

GEOCHEMISTRY

Sulfurization of dissolved organic matter in the anoxic water column of the Black Sea

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Today's oceans store as much dissolved organic carbon (DOC) in the water column as there is CO₂ in the atmosphere, and as such dissolved organic matter (DOM) is an important component of the global carbon cycle. It was shown that in anoxic marine sediments, reduced sulfur species (e.g., H₂S) abiotically react with organic matter, contributing to carbon preservation. It is not known whether such processes also contribute to preserving DOM in ocean waters. Here, we show DOM sulfurization within the sulfidic waters of the Black Sea, by combining elemental, isotopic, and molecular analyses. Dissolved organic sulfur (DOS) is formed largely in the water column and not derived from sediments or allochthonous nonmarine sources. Our findings suggest that during large-scale anoxic events, DOM may accumulate through abiotic reactions with reduced sulfur species, having long-lasting effects on global climate by enhancing organic carbon sequestration.

INTRODUCTION

Widespread ocean anoxic events (OAEs) have occurred episodically throughout Earth's history. These events have often been associated with warmer climate, rises in sea level, and episodes of enhanced volcanism and caused mass extinctions of marine life (1–3). Since the middle of the 20th century, the concentrations of O₂ in open ocean and coastal waters have been decreasing in many regions worldwide (4, 5). Ocean deoxygenation results from enhanced nutrient inputs to stratified upper ocean areas with limited exchange of oxygenated surface waters and the ocean interior (4, 6). The oceanic oxygen minimum zones (OMZs) include those marine regions where dissolved O₂ concentration falls below 20 μM (7). In extreme cases, microorganisms rely on sulfate as electron acceptor for organic carbon degradation, causing the accumulation of the product hydrogen sulfide (H₂S) in deep, stagnant basins or underneath eutrophic surface waters (8). The Black Sea is a model system in this context, because it is the largest existing anoxic marine basin in the world, and it is considered an analog to the early Eocene's abyssal ocean, which had extended periods of anoxia (9). The Black Sea has become a saline basin ca. 7000 years ago, when it received seawater from the Mediterranean Sea via the Bosphorus strait. It also receives substantial amount of freshwater from rivers, mainly the Danube River, generating a strong salinity-driven density gradient with a permanent halocline (10). This limits vertical mixing and creates permanent anoxia with sulfidic waters below ca. 150 m water depth (11–13). Dissolved organic carbon (DOC) concentrations are ca. 2.5 times higher in the deep Black Sea than in the Mediterranean source water or in any of the major ocean basins (14–17). A similar accumulation of DOC in the global ocean may have had a major influence on the Neoproterozoic carbon cycle and global climate during this period of Earth's history (18). So far, there is no consensus about the reasons

behind DOC accumulation in the Black Sea. Potential explanations include reduced microbial mineralization of DOC under anoxic conditions (9), chemosynthesis and release from the sediments (19), or downward export and accumulation of riverine, terrigenous DOC (16).

In this study, we propose the alternative hypothesis that autochthonous dissolved organic matter (DOM) abiotically reacts with reduced sulfur species in the deep water column of the Black Sea. In marine sediments, this abiotic sulfurization contributes substantially to organic matter preservation (20–23). Sulfur is also rapidly incorporated into organic particles while they sink through the anoxic water column of the Cariaco basin (24). Sulfurization of organic matter may have provided a negative feedback mechanism in the ancient climate system by enhancing organic carbon burial, thereby accelerating climate cooling and recovery from anoxic conditions (25, 26). It was previously shown that under sulfidic conditions in marine sediments and hydrothermal systems, also dissolved organic compounds sulfurize (27–31). However, it is unknown whether the vast diluted pool of oceanic DOM can abiotically react with reduced sulfur. To test our hypothesis of sulfurization in the water column of the Black Sea, we applied a combined molecular, elemental, and isotopic proxy approach. The water column and sedimentary pore-water from two distinct regions of the Black Sea, the Bosphorus strait (close to the Mediterranean Sea influx) and the Crimean shelf (close to the Danube River plume), were sampled (Fig. 1A). We also considered the source waters from the Mediterranean Sea (32) and the Danube River (33). As a representative for old refractory ocean DOM, we included a reference sample from North Equatorial Pacific Intermediate Water (NEqPIW) (34). Elemental (C, S) and stable carbon isotopic analyses (δ¹³C) (Table 1) were combined with molecular characterization of DOM via ultrahigh-resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS; table S1). By using FT-ICR-MS, we identified on average ca. 7000 molecular formulas of dissolved organic compounds per aqueous sample, including more than 1500 containing sulfur (table S2). A novel molecular index to quantify the extent of abiotic sulfurization in DOM based on molecular data was established (AbioS index; Fig. 2). On the basis of a subset of 15 characteristic dissolved organic sulfur (DOS) molecular formulas identified as exclusively produced by abiotic

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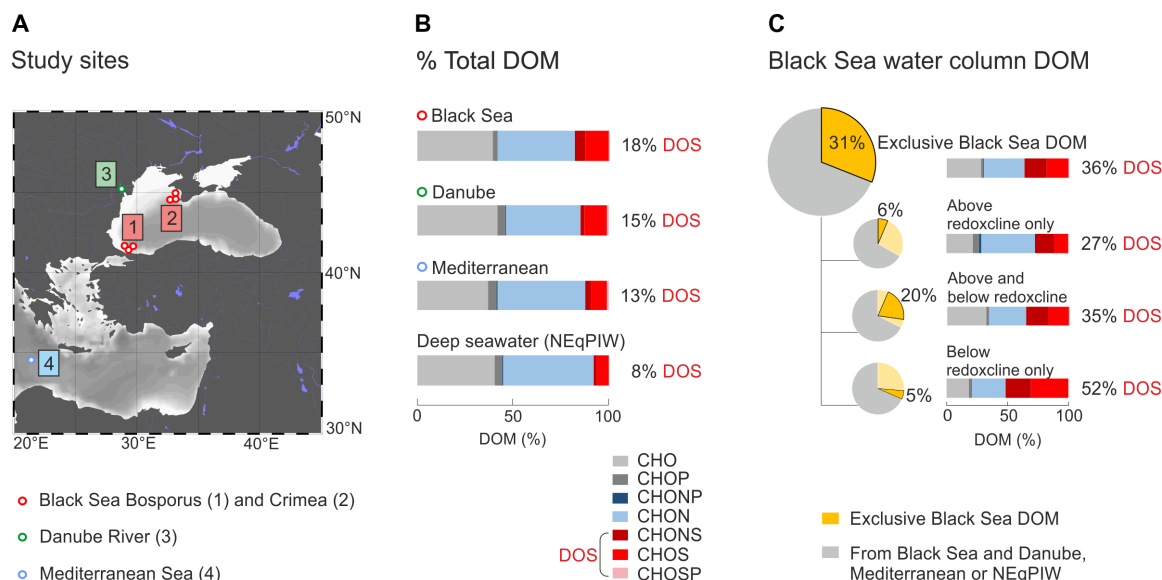


Fig. 1. DOM accumulating in the Black Sea water column is a mix of terrigenous, marine, and sulfur-enriched autochthonous compounds. (A) Study sites: Black Sea Bosphorus strait (1, red), Black Sea Crimean shelf (2, red), Danube River (3, green), and Mediterranean Sea (4, blue). (B) Summary of the molecular characterization of DOM by FT-ICR-MS. The bar plots indicate the relative abundance of the different molecular formulas identified by FT-ICR-MS. The DOS molecular formulas (red) group includes CHOS, CHONS, and CHOSP formulas. (C) Exclusive Black Sea DOM molecular formulas that were not identified in the Danube River, Mediterranean Sea, and deep ocean water (NEqPIW) differentiated (in dark yellow) between above the redoxcline (RC) only, above and below the RC, and below the RC only. Additional information on the exclusive fraction is presented in fig. S3.

sulfurization of DOM under laboratory conditions (35) and in sulfidic sediments (35, 36), the AbioS index is defined as (i) the number of detected AbioS molecular formulas (AbioS, #) and (ii) the sum of normalized signal intensities as percentage of total normalized signal intensity of FT-ICR-MS spectra (AbioS:DOM, %) (Fig. 2).

RESULTS AND DISCUSSION

DOM accumulating in the Black Sea is enriched in autochthonous sulfur compounds

Accumulation of DOC in the Black Sea water column was identified as a source-independent process. We estimated the terrigenous DOC fraction on the basis of comparison with the riverine and marine end-members by applying two independent approaches (fig. S1). The fraction of terrigenous DOC in the Black Sea was ca. 40% based on the relative contribution of dissolved black carbon (DBC; Table 1 and fig. S1). DBC represents polycondensed aromatic compounds that are largely mobilized from charred material in soils (33). Terrestrial input is a major source of DBC to the ocean (33), but a recent study revealed additional sources with a carbon isotopic signature similar to that of marine phytoplankton contributing to the oceanic DBC pool (37). Thus, the approach based on the relative contribution of DBC overestimated rather than underestimated the proportion of terrigenous DOC in the Black Sea. On the basis of the molecular DOM composition considering the percentage of total signal intensity in FT-ICR-MS spectra of aromatic compounds and molecular formula indicators of global riverine DOM [t-peaks; (38)], the terrigenous DOC fraction in the Black Sea was estimated as <25% (figs. S1 and S2). This lower percentage is in line with previous estimates based on optical analyses, suggesting 4 to 12% of terrigenous DOC in the central Black Sea (39). Consistently, all these complementary compositional characteristics suggest that the dissolved organics

accumulating in the Black Sea are a mix of terrigenous, marine, and autochthonous material (39).

The DOC and solid-phase extractable DOC (SPE-DOC) mainly accumulated above the redoxcline (RC) at depths of 0 to 150 m (above RC: DOC mean average \pm SD, $218 \pm 90 \mu\text{M}$; SPE-DOC, $128 \pm 49 \mu\text{M}$; below RC: DOC, $131 \pm 39 \mu\text{M}$; SPE-DOC, $73 \pm 37 \mu\text{M}$; Fig. 3 and Table 1), presumably due to its origin from algal primary production in surface waters (40). The proximity to the shelf was reflected in higher concentrations of DOC and SPE-DOC for Crimea water column samples compared to the more marine sites sampled in the Bosphorus region (Bosphorus: DOC, $134 \pm 29 \mu\text{M}$; SPE-DOC, $75 \pm 22 \mu\text{M}$; Crimea: DOC, $251 \pm 91 \mu\text{M}$; SPE-DOC, $145 \pm 52 \mu\text{M}$; Fig. 3 and Table 1). However, our FT-ICR-MS analysis showed that the terrestrial-derived molecular formulas were homogeneously distributed with depth in both Bosphorus and Crimea regions (fig. S2), indicating that increased DOC concentrations close to the Crimea shelf may be related to higher productivity (19). Thus, vertical distribution of DOM in the Black Sea is likely governed by in situ processes rather than reflecting a variability in the origin of waters (19). A fraction of Black Sea DOM could be derived from microbial methane oxidation, resulting in carbon products with light stable carbon isotopic composition that have been identified throughout the Black Sea water column, not only at the chemocline but also in the oxic water column (41). Not only incorporation of methane-derived carbon into DOM could be an explanation for the observed homogenous distribution of the light-isotope-enriched stable carbon isotopic signature of water column DOM (mean average \pm SD, $-26.0 \pm 0.3\text{‰}$; Table 1 and fig. S2) but also chemosynthesis would result in similar light isotopic enrichments of released DOM (19, 42). We identified almost one-third (31%) of all molecular formulas in the Black Sea water column as unique and exclusive to this biome, i.e., they were not detected in the riverine or marine end-members (Fig. 1C). The

Table 1. Summary of geochemical parameters. Values are given as mean average \pm SD; *n*, number of samples; SPE, solid-phase extract; RC, redoxcline; $\delta^{13}\text{C}$, stable carbon isotopic composition relative to Vienna Pee Dee belemnite (VPDB); DBC, dissolved black carbon; and na, not analyzed. References from the literature included in the table are *, (33); †, (32); ‡, (34); §, (43); ||, (35); and ¶, (36).

		Depth	<i>n</i>	DOC (μM)	SPE-DOC (μM)	SPE-DOS (μM)	DOS/DOC ratio (SPE, in %)	$\delta^{13}\text{C}$ (SPE, ‰ VPDB)	DBC (μM)
Bosporus water column	Bos1	Above RC (<150 m)	4	159 \pm 26	79 \pm 33	0.4 \pm 0.1	0.6 \pm 0.2	-26.0 \pm 0.1	6.2 \pm 0.5
		Below RC (>150 m)	6	117 \pm 10	57 \pm 17	0.7 \pm 0.2	1.2 \pm 0.3	-25.7 \pm 0.2	5.0 \pm 0.6
	Bos2	Above RC (<150 m)	5	170 \pm 39	100 \pm 12	0.6 \pm 0.1	0.6 \pm 0.1	-25.9 \pm 0.2	5.4 \pm 0.3
		Below RC (>150 m)	4	116 \pm 7	59 \pm 12	0.8 \pm 0.1	1.5 \pm 0.2	-25.9 \pm 0.3	4.5 \pm 0.9
	Bos3	Above RC (<150 m)	3	139 \pm 14	88 \pm 10	0.5 \pm 0.1	0.6 \pm 0.1	-26.1 \pm 0.1	5.8 \pm 0.2
		Below RC (>150 m)	6	115 \pm 2	72 \pm 2	1.0 \pm 0.3	1.4 \pm 0.4	-26.1 \pm 0.1	5.3 \pm 0.4
Crimea water column	Cri1	Above RC (<150 m)	9	258 \pm 80	193 \pm 29	1.0 \pm 0.7	0.5 \pm 0.3	-26.3 \pm 0.3	6.5 \pm 1.5
	Cri2	Above RC (<150 m)	9	215 \pm 83	122 \pm 33	0.6 \pm 0.2	0.5 \pm 0.1	-26.0 \pm 0.3	5.8 \pm 1.5
	Cri3	Above RC (<150 m)	4	347 \pm 130	108 \pm 29	0.7 \pm 0.2	0.7 \pm 0.2	-25.8 \pm 0.2	6.1 \pm 0.6
		Below RC (>150 m)	3	211 \pm 42	123 \pm 82	1.2 \pm 0.6	1.3 \pm 0.7	-25.4 \pm 0.6	6.1 \pm 2.8
Crimea porewater	Pw1 and Pw2	0–20 cmbsf	4	341 \pm 67	214 \pm 48	1.3 \pm 0.3	0.7 \pm 0.3	-26.0 \pm 0.3	7.0 \pm 1.4
	Pw3	0–20 cmbsf	2	345 \pm 114	221 \pm 0.3	5.9 \pm 2.0	2.7 \pm 0.9	-26.2 \pm 0.2	8.9 \pm 1.6
Terrigenous end-member	Danube River	0 m	1	167*	105*	na	na	-28.0	13.3
Marine end-member	Mediterranean Sea	900 m	1	46 [†]	22 [†]	na	na	-22.3	0.9
Deep ocean reference	NEqPIW	674 m	1	44 [‡]	27 [‡]	0.2 [§]	0.4 [§]	na	na
Sulfidic porewater	Tidal flat	50 cmbsf	1	1276	873	32	3.7	na	na
	Saltmarsh	50 cmbsf	1	886 [¶]	689 [¶]	10 [¶]	1.4 [¶]	na	na
Experimental abiotic sulfurization	Control	-	6	170 \pm 28	68 \pm 27	0.6 \pm 0.2	0.8 \pm 0.1	na	na
	Sulfurized	-	6	192 \pm 36	28 \pm 8	2.7 \pm 0.5	1.0 \pm 2.9	na	na

exclusive DOM fraction was more unsaturated and reduced (O-poor) below than above the RC (fig. S3). Statistical dissimilarity analyses (Bray-Curtis) based on normalized FT-ICR-MS signal intensities of all detected molecular formula revealed high similarities (<15% dissimilarity) of all Black Sea water column DOM samples, while they were ca. 40% dissimilar to DOM from Danube River or Mediterranean Sea and ca. 30% dissimilar to NEqPIW DOM (Fig. 4A and fig. S4A). Together, bulk and molecular characteristics provide conclusive evidence for a high proportion of autochthonous material in shaping the molecular composition of Black Sea DOM.

High concentration of DOS compounds was a characteristic feature of the DOM prevailing in the anoxic Black Sea. Below the RC, the Black Sea water column was enriched in DOS (SPE-DOS, 0.9 \pm 0.3 μM) compared to other oxic ocean basins [SPE-DOS < 0.2 μM (43, 44)] (Fig. 3 and Table 1). The molar DOS/DOC ratios (from quantitative element analysis of the SPE fraction) were on average

more than double in the sulfidic zone below the RC (1.30 \pm 0.4%) than above (0.56 \pm 0.2%) (mean average \pm SD; Fig. 5 and Table 1). This is opposite to depth distributions in major oxic ocean basins, where DOS accumulates preferentially in the surface waters, presumably due to microbial production of DOS near the sea surface and preferential decomposition of these compounds before export to the deep ocean (44, 45). On the molecular level, 18% of all molecular formulas and 52% of the molecular formulas that exclusively occurred in the anoxic waters of the Black Sea contained sulfur (Fig. 1, B and C). A recent study in the western Atlantic Ocean reported 6% of DOS formulas in total DOM and suggested that the SPE DOM did not capture a representative fraction of DOS (45). We detected a similar proportion of DOS formulas for the fully oxygenated deep ocean reference sample included in our study (NEqPIW DOS formulas, 8%; Fig. 1B), previously shown to mainly consist of unreactive oxidized sulfonic acids (43). Given the reproducible and consistent differences in

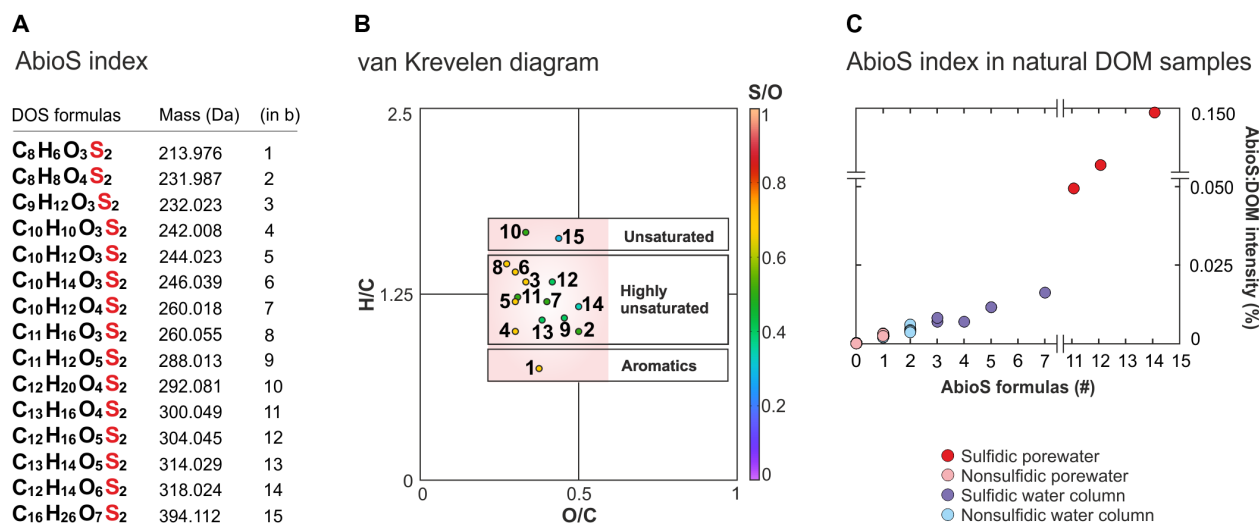


Fig. 2. Novel molecular AbioS index for the identification of abiotic sulfuration of DOM in FT-ICR-MS datasets. (A) Fifteen characteristic DOS molecular formulas derived from abiotic sulfuration under laboratory conditions [(35); see Materials and Methods]. (B) van Krevelen diagram representing the H/C (hydrogen-to-carbon ratio) and O/C (oxygen-to-carbon ratio) of AbioS formulas. The color scale illustrates the S/O (sulfur-per-oxygen ratio). Every dot represents a specific molecular formula listed in (A) (numbers presented in the third column). (C) Metrics of AbioS index in natural sulfidic and nonsulfidic water column and porewater samples. The metrics of the AbioS index considered (i) the number of detected AbioS formulas (horizontal axis) and (ii) the sum of normalized signal intensities as percentage of total signal intensity in FT-ICR-MS spectra (vertical axis). The water column (blue and purple) and nonsulfidic porewater samples (pink) are all from this study (see Figs. 3 and 5), while the sulfidic porewater (red) includes the Pw3 sample from Crimea, and two porewater samples from sulfidic sediments where abiotic sulfuration has been reported in the literature (35, 36).

DOS distribution pattern observed in the Black Sea, our study provides evidence that the SPE-DOS fraction represents relevant characteristics of DOS, in particular in aquatic environments with varying redox conditions where sulfur is a key player of biogeochemical processes [i.e., anoxic basins (46, this study), deep and coastal hydrothermal systems (28, 47), sulfidic porewaters (27, 35)]. Most of the Black Sea DOS compounds were likely autochthonous, as indicated by the high proportion of sulfur-containing molecular formulas in the exclusive Black Sea molecular formulas (36%; Fig. 1C) and by the high dissimilarity of Black Sea DOS compared to the riverine and Mediterranean end-members (ca. 60%; Fig. 4B and fig. S4B). Further evidence that the DOS accumulating in the Black Sea is not of Mediterranean origin was inferred from the decrease in DOS/DOC ratio due to seawater intrusion detected at station Bos3 (ca. 250 to 350 m depth; Figs. 3A and 5A). We conclude that DOM composition and DOS accumulation are dominantly governed by the prevailing redox conditions of the Black Sea.

Sulfuration of DOM in the anoxic waters of the Black Sea

Molecular proxies demonstrated abiotic sulfuration of DOM in the anoxic water column of the Black Sea. The molecular formulas of the AbioS index, a novel diagnostic tool for the detection of abiotic sulfuration of DOM in FT-ICR-MS datasets (Fig. 2), increased in intensities with sulfide concentrations ($r = 0.80$, $P < 0.0001$; Fig. 5), with highest significant positive correlations below the RC and excluding the samples exposed to oxygenated seawater intrusion ($r = 0.92$, $P < 0.0001$; Figs. 3 and 5). Furthermore, the AbioS formulas were considerably more abundant in sulfidic water column samples (below RC ≤ 7 AbioS formulas (of 15 molecular formulas), above RC ≤ 2 AbioS formulas; Figs. 2C and 5). The AbioS formulas cover a mass range of 213 to 394 Da (Fig. 2A) with a degree of saturation typical for natural DOM samples (highly unsaturated, 12 formulas; unsaturated, 2 formulas; aromatics, 1 formula; Fig. 2B) but with low

oxygen-to-carbon (O/C) ratios in average (0.37 ± 0.08 ; Fig. 2B), suggesting that they belong to reduced (O-poor) DOM, a characteristic feature of sulfidic porewaters, coastal, and deep-sea hydrothermal systems (28, 36, 47). We also found quantitative and qualitative evidence for abiotic sulfuration of DOM in the underlying sulfidic sediments (Pw3, 11 AbioS formulas; SPE-DOS, $5.9 \pm 2.0 \mu\text{M}$; DOS/DOC ratio, $2.7 \pm 0.9\%$; Figs. 3 and 5C and Table 1), consistent with previous studies (29, 30). On the contrary, there was no imprint of abiotic sulfuration in the DOM of nonsulfidic porewater (Pw1 and Pw2 ≤ 1 AbioS formula; SPE-DOS, $1.3 \pm 0.3 \mu\text{M}$; DOS/DOC ratio, $0.7 \pm 0.3\%$; Figs. 3 and 5C and Table 1). Release from sediments is a potential source of DOM to the anoxic deep waters of the Black Sea (19), but it is unlikely that DOS in the water column was primarily derived from sediments. The AbioS index was also increased in water layers distant from the sediments, and the DOS/DOC ratio above the seafloor was lower or similar to below the RC or at 300 m depth (Bos1, Bos2, and Bos3; Fig. 5A). Furthermore, the abundance distributions of the AbioS formulas of the most sulfidic water column samples were highly dissimilar (ca. 70%) to the potential sulfidic sedimentary end-members (Fig. 4C). DOS from the riverine and Mediterranean end-members was also highly dissimilar (ca. 60%) in its molecular composition to DOS from the Black Sea water column (Fig. 4B and fig. S4B), inferring that sulfur compounds in the Black Sea did not originate from the Danube River or the Mediterranean Sea. Hence, there is strong and previously unidentified evidence that abiotic sulfuration of DOM occurs within the anoxic water column of the Black Sea.

Stabilization of DOM via sulfuration provides an additional explanation for the extraordinarily high DOC concentration in the deep Black Sea water column. The quantitative molar DOS/DOC ratio correlated with sulfide concentration in the Black Sea water column, but with high variability ($r = 0.55$, $P < 0.0001$; Fig. 5), suggesting that other processes might complement abiotic sulfuration. In other comparable environments, such as the semi-enclosed East Japan Sea,

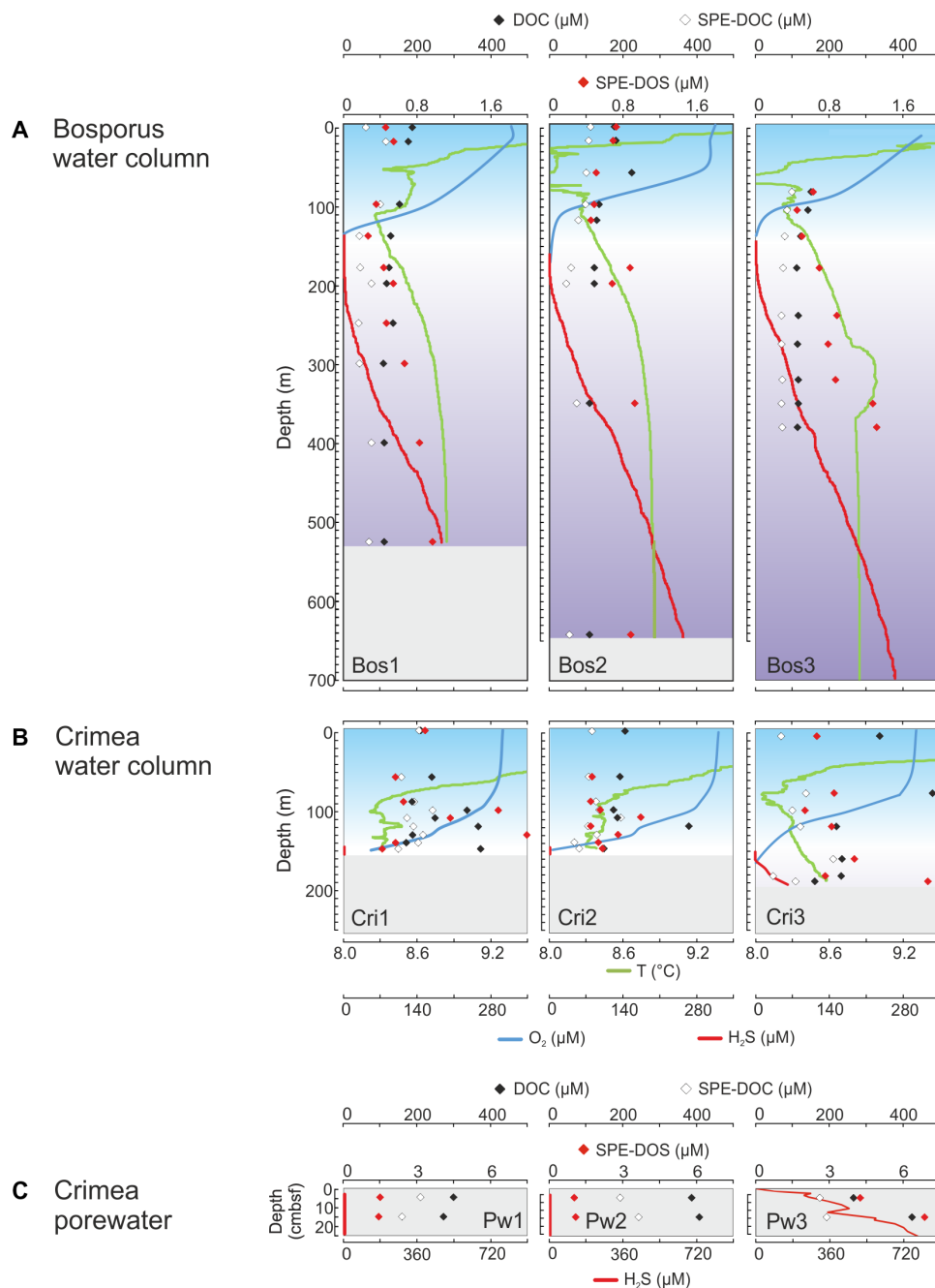


Fig. 3. Physicochemical composition of Black Sea water column and sediment samples. (A) Bosphorus water column (Bos1, Bos2, and Bos3). (B) Crimea water column (Cri1, Cri2, and Cri3). (C) Crimea porewater (Pw1, Pw2, and Pw3). Data included are DOC (black diamonds), SPE-DOC (white diamonds), SPE-DOS (red diamonds), temperature (green line), oxygen concentration (O_2 , blue line), and hydrogen sulfide concentration (H_2S , red line). According to the temperature data, a Mediterranean seawater intrusion was detected during sampling at the Bosphorus station Bos3 (ca. 250 to 350 m depth, green). Sulfidic conditions were found in the water column below the RC (150 m depth) in Bos1, Bos2, Bos3, and Cri3 and in the porewater sample Pw3 (H_2S , red line).

DOC accumulation has been attributed to low temperature in deep waters ($<1^\circ C$) and limited microbial activity (48). Such an explanation can be ruled out for the Black Sea as the temperature was $>7^\circ C$ at all depths (Fig. 3) and highly active microbial communities have been identified in the euxinic water column of the Black Sea (8). Among these, some anaerobic microorganisms [e.g., members of the Chloroflexi and Proteobacteria (49)] might actively contribute to DOS

production by microbially mediated sulfurization. The dynamics of DOS, i.e., processes that produce, transform, and degrade DOS compounds, is dominantly controlled by prevailing redox conditions [e.g., (28)]. In the Black Sea, the duration for which water masses remain anoxic and sulfidic, or are exposed to oxygen, may be influenced by dynamic hydrologic events such as internal waves (50). Experimental studies evidenced that sulfur incorporation into DOM is a fast

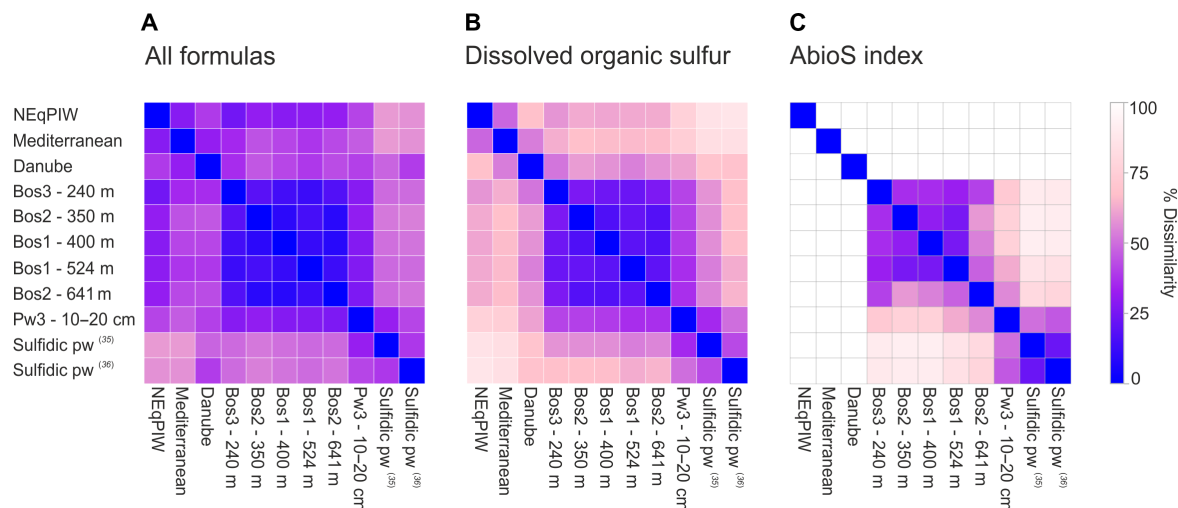


Fig. 4. Molecular dissimilarity indicates that DOS was formed in the water column and not derived from the sediments. Molecular dissimilarity analysis (Bray-Curtis) of (A) all formulas, (B) DOS formulas, and (C) specific formulas derived from abiotic sulfurization (AbioS index; Fig. 2). The analysis considered the relative signal intensities of molecular formulas detected via FT-ICR-MS, with white representing 100% dissimilar (totally different or not present at all) and blue representing 0% dissimilar (the same). Samples included deep ocean water (NEqPIW), marine, and terrigenous end-members (Mediterranean Sea and Danube River), the five water column samples with highest relative signal intensity of AbioS index molecular formulas in total DOM (Fig. 5), the sulfidic porewater sample from the Black Sea (Pw3), and two porewater samples from sulfidic sediments where abiotic sulfurization has been reported in the literature (35, 36). Same analysis with all samples grouped in those above and below RC is shown in fig. S4.

process [e.g., after 1 hour of incubation (35)], indicating that duration of exposure to sulfidic conditions very likely did not affect the extent of DOM sulfurization in the Black Sea. However, there is a lack of studies regarding the export of sulfurized DOM and its fate under oxygenated conditions. We detected large qualitative differences in DOS molecular composition above and below the RC (table S1) and quantitative DOS/DOC ratios above the RC ($0.56 \pm 0.2\%$; Fig. 5 and Table 1) were similar to those of open ocean oxic waters [ca. 0.4 to 0.6%; (36, 43, 44)]. This suggests that either sulfurized Black Sea DOM did not persist for long time under oxygenated conditions or that there was no notable export of DOS to the oxic layer through vertical mixing or diffusion, in accordance with the absence of a deoxygenation gradient above the RC (Fig. 3). Moreover, the identification of sulfurized DOM in the sulfidic samples, although the sampling and SPE were performed in the presence of oxygen, suggests that the sulfurized DOM considered in this study is not highly susceptible to oxygenation and potentially could persist in oxic waters if exported, eventually contributing to the pool of recalcitrant marine DOM. Accumulation of sulfurized DOM in the Black Sea is facilitated by the absence of light in the deep sulfidic waters. DOS compounds derived from sulfurization are highly photolabile (36), their photodegradation leading to the production of not only sulfate but also climate-active gases (51) that could eventually be released to the atmosphere due to mesoscale processes in the Black Sea, bringing the anoxic waters up to 50 m depth (8, 50). Apart from providing a potential negative feedback to increased atmospheric CO_2 and global warming through stabilization and sequestration of DOC, sulfurization of DOM also affects the distribution of toxic metals in aquatic environments via complexation. Thus, accumulation of DOS might explain the increased concentrations of methylated mercury in the anoxic waters of the Black Sea (52).

Global relevance of DOM sulfurization in a changing ocean

The quantitative molar DOC/DOS ratios of SPE DOM of the Black Sea water column above the RC were on average 200 ± 67 (mean

average \pm SD; $n = 28$), similar to ratios of the open ocean's photic zone [221 ± 31 ; $n = 129$; 0 to 105 m depth (44)]. Note that, for global comparison, we consider the inverse carbon-to-sulfur molar ratios reported in relevant previous literature [e.g., (44)]. In the deeper water column, DOC/DOS ratios of Black Sea and open ocean differ considerably. Below the RC of the Black Sea, DOC/DOS ratios were on average 85 ± 34 ($n = 19$), ca. one-third of deep global ocean estimates [261 ± 46 and 270 ± 38 ; $n = 22$; 201 to 1000 m depth; >1000 m depth (44)]. The anoxic zone in the Black Sea represents ca. 85% of the Black Sea total water volume (53). Considering the volume fraction per depth and the corresponding DOC concentrations and DOS fraction from this study and (19), we estimate that the total amount of DOC and DOS below the Black Sea RC is 720 to 776 Tg C ($n = 68$) and 24 to 26 Tg S ($n = 19$), respectively. Since the early 1960s, global oceanic oxygen reserves have been reduced by 2% and the volume of waters with anoxic conditions has more than quadrupled, mainly due to eutrophication and anthropogenic global warming (4, 5). Consequently, OMZs that now represent 8% of the total ocean volume (with 0.8% of permanently anoxic waters) are expanding (4–7). In an extreme scenario of major environmental redox change, sulfurization of DOM as observed in the Black Sea could lead to an accumulation of DOS and sequestration of stabilized DOC. For example, during the OAE 2 [ca. 94 million years ago (20)], vast amounts of particulate organic matter derived from sulfurization were sequestered in the underlying sediments, with a resemblance in sulfur speciation with modern environments suggesting similar sulfurization reactions in sinking particles from ancient and modern OMZs (24, 26). Assuming a comparable sulfide supply and effect on DOC accumulation as observed in the Black Sea, the world's oceans could potentially store 2.5 times as much DOC as they do now. Key questions to address with relevance to past and future oceans would be whether the formation of DOS via sulfurization is limited by DOC residence time, which was estimated as ca. 400 years in the deep water of the Black Sea (54), or by sulfide concentration, either that sulfurized DOM requires

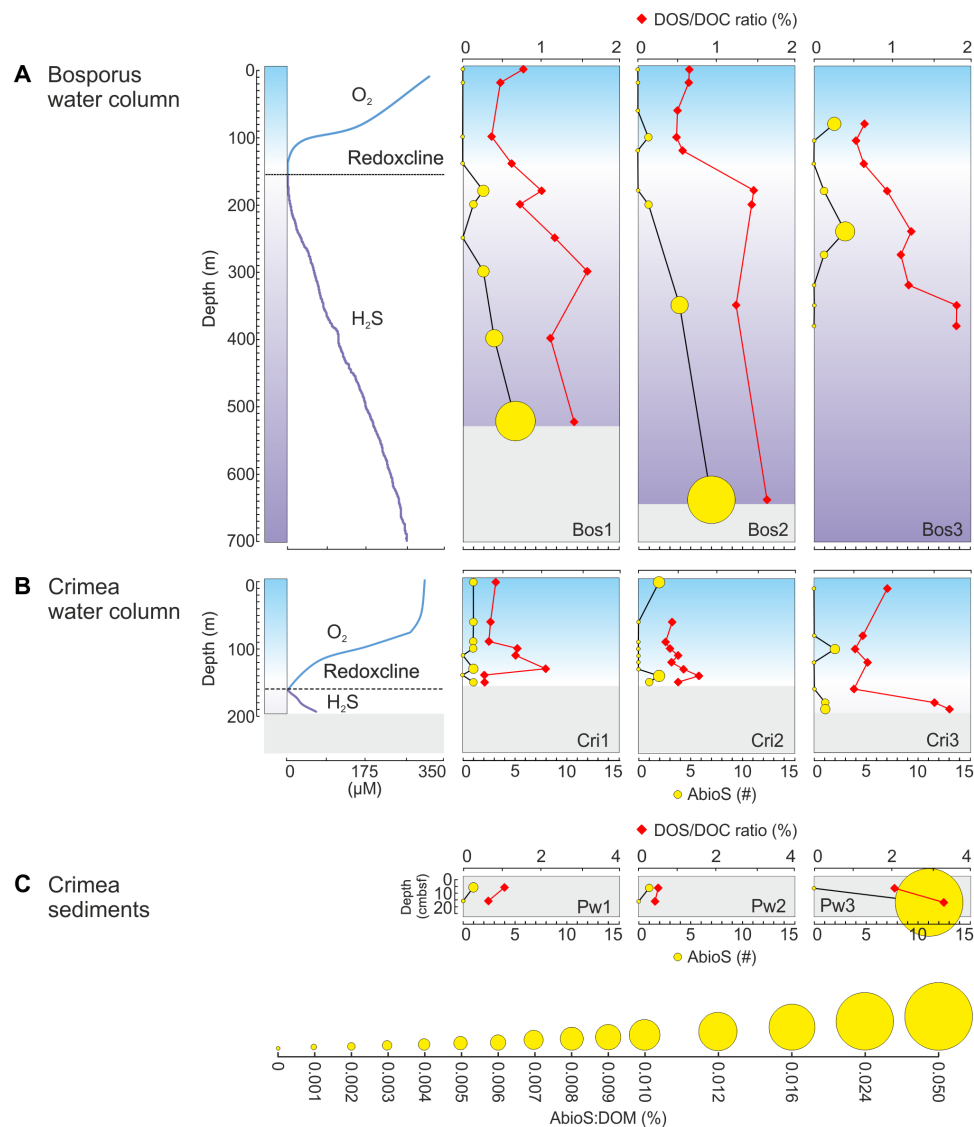


Fig. 5. Evidence for abiotic sulfurization of DOM in the sulfidic water column and sediments of the Black Sea. Quantitative, molar DOS/DOC ratios (DOS/DOC ratio of SPE DOM, in %, red diamonds). AbioS index including number of detected AbioS formulas [horizontal axis, AbioS (#)] and percentage of total FT-ICR-MS signal intensity [size of yellow circles, AbioS:DOM (%)]. O₂ and H₂S concentration profiles through the water column are shown on the left (Fig. 3). **(A)** Bosphorus water column, **(B)** Crimea water column, and **(C)** Crimea sediment porewater, only Pw3 with sulfidic conditions (Fig. 3). The background color indicates the vertical stratification, from oxic surface waters (blue) through the redoxline (white) to sulfidic conditions (purple).

a constant local maintenance of sulfidic conditions or it might be stable enough to contribute to total DOM in oxic ocean basins. In conclusion, our findings suggest that DOM may accumulate through abiotic reactions with reduced sulfur species, having long-lasting effects on global climate and providing negative feedback on global warming by enhancing organic carbon sequestration.

MATERIALS AND METHODS

Sampling and onboard measurements

All samples were collected during RV Maria S. Merian cruise MSM15-1 (12 April to 8 May 2010). Black Sea water samples (2 liter volume each) were obtained using a conductivity-temperature-depth (CTD) rosette equipped with additional sensors for oxygen (SBE 43; Seabird

Electronics) and fluorescence (ECO-AFL/FL; WET Labs). A total of 53 water column samples from six different stations in two distinct regions of the Black Sea were collected, from the southwestern Bosphorus strait (Bos1, Bos2, and Bos3) and the northwestern Crimean shelf (Cri1, Cri2, and Cri3) (Fig. 1A). The coordinates of the stations were as follows: Bos1, MSM15/181-1 (41°25.48'N, 29°17.79'E); Bos2, MSM15/219-1 (41°37.84'N, 29°1.90'E); Bos3, MSM15/272-1 (41°31.99'N, 29°16.69'E); Cri1, MSM15/382-1 (44°37.73'N, 32°54.91'E); Cri2, MSM15/403-1 (44°37.57'N, 32°55.00'E); and Cri3, MSM15/525-1 (44°35.73'N, 32°50.49'E). Immediately after sampling, water samples were frozen (−20°C) and stored in the dark. Porewater samples were obtained from sediment cores collected by a video-guided multiple corer (TV-MUC; 96-mm-inner diameter core tubes) at three stations from the Crimean shelf: Pw1, MSM15/463-1 (44°49.45'N, 33°9.26'E);

Pw2, MSM15/487-1 (44°38.78'N, 33°0.25'E), and Pw3, MSM15/528-1 (44°35.73'N, 32°49.23'E). Porewater samples were extracted at 12°C by using Rhizon samplers (pore size < 0.2 μm; Rhizosphere, The Netherlands) inserted at 0 to 20 centimeters below sea floor (cmbsf) at 5-cm intervals through predrilled holes along the side of the core liners [e.g., (55)]. Samples were combined to obtain enough volume in intervals of 0 to 10 cmbsf and 10 to 20 cmbsf. Six porewater samples were collected in total, yielding two samples per station. In the porewater samples, the total dissolved H₂S, which includes H₂S_{aq} + HS⁻ + S⁻² and the sulfidic component of polysulfides S_x⁻² was quantified using the methylene blue method (56) on a Shimadzu UV120 Spectrophotometer. Sulfide profiles were obtained from (55) taken at the same location but at different MUC deployments and thereby corresponding to the following stations in (55): Pw1-H₂S, MSM15/462-1 (44°49.45'N, 33°9.26'E); Pw2, same station coordinates (see above); and Pw3-H₂S, MSM15/448-1 (44°35.84'N, 32°49.03'E). Sulfidic conditions were only found at Pw3 (Fig. 3); here, the H₂S concentration was averaged between 0 and 10 cmbsf (234 ± 145 μM; *n* = 7) and between 10 and 20 cmbsf (567 ± 140 μM; *n* = 6) according to (55). In the water column samples, H₂S was analyzed from samples of the station Bos3 collected via pump-CTD from 145 to 330 m depth with spatial resolution of 1.5 m using the methylene blue method (56) on a spectrophotometer (GENESYS, Thermo Fisher Scientific). The stable water column stratification throughout the Black Sea resulted in a stable density-H₂S distribution [e.g., (57)]. Therefore, H₂S profiles at other stations were calculated as a function of density from a power function fitted to the density-H₂S relation from Bos3 (fig. S5). The density-H₂S data for water depths below 330 m were taken from (19) (doi.org/10.1594/PANGAEA.898717).

Geochemical analyses

After thawing, the water samples were filtered, acidified, and extracted according to (58) using SPE on styrene divinyl benzene polymer filled cartridges (1 g; Agilent Bond Elut PPL, USA). DOC concentrations were determined in triplicates from the original filtered and acidified water samples by high-temperature catalytic oxidation on a Shimadzu TOC-VCPH analyzer. For the determination of SPE-DOC concentrations, aliquots of the methanol extracts were evaporated to dryness and redissolved in ultrapure water of pH 2. The accuracy of the DOC analyses was tested in each run against deep Atlantic seawater reference material provided by the consensus reference material project (D. Hansell, University of Miami, USA). The stable carbon isotopic composition in the SPE extracts relative to Vienna Pee Dee Belemnite (δ¹³C) was determined in duplicates on an isotope ratio mass spectrometer (Finnigan MAT 252) following (59). DBC fraction, including primarily but not exclusively terrigenous DOM (33, 37), was quantified in the SPE after nitric acid digestion as benzenepolycarboxylic acids using ultrahigh performance liquid chromatography coupled with a photodiode array detector (Waters) as described by (60). SPE-DOS concentration was obtained after drying an aliquot of each methanolic extract, redissolving in nitric acid and analyzing on an inductively coupled plasma optical emission spectrometer (iCAP 6000, Thermo Fisher Scientific) following (28, 43). In accordance with previous literature, the estimates on sulfurization (see the “Sulfurization of DOM in the anoxic waters of the Black Sea” section) are presented as molar DOS-to-carbon ratios (from quantitative analyses of the SPE fraction) in percentage [amount of sulfur atoms per 100 carbon atoms; e.g., (20)]; while for comparison of global estimates (see the “Global relevance of DOM sulfurization in

a changing ocean” section), the estimates are presented in carbon-to-sulfur molar ratios [e.g., (44)].

Molecular characterization of DOM

Mass spectra were obtained in negative ionization mode using a Solarix FT-ICR-MS with a 15 T magnet (Bruker Daltonics) combined with an electrospray ionization (ESI) source (Bruker Apollo II) with a needle voltage set to -4 kV. All samples were analyzed in a 1:1 volumetric mixture of methanol and ultrapure water. The concentration was adjusted to 10 mg C liter⁻¹. The samples were directly infused into the ESI source at a flow rate of 120 μl hour⁻¹. Mass spectrometry quality and reproducibility was monitored by analyzing twice a day an internal deep-sea DOM reference sample [NEqPIW, (34)]. Two hundred transient scans in broadband mode were accumulated for each run, covering the mass range of 90 to 1500 Da. A common detection limit on the relative signal intensity scale was applied to facilitate maximum comparability among samples (61). Internal calibration of the spectra was performed using the Bruker Daltonics Data Analysis software package with the help of an established calibration list for marine DOM, consisting of more than 100 mass calibration points of known molecular formulas. The data were processed using in-house MATLAB routines, and molecular formulas with C₁₋₁₀₀, H₁₋₂₅₀, O₁₋₁₀₀, N₀₋₄, S₀₋₂, or P₀₋₁ were assigned to detected masses. Blank samples (ultrapure water pH 2) were processed and analyzed, and all of the DOM formulas detected in the blanks were removed from the dataset. The remaining detected masses were normalized to the sum of all mass intensities of the corresponding sample, and normalized intensities were used to calculate intensity-weighted averages of molar ratios [hydrogen-to-carbon (H/C), O/C, and sulfur-to-oxygen (S/O); Fig. 2 and table S1]. A total dataset of 83 individual FT-ICR-MS analyses was obtained (table S2): Black Sea water column (*n* = 53), Black Sea porewater (*n* = 6), terrigenous end-member [Danube River sample (33), analyzed twice, *n* = 2], marine end-member [Mediterranean Sea sample, station 7 at 900 m depth, (32), analyzed twice, *n* = 2], deep water reference [NEqPIW sample, (34), analyzed three times, *n* = 3], tidal flat sulfidic porewater (35) (*n* = 2), saltmarsh sulfidic porewater (36) (*n* = 3), and the samples from experimental abiotic sulfurization (35) including control (*n* = 6) and sulfurized (*n* = 6) ones. The Black Sea samples were analyzed individually per depths to obtain the highest level of detail in terms of spatial resolution along the vertical profile (e.g., Figs. 2 to 5, fig. S2, and table S2), but to provide general DOM information (e.g., relative abundances of total DOM or intensity-weighted averages), a more conservative approach was followed by combining replicates and groups of similar samples, only accepting those molecular formulas present in all of them. Namely, samples were grouped according to the study sites and whether they were above or below the RC (150 m depth) and in nonsulfidic (Pw1 and Pw2) or sulfidic sediments (Pw3) (e.g., figs. S3 and S4, Table 1, and table S1). The fraction of exclusive Black Sea DOM from the water column (Fig. 1C and fig. S3) was calculated as the molecular formulas present in at least 1 of the 10 water column groups above or below the RC (Table 1 and table S1) but not present in any of the Danube River, Mediterranean Sea, and NEqPIW samples. The proportion of exclusive formulas was calculated compared to the mean average of formulas per single water column sample identified (2217 of 7069 molecular formulas, 31%; Fig. 1C and table S2). Afterward, the exclusive Black Sea DOM formulas were subdivided into (i) those only above the RC (<150 m depth), (ii) those present in both above and

below the RC, and (iii) those detected only below the RC (>150 m depth) (Fig. 1C). The proportion of each compound group is reported as the sum of percentages of molecular formulas of a given compound group relative to the total percentage (e.g., DOS formulas in total DOM, the sum of percentages of CHOS, CHONS, and CHOSP molecular formulas) (Fig. 1, B and C, and tables S1 and S2). The normalized signal intensities were used to calculate two indices: the double bond equivalent to assess the degree of unsaturation and the modified aromaticity index [$AI_{\text{mod}} > 0.5$; (62)] to calculate the proportion of aromatic and condensed aromatic compounds in total DOM taking into consideration the abundance of carboxyl groups in natural organic matter (table S1). In function of H/C ratio and AI_{mod} , the DOM compounds were classified into aromatic ($AI_{\text{mod}} \geq 0.5$), highly unsaturated compounds ($AI_{\text{mod}} < 0.5$ and H/C ratio < 1.5), unsaturated compounds (H/C ratio ≥ 1.5 and ≤ 2), and saturated compounds (H/C ratio > 2 and ≤ 2.5) [categorization adapted from (63); fig. S3 and table S1]. The molecular approach for tracing terrigenous DOM (t-peaks) developed by (38) was applied to our dataset by analyzing the relative abundance (of the 184 t-peaks formulas) and proportion of t-peaks to the total signal intensity (figs. S1 and S2). Statistical analyses included Pearson correlations (Pearson, R software package, and corrplot package function “cor.test”) and Bray-Curtis molecular dissimilarity analysis of the normalized signal intensities [Bray-Curtis, R software package, vegan package function “vegdist”; e.g., (36)].

The AbioS index

A novel molecular index for the extent of abiotic sulfurization in SPE DOM was established (AbioS index; Figs. 2 and 5). To validate the AbioS index, additional natural DOM samples from highly sulfidic sediments where abiotic sulfurization of DOM was reported in the literature were incorporated in our dataset. This included sulfidic porewater samples from tidal flats (35) and saltmarshes (36). A total of 15 molecular formulas were identified as AbioS compounds (Fig. 2A). The metrics of the AbioS index considered (i) the number of detected AbioS molecular formulas (AbioS, #) and (ii) the sum of normalized signal intensities as percentage of total normalized signal intensity of FT-ICR-MS spectra (AbioS:DOM, %) (Fig. 2C). The AbioS index compounds were defined as sulfur-containing molecular formulas (CHOS) following these six requirements:

1) AbioS index compounds were detected in all of the six abiotically sulfurized samples under experimental conditions of (35).

2) AbioS index compounds were not detected in any of the six control samples (exposed to same laboratory conditions except the addition of sulfur) of (35).

3) AbioS index compounds followed requirements 1 and 2 in both the original FT-ICR-MS dataset analyzed in 2015 and the one reanalyzed twice in 2017. This excluded potential contamination or transformation due to long-term storage of the MeOH extracts.

4) AbioS index compounds molecular formula assignments were used if (i) directly isotopically confirmed (64) or (ii) connected through a homologous series network (CH_2) with at least one isotopically confirmed molecular formula (or member) (63). This reduced the possibility of wrong-formula assignment.

5) AbioS index compounds were not detected in samples from laboratory experiments where abiotic sulfurization was not induced, including abiotic photoincubation (36), iron-DOM coprecipitation (65), and plankton community long-term incubation.

6) AbioS index compounds were not identified in any of 288 analyses of the NEqPIW DOM reference material during 5 years (2013–2018).

This reduced the possibility of random detection due to instrument variability as the NEqPIW sample is injected twice a day for control of overall mass spectrometry quality and reproducibility at the University of Oldenburg FT-ICR-MS.

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at <http://advances.sciencemag.org/cgi/content/full/7/25/eabf6199/DC1>

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