Distribution Characteristics of Denitrifying Bacteria in the Rhizosphere of Wetland Plants in Urban Rivers of the Karst Region in Southwest China

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Abstract

The discharge of nitrogen-rich wastewater into urban rivers often leads to water eutrophication, and the construction of river wetlands is a crucial measure to mitigate this issue. Microorganisms play a significant role in the nitrogen removal processes within river ecosystems, particularly in the rhizosphere of plants where microbial activity is intense. This study investigates the distribution characteristics of denitrifying microbial communities in the rhizosphere sediments of wetland plants in the Xiaoche River urban wetland in Guiyang. High-throughput sequencing was employed to analyze the bacterial diversity in the rhizosphere and non-rhizosphere sediments of three typical wetland plants (*Acorus calamus*, *Cyperus alternifolius*, and *Echinochloa crus-galli*). Additionally, the abundance of denitrifying and anaerobic ammonium-oxidizing (anammox) bacteria in the sediments was determined using real-time quantitative PCR. Sequencing results indicated that there are 16 bacterial phyla with a relative abundance greater than 1% in both rhizosphere and non-rhizosphere sediments, with *Proteobacteria*, *Bacteroidete*s, and *Acidobacteria* being the dominant phyla, collectively accounting for over 50% of the relative abundance. The relative abundance of *Proteobacteria* was higher in the rhizosphere than in the non-rhizosphere, while *Bacteroidete*s showed higher relative abundance in the non-rhizosphere compared to the rhizosphere. There were 24 bacterial genera with relative abundance greater than 1%, and the dominant genera varied significantly among different sampling sites. Cluster analysis revealed significant differences in genus-level populations between rhizosphere and non-rhizosphere samples, with high similarity between the populations of *Acorus calamus* and *Cyperus alternifolius*. Quantitative gene results indicated that the abundance of denitrification and anammox genes was lower in the non-rhizosphere sediments than in the rhizospheres of the three plants, with anammox 16S rRNA and *nirS* gene abundance levels reaching 10^{10} copies/g dry sediment, suggesting a high richness of anammox and denitrifying bacteria in the rhizospheres of wetland plants in the Xiaoche River. Redundancy analysis (RDA) showed that the environmental factors most influencing the abundance of these two genes were total phosphorus (TP), organic matter (OM), and ammonium nitrogen (NH_4^+-N).

1 Introduction

Urban river wetlands are an integral part of ecosystems, maintaining the ecological balance of cities and improving the quality of the urban environment through their unique hydrological characteristics (McJannet et al. 2012; Yang et al. 2019). Aquatic plants are one of the foundational components of urban river wetlands; they are considered the primary producers of the entire wetland ecosystem as they generate organic matter through photosynthesis, which provides food and energy for other organisms (Deegan and Ganf, 2008; Zhao et al. 2022). Some wetland plants also play a role in purifying water quality. Species such as reeds, cattails, water hyacinths, water lilies, irises, and canna are recognized for their strong water purification capabilities and are widely used in urban sewage treatment and ecological restoration projects (Wu et al. 2013; Rai et al. 2015; Lu et al. 2018).

With the acceleration of urbanization, urban river wetlands face pollution pressures from various sources such as domestic sewage and industrial wastewater (Sarkar et al. 2021; Zhang et al. 2021b). Of particular concern is the increased nitrogen load, as nitrogen is one of the key limiting nutrients causing eutrophication in lakes (Baldantoni and Alfani 2016). Currently, the removal of nitrogen in natural environments is mainly dependent on the action of denitrifying microorganisms. The rhizosphere of wetland plants is considered an ideal environment for denitrifying microbes. Plant roots secrete organic matter, providing a rich source of carbon and energy, which promotes the growth and metabolic activity of denitrifying bacteria (Boerema et al. 2014; Wang et al. 2017; Xu et al. 2021). Denitrification and anaerobic ammonium oxidation (anammox) are the main biological nitrogen removal processes in water bodies, driven respectively by denitrifying and anammox bacteria (Thamdrup and Dalsgaard 2002; Wenk et al. 2013). The *nirS* and anammox 16S rRNA genes serve as marker genes for denitrification and anammox, respectively,

and are commonly used to determine the diversity and abundance of denitrifying and anammox bacteria (Yu et al. 2021; Zhang et al. 2021b). Wetland plants in urban river wetlands, such as reeds, water hyacinth, and water lilies, can effectively reduce the nitrogen load in water bodies through the action of rhizosphere denitrifying microorganisms, which is of significant environmental importance for enclosed or semi-enclosed water bodies like urban river wetlands (Yin et al. 2018). Studies have found that the community structure and function of rhizosphere denitrifying microorganisms vary with different types of wetland plants; the plant species directly affect the composition and structure of the rhizosphere microbial community, thereby affecting denitrification capacity (Zhao et al. 2013; Yin et al. 2018). For example, Zhao et al. (2019a) found in a freshwater lake during the summer that the diversity of anammox bacteria in the rhizosphere of submerged plants was low, and plant species differences had the greatest impact on the abundance of anammox bacteria compared to other nitrogen-cycling microbes. In addition to plant species, rhizosphere environmental factors such as pH, dissolved oxygen concentration, and root exudates also affect the activity and community structure of rhizosphere denitrifying microorganisms. For instance, Li et al. (2023b) investigated the relationship between emergent plant root exudates and rhizosphere nitrogen-cycling microorganisms and found that organic acids and amino acids were positively correlated with the abundance of rhizosphere denitrifying and anammox bacteria. Dissolved oxygen is an important factor affecting the denitrification process in the plant rhizosphere; both denitrifying and anammox bacteria have low oxygen requirements, and low-oxygen environments are more conducive to denitrification (Gruber and Galloway 2008). Therefore, studying the ecological characteristics of rhizosphere denitrifying microorganisms of wetland plants and optimizing the vegetation structure of urban river wetlands to improve the efficiency of nitrogen removal is an important approach to improving the quality of urban water environments and enhancing the ecosystem services of urban river wetlands.

Karst regions account for approximately 12% of the global terrestrial area, and the southwest region of China is the largest karst landscape area in the world (Liao et al. 2018). Karst topography is characterized by carbonate rocks, replete with numerous caves, fissures, and conduits, through which surface water can easily seep into the ground, forming underground river systems and resulting in relatively scarce surface water resources (Liu et al. 2020; Zhou et al. 2023). The soil in these regions is high in calcium content but low in nutrients, which is unfavorable for vegetation growth. The ecosystems surrounding lakes often have low species diversity and primary productivity, as well as poor resistance to disturbances (Qiao et al. 2021). The southwest region of China also belongs to the plateau, where the ecosystems of plateau rivers are relatively simple due to climatic constraints, with shorter food chains. This means that every component of the ecosystem plays a key role, and any change in a species could have a significant impact on the entire ecosystem. Once the river waters are polluted, their self-purification capacity is weak, and recovery is quite challenging (Liu et al. 2016; Chen et al. 2020).

To date, the niche differentiation and community assembly mechanisms of denitrifying microbes in the complex and variable environmental conditions of plateau river wetlands in karst areas have not been well studied. Guiyang, one of the largest cities in the karst region of southwest China, is currently undergoing a rapid urbanization process with complex hydrogeological conditions. This study takes the Xiaoche River urban river wetland in Guiyang as the research object to carry out an investigation of denitrifying bacteria in the rhizosphere of wetland plants. The research aims to fully understand the ecological mechanisms of rhizosphere denitrifying microorganisms of wetland plants, providing a scientific basis for the management and restoration of urban wetland ecosystems and promoting sustainable urban development.

2 Materials and Methods

2.1 Sample Collection

The Xiaoche River wetland is an important tributary of the Aha Lake reservoir, a significant water conservation area in Guiyang, Guizhou Province, China, which harbors a rich variety of aquatic plants. The climate in Guiyang is mainly influenced by the southeast monsoon, with an average annual precipitation of 1109 mm, over 80% of which is concentrated between May and October (Chen et al. 2015). Based on a preliminary survey of wetland plants in the Xiaoche River basin, three typical plants were selected for the study: *Acorus calamus*, *Cyperus alternifolius*, and *Echinochloa crus-galli*. In October 2019, rhizosphere sediments of these three wetland plants were collected. The plants, along with the sediment, were gently shaken to collect the rhizosphere sediment that fell off (Zhao et al. 2013), with sediment from areas of the wetland without plants serving as the non-rhizosphere control. Two sampling sites were selected for each plant species, and two for the non-rhizosphere areas as well, totaling eight sampling sites. At each sampling site, three replicate sediment samples were taken. The eight sampling sites were marked as follows: AC-A and AC-B for the two *Acorus calamus* sampling sites; CA-A and CA-B for the two *Cyperus alternifolius* sampling sites; EC-A and EC-B for the two *Echinochloa crus-galli* sampling sites; NR-A and NR-B for the two non-rhizosphere sampling sites. The collected sediment samples were placed in sterile bags, preserved in an icebox, and quickly transported back to the laboratory. Part of the sediment was stored at 4°C for the determination of basic physicochemical indicators, and the remaining sediment was stored at -80°C in an ultra-low temperature freezer for DNA extraction.

2.2 Physicochemical Property Analysis

In situ measurements of overlying water quality parameters, such as dissolved oxygen (DO), temperature (T), pH, and oxidation-reduction potential (ORP), were conducted using a portable water quality analyzer (HACH, USA). Upon returning to the laboratory, wet sediment samples were centrifuged at 4000 rpm for 10 min to collect the supernatant, which was then filtered through a 0.45 μm membrane to obtain interstitial water. Concentrations of NO_3^--N, NO_2^--N, and NH_4^+-N in the acquired interstitial water were determined using an AA3 Continuous Flow Analyzer (SEAL Analytical, Germany). The organic matter (OM), total nitrogen (TN), and total phosphorus (TP) content of the sediments were measured following the methods described by Yin et al. (2019).

2.3 Sediment Sample DNA Extraction and High-Throughput Sequencing

DNA from sediment samples was extracted following the manufacturer's instructions using the Fast DNA Spin Kit for Soil (MP bio, USA). The concentration of the extracted DNA was determined using a Nanodrop 2000 (Thermo Fisher Scientific, USA), and its quality was assessed by 1.2% agarose gel electrophoresis. DNA that passed the quality check was used for subsequent molecular experiments. Primers 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') were employed to amplify the 16S rDNA gene (Zhang et al. 2021a). The PCR amplification system included 1 μL dNTP (2.5 mmol/L), 10 μL of each forward and reverse primer (5 μmol/L), 10 μL 5×FastPfu buffer, 60 ng template DNA, 10 μL BSA, and 0.2 μL FastPfu polymerase, with ddH_2O added to bring the reaction volume to 50 μL. The amplification conditions were as follows: initial denaturation at 95°C for 5 min, followed by 15 cycles of denaturation at 95°C for 1 minute, annealing at 50°C for 1 minute, and extension at 72°C for 1 minute, with a final extension at 72°C for 7 min. PCR products were checked on a 2% agarose gel and purified using a purification kit. High-throughput sequencing was then conducted on the target products using an Illumina MiSeq Benchtop Sequencer (Illumina, USA).

To obtain high-quality sequencing data and improve the accuracy of subsequent bioinformatics analysis, low-quality bases at the ends with a quality score below 20 were first removed using TrimGalore software (Hiraoka et al. 2020). Next, paired-end sequences obtained from double-ended sequencing were merged using FLASH, and low-quality sequences after merging (where over 90% of bases had a quality score below 20) were removed (Shu et al. 2016).

Primer sequences were then identified and eliminated from the sequences using Mothur software (Zhou et al. 2020). Finally, sequences were clustered into operational taxonomic units (OTUs) at 97% similarity using USEARCH software (Yu et al. 2020). Species annotation for each sample was obtained by comparing with the Silva 16S rRNA database, as provided by the Ribosomal Database Project (RDP, Release 11.1 http://rdp.cme.msu.edu/).

2.4 Real-Time Quantitative PCR (qPCR)

Quantitative analysis of *nirS* and anammox 16S rRNA genes was conducted using the QuantStudio™ 5 Real-Time PCR System (ABI, USA). The primer sequences used for *nirS* in this study were *nirS*Cd3Af (5'-AACGYSAAGGARACSGG-3') and *nirS*R3cd (5'-GASTTCGGRTGSGTCTTSAYGAA-3') (Throbäck et al. 2004), and for anammox 16S rRNA, they were AMX809F (5'-GCCGTAAACGATGGGCACT-3') and AMX1066R (5'-AACGTCTCACGACACGAGCTG-3') (Tsushima et al. 2007). Primers were mixed with SYBR® Select Master Mix (2X) (ABI, USA) to prepare a 30 μL reaction system. The amplification system included 15 μL 2xTaqmix, 1 μL of each forward and reverse primer (10 pmol/μL), 1 μL template DNA, and 12 μL ddH_2O. The amplification program consisted of an initial denaturation step at 95°C for 15 min, followed by 40 cycles of denaturation at 95°C for 10 seconds, annealing at 56°C for *nirS* gene/59°C for anammox 16S rRNA gene for 30 seconds, and extension at 72°C for 30 seconds. Standard curves for both genes were generated using tenfold serial dilutions of known copy number plasmid DNAs. Each qPCR run included negative controls, with standard curves and all samples tested in triplicate, and the melting curves showed a single peak. The correlation coefficients (R^2) for the standard curves of the *nirS* gene and the anammox 16S rRNA gene were 0.992 and 0.994, respectively, with amplification efficiencies of 90% and 98%.

2.5 Statistical Analysis

IBM SPSS software version 20.0 (SPSS Inc., USA) was employed to conduct one-way analysis of variance (ANOVA) and Tukey's post-hoc tests, with significance evaluated by calculating *P*-values and a threshold of $P < 0.05$ indicating significant differences. Graphs were generated using Origin 9.0 software (Origin Lab Corporation, USA). Redundancy Analysis (RDA) was performed using the R software (R Core Team) to reveal correlations between gene abundance and environmental factors (Hu et al. 2020, Zhang et al. 2021b).

3 Results

3.1 Physicochemical Parameters of Sediments and Overlying Water

Table 1 displays the environmental parameters of the wetland plant rhizosphere and non-rhizosphere. The lowest contents of organic matter (OM) and total nitrogen (TN) were found in non-rhizosphere sediments, with average values of 46.95 $g \cdot kg^{-1}$ and 0.052 $g \cdot kg^{-1}$, respectively, whereas total phosphorus (TP) had the highest content, averaging 0.068 $g \cdot kg^{-1}$. *Acorus calamus* rhizosphere sediments had the highest OM and TN contents, averaging 87.12 $g \cdot kg^{-1}$ and 0.364 $g \cdot kg^{-1}$, respectively, while the lowest TP content was observed in the rhizosphere of *Echinochloa crus-galli*, averaging only 0.016 $g \cdot kg^{-1}$. The lowest concentrations of NH_4^+-N and NO_2^--N in interstitial water were found in non-rhizosphere areas, with averages of 0.065 mg L^{-1} and 0.002 mg L^{-1}, respectively. Conversely, the highest levels of NH_4^+-N and NO_2^--N in interstitial water were detected in the rhizosphere of *Cyperus alternifolius*, with averages of 0.132 $mg \cdot L^{-1}$ and 0.130 mg L^{-1}. The highest NO_3^--N concentration was found in the rhizosphere of *Echinochloa crus-galli*, with an average of 0.882 $mg \cdot L^{-1}$, whereas the lowest was in the rhizosphere of *Acorus calamus*, with an average of only 0.027 $mg \cdot L^{-1}$. The highest values of ORP, pH, DO, and T in the overlying water were recorded at the *Acorus calamus* sampling site, with averages of 126.06 mV, 7.85, 6.6 $mg \cdot L^{-1}$, and 7.2°C,

respectively. The lowest values of ORP, pH, DO, and T in the overlying water were observed at the *Cyperus alternifolius* AC-B site, with ORP even showing a negative value of -38.13 ± 7.44 mV, indicating reductive conditions in the overlying water.

3.2 Microbial Diversity Characteristics in Rhizosphere Sediments of Wetland Plants

Microorganisms in the rhizosphere and non-rhizosphere sediments of the Xiaoche River belonged to 39 phyla, among which 16 had a relative abundance > 1% (Fig. 1). The dominant bacterial phyla across the 8 sampling sites showed certain similarities, with *Proteobacteria*, *Bacteroidete*s, and *Acidobacteria* being the top three phyla, collectively accounting for approximately 50% of the total abundance. There were still variations in the relative abundance of bacterial phyla among the sampling sites. *Proteobacteria* had the highest relative abundance at all 8 sampling sites, with a lower abundance in non-rhizosphere sediments compared to the rhizosphere. *Bacteroidete*s ranked second in relative abundance but were more abundant in the non-rhizosphere than in the rhizosphere, showing a trend opposite to that of *Proteobacteria*.

Microorganisms in the rhizosphere and non-rhizosphere sediments belonged to 335 genera, with 24 genera having a relative abundance > 1% (Fig. 2). The dominant bacterial genus in the AC-A and AC-B sediment samples was Subdivision3 genera incertae sedis, with relative abundances of 4.40% and 3.26%, respectively. The most dominant genus in CA-A was Flavobacterium, with a relative abundance of 2.54%, while in CA-B, the dominant genus was Bacillariophyta, with a relative abundance of 2.47%. In EC-A, the most dominant genus was Subdivision3_genera_incertae_sedis, with a relative abundance of 3.36%, and in EC-B, the dominant genus was Bacillariophyta, with a relative abundance of 3.12%. For both NR-A and NR-B, the most dominant genus was Bacillariophyta, with relative abundances of 11.11% and 6.40%, respectively. Based on hierarchical clustering results, the microbial community structures at the genus level were more similar between *Acorus calamus* and *Cyperus alternifolius*, with greater differences observed between non-rhizosphere and the other three types of plants.

3.3 Abundance of Anaerobic Ammonium-Oxidizing Bacteria and Denitrifying Bacteria in the Rhizosphere Sediments of Wetland Plants

Quantitative results showed that the abundance of anammox 16S rRNA and *nirS* genes in the non-rhizosphere was lower than in the rhizospheres of the three types of plants (Fig. 3). The average abundance of the anammox 16S rRNA gene in the rhizosphere and non-rhizosphere sediments of *Acorus calamus*, *Cyperus alternifolius*, and *Echinochloa crus-galli* was 8.81×10^{10}, 9.66×10^{10}, 1.14×10^{11}, and 2.94×10^{10} copies/g dry sediment, respectively, while the *nirS* gene averaged 1.32×10^{11}, 9.50×10^{10}, 8.13×10^{10}, and 3.64×10^{10} copies/g dry sediment. Among the three plants, the highest abundance of anammox 16S rRNA genes was found in the rhizosphere of *Acorus calamus*, and the lowest in *Echinochloa crus-galli*, while the abundance trend of *nirS* genes was opposite, with the highest in *Echinochloa crus-galli* and the lowest in *Acorus calamus*. Overall, the abundance of anammox 16S rRNA and *nirS* genes was very high in this study, reaching the order of 10^{10} copies/g, indicating a high richness of anaerobic ammonium-oxidizing bacteria and denitrifying bacteria in the rhizosphere of wetland plants in the Xiaoche River. The results of significance testing showed that the difference in the abundance of the two denitrification genes between the rhizosphere and non-rhizosphere was significant ($P < 0.05$). The abundance of denitrification genes at the *Echinochloa crus-galli* EC-B site was significantly higher than that of the other plant rhizospheres ($P < 0.05$), with no significant difference in the abundance of denitrification genes between the remaining plant rhizospheres. The abundance of anaerobic ammonium-oxidizing genes in the rhizosphere of *Acorus calamus* was significantly higher than that of *Cyperus*

alternifolius and *Echinochloa crus-galli* ($P < 0.05$), but there was no significant difference between *Cyperus alternifolius* and *Echinochloa crus-galli*.

3.4 Relationship between the Abundance of Anaerobic Ammonium-Oxidizing Bacteria and Denitrifying Bacteria Genes and Physicochemical Factors in the Rhizosphere Sediments of Wetland Plants

According to RDA and Mantel tests, the environmental factors that significantly affect the abundance of *nirS* and anammox 16S rRNA genes were TP (r = 0.9076, $P < 0.01$), NH_4^+-N (r = 0.0446, $P < 0.05$), and OM (r = 0.5937, $P < 0.05$) (Fig. 4). The correlation results indicated that TP was negatively correlated with anammox 16S rRNA gene abundance (r=-0.959, $P < 0.01$), OM was positively correlated with the abundance of *nirS* genes (r = 0.85, $P < 0.01$), NH_4^+-N was positively correlated with the abundance of *nirS* genes (r = 0.799, $P < 0.05$), and TN was also significantly positively correlated with the abundance of *nirS* genes (r = 0.774, $P < 0.05$).

4 Discussion

In this study, the phyla *Proteobacteria*, *Bacteroidete*s, and *Acidobacteria* were overwhelmingly dominant in the Xiaoche River wetland, collectively accounting for over 50% of the relative abundance. This dominance has also been found in other environments such as the overlying water of high-altitude rivers (Zhang et al. 2020), the Qingshan Lake drinking water protection area (Zhao et al. 2019b), urban river sediments (Zhang et al. 2021b), and the Yangtze River estuary sediments (Zheng et al. 2020). *Proteobacteria* displayed a lower relative abundance in the non-rhizosphere compared to the plant rhizosphere, while *Bacteroidete*s were found to be more abundant in the non-rhizosphere. This could be attributed to differences in nutrient content within the rhizosphere, as our study results also indicated lower organic matter (OM) content in the rhizosphere. *Proteobacteria* have higher organic matter requirements, whereas *Bacteroidete*s require lower amounts of organic matter (Wu et al. 2022; Li et al. 2023a).

At the genus level, Bacillariophyta, Subdivision3 genera incertae sedis, and Flavobacterium were dominant in the Xiaoche River wetland. Bacillariophyta is a common algal genus found in drinking water sources (Zhong et al. 2022), and Flavobacterium plays an important role in dissimilatory nitrate reduction processes (Smith Garrett et al. 2018). Bacillariophyta has been found as a dominant genus in farmland soils (Li et al. 2022), Flavobacterium in the Haihe Estuary sediments of Bohai Bay (Li et al. 2019), the soils of the Tibetan Plateau (Long et al. 2017), and freshwater lakes (Wan et al. 2019), suggesting that the microbial genera identified in this study are widespread in various environments.

The abundance of *nirS* and anammox 16S rRNA genes measured in this experiment reached levels of 10^{10}-10^{11}, which indicates a relatively high abundance. The lower quantities of anaerobic ammonium-oxidizing bacteria and denitrifying bacteria in the non-rhizosphere compared to the wetland plant rhizosphere might be due to the secretion of organic acids, amino acids, and other nutrients by plant rhizosphere, which are beneficial for microbial growth (Bais et al. 2004; Haichar et al. 2014). Previous studies have similarly found higher levels of anaerobic ammonium-oxidizing bacteria in planted areas compared to unplanted areas (Li et al. 2011; Wang et al. 2012). The variation in anaerobic ammonium-oxidizing bacteria in the rhizosphere followed the pattern *Acorus calamus* > *Cyperus alternifolius* > *Echinochloa crus-galli*, while the trend for denitrifying bacteria showed the opposite pattern, with *Echinochloa crus-galli* > *Cyperus alternifolius* > *Acorus calamus*, which might be due to a competitive relationship between the two types of bacteria.

The functional role and community characteristics of denitrifying microorganisms in sediments are often closely related to their living environments. In this study, redundancy analysis (RDA) revealed that total phosphorus (TP) was significantly negatively correlated with the abundance of the anammox 16S rRNA gene ($P < 0.01$), indicating that

phosphorus may inhibit the growth of anammox bacteria. Li et al. (2018) also found a negative correlation between anammox 16S rRNA gene abundance and TP in constructed wetlands. Organic matter (OM) was positively correlated with the abundance of *nirS* genes ($P < 0.05$), but showed no significant correlation with the abundance of anammox genes. This is because most denitrifying bacteria are heterotrophic, relying heavily on organic matter, whereas anammox bacteria are autotrophic, with a low dependence on organic matter. High organic content may even inhibit the anammox process (Damashek and Francis 2018; Shen et al. 2022; Chen et al. 2023). The positive correlation between total nitrogen (TN) and *nirS* gene abundance has been confirmed in multiple environments. Zhang et al. (2021c) found a positive correlation between TN content and denitrification gene abundance (nirK, *nirS*, and nosZ) in river sediments, which significantly affected the abundance of all three genes, with *nirS* being the most affected. In this study, the concentration of ammonium nitrogen (NH_4^+-N) was positively correlated with the abundance of *nirS* genes ($P < 0.05$), a finding also observed by Yin et al. (2019) in the pore water of sediments in shallow lakes. Denitrification typically depends on nitrification to convert NH_4^+-N to NO_2^--N (Prosser 1990), and when NH_4^+-N concentrations are high, it is conducive to nitrification, which in turn promotes the $NO_2^- \rightarrow NO$ denitrification process involving the *nirS* gene, thus revealing the positive correlation between NH_4^+-N concentration and *nirS* gene abundance. Notably, in this study, NO_2^--N did not show a significant correlation with denitrification genes, which might be due to competition for the substrate NO_2^- between anammox and denitrification processes, resulting in no significant correlation between NO_2^- and either of the denitrification processes (Wang et al. 2017).

5 Conclusion

The high abundance of anaerobic ammonium-oxidizing and denitrifying bacteria in the rhizospheres of the three wetland plants in this study indicates that denitrification is a strong process in the Xiaoche River wetland. Denitrifying microorganisms exhibit different distribution characteristics in the rhizosphere and non-rhizosphere sediments of wetland plants, with significantly higher levels in the rhizosphere ($P < 0.01$). Hence, it is inferred that wetland plants may be an important factor affecting the denitrification process in sediments, and the restoration and reconstruction of urban river wetlands should fully consider the role of rhizosphere denitrifying microorganisms. The denitrifying bacteria associated with the rhizospheres of the three plants in this study did not show large differences; future research could focus on enhancing plant selection to effectively improve the denitrification efficiency of urban river wetlands by choosing suitable wetland plants for cultivation, thereby achieving the goals of purifying water bodies and restoring ecosystem health.

Declarations

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Ethical Approval The research was done according to ethical standards.

Author Contributions All authors contributed to the study conception and design. The field work was performed by Yi Li and Liangzhu Yao. Data analysis and interpretation were performed by Yi Li. The work was supervised by Xingjia

Yin. The first draft of the manuscript was written by Yi Li and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Tables

Table 1
Basic Physicochemical Properties of Wetland Plant Rhizosphere and Non-Rhizosphere Sediments, Interstitial Water, and Overlying Water

Sampling Point	Sediment			Interstitial Water			Overlying Water			
	OM (g kg^{-1})	TP (g kg^{-1})	TN (g kg^{-1})	NH_4^+-N (mg L^{-1})	NO_3^--N (mg L^{-1})	NO_2^--N (mg L^{-1})	ORP (mv)	pH	DO (mg L^{-1})	T (ºC)
AC-A	81.15 ± 3.21	0.039 ± 0.001	0.451 ± 0.030	0.141 ± 0.032	0.030 ± 0.008	0.005 ± 0.002	51.13 ± 4.05	7.25 ± 0.04	4.33 ± 0.09	7.17 ± 0.06
AC-B	93.08 ± 2.52	0.022 ± 0.002	0.276 ± 0.028	0.121 ± 0.024	0.024 ± 0.012	0.004 ± 0.002	-38.13 ± 7.44	6.70 ± 0.03	0.26 ± 0.04	6.20 ± 0.00
CA-A	87.14 ± 1.85	0.031 ± 0.009	0.311 ± 0.087	0.135 ± 0.026	0.504 ± 0.096	0.095 ± 0.022	91.05 ± 3.21	7.95 ± 0.03	5.80 ± 0.32	7.27 ± 0.06
CA-B	77.14 ± 1.23	0.025 ± 0.003	0.211 ± 0.044	0.129 ± 0.036	0.844 ± 0.110	0.165 ± 0.041	161.07 ± 5.21	7.75 ± 0.04	7.40 ± 0.41	7.13 ± 0.06
EC-A	71.75 ± 5.96	0.013 ± 0.004	0.206 ± 0.032	0.122 ± 0.023	0.807 ± 0.240	0.132 ± 0.008	43.47 ± 9.23	7.03 ± 0.02	5.25 ± 0.25	7.23 ± 0.00
EC-B	61.75 ± 2.04	0.018 ± 0.007	0.058 ± 0.016	0.054 ± 0.026	0.956 ± 0.140	0.022 ± 0.005	60.47 ± 7.21	7.39 ± 0.02	4.14 ± 0.53	6.60 ± 0.00
NR-A	50.45 ± 3.72	0.078 ± 0.022	0.045 ± 0.013	0.060 ± 0.027	0.599 ± 0.062	0.002 ± 0.001	100.50 ± 9.41	7.75 ± 0.02	5.73 ± 0.35	6.80 ± 0.12
NR-B	43.45 ± 1.51	0.058 ± 0.013	0.059 ± 0.026	0.070 ± 0.019	0.246 ± 0.052	0.001 ± 0.000	90.60 ± 8.58	7.72 ± 0.01	6.94 ± 0.05	7.00 ± 0.00

Figures

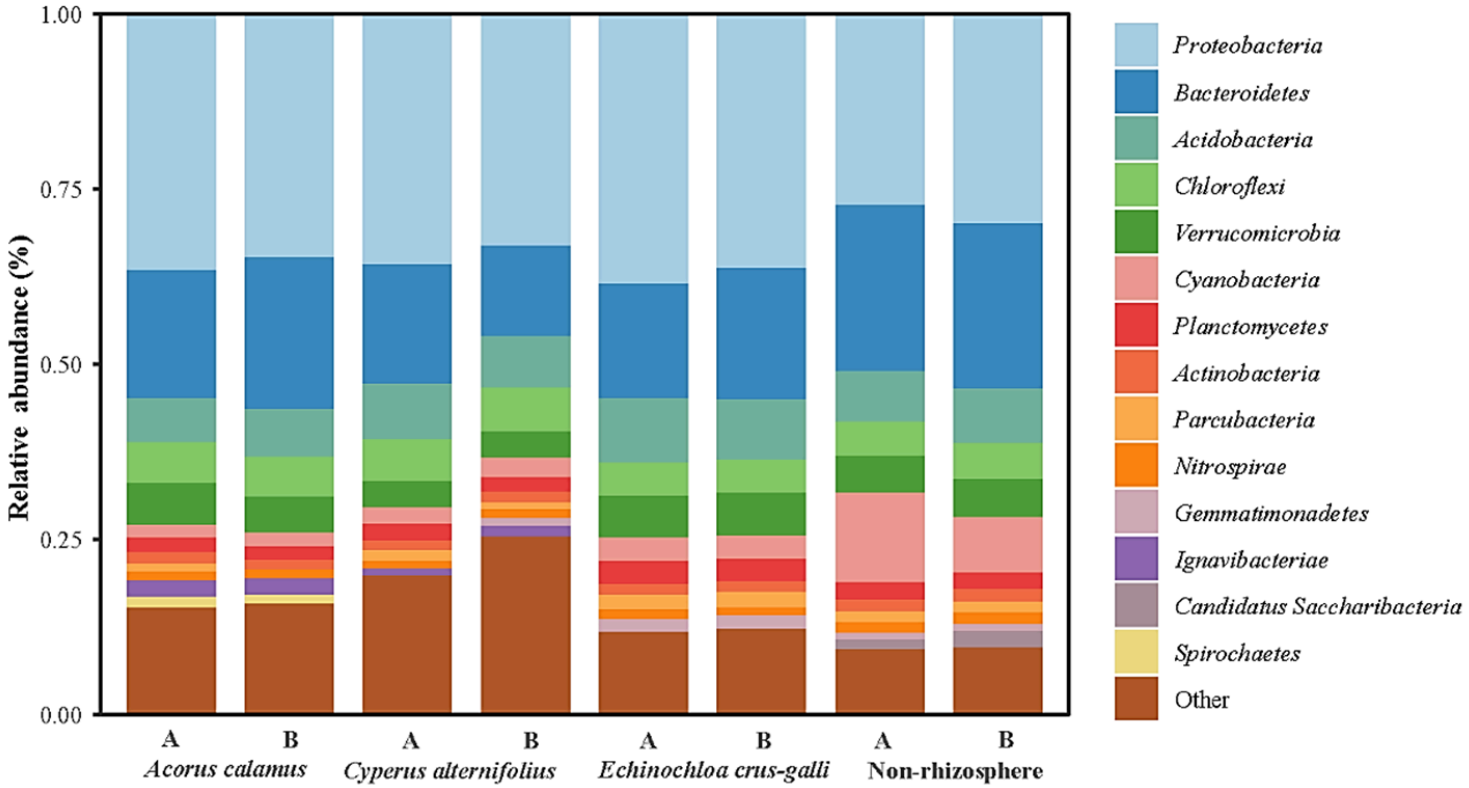

Figure 1

Relative Abundance of Microbial Phyla in the Rhizosphere of Wetland Plants at the Phylum Level

(Note: Each bar represents a sampling site, the y-axis shows relative abundance values, and the sum of relative abundances of all species in a single sampling site equals 1. Each color in the chart corresponds to a phylum, with corresponding species listed in the color legend to the right of the bar chart. The phyla listed each have a relative abundance greater than 1% in at least one sampling site, and "Other" refers to the sum of all phyla that do not exceed 1% relative abundance in any sampling site.)

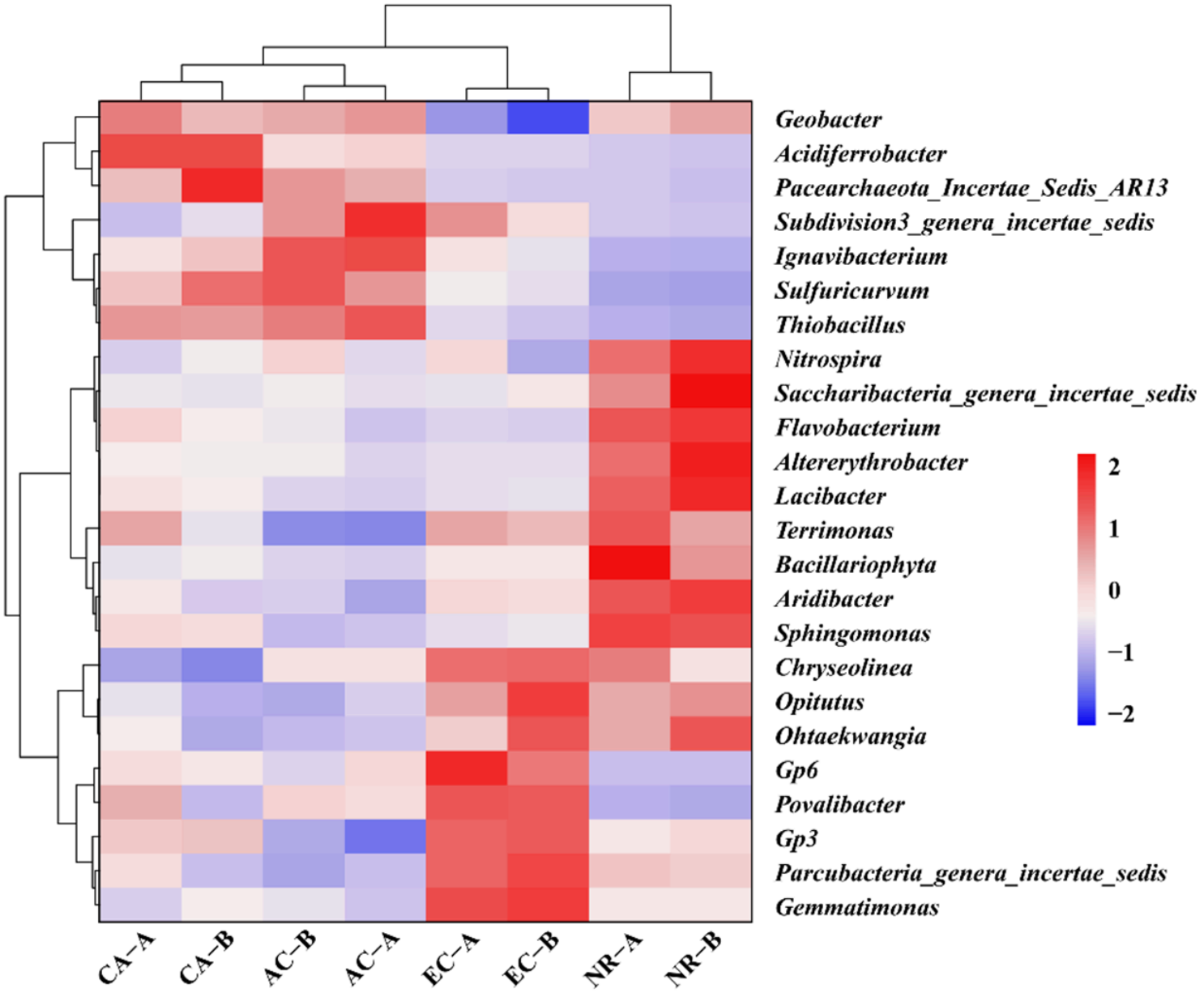

Figure 2

Heatmap of Microbial Genera at the Genus Level in the Rhizosphere of Wetland Plants

(Note: The genera listed have a relative abundance greater than 1% in at least one sampling site, with cluster analysis performed across different samples and genera.)

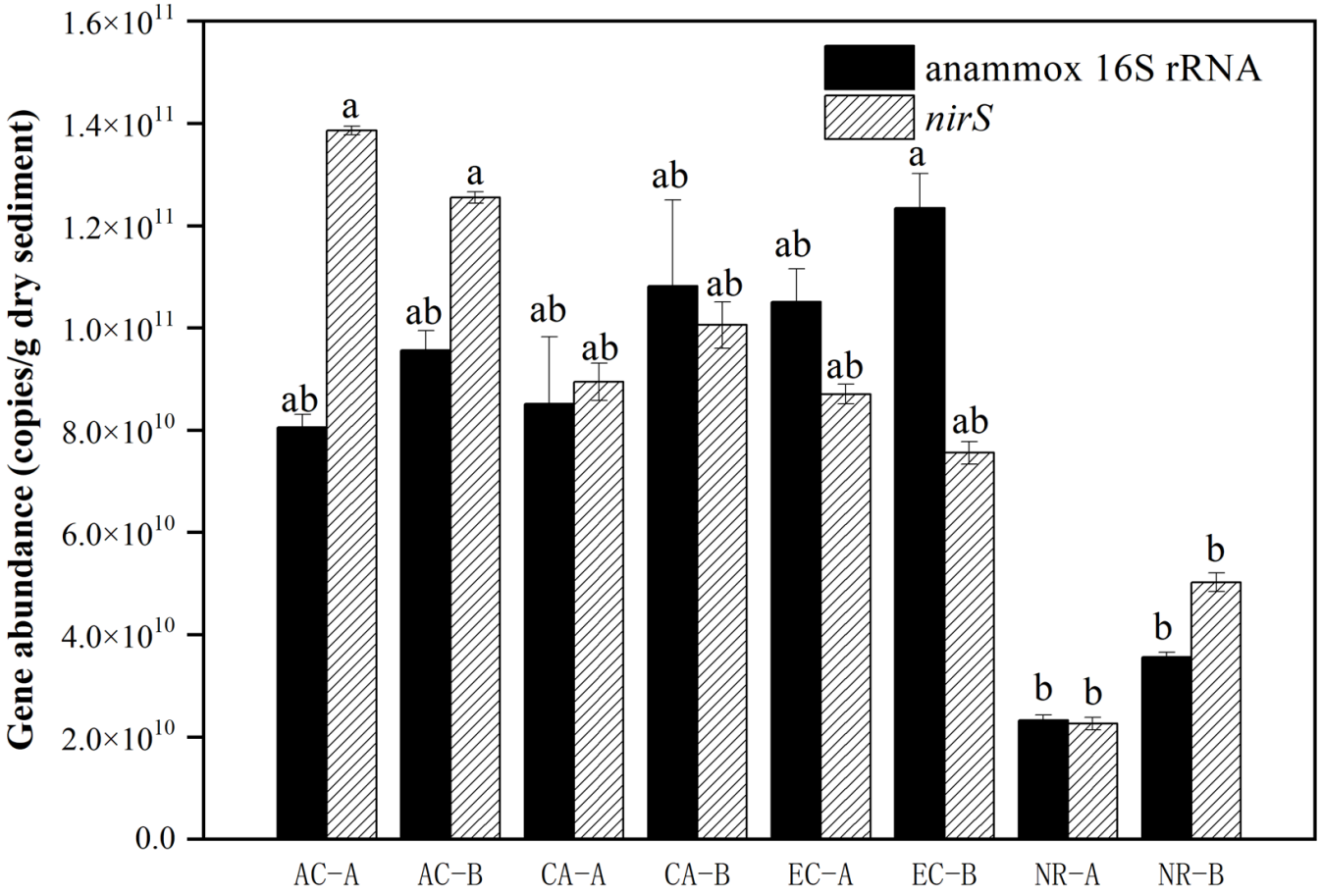

Figure 3

Abundance of Anaerobic Ammonium-Oxidizing Bacteria and Denitrifying Bacteria in the Rhizosphere of Wetland Plants

(Note: Different letters for the same gene indicate significant differences, Tukey's test, $P<0.05$).

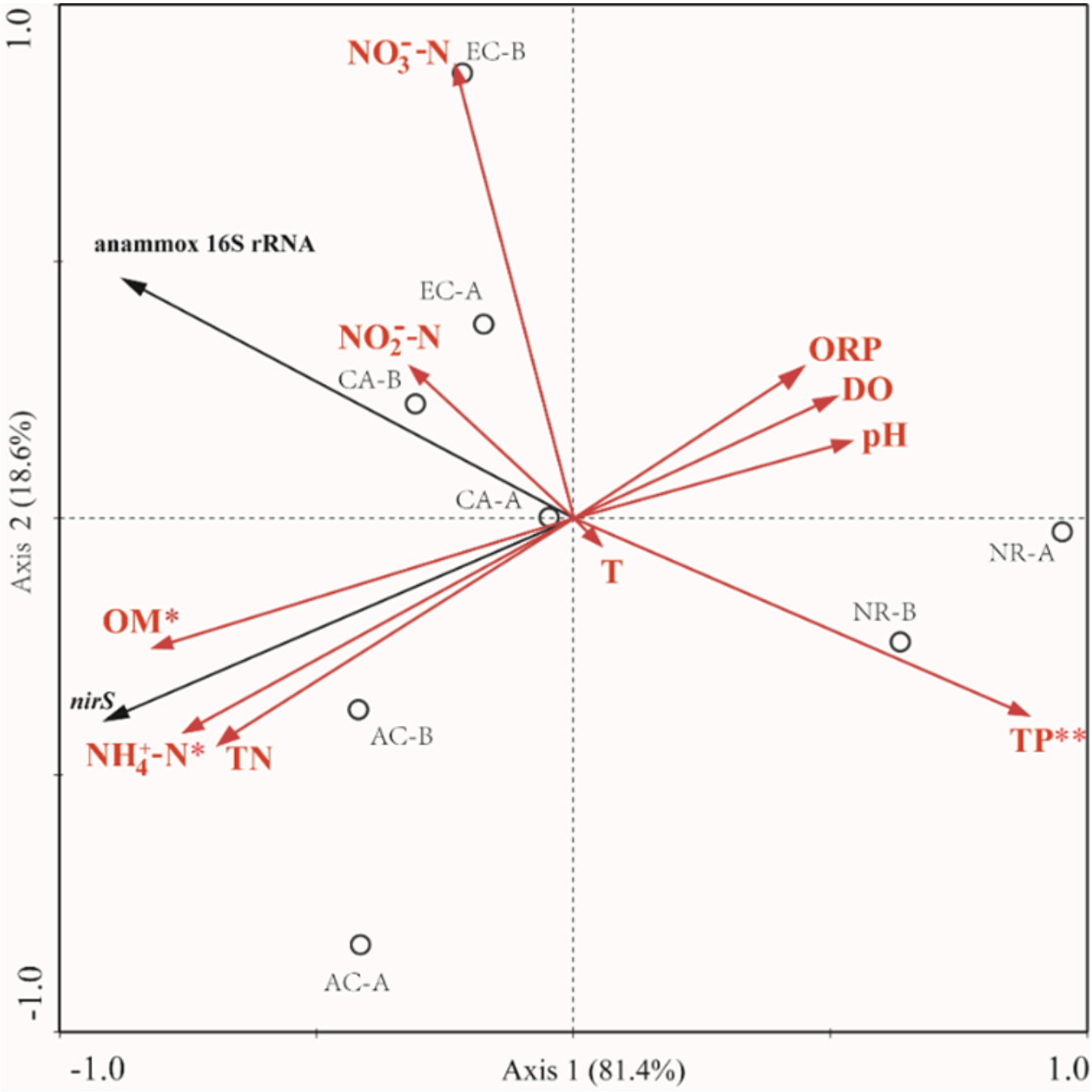

Figure 4

RDA ordination plot showing the relationship between the abundance of *nirS*and anammox 16S rRNA genes and physicochemical factors in the rhizosphere.