Perspective

Microbial sulfurization stimulates carbon sequestration in marine oxygen minimum zones

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The organosulfur cycle gradually changed with the history of oxygen on Earth, and it affected the evolution of living organisms. As one of the most ancient pathways, assimilatory sulfate reduction for sulfur amino acid synthesis could have provided up to 75% of the reduced sulfur needed for life processes in the paleo-seawater environment, which was characterized by very low sulfate concentrations. Dissimilatory reduction of sulfate to hydrogen sulfide appeared to prevail globally until the start of ocean oxygenation around 2.7 billion years ago [1], supporting a substantial portion of sedimentary sulfur pools and associated carbon mineralization. The mineralization of organic sulfur could provide additional sulfur sources for in-sediment sulfate reduction processes, in the absence of ambient sulfate, potentially contributing to 20%–100% of pyrite deposits in Neoarchean oceans [1]. The elevated levels of sulfate could cause a more significant dissimilatory sulfate reduction, which became the main source of sulfide for sedimentary pyrite and organic carbon sulfurization, especially in high-sulfate environments such as those observed in present-day oceans. On the other hand, the ocean became increasingly oxidized, and more organic matter containing oxidized sulfur could be generated by microbes. For example, sulfonates might account for a significant fraction of organic sulfur pools present in the modern marine sediments (up to 20%–40%), and they were deposited deep in the sediment columns [2]. Organic matter in black shales, deposited during the mid-Cretaceous Oceanic Anoxic Event 2 ($\sim$94 Ma), was characterized by high S:C ratios and low δ^{34}S values, suggesting that sulfur promoted organic carbon burial in the ocean [3]. Recent studies indicate that, in the oxygen minimum zones (OMZs) of the eastern tropical North Pacific, microbial sulfate reduction could enhance carbon preservation through the formation of sulfurized particulate organic carbon (POC), which primarily consists of alkyl sulfides, disulfides, aromatics, sulfate esters, sulfonates and sulfoxides [4]. In addition to POC for carbon sequestration, the microbial carbon pump (MCP) reveals the microbial transformation of labile dissolved organic carbon (DOC) into refractory dissolved organic carbon (RDOC) [5]. The accumulation of DOC with a high S:C ratio in the Black Sea has been shown to

occur in oxygen-deficient zones [6]. Thus, marine sulfur cycling deeply impacts carbon biogeochemistry in oxygen-deficient water.

Organic sulfur is widely distributed in the ocean, and it generally represents a substrate that heterotrophic bacteria utilize to obtain nutrients and for their energy metabolism [7]. The inventory of marine dissolved organic sulfur (>6700 Tg S) is far greater than that of the particulate one (200–400 Tg S) (1 Tg = 10^6 tons) (Fig. 1) [8]. In the upper ocean layer, a large amount of sulfur in the form of dissolved and particulate organic matter (600 and 720 Tg S a^{-1}, respectively), enters the microbial loop through sulfate assimilation (Fig. 1) [7]. Most of the newly generated organic sulfur is rapidly converted back into inorganic sulfate through microbial remineralization (Fig. 1). For instance, the rapid dimethylsulfoniopropionate (DMSP) cycling that occurs in this process (approximately 240 Tg S a^{-1}, and 18% of the annual production of organic sulfur) is relatively well studied due to its influence on climate regulation, acid precipitation, and sulfur transport from the marine or terrestrial environments to the atmosphere (Fig. 1) [9]. However, the DMSP was less than 1% of the total inventory of marine organic sulfur compounds, and the mechanisms that allow the maintenance of the vast and stable organic sulfur reservoirs in the deep sea remain unclear. Microorganisms have complex metabolic networks for the generation of organic sulfur that can cover more than 20 sulfur-containing chemical functional groups, including plenty of refractory compounds [7]. For example, more than 40 kinds of biogenic sulfolipids can resist decomposition and remain unaltered as they sink into the deep ocean [10]. Also, as a source of organic sulfur, the flux of marine sediments through resuspension has been estimated at around 30–200 Tg S a^{-1} [11]. The sink for marine biogenic sulfur compounds is represented by the transformation into recalcitrant ones via MCP, with an estimated annual production of approximately 1 Tg S [7] under normal oxygen levels.

In the oxygen-depleted seawaters of OMZs, the microbial sulfur cycle is a complex process in which sulfate-reducing and sulfur-oxidizing pathways are simultaneously active in confined spaces, such as within marine organic aggregates. Hydrogen sulfide, which is produced by microbial sulfate reduction, provides not only a major source of energy for inorganic carbon fixation, but also a reducing power for organic carbon synthesis (Fig. 2). Sulfate-reducing bacteria are active reactors of organic sulfur, considering that

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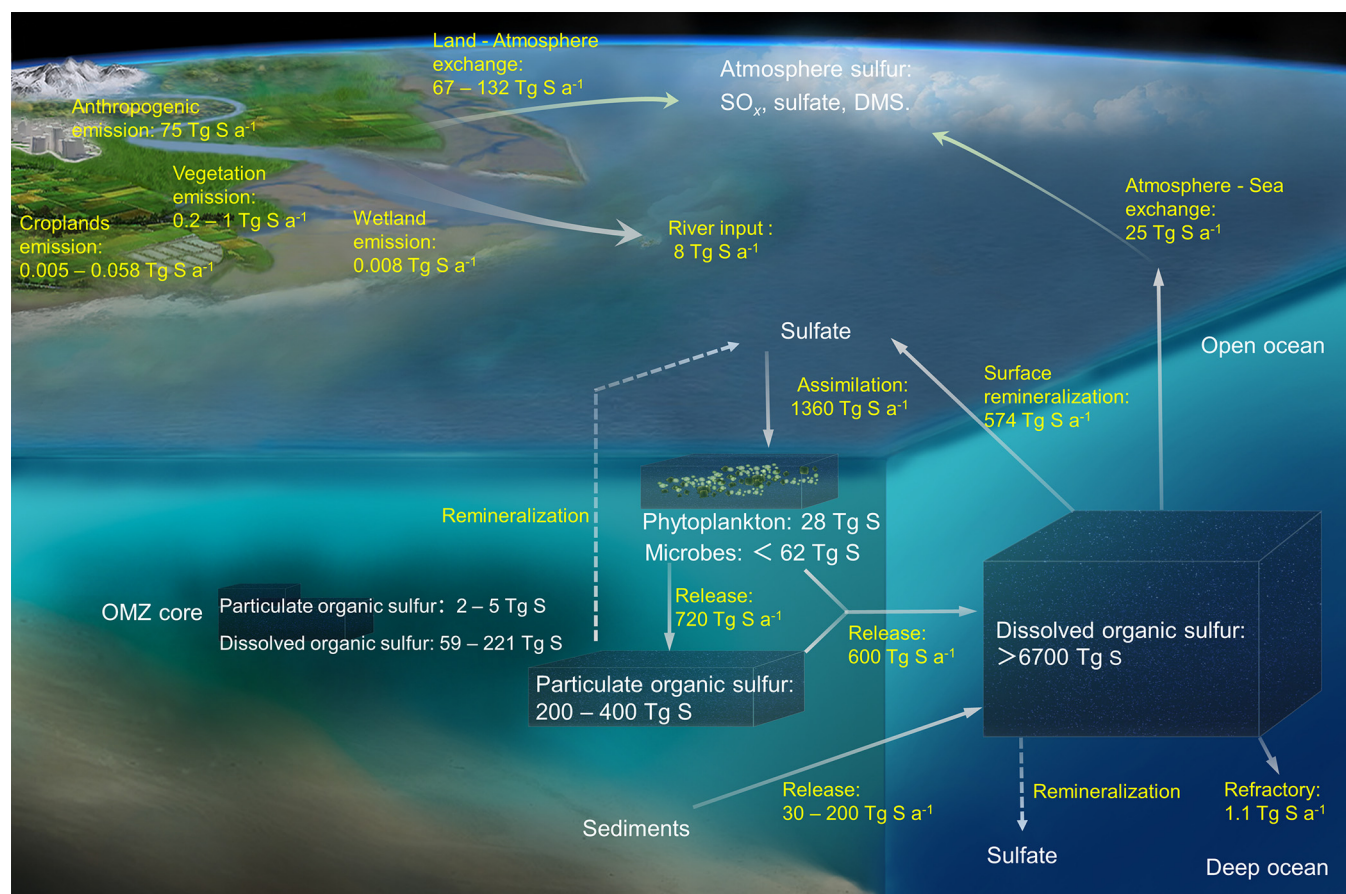 *E-mail addresses:* tangkai@xmu.edu.cn (K. Tang), jiao@xmu.edu.cn (N. Jiao).

Fig. 1. Schematic diagram of the global organosulfur cycle. White font indicates organic sulfur reservoirs; yellow font indicates the organic sulfur flux. Tg is the unit of measurement (1 Tg = 1 × 10^12 g); S represents organic sulfur. The concentration of sulfate in the global ocean is approximately 28 mmol/L. The dotted line represents an unknown flux. The OMZ core is defined by a central part with oxygen concentrations <4.5 μmol/L, and its size is estimated at about 4.61 × 10^14 m^3 in the global ocean (reference data for the estimation list are shown in the Supplementary materials). The estimation of organic sulfur content in OMZs is shown in Table S2 (online). The inventory and flux data of the marine organosulfur cycle, and the land-based sulfur release data are listed in the Table S4 (online).

locally produced sulfur compounds (sulfate and sulfide) could be incorporated into the biomass by sulfate-reducing bacteria. Sulfur-oxidizing bacteria can utilize organic polysulfides (R-Sn-H) as resources for further biosynthesis, using sulfide-quinone oxidoreductase or flavocytochrome c-sulfide dehydrogenase for sulfide oxidation, and the sulfur-oxidation system for thiosulfate oxidation [12]. Sulfur-oxidizers (such as the Gammaproteobacteria SUP05 group) have high affinities for sulfide, allowing them to maintain vanishingly low sulfide concentrations in OMZs, which in turn drive the marine cryptic sulfur cycling [13]. Furthermore, heterotrophic denitrifying (i.e., organic matter oxidation by nitrite) and anammox bacteria are recognized as major reactive nitrogen removers in OMZs [13]. Therefore, enhanced microbial processes for sulfur incorporation and nitrogen loss may lead to RDOC values characterized by increased S:C and C:N molar ratios. Sulfate-reducing, sulfur-oxidizing, denitrifying and anammox microbes are also thought to thrive in sinking particles in the OMZs. In addition to microbial sulfur transformation, the anoxic and heterogenous microenvironment associated with particles could facilitate the abiotic sulfurization of organic matter. Furthermore, in sulfidic seawaters, abiotic sulfurization of DOC could result in a S:C molar ratio of up to 15% (that of ambient seawater is between 2% and 8%), due to the fact that sulfide has a strong reducibility and nucleophilicity [11]. For instance, the widespread refractory components of carboxyl-rich aliphatic molecules could undergo a nucleophilic attack on their C=C bond by HS^- or S^{2-} to yield sulfurized compounds [3]. In OMZs, the precipitation of metal sulfides

as insoluble complexes could occur, but this process would account for only a small portion of the consumption of sulfide in anoxic seawater, due to the extremely low concentrations of reduced metals found in these zones [3]. The interactions of clay minerals with negatively charged, sulfur-containing functional groups may further promote the preservation of the organic sulfur contained in marine sediments (i.e., the coordination bond between sulfonate and the hydroxyl group on the surface of clay minerals). So far, the amount of global oceanic refractory organic sulfur has been underestimated because it still remains undetermined in OMZs, where microbes generate recalcitrant sulfur-containing compounds autonomously or in combination with abiotic processes.

The S:C ratio could reach twice or higher than that of normal seawater with the sulfurization reactions performed by microbial processes in the OMZs, compared to normal seawater (Table S1 online). The DOS inventory of the global OMZs core is estimated by the S:C ratio after organic carbon sulfurization. The estimated reservoir of organic sulfur in the OMZs core is in the teragram range (dissolved and particulate organic sulfur ranges are 59–221 and 2–5 Tg S, respectively) (Table S2 online and Fig. 1), and this is potentially similar to the amount of sulfur in marine phytoplankton and microbial biomass (>90 Tg S). In addition, according to the DOC at unit volume of OMZ core is 1.5–3 times higher than that in normal seawater which depth under euphotic zone (Table S3 online). Consistent with this, sulfide supply and the effect on DOC accumulation (2.5 times higher than normal seawater) were
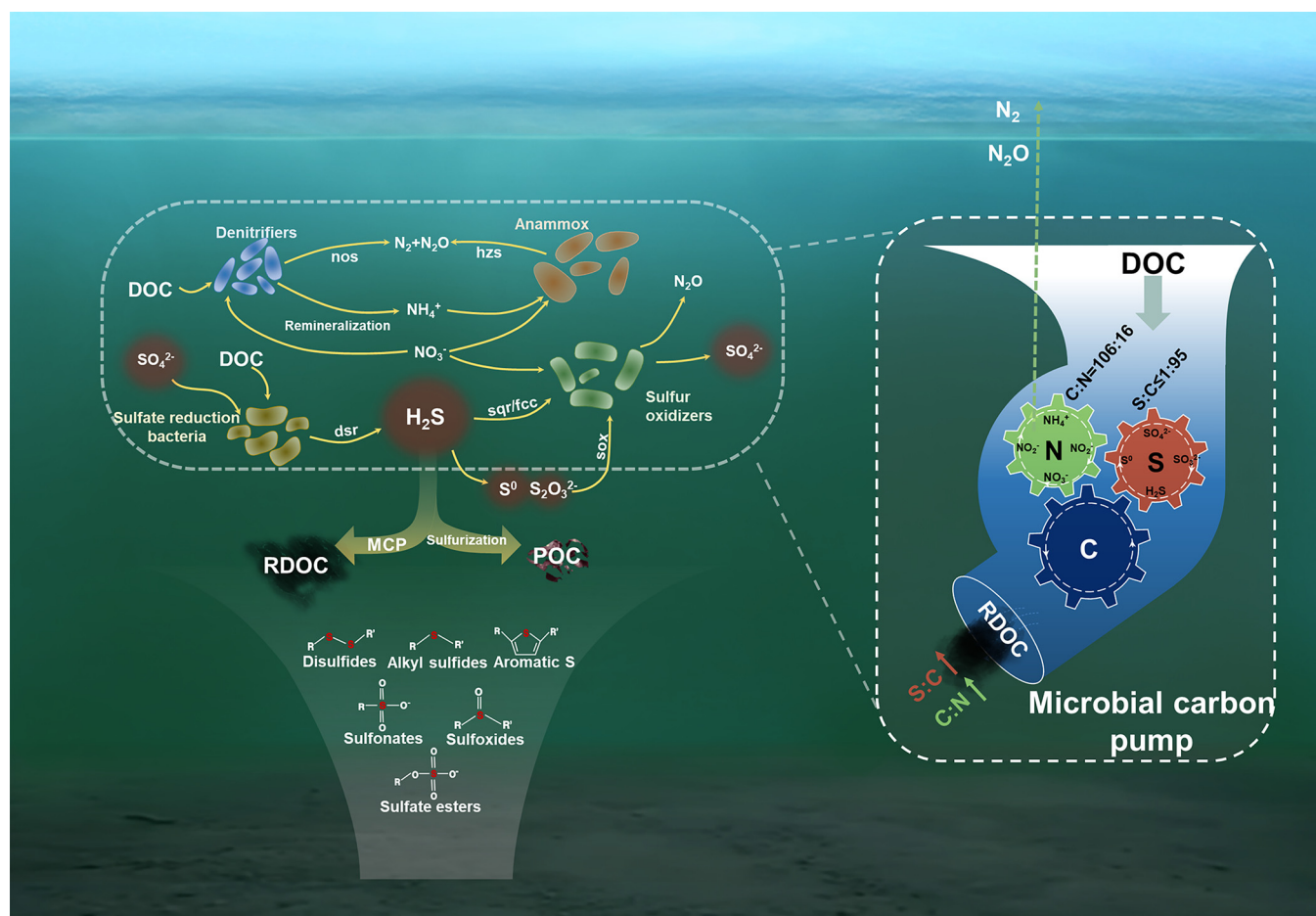
Fig. 2. Illustration of microbial carbon sequestration pathways through sulfurization in OMZs. The proposed pathways for the production of carbon sequestration are closely linked with sulfur and nitrogen transformations in OMZs, where sulfate-reducing, sulfur-oxidizing, denitrifying and anammox microbes are major players. Sulfate-reducing bacteria implement sulfate reduction and DOC mineralization processes and produce a large amount of hydrogen sulfide, while sulfur-oxidizing bacteria link to the nitrogen cycle through sulfur oxidation. A large amount of hydrogen sulfide may sulfurize RDOC and POC by infiltrating into MCP or attacking the POC, respectively. DOC, dissolved organic carbon; POC, particulate organic carbon; RDOC, refractory dissolved organic carbon; dsr, dissimilatory sulfite reductase; sqr, sulfide-quinone oxidoreductase; fcc, flavocytochrome c-sulfide dehydrogenase; sox, sulfur-oxidation system; hzs, hydrazine synthase; nos, nitrous oxide reductase.

observed in the anoxic water column of the Black Sea [6]. Through the expansion of the OMZ core and sulfurization processes, the organic carbon sequestration will increase to 90–190 Tg C by the end of this century (reference data for the estimation list are shown in the Supplementary materials). With ocean anoxic zones expanding continuously, organic carbon sequestration by sulfurization may provide more stable negative feedback on climate change [3].

Over the past 50 years the total area of open-ocean OMZs has expanded by 4.5 million square kilometers, which is equivalent to the area of the European Union, and this expansion is predicted to continue with global warming [14]. As a result, changes in the quantity and composition of marine organic sulfur are also predicted to occur [4]; therefore, monitoring these changes and predicting their impacts has become increasingly important. Due to the structural complexity of DOC, development of new methods to extract and quantify organic compounds in seawater represents one of the key challenges for future research. Advanced chemical approaches were used to target organic molecules, ultra-high resolution mass spectroscopy and X-ray absorption techniques are already available, and they are capable of characterizing the composition of marine organic sulfur. The sulfur present in dissolved organic compounds can be quantitatively determined by inductively coupled plasma-atomic emission spectroscopy; however,

the solid-phase extraction cartridge previously used for concentrating dissolved organic sulfur largely overlooks polar compounds—such as sulfonates—which are among the most abundant organic sulfur compounds present in seawater and sediments [7]. Thus, the accurate assessment of the size of organic sulfur reservoirs in marine environments relies on the development of their enrichment approaches.

The complex dynamics that encompass a variety of biotic and abiotic mechanisms, and control the production of refractory organic matter, remain poorly understood. Future research efforts should focus on the intertwined relationships between *in situ* microbial sulfur metabolic processes and variations in organic matter composition. Sulfate reduction rates in coastal and oceanic OMZs have been determined using sulfur isotope data through ship board and *in situ* measurements [4], but organic sulfur data are still not available. High-resolution, sulfur isotope nuclear magnetic resonance techniques, in combination with liquid chromatography and mass spectrometry, can directly measure metabolic fluxes in sulfur metabolisms. Additionally, the combination of Fourier transform ion cyclotron resonance mass spectrometry and multi-omic technologies (genomics, transcriptomic, proteomics, and metabolomics) provides an ideal opportunity to explore the relationships among a broad range of molecular currencies within the marine sulfur cycle, microbial population structure and metabolic traits.

Marine phytoplankton stoichiometry provides a link between carbon, nitrogen and phosphorus cycles, owing to the remineralization of the sinking POC derived from the stoichiometric preferences of phytoplankton; however, sulfur was often not incorporated into stoichiometric calculations. To understand how MCP affects variability in the S:C ratio of DOC, it is necessary to analyze the microbial cellular adjustments of nutrient allocation and sulfur in the stoichiometric ratios along the redox gradient in oxygen-depleted oceanic waters. Furthermore, a physical-biogeochemical model-based approach is also necessary to refine our view of organic matter cycling in OMZs. A model that combines nitrogen and sulfur cycles with the Regional Ocean Modeling System has been successfully applied to assess the contribution of microbial nitrate and sulfate reduction to remineralization processes in regional OMZs [15]. The developed biogeochemical models have been used to study the contribution of MCP to carbon sequestration [5]. Therefore, these biogeochemical sub-modules can be integrated with stoichiometry, and are expected to assist in the determination of carbon budget variations in OMZs in response to anthropogenic disturbance and climate warming.

In summary, OMZs have been expanding in the oceans worldwide in the past decades, attracting considerable attention from scientists and the public due to their impact on marine ecosystems. A neglected issue is the microbial-driven carbon sequestration in OMZs, which presents an underestimated component of the global carbon inventory. We propose a molecular mechanism for RDOC production based on the integration of MCP and specific sulfurization processes in OMZs. Finally, through sustained efforts to investigate microbial sulfur cycling, future investigations focusing on molecular components, metabolic fluxes, microbial stoichiometry and ecological models would expand our knowledge of the close link between carbon and sulfur cycles in OMZs now, and in the future climate change scenarios.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

Appendix A. Supplementary materials

Supplementary materials to this perspective can be found online at https://doi.org/10.1016/j.scib.2022.01.028.

References

[1] Fakhraee M, Katsev S. Organic sulfur was integral to the Archean sulfur cycle. Nat Commun 2019;10:4556.
[2] Vairavamurthy A, Zhou W, Eglinton T, et al. Sulfonates: a novel class of organic sulfur compounds in marine sediments. Geochim Cosmochim Acta 1994;58:4681–7.
[3] Raven M, Keil RG, Webb SM. Rapid, concurrent formation of organic sulfur and iron sulfides during experimental sulfurization of sinking marine particles. Glob Biogeochem Cycle 2021;35:e2021GB007062.
[4] Raven MR, Keil RG, Webb SM. Microbial sulfate reduction and organic sulfur formation in sinking marine particles. Science 2021;371:178–81.
[5] Jiao N, Cai R, Zheng Q, et al. Unveiling the enigma of refractory carbon in the ocean. Natl Sci Rev 2018;5:459–63.
[6] Gomez-Saez GV, Dittmar T, Holtappels M, et al. Sulfurization of dissolved organic matter in the anoxic water column of the Black Sea. Sci Adv 2021;7: eabf6199.
[7] Moran MA, Durham BP. Sulfur metabolites in the pelagic ocean. Nat Rev Microbiol 2019;17:665–78.
[8] Ksionzek KB, Lechtenfeld OJ, McCallister SL, et al. Dissolved organic sulfur in the ocean: biogeochemistry of a petagram inventory. Science 2016;354:456–9.
[9] Charlson RJ, Lovelock JE, Andreae MO, et al. Oceanic phytoplankton, atmospheric sulphur, cloud albedo and climate. Nature 1987;326:655–61.
[10] Gašparović B, Penezić A, Frka S, et al. Particulate sulfur-containing lipids: production and cycling from the epipelagic to the abyssopelagic zone. Deep-Sea Res Part I-Oceanogr Res Pap 2018;134:12–22.
[11] Pohlabeln AM, Gomez-Saez GV, Noriega-Ortega BE, et al. Experimental evidence for abiotic sulfurization of marine dissolved organic matter. Front Mar Sci 2017;4:364.
[12] Xin Y, Gao R, Cui F, et al. The heterotrophic bacterium *Cupriavidus pinatubonensis* JMP134 oxidizes sulfide to sulfate with thiosulfate as a key intermediate. Appl Environ Microbiol 2020;86:e01835-20.
[13] Bertagnolli AD, Stewart FJ. Microbial niches in marine oxygen minimum zones. Nat Rev Microbiol 2018;16:723–9.
[14] Stramma L, Schmidtko S, Levin LA, et al. Ocean oxygen minima expansions and their biological impacts. Deep-Sea Res Part I-Oceanogr Res Pap 2010;57:587–95.
[15] Azhar MA, Canfield DE, Fennel K, et al. A model-based insight into the coupling of nitrogen and sulfur cycles in a coastal upwelling system. J Geophys Res Biogeosci 2014;119:264–85.
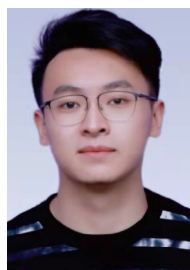
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