

Supplement of

**Potential contributions of nitrifiers and denitrifiers to
nitrous oxide sources and sinks in China's estuarine and
coastal areas**

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Table S1 (Excel file). Sampling information in the four estuaries. BS, Bohai Sea; YRE, Yangtze River Estuary; JRE, Jiulong River Estuary; PRE, Pearl River Estuary. S, surface layer; M, middle layer; B, bottom layer.

Table S3 (Excel file). Gene abundance (average and median of triplicates; copies L⁻¹) in the four estuaries. BS, Bohai Sea; YRE, Yangtze River Estuary; JRE, Jiulong River Estuary; PRE, Pearl River Estuary; AOA, ammonia-oxidizing archaea; AOB, ammonia-oxidizing bacteria; BD, below detection. S, surface layer; M, middle layer; B, bottom layer.

Table S4 (Excel file). Gene transcript abundance (transcripts L⁻¹) in the four estuaries. BS, Bohai Sea; YRE, Yangtze River Estuary; JRE, Jiulong River Estuary; PRE, Pearl River Estuary; AOA, ammonia-oxidizing archaea; AOB, ammonia-oxidizing bacteria; BD, below detection. S, surface layer; M, middle layer; B, bottom layer.

Table S5 (Excel file). Data on N₂O concentration, N₂O flux, and ΔN₂O collected from the literatures. BS, Bohai Sea; YRE, Yangtze River Estuary; JRE, Jiulong River Estuary; PRE, Pearl River Estuary. LM86, the sea-to-air N₂O flux was calculated using the transfer velocity of gas estimated by the relationship proposed by Liss and Merlivat (1986); W92, the sea-to-air N₂O flux was calculated using the transfer velocity of gas estimated by the relationship proposed by Wanninkhof (1992).

Table S2. Specific primer sets and reactions for PCR and qPCR.

Gene	Reaction	Primer	Primer sequence 5'–3'	Mixture	Program	References
Archaeal- amoA	PCR	Arch-amoAF Arch-amoAR	STAATGGTCTGGCTTAGACG GCGGCCATCCATCTGTATGT	R	R	Francis et al., 2005
	qPCR	Arch-amoAFA Arch-amoAR	GGGGTTTCTACTGGTGGT GCGGCCATCCATCTGTATGT	20 µL reaction mixture: SYBR Green II mix (Takara, Bio Inc.) 10 µL, primers 0.6 µM, BSA 8 µg, DNA/cDNA template 1µL	R	Beman et al., 2008
Bacterial- amoA	PCR	amoA-34F amoA-2R	GCGGCRAAAATGCCGCCGGAAGCG CCCCTCKGSAAAGCCTTCTTC	R	R	Molina et al., 2007 Rotthauwe and Witzel, 1997;
	qPCR	amoA-1F amoA-r New	GGGGTTTCTACTGGTGGT CCCCTCBGSAAAVCCTTCTTC	20 µl reaction mixture: SYBR Green II mix (Takara, Bio Inc.) 10 µL, primers 0.4 µM, BSA 4 µg, DNA/cDNA template 1µL	R	Rotthauwe and Witzel, 1997; Hornek et al., 2006
nirK	PCR	F1aCu R3Cu	ATCATGGTSC TGCCGCG GCCTCGATCAG(A/G)TTGTGGTT	R	R	Braker et al., 1998
	qPCR	nirK876 nirK1040	ATYGGCGGV CAYGGCGA GCCTCGATCAGRTT RTGG	20 µL reaction mixture: SYBR Green II mix (Takara, Bio Inc.) 10 µL, primers 1 µM, BSA 8 µg, DNA/cDNA template 1µL	R	Henry et al., 2004
nirS	PCR	nirS-1F	CCTAYTGGCCGCCRCART	R	R	Braker et al., 1998

		nirS-6R	CGTTGAACTTRCCGGT			
	qPCR	nirS-1F nirS-3R	CCTAYTGGCCGCCRCART GCCGCCGTCRTGVAGGAA	20 µL reaction mixture: SYBR Green II mix (Takara, Bio Inc.) 10 µL, primers 0.8 µM, BSA 4 µg, DNA/cDNA template 1µL		Braker et al., 1998
<i>nosZ I</i>	PCR	nosZ1F nosZ2R	WCSYTGTTCMTCGACAGCCAG CAKRTGCAKSGCRTGGCAGAA	R	R	Henry et al., 2006
	qPCR	nosZ2F nosZ2R	CGCRACGGCAASAAGGTSMSST CAKRTGCAKSGCRTGGCAGAA	20 µL reaction mixture: SYBR Green II mix (Takara, Bio Inc.) 10 µL, primers 1.25 µM, BSA 10 µg, DNA/cDNA template 1µL	R	Henry et al., 2006
<i>nosZ II</i>	PCR	nosZ-II-F nosZ-II-R	CTIGGICCIYTKCAYAC GCIGARCARAAITCBGTRC	R	R	Jones et al., 2013
	qPCR	nosZ-II-F nosZ-II-Rnew	CTIGGICCIYTKCAYAC KGCRTAGTGIGGYTCDCC	20 µL reaction mixture: SYBR Green II mix (Takara, Bio Inc.) 10 µL, primers 1.25 µM, BSA 10 µg, DNA/cDNA template 1µL	See the main text	Jones et al., 2013; This study

R, PCR reaction mixtures and conditions follow corresponding references.

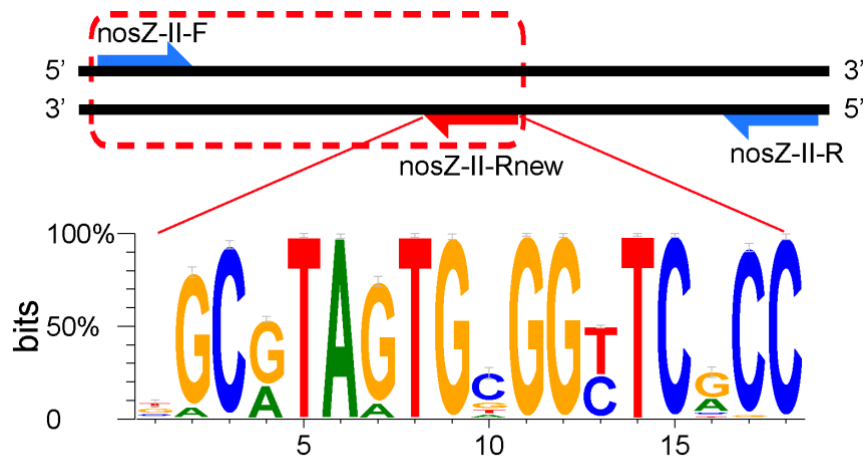


Fig. S1. Schematic representation of the clade II *nosZ* gene. The blue arrows indicate the primer binding sites reported by Jones et al. (2013). The red arrow indicates the newly designed reverse primer binding sites in this study. The consensus sequence of the new reverse primer is shown as a SeqLogo diagram, and the frequency of each base is indicated by the percentage. The dashed red box indicates the amplicon of the primer set used in this study.

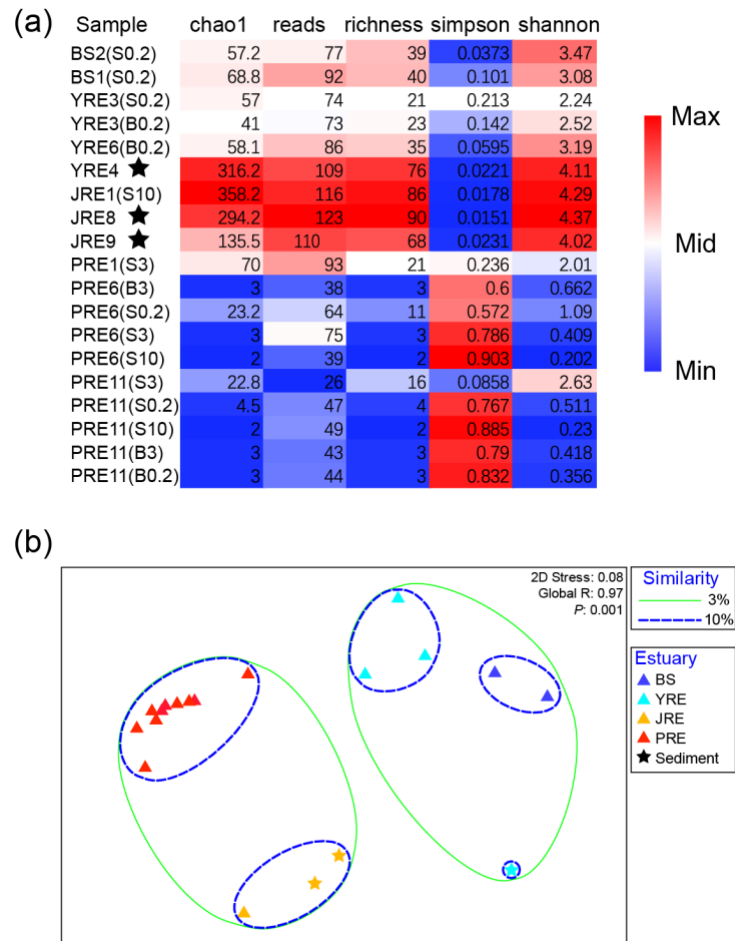


Fig. S2. (a) Alpha diversity indices of *nosZ* clade II gene clone libraries retrieved from the four estuaries. Black stars indicate sediment samples. The color bar indicates the levels of the index values. **(b)** Nonmetric multidimensional scaling (NMDS) analysis based on the Bray–Curtis similarity of the top 50 OTUs retrieved from each estuary. Each symbol represents an individual sample. BS, Bohai Sea; YRE, Yangtze River Estuary; JRE, Jiulong River Estuary; PRE, Pearl River Estuary.

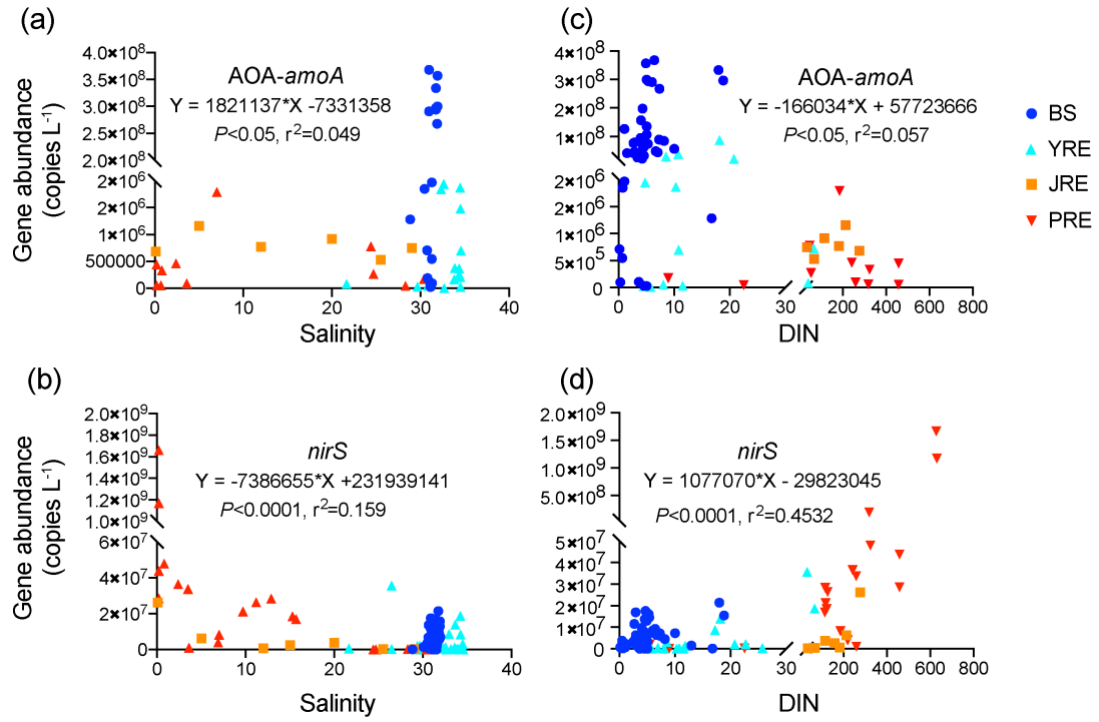


Fig. S3. Distribution of **(a, c)** archaeal-*amoA* and **(b, d)** *nirS* genes with **(a, b)** salinity and **(c, d)** the dissolved inorganic nitrogen (DIN). BS, Bohai Sea; YRE, Yangtze River Estuary; JRE, Jiulong River Estuary; PRE, Pearl River Estuary.

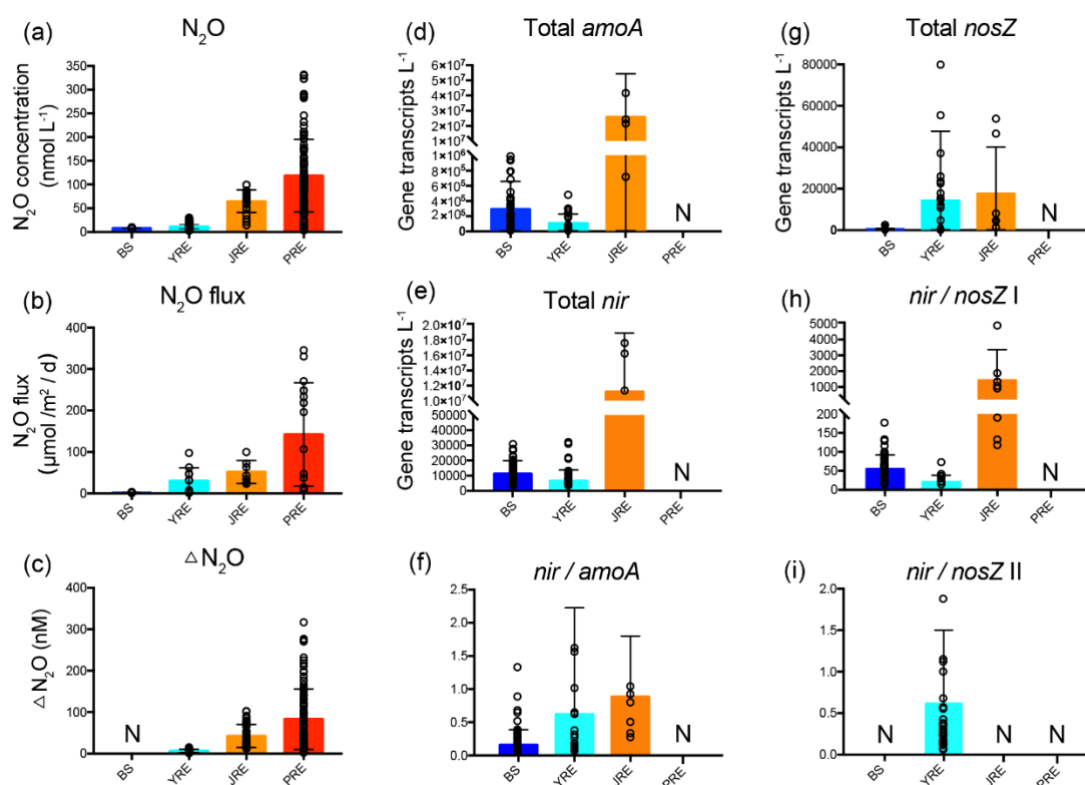


Fig. S4. The ranges of (a) N_2O concentration, (b) N_2O flux, (c) ΔN_2O , (d) total archaeal and bacterial *amoA* gene transcript abundance, (e) total *nirS* and *nirK* gene transcript abundance, (f) the ratio of total *nir* to *amoA* gene transcript abundance, (g) total *nosZ* clade I and II gene transcript abundance, (h) the ratio of total *nir* to *nosZ* clade I gene transcript abundance, and (i) ratio of total *nir* to *nosZ* clade II gene transcript abundance in the Bohai Sea (BS), Yangtze River estuary (YRE), Jiulong River estuary (JRE), and Pearl River estuary (PRE). Black circles represent the value of each sample. Bars represent the mean values. Error bars indicate standard deviation. N, no data or not determined.