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Seawall construction alters soil carbon and nitrogen dynamics and soil microbial biomass in an invasive *Spartina alterniflora* salt marsh in eastern China

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ABSTRACT

Seawalls have increasingly been constructed to control the invasion of an exotic perennial grass, *Spartina alterniflora*, in coastal wetlands of eastern China. We investigated soil organic carbon (C) and nitrogen (N), available and microbial C and N, soil microbial community composition and biomass in a seawall-reclaimed *S. alterniflora* salt marsh compared with an adjacent natural *S. alterniflora* salt marsh. Seawall reclamation in *S. alterniflora* salt marsh significantly decreased soil salinity, moisture, litter and root biomass, and strongly decreased soil total organic C by 57% and total organic N by 59%, and also lowered soil available C and N in *S. alterniflora* salt marsh. Seawall reclamation significantly decreased soil microbial biomass C and the quantities of the total phospholipid fatty acids (PLFAs), and bacterial, fungal, gram-negative bacterial, gram-positive bacterial, saturated straight-chain, and monounsaturated PLFAs in deeper soil layers (10–30 cm). Our results suggested that seawall construction could greatly decrease soil C and N accumulation of *S. alterniflora* salt marsh by decreasing *S. alterniflora* residuals input into the soil and lowering soil salinity and moisture, which further decreased soil microbial biomass by lowering soil available C and N in *S. alterniflora* salt marsh. Changes in soil microbial community composition and biomass could in turn affect soil C and N accumulation in a seawall-reclaimed *S. alterniflora* salt marsh in eastern China.

1. Introduction

Coastal wetlands (e.g., salt marshes) have been recognized as important components of 'blue carbon' (C) sinks and play vital roles in alleviating global climate change (Laffoley and Grimsditch, 2009; Bu et al., 2015). It has been suggested that C burial rate in coastal salt marshes is approximately 55 times higher than that in tropical rainforests (Macreadie et al., 2013), and their global C sequestration (preliminary estimation up to 87.2 Tg C yr^{-1}) seems to surpass that in tropical rainforests (53 Tg C yr^{-1}) (McLeod et al., 2011; Macreadie et al., 2013). Although coastal wetlands provide various ecological services, such as biodiversity preservation, C sinks, retention of pollutants, nutrient filtration, shoreline erosion control, and flood peak reduction protection (Santín et al., 2009; Borsje et al., 2011; Bu et al., 2015), an increasing number of coastal wetlands have been degraded or lost by human activity in many countries (Santín et al., 2009; Ma et al., 2014). It is estimated that approximately 50% of salt marshes worldwide have been degraded or lost primarily due to land reclamation (Barbier et al., 2011). For instance, China's coastal wetlands have been enclosed by thousands of kilometers of seawalls that cover 60% of the total length of coastline of mainland China (Ma et al., 2014). Additionally, half of coastal wetlands were lost until 2000 due to reclamation and coastal engineering (Bi et al., 2014), leading to a

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dramatic decline in biodiversity, ecological service function and changes in biogeochemical cycles (Ma et al., 2014).

In the reclamation process, coastal wetlands are usually embanked (i.e., seawall construction) to use as agricultural land or for urban purposes (Santín et al., 2009). The destruction or conversion of coastal wetlands can strongly modify the redox environment and hydrology conditions of coastal wetlands due to blocking tidal inundation after reclamation (Dick and Osunkoya, 2000). The changes in soil physico-chemical properties (Wang et al., 2014) and hydrology conditions after seawall reclamation potentially affect soil C and nitrogen (N) sinks in coastal wetlands (Bu et al., 2015). Previous studies have reported that coastal embankment significantly drives the succession of salt marshes and soil (Bozek and Burdick, 2005; Bi et al., 2014), promotes soil C pool loss and releases more CO_2 into the atmosphere from a coastal *Phragmites australis* salt marsh (Bu et al., 2015). The conversion of coastal wetlands to agricultural land has been reported to significantly decrease soil C and N accumulation during the early period of reclamation (i.e., initial 16 years), but then rapidly recover within 30 years and thereafter slow accumulated soil C and N following cultivation time until 500 years (Cui et al., 2012). However, Wang et al. (2014) have found that although organic and chemical fertilizers have been applied over a long-term period of time, 60-year agricultural reclamation significantly decreases soil C and N accumulation in the surface layer (0–30 cm), possibly because soil organic matter (SOM) output exceeds input after long-term agricultural reclamation. These contradictory findings may result from differences in reclamation history, hydrologic conditions and land use patterns after reclamation (Wang et al., 2014).

It is well known that soil microorganisms play key roles in mediating soil C and N pools, and ecosystem C and N cycling (Zeller et al., 2008; Hargreaves and Hofmockel, 2014). Coastal wetland reclamation has been shown to significantly modify soil physico-chemical properties (Li et al., 2014; Wang et al., 2014), and alter soil C and N levels (Cui et al., 2012; Bu et al., 2015). The changes in soil physico-chemical properties and available substrate induced by coastal wetland reclamation could greatly affect soil microbial community structure (Liu et al., 2013). Changes in the soil microbial community structure (e.g., the ratio of fungal:bacterial biomass) would in turn further impact soil C and N sequestration and turnover (Bailey et al., 2002). Thus, a focus on the dynamic changes in soil C and N, and the soil microbial community structure following the reclamation of coastal wetlands has important significance for predicting the effects of global climate change, especially because more intensive reclamation is expected in the foreseeable future (Ma et al., 2014; Bu et al., 2015).

Spartina alterniflora, a perennial grass that is native to North America, was introduced into China in 1979 for coastal erosion control and sediment stabilization (Yang et al., 2016a). *S. alterniflora* has many biological traits (e.g., fast growth, high leaf area index and net photosynthetic rate, well-developed root system, great clonal propagation, and high tolerance to salt and waterlogging) (Liao et al., 2007; Li et al., 2009; Yang et al., 2016a), making it a good 'ecosystem engineer' (Zhang et al., 2005; Li et al., 2009). For this reason, *S. alterniflora* has been widely introduced to the coastal wetlands of eastern China, and it has shown rampant expansion over the past 30 years, from Tianjin in the north to Beihai in the south, and covers a total area of more than 112,000 ha (An et al., 2007). Previous studies have showed that *S. alterniflora* invasion can greatly accelerate soil organic C and N sequestration by increasing *S. alterniflora* residuals input and lowering SOM decomposition in comparison with bare flat and native *Scirpus mariqueter*, *Suaeda salsa* and *P. australis* communities (Liao et al., 2007; Cheng et al., 2008; Yang et al., 2016a). Although *S. alterniflora* salt marsh strongly affects C and N sinks (Liao et al., 2007), many studies have reported that *S. alterniflora* invasion threatens native ecosystems, reduces biodiversity, alters the structure of trophic functional groups of nematode and macrobenthonic invertebrate communities, and changes the habitats of shorebirds (Li et al., 2009). The Chinese government has explored various methods, including cutting, burning and reaping young ramets, to eliminate *S. alterniflora*, and embankment has been proven to be an effective method to prevent *S. alterniflora* from expanding (An et al., 2007). Currently, coastal embankment has increasingly been used to control *S. alterniflora* invasion in coastal wetlands of eastern China (An et al., 2007).

A previous study have reported that coastal embankment significantly promotes C loss from previously sequestered soil C pool and accelerates soil respiration in a *P. australis* salt marsh (Bu et al., 2015). However, little is known about the effects of seawall construction in invasive *S. alterniflora* salt marsh on soil C and N dynamics, as well as the soil microbial community–the primary mediator of soil C and N cycling. We hypothesized that seawall construction in invasive *S. alterniflora* salt marsh would significantly decrease soil C and N accumulation by lowering plant residuals entering the soil and changing the soil physico-chemical properties, which further decrease soil microbial biomass by lowering soil available substrates. To test this hypothesis, we examined the concentrations of soil organic carbon (SOC) and organic nitrogen (SON), water-soluble organic carbon (WSOC), nitrate nitrogen (NO_3-N), ammonium nitrogen (NH_4-N), nitrite nitrogen (NO_2-N), microbial biomass carbon (MBC) and nitrogen (MBN), and examined the soil microbial community composition and biomass through phospholipid fatty acids (PLFAs) analysis in a seawall-reclaimed *S. alterniflora* salt marsh by comparing the results with an adjacent natural *S. alterniflora* salt marsh. We also identified the effects of biotic (e.g., litter and root biomass) and abiotic (e.g., soil moisture, bulk density, pH and salinity) factors on soil C and N dynamics, and soil microbial community composition and biomass in natural and seawall-reclaimed *S. alterniflora* salt marshes in a coastal wetland of eastern China.

2. Materials and methods

2.1. Study area

The study was conducted in the third core region of the Dafeng Milu National Nature Reserve (DMNNR), Jiangsu Province, China (32°59′–33°03′ N, and 120°47′–120°53′ E; Fig. 1). The DMNNR is located next to the Yellow Sea (Fig. 1). The climate in this area belongs to the typical monsoon climate transition belt from a subtropical zone to a warm temperate zone with a mean annual temperature of 14.1 °C; the mean annual precipitation exceeds 1000 mm (Liu et al., 2011). The DMNNR was established in 1986 and contains the world's largest population of wild *Elaphurus davidianus*, which have returned to nature (Liu et al., 2011). The DMNNR was recorded as part of the "Man and Biosphere Protection Network" in 1995, is a designated Ramsar Site and was listed in the directory of Wetlands of International Importance in 2002 (Wang and Wall, 2010).

S. alterniflora was intentionally introduced into the Dongtai River estuary of the intertidal zone, Dongtai city, Jiangsu province, in 1988, and it rapidly expanded northward over a distance of 100 km and occupied the coastal beach in Dafeng city, Jiangsu Province (Ding, 2009). At present, the dominant plant community of the third core area of the DMNNR is an invasive *S. alterniflora* community that occupies an area of 14 km^2, i.e., approximately 70% of the whole third core area (Ji et al., 2011). Native C_3 *S. salsa* and *P. australis* communities occupy approximately 30% of the third core area in the DMNNR. For the purposes of controlling *S. alterniflora* expansion and recovering the native salt marshes, a seawall was constructed in the third core area of the DMNNR in 2011 (Fig. 1);

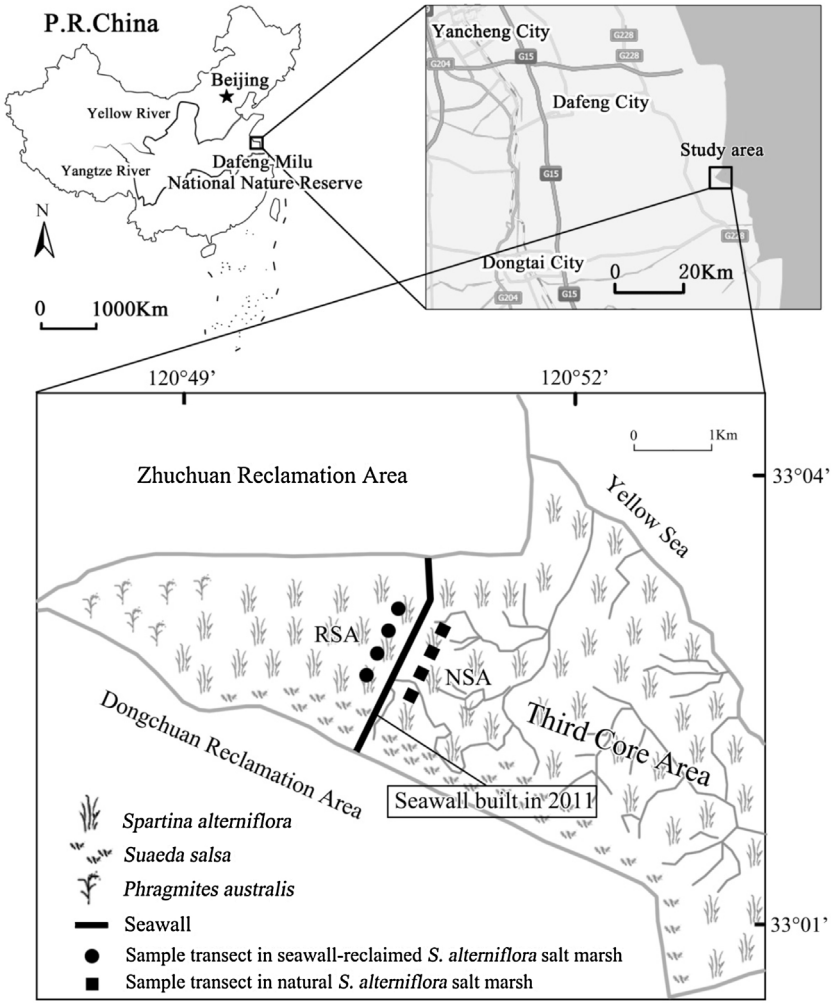

Fig 1. Location of the sampling site in the Dafeng Milu National Nature Reserve of coastal Jiangsu, China. NSA = natural *Spartina alterniflora* salt marsh; RSA = seawall-reclaimed *Spartina alterniflora* salt marsh.

this region was identified based on analyses of Thematic Mapper satellite images. This seawall was constructed using soil and covered by an impermeable cloth; consequently, both seawater and river water were blocked. The length of this seawall is approximately 2400 m, the width is approximately 2 m, and the height is approximately 2 m. Prior to embankment in 2011, the dominant specie inside and outside the seawall was *S. alterniflora* community. Approximately 5.5 km^2 of a *S. alterniflora* salt marsh was enclosed from a natural *S. alterniflora* salt marsh by seawall reclamation, and this seawall-reclaimed *S. alterniflora* salt marsh was not used as farmland or for other purposes, and was not subjected to other disturbances.

2.2. Field sample collection

In July 2013, four sample transects of 40 m × 40 m were selected in the seawall-reclaimed *S. alterniflora* salt marsh (RSA) and natural *S. alterniflora* salt marsh (i.e., the control; NSA) (Fig. 1), respectively, and there was a distance of 50 m apart between the two adjacent sample transects. The sampling transects in the seawall-reclaimed and the natural *S. alterniflora* salt marshes were symmetrically. The distance between each sampling transect and the seawall was approximately 50 m apart, and the distance of sampling transects between seawall-reclaimed and the natural *S. alterniflora* salt marshes was approximately 100 m apart (Fig. 1). Because the annual growth rate of the coastline in Dafeng is 200 m per year (Wang and Wall, 2010), the study areas including both the seawall-reclaimed and natural *S. alterniflora* salt marshes all nearby the seawall had similar marshes type and almost the same age prior to seawall reclamation. The seawall-reclaimed and natural *S. alterniflora* salt marshes in the sampling region have encroached on mud flat for approximately thirteen years; this was identified based on analyses of Thematic Mapper satellite images. We randomly selected three 2 m × 2 m plots along each transect, and three points were randomly selected for the collection of soil samples at 0–10, 10–20, and 20–30 cm soil depths. All soil samples from each soil depth in each plot were thoroughly mixed to form a composite sample. To obtain aboveground biomass, three 40 cm × 40 cm quadrats were established to collect plant leaves, stems and litter, and three soil blocks (10 cm length × 10 cm width × 30 cm depth) per transect were excavated to collect the roots of seawall-reclaimed and natural *S. alterniflora* salt marshes. All soil and plant samples were immediately transported to the laboratory at Nanjing University.

2.3. Laboratory analyses

Each soil block was placed into a 100-mesh sieve and flushed thoroughly with water; all roots that remained in the sieve were collected. All collected fresh plant samples (leaves, stems, litter and roots) were cleaned with a soft brush in the laboratory and dried at 65 °C to a constant weight. The C and N concentrations of the plant

samples were determined using an Elementar Vario Micro CHNS analyzer (Elementar Analysensystem GmbH, Germany). A fresh soil subsample was oven dried at 105 °C to determine the soil moisture (Yang et al., 2013). The bulk density was measured using the cutting ring method (Yang et al., 2016a). Plant and fauna residues in the soil samples were carefully removed, and the soil samples were then divided into three subsamples after thorough mixing. One subsample was air-dried and passed through a 1-mm sieve to measure soil pH, salinity, SOC and SON. A subsample of 2-mm sieved fresh soil was stored at 4 °C to determine WSOC, NO_3-N, NH_4-N, NO_2-N, MBC and MBN. Another subsample was passed through a 2-mm sieve and stored at −80 °C as quickly as possible after freeze-drying and was used for the analysis of PLFAs. Soil pH was measured in a 1:2.5 (soil:water) mixture with a glass membrane electrode. Soil salinity was measured in a mixture with a soil:water ratio of 1:5 using a conductivity meter (Yang et al., 2013). The SOC and SON concentrations were analyzed using an Elementar Vario Micro CHNS analyzer (Elementar Analysensystem GmbH, Germany); prior to theses analyses, approximately 10 g of dried soil subsamples were treated with 1 M HCl at room temperature for 24 h to remove any carbonate, and this fraction was interpreted to be SOM pool (Cheng et al., 2008). WSOC was extracted from 10 g of moist soil samples after the addition of 20 mL distilled water. The extracted fluid was vacuum filtered through a 0.45-μm filter, and the C concentration of the filtrate was rapidly determined using a Liqui TOCII analyzer (Elementar Analysensystem GmbH, Germany) (Yang et al., 2013). NH_4-N, NO_2-N and NO_3-N were measured using an AQ2+ Automated Discrete Analyzer (SEAL Analytical GmbH, Germany) after the samples were extracted with 2 M KCl. MBC and MBN were measured using the chloroform fumigation-extraction method (Vance et al., 1987). 25 g dry-weight-equivalent of moist soil was fumigated with ethanol-free chloroform for 48 h at 25 °C in the dark. The fumigated and un-fumigated samples were then extracted with 100 mL 0.5 M K_2SO_4 by shaking for 30 min at 200 rpm and then filtered. Organic C and TN in the K_2SO_4-extracted solution were determined using a Liqui TOCII analyzer and the Kjeldahl method, respectively. MBC and MBN were calculated according to the equation: MBC = Ec/0.38, MBN = En/0.54, where Ec and En were organic C and TN extracted from fumigated soil subtracted organic C and TN extracted from unfumigated soil, respectively (Yang et al., 2016b).

2.4. Phospholipid fatty acids analysis

The soil microbial community composition was assessed using PLFAs analysis based on the method described by Bossio and Scow (1998). Briefly, lipids were extracted from 8 g of freeze-dried soil subsamples using 23 mL of an extraction mixture of chloroform: methanol: phosphate buffer (1:2:0.8, v/v/v). The extraction was decanted into a separatory funnel that was added to12 mL of $CHCl_3$ and 12 mL of phosphate buffer after centrifugation. The separatory funnel was shaken for 2 min, and the extracts were layered overnight. The $CHCl_3$ layer was collected and dried under N_2 at 32 °C. Lipids were re-dissolved in chloroform and fractionated on a 0.5-g silica gel solid-phase extraction column (Supelco, Bellefonte, PA). Neutral and glycol lipids were eluted by 5 mL of $CHCl_3$ and 10 mL of acetone. Polar lipids were collected by 5 mL of methanol, dried under N_2 at 32 °C, and then subjected to a mild-alkali methanolysis to recover the PLFAs as methyl esters. The samples were re-dissolved in 200 mL of hexane solvent containing non-adecanoic acid methyl ester (19:0) as an internal standard. The samples were analyzed using a Hewlett-Packard 6890 Gas Chromatograph equipped with an Ultra 2-methylpolysiloxane column with N_2 as the carrier gas, and H_2 and air to support the flame. A 2-μL injection of the above dilution with a 1:50 split was employed at 250 °C for the injector and 300 °C for the detector. The oven temperature ramped from 170 °C to 300 °C at 5 °C min^{-1} and was held for 12 min. Peaks were identified using bacterial fatty acid standards and MIDI peak identification software (MIDI, Newark, DE). The concentrations of each PLFAs were calculated based on the 19:0 internal standard concentration (5 μg mL^{-1}). The quantities of the PLFAs in each sample were expressed as ng PLFAs g^{-1} dry soil and were used to estimate microbial biomass. Bacterial biomass was estimated from the concentrations of the biomarkers i14:0, i15:0, a15:0, 15:0, i16:0, i17:0, a17:0, 17:0, cy17:0, 14:1ω5c, 15:1ω6c, 16:1ω7c, and 18:1ω7c (Bossio and Scow, 1998; Bååth and Anderson, 2003; Cao et al., 2010). Fungal biomass was represented as the sum of the PLFAs 18:1ω9c, 18:2ω6,9c, and 20:1ω9c (Kourtev et al., 2002; Bååth and Anderson, 2003; Swallow et al., 2009). Gram-positive ($gram^+$) bacterial biomass was represented by the sum of the PLFAs i13:0, i14:0, i15:0, a15:0, i16:0, a16:0, i17:0, and Gram-negative ($gram^-$) bacterial biomass was estimated from the sum of the PLFAs 14:1ω5c, 15:1ω6c, 16:1ω7, 16:1ω9c, 17:1ω8c, 18:1ω7c, 12:0 2OH, 15:0 3OH, 16:1 2OH, cy17:0, cy19:0 ω8c, and 18:1ω7c 11-methyl (Kourtev et al., 2002, 2003; Sampedro et al., 2006; Cao et al., 2010). Arbuscular mycorrhizal fungal (AMF) biomass was considered to be represented by the PLFAs 16:1ω5c (Kourtev et al., 2002; Kong et al., 2008; Cao et al., 2010). Actinomycete biomass was expressed by the PLFAs 10me 16:0 and 10me 17:0 (Bossio et al., 2006). The fatty acids 14:1ω5c, 15:1ω6c, 16:1ω5c, 16:1ω7c, 16:1ω9c, 17:1ω8c, 18:1ω7c, 18:1ω9c, and 20:1ω9c were used as the biomarkers for monounsaturated PLFAs (Kourtev et al., 2003; Bossio et al., 2006; Cao et al., 2010). The fatty acids i13:0, i14:0, i15:0, a15:0, i16:0, a16:0, i17:0, a17:0, 10me 16:0, 10me 17:0, 12:0 2OH, 15:0 3OH, and16:1 2OH were chosen to represent branched PLFAs (Bossio and Scow, 1998; Bossio et al., 2006; Cao et al., 2010). The fatty acids 12:0, 13:0, 14:0, 15:0, 16:0, 17:0, 18:0, and 20:0 were considered as saturated straight-chain (SSC) PLFAs (Bossio and Scow, 1998; Cao et al., 2010). The total PLFAs of the microbial community were estimated from the sum of the fungal PLFAs, $gram^+$ bacterial PLFAs, $gram^-$ bacterial PLFAs, AMF PLFAs, actinomycete PLFAs, SSC PLFAs, and 20:4ω6,9,12,15c. The ratios fungal:bacterial PLFAs, $gram^-$:$gram^+$ and monounsaturated:branched PLFAs were calculated from the above PLFAs. The bacterial stress index (i.e., cy17:0/16:1ω7c) indicates the growth stage of the $gram^-$ bacterial community (Ponder and Tadros, 2002).

2.5. Statistical analyses

All of the statistical analyses were performed using SPSS Statistics 19 software. Data that did not meet the assumptions of normality and homogeneity of variance were log or cube root transformed prior to statistical testing. One-way analysis of variance (ANOVA) was used to determine the statistical significance of the effect of seawall reclamation on plant biomass, soil physicochemical properties, soil C and N fractions, and microbial biomass and various types of PLFAs. One-way ANOVA was also used to determine the statistical significance of soil depth on soil physicochemical properties, soil C and N fractions, and microbial biomass and various types of PLFAs. The significant differences among the group means were tested by Tukey's honest significant difference test, and the significance level was set to $P < 0.05$. Pearson's correlation analysis was performed to correlate the soil C and N fractions, and microbial biomass with the plant and soil properties. A Redundancy Analysis (RDA) was performed using CANOCO software for Windows 4.5 to test the relationship between the soil microbial community (all types of PLFAs) and environments variables. The statistical significance of the RDA was tested using the Monte Carlo permutation test (499 permutations; $P < 0.05$).

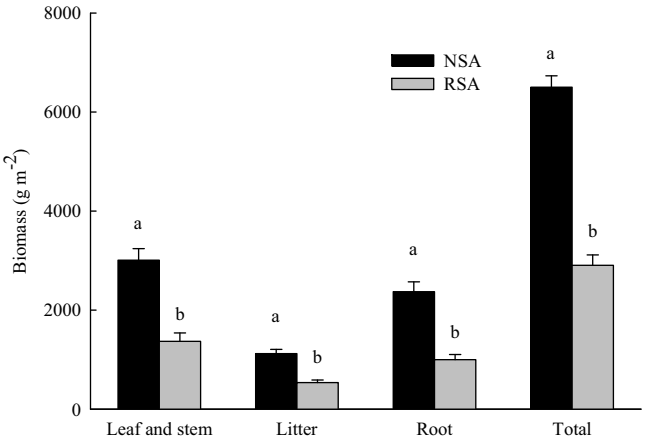

Fig. 2. Leaf and stem, litter, root and total biomass (0–30-cm soil layer) of natural and seawall-reclaimed *S. alterniflora* salt marshes. Different lowercase letters over the bars indicate statistically significant differences at $\alpha = 0.05$ level between natural and seawall-reclaimed *S. alterniflora* salt marshes for the same index and community. See Fig. 1. for abbreviations.

3. Results

3.1. Plant and soil properties

The *S. alterniflora* leaf and stem, litter, root and total biomass in the seawall-reclaimed salt marsh were significantly lower than those in the natural salt marsh (Fig. 2). Soil (0–30 cm) moisture and salinity in the seawall-reclaimed salt marsh were significantly lower than those in the natural salt marsh (Table 1). Soil bulk density and pH in the seawall-reclaimed salt marsh were significantly higher than those in the natural salt marsh except for bulk density in the 10–20-cm soil layer (Table 1). Soil moisture and salinity in the top soil layer (0–10 cm) were significantly higher than those in the deeper soil layer (20–30 cm) in both the natural and seawall-reclaimed salt marshes except for soil moisture in the seawall-reclaimed salt marsh (Table 1).

3.2. Soil carbon and nitrogen fractions

Seawall reclamation greatly affected soil C and N fractions (Table S1). The concentrations of SOC, SON, WSOC, NO_3-N, NH_4-N and NO_2-N in the 0–30-cm soil layer in the seawall-reclaimed salt marsh were significantly lower than those in the natural salt marsh (Fig. 3). The SOC and SON were significantly higher in the top soil (0–10 cm) than in the 10–30-cm soil layer in both the seawall-reclaimed and natural salt marshes (Fig. 3a and b). The concentrations of NO_3-N, NH_4-N and NO_2-N in the 0–10-cm soil layer were significantly higher than those in the 10–30-cm soil layer in the seawall-reclaimed salt marsh (Fig. 3). The WSOC, NO_3-N, NH_4-N and NO_2-N were not significantly affected by soil depth in the natural salt marsh (Fig. 3). Soil C and N fractions were significantly related to litter biomass, soil moisture and salinity, and were negatively correlated with soil pH (Table 2). The concentrations of WSOC, NO_3-N and NO_2-N were strongly associated with SOC and SON (Table 2).

3.3. Soil microbial community structure

Seawall reclamation significantly affected soil microbial biomass (e.g., MBC and the total PLFAs) (Table S2). Soil (0–30 cm) MBC concentration in the seawall-reclaimed salt marsh was significantly lower than that in the natural salt marsh (Fig. 4a), but there were no statistically significant differences in MBN and the MBC: MBN ratio in the 0–30-cm soil layer between the seawall-reclaimed and natural salt marshes (Fig. 4b and c).

The quantities of the total PLFAs and the bacterial, fungal, gram$^-$ bacterial, gram$^+$ bacterial, SSC, and monounsaturated PLFAs in the 10–30-cm soil layer in the seawall-reclaimed salt marsh were significantly lower than those in the natural salt marsh (Figs. 5 and 6). The quantities of the total PLFAs and the fungal, gram$^-$ bacterial, gram$^+$ bacterial, actinomycete, SSC and monounsaturated PLFAs in the 0–10-cm soil layer did not vary significantly between the seawall-reclaimed and natural salt marshes (Figs. 5 and 6). The fungal: bacterial PLFAs ratio in the 0–10-cm soil layer and the bacterial stress index measured in the 0–30-cm soil layer were significantly higher in the seawall-reclaimed salt marsh than those in the natural salt marsh (Figs. 5d and 6f). The quantities of the total PLFAs and the bacterial, fungal, gram$^-$ bacterial, gram$^+$ bacterial, AMF, actinomycete, SSC, monounsaturated and branched PLFAs in the 0–10-cm soil layer were significantly higher than those in the 10–30-cm soil layer in the seawall-reclaimed and natural salt marshes (Figs. 5 and 6).

3.4. Controls on soil microbial community

Ten environmental variables, including soil moisture, bulk density, pH, salinity, SOC, SON, WSOC, NO_3-N, NH_4-N, NO_2-N, explained 66.3% of the total variability in the PLFAs (Fig. 7). The variations in the PLFAs were strongly positively correlated with NO_3-N ($F = 9.46$, $P = 0.0040$) and WSOC ($F = 5.54$, $P = 0.0180$) (Fig. 7).

Table 1
Soil physico-chemical properties (mean ± SE, n = 12) in natural and seawall-reclaimed *S. alterniflora* salt marshes.

	Depth (cm)	Moisture (%)	Bulk density (g cm^{-3})	pH	Salinity (%)
NSA	0–10	54.50 ± 3.24^{Aa}	0.93 ± 0.07^{Ba}	8.33 ± 0.03^{Ba}	1.52 ± 0.06^{Aa}
	10–20	44.38 ± 1.95^{Ab}	1.07 ± 0.05^{Aa}	8.42 ± 0.04^{Ba}	1.28 ± 0.08^{Aab}
	20–30	44.23 ± 2.64^{Ab}	0.94 ± 0.08^{Ba}	8.45 ± 0.05^{Ba}	1.18 ± 0.09^{Ab}
RSA	0–10	30.73 ± 2.26^{Ba}	1.17 ± 0.04^{Aa}	9.00 ± 0.07^{Ab}	0.36 ± 0.02^{Ba}
	10–20	29.20 ± 1.57^{Ba}	1.24 ± 0.11^{Aa}	9.24 ± 0.04^{Aa}	0.27 ± 0.01^{Bb}
	20–30	27.23 ± 0.41^{Ba}	1.39 ± 0.01^{Aa}	9.38 ± 0.03^{Aa}	0.24 ± 0.01^{Bb}
Source of variation					
Seawall reclamation		108.004^{***}	25.806^{***}	463.345^{***}	528.517^{***}
Depth		5.693^{*}	1.646	15.429^{***}	9.060^{**}
Seawall reclamation × Depth		2.125	2.175	3.867^{*}	2.252

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. Different superscript uppercase letters indicate statistically significant differences at $\alpha = 0.05$ level between natural and seawall-reclaimed *S. alterniflora* salt marshes. Different superscript lowercase letters indicate statistically significant differences at $\alpha = 0.05$ level between soil depths. NSA: natural *Spartina alterniflora* salt marsh; RSA: seawall-reclaimed *Spartina alterniflora* salt marsh.

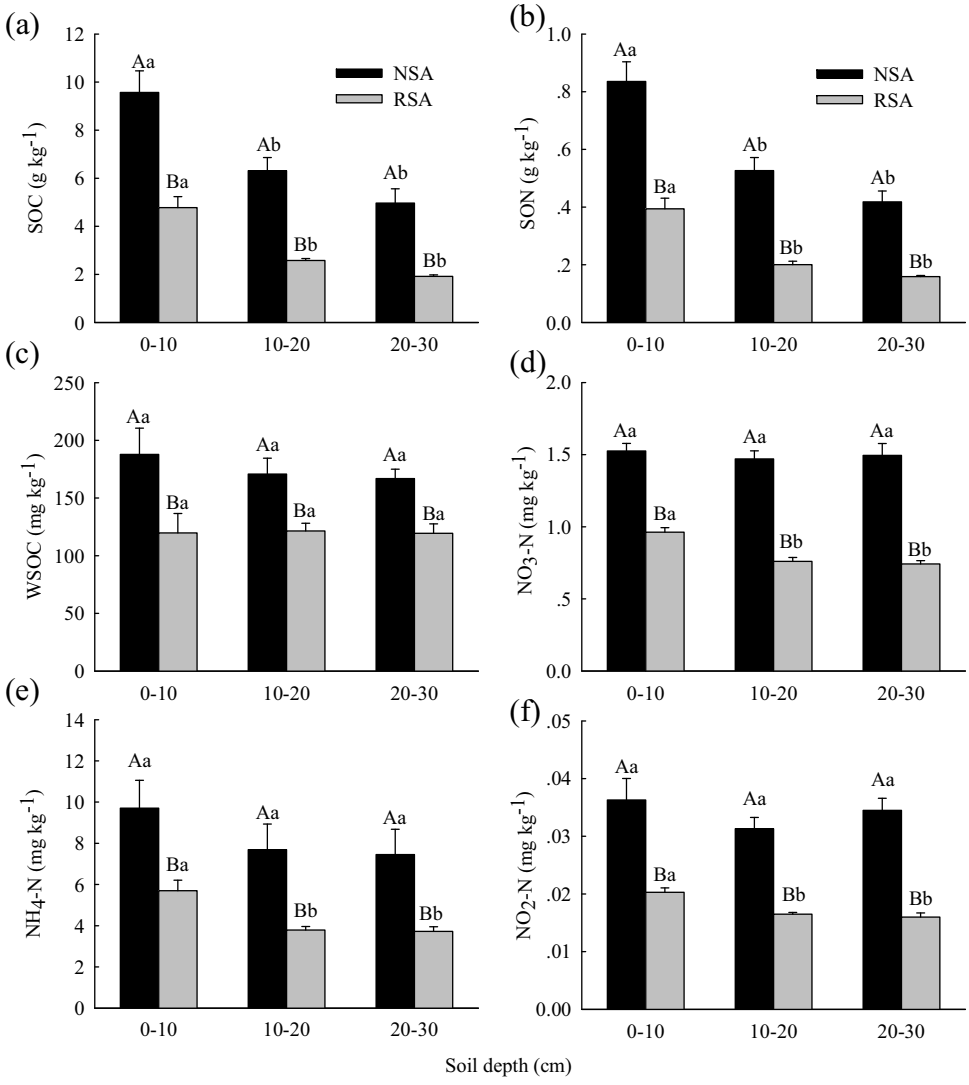

Fig. 3. (a) Soil organic carbon (SOC), (b) Soil organic nitrogen (SON), (c) Soil water-soluble organic carbon (WSOC), (d) Nitrate nitrogen (NO_3-N), (e) Ammonium nitrogen(NH_4-N), and (f) Nitrite nitrogen (NO_2-N) of natural and seawall-reclaimed *S. alterniflora* salt marshes. Different uppercase letters over the bars indicate statistically significant differences at $\alpha = 0.05$ level between natural and seawall-reclaimed *S. alterniflora* salt marshes. Different lowercase letters over the bars indicate statistically significant differences at $\alpha = 0.05$ level between soil depths. See Fig. 1. for abbreviations.

Axis 1 was highly correlated with NO_3-N and WSOC, and explained 64.5% of the total variation of the PLFAs (Fig. 7); axis 2 explained 1.7% of the total variation of the PLFAs (Fig. 7). Meanwhile, Pearson's correlation analysis showed that MBC was significantly positively correlated with soil moisture, salinity, SOC, SON, WSOC, NO_3-N, NH_4-N and NO_2-N, but they were negatively associated with soil bulk density and pH (Table 2).

4. Discussion

As expected, our study showed that the short-term seawall reclamation strongly decreased soil total organic C by 57% and total organic N by 59% in the 0–30-cm soil layer of the *S. alterniflora* salt marsh (Fig. 3a and b). Soil organic C and N sequestration is determined by plant residuals input and SOM decomposition

Table 2
Pearson correlation coefficients among soil C and N fractions, microbial biomass, plant and soil properties in the 0–30-cm soil layer of natural and seawall-reclaimed *S. alterniflora* salt marshes.

	LB	RB	Moisture	BD	pH	Salinity	SOC	SON	WSOC	NO_3-N	NH_4-N	NO_2-N	MBC
SOC	0.931**	0.557	0.963**	−0.868**	−0.965**	0.970**							
SON	0.909**	0.601	0.978**	−0.910**	−0.972**	0.984**	0.991**						
WSOC	0.831*	0.524	0.980**	−0.970**	−0.908**	0.933**	0.934**	0.956**					
NO_3-N	0.897**	0.735*	0.931**	−0.849**	−0.971**	0.976**	0.901**	0.929**	0.882**				
NH_4-N	0.748*	0.515	0.752*	−0.696	−0.765*	0.776*	0.652	0.697	0.735*	0.874**			
NO_2-N	0.904**	0.674	0.859**	−0.732*	−0.915**	0.912**	0.826*	0.837**	0.794*	0.963**	0.919**		
MBC	0.846**	0.742*	0.850**	−0.741*	−0.965**	0.926**	0.885**	0.893**	0.790*	0.936**	0.728*	0.889**	
MBN	0.512	0.442	0.292	−0.110	−0.468	0.406	0.303	0.284	0.219	0.535	0.643	0.705	0.587

* $P < 0.05$; ** $P < 0.01$. LB: litter biomass; RB: root biomass; BD: bulk density; SOC: soil organic carbon; SON: soil organic nitrogen; WSOC: soil water-soluble organic carbon; NO_3-N: nitrate nitrogen; NH_4-N: ammonium nitrogen; NO_2-N: nitrite nitrogen; MBC: microbial biomass carbon; MBN: microbial biomass nitrogen.

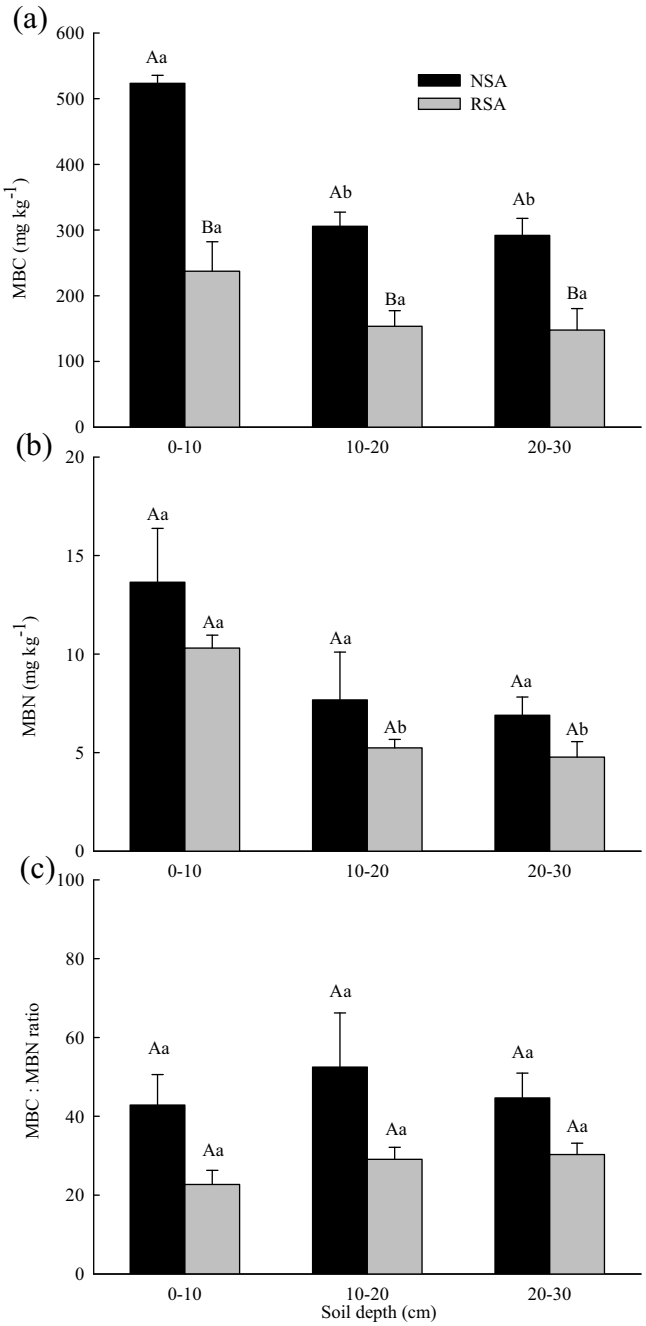

Fig. 4. (a) Soil microbial biomass carbon (MBC), (b) Soil microbial biomass nitrogen (MBN) and (c) the MBC:MBN ratio of natural and seawall-reclaimed *S. alterniflora* salt marshes. Different uppercase letters over the bars indicate statistically significant differences at $\alpha = 0.05$ level between natural and seawall-reclaimed *S. alterniflora* salt marshes. Different lowercase letters over the bars indicate statistically significant differences at $\alpha = 0.05$ level between soil depths. See Fig. 1. for abbreviations.

(Davidson and Janssens, 2006; Fissore et al., 2009). It has been suggested that embankment can inhibit *S. alterniflora* growth and prevent this species from expanding by impeding tidal flooding of the salt marsh (An et al., 2007). Previous studies have showed that moderate salinity, which relies on tidal flow input, is beneficial to *S. alterniflora* growth and can stimulate this species to complete its life history because *S. alterniflora* is unable to blossom normally and bear fruit in a freshwater environment (Zhang et al., 1985; Zhong et al., 2011). Thus, we deduced that greatly decreased soil salinity following seawall embankment may inhibited *S. alterniflora* growth and reproduction. This was confirmed by our finding that seawall reclamation significantly decreased litter, root and the total biomass in the *S. alterniflora* salt marsh (Fig. 2). Previous studies have showed that *S. alterniflora* invasion greatly increase soil organic C and N accumulation by increasing litter and roots input and decreasing SOM decomposition compared with bare flat and native plant communities in coastal wetlands of eastern China (Cheng et al., 2008; Yang et al., 2016a). The decreased soil organic C and N contents in the seawall-reclaimed *S. alterniflora* salt marsh were probably due to the lower litter and roots input into the soil (Figs. 2 and 3). This inference was supported by our results that soil organic C and N were significantly related to litter biomass (Table 2). It is well known that high moisture and/or a high water table provide anaerobic soil conditions that are conducive to SOM sequestration in the long-term (Gorham, 1991; Whitting and Chanton, 2001). A seawall can impede periodic tidal inundation of a salt marsh, resulting in a significant decrease in the soil water level (Bu et al., 2015). The soil moisture decreased dramatically in the seawall-reclaimed *S. alterniflora* salt marsh (Table 1), which could stimulate SOM decomposition and caused the loss of soil organic C and N because the SOM, which was previously sequestered under anaerobic conditions, was exposed to atmospheric oxygen (Mitra et al., 2005). Thus, the decreased soil organic C and N accumulation in the seawall-reclaimed *S. alterniflora* salt marsh (Fig. 3) could strongly attribute to changes in the soil physico-chemical properties (e.g., decreased soil moisture and salinity; Table 1) as well as decreased plant residuals entering the soil (Fig. 2).

Seawall reclamation significantly decreased the concentrations of WSOC, NH_4-N, NO_3-N and NO_2-N (Fig. 3), and thus led to low soil C and N availability in the seawall-reclaimed *S. alterniflora* salt marsh (Gao et al., 2015). WSOC is considered as the most important available substrate for soil microbial metabolism (Haynes, 2000; Vanhala et al., 2008). Previous studies have noted that higher WSOC results from concomitant increases in fresh plant residues input and the accumulation of SOM (Haynes, 2000; Liao and Boutton, 2008; Yang et al., 2013). In this study, the WSOC concentration was strongly associated with litter biomass and the concentrations of SOC and SON (Table 2). We deduced that the decreased WSOC in the seawall-reclaimed *S. alterniflora* salt marsh could be the consequence of a decrease in *S. alterniflora* residues input and the loss of SOC and SON from *S. alterniflora* soil after seawall reclamation (Figs. 2 and 3). Additionally, NH_4-N and NO_3-N are the substrates for nitrification and denitrification, respectively (Gao et al., 2015), and decreased NH_4-N, NO_3-N and NO_2-N in the seawall-reclaimed *S. alterniflora* salt marsh likely resulted in weak soil N mineralization, nitrification and denitrification after seawall reclamation (Gao et al., 2015).

Seawall reclamation significantly decreased soil various C and N fractions (Fig. 3), and also significantly lowed MBC concentration and the quantities of the total PLFAs, and bacterial, fungal, gram$^-$ bacterial, gram$^+$ bacterial, SSC, and monounsaturated PLFAs in the *S. alterniflora* salt marsh (10–30-cm soil layer) (Figs. 4a, 5 and 6). Pearson's correlation analysis indicated that the MBC concentration was significantly related to soil C and N fractions (Table 2). Soil C availability is one of the vital ecological driving factors for microbial community dynamics (Vries et al., 2012), and has a crucial effect on microbial community structure under nutrient limited conditions (Huang et al., 2015). WSOC can provide more readily available C and energy for the soil microbial community (Haynes, 2000; Yang et al., 2013). Soil available N (e.g., NO_3-N and NH_4-N) can affect the soil microbial composition by directly supplying the N resource for soil microbial metabolism (Phillips et al., 2002; Huang et al., 2014). However, soil available N can

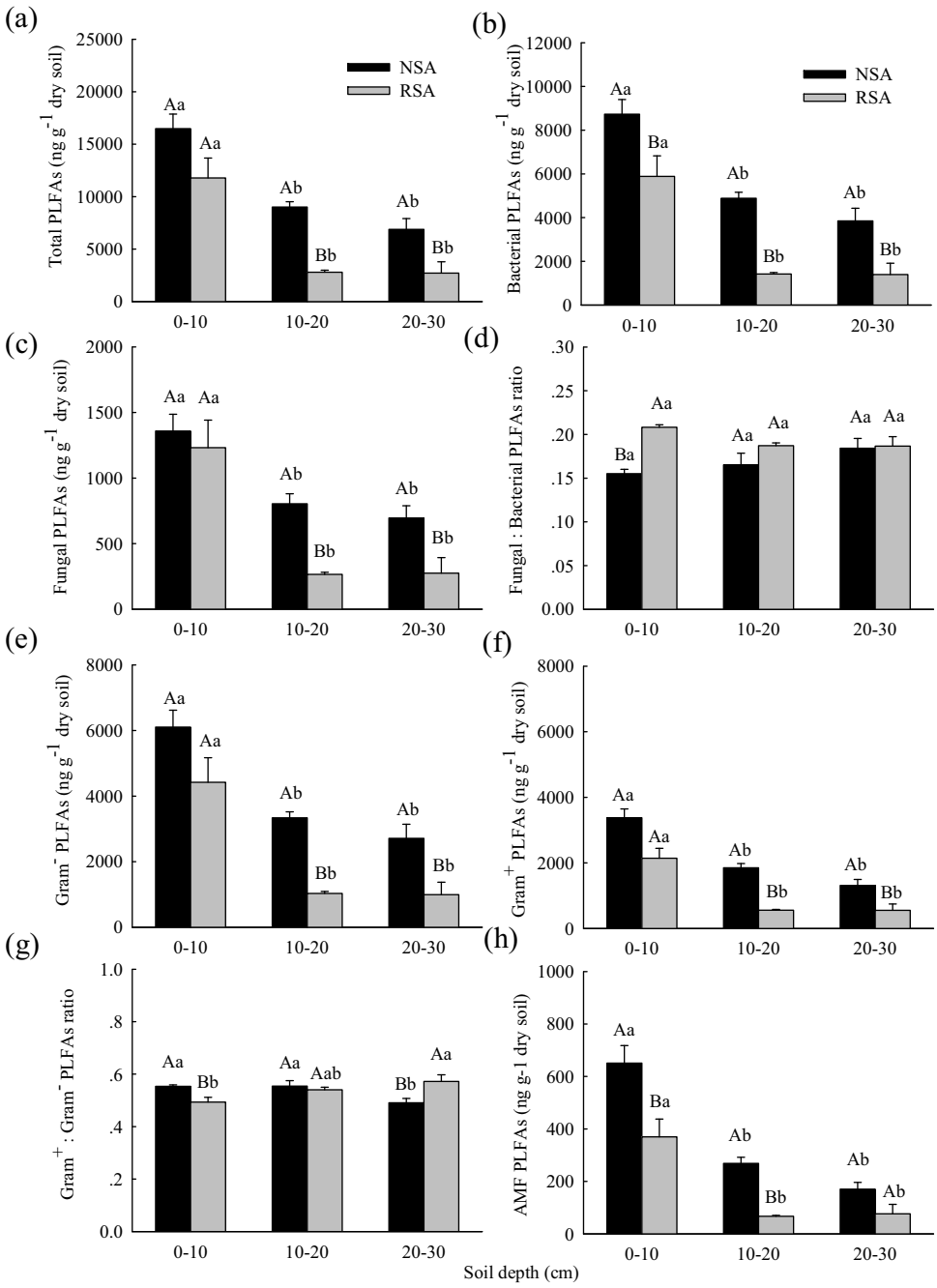

Fig. 5. (a) The total phospholipid fatty acids (PLFAs), (b) Bacterial PLFAs, (c) Fungal PLFAs concentrations; (d) Fungal:Bacterial PLFAs ratio; (e) Gram$^-$ PLFAs, (f) Gram$^+$ PLFAs concentrations, (g) Gram$^+$:Gram$^-$ PLFAs ratio and (h) the arbuscular mycorrhizal fungal (AMF) PLFAs concentrations in natural and seawall-reclaimed *S. alterniflora* salt marshes. Different uppercase letters over the bars indicate statistically significant differences at $\alpha = 0.05$ level between natural and seawall-reclaimed *S. alterniflora* salt marshes. Different lowercase letters over the bars indicate statistically significant differences at $\alpha = 0.05$ level between soil depths. See Fig. 1. for abbreviations.

seriously limit PLFAs biosynthesis when soil N is deficient (Phillips et al., 2002). The decrease in total PLFAs, and bacterial, fungal, gram$^-$ bacterial, gram$^+$ bacterial, SSC, and monounsaturated PLFAs in the seawall-reclaimed *S. alterniflora* salt marsh (10–30-cm soil layer) possibly resulted from a decrease in the readily available C and N substrates (Figs. 3, 5 and 6), which could restrict soil microbial growth and metabolism. This speculation was supported by the results of the RDA, which indicated that the PLFA variations were strongly ($P < 0.05$) related to NO_3-N and WSOC, and these factors explained 64.5% of the total variations in the PLFAs (Fig. 7). Thus, decreased soil microbial biomass (i.e., MBC and PLFAs) in the seawall-reclaimed *S. alterniflora* salt marsh was probably due to the low availability of the soil substrates (e.g., NO_3-N and WSOC).

Interestingly, seawall reclamation did not significantly affect the total PLFAs and the fungal PLFAs in the 0–10-cm soil layer (Fig. 5a and c). However, the bacterial PLFAs significantly decreased and the fungal:bacterial PLFAs ratio significantly increased in the seawall-reclaimed *S. alterniflora* salt marsh (0–10 cm soil layer)

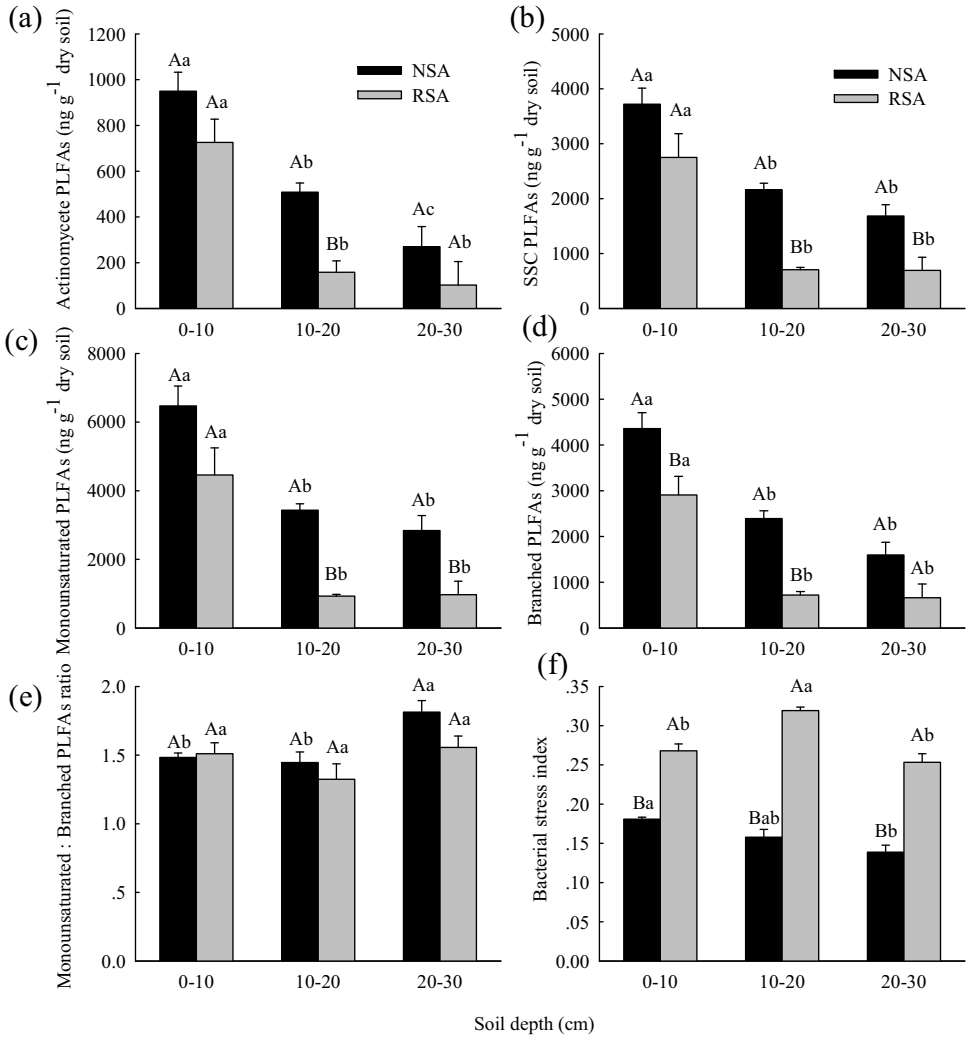

Fig. 6. (a) Actinomycete phospholipid fatty acids (PLFAs), (b) Saturated straight-chain (SSC) PLFAs, (c) Monounsaturated PLFAs, (d) Branched PLFAs concentrations, (e) Monounsaturated:branched PLFAs ratio and (f) Bacterial stress index of natural and seawall-reclaimed *S. alterniflora* salt marshes. Different uppercase letters over the bars indicate statistically significant differences at $\alpha = 0.05$ level between natural and seawall-reclaimed *S. alterniflora* salt marshes. Different lowercase letters over the bars indicate statistically significant differences at $\alpha = 0.05$ level between soil depths. See Fig. 1. for abbreviations.

(Fig. 5b and d). The fungal:bacterial PLFA ratio is usually used to indicate the responses of fungal and bacterial biomass to environmental changes (Strickland and Rousk, 2010; Dong et al., 2014). Nutrient availability has an important effect on the fungal: bacterial PLFA ratio (Suzuki et al., 2009). Dong et al. (2014) have noted that this ratio is negatively correlated with soil nutrients (e.g., NO_3-N, SOC and DOC), and the bacteria under nutrient-rich conditions are considerably more enriched than the fungi. This was consistent with our finding that seawall reclamation significantly decreased soil available C and N (e.g., WSOC, NO_3-N and NH_4-N) (Fig. 3c–e) but significantly increased the fungal:bacterial PLFAs ratio in the 0–10-cm soil layer (Fig. 5d). This likely occurred because under a low nutrient status, there is a greater decline in bacteria relative to fungi (Dong et al., 2014), and fungi are favored under low available nutrient conditions (Joergensen and Wichern, 2008).

Generally, a high bacterial stress index (cy17:0/16:1ω7c) represents a slow rate of growth and a low turnover of gram$^-$ bacteria, whereas a high value indicates a high proportion of cells in a stationary or slow phase of growth (Bossio et al., 2006). Some studies have indicated that a bacterial stress index of more than 0.5 may reveal serious environmental stress (Allison et al., 2005; Huang et al., 2015). In the present study, we found that the bacterial stress index ranged from 0.14 to 0.32 in the 0–30-cm soil layer in seawall-reclaimed and natural *S. alterniflora* salt marshes (Fig. 6f), suggesting relatively low-stress environments for bacterial growth in both of these salt marshes. However, seawall reclamation significantly increased the soil (0–30 cm) bacterial stress index (Fig. 6f), indicating slower growth rates and lower turnover rates of the gram$^-$ bacterial community in the seawall-reclaimed *S. alterniflora* salt marsh. Gram$^-$ bacteria are known to preferentially use fresh plant materials as C sources (Smith et al., 2014), whereas gram$^+$ bacteria are thought to favor older and more microbially processed SOM (Kramer and Gleixner, 2006; Potthast et al., 2012; Smith et al., 2014). We reasoned that lower growth and turnover rates of the gram$^-$ bacterial community in the seawall-reclaimed *S. alterniflora* salt marsh were probably due to a lower plant residuals input and lower soil available substrates after

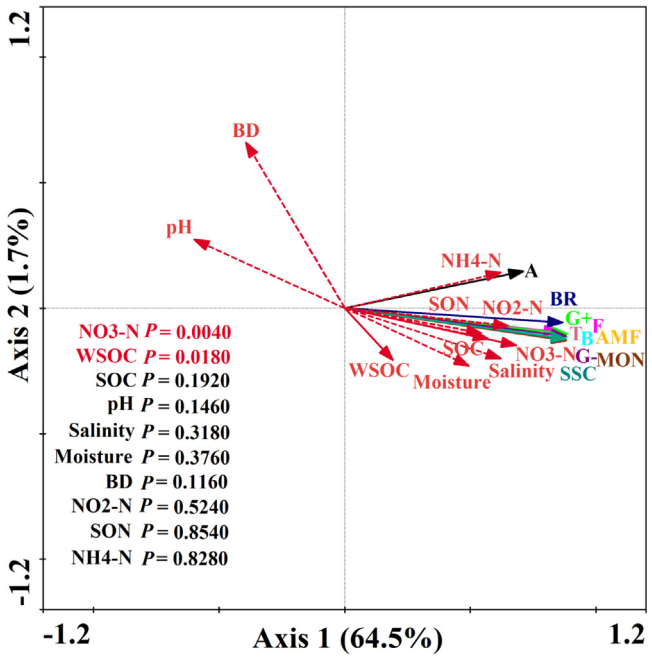

Fig. 7. RDA results of PLFAs in the soil samples and environmental variables. The explanatory variables are showed by different arrows: PLFAs profiles by colored solid arrows: total PLFAs (T); bacterial PLFAs (B); fungal PLFAs (F); gram-positive bacterial PLFAs (G^+); gram-negative bacterial PLFAs (G^-); arbuscular mycorrhizal fungal PLFAs (AMF); actinomycete PLFAs (A); saturated straight-chain PLFAs (SSC), monounsaturated PLFAs (MON); branched PLFAs (BR); and environmental variables by the red arrow: moisture, pH, salinity, bulk density (BD), soil organic carbon (SOC), soil organic nitrogen (SON), soil water-soluble organic carbon (WSOC), nitrate nitrogen(NO_3-N), ammonium nitrogen (NH_4-N), nitrite nitrogen (NO_2-N). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

seawall reclamation (Figs. 2 and 3), which caused the increase in the bacterial stress index in the seawall-reclaimed *S. alterniflora* salt marsh relative to the natural *S. alterniflora* salt marsh (Fig. 6f).

5. Conclusions

Our study provided evidence that seawall construction in invasive *S. alterniflora* salt marsh greatly decreased soil organic C and N accumulation in a coastal wetland of eastern China, which could be strongly attributed to a decline in the *S. alterniflora* residuals entering the soil and decreased soil moisture and salinity after seawall construction. Moreover, seawall reclamation significantly decreased soil available C and N, and decreased the NH_4-N, NO_3-N and NO_2-N in *S. alterniflora* salt marsh, possibly leading to weak soil N mineralization, nitrification and denitrification in the seawall-reclaimed *S. alterniflora* salt marsh. Additionally, seawall reclamation significantly decreased the MBC concentration and the quantities of the total and most types of PLFAs in the 10–30-cm soil layer possibly due to low soil available substrate in deep soil. Our results suggest that a *S. alterniflora* control project based on seawall construction could considerably weaken the C and N sinks of *S. alterniflora* salt marsh by decreasing soil organic C and N accumulation, lowering soil available C and N, and the soil microbial biomass in a coastal wetland of eastern China. These findings highlighted the evidence that seawall reclamation could effectively control *S. alterniflora* invasion, but also decrease soil C and N sequestration, which would threaten biodiversity and sustainable development of coastal wetlands (Ma et al., 2014). This in turn will provide government and policy makers with useful basic scientific information to balance seawall reclamation and *S. alterniflora* spreading in the coastal wetlands of eastern China.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apsoil.2016.11.007.

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