

# Characteristics and source tracing of organic carbon in intertidal sediments of wetland in Yangtze Estuary, China

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## Research

**Keywords:** Estuary, Sediment, Organic carbon, Biomass, Carbon stable isotope

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1       **Characteristics and source tracing of organic carbon in intertidal**  
2                               **sediments of wetland in Yangtze Estuary, China**

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22 **Abstract**

23 **Background:** Wetland ecosystem is characterized by water-land interaction and plays  
24 an important role in regional energy and material circulation. In the context of global  
25 climate change, the study of wetland carbon storage and carbon cycle has become a  
26 focus of academic attention. The characteristics of organic carbon in sediments and its  
27 source is a key problem in the study of carbon cycle in wetlands.

28 **Results:** In this study, the characteristics of total organic carbon (TOC), total nitrogen  
29 (TN) accumulation, and stable carbon isotope ( $\delta^{13}\text{C}$ ) in the vegetation and soil were  
30 investigated for the three dominant salt marsh vegetation *Phragmites australis* (PA),  
31 *Spartina alterniflora* (SA), *Scirpus mariqueter* (SM) of the coastal wetlands of  
32 Chongming Dongtan in the Yangtze River Estuary. The results showed that the mean  
33 value of TOC and TN concentrations in the surface sediments of wetland were  $1.39 \pm$   
34  $0.34\%$  and  $0.091 \pm 0.024\%$ , respectively. The carbon stable isotope ( $\delta^{13}\text{C}$ ) mean value  
35 of sediment was  $-24.17 \pm 1.51\%$ . The TOC of the sediment in the three saltmarsh plant  
36 communities was in the order of SA ( $1.76 \pm 0.38\%$ ) > PA ( $1.45 \pm 0.37\%$ ) > SM ( $0.96 \pm$   
37  $0.44\%$ ). The simulation results of the three end-member mixing equations showed that  
38 the organic carbon in sediments was mainly derived from suspended particles ( $42.44 \pm$   
39  $20.89\%$ ) and vegetation ( $34.50 \pm 25.23\%$ ). The contribution rate of microalgae is lower  
40 ( $23.06 \pm 4.62\%$ ).

41 **Conclusion:** The organic carbon in sediments of wetland in Yangtze Estuary are the  
42 result of mixed input of terrestrial organic carbon and marine organic carbon. Organic  
43 carbon in sediments was mainly derived from suspended particles and vegetations. The

44 results provide preliminary knowledge of the distribution and sources of sedimentary  
45 organic carbon for better understanding the sediment transport and deposition in this  
46 region.

47

48 **Keywords :** Yangtze Estuary, Sediment, Organic carbon, Biomass, Carbon stable  
49 isotope

50

## 51 **Background**

52 Estuary wetlands, as the interaction zone between terrestrial ecosystems and  
53 marine ecosystems, are the ecosystems with the highest ecological service value per  
54 unit area, and have various economic functions, ecological functions, and scientific  
55 values [1-3]. Under the action of physical, chemical and biological processes, a large  
56 amount of organic matter from the land and sea sources are accumulated in the  
57 ecosystem [2, 4]. Salt marsh is an important natural carbon sink, and its global average  
58 carbon fixation efficiency of surface sediments is as high as 244.7 g C/m<sup>2</sup>/a, which is  
59 helpful to alleviate global warming trends [5-10]. In addition, coastal wetland  
60 vegetation has high primary productivity, and its productivity per unit area can reach 3  
61 times than that of tropical forests, which has greatly contributed to the increase of soil  
62 carbon storage [2, 11]. It has been reported that coastal wetlands can become highly  
63 efficient carbon sinks under the combined effects of physical, chemical, and biological  
64 processes [12]. In the context of climate change, the accumulation of carbon in coastal  
65 wetland ecosystems and their sources have been received more and more attention [13].

66 There are many sources of organic carbon stored in sediments such as land sources and  
67 sea sources [14]. And the biogeochemical behaviors of different sources organic carbon  
68 vary greatly [15]. Therefore, it is significant to identify the sources of organic carbon  
69 in sediments for further understanding the carbon sequestration mechanism in wetlands.

70 Yangtze River is the third largest river in the world, and its estuary area is one of  
71 the most concerned areas for the study of land-sea interaction. Yangtze estuary wetland  
72 is an important international wetland, an important habitat for international migratory  
73 birds, and a key area for the biodiversity of China's coastal wetlands [3]. The river  
74 carries a large amount of organic matter into the estuary area, and after a series of  
75 physical actions and biogeochemical processes such as adsorption, sedimentation, and  
76 resuspension, the river eventually settles in the estuary and inshore waters [16]. Yangtze  
77 river wetland is muddy with heavy silt, strong interaction between land and sea, and the  
78 sedimentation rate is extremely high [10]. The vegetation types of wetlands are complex  
79 and diverse. Due to the introduction and rapid diffusion of C4 plants, the coverage rate  
80 of native C3 plants (*Phragmites australis* (PA), *Scirpus mariqueter* (SM) ) on the beach  
81 has decreased sharply [17], which makes the spatial distribution and source  
82 composition of organic carbon content in sediments particularly complicated. At the  
83 same time, under the common influence of natural changes and human activities, the  
84 sediments of the Yangtze River into the sea showed a decreasing trend, while the  
85 nutrient flux continued to rise [18]. Wetland dynamic changes and ecological service  
86 functions always are the focus of scholars. In the past years, many scholars have made  
87 a great deal of researches on the changes of erosion and deposition of wetland,

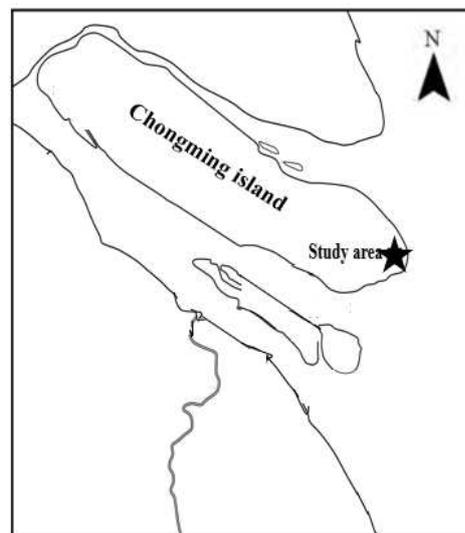
88 environmental changes, function zoning, and the impact of alien species *Spartina*  
89 *alterniflora* (SA) on the local ecosystem [19-21]. Mei et al. [22] and Yan et al. [23] have  
90 reported that the change of biomass during the growth of wetland plants will affect the  
91 spatiotemporal distribution of carbon (C) in wetland soil, and the increase of soil carbon  
92 storage has a significant linear relationship with plant biomass [23]. In addition, some  
93 studies have reported that due to tidal action, the upstream sediment will accumulate in  
94 the coastal wetland, which will also affect the C and nitrogen (N) contents of the  
95 wetland soil [24, 25]. At present, the use of tracers to explore the response of sediments  
96 in the estuary to changes in watersheds has become increasingly mature, and carbon is  
97 an important indicator of organic carbon [26, 27]. However, there is less information  
98 on the distribution characteristics and sources of organic carbon in estuarine wetland  
99 sediments using isotopic techniques. Therefore, the purpose of this study was to  
100 investigate total organic carbon (TOC), total nitrogen (TN), carbon stable isotope ( $^{13}\text{C}$ )  
101 and carbon/nitrogen ratio (C/N) in the surface sediments of estuarine wetlands, and to  
102 explore the distribution characteristics, source composition and influencing factors of  
103 organic carbon in the sediments in the study area, so as to provide references for the  
104 formulation of carbon sequestration measures in the wetland of the Yangtze river  
105 estuary.

## 106 **Materials and methods**

### 107 **Study area**

108 The study area (31°48'N ~ 31°49' N, 121°96'E ~ 121°98'E) is located Chongming  
109 Dongtan salt marsh of the Yangtze Estuary (Fig. 1). Due to at the estuary of the Yangtze

110 river, under the influence of a large amount of sediment, continuously silting, the  
111 average annual silting increase of 80 ~ 110 m [10]. The study area is one of the largest  
112 and most complete tidal flat wetlands in the estuarine area, which is subject to the  
113 deposition of land source materials carried by the runoff of the Yangtze river. The terrain  
114 is gentle, with a slope of 0.02% ~ 0.05% and a soil salinity of 0.3% ~ 0.6% [2, 3]. The  
115 climate belongs to the north sub-tropical marine climate, with the average annual  
116 temperature between 15 and 16°C, and abundant precipitation, mostly concentrated in  
117 June and October [28]. The main salt-marsh vegetation in the study area was  
118 *Phragmites australis* (PA), *Spartina alterniflora* (SA), *Scirpus mariqueter* (SM) [29].  
119 Since its large-scale introduction in 2001 and 2003, it has gradually encroached on the  
120 distribution areas of SA, PA, SM, and blackcuraria in Dongtan, Chongming, becoming  
121 one of the dominant species of salt marsh vegetation [30].



122  
123 **Fig.1.** The map of the study area.

124  
125 **Sample collection and analysis**

126 Soil sampling was conducted for different vegetation types and wetland conditions.  
127 A total of 15 sampling sites were obtained by dividing the sediment column samples.  
128 These samples cover the three typical types of vegetation, including SA, PA, and SM  
129 zones. Three 50 cm × 50 cm plant quadrats were randomly set at each sample sites, and  
130 the spacing between each quadrats was >5 m, and the living parts of plants (excluding  
131 dead plants) in the sample square were cut evenly. The plant samples (leaf, stems, and  
132 roots) were washed with tap water multiple times, and then washed with Mill-Q water  
133 3 times. The plant tissue was cut into kraft paper bags and placed in an oven, and then  
134 killed at 105 °C for 2 h, and then dried at 70 °C to a constant amount to determine the  
135 dry matter mass, and then calculate the aboveground and underground biomass of the  
136 plant per unit area. For more details of the specific methods, see “Physical and  
137 Chemical Analysis of Soils” [31]. The sediment samples were naturally air-dried and  
138 ground, and then subjected to 1.00 and 0.25 mm earth sieve. The samples were placed  
139 in polyethylene bags to preserve the sediment TOC for the determination of the samples.  
140 TOC was determined by potassium dichromate external heating method. TN was  
141 determined by kjeldahl method [31]. The C/N ratio was calculated by TOC and TN  
142 (TOC/TN ratio). <sup>13</sup>C was determined by GC-IRMS. 0.5 mol/L HCl was added to the  
143 sediment samples to remove inorganic carbonate, and the samples were washed with  
144 deionized water until the filtrate was neutral [32]. Isotope values of carbon was reported  
145 as δ<sup>13</sup>C value in ‰ relative to Vienna Pee Dee Belemnite (VPDB) according to eq. (1)  
146 [33]:

$$147 \quad \delta^{13}C = \frac{R_{sample} - R_{reference}}{R_{reference}} \times 1000 \quad (1)$$

148 where  $R_{sample}$  and  $R_{reference}$  are isotope ratios of the elements of concern in the  
149 target compound and the international reference standard (e.g., VPDB for the carbon  
150 isotope), respectively.

151

152 It has been reported that the organic carbon in salt marsh surface sediments mainly  
153 comes from plants suspended particulate matter and benthic microalgae [10, 16, 34-36].  
154 It's well known that plants can convert atmospheric CO<sub>2</sub> into organic carbon through  
155 photosynthesis and store it in sediments, which are generally considered as one of the  
156 important sources of organic carbon in sediments. Suspended particulate matter is an  
157 important source of organic carbon in salt marshes with rapid deposition. The organic  
158 primary productivity of benthic microalgae in the Yangtze Estuary accounts for about  
159 16.53% [37], and the <sup>13</sup>C field labeling method indicates that benthic microalgae is a  
160 potentially important source of organic carbon in sediments [38]. Therefore, it is  
161 assumed that the main sources of sediment in Dongtan salt marsh are salt marsh  
162 vegetation (the vegetation of the sampling site consists of SA, PA and SM) suspended  
163 sediment and benthic microalgae. Based on the <sup>13</sup>C linear mixing model, the  
164 contribution rate of organic carbon sources in sediments can be calculated by the  
165 following eq. (2-4) [39]:

$$166 \quad [TN/TOC]_{sed} = f_{plant}[TN/TOC]_{plant} + f_{SPM}[TN/TOC]_{SPM} + f_{MPB}[TN/TOC]_{MPB} \quad (2)$$

$$167 \quad \delta^{13}C_{sed} = f_{plant} \times \delta^{13}C_{plant} + f_{SPM} \times \delta^{13}C_{SPM} + f_{MPB} \times \delta^{13}C_{MPB} \quad (3)$$

$$168 \quad f_{plant} + f_{SPM} + f_{MPB} = 1 \quad (4)$$

169 Where  $f_{plant}(\%)$ ,  $f_{SPM}(\%)$  and  $f_{MPB}(\%)$  are the contribution rates of vegetation,

170 suspended particulate matter and microphytobenthos of these organic carbon sources at  
171 the sampling sites, respectively.  $\delta^{13}C_{sed}(\text{‰})$  is the  $\delta^{13}C$  value of the sediment sample.  
172  $\delta^{13}C_{plant}(\text{‰})$ ,  $\delta^{13}C_{SPM}(\text{‰})$  and  $\delta^{13}C_{MPB}(\text{‰})$  were calculated for the  $\delta^{13}C$  values of  
173 vegetation, suspended particulate matter and microphytobenthos at the sampling sites,  
174 respectively.

## 175 **Statistical analysis**

176 The mean and standard deviation values were calculated from the triplicate  
177 measurements for each sample. Linear regression analysis and Pearson correlation were  
178 used to identify relationships between TOC, TN, and plant biomass. Test results were  
179 reported with a confidence level of 95% ( $P$ -values of 0.05). All statistical analyses were  
180 performed using R statistical software.

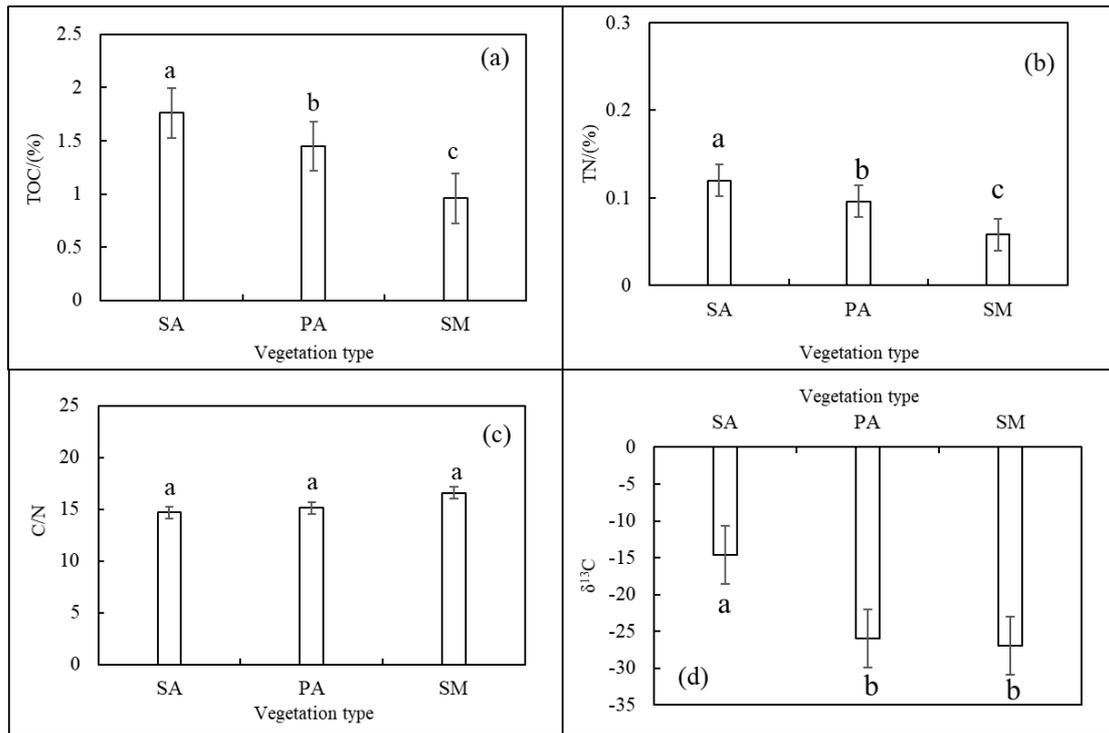
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## 182 **Results and discussion**

### 183 **Characteristics of TOC, TN, C/N and $\delta^{13}C$ in sediments**

184 The average contents of TOC and TN in the surface sediments of wetland were  
185  $1.39\% \pm 0.34\%$  and  $0.091\% \pm 0.024\%$ , respectively (Fig. 2a and b). There was a  
186 significant positive correlation between TOC and TN ( $r^2 > 0.95$ ). The C/N ratio and  
187  $\delta^{13}C$  in sediments ranged from 14.66 to 16.55 and -25.23 to -22.45‰, with an average  
188 value of 15.44 and -24.17‰, respectively (Fig. 2c and d). The contents of TOC and TN  
189 in sediments under different vegetation cover are shown in Fig. 2, and the average  
190 values of both are following in order SA > PA > SM. As shown in Fig. 2, the content of  
191 TOC in SA is the highest with a mean value of  $1.76\% \pm 0.38\%$ . The content of TOC in

192 the sediment under the SA is mediate (average value is 1.45%). The TN in sediments  
193 was similar to that of TOC (Fig. 2b). The content of TN in SA, PA, and SM is 0.120%,  
194 0.096%, and 0.058%, respectively. These results indicate that the distribution of  
195 sediment TOC and TN reserves is greatly influenced by vegetation cover type and  
196 photosynthesis [2, 43]. Plants can directly affect the carbon cycle of wetland through  
197 photosynthesis, carbon sequestration and carbon distribution [23]. The soil carbon  
198 content of SA is much higher than that of SM, which may be due to the SA is C4 plant.  
199 Compared with native C3 plants, SA is a C4 plant with a higher photosynthetic  
200 efficiency, higher net primary productivity, and aboveground and underground biomass  
201 up to 5 times and 3 times of that of hyacinth community, which is consistent with the  
202 other research results [23, 40]. In addition, the high net primary productivity of  
203 vegetation, litter yield and decomposition process are of great significance to the  
204 formation of soil carbon pool [23, 41-44]. During the plant growth, 10 to 40 percent of  
205 photosynthetic products enter soil and sediment through root secretion, while most of  
206 the rest enter the carbon sink of soil sediments in the form of litters [45, 46].  
207



208

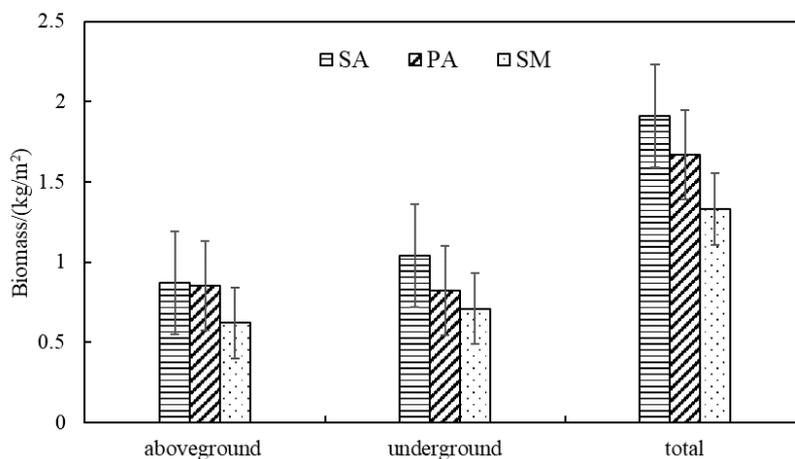
209 **Fig. 2.** Sedimentary physical properties and TOC, TN, C/N and  $\delta^{13}\text{C}$  of different vegetation zones.

210 Notes: SA presents *Spartina alterniflora*, PA presents *Phragmites australis*, SM presents *Scirpus*  
 211 *mariqueter*, the same as follows.

212 A large proportion of plant biomass (both above and below ground) inputs organic  
 213 carbon into the soil in the form of litters, and an increase in organic carbon ultimately  
 214 leads to an increase in soil carbon accumulation [47, 48], resulting in different  
 215 distribution characteristics of soil carbon stocks [23]. From Fig. 3, we found that the  
 216 vegetation biomass were also in order SA ( $1.91 \text{ kg/m}^2$ ) > PA ( $1.67 \text{ kg/m}^2$ ) > SM ( $1.33$   
 217  $\text{kg/m}^2$ ), which is similar to the other results [2]. As the pioneer species of the salt marsh  
 218 vegetation in the Yangtze estuary, SM has low productivity and only 1/4 of the biomass  
 219 per unit area of the community of SA and PA, resulting the C and N reserve are  
 220 relatively low. Furthermore, it has been reported that the invasion of the plant increased  
 221 the organic carbon content of the soil [49, 50]. These results indicated that the difference

222 of plant community productivity is the main factor influencing soil carbon storage, and  
 223 the increase of soil carbon storage is not only closely related to the physical and  
 224 chemical properties of soils, but also depends on the storage and fixation capacity of  
 225 different plant communities for carbon [23]. There was a significant linear relationship  
 226 between the sediment carbon content and the underground biomass of the three salt  
 227 marsh plant communities. With the increase of the biomass of the salt marsh plant  
 228 communities, the TOC of the sediments increased, which may be related to the plant  
 229 root growth, rhizosphere microbial activity and enzyme activity [2].

230



231

232 **Fig. 3.** The biomass of aboveground and underground biomass in different saltmarsh plant types.

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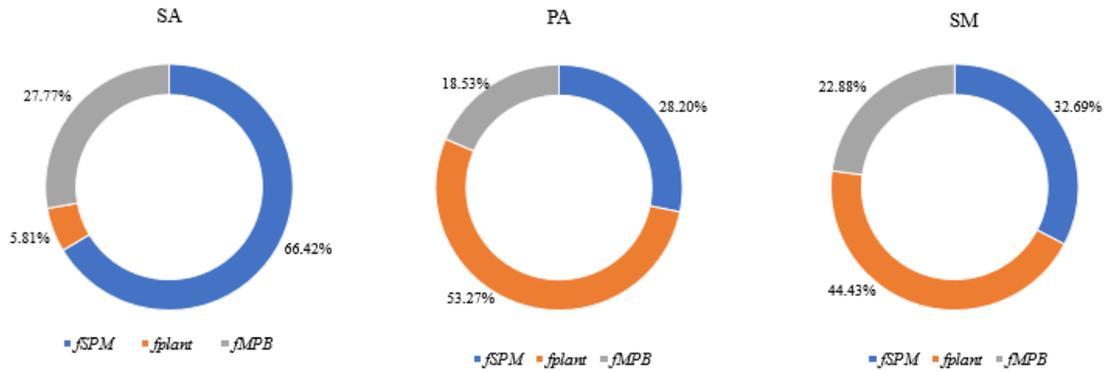
234 **Spatial distribution characteristics of organic carbon contribution rate in**  
 235 **sediments**

236 The contribution rate of organic carbon from different sources in the surface  
 237 sediments of wetland was calculated by the three end-member mixing model (Fig. 4).

238 The average contribution rate ( $f_{SPM}$ ) of all samples in the study area was  $44.44\% \pm$

239 20.89%, followed by the contribution rate of vegetation ( $f_{\text{plant}}$ ) ( $34.50\% \pm 25.23\%$ ) and  
240 contribution rate ( $f_{\text{MPB}}$ ) of benthic microalgae ( $23.06\% \pm 4.62\%$ ). The  $f_{\text{plant}}$  of organic  
241 carbon in sediments under different vegetation belts showed significant differences. As  
242 shown in Fig. 4, the organic carbon  $f_{\text{plant}}$  of the sediments in the SA was 5.81%, which  
243 was significantly lower than that in the native C3 vegetation PA (53.27%) and SM  
244 (44.43%) ( $P < 0.05$ ). The average organic carbon  $f_{\text{SPM}}$  of the sediments in the SA zone  
245 was 66.42%, which was approximately twice as high as that of PA (28.20%) and SM  
246 (32.69%) ( $P < 0.05$ ). In addition, we estimated the absolute contribution of sediment  
247 organic carbon from different vegetation types (Fig. 5), and the results showed that the  
248 absolute contribution of the vegetation to the TOC of sediments in the SA, PA, and SM  
249 zones is 0.10%, 0.77%, and 0.42%, respectively, indicating that PA belt was  
250 significantly higher than the other plant belts. However, the absolute contribution rate  
251 of suspended particulate showed opposite characteristics. The absolute contribution  
252 ( $\text{TOC} * f_{\text{SPM}}$ ) of suspended particulate matter in the SA belt to the organic carbon of  
253 sediments is the highest with a mean value of 1.16%, followed by PA belt (0.40%) and  
254 MA belt (0.31%). Generally, the absolute contribution of microphytobenthos to organic  
255 carbon were lower.

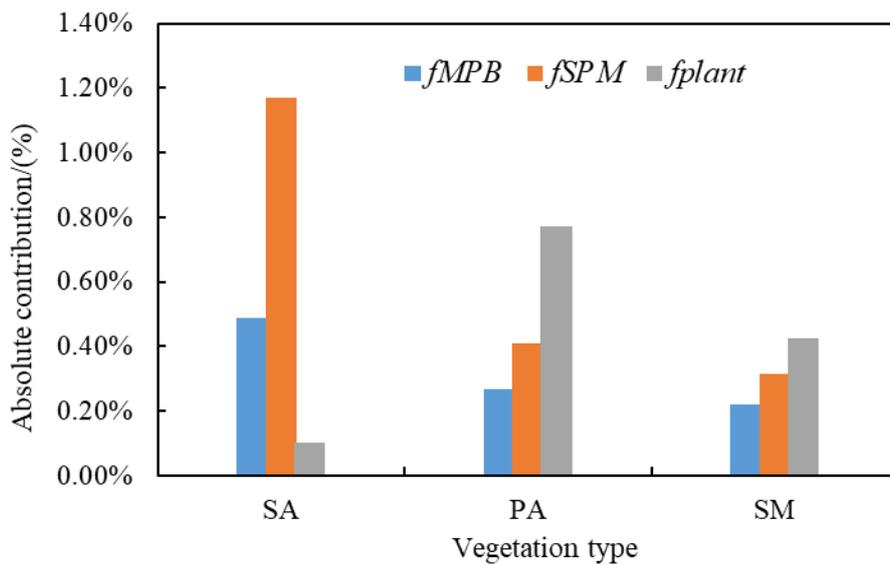
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257

258 **Fig. 4.** Contribution of organic carbon from various sources of sediments in different vegetation  
 259 belts. Notes:  $f_{plant}$ ,  $f_{MPB}$  and  $f_{SPM}$  represent the relative fraction of vegetation, suspended particulate  
 260 matter and microphytobenthos of these organic carbon sources. The same as below.

261



262

263 **Fig. 5.** Absolute contribution of organic carbon from various sources of sediments in different  
 264 vegetation belts.

265

266 **Source composition characteristics and influencing factors of organic carbon in**  
 267 **sediments**

268

Estuarine salt marshes are subject to seaborne interactions, which determines the

269 complexity of organic carbon sources in estuarine salt marshes sediments [51]. Many  
270 studies have reported the use of C/N ratio and stable isotope composition to analyze  
271 and explore the source of organic carbon in wetland sediments [52-54]. This study  
272 comprehensively analyzed the source of organic carbon in surface sediments based on  
273 the elemental and stable isotope composition characteristics of sediment samples,  
274 suspended particulate matter, vegetation and benthic microalgae in the study area. In  
275 general, the C/N of marine organic carbon is 6 ~ 9, and that of terrestrial organic carbon  
276 is  $C/N > 12$  [55, 56]. In this study, the C/N ratios were all greater than 9, indicating that  
277 the organic carbon in the sediments might be the result of mixed input of terrestrial  
278 organic carbon and Marine organic carbon.  $\delta^{13}\text{C}$  tracer results showed that the organic  
279 carbon in wetland surface sediments was mainly from suspended particulate matter and  
280 salt marsh plants, with the combined average contribution rate of TOC to sediments  
281 reaching 76.94%, indicating that suspended particles and native plants were the main  
282 sources of organic carbon in sediments, which is consistent with the other researches  
283 [10, 51].

284       Vegetation is an important biological factor for ecological succession and another  
285 important factor for controlling the composition of the salt marsh sediment source. Due  
286 to the differences in biological characteristics, the amount of organic matter input to the  
287 sediment by different vegetation varies greatly [2, 10, 57]. The differences in plant  
288 material components such as lignin content will affect the rate of plant litter degradation  
289 to other environmental media [58], and these factors interact with the time of plant  
290 community formation, and together affect the plant's contribution to sediment organic

291 carbon [10]. The process of vegetation and deposition in coastal estuarine wetlands is  
292 complex [59]. The deposition process is usually accompanied by the burial of litters,  
293 thus promoting the accumulation of carbon in the soil and sediments [24]. The dominant  
294 vegetation in the study area was mainly composed of native C3 plants (PA and SM) and  
295 exotic C4 plants (SA). It has been shown that the invasion of SA can increase the TOC  
296 content of sediments (especially the active organic carbon component) in a short period  
297 of time [60]. However, the content of N in litters was higher, the content of cellulose  
298 and lignin was lower, and its decomposability was higher than that of native plants PA  
299 and SM [2]. In addition, in terms of the grain size of sediments, the higher the elevation  
300 of the beach, the finer the matter, and vice versa [22]. Therefore, the changes of the  
301 corresponding abiotic components (such as elevation, sediment size, nutrient and  
302 salinity, etc.) and environmental factors (Season, temperature, and microbial diversity  
303 etc.) also play an important role in wetland soil carbon storage, which needs further  
304 study.

#### 305 **4. Conclusions**

306 This study investigated the distribution characteristics of carbon content in  
307 sediments in different vegetation zones of typical wetlands in the Yangtze Estuary and  
308 the sources of organic carbon. The mean value of TOC and TN concentrations in the  
309 surface sediments of wetland were  $1.39 \pm 0.34\%$  and  $0.091 \pm 0.024\%$ , respectively. The  
310 TOC of the sediment in the three saltmarsh plant communities was in the order of SA  
311 ( $1.76 \pm 0.38\%$ ) > PA ( $1.45 \pm 0.37\%$ ) > SM ( $0.96 \pm 0.44\%$ ). The mean sediment  $\delta^{13}\text{C}$   
312 value was  $-24.17 \pm 1.51\%$ , and all the C/N ratios were both greater than 9. The organic

313 carbon in sediments of wetland in Yangtze Estuary are the result of mixed input of  
314 terrestrial organic carbon and marine organic carbon. Organic carbon in sediments was  
315 mainly derived from suspended particles and vegetations. Vegetation plays an important  
316 role in the carbon storage of wetland sediments. The primary productivity of salt marsh  
317 vegetation is the main factor influencing the spatial distribution of soil carbon storage  
318 in estuarine wetland. Significant differences in TOC and TN in sediments under  
319 different vegetation, were represented as SA > PA > SM. The source of organic carbon  
320 in wetland sediments was qualitatively analyzed by C/N ratio and three-terminal model.  
321 The values of C/N were beyond 9, indicating that the organic carbon of sediments may  
322 be mixed input of terrestrial and marine organic carbon. The three end-member mixing  
323 equations showed that the organic carbon in the sediments in the study area mainly  
324 came from the suspended particles brought by rivers and the terrestrial vegetation cover  
325 in salt marshes, while the contribution of benthic microalgae was small. This study  
326 provides a preliminary knowledge of the distribution and source of organic carbon in  
327 wetland sediments, providing theoretical support for a better understanding of the  
328 circulation of carbon in sediments environment.

329

### 330 **Abbreviations**

331 TOC: total organic carbon; TN: total nitrogen;  $\delta^{13}\text{C}$ : carbon stable isotope; C/N: the  
332 ratio of carbon/nitrogen; TN/TOC: the ratio of total nitrogen/total organic carbon;  
333 TOC/TN: the ratio of total organic carbon/total nitrogen; N: nitrogen; C: carbon; SA:  
334 *Spartina alterniflora*; PA: *Phragmites australis*; SM: *Scirpus mariqueter*; MPB:

335 microphytobenthos; SPM: Suspended particulate matter.

### 336 **Ethics approval and consent to participate**

337 Not applicable.

### 338 **Consent for publication**

339 Not applicable.

### 340 **Availability of data and materials**

341 The data sets supporting the conclusions of this article are included within the article.

### 342 **Competing interests**

343 The authors declare that they have no competing interests.

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### 348 **Authors' contributions**

349 Haiyan Yu, Weiwei Li, Changxu Han, Han Fang, Xingquan Shu, and Yongfeng Liu  
350 performed the experiments. Haiyan Yu wrote the manuscript. Limin Ma and Yuwei Pan  
351 contributed to the manuscript correction. All authors read and approved the final  
352 manuscript.

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