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Microbial aerobic denitrification dominates nitrogen losses from reservoir ecosystem in the spring of Zhoucun reservoir

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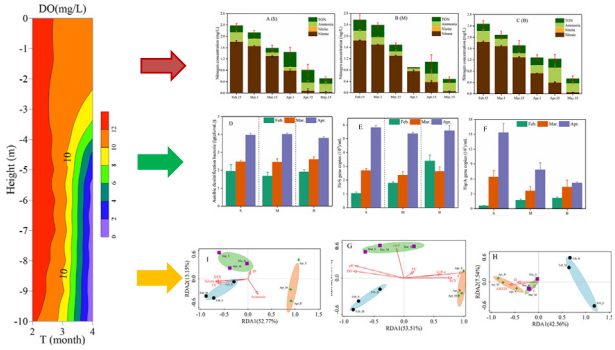
HIGHLIGHTS

- Microbial aerobic denitrification dominates nitrogen loss of water column in the spring.
- The abundance of *nir*S and aerobic denitrification bacteria increased obviously.
- The abundance of N-functional bacteria and *nap*A-type denitrification bacteria both exhibited obvious increase process.

GRAPHICAL ABSTRACT



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ABSTRACT

The mechanism and factors influencing nitrogen loss in the Zhoucun reservoir were explored during the spring. The results showed that the nitrate and total nitrogen concentration decreased from 1.84 ± 0.01 mg/L and 2.34 ± 0.06 mg/L to 0.06 ± 0.01 mg/L and 0.48 ± 0.09 mg/L, respectively. Meanwhile, the nitrate and total nitrogen removal rate reached 97.02% ± 0.25 and 79.38% ± 3.32, respectively. Moreover, the abundance of *nir*S gene and aerobic denitrification bacteria increased from 1.04–3.38 × 10^3 copies/mL and 0.71 ± 0.22 × 10^2 cfu/mL to 5.36–5.81 × 10^3 copies/mL and 8.64 ± 2.08 × 10^3 cfu/mL, respectively. The low MW fractions of DOM (<5 kDa) increased from 0.94 ± 0.02 mg/L in February to 1.51 ± 0.09 mg/L in April. E3/E4 and absorption spectral slope ratio (S_R) showed that fulvic acid accounted for the main proportion with autochthonous characteristics. These findings were consistent with the fluorescence components and fluorescence characteristic indices based on EEM-PARAFAC. Meanwhile, the microbial metabolism activity increased significantly from February to April, which contributed to the cycle of nutrients within the reservoir water system. Moreover, the abundance of the bacterial species involved in denitrification (*Exiguobacterium*, *Brevundimonas*, *Deinococcus*, *Paracoccus*, and *Pseudomonas*) increased significantly. The relative abundance of KOs related to nitrogen metabolism, were initially increased and then decreased. Specifically, K02567 (*nap*A) represented the main proportion of KOs related to denitrification. The abundance of *nap*A-type denitrifying bacteria (*Dechloromonas*, *Pseudomonas*, *Azospira*, *Rhodopseudomonas*, *Aeromonas*, *Zobellella*, *Sulfuritalea*, *Bradyrhizobium*, *Achromobacter*, *Enterobacter*, *Thauera*, and *Magnetospirillum*) increased significantly during the period of nitrogen loss. Furthermore, the levels of nitrate, T, DO, and AWCD were the most important factors affecting the N-functional bacteria composition. The systematic investigation of the nitrogen loss would provide a theoretical foundation for the remediation of the water reservoir via aerobic denitrification in the future.

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1. Introduction

Excessively high concentrations of nitrogen (N), often in the form of nitrate and ammonia, have negative effects on water quality and can result in water eutrophication. While there is still some debate regarding whether N alone is the main driver of these problems, it has been well accepted that increased N loading degrades the water quality. Traditional bio-treatment processes for nitrogen removal involve autotrophic and heterotrophic denitrification under aerobic and anoxic conditions, respectively. Because of their different oxygen requirements, these two steps are separated spatially and temporally. Given that the reaction steps are inhibited by oxygen, they rarely occur in natural waters, especially in reservoirs. However, *Thiosphaera pantotropha* (Robertson and Kuenen, 1983), the first aerobic denitrifying bacteria, highlighted a novel method of removing nitrogen in an anaerobic manner. Microbiologists have defined aerobic denitrification as the co-respiration or co-metabolism of oxygen and nitrate (Gao et al., 2010). Aerobic denitrification possesses several attractive advantages, including the fact that nitrification and denitrification can occur within the same system, and denitrification can cause sufficient alkalinity to partially balance the acidity of nitrification.

Consequently, more researchers have focused on N removal using aerobic denitrifiers in recent years. Li et al. (2012) presented the sequenced genome of *Pseudomonas stutzeri* T13, which could provide further insights into the aerobic denitrification mechanism utilized by the strain (Li et al., 2012). Immobilized *P. stutzeri* T13 can maintain a steadily high TN removal rate (Ma et al., 2015). Dissolved oxygen has influence on accumulated nitrite during heterotrophic and aerobic denitrification (Sun et al., 2015). Furthermore, Sun et al. (2017) investigated the effects of ammonium utilization on aerobic denitrification (Sun et al., 2017). Currently, several full-scale experiments involving the bio-augmentation of aerobic denitrifying bacteria have been successful in improving the treatment of wastewater, urban rivers, and river sediment remediation. For example, Duan et al. (2015) applied halophilic heterotrophic nitrifying-aerobic denitrifying SF-16 to treat saline wastewater (Duan et al., 2015). Du et al. (2017) studied the removal of N and the resulting microbial community shift in an aerobic denitrification reactor bioaugmented with a *Pseudomonas* strain for the treatment of wastewater produced by the coal-based ethylene glycol industry (Du et al., 2017). Tang et al. (2018a, b) applied aerobic denitrifiers coupled with a denitrification agent in the sediment of an urban river for the bioremediation of N pollution (Tang et al., 2018a) and explored the mechanism of aerobic denitrifiers on urban river sediment remediation through biostimulation coupled with a bioaugmentation approach (Tang et al., 2018b). It is well known that many environmental factors, including temperature, nutrients, and co-substrates, may influence the biodegradation of pollutants in the environment. Successful application of bioaugmentation technology also depends on the adaptation of microbial strains to indigenous microorganisms. Notably, the ability of the introduced microbial strains to remain viable and active is essential for the received systems.

Moreover, some researchers have shown that aerobic denitrification occurs in natural systems. For example, Gao et al. (2010, 2012) demonstrated that aerobic denitrification existed in permeable sea sediments (Gao et al., 2010) and explored the N-loss from intertidal permeable sediments of the Wadden Sea (Gao et al., 2012). Moreover, Coban et al. (2015) quantified the rates of aerobic denitrification in wetland. Marchant et al. (2017) found that denitrification occurred not only at high DO concentrations but was stimulated by frequent switches between oxic and anoxic conditions in coastal sediments, which contributed significantly to N-loss in permeable sediments making the process an important source of anthropogenic N-inputs (Marchant et al., 2017). Meanwhile, aerobic denitrifying species were also isolated from the reservoirs, and had ideal characteristics for N removal (Zhang and Zhou, 2016). Moreover, the indigenous aerobic denitrifying bacteria could be enhanced in situ using water lifting and aeration technology in an enclosure system of the reservoir, indicating ideal N removal performance (Zhou et al., 2016a, b, 2018). It has been suggested that aerobic denitrification via in situ oxygen enhancement is an effective way to decrease the endogenous N pollution in this aquatic ecosystem. Particularly in the case of drinking water obtained via reservoirs, the environmental safety issues could be addressed without adding a considerable amount of "inoculated bacterial strain" to natural water. However, there is no direct evidence confirming the occurrence of aerobic denitrification in reservoir systems.

To this end, our objectives in this study were (I) to investigate the N losses at the beginning of stratification in reservoir system; (II) to examine the changes of genes involved in denitrification and aerobic denitrifying bacteria; (III) to investigate the carbon metabolism characteristics; (IV) to examine the differences in bacterial diversity and composition; and (V) to investigate the relationship between microbial community structure and environmental factors, revealing the possible mechanisms for N removal during remediation.

2. Methods

2.1. Experimental sample collection

The experimental sample collection area was located in the main reservoir area of the Zhoucun reservoir in northern China (Fig. S1). We selected three sampling sites to investigate the extent of N removal. The coordinates of these samples sites were as follows: #1 34°56′47″N, 117°41′8″E, #2 34°56′52″N, 117°41′E, and #3 34°56′43″N, 117°40′54″E. Three rivers flow into the reservoir in this study area: the Xuwa (XW) and Xiashi (XS) Rivers, located northwest of the reservoir, and the Xijia (XJ) River, located northeast of the reservoir. The XJ, XW, and XS Rivers received much of their N loading from agricultural catchment areas and sewage, especially the XW River because of the presence of chicken farms. The average depth of the main reservoir ranged from 10.86 to 12.15 m.

Water sampling was performed monthly from February 16, 2016 to April 15, 2016. Sampling was performed with a 5-L water sampler tethered to a 20-m rope for collection at depths of 0.5, 6, 8, 10 m and bottom water at the six sites. The surface sediments (depth = 0–2 cm) were collected using a sterilized Petersen stainless steel grab sampler. The samples were stored in the dark at 4 °C and transported to the Water research laboratory (Zao Zhuang City, China). Most of the water parameters were measured within 48 h. Meanwhile, the wet sediments were air-dried, and then sieved with a standard 100-mesh sieve for analysis.

2.2. Physical and chemical analysis

The water temperature (T), dissolved oxygen (DO), pH, oxidation-reduction potential (ORP), electrical conductivity (EC), and chlorophyll-*a* (Chl-*a*) of the sampling site were determined in situ (0.5-m increments) using a multi-parameter water quality analyzer (Hydrolab DS5, HACH Company, USA). In the laboratory, the water parameters were measured using a spectrophotometer (DR6000; HACH Company, USA). Specifically, levels of nitrate, nitrite, ammonia, total N (TN), and total phosphorus (TP) were determined using the procedures detailed in the standard methods (Chinese, 2002). If the water temperature difference was exceeded by 1 °C within a 1 m depth at the metalimnion, the water column was defined as thermally stratified. The TN in the sediment was measured using the method previously outlined by Zhou et al. (2016b). Molecular weight distributions for DOC of reservoir systems were determined by ultrafiltration and were consistent with the previous study (Zhou et al., 2016b).

2.3. Spectral characteristics of DOM

The UV–vis absorption and fluorescence excitation-emission matrix (EEM of the DOM was determined by a DR6000 (HACH Company, USA;