



# Nitrogen fixation in surface sediments of the East China Sea: Occurrence and environmental implications

Rong Wang<sup>a</sup>, Xiaofei Li<sup>b</sup>, Lijun Hou<sup>a,\*</sup>, Min Liu<sup>c</sup>, Yanling Zheng<sup>c</sup>, Guoyu Yin<sup>c</sup>, Yi Yang<sup>c</sup>

<sup>a</sup> State Key Laboratory of Estuarine and Coastal Research, East China Normal University, Shanghai 200062, China

<sup>b</sup> Key Laboratory for Humid Subtropical Eco-geographical Processes of the Ministry of Education, Fujian Normal University, Fuzhou 350007, China

<sup>c</sup> Key Laboratory of Geographic Information Science (Ministry of Education), School of Geographic Sciences, East China Normal University, Shanghai 200241, China

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## ABSTRACT

Sediment nitrogen fixation and associated functional gene in the East China Sea were investigated using nitrogen-isotope tracing and molecular techniques. Potential rates of nitrogen fixation were detected, with values of 0.06–5.51 nmol N g<sup>-1</sup> h<sup>-1</sup>. Abundance of functional gene (*nifH*) ranged from 0.36 × 10<sup>6</sup> to 5.39 × 10<sup>7</sup> copies g<sup>-1</sup>. Nitrogen fixation rates were not related to the abundance of *nifH* gene but to temperature, salinity, sulfide, iron and C/N, indicating that the sediment properties rather than microbial abundance dominated the nitrogen fixation. It is also estimated that sediment nitrogen fixation annually contributed about 3.43 × 10<sup>5</sup> to 3.10 × 10<sup>7</sup> tons nitrogen to the East China Sea, which accounted for 8.2–22.6% of the total inorganic nitrogen input. Overall, this study highlights the importance of benthic nitrogen fixation in controlling nitrogen budget in the East China Sea and improves our knowledge on nitrogen cycling in the coastal marine environments.

## 1. Introduction

Nitrogen fixation is a microbially mediated process converting N<sub>2</sub> into biologically available ammonium (Hou et al., 2018). It generally contributes to most parts of nitrogen input into marine environments, compared with other nitrogen sources (Zehr, 2011; Bentzon-Tilia et al., 2015). As an important source of reactive nitrogen (Zehr and Kudela, 2011; Larmola et al., 2014; Bae et al., 2018), this process plays an important role in regulating primary productivity and nitrogen budget in coastal marine environments (Laroche and Breitbarth, 2005; Großkopf et al., 2012; Newell et al., 2016a; Benavides et al., 2018). Thus, an increasing attention to marine nitrogen fixation has been raised in recent decades.

At present, most studies have concerned about nitrogen fixation in water column because phototrophic cyanobacteria generally dominates the process of nitrogen fixation (Zehr, 2011; Andersson et al., 2014; Bentzon-Tilia et al., 2015). However, some reports indicate that heterotrophic prokaryotes have a great potential of nitrogen fixation in benthic sediments of estuarine and coastal environments (Mortazavi et al., 2012; Vieillard and Fulweiler, 2012; McCarthy et al., 2015; Gier et al., 2016; Hou et al., 2018). Also, the benthic nitrogen fixation is associated closely with environmental characteristics. For instance, Fulweiler et al. (2013) documented that benthic nitrogen fixation was

not detected in the environments where concentrations of nitrate and ammonium are > 1 μmol L<sup>-1</sup>. By contrast, some studies reported that nitrogen fixation can occur at high concentrations of 30 μmol L<sup>-1</sup> for NO<sub>3</sub><sup>-</sup> and 200 μmol L<sup>-1</sup> for NH<sub>4</sub><sup>+</sup> in marine benthic environments (Knapp, 2012). Organic matter and salinity also have great influence on nitrogen fixation via controlling composition and enzymatic activity of microbial community (Herbert, 1975; Grimm and Petrone, 1997; Marcarelli and Wurtsbaugh, 2006; Severin et al., 2012; Hou et al., 2018). Sulfide is observed to have negative or positive influences on nitrogen fixation (Gier et al., 2016; Hou et al., 2018), thus the role of sulfide in nitrogen fixation remains uncertain. Iron is another important factor for benthic nitrogen fixation because parts of iron-reducing bacterial communities can also fix nitrogen (Bertics et al., 2010; Fulweiler et al., 2013; Gier et al., 2017). The continental shelf of coastal marine environments plays a crucial role in biogeochemical cycling (Chai et al., 2006). However, the environmental characteristics in coastal marine environments are highly variable, and susceptible to hydrological fluctuations and currents (Lee et al., 2017). Diverse environmental characteristics may lead to the uncertainty of determinants regulating benthic nitrogen fixation. More studies on benthic nitrogen fixation are thus required to understand nitrogen dynamics in coastal marine environments.

Extensive continental shelf in the East China Sea develops mostly

\* Corresponding author.

E-mail address: [ljhou@sklec.ecnu.edu.cn](mailto:ljhou@sklec.ecnu.edu.cn) (L. Hou).

due to sediment deposition from the Yangtze River, hydrological fluctuations and currents (Liu et al., 2006). However, the variabilities in sediment environmental characteristics can affect the biogeochemical cycling in the continental shelf area (Lee et al., 2017). Although most studies have concerned about the nitrogen cycling recently (Deng et al., 2015; Hou et al., 2015a; Lin et al., 2016, 2017), the occurrence and biogeochemical controls of nitrogen fixation remain unclear in the continental shelf sediment of the East China Sea. In this study, the benthic nitrogen fixation was thus investigated using nitrogen-isotope tracing technique. The objectives of this study are (1) to examine the occurrence and spatial distributions of benthic nitrogen fixation in the continental shelf of the East China Sea, (2) to reveal the crucial factors controlling benthic nitrogen fixation, and (3) to estimate the contribution of benthic nitrogen fixation to nitrogen budget of the study area.

## 2. Materials and methods

### 2.1. Study area and sample collection

The continental shelf in the East China Sea is one of the largest continental shelves in the world, with an area of  $7.7 \times 10^5 \text{ km}^2$  and average water depth of 72 m (Lin et al., 2016, 2017). The continental shelf plays an important role in biogeochemical cycling since large amount of nutrients from the runoff of the Yangtze River and currents are accumulated in this region (Beardsley et al., 1985; Ichikawa and Beardsley, 2002; Lin et al., 2016). Recently, the reactive nitrogen concentration has increased approximately 10-fold, which is an important factor contributing to the eutrophication and algal blooms in the continental shelf area (Chai et al., 2006; Zheng et al., 2013).

In this study, three sampling transects were investigated, including N1–N7, M1–M7 and S1–S5 (Fig. 1). Totally, sediment samples were collected from nineteen sites by using a box corer in March and July 2016. For each site, triplicate sediment subcores (0–5 cm) were achieved and stored at 4 °C. In addition, bottom water samples were also collected with Niskin samplers and filtered immediately through 0.22 µm filters (Millipore, Bedford, USA) for nutrient analyses and incubation experiments. In the laboratory, sediment of each subcore was mixed thoroughly in a helium-filled glove bag and divided into two

parts: one was immediately incubated for measurement of potential nitrogen fixation rates and sediment characteristics, and the other was stored at  $-80 \text{ °C}$  for later analyses of molecular microbiology.

### 2.2. Analysis of site characteristics

Temperature, salinity and water depth at each site were measured *in situ* using conductivity-temperature-depth profiler (Sea-Bird 911 plus). Sediment water content was calculated according to the weight loss of fresh sediment dried at 60 °C to constant weight (Lin et al., 2017). Sediment pH was measured with Mettler-Toledo pH meter after sediment was mixed with  $\text{CO}_2$ -free deionized water at a sediment/water ratio of 1:2.5 (Hou et al., 2018). Sediment grain size was determined using LS 13320 Laser grain sizer (Hu et al., 2009). Sediment inorganic nitrogen including  $\text{NH}_4^+$  and  $\text{NO}_3^-$  (plus  $\text{NO}_2^-$ ) was extracted with  $2 \text{ mol L}^{-1}$  KCl (Hou et al., 2015b) and analyzed using continuous-flow nutrient analyzer (SAN Plus, Skalar Analytical B.V., the Netherlands), with the detection limits of  $0.5 \text{ µmol L}^{-1}$  for  $\text{NH}_4^+$  and  $0.1 \text{ µmol L}^{-1}$  for  $\text{NO}_3^-$ . Sediment organic carbon was measured using elementary analyzer (VVarioELIII, Elementary, Germany) after sediment was purified by leaching with  $0.1 \text{ mol L}^{-1}$  HCl to remove carbonate (Hou et al., 2013). Sediment amorphous Fe was extracted by using  $0.5 \text{ mol L}^{-1}$  HCl and then quantified by colorimetric (Ferrozine) analysis (Deng et al., 2015). Sediment sulfide was determined using a hydrogen sulfide sensor ( $\text{H}_2\text{S}$ -100, Unisense, Denmark), with a detection limit of  $20 \text{ nmol L}^{-1}$  (Hou et al., 2018).

### 2.3. Measurement of nitrogen fixation rate

Potential rate of nitrogen fixation was measured by sediment-slurry incubations combined with the  $^{15}\text{N}$  isotope tracing technique (Hou et al., 2018). Briefly, homogenized slurries of each site were made with fresh sediment and benthic seawater at a sediment/water ratio of 1:7, and purged with helium for 30 min to eliminate background dinitrogen gas (Li et al., 2015). The slurries were then transferred into gas-tight borosilicate vials (Labco Exetainers) in helium-filled glove box, and divided into two groups which were designated as initial and terminal samples, respectively. The vials designed as initial samples were immediately preserved with 200 µL 50%  $\text{ZnCl}_2$  solution (Lin et al., 2017), while the remaining vials were injected with 500 µL  $^{15}\text{N}_2$  (99 atom%  $^{15}\text{N}$ ; Campro Scientific, Germany) (Hou et al., 2018) and incubated in dark at site-specific temperature. After 24 h, the incubation was stopped by injecting  $\text{ZnCl}_2$ , as described for the initial samples. The concentrations of  $^{15}\text{N}$ -labeled organic nitrogen produced within the incubations were measured with membrane inlet mass spectrometer (MIMS) after the samples were oxidized by hypobromite iodine solution (Yin et al., 2014). The rates of nitrogen fixation were calculated according to the changes in the concentrations of  $^{15}\text{N}$ -labeled products during the incubation. More detailed information on this method is described by Hou et al. (2018).

### 2.4. Molecular microbial analysis

Sediment DNA was extracted using Powersoil™ DNA Isolation Kits (MOBIO, USA) as previously described (Zheng et al., 2017; Hou et al., 2018). DNA concentrations were measured with Nanodrop-2000 Spectrophotometer (Thermo, USA). The real-time fluorescent quantitative PCR (qPCR) was carried out to quantify the abundance of the sediment *nifH* gene using primers *nifH*fw (5'-GGH AAR GGH GGH ATH GGN AAR TC-3') and *nifH*rv (5'-GGC ATN GCR AAN CCV CCR CAN AC-3'), which have an extremely broad coverage for both bacterial and archaeal *nifH* genes (Mehta et al., 2003; Dang et al., 2013; Zhou et al., 2016). Triplicate sediment DNA samples from each site were conducted using an ABI7500 Sequence Detection System (Applied Biosystems, Canada) and the SYBR green qPCR method (Hou et al., 2018). The *nifH* gene qPCR condition was optimized based on previous studies by Dang

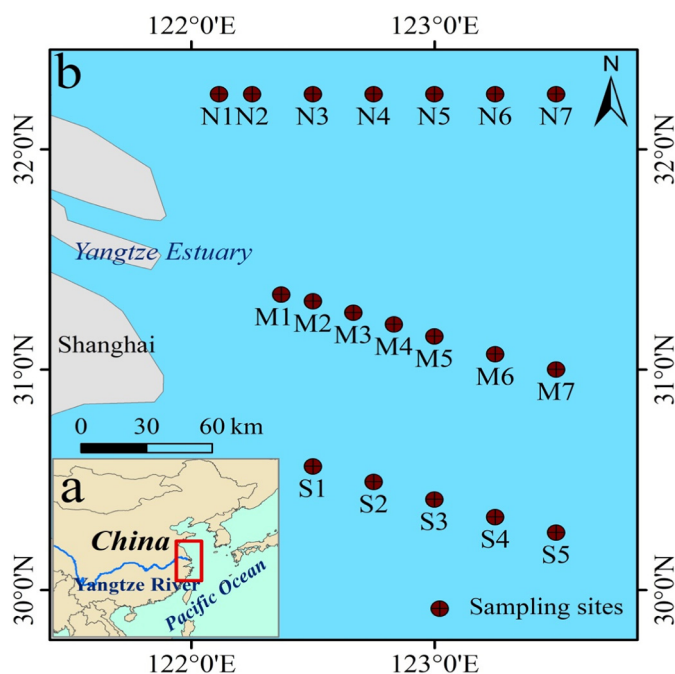


Fig. 1. Sampling sites in the East China Sea.

et al. (2013) and Zhou et al. (2016). The qPCR cycling was initiated by 50 °C 2 min, then 95 °C 30 s, followed by 30 s at 95 °C, 30 s 58 °C and 40 s 72 °C for 40 cycles, and finally 5 min at 72 °C. A qPCR standard curve, ranging from  $2.30 \times 10^4$  to  $2.30 \times 10^9$  copies per microliter, was generated with serial dilutions of linearized plasmids that contained the *nifH* gene. The amplification efficiency and correlation coefficient of qPCR were 97% and 0.994, respectively. Blank controls without template DNA were also conducted with the same qPCR procedure to exclude any possible contamination.

### 2.5. Statistical analysis

Pearson's correlation analyses were conducted to reveal the relationships among nitrogen fixation rate, abundance of *nifH* gene, and environmental factors. One-way and two-way ANOVA were performed to compare the seasonal and spatial variations of nitrogen fixation rates, abundance of *nifH* gene, and environmental factors. Redundancy analysis (RDA) was performed using CANOCO for Windows software to clarify the effects of environmental factors on nitrogen fixation rates and *nifH* gene abundance, based on the results of detrend correspondence analysis.

## 3. Results

### 3.1. Site characteristics

Sediment physicochemical characteristics of all sampling sites are shown in Table 1 and Table S1. The depth of sampling sites varied from 15 to 64 m in March and from 18 to 65 m in July. Sediment temperature was lower in March (5.1 °C to 12.6 °C) than in July (19.4 °C to 23.9 °C). An obvious salinity gradient was observed, which varied from 28.9 to 33.8‰ in March and from 8.1 to 29.6‰ in July. Sediment pH varied from 7.56 to 8.24 and from 7.21 to 8.68 in March and July, respectively. The concentrations of sediment inorganic nitrogen changed slightly in March (2.79 to 4.25  $\mu\text{g N g}^{-1}$  for  $\text{NH}_4^+$  and 0.89 to 1.36  $\mu\text{g N g}^{-1}$  for  $\text{NO}_3^-$ ), compared with July (2.41 to 9.07  $\mu\text{g N g}^{-1}$  for  $\text{NH}_4^+$  and 0.04 to 3.72  $\mu\text{g N g}^{-1}$  for  $\text{NO}_3^-$ ). Sediment  $\text{NH}_4^+$  showed significantly spatial variations (two-way ANOVA,  $F = 490.4$ ,  $df = 18$ ,  $p < 0.001$ ), but there were no significantly temporal variations (two-way ANOVA,  $F = 1.6$ ,  $df = 1$ ,  $p = 0.205$ ). Remarkable spatial and temporal variations were found for the concentrations of sediment  $\text{NO}_3^-$  (two-way ANOVA,  $F = 123.6$ ,  $df = 37$ ,  $p < 0.001$ ). The

concentrations of sulfide in sediments were relatively higher in March (25.49 to 2029.96  $\mu\text{g S g}^{-1}$ ) than in July (10.46 to 301.26  $\mu\text{g S g}^{-1}$ ) (two-way ANOVA,  $F = 1,187,971$ ,  $df = 1$ ,  $p < 0.001$ ). The contents of Fe(II) in sediments had significantly spatial and temporal heterogeneity (two-way ANOVA,  $F = 16,699$ ,  $df = 37$ ,  $p < 0.001$ ), with values of 0.62 to 3.77  $\text{mg g}^{-1}$  in March and 0.44 to 4.08  $\text{mg g}^{-1}$  in July. Likewise, significantly spatial and temporal variations were observed for the concentrations of sediment Fe(III) (two-way ANOVA,  $F = 11,298$ ,  $df = 37$ ,  $p < 0.001$ ), with values of 0.23 to 2.81  $\text{mg g}^{-1}$  in March and 0.04 to 1.45  $\text{mg g}^{-1}$  in July. The concentrations of organic carbon were in the range of 1.77 to 13.04  $\text{mg g}^{-1}$ , with relatively higher contents at low-salinity sites than at high-salinity sites ( $r = -0.396$ ,  $p = 0.000$ ). The C/N ratios of sediment ranged from 8.32 to 12.49 in March and from 6.83 to 13.36 in July. Significantly spatial variations were observed for sediment C/N ratios (two-way ANOVA,  $F = 6.34$ ,  $df = 18$ ,  $p < 0.001$ ), whereas they had no temporal variations (two-way ANOVA,  $F = 0.17$ ,  $df = 1$ ,  $p = 0.685$ ).

### 3.2. Nitrogen fixation rates

Potential rates of nitrogen fixation varied from 0.06 to 1.16  $\text{nmol N g}^{-1} \text{h}^{-1}$  in March and from 0.53 to 5.51  $\text{nmol N g}^{-1} \text{h}^{-1}$  in July (Fig. 2 and Table S2). In the study area, significantly spatial and temporal differences occurred for nitrogen fixation rates (two-way ANOVA,  $F = 46.24$ ,  $df = 18$ ,  $p < 0.01$ ). The maximal rates of nitrogen fixation appeared at sites M5 and N1 in March and July, respectively. However, the minimal rates were recorded at sites N5 and S4 in March and July, respectively. In addition, March average rate of nitrogen fixation in the transect M1 to M7 (0.51  $\text{nmol N g}^{-1} \text{h}^{-1}$ ) was greater than that in the transect N1 to N7 (0.42  $\text{nmol N g}^{-1} \text{h}^{-1}$ ) and in the transect S1 to S5 (0.32  $\text{nmol N g}^{-1} \text{h}^{-1}$ ). In contrast, higher average nitrogen fixation rate in July was observed in the transect N1 to N7 (3.01  $\text{nmol N g}^{-1} \text{h}^{-1}$ ), followed by the transect M1 to M7 (2.64  $\text{nmol N g}^{-1} \text{h}^{-1}$ ) and the transect S1 to S5 (2.01  $\text{nmol N g}^{-1} \text{h}^{-1}$ ).

### 3.3. Abundance of *nifH* gene

Abundance of *nifH* gene ranged from  $0.36 \times 10^6$  to  $4.12 \times 10^7$  copies  $\text{g}^{-1}$  and from  $2.25 \times 10^6$  to  $5.39 \times 10^7$  copies  $\text{g}^{-1}$  in March and July, respectively (Fig. 3). A significantly spatial difference in *nifH* gene abundance was observed among the sampling sites (one-way ANOVA,  $n = 114$ ,  $F = 3.240$ ,  $p < 0.001$ ). However, no temporal

**Table 1**  
Physicochemical characteristics of sampling sites in the East China Sea<sup>a</sup>.

Sites	Salinity (‰)		pH		$\text{NH}_4^+$ ( $\mu\text{g N g}^{-1}$ )		$\text{NO}_3^-$ ( $\mu\text{g N g}^{-1}$ )		Sulfide ( $\mu\text{g S g}^{-1}$ )		Fe(II) ( $\text{mg g}^{-1}$ )		Fe(III) ( $\text{mg g}^{-1}$ )		OC ( $\text{mg g}^{-1}$ )		C/N	
	March	July	March	July	March	July	March	July	March	July	March	July	March	July	March	July	March	July
N1	30.93	25.44	7.79	7.21	3.10	2.95	0.99	1.14	308.24	115.46	2.13	1.38	0.76	0.25	7.19	5.26	11.14	8.62
N2	31.42	25.65	7.89	7.45	3.26	2.93	1.04	1.48	229.45	101.26	1.70	1.63	0.73	0.04	4.26	6.26	11.04	11.90
N3	31.48	25.65	8.09	7.83	3.49	4.06	1.12	1.50	205.63	158.51	1.58	1.28	0.29	0.31	2.43	4.37	9.56	12.56
N4	32.55	27.40	8.02	8.07	3.34	3.92	1.07	0.38	226.78	35.46	1.52	0.72	0.37	0.97	2.78	3.12	8.66	9.04
N5	32.55	28.53	8.24	8.08	2.91	2.45	0.93	0.42	209.22	10.46	0.62	0.44	1.58	1.45	1.77	2.54	11.47	10.16
N6	32.05	28.02	8.06	8.09	3.03	2.32	0.97	0.38	48.24	29.46	2.64	1.81	0.95	0.74	3.86	3.41	10.06	10.66
N7	32.95	29.56	8.04	8.46	2.89	2.61	0.93	0.34	72.97	15.41	0.86	0.46	1.27	0.96	2.88	2.92	8.32	8.13
M1	29.95	8.14	7.98	8.68	3.68	2.82	1.18	1.70	41.53	156.18	2.21	2.26	1.60	0.12	13.04	9.43	12.07	10.53
M2	31.68	10.45	7.84	8.55	3.44	2.94	1.10	1.73	62.82	146.56	1.26	1.66	0.77	0.40	3.99	7.26	8.87	10.69
M3	33.21	17.59	7.77	8.35	3.31	3.35	1.06	1.73	430.87	169.99	1.39	1.69	0.63	0.26	8.69	8.26	11.00	10.93
M4	32.08	20.45	7.83	8.19	4.16	4.68	1.33	2.14	25.49	301.26	3.77	4.08	1.14	0.49	10.47	10.11	8.44	8.36
M5	32.99	22.45	8.02	8.17	4.25	2.51	1.36	1.42	36.34	56.45	1.87	2.91	1.74	0.66	5.57	4.19	11.66	8.13
M6	33.56	27.09	7.84	8.23	4.18	2.66	1.34	0.04	233.74	41.56	1.67	2.08	0.98	0.40	4.31	2.83	12.49	10.14
M7	33.72	26.57	8.03	8.31	3.91	2.48	1.31	0.21	313.14	65.14	1.22	1.50	0.23	0.48	4.56	4.28	8.35	10.19
S1	28.91	15.97	8.05	8.53	3.77	9.07	1.21	3.72	2029.96	201.22	1.39	2.05	1.91	0.35	7.56	8.15	9.89	10.21
S2	31.84	17.00	8.11	8.53	4.07	5.52	1.30	2.24	802.04	156.66	1.49	1.60	1.70	0.18	8.91	8.56	9.37	9.05
S3	33.51	24.10	7.56	8.46	3.02	2.41	0.97	0.62	78.15	81.41	78.15	1.93	2.81	0.93	3.32	3.85	9.37	6.83
S4	33.60	26.47	7.91	8.49	3.54	3.35	1.13	1.07	333.57	56.24	333.57	1.33	2.05	0.62	3.77	3.93	10.84	13.36
S5	33.84	29.36	8.14	8.43	2.79	2.71	0.89	0.81	236.75	78.56	236.75	1.57	1.05	0.68	4.84	4.21	9.89	10.83

<sup>a</sup> These data represent mean values from triplicate analyses.

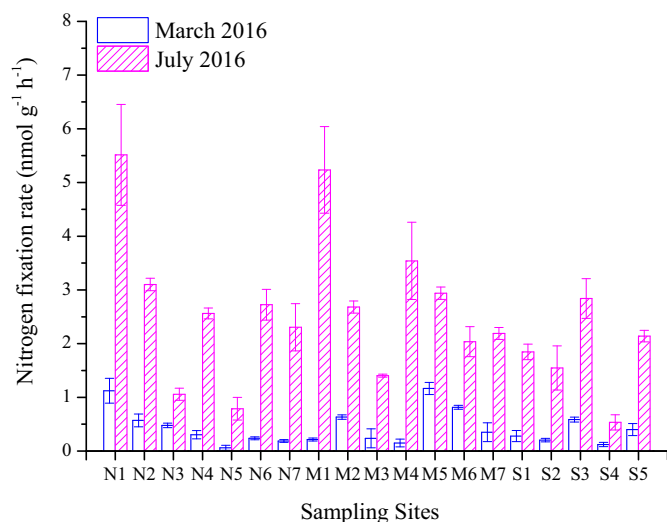


Fig. 2. Nitrogen fixation rates in surface sediments of the East China Sea. The vertical bar denotes standard error of triplicate samples.

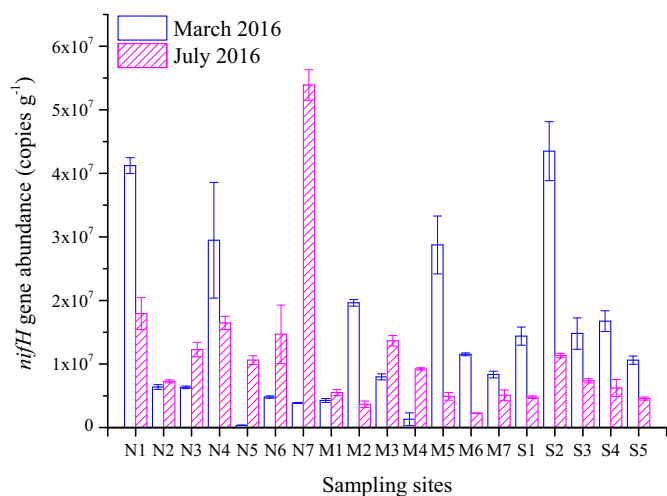


Fig. 3. Abundance of the *nifH* gene in surface sediments of the East China Sea. The vertical bar denotes standard error of triplicate samples.

variation was detected for *nifH* gene abundance (one-way ANOVA,  $n = 114$ ,  $F = 1.818$ ,  $p = 0.180$ ). In March, the highest abundance of *nifH* gene appeared at site N4, and the lowest value occurred at site M4. In July, the highest abundance of *nifH* gene was observed at site N7, and the lowest abundance of *nifH* gene was recorded at site M6.

### 3.4. Factors affecting nitrogen fixation

A RDA test was conducted to reveal the potential relationships among nitrogen fixation rates, *nifH* and environmental factors in sediment samples (Fig. 4). The first two RDA axes explained 33.7% of the cumulative variation of the nitrogen fixation rate with the abundance of *nifH* gene and environmental factors. Linear relationships between nitrogen fixation rates and different environmental factors, *nifH* gene abundance were characterized by the Pearson's correlation coefficients, as shown in Table 2. The rates of nitrogen fixation in the study area correlated significantly with sediment temperature ( $r = 0.707$ ,  $p = 0.000$ ), ferrous iron ( $r = 0.227$ ,  $p = 0.015$ ), sulfide ( $r = -0.233$ ,  $p = 0.012$ ), C/N ratio ( $r = -0.205$ ,  $p = 0.029$ ), ferric iron ( $r = -0.485$ ,  $p = 0.000$ ) and the salinity ( $r = -0.673$ ,  $p = 0.000$ ) as well as water depth ( $r = -0.213$ ,  $p = 0.023$ ). Nevertheless, there were no statistically significant correlations of nitrogen fixation rates with

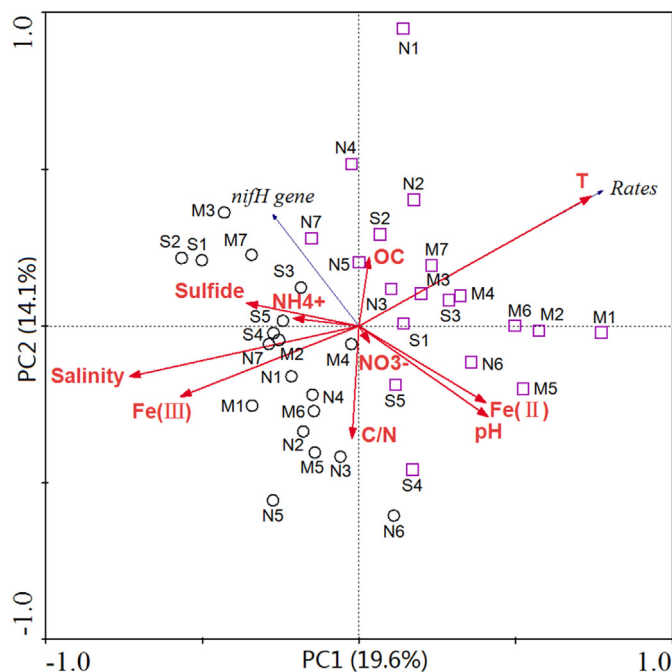


Fig. 4. Redundancy analysis (RDA) of nitrogen fixation rates and abundance of *nifH* gene with sediment characteristics.

inorganic nitrogen ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ), pH and *nifH* gene abundance in the study area.

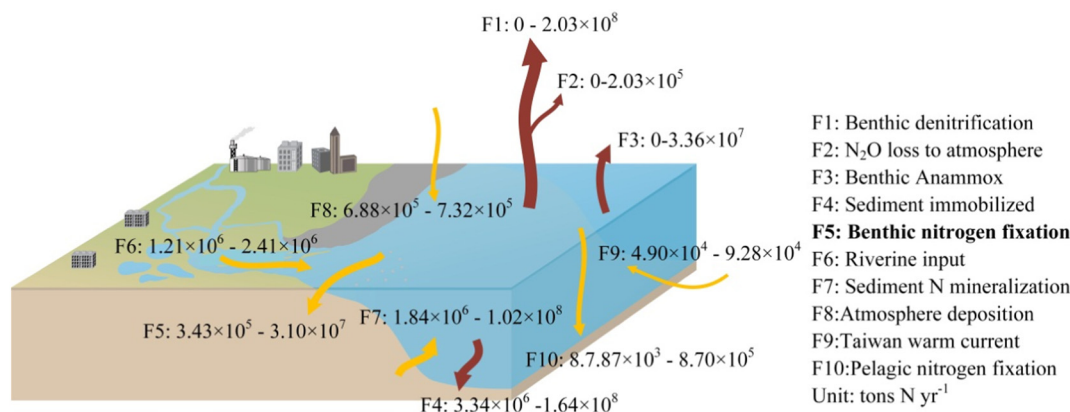
## 4. Discussion

Nitrogen transformation processes have attracted much attention because they are of importance in regulating the nitrogen budget (Yi et al., 2017). The nitrogen fixation has been examined in various environments, especially coastal marine systems (Mehta and Baross, 2006; Man-Aharonovich et al., 2007; Dang et al., 2013; McCarthy et al., 2015; Bae et al., 2018). Benthic sediment nitrogen fixation has been identified as a significant N source of coastal marine environments (Gardner et al., 2006; McCarthy et al., 2015; Newell et al., 2016a, 2016b; Hou et al., 2018). In this study, we investigated the spatial and temporal distributions of nitrogen fixation in the continental shelf sediments of the East China Sea, and their links with environmental characteristics. This study may improve understanding of the nitrogen dynamics in the coastal ecosystems.

In the present study, the *nifH* gene as the N-fixing community was quantified to reveal the microbial effect on nitrogen fixation rates. Although many studies have reported that the nitrogen fixation rate is associated closely with *nifH* gene abundance (Huang et al., 2016; Berthelot et al., 2017; Hou et al., 2018), no significant correlation between them was observed in this study ( $r = -0.044$ ,  $p = 0.641$ ), indicating that the *nifH* gene abundance may not be the crucial factor shaping the nitrogen fixation rate (Caton et al., 2018). By contrast, the activities of nitrogen fixation were significantly influenced by environmental factors, which could be the main mechanisms controlling the spatial and temporal distributions of the nitrogen fixation rates. Generally, temperature is a primary controller of nitrogen fixation because it can affect the community compositions of diazotrophs (Marcarelli and Wurtsbaugh, 2006) and enzymatic activity (Horne and Carmiggelt, 1975; Grimm and Petrone, 1997). Previous studies have documented that high temperature can increase benthic nitrogen fixation rates (Gentili et al., 2005; Houlton et al., 2008; Lindo et al., 2013; Rousk et al., 2017), with the optimal temperatures varying from 20 to 35 °C (Li et al., 2018). In our study, we also observed that nitrogen fixation rates had a significantly positive correlation with temperature,

**Table 2**Pearson's correlations of nitrogen fixation rates with physiochemical characteristics and *nifH* gene abundance (n = 114).

	<i>nifH</i> gene (Copies g <sup>-1</sup> )	Temperature (°C)	Salinity	pH	NH <sub>4</sub> <sup>+</sup> (μg g <sup>-1</sup> )	NO <sub>3</sub> <sup>-</sup> (μg g <sup>-1</sup> )	Sulfide (μg g <sup>-1</sup> )	Fe(II) (mg g <sup>-1</sup> )	Fe(III) (mg g <sup>-1</sup> )	OC (mg g <sup>-1</sup> )	C/N	Depth
Correlation	-0.044	<b>0.707</b>	<b>-0.673</b>	0.119	-0.110	0.100	<b>-0.233</b>	<b>0.227</b>	<b>-0.485</b>	0.112	<b>-0.205</b>	<b>-0.213</b>
Significance (two-tailed)	0.641	<b>0.000</b>	<b>0.000</b>	0.207	0.242	0.290	<b>0.012</b>	<b>0.015</b>	<b>0.000</b>	0.237	<b>0.029</b>	<b>0.023</b>

Note: The coefficients with significant *P* values are shown in bold.**Fig. 5.** General fluxes of nitrogen sources and sinks in the East China Sea. The area assigned for calculation is  $7.7 \times 10^5$  km<sup>2</sup>. The yellow and brown arrows represent the possible nitrogen sources and sinks, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)**Table 3**

Comparison of nitrogen fixation rates in the East China Sea with other benthic environments.

Benthic environment	N-fixation (mmol N m <sup>-2</sup> d <sup>-1</sup> )	Depth of sediment (cm)	Reference
Eutrophic estuary	0–18	0–20	Rao and Charette, 2011
Bioturbated coastal lagoon	0.8–8.5	0–10	Bertics et al., 2010
Baltic Sea, hypoxia basin	0.08–0.22	0–18	Bertics et al., 2013
Peruvian oxygen minimum zone	0.08–0.4	0–20	Gier et al., 2016
Mauritanian upwelling system	0.15	0–20	Gier et al., 2015
Atlantic ocean (2800 m)	0.00008	NA	Howarth et al., 1988
East China Sea	0.09–7.87 <sup>a</sup>	0–5	This study

<sup>a</sup> The unit of the data is converted from mmol N g<sup>-1</sup> h<sup>-1</sup> to mmol N m<sup>-2</sup> d<sup>-1</sup>, assuming that the bulk density of dry sediment is 1.19 g cm<sup>-3</sup>. NA means no data available.

showing the importance of temperature in regulating the seasonal changes of nitrogen fixation potential in the continental shelf of the East China Sea. Nitrogen fixation rates were also observed to correlate negatively with salinity in the study area ( $r = -0.673$ ,  $p = 0.000$ ), which was likely attributed to the inhibition of salinity on the metabolism of diazotrophic bacteria (Tel-or, 1980). In addition, previous studies have documented that the occurrence of nitrogen fixation is favored in neutrality condition (Belay et al., 1984; Burris, 1994). In our study, alkalinity had no significant correlation with nitrogen fixation rates, likely because the pH fluctuated slightly across the sampling sites.

Numerous studies have suggested that sulfate-reducing bacteria can be responsible for the nitrogen fixation (Bertics et al., 2013; Fulweiler et al., 2013; Gier et al., 2016). Thus, nitrogen fixation in sediments is generally coupled to sulfate reduction (Hou et al., 2018). However, a significantly negative relationship was observed between sulfide and nitrogen fixation rates in our study. This correlation implied that sulfide, as an inhibitor for the biological processes (Tam et al., 1982; Reis et al., 1992; Joye and Hollibaugh, 1995; Gier et al., 2016), may suppress the nitrogen fixation in the continental shelf sediments of the East China Sea. In addition, we observed significant correlations of the nitrogen fixation rates with the sedimentary Fe(III) ( $r = -0.485$ ,  $p = 0.000$ ) and Fe(II) ( $r = 0.227$ ,  $p = 0.015$ ). It has been reported that

iron-reducing bacteria may be responsible for the nitrogen fixation (Lovley et al., 1995; Bertics et al., 2010; Fulweiler et al., 2013; Gier et al., 2017). In the continental shelf sediments, the availability of iron may depend on sulfate reduction process (Luther III et al., 1992). The H<sub>2</sub>S and HS, as the soluble sulfur products of sulfate reduction, can reduce Fe(III) to Fe(II) and yield pyrite (FeS<sub>2</sub>) in coastal marine ecosystems (Holguin et al., 2001). Thus, we hypothesize that Fe(III) reduction to Fe(II) can counteract the toxicity of soluble sulfur, and indirectly increase nitrogen fixation rate. However, further investigation is required to confirm the hypothesis about the effects of sulfide and iron interactions on benthic nitrogen fixation.

There are diverse effects of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> on nitrogen fixation in aquatic environments. Some studies indicated that the presence of inorganic nitrogen, especially ammonium, may inhibit nitrogen fixation (Capone et al., 2005; Andersson et al., 2014; Moseman-Valtierra et al., 2016), due to the additional energy cost (Bertics et al., 2010; Knapp, 2012). However, it has also been reported that the occurrence of benthic nitrogen fixation is not inhibited by high content of ammonium (Farnelid et al., 2013; McCarthy et al., 2015; Newell et al., 2016a). These inconsistent results might be attributed to the production of excess electrons by fixing nitrogen for the diazotrophs to maintain the intracellular redox state under high ammonium concentrations (Tichy

and Tabita, 2000). In addition, the organic matter availability can counteract the inhibition of ammonium on nitrogen fixation in benthic environments (Yoch and Whiting, 1986; McGlathery et al., 1998). The microniches among sediment grains can deplete ammonium, which is favorable for the nitrogen fixation (Brandes and Devol, 2002; Bertics and Ziebis, 2010; Bertics et al., 2013). Ammonium concentrations in the continental shelf sediments of the East China Sea were generally lower than  $142 \mu\text{mol g}^{-1}$  ( $10 \mu\text{g N g}^{-1}$ ), which may be the reason why benthic nitrogen fixation was not inhibited by ammonium in this study area (Postgate, 1982; Capone, 1983; Knapp, 2012; Gier et al., 2017).

Organic matter has a great influence on nitrogen fixation because of the supplement of energy for heterotrophic organisms (Howarth et al., 1988; Fulweiler et al., 2007; Gier et al., 2016; Hou et al., 2018). Numerous studies have observed a positive relationship between nitrogen fixation and organic matter (Hartwig and Stanley, 1978; Bertics et al., 2010; Gier et al., 2016; Hou et al., 2018). In our study, however organic carbon had no significant but positive correlation with nitrogen fixation rates ( $r = 0.112$ ,  $p = 0.237$ ). As an important factor regulating nitrogen fixation, the quality of organic matter can also affect the nitrogen fixation (Westrich and Berner, 1984), which is supported by the negative relationship between the sediment C/N ratios and nitrogen fixation rates ( $r = -0.205$ ,  $p = 0.029$ ). The significant correlation between nitrogen fixation rates and water depth ( $r = -0.213$ ,  $p = 0.023$ ) indicated that the sediment characteristics and substrates availability depending on the geographical locations strongly affect nitrogen fixation capacity in the continental shelf sediments of the East China Sea (Levin et al., 2002; Gier et al., 2016).

Based on the measured nitrogen fixation rates ( $0.06\text{--}5.51 \text{ nmol N g}^{-1} \text{ h}^{-1}$ ), the area ( $7.7 \times 10^5 \text{ km}^2$ ), and the bulk sediment density ( $1.19 \text{ g cm}^{-3}$ ; Lin et al., 2016) of the East China Sea, the benthic nitrogen fixation process can fix nitrogen at the rate of  $3.43 \times 10^5\text{--}3.10 \times 10^7 \text{ tons N yr}^{-1}$  in the study area (Fig. 5). In addition, we made a comparison between the nitrogen fixation potential and other nitrogen sources and sinks to reveal the importance of benthic nitrogen fixation in the nitrogen cycling. Of these potential sources of nitrogen in the study area, the fixed nitrogen amount in sediments is higher than the inputs from the Yangtze River ( $1.21 \times 10^6\text{--}2.41 \times 10^6 \text{ tons N yr}^{-1}$ ) (Lin et al., 2016), the pelagic nitrogen fixation ( $7.87 \times 10^3\text{--}8.70 \times 10^5 \text{ tons N yr}^{-1}$ ) (Zhang et al., 2012), the atmosphere deposition ( $6.88 \times 10^5\text{--}7.32 \times 10^5 \text{ tons N yr}^{-1}$ ) and the Taiwan Warm Current ( $4.90 \times 10^4\text{--}9.28 \times 10^4 \text{ tons N yr}^{-1}$ ) (Lin et al., 2016), but lower than the input from sediment N mineralization ( $1.84 \times 10^6\text{--}1.02 \times 10^8 \text{ tons N yr}^{-1}$ ) (Lin et al., 2016). For the sinks of nitrogen, sediment nitrogen fixation capability is higher than sediment  $\text{N}_2\text{O}$  loss to atmosphere ( $0\text{--}2.03 \times 10^5 \text{ tons N yr}^{-1}$ ) (Lin et al., 2017). However, it is much lower than the removal from benthic denitrification ( $0\text{--}2.03 \times 10^8 \text{ tons N yr}^{-1}$ ) (Lin et al., 2017), sediment immobilization ( $3.34 \times 10^6\text{--}1.64 \times 10^8 \text{ tons N yr}^{-1}$ ) (Lin et al., 2016), and comparable to the loss from anammox ( $0\text{--}3.63 \times 10^7 \text{ tons N yr}^{-1}$ ) (Lin et al., 2017). Extrapolating the amounts of nitrogen sources and sinks, the sediment nitrogen fixation accounted for approximately 8.2–22.6% and 7.7–10.2% of the total inorganic nitrogen input and loss, respectively. These results suggested that sediment nitrogen fixation is an important N source and plays a significant role in controlling nitrogen budget in the continental shelf of the East China Sea (Table 3).

## 5. Conclusions

To the best of our knowledge, this is the first study on the nitrogen fixation capability and abundance of *nifH* gene in the continental shelf sediments of the East China Sea. Nitrogen fixation was detected throughout the study area, with potential rates of  $0.06\text{--}5.51 \text{ nmol N g}^{-1} \text{ h}^{-1}$  dry sediment. Abundance of *nifH* gene ranged from  $2.36 \times 10^5$  to  $7.46 \times 10^7$  copies  $\text{g}^{-1}$  dry sediment, however, it was not related to nitrogen fixation rates. Temperature and Fe (II) were the crucial factors regulating the nitrogen fixation process in

the East China Sea. Sulfide was negatively related to nitrogen fixation rates, suggesting sulfide might inhibit the nitrogen fixation. Sediment nitrogen fixation process was estimated to have a fixing nitrogen potential of  $3.43 \times 10^5\text{--}3.10 \times 10^7 \text{ tons N yr}^{-1}$ . This amount accounted for 8.2–22.6% of the total inorganic nitrogen input. Overall, these results highlight the importance of sediment nitrogen fixation in controlling nitrogen budget in the continental shelf of the East China Sea.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marpolbul.2018.10.063>.

## References

- Andersson, B., Sundbäck, K., Hellman, M., Hallin, S., Alsterberg, C., 2014. Nitrogen fixation in shallow-water sediments: spatial distribution and controlling factors. *Limnol. Oceanogr.* 59, 1932–1944.
- Bae, H.S., Morrison, E., Chanton, J.P., Ogram, A., 2018. Methanogens are major contributors to nitrogen fixation in soils of the Florida Everglades. *Appl. Environ. Microbiol.* <https://doi.org/10.1128/AEM.02222-17>.
- Beardsley, R.C., Limeburner, R., Yu, H., Cannon, G.A., 1985. Discharge of the Changjiang (Yangtze river) into the East China sea. *Cont. Shelf Res.* 4, 57–76.
- Belay, N., Sparling, R., Daniels, L., 1984. Dinitrogen fixation by a thermophilic methanogenic bacterium. *Nature* 312, 286–288.
- Benavides, M., Bonnet, S., Berman-Frank, I., Riemann, L., 2018. Deep into oceanic  $\text{N}_2$  fixation. *Front. Mar. Sci.* 5. <https://doi.org/10.3389/fmars.2018.00108>.
- Bentzon-Tilia, M., Traving, S.J., Mantikci, M., Knudsen-Leerbeck, H., Hansen, J.L., Markager, S., Riemann, L., 2015. Significant  $\text{N}_2$  fixation by heterotrophs, photoheterotrophs and heterocystous cyanobacteria in two temperate estuaries. *ISME J.* 9, 273–285.
- Berthelot, H., Benavides, M., Moisaner, P.H., Grosso, O., Bonnet, S., 2017. High-nitrogen fixation rates in the particulate and dissolved pools in the Western Tropical Pacific (Solomon and Bismarck Seas). *Geophys. Res. Lett.* 44, 8414–8423.
- Bertics, V.J., Ziebis, W., 2010. Bioturbation and the role of microniches for sulfate reduction in coastal marine sediments. *Environ. Microbiol.* 12, 3022–3034.
- Bertics, V.J., Sohm, J.A., Treude, T., Chow, C.E.T., Capone, D.G., Fuhrman, J.A., Ziebis, W., 2010. Burrowing deeper into benthic nitrogen cycling: the impact of bioturbation on nitrogen fixation coupled to sulfate reduction. *Mar. Ecol. Prog. Ser.* 409, 1–15.
- Bertics, V.J., Löscher, C.R., Salonen, I., Dale, A.W., Gier, J., Schmitz, R.A., Treude, T., 2013. Occurrence of benthic microbial nitrogen fixation coupled to sulfate reduction in the seasonally hypoxic Eckernförde Bay, Baltic Sea. *Biogeosciences* 10, 1243–1258.
- Brandes, J.A., Devol, A.H., 2002. A global marine-fixed nitrogen isotopic budget: implications for Holocene nitrogen cycling. *Glob. Biogeochem. Cycles* 16, 67–167–14.
- Burris, R.H., 1994. Comparative study of the response of *Azotobacter vinelandii* and *Acetobacter diazotrophicus* to changes in pH. *Protoplasma* 183, 62–66.
- Capone, D.G., 1983. Benthic nitrogen fixation. In: Carpenter, E.J., Capone, D.G. (Eds.), *Nitrogen in the Marine Environment*. Academic, San Diego, Calif, pp. 105–137.
- Capone, D.G., Burns, J.A., Montoya, J.P., Subramaniam, A., Mahaffey, C., Gunderson, T., Michaelis, A.F., Carpenter, E.J., 2005. Nitrogen fixation by *Trichodesmium* spp.: an important source of new nitrogen to the tropical and subtropical North Atlantic Ocean. *Glob. Biogeochem. Cycles* 19, GB2024.
- Caton, I.R., Caton, T.M., Schneegurt, M.A., 2018. Nitrogen-fixation activity and the abundance and taxonomy of *nifH* genes in agricultural, pristine, and urban prairie stream sediments chronically exposed to different levels of nitrogen loading. *Arch. Microbiol.* 200, 623–633.
- Chai, C., Yu, Z., Song, X., Cao, X., 2006. The status and characteristics of eutrophication in the Yangtze River (Changjiang) estuary and the adjacent East China Sea, China. *Hydrobiologia* 563, 313–328.
- Dang, H., Yang, J., Li, J., Luan, X., Zhang, Y., Gu, G., Xue, R., Zong, M., Klotz, M.G., 2013. Environment-dependent distribution of sediment *nifH*-harboring microbiota in the northern South China Sea. *Appl. Environ. Microbiol.* 79, 121–132.
- Deng, F., Hou, L., Liu, M., Zheng, Y., Yin, G., Li, X., Lin, X., Chen, F., Gao, J., Jiang, X., 2015. Dissimilatory nitrate reduction processes and associated contribution to nitrogen removal in sediments of the Yangtze estuary. *J. Geophys. Res. Biogeosci.* 120, 1521–1531.
- Farnelid, H., Bentzon-Tilia, M., Andersson, A.F., Bertilsson, S., Jost, G., Labrenz, M., Jürgens, K., Riemann, L., 2013. Active nitrogen-fixing heterotrophic bacteria at and below the chemocline of the central Baltic Sea. *ISME J.* 7, 1413–1423.
- Fulweiler, R.W., Nixon, S.W., Buckley, B.A., Granger, S.L., 2007. Reversal of the net dinitrogen gas flux in coastal marine sediments. *Nature* 448, 180–182.

- Fulweiler, R.W., Brown, S.M., Nixon, S.W., Jenkins, B.D., 2013. Evidence and a conceptual model for the co-occurrence of nitrogen fixation and denitrification in heterotrophic marine sediments. *Mar. Ecol. Prog. Ser.* 482, 57–68.
- Gardner, W.S., McCarthy, M.J., An, S., Sobolev, D., Sell, K.S., Brock, D., 2006. Nitrogen fixation and dissimilatory nitrate reduction to ammonium (DNRA) support nitrogen dynamics in Texas estuaries. *Limnol. Oceanogr.* 51, 558–568.
- Gentili, F., Nilsson, M.C., Zackrisson, O., Deluca, T.H., Sellstedt, A., 2005. Physiological and molecular diversity of feather moss associative  $N_2$ -fixing cyanobacteria. *J. Exp. Bot.* 56, 3121–3127.
- Gier, J., Sommer, S., Löscher, C.R., Dale, A., Schmitz, R.A., Treude, T., 2015. Nitrogen fixation in sediments along a depth transect through the Eastern Boundary Upwelling Systems off Peru and Mauritania. In: In AGU Fall Meeting Abstracts.
- Gier, J., Sommer, S., Löscher, C.R., Dale, A.W., Schmitz, R.A., Treude, T., 2016. Nitrogen fixation in sediments along a depth transect through the Peruvian oxygen minimum zone. *Biogeosciences* 13, 4065–4080.
- Gier, J., Löscher, C.R., Dale, A.W., Sommer, S., Lomnitz, U., Treude, T., 2017. Benthic dinitrogen fixation traversing the oxygen minimum zone off Mauritania (NW Africa). *Front. Mar. Sci.* 4, 390.
- Grimm, N.B., Petrone, K.C., 1997. Nitrogen fixation in a desert stream ecosystem. *Biogeochemistry* 37, 33–61.
- Großkopf, T., Mohr, W., Baustian, T., Schunck, H., Gill, D., Kuypers, M.M., Lavik, G., Schmitz, R.A., Wallace, D.W., Laroche, J., 2012. Doubling of marine dinitrogen-fixation rates based on direct measurements. *Nature* 488, 361–364.
- Hartwig, E.O., Stanley, S.O., 1978. Nitrogen fixation in Atlantic deep-sea and coastal sediments. *Deep-Sea Res.* 25, 411–417.
- Herbert, R.A., 1975. Heterotrophic nitrogen fixation in shallow estuarine sediments. *J. Exp. Mar. Biol. Ecol.* 18, 215–225.
- Holguin, G., Vazquez, P., Bashan, Y., 2001. The role of sediment microorganisms in the productivity, conservation, and rehabilitation of mangrove ecosystems: an overview. *Biol. Fertil. Soils* 33, 265–278.
- Horne, A.J., Carmiggelt, C.J., 1975. Algal nitrogen fixation in Californian streams: seasonal cycles. *Freshw. Biol.* 5, 461–470.
- Hou, L., Zheng, Y., Liu, M., Gong, J., Zhang, X., Yin, G., You, L., 2013. Anaerobic ammonium oxidation (anammox) bacterial diversity, abundance, and activity in marsh sediments of the Yangtze estuary. *J. Geophys. Res. Biogeosci.* 118, 1237–1246.
- Hou, L., Yin, G., Liu, M., Zhou, J., Zheng, Y., Gao, J., Zong, H., Yang, Y., Gao, L., Tong, C., 2015a. Effects of sulfamethazine on denitrification and the associated  $N_2O$  release in estuarine and coastal sediments. *Environ. Sci. Technol.* 49, 326–333.
- Hou, L., Zheng, Y., Liu, M., Li, X., Lin, X., Yin, G., Gao, J., Deng, F., Chen, F., Jiang, X., 2015b. Anaerobic ammonium oxidation and its contribution to nitrogen removal in China's coastal wetlands. *Sci. Rep.* 5, 15621.
- Hou, L., Wang, R., Yin, G., Liu, M., Zheng, Y., 2018. Nitrogen fixation in the intertidal sediments of the Yangtze estuary: occurrence and environmental implications. *J. Geophys. Res. Biogeosci.* 123, 936–944.
- Houlton, B.Z., Wang, Y.P., Vitousek, P.M., Field, C.B., 2008. A unifying framework for dinitrogen fixation in the terrestrial biosphere. *Nature* 454, 327–330.
- Howarth, R.W., Marino, R., Lane, J., Cole, J.J., 1988. Nitrogen fixation in freshwater, estuarine, and marine ecosystems. 1. Rates and importance. *Limnol. Oceanogr.* 33, 669–687.
- Hu, L., Guo, Z., Feng, J., Yang, Z., Fang, M., 2009. Distributions and sources of bulk organic matter and aliphatic hydrocarbons in surface sediments of the Bohai Sea, China. *Mar. Chem.* 113, 197–211.
- Huang, J., Xu, X., Wang, M., Nie, M., Qiu, S., Wang, Q., Quan, Z., Xiao, M., Li, B., 2016. Responses of soil nitrogen fixation to *Spartina alterniflora* invasion and nitrogen addition in a Chinese salt marsh. *Sci. Rep.* 6, 20384.
- Ichikawa, H., Beardsley, R.C., 2002. The current system in the yellow and East China seas. *J. Oceanogr.* 58, 77–92.
- Joye, S.B., Hollibaugh, J.T., 1995. Influence of sulfide inhibition of nitrification on nitrogen regeneration in sediments. *Science* 270, 623–625.
- Knapp, A., 2012. The sensitivity of marine  $N_2$  fixation to dissolved inorganic nitrogen. *Front. Microbiol.* 3, 374.
- Larmola, T., Leppänen, S.M., Tuittila, E.S., Aarva, M., Merilä, P., Fritze, H., Tiirola, M., 2014. Methanotrophy induces nitrogen fixation during peatland development. *Proc. Natl. Acad. Sci.* 111, 734–739.
- Laroche, J., Breitbarth, E., 2005. Importance of the diazotrophs as a source of new nitrogen in the ocean. *J. Sea Res.* 53, 67–91.
- Lee, K., Matsuno, T., Endoh, T., Ishizaka, J., Zhu, Y., Takeda, S., Sukigara, C., 2017. A role of vertical mixing on nutrient supply into the subsurface chlorophyll maximum in the shelf region of the East China Sea. *Cont. Shelf Res.* 143, 139–150.
- Levin, L., Gutiérrez, D., Rathburn, A., Neira, C., Sellanes, J., Munoz, P., Gallardo, V., Salamanca, M., 2002. Benthic processes on the Peru margin: a transect across the oxygen minimum zone during the 1997–98 El Niño. *Prog. Oceanogr.* 53, 1–27.
- Li, X., Hou, L., Liu, M., Zheng, Y., Yin, G., Lin, X., Cheng, L., Li, Y., Hu, X., 2015. Evidence of nitrogen loss from anaerobic ammonium oxidation coupled with ferric iron reduction in an intertidal wetland. *Environ. Sci. Technol.* 49, 11560–11568.
- Li, D., Zhang, Q., Xiao, K., Wang, Z., Wang, K., 2018. Divergent responses of biological nitrogen fixation in soil, litter and moss to temperature and moisture in a karst forest, southwest China. *Soil Biol. Biochem.* 118, 1–7.
- Lin, X., Hou, L., Liu, M., Li, X., Zheng, Y., Yin, G., Gao, J., Jiang, X., 2016. Nitrogen mineralization and immobilization in sediments of the East China Sea: spatio-temporal variations and environmental implications. *J. Geophys. Res. Biogeosci.* 121, 2842–2855.
- Lin, X., Liu, M., Hou, L., Gao, D., Li, X., Lu, K., Gao, J., 2017. Nitrogen losses in sediments of the East China Sea: spatiotemporal variations, controlling factors, and environmental implications. *J. Geophys. Res. Biogeosci.* 122, 2699–2715.
- Lindo, Z., Nilsson, M.C., Gundale, M.J., 2013. Bryophyte-cyanobacteria associations as regulators of the northern latitude carbon balance in response to global change. *Glob. Chang. Biol.* 19, 2022–2035.
- Liu, J.P., Li, A.C., Xu, K.H., Velozzi, D.M., Yang, Z.S., Milliman, J.D., Demaster, D.J., 2006. Sedimentary features of the Yangtze River-derived along-shelf clinoform deposit in the East China Sea. *Cont. Shelf Res.* 26, 2141–2156.
- Lovley, D.R., Phillips, E.J., Lonergan, D.J., Widman, P.K., 1995. Fe (III) and  $S_0$  reduction by *Pelobacter carbinolicus*. *Appl. Environ. Microbiol.* 61, 2132–2138.
- Luther III, G.W., Kostka, J.E., Church, T.M., Sulzberger, B., Stumm, W., 1992. Seasonal iron cycling in the salt-marsh sedimentary environment: the importance of ligand complexes with Fe (II) and Fe (III) in the dissolution of Fe (III) minerals and pyrite, respectively. *Mar. Chem.* 40, 81–103.
- Man-Aharonovich, D., Kress, N., Zeev, E.B., Berman-Frank, I., Bèjà, O., 2007. Molecular ecology of *nifH* genes and transcripts in the eastern Mediterranean sea. *Environ. Microbiol.* 9, 2354–2363.
- Marcarelli, A.M., Wurtsbaugh, W.A., 2006. Temperature and nutrient supply interact to control nitrogen fixation in oligotrophic streams: an experimental examination. *Limnol. Oceanogr.* 51, 2278–2289.
- McCarthy, M.J., Newell, S.E., Carini, S.A., Gardner, W.S., 2015. Denitrification dominates sediment nitrogen removal and is enhanced by bottom-water hypoxia in the northern gulf of Mexico. *Estuar. Coasts* 38, 2279–2294.
- McGathery, K.J., Risgaard-Petersen, N., Christensen, P.B., 1998. Temporal and spatial variation in nitrogen fixation activity in the eelgrass *Zostera marina* rhizosphere. *Mar. Ecol. Prog. Ser.* 168, 245–258.
- Mehta, M.P., Baross, J.A., 2006. Nitrogen fixation at 92 °C by a hydrothermal vent archaeon. *Science* 314, 1783–1786.
- Mehta, M.P., Butterfield, D.A., Baross, J.A., 2003. Phylogenetic diversity of nitrogenase (*nifH*) genes in deep-sea and hydrothermal vent environments of the Juan de Fuca ridge. *Appl. Environ. Microbiol.* 69, 960–970.
- Mortazavi, B., Riggs, A.A., Caffrey, J.M., Genet, H., Phipps, S.W., 2012. The contribution of benthic nutrient regeneration to primary production in a shallow eutrophic estuary, Weeks Bay, Alabama. *Estuar. Coasts* 35, 862–877.
- Moseman-Valtierra, S., Levin, L.A., Martin, R.M., 2016. Anthropogenic impacts on nitrogen fixation rates between restored and natural Mediterranean salt marshes. *Mar. Ecol. Prog. Ser.* 370, 379.
- Newell, S.E., McCarthy, M.J., Gardner, W.S., Fulweiler, R.W., 2016a. Sediment nitrogen fixation: a call for re-evaluating coastal N budgets. *Estuar. Coasts* 39, 1626–1638.
- Newell, S.E., Pritchard, K.R., Foster, S.Q., Fulweiler, R.W., 2016b. Molecular evidence for sediment nitrogen fixation in a temperate New England estuary. *Peer J* 4, e1615.
- Postgate, J.R., 1982. Biological nitrogen fixation: fundamentals. *Philos. Trans. R. Soc. B* 296, 375–385.
- Rao, A.M., Charette, M.A., 2011. Benthic nitrogen fixation in an eutrophic estuary affected by groundwater discharge. *J. Coastal Res.* 28, 477–485.
- Reis, M.A.M., Almeida, J.S., Lemos, P.C., Carrondo, M.J.T., 1992. Effect of hydrogen sulfide on growth of sulfate reducing bacteria. *Biotechnol. Bioeng.* 40, 593–600.
- Rousk, K., Pedersen, P.A., Dyrnum, K., Michelsen, A., 2017. The interactive effects of temperature and moisture on nitrogen fixation in two temperate-arctic mosses. *Theor. Exp. Plant Physiol.* 29, 25–36.
- Severin, I., Confurius-Guns, V., Stal, L.J., 2012. Effect of salinity on nitrogenase activity and composition of the active diazotrophic community in intertidal microbial mats. *Arch. Microbiol.* 194, 483–491.
- Tam, T.Y., Mayfield, C.I., Inniss, W.E., Knowles, R., 1982. Effect of sulfide on nitrogen fixation in a stream sediment-water system. *Appl. Environ. Microbiol.* 43, 1076–1079.
- Tel-Or, E., 1980. Response of  $N_2$ -fixing cyanobacteria to salt. *Appl. Environ. Microbiol.* 40, 689–693.
- Tichi, M.A., Tabita, F.R., 2000. Maintenance and control of redox poise in *Rhodobacter capsulatus* strains deficient in the Calvin-Benson-Bassham pathway. *Arch. Microbiol.* 174, 322–333.
- Viellard, A.M., Fulweiler, R.W., 2012. Impacts of long-term fertilization on salt marsh tidal creek benthic nutrient and  $N_2$  gas fluxes. *Mar. Ecol. Prog. Ser.* 471, 11–22.
- Westrich, J.T., Berner, R.A., 1984. The role of sedimentary organic matter in bacterial sulfate reduction: the G model tested. *Limnol. Oceanogr.* 29, 236–249.
- Yi, Q., Chen, Q., Hu, L., Shi, W., 2017. Tracking nitrogen sources, transformation, and transport at a basin scale with complex plain river networks. *Environ. Sci. Technol.* 51, 5396–5403.
- Yin, G., Hou, L., Liu, M., Liu, Z., Gardner, W.S., 2014. A novel membrane inlet mass spectrometer method to measure  $^{15}NH_4^+$  for isotope-enrichment experiments in aquatic ecosystems. *Environ. Sci. Technol.* 48, 9555–9562.
- Yoch, D.C., Whiting, G.J., 1986. Evidence for  $NH_4^+$  switch-off regulation of nitrogenase activity by bacteria in salt marsh sediments and roots of the grass *Spartina alterniflora*. *Appl. Environ. Microbiol.* 51, 143–149.
- Zehr, J.P., 2011. Nitrogen fixation by marine cyanobacteria. *Trends Microbiol.* 19, 162–173.
- Zehr, J.P., Kudela, R.M., 2011. Nitrogen cycle of the open ocean: from genes to ecosystems. *Annu. Rev. Mar. Sci.* 3, 197–225.
- Zhang, R., Chen, M., Cao, J., Ma, Q., Yang, J., Qiu, Y., 2012. Nitrogen fixation in the East China Sea and southern Yellow Sea during summer 2006. *Mar. Ecol. Prog. Ser.* 447, 77–86.
- Zheng, Y., Hou, L., Newell, S., Liu, M., Zhou, J., Zhao, H., You, L., Cheng, X., 2013. Community dynamics and activity of ammonia-oxidizing prokaryotes in intertidal sediments of the Yangtze estuary. *Appl. Environ. Microbiol.* 80, 408–419.
- Zheng, Y., Hou, L., Liu, M., Newell, S.E., Yin, G., Yu, C., Zhang, H., Li, X., Gao, D., Gao, J., Wang, R., Liu, C., 2017. Effects of silver nanoparticles on nitrification and associated nitrous oxide production in aquatic environments. *Sci. Adv.* 3, e1603229.
- Zhou, H., Dang, H., Klotz, M.G., 2016. Environmental conditions outweigh geographical contiguity in determining the similarity of *nifH*-harboring microbial communities in sediments of two disconnected marginal seas. *Front. Microbiol.* 7, 1111.