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Newly discovered seagrass beds and their potential for blue carbon in the coastal seas of Hainan Island, South China Sea

Zhijian Jiang[a,b,c,1], Songlin Liu[a,b,c,1], Jingping Zhang[a,b], Chunyu Zhao[a,b,c], Yunchao Wu[a,b,c], Shuo Yu[a,c], Xia Zhang[a,c], Chi Huang[d,a], Xiaoping Huang[a,c,*], Manoj Kumar[e]

[a] Key Laboratory of Tropical Marine Bio-resources and Ecology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, PR China
[b] Tropical Marine Biological Research Station in Hainan, Chinese Academy of Sciences, Sanya 572200, PR China
[c] University of Chinese Academy of Sciences, Beijing 100049, PR China
[d] College of Marine Life Science, Chinese Ocean University, Qingdao 266003, PR China
[e] Climate Change Cluster, Faculty of Science, University of Technology Sydney, Sydney, NSW 2007, Australia

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ABSTRACT

Eight new seagrass beds were discovered along the coastline of Hainan Island in South China Sea with an area of 203.64 ha. The leaf N content of all seagrasses was above the median value, indicative of N limitation, with their C:N ratio recorded significantly lower than the limiting criteria. This suggested that N is not limiting but in replete status. Further, the lower C content observed in the seagrass leaves was accompanied by higher nutrient concentration. The mean seagrass biomass C was 0.23 ± 0.16 Mg C ha^{-1}, while the average sediment organic carbon (SOC) stock was 7.02 ± 3.57 Mg C ha^{-1}. The entire SOC stock of the newly discovered seagrass beds was 1306.45 Mg C, and the overall SOC stock of seagrass bed at Hainan Island was 40858.5 Mg C. These seagrass beds are under constant threats from sea reclamation, nutrient input, aquaculture activities for oyster and snail farming, and fishing activities.

Seagrasses are monocotyledonous flowering plants that have experienced extreme evolutionary events in the angiosperm lineage before adapting to the marine habitat 130 million years ago (Olsen et al., 2016). Seagrasses are outstanding in their nutrient uptake from the water column and capturing particles to ensure water clarity. Their roots and rhizomes extend horizontally and vertically and help prevent coastal erosion by stabilizing the sediments in ocean. Seagrass bed serves as an essential habitat and a vital food source for marine fauna. Seagrasses store carbon in their living biomass through excess photosynthetic carbon fixation, and the sediments beneath them also store a large stock of organic carbon; therefore, they are considered valuable carbon sinks (Duarte et al., 2013; Fourqurean et al., 2012; Hemminga et al., 2000; Larkum et al., 2006; Miyajima et al., 2015; Phang et al., 2015; Ricart et al., 2017; Waycott et al., 2009).

There are 22 species of seagrasses belonging to 4 families and 10 genera in China, accounting for approximately 30% of known seagrass species worldwide (Huang et al., 2016; Zheng et al., 2013). Seagrasses are spatially distributed in Chinese biotas, including the South China Sea Bioregion and China's Yellow Sea and Bohai Sea Bioregion (Zheng et al., 2013); however, there is considerable lack of information for the accurate mapping of their spatial patterns and species diversity at the national scale in China. Further, an updated database on the taxonomic information of Chinese seagrass species is urgently required, considering their vital role in carbon sink. This shortage of information impedes the undertaking of national conservation and restoration programs for seagrass biodiversity (Zheng et al., 2013). Furthermore, most of the studies on seagrass distribution in China have been published in local national journals and periodicals in the Chinese language, which further constrained their inclusion in the world seagrass distribution database (Short et al., 2007). Eventually, this has affected the contribution of Chinese seagrasses to the world seagrass carbon stock (Fourqurean et al., 2012).

The area of seagrass beds in Hainan island occupy 64% of the entire area of seagrass beds in China, mostly distributed in the eastern and southern coast areas, such as Wenchang, Qionghai, Lingshui, and Sanya (Huang et al., 2006; Wang et al., 2012; Zheng et al., 2013). The major seagrass species from this region include *Enhalus acoroides*, *Thalassia hemprichii*, *Halophila ovalis*, and *Halodule uninervis*, with *T. hemprichii* as the dominant species (Huang et al., 2006; Wang et al., 2012). Because Hainan Island is a region with clearest features of tropical climate, the

* Corresponding author at: Key Laboratory of Tropical Marine Bio-resources and Ecology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, PR China.
E-mail address: xphuang@scsio.ac.cn (X. Huang).
[1] Author ZJ Jiang and SL Liu contribute equally to this article.

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area of seagrass bed and the species diversity within are the most abundant in China. However, only a few seagrass beds have been discovered in the past from western and northern coast of Hainan Island, with no records of *T. hemprichii* and other seagrasses belonging to the genus *Ruppia* (Yu and den Hartog, 2014). Moreover, there were no records on the area of seagrass beds from this region (Den Hartog and Yang, 1990; Jiang, 2012; Xu, 2011). However, recently, information on seagrass bed area being approximately 55.4 ha was recorded along the northern coast of Hainan Island (Qiu et al., 2016; Wang et al., 2012). Some marine experts have even reported that seagrass beds along the western coast of Hainan Island had disappeared (Su, 2004). The western and northern coast of Hainan Island is less frontally attacked by waves and typhoons, and the economic development level of the western coast is relatively lower than the eastern and southern coast. There is a high possibility that many hidden seagrass beds exist that are yet to be discovered in the western and northern coast. It is important to discover these seagrass beds before they "silently disappear" with the high-speed economic development. In particular, sea reclamation due to rapid real estate development and aquaculture is constantly accelerating along the whole coast of Hainan Island since it has become the International Tourism Island. Additionally, a number of anthropogenic effects (e.g., fish caging, shrimp farming, sewerage effluent release, dredging) have imported a large amount nutrients and organic matter into the coastal area along Hainan Island, threatening it with eutrophication risk (Li et al., 2010). However, the adverse effects of this high nutrient load to seagrasses remain to be explored.

Considering the above facts, we conducted field investigation along the coast of Hainan Island to discover the "hidden" seagrass beds and analyze their habitat threats. We also assessed the carbon storage potential in seagrass biomass, surface sediments and seawater. Seagrass tissue nutrients (C and N) and their ratio were also examined to evaluate the nutrient status in the seagrass beds. The results obtained in this study will contribute (1) in updating seagrass distribution along the coast of Hainan Island, (2) to their potential role as "blue carbon," and (3) in providing an overview of the nutrient status of tropical seagrass beds in the South China Sea region. It will strengthen the understanding that is needed to improve the management and protection of these ecologically and economically important marine floras.

From the available literature and other correlative data (Chen et al., 2015; Den Hartog and Yang, 1990; Huang et al., 2006; Jiang, 2012; Qiu et al., 2016; Wang et al., 2012; Xu, 2011; Zheng et al., 2013), we marked the known distribution of seagrass beds along the coast of Hainan Island (Fig. S1 in Supporting material). Then on the basis of satellite photographs, coastal geomorphic characteristics, and environmental information, we conducted field survey from June to November 2016, especially on the "blank" coastal areas where possibly some seagrass beds exist. The surveys were conducted during spring low tide to discover seagrass beds. The tangible distribution of seagrasses was recorded using GPS, and specimens were collected for seagrass species identification following the method advocated by international seagrasses researchers (Phillips and McRoy, 1990; Short and Coles, 2001). Duplicated quadrates in 25 × 25 cm or 6.7 cm in diameter were sampled for each seagrass species in each seagrass bed. In addition, duplicated water samples and sediment core samples of 5 cm depth for each seagrass species were sampled using a 5-L Niskin bottle and PVC core tube, respectively. For mixed seagrass beds, duplicated sediment samples of each seagrass species were combined for analyzing the particle size.

Samples were transported to laboratory under ice storage. Each water sample was immediately filtered through Whatman GF/F filters (0.7 μm pore size) for measuring dissolved inorganic nitrogen (DIN = nitrate + nitrite + ammonium), dissolved inorganic phosphate (DIP), dissolved silicate (DSi), dissolved organic carbon (DOC), and suspend organic carbon (POC) and nitrogen (PON). The DIN, DIP, and DSi contents of the seawater samples were measured using standard colorimetric techniques using a CANY 722s spectrophotometer. Nitrate, nitrite, ammonium, DIP, and DSi were measured using a zinc-cadmium reduction method, hydrochloride naphthodiamide method, hypobromite oxidation method, phosphorus molybdenum blue spectrophotometry, and silicon molybdenum yellow spectrophotometry, respectively (General Administration of Quality Supervision, 2008). Salinity was evaluated using a dissolved oxygen/conductivity meter (YSI, model 85, USA). DOC was determined using a Shimadzu TOC analyzer (TOC-VCPH).

The particle size of the sediment samples was analyzed using a Malvem Mastersizer 2000 laser diffractometer capable of analyzing particle sizes between 0.02 and 2000 μm. The percentages of the following three grain size groups were determined: < 4 (clay), 4–63 (silt), and > 63 μm (sand). The sediment samples were sieved through a 500-μm screen to remove coarse materials, which were weighed so their mass could be accounted for calculations. Samples were ground and homogenized with a mortar and pestle. Then they were acidified overnight with 1 N HCl at room temperature to remove carbonate, followed by washing with distilled water and drying at 40 °C in an oven. All the samples were stored in a desiccator prior to analysis. The concentrations of seawater POC and PON, sediment organic carbon (SOC), and total nitrogen were determined using a CHN Elemental Analyzer (Elementar, Vario EL-III, Germany).

We estimated the total vegetative carbon stock and total SOC stock (C_{stock}) of the top 5 cm of seagrass following the calculation (Howard et al., 2014):

$$\text{Vegetative component carbon pool (Mg C/ha)} = \text{Carbon content (kg C/m}^2) \times (\text{Mg/1000 kg}) \times (10{,}000\ \text{m}^2/\text{ha})$$

$$\text{Sediment carbon density (g/cm}^3) = \text{dry bulk density (g/cm}^3) \times (\%C_{org}/100).$$

$$\text{Amount carbon in core section (g/cm}^2) = \text{Sediment carbon density (g/cm}^3) \times \text{thickness interval (cm)}.$$

$$\begin{aligned}\text{Core carbon content (g/cm}^2) = {} & \text{Amount of carbon in core section A (g/cm}^2) \\ & + \text{Amount of carbon in core section B (g/cm}^2) \\ & + \text{Amount of carbon in core section C (g/cm}^2) \\ & + \text{....all the samples from a single core.}\end{aligned}$$

$$\begin{aligned}\text{Total core carbon (Mg C/ha)} = {} & \text{Summed core carbon (g/cm}^2) \\ & \times (1\ \text{Mg}/1{,}000{,}000\ \text{g}) \\ & \times (100{,}000{,}000\ \text{cm}^2/1\ \text{ha})\end{aligned}$$

$$\begin{aligned}\text{Average carbon in a core} = {} & \text{carbon content in core 1} \\ & + \text{carbon content in core 2} \\ & + \text{carbon content in core 3} + \text{....n/n.}\end{aligned}$$

$$\begin{aligned}&\text{Total organic carbon stock in a studied area (Mg C)} \\ &= (\text{average core carbon from Statum A (Mg C/ha)} \\ &\quad \times \text{area of Statum A (hectares)}) \\ &\quad + (\text{average core carbon from Statum B (Mg C/ha)}) \\ &\quad \times \text{area of Statum B (hectares)} + \ldots\end{aligned}$$

The means and standard errors of all variables were calculated. 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 ANOVA was used to determine whether the parameter estimates were significantly different ($p < 0.05$) among sites and seagrass species (Statistica 6.0). These parameters included SOC stock and biomass C stock. Treatment means were compared and separated by least significant difference (LSD). Pearson correlations were calculated between leaf carbon and nutrient concentration of seawater and sediment,

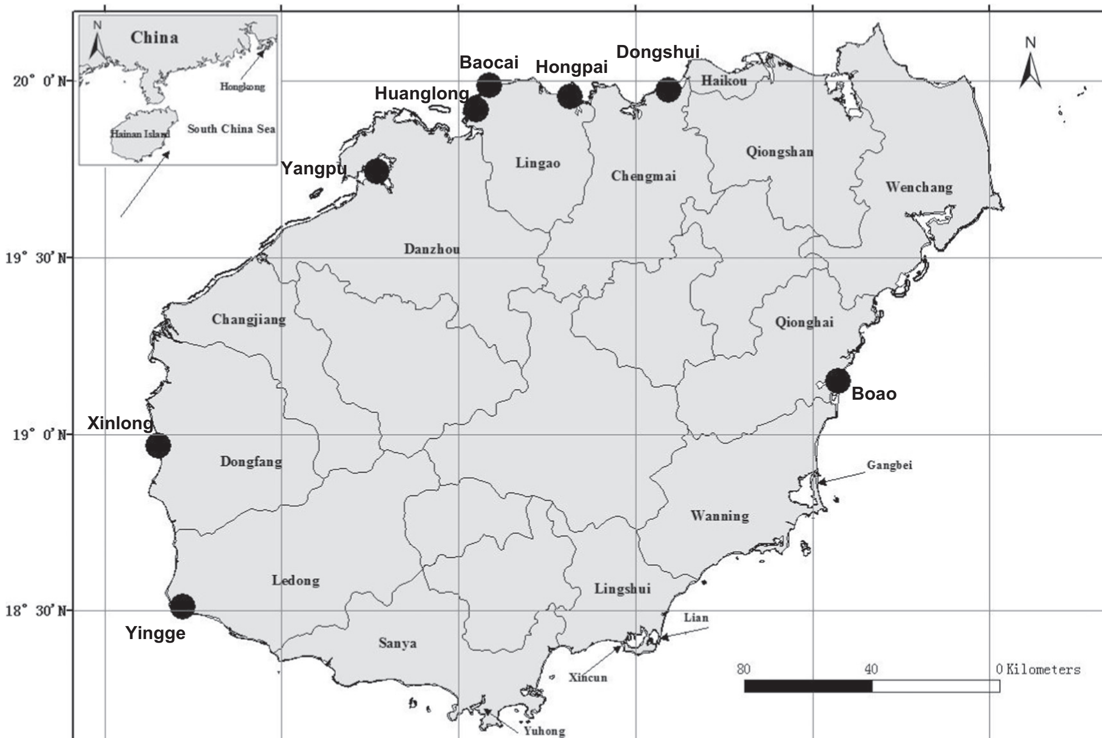

Fig. 1. Distribution of the newly discovered seagrass beds in the coastal seas of Hainan Island.

between biomass carbon content and biomass carbon stock, and between SOC and mud content (% < 0.63 μm).

Overall, eight new seagrass beds were discovered that were distributed in Dongshui, Hongpai, Baocai, Huanglong, Yangpu, Xinlong, Yingge, and Boao, with their area as 0.50, 27.42, 12.04, 8.04, 102.87, 3.72, 46.09, and 2.96 ha, respectively (Fig. 1). The entire area was recorded to be 203.64 ha, wherein four beds were recorded as mixed seagrass beds (Table 1). The seagrass species that were found in these beds included *Halophila beccarii*, *H. ovalis*, *H. uninervis*, *Halodule pinifolia*, *Ruppia brevipedunculata*, *Zostera japonica*, and *T. hemprichii*. Among them, *H. ovalis* and *H. univervis*, *H. ovalis* and *R. brevipedunculata*, *H. ovalis* and *H. pinifolia*, *Z. japonica* and *H. beccarii* were mixed together in Hongpai, Xinlong, Yingge, Boao, respectively. *H. ovalis* and *H. beccarii* had a wider distribution along the coast of Hainan Island.

In the newly discovered seagrass beds along the coast of Hainan Island, seawater salinity ranged between 2.7 and 32, with a mean of 24.8 ± 9.4, while pH ranged from 6.99 to 9.49, with a mean of 8.16 ± 0.52. Seagrass beds from Dongshui and Yangpu coast exhibited both low salinity and pH; however, Boao coast was low in salinity alone (Table 2). Seawater DIN, DIP, and DSi ranged from 1.46 to 65.51, 0.006 to 3.35, and 0.05 to 131.42 μmol/L, respectively, with their corresponding mean values as 16.94 ± 17.36, 0.74 ± 0.99, and 29.72 ± 31.44 μmol/L (Table 2). High DIN and DSi concentrations were observed in Dongshui, Yangpu, and Boao, while high DIP concentrations were observed in Dongshui and Boao. PON varied from 3.43 to 33.29 μmol/L, averaging 13.04 ± 9.12 μmol/L, while sediment total nitrogen varied between 0.011% and 0.343%, with a mean value at 0.082 ± 0.077% (Table 3).

Table 1
Distribution, species, and area of newly discovered seagrass beds.

Seagrass bed	Species	Area (ha)
Dongshui	*Halophila beccarii*	0.50
Hongpai	*Halophila ovalis, Halodule uninervis*	27.42
Baocai	*Thalassia hemprichii*	12.04
Huanglong	*H. ovalis*	8.04
Yangpu	*H. beccarii*	102.87
Xinlong	*Ruppia brevipedunculata, H. ovalis*	3.72
Yingge	*Halodule pinifolia, H. ovalis*	46.09
Boao	*Zostera japonica, H. beccarii*	2.96
Total		203.64

Sediment bulk density varied from 1.08 to 2.04 (median value: 1.47 ± 0.23), while the water content ranged from 17.62 to 62.16 (median value: 33.57 ± 12.46). For sediment particle sizes, the composition of sand, silt, and clay ranged from 12.55% to 100%, 0 to 65.40%, and 0 to 22.05%, respectively, with the corresponding median values as 71.55 ± 25.56%, 20.61 ± 18.49%, and 7.84 ± 7.43% (Table 3).

The results of the survey on the seagrass parameters are listed in Table 4. Leaf width of *H. beccarii* was similar in Dongshui, Yangpu, and Boao, while its leaf length and biomass were higher in Boao. Conversely, leaf length of *H. ovalis* was comparable in Hongpai, Huanglong, Xinlong, and Yingge, whereas the leaf was wider in Hongpai. Shoot density and biomass of *H. ovalis* were higher in Huanglong and Xinlong than in Hongpai and Yingge. Leaf length of *H. pinifolia* was longer than that of *H. uninervis*. Interestingly, seeds of *R. brevipedunculata* and *H. ovalis* were also observed on their plants.

C, N, and C/N ratio in aboveground tissue ranged from 29.36 to 48.47, 2.15 to 3.87, and 9.44 to 16.55, respectively, with their corresponding mean values as 34.08 ± 4.77, 2.86 ± 0.42, and 12.10 ± 2.10. C, N, and C/N ratio in belowground tissue varied from 22.01 to 40.22, 0.85 to 2.53, and 12.31 to 35.14, respectively, with their corresponding median values as 31.43 ± 4.94, 1.63 ± 0.45, and 20.47 ± 5.88. The C content in the aboveground tissue of these seagrasses was relatively higher than that in belowground tissue, except for *H. beccarii* in Dongshui and Yangpu and *Z. japonica* in Boao. The N content and C/N ratio in aboveground tissue were significantly higher than those in belowground tissue (Table 4). For aboveground tissue, C content showed a trend in the order *H. univervis* > *R. brevipedunculata* > *T. hemprichii* > *H. pinifolia* > *H. ovalis* > *H. beccarii* > *Z.*

Table 2
Characteristics of seawater in newly discovered seagrass beds.

Seagrass bed	Salinity	Temperature	pH	NO_3-N (μmol/L)	NH_4^+ (μmol/L)	NO_2-N (μmol/L)	DIN (μmol/L)	DIP (μmol/L)	DSi (μmol/L)	DOC (μmol/L)	POC (μmol/L)	PON (μmol/L)
Dongshui	15.75 ± 0.35	27.90 ± 0.28	7.75 ± 0.01	39.23 ± 10.02	16.87 ± 3.54	4.91 ± 0.11	61.01 ± 6.37	3.25 ± 0.14	46.54 ± 1.91	181	53.67 ± 7.39	9.83 ± 2.54
Hongpai	29.50 ± 0.71	32.35 ± 0.07	8.50 ± 0.15	0.46 ± 0.63	2.26 ± 1.14	-	2.71 ± 1.77	0.83 ± 0.09	19.35 ± 1.57	212	94.26 ± 41.99	19.69 ± 9.60
Baocai	31.90 ± 0.14	26.50 ± 0.0	8.01 ± 0.01	7.03 ± 1.08	7.13 ± 2.46	-	14.15 ± 1.38	0.260.02	9.12 ± 1.80	254.7	22.67 ± 1.11	4.47 ± 1.02
Huanglong	30.50 ± 0.71	26.00 ± 0.71	8.08 ± 0.02	2.90 ± 0.81	7.60 ± 3.83	-	10.50 ± 4.64	0.24 ± 0.17	16.16 ± 0.32	246.8	15.40 ± 6.43	18.36 ± 21.12
Yangpu	20.50 ± 0.71	29.95 ± 0.07	7.72 ± 0.04	17.70 ± 2.46	7.62 ± 0.08	0.68 ± 0.02	25.99 ± 1.25	0.70 ± 0.00	34.58 ± 7.51	206.9 ± 47.7	73.53 ± 5.11	16.53 ± 0.94
Xinlong	31.00 ± 1.00	32.97 ± 0.81	8.42 ± 0.26	2.46 ± 2.08	2.12 ± 0.58	0.03 ± 0.02	4.61 ± 0.76	0.05 ± 0.07	11.82 ± 0.55	258.3 ± 75.6	42.55 ± 2.52	9.41 ± 2.37
Yingge	30.00 ± 1.00	30.63 ± 0.57	8.33 ± 0.04	3.68 ± 2.32	4.53 ± 2.72	0.09 ± 0.04	8.31 ± 3.40	0.11 ± 0.03	19.57 ± 6.26	562.1 ± 169.3	*	*
Boao	3.80 ± 1.56	32.80 ± 0.28	8.24 ± 1.77	16.65 ± 2.73	3.76 ± 3.49	0.78 ± 0.12	21.20 ± 6.35	1.43 ± 0.67	107.59 ± 33.70	327.9 ± 155.0	*	*

"-" indicates below detection limit, "*" indicates data missing.

japonica, while C content for belowground tissue showed a trend in the order *R. brevipedunculata* > *H. univervis* > *H. beccarii* > *Z. japonica* > *H. pinifolia* > *T. hemprichii* > *H. ovalis*. On average, correlation analysis showed negative correlation of seagrass leaf carbon with seawater inorganic nitrogen and sediment nitrogen content (Fig. 2). C content in belowground tissue of *H. beccarii* exhibited a positive relationship with seawater salinity. Carbon stored in the abovegound living biomass ranged from 0.02 to 0.27 Mg C ha^{-1} with a mean of 0.11 ± 0.07 Mg C ha^{-1}, while in the belowground biomass ranged from 0.02 to 0.63 Mg C ha^{-1}, with a mean of 0.12 ± 0.14 Mg C ha^{-1}. The biomass of aboveground and belowground tissue had significant positive correlation with the biomass C stock of aboveground and belowground tissue, respectively (Fig. 3). The total seagrass biomass C stock was between 0.05 and 0.70 Mg C ha^{-1}, averaging 0.23 ± 0.16 Mg C ha^{-1}. Significant difference between species was observed for the total biomass C stock ($F = 3.615$, $p < 0.05$), with a trend in the order *T. hemprichii* > *Z. japonica* > *R. brevipedunculata* > *H. ovalis* > *H. pinifolia* > *H. beccarii* > *H. uninervis* (Fig. 4).

DOC concentration ranged from 173.2 to 882.5 μmol/L (median value: 350.6 ± 209.6 μmol/L), with the highest in Yingge, while POC ranged from 10.86 to 123.94 μmol/L (median value: 50.34 ± 31.53 μmol/L), with the highest in Hongpai. SOC varied from 0.24 to 1.67 (median value: 0.91 ± 0.46). The highest SOC was beneath *H. uninervis* in Hongpai, while the lowest SOC was beneath *T. hemprichii* in Baocai. SOC beneath *H. beccari* was higher in Dongshui than that in Yangpu and Boao. SOC beneath *H. ovalis* was lower in Xinlong than that in Hongpai, Huanglong, and Yingge. The relationship between SOC content and %mud among the newly discovered seagrass beds is shown in Fig. 5. SOC and mud contents were only slightly positively correlated, albeit insignificantly.

SOC stock of the 5-cm sediment core ranged from 1.87 to 12.85 Mg C ha^{-1}, with the mean value as 7.02 ± 3.57 Mg C ha^{-1}. There was a significant difference in the SOC stocks among the eight seagrass beds ($F = 13.68$, $p < 0.01$) (Fig. 6). The highest SOC stock was observed in Hongpai and Dongshui, while the lowest was found in Baocai and Boao. SOC stock beneath *H. beccarii* showed a trend in the order Hongpai > Yangpu > Boao.

The newly discovered seagrass beds were distributed in the reef, lagoon, river mouth, and saltwords, mainly in the intertidal zone. Most of the seagrass species were exposed to air at low tide, and three seagrass beds were located adjacent to extensive mangrove forests. We first discovered *R. brevipedunculata* in the western and northern coast of Hainan Island. Yu and den Hartog (2014) reported that the genus *Ruppia* in China was distributed from Pulandian in the temperate zone to Maoming in the subtropical zone, as well as in Yuhong in Sanya (Ito et al., 2010; Yu and den Hartog, 2014). The dimensions of the leaves of *R. brevipedunculata* in Xinlong are within the range reported earlier, which is usually less than 10 cm long and 0.3–0.5 mm wide (Yu and den Hartog, 2014). In addition, we discovered *T. hemprichii* in the coral substrate along the western coast of Hainan Island. The sediment beneath *T. hemprichii* along the eastern and southern coasts of Hainan Island was mostly recorded as coral substrate. Is there any difference in *T. hemprichii* between the two coasts? This deserves further research. Interestingly, both *H. ovalis* and *H. beccarii* were dominant species and widely distributed in the eight newly discovered seagrass beds, while *T. hemprichii* was the dominant species in the eastern and southern coast of Hainan Island. Undoubtedly, this was due to the distinct difference in sediment substrates between the two coasts. The substrate along the western and northern coast was mainly sand-clay, whereas it was chiefly coral substrate along the eastern and southern coast (Wang et al., 2012). Furthermore, *H. beccarii* was distributed in a wider range of salinity and pH. *H. beccarii* has been reported to usually occur in environments with salinity fluctuation, e.g., brackish coastal water, lagoon, and marine coastal areas. *H. beccarii* could tolerate hyposaline conditions better than hypersaline conditions and survived even in hypotonic environment to 0 psu (Fakhrulddin et al., 2013). *H. beccarii*

Table 3
Characteristics of sediments in newly discovered seagrass beds.

Seagrass bed	Water content (%)	Bulk density (g/cm^3)	Sediment particle sizes			Carbon (%)	Nitrogen (%)
			Sand (%)	Silt (%)	Clay (%)		
Dongshui	21.76 ± 0.46	1.86 ± 0.26	79.64 ± 3.24	16.55 ± 2.70	3.81 ± 0.55	1.20 ± 0.17	0.244 ± 0.141
Hongpai	27.50 ± 2.86	1.52 ± 0.16	98.16 ± 2.60	0.78 ± 1.10	1.06 ± 1.50	1.50 ± 0.31	0.012 ± 0.001
Baocai	27.37 ± 2.92	1.40 ± 0.05	99.32 ± 0.96	0.68 ± 0.96	0.00 ± 0.00	0.33 ± 0.08	0.031 ± 0.008
Huanglong	58.88 ± 4.63	1.15 ± 0.09	40.14 ± 5.13	41.94 ± 4.46	17.92 ± 0.33	1.15 ± 0.08	0.131 ± 0.002
Yangpu	20.43 ± 3.85	1.69 ± 0.04	81.52 ± 8.50	13.25 ± 4.06	5.24 ± 4.44	0.51 ± 0.23	0.051 ± 0.019
Xinlong	43.89 ± 7.07	1.29 ± 0.11	34.23 ± 30.66	50.17 ± 21.53	15.60 ± 9.13	0.87 ± 0.11	0.098 ± 0.017
Yingge	38.27 ± 1.77	1.44 ± 0.05	58.65 ± 8.42	27.36 ± 5.39	13.99 ± 3.03	1.30 ± 0.37	0.102 ± 0.058
Boao	26.30 ± 4.23	1.53 ± 0.11	80.77 ± 11.15	14.12 ± 4.19	5.11 ± 3.12	0.39 ± 0.21	0.046 ± 0.035

present in Dongshui and Yangpu had lower pH as well, suggesting that they may flourish well in futuristic oceans conditions.

High DIN and DIP concentration was found in seagrass beds in Dongshui and Boao. A large number of shrimp ponds were present near these seagrass beds, releasing plenty of nutrients into them. Furthermore, river inputs of nutrient also affected Dongshui, Yangpu, and Boao, which was confirmed by the lower salinity and higher DSi concentration. Saltwoks in Huanglong, Xinlong, and Yingge also posed eutrophication risk to these seagrasses because of limited seawater exchange. The N content of all seagrass leaves in our study was above the median value, indicating that N limited growth in seagrasses (1.8% of DW) (Duarte, 1990), and all leaf C:N ratios were significantly lower than the limiting criteria (19.75) (Duarte, 1990). The leaves of *T. hemprichii* living on carbonate-dominated meadows in Baocai also exhibited high nutrient availability. This suggested that N is not limiting but in replete status in these seagrasses owing to anthropogenic discharage. Eutrophication induced by fish caging, shrimp culture, and sewage discharge has caused seagrass decline in Xincun Bay in Hainan Island (Zhang et al., 2014). Moreover, culturing of oysters and snails in Dongshui and Yangpu, respectively, induced threat to *H. beccarii*, and fishing using electric power and shellfish collection prevail in Hongpai and Baocai. Undoubtedly, *H. beccarii* in Dongshui near Haikou may face complete disappearance in near future if we do not undertake suitable control measures to minimize real estate developments. Boao is a permanent site of the Boao Asia Forum, and their new site construction will also negatively impact the population of *Z. japonica* and *H. beccarii* in these coastal area. Considering the distinct threats to these seagrass beds (Fig. S2–S7 in Supporting material), corresponding management practices should be undertaken with immediate concern. It is crucial for the local government to adjust the development plan and minimize the destruction of the marine seagrass habitats due to rapid crazy real estate development and aquaculture activities. Moreover, the local government should urgently and comprehensively control the nutrient loading to the nearshore environment along the coast of Hainan Island to keep the important seagrass resources healthy.

Three major carbon pools can be considered in seagrass beds, including aboveground living biomass (seagrass leaves and epiphytes), belowground living biomass (roots and rhizomes), and sediment carbon (Fourqurean et al., 2012; Howard et al., 2014). Furthermore, seagrass beds can generate a substantial amount of autochthonous DOC (Barrón and Duarte, 2009; Watanabe and Kuwae, 2015). The dynamics of DOC can also play a vital role in carbon sequestration in the water columns as DOC generally dominates over the water column OC pool and includes a large proportion of refractory OC (Jiao et al., 2014; Nagata, 2008).

DOC concentration in the eight seagrass beds was almost higher than that in *Posidonia oceanica* in Sounion (Apostolaki et al., 2010), *Thalassia testudinum* in Laguna Madre (Ziegler et al., 2004), and *T. hemprichii* thriving on the coral reef in Qionghai (Table S1 in Supporting material) but was almost lower than those in Xincun (Liu et al., 2016), Lian in Lingshui, Gangbei in Wanning (Table 1 in supporting material), and Florida Bay (Chen et al., 2013). Among the eight seagrass beds, the highest DOC concentration was observed in Yingge. Yingge is a saltworks controlled by water lock, leading to poor seawater exchange. Moreover, shrimp culture around the saltworks also releases plenty of DOM, resulting in algal bloom that covers the seagrass. Thus, the release of DOC from seagrasses, algal, and shrimp culture, as well as poor seawater exchange, leads to high DOC concentrations. Furthermore, input of DOC from Longgui river, offshore shrimp culture, and seagrass also enhanced the DOC in seagrass bed in Boao. The production of autochthonous DOC and the dilution of terrestrial DOC (input from river, shrimp culture) control the dynamics of DOC in the water column in these seagrass beds. Indeed, we did not observe the refractory portion of DOC, but Watanabe and Kuwae (2015) found that seagrass directly produces refractory DOM preserved in the water column.

In our study, the DOC comprised 69.2–94.1% of the total organic carbon pool in the water column, with a DOC/POC ratio of 2.2–16.0. Similar results were also observed in the seagrass bed in Gazi Bay (Bouillon et al., 2007). The POC/PON ratio (mean value: 4.81 ± 1.46 mol mol^{-1}) in this study was lower than the terrestrial organic matter in the Furea Lagoon in Japan (Watanabe and Kuwae, 2015). This clearly indicated that phytoplankton-derived organic matter mainly contributed the POM in the water column in these newly discovered seagrass beds. This would affect the carbon sequestration in the sediment as organic matter from phytoplankton was less efficiently preserved than organic matter from terristrial input and seagrass (Watanabe and Kuwae, 2015).

The observed lower leaf C content with higher nutrient concentration from newly discovered seagrass beds suggests that high nutrients led to the reduction of seagrass leaf carbon. Under excessive nitrogen conditions, the photosynthesis of seagrass might be inhibited and carbon requirements for synthesizing amino acids may exceed the carbon fixation capacity, causing a decrease in carbon reserve (Leoni et al., 2008). The C content in the belowground tissue of *H. beccarii* reduced with decreasing salinity; this suggests the translocation of carbon reserves from belowground to aboveground tissue to adjust the osmotic pressure caused by salinity reduction (Jiang et al., 2013; Touchette, 2007). This was confirmed by the presence of the highest aboveground C content in *H. beccarii* in the lowest salinity condition in Boao. A positive correlation was observed between seagrass biomass and biomass carbon stock in this study, suggesting that seagrasses affect seagrass biomass C stock (Serrano et al., 2014). The highest biomass C stock was observed in *T. hemprichii*, which was the largest seagrass species. The mean seagrass biomass C stock in this study was similar to that in previous studies, 0.4 Mg C ha^{-1} in Abu Dhabi (Campbell et al., 2014) and 0.16 ± 0.06 Mg C ha^{-1} in Chek Jawa (Phang et al., 2015), but lower than the global average of 2.51 ± 0.49 Mg C ha^{-1} (Fourqurean et al., 2012). These distinctions were attributed to the relatively small size of seagrasses in these seagrass beds. The total seagrass biomass C stock of these newly found seagrass beds was 33.5 Mg C.

Undoubtedly, the lowest SOC content was found beneath *T.*

Table 4
Characteristics of seagrass in newly discovered seagrass beds.

Seagrass bed	Species	Leaf length (cm)	Leaf width (cm)	Shoot density (shoots/m^2)	Aboveground biomass (DW g/m^2)	Belowground biomass (DW g/m^2)	Aboveground tissue				Belowground tissue			
							C%	N%	C/N ratio	Biomass C (Mg C ha^{-1})	C%	N%	C/N ratio	Biomass C (Mg C ha^{-1})
Dongshui	*Halophila beccarii*	0.79 ± 0.11	0.16 ± 0.03	7833 ± 1650	22.14 ± 6.92	19.79 ± 6.63	30.6 ± 0.8	3.0 ± 0.2	10.2 ± 0.3	0.068 ± 0.023	35.1 ± 7.2	2.3 ± 0.2	15.5 ± 1.7	0.067 ± 0.009
Hongpai	*Halophila ovalis*	2.00 ± 0.54	1.20 ± 0.38	5850 ± 1344	35.75 ± 28.14	22.38 ± 17.47	39.3 ± 12.9	3.0 ± 0.0	13.3 ± 4.5	0.159 ± 0.157	25.1 ± 2.9	1.0 ± 0.1	26.9 ± 7.1	0.059 ± 0.050
	Halodule uninervis	3.17 ± 1.70	0.09 ± 0.01	4233 ± 2216	13.59 ± 3.01	29.03 ± 14.75	38.3 ± 11.4	2.7 ± 0.7	13.9 ± 0.4	0.050 ± 0.004	35.0 ± 1.0	1.8 ± 1.1	24.3 ± 15.3	0.101 ± 0.049
Baocai	*Thalassia hemprichii*	8.75 ± 2.59	0.82 ± 0.13	448 ± 136	26.02 ± 8.14	161.01 ± 46.12	34.8 ± 0.7	2.6 ± 0.1	13.2 ± 0.5	0.090 ± 0.027	31.5 ± 1.2	1.6 ± 0.2	19.5 ± 1.4	0.510 ± 0.165
Huanglong	*Halophila ovalis*	1.76 ± 0.13	0.71 ± 0.08	12924 ± 1081	51.92 ± 4.52	45.18 ± 9.79	33.1 ± 2.5	3.4 ± 0.3	9.8 ± 0.1	0.172 ± 0.028	24.2 ± 0.8	1.6 ± 0.4	15.7 ± 3.6	0.110 ± 0.027
Yangpu	*Halophila beccarii*	0.52 ± 0.14	0.13 ± 0.03	12500 ± 3535	15.11 ± 11.50	16.30 ± 11.70	32.2 ± 2.7	3.0 ± 0.2	10.7 ± 0.4	0.050 ± 0.041	36.5 ± 1.7	1.6 ± 0.0	22.4 ± 1.3	0.060 ± 0.045
Xinlong	*Ruppia brevipedunculata*	5.63 ± 1.53	0.04 ± 0.01	11900 ± 2970	41.56 ± 1.85	19.04 ± 1.59	36.9 ± 1.8	2.6 ± 0.6	14.7 ± 2.6	0.154 ± 0.001	35.6 ± 4.0	1.5 ± 0.1	23.6 ± 5.0	0.068 ± 0.013
	Halophila ovalis	1.89 ± 0.27	0.75 ± 0.13	11200 ± 4808	45.21 ± 36.98	54.93 ± 23.33	32.4 ± 1.1	2.7 ± 0.2	12.2 ± 0.6	0.149 ± 0.125	30.3 ± 1.1	1.6 ± 0.0	18.8 ± 0.6	0.168 ± 0.077
Yingge	*Halodule pinifolia*	6.94 ± 1.88	0.10 ± 0.01	4675 ± 601	29.44 ± 0.19	28.07 ± 0.99	34.5 ± 2.8	2.8 ± 0.2	12.2 ± 0.0	0.101 ± 0.008	31.7 ± 1.0	2.3 ± 0.1	14.0 ± 0.0	0.089 ± 0.006
	Halophila ovalis	1.66 ± 0.28	0.65 ± 0.10	6850 ± 919	11.65 ± 1.02	10.13 ± 1.90	29.6 ± 0.4	2.8 ± 0.0	10.4 ± 0.1	0.035 ± 0.003	23.9 ± 2.7	1.3 ± 0.2	18.0 ± 0.5	0.024 ± 0.007
Boao	*Zostera japonica*	21.73 ± 3.15	0.17 ± 0.04	2733 ± 450	65.52 ± 7.48	14.68 ± 0.62	32.1 ± 0.7	2.2 ± 0.1	10.2 ± 0.6	0.202 ± 0.016	34.9 ± 0.1	1.3 ± 0.2	19.2 ± 0.5	0.052 ± 0.001
	Halophila beccarii	1.26 ± 0.34	0.17 ± 0.04	10200 ± 3111	30.90 ± 12.08	42.54 ± 35.33	34.5 ± 1.7	3.4 ± 0.4	14.5 ± 0.4	0.109 ± 0.049	33.2 ± 1.2	1.7 ± 0.0	27.8 ± 3.8	0.143 ± 0.122

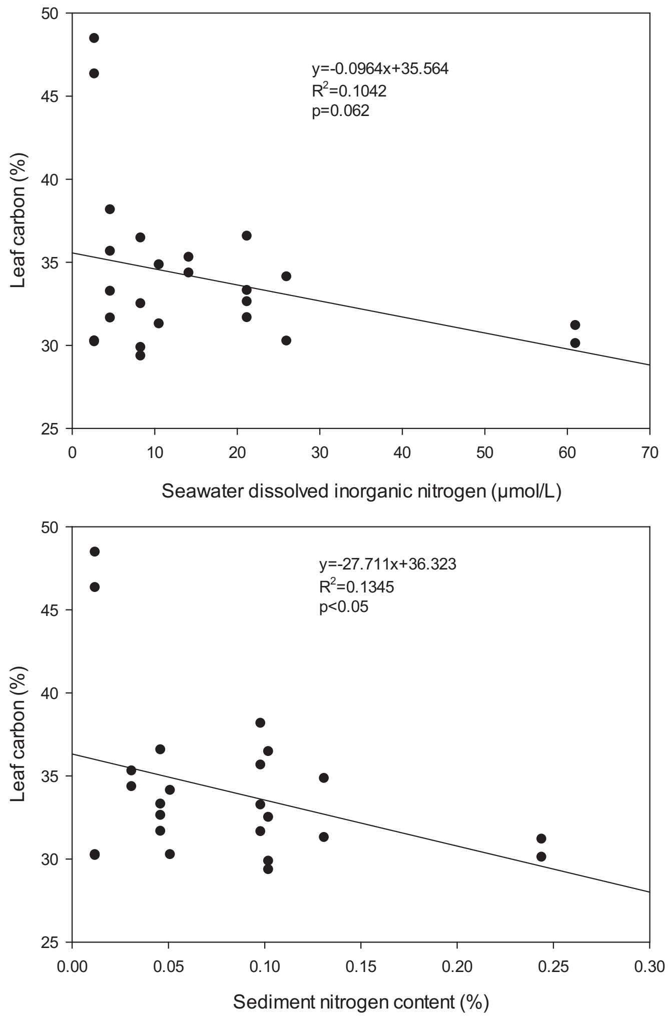

Fig. 2. Relationship between seagrass leaf carbon and seawater dissolved inorganic and sediment nitrogen in newly discovered seagrass beds in the coastal seas of Hainan Island.

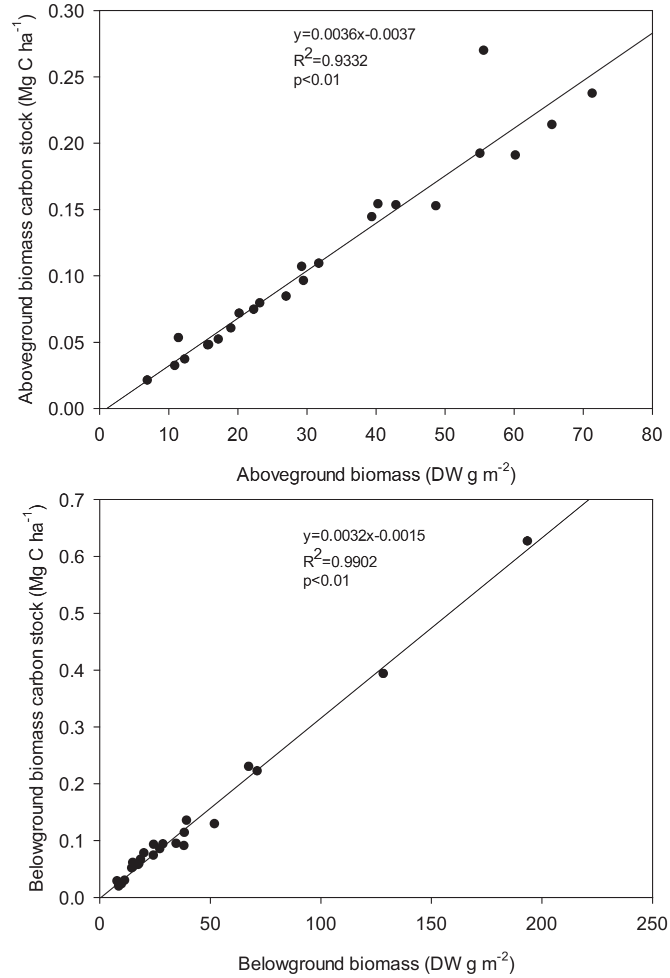

Fig. 3. Relationship between the biomass of aboveground and belowground tissue and biomass carbon stock.

hemprichii living in coral reefs. The sand composition in Baocai was the highest among the eight seagrass beds, while the silt composition was the lowest with no clay composition. Furthermore, the lower SOC was probably due to the longer leaves of *T. hemprichii* in Baocai and *Z. japonica* in Boao. Longer leaves tend to deposit more labile forms of seston. A lack of correlation between SOC and mud contents was observed in this study. Serrano et al. (2016) also reported insignificant relationship between SOC and mud content in large and long-living seagrass beds, while a positive relationship was reported between SOC and mud content in small and fast-growing meadows. Higher organic carbon was associated with the presence of fine sediment (< 63 μm) in seagrass sediment (Ricart et al., 2017). In addition to sediment characteristics and biological features, other factors including geomorphological settings (topography and hydrology) and landscape-level characteristics (patch size) also control SOC (Ricart et al., 2017; Serrano et al., 2016). For example, *H. beccarii* in Dongshui grew as a continuous meadow while it grew as patchy meadows in Yangpu and Boao. The higher SOC beneath *H. beccarii* in Dongshui might be associated with a greater capacity for the retention of more refractory autochthonous materials in continuous meadows (Ricart et al., 2017) and input of organic matter from a mangrove nearby (Chen et al., 2017).

Only half of the estimated SOC stock of the top 5 cm of sediment in the eight seagrass beds was in the range of 6–628 Mg C ha^{-1} (Fourqurean et al., 2012) and was similar to that in Xincun in Lingshui (unplublished data). The mean SOC stock in this study was relatively lower than the global average, which was due to the underestimation of the value based on data from the upper 5 cm sediment layer only. Sediment type and grain size, dry bulk density, and so on could also affect the SOC stock (Serrano et al., 2014). The lowest SOC stock was found in Baocai, where the coral reef was the seagrass substrate. The SOC stock in Dongshui was similar to that in Hongpai. Although the SOC in Dongshui was about 20% lower than that in Hongpai, the bulk density in Dongshui was about 20% higher than that in Hongpai. The whole SOC stock of the newly discovered seagrass beds was 1306.45 Mg C. From the area (5634.2 ha) of existing seagrass beds along the coast of Hainan Island (Zheng et al., 2013) and the mean SOC stock in this study, the overall SOC stock of seagrass beds in Hainan Province was 40858.5 Mg C (the upper 5 cm sediment).

This study adds new records of seagrass distribution along the coast

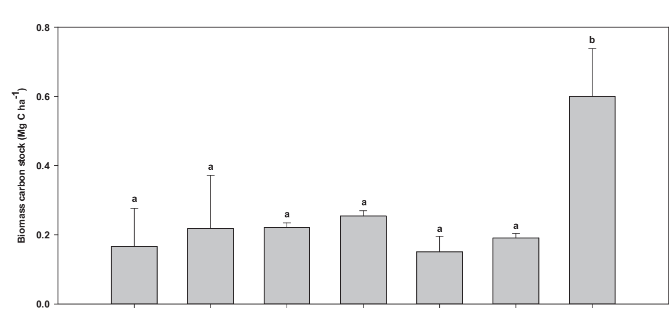

Fig. 4. Biomass carbon stock of different seagrass species in the coastal seas of Hainan Island.

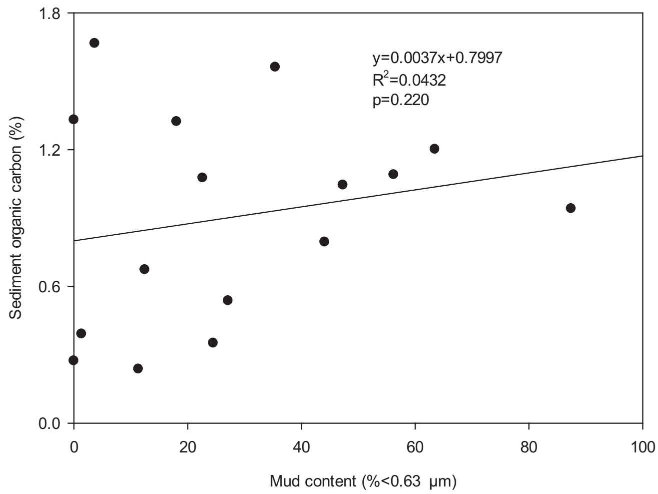

Fig. 5. Relationships between sediment organic carbon and mud content (% < 0.63 μm) in the newly discovered seagrass beds in the coastal seas of Hainan Island.

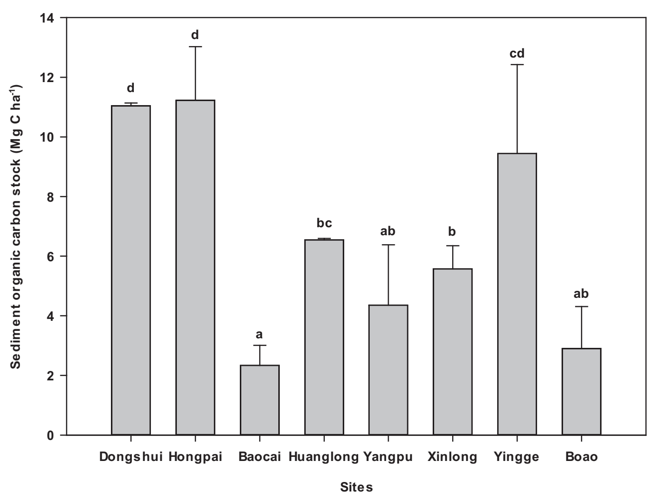

Fig. 6. Sediment organic carbon stock in newly discovered seagrass beds in the coastal seas of Hainan Island.

of Hainan Island and the knowledge of the nutrient status of tropical seagrass beds in South China Sea and contributes to the existing global database on carbon sequestration in seagrass bed. Effective protection measures are needed to control anthropogenic nutrient input and preserve these natural carbon sinks. Further research should be conducted to systematically compare the newly discovered seagrass species with the same species growing in other places from a whole organism level to cellular and biochemical level. The implementation of molecular biology techniques is also urgently required to discover new seagrass species in a rapidly changing world.

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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.2017.07.066.

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