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Primary effects of extracellular enzyme activity and microbial community on carbon and nitrogen mineralization in estuarine and tidal wetlands

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Abstract Estuarine and tidal wetlands with high primary productivity and biological activity play a crucial role in coastal nutrient dynamics. Here, to better reveal the effects of extracellular enzymes and microbial community on carbon (C) and nitrogen (N) mineralization, the incubation experiments with different C and N addition patterns to the tidal sediments of the Yangtze Estuary (China) were conducted. The results suggested a significant increase in cumulative CO_2 effluxes in the C and CN treatment experiments, while no significant difference in cumulative CO_2 effluxes between the N treatment and control (CK) experiments was observed. In addition, the nutrient addition patterns had a great influence on dissolve organic C and N levels, but a small effect on microbial biomass C and N. Microbial community composition and microbial activity were found to be positively correlated with organic C (OC) and the molar ratio of C to N (C/N). Partial correlation analysis, controlling for C/N, supported direct effects of OC on the activity of carbon-cycling extracellular enzymes (cellulase and polyphenol oxidase), while C/N exhibited negatively correlations with urease and Gram-positive bacteria to Gram-negative bacteria (G+/G−). Strong relationships were found between CO_2 efflux and mineral nitrogen with the activity of specific enzymes (sucrase, cellulase, and polyphenol oxidase) and abundances of Gram-negative bacteria, arbuscular mycorrhizal fungi, and fungi, suggesting the significant influences of microbial community and enzyme activity on C and N mineralization in the estuarine and tidal wetlands. Furthermore, this study could highlight the need to explore effects of nutrient supply on microbial communities and enzyme activity changes associated with the C and N mineralization in these wetlands induced by the climate change.

Keywords Extracellular enzyme activity · Microbial community composition · Phospholipid fatty acid (PLFA) C and N mineralization · Estuarine and tidal wetlands Yangtze estuary

Introduction

The carbon (C) pool of wetlands contains 45–70 % of all terrestrial C (Mitra et al. 2005; Morrissey et al. 2014), playing an important role in the global C cycle (Mcleod et al. 2011; Morrissey et al. 2014). Over the past several years, an increasing number of studies have shown that C inputs significantly and positively increase native sediment organic carbon (OC) decomposition (Mack et al. 2004; Shaver et al. 2006; Stern et al. 2007; Keuskamp et al. 2013), changing wetlands from a carbon sink to source due to more CO_2 release into the atmosphere. Therefore, wetlands act not only as an interceptor from land to ocean but also as a significant source of CO_2 to the atmosphere in the global C cycle.

In addition, global nitrogen (N) excessive inputs in wetlands, causing a number of environmental problems, have also received considerable attention (Wang et al. 2011; Lind et al. 2013; Deng et al. 2014). External N inputs have been reported to either increase (Mack et al. 2004) or decrease OC decomposition rates (Keuskamp et al. 2013) in

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wetland ecosystems. These inconsistencies highlight the need for further understanding of how microbial community, enzyme activity, and environmental variables affect OC decomposition, which can be best achieved by simultaneously studying CO_2 emission rates, dissolved organic carbon, as well as microbial biomass carbon.

The estuarine and tidal wetlands as a huge biological resource pool have abundant microorganisms (Hu et al. 2014), and thus microbial decomposition of organic matter is a key process of C cycling in wetland ecosystems (Stern et al. 2007). In addition, they play an important role in maintaining nutrient dynamics and degrading a wide range of contaminants, which are also strongly affected by environmental changes. Numerous studies have revealed that the climate change, plant invasion, and anthropogenic interference can strongly impact sediment microbial characteristics (Chen et al. 2012; Statham 2012; Bauer, et al. 2013), and microbial community composition can also change in response to nutrient inputs (Fang and Wang 2007). The physicochemical properties of sediment (Harrison-Kirk et al. 2013; Peralta et al. 2013), hydrological settings (Hoffmann et al. 2006; Racchetti et al. 2011), and saltwater intrusion (Ardón et al. 2013) can also potentially affect microbial C and N cycle. However, the estuarine and tidal sediments, which are the most affected by tidal changes (Sakamaki et al. 2006), have not been adequately considered to reveal how environmental variables impact microbial activity. Thus, a full understanding of C and N mineralization influenced by nutrient inputs and environmental factors is critical to clarify their feedback to the global change in estuarine and tidal wetlands.

During the past decades, the increasing quantities of nutrient influx to estuarine and tidal wetlands are attributed partly to the river runoff and coastal development (Duarte 2009; Nixon 2009). Consequently, the microbial activity induced by carbon input in sediments can change, indicating primary mechanism of carbon release from the sediments (Fang and Wang 2007). Meanwhile, shifts in microbial community composition and biomass can impact N and C cycling (Fraterrigo et al. 2006; Tavi et al. 2013), wherein Gram-positive bacteria have a primary role in C cycles (Wang et al. 2014). In return, changes in sediment OC and the C/N ratio can alter fungi/bacteria ratio (Fierer et al. 2009), and the abundance of arbuscular mycorrhizal fungi (AMF) can increase in response to humus decomposition (Moeskops et al. 2012). It has been shown that inputs of labile organic carbon (LOC) greatly enhance native OC decomposition (Blagodatskaya et al. 2007). Keuskamp et al. (2013) have recently revealed that an increase in OC mineralization rates would cause an enhanced CO_2 release. N inputs can significantly increase the microbial biomass N and microbial communities (Keuskamp et al. 2013; Peralta et al. 2013).

Enzymes also stimulate important transformation of nutrient cycling (Wallenstein and Burns 2011). The increases in activity of carbon-cycling extracellular enzymes, including cellulose, sucrase, and polyphenol oxidase, have been observed to increase the rate of C decomposition (Alster et al. 2013; Du et al. 2014), and the activity of phenol oxidase is restricted by N availability (Waldrop et al. 2004). Extracellular enzymes influencing the C decomposition have been well studied over the past several years. However, the effects of enzyme activity on N mineralization dynamics are still poorly understood. Therefore, environmental variables, enzyme activity, and microbial community composition need to be considered together to completely determine the mineralization processes in estuarine and tidal wetlands.

The Yangtze Estuary is located in the most densely populated and industrialized areas of east China and play a key role in regional microclimate regulation and maintaining high biodiversity (Tang et al. 2011a; Hou et al. 2013). In recent decades, this estuary receives large-scale anthropogenic carbon and nitrogen from agricultural activities, domestic and industrial wastewater discharge (Hou et al. 2013). Hence, the sediment respiration, CO_2 and NO_2 emission, plant invasion, and microbial nitrogen transformations have been well studied in the Yangtze Estuary (Tang et al. 2011a, b; Yu et al. 2012; Hou et al. 2013; Zheng et al. 2013; Deng et al. 2014). However, there are few studies on the effects of complicated nutrient addition, environmental variables, and sediment properties on CO_2 effluxes and mineral N dynamics in the estuarine and tidal sediments influenced by the tidal cycle. In addition, the changes of microbial biomass C and N are essential to reveal how microbial activity responds to nutrient inputs.

Here, we conducted an incubation experiment to examine whether environmental factors and/or microbial factors primarily drive the C and N mineralization in tidal sediments of the Yangtze Estuary. The explicit objectives of this study were to (1) obtain the effects of nutrient addition patterns on CO_2 flux rates and mineral N pools, (2) demonstrate whether mineral N pools reduced in respond to the increase of microbial activity, and (3) reveal the relationships of environmental factors and microbial communities with C and N mineralization in the tidal sediments of the Yangtze Estuary.

Material and methods

Study area

The Yangtze Estuary located in eastern central China is subject to the typical subtropical monsoon climate with an average temperature of 28.9 °C for summer and 5.6 °C for winter (Tang et al. 2011a) (Fig. 1). The mean annual precipitation in this region is approximately 1,100 mm, of which 60 % occurring from May to September (Tang et al. 2011a). In addition, this region can often suffer from typhoons during summer and autumn. The delta front of this estuary continues to extend

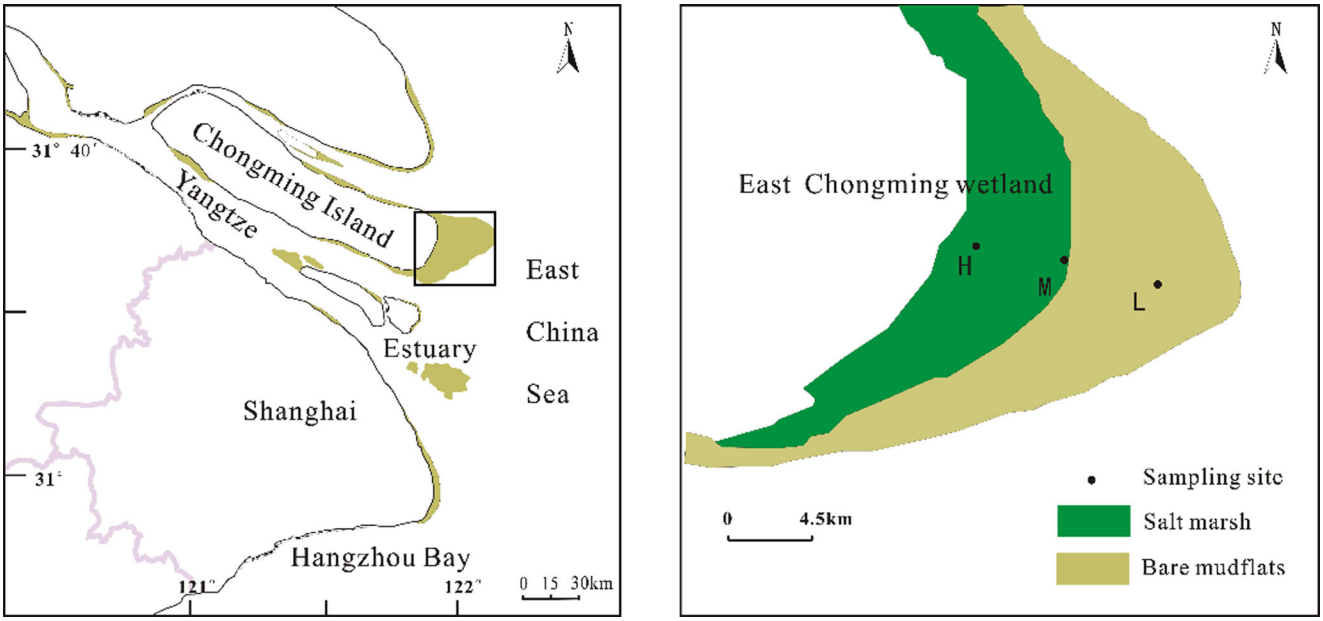

Fig. 1 The location of Yangtze Estuary (*left*) and sampling sites (*right*) in the study area

seaward rapidly due to the large amount of sediment supply from the Yangtze River (Chen and Zhong 1998; Yu et al. 2012).

The eastern Chongming wetland (31°25′–31°38′N, 121°50′–122°05′E) situated in the mouth of the Yangtze Estuary is the largest and most extensively developed wetland in the Yangtze Estuary (Fig. 1) and composed of natural high, middle, and low tidal flats (Yu et al. 2012). The low tidal flat (L) situated in the eastern fringe with an elevation of less than 2 m is characterized by bare mudflats without any plants (Wang et al. 2010). The middle tidal flat (M) between 2.0 and 2.9 m elevation is dominated by the native *Scirpus mariqueter* community and is not submerged during the neap tide and submerged for several hours during the spring tide (Wang et al. 2009). The elevation of above 2.9 m, as the high tidal flat (H), the plant communities are characterized by a native plant of *Phragmites australis*. An additional invasion species in this zone is *Spartina alterniflora*, which was introduced to the eastern Chongming wetland in 1995 (Wang et al. 2010). This species has gradually invaded a large area formerly covered by *P. australis* and has increasingly posed a threat to indigenous plants of this wetland.

Sediment sample collection and physicochemical properties analysis

Sampling was carried out in early spring of 2014. Within each of high, middle, and low tidal flats of the eastern Chongming wetland, three sampling sites were selected to account for small-scale variation. At each sampling site, surface (0–5 cm depth) sediment sample was collected with stainless steel corers and stored in a cooler (4 °C). These core sediments were transported to laboratory within 2 h after collection, homogenized immediately under a N_2 atmosphere, and stored at 4 °C until further analysis and incubation.

Sediment pH, salinity, and temperature were in situ measured using a Mettler-Toledo pH Meter and a YSI Model 30 conductivity meter, respectively. The sediment particle was measured by a laser granulometer (Beckman Coulter LS13320, USA). Sediment moisture content was determined by weight loss of a known amount of wet sediment dried at 60 °C to a constant value. Total organic C and total N concentrations in the sediment samples leached by 1 M HCl were determined using a thermal combustion furnace analyzer (Elementar analyzer vario MaxCNOHS, Germany). Exchangeable ammonium ($NH4^+$-N), nitrite (NO_2^--N), and nitrate (NO_3^--N) were extracted from fresh sediments with 2 M KCl solution and measured by a continuous flow autoanalyzer (SAN plus, *Skalar Analytical* B.V., the Netherlands) with detection limits of 0.5 μM for $NH4^+$-N and 0.1 μM for NO_2^--N and NO_3^--N (Hou et al. 2013).

Extracellular enzyme activity assay

Activities of five sediment enzymes associated with C-cycling [sucrase (SUC), cellulase (CEL) and polyphenol oxidase (PPO)], N-cycling [urease (URE)], and sediment respiration intensity [catalase (CAT)] were evaluated. The analytical methods of these enzymes were slightly modified from Tabatabai (1994), Alster et al. (2013), and Du et al. (2014). Briefly, these enzyme activities were assayed using 5 g of freeze-dried sediment with their appropriate substrate at their optimal pH and incubated for 24 h at 37 °C with gentle agitation. Subsequently, developing agent was added into the extracted enzyme solutions for colorimetric analysis. Each

assay contained substrate blank and sample blank receiving substrate and deionized water, respectively. Absorbance was detected using a colorimetric plate reader (SpectraMax M5 Microplate Spectrophotometer; Molecular Devices Corporation, Sunnyvale, CA). The slope of linear regression between standard concentrations and absorbance (all $R^2 > 0.99$) was used to calculate activity, and these values were expressed in the unit of micromole per hour per gram sediment.

Phospholipid fatty acids analysis

Microbial community compositions of all sediment samples through assessment of phospholipid fatty acids (PLFA) abundance were determined according to the method slightly adapted from White et al. (1979). Briefly, 8 g of freeze dried sediment was added into a 50-ml centrifuge tube to extract with 23 ml of the mixture solvent consisting of chloroform, methanol, and phosphate buffer (1:2:0.8) in a homothermal shaker for 2 h. Subsequently, the tube was centrifuged at 3,500×*g* for 10 min, and the supernatant was removed. The residual sediment was re-extracted by the same method for a further 30 min, and then the supernatant was removed after centrifuging. The two extracts were combined and concentrated under nitrogen condition to 1 ml, further separated on silicic acid columns, and quantified with a nonadecanoic acid standard (200 μl). After that, they were then saponified and methylated, forming fatty acid methyl esters (FAMEs). Individual FAMEs were identified by gas chromatography (Agilent 7890 gas chromatograph equipped with a flame ionization detector and an Ultra-2 column) based on their retention times and in combination with the MIDI Sherlock Microbial Identification System (MIDI Inc., Newark, DE). Five micrograms per milliliter of the methyl nonadecanoate (19:0) was used as an internal standard to quantify the contents of PLFAs.

Lipid abundance could be measured to analyze microbial community composition in sediment. In this study, the i14:0, i15:0, a15:0, i16:0, i17:0, and a17:0 of iso and anteiso branch chain fatty acids were considered as Gram-positive bacteria (Denef et al. 2009; Landesman and Dighton 2010), while Gram-negative bacteria were represented by 17:0cy, 16:1ω9c, and 19:0cy of monounsaturated and cyclopropane fatty acids (Frostegård et al. 2011; Ushio et al. 2013). 18:1ω9c was used as an indicator of fungi, whereas 16:1ω5c was used to indicate arbuscular mycorrhizal fungi (Swallow et al. 2009). In addition, the PLFA 10 Me18:0 was used to indicate actinomycetes. The total bacteria are sum of Gram-positive and Gram-negative bacteria, and total microbial biomass are sum of bacteria, fungi, arbuscular mycorrhizal fungi (AMF), and actinomycetes. The abundance of individual PLFA was calculated as a percentage of the single PLFA peak area over the total peak area and then converted to absolute content of individual PLFA (nmol g^{-1} sediment) through the internal standard concentration.

Incubation experiment

Incubations started from the next day after sampling. The incubation experiment was set up to four treatments: control (CK), glucose (C), ammonium (N), and C plus N (CN) with three replicates. For the incubations, 10 g of fresh sediment for each treatment was placed in a 500-ml Mason jar, and then 0.1 ml treatment solution per gram sediment was thoroughly mixed through the sediment. Glucose was added as a source of labile organic carbon in the C treatment (0.6 mg g^{-1} sediment) (Keuskamp et al. 2013). In the N treatment, NH_4Cl solution was added with an amount of 0.12 mg g^{-1} sediment (Keuskamp et al. 2013). The mix solution of 0.6 mg g^{-1} sediment and 0.12 mg g^{-1} sediment (5:1, Fagerbakke et al. 1996) was added in the CN treatment. Control treatment only received 1 ml of deionized water. Three additional Mason jars with a cup containing 1 ml of 1 M NaOH were sealed, serving as controls to account for the CO_2 trapped from the air (Qiao et al. 2014).

All the Mason jars with sediment were incubated in the dark for 10 d at 20 °C (Keuskamp et al. (2013). During the incubation, 1 ml of 1 M NaOH solution was placed in small cups in each incubation Mason jar to trap CO_2 and were replaced each day. CO_2 samples were trapped from each incubation Mason jar and analyzed for CO_2. Unreacted NaOH was titrated with 0.2 M HCl against the phenolphthalein endpoint (Zibilske 1994; Qiao et al. 2014).

Microbial biomass and dissolve organic carbon and nitrogen

Before and after the incubations, microbial biomass carbon (MBC) and nitrogen (MBN) were calculated as the difference between the dissolve organic carbon (DOC) and nitrogen (DON) in fumigated and non-fumigated sediments (Vance et al. 1987; Brookes et al. 1985). Briefly, 5 g of sediment was extracted with 20 ml of 0.5 M K_2SO4 solution, shaken for 30 min, and then centrifuged for 10 min at 3,000 rpm. The supernatant was filtered through 0.45 μm pore-size filter, adjusted to pH <2, and stored refrigerated in the dark at 4 °C until analysis. An additional 5 g sediment was fumigated with ethanol-free chloroform in dark for 24 h at 28 °C and then extracted again in the same way. For all the extraction, total organic C (TOC) concentrations in the K_2SO4 extracts were measured with a Dimatec-100 TOC/TIC analyzer (Dimatec Analysentechnik GmbH, Essen, Germany). TOC concentrations in the K_2SO4 extracts from non-fumigated soils were defined as DOC. Concentrations of DON were measured in fumigated and non-fumigated sediments using a continuous flow auto analyzer (SAN plus, *Skalar Analytical* B.V., the Netherlands). MBC and MBN were calculated as the difference in extractable DOC and DON before and after fumigation divided by 0.38 and 0.54, respectively (Vance et al. 1987; Brookes et al. 1985; Huang et al. 2013).

Data and statistical analyses

All statistical analyses were performed using SPSS version 17.0 for Windows (SPSS Inc., Chicago, IL, USA) or Microsoft Excel 2007. One-way analysis of variance (ANOVA), followed by Tukey's HSD test, was used to analyze the significant differences of environmental properties and PLFAs contents among the sediments and was used to test the effects of different treatments on CO_2 fluxes, DOC, DON, MBC, MBN, and sediment mineral N. Repeated measures ANOVAs over time were performed to evaluate the effects of treatments and the tidal flat sediments on CO_2 flux rates. We used Pearson correlation analysis to study the significance of relationships between CO_2 efflux and mineral N pools with microbial community and enzyme activity and environmental properties. All differences were tested for significance at $P=0.05$.

Results

Environmental properties and enzyme activity of sediment samples

Sediments were primarily consisted of clay and silt with smaller amount of sand (Table 1), and characterized by ranging from 82.6 to 97.6 % of fine fractions with the grain size of <63 μm. However, the clay and sand fractions significantly differed among the sediments. The temperature and moisture content were significant higher in the high tidal sediment compared to the middle and low tidal sediments. These results might be attributed to the tidal changes and marsh plant invasion. No significant differences of pH and salinity were found among the sediments of the high, middle, and low tidal flats ($P>0.05$).

Organic carbon, total nitrogen, and ammonium contents were higher in the middle tidal sediment than in the high and low tidal sediments. The C/N molar ratio of the high tidal sediment was the highest with a value of 29, compared with those of the middle (18) and low (24) tidal sediments. The nitrate in the low tidal sediment was three to six times higher than in the high and middle tidal sediments. Significant differences in DOC, MBC, DON, and MBN were also observed among the high, middle, and low tidal sediments ($P<0.05$).

The activity of the detected enzymes differed significantly among the sediments of the high, middle, and low tidal flats (Table 2). For the carbon-cycling enzymes (SUC, CEL, and PPO), rates were lowest at the high tidal flat (SUC= 0.471 μmol h^{-1} g^{-1} sediment, PPO=113.45 μmol h^{-1} g^{-1} sediment) and/or the low tidal flat (CEL= 0.024 μmol h^{-1} g^{-1} sediment) and highest (ca. three times greater) at the middle tidal flat (mean: SUC= 0.905 μmol h^{-1} g^{-1} sediment, CEL=0.035 μmol h^{-1} g^{-1} sediment, and PPO=358.79 μmol h^{-1} g^{-1} sediment). However,

Table 1 Environmental properties for the sampling sites

Property	H	M	L	Unit
Clay	12.75±1.24 a	26.00±3.68 b	19.36±2.15 c	%
Silt	69.95±6.52 a	71.65±6.69 a	70.00±5.87 a	%
Sand	17.40±1.84 a	2.42±0.23 b	10.73±1.73 c	%
Temperature	23.58±0.37 a	26.16±0.05 b	26.62±0.48 b	°C
Moisture content	29.34±1.96 a	38.27±3.12 b	36.09±1.16 b	%
pH	7.17±0.24 a	7.29±0.13 a	7.43±0.13 a	–
Redox	412.00±46.95 a	165.10±40.43 b	285.80±11.31 c	mV
Salinity	0.87±0.47 a	0.70±0.10 a	0.43±0.06 a	g kg soil FW^{-1}
SOC	13.61±0.88 a	16.44±0.66 b	13.90±0.27 a	g kg soil DW^{-1}
TN	0.49±0.13 a	0.90±0.14 b	0.57±0.02 a	g kg soil DW^{-1}
C/N	28.90±6.50 a	18.45±2.34 b	24.21±0.52 ab	–
DOC	0.990±0.327 a	0.827±0.231 b	0.71±0.06 b	mg C g SOC DW^{-1}
MBC	0.24±0.03 a	1.71±0.46 b	0.85±0.36 c	mg C g SOC DW^{-1}
DON	17.00±3.13 a	27.154±7.99 b	20.76±1.76 a	mg C g TN DW^{-1}
MBN	19.26±3.71 a	8.45±1.74 b	29.07±3.21 c	mg C g TN DW^{-1}
NH_4^+-N	1.51±0.64 a	1.74±0.72 a	0.87±0.25 b	mg kg soil FW^{-1}
NO_3^--N	0.33±0.04 a	0.18±0.11 a	1.20±0.31 b	mg kg soil FW^{-1}
NO_2^--N	0.10±0.002 a	0.11±0.02 a	0.11±0.02 a	mg kg soil FW^{-1}

Significant differences were indicated by the different letters after values in each row ($P<0.05$). Values are averages±standard deviations ($n=3$)

H high tidal flat, *M* middle tidal flat, *L* low tidal flat, *SOC* sediment organic carbon, *TN* total nitrogen, *C/N* molar ratio of organic carbon to nitrogen, *DOC* dissolved organic carbon, *MBC* microbial biomass carbon, *DON* dissolved organic nitrogen, *MBN* microbial biomass nitrogen

Table 2 Extracellular enzyme activity (rates in μmol h^{-1} g^{-1} sediment) in the three sediments

Enzyme activity	H	M	L
URE	0.048±0.011 a	0.078±0.014 b	0.108±0.043 c
SUC	0.471±0.026 a	0.905±0.043 b	0.647±0.124 a
CEL	0.026±0.001 a	0.035±0.000 b	0.024±0.000 a
CAT	667.83±6.43 a	492.49±6.41 b	478.95±6.88 b
PPO	113.45±5.45 a	358.79±23.56 b	167.20±24.09 a

The values are mean±SE (n=3). Different letters within a row indicate the significant difference

the URE was highest at the low tidal flat (mean: 0.108 μmol h^{-1} g^{-1} sediment) and lowest at the high tidal flat (mean: 0.048 μmol h^{-1} g^{-1} sediment), and an opposite pattern was observed for CAT [lowest at the low tidal flat (mean: 478.95 μmol h^{-1} g^{-1} sediment) and highest at the high tidal flat (mean: 667.83 μmol h^{-1} g^{-1} sediment); 1.4-fold increase].

Microbial community composition and activity

The microbial community compositions determined by PLFA are shown in Table 3. The percent mole abundance of individual lipid biomarkers, except for i14:0 (G+), cy17:0 (G−), cy19:0 (G−), and 18:1ω9c (fungi), differed in the high, middle, and low tidal sediments. In addition, the percent mole abundance of total microbial biomass, G+, and G− in the middle tidal sediment was lower than in the high and low tidal sediments. Both the high and middle tidal sediments had greater contents of all individual lipid biomarkers (except for PLFA 10 Me 18:0) than the low tidal sediment. Contents of PLFA i15:0, i17:0, and a17:0 of G+, and 16:1ω9c of G− did not differ among the sediments of the different sites. The total microbial biomass, bacteria (G+ and G−), AMF, and fungi in the middle tidal sediment were significantly higher than in the high and low tidal sediments (P<0.05), while no significant difference of actinomycetes was observed. The ratio of bacteria to fungi ratio in the middle and low tidal sediments was significantly higher than in the high tidal sediment. In contrast, the ratio of G+ to G− was significantly lower in the middle and low tidal sediments than in the high tidal sediment (P<0.05). The contents of bacteria in the sediments of all the sites were the largest, ranging from 75.97 to 82.71 % of total microbial biomass.

Microbial basal respiration (BR) rate and microbial biomass (C_{micr}) were markedly higher in the middle

Table 3 Mean percent mole abundance and concentrations of PLFAs and two PLFA ratios in the three tidal sediments

PLAF	Mole abundance (%)			Content (nmol g^{-1} sediment)		
	H	M	L	H	M	L
i14:0	3.01±0.55 a	2.37±0.33 a	2.40±0.68 a	0.47±0.08 a	0.62±0.20 a	0.28±0.07 b
i15:0	6.37±0.46 a	4.30±0.63 b	6.76±0.23 a	1.00±0.08 a	1.15±0.21 a	0.83±0.09 a
a15:0	5.89±0.40 a	4.87±0.54 b	5.66±0.23a	0.92±0.06 a	1.21±0.26 a	0.69±0.04 b
i16:0	2.43±0.20 a	1.37±0.21b	1.98±0.16 c	0.38±0.01 a	0.37±0.13 a	0.25±0.04 b
i17:0	1.92±0.06 a	1.29±0.27 b	2.30±0.46 a	0.30±0.02 a	0.35±0.15 a	0.29±0.08 a
a17:0	1.30±0.29 b	0.91±0.07 b	2.31±0.42 a	0.20±0.03 a	0.24±0.06 a	0.29±0.08 a
Gram-positive (G+)	20.92±0.89 a	15.11±0.95 b	21.42±0.27 a	3.26±0.10 a	3.94±1.00 a	2.62±0.27 b
16:1ω9c	2.46±0.62 a	1.99±0.28 b	2.75±0.25 a	0.38±0.10 a	0.53±0.17 a	0.33±0.01 a
cy17:0	2.50±0.22 a	2.58±0.34 a	2.89±0.12 a	0.39±0.02 a	0.66±0.17 b	0.35±0.05 a
cy19:0	1.06±0.32 a	1.32±0.05 a	1.05±0.11 a	0.17±0.06 a	0.33±0.06 b	0.13±0.02 a
Gram-negative (G−)	6.03±0.56 a	5.89±0.38 a	6.69±0.17 b	0.94±0.16 a	1.53±0.38 b	0.81±0.06 b
Bacteria	26.95±1.05 a	21.00±1.28 b	28.10±0.30 a	4.21±0.17 a	5.47±0.48 b	3.43±0.33 a
16:1ω5c (AMF)	1.86±0.26 a	2.99±0.26 b	2.05±0.05 a	0.29±0.06 a	0.79±0.21 b	0.25±0.02 a
10 Me 18:0 (actinomycetes)	0.56±0.14 a	0.49±0.06 a	0.89±0.03 b	0.09±0.02 a	0.13±0.04 a	0.11±0.01 a
18:1ω9c (fungi)	3.20±0.25 a	3.13±0.30 a	3.33±0.19 a	0.50±0.02 a	0.81±0.19 b	0.41±0.06 a
Microbial biomass	32.57±1.20 a	27.61±1.67 b	34.37±0.43 a	5.09±0.23 a	7.20±0.81 b	4.19±0.41 a
Bacteria/fungi				8.46±0.31 a	6.80±0.65 b	8.49±0.43 a
G+/G−				3.71±0.78 a	2.57±0.10 b	3.21±0.10 a

Data expressed as mean±SE (n=3) are reported for different taxa and two PLFA ratios (bacteria/fungi and Gram-positive/Gram-negative bacteria). AMF, G+, and G− indicate the arbuscular mycorrhizal fungi, Gram-positive, and Gram-negative bacteria, respectively. Significant differences are indicated by the different letters after values in each row (P<0.05)

Table 4 Microbial activity indicators of sediments at the high (H), middle (M), and low (L) tidal flats

Property	H	M	L	Unit
BR	1.38±0.04 a	2.81±0.33 b	1.53±0.54 a	μg CO_2-C g soil DW^{-1} h^{-1}
C_{micr}	1.34±0.29 a	12.66±4.13 b	5.12±2.01 a	μg C_{micr} g soil DW^{-1}
Q_{CO2}	1.06±0.21a	0.24±0.07 b	0.37±0.27 b	μg CO_2-C h^{-1} g C_{micr}^{-1}
C_{micr}/C_{org}	97.92±15.11a	764.69±226.26 b	369.74±151.73 a	μg C_{micr} g OC^{-1}
Qc	101.43±4.06 a	170.48±15.46 b	109.51±36.75 a	μg CO_2-C h^{-1} g SOC^{-1}

Significant differences were indicated by the different letters after values in each row ($P<0.05$). Values are averages±standard deviations ($n=3$)

tidal sediment than in the high and low tidal sediments ($P<0.05$). However, metabolic quotient (Qco_2) of the high tidal sediment was the highest compared to the middle and low tidal sediments (Table 4). In addition, the C_{micr}/C_{org} ratio of the middle tidal sediment was significantly higher than those of both the high and low tidal sediments ($P<0.05$), as were the relative carbon use (Q_C).

Effects of nutrient addition on CO_2 effluxes and nitrogen mineralization

The CO_2 efflux rates depended on nutrient amendment patterns, with an increase after glucose addition (Fig. 2). However, throughout the incubation time, specific CO_2 efflux rates within the ammonium treatment were not significantly higher than those of only water addition controls in the tidal

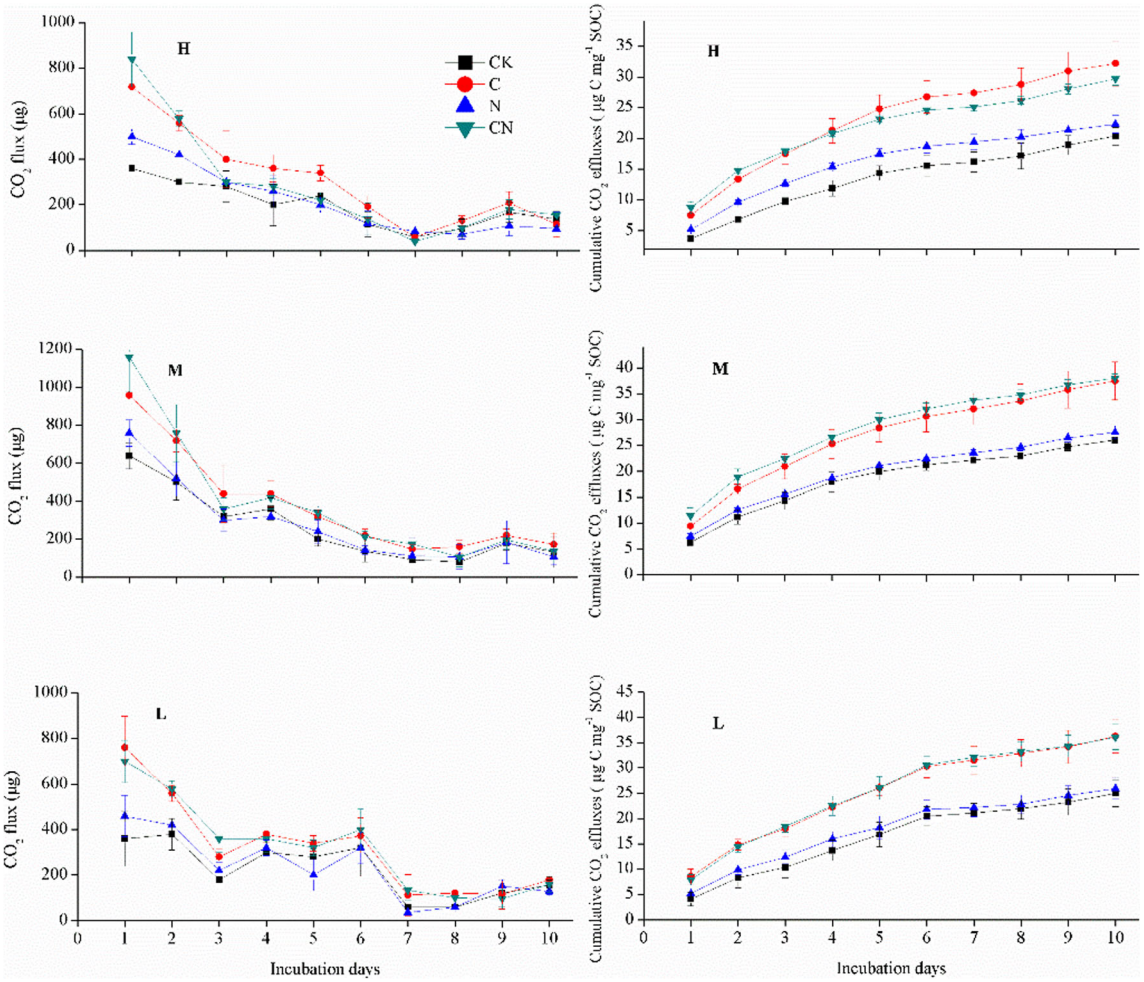

Fig. 2 CO_2 flux rates and cumulative CO_2 effluxes in the high (*H*), middle (*M*), and low (*L*) tidal sediments with different treatment patterns. Values are the means of three replicates, *bars* show standard deviations ($n=3$)

sediments (Table S1). CO_2 effluxes within the first two incubation days were higher in middle tidal flat sediment than in the high and low tidal sediments, however dramatically decreased following the incubation time. Repeated-measure ANOVA indicated that the treatment patterns and incubation time significantly affected CO_2 flux rates (Table S1 and S2).

The cumulative CO_2 effluxes induced by the C and CN additions were higher than the CK and N treatment in high, middle, and low tidal sediments. By the end of the incubations, the C and CN additions to the middle tidal sediment increased CO_2 effluxes by 5.36 and 5.27 % compared with the high tidal sediment. Compared with the high tidal sediment, those cumulative CO_2 effluxes with CK and N treatments to the middle tidal sediment increased by 5.69 and 5.27 %, and to the low tidal sediments increased by 0.99 and 1.56 %, respectively. During the incubation period, we tested if nitrogen application in sediments, without glucose addition, would induce microbial respiration, but this was not the case. In addition, addition of both nitrogen and glucose (CN) did not increased CO_2 effluxes compared to only glucose (C) addition in all sediments (Fig. 2).

At the end of incubations, a significant increase in NH_4^+-N pool was observed only in the N treatment to the high tidal flat sediment, while there was no significant difference among the CK, C, and CN treatments (Tukey's HSD test, $P<0.05$, Fig. 3a). There were no treatment differences in the middle and low tidal sediments. In addition, the NH_4^+-N pool for the N treatment was significantly higher in the high tidal sediment than in the middle and low tidal sediments (Fig. 3a). However, the CK treatment had higher NH_4^+-N pool in the middle tidal sediment than in the low tidal sediment.

A significant increase in NO_3^--N pool was observed only in the N treatment to the high and low tidal sediments, while there was no significant difference among the CK, C, and CN treatments (Tukey's HSD test, $P<0.05$, Fig. 3b). In addition, the N and CN treatments had significantly higher total mineral N pool in the high and low tidal sediments than in the middle tidal sediment, but there were no differences between the CK and C treatments (Fig. 3c). It was found that the NO_3^--N pool was the dominant form in the mineral N pools for the CK, N, and CN treatments in the high and low tidal sediments (Fig. 3b, c). It was also interesting to find that the mineral N pools did not change in the middle tidal sediment with the different treatments during incubation.

Microbial biomass and dissolved organic carbon and nitrogen budgets

In the treatments without the added glucose, DOC slightly decreased while the difference among sediment types and treatment patterns was not significant (Fig. 4a). In the glucose-amended treatments (C and CN), DOC budgets were higher as a result of the incomplete consumption of added glucose. However, during the incubation, the DOC pools in all the sediments did not change significantly with different treatments. The C treatment had significant higher MBC in the middle tidal sediment than in the high and low tidal sediments, but there were no differences in the N and

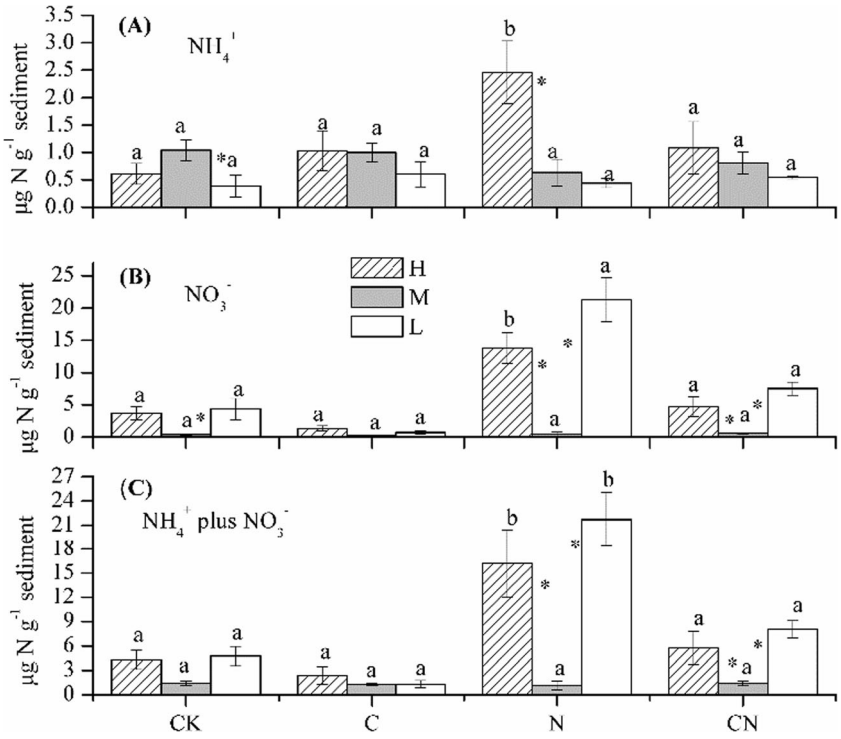

Fig. 3 Concentrations of mineral N: NH_4^+pool (**a**), NO_3^- pool (**b**), NH_4^+ plus NO_3^-pool (**c**) in the high (*H*), middle (*M*), and low (*L*) tidal sediments with different treatment patterns after a 10-day incubation period. *Bars* show standard deviation of means ($n=3$). *Different letters above columns* indicate significant difference among treatments for each sediment at $P<0.05$ level. The *asterisk above columns* indicates significant difference in mineral N contents among the high, middle, and low tidal sediments at $P<0.05$ level

CN treatments (Fig. 4b). The MBC pool increased with the treatments in the middle and low tidal sediments, while decreased in the high tidal sediment. In addition, DOC and MBC pools decreased with treatments during the incubation, exception for the low tidal sediment with the CN treatment (Fig. 4a, b; Table 1).

The changes in DON and MBN pools with the different treatments during the incubation are shown in Fig. 4c, d. DON and MBN of the tidal sediments were higher in both the C and CN treatments than in the CK treatment, indicating partial consumption of added N and the organic nitrogen release from increasing microbial biomass respiration. In addition, the high tidal sediment had the highest DOC and MBN pools, while the low tidal sediment had the lowest DOC and MBN pools (Fig. 4c, d). The MBN pools increased with different treatments during the incubation, except for the low tidal sediment (Fig. 4d; Table 1).

Direct and partial correlation analysis

The OC content was negatively correlated with Eh (r=−0.72, P=0.03) and C/N (r=−0.90, P<0.01, Table S3), and strongly correlated with C/N ratio (r=0.99, P<0.01), but unrelated to the other environmental variables (i.e., pH, salinity, all $|r|$< 0.22, P>0.39). In addition to the relationship with environmental variables, OC was strongly correlated with specific enzymes of SUC, CEL, and PPO (all r>0.81, P<0.01). Eh, a potential influencing factor, was significantly related with all enzymes except URE (all $|r|$>0.75, P<0.02). The ratios of G+ to G− and bacteria to fungi were significantly and positively correlated with the OC and C/N ratio (all $|r|$>0.69, P<0.04, Table S4). Taken together, OC and C/N were key role factors influencing enzyme activity and microbial community composition.

A partial correlation analysis was conducted to determine the extent to which OC or C/N was a direct driver of enzyme

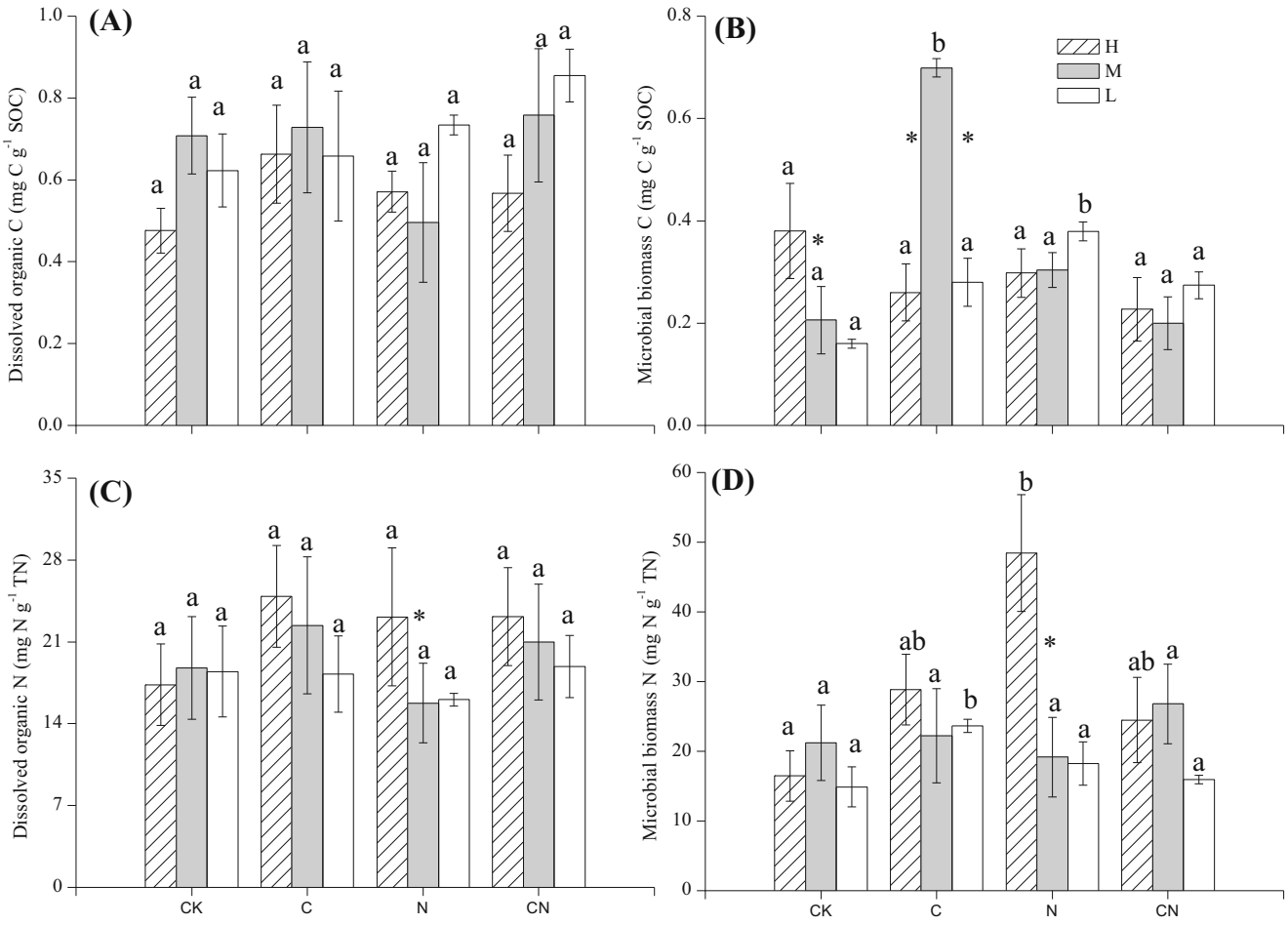

Fig. 4 Dissolved organic C (**a**), microbial biomass C (**b**), dissolved organic N (**c**), and microbial biomass N (**d**) pools of the high (*H*), middle (*M*), and low (*L*) tidal sediments after a 10-day incubation period. *Bars* show standard deviation of means (n=3). *Different letters above columns* indicate significant difference among treatments for each sediment at P<0.05 level. The *asterisk above columns* indicates significant difference in pools among the high, middle, and low tidal sediments at P<0.05 level

Table 5 Partial correlation analysis (controlling for OC or C/N) comparing enzyme activity and microbial indicators to OC and C/N

	OC		C/N	
	r	*P*	*r*	*P*
Enzyme avtivity				
URE	−0.63	0.08	−0.71	<0.05*
SUC	0.52	0.19	0.02	0.96
CEL	0.88	<0.01*	0.57	0.14
CAT	0.27	0.52	0.54	0.17
PPO	0.72	<0.05*	0.05	0.90
Microbial indicator				
G+/G−	0.43	0.29	0.80	0.02*
Bacteria	0.57	0.14	0.36	0.38
Bacteria/fungi	−0.56	0.15	−0.11	0.80
Total biomass	0.61	0.11	0.38	0.36

*$P<0.05$

activity and microbial communities (Table 5). The OC content after controlling for C/N was positively correlated with CEL ($r=0.88$, $P<0.01$) and PPO ($r=0.72$, $P<0.05$), but unrelated to the other enzymes (all $|r|<0.63$, $P>0.08$). However, C/N after controlling for OC exhibited no correlations with enzymes (all $r<0.32$, $P>0.14$) except for URE rates ($r=-0.71$, $P<0.05$). For the microbial indicators, only ratio of G+ to G− showed significantly correlated with C/N after controlling for OC ($r=0.80$, $P<0.02$), but not OC (Table 5; all $|r|<0.61$, $P>0.11$).

Correlations of the microbial indicators (enzyme activity and microbial community) with CO_2 effluxes and mineral nitrogen were also examined through direct and partial (controlling for OC) correlation analysis (Table 6). CO_2 efflux was significantly correlated with carbon-cycling enzymes (SUC, CEL, and PPO), and Gram-negative bacteria, AMF, and fungi. Mineral nitrogen was negatively and significantly related with SUC and PPO enzymes, and AMF. However, there was no significant relationship of CO_2 efflux and mineral nitrogen with enzyme activity and microbial community compositions after controlling for covariates with OC via partial correlation.

Discussion

The rhizospheric sediment microenvironments vary among different vegetation types, which alter the structure and diversity of the sediment microorganisms (Li et al. 2010; Tang et al. 2011b; Han et al. 2014). The contents of PLFAs in the low tidal sediment influenced by the tidal dynamics were lower than in the high and middle tidal sediments covered with marsh plants (Table 3). The higher ratio of bacteria to fungi in the high and middle tidal sediments indicates that rhizospheric environment has greater promotion for the growth of fungi than bacteria (Table 3), which might be attributed to the stimulation of N enrichment to the fungal activity (Garbeva et al. 2004) and the key role of saprophytic fungi in litter decomposition (Meidute et al. 2008). Bacterial community compositions also differed in the different

Table 6 Direct and partial (controlling for OC) correlation analysis comparing enzyme activity and microbial community composition to CO_2 efflux and mineral nitrogen

	CO_2 effluxes				Mineral nitrogen			
	Direct		Partial		Direct		Partial	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Enzyme activity								
URE	−0.14	0.72	−0.58	0.13	−0.01	0.98	0.08	0.85
SUC	0.79	0.01*	0.40	0.32	−0.68	0.04*	−0.24	0.57
CEL	0.91	<0.01*	0.57	0.14	−0.79	0.01	−0.38	0.35
CAT	−0.34	0.37	0.34	0.41	0.41	0.28	0.06	0.89
PPO	0.89	<0.01*	−0.02	0.96	−0.84	<0.01*	−0.42	0.31
Microbial community								
Gram-positive	0.59	0.09	0.36	0.38	−0.47	0.20	−0.13	0.76
Gram-negative	0.74	0.02*	0.33	0.43	−0.53	0.14	0.05	0.90
AMF	0.86	<0.01*	0.43	0.29	−0.70	0.03*	−0.14	0.73
Actinomycetes	0.44	0.24	0.08	0.85	−0.56	0.12	−0.39	0.34
Fungi	0.76	0.02*	0.42	0.3	−0.58	0.10	−0.07	0.87

*$P<0.05$

sedimentary environments (Table 3). The lower ratio of G+ to G− bacteria in the middle tidal sediment suggests that marsh plants affected the growth of G− and G+ bacteria because that nutrients addition (especially N input) obviously modified the bacterial community compositions (Denef et al. 2009).

Under the effects of the tidal changes and marsh plant invasion, environmental variables differed among the high, middle, and low tidal sediments. The combinations of plants and microorganisms may have more potential to nutrient cycles, possibly because the sedimentary organic carbon should maintain the balance of organic carbon input and output (Li et al. 2010). Therefore, the OC in the middle tidal sediment was significantly higher than that in the high and low tidal sediment, while C/N ratio was significantly lower due to the highest TN content (Table 1). These findings confirmed that the great microbial activity capability was observed in the middle tidal sediment (Table 4). Compared to the high and low tidal sediments, C mineralization rate in the middle tidal sediment was twice high, while organic carbon was only 1.18 to 1.21 times high, which might be attributed to the more abundance of microbial biomass measured by PLFA analysis in the middle tidal sediment (Table 3).

Keuskamp et al. (2013) have demonstrated that the microbial activity was primarily inhibited by limited energy despite a large amount of organic carbon in the mangrove sediment. Generally, higher OC and nutrient contents can increase the microbial biomass (Allen and Schlesinger 2004; Chen et al. 2012). Likewise, in this study, the highest microbial biomass (C_{micr}, Table 4) was detected in the middle tidal sediment with high OC content, whereas the lowest microbial biomass appeared in the high tidal sediment. This result was in accordance to the difference of OC contents in mangrove sediments (Keuskamp et al. 2013) as well as in forest soils (Qiao et al. 2014). The metabolic quotient (Qco_2) was significantly lower in the middle and low tidal sediments than in the high tidal sediment due to the difference in C_{micr} contents of these sediments (Keuskamp et al. 2013). In addition, microbial community composition is a crucial factor of OC mineralization (Garcia-Pausas and Paterson 2011; Tang et al. 2011b), resulting in differences of microbial activity indicators despite of the identical OC content (Tables 1 and 4). Therefore, with regard to the microbial activity associated with nutrient input, how microbial community composition in sediments responds to external C and N supply still needs further study.

Changes of microbial biomass and dissolved organic carbon and nitrogen

Dissolved organic C and N have been suggested as labile pools readily utilized by microorganisms and as indicators of external environment change effect on sediments (Strahm et al. 2009; Wickland et al. 2012). The N, which is more instable than C, is often directly lost by volatilization and leaching (Mendham et al. 2003). In this study, there were no significant differences of DOC and DON pools in all the sediments among the different treatments (except for DON in the N treatment), indicating that DOC and DON primarily come from the release of dead microorganisms and added organic carbon decomposed by microorganisms (Fontaine et al. 2003). The slight increase in DON with the treatments receiving carbon without nitrogen was likely attributed to the induction of DON release from microorganisms by carbon addition (Fontaine et al. 2003). In contrast, no significant difference in DON between the treatments receiving N and CK treatment might be attributed to the absorption of added N by microbial biomass (Keuskamp et al. 2013). However, DOC, which is sensitive to pH change, should be considered as a key driver of the change in carbon fluxes (Evans et al. 2012).

The differences in MBC and MBN under the treatments observed in present study could be associated with the differences of microbial communities in sediments because the activity of microorganisms is easily induced by available organic substrate (Keuskamp et al. 2013). In addition, MBC is sensitive in response to temperature change, which reduces with increasing temperature (Wei et al. 2014). MBN increased in the high and middle tidal sediments, mainly because added N is easily absorbed by microbial biomass (Keuskamp et al. 2013), while MBN decrease in the low tidal sediment was attributed to high nitrification (Fig. 3b). The recovery of microbial biomass is influenced by the type and rate of nutrient inputs as well as sediment characteristics (Mendham et al. 2003; Kallenbach and Grandy 2011). The Yangtze Estuary is subject to the typical subtropical monsoon climate with high temperature and precipitation. The seawater erosion, dry/wet cycles, and plant invasions might be potential factors which resulted in the minor effect of C and N additions on DOC or DON pools in the tidal sediments (Wang et al. 2010; Chen et al. 2012; Harrison-Kirk et al. 2013; Hu et al. 2014). Therefore, the significant difference in microbial communities among the sediments was probably the key reason why C and N inputs had the significant effects on MBC and MBN concentrations.

Responses of CO_2 efflux and mineral nitrogen to nutrient inputs

To the best of our knowledge, this is the first time that CO_2 flux rates and mineral nitrogen dynamics induced by different nutrient addition patterns (C, N, and CN additions) have been compared in the different tidal flat sediments of the Yangtze Estuary. We demonstrated that the addition patterns can stimulate different CO_2 productions, which strongly rely on the addition patterns receiving glucose, sediment type, and the incubation time (Fig. 2). The results of the nutrient additions to the sediments showed that the C addition is an important

driver for CO_2 production (Fig. 2). However, throughout the experiment, the N addition did not cause more cumulative CO_2 effluxes than the CK treatment in the middle and low tidal sediments, while the CN and C additions produced the same amount in the low tidal sediment (Fig. 2). These results indicated that the N addition cannot facilitate native OC mineralization, while OC decomposition can be stimulated by the C and/or CN addition (Neff et al. 2002; Ramirez et al. 2012).

The differences in CO_2 effluxes among nutrient addition patterns and sediment types could be associated with different microbial communities and activities because the activities of microorganisms are easily induced by available organic matter (Kuzyakov 2010; Wei et al. 2014; Qiao et al. 2014). Most microorganisms in the tidal flat sediments oscillate between dormant and active physiological states due to nutrient limitation, low temperature, and high moisture (Brix et al. 2001; Chmura et al. 2003; Friborg et al. 2003). After fresh organic matter input to sediments, a fraction of specialized microorganisms grow quickly and only decompose the fresh organic matter (Fontaine et al. 2003). Consequently, cumulative CO_2 effluxes increased rapidly at the beginning of incubation as added LOC induced exponentially the growing respiration rates (Keuskamp et al. 2013). The N addition cannot enhance CO_2 release because N depresses microbial activity by shifting the metabolic capabilities of bacterial communities (Ramirez et al. 2012), and added N is easily absorbed by microbial biomass (Keuskamp et al. 2013). This is apparent in the high tidal sediment because the CO_2 flux rates and cumulative CO_2 effluxes in the CN treatment were lower than in the C treatment.

CO_2 flux rates of the tidal flat sediments are also influenced by multiple environmental factors (Bauer et al. 2013; Keuskamp et al. 2013; Peralta et al. 2013). In this study, the CO_2 effluxes had significant correlation with OC, C/N ratio, Eh, moisture, and ammonium. Of all these factors, OC and TN, which play a major role in energy supply for microbial activity in sediments, have been certified as key parameters regulating microbial respiration (Tang et al. 2011b) and microbial communities (Fang and Wang 2007; Kong et al. 2011; Ramirez et al. 2012), respectively. Generally, CO_2 effluxes and BR acquired from the higher OC sites in the tidal flat of the Yangtze Estuary were significantly different from those obtained from the lower OC sites (Tables 1 and 4). Likewise, in the mangrove sediments, a dramatic increase in BR was also observed associated with OC of low (BR, 1.7 μg CO_2-C g soil DW^{-1} h^{-1}; OC, 18 g kg^{-1} DW) and high levels (BR, 4.0 μg CO_2-C g soil DW^{-1} h^{-1}; OC, 79 g kg^{-1} DW) (Keuskamp et al. 2013).

It has been reported that sediment texture and Eh might affect the microbial communities and growth by changing the microenvironment for activity and physiologies of microorganisms (Kong et al. 2011; Harrison-Kirk et al. 2013; Peralta et al. 2013). Middle tidal flat has the significant lowest redox potential than that in the high tidal flat and low tidal flat because relatively high temperature and OC content can result in decrease in Eh (Wu et al. 2012). Also, the impacts of moisture, temperature on the CO_2 release have been proposed (Tripathi et al. 2006; Jayakumar et al. 2009; Bauer et al. 2013). In the low tidal sediment of this study, NH_4^+-N and salinity had negative correlations with CO_2 effluxes. NH_4^+-N as the primary energy source might promote the microbial activity; however, previous studies also reported that high concentration of NH_4^+-N partly inhibited the microbial activity (Hatzenpichler et al. 2008; Keuskamp et al. 2013). Generally, sediment salinity suppresses the growth and metabolism of microorganisms, and an increase of sediment salinity result in a decrease of enzyme activities (Siddikee et al. 2011; Hu et al. 2014). In this study, both the high and middle tidal sediments had greater contents of all individual lipid biomarkers (except for PLFA 10 Me 18:0) than the low tidal sediment (Table 3). Consequently, CO_2 effluxes differed among the high, middle, and low tidal sediments of the Yangtze Estuary, due to the differences in environmental variables and microbial communities. However, further work is required to examine the complicated interactions between the microbial activity and the factors related to in situ hydrological conditions, such as tides, river runoff, and water mixing of estuary.

The NH_4^+-N pools in the high tidal sediment with the N treatment had significantly higher than in the middle and low tidal sediments (Fig. 3a), indicating that ammonification in response to nutrient inputs would not change within a short incubation time. The spatial distribution variations of NH_4^+-N pools were found to be in accordance with the abundance levels of ammonia-oxidizing bacteria and archaea in the study area (Zheng et al. 2013). We observed that nutrient addition patterns can induce different mineral NO_3^--N pools, which strongly depend on the addition patterns receiving nitrogen (Fig. 3b). The results of the nutrient additions to the sediments showed that the C addition is an inhibiting factor for nitrification. However, throughout the incubation experiment, all the treatments did not have a significant effect on nitrification in the middle tidal sediment (Fig. 3b), indicating that other factors complicatedly impact the sediment N mineralization. In addition, significant differences of NO_3^--N pools among sediment types compared to NH_4^+-N implied that nitrification is more sensitive to the C and N inputs than ammonification (Fig. 3). Previous studies reported that the increase in available C can contribute directly to enhanced microbial immobilization of N, resulting in less available N for nitrification (Trinsoutrot et al. 2000; Myrold and Posavatz 2007; Huang et al. 2013).

Variations in sediment microbial community composition and enzyme activity have often been suggested to be a potential link between biotic environments and C and N mineralization (Huang et al. 2013; Hou et al. 2013; Peralta et al. 2013).

In the present study, the mineral nitrogen was significantly and negatively correlated with the SUC and PPO activity and VAM fungi abundance (Table 6), which supports the argument that the differences in microbial community and enzyme types impact on the N cycling (Fraterrigo et al. 2006; Siddikee et al. 2011; Peralta et al. 2013; VanZomeren et al. 2013). Although C mineralization was significantly influenced by carbon-cycling enzymes (SUC, CEL, and PPO), these enzymes are also affected by environmental properties (e.g., C/N, Eh, and WC, Table S3) (Wallenstein and Burns 2011; Morrissey et al. 2014), indicating that a further study is still needed to reveal the complicated interactions between C mineralization and influencing factors. The Gram-negative bacteria, AMF, and fungi were found to be significantly and positively related with CO_2 efflux, supporting that specific bacteria has the function of carbon cycling. A partial correlation analysis showed that OC or C/N was a direct driver of enzyme activity of URE, CEL, and PPO, and a microbial indicator of G+/G− (Table 5). The reason is that the activities of specific enzymes may change depending on the relative availability of nutrients (Waldrop et al. 2004), and the microbial community composition may fluctuate in response to changes in contents of C and N in sediments (Fierer et al. 2009; Moeskops et al. 2012). In addition, ecology balance can be mediated by the microbial community structure and abiotic factors (Kourtev et al. 2002); thus, shifts in the microbial community have the potential to affect extracellular enzyme activity (Costa et al. 2007).

In conclusion, understanding the impacts of carbon and nitrogen inputs on the microbial activity is of increasingly specific concern as sediments are critical in global nutrients cycling due to sensitivity of coastal environment changes. Results of the present study provided evidence that, regardless of the sediment types, the microbial activity has positive responses to C inputs. N inputs appeared to decrease native OC mineralization, and the increase of microbial activity tended to reduce N mineralization. The CO_2 efflux and mineral nitrogen were found to be correlated with the activity of several extracellular enzymes and/or partial microbial communities, of which SUC, CEL, PPO, and AMF were more important in determining C and N decomposition rates. These changes might result in the enhanced sediment C sequestration under the N addition condition through mechanisms involving specific shifts in the microbial community composition (Ramirez et al. 2012). More research should consider the effects of nutrient supply on microbial communities and enzyme activity changes associated with the C and N mineralization in estuarine and tidal wetlands in a changing environment.

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