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Investigating organic sulfur in estuarine and offshore environments: A combined field and cultivation approach

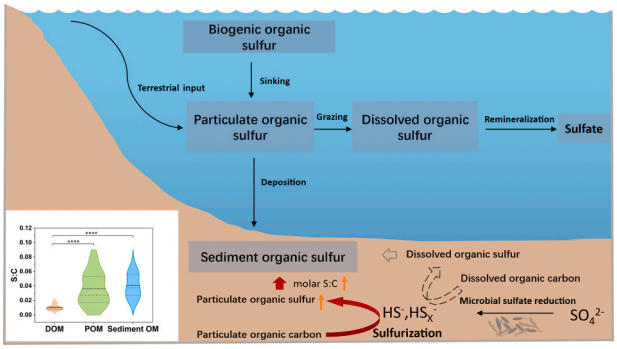
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HIGHLIGHTS

- The distribution and sources of organic sulfur in the offshore area were investigated.
- Organic sulfur showed a heterogeneous distribution with no discernable spatial pattern.
- Molar S:C ratios link particle settling to organic sulfur source in sediment.
- Sulfurization further enhances organic sulfur accumulation in sediment.

GRAPHICAL ABSTRACT



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ABSTRACT

Estuarine-offshore sediments accumulate substantial particulate organic matter, containing organic sulfur as a key component. However, the distribution and sources of organic sulfur in such environments remain poorly understood. This study investigated organic sulfur in the Yangtze River Estuary and adjacent East China Sea. Dissolved organic sulfur varied from 0.65 to 1.99 μmol/L (molar S:C 0.006–0.018), while particulate organic sulfur ranged from 0.42 to 2.69 μmol/L (molar S:C 0.007–0.082). Sedimentary organic sulfur exhibited a similar molar S:C ratio (0.014–0.071) to particulate organic sulfur in bottom water, implying that particulate matter deposition is a potential source. Furthermore, sediments exposed to frequent hypoxia harbored significantly higher organic sulfur and S:C values compared to non-hypoxic areas. Laboratory incubation experiments revealed the underlying mechanism: sustained activity of sulfate-reducing bacteria in hypoxic sediments led to a substantial increase in sedimentary organic sulfur (from 15 to 53 μmol/g) within 600 days. This microbially driven sulfurization rendered over 90 % of the organic sulfur resistant to acid hydrolysis. Therefore, this study demonstrates that, alongside particle deposition, microbial sulfurization significantly contributes to organic sulfur enrichment and likely promotes organic matter preservation in estuarine-offshore sediments, particularly

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under hypoxic conditions. This finding advances our understanding of organic sulfur sources in these vital ecosystems.

1. Introduction

The ocean is a reservoir for a variety of organic molecules, with organic sulfur receiving less attention compared to organic carbon and nitrogen. Despite its relatively low concentration in the ocean (several hundred nanomoles to several micromoles per liter), the overall oceanic inventory of organic sulfur is significant, extending to the peta-gram scale (Ksionzek et al., 2016). Controversy surrounds the primary dynamic processes driving the dissolved organic sulfur (DOS) cycle in the ocean, particularly the microbial breakdown of phytoplankton-derived organic sulfur and the "diffusion" of sulfide pore water in sediments (Dittmar et al., 2017; Koch et al., 2017; Ksionzek et al., 2016). Until recently, sulfur isotope composition variations indicated that phytoplankton are the main producers of organic sulfur in the ocean (Phillips et al., 2022). After grazing or lysis, organic sulfur is released into the water, where it is quickly used by heterotrophic microorganisms and mineralized back to sulfate (Moran and Durham, 2019). A small portion escapes and eventually reaches the sediment for preservation (Raven et al., 2016). Anoxic water promotes the formation of anaerobic "microregions" inside the particles, creating conditions for the sulfate reduction process (Raven et al., 2021). With sulfate as the electron acceptor, the reduced inorganic sulfur produced by sulfate-reducing bacteria attacks the unsaturated bonds of organic matter to form resistant organic sulfur, thereby increasing the settling flux of the particle (Pohlabeln et al., 2017; Raven et al., 2021; Raven et al., 2016). Organic matter entering the sediment undergoes further sulfurization and burial (Abdulla et al., 2020), establishing a sink of organic matter. In offshore environments, anthropogenic discharge, input of terrestrial organic matter, and high primary productivity contribute to a higher burial flux of particulate organic matter (POM) into sediments than that in the open ocean (Keil, 1995). However, the distribution and sources of organic sulfur within this system remain unknown.

This study focuses on the Yangtze River Estuary, a major conduit for nutrients and organic matter into the East China Sea (Deng et al., 2006; Jiao et al., 2018). This flux, coupled with water stratification, has led to persistent eutrophication in the Yangtze River Estuary and nearby areas (Zhu et al., 2011). This phenomenon is also associated with frequent seasonal hypoxia in bottom waters (Zhang et al., 2007). High productivity increases the deposition flux of organic carbon, contributing to the carbon-rich sediments on the eastern coastal shelf. The metabolism of settled organic matter also promotes the oxygen consumption in the bottom water (Zhu et al., 2011). Anaerobic bacteria dominate offshore surface sediments, and sulfate reduction processes are frequent (Guo et al., 2018; Liu et al., 2021). We propose that these anaerobic microbial processes, alongside settling particulate matter, serve as an additional source of organic sulfur in sediments, potentially promoting its long-term burial. In this study, we investigated the distribution and potential influencing factors of organic sulfur in estuaries and adjacent seas. Combing field investigations and cultivation experiments, we highlight a new source of organic sulfur in offshore sediments, enhancing our understanding of its sources in these areas.

2. Materials and methods

2.1. Field investigation and sampling

Sediment samples were collected from 12 locations in the Yangtze River Estuary and the adjacent East China Sea (121°6′5.6″E to

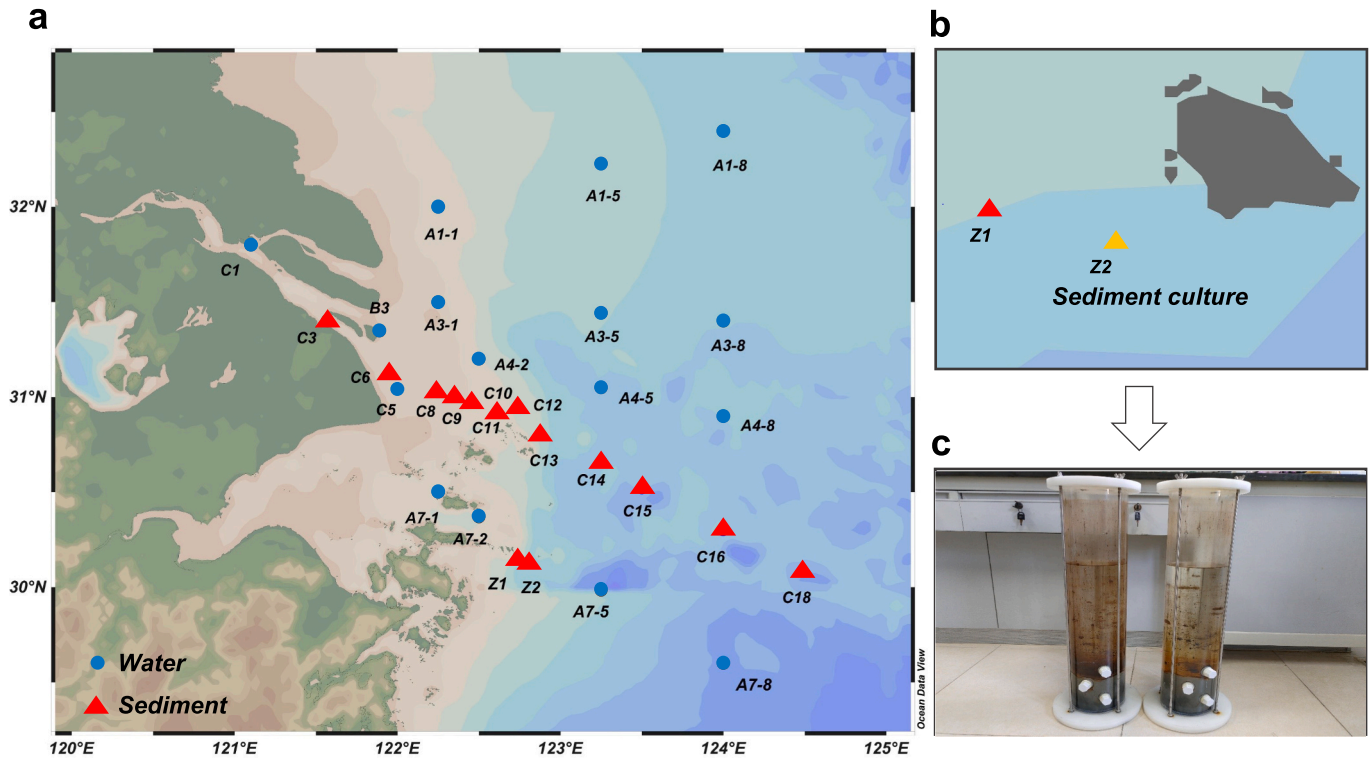


Fig. 1. Water and sediment collection stations in the Yangtze River Estuary and the adjacent East China Sea. a) In situ sample collection stations distribution. Blue closed circle, in situ water collection stations. Red triangle, surface sediment collection stations. b) Sediment collection sites for incubation experiments. Yellow triangle, in situ sediment collection and culture experimental station c) Sediment culture device.

124°29′30.6″E, 29°42′2.3″N to 31°45′58.1″N, Fig. 1a) in August 2020. Surface sediment samples (~5 cm) were collected and stored in a −80 °C refrigerator until analysis. Water samples were collected from the bottom water of 18 stations (121°5′60.0″E–124°0′0.0″E, 29°36′0.0″N–32°23′60.0″N, Fig. 1a) in July 2021. One liter of seawater was filtered through 0.7-μm glass fiber filters (47 mm diameter, Whatman) to collect particulate matter for subsequent elemental analysis of carbon, nitrogen and sulfur. The filters were immediately frozen in liquid nitrogen until further analysis. Thirty milliliters of filtrate were collected for dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) and stored in 40 mL brown glass bottles at −20 °C until further analysis. Refer to the enrichment method of marine dissolved organic sulfur (DOS) (Pohlabeln et al., 2017), 1 L seawater was filtered through 0.7-μm glass fiber filter membrane, acidified to pH 2, and enriched by PPL cartridges (200 mg, Bond Elut PPL, Agilent Technologies, USA). After enrichment, the PPL column was dried and stored in a refrigerator at-20 °C until laboratory analysis.

2.2. Sediment culture experiment

We designed a sediment anaerobic culture device to simulate the anaerobic processes of sediment. The sediments were collected from Zhoushan, located outside the Yangtze River Estuary (Fig. 1b). Sediment (0–10 cm) samples were collected on site, and large macrofauna, such as worms and crabs, were removed by visual inspection. The samples were then homogenized and filled into sediment-water interface culture devices. Two parallel culture devices were set up, named N3 and N4. The in situ bottom water was used as the overlying water for the sediment culture, and the entire process was completed within 2 h. The culture devices were placed in a dark place at room temperature and cultured for 600 days. The cultivation device monitors the dissolved oxygen state of the overlying water in the device through the dissolved oxygen patch and the optical fiber sensor (PreSens, Germany). The signal is corrected by dissolved oxygen saturated water and nitrogen deoxygenated anaerobic water until the signal is stable. Collection of sediment samples was carried out through the switchable small hole at the bottom of the culture device. The sediment pore water DOC samples were collected through the outlet hole below the culture device (Fig. 1c), using a sterile pre-pickling syringe, in a 40 mL brown glass bottle, and stored at −20 °C until analysis. The DOC in the overlying water was collected with sterile syringe through a small hole on the top of the culture device. Sediment samples for the analysis of organic carbon, nitrogen, sulfur, and microbial community structure were collected with a pre-pickled collection tube slowly inserted through a small hole on the top (reducing disturbance to the culture system). After sample collection was completed, the hole was immediately plugged, and the culture system remained anaerobic during the sampling process (except for the initial 20 days of culture).

2.3. Environmental parameters analysis

Nutrient concentrations in the field environment of the Yangtze River Estuary and its adjacent East China Sea were analyzed. The water samples for measuring the nutrient were filtered using a 0.22-μm filter membrane. Nitrate, nitrite, silicate and phosphate were detected through a standardized process (Dai et al., 2008), using the AA3 AutoAnalyzer (SEAL, Germany). Ammonium measured by spectrophotometric method with indigo carmine (Shimadzu, Japan) (Pai et al., 2001). Dissolved oxygen was determined by Winkler method (Shriwastav et al., 2010). The chlorophyll samples were filtered with a 0.7-μm glass fiber filter membrane and frozen, then determined by Hitachi 4600 molecular fluorescence spectrophotometer.

2.4. Sediment grain size analysis

An appropriate amount of sample was taken in the test tube, and 30 % H_2O_2 solution and 1 mol/L HCl solution were added respectively to remove organic matter and calcium carbonate (Vandenberghe and Jef, 2013). After standing for 24 h, the supernatant was poured out, and distilled water was added to clean until neutral. Finally, 5 mL $(NaPO_3)_6$ solution with a concentration of 0.5 % was added to the test tube to fully disperse the sample, and the particle size analysis of the sediment sample was performed using a BT-9300 ST laser grain size analyzer (Baite, China).

2.5. Particulate/dissolved organic carbon, nitrogen, sulfur analysis

Particulate organic carbon, nitrogen, sulfur (POC, PON and POS) in the field of the Yangtze River Estuary and its adjacent East China Sea were analyzed on a CHNS elemental analyzer (vario EL cube, Elementar, Germany). Before analysis, POC and PON were analyzed by treating the filters with hydrochloric acid fumes to remove inorganic carbon. Sulfate was removed from the POS samples by washing with an isotonic 3.2 % ammonium formate solution (Chen et al., 1996). Dry the filter membrane in a constant temperature oven at 60 °C. The Shimadzu TOC-VCPH analyzer was used to detect dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) by the high temperature catalytic oxidation method. Milli-Q water was used as blank subtraction in the detection process, and deep seawater (provided by the Hansell Organic Biogeochemistry Laboratory at the University of Miami, USA) was used as an additional control (Callahan et al., 2004). Before analysis, 30 mL of samples were treated with 40 μL H_3PO_4 to remove carbonate. The concentration of dissolved organic nitrogen (DON) was obtained by deducting the concentrations of nitrate, nitrite and ammonium from the concentration of TDN. The dissolved organic sulfur in seawater was enriched by solid phase extraction. DOS_{SPE} (solid phase extraction of dissolved organic sulfur) concentrations were measured on an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, 5110VDV, Agilent, USA). DOC_{SPE} (solid phase extraction of dissolved organic carbon) concentration was detected by the same TOC-VCPH analyzer as DOC. The DOS concentrations were estimated based on the extraction efficiency for DOC (that is, the percentage of DOC_{SPE} in DOC) (Dittmar et al., 2008; Ksionzek et al., 2016; Pohlabeln et al., 2017).

2.6. Sediment organic carbon, nitrogen, sulfur analysis

Freeze-dried sediment samples of 0.5–1 g were treated with 1 N hydrochloric acid to remove calcium carbonate and acid-volatile sulfur, allowing for the detection of total particulate organic carbon, nitrogen and sulfur in sediments (POC_T, PON_T, POS_T). For the acid-resistant part of the sediment, the organic carbon, nitrogen and sulfur in the particles (POC_R, PON_R, POS_R) were replaced by 6 N hydrochloric acid treatment (Raven et al., 2021). After 6–8 h of acidification, Milli-Q water was used to remove excess hydrochloric acid and inorganic salts containing carbon, nitrogen and sulfur. The samples were then dried using a freeze dryer. Accurately weighing 20–40 mg sediment samples was analyzed using a CHNS elemental analyzer (vario EL cube, Elementar, Germany).

2.7. Microbial community composition analysis

Sediment samples were collected at different periods throughout the cultivation process using sampling ports. These were then transferred to 50 mL centrifuge tubes and preserved at −80 °C until subsequent analysis. Extraction of 16S rRNA genes, representative of the bacterial community within the sediment, was performed using the PowerSoil DNA Isolation Kit (MoBio, Carlsbad, USA). Bacterial communities in the culture system were subjected to full-length 16S rRNA gene biodiversity sequencing using PacBio (Rhoads and Au, 2015). The extracted DNA was PCR amplified using full-length 16S rDNA universal primers such as 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′). Sequencing and sequence delivery were

supported by Shanghai HanYu Biotechnology Co., Ltd. The original sequencing data was processed using the SMRTLINK software to obtain circular consensus sequencing (Accuracy rate: 99 %). Following the acquisition of non-redundant sequences and removal of single-copy sequences, the processed sequences were clustered into operational taxonomic units (OTUs) using Usearch v11 (Edgar, 2010), based on a similarity threshold of 98.65 % (Kim et al., 2014), and were classified and annotated using the SILVA (138.1v) reference database (Pelin et al., 2013). The α-diversity of the bacterial community structure was calculated and analyzed using Mothur v. 1.48.0 (http://www.mothur.org). The raw sequencing data of genes obtained by Pacbio in this study have been registered in the NODE database (The National Omics Data Encyclopedia) under Project ID OEP004228 (full-length 16S rDNA, 8 samples).

2.8. Data analysis

Cluster analysis and principal component analysis (PCA) were performed on the study area based on the observed values of each station (including nutrients, chlorophyll, DOC, DON, DOS, POC, PON, POS and the molar ratio of different elements). Statistical test analysis of the in-situ survey result data was performed using one-way ANOVA in SPSS software (version R26.0.0.0). This analysis included significant tests of changes in organic carbon, organic nitrogen, organic sulfur, and element stoichiometric ratios, with a confidence interval set at 95 %.

3. Results

3.1. Spatial distribution and influencing factors of organic sulfur in bottom water

This study investigated the spatial distribution characteristics of particulate and dissolved organic matter (C, N, S) and their stoichiometric relationships (S:C, S:N, C:N) in the bottom water of the Yangtze River Estuary and the adjacent East China Sea in July 2021 (Fig. 2a–f). Compared with the organic carbon and organic nitrogen, which displayed a clear pattern of high concentration in the estuary and low concentration in the offshore area, organic sulfur showed a heterogeneous distribution with no discernable spatial pattern. Organic sulfur constitutes a small portion of the organic matter in the bottom water, with POS concentration ranging from 0.42 to 2.69 μmol S/L (average of 1.32 ± 0.515 μmol S/L) and DOS content ranging from 0.65 to l.99 μmol S/L (average 1.16 ± 0.441 μmol S/L). From an elemental stoichiometry perspective, the S:C and S:N ratios in particulate organic matter were lower in the estuary than offshore (Fig. A2). The average S:C for particulate organic matter was 0.04 ± 0.023, and the S:N was 0.59 ± 0.220. In contrast, the S:C and S:N ratios for dissolved organic matter averaged at 0.01 ± 0.004 and 0.14 ± 0.085, respectively.

A correlation analysis identified key eco-chemical indices linked to organic matter molecules in the bottom waters of Yangtze River Estuary and the adjacent East China Sea (Fig. 3a). Dissolved oxygen, nitrate, and silicate displayed similar significant positive correlations ($p < 0.05$) with POC, DOC, PON, and DON, while exhibiting a negative association with the molar ratios of carbon-nitrogen, sulfur-carbon, and sulfur-nitrogen ratios of particulate organic matter. Conversely, bottom water phosphate and ammonium displayed positive correlations with organic

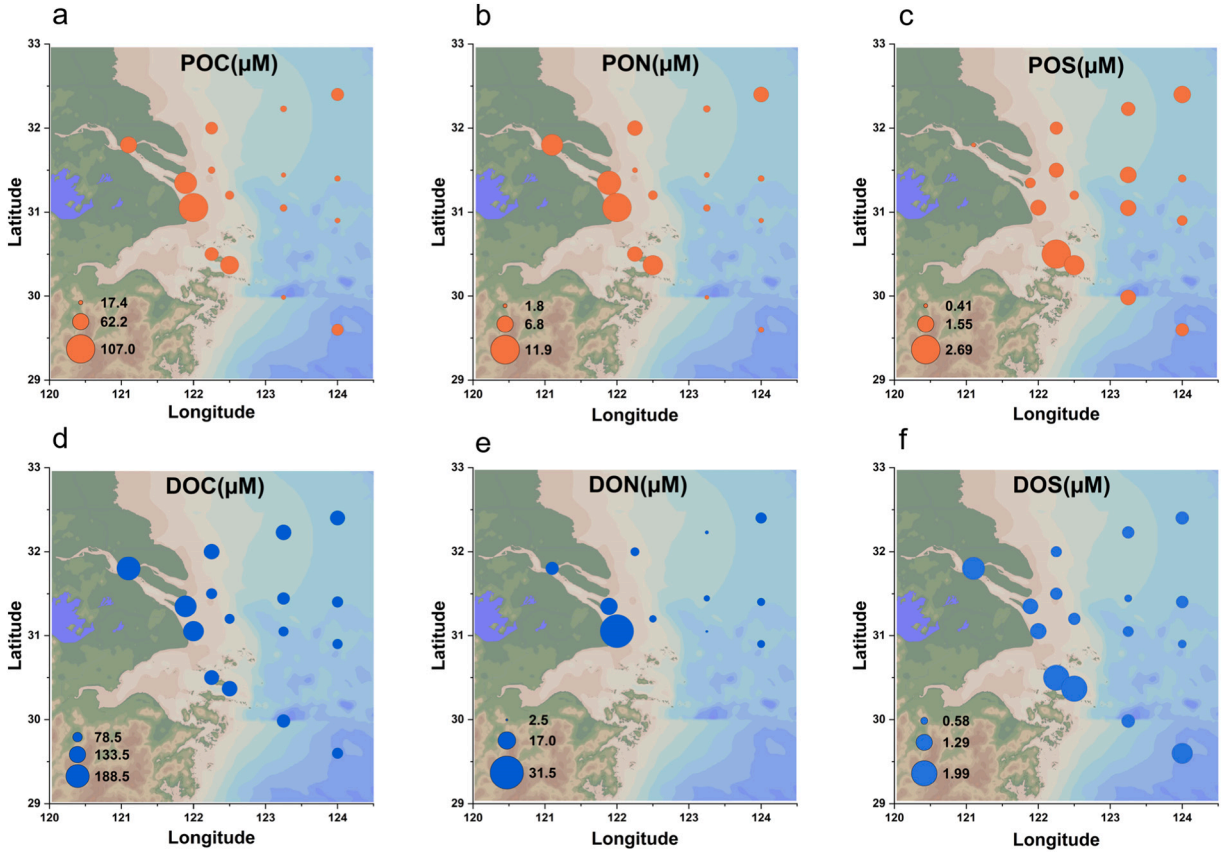


Fig. 2. Distribution characteristics of organic matter in the bottom water of the Yangtze River Estuary and the adjacent East China Sea. a–c) Representing the concentrations of POC, PON and POS in the bottom water, respectively; d–f) Representing the concentrations of DOC, DON and DOS in the bottom water, respectively.

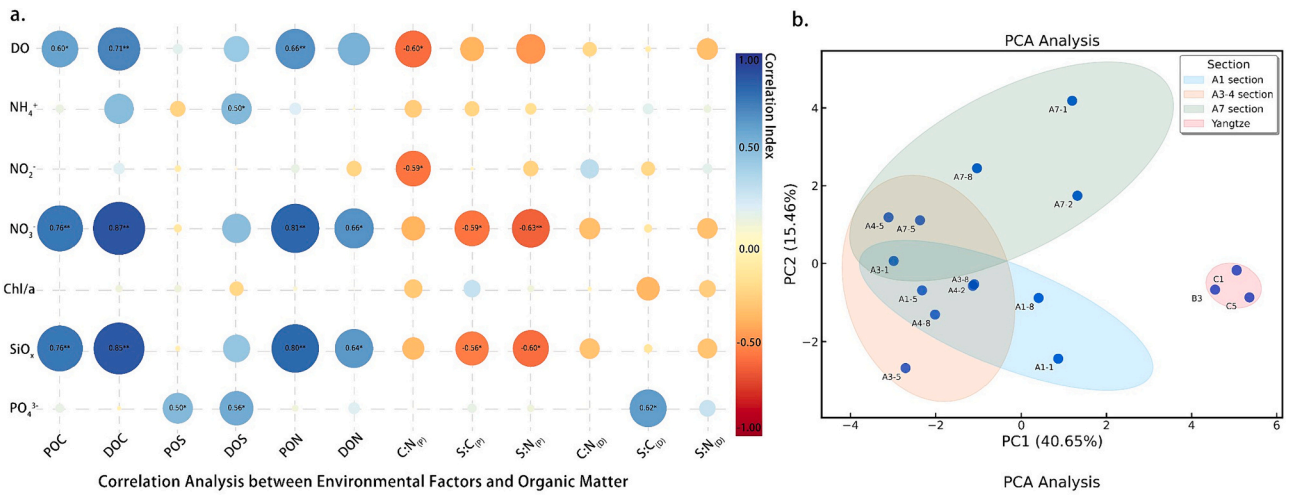


Fig. 3. Correlation and clustering analysis of particulate matter, dissolved organic matter, and environmental factors in the bottom water of the Yangtze River Estuary and the adjacent East China Sea. a) Correlation Analysis between environmental factors and organic matter. The size of the circle represents the strength of the correlation coefficient. An asterisk (*) denotes a significant correlation between the two parameters with $p < 0.05$, while two asterisks (**) indicate a highly significant correlation with $p < 0.01$ ($n = 16$). b) PCA analysis in water sample stations. The ellipses represent the 95 % confidence intervals of different sampling station groups. The legend identifies the different groups of sampling stations.

sulfur ($p < 0.05$). To distinguish differences between various water masses in the Yangtze River Estuary and the adjacent East China Sea, PCA of environmental parameters and organic matter characteristic parameters was conducted for all bottom water stations. The PCA revealed the differences between transects A1 and A7 in the sampling area of the Yangtze River Estuary and the adjacent East China Sea (Fig. 3b). The intermediate transects A3 and A4 were primarily situated between A1 and A7, while the three stations within the Yangtze River Estuary formed a separate cluster (C1, C5, and B3). This analysis shed light on the primary covariation factors connected to organic carbon in the bottom water of the estuary and adjacent seas. Notably, the PCA effectively differentiated the geographical distribution and the north-south variances in water environmental parameters within the Yangtze River Estuary and the adjacent East China Sea.

3.2. Spatial distribution of organic sulfur in sediments

The organic carbon, nitrogen and sulfur in the surface sediments of the Yangtze River Estuary and its adjacent coastal waters were analyzed (Fig. 1). The absolute quantities of particulate organic carbon, nitrogen, and sulfur in these samples varied widely, with spatial differences revealed through cluster analysis (Fig. 4). Particulate organic carbon

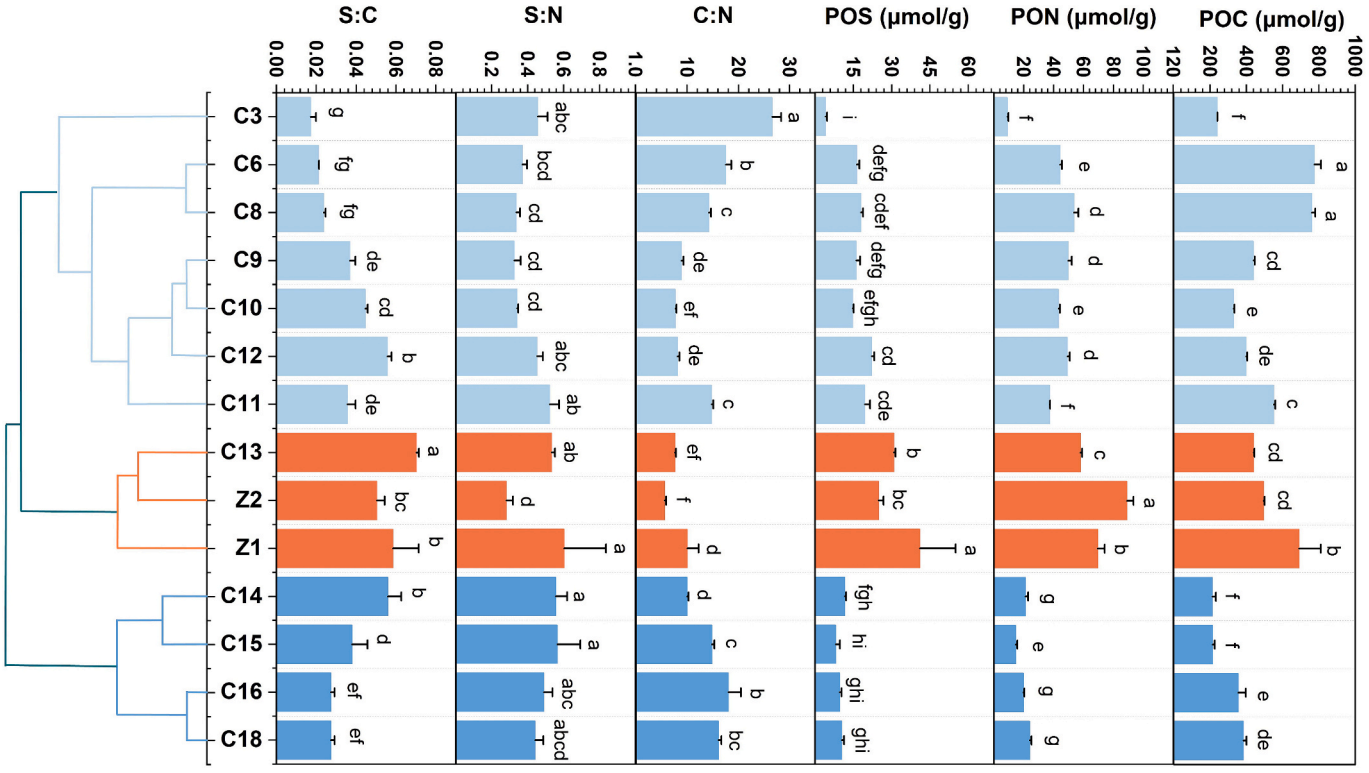


Fig. 4. Distribution and clustering characteristics of organic matter in surface sediments of the Yangtze River estuary and adjacent seas.

showed an overall decreasing trend from the estuary to the continental shelf, while particulate organic nitrogen and sulfur first increased, then decreased. The change trend of sediment particle size was opposite to that of organic sulfur content (Fig. A3). In the estuary and offshore, the sediment particle size (according to the D50 value of sediment particle size) decreased from 175.6 μm (C3) to 10.8 μm (Z2), and then increased to 203.7 μm. The elemental stoichiometric ratio of S:C in the organic matter showed paralleled the changes in POS content, ranging between 0.014 and 0.071, while the S:N range was 0.28–0.75. The C:N ratio was highest in the estuary, reaching a maximum of 27.25 and declining to 7.59 (station C13) towards the inner continental shelf, then increasing again to 10.37–18.02 (stations C14–C18) in the outer continental shelf. Station C13 had significantly higher S:C (0.07 ± 0.001), S:N (0.53 ± 0.019), and lower C:N (7.59 ± 0.22) than the surrounding stations. The area around station C13 was also identified as a frequent area of hypoxia during sampling, suggesting that dissolved oxygen levels may affect the stoichiometric relationship between organic carbon, nitrogen and sulfur in sediments.

The colors represent the different groups of stations clustered together based on their similarities in organic matter content. The letters on the right side of the column represent statistically significant differences ($p < 0.05$) between groups. Parameter values are arranged from highest to lowest based on the letter order. Stations with the same letters do not have statistically significant differences ($p > 0.05$).

In order to explore the stoichiometric differences of organic sulfur in different retention forms, the organic S:C ratio of dissolved, particulate and sediment organic matter in the bottom water of the Yangtze River estuary and adjacent seas was compared (Fig. 5). Generally, from the estuary to the offshore environment, the S:C ratio of dissolved organic matter in the bottom water shows a narrow range of variation (not exceeding 0.02), while the S:C of organic matter in bottom water particles and sediments exhibits a similar, wider range of variation, exceeding 0.06. The statistically significant difference in the stoichiometric ratio of organic matter between dissolved and particulate and sediment forms ($p < 0.0001$) indicates that the carbon and sulfur composition of organic matter components in different states is heterogeneous and may be influenced by different processes.

3.3. Dynamic changes of organic sulfur during sediment incubation

The dynamic process of organic sulfur in sediments was explored using anoxic culture (Fig. 1c). After about 20 days, both culture systems reached anoxic levels (DO < 20 μmol/L) and maintained them until the experiment's end (600 day) (Fig. A1). During the culture process, the grain size of the sediment did not change significantly. The initial rapid DOC increase in the water column resulted from DOC release from the sediment shortly after mixing. Following system stabilization, DOC in both water and sediment gradually decreased over time (Fig. A1). By the end of the 600-day incubation, the water column and sediment DOC in both experimental systems (N3/N4) had decreased to 64.88 ± 5.36 μmol/L and 69.70 ± 3.06 μmol/L, respectively. Concurrently, the initial DOC in the sediment pore water had reduced to 338.05 ± 8.50 μmol/L and 350.54 ± 21.66 μmol/L. The total decrease in DOC content in the water and sediment pore water over the 600-day period was 558.23–575.54 μmol/L, with the lost DOC accounting for 58.82 % and 57.05 % of the systems, respectively. However, the DOC retention rate of in the water column was higher, at 1.6 times that of the sediment pore water.

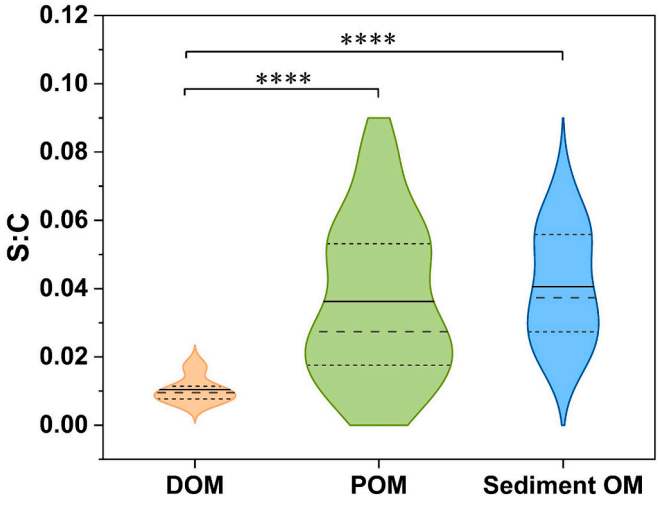


Fig. 5. Differences in S:C ratios of particulate organic matter, dissolved organic matter and sediment in the Yangtze River Estuary and the adjacent East China Sea (****, $p < 0.0001$).

The stoichiometric ratios of time-series samples from two identical sediment culture systems (N3 and N4) were analyzed (Fig. 6). The entire culture process was divided into three periods for observation: the short-term (0–25 days), the middle stage (25–70 days), and the long-term (70–600 days). The short-term period represented the culture system's gradual transition from aerobic to anoxic conditions. The middle stage corresponded to the sediment recovering and beginning to maintain anaerobic conditions, while the long-term represented the prolonged anoxic stage of the sediment. In the short-term period, especially during the first 9 days, the content of POC, PON, and POS in the sediment changed significantly. POC increased (N3, N4, $p < 0.05$), while PON and POS decreased (N4, $p < 0.05$) (Fig. 6a–f). The S:C significantly decreased (N3: 0.05 ± 0.004 to 0.033 ± 0.008, $p < 0.05$; N4: 0.05 ± 0.004 to 0.03 ± 0.007, $p < 0.05$), while there was no significant change in S:N ($p > 0.05$), and the C:N increased (N3: 5.572 ± 0.284 to 7.321 ± 0.346, $p < 0.05$; N4: 5.572 ± 0.284 to 7.385 ± 0.237, $p < 0.05$). Between 9 and 25 days, the proportional relationship between the elements did not change significantly. In the middle stage, the content of POC, PON and POS increased slightly, C:N, S:N remained stable, and S:C increased. Under long-term hypoxia, compared with POC and PON, POS content increased most significantly, and S:C increased significantly (N3: from 0.042 ± 0.002 to 0.081 ± 0.005, $p < 0.05$; N4: from 0.041 ± 0.001 to 0.048 ± 0.002, $p < 0.05$). Similarly, S: N also increased significantly (N3: from 0.315 ± 0.037 to 0.597 ± 0.007, $p < 0.05$. N4: from 0.272 ± 0.025 to 0.370 ± 0.017, $p < 0.05$), while C:N remained stable. In addition, the trend of acid-resistant organic matter is like that of total organic matter in the system. While approximately 62.3 % of POC, 56.2 % of PON, and 60.8 % of POS in the sediment organic matter before incubation could resist acid hydrolysis, the acid-resistant fraction increased rapidly during the short-term incubation process (0–9 days). Acid-resistant POC increased to 93.5 % (N3) and 97.5 % (N4), acid-resistant PON increased to 91.32 % (N3) and 93.67 % (N4), and acid-resistant POS increased to 77.31 % (N3) and 91.31 % (N4). In the middle and late stages of culture, the acid hydrolysis resistance of organic matter in the sediments remained moderate and maintained at a high proportion (>75 %) until 600 days. On average, 93.74 % of POS, 95.35 % of PON and 93.17 % of POC in the two systems showed acid hydrolysis resistance. This suggests that after long-term anoxic culture, the organic matter components in the sediments became more resistant to acid hydrolysis.

3.4. Dynamic changes of microbial community during sediment incubation

Full-length amplification and sequencing of bacterial 16S rRNA in the sediments revealed high diversity maintained over 600 days. Throughout the entire incubation period, microbial community composition remained largely stable. Although the number of complete sequence signals obtained from full-length sequencing decreased from 8423 ± 1585 initially to 6654 ± 263 at 600 days, the community exhibited a decline in α-diversity indices (Richness, Shannon, Evenness

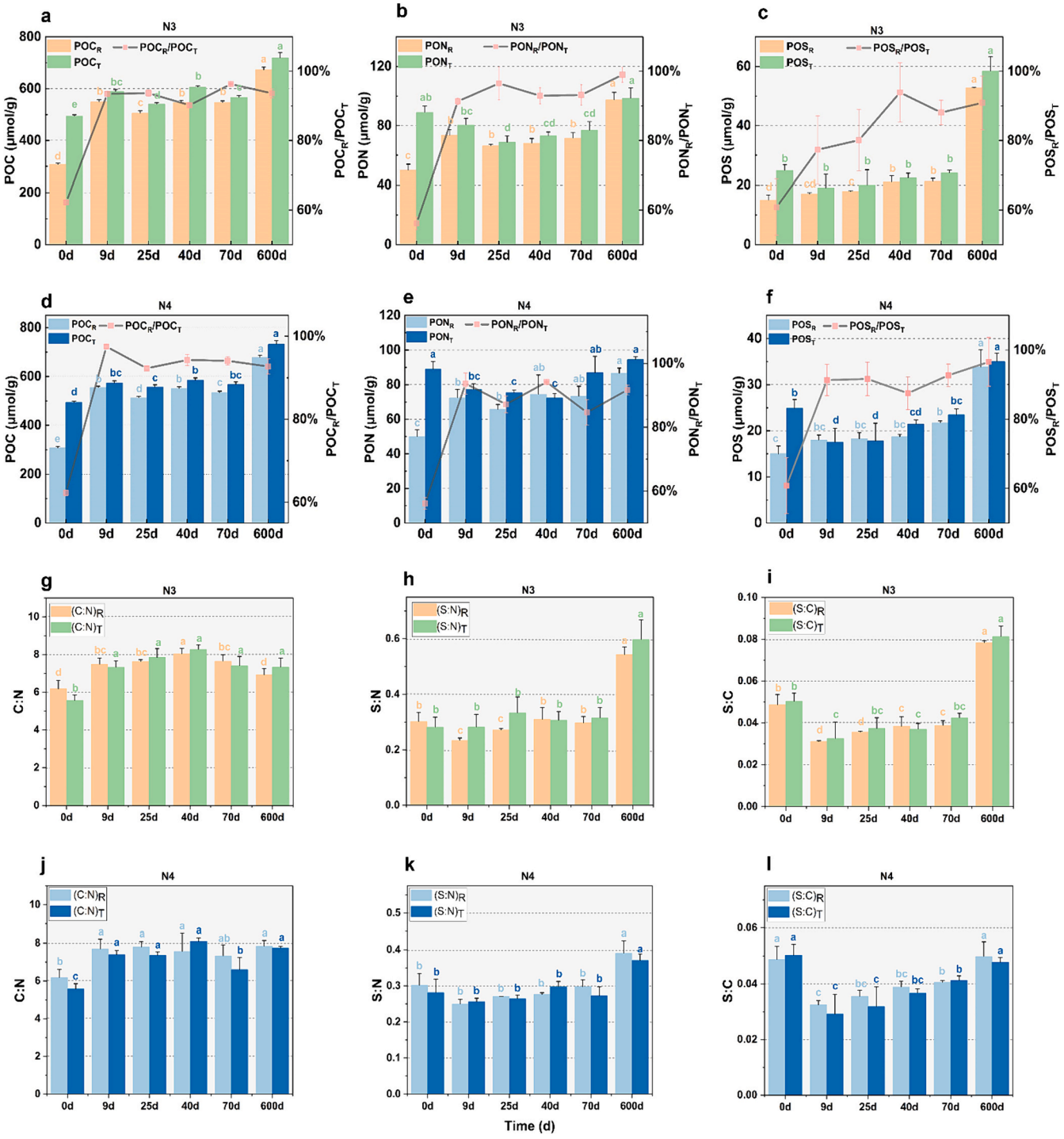


Fig. 6. Changes in organic carbon, nitrogen and sulfur in long-term culture system.
a–c) Changes in POC, PON, POS in N3 culture system. d–f) Changes of POC, PON, POS in N4 culture system. g–i) Changes in S:C, S:N, C:N in N3 culture system. j–l) Changes in S:C, S:N, C:N in N4 culture system. Subscript R represents the organic matter resistant to acid hydrolysis in sediments. Subscript T represents the total organic matter in sediments. For example, POC_R, organic carbon resistant to acid hydrolysis, POC_T, total particulate organic carbon. The letters above the column represent statistically significant differences in different periods ($p < 0.05$), and the acid-resistant hydrolyzed particulate organic matter and total particulate organic matter are distinguished by different colors. The broken line in Fig. a–f represents the proportion of acid-resistant organic matter content in the total organic matter content during the culture process.

index) (Fig. 7). This suggests that while most microbial taxa and DNA signals can persist in the environment for an extended period, some characteristic taxa gradually disappeared in the absence of a continuous supply of fresh organic matter. Throughout the incubation period, Proteobacteria, Planctomycetes, Bacteroidetes and Actinobacteria dominated the microbial composition in the sediments. In terms of relative abundance, nearly half of the core microbial taxa community proportion decreased, but some microbes adapted to microoxic, or anoxic conditions drove sulfur cycling, particularly core taxa such as, *Sulfurovum*, *Desulfatiglans*, and *Desulfuromonas*, increased their proportions by 2.81 %–4.39 %.

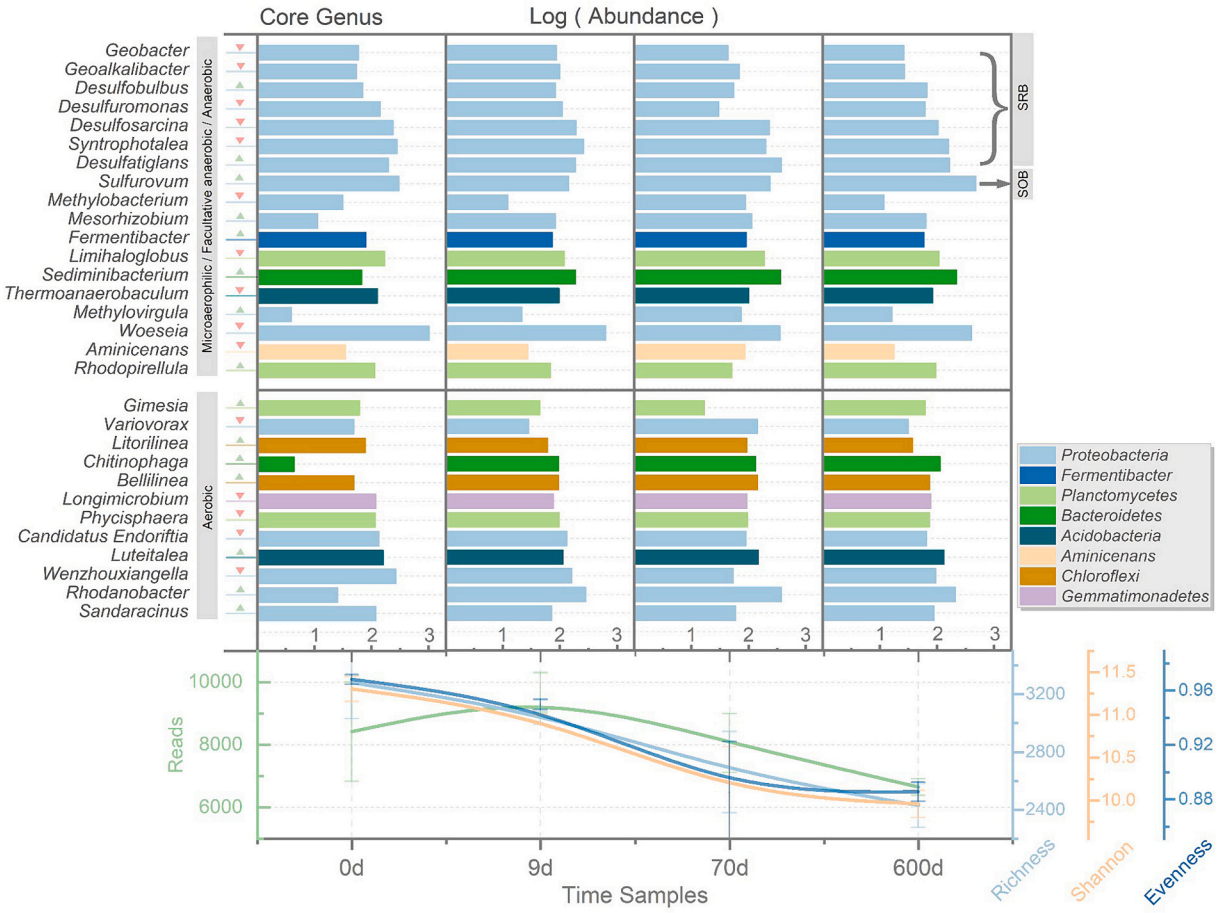


Fig. 7. Changes in microbial α-diversity and the abundance of core microbial taxa in 600-day anoxic sediment incubations. Changes in the abundance of core microbial genera (top 1 % relative abundance in each sample) throughout the incubation period (top) and the changes in α-diversity based on the 16S full-length sequencing results (bottom). Different bar colors represent different phyla to which the genera belong. Green triangles and red triangles indicate the increase and decrease, respectively, of the relative abundance of the genera from 0 to 600 days.

4. Discussion

4.1. The distribution pattern of organic sulfur in water and sediment

The complex estuarine-offshore environment, with its intricate interplay of sources and sinks for organic matter, explains the observed lack of correlation between all parameters and chlorophyll *a* in Fig. 3a. While terrestrial input contributes 7.40 Tg C to offshore carbon sequestration annually (Jiao et al., 2018), nutrient input also stimulates offshore primary production (Zhu et al., 2011). This results in a distinct distribution pattern for organic matter characteristics between the river mouth and the offshore area (Fig. 3b). The excessive nutrient input fuels rapid oxygen depletion in bottom waters due to enhanced decomposition of organic matter (Wang et al., 2012). Previous studies have indicated that seasonal hypoxia in the study area is substantially influenced by diatom growth (Zhou et al., 2020), which aligns with the observed positive correlation between silicate and organic carbon and nitrogen in our data. However, organic sulfur in the water column exhibits a different and potentially more complex distribution pattern, with distinct environmental factors influencing its distribution (Figs. 2c and f, 3a). Previous research has shown that organic sulfur undergoes faster loss through photochemical oxidation processes (Cutter et al., 2004), and the lack of correlation between organic sulfur and various environmental parameters suggests that its dynamics are more intricate than those of carbon and nitrogen (Longnecker et al., 2020). The observed positive correlation between organic sulfur and phosphate can be explained by the release of phosphate from resuspended anoxic sediments, which is often accompanied by the release of anoxic sulfurized organic matter (Pohlabeln et al., 2017).

Offshore sediments have a shallow oxygen penetration depths (Cai and Sayles, 1996), and seasonal hypoxia occurs frequently in the Yangtze River Estuary and the adjacent East China Sea (Lu et al., 2017). Notably, our on-site sediment profile revealed significantly high organic sulfur content and S:C ratios in the offshore area outside the Yangtze River estuary (core station C13, Z1 and Z2) (Fig. 4). This area persistently lacks oxygen in its upper layer (Wei et al., 2017; Zhu et al., 2011), and the surface sediment contains a higher number of sulfate-reducing gene copies (He et al., 2015). Microbial sulfate reduction promotes the formation of organic sulfur in sinking marine particles (Raven et al., 2021). We hypothesize that microbial sulfate reduction also drives organic sulfur production in the offshore environment's sediment. Sediment culture experiments were designed to observe this process.

4.2. Accumulation of organic sulfur in sedimentary culture

Microbial sulfur cycling is known to flourish under anoxic conditions (Callbeck et al., 2021), and sulfur-rich organic components have been identified in oxygen-depleted seawater/sediment environments (Gomez-Saez et al., 2021; Ksionzek et al., 2016; Raven et al., 2021). This aligns with the results of our long-term cultivation experiment. Microorganisms exhibited different sulfur metabolism at different time points. In the early stage, the homogenized sediment still contained oxygen (Fig. A1). Oxygen promoted rapid heterotrophic metabolism by microorganisms (Holmer, 1999), evidenced by the decrease in organic

nitrogen and sulfur in sediment particles (Fig. 7). However, an unexpected increase in sediment organic carbon occurred, accompanied by a decrease in DOC in the pore water. Previous studies have suggested that rapid aggregation of high DOC concentrations can explain this increase in POC (Engel et al., 2004). Additionally, abundant chemolithoautotrophic bacterial communities in the sediments (such as *Sulfurovum*) might utilize the carbon dioxide produced by heterotrophic bacteria to generate organic matter (Sun et al., 2023), further contributing to the POC elevation in the later stage. Microorganisms selectively utilize organic matter, as evidenced by the significant decrease in S:C and S:N ratios within the first 9 days, indicating that particulate organic sulfur is preferentially metabolized by heterotrophic metabolism over C:N during the initial aerobic phase. Rapid consumption of labile organic matter also led to rapid oxygen depletion in the sediment (Fig. A1), prompting some microorganisms to die off, causing the death of some aerobic microorganisms after 9 days. However, sediment anoxia provided a favorable environment for anaerobic microorganisms, such as *Desulfosarcina*, which was a major group of sulfate-reducing bacteria in the system (Fig. 7). Sulfate-reducing bacteria promote the formation of particulate organic sulfur (Raven et al., 2021), which is consistent with the gradual accumulation of organic sulfur observed in the mid-cultivation stage of this study. As the sulfate-reducing bacterial population and anaerobic environment stabilize, organic sulfur significantly accumulates in the late cultivation stage. Although the content of particulate organic carbon and nitrogen also increases in the late cultivation stage, this is mainly attributed to the decrease in dissolved organic carbon consistent with the early cultivation stage to produce aggregated particles (Engel et al., 2004). The C:N ratio stabilizes overall between 6 and 8 after the mid-cultivation stage, indicating that organic carbon and nitrogen aggregate into particles through the same process. However, the significant increase in S:C indicates that sulfurization, besides the aggregation process, is a factor promoting the increase in organic sulfur content. Furthermore, during cultivation, the acid hydrolysis resistance of organic matter including carbon, nitrogen, and sulfur improves (Fig. 6a–f), consistent with studies on sediment particles in OMZ marine areas (Raven et al., 2021), suggesting that the sulfurization process has the potential to enhance the long-term preservation of organic matter in offshore sediments.

4.3. The source of organic sulfur in offshore sediments

Sulfur-containing photosynthetic lipid particles contribute approximately 4 % of organic carbon to the deep sea through sedimentation (Gasparovic et al., 2018), Within oxygen-depleted zones, the anaerobic sulfurization process inside particles further enhances the transport of organic sulfur to sediments (Raven et al., 2021), with higher particle settling fluxes in offshore areas compared to the open ocean. Previous studies have indicated that particle settling transports organic matter to the sediment, serving as a source of organic matter burial in the estuary and nearby sea (Berner, 1982; Liu et al., 2009). In our research, the similar molar S:C characteristics between bottom water and sediment in this study also support this view (Fig. 5), the settling of particulate matter in bottom water transports organic sulfur to offshore sediments. Furthermore, the frequent hypoxia in the offshore area of the Yangtze River Estuary triggers the microbial sulfate reduction process in sediments, creating conditions for the formation of organic sulfur through sulfurization. We observed that sediments from the hypoxic core area contained higher levels of organic sulfur and a higher molar S:C ratio (Fig. 4) on-site. Further long-term anaerobic culture of sediments revealed the accumulation of organic sulfur and an increase in the molar S:C ratio (Fig. 6), indicating that besides particle settling, sulfurization processes serve as another source of organic sulfur in offshore sediments. This finding provides a new perspective on the source of organic sulfur in offshore sediment. In addition to particle settling, the process of organic sulfur production in offshore sediment is also important when studying the source of organic sulfur in offshore environments.

5. Conclusions

This study investigated the spatial distribution of organic sulfur in water and sediments of the Yangtze River Estuary and the adjacent East China Sea. The similar molar S:C ratios between particulate organic matter and sediment suggest that particle settling is a potential source of organic sulfur in sediment. Furthermore, through sediment cultivation experiments, the study explored the process of organic sulfur production within the sediment itself. These results indicate that anaerobic processes governed by microbial sulfate reduction within sediment can lead to organic sulfur production and further accumulation.

CRediT authorship contribution statement

Changjie Dong: Writing – original draft, Investigation, Formal analysis. **Quanrui Chen:** Writing – original draft, Investigation, Formal analysis. **Junhui Chen:** Writing – original draft, Investigation, Formal analysis. **Liang Dong:** Resources. **Yaojin Chen:** Resources. **Nianzhi Jiao:** Funding acquisition, Conceptualization. **Kai Tang:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

We declare that we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.171599.

References

Abdulla, H.A., Burdige, D.J., Komada, T., 2020. Abiotic formation of dissolved organic sulfur in anoxic sediments of Santa Barbara Basin. Org. Geochem. 139, 103879.
Berner, R.A., 1982. Burial of organic carbon and pyrite sulfur in the modern ocean: its geochemical and environmental significance. Am. J. Sci. 282, 451–473.
Cai, W.J., Sayles, F.L., 1996. Oxygen penetration depths and fluxes in marine sediments. Mar. Chem. 52, 123–131.
Callahan, J., Dai, M., Chen, R.F., Li, X., Lu, Z., Huang, W., 2004. Distribution of dissolved organic matter in the Pearl River Estuary, China. Mar. Chem. 89, 211–224.
Callbeck, C.M., Canfield, D.E., Kuypers, M., Yilmaz, P., Bristow, L.A., 2021. Sulfur cycling in oceanic oxygen minimum zones. Limnol. Oceanogr. 66, 2360–2392.
Chen, C.A., Lin, C., Hung, B., 1996. Stoichiometry of carbon, hydrogen, nitrogen, sulfur and oxygen in the particulate matter of the western North Pacific marginal seas. Mar. Chem. 179–190.
Cutter, G.A., Cutter, L.S., Filippino, K.C., 2004. Sources and cycling of carbonyl sulfide in the Sargasso Sea. Limnol. Oceanogr. 49, 555–565.
Dai, M., Wang, L., Guo, X., Zhai, W., Li, Q., He, B., K. S.-J., 2008. Nitrification and inorganic nitrogen distribution in a large perturbed river/estuarine system: the Pearl River Estuary, China. Biogeosciences 5, 1227–1244.
Deng, B., Zhang, J., Wu, Y., 2006. Recent sediment accumulation and carbon burial in the East China Sea. Global Biogeochem. Cycles 20, GB3014.

Dittmar, T., Koch, B., Hertkorn, N., Kattner, G., 2008. A simple and efficient method for the solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater. Limnol. Oceanogr. Methods 6, 230–235.
Dittmar, T., Stubbins, A., Ito, T., Jones, D.C., 2017. Comment on "Dissolved organic sulfur in the ocean: biogeochemistry of a petagram inventory". Science 356, 813.
Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460–2461.
Engel, A., Thoms, S., Riebesell, U., Rochelle-Newall, E., Zondervan, I., 2004. Polysaccharide aggregation as a potential sink of marine dissolved organic carbon. Nature 428, 929–932.
Gasparovic, B., Penezic, A., Frka, S., Kazazic, S., Lampitt, R.S., Holguin, F.O., Sudasinghe, N., Schaub, T., 2018. Particulate sulfur-containing lipids: production and cycling from the epipelagic to the abyssopelagic zone. Deep Sea Res. Part I: Oceanogr. Res. Papers 134, 12–22.
Gomez-Saez, G.V., Dittmar, T., Holtappels, M., Pohlabeln, A.M., Lichtschlag, A., Schnetger, B., Boetius, A., Niggemann, J., 2021. Sulfurization of dissolved organic matter in the anoxic water column of the Black Sea. Sci. Adv. 7, eabf6199.
Guo, X., Lu, D., Niu, Z., Feng, J., Chen, Y., Tou, F., Liu, M., Yang, Y., 2018. Bacterial community structure in response to environmental impacts in the intertidal sediments along the Yangtze Estuary, China. Mar. Pollut. Bull. 126, 141–149.
He, H., Zhen, Y., Mi, T., Xu, B.C., Wang, G., Zhang, Y., Yu, Z., 2015. Community composition and distribution of sulfate- and sulfite-reducing prokaryotes in sediments from the Changjiang estuary and adjacent East China Sea. Estuar. Coast. Shelf Sci. 165, 75–85.
Holmer, M., 1999. The effect of oxygen depletion on anaerobic organic matter degradation in marine sediments. Estuar. Coast. Shelf Sci. 48, 383–390.
Jiao, N., Liang, Y., Zhang, Y., Liu, J., Zhang, Y., Zhang, R., Zhao, M., Dai, M., Zhai, W., Gao, K., Song, J., Yuan, D., Li, C., Lin, G., Huang, X., Yan, H., Hu, L., Zhang, Z., Wang, L., Cao, C., Luo, Y., Luo, T., Wang, N., Dang, H., Wang, D., Zhang, S., 2018. Carbon pools and fluxes in the China seas and adjacent oceans. Sci. China (Earth Sci.) 61, 1535–1563.
Keil, H., 1995. Sedimentary organic matter preservation: an assessment and speculative synthesis. Mar. Chem. 49, 137–139.
Kim, M., Oh, H.S., Park, S.C., Chun, J., 2014. Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. Int. J. Syst. Evol. Microbiol. 64, 346–351.
Koch, B.P., Ksionzek, K.B., Lechtenfeld, O.J., McCallister, S.L., Schmitt-Kopplin, P., Geuer, J.K., Geibert, W., 2017. Response to comment on "dissolved organic sulfur in the ocean: biogeochemistry of a petagram inventory". Science 356, 813.
Ksionzek, K.B., Lechtenfeld, O.J., McCallister, S.L., Schmitt-Kopplin, P., Geuer, J.K., Geibert, W., Koch, B.P., 2016. Dissolved organic sulfur in the ocean: biogeochemistry of a petagram inventory. Science 354, 456–459.
Liu, S., Hong, G., Zhang, J., Ye, X., Jiang, X., 2009. Nutrient budgets for large Chinese estuaries. Biogeosciences 6, 2245–2263.
Liu, L., Sun, F., Zhao, H., Mi, H., He, S., Chen, Y., Liu, Y., Lan, H., Zhang, M., Wang, Z., 2021. Compositional changes of sedimentary microbes in the Yangtze River Estuary and their roles in the biochemical cycle. Sci. Total Environ. 760, 143383.
Longnecker, K., Oswald, L., Kido Soule, M.C., Cutter, G.A., Kujawinski, E.B., 2020. Organic sulfur: a spatially variable and understudied component of marine organic matter. Limnol. Oceanogr. Lett. 5, 305–312.
Lu, W., Xiang, X., Yang, L., Xu, Y., Li, X., Liu, S., 2017. The temporal-spatial distribution and changes of dissolved oxygen in the Changjiang Estuary and its adjacent waters for the last 50 a. Acta Oceanol. Sin. 36, 90–98.
Moran, M.A., Durham, B.P., 2019. Sulfur metabolites in the pelagic ocean. Nat. Rev. Microbiol. 17, 665–678.
Pai, S.C., Tsau, Y.J., Yang, T.I., 2001. pH and buffering capacity problems involved in the determination of ammonia in saline water using the indophenol blue spectrophotometric method. Anal. Chim. Acta 434, 209–216.
Pelin, Y., Wegener, P.L., Pablo, Y., Jan, G., Elmar, P., Christian, Q., Timmy, S., Jörg, P., Wolfgang, L., Oliver, G.F., 2013. The SILVA and "All-species Living Tree Project (LTP)" taxonomic frameworks. Nucleic Acids Res. 42, 643–648.
Phillips, A.A., White, M.E., Seidel, M., Wu, F., Pavia, F.F., Kemeny, P.C., Ma, A.C., Aluwihare, L.I., Dittmar, T., Sessions, A.L., 2022. Novel sulfur isotope analyses constrain sulfurized porewater fluxes as a minor component of marine dissolved organic matter. Proc. Natl. Acad. Sci. U. S. A. 119, e2209152119.
Pohlabeln, A.M., Gomez-Saez, G.V., Noriega-Ortega, B.E., Dittmar, T., 2017. Experimental evidence for abiotic sulfurization of marine dissolved organic matter. Front. Mar. Sci. 4, 364.
Raven, M.R., Sessions, A.L., Adkins, J.F., Thunell, R.C., 2016. Rapid organic matter sulfurization in sinking particles from the Cariaco Basin water column. Geochim. Cosmochim. Acta 190, 175–190.
Raven, M.R., Keil, R.G., Webb, S.M., 2021. Microbial sulfate reduction and organic sulfur formation in sinking marine particles. Science 371, 178–181.
Rhoads, A., Au, K.F., 2015. PacBio sequencing and its applications. Genomics Proteomics Bioinformatics 13, 278–289.
Shriwastav, A., Sudarsan, G., Bose, P., 2010. Modification of Winkler's method for determination of dissolved oxygen concentration in small sample volumes. J. Am. Vet. Med. Assoc. 2, 1618–1622.
Sun, Q., Xu, K., Cao, L., Du, Z., Wang, M., Sun, L., 2023. Nitrogen and sulfur cycling driven by Campylobacterota in the sediment-water interface of deep-sea cold seep: a case in the South China Sea. mBio 14, e0011723.
Vandenberghe, Jef, 2013. Grain size of fine-grained windblown sediment: a powerful proxy for process identification. Earth Sci. Rev. 121, 18–30.
Wang, B., Wei, Q., Chen, J., Xie, L., 2012. Annual cycle of hypoxia off the Changjiang (Yangtze River) Estuary. Mar. Environ. Res. 77, 1–5.
Wei, Q., Wang, B., Yu, Z., Chen, J., Xue, L., 2017. Mechanisms leading to the frequent occurrences of hypoxia and a preliminary analysis of the associated acidification off the Changjiang estuary in summer. Sci. China-Earth Sci. 60, 360–381.
Zhang, J., Liu, S., Ren, J., Wu, Y., Zhang, G., 2007. Nutrient gradients from the eutrophic Changjiang (Yangtze River) Estuary to the oligotrophic Kuroshio waters and re-evaluation of budgets for the East China Sea Shelf. Prog. Oceanogr. 74, 449–478.
Zhou, F., Chai, F., Huang, D., Wells, M., Li, H., 2020. Coupling and decoupling of high biomass phytoplankton production and hypoxia in a highly dynamic coastal system: the Changjiang (Yangtze River) estuary. Front. Mar. Sci. 7, 259.
Zhu, Z., Zhang, J., Wu, Y., Zhang, Y., Lin, J., Liu, S., 2011. Hypoxia off the Changjiang (Yangtze River) Estuary: oxygen depletion and organic matter decomposition. Mar. Chem. 125, 108–116.