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RESEARCH ARTICLE

Denitrifier abundance and community composition linked to denitrification potential in river sediments

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Abstract

Denitrification in river sediments plays a very important role in removing nitrogen in aquatic ecosystem. To gain insight into the key factors driving denitrification at large spatial scales, a total of 135 sediment samples were collected from Huaihe River and its branches located in the northern of Anhui province. Bacterial community composition and denitrifying functional genes (*nirS*, *nirK*, and *nosZ*) were measured by high-throughput sequencing and real-time PCR approaches. Potential denitrification rate (PDR) was measured by acetylene inhibition method, which varied from 0.01 to 15.69 μg N g^{-1} h^{-1}. The sequencing results based on *16S rRNA* gene found that the main denitrification bacterial taxa included *Bacillus*, *Thiobacillus*, *Acinetobacter*, *Halomonas*, *Denitratisoma*, *Pseudomonas*, *Rhodanobacter*, and *Thauera*. Therein, *Thiobacillus* might play key roles in the denitrification. Total nitrogen and N:P ratio were the only chemical factors related with all denitrification genes. Furthermore, *nirS* gene abundance could be more susceptible to environmental parameters compared with *nirK* and *nosZ* genes. Canonical correspondence analysis indicated that NO_3^-, NO_2^-, NH_4^+ and IP had the significant impacts on the *nirS*-encoding bacterial community and spatial distributions. There was a significantly positive correlation between *Thiobacillus* and *nirS* gene. We considered that higher numbers of *nosZ* appeared in nutrient rich sediments. More strikingly, PDR was positively correlated with the abundance of three functional genes. Random forest analysis showed that NH_4^+ was the most powerful predictor of PDR. These findings can yield practical and important reference for the bioremediation or evaluation of wetland systems.

Keywords River sediment · Denitrifier · Functional gene · Denitrification potential · Nutrient level · Factor

Introduction

Rivers play a substantial role in regional and global elemental biogeochemical cycles (Aufdenkampe et al. 2011), which can serve as a good sentinel of environmental changes in terrestrial and atmospheric processes (Williamson et al. 2008; Crump et al. 2009). However, nutrients originated from domestic sewage, farm drainage, industrial effluent, etc. not only lead to the deterioration of water quality in river systems, but also influence the function of river ecosystems. The nutrients in the aquatic ecosystems are foremost deposited in the sediment, harboring a lot of active and diverse microbia which affect the dynamic of nutrients (Cheng et al. 2014; Liu et al. 2014). For example, the denitrifiers dwelling in the river sediments can remove nitrate to alleviate eutrophication of river (Guo et al. 2013). The denitrifiers are widely distributed in wetland systems, including pools, rivers, lakes, and constructed wetlands (Huang et al. 2011; Ji et al. 2012;Yao et al. 2016).

Denitrification consists of many chemical steps, and each step is catalyzed by enzyme(s) from microorganisms. The reaction of reducing nitrite (NO_2^-) to nitric oxide (NO) catalyzed by nitrite reductase (encoded by *nirS* and *nirK* genes) is the central limiting step of denitrification (Kandeler et al. 2006). The terminal step of denitrificaton ($N_2O \rightarrow N_2$) is catalyzed by nitrous oxide reductases (reducing nitrous oxide to dinitrogen, encoded by *nosZ* gene). Additionally, *nirK*, *nirS*, and *nosZ* are commonly used as the marker genes to detect the microbia involved in complete denitrification and predict

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denitrification-derived N_2O emission (Myrtotsiknia, Paranychianakis, et al. 2015).

Denitrification can be achieved by phylogenetically unrelated denitrifier assemblages (Zhou et al. 2016), and there are huge differences in the physiology of denitrifying bacteria (Li et al. 2018). Hence, several previous studies have indicated that denitrifier community and denitrification rate in sediment/soil could be affected by environmental variables (Banerjee and Siciliano 2012; Myrtotsiknia, Paranychianakis, et al. 2015; Li et al. 2018). The utilization of molecular biological techniques, especially high-throughput sequencing and *q*PCR, greatly promoted studies on denitrifiers and denitrification in environmental samples (Banerjee and Siciliano 2012; Kozich et al. 2013). Simultaneously, several bacteria belonging to phylum *Proteobacteria*, including *Thauera*, *Paracoccus*, *Hypomicrobium*, and *Comamonas* can participate in denitrification process (Wang et al. 2014b). Bacteria having the potential of denitrification belong to diverse groups and possess various physiological traits and metabolic potential. Denitrifiers play a key role to sustain denitrification potential by altering environmental factors in river sediments (Bowles et al. 2012; Guo et al. 2013). The contents of ammonia, nitrate, total nitrogen, and total phosphorus might have important roles in shaping wetland denitrifier community structure (Li et al. 2018). Nitrate concentration is considered as the most important factor for controlling the denitrification process in river sediment (Piña-Ochoa, and lvarez-Cobelas, M. 2006). Meanwhile, the distribution of denitrifying functional genes *nirS* and *nirK* is environment-specific, and the relationship between *nirS*- and *nirK*-encoding denitrifiers is considered to be regulated by factors such as nutrient enrichment (Ji et al. 2012; Wang et al. 2014b). Furthermore, many studies also investigated the abundance and distribution of denitrifying genes in sediments and found that environmental factors, such as organic matter, dissolved inorganic nitrogen (DIN) (NO_3^-, NO_2^-, and NH_4^+), dissolved oxygen, and redox potential, significantly affected the distribution of denitrifying genes in sediments (Bowles et al. 2012; Myrtotsiknia, Paranychianakis, et al. 2015). Denitrifying genes have been also found to be correlated with potential denitrification rates (PDRs) (Čuhel et al. 2010; Semedo and Song 2020). Studies also indicated that the denitrification rates might been mediated by denitrifier community structure (Li et al. 2018; Xiong et al. 2017). Moreover, the sediment/soil ammonia, nitrate, nitrite, total phosphorus, and pH might exert a certain influence on denitrification rates (Xiong et al. 2017; Lisa et al. 2015). Detailed studies, therefore, are warranted to explore the relationship between denitrifier abundance and denitrification potential in the river sediments.

Huaihe River is located in plain area with the low flow velocity. An amount of nutrients including nitrogen, phosphorus, and organic matter from domestic sewage, farm drainage, industrial effluent, etc. enter into the main stream and its branches, resulting in deterioration of water quality. In the past 20 years, water pollution treatment in the Huaihe River basin has drawn great attentions of Chinese government and scientists. Although some effective measurements have been taken, the contents of nitrogen, phosphorus, and organic matter in sediments are still high. As two obligatory denitrification intermediates, NO and N_2O elicit a harmful effect on biological metabolism and the natural environment. NO, a toxic substance, is recognized as an indirect greenhouse gas (Zhou et al. 2018). The reduction reaction of NO caused by microorganisms is the main source of N_2O. And N_2O is a powerful greenhouse gas (310 times stronger than carbon dioxide's greenhouse effect), and it is also the main substance that destroys the ozone layer (Kuypers et al. 2018). In studies regarding sediment characteristics and denitrification potential, denitrifier community with functional gene abundance can be used to identify key factors to assess the function of river ecosystems, which improve river management and accelerate N loss in the form N_2 from river sediment.

In this study, river sediment samples from the main stream and 22 branches located in Anhui province with different nutrient content were collected and a series of chemical and microbial analysis were carried out. The contents of organic matter, nitrogen, and phosphorus were measured. The microbial communities were investigated by high-throughput sequencing and qPCR. We explored two questions in the current study. We first explored whether differences in nutrient level are reflected in differences in their denitrifier communities and gene abundance. Second, we aimed to identify the key biological and environmental drivers that explain the observed changes in PDR associated with nitrogen removal.

Materials and methods

Site description

The Huaihe River (30° 55′–36°36′ N, 111°55′–120°45′ E) is located in the eastern China, between the Yangtze River and the Yellow River (Jiang 2011). The Huaihe basin (approximately 270,000 km^2) is situated in a transition zone of northern-southern in china (Meng et al. 2014; He et al. 2015). The average precipitation rate in the basin is about 883mm annually. For this basin, the average annual temperature ranges 13.2–15.7°C; the annual evaporation ranges from 900 to 1500mm, and frost free period is about 200–240 days (He et al. 2015). In the basin, a complex interaction of meteorological and hydrological processes occurs which frequently trigger and exacerbate flood and drought events (Wang et al. 2014a; Zhang et al. 2015). Previous studies indicated that above 50% water resources of Huaihe River have been over-exploited (Jiang 2011). Agricultural cultivation and livestock production have been a long history in this basin.

Textile, household appliances, steel, cement, and fertilizer are the major industries located along the main stream and branches of Huaihe River, which are running through the main economic areas in the middle-eastern of China (Tian et al. 2013). In recent decades, significant amount of nutrients such as nitrogen and phosphorous from farm drainage, domestic sewage, industrial effluent, etc. had entered into the main stream and its branches that are deposited in the river sediment, which led to severe degeneration of river ecosystems.

Sample collection and pretreatment

In this study, the main stream and the leftward branches located in the Anhui province were chosen as the investigated subject. The length of main stream of Huaihe River in Anhui province is more than 400km, and its leftward branches in Anhui province mainly includes Honghe River, Guhe River, Runhe River, Shayinghe River, Xifeihe River, Cihuai River, Xinhe River, Qianhe River, Guohe River, Beifeihe River, Xiehe-Huihe River, Tuohe River, Bianhe River, and Suihe River. A total of 135 sections from main stream and its branches were chosen to collect the sediment samples (Fig. 1 and Table S1). The sampling time was from July 2015 to September 2016.

Based on different river morphological characteristics, river junctions, nearby possible pollution sources and other factors, port, and wharves with relatively slow velocity and intensive human activities were selected as sampling sections. In each sampling section, 5 subsamples of surface sediment (depth: 0–10cm) were collected by Pedersen sampler and then mixed into a sample. Immediately after collection, sediments were placed into plastic bags and held on ice until return to the laboratory. Although the study was conducted on a wide range of areas, we were able to collect river sediment samples within 2–3 days of certain city. Hence, samples were held on ice approximately 2–3 days. Centralized collection prevented the effects of long time intervals on sediment samples as much as possible. One portion of each sample was used for physical and chemical analysis, and another was stored in the refrigerating cabinet with −20 °C and used for DNA extraction.

Chemical analysis

The pH meter (Mettler Toledo FE20, $sediment_{mass}$: H_2O_{volume}=1g:5ml) was used to assess pH. The organic matter (OM) was obtained from the subsequent loss of weight after continuously drying the sample in a muffle furnace at 550 ±5°C for 6 h (Parker 1983). The total nitrogen (TN) content was measured using kjeldahl determination device (Smart et al. 1983). The mixture (5 g samples and 20% KCl) was added to the triangle bottle and then filtered after being shaken for 0.5 h. Concentrations of NH_4^+-N (phenol-sodium hypochlorite spectrophotometry), NO_3^--N (ultraviolet spectrophotometry) and NO_2^--N (diazo coupled spectrophotometry) in sediment samples were determined using the filter by a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). SMT (standard measurement and test) (Ruban et al. 2001) method was used to extract the total phosphorus and various forms of phosphorus. Total phosphorus, organic phosphorus, and inorganic phosphorus were measured by the molybdenum blue colorimetric method at 660 nm.

DNA extraction and high-throughput sequencing

Total DNA in sediment samples were extracted by using the PowerSoil® DNA isolation kit (Mo Bio Carlsbad, USA) according to the manufacturer's protocol. Each extracted genomic DNA was stored at −20 °C before further analysis.

The 16S rRNA gene was amplified by PCR for multiplexed pyrosequencing with barcoded primers, and the V3-V4 region of the bacterial 16S rDNA was amplified by primers 338F: ACTCCTACGGGAGGCAGCA and 806R: GGACTACHVGGGTWTCTAAT. The resulting amplicons were sequenced by an Ilumina Miseq PE250 machine at Shanghai Majorbio Biopharm Technology Co., Ltd (Shanghai, China).

Real-time fluorescent quantitative PCR

Quantitative analyses on samples from the denitrification were performed on gene fragments including *nirK*, *nirS*, and *nosZ* clade I (Myrtotsiknia, Paranychianakis, et al. 2015). Nitrous oxide reductase (NosZ), the enzyme associated with terminal step in denitrification, however, is not always affiliated with denitrifying microorganisms. Phylogenetic analyses have shown that clade II *nosZ* is affiliated with a much broader diversity of microorganisms than those with clade I *nosZ*, the former including both non-denitrifiers and denitrifiers (Joanne et al. 2020). The primer sequence is listed in Table S2. Amplification was performed using TaqMan Universal PCR Master Mix (Bestar Biosystem, German), and the PCR program was based on the specifications. Real-time PCRs were carried out on a Stepone real-time PCR system (Applied Biosystems USA). Each PCR mixture (10 μL) contained 5uL of Bestar® SYBR qPCR Master Mix Ex TaqTM II (2×), 0.25 μL of each primer (concentration of 10 μM), 0.2 μL of ROX reference dye (50×), 3.3 μL of ddH_2O, and 1μL of template DNA (Bestar Biosystem, German). The PCR temperature program was initiated with 2 min at 95°C, followed by 40 cycles of 10 s at 95°C and 30 s at the specific annealing temperature (Table S2), and 30 s at 72 °C. A melting curve for SYBR Green assay was generated to verify the specificity of amplification. All sediment DNA samples and negative controls were made in triplicate. After purifying PCR fragments of the respective denitrifying genes using M13 PCR from clones, standard curves were generated based on a serial

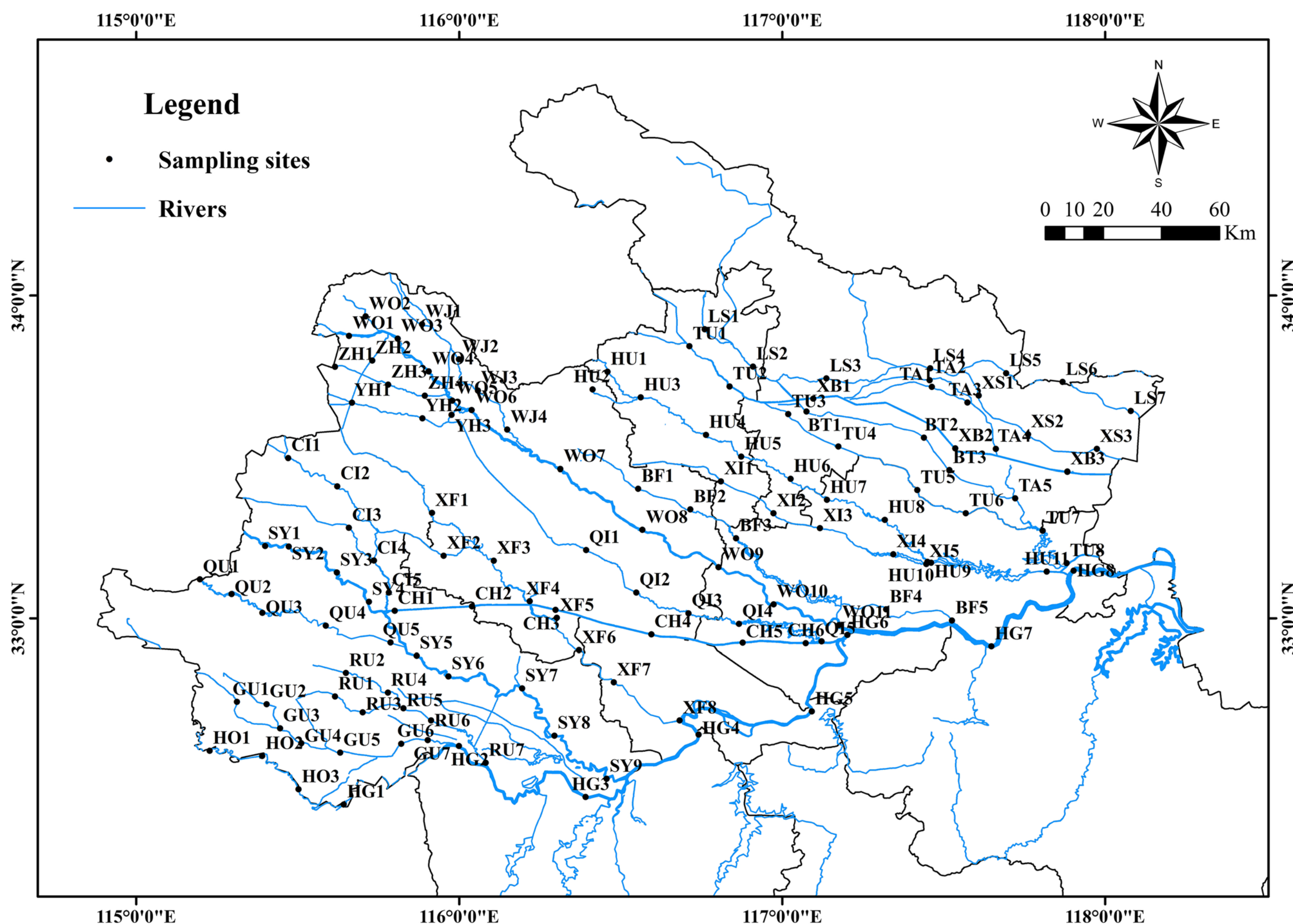

Fig. 1 Sketch map of sampling sites of rivers in northern Anhui province. Z Zhaohe River, Y Youhe River, XS Xinhe River, X Xiehe River, XF Xifeihe River, WJ Wujiahe River, W Guohe River, T Tuohe River, TA Tanghe River, S Shayinghe River, R Runhe River, Q Quanhe River, QI Qianhe River, L Suihe River, H Huihe River, HG Huaigan River, HO Honghe River, G Guhe River, CH Cihuai River, C Cihe River, XB Bianhe River, BT Beituohe River, BF Beihe River

dilution of known copies of PCR fragments. The R^2 value of each standard curve was above 0.99.

Denitrification potential

Denitrification potential was measured in triplicate sediment slurries using the acetylene inhibition technique (Magalhães et al. 2005). A total of 30mL mixture (0.18g·L^{-1} glucose and 0.1g·L^{-1} KNO_3) was used to incubate approximately 20g fresh sediment sample in 100mL sterile bottle. The bottle was flushed with nitrogen gas for 10 min brought to atmospheric pressure and add high amount of O_2-free acetylene gas (C_2H_2) to block the final denitrification step of N_2O to N_2. Therefore, the potential denitrification rate (PDR) could calculate as the increment of N_2O emissions. After culturing the sealed culture flask at 200 rpm and 25 °C for 6 h, 8 h, 12 h, and 24 h, the concentration of the produced gas N_2O was measured on a gas chromatograph equipped with an electron capture detector (ECD). The temperatures of the detector were set at 360°C. The ECD used high-purity nitrogen as a carrier gas. The column temperature was maintained at 50 °C.

Statistical analysis

Table S3 presents the information of sequence numbers from raw to filtered sequences for each sample. The clean reads were defined as sequences >200 bp and <1000 bp in length, with an average quality score >25, with filtering the chimera sequence, without ambiguous base calls, and with at least an 80% match to a previously determined 16S rRNA gene sequence by QIIME 1.8.0. SILVA database (SILVA SSU and LSU databases 128) was used for QIIME. Denitrifying floras were selected through KEGG database: First, search the name of the genus in the KEGG database, and subsequently, the genome appeared. Click in to find its keywords and finally determine its function. If the function of the genus has not been determined in the database, the references were searched to verify whether the genus could participate in the denitrification process.

Canonical correspondence analysis (CCA) was performed with R statistical platform using the “cca” and “envfit” function of the vegan package to analyze the important environmental variables of explaining changes in the denitrification community. Pearson correlation analysis was used to further explore the link between environmental factors and denitrifier abundance. $P < 0.05$ represented statistically significant.

Before the analysis, the abundance data of functional genes was logarithmically transformed to approximate normality. All data including nutrients content, gene abundance and potential denitrification rates (PDRs) were processed by column normalization. Moreover, to eliminate the interference of autocorrelation between environmental variables, partial Mantel test was employed to investigate the influence of environmental factors on denitrifying gene abundance and potential denitrification rates. Meanwhile, the relationships between the functional genes and PDR were analyzed by partial Mantel test. The results obtained were demonstrated in the “R.” Finally, random forest was used to analyze the contribution of nutrients and gene abundance to PDR changes.

Results and discussion

Chemical properties of river sediments

Table 1 presents the chemical properties of river sediments. The organic matter (OM) content varied from 10.31 to 173.09 ($g.kg^{-1}$). Similarly, ammonia nitrogen (NH_4^+-N) content varied from 2.87 to 304.46 ($mg.kg^{-1}$), and nitrate (NO_3^--N) content ranged from 0.10 to 157.48 ($mg.kg^{-1}$). While total nitrogen (TN) content showed variation from 0.01 to 4.77 ($g.kg^{-1}$), total phosphate (TP) content was in a range of 0.15 to 2.11 ($g.kg^{-1}$).

Highly correlated correlations were found among nutrients (Fig. 2) which indicated the similar environmental sources. According to the standard values of the nutrient content in the sediment (Lange 1992), the evaluation standard values of total nitrogen and total phosphorus are 0.55 g/kg and 0.60 g/kg, respectively. The higher contents of nitrogen and phosphorus in branch sediments might be mainly attributed to the terrigenous input caused by agricultural production (non-point sources) and the domestic sewage and industrial wastewater discharge (point sources) (Wang et al. 2003; Wang et al. 2013). Higher nutrient contents in the sediments of the branches might be attributed to the lower water velocity and farmland irrigation (Wang et al. 2013; Jingqiu et al. 2015). In the densely populated area, the organic matter, nitrogen, and phosphorus in domestic sewage from villages and small towns often drained into rivers with surface runoff in the flood season and deposited in the river sediments (Wang et al. 2013).

Bacterial community structure and their abundance in river sediments

Bacteria living in or on the sediments at the bottom of the aquatic system play a substantial role in the organic pollutant degradation, biogeochemical cycles, and microbial food webs (Buesing and Gessner 2006).

The sequence reads were taxonomically assigned to 55 phyla in this study. Therein, the dominant bacteria (with an average relative abundance more than 1%) mainly assigned to 12 phyla (Table S4) including phyla Proteobacteria, Chloroflexi, Bacteroidetes, Acidobacteria, and Actinobacteria, indicating that these phyla were the most important in the bacterial communities of sediments. In these dominant taxa, Firmicutes, Nitrospirae, and Cyanobacteria displayed the largest variation among 135 samples (Table S4). In parallel, α-, β-, δ-, and γ-proteobacteria (mean relative abundance) accounted for 4.19%, 16.39%, 13.35%, and 13.66%, respectively. Previous studies found that β-proteobacteria, γ-proteobacteria, δ-proteobacteria, Bacteroidetes, and Chloroflexi were dominant divisions in sediments (Haller et al. 2011; Ligi et al. 2013;Nunoura et al. 2013). In addition, 33 denitrifying genera were found, in which the average relative abundance of *Thiobacillus* (Betaproteobacteria) and *Halomonas* (Gammaproteobacteria) reached 3.739% and 2.575%, respectively (Fig. 3). In these dominant bacteria, genera *Bacillus*, *Halomonas*, *Pseudomonas*, and *Thiobacillus* have been shown to be involved in denitrification (Falk et al. 2006; Huang et al. 2011; Han and Li 2016).

Table 1 The chemical properties of sediments

Indices	pH	OM $g{\cdot}kg^{-1}$	NH_4^+-N $mg{\cdot}kg^{-1}$	NO_3^--N $mg{\cdot}kg^{-1}$	NO_2^--N $mg{\cdot}kg^{-1}$	TN $g{\cdot}kg^{-1}$	IP $mg{\cdot}kg^{-1}$	OP $mg{\cdot}kg^{-1}$	TP $mg{\cdot}kg^{-1}$	C/N ratio
Mean	7.78	38.41	62.44	22.24	0.24	0.87	471.70	85.73	675.87	79.12
Median	7.80	34.66	44.21	12.62	0.15	0.69	448.72	73.94	644.56	52.17
Minimum	6.08	10.31	2.87	0.10	0.01	0.01	92.93	2.16	152.65	21.17
Maximum	8.83	173.09	304.46	157.48	1.40	4.77	1631.96	509.17	2108.46	1184.45
CV*	5.44	56.75	86.91	124.30	94.93	85.17	39.96	65.88	39.05	145.03

Notes: *CV* coefficient of variance

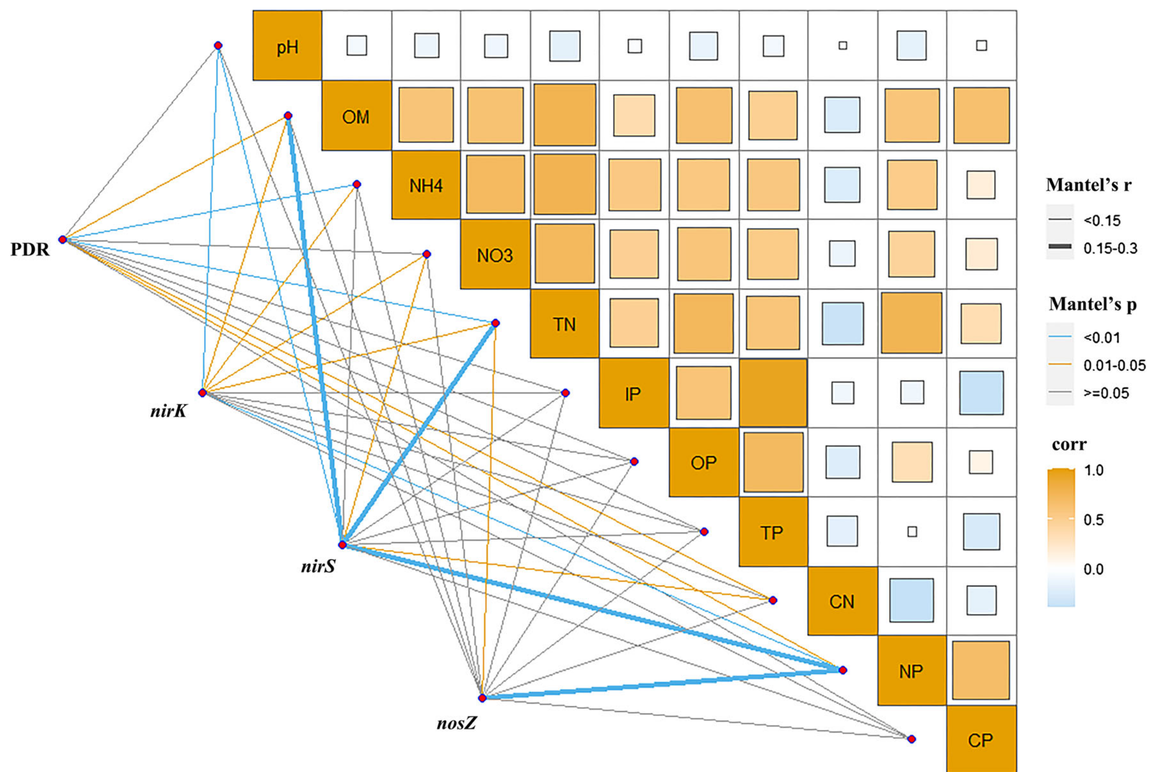

Fig. 2 The influence of environmental variables on functional gene abundance and potential denitrification rates (PDRs)

Quantities of denitrifying functional genes in river sediments

Functional genes *nirK*, *nirS*, and *nosZ* are common molecular markers for denitrifiers and widely distributed in sediments (Huang et al. 2011).

Table S5 presented the abundance of three functional genes (*nirK*, *nirS*, and *nosZ*) involved in denitrification in river sediment investigated. The abundances of *nirK*, *nirS*, and *nosZ* genes ranged from 2.17×10^6 to 9.00×10^8, 6.63×10^6 to 1.54×10^{10}, and 3.3×10^5 to 1.73×10^9 gene copies per grams of dry sediment, respectively. In this study, *nirS* displayed higher abundance than *nirK* and *nosZ* genes in most sediment samples collected, which might indicate a favorable habitat distribution for *nirS*-type denitrifiers (Myrtotsiknia, Paranychianakis, et al. 2015). The differences between denitrifying gene abundance might imply some type of preferential cooperation or provide a proxy for the contribution of the functional groups on the corresponding process (Wang et al. 2014b; Myrtotsiknia, Paranychianakis, et al. 2015). Three functional genes were detected in all sediment samples, indicating the widespread presence of denitrifying bacteria in the river sediment of the mainstream and tributaries.

Figure 2 showed that TN and N:P were found to have significant effects on the abundance of three denitrifying genes (*nirK*, *nirS*, and *nosZ*). Compared to *nirK* and *nosZ*, *nirS* was significantly correlated with a higher number of environmental variables. Specifically, *nirS* gene was significantly affected by pH, OM, TN, and N:P (Mantel's $P < 0.01$ and Mantel's $r > 0.15$). Additionally, NO_3^- and C:N were also correlated to *nirS* gene (Mantel's $P < 0.05$). However, the link between *nirK* gene and environmental factors was not strong (Mantel's $r < 0.15$). Previous studies have shown that the critical role of *nirK* and *nirS* genes in nitrogen removal in estuarine and coastal ecosystems and their abundance in sediments were affected by environmental variables (Huang et al. 2011; Wang et al. 2014b). But compared with *nirK* and *nosZ* genes, *nirS* gene could be more susceptible to environmental factors (Fig. 2), which was similar to the result of Myrtotsiknia, Paranychianakis, et al. (2015).

Studies have approved that denitrifiers containing *nirS* and *nirK* genes in environments were mainly coming from different bacterial taxa including Alphaproteobacteria, Deltaproteobacteria, Gammaproteobacteria, Actinobacteria, Firmicutes, and Nitrospirae (Green et al. 2010; Xiong et al. 2014). Bacteria containing *nirS* genes included the genera *Alcaligenes*, *Aromatoleum*, *Azoarcus*, *Bacillus*, *Brachymonas*, *Candidatus*, *Dechloromonas*, *Hahella*, *Halomonas*, *Herbaspirillum*, *Marinobacter*, *Paracoccus*, *Pseudomonas*, *Ralstonia*, *Rhodanobacter*, *Roseobacter*, *Thauera*, *Thiobacillus*, and *Thiomicrospira* (Falk et al. 2006; Huang et al. 2011). Therein, genera *Bacillus*, *Thiobacillus*, *Halomonas*, *Pseudomonas*, and *Rhodanobacter* were widely distributed in sediment samples and displayed a higher

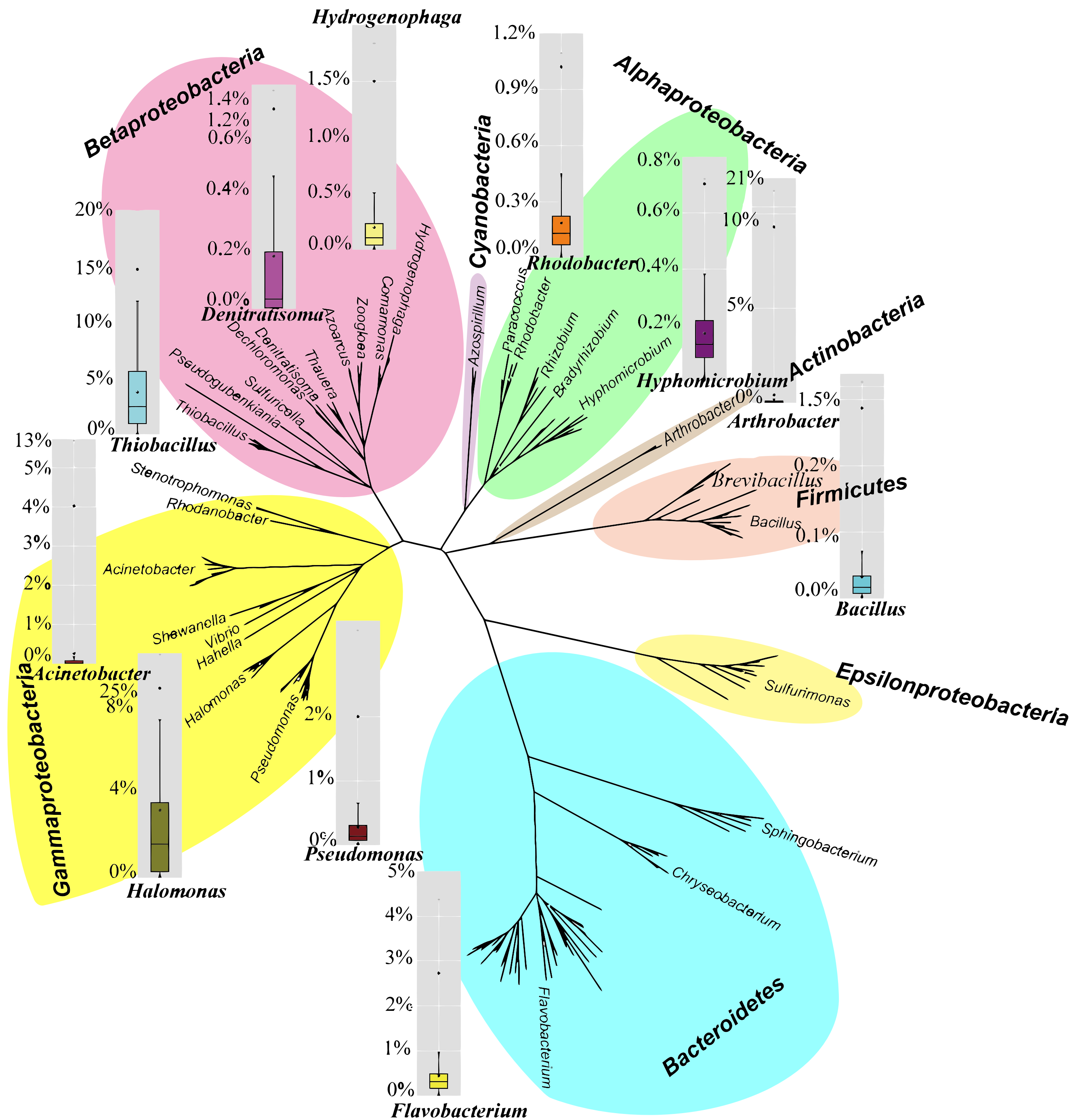

Fig. 3 Phylogenetic analysis of the abundance and community structure of denitrifying bacteria. Denitrification genera with relative abundance greater than 0.1% were displayed by box diagram

relative abundance in this study area. In parallel, correlation analysis showed that *Thiobacillus* was positively correlated with *nirS* genes (Table 2). CCA showed that among the nutrient characteristics of Huaihe River sediment, NO_3^-, NO_2^-, NH_4^+, and IP had the significant impacts on the *nirS*-encoding bacterial community structure and spatial distributions (Fig. 4), and the *nirK*-encoding denitrifiers were influenced by NO_3^-, NH_4^+, and TP (Figure S1). Organic matter (OM) was the primary electron donor for the respiratory denitrifying bacteria (Burgin and Hamilton 2008), and the study found that the relative abundance of *nirS*-encoding denitrifier community and the quantity of *nirS* were both closely related to OM in the sediment. The variation percentages in other denitrifying communities were explained by various sediment chemical properties. Figure 4 also indicated that denitrifier abundance was also significantly affected by the content of phosphorus

Table 2 Correlation analysis between denitrifiers and abundance of three functional genes

	PDR	*nirK*	*nirS*	*nosZ*
Achromobacter	0.112	0.127	−0.019	0.027
Acinetobacter	0.076	0.053	−0.046	−0.024
Arthrobacter	−0.096	−0.118	−0.103	−0.09
Azoarcus	−0.107	−0.134	−0.154	−0.116
Azospirillum	0.102	0.250**	0.067	0.047
Bacillus	−0.044	0.011	−0.098	−0.048
Bradyrhizobium	−0.039	−0.058	−0.071	−0.041
Brevibacillus	−0.004	−0.045	−0.12	−0.053
Chryseobacterium	−0.062	−0.074	−0.056	−0.011
Comamonas	−0.137	−0.022	−0.1	−0.054
Dechloromonas	0.001	0.003	0.051	0.031
Denitratisoma	0.106	0.055	0.066	−0.036
Flavobacterium	0.003	−0.148	−0.164	−0.12
Hahella	−0.093	−0.081	−0.076	−0.041
Halomonas	−0.193*	−0.165	−0.133	−0.094
Hydrogenophaga	0.043	0.033	−0.012	−0.054
Hyphomicrobium	−0.096	−0.023	−0.045	−0.045
Paracocccus	−0.052	−0.058	−0.058	0.002
Pseudogulbenkiania	0.017	0.075	0.032	0.054
Pseudomonas	−0.037	−0.072	−0.123	−0.083
Rhizobium	−0.056	−0.055	−0.053	0.009
Ralstonia	0.037	−0.081	−0.053	−0.034
Rhodanobacter	−0.012	−0.05	−0.052	−0.051
Rhodobacter	−0.147	−0.179*	−0.215*	−0.135
Shewanella	−0.095	0.002	−0.039	0.016
Sphingobacterium	−0.032	−0.097	0.002	−0.054
Stenotrophomonas	−0.053	−0.072	−0.03	−0.016
Sulfuricella	0.029	0.027	0.089	0.05
Sulfurimonas	0.044	0.067	0.104	0.122
Thauera	0.043	0.071	0.037	−0.044
Thiobacillus	0.290**	0.243**	0.202*	0.133
Vibrio	−0.071	−0.025	−0.004	−0.034
Zoogloea	−0.007	0.019	0.149	0.073

*Correlation is significant at the 0.05 level (2-tailed)

**Correlation is significant at the 0.01 level (2-tailed)

(TP and IP). TP was identified as a possible determinant of shaping denitrifier community structure in aquic brown soils in Northeast China and constructed wetland (Li et al. 2018; Yin et al. 2014). Due to the same terrestrial input, the strong correlation between P and N may partially account for this phenomenon. Another possible cause is the important role of P used as sources of energy in shaping the structure of bacterial communities (Burton and Johnston 2010; Xie et al. 2016). Nevertheless, the links between phosphorus and sediment denitrifiers remain unclear. The present study provided the evidence for the possible links of denitrifier abundance with sediment phosphorus.

Previous researches demonstrated that the abundance of *nosZ* genes was influenced by many environmental factors, such as sediment texture, carbon, total nitrogen, ammonia, nitrate, and C:N ratio (Huang et al. 2011; Myrtotsiknia, Paranychianakis, et al. 2015). Partial Mantel test presented that the abundance of *nosZ* gene was significantly affected by the contents of TN and N:P ratio in sediments, implying that the richer nitrogen nutrients can promote high gene abundance (Fig. 2). Laverman et al. (2010) considered that the higher numbers of *nosZ* were appeared in the nutrient rich sediments and possessed an efficiency of N_2 production. Studies also found that the abundance of *nosZ* genes was stimulated by the nitrogen inputs and the accelerated N_2O emissions (Myrtotsiknia, Paranychianakis, et al. 2015).

Bacterial *nosZ* is universal and abundant in sediments (Rusch and Gaidos 2013). In parallel, we observed similar impediments to functional-gene detection with commonly employed *nosZ* gene primers, which exclusively target genes from alpha-, beta-, and gamma-proteobacteria, suggesting that previous denitrifier community studies using *nosZ* analyses most likely do not elucidate the true diversity and abundance of denitrifying bacteria in the environment (Green et al. 2010). Phylogenetic analysis demonstrates that many bacteria containing *nosZ* genes are belonged to alpha-, beta-, and gamma-proteobacteria, such as genera *Alkalilimnicola*, *Bradyrhizobium*, *Brucella*, *Cupriavidus*, *Hyphomicrobium*, *Marinobacter*, *Paracoccus*, *Pseudomonas*, *Ralstonia*, *Rhodopseudomonas*, *Roseobacter*, *Silicibacter*, and *Sinorhizobium* (Henry et al. 2006; Magalhães and Hollibaugh 2008; Mills et al. 2008). This research showed that *nosZ*-type denitrifier community was mainly influenced by environmental factors such as NH_4^+, NO_3^-, TN, OM, and OP (Figure S1).

Denitrification potential of river sediments

In this study, denitrification potential rate (PDR) was expressed in N_2O production rate which exhibited a range of 0.011 to 15.694 $\mu gN \cdot g^{-1} \cdot h^{-1}$, with a mean of 1.689 $\mu gN \cdot g^{-1} \cdot h^{-1}$ and a CV of 149.99%. Theoretically, PDR in sediments was influenced by the abundance of *nirK*, *nirS*, and *nosZ* genes as well as the environmental factors. Previous studies have demonstrated that PDR was significantly positively correlated with environmental factors such as ammonia, nitrate, nitrite, total phosphorus, and pH (Laverman et al. 2007; Kanyiginya et al. 2010). Figure 2 indicated that there was significant link between PDR and nutrients (OM, TN, NH_4^+-N, C:N, and N:P ratio) in this study. And it also highlighted the key contributions of NH_4^+-N and TN to the variations of PDR. A significant correlation (Mantel's $P < 0.01$) between PDR and the abundance of functional genes was found (Fig. 5). Recent studies showed that the *nosZ* gene was correlated with PDR and hence could act as an index predicting PDR

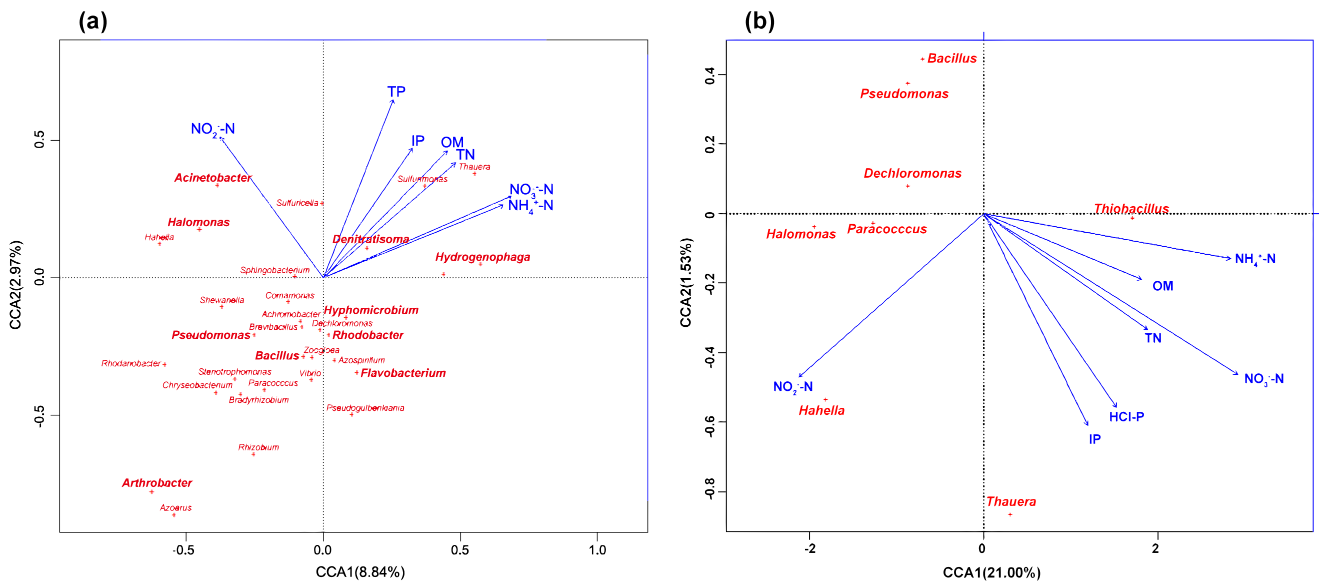

Fig. 4 The canonical correspondence analysis for chemical properties and denitrifying bacteria (**a**) as well as *nirS*-type denitrifiers (**b**)

(Hallin et al. 2009; Petersen et al. 2012). Previous studies have demonstrated that both *nirK* and *nirS* gene abundance were correlated to PDR (Morales et al. 2010). Comparing to *nirK* and *nosZ* genes, the abundance of *nirS* genes had greater significant correlation coefficient with PDR (Fig. 5), suggesting that it was more suitable using *nirS* gene abundance to predict denitrification potential in this study. And the random forest analysis also showed high influence of *nosZ* to PDR. These assays are directly associated with the release of N_2O or N_2 and the consumption of NO_3^-, hence, with the activity of denitrifying microorganisms (Xiong et al. 2017;Lisa et al. 2015). Moreover, PDR has been confirmed to be related to changes in gene abundance (Semedo and Song 2020). Petersen et al. (2012) found that functional gene abundances of denitrifiers could predict the potential rate of denitrification. Several studies have also provided evidence for distinct cooccurrence patterns of the different types of denitrifiers between habitats and gene sharing among denitrifiers (Myrtotsiknia, Paranychianakis, et al. 2015). So we have concluded that all three genes may be more suitable to predict denitrification rates, since they respond to different environmental variables, but all of them correlate strongly with PDR. Nutrient level and gene abundance were used as candidate variables in random forest analysis to PDR (Table 3), altogether explaining 14.0% the PDR variations. Random forest (RF) means predictor importance of different indices as drivers for PDR. Analysis showed that NH_4^+ was the most important contributing factor driving the change of PDR (Table 3). To date, the links of denitrification rate with sediment physicochemical variables remain obscure. Studies have proved that denitrification enzyme activity in sediment was found to be possibly affected by nitrogen contents (Lan et al. 2015). The potential influences of NH_4^+-N, NO_3^--N, and NO_2^--N on denitrification rate were also found in sediments (Li et al. 2018). This result indicated that the concentration of NH_4^+ was more important than the abundance of denitrifying genes to explain the variation of PDR. The driving force of NH_4^+ on changes in PDR is probably due to the interdependence of nitrifying and denitrifying bacteria on substrates (NH_4^+ and NO_3^-) (Myrtotsiknia, Paranychianakis, et al. 2015). Recently, the simultaneous nitrification-denitrification process has also

Table 3 Contribution of nutrients and genes abundance to PDR

Variables	Increase in MSE (%)	IncNodePurity
pH	3.50	43.82
OM	1.71	67.96
NH_4^+	7.82	80.55
NO_3^-	0.02	54.81
TN	4.78	78.19
C:N	5.55	75.55
IP	2.03	37.45
OP	3.32	45.40
TP	2.92	35.12
nirS	6.24	126.03
nirK	2.84	62.01
nosZ	6.90	72.27

Notes: random forest (RF) means predictor importance (percentage of increase of mean square error) of different indices as drivers for PDR: the increased mean square error (%IncMSE) and increased impurity index (IncNodePurity). %IncMSE measures the effect on the predictive power when the value of a specific original parameter is randomly permuted. IncNodePurity measures the total increase in the homogeneity of the data samples from splitting them on a given parameter

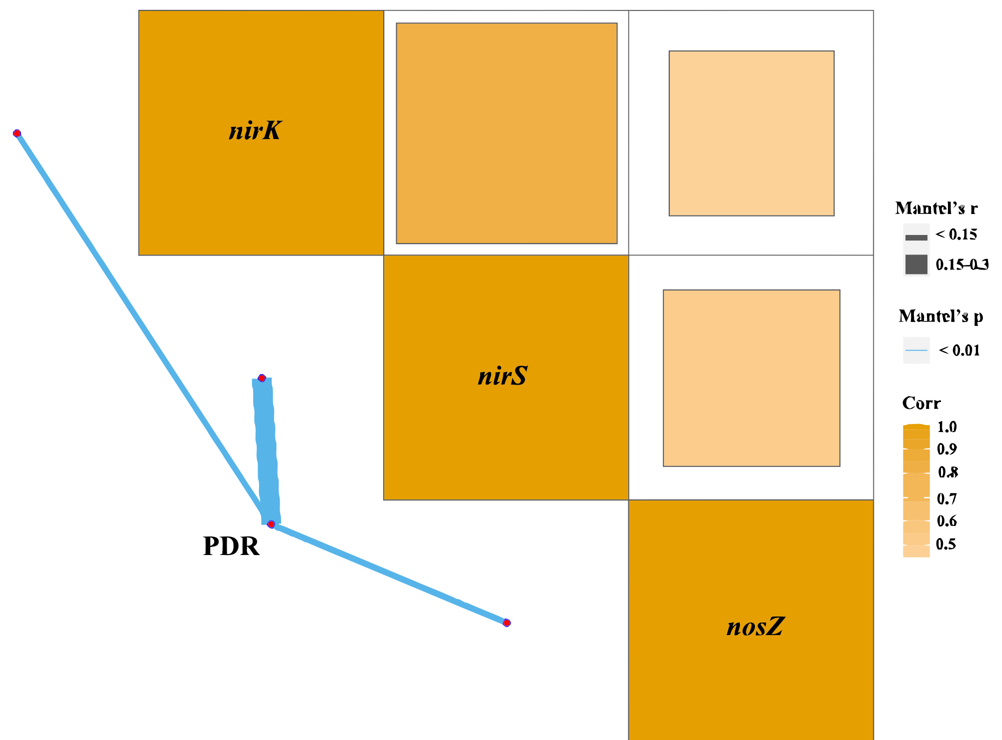

Fig. 5 Relationships between the denitrifying genes (*nirK*, *nirS*, and *nosZ*) and potential denitrification rates

been widely reported (Jia et al. 2020). The importance of NH_4^+ in this study could also suggest that nitrification-denitrification and/or nitrifier denitrification may play an important role in driving PDR (Jia et al. 2020; Xiong et al. 2017). In summary, this study indicated that variations in PDR were related to changes in nitrogen nutrient concentration and denitrifier abundance.

Further analysis (Table 2) found that there was a significantly positive correlation between PDR and abundance of genera *Thiobacillus*, which indicated that bacteria from genera *Thiobacillus* played an important role in the denitrification and might contribute to the conversion/removal of nitrogen pollutants in river sediments. However, a significantly negative correlation was also found between the PDR and *Halomonas* abundance (r = −0.193 and P= 0.025), which speculate *Halomonas* may restrain the efficiency of N_2 production. The results were instructive for possible bioremediation or evaluation of river sediments. For example, *Thiobacillus* may contribute to the conversion/removal of nitrogen pollutants in river sediments by facilitating denitrification.

Conclusions

We have concluded from our study that genera *Thiobacillus* played an important role in the denitrification of the river sediments. TN and N:P ratio were correlated with the abundance of all three denitrifying genes. Additionally, the abundance of *nirS* gene could be more easily influenced by environmental parameters, and NO_3^-, NO_2^-, NH_4^+, and IP had the significant impacts on the *nirS*-encoding bacterial community structure and spatial distributions. Quantitative response relationships between denitrification potential and functional genes were established, and PDR was positively correlated with denitrifying genes. Sediment nitrogen nutrient level and denitrifying gene abundance were shown to be tightly linked to PDR. Moreover, NH_4^+ was the key contributing factor in explaining variations in PDR. There could be a significant difference between community composition and activity throughout the depths sampled in this study as the redox conditions could change drastically, which required further study. Additionally, the physiology and ecological roles of functional genes (including other genes of nitrogen metabolism) and denitrifiers in denitrification mechanism and nitrogen oxide degradation in the environment should be of great interest for future studies.

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Availability of data and materials All data generated or analyzed during this study are included in this published article.

Author contribution MZZ was a major contributor in analyzing the data and writing the manuscript. UD provided language help. QYS originally

designed the study. PXC carried out the chemical experiments of sediments, and XHW provided help in collecting samples. All authors read and approved the final manuscript.

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Declarations

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Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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