

Effect of nutrient enrichment on the source and composition of sediment organic carbon in tropical seagrass beds in the South China Sea



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ABSTRACT

To assess the effect of nutrient enrichment on the source and composition of sediment organic carbon (SOC) beneath *Thalassia hemprichii* and *Enhalus acoroides* in tropical seagrass beds, Xincun Bay, South China Sea, intertidal sediment, primary producers, and seawater samples were collected. No significant differences on sediment $\delta^{13}\text{C}$, SOC, and microbial biomass carbon (MBC) were observed between *T. hemprichii* and *E. acoroides*. SOC was mainly of autochthonous origin, while the contribution of seagrass to SOC was less than that of suspended particulate organic matter, macroalgae and epiphytes. High nutrient concentrations contributed substantially to SOC of seagrass, macroalgae, and epiphytes. The SOC, MBC, and MBC/SOC ratio in the nearest transect to fish farming were the highest. This suggested a more labile composition of SOC and shorter turnover times in higher nutrient regions. Therefore, the research indicates that nutrient enrichment could enhance plant-derived contributions to SOC and microbial use efficiency.

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1. Introduction

Seagrasses develop organic-rich sediment composed of both autochthonous and allochthonous organic carbon (Kennedy et al., 2010; Fourqurean et al., 2012). Higher sediment organic carbon (SOC) concentrations have been observed in the sediment of seagrass beds compared to bare sediment (Boschker et al., 2000; Holmer and Frederiksen, 2007; Marbà et al., 2015). Seagrass, epiphyte, macroalgae, and suspended particulate organic matter (SPOM) trapped from the water column (Agawin and Duarte, 2002; Gacia et al., 2003) can be important sources of sediment organic carbon in seagrass beds (Kennedy et al., 2004; Papadimitriou et al., 2005; Volkman et al., 2008; Dubois et al., 2012; Miyajima et al., 2015). Kennedy et al. (2010) summarized the average contribution of seagrass to SOC to be about 50% according to a worldwide database of $\delta^{13}\text{C}$. The most active fraction of SOC is labile organic carbon (LOC), which is important in controlling ecosystem productivity and understanding the carbon transformation and biogeochemistry cycle (Cheng et al., 2008; Dodla et al., 2012). Microbial biomass carbon (MBC) is a vital fraction of LOC (Fang et al., 2005; Dodla et al., 2012; Yang et al., 2013) and serves as a sensitive indicator of change and future trends in sediment organic matter (Hicks, 2007; Yang et al., 2013).

The rapid expansion of aquaculture in global coastal zones has induced various environmental problems (Primavera, 2006; Grigorakis and Rigos, 2011; Ferriss et al., 2016). The exponential increase of aquaculture along the China coast over the last two decades has intensified the risk for degradation of sensitive marine habitats, such as mangrove, coral reef, and seagrass beds. For example, fish farming in Xincun Bay (an almost entirely closed bay and key aquaculture area in China), Hainan Island, and the South China Sea has generated a considerable amount of particulate organic waste and soluble inorganic waste that has led to the proliferation of macroalgae and epiphytes, and the decline of seagrass. As we know, there are distinct differences for the quality and quantity of organic carbon among seagrass, epiphyte, macroalgae, and SPOM (Cebrian, 1999; Banta et al., 2004). Does this alteration of primary community structure induced by nutrient loading affect the SOC sources and the LOC composition in tropical seagrass beds in Xincun Bay? Further research is required to address this question.

Seagrass species have been highlighted as factors inducing variability of sedimentary carbon stocks of seagrass beds (Lavery et al., 2013). The seagrass bed in Xincun Bay is a mixed seagrass community, with *Thalassia hemprichii* and *Enhalus acoroides* as the dominant species (Huang et al., 2006). In comparison, leaves of *E. acoroides* are wider and taller than those of *T. hemprichii*. New shoots of *T. hemprichii* stem form vertically from the creeping rhizome as short lateral branches, while *E. acoroides* has no lateral branches (Rollón, 1998). Do these differences in leaf morphology lead to significant difference on the source

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and composition of SOC between *T. hemprichii* and *E. acoroides* in Xincun Bay?

Consequently, focusing on tropical seagrass beds, the aim of this study was to examine the sources of SOC and its LOC composition beneath *T. hemprichii* and *E. acoroides* along the nutrient gradient in Xincun Bay. The results may help to elucidate the source and composition of SOC in tropical seagrass beds in the South China Sea and their controlling factors. Our data could help to predict the response of carbon biogeochemistry to anthropogenic stress in a nearly closed bay and provide policy makers along the Indo-Pacific with the knowledge needed to improve ecosystem-based management of these environmentally important marine angiosperms. This is the first report on the effect of nutrient loading on sediment LOC in seagrass beds.

2. Materials and methods

2.1. Study site

The study was performed at Xincun Bay ($18^{\circ}24'34''\text{N}$ – $18^{\circ}24'42''\text{N}$, $109^{\circ}57'42''\text{E}$ – $109^{\circ}57'58''\text{E}$), located in southeastern Hainan Island, South China Sea. Xincun Bay is an almost entirely closed bay with only one narrow channel connecting to the open sea in the southwest (Fig. 1). The substrate was mainly composed of medium-sized sand (China's bay compilation committee, 1999). Dominant seagrass species of *T. hemprichii* and *E. acoroides* occupy an area of approximately 200 ha in the shallow waters in the south of the bay (Huang et al., 2006). In recent years, cage aquaculture has developed rapidly, so that more than 450 floating cage units are located near the entrance of the bay (Zhang et al., 2014).

2.2. Field sample collection

Our sampling sites are shown in Fig. 1. According to the distance to the fish cage culture area, three transects were selected. Transect 1 was located near the bay's entrance and at a distance of about 800 m from the fish cage culture systems, while transect 3 was located far from (about 3 km) the fish cage culture systems. Transect 2 was

between them. The distance between the two transects was about 1 km. Three sampling positions were selected in each transect.

Seawater samples were collected to analyze inorganic nutrients in 2012 (December) and in 2013 (August and December). We used the Ruttner 11.004 organic glass hydrophore (KC Denmark A/S Co., Denmark) to collect water samples 50 cm below the surface during the high tide period (water depths about 1.0–1.5 m). In December 2013, *T. hemprichii* and *E. acoroides* shoot densities were determined by counting the abundance of shoots of the different species present in six quadrats (0.25 m^2) within each transect during low tide (water depths about 0–0.3 m). After that, all the seagrass plants in the quadrats were collected for subsequent analysis. In addition, macroalgae (*Ulva pertusa* and *Hypnea boergesenii*) were also collected in six quadrats (0.25 m^2) within each transect. Meanwhile, duplicated sediment (0–3 cm) samples were also collected in each sampling position where *T. hemprichii* and *E. acoroides* grew in these three transects. These sediment and macrophyte samples were stored in plastic bags. All the samples were stored in an ice chest immediately after sampling until being transported to the laboratory within a few hours.

2.3. Sample preparation and analysis

The seawater was filtrated onto pre-combusted GF/F filters (Whatman, $450\text{ }^{\circ}\text{C}$, 3 h). Filters were immediately packaged in tin foil and stored in plastic bags for subsequent SPOM analysis. The filtered seawater was analyzed for dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphate (DIP) using an AQ-2 Automated Discrete Analyzer (Seal Analytical Inc.).

For each sampling point, a sediment subsample was freeze-dried, composited by plot and sieved through a $500\text{ }\mu\text{m}$ screen to remove coarse materials. The samples were then ground and homogenized with a mortar and pestle, acidified (1 N HCl) overnight at room temperature to remove carbonate, followed by washing with distilled water and drying at $40\text{ }^{\circ}\text{C}$ in an oven. Seagrass leaves, rhizomes, and macroalgae were cleaned by distilled water to remove detritus and attached animals. Epiphytes were removed from seagrass leaves using a scalpel blade and transferred to watch-glasses. The seagrass leaves, rhizomes, epiphytes, and macroalgae were dried at $60\text{ }^{\circ}\text{C}$ for 24 h and weighed. The average

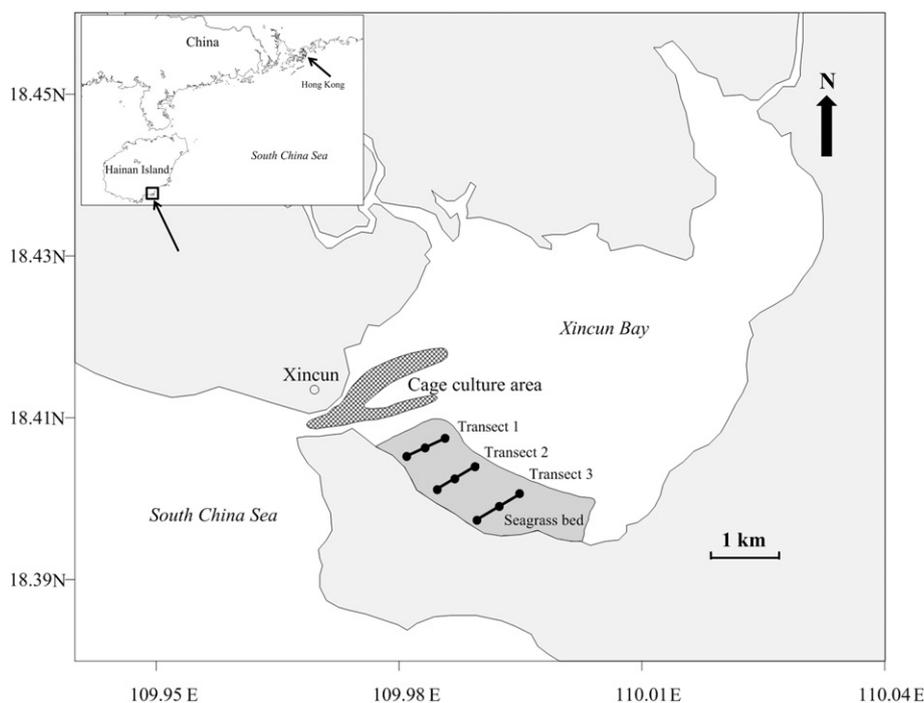


Fig. 1. Sampling sites in Xincun Bay, Hainan Island, South China Sea.

Table 1
Biological traits of seagrass, epiphytes, and macroalgae among three transects (mean \pm S.D.).

Parameters		Transect 1	Transect 2	Transect 3
<i>Thalassia hemprichii</i>	Density (shoots/m ²)	217.33 \pm 82.29 ^a	206.67 \pm 94.18 ^a	310.67 \pm 24.44 ^a
	Aboveground biomass (g DW/m ²)	29.32 \pm 11.91 ^a	43.19 \pm 16.38 ^{ab}	57.32 \pm 3.90 ^b
	Leaf OC content (%)	36.20 \pm 0.78 ^a	36.85 \pm 0.72 ^a	36.90 \pm 0.73 ^a
	Leaf N content (%)	3.20 \pm 0.12 ^a	3.23 \pm 0.07 ^a	3.12 \pm 0.07 ^a
	Leaf C/N (mol/mol)	13.22 \pm 0.20 ^a	13.33 \pm 0.04 ^a	13.82 \pm 0.19 ^a
	Epiphyte biomass (g DW/m ²)	25.77 \pm 15.20 ^a	14.16 \pm 9.14 ^a	18.25 \pm 9.51 ^a
<i>Enhalus acoroides</i>	Density (shoots/m ²)	81.33 \pm 40.07 ^a	110.00 \pm 42.43 ^a	124.00 \pm 16.97 ^a
	Aboveground biomass (g DW/m ²)	47.56 \pm 26.49 ^a	52.98 \pm 18.53 ^a	92.12 \pm 20.76 ^a
	Leaf OC content (%)	35.69 \pm 0.76 ^a	34.82 \pm 0.26 ^a	36.74 \pm 0.72 ^a
	Leaf N content (%)	3.16 \pm 0.08 ^b	2.66 \pm 0.01 ^a	2.84 \pm 0.10 ^a
	Leaf C/N (mol/mol)	13.18 \pm 0.06 ^a	15.27 \pm 0.08 ^b	15.11 \pm 0.16 ^b
	Epiphyte biomass (g DW/m ²)	15.77 \pm 12.53 ^a	7.07 \pm 5.57 ^a	4.44 \pm 1.35 ^a
Macroalgae	Biomass (g DW/m ²)	19.18 \pm 10.09 ^b	5.73 \pm 1.67 ^{ab}	3.59 \pm 2.99 ^a

The different lower case letters indicated significant differences among the three transects (Tukey post hoc test, $p < 0.05$).

biomass of the seagrass leaves, epiphytes, and macroalgae was then converted to the biomass of the species with average abundance data. Carbonate materials were removed with HCl (10%). Samples were then dried at 40 °C in an oven and ground into fine powder. Filters of SPOM were acid fumed (concentrated HCl) overnight to remove carbonates, and then dried at 40 °C. All samples were stored in a desiccator prior to analysis. $\delta^{13}\text{C}$ was analyzed on an isotope ratio mass spectrometer (Thermo Scientific MAT 253) to determine the sources of SOC (Kennedy et al., 2004; Dubois et al., 2012). All isotopic data were expressed in the conventional delta notation (‰): $\delta^{13}\text{C}_{\text{sample}} = (R_{\text{sample}}/R_{\text{reference}} - 1) \times 1000$ where $R = {}^{13}\text{C}/{}^{12}\text{C}$. The reference standard was Peedee Belemnite and the analysis uncertainty was $\leq 0.2\text{‰}$. In addition, organic carbon (OC) and total nitrogen (TN) were determined from sediment and seagrass leaves using an elemental analyzer (Vario EL).

The other moist subsample sediment was stored in the dark at 4 °C and used to analyze for bulk density, electrical conductivity, and the LOC composition (salt-extractable carbon (SEC) and MBC). Bulk density was determined after drying at 105 °C for 24 h. Sediment electrical conductivity was determined on a 1:5 (air-dried sediment: water) mixture using a conductivity meter. SEC, an immediate source of carbon for sediment microbial metabolism (Rochette and Gregorich, 1998), was extracted from triplicate 10 g moist sediment samples with the addition of 30 ml 0.5 M K_2SO_4 (Fang et al., 2005; Dodla et al., 2012). The mixture was shaken for 30 min and then centrifuged for 10 min. The supernatant was vacuum filtered through pre-combusted GF/F filters (Whatman, 450 °C, 3 h) into a pre-combusted apragaz bottle (450 °C, 3 h) for total OC analysis. MBC was determined following the chloroform fumigation–extraction method (Vance et al., 1987; Yang et al., 2013). Three sub-samples of 10 g moist sediment were fumigated in an ethanol-free chloroform atmosphere for 24 h in a 25 °C dark room, then were quickly extracted with 0.5 M K_2SO_4 as above. The filtrate

for the fumigation-treated and untreated sediments were determined rapidly by a Shimadzu TOC analyzer (TOC-V_{CPH}). Sediment MBC was calculated according to the equation: $\text{MBC} = \text{Ec}/0.38$, where Ec was the difference between filtrate SEC in fumigated and no fumigation SEC.

2.4. Statistical analysis

All data were first tested to determine if the assumptions of homogeneity and normality were met. Where these assumptions were not met, the raw data were transformed and further statistical analysis was conducted using the dataset that fulfilled the assumptions. One-way analysis of variance (ANOVA) was performed to determine the statistical significance of seawater inorganic nutrient, seagrass density, seagrass aboveground biomass, epiphyte biomass, macroalgae biomass, seagrass leaves OC, TN, and the ratios of leaves OC to TN (C/N) among the three transects. The linear mixed model (three transects and two seagrass communities as the fixed effects; the distance to the shore of the sampling sites as a random factor) was used to test for significance of the differences of sediment bulk density, electrical conductivity, SOC, sediment TN, sediment $\delta^{13}\text{C}$, SEC, MBC, SEC to SOC (SEC/SOC), and MBC to SOC (MBC/SOC). Nine possible sources, including leaves of *T. hemprichii* and *E. acoroides*, rhizomes of *T. hemprichii* and *E. acoroides*, epiphyte communities growing on *T. hemprichii* and *E. acoroides* leaves, macroalgae (*U. pertusa* and *H. boergesenii*) and SPOM, were considered as SOC sources based on $\delta^{13}\text{C}$. Isotopic mixing models, including a Bayesian approach, were applied with the software SIAR (Parnell et al., 2010) to estimate the proportional contribution of sources to the SOC. One-way ANOVA was applied to gather similar sources into groups based on the isotopic values of possible SOC sources. According to the previous studies, the sources of seagrass bed SOC were mainly composed of seagrass, benthic microalgae, epiphytes, macroalgae, and SPOM (Thimdee et al., 2003; Kennedy et al., 2004; Dubois et al., 2012; Miyajima et al., 2015). Unfortunately, in this study, we did not collect the benthic microalgae, which contribute partially to SOC in seagrass beds. The mentioned statistical analysis was performed with IBM SPSS Statistics 19.0 software.

3. Results

3.1. Variations of nutrients and plant biological traits among the transects

The concentrations of DIN and DIP in the seawater ranged from 1.41 to 12.67 μM and 0.11 to 1.22 μM , respectively. The mean concentrations of DIN and DIP in the seawater were 7.01 ± 2.93 and 0.63 ± 0.42 μM , 4.89 ± 3.23 and 0.51 ± 0.40 μM , and 3.38 ± 1.09 and 0.50 ± 0.33 μM in transects 1, 2, and 3, respectively. Statistically significant difference was observed for DIN concentrations among the three transects ($p < 0.05$), but not for DIP ($p > 0.05$). The DIN and DIP concentrations both showed a decreasing trend from transect 1 to 3.

Table 2
Statistical analysis of the effects of the seagrass community, transect, and distance to shore on sediment parameters using the linear mixed model (three transects and two seagrass communities as the fixed effects, the distance to the shore of the sampling sites as a random factor).

Sediment parameters	Community	Transect	Distance to shore
Bulk density	0.115	72.010 ^{**}	1.734
Electric conductivity	0.332	1.574	1.293
SOC	5.302	7.466 [*]	1.164
Sediment TN	1.041	11.124 [*]	1.682
SEC	1.429	1.129	0.176
MBC	1.412	76.626 [*]	0.401
SEC/SOC	0.915	46.941 ^{**}	0.867
MBC/SOC	1.426	14.862 [*]	0.016
Sediment $\delta^{13}\text{C}$	0.034	7.688 [*]	0.275

* $p < 0.05$.

** $p < 0.01$.

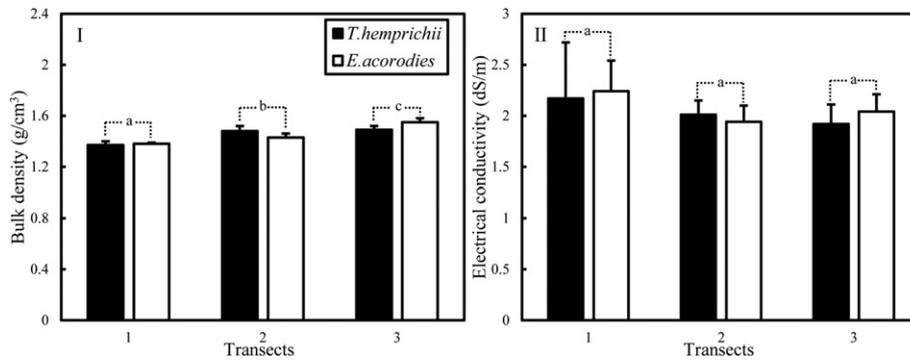


Fig. 2. Sediment bulk density (I) and electric conductivity (II) beneath the *T. hemprichii* and *E. acoroides* communities among the three transects (mean ± S.D.). The different lower case letters over the bars indicate significant differences among the three transects (Tukey post hoc test, $p < 0.05$).

Biological traits of seagrass, macroalgae, and epiphytes are shown in Table 1. The leaf TN of *E. acoroides* and macroalgae biomass were significantly higher in transect 1 than in transect 3. Conversely, aboveground biomass of *T. hemprichii* was observed markedly higher in transect 3 than in transect 1. Moreover, the density and aboveground biomass of *E. acoroides* all showed a trend of transect 1 < transect 2 < transect 3, while epiphyte biomass on *E. acoroides* presented the opposite trend.

3.2. Sediment physical properties, sediment organic carbon, and its fractions

Sediment bulk density and electrical conductivity were not affected by seagrass communities ($p > 0.05$, Table 2). There was also no significant difference in sediment electrical conductivity among the three transects ($p > 0.05$, Table 2), but not for sediment bulk density

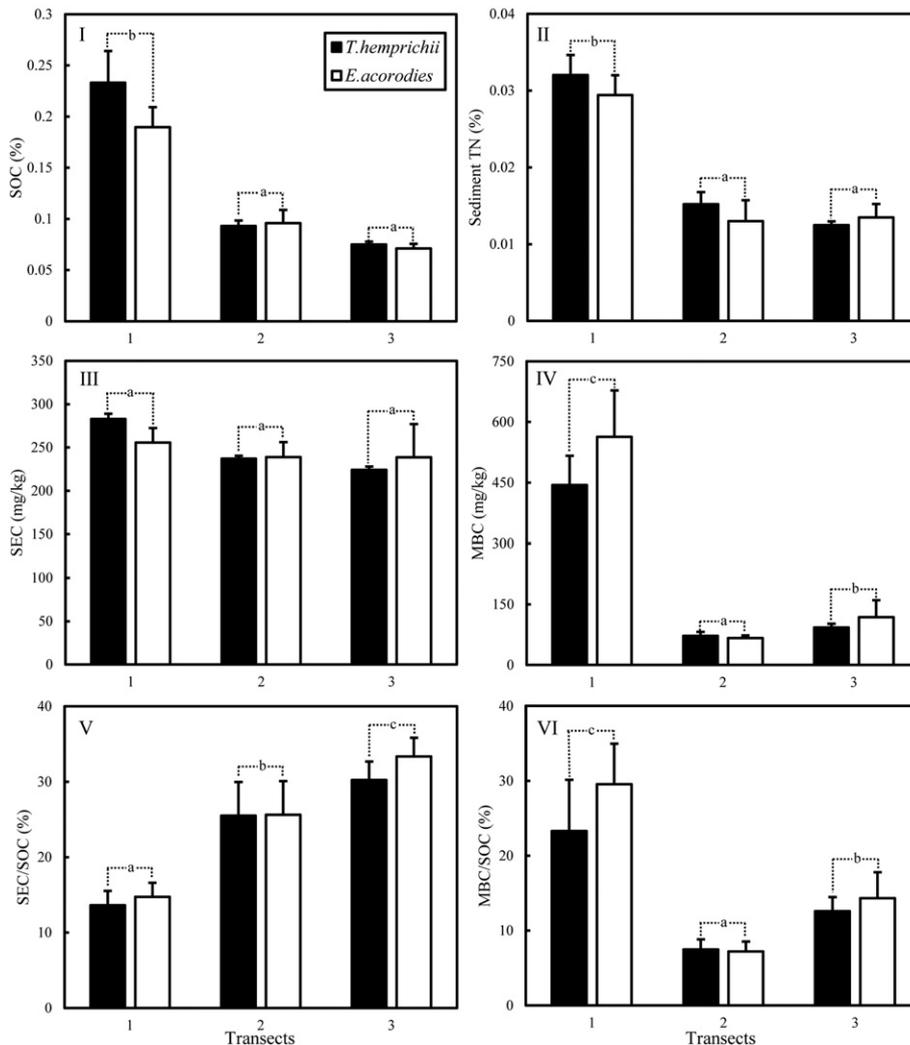


Fig. 3. Variations of the SOC content (I), the TN content (II), the SEC content (III), the MBC content (IV), the SEC/SOC (V), and the MBC/SOC (VI) under the two seagrass communities among the three transects (mean ± S.D.). The different lower case letters over the bars indicate significant differences among the three transects (Tukey post hoc test, $p < 0.05$).

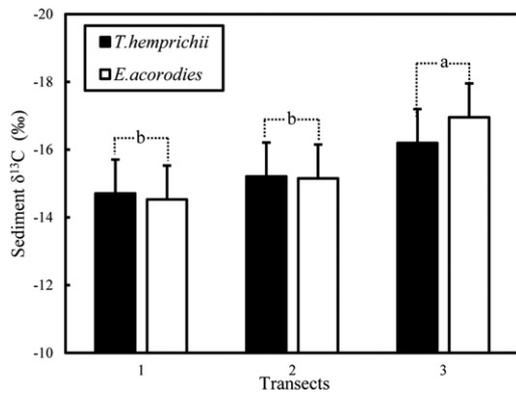


Fig. 4. Variations of the sediment $\delta^{13}\text{C}$ (‰) among the three transects (mean \pm S.D.). The different lower case letters over the bars indicate significant differences among the three transects (Tukey post hoc test, $p < 0.05$).

($p < 0.05$, Table 2). In addition, sediment bulk density showed an increasing trend from transect 1 to transect 3 (Fig. 2 I).

Differences of the contents of SOC, sediment TN, SEC, MBC, SEC/SOC ratio, and MBC/SOC ratio under the two seagrass communities were not significant ($p > 0.05$, Table 2). The SOC and sediment TN contents in transect 1 were significantly higher than in the other two transects ($p < 0.05$, Fig. 3 I and II), while SEC content was similar among the three transects (Fig. 3 III). The MBC content in transect 1 was significantly higher, which was seven times and five times higher than that in transects 2 and 3, respectively ($p < 0.05$, Fig. 3 IV). Furthermore, the ratio of SEC/SOC in the transect 1 was markedly lower than that in transects 2 and 3 ($p < 0.05$, Fig. 3 V), while the MBC/SOC ratio in transect 1 was significantly higher than in the other two transects ($p < 0.05$, Fig. 3 VI).

3.3. $\delta^{13}\text{C}$ of sediment organic carbon and its source

The average $\delta^{13}\text{C}$ of SOC was -15.44% in Xincun Bay. There was no significant difference in sediment $\delta^{13}\text{C}$ between the two seagrass communities (Table 2), while a significant difference was observed for the $\delta^{13}\text{C}$ of SOC among the three transects ($p < 0.05$), with the mean value as -14.62% , -15.18% , and -16.52% in transects 1, 2, and 3, respectively (Fig. 4).

The $\delta^{13}\text{C}$ of SOC possible sources ranged between -20.39% and -7.39% (Fig. 5). SPOM had the lowest $\delta^{13}\text{C}$ values, while the seagrass rhizomes had the highest $\delta^{13}\text{C}$ values. Furthermore, the average $\delta^{13}\text{C}$ values of macroalgae and epiphytes were similar, which was combined into one source as macroalgae and epiphytes. Although we did not

collect the benthic microalgae, previous studies have shown the benthic microalgae $\delta^{13}\text{C}$ values ranged from -16.90% to -12.06% , with a mean approximately -14.00% , in tropical and subtropical seagrass beds (Moncreiff and Sullivan, 2001; Abed-Navandi and Dworschak, 2005; Vaslet et al., 2012). $\delta^{13}\text{C}$ of benthic microalgae were similar to that of epiphytes and macroalgae in this study. Therefore, we separated the sources into seagrass, macroalgae and epiphytes, and SPOM to calculate their contribution based on variations of $\delta^{13}\text{C}$.

The contribution of seagrass, macroalgae and epiphytes, and SPOM to SOC were 0–31%, 0–60%, and 29–96%, respectively, with the average contribution as 11%, 26%, and 63%, respectively (Table 3). Furthermore, the relative contribution of seagrass, and macroalgae and epiphytes to SOC increased from transect 3 to 1, with the relative contribution of macroalgae and epiphytes increasing by 16%, while that of SPOM decreased.

4. Discussion

4.1. Nutrient load in the seagrass bed

In this study, the seawater nutrient concentrations were generally higher than in the seagrass bed in the Aegean Sea, Greece (DIN: 0.39–3.17 μM , DIP: 0.06–0.64 μM ; Apostolaki et al., 2010) and in Cape Bolinao, NW Philippines (DIN: 1.9–2.8 μM , DIP: 0.1–0.2 μM ; Agawin and Duarte, 1996). The high nutrients in this seagrass bed were mainly due to the fact that Xincun Bay is a nearly closed bay with large amounts of floating fish cage units. The nutrient concentrations in the seawater and sediment were both higher in the area close to the fish farming area, which was similar to that observed in previous studies carried out in Xincun Bay (Zhang et al., 2014). Moreover, significantly higher leaf TN of *E. acoroides* and macroalgae biomass, as well as markedly lower aboveground biomass of *T. hemprichii*, were observed near the fish farms. Together, our data indicate that aquaculture has introduced high nutrient loads into seagrass beds and has changed the community structure of the primary producers.

4.2. Characteristics of sediment organic carbon sources and composition

The results showed that sediment $\delta^{13}\text{C}$ ranged from -17.88% to -13.98% , respectively, which is consistent with marine-originated organic matter $\delta^{13}\text{C}$ values (-19% to -12%) (Thimdee et al., 2003; Dubois et al., 2012). This indicates that the SOC source in seagrass beds was of marine autochthonous origin. The contribution of seagrass to SOC (11%) was much lower than that (50%) summarized by Kennedy et al. (2010), but similar to the study of Miyajima et al. (2015) (4–34%) in the Southeast Asian region. Furthermore, the contribution of seagrass to SOC was less than that of SPOM, and macroalgae

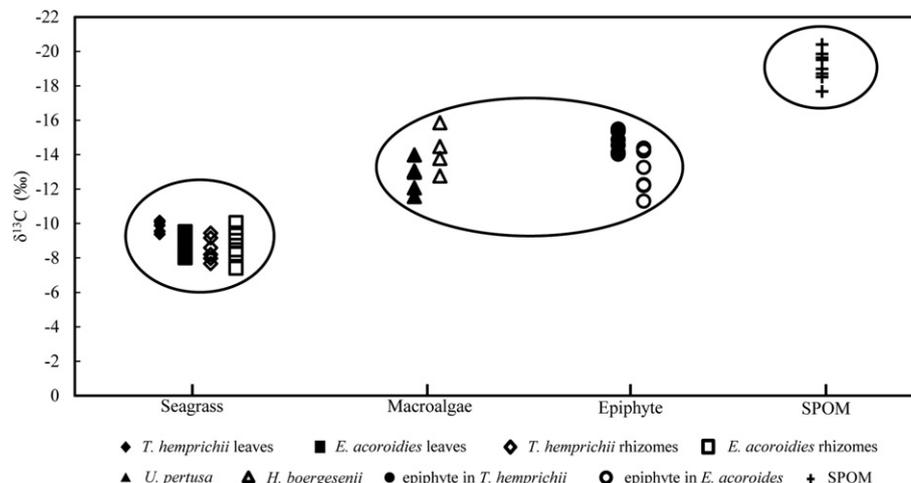


Fig. 5. $\delta^{13}\text{C}$ of SOC possible sources. These possible sources could be divided into three groups based on ANOVA (Bonferroni's test, $p < 0.05$).

Table 3

Isotopic mixing models results based on $\delta^{13}\text{C}$ (‰) values, the estimated 95% confidence intervals and mean are given for each possible sources.

Transect	Source	Low 95%	High 95%	Mean
1	Seagrass	0%	31%	14%
	Macroalgae and epiphytes	0%	60%	33%
	SPOM	29%	78%	53%
2	Seagrass	0%	27%	12%
	Macroalgae and epiphytes	0%	57%	29%
	SPOM	34%	84%	59%
3	Seagrass	0%	18%	7%
	Macroalgae and epiphytes	0%	43%	17%
	SPOM	52%	96%	76%

and epiphytes. The contribution of macroalgae and epiphytes (26%) and SPOM (63%) to SOC was similar to that in *E. acoroides* meadows in the East and Southeast Asian areas (mean 29–35%; Miyajima et al., 2015) and many previous studies (Gacia et al., 2002; Kennedy et al., 2004; Papadimitriou et al., 2005), respectively. This indicates a strong contribution of the particles trapped by the seagrass canopy and plants on seagrass leaves (Kennedy et al., 2004). The surface OC would accumulate and bury with deposition of sediment (Kennedy et al., 2004; Miyajima et al., 2015) and thus could influence the total SOC stability due to different OC sources burial efficiencies (Macreadie et al., 2012). But this is not to imply that seagrass OC is not an important component in sediment, since much of the stored carbon in seagrass beds is located deep in the sediment layer and could be enhanced through the persistence of their roots and rhizomes (Kennedy et al., 2004; Volkman et al., 2008; Fourqurean et al., 2012).

The contents of SOC in Xincun Bay ranged 0.07–0.28%. This was similar to that in the seagrass bed of Ishigaki Island and Southern Thailand (0.2–0.5%) (Miyajima et al., 2015) but was lower than the mean SOC contents ($1.80 \pm 0.30\%$) in seagrass beds summarized by Kennedy et al. (2010). The sand substrate in the seagrass beds of this study may be mainly responsible for the low SOC content in our study. Furthermore, greater oxygen penetration in sandy sediments, than in fine-grained, muddy sediments, could account for a lower SOC content as well (Reimers et al., 2004; Rusch et al., 2006). The SEC content of our study (212.16–291.92 mg/kg) was higher than that in a Florida seagrass bed (24–62 mg/kg) (Hicks, 2007), while MBC (57.33–694.41 mg/kg) was similar to that in a European seagrass bed (193–715 mg/kg) (Boschker et al., 2000). However, the SEC/SOC and MBC/SOC ratios were both higher than those in other seagrass bed sediment (Boschker et al., 2000; Hicks, 2007). This suggested that SOC was more active in this seagrass bed.

Similar sediment $\delta^{13}\text{C}$ beneath *T. hemprichii* and *E. acoroides* communities suggested the sources for SOC were alike. The SOC sources preferred to be closely associated with the geographic variability (Kennedy et al., 2010; Miyajima et al., 2015), rather than the different seagrass communities in a small area. Similar SOC content and MBC were also observed between *T. hemprichii* and *E. acoroides* communities. Though leaves of *E. acoroides* are taller and wider than those of *T. hemprichii*, more shoot branches of *T. hemprichii* compensated for the sediment deposition. This was confirmed by the observation that the density of *T. hemprichii* was 2 times higher than that of *E. acoroides*. Furthermore, the OC and $\delta^{13}\text{C}$ contents of *T. hemprichii* were also identical to those of *E. acoroides*. Therefore, the comparable OC content and $\delta^{13}\text{C}$ of the two seagrass species and the similar sediment deposition in a small area (Gacia et al., 2003; Kennedy et al., 2004) may account for the parallel source and composition of SOC between the two seagrass communities.

4.3. Responses of sediment organic carbon sources and composition to nutrient load

The relative contribution of seagrass, and macroalgae and epiphytes to SOC, both gradually increased with decreasing distance to fish farms

in Xincun Bay. The changes of SOC sources were mostly due to the alteration in the community structure of primary producers. High inorganic nutrient near fishing cages resulted in the proliferation of macroalgae and epiphytes, but inhibited seagrass growth in this study, which was a similar finding to previously published data (Burkholder et al., 2007; Schmidt et al., 2012; Zhang et al., 2014). Eutrophication in the *Ruppia megacarpa* meadow in Wilson Inlet also induced high contribution of algae to SOC (Volkman et al., 2008). Additionally, the low seagrass biomass seemed to contribute less of seagrass to SOC. But high nutrients stimulated leaf litter production of seagrass as well (Apostolaki et al., 2009; Zhou et al., 2014). The seagrass leaf litter mostly retained *in situ*, a reflection that Xincun Bay is a nearly closed bay, with only one narrow channel connecting to the open sea. The increased amounts of seagrass leaf litter, and macroalgae and epiphytes input, heightened the relative contribution of these potential sources to SOC. Thus, elevated SOC was also observed in the high nutrient region. The high SOC content did not indicate high OC storage. Large amounts of macroalgae and epiphytes input resulted in abundant LOC incoming (Duarte and Cebrián, 1996) and consequently elevated the MBC levels in the closing aquaculture area. More SOC stored as MBC implied short turnover times and a more labile microbial community in neighboring, rather than distant fish farms. It has been shown that high microbial activity will accelerate the use of SOC (López et al., 1998). SEC represents soluble carboxyl carbon compounds that are susceptible to microbial degradation (Dodla et al., 2012), and the low SEC/SOC ratios in this study further reflect the high microbial activity in the high nutrient district. Increased inorganic nutrients could also significantly stimulate microbial activity (López et al., 1998). Meanwhile, the nutrient-induced loss of seagrass would not only decrease the organic carbon storage in living biomass of seagrass (Russell et al., 2013), but also trigger the erosion of historic carbon deposits (Macreadie et al., 2014; Marbà et al., 2015). However, does the higher microbial use efficiency of SOC induced by nutrient enrichment reduce the SOC storage? This requires further research, including more detailed studies of sediment microbial community structure, enzyme activity, and sediment core OC characteristics.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.marpolbul.2016.06.054>.

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