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DISTRIBUTION AND STRUCTURE CHARACTERISTICS OF DISSOLVED ORGANIC MATTER IN SPARTINA ALTERNIFLORA WETLAND SOILS

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Abstract: The invasion of *Spartina alterniflora* has damaged coastal ecosystems in China. Current research focuses primarily on the total organic carbon stock and distribution in *Spartina alterniflora* wetlands, lacking a systematic exploration of the composition and characteristics of dissolved organic matter (DOM) in the soil. Therefore, *Spartina alterniflora* wetlands along China's coast were set as our research area. We determined the content and composition of the DOM in soils using UV-Vis absorption spectroscopy and three-dimensional fluorescence spectroscopy. The results show significant spatial differentiation in DOC content in *Spartina alterniflora* wetland soils across the study area. A positive correlation exists between CDOM and DOC content, indicating a synergistic effect between the formation of colored dissolved components and the release of organic carbon. DOM in *Spartina alterniflora* wetland soils is mainly composed of humic substances and protein-like substances, exhibiting high biological activity and a significant terrestrial-dominated characteristic. These findings provide a new perspective for assessing the carbon cycle effects of *Spartina alterniflora* invasion and offer a scientific basis for the ecological restoration and carbon sequestration management of coastal wetlands.
Keywords: Dissolved organic carbon; Wetland; Composition

1 INTRODUCTION

Estuarine wetland is a key carbon sink area, and the *Spartina alterniflora* of invasive plants has a profound impact on soil carbon sequestration in wetland ecosystems [1]. The spread area of *Spartina alterniflora* in China has reached 519.70 km^2 by 2020 [2]. Its invasion not only damages native biodiversity but also indirectly affects the overall carbon sequestration capacity by changing the soil carbon balance [3]. Different plant species affect soil organic carbon (SOC) sequestration mainly by regulating root turnover rate and changing root exudate characteristics [4]. The *Spartina alterniflora* significantly improves SOC sequestration efficiency with higher root biomass and faster root turnover [5]. It releases significantly higher amounts of root exudates into the soil environment, thereby effectively increasing the active organic carbon content [6]. Due to its important role in responding to climate change, the mechanism of carbon sequestration in *Spartina alterniflora* wetlands should be analyzed in depth. It is of great scientific significance to accurately assess the carbon sink function of coastal wetlands and to develop management strategies for invasive plants.

Although a large number of studies have shown that *Spartina alterniflora* has a significant impact on the dynamics of organic carbon in coastal wetlands, their impact on the mobile carbon pool of dissolved organic matter (DOM) has not been systematically explored. DOM is widely found in soils, sediments, and natural waters [7], and its biomineralization process synergizes with anthropogenic carbon emissions, resulting in a continuous increase in atmospheric carbon dioxide concentrations [8]. As the most active component of the soil organic carbon pool [9], the dynamic changes of DOM directly reflect the carbon turnover efficiency of ecosystems [10]. DOM plays a key role in natural ecosystems due to its involvement in diverse environmental processes. As a strong metal chelating agent, DOM significantly affects the solubility, migration path, and biological toxicity of heavy metals [11]. At the same time, DOM plays an important role in the transport of organic pollutants [7], photochemical reactions in water [12], nutrient cycling, and bioavailability [13]. UV-Vis spectroscopy and three-dimensional fluorescence spectroscopy have the advantage of being low-cost, informative, and not damaging to the structure of natural organic substances [14]. It has been successfully applied to source identification and macroscopic chemical property characterization of DOM in lakes [15], oceans and reservoirs [16-17]. These advanced techniques have also been used to characterize the composition and structure of DOM in forest soils [18], croplands [19], and sediments [20]. Therefore, the combined application of multiple spectral techniques helps reveal the structural characteristics of soil DOM in *Spartina alterniflora* wetlands. DOM is one of the basic energy sources of ecosystems [21]. However, the spatial distribution and structural characteristics of DOM in *Spartina alterniflora* wetlands are still not fully understood.

In this study, the typical *Spartina alterniflora* invading coastal wetlands in China was selected as the target area, and the theoretical framework and technical methods of ecology and geochemistry were comprehensively applied. UV-Vis spectroscopy and three-dimensional fluorescence spectroscopy were integrated to systematically analyze the content distribution and composition of soil DOM. The results of this study will deepen the understanding of the impact mechanism of *Spartina alterniflora* invasion on the carbon cycle process in coastal wetlands. It provides a key scientific basis for building a more accurate assessment model of carbon storage in coastal wetlands, formulating invasive plant management strategies based on DOM Dynamics, and improving wetland carbon sink capacity.

2 MATERIALS AND METHODS

2.1 Researching Area

The *Spartina alterniflora* wetlands in China are mainly distributed naturally in the coastal areas of Tianjin, Shandong, Jiangsu, Shanghai, Zhejiang, Fujian, and Guangdong provinces[22]. Typical *Spartina alterniflora* wetlands in China were selected as the study areas (Figure 1), including Tanggu Coastal Wetland (TG) in Tianjin, Kendong Wetland in Dongying city (DY) of Jiangsu province, Fangtang Estuary Wetland in Yancheng city (YC) of Jiangsu province, Chongming Dongtan Wetland (CM) in Shanghai, Xuanmen Bay National Wetland in Yueqing city (YQ) of Zhejiang province, Beishan Village Mangrove Wetland in Luoyuan Bay (LY) of Fujian province, Zhangjiang Estuary Wetland in Yunxiao city (YX) of Guangdong province, Qi'ao Mangrove Wetland in Zhuhai city (ZH) of Guangdong province, and Leizhou Bay Mangrove Wetland (LZ) in Zhanjiang of Guangdong province. Surface sediment (0-5 cm) samples were collected using a stainless steel sampler and sampled in 3 independent replicates by the diagonal point method. Samples (n=27) were transferred to self-sealing bags immediately after collection, transported back to the laboratory within 48 hours, and stored in a refrigerator at -18 °C. Samples were freeze-dried, removed of plant residues and gravel, ground through a 0.149 mm sieve (100 mesh), and stored in a dryer for testing.

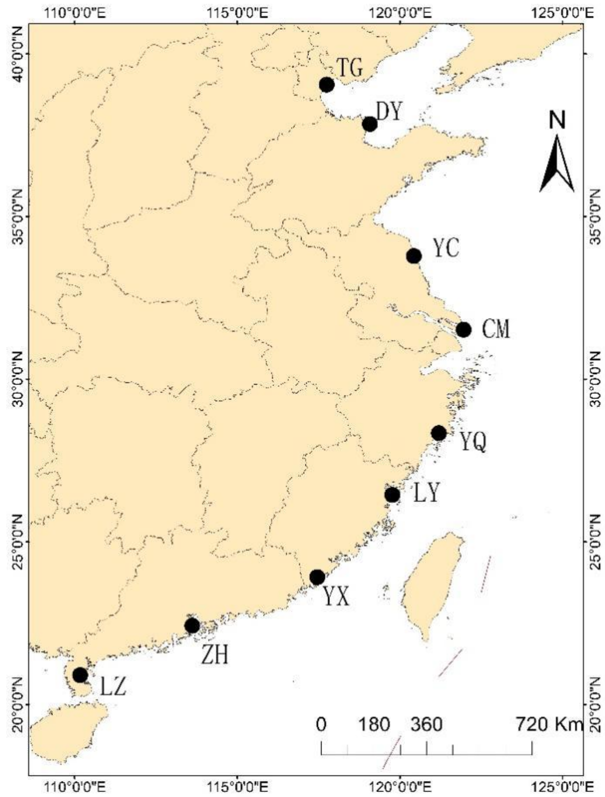

Figure 1 Distribution Map of the Study Area

Note: TG = Tanggu; DY = Dongying; YC = Yancheng; CM = Chongming; YQ = Yueqing; LY = Luoyuan; YX = Yunxiao; ZH = Zhuhai; LZ = Leizhou. The sampling map was compiled with ArcGIS 20.0, relying on public cartographic data issued by the Ministry of Natural Resources of China.{https://cloudcenter.tianditu.gov.cn/ administrativeDivision}

2.2 DOM Samples Preparation

Accurately weigh 0.5 g of soil sample dried by a 0.149 mm nylon sieve, place it in a 50 mL polypropylene centrifuge tube, and add 20 mL ultrapure water (resistivity ≥18.2 MΩ·cm). The samples were transferred to a variable-frequency shaker for constant-temperature shaking at 25 ± 0.5 °C, 150 r/min for 24 h with full light avoidance operation to inhibit photochemical degradation. The samples were centrifuged for 15 min at 25 °C at 8000 r/min. The supernatant was sequentially filtered through a 0.7 μm GF/F glass fiber membrane (Whatman, UK) and a 0.22 μm PES needle filter (Millipore, USA) to effectively remove microbial and suspended matter interference. After filtration, the samples were transferred to amber glass bottles, kept at 4 °C in the dark, and analyzed within 48 h. The concentration of dissolved organic carbon acid (DOC) in the sample solution was determined by a total organic carbon analyzer (TOC-LCPH, Shimadzu, Japan) after adjusting pH < 2 [23].

2.3 Determination of DOM composition

UV-Visible photometer (Xipu, Shanghai) was used for full-wavelength scanning (1 nm resolution) at 200-800 nm to obtain the UV-Vis absorption spectrum. Excitation-emission matrix spectra (EEMs) were acquired using a three-dimensional fluorescence spectrometer (Tianmei, Shanghai), with parameters set to excitation wavelength (Ex) from 250 to 500 nm (5 nm interval), emission wavelength (Em) from 280 to 600 nm (2 nm interval), scan speed 1200 nm/min, response time 0.5 s. The fluorescence data were deducted by Milli-Q ultrapure water blank, normalized by the water Raman peak excited at 350 nm, and combined with the UV absorption data to correct the internal filtering effect. Raman and Rayleigh package of R language version 4.1.1 are used to remove scattering interference. The fluorescent dissolved organic matter (FDOM) composition was resolved by parallel factor analysis (PARAFAC) of the DOMFluor toolbox on the MATLAB 2018b platform [24], and the relative content of each component was quantified as maximum fluorescence intensity (Fmax) expression.

Combining UV-Vis absorption and three-dimensional fluorescence spectral data, DOM properties were analyzed and evaluated. The absorption coefficient a(λ) represents chromophoric dissolved organic matter (CDOM), its concentration is characterized by the absorption coefficient a_{355} [25]; Specific ultraviolet absorbance (SUVA) is appiled with $SUVA_{254}$(ratio of 254 nm absorption coefficient to DOC concentration), reflecting DOM aromaticity [26]; The spectral slope $S_{275-295}$ indicates the DOM molecular mass character [27]. Fluorescence index (FI) is determined by FI $\leq$1.4 and FI $\geq$1.9 corresponding to terrestrial and endogenous DOM, respectively [28]; The Humification index (HIX) is calculated to characterize the degree of DOM Humification [29], and the Biological Index (BIX) is calculated to reflect the contribution of microbial source DOM [30].

2.4 Statistics and Analysis of Data

One-factor variance analysis (ANOVA) was used to test differences in DOM characteristic indicators in wetland soils. Variance analysis and Tukey post-test were used for data that met variance uniformity. Welch variance analysis and Dunnett T3 test were used for data that did not meet variance uniformity, and Spearman correlation was used to analyze correlations between DOC content, CDOM content, and spectral indicators. Primary component analysis (PCA) was used to explore the overall characteristics of the DOM database. SPSS 22.0 and R 4.1.1 software were used for data analysis.

3 RESULTS

3.1 DOM Contents

DOC concentrations averaged to 6.13±1.50 g/kg in the *Spartina alterniflora* wetland soils (Figure 2a). The DOC content in ZH wetland soils (12.96±2.98 g/kg) was significantly higher than that in other wetland soils, followed by YC and YQ, while the DOC content in the DY wetland soil (1.64±0.27 g/kg) was the lowest. The DOC/SOC ratio ranged from 38.93±26.66% to an average of 41.85%, showing high volatility overall. The DOC/SOC ratio in the YX (65.60±1.90%) wetland soil was abnormally high. The DOC/SOC ratios in the LZ (55.71±4.22%), YQ (54.80±0.80%), ZH (54.36±6.39%) wetlands were relatively high. The DOC/SOC ratio the DY wetlands (12.27±1.57%) and YC wetlands (16.83±8.03%) were relatively low. The average CDOM content in the *Spartina alterniflora* wetland soil was 19.24 m^{-1}(Figure 2c), with the highest CDOM concentration in LY wetland soils (60.68±8.64 m^{-1}), indicating its strongest absorption capacity. The CDOM concentrations in other wetland soils were generally low. There was a positive correlation between CDOM and DOC content (ρ= 0.039, $p < 0.05$; Figure 2d).

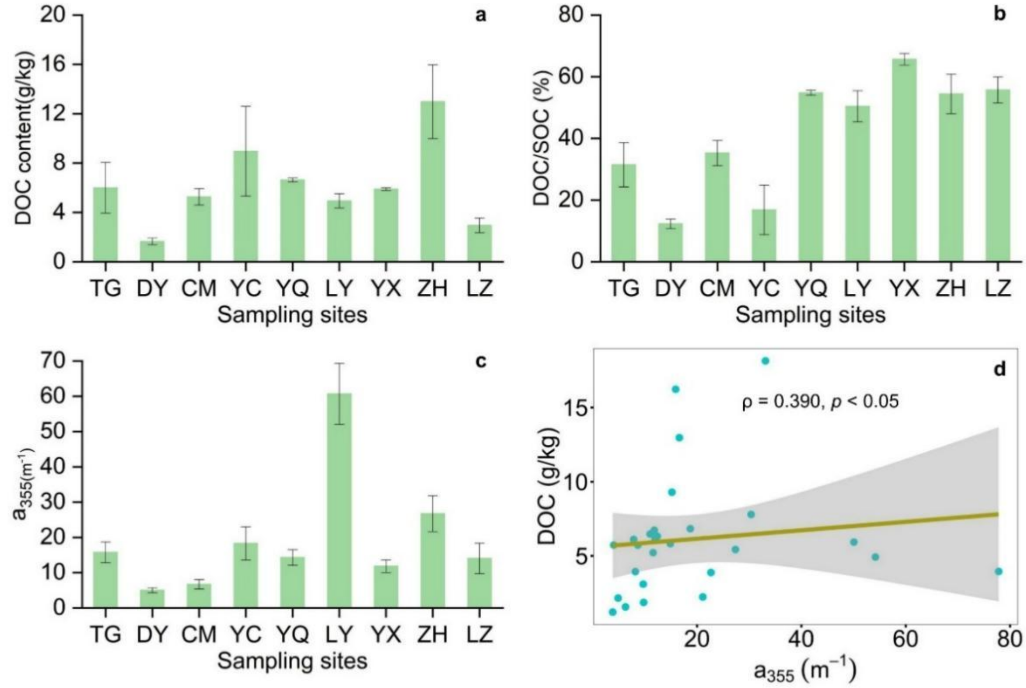

Figure 2 Quantitative Characteristics of DOM in *Spartina Alterniflora* Wetlands

Note: (a) DOC contents; (b) DOC/SOC; (c) CDOM concentration; (d) Correlation between CDOM and DOC; 95% grey confidence band; ρ= Spearman correlation coefficient; p = significance level.

3.2 DOM Compositions

According to EEMs analysis, DOM in *Spartina alterniflora* wetland soils was mainly composed of humic-like substances and protein-like substances (Figure 3–4, Table 1), UVA humic substances (C1), UVC humic substances (C2), UVC humic substances (C3), tryptophan-like substances (C4). The UVA humic-like component (C1) has a maximum excitation wavelength (Ex) of 296(275) nm and a maximum emission wavelength (Em) of 409 nm. The UVC humic-like component (C2) has a maximum excitation wavelength (Ex) of 251(320) nm and a maximum emission wavelength (Em) of 425(561) nm. The UVC humic-like component (C3) has a maximum excitation wavelength (Ex) of 356 nm and a maximum emission wavelength (Em) of 469 nm. Tryptophan-like (C4) has an excitation (Ex) maximum of 272 nm and an emission (Em) maximum of 331(473) nm. The proportions of each component were 29.12 ± 2.56%, 40.74 ± 2.44%, 12.10 ± 3.48%, and 15.94 ± 6.06% of the total, respectively (Figure 5a) . These components are characterized by terrigenous, marine or anthropogenic sources.

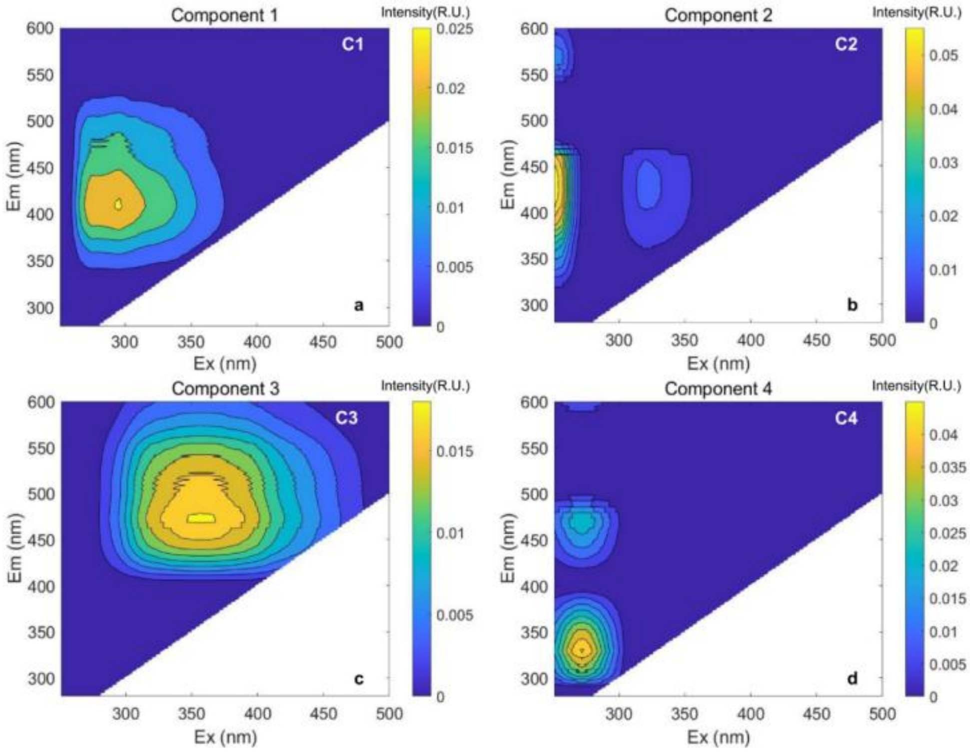

Figure 3 Fluorescence Spectra of Soil DOM Components in *Spartina Alterniflora* Wetlands
Note: Component 1(C1); Component 2(C2); Component 3(C3); Component 4(C4).

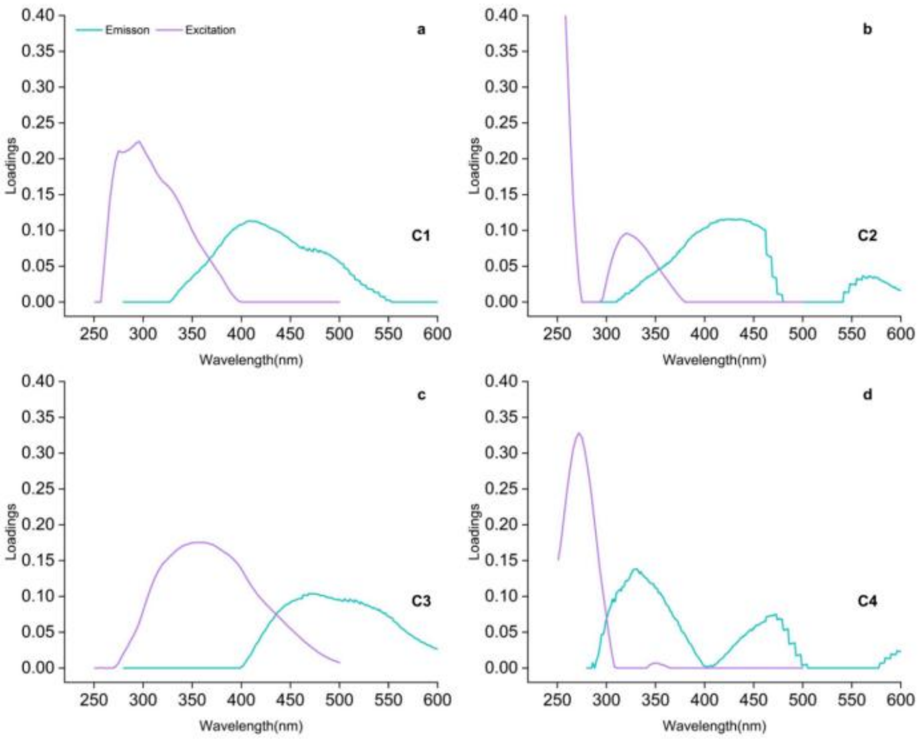

Figure 4 DOM Component Loading in *Spartina alterniflora* Wetlands
Note: Component 1(C1); Component 2(C2); Component 3(C3); Component 4(C4)

Table 1 Identification of Excitation-Emission Matrix Peaks

This study					Source	Previous study			
Components	Peak	Label	Ex (nm)	Em (nm)		Peak	Ex (nm)	Em (nm)	References

UVA humic substances	M	C1	296 (275)	409	Allochthonous or anthropogenic source	A, C	<250–280 (325–360)	370–480	[31]
						M	290–325(<250)	370–430	[32]
UVC humic substances	A	C2	251 (320)	425 (561)	Allochthonous source	/	250 (350)	450	[33]
						A	260	380–460	[34]
UVC humic substances	C	C3	356	469	Allochthonous source	C	320–360	420–460	[35]
						C	350	420–480	[34]
Tryptophan-like substances	T	C4	272	331 (473)	Marine source	T	270–280 (<240)	330–368	[36]
						T	275	340	[34]

Note: The emission wavelengths in parentheses are secondary peaks. "/" indicates none.

3.3 Fluorescence Parameters of DOM

UVC humic substances (C2) accounted for the highest proportion of the total DOM fraction, followed by UVA humic substances (C1) (Figure 5a). UVC humic-like components (C3) and tryptophan-like components (C4) accounted for a low proportion of the total components. DOM with a high UV absorbance $SUVA_{254}$ contains more aromaticity. As shown in Figure 5b, the aromaticity of DOM was shown at 2.67 ± 1.9 L mg C^{-1} m^{-1}, and the aromaticity of DOM in the LY wetland soils was the highest at 4.57± 1.10 L mg C^{-1} m^{-1}. The results indicated that the DOM aromatic compounds and unsaturation were higher in LY wetland soils. In Figure 5c, the molecular weights of DOM ranged from 6.58×10^{-3}–23.38×10^{-3} nm^{-1}. The spectral slope $S_{275-295}$ was lower, which indicated that the molecular weight of DOM was lower and the humification degree was lower. As a key parameter in DOM fluorescence spectroscopy analysis, FI values were used to characterize the source of DOM. 1.4≤ FI values ≤1.7 represent both terrestrial and endogenous DOM signatures [37]. In this study, the FI value was 1.63±0.17 (Figure 5d), indicating that the DOM in the *Spartina alterniflora* wetland soils exhibited both terrestrial and endogenous characteristics. The HIX value indicates the degree of humification of the DOM [29]. The degree of humification of DOM is 1.81±0.67, as shown in Figure 5e. BIX values represent microbial source for DOM freshness and biological activity [38]. The BIX value is lower than 0.7, which means that the structure of microbial DOM is complex, within difficult microbial degradation and lower biological activity. The BIX value was 0.73±0.07, indicating that the *Spartina alterniflora* wetland soil had a high content of endogenous DOM and high biological activity (Figure 5f).

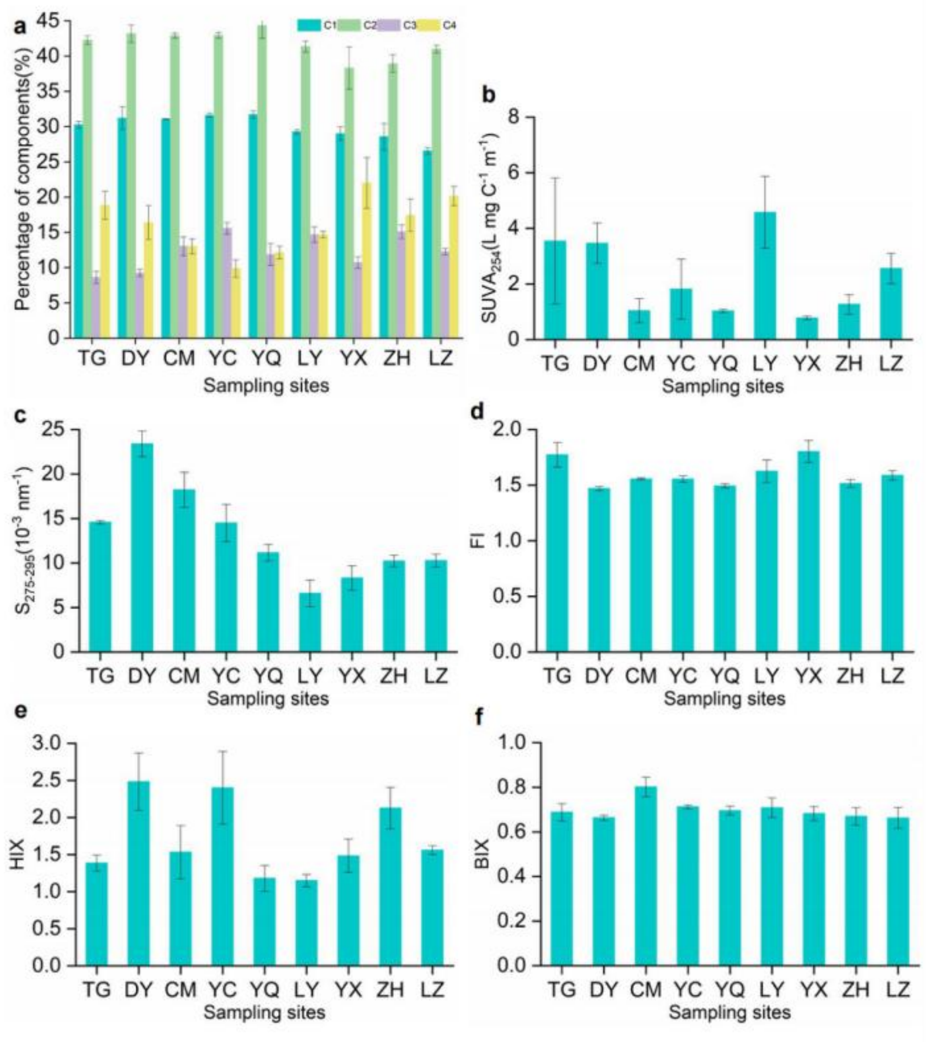

Figure 5 DOM Composition in Spartina Alterniflora Wetlands

Note: (a) FDOM content.; (b) $SUVA_{254}$; (c)$S_{275–295}$; (d) FI; (e) HIX; (f) BIX

3.4 Characteristics of the DOM Pool

To further analyze the overall characteristics of the *Spartina alterniflora* wetland DOM pool, principal component analysis (PCA) was conducted (Figure 6). The results showed that the characteristics of the wetland DOM pool corresponded to its geographical distribution area. The DOM pool could be divided into three groups according to its coastal distribution area, i.e., Group 1–Northern region (TG, DY), Group 2–Central region (CM, YC, YQ), and Group 3–Southern region (LY, YX, ZH, LZ). Based on the variance interpretation rate (PC1 = 33.3%, PC2 = 23.8%), the first principal component (PC1) was mainly driven by UVA-like humic substances (C1) and UV absorption parameters (a_{355}). PC1 was mainly driven by UVA-like humic substances (C1) and UV absorption parameters (a_{355}). It is suggested that DOM fractions with high humification degree and strong UV absorption characteristics are clustered in the positive direction, which may be closely related to terrestrial inputs such as organic matter carried by soil erosion. The second principal component (PC2) was dominated by UVC humic substances (C3) and molecular weight parameters ($S_{275-295}$). DOM samples with negative direction distribution showed high molecular weight DOM characteristics, suggesting that they may undergo microbial degradation or chemical polymerization process.

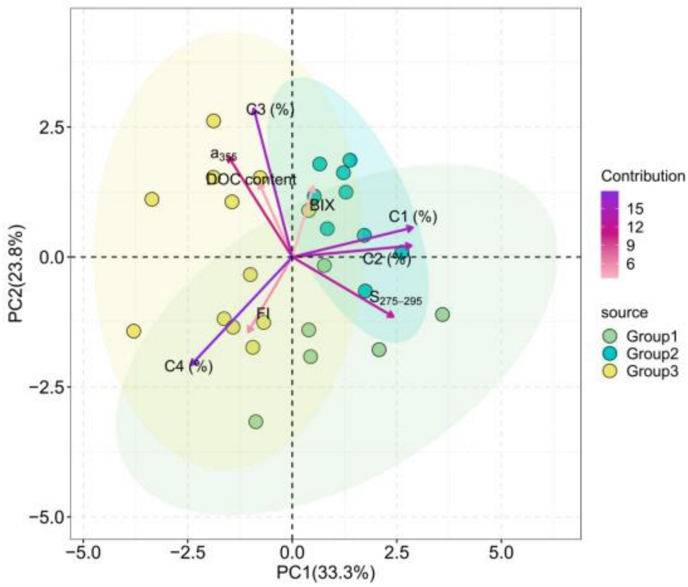

Figure 6 Results of Principal Component Analysis (PCA) based on DOM Features.
Note: Colored dots are DOM samples; Groups 1-3 are the northern, central, and southern segments of the study area; Variables significantly associated with DOM are shown ($p < 0.05$). TG = Tanggu; DY = Dongying; YC = Yancheng; CM = Chongming; YQ = Yueqing; LY = Luoyuan; YX = Yunxiao; ZH = Zhuhai; LZ = Leizhou

4 DISCUSSION

4.1 Mobile Carbon Sinks in Wetlands

The DOC and DOC/SOC contents at various stations in the *Spartina alterniflora* wetlands showed significant spatial differentiation (Figure 2). In the high DOC area, the DOC content in YC wetlands was 8.96±3.63 mg/L, which was affected by agricultural/estuarine input; the DOC content in ZH wetlands was 12.96±2.98 mg/L, which may be driven by terrestrial input [39]. In the low DOC area, the DOC content of the soil in DY wetlands was 1.64±0.27 mg/L, which showed typical low DOC characteristics of estuaries [40]. The DOC/SOC patterns showed that YX (65.60±1.90%), ZH (54.36±6.39%), and LZ (55.71±4.22%) wetlands showed high levels of active organic carbon. The soil DOC/SOC ratios in the Pearl River Delta region (YQ, YX and LZ) were also generally higher than 50%. Such high DOC/SOC ratios may be related to their reliance on the reducing environment of tidal flats to promote complexation and fixation. The high proportion of DOC also reflects the active decomposition of soil organic matter and the efficient utilization of carbon sources by microbial communities, which may be related to the high temperature and humidity climate or frequent tidal disturbances [41]. The DOC content in the TG and DY wetlands showed a moderate level (1.64-6.00 mg/L), where DOC/SOC (12.28–31.45%) may reflect the dilution effect of brackish water mixing on organic matter [42], as well as the dynamic balance between land-based input and marine degradation. In addition, the synergistic moderate level of DOC/SOC (35.28±4.05%) and DOC content (5.26±0.66 mg/L) in CM wetland soils reflects the filter-storage function of wetland for organic carbon, but the risk of carbon loss caused by reclamation activities should be noted. The well-developed root system of *Spartina alterniflora* directly increases the bioavailability of soil DOC by secreting organic substances such as sugars and amino acids [43]. The DOC may flow through the following pathways, a part of the short-term carbon source can be rapidly decomposed by microorganisms into CO_2 [44]; another part acts as a long-term carbon sink, and the carbon source can be decomposed by microorganisms into CO_2, some DOC combines with iron oxides or clays to form stable organic carbon [45]. DOC in *Spartina alterniflora* wetlands have a source-sink conversion threshold, and their dynamics may also be tidal-regulated. DOC can be rapidly discharged from the system through tidal transport, reducing carbon sink efficiency. DOC in wetland soils also promote the stabilization of mobile carbon pools due to their prolonged residence time. Therefore, DOC in *Spartina alterniflora* wetlands are both a transfer station for carbon sinks and a risk source for carbon loss.

4.2 Implications of the DOM Pool for the Coastal Wetlands

Through integrated data analysis, the characteristics of the DOM pool in *Spartina alterniflora* wetlands were revealed (Figure 6). The results are closely related to regional geographical features and the intensity of human activities. The DOM pool in *Spartina alterniflora* wetlands exhibits a spatial differentiation mechanism. The characteristics of the DOM pool can be divided into three categories according to the coastal distribution area: the northern region (TG, DY), the central region (CM, YC, YQ), and the southern region (LY, YX, ZH, LZ).

In the northern region, the DOM pool in wetlands is dominated by terrestrial sources. PC1 shows a high value cluster, that is, UVA humus is significantly positively correlated with CDOM (a_{355}), indicating that DOM is mainly terrestrial input, possibly affected by coastal agricultural non-point sources (such as farmland runoff) and industrial emissions, with the higher SOC content [45]. The offshore sediments of TG wetlands and the DY oil field activity may contribute to UVC humic substances, but the weak negative directional projection of PC2 indicates limited anthropogenic interference [46]. The section from TG district in TJ to DY wetlands in Jiangsu province is located on the coast of the Yellow Sea. Affected by the runoff of the Yellow River and the Yangtze River, the input of organic matter in the sediments is dominated by terrigenous debris [47].

In the middle region, the DOM pool shows a transitional characteristics. PC1 shows a median value, while PC2 shows a negative extension, exhibiting characteristics of both terrestrial and endogenous DOM. The CM wetlands may produce high molecular weight DOM through microbial degradation [48]. Humic-like substances are released from YC wetlands, where tidal flat sediments and freshwater input from the YQ estuary form a mixed state [49]. The CM wetlands in Shanghai and YQ wetlands in Zhejiang are located between the Yangtze River estuary and the Oujiang River estuary. They are affected by both runoff and tidal currents, resulting in high organic carbon content in the sediments [50]. This hydrological connectivity affects the molecular weight distribution balance of DOM. The plants of *Spartina alterniflora* in this area still exhibits fragmented distribution. Soil DOM in the middle estuary area shows bidirectional land-sea input characteristics, and wetland protection and management should be strengthened to regulate DOM migration.

In the southern region, PC2 dominated the endogenous characteristics, UVC humic substances coupled with high values of BIX, indicating the absolute dominance of microbial-derived DOM [51]. Warm climates promote microbial decomposition to produce soluble organic matter such as tryptophan-like [52], consistent with tropical-subtropical estuarine characteristics. This is related to differences in regional DOC dynamics in the southern region[53]. DOC concentration in the offshore area of ZH wetlands may fluctuate seasonally under the influence of saltwater-freshwater mixing [54], while the ecosystem in Leizhou Bay, a closed bay, maintains high DOC stability through litter input[55]. The plant of *Spartina alterniflora* show that it replaces mangroves through strong competitiveness, with wide range of salinity adaptation and high reproductive efficiency [56]. Its invasion can significantly weaken the blue carbon function of mangroves by reconstructing DOM sources, changing microbial metabolism pathways, and hydrological connectivity. Rapid *Spartina alterniflora* expansion could reduce the carbon sink capacity of native wetlands. It is urgent to pay attention to ecological restoration of mangroves and maintain blue carbon sink function of mangroves.

5 CONCLUSION

This study investigated the distribution and structural characteristics of DOM from *Spartina alterniflora* wetland soils along the Chinese coast. The DOC content in *Spartina alterniflora* wetlands soils showed significant spatial differentiation. The high temperature and humidity of tropical regions facilitated efficient utilization of carbon sources by microbial communities, accelerating the decomposition of organic matter and resulting in a higher proportion of active organic carbon. A positive correlation was found between CDOM and DOC content, indicating a synergistic effect between the generation of colored dissolved components and the release of organic carbon. DOM in *Spartina alterniflora* wetlands soil was mainly composed of humic substances and protein-like substances with high biological activity, exhibiting a significant terrestrial-dominated characteristic. Based on the spectral characteristics of DOM, the study reflects the multidimensional regulatory effect of the dynamic changes in the soil DOM pool of coastal wetlands on ecosystem functions. Restoration plans should be designed based on DOM characteristics, ultimately combining physical intervention and ecological substitution to rebuild a highly humified and highly stable DOM pool, providing scientific support for carbon neutrality in coastal wetlands.

COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

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AUTHOR CONTRIBUTIONS

Ruping Wu, KaiQi Li, Yuxin Zheng conducted Sample collection and field investigation; KaiQi Li, Yuxin Zheng performed laboratory analysis and data processing. Shengjie Wu led the writing of the original draft. All authors contributed critically to the drafts and gave final approval for publication.

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