

Response of benthic nitrogen cycling to estuarine hypoxia

Guodong Song^{1b},^{1,2} Sumei Liu,^{1,2*} Jing Zhang,³ Zhuoyi Zhu,³ Guiling Zhang,^{1,2} Hannah K. Marchant,⁴ Marcel M. M. Kuypers,⁴ Gaute Lavik⁴

¹Frontiers Science Center for Deep Ocean Multispheres and Earth System, and Key Laboratory of Marine Chemistry Theory and Technology, Ministry of Education, Ocean University of China, Qingdao, China

²Laboratory for Marine Ecology and Environmental Science, Qingdao National Laboratory for Marine Science and Technology, Qingdao, China

³State Key Laboratory of Estuarine and Coastal Research, East China Normal University, Shanghai, China

⁴Department of Biogeochemistry, Max Planck Institute for Marine Microbiology, Bremen, Germany

Abstract

The effects of bottom water oxygen concentration on sediment oxygen uptake, oxygen penetration depth, nitrate and ammonium fluxes, anammox, denitrification, dissimilatory nitrate reduction to ammonium, nitrification, and mineralization were investigated off the Changjiang estuary and its adjacent East China Sea, by combining a seasonal comparison with three artificially induced bottom water oxygen conditions (oxic, ambient, and severe hypoxia). A 50% decrease in in-situ bottom water oxygen concentrations between May and August, led to decreases in the average sediment oxygen uptake and oxygen penetration depth by 23% and 29%, respectively. Anammox rates decreased by a factor of 2.5, and the relative contribution of anammox to the total benthic N-loss decreased from 20% to 7.4%. However, denitrification rates increased, leading to an overall benthic N-loss rate of $0.85 \text{ mmol N m}^{-2} \text{ d}^{-1}$. At the same time, an increasing contribution of dissimilatory nitrate reduction to ammonium to total nitrate reduction led to higher recycling of inorganic nitrogen during hypoxia in August. Under artificially induced conditions of severe hypoxia, there was a sharp decrease in both sediment oxygen uptake and benthic N-loss rates by 88% and 38%, respectively. Nitrate and ammonium fluxes showed complex behavior at different sites which could be related to the repression of sedimentary nitrification below a bottom water oxygen threshold of $9.7 \mu\text{M}$ and increasing dissimilatory nitrate reduction to ammonium. Taken together, our results indicate that changes in benthic nutrient cycling under seasonal hypoxia enhance the retention of both organic and inorganic nitrogen, thereby exacerbating oxygen deficiency.

From a global perspective, nitrogen is usually considered as a limiting factor for primary production in marine environments (Falkowski 1997; Gruber 2008). Its availability depends on the balance between sources and sinks, primarily nitrogen fixation as a source, and denitrification and anammox as sinks (Brandes et al. 2007). However, with intensifying human activity, excess anthropogenically fixed nitrogen is entering estuaries and coastal systems, enhancing eutrophication and/or hypoxia (Diaz and Rosenberg 2008; Zhang et al. 2010). Although a substantial amount of fixed nitrogen can be removed via denitrification and anammox (Seitzinger 1988; Hulth et al. 2005), the nitrogen cycle has been intensively disrupted and is currently functioning far outside the safe operating space for humanity (Steffen et al. 2015).

Marine sediments are hotspots of nitrogen cycling and it is estimated that more than 60% of marine fixed nitrogen loss occurs within them (Brandes et al. 2007; Eugster and

Gruber 2012). The complete benthic nitrogen cycle includes ammonium release during aerobic mineralization, ammonia and nitrite oxidation in the oxic part of the sediment, and dissimilatory nitrate reduction to ammonium, denitrification and anammox in the anoxic part of the sediment (Thamdrup 2012; Kuypers et al. 2018). Of these nitrate reduction processes, denitrification and anammox form N_2 , leading to N-loss, while dissimilatory nitrate reduction to ammonium retains fixed nitrogen. Ammonium that is formed in the sediment, and not ultimately converted to N_2 by anammox or denitrification is released to the water column where it supports pelagic primary productivity.

Several studies have investigated the response of the benthic nitrogen cycle to changes in bottom water oxygen (Rysgaard et al. 1994; Neubacher et al. 2011, 2013; Caffrey et al. 2019), however, even similar studies in the same area have conflicting results (Neubacher et al. 2011, 2013). Hence, we are still unable to predict the response of the benthic nitrogen cycling to expanding coastal hypoxia (Breitburg

*Correspondence: sumeiliu@ouc.edu.cn

et al. 2018). This has far reaching consequences, as shifts in the benthic nitrogen cycle consequently influence pelagic primary productivity, and therefore feedbacks to global climate change. Until now, most previous studies have focused mainly on the responses of denitrification and anammox to changing bottom water oxygen concentrations. However, it is becoming clear that dissimilatory nitrate reduction to ammonium must also be taken into account to understand the changing benthic nitrogen cycle (Song et al. 2013; Bonaglia et al. 2017). Therefore, comprehensive investigation of the response of all three processes to bottom water oxygen concentrations is required.

The Changjiang (Yangtze River) estuary and its adjacent East China Sea is close to the Changjiang Delta region, which has one of the highest population densities in China (Dai et al. 2011; Hu et al. 2014). With the rapid development of China's economy over the past 40 years, the total nitrogen inputs to the Changjiang basin have increased by a factor of 3 from the end 1960s to 1990s (Yan et al. 2003), with net anthropogenic nitrogen inputs doubling in the 1980–2012 period (Chen et al. 2016). As a result, the nitrogen flux from the Changjiang River into the East China Sea has increased more than two-fold from the early 1980s to 2000 (Li et al. 2007) and the dissolved inorganic nitrogen concentration in the Changjiang estuary has increased by a factor of 2 since 1980s to 2010s (Wang 2006). Consequently, frequent red tides and hypoxia (dissolved oxygen < 62.5 μM , Rabalais et al. 2010) have been reported in the Changjiang estuary and the East China Sea during the past decade (Li et al. 2002; Zhou et al. 2008; Zhu et al. 2011). Considering that the urban population in this region is still growing (Liang et al. 2014), it is unlikely that the situation in the Changjiang estuary and its adjacent East China Sea will improve in the coming years, despite regional improvements in energy-conservation, emission-reduction, and land-based pollutant discharge.

Thus, the Changjiang estuary and its adjacent East China Sea supply an environmentally relevant field site to study the effects of changing bottom water oxygen concentrations on the benthic nitrogen cycle. In this study, we use the ^{15}N isotope pairing technique to investigate the response of the benthic nitrogen cycle to different bottom water oxygen levels caused by both natural decrease and artificially controlled experiments. We investigated how changing bottom water oxygen concentrations impacted (1) benthic O_2 dynamics, (2) benthic nitrogen fluxes, (3) mineralization and nitrification, and (4) anammox, denitrification and, dissimilatory nitrate reduction to ammonium rates.

Materials and methods

Sites description and sampling

Sampling and experiments were conducted during two cruises to the Changjiang estuary and the adjacent East China Sea (Fig. 1; Table 1). From 11 May to 7 June 2011, the cruise

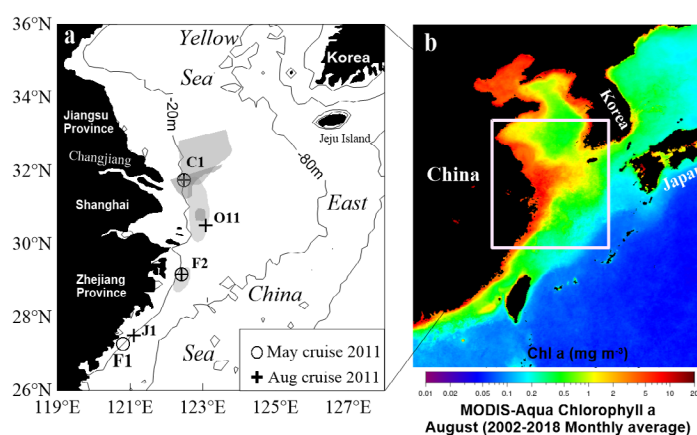


Fig 1. (a) Locations of investigation sites off the Changjiang estuary and its adjacent East China Sea. Historically reported hypoxic zones are indicated by shading, more detailed information can be found in Zhu et al. (2011). **(b)** Average Chlorophyll a concentration in August (2002–2018) in the East China Sea. Source: https://oceancolor.gsfc.nasa.gov/showimages/MODISA/IMAGES/CHL/L3/2002/213/A20022132018243.L3m_MC_CHL_chlor_a_4km.nc.png.

was conducted by *R/V Shiyan 3*, and from 11 to 30 August 2011, the cruise was conducted by *R/V Beidou*. The response of the benthic nitrogen cycle to bottom hypoxia was investigated at five sites. Sites C1 and F2 were sampled on both cruises. Sites F1 and J1 were sampled in May and August respectively, but as they were close to each other and had similar water depths and basic water column and sediment characteristics are treated as repeated sites. Site O11 was only sampled in August. All five sites were located in an area where the probability of hypoxia between depths of 25 and 60 m in summer was high (Zhu et al. 2011). However, there was an unexpected mixing of the usually stratified water column by the Typhoon Muifa before the August cruise, and therefore no strict hypoxia was observed (Chen et al. 2012). Thus, in August we performed additional experiments where we artificially induced severe hypoxia, which was compared to experiments under ambient and oxic conditions, for integrated comparison (see below).

A multi-corer (length = 60 cm, id = 9.5 cm) was used for sediment core collection. Overlying water in the cores was filtered through a 0.45 μm syringe filter into precleaned PE bottles and preserved with saturated HgCl_2 for later nutrient analysis. Sub-cores for oxygen profiles were sampled using a cutoff 60 mL plastic syringe with clear overlying water. Sediment oxygen profiles were measured in duplicates or triplicate within 2 h, using an oxygen microelectrode (OX-25, Unisense, Denmark) installed on a micromanipulator with a resolution of 100 μm . Porewater samples for nutrient analysis were extracted using Rhizons according to Song et al. (2013) and stored in the dark after preservation with HgCl_2 .

Sediments were collected by box corer for slurry, intact core incubations and surface sediment characteristics (porosity,

Table 1. Investigation sites locations and basic characteristics of bottom water and surface sediments.

Site	Cruise	Long. (N)	Lat. (E)	Water				Ambient oxygen (μM)	Ambient NO_x^- (μM)	Ambient NH_4^+ (μM)	Oxygen saturation (%)	Sediment type	Porosity	TOC (%)
				depth (m)	Temp. ($^\circ\text{C}$)	Surfacesalinity (PSU)	Ambient oxygen (μM)							
F1	May	120°48.3'	27°29.6'	25.1	19.1	29.8	184	11.8	1.15	78	Silt-clay	0.77	-	
J1	August	121°06.6'	27°15.6'	27.3	24.7	25.7	84.8	8.6	1.85	40		0.70	0.50	
F2	May	122°24.7'	27°10.3'	39.0	18.1	31.0	160	17.3	0.93	66	Silt-clay	0.80	0.58	
F2	August	122°24.6'	29°10.1'	33.3	23.5	28.1	128	15.6	1.83	59		0.75	0.47	
C1	May	122°28.4'	31°44.6'	26.0	17.2	30.9	235	25.6	0.64	95	Silt-clay-sand	0.62	0.29	
C1	August	122°29.1'	31°44.7'	26.4	22.6	24.6	110	18.6	0.85	49		0.70	0.43	
O11	August	123°05.0'	30°30.4'	59.0	19.5	25.8	87.1	15.6	1.16	37	Silt-clay-sand	0.70	0.24	

total organic carbon). Sub-cores (length = 30 cm, id = 5 cm) were adjusted to ~ 13 cm, sealed with a rubber stopper, capped with lids with magnetic stirring bars and placed in a dark, temperature-controlled container for measurement of intact fluxes and nitrogen transformations (see below). Surface (~ 0–3 cm) sediments for porosity and total organic carbon (TOC) determination were collected using box corer and stored in zip-lock PE bags and frozen at -20°C .

Bottom water was collected from ~ 2 m above the seafloor using Niskin bottles on a CTD-rosette system or using a 60-liter water sampler. Water temperature and salinity were simultaneously measured by CTD. Ambient bottom water dissolved oxygen was analyzed by Winkler titration on board. Water samples for ambient NH_4^+ , NO_3^- , and NO_2^- concentrations analysis were filtered by a $0.45 \mu\text{m}$ syringe filter, and preserved with saturated HgCl_2 in dark. Bottom water for sediment slurry and intact core incubation was distributed into 10-liter PE barrels wrapped in black cloth and stored in the dark at ambient water temperature.

Measuring oxygen uptake and nutrient fluxes with intact core incubations

On the August cruise, three different oxygen conditions were applied to investigate the response of sediment oxygen uptake and nutrient flux to reduced oxygen. Oxic, ambient and severe hypoxia conditions were achieved by continuously bubbling bottom water with air, air + nitrogen or, nitrogen only. Oxygen concentrations in each condition was monitored by O_2 microelectrode (OX-25, Unisense, Denmark) every 2–4 h. The oxygen concentration in the severe hypoxia condition ranged from 11 to $22 \mu\text{M}$ with an average of $16 \mu\text{M}$, corresponding to an oxygen saturation of 7%, far less than the hypoxic criteria. The incubation procedure was similar to Rysgaard et al. (2004) and Song et al. (2016a). For each oxygen condition, 10 sub-cores were pre-incubated for a 6–8 h in the dark by circulation with oxygen adjusted bottom water. Subsequently, intact core incubations were carried out for 16–24 h. Dependent on the change in oxygen concentration (Song et al. 2016a), duplicates or triplicate samples for oxygen and nutrients were taken at 4–8 h intervals. Oxygen was determined by Winkler titration with a precision better than 1% (Song et al. 2016a). Nutrient samples were filtered and preserved as described above. Bottom water from the black barrel was also sampled as control at the same time points. For the May cruise, incubations were conducted only at the ambient oxygen level.

Slurry and intact core incubations to determine benthic anammox, denitrification, and dissimilatory nitrate reduction to ammonium rates

Slurry incubations were carried out to measure anammox, denitrification, and dissimilatory nitrate reduction to ammonium potential rates, the relative contribution of anammox to the benthic N-loss (ra) and the relative contribution of

dissimilatory nitrate reduction to ammonium to the total nitrate reduction according to Thamdrup and Dalsgaard (2002) and Song et al. (2013, 2016b). Briefly, 60 mL of sediment each from 0 to 4 cm was added to three gas-impermeable bags and mixed with 250 mL He-flushed anoxic bottom water. Each bag was then labeled with one of three different tracer combinations ($^{15}\text{NH}_4^+$, $^{15}\text{NH}_4^+ + ^{14}\text{NO}_3^-$, or $^{15}\text{NO}_3^-$), each of which was injected into each bag to a final concentration of 100 μM . Five samples were withdrawn over 8–10 h and preserved in 5.9 mL Exetainer vials (Labco, United Kingdom) pre-filled with 0.1 mL saturated HgCl_2 solution for ^{15}N labeled N_2 and $^{15}\text{NH}_4^+$ analysis.

Intact core incubations to determine benthic anammox, denitrification and dissimilatory nitrate reduction to ammonium fluxes were carried out according to Risgaard-Petersen et al. (2004) and Rysgaard et al. (2004). Briefly, after net flux determination without destructive sampling (see section "Measuring oxygen uptake and nutrient fluxes with intact core incubations"), all cores were pre-incubated again for another 6–8 h under the three oxygen conditions. Then $^{15}\text{NO}_3^-$ tracer was injected into the cores using gas-tight syringe from a stock solution (100 mM, $\text{Na}^{15}\text{NO}_3$, 98% ^{15}N atom%, Sigma-Aldrich) to a final concentration of $\sim 100 \mu\text{M}$. After 0.5–1 h equilibration (to allow establishment of a stable NO_3^-), duplicates or triplicate cores were sacrificed at 2–3 h intervals over the next 8–10 h. Subsamples were collected and preserved as described by Risgaard-Petersen et al. (2004) using 12 mL Exetainer vials (Labco, United Kingdom) for ^{15}N labeled N_2 and $^{15}\text{NH}_4^+$ analysis.

Analytical methods

Samples for nutrient concentrations were analyzed spectrophotometrically on return to the laboratory using an auto-analyzer with a precision of $\sim 5\%$ (Song et al. 2013). Dissolved organic nitrogen (DON) was only measured in August, by the alkaline $\text{K}_2\text{S}_2\text{O}_8$ digestion method (Koroleff 1983).

Sediment porosity was calculated by the weight difference before and after freeze-drying, assuming a sediment particle density of 2.65 g cm^{-3} and seawater density of 1.02 g cm^{-3} . TOC content was measured by an elemental analyzer (Vario EL-III, Elemental Analyzer, Germany) (Zhang et al. 2007).

Samples for $^{29}\text{N}_2$ and $^{30}\text{N}_2$ concentrations were measured using the static headspace method on a GC-IRMS (Holtappels et al. 2011; Song et al. 2013). Samples for $^{15}\text{NH}_4^+$ concentrations from the $^{15}\text{NO}_3^-$ experiment were measured after conversion using hypobromite oxidation, followed by GC-IRMS analysis (Warembourg 1993; Song et al. 2013).

Rate calculations

Sediment oxygen uptake rate and nutrient flux were calculated by linear regression of measured concentration against time (Song et al. 2016a). The units for sediment oxygen uptake and all nitrogen flux were $\text{mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$ and $\text{mmol N m}^{-2} \text{ d}^{-1}$, respectively.

Production rates of $^{29}\text{N}_2$ (P_{29}), $^{30}\text{N}_2$ (P_{30}), and $^{15}\text{NH}_4^+$ ($P_{15}\text{NH}_4^+$) from intact cores amended with $^{15}\text{NO}_3^-$ were calculated by linear regression of measured concentration against time. Subsequently, benthic N-loss rate (P_{14}) was calculated according to Risgaard-Petersen et al. (2003).

$$P_{14} = 2 \times r_{14} \times [P_{29} + P_{30} \times (1 - r_{14})] \quad (1)$$

Where, r_{14} is the ratio of $^{14}\text{NO}_3^-$ to $^{15}\text{NO}_3^-$ undergoing nitrate reduction, which is derived by ra in slurry incubation, and the ratio of $^{29}\text{N}_2$ to $^{30}\text{N}_2$ (R_{29}) in core incubation according to Risgaard-Petersen et al. (2003).

Then anammox and denitrification rates from the core incubations were calculated by,

$$\text{anammox} = P_{14} \times ra \quad (2)$$

$$\text{denitrification} = P_{14} \times (1 - ra) \quad (3)$$

Dissimilatory nitrate reduction to ammonium rate from $^{14}\text{NO}_3^-$ was calculated according to Trimmer and Nicholls (2009).

Coupled nitrification N-loss (P_n) and N-loss supported by $^{14}\text{NO}_3^-$ from the overlying water (P_w) were calculated according to Risgaard-Petersen et al. (2003). Estimated nitrification and mineralization rates were calculated according to Neubacher et al. (2011). If the estimated nitrification and mineralization rates show a negative value, we set it to zero.

Statistical treatments

Differences between benthic fluxes for season (May, August) under ambient oxygen level and artificial oxygen levels (oxic, ambient, and severe hypoxia) in August, were treated by one-way ANOVA (ANOVA) with critical p value of 0.05. Correlations between benthic fluxes and environment parameters were determined using Pearson's correlation analysis with p value of 0.05. All the statistical treatments were run in the Sigmaplot software.

Results

General characteristics

During the May cruise, no water column hypoxia was observed. Bottom waters were well-oxygenated, with an average saturation of more than 80%, except F2 which had a saturation of 66%. On the August cruise, bottom water temperatures were comparatively higher, while surface salinity had decreased and water stratification was enhanced. Consequently, average bottom water oxygen concentrations and saturations decreased from ~ 200 to $\sim 100 \mu\text{M}$ and from 80% to 45%, respectively (Table 1). No strict hypoxia was observed on the August cruise due to the Typhoon Muifa just before our investigation (Chen et al. 2012). Nitrate concentrations in bottom water was 10–27% lower on the August cruise while ammonium concentrations were significantly higher by a

factor of 1.3–2.0. Sediment TOC at F2 was lower, while at C1 it was higher (Table 1).

Pore water profiles of oxygen and nitrate

On the May cruise, oxygen profiles were only measured at C1 and F2 (Fig. 2). On the August cruise, oxygen profiles were measured at all four studied sites with penetration depths ranging from 1.6 ± 0.1 to 3.8 ± 0.1 mm. For the repeated sites C1 and F2, oxygen penetration depths decreased significantly between May and August, from 4.0 ± 0.1 mm to 1.6 ± 0.1 mm at C1 and from 4.3 ± 0.2 mm to 3.0 ± 0.1 mm at F2 (Figs. 2, 3). On average, therefore, oxygen penetration depths decreased by 29% in August compared to May.

Nitrate concentrations within the sediment decreased sharply to a concentration less than $0.5 \mu\text{M}$ at 2 cm at both C1 and F2 on the May cruise, however, a nitrate peak in surface 0–1 cm at C1 was observed indicating active nitrification (Fig. 4). At site F1, nitrate also showed a decreasing pattern with increasing sediment depth, however, it was never less than $0.5 \mu\text{M}$ even down to 9 cm and in some layers, disorderly peaks appeared (Fig. 4). On the August cruise, nitrate profiles at all sites exhibited a sharp decline down to 0.5 – $1 \mu\text{M}$ at 2 cm depth and there were no pronounced nitrification layers found, constraining to less than $0.5 \mu\text{M}$ (Fig. 4).

Sediment oxygen uptake

In the incubations carried out at ambient oxygen concentrations, sediment oxygen uptake decreased significantly

between May and August at stations J1 and F2 by a factor of 47% and 23%, respectively (from 11.6 and $17.6 \text{ mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$ in May to 6.1 and $13.6 \text{ mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$ in August, Fig. 5). On the contrary, sediment oxygen uptake increased by 40% at C1, from 12 to $17 \text{ mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$ (Fig. 5).

For the incubations carried out at different oxygen levels during the August cruise, sediment oxygen uptake was much lower in the severe hypoxia condition compared to other two oxygen conditions at all sites (ANOVA, $p < 0.001$). Compared to the ambient oxygen condition, sediment oxygen uptake decreased in the severe hypoxia condition from 13.6 to $2.0 \text{ mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$ at F2, from 5.4 to $3.1 \text{ mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$ at O11 and from 17.0 and $6.1 \text{ mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$ to zero at J1 and C1, respectively (Fig. 5). On average this represented a decrease of 88%.

Partitioning of anammox, denitrification, and dissimilatory nitrate reduction to ammonium in slurry incubations

In May, ra in the slurry incubations ranged from 13.3% to 30.4% with an average of 20% (Fig. 6). While dissimilatory nitrate reduction to ammonium accounted for 3.9–5.1% with an average of 4.5% in total nitrate reduction (anammox + denitrification + dissimilatory nitrate reduction to ammonium) (Fig. 6). In August, ra was lower, ranging from 2.5% to 17.1% with an average of 7.4%. In August, dissimilatory nitrate reduction to ammonium contributed 3.5–35.9% with an average of 15.4%, which was higher than that in May (ANOVA, $p < 0.05$).

Benthic N-loss and dissimilatory nitrate reduction to ammonium rates in core incubations

In the incubations carried out at ambient oxygen concentrations, in May, benthic N-loss rates in the core incubations ranged from 0.62 to $0.92 \text{ mmol N m}^{-2} \text{ d}^{-1}$ with an average of $0.78 \text{ mmol N m}^{-2} \text{ d}^{-1}$. Coupled nitrification N-loss (P_n) accounted for 56–87% of total benthic N-loss, with an average of 67%. Dissimilatory nitrate reduction to ammonium rates measured at the same time were much lower, and fluctuated between 0.01 and $0.04 \text{ mmol N m}^{-2} \text{ d}^{-1}$, with an average value of only $0.02 \text{ mmol N m}^{-2} \text{ d}^{-1}$ (Fig. 7). In August, benthic N-loss rates ranged from 0.72 to $1.13 \text{ mmol N m}^{-2} \text{ d}^{-1}$ with an average of $0.85 \text{ mmol N m}^{-2} \text{ d}^{-1}$. Denitrification rates ranged from 0.61 and $1.09 \text{ mmol N m}^{-2} \text{ d}^{-1}$, and anammox rates were between 0.02 and $0.12 \text{ mmol N m}^{-2} \text{ d}^{-1}$. P_n was more variable than in May, from 12% to 89% with an average of 50% (Fig. 7). Dissimilatory nitrate reduction to ammonium rates ranged from 0 to $0.21 \text{ mmol N m}^{-2} \text{ d}^{-1}$ with an average value of $0.10 \text{ mmol N m}^{-2} \text{ d}^{-1}$. There was no significant difference between benthic N-loss rates at ambient oxygen conditions in May and August (ANOVA, $p = 0.595$), although the average anammox rates were 2.5-fold lower in August (ANOVA, $p = 0.038$). Dissimilatory nitrate reduction to

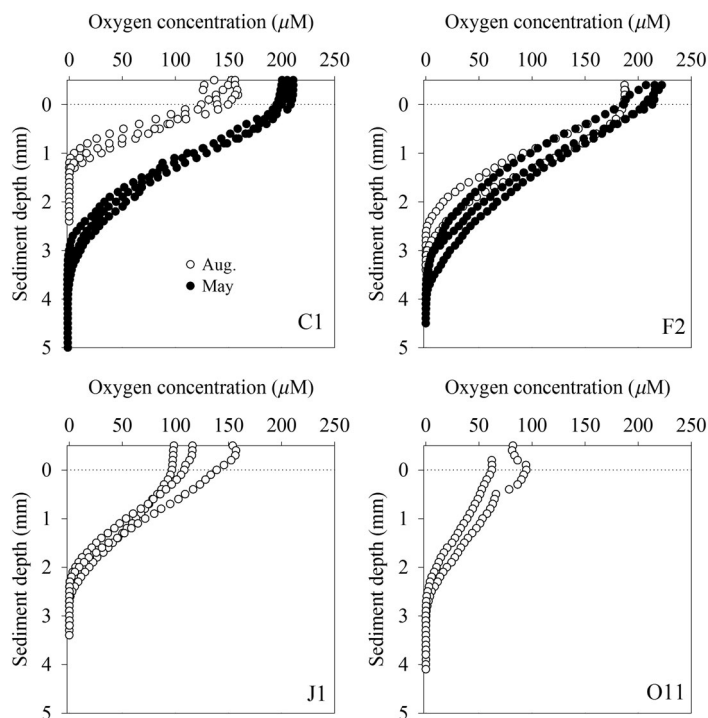


Fig. 2. Oxygen profiles in sediments measured under ambient oxygen condition.

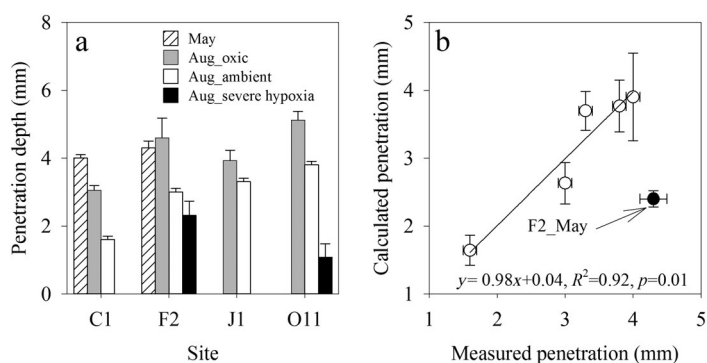


Fig 3. (a) Comparison of sediment oxygen penetration depth in different conditions and (b) comparison of oxygen penetration depths measured by microelectrode and calculated from sediment oxygen uptake. Oxygen penetration depths were calculated using the empirical formula of Cai and Sayles (1996). In panel (a) ambient oxygen data were measured values, while the penetration depths in the oxic and severe hypoxia conditions were calculated values according to linear equation in (b).

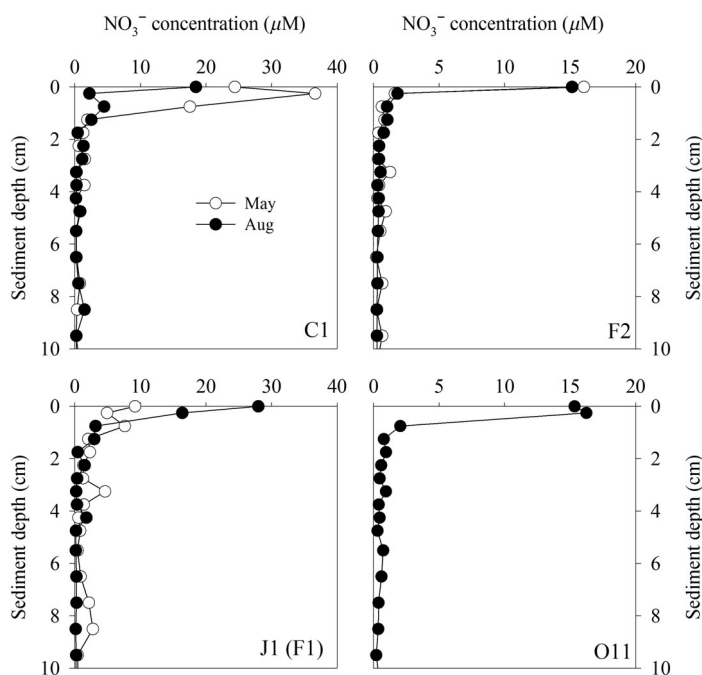


Fig 4. Nitrate profiles in sediments from two cruises.

ammonium rates increased significantly at C1 from 0.011 to 0.21 $\text{mmol N m}^{-2} \text{d}^{-1}$, whereas there was no change at J1 and F2. Dissimilatory nitrate reduction to ammonium was not measured in May at O11, therefore no comparison can be made.

In the incubations carried out under oxic and ambient oxygen conditions in August, there were no significant differences at any of the stations, or overall, in benthic N-loss or dissimilatory nitrate reduction to ammonium rates (ANOVA, $p = 0.343$ for benthic N-loss and $p = 0.431$ for dissimilatory nitrate reduction to ammonium).

In the incubations carried out under severe hypoxia in August, compared with ambient oxygen condition, benthic N-loss rates from both denitrification and anammox decreased by 32–44%, with an average of 38% at all sites (Fig. 7). Similarly, P_n showed a pronounced decrease under severe hypoxia. At C1 and O11, P_w was the main contributor to the benthic N-loss with a fraction of 86% (Fig. 7). Compared to the ambient condition, dissimilatory nitrate reduction to ammonium rates increased in the severe hypoxia condition by a third at C1 from 0.21 to 0.32 $\text{mmol N m}^{-2} \text{d}^{-1}$, from non-detectable to 0.04 $\text{mmol N m}^{-2} \text{d}^{-1}$ at F2, remained the same at J1 and dropped from 0.13 to 0.02 $\text{mmol N m}^{-2} \text{d}^{-1}$ at O11 (Fig. 7). Overall, the contribution of dissimilatory nitrate reduction to ammonium to total nitrate reduction increased in the severe hypoxia condition from 5% to 30% at C1, from 0% to 7% at F2, from 0% to 5% at J1 and decreased from 13% to 9% at O11.

Nutrient net fluxes, nitrification and mineralization

In the incubations carried out under ambient oxygen concentrations, benthic NH_4^+ flux varied between sites and seasons. In May, the only detectable NH_4^+ flux was a net efflux at J1 (0.04 $\text{mmol N m}^{-2} \text{d}^{-1}$, Fig. 5). While in August, there was a net NH_4^+ efflux at C1 and F2. A net NH_4^+ influx into the sediment was observed at J1 and there was no detectable NH_4^+ flux at O11 (Fig. 5). In May, sediments were a source of nitrate to overlying water, with effluxes of 0.3 and 0.5 $\text{mmol N m}^{-2} \text{d}^{-1}$ at C1 and J1 and were a sink of nitrate at F2 with an influx of 0.4 $\text{mmol N m}^{-2} \text{d}^{-1}$. In August, there was an influx of NO_3^- at C1 and O11 (0.5 and 0.2 $\text{mmol N m}^{-2} \text{d}^{-1}$, respectively). There was a net efflux of NO_3^- (0.6 $\text{mmol N m}^{-2} \text{d}^{-1}$) at both F2 and J1 (Fig. 5).

In the incubations carried out at different oxygen concentrations in August, there was no detectable NH_4^+ flux under oxic conditions at any sites except for O11 (Fig. 5). However, in the severe hypoxia conditions, sediments were a source of NH_4^+ , with pronounced effluxes at all sites ranging between 0.4 and 0.8 $\text{mmol N m}^{-2} \text{d}^{-1}$ (Fig. 5).

Under oxic conditions, sediments were a source of nitrate at all sites except C1, with effluxes ranging from 0.1 to 0.6 $\text{mmol N m}^{-2} \text{d}^{-1}$. In contrast, at C1 there was a net uptake of NO_3^- into the sediment of 0.4 $\text{mmol N m}^{-2} \text{d}^{-1}$ (Fig. 5). In severe hypoxia conditions, there was an influx of NO_3^- into the sediment at all sites with an average of 1 $\text{mmol N m}^{-2} \text{d}^{-1}$ (Fig. 5).

Under the oxic and hypoxic conditions, DON fluxes showed a large variation with influx, efflux or no detected flux at different sites (Fig. 5). However, in the ambient condition, DON consistently showed influx at all sites ranging from 0.66 to 1.68 $\text{mmol N m}^{-2} \text{d}^{-1}$ with an average of 1.13 $\text{mmol N m}^{-2} \text{d}^{-1}$ (Fig. 5).

The estimated nitrification rates decreased sharply in the severely hypoxic conditions compared to the oxic and/or the

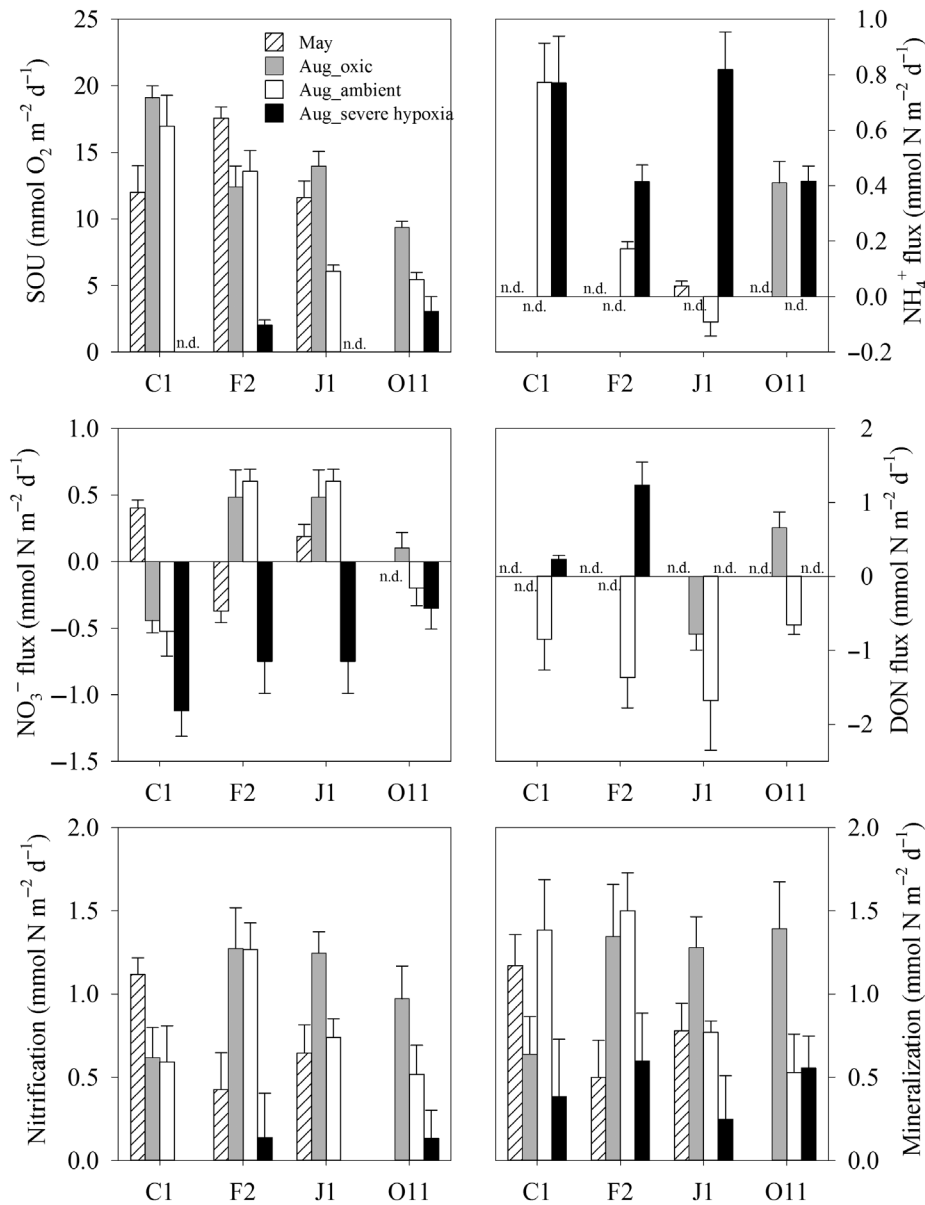


Fig 5. Sediment oxygen uptake (SOU), net flux of NH_4^+ , NO_3^- , DON, nitrification, and mineralization rates from intact core incubation. n.d.: None detectable. For nutrient flux, positive values represent release from sediment to overlying water and vice versa. The nitrification and mineralization rates were estimated by the approach of Gihring et al. (2010) and Neubacher et al. (2011), respectively. Minus nitrification value indicated nitrification was broken down under severe hypoxia.

ambient conditions (Fig. 5). The estimated mineralization rates showed a similar variation as nitrification.

Correlations among environmental parameters and benthic nitrogen transformation rates

Pearson correlation (Table 2) revealed that sediment oxygen uptake was positively correlated with bottom water DO, while NH_4^+ flux was negatively correlated with bottom water DO. NO_3^- flux was positively correlated with bottom water DO, and negatively correlated with NH_4^+ flux. There was no significant correlation between DON flux and any environmental parameters or benthic nitrogen transformation rates.

Benthic N-loss rates were positively correlated with bottom water DO and sediment oxygen uptake, while denitrification rate was positively correlated with sediment oxygen uptake and benthic N-loss rate. Anammox rate was positively correlated with P_n and negatively correlated with dissimilatory nitrate reduction to ammonium rate and P_w . Dissimilatory nitrate reduction to ammonium rate was positively correlated with NH_4^+ flux, and negatively correlated with NO_3^- flux. Both nitrification and mineralization rates were positively correlated with bottom water DO, sediment oxygen uptake, NO_3^- flux and P_n . A high positive correlation between nitrification and mineralization rates was found.

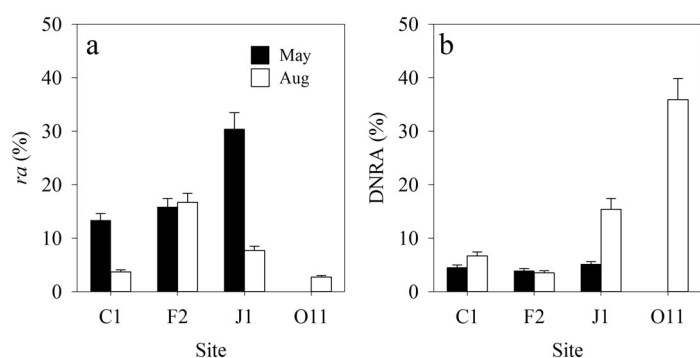


Fig 6. The relative contribution of anammox to the benthic N-loss ($ra\%$) (a) and dissimilatory nitrate reduction to ammonium to total benthic nitrate reduction (DNRA%) (b) in two cruises.

Discussion

Benthic oxygen and nitrogen transformations off the Changjiang estuary under ambient oxygen concentrations

In the present study, sediment oxygen uptake at ambient oxygen concentrations ranged from 5.44 to 17.56 $\text{mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$, which is consistent with a previous study in the East China Sea (Song et al. 2016a), and studies from other continental shelves (Glud 2008). Sediment oxygen uptake is an important proxy of benthic respiration, and our study implies that the East China Sea has a similar remineralization activity to other regions in the world. However, benthic fluxes of NO_3^- and NH_4^+ were lower than expected when compared to the predicted flux from sediment oxygen uptake if we assume organic matter oxidation follows the Redfield ratio between oxygen and nitrogen. Furthermore, NO_3^- and NH_4^+ fluxes showed large fluctuations between different stations and seasons (Fig. 5). This prevented us evaluating the contribution of benthic nitrogen regeneration to pelagic productivity. Taken together these results highlight the need for high temporal and spatial resolution measurements of not just sediment oxygen uptake, but also benthic N-fluxes if we are to fully understand benthic-pelagic coupling. Nevertheless, we were able to investigate the impact of different nitrogen cycling processes under ambient conditions.

Benthic nitrogen loss (via denitrification and anammox) and dissimilatory nitrate reduction to ammonium are important regulatory mechanisms within ecosystems, because the former can reduce and the latter can enhance eutrophication (Seitzinger 1988; Gardner et al. 2006; McCarthy et al. 2015). The co-existence of denitrification, anammox and dissimilatory nitrate reduction to ammonium has been confirmed previously in the Changjiang estuary and the East China Sea using the ^{15}N isotope pairing technique in slurry incubations, however, only potential rates were reported (Song et al. 2013; Deng et al. 2015). To our knowledge, denitrification, anammox, and dissimilatory nitrate reduction to ammonium rates measured using intact core incubations are very scarce

in this area. We found a relatively small variation in the benthic N-loss rates between stations and seasons (19–22%), which implies that benthic N-loss is relatively stable in this region. Thus, we scaled up the measured benthic N-loss rates to the inner shelf of the East China Sea with water depths less than 60 m (area = 1.2×10^5 km^2). The benthic N-loss was estimated as $8.86 \pm 1.73 \times 10^9$ mol N in summer (June to August), which is around one third of the net nitrogen load from Changjiang of 2.66×10^{10} mol N in the summer season (Liu et al. 2009). The same procedure revealed that ammonium fluxes through dissimilatory nitrate reduction to ammonium from the sediment to the water column were around $6.70 \pm 8.53 \times 10^8$ mol N, only 7.6% of the benthic N-loss. Although, there is more uncertainty surrounding the dissimilatory nitrate reduction to ammonium flux, our results clearly demonstrate that benthic nitrogen transformation is a sink for nitrogen in the East China Sea.

Response of oxygen and nitrogen transformations to hypoxia off the Changjiang estuary and its adjacent East China Sea

Dissolved oxygen is usually regarded as the terminal electron acceptor for the oxidation of organic matter in sediments, thus, changes in its concentration/flux into the sediment will impact a series of biogeochemical parameters (Middelburg and Levin 2009). The most direct parameters are sediment oxygen uptake and oxygen penetration depth, which typically decrease under hypoxia (Rasmussen and Jørgensen 1992; Lohse et al. 1996; Rowe et al. 2002; Neubacher et al. 2011), a response that we could confirm in this study, both from repeated measurements in May and August, and by experimentally manipulating the oxygen concentrations in water overlying sediment cores (Fig. 3).

The transformation of inorganic nitrogen species also responded to changing bottom water oxygen concentrations. Changes in NO_3^- and NH_4^+ fluxes, as well as the partitioning of benthic N-loss (via denitrification and anammox) vs. nitrogen retention (through dissimilatory nitrate reduction to ammonium), were observed, when comparing the oxic and severe hypoxia conditions (Figs. 5, 7). For example, there was an NH_4^+ efflux from the sediment at all sites in severe hypoxia condition, and insignificant fluxes in either direction in the oxic condition. Benthic nitrogen fluxes in the ambient condition were less predictable, and both influx and efflux of NH_4^+ were observed. Overall, a negative correlation between NH_4^+ flux and bottom water DO indicates that severe hypoxia promotes NH_4^+ release from sediment (Table 2). The NH_4^+ efflux likely resulted from the increased dissimilatory nitrate reduction to ammonium rates observed at two of the four stations coupled to a decrease in nitrification rates (see below). At the same time, denitrification rates decreased in the severe hypoxia condition (Fig. 7), which was likely due to the tight coupling between benthic N-loss and aerobic mineralization

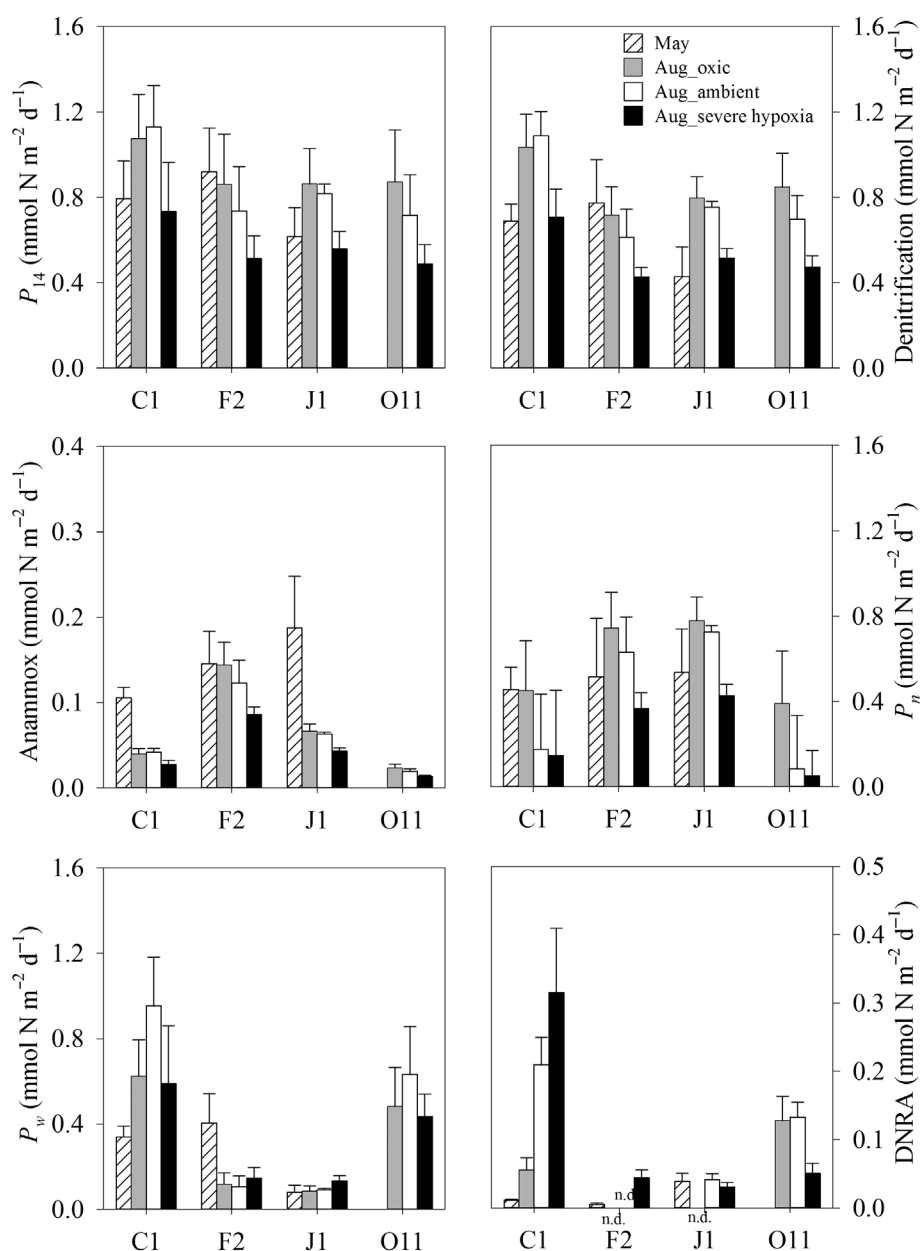


Fig 7. Benthic N-loss (P_{14}), denitrification, anammox, dissimilatory nitrate reduction to ammonium (DNRA), benthic N-loss coupled nitrification (P_n) and supported by overlying water $^{14}\text{NO}_3^-$ (P_w) from intact core incubation in May and August. n.d.: None detectable.

within the sediment (indicated by the positive correlation between P_{14} and sediment oxygen uptake). Overall, the contribution of dissimilatory nitrate reduction to ammonium to benthic nitrate reduction was higher in the severe hypoxia condition compared to the ambient condition, and was as high as 30% at station C1. Together, these results indicate that severe hypoxia can suppress nitrogen loss and increase the recycling of inorganic nitrogen. The influx of DON observed under ambient oxygen concentrations in August indicates that DON is an important participant in the benthic nitrogen

cycle, consistent with the previous observations (Gihring et al. 2010), however the response of DON fluxes to different oxygen concentrations was unclear and warrants more investigation.

Mineralization and nitrification are also integral to the benthic nitrogen cycle and can be impacted by changing oxygen levels. Under oxic conditions, mineralization produces NH_4^+ for nitrification, and the latter can both release NO_3^- to the overlying water and anoxic sediment below. We observed, that upon the onset of severe hypoxia, both mineralization

Table 2. Pearson correlation analysis between bottom oxygen concentrations and measured or predicted nitrogen fluxes. Asterisks denote significance $p < 0.05$. The unit of sediment oxygen uptake is $\text{mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$, and all nitrogen related rates and fluxes units are in $\text{mmol N m}^{-2} \text{ d}^{-1}$. SOU denotes sediment oxygen uptake. Denit., Amox. and DNRA denote denitrification, anammox and dissimilatory nitrate reduction to ammonium rates, respectively. Nit. and Min. denote nitrification and mineralization rates, respectively.

	T (°C)	NO _x ⁻ (μM)	NH ₄ ⁺ (μM)	TOC (%)	SOU	NH ₄ ⁺ flux	NO ₃ ⁻ flux	DON flux	P ₁₄	Denit.	Amox	DNRA	P _n	P _w	Nit.	Min.
DO (μM)	-0.73	0.62	-0.59	-0.14	0.81*	-0.64*	0.61*	-0.15	0.61*	0.47	0.43	-0.35	0.54*	-0.05	0.79*	0.60*
T (°C)		-0.59	0.79*	0.37	-0.19	0.30	0.32	-0.91	0.23	0.28	-0.40	0.21	0.29	-0.10	0.20	0.40
NO _x ⁻ (μM)			-0.76*	-0.43	0.44	0.25	-0.24	0.78	0.30	0.28	-0.03	0.03	-0.38	0.44	0.30	0.36
NH ₄ ⁺ (μM)				0.35	-0.43	-0.27	0.63	-0.88	-0.33	-0.29	-0.07	-0.29	0.56	-0.58	0.30	0.09
TOC (%)					0.51	0.05	-0.01	-0.84	0.38	0.15	0.55	-0.31	0.64	-0.32	-0.15	-0.02
SOU flux						-0.49	0.41	-0.36	0.79*	0.63*	0.41	-0.29	0.41	0.19	0.62*	0.56*
NH ₄ ⁺ flux							-0.72*	0.39	-0.19	-0.04	-0.48	0.62*	-0.57*	0.37	-0.61*	-0.21
NO ₃ ⁻ flux								-0.59	0.16	0.02	0.42	-0.60*	0.71*	-0.52*	0.89*	0.68*
DON flux									-0.33	-0.28	-0.14	0.17	-0.34	0.09	-0.44	-0.31
P ₁₄										0.96*	-0.03	0.19	0.21	0.51	0.44	0.46
Denit.											-0.31	0.33	0.03	0.64*	0.31	0.37
Amox.												-0.53*	0.60*	-0.54*	0.38	0.22
DNRA													-0.66*	0.71*	-0.44	-0.17
P _n														-0.74*	0.66*	0.42
P _w															-0.28	-0.05
Nit.																0.87*

and nitrification were quickly inhibited, and denitrification decreased as only nitrate from the overlying water was available as a substrate. Interestingly, this decrease in coupled nitrification–denitrification only occurred in the severely hypoxic condition and no change was observed between the oxic and ambient oxygen conditions. The oxygen level of the ambient condition in this study was significantly higher than that of hypoxia but lower than in the oxic condition, which implies that there might be a transitional state for the benthic nitrogen transformation. Our results indicate that nitrification rates increased with oxygen concentrations until a value around $117 \mu\text{M}$ (Fig. 8). Above this, nitrification rates showed no clear increase or decrease, and were relatively stable with an average value of $0.91 \pm 0.32 \text{ mmol N m}^{-2} \text{ d}^{-1}$. Below oxygen concentrations of $117 \mu\text{M}$, nitrification rates decreased in concert with oxygen and could be predicted by a linear regression ($y = 0.0085x - 0.0823$, $R^2 = 0.862$, $p = 0.0009$) (Fig. 8). The oxygen concentration corresponding to the intersection of the regression line and $y = 0.91$ line could therefore be regarded as the threshold below which nitrification responds to decreases in oxygen, which is $117 \mu\text{M}$, a value very close to the ambient oxygen condition ($\sim 102 \mu\text{M}$). The linear relationship also indicated that below a threshold of $9.7 \mu\text{M}$ oxygen in the bottom water, nitrification activity in the sediment ceased. Below this threshold, therefore, the overlying water becomes the only significant source of nitrate to fuel nitrate reduction, thus the influx of NO_3^- under the severe hypoxia can be explained (Fig. 5). Moreover, as the oxygen concentration in the ambient oxygen condition lay between the two thresholds, the cause of the variability in NO_3^- and NH_4^+ fluxes in that condition becomes clearer (Fig. 5). The combined results implied that the benthic nitrogen cycle was in a transitional state under the ambient conditions. It is

important to note that there are certain uncertainties in the upper and lower thresholds corresponding to the nitrification activity due to the discrete experimental data. For example, for the upper threshold, the fluctuation is at least $\pm 30\%$. Therefore, when using the threshold to explain the impact of oxygen on benthic nitrogen cycle, special care needs to be taken, and it should be carried out according to the actual situation. Based on these results, a clearer picture of the benthic nitrogen cycle off the Changjiang estuary and its adjacent East China Sea is emerging (Fig. 9).

The global implication of oxygen impact on the benthic nitrogen cycle

Although this study focuses on the effect of hypoxia on nitrogen transformation in the Changjiang estuary and its adjacent East China Sea, it can be expanded to a more general topic, i.e., the response of benthic nitrogen cycle to changes in bottom water oxygen concentrations. So far, tens of thousands of hypoxic sites have been reported (Diaz and Rosenberg 2008; Breitburg et al. 2018), and it is not feasible to check the oxygen response of each one by one. Alternatively, several studies have examined the response of benthic nitrogen cycle to oxygen in different hypoxic systems. These studies cover normoxic or mildly hypoxic (Neubacher et al. 2011), seasonally hypoxic (this study), and persistently anoxic bottom water types (De Brabandere et al. 2015), three typical hypoxic systems according to Diaz and Rosenberg (2008).

Figure 10 details the responses of the benthic nitrogen cycle to changes in oxygen within different hypoxic systems. In normoxic or mildly periodic hypoxic systems, such as the North Sea, benthic nitrogen loss is mainly supported by coupled nitrification–denitrification, where the contribution of P_n to the benthic nitrogen loss can be more than 90% (Weston et al. 2008; Marchant et al. 2016). In such a system, bottom water oxygen decreases will lead to shallower the oxygen penetration depths, less nitrification and therefore less coupled nitrification–denitrification. Neubacher et al. (2011) demonstrated that experimentally induced changes in oxygen concentrations from almost fully oxic conditions (90%) to mildly hypoxic conditions (33% or $\sim 93 \mu\text{M}$), increased the proportion of denitrification supported by water column nitrate. Considering our results, nitrification is not necessarily repressed at this concentration, meaning that the shallower depth of oxygen penetration depth will not cause the magnitude of nitrate downward diffusion flux to change. As a result, we expect that the benthic N-loss will increase slightly in systems exposed to mildly hypoxic conditions compared to normoxic conditions.

For seasonally hypoxic systems, as we have discussed above, hypoxia reduces nitrification and subsequently benthic nitrogen loss, and the relative contribution of dissimilatory nitrate reduction to ammonium increases. Similar observations were reported by Jäntti and Hietanen (2012) who found that the contribution of dissimilatory nitrate reduction to

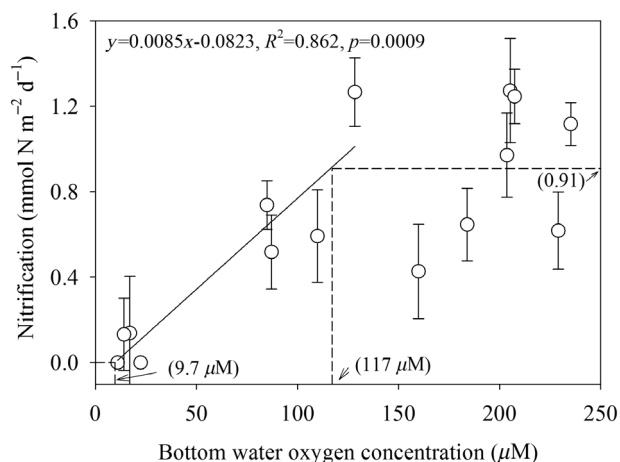


Fig 8. Relationship between estimated nitrification rate and bottom water oxygen concentration. A linear relationship between nitrification and oxygen concentration was found at concentrations lower than $117 \mu\text{M}$. At bottom water oxygen concentrations less than $9.7 \mu\text{M}$, nitrification ceased.

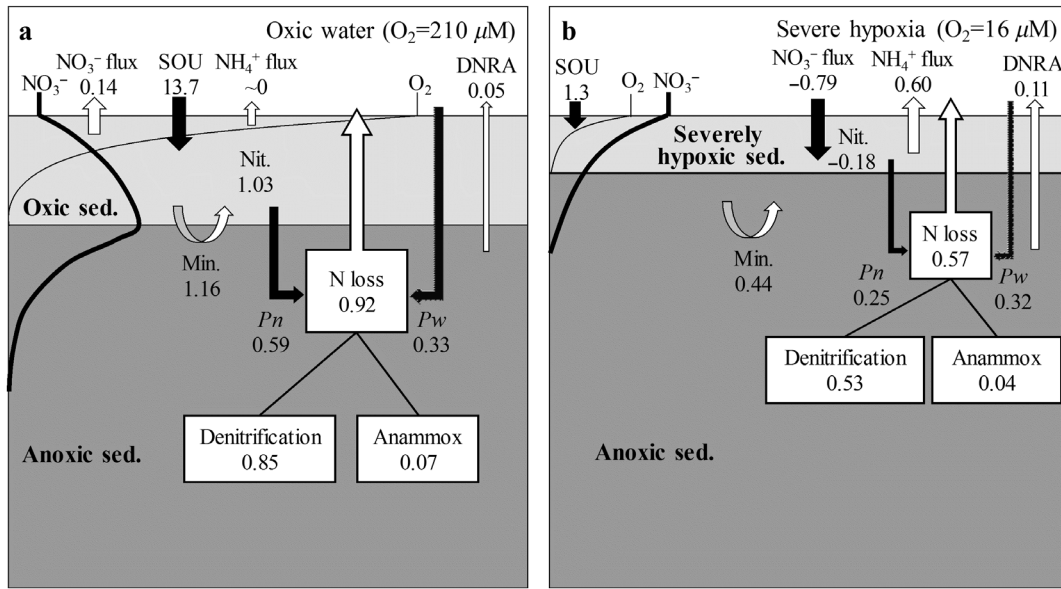


Fig 9. Summary schematic diagram of benthic nitrogen cycle off the Changjiang estuary and its adjacent East China Sea with average rates from two contrasting bottom oxygen conditions: oxic water **(a)** and severe hypoxia **(b)**. Unit: $\text{mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$ for sediment oxygen uptake (SOU), $\text{mmol N m}^{-2} \text{ d}^{-1}$ for all related benthic nitrogen transformations. DNRA denotes dissimilatory nitrate reduction to ammonium.

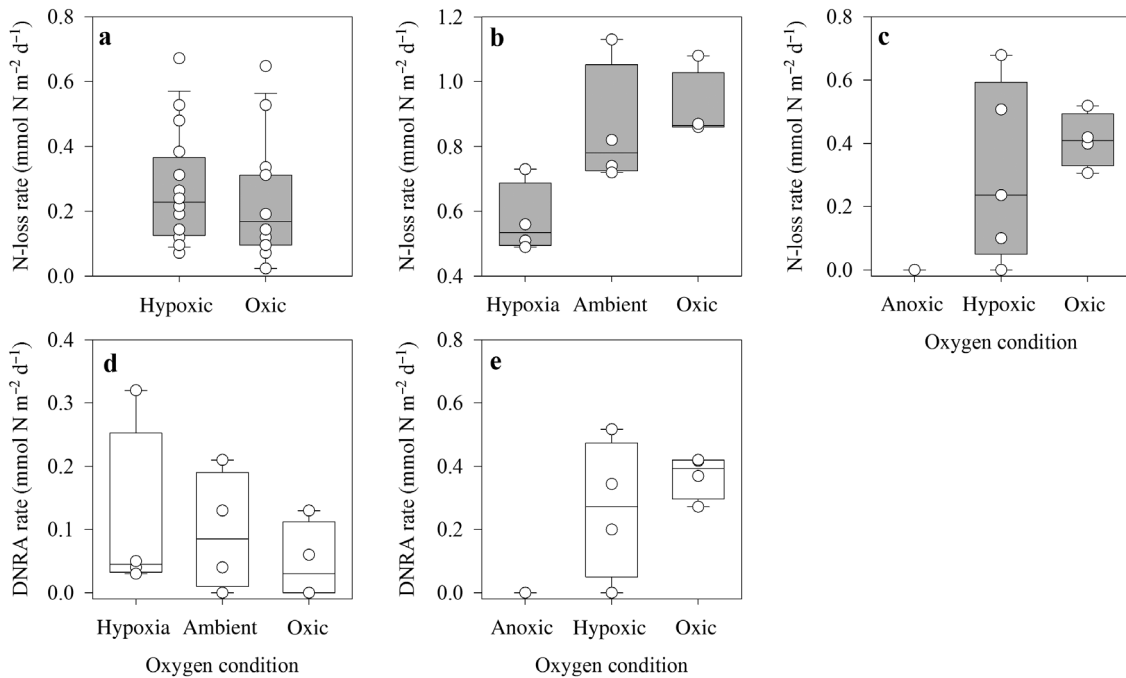


Fig 10. Compiled literature values for the response of benthic nitrogen transformation to bottom water oxygen change. **(a)** the North Sea, a normoxic or mild hypoxic system (Neubacher et al. 2011), **(b and d)** the Changjiang estuary and its adjacent East China Sea, a seasonal hypoxic system (this study), and **(c and e)** the by Fjord on the Swedish west coast, a persistent anoxic bottom water (De Brabandere et al. 2015). In **(c and e)**, only the data from persistent anoxic sites were used. DNRA denotes dissimilatory nitrate reduction to ammonium.

ammonium to nitrate reduction in the Baltic Sea sediment was promoted when bottom water oxygen was less than $110 \mu\text{M}$. Furthermore, when we re-analyzed the data from

Lehrter et al. (2012), we found a positive correlation between benthic N-loss rate and bottom water oxygen that implies hypoxia weakened the benthic N-loss rate on the Louisiana

continental shelf of the Gulf of Mexico. In contrast to Lehrter et al. (2012), McCarthy et al. (2015) found a significant negative correlation between the benthic N-loss rate and bottom water oxygen content in the Gulf of Mexico. This could be due to the influence of other factors such as temperature, organic carbon or nitrate concentration. To test the influence of these, single-factor controlled incubation experiments are required. However, if we want to understand the synergy or coupled effects of various environmental factors changes on the benthic nitrogen cycle, multi-factor-controlled experiments should not be neglected.

In summary, our synthesized analysis indicates that the response of the benthic nitrogen cycle to oxygen under different hypoxic/anoxic scenarios shows different patterns. However, if hypoxia intensifies and persists for a long time, the change in the benthic nitrogen cycle tends to be consistent. That is, the nitrogen loss activity may decrease and the recycling activity is enhanced, which may further aggravate the degree of eutrophication and hypoxia, eventually leading to a water column where oxygen levels are low enough for N-loss to occur. Currently, studies of water column nitrogen loss in seasonally hypoxic waters are rare, but some studies show that the transition from fully oxic to anoxic/sulfidic can occur rapidly in shallow coastal waters (Naqvi et al. 2009). We suggest strengthening this research in the future, as the oxygen content in some seasonally hypoxic waters is already below the oxygen tolerance threshold for anaerobic ammonium oxidation or denitrification, making these waters a potential hot spot for nitrogen loss (Kalvelage et al. 2011; McCarthy et al. 2015).

In addition, our research puts forward higher requirements for evaluating nitrogen budgets of different coastal shelf systems in the future, and also provides a reference for setting parameters in model study. However, the synthesized analysis summarized here assume that different research results are sufficiently representative for each scenario. Furthermore, we currently do not know enough about how methodology impacts results, i.e., long-term vs. short-term oxygen-manipulations (Neubacher et al. 2011, 2013), or slurry vs. intact core incubations (e.g., Caffrey et al. 2019; Cojean et al. 2019). We call for international comparative studies on the response of benthic nitrogen cycle to oxygen change in the future, so that we can better elucidate the benthic nitrogen cycle, especially in the current of exacerbating hypoxia.

References

- Bonaglia, S., and others. 2017. The fate of fixed nitrogen in marine sediments with low organic loading: An in situ study. *Biogeosciences* **14**: 285–300. doi:10.5194/bg-14-285-2017
- Brandes, J., A. Devol, and C. Deutsch. 2007. New developments in the marine nitrogen cycle. *Chem. Rev.* **107**: 577–589. doi:10.1021/cr050377t
- Breitburg, D., and others. 2018. Declining oxygen in the global ocean and coastal waters. *Science* **359**: eaam7240. doi:10.1126/science.aam7240
- Caffrey, J. M., S. Bonaglia, and D. J. Conley. 2019. Short exposure to oxygen and sulfide alter nitrification, denitrification, and DNRA activity in seasonally hypoxic estuarine sediments. *FEMS Microbiol. Lett.* **366**: fny288. doi:10.1093/femsle/fny288
- Cai, W.-J., and F. L. Sayles. 1996. Oxygen penetration depths and fluxes in marine sediments. *Mar. Chem.* **52**: 123–131. doi:10.1016/0304-4203(95)00081-X
- Chen, J. Y., X. B. Ni, Z. H. Mao, Y. F. Wang, L. J. Liang, and F. Gong. 2012. Remote sensing and buoy based effect analysis of typhoon on hypoxia off the Changjiang (Yangtze) estuary, p. 853211. *In* Remote sensing of the ocean, sea ice, coastal waters, and large water regions 2012, v. **8532**. International Society for Optics and Photonics.
- Chen, F., and others. 2016. Net anthropogenic nitrogen inputs (NANI) into the Yangtze River basin and the relationship with riverine nitrogen export. *J. Geophys. Res.-Biogeosci.* **121**: 451–465. doi:10.1002/2015JG003186
- Cojean, A. N., J. Zopfi, A. Gerster, C. Frey, F. Lepori, and M. F. Lehmann. 2019. Direct O₂ control on the partitioning between denitrification and dissimilatory nitrate reduction to ammonium in lake sediments. *Biogeosciences* **16**: 4705–4718. doi:10.5194/bg-16-4705-2019
- Dai, Z. J., J. Z. Du, X. L. Zhang, N. Su, and J. F. Li. 2011. Variation of riverine material loads and environmental consequences on the Changjiang (Yangtze) Estuary in recent decades (1955–2008). *Environ. Sci. Technol.* **45**: 223–227. doi:10.1021/es103026a
- De Brabandere, L., S. Bonaglia, M. Kononets, L. Viktorsson, A. Stigebrandt, B. Thamdrup, and P. J. Hall. 2015. Oxygenation of an anoxic fjord basin strongly stimulates benthic denitrification and DNRA. *Biogeochemistry* **126**: 131–152. doi:10.1007/s10533-015-0148-6
- Deng, F. Y., and others. 2015. Dissimilatory nitrate reduction processes and associated contribution to nitrogen removal in sediments of the Yangtze Estuary. *J. Geophys. Res.-Biogeosci.* **120**: 1521–1531. doi:10.1002/2015JG003007
- Diaz, R. J., and R. Rosenberg. 2008. Spreading dead zones and consequences for marine ecosystems. *Science* **321**: 926–929. doi:10.1126/science.1156401
- Eugster, O., and N. Gruber. 2012. A probabilistic estimate of global marine N-fixation and denitrification. *Global Biogeochem. Cycles* **26**: GB4013. doi:10.1029/2012GB004300
- Falkowski, P. 1997. Evolution of the nitrogen cycle and its influence on the biological sequestration of CO₂ in the ocean. *Nature* **387**: 272–275. doi:10.1038/387272a0
- Gardner, W., M. McCarthy, S. An, D. Sobolev, K. Sell, and D. Brock. 2006. Nitrogen fixation and dissimilatory nitrate

- reduction to ammonium (DNRA) support nitrogen dynamics in Texas estuaries. *Limnol. Oceanogr.* **51**: 558–568. doi:[10.4319/lo.2006.51.1_part_2.0558](https://doi.org/10.4319/lo.2006.51.1_part_2.0558)
- Gihring, T., G. Lavik, M. Kuypers, and J. Kostka. 2010. Direct determination of nitrogen cycling rates and pathways in Arctic fjord sediments (Svalbard, Norway). *Limnol. Oceanogr.* **55**: 740–752. doi:[10.4319/lo.2009.55.2.0740](https://doi.org/10.4319/lo.2009.55.2.0740)
- Glud, R. N. 2008. Oxygen dynamics of marine sediments. *Mar. Biol. Res.* **4**: 243–289. doi:[10.1080/17451000801888726](https://doi.org/10.1080/17451000801888726)
- Gruber, N. 2008. The marine nitrogen cycle: Overview and challenges, p. 1–50. *In* D. G. Capone, D. A. Bronk, M. R. Mulholland, and E. J. Carpenter [eds.], *Nitrogen in the marine environment*. Elsevier.
- Holtappels, M., G. Lavik, M. M. Jensen, and M. M. M. Kuypers. 2011. ¹⁵N-labeling experiments to dissect the contributions of heterotrophic denitrification and anammox to nitrogen removal in the OMZ waters of the ocean, p. 223–251. *In* M. G. Klotz [ed.], *Methods in enzymology*. Elsevier. doi:[10.1016/B978-0-12-381294-0.00010-9](https://doi.org/10.1016/B978-0-12-381294-0.00010-9)
- Hu, J. L., Y. G. Wang, Q. Ying, and H. L. Zhang. 2014. Spatial and temporal variability of PM_{2.5} and PM₁₀ over the North China Plain and the Yangtze River Delta, China. *Atmos. Environ.* **95**: 598–609. doi:[10.1016/j.atmosenv.2014.07.019](https://doi.org/10.1016/j.atmosenv.2014.07.019)
- Hulth, S., and others. 2005. Nitrogen removal in marine environments: Recent findings and future research challenges. *Mar. Chem.* **94**: 125–145, doi:[10.1016/j.marchem.2004.07.013](https://doi.org/10.1016/j.marchem.2004.07.013)
- Jääntti, H., and S. Hietanen. 2012. The effects of hypoxia on sediment nitrogen cycling in the Baltic Sea. *Ambio* **41**: 161–169. doi:[10.1007/s13280-011-0233-6](https://doi.org/10.1007/s13280-011-0233-6)
- Kalvelage, T., and others. 2011. Oxygen sensitivity of Anammox and coupled N-cycle processes in oxygen minimum zones. *PLoS One* **6**: e29299. doi:[10.1371/journal.pone.0029299](https://doi.org/10.1371/journal.pone.0029299)
- Koroleff, F. 1983. Determination of nutrients, p. 125–187. *In* K. Grasshoff, M. Eberhardt, and K. Kremling [eds.], *Methods of seawater analysis*. Verlag Chemie.
- Kuypers, M. M., H. K. Marchant, and B. Kartal. 2018. The microbial nitrogen-cycling network. *Nat. Rev. Microbiol.* **16**: 263–276. doi:[10.1038/nrmicro.2018.9](https://doi.org/10.1038/nrmicro.2018.9)
- Lehrter, J. C., D. L. Beddick, R. Devereux, D. F. Yates, and M. C. Murrell. 2012. Sediment-water fluxes of dissolved inorganic carbon, O₂, nutrients, and N₂ from the hypoxic region of the Louisiana continental shelf. *Biogeochemistry* **109**: 233–252. doi:[10.1007/s10533-011-9623-x](https://doi.org/10.1007/s10533-011-9623-x)
- Li, D. J., J. Zhang, D. J. Huang, Y. Wu, and J. Liang. 2002. Oxygen depletion off the Changjiang (Yangtze River) estuary. *Sci. China. Ser. D* **45**: 1137–1146. doi:[10.1360/02yd9110](https://doi.org/10.1360/02yd9110)
- Li, M., K. Xu, M. Watanabe, and Z. Chen. 2007. Long-term variations in dissolved silicate, nitrogen, and phosphorus flux from the Yangtze River into the East China Sea and impacts on estuarine ecosystem. *Estuar. Coast. Shelf Sci.* **71**: 3–12. doi:[10.1016/j.ecss.2006.08.013](https://doi.org/10.1016/j.ecss.2006.08.013)
- Liang, Z., Z. Li, and Z. D. Ma. 2014. Changing patterns of the floating population in China, 2000–2010. *Popul. Dev. Rev.* **40**: 695–716. doi:[10.1111/j.1728-4457.2014.00007.x](https://doi.org/10.1111/j.1728-4457.2014.00007.x)
- Liu, S. M., G. H. Hong, J. Zhang, X. W. Ye, and X. L. Jiang. 2009. Nutrient budgets for large Chinese estuaries. *Biogeosciences* **6**: 2245–2263. doi:[10.5194/bg-6-2245-2009](https://doi.org/10.5194/bg-6-2245-2009)
- Lohse, L., E. H. G. Epping, W. Helder, and W. Van Raaphorst. 1996. Oxygen pore water profiles in continental shelf sediments of the North Sea: Turbulent versus molecular diffusion. *Mar. Ecol. Prog. Ser.* **145**: 63–75. doi:[10.3354/meps145063](https://doi.org/10.3354/meps145063)
- Marchant, H. K., M. Holtappels, G. Lavik, S. Ahmerkamp, C. Winter, and M. M. M. Kuypers. 2016. Coupled nitrification–denitrification leads to extensive N loss in subtidal permeable sediments. *Limnol. Oceanogr.* **61**: 1033–1048. doi:[10.1002/lno.10271](https://doi.org/10.1002/lno.10271)
- McCarthy, M. J., S. E. Newell, S. A. Carini, and W. S. Gardner. 2015. Denitrification dominates sediment nitrogen removal and is enhanced by bottom-water hypoxia in the Northern Gulf of Mexico. *Estuar. Coast.* **38**: 2279–2294. doi:[10.1007/s12237-015-9964-0](https://doi.org/10.1007/s12237-015-9964-0)
- Middelburg, J., and L. Levin. 2009. Coastal hypoxia and sediment biogeochemistry. *Biogeosciences* **6**: 1273–1293. doi:[10.5194/bg-6-1273-2009](https://doi.org/10.5194/bg-6-1273-2009)
- Naqvi, S. W. A., and others. 2009. Seasonal anoxia over the western Indian continental shelf, Indian Ocean: Biogeochemical processes and ecological variability, p. 333–345. *In* J. D. Wiggert, R. R. Hood, S. W. A. Naqvi, K. H. Brink, and S. L. Smith [eds.], *Geophysical Monograph Series*, v. 185. AGU.
- Neubacher, E. C., R. E. Parker, and M. Trimmer. 2011. Short-term hypoxia alters the balance of the nitrogen cycle in coastal sediments. *Limnol. Oceanogr.* **56**: 651–665. doi:[10.4319/lo.2011.56.2.0651](https://doi.org/10.4319/lo.2011.56.2.0651)
- Neubacher, E. C., R. E. Parker, and M. Trimmer. 2013. The potential effect of sustained hypoxia on nitrogen cycling in sediment from the southern North Sea: A mesocosm experiment. *Biogeochemistry* **113**: 69–84. doi:[10.1007/s10533-012-9749-5](https://doi.org/10.1007/s10533-012-9749-5)
- Rabalais, N., R. Diaz, L. Levin, R. Turner, D. Gilbert, and J. Zhang. 2010. Dynamics and distribution of natural and human-caused hypoxia. *Biogeosciences* **7**: 585–619. doi:[10.5194/bg-7-585-2010](https://doi.org/10.5194/bg-7-585-2010)
- Rasmussen, H., and B. B. Jørgensen. 1992. Microelectrode studies of seasonal oxygen uptake in a coastal sediment: Role of molecular diffusion. *Mar. Ecol. Prog. Ser.* **81**: 289–303. doi:[10.3354/meps081289](https://doi.org/10.3354/meps081289)
- Risgaard-Petersen, N., L. P. Nielsen, S. Rysgaard, T. Dalsgaard, and R. L. Meyer. 2003. Application of the isotope pairing technique in sediments where anammox and denitrification co-exist. *Limnol. Oceanogr.: Methods* **1**: 63–73. doi:[10.4319/lom.2003.1.63](https://doi.org/10.4319/lom.2003.1.63)
- Risgaard-Petersen, N., and others. 2004. Anaerobic ammonium oxidation in an estuarine sediment. *Aquat. Microb. Ecol.* **36**: 293–304. doi:[10.3354/ame036293](https://doi.org/10.3354/ame036293)

- Rowe, G. T., M. E. C. Kaegi, J. W. Morse, G. S. Boland, and E. G. Escobar Briones. 2002. Sediment community metabolism associated with continental shelf hypoxia, northern Gulf of Mexico. *Estuar. Coast.* **25**: 1097–1106. doi:10.1007/BF02692207
- Rysgaard, S., N. Risgaard-Petersen, N. P. Sloth, K. Jensen, and L. P. Nielsen. 1994. Oxygen regulation of nitrification and denitrification in sediments. *Limnol. Oceanogr.* **39**: 1643–1652. doi:10.4319/lo.1994.39.7.1643
- Rysgaard, S., R. Glud, N. Risgaard-Petersen, and T. Dalsgaard. 2004. Denitrification and anammox activity in Arctic marine sediments. *Limnol. Oceanogr.* **49**: 1493–1502. doi:10.4319/lo.2004.49.5.1493
- Seitzinger, S. 1988. Denitrification in freshwater and coastal marine ecosystems: Ecological and geochemical significance. *Limnol. Oceanogr.* **33**: 702–724. doi:10.4319/lo.1988.33.4_part_2.0702
- Song, G. D., S. M. Liu, H. Marchant, M. M. M. Kuypers, and G. Lavik. 2013. Anammox, denitrification and dissimilatory nitrate reduction to ammonium in the East China Sea sediment. *Biogeosciences* **10**: 6851–6864. doi:10.5194/bg-10-6851-2013
- Song, G. D., S. M. Liu, Z. Y. Zhu, W. D. Zhai, C. J. Zhu, and J. Zhang. 2016a. Sediment oxygen consumption and benthic organic carbon mineralization on the continental shelves of the East China Sea and the Yellow Sea. *Deep-Sea Res. II Top. Stud. Oceanogr.* **124**: 53–63. doi:10.1016/j.dsr.2015.04.012
- Song, G. D., S. M. Liu, M. M. M. Kuypers, and G. Lavik. 2016b. Application of the isotope pairing technique in sediments where anammox, denitrification, and dissimilatory nitrate reduction to ammonium coexist: DNRA and isotope pairing. *Limnol. Oceanogr.: Methods* **14**: 801–805. doi:10.1002/lom3.10127
- Steffen, W., and others. 2015. Planetary boundaries: Guiding human development on a changing planet. *Science* **347**: 1259855. doi:10.1126/science.1259855
- Thamdrup, B. 2012. Novel pathways and organisms in global nitrogen cycling. *Annu. Rev. Ecol. Evol. S.* **43**: 407–428. doi:10.1146/annurev-ecolsys-102710-145048
- Thamdrup, B., and T. Dalsgaard. 2002. Production of N₂ through anaerobic ammonium oxidation coupled to nitrate reduction in marine sediments. *Appl. Environ. Microbiol.* **68**: 1312–1318. doi:10.1128/AEM.68.3.1312-1318.2002
- Trimmer, M., and J. Nicholls. 2009. Production of nitrogen gas via anammox and denitrification in intact sediment cores along a continental shelf to slope transect in the North Atlantic. *Limnol. Oceanogr.* **54**: 577–589. doi:10.4319/lo.2009.54.2.0577
- Wang, B. 2006. Cultural eutrophication in the Changjiang (Yangtze River) plume: History and perspective. *Estuar. Coast. Shelf Sci.* **69**: 471–477. doi:10.1016/j.ecss.2006.05.010
- Warembourg, F. R. 1993. Nitrogen fixation in soil and plant systems, p. 127–156. *In* R. Knowles and T. H. Blackburn [eds.], *Nitrogen Isotope Techniques*. Academic Press.
- Weston, K., L. Fernand, J. Nicholls, A. Marca-Bell, D. Mills, D. Sivyver, and M. Trimmer. 2008. Sedimentary and water column processes in the oyster grounds: A potentially hypoxic region of the North Sea. *Mar. Environ. Res.* **65**: 235–249. doi:10.1016/j.marenvres.2007.11.002
- Yan, W. J., S. Zhang, P. Sun, and S. P. Seitzinger. 2003. How do nitrogen inputs to the Chengjiang basin impact the Changjiang River nitrate. *Global Biogeochem. Cycles* **17**: 1091. doi:10.1029/2002GB002029
- Zhang, J., Y. Wu, T. Jennerjahn, V. Ittekkot, and Q. He. 2007. Distribution of organic matter in the Changjiang (Yangtze River) Estuary and their stable carbon and nitrogen isotopic ratios: Implications for source discrimination and sedimentary dynamics. *Mar. Chem.* **106**: 111–126. doi:10.1016/j.marchem.2007.02.003
- Zhang, J., and others. 2010. Natural and human-induced hypoxia and consequences for coastal areas: Synthesis and future development. *Biogeosciences* **7**: 1443–1467. doi:10.5194/bg-7-1443-2010
- Zhou, M. J., Z. L. Shen, and R. C. Yu. 2008. Responses of a coastal phytoplankton community to increased nutrient input from the Changjiang (Yangtze) River. *Cont. Shelf Res.* **28**: 1483–1489. doi:10.1016/j.csr.2007.02.009
- Zhu, Z. Y., J. Zhang, Y. Wu, Y. Y. Zhang, J. Lin, and S. M. Liu. 2011. Hypoxia off the Changjiang (Yangtze River) Estuary: Oxygen depletion and organic matter decomposition. *Mar. Chem.* **125**: 108–116. doi:10.1016/j.marchem.2011.03.005

Acknowledgments

The captains and crew of *R/V Shiyang 3* and *Beidou* are thanked for their help with field sampling. Song, G. D. (not the author), Jiang H. H., Ge C. F., Sun J. Q., Hu J., Li R. H., Wu B., Gabriele Klockgether, Kalvelage T. and Füssel J. are thanked for their help in the field or the laboratory for samples analysis. This study is financially supported by the National Science Foundation of China (NSFC: 41606093, U1806211, 42076035), the Youth Talent Support Program of the Laboratory for Marine Ecology and Environmental Science, Pilot National Laboratory for Marine Science and Technology (Qingdao) (LMEES-YTSP-2018-02-04), the National Research and Development program of China (2016YFA0600902 and 2016YFA0601302), the Ministry of Science & Technology of P. R. China (2011CB409802), Aoshan Talents Program Supported by Qingdao National Laboratory for Marine Science and Technology (2015ASTP-OS08), the Taishan Scholars Programme of Shandong Province. This study is a contribution to the international IMBeR project.

Conflict of Interest

None declared.

Submitted 08 October 2019

Revised 20 May 2020

Accepted 19 September 2020

Associate editor: Bo Thamdrup