Nitrogen removal characteristics of indigenous aerobic denitrifiers and changes in the microbial community of a reservoir enclosure system via *in situ* oxygen enhancement using water lifting and aeration technology

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Abstract: Indigenous aerobic denitrifiers of a reservoir system were enhanced *in situ* by water lifting and aeration technology. Nitrogen removal characteristics and changes in the bacterial community were investigated. Results from a 30-day experiment showed that the TN in the enhanced water system decreased from 1.08–2.02 to 0.75–0.91 mg/L and that TN removal rates varied between 21.74–52.54% without nitrite accumulation, and TN removal rate of surface sediments reached 41.37 ± 1.55%. The densities of aerobic denitrifiers in the enhanced system increased. Furthermore, the enhanced system showed a clear inhibition of Fe, Mn, and P performances. Community analysis using Miseq showed that diversity was higher in the *in situ* oxygen enhanced system than in the control system. In addition, the microbial composition was significantly different between systems. It can be concluded that *in situ* enhancement of indigenous aerobic denitrifiers is very effective in removing nitrogen from water reservoir systems.

Keywords: aerobic denitrification; *in situ*; nitrogen removal; Miseq high-throughput sequencing technique; water reservoir

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1. Introduction

Excessive nitrogen concentration, often in the form of nitrate and ammonia, leads to poor water quality and has recently become a growing concern. Increasing N export has been related to water eutrophication in coasts, lakes, and especially in drinking water reservoirs. There is still some debate over whether N alone is the main driver of these problems, but there is no doubt that an increase in N loading causes water quality degradation. In the past few years, bioremediation has attracted growing attention because it has lower maintenance costs and is more efficient at removing pollutants than other methods (Zhu et al. 2008). Traditional biotreatment processes for nitrogen removal involve autotrophic nitrification and heterotrophic denitrification. Nitrification is achieved under aerobic conditions, while denitrification requires anaerobic and anoxic conditions through a sequence of intermediates (nitrate, nitrite, nitric oxide, and nitrous oxide), resulting in nitrogen gas. Because of their different oxygen requirements, these two steps are separated spatially and temporally. Since oxygen inhibits the reaction steps, traditional processes are impractical in natural waters, especially in reservoirs. The discovery of the first aerobic denitrifying bacteria, *Thiosphaera pantotropha* (Robertson and Kuenen 1983), led to a novel method for removing nitrogen which is not limited by oxygen. Moreover, aerobic denitrification occurs in natural systems. Gao et al. (2010) demonstrated that aerobic denitrification exists in permeable sea sediments (Gao et al. 2010), and Coban et al. (2015) quantified the rates of aerobic denitrification (Coban et al. 2015a).

Microbiologists have defined aerobic denitrification as the co-respiration or co-metabolism of oxygen and nitrate. Aerobic denitrification has attractive advantages: nitrification and denitrification can occur in the same system, and denitrification can cause sufficient alkalinity to partly balance the acidity of nitrification. Consequently,

more researchers in recent years have focused on nitrogen removal using aerobic denitrifiers. Some full-scale bioaugmentation experiments with aerobic denitrifying bacteria have been conducted successfully. For example, Robertson et al.(1989) applied bioaugmentation to biologically remove nitrogen from wastewater (Robertson et al. 1989). These authors introduced and maintained aerobic denitrifiers in a complex nitrifying community, allowing nitrification and denitrification to occur concurrently in the same aerobic unit. Patureau et al. (1997) successfully combined an aerobic denitrifier, *Microvirgula aerodenitrificans* (Patureau et al. 1997), with a nitrifying consortium, despite the fact that the denitrifying activity of the aerobic bioreactor declined over time. Cattaneo et al. (2003) studied the performance of *Pseudomonas denitrificans* in a fluidized bed and in a stirred tank reactor, and found that bacteria successfully removed nitrogen in the fluidized bed reactor (Cattaneo et al. 2003). Recently, Chen et al. (2015) found that PCN bacteria capable of nitrogen removal could be used to treat municipal wastewater in a pilot scale SBR (Chen et al. 2015). This approach was able to meet the strict requirements of the National Municipal Wastewater Discharge Standards of China (chemical oxygen demand (COD) < 50 mg/L, total nitrogen (TN) < 15 mg/L, total phosphorus (TP) < 0.5 mg/L). It is well known that the successful application of bioaugmentation technology depends on the adaptation of microbial strains to indigenous microorganisms, which means the introduced microbial strains should survive and remain active in the receiving systems. Although bioaugmentation seems simple at first, many attempts to use bioaugmentation have failed owing to the poor *in situ* survival or low activity of bioaugmentation strains (Thompson et al. 2005). Problems concerning the adaptation of inoculated microorganisms, insufficient substrate, competition between introduced species and indigenous biomass, and grazing by protozoa have been suggested as possible reasons

for experimental failure. Moreover, when adding a considerable amount of "inoculated bacteria strains" to natural water, especially in drinking water reservoirs, environmental safety cannot be ignored (Wu et al. 2014).

Research on bacterial inoculation or *in situ* enhancement of indigenous aerobic denitrifying bacteria for nitrogen pollution removal or bioremediation of reservoir systems is scarce when compared to research on bioaugmentation in soils, municipal wastewater, and groundwater systems. It is known that the quality of water reservoirs is affected by endogenous pollutants released into the overlying water under anoxic conditions (Gantzer et al. 2009). Many studies demonstrated that anoxia could re-introduce N, P, Fe, and Mn from sediments into overlying water layers (Gantzer et al. 2009, Chai et al. 2011). To effectively control pollutants released from sediments, aerobic conditions in reservoirs must be kept using hypolimnetic oxygenation (Beutel and Horne 1999). During the past few decades, WLA (water lifting and aeration) technology has been developed and used effectively to increase dissolved oxygen concentration and improve water quality of micro-polluted drinking water reservoirs (Cong et al. 2006, Cong et al. 2009, Bryant et al. 2011, Gerling et al. 2014). Meanwhile, aerobic denitrifying species with nitrogen removal characteristics have also been isolated from reservoirs (Wei et al. 2010, Guo et al. 2013, Huang et al. 2015a, Huang et al. 2015b). This suggests that aerobic denitrification is an effective way to decrease endogenous nitrogen pollution in aquatic ecosystems. However, the underlying mechanisms are not well understood (Gao et al. 2010, Coban et al. 2015b).

In the present study, indigenous aerobic denitrifiers from the enclosure system of Zhoucun drinking water reservoir were enhanced *in situ* using WLA technology. WLA is used to mix and oxygenate water, facilitating the growth of aerobic denitrifiers and enhancing the water denitrification by aerobic microorganisms (Huang et al. 2012). The

objectives of this study were: (i) to investigate the feasibility and efficiency of nitrogen removal by indigenous aerobic denitrifiers and the inhibition of Fe, Mn, and P pollutants via *in situ* oxygenation; (ii) to examine the bacterial diversity and abundance, and to find out which genera of bacterioplankton are present at different times in enhanced and control systems; and (iii) to investigate the relationship between the bacterioplankton community structure and environmental driving factors, focusing especially on the bacteria involved in nitrogen cycling across the whole experimental period.

2. Methods

2.1 Experimental system

Enhanced system. An enhanced system was used to simulate the WLA (Water lifting and aeration) technology system. Compressed air was released in the bottom of the enhanced experimental system in the form of small bubbles, which increased dissolved oxygen concentration through direct mixing and oxygenation (Supplementary Figure S1). **Control system.** An experimental system without aeration, placed at the bottom of the reservoir, was used as control group (Supplementary Figure S2).

2.2 Nitrogen removal in the enhanced system

The enhanced experiment was conducted in three periods with three different oxygen concentration levels (high oxygen concentration, medium oxygen concentration, and low oxygen concentration). In order to study the nitrogen removal performance, nitrate, nitrite, ammonia, TN (total nitrogen), and TDN (total dissolved nitrogen) concentrations were measured in each period. All parameters were measured in triplicate (n = 3). In order to access the inhibition of TN in sediments, the TN of surface sediments was also measured at specific times in both enhanced and control systems.

2.3 Changes of oligotrophic aerobic denitrifiers

In order to investigate whether the ability of indigenous bacteria to remove nitrogen could be improved in the enhanced system, the numbers of oligotrophic aerobic

denitrifiers were measured in enclosure systems. The density of oligotrophic aerobic denitrification bacteria in the experimental systems were measured by plate counts (Huang et al. 2012, Huang et al. 2015c). The numbers of aerobic denitrification bacteria of water samples (0.5 m, 5.0 m, 7.5 m, 10.0 m, and 13.0 m) were tested via gradient dilution. The gradient dilutions were as follows: 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}, respectively. Then 0.2 mL diluents were streaked onto a solid screening medium (included (g/L): CH_3COONa, 0.10; $NaNO_3$, 0.02; $K_2HPO_4 \cdot 3H_2O$, 0.02; $CaCl_2$, 0.01; $MgCl_2 \cdot 6H_2O$, 0.01; and agar, 20; pH 7.2.) in triplicate and incubated at 30 °C for 5 days. Prominent single colonies were harvested and calculated.

2.4 Quantification of nirS and nirK genes abundance

Quantitative PCR was used to estimate the numbers of nirS and nirK copies in water and sediment systems collected at 20 sites during the experimental period of Oct.16-Nov. 14. DNA was extracted from an approximately 2 L water sample (every water sample) and ~ 50 mL of surface sediment (0-2 cm) for water and soil samples. Primers used for nirK quantification (Zhou et al. 2016) were F1aCu, 5'-ATYGGCGGVCAYGGCGA-3' and R3Cu, and 5'-GCCTCGATCAGRTTRTGGTT-3'. Primers used for nirS quantification (Zhou et al. 2016) were cd3aF, 5'-GTSAACGTSAAGGARACSGG-3' and R3cd, and 5'-GASTTCGGRTGSGTCTTGA-3'. Real-time PCR (qPCR) was performed on an ABI 7500 real-time system (Life Technologies, USA). And the PCR reaction mix according to (Zhou et al. 2016).

2.5 Inhibition of Fe, Mn, and P in the enhanced system

In order to investigate the inhibition of Fe, Mn, and P, the enhanced system simulated the WLA technology system. The concentrations of Fe, Mn, and P in water and surface sediment samples were measured in both enhanced and control systems.

2.6 Microbial DNA Extraction

In order to obtain total DNA, all water samples (each 2 L) were filtered with a 0.22 μm cellulose acetate membrane filter and ~50 mL surface sediment (0 - 2 cm) was collected. The whole microbial genomic DNA was extracted using the Water and Soil DNA Kit (OMEGA, Irving, TX, USA) according to the manufacturer's instructions. DNA was purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.) according to the manufacturer's instructions. The extracted DNA was stored at -80 ℃ for PCR amplification analysis.

2.7 Microbial community analysis using Illumina Miseq Sequencing

To explore the water and surface sediment microbial community composition in the enclosure systems, the Illumina Miseq Sequencing platform was used. DNA extracted from water and surface sediment samples (as described above) was amplified by PCR using primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 338R (5'-TGCTGCCTCCCGTAGGAGT-3') targeting the V2 regions of bacterial 16S rRNA genes (Ravel et al. 2011). All PCR products were sequenced using an Illumina Miseq Sequencing platform according to the standard protocols of the Shanghai Majorbio Bio-pharm Technology Co., Ltd, China. Sequences shorter than 200 bps and low quality sequences (quality score <25) were removed (Quince et al. 2011). The taxonomic classification of effective sequences was determined using the RDP (Ribosomal Database Project) database (http://rdp.cme.msu.edu/).

2.8 Analysis

Physical and Chemical analysis. The parameters of enclosure systems were measured in situ at 0.5-m increments using a multi parameter water quality analyzer (Hydrolab DS5, HACH Company, USA). In detail, T (Temperature), DO (Dissolved oxygen), pH, ORP (Oxidation-reduction potential), EC (Electrical conductivity), and CHl-a (Chlorophyll-a). The water parameters of the enclosure system were measured by using a spectrophotometer (DR6000; HACH Company, USA). Specifically the TN and

nitrate concentrations were measured using hydrochloric acid photometry (Chinese 2002). The nitrite concentration was determined by utilizing N-(1-naphthalene)-diaminoethane photometry (Chinese 2002). Ammonium-N concentration was determined using Nessler's reagent spectrophotometry (Chinese 2002). TP concentration was measured by ammonium molybdate spectrophotometric method (Chinese 2002). Fe concentration was measured by phenanthroline spectrophotometry (Chinese 2002). Mn concentration was measured by potassium periodate spectrophotometric method (Chinese 2002). The TN and TP (surface sediment) were determined by persulfate. Samples of nitrate, nitrite, ammonia, and TDN were filtered using a 0.45 μm cellulose-acetate filter. Finally, surface sediments were collected at a deep layer of 0 - 2 cm using a sterilized Petersen stainless steel grab sampler.

Data statistical analysis. The fundamental analysis, Rarefaction curves (RC), abundance-based coverage estimators (ACE), Chao richness estimator, Coverage, the Shannon and Simpson diversity were calculated by MOTHUR (Loman et al. 2012); the advanced analysis, the microbial community, Heatmap, PCA, PCoA, Hcluster, and Venn of OTU distribution. The gradient length of the longest axis explored by detrended correspondence analyses (DCA) was shorter than 3 SD (standard deviation) units. This indicates that species exhibited liner responses to environmental gradients and thus further analyses were conducted using redundancy analysis (RDA) ordination response models.

3. Results and discussion

3.1 Spatial and temporal environmental heterogeneity in enclosure systems

As shown in Table 1, the DO (dissolved oxygen) concentration in the enhanced system was divided into three time periods corresponding to different oxygen concentration levels (high oxygen concentration, Oct.16 - Oct.21; medium oxygen

concentration, Oct.21 - Oct.29; and low oxygen concentration, Oct.29 - Nov.14). The high oxygen concentration level was maintained at 11.49 ± 0.81 mg/L (DO%, 118%; T, 17.51 °C), the medium level was maintained at 8.19 ± 1.01 mg/L (DO%, 96%; T, 16.92 °C), and the low level was maintained at 4.76 ± 0.79 mg/L (DO%, 52%; T, 15.8 °C). The DO concentration of the bottom water layer was maintained at 0.36 ± 0.19 mg/L (DO%, 5%; T, 14.0 °C). The DO concentration of control system was maintained at 0.21 ± 0.09 mg/L (DO%, 3%; T, 13.8 °C; Oct.19 - Nov.14). At the bottom of the enclosure systems, the DO concentration was maintained under anaerobic conditions. Water temperature in the enclosure systems ranged from 21.2 °C to 12.5 °C and all water depth layers were maintained at a fixed state on Oct. 5.

In addition, the pH, ORP (oxidation-reduction potential), EC (electrical conductivity), and CHl-a (chlorophyll-a) in the enhanced system showed similar trends in all water layers. In this system, pH ranged from 7.62 ± 0.28 (0-12.0 m, n = 27, Oct. 16) and 9.35 ± 0.20 (12.0-13.0 m, n = 7, Oct. 16) to 7.47 ± 0.16 (0-13.0 m, n = 26, Oct. 19), 7.31 ± 0.18 (0-13.0 m, n = 52, Oct. 29), and 7.94 ± 0.02 (0-13.0 m, n = 26, Nov. 14). In the control system, pH increased from 7.62 ± 0.28 (0-12.0 m, n = 27, Oct. 16) and 9.35 ± 0.20 (12.0-13.0 m, n = 7, Oct. 16) to 8.31 ± 0.45 (0-13.0 m, n = 23, Oct. 19), 8.60 ± 0.31 (0-13.0 m, n = 20, Oct. 29), and 8.60 ± 0.26 (0-13.0 m, n = 20, Nov. 14). Denitrification of indigenous aerobic denitrifiers occurred in the enhanced system and, therefore, the alkalinity generated during denitrification could partly balance the acidity of nitrification. Hence, the pH in the enhanced system could maintain a steady state. The ORP in the enhanced and control systems decreased from 326.96 ± 9.77 (0-12.0 m, n = 27, Oct. 16) to 271.93 ± 2.35 mv (n = 27, Nov. 14) and 76.85 ± 32.92 mv (n = 27, Nov. 14), respectively. The EC in the enhanced system increased from 345.94 ± 33.66 (n = 34, Oct. 16) to 369 ± 0 us/cm (n = 27, Nov. 14), whereas the EC in the control

system increased from 345.94 ± 33.66 (n = 34, Oct. 16) to 342.41 ± 5.08 us/cm (n = 27, Nov. 14). The CHl-a concentration in enhanced and control systems decreased from 21.69 ± 12.66 (n = 34, Oct. 16) to 4.33 ± 0.27 μg/L (n = 27, Nov. 14) and 4.06 ± 0.54 μg/L (n = 27, Nov. 14), respectively.

3.2 Nitrogen removal in the enhanced system

Nitrogen is a key biogenic element in freshwater ecosystems, especially in reservoir systems. In recent years, an increasing amount of nitrogen has been discharged into the Zhoucun Reservoir, strongly increasing eutrophication. During periods of phytoplankton blooms (summer and fall), nitrogen addition stimulates phytoplankton growth. Therefore, nitrogen removal will limit eutrophication and phytoplankton growth, as suggested by Xu et al. (Xu et al. 2010). These authors showed that nitrogen and phosphorus inputs controlled phytoplankton growth and eutrophication (Lake Taihu, near Shanghai).

During the high oxygen concentration period (Oct. 16 - Oct. 21), the nitrate concentration of the enhanced system declined from 0.40 ± 0.04 to 0.02 ± 0.02 mg/L (Fig. S3-A). During the medium oxygen concentration period (Oct. 21-29), nitrate concentration ranged from 0.02 ± 0.02 to 0.09 ± 0.05 mg/L, while during the low oxygen concentration period (Oct. 29 - Nov. 14), nitrate concentration increased from 0.09 ± 0.05 to 0.37 ± 0.11 mg/L. In the latter period, nitrate concentration did not change much, and nitrate production and removal remained in a state of dynamic equilibrium. This was consistent with the decrease in nitrogen concentration in the sediment surface.

The nitrite concentration in the enhanced system was maintained at 0-0.09 mg/L, whereas that in the control system was 0-0.04 mg/L (Fig. S3-B). On Nov. 2, the nitrite concentration in the enhanced system initially increased and then decreased, after which

a peak occurred, reaching 0.08 ± 0.01 mg/L in all water layers. The control system, however, exhibited only a downward trend and the nitrite concentration decreased from 0.04 ± 0.01 (n = 5, Oct. 16) to 0 mg/L (n = 70, Oct. 16 - Nov. 14).

The ammonia concentration in the 10.0-13.0 m deep layers of the enhanced system continuously decreased, from 1.01-1.56 (Oct. 16, start) to 0.27-0.32 mg/L (Nov. 14, end) (Fig. S3-C). The ammonia concentration in control system ranged from 0.74-1.84 (Oct. 16, start) to 1.23-1.74 (Nov. 3), to 0.58-0.51 mg/L (Nov. 14). However, because a series of changes (i.e. nitrification and denitrification, and release from the surface sediment) occurred simultaneously in the enhanced system, the trend in ammonia concentration in the 0.5-10.0 m water layer showed ups and downs (Oct. 16 - Nov. 14). In the control system, ammonia concentration increased until a maximum and the decreased (Oct. 16 - Nov. 14).

Changes in water and surface sediment TN concentration in enhanced and control systems reflected the accumulation and removal of nitrogen sources. Changes in TN and TDN concentration in the water ecosystem are depicted in Fig. 1 and Fig. S4, respectively. During the high oxygen concentration period (Oct. 16 - Oct. 21), TN removal rates in the enhanced system (0-10.0 m) were higher than those of the control system (Fig. 1). Afterwards, TN concentrations in the enhanced system began to decrease while those of the control system gradually increased. At 0.5 m, the TN concentration in the enhanced system decreased from 1.08 to 0.82 mg/L and the TN removal rate reached 23.64%, while the concentration in the control system increased from 0.93 to 1.20 mg/L and the TN removal rate reached 28.89%. At 5.0 m, the TN concentration in the enhanced system decreased from 1.52 to 0.88 mg/L and the TN removal rate reached 41.94%, whereas the concentration in the control system decreased from 1.25 to 1.23 mg/L and the rate reached 1.96%. At 7.5 m, the TN

concentration in the enhanced system decreased from 1.31 to 0.82 mg/L and the TN removal rate reached 36.84%, whereas the concentration in the control system decreased from 1.32 to 1.24 mg/L and the rate reached 6.30%. At 10.0 m, the TN concentration in the enhanced system decreased from 1.83 to 0.91 mg/L and the TN removal rate reached 50.27%, whereas the concentration in the control system decreased from 1.64 to 1.30 mg/L and the rate reached 20.53%. At 13.0 m, the TN concentration in the enhanced system declined from 2.02 to 0.75 mg/L and the TN removal rate reached 62.90%, while the concentration in the control system decreased from 1.84 to 1.37 mg/L and the rate reached 25.41%.

Changes in TDN concentration and removal rate in the enclosure systems are shown in Fig. S4. The TDN concentration of all water layers (0.5-13.0 m) in enhanced and control systems ranged from 0.87-1.63 mg/L and 0.87-1.43 mg/L to 0.62-0.72 mg/L and 0.79-0.85 mg/L, respectively. The TDN removal rates in the enhanced system (0.5-13.0 m) varied between 17.98-58.04%, whereas those of the control system ranged from 8.99-41.03%.

TN concentrations in surface sediments, in both enhanced and control systems, are presented in Fig. S5. The TN concentration in the enhanced system decreased from 4040.24 ± 38.65 to 2368.63 ± 62.63 μg/g and the TN removal rate reached 41.37 ± 1.55%. In the control system, concentrations ranged from 4564.87 ± 199.07 to 3935.79 ± 260.11 μg/g and the removal rate reached 13.78 ± 5.70%.

To our knowledge, studies on aerobic denitrification in natural environments are rare: only two studies have been found, one in marine sediments (Gao et al. 2010) and one in constructed wetlands (CW) (Coban et al. 2015b, Coban et al. 2014). The present study is the first report showing nitrogen removal by indigenous aerobic denitrifiers in water reservoir systems via *in situ* oxygen enhancement. Coban et al. (2014) saw that the

average ammonia removal rate in CW was 0.63 g ammonia-N m^{-2} d^{-1} in the summer, 0.37 g ammonia-N m^{-2} d^{-1} in the autumn, and 0.25 g ammonia-N m^{-2} d^{-1} in spring. In the field, most of the ammonia was simultaneously removed via nitrification-denitrification, which occurred throughout the year (Coban et al. 2015b, Coban et al. 2014). However, no accumulation nitrate was observed. These results are consistent with the results from the *in situ* enhancement experiment of the present study. A comparison of the changes in nitrogen concentration (TN, TDN, ammonia, nitrate, and nitrite) in the reservoir water system are shown in Supplementary Table S1. The TN concentration in all water layers (except 13.0 m water layer) increased, and the TN concentrations of the enhanced system were lower than those of the reservoir water system. The TN concentration at 0.5 m, 5.0 m, 7.5 m, 10.0 m, and 13.0 m ranged from 0.74, 0.69, 0.72, 0.77, and 2.01 mg/L to 1.14, 1.00, 1.01, 1.38, and 1.48 mg/L, respectively. The TDN concentration ranged from 0.52, 0.48, 0.45, 0.60, and 1.84 mg/L to 1.05, 0.95, 0.95, 1.09, and 1.35 mg/L, respectively. Based on these results, *in situ* enhancement could strengthen the ability of indigenous aerobic denitrifiers to remove nitrogen from micro-polluted water sources.

3.3 Growth of aerobic denitrifiers in the enhanced system

The changes in the number of oligotrophic denitrification bacteria in both enhanced and control systems during the whole experimental period can be seen in Fig. 2. Meanwhile, statistical significance of the data was presented in different lower case letters. During the early stages of the *in situ* enhanced experiment, the relatively abundant organic nutrients supplied energy and electron donors to microbial growth, especially to the oligotrophic aerobic denitrification bacteria present in the enclosure systems.

As shown in Fig. 2, the densities of oligotrophic AD (aerobic denitrification) bacteria in enhanced and control systems increased from $3.76 \pm 0.52 \times 10^4$ (n = 5, Oct. 16) and

7.70 ± 3.78 × 10^4 cfu/mL (n = 5, Oct. 16) to 1.81 ± 0.34 × 10^6 (n = 5, Oct. 21) and 1.50 ± 0.32 × 10^6 cfu/mL (n = 5, Oct. 21). The density of AD bacteria in all water layers (0.5-13.0 m, Oct. 21 - Nov. 8) was relatively constant: in the enhanced system, density was maintained at 3.35-6.57 × 10^6 cfu/mL, whereas in the control system, density was 0.93-2.11 × 10^6 cfu/mL. On Nov. 8, the low temperature and lack of organic matter caused a rapid decrease in AD bacteria numbers, which was consistent with changes in TOC in the enclosure systems (Supplementary Table S2). The AD bacteria density in the enhanced system reached 5.66 × 10^5 cfu/mL, whereas that of the control system was 2.18 × 10^5 cfu/mL. TOC suddenly increased from Nov. 8 - Nov. 14, due to the death of part of the bacteria (Table S2). The remaining bacteria continued to utilize the organic matter as substrate for growth and nitrogen removal, and became adapted to low temperature conditions. Thereafter, AD bacteria density in both enhanced and control systems increased to 1.95 ± 0.71 × 10^6 cfu/mL (n = 10, Nov. 10 - Nov. 14) and 0.59 ± 0.18 × 10^6 cfu/mL (n = 10, Nov. 10 - Nov. 14), respectively. Overall, the number of AD bacteria indicated the ability to remove nitrogen. During the middle period, AD bacteria gradually adapted to the oligotrophic conditions, resulting in a small change in numbers due to bacteria growth and death at a dynamic equilibrium state. This was consistent with previous findings (Huang et al. 2012). In the natural reservoir water system, the density of aerobic denitrifying bacteria ranged from 0.38 ± 0.61 × 10^4 cfu/mL (0 - 13.0 m, Oct. 15) to 1.34 ± 0.44 × 10^4 cfu/mL (0 - 13.0 m, Nov. 14) (Supplementary Table S3). Clearly, the AD bacteria density in the enhanced system was ~2 fold higher than that the density in the natural water system. This indicates that the enhanced system was better at removing nitrogen.

It is known that nitrite reductase occurs in two structurally different but functionally equivalent forms: nirK and nirS. Quantification of bacteria capable of denitrification is

critical for a better understanding of denitrifying activity in the natural environment. Real-time PCR served to quantify the denitrifying nitrite reductase genes (nirK and nirS) (Table 2). In the control water system (0-13 m), the functional genes (nirK and nirS) first increased and then decreased, increasing from $1.82 \pm 0.36 \times 10^4$ (Oct. 16) and $5.01 \pm 1.54 \times 10^5$ copies/μL (Oct. 16) to $6.31 \pm 1.48 \times 10^4$ (Oct. 29) and $6.03 \pm 1.26 \times 10^5$ copies/μL (Nov. 3), and dropping to $1.95 \pm 1.34 \times 10^4$ (Nov. 14) and $1.26 \pm 1.54 \times 10^5$ copies/μL (Nov. 14), respectively. In the enhanced water system, however, the denitrification functional genes (nirK and nirS) presented an increased trend from $1.55 \pm 1.12 \times 10^4$ (Oct. 16) and $1.91 \pm 1.78 \times 10^5$ copies/μL (Oct. 16) to $3.55 \pm 1.45 \times 10^4$ (Nov. 14) and $4.07 \pm 1.48 \times 10^5$ copies/μL (Nov. 14), respectively. In the enhanced surface sediment system, nirK and nirS increased ~6 and 3 times, respectively, in relation to their initial number, whereas in the control system these genes decreased to about 1/7 and 1/3, respectively, of their initial number. As described above, indigenous aerobic denitrifying bacteria were enhanced via *in situ* oxygen enhancement. In order to further investigate the mechanisms behind this enhancement, changes in the denitrifying bacterial community structure due to the nitrogen removal process should be studied in the future.

3.4 Inhibition of Fe, Mn, and P in the enhanced system

The Fe concentration in the enhanced system ranged from 0.09 ± 0.03 to 0.10 ± 0.01 mg/L (Supplementary Table S4). The control system always maintained an anaerobic environment (Oct. 19 - Nov. 14), and the ORP always gradually declined. Fe was released from the sediment and its concentration in the control system increased from 0.09 ± 0.03 to 0.25 ± 0.06 mg/L. Mn concentration in the enhanced system ranged from 0.17 ± 0.07 (start, not including 13.0 m, 0.76 mg/L, Oct. 16) to 0.20 ± 0.02 mg/L (end, Nov. 14), whereas Mn concentration in the control system increased from 0.13 ± 0.07

(start, not including 13.0 m, 0.76 mg/L, Oct. 16) to 0.83 ± 0.03 mg/L (end, Nov. 14) (Supplementary Table S4). At the same time, the TP (total phosphorus) concentration in the enhanced system ranged from 0.11 ± 0.04 (n = 5, Oct. 16) to 0.14 ± 0.06 mg/L (n = 5, Nov. 14), whereas that of the control system increased from 0.14 ± 0.04 (n = 5, Oct. 16) to 0.21 ± 0.09 mg/L (n = 5, Nov. 14) (Supplementary Table S4).

The TP concentration in surface sediments of both enhanced and control systems is presented in Fig. S5. TP concentration in surface sediments decreased from 1065.77 ± 14.39 to 858.53 ± 26.30 μg/g in the enhanced system and from 1939.66 ± 75.18 to 909.98 ± 37.05 μg/g in the control system. The TP release in surface sediments was lower in the enhanced system than in the control system. These results show that the enhanced system is better at reducing Fe, Mn, and P in micro-polluted water than the control system.

3.5 Pyrosequencing overview

Using Miseq high-through sequencing, a total of 584852 sequences with an average length of 319.27 bp were obtained, after quality trimming, for 40 samples (30 water samples and 10 surface sediment samples analyzed in triplicate). Sequencing revealed a total of 31647 OTUs (operational taxonomic units) with 97% similarity (Table 3). The highest number of OTUs in the water system was found at 7.5 m in the enhanced system (over the whole experimental period). The highest OTU number in the water control system changed from 13 m (period 1-2) to 7.5 m (period 3-4) and afterwards to 0.5 m (period 3). OTU numbers in surface sediments first decreased and then increased in both enhanced and control systems. The ACE and Chao diversity estimators in the water system varied among depths, ranging from 545 and 374 to 992 and 870, respectively, in the enhanced system, and from 475 and 405 to 1044 and 897, respectively, in the control system. In surface sediments, ACE and Chao diversity estimators were higher in

the enhanced system than in the control system. Shannon richness in the water and surface sediments was also higher in the enhanced system than in the control system. The average coverage of enhanced and control systems was 0.968 and 0.969, respectively. These results seem to reflect the real structure of microbial communities (Luo et al. 2013).

Rarefaction curves of the number of OTUs at 97% similarity showed that a number of reads were sampled (Supplementary Figure S6). The distribution of microbial phyla in water and sediment systems is shown in Figure S7. The 31647 OTUs with 97% similarity were affiliated with 15 main phyla including: Acidobacteria, Actinobacteria (dominant 2), Armatimonadetes, Bacteria_unclassified, Bacteroidetes (dominant 3), Caldiserica, Chlorobi, Chloroflexi, Cyanobacteria, Firmicutes (dominant 4), Gemmatimonadetes, Nitrospirae, Planctomycetes, Proteobacteria (dominant 1), and Spirochaetae. Distinct communities in the enhanced and control systems of water and surface sediments were identified at the phylum level (Supplementary Figure S7).

3.6 Spatial and temporal changes of microbial community

The microbial compositions were significantly different in different periods (Figure S7 and S8). For example, Proteobacteria declined from 70-80% to 40-50% in enhanced and control water systems. In addition, Proteobacteria in enhanced sediment samples increased from 39.91% to 42.16%, while those in the control decreased from 44.04% to 30.57%. Bacteroidetes increased from ~0.65% (0.5 m, period 1) and ~1.55% (7.5 m, period 1) to 15.47% (0.5 m, period 5) and 16.17% (7.5 m, period 5) in enhanced and control water systems, respectively. In enhanced surface sediment systems, although Firmicutes accounted for 10.34% of the sequences in period 1, a consistent decrease was observed afterwards, reaching 4.92% at the end of the experiment (period 5).

Firmicutes in the control system, on the other hand, increased from 2.31% (period 1) up to 23.89% (period 5).

In both enhanced and control water systems, Proteobacteria decreased although densities were higher in the enhanced system than in the control system. In the water control system, sequences of β-Proteobacteria and γ-Proteobacteria were the most abundant Proteobacteria sequences during period 1, after which β-Proteobacteria sequences became the most abundant (period 3); during periods 4 and 5, most sequences were of β-Proteobacteria and ε-Proteobacteria. In the enhanced water system, main Proteobacteria sequences changed from β-Proteobacteria and γ-Proteobacteria during period 1 to α-Proteobacteria and β-Proteobacteria during periods 3-5. α-Proteobacteria in the enhanced water system reached 51.48-60.18% at 0.5-13 m during period 3 and 45.49-49.18% at 0.5-13 m during period 4. However, in the control system, α-Proteobacteria accounted for 12.97-15.97% at 0.5-13 m during period 3 and 7.98-8.90% at 0.5-13 m during period 4. In surface sediments, the main Proteobacteria sequences in enhanced and control systems were β-Proteobacteria (~20%) and γ-Proteobacteria (~13%) during the whole period. Actinobacteria were also present during the whole period in both enhanced and control water and sediment systems (Supplementary Figure S8). The Flavobacteriia class accounted for most sequences of the Bacteroidetes phylum during the whole experimental period (1-5) and at all water depth layers in enhanced and control systems. However, in surface sediments, the main sequences of the Bacteroidetes phylum were from the Sphingobacteriia class in both enhanced and control systems (Supplementary Figure S8).

The abundance of the dominant bacterial genus in enhanced and control water and surface sediment systems during the different periods is shown in Figure 3. In the enhanced water system, *Rheinheimera* (28.38 ± 8.90%), *Comamonas* (29.36 ± 6.03%),

and *Pseudomonas* (16.39 ± 1.00%) dominated all water layers (0.5-13 m) during period 1. During period 2 the main genus present were *Acidovorax* (20.37 ± 1.23%), *Flavobacterium* (16.51 ± 0.18%), and *Novosphingobium* (11.78 ± 0.39%), whereas during period 3, *Novosphingobium* (45.03 ± 6.43%), *hgcI_clade* (10.26 ± 2.76%), and *Methyloversatilis* (6.48 ± 0.94%) were the most abundant genus. *Novosphingobium* (37.85 ± 2.80%), *hgcI_clade* (12.51 ± 1.52%), and *Acidovorax* (9.20 ± 0.40%) dominated during period 4, whereas *Acidovorax* (14.87 ± 5.00%), *hgcI_clade* (13.79 ± 5.02%), and *Hydrogenophaga* (7.31%, in 7.5m water layer) dominated all water layers during period 5. In all water layers of the control system, *Acidovorax* (21.90 ± 6.05%) and *Rheinheimera* (13.12 ± 4.62%) dominated during period 1; *Acidovorax* (38.43 ± 5.39%), *Caulobacter* (9.44 ± 1.06%), and *Hydrogenophaga* (7.68 ± 0.36%) were the main genus present during period 3; and *Arcobacter* (14.81 ± 10.25%) and *Hydrogenophaga* (12.78 ± 7.26%) dominated during period 5. In enhanced and control surface sediment systems, the main genus present largely varied between periods: e.g., *Lactococcus* in the enhanced system decreased from 4.40% during period 1 to 2.28% during period 5, while in the control system it increased from 0.40% (period-1) to 13.22% (period-5) (Supplementary Table S5).

3.7 Monitoring of N-functional bacteria

In order to prevent contamination of drinking water and reduce the proliferation of harmful algal blooms (especially cyanobacteria blooms), an appropriate nutrient (N and P) control strategy is highly necessary. N may occur in gaseous form and may be released to the atmosphere through denitrification, whereas P may be recycled internally (water-sediment) in the water reservoir system (Paerl et al. 2014). For example, toxic cyanobacteria genus (such as *Microcystis* spp.) without N_2-fixing ability often dominate in nutrient-sensitive (P-focused controls well) systems; in 2007, massive *Microcystis*

blooms (observed in Taihu, China) have cut off drinking water supply (10 million local residents). Therefore, N is a critical factor limiting *Microcystis* blooms. This is consistent with the findings of Howarth and Marino (2006) who proposed N as the limiting nutrient for eutrophication (Howarth and Marino 2006). In the present study, the enhanced system showed perfect nitrogen removal performances via *in situ* oxygen enhancement of indigenous aerobic denitrifiers in the water reservoir. Therefore, it is important to analyze the changes in N-functional bacterial species.

In a previous study (Yan et al. 2015), it was shown that a large amount of Proteobacteria (such as *Hydrogenophaga* and *Acidovorax*) were involved in nitrogen cycling (Yan et al. 2015). The dominant Proteobacteria genus in the enhanced system (all water layers, 0.5-13 m) during period 2 were *Acidovorax* (20.37 ± 1.23%), *Novosphingobium* (45.03 ± 6.43%), and *Novosphingobium* (37.85±2.80%), and, during period 5, *Acidovorax* (14.87±5.00%). The aerobic denitrifying bacteria ZHF2 (GenBank no. KP717095) and ZHF8 (GenBank no. KP717087), belonging to *Novosphingobium*, were isolated from the water reservoir. The abundance of *Hydrogenophaga* in the enhanced system increased from 0.73% (period 1) to 7.35% (period 5), whereas the abundance of *Acinetobacter* and *Zoogloea* increased from <0.01% (period 1) and 0.03% (period 1) to <0.40% (period 5) and 0.37% (period 5), respectively. G107 (GenBank no. KP717096), 81Y (GenBank no. KP717097), and N299 (GenBank no. KP717093) were also aerobic denitrifiers (Zhou et al. 2016). The dominant Bacteroidetes in the enhanced system were *Sphingomonadaceae*, which increased from <0.01% during period 1 to 1.6% during period 5. This genus was identical to the aerobic denitrifying bacteria ZMF6 (GenBank no. KP717084).

The enhanced system showed perfect nitrogen removal performances via *in situ* oxygen enhancement of indigenous aerobic denitrifiers. Therefore, denitrification

played a critical role in the N cycle. Future research based on high-throughput GeoChip functional gene microarray analysis is necessary to further study the community structure and function of indigenous aerobic denitrifying bacteria via *in situ* oxygen enhancement. This will allow assessing denitrifier diversity, denitrifier density, denitrification activity, denitrifying enzymes, the link between activity and diversity, and nitrogen metabolic genes (haoA, amoA, nosZ, nirK, and nirS) in the water and sediment surface during a period of water lifting aeration (WLAs).

3.8 Microbial community structure comparison

Several statistical methodologies were used to identify the bacterial community relationship among water and surface sediment samples collected from enhanced and control systems. Community structure comparisons were done by principal component analysis (PCA). Results revealed that the first two principle components (PC1 and PC2) explained 64.99% and 69.55% of the variability in the water and surface sediment, respectively (Figure 4). The accumulated contribution ratios of PC1 and PC2 in water achieved 38.61% and 26.38%, respectively, whereas, in the surface sediment system, the accumulated contribution ratios of PC1 and PC2 achieved 57.35% and 12.2%, respectively (Figure 4). Microflora were well separated at different time periods in both enhanced and control systems. In the enhanced system, H water samples were all located in quadrants 1 and 4, while in the control system they were located in quadrants 2 and 3. Samples of the enhanced sediment system were located in quadrant 3, while those of the control were in located in quadrants 1 and 2. The hierarchical clustering based on OTU information was also generated (Supplementary Figure S9). Water samples from the same system and period tightly grouped, and water and surface sediment samples could be well separated (ten groups were discriminated in the water and five in the sediment surface).

3.9 Relationship between the microbial community and environmental variables

In order to explore the effects of water quality on the bacterial community, multiple statistical analyses (RDA) were used to identify the relationship between the microbial functional community and environmental variables based on the genus level. This approach allows finding the missing link between diversity and activity using denitrifying bacteria as a model organism.

The different bacterial communities present in enhanced and control water samples were well discriminated at the genus level (Figure 5A and 5B). The first two RDA dimensions using the 16 parameters (VIF <20, except Mn = 37) explained 48.78% of the microbial community variation (Figure 5A, F = 3.97, P = 0.002). Physical and chemical parameters including TN, Nitrite, TDN, TOC, Fe, Mn, DO, pH, ORP, and CHl-a significantly influenced water bacterial community composition (Figure 5A and Table S6). In surface sediments, the first two RDA dimensions explained 24.87% of the bacterial community variation (Figure 5D, F = 0.83, P = 0.75), and RDA analysis revealed that TN-S, TP-S, and DO were the critical environmental factors influencing the spatial and temporal variation of the bacterial community. During the different periods, the microbial community in the enhanced water system (negative values of the RDA x-axis, Figure 5A) was relatively different from the one in the control system (positive values of the RDA x-axis, Figure 5A). Similarly, the microbial community in the enhanced sediment system (quadrant 1 and 3, Figure 5B) was different from the one in the control system (quadrant 3 and 4, Figure 5B). From this study it is clear that the nitrogen source (TN, TDN, ammonia, nitrate, and nitrite) was the most important factor affecting the bacterial community function and composition. Therefore, changes in N-

functional bacteria cause important implications in the geochemical cycle. Future research is needed to further explore the mechanism of nitrogen removal.

4. Conclusions

The enhanced system showed perfect nitrogen removal and pollutant inhibition performances via *in situ* oxygen enhancement of indigenous aerobic denitrifiers. The densities of aerobic denitrifiers in the enhanced system increased. In the *in situ* oxygen enhanced system, diversity was higher and the microbial composition was significantly different compared to that in the control system. N, Fe, Mn, and DO were the most important factors affecting the bacterial community function and composition. Results suggest that nitrogen removal via *in situ* enhancement of indigenous aerobic denitrifying bacteria is possible, which may provide a technical support for future field tests.

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Figure legends

Figure 1. Changes in TN concentration in enhanced and control systems. (A, 0.5 m water layer; B, 5.0 m water layer; C, 7.5 m water layer; D, 10.0 m water layer; E, 13.0 m water layer; H, enhanced system; B, control system)

Figure 2. Changes in aerobic denitrifying bacteria in enhanced and control systems. Data are mean ± SD of all depth layers. Analysis of variance (ANOVA, P = 0.05) was used to examine differences among site parameters. Different lower case letters indicate significant differences among the sites. The numbers 1, 2, and 3 correspond to the high, medium, and low DO periods, respectively. H refers to the enhanced system and B refers to the control system.

Figure 3. Abundance of dominating bacterial genus in enhanced and control water systems and in the surface sediment system. (A) Abundance of dominating bacterial genus at the water surface (0.5 m), (B) abundance of dominating bacterial genus in the medium (7.5 m) water layer, (C) abundance of dominating bacterial genus in the bottom (13 m) water layer, and (D) abundance of dominating bacterial genus in surface sediments.

Figure 4. Principal component analysis (PCA) of water and surface sediments in enhanced and control systems. (A) Enhanced and control water systems and (B) enhanced and control surface sediment systems.

Figure 5. Redundancy analyses of water and surface sediment bacterial communities in enhanced and control systems. (A) RDA of water bacterial communities based on genus distribution and (B) RDA of surface sediment bacterial communities based on genus distribution. For other details please refer to the above descriptions.

Table legends

Table 1. Changes in DO, T, pH, ORP, EC, and CHl-a concentration at different periods in enhanced and control systems.

Table 2. Changes in the functional genes nirS and nirK in enhanced and control systems.

Table 3. Spatial and temporal distribution of microbial community diversity and richness estimators in water and surface sediment systems of the enclosure experiment.

Table 1

Parameters	Enhanced system			Control system		
	Surface (0-5m)	Medium (5-10m)	Bottom (10-13m)	Surface (0-5m)	Medium (5-10m)	Bottom (10-13m)
DO(mg/L)						
Oct.16–21	10.91±0.76	12.06±0.25	0.25±0.18	3.11±4.37	1.37±2.36	0.10±0.05
Oct.21-29	7.06±1.43	7.42±1.91	0.30±0.02	0.23±0.08	0.14±0.01	0.10±0.00
Oct.29-Nov.14	5.02±0.75	4.61±0.75	0.39±0.20	0.29±0.08	0.18±0.02	0.14±0.02
T(°C)						
Oct.16–21	18.36±0.83	18.12±0.59	13.27±1.58	19.93±1.06	18.99±1.01	12.69±0.93
Oct.21-29	17.72±0.48	17.53±0.34	12.47±0.35	18.08±0.39	17.80±0.13	12.67±0.21
Oct.29-Nov.5	16.55±0.38	16.45±0.45	13.82±0.67	16.67±0.40	16.75±0.28	13.96±0.98
Oct.5-Nov.14	14.82±0.57	14.67±0.60	14.40±0.70	14.52±0.50	14.37±0.51	14.14±0.62
pH						
Oct.16	7.82±0.05	7.44±0.25	8.85±1.01	7.82±0.05	7.44±0.25	8.85±0.98
Oct.19	7.51±0.19	7.39±0.01	7.49±0.15	7.96±0.08	8.20±0.09	8.86±0.41
Oct.29	7.46±0.15	7.25±0.03	7.11±0.06	8.34±0.20	8.81±0.11	9.05±0.03
Nov.14	7.95±0.01	7.94±0.01	7.92±0.01	8.48±0.10	8.81±0.10	9.09±0.10
ORP(mv)						
Oct.16	320.20±2.11	333.1±7.84	21.29±16.13	320.20±2.11	333.1±7.84	21.29±16.13
Oct.19	229.73±23.89	248.22±0.44	230.67±30.02	180.33±13.18	148.57±11.73	70.86±34.23
Oct.29	237.62±18.52	267.47±4.09	281.58±3.42	134.33±25.10	73.15±16.42	42.00±2.83
Nov.14	269.73±2.05	273.10±0.99	274.00±0.00	110.18±12.42	66.60±12.20	32.83±9.02
EC(us/cm)						
Oct.16	325.81±0.54	332.11±4.83	395.56±29.26	324.61±0.84	331.31±2.83	392.24±20.26
Oct.19	349.87±0.35	350.00±0.00	350.00±0.00	331.00±0.00	331.43±0.79	369.57±29.25
Oct.29	349.00±0.00	348.67±0.49	346.25±0.97	339.00±0.00	341.38±2.63	347.00±0.00
Nov.14	369.00±0.00	369.00±0.00	369.00±0.00	339.00±0.00	341.30±2.11	350.50±4.42
CHl-a(μg/L)						
Oct.16	33.14±2.25	16.97±5.83	2.78±0.33	34.17±2.15	18.87±3.81	3.78±0.52
Oct.19	5.28±0.45	5.55±0.30	5.55±0.23	4.54±0.32	4.42±0.12	2.98±0.57
Oct.29	4.15±0.42	4.25±0.32	4.07±0.57	4.49±0.28	3.67±0.27	3.28±0.05
Nov.14	4.30±0.19	4.48±0.12	4.12±0.42	4.55±0.28	3.93±0.35	3.38±0.10

Note: DO(oct.16-21), (oct.21-29) and (oct.29-nov.14) means high DO period, medium DO period, and low DO period, respectively; Enhanced system means enclosure system with aeration equipment, control system means without aeration equipment.

Table 2

System	nirK genes (copies/μl)				nirS genes (copies/μl)			
	Water system		Sediment system		Water system		Sediment system	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Control system								
Oct.16	4.26	0.09	5.44	0.02	5.62	0.14	5.92	0.02
Oct.19	4.76	0.14	4.99	0.02	5.71	0.17	5.70	0.04
Oct.29	4.80	0.17	4.92	0.04	5.46	0.15	5.65	0.08
Nov.3	4.56	0.15	4.74	0.02	5.78	0.10	5.09	0.02
Nov.14	4.29	0.13	4.56	0.03	5.10	0.14	4.41	0.01
Enhanced system								
Oct.16	4.19	0.30	4.42	0.01	5.28	0.25	5.53	0.01
Oct.19	4.16	0.08	4.44	0.00	5.32	0.05	6.15	0.04
Oct.29	4.51	0.22	4.87	0.04	5.43	0.14	6.58	0.04
Nov.3	4.75	0.18	4.33	0.01	5.37	0.07	5.48	0.00
Nov.14	4.55	0.16	5.27	0.01	5.61	0.17	6.16	0.01

Note, nirK and nirS mean denitrification function genes; Water system means 0-13 m water layer; Sediment system means 0-2 cm sediment; Data means Mean (lg(nirK or nirS)) ± S.D. (Standard Deviation)

Table 3

Period	Water Depth	Enhance system							control system						
		Reads Number	0.97 Level						Reads Number	0.97 Level					
			OTUs	Diversity		coverage	Richness			OTUs	Diversity		coverage	Richness	
				ACE	Chao1		Shannon	Simpson			ACE	Chao1		Shannon	Simpson
1	0.5 m	11322	235	545	374	0.9906	2.43	0.2003	12026	274	475	405	0.9914	3.04	0.1066
	7.5 m	13670	418	837	717	0.9867	2.97	0.1415	15466	418	809	650	0.9886	3.13	0.1065
	13 m	8920	343	841	620	0.9813	2.81	0.1558	13266	466	683	660	0.9865	3.55	0.0847
	sediment	14707	2132	3260	3136	0.9394	6.3	0.0061	10455	1746	3332	2650	0.9260	6.15	0.007
2	0.5 m	15944	374	644	537	0.9917	3.68	0.0599	15470	413	787	612	0.9897	3.37	0.1121
	7.5 m	14894	377	768	643	0.9899	3.65	0.0646	11285	349	706	570	0.9876	3.32	0.1209
	13 m	9122	330	709	583	0.9845	3.67	0.0606	12785	471	831	692	0.9859	3.49	0.1137
	sediment	10304	1876	4178	3196	0.9106	6.21	0.0067	8685	1692	3985	2923	0.9010	5.99	0.0133
3	0.5 m	12584	346	604	520	0.9902	3.36	0.1251	12057	394	734	576	0.9873	3.18	0.1697
	7.5 m	16395	359	668	526	0.9920	3.07	0.1609	11615	447	944	673	0.9840	3.59	0.1081
	13 m	11880	308	583	474	0.9899	2.91	0.1771	10539	411	871	674	0.9831	3.21	0.1648
	sediment	8577	1695	3954	2909	0.8993	6.06	0.0099	8472	1619	3634	2738	0.9053	5.95	0.0128
4	0.5 m	8973	392	702	602	0.9828	3.45	0.1079	10639	410	851	652	0.9836	3.45	0.0919
	7.5 m	11376	492	992	870	0.9817	3.68	0.0931	15489	446	824	721	0.9886	3.59	0.0742
	13 m	11855	474	846	702	0.9849	3.61	0.1038	9564	352	676	573	0.9847	3.34	0.1036
	sediment	10783	2067	4022	3226	0.9124	6.41	0.0061	14235	2290	3589	3524	0.9302	6.37	0.0072
5	0.5 m	10906	477	921	826	0.9825	4.17	0.0426	16012	609	886	867	0.9861	3.82	0.0866
	7.5 m	13088	551	934	817	0.9848	4.32	0.0388	9554	470	868	685	0.9798	4.14	0.0331
	13 m	8295	446	803	633	0.9799	4.19	0.0453	14042	603	1044	897	0.9835	4.05	0.0596
	sediment	11280	2175	4122	3323	0.9138	6.51	0.0052	10780	1900	2814	2741	0.9274	6.19	0.0142

Abbreviation: ACE, abundance based-coverage estimator; OTUs, operational taxonomic units; Diversity, Diversity Estimator; Richness, Richness Estimator

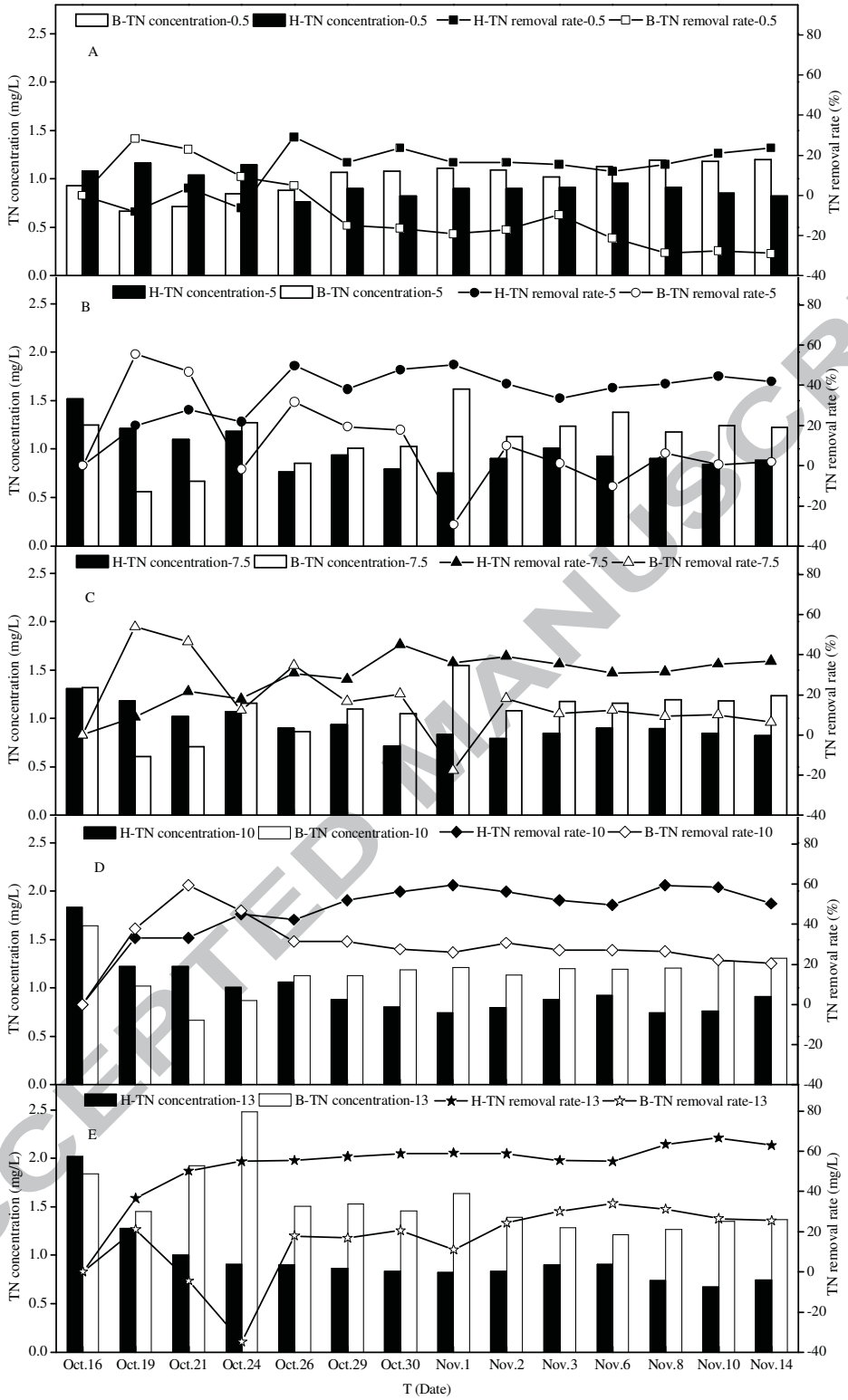

Figure 1

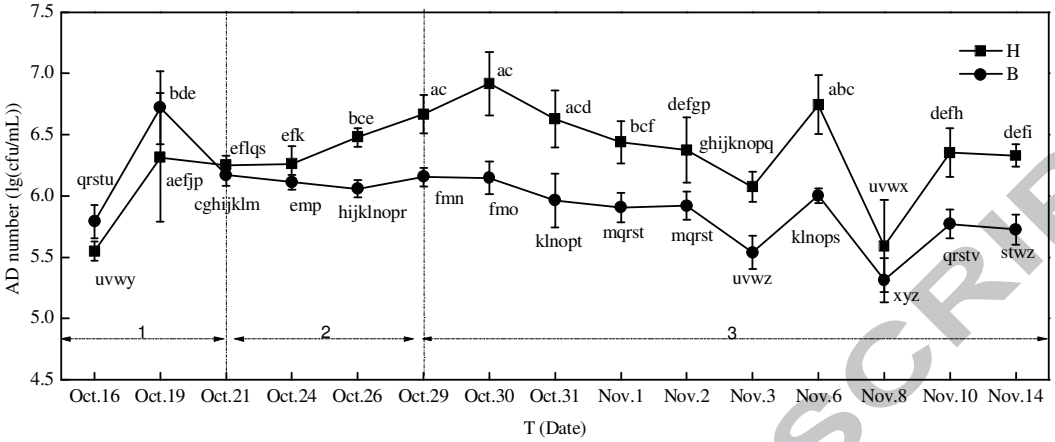

Figure 2

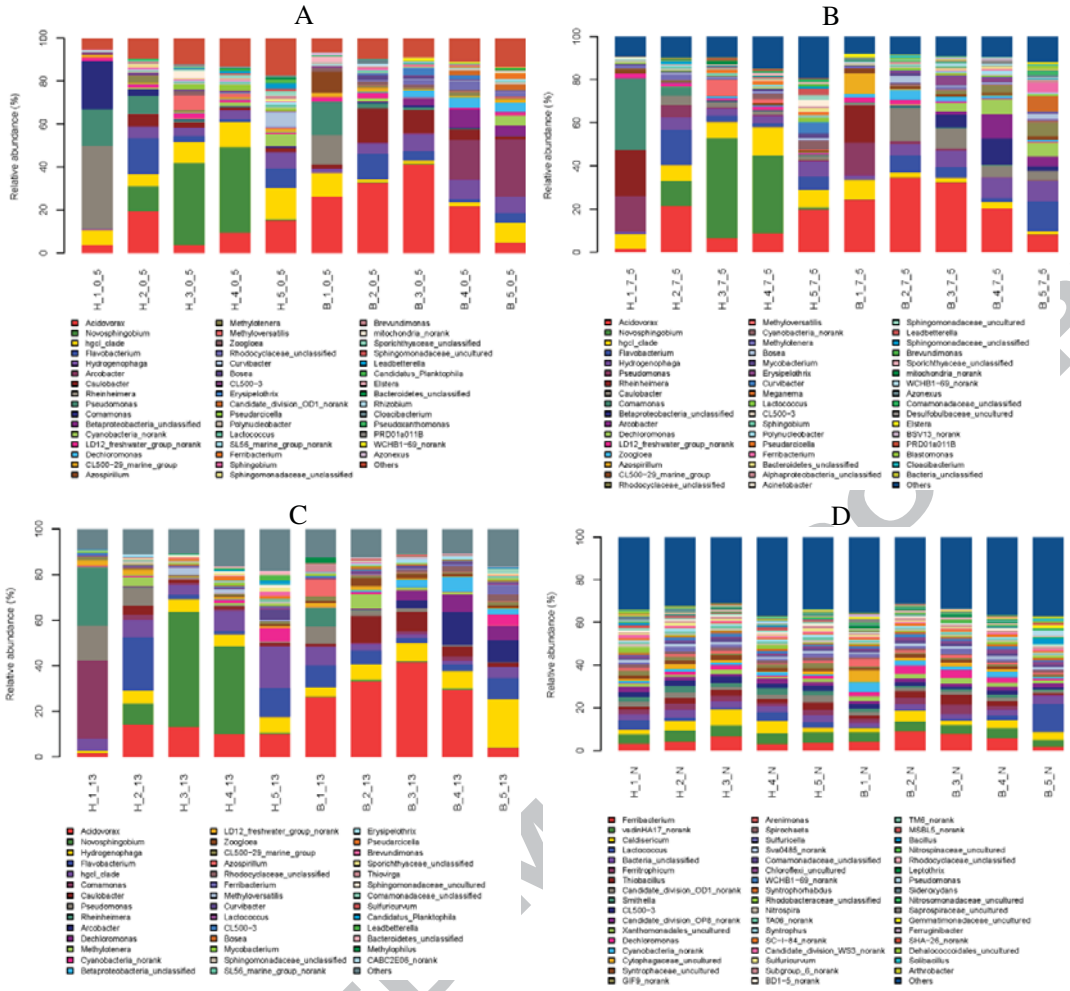

Figure 3

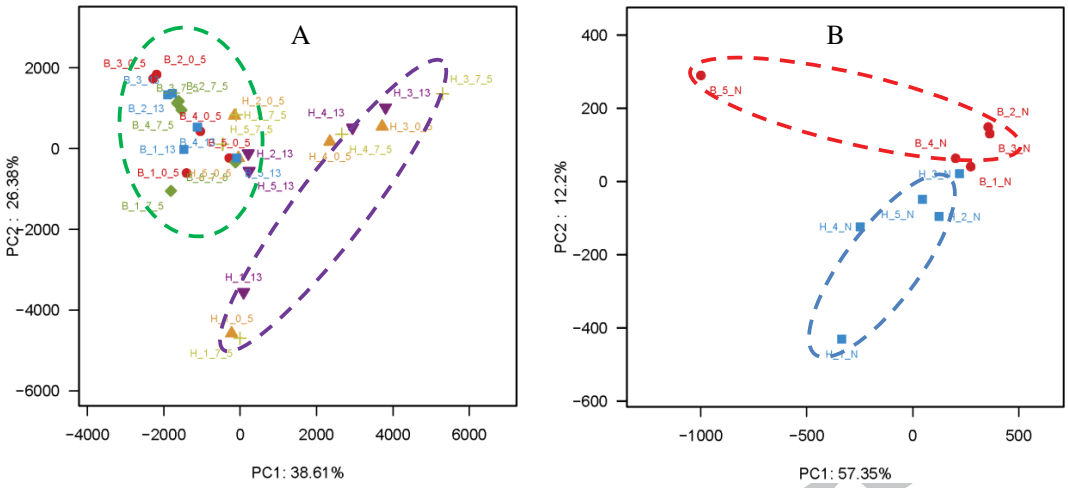

Figure 4

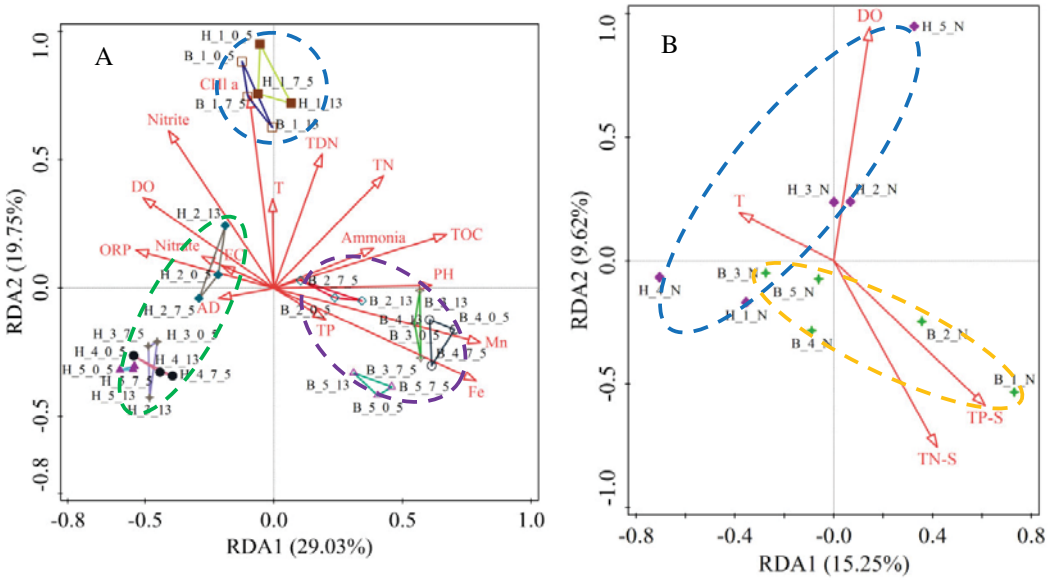

Figure 5

Hightlights

- The indigenous aerobic denitrifiers were enhanced *in situ.*
- The enhanced system performed very well in terms of nitrogen removal and the inhibition of Fe, Mn and P pollutants.
- The N-functional bacteria were obviously increased via in situ oxygen enhanced.
- Nitrogen source, Fe, Mn, DO were the most important factors affecting the bacterial community function and composition.