *Ideonella* has been discovered to be excellent microbial reducers of perchlorate in mesophilic freshwater environments [51]. Perchlorate is a recognised chemical and well-known pollutant. It can be chemically made or can occur from natural sources. It can be naturally introduced into water bodies from fertilizer run-offs and potash ores, unfortunately, perchlorates are highly stable and persistent in the environment and can cause a disruption in the functionality of the thyroid gland when ingested in humans at high concentrations. Perchlorates cause disruption by blocking the protein responsible for pumping iodine, hence inhibit the uptake of iodine into the body [52] [53] [54]. *Magnetospirillum*, another prevalent microbe discovered at both docking sites, belongs to the diverse group of Magnetotactic bacteria (MTB). Most MTB has been associated with several classes of Proteobacteria such as Alpha, Beta, and Gammaproteobacteria [55]. They can make use of the earth's magnetic field to accurately locate environments with a reduced amount of oxygen, (as they are micro-aerophilic in nature) especially in freshwaters, using a special organelle called magnetosomes [56]. It has been reported as ubiquitous in freshwater and marine habitats [57]

[58]. The discovery of *Magnetospirillum* in Idah River is in agreement with [59] who reported the presence of this MTB as well in a freshwater lake in Northern China. In addition, *Magnetospirillum* has also been identified in freshwater sediments such as in reports of [60] [61] and [62]. Included among prevalent taxa in these sampled sites (DPA and DPB) were ASVs closely related to *Polynucleobacter*. *Polynucleobacter* is a member of the Betaproteobacteria class, belonging to the family Burkholderiaceae. It has been heavily associated with freshwater lakes and rivers, constituting more than 10% of bacterioplankton in freshwater habitats [63]. Our finding is therefore consistent with previous studies, in which the abundance of *Polynucleobacter* was also recognised in freshwater habitats [64] [65].

Comparing sample sites IAC and IAM, 42.7% of the total bacterial genera identified from these sites were shared (**Figure 4(b)**). Among those prevalent were *Geothrix*, *Polynucleobacter*, CL500-29 marine group, hgcI clade and *Ideonella*. These sample sites were selected to account for microbial assemblages that could be transient, residential, or free-living, further away from the docking points of the river. These sites are characterised by constant water movement and scanty plant growth amid the flowing river.

The presence of some bacterial genera identified by 16S rRNA sequencing of these sites points to a contamination input into the river. *Geothrix*, particularly *G. fermentas* is known to be present in petroleum-contaminated sediments, freshwaters, and even aquifers [66]. They have specifically been recognised for the role they play in purifying petroleum-contaminated environments and metal recycling within the environment [67]. Likewise, *Ideonella* is known to be a degrader of plastics. In addition to these degraders, CL500-29 marine group, belonging to the large phylum of the Actinobacteria was also prevalent. Previously, this group of bacteria were only found majorly in marine habitats, however, several studies have proven that they have also been found to thrive in freshwater habitats such as lakes and river bodies [68] [69]. Interestingly, our finding is in concordance with studies of [70] who similarly, found CL500-29 among bacteria consortia from a river plagued with assorted types of domestic sewage.

We also compared all sample sites and discovered 12.9% of the total Amplicon Sequence Variants (ASVs) were present across all sampled sites. Among these were ASVs associated with *Erysipelothrix*, *Novosphingobium*, *Cyanobium PCC-6307* and *WCHB1-32*. These bacterial taxa have also been detected in freshwater habitats [71] [72] [73]. Among these identified taxa were bacterial pathogens such as *Erysipelothrix*, known to be associated with animal waste [71]. Its ASV detected across all sample sites is not surprising as [10] had previously reported abattoir effluents being channelled into Idah River. This pathogen was however not detected using conventional methodology for identification. On the other hand, *Cyanobium PCC-6307*, classified within the Picocyanobacteria, has been implicated in generating algal blooms, while clades of *Novosphingobium* have been documented to be excellent degraders of various xe-

nobiotic compounds and catabolism of aromatic compounds [74] [75]. These findings are in agreement with previous studies in which *Novosphingobium* and *Cyanobium PCC-6307* were recognised among freshwater environmental sequences [73] [75] [76].

5. Conclusion

Using 16S rRNA metagenomic techniques, this work represents a detailed study of the bacterial community that characterises Idah River, located in Kogi State, Nigeria. Our findings buttress those generated in our previous report. Deep sequencing employed in this study, however, helped in identifying several bacteria that would have been difficult to grow using standard laboratory media, for example, *Polynucleobacter* and the MTB group of bacteria. This method had also revealed several degraders that were not detected previously, apparently showcasing the superiority of culture-independent techniques over culture-dependent techniques. We also stated several unknown sequences at the genus level, which might be novel bacteria, and might be classified in the nearest future. The consortia of ASVs closely related to pathogens and degraders seems to buttress the point that Idah River is contaminated with both animal and man-made products (most likely through anthropogenic activities involving agricultural and non-agricultural wastes being released into the river). Such activities occurring in and around the river that influenced the bacteria abundance and diversity may also play a significant role in determining the microbial communities of the aquatic animals in the river. With the use of microbiome analysis in this study, we can now gain a better insight into these bacteria that may be linked to the health and productivity of aquatic life in the Idah River.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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