

1 **Rapid, concurrent formation of organic sulfur and iron sulfides during experimental sulfurization**  
2 **of sinking marine particles**

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10 **Key Points:**

- 11 **1.** Organic matter in sinking marine particles sulfurizes rapidly in the presence of polysulfides, tripling its  
12 S:C ratio in 48 hours.  
13 **2.** Iron monosulfides form from iron oxyhydroxide particles on the same timescale as organic sulfur.  
14 **3.** Organic matter sulfurization in sinking particles may increase carbon burial in sediments, impacting  
15 sedimentary records and climate.

16 **Abstract**

17       Organic matter (OM) sulfurization can enhance the preservation and sequestration of carbon in  
18 anoxic sediments, and it has been observed in sinking marine particles from marine O<sub>2</sub>-deficient zones. The  
19 magnitude of this effect on carbon burial remains unclear, however, because the transformations that occur  
20 when sinking particles encounter sulfidic conditions remain undescribed. Here, we briefly expose sinking  
21 marine particles from the eastern tropical North Pacific O<sub>2</sub>-deficient zone to environmentally relevant  
22 sulfidic conditions (20°C, 0.5 mM [poly]sulfide, two days) and then characterize the resulting solid-phase  
23 organic and inorganic products in detail. During these experiments, the abundance of organic sulfur in both  
24 hydrolyzable and hydrolysis-resistant solids roughly triples, indicating extensive OM sulfurization. Lipids  
25 also sulfurize on this timescale, albeit less extensively. In all three pools, OM sulfurization produces organic  
26 monosulfides, thiols, and disulfides. Hydrolyzable sulfurization products appear within ≤ 200-μm regions  
27 of relatively homogenous composition that are suggestive of sulfurized extracellular polymeric substances  
28 (EPS). Concurrently, reactions with particulate iron oxyhydroxides generate low and fairly uniform  
29 concentrations of iron sulfide (FeS) within these same EPS-like materials. Iron oxyhydroxides were not  
30 fully consumed during the experiment, which demonstrates that organic materials can be competitive with  
31 reactive iron for sulfide. These experiments support the hypothesis that sinking, OM- and EPS-rich particles  
32 in a sulfidic water mass can sulfurize within days, potentially contributing to enhanced sedimentary carbon  
33 sequestration. Additionally, sulfur-isotope and chemical records of organic S and iron sulfides in sediments  
34 have the potential to incorporate signals from water column processes.

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36 **Plain Language Summary**

37       Vast amounts of organic carbon are stored in sediments on the ocean floor. This organic carbon is  
38 potentially food for macro- and microorganisms, and yet, under specific environmental conditions, it can  
39 escape being eaten and instead persist in sediments and rocks for millions of years. Here, we conduct  
40 experiments that test how the organic and inorganic materials in sinking marine particles can be

41 transformed by two days of exposure to sulfidic environmental conditions, which are often associated  
42 with high rates of organic carbon burial in sediments. We find that these sulfidic conditions substantially  
43 alter the chemistry of (“pickle”) particle organic materials, yielding products that resemble preserved  
44 organic materials in ancient deposits. Marine particles that encounter sulfidic conditions in the  
45 environment are therefore more likely to be preserved and buried in sediments, sequestering carbon out of  
46 the ocean and atmosphere. This process, called ‘sulfurization,’ may act as a stabilizing feedback in the  
47 carbon cycle as ocean anoxic zones expand in response to ongoing climate change.

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## 50 **1. Introduction**

51 In most of the surface ocean today, photosynthetic algae and bacteria produce organic matter (OM)  
52 that is cycled efficiently and locally through metabolic networks linking bacteria, viruses, zooplankton, and  
53 their exudates. OM may also become incorporated into aggregates with sufficient density to sink, or it can  
54 be transported out of the mixed layer by other particle “pumps” (Boyd et al., 2019). Large ( $\geq \sim 1$  mm),  
55 sinking particles may travel thousands of meters to the seafloor in a few days (La Rocha and Passow, 2007).  
56 As particles sink, they are continually used as a food source, so the downward flux of sinking particulate  
57 OM is strongly attenuated with depth due to oxic respiration. As a result, only a tiny fraction ( $\sim 1.5\%$ ) of  
58 global marine primary production is buried in sediments. In contrast, the efficiency of OM burial can be  
59 much higher in certain types of near-shore (coastal, shelf, or borderland basin) environments (Dunne et al.,  
60 2007; Bianchi et al., 2018), especially those with low dissolved  $O_2$  concentrations like the  $O_2$ -deficient  
61 zones (ODZs) of the Eastern Tropical Pacific and Arabian Sea (Martin et al., 1987; Devol and Hartnett,  
62 2001; B. Van Mooy et al., 2002; Hartnett and Devol, 2003; Keil et al., 2016). Under the more strongly  
63 reducing, frequently sulfidic conditions found in the southern North Atlantic during the Cretaceous ocean  
64 anoxic events, OM burial in sediments served as a major sink for  $CO_2$  and likely mitigated a hothouse  
65 climate (Arthur et al., 1988; Sinninghe Damsté and Köster, 1998; Hülse et al., 2019). Nonetheless, without

66 a more mechanistic understanding of the underlying causes of enhanced sinking particle fluxes in anoxic  
67 environments, we are unable to quantitatively predict how ongoing ODZ expansion and other changes in  
68 marine O<sub>2</sub> availability (Deutsch et al., 2011; Stramma et al., 2011; Schmidtko et al., 2017; Breitburg et al.,  
69 2018; Takano et al., 2018) will impact carbon fluxes to the sediments.

70 Multiple physical, chemical, and biological mechanisms contribute to the enhanced sinking organic  
71 particle flux through anoxic water columns (Keil et al., 2016). Especially in anoxic environments, clays and  
72 other minerals physically protect sedimentary OM by occlusion or sorption onto surfaces (Salmon et al.,  
73 2000; Arnarson and Keil, 2007). Anaerobic microorganisms also gain less energy from the oxidation of  
74 organic matter than aerobic organisms (Froelich et al., 1979), and some individual organic molecules may  
75 become energetically inaccessible at certain redox potentials (Boye et al., 2017). However, anoxic  
76 sedimentary systems often preserve greater quantities of OM than can be explained by surface protection,  
77 microbial energetics, or the availability of alternative electron acceptors like sulfate (Arndt et al., 2013),  
78 indicating that there is a role for condensation and kerogenization reactions that render OM inaccessible to  
79 microbes and their exoenzymes. A special category of kerogenization reactions that is specific to anoxic  
80 environments, OM sulfurization, was observed in sinking ODZ particles under *in-situ* conditions and could  
81 be a significant contributor to OM burial (Raven et al., 2021).

82 OM sulfurization reduces the effective lability of OM by replacing certain functional groups (e.g.,  
83 aldehydes, conjugated double bonds) with organic S functionalities and by bridging molecules together,  
84 increasing their molecular weight (Sinninghe Damsté et al., 1988; Kohnen et al., 1989; Kutuzov et al.,  
85 2020). Sulfurized OM is thus less susceptible to breakdown by microbial exoenzymes than fresh or  
86 degraded algal biomass (Boussafir and Lallier-Verges, 1997; Sinninghe Damsté and Köster, 1998). The  
87 reactants for sulfurization on timescales of days or less appear to be polysulfides (S<sub>x</sub><sup>2-</sup>, 2 ≤ x ≤ 8), which  
88 form spontaneously in the presence of dissolved sulfide (HS<sup>-</sup>) and elemental S (S<sup>0</sup>) or other oxidants  
89 (Kamyshny et al., 2004; Rickard and Luther, 2007). In experiments, algal biomass has been shown to  
90 sulfurize rapidly in the presence of dissolved polysulfides, producing pyrolysates interpreted as deriving

91 from carbohydrates cross-linked with organic sulfides and polysulfides (Gelin et al., 1998; Kok, Schouten,  
92 et al., 2000; Pohlabein et al., 2017). Experiments with model compounds have shown similar cross-linking  
93 following polysulfide exposure (van Dongen et al., 2003; Amrani and Aizenshtat, 2004a). Over the past  
94 few years, OM sulfurization has been reported across a growing diversity of environments, including  
95 coastal mangrove forests, hydrothermal systems, marine surface sediments exposed to variable redox  
96 conditions (Gomez-Saez et al., 2016; Jessen et al., 2017; Raven, Fike, Gomes, et al., 2019; Gomez-Saez et  
97 al., 2021), and sinking marine particles in both sulfidic basins and anoxic (non-sulfidic) ODZs (Raven,  
98 Sessions, Adkins, et al., 2016; Raven et al., 2021). The sulfurization of OM in sinking marine particles  
99 could have a particularly large effect on fluxes in the marine carbon cycle because it impacts a relatively  
100 large and reactive pool of sinking biomass, where moderate changes in preservation efficiency can translate  
101 into substantial changes in the rates of sedimentary OM burial (Raven et al., 2018).

102 In this study, we investigate how sinking marine particles from the eastern tropical North Pacific  
103 ODZ respond to a brief exposure to environmentally relevant sulfidic conditions. The 48-hour duration of  
104 these experiments could be analogous to, for example, the experience of a large particle sinking through a  
105 polysulfide-rich chemocline in the water column. Previous sulfurization experiments that demonstrated  
106 rapid OM sulfurization generally used model compounds (Amrani and Aizenshtat, 2004b) or elevated  
107 temperatures, phase transfer agents, and/or elevated polysulfide concentrations that make them challenging  
108 to directly compare with modern marine environments (e.g., 50°C, 13 mM [poly]sulfide, 30 days) (Gelin  
109 et al., 1998; Kok, Rijpstra, et al., 2000; van Dongen et al., 2003). Here, we conduct two-day experiments  
110 with natural particle samples under environmental conditions (20°C, 0.5 mM [poly]sulfide), and use an  
111 expanded suite of X-ray spectroscopic techniques, to investigate how sulfidic conditions transform sinking  
112 marine particles.

113

## 114 **2. Materials and Methods**

115

116        2.1 Polysulfide solution preparation

117 Polysulfide solutions were prepared by combining 50 mg of natural-abundance  $S^0(s)$  (Sigma Aldrich, trace  
118 metal grade) and 1 mg of  $^{34}S$ -labeled ( $\geq 98\%$ )  $^{34}S^0(s)$  in  $O_2$ -free ultra-pure water with 1.25 mL of 400-mM  
119 sodium sulfide solution adjusted to pH 8 (with HCl). Solutions were equilibrated in the presence of this  
120 excess  $S^0(s)$  at  $18^\circ C$  in the dark for  $>60$  days before use, at which point S-isotope compositions in the  
121 aqueous phase were stable and fully equilibrated with solids. The total concentration of dissolved  
122 (poly)sulfide S in solution under these conditions was  $\sim 12$  mM.

123 In a fully aqueous system, polysulfide speciation would be controlled primarily by pH and the relative  
124 abundances of sulfur and sulfide precursors. In the presence of excess  $S^0(s)$ , however, the major species  
125 present in mixed polysulfide solutions are primarily a function of pH. At pH 8, experimental polysulfide  
126 solutions are largely composed of bisulfide anions and mid-chain-length polysulfides:  $HS^- \rightarrow S_5^{2-} \rightarrow S_6^{2-} \rightarrow S_4^{2-}$   
127 (Rickard and Luther, 2007). Solid-phase  $S^0$  was removed by in-line filtration during injection of the  
128 polysulfide solution into experiments.

129        2.2 Sampling site

130        Samples (IGSN: IEMRRETNP) were collected from the eastern tropical North Pacific ODZ in  
131 spring 2018 as part of cruise RR1807 on the R/V Roger Revelle. We deployed a surface-float-tethered  
132 particle trap with a 2-meter-diameter, 50- $\mu m$ -mesh net (Van Mooy and Keil, 2015) at two sites: a relatively  
133 low particle flux site ('P2,' 200 km from the Mexican coast,  $17.0^\circ N \times 107.0^\circ W$ ,  $\sim 3500$  m water depth), and  
134 a relatively high particle flux site ('P1,'  $\sim 50$  km from shore,  $20.3^\circ N \times 106.1^\circ W$ ,  $\sim 1500$  m water depth).  
135 This same population of samples was also used for radiosulfur measurements of microbial sulfate reduction  
136 rates and the identification of *in-situ* organic S formation (Raven et al., 2021). Particles were trapped at the  
137 depth of the secondary nitrite maximum (120-143 m at P1 and 147 m at P2) for approximately 48 hrs. After  
138 recovery, the 2-m-diameter net, which closed *in-situ* before recovery, was rinsed with filtered surface  
139 seawater to collect particles. Samples for this study (Table S1) include one sample from P2 ('F') and five  
140 samples from P1 ('A' through 'E'), all of which were collected from the 'net wash.' During processing, the

141 experimental sample from P2 was lost due to an unfortunate wind incident. Aliquots for controls ('A<sub>C</sub>,' B<sub>C</sub>,'  
142 etc.) were syringe-filtered in a N<sub>2</sub>-filled glovebag onto pre-combusted, 0.7 μm (GF-F) glass fiber filters and  
143 immediately frozen under N<sub>2</sub> headspace at -20°C. Aliquots for polysulfide exposure experiments ('A<sub>Sx</sub>,'  
144 B<sub>Sx</sub>,' etc.) were transferred to 250-mL serum bottles and sparged with N<sub>2</sub>. Each experiment received 10 mL  
145 of the 12-mM, filtered, <sup>34</sup>S-labeled, mixed polysulfide solution yielding a total reduced sulfur concentration  
146 in experiments of 0.52 mM. Bottles were incubated for 48 hrs at ~20°C in the dark. After incubation, 1-mL  
147 aliquots of seawater were filtered through GF-F filters into vials containing concentrated HCl to volatilize  
148 H<sub>2</sub>S and then preserved with BaCl<sub>2</sub> for sulfate S-isotope analysis. Particle solids were collected anoxically  
149 onto pre-combusted GF-F filters and frozen under N<sub>2</sub> at -20°C.

### 150 *2.3 Sample collection, handling, and processing*

151 Particle samples were subdivided into three pools for analysis: extractable lipids (OM<sub>Lipid</sub>), acid-  
152 soluble/volatile materials (OM<sub>Hyd</sub>), and acid-resistant organics (OM<sub>Res</sub>), as diagrammed in Figure S1. After  
153 filters were washed with N<sub>2</sub>-sparged pH 7.8 tris buffer solution to remove inorganic sulfate and lyophilized,  
154 splits were set aside for 'whole particle' spectroscopy, and selected controls with sufficient particle material  
155 were split to allow elemental and isotopic analysis of 'whole particles' with minimal disruption. Remaining  
156 particles were microwave-extracted (CEM MARS-6) twice in 9:1 dichloromethane:methanol. Solvent  
157 extracts were concentrated under N<sub>2</sub> and exposed to activated Cu<sup>0</sup> for 12 hrs to remove elemental S. Lipid  
158 extract aliquots for XAS were dried onto quartz slides, and the remaining material was trapped onto washed  
159 and dried silica gel for elemental analysis. Splits of solvent-extracted particle filters were set aside for X-  
160 ray absorption spectroscopy and X-ray fluorescence mapping (XAS/XRF), and experimental samples with  
161 sufficient material were split for elemental analysis. Experimental particles were split after solvent  
162 extraction to ensure removal of reactant polysulfide before S quantification.

163 All solvent-extracted particles were subjected to acid-volatile sulfide (AVS) extraction with hot  
164 (~70°C) 6N hydrochloric acid under flowing N<sub>2</sub> (Rickard and Morse, 2005; Raven, Fike, Gomes, et al.,  
165 2019). In addition to volatilizing sulfides from FeS, this method solubilizes a large proportion of the

166 carbohydrates and proteins in OM (Hill, 1965). After AVS hydrolysis, remaining solids were washed in  
167 ultra-pure water and divided into splits for XAS and for elemental analysis.

#### 168 *2.4 EA-IRMS analysis*

169 Carbon isotopes and S:N:C elemental ratios of lipid extracts and whole and AVS-extracted particles  
170 were analyzed at UCSB with an Elementar Vario Isotope Select elemental analyzer (EA), which includes  
171 a ramped-temperature column to improve SO<sub>2</sub> peak shape, coupled to a Nu Horizon isotope ratio mass  
172 spectrometer (IRMS). C-isotope data were internally standardized to CO<sub>2</sub> gas standards and calibrated to  
173 VPDB using the caffeine isotope standards USGS-61, -62, and -63. Reported uncertainties reflect long-  
174 term uncertainties for replicate sulfanilamide standards. Whole particle samples before acidification retain  
175 some seawater sulfate, as quantified by XAS (below); reported S:C ratios for ‘whole particle’ and ‘solvent-  
176 extracted’ OM were corrected (uncorrected data included in Table S2) to remove the proportional  
177 contributions from inorganic phases (sulfate and FeS) based on the relative abundances of these phases in  
178 XAS spectra. S-isotope values for dissolved sulfate at the end of the polysulfide exposure experiment and  
179 for the initial polysulfide spike were measured by EA-IRMS as barium sulfate and zinc sulfide,  
180 respectively. Samples contained WO<sub>3</sub> as a combustion aid, and S-isotope values were calibrated to VCDT  
181 using the isotope standards IAEA-S1, S2, S3, and S5. The  $\delta^{34}\text{S}$  values for the <sup>34</sup>S-labeled polysulfide spike  
182 are estimates because they exceed the calibration range of these standards.

#### 183 *XAS/XRF analysis and data processing*

184 The redox speciation and bonding environment of sulfur and iron in the filter-bound particles were  
185 analyzed at the Stanford Synchrotron Radiation Lightsource (SSRL). Glass fiber filter pieces were adhered  
186 onto Saint Gobain M60 S-free polyester tape and covered in 5- $\mu\text{m}$ -thick SPEX 3520 polypropylene XRF  
187 film. ‘Bulk’ sulfur K-edge spectra (500  $\mu\text{m}^2$  spot size) were collected on beam line 14-3 on whole particles,  
188 solvent-extracted particles, HCl-extracted particles, and lipid extracts, before and after copper exposure.  
189 Additionally, a micro-focused X-ray beam was used to map S and Fe species by rastering over selected  
190 mapping areas at specific energies (for sulfur: 2472.0, 2472.9, 2473.9, 2474.25, 2476.15, 2477.8, 2481.4,

191 2482.6, and 2486.0 eV; for iron: 7116.0, 7128.0, 7133.0, 7139.0, and 7147.0 eV) to create elemental and  
192 chemical distribution maps. Full XAS spectra were collected from 2460 to 2540 eV (sulfur) or 6900 to 7500  
193 eV (iron) at selected spots.

194 Sulfur data were collected at SSRL beam line 14–3, which is equipped with a Si(111) ( $\Phi = 90$ ) double  
195 crystal monochromator and calibrated to the thiol pre-edge peak of thiosulfate at 2472.02 eV. The S  $K\alpha$   
196 fluorescence line was measured with a Si Vortex Si drift detector (Hitachi) using Xspress3 pulse processing  
197 electronics (Quantum Detectors). The X-ray beam was focused using an axially symmetric focusing mirror  
198 (SIGRAY) to a size of  $5 \times 5 \mu\text{m}$  at a flux of  $\sim 8 \times 10^{10}$  photons per second; maps were collected at a resolution  
199 of  $5 \mu\text{m}^2$ . Sulfur XAS spectra were processed in the SIXPACK (Webb, 2005; Webb, 2020) software  
200 package using a K-edge E0 of 2473 and pre-edge and post-edge linear normalization ranges of  $-20$  to  $-7$   
201 and  $35$  to  $70$  eV, respectively. Sulfur K-edge fitting standards are shown in Figure S2. Uncertainties  
202 reported in Table S3 refer to the confidence in the linear combination fit calculated in SIXPACK. Iron data  
203 were collected at SSRL beam line 2–3, a bending magnet workstation equipped with a Si(111) ( $\Phi = 0$ )  
204 double crystal monochromator calibrated such that the first derivative of an Fe metal foil was set to 7112  
205 eV. The beam line uses an axially symmetric focusing mirror (SIGRAY) to achieve a spot size of  $5 \times 5 \mu\text{m}$   
206 at a flux of  $\sim 5 \times 10^8$  photons per second at 7100 eV, and uses a similar fluorescence data collection system  
207 as above with 14-3 to collect K-edge Fe spectra from 6900 to 7500 eV and elemental maps of Ca, P, Mn,  
208 Ti, S, and other metals at  $5\text{-}\mu\text{m}$  resolution. XRF maps from both beam lines were processed using the  
209 MicroAnalysis Toolkit (SMAK; (Webb et al., 2011)). Sulfur XANES fitting used 3-pt blurred maps  
210 (standard deviation 0.5) and a set of six standard spectra (FeS, methionine, glutathione disulfide, methionine  
211 sulfoxide, cysteic acid / sulfonate, and sulfate ester, Fig. S2).

212

### 213 **3. Results**

#### 214 **3.1 EA–IRMS results**

215 The carbon-isotope compositions of sinking particle materials are similar for samples from both  
216 the high- and low-particle-flux sites (A–E and F, respectively; Fig. 1A and Table S2). Whole washed  
217 particles before acidification, which may contain both organic C and calcium carbonate, have  $\delta^{13}\text{C}$  values  
218 between  $-26.0$  and  $-23.7\text{‰}$  (mean  $-24.6\text{‰}$ ), while lipid extracts have relatively  $^{13}\text{C}$ -depleted compositions  
219 (Hayes, 2001) between  $-32.9$  and  $-28.3\text{‰}$  (mean  $-30.4\text{‰}$ ). Accordingly, the  $\delta^{13}\text{C}$  values for  $\text{S}_x$ -exposed,  
220 solvent-extracted particles are higher (mean  $-22.0\text{‰}$ ) than those for whole particle controls due to the  
221 removal of  $^{13}\text{C}$ -depleted lipids by solvent extraction (Fig. 1A). After both lipid extraction and strong  
222 acidification (6N HCl,  $70^\circ\text{C}$ , 2 hrs), residual particle material ( $\text{OM}_{\text{Res}}$ ) from both experiments and controls  
223 has a  $\delta^{13}\text{C}$  value between  $-27.4$  and  $-25.0$  (mean  $-25.7\text{‰}$ ). There is no significant change in the C-isotope  
224 composition of either  $\text{OM}_{\text{Res}}$  or  $\text{OM}_{\text{Lipid}}$  associated with  $\text{S}_x$  exposure.

225 The nitrogen contents of whole particles, lipids, and  $\text{OM}_{\text{Res}}$  primarily track the abundance of protein  
226 in each pool (Fig. S3). Whole particles N:C ratios (8.9 – 15.3 mol%) are typical for protein-rich, primary  
227 producer biomass that has experienced some degradation (16:117 = 13.7%; (L. Anderson and Sarmiento,  
228 1994)), while lipid extracts have lower N:C ratios (0.6 – 3.8 mol%). Molar N:C ratios in  $\text{OM}_{\text{Res}}$  controls  
229 are between 2.5 and 4.7 mol%. In some cases,  $\text{S}_x$ -exposed  $\text{OM}_{\text{Res}}$  contains significantly more N than  $\text{OM}_{\text{Res}}$   
230 controls, with N:C ratios of up to 7.8 mol% (sample  $\text{D}_{\text{Sx}}$ ; Fig. S3).

231 Sulfur-isotope compositions of dissolved sulfate in experimental bottles are between 22.6‰ and  
232 24.3‰, summarized in Table S2. Replicates of the polysulfide spike were trapped as zinc sulfide and thus  
233 reflect thio sulfur (bisulfide and roughly half of polysulfide S); the effect of excluding zero-valent  
234 polysulfide S is negligible in this case given the much larger uncertainties from standard extrapolation.  
235 Spike  $\delta^{34}\text{S}$  values average 342.2‰ (Table S3).

236 Particle S:C ratios (Fig. 1B) increase in response to  $\text{S}_x$  exposure, reflecting the addition of  
237 (poly)sulfide S to particulate OM. In controls, the S:C ratio of organic materials in whole particles is 0.64–  
238 0.74 mol% at high-flux site P1 and 1.3 mol% in one sample from low-flux site P2 (Table S2).  $\text{OM}_{\text{Res}}$  and  
239 lipids have lower S:C ratios, averaging 0.3 mol% and 0.4 mol%, respectively. After sulfurization, organic

240 materials in whole and solvent-extracted particles from P1 have S:C ratios between 1.7 and 2.1 mol%, an  
241 approximately 2.8-fold increase over P1 controls. Similarly, average  $S_x$ -exposed  $OM_{Res}$  S:C ratios average  
242 1.1% (range 0.5 – 1.7%), a roughly 3.3-fold increase over P1 controls. Lipid extract S:C ratios are variable  
243 among samples (0.1 – 1.1%) and do not differ systematically between controls and  $S_x$ -exposed samples.

244

### 245 3.2 Bulk Particle XAS Speciation

246 The redox speciation of sulfur in particles varies systematically among lipid, hydrolyzable, and  
247 hydrolysis-resistant materials, and these distributions are consistent across samples from both sites and all  
248 depths (Fig. 2). Broadly speaking, the organic S (OS) in whole (control) particles from the ETNP is  
249 approximately 60% oxidized (sulfonates and sulfate esters) and 40% reduced (sulfides, disulfides, and  
250 aromatics). Organic solvent extracts are predominantly (58–78%) sulfate esters with up to 16% sulfonates,  
251 and the remaining 3.2 – 16.1% of the lipid OS pool is reduced. Hot acidification (6N HCl, 70°, 2 hrs)  
252 removed approximately 85% of the total sulfur in the particles, which included most of the non-lipid  
253 oxidized OS as sub-equal pools of sulfate esters and sulfonates. A reduced OS component is also removed  
254 by acidification that is best fit as aromatic S. After acidification, residual solids ( $OM_{Res}$ ) contain sulfur  
255 predominantly as sulfides and disulfides, with smaller amounts of aromatics and oxidized forms, as was  
256 previously reported for parallel experiments with this population of particles (Raven et al., 2021).

257 After exposure to polysulfides for ~48 hrs, the speciation of sulfur in all five of the particle samples  
258 from site P1 was transformed, as summarized on the right-hand side of Fig. 2. Compared to controls,  $S_x$ -  
259 exposed particles contain a larger proportion of reduced species (sulfides and disulfides) and iron sulfides.  
260  $S_x$ -exposed whole particles contain some elemental sulfur derived from the polysulfide reactant solution  
261 that was subsequently removed by solvent extraction and copper exposure. Copper-treated lipids after  
262 polysulfide exposure contained nearly 50% reduced OS in addition to the sulfate esters and sulfonates  
263 observed in the  $OM_{Lipid}$  controls. Reduced OS in the  $S_x$ -exposed lipids is composed of sulfides and  
264 disulfides with some zero-valent S. Particle materials lost during acidification include iron sulfides (AVS)

265 and roughly sub-equal pools of reduced and oxidized OS ( $OM_{Hyd}$ ). OS in  $OM_{Res}$ , on the other hand, is  
266 almost exclusively reduced (sulfides and disulfides). Oxidized OS thus makes a smaller contribution to  
267 total OS in the experimental particles than in corresponding controls.

268

### 269 3.3 Particle XRF Maps

270 To examine the spatial variability in particle OS speciation, we mapped particles at 5-to-7- $\mu m$   
271 resolution using X-ray fluorescence imaging. Figure 3 presents maps of sulfur speciation in whole, buffer-  
272 washed particles from site P1 at 123 m depth (sample 'A<sub>C</sub>'). Sulfur was not detected in filter backgrounds.  
273 Organic sulfur speciation is spatially heterogeneous in control particles, with separate regions that are rich  
274 in reduced versus oxidized organic S. The abundance of reduced organic S at specific spots ranges from  
275 20.5% (spot 8) to 72.5% (spot 2). Reduced organic S, including organic monosulfides, thiols, aromatics,  
276 and disulfides, appears as localized concentrations ranging from  $\leq 7 \mu m$  (single pixel) to nearly 80  $\mu m$  in  
277 diameter. Oxidized components (sulfonates and sulfate esters) are also found in discrete regions up to  
278 several hundred microns in size.

279 After exposure to polysulfides, particles accumulate organic monosulfides and disulfides (Fig. 4).  
280 The proportion of OS in reduced forms (sulfides, disulfides, and aromatics) ranges from 59.3 to 78.3% (Fig.  
281 4A and Table S5), and the overall proportion of reduced S is higher, consistent with the results for bulk  
282 speciation (Fig. 2). Newly formed disulfides appear as irregularly sized splotches that are generally but not  
283 exclusively associated with other forms of organic S, especially organic monosulfides (e.g., spot 7). Regions  
284 that are relatively rich in oxidized OS are discrete and 100–200  $\mu m$  in size, similar to those observed in  
285 controls. In contrast, iron monosulfides are found throughout sulfurized particle materials and do not  
286 generally accumulate as singular particulates. Despite its relatively low abundance, the presence of FeS in  
287 these samples is confirmed by the characteristic pre-edge peak near 2470 eV in the XAS spectra from Fig.

288 4A (shown in Fig. S4). Gypsum (calcium sulfate) was also detected as an individual 25- $\mu$ m-diameter  
289 particulate (spot 6).

290 Most of the iron on the particle filters is present as discrete, 15–40  $\mu$ m particulates (Fig. 5). Prior  
291 to polysulfide exposure, iron oxyhydroxides are scattered throughout the samples and are not spatially  
292 associated with either carbonates or organic matter (P or S). After polysulfide exposure, some of these  
293 discrete iron particulates remain (e.g., C<sub>Sx</sub>, Fig. 5), but iron also accumulates throughout the particles as a  
294 low, uniform abundance phase that is broadly co-located with sulfur. Based on XAS spectra in Fig. 4, at  
295 least some of this material is FeS (e.g., mackinawite).

296

## 297 4. Discussion

### 298 4.1 *Controls: Organic sulfur speciation in sinking marine particles*

299 Sulfur is a major component of biomass. Molar S:C ratios for marine biomass are typically 0.5–  
300 1%, although they can be lower in woody plants and higher in some S-cycling microorganisms (Matrai and  
301 Eppley, 1989; Chen et al., 1996). The speciation of organic sulfur in particles (Fig. 2) reflects the  
302 contributions of various compound classes to functionally defined categories of OM, as well as any  
303 subsequent transformations of that OM due to enzymatic degradation, condensation, oxidation, and/or  
304 sulfurization.

305 Sinking particles from the ETNP ODZ contain the full suite of reduced and oxidized OS moieties  
306 that have been previously described for proteins, lipids, and carbohydrates. A large proportion (42–65%)  
307 of the assimilatory S in microplankton is typically found as proteins and polypeptides (Cuhel et al., 1982),  
308 specifically the amino acids cysteine, which is a thiol, and methionine, which is an organic monosulfide.  
309 Cysteine and methionine are highly susceptible to oxidation, both in the environment and during laboratory  
310 handling, which will produce sulfoxide (Vogt, 1995) and/or sulfonate (Phillips et al., 2021). The AVS  
311 hydrolysis method used here to isolate OM<sub>Res</sub> is less intense (shorter duration and lower temperature) but

312 otherwise similar to some early methods for protein hydrolysis (e.g., 24 hrs, 110°, 6N HCl) (Hill, 1965).  
313 Therefore, AVS hydrolysis is likely to solubilize many proteins in our particles, which is supported by the  
314 drop in molar N:C ratios from whole particles (averaging 11.6%) to OM<sub>Res</sub> (averaging 4.5%; Fig. S1).  
315 However, we find that most of the OS in the OM<sub>Hyd</sub> pool is relatively oxidized (Fig. 2), suggesting that  
316 cysteine and methionine are not major contributors to OM<sub>Hyd</sub>. (We calculate speciation by comparing solids  
317 before and after hydrolysis, so the lack of reduced S in OM<sub>Hyd</sub> is not caused by amino acid oxidation during  
318 hydrolysis.) Instead, the reduced OS species in OM<sub>Hyd</sub> are best fit as aromatic, and the main peak in their  
319 XAS spectra at ~2473.5 eV is resolvably shifted relative to cysteine and methionine. Although aromatic  
320 OS compounds have been attributed to rapid OM sulfurization in a few cases (i.e., phytol thiophene,  
321 (LaLonde et al., 1987; Raven, Sessions, Adkins, et al., 2016)), aromatic OS is generally rare in modern  
322 samples, and we do not observe aromatic OS formation during polysulfide experiments (below). The  
323 immediate provenance of apparent aromatic OS in OM<sub>Hyd</sub> from untreated particles is thus not yet known.  
324 Rather than appearing in OM<sub>Hyd</sub>, organic monosulfides and thiols account for ~80% of the S in OM<sub>Res</sub> from  
325 control particles. These functional groups are localized in cell-sized ( $\leq 20$   $\mu\text{m}$ ) structures, which suggests  
326 they may be proteinaceous (Raven et al., 2021). In addition to amino acids, these structures contain organic  
327 disulfides that may reflect amino acid dimers, like cystine. Finally, even in these unamended ‘control’  
328 samples, we expect to have at least trace contributions of sulfides and/or disulfides to OM<sub>Res</sub> from *in-situ*  
329 OM sulfurization, as we observed using radiolabels in Raven et al. (2021).

330       Oxidized OS compounds comprise the majority of total OS in lipids, OM<sub>Hyd</sub>, and whole particles.  
331 Lipid extracts are particularly rich in sulfate esters, which could represent sulfated hormones (e.g.,  
332 cholesterol sulfate) and/or various sulfoglycolipids common in animals (Benson et al., 1959; R. Anderson  
333 et al., 1978; Ishizuka, 1997). Lipids also contain sulfonates, which could reflect contributions from common  
334 S-bearing lipids like sulfoquinovosyl diacylglycerides (SQDGs). In OM<sub>Hyd</sub>, carbohydrates appear to be  
335 major sources of oxidized OS, especially sulfate esters (Fig. 2). Exudates from macrophytoplankton can be  
336 major sources of such sulfate-ester-bearing polysaccharides (Ramus and Groves, 1974; Percival et al.,

337 1980) and are likely to be particularly important here, because these extracellular polysaccharides, which  
338 can be produced in vast quantities by diatoms, are thought to contribute directly to the formation of large,  
339 sinking particles (Alldredge and Silver, 1988; La Rocha and Passow, 2007; Arnosti et al., 2021; Vidal-  
340 Melgosa et al., 2021). Hydrolyzable sulfate esters are also frequently localized in irregularly sized particles  
341 (Fig. 3) that could represent detritus from plants and animals and/or sulfated polysaccharides from algal  
342 exudates (Vidal-Melgosa et al., 2021). Overall, these XAS data underscore the substantial contributions of  
343 oxidized OS species to lipids and carbohydrates in marine particles, which can be clearly distinguished  
344 from amino acids and the products of abiotic OM sulfurization.

345

#### 346 4.2 *Experiments: Organic products of particle sulfurization reactions*

347 In a separate study (Raven et al., 2021), we used radiolabeled sulfate to identify organic S formation  
348 in sinking marine particles under anoxic, sulfide-limited, ODZ-like conditions. Here, we investigate how  
349 this same population of particles would be transformed by short-term exposure to more strongly reducing,  
350 sulfidic conditions. Polysulfide concentrations in our experiments (~0.5 mM) are equivalent to or slightly  
351 higher than reported concentrations in a range of modern environments: the Great Salt Marsh (Boulegue et  
352 al., 1982; Luther et al., 1986), sulfidic lakes like Mahoney Lake (Overmann et al., 1996) and Fayetteville  
353 Green Lake (Zerkle et al., 2010), and the Black Sea (Holmkvist et al., 2011). Polysulfide concentrations  
354 can be even higher in specific environments like microbial mats, where up to 100s of mM polysulfides have  
355 been reported (Findlay, 2016). Conditions in experimental bottles therefore coarsely reproduce the  
356 experience of particles in certain modern and ancient Earth environments.

357 Experiments with particles and (poly)sulfide generated organic S in the proto-kerogen,  
358 hydrolysable, and lipid pools. Based on XAS fits, organic S accounts for the majority (67 to 82%) of the  
359 newly formed non-lipid particle solids (Fig. 6); inorganic products (iron sulfides) are discussed in Section  
360 4.3, below. The initial molar S:C ratios in total particle OM from high-flux site P1 average 0.69% (range  
361 0.64 – 0.74%), and these ratios increase after 48 hours of polysulfide exposure to an average of 1.9% (range

362 1.7 – 2.1%). OM S:C ratios are somewhat lower in the OM<sub>Res</sub> pool, averaging 0.33% before, and 1.1% after,  
363 polysulfide exposure (Fig. 1B). These S:C ratios are similar to those found in OM<sub>Res</sub> in sediments from O<sub>2</sub>-  
364 limited continental margin sediments, including the Santa Barbara Basin (OM S:C ratios average 2.1 mol%  
365 in the upper 50 cm; (Raven, Sessions, Fischer, et al., 2016)), the Peru Margin (0.5 – 2.3% in the upper meter  
366 of sediments; (Mossman et al., 1991; Suits and Arthur, 2000), and the Namibian Margin (OM S:C ratios  
367 average 2.3% for all data; (Dale et al., 2009)). But, sulfurized particle S:C ratios remain below those  
368 observed for OM in sulfidic basins like the Cariaco Basin (~4 mol%; (Werne et al., 2003)). It is likely that  
369 longer-term exposure to polysulfides would further increase the S content of particle OM, eventually  
370 reaching ‘saturation’ or full sulfurization of the functional groups that are reactive on the timescale of  
371 interest, as modified by other environmental factors (Amrani et al., 2007). The change in particle S:C ratios  
372 as a result of sulfurization indicates that organic precursor molecules contained at least that density of  
373 rapidly sulfurizable functional groups (aldehydes, ketones, certain re-arrangeable alcohols, and conjugated  
374 double bonds (Kutuzov et al., 2020)). Primary biogenic molecules can also gain sulfide-reactive functional  
375 groups like carbonyls through photochemical reactions in the euphotic zone (Amrani and Aizenschtat  
376 2004c).

377         The short duration of these 48-hour experiments makes it possible to investigate potential OM  
378 preservation processes on the same timescale as particle OM breakdown and remineralization. Typical  
379 sinking particle OM remineralization rates are ~12% per day (Iversen and Ploug, 2013; Cavan et al., 2017),  
380 which means that reactions that transform particle OM within days are particularly important for impacting  
381 the extent of OM remineralization in sinking particles and, by extension, carbon fluxes to the sediments.  
382 Additionally, the large changes in organic S chemistry observed within 48 hours in these experiments  
383 demonstrate that even intermittently sulfidic conditions – on the timescale of hours to days – can have a  
384 dramatic effect on the composition of particulate OM.

385         The initial products of particle sulfurization are primarily organic monosulfides and disulfides (Fig.  
386 6). Although three of the five sulfurized samples also contained more sulfonates or sulfate esters than their

387 respective controls, this likely represents heterogeneity in the distribution of assimilatory OS particles  
388 among control and experiment filter aliquots. In Figure 6, the speciation of newly formed materials is  
389 calculated by assuming that sulfurization adds new sulfur to an unchanging pool of biogenic OS, as  
390 measured in the control sample. The calculated, newly formed OS is very similar to the overall speciation  
391 of  $S_x$ -exposed  $OM_{Res}$  (Fig. 2) and is consistent with observations from the sulfurization of standard  
392 compounds under conditions similar to those investigated here (Amrani and Aizenshtat, 2004b). In those  
393 experiments,  $\alpha$ ,  $\beta$ -unsaturated aldehydes, including the chlorophyll-derived  $C_{20}$  isoprenoid phytenal, were  
394 exposed to a polysulfide solution and the products were identified as disulfide-bridged oligo-polymers.  
395 Nucleophilic polysulfides attacked the conjugated double bond rapidly (within hours) and the carbonyl  
396 group more slowly, leading to carbon skeletons cross-linked by two or more  $S_x$  (e.g., polysulfide) bridges  
397 within days to weeks (Amrani and Aizenshtat, 2004b). Similar mechanisms could explain the observed  
398 rapid formation of organic sulfides, disulfides, and polysulfides ( $S_{x \geq 3}$ ) during the sulfurization of sinking  
399 marine particles.

400 Newly formed organic disulfides appear within certain particle regions that range from 30 to 300  
401  $\mu m$  in diameter (Fig. 4). These ‘strongly sulfurized’ regions often envelop clusters of small (single-pixel;  $\leq$   
402 5  $\mu m$ ), sulfide-rich particulates that are interpreted as cells. And, they are also frequently associated with  
403 the larger (20–200  $\mu m$ ), sulfate-ester-rich irregular particles that may represent concentrations of  
404 polysaccharide exudates or contributions from plant or animal detritus. These spatial relationships suggest  
405 that sulfurization affects a ubiquitous particle component that naturally contains a lower concentration of  
406 organic S than other forms of biomass. Exopolymeric substances (EPS) are a leading candidate for this  
407 component. EPS is a loosely-defined blend of polysaccharides, proteins, nucleic acids, and lipids, with  
408 carboxylate, amine, hydroxyl, sulfate, and phosphate functional groups (Alvarado Quiroz et al., 2006;  
409 Braissant et al., 2007). EPS is an important contributor to the formation of large, sinking particles, building  
410 particle size and density by binding organic and inorganic solid materials together (Alldredge and Silver,

411 1988; Passow et al., 1994; Bhaskar and Bhosle, 2005). The abundance of EPS in large sinking particles  
412 may make these organic materials particularly susceptible to rapid sulfurization.

413 XAS results strongly indicate that lipids sulfurize alongside non-lipid OM over 48 hours of  
414 polysulfide exposure, despite the lack of consistent trends in lipid S:C ratios. Variable lipid S:C ratios  
415 among samples, both before and after sulfurization, most likely reflect the heterogeneous distributions of  
416 specific particle components (e.g., animal detritus; Ishizuka 1997) that are key sources of sulfated lipids.  
417 Sulfurization may also reduce the solubility of lipid molecules and transfer them functionally to the OM<sub>Res</sub>  
418 pool, although here this process was insufficient to significantly lower the C-isotope composition of OM<sub>Res</sub>  
419 (Fig. 1A). Newly formed lipid OS is compositionally similar to newly formed non-lipid OS, with varying  
420 proportions of organic sulfides, disulfides, and oxidized species. Sulfurized lipids also contain zero-valent  
421 S that may represent the S<sup>0</sup> atoms in S<sub>3</sub> and longer organic polysulfides, which may therefore be more  
422 important in lipids than for other organic precursors (Fig. 6). Rapid lipid sulfurization has been documented  
423 for specific lipid molecules both experimentally and in the environment (Van Mooy et al., 2002; Amrani  
424 and Aizenshtat, 2004b; Raven, Sessions, Adkins, et al., 2016), while other specific lipids are known to  
425 sulfurize over thousands of years (Kok, Rijpstra, et al., 2000; Werne et al., 2000). The observed changes in  
426 the speciation of total extractable lipid OS help to scale up these compound-specific observations and  
427 indicate that rapid sulfurization can impact a substantial proportion of the bulk sedimentary lipid pool.

428

### 429 4.3 *Experiments: Competitive sinks for polysulfides*

430 Dissolved sulfide (H<sub>2</sub>S/HS<sup>-</sup>) has many possible reaction pathways in real, complex marine  
431 particles. In addition to reactions with OM, both microbial sulfide oxidation and iron sulfidization can occur  
432 rapidly, generating inorganic sulfur species with redox states ranging from S<sup>0</sup> to sulfate, and iron sulfides,  
433 respectively.

434 We use the appearance of  $^{34}\text{S}$ -labeled sulfate to estimate the scale of polysulfide oxidation during  
435 the 48-hour experiment. The  $\delta^{34}\text{S}$  value of seawater sulfate increased during the experiment to values  
436 between  $22.6 \pm 0.4\text{‰}$  and  $24.3 \pm 0.4\text{‰}$ , a significant change from initial sulfate at  $\sim 21\text{‰}$ . Given a 28 mM  
437 concentration of seawater sulfate, these values indicate the addition of between 140 and 295  $\mu\text{M}$  sulfate  
438 with a  $\delta^{34}\text{S}$  value matching the polysulfide spike ( $\sim 342\text{‰}$ ), which represents a substantial proportion (27 –  
439 57%) of the 520  $\mu\text{M}$  polysulfide solution originally added to each experiment. Some of this (poly)sulfide  
440 oxidation may have occurred abiotically through reaction with any dissolved  $\text{O}_2$  that was introduced during  
441 on-deck handling of these ‘net wash’ samples and incompletely removed during gentle sparging with  $\text{N}_2$ .  
442 However, even dissolved  $\text{O}_2$  concentrations of as much as 10  $\mu\text{M}$  would account for only a few percent ( $\sim 5$   
443  $\mu\text{M}$ ) of this sulfate production. Instead, most (poly)sulfide oxidation likely occurred through microbial  
444 processes, which can be highly efficient at drawing down limiting sulfide concentrations, generating a  
445 tightly coupled and often cryptic sulfur cycle in sediments (Canfield et al., 1992; Jorgensen, 2019) and the  
446 water column (Canfield et al., 2010; Johnston et al., 2014). Given ETNP ODZ conditions, nitrate is likely  
447 to be a main oxidant powering sulfide oxidation in the dark (Devol et al., 2019). Net rates of (poly)sulfide  
448 oxidation in our experimental bottles were  $\sim 100 \mu\text{M}/\text{day}$ , which is similar to sulfide oxidation rates reported  
449 for very different environments like shallow marine sediments (Findlay et al., 2020). Notably, OM  
450 sulfurization occurs in particles despite this active competition for (poly)sulfide from oxidative sinks.  
451 Although this oxidative cycle likely generated some quantity of more oxidized inorganic sulfur species (e.g.  
452 thiosulfate), the key reactant for OM sulfurization is still most likely polysulfide because this matches the  
453 redox state of the newly formed organic S.

454 Another important sink for (poly)sulfide in particles is the formation of iron sulfides. The initial  
455 product of the reaction between  $\text{Fe}^{2+}$  and  $\text{S}^{2-}$  is an iron monosulfide (e.g.,  $\text{FeS}(\text{aq})$ , mackinawite). Given  
456 unlimiting sulfide, the rate of this reaction depends on the availability of  $\text{Fe}^{2+}$ , which is typically sourced  
457 from the reduction of Fe(III)-oxyhydroxides and other reactive Fe(III) species. Marine particles from ODZs  
458 frequently contain  $\text{Fe}^{3+}$  in the form of iron(III) oxyhydroxides, which appear to be actively recycled between

459 dissolved  $\text{Fe}^{2+}$  and particulate Fe(III) minerals (Resing et al., 2015; Heller et al., 2017). We observe similar  
460 iron species in our control particle samples; the first-derivative X-ray spectra for iron in these particulates  
461 (Fig. 5) have similar spectral features to Fe(III) oxyhydroxide standards. Before  $\text{S}_x$  exposure, these iron  
462 oxyhydroxides are found in discrete, 10–50  $\mu\text{m}$ -diameter particles with a broadly round morphology. These  
463 iron-bearing particulates are found throughout the mapped samples (A, B, and C) and are not spatially  
464 associated with calcium, phosphorus, or total sulfur.

465         After 48 hours of exposure to polysulfides, iron sulfides (FeS) form that have a distinctly different  
466 distribution than their Fe(III) precursors. FeS does not appear as discrete, resolvable particles and instead  
467 accumulates to low, uniform concentrations throughout the same,  $\sim 200\text{-}\mu\text{m}$ -scale particle regions that  
468 accumulate organic S (Fig. 5). Although Fe(III) particulates do not appear to be local FeS formation  
469 hotspots, they must be the source of iron for FeS formation because dissolved  $\text{Fe}^{2+}$  concentrations in the  
470 ETNP ODZ are only  $\sim 2$  nM (*Bolster et al., under review GCA*) and iron backgrounds in the EPS from  
471 controls are low (Fig. 5). FeS formation therefore most likely proceeded through the reductive dissolution  
472 of Fe(III) oxyhydroxides by sulfide to dissolved  $\text{Fe}^{2+}$ , which was subsequently precipitated from solution  
473 as FeS. Iron-cycling microbes may also play a role in the generation of dissolved  $\text{Fe}^{2+}$ . Although greater  
474 temporal resolution is needed to evaluate the kinetic competition between sulfurizable organic moieties and  
475  $\text{Fe}^{2+}$  for sulfide, the concurrent and co-located formation of FeS and OS within 48 hrs illustrates the tightly  
476 coupled formation of both inorganic and organic sulfur phases in sedimentary systems.

477         Sulfide-derived organic S has been seen to accumulate prior to the complete consumption of iron  
478 oxyhydroxides across diverse marine and lacustrine environments (Francois, 1987; Hartgers et al., 1997;  
479 Urban et al., 1999; Filley et al., 2002; van Dongen et al., 2003; Dale et al., 2009; Raven, Sessions, Fischer,  
480 et al., 2016). At the same time, environments with abundant reactive iron and active iron cycling can  
481 suppress the formation of organic and inorganic S (Shawar et al., 2018). The relative rates of formation for  
482 organic and inorganic S are complex and will depend on the identities and morphologies of organic and  
483 inorganic precursors, local geochemical conditions, and spatial relationships between sulfide sources and

484 potential sinks. It is clear, however, that organic S has the potential to preserve S-isotope signals that reflect  
485 a water column, particle-hosted sulfur cycle. We speculate that relatively rapid microbial sulfate reduction  
486 with fresh, particle OM may generate smaller S-isotope fractionations between sulfate and sulfide and  
487 therefore more  $^{34}\text{S}$ -enriched dissolved sulfide, OS, and FeS than equivalent metabolisms in near-surface  
488 sediments. The S-isotope composition of sulfide in particles may also be impacted by closed-system  
489 processes within diffusively-limited particle microenvironments, and by the activity of tightly coupled  
490 microbial metabolisms in addition to microbial sulfate reduction. In all of these cases, OS  $\delta^{34}\text{S}$  values can  
491 be influenced by processes outside of a traditional, one-dimensional sedimentary diagenetic framework.

492

#### 493 **4.4 Implications for the long-term preservation of sulfurized OM**

494 Because particle OM can sulfurize rapidly, even brief periods of sulfidic conditions in the  
495 environment have the potential to transform the chemical structure of sinking particulate OM and impact  
496 its lability. OM sulfurization is thus capable of transforming OM in temporally dynamic systems with only  
497 intermittently sulfidic conditions, ranging from tidally and photosynthetically cyclic systems like microbial  
498 mats and inter-tidal habitats to environments with strong seasonal upwelling. In sulfidic lakes and basins,  
499 sinking particles that encounter a layer of polysulfide-rich water near the  $\text{O}_2$ - $\text{H}_2\text{S}$  chemocline (Overmann  
500 et al., 1996; Li et al., 2008) are likely to carry a signal of rapid OM sulfurization reactions to underlying  
501 sediments, similar to interpretations of pyrite  $\delta^{34}\text{S}$  values from the Black Sea and Cariaco Basin (Lyons,  
502 1997; Lyons et al., 2003). The isotopic composition and speciation of organic sulfur preserved in sediments  
503 will in part reflect rapid reactions in polysulfide-rich hotspots.

504 Prior to long-term burial, however, the initial products of particle sulfurization may experience  
505 additional condensation reactions, enzymatic attack, and changing environmental conditions that could  
506 further alter their chemistry. Organic di- and poly-sulfides may be particularly susceptible to isotope  
507 exchange with inorganic polysulfides and chemical maturation – the rearrangement of bonds to more  
508 energetically stable forms during sedimentary diagenesis (Canfield et al., 1998; Amrani et al., 2006).

509 Organic polysulfide maturation would decrease the proportion of organic polysulfides and disulfides over  
510 time and increase the abundance of monosulfidic or aromatic moieties (Kohnen et al., 1991; Amrani et al.,  
511 2006), which are more common in ancient deposits. S-isotope exchange between organic and inorganic  
512 polysulfides could also help explain puzzling S-isotope distributions among sedimentary phases in shallow  
513 anoxic sediments (i.e., (Dale et al., 2009; Raven, Sessions, Fischer, et al., 2016)). Here, our experiments  
514 with relatively high concentration of polysulfides strongly favor the formation of organic di- and poly-  
515 sulfides relative to reactions with sulfide (Kohnen et al., 1989). As a result, OM contains an average of  
516 52.4% disulfides (range 46.8 to 67.4%; Fig. 6, excluding FeS), which is significantly higher than control  
517 OM<sub>Res</sub> from untreated particles, which averages 31.3% (range 23.4 to 47.7%) disulfides, or than kerogens  
518 from 100-million-year-old black shales, which have a maximum reported disulfide content of ~28%  
519 (Raven, Fike, Bradley, et al., 2019; Raven et al., 2021). Depending on the scale and timing of organic  
520 polysulfide exchange and maturation in the environment, the  $\delta^{34}\text{S}$  values recorded in sedimentary OM may  
521 partially reflect later generations of environmental (poly)sulfide rather than the S-isotope composition of  
522 the (poly)sulfide that initially drove OM sulfurization. Further work is needed to better understand the  
523 stability of OM  $\delta^{34}\text{S}$  values during very early diagenesis and the preservation potential of rapidly-formed  
524 organic polysulfides. Organic polysulfide maturation could also serve as a source of sulfur to other  
525 sedimentary reactions, including the conversion of iron monosulfides to pyrite, S<sup>0</sup> disproportionation, or  
526 gradual lipid sulfurization.

527 OM sulfurization in sinking marine particles has substantial implications for the carbon cycle, both  
528 in response to anthropogenic climate change and during periods of Earth history with relatively widespread  
529 sulfidic conditions. In the modern ocean, sinking particle sulfurization could help explain the observation  
530 that sediments below a water column ODZ can have higher carbon contents than those under water columns  
531 without a strong O<sub>2</sub> minimum, even when bottom water is oxygenated (Lückge et al., 1996; Devol and  
532 Hartnett, 2001; B. Van Mooy et al., 2002; Keil et al., 2016). Due to the potential for rapid OM sulfurization  
533 in the water column, the ongoing expansion of ODZs (Schmidtke et al., 2017) may increase OM burial in

534 even deep-water, O<sub>2</sub>-exposed sediments. OM burial during Ocean Anoxic Event 2 (~94 Mya) was also  
535 likely enhanced due to water column particle sulfurization, drawing down atmospheric CO<sub>2</sub> and impacting  
536 climate (Sinninghe Damsté and Köster, 1998; Hülse et al., 2019; Raven, Fike, Bradley, et al., 2019). On  
537 even longer timescales, there is widespread evidence for locally sulfidic conditions at intermediate water  
538 depths throughout the Proterozoic (Lyons et al., 2014; van de Velde et al., 2020). OM sulfurization may  
539 have influenced the efficiency of carbon burial throughout this period, modifying the organic carbon burial  
540 processes that contributed to the oxygenation of the surface Earth. On all of these timescales, particle OM  
541 sulfurization in the water column is a powerful lever connecting changes in local redox state to substantial  
542 transformations in the pool of OM delivered to, and preserved in, marine sediments.

543

## 544 **5. Conclusions**

545 Sinking, OM-rich marine particles are transformed by reactions with polysulfides within 48 hours.  
546 Organic materials in the acid-soluble, acid-resistant, and lipid pools can all sulfurize on this timescale. Iron  
547 monosulfides (FeS) also form concurrently, prior to the complete consumption of Fe(III) minerals, which  
548 indicates that organic matter and iron minerals can be competitive sinks for (poly)sulfide over short (day)  
549 timescales. Both organic S and FeS phases appear within regions of particles that are suggestive of  
550 extracellular polymeric substances (EPS), which may be particularly important substrates for sinking  
551 particle sulfurization. Rapid particle-hosted OM sulfurization has the potential to enhance total organic  
552 carbon burial in sediments and to help explain why marine sediments in sulfidic environments often  
553 preserve abundant OM.

554 The initial products of particle OM sulfurization are primarily organic monosulfides and disulfides.  
555 Subsequent transformations of this sulfurized OM during sedimentation and early diagenesis could further  
556 transform the speciation and/or isotopic composition of organic S. In ancient deposits, the S-isotope  
557 compositions of organic S and iron sulfides will depend on the availability and speciation of iron and  
558 organic reactants during their formation – potentially in the water column – as well as these later reactions.

559

560

561

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576

577 **Open Research / Data Availability**

578 All of the processed data used in this manuscript are presented in the main text and supporting information.  
579 Data files are also archived on FigShare (10.6084/m9.figshare.16550790).

580

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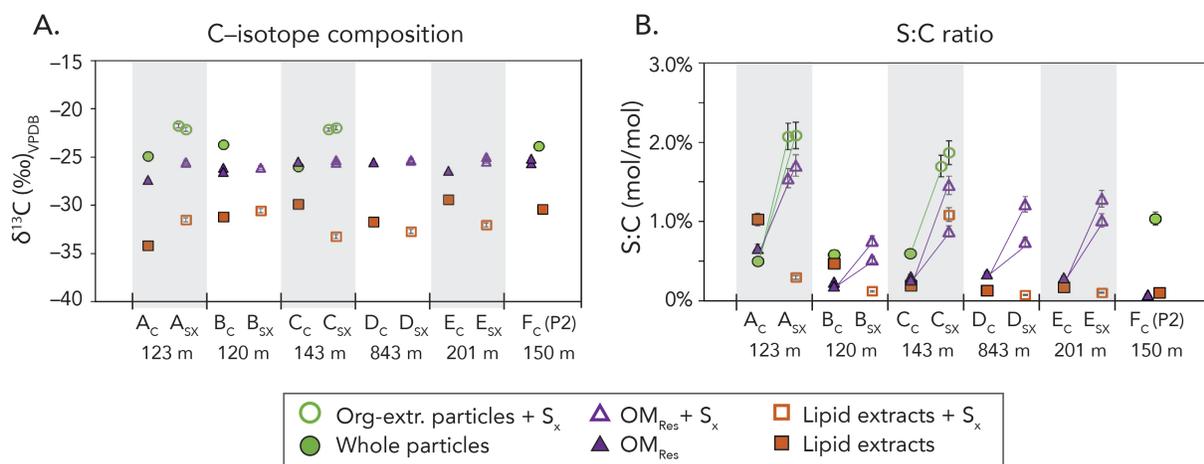
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855

856

857

858 FIGURE CAPTIONS



859

860 **Fig. 1: Carbon-isotope composition and molar S:C ratio of particle materials, before and after  $S_x$**

861 **exposure.** S:C ratios exclude inorganic phases (sulfate and FeS) quantified by XAS; uncorrected ratios are

862 reported in Table S2. Filled symbols represent controls (e.g., 'A<sub>C</sub>'), and hollow symbols represent

863 experiments (e.g., 'A<sub>S<sub>x</sub></sub>'), as detailed in Table S2. The six discrete sets of samples are listed on the x-axis

864 in arbitrary order. Whole and organic-solvent-extracted particles represent the combination of OM<sub>Hyd</sub> and

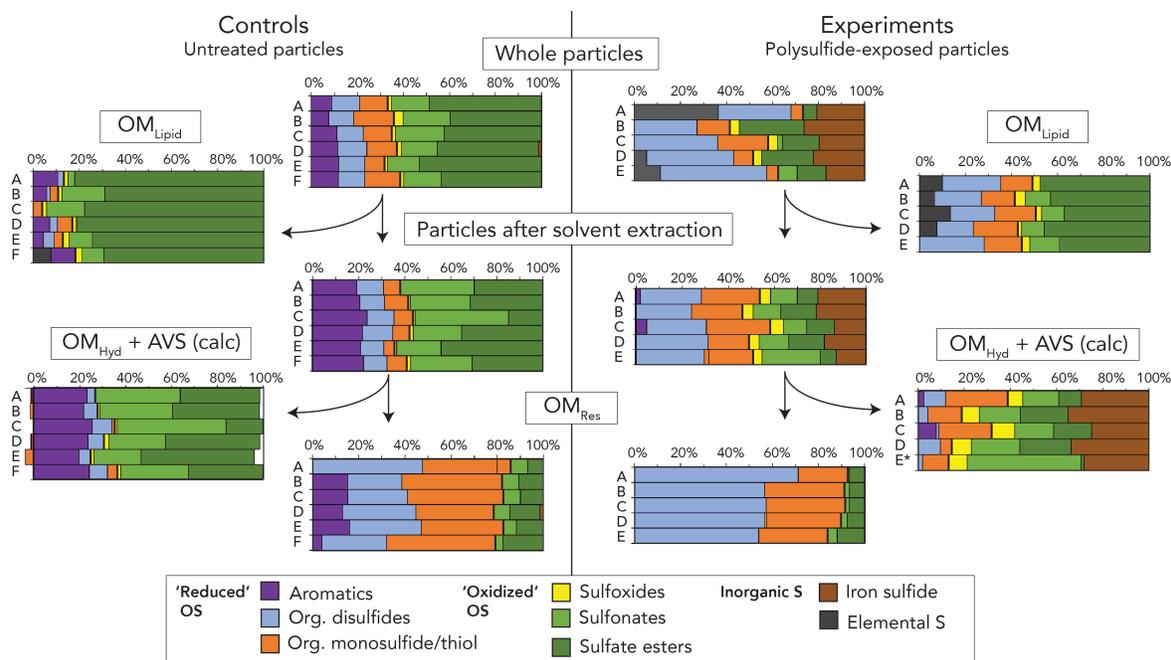
865 OM<sub>Res</sub>. Samples with multiple symbols represent discrete filter splits rather than replicates of homogenized

866 samples. Error bars indicate the long-term reproducibility of standards ( $2\sigma$ ). In panel B, purple and green

867 lines highlight the consistent increase in the S:C ratio of OM<sub>Res</sub> and whole particle samples following

868 sulfurization; bulk lipids do not show a consistent trend.

869



870

871 **Fig. 2 Sulfur speciation in sinking ETNP particles, with and without polysulfide exposure.** Samples

872 A through F represent six separate trap deployments (see Table S1). Heavy black lines between the orange

873 and yellow bars broadly separate 'reduced' from 'oxidized' organic sulfur species. Inorganic sulfate was

874 also detected in samples before hot acidification and is excluded from normalization. Non-sulfate materials

875 lost during hot acidification are calculated by difference using X-ray spectrum step heights and are subject

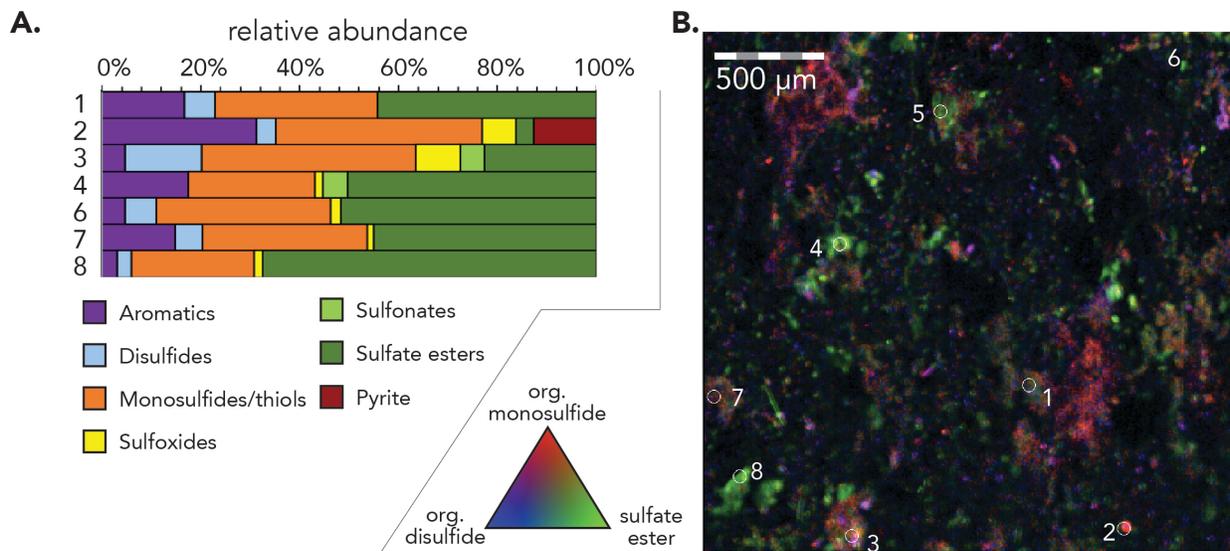
876 to errors of 5–10%. One sample labeled E\* used an assigned step height. Fit uncertainties on each

877 component are typically <2% (see Table S4). The elemental S detected in experimental whole particles

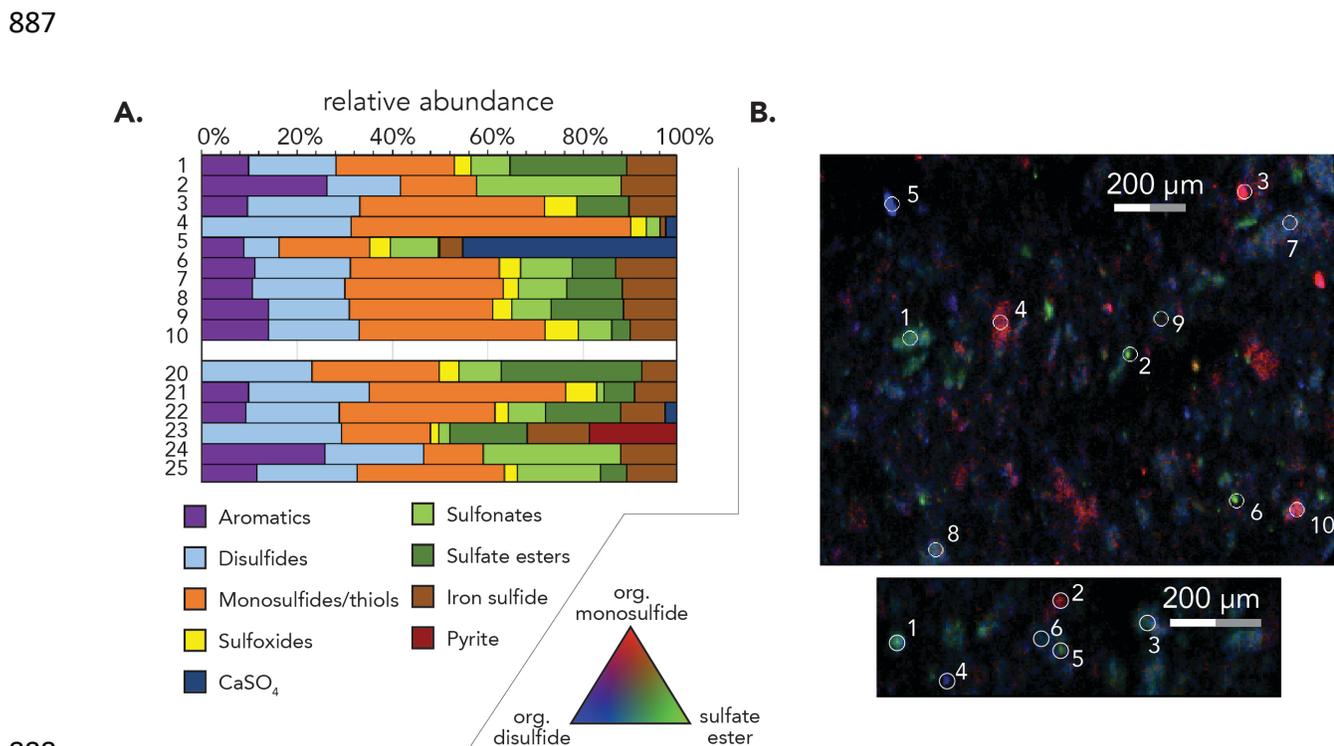
878 (grey) may derive from polysulfide reactants; this was removed from lipid extracts before analysis by Cu

879 exposure.

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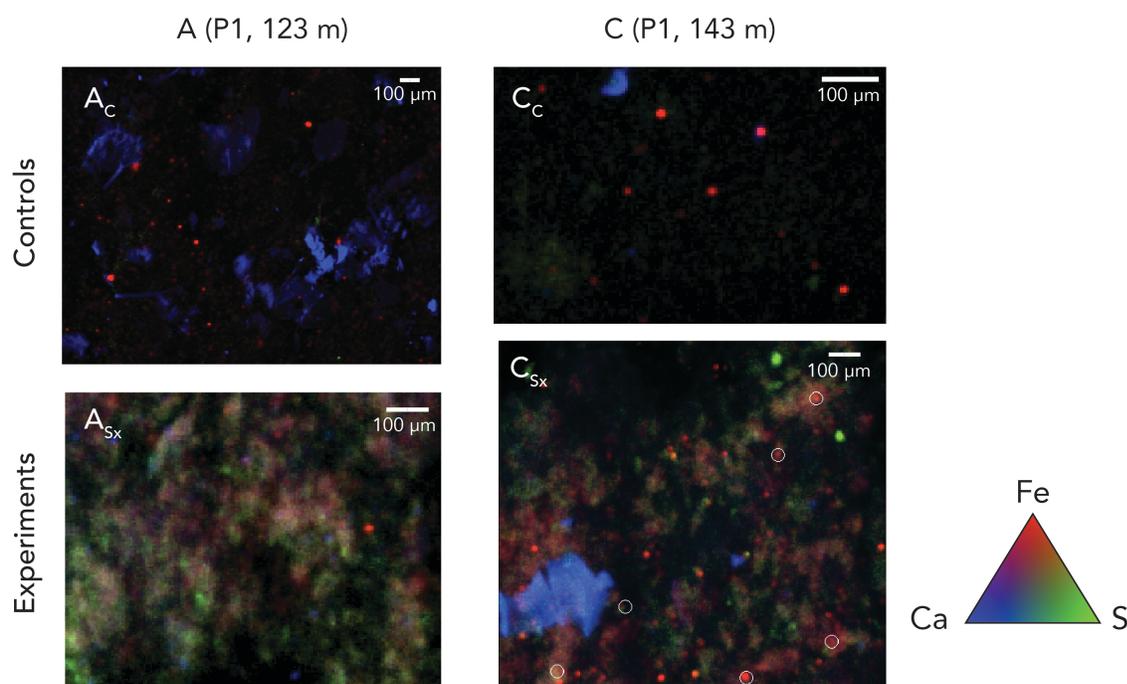


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 882 **Fig. 3 Sulfur speciation of whole particle controls by XAS and XRF.** Particles were collected from  
 883 site P1 (123 m, sample 'Ac') and are mounted on GFF filters. Panel A: Fitted XAS spectra for specific (~1  
 884  $\mu\text{m}^2$ ) spots, numbered at right. Uncertainties are typically <2%, see Table S5. Panel B: Tri-color XANES  
 885 fits to multiple-energy maps showing organic monosulfides and thiols (red), disulfides (blue), and sulfate  
 886 esters (green). Map step size = 7  $\mu\text{m}$ .

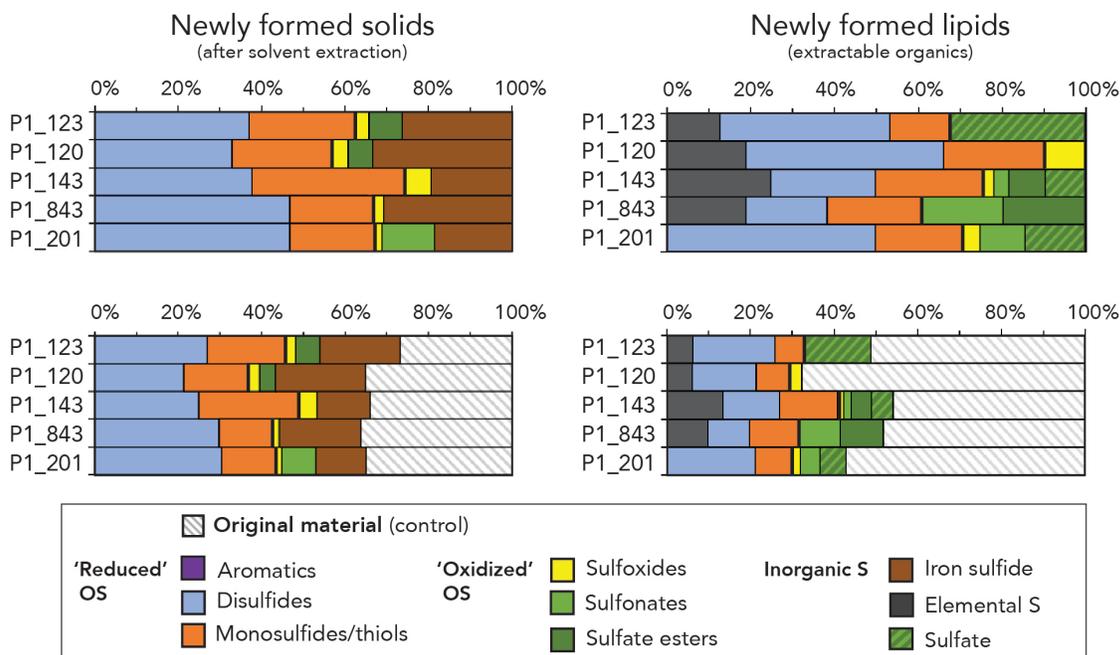


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889 **Fig. 4 Sulfur speciation maps of polysulfide-exposed particles by XAS and XRF.** As in Fig. 3,  
 890 particles were collected from site P1 (123 m, sample 'A<sub>Sx</sub>') and are mounted on GFF filters. Panel A: Fitted  
 891 XAS spectra for specific (~1 μm<sup>2</sup>) spots, numbered at right. Uncertainties are typically <2%, see Table S5.  
 892 Panel B: Tri-color XANES fits to multiple-energy maps from two adjacent filter regions, showing organic  
 893 monosulfides and thiols (red), disulfides (blue), and sulfate esters (green). Step size = 5 μm. Newly formed  
 894 disulfides appear as 50-to-100-μm regions surrounding more discrete particles containing various forms of  
 895 organic S.  
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 898 **Fig. 5: Maps of iron, calcium, and sulfur on particle filters by XRF.** Samples were prepared by washing  
 899 with buffer under anoxic conditions; sulfur maps in all panels thus include trace inorganic sulfate. Maps  
 900 show representative regions from sample splits, not the same regions after treatment. Pixels are 5 μm<sup>2</sup>.  
 901 Colors show iron at 7133 eV (red), total calcium (blue), and total sulfur (green).  
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904 **Fig. 6 Speciation of products formed during polysulfide exposure.** Heavy black lines broadly separate  
 905 'reduced' from 'oxidized' organic sulfur species. Results were calculated by linear combination fitting of  
 906  $S_x$ -exposed sample spectra for solvent-extracted particles ( $OM_{Res} + OM_{Hyd}$ , left) and lipid extracts (right)  
 907 using the control spectra from each sample as a component. Upper and lower panels show the same data,  
 908 but the lower panel highlights the proportion of  $S_x$ -exposed materials that were attributed to pre-existing  
 909 (control) materials. Newly formed organic S in both pools is largely sulfides and disulfides.

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