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ENVIRONMENTAL BIOTECHNOLOGY

# *nirS*-Encoding denitrifier community composition, distribution, and abundance along the coastal wetlands of China

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**Abstract** For the past few decades, human activities have intensively increased the reactive nitrogen enrichment in China's coastal wetlands. Although denitrification is a critical pathway of nitrogen removal, the understanding of denitrifier community dynamics driving denitrification remains limited in the coastal wetlands. In this study, the diversity, abundance, and community composition of *nirS*-encoding denitrifiers were analyzed to reveal their variations in China's coastal wetlands. Diverse *nirS* sequences were obtained and more than 98 % of them shared considerable phylogenetic similarity with sequences obtained from aquatic systems (marine/estuarine/coastal sediments and hypoxia sea water). Clone library analysis revealed that the distribution and composition of *nirS*-harboring denitrifiers had a significant latitudinal differentiation, but without a seasonal shift. Canonical correspondence analysis showed that the community structure of *nirS*-encoding denitrifiers was significantly related to temperature and ammonium concentration. The *nirS* gene abundance ranged from $4.3 \times 10^5$ to $3.7 \times 10^7$ copies $g^{-1}$ dry sediment, with a significant spatial heterogeneity. Among all detected environmental factors, temperature was a key factor affecting not only the *nirS* gene abundance but also the community structure of *nirS*-type denitrifiers. Overall, this study significantly enhances our understanding of the structure and dynamics of denitrifying communities in the coastal wetlands of China.

**Keywords** Denitrification · *nirS* gene · Coastal wetlands · Temperature · Abundance · Community structure

## Introduction

Over the past several decades, reactive nitrogen (Nr) input has incredibly increased because of human activities (Galloway and Cowling 2002; Galloway et al. 2008). By 2050, the nitrogen delivery in most regions is predicted to reach 50 kg N $ha^{-1}$ $year^{-1}$ to afford the still-growing human population (Galloway et al. 2004). The acceleration of Nr is attributed mainly to industrial production of nitrogen fertilizer and combustion of fossil fuel which contribute about $1.48 \times 10^{13}$ mol N $year^{-1}$ (Canfield et al. 2010; Vitousek et al. 1997). Although the increasing input of Nr has enabled humans to greatly improve crop yield, considerable Nr is eventually transported into estuarine and coastal wetlands through river flow, groundwater discharge and atmospheric deposition (Boyer et al. 2006; Seitzinger 2008), which has caused a series of environmental problems such as aquatic eutrophication, harmful algal bloom and hypoxia (Earl et al. 2006; Gruber and Galloway 2008; Howarth 2008). Therefore, it is of significance to study the nitrogen removal and associated microbial mechanisms for protecting and improving water quality in estuarine and coastal environments.

Denitrification, the dissimilatory reduction of oxidized N compounds ($NO_3^-$ and $NO_2^-$) to gaseous nitrogen (including

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NO, $N_2O$, and $N_2$), has been considered an effective microbial nitrogen removal process (Falkowski et al. 2008; Zumft 1997). This process contributes more than 70 % of the nitrogen loss in natural ecosystems, which is much higher than anammox process (Babbin et al. 2014; Dalsgaard et al. 2012). Thus denitrification plays a crucial role in controlling nitrogen fate (van Breemen et al. 2002). Diverse types of metabolic enzymes, including nitrate reductases (Nar), nitrite reductases (Nir), nitric oxide reductases (Nor), and nitrous oxide reductases (Nos), catalyze the denitrification process (Zumft 1997). In particular, Nir catalyzes the rate-limiting step in denitrification, which is encoded by *nirK* and/or *nirS* genes (Braker et al. 2001). Although these two genes are structurally different, enzyme types are functionally and physiologically similar (Coyne et al. 1989). Compared with *nirK* which is also contained in nitrifiers (Cantera and Stein 2007), *nirS* is found to be more widely distributed in natural environments (Braker et al. 1998). Therefore, *nirS* has been most frequently used for functional biomarkers of denitrifying community.

China has contributed far more Nr than other countries, due to large population and rapid agricultural and economic development (Cui et al. 2013). Over the past century, Nr emission had increased from 9.2 to 56 Tg $year^{-1}$ in China (Cui et al. 2013), and the majority of the Nr is delivered into the environment (Galloway and Cowling 2002; Wang and Wang 2009). At present, the estuaries and coastal seas of China have greatly suffered from the severe Nr pollution (Cui et al. 2013; Zhao et al. 2012). Although the important role of denitrification in the nitrogen removal is identified, little is known about the dynamics of denitrifiers in the nitrogen-enriched environments. The objectives of this study are to investigate the abundance, diversity, and distribution of *nirS*-harboring denitrifiers along the coastal wetlands of China and to explore the underlying interactions among the dynamics of denitrifiers, environmental parameters, and denitrifying activities in the wetland ecosystem.

## Materials and methods

### Study area

The coastal regions of China cover an area over three million square kilometers, with a 32,000 km coastline (Wang 1992). In China, the development of coastal regions has made a significant contribution to the national economy, harboring more than 60 % of China's gross domestic product (He et al. 2014). Over the past two decades, the coastal wetland areas have decreased $3.38 \times 10^6$ ha, with a total loss rate of 9.33 % (Wetland China 2014). The loss of the coastal wetlands is mainly attributed to the increasing growth of population in the coastal zone, which was coupled with rising coastal urban areas, economic growth, rapid urbanization, and infrastructure development (He et al. 2014; Shi et al. 2015). In addition, the interference of human activities has caused a series of ecological changes in China's coastal wetland ecosystems, such as the losses of biological habitat, productivity, and diversity (Lin et al. 2007; Sun et al. 2015; Xie et al. 2010).

### Sample collection

In this study, sediment samples were collected from eleven sites (P1 to P11) located in the coastal wetlands of China (including Dandong, Tangshan, Weifang, Qingdao, Lianyungang, Shanghai, Wenzhou, Fuzhou, Shantou, Zhuhai, and Beihai) (Fig. 1), ranging from high latitude sites (P1 to P6) to low latitude sites (P7 to P11). The numbering scheme for sampling sites decreases with latitude. Field work was conducted in winter (January) and summer (August) 2014, respectively. Triplicate sediment samples (0-5 cm) were collected with PVC cores at each site. The core sediments were transported to the lab on ice and immediately homogenized as one composite sample under helium condition. A fraction of the composite sample was archived at −80 °C until DNA isolation and used for microbial molecular analysis. The remaining sample was stored at 4 °C for analyses of sediment physicochemical parameters and denitrification activity. The data on sediment properties and denitrification rates in these collected samples have been given in Hou et al. (2015) (Table S1 and Fig. S1).

### PCR amplification, cloning, and phylogenetic analysis

Total genomic DNA was extracted from ~0.3 g sediment with PowerSoil DNA isolation kits (MoBio, USA). The *nirS* gene fragment (encoding cytochrome *cd*1-containing nitrite reductase ~840–890 bp) was amplified from sediment DNA extracts using primers *nirS*-1F and *nirS*-6R (Braker et al.

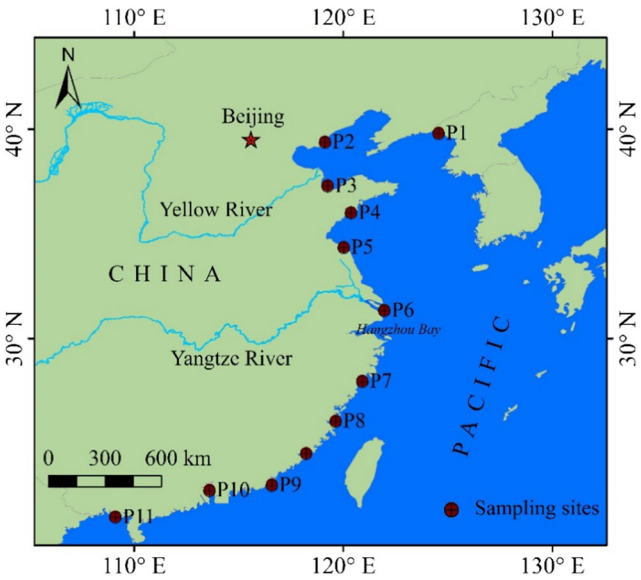


**Fig. 1** Location of sampling sites within the coastal wetlands of China

1998). The details of the primers and PCR conditions are shown in Table S2. The amplification products were visualized by electrophoresis on 1.0 % agarose gels. The obtained PCR products were purified using the Gel Advance gel extraction system (Viogene, China), and cloned into the pUCm-T Vector (Sangon, China). Then insert-containing transformants were transformed into *E. coli* XL1-Blue for growth. Approximate 100 clones were randomly screened from each sample and sequenced with an ABI 3370XL Prism genetic analyzer (Applied Biosystems, Canada).

Sequences were assembled, edited and put in order with DNAstar Lasergene (DNAstar, USA). The *nirS* sequences were analyzed in GenBank by BLAST (http://blast.st-va.ncbi.nlm.nih.gov/Blast.cgi) to obtain reference sequences. All of the obtained sequences were aligned with the ClustalX software (Thompson et al. 1997). The Mothur program (http://www.mothur.org/) was used to classify the sequences into one operational taxonomic unit (OTU) with >95 % identity. Phylogenetic tree was created by Mega 5 program with the neighbor-joining method (Kumar et al. 2004). The reliability of the tree topologies was estimated by performing 1000 bootstrapping replicates (Tamura et al. 2007).

The unique nucleotide sequences obtained in this study are available, and have been deposited in GenBank database under accession numbers of KU995338 to KU996352.

### qPCR of *nirS* gene

The abundance of *nirS* gene fragment (425 bp) in sediments was quantified in triplicate with primers cd3aF and R3cd (Throback et al. 2004). The details of the primers and PCR conditions are shown in Table S2. Plasmids containing cloned *nirS* PCR amplicons were generated with *Escherichia coli* hosts by using Qiagen Miniprep Spin Kit. The plasmids were diluted into a series of gradient as standard curves. The concentration of original plasmid was estimated with a Nanodrop-2000 Spectrophotometer (Thermo, USA). Quantification standard curves were formed via platting the threshold cycle (Ct) versus the $\log_{10}$ values of *nirS* gene copy numbers carried by the standard plasmids solutions, with strong linear relationship ($R^2 = 0.9996$) and high amplification efficiency (94.7 % in winter and 100.1 % in summer). The standard curve ranged over 7 orders of magnitude of the standard plasmid's concentrations ($1.71 \times 10^3$ to $1.71 \times 10^9$ copies per microliter). The melt curve for standards and samples only had a single peak at 85.4 °C, indicating that the fluorescent signals were obtained from specific DNA samples in all the process of quantitative PCR.

### Statistics analysis

The biodiversity indicators (Shannon-Weiner and Simpson), species richness Chao 1 estimator, and the rarefactions curves were obtained with the Mothur program (Schloss et al. 2009). The coverage for each constructed clone library was calculated using the follow method: the obtained OTUs number divided by chao1 indicator (Mohamed et al. 2010). Relationships between denitrifying bacterial community compositions and environmental indices were analyzed by the Canoco (version 4.5) software using canonical correspondence analysis (CCA) on the basis of the results of detrended correspondence analysis (DCA) (Danovaro and Gambi 2002; ter Braak 1988). Community classifications of sediment *nirS*-based denitrifiers were explored with principal coordinates analysis (PCoA) by the Qiime 1.9.0 software (Caporaso et al. 2010). Pearson correlation analyses were conducted with SPSS (version 16.0) software to explore correlations of environmental variables with the richness and abundance of *nirS*-harboring denitrifiers. Additionally, one-way analysis of variance (ANOVA) was performed to compare spatial and seasonal differences in *nirS*-encoding denitrifiers.

## Results

### *nirS*-based denitrifier diversity

The *nirS* gene sequences were successfully recovered from sediment DNA extracts at all sampling sites. Clone libraries were constructed for each site, containing 73 to 96 clones per library (Table 1). This resulted in a total database of 1942 *nirS* gene clones (Table 1). To date, these represented the most widespread *nirS* gene clone library in China's coastal wetlands. To analyze the diversity of *nirS*-based denitrifiers, 5 % divergence at the nucleotide level was used for *nirS* sequences to define OTU. In this study, 13 to 33 OTUs were obtained in each individual clone library (Table 1), and a total of 493 OTUs were identified. The library coverage was estimated at between 91.5–99.2 %. The high coverage indicates that the majority of the *nirS*-based denitrifier diversity was obtained, which is further confirmed by the rarefaction analysis (Fig. 2).

Based on the Shannon-Weiner and Simpson indices (Table 1), the maximal *nirS*-type denitrifier richness was found at site P6 in summer and P1 in winter where 30 and 33 OTUs were observed, respectively. The lack of significant curvature (Fig. 2) indicated that the richness of distinct *niS*-based sequences was not yet saturated in those two libraries. The second high richness of *nirS*-type denitrifiers appeared at site P10 in summer where 28 OTUs were obtained. Additionally, relatively low biodiversity of *nirS* gene was recorded at site P8 in summer where only 13 OTUs were found. The other 18 clone libraries had intermediate diversity, with an average Shannon-Weiner index of 2.58. Overall, the diversity of *nirS* gene showed a significant spatial difference (one-way ANOVA, $P = 0.001$) along the coastal wetlands of China. However, no distinctive seasonal shift was found between

**Table 1** Diversity estimators of *nirS*-encoding denitrifiers in the coastal wetlands of China

| Seasons | Sites | No. of clones | OTUs[a] | Shannon[b] | Chao 1[c] | 1/Simpson[d] | Coverage (%)[e] |
|---|---|---|---|---|---|---|---|
| winter | P1 | 85 | 33 | 3.17 | 36.1 | 21.12 | 91.5 |
| | P2 | 86 | 15 | 1.94 | 15.9 | 4.32 | 94.6 |
| | P3 | 93 | 20 | 2.59 | 21.3 | 11.50 | 94.1 |
| | P4 | 90 | 20 | 2.20 | 21.4 | 5.40 | 93.6 |
| | P5 | 87 | 25 | 2.88 | 25.8 | 15.52 | 97.0 |
| | P6 | 96 | 19 | 2.50 | 20.2 | 9.01 | 94.1 |
| | P7 | 85 | 18 | 2.19 | 19.1 | 5.61 | 94.2 |
| | P8 | 94 | 15 | 2.04 | 15.1 | 5.27 | 99.2 |
| | P9 | 90 | 26 | 2.84 | 27.2 | 12.75 | 95.8 |
| | P10 | 88 | 23 | 2.90 | 23.7 | 18.23 | 97.2 |
| | P11 | 89 | 30 | 3.00 | 30.5 | 14.50 | 98.5 |
| Summer | P1 | 88 | 23 | 2.71 | 23.5 | 11.60 | 98.0 |
| | P2 | 94 | 25 | 2.89 | 25.8 | 16.43 | 96.8 |
| | P3 | 87 | 20 | 2.45 | 20.6 | 7.68 | 97.1 |
| | P4 | 90 | 26 | 2.89 | 26.4 | 14.56 | 98.6 |
| | P5 | 73 | 24 | 2.93 | 25.0 | 16.95 | 96.0 |
| | P6 | 79 | 30 | 3.23 | 31.4 | 29.34 | 95.5 |
| | P7 | 89 | 14 | 1.68 | 14.1 | 2.84 | 99.0 |
| | P8 | 93 | 13 | 1.28 | 13.4 | 1.97 | 97.2 |
| | P9 | 91 | 23 | 2.72 | 24.1 | 11.31 | 95.4 |
| | P10 | 85 | 28 | 3.06 | 28.6 | 19.40 | 97.8 |
| | P11 | 90 | 23 | 2.66 | 24.4 | 11.06 | 94.4 |

[a] OTUs are defined based on 5 % nucleotide acid divergence

[b] Shannon–Weiner index. Higher number represents more diversity

[c] Nonparametric statistical predictions of total richness of OTUs based on distribution of singletons and doubletons

[d] Reciprocal of Simpson's diversity index. Higher number represents more diversity

[e] Percentage of observed number of OTUs divided by Chao1 estimate

summer and winter (one-way ANOVA, $P = 0.919$), with average Shannon-Weiner indices of 2.59 and 2.57, respectively.

## Community composition and distribution of *nirS*-encoding bacteria

For the phylogenetic analysis, the *nirS* gene sequences were grouped into 10 distinctly defined clusters (I to X) on the basis of evolutionary distance (Fig. 3). In this study, the levels of nucleotide clone identity ranged from 45 % to 100 %. The 1942 *nirS* gene sequences were aligned with representative database sequences and had high degree of identity (93.0–100.0 %) to the closest matched sequences retrieved from the GenBank. Phylogenetic analysis showed that a large proportion of the unique OTUs matched with uncultured environmental *nirS* assemblages. The majority of the closely matched sequences in the GenBank were retrieved from sediment environments, including Haihe River estuary (KC106788), Yangtze estuary (KF363218; EU236006; KM892169; EU235754) (Zhang et al. 2014; Zheng et al. 2015), Bahia del Tobari estuary (KC614247), San Francisco Bay estuary (GQ453730) (Mosier and Francis 2010), Bohai Gulf (JN257854), East Pacific sea (GU348415), coastal sea in France (KJ640012) (Stauffert et al. 2014), as well as coastal regions in Baltic sea (DQ072204) (Hannig et al. 2006) and the South Pacific (AJ811468) (Castro-Gonzalez et al. 2005). The *nirS* gene sequences of cluster IX were closely related to the cultivated denitrifiers of *Proteobacteria Pseudomonadales* (CP000304) (90 % identity) (Yan et al. 2008).

All of the ten clusters corresponded to distinct groups in the phylogenetic tree (Fig. 3). The cluster II contained the maximal amount of *nirS* gene sequences, occupying 26.6 % of the total sequences (Fig. 4). However, the cluster V only accounted for 0.6 % (Fig. 4). The clusters I, II, and IV were discovered in all the 22 built clone libraries, including 1.1 % to 65.2 % of *nirS* gene sequences. Of these ten clusters, no one was unique to a particular site, and all the clusters included sequences from four or more samples. The *nirS* sequences

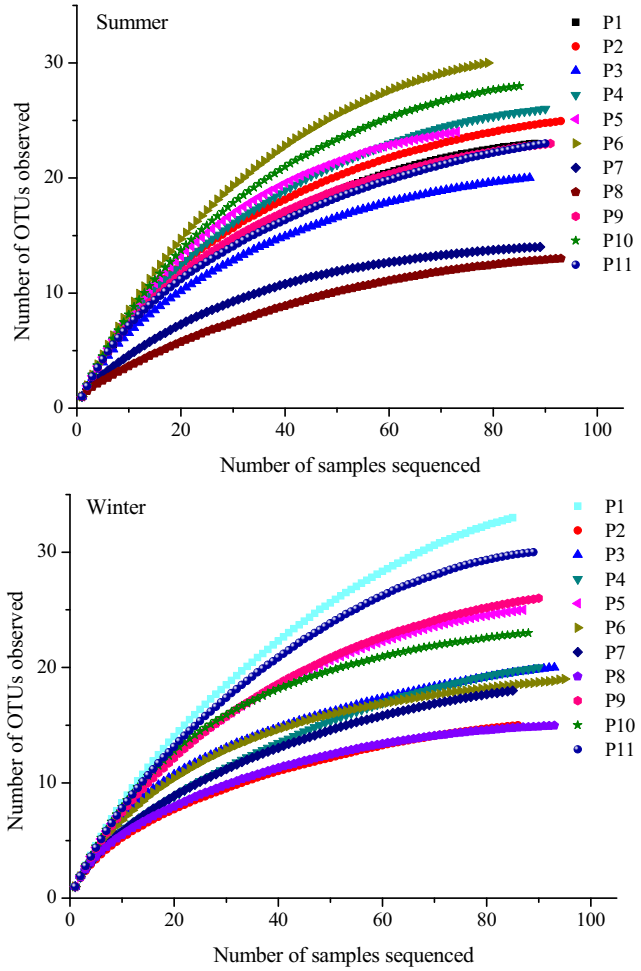


**Fig. 2** Rarefaction analysis of *nirS*-encoding denitrifier communities in summer and winter, respectively. OTUs were defined by <5 % divergence in nucleotide sequence

from high latitude habitats (P1 to P6) were the dominant type in the clusters II, V, VIII, and X (Table S3), accounting for 61.0 % to 83.3 % of the *nirS*-based denitrifiers. However, in the clusters VI and IX, the *nirS* sequences were co-dominated by the low latitude sites (P7 to P11), accounting for 71.1 % to 84.8 %. Among all the 22 clone libraries, no one library was dispersed throughout the phylogenetic tree, which generally occupied 5 to 8 clusters.

Spatial and temporal distributions of *nirS*-harboring denitrifier community were statistically compared with the weighted UniFrac PCoA analysis (Fig. 5). The first two principal coordinates explained 42.91 % of the *nirS*-based denitrifier community changes among all the sampling sites. The distribution of *nirS*-based bacterial community did not show a significant seasonal difference between summer and winter clone libraries (Fig. S2), indicating that the *nirS*-encoding bacterial community compositions were relatively stable in China's coastal wetland sediments. However, a significant latitudinal shift was characterized in the distribution of denitrifier assemblages along the coastal wetlands (Fig. 5).

## Quantification of *nirS*-based denitrifiers

The qPCR results indicated a geographically heterogeneous distribution of *nirS*-encoding denitrifier abundance along the coastal wetlands of China (one-way ANOVA, $P < 0.001$) (Fig. 6). The copy numbers of *nirS* gene ranged from $3.1 \times 10^6$ to $3.7 \times 10^7$ copies $g^{-1}$ dry sediment in summer and $4.3 \times 10^5$ to $1.2 \times 10^7$ copies $g^{-1}$ dry sediment in winter. The highest copy number was found at the low latitude site P7 in summer, and the lowest copy number was detected at the high latitude site P5 in winter. The average copy number of *nirS*-encoding denitrifying community was slightly higher at the low latitude sites (P7 to P11) than at the high latitude sites (P1 to P6) (Fig. 6). Although no significant seasonal shift was found (one-way ANOVA, $P = 0.079$), the *nirS*-encoding bacterial abundance tended to be higher in summer than in winter (except for sites P6 and P11), with respective average abundance of $9.0 \times 10^6$ and $2.9 \times 10^6$ copies $g^{-1}$ dry sediment.

## Relationships of *nirS*-encoding denitrifier community dynamics with environmental factors

The relationships between the *nirS*-type denitrifier communities with environmental variables were tested by the canonical correspondence analysis (CCA) (Fig. 7). The environmental parameters in the first two CCA dimensions (CCA1 and CCA2) provided 49.9 % of the cumulative variance of the *nirS*-type denitrifying community-environment correlation. The results showed that the *nirS*-based denitrifying community structures in the sediments of China's coastal wetlands were significantly correlated to ammonium ($P = 0.017$, $F = 2.89$, 1000 Monte Carlo permutations) and temperature ($P = 0.048$, $F = 1.63$, 1000 Monte Carlo permutations), which accounted for 37.0 % of the total CCA expositive power. Although the contribution of other investigated environmental variables (including nitrate, nitrite, organic carbon, sulfide, salinity, organic nitrogen, median size, pH, and C:N ratios) were not significant ($P > 0.05$, 1000 Monte Carlo permutations), the union of these environmental factors accounted for additional 45.0 % of the entire CCA expositive power.

The relationships of *nirS*-based bacteria diversity with environmental parameters were also analyzed with SPSS software. The results showed that the diversity of *nirS*-based denitrifying bacteria was positively correlated to organic nitrogen ($R = 0.458$, $P = 0.032$, $N = 22$) and negatively correlated to C:N ratios ($R = -0.540$, $P = 0.009$, $N = 22$) (Table S4). However, no significant correlations were found between *nirS*-based denitrifier diversity and other environmental variables (including temperature, salinity, pH, sediment median size, organic carbon, sulfide, ammonium, nitrite, and nitrate) ($P > 0.05$).

In addition, Pearson correlation analyses indicated that the *nirS*-based denitrifier abundance was only correlated with

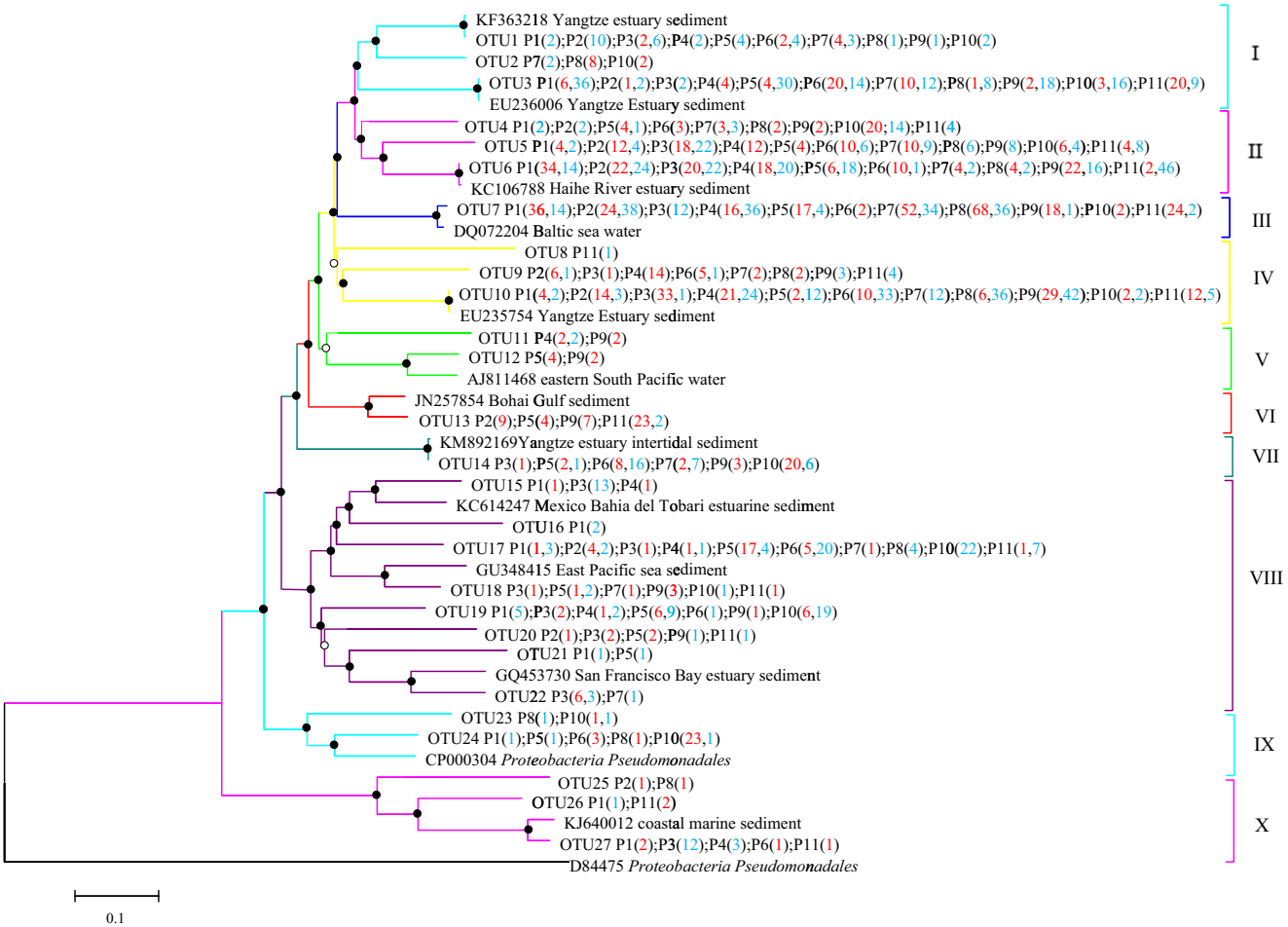


**Fig. 3** Neighbor-joining phylogenetic tree of *nirS* sequences in China's coastal wetland sediments. This showed affiliations between *nirS* gene fragments derived from the coastal wetlands of China and related matches, with the *Proteobacteria Pseudomonadales* (accession no. D84475) used as the outgroup. Clone names include the sample name and the number of sequences recovered from each sampling site in summer (red) and winter (blue). Bootstrap values greater than 50 % ($n$ = 1000) are shown with solid circle, and those less than 50 % are shown with open circle on the corresponding nodes

temperature ($R$ = 0.420, $P$ = 0.050), as compared with other environmental variables (Table S4).

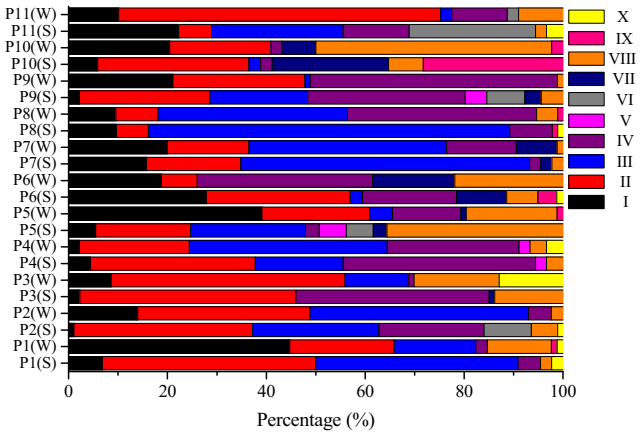


**Fig. 4** The community composition of *nirS*-encoding denitrifiers. S and W in brackets represent summer and winter, respectively

## Discussion

This study examined the *nirS*-harboring denitrifier community diversity, abundance, and distribution, which provides novel insights into the denitrifier community dynamics and associations with environment factors in the coastal wetlands of China. The diversity of *nirS*-based denitrifiers in this study was consistent with previous reports from other environmental ecosystems (Francis et al. 2013; Li et al. 2013). The majority of detected *nirS* sequences fell into numerous novel phylogenetic lineages and OTUs, most of which might represent coastal wetland-specific *nirS*-encoding denitrifiers. All *nirS* sequences shared considerable phylogenetic similarity with sequences obtained from aquatic systems (marine/estuarine/coastal sediments and hypoxia sea water; Fig. 3), suggesting that all the microorganisms in this study derived from coastal rather than terrestrial environments. The only cultivated sequence is *Proteobacteria Pseudomonadales* (Yan et al. 2008). However, most of the *nirS*-encoding

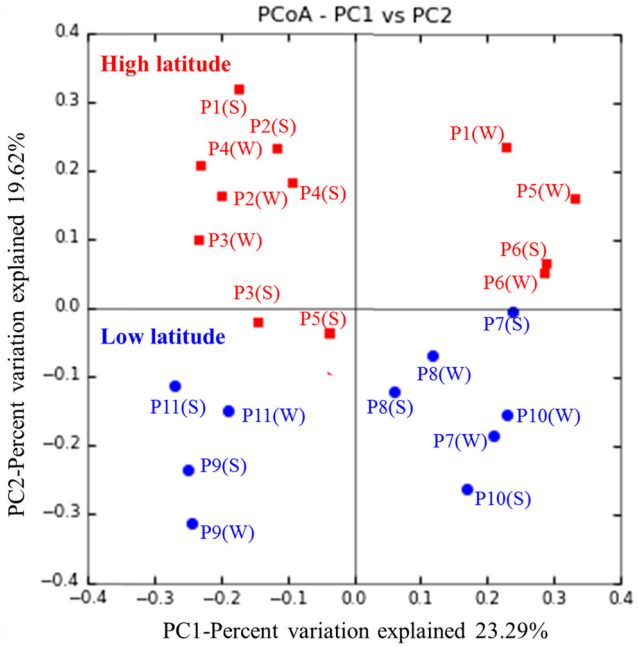


**Fig. 5** The UniFrac weighted PCoA analysis of *nirS*-encoding denitrifier communities. S and W represent summer and winter samples, respectively. Red and blue font represent samples from high and low latitude sites, respectively

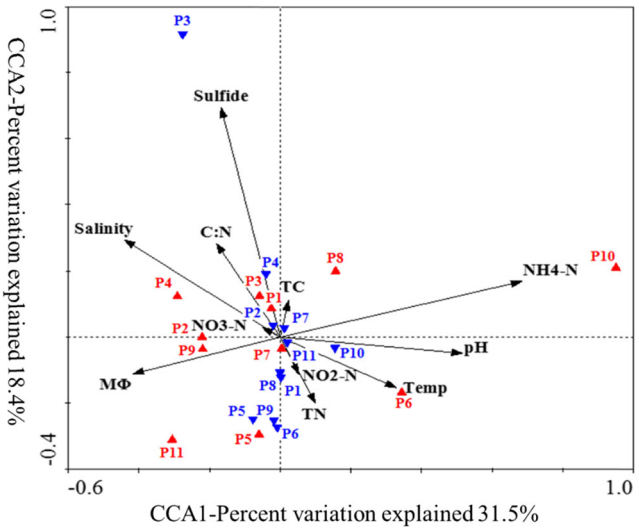


**Fig. 7** Canonical correspondence analysis for the correlations of environment factors with the community structure of *nirS*-encoding denitrifiers in both summer (red up-triangle) and winter (blue down-triangle). Temp, OC, C:N, MΦ, NO3-N, NO2-N, ON, and NH4-N represent temperature, organic carbon, C:N ratios, sediment mean size, nitrate, nitrite, organic nitrogen, and ammonium, respectively

genotypes observed in the present study were affiliated with uncultured denitrifying strains. Within all the ten clusters, several clusters (II, V, VI, VIII, IX, and X) showed a distinctive latitudinal differentiation along China's coastal wetlands (Table S3), probably suggesting that the diverse denitrifiers have different environmental adaptation strategies. Furthermore, the community structure and distribution of *nirS*-encoding denirifiers showed obvious latitudinal heterogeneity along the coastal wetlands of China on the basis of statistical analyses (Fig. 5). This result indicates that temperature may be an important environmental parameter shaping the biogeographical distribution and composition of *nirS*-based denitrifying communities in the coastal wetlands of China. Furthermore, the latitudinal distribution pattern was also supported by the CCA results (Fig. 7). Previous studies have found that salinity is a dominant environmental variable shaping the biogeographical distribution of denitrifiers (Abell et al. 2010; Francis et al. 2013; Yoshie et al. 2004; Zheng et al. 2015), mainly by comparing different-salinity habitats. However, samples in this study were collected from coastal wetland sediments with relatively consistent salinity. Therefore, we concluded that temperature rather than salinity significantly contributed to the latitudinal distribution of *nirS*-based denitrifiers. Interestingly, a similar distribution pattern was found for the anaerobic ammonium oxidation (anammox) community as well (Hou et al. 2015).

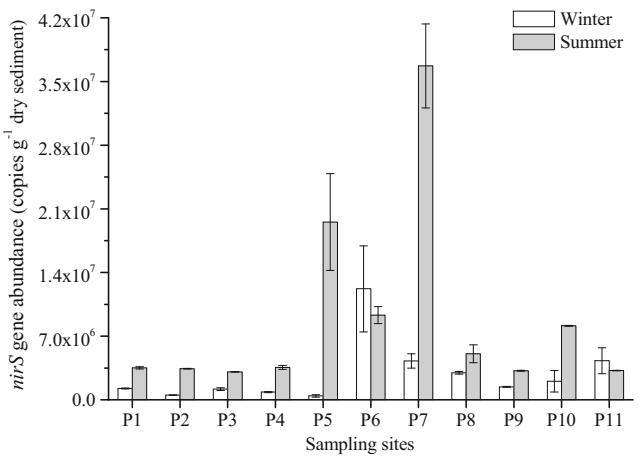


**Fig. 6** The abundance of *nirS*-based denitrifiers in the coastal wetlands of China. Vertical bars show standard error ($n = 3$)

In addition to temperature, other biochemical indices can also influence the distribution and diversity of *nirS*-encoding bacterial communities. It should be noted that a diverse of environmental factors may be significant in shaping the coastal wetland denitrifiers with complex interactions (Dang et al. 2009; Francis et al. 2013; Bulow et al. 2008). In the present study, the ammonium concentriation also had significant contribution to *nirS*-based denirifier community structure, which may be attributed to the increased supply of oxidized nitrogen through nitrification process and thus provides the electron acceptor for denitrification (Avrahami et al. 2002). Similar results have also been observed in the Yangtze estuary and Jiaozhou Bay (Dang et al. 2009; Zheng et al. 2015). Additionally, C:N ratios has been reported to have significant impact on the denitrification activity, nitrite accumulation, and microbial community composition (Her and Huang 1995; Kim et al. 2008; Mosier and Francis 2010). The diversity of

*nirS*-based denitrifiers was also observed to negatively correlate with C:N ratios in this study. In addition, the *nirS*-based denitrifier diversity was positively affected by organic nitrogen. Therefore, sediment with high nitrogen content may support the coexistence of diverse denitrifying bacteria. Also, the effect of pH on shaping *nirS*-harboring community composition has been documented by Hallin et al. (2009), which is likely due to the narrow pH ranges for optimal growth of microorganisms (Rousk et al. 2010). However, no significant relationship between pH and *nirS*-encoding denirifier composition was found in the present study. Bulow et al. (2008) have indicated that the greatest control on the diversity of denitrifying communities is environmental stability. Along the coastal wetlands of China, the community of *nirS*-based denitrifying bacteria did not display a statistically significant seasonal shift between summer and winter, indicating that the denitrifiers distribution reflects an adaption to site-specific features.

The abundance of *nirS*-based denitrifiers demonstrated significant spatiotemporal fluctuations in the coastal wetland sediments of China. The copy numbers of *nirS* gene ranged from $4.3 \times 10^5$ to $3.7 \times 10^7$ copies $g^{-1}$ dry sediment. The values are within the ranges reported in the Colne estuary (~$10^4$ to $10^7$ copies per gram of sediment), Chesapeake Bay ($2 \times 10^5$ to $7 \times 10^7$ copies $g^{-1}$ dry sediment), and San Francisco Bay ($5.4 \times 10^5$ to $5.4 \times 10^7$ copies $g^{-1}$ dry sediment) (Bulow et al. 2008; Mosier and Francis 2010; Smith et al. 2007). Similar to the community structure of *nirS*-harboring denitrifiers, *nirS* gene abundance was also observed to be positively correlated with temperature. Braker et al. (2010) reported that temperature exerts a significant effect on the abundance and composition of denitrifying communities in agricultural soil. In general, temperature could either modify the function of existing microorganisms or rebuild microbial communities, and thus changes the fundamental physiologies which drive biogeochemical processes (Schimel and Gulledge 1998). Although it has been reported that multiple environmental factors (e.g., organic matter, pH, nitrate, ammonium, and sediment water) can affect the abundance of *nirS* gene (Dandie et al. 2011; Dong et al. 2009; Kandeler et al. 2006), no significant relationships were found between the *nirS*-encoding denitrifier abundance and other environmental variables detected in this study (including ammonium, C:N ratios, nitrate, nitrite, organic carbon, sulfide, salinity, organic nitrogen, median size, and pH). In addition, denitrifying bacteria abundance tended to be slightly higher in summer than in winter, even though there was no significant seasonal variation in the *nirS* gene abundance. These results further demonstrate the importance of temperature in shaping the dynamics of denitrifiers in the coastal wetlands of China.

Hou et al. (2015) reported that the denitrification rates showed significant latitudinal heterogeneity along the coastal wetlands of China (one-way ANOVA, $p < 0.05$) (Fig. 8), with

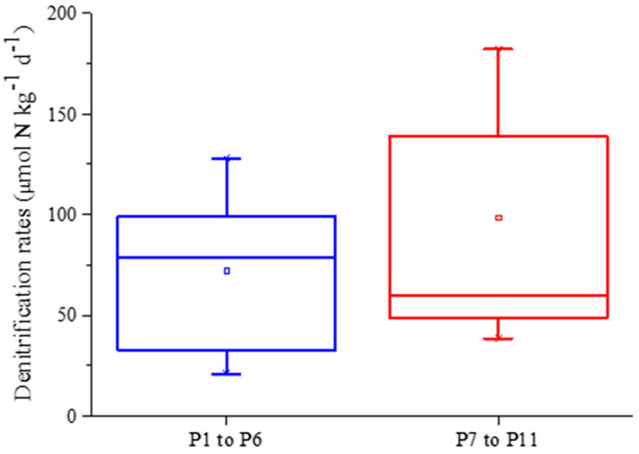


**Fig. 8** Spatial variation of denitrification rates in sediments. The squares represent means and solid lines represent median values. Boxes enclose the interquartile range, and whiskers show the full range

average rates of 98.82 μmol N $kg^{-1}$ $day^{-1}$ at the low latitude sites (P7 to P11) and 72.39 μmol N $kg^{-1}$ $day^{-1}$ at the high latitude sites (P1 to P6). Interestingly, it was observed that the denitrification rates were significantly correlated with the *nirS*-encoding denitrifier abundance ($R = 0.548$, $P < 0.001$) (Fig. S3). The result is consistent with the previous report in the San Francisco Bay estuary (Mosier and Francis 2010). However, the denitrification rates were not significantly related to the denitrifying community composition ($R = -0.126$, $P = 0.57$) (Fig. S4). These relationships imply that the abundance of denitrifiers, more than structure and diversity, predicts the activity of denitrifying community in the coastal wetlands of China.

In conclusion, this study demonstrated the abundance, composition, and distribution of *nirS*-based denitrifying communities along the coastal wetlands of China. To date, the present work represents the most systematic characterization of *nirS*-type denitrifiers based on molecular effort in China's coastal wetland ecosystems. The composition and structure of *nirS*-based denitrifier communities showed distinctive latitudinal heterogeneity along the coastal wetlands of China. The abundance of *nirS* gene varied between $4.3 \times 10^5$ and $3.7 \times 10^7$ copies $g^{-1}$ dry sediment, with significant spatial heterogeneity. However, there was no significant seasonal shift in the abundance, structure, and distribution of *nirS*-encoding denitrifiers. In this study, temperature was characterized as a key parameter in regulating the latitudinal distribution of denitrifier community abundance, composition, and distribution. This research provides new insights into the dynamics of *nirS*-encoding denitrifiers in China's coastal wetland ecosystems.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** This article does not contain any studies with human participants or animals.

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