

Article

In-Situ Improvement of the Sediment Microenvironment by Nitrate in Tailwater of Wastewater Treatment Plants Combined with Aerobic Denitrifying Bacteria under Low-DO Regulation

Junyi Chen ¹, Chao Zhang ², Yun Liu ³, Jie Tian ^{4,*}  and Jianbo Guo ⁵

¹ National Engineering Laboratory for Lake Pollution Control and Ecological Restoration, Chinese Research Academy of Environmental Sciences, Beijing 100012, China; chenji@craes.org.cn

² Tianjin Academy of Eco-Environmental Sciences, Tianjin 300191, China; zc2017@tju.edu.cn

³ Key Laboratory for Northern Urban Agriculture of Ministry of Agriculture and Rural Affairs, Beijing University of Agriculture, Beijing 102206, China; yun_liu810@163.com

⁴ Tianjin Huanke Environmental Consulting Co., Ltd., Tianjin 300191, China

⁵ Zhongtai Bochao Intelligent Environmental Protection Technology (Suzhou) Co., Ltd., Suzhou 215128, China; jianbguo@163.com

* Correspondence: 15614136092@163.com

Abstract: Preventing the rebound of black and odorous water bodies is critical for improving the ecological environment of water bodies. This study examined the effect and underlying mechanism of in-situ improvement of the sediment microenvironment by nitrate in the tailwater of wastewater treatment plants combined with aerobic denitrifying bacteria under low-DO regulation (TailN + CFM + LDO). On the 60th day of remediation, the levels of dissolved oxygen and oxidation–reduction potential in the overlying water rose to 5.6 mg/L and 300 mV, respectively, the concentration of acid volatile sulfide within the sediment significantly decreased by 70.4%, and the organic matter content in the sediment was reduced by 62.7%, in which the heavy fraction organic matter was degraded from 105 g/kg to 56 g/kg, and the potential risk of water reverting to black and odorous conditions significantly decreased. Amplicon sequencing analysis revealed that the relative abundance of the electroactive bacteria *Thiobacillus* and *Pseudomonas* with denitrification capacity was found to be significantly higher in the TailN + CFM + LDO group than in the other remediation groups. Functional prediction of the 16S sequencing results indicated that both the quantity and activity of critical microbial enzymes involved in nitrification and denitrification processes could be enhanced in the TailN + CFM + LDO group. These results improved our understanding of the improvement of the sediment microenvironment and could thus facilitate its application.

Keywords: black and odorous water bodies; microenvironment; sulfur autotrophic denitrification; electroactive bacteria; functional gene



Citation: Chen, J.; Zhang, C.; Liu, Y.; Tian, J.; Guo, J. In-Situ Improvement of the Sediment Microenvironment by Nitrate in Tailwater of Wastewater Treatment Plants Combined with Aerobic Denitrifying Bacteria under Low-DO Regulation. *Water* **2024**, *16*, 1000. <https://doi.org/10.3390/w16071000>

Academic Editor: Alejandro Gonzalez-Martinez

Received: 15 February 2024

Revised: 20 March 2024

Accepted: 27 March 2024

Published: 29 March 2024



Copyright: © 2024 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/>).

1. Introduction

Tailwater of wastewater treatment plants, as a potential source of pollution in urban receiving water bodies, has a significant impact on urban river ecosystems [1]. Understanding the impact of wastewater treatment plant tailwater on receiving water bodies and the associated mechanisms is crucial for promoting the comprehensive utilization of sewage resources. Sediments in water bodies have a high total organic matter (TOM) content and insufficient hydrodynamics, rendering them susceptible to the proliferation of anaerobic microorganisms like sulfate-reducing bacteria, anaerobic digestion bacteria, and iron-reducing bacteria. These microorganisms produce substances such as FeS, NH₃-N, and acid volatile sulfide (AVS) that cause black and odorous conditions in water bodies [2]. Therefore, reducing the TOM in sediments and improving the sediment microecological environment have emerged as primary strategies to prevent the recurrence of black and odorous water.

The gradual decrease of TOM in sediments and the production of reducing substances are mainly due to inadequate microbial activity and a lack of electron acceptors in sediments [3,4]. Previous studies have indicated that effective remediation outcomes can be achieved through the utilization of either $\text{Ca}(\text{NO}_3)_2$ or domesticated composite microorganisms in sediments. $\text{Ca}(\text{NO}_3)_2$ acts as a source of electron acceptors for microorganisms, while composite microorganisms can enhance microbial activity and establish stable ecosystems, effectively degrading nitrogen, phosphorus, sulfides, etc. [5,6]. $\text{Ca}(\text{NO}_3)_2$, as an economical and efficient electron acceptor, has been extensively used in the remediation of black and odorous water bodies, effectively reducing AVS in water bodies and improving the redox environment [7,8]. However, excessive use of $\text{Ca}(\text{NO}_3)_2$ can pose environmental risks to water bodies [9]. To minimize these risks, it may be preferable to introduce nitrate-containing tailwater from wastewater treatment plants into the receiving water body using a continuous flow rate. This approach helps to mitigate the negative effect of excess nitrate on the water body, as opposed to directly adding $\text{Ca}(\text{NO}_3)_2$ to the water.

Due to the long-term anaerobic or hypoxic conditions in sediments, excess NO_3^- -N under microbial influence can undergo dissimilatory nitrate reduction to ammonium (DNRA). The release of NH_3 -N may pose potential environmental hazards [10]. Previous studies have shown that employing low-intensity or intermittent aeration can effectively increase DO and oxidation–reduction potential (ORP) levels. This aeration technique is also found to be particularly effective in inhibiting the DNRA process [11,12]. Aerobic denitrifying bacteria such as microorganisms prevalent in water bodies prefer aerobic environments (DO of 0.2–5.9 mg/L). However, there is limited research reporting the application of tailwater NO_3^- -N + aerobic denitrifying bacteria + low-DO regulation group to prevent the rebound of black and odorous water bodies. Additionally, the influencing mechanism of wastewater treatment plant tailwater on the microbial ecological environment of water bodies has not been reported.

This study aimed to investigate the inhibitory effect of using NO_3^- -N in wastewater treatment plant tailwater combined with aerobic denitrifying bacteria under low-DO conditions on the rebound of black and odorous water bodies. We discuss the impact of various remediation conditions on DO, ORP, NH_3 -N, NO_3^- -N, NO_2^- -N in the overlying water and AVS in the sediment. Additionally, we investigated changes in sediment microbial community structure with the aid of high-throughput sequencing technology. We aimed to explore the changes in the microbial ecological environment of water bodies and the mechanisms that inhibited the rebound of black and odorous water bodies. Through this exploration, we aim to offer practical insights for the remediation of urban water bodies contaminated with black and odorous water.

2. Materials and Methods

2.1. Sediments and Overlying Water Sources

The sediment and overlying water used in this experiment were collected from a former black water body. This typical water body received inputs from municipal wastewater treatment plant tailwater and runoff discharge. Sediment was collected using a column sampler from the 0–30 cm depth of a river. After collection, any plastic, stone, or glass debris present in the sediment was promptly removed. The sediment was then transferred to plastic buckets, sealed with lids to prevent air interference, and immediately sent to the laboratory. It was stored in a dark environment at 4 °C. Subsequently, overlying water samples were collected from a section of the river that was unaffected by sediment disturbance. The overlying water characteristics were as follows: ORP 100.15 mV, pH 7.45, DO 2.10 mg/L, chemical oxygen demand (COD) 43.00 mg/L, NH_3 -N 4.20 mg/L, and total nitrogen (TN) 4.50 mg/L. The wastewater treatment plant tailwater exhibited the following characteristics: ORP 200.34 mV, pH 7.12, DO 2.10 mg/L, COD 47.00 mg/L, NH_3 -N 4.30 mg/L, and TN 9.50 mg/L. The primary indicators of the sediment included a TOM content of 150 g/kg, a heavy fraction organic matter (HFOM) content of 105 g/kg, a light fraction organic matter (LFOM) content of 45 g/kg, and an AVS content of 3010 mg/kg.

2.2. Experimental Design

The experimental setup consisted of a custom-made water tank with dimensions of 40 cm (length) $\times$ 40 cm (width) $\times$ 100 cm (height) (Figure S1). Approximately 3.2 L of sediment (20 cm in height) was filled into the tank, and about 12.8 L of overlying water (80 cm in height) was gradually added to the tank using the siphoning method. The experiment modeled the heights and proportions of sediment and overlying water based on a former black and odorous water body, which is a common type of water body receiving municipal wastewater treatment plant tailwater. The experimental groups were as follows: blank group (CN), tailwater NO_3^- -N group (TailN), tailwater NO_3^- -N + aerobic denitrifying bacteria group (TailN + CFM), and tailwater NO_3^- -N + aerobic denitrifying bacteria + low-DO regulation group (TailN + CFM + LDO). The blank group was supplied with overlying water from the river, whereas the remediation groups were supplied with tailwater from the specified wastewater treatment plant. The water supply had a flow rate of $0.005 \text{ m}^3/\text{s}$. Aerobic denitrifying bacteria were *Citrobacter* sp. isolated and screened from sediments. The aerobic denitrifying bacteria dosage was established at 1 g/month based on preliminary experiment optimization results. In the TailN + CFM + LDO group, intermittent aeration ($0.1 \text{ m}^3/\text{h}$, 3 h/d) was employed. The overlying water samples were collected every 5 days, whereas sediment samples were obtained every 15 days based on the respective indicators. The microbial community structure analysis of sediment samples was conducted on the 60th day of the remediation process.

2.3. Analytical Methods

A multi-parameter water quality analyzer (WTW-Multi360 IDS, Beijing, China) was used to measure the DO and ORP in both the overlying water and interstitial water. Before analysis, overlying water samples for TN, $\text{NH}_3\text{-N}$, NO_3^- -N, and NO_2^- -N were centrifuged at 8000 rpm for 10 min, and the resulting supernatant was filtered through a $0.45 \mu\text{m}$ membrane filter. The potassium dichromate oxidation method was used to determine the COD of the overlying water. Additionally, the sulfate (SO_4^{2-}) content in the overlying water was measured using ion chromatography (Dionex 1100, Waltham, MA, USA). TOM in the sediment was determined by the potassium dichromate oxidation method, whereas the total nitrogen in the sediment was measured by the alkaline potassium persulfate method. HFOM and LFOM were separated by the density method; it is generally accepted that TOM having a density $\rho < 1.7 \text{ g/cm}^3$ belongs to LFOM, while TOM having a density $\rho > 1.7 \text{ g/cm}^3$ belongs to HFOM. The AVS in the sediment was determined using the methylene blue spectrophotometric method [13].

The V3-V4 region of the 16S rDNA gene was subject to high-throughput sequencing on the sediment and activated sludge samples using amplicon sequencing technology. The primer sequences used were ACTCCTACGGGAGGCAGCAG and GGACTACHVGGGTWTCTAAT. The bioinformatics analysis of the sequencing data was conducted using the R language. Microbial alpha (α) diversity and beta (β) diversity were calculated using Mothur and the “vegan” package in the R language, respectively. The microbial co-occurrence network was developed using the “WGCNA” package in R 3.3.2, where nodes represented operational taxonomic units (OTUs) and edges represented correlations between OTUs. For the purpose of multiple testing, all p -values were adjusted using the Multtest and Hochberg false discovery rate (FDR) control procedures.

The abundance of functional genes revealed the elemental metabolic pathways in different ecosystems [14,15]. Tax4Fun has become a widely used and robust tool for analyzing complex communities' functional genes [16,17]. The normalized bacterial OTUs table was imported to Tax4Fun to predict the microbial metabolic function [18]. Tax4Fun function predictions based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) metabolic pathway database analysis were used to predict functional genes [16].

2.4. Statistical Analysis

To estimate the random error of the measurements, all samples were analyzed in triplicate. The means, standard deviations, and analysis of variance (ANOVA) were calculated

with the aid of SPSS 22.0. The means were compared using a one-way ANOVA, with the significance level set at $p < 0.05$. The data for normality was analyzed to verify the appropriateness of the chosen statistical method.

3. Results and Discussion

3.1. Physiochemical Parameters

DO and ORP are vital indicators for evaluating the presence of black and odorous water. The impact of various remediation strategies on the DO and ORP in the overlying water is illustrated in Figure 1. As shown in Figure 1a, after 60 days of remediation, the DO in the overlying water gradually rose from 2.1 mg/L to 3.0 mg/L (TailN), 3.1 mg/L (TailN + CFM), and 5.6 mg/L (TailN + CFM + LDO). In contrast, the DO in the blank group (CN) exhibited a gradual decrease from the initial 2.1 mg/L to 1.4 mg/L. The changes in the ORP followed a similar pattern. After 60 days of remediation, the ORP exhibited a gradual increase from the initial 100.2 mV to 181.6 mV (TailN), 189.5 mV (TailN + CFM), and 300.0 mV (TailN + CFM + LDO) (Figure 1b). In contrast, the ORP in the overlying water of the CN exhibited a gradual decrease from the initial 100.2 mV to -15.2 mV. Li et al. (2019) observed similar findings regarding the role of intermittent aeration and $\text{Ca}(\text{NO}_3)_2$ in increasing DO and ORP levels in the overlying water. However, the increase in the DO level during the present study was significantly higher compared to that in previous studies [5] conducted under the same aeration condition. This difference can be mainly attributed to the varying levels of reducing substances present in the water bodies [19]. Based on the changes in the DO and ORP, it was evident that the water quality in the CN gradually deteriorated to a mild black and odorous level, while the water quality gradually improved, and the occurrence of black and odorous water was inhibited in the groups receiving the tailwater with NO_3^- -N. NO_3^- -N, as an oxidant, can oxidize reducing substances in the water, including easily oxidizable TOM, S^{2-} , and Fe^{2+} through the influence of heterotrophic denitrifying bacteria, sulfur autotrophic denitrifying bacteria, and iron autotrophic denitrifying bacteria, thereby improving water quality [3,4]. It is worth noting that the introduction of aerobic denitrifying bacteria in the TailN + CFM group had minimal effect on the levels of DO and ORP. This can be attributed to the fact that DO and ORP are associated with the presence of reducing substances, such as AVS and Fe^{2+} , in the water bodies [19]. The aerobic denitrifying bacteria, however, have a lesser effect on the concentrations of AVS and Fe^{2+} . As a result, the addition of aerobic denitrifying bacteria did not lead to significant changes in the DO and ORP levels. In the TailN + CFM + LDO group, the introduction of oxygen significantly increased both the DO and ORP in the overlying water. This study demonstrated that a high content of oxygen can enhance the activity of facultative microorganisms at the water–sediment interface and promote the decomposition and transformation of TOM or reducible substances, thereby enhancing the self-purification capacity of the water [5]. Thus, low-oxygen aeration is beneficial for improving the water–sediment microecosystem.

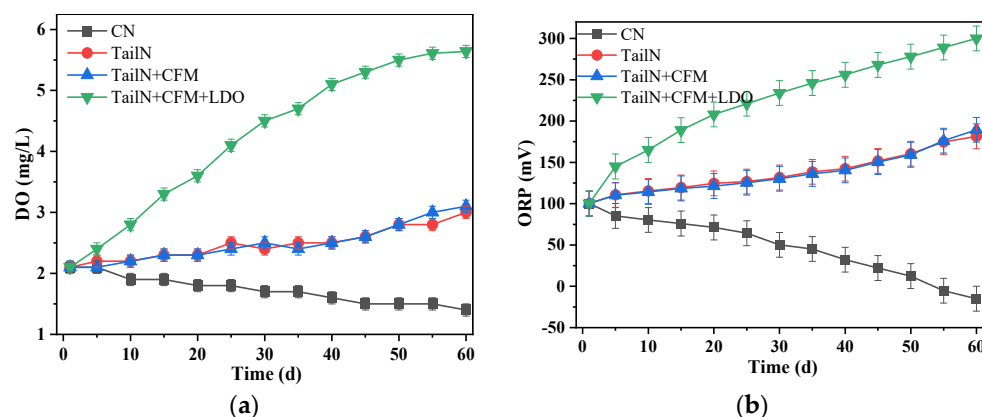


Figure 1. Variation of DO (a) and ORP (b) in the overlying water.

3.2. Changes in Sulfur Forms in Water

The primary cause of black-odorous water is closely associated with the presence of the sulfur element in water. Sulfides are the primary source of odor in water. Furthermore, S^{2-} combines with manganese and iron in the water to form MnS and FeS , which are the leading causes of water blackening [2]. Consequently, to study the inhibitory effects of various remediation strategies on black-odorous water, the changes in AVS in sediment and the concentration of SO_4^{2-} in the overlying water were analyzed (Figure 2).

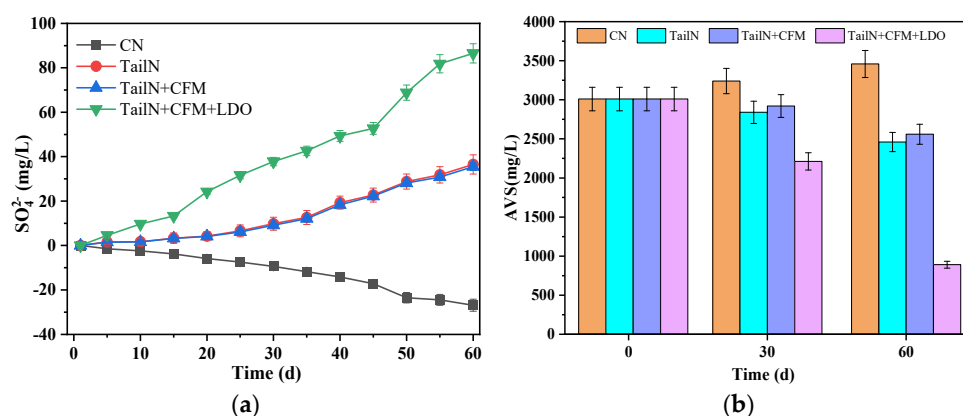


Figure 2. Variation of overlying water SO_4^{2-} (a) and sediment AVS (b) during restoration.

The changes in SO_4^{2-} concentration in the overlying water of varying remediation groups are shown in Figure 2a. After 60 days of remediation, the SO_4^{2-} concentration in the overlying water of the CN decreased by 26.9 mg/L, while in the other remediation groups, it exhibited a gradual increase to 36.5 mg/L (TailN), 35.5 mg/L (TailN + CFM), and 86.5 mg/L (TailN + CFM + LDO). From the perspective of the increased SO_4^{2-} concentration, under low oxygen conditions, there was a huge rise in the SO_4^{2-} concentration in the overlying water. This study indicates that the impact of NO_3^- -N, SO_4^{2-} in the overlying water was mainly produced through sulfur autotrophic denitrification [9]. At the same time, with the introduction of oxygen, the DO and ORP in the overlying water were significantly increased, and the activity of sulfur-oxidizing bacteria at the water–sediment interface was further enhanced, increasing the SO_4^{2-} concentration in the overlying water [5]. In the blank group, as the DO in the overlying water decreased, the concentration of SO_4^{2-} also decreased. This study indicated that the activity of anaerobic bacteria in the sediment gradually increased, and sulfate-reducing bacteria, as the main anaerobic bacteria in the sediment, participated in sulfate reduction reactions. This ultimately led to the reduction of SO_4^{2-} in the overlying water [7].

The changes in the AVS concentration in the sediment of different remediation groups are illustrated in Figure 2b. After 60 days of remediation, the AVS concentration in the remediation groups decreased to 2460 g/kg (TailN), 2560 g/kg (TailN + CFM), and 890 g/kg (TailN + CFM + LDO), reflecting declines of 18.3%, 15.3%, and 70.4%, respectively. In contrast, the AVS concentration in the blank group increased from 3010 g/kg to 3458 g/kg. According to studies, the reduction of reducible sulfur in sediment is mainly achieved through sulfur autotrophic denitrification reactions with NO_3^- -N [3]. The increase in AVS concentration in the blank group's sediment is primarily associated with decreases in DO and ORP in the overlying water. As the reducing environment of the water body strengthens, the activity of sulfate-reducing bacteria in the sediment increases, resulting in an increase in AVS concentration [20].

3.3. Changes in the Morphology of TOM in the Sediment

The changes in the sediment TOM concentration in the different remediation groups are shown in Figure 3. After 60 days of remediation, the sediment TOM in the remediation group was reduced to 112 g/kg (TailN), 98 g/kg (TailN + CFM), and 56 g/kg (TailN + CFM

+ LDO), which were 25.3% (TailN), 34.7% (TailN + CFM), and 62.7%, and 62.7% (TailN + CFM + LDO), respectively, however, the sediment TOM in the CN was reduced by only 10%. In the absence of remediation, the TOM in the sediments was degraded mainly by sulfate-reducing bacteria, heterotrophic iron-reducing bacteria, and electroactive bacteria. Since this study did not use isotopic labeling to calculate the amount of TOM consumed by the sulfate-reducing process and heterotrophic iron-reducing bacteria process, the generated AVS and Fe(II) may have been overlooked for re-oxidation [21], and therefore, the amount of TOM degraded by the sulfate-reducing process and heterotrophic iron-reducing bacteria process could not be accurately calculated.

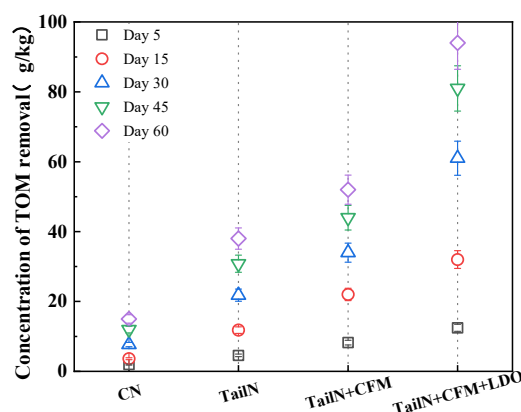


Figure 3. Concentration of TOM removal.

To deeply explore the change rule of TOM, the changes of LFOM and HFOM during the remediation process were analyzed; the results are shown in Figure 4. On the 60th day of remediation, the LFOM in the sediment dropped from the initial 45 g/kg to 10 g/kg (TailN), 2 g/kg (TailN + CFM), and 0 g/kg (TailN + CFM + LDO), while the LFOM in the sediment of the CN group only degraded to 32 g/kg. The HFOM in the sediment decreased from the initial 105 g/kg to 102 g/kg (TailN), 96 g/kg (TailN + CFM), and 56 g/kg (TailN + CFM + LDO), while only 2 g/kg of HFOM was degraded in the CN group. The results showed that the LFOM was preferentially degraded in the remediation groups, with significant degradation of the HFOM in the TailN + CFM + LDO group. However, the degradation of the HFOM could not be achieved without the participation of electroactive bacteria, suggesting that the TailN + CFM + LDO group may have promoted the degradation of the HFOM by inducing electroactive bacteria. The main reason for this was that the extracellular electron transfer of electroactive bacteria enhanced HFOM degradation due to factors such as spatial site resistance or mass transfer [22,23]. Under aeration and denitrification, the DO and ORP in the water column were effectively enhanced, which increased the Fe(III) concentration in the water bodies and induced electroactive bacteria such as *Pseudomonas* and *Thiobacillus*.

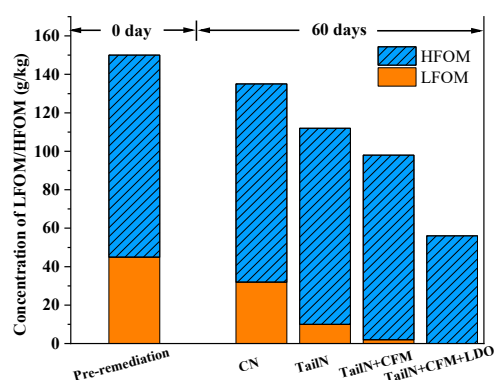


Figure 4. Concentration of LFOM/HFOM.

3.4. Analysis of Sediment Microbial Community Structure

The 16S amplicon sequencing analysis was performed on sediment samples collected on day 60 to identify the changes in the sediment microbial community structure. The results are shown in Figure 5. The relative abundance of *Sterolibacterium* [24], *Brevundimonas* [25], *Silanimonas* [26], *Thiobacillus* [20], and *Denitratisoma* [24], which are bacterial genera with denitrification functions, increased in the TailN, TailN + CFM, and TailN + CFM + LDO groups. Among them, the relative abundance of *Sterolibacterium* increased from 0.24% (the original sample) to 0.64% (CN), 1.37% (TailN), 1.00% (TailN + CFM), and 3.21% (TailN + CFM + LDO). The results indicated that the addition of NO_3^- -N in the tailwater increased the relative abundance of denitrifying bacteria genera. It is worth noting that the relative abundance of *Sterolibacterium* increased the most under low oxygen conditions, and previous studies have shown that low oxygen conditions can effectively stimulate the activity of denitrifying bacteria [5]. Additionally, two microorganisms capable of eliminating black and odor, *Rhizobium* [27] and *Mycobacterium* [28], were detected in the remediation groups. Their relative abundance increased compared to the blank group, with the highest increase observed under low oxygen conditions. The relative abundance of the sulfate-reducing bacterium *Syntrophus* [29] in the sediment decreased significantly with the introduction of nitrate in the tailwater, dropping from 1.56% (the original sample) to 1.39% (CN), 1.27% (TailN), 1.37% (TailN + CFM), and 0.90% (TailN + CFM + LDO).

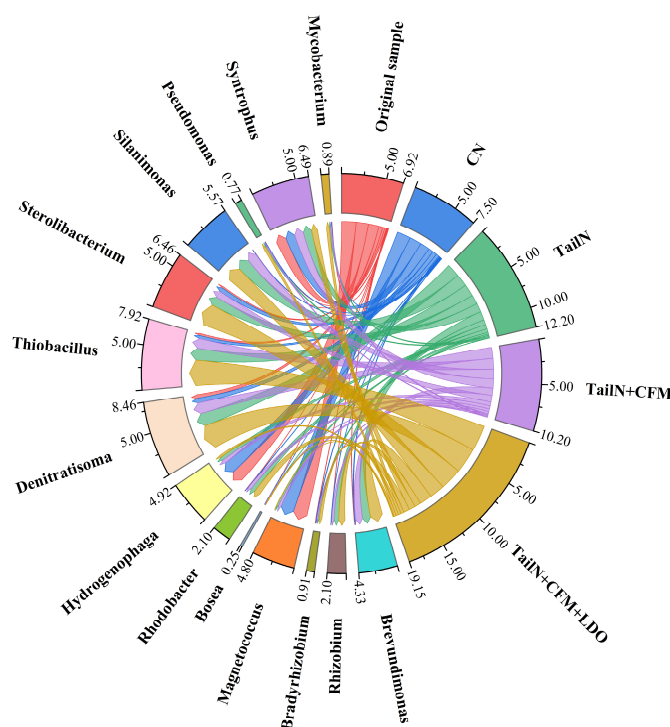


Figure 5. Composition and abundance of the sediment samples at genus levels.

The relative abundance of electroactive bacteria *Thiobacillus* [20] and *Pseudomonas* [30,31] with denitrification ability increased in the remediation group, with the relative abundance of *Thiobacillus* increasing from 0.32% (the original sample) to 0.45% (CN), 1.79% (TailN), 2.35% (TailN + CFM), and 3.84% (TailN + CFM + LDO). The microbial community structure indicated that the addition of NO_3^- -N to the tailwater greatly enhanced the microbial community structure of the sediment, with the greatest improvement observed under low oxygen conditions.

To gain deeper insights into the impact of TailN + CFM + LDO on the microbial ecological environment of the sediment, stratified sampling of the TailN + CFM + LDO sediment was performed on the 60th day. The collected sediment samples were then subjected to microbial community structure analysis. The results are shown in Figure 6. In the shallow

layer (0~4 cm), the relative abundance of bacteria genera with denitrification function, including *Sterolibacterium* [24], *Brevundimonas* [25], *Silanimonas* [26], *Thiobacillus* [20], and *Denitratisoma* [24], was significantly higher than that of the bacteria genera with denitrification function in the deep layer. Furthermore, it exhibited a decreasing trend with increasing depth. Additionally, *Rhizobium* [27] and *Mycobacterium* [28], which are microorganisms capable of eliminating black and odor, and *Thiobacillus* [20], a sulfur autotrophic denitrifying bacterium, exhibited similar patterns of relative abundance changes. The relative abundance of *Syntrophus* [29], a sulfate-reducing bacterium, in the sediment exhibited an opposite pattern of change. Combining the changes in concentrations of DO, ORP, $\text{NH}_3\text{-N}$, $\text{NO}_3^- \text{-N}$, $\text{NO}_2^- \text{-N}$, and SO_4^{2-} at different depths in the sediment (Figure S2), it could be inferred that denitrification mainly occurred within a range from −8 cm to 0 cm, whereas sulfate reduction mainly occurred within a range from −16 cm to −8 cm. The results indicated that the microbial community structure in the sediment within a range from −8 to 0 cm was significantly improved by the TailN + CFM + LDO treatment.

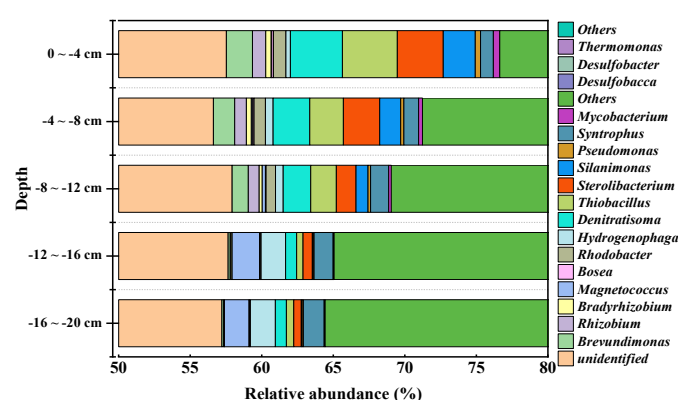


Figure 6. Downcore distribution of composition and abundance in different sediment sections at genus levels.

3.5. Mechanisms of Improvement in Sediment Microbial Ecological Environment

To further analyze the mechanisms of improvement in the sediment microbial ecological environment, the PICRUSt software (Version 1.1.2) was used to predict the relative abundance of nitrogen and sulfur metabolism pathways, as well as essential genes, in various sediment layers of the TailN + CFM + LDO group. The results are shown in Figure 7. The key genes present in nitrogen cycling included denitrification genes (such as *narH*, *napA*, *nirK*, *nosZ*, and *norB*), nitrogen fixation genes (such as *nifD* and *nifH*), ammonia oxidation genes (such as *amoA* and *amoB*), and DNRA genes (such as *nirB* and *nirD*) [32,33]. Nitrogen fixation and DNRA genes facilitate the formation of $\text{NH}_3\text{-N}$, whereas denitrification and ammonia oxidation genes promote nitrogen removal [34,35]. The relative abundance of nitrogen fixation genes and DNRA genes in the deep sediment layer (−20~−12 cm) was significantly higher than that in the shallow sediment layer (−12~0 cm). The relative abundance of denitrification and ammonia oxidation genes in the shallow sediment layer (−12~0 cm) was considerably higher than that in the deep sediment layer (−20~−12 cm). The key sulfur cycling processes include sulfur oxidation (such as *Sox*, *SoxA*, *SoxB*, *SoxD*, and *SoxG*), dissimilatory sulfate reduction (such as *dsrB*, *dsrC*, *aprA*, and *aprB*), and assimilatory sulfate reduction (such as *sat*, *cysC*, *cysN*, and *sir*) [36]. The increase in the relative abundance of *Thiobacillus*, a sulfur autotrophic denitrifying bacterium, indicated that sulfur autotrophic denitrification was the primary mechanism in the shallow sediment layer (−4~0 cm) [37,38]. The presence of a high concentration of sulfide in sediment promotes the utilization of nitrogen and sulfur by sulfur bacteria [5,39]. Researchers discovered that the degradation of AVS in the sediment is primarily driven by sulfur-driven autotrophic denitrification [40,41]. The relative abundance of sulfur oxidation genes in the shallow sediment layer (−12~0 cm) was substantially higher than that in the deep sediment layer (−20~−12 cm). The relative abundance of dissimilatory sulfate reduction genes in the

shallow sediment layer (−12~0 cm) was significantly lower than that in the deep sediment layer (−20~12 cm). Dissimilatory sulfate reduction is the primary biochemical reaction responsible for H_2S production in anaerobic environments, while assimilatory sulfate reduction results in the production of organic sulfur compounds such as cysteine and methionine. In summary, under the conditions of TailN + CFM + LDO, the enhancement of sulfur oxidation in the shallow sediment layer (−4~0 cm) inhibited dissimilatory sulfate reduction, thereby promoting the oxidation of AVS in the sediment. This process promotes sulfur-driven autotrophic denitrification [38,42].

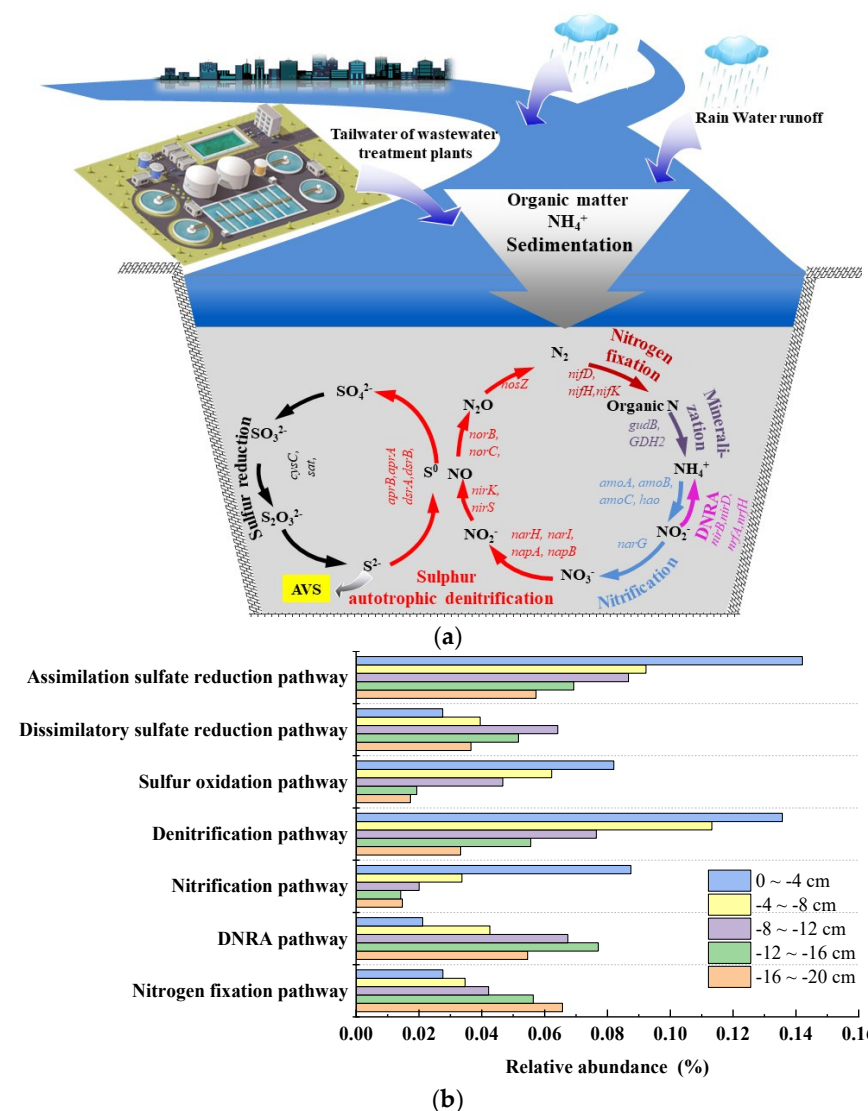


Figure 7. Nitrogen and sulfur metabolic pathways (a) and relative abundance of key genes (b) in sediments.

4. Conclusions

In this study, it was discovered that the nitrate remediation measure in the tailwater of wastewater treatment plants combined with aerobic denitrifying bacteria under low-DO regulation effectively increased the DO concentration and ORP in the overlying water, thereby enhancing the activity of electroactive bacteria, denitrifying and sulfur-oxidizing microorganisms in the sediment. This increased the quantity and activity of key enzymes present in the nitrification and denitrification processes. With the improvement of the microbial ecological environment, the AVS in the sediment dropped by 70.4%, and the TOM present in the sediment experienced degradation of 62.7%, in which the HFOM was

degraded from 105 g/kg to 56 g/kg, and the potential risk of water reverting to black and odorous conditions significantly decreased. The results indicated that TailN + CFM + LDO has a promising remediation effect on the sediment microenvironment.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/w16071000/s1>. Figure S1: Schema; Figure S2 Downcore distribution of $\text{NH}_3\text{-N}$, $\text{NO}_2^- \text{-N}$, $\text{NO}_3^- \text{-N}$, SO_4^{2-} ORP and DO in different sediment sections of TailN + CFM + LDO on day 60.

Author Contributions: Conceptualization, J.C. and J.T.; Methodology, C.Z.; Validation, J.T., J.G. and J.C.; Formal Analysis, J.C.; Investigation, C.Z.; Resources, Y.L.; Data Curation, J.C.; Writing—Original Draft Preparation, J.T.; Writing—Review & Editing, J.G.; Visualization, J.T.; Project Administration, J.G.; Funding Acquisition, Y.L. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the National Natural Science Foundation of China (40871232); Beijing Natural Science Foundation-Joint key projects of the Education Commission (KZ201810020025).

Data Availability Statement: The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

Conflicts of Interest: Author Jie Tian was employed by Tianjin Huanke Environmental Consulting Co., Ltd., Author Jianbo Guo was employed by Zhongtai Bochao Intelligent Environmental Protection Technology (Suzhou) Co., Ltd. Other authors declare no conflict of interests.

References

- Gong, S.; Donde, O.O.; Cai, Q.; Wu, X.; Song, K.; Wang, C.; Hong, P.; Xiao, B.; Tian, C. Improved lakeshore sediment microenvironment and enhanced denitrification efficiency by natural solid carbon sources. *Int. J. Sediment Res.* **2022**, *37*, 823–832. [CrossRef]
- Cao, J.; Sun, Q.; Zhao, D.; Xu, M.; Shen, Q.; Wang, D.; Wang, Y.; Ding, S. A critical review of the appearance of black-odorous waterbodies in China and treatment methods. *J. Hazard. Mater.* **2020**, *385*, 121511. [CrossRef] [PubMed]
- Yin, H.; Yang, P.; Kong, M. Effects of nitrate dosing on the migration of reduced sulfur in black odorous river sediment and the influencing factors. *Chem. Eng. J.* **2019**, *371*, 516–523. [CrossRef]
- Wang, Y.; Wang, W.H.; Yan, F.L.; Ding, Z.; Feng, L.L.; Zhao, J.C. Effects and mechanisms of calcium peroxide on purification of severely eutrophic water. *Sci. Total Environ.* **2019**, *650*, 2796–2806. [CrossRef] [PubMed]
- Li, W.; Zhang, S.; Zhang, L.; Li, X.; Wang, F.; Li, G.; Li, J.; Li, W. In-situ remediation of sediment by calcium nitrate combined with composite microorganisms under low-DO regulation. *Sci. Total Environ.* **2019**, *697*, 134109. [CrossRef] [PubMed]
- Zhang, C.; Li, M.; Sun, J.; Zhang, S.; Huang, J. The mechanism of C–N–S interconnection degradation in organic-rich sediments by $\text{Ca}(\text{NO}_3)_2$ –Ca–O₂ synergistic remediation. *Environ. Res.* **2022**, *214*, 113992. [CrossRef] [PubMed]
- Liu, X.; Tao, Y.; Zhou, K.; Zhang, Q.; Chen, G.; Zhang, X. Effect of water quality improvement on the remediation of river sediment due to the addition of calcium nitrate. *Sci. Total Environ.* **2017**, *575*, 887–894. [CrossRef] [PubMed]
- Lu, S.; Zhang, X.; Xue, Y. Application of calcium peroxide in water and soil treatment: A review. *J. Hazard. Mater.* **2017**, *337*, 163–177. [CrossRef] [PubMed]
- He, Z.; Huang, R.; Liang, Y.; Yu, G.; Chong, Y.; Wang, L. Index for nitrate dosage calculation on sediment odor control using nitrate-dependent ferrous and sulfide oxidation interactions. *J. Environ. Manag.* **2018**, *226*, 289–297. [CrossRef] [PubMed]
- Rahman, M.M.; Roberts, K.L.; Grace, M.R.; Kessler, A.J.; Cook, P.L.M. Role of organic carbon, nitrate and ferrous iron on the partitioning between denitrification and DNRA in constructed stormwater urban wetlands. *Sci. Total Environ.* **2019**, *666*, 608–617. [CrossRef] [PubMed]
- Chang, Y.K.; Hou, L.J.; Gao, D.Z.; Liu, M.; Liu, C.; Zhang, Z.X. Organic matter degradation state affects dissimilatory nitrate reduction processes in Knysna estuarine sediment, South Africa. *J. Soils Sed.* **2021**, *21*, 3202–3212. [CrossRef]
- Wang, F.; Yang, F.; Gao, H.; Bai, Y.; Liao, H.; Li, H. Simulation of Denitrification Process of Calcium Nitrate Combined with Low Oxygen Aeration Based on Double Logarithm Mode. *Water* **2022**, *14*, 269. [CrossRef]
- Zhang, C.; Li, M.; Zhang, S.; Qi, Z.; Huang, J.; Sun, J. An improved method of fluorescein diacetate determination for assessing the effects of pollutants on microbial activity in urban river sediments. *J. Soils Sed.* **2022**, *22*, 2792–2801. [CrossRef]
- Shu, D.; He, Y.; Yue, H.; Wang, Q. Metagenomic and quantitative insights into microbial communities and functional genes of nitrogen and iron cycling in twelve wastewater treatment systems. *Chem. Eng. J.* **2016**, *290*, 21–30. [CrossRef]
- Nelson, M.B.; Martiny, A.C.; Martiny, J.B.H. Global biogeography of microbial nitrogen-cycling traits in soil. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 8033–8040. [CrossRef] [PubMed]

16. Liu, L.; Sun, F.; Zhao, H.; Mi, H.; He, S.; Chen, Y.; Liu, Y.; Lan, H.; Zhang, M.; Wang, Z. Compositional changes of sedimentary microbes in the Yangtze River Estuary and their roles in the biochemical cycle. *Sci. Total Environ.* **2021**, *760*, 143383. [[CrossRef](#)] [[PubMed](#)]
17. Zhang, C.; Li, M.; Sun, J.; Huang, J.; Chang, S. Fluorescein diacetate hydrolytic activity as a sensitive tool to quantify nitrogen/sulfur gene content in urban river sediments in China. *Environ. Sci. Pollut. Res.* **2023**, *30*, 62544–62552. [[CrossRef](#)] [[PubMed](#)]
18. Aßhauer, K.P.; Wemheuer, B.; Daniel, R.; Meinicke, P. Tax4Fun: Predicting functional profiles from metagenomic 16S rRNA data. *Bioinformatics* **2015**, *31*, 2882–2884. [[CrossRef](#)] [[PubMed](#)]
19. Malecki, L.M.; White, J.R.; Reddy, K.R. Nitrogen and phosphorus flux rates from sediment in the Lower St. Johns River estuary. *J. Environ. Qual.* **2004**, *33*, 1545–1555. [[CrossRef](#)] [[PubMed](#)]
20. Lin, W.; Long, X.; Chong, Y.; Yu, G. Potential risk assessment of heavy metals in sediments during the denitrification process enhanced by calcium nitrate addition: Effect of AVS residual. *Ecol. Eng.* **2016**, *87*, 333–339.
21. Luo, M.; Zhu, W.; Huang, J.; Liu, Y.; Duan, X.; Wu, J.; Tong, C. Anaerobic organic carbon mineralization in tidal wetlands along a low-level salinity gradient of a subtropical estuary: Rates, pathways, and controls. *Geoderma* **2019**, *337*, 1245–1257. [[CrossRef](#)]
22. Tan, W.B.; Zhao, X.Y.; Dang, Q.L.; Cui, D.Y.; Xi, B.D. Microbially reducible extent of solid-phase humic substances is governed by their physico-chemical protection in soils: Evidence from electrochemical measurements. *Sci. Total Environ.* **2020**, *708*, 134683. [[CrossRef](#)] [[PubMed](#)]
23. Li, M.; Sun, J.M.; Liu, C.; Tang, Y.Q.; Huang, J.J. The remediation of urban freshwater sediment by humic-reducing activated sludge. *Environ. Pollut.* **2020**, *265*, 115038. [[CrossRef](#)] [[PubMed](#)]
24. Yu, J.; Fan, C.; Zhong, J.; Zhang, Y.; Wang, C.; Zhang, L. Evaluation of in situ simulated dredging to reduce internal nitrogen flux across the sediment-water interface in Lake Taihu, China. *Environ. Pollut.* **2016**, *214*, 866–877. [[CrossRef](#)] [[PubMed](#)]
25. Zhou, S.; Zhang, Y.; Huang, T.; Liu, Y.; Fang, K.; Zhang, C. Microbial aerobic denitrification dominates nitrogen losses from reservoir ecosystem in the spring of Zhoucun reservoir. *Sci. Total Environ.* **2019**, *651*, 998–1010. [[CrossRef](#)] [[PubMed](#)]
26. Manucharova, N.A.; Vlasenko, A.N.; Tourova, T.P.; Panteleeva, A.N.; Stepanov, A.L.; Zenova, G.M. Thermophilic chitinolytic microorganisms of brown semidesert soil. *Microbiology* **2008**, *77*, 610–614. [[CrossRef](#)]
27. Su, J.F.; Zhang, Y.M.; Liang, D.H.; Wang, J.X.; Li, M. Performance and microbial community of an immobilized biofilm reactor (IBR) for Mn(II)-based autotrophic and mixotrophic denitrification. *Bioresour. Technol.* **2019**, *286*, 121407. [[CrossRef](#)] [[PubMed](#)]
28. Sasaki, Y.; Oguchi, H.; Kobayashi, T.; Kusama, S.; Sugiura, R.; Moriya, K.; Hirata, T.; Yukioka, Y.; Takaya, N.; Yajima, S. Nitrogen oxide cycle regulates nitric oxide levels and bacterial cell signaling. *Sci. Rep.* **2016**, *6*, 22038. [[CrossRef](#)] [[PubMed](#)]
29. Keshri, J.; Ram, A.S.P.; Sime-Ngando, T. Distinctive Patterns in the Taxonomical Resolution of Bacterioplankton in the Sediment and Pore Waters of Contrasted Freshwater Lakes. *Microb. Ecol.* **2018**, *75*, 662–673. [[CrossRef](#)] [[PubMed](#)]
30. Su, W.; Zhang, L.; Li, D.; Zhan, G.; Qian, J.; Tao, Y. Dissimilatory nitrate reduction by *Pseudomonas alcaliphila* with an electrode as the sole electron donor. *Biotechnol. Bioeng.* **2012**, *109*, 2904–2910. [[CrossRef](#)]
31. Zhang, W.; Zhang, Y.; Su, W.; Jiang, Y.; Su, M.; Gao, P.; Li, D. Effects of cathode potentials and nitrate concentrations on dissimilatory nitrate reductions by *Pseudomonas alcaliphila* in bioelectrochemical systems. *J. Environ. Sci.* **2014**, *26*, 885–891. [[CrossRef](#)] [[PubMed](#)]
32. Guo, J.; Peng, Y.; Fan, L.; Zhang, L.; Ni, B.-J.; Kartal, B.; Feng, X.; Jetten, M.S.M.; Yuan, Z. Metagenomic analysis of anammox communities in three different microbial aggregates. *Environ. Microbiol.* **2016**, *18*, 2979–2993. [[CrossRef](#)] [[PubMed](#)]
33. Zhang, H.; Sun, L.; Li, Y.; Zhang, W.; Niu, L.; Wang, L. The bacterial community structure and N-cycling gene abundance in response to dam construction in a riparian zone. *Environ. Res.* **2021**, *194*, 110717. [[CrossRef](#)] [[PubMed](#)]
34. Hu, Y.; Hong, Y.; Ye, J.; Wu, J.; Wang, Y.; Ye, F.; Chang, X.; Long, A. Shift of DNRA bacterial community composition in sediment cores of the Pearl River Estuary and the impact of environmental factors. *Ecotoxicology* **2021**, *30*, 1704. [[CrossRef](#)]
35. Wang, S.; Wu, B.; Wang, Y.; Wang, X. Nitrate Migration and Transformation in Low Permeability Sediments: Laboratory Experiments and Modeling. *Water* **2023**, *15*, 2528. [[CrossRef](#)]
36. Zhang, M.; Li, Y.; Sun, Q.; Chen, P.; Wei, X. Correlations of functional genes involved in methane, nitrogen and sulfur cycling in river sediments. *Ecol. Indic.* **2020**, *115*, 106411. [[CrossRef](#)]
37. Chen, M.; Zhou, X.-F.; Yu, Y.-Q.; Liu, X.; Zeng, R.J.-X.; Zhou, S.-G.; He, Z. Light-driven nitrous oxide production via autotrophic denitrification by self-photosensitized *Thiobacillus denitrificans*. *Environ. Int.* **2019**, *127*, 353–360. [[CrossRef](#)] [[PubMed](#)]
38. Gao, S.; Li, Z.; Hou, Y.; Wang, A.; Liu, Q.; Huang, C. Effects of different carbon sources on the efficiency of sulfur-oxidizing denitrifying microorganisms. *Environ. Res.* **2022**, *204*, 111946. [[CrossRef](#)]
39. Guo, G.; Ekama, G.A.; Wang, Y.; Dai, J.; Biswal, B.K.; Chen, G.; Wu, D. Advances in sulfur conversion-associated enhanced biological phosphorus removal in sulfate-rich wastewater treatment: A review. *Bioresour. Technol.* **2019**, *285*, 121303. [[CrossRef](#)] [[PubMed](#)]
40. Li, S.; Pang, Y.; Ji, G. Increase of N₂O production during nitrate reduction after long-term sulfide addition in lake sediment microcosms. *Environ. Pollut.* **2021**, *291*, 118231. [[CrossRef](#)] [[PubMed](#)]

41. Pang, Y.; Wang, J.; Li, S.; Ji, G. Long-term sulfide input enhances chemoautotrophic denitrification rather than DNRA in freshwater lake sediments. *Environ. Pollut.* **2021**, *270*, 116201. [[CrossRef](#)] [[PubMed](#)]
42. Huang, C.; Liu, Q.; Wang, H.; Gao, L.; Hou, Y.-N.; Nan, J.; Ren, N.; Li, Z.-L. Influence of microbial spatial distribution and activity in an EGSB reactor under high- and low-loading denitrification desulfurization. *Environ. Res.* **2021**, *195*, 110311. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.