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EDITED BY
Zhanfei Liu,
University of Texas at Austin,
United States

REVIEWED BY
Shuting Liu,
Kean University, United States
Aliya Naz,
O. P. Jindal Global University, India
Hualong Hong,
Xiamen University, China

*CORRESPONDENCE
You-Shao Wang
yswang@scsio.ac.cn
Hao Cheng
chenghao@scsio.ac.cn

†These authors have contributed
equally to this work

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Dynamics of extracellular polymeric substances and soil organic carbon with mangrove zonation along a continuous tidal gradient

Dong-Xi Liu^{1,2,3,4,5†}, Zhi-Mao Mai^{1,2,3†}, Cui-Ci Sun^{1,2,3,4†},
Yan-Wu Zhou^{1,2,3}, Hui-Huang Liao^{1,2,3,4,5},
You-Shao Wang^{1,2,3,4*} and Hao Cheng^{1,2,3*}

¹State Key Laboratory of Tropical Oceanography, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou, China, ²Key Laboratory of Tropical Marine Bioresources and Ecology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou, China, ³Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou), Guangzhou, China, ⁴Daya Bay Marine Biology Research Station, Chinese Academy of Sciences, Shenzhen, China, ⁵School of Earth and Planetary, University of Chinese Academy of Sciences, Beijing, China

The importance of mangroves in blue carbon storage has been widely reported. However, the potential contribution of microbial extracellular polymeric substances (EPS) to the carbon pool is still poorly understood in mangrove ecosystems. Thus, a natural mangrove reserve located in Gaoqiao was chosen to investigate the linkage between microbial EPS and sediment organic carbon. Sequential resin extraction, Fourier transform infrared spectroscopy (FTIR), and 16S high-throughput sequencing were employed to determine the variations in EPS and bacterial taxa in different mangrove communities. Both EPS and EPS-C increased significantly with mangrove zonation from seaward to landward, irrespective of the EPS subfractions (colloidal and bound EPS) and components (extracellular protein and polysaccharide). Moreover, both EPS and EPS-C were found to be positively correlated with soil organic carbon. The present data further showed that EPS-C accounted for 1.84~10.69% of TOC in surface sediments. Multiple functional groups (e.g., O-H, N-H, and C=O), which may provide ligands for particle adsorption and complexation, were identified by FTIR. Consistent with the ascend of EPS with mangrove zonation from seaward to landward, the highest transmittance intensity for all functional groups was consistently exhibited in EPS isolated from landward Rhizophoraceae forest. In addition, the present data also indicated an interesting positive linkage between EPS and the abundance of some specific bacterial taxa, such as Rhizobiales, Corynebacteriales, and Gaiellales. In summary, this study claims the importance of EPS in the carbon pool in mangrove ecosystems. The present study may provide a better understanding of the functions of mangroves in carbon stocks.

KEYWORDS

mangroves, mangrove zonation, extracellular polymeric substances, organic carbon, microbial community

Introduction

As special coastal guardians along tropical and subtropical shorelines, mangroves play a crucial role in windbreaks, siltation promotion and environmental purification (Atwood and Hammill, 2018). Mangrove ecosystems are also famous for their high productivity and carbon density, although the carbon stock in mangroves may vary significantly among different regions and communities (Komiyama et al., 2008; Alongi, 2014b; Wang, 2019; Wang and Gu, 2021). A previous study reported that approximately 75% of organic carbon in mangrove ecosystems was sequestered in sediments (Alongi, 2014a). It would be of interest to further explore the potential factors that may play crucial roles in carbon storage in mangrove sediments.

Extracellular polymeric substances (EPS), a kind of microbial secretion, have been widely found in aquatic habitats (Zhang et al., 2015b). Proteins and polysaccharides are the two dominant components of EPS (Frolund et al., 1995). As a buffer interface around microorganisms, EPS acts as an extra carbon and nutrition pool for microorganisms (Urrea et al., 2017; Tian et al., 2020). EPS-induced biofilms further facilitate the formation of microbial aggregates (Wingender et al., 1999). A large number of studies have clarified that EPS can protect cells and microorganisms from external adversities such as heavy metals (Hou et al., 2013; Kang et al., 2014) and persistent organic pollutants (Kang and Park, 2010; Yang et al., 2020). Moreover, EPS can promote the adsorption of macromolecular organic matter with the extracellular matrix due to its viscosity (Li and Ganczarczyk, 1990). A study of activated sludge treatment of sewage confirmed that EPS also contributed to flocculation settlement by aggregating suspended solids in water (Wilén et al., 2000). Zhu and Yan (2022) further indicated that the contribution of EPS to the carbon stock may far exceed than that of microbial biomass carbon (MBC) itself.

The distribution of mangroves in intertidal regions is highly correlated with geomorphological conditions (Fromard et al., 1998; Ellison et al., 2000; Chowdhury and Maiti, 2016). In southern China, the mangrove community usually possesses an interesting sequential zonation following the variation in environmental factors in the intertidal zones. Generally, pioneer mangrove species (e.g., *Avicennia marina*) often occur in seaward zones with low tidal gradients where the sediment pore water has relatively high salinity but low nutrients, while Rhizophoraceae mangroves are often located in the middle and/or landward areas with lower flooding stress (Cheng et al., 2020).

Abbreviations: BM, bare mudflat; AM, *Avicennia marina*; AC, *Aegiceras corniculatum*; MF, mixed Rhizophoraceae forest; EPS, extracellular polymeric substances; EPS-PT, extracellular protein; EPS-PSC, extracellular polysaccharide; EPS-C, EPS carbon; FTIR, Fourier transform infrared spectroscopy; TOC, total organic carbon; Sal, salinity; TN, total nitrogen; TP, total phosphorus.

Furthermore, carbon stock and sequestration potential of sediments may vary significantly among different mangrove communities along a continuous tidal gradient during mangrove zonation (Feng et al., 2019; Chen et al., 2021a). Moreover, both EPS contents and producers (e.g., bacteria, fungi, and benthic diatoms) may also vary significantly with the variation in aboveground mangroves (Xiao and Zheng, 2016; Wang et al., 2019b; Mai et al., 2022). Unfortunately, the dynamics of microbial EPS and their potential functions on carbon stock and sequestration in mangrove ecosystems are still poorly understood.

Therefore, a natural mangrove reserve with a three-zonation stage was chosen to (1) investigate the microbial community and distribution of EPS in different mangrove zonation gradients; (2) explore the potential effect of EPS on the carbon stock in mangrove ecosystems; and (3) investigate the relationships among mangrove zonation, microbial structure, EPS, and carbon stock. To the best of our knowledge, this is the first study to explore the carbon stock from the aspect of microbial EPS and provide a new perspective to realize variation in mangrove carbon storage in different mangrove communities.

Materials and methods

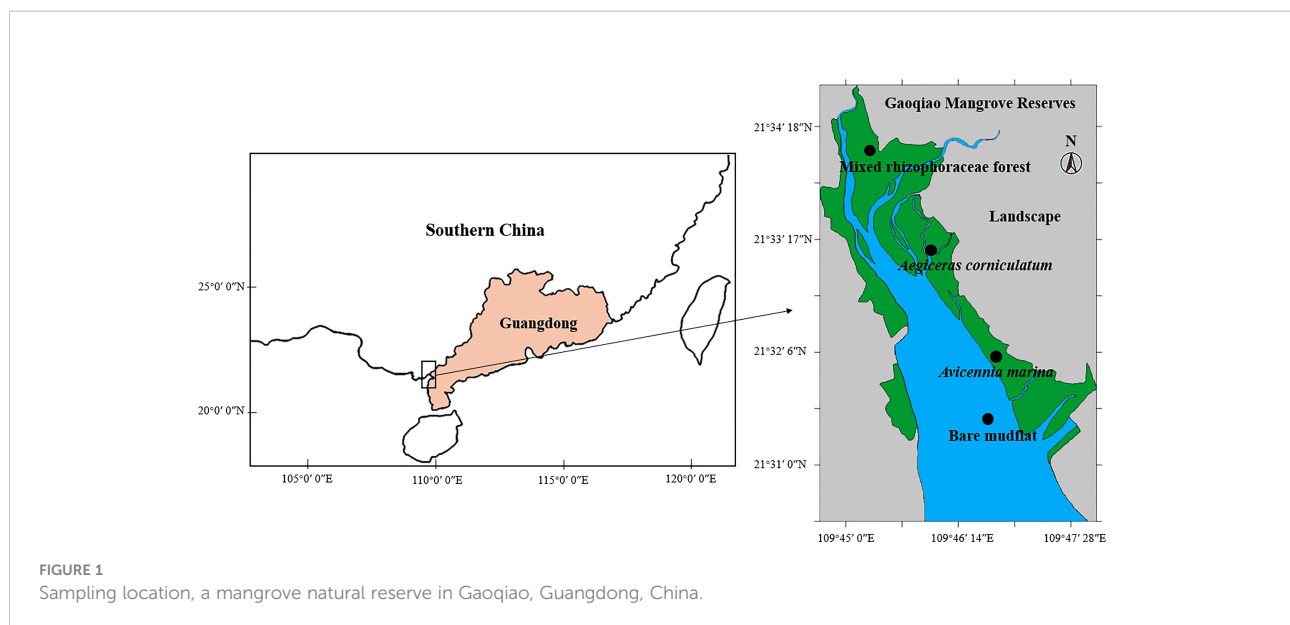
Sediment sampling

A natural mangrove reserve located in Gaoqiao, Zhanjiang City, Guangdong Province, China, was selected since it is one of the largest mangrove natural reserves in China. This region has a subtropical monsoon climate, with an average annual temperature of 23.4 °C and average annual precipitation of 1816 mm (mainly from April to September). Mangrove species colonization is partially controlled by terrain and tide.

In August 2021, surface sediment samples (0–5 cm) were collected from the Gaoqiao mangrove reserve. There were four sampling sites from seaward to landward (Figure 1), which represent the locations of bare mudflat (BM), *Avicennia marina* (AM, seaward pioneer mangrove), *Aegiceras corniculatum* (AC, medium transitional mangroves), and mixed Rhizophoraceae forest (MF, landward mixed rhizophoraceae forest, mainly occupied by *Rhizophora stylosa* and *Bruguiera gymnorrhiza*). At each sampling site, five surface sediment samples, with an interval of no less than 50 m, were collected. Thus, 20 packs of fresh sediment samples were acquired. Samples were transferred (stored with dry ice) to the laboratory in two days after field sampling.

Analysis of sediment physicochemical parameters

For total organic carbon (TOC) measurement approximately 0.05 g air-dried sediment was incubated in 0.5



mL 2 M HCl to remove carbonates (Cheng et al., 2020), then the samples were dried in the analyzer reactor with 500 mL/min air flow. The content of TOC was then determined using an elemental analyzer (TOC-V, CPH, Shimadzu, Japan) equipped with a solid sample module (SSM-5000A, Shimadzu, Japan).

For other physicochemical parameters, a pH meter (S210-KCN, Mettler Toledo, Germany) was employed to determine the pH of a 1:2.5 soil-to-water ratio turbid liquid, while salinity was measured in a 1:5 soil-to-water ratio turbid liquid using a salinity analyzer (PNT3000, STEPS, Nuremberg, Germany) (Wang et al., 2019a). Total nitrogen (TN) and total phosphorus (TP) analyses were performed using the modified Kjeldahl method and the molybdate reduction method according to the standard methods (HJ 717-2014 and HJ 632-2011), respectively.

EPS analysis and determination

EPS was extracted using cation exchange resin as described by previous investigators with minor modifications (Takahashi et al., 2009; Mai et al., 2022). In brief, artificial seawater (30‰ salinity) was added to tubes containing 30 g of fresh sediment and mixed in a shaker for 1 hour at 4°C in the dark. The supernatant was collected after centrifugation at 3500 rpm at 4°C for 10 min to obtain colloidal EPS. Then, 20 mL of artificial seawater and 2 g of activated cation exchange Dowex 50 WX8 resin (hydrogen form, 200-400 mesh, Sigma-Aldrich, MO, USA) were added to the remaining sediment, mixed for 1 hour at 4°C in the dark and then centrifuged at 3500 rpm at 4°C for 10 min to obtain bound EPS. Both the supernatants of the two EPS subfractions were purified using dialysis bags (3.5 kD, incubating at 4°C for 24 hours). The purified EPS samples were then freeze-dried.

The freeze-dried EPS powder was placed in an attenuated total reflection (ATR, Miracle, Pike, America) instrument after meticulously grinding. Analysis was conducted at 25 °C with Fourier transform infrared spectroscopy (FTIR, IR Affinity-1, Shimadzu, Japan) to record the functional group composition of the two EPS subfractions. A total of 25 scans with a resolution of 4 cm⁻¹ and a scanning range of 500–4000 cm⁻¹ were performed (Yang et al., 2018). The atmosphere was used as a blank, and the transmittance of air was subtracted for each testing sample. The inverted peaks thus appeared near 2400 cm⁻¹ due to CO₂ in the air.

For the measurements of extracellular protein (EPS-PT) and polysaccharide (EPS-PSC), the freeze-dried colloidal and bound EPS powder was redissolved in 10 mL of deionized water, respectively. The protein concentration was determined using a modified bicinchoninic acid protein assay kit (Sangon Biotech, Shanghai, China) with bovine serum albumin as the standard (Smith et al., 1985). The carbon ratio of EPS-PT was estimated to be an average of 40% according to the data reported by Bura et al. (1998). Polysaccharide content was determined using the phenol–sulfuric acid method with glucose as the standard (DuBois et al., 1956). Polysaccharides were assumed to be all converted to furfural (C₅H₄O₂), so the carbon ratio of EPS-PSC was estimated to be 62.5% (DuBois et al., 1956). An elemental analyzer (TOC-V, CPH, Shimadzu, Japan), which was configured with an automatic sampler, was employed to measure dissolved organic carbon in EPS solution (Jiang et al., 2006).

16S illumina sequencing

Total DNA in 1 g of sediment was extracted by the Soil DNA Extraction Kit (Omega, United States). The 16S rRNA gene was

amplified using 338F (5'-ACTCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') primers. Polymerase chain reaction (PCR) was performed in a 50 μ L reaction system. The PCR program was as follows: pre-denaturation at 98°C for 2 min; 30 cycles of denaturation at 95°C for 1.5 min, annealing at 55°C for 30 s and extension at 72°C for 45 s; and final extension at 72°C for 10 min and preservation at 4°C. The PCR product was purified by agarose gel electrophoresis and recovered *via* a DNA Gel Extraction Kit (Omega, D2500-01, United States). The Illumina HiSeq platform (Illumina, San Diego, CA, United States) was employed for paired-end sequencing according to standard protocols. All sequences were submitted to the National Center for Biotechnology Information (NCBI) database.

Raw sequences were processed using Trimmomatic (Bolger et al., 2014), and merged using FLASH (version 1.2.11) (Magoč and Salzberg, 2011). The flashed reads were analyzed with the Quantitative Insights into Microbial Ecology (QIIME) (version 1.9.1) (Caporaso et al., 2010) and compared with a reference database (Gold database, http://drive5.com/uchime/uchime_download.html) (Haas et al., 2011) using the UCHIME algorithm to obtain effective tags (Edgar et al., 2011). Sequence analysis was processed using the UPARSE software (Uparse v7.0.1001), and 97% similarity was considered to indicate the same OTUs. For each representative sequence, taxonomic information with a confidence threshold of ≥ 0.5 was annotated using the Silva reference databases (Quast et al., 2013).

Data analysis

The data were tested for normality (Kolmogorov-Smirnov test, SPSS, version 22.0), and one-way analysis of variance (least significant difference *post hoc* test, LSD) was performed to evaluate significant differences. Kaiser-Meyer-Olkin (KMO) test was employed to analyze the correlation coefficient between all the variations before the principal component analysis (PCA) was performed. PCA and Pearson correlation matrix were performed using the software of Origin to assess the relationship between EPS-PT and EPS-PSC, EPS-C and sediment physicochemical properties (TOC, TN, TP, pH, salinity).

The raw bacterial reads were uploaded to NCBI (PRJNA839550). Data were analyzed base on the Majorbio

cloud platform (www.majorbio.com). Analysis of similarities (ANOSIM) was performed to determine the statistical significance of differences of all OTUs between samples. Principal coordinate analysis (PCoA) was used to investigate the association with microbial communities among sites. Kruskal Wallis H test was applied in the analysis of the significant differences of species in four groups of sediment samples and their comparison between each pair (Wei et al., 2014). Correlation analysis among sediment physicochemical properties and bacterial community structure (using the relative abundance of microbial taxa) was demonstrated by a two-matrix correlation heatmap.

Results

Soil TOC and other physicochemical parameters

According to Table 1, TOC concentrations ranged from 3.22 to 39.57 g/kg, and the highest TOC concentration was found in MF, followed by AC, AM, and BM. Similar distribution patterns were also found in TN and TP, with the highest values in MF but the lowest in BM. In contrast, the maximum salinity was observed in BM, while the minimum salinity was observed in MF. The pH values ranged from 6.40 to 7.20, and the highest pH was found in BM, followed by AC, MF, and AM.

EPS subfractions and components

The concentration of total EPS-PSC ranged from 18.04 to 158.64 mg/kg. As shown in Table 2, the maximum EPS-PSC is detected in MF, followed by AC, AM, and BM. Consistent with EPS-PSC, similar distribution patterns were also observed in colloidal and bound EPS-PSC.

The concentration of total EPS-PT ranged from 66.97 to 171.31 mg/kg (Table 2). Similarly, higher concentrations of bound EPS-PT were also detected in mangrove sediments than in bare mudflat. However, except for AM, the contents of colloidal EPS-PT were comparable between mangrove and mudflat sediments.

TABLE 1 Variation in sediment physicochemical properties at different sampling sites. Mean \pm SE, small letters represent significant differences ($p < 0.05$) among the four sites.

Sampling sites	pH	Salinity (g/L)	TN (g/kg)	TP (g/kg)	TOC (g/kg)
BM	7.20 \pm 0.05 ^a	0.91 \pm 0.08 ^a	0.50 \pm 0.15 ^d	0.19 \pm 0.07 ^c	3.22 \pm 0.30 ^c
AM	6.40 \pm 0.06 ^c	0.91 \pm 0.06 ^a	1.64 \pm 0.9 ^b	0.61 \pm 0.04 ^b	21.16 \pm 2.29 ^b
AC	7.10 \pm 0.04 ^a	0.68 \pm 0.02 ^b	1.30 \pm 0.13 ^c	0.63 \pm 0.07 ^b	19.96 \pm 1.99 ^b
MF	6.80 \pm 0.04 ^b	0.63 \pm 0.03 ^b	2.34 \pm 0.03 ^a	1.00 \pm 0.04 ^a	39.57 \pm 1.32 ^a

TABLE 2 Variation in EPS at different sampling sites. Mean \pm SE, small letters represent significant differences ($p < 0.05$) among the four sites.

Sampling sites	Colloidal (mg/kg)		Bound (mg/kg)	
	Protein	Polysaccharide	Protein	Polysaccharide
BM	20.20 \pm 6.59 ^b	7.51 \pm 0.52 ^c	46.77 \pm 1.9 ^c	10.53 \pm 0.72 ^c
AM	61.12 \pm 5.81 ^a	23.17 \pm 4.98 ^{bc}	91.96 \pm 19.06 ^b	23.56 \pm 2.96 ^{bc}
AC	21.21 \pm 6.72 ^b	41.91 \pm 10.26 ^b	144.53 \pm 44.19 ^a	28.26 \pm 6.15 ^b
MF	27.11 \pm 5.21 ^b	105.57 \pm 17.19 ^a	144.20 \pm 14.15 ^a	53.07 \pm 10.42 ^a

EPS-C

In total, EPS-C ranged from 0.33 to 0.73 g/kg, much higher EPS-C was detected in mangrove sediments than in mudflat, and the highest EPS-C was observed in MF. However, the bare mudflat exhibited a higher EPS-C/TOC ratio (10.69%) than the mangrove sediments (1.84 ~ 2.98%) (Figure 2).

Detailed information for colloidal and bound EPS-C is shown in Figure S1. Both the contents of colloidal and bound EPS-C increased significantly ($p < 0.05$) with mangrove zonation from seaward to landward. The highest bound EPS-C ratio was also found in MF, followed by AC, AM, and BM. For the component of EPS-C, protein and polysaccharide occupied 8.10~11.77% and 3.41~13.78% of EPS-C, respectively, approximately equal to the ratio of these two components in total EPS (Table S1).

FTIR spectra of EPS

Similar infrared bands of EPS were observed, regardless of colloidal or bound subfraction. The results from Figure 3 show five dominant peak segments: 3520-3200 cm^{-1} in region I (O-H stretching associated with oxhydrolyl), 1800-1600 cm^{-1} in region II (C=C, C=O stretching, C=N, N-H vibrations from proteins), 1600-1400 cm^{-1} in region III (stretching of O-alkyl group, methyl and methylene which may be attributed to proteins), 1200-1000 cm^{-1} in region IV (ring vibrations of P=O, P-O, C-O-C, C-O-P partially attributed to polysaccharides) and 650-400 cm^{-1} in region V (region without functional groups, stretching of C-H). In terms of the difference in FTIR transmittance intensity among EPS extracted from the four sampling sites, the highest transmittance intensity for all infrared bands was consistently exhibited in MF, followed by AC, AM, and BM.

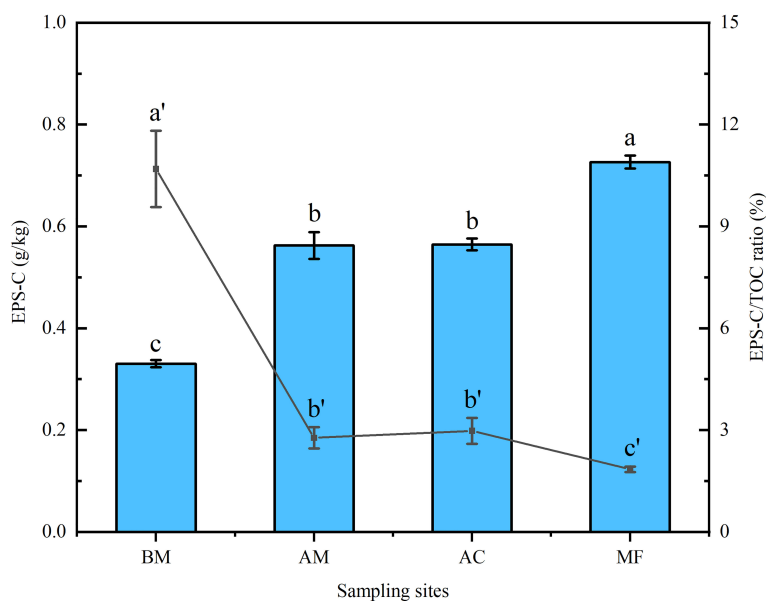


FIGURE 2

Variation in EPS-C content and EPS-C/TOC ratio in sediments associated with four sampling sites. The bar chart (relevant to the left Y-axis) and line graph (relevant to the right Y-axis) represent the values of EPS-C and the EPS-C/TOC ratio, respectively. Mean \pm SE, different letters show significant differences ($p < 0.05$) among the four different sampling sites.

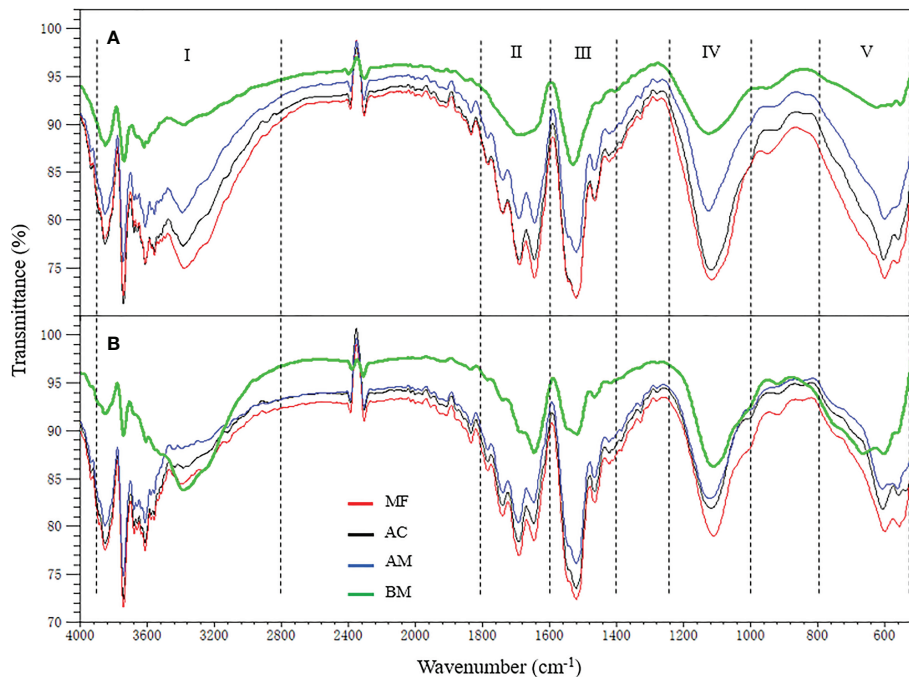


FIGURE 3
FTIR spectra of bound EPS (A) and colloidal EPS (B) isolated from four sampling sites.

Microbial community composition

The ANOSIM results showed that significant differences were observed in the microbiome among BM, AM, AC, and MF (ANOSIM $R = 0.7527$, $p = 0.001$). The results of Figure 4A illustrate the differences in microbial composition among the four sampling sites at the class level. Compared with BM, higher abundances of Alphaproteobacteria, Actinobacteria, and Thermoleophilia were detected in mangrove sediments, especially in the sediment associated with MF.

Figure 4B lists the discrepancy in microbial composition at the order level. Specifically, the sediment of MF exhibited the highest abundance of Rhizobiales and Solirubrobacterales in comparison with the other three sampling sites. Similarly, Corynebacteriales and Gaiellales also showed the highest abundance in MF sediment, whereas the lowest abundance was found in BM sediment. More detailed comparisons between each pair of bacteria are presented in Figures S2, S3.

Relationships between microbial composition, EPS, EPS-C, TOC, and other soil physicochemical properties

According to the PCoA results (Figure S4), microbial communities showed a similarity in AM and AC, while microbial communities of BM and MF were quite different. As shown in

Figure 5, a significant positive relationship was observed between TOC and EPS-C, with a correlation coefficient of 0.92. Furthermore, both TOC and EPS-C were positively correlated with EPS-PT and EPS-PSC. In addition, TN and TP were also positively correlated with TOC and EPS-C.

As shown in Figure 6A, at the class level, Alphaproteobacteria, Actinobacteria, Vicinamibacteria, and Thermoleophilia were positively correlated not only with TOC, TN and TP but also with EPS (e.g., EPS-PSC and EPS-PT) and EPS bound carbon (EPS-C). More detailed correlation analysis at the order level is presented in Figure 6B. Specifically, Rhizobiales, Microtrichales, Corynebacteriales, and Gaiellales were positively associated with EPS components and EPS-C. Positive correlations between those bacterial orders and sediment nutrient parameters (e.g., TN, TP, and TOC) were also observed. More information on the correlation analyses between EPS subfractions and the bacterial community at the class and/or order level is displayed in Figure S5.

Discussion

Variation in carbon storage in sediments with mangrove zonation along a continuous tidal gradient

In this study, the content of TOC in the Gaoqiao mangrove reserve ranged from 19.96 to 39.57 g/kg, higher

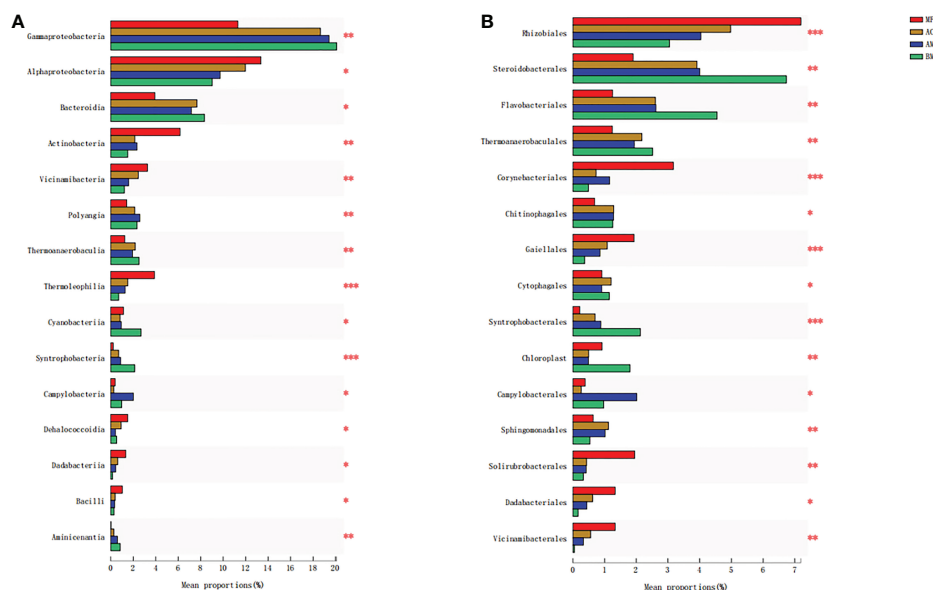


FIGURE 4 Significant difference analyses of the majority of bacterial taxa at the class level (A) and order level (B) from the four sampling sites. Meaning of asterisks, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

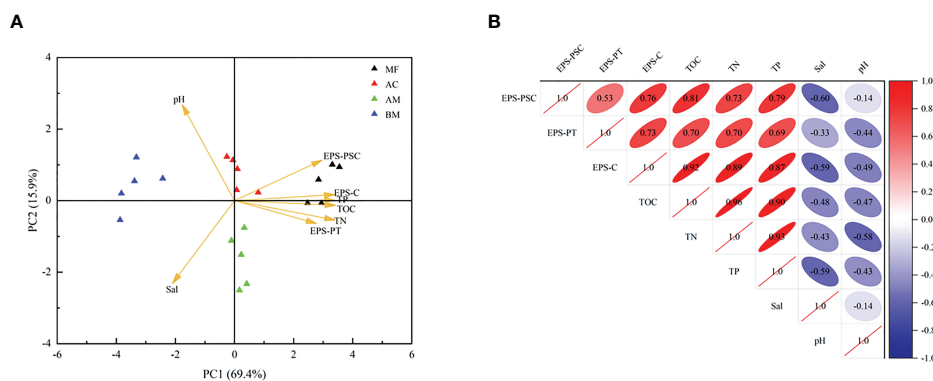
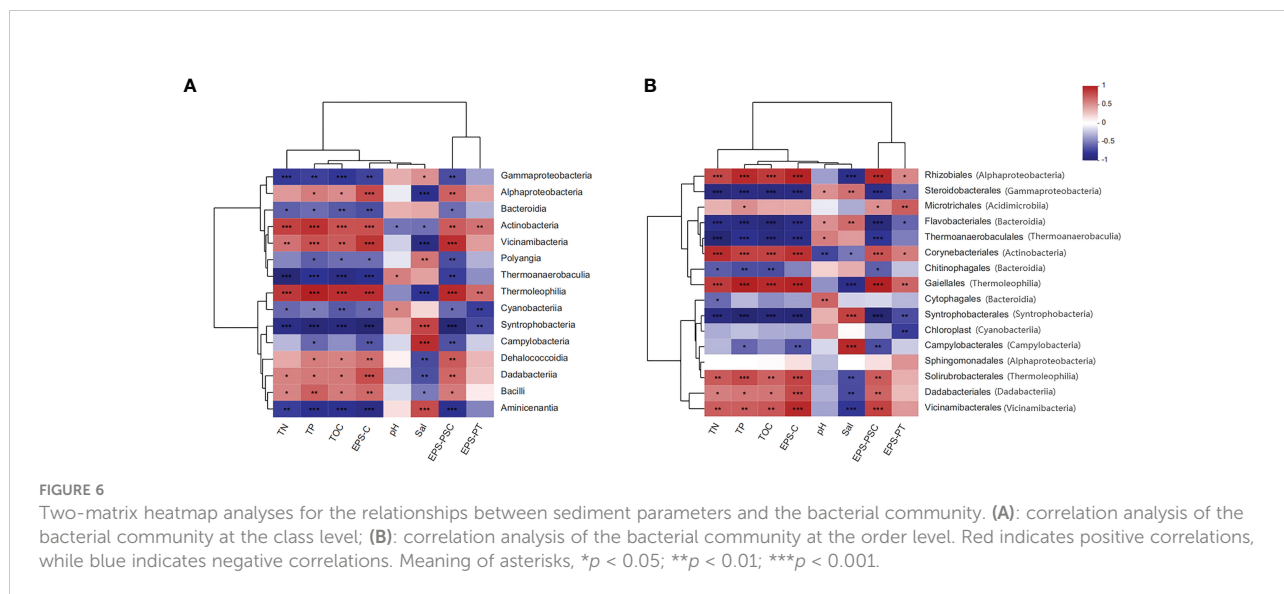


FIGURE 5 Principal component analysis and Pearson's correlation analysis of sediment physicochemical properties (KMO value = 0.690; $n = 20$). (A): Triangle scatters in different colors represent the four sampling sites. (B): Positive correlations are identified in red, while negative correlations are identified in blue.

than that of broad-leaved forests in northeast Guangdong Province (ranging from 16.75 to 22.14 g/kg) (Lin and Gao, 2009). The contents of TOC, TN, and TP also increased significantly ($p < 0.05$) with the elevation of the tidal gradient during mangrove zonation (Table 1). Consistent with previous findings (Wang et al., 2013), the present data also found that landward mangrove sediment has a higher soil organic carbon concentration.

Organic carbon concentrations in sediments may vary significantly between different mangrove communities due to differences in tidal gradients, plant diversity, and deposition rates (Gleason and Ewel, 2002; Ceron et al., 2011). In this study, the seaward pioneer *Avicennia marina* and transition mangroves (*Aegiceras corniculatum*) exhibited very low plant diversity (almost pure forest). Previous studies have shown a positive correlation between mangrove diversity and



sediment carbon storage (MacKenzie et al., 2016; Lamont et al., 2019). Rhizophoraceae plants, which occur in the middle and/or landward regions along the southern China coast, often constitute the climax mangrove community with the highest plant productivity and diversity (Bai et al., 2021). The enhancement of soil organic carbon in landward MF in this study may also be partly ascribed to its higher plant diversity. Therefore, the application of a multispecies planting strategy in mangrove restoration may improve biodiversity and blue carbon storage simultaneously (Alongi, 2011).

Variation in microbial community structure and EPS content with mangrove zonation

The data presented in Table 2 show that the EPS content increased significantly with mangrove zonation from seaward to landward. This increase may partly be ascribed to the improvement of sediment nutrient concentrations (such as TOC, TN, and TP) (Table 1) and some specific microbial taxa (Figures 4, S2, S3) during mangrove zonation in intertidal regions (Figure 5). In this study, the highest abundances of Alphaproteobacteria and Actinobacteria were found in the sediment of MF (Figure 4A).

A positive correlation was observed between these two classes of bacteria and EPS (Figure 6A). It was reported that Alphaproteobacteria and Actinobacteria could secrete EPS to ameliorate the rhizosphere microenvironment and increase water retaining capacity (Marasco et al., 2022). Previous studies also claimed that Alphaproteobacteria were the main EPS-producing bacteria in membrane bioreactors (Neoh et al.,

2017). In addition, these two bacterial taxa were found to participate in the degradation of litterfall, rhizosphere metabolites, and recalcitrant organic carbon (Zhang et al., 2020). Increased TOC induced with the elevation of the tidal gradient along mangrove zonation may also promote the growth of these two bacterial taxa.

Correlation analysis at the order level (Figure 6B) suggested that increasing Rhizobiales, Corynebacteriales and Gaiellales abundances from seaward to landward may enhance EPS secretion. Rhizobiales are known as beneficial partners in interactions (Liu et al., 2022). Rhizobiales in carbon-rich substrates may accelerate the decomposition of amino acids and phenols due to their abundant extracellular carboxylase and oxidase enzymes (Mai et al., 2021; Yu et al., 2022). Moreover, Rhizobiales are regarded as one of the important producers of extracellular polymers in membrane bioreactors. EPS secreted by Rhizobiales are crucial for their stabilization and reproduction (Santaella et al., 2008; Cydzik-Kwiatkowska, 2015). Previous investigators also claimed that Solirubrobacteriales promoted the formation of soil aggregates synergizing with Rhizobiales (Marcos et al., 2019). Guo et al. (2020) claimed the importance of Corynebacteriales in carbon metabolism and cellulose biodegradation. Chen et al. (2021b) also reported that Gaiellales could accelerate the formation process of soil aggregates through EPS secretion. The results showed that the abundance of some other bacteria (e.g., Steroidobacteriales, Flavobacteriales, Syntrophobacteriales, etc) was negatively correlated with sediment parameters (e.g., saline and pH) and EPS (Figure 6). The growth of these bacterial taxa may be limited by abiotic factors in mangrove sediment (e.g., high salinity, anaerobic and acidic conditions) (Wu et al., 2016), leading to a lower contribution to EPS content. In addition, according to the difference analysis of EPS in a diatom-dominated intertidal

mudflat (Marennes–Oléron, France), the EPS content was higher in winter than that in summer (Pierre et al., 2014).

In summary, the dynamics of EPS are jointly regulated by the bacterial community. However, the enriched microbial taxa can either be EPS producing taxa or benefit from other organic substrates released by other organisms. The mechanisms of EPS secretion from microbes, especially for bacteria isolated from mangrove sediments, are still poorly understood. Investigation for screening EPS-producing bacterial taxa needs to be further conducted. In addition, similar studies on the relationships between EPS and other microorganisms, such as algae and fungi, should also be conducted.

Linkage between EPS and carbon storage and transportation in mangrove ecosystems

The present data showed that both EPS and EPS-C were positively correlated with TOC (Figure 5), and a similar positive relationship between glomalin-related soil protein and soil organic carbon was also reported (Zhang et al., 2015a; Wang et al., 2018). In this study, most of the EPS-C may come from the EPS itself because most organic particles attached to the EPS were removed during EPS extraction and purification, except for some soluble organic colloids (Yang et al., 2020). The present data further indicated a linkage between mangrove zonation and increased EPS-C. As shown in Figure 2, EPS-C contributed 1.84–10.69% of TOC, which is much higher than that of MBC in tropical (subtropical) forests (1.5%) and mangroves (0.45%) (Xu et al., 2013; Wang et al., 2018).

In this study, both colloidal and bound EPS-C increased significantly with mangrove zonation from seaward to landward (Figure S1). Soluble colloidal EPS may be easily degraded and utilized by bacteria and algae (McKew et al., 2013), while bound EPS is generally considered recalcitrant carbon and may be subjected to long-term retention and diagenesis in sediments (Evrard et al., 2008; Luo et al., 2020). Increased EPS (especially the increased bound EPS-C ratio, Figure S1B) favored by mangrove zonation may thus contribute to the burial of stable organic carbon in the sediment of landward MF. For EPS-C components, the present data showed that protein and polysaccharide only occupied a part of EPS-C, approximately equal to the ratio of these two components in total EPS (Table S1). The contributions of other EPS components (e.g., nucleic acids, lipids and humus) to EPS-C and the turnover rate of different EPS sub-fractions and components, as well as their potential contribution to the long-term storage of sediment carbon stock, are worth further investigation (Flemming and Wingender, 2001).

As shown in Figure 3, EPS isolated from mangrove sediments contained multiple functional groups (e.g., O-H, N-H, -COO-, and P-O). Similar spectroscopic characterization and functional traits

were also observed in EPS isolated from single bacteria and/or activated sludge (Guibaud et al., 2005; Badireddy et al., 2008). Although the spectroscopic characterization and functional traits of EPS were similar, the spectral absorption peaks of these functional groups appeared to increase with the increase in tidal gradient along mangrove zonation, consistent with increased EPS content. Increased EPS and functional groups may facilitate biofloculation and sedimentation through interparticle bridging and biosorption, leading to more organic particles precipitation during tidal flooding (Gerbersdorf and Wieprecht, 2015; Xu et al., 2017). Besides, increased EPS may also facilitate the formation process of soil aggregates and thus may partly protect soil organic carbon from mineralization (Degens, 1997; DeKa et al., 2019). Moreover, interattraction between charged EPS functional groups and mineral particles may promote the formation of mineral-associated organic carbon (Lin et al., 2016; Liu et al., 2022). In addition, our previous study indicated that increased EPS favored by mangrove restoration can promote sediment anti-scourability (Mai et al., 2022). Thus, higher EPS in landward MF sediment may not only benefit for TOC preservation in sediments, but also reduce soil TOC erosion simultaneously. More detailed mechanisms of EPS on soil carbon sequestration in mangroves are worthy of further investigation.

Nevertheless, it was interesting that bare mudflat sediments possessed a higher ratio of EPS-C/TOC than mangrove sediments, although a much lower content of EPS-C was detected in the sediment of the bare mudflat (Figure 2). The origin of sediment organic carbon is dominated by autochthonous carbon favored by mangrove litters (Xiong et al., 2018). The lower EPS-C/TOC ratio in mangrove sediments may be partly ascribed to the increment of vegetation carbon inputs driven by plant litters from aboveground mangroves (Feng et al., 2017). In the bare mudflat, almost 11% of sediment organic carbon was contributed by EPS-C. A previous study inferred that the majority of mangrove dissolved organic carbon will be exported into the estuary (up to 65%) (Romigh et al., 2006). Frequent tidal flooding may further enhance carbon exchange between mangrove sediments and their neighboring mudflats (Bouillon et al., 2007). Generally, the function of mudflats in carbon storage and sequestration is often ignored. Phang et al. (2015) estimated that mudflat carbon storage in the Chek Jawa intertidal zone in Singapore is equivalent to 29% of local mangrove carbon storage. Moreover, The amassment of nutrients in mudflats may benefit the invasion of pioneer mangrove species. Further studies focused on mangrove carbon export/import, such as the application of isotopic tracing and biomarker analyses, need to be conducted in the future.

Conclusion

This study provided evidence for a linkage between mangrove zonation and increased microbial EPS that may contribute to carbon storage in sediments. The data showed that EPS-C ranged from 0.33 to 0.73 g/kg and accounted for 1.84–10.69% of the total organic

carbon in surface sediments. Moreover, the content of soil organic carbon was found to be positively related to EPS and EPS-C. Multiple functional groups (e.g., stretching of O-H, N-H, and C=O) in EPS that may facilitate the adsorption of organic materials were identified by FTIR. Consistent with the increasing EPS contents with mangrove zonation from seaward to landward, the highest transmittance intensity of all functional groups was also observed in the landward Rhizophoraceae forest. In addition, the results showed a positive correlation between the EPS content and the abundance of certain bacterial taxa, such as Rhizobiales, Corynebacteriales, and Gaiellales. This study indicated the importance of microbial EPS in the carbon stock in mangrove sediments.

Data availability statement

The data presented in the study are deposited in the NCBI (<https://www.ncbi.nlm.nih.gov/>) repository, accession number PRJNA839550.

Author contributions

D-XL contributed to the experiments, data analysis and the first draft of the manuscript; Z-MM and C-CS contributed to microbial analysis; Y-SW and HC contributed to conception and design of the study; H-HL and Y-WZ conducted field sample collection. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2022.967767/full#supplementary-material>

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