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Water Quality and Microbial Community Changes in an Urban River after Micro-Nano Bubble Technology *in Situ* Treatment

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Abstract: Currently, black-odor river has received great attention in China. In this study, the micro-nano bubble technology (MBT) was used to mitigate the water pollution rapidly and continuously by increasing the concentration of dissolved oxygen (DO) in water. During treatment, the concentration of DO increased from 0.60 mg/L to over 5.00 mg/L, and the oxidation reduction potential (ORP) also changed from a negative value to over 100.00 mV after only five days aeration. High throughput pyrosequencing technology was employed to identify the microbial community structure. At genus level, the dominant bacteria were anaerobic and nutrient-loving microbes (e.g., *Arcobacter sp.*, *Azonexus sp.*, and *Citrobacter sp.*) before, and the relative abundances of aerobic and functional microbes (e.g., *Perlucidibaca sp.*, *Pseudarcicella sp.*, *Rhodoluna sp.*, and *Sediminibacterium sp.*) were increased after treatment. Meanwhile, the water quality was significantly improved with about 50% removal ratios of chemical oxygen demand (COD_{Cr}) and ammonia nitrogen (NH_4^+-N). Canonical correspondence analysis (CCA) results showed that microbial community structure shaped by COD, DO, NH_4^+-N, and TP, CCA1 and CCA2 explained 41.94% and 24.56% of total variances, respectively. Overall, the MBT could improve the water quality of urban black-odor river by raising the DO and activate the aerobic microbes.

Keywords: long term performance; black-odor urban river; micro-nano bubble technology; *in situ* treatment; biodiversity

1. Introduction

Urban watercourses are essential and important to the urban ecosystem, landscape ecology and citizen's life. However, in recent decades, rapid industrialization and urbanization has accelerated the worsening waters in urban area in China. Urban rivers are usually used as drains for sewage, industrial wastewater and even domestic garbage, which contained various pollutants. When organic pollutants are discharged to rivers, part of them is precipitated in the sediment and the others are metabolized by microbes [1,2]. During the metabolism, the microbes exhaust the dissolved oxygen in the water and make the aquatic environment anoxic, which promotes the growth of anaerobic microbes. Meanwhile, the anaerobic digestion produces odor gases (e.g., H_2S) [3] and black substances

(e.g., FeS) [4,5]. As a result, the river is in anoxic condition, showing black appearance and stinking smell. These black-odor rivers flows through the cities affect residents' life and pose a serious threat to human health [6]. Thus, treating the polluted urban rivers and recovering their ecological function have attracted much attention in China.

It is well known that microbes play an important role in biogeochemical cycling of aquatic ecosystem, which could decompose, transform and mineralize organic or inorganic matters in urban rivers [7]. Additionally, microbes are the first that interacted with dissolved substances and severely impacted by perturbations of the water quality [8,9]. Previous study showed that although microbes could adapt to the environment fluctuation to some extent, the microbial structure will shift significantly with intensive change of the ambient [10]. Thereby, microbes could be used to monitor and evaluate the pollution of water, and increasing the concentration of dissolved oxygen in the water body is the primary method to alleviate the pollution of urban streams.

Many physiochemical or biological treatment methods, which involve plants, aeration, chemical agents and bacterial inoculation exclusively or in combination to remove or metabolize the pollutants [11–13], and to mitigate the pollution in different extents. Lately, the micro-nano bubble technology (MBT) has been applied in water treatment, resulting in good effects. Micro-nano bubbles are small bubbles with diameters of several micrometers and nanometers, with high self-pressurization and longer lifetime [14,15], which have recently been explored as promising candidates for aeration. The primary application of micro-nano bubble was focused on activating the microorganisms, flotation, water treatment, and aeration [14,16,17]. Unlike the normal macro bubbles (with diameter 1–10 mm), the micro-nano bubbles would swell and burst in liquid with high mass transfer [15], and significantly increase the concentration of dissolved oxygen (DO) in water, reaching to the possible oversaturation. For these excellent properties, MBT would resolve the problem of hypoxia in polluted river efficiently. Recently, micro-nano bubbles were used to mitigate the pollution by enhancing the concentration of DO and eliminating the impurities in the river [18].

In this study, MBT was used for aeration and enhance the concentration of DO in an urban black-odor river, and the long term performance of MBT treatment was investigated. In the Pearl River Delta, featuring highly-developed manufacturing industry, a river was picked to be the trial target of MBT experiment. The object was figured out the MBT influence on microbial community structure, explored the effect on DO, oxidation reduction potential (ORP), chemical oxygen demand (COD_{Cr}), ammonia nitrogen (NH_4^+-N), pH, and total phosphorus (TP) [19]. The relationship between microbes and physicochemical factors of water was also investigated.

2. Material and Methods

2.1. Study Site and Sampling

The urban river lies between 22°45′24″ N, 113°47′9″ E and 22°46′13″ N, 113°46′52″ E, the city of Dongguan, Guangdong Province, South China. This area is dominated by a typical subtropical marine climate with annual average temperature around 22.2 °C, data are given in Figure S1 in the Supplementary Information (SI). This river is a tributary of Maozhou River, which is connected to the South China Sea.

Considering the past influences of the drain outlets along the rivers and tidal water, we set up the sampling sites at the upper reaches of the river. In this study, three sampling sites (marked as S1 (22°46′8″ N, 113°46′51″ E), S2 (22°45′53″ N, 113°46′53″ E), and S3 (22°45′43″ N, 113°46′57″ E)) were picked at the deep areas along the river, with an over 300 m distance from each other (Figure 1). To study the long-term performance of MBT, the sampling work lasted for 12 months from 19 July 2017 to 27 August 2018, including 3 periods. The first period was from day 0 to day 3, which showed the original physiochemical properties of the river before treatment. The second period was from day 4 to day 39, which was the continuous aeration period (aeration for 24 h per day). The third period was from day 40 to day 157, which was intermittent aeration (aeration for 6 h per day). The work was paused

during February (28 days), 2018 for the Spring Festival. During the treatment, we sampled every day before day 40, and sampled every Monday, Thursday, and Saturday from day 40 to day 157. Samples for microbial community research were collected on 20 July (d1), 25 August (d20), 30 September (d37), 30 October (d67), 30 April (d133), and 31 May (d146), respectively. The temperature was around 25~35 °C when taken with the microbial samples to minimize the influence of temperature. Samples for COD_{Cr}, NH_4^+-N and TP analysis were collected with a van Dorn bottles under the surface of river (0.5 m) and sent to the laboratory immediately. The DO, pH, and ORP were measured *in situ* at the subsurface using a multipara meter quality detector (Hydrolabi DS5, HACH, USA). The concentration of TP, COD and NH_4^+-N were measured according to the standard methods (APHA, 2012) [20]. The ORP was only monitored from day 0 to day 39 for confirming its relationship with DO. Before the treatment, all the drain outlets were blocked.
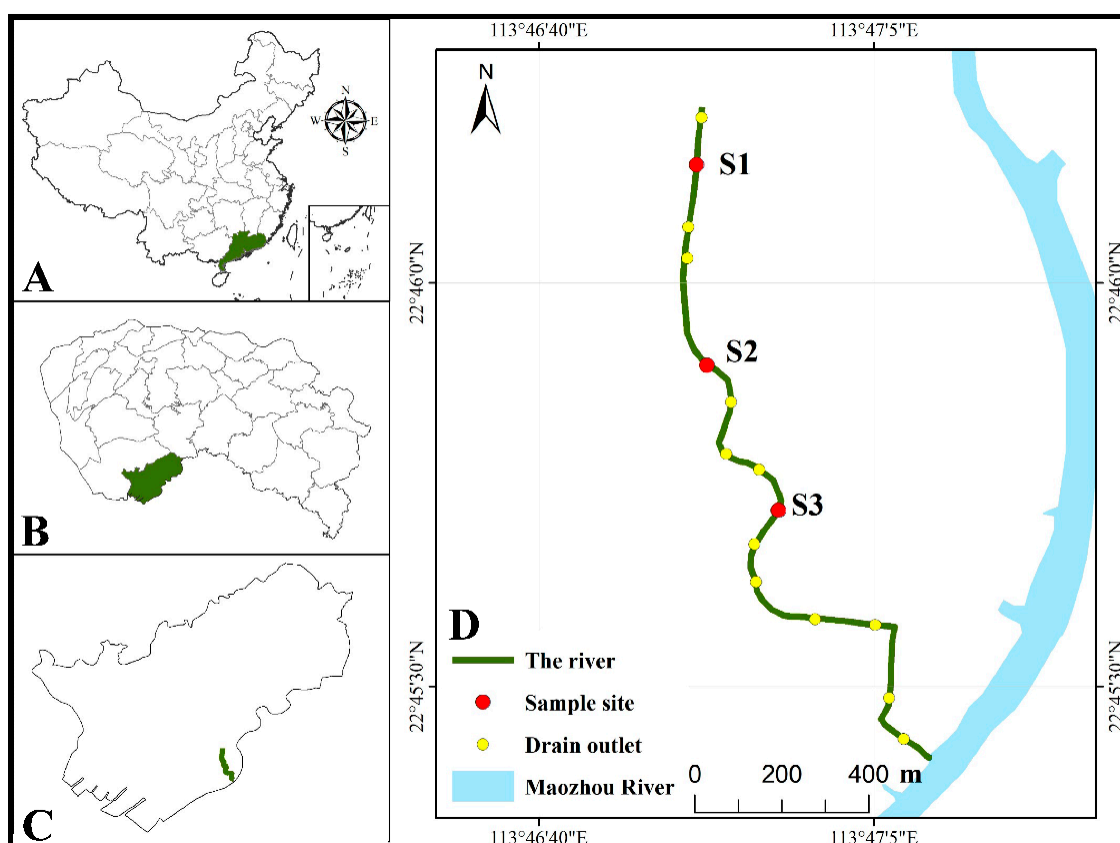

Figure 1. The location of Dongguan City in China (**A**), the location of Changan Town in Dongguan City (**B**), the location of the river in Changan Town (**C**), and the sampling sites of this study (**D**).

2.2. DNA Extraction and Illumina Miseq Sequencing

An E.Z.N.ATM Mag-Bind Soil DNA Kit (Omega, Bio-Tek, Norcross, GA, USA) was employed to extracted DNA by following the manufacturer's instructions. The PCR amplification was performed according to the Illumina 16S Metagenomic Sequencing Library preparation guide (Illumina). The V3-V4 hypervariable regions of the bacteria 16S rRNA gene were amplified with primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 805R (5′-GACTACHVGGGTATCTAATCC-3′) by the thermocycler PCR system. The purified amplicons were measured by Qubit dsDNA HS Assay Kit (Life Technologies, Eugene, OR, USA). The purified amplicons were pooled in equimolar, using the Illumina MiSeq system by Sangon Biotech (Shanghai, China). Sequences containing ambiguous bases and any longer than 480 base pairs (bp) were dislodged and those with a maximum homopolymer length of 6 bp were allowed [21]. Any sequence shorter than 200bp were removed.

2.3. Statistical and Bioinformatics Analysis

Raw fastq files were de-multiplexed and quality-filtered using QIIME (version 1.17). The OTUs were clustered with 97% similarity [22] by UPARSE (version 7.1) [23], and chimeric sequences were identified and removed using UCHIME [24]. In this study, MOTHUR [25] was used to calculate the Shannon's diversity, Simpson index, ACE and Chao 1 for each sample. Coverage is defined as: $C = 1 - \frac{n_1}{N}$, where n_1 refers to the number of the singleton OTU, N is the number of the total sequences. ACE and Chao 1 can estimate the species richness.

The hierarchical clustering tree on OTU level was established based on Bray-Curtis distance with Vegan package within R. The principal coordinate analysis was based on the UniFrac dissimilarity values and performed to interpret the microbial communities' relative similarity from each sample. The Canonical correspondence analysis (CCA) was used to determine the correlations between microbial communities and environmental factors by Vegan package within R. The microbial community functions were predicted by Clusters of Orthologous Group (COG).

3. Results and Discussion

3.1. Water Quality Improvement of Black-Odor River

Selected water physicochemical indexes, such as DO, ORP, COD and so on, were used to evaluate the river condition and impact assessment. The result showed that the river was polluted with low DO (0.60 mg/L–1.02 mg/L) and ORP (around −140.00 mV) levels before treatment, and then the DO and ORP values were significantly increased during MBT treatment and stabilizes gradually (Figure 2). During the continuous aeration period, the DO increased to 4.00 mg/L–7.50 mg/L, and then were stable around 4.00 mg/L during intermittent aeration (Figure 2A). In like manner, the ORP value increased from negative values to around 130.00 mV during continuous aeration period, and then stable. The result in March 1 showed that the DO was decreased after one month without aeration due to the Spring Festival, while it was recovered to previous values after several days of intermittent aeration. The sharp decreasing of DO and ORP values at day 20 might due to the discharge of waste-water when sampling. Meanwhile, the pH was around 7.00 during the treatment, which was not affected by MBT. In addition, the micro-nano bubbles could combine the impurities in the river, then float upon water and been eliminated, for which, the turbidity of the river was decreased from 148.00 NTU (d1) to around 21.00 NTU (d157) (data not shown).
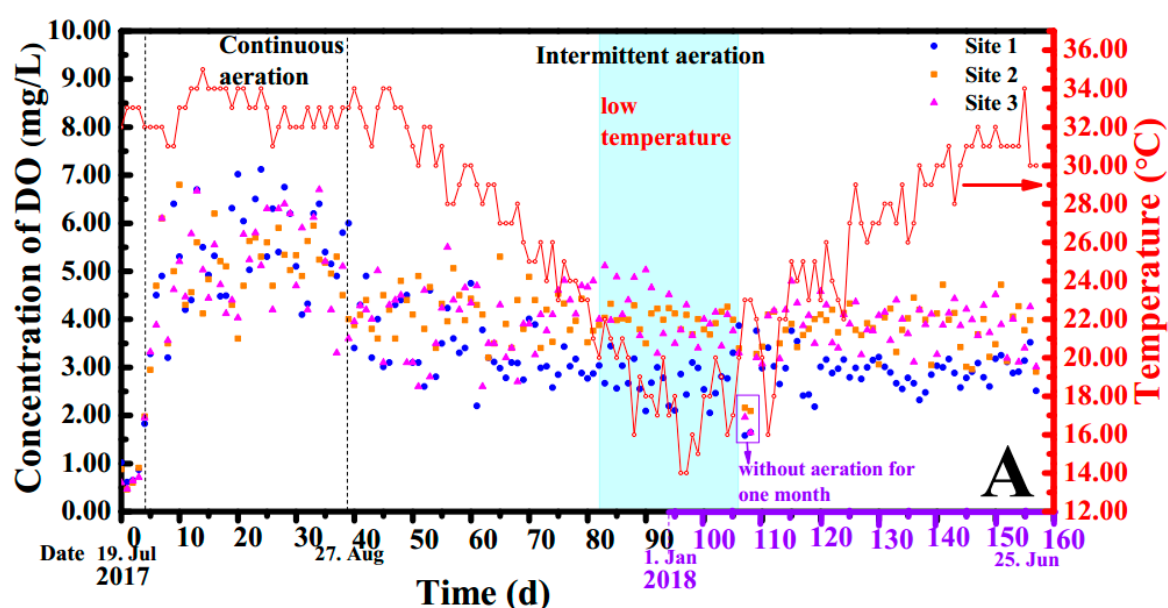

Figure 2. *Cont.*
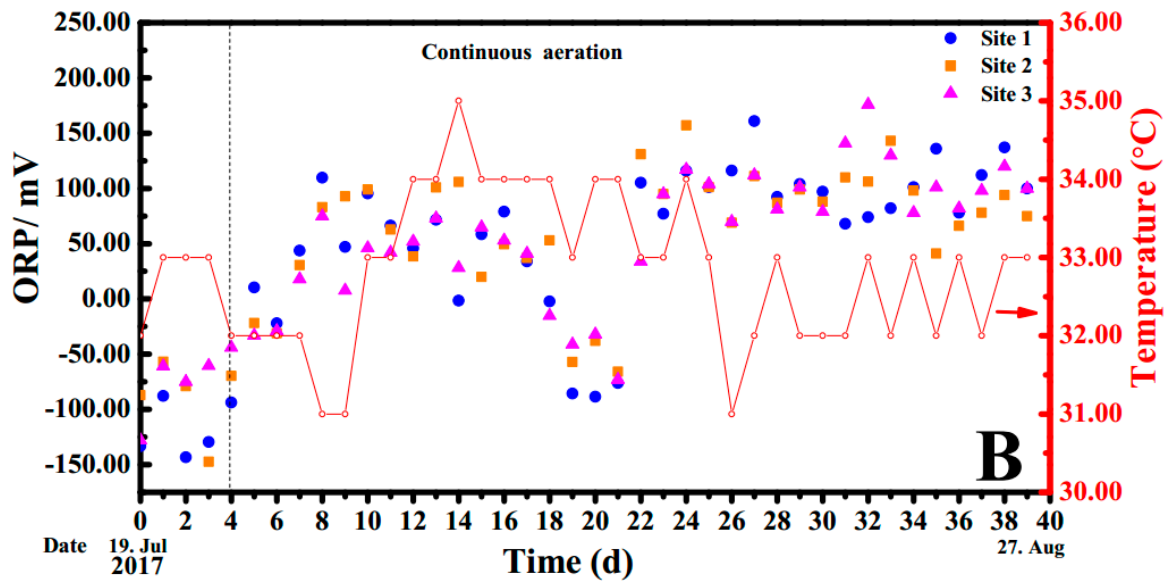
Figure 2. The DO and ORP improvement of river (**A**: DO, **B**: ORP).

A massive amount of nitrogen and phosphorous in aquatic environment led to eutrophication of urban rivers. In this study, the concentration of NH_4^+-N was increased at the beginning of aeration and then decreased to a lower value (Figure 3A). The change from continuous aeration to intermittent aeration had affected the concentration of NH_4^+-N, which was steady gradually at the following time. The concentration of NH_4^+-N was increased during the cold days, which might relate to the low activity of microbes with low temperatures [26]. Site 3 was closed to the Maozhou River, and diluted by the tidal river, resulting in lower concentration of NH_4^+-N after aeration. During the sampling period, the concentration of TP didn't decreased as other factors. The aeration even promoted the phosphorus release from sediment and caused the values a little higher than before, data are given in Figure S2 in SI. Meanwhile, the COD_{Cr} value could reflect the extent of river pollution and pollution level. After treatment, the COD_{Cr} of the river decreased significantly (Figure 3B) and stayed at a low level around 45.00 mg/L. The COD_{Cr} value increased after February and decreased to former concentration in a few days with intermittent aeration. The results showed that the intermittent aeration is necessary for guaranteeing the water quality.
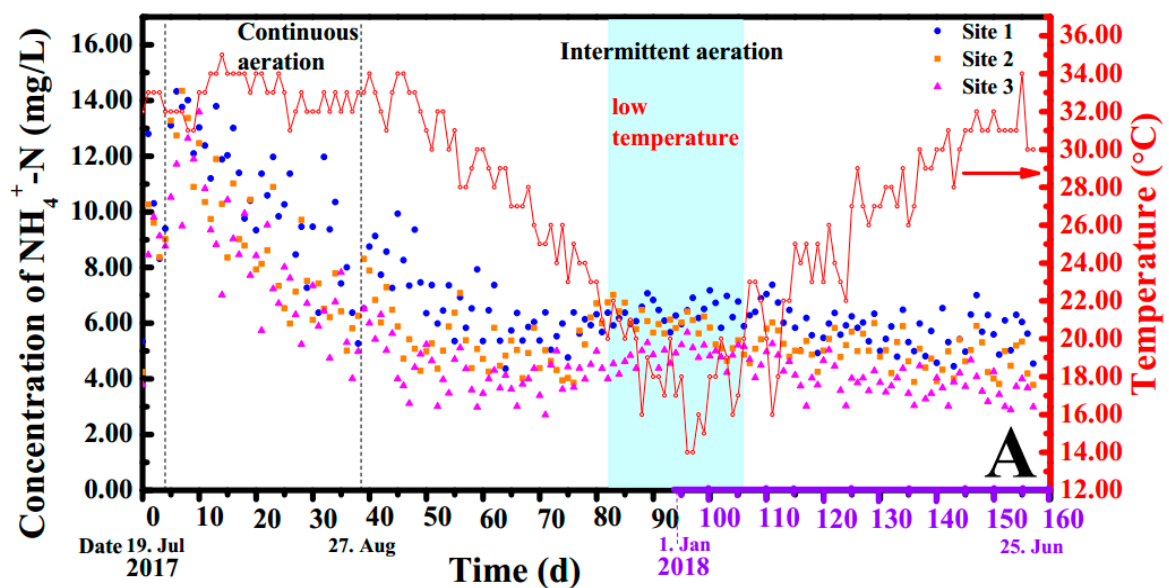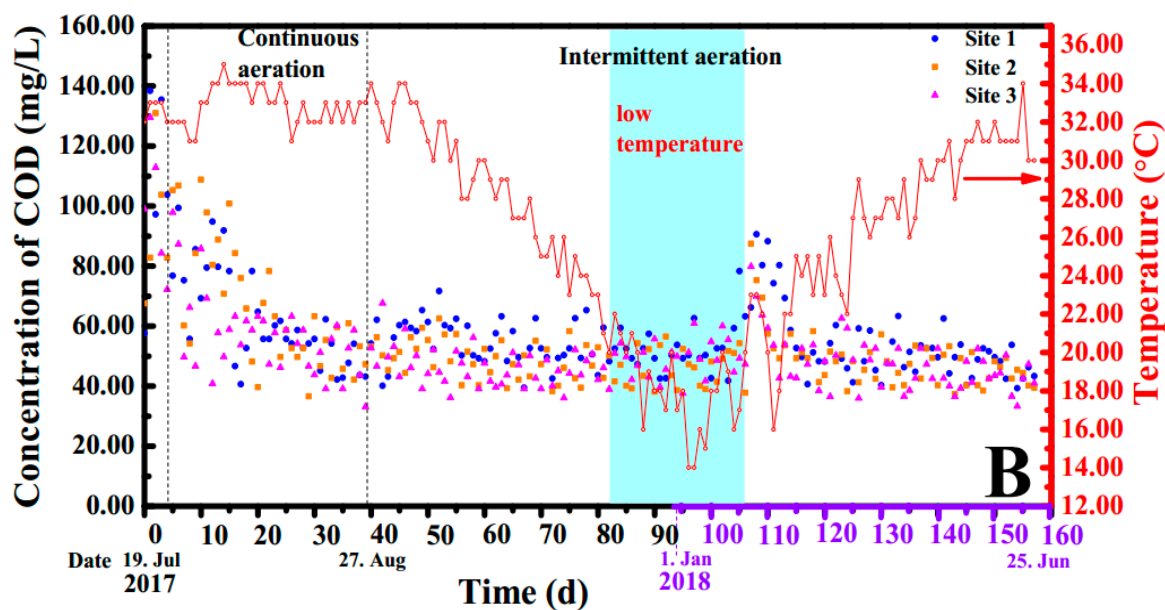

Figure 3. *Cont.*

Figure 3. The NH$_4^+$-N and COD improvement of river (**A**: NH$_4^+$-N, **B**: COD).

At the beginning of aeration, the value of DO and ORP was significantly increased, meanwhile the aeration also promoted the organic matters releasing from sediment, therefore, the concentration of organic matters was increased first and was then followed by a decreasing trend. Finally, the physicochemical properties of river were improved and stabilized gradually at the later stage of treatment. It should be noted that the long-term intermittent aeration would be necessary to ensure the water quality of the polluted river if the internal pollution was not done.

3.2. Change of Microbial Profile Alongside MBT Treatment

The microbes would respond to the change of aquatic environment, such as the physicochemical properties, organic and inorganic matters. The number of clean sequencing was changed from 59,485 to 32,237, and the coverage was more than 98.50%, which presented the real microbial information in the river. Before the treatment, there were 1845 of OTUs in the river (day 1); at day 37, there were 1603 of OTUs; at day 146, there were 968 of OTUs. The results showed that the OTU number was changed with the water quality of the river, which showed a decreasing trend. The Chao 1 and ACE indices showed that the microbial community richness was decreased after MBT treatment, data are given in Table S1 in SI. However, the Shannon and Simpson indices had little change, indicating that the microbial evenness was higher after treatment. There were 224 to 565 unique OTUs were detected during the treatment, indicating that the MBT treatment activated some indigenous microorganism at different stage of the treatment. The decreasing trend might be related to the decreasing concentration of organic matters, the lower nutrition for microbial growth in the river, the lower microbes' richness and diversity. At the same time, the microbes, which adapted to the condition of river, would become the new dominant species.

To illustrate the relationship among microbial communities from different samples, the cluster tree using Bray-Crutis were constructed according to the OTU distribution at 97.00% similarity level. According to Figure 4, the six samples were clustered into four major groups: The first group was composed of d1 and d20, d67, and d133 was the second and third group separately, d37 and d146 were the fourth groups. According to the previous study, the microbial community would shift with the ambient [27], which demonstrated that the river conditions were similar in every single group. In total, the results showed that microbial community would shift with the changing environment in urban river during the treatment by MBT, and the biodiversity showed a decreasing trend with the improvement of river quality.
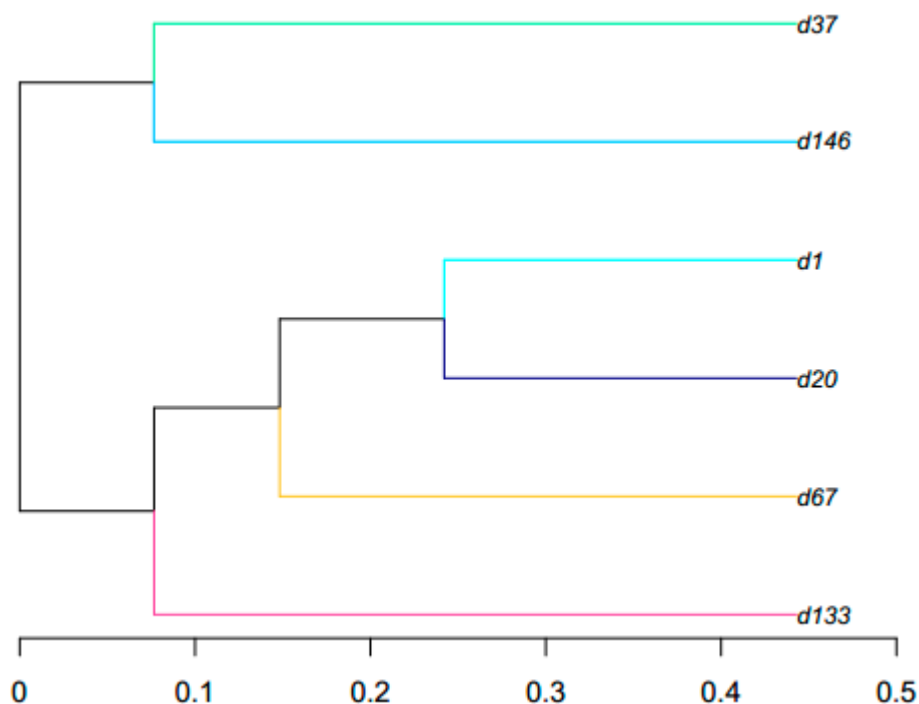
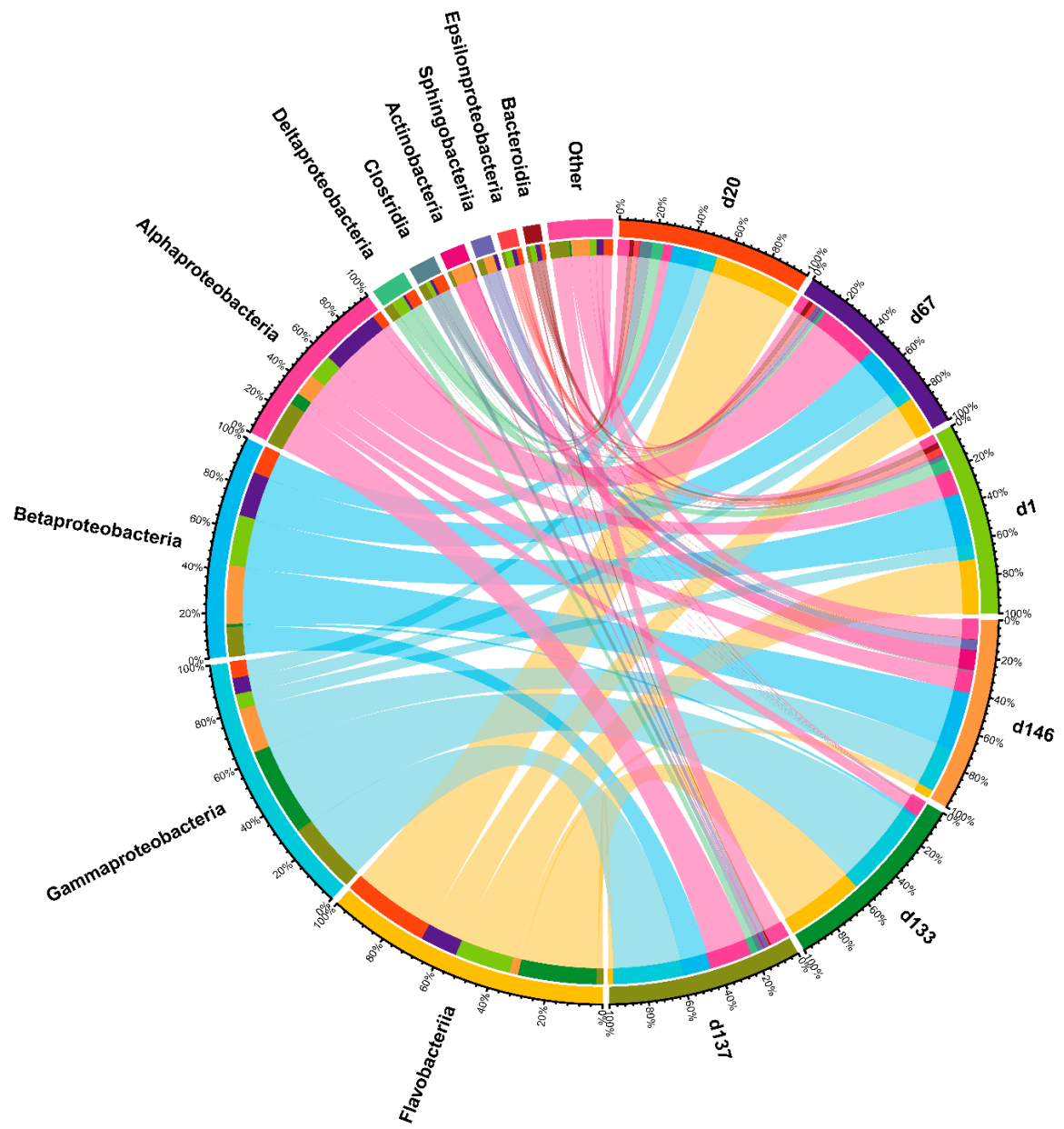**Figure 4.** Bray-Curtis tree based on the 97% similarity of the OTUs.

3.3. Change of the Microbial Community Structure

The change of microbial community composition is the key point of microbial impact of MBT. For better understanding the succession of microbial community during the treatment, the taxonomic affiliation at class level and genus level were analyzed.

3.3.1. The Microbial Community Succession at Class Level

The Figure 5A shows the distribution of microbes at class level (abundance $\geq$1%). The *Alphaproteobacteria*, *Betaproteobacteria*, *Deltaproteobacteria*, *Epsilonproteobacteria*, *Gammaproteobacteria*, *Flavobacteriia*, *Actinobacteria*, *Clostridia*, *Sphingobacteriia*, and *Bacteroidia* were dominant microbes at class level. The Alphaproteobacteria, Gammaproteobacteria and Deltaproteobacteria are usually abundant in marine environment [28]. The tidal water brought seawater and marine microbes, affecting the microbial community structure in the river. For other microbes, though they were still abundant after treatment, the relative abundance was changed. The relative abundance of *Actinobacteria*, *Cytophagia* and *Sphingobacteriia* were increased significantly after MBT treatment, which were found in oligotrophic aquatic environment and able to degrade biopolymer [29]. Furthermore, it was reported that *Actinobacteria* and *Verrucomicrobiae* are more abundant in sediment samples than water [30]. The increased abundance of *Actinobacteria* and *Verrucomicrobiae* indicated that MBT might encourage the microorganisms migrated with nutrients from sediment to water.

The *Bacteroidia*, *Clostridia*, *Deltaproteobacteria*, *Flavobacteriia* and *Synergistia* were decreased after MBT treatment, most of which were reported as potential opportunistic pathogens or nutrient-loving microbes under anaerobic condition. For example, *Bacteroidia* and *Flavobacteriia* were reported as potential fecal indicator bacteria [31], *Deltaproteobacteria* could metabolize sulfate in anaerobic environment [30]. The decreased trend of these microbes suggests that after MBT treatment, the aquatic environment was not suitable for these microbes. In all, the result showed that MBT would influence the abundance of microbes, which was mainly related to the concentration of DO and organic matters.
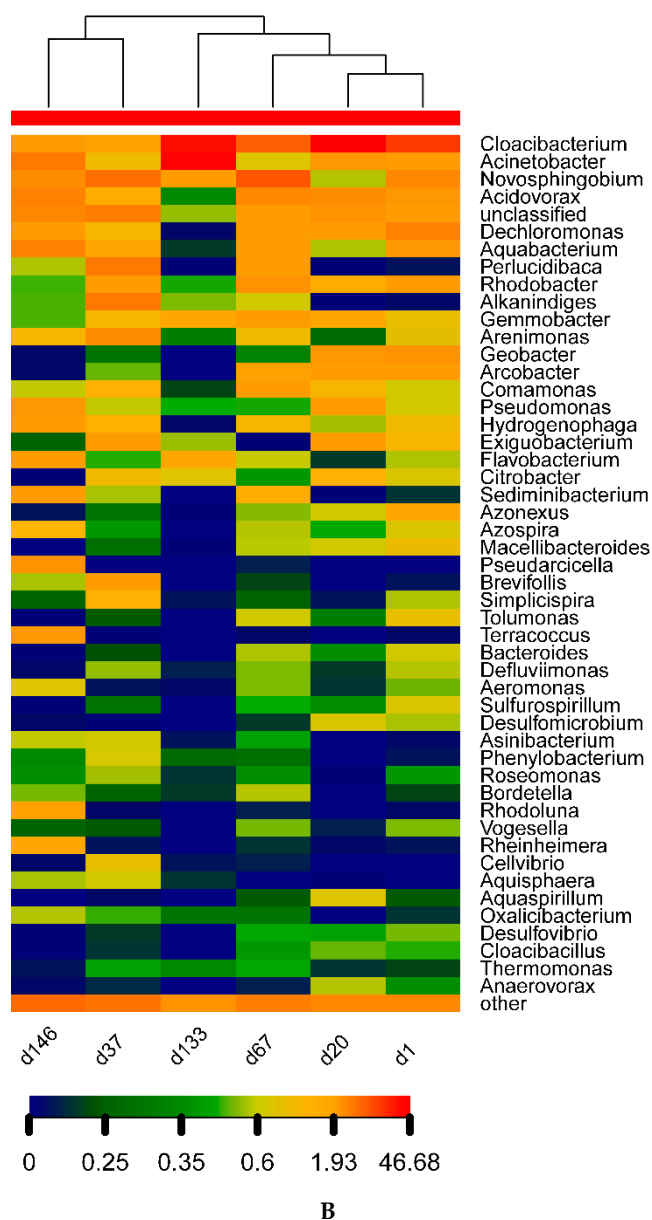
A

Figure 5. *Cont.*

Figure 5. Community structure and distribution of samples (**A**: At class level by Circos analysis; **B**: At genus level by heatmap).

3.3.2. The Microbial Community Succession at Genus Level

The Figure 5B showed the response of predominant genera (abundance ≥ 0.1%) to MBT treatment, *Acidovorax*, *Acinetobacter*, *Cloacibacterium*, and *Novosphingobium* were abundant before and after MBT treatment, living in fresh water commonly [32]. The abundance of *Arcobacter sp.*, *Azonexus sp.*, *Citrobacter sp.*, *Dechloromonas*, *Flavobacterium*, *Rhodobacter sp.*, and *Geobacter sp.* were decreased after treatment, which might be related to the increasing DO and the decreasing concentration of organic matters [33,34]. In contrast, the abundance of *Perlucidibaca sp.*, *Pseudarcicella sp.*, *Rhodoluna sp.*, and *Sediminibacterium sp.*, were higher after MBT treatment. Most of these genera were aerobic bacteria and functional microbes, such as *Sediminibacterium sp.* and *Rheinheimera*, which were aerobic microbes and predominant in freshwater, marine and sediment [35,36].

At different stages of treatment, the dominant microbes were different. For example, before treatment, the dominant microbes prefer anaerobic and eutrophic condition (e.g., *Dechloromonas* and *Flavobacterium*); during the treatment, the abundance of sediment microbes were increased

(e.g., *Actinobacteria* and *Verrucomicrobiae*); and after treatment, the abundant microbes were adapted to aerobic and oligotrophic environment (e.g., *Actinobacteria*, *Cytophagia* and *Sphingobacteriia*). It is clear that the microbial community would shift with the concentration of pollutants and changing environment [37].

3.4. Correlation of Microbial Community and Physiochemical Characteristics

The Canonical correspondence analysis (CCA) was used to determine the relationship between microbial community and physiochemical properties in the river, and five variables (COD_{Cr}, NH_4^+-N, pH, DO and TP) were selected to form a significant CCA model ($p < 0.05$). CCA1 and CCA2 presented 41.94% and 24.56% of total variances, respectively (Figure 6), indicating that these five variables were the major factors shaping microbial community.

In Figure 6, DO, COD and pH were the major factors shaping the microbial community structure, NH_4^+-N and TP were also involved. The previous study showed the importance of DO for microbial activity in aquatic environment [38], and the concentration of COD and NH_4^+-N would also influence the microbes [39]. The six samples were separated to four groups, showed the difference of microbial community at different stage during the treatment. Considering the marine microbes found in the river and the dilution of pollutants, tidal water was also involved in the development of microbial community. Therefore, the physiochemical factors and pollutants of the urban river worked together to determine the microbial community structure in the urban river.
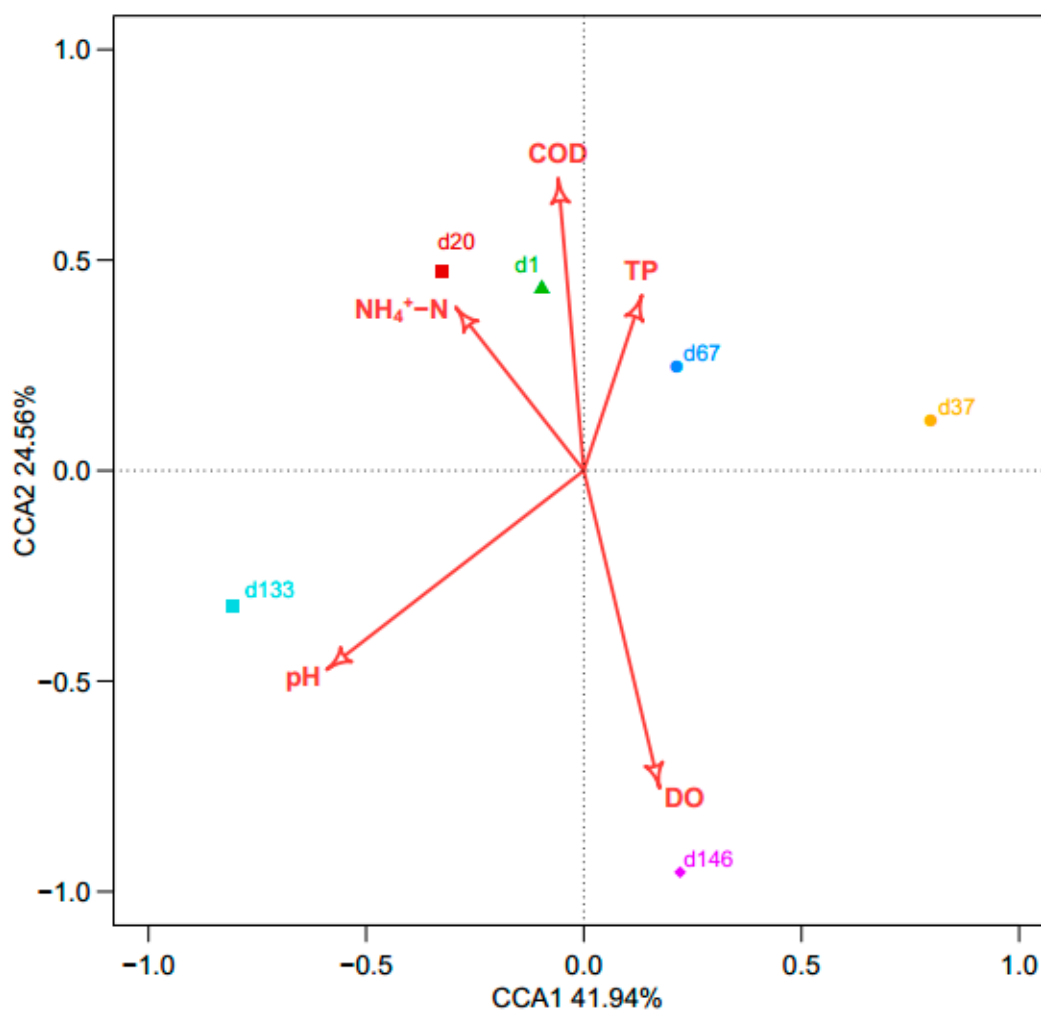

Figure 6. Canonical correspondence analysis (CCA) of microbial community and physiochemical properties in the river.

In summary, MBT changed the physical and chemical conditions of the river mainly by increasing the concentration of DO, and then the aerobic microbes were stimulated and activated at degradation of pollutants. Thus, the microbial community would shift and the water quality was improved in this river.

3.5. Microbial Function during MBT Treatment

The microbial community functions were predicted by COG in Figure 7. According to the result, the COG categories related to T (Signal transduction mechanisms), M (Cell wall/membrane/envelope biogenesis), N (Cell motility), J (Translation, ribosomal structure), P (Inorganic ion transport and metabolism), H (Coenzyme transport and metabolism), C (Energy production and conversion), U (Intracellular trafficking, secretion), and F (Nucleotide transport and metabolism) were more abundant before MBT treatment. This might because that the urban black-odor river with high concentration of COD and NH_4^+-N was suitable for microbes to grow and reproduce, which was matched with the high microbial abundance of sample d1.
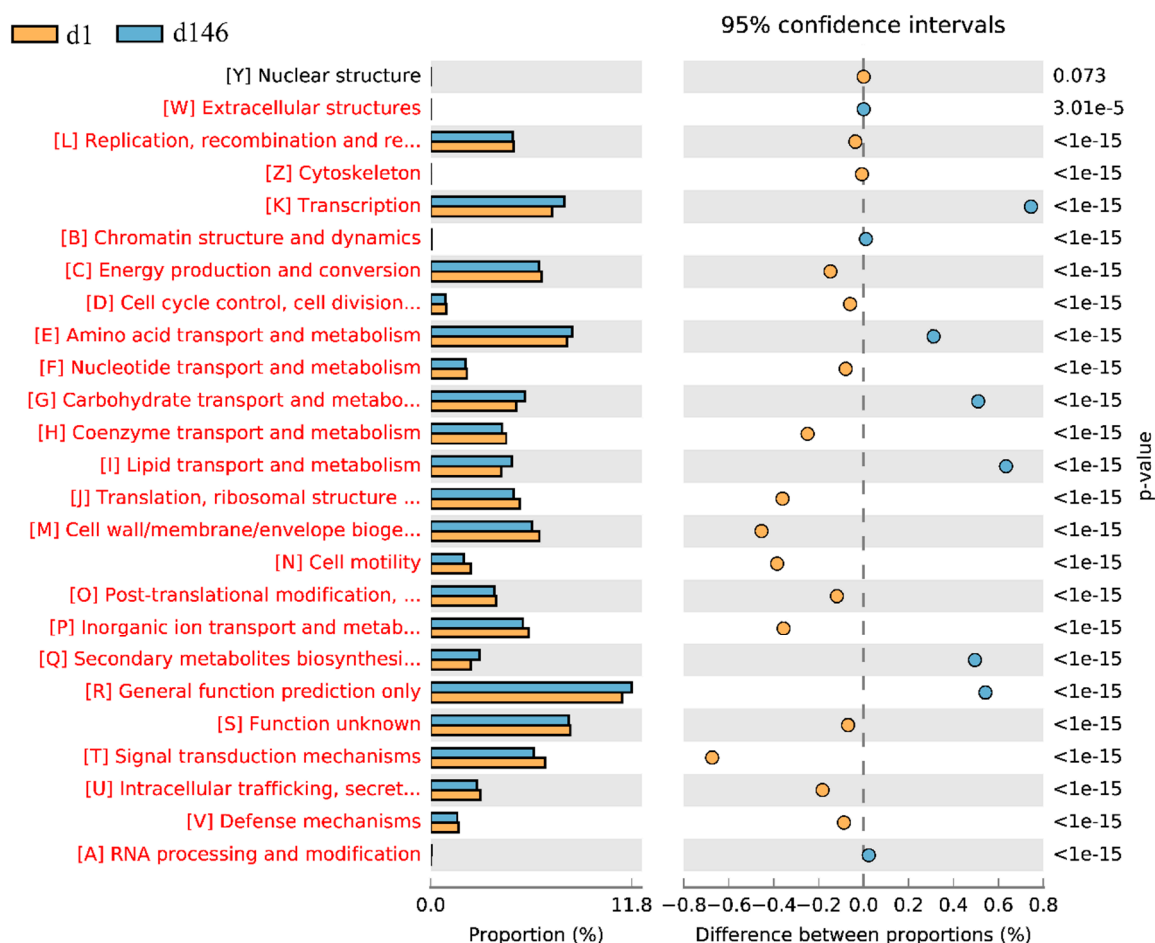

Figure 7. Functional groups by COG categories showed the difference of microbial function before and after MBT treatment in the river (The left was the abundance of different functional groups; the middle was the percentage of functional group abundance with 95% confidence intervals; the *p* value was shown in the rightmost; *p* < 0.05, means significant difference).

Meanwhile microbes responsible to K (transcription), E (Amino acid transport and metabolism), G (carbohydrate transport and metabolism), I (lipid transport and metabolism) and Q (secondary metabolites biosynthesis) were enriched after MBT treatment. It has been reported that the secondary metabolism are well known for the ability to inhibit other microorganisms [40]. Additionally, the result

might due to the reduction of nutrient in the river after MBT treatment, the microorganisms had to compete for resources. The changes of microbial community function showed that the MBT treatment could mitigate the pollution of river.

4. Conclusions

MBT was applied to mitigate the pollution of an urban black-odor river in South China. After the MBT treatment, the concentration of DO was increased and the microbial community was changed, which led to improvement of water quality in the river. The result indicated that MBT is suitable for *in situ* treatment to mitigate the pollution of the urban black-odor river. Microbial community was shifted during the treatment, and aerobic microbes were dominant at the end of treatment. The relative abundance of dominant classes and genera were changed with the physiochemical conditions and pollution degree in the process of MBT treatment. Thus, MBT could be a possible solution to counteract the deterioration of urban rivers in South China before starting an interception project.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4441/11/1/66/s1, Figure S1: Monthly mean temperature of Dongguan City (2016–2018), Figure S2. The Concentration of TP (mg/L) during remediation, Table S1. The ACE and Chao 1 index of samples.

Author Contributions: Y.W. has conceived the study. She also carried out the experiment, data collection, analysis of data, and prepared the first edition of the manuscript. S.L., Y.H., H.L., W.Y. and S.S. participated in the design of the study and helped to draft and edit the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

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