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Marine dissolved organic sulfur –

Sources, fate, and structural characteristics

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“It always seems impossible until it is done.”

– Nelson R. Mandela

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Abstract

Dissolved organic matter (DOM) is one of the largest reservoirs of organic carbon on Earth. It mediates the fluxes of carbon, nutrients, and also trace elements. The sulfur-containing compounds within the DOM mixture are called dissolved organic sulfur (DOS). DOS is an important part of the global sulfur cycle and it is the largest reservoir of marine organic sulfur, yet knowledge on its molecular composition is fragmentary. Sulfidic marine environments are potential hotspots of DOS formation, because reactive reduced sulfur species are known to abiotically react with natural organic matter. The aim of this thesis was to quantitatively and molecularly characterize DOS from different environments and to find indication for its sources, pathways, and sinks in marine systems using laboratory simulation experiments of natural processes.

DOM samples from contrasting environmental conditions were studied: open ocean DOM from the North Sea surface and deep sea water from the North Equatorial Pacific, pore water from sediments from the German Wadden Sea, and four depths of the Black Sea water column representing the different oxidation states therein (oxic in the photic zone, as well as oxic, anoxic, and sulfidic in the aphotic zone). Solid-phase extractable DOS concentrations were analyzed and bulk DOS concentrations were estimated based on the extraction efficiencies obtained for dissolved organic carbon (DOC). In order to analyze the structural characteristics of DOS, methods were developed to particularly test for specific sulfur-containing functional groups. For this, selective wet-chemical alteration experiments targeting the sulfur-containing functional groups were applied to the samples prior to Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS). These experiments included harsh hydrolysis, selective derivatization, oxidation, and deoxygenation to test for thioesters, sulfonic acid esters, alkylsulfates, thiols, non-aromatic thioethers, and sulfoxides. Additionally, collision-induced fragmentation experiments were applied to test for sulfonic acids in DOS. In order to investigate potential sources for the marine DOS, two DOM samples, one from the North Sea and one freshly phytoplankton-produced from mesocosms, were incubated with inorganic reduced sulfur in seawater for four weeks to simulate natural sulfidic environmental conditions. The sulfurization products were analyzed by FT-ICR-MS. To identify a possible sink, a solar irradiation experiment was conducted to test for potential light induced alteration- and degradation reactions of DOS compounds. For this, DOM pore water samples from a sulfidic salt marsh system were exposed to oxygen, metal co-precipitation and incubated in a solar irradiation simulator before optical, geochemical, and FT-ICR-MS analyses.

DOS concentrations were generally found to be higher in sulfidic compared to oxic environments and DOS concentrations were also higher in surface waters compared to the deep sea. The values of the North Sea, the North Equatorial Pacific and the non-sulfidic depths of the Black Sea were between 0.18 and 0.99 µmol L^{-1}. In the sulfidic regime of the Black Sea

and in the sulfidic pore water, values were higher between 1.52 and 47.29 µmol L^{-1}. Similar trends were observed regarding the DOS/DOC concentration ratios, i. e. higher ratios in sulfidic compared to oxic environments. While thioesters, sulfonic acid esters, alkylsulfates, thiols, and sulfoxides were not detected in any of the samples, sulfonic acid functional groups were found in all analyzed samples and are thus likely a ubiquitous structural feature of DOS. Thioethers were exclusively detected in highly sulfidic pore water and in the sulfurized sample of the freshly produced DOM from a mesocosm. This indicates that this kind of reduced DOS is only present in highly sulfidic marine environments that are supplied by fresh and reactive organic matter. The sulfurization experiment confirmed that DOS compounds can be abiotically produced and that the incorporation of reduced inorganic sulfur species is an unselective, complex and rather fast process that likely is affected by follow-up reactions such as oxidation. Comparison of the artificially produced DOS from laboratory experiments with the natural pore water DOS revealed a distinct similarity in the molecular composition suggesting that the simulated processes are a source for DOS in nature, too. Furthermore, the global transport of DOS from sulfidic sediments to the open ocean by benthic fluxes was estimated to be 45 – 120 Tg S a^{-1}. This is about ten times bigger than the riverine input. In the solar irradiation experiment, mainly compounds typical for vascular plant debris, in particular polyphenols, were degraded but also approximately 10 % of the DOS compounds. The results showed that part of the DOS compounds from sulfidic pore waters are photo-reactive indicating that photodegradation is a key factor controlling the stability and fate of marine DOS.

In conclusion, this thesis provides novel insights into the distribution and molecular composition of DOS compounds in marine systems. Furthermore, it gives evidence that abiotic sulfurization is taking place in marine sulfidic environments thereby producing reduced DOS compounds that likely get oxidized once they are exposed to oxic conditions in the water column. The input of DOS by sulfurization and benthic fluxes is likely the single most important source to the ocean and photodegradation is a potential sink for this DOS.

Zusammenfassung

Gelöstes organisches Material (engl. „dissolved organic matter", DOM) ist eines der größten organischen Kohlenstoffreservoirs der Erde. Es vermittelt die Stoffflüsse von Kohlenstoff, Nährstoffen und auch Spurenelementen. Die schwefelhaltigen Verbindungen innerhalb des DOM Gemisches werden als gelöster organischer Schwefel (engl. „dissolved organic sulfur", DOS) bezeichnet, welcher eine entscheidende Rolle im globalen Schwefelkreislauf spielt und das größte organische Schwefelreservoir im Ozean darstellt. Dennoch ist das Wissen über dessen molekulare Zusammensetzung sehr begrenzt. Sulfidische, marine Milieus sind potentielle Hotspots für die Produktion von DOS, da reaktive, reduzierte Schwefelspezies dafür bekannt sind, abiotisch mit natürlichem organischem Material zu reagieren. Das Ziel dieser Dissertation war die quantitative und molekulare Charakterisierung von DOS aus verschiedenen natürlichen Milieus und Hinweise auf die Quellen und Pfade von DOS in marinen Systemen durch Laborsimulationen von natürlichen Prozessen zu finden.

DOM Proben aus unterschiedlichen natürlichen Mileus wurden untersucht: ozeanisches DOM von der Nordseeoberfläche und aus der Tiefsee des nordäquatorialischen Pazifiks, Porenwasser aus Sedimenten des deutschen Wattenmeeres und vier Tiefen der Wassersäule des Schwarzen Meeres, die die verschiedenen Oxidationstufen widerspiegeln (oxisch aus der photischen Zone, sowie oxisch, anoxisch und sulfidisch aus der aphotischen Zone). Die Konzentrationen des Festphasen-extrahierbaren DOS wurden ermittelt und die DOS Gesamtwasserkonzentrationen mithilfe der für gelösten organischen Kohlenstoff (dissolved organic carbon, DOC) ermittelten Extraktionseffizienzen abgeschätzt. Zur Untersuchung der strukturellen Eigenschaften des DOS, wurden Methoden entwickelt, um spezifisch auf bestimmte schwefelhaltige funktionelle Gruppen zu testen. Hierfür wurden die Proben selektiven, nasschemischen Umwandlungsexperimenten, die auf die schwefelhaltigen funktionellen Gruppen abzielten, unterzogen bevor sie mithilfe von Fourier-Transformation Ionen Zyklotron Resonanz Massenspektrometrie (FT-ICR-MS) untersucht wurden. Diese Experimente beinhalteten Hydrolyse, selektive Derivatisierung, Oxidation und Deoxygenierung um auf Thioester, Sulfonsäureester, Alkylsulfate, Thiole, nicht aromatische Thioether und Sulfoxide zu testen. Zusätzlich wurden eine kollisionsinduzierte Fragmentierungsexperimente durchgeführt, um DOS auf Sulfonsäuren zu testen. Zur Untersuchung potentieller Quellen des marinen DOS wurden zwei DOM Proben, eine von der Nordsee und eine frisch phytoplanktonproduzierte Mesokosmus-Probe, zusammen mit anorganischem, reduzierten Schwefel in Meerwasser für vier Wochen inkubiert, um die Bedingungen einer natürlichen, sulfidischen Umgebung zu simulieren. Die Sulfurisierungsprodukte wurden mithilfe der FT-ICR-MS untersucht. Zur Bestimmung einer möglichen Senke wurde ein Sonnenbestrahlungsexperiment durchgeführt, um potentielle lichtinduzierte Umwandlungs- und Abbaureaktionen der DOS Verbindungen aufzudecken.

Hierfür wurden DOM Sedimentproben aus einer sulfidischen Salzwiese Sauerstoff und Metall-Co-Ausfällung ausgesetzt und dann in einem Sonnenstrahlungssimulator inkubiert, bevor sie optisch, geochemisch und mithilfe von FT-ICR-MS untersucht wurden.

Die DOS Konzentrationen waren im Allgemeinen höher in sulfidischen Mileus verglichen mit den oxischen, ebenso waren die Konzentrationen höher in Oberflächengewässern als in der Tiefsee. Die Werte für die Nordsee, das tiefe nordäquatorialische Pazifikwasser und die nicht sulfidischen Tiefen des Schwarzen Meeres lagen zwischen 0.18 bis 0.99 µmol L^{-1}. In den sulfidischen Mileus lagen die Werte mit 1.52 bis 47.29 µmol L^{-1} höher. Die gleichen Trends wurden auch für die DOS/DOC Konzentrationsverhältnisse beobachtet. Auch hier zeigten die sulfidischen Proben höhere Verhältnisse als die oxischen. Während Thioether, Sulfonsäureester, Alkylsulfate, Thiole und Sulfoxide in keiner der Proben detektiert wurden, wurde die Sulfonsäuregruppe in allen Proben detektiert und ist daher wahrscheinlich eine universelle Struktureigenschaft des DOS. Thioether wurden nur in der stark sulfidischen Porenwasserprobe und dem sulfurisierten, frisch produzierten Mesokosmus-DOM gefunden. Dies deutet darauf hin, dass diese Form des reduzierten DOS nur in stark sulfidischen, marinen Mileus, die mit frischem und reaktivem organischen Material versorgt werden, vorhanden ist. Das Sulfurisierungsexperiment bestätigte, dass DOS Verbindungen abiotisch produziert werden können, und dass der Einbau von reduziertem, anorganischem Schwefel ein unselektiver, komplexer und recht schneller Prozess ist, der wahrscheinlich durch Folgereaktionen wie Oxidation beeinflusst wird. Der Vergleich von künstlich produziertem DOS aus dem Laborexperiment mit natürlichem Porenwasser DOS zeigte große Übereinstimmung der molekularen Zusammensetzung. Dies deutet darauf hin, dass die simulierten Prozesse auch in der Natur eine Quelle für DOS sein können. Der globale Transport von DOS aus sulfidischen Sedimenten zum offenen Ozean geschieht durch benthische Ströme und wurde mit 45 – 120 Tg S a^{-1} bestimmt. Dies ist in etwa zehn mal so viel wie der entsprechende Flusseintrag. Im Sonnenbestrahlungsexperiment wurden hauptsächlich die Verbindungen abgebaut, die typisch sind für vaskuläre Pflanzenfasern, aber auch ungefähr 10 % der DOS Verbindungen. Die Ergebnisse zeigen, dass DOS Verbindungen sehr photoreaktiv sind, was darauf hindeutet, dass Photoabbau ein Hauptkontrollfaktor für die Stabilität und den Verbleib von marinem DOS darstellt.

Diese Dissertation bietet insgesamt neuartige Einblicke in die Verteilung und molekulare Zusammensetzung von DOS Verbindungen. Desweiteren zeigt sie, dass abiotische Sulfurisierung in marinen, sulfidischen Milieus stattfindet und dabei reduzierte DOS Verbindungen produziert werden, die wahrscheinlich oxidiert werden, sobald sie den oxischen Bedingungen der Wassersäule ausgesetzt sind. Der Eintrag von DOS durch Sulfurisierung und benthische Ströme ist wahrscheinlich die wichtigste Quelle für die Ozeane und Photoabbau ist eine potentielle Senke dieses DOS.

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Thank you all so very much!

List of Abbreviations

AI	Aromaticity index
BS	Black Sea
CDOM	Chromophoric dissolved organic matter
CID	Collision induced dissociation
CRAM	Carboxylic-rich alicyclic molecules
DBE	Double-bond equivalent
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
DON	Dissolved organic nitrogen
DOP	Dissolved organic phosphorus
DOS	Dissolved organic sulfur
ESI	Electrospray Ionization
FDOM	Fluorescent dissolved organic matter
FT-ICR-MS	Fourier-transform ion cyclotron resonance mass spectrometry
H/C	Hydrogen-to-carbon-ratio
JS	Janssand
m/z	mass to charge ratio
MS/MS	Tandem mass spectrometry
NEqPIW	North Equatorial Pacific intermediate water
NMR	Nuclear magnetic resonance spectroscopy
NPDW	North pacific deep water (see NEqPIW)
NS	North Sea
O/C	Oxygen-to-carbon-ratio
POM	Particulate organic matter
S	Sulfur
S/C	Sulfur-to-carbon-ratio
SPE	Solid-phase extraction
UV	Ultra-violet
XANES	X-ray absorption near edge structure

1. Introduction

1.1. Sulfur cycle

Sulfur is essential to living organisms as it is a component of amino acids, vitamins, and coenzymes. Sulfur is among the ten most abundant elements in the earth's crust and has stable valence states ranging from -2 to +6 (Atlas and Bartha, 1987). Furthermore, it plays an important role in many ecological and environmental transformations (Vairavamurthy et al., 1995 [a]). The major global pools today are metal sulfides such as pyrite and gypsum in rock (7.4×10^9 Tg S), sulfate dissolved in seawater (1.3×10^9 Tg S), sulfate buried in evaporites derived from ocean water ($1.3\text{-}8 \times 10^9$ Tg S), and sulfur-containing buried organic matter (Atlas and Bartha, 1987; Bottrell and Newton, 2006; Schlesinger and Bernhardt, 2013). The major sources of marine sulfate are riverine input, direct oxidation of sulfide, and elemental sulfur disproportionation. Eruptive and post-volcanic activity introduce sulfur into the ecosphere at low rates (29 Tg S a^{-1}; Ivanov, 1981).

It is assumed that some of the earliest organisms on earth gained energy from metabolism using sulfur compounds before the evolution of oxygenic photosynthesis. The microbial processes in those times included dissimilatory elemental sulfur reduction, dissimilatory sulfate reduction, and anoxygenic photosynthesis producing organic matter and oxidized sulfur species; sulfate reduction was not occurring at this time (Canfield and Raiswell, 1999). These microorganisms can still be found in modern sulfide-rich hydrothermal systems where they usually oxidize metal sulfides released from the vents to elemental sulfur and ultimately to sulfate in oxygenated seawater (Jannasch, 1985; Sievert and Vetriani, 2012).

In the modern oceans, sulfur species still play a central role in anaerobic and also aerobic processes (Fig. 1.1). Plants, algae, and many heterotrophic microorganisms assimilate sulfur as sulfate. During anaerobic organic matter oxidation, sulfate and also elemental sulfur serve as electron acceptors. The desulfurization process releases sulfide during the decomposition of reduced organic sulfur. Sulfide can be oxidized to elemental sulfur and sulfate by microbes or chemically or photochemically by atmospheric oxygen (Atlas and Bartha, 1987). Furthermore, sulfide can react abiotically with organic matter to form organic sulfur compounds (Vairavamurthy et al., 1995 [a]).

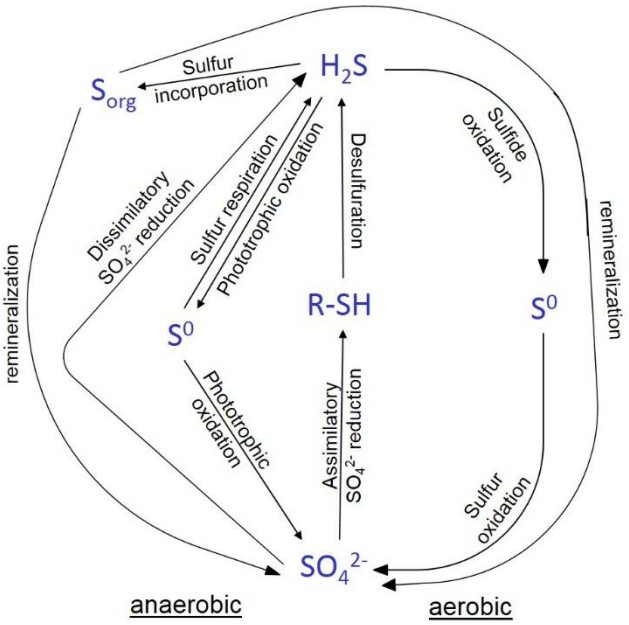

Figure 1.1: Schematic sulfur cycle showing biogeochemical transformations of oxidized and reduced forms of sulfur in anaerobic and aerobic systems. R-SH represents sulfuryl groups of cell protein and S_{org} represents organic sulfur compounds, based on Atlas and Bartha, 1987.

Several processes drive the fluxes between the sulfur reservoirs. Sulfate is an electron acceptor in dissimilatory bacterial sulfate reduction while remineralization of organic matter in sediments. In 2009, 220 species of 60 genera of sulfate-reducing bacteria were known (Barton and Fauque, 2009).The sulfate reduction can occur over a wide range of pH, pressure, temperature, and salinity conditions but only few compounds can serve as electron donors (most common: pyruvate, lactate, and H_2) so that sulfate reduction is often carbon limited (Atlas and Bartha, 1987). Bacterial sulfate reduction fractionates the stable sulfur isotopes ^{32}S and ^{34}S because the ^{32}S isotope is preferentially reduced to sulfide due to the slightly weaker atomic bond of the lighter isotope (Bottrell and Newton, 2006). Most of the produced sulfide (~75-90 %) is re-oxidized to sulfate under oxic conditions in the seawater. Part of the sulfide is incorporated into organic matter forming organic sulfur compounds during early stages of diagenesis (Vairavamurthy et al., 1995 [a]; Kang et al., 2014). Another part of the sulfide reacts with iron species forming various forms of ferrous sulfide which are not thermally stable and eventually react to pyrite that is buried in sediments (Jørgensen, 1977). The net burial rate of sulfur in this pathway is about 60 Tg S a^{-1} (Bottrell and Newton, 2006). The fixed pyrite-sulfur creates a large reservoir of ^{34}S-depleted sulfur (-10 to -12 ‰) that is balanced by ^{34}S-enriched sulfate in the ocean (+21 ‰) (Bottrell and Newton, 2006; Schlesinger and Bernhardt, 2013). Thus, the relative abundance of ^{32}S and ^{34}S isotopes allows for differentiation of biologically generated sulfide and sulfide from strictly geochemical processes (Atlas and Bartha, 1987; Brüchert and Pratt 1996; Giesler et al., 2009; Kang et al., 2014). The sulfide buried in sediments can be uplifted by tectonic processes and then be affected by weathering of

sediments. When oxygen is present, even pyrite can be oxidized so that sulfate is released from the weathering processes to the rivers and finally transported to the ocean. The total natural riverine flux of sulfur was estimated as 104 Tg S a^{-1} (Ivanov, 1981; Bottrell and Newton, 2006). Further processes driving the fluxes are formation of sulfate evaporites, sulfate incorporation into marine carbonates, burial of organic sulfur compounds, and hydrothermal interactions (Bottrell and Newton, 2006). In hot anoxic hydrothermal systems, geothermal energy is used to reduce sulfate from the sediments or oceanic sulfate that entered the crust through cracks to sulfide and elemental sulfur (Jannasch, 1985; Vairavamurthy et al., 1995 [a]). The sulfur species enter the cooler, oxygenated seawater via the hydrothermal fluids and precipitate as elemental sulfur or metal sulfides that form plumes called white or black smokers. The microbial community attached to the vents is energetically supported by chemoautotrophic oxidation of reduced sulfur species (Jannasch and Wirsen, 1979; Jannasch and Mottl, 1985; Atlas and Bartha, 1987). The oceanic sulfur cycle further includes assimilatory sulfate reduction (incorporation of sulfur into biomass of sea plants, ~360 Tg S a^{-1}) which is mostly directly remineralized to sulfate or buried in the sediments where it is then largely remineralized, except for ~2 Tg S, which is buried as organic sulfur. Also, sulfate is buried in sediments as part of skeletons and shells (~30 Tg S a^{-1}; Ivanov, 1981).

Sulfur gases emitted from seawater, freshwater wetlands, volcanos, and fossil fuel burning to the atmosphere have an impact on the global climate. Besides carbonylsulfide and carbon disulfide, dimethylsulfide is the main sulfur gas in the atmosphere with mean residence times of less than two days due to quick oxidation to sulfur dioxide and sulfuric acid which leads to aerosol and then cloud formation that finally precipitates as acid rain. The induced cloud formation also increases the earth's albedo leading to a global cooling effect (Ivanov, 1981; Atlas and Bartha, 1987; Schlesinger and Bernhardt, 2013).

The anthropogenic impact on the global sulfur cycle by mining, use of fertilizers, and fossil fuel burning has destructive consequences. The amount of oceanic sulfate increases while the reduced sulfur stored in the earth's crust decreases (Schlesinger and Bernhardt, 2013). The total input of sulfur from anthropogenic pollution was estimated as 120 Tg S a^{-1} in 1981 (Ivanov, 1981) and recently as 60-100 Tg S a^{-1} (Roberts, 2015). This decrease was likely due to stricter regulations on fertilization and emissions. However, as a result of fossil fuel burning, areas downwind of industrial regions are still affected by acidic deposition that possibly impacts rock weathering, forest growth, and ocean productivity (Schlesinger and Bernhardt, 2013).

Marine organic sulfur can not only be found as biomass. The major oceanic reservoir of organic sulfur was recently estimated as >6.7 Pg S in the form of dissolved organic sulfur (DOS) (Ksionzek et al., 2016). Due to the ability of DOS to complex heavy metals like mercury, it has great influence on metal mobilization, and thus, their global cycles. An overview of the inorganic and organic sulfur reservoirs and fluxes is shown in Figure 1.2.

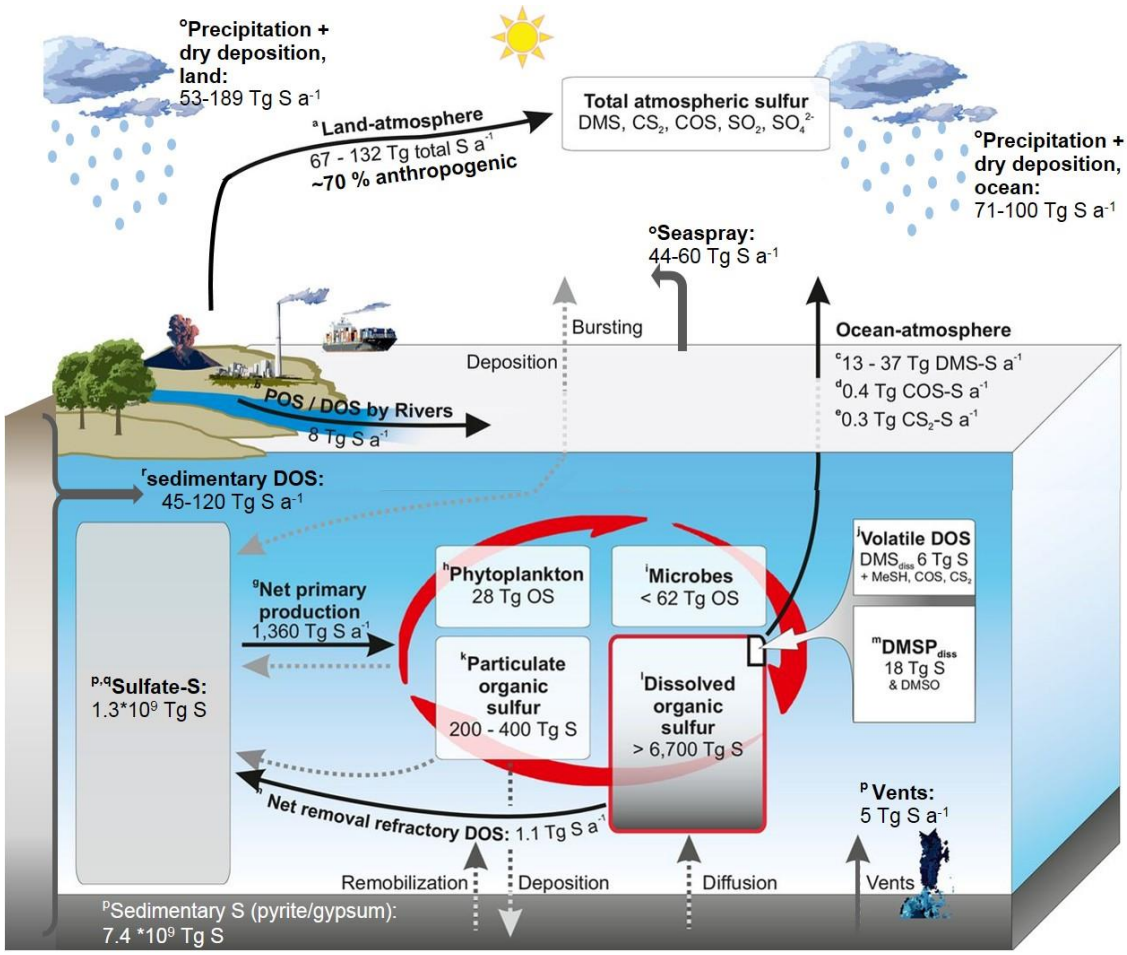

Figure 1.2: Schematic overview of a simplified marine organic sulfur cycle showing organic sulfur reservoirs and fluxes. Additional information on S reservoirs and fluxes were added from (o) Ivanov (1981), (p) Schlesinger and Bernhardt (2013), (q) Bottrell and Newton (2006), and (r) this thesis (Chapter 3). Known and calculated organic sulfur fluxes are presented as solid lines and unknown fluxes as dotted lines. The red circle indicates the cycling of labile DOS compounds such as DMSP (depicted in the small white box). Adapted from Ksionzek et al., 2016. ©AAAS

1.2. Dissolved organic sulfur as part of dissolved organic matter

Dissolved organic sulfur is part of the so-called dissolved organic matter (DOM). It is the fraction of DOM that contains sulfur. Other fractions are dissolved organic nitrogen (DON) and dissolved organic phosphorus (DOP). These three fractions describe the heteroatom-containing part of the DOM. The bulk DOM however, consists of only carbon, hydrogen, and oxygen. General definitions and features of DOM as a whole also apply to the subfractions.

DOM is an important part of the carbon cycle and it is one of the largest reservoirs of organic carbon on earth's surface with approximately 55 Pmol carbon. This is about the same amount of carbon that is bound as carbon dioxide in the atmosphere or that is present in all

living biomass on land and in the ocean combined (Hedges 1992; Hansell et al., 2009). Due to its enormous size it inheres a great potential to influence global biogeochemical cycles and changes in DOM respiration could influence the global climate (Dittmar and Stubbins, 2014). DOM is a main mediator for the energy flux from autotrophs to microbial heterotrophs in the ocean (Carlson et al., 2007). Furthermore, DOM mediates the fluxes of carbon, nutrients, and also trace elements by ligand associations (Hedges, 1992). Despite the great size and influence of DOM, knowledge on it is limited. The molecular composition of DOM is still largely unknown and the reasons behind its long-term accumulation in the ocean remain to be resolved (Dittmar and Stubbins, 2014).

1.2.1. Defining DOM – Size and reactivity classifications

The classic definition of dissolved organic matter is an operational one stating that all organic matter that passes a filter with a pore size of 0.7 µm or less is termed “dissolved”. DOM is further categorized into high molecular weight (HMW, <10 kDa and >3 kDa) and low molecular weight (LMW, < 1 kDa) DOM. The LMW DOM fraction accounts for approximately 65-80 % of the bulk DOC in the ocean and increases in proportion with depth. Thus, the majority of DOM in the deep ocean is LMW DOM (Ogawa and Tanoue, 2003).

DOM is also categorized into reactivity fractions based on their turnover rates in the ocean. There are three broad categories: labile, semi-labile, and refractory DOM (Kirchman et al., 1993). Lately, a more differentiated classification was suggested. Here, the DOM is categorized into labile and recalcitrant DOM. The latter is further categorized into semi-labile, semi-refractory, refractory, and ultra-refractory DOM (Hansell, 2013). The labile DOM is the smallest and most reactive fraction with turnover rates of hours to days. It can only be found in the surface ocean and it does not accumulate. The semi-labile and semi-refractory DOM only accumulate with development of a seasonal pycnocline or above the main pycnocline, respectively, and can only be found in the upper 1000 m of the water column. Refractory and ultra-refractory DOM are present in the whole water column. Refractory DOM has relevance on a climate time scale (centuries to millennia) with bulk ages of 4000-6000 years. The reasons behind this long-term stability are still matter of debate (Dittmar, 2015). A fraction of DOM might be intrinsically resistant due to its molecular structure of mainly thermogenic origin (Dittmar and Paeng, 2009), while other compounds may be too dilute in seawater to be efficiently taken up by microbes (Arrieta et al., 2015). A recent study on the relationship between organic matter size, radiocarbon age, and chemical composition (carbon-to-nitrogen ratios) found that small molecules are more persistent in the ocean than larger ones and that the chemical composition is primarily controlling the bioavailability of particulate and dissolved organic matter (Walker et al., 2016).

Each reactivity fraction has distinct roles in oceanic carbon cycling. The labile DOM feeds the microbial loop which produces biomass available for higher trophic levels (Azam et

al., 1983). The semi-labile and semi-refractory DOM constitute the dissolved phase of the biological pump which describes the ocean's mechanism of biological driven carbon sequestration (Sigman and Haug, 2003; Jiao and Zheng, 2011). The refractory DOM could be a source and sink for variations in paleoclimate. Ultra-refractory DOM is a small but global pool with turnover rates bigger than the one of the ocean circulation. It may have a role in controlling the inventory of atmospheric carbon dioxide on geological timescales. Both biotic and abiotic processes are likely responsible for the removal and transformation of DOM. The abiotic removal processes include photolysis by UV irradiation, transformation into particles or gels, and scavenging onto sinking particles (Hansell, 2013).

The size-reactivity continuum model (Fig. 1.3) is an approach to unite these observations about size, reactivity, and age of DOM in the ocean (Amon and Benner, 1996; Benner and Amon, 2015). It describes the flow of organic matter as a continuum from a highly structured to a more complex state. Thereby, biodegradation is the dominant process shaping the size distribution of organic matter in seawater being even more important than biosynthesis (Benner and Amon, 2015). The rates of microbial degradation were found to be higher in incubations with HMW DOM indicating that it is more bioreactive than LMW DOM (Amon and Benner, 1996). This phenomenon is not solely based on the size per se but different size classes of organic matter have distinct chemical compositions that influence microbial degradation. Isotopic analyses of the different size classes confirmed the size-reactivity continuum model showing that particulate organic matter is on average contemporary whereas DOM is on average millennial with HMW DOM being decadal to centennial (Benner and Amon, 2015). Burdige and Gardener (1998) adapted this model to pore water. Their model depicts the degradation of particulate organic matter to inorganic nutrients as a series of hydrolytic or oxidative, fermentative, and eventually respiratory processes with increasingly smaller molecules involved.

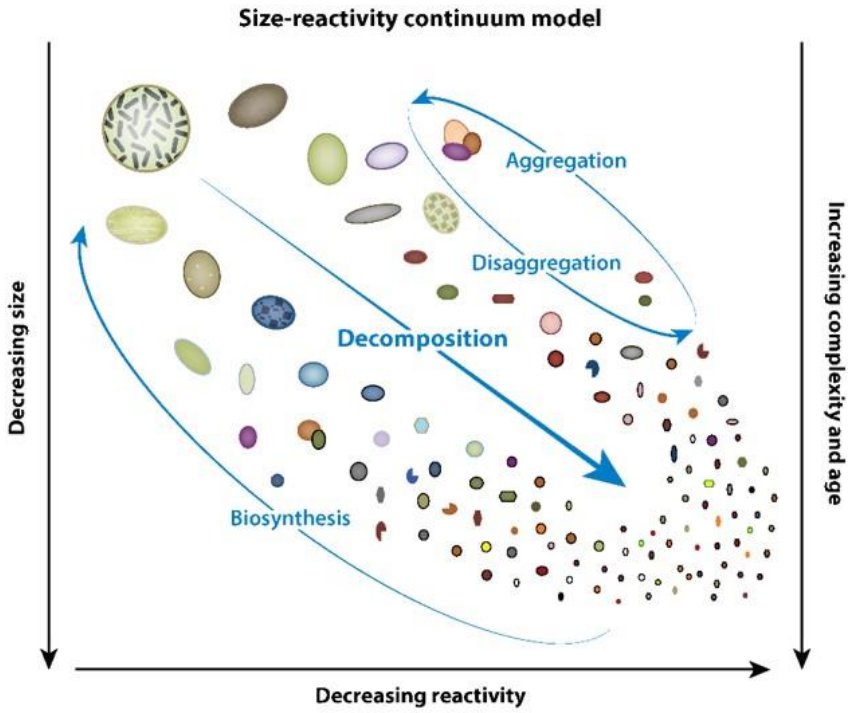

Figure 1.3: Model of the size-reactivity continuum, showing the net flow of organic carbon from larger to smaller size. With increasing decomposition the chemical complexity of organic matter increases, biological reactivity declines, and the radiocarbon ages increase. Small organic molecules can be transformed into larger size classes by biosynthesis. The effect of aggregation and disaggregation processes is not well understood. Reprinted with permission from Benner and Amon (2015).

1.2.2. Sources and sinks of DOM

There are several sources of DOC to the ocean. Riverine discharge transports DOM and particulate organic matter (POM) to the ocean. This total carbon input is about 0.4 Pg C a^{-1} (Schlesinger and Melack, 1981) with roughly half (0.25 Pg C a^{-1}, Hedges et al., 1997) being dissolved. Sediment pore waters transport labile and refractory DOM (Burdige, 2002).

The global DOC input to the ocean from intertidal sediments, coastal and continental margin sediments (0-2000 m water depth), and sediments >2000 m water depth was estimated as in total ~450 Tg C a^{-1} (Burdige et al., 1999; Dunne et al., 2007; Maher and Eyre, 2010; Burdige and Komada, 2015). However, compared to the major source of DOC to the ocean which is autotrophic production in the euphotic zone, river and sediment discharge have minor significance to the total carbon input. The net primary production is 104.9 Pg C a^{-1} with roughly equal contributions from land (56.4 Pg C a^{-1}) and ocean (48.5 Pg C a^{-1}) (Field et al., 1998). Typically, 13 % of the primary production is extracellularly released as DOM but this value is highly variable (Carlson, 2002). Grazing-induced DOM

production describes the pathway of organic matter from phytoplankton to bacteria via the byproducts of zooplankton ingestion and digestion. The zooplankton grazers can transform POM into DOM via a variety of processes including sloppy feeding, egestion, and excretion (Carlson, 2002, Jiao et al., 2010). Further DOM sources are viral cell lysis ("viral shunt", Wilhelm and Suttle, 1999) and POM solubilization by bacterial and archaeal ectohydrolyses with preferential solubilization of DON and DOP (Carlson, 2002, Jiao et al., 2010). Heterotrophic prokaryotes (bacteria and archaea) consume the produced DOM and are subsequently consumed by small grazers (protists) thus connecting DOM to the food web (Weinbauer et al., 2011). This process is the microbial loop (Azam et al., 1983). In total 10-50 % of the carbon fixed by photosynthesis is utilized by bacteria (Azam et al., 1983).

However, not all of the utilized DOM is brought back to the food web. Part of the DOM utilized by bacteria is transformed to refractory DOM that is stored for millennia in the ocean. This process has been referred to as microbial carbon pump (Jiao et al., 2010). The major sink for the produced DOM is microbial mineralization (Hansell et al., 2009). A minor sink is the alteration of DOM while circling through high temperature hydrothermal ridge-flanks (Lang et al., 2006). DOC concentrations are lower in the outflow of the vents compared to the surrounding water. The removal rate of this process was estimated as 70-140 Tg C a^{-1} (Lang et al., 2006). Another sink is organic gel formation of DOM leading to particle formation or adsorption onto particles and consequently, sinking to sediments. The removal rate of this process ranges between 1.4-2.8 nmol C kg^{-1} a^{-1} (Hansell et al., 2009).

The eventual fate of most of this exported material is remineralization to CO_2. The whole process of carbon fixation in the upper ocean by primary production to the sinking and burial of POM is the biological pump (Jiao et al., 2010). Therefore, the fundamental driver of the biological pump is primary production whereas heterotrophic microbes drive the microbial carbon pump. The biological pump and the microbial pump are connected by the microbial loop (Fig. 1.4). In eutrophic waters with high primary production, POM export by the biological pump is likely exceeding the carbon storage mediated by the microbial pump, whereas the microbial pump is likely stronger in oligotrophic waters with strong stratification and high bacterial activity. Both processes are probably coupled down in the mesopelagic and bathypelagic zone (Jiao et al., 2010).

In the sediments, two common models for carbon preservation exist (Burdige, 2002). The geopolymerization model involves the condensation reaction of LMW DOM to HMW DOM and eventually POM (Nissenbaum et al., 1972; Tissot and Welte, 1978). The mesopore protection model describes the sorption of DOM on mineral surfaces in small mesopores where it is physically protected from attacks by microbial enzymes (Mayer, 1994; Hedges et al., 1999). These effects might be combined since rates of abiotic condensation may be accelerated in mesopores by steric- or concentration-related phenomena which would further enhance the preservation of sorbed DOM.

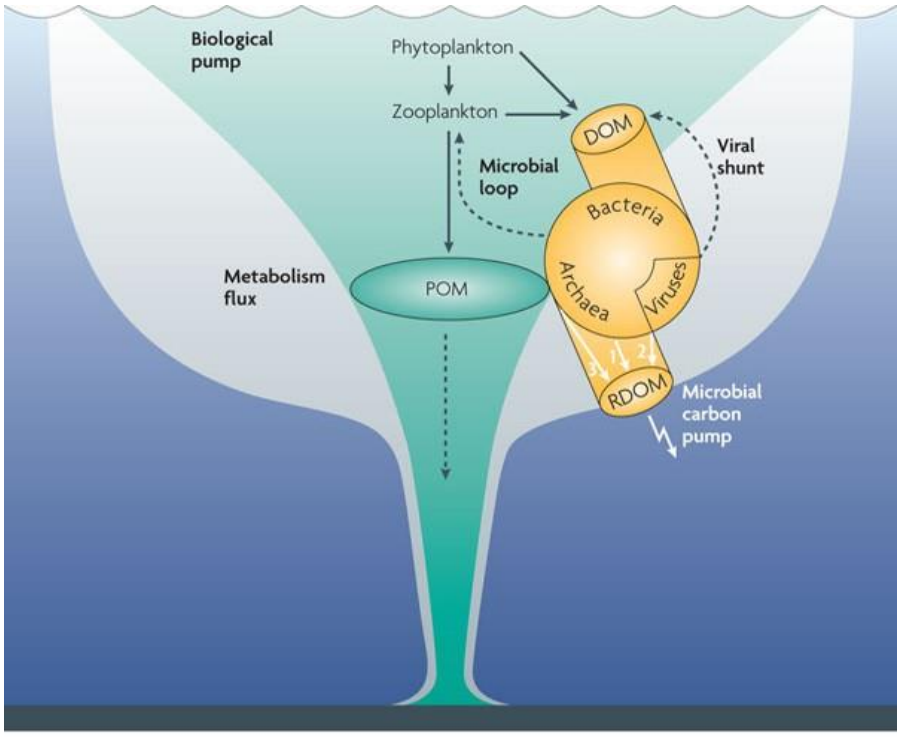

Figure 1.4: Major biological processes involved in carbon cycling in the ocean. The biological pump is the process in which CO_2 is fixed by primary production in the upper ocean and transported as sinking biogenic particles to the deep ocean. The microbial loop is the process in which DOM is taken up by bacteria and archaea which are then consumed themselves thereby linking the DOM to the food web. The viral shunt returns the POM to the DOM pool. The microbial carbon pump was proposed to convert SLDOM and SRDOM into refractory DOM. Reprinted with permission from Jiao et al. (2010).

Another important sink in the photic zone are photochemical reactions (Mopper et al., 1991). Two photochemical oxidation/degradation pathways exist. The abiotic pathway represents the direct photochemical remineralization. The sequential photochemical/biotic pathway involves the photodegradation of DOM to LMW substrates which are then consumed by microbes (Mopper and Kieber, 2002). The abiotic pathway also results in the emission of climate relevant gases like carbon monoxide, carbonylsulfide, and dimethylsulfide. Furthermore, photodegradation is the primary sink for refractory dissolved black carbon (Stubbins et al., 2012). In the sequential photochemical/biotic pathway, photodegradation processes are able to enhance the bioavailability of old, biorefractory DOM (Mopper and Kieber, 2002). Most studies of irradiation experiments followed by bacterial consumption showed positive effects on microbial activity, others showed a negative, mixed, or no effect at all (e.g. Judd et al., 2007; Abboudi et al., 2008; Mopper et al., 2015; Riedel et al., 2016). It is hypothesized that photoalteration renders freshly produced algal-derived DOM in surface waters less available while humic-rich deep water DOM becomes more available to bacteria (Benner and Biddanda, 1998). The efficiency of photochemical processes is expressed

as quantum yield. It is a unitless ratio of the number of moles of species formed or photolyzed divided by the number of moles of photons absorbed at any one wavelength (Mopper et al., 2015). The estimated loss of direct photochemical mineralization is 12-16 Pg C a^{-1} (Moran and Zepp, 1997).

For DOS two potential sources are discussed: Firstly, ^{34}S-enriched biosynthetic organic sulfur derived from aquatic vascular plants or from dissolved sulfate assimilated by algae and secondly, ^{34}S-depleted diagenetic organic sulfur from reactions of organic matter with sulfide (produced by bacterial sulfate reduction where ^{32}S is preferred, Anderson and Pratt, 1995) (Brüchert and Pratt, 1996). It has been argued that it is unlikely that biosynthetic production can account for all the organic sulfur present in sedimentary and fossil organic matter because the abundance of sulfur in biomass is very low (Aizenshtat et al., 1995). Furthermore, sedimentary organic sulfur is ^{32}S-enriched by >10 % relative to biomass sulfur (Anderson and Pratt, 1995; Aizenshtat et al., 1995). Therefore, it is generally accepted that marine organic sulfur is also produced by abiotic incorporation of inorganic reduced sulfur species into organic matter, the so-called sulfurization (Sinninghe Damsté et al., 1989; Vairavamurthy et al., 1995 [b]; Schmidt et al., 2009). Sulfurization is likely to happen in sulfidic environments, where sulfide species are present. The reaction mechanism of this incorporation is likely the nucleophilic Michael addition as sulfidic systems are slightly basic. Thus, the organic molecule should have an activated unsaturated bond as in α,β-unsaturated carbonyl compounds (Aizenshtat et al., 1995). The sinks for DOS are the same as presented above for DOC: remineralization, incorporation into microbial biomass, and photodegradation (Hertkorn et al., 2013; Ksionzek et al., 2016).

1.2.3. Inventory and fluxes of DOM in marine systems

Historically, marine DOM had been considered a spatially constant, refractory pool of carbon that is uniformly distributed throughout the deep sea (Hansell, 2009). Since then, it has been consistently observed that elevated concentrations of DOC as a proxy for DOM appear in the upper water column which decrease with depth, followed by a low, uniform value in the deep water ranging in total from 34 to ~80 µmol C L^{-1} (Barber, 1968; Hansell, 2009). Half of the primary production in in the upper water column is funneled through DOM into the microbial loop. This labile DOC represents a large flux of carbon although it ends up as only a small fraction of the oceanic DOC inventory (Hansell, 2009). Ocean circulation combined with the reactivity fractions of DOM determine further trends in global DOC distribution (Fig. 1.5). In the Atlantic, DOC rich surface waters (>50 µmol C L^{-1}) are exported with North Atlantic Deep Water (NADW) formation. This is followed by a decline in DOC concentrations along intermediate and deep ventilation pathways towards the South Atlantic to concentrations of 40-50 µmol C L^{-1} at the equator and to ~39 µmol C L^{-1} in deep, circumpolar Antarctic waters. In the Pacific, relatively DOC-rich circumpolar deep water

invades northward along the bottom (LCDW) while DOC is slowly removed. The DOC-depleted water then returns southward at mid-depths (PDW) (Hansell, 2009 and 2013). Back at Antarctica it partially rises to the surface and is redistributed through the Antarctic Circumpolar Current into the major global ocean basins. This global distribution of water masses from the North Atlantic into the North Pacific and back to Antarctica takes more than 1000 years (Dittmar and Stubbins, 2014). While circulating, heterotrophic microbes slowly consume DOM explaining the drop of DOC concentrations from >50 µmol C L^{-1} in the deep North Atlantic to ~34 µmol C L^{-1} in the oldest water at mid depth in the South Pacific (Dittmar and Stubbins, 2014).

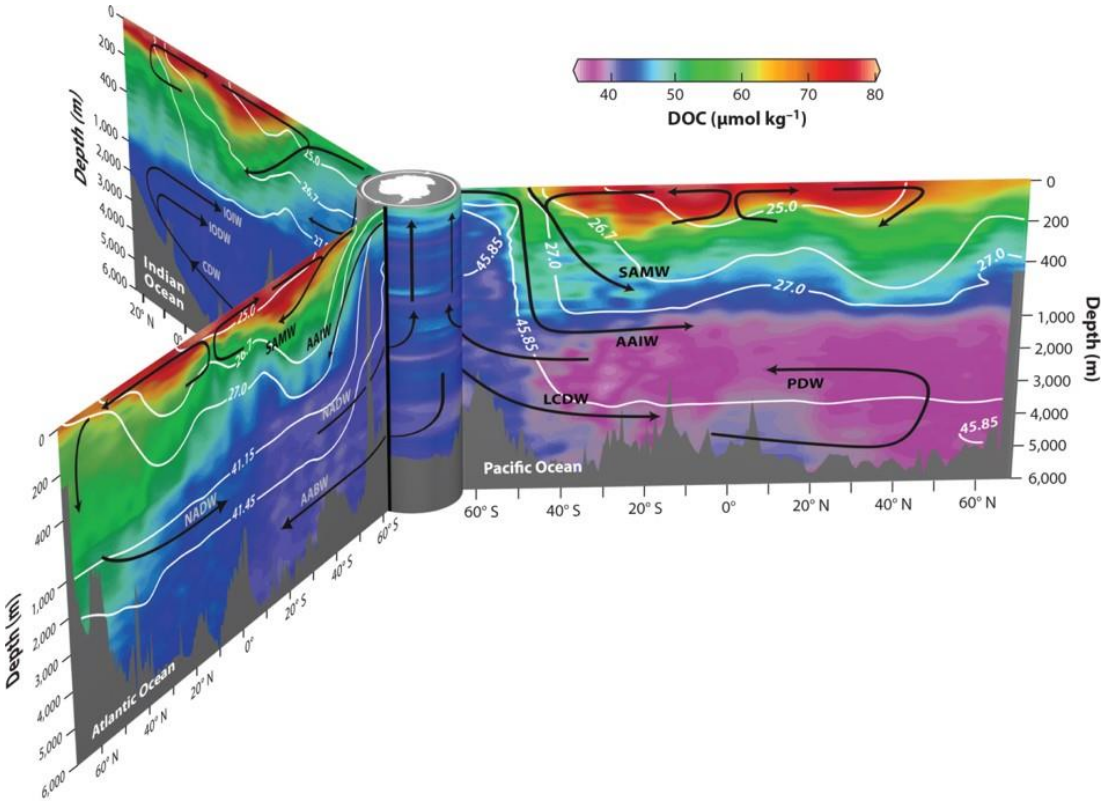

Figure 1.5: DOC concentration observations in the central Atlantic, central Pacific, and eastern Indian Oceans with all lines connected via the Antarctic circumpolar currents. Black arrows depict water mass renewal and circulation, white lines indicate selected isopycnal surfaces. Abbreviations: AABW = Antarctic Bottom Water, AAIW = Antarctic Intermediate Water, CDW = Circumpolar Deep Water, IODW = Indian Ocean Deep Water, IOIW = Indian Ocean Intermediate Water, LCDW = Lower Circumpolar Deep Water, NADW = North Atlantic Deep Water, PDW = Pacific Deep Water, SAMW = Subantarctic Mode Water. Reprinted with permission from Hansell (2013).

Vertical transport of biogenic carbon from the surface euphotic zone to the ocean's interior is controlled by the biological pump which combines the processes of passively sinking

particulate organic matter, active vertical transport by zooplankton, and diffusion. Highest surface DOC concentrations occur in tropical and subtropical systems (70-80 µmol C L^{-1}) where vertical stratification of the upper water column supports accumulation of biologically resistant organic matter (Hansell, 2009). From the tropical systems DOC is transported with the surface currents to the subpolar seas and the circum-polar Southern Ocean where lower DOC concentrations are present (~40-50 µmol C L^{-1}) because the surface waters are readily mixed with low-DOC deep ocean waters which provides the transport of DOC to greater depths (Hansell, 2009 and 2013).

DOM accumulates in the subtropical gyres as they cycle in upon themselves. The DOM is exported therein by Ekman convergence moving the DOC-enriched waters to depths of a few hundred meters (Hansell, 2013). DOM mineralization is inhibited in the gyres maybe due to low nutrient concentrations or photodegradation that alters the molecules in a way that it becomes unusable for microbes (Dittmar and Stubbins, 2014).

Coastal waters which are greatly influenced by terrestrial input and upwelling events show high DOC concentrations with large variability. This variability is caused by the high variations in space and time of the continental fluxes. In tropical and artic latitudes, riverine discharge of water and its constituents is highest whereas little discharge occurs in temperate and Antarctic latitudes. However, seasonal variability is less in tropical rivers compared to the other regions (Dittmar and Stubbins, 2014).

For only the sulfur-containing fraction of the DOM less information is available. DOS concentration analysis in marine water is hampered by sulfate which exceeds the concentration of DOS five orders of magnitude (Ksionzek et al., 2016). Recently, the global minimum DOS inventory was estimated as 6.7 Pg S (maximum value when a phytoplankton C/S ratio is assumed: 18.6 Pg S) (Ksionzek et al., 2016). DOS concentrations decrease with depth like DOC but DOC/DOS ratios increase with depth indicating that DOS declines more rapidly than DOC with depth (Hertkorn et al., 2013; Ksionzek et al., 2016).

In marine pore waters DOC concentrations are generally and up to one order of magnitude higher than in the overlying bottom water and have commonly higher values in anoxic sediments (Burdige, 2002). Within most sediments, DOC concentrations increase with depth (e.g. Burdige and Gardener, 1998). These depth profiles can further be divided into two categories: In anoxic, sulfate reduction dominated sediments, DOC concentrations of the pore water generally increase. In oxic sediments however, DOC concentrations are still higher than in the overlying bottom water but also show more constant values with depth in the upper layers of the sediments (Burdige, 2002). The global DOC flux from intertidal sediments, coastal and continental margin sediments (0-2000 m water depth), and sediments >2000 m water depth was estimated as in total ~450 Tg C a^{-1} (Burdige et al., 1999; Dunne et al., 2007; Maher and Eyre, 2010; Burdige and Komada 2015) which is higher than the riverine input (~210 Tg C a^{-1}, Ludwig and Probst, 1996) indicating a high influence of sedimentary DOC on the ocean.

1.3. Molecular characterization of DOM and DOS

The structural characterization of DOM would help to better identify sources, sinks, and turnover processes of DOM. However, this is a major challenge due to the great complexity of DOM, the low concentration of each individual compound, and the multiplicity of structural isomers for each molecular formula (Dittmar and Stubbins, 2014). Even modern chromatographic techniques can only separate the DOM into fractions according to polarity or molecular size which still leads to too complex mixtures for available analytical techniques (Dittmar et al., 2007; Koch et al., 2008). So far, only a few defined building blocks such as amino acids, sugars, and lignin-derived phenols could be isolated and quantified. However, these molecular building blocks account for only <7 % of the DOM (Kaiser and Benner, 2009, 2012; Dittmar and Stubbins 2014). Most of the DOM thus, remains molecularly uncharacterized and the knowledge on DOS molecular characteristics is even scarcer (Lechtenfeld et al., 2011; Hertkorn et al., 2013). X-ray absorption near edge structure (XANES) analysis of Peru margin sedimentary organic sulfur showed based on its oxidation state that it mainly consists of thioether and to a lower level thiophenes but also of sulfonic acids and minor amounts of sulfoxides (Eglinton et al., 1994). Another study on organic sulfur in sediments of the Peru margin as well as of the coast of New York revealed the presence of sulfonates in rather shallow depths and also reduced sulfur compounds in greater depths (Vairavamurthy et al., 1994). XANES analysis of humic and fulvic sulfur in Jiaozhou Bay sediments also showed strongly reduced sulfur species like thiols or sulfides as well as strongly oxidized species like sulfonates or ester-sulfates (Zhu et al., 2014). A study on open South Atlantic Ocean DOS compounds with FT-ICR-MS revealed a hydrogen- and oxygen-deficiency in DOS compounds in the abyssal ocean suggesting the prevalence of reduced sulfur compounds like thiols, sulfides, or thioethers there (Hertkorn et al., 2013).

A possible technique to analyze DOM molecular characteristics are e.g. optical techniques. UV-visible absorption and fluorescence analyses of DOM obtain qualitative information on DOM that allow inference on its source, diagenetic state, reactivity, ecological function, and chemistry (Coble et al., 1990; Fellman et al., 2010; Stubbins et al., 2014). However, this information is limited to the chromophoric (chromophoric dissolved organic matter, CDOM) and therein the fluorescent (fluorescent dissolved organic matter, FDOM) fraction of the DOM pool.

The most widely applied technique for the structural characterization of DOM is nuclear magnetic resonance (NMR) spectroscopy (Lam and Simpson, 2008; Dittmar and Stubbins, 2014). One-dimensional ^{1}H- and ^{13}C-NMR and also combined as two-dimensional spectra deliver information on atomic bonds and functional groups. NMR is not limited by molecular sizes and can be performed in solution- and solid-state (Simpson et al., 2011). The use of NMR on DOM samples obtained novel insights into the structures in the DOM pool. For example, Hertkorn et al. (2006) found a new class of carboxylic-rich alicyclic molecules (CRAM) that

constitute ~8 % of the DOM pool. Usage of two-dimensional NMR spectra revealed in South Atlantic Ocean DOM samples of several depths from surface to deep sea a higher abundance of olefinic protons and carbons than aromatic ones (Hertkorn et al., 2013). However, NMR is not well suited for DOS analysis because ^{33}S is the only naturally occurring NMR-active sulfur isotope which also has very low natural abundance (0.76 %). It is quadrupolar, resulting in low sensitivity and very broad signals which is the reason why it is of limited use for the analysis of organosulfur compounds (O'Dell and Moudrakovski, 2010).

Ultrahigh resolution mass spectrometry coupled to electrospray ionization (ESI) has become one of the most powerful techniques for the molecular characterization of DOM (e.g. Koch et al., 2005; Dittmar and Paeng, 2009, Stenson et al., 2002 and 2003). This technique enables the analysis of molecular formulae of intact molecules and was therefore chosen for the analysis of DOS in this thesis. A detailed description of this technique can be found in the following chapter.

These analytical methods require salt-free samples with high DOM concentrations. To analyze marine water, it is therefore necessary to concentrate the samples and to also eliminate the contained salts. A possible isolation method is ultra-filtration with a 1-kDa cutoff which isolates up to 30 % of marine DOM (Amon and Benner, 1996; Benner et al., 1997). Another more efficient method with DOC recoveries exceeding 60 % is a sequential combination of reversed osmosis and electrodialysis (Vetter et al., 2007). However, the isolates from these methods still contain salt which can be problematic for DOM molecular analyses (Dittmar et al., 2008). Solid-phase extraction (SPE) is the most commonly used isolation method. SPE with commercially available cartridges filled with a styrene divinyl benzene polymer sorbent (Varian PPL) is an easy to handle method that can even be performed in field. It obtains DOC extraction efficiencies of around 60 % with no salt left in the extract. The PPL resin is able to retain highly polar to nonpolar substances and therefore covers the entire polarity range of the DOM pool (Dittmar et al., 2008). However, for all of the presented isolation methods a major part of the DOM is lost from the analytical window. This means, as all mass spectrometric analyses in this thesis were obtained from solid-phase extracted DOM samples, all obtained results were only valid for SPE-DOM.

1.3.1. Mass spectrometric analysis

Developments in ultrahigh resolution mass spectrometry made it possible to resolve the enormous molecular complexity of DOM. Soft ionization techniques such as electrospray ionization (ESI) coupled to the mass spectrometers enables the analysis of intact molecules. Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) is able to detect several tens of thousands of compounds simultaneously and thus making chromatographic separation prior to analysis unnecessary. It further exhibits an accuracy better than 0.1 mDa which is less than the mass of a single electron (Dittmar and Stubbins, 2014). This high mass

accuracy allows to assign molecular formulae to the detected masses utilizing the mass defect within an error of <1 ppm (Koch et al., 2005; Stenson et al., 2003). Analysis of DOM samples with FT-ICR-MS revealed that the SPE-DOM molecules occupy a small mass range from mainly 250 to 550 Da (Fig. 1.6) which indicates that it is mainly composed of microbial metabolites (Dittmar and Stubbins, 2014). The analysis of DOM further detected more than 10,000 molecular formulae and thus confirmed that DOM is among the most complex molecular mixtures on the planet. The molecular formulae level information on DOM obtained by FT-ICR-MS shows that the compounds are composed of carbon (C), hydrogen (H), and oxygen (O) and to a much smaller number also contain nitrogen (N), sulfur (S), or phosphorus (P) (Schmidt et al., 2009, 2011).

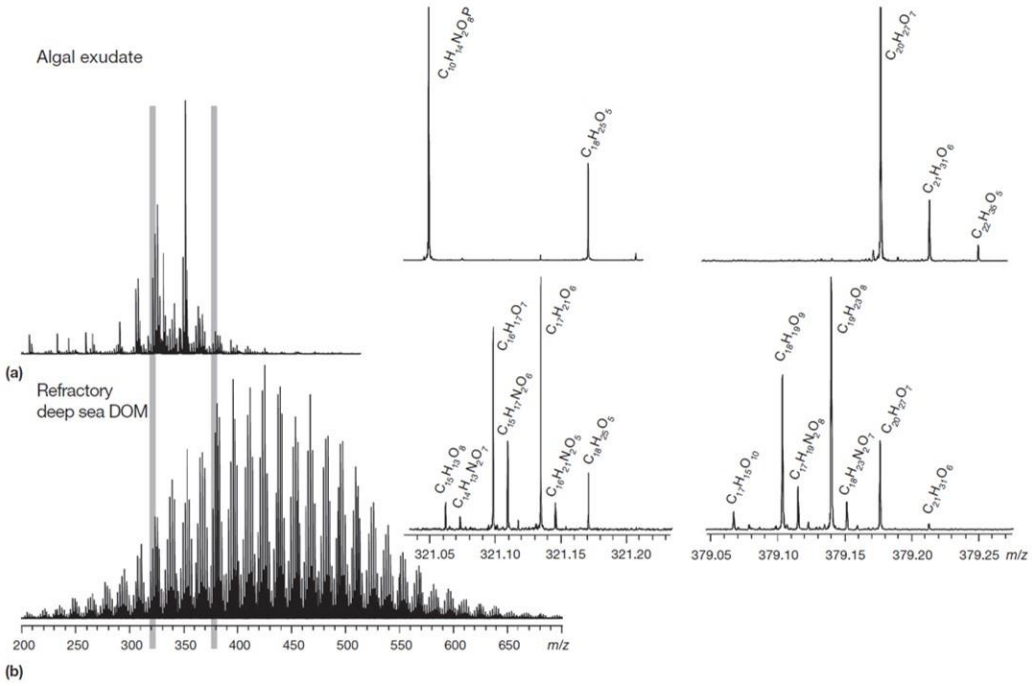

Figure 1.6: FT-ICR mass spectra of (a) labile DOM from an algal exudate and (b) refractory DOM from a deep sea sample. The whole mass spectra and two exemplary masses (*m/z* = 321 and 379 Da) are shown each peak representing a singly charged intact molecule. The high mass accuracy allows to assign molecular formulae to the detected masses. While the refractory DOM is characterized by a very high molecular diversity, the labile DOM is less diverse and shows a less regular mass spacing pattern. Reprinted with permission from Dittmar and Stubbins (2014).

A commonly used visualization tool to outline the molecular composition of a DOM sample is the van Krevelen diagram. In this diagram the hydrogen-to-carbon-ratio (H/C) of the detected molecular formulae is plotted against the oxygen-to-carbon-ratio (O/C). Different areas in the van Krevelen plot can be assigned to different compound classes according to their saturation and oxidation state (Fig. 1.7). The pattern of the compounds in a van Krevelen

diagram can also reflect the sources and reaction pathways of the DOM like e.g. oxidation, reduction, methylation, or hydration (Kim et al., 2003).

Based on the molecular formulae some structural features can be inferred. High saturation (H/C > ~1.5) expresses the presence of aliphatic structures (Stubbins et al., 2010), low saturation on the opposite expresses aromatic structures and even condensed polycyclic aromatics (Koch and Dittmar, 2006). Further structural features that can be calculated from the molecular formulae are the double-bond equivalent (DBE, Stenson et al., 2003) and the aromaticity index (AI, Koch and Dittmar, 2006, 2015). Nevertheless, complete structural information cannot be obtained from the FT-ICR-MS analyses because each molecular formulae can consist of millions of structural isomers (Hertkorn et al., 2007). Thus, DOM could consist of billions of different molecules. Further analysis tools or experiments are therefore necessary to obtain more information on structural features of DOM.

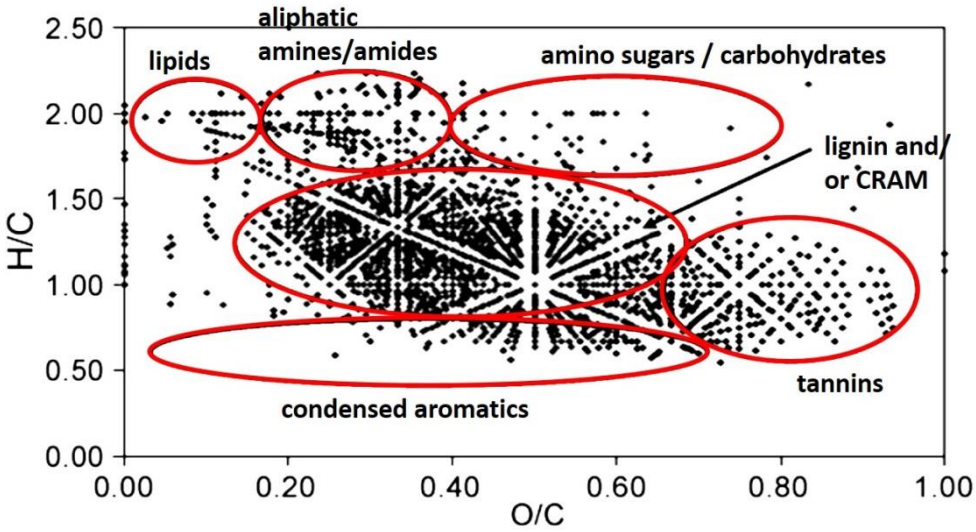

Figure 1.7: Exemplary van Krevelen diagram showing the molecular composition of a DOM sample from the Great Dismal Swamp in Virginia, USA. Each dot represents an individual molecular formula that was assigned to a mass detected by FT-ICR-MS. Highlighted in red are the different compound classes. Adapted from: Sleighter and Hatcher (2008). ©Elsevier

1.3.1.1. Tandem FT-ICR-MS

Tandem FT-ICR-MS (MS/MS) enables insight into structural features of the DOM compounds. Tandem mass spectrometry is a technique where mass-selected ions are subject to a second mass spectrometric analysis. There are two basic concepts for MS/MS: tandem in space and tandem in time. FT-ICR-MS has a single *m/z* analyzer and is therefore a tandem in time mass spectrometer. The collision-induced dissociation (CID) is the most important activation method and enables the fragmentation of stable molecules in the gas phase. The energy that is brought into the ion by the collision with a neutral molecule leads to

dissociation into substructures. The fragmentation spectra can be considered as structural fingerprints of organic compounds. The neutral losses can be characteristic for specific functional groups and can thus provide information on structural characteristics of the fragmented compound (Gross, 2011). Thanks to the high mass accuracy of the FT-ICR-MS it is even possible to isolate a single mass for in-cell fragmentation (FT-ICR in-cell MS/MS, Fig. 1.8). However, by decreasing the mass window also the intensity of the mass peak decreases which is a problem for the already low-intensity sulfur-containing compound mass peaks (Witt et al., 2009). But even if a bigger mass window of one nominal mass is isolated, fragment ions can still be assigned to the corresponding parent ion due to the high mass accuracy. Fragmentation experiments on a Suwannee River fulvic acid standard revealed that the most abundant neutral losses in this natural organic matter sample were CO_2, H_2O, CO, and CH_4 which indicate the presence of several carboxyl groups (Stenson et al., 2003; Witt et al., 2009; Fig. 1.8). It is crucial to choose the right energy for the CID so that the parent ions fragment but also that not too large, uncharacteristic losses occur due to secondary fragmentation (Stenson et al., 2003).

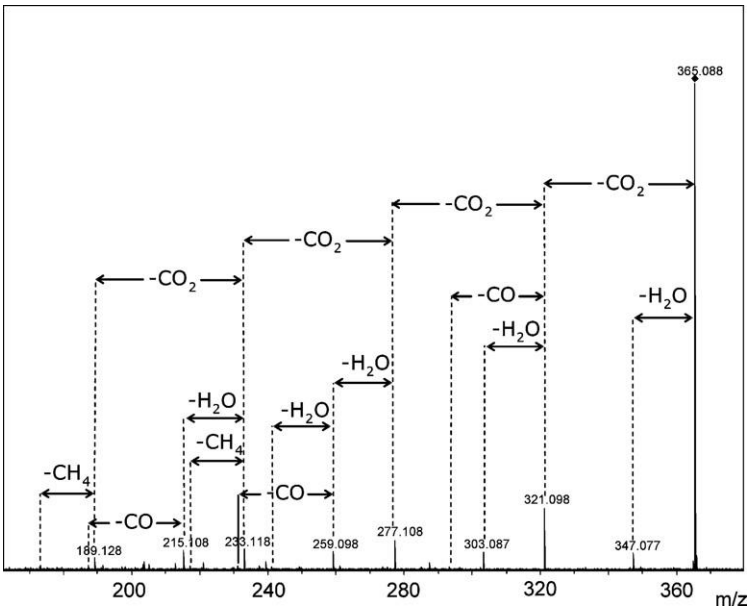

Figure 1.8: FT-ICR-MS in-cell CID fragmentation spectrum of the Suwannee River fulvic acid standard. The single isolated parent ion *m/z* = 365.088 ($C_{17}H_{17}O_9^-$) was fragmented and neutral losses were assigned to the fragment mass peaks. Reprinted with permission from: Witt et al. (2009). Copyright (2009) American Chemical Society.

Very little focus has been put on the fragmentation features of the sulfur-containing compounds in organic matter. A major problem here is the low intensity of the DOS mass peaks (Rossel et al., 2013; Seidel et al., 2014). Lin et al. (2012) detected organosulfates in aerosols from East Asia using ultrahigh resolution MS/MS. Cortes-Francisco and Caixach

(2015) analyzed the Suwannee River fulvic acid standard but did not find any indication for the presence of organic sulfites or sulfates. In this thesis optimized instrument settings at the FT-ICR-MS and high DOC concentrations for the fragmentation experiments were successfully used to detect the sulfonic acid functional group in different marine DOM samples (Pohlabeln and Dittmar, 2015; Fig. 1.9).

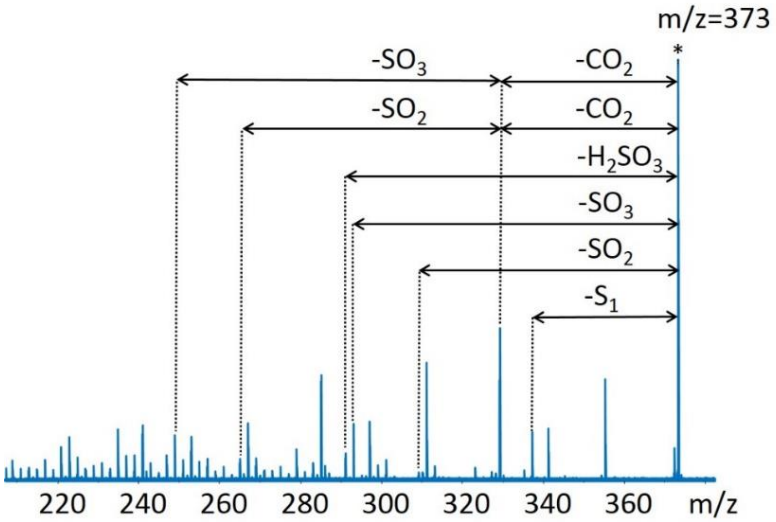

Figure 1.9: FT-ICR-MS CID fragmentation spectrum of a North Sea surface sample. The parent ions of nominal mass *m/z* = 373 were fragmented and their sulfur-containing neutral losses were assigned to the fragment mass peaks. Adapted from: Pohlabeln and Dittmar (2015). ©Elsevier

1.3.2. Alteration reactions

From FT-ICR-MS analysis alone it is not possible to get significant structural information. Tandem mass spectrometry enables first insights into functionalities but is still limited. Therefore, further functional group targeted analysis methods were necessary to obtain more information on DOS structural characteristics. The basic principle in this thesis to analyze sulfur-containing functional groups besides the tandem mass spectrometry is to apply functional group selective alteration reactions to DOM samples and to analyze the mass spectra before and after the treatment for differences which would indicate the presence of the targeted functional group. No such alteration reactions were available for DOM analysis and were thus developed within the scope of this thesis (Chapter 2). For this, published procedures for alteration reactions of sulfur-containing functional groups in the fields of medicine or chemistry were adapted to DOM analysis. The major challenge here was to find highly selective alteration reactions to cope with the high complexity of functionalities present in the DOM mixture so that a positive result in an alteration experiment could be unambiguously assigned to one sulfur-containing functional group. Some sulfur

functionalities could be excluded a priori from our investigation due to their instability such as thioaldehydes (Hirota et al., 1996), thioketones (Bruno et al., 1983), sulfenic acids (Aversa et al., 2007), and sulfinic acids (Oae, 1977). Others, namely thiophenes and sulfones, could not be included in the analysis due to their high unreactivity that hinders selective alteration reactions and besides that these functionalities do not show unambiguous, characteristic losses in fragmentation experiments. The final developed methods were a derivatization reaction with 2-bromo-1,4-naphthoquinone to test for thiols, an acidic hydrolysis to test for thioesters, sulfonic acids esters, and alkylsulfates, an oxidation for thioether analysis and a deoxygenation for sulfoxide analysis. The applicability of all developed alteration reactions was thoroughly tested with model compounds that bore the respective sulfur-functionality also within a DOM matrix. A schematic overview of the procedure of all alteration reactions can be found in Figure 1.10.

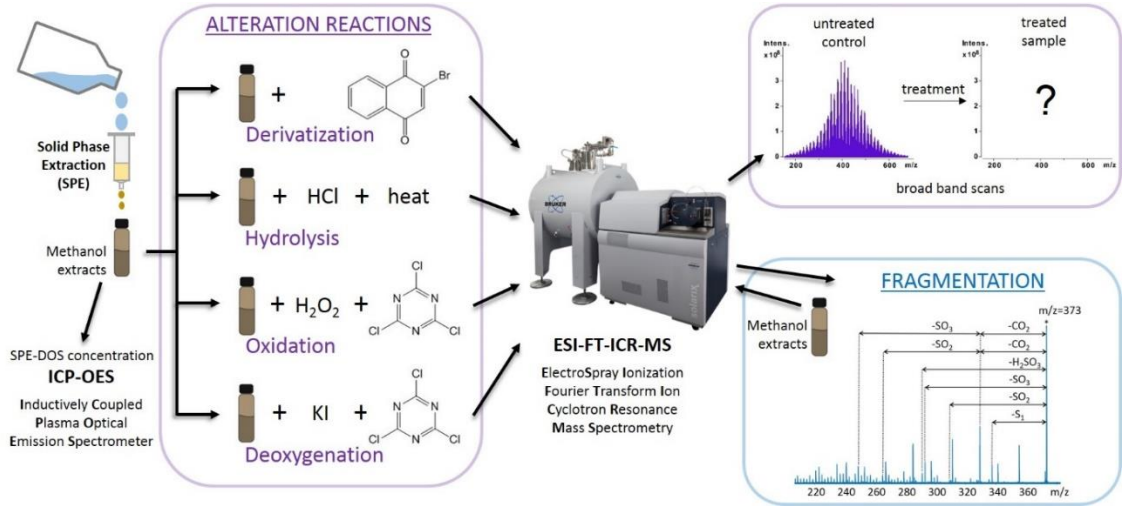

Figure 1.10: Schematic summary of the methodological approaches including analysis techniques.

1.3.3. DOS concentration analysis

The analysis of the DOS concentration in marine water samples is hindered by the presence of sulfate. Therefore, DOS water concentrations can only be determined with complex sulfate removal steps or by measuring the DOS content in the solid-phase extracts (SPE-DOS) and calculate the bulk DOS concentration using the extraction efficiency determined by bulk DOC and SPE-DOC concentration measurements of the sample. To verify that DOS compounds show similar extraction features as DOC in general, extraction efficiencies of sulfur-containing model compounds were tested in the framework of this thesis. The organic sulfur model compounds included thiols, sulfonic acids, sulfones,

thioethers, and thiophenes with molecular weights ranging from 154 to 342 Da. Stock solutions of the model compounds were prepared and extracted using the same procedure as for DOM samples (Dittmar et al., 2008). DOC analysis of the stock solution and the extract provided the extraction efficiencies. None of the extraction efficiencies was found to be below previously described DOC recoveries of around 40-65 % (Dittmar et al., 2008). Very little information is available on marine DOS concentrations. So far values have been published on the surface waters of the Central South Atlantic (0.33-0.88 µM), the Antarctic (0.44-1.17 µM), the Sargasso Sea (0.04-0.40 µM), the East Atlantic (0.34 µM), and the Atlantic sector of the Southern Ocean (0.19 µM) (Cutter et al., 2004; Lechtenfeld et al., 2011; Ksionzek et al., 2016). This thesis provides DOS concentration values for various marine environments namely the North Sea surface, North equatorial intermediate water, the Black Sea, and German Wadden Sea pore water.

1.4. Study sites

In this thesis DOS samples from contrasting environments with different physicochemical properties were analyzed to obtain representative information about DOS characteristics. The investigated locations were two open ocean samples, one from the surface water of the North Sea and the other one from the North Equatorial Pacific Intermediate Water (NEqPIW) near Hawaii. Furthermore, sulfidic pore waters from an intertidal coastal sediment near the German North Sea coast and from a subtropical salt marsh system at the coast of Georgia, USA, were investigated. Additionally, a depth profile of the Black Sea consisting of four different depths representing different redox states was analyzed.

1.4.1. North Sea and North Equatorial Intermediate Water – Open Ocean

The North Equatorial Intermediate Water (NEqPIW) was collected at the Natural Energy Laboratory of Hawaii Authority (NELHA) on the island of Hawaii, USA, which provides resources and facilities for energy and ocean-related research, education, and commercial activities (www.nelha.org). At this station water out of 674 m depth is continuously pumped up with a flow rate of 0.5 $m^3 s^{-1}$ (Green et al., 2014). The deep Pacific water is considered one of the oldest water masses on earth (Stuiver et al., 1983) and therefore dominated by refractory DOM (Hansell et al., 2013).

The North Sea sample was taken near the island of Helgoland in the German Bight. The Southern North Sea is characterized by the strong interaction with its bottom water that is at a depth of less than 100 m (near Helgoland less than 40 m). The dominant features in its dynamics are the tides which produce a turbulent exchange and also effect the currents at the bottom of the sea. At least three different types of fronts can be observed in the southern

North Sea: tidal mixing fronts, river plume fronts, and upwelling fronts. The sea surface salinity varies from less than 31 near the coast to 33-35 in the German Bight (Otto et al., 1990). The area near Helgoland is affected by fresh water input mainly by the rivers Elbe and Weser that transport terrestrial organic matter (Atlas and Bartha, 1987; Lucas et al., 2016). Furthermore, the coastal North Sea is supplied by the Wadden Sea which exhibits high primary productivity producing organic matter that is leached out by the tidal currents (Beck and Brumsack, 2012).

The DOM samples from the North Sea surface and NEqPIW were used in this study to develop and evaluate sulfur-containing functional group targeted analysis methods which obtained insight into the structural characteristics of DOS compounds in these systems. Furthermore, DOS concentrations were determined (Chapter 2).

1.4.2. Janssand – Intertidal sediment pore water

The Janssand belongs to the German Wadden Sea system which is one of the largest continuous tidal flat areas in the world. Although organisms living here have to tolerate extreme environmental gradients in salinity, light, oxygen availability, and temperature, it exhibits high rates of primary production. The high productivity is enhanced by nutrient supply due to rapid organic matter remineralization (Beck and Brumsack, 2012). In permeable sands organic matter is aerobically degraded whereas in the deeper parts of the sediments anaerobic microbial processes occur (Røy et al., 2008). Tidal pumping induces pore water exchange with North Sea water leading to mutual influencing (Beck and Brumsack, 2012).

The Janssand is a creek bank located at the back barrier basin of Spiekeroog Island at the northwestern coast of Germany (Fig. 1.11). At low tide it has a surface area of ca. 3 km² and the flat central area is ca. 1.9 m elevated above the low water level (Røy et al., 2008). During high tide it is covered by 1-2 m seawater and exposed for up to six hours during low tide. The Janssand site is dominated by fine-grained sands and consists mainly of marine deposits in the upper three meters with more muddy deposits below (Seidel et al., 2014). The upper sandy sediments are mostly advection-dominated, whereas the deeper muddy sediments are diffusion-dominated (Riedel et al., 2011). The pore water turns anoxic within 10 cm depth and at the low water line sulfidic below 10 cm depth with high sulfide concentrations of up to mM values (Jansen et al., 2009). The pore water can escape from the sediments by skin (rapid, pressure gradient induced flow through upper most sediment layer) and body circulation (slow, hydraulic gradient induced flow through deeper sediment layer). These flows supply the deeper permeable sediments with nutrients and organic matter (Beck et al., 2009; Billerbeck et al., 2006). The time it takes a water load to flow from the surface through the sediment and out of the seep varies with the depth of penetration from 4 to up to 285 years (Røy et al., 2008).

In this thesis Janssand pore water samples from different seasons (March, May, July, and November, Fig. 1.11) and from those depths and sites that showed the largest number of sulfur-containing molecular formulae in a previous study (Seidel et al., 2014) were pooled and analyzed for their DOS concentration and structural characteristics (Chapter 3 and 4).

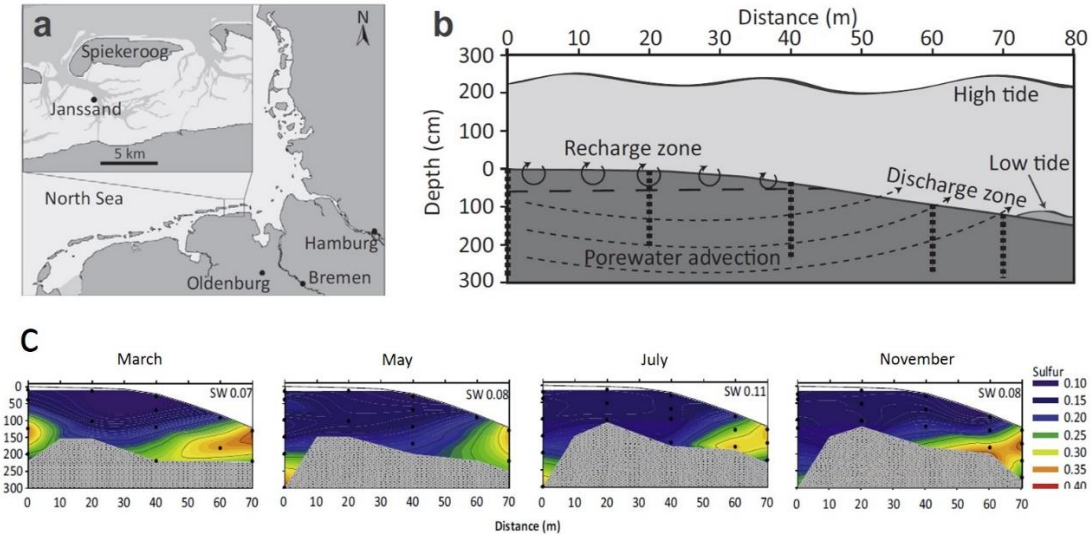

Figure 1.11: (a) Map of the Janssand near the island of Spiekeroog. (b) Profile of the Janssand showing water levels at high and low tide and water streams. (c) Weighted averages of numbers of sulfur atoms of all identified formulae in the pore water and seawater DOM along the sampled transect at different seasons. Adapted from: Seidel et al. (2014). ©Elsevier

1.4.3. Georgia salt marsh – Intertidal sediment pore water

Great parts of the North American Atlantic coast are bordered by salt marshes that are dominated by *spartina* spp. grasses. These marshes are extremely productive environments. Variations in tidal regime, temperature, topography, hydrology, vegetation, infauna, and microbiota influence geochemical cycling and turn salt marshes into complex systems (Hines et al., 1989; Kostka and Luther, 1995; Bull and Taillefert, 2001). Their sediments are anoxic near the surface and organic matter oxidation occurs primarily via dissimilatory sulfate reduction (Hines et al., 1989; Caffrey, 2004; Hyun et al., 2007). Tidal forces induce pore water exchange with the overlying water (Taillefert et al., 2007).

The Saltmarsh Ecosystem Research Facility (SERF) on the campus of the Skidaway Institute of Oceanography (SkIO) on Skidaway Island, Georgia, USA, is a 213 m long boardwalk providing direct access to the saltmarsh. The surrounding environments around SERF range from an inland saltmarsh meadow to a tidal creek, and are covered with tall *Spartina alterniflora* with some free flats in between (Taillefert et al., 2007). The sediments turn anoxic

within few millimeters depth with highly sulfidic conditions starting at only few centimeters depth (Bull and Taillefert 2001). During low tide, the sediment at the sampling location is exposed to air for 5 to 7 h and covered by up to 60 cm of water during high tide (Taillefert et al., 2007).

In this thesis, the pore water samples from the SERF salt marsh were affected by solar irradiation in a laboratory experiment. DOS concentrations and molecular characteristics were analyzed during the experiment. Additionally to the pore water, water of the nearby Skidaway River and from one of its creeks was analyzed as well as ocean surface water ~50 km and ~100 km off Georgia coast (Chapter 5).

1.4.4. Black Sea – Depth profile

The Black Sea is a sea in Southeastern Europe that is surrounded by Europe, Anatolia, and the Caucasus. It has a surface area of 4.2×10^5 km², a maximum depth of 2200 m, and a volume of 5.3×10^5 km³, representing the largest land-locked marine basin in the world (Özsoy and Ünlüata, 1997). Furthermore, the Black Sea is the world's largest anoxic basin. It has a strong halocline formed between deep, salty water from the Mediterranean Sea which enters through the Bosporus and less salty surface waters which are diluted by freshwater river runoff from mainly the Danube, the Dnepr, and the Dnestr forming a permanently stratified water column. At the Bosporus a two-layer flow is observed. The surface outflow is approximately 604 km³ a^{-1} and has a salinity of 17.5 which is lower than the Black Sea surface (~18.5). The Mediterranean inflow is approximately 304 km³ a^{-1} and has a salinity of 35-38 which is higher than the Black Sea deep water (~22.3) (Oguz et al., 2004). These differences imply a limited vertical mixture of water masses within the Black Sea (Albert et al., 1995; Murray et al., 2007). Seasonal and interannual variability extends to depths of around 500 m. Below the halocline, the intrusion of the Mediterranean water drives the interior mixing (Özsoy and Ünlüata, 1997). This restricted mixing provides a permanent chemical stratification showing an oxic layer (<100 m depth), a suboxic/anoxic layer (~100-120 m), and a sulfidic layer (>120 m) (Luther et al., 1991; Mopper and Kieber, 1991; Ducklow et al., 2007). It is estimated that the residence time for the deep water layer is around 600 years and around 5 years for the suboxic zone (Lee et al., 2002). Oxygen is not able to penetrate below ca. 100 m due to the density stratification. This provides ideal conditions for a variety of bacteria that are not usually found in the marine environment (Coble et al., 1991). This variety of microbial activity leads to different turnover rates of organic matter. While rates are faster in the surface area, they are 1-3 orders of magnitude lower in the anoxic layer (Mopper and Kieber, 1991). The DOC concentrations in the deep Black Sea are approximately 2.5 times higher than found in the global ocean due to the high terrestrial input leading to the estimate that more than half of the DOC in the deep Black Sea is terrigenous (Margolin et al., 2016).

Removal processes of the terrigenous DOC are mainly photooxidation, particle flocculation, co-precipitation, and heterotrophic respiration (Margolin et al., 2016).

Four different depths (oxic with light influence: 2 m depth; oxic: 20 m depth; suboxic/anoxic: 100 and 120 m depth, pooled; sulfidic: 350 and 641 m depth, pooled) of the Black Sea Bosporus area were analyzed for their DOS concentration and structural characteristics (Chapter 4).

1.5. Objectives

The aim of this thesis was to gain more information on the sources, pathways and composition of DOS using natural DOM samples from various environments. The following research questions were approached in this regard:

1) Is abiotic sulfurization of organic matter in sulfidic marine environments a possible source for the DOS present in the open oceans?
2) What structural characteristics do the DOS compounds have?
3) Are there spatial differences in the concentration and/or structural composition of DOS compounds?
4) Is the oxidation state of the DOS functionalities a function of the redox conditions of the environment they are in?
5) Is DOS affected by photochemically induced alteration and/or degradation reactions?

Chapter 2 describes the in this thesis developed alteration reactions to analyze DOM samples for specific sulfur-containing functional groups including the fragmentation studies and shows the findings for a North Sea surface and a Pacific deep water DOM sample.

Chapter 3 aims to answer the question of the source of the DOS in the oceans. In an artificial sulfurization experiment two different DOM samples were mixed with inorganic reduced sulfur species under anoxic conditions. The resulting DOS products from the experiment were compared to a natural DOM sample from a sulfidic environment obtaining striking similarities.

Chapter 4 illustrates the analyses of DOM samples from different locations. DOS concentrations and structural information based on the methods described in Chapter 2 were compared among samples and put into relation to their environmental conditions.

Chapter 5 presents a study on photochemical degradation as a potential sink for DOS. It aims to show the alteration and degradation processes in pore water DOS induced by solar irradiation on its pathway from production in sulfidic environments towards the open ocean. Various samples from a transect representing the natural route of the pore water DOS were analyzed in comparison to the artificially conducted processes.

1.6. List of Manuscripts and Contributions to Publications

This thesis includes complete versions of four manuscripts. Chapter 2 includes a published manuscript, Chapter 3 includes a manuscript that is under review, and Chapters 4 and 5 include manuscripts close to submission. All manuscripts are presented unchanged in content but with adjusted formatting according to the style of this thesis.

Chapter 2 – Novel insights into the molecular structure of non-volatile marine dissolved organic sulfur
(published manuscript)

Anika M. Pohlabeln and Thorsten Dittmar

This study was planned by A.M.P. and T.D.. Method development and evaluation and conduction of experiments on natural samples was performed by A.M.P.. Mass spectrometric analyses and data interpretation were conducted by A.M.P., significantly advised by T.D.. The manuscript was written by A.M.P. with input from T.D..

The manuscript is published in Marine Chemistry, Vol. 168, pages 86-94,
doi: 10.1016/j.marchem. 2014.10.018

Chapter 3 – Evidence for abiotic sulfurization of marine dissolved organic matter in sulfidic environments
(under review)

Anika M. Pohlabeln, Gonzalo V. Gomez-Saez, Beatriz E. Noriega-Ortega, and Thorsten Dittmar

This study was initiated and designed by A.M.P. and T.D.. Sample preparation and all experiments including mesocosm culture, sulfurization, and functional group targeted alteration reactions were carried out by A.M.P.. Mass spectrometric analyses of the obtained samples were done by A.M.P.. G.V.G.-S. performed statistical analysis of sulfurization pathways. Bacterial analysis was conducted by B.E.N.O. and A.M.P.. All authors contributed to the data interpretation. The manuscript was written by A.M.P. with significant input from G.V.G.-S. and T.D..

The manuscript is under review at Proceedings of the National Academy of Sciences of the United States of America (PNAS).

Chapter 4 – Molecular clues for a pathway of dissolved organic sulfur produced in sulfidic sediments to the open ocean (manuscript in preparation)

Anika M. Pohlabeln, Jutta Niggemann, and Thorsten Dittmar

This study was planned by A.M.P., T.D., and J.N.. Functional group targeted alteration reactions were carried out by A.M.P.. Mass spectrometric analyses were performed by A.M.P.. Data interpretation was done by A.M.P. and T.D. with input by J.N.. The manuscript was written by A.M.P. with input from J.N. and T.D..

The manuscript is intended for submission to Marine Chemistry.

Chapter 5 – Photochemical alteration of dissolved organic sulfur from sulfidic pore water (manuscript in preparation)

Gonzalo V. Gomez-Saez, Anika M. Pohlabeln, Aron Stubbins, and Thorsten Dittmar

This study was planned by A.M.P., T.D., and A.S.. Sampling of all DOM samples was done by A.M.P.. Laboratory photodegradation experiments and optical analyses were carried out by A.M.P.. Analysis of inorganic compounds was conducted by G.V.G.-S.. Mass spectrometric analyses were done by G.V.G.-S. and A.M.P.. All authors contributed to the data interpretation. The manuscript was written by G.V.G.-S. with input from A.M.P., A.S. and T.D..

The manuscript is intended for submission to Environmental Science and Technology.

2. Manuscript I

Novel insights into the molecular structure of non-volatile marine dissolved organic sulfur

Anika M. Pohlabeln and Thorsten Dittmar

published in Marine Chemistry (2015), Vol. 168, pages 86-94
doi: 10.1016/j.marchem.2014.10.018

Research Group for Marine Geochemistry (ICBM-MPI Bridging Group), Carl von Ossietzky University Oldenburg, Institute for Chemistry and Biology of the Marine Environment (ICBM), Carl-von-Ossietzky Straße 9-11, D-26129 Oldenburg, Germany

2.1. Highlights

- Non-volatile marine dissolved organic sulfur (DOS) was molecularly characterized
- DOS does not contain reduced (e.g. thiols) or hydrolysable (e.g. thioesters) groups
- DOS contains sulfonic acids, and possibly thiophenes
- DOS is highly resistant against oxidation and hydrolyses
- The molecular structure of DOS seems universal for the oxic water column

2.2. Abstract

Abiotic sulfurization likely contributes to the preservation of organic matter in fossil deposits. It is unknown whether this mechanism also stabilizes dissolved organic matter (DOM) in the oceanic water column. Knowledge of the structure of the sulfur-containing compounds in DOM could help to understand the observed stability of at least a fraction of refractory DOM in the ocean. Structural analysis of dissolved organic sulfur (DOS) is complicated by the great molecular diversity and the low concentration of each compound. Two contrasting marine DOM samples, one deep-sea sample from the North Pacific Ocean and one surface sample from the marginal North Sea (Germany), were examined in this study. Selective alteration experiments targeting different sulfur-containing functional groups were applied prior to Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS). All treatments were also carried out with corresponding model compounds, in order to test for the applicability of the chosen reactions and for matrix effects, and to confirm the types of changes caused by the treatments. Alteration experiments comprised harsh hydrolysis, selective derivatization of thiols, oxidation, and deoxygenation. None of these treatments induced detectable changes to DOS, indicating the absence of thioesters, sulfonic acid esters, alkylsulfates, thiols, non-aromatic thioethers, and sulfoxides in solid-phase-extractable DOM (SPE-DOM). Collision-induced fragmentation of isolated nominal masses in the FT-ICR-MS confirmed these findings and revealed the presence of sulfonic acids in both samples. Additionally, the presence of thiophenes and possibly also sulfones is likely, while the presence of any other form of sulfur in SPE-DOM can be ruled out based on our results. In conclusion, only unreactive (fully oxidized and hydrolyzed) sulfur compounds resist in the water column as part of refractory DOM. There was no detectable difference in molecular structure between DOS of the marginal North Sea and the deep North Pacific, indicating that the chemical inertness and the presence of sulfonic acids are global features of DOS residing in the oxic water column.

2.3. Introduction

Dissolved Organic Matter (DOM) in aquatic environments is a complex mixture of at least many thousands of organic compounds (Dittmar, 2015). It is ubiquitous in seawater and is one of the largest reservoirs of organic carbon on Earth (Hedges, 1992). Its concentration and distribution in the global oceans varies in the water column as the concentration decreases with increasing water depth with a low and almost uniform value in the deep water (Barber, 1968; Hansell et al., 2009). DOM is often operationally divided into categories of different turnover-rates (Hansell et al., 2009). Labile DOM is the smallest fraction and has a rapid turnover time of minutes to days (Hansell et al., 2009) and semi-labile DOM has a longer turnover time of months to a year (Kirchmann et al., 1993). These two fractions can be found mainly in the surface ocean due to primary production and microbial turnover of organic matter (Barber, 1968). The third and largest pool of DOM turns over in about 16,000 years (Hansell, 2013) and is thus named refractory DOM (Williams and Druffel, 1987). It can be found in the whole water column and is the only fraction that occurs in the deep sea (Ogawa and Tanoue, 2003). The reasons behind the stability of DOM are unknown (Dittmar, 2015).

Abiotic sulfurization can contribute to the stabilization of organic matter in fossil deposits (Sinninghe Damsté et al., 1998; Kok et al., 2000; Amrani et al., 2007; Bushnev and Burdel´naya, 2008). It is unknown whether similar mechanisms contribute to the stability of DOM in the ocean. Knowledge of the structure of dissolved organic sulfur (DOS) could thus help to understand the observed stability of at least a fraction of DOM in the ocean. DOS, as part of DOM, potentially plays a significant role in the marine environment. Some sulfur compounds, in particular thiols, determine the mobility of essential trace elements (Dupont et al., 2006) and hazardous elements such as mercury in aquatic systems (Skyllberg et al., 2003; Ravichandran, 2004). Cutter et al. (2004) demonstrated photoproduction of carbonyl sulfide from DOS. Carbonyl sulfide is responsible for maintaining the stratospheric sulfate aerosol layer, and it can decay into dissolved sulfide in the ocean, affecting the cycling of many trace metals. Also other volatile sulfur compounds of climate relevance may originate from DOS. Thus, the marine cycling of DOS has both atmospheric and oceanic consequences (Cutter et al., 2004, and references therein). DOS could, at least partially, be a biological product in the ocean. For instance dimethylsulphoniopropionate (DMSP) is an abundant algal product that quickly decays in the ocean by enzymatic cleavage into dimethyl sulfide (DMS). These two biogenic organic sulfur compounds have received considerable attention in the scientific literature, because DMS is volatile and quickly oxidizes into sulfate in the atmosphere forming there condensation nuclei for water droplets (e.g., Simó and Pedros-Alio, 1999; Andreae et al., 2003). Also peptides and other common biomolecules contain sulfur (Dupont et al., 2006), and as for the bulk of DOM some of these compounds may be preserved for unknown reasons in the ocean (Dittmar, 2015). On the other hand, the molecular resemblance of DOS with bulk DOM, on a molecular formulae level, has been interpreted as evidence for the abiotic and

nonspecific incorporation of sulfur into DOM in a freshwater wetland (Sleighter et al., 2014). In line with this hypothesis, there is evidence for the formation of DOS in coastal marine sediments, where bacterial dissimilatory sulfate reduction causes high concentrations of dissolved sulfide species in pore waters (Seidel et al., 2014).

Due to the great complexity of DOM, the low concentration of each individual compound, and the multiplicity of structural isomers for each molecular formula, structural analysis of DOM in general is a major challenge. The molecular structure of only a minor fraction of DOM (<5%) is known (Dittmar and Stubbins, 2014). The knowledge on dissolved organic sulfur (DOS) in the water column is even scarcer (Lechtenfeld et al., 2011; Gonsior et al., 2011; Hertkorn et al., 2013), and the molecular structure of non-volatile DOS is unknown. More is known about organic sulfur compounds in marine sediments. Sulfate-reducing bacteria in sediments provide inorganic reduced sulfur species which are assumed to abiotically react with organic matter to give organic sulfur compounds (Sinninghe Damsté et al., 1989; Vairavamurthy et al., 1995 and references therein; Schouten et al., 1994; Schneckenburger et al., 1998; Schmidt et al., 2009). Organic sulfur in marine sediments is mainly composed of strongly reduced molecules like sulfides, but also strongly oxidized compounds like sulfonates occur in sediments (Eglinton et al., 1994; Vairavamurthy et al., 1994; Zhu et al., 2014).

Objective of this study was to obtain fundamental molecular information on marine DOS, in order to lay a molecular foundation for assessing the source and fate of DOS in marine systems. For this purpose we structurally characterized DOS from two sharply contrasting marine systems, the deep North Pacific off Big Island (Hawaii) and the open North Sea (Germany). Ultrahigh-resolution Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) has become one of the most powerful techniques for the molecular characterization of DOM (e.g., Koch et al., 2005; Dittmar and Paeng, 2009; Hertkorn et al., 2013; Osterholz et al., 2014). Through this technique, molecular formulae of intact DOS compounds can be readily obtained. To obtain structural information beyond the molecular formulae level we performed selective chemical reactions prior to FT-ICR-MS analysis to target specific functional groups that potentially occur in DOS. Some functional groups could be excluded a priori from the study due to their instability in aqueous solution that makes them unlikely to occur in the ocean, e.g. thioaldehydes (Okazaki, 1995), thioketones (Okazaki, 1995) or sulfinic acids (Oae, 1977). For the more stable sulfur-containing functional groups we hypothesized that they are possibly occurring in the ocean. The presence of thiols was analyzed by usage of the derivatization reagent 2-bromo-1,4-naphthoquinone that selectively adds to thiol groups (Lobo et al., 2007). To test for non-aromatic thioethers an oxidation reaction was applied and for sulfoxides a deoxygenation reaction (Bahrami et al., 2011). Hydrolysis was used to test for thioesters (Bracher et al., 2011) as well as sulfonic acid esters (Hemond and Fechner, 2000) and alkylsulfates (Hu et al., 2011). Collision fragmentation experiments were performed within the FT-ICR-MS to collect independent evidence for some

of the above functional groups and to search for sulfonic acid for which no selective derivatization method exists.

2.4. Material and Methods

2.4.1. Sampling, sample preparation and general analyses

North Pacific Deep Water (NPDW) was taken at the National Energy Laboratory of Hawaii Authority in Kailua-Kona, Hawaii in 2009 (Green et al., 2014). The open North Sea (Germany) was sampled at the sea surface near the island of Helgoland in winter 2009, before the occurrence of spring blooms and when DOM was not directly affected by fresh input. Dissolved organic carbon (DOC) concentrations of the samples were determined by high-temperature catalytic oxidation on a Shimadzu TOC-VCPH analyzer. Prior to further analysis, the samples were desalted via solid-phase extraction (SPE) after Dittmar et al. (2008). Briefly, the NPDW sample was filtered through a 0.2 µm polyethersulfone filter (Infiltec, Causa-PES, Germany) and the North Sea sample through a 0.7 µm precombusted glass fiber filter (Whatman GF/F, USA). The filtered samples were acidified with HCl (p.a., Roth Germany) to pH=2, and passed through a modified styrene divinyl benzene polymer resin (Bond Elut-PPL, Varian Inc., USA). Prior to use, the PPL resin was soaked overnight in methanol (absolute ULC/MS 99.98 %, Biosolve BV, Netherlands), and afterward sequentially rinsed with methanol and 0.01 M HCl. After loading the sample on the resin, it was desalted with 0.01 M HCl, dried under a stream of purified compressed air (NPDW) or nitrogen (North Sea). The samples were then eluted off the resin with methanol. Solid-phase extractable DOC (SPE-DOC) was determined after drying a small aliquot (100 µL) of the methanol extract (12 hours at 80 °C) and redissolving it in ultrapure water (10 mL). More than 60% of DOC was recovered from the two seawater samples through SPE. Sulfur concentrations were measured in SPE-DOM on an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, iCAP 6000, Thermo, Bremen, Germany).

FT-ICR-MS measurements were performed with a solariX Fourier-transform ion cyclotron resonance mass spectrometer with a 15 Tesla magnet system (Bruker Daltronik GmbH, Bremen, Germany). A Bruker Daltonik Apollo II atmospheric pressure electrospray ionization unit (ESI) was used as the external ion source in negative ionization mode, which is particularly well suited to ionize the targeted organic sulfur compounds (Gross, 2011). All samples were analyzed in a 1:1 volumetric mixture of methanol and ultrapure water. The concentration was adjusted to 20 mg carbon/L for DOM samples and 50 mg/L for model compounds. The samples were directly infused into the ESI source at a flow rate of 120 µL/h. For fragmentation experiments, the model compound concentration was 50 mg/L and the DOC concentration was 150 mg/L with a flow rate of 360 µL/h. Reproducibility was monitored by analyzing NPDW DOM twice a day, every morning and evening. 500 transient scans in broadband mode were accumulated for each run, covering the mass range of 150-2000 Da.

Only molecular masses detected with a signal-to-noise ratio (S/N) greater than five and reproducibly in at least two procedural replicates were considered. All detected ions were singly-charged. After internal calibration, the mass error was <100 ppb. At this high mass accuracy, molecular formulae were unambiguously assigned to all compounds containing the elements C, H, O and S. Formula assignment for each detected mass was done after Rossel et al. (2013). For analysis, the detected masses were normalized to the sum of all mass intensities of the corresponding sample.

2.4.2. Thiol derivatization

To detect thiol groups in the DOM samples, we added 2-bromo-1,4-naphthoquinone (NQBr, 98 %, Sigma-Aldrich, Germany) which selectively adds to thiol groups (Lobo et al., 2007) (Fig. 2.1). Three steps were conducted: Firstly, model compounds were analyzed to prove the method. For this, the model compounds were dissolved in acetonitrile (99.9 % HPLC gradient grade, Roth, Germany) and the derivatization reagent NQBr was added in a molar excess of 1.1:1 over the thiol compound. The model thiol compounds were: 4-phenylthiazole-2-thiol (PTT, 90 %, Sigma-Aldrich, Germany), thiosalicylic acid (TA, 97 %, Sigma-Aldrich, Germany), 2-mercapto-4-methyl-5-thiazoleacetic acid (MTA, 98 %, Sigma-Aldrich, Germany), and mercaptosuccinic acid (MSA) (≥ 99.0 % HPLC grade, Sigma-Aldrich, Germany). Salicylic acid (SA, ≥ 99.0 % ACS grade, Sigma-Aldrich, Germany) was used to test the selectivity of the reagent. Secondly, the method was applied to the two natural DOM samples. For this, the DOM samples were dissolved in a NQBr-acetonitrile solution (100 mg/L). Lastly, the setup was checked for matrix effects. For this, three model compounds (TA, MTA, and MSA) were added to the DOM samples prior to the addition of the reagent. All reactions were performed in duplicates. After 30 minutes of reaction time at room temperature, the reaction mixtures were diluted 1:1 with ultrapure water and analyzed via FT-ICR-MS. As procedural controls, all samples were also analyzed without addition of the derivatization reagent, but including all other procedural steps of the derivatization procedure.

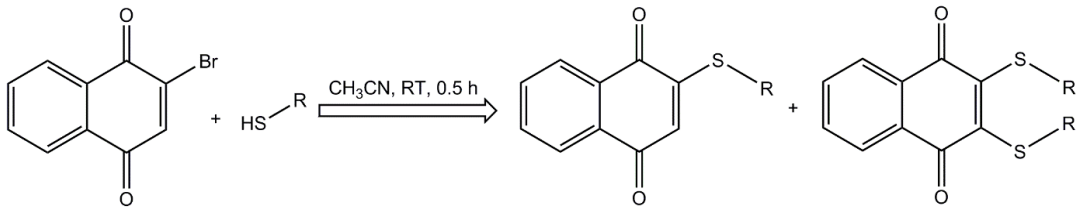

Figure 2.1: Reaction pathway of derivatization reagent 2-bromo-1,4-naphthoquinone and thiol to the corresponding mono-reacted and di-reacted product. Reaction efficiency was reported to be 92-96 % (Lobo et al., 2007).

2.4.3. Hydrolysis

To analyze the two DOM samples for thioesters/sulfonic acid esters/alkylsulfates, the gently dried samples (50°C, 16 hours) were dissolved in hydrochloric acid (25 % p.a., Roth, Germany) at different concentrations (0.1, 1, and 6 M) (Fig. 2.2). The solutions were sparged in glass ampoules (1 mL) with argon gas via a PTFE-tube to prevent oxidation processes. Immediately after that, the ampules were fire-sealed and heated in a high pressure reaction vessel to 110 °C for 24 hours. After hydrolysis, the pH of the samples was adjusted to pH=2 with a NaOH solution (32 %p.a., Roth, Germany). The reaction mixtures were extracted via PPL-SPE as described above to remove the salt generated from pH adjustment prior to FT-ICR-MS analysis. As procedural controls, all samples were exposed to the same procedure without addition of acid, and, in addition, without heating. All reactions were performed in duplicates.

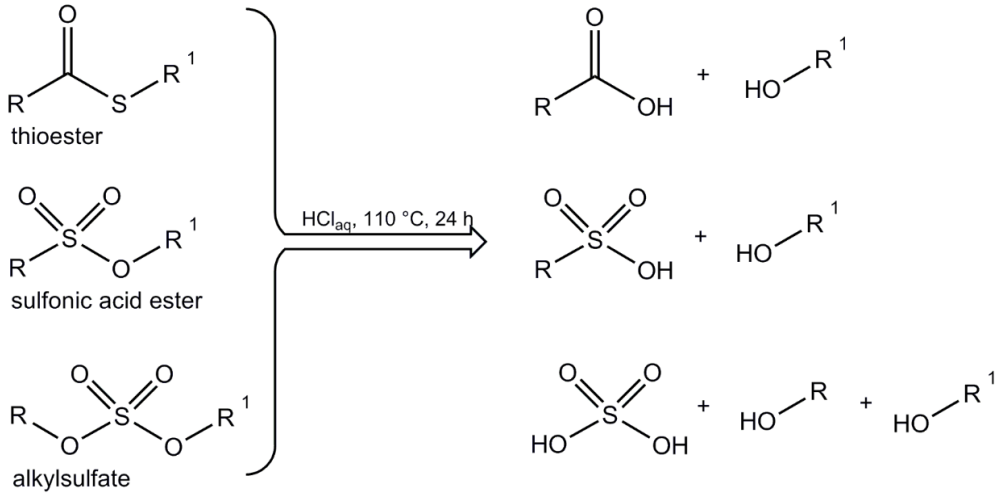

Figure 2.2: Reaction pathways of thioesters, sulfonic acid esters, and alkylsulfates in acidic hydrolysis.

2.4.4. Oxidation and deoxygenation

For the detection of non-aromatic thioethers an oxidation experiment was performed, and sulfoxides were detected with a deoxygenation experiment that does not affect sulfones (Bahrami et al., 2011) (Fig. 2.3). Again, three steps were performed: Firstly, the method was tested with the model compounds 3,3'-thiodipropionic acid (TPA, 99 %, Acros Organics, Belgium), 4,4'-thiodiphenol (TDP, 99 %, Acros Organics, Belgium), DL-methionine sulfoxide (MSO, 99 %, Acros Organics, Belgium), and Ricobendazole (RB, ≥98 % HPLC, Sigma-Aldrich, Germany). TPA and TDP were used to verify the oxidation, MSO and RB for deoxygenation. For this, the model compounds were dissolved in acetonitrile (HPLC, isocratic grade, VWR International, France) and a solution of cyanuric chloride (99 %, Acros Organics, Belgium) in acetonitrile was added. For oxidation, an aqueous hydrogen peroxide solution (30 %,

Rotipuran p.a., stabilized, Roth, Germany) was added (molar ratio thioether/cyanuric chloride/H_2O_2 = 1:1:2). For deoxygenation, an acetonitrile solution of potassium iodide (≥99 %, Roth, Germany) was added (molar ratio: sulfoxide/cyanuric chloride/KI = 1:1:2.5). The reaction mixtures were allowed to stand at room temperature for two hours, then the acetonitrile was gently evaporated at 40°C and the residue was dissolved in ultrapure water and extracted via PPL-SPE to remove the salt. Secondly, the method was applied to both DOM samples. For this, the DOM samples were dissolved in acetonitrile and cyanuric chloride and H_2O_2 or KI, respectively, were added. The reaction mixtures were treated like described for the model compounds. Lastly, the setup was checked for matrix effects. For this, the model compounds were added to the DOM samples prior to any reagent addition and then treated like described for the model compounds. All samples were performed in duplicates. Additionally, procedural controls (without oxidation or deoxygenation reagents) for both DOM samples and the model compounds were conducted.

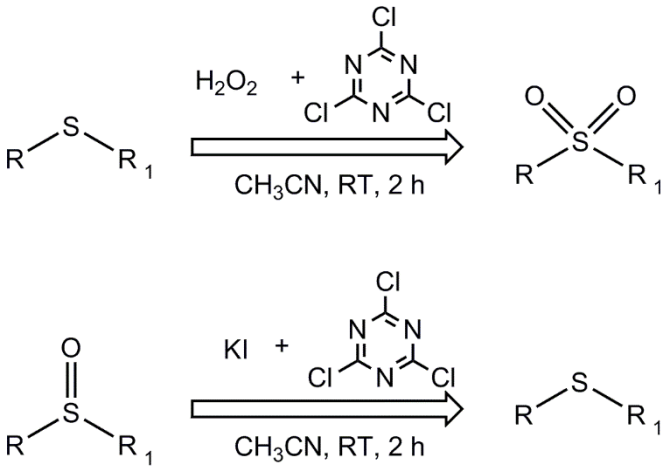

Figure 2.3: Reaction pathway of oxidation of thioethers with hydrogen peroxide and deoxygenation of sulfoxides with potassium iodide under the presence of cyanuric chloride. Reaction efficiency was reported to be 92-98 % for oxidation and 93-99 % for deoxygenation (Bahrami et al., 2011).

2.4.5. Collision-induced fragmentation

For fragmentation experiments, different nominal masses consisting of three CH_2-homologous series (Stenson et al., 2002) were analyzed for both DOM samples representatively for the entire sample (Tab. 2.1). For each nominal mass 2-3 sulfur-containing exact masses were analyzed. These sulfur compounds differed from the mass of CH_2 (14 Da) between the nominal masses within a series (e.g. 373 to 387). For each nominal mass, at first an isolation spectrum was measured to ensure that only compounds of the targeted nominal mass were fragmented. The Q_1 mass of the quadrupole unit of the FT-ICR-MS was chosen at

the *m/z* value of the analyzed nominal mass with a mass window of 0.7 amu. Next, all isolated ions were fragmented in the hexapole collision cell via collision with neutral argon gas. The collision energy was optimized to obtain a maximum daughter to parent ion ratio which was achieved for all samples and model compounds at a fragmentation voltage of 15 V. As model compounds, two sulfonic acid model compounds, one aromatic sulfonic acid salt (alizarin red S, ARS, pure, Fisher Scientific, UK) and one aliphatic sulfonic acid (camphorsulfonic acid, CSA, > 98 %, Fluka, Switzerland) were used. For the model compounds, the fragmentation experiments were carried out in duplicates.

Table 2.1: Observed neutral losses from isolated masses from the North Sea DOM sample (NPDW DOM results in parentheses). Only sulfur containing molecular formulae were considered. The ✓ symbolizes a found fragment and the X no found fragment.

nominal mass	considered molecular formulae	SO_3	CO_2+SO_2	CO_2+SO_3	H_2SO_3	SO_2	S
m/z 373	$C_{15}H_{17}O_9S$ $C_{16}H_{21}O_8S$	✓(✓)	✓(✓)	✓(✓)	✓(✓)	✓(✓)	✓(✓)
m/z 387	$C_{16}H_{19}O_9S$ $C_{17}H_{23}O_8S$	✓(✓)	✓(✓)	✓(✓)	✓(✓)	✓(✓)	✓(✓)
m/z 401	$C_{16}H_{17}O_{10}S$ $C_{17}H_{21}O_9S$ $C_{18}H_{25}O_8S$	✓(✓)	✓(✓)	✓(✓)	✓(✓)	✓(✓)	✓(✓)
m/z 415	$C_{17}H_{19}O_{10}S$ $C_{18}H_{23}O_9S$ $C_{19}H_{27}O_8S$	✓(✓)	✓(✓)	✓(✓)	✓(✓)	✓(✓)	✓(✓)
m/z 375	$C_{14}H_{15}O_{10}S$ $C_{15}H_{19}O_9S$ $C_{16}H_{23}O_8S$	✓(✓)	✓(✓)	✓(✓)	✓(✓)	✓(X)	✓(✓)
m/z 389	$C_{15}H_{17}O_{10}S$ $C_{16}H_{21}O_9S$ $C_{17}H_{25}O_8S$	✓(✓)	✓(✓)	✓(✓)	✓(✓)	X(✓)	✓(✓)
m/z 403	$C_{16}H_{19}O_{10}S$ $C_{17}H_{23}O_9S$	✓(✓)	✓(✓)	✓(✓)	✓(✓)	✓(✓)	✓(✓)
m/z 417	$C_{16}H_{17}O_{11}S$ $C_{17}H_{21}O_{10}S$ $C_{18}H_{25}O_9S$	✓(✓)	✓(✓)	✓(✓)	✓(✓)	X(✓)	✓(✓)
m/z 377	$C_{14}H_{17}O_{10}S$ $C_{15}H_{21}O_9S$ $C_{16}H_{25}O_8S$	✓(✓)	X(X)	✓(✓)	✓(✓)	X(X)	X(X)
m/z 391	$C_{15}H_{19}O_{10}S$ $C_{16}H_{23}O_9S$	✓(✓)	X(X)	✓(✓)	✓(✓)	X(X)	X(X)
m/z 405	$C_{15}H_{17}O_{11}S$ $C_{16}H_{21}O_{10}S$ $C_{17}H_{25}O_9S$	✓(✓)	✓(X)	✓(✓)	✓(✓)	X(X)	✓(✓)
m/z 419	$C_{16}H_{19}O_{11}S$ $C_{17}H_{23}O_{10}S$	✓(✓)	X(✓)	✓(✓)	✓(✓)	X(X)	X(✓)

2.4.6. Statistical analysis of FT-ICR-MS data

To test whether discrepancies between the spectra of the derivatization and hydrolysis experiments were actually based on the reaction processes or just due to measurement variations a variance test was performed. For the variance test the normalized intensities of all *m/z*-ratios after any kind of reaction were subtracted from the normalized intensities prior to any kind of reaction (i.e., the respective procedural controls). As each sample was analyzed in duplicates a multiplicity of mathematical differences was obtained by doing all possible subtractions. Only those *m/z*-ratios were further considered that showed a consistent trend in the differences prior to after reaction, i.e., the differences of replicate analyses were both either negative or positive. The same trend analysis was done for the NPDW reference sample that was run twice a day. Here, the evening-measurement was treated as the sample after any reaction and the morning-measurement as the sample prior to any reaction. The intensities of the *m/z*-values that showed a consistent trend were then normalized by dividing the noise (the lowest measured intensity of all samples prior to application of the S/N filter to the data set) by the intensity of the respective *m/z* value. The respective values for the NPDW reference sample represented the instrument variations. This second normalization was necessary to achieve comparability between the sample and the reference values. Only differences between treated and untreated sample that were bigger than the differences caused by the instrument were further considered.

Compared to the derivatization and hydrolysis, the oxidation and deoxygenation experiments do not lead to a clear separation of reaction products. While there is a characteristic molecule addition in the thiol-derivatization and molecule cleavage in hydrolysis, there is only a small (one or two oxygen atoms) increase or decrease in oxygen content for a sulfur-containing molecule in the oxidation and deoxygenation experiments. To determine and visualize even slight differences between the spectra of the oxidation and deoxygenation experiments, the mass spectra were interpreted via multivariate statistical methods. The Bray Curtis dissimilarity between spectra was first assessed. To identify those compounds that mainly contributed to the variation displayed in the Bray Curtis Dissimilarity, a principal component analysis (PCA) was conducted. The compounds with highest absolute correlation values (“loadings”) with the synthetic variables (principal components) contributed most to the Bray Curtis dissimilarity (Ramette, 2007). All multivariate statistical analyses were performed with help of the R software package (version 2.0).

2.5. Results and Discussion

DOC concentrations in the sampled water were 94 µM for the North Sea sample and 44 µM for the NPDW sample. The concentrations of solid-phase extractable DOS were 0.84 µM for the North Sea sample and 0.18 µM for the NPDW sample. These concentrations result in molar DOS/DOC ratios of 0.0089 for the North Sea sample and 0.0041 for the NPDW sample. These values are in accordance with DOS concentration values published for surface waters from the South Atlantic (Lechtenfeld et al., 2011) and values for Canadian lakes (Houle et al., 1995). Similar element ratios were detected via FT-ICR-MS. A total of 173 different DOS molecular formulae were detected in the NPDW and 382 in the North Sea sample. Under consideration of the number of carbon and sulfur atoms in all molecular formulae, the intensity weighted S/C-ratios were 0.0051 for the North Sea sample and 0.0016 for the NPDW sample. This overall agreement (in terms of trend and order of magnitude) between bulk elemental analysis and molecular analysis is encouraging, and indicates that FT-ICR-MS was capable to at least semi-quantitatively characterize a representative fraction of DOM.

2.5.1. Thiol derivatization

Our tests with thiol model compounds showed that NQBr was a selective derivatization reagent for thiol functional groups, also in presence of a complex DOM matrix. In our tests, NQBr did not react with hydroxyl or carboxyl groups (tested with salicylic acid, data not shown). These findings are consistent with Lobo et al. (2007) who also found that NQBr is a selective reagent for thiols in complex matrices. Interestingly, the reagent did not only give the mono-reacted product but also the di-reacted one (Fig. 2.1) which was also observed by Gracheva et al. (2004). These two possible products were considered during the analysis of the DOM experiment results. The comparison of the DOM spectra with no NQBr added and NQBr added revealed no detectable difference in the distribution of sulfur-containing compounds for both, North Sea DOM and NPDW DOM (Fig. 2.4). There are traces of thiols in seawater (Al-Farawati and van den Berg, 2001; Dupont et al., 2006; Laglera et al., 2014), but our results indicate that they are not a major fraction of DOS that is recovered via SPE from marine waters.

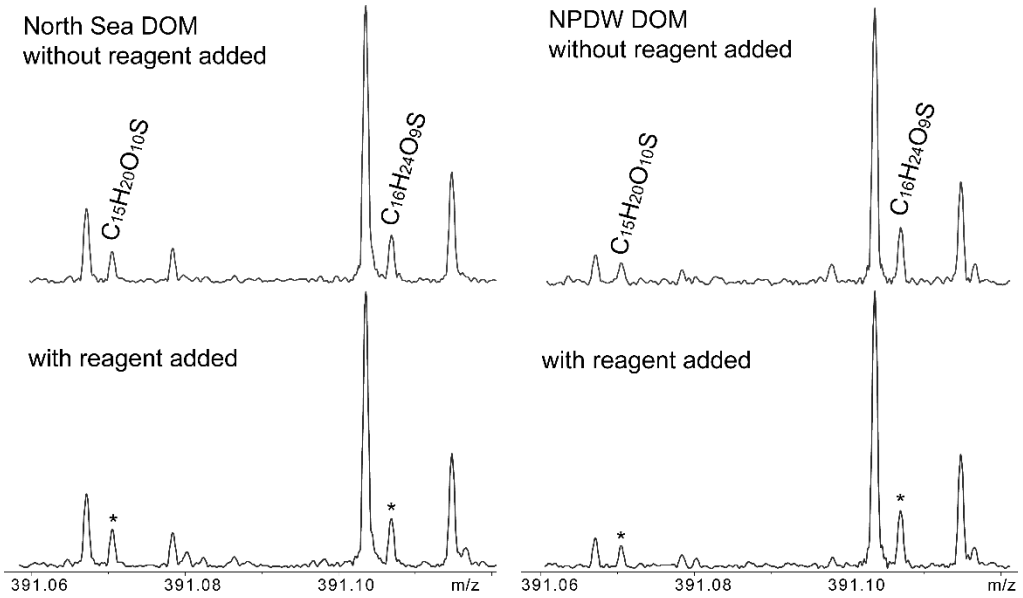

Figure 2.4: Exemplary sections of FT-ICR-MS spectra for the thiol derivatization experiment. Comparison of one nominal mass (*m/z* = 391) out of the FT-ICR mass spectra of the North Sea (left) and NPDW (right) DOM. The spectra are scaled to the same intensity level. The sulfur containing masses showed no significant difference between control and experiment, i.e. the sample without reagent (above) and with reagent (below) added. A variance test confirmed this lack of differences for all detected masses (for details see methods section).

2.5.2. Hydrolysis

Thioesters, sulfonic acid esters or alkylsulfates in DOM were expected to hydrolyze under the harsh hydrochloric acid treatment under oxygen-free conditions in the heat (Bracher et al., 2011; Hemond and Fechner, 2000; Hu et al., 2011). For both DOM samples no detectable difference was observed between the hydrolysis experiment and the controls for all acid concentrations (Fig. 2.5). Because of the extreme resistance to acidic hydrolysis, we conclude that thioesters, sulfonic acid esters or alkylsulfates do not significantly contribute to solid-phase extractable DOS. Furthermore, this observed resistance to hydrolysis is consistent with the refractory character of the compounds in the aqueous environments of the ocean.

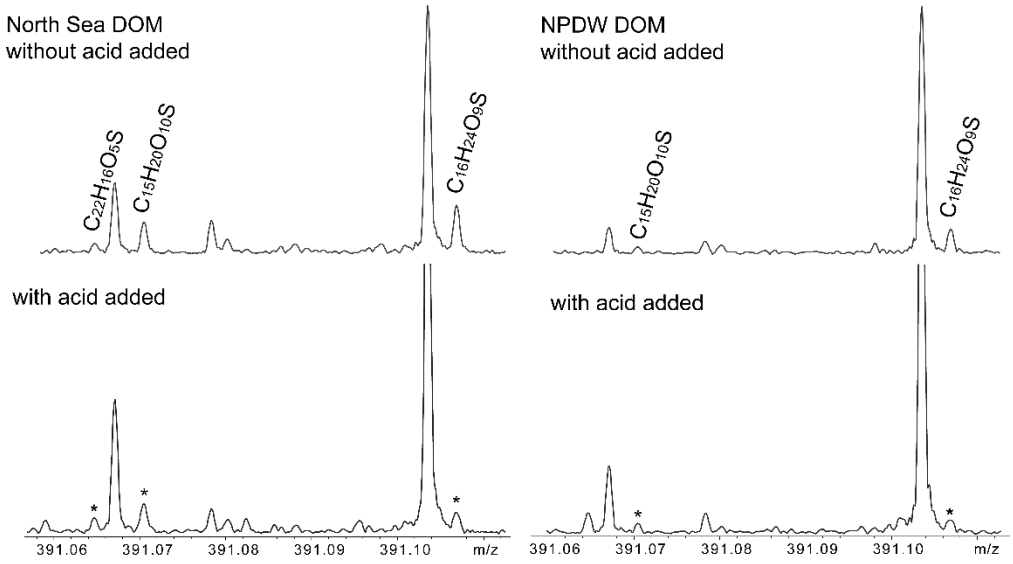

Figure 2.5: Exemplary section of FT-ICR-MS spectra for the hydrolysis experiment. Comparison of one nominal mass (*m/z* = 391) out of the FT-ICR mass spectra of the North Sea (left) and NPDW (right) DOM. The spectra are scaled to the same intensity level. The sulfur containing masses showed no significant difference between control and experiment, the sample without acid or heat (above) and with acid added and heated (below). A variance test confirmed this lack of difference for all detected masses (for details see methods section).

2.5.3. Oxidation and Deoxygenation

Oxidation and deoxygenation experiments with non-aromatic thioether and sulfoxide model compounds, respectively, yielded the expected reaction products in both the pure acetonitrile and the acetonitrile/extracted DOM matrix. Further tests with model compounds that do not contain sulfur containing functional groups revealed slight side reactions as, e.g., oxidation of hydroxyl groups or deoxygenation of ketone groups (data not shown). These findings question the selectivity of the applied method which was not reported before (Bahrami et al. 2011). We did not observe these kinds of side reactions for the DOM samples, suggesting that the respective functional groups therein do not react with the reagents used. Therefore, these side reactions can be considered of little importance for our analysis of marine DOM.

In contrast to the model compounds, for both DOM samples no detectable difference was observed between the experiment and the controls (Fig. 2.6). Also the more specific comparison of the intensity-weighted oxygen content of all sulfur compounds of the samples showed no increase after oxidation or decrease after deoxygenation. Consistent with this observation, the Bray Curtis dissimilarity between mass spectra did not increase due to the treatment with the oxidation or deoxygenation reagent, neither for the entire data set nor for the subset of the 616 molecular formulae (and their intensities) that contained sulfur. This

is, the variability between the untreated DOM samples (controls) for both North Sea and NPDW and the respective oxidized sample was within the procedural variability and therefore not significant. The same was true for the variability between the untreated and the deoxygenated DOM for both samples. The lack of any significant difference between controls and oxidized and deoxygenated samples is evidence for the absence of non-aromatic thioethers or sulfoxides in SPE-DOM. The analyzed DOM samples were taken from oxic marine environments. As non-aromatic thioethers as well as sulfoxides are sensitive to oxygen (Breitmaier and Jung, 2012) it stands to reason that they are not stable in the oxic water column. This is in accordance with our results.

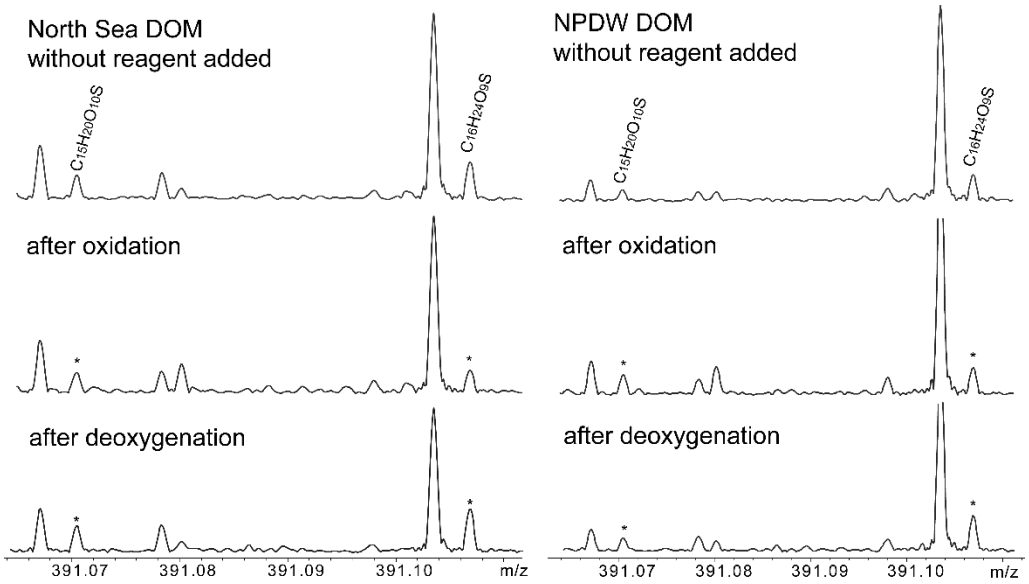

Figure 2.6: Exemplary sections of FT-ICR-MS spectra for the oxidation and deoxygenation experiments. Comparison of one nominal mass (*m/z* = 391) out of the FT-ICR mass spectra of the North Sea (left) and NPDW (right) DOM. The spectra are scaled to the same intensity level. The sulfur containing masses showed no significant difference between control and experiment, i.e. the sample without reagent (above) and with reagent added (below, oxidation in the middle, deoxygenation at the bottom). The Bray Curtis Dissimilarity analysis showed that the variability between the samples was within the procedural variability and therefore not significant (for details see corresponding section in the text).

2.5.4. Collision-induced fragmentation

Isolation spectra analyses prior to fragmentation ensured that only compounds of the targeted nominal mass were included (Fig. 2.7). The two sulfonic acid model compounds, the aromatic sulfonic acid salt (ARS) and the aliphatic sulfonic acid (CSA) showed characteristic neutral losses as expected. These indicative losses were SO_2, H_2SO_3 and for ARS additionally SO_3. The fragmentation spectra of the two natural DOM samples were analyzed for these indicator losses. All considered compounds showed common neutral losses (Stenson et al.,

2003; Witt et al., 2009) such as CO_2, H_2O, CH_3OH, and O_2 and also the indicator neutral losses (SO_2, H_2SO_3, and SO_3) were found in both DOM samples (Table 2.1). This suggests the presence of sulfonic acids (or their respective salts) in SPE-DOM. In addition, the sulfur-containing compounds must also have a carboxyl group as they lost also CO_2. Additionally, at only some nominal masses there were losses of a single sulfur atom (S_1, Fig. 2.8, Table 2.1). The loss of S_1 might be released from slightly reduced sulfur-containing functional groups with an oxidation state ≤0, such as thiophenes. Other slightly reduced forms, aside thiophenes, like thioethers, sulfoxides, and thioesters could be ruled out by our previous experiments.

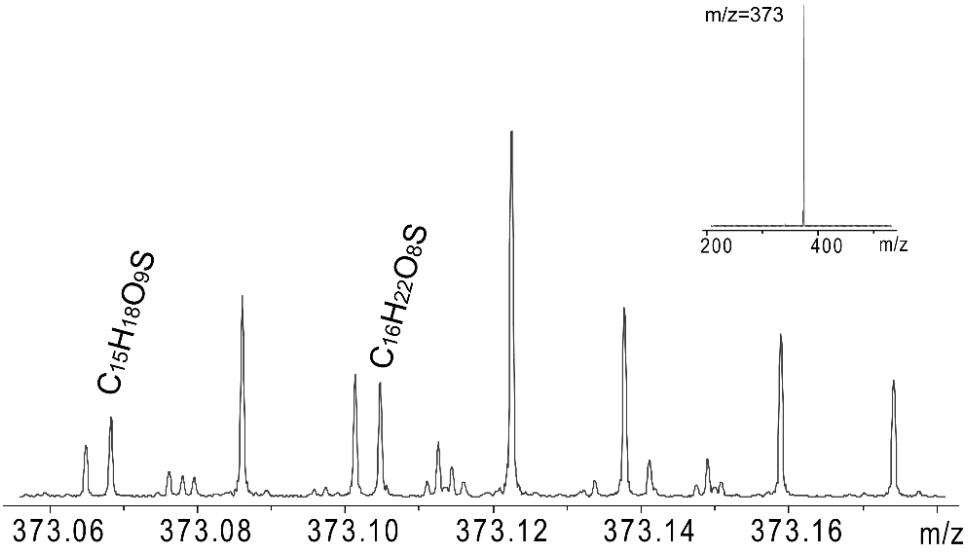

Figure 2.7: Exemplary section of a FT-ICR-MS isolation spectrum of North Sea DOM; NPDW showed similar results. Shown is a part of the full range mass spectrum of the isolation of nominal mass 373 (upper right corner) and an enlarged view of the nominal mass showing the single mass-to-charge-ratios of the nominal mass.

Concerning the CH_2-homologous series analyzed, sulfur-containing compounds of the same CH_2-homologous series were more similar with respect to their fragmentations patterns, than compounds belonging to different series. This discrepancy suggests that the sulfur-containing functional group is of the same nature within a CH_2-homologous series which could be a hint for similar production-pathways for a large variety of compounds in DOM. Consistently, sulfur-containing compounds within a nominal mass also differed with respect to the presence of those neutral losses that did not contain sulfur. A loss of H_2O e.g. could be found for one compound but not for another of the same nominal mass.

Consistent with our findings for marine SPE-DOM, sulfonic acids can be main part of organic sulfur compounds in marine sediments (Eglinton et al. 1994; Vairavamurthy et al. 1994; Zhu et al. 2014). Sulfonic acids may be introduced into the water column via different pathways. Sulfurization in reduced sediments may introduce sulfur into sedimentary or dissolved organic matter in first place. If these compounds become soluble through additional

alteration processes they may diffuse towards the surface of the sediments and into the water column. A more efficient transport process than diffusion is the advective transport of DOM through reduced sediments, as it occurs in tidal systems at coastal margins or hydrothermal systems in the deep sea. Reduced sulfur compounds may quickly oxidize to form sulfonic acids once they reach the oxic sediment surface or water column. Neither the pathways of sulfurization nor of oxidation to sulfonic acids whether in the sediment or in the water column are fully understood (Zhu et al., 2014).

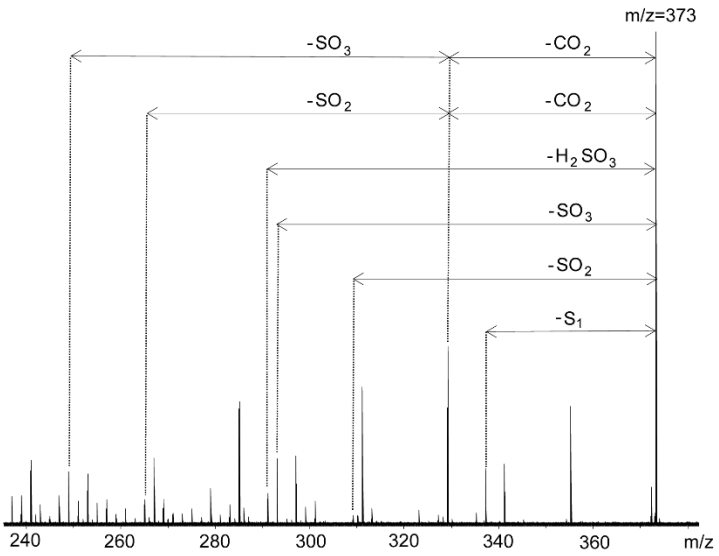

Figure 2.8: Exemplary section of a FT-ICR-MS collision-induced fragmentation spectrum of North Sea DOM; NPDW DOM showed very similar results. Shown is the fragmentation pattern of nominal mass *m/z* = 373 (considered molecular formulae: $C_{15}H_{18}O_9S$ and $C_{16}H_{22}O_8S$) with the corresponding neutral losses. Each peak visible here actually consists of several resolved peaks.

2.6. Conclusions

This study revealed basic structural information on sulfur-containing compounds in DOM of two different sampling sides, one from the Pacific deep sea and one from the open North Sea surface (Table 2.2). By usage of different functional-group selective alteration reactions we were able to rule out thioesters, sulfonic acid esters, alkylsulfates, thiols, sulfoxides and non-aromatic thioethers as major structural component of SPE-DOM from the oceanic water column. We cannot fully rule out steric hindrance that may inhibit reactions in the experiments and in nature, but such mechanism is not described in the literature for the functional groups and experimental conditions considered in this study. With collision-induced fragmentation experiments we detected sulfonic acids as a main component of the analyzed DOS. These findings contradict an earlier suggestion that thiols and thioethers could be part of SPE-DOM (Hertkorn et al., 2013). It is possible that thiols and thioethers are firstly

produced by reaction of reduced inorganic sulfur compounds with organic matter (Sinninghe Damsté et al., 1989; Aizenshtat et al., 1995; Schneckenburger et al., 1998) and then rapidly degraded to the sulfur compounds we found. We also found some evidence for aromatic sulfur heterocycles (thiophene derivatives) which are indeed plausible reduced structural feature in DOM because of their inert character. Because of their chemical inertness there is no selective reaction pathway available for the selective and targeted detection of thiophenes. Earlier studies described thiophene derivatives as products of sulfur incorporation into sedimentary organic material in oxygen minimum zones (Krein and Aizenshtat, 1995; Aizenshtat et al., 1995; Eglinton and Repeta, 2014) which could then be transported into the oxic water column. Thioethers had also been suggested as products of sulfur incorporation into organic material (Krein and Aizenshtat, 1995; Eglinton and Repeta, 2014), but we did not find any evidence for their presence in DOS. Sulfones are likely oxidation products of thioethers, but, unfortunately, they are too unreactive for selective alteration reactions for unambiguous detection.

Table 2.2: Summary of results obtained from the experiments of North Sea and NPDW DOM.

Compound	Applied experiment	Results for North Sea and NPDW DOM samples
R-SH	derivatization	thiols unlikely, because no derivatization-reagent addition observed
R-CO-S-R‘ / R-SO_2-O-R‘ / R-O-SO_2-O-R’	hydrolysis	thioesters/sulfonic acid esters/alkylsulfates unlikely, because no molecule cleavage observed
R-S-R’	oxidation	non-aromatic thioethers unlikely, because no oxidation products found
R-SO-R’	deoxygenation	sulfoxides unlikely, because no deoxygenation products found
R-SO_3H	fragmentation	existence of sulfonic acids proven, because indicative neutral losses found

In conclusion, the detected and assumed functional groups in DOM are sulfonic acids and thiophenes, and possibly also sulfones. These sulfur compounds may be rather unreactive, because they cannot be further oxidized or hydrolyzed, which is consistent with their presence in refractory deep-sea DOM, though experimental data on the reactivity of individual DOS compounds are lacking. The two DOM samples considered in this study originated from very different marine systems and water depths, exhibited different DOC and DOS concentrations, but were very similar with respect to DOS molecular structure. It is

therefore likely that the molecular features discovered in this study are universal and representative for the oxic marine water column in general.

2.7. Acknowledgments

The authors are most grateful to Jutta Niggemann and Jens Christoffers for helpful advice during this study. We would like to thank Katrin Klaproth for support in FT-ICR-MS analyses, Bernhard Schnetger and Eleonore Gründken for ICP-OES analyses and Matthias Friebe for DOC measurements. We also thank the two anonymous reviewers whose comments helped to improve an earlier version of this manuscript.

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3. Manuscript II

Experimental evidence for abiotic sulfurization of marine dissolved organic matter

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3.1. Abstract

Dissolved organic sulfur (DOS) is the largest pool of organic sulfur in the oceans, and as such an important component of the global sulfur cycle. DOS in the deep ocean is resistant against microbial degradation and turns over on a millennium time scale. DOS sources and the mechanisms behind its stability are largely unknown. Here, we hypothesize that in sulfate-reducing sediments sulfur is abiotically incorporated into dissolved organic matter (DOM) and released to the open ocean. We mimicked the environment in sediments and exposed natural seawater and the filtrate of a plankton culture to sulfidic conditions. Already after one hour at 20 °C, DOS concentrations had increased 4-fold in these experiments, and 14-fold after 4 weeks at 50 °C. Molecular analysis via ultrahigh-resolution mass spectrometry showed that sulfur was covalently bond to DOM and that the incorporation of sulfur was to a large degree molecularly unselective. Incorporation of sulfur was reductive, but the initial products were apparently sensitive to oxidation so that most sulfur was present as stable sulfonic acids. Experimentally produced and natural DOS from sediments were highly similar on a molecular formula and structural level which confirms the authenticity of our experiments. By combining our data with published benthic DOC flux values we estimate that 45 – 120 Tg DOS are annually transported from sulfate reducing sediments to the oceans. This flux is about one order of magnitude larger than that via rivers and by far sufficient to balance the estimated global net removal of refractory DOS.

3.2. Significance

Sulfur is essential for life. It also exerts major influence on Earth's climate because as sulfate it creates condensation nuclei in the atmosphere through which clouds form. Marine life in the sunlit ocean plays a major role in this context because it continuously takes up and releases various forms of sulfur. In the deep sea, however, dissolved organic sulfur compounds, that are presumed to originate from organisms, have accumulated over millennia to the largest pool of organic sulfur in the oceans. Here we discovered that a major source of this mysterious and stable pool of organic sulfur in the ocean are chemical reactions in marine sediments. These abiotic processes seem to be essential in the global cycle of sulfur.

3.3. Introduction

Dissolved organic matter (DOM) is a complex mixture of thousands of organic compounds (1). It is ubiquitous in aquatic environments, in surface and deep sea (2), and also in sediments (3, 4). Many compounds within the DOM mixture contain sulfur (dissolved organic sulfur, DOS), and in sum they make up the largest reservoir of organic sulfur in the ocean (global inventory of >6.7 Pg S, 5). Despite the relevance of DOS in global

biogeochemical cycles, knowledge on its sources and turnover, and its molecular composition is scarce. Marine primary production is considered a major source of organic sulfur, explaining elevated DOS concentrations in surface waters (5). Yet, this organic sulfur is part of a rapidly cycled, labile DOS pool (5), and presumably contributes little to the pool of the long-lived refractory DOS in the oceans' interior. The source of the large refractory DOS pool which is evenly distributed in the water column is unknown.

It is generally accepted that, in sediments, inorganic reduced sulfur species are abiotically incorporated into organic matter during early stages of diagenesis forming organic sulfur compounds (e.g. 3, 6, 7). Several studies observed such incorporation into different types of organic compounds. Some tested the reaction of specific alkenes or aldehydes with sulfide (NaSH) and elemental sulfur (S) in organic solvents (8, 9, 10). Others mixed carbohydrates or algal material with NaSH and S in (sea)water to simulate natural conditions in sediments (11, 12). There are also indications that natural DOM may be sulfurized in sulfidic marine systems because DOS concentrations were found to be distinctly higher in sulfidic environments than in oxic ones (4, 13). The incorporation of sulfur likely protects labile organic matter from microbial alterations (14) but it is unknown whether similar mechanisms contribute to the stability of DOM in marine systems.

The aim of this study was to experimentally test the hypothesis that abiotic sulfurization of DOM occurs in marine sulfidic environments making them a potential formation site for DOS in the ocean. Sulfurization experiments were done in triplicate, each with freshly produced DOM from in-house mesocoms where natural marine planktonic communities were grown, and with natural DOM samples from the North Sea. Sulfurization was tested by addition of NaSH and S to the samples as done previously for particulate organic matter (11). This experimental procedure was chosen because sulfide and S are present in sulfidic seawater and together form polysulfides (15). Polysulfides are the most nucleophilic species of reduced sulfur and likely the most important sulfur species for diagenetic sulfur incorporation into organic matter (10, 16). Long-term (4 weeks) sulfurization experiments were complemented by a very short (1 hour) incubation.

The sulfurized samples were compared on a most detailed molecular level to DOM from sulfidic sediments in the German Wadden Sea (Janssand) that is likely an area where natural sulfurization occurs (4). For the molecular analysis of DOM prior and after the sulfurization experiment we used ultrahigh-resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS). DOM comprises are largely inseparable mixture of compounds and is as such only partially accessible to conventional chromatographic analytical techniques. FT-ICR-MS coupled to soft electrospray ionization enables the analysis of individual, intact molecules in the otherwise inseparable DOM mixture. The high mass accuracy of FT-ICR-MS allows the assignment of molecular formulae to the detected masses (e.g. 17, 18, 19). Additionally, we performed a series of analytical experiments prior to mass spectrometry analysis and within the FT-ICR-MS to determine the molecular structure of S-containing

functional groups (20). Molecular analyses were accompanied by quantitative element analysis.

3.4. Results and Discussion

3.4.1. Artificial production of DOS

DOS production was evident by a distinct increase of bulk DOS concentrations after sulfurization (Table 3.1). Mesocosm DOS concentrations increased from ~1 µmol L^{-1} (controls) to 10-21 µmol L^{-1} after sulfurization (increase by factor 14, on average). North Sea values increased from ~1.5 µmol L^{-1} (controls) to 16-23 µmol L^{-1} after sulfurization (increase by factor 14, on average). The DOS/DOC concentration ratios also strongly increased to a similar degree due to sulfurization (Table 3.1).

With help of FT-ICR-MS we detected 7,177 to 13,948 DOM molecular formulae in the mesocosms and 11,165 to 16,472 in the North Sea whereas the sulfurized samples had on average 50 % more molecular formulae than the controls (Table 3.1). Furthermore, due to sulfurization, the number of S-containing compounds increased in the mesocosms from 1,925 to 5,609 on average (increase by factor 2.9) and in the North Sea from 3,434 to 6,193 on average (increase by factor 1.8). Also the percentage of S-containing molecular formulae increased in the mesocosms from 25 % to 45 % on average (increase by factor 1.8) and in the North Sea from 29 % to 38 % on average (increase by factor 1.3) (Fig. 3.1, 3.2). The FT-ICR-MS signal intensity-weighted sulfur-to-carbon ratio (S/C) was distinctly higher after sulfurization (factor 5.3 for mesocosm, factor 1.8 for North Sea). The sulfurization led to a higher number of shared DOS compounds between North Sea and mesocosm samples. They shared 1,148 DOS formulae before incubation and 3,371 DOS formulae after sulfurization (Fig. 3.3 a, b), indicating a high similarity of DOS on a molecular level.

These detected changes in DOS content must result from abiotic reactions as cell counts of all samples in the sulfurization experiment revealed no bacterial growth. Furthermore, we excluded the possibility of simple sulfur adducts formed in solution or during ionization by an adduct test where we added sulfide (NaSH) to the control samples immediately prior to FT-ICR-MS analysis. This test verified the covalent incorporation of inorganic sulfur into organic matter in our sulfurization experiment.

In natural sulfidic environments as well as in our experiments, sulfide concentrations are in excess compared to the susceptible organic molecules. Thus, the sulfurization reaction follows a pseudo first order kinetic, which is largely independent of the concentration of the reactant. In our experiments we chose a higher reaction temperature than in nature to enhance the reaction rate. We did this to simulate the longer residence time of DOM in natural sulfidic environments, that can be up to decades in coastal tidal flats (e.g. Janssand, 21), in only 4 weeks of incubation. To obtain general information on the speed of the

sulfurization reaction of DOM, we incubated a North Sea water sample for only 1 hour at 20 °C in comparison to the 4 weeks incubation at 50 °C. Even for the short-term sulfurization experiment an increase in bulk DOS concentration was detected. DOS concentrations increased from ~3 µmol DOS L^{-1} in the controls to 12 µmol DOS L^{-1} after sulfurization (increase by factor 4.0). Also the number of sulfur-containing molecular formulae detected by FT-ICR-MS increased by factor 1.3.

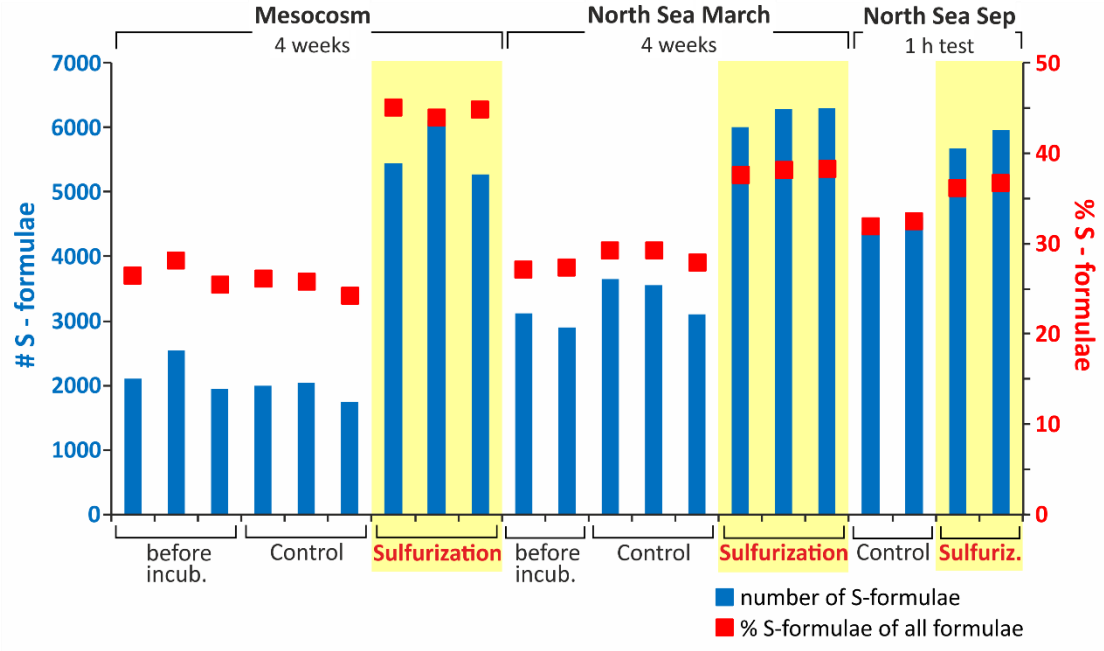

Figure 3.1: Number and proportion of DOS molecular formulae prior to and after sulfurization. The blue bars show the number of S-containing molecular formulae and the red dots the relative percentage of S-containing formulae of all assigned molecular formulae. Plotted are the short- and long-term sulfurization samples. A distinct increase in DOS content is visible after sulfurization for mesocosm and North Sea DOM even after only one hour of reaction time. In the original (untreated) sample of the 1 hour experiment the total number of DOS compounds was higher compared to the 4 weeks approach, but this is only because the North Sea samples were taken at different seasons and tides. This difference reflects the highly dynamic nature of the coastal North Sea. For quantitative data from element analyses we refer to Tab. 3.1.

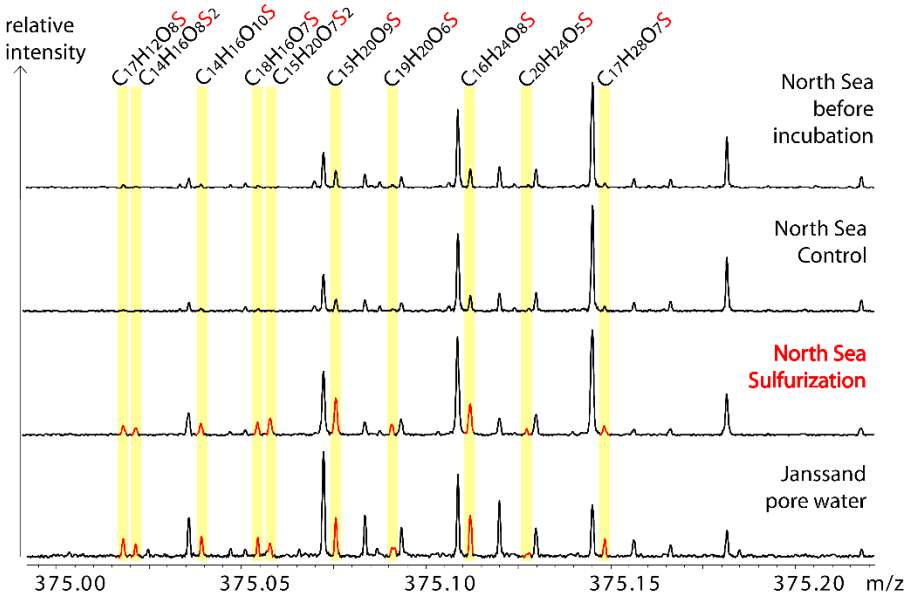

Figure 3.2: Molecular similarity of sulfurized and pore water DOM. Exemplary sections of the FT-ICR-MS spectra. Comparison of one nominal mass (*m/z* = 375) of the North Sea sample before incubation, control, and after sulfurization with the natural pore water sample (Janssand). The spectra are scaled to the same intensity level. Neutral S-containing molecular formulae of detected ions are assigned and the corresponding FT-ICR-MS peaks are highlighted.

Even on a molecular level, the sulfurization products after 1 hour of incubation were very similar to those after 4 weeks of incubation. From the DOS compounds that increased in intensity after 1 hour, 93 % also increased in the long-term incubations (either in mesocosm or in North Sea). Of the molecular formulae that increased in intensity after incubating 4 weeks, 55 % (mesocosm) and 66 % (North Sea) also increased in the 1 hour approach (Fig. 3.3 c). Thus, sulfur incorporation into DOM happens fast, indicating that organic matter does not need long residence times in natural sulfidic environments to be affected by sulfurization.

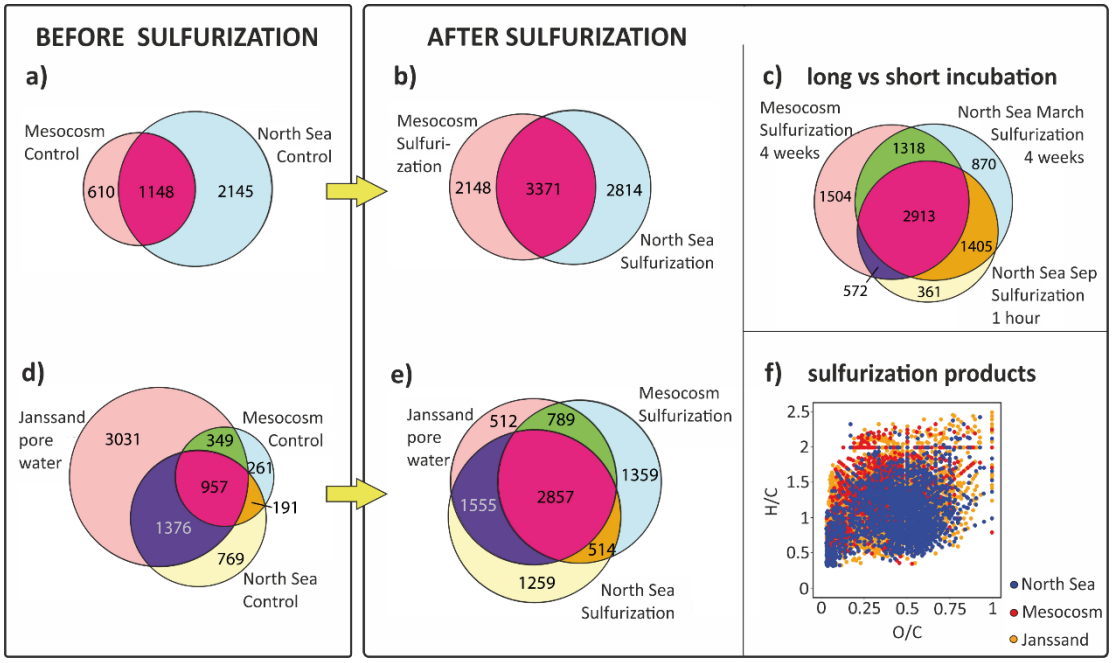

Figure 3.3: Molecular similarity of sulfurized and pore water DOM. Sulfurization increased the number of shared S-containing molecular formulae compared to DOS from sulfidic pore water (Janssand). Venn diagrams including only S-containing formulae of a) mesocosms and North Sea controls, b) mesocosms sulfurized and North Sea sulfurized, c) S-containing formulae that increased in relative intensity after sulfurization for long (4 weeks) and short (one hour) terms, d) mesocosms controls, North Sea controls and pore water, and e) mesocosms sulfurized, North Sea sulfurized and pore water. Only formulae were counted that appeared in at least two out of three replicates or in some cases for both duplicates. The number of shared DOS-formulae increases after sulfurization (pink area) showing higher similarity among samples. f) van Krevelen diagram (hydrogen-to-carbon (H/C) and oxygen-to-carbon (O/C) ratios) showing the S-containing formulae produced by sulfurization (present in the sulfurized but not in control samples) in the mesocosms and North Sea together with the S-containing formulae in the pore water.

3.4.2. Molecular similarity of natural and artificial DOS

Our experimental setup mimicked an aquatic, anoxic, and highly sulfidic environment similar to natural conditions in marine sediments. The DOS/DOC concentration ratios of the sulfurized samples (mesocosms: 0.105, North Sea: 0.095) were higher than in the pore water (0.037) (Table 3.1). This is possibly due to the stable and uninterrupted reaction conditions in the artificial sulfurization experiment. In nature, H_2S concentrations in pore waters are dynamic (22), and advective pore water transport across redox gradients or sediment reworking may temporarily interrupt sulfurization. Despite these higher reaction yields in our experiment, the artificial and natural DOS were very similar in their molecular composition. As a result of sulfurization, the total and relative number of DOS compounds and the intensity-

weighted S/C ratio converged to the values of the pore water DOM (Table 3.1). Furthermore, sulfurization led to an almost undistinguishable molecular pattern compared to the pore water (Fig. 3.2, Fig. 3.3 f and 3.7). Comprehensive, presence-absence analysis showed an increase in number of shared DOS compounds of our samples and the pore water due to sulfurization (Fig. 3.3 d, e). The number of DOS compounds shared with pore water increased for the North Sea sample from 2,333 to 4,412 (increase by factor 1.9) after sulfurization and for the mesocosm sample from 1,306 to 3,646 (increase by factor 2.8). In addition to the presence-absence analysis of molecular formulae, a statistical Bray Curtis dissimilarity analysis was performed that takes into account not only all DOS molecular formulae, but also their respective FT-ICR-MS signal intensities, a semi-quantitative analytical information. These most detailed molecular fingerprint analyses confirmed the high level of molecular similarity between artificial and natural DOC. The sulfurized mesocosm and North Sea samples were more similar to the pore water than the controls (Fig. 3.8 a).

So far, we discussed molecular similarity on a molecular formula level. Behind each molecular formula, however, a large structural diversity can be hidden, and identical molecular formulae may be represented by different isomers. To test for this possibility, we performed extensive structural analysis of the sulfur-containing functional groups in each sample (20). Even on a structural basis, the sulfurized samples and the pore water DOS showed a high level of similarity: sulfonic acids were the predominant functionality in all analyzed experimental and natural samples (Table 3.3 and 3.4), and none of the samples contained detectable amounts of thiols, thioesters, sulfonic acid esters, alkylsulfates, or sulfoxides. A slight difference between the samples was observed in the molecular fragmentation experiments in the FT-ICR-MS for the neutral loss of H_2S. These H_2S fragments occurred in all sulfurized and the pore water samples, but not in any of the controls (Table 3.4). The loss of H_2S is not indicative for an explicit functional group (23) but it is an indicator for reduced sulfur compounds (sulfur oxidation state ≤0). This trend is reasonable as reduced inorganic sulfur is incorporated into DOM during sulfurization.

Similar to the statistical comparison on a molecular formula level, we used the molecular fragmentation pattern as structural fingerprints for Bray Curtis dissimilarity analyses. The dissimilarity analysis was done with the FT-ICR-MS signal intensities of those fragments that lost SO_3 and H_2SO_3 (from sulfonic acids) which were normalized to the intensity of the respective precursor-ion. Again, mesocosm and North Sea DOS was more similar to the pore water after sulfurization than in the controls (Fig. 3.8 b). This further confirms the structural similarity of DOS produced by artificial sulfurization compared to the DOS in natural pore water. The similarity of the artificially sulfurized DOM to the naturally sulfurized pore water supports the authenticity of our experimental setup.

3.4.3. Potential reaction pathways of sulfur incorporation

The detailed mechanism of sulfur incorporation in reduced sediments is unknown, but the most discussed possible mechanism for the sulfur incorporation is the Michael addition (10, 16, 24) that describes a nucleophilic addition to a α,β-unsaturated carbonyl compound resulting in thiols. As thiols were not detected here, it is likely that the thiols which are reactive nucleophiles themselves (25, 26) incur a second Michael addition intra- or intermolecularly to form thioethers. However, no evidence for thioethers was found, with the exception of the mesocosm DOM. In this experiment, a minor contribution of thioethers is likely, because of the more than 3,500 sulfur-containing molecular formulae that were produced by sulfurization of mesocosm DOM (Tab. 3.1), 264 could be chemically oxidized (following the procedure as in ref. 20). These oxidizable DOS compounds are likely thioethers (20). The lack (or low abundance) of thioethers is evidence for additional reactions after the incorporation of sulfur. Possibly, the sulfurized North Sea DOM (and part of the mesocosm DOM) molecules react in an intramolecular fashion producing thiophenes which we are not able to unambiguously detect because thiophenes are very unreactive and behave uncharacteristic in fragmentation. Furthermore, the abundance of the sulfonic acid group and the essential lack of chemically oxidizable sulfur functionalities in all samples indicate almost complete secondary oxidation after sulfurization. Thiols and thioethers are in general unstable under the presence of oxygen (27) and had likely been oxidized prior to analysis. In analogy, reduced sulfur-containing compounds are likely quickly oxidized once they escape into the oxic open ocean. The main oxidation products are apparently sulfonic acids which we found in all our so far analyzed environments even in the deep sea (20). Sulfonic acids are very stable compounds and it has been suggested that sulfur incorporation preserves organic matter from microbial degradation (28, 29).

Another interesting result of our sulfurization experiment was the non-selectivity of sulfur incorporation. Sulfurization occurred irrespective of saturation, aromaticity, degree of oxidation or heteroelement content (e.g. nitrogen) of the precursor compounds (e.g. Fig. 3.6). The sulfurized compounds had molecular formulae characteristic for polyphenols, condensed polycyclic aromates, peptides, carbohydrates and other unsaturated compounds. No selectivity was observed for any of these tentatively assigned compound groups. Thus, sulfur incorporation was not selective to a specific compound class but affected the entire DOM pool. This makes sulfurization fundamentally different from other abiotic transformations of DOM like photodegradation (selective towards aromatic compounds, 30) or adsorption onto iron minerals (selective towards carboxylic-rich aromates, 31).

The variety and complexity of potential sulfurization pathways and products became also apparent when analyzing potential precursor-product-relationships among the molecular formulae obtained by FT-ICR-MS. This approach identifies the potential for reaction pathways, or the absence thereof, based on the presence and absence of specific molecular

formulae prior and after the reaction. Potential precursors were identified according to 20 hypothetical sulfurization reactions (Table 3.2). In general, the same potential reaction patterns were observed between the mesocosms and North Sea samples. The most effective potential sulfurization reactions (precursors for over 80 % of DOS formulae) were those exchanging one or two H_2 by a H_2S molecule (Table 3.2) which would not represent the Michael addition mechanism. However, we also found strong indication for the Michael pathway (+H_2S, ~70 %, Table 3.2) and potential reactions supporting the high abundance of the sulfonic acid groups (+O_2 or +H_2O reactions, ~50-80 %, Table 3.2).

3.4.4. Global relevance of sedimentary DOS flux

The global input of DOS from sulfate reducing sedimentary pore waters can be estimated based on benthic DOC fluxes from sediments. This flux from intertidal sediments, coastal and continental margin sediments (0-2000 m water depth), and sediments >2000 m water depth is estimated as ~450 Tg C a^{-1} in total (32, 33, 34, 35). We estimate a global sedimentary DOS flux by using S/C ratios of pore water DOM and from our sulfurization experiments. On the one hand, the minimum DOS flux is based on the quantitatively analyzed natural S/C ratio from Janssand pore water DOM (0.037; Table 3.1). To the best of our knowledge, this is the only quantitative number available for S/C ratios in sedimentary DOM. This ratio is comparable to the few reported S/C ratios in sedimentary solid organic matter, e.g. at the Peru margin (0.038 ± 0.026) (36), at the Cariaco Basin (0.048 ± 0.011) (37), at the coast of British Columbia (0.041 ± 0.011) (38), or the Delaware salt marsh (0.038 ± 0.020) (39) which show similar S/C ratios even though they came from very different locations. On the other hand, we consider for the maximum flux estimate the average S/C ratio obtained by our sulfurization experiments (mesocosms: 0.105, North Sea: 0.095). Thus, the global sedimentary DOS flux can be estimated as 45 – 120 Tg DOS a^{-1}. Owing to the scarcity of global data on sedimentary DOS, the uncertainty of our estimate is inherently large. But this first attempt illustrates that this benthic DOS flux is potentially one order of magnitude larger than the riverine organic sulfur input to the ocean (8 Tg S a^{-1}, 5) and by far sufficient to balance the estimated global net removal of refractory DOS (1.1 Tg S a^{-1}, 5). Based on our estimate we suggest that sulfurization in sulfidic environments is the single most important source mechanism of refractory DOS to the oceans (Fig. 3.4). This pathway is not considered in current models of the global sulfur cycle (5). We just started to disentangle the marine sulfur cycle, and in this study we closed one major gap with respect to the sources of DOS to the ocean. A significant lack of knowledge still exists with respect to the reactivity and stability of the various DOS fractions in sulfidic and open ocean waters which should be target of future studies.

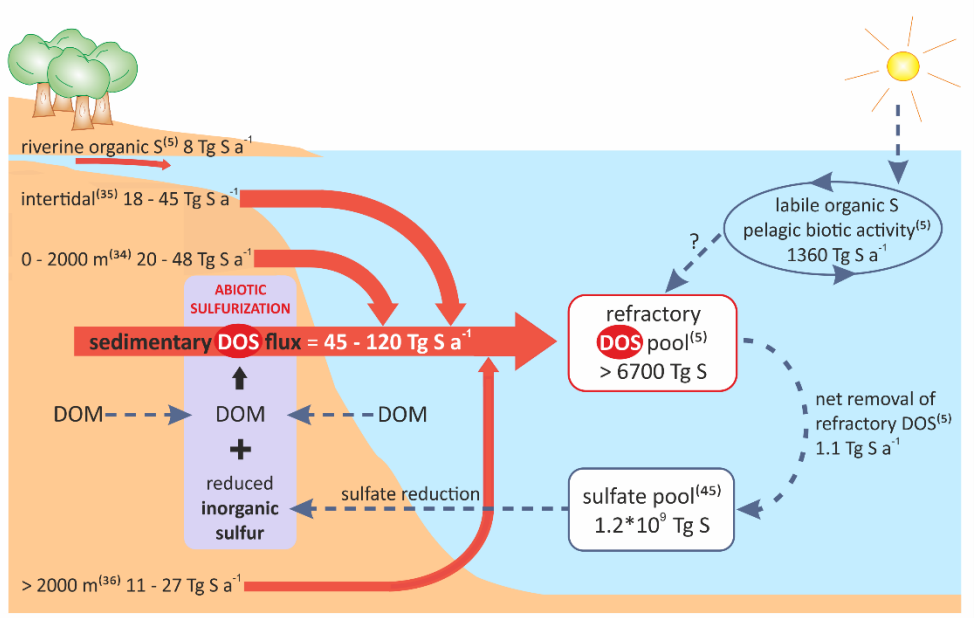

Figure 3.4: Conceptual scheme of the main pathways of the organic sulfur cycle in the ocean. Based on our results we propose that benthic DOS flux is a major pathway of DOS input to the ocean, a pathway that is not considered in current models of the sulfur cycle. DOS is formed in sulfate-reducing sediments through the abiotic reaction of organic matter with reactive inorganic sulfur species. Production and decomposition rates are not balanced in this scheme, which illustrates our current lack of knowledge with respect to the reactivity and stability of the various DOS fractions in the ocean. For simplicity, atmospheric exchange is not shown. For references and details on our estimates we refer to the main text.

3.5. Material and Methods

Details on Material and Methods can be found in the supplement. A general overview is given in the following.

The mesocosm experiments were conducted in triplicates after ref. 40. Bulk DOC concentrations after 18 days of incubation ranged between 100-150 µmol C L^{-1}. Growing was stopped then by filtering and acidifying to pH 2. The North Sea surface water sample was taken near Spiekeroog Island, Germany, filtered and acidified to pH 2 with HCl. It was stored at 4 °C until the experiment. Due to the acidification to pH 2 before sulfurization, all samples were sterile and microbial growth was further monitored during the sulfurization experiment via cell counts. The sulfurization experiments were done in 2.5 L amber glass bottles (Fig. 3.5). Prior to the experiments, the pH was adjusted to pH 8 with NaOH to simulate the natural seawater pH and left overnight. Still at pH 8, each sample was bubbled the next day with argon to expel all O_2 from the bottles. No further treatments were conducted with the "control" samples. For the sulfurization approach, inorganic sulfur compounds (10 g NaSH and 290 mg sulfur, 11) were added to the samples that we refer to as "Sulf" samples in the

following. A reaction blank consisted of ultrapure water that was bubbled with argon and to which the sulfur reagents were added as for the samples. All bottles - controls, Sulfs and blank - were incubated in ovens at 50 °C for 4 weeks (11). The “Sulf” samples were shaken daily to disperse the inorganic sulfur compounds. After 4 weeks at 50 °C, the samples were acidified to pH 2 with HCl and filtered. The short term experiment (1 hour) was done in the same fashion, but the duration was reduced to one hour and the temperature to 20°C.

All samples were extracted using solid phase extraction (SPE, 41). Bulk- and SPE-DOC and DOS concentrations prior to and after incubation were determined (20). FT-ICR-MS broad band scans and fragmentation experiments were performed with a 15 Tesla Solarix instrument (Bruker Daltonik, Germany) (20). Each sample from the sulfurization experiment, controls and “Sulfs”, were analyzed for their S-containing functional groups. For this purpose, the functional group selective wet-chemical alteration reactions were conducted after ref. 20. Collision-induced fragmentation experiments were done on selected nominal masses (20). All analyses were done at least in duplicate.

To ensure that the observed sulfurization products originate from covalent incorporation of inorganic sulfur into organic molecules and not only from sulfide adducts, an adduct test was conducted. For this the SPE extract of a North Sea control sample was mixed with an aqueous solution of NaSH and immediately analyzed at the FT-ICR-MS. A broad band mass spectrum was recorded and additionally two nominal masses (*m/z* = 377 and 389) were fragmented. For comparison, the same analysis was done with the same sample without addition of inorganic sulfur.

Molecular formulae assignment was done after ref. 42. Molecular formulae detected in the reaction blanks of the sulfurization experiments were disregarded from further consideration. The identified molecular formulae were tentatively assigned to compound groups based on molar ratios, aromaticity index, and heteroatom content (4, 43). These compound groups were polycyclic aromatics, polyphenols, sugars, and peptides. Potential reaction pathways of sulfur incorporation were tested in the FT-ICR-MS dataset on the basis of relationships of molecular formulae following nine possibilities of S addition while adding/removing H and/or O (e.g. 13, 44).

For statistical analysis, the detected masses were normalized to the sum of all mass intensities of the corresponding sample. For presence-absence analysis only masses were considered when present in two out of three triplicates or in both of duplicates. Further multivariate statistical analysis was done on the normalized data (Bray-Curtis dissimilarity). For the analysis of the derivatization and hydrolysis alteration reactions a variance test was performed as described in ref. 20.

3.6. Acknowledgments

We are grateful to K. Klaproth for support in FT-ICR-MS analyses, B. Schnetger and E. Gründken for ICP-OES analyses, and to M. Friebe and I. Ulber for help with the experiments and DOC measurements. We thank A. Braun for taking North Sea samples, A. Mentges for MATLAB help and M. Wolterink for support in cell counts.

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3.8. Supplementary

3.8.1. Material and Methods

3.8.1.1. Mesocosm experiments and samplings

The mesocosm experiment was conducted as in ref. 40. The mesocosms were set up in triplicates (M1, M2, M3) each consisting of 4.95 L artificial nutrient-enriched seawater (DOC 18 µmol C L^{-1}) mixed with 0.05 L prefiltered (poresize: 100 µm) coastal North Sea water containing the natural communities of phyto- and bacterioplankton as inoculum (Spiekeroog, Germany, March 18th 2015, 53°01.30' N, 8°27.10'E, low tide, DOC 157 µmol C L^{-1}) in acid-rinsed 5 L glass bottles. The mesocosms were incubated at approximately 17 °C and illuminated for 12 hours per day (400-700 nm) while the water was constantly stirred. After 18 days the before dispersed algae had clustered together and the DOC concentration of the mesocosm water ranged between 100-150 µmol C L^{-1}. At this time point the incubation was stopped and the mesocosm water was filtered sequentially through glass microfiber filters (2 µm, GMF, Whatman, USA) and glass fiber filters (0.7 µm, GFF, Whatman, USA) and acidified to pH 2 with hydrochloric acid (p.a., Merck, Germany) to stop any microbial activity.

The North Sea sample for the long-term sulfurization experiment was taken on March 3rd 2015 at low tide and the sample for the one-hour incubation on September 15th 2015 at high tide, both at the same location as the mesocosm inoculum. Samples were filtered and acidified as described above and stored at 4 °C until the experiment.

3.8.1.2. Sulfurization experiments

The filtered and acidified mesocosm and North Sea samples were transferred to 2.5 L amber glass bottles (Fig. 3.5). For each sample the pH was adjusted to pH 8 with NaOH (p.a. Roth, Germany) to simulate the natural seawater pH and left overnight. The pH was checked at the next day and each sample was bubbled with argon for 20 minutes to expel all O_2 from the bottles. No further treatments were conducted with the "control" samples. For the sulfurization approach, inorganic sulfur compounds (10 g NaSH and 290 mg sulfur, after ref. 11) were added to the samples that we refer to as "Sulf" samples in the following. Sulfur reagents were analytical grade (p.a. Sigma Aldrich). A reaction blank consisting of one liter of ultrapure water was bubbled with argon and 4 g NaSH and 0.12 g elemental sulfur were added. All bottles - controls, Sulfs and blank - were placed in ovens at 50 °C for 4 weeks (following ref. 11). The "Sulf" samples were shaken daily to disperse the inorganic sulfur compounds. After 4 weeks at 50 °C the samples were acidified and filtered as described above.

For the one-hour sulfurization experiment, the pH of the sample was also adjusted to pH 8 and the sample was split (540 ml each) in two controls and two "Sulf" samples. The

samples were purged with Argon gas and 2 g NaSH and 60 mg sulfur were added to the "Sulf" samples. The solutions were kept dark at room temperature for one hour with two intervals for shaking the samples, then they were acidified and filtered as described above.

To ensure that the observed sulfurization products originate from covalent incorporation of inorganic sulfur into organic molecules and not only from hydrogensulfide ion adducts an adduct test was conducted. For this, the DOM methanol extract sample "North Sea Control 1" was mixed with an aqueous solution of NaSH (DOC-to-sulfur ratio: 10:1, final DOC concentration: 15 ppm) and immediately analyzed at the FT-ICR-MS. Adducts of elemental sulfur or polysulfides did not occur in our sulfurization experiments because all detected sulfur compounds contained not more than two sulfur atoms in their molecular formula. A broad band mass spectrum was recorded after the fast addition of NaSH and additionally two nominal masses (*m/z* = 377 and 389) were fragmented. For comparison, the same FT-ICR-MS analysis was done with the "North Sea Control 1" extract without addition of inorganic sulfur.

3.8.1.3. Sample preparation and elemental and molecular formula analyses

All samples including the blank were extracted according to ref. 41 using solid phase extraction (SPE) on styrene divinyl benzene polymer filled cartridges (1 g, Agilent Bond Elut PPL, USA). Bulk DOC concentrations of the samples prior to and after incubation were determined by high-temperature catalytic oxidation on a Shimadzu TOC-VCPH analyzer. Accuracy of the DOC determination was validated by analyzing the deep sea reference sample provided by D. Hansell and colleagues (University of Miami, USA). DOC concentrations of all methanol extracts obtained by SPE were determined by taking an aliquot of the extract, removing the methanol by evaporation and dissolving the residue in 0.01 M HCl which was then analyzed on the same Shimadzu TOC-VCPH analyzer. From those values the extraction efficiencies were calculated. DOS concentrations of the SPE-extracts were measured on an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, iCAP 6000, Thermo Fisher Scientific GmbH, Bremen, Germany) and bulk DOS concentrations were estimated based on the extraction efficiency for DOC as done previously (5, 20).

FT-ICR-MS measurements were performed with a solariX Fourier-transform ion cyclotron resonance mass spectrometer with a 15 T magnet system (Bruker Daltonik GmbH, Bremen, Germany). A Bruker Daltonik Apollo II atmospheric pressure electrospray ionization unit (ESI) was used as the external ion source in negative ionization mode. All samples were analyzed in a 1:1 volumetric mixture of methanol (or acetonitrile for derivatization experiment) and ultrapure water. The DOC concentration was adjusted to 15 mg C L^{-1}. The samples were directly infused into the ESI source at a flow rate of 120 µL h^{-1}. For fragmentation experiments, the DOC concentration was adjusted to 100 mg C L^{-1}, and a flow rate of 360 µL h^{-1} was used for ESI. Reproducibility was monitored by analyzing an in-house

reference sample from North Equatorial Pacific Intermediate water (46) every morning and evening. Five hundred transient scans in broadband mode were accumulated for each run, covering the mass range of 150-2000 Da. A method detection limit was applied to remove noise peaks from the data set (47). All detected ions were singly charged. After internal calibration, the mass error was <100 ppb. At this high mass accuracy, molecular formulae were unambiguously assigned to all compounds containing the elements C, H, O, S, N and P. Formula assignment for each detected mass was done after ref. 42 but the allowed number of nitrogen atoms was increased to four. The identified molecular formulae were tentatively assigned to compound groups based on their molar ratios, aromaticity index, and heteroatom content (4, 43). These compound groups were polycyclic aromatics, polyphenols, sugars, and peptides. Because of the multitude of possible isomers behind a given molecular formula, these assignments are not unambiguous but they provide a reasonable overview of possibly structures behind the cocktail of detected molecular formulae.

3.8.1.4. Molecular analysis of sulfur functional groups

Each sample from the sulfurization experiment, controls and “Sulfs”, were analyzed for their S-containing functional groups. For this purpose, the functional group selective wet-chemical alteration reactions were conducted after (20), and samples were analyzed in broadband mode on the FT-ICR-MS before and after the alteration reaction as described above. Each sample was analyzed at least in duplicate. In brief, to test for thiols SPE-DOM samples were dried and dissolved in an acetonitrile solution of the thiol-selective reagent 2-bromo-1,4-naphthoquinone for 30 minutes at 20 °C. For hydrolysis, dried SPE-DOM samples were dissolved in hydrochloric acid (25 %, p.a., Merck, Germany) and heated to 110 °C for 24 hours. For oxidation and deoxygenation experiments, DOM samples were dissolved in acetonitrile and cyanuric chloride and hydrogen peroxide or potassium iodide, respectively, were added. Then, the mixture was allowed to stand at 20 °C for 2 hours.

In addition, collision-induced fragmentation experiments were performed on selected nominal masses, following the procedure as in ref. 20: twelve nominal masses (for M2 Sulf only 6 masses due to shortage of sample) consisting of three CH_2-homologuous series were analyzed. For each nominal mass, 4-7 sulfur-containing molecular formulae were fragmented.

For the statistical interpretation of the functional group selective alteration reactions a variance test was performed as described in ref. 20. With this approach it was tested whether discrepancies between the mass spectra of the derivatization and hydrolysis experiments were actually based on the reaction processes or just due to measurement variations. Briefly, the spectra prior to and after reaction were screened for trends in the FT-ICR-MS signal intensities of the *m/z*-ratios (decreasing or increasing trend after reaction). The same analysis was done for the spectra of the reference sample that was measured every morning and evening and thus represents the instrument variability. The detected variance in the

reference material was set as threshold. Only variance between treated and untreated DOM sample that were above the threshold (higher than instrument variability) were considered as reaction-induced. Compared to the derivatization and hydrolysis, the oxidation and deoxygenation experiments do not lead to a clear separation of reaction products. While there is a characteristic molecule addition in the thiol derivatization and molecule cleavage in hydrolysis, there is only a small (one or two oxygen atoms) increase or decrease in oxygen content for a S-containing molecule in the oxidation and deoxygenation experiment. To determine and visualize even slight differences between the spectra of the oxidation and deoxygenation experiments, the mass spectra were interpreted via multivariate statistical analysis (Bray Curtis dissimilarity).

3.8.1.5. Microbial analysis

To verify the lack of microbial activity during sulfurization, samples for cell counts were taken prior to the experiments and afterwards. The samples were fixed with glutardialdehyde (1 % final concentration, Carl Roth, Germany) and cells were counted with a BD accuri C6 Flow Cytometer (BD Biosciences, USA) using SybrGreen (Invitrogen, United Kingdom). Fluorescent microscopy was also used to verify the negative results from the flow cytometer. Samples were filtered (0.2 μm polycarbonate) and SybrGreen was added. After 30 minutes dark incubation, the filters were analyzed under the microscope.

3.8.1.6. Potential sulfurization reactions analysis

In this study, 20 potential sulfurization reactions were chosen after (13), following nine possibilities of S addition while adding/removing H and/or O: $+ S_1$; $+ S_1/ - H_n$; $+ H_nS_1O_n$; $+ S_1O_n$; $+ S_1O_n/ - H_n$; $+ H_nS_1$; $+S_1/ - H_nO_n$; $+ S_1/ - O_n$; $+ H_nS_1/ - O_n$. The corresponding potential reactions of S addition 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 compounds with S_1 were obtained (Table 3.2). The effectiveness of the potential reactions was considered as a percentage of S_1 formulae present in the sulfurized samples with one potential precursor following the corresponding reaction (13). Additionally, two different groups of sulfur compounds were targeted: the sulfurization produced DOS formulae (Table 3.2a) and those DOS formulae already present in the samples before incubation (Table 3.2b). In this second case, two criteria were followed: 1) The intensity of the DOS compound's mass peaks increased after sulfurization and 2) the mass peaks of the corresponding CHO precursor of the DOS compounds decreased in intensity.

3.8.2. Figures

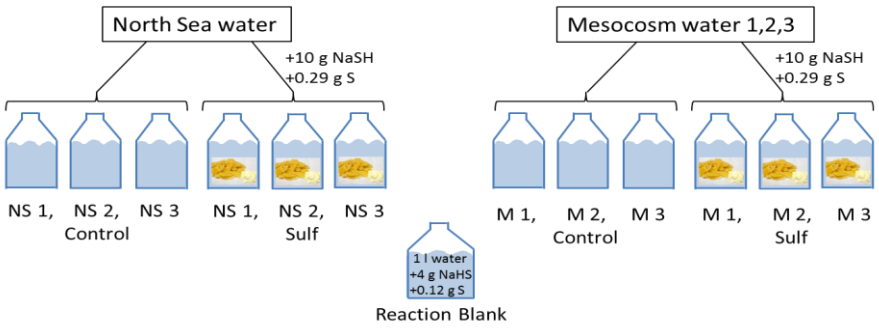

Figure 3.5: Scheme of the setup for the sulfurization experiment. Two types of DOM samples were used, each in triplicate, North Sea and freshly produced mesocosm DOM. Both were divided into controls and those that were mixed with NaSH and S. Additionally, a reaction blank was prepared that consisted of ultrapure water mixed with NaSH and S.

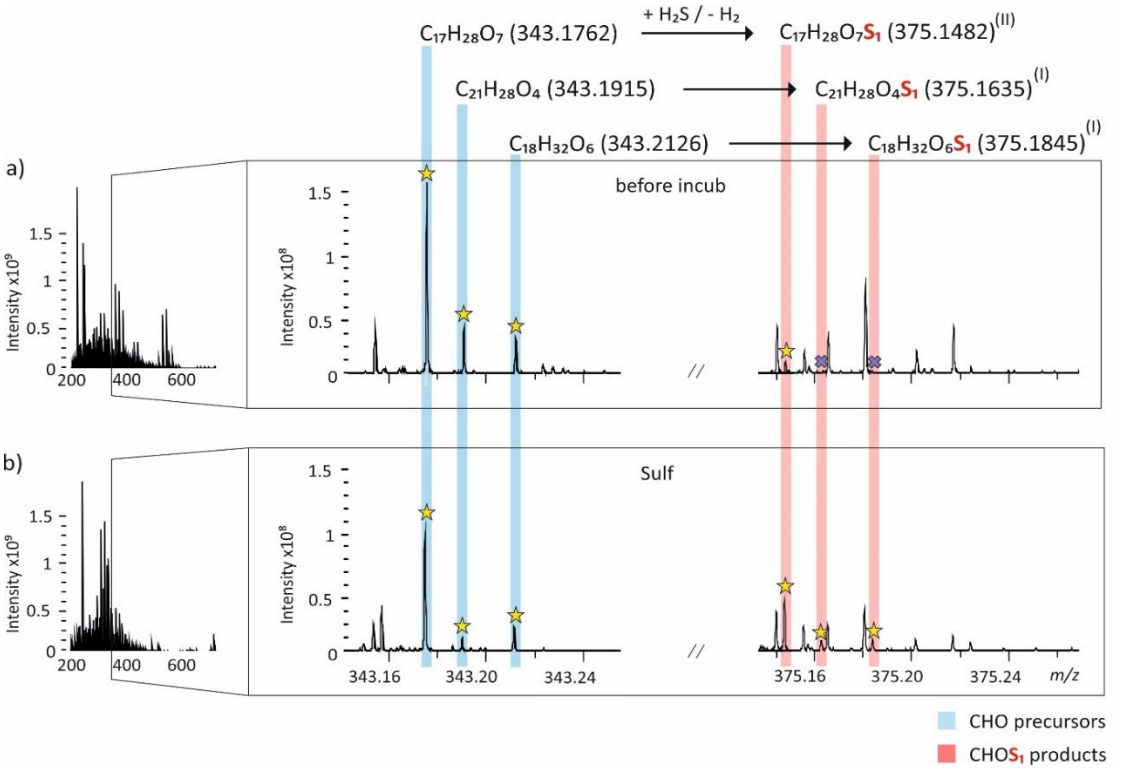

Figure 3.6: Potential sulfurization pathways. Example of potential sulfurization reactions in mesocosm FT-ICR-MS spectra (a) before and (b) after sulfurization. The potential CHO-precursors (blue) following the sulfurization reaction of +H2S / H2 would give the DOS compounds detected after sulfurization (red) as products. The DOS products were targeted in two different categories being (I) exclusively produced after sulfurization (Table 3.2a) and (II) present before incubation with increased intensity after sulfurization and whose precursors decreased in intensity (Table 3.2b).

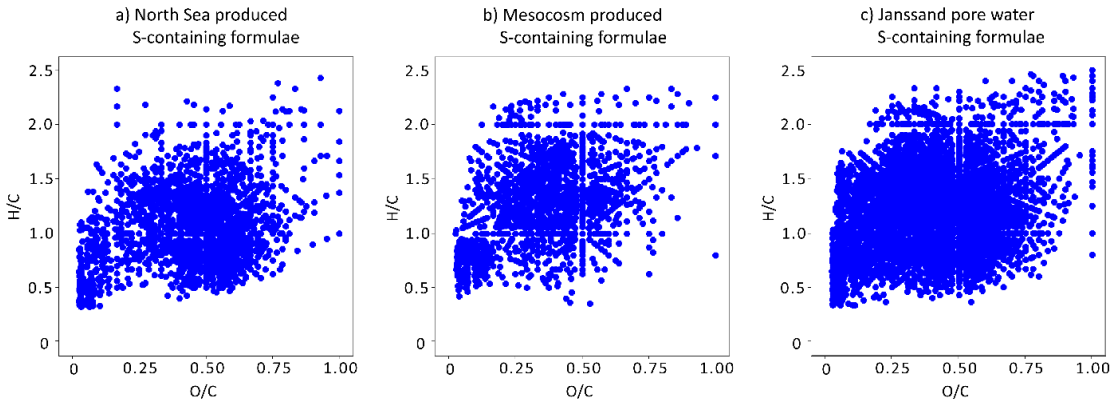

Figure 3.7: Molecular similarity of sulfurization products to sulfidic pore water DOS. (a) DOS molecular formulae produced after sulfurization for the North Sea samples, (b) DOS molecular formulae produced after sulfurization for the mesocosm sample and (c) DOS molecular formulae detected in the Janssand pore water sample.

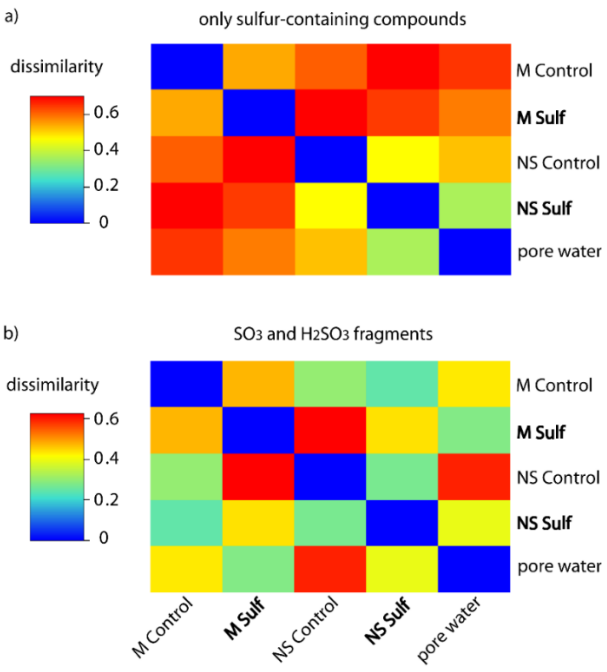

Figure 3.8: Molecular similarity of sulfurization products to the pore water sample. Bray Curtis dissimilarity analysis of the FT-ICR-MS data of the mesocosm ("M") and North Sea ("NS") controls and sulfurized samples (4 weeks) and the Janssand pore water sample. (a) Bray Curtis molecular dissimilarity based on FT-ICR-MS signal intensities of S-containing molecular formulae, (b) Bray Curtis molecular dissimilarity based on the abundance of the SO3- and H2SO3-fragments detected over all nominal masses (FT-ICR-MS signal intensity of fragment-ion divided by intensity of mother-ion). A higher value means higher dissimilarity between the samples. After sulfurization, an increase in similarity between mesocosm as well as North Sea DOS and pore water DOS occurred. For comparison, the dissimilarity between replicates of the fragmentation experiment ranged between 10-25 %.

3.8.3. Tables

Table 3.1: Element concentrations and ratios for all samples obtained from elemental analysis and FT-ICR-MS assigned molecular formulae (intensity weighted = number of carbon or sulfur atoms, respectively, for a detected molecular formula multiplied by its mass intensity divided by the sum of all mass intensities).

Sample	Bulk DOC [µM]	SPE-DOC [µM]	Bulk DOS [µM]	DOS/ DOC (conc.)	No. all formulae (FT-ICR-MS)	No. S-formulae (FT-ICR-MS)	No. S-formulae/ No. all formulae (FT-ICR-MS) [%]	intensity-weighted S/C (FT-ICR-MS) x1000
M1 Con	138	39	1.5	0.011	7658	1995	26.05	6.8
M2 Con	132	42	1.0	0.008	7949	2045	25.73	5.8
M3 Con	179	51	1.2	0.007	7177	1736	24.19	6.1
M1 Sulf	150	32	10.3	0.069	12086	5438	44.99	32.4
M2 Sulf	143	15	21.3	0.149	13948	6122	43.89	32.2
M3 Sulf	206	24	19.7	0.096	11757	5268	44.81	34.3
NS1 Con	188	98	1.4	0.008	12514	3645	29.13	7.0
NS2 Con	191	88	1.6	0.008	12180	3550	29.15	7.0
NS3 Con	192	89	1.5	0.008	11165	3107	27.83	6.7
NS1 Sulf	217	37	16.2	0.075	15970	6003	37.59	12.2
NS2 Sulf	209	29	22.8	0.109	16467	6280	38.14	12.4
NS3 Sulf	228	31	23.2	0.102	16472	6295	38.22	12.7
1h Con A	181	61	3.1	0.017	13724	4376	31.89	7.7
1h Con B	180	65	2.8	0.016	14191	4608	32.47	7.8
1h Sulf A	203	40	10.2	0.050	15735	5675	36.07	10.1
1h Sulf B	205	32	13.4	0.065	16236	5956	36.68	10.3
Pore water	1276	873	47.3	0.037	14285	5713	39.99	17.3

The total number of non-S-containing compounds also increased slightly (mesocosm: +16 %; North Sea: +24 %). Furthermore, a decrease of DOC extraction efficiency after sulfurization was observed. As the bulk DOC concentrations after sulfurization did not decrease, this decrease in extraction efficiency cannot be due to coagulation with inorganic sulfur particles. It is possible though, that while sulfur was incorporated into DOM or due to second step rearrangement reactions like cyclisation reactions (48), small non-solid-phase-extractable organic compounds were eliminated from the molecules. Alternatively, this might be degradation of DOM due to the elevated temperature (50 °C) in the experiment (49).

Table 3.2: Potential reactions of sulfur incorporation adding a H_2S molecule and adding/removing one or two H_2, O_2 and/or H_2O tested for mesocosm and North Sea samples before and after sulfurization. a) percentage of precursors of exclusively after sulfurization visible products (Fig. 3.6.I.) and b) percentage of precursors decreasing in intensity after sulfurization with simultaneously increasing intensity of sulfurization products (Fig. 3.6.II). The different percentages obtained for compounds with only S as heteroatom ($CHOS_1$) and also for those with N and/or P ($CHO(NP)S_1$) are included for every potential reaction.

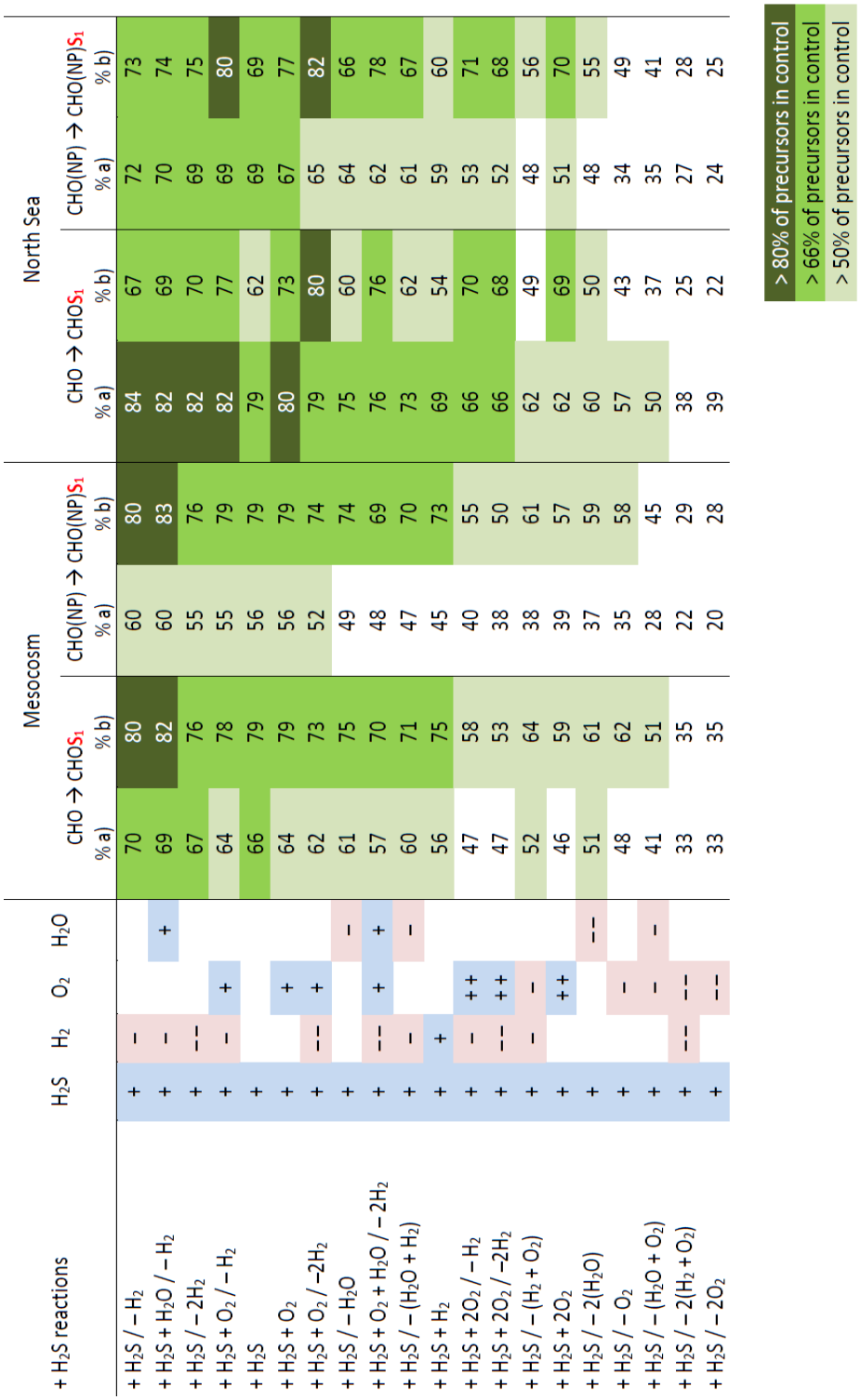

Table 3.3: Considered sulfur-containing molecular formulae of the respective nominal masses in fragmentation experiment analysis.

nominal mass	considered molecular formulae				
	Mesocosm Control	Mesocosm Sulf	North Sea Control	North Sea Sulf	Janssand pore water
m/z 373	$C_{14}H_{13}O_{10}S^-$	$C_{18}H_{13}O_7S^-$	$C_{14}H_{13}O_{10}S^-$	$C_{14}H_{13}O_{10}S^-$	$C_{15}H_{17}O_9S^-$
	$C_{18}H_{13}O_7S^-$	$C_{15}H_{17}O_9S^-$	$C_{18}H_{13}O_7S^-$	$C_{18}H_{13}O_7S^-$	$C_{16}H_{21}O_8S^-$
	$C_{15}H_{17}O_9S^-$	$C_{16}H_{21}O_8S^-$	$C_{15}H_{17}O_9S^-$	$C_{15}H_{17}O_9S^-$	$C_{17}H_{25}O_7S^-$
	$C_{16}H_{21}O_8S^-$	$C_{17}H_{25}O_7S^-$	$C_{16}H_{21}O_8S^-$	$C_{16}H_{21}O_8S^-$	$C_{14}H_{13}O_{10}S^-$
	$C_{17}H_{25}O_7S^-$		$C_{17}H_{25}O_7S^-$	$C_{17}H_{25}O_7S^-$	$C_{18}H_{13}O_7S^-$
					$C_{19}H_{15}O_7S^-$
m/z 387	$C_{15}H_{15}O_{10}S^-$	$C_{15}H_{15}O_{10}S^-$	$C_{18}H_{11}O_8S^-$	$C_{18}H_{11}O_8S^-$	$C_{15}H_{15}O_{10}S^-$
	$C_{19}H_{15}O_7S^-$	$C_{19}H_{15}O_7S^-$	$C_{15}H_{15}O_{10}S^-$	$C_{15}H_{15}O_{10}S^-$	$C_{16}H_{19}O_9S^-$
	$C_{16}H_{19}O_9S^-$	$C_{16}H_{19}O_9S^-$	$C_{19}H_{15}O_7S^-$	$C_{19}H_{15}O_7S^-$	$C_{17}H_{23}O_8S^-$
	$C_{17}H_{23}O_8S^-$	$C_{17}H_{23}O_8S^-$	$C_{16}H_{19}O_9S^-$	$C_{16}H_{19}O_9S^-$	$C_{18}H_{27}O_7S^-$
	$C_{18}H_{27}O_7S^-$	$C_{18}H_{27}O_7S^-$	$C_{17}H_{23}O_8S^-$	$C_{17}H_{23}O_8S^-$	$C_{18}H_{11}O_8S^-$
			$C_{18}H_{27}O_7S^-$	$C_{18}H_{27}O_7S^-$	$C_{19}H_{15}O_7S^-$
m/z 401	$C_{19}H_{13}O_8S^-$	$C_{16}H_{17}O_{10}S^-$	$C_{19}H_{13}O_8S^-$	$C_{19}H_{13}O_8S^-$	$C_{16}H_{17}O_{10}S^-$
	$C_{16}H_{17}O_{10}S^-$	$C_{17}H_{21}O_9S^-$	$C_{16}H_{17}O_{10}S^-$	$C_{16}H_{17}O_{10}S^-$	$C_{17}H_{21}O_9S^-$
	$C_{17}H_{21}O_9S^-$	$C_{18}H_{25}O_8S^-$	$C_{17}H_{21}O_9S^-$	$C_{17}H_{21}O_9S^-$	$C_{18}H_{25}O_8S^-$
	$C_{18}H_{25}O_8S^-$	$C_{19}H_{29}O_7S^-$	$C_{18}H_{25}O_8S^-$	$C_{18}H_{25}O_8S^-$	$C_{19}H_{29}O_7S^-$
	$C_{19}H_{29}O_7S^-$		$C_{19}H_{29}O_7S^-$	$C_{19}H_{29}O_7S^-$	$C_{19}H_{13}O_8S^-$
m/z 415	$C_{16}H_{15}O_{11}S^-$	$C_{17}H_{19}O_{10}S^-$	$C_{16}H_{15}O_{11}S^-$	$C_{16}H_{15}O_{11}S^-$	$C_{17}H_{19}O_{10}S^-$
	$C_{20}H_{15}O_8S^-$	$C_{18}H_{23}O_9S^-$	$C_{20}H_{15}O_8S^-$	$C_{20}H_{15}O_8S^-$	$C_{18}H_{23}O_9S^-$
	$C_{17}H_{19}O_{10}S^-$	$C_{19}H_{27}O_8S^-$	$C_{17}H_{19}O_{10}S^-$	$C_{17}H_{19}O_{10}S^-$	$C_{19}H_{27}O_8S^-$
	$C_{18}H_{23}O_9S^-$	$C_{20}H_{31}O_7S^-$	$C_{18}H_{23}O_9S^-$	$C_{18}H_{23}O_9S^-$	$C_{20}H_{31}O_7S^-$
	$C_{19}H_{27}O_8S^-$		$C_{19}H_{27}O_8S^-$	$C_{19}H_{27}O_8S^-$	$C_{16}H_{15}O_{11}S^-$
	$C_{20}H_{31}O_7S^-$		$C_{20}H_{31}O_7S^-$	$C_{20}H_{31}O_7S^-$	$C_{20}H_{15}O_8S^-$
m/z 375	$C_{17}H_{11}O_8S^-$	$C_{17}H_{11}O_8S^-$	$C_{17}H_{11}O_8S^-$	$C_{17}H_{11}O_8S^-$	$C_{15}H_{19}O_9S^-$
	$C_{14}H_{15}O_{10}S^-$	$C_{18}H_{15}O_7S^-$	$C_{14}H_{15}O_{10}S^-$	$C_{14}H_{15}O_{10}S^-$	$C_{16}H_{23}O_8S^-$
	$C_{18}H_{15}O_7S^-$	$C_{15}H_{19}O_9S^-$	$C_{18}H_{15}O_7S^-$	$C_{18}H_{15}O_7S^-$	$C_{17}H_{27}O_7S^-$
	$C_{15}H_{19}O_9S^-$	$C_{16}H_{23}O_8S^-$	$C_{15}H_{19}O_9S^-$	$C_{15}H_{19}O_9S^-$	$C_{17}H_{11}O_8S^-$
	$C_{16}H_{23}O_8S^-$	$C_{17}H_{27}O_7S^-$	$C_{16}H_{23}O_8S^-$	$C_{16}H_{23}O_8S^-$	$C_{14}H_{15}O_{10}S^-$
	$C_{17}H_{27}O_7S^-$		$C_{17}H_{27}O_7S^-$	$C_{17}H_{27}O_7S^-$	$C_{18}H_{15}O_7S^-$
m/z 389	$C_{15}H_{17}O_{10}S^-$	$C_{15}H_{17}O_{10}S^-$	$C_{18}H_{13}O_8S^-$	$C_{18}H_{13}O_8S^-$	$C_{15}H_{17}O_{10}S^-$
	$C_{19}H_{17}O_7S^-$	$C_{19}H_{17}O_7S^-$	$C_{15}H_{17}O_{10}S^-$	$C_{15}H_{17}O_{10}S^-$	$C_{16}H_{21}O_9S^-$
	$C_{16}H_{21}O_9S^-$	$C_{16}H_{21}O_9S^-$	$C_{19}H_{17}O_7S^-$	$C_{19}H_{17}O_7S^-$	$C_{17}H_{25}O_8S^-$
	$C_{17}H_{25}O_8S^-$	$C_{17}H_{25}O_8S^-$	$C_{16}H_{21}O_9S^-$	$C_{16}H_{21}O_9S^-$	$C_{18}H_{29}O_7S^-$
	$C_{18}H_{29}O_7S^-$	$C_{18}H_{29}O_7S^-$	$C_{17}H_{25}O_8S^-$	$C_{17}H_{25}O_8S^-$	$C_{18}H_{13}O_8S^-$
	$C_{18}H_{13}O_8S^-$	$C_{18}H_{13}O_8S^-$	$C_{18}H_{29}O_7S^-$	$C_{18}H_{29}O_7S^-$	$C_{19}H_{17}O_7S^-$
m/z 403	$C_{19}H_{15}O_8S^-$	$C_{19}H_{15}O_8S^-$	$C_{15}H_{15}O_{11}S^-$	$C_{15}H_{15}O_{11}S^-$	$C_{16}H_{19}O_{10}S^-$
	$C_{16}H_{19}O_{10}S^-$	$C_{16}H_{19}O_{10}S^-$	$C_{19}H_{15}O_8S^-$	$C_{19}H_{15}O_8S^-$	$C_{17}H_{23}O_9S^-$
	$C_{17}H_{23}O_9S^-$	$C_{17}H_{23}O_9S^-$	$C_{16}H_{19}O_{10}S^-$	$C_{16}H_{19}O_{10}S^-$	$C_{18}H_{27}O_8S^-$
	$C_{18}H_{27}O_8S^-$	$C_{18}H_{27}O_8S^-$	$C_{17}H_{23}O_9S^-$	$C_{17}H_{23}O_9S^-$	$C_{18}H_{11}O_9S^-$
	$C_{19}H_{31}O_7S^-$	$C_{19}H_{31}O_7S^-$	$C_{18}H_{27}O_8S^-$	$C_{18}H_{27}O_8S^-$	$C_{15}H_{15}O_{11}S^-$
			$C_{19}H_{31}O_7S^-$	$C_{19}H_{31}O_7S^-$	$C_{19}H_{15}O_8S^-$
					$C_{20}H_{19}O_7S^-$
					$C_{19}H_{31}O_7S^-$

m/z 417	$C_{19}H_{13}O_9S^-$	$C_{20}H_{17}O_8S$	$C_{19}H_{13}O_9S^-$	$C_{19}H_{13}O_9S^-$	$C_{16}H_{17}O_{11}S^-$
	$C_{16}H_{17}O_{11}S^-$	$C_{17}H_{21}O_{10}S^-$	$C_{16}H_{17}O_{11}S^-$	$C_{16}H_{17}O_{11}S^-$	$C_{17}H_{21}O_{10}S^-$
	$C_{20}H_{17}O_8S$	$C_{18}H_{25}O_9S^-$	$C_{20}H_{17}O_8S$	$C_{20}H_{17}O_8S$	$C_{18}H_{25}O_9S^-$
	$C_{17}H_{21}O_{10}S^-$	$C_{19}H_{29}O_8S^-$	$C_{17}H_{21}O_{10}S^-$	$C_{17}H_{21}O_{10}S^-$	$C_{19}H_{29}O_8S^-$
	$C_{18}H_{25}O_9S^-$		$C_{18}H_{25}O_9S^-$	$C_{18}H_{25}O_9S^-$	$C_{19}H_{13}O_9S^-$
	$C_{19}H_{29}O_8S^-$		$C_{19}H_{29}O_8S^-$	$C_{19}H_{29}O_8S$	$C_{20}H_{17}O_8S$
m/z 377	$C_{17}H_{13}O_8S^-$	$C_{17}H_{13}O_8S^-$	$C_{17}H_{13}O_8S^-$	$C_{17}H_{13}O_8S^-$	$C_{14}H_{17}O_{10}S^-$
	$C_{14}H_{17}O_{10}S^-$	$C_{18}H_{17}O_7S^-$	$C_{14}H_{17}O_{10}S^-$	$C_{14}H_{17}O_{10}S^-$	$C_{15}H_{21}O_9S^-$
	$C_{18}H_{17}O_7S^-$	$C_{15}H_{21}O_9S^-$	$C_{18}H_{17}O_7S^-$	$C_{18}H_{17}O_7S^-$	$C_{16}H_{25}O_8S^-$
	$C_{15}H_{21}O_9S^-$	$C_{19}H_{21}O_6S^-$	$C_{15}H_{21}O_9S^-$	$C_{15}H_{21}O_9S^-$	$C_{17}H_{29}O_7S^-$
	$C_{19}H_{21}O_6S^-$	$C_{16}H_{25}O_8S^-$	$C_{19}H_{21}O_6S^-$	$C_{19}H_{21}O_6S^-$	$C_{17}H_{13}O_8S^-$
	$C_{16}H_{25}O_8S^-$	$C_{17}H_{29}O_7S^-$	$C_{16}H_{25}O_8S^-$	$C_{16}H_{25}O_8S^-$	$C_{18}H_{17}O_7S^-$
	$C_{17}H_{29}O_7S^-$		$C_{17}H_{29}O_7S^-$	$C_{17}H_{29}O_7S^-$	$C_{19}H_{21}O_6S^-$
m/z 391	$C_{15}H_{19}O_{10}S^-$	$C_{15}H_{19}O_{10}S^-$	$C_{17}H_{11}O_9S^-$	$C_{17}H_{11}O_9S^-$	$C_{15}H_{19}O_{10}S^-$
	$C_{17}H_{11}O_9S^-$	$C_{17}H_{11}O_9S^-$	$C_{18}H_{15}O_8S^-$	$C_{18}H_{15}O_8S^-$	$C_{16}H_{23}O_9S^-$
	$C_{18}H_{15}O_8S^-$	$C_{18}H_{15}O_8S^-$	$C_{15}H_{19}O_{10}S^-$	$C_{15}H_{19}O_{10}S^-$	$C_{17}H_{27}O_8S^-$
	$C_{19}H_{19}O_7S^-$	$C_{19}H_{19}O_7S^-$	$C_{19}H_{19}O_7S^-$	$C_{19}H_{19}O_7S^-$	$C_{17}H_{11}O_9S^-$
	$C_{16}H_{23}O_9S^-$	$C_{16}H_{23}O_9S^-$	$C_{16}H_{23}O_9S^-$	$C_{16}H_{23}O_9S^-$	$C_{18}H_{15}O_8S^-$
	$C_{17}H_{27}O_8S^-$	$C_{17}H_{27}O_8S^-$	$C_{17}H_{27}O_8S^-$	$C_{17}H_{27}O_8S^-$	$C_{19}H_{19}O_7S^-$
	$C_{18}H_{31}O_7S^-$	$C_{18}H_{31}O_7S^-$	$C_{18}H_{31}O_7S^-$	$C_{18}H_{31}O_7S^-$	$C_{18}H_{31}O_7S^-$
m/z 405	$C_{15}H_{17}O_{11}S^-$	$C_{19}H_{17}O_8S^-$	$C_{18}H_{13}O_9S^-$	$C_{18}H_{13}O_9S^-$	$C_{15}H_{17}O_{11}S^-$
	$C_{19}H_{17}O_8S^-$	$C_{16}H_{21}O_{10}S^-$	$C_{15}H_{17}O_{11}S^-$	$C_{15}H_{17}O_{11}S^-$	$C_{16}H_{21}O_{10}S^-$
	$C_{16}H_{21}O_{10}S^-$	$C_{17}H_{25}O_9S^-$	$C_{19}H_{17}O_8S^-$	$C_{19}H_{17}O_8S^-$	$C_{17}H_{25}O_9S^-$
	$C_{17}H_{25}O_9S^-$	$C_{18}H_{29}O_8S^-$	$C_{16}H_{21}O_{10}S^-$	$C_{16}H_{21}O_{10}S^-$	$C_{18}H_{29}O_8S^-$
	$C_{18}H_{29}O_8S^-$		$C_{17}H_{25}O_9S^-$	$C_{17}H_{25}O_9S^-$	$C_{18}H_{13}O_9S^-$
	$C_{18}H_{13}O_9S^-$		$C_{18}H_{29}O_8S^-$	$C_{18}H_{29}O_8S^-$	$C_{19}H_{17}O_8S^-$
m/z 419	$C_{16}H_{19}O_{11}S^-$	$C_{20}H_{19}O_8S^-$	$C_{19}H_{15}O_9S^-$	$C_{19}H_{15}O_9S^-$	$C_{16}H_{19}O_{11}S^-$
	$C_{20}H_{19}O_8S^-$	$C_{17}H_{23}O_{10}S^-$	$C_{16}H_{19}O_{11}S^-$	$C_{16}H_{19}O_{11}S^-$	$C_{17}H_{23}O_{10}S^-$
	$C_{17}H_{23}O_{10}S^-$	$C_{18}H_{27}O_9S^-$	$C_{20}H_{19}O_8S^-$	$C_{20}H_{19}O_8S^-$	$C_{18}H_{27}O_9S^-$
	$C_{18}H_{27}O_9S^-$	$C_{19}H_{31}O_8S^-$	$C_{17}H_{23}O_{10}S^-$	$C_{17}H_{23}O_{10}S^-$	$C_{19}H_{15}O_9S^-$
	$C_{19}H_{31}O_8S^-$		$C_{18}H_{27}O_9S^-$	$C_{18}H_{27}O_9S^-$	$C_{20}H_{19}O_8S^-$
			$C_{19}H_{31}O_8S^-$	$C_{19}H_{31}O_8S^-$	$C_{19}H_{31}O_8S^-$

Table 3.4: Observed neutral losses from isolated masses (*m/z*) of the mesocosm and North Sea samples (controls and sulfurized ones). Only sulfur-containing molecular formulae were considered. The ✓ symbolizes a found fragment and the X no found fragment. For the "M2 Sulf" sample only 6 nominal masses could be analyzed due to shortage of sample.

	neutral losses					neutral losses				
sample	Nominal mass	SO_3	H_2SO_3	SO_2	H_2S	Nominal mass	SO_3	H_2SO_3	SO_2	H_2S
M1 Control		✓	✓	✓	X		✓	✓	X	X
M2 Control		✓	✓	✓	X		✓	✓	X	X
M3 Control		✓	✓	✓	X		✓	✓	X	X
M1 Sulf		✓	✓	✓	✓		✓	✓	X	✓
M2 Sulf		✓	✓	✓	✓		-	-	-	-
M3 Sulf		✓	✓	✓	X		✓	✓	X	✓
NS1 Control	*m/z* 373	✓	✓	✓	X	*m/z* 401	✓	✓	✓	X
NS2 Control		✓	✓	✓	X		✓	✓	✓	X
NS3 Control		✓	✓	✓	X		✓	✓	✓	X
NS1 Sulf		✓	✓	✓	✓		✓	✓	✓	✓
NS2 Sulf		✓	✓	✓	✓		✓	✓	✓	✓
NS3 Sulf		✓	✓	✓	✓		✓	✓	✓	✓
Pore water		✓	✓	✓	✓		✓	✓	✓	✓
M1 Control		✓	✓	✓	X		✓	✓	X	X
M2 Control		✓	✓	X	X		✓	✓	X	X
M3 Control		✓	✓	X	X		✓	✓	X	X
M1 Sulf		✓	✓	✓	X		✓	X	X	X
M2 Sulf		✓	✓	✓	✓		-	-	-	-
M3 Sulf		✓	✓	X	✓		✓	✓	X	X
NS1 Control	*m/z* 375	✓	✓	✓	X	*m/z* 403	✓	✓	✓	X
NS2 Control		✓	✓	✓	X		✓	✓	✓	X
NS3 Control		✓	✓	✓	X		✓	✓	✓	X
NS1 Sulf		✓	✓	✓	✓		✓	✓	✓	✓
NS2 Sulf		✓	✓	✓	✓		✓	✓	✓	✓
NS3 Sulf		✓	✓	✓	✓		✓	✓	✓	✓
Pore water		✓	✓	✓	✓		✓	✓	✓	✓
M1 Control		✓	✓	X	X		X	✓	X	X
M2 Control		✓	✓	X	X		X	✓	X	X
M3 Control		✓	✓	✓	X		✓	X	X	X
M1 Sulf		✓	✓	✓	✓		X	✓	X	X
M2 Sulf	*m/z* 377	✓	✓	✓	✓	*m/z* 405	-	-	-	-
M3 Sulf		✓	✓	X	✓		✓	X	X	X
NS1 Control		✓	✓	✓	X		✓	✓	✓	X
NS2 Control		✓	✓	✓	X		✓	✓	✓	X

NS3 Control		✓	✓	✓	X		✓	✓	✓	X
NS1 Sulf		✓	✓	✓	✓		✓	✓	✓	✓
NS2 Sulf		✓	✓	✓	✓		✓	✓	✓	✓
NS3 Sulf		✓	✓	✓	✓		✓	✓	✓	✓
Pore water		✓	✓	✓	✓		✓	✓	✓	✓
M1 Control		✓	✓	X	X		✓	✓	X	X
M2 Control		✓	✓	X	X		✓	✓	X	X
M3 Control		✓	✓	X	X		✓	✓	X	X
M1 Sulf		✓	✓	X	✓		✓	X	X	X
M2 Sulf		✓	✓	✓	✓		-	-	-	-
M3 Sulf		✓	✓	X	X		✓	X	X	✓
NS1 Control	*m/z* 387	✓	✓	✓	X	*m/z* 415	✓	✓	✓	X
NS2 Control		✓	✓	✓	X		✓	✓	✓	X
NS3 Control		✓	✓	✓	X		✓	✓	✓	X
NS1 Sulf		✓	✓	✓	✓		✓	✓	✓	✓
NS2 Sulf		✓	✓	✓	✓		✓	✓	✓	✓
NS3 Sulf		✓	✓	✓	✓		✓	✓	✓	✓
Pore water		✓	✓	✓	✓		✓	✓	✓	✓
M1 Control		✓	✓	X	X		✓	✓	X	X
M2 Control		✓	✓	✓	X		✓	✓	X	X
M3 Control		✓	✓	X	X		✓	✓	X	X
M1 Sulf		✓	✓	✓	X		✓	✓	X	X
M2 Sulf		✓	✓	✓	✓		-	-	-	-
M3 Sulf		✓	✓	X	X		✓	✓	X	✓
NS1 Control	*m/z* 389	✓	✓	✓	X	*m/z* 417	✓	✓	✓	X
NS2 Control		✓	✓	✓	✓		✓	✓	✓	X
NS3 Control		✓	✓	✓	X		✓	✓	✓	X
NS1 Sulf		✓	✓	✓	✓		✓	✓	✓	✓
NS2 Sulf		✓	✓	✓	✓		✓	✓	✓	✓
NS3 Sulf		✓	✓	✓	✓		✓	✓	✓	✓
Pore water		✓	✓	✓	✓		✓	✓	✓	✓
M1 Control		✓	✓	X	X		X	X	X	X
M2 Control		✓	✓	X	X		✓	X	X	X
M3 Control		✓	✓	✓	X		✓	✓	X	X
M1 Sulf		✓	✓	X	X		X	X	X	X
M2 Sulf		✓	✓	X	X		-	-	-	-
M3 Sulf	*m/z* 391	✓	✓	✓	X	*m/z* 419	✓	✓	X	X
NS1 Control		✓	✓	✓	X		✓	✓	✓	X
NS2 Control		✓	✓	✓	X		✓	✓	✓	X
NS3 Control		✓	✓	✓	X		✓	✓	✓	X
NS1 Sulf		✓	✓	✓	✓		✓	✓	✓	✓
NS2 Sulf		✓	✓	✓	✓		✓	✓	✓	✓

NS3 Sulf		✓	✓	✓	✓		✓	✓	✓	✓
Pore water		✓	✓	✓	✓		✓	✓	✓	✓

4. Manuscript III

Molecular clues for a pathway of dissolved organic sulfur produced in sulfidic sediments to the open ocean

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manuscript in preparation

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4.1. Abstract

Dissolved organic sulfur (DOS) is a notably understudied component of marine dissolved organic matter (DOM) although it is the largest reservoir of marine organic sulfur. Knowledge on the sources, turnover, and molecular composition of DOS is fragmentary, and consequently, the role of DOS in marine sulfur cycling is not well understood. Sulfidic marine environments are potential hotspots of DOS formation, because reactive reduced sulfur species are known to abiotically react with natural organic matter. The objective of this study was to quantify and molecularly characterize solid-phase-extractable DOS from a range of oxygen-rich to oxygen-depleted marine environments and to identify molecular clues for the pathways of DOS in the marine environment. For this purpose, DOS from two contrasting environments, the German Wadden Sea sediment and the Black Sea at various water depths (oxic and anoxic), were examined and its molecular composition compared to our previous results from the open North Sea off Germany and the deep North Pacific. For the molecular characterization of DOS, selective wet-chemical alteration experiments targeting different sulfur-containing functional groups were applied prior to Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS). These experiments included harsh hydrolysis, selective derivatization of thiols, oxidation, and deoxygenation to test for thioesters, sulfonic acid esters, alkylsulfates, thiols, non-aromatic thioethers, and sulfoxides. Additionally, collision-induced fragmentation experiments were applied to test for sulfonic acids. Highest concentrations of DOS and highest S/C ratios in DOM were found in the sulfate-reducing environments. Most sulfur was present as sulfonic acid, independent of the environmental conditions. DOS from the oxic open ocean and sulfate-reducing environments was undistinguishable on a molecular formula and also on a structural level. One exception were non-aromatic thioethers that were found in low abundance only in sediment porewater. Thioethers are most likely not stable on a long term in oxic waters. The molecular similarity of DOS and the systematic differences in DOS concentration indicate that reducing sediments are a main source of marine dissolved organic sulfur.

4.2. Introduction

Marine dissolved organic matter (DOM) is a complex mixture of thousands of organic compounds (Dittmar, 2015), and it forms one of the largest reservoirs of organic carbon on Earth's surface (Hansell et al., 2009). DOM is often operationally divided into categories of different turn-over rates with the most stable and biggest fraction showing a turn-over rate of about 16,000 years which is thus named refractory DOM (Hansell, 2013). The reasons behind this long-term stability are still matter of debate (Dittmar, 2015), a fraction of DOM might be intrinsically resistant due to its molecular structure (Dittmar and Paeng, 2009), while other compounds are too dilute in seawater to be efficiently taken up by microbes (Arrieta et

al., 2015). Knowledge on the molecular structures of the compounds in the DOM mixture is essential to understand their reactivity. Structural analysis of DOM in general is a major challenge due to the great complexity, the low concentration of each individual compound, and the multiplicity of structural isomers for each molecular formula. So far only a minor fraction (<5%) of DOM is characterized on a molecular compound level (Dittmar and Stubbins, 2014), and sulfur-containing compounds are among the most understudied constituents of DOM.

Marine organisms biosynthesize and actively excrete sulfur-containing organic compounds. For instance, all marine algae produce sulfated polysaccharides as major extracellular matrix components (Popper et al., 2011). Another common sulfur-containing compound in marine organisms is dimethylsulfoniopropionate (DMSP). DMSP functions as osmolyte in plankton and macroalgae, and it is one of the most significant single substrates for bacterioplankton in the upper ocean (Kiene et al., 2000; Howard et al., 2006). One of the main degradation products is the volatile dimethyl sulfide (DMS) which quickly oxidizes in the atmosphere to form sulfate, and as such it plays a major role in the formation of condensation nuclei and clouds (Schlesinger and Bernhardt, 2013). In sediments, reactive reduced sulfur species, derived from microbial sulfate reduction, are known to abiotically react with natural organic matter which may contribute to organic matter preservation in sediments (Sinninghe Damsté et al., 1989; Vairavamurthy et al., 1995 and references therein; Schouten et al., 1994; Schneckenburger et al., 1998; Schmidt et al., 2009). In laboratory experiments we found evidence for abiotic sulfurization of DOM forming dissolved organic sulfur (DOS) which highly resembles natural pore water DOS (Pohlabeln et al., submitted). In the water column, DOS forms the largest pool of organic sulfur with a global inventory of >6.7 Pg S (Ksionzek et al., 2016). Despite its ubiquity and the well-established linkages between the biogeochemical cycling of sulfur, carbon and other elements and also the global climate system, our knowledge on the sources, turnover and molecular composition of DOS is at best fragmentary (Lechtenfeld et al., 2011; Gonsior et al., 2011; Levine, 2016). Consequently, the marine organic sulfur cycle is poorly understood as a whole.

In a previous study (Pohlabeln and Dittmar, 2015) we provided novel insights into the structure of non-volatile solid-phase extractable DOS (SPE-DOS, Dittmar et al., 2008). We applied selective chemical treatments prior to Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) to two marine DOM samples, one from the open North Sea surface (Germany) and one from North Equatorial Pacific Intermediate Water (NEqPIW, Green et al., 2014). Targets were specific sulfur-containing functional groups that potentially occur in DOS. In both samples the only observed functional group was the sulfonic acid group which indicates that the sulfur-containing compounds in the water column of the ocean are fully oxidized and rather stable species. In another study, we found indications that DOS compounds were produced in sulfidic sediments and show a reduced character (Pohlabeln et al., submitted). Additionally, elevated concentrations of DOS in anoxic coastal and shelf

sediments had previously been interpreted as evidence for DOS originating in these environments (Schmidt et al., 2009; Seidel et al., 2014). Wave and tide induced advective flux through permeable coastal sediments as well as submarine groundwater discharge may carry DOS from its anoxic formation site into the ocean water column (Billerbeck et al., 2006; Beck et al., 2009; Seidel et al., 2014). We hypothesize that sulfate-reducing zones in the water column may also be hotspots of abiotic DOM sulfurization.

The objective of this study was to quantify and molecularly characterize solid-phase extractable DOS from a range of oxygen-rich to oxygen-depleted marine environments and to identify molecular clues for the pathway of DOS to the open ocean. We chose two very different study sites to investigate whether the structure of sulfur-containing compounds in DOM depends on the prevailing environmental conditions: a well-studied sandbank in the German Wadden Sea (Janssand; Seidel et al., 2014) to investigate DOS in sulfate-reducing sediment porewater, and the Black Sea to investigate DOS in the water column under different redox conditions. At Janssand, DOM is largely derived from microbial reworking of marine sedimentary deposits, and DOC concentrations reach up to 2.7 mM in the sulfate-reducing porewater (Seidel et al., 2014). The Black Sea is the world's largest anoxic marine basin. Its water column has a permanent chemical stratification with oxic (<100 m), anoxic (~100-120 m), and sulfidic (>200 m) layers (Özsoy and Ünlüata, 1997). The deep basin of the Black Sea is oxygen-free, however, also sulfate reduction rates are low (Albert et al., 1995). Concentrations of dissolved organic carbon are high (~120 µM, Ducklow et al., 2007; Margolin et al., 2016) compared to the deep open ocean (~40 µM, Hansell et al., 2009), possibly due to downward export of terrigenous DOM from riverine inputs (Margolin et al., 2016).

Analysis was conducted as outlined in Pohlabeln and Dittmar (2015) via a combination of ultrahigh-resolution FT-ICR-MS and targeted wet-chemical reactions. FT-ICR-MS has become one of the most powerful techniques for the molecular characterization of DOM because it enables the obtainment of thousands of molecular formulae of intact DOM compounds within the complex DOM mixture (e.g., Koch et al., 2005; Dittmar and Paeng, 2009; Hertkorn et al., 2013; Osterholz et al., 2014). To obtain structural information on DOS groups beyond the molecular formula level, we applied selective chemical alteration reactions prior to the analysis with FT-ICR-MS. To test for the presence of thiols, the selective derivatization reagent 2-bromo-1,4-naphthoquinone was used. Hydrolysis was conducted to screen for thioesters as well as for sulfonic acid esters and alkylsulfates. To analyze for non-aromatic thioethers an oxidation was performed and a deoxygenation for possibly occurring sulfoxides. Collision fragmentation experiments within the FT-ICR-MS were done to test for sulfonic acids for which no selective derivatization reaction exists.

4.3. Material and Methods

4.3.1. Study sites

The sandbank Janssand belongs to the Wadden Sea and is located at the back barrier basin of Spiekeroog Island at the northwestern coast of Germany. This site has been studied before and detailed description can be found in e.g. Billerbeck et al. (2006), Roy et al. (2008), and Seidel et al. (2014). Janssand is an advection-dominated system that is covered by 1-2 m water during high tide and exposed for up to 6 h during low tide. Janssand sediment is dominated by fine-grained sands and consists mainly of marine deposits in the upper 3 m. The sediment becomes anoxic at <10 cm depth (Seidel et al., 2014).

The Black Sea is a marginal sea that is surrounded by Europe, Anatolia, and the Caucasus. It has a surface area of $4.2x10^5$ km², a maximum depth of 2200 m, and a volume of $5.3x10^5$ km³, representing the largest land-locked marine basin in the world (Özsoy and Ünlüata, 1997). The Black Sea is almost completely anoxic, containing oxygen only in the upper water layers (<100 m) and sulfide in deeper waters with a permanent halocline separating oxic and anoxic waters. The Black Sea is fed by freshwater inflow from several rivers mainly the Danube, Dnepr, and Dnestr and by saline water from the Mediterranean Sea via the Bosporus (Özsoy and Ünlüata, 1997). At the Bosporus, a two-layer flow is observed. The upper stream going southward has a salinity of 17.5 which is lower than the Black Sea surface, and the lower stream going northward has a salinity of 35-38 which is higher than the Black Sea deep water. These differences imply a mixture of water masses within the Black Sea (Albert et al., 1995). Nevertheless, vertical mixing is limited. Seasonal and interannual variability extends to depths of around 500 m. Below the halocline, the intrusion of the Mediterranean water drives the interior mixing (Özsoy and Ünlüata, 1997). This restricted mixing provides a permanent chemical stratification showing an oxic layer (<70 m depth), an anoxic layer (70-120 m), and a sulfidic layer (>120 m) (Luther et al., 1991; Mopper and Kieber, 1991; Ducklow et al., 2007).

For comparison, we include published data for the coastal North Sea (Germany) and for North Equatorial Pacific Intermediate Water (NEqPIW), which is one of the oldest oceanic water masses (Pohlabeln and Dittmar, 2015).

4.3.2. Sampling, sample preparation and bulk parameters

Pore water samples were taken from Janssand in 2010 (Seidel et al., 2014). A 70 m long transect was sampled at various depths during low tide for one year (March, May, July, and November), for details see Seidel et al. (2014). Briefly, samples were taken with help of stainless steel tubes that were pushed into the sediment, and water was pumped with vacuum hand pumps or PE syringes, and filtered through glass microfiber filters (GMF, 2 µm, Whatman, USA) and glass fiber filters (GF/F, 0.7 µm, Whatman, USA) that were combusted to

remove organic contaminants (4h, 400°C). The filtered samples were acidified to pH 2 with hydrochloric acid (p.a., Merck, Germany) and extracted according to Dittmar et al. (2008) using solid phase extraction (SPE) on styrene divinyl benzene polymer filled cartridges (1 g, Agilent Bond Elut PPL, USA). For the experiments in this study, samples from different seasons were pooled from those depths and sites were the largest number of sulfur-containing DOM molecular formulae was previously detected, which coincided with anoxic conditions near the low tide water line (Seidel et al., 2014). Thus, the pooled sample contained porewater from the sediment surface down to 1 m below sediment surface.

Black Sea water samples were taken at the south-western region of the basin (41° 37.84' N, 29° 1.90' E) using a CTD during the cruise MSM15-1 with RV Meteor in April 2010. The sampled depths were 2 m, 20 m, 100 m, 120 m, 350 m, and 641 m. Immediately after sampling, water samples were frozen and transported to Oldenburg, Germany. After unfreezing, the samples were filtered, acidified, and extracted as outlined above for the Janssand sample. The extracts from 2 m depth is referred to as "oxic+photo" due to the additional effect by exposure to light, the extract from 20 m depth is referred to as "oxic", extracts from 100 and 120 m depth were pooled and named "anoxic", and extracts from 350 and 641 m depth were pooled and named "sulfidic", reflecting the environmental conditions at the respective depth.

DOC concentrations were determined in the original filtered and acidified water samples and solid-phase extracts by high-temperature catalytic oxidation on a Shimadzu TOC-VCPH analyzer. For the determination of DOC in the extracts, aliquots of the methanol extracts were evaporated to dryness and redissolved in ultrapure water of pH 2. The accuracy of the DOC measurements was validated with help of the deep sea reference samples provided by D. Hansell (University of Miami, USA). DOS concentration in the SPE-extracts was measured on an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, iCAP 6000, Thermo, Bremen, Germany). DOS concentrations for the bulk water were approximated under consideration of the extracted volumes and the respective extraction efficiency for DOC (67 % for Janssand, 60-67 % for Black Sea). In extraction tests with the model compounds used in Pohlabeln and Dittmar (2015) bearing different sulfur-containing functional groups we found that all DOS compounds showed similar extraction efficiencies.

4.3.3. Molecular analysis via FT-ICR-MS

FT-ICR-MS analyses were performed on a solariX FT-ICR-MS with a 15 T magnet system (Bruker Daltonik GmbH, Bremen, Germany). A Bruker Daltonik Apollo II atmospheric pressure electrospray ionization unit (ESI) was used as the external ion source in negative ionization mode. All samples were analyzed in a 1:1 volumetric mixture of methanol (or acetonitrile for derivatization experiment) and ultrapure water. The concentration was adjusted to 20 mg carbon/L. The samples were directly infused into the ESI source at a flow rate of 120 µL/h. For

fragmentation experiments, the concentration ranged between 120 to 150 mg carbon/L at a flow rate of 360 µL/h. Reproducibility was monitored by analyzing an internal deep-sea DOM reference sample (NEqPIW, Green et al., 2014) every morning and evening. Five hundred transient scans in broadband mode were accumulated for each run, covering the mass range of 150-2000 Da. For the alteration experiments (derivatization, hydrolysis, oxidation, and deoxygenation) only molecular masses were considered that were detected with a signal-to-noise ratio (S/N) greater than five and detection in at least two procedural replicates. The same signal-to-noise-ratio was applied to the fragmentation spectra. Limited amount of sample prohibited replicate measurements for the bulk (untreated) samples and thus, instead of the replicate detection criterion, a method detection limit (Riedel and Dittmar, 2014) was applied to reliably remove noise peaks from the data set. All detected ions were singly charged. After internal calibration, the mass error was <100 ppb. At this high mass accuracy, molecular formulae were unambiguously assigned to all compounds containing the elements C, H, O, S, N and P. Formula assignment for each detected mass was done after Rossel et al. (2013) but the allowed number of nitrogen atoms was increased to four. For statistical analysis, the detected masses were normalized to the sum of all mass intensities of the corresponding sample. For the calculation of intensity-weighted carbon-to-sulfur ratios of individual samples we first multiplied the number of carbon or sulfur atoms, respectively, for each detected molecular formula with the respective signal intensity. The resulting values were summed up across all detected molecular formulae for carbon and sulfur, respectively. The intensity weighted carbon-to-sulfur ratios were then calculated from these values.

The functional group selective wet-chemical alteration reactions were conducted as in our previous study in which also the applicability of the methods was tested (Pohlabeln and Dittmar, 2015). Briefly, for the thiol derivatization, dried DOM samples (duplicates) were dissolved in an acetonitrile solution of the thiol-selective reagent 2-bromo-1,4-naphthoquinone for 30 minutes at 20 °C. For the hydrolysis, dried DOM samples (duplicates) were dissolved in hydrochloric acid (25 %) and heated to 110 °C for 24 hours. For the oxidation and deoxygenation experiment, dried DOM samples (triplicates) were dissolved in acetonitrile and cyanuric chloride and hydrogen peroxide or potassium iodide, respectively, were added and the mixture was allowed to stand at 20 °C for 2 hours. In all these experiments DOM samples without ("untreated") and with described treatment were diluted 1:1 with ultrapure water prior to FT-ICR-MS analysis. For the collision-induced fragmentation twelve nominal masses consisting of three CH_2-homologuous series (Stenson et al., 2002) were analyzed representatively for the entire sample. For each nominal mass 3-7 sulfur-containing exact masses were analyzed.

4.3.4. Statistical analysis of FT-ICR-MS data

To test whether observed differences between the spectra of the derivatization and hydrolysis experiments were actually based on the reaction processes or just due to measurement variations, a statistical variance test was performed as described in Pohlabeln and Dittmar (2015). Briefly, the spectra prior to and after reaction were screened for trends in the signal intensities of all detected masses (decreasing or increasing trend after reaction). The same analysis was done for the spectra of the reference material that was measured every morning and evening and thus represents the instrument variability. The detected variance in the reference material was set as the threshold. Only variance between treated and untreated DOM sample that was above the threshold (higher than instrument variability) was considered as reaction-induced.

In contrast to derivatization and hydrolysis, the oxidation and deoxygenation experiments do not lead to a clear separation of reaction products. While there is a characteristic molecule addition in the thiol derivatization and molecule cleavage in hydrolysis, there is only a small (one or two oxygen atoms) increase or decrease in oxygen content for a sulfur-containing molecule in the oxidation and deoxygenation experiments. To determine and visualize even slight differences between the spectra induced in the oxidation and deoxygenation experiments, the mass spectra were interpreted via multivariate statistical analysis (Bray Curtis dissimilarity). All multivariate statistical analyses were performed with help of the R software package (version 2.0).

4.4. Results

4.4.1. Bulk parameters

DOC concentration in the Janssand pore water was very high (1276 µM) and distinctly lower in the Black Sea, where it decreased with water depth (from 177 to 109 µM; Table 4.1). The concentration of DOS was also highest at Janssand (47.3 µM) and distinctly lower in the Black Sea, increasing with water depth from 1.0 to 1.5 µM. The corresponding organic sulfur-to-organic carbon ratio in SPE-DOM was higher at Janssand (37 $x10^{-3}$) compared to the Black Sea where the ratio increased from 5.6 $x10^{-3}$ at the sea surface to 14 $x10^{-3}$ in the sulfidic deep layer. Intensity-weighted element ratios calculated from the molecular formulae detected via FT-ICR-MS showed a similar overall trend and were in the same order of magnitude (Table 4.1).

Table 4.1: Element concentrations and ratios for all samples obtained from elemental analysis and FT-ICR-MS assigned molecular formulae (intensity weighted = number of carbon or sulfur atoms, respectively, for a detected molecular formula multiplied by its mass intensity divided by the sum of all mass intensities).

Sample	DOC [µM]	DOS [µM]	DOS/DOC (conc.) x1000	No. all formulae (FT-ICR-MS)	No. S-formulae (FT-ICR-MS)	No. S-formulae/ No. all formulae (FT-ICR-MS) [%]	intensity-weighted S/C (FT-ICR-MS) x1000	intensity-weighted S/C of common molecular Formulae (FT-ICR-MS) x1000
NEqPlW	44	0.18	4.09	5568	1196	21.5	5.79	2.47
North Sea	94	0.84	8.94	6248	1495	24.0	6.51	2.71
Janssand, pore water	1276	47.29	37.06	5420	1563	28.8	13.98	5.44
BS oxic+photo	177	0.99	5.59	5561	1274	22.9	6.14	2.13
BS oxic	178	0.96	5.39	6869	1766	25.7	7.59	2.55
BS anoxic	124	0.77	6.21	6126	1432	23.4	6.05	2.19
BS sulfidic	109	1.52	13.94	6094	1481	24.3	6.14	2.27

4.4.2. General molecular trends on a molecular formula level

Via broad band FT-ICR-MS analysis 5,420 – 6,869 molecular formulae were detected in each sample, 1,196 – 1,766 of them contained sulfur (Table 4.1). Although the Black Sea oxic sample showed the highest number of sulfur-containing compounds, the percentage of sulfur-containing molecular formulae was highest in the pore water sample (29 %). When weighted by FT-ICR-MS signal intensity, this trend was even more pronounced. Of all detected sulfur-containing molecular formulae, 299 co-occurred in all samples. Janssand had the highest number of exclusive sulfur-containing molecular formulae (n = 466) that did not occur in any of the other samples which could be seen directly from the spectra (Fig. 4.1). Overall, the water column samples showed a higher level of similarity among themselves than compared with Janssand. The water column samples shared 533 common sulfur-containing molecular formulae, of which 234 did not occur at Janssand. This trend was also apparent when FT-ICR-MS signal intensities were taken into account. A Bray-Curtis dissimilarity analysis based on the signal intensities of the detected masses (Fig. 4.2 a-d) revealed sharp dissimilarities of pore water DOM, compared to all other samples. This difference was apparent when all masses, all common masses, all S-containing molecular formulae and all common S-containing molecular formulae were considered. For most of these categories, the Black Sea samples showed a high similarity in between themselves, and the Black Sea was more similar to the North Sea than to North Equatorial Pacific Intermediate Water (NEqPIW).

4.4.3. Trends in sulfur functionalities

Thiols: The comparison of the DOM spectra with no NQBr added and NQBr added revealed no detectable difference in the distribution of sulfur-containing compounds for all tested samples (variance test, see Material and methods). For this comparison we not only screened for the mono- but also for the di-reacted products of the thiol derivatization reaction. We conclude that thiols did not significantly contribute to solid-phase-extractable DOS in our samples.

Thioesters, sulfonic acid esters, and alkylsulfates: In the hydrolysis experiment none of the analyzed samples, showed any significant difference in the distribution of sulfur-containing compounds between the hydrolysis experiment and the control spectra of untreated sample (variance test). Based on this, thioesters, sulfonic acid esters, and alkylsulfates did not significantly contribute to solid-phase-extractable DOS in our samples.

Thioethers and sulfoxides: In the deoxygenation experiment to test for sulfoxides, no significant difference (variance test) in the distribution of the sulfur-containing compounds in the DOM spectra with and without reagents were detected for any sample. For the oxidation experiment, only the pore water sample from Janssand showed the presence of non-aromatic thioethers (Fig. 4.3). On average, in the Janssand sample, sulfur-containing molecular formulae contained 14 % more oxygen after oxidation. In all other samples, including the

Black Sea sulfidic sample, the distribution of the sulfur-containing compounds did not change due to oxidation. Thus, the only sample that contained detectable amounts of thioethers was Janssand pore water.

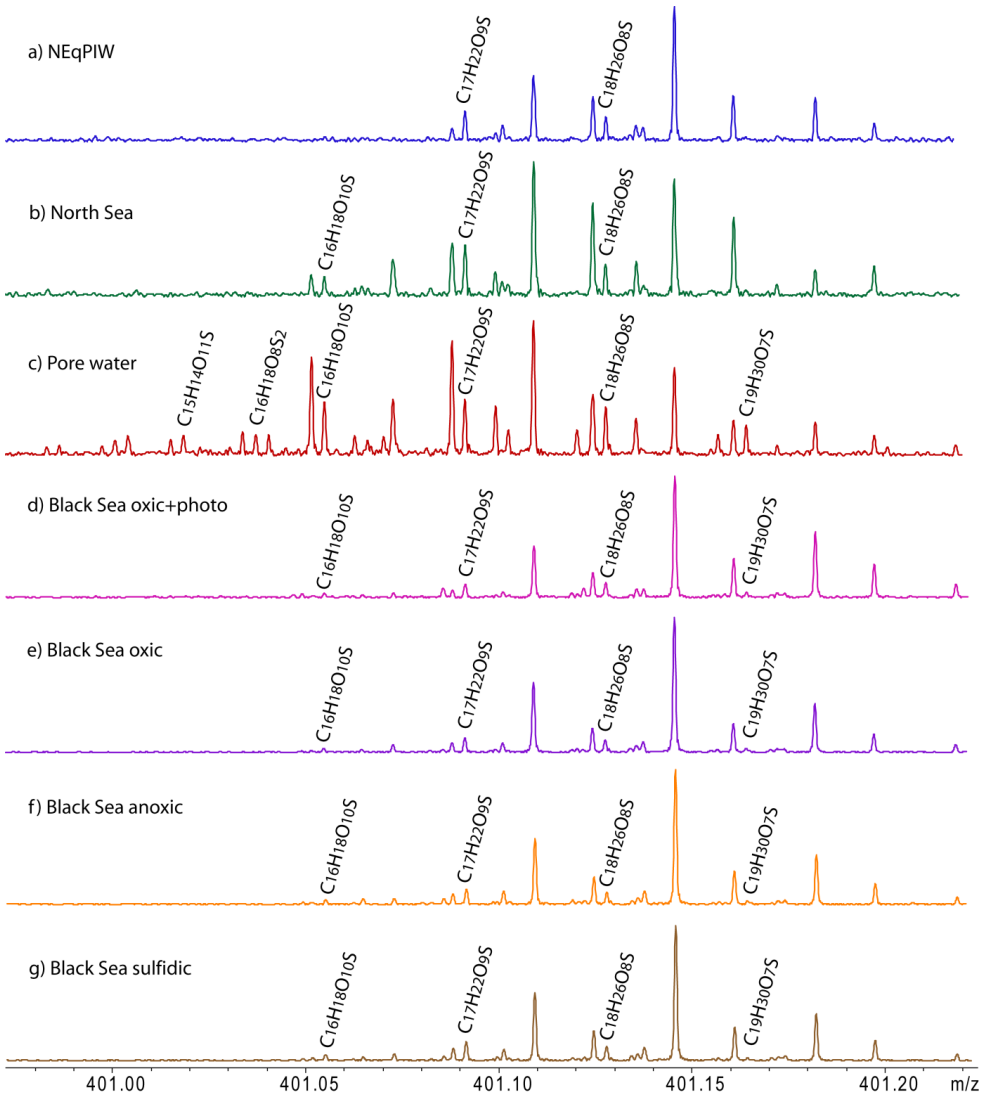

Figure 4.1: Exemplary sections of the FT-ICR mass spectra for all analyzed environments. Comparison of one nominal mass (m/z = 401) out of the FT-ICR mass spectra of the a) North equatorial intermediate water (NEqPIW), b) North Sea, c) Janssand pore water, and Black Sea water column (d) oxic+photo, e) oxic, f) anoxic, g) sulfidic. The neutral sulfur-containing molecular formulae of the detected ions were assigned to the corresponding peaks. The sulfur-containing compounds exhibit similar signal intensities but the number of sulfur-containing compounds is higher in the pore water than in the other samples whereas the deep sea sample (NEqPIW) shows the lowest number.

Sulfonic acids: The fragmentation spectra were screened for neutral losses that were indicative for the presence of sulfonic acids, i.e. SO_2, H_2SO_3, and SO_3. For each analyzed nominal mass in all samples neutral losses of CO_2, H_2O, and CH_3OH, and combinations thereof, were found. The sulfonic acids indicator neutral losses were also found in all our samples (Tables 4.2 and 4.3). This is evidence for the presence of sulfonic acids in the tested SPE-DOM. Furthermore, the sulfur-containing compounds must also have carboxyl groups as they also lost CO_2 and H_2O. In addition, losses of a single sulfur atom were observed for all analyzed samples. The loss of S_1 might result from slightly reduced sulfur-containing functional groups with a sulfur oxidation state ≤0. For the Janssand pore water sample these losses could originate from the detected thioethers. As no thioethers were detected in the Black Sea samples the losses might originate from thiophenes. Bray Curtis dissimilarity analyses performed on FT-ICR-MS signal intensities that were normalized to the signal intensity of the respective parent ion revealed a contrasting picture. When all sulfur-containing formulae were taken into account, the pore water sample is the most dissimilar (Fig. 4.4 a, b). However, when only those sulfur-containing formulae that all samples share were taken into account, the North Sea sample is slightly the most dissimilar one (Fig. 4.4 c, d).

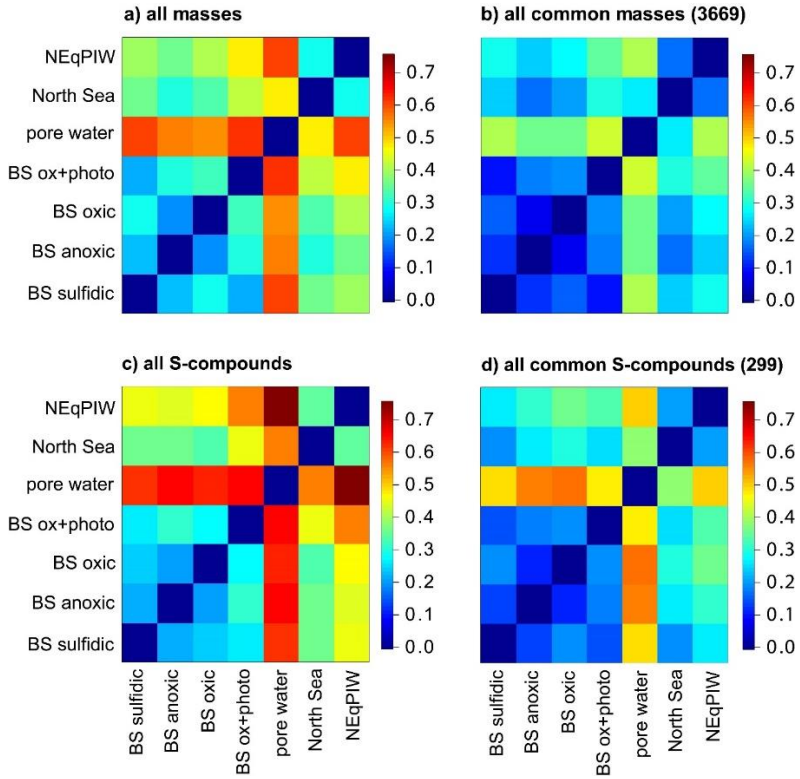

Figure 4.2: Bray Curtis dissimilarity analysis of all analyzed environments based on molecular composition derived from FT-ICR-MS analysis; a) all masses that were detected for the corresponding sample, b) only those masses that all analyzed samples had in common (3669), c) only the sulfur-containing molecular formulae of the corresponding sample, d) the sulfur-containing molecular formulae that all samples had in common (299). A higher value indicates higher dissimilarity between the respective samples.

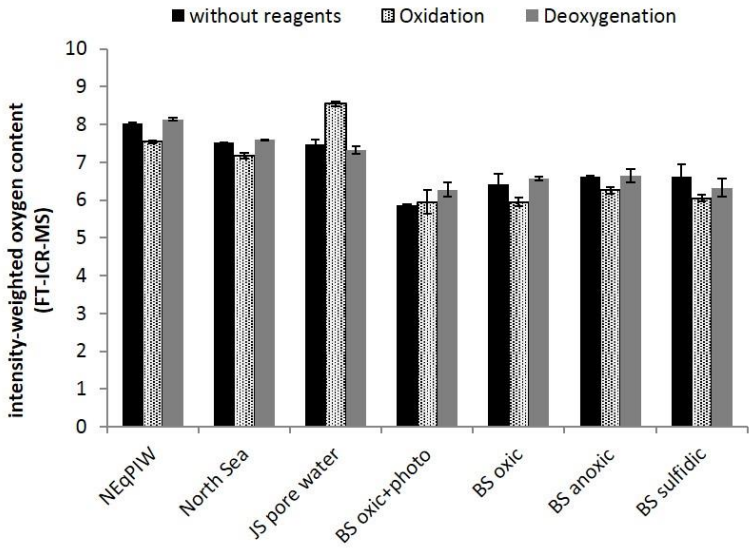

Figure 4.3: Intensity-weighted oxygen content (number of oxygen atoms in the molecular formulae) of the sulfur-containing molecular formulae detected by FT-ICR-MS for all samples. The black bars represent the pure DOM sample without added reagents, the dotted bars the DOM sample where the oxidation reagents were added, and the grey bars represent the DOM sample where the deoxygenation reagents were added. The error bars show the standard deviation of the procedural replicates (n=3). There are no significant differences between the untreated and the deoxygenation samples no matter for what environment. For the oxidized samples a clear increase in oxygen content is visible only for the Janssand pore water sample.

4.5. Discussion

4.5.1. DOS enrichment in sulfidic environments

DOC and also DOS concentrations were very different in the different systems (Table 4.1). DOC concentrations were highest in the pore water (1276 µM) and in accordance with previous studies of the Janssand (Billerbeck et al., 2006; Seidel et al., 2014). The Black Sea showed DOC concentrations higher than the values found previously for the North Sea and NEqPIW (Pohlabeln and Dittmar, 2015). Furthermore, DOC concentrations were decreasing with depth (from 177 to 109 µM) which is in accordance with earlier studies (Ducklow et al., 2007; Margolin et al., 2016). DOS concentrations have not been analyzed before in either of the systems presented here and provide novel information on the influence of redox regimes on DOM composition. The non-sulfidic regimes (North Sea, NEqPIW, Black Sea oxic+photo/oxic/anoxic) had rather low DOS concentrations compared to the sulfidic regimes (Janssand pore water, Black Sea sulfidic; Table 4.1).

The organic sulfur-to-organic carbon ratio (S/C) derived from elemental analysis of SPE-DOM was four times higher in the pore water (37.06) than in the open North Sea (8.94),

indicating that coastal sediments are a possible source of DOS to the ocean. The same trend in the S/C-ratio was found for the molecular formulae detected by FT-ICR-MS analysis, proving the suitability of the FT-ICR-MS method for DOS analysis. For the Black Sea, S/C-concentration-ratios were quite similar among the oxic and anoxic samples and in the range of the North Sea and NEqPIW sample (on average 5.73) but higher for the sulfidic bottom layer (13.94). Our findings indicate that DOM in sulfidic environments is enriched in DOS compared to oxic environments.

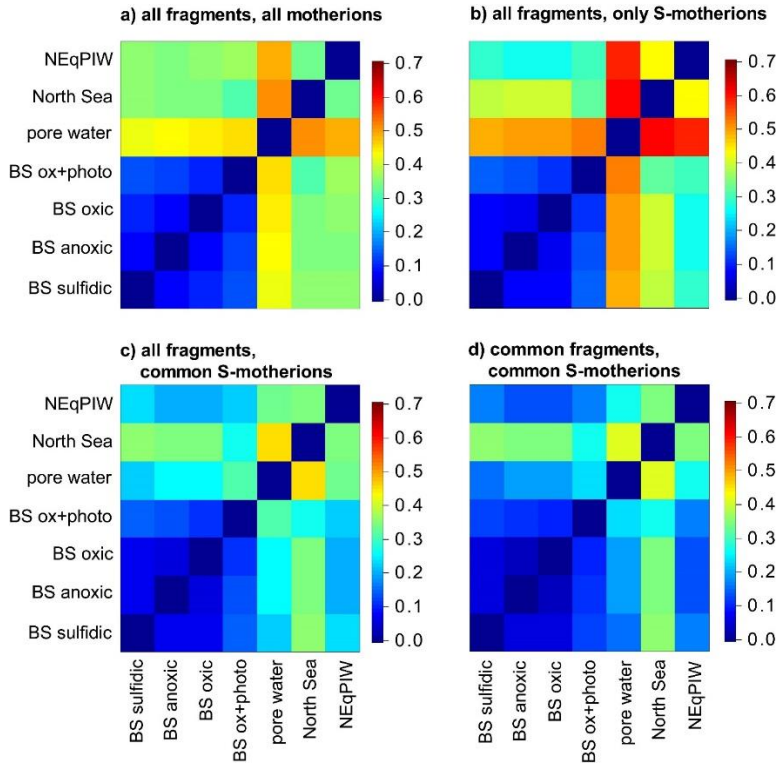

Figure 4.4: Bray Curtis dissimilarity analysis of all analyzed environments based on molecular composition derived from FT-ICR-MS analysis; a) all parent ions of the corresponding sample were considered in the analysis, b) only the sulfur-containing parent ions, c) only the sulfur-containing parent ions that all samples had in common, d) only the common sulfur-containing parent ions and also only the common fragments were considered. The values for the statistical analysis were obtained by normalizing the single fragment intensities by the sum of all considered fragment intensities plus the parent ion intensity belonging to the fragments (non-sulfur- and/or sulfur-containing formulae). The considered fragments were H_2O, CO_2, $2xCO_2$, CO_2+H_2O, CO_2+2xH_2O, CO_2+3xH_2O, CH_4O, SO_2, SO_3, H_2SO_3, CO_2+SO_2, and CO_2+SO_3.

A Bray Curtis dissimilarity analysis of all tested environments (Fig. 4.2) revealed a rather high similarity among the Black Sea water samples. The oxic+photo depth (2 m depth) showed highest dissimilarity compared to the other Black Sea samples and also compared to the other systems (North Sea, NEqPIW, and pore water). This is likely due to the high terrestrial riverine

input into this topmost layer of the Black Sea and the low vertical mixing therein (Ducklow et al., 2007; Margolin et al., 2016). The two open ocean samples (North Sea and NEqPIW) are quite similar whereas the pore water sample is very different from all other samples. The outcome of the Bray Curtis dissimilarity analysis was similar no matter if all detected masses were analyzed (Fig. 4.2 a), or only those masses that all samples had in common (Fig. 4. b), or only the sulfur-containing compounds in the samples (Fig. 4.2 c, d). This indicates that the differences among samples are mainly derived from the DOS compounds in the samples.

4.5.2. Redox conditions affect DOS composition

Structural analysis of the samples was conducted with functional group targeting alteration reactions and collision-induced fragmentation. In our previous study on open ocean DOM (North Sea and NEqPIW) the only detected functional group was the sulfonic acid group (Pohlabeln and Dittmar, 2015). Thus, we suggested that all DOS compounds are fully oxidized and rather stable. However, the molecular composition of DOS in sulfidic environments was unclear and could be reduced due to the reducing redox conditions. Similar to the North Sea and NEqPIW, all Black Sea water samples showed only the sulfonic acid group. In contrast, the pore water sample from a reducing environment showed beside the presence of sulfonic acids also the presence of reduced DOS compounds, namely thioethers. Earlier studies also found that sedimentary DOS is mainly composed of strongly reduced molecules like sulfides, but also strongly oxidized compounds like sulfonates (Eglington et al., 1994; Vairavamurthy et al., 1994; Zhu et al., 2014). It is interesting that an oxidized functional group like the sulfonic acid group is found in the anoxic environment of the Janssand pore water. This is possibly a consequence of sample processing whereby the sample gets into contact with oxygen. However, Zhu et al. (2014) and Vairavamurthy et al. (1994) avoided oxygen contact in their sample processing and still found oxidized species. Thus, the more likely explanation for oxidized DOS species being present in the pore water is that the DOS in the upper sediment layer already gets into contact with oxygen by oxygen penetration into the pores (Billerbeck et al., 2006; Roy et al., 2008). Another suggestion was made by Vairavamurthy et al. (1994). They described the possible reaction of organic matter with sulfite or thiosulfate to directly form sulfonic acids. However, the sulfonic acids could be an artifact of open ocean DOS containing sulfonic acids that entered the sediment via advective transport.

It is surprising that we did not find reduced DOS compounds in the sulfidic depth of the Black Sea like in the sulfidic pore water of the Janssand. This difference between the two sulfidic environments can be due to two reasons: either reduced DOS compounds like thioethers are slowly produced in the deep Black Sea and rather quickly discharged from the system by mixing induced by the intrusion of Mediterranean water (Özsoy and Ünlüata, 1997) or altered into other sulfur-containing functional groups (e.g. into thiophenes, Krein and Aizenshtat 1995) before they can accumulate or secondly, they are not produced at all.

Moreover, it cannot be fully excluded that these reduced compounds are lost by oxidation during sample processing (sampling was not conducted with exclusion of oxygen). In our earlier study we found indication that the content of reduced inorganic sulfur and also of reactive organic matter in a system likely controls the production of DOS (Pohlabeln et al., submitted). Reduced inorganic sulfur originates from microbial sulfate reduction. Sulfate reduction rates in the Black Sea were found to be around 0.71 mmol m^{-2} d^{-1} (depth integrated average of two sampling sites, Albert et al., 1995) and rates in the Janssand around 4.92 mmol m^{-2} d^{-1} (annual average at lower sand flat, Billerbeck et al., 2006). As a function of sulfate reduction rate, H_2S concentrations in the Janssand are much higher than in the Black Sea. Jansen et al. (2009) found values of around 500 µM H_2S at the low water line of the Janssand and Ducklow et al. (2007) found values of maximum 150 µM H_2S in the deep Black Sea. Higher H_2S concentrations lead to a higher redox potential. Therefore, the Janssand environment has a greater reducing ability than the deep Black Sea. Furthermore, the supply of organic matter is different in the two environments. Only matured sinking particulate organic matter (Mopper and Kieber, 1991; Albert et al., 1995) with a significant fraction being of terrestrial origin (Özsoy and Ünlüata, 1979) is brought into the depth of the Black Sea. In situ production of fresh organic matter is limited to microbial activity, e.g. heterotrophic processing of organic matter or autotrophic primary production (Mopper and Kieber, 1991). The Janssand sediment, though, is additionally supplied by fresh organic matter produced by pelagic and benthic organisms that is infiltrated into the sediment by advective flow. In our earlier study we also observed the production of thioethers only for the freshly produced DOM sample not for the more mature sample (Pohlabeln et al., submitted). Nevertheless, conditions for sulfurization are given in the Black Sea albeit less expressed than in the Janssand. Hence, it is likely that reduced DOS compounds like thioethers are produced in the sulfidic depth of the Black Sea but to such a low level that they are discharged from the system by mixing induced by the intrusion of Mediterranean water (Özsoy and Ünlüata, 1997) or altered (e.g. through intramolecular reaction to form thiophenes; Krein and Aizenshtat 1995) before they can accumulate. Therefore, they are not detectable whereas in the Janssand more reduced DOS compounds are produced than can be discharged or altered quickly enough so that they can be detected.

Furthermore, the fragmentation experiment revealed that there is a difference in the losses of fragments and thus in the relative abundance and/or structure of compounds (Fig. 4.4 a-d; Fig. 4.7). Similar to the Bray Curtis analysis of masses and sulfur-containing molecular formulae, the pore water sample is the sample with highest dissimilarity when all parent ions (compounds that get fragmented) are considered (Fig. 4.4 a) and especially when only sulfur-containing parent ions were considered (Fig. 4.4 b). When only those sulfur-containing parent ions were considered that were common among all samples (Fig. 4.4 c) the pore water sample is much more similar to the other environments meaning that the dissimilarity of the pore water sample is driven by its exclusively present sulfur-containing compounds. The similarity

of all open ocean samples and the pore water dissimilarity based on the pore water exclusive sulfur-containing formulae is even evident from the fragmentation mass spectra themselves (Fig. 4.7). In fact, in the analysis of the common sulfur-containing parent ions, the North Sea sample is a little more dissimilar compared to the other environments (Fig. 4.4 c). Because the North Sea dissimilarity remains even when only common parent ions and common neutral losses were considered (Fig. 4.4 d) this observed dissimilarity of the North Sea sample could be an analytical artifact.

4.5.3. Implications from differences between environments

The sulfur-containing molecular formulae of the different samples were compared in a van Krevelen diagram regarding open ocean versus pore water DOS (Fig. 4.5). The sulfur-containing compounds that were exclusively present in the open ocean samples, showed higher and also lower O/C-ratios than the pore water DOS (Fig. 4.5, red dots). Furthermore, there was a high number of DOS compounds that were exclusively present in the pore water (Fig. 4.5, dark blue dots). The differences between open ocean and pore water DOS were also visible in the FT-ICR mass spectra themselves (Fig. 4.1) where the pore water sample showed more sulfur-containing mass peaks. The disappearance of sulfur-containing compounds from pore water to the ocean could be due to three reasons: 1) oxidation of reduced compounds after entering the oxic ocean (Gomez-Saez et al., 2016), 2) degradation processes whether biotic (e.g. Weinbauer et al., 2011) or abiotic (e.g. Mopper et al., 1991), 3) extensive dilution that puts the already low concentrated sulfur-containing compounds below analytical detection. Options 2 and 3 are supported by the observed decrease of the ratio of the number of sulfur-containing molecular formulae to the number of all assigned molecular formulae from pore water towards deep water, i.e. decreasing fraction of sulfur-containing compounds in DOM (Table 4.1). This trend is also visible for the intensity-weighted S/C-ratio (Table 4.1). Option 1 is supported by the oxidability of the pore water DOS as it was shown in our oxidation experiment and by an earlier study on hydrothermal DOS (Gomez-Saez et al., 2016). Thus, it is likely that all three options account for the disappearance of sulfur-containing compounds.

Similar to the sulfidic pore water, the sulfidic depth of the Black Sea showed in total more exclusive sulfur-containing compounds than the other depths. This means that not only the S/C-concentration ratio was higher in the sulfidic water of the Black Sea but also the diversity of the sulfur-containing compounds. Thus, we conclude that the DOS content and its diversity is higher in sulfidic than in oxic environments.

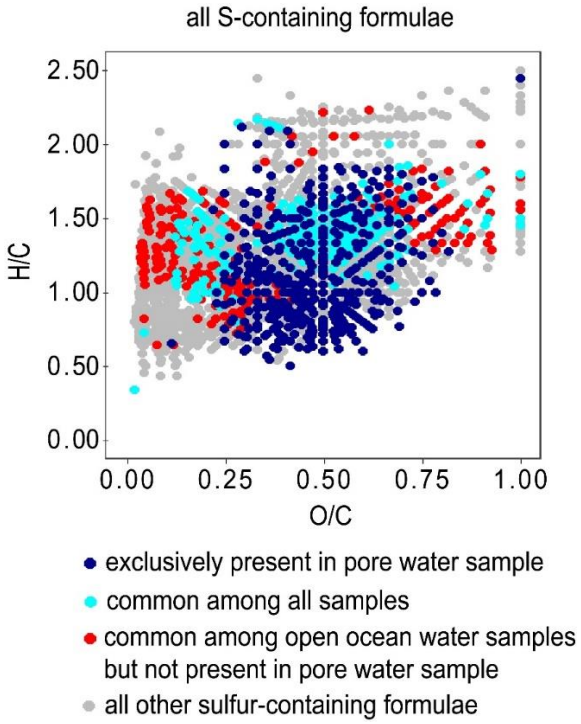

Figure 4.5: Van Krevelen diagrams, including only sulfur-containing molecular formulae that are exclusively present in the Janssand pore water sample (dark blue), common among all samples (cyan), common among all open ocean water samples but not present in the pore water sample (red), or the remaining sulfur-containing formulae (grey).

4.5.4. DOS on a global scale

The DOS concentrations and sulfur-to-carbon ratios obtained from all tested environments can be interpreted as a general dilution-gradient of DOS from sulfidic waters to the deep open ocean. This supports the hypothesis that sulfidic waters are the source of DOS. Through hydrodynamic processes (Billerbeck et al., 2006; Roy et al., 2008; Beck et al., 2009) the pore water DOS enters the open ocean. We found that the flux of DOS from sulfidic sediments to the open ocean ranges between 45 – 120 Tg S a^{-1} (Pohlabeln et al., submitted) which is approximately ten times bigger than the global riverine input (Ksionzek et al., 2016). In the open ocean, the reduced DOS from the sediments is subject to oxic conditions. The oxic environment could lead to an oxidation of the reduced DOS so that thioethers are oxidized, what would explain that they are not found in the North Sea sample.

We found a decrease in S/C-ratio in the open ocean with depth not only for elemental concentrations but also for FT-ICR-MS derived molecular formula as it was similarly observed by Hertkorn et al. (2013). They analyzed differences in molecular formulae derived from FT-ICR mass spectra of DOM samples from increasing water depths of the open South Atlantic Ocean. Further, they found increasing counts of oxygen atoms (based on FT-ICR-MS derived molecular formulae) indicating progressive oxidation with depth. This supports our hypothesis of reduced compounds entering the ocean and becoming oxidized therein.

Accordingly, the intensity-weighted oxygen content (i.e. number of oxygen atoms in the FT-ICR-MS derived molecular formulae) of the (sulfur-containing) compounds of our tested environments shows an increase in oxygen content of the compounds with increasing water column depth (Fig. 4.6). Interestingly, this trend is also slightly visible when only sulfur-containing compounds were plotted indicating that the reduced sulfur-containing compounds are susceptible to oxygen and not stable in the open ocean. Gomez-Saez et al. (2016) analyzed sulfidic hydrothermal systems as a possible source for DOS to the ocean. They found that the DOS escaping from the vents had on average lower O/C-ratios than the surrounding seawater or NEqPIW. These studies agree with our observations indicating that DOS is produced in sulfidic environments and then transported to the open oxic oceans where it becomes oxidized forming the DOS compounds that can be found throughout the water column.

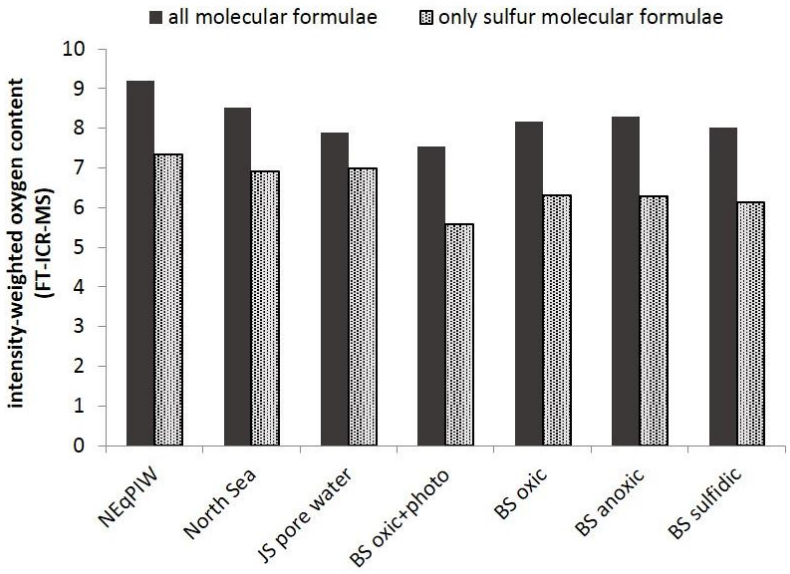

Figure 4.6: Intensity-weighted oxygen content (number of oxygen atoms in the molecular formulae) of all (black bars) and only sulfur-containing (dotted bars) molecular formulae detected by FT-ICR-MS. An increasing oxygen content trend with depth is visible for both, coastal sediments towards deep ocean and also within the Black Sea.

4.6. Conclusion

Our objective was to test whether the structure of dissolved organic sulfur compounds depends on the prevailing redox conditions in different aquatic systems. Further, we investigated whether the DOS produced in sulfidic waters is the source of the DOS found in the open ocean. We used elemental analysis, functional group targeted alteration reactions, and ultrahigh resolution mass spectrometry to compare DOM samples from different environments: pore water from the German Wadden Sea, four different water depths from

the Black Sea, and open ocean water from surface and depth. We found that DOS concentrations were distinctly higher in sulfidic waters than in oxic waters, indicating that DOS is produced in these sulfidic environments and released into the open oceans. Regarding the structure, we found that the sulfonic acid group is ubiquitous in all samples. As the sulfonic acid group is a rather stable group it is reasonable to find it in refractory DOM. The observed similarity of DOS composition between different environments indicates similar formation and alteration processes of DOS on a global scale. One sulfur-containing functional group was exclusively found in the anoxic pore water: non-aromatic thioethers. This reduced functional group is likely the product of abiotic sulfurization taking place in the sulfidic environment of the coastal sediment. Thioethers were not found for the sulfidic depth of the Black Sea. However, we found indication for sulfurization taking place also in this reducing environment of the Black Sea but to a lower extent likely due to lower H_2S concentrations and less reactive organic matter. We conclude that DOS is produced in sulfidic environments and released to the open ocean where it is modified by oxidation processes to mainly sulfonic acids, which contribute to the stable fraction of marine DOM.

4.7. Acknowledgments

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4.9. Supplementary

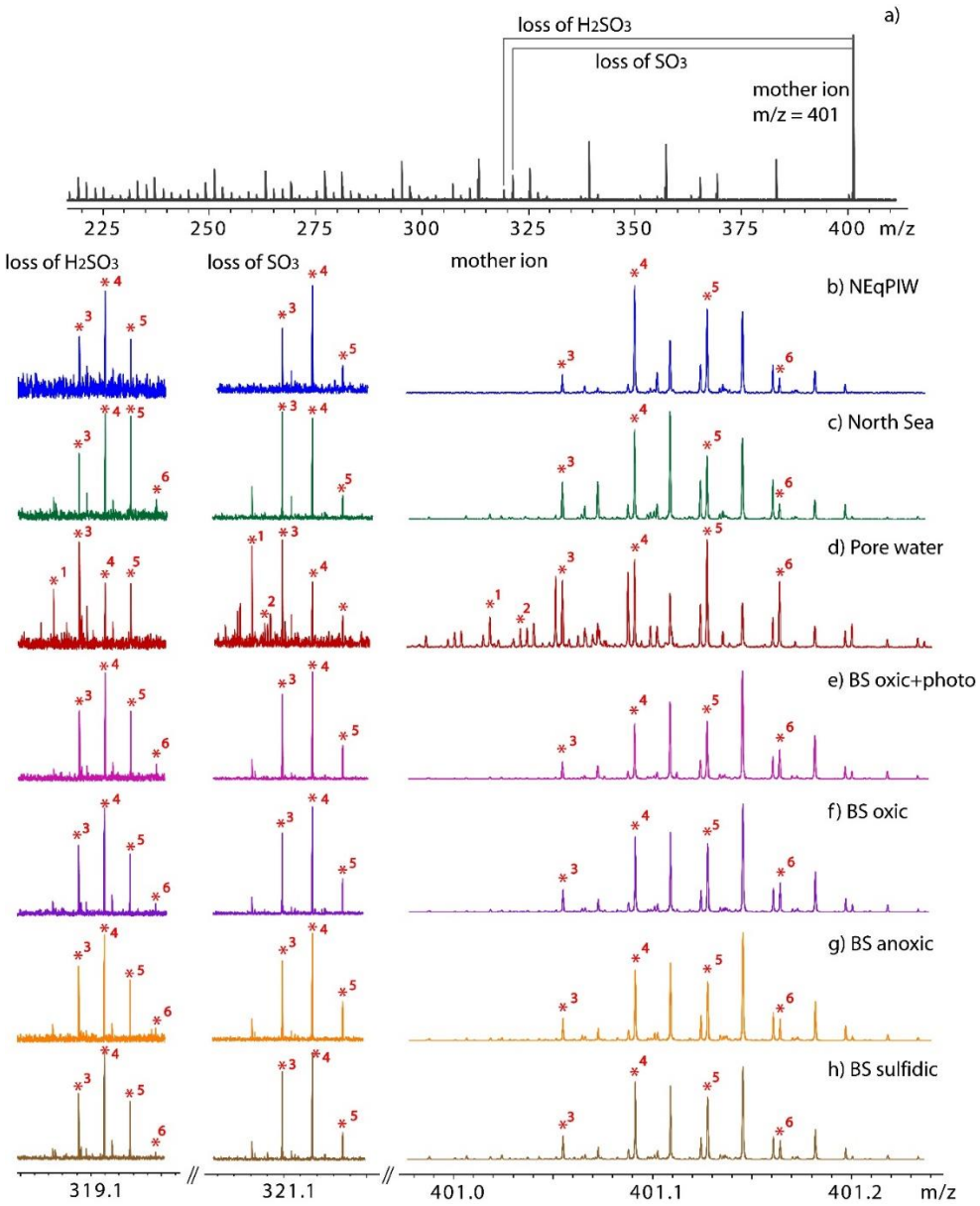

Figure 4.7: Exemplary sections of the FT-ICR mass spectra for all analyzed environments in the fragmentation experiment: b) NEqPIW, c) North Sea, d) Janssand pore water, e) Black Sea oxic+photo, f) Black Sea oxic, g) Black Sea anoxic, h) Black Sea sulfidic. The uppermost row (a) shows the full range fragmentation spectrum of the North Sea sample for the isolated nominal mass 401. Below that are the zoomed in areas of the respective parent ion m/z (m/z = 401), the m/z of the fragments after losing SO_3 (m/z = 321), and the m/z of the fragments after losing H_2SO_3 (m/z = 319). The red stars indicate the parent ion masses with sulfur-containing molecular formulae assigned or the mass after a loss of SO_3 or H_2SO_3, respectively. The little red numbers next to the stars indicate which fragment peak belongs to which parent ion peak. The number of these DOS compounds and also the intensity ratios are slightly different than in Figure 1 because the analysis at the FT-ICR-MS was different here (isolated nominal mass and fragmentation mode).

Table 4.2: Considered sulfur-containing molecular formulae of the respective nominal masses in analysis of fragmentation.

nominal mass	considered molecular formulae	
	Black Sea	pore water
m/z 373	$C_{15}H_{17}O_9S^-$	$C_{15}H_{17}O_9S^-$
	$C_{16}H_{21}O_8S^-$	$C_{16}H_{21}O_8S^-$
	$C_{17}H_{25}O_7S^-$	$C_{17}H_{25}O_7S^-$
	$C_{22}H_{29}O_3S^-$	$C_{14}H_{13}O_{10}S^-$
	$C_{22}H_{13}O_4S^-$	$C_{18}H_{13}O_7S^-$
		$C_{19}H_{15}O_7S^-$
m/z 387	$C_{15}H_{15}O_{10}S^-$	$C_{15}H_{15}O_{10}S^-$
	$C_{16}H_{19}O_9S^-$	$C_{16}H_{19}O_9S^-$
	$C_{17}H_{23}O_8S^-$	$C_{17}H_{23}O_8S^-$
	$C_{18}H_{27}O_7S^-$	$C_{18}H_{27}O_7S^-$
		$C_{18}H_{11}O_8S^-$
		$C_{19}H_{15}O_7S^-$
m/z 401	$C_{16}H_{17}O_{10}S^-$	$C_{16}H_{17}O_{10}S^-$
	$C_{17}H_{21}O_9S^-$	$C_{17}H_{21}O_9S^-$
	$C_{18}H_{25}O_8S^-$	$C_{18}H_{25}O_8S^-$
	$C_{19}H_{29}O_7S^-$	$C_{19}H_{29}O_7S^-$
		$C_{19}H_{13}O_8S^-$
m/z 415	$C_{17}H_{19}O_{10}S^-$	$C_{17}H_{19}O_{10}S^-$
	$C_{18}H_{23}O_9S^-$	$C_{18}H_{23}O_9S^-$
	$C_{19}H_{27}O_8S^-$	$C_{19}H_{27}O_8S^-$
	$C_{20}H_{31}O_7S^-$	$C_{20}H_{31}O_7S^-$
		$C_{16}H_{15}O_{11}S^-$
		$C_{20}H_{15}O_8S^-$
m/z 375	$C_{15}H_{19}O_9S^-$	$C_{15}H_{19}O_9S^-$
	$C_{16}H_{23}O_8S^-$	$C_{16}H_{23}O_8S^-$
	$C_{17}H_{27}O_7S^-$	$C_{17}H_{27}O_7S^-$
	$C_{22}H_{15}O_4S^-$	$C_{17}H_{11}O_8S^-$
		$C_{14}H_{15}O_{10}S^-$
		$C_{18}H_{15}O_7S^-$
m/z 389	$C_{22}H_{13}O_5S^-$	$C_{15}H_{17}O_{10}S^-$
	$C_{15}H_{17}O_{10}S^-$	$C_{16}H_{21}O_9S^-$
	$C_{16}H_{21}O_9S^-$	$C_{17}H_{25}O_8S^-$
	$C_{17}H_{25}O_8S^-$	$C_{18}H_{29}O_7S^-$
	$C_{18}H_{29}O_7S^-$	$C_{18}H_{13}O_8S^-$
		$C_{19}H_{17}O_7S^-$
m/z 403	$C_{16}H_{19}O_{10}S^-$	$C_{16}H_{19}O_{10}S^-$
	$C_{17}H_{23}O_9S^-$	$C_{17}H_{23}O_9S^-$
	$C_{18}H_{27}O_8S^-$	$C_{18}H_{27}O_8S^-$
	$C_{23}H_{15}O_5S^-$	$C_{18}H_{11}O_9S^-$
		$C_{15}H_{15}O_{11}S^-$
		$C_{19}H_{15}O_8S^-$
		$C_{20}H_{19}O_7S^-$
		$C_{19}H_{31}O_7S^-$
m/z 417	$C_{16}H_{17}O_{11}S^-$	$C_{16}H_{17}O_{11}S^-$
	$C_{17}H_{21}O_{10}S^-$	$C_{17}H_{21}O_{10}S^-$
	$C_{18}H_{25}O_9S^-$	$C_{18}H_{25}O_9S^-$
	$C_{19}H_{29}O_8S^-$	$C_{19}H_{29}O_8S^-$
		$C_{19}H_{13}O_9S^-$
		$C_{20}H_{17}O_8S$
m/z 377	$C_{14}H_{17}O_{10}S^-$	$C_{14}H_{17}O_{10}S^-$
	$C_{15}H_{21}O_9S^-$	$C_{15}H_{21}O_9S^-$
	$C_{16}H_{25}O_8S^-$	$C_{16}H_{25}O_8S^-$
	$C_{17}H_{29}O_7S^-$	$C_{17}H_{29}O_7S^-$
	$C_{21}H_{13}O_5S^-$	$C_{17}H_{13}O_8S^-$
	$C_{22}H_{17}O_4S^-$	$C_{18}H_{17}O_7S^-$
		$C_{19}H_{21}O_6S^-$
m/z 391	$C_{22}H_{15}O_5S^-$	$C_{15}H_{19}O_{10}S^-$
	$C_{15}H_{19}O_{10}S^-$	$C_{16}H_{23}O_9S^-$

	$C_{16}H_{23}O_9S^-$	$C_{17}H_{27}O_8S^-$
	$C_{24}H_{23}O_3S^-$	$C_{17}H_{11}O_9S^-$
	$C_{17}H_{27}O_8S^-$	$C_{18}H_{15}O_8S^-$
		$C_{19}H_{19}O_7S^-$
		$C_{18}H_{31}O_7S^-$
m/z 405	$C_{15}H_{17}O_{11}S^-$	$C_{15}H_{17}O_{11}S^-$
	$C_{23}H_{17}O_5S^-$	$C_{16}H_{21}O_{10}S^-$
	$C_{16}H_{21}O_{10}S^-$	$C_{17}H_{25}O_9S^-$
	$C_{17}H_{25}O_9S^-$	$C_{18}H_{29}O_8S^-$
	$C_{18}H_{29}O_8S^-$	$C_{18}H_{13}O_9S^-$
		$C_{19}H_{17}O_8S^-$
m/z 419	$C_{16}H_{19}O_{11}S^-$	$C_{16}H_{19}O_{11}S^-$
	$C_{17}H_{23}O_{10}S^-$	$C_{17}H_{23}O_{10}S^-$
	$C_{18}H_{27}O_9S^-$	$C_{18}H_{27}O_9S^-$
	$C_{23}H_{15}O_6S^-$	$C_{19}H_{15}O_9S^-$
		$C_{20}H_{19}O_8S^-$
		$C_{19}H_{31}O_8S^-$

Table 4.3: Observed neutral losses from isolated masses (*m/z*) from the Black Sea depths (sulfidic, anoxic, oxic, oxic+photo) and the pore water DOM. Only sulfur-containing molecular formulae were considered. The ✓ symbolizes a found fragment and the X no found fragment.

		neutral losses					neutral losses			
sample	Nominal mass	SO_3	H_2SO_3	SO_2	S_1	Nominal mass	SO_3	H_2SO_3	SO_2	S_1
sulfidic		✓	✓	✓	✓		✓	✓	✓	✓
anoxic		✓	✓	✓	✓		✓	✓	✓	✓
oxic	*m/z* 373	✓	✓	✓	✓	*m/z* 401	✓	✓	✓	✓
oxic+photo		✓	✓	✓	✓		✓	✓	✓	✓
pore water		✓	✓	✓	✓		✓	✓	✓	✓
sulfidic		✓	✓	✓	✓		✓	✓	✓	✓
anoxic		✓	✓	✓	✓		✓	✓	✓	✓
oxic	*m/z* 375	✓	✓	✓	✓	*m/z* 403	✓	✓	✓	✓
oxic+photo		✓	✓	✓	✓		✓	✓	✓	✓
pore water		✓	✓	✓	✓		✓	✓	✓	✓
sulfidic		✓	✓	✓	✓		✓	✓	✓	✓
anoxic		✓	✓	X	✓		✓	✓	✓	✓
oxic	*m/z* 377	✓	✓	X	✓	*m/z* 405	✓	✓	✓	✓
oxic+photo		✓	✓	X	✓		✓	✓	✓	✓
pore water		✓	✓	✓	✓		✓	✓	✓	✓
sulfidic		✓	✓	✓	✓		✓	✓	✓	✓
anoxic		✓	✓	✓	✓		✓	✓	✓	✓
oxic	*m/z* 387	✓	✓	✓	✓	*m/z* 415	✓	✓	✓	✓
oxic+photo		✓	✓	✓	✓		✓	✓	✓	✓
pore water		✓	✓	✓	✓		✓	✓	✓	✓
sulfidic		✓	✓	✓	✓		✓	✓	✓	✓
anoxic		✓	✓	✓	✓		✓	✓	✓	✓
oxic	*m/z* 389	✓	✓	✓	✓	*m/z* 417	✓	✓	✓	✓
oxic+photo		✓	✓	✓	✓		✓	✓	✓	✓
pore water		✓	✓	✓	✓		✓	✓	✓	✓
sulfidic		✓	✓	✓	✓		✓	✓	✓	✓
anoxic		✓	✓	✓	✓		✓	✓	✓	✓
oxic	*m/z* 391	✓	✓	✓	✓	*m/z* 419	✓	✓	✓	✓
oxic+photo		✓	✓	✓	✓		✓	✓	✓	✓
pore water		✓	✓	✓	✓		✓	✓	✓	✓

5. Manuscript IV

Photochemical alteration of dissolved organic sulfur from sulfidic pore water

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manuscript in preparation

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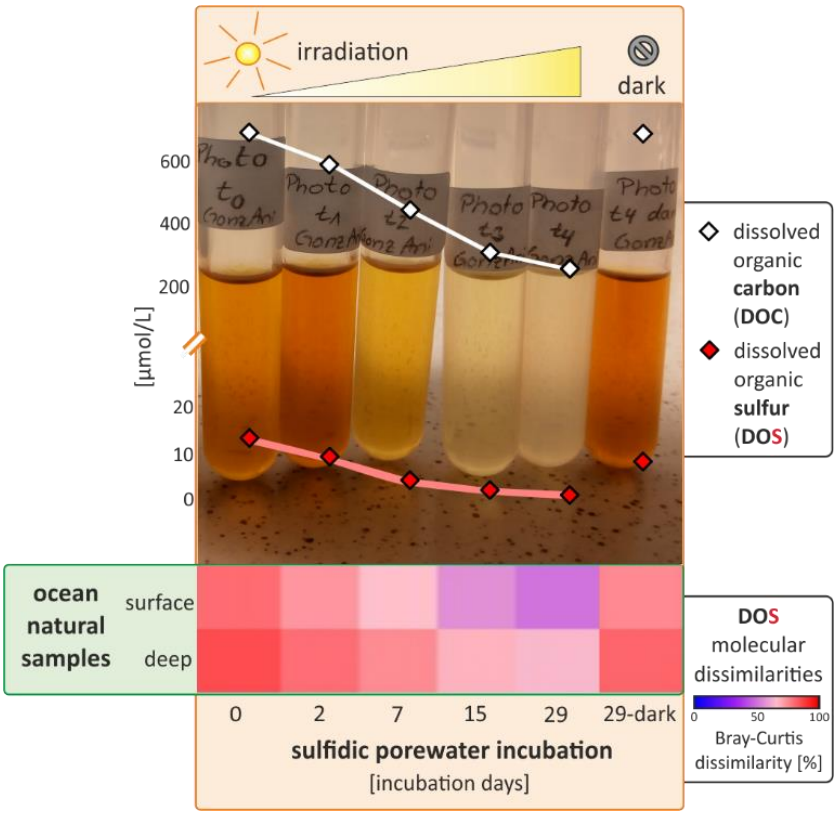
5.1. Abstract

Benthic pore water flux and potentially submarine groundwater discharge are major pathways to transport sulfidic pore water and dissolved organic sulfur (DOS) from sediments into the water column. However, the fate of the sulfidic pore water in the sunlit and oxic water column is unknown. In this study, we hypothesize that photodegradation after discharge from the dark sedimentary environment is a relevant process for DOS molecular transformation and decomposition. We compared experimental and environmental data to analyze DOS from a subtropical saltmarsh system combining ultra-high resolution mass spectrometry with optical and geochemical analyses. Sulfidic pore water was exposed to oxygen, metal co-precipitation and solar irradiation in the laboratory to simulate potential abiotic transformations in the water column. After 29 days of irradiation, photochemical degradation affected mainly aromatic compounds but also ~10 % of DOS molecules. This was consistent with the trends observed for the natural samples showing ~20 % decrease of DOS content in dissolved organic matter (DOM) from pore water to open ocean. In addition, solar irradiation increased the molecular similarity of pore water DOS to saltmarsh and oceanic water column DOS. Therefore, we conclude that DOS from sulfidic coastal sediments is preferentially photolabile and solar irradiation is a key factor controlling the stability and fate of pore water DOS.

5.2. Visual abstract



5.3. Introduction

Dissolved organic matter (DOM) is a complex mixture of thousands of organic compounds with an essential role in global biogeochemistry.[1] The importance of DOM 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,[2] and similar to all atmospheric CO_2.[3] DOM is categorically divided into reactivity fractions depending on its turnover time: minutes to days for labile DOM, weeks to one year for semi-labile DOM, and thousands of years for refractory DOM.[2,4] However, the reasons behind DOM stability remain unknown.[5]

Many compounds within the DOM mixture contain sulfur (dissolved organic sulfur, DOS), and in sum they make up the largest reservoir of organic sulfur in the ocean (global inventory of >6.7 Pg S [6]). While a fraction of DOS is small, rapidly cycled, and representative of the labile DOS pool,[6] the majority of DOS compounds in the ocean are unreactive and fully oxidized components of the refractory DOM.[7] However, knowledge on DOS molecular composition, sources, and turnover is scarce, and therefore the connection between the labile and non-labile DOS pools remains unclear.[8] Sulfidic environments represent a source of potentially labile and reduced DOS to the ocean, as inorganic sulfur species get abiotically incorporated into DOM producing DOS compounds.[9] Benthic pore water flux and potentially submarine groundwater discharge are major pathways to transport sulfidic pore water and DOS from sediments into the water column, representing at least 5 times the annual export of riverine organic sulfur into the ocean.[6,9] Once these DOS compounds leave the sediments, different biogeochemical transformations potentially change their properties and structures.[10–13] However, the fate of the sulfidic pore water in the sunlit and oxic water column is unknown.[9]

One factor controlling oxidation states and organic matter transformations are photochemical alteration reactions induced by solar irradiation.[14] The optical properties of DOM in aquatic environments are categorized into two groups. On the one hand, the chromophoric or colored DOM (CDOM) influences ocean color [15] and is responsible for nearly 90 % of potentially harmful UV radiation attenuation in the global ocean.[16] On the other hand, the fluorescent DOM (FDOM) is able to emit light and can be even more sensitive to photodegradation than CDOM.[17] Photochemical reactions of DOM result in a partial or complete remineralization of DOM, leading to new, stable products or a suite of reactive species that could initiate secondary reactions.[17] The influence of photochemistry in the ocean is not restricted to the photic zone due to vertical export of DOM previously modified at the surface.[18] Photodegradation of DOS compounds can affect the global climate by releasing climate impacting gases like carbonylsulfide or dimethylsulfide.[19] Furthermore, DOS compounds bind type B metals like mercury that could be released to aquatic systems as a consequence of photochemical degradation.[20]

Recent advances in mass spectrometry allow characterization of the complex mixture of DOM at a molecular level. The use of Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) brings a major fraction of DOM into the analytical window that can be related to diverse biogeochemical transformations.[1,21–23] Several studies linking photochemical reactivity and molecular composition of DOM have been reported.[18,24–32] The photolabile fraction of natural DOM samples was predominantly aromatic, including compounds typical for vascular plant debris, in particular polyphenols, and thereby relating to the degree of condensation of the molecules.[24–26,32] As a consequence, a shift from terrestrial to marine molecular signature of DOM during solar irradiation experiments on riverine DOM has been observed.[26,32] Besides aromatics, the photolabile fraction of DOM can also be enriched in DOS formulae.[27,30] However, to the best of our knowledge there has not been a study yet characterizing the photochemical transformations of DOS from sulfidic pore water at a molecular level.

In this study, we aim to analyze the impact of solar irradiation on pore water DOM from a sulfidic saltmarsh system during its simulated way to the open ocean, focusing on the fate of the DOS components. We hypothesize that photodegradation after discharge from the dark sedimentary environment is a relevant process for DOS molecular transformation and decomposition. In order to test this hypothesis, we compared experimental and environmental data to analyze DOS from a subtropical saltmarsh system combining FT-ICR-MS with optical and geochemical analyses. Sulfidic pore water was exposed to oxygen, metal co-precipitation and solar irradiation in the laboratory to simulate potential abiotic transformations in the water column. The results were then compared to natural samples of the surrounding saltmarsh and the open ocean at a molecular level.

5.4. Materials and Methods

5.4.1. Study site

Our sampling site was a subtropical saltmarsh system. Pore water sampling was conducted at the Saltmarsh Ecosystem Research Facility (SERF) on the campus of the Skidaway Institute of Oceanography (SkIO) on Skidaway Island, Georgia, USA. This facility is a 213 m long boardwalk providing direct access to the saltmarsh. The surrounding environments around SERF ranged from an inland saltmarsh meadow to a tidal creek, and were covered with tall *Spartina alterniflora*.[33] The sampling location was an unvegetated flat around 30 m from the island (31°58´31.4´´N, 81°01´50.5´´W), where sediments turned anoxic within few millimeters depth with highly sulfidic conditions starting at only few centimeters depth. During low tide, the sediment at the sampling location was exposed to air for 5 to 7 h [33] and covered by up to 60 cm of water during high tide. The surficial water above the pore water sampling spot was sampled twice during high tide. One time early in the morning when

it was not yet affected by direct solar irradiation, and one more time at noon when sunlight was shining directly on the water ("surficial water dawn and noon" samples; Table 5.1). Estuarine samples were taken from the Skidaway River which was accessible by the dock (31°59´24.5´´N, 81°01´20.4´´W) on the campus of Skidaway Institute ("estuary" sample; Table 5.1). Creek water samples were taken at low and high tides in Groves Creek (31°58´16.8´´N, 81°01´37.5´´W), that was an intertidal saltmarsh system close to Wilmington River on Skidaway Island with tidal range of up to 3 m ("creek LT and HT" samples; Table 5.1). The open ocean samples were taken during a cruise with the RV Savannah on August 2016. Surface seawater (<2 m depth) was sampled at two different spots: ~50 km distance from the coast (31°33‘63‘‘N, 80°31‘83‘‘W) where the water column can be up to 25 m depth and ~100 km from the coast (31°02‘84‘‘N, 80°13‘88‘‘W) where the water column can reach up to 40 m depth ("ocean 50 km and 100 km" samples; Table 5.1).

5.4.2. Field sampling and geochemical analyses

Pore water samples were taken during low tide at the SERF dock in July and August 2016. The pore water was sampled using 70 cm long stainless steel push point samplers as described by [34]. Summarizing, the point samplers were pushed into the sediment to ~50 cm depth and then polyethylene syringes were connected with Viton tubes to the samplers. The first aliquots of pore water were discharged to ensure sufficient flushing of the sampling system.[34] In total 1 L of pore water was taken for each sample. The pore water obtained was filtered in the field with pre-combusted glass microfiber filters (2 µm, GMF, Whatman, USA) and glass fiber filters (0.7 µm, GF/F, Whatman, USA). For the anoxic sampling the acid-washed sample bottles (HDPE, Thermo Scientific) were equipped with a self-designed tubing system that was gas-tight and contained vacuum during sampling to avoid any oxygen contact with the sample. The pore water samples were directly taken to the laboratory after sampling. In total four approaches were conducted: (a) for one aliquot oxygen contact was prevented during extraction using argon gas ("+Ar" sample; Table 5.1), (b) the remaining pore water was exposed to oxygen overnight to simulate potential DOM oxidation occurring in natural aquatic environments ("O_2" sample; Table 5.1), (c) part of the oxidized pore water was filtered again (0.7 µm, GF/F, Whatman, USA; "metals precip" sample; Table 5.1) to remove potential metal and organic matter co-precipitation particles, and (d) the oxidized and once more filtered (0.2 µm, GHP, Whatman, USA) pore water sample was incubated in the solar irradiation experiment ("solar irradiation" samples; Table 5.1; see 5.4.3).

On the other hand, natural samples from the water column of the saltmarsh and the ocean were directly filled in acid-washed bottles and immediately transported to the laboratory and filtered there (pre-combusted GMF and GF/F, Whatman, USA) ("saltmarsh and ocean" samples; Table 5.1). Aliquots for sulfide (H_2S), sulfate (SO_4^{2-}), chloride (Cl^-), dissolved iron (Fe) and manganese (Mn) analyses were taken from the filtered samples. For H_2S analysis

5 mL of only anoxically sampled pore water as well as overlying waters were transferred to a pre-combusted amber-glass vial containing 0.5 mL of 5 % (wt/wt) $ZnCl_2$ (Fisher Scientific). For SO_4^{2-} and Cl^- analyses, 5 mL of sample were 0.2 µm filtered (GHP filters, Pall Life Science) and transferred to pre-combusted amber-glass vials. For Fe and Mn analysis, 100 mL of sample were transferred to acid-washed polyethylene bottles and acidified to pH 2 by adding distilled HCl (6 M, Fisher Scientific). The Fe and Mn samples were kept at 4 °C until analysis by inductively coupled plasma mass spectrometry (Perkin Elmer NexION 300D, Dynamic Reaction cell mode) at SkIO. For H_2S, SO_4^{2-} and Cl^- analyses, 5 ml aliquots were kept frozen until analysis in Germany. SO_4^{2-} and Cl^- were determined by suppressed ion chromatography using a Metrohm 761 Compact IC (Metrohm A Supp 5 column; 3.2 mM Na_2CO_3 and 1 mM $NaHCO_3$ eluent; 20 µL sample loop) with CO_2 suppression and online removal of zinc (Metrohm A PPC 1 HC matrix elimination column). Standards were prepared from a Merck Certified Sulfate Standard solution (1001 ± 2 mg SO_4^{2-} L^{-1}). IAPSO seawater was employed as a secondary reference standard. Total dissolved H_2S, which includes H_2S_{aq} + HS^- + S^{2-} and the sulfidic component of polysulfides S_x^{2-}, was measured using the methylene blue method according to [35], on a Shimadzu UV120 Spectrophotometer.

After subsampling aliquots for geochemical and optical analyses, the remaining samples were acidified to pH 2 using HCl (6 M, Fisher Scientific). For analysis of dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) 10 mL of acidified sample (in triplicates) were transferred to pre-combusted glass vials and stored at 4 °C until analysis via high temperature catalytic combustion using a Shimadzu TOC-VCPH/CPN Total Organic Carbon Analyzer. For DOM analysis by FT-ICR-MS, the remaining acidified samples (~800 mL for pore water and ~1300 mL for saltmarsh and ocean samples) were extracted according to [36] using solid phase extraction (SPE) on styrene divinyl benzene polymer filled cartridges (Agilent Bond Elute PPL, 1 g). The DOC_{SPE} extracts were stored frozen and in the dark until analysis in Germany (see 5.4.4).

5.4.3. Optical analyses

After anoxic sampling, oxidation and filtration (0.2 µM), the remaining pore water samples were irradiated. Firstly, they were transferred to 1 L pre-combusted, UV-C sterilized spherical quartz irradiation flasks. All samples were then placed under a solar simulator fitted with 12 UVA-340 bulbs (Q-Panel), which provide a spectral shape and flux closely approximating natural sunlight from 295 to 365 nm [37], the main wavelength range for environmental photochemical reactions involving CDOM.[20] The integrated irradiance quantified in the solar simulator was ~14.4 ± 0.7 W m^{-2} as determined using a spectro-radiometer (OL756, Optronic Laboratories) fitted with a quartz fiber optic cable and 2" diameter integrating sphere and calibrated with a NIST standard lamp (OL752-10 irradiance standard [38]). Samples were irradiated at 25 – 30 °C. One day of irradiation using this solar

simulator is equivalent to approximately one day of solar irradiance during July at the SkIO based upon irradiance modeled using the System for Transfer of Atmospheric Radiation (STAR) [39]. Subsamples at 0 days ("t0" sample), 2 days ("t1" sample), 7 days ("t2" sample), 15 days ("t3" sample) and at 29 days ("t4" sample) were considered (Table 5.1). In addition, CDOM and FDOM were analyzed each day the first week (Fig. 5.1) and a control sample was wrapped in aluminum foil to prevent solar irradiation and incubated with the other samples during the 29 days of experiment ("t4 dark" sample; Table 5.1). Following irradiation, aliquots were transferred from the flasks to a 1 cm quartz absorbance cuvette (Starna Cells) using pre-combusted Pasteur pipettes. The cuvette was then situated in the fluorescence spectrophotometer (Horiba Jobin-Yvon AquaLog) with excitation and emission ranging from 242 to 842 nm. An aliquot of temperature equilibrated ultrapure water was run immediately before the samples to provide a blank. Excitation Emission Matrix (EEM) spectra were corrected for Rayleigh light scattering, inner filter effects, instrument-specific correction files, and Raman-normalized.[40] Cuvette checks, as well as lamp and Raman water scans, were performed prior to fluorescence analysis to ensure instrument reproducibility. Data output from the spectrophotometer were in the form of dimensionless absorbance (A) and were converted to the Napierian absorption coefficient, a (m^{-1}).[41] Spectral slopes (nm^{-1}) in the ranges 275 to 295 nm and 350 to 400 nm, together with the ratio of these slopes (Slope ratio, SR) were calculated following [42]. The carbon-normalized light absorbance at 254 nm ($SUVA_{254}$; L mg-C^{-1} m^{-1}) was then calculated by dividing the Decadic light absorption coefficient at 254 nm (m^{-1}) by the DOC concentration (mg C L^{-1}).[43] Intensities across set wavelength ranges were used to assess the fluorescence quality, differentiating between humic and protein-like peaks following established parameters by [44].

To verify the lack of microbial activity during irradiation, samples for cell counts were taken at each time point (t0 – t4, t3-dark, and t4-dark). The samples were fixed with glutardialdehyde (0.1 % final concentration, Fisher Scientific) and cells were counted with a BD accuri C6 Flow Cytometer (BD Biosciences, USA) using SybrGreen (life tech., USA).

5.4.4. FT-ICR-MS analysis

Aliquots of the SPE-DOM extracts were used to determine the SPE-DOS concentration. Sulfur concentrations were measured using an ICP-MS at the University of Oldenburg (Germany) following established procedures for DOS_{SPE} quantification.[7,12] 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.[45,46] A common detection limit on the relative signal intensity scale was applied to facilitate maximum comparability among samples.[46] 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 formulae. The data were processed using in-house Matlab routines, and molecular formulae with $C_{1\text{-}100}$ $H_{1\text{-}250}$ $O_{1\text{-}100}$ $N_{0\text{-}4}$ $S_{0\text{-}2}$ or $P_{0\text{-}1}$ were assigned to peaks following [46]. Only those molecular formulae detected in all analytical replicates were accepted, which decreased the number of formulae but efficiently removed non-analytes from the data set that only result from noise in the mass spectra.[46] Additionally, we analyzed blank samples and all of the DOM formulae detected in the blanks were removed from our DOM dataset.

The molecular formulae were used to calculate two indexes: the Double Bond Equivalence Index (DBE) in order to assess the degree of unsaturation, and the modified Aromaticity Index (AI_{mod}) to estimate the presence and abundance of aromatics ($AI_{mod} \geq 0.5$) and condensed aromatics ($AI_{mod} \geq 0.66$), taking into consideration the abundance of carboxyl groups in natural organic matter.[47] Signal intensities of individual signals were converted into normalized intensities relative to total signal intensity. These relative signal intensities were used to calculate intensity-weighted averages of DBE, AI_{mod} and molar ratios (H/C, O/C, S/C).[10–12,48] Molecular formulae were categorized as photolabile, photoresistant and photoproduced depending on their presence or absence in the FT-ICR-MS spectra along the solar irradiation incubation.[26,27,30] Furthermore, we investigated the molecular dissimilarity of DOM_{SPE} with respect to the relative signal intensities of the molecular formula using multivariate statistical analysis on the normalized data (Bray-Curtis dissimilarity analysis, vegan package function vegdist; Fig. 5.4), with help of the R software package (version 3.2.5).

5.5. Results and Discussion

5.5.1. Photodegradation of DOM from sulfidic pore water

In this study we investigated the impact of solar irradiation on pore water DOM from a sulfidic saltmarsh system during its simulated way to the open ocean, focusing on the fate of the DOS components. While the pH was slightly alkaline in all samples, the geochemical composition of pore water was considerable different from the saltmarsh and oceanic ones. For example, the DOC, TDN and DOS concentration were considerably higher in pore water (Table 5.1). In the transect from pore water to open ocean, an enrichment in salinity and Cl^- concentration, and a depletion in dissolved Fe and Mn concentration were detected (Table 5.1). We thereby had a study site with a clear gradient from pore water to the water column, that could be linked to the vertical advection of pore water previously reported in this saltmarsh system.[33] The potential key role of sulfur biogeochemistry in our study site was suggested by high sulfidic conditions in pore water, with H_2S concentration of >900 µM and

SO_4^{2-} concentration of ~20 mM. In contrast, H_2S was not detected in the water column samples and SO_4^{2-} concentration ranged from >20 mM in the saltmarsh to >30 mM in the ocean (Table 5.1).

In our study, we simulated potential abiotic transformations occurring to pore water on its way to the open ocean. Prior to solar irradiation, pore water samples were induced to oxidation and co-precipitation of DOM and metals.[48,49] Consequently, some variation in the geochemical parameters were observed (e.g. depletion in dissolved Fe and Mn; Table 5.1), but major variations in the bulk measurement ratios or the molecular atomic composition of DOM_{SPE} were not detected (Fig. 5.2, 5.3 a). In contrast, after 29 days of solar irradiation photochemical degradation of DOM_{SPE} was indicated by >80 % bleaching of CDOM occurring together with >60 % depletion of DOC_{SPE} in pore water (Fig. 5.1 a, b). This was consistent with previous studies where CDOM photobleaching was generally observed after solar irradiation experiments in freshwater DOM,[50–52] estuarine or riverine DOM,[32,53] plant leachate-derived DOM [29,51], or deep-sea DOM studies.[27,30] In the case of pore water, once DOM is released from sediments into the water column many of the terrigenous compounds can be quickly degraded by microbes.[54] However, some terrestrial DOM escapes microbial degradation and is released into aquatic environments, being usually aromatic in nature.[32] Aromatic compounds act as primary chromophores and initiators of photoreactions in natural waters.[37,55] In our study, photodegradation affected mainly the terrestrially-derived DOM_{SPE} as suggested by ~20 % decrease of aromatics (Fig. 5.3 b) implying a decrease in aromaticity and DBE indexes (Table 5.1), a >50 % increase of the slope ratio (S_R), a >50 % decrease of $SUVA_{254}$ and almost total photobleaching of humic-like compounds of FDOM (Table 5.1; Fig. 5.1 b, c; Fig. 5.3 b). Potential microbial degradation of aromatics was ruled out as cell counts revealed no bacterial activity during solar irradiation, and the emission of protein-like compounds in FDOM was very low during our incubation (Fig. 5.1 c). The preferential photo-degradation of aromatic compounds was consistent with previous FT-ICR-MS studies in which the photolabile fraction was predominantly aromatic including compounds typical for vascular plant debris, in particular polyphenols.[24–26,29,32]

Despite of the high photolability of terrestrial DOM_{SPE} detected in our study, ~20 % of aromatic compounds including ~10 % of condensed aromatic ones were not degraded by irradiation (Fig. 5.3 b, c). This is in accordance with previous studies suggesting that photo-chemical stability of condensed molecules may determine the long-term refractory character of a group of terrigenous DOM_{SPE} in the ocean.[26,27,29,32] For instance, some highly condensed DOM compounds derived from land fires, such as dissolved black carbon, were detected in deep-sea DOM_{SPE} although they are considered highly photo-reactive [27] and able to accumulate over thousands of years in the ocean.[56] The photolabile fraction of deep ocean DOM_{SPE} contains not only aromatic compounds but also can be enriched in S-containing formulae.[30] While photodegradation of aromatic and condensed aromatic compounds has been extensively discussed in the literature, the reasons behind the potential photolabile

character of DOS are not so well documented.[20] We thereby discuss in the following sections irradiation-induced DOS molecular transformations.

Table 5.1: Physicochemical composition of DOM including optical, geochemical and FT-ICR-MS data in all samples from laboratory pore water incubations and natural saltmarsh and ocean samples, with "-" representing not analyzed.

	Laboratory incubations									Natural samples							
				Porewater						Saltmarsh			Creek		Ocean		
				Solar irradiation (incubation days)						Surficial water							
	+Ar	+O_2	Metals precip.	t0 (0d)	t1 (2d)	t2 (7d)	t3 (15d)	t4 (29d)	dark (29d)	Dawn	Noon	Estuary	Low tide	High tide	50 km	100 km	NEqPIW
Optical																	
$CDOM_{\lambda 300}$ (m^{-1})	-	-	-	66	58	30	12	7	67	-	-	-	-	-	-	-	-
$S_{275-295}$ (nm^{-1})	-	-	-	0.016	0.018	0.022	0.027	0.032	0.015	-	-	-	-	-	-	-	-
$S_{350-400}$ (nm^{-1})	-	-	-	0.016	0.014	0.016	0.018	0.019	0.016	-	-	-	-	-	-	-	-
Slope ratio (S_R)	-	-	-	0.98	1.24	1.39	1.50	1.76	0.96	-	-	-	-	-	-	-	-
$SUVA_{254}$ ($Lmg\text{-}C^{-1}m^{-1}$)	-	-	-	57	54	33	19	14	53	-	-	-	-	-	-	-	-
Geochemistry																	
Salinity	25	25	25	25	-	-	-	-	-	29	30	28	32	33	36	36	-
pH	8	8	8	8	-	-	-	-	-	8	8	8	8	8	8	8	-
DOC (µM)	886	897	1069	897	1035	867	866	1037	1453	436	562	323	288	245	78	78	-
TDN (µM)	201	202	201	226	207	192	216	208	200	36	21	17	13	13	4	5	-
DOS (µM)	12	11	15	17	16	8	7	6	17	3	4	2	2	2	0.6	0.5	
DOC_{SPE} (µM)	689	683	641	678	581	436	304	250	675	313	294	208	201	163	43	43	-
DON_{SPE} (µM)	23	21	21	22	19	15	11	10	22	13	13	9	10	8	3	3	-
DOS_{SPE} (µM)	10	8	7	13	9	4	2	1	8	2	2	1	1	1	0.3	0.3	-
N/C_{SPE} ratio	0.033	0.031	0.033	0.032	0.033	0.034	0.036	0.040	0.033	0.042	0.044	0.043	0.050	0.049	0.070	0.070	-
S/C_{SPE} ratio	0.014	0.012	0.014	0.019	0.015	0.009	0.008	0.006	0.012	0.007	0.007	0.007	0.007	0.007	0.008	0.006	-
H_2S (µM)	924	-	-	-	-	-	-	-	-	<0.1	<0.1	-	-	-	-	-	-
Fe (µM)	0.5	0.5	0.1	-	-	-	-	-	-	0.3	0.1	<0.1	<0.1	<0.1	<0.1	<0.1	-
Mn (µM)	8.8	8.7	7.8	-	-	-	-	-	-	3.5	2.9	0.2	1.6	<0.1	<0.1	<0.1	-
Cl^- (mM)	415	405	400	-	422	-	-	411	426	478	508	452	558	556	587	591	-
SO_4^{2-} (mM)	18	17	17	-	18	-	-	19	19	24	26	23	29	28	31	31	-
FT-ICR-MS																	
Total formulas	6451	6377	6621	4540	6098	7299	6611	4740	6008	7064	6372	5665	5550	7231	8214	8152	7175
H/C ratio	1.05	1.05	1.06	1.06	1.10	1.16	1.22	1.27	1.08	1.12	1.13	1.15	1.15	1.17	1.26	1.26	1.27
O/C ratio	0.41	0.41	0.40	0.39	0.41	0.46	0.47	0.45	0.40	0.45	0.45	0.44	0.43	0.47	0.49	0.49	0.46
N/C ratio	0.019	0.020	0.020	0.019	0.020	0.023	0.025	0.026	0.021	0.027	0.028	0.027	0.030	0.026	0.029	0.029	0.024
S/C ratio	0.021	0.024	0.025	0.027	0.025	0.023	0.017	0.013	0.025	0.009	0.010	0.008	0.008	0.006	0.004	0.003	0.003
P/C ratio	<0.001	<0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	<0.001	<0.001	0.001	0.001	<0.001	0.002	0.003	0.004
DBE index	7.6	10.0	10.0	9.5	9.2	8.7	8.2	7.6	9.5	9.4	9.3	9.1	8.9	9.4	9.0	9.0	9.7
AI_{mod} index	0.31	0.39	0.38	0.39	0.36	0.30	0.26	0.24	0.37	0.33	0.33	0.33	0.32	0.29	0.22	0.22	0.22

5.5.2. Preferential photo-lability of pore water DOS

After 29 days of solar irradiation, we detected a clear depletion in bulk concentration of DOS_{SPE}, S/C_{SPE} ratios and ~10 % decrease of the relative abundance of DOS_{SPE} molecular formulae in total DOM_{SPE} suggesting preferential photolability of DOS (Table 5.1; Fig. 5.1 a; Fig. 5.2 a; Fig. 5.3 a). Furthermore, the number of photolabile molecular formulae containing S as unique heteroatom ($CHOS_{1-2}$) was more than double the number of those without heteroatoms (CHO) and one order of magnitude higher than N-containing compounds ($CHON_{1-4}$; $CHON_{1-4}S_{1-2}$; Fig. 5.3 c). Photolabile DOS has been reported in studies regarding deep sea DOM [27,30] or acid mine drainage DOM.[57] One potential photochemical sink for DOS would be subsequent oxidation to SO_4^{2-}.[30,57] However, we did not observe a significant increase in SO_4^{2-} concentration along the solar irradiation experiment (Table 5.1). Another potential DOS sink would be photoproduction of climate impacting gases like carbonylsulfide.[30] While DOS molecules containing only S as heteroatom are not considered efficient precursors of carbonylsulfide, those ones containing both S and N (e.g. glutathione and cysteine) are efficient photo-producers.[19] However, in our study the photolability of DOS_{SPE} compounds containing both S and N was not as clear as those containing only S as heteroatom (Fig. 5.3 a, c). Furthermore, the photodegradation of DOS compounds, complexing toxic metals like mercury especially via thiol groups, can lead to a release of the metals.[20,58] In our study, the photolabile fraction of DOS_{SPE} included predominantly molecules with a low oxygen to carbon ratio (O/C; Fig. 5.3 c), suggesting reduced functional groups like thiols. However, thiol groups have not been detected in our previous analysis of natural sulfidic pore water.[13] Further studies on the structural characteristics of pore water DOS and the potential complexation of mercury and its release upon photodegradation are necessary.

In contrast to DOS, the DON_{SPE} compounds showed different trends after irradiation as suggested by an increase in the N/C_{SPE} ratio, ~10 % more DON_{SPE} compounds in total DOM_{SPE} and more than double the amount of photoproduced than photolabile N-compounds (Fig. 5.2 b; Fig. 5.3 c). These increases in relative abundance of DON_{SPE} and photoproduced formulae after irradiation were consistent with previous FT-ICR-MS studies.[29,32] Potential explanations could be photonitration [59] or photochemical reactions incorporating ammonium into dissolved organic forms.[60] A potential sink of these photoproduced DON_{SPE} compounds could be posterior microbial degradation.[32] However, the bulk DON_{SPE} concentration depleted similarly to DOS_{SPE} after solar irradiation (22 to 10 µM; Table 5.1; Fig. 5.1 a). Thus, we suggest that some DON_{SPE} molecules were photoproduced but overall the DON_{SPE} pool in pore water was photodegraded, probably not as fast as compounds without heteroatoms or DOS ones.

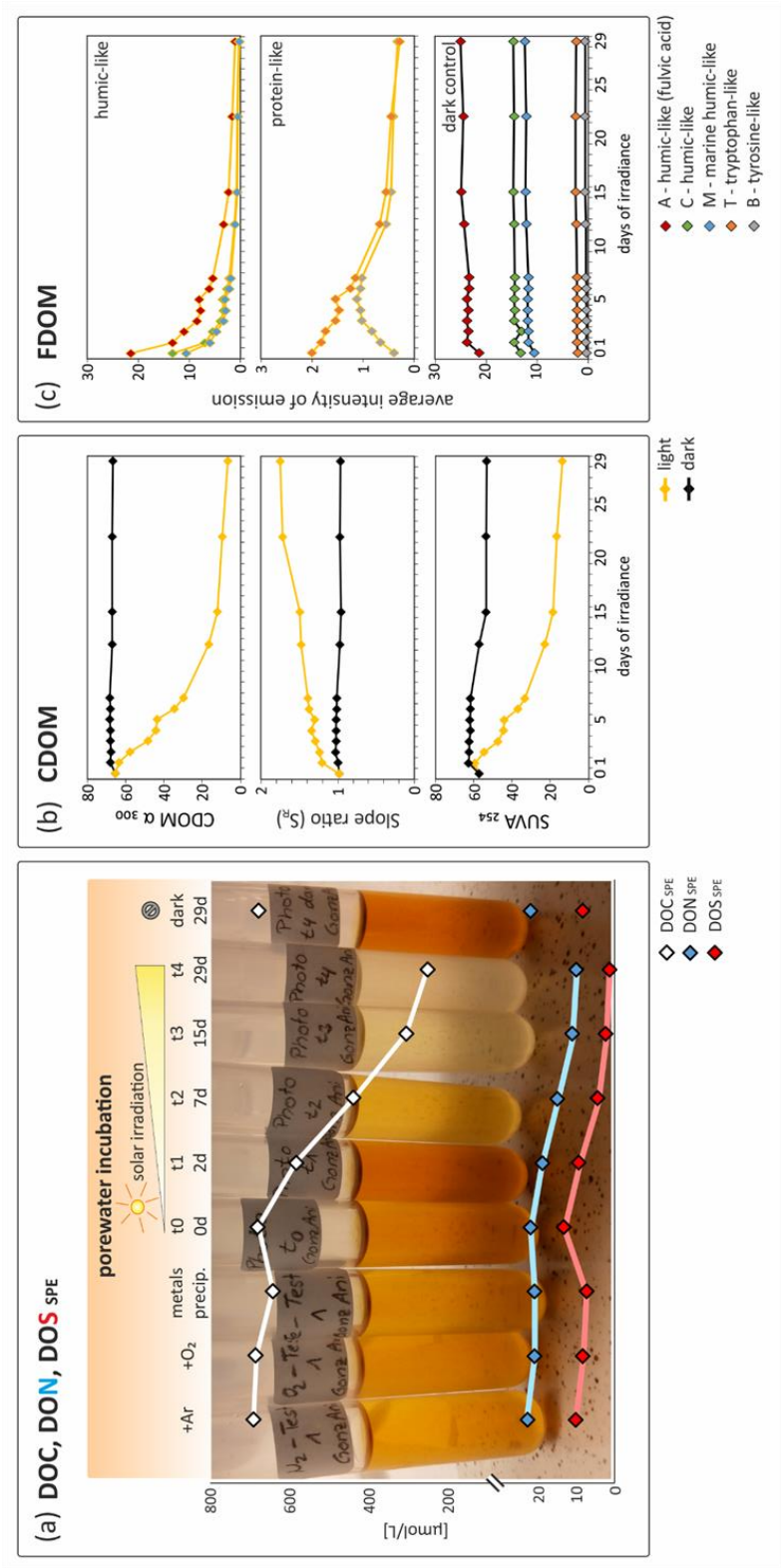


Figure 5.1: Solar irradiation effects on sulfidic pore water; (a) bulk measurements of DOC_{SPE}, DON_{SPE} and DOS_{SPE} overlying the photography of DOM_{SPE} samples with visible photobleaching, (b) CDOM parameters such as $CDOM\alpha_{300}$, slope ratios (S_R) and $SUVA_{254}$, and (c) FDOM humic-like and protein-like parameters. The values for the light-exposed (yellow) and dark-isolated (black) samples were plotted for the 29 days of irradiation incubation.

This is consistent with previous studies showing preferential and faster degradation of DOC compounds without heteroatoms over heteroatomic ones in coastal gradients [60] or river to ocean transects.[61] Our controversial results regarding DON_{SPE} quantitative measurements and relative abundances in total DOM_{SPE} could be related to the fact that heteroatom-compounds usually result in small FT-ICR-MS signal intensities.[29] As photodegradation preferentially affects large molecules,[42] a decrease in molecular weight of total DOM_{SPE} signature is generally observed. If DON_{SPE} low weight compounds were photodegraded more slowly than compounds without heteroatoms, then higher relative abundances of DON_{SPE} and higher N/C_{SPE} ratios after irradiation could be detected. In general terms, our study suggested FT-ICR-MS technique as a good approach to estimate heteroatomic trends in total DOM_{SPE}.[7,12] However, we also highlighted that relative numbers may lead to misinterpretation of the detected trends if they are not combined with quantitative measurements (Fig. 5.2). This should be considered by further studies establishing molecular categorizations of photolabile or photoproduced heteroatomic compounds in natural DOM samples.

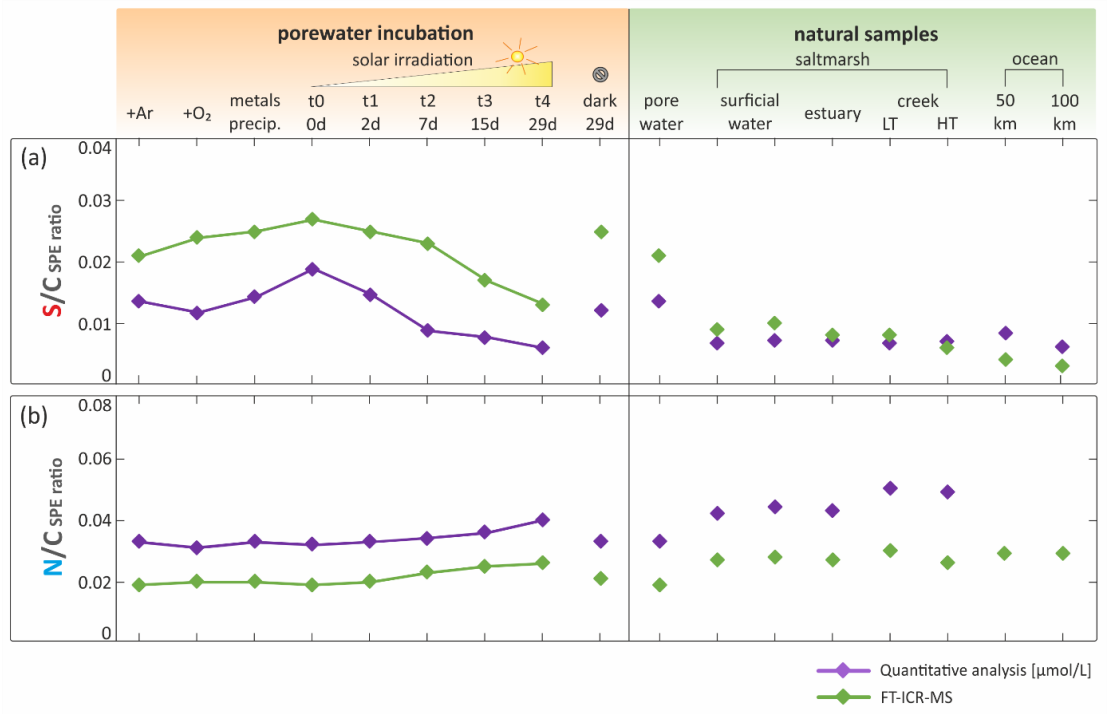


Figure 5.2: Ratios of (a) sulfur-per-carbon S/C_{SPE}, and (b) nitrogen-per-carbon N/C_{SPE}, obtained for the irradiated pore water samples and the natural saltmarsh and ocean samples. Similar trends were observed using FT-ICR-MS (green) or quantitative measurements (purple).

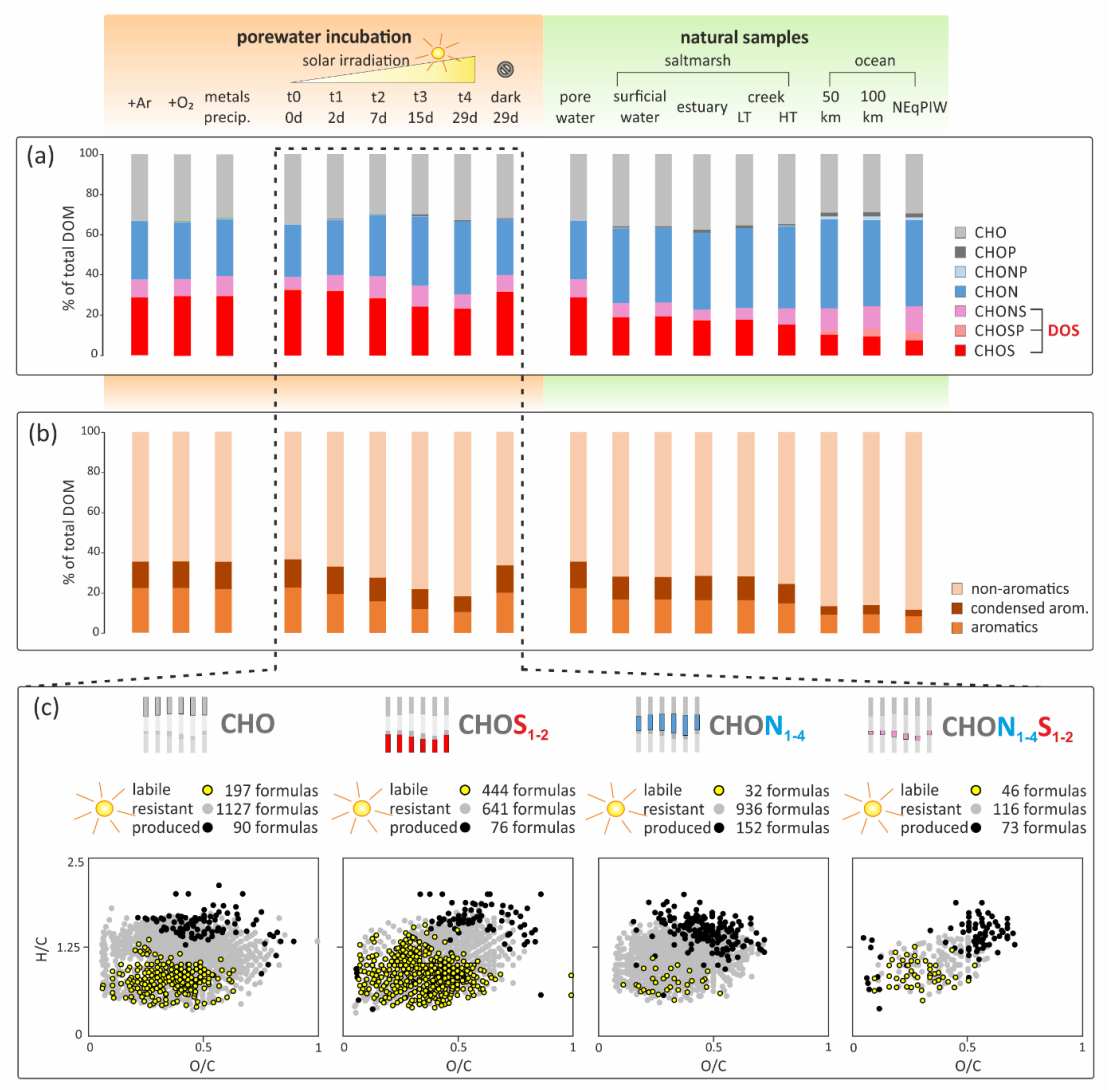


Figure 5.3: DOM_{SPE} molecular compounds identified by FT-ICR-MS. Different compound categories are shown as a percentage of all molecular formulae detected for the respective sample: (a) molecular formulae highlighting the DOS_{SPE} categories CHOS (light red), CHONS (purple) and CHOSP (dark red); (b) aromatic compounds (orange), condensed aromatics ones (dark brown) and non-aromatic compounds (light brown); (c) van Krevelen diagrams representing the O/C (oxygen-to-carbon ratio) and H/C (hydrogen-to-carbon ratio) of the solar irradiated pore water, differentiating between photolabile (yellow), photoproduced (black) and photoresistant (grey) compounds.

5.5.3. Solar irradiation minimized differences between pore water and oceanic DOS

We observed similar trends for DOS_{SPE} in our experiment and in the natural DOM_{SPE} samples. From pore water to the ocean, DOS_{SPE} concentrations and S/C_{SPE} ratios were depleted and the percentage of DOS_{SPE} of total DOM_{SPE} decreased by ~20 %, suggesting

photodegradation processes along this transect (Table 5.1; Fig. 5.2 a; Fig. 5.3 a). Furthermore, we performed a Bray-Curtis dissimilarity analysis[32] on the DOM_{SPE} compounds detected by FT-ICR-MS considering not only the presence and absence of molecular formulae, but also their signal intensity distribution (Fig. 5.4). Comparing pore water, saltmarsh and ocean samples, molecular dissimilarities >80 % between DOS_{SPE} compounds were initially detected (red colors of CHOS, CHONS; Fig. 5.4). In contrast, molecular formulae containing only N or no heteroatom were considerably less dissimilar (blue-purple colors of CHON, CHO; Fig. 5.4). Interestingly, solar irradiation minimized differences in DOS_{SPE} compounds. The long-time irradiated pore water DOS_{SPE} (t3 and t4; Fig. 5.4) was distinctly more similar to saltmarsh and oceanic DOS_{SPE}, even clustering more similar to the saltmarsh samples than to the precursor pore water DOS_{SPE} with <7 days of light irradiation (dendogram CHOS; Fig. 5.4). This transformation at a molecular level was not observed in DOS_{SPE} compounds after oxidation, co-precipitation with metals or incubation in the dark (+O_2, metals precip., dark 29 d; Fig. 5.4), indicating specific photochemical transformations of pore water DOS.

Photochemical reactions resulting in homogenization of the molecular fingerprint of DOM and potential recalcitrance in the environment of photoproduced DOM have been previously suggested,[26] without focusing on specific DOS transformation and its potential key role in DOM stability. For example, it has been proposed that microbial and photodegradation of DOM_{SPE} may result in the accumulation of refractory compounds that share very similar molecular structures, independent of their original source.[62] This was consistent with studies of the optical characteristics of extensively bleached DOM collected at the thermocline level of the North Pacific that were comparable to those of surface waters,[63] or studies considering extensive microbial and photodegradation, where a high percentage of shared formulae was found between deep-sea and wetland plant-derived DOM_{SPE}.[29] A recent study showed that riverine DOM_{SPE} resembled marine DOM_{SPE} molecular signature.[32] However, high dissimilarities between degraded riverine and deep-sea DOM_{SPE} suggested that convergence into an universal molecular fingerprint of DOM in the course of degradation would need additional processes than microbial and photodegradation.[32] In contrast, our study indicated lower molecular dissimilarities in DOS_{SPE} compounds linked to exclusively photochemical transformations. Therefore, photochemical alteration of pore water DOS may result in long-term persistent DOS molecules found to be ubiquitous in aquatic environments.[7] Our findings are also in accordance with previous studies suggesting that intensively photodegraded DOM shares common molecular properties independent of its origin.[29,62] We therefore conclude that DOS from sulfidic pore water is preferentially photolabile and solar irradiation is a key factor controlling the stability and fate of pore water DOS.

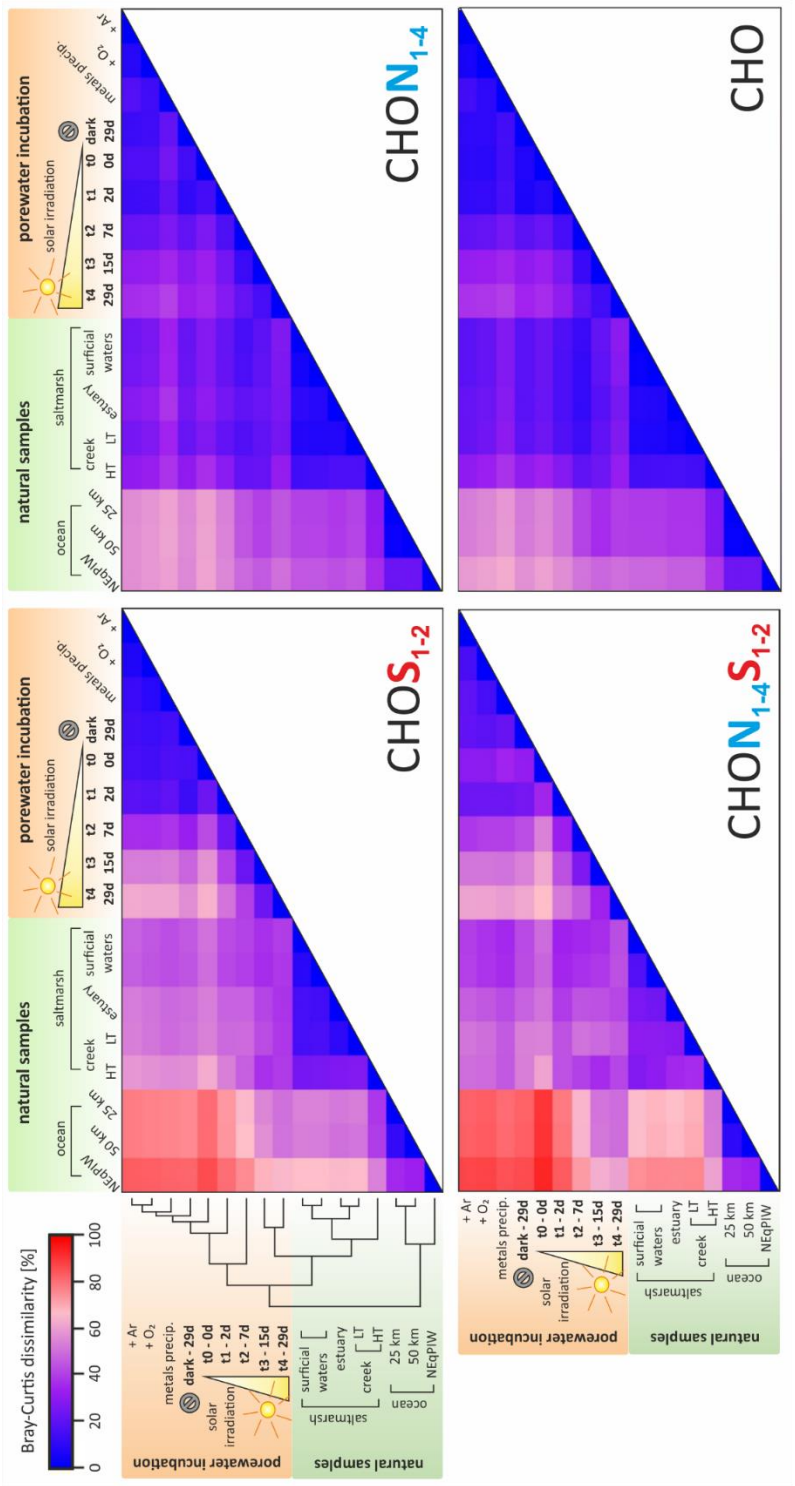


Figure 5.4: Bray Curtis dissimilarity analysis showing that solar irradiation increased the molecular similarity of pore water DOS to saltmarsh and oceanic water column DOS. The analysis considered molecular formulae detected in the different samples under consideration of FT-ICR-MS signal intensities, being red 100 % dissimilar and blue 0 % dissimilar. The four major groups of formulae identified by FT-ICR-MS (Fig. 3a) were plotted here, showing DOS formulae on the left side (CHOS and CHONS formulae) and non-DOS formulae of the right side (CHON and CHO formulae).

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6. Concluding Remarks and Perspectives

6.1. Conclusion

The aim of this thesis was to obtain quantitative and qualitative information on marine dissolved organic sulfur to better understand its role in the global sulfur cycle. Concentration analyses of solid-phase extractable DOS was used to estimate the bulk DOS concentrations in different environments. Methods were developed to selectively test diverse DOM samples for various possible sulfur-containing functional groups. A laboratory simulation of the sulfurization process of DOM was investigated as a possible source for the global DOS. Additionally, the effects of solar irradiation on a DOS sample from a sulfidic pore water were investigated by a laboratory irradiation experiment. The results of this work provide answers to the previously stated research questions:

1) Is abiotic sulfurization of organic matter in sulfidic marine environments a possible source for the DOS present in the open oceans?

The artificial sulfurization experiment, in which DOM was mixed with NaSH and elemental sulfur in seawater and incubated at 50 °C for four weeks, showed abiotic incorporation of inorganic reduced sulfur into DOM compounds producing DOS. The process was found to be non-selective regarding compound classes like peptides or sugars and to happen rather fast as DOS production was already observed after one hour of incubation. A comparison of the artificially produced DOS with DOS from natural sulfidic pore water showed high similarity indicating that similar processes like the one that was simulated in the laboratory are happening in nature, too. Analysis of potential sulfur incorporation pathways indicated a high variety and complexity of the sulfurization reaction and possible follow-up alteration reactions including inter- and intramolecular addition reactions and oxidation reactions. The latter would happen when DOS produced in sulfidic environments escapes to the open oxic ocean. This flux of DOS from sulfidic sediments to the open ocean was estimated as 45 – 120 Tg S a^{-1}. This number is about one order of magnitude higher than the riverine organic sulfur input to the ocean (8 Tg S a^{-1}, Ksionzek et al., 2016) and by far sufficient to balance the estimated global net removal of non-labile oceanic DOS (1.1 Tg S a^{-1}, Ksionzek et al., 2016). DOS input via sulfurization and benthic fluxes could be the single most important source of refractory DOS to the ocean (Chapter 3). If not only diffusive input is taken into account but also transport via submarine groundwater discharge, the herein estimated input of sulfurization-produced DOS from sediments to the ocean would be even higher.

2) What structural characteristics do the DOS compounds have?

In this thesis, DOM samples from different locations with different environmental conditions were investigated regarding their sulfur-containing functional groups. Some sulfur-functionalities could be excluded a priori from the analysis due to their instability in aqueous solutions such as thioaldehydes, thioketones, sulfenic acids and sulfinic acids. Others, namely sulfones and thiophenes were to unreactive for selective alteration reactions and do not show characteristic features in fragmentation experiments and therefore, could not be included in the analysis. However, samples were tested for a wide set of possibly occurring sulfur-containing functional groups. A derivatization reaction with 2-bromo-1,4-naphthoquinone was used to test for thiols, an acidic hydrolysis was used to test for thioesters, sulfonic acids esters, and alkylsulfates, an oxidation was used for thioether analysis and a deoxygenation for sulfoxide analysis. Furthermore, fragmentation experiments were conducted to test for sulfonic acids. While thiols, thioesters, sulfonic acid esters, alkylsulfates, and sulfoxides were not detected in any analyzed DOM sample, sulfonic acids were present in all of them. This indicates that sulfonic acids are an ubiquitous structural feature in global DOS. Thioethers were exclusively found for the highly sulfidic pore water sample and for the DOS compounds produced by the artificial sulfurization experiment on freshly produced mesocosm DOM. Thioethers were not found in any DOM sample from an oxic regime but also not in the sulfidic depth of the Black Sea. Also the artificially sulfurized North Sea DOM sample showed no production of thioethers. These different findings on the presence of thioethers indicate that thioethers are likely the product of reduced sulfur incorporation into rather fresh organic matter. Additionally, thioethers are susceptible to oxidation so that once they leave anoxic conditions and enter oxic waters, they form sulfones or eventually even sulfonic acids (Chapters 2, 3, 4).

The absence of thiols in all tested samples was surprising as other studies found thiols in seawater samples (Al-Farawati and van den Berg, 2001; Dupont et al., 2006; Laglera et al., 2014). These studies mainly found thiols in the form of small polar compounds like cysteine or glutathione and also reported that the detected thiols are able to complex metal atoms. It is very likely, that these small polar thiols are not retained in the solid-phase extraction and thus not present in the SPE-DOS that was analyzed in this thesis. Furthermore, the thiols that complex metals are likely not able to freely react with the derivatization reagent and thus, are not detectable with the applied method. However, the results from the derivatization experiment showed that there are no free thiols in the solid-phase extractable DOS. The absence of thiols in the artificial sulfurization products is very likely due to second step reactions after the sulfide gets incorporated into DOM forming thiols at first which can then form thioethers or thiophenes.

3) Are there spatial differences in the concentration and/or structural composition of DOS compounds?

In this thesis only few different locations were analyzed. Even with this low spatial resolution, DOS concentration results show a trend of higher values in the sulfidic environments of the pore water and also in the sulfidic Black Sea water compared to the oxic environments. Furthermore, higher DOS concentrations were observed in the surface water (North Sea) than in deeper water (NEqPIW) (Table 6.1) which is in accordance with published values (Ksionzek et al., 2016). The DOS/DOC concentration ratio is higher in sulfidic environments than in oxic ones especially for the pore water sample. The sedimentary S/C-ratio found in this study is in accordance to S/C-ratios published for different sedimentary organic matter samples which range between 0.0375 to 0.0475 (e.g. Mossmann et al., 1991; Quijada et al., 2016). The water column S/C concentration ratios were found to be lower by approximately factor 10 in this thesis and in the literature (Ksionzek et al., 2016).

No indication for spatial differences regarding the sulfur-containing functional groups was observed for the samples analyzed in this study. Sulfonic acids were detected in all analyzed samples and are likely an ubiquitous sulfur functionality in global DOS. The only differences regarding structure were found to be a function of the redox conditions of the environments they are in (Chapter 4).

Table 6.1: Concentration values for dissolved organic carbon and dissolved organic sulfur and their ratio for the DOM samples analyzed in this thesis.

Sample	DOC [µM]	DOS [µM]	DOS/DOC
NEqPIW	44	0.18	0.0041
North Sea	94	0.84	0.0089
Janssand pore water	1276	47.29	0.0371
Black Sea, oxic+photo (2 m depth)	177	0.99	0.0056
Black Sea, oxic (20 m depth)	178	0.96	0.0054
Black Sea, anoxic (100-120 m depth)	124	0.77	0.0062
Black Sea, sulfidic (350-641 m depth)	109	1.52	0.0139

4) Is the oxidation state of the DOS functionalities a function of the redox conditions of the environment they are in?

The results of this study indicate that DOS compounds in sulfidic environments are more reduced than DOS compounds in oxic environments. The presence of thioethers, a reduced sulfur-containing functional group, was found exclusively for highly sulfidic environments. Furthermore, results from the fragmentation experiments indicate similar trends. The loss of H_2S which can be seen as an indicator loss of reduced sulfur functionalities (oxidation state ≤ 0, Pretsch et al., 2009) was only found for the highly sulfidic pore water sample and for the artificial sulfurization products. It is likely that the reduced DOS compounds get oxidized once they enter oxic waters and form the DOS compounds that are found in the open water column (Chapters 3, 4).

However, the data set of this thesis is limited. The interpretations regarding the reduced character of DOS compounds in highly sulfidic environments are based on only one sulfidic pore water sample. More sulfur functional group tests on DOS from sulfidic environments are necessary to confirm the results found in this thesis.

5) Is DOS affected by photochemically induced alteration and/or degradation reactions?

A sulfidic pore water sample from a subtropical salt marsh system was exposed to oxygen, metal co-precipitation, and solar irradiation in the laboratory in order to simulate the potential abiotic processes the pore water DOM would undergo while leaving the sediment towards the open ocean. While the concentrations of solid-phase extractable DOC and DOS were hardly affected by the oxidation and co-precipitation, values significantly decreased during the irradiation experiment. Mainly compounds typical for vascular plant debris, in particular polyphenols, were degraded but also approximately 10 % of the DOS compounds. Similar trends were observed when the pore water DOM was compared to the estuary and open ocean DOM samples. The loss of aromatic and DOS compounds was even more pronounced along this transect. These results showed that part of the DOS compounds from sulfidic pore waters are photo-reactive indicating that solar irradiation induced alteration reactions are a key factor controlling the stability and fate of marine DOS. It is unclear so far, if the photo-altered DOS compounds are more or less bio-available. However, the increased similarity of artificially irradiated pore water DOS to open ocean DOS indicates that photo-alteration of DOS produced in sulfidic pore waters by sulfurization leads to marine refractory DOS (Chapter 5).

6.2. Future Perspectives

This thesis provides information on several aspects of DOS and research interest and thus the knowledge on DOS is growing, but there are still open questions. An important issue is the turnover rate of DOS compounds. It is reasonable that the same categories (labile, semi-labile, semi-refractory, refractory, and ultra-refractory (Hansell, 2013)) of DOM in general also apply to DOS in particular. Biosynthetic DOS is likely labile but may become refractory through the microbial carbon pump. We found that abiotic sulfurization of organic matter in sulfidic systems is a main source for DOS to the ocean. However, it is unknown if the DOS produced via sulfurization is mainly labile or refractory. It was proposed that the incorporation of sulfur enhances the stability of organic matter in fossil deposits (Sinninghe Damsté et al., 1998; Kok et al., 2000; Amrani et al., 2007; Bushnev and Burdel´naya, 2008) but it is uncertain if this also applies to dissolved organic matter. In this context, studies on the interaction between DOS from environments of different redox conditions (oxic, anoxic, sulfidic) and microbial communities would be of avail.

Another interesting aspect of DOS is its ability to complex metals in particular mercury. A better understanding of the DOS-metal-interactions would help to unite the global sulfur cycle with for example the mercury cycle. We found that part of the DOS is photodegradable. Thus, it is important to know if photolabile DOS compounds can complex with metals before they are degraded by solar irradiation or if metal complexation only happens with DOS compounds that survived solar irradiation. Furthermore, metallo-sulfur complexes are likely stabilizing the sulfur compounds to thermal oxidation but destabilizing to photodegradation due to ligand-metal-charge-transfer reactions (Mopper et al., 2015). Thus, photodegradation of metallo-sulfur complexes could release free toxic metals to the environment.

A methodical challenge is the concentration analysis of bulk DOS directly from a seawater sample. So far, only few values on the global DOS concentrations are available because DOS analysis is hindered by the high sulfate concentration in seawater. Most commonly, solid-phase extraction is used to remove the sulfate and then the extract is analyzed for SPE-DOS. From that the bulk DOS concentration is estimated using the extraction efficiency values detected for DOC. For this, similar extraction efficiencies of DOC and DOS are assumed but the validity of this approach cannot be fully assured. Cutter et al. (2004) developed a method for selective sulfate removal followed by determination of total dissolved sulfur. The sulfate is removed by pumping the seawater sample through a cartridge that contains barium immobilized on a cation exchange resin ($BaSO_4$ precipitation), an Ag/cation exchange resin cartridge (AgCl precipitation), and finally through a strong anion exchange resin that removes most of the remaining sulfate. Tests of this approach with sulfur-containing model compounds revealed recoveries of >95 %. Although this new method is promising, it still involves many extraction steps that may change the DOS content.

Large-scale isotopic analyses on the DOS isotopic composition would provide information that would help to enhance the understanding on the global sources and pathways of DOS. DOS from biosynthetic origin (assimilatory sulfate reduction) is ^{34}S-enriched because ^{32}S-sulfate is preferentially used in bacterial sulfate reduction leaving the remaining oceanic sulfate ^{34}S-enriched (Bottrell and Newton, 2006; Schlesinger and Bernhardt, 2013). Therefore, the sulfide produced by bacterial sulfate reduction is ^{32}S-enriched. When this sulfide is abiotically incorporated into organic matter it produces ^{32}S-enriched DOS. In this way, the isotopic composition of any DOS sample could give indication on its origin and from that on the pathway it undertook. This approach was already used for small scale systems in stream waters of forested areas (Giesler et al., 2009; Kang et al., 2014). A similar approach was done for the organic sulfur present in petroleums, kerogens, and sediments (Dinur et al., 1980; Mossmann et al., 1991; Raven et al., 2016). There the organic sulfur was enriched in the lighter isotope by >10 % relative to the sulfur in biomass indicating that the sedimentary organic sulfur cannot completely result from biomass burial. However, organically bound sulfur was found to be heavier than co-existing pyrite indicating that it also does not completely result from abiotic pathways (Mossmann et al., 1991). Brüchert and Pratt (1996) also found indication that the organic sulfur present in an estuarine sediment sample from a Florida Bay is a mixture of ^{34}S-depleted sulfur from bacterial reduction derived sulfide and ^{34}S-enriched biosynthetic sulfur.

Information from future studies of the issues described above would help to fill the current knowledge gaps on DOS and increase our understanding of the global sulfur cycle not only qualitatively but also quantitatively.

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8. Co-author publication

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

Gomez-Saez, G. V., Niggemann, J., Dittmar, T., **Pohlabeln, A. M.**, Lang, S. Q., Noowong, A., Pichler, T., Wörmer, L., Bühring, S. I.

Abstract:

Shallow submarine hydrothermal systems are extreme environments with strong redox gradients at the interface of hot, reduced fluids and cold, oxygenated seawater. Hydrothermal fluids are often depleted in sulfate when compared to surrounding seawater and can contain high concentrations of hydrogen sulfide (H_2S). It is well known that sulfur in its various oxidation states plays an important role in processing and transformation of organic matter. However, the formation and the reactivity of dissolved organic sulfur (DOS) in the water column at hydrothermal systems are so far not well understood. We investigated DOS dynamics and its relation to the physicochemical environment by studying the molecular composition of dissolved organic matter (DOM) in three contrasting shallow hydrothermal systems off Milos (Eastern Mediterranean), Dominica (Caribbean Sea) and Iceland (North Atlantic). We used ultra-high resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) to characterize the DOM on a molecular level. The molecular information was complemented with general geochemical data, quantitative dissolved organic carbon (DOC) and DOS analyses as well as isotopic measurements (δ^2H, $\delta^{18}O$, $F^{14}C$). In contrast to the predominantly meteoric fluids from Dominica and Iceland, hydrothermal fluids from Milos were mainly fed by recirculating seawater. The hydrothermal fluids from Milos were enriched in H_2S and DOS, as indicated by high DOS/DOC ratios and by the fact that 93.5% of all assigned DOM formulae that were exclusively present in the fluids contained sulfur. In all three systems, DOS from hydrothermal fluids had on average lower O/C ratios (0.31 − 0.33) than surrounding surface seawater DOS (0.47 − 0.49), suggesting shallow hydrothermal systems as a source of reduced DOS, which will likely get oxidized upon contact with oxygenated seawater. Evaluation of hypothetical pathways suggests DOM reduction and sulfurization during seawater recirculation in Milos seafloor. The four most effective pathways 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 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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Popular Summary

Dissolved organic matter (DOM) describes all organic molecules that are dissolved in water. Thus, it can casually be called the “tea of the sea”. Although DOM can be found in aquatic systems all over the world, the knowledge on DOM is limited. So far, no analysis method is able to separate the molecules in the complex DOM mixture so that they could be investigated individually. Furthermore, the concentration of the single compounds is very low. Thus, trying to get information on specific compounds in the DOM mixture is like looking for a specific piece of hay in a haystack. An impressive method to get some insights into the composition of DOM is Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) that enables the detection of several tens of thousands of compounds simultaneously making chromatographic separation unnecessary. The mass accuracy is less than 0.1 mDa which is less than the mass of a single electron which is 9.11 $x10^{-31}$ kg. This number has 30 zeros after the comma. Based on this high mass accuracy it is possible to assign the molecular formulae to the detected masses. However, each of these molecular formulae can consist of millions of possible structures (isomers) because the atoms can be combined in many ways. To get information on the structural level, further methods have to be applied.

In this thesis selective alteration reactions were developed to target this problem. With these methods we were able to test DOM samples for specific potentially present functional groups. For some a molecule was added to the targeted functional group, for others it was cleaved. The untreated and treated sample were then analyzed at the FT-ICR-MS and the obtained spectra were compared. If they showed differences this would mean that the targeted functional group was present in the sample. Collision-induced fragmentation experiments were also used to look for indicative losses representing specific functional groups. Imagine this process as shooting with argon atoms at the DOM molecules. The impact results in a characteristic breaking of the molecules.

In our analyses we focused on the sulfur-containing compounds within the DOM mixture called dissolved organic sulfur (DOS). Knowledge on them is very scarce. This lack of studies on DOS might be due to the smelly character of sulfur or because DOS is only a subfraction of DOM meaning that it is even less concentrated in the water and thus, harder to investigate. Nevertheless, we were undeterred by that and analyzed DOS from different locations across the planet: open ocean DOM from the North Sea surface and deep sea water from the North Equatorial Pacific, four depths of the Black Sea water column, as well as pore water from sediments from the German Wadden Sea and a subtropical salt marsh in the USA.

We found that DOS concentrations were generally higher in surface waters than in deeper waters and also higher in sulfidic environments compared to oxic ones. A common structural feature of the DOS samples was the fully oxidized and hydrolyzed sulfonic acid group. Only for the highly sulfidic pore water sample we observed reduced DOS functionalities like thioethers indicating special processes occurring there. To examine where DOS originates,

we mixed DOM with reactive sulfur species for four weeks. We found an increase in the number of DOS compounds afterwards, meaning that the sulfur got incorporated into the DOM compounds producing DOS. This process is called sulfurization. The produced DOS looked just like the DOS that we found in natural sulfidic pore water. Thus, similar processes like the one we simulated in the laboratory must happen there, too. The global input of DOS produced by sulfurization and transported to the ocean by benthic fluxes could be estimated as 45 – 120 $x10^{12}$ g sulfur per year. This would be more than two million trailer trucks full of sulfur. This input is bigger than the DOS input by rivers and could be the single most important source to the ocean. A possible removal process for this DOS in the ocean is photodegradation. The energy input by solar irradiation results in the breaking of the DOS molecules. Humans can experience this kind of solar energy as sunburn.

In conclusion, this thesis provides novel insights into the distribution of DOS compounds in marine systems and their molecular composition. Furthermore, it gives evidence for a source (sulfurization) and a sink (photodegradation) of DOS in the global sulfur cycle.

Populärwissenschaftliche Zusammenfassung

Gelöstes organisches Material (engl. „dissolved organic matter“, DOM) beschreibt alle organischen Moleküle, die in Wasser gelöst sind. Daher wird es informell als „Tee des Meeres“ bezeichnet. Obwohl DOM in wässrigen Systemen auf der ganzen Welt zu finden ist, ist das Wissen über DOM sehr begrenzt. Bis jetzt gibt es keine Analysemethode, die in der Lage wäre, die Moleküle in dem komplexen DOM Gemisch aufzutrennen, um sie einzeln zu untersuchen. Desweiteren sind die Konzentrationen der einzelnen Verbindungen sehr gering. Daher ist der Versuch Informationen über spezifische Verbindungen im DOM Gemisch zu erhalten wie die Suche nach einem speziellen Heuhalm im Heuhaufen. Eine beeindruckende Methode, um Einblicke in die Zusammensetzung des DOM zu erhalten, ist die Fourier-Transformation Ionen Zyklotron Resonanz Massenspektrometrie (FT-ICR-MS), welche die Ermittlung von mehreren tausend Verbindungen gleichzeitig ermöglicht, sodass eine chromatografische Trennung unnötig wird. Die Massengenauigkeit liegt bei unter 0.1 mDa, was weniger als die Masse eines einzelnen Elektrons ist, die 9.11×10^{-31} kg beträgt. Diese Zahl hat 30 Nullen nach dem Komma. Aufgrund dieser hohen Massengenauigkeit können die Summenformeln den ermittelten Massen zugeordnet werden. Allerdings kann jede dieser Summenformeln Millionen von möglichen Strukturen (Isomere) haben, da die Atome auf verschiedene Weise zusammengesetzt werden können. Um Informationen auf struktureller Ebene zu erhalten, müssen daher weitergehende Methoden angewendet werden.

Im Rahmen dieser Arbeit wurden selektive Umwandlungsreaktionen entwickelt, um dieses Problem anzugehen. Diese Methoden ermöglichten es, DOM Proben auf spezifische, potentiell präsente, funktionelle Gruppen hin zu untersuchen. Für manche wurde ein Molekül an die angezielte funktionelle Gruppe addiert, für andere wurde es gespalten. Die unbehandelte und die behandelte Probe wurden dann mit der FT-ICR-MS untersucht und die erhaltenen Spektren verglichen. Wenn sie Unterschiede aufwiesen, bedeutete dies, dass die anvisierte funktionelle Gruppe in der Probe vorhanden war. Es wurden auch kollisionsinduzierte Fragmentierungsexperimente verwendet, in denen nach indikativen Abspaltungen für spezielle funktionelle Gruppen gesucht wurde. Diesen Prozess kann man sich so vorstellen, dass Argonatome auf die DOM Moleküle geschossen werden und dieser Einschlag dann zu charakteristischen Aufspaltungen des Moleküls führt.

In unseren Analysen konzentrierten wir uns auf die schwefelhaltigen Verbindungen im DOM Gemisch, die als gelöster organischer Schwefel (engl. „dissolved organic sulfur“, DOS) bezeichnet werden. Über diese Verbindungen ist noch sehr wenig bekannt. Dieser Mangel an Studien über DOS könnte an dem übelriechenden Wesen des Schwefels liegen oder aber daran, dass DOS nur eine Unterfraktion des DOM ist, was bedeutet, dass es noch geringer in Gewässern vorhanden und daher noch schwieriger zu untersuchen ist. Trotzdem haben wir uns davon nicht abschrecken lassen und DOS von verschiedenen Orten der Welt untersucht: ozeanisches DOM von der Nordseeoberfläche und aus der Tiefsee des nordäquatorialischen

Pazifiks, vier Tiefen der Wassersäule des Schwarzen Meeres, sowie Porenwasser aus Sedimenten des deutschen Wattenmeeres und einer subtropischen Salzwiese in den USA.

Wir fanden heraus, dass die DOS Konzentrationen generell höher in Oberflächengewässern als in der Tiefsee waren, ebenso waren sie höher in sulfidischen Mileus als in oxischen. Eine universelle Struktureigenschaft der DOS Proben war die vollständig oxidierte und hydrolysierte Sulfonsäuregruppe. Nur in der stark sulfidischen Porenwasserprobe fanden wir reduzierte DOS Funktionalitäten wie Thioether, was auf spezielle Prozesse dort hindeutet. Um heraus zu finden, wo das DOS entspringt, vermischten wir DOM vier Wochen lang mit reaktiven Schwefelspezies. Wir entdeckten nach dieser Zeit einen Anstieg an DOS Verbindungen, was bedeutet, dass der Schwefel in das DOM eingebaut wurde, wobei DOS produziert wurde. Diesen Prozess bezeichnet man als Sulfurisierung. Die produzierten DOS Verbindungen sahen dem DOS in dem natürlichen Porenwasser sehr ähnlich. Daher müssen gleiche Prozesse wie der, den wir im Labor simuliert haben, auch dort stattfinden. Wir konnten den globalen Eintrag von DOS, welches durch Sulfurisierung produziert und dann durch benthische Flüsse in den Ozean transportiert wird, auf 45 - 120 $x10^{12}$ g Schwefel pro Jahr bestimmen. Dies entspräche mehr als zwei Millionen Sattelzügen. Dieser Eintrag ist größer als der globale DOS Eintrag durch Flüsse und könnte die wichtigste Quelle für den Ozean sein. Ein möglicher Abbauprozess für DOS im Ozean ist der Photoabbau. Der Energieeintrag durch Sonneneinstrahlung führt zum Zerbrechen der DOS Moleküle. Menschen erleben diese Form der Sonnenenergie als Sonnenbrand.

Diese Dissertation bietet insgesamt neue Einblicke in die Verteilung von DOS Verbindungen in marinen Systemen und ihre molekulare Zusammensetzung. Außerdem liefert sie Beweise für eine Quelle (Sulfurisierung) sowie eine Senke (Photoabbau) des DOS im globalen Schwefelkreislauf.

Curriculum Vitae

Name	Anika Maria Pohlabeln
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Education

Since 11/2013	Ph.D. student Research Group for Marine Geochemistry (ICBM-MPI Bridging Group), University of Oldenburg (Germany)
2011 – 2013	M.Sc. in Chemistry, University of Oldenburg (Germany)
2008 – 2011	B.Sc. in Chemistry, University of Oldenburg (Germany)

Stipends and Awards

2016	German Academic Exchange Service (DAAD) Ph.D. Student Stipend
2015	Wolfgang Schulenberg Stipend
2015	Graduate School Science and Technology travel grant
2015	Student Presentation Award of the Association for the Sciences of Limnology and Oceanography (ASLO)
2009, 2010, 2013	Federal state stipends of Lower Saxony, Germany

Publications

Pohlabeln, A. M., Dittmar, T., 2015. Novel insights into the molecular structure of non-volatile marine dissolved organic sulfur. Mar. Chem. 168, pp. 86-94.

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Pohlabeln, A. M., Niggemann, J., Dittmar, T. Molecular clues for a pathway of dissolved organic sulfur produced in sulfidic sediments to the open ocean. in prep.

Gomez-Saez, G. V., **Pohlabeln, A. M.**, Stubbins, A., Dittmar, T. Photochemical alteration of dissolved organic sulfur from sulfidic pore waters. in prep.

Gomez-Saez, G. V., Niggemann, J., Dittmar, T., **Pohlabeln, A. M.**, Lang, S. Q., Noowong, A., Pichler, T., Wörmer, L., Bühring, S. I., 2016. Molecular evidence for abiotic sulfurization of dissolved organic matter in marine shallow hydrothermal systems. Geochim. Cosmochim. Ac., 190, 35-52.

Conference contributions

Pohlabeln, A. M., Niggemann, J., Dittmar, T., 2016. Evidence for abiotic sulfurization of marine dissolved organic matter in sulfidic environments. American Geophysical Union, Ocean Sciences Meeting, 21-26 Feb., New Orleans, USA (Talk).

Gomez-Saez, G. V., Niggemann, J., Dittmar, T., **Pohlabeln, A. M.**, Lang, S. Q., Noowong, A., Pichler, T., Wörmer, L., Bühring, S. I., 2016. Sources and fate of dissolved organic sulfur at the redox interface of marine shallow hydrothermal systems. American Geophysical Union, Ocean Sciences Meeting, 21-26 Feb., New Orleans, USA (Talk).

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Pohlabeln, A. M., Niggemann, J., Christoffers, J., and Dittmar, T., 2013. Dissolved organic sulfur in the ocean – A mass spectrometric investigation, YOUMARES 4. German Young Marine Scientist Meeting, Oldenburg, Germany, 11.-13. September (Poster).

Fieldwork

Jul – Sep 2016	Research project on photodegradation of dissolved organic sulfur in Prof. Dr. Aron Stubbins laboratory at Skidaway Institute of Oceanography in Savannah, Georgia, USA

Author’s declaration

I hereby declare that I wrote this thesis on my own and without any unreferenced sources or aid. I confirm, that I followed the general principles of good scientific work and publishing, as they are specified in the guidelines of good scientific practice of the Carl von Ossietzky University of Oldenburg. Furthermore, I declare that this thesis neither completely nor partly is and was not submitted to another university for assessment in a dissertation procedure.

Eidesstattliche Erklärung

Hiermit versichere ich, dass ich diese Arbeit selbstständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet habe. Außerdem versichere ich, dass ich die allgemeinen Prinzipien wissenschaftlicher Arbeit und Veröffentlichung, wie sie in den Leitlinien guter wissenschaftlicher Praxis der Carl von Ossietzky Universität Oldenburg festgelegt sind, befolgt habe. Desweiteren erkläre ich, dass diese Dissertation weder in ihrer Gesamtheit noch in Teilen einer anderen Hochschule zur Begutachtung in einem Promotionsverfahren vorliegt oder vorgelegen hat.

Oldenburg, den 16. Dezember 2016
(Anika Maria Pohlabeln)