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Original Research

Comparing Bacterial Diversity in Two Full-Scale Enhanced Biological Phosphate Removal Reactors Using 16S Amplicon Pyrosequencing

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Abstract

Despite their stability and the widespread use of enhanced biological phosphorus removal (EBPR), little is known about their microbial composition and activity. In our study we investigated high-throughput pyrosequencing of bacterial communities from two full-scale EBPR reactors of South Africa. Findings indicated that both EBPRs harboured high bacterial similarity, ranging from 83 to 100% with a diverse community dominated by *Proteobacteria* (57.04 to 79.48% for failed EBPR and 61.7 to 85.39% for successful EBPR) throughout the five selected treatment zones with the exception of the fermenter (*Bacteroidetes*: 55.84%) from the successful EBPR. However, a lower dissimilarity was observed with the presence of 70 unique bacterial genera from successful EBPRs belonging to *Gammaproteobacteria*, *Betaproteobacteria*, and *Actinobacteria*, while 69 unique genera from failed EBPR belonged to *Alphaproteobacteria*, *Betaproteobacteria*, and *Clostridia*. The failed EBPR (54.58%) revealed less fermenting bacteria in the fermenter as compared to the successful EBPR (73.58%). More detrimental organisms and less nitrifying/denitrifying bacteria were also found in failed EBPR than in the successful EBPR, as well as phosphate-accumulating bacteria. Canonical correspondence analysis (CCA) displayed a very low relationship between microbial patterns, pH and DO – suggesting that these environmental factors played a major role in community dissimilarity. Aerobic zones appeared to have the highest dissimilarity between both EBPRs, with the failed EBPR predominated by *Acidovorax* (26.2%) and the successful EBPR with unclassified *Rhodocyclaceae* (37.24%). Furthermore, 21.47% of readings (failed EBPR) and 17.18% of readings (successful EBPR) could not be assigned to taxonomic classifications, highlighting the high diversity level of novel microbial species in such an environment.

Keywords: EBPR, microbial diversity, aerobic zone, anaerobic zone, pyrosequencing

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Background

Pollution of water sources is currently a global concern due to the detrimental effects of pollutants on both human and animal health [1]. In South Africa, like in many other developing countries, there is a problem with an influx of population into urban areas. This together with the growth of major cities and industrial activities has led to an increase in the municipal biomass waste in effluents being discharged into the environment with the contamination of water sources. Moreover, the volume of wastes that goes into wastewater treatment plants is enormous, while the treatment efficiency is hampered by a number of factors such as high capital and operating costs, which render some technologies unfeasible – primarily for application in rural areas [2].

To deal with this issue, several technologies have been developed and these include chemical precipitation, chemical oxidation, ion exchange, etc. Due to their advantages over the conventional methods, biological treatment methods such as enhanced biological phosphorus removal (EBPR) have been widely seen as the most effective and eco-friendly option of the available treatment processes of wastewater [3]. This process requires the presence of diverse microorganisms to remove organic contents and nutrients as well as other toxic materials from domestic and industrial wastewater and to protect receiving water bodies from eutrophication [4-5]. Although the EBPR process is regarded as one of the most successful for the removal of phosphorus from wastewater, the phosphorus removal capacity can also be prone to apparent instability and unreliability, and deterioration is often attributed to detrimental bacteria present in the system [4].

More than three decades ago, researchers made the first attempts to identify these microorganisms involved in EBPR based on culture-dependent techniques [6-7]. It has then been reported that the naturally occurring polyphosphate-accumulating organisms (PAOs) were responsible for phosphorus removal, whereas the presence of glycogen-accumulating organisms (GAOs) in the EBPR process was the main cause of EBPR deterioration as the latter also compete for volatile fatty acid (VFA) as a substrate for energy [5]. However, the advent of molecular tools showed that several pure cultures of PAOs such as *Acinetobacter*, *Microlunatus*, *Tetrasphaera*, and *Lampropedia* genera were not having great effect in full-scale wastewater treatment plants (WWTPs), and a high diversity of phylogenetic groups involved in the removal of phosphorus were present in EBPR sludge [8]. Furthermore, PAOs such as *Candidatus Accumulibacter phosphatis* and the actinobacterial genus *Tetrasphaera* that enable enhanced biological phosphorus removal in full-scale WWTPs have been identified and characterized [7, 9].

While it remains a challenge to identify and isolate the PAOs or GAOs in pure cultures using alternative molecular analyses, metagenomic analysis using next-generation sequencing appears to be more successful for characterizing the microbial ecology of the EBPR systems as it offers the possibility of studying the diversity of phylogenetic groups and the functional potential of a complex community [10]. Furthermore, their success in profiling microbial communities in a wider range has been used as a blueprint by which more studies can be designed and comprehensive hypotheses generated [11]. Recently, very few metagenomic studies using next-generation sequencing have been carried out on metagenomes from a full-scale EBPR [4, 10, 12-13]. In addition, very few studies have investigated the microbial community throughout all treatment zones of the EBPR. Since each EBPR appeared to be unique, this study investigated for the first time the microbial community of the targeted full-scale EBPRs using a pyrosequencing platform. The microbial diversity of the two full-scale EBPRs (Olifantsvlei Wastewater Treatment Works and the Bushkoppies Wastewater Treatment Works) was compared in order to provide a more detailed understanding of their relationships to operational variables and design in complex habitats. Despite the fact that Olifantsvlei Wastewater Treatment Works and the Bushkoppies Wastewater Treatment Works have been similarly designed with the use of activated sludge, BNR, extended aeration, and maturation ponds (except for the absence of a pre-anoxic zone at the latter, and also since these plants receive the same type of influents), the Bushkoppies Wastewater Treatment works showed consistent noncompliance as compared to the Olifantsvlei Wastewater Treatment Works.

Materials and Methods

Study Area and Wastewater Sample Collection

Wastewater samples were collected in April 2015 from two treatment plants, namely at the Olifantsvlei Wastewater Treatment Works (26.321864S, 27.901647E) and the Bushkoppies Wastewater Treatment Works (26.311111S, 27.935E) in Johannesburg. A comparison of these two treatment works was performed in order to assess the consistent noncompliance of the latter. These EBPRs use activated sludge, BNR, extended aeration, and maturation ponds, and receive the same type of influents and are subjected to the same climatic conditions and are configured as Johannesburg and anaerobic-oxic process reactors and combined in the secondary clarifier. The fermenter responsible for fermenting organic matter for producing VFA is a combined system that recycled the sludge back to each train (Fig. 1). Due to the above and for the sake of this study, the targeted plants were referred to as "failed unit" for Bushkoppies and "successful unit" for Olifantsvlei. Samples of 1 L were collected in sterile plastic sampling bottles in triplicate from the primary settling tank (PST), fermenter, anaerobic zone, anoxic zone, and aerobic zone, and immediately placed in a cooler box (4°C) for transportation to the laboratory for physicochemical analyses and microbial diversity

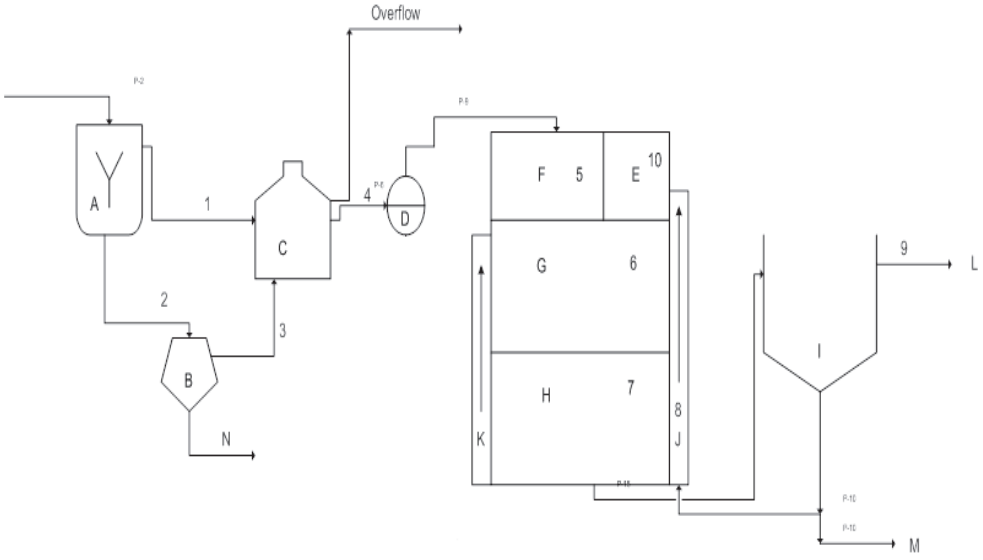

Fig. 1. Schematic design of the EBPR.
Legend: Successful EBPR
A: Primary settling tanks (2 x); B: Fermenter (2 tanks). Sludge from PST is pumped into one tank. Remains for 4 – 6 days. Waste out with settled sewerage from PST. Other tank is then filled up.; C: Balancing tank; D: Division box, out flow from balancing tank.; E: Pre-anoxic. Receives the RAS; F: Anaerobic zone. Receives outflow from balancing tank.; G: Anoxic zone. Receives recycle from aerobic zone; H: Aerobic zone; I: Secondary settling tank. Suction lift tanks (3 x). Difficult to control.; J: Return activated sludge (RAS) from the bottom of the SST's; K: Recycle stream from aerobic to anoxic.; L: Water effluent from SST goes to disinfection and river; M: Waste activated sludge. Wasted directly from aeration tank.; N: Anaerobic digestion.
Failed EBPR:
B: Fermenter, Receives sludge from the PST. Kept it settle for 4 days. E: Pre-anoxic not present, F: Anaerobic zone. Excessive "bubbling" where the water enters the zone.; I: Secondary settling tank. Mechanical scrapped tanks. Removes sludge better.

studies. No specific permit was needed for collecting the wastewater samples in the described sample area, and this study did not involve endangered or protected species. However, responsible officers with informed consent assisted with the collection of wastewater samples. In order to assess environmental variables, samples were homogenously mixed and filtered using No. 1 filter paper (Whatman). The filtered samples were then used to determine chemical oxygen demand (COD), nitrate, and phosphate. To test for NO_3^{-1}, the sodium salicylate method was used as reported by Monteiro et al. [14]. Briefly, 50 ml of the samples were pippeted into a ml beaker and mixed with 1 ml of the salicylate solution. The mixture was dried in an oven at 105°C to allow the formation of NO_2^{+1} from NO_3^{-1}. Then 1 ml of sulfuric acid was added and allowed to cool for 10 min, and 7 ml of the solution containing sodium hydroxide and sodium and potassium tartrate were later added. The solution was later made up with water and analyzed in a spectrophometer [14]. For PO_4^{-3} we used the 424f standard method as reported by APHA [15]. 424f uses ammonium molybdate and potassium antimonyl tartrate in order to react in an acidic medium with orthophosphate to form a heteropoly acid (phosphomolybdic acid) that is reduced to intensely coloured molybdenum blue by ascorbic acid. The closed reflux method was also used to measure COD concentration [15], whereas pH, DO, electrical conductivity (EC), and temperature were measured on-site using specific probes (HACH, Germany).

Metagenomic DNA Extraction, Amplification, and Pyrosequencing of Bacterial 16S RNA Genes

For microbial analysis, unfiltered samples were homogenously mixed and a 50 mL aliquot was taken and centrifuged at 10,000 x g for five minutes at 4ºC. The harvested cell pellets were re-suspended in 1x TE buffer (pH 8.0). The suspensions were well mixed and microbial DNA was extracted with the ZR Fungal/Bacterial DNA Kit™ (Zymo Research Corporation, USA) according to the procedures provided by the manufacturer. In order to monitor/assess for any contamination, a series of negative controls containing no DNA templates was also used throughout this experiment. The integrity and purity of the metagenomic DNA was later assessed on the 0.8% agarose gel and NanoDrop™ spectrophotometer (NanoDrop™ 2000, Thermo Scientific, Japan). The PCR reaction was performed on all the extracted metagenomic DNA samples as well as negative controls using the universal primer 27F and 518R [5]. This primer pair amplifies approximately the 500 bp of 16S rRNA gene sequence targeting variable region V1 to V3 of the 16S rRNA gene. Each PCR reaction contained 25 µL of 2X DreamTaq Green Master Mix (DNA polymerase, dNTPs and 4 mM MgCl2), 22 µL of nuclease-free water, 1 µL of forward primer (0.2 µM), 1µL of reverse primer (0.2 µM), and 1µL of metagenomic DNA (50-100 ng μL^{-1}) to make up a volume of 50 µL.

All PCR reactions were performed on ice to minimize non-specific amplification and primer dimerization. The PCR cycle started with an initial denaturation step at 94ºC for five minutes, followed by 30 cycles of denaturation at 94ºC for one minute, annealing at 55ºC for 30 seconds and extension at 72ºC for one minute and 30 seconds, and a final extension at 72ºC for 10 minutes, followed by cooling to 4ºC. The PCR product (10 µL) was loaded in 1% (m/v) agarose gel (Merck, South Africa) stained with 5% of 10 mg mL^{-1} ethidium bromide (Merck, South Africa) and visualised under an ultraviolet transilluminator (InGenius Bio Imaging System, Syngene, Cambridge, UK). The correct PCR amplicons were excised and purified using the DNA clean and concentrator kits (Zymo Research Corporation, USA). The purified DNA samples were then quantified using a NanoDrop spectrophotometer. The used primers contained the appropriate adaptor and barcode sequences necessary for running the samples on the GS-FLX-Titanium (Roche). Prior to the paired-end pyrosequencing services provided by Inqaba Biotechnology Industries (South Africa) using the GS-FLX-Titanium series (Roche, Switzerland), replicates for respective samples were pooled together at approximately equimolar concentrations based on the library concentrations and calculated amplicon sizes.

Processing of Pyrosequencing Data and Statistical Analysis

The raw sequences generated from pyrosequencing were processed in a Mothur pipeline for quality control (QC) to remove artificial replicate sequences produced by sequencing artifacts (primers, barcodes, adaptor sequences, etc.) and low-quality sequences [16]. Sequences that passed QC were further pre-screened for identification of ribosomal readings (at least 80% identity) and removal of non-ribosomal readings using qiime-uclust and the following RNA databases: Greengenes, LSU, SSU, and RDP. Moreover, chimeric sequences were identified from ribosomal sequences and removed using UCHIME according to the *de novo* method [17]. The non-chimeric rRNA sequences obtained were analyzed using the Ribosomal Database Project (RDP) Classifier tool of RDP pyrosequencing pipeline [18] and sequences were classified with a 97% confidence threshold. These meta-sequences were later aligned using the RDP align tool in the same pipeline, and the cluster files were generated for each sample with the RDP complete linkage clustering tool. Furthermore, the rarefaction curves were created by using the RDP rarefaction tool. Genetic distance was determined and sequences clustered into operational taxonomic units (OTUs) at 3% genetic distance using the nearest-neighbour method, and a representative sequence was chosen for each cluster/OTU at each distance. The choice of nucleotide cutoff in this study was based on Brown et al. [12], who reported that the bacterial diversity could decrease with an increase of cutoff. Furthermore, the default mismatch cutoff at 3% for cdhit-454 was also set in order to recover approximately 95% of readings. Since increasing the cutoff strongly affects the overall number of readings assigned to a specific taxon, in this study the default genetic distance cutoff of 3% was used. The Shanon diversity index and the Chao1 richness estimator were calculated for each sample. The relative abundance (%) of individual taxa within each community was calculated by comparing the number of sequences assigned to a specific taxon against the number of total sequences obtained for that sample. The similarity and dissimilarity in bacterial community structure within both wastewater treatment plants was analyzed using the Jaccard-Sørensen index [18]. Statistically, we should indicate that both indexes can be shown as similarity or dissimilarity indexes. Canonical correspondence analysis (CCA) for the environmental parameters was also performed to explore the interrelationships between them and their possible effect on a bacterial community. PAST 2.06 statistical software was used to generate CCA bi-plots [19]. Generated data was later made publicly available at the DDBJ Sequence Read Archive (DRA) under the accession number PRJDB4407.

Results and Discussion

Sequencing

The enhanced biological phosphorus removal processes have been seen widely as the most reliable approach for the treatment of wastewater generated from both municipal and industrial activities [20]. Among its microbial population, bacteria have been reported to play the most essential and critical role for the removal of pollutants [21]. In the present study, the bacterial community structure and species richness, from five zones of two wastewater treatment plants situated in Johannesburg, were analyzed using pyrosequencing of the 16S rRNA gene. Extracted rDNA was found to be of high quality, with A260/280 ratios of approximately 1.8 for all samples. As activated sludge samples are seen as complex samples due to the presence of several inhibitors, the purity, integrity, and size of the rDNA from such a matrix are critical and play a major role in the specific yield (rDNA/g sample) of rDNA by later impacting the accuracy of the results of metagenomic studies, especially in high-diversity communities [21-22]. When investigating the microbial community of a full-scale EBPR, Albertsen et al. [4] revealed that some microbial phyla such as *Actinobacteria* (*Tetra-sphaera*) and *Chloroflexi* have been underrepresented from the metagenome datasets due to the DNA extraction bias.

Pyrosequencing of DNA amplicons from high-quality rDNA generated a total range of 1,120 to 2,436 readings from treatment zones (aerobic, anoxic, anaerobic, fermenter, and PST) with Anoxic A (2,030 readings) and Anaerobic B (2,436 readings) having the highest readings from both failed and successful EBPRs, respectively (Table 1).

Table 1. Sequencing statistics from wastewater samples.

WWTWs	Sampling zone	No. of seq.	Average length (bp)	QC passed		rRNA seq		No. of non-chimeric seq.	
				No.	%	No.	%	No.	%
Failed EBPR	Aerobic A	1,950	270.59±158.18	1,433	73.49	1,180	60.51	1,152	59.08
	Anoxic A	2,030	329.17±188.14	1,807	89.01	1,530	75.37	1,227	60.44
	Anaerobic A	1,908	339.27±186.92	1,658	86.9	1,420	74.42	1,118	58.60
	Fermenter A	1,705	341.84±192.90	1,544	90.56	1,375	80.65	1,121	65.75
	PSTA	1,120	274.19±152.80	872	77.86	823	73.48	736	65.71
Successful EBPR	Aerobic B	1,750	318.32±187.47	1,568	89.6	1,360	77.71	1,217	69.54
	Anoxic B	1,876	316.83±185.12	1,682	89.66	1,523	81.18	1,166	62.15
	Anaerobic B	2,436	303.63±181.97	2,203	90.44	1,790	73.48	1,556	63.88
	Fermenter B	1,748	366.81±187.09	1,610	92.11	1,496	85.58	1,217	69.62
	PSTB	1,269	272.70±162.78	978	77.07	976	76.91	810	63.83

Note: All percentages of sequences refer to the total sequences of each sampling site.

Of the obtained readings, 76.17-90.56% were for failed the EBPR and 77.07-92.11% were for the successful EBPR and were able to meet the quality criteria (QC). Sequences up to 80.65% from the failed EBPR and 85.58% from the successful EBPR were found to be of ribosomal origin. The ribosomal sequences were then analyzed for the presence of chimeric sequences, and 520-1,227 and 810-1,556 high-quality non-chimeric rRNA readings

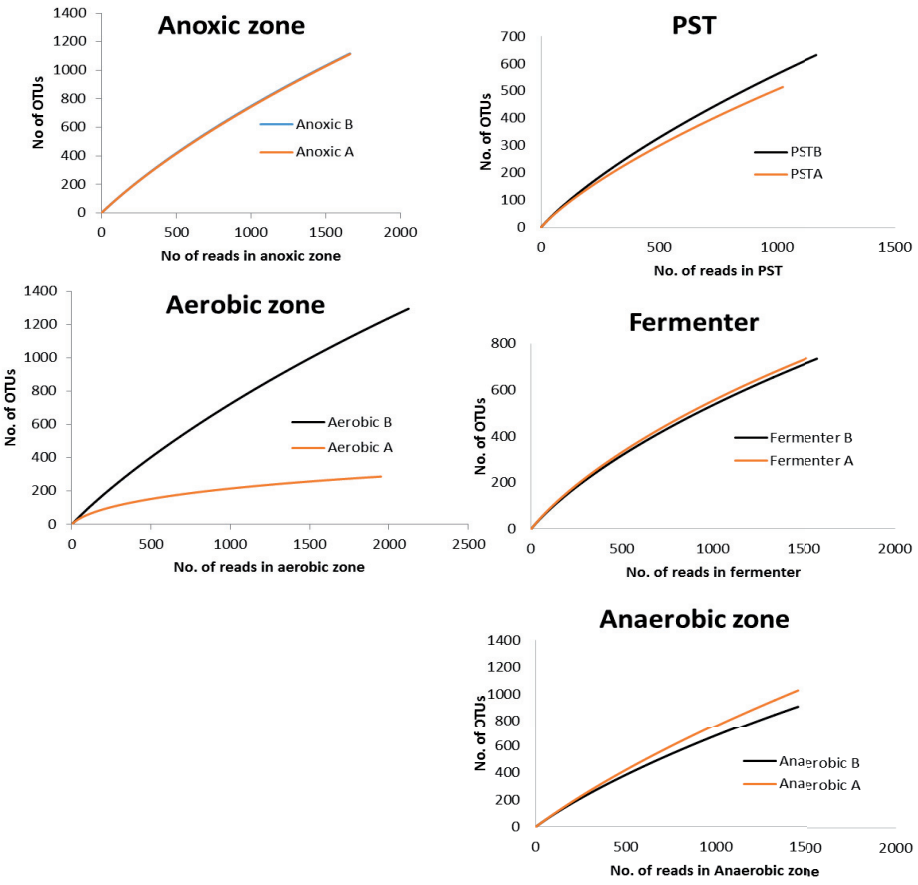

Fig. 2. Rarefaction curves dissimilarity levels at failed EBPR (Aerobic A, Anoxic A, Anaerobic A, Fermenter A, and PSTA) and successful EBPR (Aerobic B, Anoxic B, Anaerobic B, Fermenter B, and PSTB)

Table 2. Diversity indices from 10 sampling points of two wastewater treatment works.

	Samples	OTU	Shannon index	Chao1
Failed EBPR	Aerobic A	287	5.36	1,025.14
	Anoxic A	1,216	6.84	3,526.05
	Anaerobic A	1,195	6.86	3,871.12
	Fermenter A	737	6.12	1,672.36
	PSTA	516	5.64	1,533.9
Successful EBPR	Aerobic B	1,296	6.83	3,496.38
	Anoxic B	1,118	6.8	3,519.88
	Anaerobic B	907	6.48	2,661.09
	Fermenter B	735	5.95	1,749.55
	PSTB	633	5.94	1,965.66

from failed and successful EBPRs respectively were obtained and considered for further analysis. All non-chimeric readings of an average between 270.59±158.18 bp and 366.81±187.09 bp were successfully classified at the domain level. Previous studies further revealed that the accurate classification of 16S rDNA sequences to the perceived diversity of the microbial community is strongly dependent on the high quality of the 16S database [11, 23], which in return can be influenced by sample preparation, primer selection, and the formation of chimeric 16S amplification products [24].

Diversity Indices and Community Species Richness

During the last decade, studies on diversity level have reported the potential positive correlation between biodiversity and micro-ecosystem function [25]. It has also been stated that the stability of a micro-ecosystem function increases as biodiversity increases [26]. According to Saikaly et al. [27], minimum species richness is needed in order to maintain ecosystem function in a particular environment, while a large microbial diversity and species richness is required in order to maintain a stable micro-ecosystem in a varying environment. In this study, the RDP pyrosequencing pipeline was used to determine a bacterial community from two EBPRs by assigning the read tags to different operational taxonomic units (OTUs) at 3% nucleotide cutoff. As the number of OTUs gives an approximation of species diversity in a sample [28], a total of 8,640 OTUs were recovered from the 10 sampling points. It was noted that the successful wastewater treatment plant had the highest OTUs of 1,296 at an aerobic zone (aerobic B). Regardless of the fact that the successful EBPR was having more OTUs and readings than the failed EBPR, the rarefaction analysis has revealed no significant difference in the number of readings and OTUs at the same sampling zone of both EBPRs. This indicates a similar level of diversity with the exception of the aerobic zone, where the level of bacterial diversity was apparently not similar. Furthermore, the rarefaction curves showing the plot of number of readings versus the number of OTUs is illustrated in Fig. 2.

In order to ascertain microbial complexity within each sampling point, the Shannon-Weaver index and Chao1 richness estimator were determined at 3% cutoff (Table 2). The Shannon-Weaver diversity index ranges from 0 (or 0%), indicating pronounced dominance, to 1 (or 100%), indicating equal abundance of all species [29]. In the present study, the diversity index (Shannon) revealed a very close level of diversity between both wastewater treatment plants with 30.82 for failed EBPR and 32 for successful EBPR. Furthermore, the diversity index within individual sampling points appeared not to

Table S1 Pairwise bacterial community similarity between the sampling points using Jaccard index (light grey) and Sørensen index (dark grey) at 3% nucleotide cutoff level

	Aerobic _A	Aerobic _B	Anaerobic _A	Anaerobic _B	Anoxic _A	Anoxic _B	Fermenter _A	Fermenter _B	PST_A	PST_B
Aerobic_A	0.00	96	66	99	93	98	97	100	94	96
Aerobic_B	98	0.00	94	86	90	84	99	99	94	48
Anaerobic_A	80	97	0.00	91	83	86	98	99	95	98
Anaerobic_B	99	93	95	0.00	95	74	99	100	93	98
Anoxic_A	96	95	90	97	0.00	83	98	98	95	99
Anoxic_B	99	91	92	85	91	0.00	98	99	100	95
Fermenter_A	98	100	99	100	99	99	0.00	91	98	99
Fermenter_B	100	100	100	100	99	99	95	0.00	95	99
PST_A	97	97	97	97	97	100	99	98	0.00	95
PST_B	98	65	99	99	99	98	100	100	97	0.00

Note: values are expressed in percentage (%)

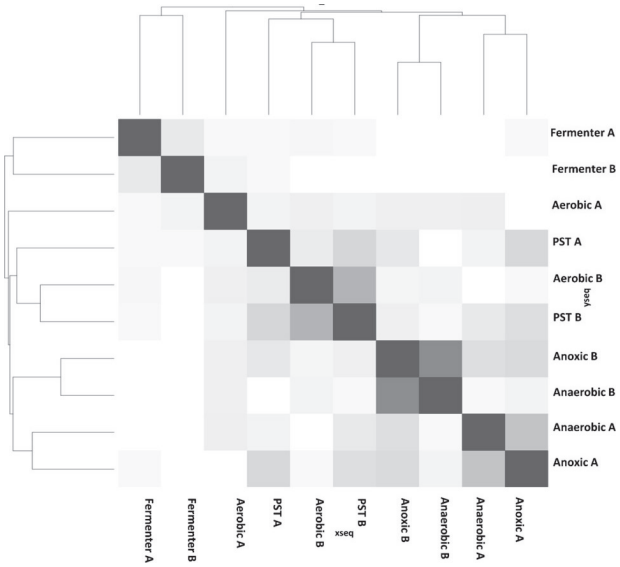

Fig. 3. Heat map generated from Sørensen index highlighting the similarity at 3% cutoff level among the 10 samples collected from both EBPR treatment plants. The higher the colour intensity, the higher the bacterial similarity between the pair.

be significantly different ($p>0.05$) within both successful and failed EBPRs.

However, the species richness from both plants was also determined using the Chao1 estimator at 3% nucleotide cutoff. Similar to the Shannon index, the observed and estimated richness index indicated higher bacterial richness at 3% cutoff at the successful EBPR when compared to the failed EBPR. The Chao1 richness estimator revealed a species richness ranging between 1,025.14 and 3,871.12, and 1,749.55 and 3,519.88 from failed and successful EBPR, respectively. In the failed wastewater treatment plant, the anaerobic zone appeared to have the highest bacterial species richness, whereas in the successful wastewater treatment plant, the anoxic zone had the highest bacterial species richness. The pairwise bacterial community similarity between the sampling points of each wastewater treatment plant was confirmed using the Jaccard and Sørensen index based on the presence and absence of each OTU (Table S1). The Jaccard and Sørensen index revealed no or slightly similar levels of diversity between each pair of the same sampling points and between both plants.

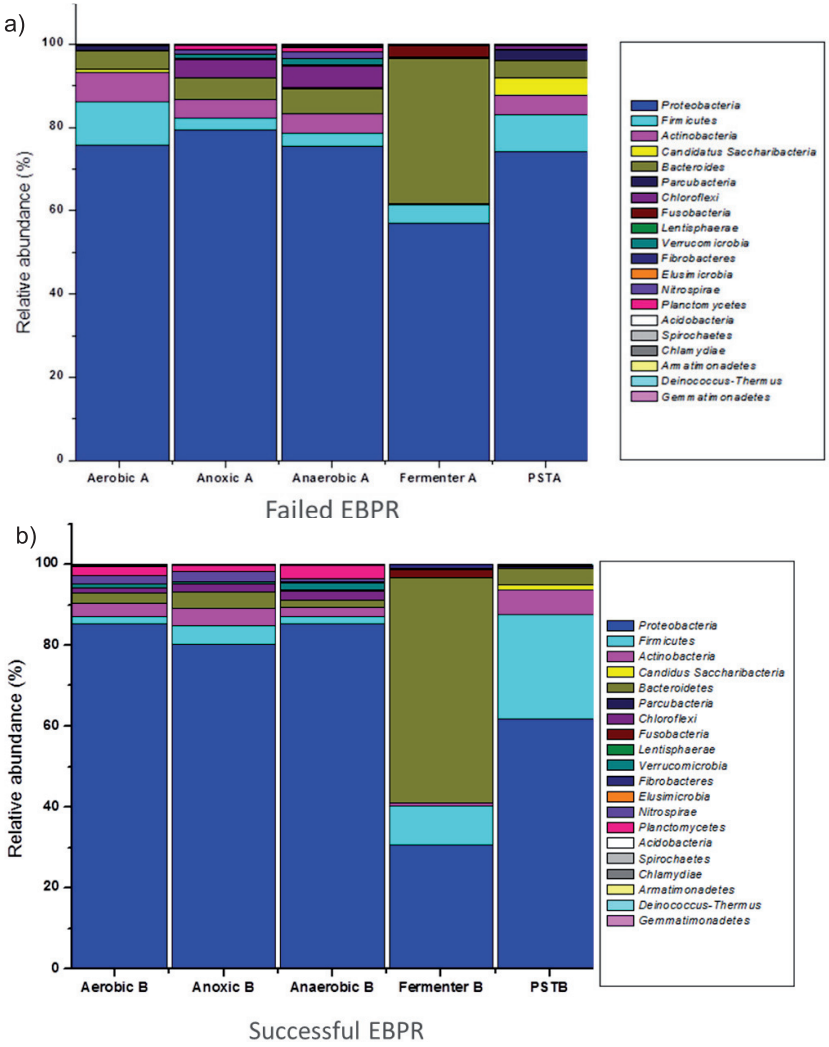

Fig. 4. Taxonomic distribution of different bacterial phyla in both treatment plants from the Johannesburg area: a) the zone belonging to failed EBPR and b) the zone belonging to the successful EBPR.

Table S2. Unifrac metric p values.

	Aerobic _A	Aerobic _B	Anaerobic _A	Anaerobic _B	Anoxic _A	Anoxic _B	Fermenter _A	Fermenter _B	PST_A	PST_B
Aerobic_A		0.02	0.09	0.05	0.04	0.01	**0.00**	0.02	0.05	0.01
Aerobic_B	0.02		0.04	0.01	0.30	0.03	0.03	0.03	0.01	0.07
Anaerobic_A	0.09	0.04		0.01	0.02	0.02	0.03	0.02	0.01	0.03
Anaerobic_B	0.05	0.01	0.01		0.02	**0.00**	0.03	0.04	**0.00**	0.03
Anoxic_A	0.04	0.30	0.02	0.02		0.01	0.03	0.03	0.02	0.02
Anoxic_B	0.01	0.03	0.02	**0.00**	0.01		0.04	0.04	0.02	0.03
Fermenter_A	**0.00**	0.03	0.03	0.03	0.03	0.04		**0.00**	0.01	0.02
Fermenter_B	0.02	0.03	0.02	0.04	0.03	0.04	**0.00**		0.01	0.03
PST_A	0.05	0.01	0.01	**0.00**	0.02	0.02	0.01	0.01		0.03
PST_B	0.01	0.07	0.03	0.03	0.02	0.03	0.02	0.03	0.03	

Similar observation was noted when comparing sampling zones in the order the wastewater flows through EBPR treatment process. Most of the Sørensen values from each pair were closely ranged 83-100% with the exception of the pairs anaerobic B and anoxic B, anaerobic A and aerobic A, and PST B and aerobic B, which were found

Table S3 Relative abundance (%) of major phylogenetic groups within treatment zones of each wastewater treatment plant (EBPR)

	Failed EBPR					Successful EBPR				
Name	Aerobic A	Anoxic A	Anaerobic A	Fermenter A	PSTA	Aerobic B	Anoxic B	Anaerobic B	Fermenter B	PSTB
Firmicutes	10.48	2.81	3.13	4.48	9.07	1.81	4.58	1.74	9.67	26.03
Proteobacteria	75.81	79.48	75.42	57.04	74.09	85.39	80.29	85.36	30.7	61.7
Actinobacteria	6.85	4.36	4.81	0.24	4.53	3.11	4.2	2.23	0.54	5.96
Candidatus Saccharibacteria	0.81	0.07	0.00	0.00	4.15	0.11	0.00	0.17	0	1.15
Bacteroidetes	4.44	5.27	6.03	34.91	4.27	2.49	4.05	1.74	55.84	4.24
Parcubacteria	1.21	0.00	0.08	0.08	2.46	0.06	0.00	0.00	0.00	0.34
Chloroflexi	0.2	4.43	5.34	0.00	1.04	1.13	2.06	2.23	0	0.23
Fusobacteria	0.2	0.14	0.15	2.93	0.13	0.11	0.15	0.17	2.09	0.11
Lentisphaerae	0.00	0.00	0.00	0.08	0.00	0.00	0.00	0.00	0.08	0.00
Verrucomicrobia	0.00	1.05	1.53	0.16	0.13	0.91	0.31	1.9	0.08	0.00
Fibrobacteres	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.08	1.01	0.00
Elusimicrobia	0.00	0.00	0.00	0.00	0.13	0.00	0.08	0.00	0.00	0.00
Nitrospirae	0.00	1.19	1.53	0.00	0.00	2.21	2.37	0.83	0.00	0.00
Planctomycetes	0.00	0.91	1.15	0.00	0.00	2.21	1.53	3.31	0.00	0.11
Acidobacteria	0.00	0.07	0.31	0.00	0.00	0.34	0.08	0.17	0.00	0.00
Spirochaetes	0.00	0.00	0.31	0.00	0.00	0.00	0.15	0.00	0.00	0.00
Chlamydiae	0.00	0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Armatimonadetes	0.00	0.07	0.08	0.00	0.00	0.00	0.08	0.08	0.00	0.00
Deinococcus-Thermus	0.00	0.07	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.11
Gemmatimonadetes	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table S4A. Abundances of all class in each sample of the Failed EBPR. The abundance is presented in terms of percentages of the total sequences in a sample.

Name	Aerobic_A	Anaerobic_A	Anoxic_A	Fermenter_A	PST_A
Betaproteobacteria	44.35	40.41	45.01	13.45	44.24
Alphaproteobacteria	12.11	5.42	5.34	0.57	11.79
Gammaproteobacteria	14.37	4.74	6.05	30.07	12.48
Epsilonproteobacteria	0.21	0.61	0.14	3.83	0.28
Deltaproteobacteria	0.00	4.81	3.02	0.00	0.28
Actinobacteria	6.98	4.81	4.36	0.24	4.72
Bacilli	0.41	0.00	0.07	0.24	0.83
Erysipelotrichia	0.41	0.00	0.28	0.00	0.28
Clostridia	9.24	2.83	2.11	2.85	8.18
Negativicutes	0.00	0.15	0.14	1.22	0.00
Flavobacteriia	4.31	0.53	0.91	1.30	3.19
Sphingobacteriia	0.21	0.92	0.98	0.00	0.00
Cytophagia	0.00	0.31	0.07	0.00	0.00
Bacteroidetes_incertae_sedis	0.00	0.08	0.00	0.00	0.00
Bacteroidia	0.00	0.38	0.14	23.15	0.00
Fusobacteriia	0.21	0.15	0.14	2.85	0.14
Anaerolineae	0.21	4.51	4.15	0.00	0.55
Caldilineae	0.00	0.38	0.07	0.00	0.42
Chloroflexia	0.00	0.23	0.07	0.00	0.14
Nitrospira	0.00	1.53	1.20	0.00	0.00
Planctomycetia	0.00	1.15	0.91	0.00	0.00
Verrucomicrobiae	0.00	0.38	0.07	0.00	0.14
Subdivision3	0.00	0.38	0.07	0.00	0.00
Opitutae	0.00	0.61	0.77	0.16	0.00
Acidobacteria_Gp4	0.00	0.08	0.00	0.00	0.00
Holophagae	0.00	0.15	0.00	0.00	0.00
Acidobacteria_Gp3	0.00	0.00	0.07	0.00	0.00
Spirochaetia	0.00	0.31	0.00	0.00	0.00
Chlamydiia	0.00	0.15	0.00	0.00	0.00
Fimbriimonadia	0.00	0.00	0.07	0.00	0.00
Deinococci	0.00	0.00	0.07	0.00	0.00
Gemmatimonadetes	0.00	0.00	0.07	0.00	0.00
Lentisphaeria	0.00	0.00	0.00	0.08	0.00
Chloroplast	0.00	0.00	0.00	0.08	0.00
Elusimicrobia	0.00	0.00	0.00	0.00	0.14
unclassified_*Firmicutes*	0.62	0.15	0.21	0.16	0.55
unclassified_*"Proteobacteria"*	6.37	19.48	19.97	9.21	10.12
unclassified_*"Bacteroidetes"*	0.00	3.90	3.16	10.51	1.39
unclassified_*"Acidobacteria"*	0.00	0.08	0.00	0.00	0.00

Table S4A. Continued.

unclassified_*"Chloroflexi"*	0.00	0.23	0.14	0.00	0.00
unclassified_*"Verrucomicrobia"*	0.00	0.15	0.14	0.00	0.00
unclassified_*"Actinobacteria"*	0.00	0.00	0.00	0.00	0.14

Table S4B. Abundances of all class in each sample of the Successful EBPR. The abundance is presented in terms of percentages of the total sequences in a sample.

Name	Aerobic_B	Anaerobic_B	Anoxic_B	Fermenter_B	PST_B
Planctomycetia	2.21	3.24	1.53	0.00	0.12
Phycisphaerae	0.00	0.08	0.00	0.00	0.00
Betaproteobacteria	45.74	61.41	37.23	8.19	35.39
Gammaproteobacteria	14.98	4.56	19.27	16.23	14.78
Alphaproteobacteria	6.24	1.16	4.43	0.31	5.59
Deltaproteobacteria	1.65	2.57	1.76	0.08	0.00
Epsilonproteobacteria	0.00	0.08	0.92	1.16	0.00
Anaerolineae	0.91	1.83	1.68	0.00	0.00
Caldilineae	0.11	0.33	0.31	0.00	0.23
Chloroflexia	0.00	0.00	0.08	0.00	0.00
Fusobacteriia	0.11	0.17	0.15	2.09	0.12
Flavobacteriia	0.34	0.17	0.38	0.77	3.49
Sphingobacteriia	0.34	0.41	0.23	0.00	0.58
Bacteroidia	0.11	0.17	1.30	38.49	0.00
Bacteroidetes_incertae_sedis	0.11	0.00	0.08	0.08	0.00
Cytophagia	0.00	0.00	0.23	0.00	0.00
Nitrospira	2.21	0.83	2.37	0.00	0.00
Actinobacteria	3.06	2.16	4.20	0.54	5.94
Subdivision3	0.23	0.25	0.08	0.00	0.00
Verrucomicrobiae	0.28	0.25	0.15	0.00	0.00
Opitutae	0.34	1.24	0.08	0.08	0.00
Acidobacteria_Gp3	0.11	0.00	0.08	0.00	0.00
Acidobacteria_Gp6	0.06	0.00	0.00	0.00	0.00
Acidobacteria_Gp4	0.00	0.17	0.00	0.00	0.00
Clostridia	1.53	1.66	3.44	7.81	22.12
Bacilli	0.11	0.00	0.15	0.00	1.51
Erysipelotrichia	0.06	0.08	0.08	0.15	1.16
Negativicutes	0.00	0.00	0.31	1.08	0.00
Deinococci	0.06	0.00	0.00	0.00	0.12
Fibrobacteria	0.00	0.08	0.08	1.00	0.00
Armatimonadia	0.00	0.08	0.00	0.00	0.00
Elusimicrobia	0.00	0.00	0.08	0.00	0.00
Spirochaetia	0.00	0.00	0.15	0.00	0.00

Table S4B. Continued.

Lentisphaeria	0.00	0.00	0.00	0.08	0.00
unclassified_"*Proteobacteria*"	16.91	15.68	16.67	4.71	6.87
unclassified_"*Bacteroidetes*"	1.65	1.00	1.83	16.54	0.23
unclassified_"*Chloroflexi*"	0.11	0.08	0.00	0.00	0.00
unclassified_"*Acidobacteria*"	0.17	0.00	0.00	0.00	0.00
unclassified_"*Verrucomicrobia*"	0.06	0.17	0.00	0.00	0.00
unclassified_*Firmicutes*	0.11	0.00	0.61	0.62	1.63
unclassified_"*Actinobacteria*"	0.06	0.08	0.00	0.00	0.12
unclassified_"*Armatimonadetes*"	0.00	0.00	0.08	0.00	0.00

to be below 80%, highlighting a slightly similar level of diversity between pairs (Fig. 3). Similarly, the Jaccard values also showed results ranging 65-100%. Another statistical analysis using the UniFrac metric analysis was performed to further analyse the similarity between samples by taking into consideration not only the presence or abundance of OTUs, but also the bacterial phylogeny [30]. Similarly to the Jaccard and Sørensen index, UniFrac metrics revealed a high significant shift ($p>0.05$) among the microbial community of sampling zones with the exception of anaerobic_A/aerobic_A and PST_B / aerobic_B, highlighting no or slightly similar levels of diversity (Table S2). This finding disagrees with the results by Zhang et al. [31], who reported a similar level of diversity between the bacterial structure and activity of 14 EBPR. Lawson et al. [32] also stated that functional similarity in several EBPRs is mostly translated by the number of OTUs having similar genus-level affiliation. A significant shift of the dominant microbial community highlighting high dissimilarity between anaerobic-anoxic has also been reported by Lv et al. [21] when comparing the bacterial community in EBPRs.

Bacterial Community Structure in Both Wastewater Treatment Plants

In order to comparatively analyse the bacterial community from the two different wastewater treatment plants, the RDP classifier was used to assign the readings to specific phylogenetic bacterial taxa. The results showed 19 bacterial phyla with the exception of unclassified bacterial readings from both failed and successful EBPRs. Both EBPRs had 17 shared phyla and two unique phyla for failed EBPRs (*Chlamydiae and Gemmatimonadetes*) and two unique phyla for successful EBPRs (*Microgenomates and Fibrobacteres*) (Table S3, Fig. 4). The observed unique phyla have similarly been reported elsewhere in low proportions in activated sludge [5, 8, 32]. In terms of their abundance in both plants separately, five phyla – *Proteobacteria* (72.37%), *Bacteriodetes*, *Firmicutes*, *Actinobacteria*, and *Chloroflexi* – occupied approximately 95.71% of all classified bacteria from the failed EBPR, while in the successful EBPR *Proteobacteria*, *Bacteriodetes*, *Firmicutes*, *Actinobacteria*, and *Planctomycetes* were the most abundant with approximately 95.77% of the entire classified bacterial population (Fig. 3). This study is in agreement with Tian et al. [32], who also reported the predominance of *Proteobacteria*, *Bacteriodetes*, *Firmicutes*, *Actinobacteria*, and *Planctomycetes* in wastewater treatment plants containing approximately 51 phyla and 900 genera. Bond et al. [8], in their study investigating the bacterial community structures of phosphate-removing and non-phosphate-removing activated sludges from sequencing batch reactors, also revealed that *Proteobacteria, Planctomycete, Flexibacter, Cytophaga, and Bacteroides* were the dominant microbial groups.

The presence of *Proteobacteria* in EBPR as the most predominant phylum followed by *Bacteriodetes* as the second most predominant phylum has also been reported by Albertsen et al. [4]. This has been confirmed by Ye and Zhang [20], who reported *Proteobacteria* as the predominant bacterial phylum in several activated sludge samples. Furthermore, the predominance of *Bacteriodetes* over *Proteobacteria* in EBPR or activated sludge has not been intensively reported [32-33].

It was also noted that the bacterial community from both plants become more diverse as the sequences were classified into lower taxonomic levels (Table S4-7). Despite the high diversity, many readings could not be classified into a lower taxonomic level. This could be explained as Jin et al. [34] reported that up to 80-90% of microorganisms in activated sludge cannot be cultured and that the sludge harbours a community of largely uninvestigated novel species. In the present study, a total of approximately 49.64% of readings and 58.52% of readings from failed and successful EBPR plants, respectively, could not be classified up to a genus level (Table S3-S7). In addition, in both plants 21.47% of readings (1,430) from failed EBPR and 17.18% of readings (1,337) from successful EBPR could also not be classified at the phylum level. This result was in disagreement with the microbial pattern as reported by Feng et al. [35].

Table S5A. Abundances of all order in each sample of the Failed EBPR. The abundance is presented in terms of percentages of the total sequences in a sample

Name	Aerobic_A	Anaerobic_A	Anoxic_A	Fermenter_A	PST_A
Burkholderiales	43.05	8.50	8.85	9.67	42.50
Rhodocyclales	0.88	22.27	25.62	1.43	1.26
Neisseriales	0.00	0.30	0.00	0.51	0.16
Ferrovales	0.00	0.51	0.00	0.00	0.00
Nitrosomonadales	0.00	0.51	0.74	0.00	0.32
Rhizobiales	1.55	1.92	2.12	0.10	2.37
Caulobacterales	5.96	0.10	0.00	0.00	4.42
Sphingomonadales	0.66	0.10	0.46	0.00	1.58
Rhodobacterales	0.88	0.91	1.38	0.61	1.26
Rhodospirillales	0.22	0.40	0.46	0.00	0.63
Enterobacteriales	3.09	0.20	0.28	1.83	0.95
Pseudomonadales	5.30	1.62	1.94	13.24	5.21
Oceanospirillales	0.22	0.00	0.00	0.00	1.11
Alteromonadales	0.66	0.00	0.00	0.00	0.79
Xanthomonadales	0.44	0.51	1.47	0.00	0.16
Aeromonadales	0.22	0.71	0.92	7.84	0.16
Chromatiales	0.00	0.00	0.00	0.00	0.16
Thiotrichales	0.00	0.00	0.00	0.00	0.16
Campylobacterales	0.22	0.71	0.18	4.79	0.32
Myxococcales	0.00	5.67	3.32	0.00	0.00
Bdellovibrionales	0.00	0.00	0.00	0.00	0.32
Actinomycetales	6.18	3.54	3.41	0.20	4.74
Bifidobacteriales	0.00	0.20	0.00	0.10	0.00
Coriobacteriales	0.22	0.00	0.00	0.00	0.00
Solirubrobacterales	0.44	0.00	0.00	0.00	0.00
Acidimicrobiales	0.22	1.42	1.11	0.00	0.00
Lactobacillales	0.22	0.00	0.09	0.31	0.32
Bacillales	0.22	0.00	0.00	0.00	0.47
Erysipelotrichales	0.44	0.00	0.37	0.00	0.32
Clostridiales	9.93	3.74	2.76	3.56	9.32
Selenomonadales	0.00	0.20	0.18	1.53	0.00
Flavobacteriales	4.64	0.71	1.20	1.63	3.63
Sphingobacteriales	0.22	1.21	1.29	0.00	0.00
Cytophagales	0.00	0.40	0.09	0.00	0.00
Bacteroidales	0.00	0.51	0.18	28.92	0.00
Fusobacteriales	0.22	0.20	0.18	3.56	0.16
Anaerolineales	0.22	5.97	5.44	0.00	0.63
Caldilineales	0.00	0.51	0.09	0.00	0.47
Chloroflexales	0.00	0.30	0.09	0.00	0.00

Table S5A. Continued.

Herpetosiphonales	0.00	0.00	0.00	0.00	0.16
Nitrospirales	0.00	2.02	1.57	0.00	0.00
Planctomycetales	0.00	1.52	1.20	0.00	0.00
Verrucomicrobiales	0.00	0.51	0.09	0.00	0.16
Opitutales	0.00	0.81	0.92	0.20	0.00
Holophagales	0.00	0.20	0.00	0.00	0.00
Spirochaetales	0.00	0.40	0.00	0.00	0.00
Chlamydiales	0.00	0.20	0.00	0.00	0.00
Fimbriimonadales	0.00	0.00	0.09	0.00	0.00
Gemmatimonadales	0.00	0.00	0.09	0.00	0.00
Victivallales	0.00	0.00	0.00	0.10	0.00
Elusimicrobiales	0.00	0.00	0.00	0.00	0.16
unclassified_Alphaproteobacteria	3.75	3.74	2.58	0.00	3.16
unclassified_Gammaproteobacteria	5.52	3.24	3.32	14.66	5.53
unclassified_Betaproteobacteria	3.75	21.46	23.78	5.19	6.16
unclassified_Deltaproteobacteria	0.00	0.71	0.65	0.00	0.00
unclassified_Epsilonproteobacteria	0.00	0.10	0.00	0.00	0.00
unclassified_Rubrobacteridae	0.00	0.10	0.00	0.00	0.00
unclassified_Actinobacteria	0.44	1.11	1.20	0.00	0.63
unclassified_Bacilli	0.00	0.00	0.00	0.00	0.16
unclassified_Opitutae	0.00	0.00	0.09	0.00	0.00
unclassified_Acidobacteria_Gp3	0.00	0.00	0.09	0.00	0.00
unclassified_Deinococci	0.00	0.00	0.09	0.00	0.00

Table S5B. Abundances of all order in each sample of the Successful EBPR. The abundance is presented in terms of percentages of the total sequences in a sample.

Name	Aerobic_B	Anaerobic_B	Anoxic_B	Fermenter_B	PST_B
Planctomycetales	2.75	3.82	1.90	0.00	0.13
Phycisphaerales	0.00	0.10	0.00	0.00	0.00
Rhodocyclales	24.45	34.17	15.92	0.50	0.77
Burkholderiales	8.32	11.56	8.72	6.73	32.57
Nitrosomonadales	0.49	1.01	0.57	0.00	0.00
Ferrovales	0.14	0.20	0.00	0.00	0.00
Neisseriales	0.00	0.00	0.09	0.40	0.00
Enterobacteriales	7.68	0.00	9.67	0.89	0.77
Pseudomonadales	0.85	0.30	1.23	9.80	1.92
Xanthomonadales	0.21	0.10	0.47	0.20	0.38
Aeromonadales	0.28	0.00	0.47	4.36	1.02
Oceanospirillales	0.00	0.00	0.00	0.00	1.02
Alteromonadales	0.00	0.00	0.00	0.00	1.66

Table S5B. Continued.

Rhizobiales	1.55	0.30	1.61	0.10	1.02
Rhodobacterales	2.18	0.10	0.85	0.20	1.02
Sphingomonadales	0.63	0.50	0.28	0.00	0.51
Rhodospirillales	0.21	0.00	0.09	0.00	0.13
Caulobacterales	0.07	0.00	0.09	0.00	2.04
Myxococcales	1.69	2.41	1.52	0.00	0.00
Bdellovibrionales	0.14	0.30	0.19	0.00	0.00
Desulfovibrionales	0.00	0.10	0.00	0.10	0.00
Campylobacterales	0.00	0.10	1.14	1.49	0.00
Anaerolineales	1.13	2.21	2.09	0.00	0.00
Caldilineales	0.14	0.40	0.38	0.00	0.26
Chloroflexales	0.00	0.00	0.09	0.00	0.00
Fusobacteriales	0.14	0.20	0.19	2.67	0.13
Flavobacteriales	0.42	0.20	0.47	0.99	3.83
Sphingobacteriales	0.42	0.50	0.28	0.00	0.64
Bacteroidales	0.14	0.20	1.61	49.31	0.00
Cytophagales	0.00	0.00	0.28	0.00	0.00
Nitrospirales	2.75	1.01	2.94	0.00	0.00
Acidimicrobiales	0.78	0.20	0.57	0.00	0.26
Actinomycetales	2.47	1.71	3.89	0.59	6.00
Bifidobacteriales	0.07	0.00	0.09	0.00	0.00
Solirubrobacterales	0.14	0.00	0.19	0.00	0.00
Coriobacteriales	0.00	0.00	0.00	0.10	0.00
Verrucomicrobiales	0.35	0.30	0.19	0.00	0.00
Opitutales	0.42	1.51	0.09	0.00	0.00
Clostridiales	1.90	2.01	4.27	9.90	24.27
Lactobacillales	0.14	0.00	0.09	0.00	0.26
Bacillales	0.00	0.00	0.09	0.00	1.40
Erysipelotrichales	0.07	0.10	0.09	0.20	1.28
Selenomonadales	0.00	0.00	0.38	1.39	0.00
Deinococcales	0.00	0.00	0.00	0.00	0.13
Fibrobacterales	0.00	0.10	0.09	1.29	0.00
Armatimonadales	0.00	0.10	0.00	0.00	0.00
Elusimicrobiales	0.00	0.00	0.09	0.00	0.00
Spirochaetales	0.00	0.00	0.19	0.00	0.00
Victivallales	0.00	0.00	0.00	0.10	0.00
unclassified_Planctomycetia	0.00	0.10	0.00	0.00	0.00
unclassified_Betaproteobacteria	23.40	27.44	20.85	2.87	5.49
unclassified_Gammaproteobacteria	9.58	5.13	12.04	5.54	9.45
unclassified_Alphaproteobacteria	3.10	0.50	2.56	0.10	1.40

Table S5B. Continued.

unclassified_Deltaproteobacteria	0.21	0.30	0.47	0.00	0.00
unclassified_Actinobacteria	0.35	0.70	0.47	0.00	0.26
unclassified_Opitutae	0.00	0.00	0.00	0.10	0.00
unclassified_Acidobacteria_Gp3	0.14	0.00	0.09	0.00	0.00
unclassified_Deinococci	0.07	0.00	0.00	0.00	0.00
unclassified_Clostridia	0.00	0.00	0.00	0.10	0.00

Table S6A. Abundances of all family in each sample of the Failed EBPR. The abundance is presented in terms of percentages of the total sequences in a sample.

Name	Aerobic_A	Anaerobic_A	Anoxic_A	Fermenter_A	PST_A
Comamonadaceae	38.01	4.95	4.73	11.29	28.09
Burkholderiaceae	4.34	0.00	0.27	0.00	12.55
Oxalobacteraceae	2.30	0.00	0.00	0.00	3.18
Burkholderiales_incertae_sedis	1.28	2.62	2.97	0.00	1.31
Alcaligenaceae	0.00	0.00	0.41	0.00	0.19
Rhodocyclaceae	1.02	32.02	37.57	1.78	1.50
Neisseriaceae	0.00	0.44	0.00	0.63	0.19
Ferrovaceae	0.00	0.73	0.00	0.00	0.00
Nitrosomonadaceae	0.00	0.73	1.08	0.00	0.37
Phyllobacteriaceae	0.26	0.29	0.27	0.00	0.75
Methylobacteriaceae	0.51	0.00	0.00	0.00	0.19
Hyphomicrobiaceae	0.26	0.00	0.27	0.13	0.19
Bradyrhizobiaceae	0.00	0.87	0.95	0.00	0.19
Rhizobiaceae	0.00	0.15	0.00	0.00	0.00
Methylocystaceae	0.00	0.00	0.14	0.00	0.00
Beijerinckiaceae	0.00	0.00	0.00	0.00	0.19
Caulobacteraceae	6.89	0.15	0.00	0.00	5.24
Sphingomonadaceae	0.77	0.15	0.41	0.00	1.69
Erythrobacteraceae	0.00	0.00	0.00	0.00	0.19
Rhodobacteraceae	1.02	1.31	2.03	0.76	1.50
Acetobacteraceae	0.26	0.29	0.27	0.00	0.00
Rhodospirillaceae	0.00	0.29	0.41	0.00	0.75
Enterobacteriaceae	3.57	0.29	0.41	2.28	1.12
Pseudomonadaceae	5.61	0.87	1.76	0.89	4.12
Moraxellaceae	0.51	1.31	0.95	15.23	1.87
Halomonadaceae	0.26	0.00	0.00	0.00	1.31
Shewanellaceae	0.77	0.00	0.00	0.00	0.75
Xanthomonadaceae	0.51	0.73	2.16	0.00	0.19
Aeromonadaceae	0.26	1.02	1.35	9.77	0.19
Chromatiaceae	0.00	0.00	0.00	0.00	0.19

Table S6A. Continued.

Thiotrichaceae	0.00	0.00	0.00	0.00	0.19
Campylobacteraceae	0.26	1.02	0.14	5.84	0.37
Polyangiaceae	0.00	1.89	0.54	0.00	0.00
Nannocystaceae	0.00	3.78	2.84	0.00	0.00
Haliangiaceae	0.00	0.15	0.00	0.00	0.00
Kofleriaceae	0.00	0.15	0.14	0.00	0.00
Bacteriovoracaceae	0.00	0.00	0.00	0.00	0.19
Bdellovibrionaceae	0.00	0.00	0.00	0.00	0.19
Geodermatophilaceae	0.26	0.00	0.00	0.00	0.94
Nocardiaceae	0.26	0.15	0.00	0.00	0.00
Mycobacteriaceae	0.00	0.00	0.54	0.00	0.00
Corynebacteriaceae	0.00	0.00	0.00	0.00	0.19
Microbacteriaceae	1.02	0.15	0.00	0.00	0.75
Intrasporangiaceae	0.00	1.16	0.54	0.00	0.00
Dermacoccaceae	0.00	0.15	0.00	0.00	0.00
Micrococcaceae	0.00	0.00	0.00	0.00	0.19
Nocardioidaceae	0.26	0.87	0.54	0.00	0.94
Propionibacteriaceae	0.00	0.00	0.14	0.13	0.37
Actinomycetaceae	0.26	0.00	0.00	0.00	0.19
Bifidobacteriaceae	0.00	0.29	0.00	0.13	0.00
Coriobacteriaceae	0.26	0.00	0.00	0.00	0.00
Iamiaceae	0.00	0.87	0.00	0.00	0.00
Acidimicrobiaceae	0.00	0.29	0.41	0.00	0.00
Streptococcaceae	0.26	0.00	0.14	0.38	0.19
Bacillaceae 1	0.00	0.00	0.00	0.00	0.56
Erysipelotrichaceae	0.51	0.00	0.54	0.00	0.37
Peptostreptococcaceae	4.08	1.46	1.35	0.63	4.12
Clostridiaceae 1	5.61	1.46	1.35	0.89	3.00
Ruminococcaceae	0.77	0.29	0.14	1.65	1.50
Lachnospiraceae	0.00	0.87	0.41	0.63	0.94
Acidaminococcaceae	0.00	0.15	0.00	0.76	0.00
Veillonellaceae	0.00	0.00	0.14	0.76	0.00
Flavobacteriaceae	5.36	1.02	1.49	1.90	4.12
Chitinophagaceae	0.26	1.46	1.08	0.00	0.00
Saprospiraceae	0.00	0.29	0.41	0.00	0.00
Sphingobacteriaceae	0.00	0.00	0.27	0.00	0.00
Cytophagaceae	0.00	0.58	0.00	0.00	0.00
Prevotellaceae	0.00	0.58	0.14	21.95	0.00
Bacteroidaceae	0.00	0.00	0.00	0.51	0.00
Porphyromonadaceae	0.00	0.00	0.00	0.25	0.00
Leptotrichiaceae	0.00	0.15	0.14	1.02	0.00

Table S6A. Continued.

Fusobacteriaceae	0.00	0.00	0.14	1.02	0.19
Anaerolineaceae	0.26	8.59	7.97	0.00	0.75
Caldilineaceae	0.00	0.73	0.14	0.00	0.56
Chloroflexaceae	0.00	0.29	0.00	0.00	0.00
Herpetosiphonaceae	0.00	0.00	0.00	0.00	0.19
Nitrospiraceae	0.00	2.91	2.30	0.00	0.00
Planctomycetaceae	0.00	2.18	1.76	0.00	0.00
Verrucomicrobiaceae	0.00	0.73	0.14	0.00	0.19
Opitutaceae	0.00	1.16	1.35	0.25	0.00
Holophagaceae	0.00	0.29	0.00	0.00	0.00
Leptospiraceae	0.00	0.58	0.00	0.00	0.00
Fimbriimonadaceae	0.00	0.00	0.14	0.00	0.00
Gemmatimonadaceae	0.00	0.00	0.14	0.00	0.00
Victivallaceae	0.00	0.00	0.00	0.13	0.00
Chloroplast	0.00	0.00	0.00	0.13	0.00
Elusimicrobiaceae	0.00	0.00	0.00	0.00	0.19
unclassified_Burkholderiales	3.83	4.66	4.59	0.76	5.06
unclassified_Rhizobiales	0.77	1.46	1.49	0.00	1.31
unclassified_Sphingomonadales	0.00	0.00	0.27	0.00	0.00
unclassified_Pseudomonadales	0.00	0.15	0.14	0.38	0.19
unclassified_Alteromonadales	0.00	0.00	0.00	0.00	0.19
unclassified_Campylobacterales	0.00	0.00	0.14	0.13	0.00
unclassified_Sorangiineae	0.00	0.29	0.14	0.00	0.00
unclassified_Nannocystineae	0.00	0.15	0.14	0.00	0.00
unclassified_Myxococcales	0.00	1.75	1.08	0.00	0.00
unclassified_Corynebacterineae	1.28	0.29	0.68	0.00	0.19
unclassified_Micrococcineae	0.00	0.00	0.27	0.13	0.37
unclassified_Actinomycetales	3.83	2.33	2.30	0.00	1.50
unclassified_Solirubrobacterales	0.51	0.00	0.00	0.00	0.00
unclassified_"Acidimicrobineae"	0.26	0.87	1.22	0.00	0.00
unclassified_Bacillales	0.26	0.00	0.00	0.00	0.00
unclassified_Lactobacillales	0.00	0.00	0.00	0.00	0.19
unclassified_Clostridiales	1.02	1.31	0.81	0.63	1.50
unclassified_Selenomonadales	0.00	0.15	0.14	0.38	0.00
unclassified_"Flavobacteriales"	0.00	0.00	0.27	0.13	0.19
unclassified_"Sphingobacteriales"	0.00	0.00	0.14	0.00	0.00
unclassified_Cytophagales	0.00	0.00	0.14	0.00	0.00
unclassified_"Bacteroidales"	0.00	0.15	0.14	13.32	0.00
unclassified_"Fusobacteriales"	0.26	0.15	0.00	2.41	0.00
unclassified_Chloroflexineae	0.00	0.15	0.14	0.00	0.00
unclassified_Chlamydiales	0.00	0.29	0.00	0.00	0.00

Table S6B. Abundances of all family in each sample of the Successful EBPR. The abundance is presented in terms of percentages of the total sequences in a sample.

Name	Aerobic_B	Anaerobic_B	Anoxic_B	Fermenter_B	PST_B
Planctomycetaceae	4.35	5.80	2.99	0.00	0.15
Phycisphaeraceae	0.00	0.15	0.00	0.00	0.00
Rhodocyclaceae	38.73	51.91	25.07	0.54	0.92
Burkholderiales_incertae_sedis	3.46	5.04	2.39	0.00	0.31
Comamonadaceae	4.80	5.80	5.67	7.27	19.14
Burkholderiaceae	0.11	0.61	0.60	0.00	15.01
Alcaligenaceae	0.22	0.00	0.00	0.00	0.00
Oxalobacteraceae	0.11	0.31	0.15	0.00	1.53
Nitrosomonadaceae	0.67	1.53	0.90	0.00	0.00
Ferrovaceae	0.22	0.31	0.00	0.00	0.00
Neisseriaceae	0.00	0.00	0.15	0.43	0.00
Enterobacteriaceae	12.17	0.00	15.22	0.98	0.92
Pseudomonadaceae	0.56	0.00	0.90	0.00	1.53
Moraxellaceae	0.33	0.00	0.15	10.09	0.61
Xanthomonadaceae	0.22	0.15	0.60	0.22	0.46
Sinobacteraceae	0.11	0.00	0.15	0.00	0.00
Aeromonadaceae	0.45	0.00	0.45	4.77	1.23
Succinivibrionaceae	0.00	0.00	0.30	0.00	0.00
Halomonadaceae	0.00	0.00	0.00	0.00	1.23
Shewanellaceae	0.00	0.00	0.00	0.00	1.68
Bradyrhizobiaceae	0.89	0.00	0.60	0.00	0.15
Rhizobiales_incertae_sedis	0.11	0.00	0.00	0.00	0.00
Methylocystaceae	0.11	0.15	0.00	0.00	0.00
Hyphomicrobiaceae	0.11	0.00	0.15	0.00	0.00
Phyllobacteriaceae	0.00	0.00	0.15	0.00	0.31
Rhodobacteraceae	3.46	0.15	1.34	0.22	1.23
Sphingomonadaceae	0.89	0.61	0.30	0.00	0.61
Acetobacteraceae	0.11	0.00	0.15	0.00	0.00
Rhodospirillaceae	0.22	0.00	0.00	0.00	0.15
Caulobacteraceae	0.11	0.00	0.15	0.00	2.45
Polyangiaceae	0.89	3.05	0.75	0.00	0.00
Nannocystaceae	1.00	0.15	1.34	0.00	0.00
Cystobacteraceae	0.11	0.00	0.15	0.00	0.00
Bdellovibrionaceae	0.22	0.46	0.30	0.00	0.00
Desulfomicrobiaceae	0.00	0.15	0.00	0.00	0.00
Desulfovibrionaceae	0.00	0.00	0.00	0.11	0.00
Campylobacteraceae	0.00	0.15	1.79	1.63	0.00
Anaerolineaceae	1.79	3.36	3.28	0.00	0.00
Caldilineaceae	0.22	0.61	0.60	0.00	0.31

Table S6B. Continued.

Chloroflexaceae	0.00	0.00	0.15	0.00	0.00
Leptotrichiaceae	0.00	0.15	0.00	0.65	0.15
Fusobacteriaceae	0.00	0.00	0.30	0.00	0.00
Flavobacteriaceae	0.22	0.31	0.30	0.98	4.59
Chitinophagaceae	0.56	0.76	0.30	0.00	0.61
Porphyromonadaceae	0.11	0.15	0.00	0.33	0.00
Prevotellaceae	0.00	0.00	1.34	38.07	0.00
Bacteroidaceae	0.00	0.00	0.45	0.43	0.00
Cytophagaceae	0.00	0.00	0.45	0.00	0.00
Nitrospiraceae	4.35	1.53	4.63	0.00	0.00
Iamiaceae	0.45	0.15	0.45	0.00	0.15
Acidimicrobiaceae	0.22	0.00	0.30	0.00	0.00
Intrasporangiaceae	1.56	0.61	2.09	0.00	0.00
Micrococcaceae	0.00	0.00	0.00	0.00	0.46
Microbacteriaceae	0.00	0.00	0.00	0.00	0.15
Nocardioidaceae	0.33	0.31	0.60	0.00	1.23
Propionibacteriaceae	0.00	0.00	0.00	0.33	0.15
Mycobacteriaceae	0.11	0.00	0.00	0.00	0.00
Nocardiaceae	0.00	0.00	0.15	0.00	0.15
Corynebacteriaceae	0.00	0.00	0.00	0.00	0.46
Micromonosporaceae	0.00	0.00	0.00	0.00	0.15
Nakamurellaceae	0.00	0.00	0.00	0.00	0.15
Geodermatophilaceae	0.00	0.00	0.00	0.00	0.15
Bifidobacteriaceae	0.11	0.00	0.15	0.00	0.00
Conexibacteraceae	0.22	0.00	0.30	0.00	0.00
Coriobacteriaceae	0.00	0.00	0.00	0.11	0.00
Verrucomicrobiaceae	0.56	0.46	0.30	0.00	0.00
Opitutaceae	0.67	2.29	0.15	0.00	0.00
Lachnospiraceae	0.22	0.00	0.45	3.90	0.61
Clostridiaceae 1	0.45	0.61	0.90	0.76	15.62
Peptostreptococcaceae	1.67	1.07	2.84	0.43	8.58
Ruminococcaceae	0.22	0.31	0.30	2.82	1.38
Clostridiales_Incertae Sedis XIII	0.11	0.00	0.00	0.00	0.00
Clostridiales_Incertae Sedis XI	0.00	0.00	0.15	0.00	0.00
Streptococcaceae	0.11	0.00	0.15	0.00	0.15
Enterococcaceae	0.11	0.00	0.00	0.00	0.00
Leuconostocaceae	0.00	0.00	0.00	0.00	0.15
Bacillaceae 1	0.00	0.00	0.15	0.00	1.07
Bacillaceae 2	0.00	0.00	0.00	0.00	0.31
Erysipelotrichaceae	0.11	0.15	0.15	0.22	1.53

Table S6B. Continued.

Acidaminococcaceae	0.00	0.00	0.15	0.43	0.00
Veillonellaceae	0.00	0.00	0.45	0.65	0.00
Deinococcaceae	0.00	0.00	0.00	0.00	0.15
Fibrobacteraceae	0.00	0.15	0.15	1.41	0.00
Armatimonadaceae	0.00	0.15	0.00	0.00	0.00
Elusimicrobiaceae	0.00	0.00	0.15	0.00	0.00
Spirochaetaceae	0.00	0.00	0.15	0.00	0.00
Leptospiraceae	0.00	0.00	0.15	0.00	0.00
Victivallaceae	0.00	0.00	0.00	0.11	0.00
unclassified_Burkholderiales	4.46	5.80	4.93	0.11	3.06
unclassified_Nitrosomonadales	0.11	0.00	0.00	0.00	0.00
unclassified_Pseudomonadales	0.45	0.46	0.90	0.65	0.15
unclassified_Alteromonadales	0.00	0.00	0.00	0.00	0.31
unclassified_Rhizobiales	1.23	0.31	1.64	0.11	0.77
unclassified_Sphingomonadales	0.11	0.15	0.15	0.00	0.00
unclassified_Myxococcales	0.33	0.15	0.15	0.00	0.00
unclassified_"Fusobacteriales"	0.22	0.15	0.00	2.28	0.00
unclassified_"Flavobacteriales"	0.45	0.00	0.45	0.11	0.00
unclassified_"Sphingobacteriales"	0.11	0.00	0.15	0.00	0.15
unclassified_"Bacteroidales"	0.11	0.15	0.75	15.18	0.00
unclassified_"Acidimicrobineae"	0.56	0.15	0.15	0.00	0.15
unclassified_Micrococcineae	0.22	0.15	0.60	0.00	0.31
unclassified_Corynebacterineae	0.22	0.15	0.15	0.00	1.68
unclassified_Actinomycetales	1.45	1.37	2.54	0.33	2.14
unclassified_Clostridiales	0.33	1.07	2.09	2.93	2.91
unclassified_Bacillales	0.00	0.00	0.00	0.00	0.31
unclassified_Selenomonadales	0.00	0.00	0.00	0.43	0.00
unclassified_Cystobacterineae	0.11	0.00	0.00	0.00	0.00
unclassified_Nannocystineae	0.22	0.15	0.00	0.00	0.00
unclassified_Sorangiineae	0.00	0.15	0.00	0.00	0.00

Table S7A. Abundances of all genus in each sample of the Failed EBPR. The abundance is presented in terms of percentages of the total sequences in a sample.

Name	Aerobic_A	Anaerobic_A	Anoxic_A	Fermenter_A	PST_A
Acidovorax	26.20	0.50	0.78	3.72	17.05
Delftia	1.41	0.00	0.00	0.00	1.14
Alicycliphilus	0.56	0.50	0.31	0.78	0.38
Comamonas	0.28	0.33	0.00	0.16	0.00
Caenimonas	0.00	0.17	0.78	0.00	0.19
Albidiferax	0.00	0.17	0.00	0.00	0.00
Curvibacter	0.00	0.33	0.00	0.00	0.00
Ramlibacter	0.00	0.00	0.00	0.00	0.19
Chitinimonas	0.56	0.00	0.31	0.00	0.00
Pandoraea	3.38	0.00	0.00	0.00	10.61
Ralstonia	0.28	0.00	0.00	0.00	0.19
Cupriavidus	0.28	0.00	0.00	0.00	1.52
Burkholderia	0.00	0.00	0.00	0.00	0.19
Massilia	1.41	0.00	0.00	0.00	1.14
Naxibacter	0.56	0.00	0.00	0.00	0.00
Noviherbaspirillum	0.00	0.00	0.00	0.00	0.57
Aquincola	0.28	0.00	0.00	0.00	0.19
Ideonella	0.00	0.17	0.00	0.00	0.00
Rivibacter	0.00	0.17	0.00	0.00	0.00
Inhella	0.00	0.17	0.78	0.00	0.00
Rubrivivax	0.00	0.00	0.16	0.00	0.00
Xylophilus	0.00	0.00	0.16	0.00	0.00
Aquabacterium	0.00	0.00	0.16	0.00	0.19
Derxia	0.00	0.00	0.47	0.00	0.00
Alcaligenes	0.00	0.00	0.00	0.00	0.19
Propionivibrio	0.28	4.17	5.49	0.00	0.19
Ferribacterium	0.00	0.33	0.31	0.00	0.00
Sulfuritalea	0.00	1.67	0.94	0.00	0.00
Methyloversatilis	0.00	0.33	0.16	0.00	0.19
Zoogloea	0.00	0.83	0.31	0.16	0.19
Dechloromonas	0.00	0.17	0.16	0.31	0.00
Thauera	0.00	0.00	0.16	0.31	0.00
Rhodocyclus	0.00	0.00	0.31	0.00	0.00
Uruburuella	0.00	0.33	0.00	0.00	0.19
Laribacter	0.00	0.17	0.00	0.31	0.00
Ferrovum	0.00	0.83	0.00	0.00	0.00
Mesorhizobium	0.00	0.17	0.00	0.00	0.00
Microvirga	0.56	0.00	0.00	0.00	0.19
Devosia	0.28	0.00	0.00	0.00	0.00

Table S7A. Continued

Prosthecomicrobium	0.00	0.00	0.31	0.00	0.00
Aquabacter	0.00	0.00	0.00	0.16	0.00
Hyphomicrobium	0.00	0.00	0.00	0.00	0.19
Afipia	0.00	0.17	0.00	0.00	0.00
Oligotropha	0.00	0.50	0.16	0.00	0.00
Brevundimonas	6.76	0.00	0.00	0.00	4.36
Phenylobacterium	0.28	0.17	0.00	0.00	0.19
Sphingomonas	0.28	0.00	0.00	0.00	0.76
Sphingorhabdus	0.00	0.17	0.00	0.00	0.00
Pannonibacter	0.56	0.00	0.00	0.00	0.38
Amaricoccus	0.28	0.00	0.00	0.16	0.19
Gemmobacter	0.00	0.00	0.16	0.00	0.19
Paracoccus	0.00	0.00	0.00	0.47	0.38
Roseomonas	0.28	0.00	0.00	0.00	0.00
Stella	0.00	0.17	0.00	0.00	0.00
Magnetospirillum	0.00	0.17	0.00	0.00	0.00
Defluviicoccus	0.00	0.17	0.00	0.00	0.00
Azospirillum	0.00	0.00	0.00	0.00	0.19
Desertibacter	0.00	0.00	0.00	0.00	0.38
Enterobacter	0.28	0.00	0.00	0.00	0.00
Proteus	0.00	0.00	0.00	0.31	0.19
Citrobacter	0.00	0.00	0.00	0.16	0.00
Pseudomonas	0.56	0.00	0.00	0.31	0.19
Cellvibrio	0.00	1.00	1.73	0.00	0.00
Serpens	0.00	0.00	0.16	0.00	0.19
Acinetobacter	0.56	0.67	0.31	11.94	0.57
Enhydrobacter	0.00	0.33	0.31	2.79	0.57
Alkanindiges	0.00	0.00	0.00	0.31	0.19
Halomonas	0.28	0.00	0.00	0.00	0.38
Shewanella	0.85	0.00	0.00	0.00	0.76
Lysobacter	0.28	0.00	0.00	0.00	0.00
Dokdonella	0.00	0.50	0.78	0.00	0.00
Thermomonas	0.00	0.00	0.16	0.00	0.00
Aquimonas	0.00	0.00	0.31	0.00	0.00
Aeromonas	0.28	0.50	1.10	8.53	0.19
Tolumonas	0.00	0.50	0.31	2.48	0.00
Rheinheimera	0.00	0.00	0.00	0.00	0.19
Thiothrix	0.00	0.00	0.00	0.00	0.19
Arcobacter	0.28	1.17	0.16	6.98	0.38
Sulfurospirillum	0.00	0.00	0.00	0.16	0.00
Byssovorax	0.00	0.83	0.16	0.00	0.00

Table S7A. Continued

Nannocystis	0.00	2.50	2.35	0.00	0.00
Haliangium	0.00	0.17	0.00	0.00	0.00
Kofleria	0.00	0.17	0.16	0.00	0.00
Bacteriovorax	0.00	0.00	0.00	0.00	0.19
Vampirovibrio	0.00	0.00	0.00	0.00	0.19
Blastococcus	0.28	0.00	0.00	0.00	0.95
Mycobacterium	0.00	0.00	0.63	0.00	0.00
Corynebacterium	0.00	0.00	0.00	0.00	0.19
Microbacterium	0.28	0.00	0.00	0.00	0.19
Leucobacter	0.28	0.00	0.00	0.00	0.00
Agrococcus	0.00	0.00	0.00	0.00	0.19
Tetrasphaera	0.00	1.34	0.63	0.00	0.00
Dermacoccus	0.00	0.17	0.00	0.00	0.00
Nesterenkonia	0.00	0.00	0.00	0.00	0.19
Nocardioides	0.00	0.17	0.16	0.00	0.00
Marmoricola	0.00	0.33	0.47	0.00	0.57
Pimelobacter	0.00	0.17	0.00	0.00	0.00
Brooklawnia	0.00	0.00	0.00	0.16	0.00
Micropruina	0.00	0.00	0.00	0.00	0.19
Actinomyces	0.28	0.00	0.00	0.00	0.19
Bifidobacterium	0.00	0.33	0.00	0.16	0.00
Collinsella	0.28	0.00	0.00	0.00	0.00
Iamia	0.00	1.00	0.00	0.00	0.00
Ilumatobacter	0.00	0.33	0.16	0.00	0.00
Streptococcus	0.28	0.00	0.16	0.47	0.00
Aeribacillus	0.00	0.00	0.00	0.00	0.57
Turicibacter	0.56	0.00	0.47	0.00	0.38
Erysipelotrichaceae_incertae_sedis	0.00	0.00	0.16	0.00	0.00
Clostridium XI	3.66	1.34	0.94	0.31	1.89
Proteocatella	0.00	0.00	0.16	0.00	0.00
Peptostreptococcaceae_incertae_sedis	0.00	0.00	0.00	0.00	0.19
Clostridium sensu stricto	2.82	0.33	0.31	0.47	1.89
Anaerobacter	0.00	0.17	0.00	0.00	0.00
Sarcina	0.00	0.00	0.16	0.00	0.00
Faecalibacterium	0.28	0.00	0.00	1.09	0.57
Oscillibacter	0.00	0.00	0.00	0.31	0.00
Subdoligranulum	0.00	0.00	0.00	0.16	0.00
Saccharofermentans	0.00	0.00	0.00	0.00	0.38
Lachnospiracea_incertae_sedis	0.00	0.17	0.00	0.00	0.00
Ruminococcus2	0.00	0.17	0.00	0.00	0.19
Roseburia	0.00	0.00	0.00	0.16	0.00

Table S7A. Continued

Blautia	0.00	0.00	0.00	0.00	0.19
Phascolarctobacterium	0.00	0.00	0.00	0.16	0.00
Succinispira	0.00	0.00	0.00	0.16	0.00
Megasphaera	0.00	0.00	0.16	0.16	0.00
Mitsuokella	0.00	0.00	0.00	0.47	0.00
Parcubacteria_genera_incertae_sedis	1.69	0.17	0.00	0.16	3.60
Flavobacterium	3.94	0.50	0.94	0.47	3.03
Cloacibacterium	0.00	0.00	0.16	1.09	0.38
Niastella	0.28	0.00	0.00	0.00	0.00
Terrimonas	0.00	0.33	0.16	0.00	0.00
Sediminibacterium	0.00	0.17	0.47	0.00	0.00
Ferruginibacter	0.00	0.17	0.00	0.00	0.00
Haliscomenobacter	0.00	0.33	0.47	0.00	0.00
Arcticibacter	0.00	0.00	0.16	0.00	0.00
Pedobacter	0.00	0.00	0.16	0.00	0.00
Leadbetterella	0.00	0.33	0.00	0.00	0.00
Runella	0.00	0.33	0.00	0.00	0.00
Ohtaekwangia	0.00	0.17	0.00	0.00	0.00
Prevotella	0.00	0.17	0.16	3.72	0.00
Xylanibacter	0.00	0.00	0.00	3.57	0.00
Bacteroides	0.00	0.00	0.00	0.62	0.00
Saccharibacteria_genera_incertae_sedis	1.13	0.00	0.16	0.00	6.06
Longilinea	0.00	0.17	0.00	0.00	0.00
Litorilinea	0.00	0.17	0.00	0.00	0.00
Caldilinea	0.00	0.17	0.16	0.00	0.19
Roseiflexus	0.00	0.17	0.00	0.00	0.00
Herpetosiphon	0.00	0.00	0.00	0.00	0.19
Nitrospira	0.00	3.34	2.67	0.00	0.00
Gemmata	0.00	0.67	0.47	0.00	0.00
Planctomyces	0.00	0.50	0.16	0.00	0.00
Aquisphaera	0.00	0.00	0.16	0.00	0.00
Prosthecobacter	0.00	0.67	0.00	0.00	0.19
Luteolibacter	0.00	0.17	0.00	0.00	0.00
Subdivision3_genera_incertae_sedis	0.00	0.83	0.16	0.00	0.00
Opitutus	0.00	1.00	1.57	0.31	0.00
Blastocatella	0.00	0.17	0.00	0.00	0.00
Turneriella	0.00	0.67	0.00	0.00	0.00
Armatimonadetes_gp5	0.00	0.17	0.00	0.00	0.00
Fimbriimonas	0.00	0.00	0.16	0.00	0.00
Gemmatimonas	0.00	0.00	0.16	0.00	0.00
Victivallis	0.00	0.00	0.00	0.16	0.00

Table S7A. Continued

Bacillariophyta	0.00	0.00	0.00	0.16	0.00
Elusimicrobium	0.00	0.00	0.00	0.00	0.19
unclassified_Comamonadaceae	13.52	3.67	3.61	9.15	9.47
unclassified_Oxalobacteraceae	0.56	0.00	0.00	0.00	1.52
unclassified_Burkholderiales_incertae_sedis	1.13	2.50	2.20	0.00	0.95
unclassified_Burkholderiaceae	0.28	0.00	0.00	0.00	0.19
unclassified_Rhodocyclaceae	0.85	29.22	35.79	1.40	0.95
unclassified_Neisseriaceae	0.00	0.00	0.00	0.47	0.00
unclassified_Nitrosomonadaceae	0.00	0.83	1.26	0.00	0.38
unclassified_Phyllobacteriaceae	0.28	0.17	0.31	0.00	0.76
unclassified_Bradyrhizobiaceae	0.00	0.33	0.94	0.00	0.19
unclassified_Rhizobiaceae	0.00	0.17	0.00	0.00	0.00
unclassified_Methylocystaceae	0.00	0.00	0.16	0.00	0.00
unclassified_Beijerinckiaceae	0.00	0.00	0.00	0.00	0.19
unclassified_Caulobacteraceae	0.56	0.00	0.00	0.00	0.76
unclassified_Sphingomonadaceae	0.56	0.00	0.47	0.00	0.95
unclassified_Erythrobacteraceae	0.00	0.00	0.00	0.00	0.19
unclassified_Rhodobacteraceae	0.28	1.50	2.20	0.31	0.38
unclassified_Acetobacteraceae	0.00	0.17	0.31	0.00	0.00
unclassified_Rhodospirillaceae	0.00	0.00	0.47	0.00	0.19
unclassified_Enterobacteriaceae	3.66	0.33	0.47	2.33	0.95
unclassified_Pseudomonadaceae	5.63	0.00	0.16	0.78	3.79
unclassified_Moraxellaceae	0.00	0.50	0.47	3.57	0.57
unclassified_Halomonadaceae	0.00	0.00	0.00	0.00	0.95
unclassified_Xanthomonadaceae	0.28	0.33	1.26	0.00	0.19
unclassified_Aeromonadaceae	0.00	0.17	0.16	0.93	0.00
unclassified_Polyangiaceae	0.00	1.34	0.47	0.00	0.00
unclassified_Nannocystaceae	0.00	1.84	0.94	0.00	0.00
unclassified_Nocardiaceae	0.28	0.17	0.00	0.00	0.00
unclassified_Microbacteriaceae	0.56	0.17	0.00	0.00	0.38
unclassified_Nocardioidaceae	0.28	0.33	0.00	0.00	0.38
unclassified_Propionibacteriaceae	0.00	0.00	0.16	0.00	0.19
unclassified_Acidimicrobiaceae	0.00	0.00	0.31	0.00	0.00
unclassified_Streptococcaceae	0.00	0.00	0.00	0.00	0.19
unclassified_Clostridiaceae 1	3.38	1.17	1.10	0.62	1.14
unclassified_Peptostreptococcaceae	0.85	0.33	0.47	0.47	2.08
unclassified_Ruminococcaceae	0.56	0.33	0.16	0.47	0.57
unclassified_Lachnospiraceae	0.00	0.67	0.47	0.62	0.57
unclassified_Acidaminococcaceae	0.00	0.17	0.00	0.62	0.00
unclassified_Veillonellaceae	0.00	0.00	0.00	0.31	0.00
unclassified_Flavobacteriaceae	1.97	0.67	0.63	0.78	0.76

Table S7A. Continued.

unclassified_Chitinophagaceae	0.00	1.00	0.63	0.00	0.00
unclassified_"Prevotellaceae"	0.00	0.50	0.00	19.53	0.00
unclassified_"Porphyromonadaceae"	0.00	0.00	0.00	0.31	0.00
unclassified_"Leptotrichiaceae"	0.00	0.17	0.16	1.24	0.00
unclassified_"Fusobacteriaceae"	0.00	0.00	0.16	1.24	0.19
unclassified_Anaerolineaceae	0.28	9.68	9.26	0.00	0.76
unclassified_Caldilineaceae	0.00	0.50	0.00	0.00	0.38
unclassified_Chloroflexaceae	0.00	0.17	0.00	0.00	0.00
unclassified_Planctomycetaceae	0.00	1.34	1.26	0.00	0.00
unclassified_Verrucomicrobiaceae	0.00	0.00	0.16	0.00	0.00
unclassified_Opitutaceae	0.00	0.33	0.00	0.00	0.00
unclassified_Holophagaceae	0.00	0.33	0.00	0.00	0.00

Table S7B. Abundances of all genus in each sample of the Successful EBPR. The abundance is presented in terms of percentages of the total sequences in a sample.

Name	Aerobic_B	Anaerobic_B	Anoxic_B	Fermenter_B	PST_B
Planctomyces	0.62	1.68	0.70	0.00	0.17
Schlesneria	0.12	0.17	0.00	0.00	0.00
Pirellula	0.12	0.17	0.17	0.00	0.00
Gemmata	0.00	0.17	0.52	0.00	0.00
Phycisphaera	0.00	0.17	0.00	0.00	0.00
Sulfuritalea	1.36	0.51	0.17	0.00	0.00
Propionivibrio	3.45	8.59	2.61	0.00	0.34
Methyloversatilis	0.25	0.00	0.17	0.00	0.00
Dechloromonas	0.12	0.17	0.35	0.14	0.17
Ferribacterium	0.12	0.00	0.35	0.00	0.00
Zoogloea	0.12	0.17	0.17	0.56	0.00
Thauera	0.12	0.17	0.00	0.00	0.00
Piscinibacter	0.12	0.00	0.00	0.00	0.00
Aquabacterium	0.49	0.34	0.17	0.00	0.00
Inhella	0.49	0.67	0.17	0.00	0.00
Ideonella	0.00	0.17	0.17	0.00	0.00
Rubrivivax	0.00	0.00	0.17	0.00	0.00
Caenimonas	1.36	1.01	1.05	0.00	0.00
Ottowia	0.12	0.00	0.00	0.00	0.00
Simplicispira	0.12	0.17	0.00	0.00	0.00
Comamonas	0.37	0.00	0.17	0.56	1.02
Rhodoferax	0.12	0.00	0.00	0.00	0.00
Alicycliphilus	0.12	0.00	0.35	0.28	0.00
Albidiferax	0.12	0.00	0.00	0.00	0.00

Table S7B. Continued

Curvibacter	0.12	0.17	0.17	0.00	0.00
Acidovorax	0.12	0.51	0.17	2.36	13.44
Delftia	0.12	0.00	0.00	0.00	0.51
Malikia	0.00	0.00	0.17	0.00	0.00
Macromonas	0.00	0.00	0.17	0.00	0.00
Pseudorhodoferax	0.00	0.00	0.17	0.00	0.00
Chitinimonas	0.12	0.51	0.70	0.00	0.00
Pandoraea	0.00	0.00	0.00	0.00	14.63
Cupriavidus	0.00	0.00	0.00	0.00	1.02
Ralstonia	0.00	0.00	0.00	0.00	0.17
Derxia	0.25	0.00	0.00	0.00	0.00
Undibacterium	0.12	0.34	0.17	0.00	0.00
Massilia	0.00	0.00	0.00	0.00	1.02
Ferrovum	0.25	0.34	0.00	0.00	0.00
Uruburuella	0.00	0.00	0.00	0.14	0.00
Laribacter	0.00	0.00	0.00	0.28	0.00
Proteus	6.41	0.00	4.88	0.14	0.17
Escherichia/Shigella	0.12	0.00	0.52	0.00	0.00
Yokenella	0.25	0.00	0.00	0.14	0.00
Citrobacter	0.37	0.00	0.35	0.00	0.00
Morganella	0.12	0.00	0.35	0.00	0.00
Enterobacter	0.00	0.00	0.17	0.00	0.17
Cosenzaea	0.00	0.00	0.35	0.00	0.00
Pluralibacter	0.00	0.00	0.17	0.00	0.00
Serratia	0.00	0.00	0.17	0.00	0.00
Rhizobacter	0.49	0.00	0.35	0.00	0.00
Pseudomonas	0.12	0.00	0.35	0.00	0.51
Cellvibrio	0.00	0.00	0.17	0.00	0.00
Enhydrobacter	0.25	0.00	0.17	1.25	0.34
Acinetobacter	0.12	0.00	0.00	8.34	0.34
Dokdonella	0.12	0.00	0.35	0.00	0.00
Stenotrophomonas	0.00	0.00	0.00	0.14	0.17
Thermomonas	0.00	0.00	0.00	0.14	0.00
Lysobacter	0.00	0.00	0.00	0.00	0.17
Pseudoxanthomonas	0.00	0.00	0.00	0.00	0.17
Steroidobacter	0.12	0.00	0.17	0.00	0.00
Aeromonas	0.49	0.00	0.35	3.62	1.19
Tolumonas	0.00	0.00	0.17	1.81	0.00
Succinivibrio	0.00	0.00	0.17	0.00	0.00
Halomonas	0.00	0.00	0.00	0.00	0.17
Shewanella	0.00	0.00	0.00	0.00	1.87

Table S7B. Continued

Rhodopseudomonas	0.25	0.00	0.17	0.00	0.00
Vasilyevaea	0.12	0.00	0.00	0.00	0.00
Methylocystis	0.12	0.00	0.00	0.00	0.00
Prosthecomicrobium	0.12	0.00	0.00	0.00	0.00
Hyphomicrobium	0.00	0.00	0.17	0.00	0.00
Rhodobacter	0.12	0.00	0.00	0.00	0.00
Amaricoccus	0.00	0.00	0.17	0.00	0.17
Gemmobacter	0.00	0.00	0.00	0.14	0.00
Paracoccus	0.00	0.00	0.00	0.14	0.00
Pannonibacter	0.00	0.00	0.00	0.00	0.34
Sphingomonas	0.12	0.00	0.00	0.00	0.51
Sphingorhabdus	0.12	0.34	0.17	0.00	0.00
Blastomonas	0.12	0.00	0.00	0.00	0.00
Defluviicoccus	0.25	0.00	0.00	0.00	0.00
Skermanella	0.00	0.00	0.00	0.00	0.17
Caulobacter	0.12	0.00	0.17	0.00	0.00
Brevundimonas	0.00	0.00	0.00	0.00	2.21
Byssovorax	0.12	1.18	0.00	0.00	0.00
Nannocystis	0.49	0.00	1.22	0.00	0.00
Bdellovibrio	0.25	0.51	0.35	0.00	0.00
Desulfomicrobium	0.00	0.17	0.00	0.00	0.00
Arcobacter	0.00	0.17	2.09	2.09	0.00
Caldilinea	0.00	0.00	0.17	0.00	0.34
Cloacibacterium	0.12	0.00	0.00	0.00	0.17
Flavobacterium	0.00	0.17	0.17	0.56	4.08
Chryseobacterium	0.00	0.00	0.00	0.14	0.00
Terrimonas	0.00	0.51	0.00	0.00	0.00
Ferruginibacter	0.00	0.34	0.00	0.00	0.00
Hydrotalea	0.00	0.00	0.00	0.00	0.68
Paludibacter	0.00	0.17	0.00	0.14	0.00
Parabacteroides	0.00	0.00	0.00	0.14	0.00
Prevotella	0.00	0.00	0.35	7.09	0.00
Xylanibacter	0.00	0.00	0.35	0.83	0.00
Bacteroides	0.00	0.00	0.52	0.56	0.00
Ohtaekwangia	0.25	0.00	0.17	0.14	0.00
Runella	0.00	0.00	0.35	0.00	0.00
Leadbetterella	0.00	0.00	0.17	0.00	0.00
Nitrospira	4.81	1.68	5.40	0.00	0.00
Iamia	0.49	0.17	0.52	0.00	0.17
Ilumatobacter	0.25	0.00	0.17	0.00	0.00
Tetrasphaera	1.48	0.51	2.09	0.00	0.00

Table S7B. Continued

Nesterenkonia	0.00	0.00	0.00	0.00	0.34
Micrococcus	0.00	0.00	0.00	0.00	0.17
Microbacterium	0.00	0.00	0.00	0.00	0.17
Marmoricola	0.25	0.34	0.17	0.00	0.85
Nocardioides	0.00	0.00	0.17	0.00	0.17
Tessaracoccus	0.00	0.00	0.00	0.14	0.00
Micropruina	0.00	0.00	0.00	0.14	0.00
Brooklawnia	0.00	0.00	0.00	0.00	0.17
Mycobacterium	0.12	0.00	0.00	0.00	0.00
Skermania	0.00	0.00	0.17	0.00	0.00
Gordonia	0.00	0.00	0.00	0.00	0.17
Corynebacterium	0.00	0.00	0.00	0.00	0.51
Nakamurella	0.00	0.00	0.00	0.00	0.17
Blastococcus	0.00	0.00	0.00	0.00	0.17
Bifidobacterium	0.12	0.00	0.17	0.00	0.00
Conexibacter	0.25	0.00	0.35	0.00	0.00
Subdivision3_genera_incertae_sedis	0.49	0.51	0.17	0.00	0.00
Roseimicrobium	0.12	0.00	0.00	0.00	0.00
Prosthecobacter	0.37	0.34	0.00	0.00	0.00
Opitutus	0.62	2.36	0.17	0.00	0.00
Gp6	0.12	0.00	0.00	0.00	0.00
Gp4	0.00	0.34	0.00	0.00	0.00
Saccharibacteria_genera_incertae_sedis	0.25	0.34	0.00	0.00	1.70
Blautia	0.00	0.00	0.17	0.00	0.00
Coprococcus	0.00	0.00	0.00	0.14	0.00
Roseburia	0.00	0.00	0.00	0.14	0.17
Cellulosilyticum	0.00	0.00	0.00	0.00	0.17
Sarcina	0.12	0.00	0.17	0.00	0.00
Clostridium sensu stricto	0.00	0.34	0.35	0.28	7.31
Proteiniclasticum	0.00	0.17	0.00	0.00	0.00
Anaerobacter	0.00	0.00	0.00	0.00	0.17
Clostridium XI	1.36	0.67	2.44	0.28	6.12
Faecalibacterium	0.00	0.00	0.35	2.09	0.85
Oscillibacter	0.00	0.00	0.00	0.14	0.00
Ruminococcus	0.00	0.00	0.00	0.28	0.00
Anaerofilum	0.00	0.00	0.00	0.14	0.00
Mogibacterium	0.12	0.00	0.00	0.00	0.00
Gallicola	0.00	0.00	0.17	0.00	0.00
Streptococcus	0.12	0.00	0.17	0.00	0.17
Enterococcus	0.12	0.00	0.00	0.00	0.00
Leuconostoc	0.00	0.00	0.00	0.00	0.17

Table S7B. Continued

Bacillus	0.00	0.00	0.17	0.00	0.00
Aeribacillus	0.00	0.00	0.00	0.00	1.19
Paraliobacillus	0.00	0.00	0.00	0.00	0.17
Turicibacter	0.12	0.00	0.00	0.28	1.53
Catenibacterium	0.00	0.17	0.17	0.00	0.00
Erysipelotrichaceae_incertae_sedis	0.00	0.00	0.00	0.00	0.17
Allisonella	0.00	0.00	0.35	0.00	0.00
Megasphaera	0.00	0.00	0.17	0.28	0.00
Mitsuokella	0.00	0.00	0.00	0.14	0.00
Microgenomates_genera_incertae_sedis	0.12	0.00	0.00	0.00	0.00
Parcubacteria_genera_incertae_sedis	0.12	0.00	0.00	0.00	0.51
Fibrobacter	0.00	0.17	0.17	1.81	0.00
Armatimonas/Armatimonadetes_gp1	0.00	0.17	0.00	0.00	0.00
Elusimicrobium	0.00	0.00	0.17	0.00	0.00
Turneriella	0.00	0.00	0.17	0.00	0.00
Victivallis	0.00	0.00	0.00	0.14	0.00
unclassified_Planctomycetaceae	3.95	4.21	2.09	0.00	0.00
unclassified_Rhodocyclaceae	37.24	47.64	25.44	0.00	0.51
unclassified_Burkholderiales_incertae_sedis	2.71	4.38	2.09	0.00	0.34
unclassified_Comamonadaceae	2.59	4.55	4.18	6.12	6.29
unclassified_Burkholderiaceae	0.00	0.17	0.00	0.00	0.85
unclassified_Oxalobacteraceae	0.00	0.00	0.00	0.00	0.68
unclassified_Nitrosomonadaceae	0.74	1.68	1.05	0.00	0.00
unclassified_Neisseriaceae	0.00	0.00	0.17	0.14	0.00
unclassified_Enterobacteriaceae	6.17	0.00	10.80	0.97	0.68
unclassified_Pseudomonadaceae	0.00	0.00	0.17	0.00	1.19
unclassified_Moraxellaceae	0.00	0.00	0.00	3.34	0.00
unclassified_Xanthomonadaceae	0.12	0.17	0.35	0.00	0.00
unclassified_Succinivibrionaceae	0.00	0.00	0.17	0.00	0.00
unclassified_Aeromonadaceae	0.00	0.00	0.00	0.70	0.17
unclassified_Halomonadaceae	0.00	0.00	0.00	0.00	1.19
unclassified_Bradyrhizobiaceae	0.74	0.00	0.52	0.00	0.17
unclassified_Methylocystaceae	0.00	0.17	0.00	0.00	0.00
unclassified_Phyllobacteriaceae	0.00	0.00	0.17	0.00	0.34
unclassified_Rhodobacteraceae	3.70	0.17	1.39	0.00	0.85
unclassified_Sphingomonadaceae	0.62	0.34	0.17	0.00	0.17
unclassified_Acetobacteraceae	0.12	0.00	0.17	0.00	0.00
unclassified_Caulobacteraceae	0.00	0.00	0.00	0.00	0.51
unclassified_Polyangiaceae	0.86	2.19	0.87	0.00	0.00
unclassified_Nannocystaceae	0.62	0.17	0.35	0.00	0.00
unclassified_Cystobacteraceae	0.12	0.00	0.17	0.00	0.00

Table S7B. Continued

unclassified_Desulfovibrionaceae	0.00	0.00	0.00	0.14	0.00
unclassified_Anaerolineaceae	1.97	3.70	3.83	0.00	0.00
unclassified_Caldilineaceae	0.25	0.67	0.52	0.00	0.00
unclassified_Chloroflexaceae	0.00	0.00	0.17	0.00	0.00
unclassified_"Leptotrichiaceae"	0.00	0.17	0.00	0.83	0.17
unclassified_"Fusobacteriaceae"	0.00	0.00	0.35	0.00	0.00
unclassified_Flavobacteriaceae	0.12	0.17	0.17	0.56	0.85
unclassified_Chitinophagaceae	0.62	0.00	0.35	0.00	0.00
unclassified_"Porphyromonadaceae"	0.12	0.00	0.00	0.14	0.00
unclassified_"Prevotellaceae"	0.00	0.00	0.87	40.89	0.00
unclassified_Acidimicrobiaceae	0.00	0.00	0.17	0.00	0.00
unclassified_Intrasporangiaceae	0.25	0.17	0.35	0.00	0.00
unclassified_Nocardioidaceae	0.12	0.00	0.35	0.00	0.34
unclassified_Propionibacteriaceae	0.00	0.00	0.00	0.14	0.00
unclassified_Micromonosporaceae	0.00	0.00	0.00	0.00	0.17
unclassified_Coriobacteriaceae	0.00	0.00	0.00	0.14	0.00
unclassified_Verrucomicrobiaceae	0.12	0.17	0.35	0.00	0.00
unclassified_Opitutaceae	0.12	0.17	0.00	0.00	0.00
unclassified_Lachnospiraceae	0.25	0.00	0.35	4.73	0.34
unclassified_Clostridiaceae 1	0.37	0.17	0.52	0.70	9.86
unclassified_Peptostreptococcaceae	0.49	0.51	0.87	0.28	3.40
unclassified_Ruminococcaceae	0.25	0.34	0.00	0.97	0.68
unclassified_Bacillaceae 2	0.00	0.00	0.00	0.00	0.17
unclassified_Acidaminococcaceae	0.00	0.00	0.17	0.56	0.00
unclassified_Veillonellaceae	0.00	0.00	0.00	0.42	0.00
unclassified_Deinococcaceae	0.00	0.00	0.00	0.00	0.17
unclassified_Spirochaetaceae	0.00	0.00	0.17	0.00	0.00

Apart from the similarity in containing more unclassified bacterial communities (on a genus level) than classified ones, the failed plants differ from successful wastewater treatment plants in the composition of their bacterial communities as well as the presence of unique bacterial species. Furthermore, more than 70 unique genera were only found in successful EBPR with relative abundance ranging from 2 to 6%, while failed EBPR also revealed 69 unique genera at relative abundance ranging from 1 to 5.8% of the total microbial population (Fig. 5). It was noted that most of these unique genera from the successful EBPR belonged to *Gammaproteobacteria*, *Betaproteobacteria*, and *Actinobacteria*, while those from the failed EBPR belonged to *Alphaproteobacteria*, *Betaproteobacteria,* and *Clostridia*. The microbial composition of activated sludge has been seen as critical since they play a major role in the treatment of wastewater [21]. In order to enhance the wastewater treatment efficiency in removing phosphate, fermenter has been incorporated in the EBPR systems. The fermenter plays an important role in augmenting the availability of VFA needed by phosphate-accumulating organisms to ensure reliable phosphorus removal [5]. In the present study, fermenting bacterial genera such as *Acidovorax* (2.36%), *Enhydrobacter* (1.25%), *Aeromonas* (3.62%), *Tolumonas* (1.81%), *Prevotella* (7.09%), *Faecalibacterium* (0.56%), *Fibrobacter* (1.81%), unclassified *Comamonadaceae* (6.12%), unclassified *Moraxellaceae* (3.34%), unclassified *Prevotellaceae* (40.89%), and unclassified *Lachnospiraceae* (4.73%) were found in higher abundance in the successful EBPR as compared to the failed EBPR (Table S7), and are seen as crucial for VFA production [36-37]. However, in the failed EBPR, *Acinetobacter* known to be part of the non-fermenting bacteria [13,37] were also reported as the second most abundant genus in contrast to BWTW. In order to

remove pollutants using conventional EBPR processes, activated sludge is cycled under sequential anaerobic, anoxic, and aerobic conditions that promote the growth of specific microorganisms respective of each condition [10]. Under anaerobic conditions, the present study has shown that the bacterial community of successful EBPR was predominantly containing phosphate-accumulating bacteria such as unclassified *Rhodocyclaceae* (47.64%) [8, 38], followed by glycogen-accumulating organisms (GAOs) such as *Propionivibrio* (8.59%) [39]. Even though many EBPR wastewater treatment plants run well, the deterioration of their pollutant removal capacity due to the competition between beneficial (PAOs) and detrimental (GAOs) organisms have also been reported [5, 33]. It was further observed that the anaerobic zone of the successful EBPR also had high abundance of an additional bacterial community responsible for nitrification and denitrification, such as unclassified *Comamonadaceae* (4.55), unclassified *Burkholderiales* incertae sedis (4.38), *Planctomyces* (1.68), *Nitrospira* (1.68), unclassified *Nitrosomonadaceae* (1.68), unclassified *Anaerolineaceae* (3.7), and so on [3, 7, 33]. In contrast, despite the similarity

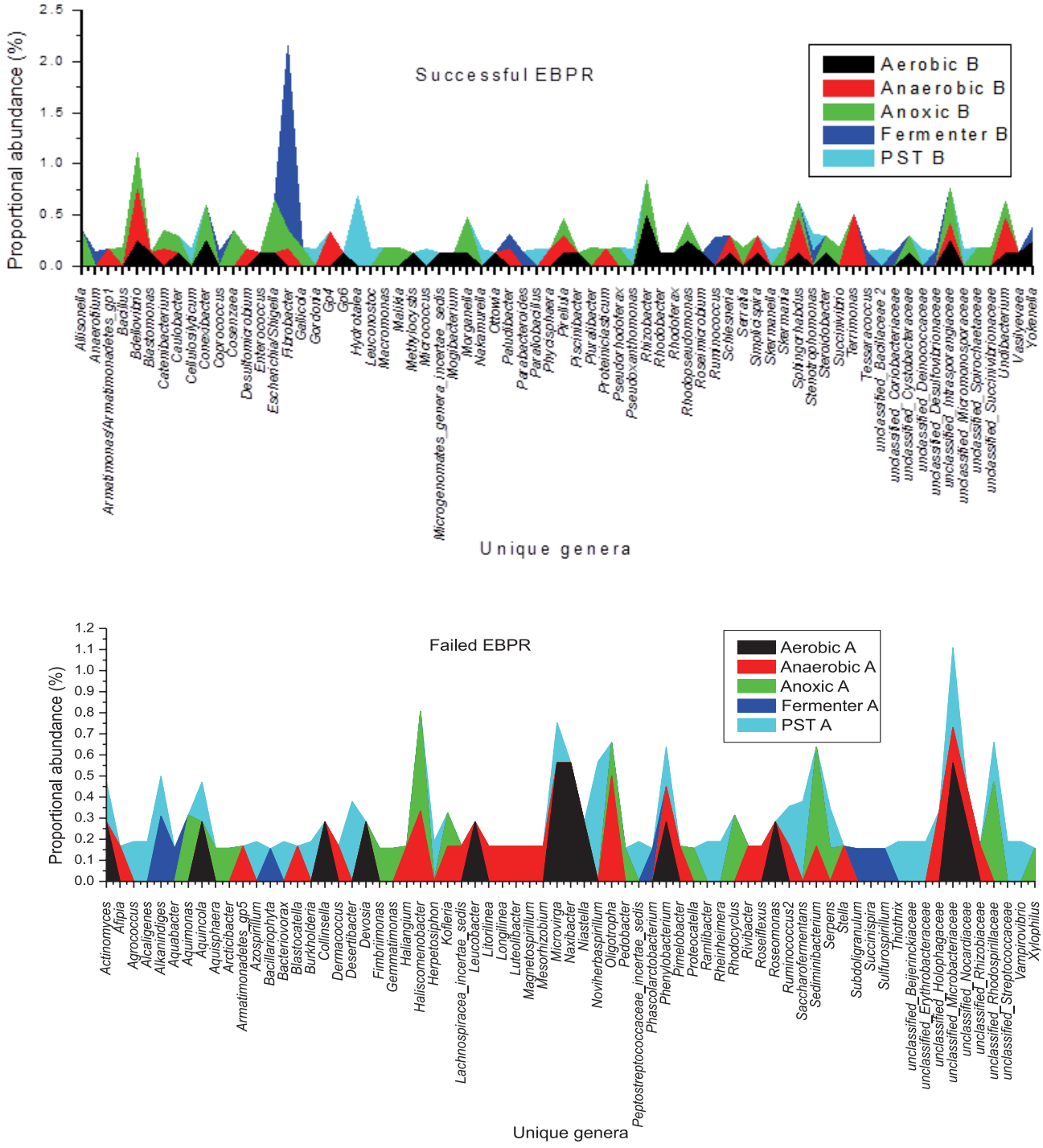

Fig. 5. Relative abundance of unique genera from failed EBPR and successful EBPR wastewater treatment plants.

of bacterial communities between anaerobic zones of both plants, the abundance of phosphate-accumulating, -nitrifying and -denitrifying bacteria at the failed EBPR were found to be very low compared to the successful EBPR. Sidat et al. [40] reported that the presence of P-removing bacteria under anaerobic conditions is due to the fact that they are needed to convert VFAs synthesised in the zone by fermenters to polyhydroxybutyrate (PHB), which is stored intracellularly. Under anoxic conditions, a significant decrease of unclassified *Rhodocyclaceae* (25.44% – over 50% compared to their abundance in the anaerobic zone) was noted, while a drastic increase of unclassified *Enterobacteriaceae* (10.8% – considered a special species due to their ability to remove both nitrate and phosphate under anoxic conditions) was also observed in the successful EBPR [3-4]. Furthermore, a large number of nitrifying/denitrifying bacteria such as *Nitrospira* (5.4%), *Proteus* (4.88%), unclassified *Comamonadaceae* (4.18%), unclassified *Anaerolineaceae* (3.83%), *Clostridium* XI (2.44%), *Arcobacter* (2.09%), *Tetrasphaera* (2.09%), unclassified *Planctomycetaceae* (2.09%), unclassified *Burkholderiales* incertae sedis (2.09%), unclassified *Rhodobacteraceae* (1.39%), *Nannocystis* (1.22%), *Caenimonas* (1.05%), and unclassified *Nitrosomonadaceae* (1.05%) was also noted. In contrary to the successful EBPR, the failed EBPR showed an increase in abundance of unclassified *Rhodocyclaceae* (35.79%), unclassified *Anaerolineaceae* (9.26%), and *Propionivibrio* (5.49%) when compared to anaerobic and anoxic zones. In addition, the anoxic zone of the failed EBPR also showed a diverse nitrifying and denitrifying bacterial community despite the fact that there was less abundance compared to the anoxic zone of the successful EBPR. It has been proven that anaerobic and anoxic conditions are designed to promote nitrification and denitrification, respectively [5].

Under subsequent aerobic conditions, the present study revealed that both plants had a significant difference in terms of predominant microbial genera. The successful EBPR was found to have high relative abundance of PAOs with unclassified *Rhodocyclaceae* (37.24%) as the most dominant one, followed by *Proteus* (6.41%). In the failed EBPR however, *Acidovorax* (26.2%) was reported to be the most abundant genera, followed by unclassified *Comamonadaceae* (13.52%) and *Brevundimonas* (6.76%). Despite the fact that *Acidovorax* is not so common in the removal of phosphate, Ren et al. [41] reported that this genus can participate in the removal of both phosphate and nitrite. Gonzalez-Gil and Holliger [42] further reported that most of the species belonging to *Acidovorax* are capable of heterotrophic denitrification of nitrate and may utilise acetate, propionate, and PHB from decomposing cells as the carbon source for denitrification. However, this was in disagreement with Siezen and Galardini [43], who reported that species belonging to *Acidovorax* are mostly responsible for biofilm formation and flocculation. The second most abundant genus (*Comamonadaceae*) at the failed EBPR has also been reported by Ong et al. [44] as being strictly aerobic, non-fermentative, and capable of accumulating PHB. Moreover, the present study also revealed the presence of nitrifying and denitrifying bacteria in the aerobic zone (Table S7). Fu et al. [45] reported that a denitrifier's ability to grow in aerobic conditions is probably attributed to the variation of DO concentration gradient in the biofilm, creating the anoxic micro-environment in the deeper layer of biofilm, which is beneficial for the growth of denitrifiers. Bai et al. [3] also stated that the coexistence of nitrifying and denitrifying bacteria in the aerobic zone is beneficial as it facilitates simultaneous nitrification and denitrification occurring in the zone. In the present study, most of the dominant microorganisms recovered have been reported previously for their involvement in the removal of phosphate and nitrate from wastewater [7, 46-48]. Furthermore, the microbial pattern noted in the present study is also similar to previous observations [4, 41, 49]. It should be mentioned that not many studies have been carried out to investigate microbial ecology from the full-scale EBPR. In addition, this study corroborates with the findings by Zhang et al. [31] in reporting some rare bacterial classes: *Acidobacteria* Gp3, *Acidobacteria* Gp6, and *Acidobacteria* Gp4. However, the study disagrees with the findings of Martín et al. [50] and Albertsen et al. [4] by revealing that PAOs populating the selected EBPRs were closely related to *Rhodocyclus* bacteria and *Actinobacteria* instead of *Candidatus Accumulibacter phosphatis*. The results showed that most of the samples from failed and successful EBPRs had almost the same composition as they were clustered together with Jaccard values ranging from 65 to 100%. These results were confirmed by the rarefaction curves that showed little to no existing dissimilarity between treatment zones of both plants with the exception of the aerobic zone. However, Lawson et al. [32] also revealed low dissimilarity between the bioreactor representing the lab-scale EBPR.

Environmental Variables

The results of the physicochemical parameters of wastewater samples collected from different zones (PST, fermenter, anaerobic, anoxic, and aerobic) of the failed and successful EBPR treatment plants are shown in Table S8. As a phosphorus removal plant, the results revealed a decrease in phosphorus content from 33.35 mg L^{-1} to 0.97 mg L^{-1}, and 18.25 mg L^{-1} to 1.91 mg L^{-1} from the successful and failed EBPRs, respectively, as wastewater was subjected to anaerobic and aerobic treatment from EBPRs. A similar observation was noted with COD, which decreased from both EBPRs. The collected wastewater samples appeared to be neutral to slightly alkaline (Table S8). However, temperatures ranged 22.2-23.5ºC for the successful EBPR and 22.2-23.4ºC for the failed EBPR, showing that both reactors had similar temperature conditions.

DO depletion from aerobic to anaerobic zones highlighted the good performance of the treatment plants. Electrical conductivity (EC) as salinity has been reported as a significant parameter in the bacterial community as

Table S8: Environmental variables of the wastewater samples

	Parameters	PST	Fermenter	Anaerobic	Anoxic	Aerobic
Successful EBPR	Phosphate(mg L^{-1})	33.35±4.2	22.13±1.02	26.38±2.93	16.89±0.97	0.97±0.01
	Nitrate (mg L^{-1})	3.23±0.89	5.20±0.75	3.17±1.34	3.87±0.83	3.07±0.07
	COD (mg L^{-1})	198.50±25.47	164.33±7.97	60.17±7.45	43.95±2.84	27.67±0.98
	EC (µS cm^{-1})	634.00±94.15	639.00±34.75	827.00±41.009	606.00±19.65	589.00±47.81
	Temperature (ºC)	23.00±2.92	22.20±2.94	22.80±2.84	22.60±2.89	23.50±3.67
	DO (mg L^{-1})	6.10±1.71	1.64±0.92	0.09±0.01	0.27±0.02	2.36±0.05
	pH	7.21±0.23	7.08±1.54	7.00±1.62	6.99±0.95	7.27±0.07
Failed EBPR	Phosphate(mg L^{-1})	18.25±2.27	25.63±5.03	26.65±2.64	18.61±2.84	1.91±0.01
	Nitrate (mg L^{-1})	3.79±0.21	3.19±1.84	2.01±0.81	3.35±0.92	4.11±0.03
	COD (mg L^{-1})	225.17±17.07	181.83±43.54	96.83±17.94	18.50±1.67	35.17±2.74
	EC (µS cm^{-1})	655.00±26.93	664.00±39.65	947.00±25.84	753.00±52.93	755.00±21.93
	Temperature (°C)	23.00±1.98	22.20±3.29	22.80±3.91	23.40±2.91	22.30±1.95
	DO (mg L^{-1})	3.95±0.98	1.65±0.10	0.02±0.00	0.38±0.03	1.09±0.01
	pH	7.00±1.69	7.01±0.81	6.98±0.08	7.23±0.92	7.08±0.02

it regulates their composition and diversity [13, 51-52]. Similar to EC, pH was also seen as an important factor with temperature and DO regulating the overall diversity and composition level of bacteria in aquatic environments [13]. Based on the variance inflation factors, Wang and co-authors [13] revealed that COD, total nitrogen, total phosphorus, DO, pH, and temperature variations were relatively correlated to the bacterial communities. In their study investigating the impact of temperature on microbial communities, Siggins et al. [53] also revealed that temperature is one of the most important parameters influencing bacterial composition in the environment. This was also revealed by the present finding when performing the canonical correspondence analysis (CCA) to assess the correlation between environmental factors and bacterial communities (Fig. 6). The CCA diagram displayed 99.97% of the variance in the bacteria-environmental relationships at the first axis and 0.033% of the variance at the second axis. However, the relationship between environmental factors and bacterial shift (pyrosequencing data) was significant at both the first and the second axis ($p<0.05$). Environmental factors – namely COD, DO, pH, temperature, and PO_4^{-3} – showed a strong positive correlation with the first axis and a negative correlation with the second axis, while NO_3^- showed a strong positive correlation with both first and second axes. Electrical conductivity showed a strong negative correlation with both the first and second axes. While comparing EBPR zones, anoxic as well as anaerobic zones from both successful and failed EBPRs were primarily linked to EC, while fermenters linked to nitrate. Not surprisingly, the aerobic zone from the successful EBPR appeared to be linked to COD, DO, pH, temperature, and PO_4^{-3} load, while the aerobic zone from the failed EBPR was only linked to nitrate load, which highlighted the possible dissimilarity. Furthermore, temperature and pH appeared to have low or no significant impact on bacterial community structures in both EBPRs despite being positively correlated with the first axis and ne-gatively correlated with the second. This study is in agreement with the findings of Wang et al. [10], who reported the possible links between COD, DO, and temperature with microbial community functional structures in EBPRs.

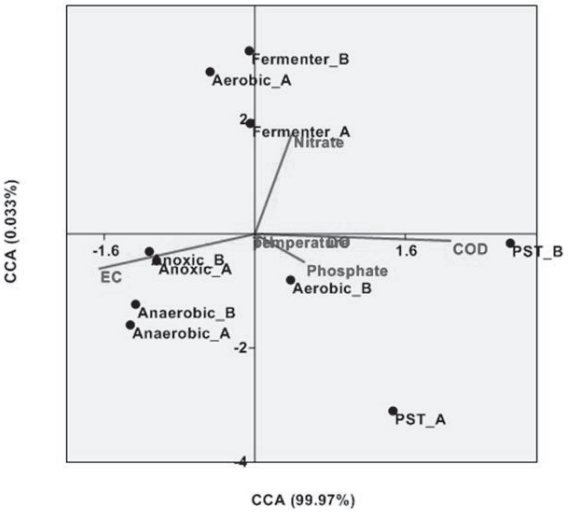

Fig. 6. Canonical correspondence analysis (CCA) comparing the variation of environmental parameters (EC: electrical conductivity) from each sampling point (PST, fermenter, anaerobic, anoxic, and aerobic) and their possible effects on a bacterial community. The CCA first axis (99.97%) and the second axis (0.033%) show the observed variation between parameters.

Conclusion

This study investigated the bacterial community of two full-scale EBPR wastewater treatment plants using a pyrosequencing platform. A comparison of the bacterial diversity of the two full-scale EBPRs provided links between the bacterial community structures from both successful and failed EBPRs. It was observed that *Proteobacteria* was the most predominant phylum of both EBPRs within each sampling zone, except in fermenters, where *Bacteriodetes* was the dominant one. Apart from the 18 phyla shared between both EBPRs, two unique phyla were noted for successful EBPR (*Chlamydiae and Gemmatimonadetes)* and failed EBPR (*Microgenomates* and *Fibrobacteres*). In the present study the constant failure of Bushkoppies was noted to be due to less abundance of fermenting bacteria from the fermenter and less abundance of nitrifying/denitrifying as well as phosphate-accumulating bacteria in the bioreactor. Furthermore, the present study suggested that the high abundance of *Acidovorax* in the Bushkoppies aerobic zone could have also been the cause of the constant failure of this EBPR. The study also showed a relatively high abundance of detrimental bacteria such as GAOs throughout all zones of this bioreactor when compared to the successful EBPR. However, bacterial communities from both EBPRs showed a similar correlation with EC in fermenters and anaerobic zones, while temperature and pH showed a similar correlation throughout all the EBPRs. The difference between the successful and failed EBPRs was most apparent in the aerobic zones, as this zone from both EBPR was linked with different environmental factors.

Authors' Contributions

IK Conceived and designed the experiments; IK MT Contributed reagents/materials/analysis tools; IK carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript; and IK SA MT critically reviewed the manuscript. The three authors read and approved the final manuscript.

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