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Community Structure of Nitrifying and Denitrifying Bacteria from Effluents Discharged into Lake Victoria, Kenya

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Abstract

An active microbial community of nitrifying and denitrifying bacteria is needed for efficient utilization of nitrogenous compounds from wastewater. In this study, we explored the bacterial community diversity and structure within rivers, treated and untreated wastewater treatment plants (WWTPs) discharging into Lake Victoria. Water samples were collected from rivers and WWTPs that drain into Lake Victoria. Physicochemical analysis was done to determine the level of nutrients or pollutant loading in the samples. Total community DNA was extracted, followed by Illumina high throughput sequencing to determine the total microbial community and abundance. Enrichment and isolation were then done to recover potential nitrifiers and denitrifiers. Physicochemical analysis pointed to high levels total nitrogen and ammonia in both treated and untreated WWTPs as compared to the samples from the lake and rivers. A total of 1,763 operational taxonomic units (OTUs) spread across 26 bacterial phyla were observed with the most dominant phylum being Proteobacteria. We observed a decreasing trend in diversity from the lake, rivers to WWTPs. The genus *Planktothrix* constituted 19% of the sequence reads in sample J2 collected from the lagoon. All the isolates recovered in this study were affiliated to three genera: *Pseudomonas*, *Klebsiella* and *Enterobacter* in the phylum Proteobacteria. A combination of metagenomic analysis and a culture-dependent approach helped us understand the relative abundance as well as potential nitrifiers and denitrifiers present in different samples. The recovered isolates could be used for in situ removal of nitrogenous compounds from contaminated wastewater.

Introduction

Over the past few decades, global population and anthropogenic activities such as crop production, sewage discharge, fossil fuel combustion and fertilizer use in cropland have substantially intensified [1]. Nowadays, human activities greatly contribute to the rate at which nitrogen enters the terrestrial ecosystem as compared to preindustrial era [2]. Nitrogen load to aquatic ecosystems from both diffuse and point sources in the period 2002 to 2010 was estimated at 32.6 million tons per year, of which 24.4 million tons were from agriculture [3]. The uncontrollable release of nitrogenous compounds into the coastal seas causes ecological damage due to the shift in nutrient balance resulting in eutrophication [4]. Eutrophication promotes the production of water hyacinth, green macroalgae blooms and toxin-producing algal blooms which leads to anoxia and hypoxia, causing mass mortality of fish and health risks [5]. For example, the water quality in Lake Victoria, Kenya has deteriorated over the years due to nutrient loading and this poses a major threat to the ecological functions offered by the lake ecosystem [6]. Eutrophication has resulted in the reiterative proliferation of aquatic weeds such as water hyacinth and cyanobacteria in the Lake Victoria basin [7]. Mechanical, physical or biological techniques have been some of the methods used to mitigate against water hyacinth [8]. Toxic cyanobacteria blooms have flourished in the lake, specifically in the bays and gulfs [9].

On account of the adverse effects of nitrate pollution, it has become an issue of global concern to remediate the levels of nitrate in the ecosystem [10]. Nitrate utilization through traditional biological methods such as sequencing batch reactor, anaerobic–anoxic–oxic process and Bardenpho process requires a large space and they are time-consuming due to temporal or spatial separation, hence

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increasing the cost and energy for wastewater treatment plants [11]. By ensuring high efficiency of nitrification and denitrification during biological nitrogenous compound utilization, the risk of eutrophication in receiving water bodies is kept at minimal levels [12]. The effectiveness of nitrifiers and denitrifiers depends on the activity, abundance, and diversity of bacterial species, which in turn are affected by environmental parameters such as biochemical oxygen demand, total dissolved solids, total nitrogen, carbon source, pH, and temperature [13]. Many studies aim at assessing the total bacterial communities as well as a microbial composition based on the 16S rRNA gene in wastewater treatment [14]. Currently, research has shifted to aerobic denitrifiers since they can simultaneously exploit both oxygen and nitrate as the terminal electron acceptors [15]. They can overcome the gap created by the traditional technique by boosting the efficiency for nitrogenous compounds utilization, reducing the space, and cost due to their potential to perform simultaneous nitrification and denitrification in a single reactor [16, 17]. Aerobic denitrifiers possess the ability to carry out coupled nitrification and denitrification, hence they are often referred to as heterotrophic nitrification and aerobic denitrification (HN-AD) bacteria. So far, various HN-AD bacteria have been isolated and utilized in the treatment of wastewater plants: *Alcaligenes faecalis* [17], *Pseudomonas balearica* strain RAD-17 [18]. However, more studies are still needed to fully comprehend the microbial community structure and potential application of aerobic denitrifiers in wastewater treatment. The aim of this study was to analyze the microbial diversity and abundance in the WWTPs and rivers that drain to Lake Victoria.

Materials and Methods

Sample Collection and Physiochemical Analysis

Eight surface water samples were collected in 500 ml sterile bottles from rivers and WWTPs that drain into Lake Victoria (Supplementary Fig. 1). The sample collection sites were: Dunga beach sites A and B, Homabay beach, River Sondu, River Nyando, Kisat WWTP, Lagoon WWTP, and Homabay WWTP (Table 1). The samples were packed in cool boxes and transported to Microbiology Laboratory. Physiochemical analysis was done at the Standard Global Services Kenya Limited laboratories. The parameters analyzed were: pH, total suspended solids, biochemical oxygen demand, total nitrogen, nitrate, ammonia, nitrite and chloride. Nitrate was analyzed using the APHA-4500-NO3 method, nitrite was analyzed using the APHA-4500-NO2, while ammonia was analyzed using the APHA-4500- NH3 F method [19].

Table 1 Sample collection points and Physicochemical analysis of water samples

Sample origin			Rivers		Treated WWTP	Untreated WWTPs		Lake		
Parameters	Method	Units	(J1) River sondu	(J8) River nyando	(J7) kisat WWTP	(J2) lagoon WWTP	(J4) homabay WWTP	(J3) homabay B	(J5) dunga A	(J6) dunga B
Sampling points	GPS	Latitude	0° 22′ 46″	0° 0′ 57″	0° 4′ 57″	0° 28′ 35″	0° 31′ 27″	0° 30′ 41"	0° 8′ 43"	0° 8′ 20″
		Longitude	34° 49′ 21″	35° 16′ 32″	34° 44′ 58″	34° 30′ 42″	34° 26′ 53″	34° 28′ 34"	34° 44′ 13.2"	34° 44′ 11"
pH	APHA 4500 H +		6.27	6.78	6.76	6.71	7.46	7.42	7	6.44
Total nitrogen	Calculation	mg/l	1.23	1.34	24.64	18	45.43	1.57	3.36	3.92
TDS	APHA 2540 C	mg/l	45	144	661	553	1308	264	276	242
Nitrite as N	APHA-4500-NO2	mg/l	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chloride	APHA-4500-CL B	mg/l	7.88	12.32	62.09	60.12	146.35	10.35	9.36	11.83
Ammonia as N	APHA-4500-NH3 F	mg/l	<0.02	<0.02	19.49	10.77	38.69	<0.02	2.84	2.55
Nitrate as N	APHA-4500-NO3	mg/l	0.11	0.22	<0.01	0.08	0.07	0.45	<0.01	<0.01
BOD5 @ 20 °C	APHA 5210	mg/l	45	30	153	60	126	63	73	183

Microbial Community Analysis

DNA Isolation, Amplicon Library Preparation and Sequencing

Total microbial community DNA was isolated using the phenol–chloroform protocol as described [20]. Obtained DNA pellets were air-dried and resuspended in 30.0 µl of PCR water. The quality of the extracted DNA was checked by separating 4 µl on 1% gel at 115 V for 40 min. Purified DNA (25 µl) was stabilized using DNAstable® (Biomatrica), air-dried, and shipped to MR DNA Research Laboratory, USA for sequencing. The 16S rRNA gene V4-V7 region was amplified from the extracted DNA using barcoded primers 515F (GTGCCAGCMGCCGCGGTAA) and 806R (GGA CTACHVGGGTWTCTAAT) [21]. Amplicons were generated using HotStarTaq Plus Master Mix Kit (Qiagen, USA). PCR amplification was performed as follows: initial denaturation step at 94 ℃ for 3 min, followed by 30 cycles of denaturation at 94 ℃ for 30 s, annealing at 53 ℃ for 40 s and extension at 72 ℃ for 1 min. A final extension step at 72 ℃ for 5 min was included. After amplification, all PCR products were checked in 2% agarose gel to determine the success of amplification and the relative intensity of bands. Barcoded samples were pooled together in equimolar ratios according to their molecular weight and DNA concentration from the gel images. Calibrated Ampure XP beads were used to purify pooled samples for use in library preparation. The Illumina TruSeq DNA library protocol was used to prepare the DNA library using the pooled and purified PCR products. Sequencing was done at Molecular Research DNA (www.mrdnalab.com, Shallowater, TX, USA) on a MiSeq 2 × 300 bp Version 3 following the manufacturer's guidelines.

Analysis of Sequences Data and Taxonomic Classification

The Q25 sequence data derived from the sequencing process was processed using the MR DNA ribosomal and functional gene analysis pipeline (www.mrdnalab.com, MR DNA, Shallowater, TX). Sequences were depleted of primers, reads < 250 bp removed, and sequences with ambiguous base calls removed. The obtained reads were quality filtered using a maximum expected error threshold of 1.0. The dereplicated sequences were denoised and any chimeric sequences removed using Uchime [22]. Operational taxonomic units (OTUs) were picked at 3% divergence, which is equivalent to 97% similarity. Taxonomic assignment of each OTUs was done using BLASTn against a curated database derived from NCBI (www.ncbi.nlm.nih.gov). Richness, Shannon and Simpson indices were calculated from the resulting OTUs using *microbiomeSeq* [23], phyloseq [24] and *vegan* [25] R packages. Heatmaps were generated using gplots package in R [26]. Principle component analysis (PCA) was done to explore the effect of environmental variable on the microbial community using AmpViz2 package in R [27]. The assembled sequence reads have been deposited into the SRA under the accession PRJNA824944.

Enrichment and Isolation

Enrichment and isolation of bacterial denitrifiers was done using a screening medium and solid bromothymol blue medium. The screening medium (pH 7.2) consisted of the following reagents per liter: sodium succinate (2.84 g), $NaNO_3$ (0.84 g), KH_2PO_4 (1.36 g), $(NH_4)2SO_4$ (0.27 g), Yeast extract (1 g), $MgSO_4.7H_2O$, (0.19 g), TE (trace element) solution (1 ml). For nitrification, $(NH_4)_2SO_4$ was used as the sole nitrogen source, while for denitrification, $NaNO_3$ was used as the sole nitrogen source. The effluent samples (50 ml) were used to inoculate 100 ml of screening medium in a sterilized 500 ml Erlenmeyer flasks in triplicate and incubated on a rotary shaker at 150 rpm, 30 °C for 3 days. This was done to specifically enrich for denitrifying bacteria. After the growth period, the enrichment samples were serially diluted, one hundred microliters from 10^8, 10^9, to 10^{10} spread plated on solid bromothymol blue medium and incubated at 30 °C for 48 h. Colonies showing a blue cloudy appearance were picked and purified by repeated streaking on fresh solid bromothymol blue medium plates. Based on the data obtained, only 12 bacterial isolates were selected for further study since they exhibited efficient heterotrophic nitrification and aerobic denitrification capabilities in both screening media and wastewater.

Identification of Bacterial Isolates

Selected pure isolates were grown overnight at 30 °C in 20 ml of the LB media in a rotary shaker. Cells for nucleic acid extraction were harvested by centrifuging 1 ml of the broth cultures. Bacterial genomic DNA was isolated using the phenol–chloroform protocol as described [20]. The 16S rRNA gene was amplified using bacterial universal primers pair 8F (5′-AG (A/G) GTTTGATCCTGGCT-3′) and 1492R reverse, (5′-CGGCTACCTTGTTACGACTT-3′) on a Sure cycler 8800 (Agilent Technologies). The PCR mix was prepared in a total reaction volume of 50 µl (30.0 µl of Molecular biology grade water, 1.0 µl of genomic DNA, 2.5 µl of each primer, 10.0 µl polymerase buffer, 1.0 µl of dNTPs, 1.0 µl of MgCl2, 1.5 µl of dimethyl sulfoxide (DMSO) and 0.3 µl Taq polymerase). The following PCR reaction conditions were used: 95 °C for 5 min, followed by 35 cycles (1 min of denaturing at 94 °C, 1 min of annealing at 53 °C, 1 min of extension at 72 °C) and final extension step at 72 °C for 5 min. The presence and quantity of PCR products were checked on 1% agarose gel at 115 V for

35 min in 1 × TAE buffer, followed by staining with fluorescent dye to enhance visualization under UV. Cleaning of the amplified fragments was done by mixing 12.5 µl of PCR product with 2.5 µl of ExoSAP-IT™ (Thermo Fisher Scientific), incubated at 37 °C for 30 min, followed by heat inactivation at 95 °C for 5 min to stop the reaction. Similar universal primers 8F and 1492 R were used to sequence PCR products at Inqaba biotech, South Africa. The sequenced amplicons were checked for quality and edited using Chromas Lite 2.0.1 (https://technelysium.com.au/wp/chromas). Sequence similarity level to existing type strains was done on EzBioCloud server [28]. Phylogenetic analysis was done using Molecular Evolutionary Genetics Analysis (MEGA) version X [29]. The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) were shown next to the branches. The evolutionary distances were computed using the Maximum Composite Likelihood method using *Bacillus sp. CK-8* as the outgroup. The isolate sequences have been submitted to NCBI under the accession numbers ON227417 to ON227434.

Results

Samples Collection and Physicochemical Analyses

A total of eight samples were analyzed. The range of the various physicochemical parameters is shown in Table 1. Total nitrogen was high in both treated and untreated WWTPs (Kisat WWTPs, 24.64 mg/l, Homabay WWTP, 45.43 mg/l, Lagoon WWTP 18 mg/l) while elevated levels of ammonia were also detected in WWTPs (Kisat WWTP, 19.49 mg/l, Homabay WWTP, 38.69 mg/l, Lagoon WWTP, 10.77 mg/l). However, both lakes and river samples had relatively low levels of total nitrogen and ammonia. Higher level of chloride was detected in both treated and untreated WWTPs than the rivers and lake samples. The pH of all samples was found to range between 6.5 and 8.5 except for River Sondu (6.25) and Dunga B (6.46) which were slightly lower (Table 1).

Microbial Community Diversity

A total of 207,924 high quality sequences were obtained after quality filtering. The number of sequences per sample ranged between 18,205 and 29,531 while OTUs per sample ranged from 702 to 1,241 (Table 2). A total of 26 bacterial phyla were identified across the samples. The most abundant number of OTUs were affiliated to the phylum Proteobacteria (60% of all the reads) followed by Actinobacteria, Bacteroidetes, Firmicutes, Verrucomicrobia and Cyanobacteria which occurred at varying percentages across the samples (Figs. 1, 2). The major genera represented in the phylum Proteobacteria were *Dechloromonas* and *Hydrogenophaga* as shown in Fig. 2. Some of the species in these two genera are known to perform denitrification [30]. Sequences related to the genus *Arcobacter* in the Class *Epsilonproteobacteria* were highly abundant in sample J5 (Dunga A). The Bacteroidetes group was the second most abundant phylum with 1.8% of the total reads affiliating to the genus *Bacteroides.* Some species in this genus have been shown to have nitrate reduction capabilities [31]. The phylum *Firmicutes* was largely represented by obligate anaerobes of the genus *Clostridium* (2.1% of all bacterial sequences). Some *Clostridium spp.* have been reported to convert benzaldehyde to benzoate and benzyl alcohol [32]. We noted that the cyanobacterial genus *Planktothrix agardhii* was highly abundant in sample J2 (Lagoon WWTP) accounting for 18.9% of the total sequence reads in this sample (Fig. 1). *Planktothrix agardhii* is a toxin-producing strain of *Cyanobacteria* that possesses a potential health risk to human populations, fish and other aquatic organisms [33].

Diversity Index Analysis

Diversity Index is a crucial tool in quantification of diversity in a community and it also describes its numerical structure [34]. High values of diversity indices represent a rich microbial diversity. The sequences, OTUs and α-diversity indices of the microbial communities in the different samples are presented in Table 2. The number of OTUs and diversity indices revealed a decreasing trend in diversity

Table 2 Sequences, OTUs and α-diversity indices of microbial communities obtained from different samples

Sample ID	Site	No. of sequences	OTUs	Chao1	Ace	Simpson	Shannon
J1	River sondu	23,730	1,202	1361.56	1355.19	0.99	8.53
J2	Lagoon WWTP	29,531	883	1097.03	1055.39	0.96	6.94
J3	Homabay B	23,596	1,241	1456.78	1401.15	0.99	8.30
J4	Homabay WWTP	29,354	732	1022.38	1050.56	0.96	6.28
J5	Dunga site A	29,380	995	1199.48	1191.98	0.97	6.50
J6	Dunga site B	18,205	1,033	1368.41	1392.83	1.00	8.53
J7	Kisat WWTP	29,518	702	1032.31	945.88	0.98	7.11
J8	River nyando	24,610	1,138	1343.11	1301.36	0.99	8.03

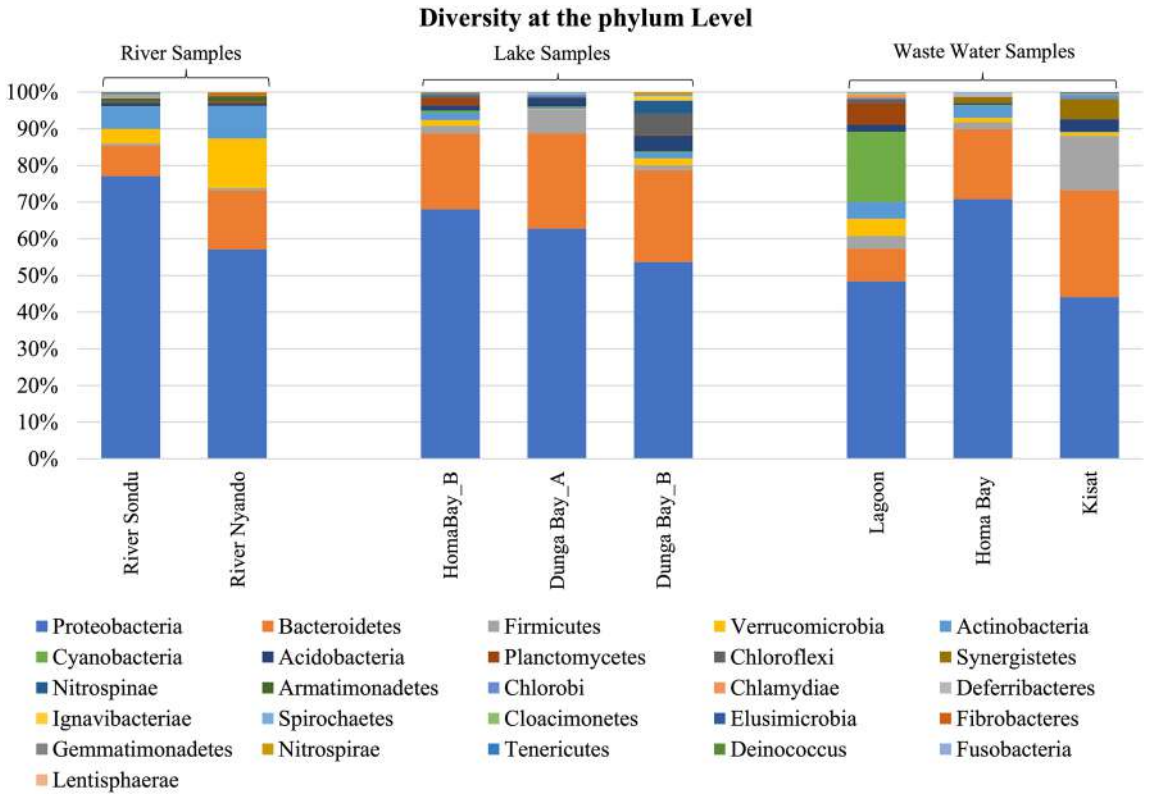

Fig. 1 The relative abundance of different phyla across the eight samples. Each phylum in the graph is designated with a unique color code (Color figure online)

from the lake, rivers to WWTPs (Fig. 3). The sample with the richest biodiversity was reported in sample J3 (Homabay beach) with a Shannon diversity value of 7.9, whereas sample J4 (Homabay WWTP) had the least biodiversity with Shannon Diversity Index of 5.9. Sample J7, Kisat-treated WWTPs, had relatively higher diversity than the two untreated WWTPs (Homabay WWTP and Lagoon WWTP) with a Shannon Diversity Index of 6.7 and Richness Index of 576.87. Beta diversity analysis showed that samples with similar water chemistry and microbial diversity clustered together (Fig. 4). The ordination method used highlights the differences between the different sites based on their microbial community composition.

The lake samples (Dunga beach site A&B and Homabay beach) are clustered together separately, similar to rivers (River Sondu and River Nyando), whereas in WWTPs the trend is different. Untreated WWTPs (Homabay and Lagoon WWTPs) are ecologically far apart from the treated WWTP (Kisat WWTP).

Phylogenetic Analysis of 16S rRNA Gene Libraries

Phylogenetic analysis based on 16S rRNA marker gene showed that the isolates separated into two clusters: The first cluster consists of members of the *Enterobacter* and *Klebsiella* and the other cluster has *Pseudomonas* members. (Fig. 5). Based on the analysis, the sequences revealed that the recovered isolates had the closest matches to the *Gammaproteobacteria* group (Table 3).

Discussion

The study explored the diversity of bacterial communities between eight water samples collected from Rivers and wastewater treatment plants draining into Lake Victoria. The results were compared to the samples collected from the Lake. WWTPs recorded higher levels of pollutants than rivers and lake waters, which could be responsible for the significantly reduced bacterial diversity observed in the WWTPs. These results were consistent with the current study on the negative effects of pollutants on microbial diversity and community functions [35]. Environmental parameters such as high biochemical oxygen demand may lower the amount of dissolved oxygen hence creating an anoxic environment that may significantly influence bacterial density in WWTPs [36]. In addition, high abundance of *Planktothrix agardhii* observed in sample J2 (Lagoon

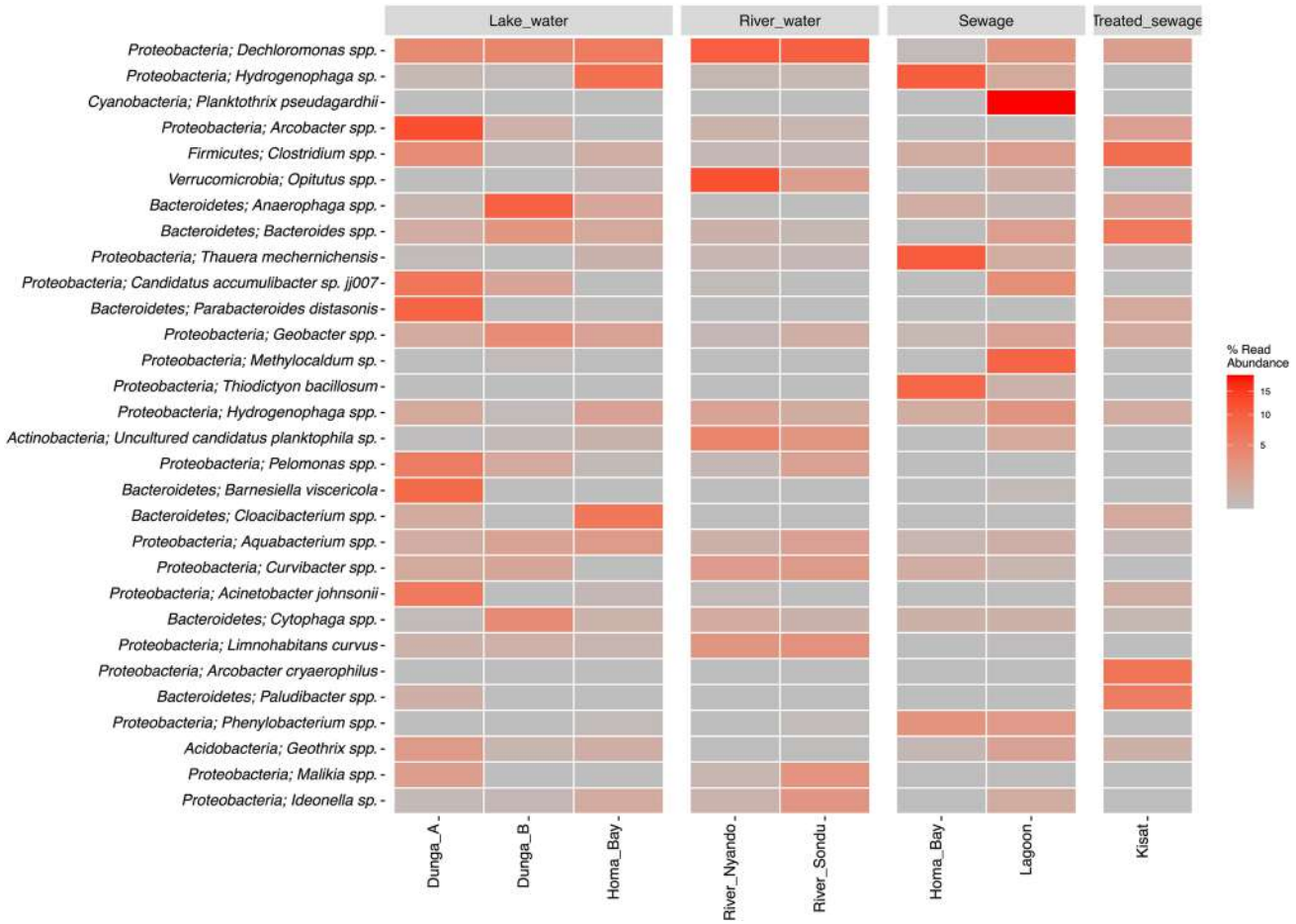

Fig. 2 Read abundance at species level across the eight samples. Read affiliated to phylum Proteobacteria were the most abundant (Color figure online)

WWTP) may be a result of nutrient loading. *Cyanobacteria* is a widely and ubiquitously distributed group and can be used as a bioindicator for monitoring eutrophication [37]. The high abundance of reads affiliated to Proteobacteria, followed by Bacteroidetes and Firmicutes is consistent with other studies on wastewater treatment [38]. The Classes *Betaproteobacteria* and *Alphaproteobacteria* groups were enriched in freshwater samples such as (J1 River Sondu). Their abundance in freshwater ecosystems is significantly influenced by pH and nutrients availability [39]. Bacterial communities enriched in WWTPs were mainly limited to *Gammaproteobacteria* and studies have reported their competence to perform denitrification process in biological wastewater treatment plants [40]. *Nitrospira* was reported from the study and is known to be a widespread nitrifying bacterium [41]. The relative abundance of *Nitrospira* decreased from 0.6% in sample J6 to 0.0% in samples J2, J4, J5 and J7. This is consistent with other findings where the abundance of *Nitrospira* has been reported to be negatively correlated to high ammonia concentrations [42]. The isolates recovered are affiliated to species known to exhibit efficient heterotrophic nitrification and aerobic denitrification capabilities. *Enterobacter cloacae* for example can simultaneously denitrify and remove phosphorus and also carry out heterotrophic nitrification and aerobic denitrification process [43]. *Klebsiella pneumonia* and *Klebsiella variicola* have been well documented with the ability to carry out coupled heterotrophic nitrification and aerobic denitrification for bioremediation of wastewater [44]. A recent study reported *Pseudomonas guguanensis* strain 4-n-1 with the ability to remove ammonium sulfate and *Pseudomonas guariconensis* revealed efficient denitrification [45]. *Pseudomonas putida* and *Pseudomonas alcaliphilia* have frequently been used in the bioremediation of contaminated water as potential heterotrophic nitrifiers and aerobic denitrifiers [18, 46, 47].

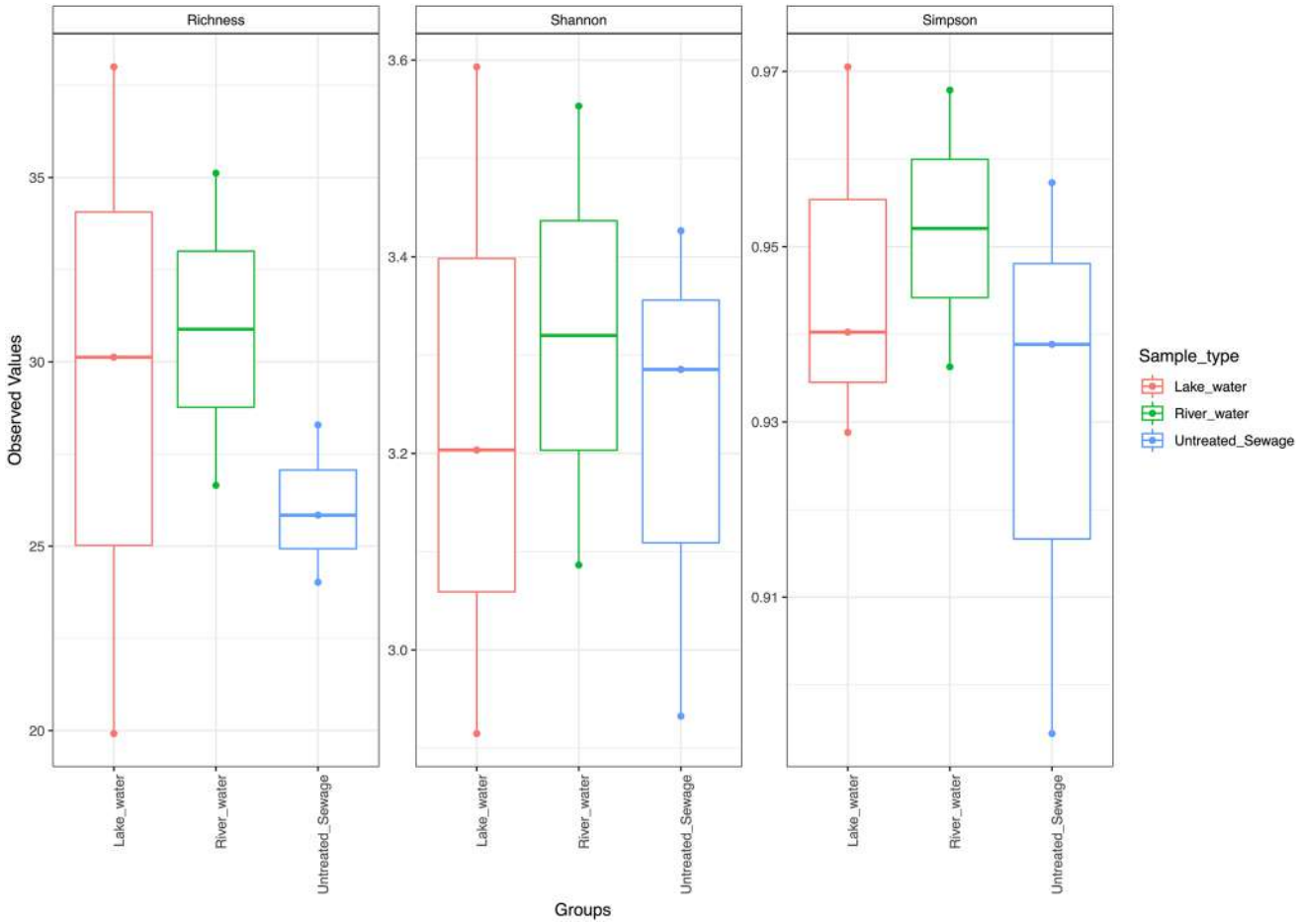

Fig. 3 Alpha diversity comparisons between different sample types and sampling sites. Statistical analysis was performed through three species richness estimators: Richness, Simpson, and Shannon diversity index (Color figure online)

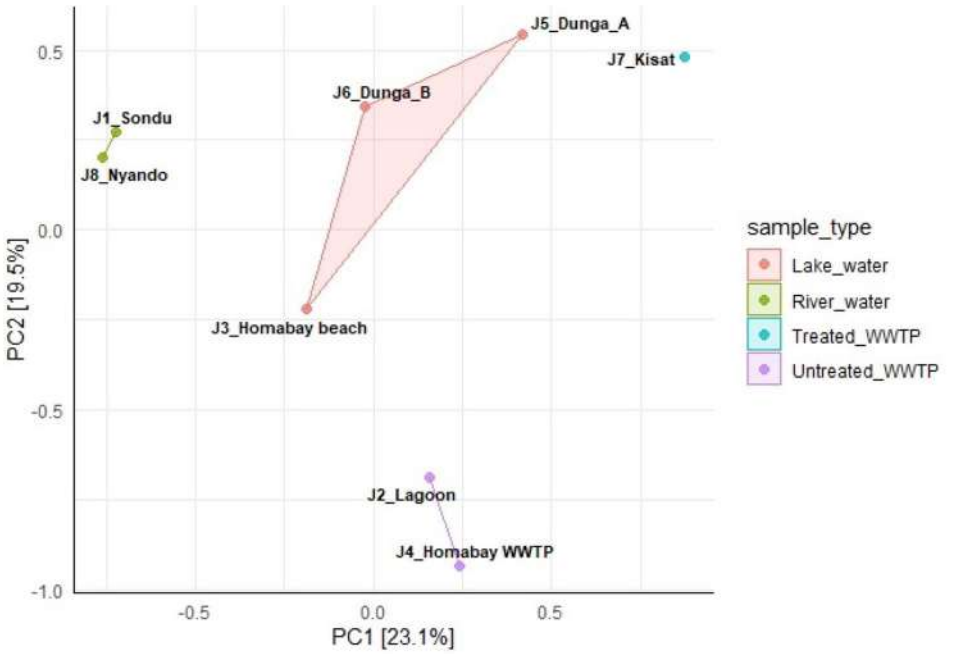

Fig. 4 Principal component analysis showing the ecological distance between sampling sites based on the microbial composition in each sample. Samples are plotted as points in the x/y-plane

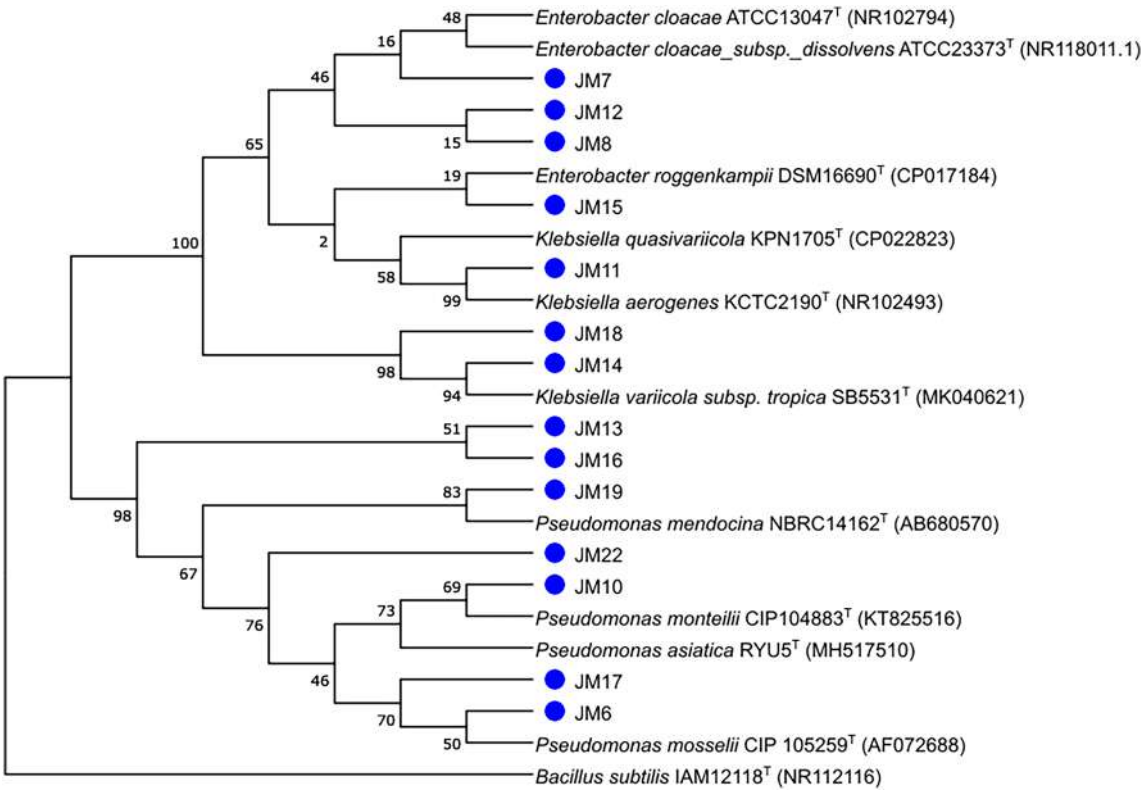

Fig. 5 Evolutionary relationships of taxa. The evolutionary history was inferred using the Neighbor-Joining method. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. Evolutionary analyses were conducted in MEGA X software and rooted using *Bacillus sp*. CK-8 as the out-group

Table 3 Isolates obtained through culture dependent methods and their similarities to known type strains

Sample name	Sample accession number	Related type strain	Type Strain accession number	Similarity to type strain	Sequence length (bp)
JM2	ON227428	*Klebsiella quasivariicola*	KPN1705	99.84	670
JM4	ON227429	*Pseudomonas citronellolis*	NBRC 103043	100	457
JM5	ON227430	*Pseudomonas mosselii*	CIP 105259	100	468
JM6	ON227431	*Pseudomonas mosselii*	CIP 105259	100	531
JM7	ON227432	*Enterobacter cloacae subsp. dissolvens*	LMG 2683	99.86	715
JM8	ON227433	*Enterobacter cloacae subsp. dissolvens*	LMG 2683	100	850
JM10	ON227417	*Pseudomonas monteilii*	NBRC 103158	100	788
JM11	ON227418	*Klebsiella aerogenes*	KCTC 2190	100	834
JM12	ON227419	*Enterobacter cloacae subsp. dissolvens*	LMG 2683	100	751
JM13	ON227420	*ATKM_s*	P818	100	759
JM14	ON227421	*Klebsiella variicola subsp. tropica*	SB5531	99.84	626
JM15	ON227422	*Enterobacter roggenkampii*	EN-117	100	660
JM16	ON227423	*ATKM_s*	P818	99.11	803
JM17	ON227424	*Pseudomonas mosselii*	CIP 105259	100	568
JM18	ON227425	*Klebsiella variicola subsp. tropica*	SB5531	99.87	801
JM19	ON227426	*Pseudomonas mendocina*	NBRC 14162	100	710
JM22	ON227427	*Pseudomonas asiatica*	RYU5	100	748

Conclusion

Proteobacteria was the richest phylum that was largely represented by *Betaproteobacteria, Alphaproteobacteria* and *Gammaproteobacteria*. *Alphaproteobacteria* and *Betaproteobacteria* were rich in freshwaters, unlike *Gammaproteobacteria* which dominated in the WWTPs. This study reveals a richer bacterial density in rivers and lake waters than WWTPs, and this could be attributed to elevated levels of organic and inorganic pollutants present in WWTPs. Based on culture-independent 16S rRNA gene sequencing, all isolated bacteria were closest matches to *Gammaproteobacteria.* Members of *Gammaproteobacteria* are well adapted to high environmental stressors and possess efficient biodegradation capacity. This unique feature makes *Gammaproteobacteria* more fit for the bioremediation of contaminated sites. The microbes isolated from these ecosystems can be maintained and exploited for the management of high nutrient loadings from contaminated wastewaters.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00284-022-02950-1.

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Author Contributions JMW did the experiments, data collection and wrote the manuscript. DM assisted in data collection. MT and CM designed the experiments, guided sample collection, and proofread the manuscript. RM-guided data analysis, manuscript writing and proofreading. All the authors read and approved the final manuscript.

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Data Availability The Raw sequence reads have been deposited into the SRA under the accession PRJNA824944. Sequence reads from the Illumina sequencing are under the Biosample SUB170771 while the isolate sequences are under the Biosample SUB11316194 (Accession numbers ON227417-ON227434).

Declarations

Conflict of interest The authors declare that there is no conflict of interest.

Consent for Publication The authors give consent for publication.

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