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Take down policy

Molecular evidence for abiotic sulfurization of dissolved organic matter in marine shallow hydrothermal systems

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1. INTRODUCTION

Dissolved organic matter (DOM) is defined as the organic components in water that pass through a $\leqslant 0.7$ μm filter. The importance of DOM in global geochemistry relies on the enormous amount of carbon that is dissolved in the oceans, quantified as more than 200 times the carbon of all living marine biomass (Hansell et al., 2009) and being similar to all atmospheric CO_2 (Hedges, 1992). Thus, changes in DOM dynamics may have implications for local and global carbon cycling processes (Battin et al., 2009; Dittmar and Stubbins, 2014; German et al., 2015). While molecular DOM characterization of a range of marine habitats has become available in the last years (e.g. Lechtenfeld et al., 2014; Hansman et al., 2015), there exists a striking scarcity of studies targeting specifically the role of organic sulfur species in DOM (e.g. Lechtenfeld et al., 2011; Pohlabeln and Dittmar, 2015). Sulfur plays a considerable role in the various transformations of organic matter, from early diagenesis to the late stage of catagenesis, due to its ability to exist in many different oxidation states (Aizenshtat et al., 1995; Sleighter et al., 2014). In marine sediments, organic sulfur is quantitatively the second most important sulfur pool only behind pyrite, frequently accounting for 35% of total sedimentary sulfur in many marine environments (Zaback and Pratt, 1992; Cutter and Kluckhohn, 1999; Werne et al., 2004; Zhu et al., 2014). Abiotic sulfurization can contribute to the stabilization of organic matter (Sinninghe Damsté et al., 1989; Sinninghe Damsté and de Leeuw, 1990) but whether it also contributes to the stability of DOM in the oceans is still an open research question (Dittmar, 2015).

A significant contribution to the understanding of dissolved organic sulfur (DOS) origin and fate may be achieved through the investigation of DOS biogeochemistry at hydrothermal systems. Hydrothermal activity has been operating for most of the Earth´s history, occurring over a wide depth range in the oceans, from intertidal to the abyss (Sander and Koschinsky, 2011). Hydrothermal vents have been postulated as possible sites for the first steps of organic chemical evolution, where sulfur reduction might have played a role in prebiotic chemical processes occurring in sulfide-rich environments (Russell and Hall, 1997; Cody et al., 2000; Hebting et al., 2006; McCollom and Seewald, 2007). The hydrothermal vents located at less than 200 m water depth are categorized as shallow-water hydrothermal systems (Tarasov et al., 2005). They are easily accessible extreme environments with strong redox gradients and unique biogeochemical conditions due to the interaction of hot, reduced fluids and cold, oxygenated seawater (e.g. Dando et al., 1999; Tarasov et al., 2005). The main group

of solid phase extractable (SPE) DOS in the open ocean was recently identified as unreactive sulfonic acids (Pohlabeln and Dittmar, 2015). In hydrothermal systems, reduced sulfur compounds are expected to be released and quickly oxidized to form sulfonic acids once they reach the oxic sediment surface or water column. Some functional groups like thiols and thioethers could be produced by reaction of reduced inorganic sulfur compounds with organic matter (Sinninghe Damsté et al., 1989; Aizenshtat et al., 1995; Schneckenburger et al., 1998; Hertkorn et al., 2013; Sleighter et al., 2014) and then be rapidly oxidized to sulfonic acids as well (Pohlabeln and Dittmar, 2015). However, neither the pathways of sulfurization nor of oxidation of DOS at hydrothermal systems are well understood (Zhu et al., 2014; Pohlabeln and Dittmar, 2015).

Recent advances in mass spectrometry allow characterization of the complex mixture of DOM at a molecular level. Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) in combination with soft ionization techniques such as electrospray ionization (ESI) provides molecular information on individual compounds without prior chromatographic separation. Thousands of molecular formulas containing C, H, O, N, S or P can be attributed to a single water sample (e.g. Marshall et al., 1998; Kujawinski et al., 2002; Kujawinski and Behn, 2006; Schmidt et al., 2014; Zark et al., 2015), providing unique data sets to study the multitude of processes that affect the composition of the DOM pool in the oceans. The aim of this paper is to characterize at a molecular level DOS variations at the interface between hot, reduced hydrothermal fluids and cold, oxygenated seawater in three contrasting marine shallow-water hydrothermal systems off the coast of Milos (Eastern Mediterranean), Dominica (Caribbean Sea) and Iceland (North Atlantic) (Fig. 1). We hypothesize that in shallow hydrothermal systems (1) the reduced DOS released from hydrothermal fluids is oxidized upon contact with oxygenated seawater and (2) there is DOM reduction and sulfurization during seawater recirculation through the subsurface. Therefore, within a robust geochemical data set we specifically investigated (1) the impact of physico-chemical properties on the DOM signature and (2) the molecular similarities and differences in DOS between shallow hydrothermal fluids and surrounding surface seawater samples.

2. STUDY SITES

2.1. Milos

Milos Island is located in the tectonically active region of the Hellenic Volcanic Arc in the Eastern Mediterranean
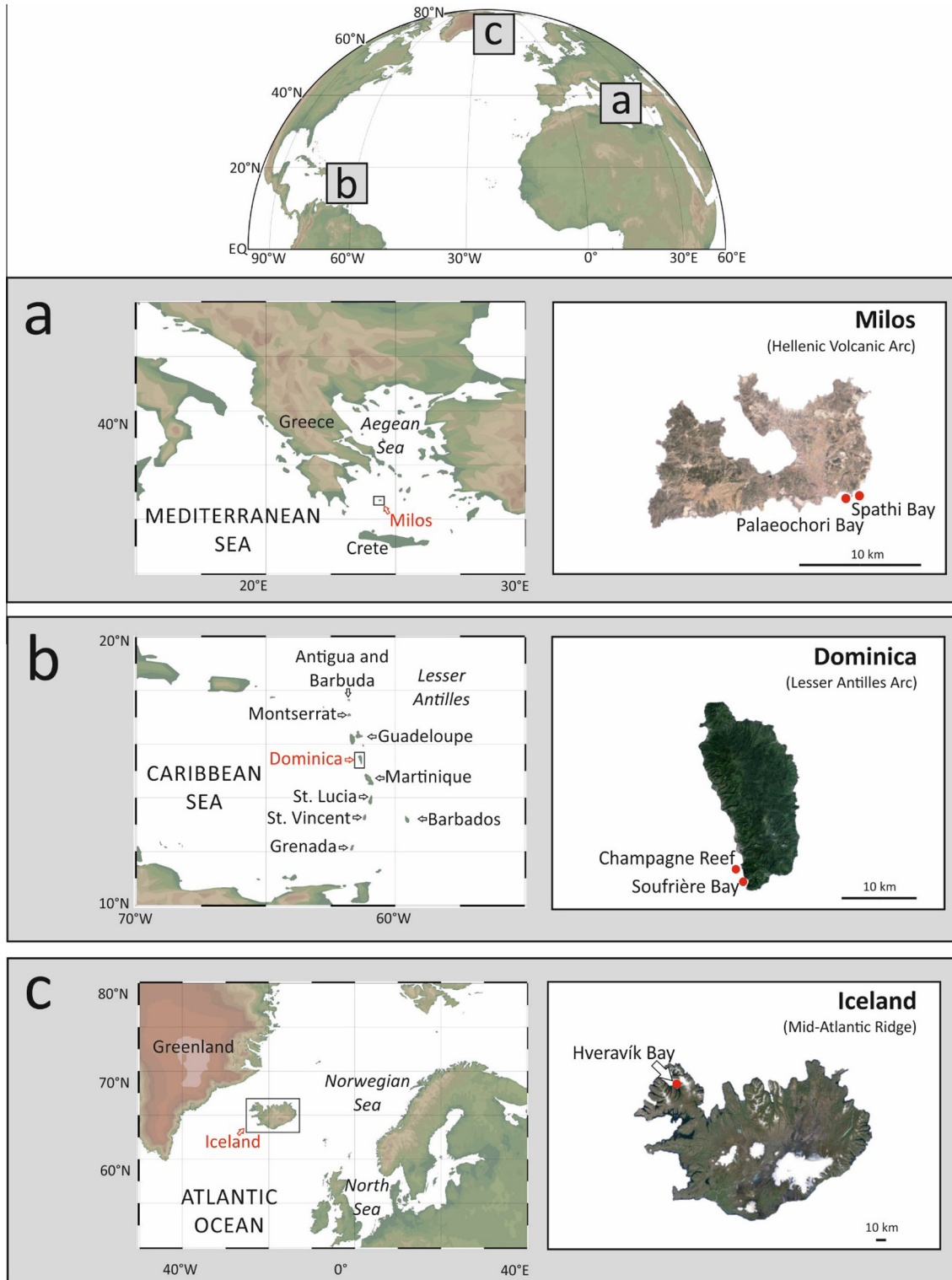
Fig. 1. Shallow-water hydrothermal systems studied: (a) Milos (Hellenic Volcanic Arc, Eastern Mediterranean), (b) Dominica (Lesser Antilles Arc, Caribbean Sea) and (c) Iceland (Mid-Atlantic Ridge, North Atlantic). Maps were created using Ocean Data View (R. Schlitzer, http://odv.awi.de) and Google Earth (http://earth.google.com).

(Dando et al., 1999; Fig. 1a). The volcanism of the Hellenic Volcanic Arc is linked to the subduction of the African plate beneath the Aegean micro plate. It started during the Early to Middle Pliocene and the last eruption occurred 90,000 years ago (McKenzie, 1972). Milos hydrothermal activity occurs on shore but also in the shallow waters off

the coast (Kleint et al., 2015). The marine shallow hydrothermal vents off Milos cover an area of about 35 km² and hot fluids can reach temperatures up to 150 °C (Dando et al., 1995, 1999). Two types of venting activity are observed in Milos hydrothermal system: a gas-dominated focused flow with visible gas bubbles, containing mainly CO_2, plus minor contributions from H_2, H_2S and CH_4, and a brine-rich fluid seep enriched in Ca, Na, K, SiO_2, Mn and NH_3 (Dando et al., 1995; Fitzsimmons et al., 1997; Yücel et al., 2013). Abundant yellow, white and orange patches appear in Milos sediment as a result of elemental sulfur or arsenic sulfide precipitates (Price et al., 2013). In this study, the hydrothermal fluids released at the most intense submarine venting area in the south-east of Milos, at Palaeochori and Spathi bays (Fig. 1a), were investigated.

2.2. Dominica

Dominica has been the volcanically most productive island in the Lesser Antilles arc over the last 100,000 years and one of the most productive worldwide (Wadge, 1984; Lindsay et al., 2005). It belongs to the Lesser Antilles archipelago, between the Atlantic Ocean and the Caribbean Sea, which represents one of only two active arc systems in the Atlantic Ocean (Fig. 1b). Most of the Lesser Antilles islands in the Caribbean Sea only have a single vent, but Dominica has nine potentially active volcanic centres (Lindsay et al., 2005; Joseph et al., 2011). Quaternary volcanic activity on Dominica has been dominated by intermediate to felsic magmas, erupted as large-volume ignimbrites and dome complexes (Lindsay et al., 2005; Kleint et al., 2015). Dominica submarine hydrothermal venting occurs mainly along the submerged flank of the Plat Pays Volcanic Complex in the south-west of the island, with fluid temperatures ranging between 44 and 75 °C (McCarthy et al., 2005; Gomez-Saez et al., 2015). The hydrothermal fluids are characterised by high concentrations of ferrous iron, which is immediately oxidized upon contact with oxygenated seawater, leading to the formation of orange patches in the sediment composed of hydrous ferric oxide precipitates (McCarthy et al., 2005; Gomez-Saez et al., 2015). For this study, the hydrothermal fluids released at Champagne Reef and Soufrière Bay submarine venting systems in the south-west of Dominica (Fig. 1b) were investigated.

2.3. Iceland

Iceland is located on the volcanically active Mid-Atlantic ridge system and represents the largest area of sub-aerially exposed mid-ocean ridge on Earth (Höskuldsson et al., 2007). In northern Iceland, several localities are known with submarine geothermal activity in basaltic lava of 6–12 million years of age (Marteinsson et al., 2001). The low-temperature systems in Iceland have by definition a reservoir temperature below 150 °C at 1 km depth and are mainly located outside the volcanic zone that passes through Iceland (Axelsson et al., 2010). The hot springs in the north-west region of the Reykjanes peninsula are considered as one of the most extreme microbial environments on Earth (Hobel et al., 2005). The Reykjanes geothermal system is supplied with deep fresh and slightly alkaline groundwater, with low sulfide concentrations and fluid temperatures ranging between 45 and 95 °C (Kristjansson et al., 1986). Freshwater hot springs have been described on the seashore, from the tidal zone to about 100 m off the coast (Hobel et al., 2005). The hydrothermal vents in this area are influenced by tides as high as 4 m, leading to almost 100 °C temperature fluctuations implying severe changes in salinity, light penetration and oxygen concentration (Hobel et al., 2005). In this study, the Hveravík Bay hydrothermal system in the Reykjanes peninsula in north-west Iceland was investigated (Fig. 1c). We sampled a shallow vent, which was air-exposed under low tide conditions (Hveravík-1) and another permanently submerged vent (Hveravík-2).

3. MATERIALS AND METHODS

3.1. Field work

The hydrothermal fluids and seawater samples for this study were taken during field expeditions to Milos (11th–30th of May 2012), Dominica (9th–24th of April 2013) and Iceland (15th–30th of June 2014). Identification of hot-fluid spots in the area were carried out by SCUBA diving using the same *in situ* temperature probes as in previous studies (e.g. Price et al., 2013; Gomez-Saez et al., 2015). Fluid samples were collected with a funnel placed on the vent orifice which opened into food-grade large volume nylon bags. On shore, 2 L polycarbonate bottles (Nalgene, USA), previously cleaned with ultrapure pH 2 water, were filled with the hydrothermal fluids up to the top to prevent headspace, immediately closed and kept in the dark until filtration and acidification. Surface seawater samples were taken directly into the 2 L bottles. Samples were filtered using pre-combusted glass microfiber filters (GF/F, pore size: 0.7 μm, diameter: 47 mm; Whatman), acidified to pH 2 (HCl 25% p.a., Carl Roth, Germany) and kept at 4 °C in the dark until extraction.

3.2. Geochemical parameters

Temperature, pH and salinity were measured directly in the field. The pH and salinity were determined using a WTW pH meter 3210 with Mic-D electrode. Aliquots of 4 mL filtered (0.2 μm, GHP Pall Acrodisc) vent fluids were kept sealed in cold storage at 4 °C for transport to the laboratory. Analyses of inorganic geochemical parameters of Milos and Dominica samples were performed at the University of Bremen (Germany) and in the case of Iceland samples at Jacobs University (Germany), following established procedures for hydrothermal fluids from marine shallow-water hydrothermal systems (e.g. McCarthy et al., 2005; Price et al., 2013; Gomez-Saez et al., 2015; Kleint et al., 2015). Aliquots for H_2S analysis were preserved by precipitation of ZnS and analyzed using a Merck photometer at a wavelength of 670 nm (e.g. Price et al., 2013). The anions Br, Cl, F and SO_4 were determined by

ion chromatography using a Metrohm system (Switzerland). The cations of As, Ca, Fe, K, Li, Mg, Mn, Na, Si and Sr were measured by inductively coupled plasma-optical emission spectroscopy (ICP-OES) using a Perkin Elmer Optima 7300 (USA). Oxygen and deuterium isotopic analyses were performed on filtered samples (0.2 μm, GHP Pall Acrodisc) using a LGR liquid water isotope analyzer (LWIA-24d) at the University of Bremen (Germany) (e.g. McCarthy et al., 2005; Price et al., 2013). All isotopic values are reported using the standard delta notation. Both $\delta^{18}O$ and $\delta^{2}H$ are expressed relative to Vienna Standard Mean Ocean Water (VSMOW; Craig, 1961).

3.3. Dissolved organic matter

Aliquots of 20 mL (triplicates per sample), filtered (0.7 μm; GF/F; Whatman) and acidified to pH 2, were analyzed for dissolved organic carbon (DOC) concentration via high temperature catalytic combustion using a Shimadzu TOC-VCPH/CPN Total Organic Carbon Analyzer at the University of Oldenburg (Germany). Accuracy of DOC determination was checked daily by analyzing the deep-sea reference provided by D. Hansell (University of Miami, USA). DOM was extracted from filtered and acidified samples by solid phase extraction (SPE) using divinyl benzene polymer in pre-packed cartridges (1 g PPL, Agilent, USA) according to Dittmar et al. (2008). Acidification of the sample to pH 2 is recommended for DOM extraction of natural water samples in FT-ICR-MS analysis, because the majority of natural DOM is assumed to consist of organic acids (Dittmar et al., 2008). Although low pH may decrease binding strengths, protonation of the binding sites is not high enough to completely dissociate metals like iron from acid-stable metal–organic complexes (Waska et al., 2015). Prior to extraction, the cartridges were soaked with methanol for 24 h and rinsed afterwards twice each, with ultrapure water, methanol (VWR, USA) and ultrapure water at pH 2. After loading DOM onto the adsorbing resin (PPL) of the SPE cartridge, the remaining salts were removed by rinsing with ultrapure water at pH 2 and dried by pressing air through the cartridge with a clean air-syringe (20 mL, Braun, Germany). DOM was eluted with 6 mL of methanol (HPLC-grade, Sigma–Aldrich, USA) and the SPE-DOM extracts were stored in amber vials at −20 °C. The extraction efficiency of PPL adsorber is on average 62% for marine samples (Dittmar et al., 2008). However, high-temperature fluid samples usually have lower SPE-DOC concentrations and lower extraction volumes (Hawkes et al., 2015, 2016; Rossel et al., 2015). Accordingly, we found on average lower SPE extraction efficiencies in hydrothermal fluids (35 ± 5%) than in surface seawater samples (45 ± 31%). Aliquots of the SPE-DOM extracts were used to determine the SPE-DOS concentration and ^{14}C content. Sulfur concentrations were measured using an ICP-OES (iCAP 6000, Thermo, Bremen, Germany) at the University of Oldenburg (Germany) according to Pohlabeln and Dittmar (2015). The radiocarbon analyses of Milos SPE-DOC were carried out by Accelerator Mass Spectrometry (AMS) at the Institute of Particle Physics and the Geological Institute of the ETH Zürich

(Zürich, Switzerland). Aliquots of the SPE-DOM extracts were transferred to pre-combusted quartz tubes (850 °C, 5 h), dried under high purity helium, and flame sealed with combusted CuO under vacuum. CO_2 was cryogenically captured and measured using a microscale radiocarbon dating system and gas feeding system (MICADAS; Synal et al., 2007; Ruff et al., 2007, 2010; Wacker et al., 2010). Processing blanks, where ultrapure water was solid phase extracted in an identical manner, were also analyzed but contained insufficient carbon for ^{14}C analysis. Data are reported as fraction modern ($F^{14}C$) after Reimer et al. (2004) to differentiate between dead ($F^{14}C = 0$) and modern ($F^{14}C = 1$) radiocarbon. The total measured $F^{14}C$ value is a mixture of the sample and blank contributions: $F_T * C_T = F_S * C_S + F_{EX} * C_{EX}$, where F is the $F^{14}C$ fraction modern radiocarbon content and C is the amount of carbon of the total measurement (subscript T), the sample (subscript S), and the extraneous carbon (subscript EX) (Lang et al., 2013).

For characterization of DOM molecular composition, mass spectra were obtained in negative ionization mode using the 15 Tesla FT-ICR-MS (Solarix, Bruker Daltonik, Bremen, Germany) at the University of Oldenburg (Germany) combined with an ESI device (Bruker Apollo II) with a needle voltage set to – 4 kV. A total of 500 scans were accumulated per run. The mass-to-charge window was set to 150–2000 Da. For control of overall mass spectrometry quality and reproducibility, twice a day an in-house reference of DOM from North Equatorial Pacific Intermediate Water (NEqPIW) was analyzed (Green et al., 2014; Riedel and Dittmar, 2014). A common detection limit on the relative signal intensity scale was applied to facilitate maximum comparability among samples (Riedel and Dittmar, 2014), and this explains the relatively low number of compounds detected in NEqPIW compared to previous studies (Table 2). Internal calibration of the spectra was performed using the Bruker Daltonics Data Analysis software package with help of an established calibration list for marine DOM, consisting of >100 mass calibration points of known molecular formulas. The data were processed using in-house Matlab routines (Riedel et al., 2012) and molecular formulas with C_{1-100} H_{1-250} O_{1-100} N_{0-4} S_{0-2} or P_{0-1} were assigned to peaks with a minimum signal-to-noise ratio of four according to Koch et al. (2007). For the Iceland samples, where duplicates or triplicates were available, only those molecular formulas detected in all analytical replicates were accepted (80–91% of total formulas), which decreased the number of formulas but efficiently removed non-analytes from the data set that only result from noise in the mass spectra (Riedel and Dittmar, 2014). Additionally, we analyzed blank samples and all of the DOM formulas detected in the blanks were removed from our DOM dataset. Since the hydrothermal fluids sampling methodology was identical for the three study sites (Milos, Dominica and Iceland), the exclusive DOM or DOS fraction from the hydrothermal fluids was unlikely affected by the sampling procedure (food-grade large volume nylon bags).

The molecular formulas were used to calculate two indexes: the Double Bond Equivalence Index (DBE = 1

Table 1

Physicochemical composition of Milos, Dominica and Iceland surface seawater and shallow hydrothermal vent fluids. Being "n.a." not analyzed and "b.d." below detection limit. The Na – K geothermometer is in the form from Fournier (1979): T (°C) = (1217/log(Na/K) + 1.483) − 273.15; The Na – K – Ca geothermometer equation is in the form from Henley et al., (1984): T (°C) = (1647/(log(Na/K) + 0.33 (log(Ca) 1/2Na) + 2.06) + 2.47)) − 273.15. Concentrations in geothermometer calculations are in mg/L.

		Seawater			Hydrothermal fluids					
		Milos	Dominica	Iceland	Milos		Dominica		Iceland	
					Palaeochori	Spathi	Champagne	Soufrière	Hveravík-1	Hveravík-2
	Water depth (m)	0	0	0	5	12	5	5	0	2
	Temperature (°C)	20	28	11	81	65	75	55	86	80
	pH	8.2	7.9	8.2	4.9	5.7	6.3	6.3	8.0	8.3
	Salinity	38	34	31	56	33	17	11	2	5
	δ^2H	8.6	6.3	−7.9	5.0	8.9	−1.9	−5.5	−76.6	−67.6
	$\delta^{18}O$	1.3	0.8	−0.4	2.6	1.2	−1.0	−1.3	−10.3	−9.4
	$F^{14}C_{SPE}$	1.02–1.03	n.a.	n.a.	0.78–0.81	0.77–0.79	n.a.	n.a.	n.a.	n.a.
	DOC (μM)	60	99	183	21	41	22	27	112	142
	DOS (μM)	1.8	0.2	0.5	0.8	1.3	0.1	0.1	0.1	0.2
	DOS/DOC ratio	0.0296	0.0024	0.0030	0.0375	0.0317	0.0026	0.0051	0.0009	0.0013
Major elements and molecules (mM)	Br	0.97	0.83	0.98	1.23	0.92	0.40	0.20	0.01	0.06
	Ca	10.7	11.0	7.86	31.8	20.1	12.2	3.97	4.64	5.19
	Cl	664.0	569.6	623.1	947.8	633.6	273.5	141.8	22.4	85.0
	K	10.8	10.6	4.40	75.1	19.4	6.55	3.19	0.09	0.67
	Mg	57.2	60.3	48.1	25.9	99.8	29.4	18.2	0.10	6.37
	Na	503.2	513.6	397.6	673.7	893.8	314.3	149.3	13.9	65.3
	SO_4	29.9	26.1	26.7	8.36	29.7	10.4	7.42	0.95	5.43
Trace elements and molecules (μM)	B	463.3	409.7	586.5	2434.9	457.9	1119.5	543.2	321.9	414.4
	Fe	b.d.	b.d.	b.d.	229.7	24.2	88.2	214.7	b.d.	b.d.
	F	36.8	b.d.	239.5	52.6	31.6	b.d.	b.d.	14.3	19.7
	H_2S	b.d.	b.d.	b.d.	498.0	255.6	0.3	1.3	1.4	1.4
	Li	47.2	45.5	15.8	5296.8	129.6	256.8	64.3	1.15	4.03
	Mn	7.22	b.d.	b.d.	57.4	17.7	b.d.	b.d.	b.d.	b.d.
	Si	b.d.	b.d.	294.8	274.8	639.4	226.2	665.9	1438.2	1324.3
	Sr	84.1	88.3	69.5	236.3	80.1	71.2	26.4	4.79	12.7
Geothermometers (°C)	Na/K	–	–	–	279	144	142	143	78	102
	Na – K – Ca	–	–	–	276	179	164	162	79	115

Table 2

Summary of molecular composition of the DOM as characterized by FT-ICR-MS, given in percentages of the relative abundances of all identified formulas, and as intensity-weighted average values, where indicated.

	Seawater				Hydrothermal fluids						Exclusive DOM of hydrothermal fluids		
					Milos		Dominica		Iceland				
	NEqPIW	Milos	Dominica	Iceland	Palaeochori	Spathi	Champagne	Soufrière	Hveravík-1	Hveravík-2	Milos	Dominica	Iceland
Total formulas	2418	3266	2538	1815	3430	3505	2852	2840	2001	2127	322	223	124
H/C ratio$_{average}$	1.25	1.25	1.25	1.20	1.25	1.25	1.21	1.18	1.13	1.20	1.37	1.03	1.51
O/C ratio$_{average}$	0.49	0.49	0.49	0.49	0.43	0.47	0.45	0.43	0.43	0.47	0.32	0.32	0.25
S/C ratio$_{average}$	0.003	0.003	0.002	0.003	0.009	0.007	0.003	0.005	0.005	0.005	0.062	0.022	0.013
DBE index$_{average}$	8.9	8.4	8.4	8.8	7.9	8.1	9.0	9.3	9.4	8.9	6.3	11.0	7.7
AI$_{mod}$ index$_{average}$	0.2	0.2	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.4	0.2
Aromatics %	7	6	9	13	6	6	12	12	21	15	5	35	9
Condensed aromatics %	<1	<1	1	4	<1	<1	2	2	5	3	0	5	0

$+ \emptyset \, (2C - H + N + P))$ in order to assess the degree of unsaturation, and the modified Aromaticity Index ($AI_{mod} = (1 + C - \emptyset O - S - \emptyset(N + P + H)/(C - \emptyset O - S - N - P)))$ to estimate the presence and abundance of aromatics ($AI_{mod} \geqslant 0.5$) and condensed aromatics ($AI_{mod} \geqslant 0.66$), taking into consideration the abundance of carboxyl groups in natural organic matter (Koch and Dittmar, 2006). Condensed aromatics containing nitrogen, sulfur or phosphorus were excluded for data interpretation as they were not specifically confirmed by the presence of the respective ^{13}C isotopologs due to their low abundance in the mass spectra. Signal intensities of individual signals were converted into percentages relative to total signal intensity of each sample. These relative signal intensities were used to calculate intensity-weighted averages of DBE, AI_{mod} and molar ratios (H/C, O/C, S/C) (e.g. Schmidt et al., 2009; Seidel et al., 2014).

Potential sulfurization reactions were tested in the FT-ICR-MS dataset on the basis of relationships of molecular formulas and the geochemical context (e.g. Schmidt et al., 2014). The following nine possibilities of S addition while adding/removing H and/or O were tested with 27 potential reactions (Table 3), differentiating between oxidative and reductive ones: oxidative sulfurization reactions structure: $+ S_1; + S_1/ - H_n; + H_nS_1O_n; + S_1O_n; + S_1O_n/ - H_n$; reductive sulfurization reactions structure: $+ H_nS_1; + S_1/ - H_nO_n; + S_1/ - O_n; + H_nS_1/ - O_n$. The effectiveness of the potential sulfurization reactions was considered as a percentage of mono-S compounds ($CHOS_1$) exclusively present in hydrothermal fluids that can be considered the product of a seawater DOM precursor (CHO) following the corresponding reaction of S addition. All the reactions with >50% of potential precursors in seawater were proposed as the equivalent $+ H_2S$ reaction. They were exchanging H_2O, H_2 and/or O_2 by a H_2S molecule and accordingly an exclusive $CHOS_1$ formula from the hydrothermal fluids was obtained.

4. RESULTS

4.1. Hydrothermal fluids imprint on general geochemistry

Hydrothermal fluids and seawater differed substantially in temperature and pH. Hydrothermal fluid temperatures ranged from 55 to 86 °C and seawater from 11 to 28 °C at the three sampling sites (Table 1). Fluids in Milos and Dominica were acidic compared to seawater, in contrast to Iceland hydrothermal fluids that were slightly alkaline (Table 1). Trace elements and molecules that were likely of hydrothermal origin, as they were detected in the fluids but not in the surrounding seawater, were H_2S in Milos and dissolved Fe and Si in both Milos and Dominica (Table 1).

Salinity, major element concentrations and isotopic composition indicated substantial differences in the origin of the fluids at the three sites. Salinity and major element concentrations were similar or higher in Milos fluids as compared to seawater, but lower in Dominica and Iceland hydrothermal fluids (Table 1). The isotopic composition of the fluids (δ^2H and $\delta^{18}O$) from Milos, mainly from Palaeochori Bay,

Table 3

Theoretical sulfurization reactions tested in this study, differentiating between oxidative (blue) and reductive (red) reactions. The nine possibilities of adding one S-atom and adding/removing H and/or O were tested. The effectiveness of the potential reactions was considered as a percentage of mono-S compounds ($CHOS_1$) exclusive in hydrothermal fluids that can be considered the product of a seawater DOM precursor following the corresponding reaction. The reactions with >50% of precursors found in seawater are highlighted in light green, >75% in green and >85% in dark green. All the reactions giving at least >50% were potential + H_2S incorporation reactions, correspondingly exchanging H_2O, H_2 and/or O_2 by a H_2S molecule. For example, the reductive reaction of substituting one O atom by one S atom is equivalent to the reaction substituting one H_2O by one H_2S molecule and could provide a precursor in seawater DOM formulas to 87% of Milos $CHOS_1$-compounds exclusive from hydrothermal fluids. An example of this procedure is visualized in Fig. 6.

	+ S reactions	% of precursors in seawater			equivalent + H_2S reactions	− H_2	− O_2	− H_2O
		Milos	Dominica	Iceland				
OXIDATIVE reactions	+ S	87	21	8	+ H_2S / − H_2			
	+ S/ − H_2	84	21	3	+ H_2S / − $2H_2$			
	+ S/ − H_4	70	20	0	+ H_2S / − $4H_2$			
	+ H_2OS	82	44	20	+ H_2S + H_2O / − H_2			
	+ H_2SO_2	60	48	13	+ H_2S + O_2			
	+ H_2SO_4	9	20	13				
	+ SO	82	26	13	+ H_2S + H_2O / − $2H_2$			
	+ SO_2	64	42	10	+ H_2S + O_2 / − H_2			
	+ SO_3	33	49	23				
	+ SO_4	15	31	15				
	+ SO_5	4	10	5				
	+ SO/ − H_2	66	30	0	+ H_2S + O_2/ − (H_2 + H_2O)			
	+ SO_2/ − H_2	60	43	0	+ H_2S + O_2/ − $2H_2$			
	+ SO_4/ − H_2	16	25	5				
REDUCTIVE reactions	+ H_2S	86	32	18	+ H_2S			
	+ H_4S	74	33	20	+ H_2S + H_2			
	+ S/ − H_2O	83	16	8	+ H_2S / − (H_2O + H_2)			
	+ S/ − H_2O_2	82	24	10	+ H_2S / − 2 (H_2O)			
	+ S/ − H_2O_4	76	62	30	+ H_2S/ − 2 (H_2 + O_2)			
	+ S/ − O	87	21	10	+ H_2S / − H_2O			
	+ S/ − O_2	87	33	18	+ H_2S / − (H_2 + O_2)			
	+ S/ − O_3	85	41	23	+ H_2S/ − (H_2O + O_2)			
	+ S/ − O_4	82	58	35	+ H_2S/ − (H_2 + $2O_2$)			
	+ S/ − O_5	76	70	48	+ H_2S/ − (H_2O + $2O_2$)			
	+ H_2S/ − O	80	28	23	+ H_2S + H_2O/ − (O_2 + H_2)			
	+ H_2S/ − O_2	75	32	18	+ H_2S / − O_2			
	+ H_2S/ − O_4	80	56	45	+ H_2S / − $2O_2$			

> 85% of precursors in seawater
> 75% of precursors in seawater
> 50% of precursors in seawater

was far off from the estimated Mediterranean Sea meteoric water line, whereas Dominica and Iceland fluids plotted close to the global and the estimated Caribbean Sea meteoric water line (Fig. 2; Table 1). Dominica and Iceland hydrothermal fluids thus seem to be composed mainly of meteoric water, while Milos hydrothermal fluids were predominantly fed by recirculating seawater.

The hydrothermal fluids had lower DOC concentrations than corresponding surface seawater in all three systems. The DOC of hydrothermal fluids was 21–41 μM in Milos, 22–27 μM in Dominica and 112–142 μM in Iceland, and seawater DOC concentrations were 60 μM, 99 μM and 183 μM in Milos, Dominica and Iceland, respectively (Table 1). The Milos SPE-DOC contained less ^{14}C than
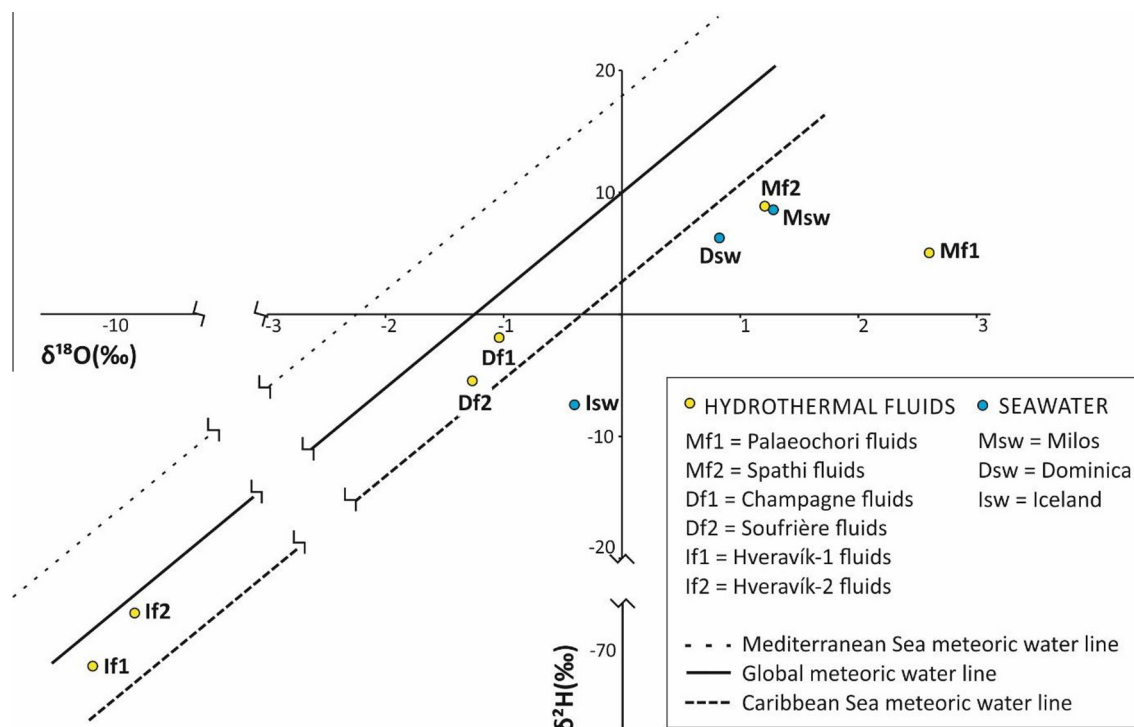
Fig. 2. Diagram of $\delta^2 H$ and $\delta^{18}O$ isotopic values of shallow hydrothermal fluids (yellow) and surface seawater (blue) samples. Both $\delta^{18}O$ and $\delta^2 H$ are expressed relative to Vienna Standard Mean Ocean Water (VSMOW; Craig, 1961). Caribbean Sea meteoric water line adapted from McCarthy et al. (2005) and Mediterranean Sea meteoric water line from Price et al. (2013). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

near-by seawater. Samples had 77–167 µg C while SPE process blanks contained <2 µg C. Because the AMS requires at least 5 µg C for analysis, the ^{14}C content of the SPE process blank was not obtained. The sample SPE data are therefore reported as a range that reflects the possibility that the contribution of 2 µg C of extraneous carbon from the blank was either entirely modern ($F^{14}C = 1$) or entirely ^{14}C free ($F^{14}C = 0$). Even with this extreme assumption, the data clearly demonstrate that Milos seawater contains organic matter with a modern $F^{14}C$ signature (1.02–1.03) while the fluids from the Palaeochori (0.78–0.81) and Spathi (0.77–0.79) vents contain material that has less ^{14}C (Table 1). The isotopic results were consistent with the estimated reservoir temperature of the fluids, showing the highest values in Milos fluids from Palaeochori (276–279 °C) as compared to rest of the samples (<180 °C; Table 1). Assuming the same extraction efficiency for SPE-DOS as for SPE-DOC (e.g. Lechtenfeld et al., 2011; Pohlabeln and Dittmar, 2015), the DOS concentrations in the hydrothermal fluids were 0.8–1.3 µM in Milos, 0.1 µM in Dominica and 0.1–0.2 µM in Iceland; and seawater DOS concentrations were 1.8 µM in Milos, 0.2 µM in Dominica and 0.5 µM in Iceland. Accordingly, molar DOS/DOC ratios were one order of magnitude higher in Milos compared to Dominica and Iceland (Table 1).

4.2. Hydrothermal fluids imprint on DOM signature

Using FT-ICR-MS, more formulas were found in hydrothermal fluids than in surrounding seawater: a total

of 3266–3505 molecular formulas were identified in Milos samples, 2538–2852 in Dominica, 1815–2127 in Iceland and 2418 formulas in the NEqPIW sample included as DOM reference (Table 2). The DOM of the hydrothermal fluids was on average more reduced than seawater DOM (O/C ratio; Table 2). The degree of unsaturation of the DOM molecules, as assessed by weighted averages of H/C ratios, DBE, and the relative abundance of aromatics, differed at the three sites (Table 2). On Milos, seawater and hydrothermal fluids showed a similar degree of unsaturation, whereas on Dominica, lower H/C ratio, higher DBE and higher abundances of aromatics indicated more unsaturated molecules in the hydrothermal fluids than in the seawater. On Iceland, a similar degree of unsaturation was found for seawater and fluids from the permanently submerged hydrothermal vent (Hveravík-2), while more unsaturated molecules were found in the air-exposed hydrothermal vent (Hveravík-1).

Another characteristic feature was the high weight averaged S/C ratio in the DOM of Milos hydrothermal fluids (Table 2). The most obvious difference in molecular DOM composition between hydrothermal fluids and seawater on Milos was the number of sulfur containing formulas (CHOS; Fig. 3). Hydrothermal fluids from Milos contained 8% more CHOS compounds than the corresponding seawater (hydrothermal fluids 23%; seawater 15%; Fig. 3). In the other two systems, molecular composition of hydrothermal fluids and seawater were relatively similar regarding CHOS compounds. On Dominica, the dominant groups of formulas were CHO (~50%) and
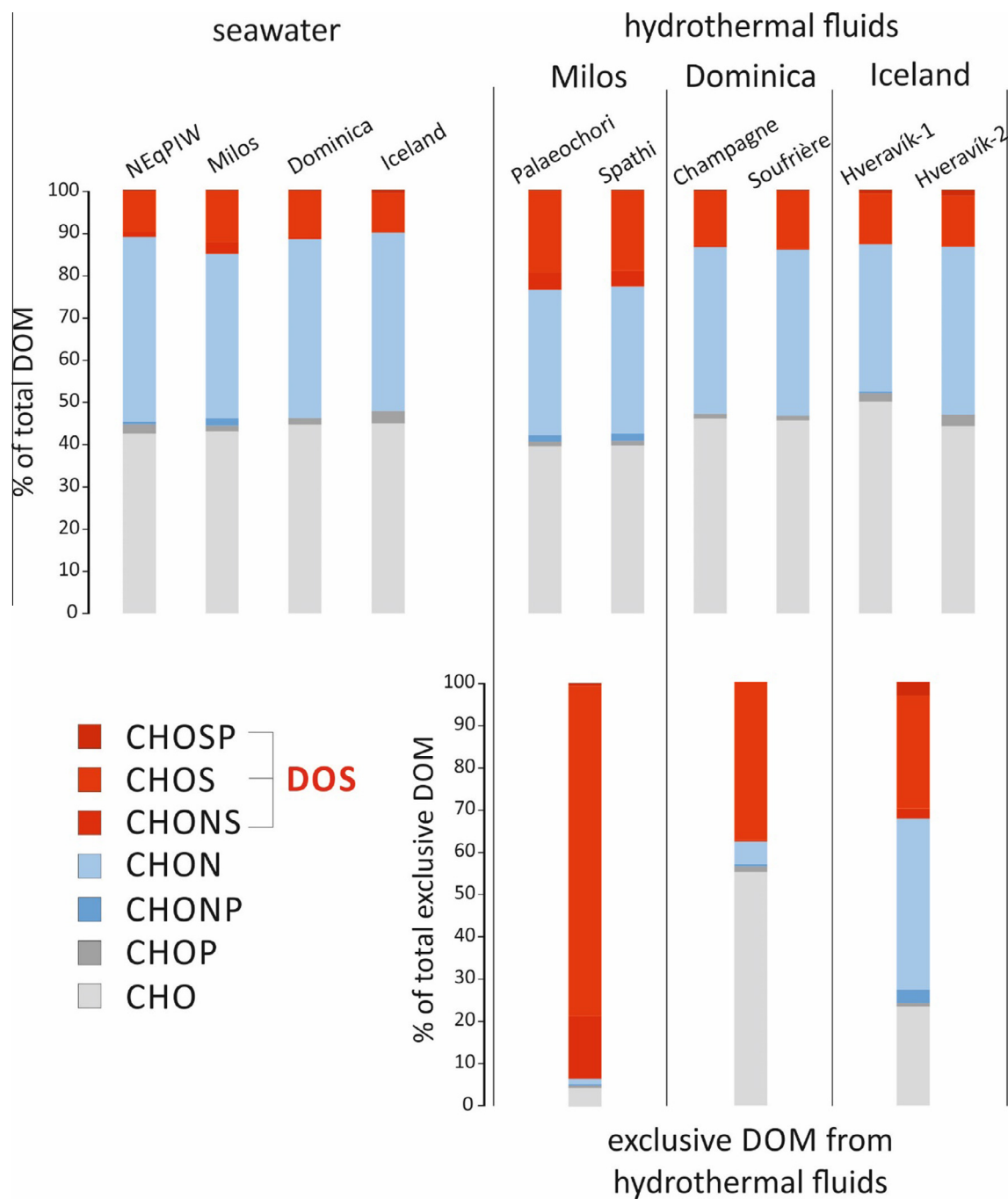
Fig. 3. DOM molecular compounds identified by FT-ICR-MS as a percentage of all molecular formulas detected for the respective sample: surface seawater samples (upper left), hydrothermal fluids (upper right) and exclusive formulas in hydrothermal fluids DOM (down right). Formulas containing sulfur (DOS) are highlighted in red and included CHOS, CHONS and CHOSP formulas. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

CHON (~40%), while CHOS accounted for ~11% and CHOP for <2%. On Iceland, CHO and CHON formulas were also similarly abundant (~40%), while CHOS contributed ~14% and CHOP ~5% to the identified formulas. Besides the higher number of CHOS formulas in Milos fluids, the sum of intensities of these CHOS compounds was also highest in these systems (11–14% of total intensities; Fig. 4). In the seawater samples of Milos and Dominica,

CHOS compounds summed up to only 5% of total intensity, in Iceland seawater to 7%, respectively. The hydrothermal fluids of Dominica (5–7% of total intensity) and Iceland (8%) were only slightly enriched in CHOS compounds compared to seawater (Fig. 4).

The differences in molecular DOM composition of hydrothermal fluids and seawater were also manifested in occurrence of exclusive formulas only present in the
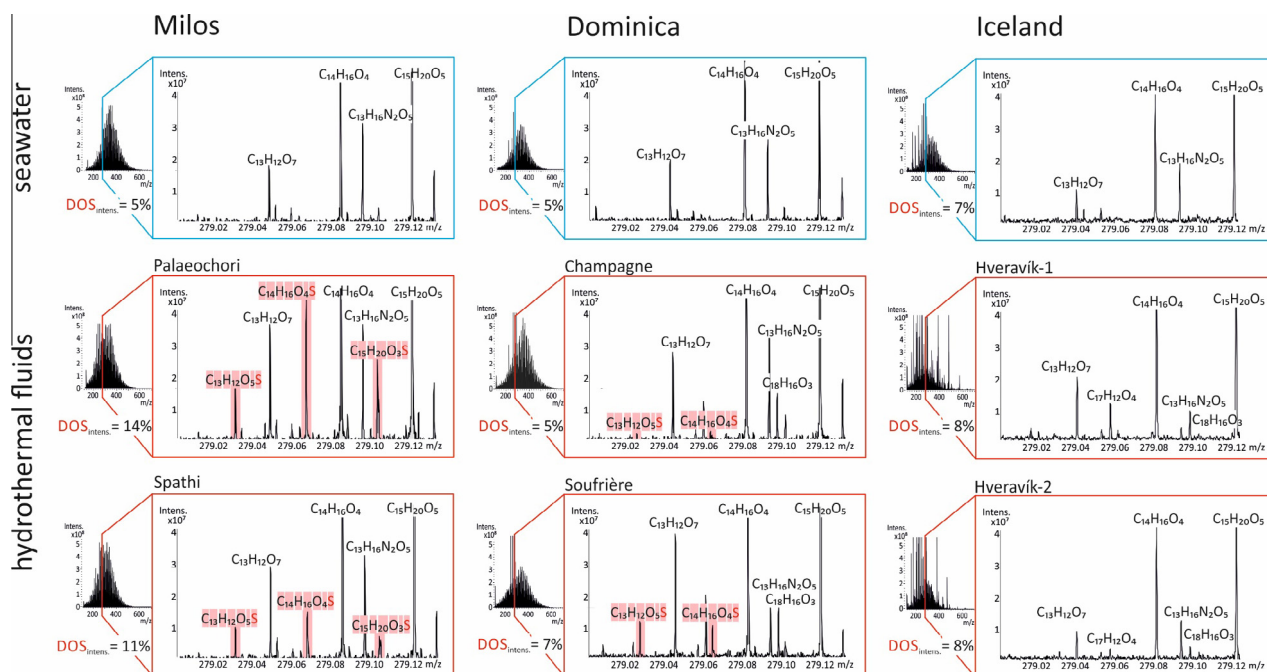
Fig. 4. FT-ICR mass spectra (ESI negative mode) of seawater (blue) and hydrothermal fluids (red) samples. Expanded section of the mass spectra at 279.00–279.14 Da with the identified molecular formulas. DOS formulas highlighted in red provide evidence for the relevance of S-containing compounds in Milos hydrothermal fluids. DOS$_{intens.}$ gives the percentage of summed intensities of all DOS compounds relative to total intensity of the respective sample. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

hydrothermal fluids. On Milos, these exclusive formulas accounted for 9% (322 formulas) of the formulas in the hydrothermal fluids, in Dominica for 8% (223 formulas) and in Iceland for 6% (124 formulas), respectively (Table 2). Regarding their molecular composition, major differences were observed between sites. The exclusive formulas represented mainly reduced compounds with very low O/C ratio (0.25–0.32; Table 2; Fig. 5a), predominantly saturated in Milos and Iceland, but mainly unsaturated in Dominica with more than one third being aromatics (Table 2; Fig. 5a). The exclusive formulas of all hydrothermal fluids were on average strongly enriched in sulfur (S/C 0.013–0.062) when compared to total DOM of both fluids and seawater (maximum 0.009; Table 2). On Milos, almost all exclusive formulas in the hydrothermal DOM contained sulfur (>90%; Fig. 3), for Dominica and Iceland the respective percentages were considerably lower but still elevated (∼38% and ∼30%, respectively; Fig. 3).

4.3. Molecular variations of exclusive DOS from hydrothermal fluids

The molecular composition of the exclusive DOS from hydrothermal fluids (Fig. 5b) was substantially different from the DOS compounds from seawater (Fig. 5c). These differences were most evident from the degree of oxidation. In the three systems, the specific DOS of hydrothermal fluids had lower O/C ratios on average (0.26–0.34; Fig 5b) than the surrounding surface seawater or NEqPIW DOS (0.45–0.52; Fig 5c). A closer look into the exclusive DOS

from hydrothermal fluids was then obtained by searching for the presence of potential precursors for the individual CHOS$_1$ formulas in the seawater DOM (Fig. 6). Potential precursors were identified according to the 27 hypothetical sulfurization reactions shown in Table 3. On Dominica, only four reductive reactions provided potential precursors for more than 50% of exclusive mono-S compounds (CHOS$_1$) from hydrothermal fluids. None of the 27 potential sulfurization reactions achieved this 50% threshold value on Iceland. In the DOS-enriched system of Milos, almost all potential sulfurization reactions provided precursors for more than 50% of exclusive CHOS$_1$ compounds, many of them for more than 75% and up to 87% (Table 3; Fig. 6). Furthermore, a clear trend was observed regarding these reactions. All those that could be categorized as reductive ones provided a potential precursor for at least 74% of all specific CHOS$_1$ compounds from Milos fluids (Table 3). The four most effective potential sulfurization reactions (precursors for over 85% of exclusive formulas) were those exchanging an O atom by one S atom in the formula or the equivalent + H$_2$S reaction, correspondingly exchanging H$_2$O, H$_2$ and/or O$_2$ by a H$_2$S molecule (Table 3; Fig. 6).

5. DISCUSSION

5.1. Origin of the hydrothermal fluids

Understanding the origin of hydrothermal fluids is an essential step to decipher potential subsurface processes
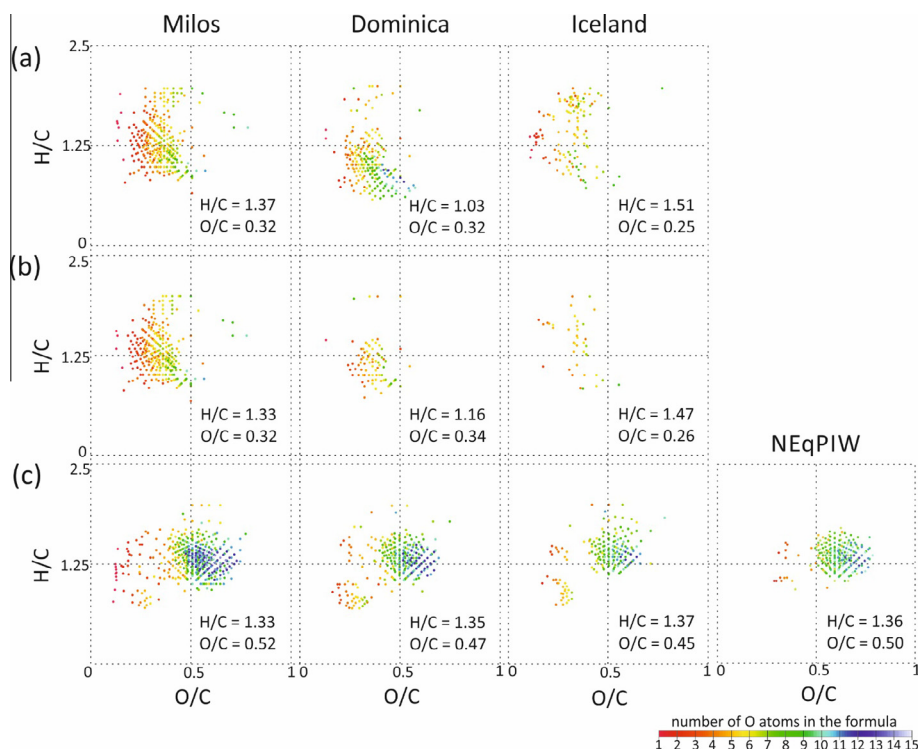
Fig. 5. Van Krevelen diagrams representing the O/C (oxygen-to-carbon ratio) and H/C (hydrogen-to-carbon ratio) in Milos, Dominica, Iceland and NEqPIW DOM samples: (a) exclusive DOM compounds from hydrothermal fluids, (b) exclusive DOS compounds from hydrothermal fluids (those formulas of "a" containing one or two S atoms) and (c) seawater DOS compounds (formulas containing S atoms in Milos, Dominica, Iceland and NEqPIW surface seawater samples). Every dot represents a specific molecular formula in the FT-ICR-MS dataset. The color scale illustrates the number of O atoms in every formula providing visual evidence of more oxidized compounds (blue dots) in seawater DOS (c) than in exclusive hydrothermal DOS (b), as represented also by the O/C and H/C intensity-weighted averages in each diagram. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(McCarthy et al., 2005). Fluids in a shallow-water hydrothermal system may be derived from one or from a combination of the following sources: meteoric water, seawater and/or magmatic water (Pichler, 2005). While deep-sea hydrothermal systems most likely derive all of their fluids from seawater, on-land hydrothermal fluids are originated mainly from meteoric sources along with possible magmatic contributions (Giggenbach, 1992). The three shallow-water systems in this study have hydrothermal fluids with different geochemical and isotopic composition indicating different potential sources.

Dominica and Iceland hydrothermal fluids were a mixture of mainly meteoric water and seawater. Meteoric waters are generally depleted in the heavy isotopic species of hydrogen and oxygen relative to ocean waters (Craig, 1961). In our study, the δ^2H and $\delta^{18}O$ isotopic values of Dominica and Iceland fluids were depleted and closely aligned between the global meteoric water line and the estimated Caribbean Sea meteoric water line (McCarthy et al., 2005; Fig. 2; Table 1), suggesting local meteoric origin, although discharging in a submarine environment (Pichler, 2005). Similar isotopic values were observed in a global marine precipitation survey (IAWA/WMO since 1961) at the station closest to the Dominica hydrothermal vents (−1.0 δ^2H, −0.9 $\delta^{18}O$ in Barbados, Lesser Antilles; Rozanski et al., 1993). The low salinity and major element

concentrations were also consistent with a mainly meteoric origin of Dominica and Iceland fluids.

In contrast, the fluids discharging on Milos were predominantly recirculating seawater, as evidenced by the high salinity and major element concentrations and by the isotopic values of δ^2H and $\delta^{18}O$ that were far off from the estimated Mediterranean Sea meteoric water line (Price et al., 2013; Fig. 2; Table 1), as well as from values of the closest station in the global precipitation survey (−6.3 δ^2H, −32.8 $\delta^{18}O$ in Heraklion, Aegean Sea; Rozanski et al., 1993). This is consistent with a higher estimated reservoir temperature obtained for Milos samples (Table 1), suggesting that the endmember fluids were likely a mixture of seawater and andesitic water with minor meteoric water content (e.g. Yamanaka et al., 2013). Potentially high temperature of Milos reservoir might imply abiotic origin of H_2S during the final stages of kerogen formation (temperature required between 150 and 200 °C; McCarthy et al., 2011a). Magmatic contribution in Milos hydrothermal fluids composition was previously confirmed by stable isotope studies on the released gases (Botz et al., 1996) and potentially hot magma was indicated by direct drilling of the underlying hydrothermal reservoir, detecting 318 °C at 1200 m depth (Vrouzi, 1985; Dando et al., 1999) as well as reservoir temperature estimations of up to 325 °C (Fitzsimmons et al., 1997). Consequently, subsurface seawater recirculation
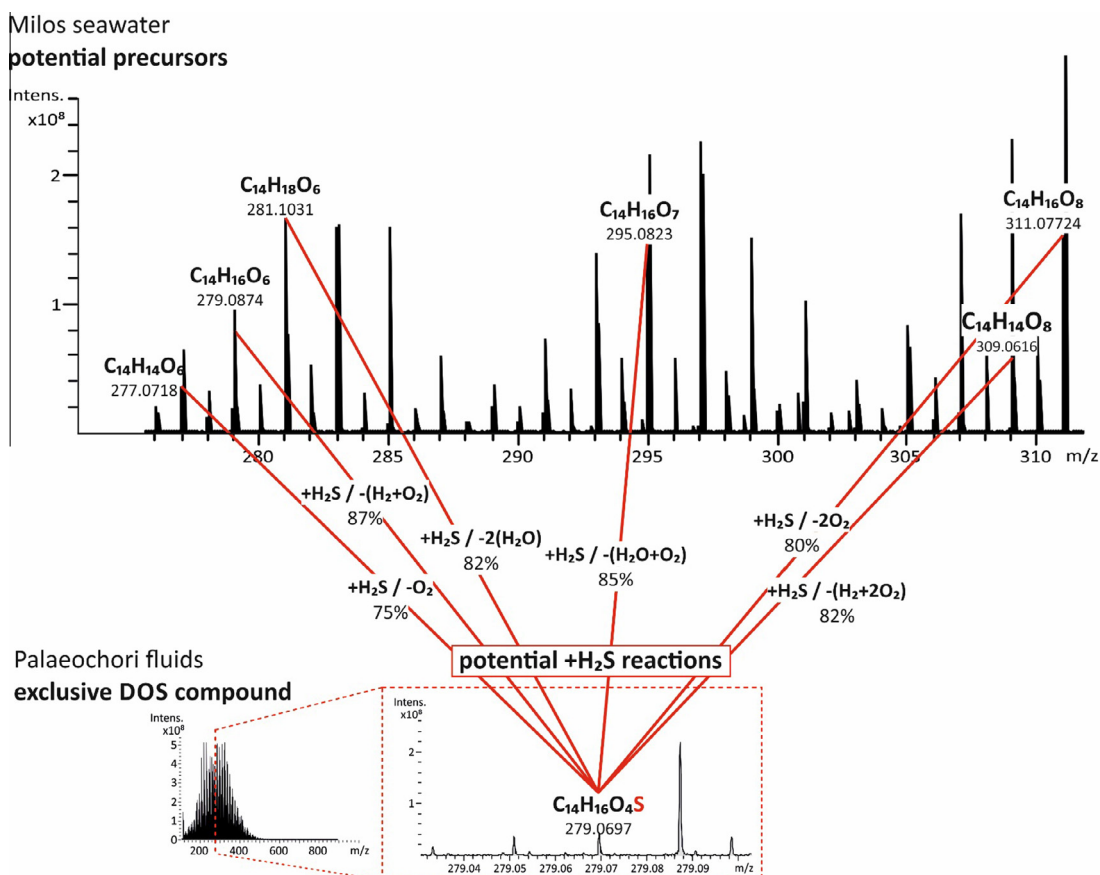
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Fig. 6. Example of potential sulfurization reactions tested in this study (Table 3), showing an exclusive DOS compound of Milos hydrothermal fluids from Palaeochori Bay (down) and the potential precursors in the FT-ICR mass spectra of Milos seawater DOM (up). Neutral molecular formulas of detected ions are presented. The potential proposed reactions as $+H_2S$ incorporation reactions, correspondingly exchanging H_2O, H_2 and/or O_2 by a H_2S molecule, are represented in red including the percentage of precursors found in seawater following the corresponding reaction.

may have been favored by magmatic input, because heat makes the water less buoyant, driving the water flow. Our ^{14}C isotopic analysis of the SPE-DOM indicated a contribution of non-modern carbon. One possible source is the desorption of pre-aged organics from the sediments (Schillawski and Petsch, 2008). Alternatively, primary production could convert CO_2 with a non-modern signature, such as ^{14}C-free mantle CO_2 or pre-aged CO_2 remineralized from the sediment, into organic molecules. The seawater at Milos was entirely modern. If the only other SPE-DOM carbon contribution to the Milos hydrothermal fluids was ^{14}C-free, then the organics were composed of approximately 80% modern and 20% dead radiocarbon material (Table 1). If the sediments contributed significant carbon to the fluids they would have also have this mixed radiocarbon signature. Similar observations have been made in deep hydrothermal systems, for example, the $F^{14}C$ values of ultra-filtrated (>1000 Da) DOC in axial diffuse hydrothermal fluids from the Juan de Fuca Ridge ranged from 0.48 to 0.55, comparable to local deep seawater with an $F^{14}C$ of 0.56 (McCarthy et al., 2011b). The fact that Milos fluids may be more influenced by magmatic contributions than Dominica and Iceland is also relevant to the potential

sulfurization of seawater DOM and other subsurface processes. In contrast, the higher meteoric content in Dominica and Iceland fluids likely favor processes occurring in terrestrial environments that may subsequently affect the DOM molecular composition.

5.2. DOC depletion in the fluids and DOS enrichment in Milos

Bulk DOC concentrations provide an integrated view of sources and processes in hydrothermal venting sites (Lang et al., 2006; McCarthy et al., 2011b). In the three systems included in this study, DOC concentrations in hydrothermal fluids were lower than in surface seawater (Table 1). The surface seawater DOC concentration in Milos was within the range of regular open ocean surface water (40–80 µM; Hansell et al., 2009). In case of Dominica and Iceland it was higher but still representative for coastal regions (Dittmar and Stubbins, 2014) where shallow-water hydrothermal vents are located. Given the close proximity to the island, an input of terrigenous organic matter is a likely explanation for the enhanced DOC concentrations in the surface seawater samples through rivers,

rain-washing or submarine groundwater discharge via aquifers (Pichler, 2005). This is supported by a higher percentage of aromatics in Dominica and Iceland surface seawater DOM signature than in the reference DOM from the deep ocean (NEqPIW), which might reflect terrestrial plant derived compounds like lignin-phenols (Dittmar and Stubbins, 2014). Alternatively, increased DOC concentrations might be derived from recent primary production, which is the biggest source of organic carbon in the oceanic epipelagic zone (0–200 m water depth; Dittmar and Stubbins, 2014). Our three study sites were located in the shallow, euphotic and continuously mixed water column, with close bentho-pelagic coupling, and typically high rates of primary production (Behrenfeld et al., 2005; Cloern et al., 2014). Therefore, higher DOC concentrations in surface seawater of Milos, Dominica and Iceland as compared to the hydrothermal fluids could be explained by both terrestrial input and/or autochtonous production in the coastal waters.

The most likely explanation for the low DOC concentrations (Table 1) in Dominica and Iceland hydrothermal fluids may be the strong contribution of low-DOC meteoric water. In addition, different abiotic and biotic processes during seawater recirculation might contribute to DOC depletion in hydrothermal fluids. In the deep ocean, seawater DOC can be removed by hydrothermal circulation within Earth´s crust (Lang et al., 2006; Lin et al., 2012; Hansell, 2013; Hawkes et al., 2015). Potential explanations are microbial consumption driven by vent communities, interactions with minerals in the subsurface leading to DOC sorption or direct abiotic thermal degradation of DOC (Lang et al., 2006; Santelli et al., 2008; Hawkes et al., 2015, 2016). The possibilities of heterotrophic consumption and organic matter adsorption to mineral surfaces may be favored by high availability of organic carbon in shallow hydrothermal systems (e.g. Svensson et al., 2004) and high porosity of Milos and Dominica sediment, respectively. In addition, co-precipitation of DOM with minerals can occur at the redox interface and subsequently remove a DOC fraction from the fluids (Riedel et al., 2013; Gomez-Saez et al., 2015). However, thermal degradation of SPE-DOM was recently detected at temperatures above 68 °C (Hawkes et al., 2016) and therefore represents, together with the low-DOC meteoric water input, the most likely explanation of the lower DOC concentration detected in Milos, Dominica and Iceland shallow hydrothermal fluids compared to surface seawater.

Information on DOS concentrations in marine systems is still scarce (Lechtenfeld et al., 2011; Pohlabeln and Dittmar, 2015). Compared to the few published data, the DOS concentrations in the Milos hydrothermal system were distinctly higher (0.8–1.8 μM) than in other marine environments, (0.8 μM North Sea; 0.3–0.9 μM South Atlantic central water; 0.4–1.2 μM Antarctic surface water; Lechtenfeld et al., 2011; Pohlabeln and Dittmar, 2015), whereas Dominica and Iceland DOS concentrations were similar to values reported for open ocean waters (Cutter et al., 2004; Lechtenfeld et al., 2011; Pohlabeln and Dittmar, 2015). Accordingly, the molar DOS/DOC ratio in Milos was one order of magnitude higher than in the

other studied marine systems. FT-ICR-MS data were consistent with the ICP-OES based quantification. Nearly all DOM molecular formulas exclusively present in the Milos fluids contained sulfur. Moreover, the percentage of CHOS-compounds in total DOM on Milos was one of the highest ever observed through DOM characterization by FT-ICR-MS analysis of natural samples (e.g. Riedel et al., 2013) and distinctly higher than the sum of CHOS intensities observed for Dominica and Iceland. Our findings thereby support FT-ICR-MS as a capable technique to semi-quantitatively characterize the fraction of DOM containing DOS and identify potential DOS-enriched scenarios in marine environments.

5.3. Redox transformations of hydrothermal DOM

Our data identify shallow-water hydrothermal fluids as a source of reduced DOM and reduced DOS to the oceans. On Milos, where seawater recirculation through the sediment accounted for most of the fluids composition, variations in O/C ratios suggested DOM reduction during recirculation and subsequent oxidation upon contact with oxygenated seawater. Formation of organically bound sulfur is promoted by the availability of reactive organic matter, an excess of reduced sulfur species and a limited supply of reactive iron (Eglinton and Repeta, 2014). In hydrothermal fluids from Palaeochori Bay (Milos) and Soufrière Bay (Dominica), dissolved iron was much higher than in the vents from Spathi Bay (Milos) and Champagne Reef (Dominica). In Iceland fluids, dissolved iron was below detection limit (Table 1). Nevertheless, DOS concentration and CHOS-compounds abundance was similarly high in both Milos fluids and lower in Dominica and Iceland fluid samples, respectively. Thus, in our study dissolved iron apparently was not linked to DOS abundance.

In contrast, clear trends were observed in H_2S concentration and DOS abundance. In Milos hydrothermal fluids, we detected high H_2S concentration together with high abundance of DOS, and vice versa in Dominica and Iceland. High DOS content in DOM from pore water in anoxic coastal sediments enriched in H_2S and reduced sulfur species has been demonstrated before (Schmidt et al., 2009; Seidel et al., 2014). Elevated H_2S concentration in interstitial water coinciding with increasing abundance of DOS compounds (in number and intensity), probably resulted from sulfurization reactions during the early stages of sedimentary diagenesis (e.g. Black Sea; Schmidt et al., 2014). This could suggest that the exclusive fraction of DOS detected in our hydrothermal fluid samples may be at least partially originated from sedimentary organic matter. On the other hand, our results indicated endmember fluids in Milos to be highly influenced by seawater recirculation and sulfurization reactions have been detected even at the sediment–water interface (Sinninghe Damsté and de Leeuw, 1990; Wakeham et al., 1995). Therefore, potential DOS origin in DOM sulfurization processes during seawater recirculation may be reasonable in our study and we discuss potential sulfurization reactions at a molecular level.

Some organic compounds can react with reduced sulfur species as hydrogen sulfides or polysulfides binding to

carbon–carbon double bonds by nucleophilic addition (Vairavamurthy and Mopper, 1987; Kohnen et al., 1989; Sleighter et al., 2014). Unequivocal precursor-product relationships cannot be established without detailed structural information, but potential reactions can be explored on the basis of relationships of molecular formulas and the geochemical context. For example, Schmidt et al. (2014) tested in a FT-ICR-MS dataset the possible sulfurization reaction of exchanging one O atom by one S atom and two H atoms. In our study, we observed reductive sulfurization of seawater DOM recirculating through Milos sediment, most likely substituting one O atom by one S atom or correspondingly exchanging H_2O, H_2 and/or O_2 by a H_2S molecule (Table 3). Hydrothermal vents have been postulated as possible sites for the first steps of organic chemical evolution, where sulfur reduction might have played a role in prebiotic chemistry occurring in sulfide-rich environments (Russell and Hall, 1997; Cody et al., 2000; Hebting et al., 2006). McCollom and Seewald (2007) described a potential mechanism to convert simple organic compounds to complex organic matter in sulfur-rich hydrothermal environments, by progressive elongation of alkyl carbon chains and formation of complex organic compounds through involvement of a thiol intermediate. Our results from natural environments support the last step of their hypothetical reaction which was the addition of a H_2S molecule instead of H_2O (McCollom and Seewald, 2007).

In the water column, the main compound group in DOS was recently identified as unreactive sulfonic acids, which are fully oxidized molecules with low reactivity and a component of the long-time persistent fraction of DOM in the oceans (Pohlabeln and Dittmar, 2015). This is consistent with the O/C ratio variations that we observed in our study. While our surface seawater samples contained only oxidized DOS compounds with high O/C ratios, the hydrothermal fluids not only contained higher percentages of DOS (Fig. 3), but included oxidized DOS compounds and a fraction of reduced DOS exclusive from the fluids (Fig. 5). Therefore, our results suggested potential transformation of reduced DOS molecules exclusively from the fluids to more oxidized DOS compounds abundant in seawater, which were not removed during hydrothermal circulation through the subsurface. At the same time, a new pool of reduced DOS was generated either through sulfurization of seawater DOM or release of DOS from sedimentary organic matter. This supports the scenario that functional groups like thiols and thioethers could be produced in hydrothermal systems by reactions of reduced inorganic sulfur compounds with organic matter (Sinninghe Damsté et al., 1989; Aizenshtat et al., 1995; Schneckenburger et al., 1998; Hertkorn et al., 2013 Sleighter et al., 2014) and then be rapidly oxidized to unreactive sulfonic acids once exposed to oxygenated seawater (Pohlabeln and Dittmar, 2015).

From a biotic perspective, organic matter can react with H_2S and other reduced sulfur species (e.g. Cutter and Kluckhohn, 1999; Schmidt et al., 2009, 2014) produced from the activity of sulfate-reducing bacteria (SRB) or dissimilatory iron-reducing bacteria (DIRB), which were

found in relatively large number in Palaeochori Bay (Sievert et al., 1999). Furthermore, the reduced inorganic sulfur compounds in Milos hydrothermal fluids, mainly H_2S, would provide the necessary electron donor for sulfur-oxidizing bacteria (SOB), which contribute to primary production in the Milos system (Sievert et al., 1999). Analysis of the structural information (e.g. Pohlabeln and Dittmar, 2015) of the reduced organosulfur compounds that were detected in hydrothermal fluids prior to oxidation upon contact with oxygenated seawater, isotopic investigation (e.g. $\delta^{34}S$; Raven et al., 2015) to provide insights into the CHOS-compounds origin and also research on sulfur-related microbes and their linkage to molecular DOM composition (e.g. Osterholz et al., 2016) in marine shallow hydrothermal systems will further improve our understanding of the underlying mechanisms that control global organic sulfur biogeochemistry.

6. CONCLUSION

We investigated DOS dynamics and its relation to the physicochemical environment by studying the molecular composition of DOM in three contrasting shallow hydrothermal systems off the coast of Milos (Eastern Mediterranean), Dominica (Caribbean Sea) and Iceland (North Atlantic). In all three systems, exclusive DOS from hydrothermal fluids had on average lower O/C ratios (0.26–0.34) than surrounding surface seawater or NEqPIW DOS (0.45–0.52). Therefore, shallow-hydrothermal systems can be considered a source of reduced DOS, which will get oxidized upon contact with oxygenated seawater. Our results suggested that the more oxidized DOS compounds abundant in seawater were not removed during hydrothermal circulation through the subsurface. At the same time, a new pool of reduced DOS was generated either through sulfurization of seawater DOM or release of DOS from sedimentary organic matter. This supports the scenario that functional groups like thiols and thioethers could be produced in hydrothermal systems and then be rapidly oxidized to form unreactive sulfonic acids, which were recently reported as the main group of DOS in the oceans and part of the long-time persistent fraction of marine DOM.

In contrast to the predominantly low-DOC meteoric fluids from Dominica and Iceland, the hydrothermal fluids from Milos were mainly fed by recirculating seawater. Furthermore, the hydrothermal fluids from Milos were enriched in H_2S and DOS, as indicated by DOS/DOC ratio one order of magnitude higher than for Dominica and Iceland systems, and by the fact that >90% of all assigned DOM formulas exclusively present in the fluids contained sulfur. Evaluation of hypothetical sulfurization reactions during seawater recirculation in Milos sediments suggested DOM reduction and sulfurization. The four most effective potential sulfurization reactions (precursors for over 85% of exclusive DOS formulas) were those exchanging an O atom by one S atom in the formula or the equivalent $+ H_2S$ reaction, correspondingly exchanging H_2O, H_2 and/or O_2 by a H_2S molecule. Our results thereby support the last step of hypothetical abiotic reactions proposed

earlier for sulfide-rich hydrothermal systems, which was the replacement of a H_2O molecule by H_2S. In conclusion, our study reveals novel insights into DOS dynamics in marine hydrothermal environments and provides a conceptual framework for molecular-scale mechanisms in organic sulfur geochemistry.

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