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A Study on the Characteristics of Nitrification and Denitrification of Three Small Watersheds During the Wet and Dry Seasons with Various Sources of Pollution: A Case Study of the Jinjing Basin

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Abstract: Nitrogen cycling in freshwater ecosystems is critical for maintaining water quality, and understanding the processes of nitrification and denitrification is essential for effective nitrogen management, particularly in areas with diverse pollution sources. This study investigated the nitrification and denitrification processes in three tributaries of the Jinjing River—Tuojia (agricultural), Jinjing (residential), and Guanjia (woodland)—during both the wet and dry seasons. The potential nitrification rates (PNRs) and potential denitrification rates (PDNRs) were measured across these sites. The highest rates were observed in Tuojia during the wet season, with the PNR reaching 39.7 $\mu g \cdot kg^{-1}\ h^{-1}$ and the PDNR reaching 3.25 $mg \cdot kg^{-1} \cdot h^{-1}$, while the rates were considerably lower in Jinjing and Guanjia. The ammonia-oxidizing archaea (AOA) abundance was higher than the ammonia-oxidizing bacteria (AOB) abundance at all sites, with Tuojia exhibiting the highest AOA abundance (5.9×10^7 copies$\cdot g^{-1}$) during the wet season. The nitrate-nitrogen (NO_3^--N) content was a key factor influencing denitrification, and the AOA abundance was significantly correlated with nitrification rates ($r = 0.69$; $p < 0.05$). These findings highlight the spatial and seasonal variability in nitrogen cycling and emphasize the importance of developing targeted nitrogen management strategies in regions with mixed land uses and pollution sources.

Keywords: potential nitrification rates; potential denitrification rates; seasonal variability; microbial functional genes

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1. Introduction

With the rapid development of industrial and agricultural sectors in recent decades, there has been a substantial influx of exogenous nitrogen into water bodies, posing significant threats to water quality, particularly in river basins. In North America, agricultural activities, especially the use of synthetic fertilizers and livestock manure, are the primary contributors to nitrogen pollution [1]. Similarly, in European countries with high livestock densities, such as the Netherlands, Denmark, and France, the issue of nitrogen surplus is particularly acute. Excess nitrogen from agricultural inputs results in soil acidification, biodiversity loss, and the eutrophication of freshwater and marine systems [2]. In light of these challenges,

there is a growing need to improve our understanding of nitrogen dynamics in river basins, particularly focusing on the processes of nitrification and denitrification in sediments, which play a crucial role in nitrogen removal.

Nitrogen pollution has been a longstanding issue, and various studies have demonstrated that microbial processes, particularly those occurring in sediments, are essential for mediating nitrogen transformation and removal. Nitrogen removal in aquatic ecosystems is predominantly driven by microbial processes, including nitrification and denitrification. A key area of research has focused on the role of nitrifying and denitrifying microorganisms in sediments, which are responsible for converting nitrogen compounds into less harmful forms. Studies on microbial nitrogen cycling have emphasized the importance of anammox (anaerobic ammonium oxidation) bacteria in nitrogen removal in some systems [3], though nitrification and denitrification remain the dominant pathways for nitrogen removal in most freshwater and marine ecosystems.

In river basins, including those in China, nitrogen pollution levels often vary across sub-basins, with differing rates of nitrogen purification linked to the microbial activity in the sediments. Previous research has shown that microbial communities involved in nitrification and denitrification are critical to regulating nitrogen levels [4,5]. However, there remains a limited understanding of how these processes vary seasonally or in response to changes in the physical and chemical properties of water and sediment. Research on the microbial abundance, diversity, and functional gene expression of nitrification and denitrification microorganisms in river basin sediments remains sparse, especially in small-watershed systems.

The Jinjing River Basin, a small watershed in Hunan Province, China, serves as an ideal case study for exploring these dynamics. Preliminary studies have indicated that nitrogen pollution levels in the Jinjing River differ significantly across its sub-basins, possibly due to varying rates of microbial nitrogen removal [6] However, there is still a knowledge gap in understanding how microbial activity and community structure are influenced by environmental variables, such as temperature, organic matter content, and nitrogen levels. Recent studies have highlighted the potential of measuring the potential nitrification rate (PNR) and potential denitrification rate (PDNR) to assess microbial activity in nitrogen removal [7,8]. Moreover, the use of molecular markers, like the amoA gene for nitrifiers, and the narG, nirS, and nirK genes for denitrifiers, has enabled more precise studies of microbial abundance and function [4,9].

This study aimed to address these gaps by investigating the nitrification and denitrification potential in the water and sediments of the Jinjing River Basin. We focused on seasonal variations in microbial activity, functional gene abundance, and the correlations with the physicochemical properties of the water and sediments. This approach will help us better understand the nitrogen transformation processes in this basin and their implications for nitrogen pollution management. Specifically, by quantifying microbial activity and linking it to environmental variables, we seek to provide more accurate insights into the nitrogen removal pathways, which are essential for mitigating nitrogen pollution and its ecological consequences in the basin [10].

2. Materials and Methods

2.1. Sample Collection and Processing

Three tributaries with different sources of pollution were chosen. These included (i) residential areas (Jinjing River); (ii) woodland or mountain watersheds with relatively little anthropogenic impact (Guanjia River); and (iii) farmland runoff (Tuojia River). Fifteen sampling sites were set up in the upper, middle, and lower reaches of the Jinjing River, Guanjia River, Tuojia River, and their tributaries as well. Three samples were collected from all fifteen sites.

2.1.1. Water Sample Collection and Pretreatment

The water samples were collected in December 2021 (the dry season) and April 2022 (the wet season) at the selected sampling sites. The water was collected in 200 mL polyvinyl plastic bottles at each site. The samples were stored in a 4 °C refrigerator for 2 days to preserve their integrity before the analysis of their physical and chemical parameters. The water quality was monitored at each sampling site using a portable water quality multi-parameter tester (SG68, METTLER-TOLEDO, Greifensee, Switzerland) to measure the temperature (T), pH, and dissolved oxygen (DO). The flow rate (V) was measured in situ with a current meter, and the river width was recorded to provide context for the water flow dynamics. These sampling conditions were chosen to ensure an accurate representation of the water quality during seasonal changes, which may influence microbial activity and nitrogen cycling.

2.1.2. Sediment Collection and Pretreatment

The sediment samples were collected simultaneously with the water samples at each site. A three-point sampling method was used to collect surface sediment (0–5 cm depth) from the riverbed. This depth was selected because it represented the zone of active microbial processes in the sediment. The sediment was stored in sterile zip-lock bags and subsequently divided into four samples: one for microbial activity determination, one for microbial gene abundance analysis, one for physical and chemical property assessment, and one for retention. The division of the sediment into multiple samples ensured a comprehensive analysis of the sediment's microbial and physicochemical characteristics.

2.2. Determination of Potential Rates of Nitrification and Denitrification

2.2.1. Potential Nitrification Rate (PNR)

To determine the potential nitrification rate (PNR), 5 g of fresh sediment was placed into a 50 mL centrifuge tube, to which 20 mL of PBS solution containing 10 mmol·L^{-1} $(NH_4)_2SO_4$ and 100 mmol·L^{-1} $KClO_3$ were added. The 5 g sample size was selected based on previous studies to ensure sufficient microbial biomass for accurate rate measurements. The samples were shaken at 180 r·min^{-1} under dark conditions for 1, 4, 12, and 24 h to assess the temporal changes in nitrification. After the incubation period, 5 mL of a 2 mol·L^{-1} KCl solution was added to extract nitrite (NO_2^--N). The concentration of NO_2^--N was determined using an upper flow analyzer (AA3, SEAL Analytical, Norderstedt, Germany). The linear increment in the NO_2^--N concentration over time was used to calculate the potential nitrification rate (PNR), as described by [11].

2.2.2. Potential Denitrification Rate (PDNR)

For the potential denitrification rate (PDNR) determination, 5 g of fresh sediment was placed into a 150 mL wide-mouth bottle and left overnight at 25 °C to equilibrate. The 5 g sediment sample size was chosen based on previous experiments, ensuring a sufficient volume of sediment to yield measurable N_2O production. On the second day, 15 mL of a substrate solution containing 1 mmol·L^{-1} glucose and 1 mmol·L^{-1} KNO_3 was added to the bottle. The bottle was then sealed, and a vacuum was applied to remove the air. Nitrogen gas was purged from the bottle three times to ensure the absence of oxygen, followed by the addition of 10% acetylene gas to a final pressure of 1 atmosphere. The culture was incubated at 25 °C with shaking at 225 r·min^{-1} for 6 h. Gas samples (1 mL) were collected every hour during the incubation period. The concentration of N_2O in the gas was analyzed using a gas chromatograph (GC-2014, Shimadzu, Tokyo, Japan). The linear increase in the N_2O concentration was used to calculate the potential denitrification rate (PDNR) [12].

2.3. DNA Extraction and Real-Time Fluorescence Quantification

A 0.5 g aliquot of fresh sediment was weighed, and DNA was extracted using the Fast DNA® SPIN Kit for Soil (MP Biomedicals, LLC, Solon, OH, USA), following the

manufacturer's protocol. This sample size was chosen based on the optimal DNA yield reported for microbial communities in sediment samples. The DNA concentration and quality were determined using a Nanodrop ND-1000 UV–vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The DNA was then diluted and stored at −20 °C for further analysis.

To quantify the abundance of key denitrification functional genes, standard curves were constructed using the plasma DNA of the target genes. The plasmids were diluted to produce a series of standards with concentrations ranging from 10^{-2} to 10^{-8} copies per μL. Real-time quantitative PCR (qPCR) was performed using an ABI7900 PCR system (Applied Biosystems, Foster City, CA, USA), with three technical replicates per sample. Sterile water was used as a negative control. The amplification conditions were as follows: pre-denaturation at 95 °C for 30 s, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 55 °C (16S rRNA, AOA-amoA, AOB-amoA, and narG) or 57 °C (nirS and nirK) for 20 s, and extension at 72 °C for 20 s. A melting curve (95 °C for 15 s, 60 °C for 15 s, and 95 °C for 15 s) was included to verify the specificity of the amplification. The standard curve, negative control, and sample amplification were conducted on the same 384-well plate to minimize the inter-batch variability. The amplification efficiency of each gene was confirmed to range from 90% to 110%, with an R^2 value ≥ 0.99, indicating reliable quantification.

The qPCR system was modified according to the SYBR® Premix Ex Taq™ (TakaRa, Tokyo, Japan) specifications. Each reaction mixture contained 0.4 μL of an upstream primer (10 μM), 0.4 μL of a downstream primer (10 μM), 5.0 μL of Premix, 3.2 μL of water, and 1.0 μL of the DNA template (5 ng/μL). The primer sequences are listed in Table 1.

Table 1. Functional gene primer sequences.

Gene	Primers	Primer Sequence	Data Source
16S *rRNA*	1369F	CGGTGAATACGTTCYCGG	[13]
	1492R	GGWTACCTTGTTACGACT	
AOB *amoA*	*amoA*-1F	GGGGTTTCTACTGGTGGT	[14]
	amoA-2R	CCCCTCKGSAAAGCCTTCTTC	
AOA *amoA*	23F	ATGGTCTGGCTWAGACG	[15]
	616R	GCCATCCATCTGTATGTCCA	
narG	*narG*-517F	CCGATYCCGGCVAT-GTCSAT	[16]
	narG-773R	GGNACGTTNGADCCCCA	
nirS	*nirS*-cd3aF	GTSAACGTSAAGGARACSGG	[17]
	nirS-R3cd	GASTTCGGRTGSGTCTTGA	
nirK	*nirK*-876F	ATYGGCGGVCAYGGCGA	[18]
	nirK-1040R	GCCTCGATCAGRTTRTGGTT	

Note: F and R represent the upstream and downstream primers, respectively; S = C or G; Y = C or T; R = A or G; D = A, G, or T; V= A, C, or G; B = C, G, or T; N = A, C, T, or G.

2.4. Statistical Analysis

Microsoft Excel 2021, Minitab 21, and the graghpad prism 10 software package was used for preliminary data processing, where data analysis, data plotting, one-way analysis of variance, and paired *t*-tests were used to compare the significant differences between the different data groups, with a statistical significance level of 0.05 ($p < 0.05$).

3. Results

3.1. Microbial Activities of Nitrification and Denitrification of Sediment in Small Watershed

The nitrification and denitrification rates of the sediment in the small watershed are presented in Table 2 and Figure 1. For the PNR, there was considerable spatial and temporal variability. Spatially, the PNR of the sediment followed the following pattern:

Tuojia River (farmland) > Jinjing River (residential area) > Guanjia River (woodland), with ranges of 0.48–39.70 $\mu g \cdot kg^{-1} \cdot h^{-1}$, 0.43–6.21 $\mu g \cdot kg^{-1} \cdot h^{-1}$, and 0.27–5.37 $\mu g \cdot kg^{-1} \cdot h^{-1}$, respectively. During the wet season, the PNR in the Tuojia River (farmland) sediment was significantly higher than in the Jinjing River (residential area) and Guanjia River (woodland) sediments. Temporally, the PNR in the Tuojia River (farmland) was significantly higher in the wet season compared with the dry season, while no significant seasonal difference was observed in the Jinjing River and Guanjia River. This pattern in the Tuojia River may be due to frequent wet–dry cycles in farmland areas during the wet season, increasing sediment–air contact and promoting the growth of nitrifying microorganisms, thus enhancing nitrification.

Table 2. Potential nitrification and potential denitrification in sub-basin sediment of Jinjing watershed.

Location	Site	PNR ($\mu g \cdot kg^{-1} \cdot h^{-1}$)		PDNR ($mg \cdot kg^{-1} \cdot h^{-1}$)	
		Wet Season	Dry Season	Wet Season	Dry Season
Jinjing River (residential area)	A1	6.21	3.14	0.28	0.51
	A2	4.56	4.32	1.15	1.01
	A3	0.43	4.12	1.02	1.04
Guanjia River (woodland)	B1	4.19	3.85	0.78	0.73
	B2	0.38	0.27	0.32	0.33
	B3	5.32	4.92	0.22	0.24
Guanjia River sub-stream	D1	5.37	4.92	0.98	0.53
	D2	0.32	0.27	0.72	0.13
	D3	1.29	1.47	0.62	0.17
Tuojia River (farmland)	C1	39.7	4.06	3.25	2.71
	C2	11.2	2.58	1.73	0.57
	C3	2.30	3.39	0.39	0.12
Tuojia River sub-stream	E1	14.1	0.48	2.89	0.21
	E2	6.93	1.31	1.36	0.25
	E3	5.84	2.78	2.36	1.13

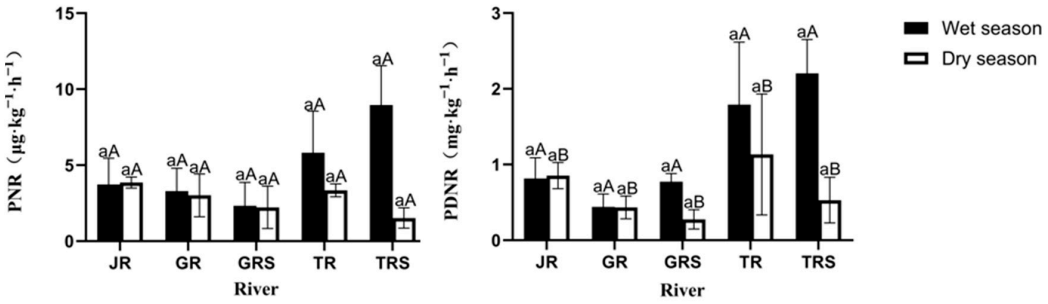

Figure 1. PNRs and PDNRs in sub-basin sediment of Jinjing watershed. Data are represented as means ± SEM (n = 3). Same letters indicate no significant difference ($p > 0.05$). Small letters represent rivers, and capital letters represent seasons. JR = Jinjing River, GR = Guanjia River, GRS = Guanjia sub-stream, TR = Tuojia River, and TRS = Tuojia River sub-stream.

The PDNR of the sediment followed a similar spatial trend: Tuojia River (farmland) > Jinjing River (residential area) > Guanjia River (woodland), with PDNR ranges of 0.25–3.25 $mg \cdot kg^{-1} \cdot h^{-1}$, 0.28–1.15 $mg \cdot kg^{-1} \cdot h^{-1}$, and 0.13–0.78 $mg \cdot kg^{-1} \cdot h^{-1}$, respectively. Spatially, the PDNR in the Tuojia River (farmland) sediment was significantly higher than in the Jinjing River and Guanjia River during the wet season ($p < 0.05$). Additionally, the PDNR in the Tuojia River sediment was significantly higher in the wet season than in the dry season, whereas no significant seasonal difference in the PDNR was found in the Jinjing and Guanjia Rivers. The PDNR pattern aligned with the spatial distribution of the TN, SOM, and DOC contents in the sub-basins of the Jinjing River Basin.

The influence of the physical and chemical properties of the water and sediment on the potential nitrification rate (PNR) and potential denitrification rate (PDNR) of the sediment was analyzed, as shown in Table 3. Pearson correlation analysis indicated that different physicochemical properties had varying effects on the PNR and PDNR in the Jinjing River Basin. For the PNR, there was a significant positive correlation with the dissolved organic carbon (DOC), temperature (T), and NH_4^+-N in the water environment. In contrast, in the sediment environment, the PNR was significantly positively correlated with total nitrogen (TN) and sediment organic matter (SOM) ($p < 0.05$), suggesting that the effects of the physicochemical properties on the PNR varied between the environments.

Table 3. Pearson correlation analysis of PNR and PDNR with environmental variables.

	Environmental Factor	PNR	PDNR
Waterbody	NH_4^+-N	0.733 **	−0.088
	NO_3^--N	−0.153	0.647 **
	TN	−0.216	0.379 *
	TP	−0.047	0.358 *
	DOC	0.362 *	0.287
	T	0.429 *	0.267
	DO	0.294	−0.352 *
	pH	−0.214	−0.136
	Eh	0.157	0.214
Sediment	NH_4^+-N	0.070	0.035
	NO_3^--N	−0.158	0.111
	TN	0.439 *	0.712 **
	SOM	0.605 **	0.792 **
	DOC	0.269	0.029
	pH	−0.213	−0.416 *

Note: * indicates a significant correlation at the 0.05 level; ** indicates a very significant association at the 0.01 level, the same below.

For the PDNR, there was a positive correlation with the TN and total phosphorus (TP) in the water ($p < 0.05$), as well as a significant positive correlation with NO_3^--N ($p < 0.01$) and a negative correlation with dissolved oxygen (DO) ($p < 0.05$). In the sediment environment, the PDNR showed a negative correlation with sediment pH ($p < 0.05$) and positive correlations with TN and SOM ($p < 0.01$).

3.2. Gene Abundance of Microorganisms for Nitrification and Denitrification in Sediment of Small Watershed

Figure 2 shows the total bacterial abundance in the sediment across the different rivers in the basin. In the Tuojia River (farmland), the bacterial counts ranged from 6.7×10^9 to 4.4×10^{10} copies·g^{-1}, with an average of 2.23×10^{10} copies·g^{-1}. In the Jinjing River (residential area), the bacterial abundance ranged from 3.0×10^9 to 3.8×10^{10} copies·g^{-1}, with a mean value of 1.18×10^{10} copies·g^{-1}. The Guanjia River (woodland) showed bacterial counts ranging from 1.5×10^9 to 5.4×10^{10} copies·g^{-1}, with an average of 1.45×10^{10} copies·g^{-1}.

Overall, the total bacterial abundance followed the following trend: Tuojia River (farmland) > Jinjing River (residential area) > Guanjia River (woodland). The bacterial counts in the Tuojia River were significantly higher than those in the Guanjia River, which, in turn, were significantly higher than those in the Jinjing River ($p < 0.05$).

In terms of seasonal variations, the total bacterial abundances in the Tuojia River (farmland) and Guanjia River (woodland) were higher during the wet season compared with the dry season. Conversely, the Jinjing River (residential area) showed significantly higher bacterial counts in the dry season than in the wet season. This seasonal pattern generally followed the following trend: Tuojia River (farmland) > Guanjia River (woodland) > Jinjing River (residential area), with the wet season consistently showing a higher bacterial abundance, except in the Jinjing River.

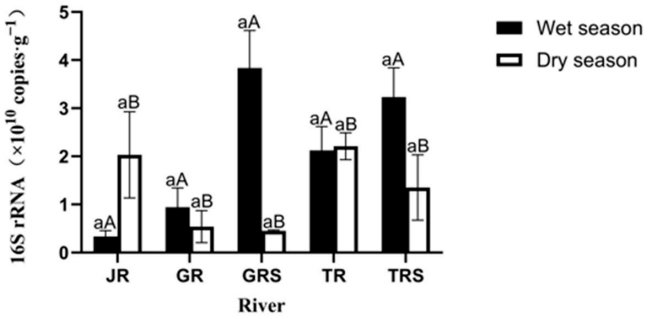

Figure 2. Total bacterial abundances in sediment of Jinjing watershed. Data are represented as means ± SEM (n = 3). Same letters indicate no significant differences ($p > 0.05$). Small letters represent rivers, and capital letters represent seasons. JR = Jinjing River, GR = Guanjia River, GRS = Guanjia sub-stream, TR = Tuojia River, and TRS = Tuojia River sub-stream.

The abundances of AOA and AOB in the sediments across the different regions of the river basin are illustrated in Figure 3. In the Tuojia River (farmland), the AOA and AOB abundances ranged from 1.4×10^6 to 5.9×10^7 copies·g^{-1} and 1.4×10^5 to 1.1×10^7 copies·g^{-1}, respectively, with mean values of 2.4×10^7 and 4.5×10^5 copies·g^{-1}. The AOA abundance in the bottom sediment of the Tuojia River was higher than the AOB abundance during the wet season, while the opposite was observed in the dry season. Both the AOA and AOB abundances in the Tuojia River sediment were higher in the wet period compared with the dry period.

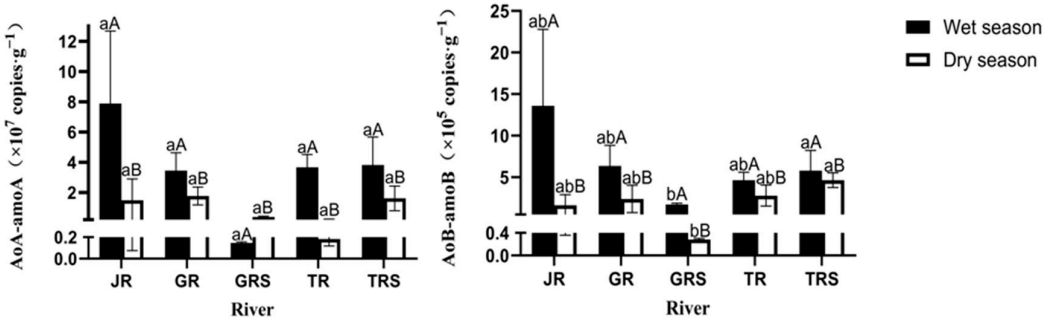

Figure 3. Abundances of AOA (left) and AOB (right) in sediment of Jinjing watershed. Data are represented as means ± SEM (n = 3). Same letters indicate no significant difference ($p > 0.05$). Small letters represent rivers, and capital letters represent seasons. JR = Jinjing River, GR = Guanjia River, GRS = Guanjia sub-stream, TR = Tuojia River, and TRS = Tuojia River sub-stream.

In the Jinjing River (residential area), the AOA and AOB abundances ranged from 4.0×10^5 to 1.7×10^8 copies·g^{-1} and 1.4×10^4 to 3.1×10^6 copies·g^{-1}, respectively, with mean values of 4.7×10^7 and 7.3×10^5 copies·g^{-1}. The AOA abundance was higher than the AOB abundance, and both were significantly higher during the wet season than the dry season.

In Guanjia (woodland), the AOA and AOB abundances ranged from 1.3×10^6 to 5.7×10^7 copies·g^{-1} and 2.5×10^4 to 1.1×10^6 copies·g^{-1}, respectively, with mean values of 1.4×10^7 and 2.7×10^5 copies·g^{-1}. The AOA abundance exceeded the AOB abundance in the dry season, while the opposite trend was observed in the wet season. Overall, the AOA and AOB abundances in Guanjia (woodland) were higher in the wet season than in the dry season.

Across all three rivers, the abundances of AOA and AOB followed the following pattern: Jinjing River (residential area) > Tuojia River (farmland) > Guanjia (woodland). The AOA abundance was generally higher than the AOB abundance, except in the Tuojia River (farmland) at specific times. Overall, the seasonal variations showed that both the AOA and AOB abundances were consistently higher in the wet season compared with the dry season across all regions.

The abundances of the nirS, nirK, and narG genes in the sediments across the study area are illustrated in Figure 4. In Jinjing (residential area), Tuojia (farmland), and Guanjia (woodland), the nirS gene abundance ranged from 3.0×10^7 to 9.4×10^8, 4.0×10^7 to 1.8×10^9, and 2.7×10^7 to 9.9×10^8 copies·g^{-1}, respectively, with average values of 2.1×10^8, 4.2×10^8, and 2.7×10^8 copies·g^{-1}. The NirS gene abundance was highest in Tuojia (farmland), particularly during the wet season, and was significantly higher than in Jinjing (residential area) and Guanjia (woodland) ($p < 0.05$).

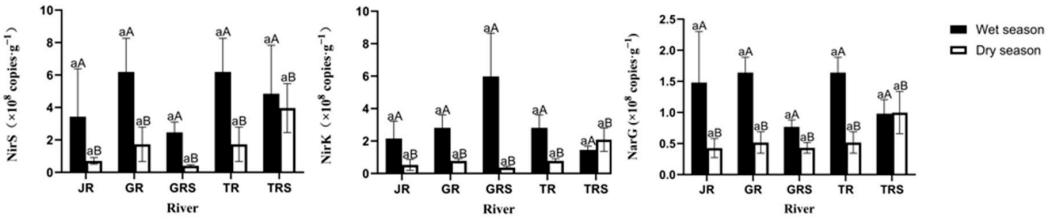

Figure 4. Gene abundances of nirS nirK and narG in sediment of Jinjing watershed. Data are represented as means ± SEM (n = 3). Same letters indicate no significant difference ($p > 0.05$). Small letters represent rivers, and capital letters represent seasons. JR = Jinjing River, GR = Guanjia River, GRS = Guanjia sub-stream, TR = Tuojia River, and TRS = Tuojia River sub-stream.

For the nirK gene, the abundance in Jinjing, Tuojia, and Guanjia ranged from 0.9×10^7 to 4.2×10^8, 0.7×10^7 to 3.8×10^8, and 0.2×10^7 to 1.9×10^8 copies·g^{-1}, respectively, with averages of 1.3×10^8, 1.8×10^8, and 2.5×10^8 copies·g^{-1}. Guanjia had the highest nirK abundance during the wet season, with no significant difference between regions ($p > 0.05$); however, in the dry season, nirK's abundance in Tuojia was significantly higher than in Guanjia and Jinjing ($p > 0.05$).

For the narG gene, the gene abundance ranged from 1.2×10^7 to 3.1×10^8, 5.2×10^7 to 3.1×10^8, and 2.4×10^7 to 1.9×10^8 copies·g^{-1} in Jinjing, Tuojia, and Guanjia, respectively, with averages of 1.0×10^8, 1.0×10^8, and 0.8×10^8 copies·g^{-1}. Jinjing exhibited the highest narG abundance during the wet season, but no significant differences were observed between regions ($p > 0.05$). However, narG's abundance in Tuojia was significantly higher than in Jinjing and Guanjia ($p > 0.05$). Overall, the nirS, nirK, and narG gene abundances were generally higher in the wet season compared with the dry season.

The Pearson correlation analysis of the gene abundances and the physicochemical property of each microorganism is presented in Table 4. The 16S rRNA gene showed a positive correlation with TN, SOM, and PDNR ($p > 0.01$) but was significantly negatively correlated with pH ($p < 0.01$). Notably, 16S rRNA was not significantly correlated with the water's physical and chemical properties ($p < 0.05$), suggesting that sediment characteristics have a greater influence on microorganisms.

For the nitrification genes, AOA was negatively correlated only with NH_4^+-N in the water ($p < 0.05$), while AOB showed positive correlations with NH_4^+-N and temperature ($p < 0.05$) and a negative correlation with DO ($p < 0.05$). The lack of significant correlations between AOA, AOB, and sediment properties ($p < 0.05$) indicates that water properties exert a stronger influence on nitrifying microorganisms.

Regarding the denitrification genes, nirS was positively correlated with sediment TN, SOM, and PDNR ($p < 0.05$). The nirK gene showed positive correlations with sediment PDNR, TN, and temperature ($p < 0.05$). The narG gene was positively correlated with water temperature ($p < 0.01$) but negatively correlated with DO and pH ($p < 0.05$). Overall, nitrification genes were primarily influenced by water properties, while denitrification genes were significantly influenced by both sediment and water properties, with different factors playing key roles in varying environments.

Table 4. Pearson correlation analysis between gene abundance and physicochemical properties.

		16S *rRNA*	AOA	AOB	*nirS*	*nirK*	*narG*
Sediment	NH_4^+-N	−0.071	0.231	0.335	0.062	0.159	0.171
	NO_3^--N	−0.035	−0.057	−0.012	−0.139	−0.078	0.011
	TN	0.632 **	−0.193	−0.179	0.399 *	0.303	0.103
	SOM	0.601 **	−0.140	−0.099	0.394 *	0.339	0.178
	DOC	−0.065	−0.221	−0.275	−0.104	−0.091	−0.345
	pH	−0.623 **	−0.326	−0.205	−0.155	−0.111	−0.178
	PNR	−0.007	−0.052	−0.018	0.161	0.001	0.237
	PDNR	0.581 **	−0.133	−0.053	0.405 *	0.405 *	0.332
Waterbody	NH_4^+-N	0.251	−0.381 *	0.376 *	0.478*	0.347	0.030
	NO_3^--N	0.005	0.039	0.235	0.103	0.173	0.074
	TN	0.250	−0.020	0.090	0.494 **	0.468 *	0.173
	TP	0.322	0.105	0.143	0.250	0.373	0.147
	DOC	0.120	0.064	0.313	0.145	0.285	0.249
	T	0.089	0.326	0.399 *	0.399 *	0.408 *	0.671 **
	DO	−0.160	−0.294	0.412 *	−0.194	−0.240	−0.492 **
	pH	0.095	0.045	−0.056	−0.246	−0.203	−0.455 *

Note: * indicates a significant correlation at the 0.05 level; ** indicates a very significant association at the 0.01 level, the same below.

3.3. Analysis of Influencing Factors

The structural equation model in Figure 5 shows the influence of physical and chemical properties on the potential nitrification rate (PNR) of the sediment. The direct effect of the AOA gene abundance on the PNR was 0.687, with an additional indirect effect of 0.06 via the AOA → WAN → PNR pathway, giving a total impact of 0.693. This indicates that the AOA abundance explained 69.3% of the PNR variation in the sediment. Similarly, the AOB gene abundance had a direct effect of 0.364 and an indirect effect of −0.05 through the AOB → WAN → PNR pathway, resulting in a total effect of 0.359, accounting for 35.9% of the PNR variation.

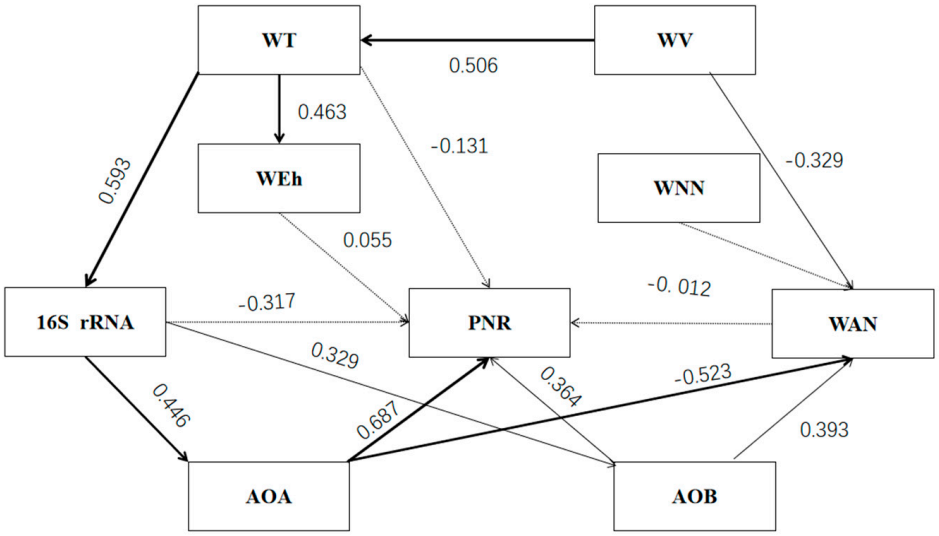

Figure 5. Structural equation model of effects of different environmental factors on nitrification potential of sediment. Dotted lines mean insignificant; solid lines mean significant at 0.05 level; numbers represent path coefficients between variables; and WT, WV, WEh, and WAN and WNN represent water temperature, flow rate, REDOX potential, and NH_4^+-N and NO_3^--N concentrations, respectively.

The direct effect of 16S rRNA on the PNR was −0.137, with indirect effects of 0.306 via the 16S rRNA → AOA → PNR pathway and 0.116 via the 16S rRNA → AOB → PNR pathway, totaling 0.285. This suggests that 16S rRNA explained 28.5% of the PNR variation. Overall, the AOA and AOB gene abundances were the primary drivers of the PNR in the sediment. The path coefficient for the WT → 16S rRNA → AOA pathway was 0.27, indicating that water temperature was a significant physicochemical factor affecting AOA. Additionally, the direct effects of 16S rRNA on AOA and AOB, with path coefficients of 0.446 and 0.319, respectively, highlight the dominant role of AOA genes among nitrification-related genes.

The structural equation model illustrating the impacts of various physical and chemical properties on the sediment denitrification potential (PDNR) is shown in Figure 6. The path coefficient for NO_3^--N's direct effect on the PDNR was 0.715, indicating that NO_3^--N accounted for 71.5% of the variation in the PDNR, highlighting it as the primary factor influencing denitrification. The direct effect of sediment organic matter (SOM) on the PDNR was 0.200, with an additional indirect effect of 0.007 via the SOM → 16S rRNA → PDNR pathway, yielding a total impact of 0.207, or 20.7%, of the PDNR variation explained by SOM.

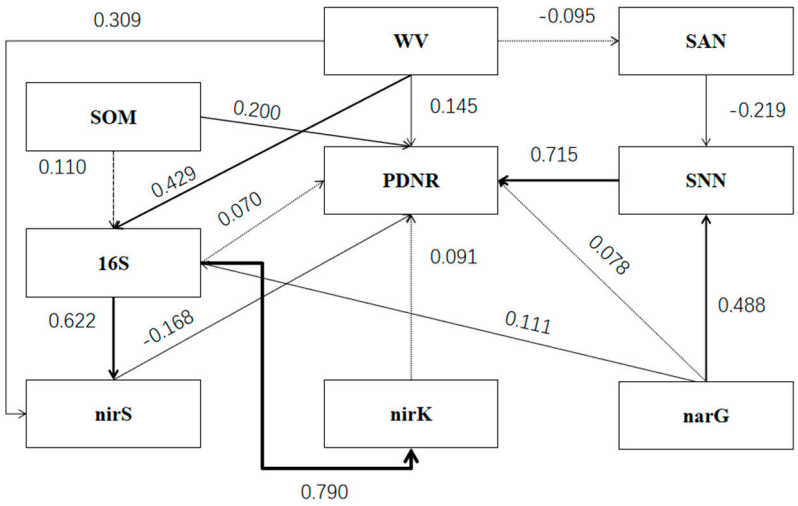

Figure 6. Structural equation model of effects of different environmental factors on denitrification potential of sediment. Dotted lines mean insignificant; solid lines mean significant at level of 0.05; numbers represent path coefficients between variables; and WV, SOM, and SAN and SNN represent water velocity, organic matter content in sediment, and NH_4^+-N and NO_3^--N concentrations in sediment, respectively.

For functional genes, the nirS gene had a negative effect on the PDNR, with a path coefficient of −0.168, explaining 16.8% of the PDNR variation, while nirK contributed 0.091, or 9.1%, of the variation. The narG gene had a direct effect of 0.078 and an indirect effect of 0.349 through the narG → SNN → PDNR pathway, with a total impact of 0.427, accounting for 42.7% of the PDNR variability. These results underscore that the NO_3^--N content in sediment was the most significant factor affecting the PDNR, followed by the narG gene abundance.

4. Discussion

This study revealed significant spatial and temporal variations in the potential nitrification (PNR) and denitrification (PDNR) across the Jinjing River Basin. The PNR in the basin ranged from 0.27 to 39.71 $\mu g \cdot kg^{-1} \cdot h^{-1}$, averaging at 5.0 $\mu g \cdot kg^{-1} \cdot h^{-1}$, which is lower than in other river systems [19–21]. Nitrification in sediment is often limited by dissolved oxygen (DO), with farmland areas like the Tuojia sub-basin exhibiting the highest PNR due to increased DO from dry–wet cycles, which boosts nitrifying microbial activity [22].

Temporal analysis showed that the PNR was generally higher in the wet season in farmland areas, aligning with studies showing that alternating wet and dry conditions can stimulate nitrification ($p < 0.05$).

The PDNR values, ranging from 0.12 to 3.25 $mg \cdot kg^{-1} \cdot h^{-1}$, with an average of 0.93 $mg \cdot kg^{-1} \cdot h^{-1}$, were highest in the Tuojia River sub-basin during the wet season. These results, comparable to findings in the Huaihe River [23] and other regions [24,25], indicate a typical range for agricultural sediments. Seasonal increases in the PDNR are likely influenced by the anaerobic conditions necessary for denitrification, with higher NO_3^--N and SOM levels providing essential substrates for microbial activity [26]. In a study on Taihu Lake, it was found that with a decrease in the nitrate-nitrogen content, the denitrification rate also decreased [27].

The microbial nitrification characteristics showed a predominance of ammonia-oxidizing archaea (AOA) over ammonia-oxidizing bacteria (AOB) in the basin, especially in lower-NH_4^+-N conditions, indicating that AOA are better suited to these environments [28,29]. The observed higher abundance of AOA, particularly in low-nutrient environments, is likely due to their metabolic advantages, including their ability to thrive at lower ammonia concentrations and their greater efficiency in energy production in nutrient-limited conditions. The ammonia-oxidizing archaea and AOB gene abundances were generally higher in residential areas, suggesting greater microbial adaptation to pollution [30]. Seasonal variations in gene abundances were evident, with both AOA and AOB abundances being higher in the wet season, possibly due to increased surface runoff, which brings additional nitrogen and phosphorus into the rivers [31]. The positive correlation of AOB abundance with NH_4^+-N and DO supports their aerobic adaptation, while AOA abundance was more pronounced in low-ammonium settings, consistent with previous findings [32,33].

The denitrification gene analysis highlighted that the nirS gene abundance exceeded that of nirK and narG across most sites, particularly in farmland areas. This suggests nirS's dominance in denitrification, as observed in similar environments [34–36]. Conversely, studies in estuarine and wetland sediments have identified nirK or narG as dominant genes, indicating ecosystem-dependent variability [37,38]. In the Jinjing Basin, the higher denitrification gene abundance in the wet season suggests enhanced microbial growth due to fluctuating water levels, facilitating denitrifying bacteria [39].

The main factors influencing the PNR and PDNR were NH_4^+-N, temperature, TN, and SOM. Specifically, NH_4^+-N significantly affected AOB, aligning with findings that AOB activity increases with higher ammonium concentrations [28]. In agricultural zones, nitrogen fertilization practices often result in elevated ammonium concentrations, which could stimulate AOB activity, making these regions hotspots for nitrification. Therefore, land-use management practices that reduce nutrient runoff, such as the implementation of buffer zones or controlled fertilization schedules, could significantly mitigate excessive nitrification and reduce the downstream impact on water quality. Structural equation modeling revealed that AOA and AOB accounted for 69.3% and 35.9% of the PNR variability, respectively, underscoring their roles in sediment nitrification [40]. For denitrification, NO_3^--N was the primary factor influencing the PDNR, explaining 71.5% of its variability, while narG's abundance was also strongly correlated, as seen in other nitrogen-rich systems [41,42]. This suggests that nitrate availability is a key determinant for denitrification processes in agricultural systems, where excess nitrogen from fertilizers can stimulate denitrification, potentially leading to the loss of nitrogen to the atmosphere as N_2O or N_2.

This study demonstrates that both land use and seasonal changes influence nitrification and denitrification dynamics, with nitrogen availability, oxygen levels, and microbial abundance playing important roles. These findings have significant implications for watershed management, particularly in agricultural zones. Understanding the dynamics of nitrification and denitrification allows land-use practices to be tailored to reduce nutrient runoff, optimize fertilizer use, and mitigate nitrogen pollution, thereby improving water quality and maintaining the ecological balance of river basins.

5. Conclusions

This study revealed notable spatial and seasonal variations in nitrification and denitrification across three sub-basins in the Jinjing River Basin, with farmland areas showing the highest activity. Potential nitrification (PNR) and denitrification (PDNR) rates were consistently higher in the wet season, likely due to increased microbial activity from temperature and nutrient influx. Ammonia-oxidizing archaea (AOA) were more abundant than bacteria (AOB) across all sites, and nitrate-nitrogen (NO_3^--N) emerged as the dominant factor influencing the PDNR. These findings highlight the influences of environmental conditions and nutrient levels on nitrogen cycling, emphasizing the need for targeted nitrogen management in agricultural watersheds.

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