

Article

Effect of the Aerobic Denitrifying Bacterium *Pseudomonas furukawaii* ZS1 on Microbiota Compositions in Grass Carp Culture Water

Wangbao Gong^{1,2}, Shuwei Gao¹, Yun Zhu¹, Guangjun Wang^{1,2}, Kai Zhang^{1,2}, Zhifei Li^{1,2}, Ermeng Yu^{1,2} ,
Jingjing Tian^{1,2}, Yun Xia^{1,2}, Jun Xie^{1,2,*} and Jiajia Ni^{3,4,*} 

- ¹ Key Laboratory of Tropical & Subtropical Fishery Resource Application & Cultivation, Ministry of Agriculture, Pearl River Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou 510380, China; gongwb@prfri.ac.cn (W.G.); 15036954469@163.com (S.G.); zhuyun0513@163.com (Y.Z.); gjwang@prfri.ac.cn (G.W.); zk@prfri.ac.cn (K.Z.); lzf@prfri.ac.cn (Z.L.); yem@prfri.ac.cn (E.Y.); tianjj@prfri.ac.cn (J.T.); xiayun412@126.com (Y.X.)
- ² Guangdong Ecological Remediation of Aquaculture Pollution Research Center, Guangzhou 510380, China
- ³ Dongguan Key Laboratory of Medical Bioactive Molecular Developmental and Translational Research, Guangdong Medical University, Dongguan 523808, China
- ⁴ Research and Development Center, Guangdong Meilikang Bio-Science Ltd., Dongguan 523808, China
- * Correspondence: xj@prfri.ac.cn (J.X.); nijiajia2005@126.com (J.N.)



Citation: Gong, W.; Gao, S.; Zhu, Y.; Wang, G.; Zhang, K.; Li, Z.; Yu, E.; Tian, J.; Xia, Y.; Xie, J.; et al. Effect of the Aerobic Denitrifying Bacterium *Pseudomonas furukawaii* ZS1 on Microbiota Compositions in Grass Carp Culture Water. *Water* **2021**, *13*, 1329. <https://doi.org/10.3390/w13101329>

Academic Editors: Pei Xu and Yanyan Zhang

Received: 8 April 2021

Accepted: 7 May 2021

Published: 11 May 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: Background: Although functional bacteria are widely used in aquaculture water treatment, whether they affect the indigenous microbiota and whether the impact is persistent remain unclear. Therefore, we aimed to explore the denitrification effect of *Pseudomonas furukawaii* ZS1 isolated from a grass carp culture pond in nitrogen-rich aquaculture water, and determine whether its effect on the microbiota structure of the aquaculture water was persistent. Methods: Three each of treatment and control groups were set up, and *P. furukawaii* ZS1 was added to the treatment group. The concentrations of ammonia nitrogen, nitrite, and nitrate, and the pH of each sample were measured for eight consecutive days. Changes of microbiota composition in the water were analysed via high-throughput sequencing. Results: Ammonia, nitrite, and nitrate concentrations were substantially lower in the treatment group than in the control group. There were significant differences in the microbiota structure between treatment and control groups, especially on days 2–7 after adding *P. furukawaii* ZS1. Furthermore, significantly enriched bacterial genera in the treatment group were initially higher in number than inhibited genera, but subsequently reverted to being lower in number. Conclusions: These results provide theoretical guidance for the effective use of *P. furukawaii* ZS1 to control aquaculture water.

Keywords: pond microbiota; *Pseudomonas furukawaii*; nitrogen cycle; grass carp culture; high-throughput sequencing; aquaculture

1. Introduction

The majority of aquatic products are obtained from aquaculture, owing to a decrease in wild fishery resources. In 2018, the total output of aquatic products in China was 64.5766 million tons, of which 49.9106 million tons (i.e., 77.3%) was derived from aquaculture [1]. This high-density and intensive culture method uses large amounts of bait and generates a substantial accumulation of fish excreta, thereby deteriorating water quality, and consequently negatively affecting the quality of aquatic products as well as polluting the environment [2–4]. Approximately 75% of the feed nitrogen are not utilized and remain as waste in the water [5]. Therefore, there is an urgent need for a nitrogen-control and pollution-free production system in aquaculture [5].

Microbial metabolism regulates material circulation in water and plays an important role in the purification and environmental restoration of pond water [6,7]. Microorganisms

participate in the cycling of carbon, nitrogen, phosphorus, and other important elements through various metabolic pathways, as well as substantially contribute to the degradation of pollutants [8]. Ammonia- (NH_4^+ -N), nitrite- (NO_2^- -N), nitrate-nitrogen (NO_3^- -N), and organic nitrogen can be removed from water via nitrification and denitrification [7]. Chen et al. [9] showed that following the addition of compound probiotics (yeast, lactobacillus, and bacillus) and nutrients to *Litopenaeus vannamei* and *Acanthopagrus latus* polyculture ponds, the diversity of microbiota markedly increased, while the concentration of harmful *Vibrio* bacteria, ammonia-, and nitrite-nitrogen decreased to the appropriate range for these aquatic species. Bacteria in *Pseudomonas* are commonly reported as aerobic denitrification bacteria, such as *Pseudomonas sihuiensis* [10], *Pseudomonas hussainii* strain MB3 [11], *Pseudomonas monteilii* strain H97 [12], and *Pseudomonas mendocina* [13]. *Pseudomonas furukawaii* ZS1 isolated from a grass carp culture pond was an aerobic denitrifying bacterium that effectively removes nitrogen [14].

Although functional microorganisms play an important role in maintaining nitrogen balance in aquaculture water, the overall structure of the microbial community also plays an important role in maintaining the pollution level of aquaculture water [2,3]. Although extensive research has confirmed that adding probiotics to aquaculture water can effectively improve water quality and microbiota characteristics, as well as promote healthy and pollution-free aquaculture, it remains unclear whether the impact of probiotics on microbiota structure in aquaculture water is long-lasting. Conclusive data would determine whether it is necessary to regularly supplement aquaculture water with probiotics and would indicate the optimum interval between two consecutive doses. A previous study in another aquaculture water treatment system [2] showed that the microbial community structure of aquaculture water recovered after approximately 1 week of artificial disturbance. *P. furukawaii* ZS1 effectively removes nitrogen from aquaculture water [14]. Therefore, we hypothesized that *P. furukawaii* ZS1 could promote the removal of nitrogen by microbiota in the aquaculture water, and the effect on the microbiota structure would last one week. The aim of this study was to evaluate the effect of probiotics on water quality and on the microbiota structure of grass carp culture water, by adding the aerobic denitrifying bacterium *P. furukawaii* ZS1 to the aquaculture water. These results provide theoretical guidance for the effective use of *P. furukawaii* ZS1 to control aquaculture water.

2. Materials and Methods

2.1. Bacterial Strain and Experimental Design

The aerobic denitrifying bacterium *P. furukawaii* ZS1 used in this experiment was collected from a grass carp culture pond at Huachen farm in Zhongshan City, China [14]. Before the experiment, *P. furukawaii* ZS1 preserved in glycerin at -80°C were cultured for 24 h using denitrifying enrichment medium (containing 3.0 g yeast extract, 5.0 g peptone, 1.0 g KNO_3 , and 1000 mL distilled water, $\text{pH} = 7.6$) in a constant temperature shaker (180 r/min) at 30°C . Then, the bacterial culture was divided into 10 conical flasks with 5 L capacity, and 2 L denitrifying enrichment medium was added to each flask. The bacterial cells were cultured in a constant temperature shaker (180 r/min) for 24 h at 30°C .

Six 25 L rectangular glass tanks were cleaned and disinfected with potassium permanganate prior to the experiment. Next, 15 L of culture water from a grass carp culture pond in the Pearl River Fisheries Research Institute was transferred into each glass tank. Potassium nitrate (KNO_3) was added to the water to bring the concentration of NO_3^- -N to 50 mg/L, and sodium acetate was added as a carbon source to achieve a C/N ratio of 5:1. *P. furukawaii* ZS1 was cultured and added to the three treatment tanks at 9:30 a.m. every day during the experiment, to a final concentration of 4×10^5 CFU/mL. An equal volume of sterile water was added to the three control tanks. All experimental tanks were continuously aerated air gas during the experiment. The experiment was carried out in the laboratory experimental base of the Pearl River Fisheries Research Institute, and water temperature was maintained at 30°C .

2.2. Sample Collection and Assessment of Water Quality

According to a previous study in another aquaculture water treatment system [2], the microbial community structure of aquaculture water recovered after approximately 1 week of artificial disturbance. Therefore, the experiment lasted 8 days. Water temperature, pH, as well as NH_4^+ -N, NO_3^- -N, and NO_2^- -N concentrations of the treated and control water samples were measured at 9:00–9:30 a.m. every day, as previously described [3, 15]. Briefly, water temperature and pH were measured in situ using a digital multi-meter (WTW, Weilheim, Germany). NH_4^+ -N concentration was measured using the Nash reagent photometry method, and NO_3^- -N, and NO_2^- -N concentrations were determined using n-(1-naphthyl)-ethylenediamine dihydrochloride spectrophotometric method and zinc/chromium (II) reduction method, respectively [3,15]. Approximately 500 mL of water was simultaneously collected from the middle position of each tank and filtered through glass-fiber membranes (GF/C) with 0.22 μm apertures to collect microbiota for DNA extraction, as previously described [16]. The recovered filter membranes were stored at -80°C for subsequent DNA extraction.

2.3. Microbial Composition Analysis via High-throughput Sequencing

Before DNA extraction, the recovered filter membranes were cut into pieces with sterilized scissors. Genomic DNA of the water microbiota was extracted from the filter membranes using a PowerSoil DNA isolation kit (QIAGEN, Hilden, Germany). The V4–V5 hypervariable region of the prokaryotic 16S rRNA gene was amplified using the universal primer pair 515F (5'-GTGYCAGCMGCCGCGGTA-3') and 909R (5'-CCCGYCAATTCMTTTRAGT-3'), as previously reported [17–19]. The 5'-end of primer 515F included a 12-nt sample-specific barcode sequence to distinguish between samples. Each sample was amplified in duplicate and the PCR products were mixed and purified using an AxyPrep DNA gel extraction kit (Axygen, Suzhou, China). All purified DNA was mixed in equal amounts and sequenced on the Illumina MiSeq platform with 250 bp paired-end sequencing. High-throughput sequencing was performed by Guangdong Meilikang Bio-Sciences Ltd., China.

Raw reads were spliced using the FLASH 1.2.8 software [20], and QIIME 1.9.0 [21] was used to remove the low-quality sequences, as previously described [18,22]. The UCHIME program was used to detect and remove chimeric sequences, and sequences with more than 97% similarity were assigned to the same operational taxonomic unit (OTU) using the UPARSE software [23]. Taxonomic annotation of OTUs was conducted using the Ribosomal Database Project classifier [24] with the greengenes gg_13_8 dataset as a reference.

2.4. Data Analysis

Correspondence analysis (CA), canonical correspondence analysis (CCA), and non-parametric multivariate analysis of variance (PERMANOVA) [25] were conducted using R software with the vegan package [26]. Welch's *t*-test was employed to screen the significantly different OTUs, using the statistical analysis of metagenomic profiles software [27]. Welch's *t*-test was also used to compare aquatic environmental factors using R software.

3. Results

3.1. *P. furukawaii* ZS1 Promoted Nitrogen Removal from Aquaculture Water

The concentration of NH_4^+ -N in both the treatment and control groups was lower than 0.500 mg/L on days 1–4 of the experiment. On day 5, the concentration of NH_4^+ -N increased sharply from 1.728 mg/L to 36.812 mg/L in the control group, and reached a highest concentration of 39.574 mg/L on day 7, before beginning to decline. The concentration of NH_4^+ -N in the treatment group increased from 1.290 mg/L to 29.706 mg/L, and began to decline after reaching the highest concentration of 33.722 mg/L on day 7 (Figure 1A). Except for days 3 and 4 following the addition of *P. furukawaii* ZS1, the concentrations of ammonia nitrogen in the treatment group were significantly lower than those in the control groups (Figure 1A). A gradual decrease in NO_3^- -N concentration was initially observed, at a speed significantly faster in the treatment group than in the control

group (Figure 1B). NO_2^- -N concentrations in the treatment and control groups were low at 0.274 mg/L and 0.269 mg/L, respectively. The addition of *P. furukawaii* ZS1 caused a rapid increase in NO_2^- -N on day 2, and concentrations remained high until day 5, with a higher rate of decline observed in the treatment group compared to the control group (Figure 1C). The water pH of the treatment group was initially significantly lower than that of the control group on days 1–6, following the addition of *P. furukawaii* ZS1, but was subsequently significantly higher than that of the control group (Figure 1D). These results showed that *P. furukawaii* ZS1 significantly improved the denitrification process of microbiota in aquaculture water, thus promoting nitrogen removal from the medium.

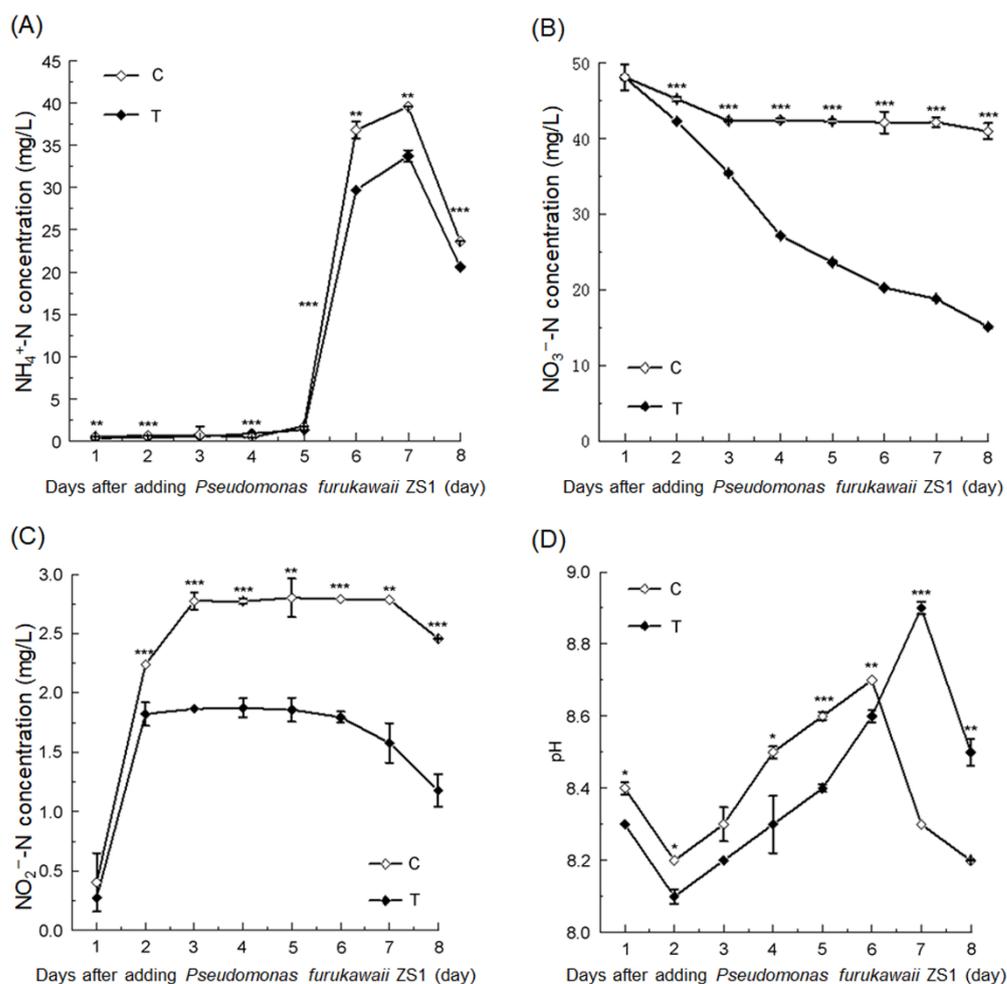


Figure 1. Changes in ammonia nitrogen (A), nitrate (B), and nitrite (C) concentrations, as well as in pH (D) in tested water samples during the experimental process. The treatment group contained added *Pseudomonas furukawaii* ZS1. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

3.2. *P. furukawaii* ZS1 Temporarily Altered the Microbiota Composition in Aquaculture Water

A total of 4,636,002 high-quality sequences were obtained from 48 samples using high-throughput sequencing. To exclude the influence of sequencing depth on the results, 31,517 sequences were randomly selected from each sample for subsequent analysis. A total of 12,885 OTUs were obtained from these sequences. Except for a small number of OTUs (corresponding to 0.01% of the total sequences that were analysed), the remaining were classified into 40 phyla (Table S1). Proteobacteria (92.65–22.13%), Bacteroidetes (66.60–1.67%), Cyanobacteria (50.1–0.53%), Firmicutes (11.66–0.45%), Verrucomicrobia (66.24–0.05%), Planctomycetes (10.00–0.02%), Actinobacteria (50.80–0.45%), Chloroflexi (5.11–0.05%), and Tenericutes (1.48–0.00%) dominated the microbiota. Although the dominant phyla (Proteobacteria, Bacteroidetes, Verrucomicrobia, Planctomycetes, Actinobacteria, and

Tenericutes) showed significant differences in their relative abundance on the first two days after exposure to *P. furukawaii* ZS1 (Figure 2A), the CA profile based on the compositions of dominant genera in each sample indicated that differences in the water microbiota occurred at all stages of the process, and exhibited a similar changing trend during the experiment (PERMANOVA, $F = 4.31$, $P = 0.005$; Figure 2B).

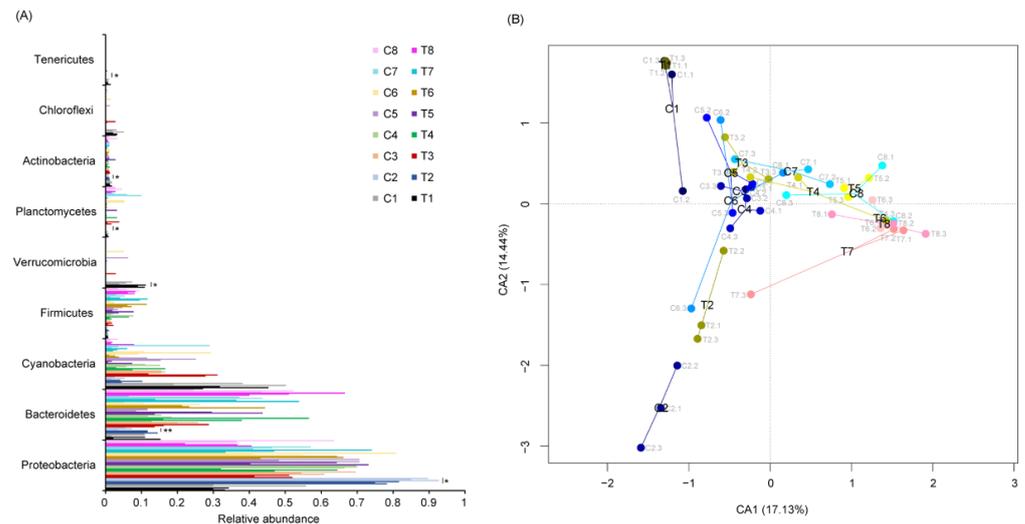


Figure 2. Varying dominant phyla between treated and control water samples on different days after adding *Pseudomonas furukawaii* ZS1 (A) and PCA profile based on OTU compositions of each water sample (B). Different colorful circles in the panel (B) indicate different groups collected different days after adding *P. furukawaii* ZS1. Mid-night blue, navy blue, slate blue, royal blue, blue, dodger blue, deep sky blue, and cyan indicate controls collected at first, second, third, fourth, fifth, sixth, seventh, and eighth day after adding *P. furukawaii* ZS1, respectively; and saddle brown, dark olive green, dark goldenrod, gold, yellow, bisque, coral, and dark salmon indicate treatments collected at first, second, third, fourth, fifth, sixth, seventh, and eighth day after adding *P. furukawaii* ZS1, respectively. *, $p < 0.05$; **, $p < 0.01$.

To determine the effect of *P. furukawaii* ZS1 on the microbiota of culture water, changes in microbiota between the treatment and control groups were compared and analysed. On days 1 and 8 of the experiment, only two bacterial genera were detected; on days 1 and 2, the number of bacterial genera that were notably enriched in the treatment group was significantly higher than that of markedly inhibited bacteria. On days 3 and 4, the number of bacterial genera that were significantly enriched and inhibited in the treatment group remained essentially similar. On days 5–7, the number of bacterial genera that were notably inhibited was significantly higher than that of the bacterial genera markedly enriched (Figure 3). This change was consistent with the concentrations of ammonia- and nitrite-nitrogen detected in the water. The bacterial genera exhibiting significant differences in the microbiota also depicted a regularity of alteration. *Zoogloea* was significantly enriched on days 2 and 3, whereas *Leptotrix* was significantly inhibited on days 3 and 4. *Pseudomonas*, *Paenibacillus*, and *Brevundimonas* were significantly inhibited on days 4 and 5, 5 and 6, and 4–6, respectively (Figure 3).

To examine the relationship between genera and ammonia nitrogen, nitrate nitrogen, nitrite nitrogen, and pH value, CCA was used to analyse the relationship between OTUs, genera, and dominant genera and these indicators. The results obtained based on OTU, genus, and dominant genus compositions of the microbiota were essentially consistent, and ammonia-, nitrate-, and nitrite-nitrogen were significantly correlated with the microbiota compositions (Figure 4).

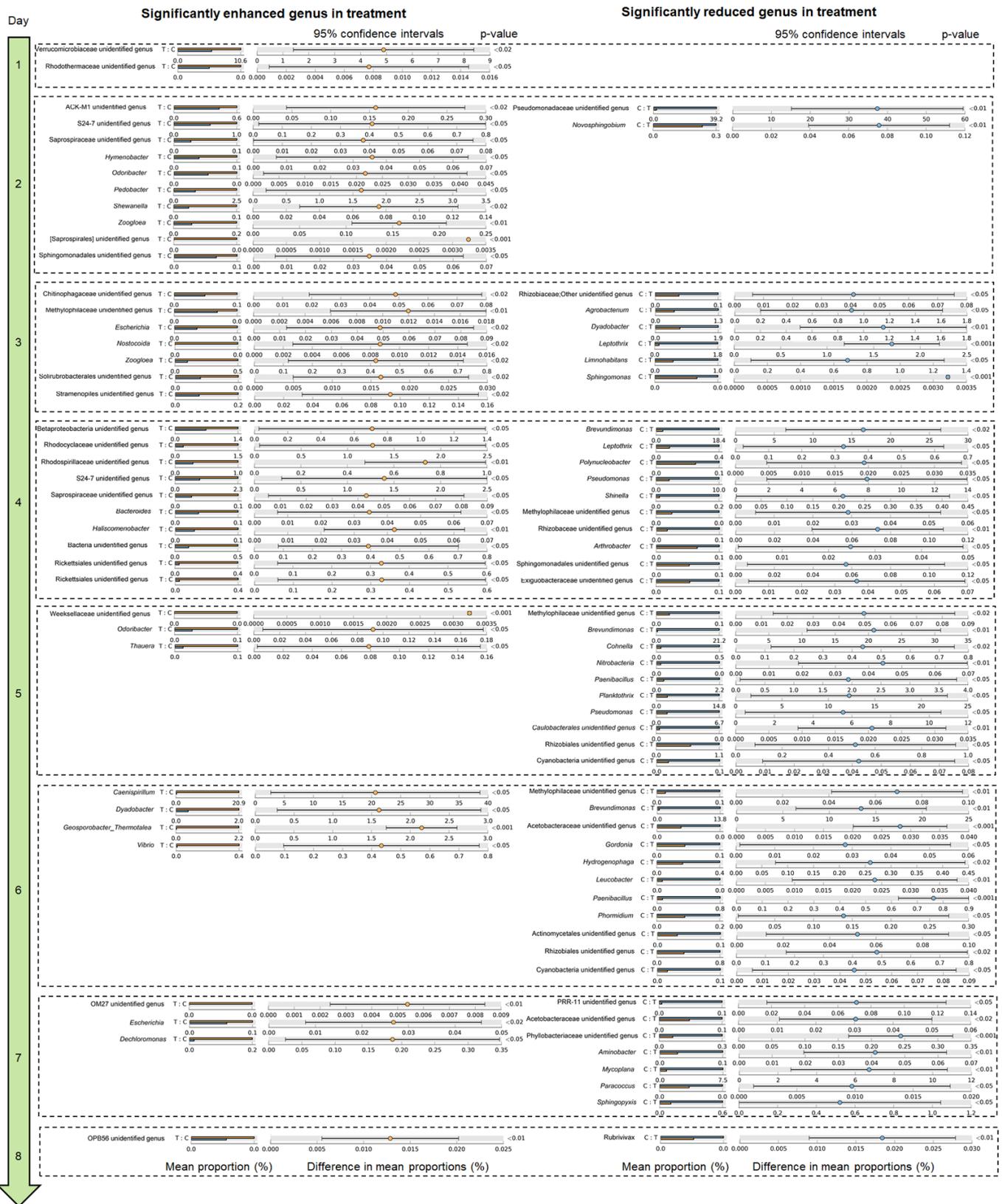


Figure 3. Differences in water microbiota between the treatment and control groups at different days following the start of the experiment. Post-hoc plots were created using the statistical analysis of metagenomic profiles software.

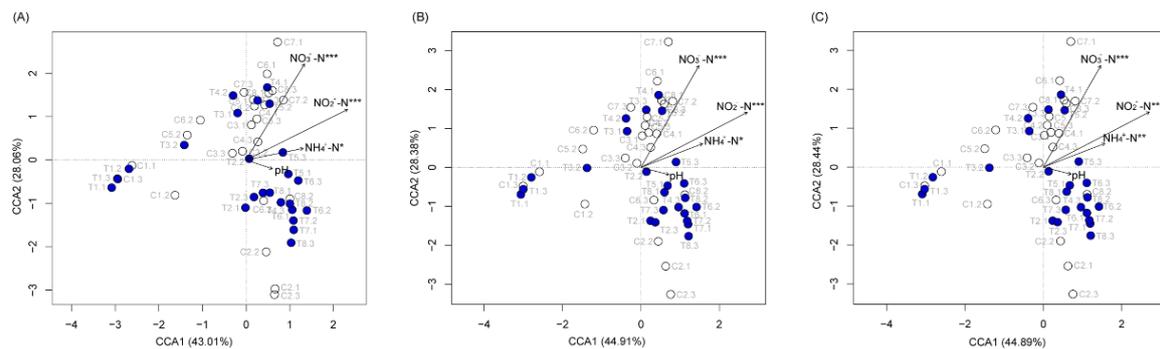


Figure 4. Canonical correspondence analysis profiles based on OTUs (A), genera (B), and dominant genera (C) compositions of water microbiota and on aquatic environmental factors. Dark blue circles indicate samples treated with *Pseudomonas furukawaii* ZS1. White circles indicate samples of control. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

4. Discussion

The control of nitrogen pollutants is the focus of water treatment in aquaculture [2]. Microbiota play a key role in the transformation and circulation of nitrogen, which can reduce ammonia nitrogen, nitrite, and nitrate levels from pond aquaculture water [28–30]. Bacteria in *Pseudomonas* are commonly reported as aerobic denitrification bacteria, such as *Pseudomonas sihuiensis* [10], *Pseudomonas hussainii* strain MB3 [11], *Pseudomonas monteilii* strain H97 [12], and *Pseudomonas mendocina* [13]. Our previous study confirmed *P. furukawaii* ZS1 as an aerobic denitrifying bacterium that effectively removes nitrogen [14]. In present study, our results also showed that *P. furukawaii* ZS1 could effectively promote the removal of inorganic nitrogen in aquaculture water.

The addition of probiotics altered the water microbiota structure and improved the regulation of aquaculture water quality. Li et al. [31] reported that mature biological flocs could reduce the contents of ammonia nitrogen, nitrite, and nitrate in water, substantially increase the content of suspended solids in water, and increase the metabolic intensity of polymers and carbohydrates. Wang et al. [30] found that the microecological agents MP-1 and MP-2 could markedly alter the water quality of shrimp ponds in the later stages of aquaculture, and speculated that the water purification effect might be caused by a joint action of these agents, after changing the microbiota structure. Li et al. [32] added *Bacillus* to shrimp culture ponds, and their results implied that the bacteria could modify water quality by substantially altering the microbiota structure. Xiong [33] observed that *Bacillus subtilis*, *Lactobacillus casei*, and *B. pumilus* reduced the nitrite content in water, as well as altered the water microbiota structure. The present study similarly revealed that added *P. furukawaii* ZS1 modified the microbiota structure of aquaculture water. The results also indicated that *P. furukawaii* ZS1 was highly effective at controlling water quality. However, as it remained unclear whether the reduced nitrogen levels were a result of added probiotics or caused by indigenous microbiota, further studies are required.

Although fluctuations occur in microbial communities, aquatic ecosystems are generally relatively stable. Li et al. [2] reported that, although the community structure of biofilms in aquaculture ponds was strongly affected by the addition of avermectin B1, recovery was complete approximately one week later. The findings also indicated that the addition of *P. furukawaii* ZS1 significantly altered the structure of the water microbiota, and that the changes gradually dissipated after a week.

Although previous studies have shown that the nutrient content in aquaculture water is an important factor that impacts microbiota composition, the relationship between aquatic environmental factors and microbial community varies among different habitats. Ni et al. [3] reported that water temperature, chemical oxygen demand, nitrate concentration, and pH in subtropical ponds were markedly correlated with the composition of microbial communities. Although increment in pH was observed in the present study, the reason caused the increment needs to be further study. Wu et al. [34] reported that the

correlations between environmental factors and various microbiota in the Chaohu Lake and three urban rivers in China were dissimilar. The results showed that the concentrations of ammonia nitrogen, NO_3^- -N, and NO_2^- -N in the tested aquaculture water were significantly correlated with the microbiota composition (Figure 4).

Water temperature, dissolved oxygen, chemical oxygen demand, pH, and concentrations of ammonia nitrogen, NO_3^- -N, and NO_2^- -N not only influence the structure of aquaculture water microbiota [3,35], but also affect the denitrification efficiency [36,37]. Dissolved oxygen and C/N ratio were considered as two most important environmental parameters [36,37]. The optimal C/N ratios of different species of aerobic denitrifying bacteria are different, but most studies show that the optimal C/N ratio is 4.0–5.0. [36,37]. Therefore, at the beginning of this experiment, the C/N ratio was artificially adjusted to 5.0. Dissolved oxygen is another environmental parameter that most influences the denitrification efficiency. It is generally believed that the aerobic denitrification process is limited when the dissolved oxygen is less than 3.0 mg/L [36]. In order to ensure that the experiment process was aerobic denitrification process rather than anaerobic denitrification process, we fully aerated air gas to the tanks continuously during the experiment.

Practical Implications of this Study

Extensive research has confirmed that adding probiotics to aquaculture water can effectively improve water quality and microbiota characteristics, as well as promote healthy and pollution-free aquaculture. In the present study, our results also showed that *P. furukawaii* ZS1 could effectively promote the removal of inorganic nitrogen in aquaculture water. Moreover, there were significant differences in the microbiota structure between treatment and control groups, especially on days 2–7 after adding *P. furukawaii* ZS1. These results provide theoretical guidance for the effective use of *P. furukawaii* ZS1 to control aquaculture water. Whether the weekly use of *P. furukawaii* ZS1 can maintain the high nitrogen removal efficiency in grass carp culture pond water needs to be further verified in the culture process.

5. Conclusions

The addition of *P. furukawaii* ZS1 to grass carp culture water significantly reduced the concentrations of NO_3^- -N, NO_2^- -N, and ammonia nitrogen in the water, and the water microbiota structure was altered for a short period of time. This change included a transformation process that promoted the enrichment of certain specific bacterial genera while inhibiting the abundance of others. These results provide theoretical guidance for the effective use of *P. furukawaii* ZS1 to control aquaculture water. However, the molecular mechanism by which *P. furukawaii* ZS1 promotes nitrogen removal from water requires yet further study.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/w13101329/s1>, Table S1: Phylum composition of the pond microbiota.

Author Contributions: Conceptualization, W.G., J.X. and J.N.; methodology, W.G., S.G., Y.Z., G.W., K.Z., Z.L., E.Y., J.T. and Y.X.; software, W.G. and J.N.; validation, S.G., Y.Z., G.W., K.Z., Z.L. and J.N.; formal analysis, W.G., S.G., J.X. and J.N.; investigation, W.G., S.G., Y.Z., G.W., K.Z., Z.L., E.Y., J.T. and Y.X.; resources, J.X.; data curation, W.G., S.G. and Y.Z.; writing—original draft preparation, W.G., S.G. and J.N.; writing—review and editing, J.X.; visualization, W.G. and J.N.; supervision, Z.L.; funding acquisition, J.X. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Special Fund for Fishery Economic Development of Guangdong Province, grant number 2019B13, the National Key Research and Development Project, grant number 2019YFD0900302 and the Natural Science Foundation of Guangdong Province, grant number 2018A030313412.

Informed Consent Statement: Not applicable.

Data Availability Statement: The merged sequences were submitted to the Genome Sequence Archive database (GSA, <https://bigd.big.ac.cn/gsa/> (accessed on 12 January 2021)) under the accession number CRA003732.

Acknowledgments: We would like to thank anonymous technicians at Guangdong Meilikang Bio-Science Ltd., China for assistance with data analysis.

Conflicts of Interest: The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

- China National Bureau of Statistics. *China Fishery Statistical Yearbook 2019*; China Agricultural Press: Beijing, China, 2019; pp. 1–138.
- Li, Z.; Che, J.; Xie, J.; Wang, G.; Yu, E.; Xia, Y.; Yu, D.; Zhang, K. Microbial succession in biofilms growing on artificial substratum in subtropical freshwater aquaculture ponds. *Fems Microbiol. Lett.* **2017**, *364*, fnx017. [[CrossRef](#)]
- Ni, J.J.; Li, X.J.; Chen, F.; Wu, H.H.; Xu, M.Y. Community structure and potential nitrogen metabolisms of subtropical aquaculture pond microbiota. *Appl. Ecol. Environ. Res.* **2018**, *16*, 7687–7697. [[CrossRef](#)]
- Li, Z.; Wang, G.; Yu, E.; Zhang, K.; Yu, D.; Gong, W.; Xie, J. Artificial substrata increase pond farming density of grass carp (*Ctenopharyngodon idella*) by increasing the bacteria that participate in nitrogen and phosphorus cycles in pond water. *PeerJ* **2019**, *7*, e7906. [[CrossRef](#)]
- Piedrahita, R.H. Reducing the potential environmental impact of tank aquaculture effluents through intensification and recirculation. *Aquaculture* **2003**, *226*, 35–44. [[CrossRef](#)]
- Attramadal, K.J.K.; Salvesen, I.; Xue, R.; Øie, G.; Størseth, T.R.; Vadstein, O.; Olsen, Y. Recirculation as a possible microbial control strategy in the production of marine larvae. *Aquac. Eng.* **2012**, *46*, 27–39. [[CrossRef](#)]
- Feng, Y.; Zhang, R.; Shi, C.; Li, L.; Ma, Y. Study of microbial community participating in water cycle. *J. Anhui. Agric. Sci.* **2016**, *44*, 132–134.
- Zheng, H.; Zhou, Q. Function of microorganisms in bioremediation of eutrophic water. *Chin. J. Eco-Agricul.* **2009**, *17*, 197–202. [[CrossRef](#)]
- Chen, J.; Zheng, J.; Mao, Y.; Su, Y.; Wang, J. Effects of probiotics and compound nutrients on water quality and community structure of bacteria in fish-shrimp farming pond. *J. Trop. Biol.* **2019**, *10*, 6–13.
- Hong, P.; Huang, Y.; Chen, M.; Xiao, B. Efficacy of inorganic nitrogen removal by a salt-tolerant aerobic denitrifying bacterium, *Pseudomonas Sihuiensis* LK-618. *Bioprocess Biosyst. Eng.* **2021**. [[CrossRef](#)]
- Jaiswal, S.K.; Saxena, R.; Mittal, P.; Gupta, A.; Sharma, V.K. Draft genome sequence of *Pseudomonas hussainii* strain MB3, a denitrifying aerobic bacterium isolated from the rhizospheric region of mangrove trees in the Andaman Islands, India. *Genome Announc.* **2017**, *5*, e01527-16. [[CrossRef](#)]
- Cai, X.; He, T.; Ye, Q.; Li, Z. Identification and characterization of a hypothermic alkaliphilic aerobic denitrifying bacterium *Pseudomonas monteillii* strain H97. *Environ. Sci.* **2018**, *39*, 3314–3320.
- Zhang, W.; Yan, C.; Shen, J.; Wei, R.; Gao, Y.; Miao, A.; Xiao, L.; Yang, L. Characterization of aerobic denitrifying bacterium *Pseudomonas mendocina* strain GL6 and its potential application in wastewater treatment plant effluent. *Int. J. Environ. Res. Public Health* **2019**, *16*, 364. [[CrossRef](#)]
- Zhu, Y.; Gong, W.; Xie, J.; Wang, G.; Yu, D.; Li, Z.; Zhang, K.; Tian, J. Identification and denitrification characteristics of the aerobic denitrifying bacteria *Pseudomonas furukawaii* ZS1. *Acta Hydrobiol. Sin.* **2020**, *44*, 895–903.
- Huang, X.F. *Survey, Observation and Analysis of Lake Ecology*; China Standard Press: Beijing, China, 2000.
- Ni, J.; Yu, Y.; Feng, W.; Yang, Q.; Pan, G.; Yang, B.; Zhang, X.; Li, X. Impacts of algal blooms removal by chitosan-modified soils on zooplankton community in Taihu Lake. *China J. Environ. Sci.* **2010**, *22*, 1500–1507. [[CrossRef](#)]
- Ni, J.; Li, X.; He, Z.; Xu, M. A novel method to determine the minimum number of sequences required for reliable microbial community analysis. *J. Microbiol. Methods* **2017**, *139*, 196–201. [[CrossRef](#)]
- Ni, J.; Huang, R.; Zhou, H.; Xu, X.; Li, Y.; Cao, P.; Zhong, K.; Ge, M.; Chen, X.; Hou, B.; et al. Analysis of the relationship between the degree of dysbiosis in gut microbiota and prognosis at different stages of primary hepatocellular carcinoma. *Front. Microbiol.* **2019**, *10*, 1458. [[CrossRef](#)] [[PubMed](#)]
- Huang, R.; Li, T.; Ni, J.; Bai, X.; Gao, Y.; Li, Y.; Zhang, P.; Gong, Y. Different sex-based responses of gut microbiota during the development of hepatocellular carcinoma in liver-specific *Tsc1*-knockout mice. *Front. Microbiol.* **2018**, *9*, 1008. [[CrossRef](#)]
- Magoč, T.; Salzberg, S.L. FLASH: Fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* **2011**, *27*, 2957–2963. [[CrossRef](#)]
- Caporaso, J.G.; Kuczynski, J.; Stombaugh, J.; Bittinger, K.; Bushman, F.D.; Costello, E.K.; Fierer, N.; Peña, A.G.; Goodrich, J.K.; Gordon, J.I.; et al. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* **2010**, *7*, 335–336. [[CrossRef](#)]
- Xiang, J.; He, T.; Wang, P.; Xie, M.; Xiang, J.; Ni, J. Opportunistic pathogens are abundant in the gut of cultured giant spiny frog (*Paa spinosa*). *Aquacul. Res.* **2018**, *49*, 2033–2041. [[CrossRef](#)]
- Edgar, R.C. UPASE: Highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods* **2013**, *10*, 996–998. [[CrossRef](#)]

24. Wang, Q.; Garrity, G.M.; Tiedje, J.M.; Cole, J.R. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* **2007**, *73*, 5261–5267. [[CrossRef](#)] [[PubMed](#)]
25. Anderson, M.J. A new method for non-parametric multivariate analysis of variance. *Austral. Ecol.* **2001**, *26*, 32–46.
26. Dixon, P. VEGAN, a package of R functions for community ecology. *J. Veg. Sci.* **2003**, *14*, 927–930. [[CrossRef](#)]
27. Parks, D.H.; Beiko, R.G. Identifying biologically relevant differences between metagenomic communities. *Bioinformatics* **2010**, *26*, 715–721. [[CrossRef](#)] [[PubMed](#)]
28. Hargreaves, J.A. Nitrogen biogeochemistry of aquaculture ponds. *Aquaculture* **1998**, *166*, 181–212. [[CrossRef](#)]
29. Hu, Z.; Lee, J.W.; Chandran, K.; Kim, S.; Sharma, K.; Brotto, A.C.; Khanal, S.K. Nitrogen transformations in intensive aquaculture system and its implication to climate change through nitrous oxide emission. *Bioresour. Technol.* **2013**, *130*, 314–320. [[CrossRef](#)]
30. Wang, X.; Wu, Y.; Yao, X.; Liu, Y.; Sun, H.; Tang, J. Effect of probiotics on water quality and bacterial community in shrimp pools at later stage of cultivation. *Acta. Agricul. Zhejiangensis* **2014**, *26*, 1–47.
31. Li, Z.; Wang, G.; Yu, D.; Lu, B.; Xie, J.; Yu, E.; Xia, Y.; Chong, X. Effect of bioflocs on water quality and metabolic functions of microbial community in zero-water exchange tanks. *J. Shanghai Fish. Univ.* **2015**, *24*, 503–512.
32. Li, Z.; Lin, L.; Yang, Y.; Lin, X. Influence of *Bacillus* on the microbial communities in shrimp ponds. *J. Agro-Environ. Sci.* **2007**, *26*, 1183–1189.
33. Xiong, H. *Preliminary Study on the Effect of External Bacteria on Water Quality and Microbial Community Structure of Aquaculture Water*; Huazhong Agricultural University: Wuhan, China, 2011.
34. Wu, L.; Shu, F.; Ou, Z.; Chen, Q.; Wang, L.; Wang, H.; Xu, Z. Compositions of prokaryote communities and their relationship to physiochemical factors in December in Chaohu Lake and three urban rivers in China. *Appl. Ecol. Environ. Res.* **2019**, *17*, 7265–7281. [[CrossRef](#)]
35. Betiku, O.C.; Sarjeant, K.C.; Ngatia, L.W.; Aghimien, M.O.; Odewumi, C.O.; Latinwo, L.M. Evaluation of microbial diversity of three recreational water bodies using 16S rRNA metagenomic approach. *Sci. Total Environ.* **2021**, *771*, 144773. [[CrossRef](#)]
36. Huang, H.K.; Tseng, S.K. Nitrate reduction by *Citrobacter diversus* under aerobic environment. *Appl. Microbiol. Biotechnol.* **2001**, *55*, 90–94. [[CrossRef](#)] [[PubMed](#)]
37. Bernat, K.; Wojnowska-Baryła, I. Carbon source in aerobic denitrification. *Biochem. Eng. J.* **2007**, *36*, 116–122. [[CrossRef](#)]