

Macrophytes and crabs affect nitrogen transformations in salt marshes of the Yangtze River Estuary

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ABSTRACT

Nitrogen (N) cycling is important in determining ecosystem primary productivity and the succession of plant communities in coastal marshes. In order to examine the effects of biotic disturbances (i.e. vegetation change and crab burrowing) on N transformations, we conducted a field experiment in which crabs were either removed or left intact in three marsh types (bare flat, *Phragmites australis* marsh and *Spartina alterniflora* marsh) at Chongming Dongtan in the Yangtze River Estuary. The potential rates of soil gross nitrogen mineralization (GNM), gross ammonium immobilization (GAI), gross nitrification and gross nitrate consumption were determined using an *ex situ* incubation approach. Gross N mineralization was significantly lower in vegetated marshes than in the bare flat, probably because dissolved organic carbon (the main energy source for microbes) was lower in vegetated marshes. Less dissolved organic carbon in vegetated marshes may be attributable to high microbial demand for labile carbon relative to the supply of labile carbon in the rhizosphere. The presence of crabs increased the GAI:GNM ratio, indicating that crabs may increase the soil microbial demand for ammonium. Ammonium immobilization competed with nitrification for ammonium, and limited nitrification in crab-present plots. Furthermore, the lower nitrate production by nitrifiers in crab-present plots resulted in lower rates of nitrate consumption. These results highlight the role of labile carbon and N in mediating the effects of macrophyte and soil fauna on N transformations.

1. Introduction

Nitrogen (N) is usually a limiting nutrient for plant growth in coastal salt marshes, and N availability is considered to have major impacts on ecosystem structure and primary productivity (Herbert, 1999). Nitrogen transformations, such as N mineralization, nitrification and denitrification, are important in determining N availability in coastal ecosystems (Fanjul et al., 2011). These processes can be influenced by biotic disturbances caused by vegetation changes and soil fauna activities (Gribsholt and Kristensen, 2002; Lohrer et al., 2004).

Macrophytes and soil fauna affect ecosystem N cycling by altering physio-chemical characteristics and microbial activities in the sediment (Osler and Sommerkorn, 2007). Vegetation change can alter carbon and N accumulation in ecosystems because plant species differ in photosynthetic and N-fixing capabilities (DeMeester and Richter, 2010; McCulley and Jackson, 2012; Huang et al., 2016). Macrophytes

assimilate inorganic N (mainly as ammonium) from the sediment (Magri et al., 2018), competing with bacteria for available N (Booth et al., 2005; Petersen and Jensen, 1997). Macrophytes also release root exudates, which stimulate microbial processes such as mineralization, nitrification, and denitrification (Soana and Bartoli, 2013; Vila-Costa et al., 2016; Windham and Meyerson, 2003). Burrowing crabs can alter sediment texture, soil particle distribution and oxygen availability (Bertics and Ziebis, 2010; Wang et al., 2010), and can also stimulate organic matter decomposition, as well as nutrient fluxes (Kristensen et al., 2000; Fanjul et al., 2015).

Soil N transformations may be limited by availability of labile carbon and nitrogen (Schimel and Bennett, 2004; Osler and Sommerkorn, 2007). The energy required for N transformations can be supplied by labile carbon, which is regulated by soil microbial activities and plant exudates (Fenner et al., 2004). Nitrogen mineralization supplies ammonium for nitrification, which in turn produces nitrate for

Abbreviations: GNM, gross nitrogen mineralization; GAI, gross ammonium immobilization; GN, gross nitrification; GNC, gross nitrate consumption

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denitrification. Nitrifiers will thus decrease when ammonium availability cannot meet the total demand by plants and bacteria. This will lead to decreases in the production and subsequent consumption of nitrate (Tang et al., 2018). However, it remains unclear whether labile carbon and nitrogen may mediate changes in soil nitrogen transformations caused by macrophyte and soil fauna disturbances.

This study aimed to examine how marsh macrophytes and crab activities affect the soil N transformations in the intertidal zone of the Yangtze River Estuary. A field experiment was conducted in three marsh types (bare flat, *Phragmites australis* marsh, and *Spartina alterniflora* marsh) in which crabs were either removed or left intact. We compared the rates of gross N mineralization (GMN), gross nitrification (GN), gross ammonium immobilization (GAI), and gross nitrate consumption (GNC) among marsh types and between crab pressures. We then explored the relationships between rates of N transformations and contents of labile carbon and nitrogen in soils for understanding how bioturbators may control the N cycling via altering the availability of labile carbon and nitrogen. We tested the hypotheses that (1) the transformations of various N forms show different patterns in response to macrophyte and crab disturbances, and (2) the labile carbon and nitrogen play a critical role in controlling nitrogen transformations under bioturbances.

2. Materials & methods

2.1. Study site and experimental design

The study was carried out over the growing season (April–September) of 2009 at Chongming Dongtan in the Yangtze River estuary, Shanghai, China (31°25′–31°38′N, 121°50′–122°05′E). Dongtan salt marsh occupies an area of about 230 km², with soil pore water salinity ranging from 5 to 10‰ (Wang et al., 2008). Soil temperature varies between 19.7 and 31.5 °C during the growing season (Chen et al., 2007). The mean annual precipitation is 1123.7 mm, most of which falling in summer. The high- and mid-tidal marshes are respectively dominated by native *P. australis* and invasive *S. alterniflora*, with mosaic bare patches (Li et al., 2009). Crab species dwelling in the estuary consist mainly of *Helice tientsinensis*, *Sesarma dehaani*, *Parasesarma plicatum*, and *Uca arcuata*, with the average crab density being about 20 ind. m⁻² (Qin et al., 2010).

We conducted a field manipulation experiment to examine the effects of marsh types and crabs on nitrogen dynamics in the sediment. Four pairs of side-by-side 1.5 × 1.5 m plots were established in each of the three marsh types (i.e. bare flat, *P. australis* marsh, *S. alterniflora* marsh) (24 in total) (Fig. 1a) in April. The distance between plot pairs was about 100–200 m. Crabs in one plot of each pair were removed using traps (hereafter “crab-removed plot”), and those in the other plot of the same pair were kept undisturbed (“crab-present plot”). The crab-removed plots were enclosed by nylon nets of 1 mm² mesh. These nets were set up with an aboveground height of 70 cm and a belowground depth to 30 cm to prevent crabs from moving into or out of plots (Fig. 1b). Two traps were deployed in each plot by inserting into the sediment a cylindrical PVC bucket (25 cm in diameter, 25 cm in depth) in two corners along a diagonal, with the upper edge of the buckets being leveled to the soil surface (Fig. 1c). Holes were bored at the bottom of the traps, allowing for natural tidal fluctuations in water level but preventing trapped crabs from escaping. Trapped crabs were hand-removed biweekly. The crab-present plot of each pair was established within 2 m of the crab-removed plot of the same pair. They were only enclosed by aboveground nylon nets, which created similar microhabitats to those in the crab-removed plots but allowed crabs to move in and out freely. Plant and soil samples were collected in a subplot of 1 × 1 m in the center of each plot to avoid potential edge effects.

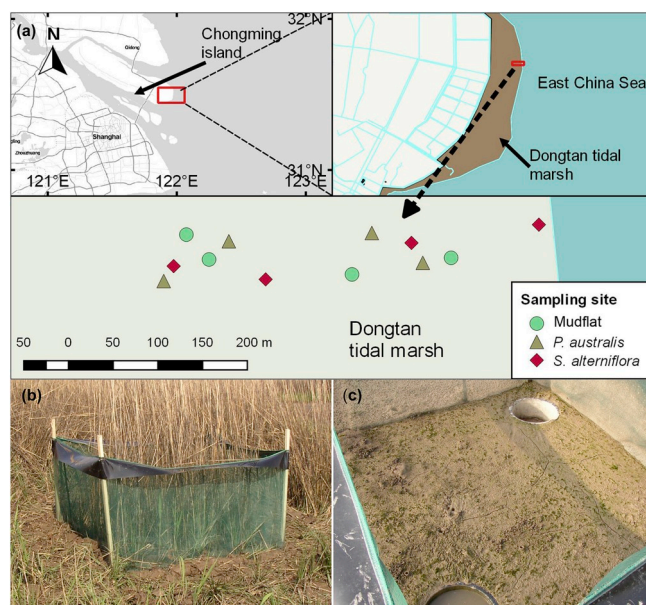


Fig. 1. Study site and experimental plots in the Yangtze River Estuary (a), a plot enclosed by nylon nets (b), and traps in a crab-removed plot (c).

2.2. Macrophyte and crab characteristics

Aboveground plant parts were harvested in late September in all plots. At the same time, belowground biomass was estimated by sampling five soil cores of 34 mm in diameter and 30 cm in depth in each plot. The belowground plant tissues were flushed with running water. Both above- and belowground plant parts were weighed after oven-drying at 60 °C to constant weight. Total plant biomass was calculated as the sum of above- and belowground biomass. The intensity of disturbance induced by crabs was estimated by the density and mean diameter of crab burrows, which were recorded as soon as the aboveground plant parts were harvested.

2.3. Soil properties

Five soil cores were sampled to a depth of 30 cm from each plot and mixed. Subsamples of fresh soil were passed through a 2-mm sieve and stored at 4 °C, and were then used in determining dissolved total nitrogen (DTN), dissolved organic carbon (DOC), soil microbial biomass carbon and nitrogen (MBC and MBN), ammonium nitrogen (NH₄-N) and nitrate nitrogen (NO₃-N). Approximately 10 g soil was extracted with 0.5 mol L⁻¹ K₂SO₄ (1:4, w/w) on a shaker at 200 rpm and 30 °C for 1 h and filtered through medium-speed qualitative filter paper. Contents of NH₄-N and NO₃-N were determined for the filtrates with a SmartChem® 200 Discrete Analyzer (WESTCO Scientific Instruments Inc., Brookfield, USA). Soil MBC and MBN were extracted with 0.5 M K₂SO₄ solutions (1:4 w/w) from the fumigated (24 h) and non-fumigated soils using chloroform fumigation-extraction method with a conversion factor 0.45 (Vance et al., 1987). DTN, DOC, MBN and MBC were determined for the same filtrates with the multi N/C 3100 Analyzer (Analytik Jena AG, Jena, Germany). DON was calculated as the difference between DTN and inorganic nitrogen (NH₄-N and NO₃-N).

2.4. Potential rates of gross nitrogen transformations

The potential rates of gross N transformations were determined by the ¹⁵N isotopic pool dilution technique (Cookson et al., 2007). About 10 g fresh soil samples were labeled with 99% enrichment ¹⁵NH₄Cl (determine NH₄-N production and consumption) or K¹⁵NO₃ (determine NO₃-N production and consumption) in 100 mL plastic vials to a level

of ca. $1 \mu\text{g }^{15}\text{N g}^{-1}$ soil. The vials were incubated at 25°C in darkness. After 4 and 28 h, subsamples of ca. 5 g soil were picked from each vial and immediately extracted with $0.5 \text{ mol L}^{-1} \text{ K}_2\text{SO}_4$ (1:4 w/w) on a shaker at 200 rpm and 30°C for 1 h. Contents of $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ in extracts were determined as described above (see 2.3). The ^{15}N abundance of $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ in the K_2SO_4 extracts was measured separately by a two-stage diffusion method (Brooks et al., 1989) and determined with Flash-EA1112 elemental analyzer coupled to a Thermo Finnigan Deltaplus XP mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). The potential rates of gross N transformations were calculated following Eqs. (1) and (2) (Kirkham and Bartholomew, 1954).

$$m = \frac{M_0 - M}{t} \times \frac{\log M_0 \times M/H \times M_0}{\log M_0/M} \quad (1)$$

$$c = \frac{M_0 - M}{t} \times \frac{\log H_0/H}{\log M_0/M} \quad (2)$$

where m is the rate of GNM or GN ($\mu\text{g N g}^{-1} \text{ d}^{-1}$), c the rate of gross $\text{NH}_4\text{-N}$ consumption or the GNC ($\mu\text{g N g}^{-1} \text{ d}^{-1}$), t the time (24 h in this study), M_0 the initial $^{14+15}\text{N}$ pool ($\mu\text{g N g}^{-1}$), M the post-incubation $^{14+15}\text{N}$ pool ($\mu\text{g N g}^{-1}$), H_0 the initial ^{15}N pool ($\mu\text{g N g}^{-1}$), H the post-incubation ^{15}N pool ($\mu\text{g N g}^{-1}$). GAI was calculated as the difference between gross $\text{NH}_4\text{-N}$ consumption and GN.

2.5. Statistical analysis

All statistical analyses were performed with the software R 3.4.2 (R Core Team, 2017). The effects of marsh types and crab treatments on rates of gross N transformation and other soil properties were examined using two-way analysis of variance (ANOVA), followed by multi-comparisons using the LSD method with the “agricolae” package (Mendiburu, 2017). Pearson's correlation was used to test the relationships among variables. Data were log-transformed to meet the assumptions for parametric tests.

3. Results

3.1. Characteristics of macrophytes and crab burrows

The density and diameter of crab burrows in crab-present plots were significantly higher than those in crab-removed plots (Table 1, diameter: $F_{1,18} = 35.51$, $P < 0.01$; density: $F_{1,18} = 10.73$, $P < 0.01$). Crab-removed plots had higher above- and belowground biomass than crab-present plots in both *P. australis* and *S. alterniflora* marshes (Table 1). However, neither above- nor belowground biomass showed significant difference between the two vegetated marshes (Table 1, aboveground biomass: $F_{1,12} = 2.90$, $P = 0.12$; belowground biomass: $F_{1,12} = 0.75$, $P = 0.40$).

3.2. Potential rates of gross nitrogen transformation

The rate of GNM differed significantly among marsh types, while the rate of GN and GNC were significantly affected by crab treatments

Table 1

Characteristics of macrophytes and crab burrows (mean \pm SE) in three marsh types with or without crabs.

Marsh type	Crab treatment	Crab burrow diameter (cm)	Crab burrow density (ind. m^{-2})	Aboveground biomass (g m^{-2})	Belowground biomass (g m^{-2})
Bare flat	removed	$2.00 \pm 0.11^{\text{d1}}$	$5.49 \pm 1.18^{\text{ab}}$	0	0
	present	$3.06 \pm 0.25^{\text{bc}}$	$8.11 \pm 3.88^{\text{a}}$	0	0
<i>P. australis</i>	removed	$2.66 \pm 0.34^{\text{cd}}$	$2.44 \pm 0.46^{\text{b}}$	$606.39 \pm 55.53^{\text{a}}$	$1966.26 \pm 458.38^{\text{a}}$
	present	$3.83 \pm 0.21^{\text{a}}$	$8.67 \pm 1.29^{\text{a}}$	$429.16 \pm 52.91^{\text{ab}}$	$871.40 \pm 321.45^{\text{a}}$
<i>S. alterniflora</i>	removed	$2.33 \pm 0.19^{\text{cd}}$	$1.89 \pm 0.11^{\text{b}}$	$441.02 \pm 78.17^{\text{ab}}$	$2156.89 \pm 964.44^{\text{a}}$
	present	$3.71 \pm 0.29^{\text{ab}}$	$8.11 \pm 1.77^{\text{a}}$	$363.52 \pm 80.16^{\text{b}}$	$1894.55 \pm 844.86^{\text{a}}$

1. The lowercase superscript letters indicate significance at the 0.05 level for the differences among marsh types and crab treatments ($n = 4$).

(Table 2). Gross N mineralization was higher in the bare flat than in vegetated marshes, while there was no significant difference in GNM between the *P. australis* marsh and the *S. alterniflora* marsh (Fig. 2a). Gross ammonium immobilization was positively correlated with GNM (Table 3). Both GNM and GAI were higher in crab-removed plots than in crab-present plots in bare flat, but the opposite pattern was observed in vegetated marshes (Fig. 2a and b), despite an insignificant interaction between marsh types and crab treatments (Table 2). Both GN and GNC were significantly higher in crab-removed plots than in crab-present plots (Fig. 2c and d), and were negatively correlated with GAI (Table 3). In addition, GNC was positively correlated with GN (Table 3).

3.3. Labile soil carbon and nitrogen contents

A negative correlation was found between soil DOC and MBC ($r = -0.40$, $P = 0.052$), and both of them differed significantly among marsh types (Table 2). Soil DOC content was higher in the bare flat than in vegetated marshes (Fig. 3a). Soil MBC was significantly affected by the interaction between marsh types and crab treatments (Table 2), and showed highest values in the *S. alterniflora* marsh with crabs and lowest values in the bare flat with crabs (Fig. 3e).

Soil $\text{NH}_4\text{-N}$ content was significantly higher in vegetated marshes than in the bare flat (Fig. 3c), and showed a negative correlation with MBN ($r = -0.41$, $P < 0.05$). Soil $\text{NO}_3\text{-N}$ content was significantly affected by both marsh types and crab treatments, as well as their interaction (Table 2). Soil $\text{NO}_3\text{-N}$ was higher in crab-present plots in both the bare flat and the *P. australis* marsh, but did not differ significantly between crab treatments in the *S. alterniflora* marsh (Fig. 3d).

3.4. Relationships between rates of gross nitrogen transformations and soil biofactors

GNM was positively correlated with DOC ($r = 0.55$, $P < 0.01$). Both GNM and DOC were negatively correlated with total plant biomass (Fig. 4a and b), which showed a marginally significant positive correlation with MBC across the three marsh types (Fig. 4c). However, for the soil samples only from vegetated marshes, DOC was positively correlated with total plant biomass (Fig. 4b), but neither GNM nor BMC was correlated with plant biomass. No significant relationship was observed between GNM and other environmental variables, i.e., the diameter and density of crab burrows, soil DON, inorganic nitrogen and MBN ($P > 0.1$).

The rates of both GN and GNC were negatively related to the diameter of crab burrows (Fig. 4d and e), and GNC was also negatively correlated with the density of crab burrows ($r = -0.42$, $P < 0.05$). Meanwhile, the ratio of GAI to GNM was positively correlated with the diameter of crab burrows (Fig. 4f). Neither GN nor GNC was correlated with plant biomass, labile soil carbon and nitrogen ($P > 0.1$).

4. Discussion

Our study in coastal marshes in the Yangtze River Estuary suggests that macrophytes and crabs can effect N transformations via altering labile soil carbon and nitrogen (Fig. 5). Nitrogen mineralization was

Table 2
Two-way ANOVAs for testing the effects of marsh types and crab removal on soil variables.

Variable ¹	Marsh type			Crab treatment			Marsh type × Crab treatment		
	df	F	P	df	F	P	df	F	P
GNM	2,18	5.855	0.011	1,18	0.126	0.727	2,18	2.279	0.131
GAI	2,18	0.949	0.406	1,18	1.059	0.317	2,18	2.697	0.095
GN	2,18	2.030	0.160	1,18	5.516	0.031	2,18	0.076	0.927
GNC	2,18	0.157	0.856	1,18	6.902	0.017	2,18	0.437	0.653
DOC	2,18	5.253	0.016	1,18	1.054	0.318	2,18	0.391	0.682
DON	2,18	3.598	0.049	1,18	0.249	0.623	2,18	2.548	0.106
NH ₄ -N	2,18	12.915	< 0.001	1,18	0.026	0.874	2,18	1.055	0.369
NO ₃ -N	2,18	5.596	0.013	1,18	8.368	0.010	2,18	4.861	0.021
MBC	2,18	7.220	< 0.01	1,18	0.435	0.518	2,18	5.031	0.018
MBN	2,18	0.896	0.426	1,18	0.001	0.976	2,18	0.972	0.397

1. GNM: gross nitrogen mineralization; GAI: gross ammonium immobilization; GN: gross nitrification; GNC: gross nitrate consumption; DOC: dissolved organic carbon; DON: dissolved organic nitrogen; NH₄-N: ammonium nitrogen; NO₃-N: nitrate nitrogen; MBC: microbial biomass carbon; MBN: microbial biomass nitrogen.

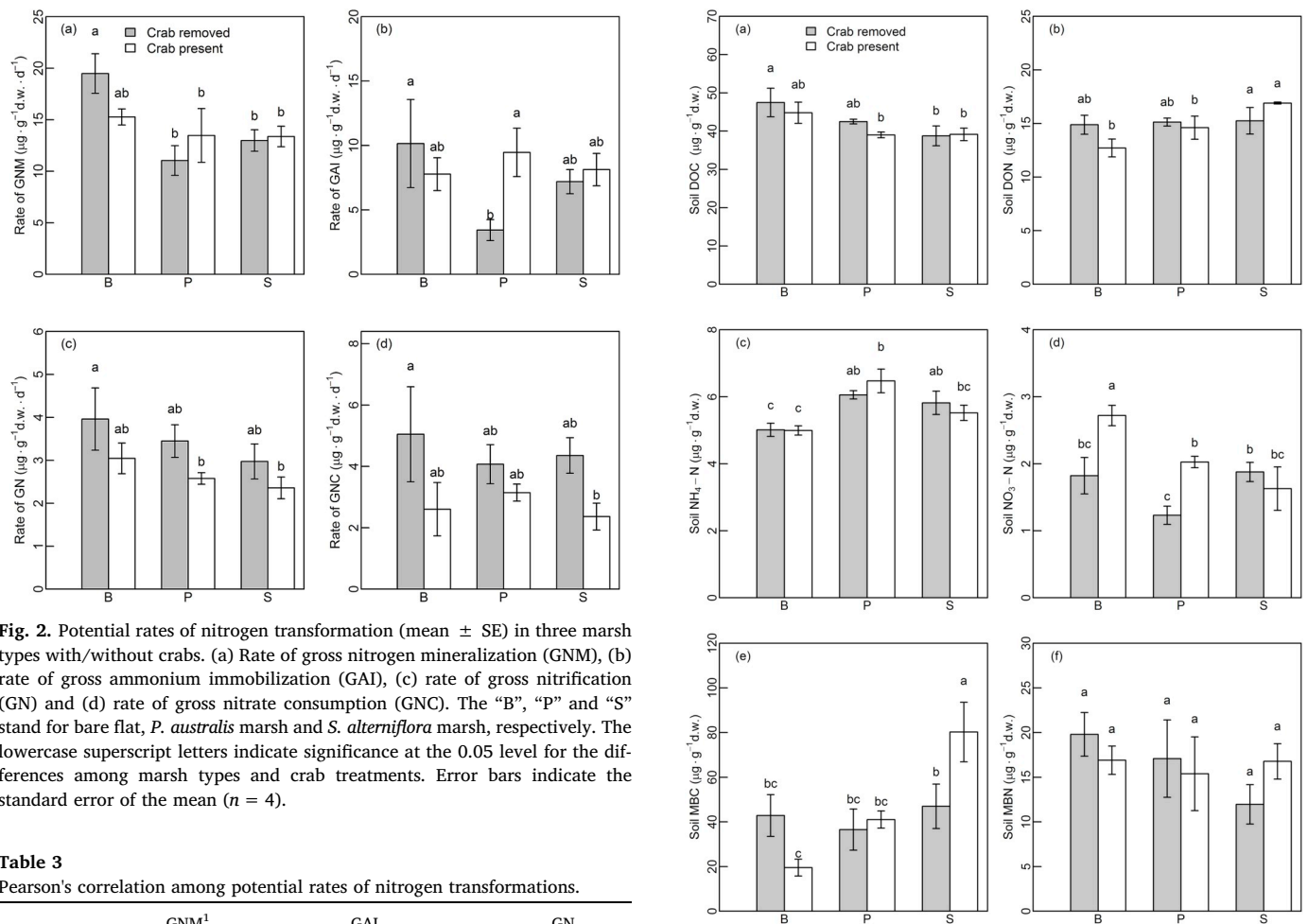


Fig. 2. Potential rates of nitrogen transformation (mean ± SE) in three marsh types with/without crabs. (a) Rate of gross nitrogen mineralization (GNM), (b) rate of gross ammonium immobilization (GAI), (c) rate of gross nitrification (GN) and (d) rate of gross nitrate consumption (GNC). The “B”, “P” and “S” stand for bare flat, *P. australis* marsh and *S. alterniflora* marsh, respectively. The lowercase superscript letters indicate significance at the 0.05 level for the differences among marsh types and crab treatments. Error bars indicate the standard error of the mean ($n = 4$).

Table 3
Pearson's correlation among potential rates of nitrogen transformations.

	GNM ¹	GAI	GN
GAI	0.680***		
GN	0.168	-0.549***	
GNC	0.157	-0.530**	0.882***

1. “***”, “**” indicates the significance levels at 0.01, and 0.001, respectively ($n = 24$). GNM: gross nitrogen mineralization; GAI: gross ammonium immobilization; GN: gross nitrification; GNC: gross nitrate consumption.

driven primarily by DOC, which was lower in vegetated marshes. Macrophytes might promote soil microbes and increase MBC in the rhizosphere. Higher MBC in vegetated marshes led to lower soil DOC content, and thus lower GNM compared to the bare flat (Fig. 2a). Meanwhile, crab activities enhanced the proportion of GAI in NH₄-N

Fig. 3. Labile soil nitrogen and carbon contents (mean ± SE) in three marsh types with or without crabs. (a) Soil dissolved organic carbon (DOC), (b) soil dissolved organic nitrogen (DON), (c) soil ammonium nitrogen (NH₄-N), (d) soil nitrate nitrogen (NO₃-N), (e) soil microbial biomass carbon (MBC), (f) soil microbial biomass nitrogen (MBN). The “B”, “P” and “S” stand for the bare flat, *P. australis* marsh and *S. alterniflora* marsh, respectively. The lowercase superscript letters indicate significance at the 0.05 level for the differences among marsh types and crab treatments. Error bars indicate the standard error of the mean ($n = 4$).

consumptions, as indicated by the positive relationship between the GAI:GNM ratio and the diameter of crab burrows (Fig. 4f). The NH₄-N immobilization in crab-present plots might be activated by soil

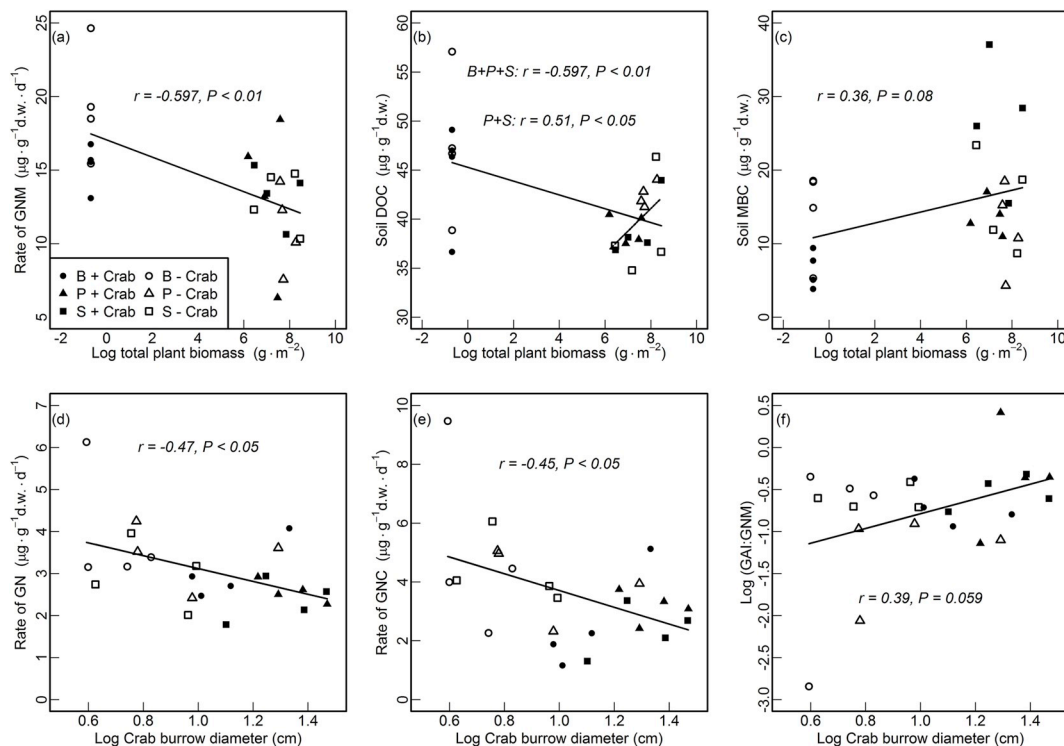


Fig. 4. Relationships between biofactors and soil variables. Relationships between log-transformed total plant biomass and (a) the rate of gross nitrogen mineralization (GNM), (b) soil dissolved organic carbon (DOC), (c) soil microbial biomass carbon (MBC), log-transformed crab burrow diameter and (d) the rate of gross nitrification (GN), (e) the rate of gross nitrate consumption (GNC) and (f) the log-transformed ratio of GAI to GNM. The r and P value indicate the Pearson's correlation and significant level respectively. In plot (b), the “B + P + S” stands for “bare flat + *P. australis* marsh + *S. alterniflora* marsh” and “P + S” stands for “*P. australis* marsh + *S. alterniflora* marsh”. For other plots, r and P values were calculated with pooled data across three marsh types.

microbes, and resulted in the $\text{NH}_4\text{-N}$ -limitation to nitrification. In addition, $\text{NO}_3\text{-N}$ consumption was highly coupled with nitrification (Table 3), since GNC was restricted by $\text{NO}_3\text{-N}$ availability in crab-present plots (Fig. 2d).

4.1. Soil DOC and MBC mediated the effects of macrophytes on N mineralization

Our results suggest that macrophytes including *P. australis* and *S. alterniflora* may drive the soil N mineralization by altering labile carbon (Chu and Grogan, 2010; Cookson et al., 2007). Rate of GNM was lower in vegetated marshes than in the bare flat (Fig. 2a), and was negatively correlated with total plant biomass across the three marsh types (Fig. 4a). This pattern is contrary to many other studies in which N mineralization was promoted by rhizosphere microbes, as root exudates

can provide abundant labile carbon for bacterial mineralization (Carrillo et al., 2011; Fanjul et al., 2011; Olsen et al., 2011). Lower GNM in vegetated marshes can be attributed to lower DOC (Fig. 3a), which serves as the primary energy source for microbial processes (Chu and Grogan, 2010; Cookson et al., 2007). The driving of N mineralization by DOC was supported by the positive relationship between GNM and DOC ($r = 0.55, P < 0.01$), and has also been found in N-limited forests (Magill and Aber, 2000; Schmidt et al., 2011).

Lower DOC in vegetated marshes found here may be due in part to the decreases in root-derived carbon during the late growing-season (September) (Vacheron et al., 2013). Labile soil carbon may be quickly depleted by microbes in the rhizosphere, resulting in the higher MBC but lower DOC in vegetated marshes (Fig. 3a and e). Total plant biomass was positively correlated with MBC (Fig. 4c), but negatively correlated with DOC (Fig. 4b) across the three marsh types. This

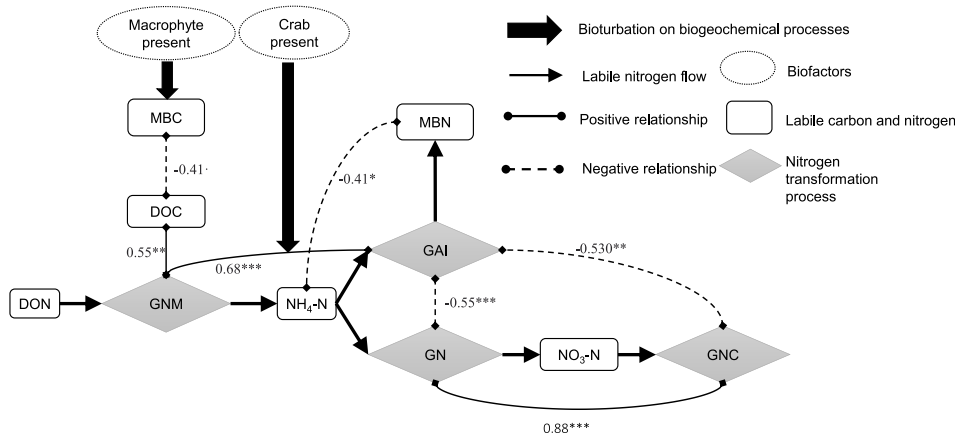


Fig. 5. Graphical summary of bioturbation effects on N transformation processes in coastal marshes in the Yangtze River Estuary. The values present the Pearson's correlation, and “”, “*”, “**”, “***”, “****”, “*****” indicate the significance at 0.1, 0.05, 0.01 and 0.001 levels, respectively. GNM: gross nitrogen mineralization; GAI: gross ammonium immobilization; GN: gross nitrification; GNC: gross nitrate consumption; DOC: dissolved organic carbon; DON: dissolved organic nitrogen; $\text{NH}_4\text{-N}$: ammonium nitrogen; $\text{NO}_3\text{-N}$: nitrate nitrogen; MBC: microbial biomass carbon; MBN: microbial biomass nitrogen.

suggests that the consumption of DOC in the rhizosphere may be faster than its supply by roots during the late growing-season. Labile carbon limitation to heterotrophic microorganisms has been observed in wetlands, and the carbon was likely preferentially allocated to bacterial growth under low C:N conditions (Grebliunas and Perry, 2016). The DOC:DON ratio at the study site was 3.35 ± 0.11 , 2.76 ± 0.14 and 2.43 ± 0.09 in the bare flat, *P. australis* marsh and *S. alterniflora* marsh respectively. These values were much lower than those reported for other coastal and inland wetlands with the DOC: DON > 10 (Petroni et al., 2009; Lønborg and Søndergaard, 2009; Bernal et al., 2005). Therefore, the DOC deficiency may have occurred at the study site, especially in vegetated marshes. In addition, DOC increased with total plant biomass in *S. alterniflora* and *P. australis* marshes (Fig. 4b), indicating that plant biomass could increase DOC to some extent. In this case, the supply of labile carbon may have met the demand by bacteria in the rhizosphere, as indicated by a lack of correlation between MBC and total plant biomass in vegetated marshes (Fig. 4c).

Previous studies showed that the soil N mineralization can be promoted by the invasive *S. alterniflora*, because its invasion led to increases in organic carbon and thus provided energy for bacterial mineralization (Zhang et al., 2016). However, GNM did not differ between the *S. alterniflora* marsh and the *P. australis* marsh (Fig. 2a) in this study, as DOC was similar between the two marsh types (Fig. 3a). Some studies reported that the invasion of *S. alterniflora* in coastal marshes promoted the accumulation of recalcitrant carbon (e.g. lignin) more than labile carbon (Yang et al., 2015) in the short term (< 10 years). Therefore, we speculated that the labile carbon accumulation caused by *S. alterniflora* invasions had not yet become observable due to the short invasion history. Another possibility is that the labile carbon accumulated by *S. alterniflora* invasion had been preferentially utilized by soil microbes under the pressure of soil fauna activities, as MBC showed the highest values in the *S. alterniflora* marsh in the presence of crabs (Fig. 3e). Gross N mineralization was higher in crab-removed plots than in crab-present plots in the bare flat (Fig. 2a), while the opposite pattern was observed in vegetated marshes. Dissolved organic carbon in crab-removed plots was higher than in crab-present plots in the bare flat (Fig. 3a). One possible explanation for this difference was that tidal flush may cause more labile carbon losses from the disturbed sediment which is characterized by a high surface area (Tzortziou et al., 2011). Therefore, DOC was likely responsible for differences in GNM between crab treatments in the bare flat. The higher GNM in crab-present plots in vegetated marshes may be attributed to more active mineralizers as a result of crab activities (Fanjul et al., 2011).

4.2. Crab effects on nitrification and nitrate consumption via altering the GAI:GNM ratio

The bacteria in crab-present plots may have higher activities (Thomas and Blum, 2010) and higher metabolic demand for ammonium, especially in nitrogen limiting systems such as coastal marshes (Bai et al., 2012; Fanjul et al., 2011). We found that crabs may activate soil microbes and promote ammonium immobilization, as indicated by increases in the GAI:GNM ratio with the diameter of crab burrows (Fig. 4f). In addition, the rate of GAI was positively correlated with that of GNM (Table 3), and showed similar patterns among marsh types and between crab treatments (Fig. 2b). This indicated that ammonium immobilization was also driven by N mineralization, which was indirectly affected by plants.

Rate of GN was lower in crab-present than in crab-removed plots (Fig. 2c), and was negatively correlated with the diameter of crab burrows (Fig. 4d). Ammonium immobilization and nitrification appeared to compete for $\text{NH}_4\text{-N}$ during incubation, since GN was negatively correlated with GAI (Table 3). The negative relationship between MBN and $\text{NH}_4\text{-N}$ contents (Fig. 5) also indicated that the microbial utilization of labile N reduced the $\text{NH}_4\text{-N}$ availability. The presence of crabs may indirectly inhibit nitrification via enhancing the GAI:GNM

ratio, and thus reduce the ammonium available to nitrification. We found that $63.71 \pm 15.42\%$ of produced $\text{NH}_4\text{-N}$ by GNM was immobilized by bacteria in crab-present plots, compared to $44.88 \pm 10.04\%$ in crab-removed plots; and $20.05 \pm 3.54\%$ of produced $\text{NH}_4\text{-N}$ was transformed to $\text{NO}_3\text{-N}$ in crab-present plots, compared to $25.64 \pm 4.47\%$ in crab-removed plots. Previous studies provided evidence that $\text{NO}_3\text{-N}$ taken up by bacteria accounts for a proportion of 15–40% in marine and forest ecosystems (Allen et al., 2002; Myrold and Posavatz, 2007). We suggest that the ammonium utilized by bacteria is also an important process in nitrogen cycling in tidal marshes, and may regulate nitrification under crab disturbance. Some studies reported that nitrification could be promoted by benthic fauna, as their burrowing activities could increase oxygen content in soils (Fanjul et al., 2011). However, this study determined the rates of GN using an *ex situ* method, by which the heterogeneity of soil oxygen content in the field may be eliminated due to the destruction of soil cores. Therefore, enhanced soil oxygen content by crab burrowing was not likely to be responsible for differences in GN between crab treatments.

Gross nitrate consumption was positively correlated with GN (Table 3), suggesting that nitrate consumption was limited by $\text{NO}_3\text{-N}$, which is in turn produced by nitrification. The main pathways of nitrate consumption include microbial immobilization of nitrate, dissimilatory nitrate reduction to ammonium and denitrification, and denitrification has been reported to depend on nitrate production (Dornhoffer et al., 2015; Fanjul et al., 2011; Lunstrum et al., 2017). Our results are in line with these studies in that nitrate availability was the primary driver of nitrate consumption in coastal ecosystems. To sum up, crab activities may indirectly depress nitrate consumption via reducing the availability of $\text{NO}_3\text{-N}$ in the Dongtan marsh.

5. Conclusions

We found that biotic disturbances (i.e. macrophyte and crab activities) affected N transformations in the Yangtze River estuary via altering the availability of labile carbon and nitrogen. The microbial demand for labile carbon might exceed the supply of labile carbon in the rhizosphere in the late growing-season, and thus limit N-mineralization in vegetated marshes. Crabs may enhance the demand for $\text{NH}_4\text{-N}$ by soil bacteria, and therefore depress nitrification and nitrate consumption. Our results could help understand the nitrogen cycling in coastal marshes experiencing biotic disturbances. It is worthy of note that this study only estimated the potential rates of gross N transformations. Future studies with *in situ* measurements of N transformation rates are needed for examining the response of N cycling to vegetation changes and soil fauna disturbances.

Declarations of interest

None.

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