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Linking Improvement of Soil Structure to Soil Carbon Storage Following Invasion by a C_4 Plant *Spartina alterniflora*

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Abstract

Coastal wetlands are increasingly recognized as important ecosystems for long-term carbon (C) storage. However, how soil aggregation mediates C accumulation and sequestration in these ecosystems remains unclear. Using the ^{13}C isotope tracer from the invasion of a C_4 plant, *Spartina alterniflora*, into the native ecosystem originally covered by C_3 plants across Eastern Chinese coastal wetlands, we investigated a potential C stabilization process via soil structural protection. We quantified changes in soil aggregates, soil organic carbon (SOC), soil total nitrogen (STN), and natural ^{13}C isotope abundance within aggregate fractions across a chronosequence of 0-, 4-, 8-, and 12-year *S. alterniflora* invasion. Our results showed that soil aggregate stability increased significantly along the chronosequence. Meanwhile, SOC and STN concentrations increased with invasion time in the whole soil and aggregate fractions, which were linked to increasing soil aggregate stability. The contribution of *S. alterniflora*-derived SOC increased from 18.96 to 40.24% in the 0–20 cm layer and from 4.66 to 32.04% in the 20–40 cm layer across the chronosequence from 4 to 12 years with the highest proportion observed in macro-aggregates. Our results indicate that invasion of *S. alterniflora* to coastal wetlands can sequester more C largely due to formation and stabilization of soil aggregates by soil structural protection.

Key words: coastal wetland; *Spartina alterniflora*; soil aggregates; soil organic carbon; soil total nitrogen; stable carbon isotope.

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Introduction

The increasing atmospheric concentrations of greenhouse gases (GHGs) from human activities are the main drivers of observed climate change

(IPCC 2013). Strategies to mitigate GHG emissions have focused on the conservation of terrestrial carbon (C) sinks (Agrawal and others 2011). Coastal vegetated ecosystems rank among the most efficient C sinks in the biosphere, and conserving and restoring ''blue carbon'' sinks has been viewed as a promising strategy (Duarte and others 2005). The overall average rate of C sequestration was 210 g CO_2 m^{-2} y^{-1} in coastal wetlands, which is an order of magnitude greater than peatlands (Roulet 2000; Chmura and others 2003). Moreover, coastal wetlands are not a significant source for atmospheric methane (CH_4) compared with non-tidal wetlands, due to the presence of abundant sulfate (SO_4^{2-}) which inhibits CH_4 production (Choi and Wang 2004). Due to the higher rate of C sequestration, lower CH_4 emissions, and other ecosystem services they provide (for example, buffering the impacts of sea-level rise and dissipating wave energy), coastal wetlands are among the most valuable ecosystems for mitigating climate change (Mcleod and others 2011; Kirwan and Megonigal 2013).

Given the importance of coastal wetlands to atmospheric C budget, understanding the processes of C accumulation and mechanisms underlying soil organic carbon (SOC) stabilization provides a basis for coastal wetland management and conservation. However, substantial uncertainties exist in the quantification of SOC sources in coastal wetlands due to complicated environment (Harvey and Mannino 2001). SOC in coastal wetlands comes from both external and internal sources, including relict organic materials such as allochthonous particulate matter and dissolved organic matter, and newly produced organic materials from the primary production of local plants (Neubauer 2008; Kennedy and others 2010; Mcleod and others 2011). Recent works have demonstrated the importance of the burial organic C from plant communities in coastal wetlands (Chmura and others 2003; Donato and others 2011; Kelleway and others 2016). For example, an estimate of nearly 50% of C fixed in marine sediments is thought to be from the internal origin at the global level (Duarte and others 2005). Bull and others (1999) reported that the *Spartina anglica* biomass contributed 37–100% to salt marsh sediments at Wytch Farm, Dorset, UK, as a result of local organic matter burial into the sediments with low oxygen conditions (Alongi 2002; Kristensen and others 2008; Fourqurean and others 2012). However, other studies suggested that external C input was the primary source of the C accumulation in coastal wetlands (Kennedy and others 2010), which was ascribed to marsh accretion and lateral detritus transported by strong and frequent surface water movements (Hemminga and others 1996; Neubauer 2008). These controversial results may limit our ability to predict future C sequestration and stabilization in coastal ecosystems. Therefore, it is necessary to probe the potential mechanisms underlying the SOC accumulation in coastal wetlands.

Soil C storage and turnover are largely determined by soil structure in upland systems (Golchin and others 1997; Wilson and others 2009). SOC is incorporated into the soil matrix in different sizes and shapes through flocculation, cementation, and arrangement of soil particles (Duiker and others 2003). The position of SOC within different physical fractions causes differential accessibility to microbial decomposers and leads to a wide range of SOC pools that vary in stability and dynamics (Six and others 2000a; Liao and others 2006). For example, the macro-aggregates are formed around the fresh organic matter, which serve as a C source for microbial activity and the production of microbial-derived binding agents (Jastrow 1996; Six and others 1998, 2000b). However, the micro-aggregates, which are bound together by roots, fungal hyphae, or polysaccharides generated by the decomposition of organic inputs, contained a greater proportion of older organic matters that is more resistant to decay (Six and Jastrow 2002). Therefore, the dynamics of aggregate formation are closely linked with the stabilization of soil organic matter (Salome and others 2010). Although numerous studies in upland ecosystems have shown that soil aggregation provides physical protection of SOC against rapid decomposition and plays a critical role in SOC turnover (Christensen 2001; Six and others 2004; Abiven and others 2009), the importance of soil structure controlling the SOC sequestration in coastal wetlands remains largely unknown.

Spartina alterniflora was introduced to Chinese coastal wetlands for the purpose of ecological engineering, in 1979 from the USA (Wang and others 2006; An and others 2007). The C_4 plant *S. alterniflora* has spread rapidly and becomes the dominant vegetation type since it was transplanted to the Dongtan wetlands, which were previously dominated by C_3 vegetation such as *Scirpus mariqueter* and *Phragmites australis* (Chen and others 2004). This shift in vegetation structure provides a unique opportunity to examine the contribution of coastal vegetation to SOC accumulation and to seek the mechanism of SOC stabilization mediated by soil aggregation in coastal wetlands, although salinity, hydrology and oxygen availability may

influence soil C accumulation (Chambers and others 2016; Graves and others 2016). Furthermore, the chronosequence created by the *S. alterniflora* invasion provides a temporal dimension for exploring the mechanisms contributing to the accumulation and stabilization of SOC. In this study, we hypothesized: (1) the *S. alterniflora* invasion would increase soil aggregate stability and soil C and N contents in the whole soil and aggregate fractions and (2) the increasing SOC was mainly derived from inputs of *S. alterniflora*. The specific objectives of this study were to: (1) evaluate the influence of *S. alterniflora* invasion on soil aggregate stability and C and N pools in aggregate fractions and (2) partition C contributions from *S. alterniflora* in the whole soil and aggregate fractions.

Materials and Methods

Study Area

This study was conducted at Dongtan wetland of Chongming Island, the largest wetland in the Yangtze River estuary, China (31°03′–31°38′ N, 121°47′–122°05′ E, Figure 1). The local climate is a subtropical monsoon, characterized by a mean annual temperature of 15.2°C and a total annual precipitation of 1025 mm. Dongtan wetland is expanding eastward to the sea at a rate of more than 150 m per year due to the continuous silt sedimentation from the Yangtze River (Zhao and others 2009). The dominant vegetation species were the C_3 plants *Scirpus mariqueter* and *Phragmites australis* before the 1980s. The invasive C_4 plant *Spartina alterniflora*, which was intentionally introduced from the USA to Chinese coastal wetlands in 1979 (Qin and Zhong 1992), was transplanted into Dongtan in May 2001 (Chen and others 2004). Since then, it has been rapidly spreading along the Dongtan wetlands.

Soil and Plant Sampling

Soil and plant samples were collected from *S. alterniflora* communities in June 2013. The invasion time of *S. alterniflora* within the sample region was identified based on Thematic Mapper (TM) satellite images (Wang and others 2015) and historical records. The chronosequence consisted of *S. alterniflora* invaded in 2001 (SA-12), 2005 (SA-8), 2009 (SA-4), and the bare flat (BF, Figure 1). The sampled region was reasonably flat with a less than 0.5 degree slope. Tidal fluctuation at this tested area is regular and semidiurnal.

Soil samples were collected at four sites (BF, SA-4, SA-8, and SA-12) along the chronosequence (Figure 1). At each site, three sampling plots (3 × 3 m) were randomly established, and three soil samples were taken at each plot by using a 3-cm-diameter stainless steel sampler from the 0–20 and 20–40 cm layers, respectively. Soil samples within the same layer from each plot were mixed thoroughly. Meanwhile, three replicates of plant samples, including leaves, litters, and roots, were collected at each site. All soil and plant samples were filled in sample sack and kept on ice in coolers, immediately transported to the laboratory at Fudan University, and stored at 4°C for further analysis.

Laboratory Analyses

Every soil sample was physically cleaned of plant and organic debris with tweezers carefully and divided into two subsamples. One subsample was air-dried for 20 days to analyze for aggregate fractions. Another subsample was dried at 55°C for measuring soil organic carbon (SOC), soil total nitrogen (STN), and stable carbon isotopic composition ($\delta^{13}C$) of the SOC. The plant samples were dried at 65°C, ground by a planetary ball mill, and passed through a 100-mesh sieve for carbon isotopic analysis by using a MAT 253 isotope ratio mass spectrometer (Thermo Electron, Bremen, Germany).

Soil samples were treated with 1 N HCl for 24 h at room temperature to remove the inorganic carbon before further measurements (Lin and others 1999). The $\delta^{13}C$ value of each HCl-treated soil and plant samples was measured on a MAT 253 isotope ratio mass spectrometer (Thermo Electron, Bremen, Germany). Precision for $\delta^{13}C$ was ± 0.10‰ according to repeated measurements of a laboratory working standard (Shenyang Institute of Applied Ecology, Chinese Academy of Sciences). The $\delta^{13}C$ of the organic matter is calculated from:

$$\delta^{13}C = \left[R_{sample}/R_{PDB} - 1\right] \times 1000 \qquad (1)$$

where R_{sample} and R_{PDB} are the $^{13}C/^{12}C$ ratio of the sample and the Pee Dee Belemnite (PDB) standard (Desjardins and others 1996; Wooller and others 2003), respectively.

Soil Physical Fractionation

The size distribution of water-stable aggregates (WSAs) and the stability of moist soil were determined using fast wetting (NVFW) techniques under ambient atmospheric pressure (Kemper and Rosenau 1986; Sun and others 2014). A 100-g soil sample was placed on the top of a nest of five sieves

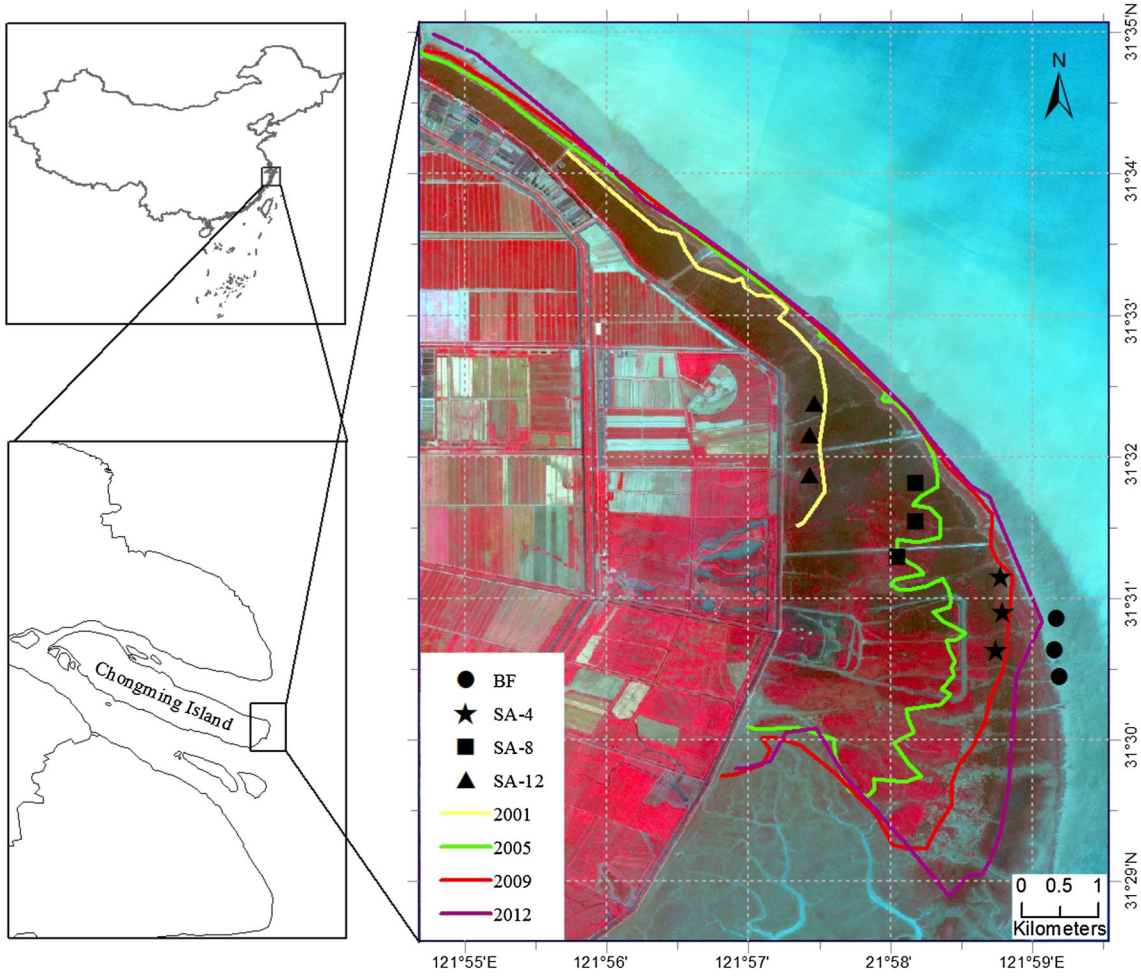

Figure 1. Location of the sampling sites at Dongtan wetland, Chongming Island, Yangtze River estuary, China. SA-4, SA-8, and SA-12 represent *S. alterniflora* community invaded in 2009, 2005, and 2001, respectively; BF represents bare flat.

with 2, 1, 0.5, 0.25, and 0.106 mm openings and submerged in tap water for 2 min at room temperature. The wet sieve instruments (Model: DIK-2012, Daiki Rika Kogyo Co. Ltd., Japan) were then set in oscillating motion 3 cm up and down at a frequency of 0.5 cycle s^{-1} for 10 min. After that, the resistant fractions (> 2 mm aggregates were not found) on the top of each sieve and the unstable (< 0.106 mm) aggregates were transferred into beakers. Each aggregate size fraction was dried at 65°C to constant weight. The mean weight diameter (MWD, mm) of soil aggregates is calculated as follows:

$$\mathrm{MWD} = \frac{\sum_{i=1}^{5} M_i \times D_i}{\sum_{i=1}^{5} M_i} \qquad (2)$$

where within each of the five classes of diameter (i = 1–5), D_i [mm] is the central diameter of aggregates in each size class (approached as mean size of upper and lower limits of sieve size used: 1.5, 0.75, 0.375, 0.178, and 0.053 mm, respectively, Barto and others 2010); M_i [g] is the mass of stable soil aggregates isolated within the ith diameter class. The fractions above 1 mm were corrected by removing the mass of marly platelets.

Calculation of Carbon Inputs

The proportion f (%) of *S. alterniflora*-derived C in the soil fractions was calculated using a two-source mixing model (Chiang and others 2004; Cheng and others 2006) as follows:

$$f = \left(\delta^{13}\mathrm{C_{new}} - \delta^{13}\mathrm{C_{old}}\right) / \left(\delta^{13}\mathrm{C_{sa}} - \delta^{13}\mathrm{C_{old}}\right) \times 100 \qquad (3)$$

where $\delta^{13}C_{sa}$ is the mean δ^{13}C of *S. alterniflora* plant materials, including litters, rhizomes, and roots; $\delta^{13}C_{new}$ is the mean δ^{13}C of SOC in the soil fractions after the invasion of *S. alterniflora*; and $\delta^{13}C_{old}$ is the mean δ^{13}C of SOC in the soil fractions before

the invasion of *S. alterniflora*; here, it represents the mean $\delta^{13}C$ of SOC in the bare tidal mudflat soil fractions.

The accumulation rate (R, g C kg^{-1} soil y^{-1}) of SOC or *S. alterniflora*-derived C in soil physical fractions is calculated as:

$$R = C_{in}/t \quad (4)$$

where C_{in} is assumed to be the increased storage of SOC or *S. alterniflora*-derived C in soil fractions, and t is the invasion time.

Statistical Analyses

One-way analysis of variance (ANOVA) was performed to determine differences in soil physical and chemical characteristics in the whole soil and aggregate fractions among each site. Significant differences among different sites were assessed by Fisher's protected least significant difference (LSD) test at $P = 0.05$ for all the variables. Regression analyses were used to determine the relationships of MWD, SOC, STN, accumulative rates of SOC and *S. alterniflora*-derived C against plant invasion time. The ANOVA and regression analysis were carried out in SPSS Statistics 23 (SPSS. Inc., Chicago, IL, USA).

Results

Soil Physicochemical Properties and Soil Aggregate Stability

Along the chronosequence of *Spartina alterniflora* invasion from bare flat to 12-year invasion, soil pH in the *S. alterniflora* community ranged from 8.11 to 8.49 but with no significant difference. However, soil electrical conductivity and salinity in the *S. alterniflora* community were higher than those in bare flat (Table 1). The $\delta^{13}C$ values of *S. alterniflora* ranged from − 13.45 to − 14.44‰ with a mean value of − 13.86‰ ± 0.10 (mean ± SE, Table S1), showing no significant difference among leaves, litter, and roots. The $\delta^{13}C$ values of SOC in the whole soil and aggregate fractions increased along the chronosequence of *S. alterniflora* invasion from bare flat to 12 years in both the surface (0–20 cm) and deep soil layers (20–40 cm). Meanwhile, within aggregate fractions, the $\delta^{13}C$ values increased with the particle size at each site, but not at the bare flat (Table 2).

The distribution of soil aggregate fractions as a proportion of the whole-soil mass varied significantly from bare flat to the invasion of *S. alterniflora* with 4–12 years (Figure 2). The mass proportion of macro-aggregates (> 0.25 mm) increased along the chronosequence of the *S. alterniflora* invasion, but decreased for fine aggregates (< 0.106 mm), and showed no significant change in medium aggregates (0.106–0.25 mm) in both soil layers (Figure 2A, C). Meanwhile, soil aggregate stability (as quantified by the mean weight diameter, MWD) increased linearly with the chronosequence of the *S. alterniflora* invasion in surface layer ($R^2 = 0.99$, $P < 0.01$) and deep layer ($R^2 = 0.93$, $P = 0.03$, Figure 2B, D).

Table 1. Soil Physicochemical Characteristics of *Spartina alterniflora* Communities in Dongtan Wetlands, Shanghai, China

Site	pH	EC (ms/cm)	Salinity (PPT)
Bare flat	8.49 ± 0.08[a]	1.96 ± 0.14[a]	5.96 ± 0.46[a]
SA-4	8.11 ± 0.15[a]	2.76 ± 0.03[bc]	8.55 ± 0.09[b]
SA-8	8.38 ± 0.04[a]	2.19 ± 0.21[ab]	6.74 ± 0.68[ab]
SA-12	8.33 ± 0.13[a]	2.82 ± 0.26[c]	8.76 ± 0.87[b]

The values are means (n = 3) with standard errors.
EC electrical conductivity.
Different letters in the same column represent significant differences at P < 0.05 level among sampling sites.

SOC and STN Concentrations in the Whole Soil and Aggregate Fractions

Along the chronosequence following *S. alterniflora* invasion, SOC and STN concentrations linearly increased by twofold in the whole soil and aggregate fractions, but the C/N ratio had no significant trend (Figure 3). The concentration of SOC in the whole soil increased from 6.73 ± 0.67 g C kg^{-1} soil in bare flats to 20.31 ± 1.03 g C kg^{-1} soil in the 12-year invaded soil by *S. alterniflora* (SA-12) in surface layer (0–20 cm) and increased from 6.00 ± 0.46 to 13.26 ± 0.86 g C kg^{-1} soil in deep layer (20–40 cm, Figure 3C). Changes in N concentration paralleled those in C with an increase from 0.86 ± 0.06 to 2.28 ± 0.25 g N kg^{-1} soil in surface layer and an increase from 0.75 ± 0.03 to 1.55 ± 0.08 g N kg^{-1} soil in deep layer (Figure 3F). Within aggregate fractions, the largest SOC concentration was observed in the large macro-aggregates (> 1 mm), which increased from 10.82 ± 1.27 to 24.10 ± 1.64 g C kg^{-1} soil in surface layer and from 10.52 ± 0.22 to 16.27 ± 0.89 g C kg^{-1} soil in deep layer. The smallest SOC concentration occurred in fine aggregates (< 0.106 mm), which increased from 5.73 ± 0.77 to 15.09 ± 0.89 C kg^{-1} soil in surface layer and from 4.76 ± 0.26 to 10.47 ± 1.02 g C kg^{-1} soil in

Table 2. Variation in $\delta^{13}C$ Values of SOC Within Different Soil Fractions in *Spartina alterniflora* (SA) Communities and Bare Flat (BF) at Dongtan Wetlands, Shanghai, China

	0–20 cm			
	BF $\delta^{13}C$ (‰)	**SA-4** $\delta^{13}C$ (‰)	**SA-8** $\delta^{13}C$ (‰)	**SA-12** $\delta^{13}C$ (‰)
> 1 mm	− 23.99 ± 0.28aA	− 20.14 ± 0.70aB	− 19.70 ± 0.31aBC	− 18.57 ± 0.31aC
0.5–1 mm	− 23.89 ± 0.07aA	− 21.00 ± 0.56abB	− 20.32 ± 0.38aBC	− 19.06 ± 0.28abC
0.25–0.5 mm	− 23.83 ± 0.30aA	− 21.59 ± 0.50abB	− 20.41 ± 0.44aBC	− 19.36 ± 0.38bC
0.106–0.25 mm	− 24.05 ± 0.32aA	− 21.77 ± 0.56abB	− 20.37 ± 0.36aBC	− 19.15 ± 0.54abC
< 0.106 mm	− 23.82 ± 0.06aA	− 22.81 ± 0.34bA	− 21.18 ± 0.49aB	− 20.70 ± 0.41cB
Whole soil	− 23.83 ± 0.13A	− 21.94 ± 0.49B	− 20.58 ± 0.43C	− 19.38 ± 0.31D
	20–40 cm			
	BF $\delta^{13}C$ (‰)	**SA-4** $\delta^{13}C$ (‰)	**SA-8** $\delta^{13}C$ (‰)	**SA-12** $\delta^{13}C$ (‰)
> 1 mm	− 24.28 ± 0.18aA	− 22.74 ± 0.25aB	− 20.71 ± 0.52aC	− 19.44 ± 0.34aD
0.5–1 mm	− 24.22 ± 0.27aA	− 22.89 ± 0.07abB	− 21.13 ± 0.68abC	− 20.02 ± 0.33aC
0.25–0.5 mm	− 24.33 ± 0.29aA	− 23.41 ± 0.16cbA	− 21.30 ± 0.81abB	− 20.45 ± 0.52aB
0.106–0.25 mm	− 24.42 ± 0.18aA	− 23.31 ± 0.17cbA	− 20.98 ± 0.89abB	− 20.00 ± 0.55aB
< 0.106 mm	− 23.87 ± 0.04aA	− 23.66 ± 0.08cA	− 23.04 ± 0.03bB	− 21.87 ± 0.42bB
Whole soil	− 23.97 ± 0.07A	− 23.50 ± 0.13A	− 22.22 ± 0.35B	− 20.72 ± 0.33C

The values are means with standard errors (n = 3).
Different lowercase letters among soil fractions indicate significant differences at $P < 0.05$. Different uppercase letters indicate significant differences at $P < 0.05$ level among sampling sites.

deep layer. Similar patterns were observed in STN concentration in soil fractions.

To evaluate the changes in SOC and STN in soil aggregate fractions, we calculated the mean accumulation rate of SOC and STN among different aggregate size classes. SOC accumulation rate increased along the chronosequence of *S. alterniflora* invasion for all fractions in surface soil layer, although the trends were not all statistically significant. However, in the deep soil layer, the highest SOC accumulation rate was observed in the period of 4–8 years except for fine aggregates, which increased with the invasion time. N accumulation rate showed a similar pattern as C accumulation rate (Figure S1).

The concentrations of SOC and STN in the whole soil were strongly linked to the soil aggregate stability (MWD). Soil organic C concentration increased linearly with MWD in 0–20 cm ($R^2 = 0.78$, $P < 0.01$) and 20–40 cm ($R^2 = 0.80$, $P < 0.01$) soil layer (Figure 4A). Changes in N concentration paralleled those of SOC in 0–20 cm layer ($R^2 = 0.59$, $P < 0.01$) and 20–40 cm layer ($R^2 = 0.81$, $P < 0.01$, Figure 4B).

Spartina alterniflora-Derived C in the Whole Soil and Aggregate Fractions

The proportion of *S. alterniflora*-derived C increased along the chronosequence following *S. alterniflora* invasion (Figure 5). Soil organic C derived from *S. alterniflora* increased from 1.60 to 9.13 g C kg^{-1} soil in 0–20 cm layer and 0.31 to 4.31 g C kg^{-1} soil in 20–40 cm layer after the 4–12 years of plant invasion, accounting for 18.96–40.24% and 4.66–32.04% of total SOC in the whole soil, respectively (Figures 5 and 6). The relative proportion of *S. alterniflora*-derived C was higher in 0–20 cm than 20–40 cm layer (Figure 5B, D). *S. alterniflora*-derived C in all aggregate fractions increased along the chronosequence. The largest proportion of *S. alterniflora*-derived C was observed in large macroaggregates (> 1 mm), and the smallest was in the fine aggregates (< 0.106 mm, Figure 5A, C).

To evaluate the changes in *S. alterniflora*-derived C in soil fractions, we calculated the mean accumulation rate of *S. alterniflora*-derived C among different aggregate size classes during the three invasion

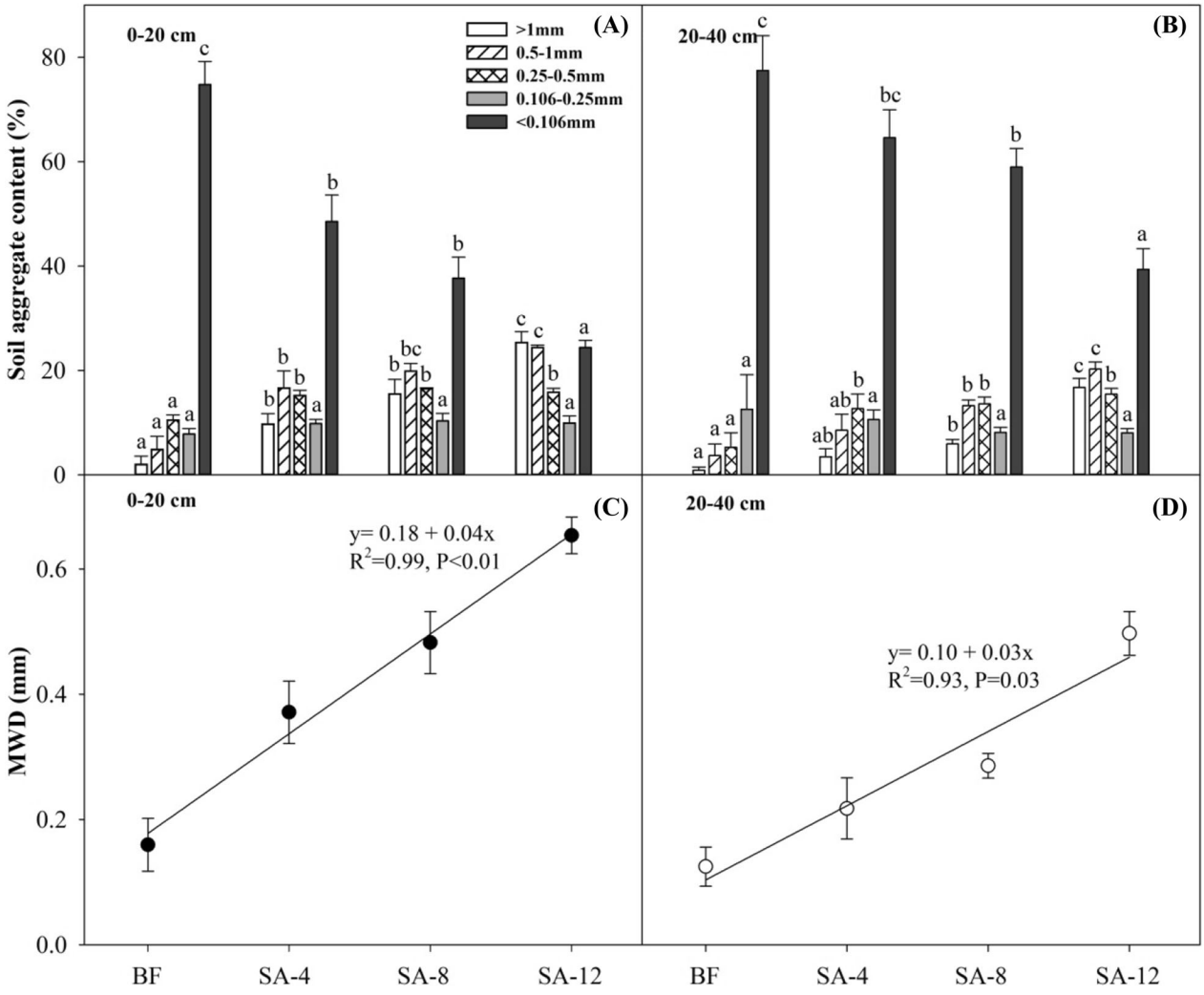

Figure 2. Proportion of water-stable aggregates along *S. alterniflora* invasion chronosequence in 0–20 cm (**A**) and 20–40 cm (**B**) soil layers, and influence of invasion year on soil aggregate stability (mean weight diameter; MWD, **C** and **D**). Vertical bars denote standard errors of means (n = 3). Different letters over the bars indicate significant differences among sampling site at $P < 0.05$.

stages (0–4, 4–8, and 8–12 years). The highest accumulation rate was observed in the 8- to 12-year stage in surface soil layer, whereas the smallest occurred in the 0- to 4-year stage in the deep soil layer (Figure S2). The accumulation rate in macro- and medium aggregates showed no significant difference between the 0- to 4-year and 4- to 8-year stages, but increased at the 8- to 12-year stage in the surface layer. In the deep layer, the accumulation rate in large macro-aggregates increased significantly from 0.39 ± 0.03 to 0.91 ± 0.08 g C kg^{-1} soil y^{-1}, and the same patterns were observed in other size aggregates. However, there is no apparent difference between the 4- to 8-year and 8- to 12-year stages in macro- and medium aggregates except for the fine aggregates (Figure S2).

Discussion

Effects of *S. alterniflora* Invasion on Soil Aggregate Stability

Soil aggregate stabilization is driven by both abiotic and biotic factors, which are related to soil properties, vegetation characteristics, land management, and climate (Bronick and Lal 2005; Cécillon and others 2010). Our results showed that soil aggregate stability increased with invasion time of *S. alterniflora* in Dongtan coastal wetlands (Figure 2). Generally, vegetation characteristics illustrated the positive effects on soil aggregate stability, which was potentially driven by above- and below-ground linkage. Above-ground stems, leaves, and litters from plants protect the soil from being ero-

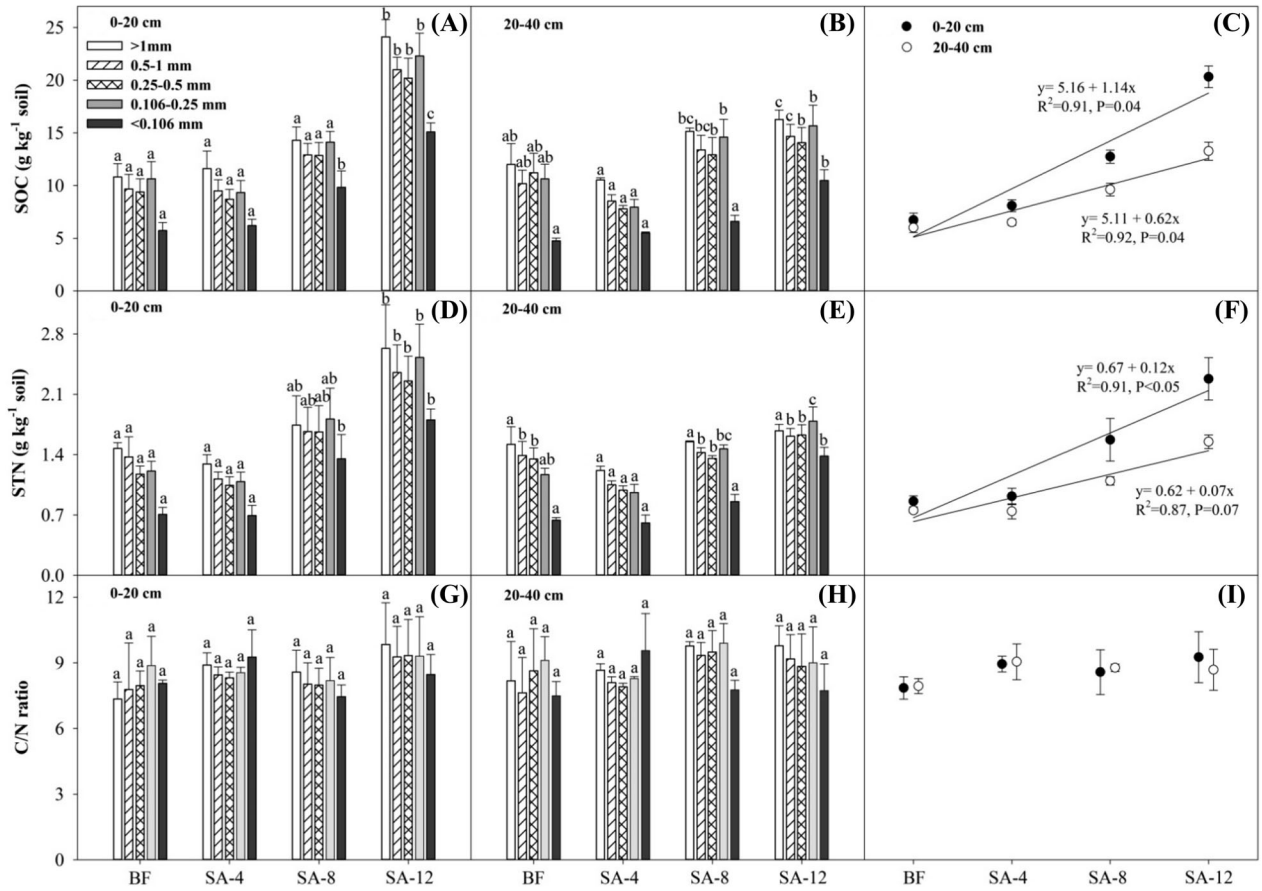

Figure 3. Concentration of SOC, STN, and C/N ratio in soil fractions in 0–20 cm (**A**, **D**, **G**) and 20–40 cm (**B**, **E**, **H**) soil layers, and whole soil (**C**, **F**, **I**). Vertical bars denote standard errors of means (n = 3). Different letters over the bars indicate significant differences among sampling site at $P < 0.05$.

ded by dissipating the wave energy (Duarte and others 2013) and counteracting the raindrop energy of rainfall (Zuazo and Pleguezuelo 2008). Meanwhile, organic compounds, which are left over from litter decomposition, could hold soil aggregates together as stabilizing agents (Liao and others 2006; Abiven and others 2009). Belowground roots facilitate the formation and stabilization of soil aggregates through a variety of ways, including providing mechanical entanglement, changing soil density via root penetration, and acting as a binding agent by roots exudates (Jastrow and others 1998; Denef and others 2002; Bronick and Lal 2005). For example, Six and others (2004) reported that root exudations (for example, polysaccharides) acted as binding agents to hold the macro-aggregate together. Morel and others (1991) suggested that mucilage released by roots could stick soil particles together directly.

Our results also showed that macro-aggregates (> 0.25 mm) increased, whereas fine aggregates (< 0.106 mm) decreased along the chronosequence (Figure 2). This finding was consistent with the results from Zhang and others (2010), who reported that the macro-aggregates increased with *S. alterniflora* invasion in an Eastern Chinese estuarine wetland, probably attributed to greater organic matter inputs (Liao and others 2006). Macro-aggregates are generally formed around temporary binding agents such as roots, fungal hyphae, and transient agents (for example, polysaccharides, Oades and Waters 1991; Six and others 2000b). Therefore, the increasing formation of macro-aggregates in coastal wetlands could be facilitated by the increasing root biomass (Liao and others 2007; Zhang and others 2010) and soil microbial biomass (Yang and others 2016) following *S. alterniflora* invasion. The simultaneous decrease in the fine aggregate-sized fraction indicated that fine aggregates are being bound into macro-aggregates in coastal wetlands. Thus, the soil aggregate stability in coastal wetlands increased along the chronosequence of *S. alterniflora* invasion, which would be of major significance in reducing erosion, sequester-

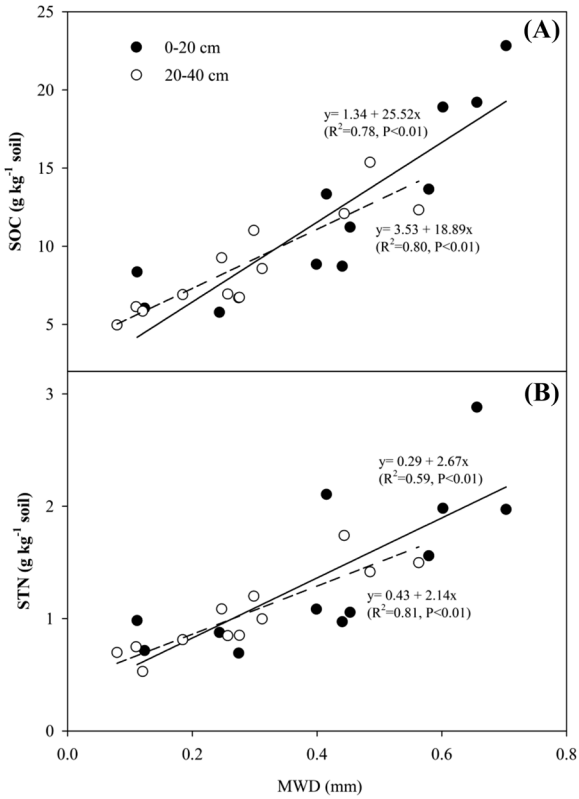

Figure 4. SOC (**A**) and STN (**B**) versus soil aggregate stability (MWD) in 0–20 and 20–40 cm soil layers.

ing carbon, and mitigating the sea-level rise (Deegan and others 2012; Kirwan and Megonigal 2013).

Dynamics of SOC and STN in the Whole Soil and Aggregate Fractions

The high productivity (especially roots) and structural complexity of *S. alterniflora* predispose coastal wetlands to be highly efficient in sequestering organic matters (Mcleod and others 2011). Our results showed that SOC and STN concentrations in the whole soil and aggregate fractions increased along the chronosequence of *S. alterniflora* invasion, but C/N ratio had no significant changes (Figure 3). Our findings were consistent with previous studies that plant invasion profoundly influenced soil C and N pools through changes in the quantity and quality of organic matter entering soils (Windgam and Ehrenfeld 2003; Liao and others 2008). Zhang and others (2010) have reported that the above- and below-ground biomass increased with *S. alterniflora* invasion, which left distinct imprints on soil C and N pools in Eastern Chinese coastal wetlands. Organic inputs of high substrate from *S. alterniflora* induced more labile C content in the soils (Cheng and others 2008), which in turn could enhance the recalcitrant C pool (Rovira and Vallejo 2002). Meanwhile, the residues of *S. alterniflora* were resistant to decay due to the high C/N ratio and K and Na contents (Yang and others 2009). Thus, continuous inputs, low quality of litters, and the physical protection offered by aggregates lead to SOC and STN accumulation in the whole soil and aggregate fractions along the chronosequence. Furthermore, Cheng and others (2006) documented that C and N concentrations were closely related to root distribution following *S. alterniflora* invasion. Roots and their associated microorganisms played dominate roles in soil C accumulation (Clemmensen and others 2013). However, we still lack sufficient data to differentiate the relative contribution of roots and shoots, which deserves future studies.

Our results showed that the SOC and STN concentrations in fine aggregates (< 0.106 mm) were smaller than in macro- (> 0.25 mm) and medium (0.106–0.25 mm) aggregates, which all increased with the chronosequence. This phenomenon may be induced by the process of soil aggregate formation, in which exogenous organic matter is first incorporated into the macro-aggregate fraction and the new C-enriched relative to smaller fractions (Gale and others 2000). Thus, the increasing soil aggregate stability represents an important contribution to the C and N accumulations in coastal wetlands (Figure 4). The sustained increase in SOC and STN along the chronosequence following *S. alterniflora* invasion at Dongtan wetlands suggested the large potential to sequestrate more C after a 12-year invasion. Therefore, the *S. alterniflora* expansion, which can sequestrate soil C efficiently, may be a promising strategy as an eco-engineering solution for C mitigation.

In addition to plant invasion inputs by *S. alterniflora*, we found that parts of the increased SOC were derived from extraneous inputs (Figure 6). Our finding was consistent with previous observations in a variety of coastal wetlands, indicating that the extraneous inputs mainly came from sediments or phytoplankton (Cheng and others 2006; Gebrehiwet and others 2008; Kelleway and others 2016). For example, Kelleway and others (2016) reported that sedimentation may largely control the below-ground C stocks of salt marshes, which was attributed to the allochthonous inputs and/or the increasing C preservation capacity of fine sediments. Meanwhile, Gebrehiwet and others (2008) suggested that phytoplankton partly contributed to the soil organic matter in a *S. alterniflora*-vegetated wetland. In our study, the sediments and marine phytoplankton may con-

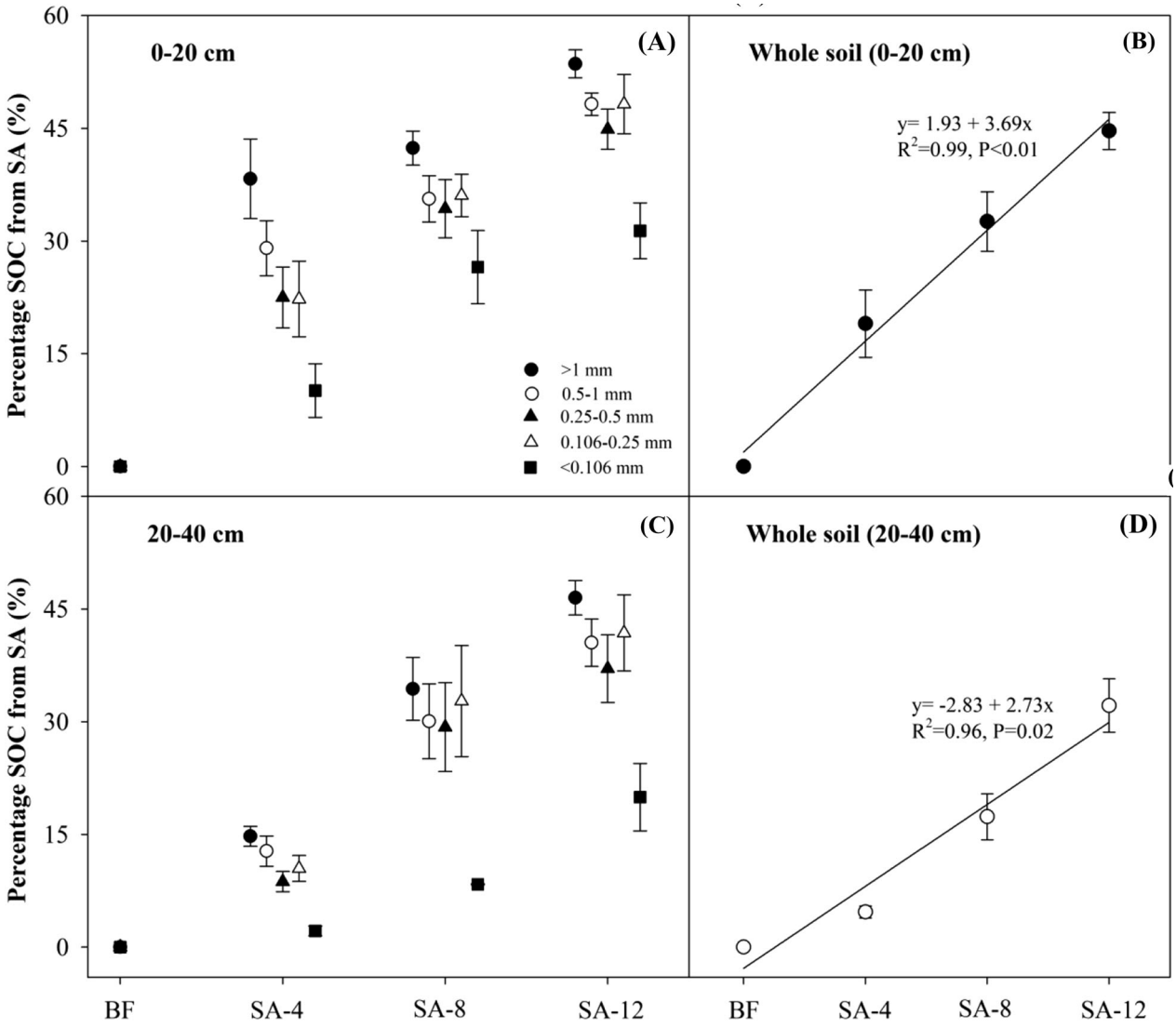

Figure 5. Distribution proportion of SOC derived from *S. alterniflora* in soil fraction and whole soil along the chronosequence in 0–20 cm (**A**, **B**), 20–40 cm (**C**, **D**) soil layers.

tribute to the SOC accumulation to some degree, especially in the upper soil. Therefore, further studies are necessary to quantify the relative contribution of multiple sources to SOC accumulation.

Dynamics of *S. alterniflora*-Derived C in Soil Aggregate Fractions

Spartina alterniflora-derived C increased along the chronosequence, but exhibited different patterns in soil aggregate fractions (Figures 5, 6). The highest proportion of *S. alterniflora*-derived C occurred in coarse macro-aggregates (> 1 mm), and the lowest was in the fine fraction (< 0.106 mm) both in 0–20 and 20–40 cm soil layers (Figures S3–S7). The decreased proportion of *S. alterniflora*-derived C from the coarse to the fine fractions indicated that *S. alterniflora* residues were initially incorporated into the coarse fraction and then turned into the fine fraction in coastal wetlands (Desjardins and others 2006; Zhang and others 2010), achieving the long-term SOC sequestration. The fresh residues of *S. alterniflora* derived the formation of macro-aggregates, becoming coarse intra-aggregate particulate organic matter (POM). The intra-aggregate particulate POM was encrusted with microbial products and soil clay particulates, forming micro-aggregates within macro-aggregates. Previous studies have shown that C within silt and clay was more stable and highly humified (Balesdent and others 1988), and thus, the increasing rate of *S. alterniflora*-derived C in fine fractions was relatively low.

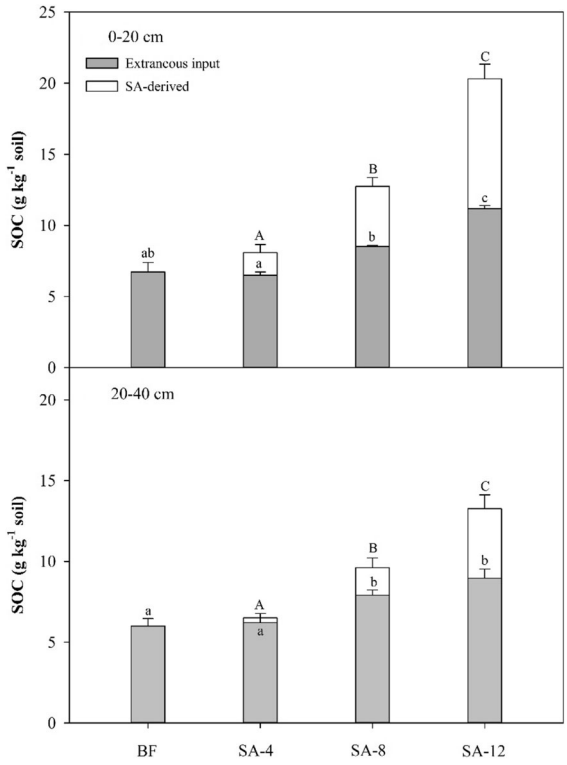

Figure 6. Concentration of SOC derived from *S. alterniflora* and extraneous input along the chronosequence. Different upper letters over the bars indicate significant differences of *S. alterniflora*-derived C among sampling site at $P < 0.05$. Different lower letters over the bars indicate significant differences of extraneous input-derived C among sampling site at $P < 0.05$.

The *S. alterniflora*-derived C in macro-aggregates represented an important contribution to C accumulation, even though this pool generally does not exhibit long-term stability (Tisdall and Oades 1982). Numerous studies have shown that macro-aggregates are sensitive to changes in SOM because soil structure is dependent on live binding agents (Christensen 2001; Six and others 2002; Liao and others 2006). However, macro-aggregates induced compartmentalization of substrate and soil microbes and blocked diffusion of oxygen into macro-aggregates (Killham and others 1993; Six and Jastrow 2002), thus slowing down the decomposition rate of original inputs. Meanwhile, the increasing macro-aggregates would accelerate the formation of micro-aggregates, which would be more stable than macro-aggregates (Tisdall and Oades 1982; Six and others 2000b).

The physical protection of organic matter in soil micro-aggregates was greater than that provided by macro-aggregates (Krull and others 2003). Jastrow and others (1996) found that the average turnover time of organic C in soil micro-aggregates was 412 years, which is considerably longer than that in macro-aggregates with only 140 years. Meanwhile, the silt and clay content influenced the physical protection indirectly by affecting aggregate dynamics (Six and others 2002). Previous studies documented that physical protection of organic C within soil aggregates increased with clay content (Franzluebbers and Arshad 1997). Buyanovsky and others (1994) calculated that the mean residence time of C associated with silt and clay was more than 1000 years. The silt and clay fraction was thus regarded as a long-term pool for soil C storage (Liao and others 2006). In our study, the *S. alterniflora*-derived C accumulation rates increased along the chronosequence of plant invasion in micro-aggregates and silt + clay fractions (< 0.25 mm). These results indicated that *S. alterniflora*-derived C was protected by stabilization within soil micro-aggregates and in association with silt + clay fractions. Therefore, the structural protection may play a key role in SOC sequestration in coastal wetlands.

Implications

Our results from the chronosequence of *S. alterniflora* invasion may provide some insights into the regulation of soil aggregates on C accumulation and offer implications for conservation of coastal wetlands and development of future earth system models. In this study, soil C sequestration was largely derived from internal source inputs (that is, plants) in Dongtan coastal wetlands, and the enhanced soil C storage following *S. alterniflora* invasion was linked with improvement of soil structure. If increasing C sequestration is a strategic goal, conservation and management intervention should be prioritized for the vegetation of coastal ecosystems, as massive coastal reclamation, engineering, and urbanization dramatically degrade biodiversity and destroy coastal wetlands (Green and Short 2003; Ma and others 2014).

Our study also pointed out the importance of soil structure on SOC accumulation in coastal ecosystems, which was usually overlooked in current Earth system models. Such models (for example, Century) are built around pools that cannot be separated physically or chemically, or even quantified directly (Jenkinson and Coleman 2008; Koven and others 2013; Luo and others 2015). Recently, Abramoff and others (2018) proposed a conceptual model with five measurable soil C pools, in which aggregate C was included as well as mineral-associated organic matter, particulate organic matter, low molecular weight C, and micro-

bial biomass C. Our study highlights the suitability using aggregate C for upscaling field-based measurements to regional blue C inventories and for modeling soil C stocks across space and through time under global change scenarios.

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