Carl von Ossietzky Universität Oldenburg

Fakultät für Mathematik und Naturwissenschaften

The molecular geography of dissolved organic matter in the Atlantic and Southern Ocean

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Maren Seibt

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1. Gutachter: Prof. Dr. Thorsten Dittmar
2. Gutachterin: Dr. Katharina Pahnke-May

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ABSTRACT

Dissolved organic matter (DOM) is a vast reservoir of carbon comparable in size to atmospheric CO_2. It is a key component in the marine carbon cycle and therefore also relevant in the global climate system. The millennial stability of DOM has puzzled oceanographers in the past decades and the reasons behind it are subject to an ongoing and controversial scientific debate. Because the distribution and molecular composition of DOM determine its biological availability and residence time in the ocean, it is the overall goal of this thesis to identify distinct patterns in the molecular composition and relate it to biotic and abiotic processes. For that purpose, more than 350 samples taken in the Atlantic and Southern Ocean were analyzed via ultrahigh resolution mass spectrometry (Fourier-transform ion cyclotron resonance mass spectrometry; FT-ICR-MS). The main results of this thesis are summarized in three chapters, each of which corresponds to a manuscript prepared for submission to a scientific journal.

In the first manuscript (second chapter), two process-related indices revealing the molecular signature of photodegradation and bioproduction embedded in marine DOM are introduced. Both indices are calculated with relative signal intensities of process-specific marker compounds, which were identified in molecular data sets from two laboratory experiments, that were published recently. The application of the indices to DOM sample sets from the Pacific, Atlantic and Southern Ocean traces the characteristic signature of both processes in natural environments and makes it possible to assess the relative extent of both processes.

In the second manuscript (third chapter), the major factors influencing the molecular geography of DOM in the Atlantic and Southern Ocean are identified. For that purpose, a simple two-source mixing model is constructed using the molecular signatures of two characteristic endmembers: Microbially produced DOM obtained during a previously published laboratory experiment and refractory deep sea DOM from the Central North Pacific were mixed proportionally with increasing amounts of fresh DOM on top of the refractory background. In a second step, the modeled fingerprints with the highest molecular similarity to DOM from the Atlantic and Southern Ocean were identified. The results demonstrate that most of the molecular variability of DOM can be explained by mixtures of these two endmembers, which

leads to the conclusion that microbial production mainly shapes the molecular composition and that water mass mixing is mostly responsible for transporting the characteristic signature into deeper water layers.

The third manuscript (fourth chapter) focuses on the distribution and molecular composition of solid-phase extractable dissolved organic nitrogen (SPE-DON) in the Atlantic and Southern Ocean and the associated biotic and abiotic processes. Specific patterns of the molecular geography of SPE-DON were identified and linked to published data focusing on the specific microbial community compositions and the prevailing nutrient regimes. Especially the oligotrophic gyres are potential hotspots of DON production and turnover. The molecular data of SPE-DON provides complementary information to several already published studies targeting the turnover of bulk DON in the global ocean.

ZUSAMMENFASSUNG

Das gelöste organische Material (engl. dissolved organic matter; DOM) beschreibt ein Reservoir organischen Kohlenstoffs im Ozean, das in seiner Größe mit der Menge des atmospherischen CO_2 vergleichbar ist. DOM ist eine Schlüsselkomponente des marinen Kohlenstoffkreislaufs und damit auch ein wichtiger Baustein im globalen Klimasystems. Die enorme Langlebigkeit des DOM über einen Zeitraum von Jahrtausenden verblüfft Ozeanographen seit Jahrzenten. Die Gründe für diese Langlebigkeit sind Gegenstand zahlreicher kotrovers geführter wissenschaftlicher Debatten. Die Verteilung sowie die molekulare Zusammensetzung des DOM bestimmt dabei maßgeblich die biologische Verfügbarkeit und Aufenthaltsdauer im Ozean. In diesem Zusammenhang steht das übergeordnete Ziel der vorliegenden Arbeit, bestimmte Muster in der molekularen Zusamensetzung zu erkennen und diese mit biotischen und abiotischen Prozessen in Verbindung zu bringen. Zu diesem Zweck wurden mehr als 350 DOM Proben im Atlantik und im Südpolarmeer gesammelt und mittels ultrahochauflösender Fourier-Transformation Ionencyclotronresonanz Massenspektrometrie (FT-ICR-MS) analysiert. Die wichtigsten Ergebnisse dieser Arbeit sind in drei Kapiteln zusammegefasst, wobei jedes dieser Kapitel einem Manuskript entspricht, das zur Publikation in einer wissenschaftlichen Zeitschrift vorbereitet wurde.

Im ersten Manuskript werden zwei Indizes vorgestellt, die die molekulare Signatur von Photoabbau und biologischer Produktion in der Zusammensetzung von DOM offenlegen. Beide Indizes werden mit den relativen Signalintensitäten von prozessspezifischen „Marker"-Molekülen berechnet, die mit Hilfe zweier vor kurzem publizierter experimenteller Datensätze identifiziert wurden. Durch die Anwendung der beiden Indizes in DOM Proben aus dem Atlantik, Pazifik und Südpolarmeer kann die Spur von Photoabbau und biologischer Produktion in der Umwelt verfolgt und das relative Ausmaß beider Prozesse auf die molekulare Zusammensetzung untersucht werden.

Im zweiten Manuskript werden Faktoren identifiziert, die die molekulare Geographie der DOM Moleküle maßgeblich beeinflussen. Zu diesem Zweck wurde ein einfaches zwei-Quellen Mischungsmodell mit zwei charakteristischen „Endmem-

ber“ konstruiert: Mikrobiell produziertes DOM, das während einer bereits publizierten Studie gewonnen wurde, und refraktäres DOM aus dem tiefen Nord-Pazifik wurden proportional mit aufsteigender Menge frisch produzierten Materials als Zusatz zu refraktärem Material gemischt. In einem zweiten Schritt wurden die modelierten molekularen Fingerabdrücke, die die höchste Ähnlichkeit zu natürlichen molekularen DOM Fingerabdrücken aus dem Atlantik und Südpolarmeer aufweisen, identifiziert. Die Ergebnisse zeigen, dass ein großer Teil der molekularen Variabilität durch die Mischung dieser beiden „Endmember“ erklärt werden kann. Dies bedeutet, dass mikrobielle Produktion maßgeblich die molekulare Zusammensetzung des DOM beeinflusst und dass eine Durchmischung verschiedener Wassermassen diese charakteristische Signatur in tiefere Wasserschichten transportiert.

Das dritte Manuskript beschreibt die Verteilung des durch Festphasen-Extraktion gewonnenen gelösten organischen Stickstoffs (engl. solid-phase extractable dissolved organic nitrogen; SPE-DON) im Atlantik und Südpolarmeer und die damit verbundenen biotischen und abiotischen Prozesse. Spezielle Muster in der molekularen Geographie von SPE-DON wurden identifizert und mit publizierten Studien verknüpft, die die Zusammensetzung der mikrobiellen Gemeinschaften und die Verfügbarkeit von Nährstoffen in den Probenahmegebieten beschreiben. Besonders die oligotrophen Wirbel scheinen „Hotspots“ für mikrobielle Produktion und Umsatz zu sein. Die molekularen SPE-DON Daten bieten zusätzliche Informationen zu vielen bereits publizierten Studien, die den Umsatz des gesamten DON im globalen Ozean beschreiben.

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LIST OF ABBREVATIONS

AABW	Antarctic Bottom Water
AAIW	Antarctic Intermediate Water
AASW	Antarctic Surface Water
AI	Aromaticity Index
CDOM	Chromophoric DOM
CDW	Circumpolar Deep Water
Da	Dalton
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
DON	Dissolved organic nitrogen
DOP	Dissolved organic phosphorus
DOS	Dissolved organic sulfur
EMW	Eurafrican Mediterranean Water
FT-ICR-MS	Fourier Transform Ion Cyclotron Resonance Mass Spectrometry
HLNC	High nutrient-low chlorophyll
HMW	High molecular weight
LMW	Low molecular weight
m/z	Mass to charge ratio
NACW	North Atlantic Central Water
NADW	North Atlantic Deep Water
NEqPIW	North Equatorial Pacific Intermediate Water
NPP	Net primary production
POM	Particulate organic matter
SACW	South Atlantic Central Water
SASW	Subantarctic Surface Water
SML	Surface mixed layer of the Atlantic
SPE	Solid-phase extraction
STSW	Subtropical Surface Water
TEP	Transparent exopolymer particles
WDW	Weddell Sea Warm Deep Water
WSDW	Weddell Sea Deep Water

1. INTRODUCTION

The global carbon cycle describes the exchange of carbon between the Earth's major carbon reservoirs, i.e. the atmosphere, the ocean, the land and the fossil reservoirs. Fluxes between each reservoir and changes in its size impact the global climate. Due to the fundamental shift in land use and mankind's extensive burning of fossils fuels, large proportions of the fossil reservoir are transferred to the atmosphere in the form of the greenhouse gas CO_2 (Figure 1.1), leading to a changing global climate. The rate at which the atmospheric CO_2 increases is influenced by terrestrial and oceanic processes, which can either act as an additional source or sink of atmospheric CO_2. About one quarter of the CO_2 produced by anthropogenic activities is dissolved in the ocean (Le Quéré et al., 2015), clearly showing the potential of the ocean in mitigating global climate change.

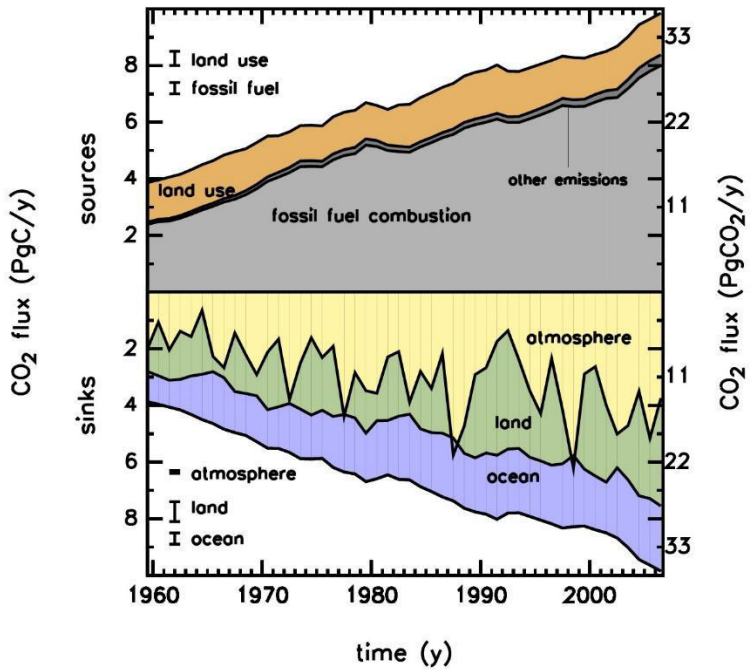

Figure 1.1 CO_2 budget from 1959 to 2006. The upper panel shows CO_2 emissions to the atmosphere (sources) as the sum of fossil fuel combustion, land-use change, and other emissions, which are primarily from cement production. The lower panel shows the fate of the emitted CO_2, including the increase in atmospheric CO_2 plus the sinks of CO_2 on land and in the ocean. Flux is in Pg y^{-1} carbon (left axis) and Pg y^{-1} CO_2 (right axis). Reprinted from Canadell et al. (2007).

One of the largest active carbon pools within the global carbon cycle and subject of key biogeochemical processes in the marine carbon cycle is marine dissolved organic matter (DOM). It is involved in biological processes as supplier of energy and nutrients to marine microorganisms and therefore forms the basis of the marine food web, but it also acts as a long-time carbon storage system in the deep sea, where it resides in mostly biologically inert forms for thousands of years.

The distribution and molecular composition of DOM determine the residence time and biological availability of DOM in the ocean. Hence, deeper knowledge of both, distribution and composition, will enlighten the role of DOM in the global carbon cycle and consequently its function within the global climate cycle. The aim of this thesis is to investigate the molecular DOM composition in the Atlantic and Southern Ocean and to identify factors influencing composition, residence time and biological availability of this important carbon pool. The results will contribute to our understanding of interactions between the DOM pool and the environment (biotically and abiotically) and will add information to the present discussion about how changes in DOM pool size or reactivity will impact other carbon reservoirs on land, in the ocean or the atmosphere (Moran et al., 2016).

1.1 Dissolved organic matter in the ocean

DOM is found in marine and terrestrial ecosystems (i.e. lakes, rivers and soils). The focus of this thesis is on marine DOM and the characteriziation, production and degradation mechanisms of terrestrial DOM are therefore out of scope and not discussed in the following sections.

1.1.1 What is dissolved organic matter?

Dissolved organic matter (DOM) in the ocean is operationally defined as the fraction of organic compounds, which passes through a filter with a pore size of < 0.7 µm (Ogawa and Tanoue, 2003). As the term “dissolved” is operationally defined, DOM also includes viruses and small bacteria, which are not retained on the filters. The main elemental constituents of DOM are carbon, oxygen and hydrogen, but other elements such as nitrogen, phosphorus and sulfur are also bound in DOM forming the sub-pools of dissolved organic carbon (DOC), dissolved organic nitrogen (DON), dissolved organic phosphorus (DOP) and dissolved organic sulfur

(DOS). The presence of these elements makes DOM an essential source of nutrients to marine organisms.

The ocean holds about 1000 Gt of organic carbon, which is about the same amount as all living biomass in terrestrial ecosystems combined (600 –1000 Gt; Falkowski et al., 2000). The largest fraction of marine organic carbon is bound in DOM, which holds about 662 Gt of carbon (Hansell, 2013).

As DOC is the principal component of DOM, it can be used as a proxy for DOM concentrations in the ocean. In the surface ocean DOC concentrations are variable, but mostly range between 40 – 80 µmol l^{-1} with the highest concentrations in the subtropical and tropical surface ocean due to the strong stratification of the water column, which favors the accumulation of DOM compounds (Hansell et al., 2009). Lowest concentrations of surface DOC are found in the polar regions, where upwelling deep waters with low DOC concentrations mix with surface waters. In the deep sea, DOM concentrations are low and mostly homogenously distributed throughout the global deep ocean with concentrations ~ 40 µmol l^{-1} (Barber, 1968; Hansell et al., 2009; Figure 1.2).

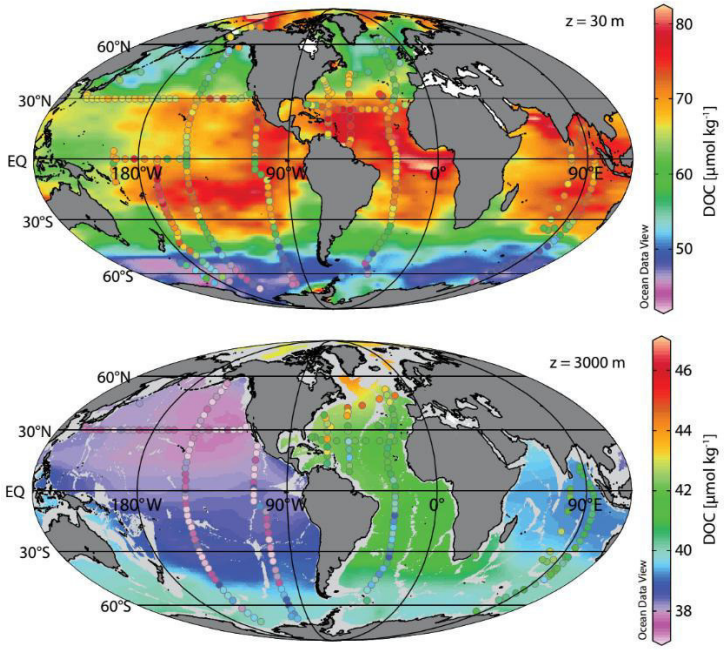

Figure 1.2 Concentration of DOC in the global ocean at 30 m (upper panel) and at 3000 m (lower panel). The dots represent measured concentrations, while the background field is modeled. Reprinted from Hansell et al. (2009).

DON in the surface ocean is the dominant form of nitrogen with concentrations ranging between 2 to 7 µmol l^{-1} (Letscher et al., 2013) and decreasing concentrations with depth as nitrate becomes the more abundant form of nitrogen (Torres-Valdés et al., 2009). DOS and DOP are the smallest sub-pools of DOM. Ksionzek et al. (2016) estimated minimum DOS concentrations of 0.31 ± 0.09 µmol l^{-1} in the surface ocean and 0.17 ± 0.03 µmol l^{-1} in the deep ocean with an estimated total inventory of 6.7 Gt. The concentration of DOP is ~ 0.2 µmol l^{-1} in the surface ocean and < 0.1µmol l^{-1} in the deep ocean (Karl and Björkman, 2015).

To understand the cycling of DOM in the ocean, several approaches were developed to classify DOM into categories of characteristic features. These categories include the classification along molecule size, origin, reactivity or compound group. The molecule size can be roughly split into two main categories, the high molecular weight compounds (HMW DOM), which have a molecule size of > 1 kDa and the low molecular weight compounds (LMW DOM) with a molecule size < 1 kDa. HMW DOM is mostly found in terrestrial aquatic ecosystems (> 80 % in Amazon River samples; Amon and Benner, 1996), whereas LMW DOM is mostly found in marine ecosystems (65 – 80 %; Ogawa and Tanoue, 2003). Since functional features are not represented by size alone, Amon and Benner (1996) proposed a size continuum model, which accounts for the fact that many DOM fractions sharing a functionality (e.g. the marine gel phase), can span a wide range of sizes classes (Verdugo et al., 2004).

Based on the residence time of DOM in the ocean, DOM can be classified into five main reactivity fractions: labile, semi-labile, semi-refractory, refractory and ultra-refractory DOM (Hansell, 2013). Labile DOM consists of sugars and short-chain organic acids. It is turned over rapidly within minutes to days in the upper water column and does not accumulate in the ocean. Therefore, it is found in very low concentrations (Hansell, 2013) or escapes analytical detection completely. Semi-labile DOM has turnover rates of months to several years and, contrary to labile DOM, can be exported horizontally and vertically from its region of formation (Hansell, 2013). It is observable as seasonal variability in DOC concentrations above the pycnocline in the euphotic zone. Its turnover largely depends on its molecular composition, on nutrient availability and microbial community structure

(Carlson et al., 2004). Labile and semi-labile DOM together form the basis of the marine food web in the euphotic zone. After export to the mesopelagic zone, semi-labile DOM fuels the subsurface microbial production (Hansell, 2013). Semi-refractory DOM has a turnover time of decades and requires the presence of a permanent pycnocline (Hansell, 2013). It is therefore only observable in strongly stratified water columns such as the Atlantic Ocean. Refractory DOM is the most abundant form of DOM with an inventory of 630 ± 32 Gt and a residence time of millennia (Hansell, 2013). Refractory DOM is ubiquitously found in all water depths (Bauer et al., 1992). Due to the extremely long residence time, it plays a significant role in the global climate cycle. The most stable DOM pool is the ultra-refractory DOM with a pool size of > 12 Gt C and a residence time in the ocean of about 40,000 years (Hansell, 2013). These ultra-stable carbon compounds are thought to be of mostly thermogenic origin and represent the link between the biological and geological realm (Hansell, 2013).

The categorization of DOM into different compound classes can help to gain some insights into structural properties of DOM. Amino acids, carbohydrates, and fatty acids are labile DOM components and can be directly quantified via analytical methods, because their structure is well known. However, as mentioned above, these compounds classes have a very transient nature and often escape analytical detection. If detected, they can give valuable insights into early DOM diagenesis (e.g. Dauwe et al., 1999). Compound classes can also help to trace back the molecules to the place of their production; hence they can be used as biomarkers. Lignin phenols, for example, are a characteristic feature of vascular plants only found in terrestrial ecosystems and are detected in almost all areas of the global ocean (Hernes and Benner, 2006). Another marker compound group is black carbon, which is the product of incomplete combustion and is transported to the ocean via rivers (Dittmar et al., 2012). Black carbon belongs to the pool of ultra-refractory DOM (Hansell, 2013) and accounts for ~ 10 % of the global riverine DOC flux to the ocean (Jaffé et al., 2013).

1.1.2 Sources, turnover and sinks of organic carbon in the ocean

The ultimate source of marine dissolved organic matter (DOM) is primary production in the surface layer of the global ocean. Photoautotrophic organisms in the euphotic zone take up water and CO_2 from the atmosphere and build up biomass using energy from sunlight.

About half of the global net primary production (NPP; 104.9 Gt C year^{-1}) occurs in the global ocean (~ 48 Gt C year^{-1}) with the largest fraction (27.4 Gt C year^{-1}) being produced in the mesotrophic areas (Field et al., 1998). Although the oligotrophic ocean is limited by nutrients, primary production rates are second highest (11 Gt C year^{-1}) due to the vast size of these biogeographic areas (Figure 1.3).

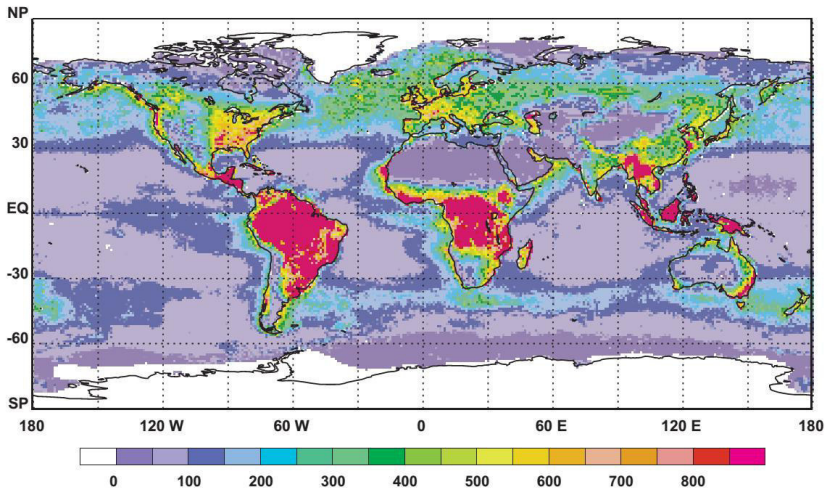

Figure 1.3 Global annual NPP in g of C per m^2 per year. Global NPP is 104.9 Gt of C year^{-1}, with 46.2 % contributed by the oceans and 53.8 % contributed by the land. Abbreviations: NP = North Pole, EQ = equator, SP = South Pole. Reprinted from Field et al. (1998).

Marine primary production is a highly dynamic process considering that the annual NPP of ~ 48 Gt C is carried out by a phytoplankton biomass of only about 1 Gt (Carr et al., 2006). Most of the fixed carbon is again lost to the atmosphere via respiration (Del Giorgio and Duarte, 2002); the remaining parts are either taken up by organisms of higher trophic levels, transferred to the POM pool or reach the DOM pool via various processes: extracellular release by phytoplankton (e.g.Wetz and Wheeler, 2007), grazing by zooplankton (sloppy feeding; e.g. Nagata and Kirchman, 1992), bacterial and viral cell lysis (e.g. Lønborg et al., 2013), and dissolution of particles and heterotrophic turnover (e.g. Arnosti et al., 2011). Other

sources of DOM to the ocean are fluvial, tidal or riverine input (Dittmar and Stubbins, 2014), but these terrestrial sources play only minor roles in the marine carbon cycle (Hansell et al., 2009; Hansell, 2013).

Due to the small size of individual DOM molecules, DOM is largely unavailable to most marine organisms and its uptake is mostly limited to microorganisms. The microbial loop, in which heterotrophic bacteria utilize DOM to build up biomass, keeps the carbon fixed by primary production within the marine food web (Azam et al., 1983). It also helps to regenerate nutrients, which would otherwise be lost from the food chain. These regenerated nutrients can then fuel production in nutrient-limited ecosystems such as the subtropical gyres.

About a third (~ 16 Gt C) of the global primary production is exported to the deep sea (biological pump; Figure 1.4) and effectively sequestered from the atmosphere for centuries to millennia (Falkowski et al., 1998). Non-living particles (e.g. fecal pellets, dead cells or aggregates, together termed as "marine snow") can be exported from the euphotic layers of the ocean into the deep sea (Alldredge and Silver, 1988) and serve as hot spots for microbial activity (Simon et al., 2002).

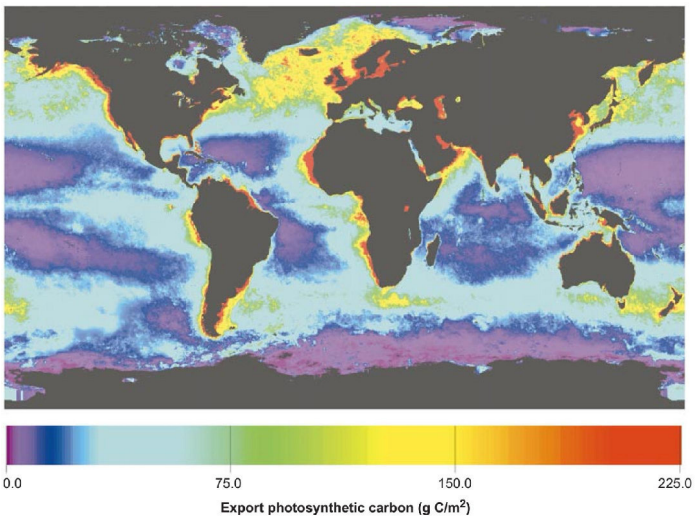

Figure 1.4 Map of the annual mean export production of the world ocean. The export production is also termed the biological pump. The influence of coastal upwelling in supporting high export production is especially apparent. Reprinted from Falkowski et al. (1998).

It was hypothesized that bio-resistant molecules are produced as a byproduct of heterotrophic processing of labile DOM within the microbial loop, contributing to the pool of refractory DOM and, thus, to the long-term storage of DOM in the ocean

(Ogawa et al., 2001). This concept was termed the microbial carbon pump (Jiao et al., 2010). Figure 1.5 summarizes the biological sources, sinks and turnover in the marine carbon cycle.

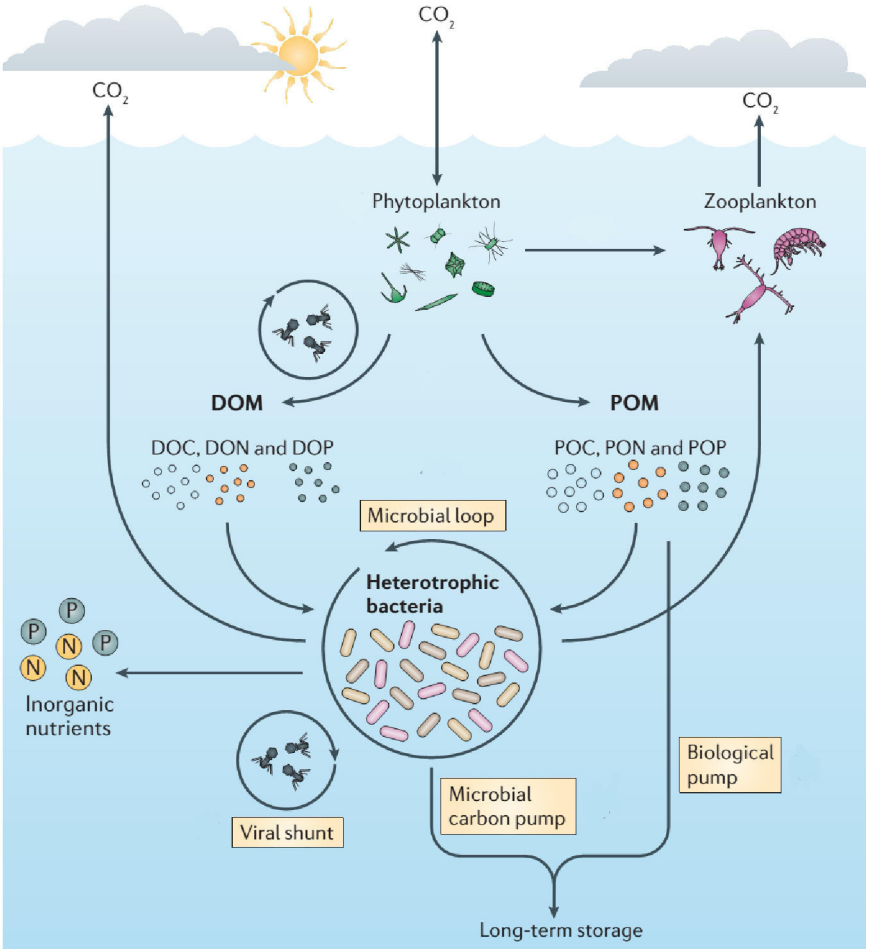

Figure 1.5 Schematic summary of sources, sinks and turnover of marine organic carbon. Carbon, fixed by phytoplankton, is either respired, taken up by organisms of higher trophic levels, dissolved and channeled into the DOM pool or exported to the deep sea via POM (biological pump). Within the microbial loop, microorganisms take up carbon from the DOM and POM pool keeping the energy in the marine food web and regenerating nutrients. During photosynthesis, at higher trophic levels and within the microbial loop carbon is lost from the marine carbon pool via respiration. The microbial carbon pump is the conceptual framework summarizing microbial processes producing refractory DOM, which is mostly bio-resistant and remains in the ocean over thousands of years. Reprinted from Buchan et al. (2014).

Along with biological mechanisms acting as sinks for organic carbon in the ocean, several abiotic processes modify DOM, which is either lost from the DOM pool or transformed. These processes include the adsorption to particles (Satterberg et al.,

2003) and photodegradation (Mopper et al., 1991). It has been shown that photodegradation of DOM can act as a sink for DOM (Moran et al., 2000), enhance (Moran and Zepp, 1997) or reduce the biological availability (Naganuma et al., 1996), or can have no impact at all (Thomas and Lara, 1995). The fundamentally different conclusions from these studies show that photodegradation is a very complex process with no clear outcome and no specific signature in the molecular DOM composition. Tranvik and Bertilsson (2001) found that the impact of photodegradation is directly linked to DOM origin and quality and may differ between DOM compound classes (e.g. humic substances and algal-derived DOM). The abiotic formation of aggregates and gels (e.g. TEP) constitutes another removal mechanism of DOM (Alldredge et al., 1993). Larger particles formed by aggregation of DOM can bridge the gap between DOM and POM. Analogous to the microbial loop, this "abiotic loop" keeps the energy within the marine food web as the aggregates are large enough to be taken up by marine organisms.

1.2 Characterizing marine dissolved organic matter

The characterization of DOM is a challenging task, since its molecular composition consists of presumably millions of different compounds (Dittmar, 2015) with an even higher number of potential structural isomers (Hertkorn et al., 2008). Several compound classes with known structures can be targeted separately such as amino acids, sugars and short-chain fatty acids, but these quantifiable organic compounds account for less than 2 % of bulk DOM in the deep sea (Kaiser and Benner, 2009). The approaches for studying the marine DOM cycle include the characterization of bulk DOM (DOC concentration, isotopic composition and optical properties) as well as ultrahigh-resolution techniques aiming at elucidating the DOM composition on the molecular level. In the following chapters the discussion is limited to the isotopic characteristics of DOM, the ultrahigh-resolution mass spectrometric analysis of DOM and the statistical approaches used for DOM data evaluation. The discussed features and techniques were either applied in this thesis or relevant for data interpretation, but this discussion is by no means exhaustive and should rather provide an overview over current scientific approaches. For further information about the optical properties of DOM (chromophoric DOM (CDOM) and fluorescent

DOM (FDOM)) and photochemical processes see Mopper et al. (2015). For a comprehensive review on current analytical techniques for characterizing DOM see Nebbioso and Piccolo (2013).

1.2.1 Isotopic characteristics of DOM

Carbon has two stable isotopes, the abundant and lighter ^{12}C and the less abundant and heavier ^{13}C. The atmospheric CO_2 contains 98.9 % ^{12}C and 1.1 % ^{13}C (Farquhar et al., 1989). During photosynthesis, $^{12}CO_2$ is preferably used, but some phototrophic organisms use both isotopes in slightly different proportions. This imbalance leads to different isotope ratios ($\delta^{13}C$) characteristic for specific types of phototrophs providing information about what type of plant fixed a given organic molecule. The most significant differences in $\delta^{13}C$ occur between classes of C3 and C4 land plants (-33 ‰ to -24 ‰ for C3 plants and -16 ‰ to -10 ‰ for C4 plants). Fixed carbon from terrestrial ecosystems with these specific isotope signatures can be transported via rivers to the ocean.

The lowest $\delta^{13}C$ values with up to -28 ‰ are found for marine DOM produced in polar waters, which is, due to the high pCO_2 in polar regions, favoring greater autotrophic fractionation and the production of ^{13}C-depleted organics (Rau et al., 1982). Excluding the low $\delta^{13}C$ DOM values in polar waters, the variance in $\delta^{13}C$ is only between -23 ‰ and -18 ‰ for marine DOM. This small range makes it very difficult to distinguish DOM sources in the global ocean. However, the great difference between $\delta^{13}C$ values of DOM produced in terrestrial ecosystems and marine DOM makes $\delta^{13}C$ a great tool to distinguish marine from terrestrial sources.

In addition to the two stable isotopes, carbon also has a radioactive isotope (^{14}C). Radiocarbon is produced at constant rates in the upper atmosphere when cosmic rays enter the atmosphere and collide with atoms producing free neutrons. These neutrons are absorbed by nitrogen atoms to produce ^{14}C and protons. Due to its radioactive nature, radiocarbon decays with a half-life of 5730 ± 40 years (Godwin, 1962) and is therefore a valuable tool in estimating the age of carbon-containing materials. An autotrophic organism takes up atmospheric CO_2 and its isotopic signature is therefore the same as the surrounding atmosphere. Once the organism dies, this exchange stops and due to its radioactive nature, the ^{14}C content decreases while

the stable ^{12}C content remains the same. This drop in the ratio between ^{14}C and ^{12}C is expressed as $\Delta^{14}C$ and measured in part per million (ppm). The lower the ratio, the older the organic remains of an organism. Because the atmospheric production of ^{14}C is relatively constant, it is assumed that the production rate was constant in the past. However, the testing of nuclear bombs in the 1950s and 1960s lead to an abrupt rise in atmospheric ^{14}C. Hence, the age of a carbon pool is given as years before present (ybp) with a correction for anthropogenically produced radiocarbon.

DOM is a complex mixture of thousands of different compounds, all possibly having different ages. The bulk ^{14}C age of any given DOM sample is therefore only an average of all the compound ages present in that sample. In general, $\Delta^{14}C$ values of DOM are highest in the surface waters (youngest material) and lowest in the deep sea (oldest material). However, $\Delta^{14}C$ values in the surface DOM never reach the value of atmospheric CO_2 indicating that surface DOM consists of a mixture of freshly produced DOM and older deep sea DOM. The analysis of DOM from the deep Pacific revealed a radiocarbon age of > 3000 ybp (Williams et al., 1969), exceeding the time a water parcel needs to fulfill a full transit of the thermohaline circulation. This exceptionally high age gave a first indication that some DOM molecules are biologically inert and are effectively removed from active cycling for the time of millennia.

1.2.2 Ultrahigh-resolution mass spectrometry

Most chemical approaches (e.g. chromatography) can separate the DOM pool into DOM sub-pools of the same physicochemical properties (i.e. polarity or molecule size), whereas DOM appears to be physically inseparable on the molecular level so far (Dittmar and Stubbins, 2014). This property makes it difficult to study the turnover on the level of individual compounds. The advent of ultrahigh-resolution mass spectrometry made it possible for the first time to resolve the complex mixture of DOM on the bulk level (Koch et al., 2007). Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) is capable of resolving the mass of an intact DOM molecule with an accuracy of less than 0.1 mDa (Dittmar and Stubbins, 2014). For comparison, the mass of an electron is 0.5 mDa. If FT-ICR-MS is used with soft ionization techniques, it can be used to detect thousands of different DOM

molecules simultaneously, making upstream chromatographic methods superfluous.

The analysis via FT-ICR-MS requires the concentration and desalting of oceanic water samples because salt disturbs analytical detection. Dittmar et al. (2008) proposed a simple solid-phase extraction (SPE) method which makes sample handling easy and allows for a high sample throughput even for ship-based sampling campaigns. DOM analysis via FT-ICR-MS enabled the identification of molecular formulae of more than 10,000 different compounds in marine DOM (Hertkorn et al., 2006). Most of the molecules detected via FT-ICR-MS are small molecules with masses between 250 – 650 Da (Figure 1.6) and are therefore small enough to be taken up directly by microorganisms (Carlson et al., 2007). Although FT-ICR-MS provides new insights into the complexity of DOM, the mass spectrometric data is restricted to information about molecular masses and formulae with only limited information about the molecular structure behind the detected molecules. The lack of structural information makes it impossible to determine the exact number of structural isomers behind each detected molecular formula. A first approach for estimating the number of structural isomers in marine DOM via fragmentation, however, clearly showed that behind 10,000 detected molecular formulae more than 100,000 different compounds may be hidden (Zark et al., 2017).

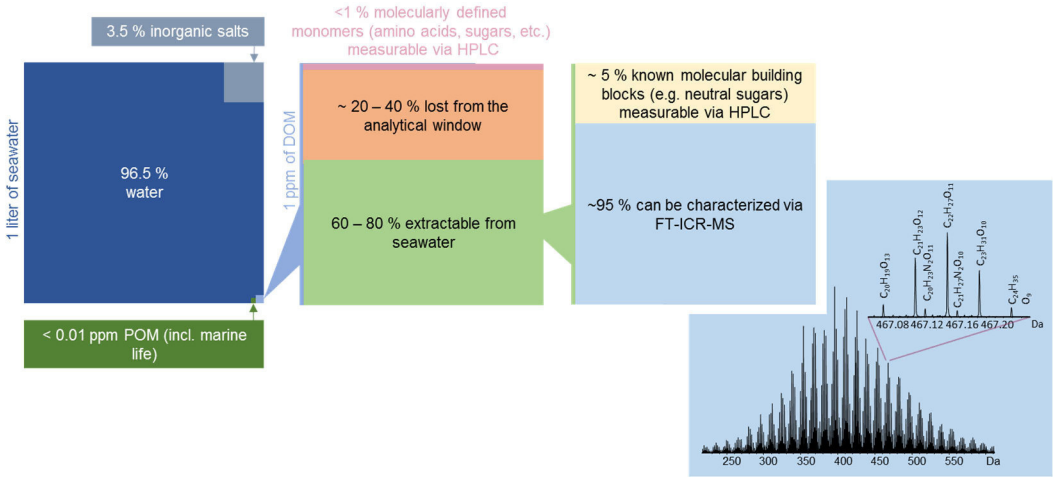

Figure 1.6 One liter of deep seawater contains water, inorganic salts, POM, and DOM. Less than 1 % of DOM consists of directly measurable intact moieties such as organic acids and sugars. About 20 – 40 % of the complete DOM pool is lost from the analytical window. Of the remaining 60 – 80 % of DOM, another 5 % become accessible after hydrolysis or oxidation and molecular building blocks such as neutral sugars, benzenepolycarboxylic acids (BPCAs), amino acids and sugars and lignin-derived phenols can by analyzed via HPLC. The remaining ~ 95 % can be characterized on the molecular level via FT-ICR-MS after concentration and desalting. Modified after Dittmar and Stubbins (2014).

A helpful tool to extract basic information from the molecular analysis via FT-ICR-MS is the Van Krevelen diagram. Based on FT-ICR-MS molecular data, the atomic ratios of hydrogen to carbon (H/C) and oxygen to carbon (O/C) can be calculated for all molecular formulae and plotted against each other in a Van Krevelen diagram. This graphical evaluation of the molecular data gives first indications about compound characteristics of the molecular data (Figure 1.7).

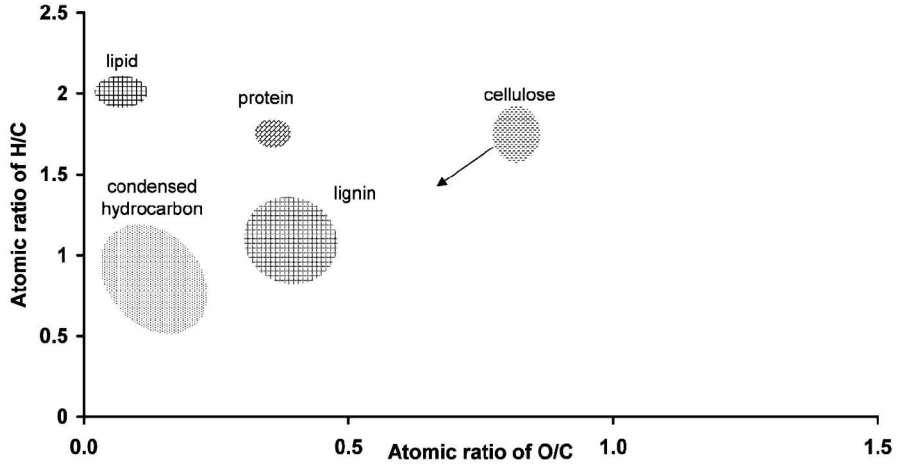

Figure 1.7 Schematic Van Krevelen plot of molecular DOM data. Shaded areas indicate regions where compounds of individual biomolecular building blocks occur. The arrow designates a pathway for a condensation reaction. Reprinted from Kim et al. (2003).

Other tools for extracting information from mass spectrometric DOM data is the calculation of the double bound equivalent (DBE) and the aromaticity index (AI; Koch and Dittmar, 2006). They help to identify aromatics and condensed aromatics in DOM solely from individual molecular formulae. With the information obtained by the elemental composition, the atomic H/C and O/C ratios and the aromaticity index, mass spectrometric molecular data can be classified into compound groups such as peptide-like compounds, carbohydrate-like compounds, saturated fatty acids, unsaturated aliphatic compounds, highly unsaturated compounds, phenols and polyphenols (Romano et al., 2014). However, this classification is not unambiguous and other structures may exist for any compound assigned to one of these groups.

1.2.3 Statistical methods in DOM research

Identifying significant trends in a sample set is difficult considering the amount of data provided by ultrahigh resolution mass spectrometry. For example, the data set collected to conduct this thesis comprises > 350 DOM samples with > 3000 molecular formulae identified per sample resulting in a dataset of more than 1,000,000 data points. To extract information from large data sets such as this, multivariate statistical approaches are often used in DOM research (e.g. Lechtenfeld et al., 2014; Sleighter et al., 2010). Multivariate statistical methods can help to reduce

data complexity by identifying major patterns and may also simplify graphical visualization. There are numerous statistical methods available and the application of specific methods to any given data set is driven by the structure of the data set and the scientific question. A review of statistical methods in microbial ecology was published by Ramette (2007) and most of the approaches can also be applied in DOM research.

The main tool used in this thesis is the Bray-Curtis dissimilarity (Bray and Curtis, 1957). The Bray-Curtis dissimilarity is a non-Euclidian measure, which means that it does not conform to the physical concept of distance (the Bray-Curtis dissimilarity is therefore non-metric). It is often used when analyzing ecological abundance data from different sample locations, because it quantifies the differences between samples. When applying the Bray-Curtis dissimilarity to DOM data, the mass spectrometric data is used in the same sense than ecological data, where samples are the observed entities and the peak intensities represent the abundance of single species. Hence, each observed compound signal is treated as a single species, which may be absent or present in specific abundances in different samples depending on environmental conditions, sampling location or biological influencing factors such as microbial community composition.

The Bray-Curtis dissimilarity assumes values between 0 and 1, with two samples being completely identical when the Bray-Curtis dissimilarity is 0 and completely different if the Bray-Curtis dissimilarity is 1. When transformed into percentage, it gives the percental value of difference between two samples. The Bray-Curtis dissimilarity (d) between two samples a and b is calculated as follows, supposing that n_{aj} and n_{bj} are the peak intensities in sample a and b and n_{a+} and n_{b+} the sum of the all peak intensities in the respective sample:

$$d_{ab} = \frac{\sum_{j=1}^{J} |n_{aj} - n_{bj}|}{n_{a+} + n_{b+}} \quad (1)$$

1.3 Processes driving the molecular geography of dissolved organic matter in the global ocean

The term “molecular geography of DOM” describes the distribution of DOM molecules in geographic space in relation to the respective environmental conditions,

analogous to the concept of biogeography (study of species and ecosystems in geographic space). It aims at identifying patterns in the molecular DOM composition representative for e.g. water masses, nutrient availability and microbial community composition in the ocean. The focus of this thesis is the molecular geography of the Atlantic and Southern Ocean. Most of the processes shaping the DOM molecular geography in the ocean are not confined to the Atlantic and Southern Ocean, but occur in various marine environments. For this reason, the results of several studies targeting specific processes, which influence the molecular geography in the global ocean, are summarized in this chapter.

1.3.1 Physicochemical properties of the Atlatic and Southern Ocean

The Atlantic Ocean is characterized by a strong stratification of the water column, which is driven by high temperatures and salinity gradients. The Southern Ocean on the other hand is well mixed with almost uniformly lower salinities and temperatures. Due to the mixing, surface waters in the Southern Ocean containing higher DOC concentrations are diluted with deep sea waters carrying lower DOC concentrations, resulting in largely uniform DOC concentrations throughout the water column (Hansell et al., 2009). DOM in the Atlantic Ocean, however, can accumulate above the seasonal and permanent pycnocline, which leads to high DOC concentrations in the surface waters. In contrast to the Southern Ocean, where DOM export is mainly directed vertically, DOC in the Atlantic Ocean can be exported vertically as well as horizontally (Hansell, 2013) and therefore fuels microbial production in nutrient-depleted environments (Torres-Valdés et al., 2009).

1.3.2 DOM in the euphotic water layer

Most of the DOM production occurs in the surface water masses. Hence, DOM concentration and composition is highly variable there and influenced by nutrient availability, microbial community composition, and other environmental factors. Phytoplankton communities produce and release a large suite of several hundreds of different organic molecules (Becker et al., 2014; Bittar et al., 2015) into the water column, all of which are channeled through the microbial loop on very short time spans. Since the demand of these labile compounds is larger than their supply, their

detection against the background of the more persistent DOM fractions is challenging. The chemical characteristics of the phytoplankton-derived DOM vary with the growth stage of the cells (Barofsky et al., 2009) and the producing taxa, but closely related phytoplankton strains tend to produce organic compound suites of a more similar chemical composition (Becker et al., 2014). About 20 % of the photosynthetically fixed carbon is excreted via extracellular release into the DOM pool (Marañón et al., 2005), underlining the importance of phytoplankton-derived DOM for fueling secondary production. Some of the DOM compounds produced by phytoplankton are more resistant to microbial oxidation and may remain in the surface ocean on longer timescales (Aluwihare and Repeta, 1999). Phytoplankton-derived DOM is the basis of bacterial production in the upper ocean; bacterial transformations then increase the molecular diversity of the DOM pool, partly because many bacterially-produced DOM compounds are synthesized for purposes beyond the cell wall (e.g. extracellular enzymes; Moran et al., 2016). A study targeting the ecological significance of a bacterial strain found that it solely consumed all labile DOC within 5 days of a laboratory experiment, whereas a more diverse assemblage continued to degrade semi-labile DOC during the remaining time span of the experiment (> one year), showing that microbes can have a multitude of metabolic capabilities and ecological functions with different impacts on the marine carbon cycle (Pedler et al., 2014). A study targeting the carbon flow between phytoplankton species and heterotrophic microorganisms suggests that although some bacterial groups are highly specialized in assimilating specific substrates, the direct carbon flow between phytoplankton species and the majority of the microbial community is rather weak indicating that the substrate diversity promotes the observed bacterial diversity in the open ocean (Sarmento and Gasol, 2012). In the coastal ocean, metagenomic analysis indicates that most of the microbial species are generalists with the capability of processing a large variety of substrates (Mou et al., 2008). All these studies show the diversity of phyto- and bacterioplankton interactions and the variety of DOM transformation processes shaping the DOM molecular composition in the surface ocean, but to date the picture is by no means complete. Particularly the fact that the specific substrate requirements for most heterotrophic marine microbes are not known so far, including many of the most abundant marine bacteria and archaea, complicates our understanding of the cycling of DOM in the ocean

(DeLong et al., 2006; Morris et al., 2002). With the development of the "omics" tools (i.e. genomics, transcriptomics, proteomics, and metabolomics), large amounts of data are now available to study the links between phytoplankton and bacterial communities and the DOM pool (e.g. McCarren et al., 2010; Osterholz et al., 2016; Poretsky et al., 2010).

DOM produced in the euphotic zone of the ocean is not only subject to biological transformation processes, but is also abiotically transformed by photochemical reactions. Some fractions of the DOM pool are converted into inorganic carbon compounds (dissolved inorganic carbon and carbon monoxide) and therefore lost from the DOM pool. Other fractions are transformed from refractory into bioavailable forms of DOM and subsequently turned over by marine microorganisms (Bushaw-Newton and Moran, 1999; Miller and Moran, 1997). But also, the opposite transformation has been observed: The conversion of bioavailable into bio-resistant forms removing DOM from the most active carbon cycle (Benner and Biddanda, 1998). Photochemical modifications of DOM can also lead to the loss of color and therefore alter the optical properties of sea water and the penetration depth of ultraviolet and photosynthetically active wavelengths (Vodacek et al., 1997). This change in light regime can have positive as well as negative effects on the marine microbial activity (Herndl et al., 1997). Stedmon and Markager (2005) studied the production and utilization of photoactive DOM in a laboratory-based experiment. They found that the nutrient regime had a significant influence on the quality of the produced DOM and that photodegradation was an important sink for microbially derived humic material. DOM molecules produced during photodegradation are mostly aliphatic compounds and the overall DOM pool shows a decrease of structural diversity in the molecular DOM composition (Stubbins et al., 2010). DOM compounds containing nitrogen seem to be exceptionally photo-labile as shown by photodegradation experiments with deep sea DOM (Stubbins and Dittmar, 2015). The release of nitrogen-rich compounds from DOM during photochemical transformation fuels bacterial productivity in the surface ocean by providing nutrients to nitrogen-limited environments (Bushaw et al., 1996).

Although DON serves as a source of nitrogen to many marine organisms (Bronk et al., 1994), biological turnover can also transform it to biologically inert forms,

which are stable in the deep sea over long timescales (McCarthy et al., 1997). Letscher et al. (2013) found that some DON fractions in the surface ocean seem to be recalcitrant to microbial utilization, but when exposed to a microbial community in the mesopelagic, DON remineralization occurred, emphasizing that DON cycling in the ocean is strongly influenced by the prevailing microbial community composition. DON cycling seems to be partly decoupled from bulk DOM cycling as indicated by a different C:N production and decomposition stoichiometry of labile and refractory DOM (199:20 and 3511:202, respectively; Hopkinson and Vallino, 2005).

1.3.3 Long-term stability of DOM in the deep ocean

DOM is the basis of most marine food webs. However, a small fraction escapes rapid mineralization and accumulates as refractory DOM in the global ocean (Hansell, 2013). Marine microorganisms can produce refractory DOM that persists in the ocean on long timescales (Lechtenfeld et al., 2015; Ogawa et al., 2001). Osterholz et al. (2015) showed experimentally that a phytoplankton and microbial community taken from the coastal North Sea channeled <0.4 % of its net community production (NCP) into the refractory DOM pool and further produced DOM compounds, which are molecularly indistinguishable from refractory DOM but vary in relative abundance. In the deep sea, where water masses have long been separated from the productive surface layers, DOM removal processes are rather slow, which is also reflected in the uniformly low DOC concentrations (Figure 1.2 lower panel). Lechtenfeld et al. (2014) studied SPE-extracted DOM in the Eastern Atlantic and Southern Ocean and found residence times of ~ 100 ka for some DOM compounds emphasizing the long-term stability of DOM in the ocean. Although water masses carry characteristic microbial communities (e.g. Agogué et al., 2011), DOM on the molecular level seems to be exceptionally similar, lacking any specific imprint of characteristic microbial communities (Hansman et al., 2015). A degradation index (I_{deg}) introduced by Flerus et al. (2012) revealed a degradation continuum for SPE-DOM samples in the eastern Atlantic with varying degradation rates for different DOM compounds. This explains the contrast between the bulk age of the DOM pool of 4000 – 6000 years (Druffel et al., 1992) and the exceptional high residence times of individual compounds (Lechtenfeld et al., 2014). Recent studies

on the age of DOC (Druffel and Bauer, 2000) and the molecular DOM composition (Hansman et al., 2015) suggest that most DOM is transported conservatively along the global conveyer belt and variations in composition are due to the conservative mixing of different water masses. Hansell and Carlson (2013) postulated regional DOM sinks in the deep sea, which account for the small DOC concentration differences along the global conveyer belt. Slow biological DOM transformation processes in the deep sea, however, change the molecular composition, as seen by a close correlation between the apparent oxygen utilization (AOU) and the production of chromophoric dissolved organic matter (CDOM) as a byproduct of the oxidation of organic matter from sinking particles in the deep Pacific (Nelson et al., 2010).

Considering the large amount of nutrients stored in DOM and its importance as substrate for heterotrophic microorganisms, it is surprising that DOM exists in the observed high concentrations and is persistent over exceptionally long time spans. This paradox situation, in which marine organisms are surrounded by an excess concentration of substrate without using it on a millennial time scale, has puzzled oceanographers for a long time and the mechanisms behind this long-term stability of DOM are still not very well understood. Benner and Amon (2015) provided a conceptual framework for linking DOM decomposition with size, reactivity, complexity and age with the size-reactivity continuum model: A decrease in reactivity and size (spanning the range from aggregates to LMW DOM molecules) goes along with an increase in complexity and age. Mentges et al. (2017) showed that the functional diversity in open ocean DOM decreases with increasing degradation state. In this study, the term “functional diversity” does not refer to the diversity of functional groups in a chemical sense. It is rather used in an ecological context, where the authors assume that compounds reacting in a similar way also share similar chemical properties. Combining the size-reactivity continuum model (Benner and Amon, 2015) with the findings of Mentges et al. (2017) it can be concluded that although complexity of organic material increases with degradation, the ecological functionality and therefore the biological reactivity of DOM decreases significantly. However, the decrease in functional diversity does not imply a decrease in structural diversity in DOM (Mentges et al., 2017).

Dittmar (2015) summarized the current hypotheses as to why DOM accumulates in the ocean: First, the "environment hypothesis" attributes the long-term stability of DOM to environmental factors such as the lack of nutrients, essential metabolites (vitamins) or electron acceptors. Environmental factors, however, only preserve DOM on shorter timescales (< 10 years), because a shift in environmental conditions can lead to a decomposition of DOM (Carlson et al., 2002). Second, the "intrinsic stability hypothesis" links DOM stability to its inherent molecular structure. Biotic (e.g. Ogawa et al., 2001) or abiotic transformation (e.g. Dittmar and Paeng, 2009) processes form refractory DOM compounds, which are no longer available as substrate leading to the accumulation of bio-resistant DOM in the global ocean. Third, the "molecular diversity hypothesis" accounts for the fact that DOM is a complex mixture of possibly thousands of different compounds (Dittmar and Stubbins, 2014) with an even higher number of structural isomers, each present at very low concentrations (Zark et al., 2017). Although the total concentration of DOM is high enough in the deep sea, the concentration of a single suitable substrate compound might be so low that a heterotrophic cell only rarely encounters it, limiting its assimilation and decomposition (Dittmar, 2015). Arrieta et al. (2015) could indeed show that refractory deep sea DOM induced microbial growth if present in higher than in situ concentrations leading to the conclusion that concentration rather than recalcitrance limits microbial turnover in the deep sea. If extreme dilution of single substrates is the driving factor behind the stability of DOM in the deep sea, the addition of freshly produced compounds in the sunlit surface layers should elevate substrate concentration levels above the respective threshold, causing a simultaneous degradation of fresh and old DOM (Middelburg, 2015). Based on radiocarbon dating of bacterial nucleic acids, the co-cycling of older and younger DOM has been shown for the eastern North Pacific (Cherrier et al., 1999). Definite empirical evidence whether none, one or a combination of the above described hypotheses drives the millennial stability of DOM is still open to the debate (Dittmar, 2015).

1.4 Objectives

DOM is the largest and most active pool of organic carbon in the ocean and the basis for all marine life. The two most important ecosystem functions of this highly diverse pool of organic carbon are (1) the maintenance of microbial production and thereby keeping the energy fixed via photosynthesis in the marine food web and (2) the long-term storage and sequestration of organic carbon in the deep sea and consequently removing it from the active carbon cycle, which directly influences the global climate. The turnover time of DOM varies between minutes to millennia and the factors determining the stability of DOM compounds in the environment are subject of current research (see chapter 1.3).

Over a distance spanning more than 15,000 km – including polar, subpolar, temperate, subtropical and tropical biogeographic provinces – a comprehensive data set of > 350 DOM samples from various water depths was collected in the Atlantic and Southern Ocean for ultrahigh resolution analysis of the molecular DOM composition. The detailed molecular characterization of marine DOM gives valuable insights into the distribution patterns of DOM molecules. Knowing their distribution is key to understanding their role in biogeochemical processes. Hence, this thesis investigates the following superordinate research question:

What drives the molecular geography in the Atlantic and Southern Ocean?

Specifically, the following hypotheses are addressed:

1. Photodegradation and bioproduction leave characteristic imprints in the DOM molecular composition of the Atlantic and Southern Ocean.

   MANUSCRIPT I: "Natural transformation of marine dissolved organic matter: Process-related molecular indices for bioproduction and photodegradation."

2. Water mass mixing drives the molecular geography of DOM in the Atlantic and Southern Ocean.

   MANUSCRIPT II: "The molecular geography of dissolved organic matter in the Atlantic Ocean can largely be explained by a simple two-source mixing model."

3. The distribution of DON in the Atlantic and Southern Ocean depends mostly on nutrient availability and microbial community composition.

MANUSCRIPT III: "Molecular geography of the Atlantic and Southern Ocean: Distribution patterns of organically bound dissolved nitrogen and biogeochemical implications."

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MANUSCRIPT I

Natural transformation of marine dissolved organic matter: Process-related molecular indices for bioproduction and photodegradation

Maren Seibt[1], Thorsten Dittmar[1] and Jutta Niggemann[1]

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

Photodegration and bioproduction are two of the major processes shaping the molecular DOM composition in the ocean. In manuscript I the characteristic molecular signatures of both processes were captured in two process-related indices based on molecular data from already published laboratory experiments targeting exclusively bioproduction and photodegradation of DOM. The indices were then applied to a large data sets from the Atlantic, Pacific and Southern Ocean, proving their validity in a large variety of natural marine environments.

All authors designed the study. M. Seibt collected the samples from the Atlantic and Southern Ocean. M. Seibt performed the data evaluation and analyzed the published data for calculation of the process-related indices together with J. Niggemann and T. Dittmar. M. Seibt wrote the manuscript with input from all authors.

The manuscript is close to submission to *Limnology and Oceanography* and presented with adjusted formatting according to the style of this thesis.

2.1 Abstract

Dissolved organic matter (DOM) holds the largest amount of organic carbon in the ocean, most of it residing in the deep ocean with radiocarbon ages of thousands of years. Mechanisms responsible for the millennial stability of deep sea DOM and the deferral from biologically labile to refractory DOM are still largely unknown. Microbial production and photodegradation in the surface layers of the global ocean are two of the major processes shaping the molecular composition of DOM. Here, we used molecular data obtained via ultrahigh resolution mass spectrometry (Fourier-transform ion cyclotron resonance mass spectrometry, FT-ICR-MS) from two separate laboratory studies that focused on (1) microbial production of fresh DOM and (2) photodegradation of deep sea DOM to derive independent process-related molecular indices for biological production ($I_{bioprod}$) and photodegradation (I_{photo}). We hypothesize that these indices disentangle and assess the influence of bioproduction and photodegradation on the molecular DOM composition in natural environments. Both indices were successfully applied to large global data sets of > 400 DOM samples spanning major biogeographical provinces and up to 5000 m water depths in the Atlantic, Pacific and Southern Ocean. The observed distribution of I_{photo} and $I_{bioprod}$ was consistent with increased photodegradation and bioproduction in sunlit surface waters of the tropical Atlantic Ocean. Combined application of I_{photo} and $I_{bioprod}$ clearly distinguished the effect of the two processes and the relative extent of the induced molecular changes, demonstrating that both indices provide valuable novel tools to unravel and quantitatively evaluate processes that ultimately determine the stability of deep sea DOM.

2.2 Introduction

Marine dissolved organic matter (DOM) represents one of the biggest active carbon reservoirs on Earth (662 ± 32 Gt C; Hansell et al., 2009). A large fraction (630 ± 30 Gt C; refractory DOM) resides in the deep ocean and is mostly unavailable for immediate biological turnover as manifested in its millennial stability (Hansell, 2013). Reasons for this observed stability are poorly understood (e.g. Ogawa and Tanoue, 2003) and underlying processes are still under debate (e.g. Dittmar, 2015; Loh et al., 2004). The most reactive and smallest fraction of DOM is the labile DOM pool with an inventory of < 0.2 Gt C (Hansell, 2013). It is the most fundamental form and the starting point for any biotic or abiotic transformation process on DOM.

Marine DOM is mainly produced via photosynthesis by phytoplankton at a rate of ~ 50 Gt C yr^{-1} (Behrenfeld and Falkowski, 1997; Morel and Antoine, 2002) and processed by heterotrophic microorganisms retaining part of the energy of primarily fixed carbon in the marine food web (microbial loop; Azam et al., 1983; Pomeroy, 1974). The manifold production and degradation processes, the variety of ages, reactivities, and histories of marine DOM result in a complex mixture with thousands to millions of different molecules (Hertkorn et al., 2007; Zark et al., 2017). Processes adding or removing molecules from the marine DOM pool include photosynthetic (e.g. Fogg, 1983) and microbial production (e.g. Ogawa et al., 2001), microbial (e.g. Koch et al., 2014) and photo-induced (e.g. Stubbins and Dittmar, 2015) degradation, water mass mixing, (e.g. Hansman et al., 2015), aggregation (e.g. Verdugo et al., 2004) and adsorption and desorption to and from particles (e.g. Davis, 1982).

The complexity of marine DOM makes its detailed chemical characterization challenging. The advent of ultra-high resolution analytical techniques such as Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) enables attainment of molecular information on the DOM composition in unprecedented detail. Combined with a soft ionization method (electrospray ionization; ESI) it is possible to resolve and detect tens of thousands of different molecular masses within one DOM sample and due to the ultra-high resolution, molecular formulae can be assigned to >70 % of the detected masses (e.g. Stenson et al., 2003).

DOM forms the basis of microbial life in the ocean. For this reason, it is puzzling that more than 90 % of marine DOM resides in the deep sea largely resisting biological utilization (Hansell, 2013). For understanding the conversion of DOM from highly reactive (labile) to mostly unreactive (refractory), it is crucial to identify the involved processes and to assess their relative impact on the molecular composition of DOM. In natural environments, a huge variety of production, transformation and degradation processes simultaneously act on the DOM pool on very different temporal and spatial scales. Therefore, studies of specific processes are mostly restricted to controlled laboratory experiments and limited timespans (e.g. Ogawa et al., 2001; Stubbins et al., 2010). Our current understanding on how the composition of marine DOM is shaped over time and space in the world's ocean is mostly deduced from observed distribution patterns. The general degradation state of DOM can be described by the amino acid based degradation index (DI) that links systematic changes in amino acids composition to the reactivity of bulk organic matter (Dauwe et al., 1999). Previous studies successfully applied the DI to quantitatively assess the general degradation state of DOM (e.g. Amon et al., 2001; Dittmar et al., 2001). Flerus et al. (2012) introduced a degradation index (I_{deg}) based on the molecular fingerprints of DOM obtained via FT-ICR-MS. They correlated intensities of mass peaks in marine DOM samples with the radiocarbon age of the respective sample and identified peaks systematically increasing and decreasing in intensity with age. With this index, a DOM sample can be classified regarding its "freshness" or "age" based on the molecular composition obtained via FT-ICR-MS. The amino acid based DI (Dauwe et al., 1999) and the I_{deg} (Flerus et al., 2012) are valuable complementary tools to assess the DOM degradation stage, but their application does not provide information about the processes driving the transformation from biologically available to the biologically more resistant forms of DOM.

In this study, we introduce two process-related indices for DOM production (i.e. bioproduction) and DOM degradation (i.e. photodegradation) derived from the molecular DOM data obtained via FT-ICR-MS. We used data sets of two controlled experiments on microbial production (Osterholz et al., 2015) and photodegradation (Stubbins and Dittmar, 2015) of DOM. The two indices do not only provide general information about the state of a given DOM sample, but also disclose the respective process driving the observed molecular changes. We successfully applied the newly

developed indices to extensive data sets comprising > 400 samples from the Atlantic and Southern Ocean (own data) and the North Pacific Ocean (Medeiros et al., 2015). We demonstrate that the process-related indices are applicable to marine samples from a variety of oceanic environments distributed across major global oceans.

2.3 Material and methods

DOM samples – The process-related indices introduced in this study were derived using data from a photodegradation experiment on North Atlantic Deep Water (NADW; Stubbins and Dittmar, 2015) and a laboratory mesocosm experiment studying the natural microbial production of DOM over the course of three years (Osterholz et al., 2015). The new indices were then applied to a data set from the North Pacific (Medeiros et al., 2015) and a data set obtained in the Atlantic and Southern Ocean (own data). Samples taken in the Pacific were also used for photodegradation experiments, exposing DOM to irradiation in a solar simulator and to natural sunlight (Medeiros et al., 2015). For detailed description of experimental set ups, sample handling and data evaluation refer to the respective publications.

Atlantic and Southern Ocean DOM samples were taken during three RV Polarstern cruises ANT-XXVIII/2 (Atlantic sector of the Southern Ocean; 39.2° S to 70.5° S), ANT-XXVIII/4 (Drake Passage and Antarctic Peninsula; 56.1° S to 62.4°S) and ANT-XXVIII/5 (Atlantic; 51° S to 47° N) in austral spring and summer (Dec 2011 - May 2012; Figure 2.1 A). All samples were directly filtered from a rosette sampler via gravity flow. Samples were taken in high resolution in the upper 200 m of the water column (20 m, 40 m, 60 m, 100 m, 200 m and in the fluorescence maximum, if present). Deeper samples were taken from major water masses (Figure 2.1 B). Samples for DOM analysis were extracted according to the solid-phase extraction (SPE-) method published by Dittmar et al. (2008). In short, 4 l of sea water were filtered through a pre-combusted (400 °C, 4 h) 0.7 µm glass fibre filter (GF/F, Whatman, United Kingdom) and acidified to a final pH of 2 (HCl, 25%, p.a., Carl Roth, Germany). The samples were extracted on commercially pre-packed cartridges (1 g of sorbent, PPL, Agilent, USA) via gravity flow. After extraction, the cartridges were deionized by rinsing them with two cartridge volumes of ultrapure water (pH 2). The cartridges were then dried with nitrogen gas and immediately

eluted with 6 ml of methanol (HPLC-grade, Sigma-Aldrich, USA) into pre-combusted amber vials and DOM extracts were stored in the dark at -20°C until further analysis in the laboratory.

The carbon-based extraction efficiencies of all sample sets were in the same range, indicating that the extracted fraction of DOM (i.e. SPE-DOM) is reproducible and comparable between different sampling campaigns. The carbon-based extraction efficiency was 53 ± 9 % for Atlantic and Southern Ocean DOM, 58 ± 6 % for Pacific Ocean DOM (Medeiros et al., 2015) and 70 % (Osterholz et al., 2015) and 67-74 % (Stubbins and Dittmar, 2015) for DOM of the experimental data sets used for index calculation.

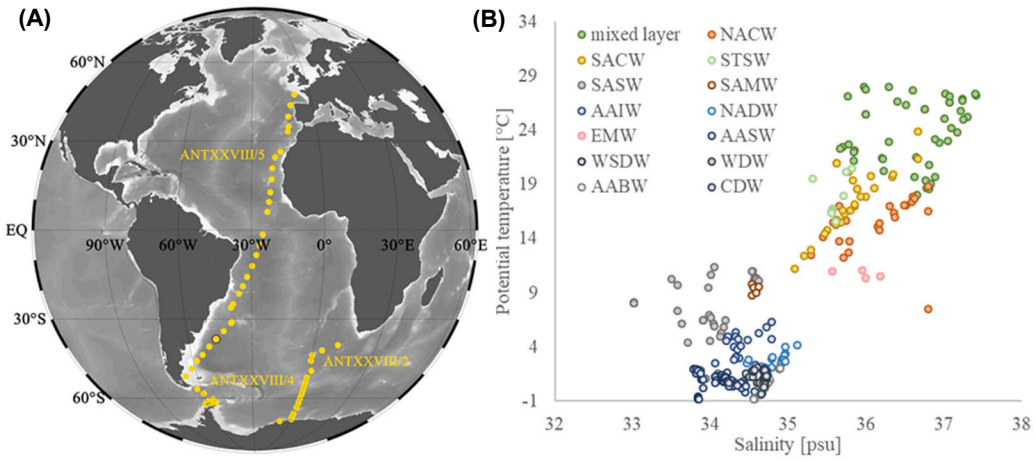

Figure 2.1 Cruise tracks in the Atlantic and Southern Ocean **(A)** and temperature-salinity diagram with all sampled water masses **(B)**. Abbreviations in alphabetical order are: Antarctic Bottom Water (AABW), Antarctic Surface Water (AASW), Antarctic Intermediate Water (AAIW), Circumpolar Deep Water (CDW), Eurafrican Mediterranean Water (EMW), North Atlantic Central Water (NACW), North Atlantic Deep Water (NADW), South Atlantic Central Water (SACW), Subantarctic Mode Water (SAMW), Subantarctic Surface Water (SASW), Subtropical Surface Water (STSW), Weddell Sea Warm Deep Water (WDW) and Weddell Sea Deep Water (WSDW).

Molecular composition of DOM - All DOM extracts, including experimental data sets as well as application data sets, were analyzed on a solariX FT-ICR-MS (Bruker Daltonik GmbH, Bremen, Germany) equipped with a 15 Tesla superconducting magnet (Bruker Biospin, Wissembourg, France) and an electrospray ioni-

zation source (ESI; Apollo II ion source, Bruker Daltonik GmbH, Bremen, Germany). For analysis, all DOM extracts were mixed with ultrapure water and methanol (MS grade, 1:1 v/v) to a final carbon concentration of 10 ppm. For analysis validation, an in-house reference sample (North Equatorial Pacific Intermediate Water (NEqPIW); Bostock et al., 2010), collected at the Natural Energy Laboratory of Hawaii Authority in 2009 (Green et al., 2014), was measured twice a day.

Mass spectra were recorded in a mass range of 150 – 2000 Da with 500 acquired scans per sample. Instrument settings were as outlined in Seidel et al. (2014). All mass spectra were internally calibrated using a list of > 50 known molecular formula mass peaks from the NEqPIW reference sample. All detected ions were singly charged and the mass error allowed for the internal calibration was < 0.1 ppm. The total ion current and the overall intensity distribution were in the same order of magnitude for all samples. The mass to charge ratio, resolution and peak intensity were exported and processed using in-house Matlab routines. Molecular formulae were assigned to peaks with a minimum signal-to-noise ratio of 4 following the rules published by Koch et al. (2007) with following restrictions: $^{12}C_{1\text{-}130}{}^{1}H_{1\text{-}200}O_{1\text{-}50}{}^{14}N_{0\text{-}4}S_{0\text{-}2}P_{0\text{-}2}$. Only compounds with an assigned molecular formula were considered for further evaluation. All mass spectrometric data sets (i.e. photodegradation and mesocosm experiment and Atlantic and Southern Ocean DOM data) were merged into one comprehensive data set. This approach resulted in a data set comprising a total of 4705 assigned molecular formulae. Normalization was done on the reduced data set as the very last step of data processing: Each peak intensity present in a sample was divided by the sum of all peak intensities of the respective sample, considering only peaks with assigned molecular formula.

Bioproduction index ($I_{bioprod}$) and photodegradation index (I_{photo}) - The bioproduction index ($I_{bioprod}$) was developed based on results of a 3-year lasting mesocosm experiment on DOM production by a natural microbial community of phyto- and bacterioplankton (Osterholz et al., 2015). In this experiment, photodegradation of DOM was negligible, because the light provided to the mesocosms excluded UV-light (400 – 700 nm; Osterholz et al., 2015), which is mainly responsible for the breakdown of photo-reactive DOM (e.g. Mopper et al., 1991). Therefore,

changes in DOM composition in this experiment can exclusively be attributed to microbial bioproduction of DOM. We used an integrated sample over the course of the second year of the experiment, assuming that the DOM contains a mixture of freshly produced and slightly transformed microbial DOM that meets the reactivity criteria for labile, semi-labile and semi-refractory marine DOM with lifetimes of hours to decades (Hansell, 2013).

For the derivation of our process-related bioproduction index, we assume that the processes shaping the DOM composition in the mesocosms are representative for the natural open ocean environment and that the results from the laboratory study can be scaled up to global dimensions. This approach implies that microbially produced and transformed DOM shares unique characteristics independent of community composition, available substrates and prevailing growth conditions. Previous studies have shown that even single bacterial strains release very different DOM depending on growth conditions (e.g. Romano et al., 2014; Noriega-Ortega et al., in preparation). However, it has been proposed that the main factor shaping the global marine DOM composition is not primarily the prevailing microbial community composition but rather the functional diversity within the community and the multitude of possible transformation processes (Osterholz et al., 2016). Laboratory-based experiments support this view, demonstrating that more complex microbial communities transform bioavailable substrates into DOM, which is exceptionally similar to natural marine DOM (Koch et al., 2014; Osterholz et al., 2015).

The photodegradation index (I_{photo}) was derived from data obtained during a photodegradation experiment of North Atlantic Deep Water (NADW) sampled at the Bermuda Atlantic Time Series site (BATS; Stubbins and Dittmar, 2015). For this photodegradation experiment on natural deep sea DOM, a solar simulator emitting high energy irradiance was used, including UV-light in the range 295 to 365 nm, which inhibited microbial growth. Therefore, the dominant process shaping DOM composition in this experiment was photo-degradation of natural DOM.

Previously published indices based on FT-ICR-MS molecular data describing the state of a given DOM sample were derived by correlating intensities of mass peaks with specific sample characteristics like radiocarbon age (Flerus et al., 2012) or fraction of terrestrial material ($\delta^{13}C$; Medeiros et al., 2016). These indices are based

on a few "marker peaks" that changed systematically with the chosen parameters. The $I_{bioprod}$ and the I_{photo} are also based on distinct "marker peaks", but rather than describing the current state of the DOM composition, these novel indices reveal the dominant processes that led to this current state.

To derive the bioproduction index we compared the DOM produced in the mesocosm experiment to a natural deep sea sample taken from Equatorial Pacific Intermediate Water (NEqPIW; Bostock et al., 2010). Deep sea DOM is considered refractory and stable over the timescale of millennia (Hansell, 2013). Therefore, mass peaks in the mesocosm (fresh) sample that showed a higher relative intensity than the deep sea sample were considered potential "marker peaks" for bioproduction. Mass peaks selected for the bioproduction index ($I_{bioprod}$) had to fulfill the following three criteria: First, selected peaks must have an intensity > 5 % of the maximum peak intensity in the respective sample, which makes their occurrence more likely in a larger variety of environments and also reduces the variability in the calculated index ("intensity criterium"). Second, the intensity of selected peaks in the integrated mesocosm sample must be at least 30 % higher than the respective peaks in the NEqPIW sample ("production criterium"; Table 2.1). Third, the selected peaks must be unsusceptible to photodegradation, hence their intensity during the photodegradation experiment had to remain unchanged before and after irradiation ("process criterium bioproduction"). A total of 31 mass peaks of our data set met the above criteria and were considered to be characteristic for bioproduction.

A similar set of conditions had to be met for mass peaks used for the photodegradation index (I_{photo}): First, the "intensity criterium" had to be fulfilled in the same way, i.e. the selected mass peaks must have an intensity > 5 % of the maximum peak intensity in the respective sample. Second, the intensities of the selected mass peaks must be at least 30 % lower in the irradiated sample than in the sample prior to irradiation ("degradation criterium"; Table 2.1). Third, the influence of bioproduction on the selected mass peaks must be negligible, meaning that the relative peak intensity in the integrated mesocosm sample did not deviate from the NEqPIW reference sample ("process criterium photodegradation"). A total of 14 mass peaks of our data set met the above criteria and were considered to be characteristic for photodegradation.

2.4 Results

For the definition of each of the two indices, we selected five of the mass peaks that met the criteria for the respective process and covered the maximum possible mass range (Table 2.1; P1 – P5 for I_{photo} and B1 – B5 for $I_{bioprod}$). The peaks selected for I_{photo} cover a wider mass range (~300 – 450 Da) than the peaks selected for the $I_{bioprod}$ (~250 – 360 Da). Stubbins et al. (2010) showed that photo-labile DOM compounds generally have a higher molecular weight than the photo-resistant compounds. In contrast, the most prominent signature of biological production and transformation of DOM is mostly found in the lower mass range (Riedel et al., 2016), especially when compared to refractory DOM (Osterholz et al., 2015).

The rationale for selecting five mass peaks for index calculation is that the indices should be applicable in a maximum variety of different environments. The more peaks are chosen, the higher the probability of one not being present in a sample of interest. Likewise, the I_{deg} of Flerus et al. (2012) is also based on 5 peaks and its applicability has been demonstrated for different water masses of the Atlantic Ocean.

For index calculation, the intensity of the five respective peaks was summed up and divided by the sum of five mass peaks, which were neither influenced by photodegradation nor bioproduction, because they had the same relative intensity in the integrated mesocosm sample, the photodegradation experiment samples and the NEqPIW reference sample and fulfilled the intensity criterium

.

Table 2.1 Selected mass peaks, assigned elemental formulae, m/z, the factor of peak intensity change between untreated and photodegraded NADW (I_{photo}) and between NEqPIW and mesocosm DOM ($I_{bioprod}$) and the respective calculation of the indices. The factor of relative peak intensity change was calculated by dividing the respective peak intensity of the irradiated sample by the untreated sample for the I_{photo} and the respective mesocosm peak intensity by the NEqPIW peak intensity for the $I_{bioprod}$ (see also Table S2.1).

	Peak No.	Elemental Formula	m/z	Factor of relative peak intensity change	Index
Photodegradation	P1	$C_{16}H_{16}O_7$	319.0823	0.60	$I_{photo} = \frac{(P1 + P2 + P3 + P4 + P5)}{(D1 + D2 + D3 + D4 + D5)}$
	P2	$C_{19}H_{20}O_7$	359.1136	0.67	
	P3	$C_{16}H_{20}N_2O_9$	383.1096	0.69	
	P4	$C_{23}H_{26}O_8$	429.1555	0.67	
	P5	$C_{24}H_{30}O_8$	445.1868	0.67	
Bioproduction	B1	$C_{13}H_{18}O_5$	253.1081	10.46	$I_{bioprod} = \frac{(B1 + B2 + B3 + B4 + B5)}{(D1 + D2 + D3 + D4 + D5)}$
	B2	$C_{13}H_{16}O_6$	267.0874	4.34	
	B3	$C_{13}H_{19}NO_6$	284.1140	6.22	
	B4	$C_{13}H_{17}NO_7$	298.0932	3.14	
	B5	$C_{18}H_{28}O_7$	355.1762	4.13	
	D1	$C_{14}H_{16}O_8$	311.0772	1.03	
	D2	$C_{17}H_{24}O_8$	355.1398	1.00	
	D3	$C_{16}H_{22}O_9$	357.1191	0.98	
	D4	$C_{19}H_{28}O_9$	399.1661	1.01	
	D5	$C_{19}H_{28}O_{10}$	415.1610	1.00	

Prerequisite for a universally applicable index based on mass spectrometric molecular data is the occurrence of the selected mass peaks in a wide variety of environments. The bioproduction ($I_{bioprod}$) and photodegradation (I_{photo}) indices were applied to a large variety of DOM samples from the Pacific, Atlantic and Southern Ocean. Hence, the newly developed indices were applied to > 400 marine samples, comprising surface samples from different biogeographic zones as well as deep ocean samples from all major water masses of the Atlantic and Southern Ocean.

The I_{photo} of untreated and photodegraded NADW DOM was 0.24 and 0.16, respectively (Figure 2.2 A). Hence, the more photodegraded a DOM sample is, the lower is the I_{photo}. The $I_{bioprod}$ was 0.19 for the NEqPIW sample and 1.05 for the integrated mesocosm sample, respectively (Figure 2.2 B). Therefore, if the influence of bioproduction is high the $I_{bioprod}$ is high as well. The calculated indices for the endmembers of both processes are indicated as the upper and lower boundaries of the blue and green box in Figure 2.2 A and B, respectively.

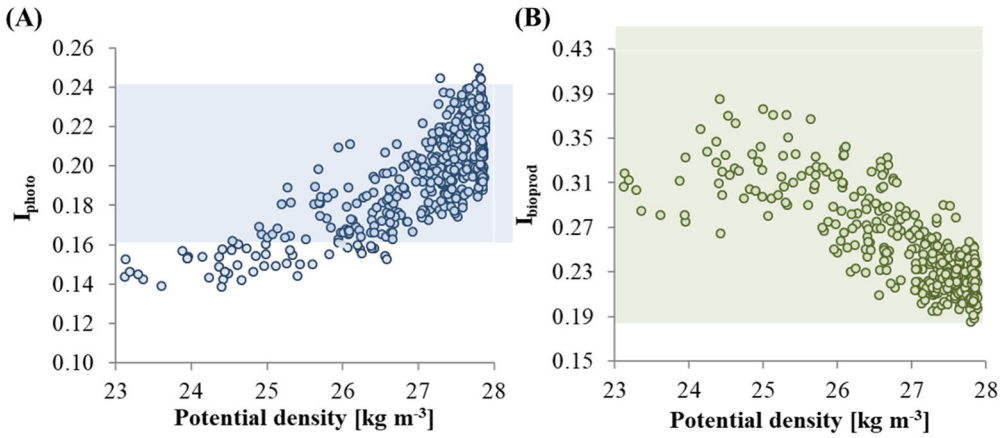

Figure 2.2 The I_{photo} **(A)** and $I_{bioprod}$ **(B)** for all Atlantic and Southern Ocean samples along water density. The upper and lower boundaries of the blue box represent the I_{photo} of untreated and photodegraded NADW DOM (I_{photo} = 0.24 and 0.16, respectively). The lower boundary of the green box represents the $I_{bioprod}$ of NEqPIW DOM ($I_{bioprod}$ = 0.19). Please note that the $I_{bioprod}$ of mesocosm DOM is 1.05 and therefore not shown on the y-axis. Please note the different y-axis scale.

Application of I_{photo} to environmental samples: The I_{photo} for Atlantic and Southern Ocean DOM was lowest in the surface mixed layer (0.14 – 0.20) in the tropical and subtropical Atlantic Ocean. Highest values for the I_{photo} (up to 0.25) were found

throughout the Southern Ocean, where solar irradiance is weaker than at lower latitudes (Figure 2.3 A and B).

In the North Pacific, the I_{photo} was between 0.17 and 0.20 for surface samples and between 0.23 and 0.24 for deep sea samples (Figure 2.5 A, blue dots). It is noteworthy that the highest I_{photo} values in the North Pacific data set were found for surface samples at station 17 and station 24 (average I_{photo} = 0.20), which had elevated CDOM concentrations (Medeiros et al., 2015). For the surface samples at station 29 and 8, where CDOM concentration was generally lower (Medeiros et al., 2015), the I_{photo} was lowest with an average of 0.17.

After photodegradation of the North Pacific DOM, the I_{photo} of surface and deep sea samples were lowered to 0.16 – 0.18 and to 0.19 – 0.20, respectively (Figure 2.5 A, red dots), with a more pronounced decrease of the I_{photo} at stations with a higher CDOM concentration, consistent with the susceptibility of CDOM to photodegradation (e.g. Helms et al., 2013).

Application of $I_{bioprod}$ to environmental samples: The $I_{bioprod}$ was highest in the warm and productive surface layers in the Atlantic ($I_{bioprod}$ = 0.20 – 0.39) and lowest in the deep sea and in the Southern Ocean ($I_{bioprod}$ = 0.19 – 0.29; Figure 2.4 A and B). The $I_{bioprod}$ was particularly high in the surface mixed layer in the South Atlantic Gyre. It is noteworthy that the highest calculated $I_{bioprod}$ for the Atlantic was still significantly lower than the $I_{bioprod}$ calculated for the mesocosm DOM ($I_{bioprod}$ = 1.05).

The $I_{bioprod}$ for the North Pacific samples was high (0.31 – 0.34) for surface samples and generally low for samples below 1000 m (0.19 – 0.21) following the general trend of $I_{bioprod}$ also observed for Atlantic DOM, with elevated values in the productive surface layers and lower values in the deep sea (Figure 2.5 B, blue dots). After photodegradation, the $I_{bioprod}$ of some deep water samples increased slightly (Figure 2.5 B, red dots), but were still significantly lower than the observed values for the surface water samples.

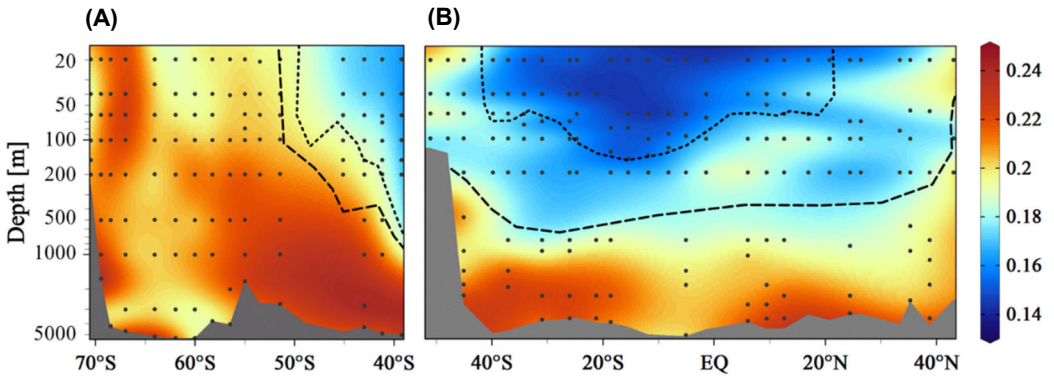

Figure 2.3 The I_{photo} in the Southern Ocean (ANTXXVIII/2; **A**) and the Atlantic Ocean (ANTXXVIII/5; **B**). The dashed lines indicate the upper and lower boundary of the pycnocline. The indices calculated for DOM samples taken on cruise ANTXXVIII/4 correspond very well to the index calculated for DOM samples taken on cruise ANTXXVIII/2 (see also Figure 2.2) and are therefore not shown. Please note logarithmic depth scale.

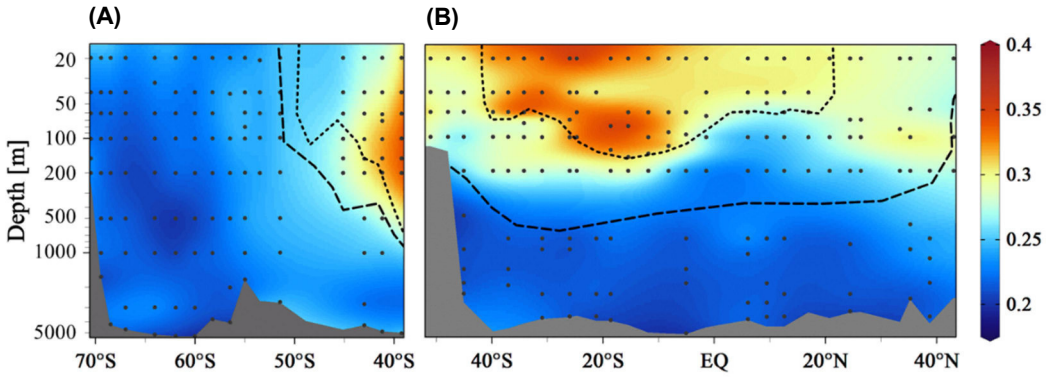

Figure 2.4 The $I_{bioprod}$ in the Southern Ocean (ANTXXVIII/2; **A**) and the Atlantic Ocean (ANTXXVIII/5; **B**). The dashed lines indicate the upper and lower boundary of the pycnocline. The indices calculated for DOM samples taken on cruise ANTXXVIII/4 correspond very well to the index calculated for DOM samples taken on cruise ANTXXVIII/2 (see also Figure 2.2) and are therefore not shown. Please note logarithmic depth scale.

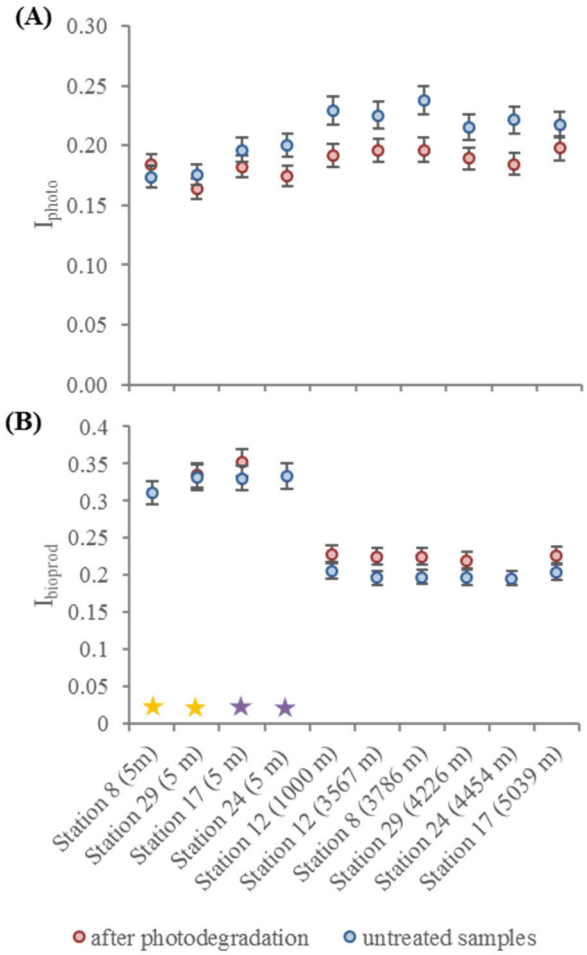

Figure 2.5 The Iphoto **(A)** and Ibioprod **(B)** for untreated (blue) and photodegraded (red) North Pacific DOM from different stations and various water depths. Error bars indicate the variation of the indices when calculated for repeated FT-ICR-MS analysis of the same sample (n=11; max. 5 %). For location of the stations refer to Medeiros et al. (2015). The yellow and purple stars indicate stations with low and high surface CDOM concentrations, respectively (Medeiros et al., 2015)

2.5 Discussion

By applying the I_{photo} and the $I_{bioprod}$ to a wide range of environmental samples, we demonstrate that both indices are valuable tools for distinguishing two of the major processes, which shape the molecular composition of oceanic DOM. The addition of various amounts of freshly, microbially produced DOM to a refractory background can indeed explain most of the molecular variability in the DOM composition of the Atlantic and Southern Ocean as shown in the following chapter of this

thesis. Most freshly produced DOM in the ocean does not accumulate and has a very short turnover time (Hansell, 2013): it is either taken up quickly by heterotrophic microorganisms or diluted by mixing water masses. Pure mesocosm DOM, which consists mainly of semi-labile and semi-refractory DOM (Osterholz et al., 2015), has a high $I_{bioprod}$ not found in any sample from the Atlantic, Pacific or Southern Ocean. Apparently, the signature of bioproduction in the DOM composition of natural oceanic environments is diluted by the refractory DOM background present in each marine DOM sample resulting in overall lower $I_{bioprod}$. However, the high $I_{bioprod}$ in the mesocosms clearly suggests that in DOM production hot spots such as phytoplankton blooms or coastal areas, the $I_{bioprod}$ could be considerably higher than observed in the Atlantic, Pacific and Southern Ocean, where no pronounced phytoplankton bloom was encountered during sampling.

The mesocosm and photodegradation experiments were conducted to study two specific processes: the exclusive production of microbial DOM in the mesocosm experiment and the maximum possible impact of photodegradation on the molecular composition of deep sea DOM in the photodegradation experiment. To observe any changes in composition a reference sample for comparison is needed. In both cases, we chose a photo-chemically untreated and biologically largely inert deep sea DOM sample to asses any qualitative compositional changes during the experiments. For approximating the range both indices can cover, the I_{photo} and the $I_{bioprod}$ were calculated for their respective experimental data set, because we assume that our choice of endmembers (i.e. mesocosm DOM for bioproduction and photodegraded NADW for photodegradation) covers a maximum range of possible changes in the molecular DOM composition. However, the I_{photo} for DOM in the surface mixed layer of the subtropical Atlantic Ocean is even lower than the I_{photo} of photodegraded NADW DOM (Figure 2.2; I_{photo} values below the blue box). The deep ocean is a unique setting were photodegradation does not occur naturally and bioproduction plays only a minor role, leading to an environment where processing of DOM happens on very slow timescales (Hansell et al., 2012) and is probably mostly driven by abiotic processes such as sorption and desorption to particles (Dittmar and Stubbins, 2014). In contrast, in the surface ocean DOM turnover is fast and both, bioproduction and photodegradation, play important roles in shaping

the molecular DOM composition. The I_{photo} is calculated based on an experiment with deep sea DOM, neglecting photodegradation of fresh DOM. Thus, the I_{photo} in the surface mixed layer can deviate from the calculated range due to the presence of fresh DOM, which changes the molecular composition and its susceptibility to photodegradation.

Both considered processes have their maximum impact in the sunlit, warm and productive surface layer, which also explains the covariance of both indices, since bioproduction and photodegradation depend on solar irradiance. Especially in the South Atlantic Gyre both, photodegradation and bioproduction, have a strong effect on shaping the molecular DOM composition. Because both, semi-labile and semi-refractory DOM, are persistent on timescales greater than one year, accumulation of these DOM fractions is possible (Hansell, 2013) and their contribution to the overall DOM pool is detectable with the $I_{bioprod}$. Photodegradation and bioproduction can be mutually dependent (Amado et al., 2007; Kramer and Herndl, 2004; Tranvik and Bertilsson, 2001). Photodegradation of surface DOM can either lead to an enhanced or decreased biological availability for some of the DOM molecules (Obernosterer et al., 2001). Cherrier et al. (1999) showed that open ocean bacteria also assimilate, along with newly fixed carbon, isotopically old carbon as measured by the radiocarbon age of their nucleic acids. This is either possible via co-metabolism with labile DOM, which has been shown in various cases (e.g. Bianchi, 2011) or via photo-oxidation of otherwise bio-resistant DOM. Extensive photodegradation as indicated by the extremely low I_{photo} in the South Atlantic Gyre likely goes along with the breakdown of otherwise bio-resistant compounds into bioavailable forms, which in turn sustain a high bioproduction as indicated by the high $I_{bioprod}$.

The mixed layer pump exports organic material from the euphotic into the mesopelagic zone and sustains heterotrophic production in water depths between 100 – 1000 m (Dall'Olmo et al., 2016; Gardner et al., 1995). The characteristic molecular signatures of processes shaping the DOM molecular composition in the euphotic zone are exported to deeper layers via the mixed layer pump as seen by lower I_{photo} values in the mesopelagic than in the bathypelagic water samples from the Atlantic (Figure 2.3 B). The molecular signature of bioproduction ($I_{bioprod}$), how-

ever, is mostly restricted to the upper 200 m (Figure 2.4 B). The $I_{bioprod}$ is an indicator for microbial production of DOM mostly belonging to the semi-persistent DOM fractions. Semi-labile and semi-refractory DOM sustains the subsurface microbial loop in the mesopelagic water depths (Hansell, 2013) acting as a sink for DOM produced in the euphotic zone and exported to greater water depths via the mixed layer pump. As a consequence, the characteristic signature of bioproduction in the DOM molecular composition is lost below the surface mixed layer.

In the Pacific Ocean, surface samples with higher CDOM concentrations also had higher I_{photo} values, indicating their potential for further photodegradation. After irradiation of surface DOM samples with high CDOM concentrations in the solar simulator, the I_{photo} was in the same range as untreated surface samples with low CDOM concentrations, which most likely already underwent extensive photodegradation (Figure 2.5 A). The I_{photo} for deep Pacific samples (> 1000 m) is higher (> 0.21) than for the surface samples (< 0.21). When the deep Pacific samples are exposed to irradiation (either in the solar simulator or to natural sunlight) the I_{photo} is lowered (0.19 – 0.20), but does not fall below I_{photo} values of the surface samples (Figure 2.5 A). The presence of fresh DOM in the surface waters seems to have the same effect also observed for Atlantic DOM samples: It changes the molecular composition and consequently its susceptibility to photodegradation.

After photodegradation, the $I_{bioprod}$ of some deep water samples increased slightly (Figure 2.5 B, red dots), which is mainly caused by the increase of the relative peak intensity of bioproduction peak B5 ($C_{18}H_{28}O_7$, Table 2.1) during photodegradation. The intensity increase of peak B5 might be the result of the breakdown of a photo-labile compound during photodegradation producing B5 as successor compound. In the North Pacific, CDOM concentrations are higher than in the Atlantic (Nelson and Siegel, 2013), which could indicate that the precursor compound of B5 is a CDOM compound that was not present in the deep Atlantic sample used for identifying suitable peaks for the indices. The relative intensity of peak B5 also increases after photodegradation of Congo River DOM, which is of terrestrial origin and overall very photo-labile (Stubbins et al., 2010). We therefore conclude that for samples with high CDOM concentrations, the $I_{bioprod}$ should be reviewed critically. However, after photodegradation the $I_{bioprod}$ in the deep North Pacific is still in the

same range as the $I_{bioprod}$ for deep Atlantic samples indicating that although the $I_{bioprod}$ increases slightly after photodegradation, the validity of the $I_{bioprod}$ is not diminished.

The degradation index (I_{deg}) published by Flerus et al. (2012) assesses the degradation state of a DOM sample based on the age of the bulk DOM sample. The more degraded (and therefore older) a sample is, the higher is the resulting I_{deg}. If DOM underwent extensive photodegradation, the I_{photo} is low. In the deep sea, DOM occurs in its most degraded forms and photodegradation has no impact. Hence, the I_{deg} and I_{photo} are both high in the deep sea DOM samples (Figure 2.6 A) compared to the sunlit surface waters, where DOM is overall younger and photodegradation impacts the molecular DOM composition. I_{deg} and $I_{bioprod}$ are negatively correlated (Figure 2.6 B), because bioproduction is negligible in the deep sea, but impacts the molecular DOM composition of the younger and less degraded samples.

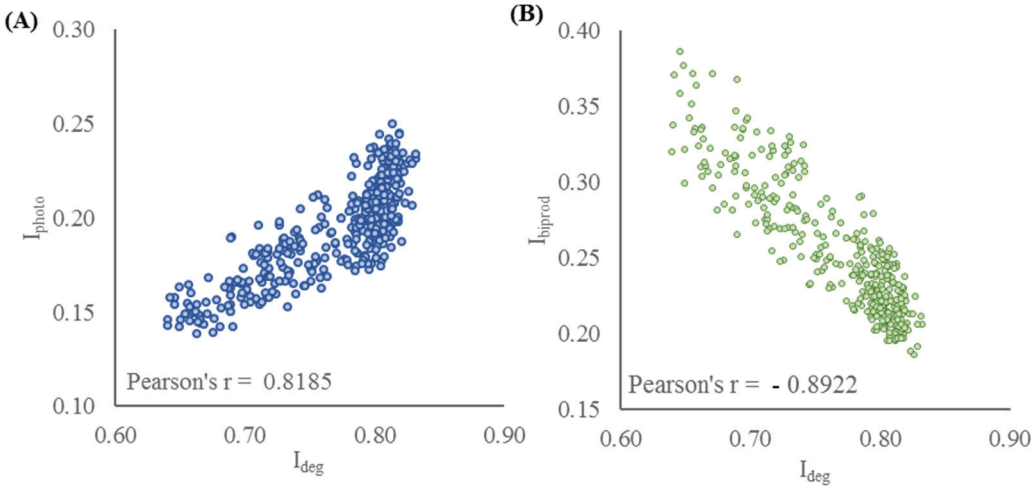

Figure 2.6 Correlation between I_{photo} **(A)** and $I_{bioprod}$ **(B)** with the I_{deg} (Flerus et al., 2012)

A direct comparison of the I_{deg} and I_{photo} shows, that the I_{deg} of the photodegradation data set (Stubbins and Dittmar, 2015) stays about the same (0.86 and 0.82 before and after irradiation, respectively), clearly showing that photodegradation is not the major process driving the I_{deg}. Based on this finding, we conclude that the I_{photo} is

not biased by other degradation processes but is rather a unique indicator for photodegradation. The I_{deg} for DOM produced in the mesocosms (bioproduction) is 0.15, indicating that DOM in the mesocosms is only little degraded and that degradation is an almost negligible process in this experiment. The I_{deg} and $I_{biobrod}$ provide complementary information on DOM aging and the production of DOM by microbial communities, respectively.

2.6 Conclusion

The process-related indices introduced in this study are based on controlled laboratory experiments, because the distinction between processes and the development of process-related indices is not achievable with a natural sample set. The $I_{bioprod}$ and the I_{photo} provide novel tools for assessing processes behind observed changes in the natural DOM composition. Both indices disclose not only information about the respective process but also differentiate and quantitatively assess the changes both processes cause in the molecular DOM composition. The information provided by both indices is crucial for disentangling the mechanisms controlling the molecular composition of this huge carbon reservoir and is therefore of relevance for a comprehensive understanding of DOM and its millennial stability in the deep ocean. The application of both indices to a large global sample set conclusively demonstrates that they yield reasonable and comprehensible results for natural DOM samples.

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2.9 Supplementary materials

Table S2.1 Elemental formulae, m/z and the peak intensities of the peaks chosen for calculation of the I_{photo} (P1 – P5 and D1 – D5; untreated and photodegraded NADW DOM) and the $I_{bioprod}$ (B1 – B5 and D1 – D5; NEqPIW and mesocosm DOM) and the calculation of the factor of relative peak intensity change (see also Table 2.1)

			I_{photo}		$I_{bioprod}$		
Peak No.	**Elemental Formula**	**m/z**	**Peak intensity in NADW sample (untreated)**	**Peak intensity in NADW sample (photodegraded)**	**Peak intensity in NEqPIW sample**	**Peak intensity in integrated mesocosm sample**	**Calculation of factor for relative peak intensity change**
P1	$C_{16}H_{16}O_7$	319.0823	0.3164	0.1885	0.3596	0.3880	Factor = 0.1885/0.3164
P2	$C_{19}H_{20}O_7$	359.1136	0.2970	0.1990	0.4039	0.4047	Factor = 0.1990/0.2970
P3	$C_{16}H_{20}N_2O_9$	383.1096	0.2553	0.1774	0.2693	0.2571	Factor = 0.1774/0.2553
P4	$C_{23}H_{26}O_8$	429.1555	0.2675	0.1797	0.2767	0.2664	Factor = 0.1797/0.2675
P5	$C_{24}H_{30}O_8$	445.1868	0.4455	0.2985	0.4110	0.4290	Factor = 0.4455/0.2985
B1	$C_{13}H_{18}O_5$	253.1081	0.3119	0.3094	0.3697	3.8675	Factor = 3.8675/0.3697
B2	$C_{13}H_{16}O_6$	267.0874	0.3695	0.3713	0.4272	1.8540	Factor = 1.8540/0.4272
B3	$C_{13}H_{19}NO_6$	284.114	0.0789	0.0800	0.1030	0.6412	Factor = 0.6412/0.1030
B4	$C_{13}H_{17}NO_7$	298.0932	0.1403	0.1408	0.1890	0.5933	Factor = 0.5933/0.1890
B5	$C_{18}H_{28}O_7$	355.1762	0.4434	0.4528	0.5285	2.1822	Factor = 2.1822/0.5285
D1	$C_{14}H_{16}O_8$	311.0772	0.5165	0.5197	0.6140	0.6512	Factor = (0.5197/0.5165 + 0.6512/0.6140)/2
D2	$C_{17}H_{24}O_8$	355.1398	2.1211	2.1366	3.0489	3.0650	Factor = (2.1366/2.1211 + 3.0650/3.0489)/2
D3	$C_{16}H_{22}O_9$	357.1191	1.4340	1.4880	1.9826	1.8309	Factor = (1.4880/1.4340 + 1.8309/1.9826)/2
D4	$C_{19}H_{28}O_9$	399.1661	1.4453	1.3841	1.7431	1.8609	Factor = (1.3841/1.4453 + 1.8609/1.7431)/2
D5	$C_{19}H_{28}O_{10}$	415.161	1.0417	1.0112	1.2703	1.3158	Factor = (1.0112/1.0417 + 1.3158/1.2703)/2

MANUSCRIPT II

The molecular geography of dissolved organic matter in the Atlantic Ocean can largely be explained by a simple two-source mixing model

Maren Seibt[1], Jutta Niggemann[1], Helena Osterholz[1] and Thorsten Dittmar[1]

A modified version of this chapter will be submitted to *Nature*

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

The DOM molecular composition in the Atlantic and Southern Ocean is exceptionally similar across a large spatial scale. Highest dissimilarities in the molecular composition are found between the surface mixed layer and the deep sea. In this chapter, we arithmetically constructed a two-source mixing model with two disitinct endmembers (microbially produced DOM in a laboratory experiment and refractory DOM from the deep North Pacific) and compared the model to natural molecular fingerprints of Atlantic and Southern Ocean DOM. The comparison showed that most of the molecular variability in DOM from both oceans is explained by mixtures of variable input of fresh DOM.

All authors designed the study. M. Seibt collected the samples from the Atlantic and Southern Ocean. H. Osterholz perfomed the experiment and analyzed the data of the microbial DOM production experiment. M. Seibt performed data analysis of Atlantic and Southern Ocean DOM and calculated the two-source mixing model together with H. Osterholz, J. Niggemann and T. Dittmar. M. Seibt wrote the manuscript with input from all authors.

The manuscript will be modified for submission to *Nature* and is presented here in more detail and with adjusted formatting according to the style of this thesis.

3.1 Abstract

Marine dissolved organic matter (DOM) is a structurally diverse mixture of thousands of different molecules resulting in extremely low concentrations (< picomolar) of individual compounds. However, the multitude of compounds adds up to one of the largest pools of reduced carbon in the world (662 Gt C) making DOM a significant component of the global carbon and climate cycle. Despite the geographical diversity of primary producers, microbial community structures, variety of abiotic conditions and ecosystem types in the global ocean, we found that marine molecular DOM composition is exceptionally similar across the globe (maximum of 14 % dissimilarity on a Bray-Curtis scale between surface and deep sea DOM in the Atlantic) when analyzed at ultrahigh analytical resolution using Fourier-Transform Ion Cyclotron Resonance Mass Spectrometry. Based on a two-source mixing model, constructed by arithmetically mixing increasing amounts of fresh with refractory DOM, we show that most of the observed molecular differences in DOM composition in more than 380 DOM samples from the Atlantic and Southern Ocean can be explained by the simple mixing of water masses carrying variable amounts of fresh DOM. We find that most of the compositional differences of marine DOM in these two global oceans are captured by only 27 modeled fingerprints, implying that the molecular signature of heterotrophic microbial processing is to some extent universal. Based on DOM molecular composition, we are for the first time able to quantify the contribution of microbially processed DOM in the open ocean, which is less than 4 µM in the deep ocean and between 5 and 12 µM in the surface mixed layer. Our results grant new insights into the cycling of DOM and identifies microbial production as one of the major drivers behind the molecular geography of DOM in the global ocean.

3.2 Introduction

Dissolved organic matter (DOM) is a complex mixture of thousands of different compounds and an even higher number of structural isomers (Hertkorn et al., 2007). The largest portion of the global DOM pool with uniformly low concentrations of 34 – 45 µmol C l^{-1} (Druffel et al., 1992; Hansell et al., 2012) is found in the deep sea (630 ± 32 Gt C, Hansell, 2013), which is characterized by little DOM production and slow transformation rates (Reinthaler et al., 2006). Deep sea DOM is the result of extensive heterotrophic processing regarded as the recalcitrant leftover of biological activity (Jiao et al., 2010) and persists over the timescale of millennia in the deep ocean (Bauer et al., 1992; Williams and Druffel, 1987). In contrast, autotrophic production, heterotrophic turnover and abiotic processes such as photodegradation make the euphotic surface layer a much more variable environment concerning DOM pool size and reactivity. Higher DOC concentrations (up to 80 µmol C l^{-1}, Hansell et al., 2009) are hypothesized to be due to the addition of freshly produced DOM (including labile, semi-labile and semi-refractory reactivity fractions of DOM) on top of a significant amount of refractory DOM and the accumulation of DOM above physical barriers such as the pycnocline (Hansell, 2013). The most labile part of DOM (containing amongst others amino acids and sugars) has turnover times of minutes to days (Hansell, 2013), making it rather easy to study its cycling in situ but also in laboratory experiments (Amon and Benner, 1994; Cherrier et al., 1996; Jorgensen et al., 2014). The much longer turnover time of years to millennia of the semi-labile, semi-refractory and refractory DOM pools (Hansell, 2013) makes it, however, very difficult to study their cycling in laboratory experiments. Due to its estimated age of millennia, the most refractory part of the DOM pool survives several cycles of the thermohaline circulation and forms the refractory background present in each marine DOM sample. The advent of ultrahigh resolution techniques such as Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) enables us to characterize the molecular DOM composition in unprecedented detail. Studies examining the molecular transformation of refractory DOM in the ocean find a continuum of DOM degradation states (Flerus et al., 2012; Lechtenfeld et al., 2014), emphasizing the complex processes shaping the molecular DOM composition. Knowing the turnover mechanisms and factors

influencing the molecular composition of this vast pool of organic carbon is, however, crucial for understanding its role in the global carbon and climate cycle.

Prokaryotic life in the ocean occurs at high abundance with an estimated total number of 1.2×10^{29} cells (Whitman et al., 1998) and estimates of the number of different species go as high as 10^6 (Curtis et al., 2002). Microbial community structures in the global oceans vary substantially, strongly depending on prevailing environmental conditions such as depth, temperature, salinity and availability of nutrients (e.g. Crump et al., 2004; Fuhrman et al., 1993). It was shown that deep water masses in the North Atlantic Ocean carry distinct microbial communities characteristic for each water mass (Agogué et al., 2011). Different microbial community structures and their associated metabolic pathways result in a complex mixture of organic compounds, which should be characteristic for the producing community. However, across the Atlantic and Southern Ocean, DOM molecular composition is exceptionally similar on a large latitudinal range (Flerus et al., 2012; Hansman et al., 2015; Lechtenfeld et al., 2014), with only little compositional differences on the molecular level. Major differences are the higher hydrogen to carbon ratios (Chen et al., 2014) and a smaller average molecule size of surface DOM when compared to deep sea DOM (Flerus et al., 2012). One reason for the missing direct link between the molecular DOM composition and the microbial community in the ocean could be the large discrepancy between observed time scales: Microbial turnover is an immediate process adding younger signatures to an already existing older DOM pool (Loh et al., 2004; Repeta and Aluwihare, 2006). These complex mixtures of degradation states and ages in marine DOM likely blur characteristic imprints of the producing community. Although an immediate connection between DOM composition and microbial community structure is difficult to observe, heterotrophic microbial activity is one of the major factors driving DOM composition (and vice versa) in the ocean (Amon and Benner, 1996; Landa et al., 2014; McCarren et al., 2010; Osterholz et al., 2014).

Osterholz et al. (2015) showed in a study with three mesocosms, each containing the same natural phytoplankton and microbial community at the beginning of the experiment, that the microbial community structure strongly diverged in the mesocosms over an incubation time of three years, whereas the molecular composition

of DOM converged into similar signatures as complex as marine DOM, but yet different in the relative abundance of compounds. The results of this study indicate that the molecular signature of heterotrophic processing is to some extent universal and requires only a complex enough microbial community allowing for a multitude of possible interactions.

In this study, we follow the signature of microbial activity in the open ocean and test whether the molecular geography (i.e. the global distribution patterns of organic molecules) of the Atlantic and Southern Ocean can be explained by mixing the fresh signature of microbial processing with refractory background DOM. This would indicate that the observed compositional differences between surface and deep sea DOM is the result of simple mixing of waters carrying specific DOM signatures with variable contributions of freshly produced DOM. We test this hypothesis by constructing a two-source mixing model with modeled DOM compositions containing increasing amounts of fresh DOM. The two used DOM endmembers are the molecular signature of fresh DOM produced in a laboratory experiment (Osterholz et al., 2015) and the molecular signature of degraded DOM (North Equatorial Pacific Intermediate Water; NEqPIW). The modeled DOM compositions of this two-source mixing model are then compared to Atlantic and Southern Ocean DOM and their molecular dissimilarity evaluated. All samples, including both endmembers as well as the whole data set from the Atlantic and Southern Ocean, were analyzed via FT-ICR-MS with the same instrument settings, making a direct comparison of the ultrahigh resolution fingerprints possible.

3.3 Material and methods

Sampling – For a detailed description of the mesocosm experiment and the sampling procedure refer to Osterholz et al. (2015). Atlantic and Southern Ocean DOM was sampled during three cruises of R/V Polarstern to the Atlantic sector of the Southern Ocean (39° S to 71° S; ANT-XXVIII/2), to the Drake Passage and Antarctic Peninsula (56° S to 62° S, ANT-XXVIII/4) and the Atlantic Ocean (51° S to 47° N; ANT-XXVIII/4) between December 2011 and May 2012 (Figure 3.1 A). Samples were taken in high resolution in the surface water masses (20 m, 40 m, 60 m, 100 m, 200 m and in the fluorescence maximum, if present) and in lower

resolution in the deeper water masses (Figure 3.1 B, physical and other sample properties from cruise ANTXXVIII/4 correspond very well with samples of transect ANTXXVIII/2 and are therefore not visualized). The position of the upper and lower boundary of the pycnocline was identified during the CTD casts (water layer with rapid change of density) and following the 27.0 isopycnal, respectively. In total, 383 DOM samples were taken in the Atlantic and Southern Ocean. All samples were extracted with a solid-phase extraction (SPE) method according to Dittmar et al. (2008). In brief, 4 l of sea water were filtered through pre-combusted (400 °C, 4 h) 0.7 µm glass fibre filters (GF/F, Whatman, United Kingdom), acidified to a final pH of 2 (HCl, 25%, p.a., Carl Roth, Germany) and extracted with commercially pre-packed cartridges (1 g of sorbent, PPL, Agilent, USA). Following extraction, all samples were de-salted, dried and eluted with 6 ml methanol (HPLC-grade, Sigma-Aldrich, USA). Extracts were frozen at -20 °C until analysis in the laboratory in Oldenburg. Dissolved organic carbon (DOC) concentrations of the extracts were quantified via the high temperature catalytic oxidation method (HTCO) on a Shimadzu TOC-VCPH/CPN Total Organic Carbon Analyzer equipped with an ASI-V autosampler. The carbon-based extraction efficiency was 53 ± 9 % for all Atlantic and Southern Ocean samples.

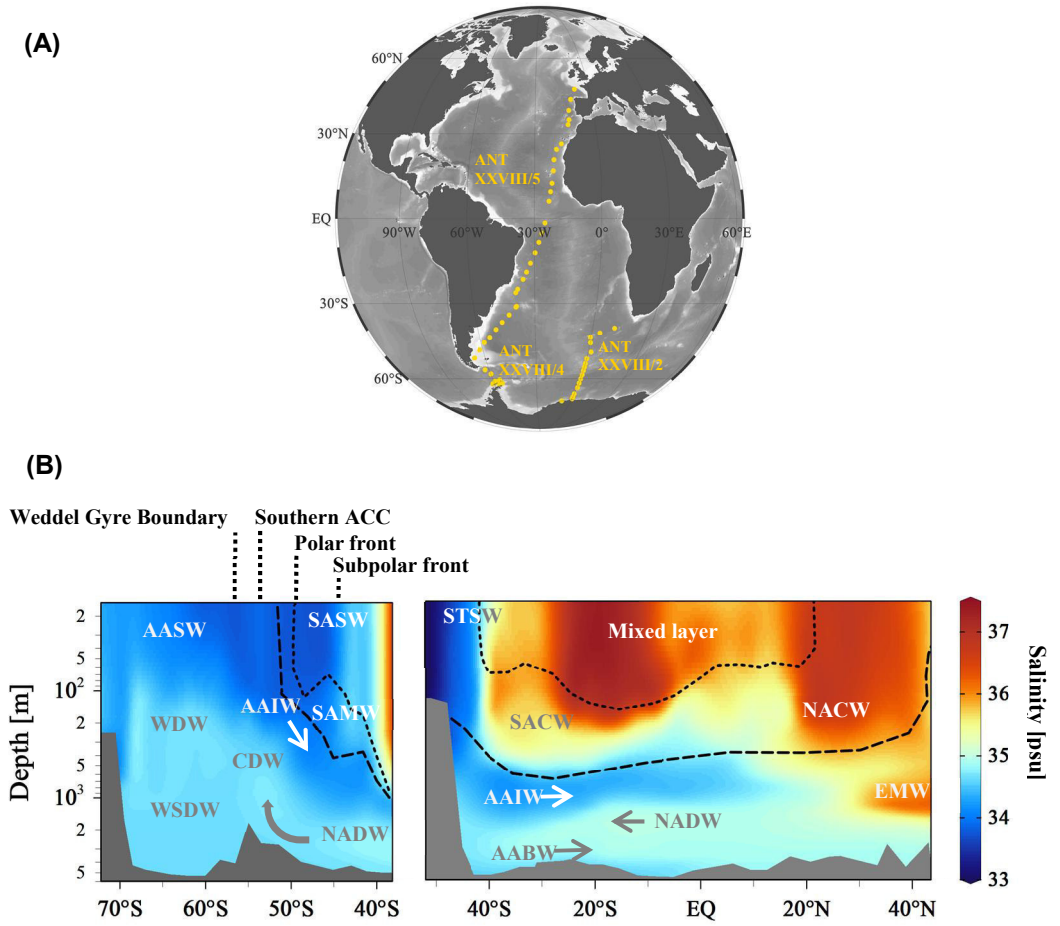

Figure 3.1 Sampling transects in the Atlantic and Southern Ocean (ANTXXVIII/2 from Cape Town/South Africa to Antarctica; ANTXXVIII/4 from Punta Arenas/Chile to the Antarctic Peninsula; ANTXXVIII/5 from Punta Arenas/Chile to Bremerhaven/Germany) **(A)** and salinity profiles along ANTXXVIII/2 (left) and ANTXXVIII/5 (right) with major water masses and frontal systems (data of ANTXXVIII/4 corresponds well to data of ANTXXVIII/2 and are not shown) **(B)**. Dashed lines indicate the upper and lower boundary of the pycnocline and arrows the general flow direction of water masses. Please note the logarithmic depth scale. Abbreviations and number of samples for each water mass are as follows (in alphabetical order): Antarctic Bottom Water (AABW, n = 23), Antarctic Intermediate Water (AAIW, n = 19), Antarctic Surface Water (AASW, n = 111), Circumpolar Deep Water (CDW, n = 26), Eurafrican Mediterranean Water (EMW, n = 4), surface mixed layer of the Atlantic (mixed layer, n = 47), North Atlantic Central Water (NACW, n = 23), North Atlantic Deep Water (NADW, n = 22), South Atlantic Central Water (SACW, n = 30), Subantarctic Surface Water (SASW, n= 35), Subantarctic Mode Water (SAMW, n = 5), Subtropical Surface Water (STSW, n = 11), Weddell Sea Warm Deep Water (WDW, n = 19) and Weddell Sea Deep Water (WSDW, n = 8). All cross-section plots were produced with Ocean Data View (Schlitzer, 2015).

Molecular DOM composition – All SPE-DOM samples were analyzed via FT-ICR-MS (Bruker Daltonik GmbH, Bremen, Germany) equipped with a 15 T superconducting magnet (Bruker Biospin, Wissembourg, France) and an electrospray ionization source (ESI; Apollo II ion source, Bruker Daltonik GmbH, Bremen, Germany). ESI is a soft ionization technique, which allows the molecules to

stay intact and is widely used in the analysis of natural organic mixtures (D'Andrilli et al., 2010; Dittmar and Koch, 2006; Kujawinski and Behn, 2006). Prior to analysis all extracts were mixed with ultrapure water and methanol (MS grade, 1:1 v/v) to a final carbon concentration of 10 ppm, filtered (0.2 μm) and measured in negative ion mode. All mass spectra were analyzed with the Data Analysis version 4.0 SP4 software package (Bruker Daltonik GmbH) and recorded in a mass range of 150 - 2000 Da with 500 acquired scans. All spectra were internally calibrated using a list of > 50 known molecular formula mass peaks from a marine in-house reference sample. All ions were singly charged and the allowed error for ions used for the internal calibration was less than 0.1 ppm. The mass to charge ratio (m/z), resolution and peak intensity were exported and processed using in-house Matlab routines. Molecular formulae were assigned to peaks with a minimum signal-to-noise ratio of 4 following the rules published by Koch et al. (2007) with following restrictions: $^{12}C_{1\text{-}130}{}^{1}H_{1\text{-}200}O_{1\text{-}50}{}^{14}N_{0\text{-}4}S_{0\text{-}2}P_{0\text{-}2}$. All mass-spectra were normalized by dividing each FT-ICR-MS signal intensity by the sum of all signal intensities present in the sample. All statistical analyses of FT-ICR-MS data were performed with the software "R" (Version 3.2.0, package "vegan"; Oksanen et al., 2015).

To account for analytical variance, we measured an in-house reference sample twice a day during the whole measuring campaign and determined the analytical variance by calculating a Bray-Curtis dissimilarity matrix for this reference data set. The Bray-Curtis dissimilarity is a measure of the molecular differences within a sample set and ranges from 0 % (identical sample) to 100 % (completely different sample). The determined analytical variance of 3.5 % was subtracted from Bray-Curtis dissimilarities matrices calculated for the mixing model and Atlantic and Southern Ocean DOM.

Based on their molecular properties, all identified compounds were assigned to molecular categories (polyphenols, phenols, highly unsaturated compounds, unsaturated aliphatics and peptide-like molecular formulae) following the rules described by Romano et al. (2014) and their relative contribution to the overall intensity was calculated. Because a multitude of possible structural isomers exists behind every identified molecular formula (Hertkorn et al., 2007), the classification into molecular categories is not unambiguous and an affiliation to another compound group

may also be possible. For the ease of reading we will regard the sorting into molecular categories to be universally applicable neglecting the possibility of any other compound affiliation.

Flerus et al. (2012) showed that marine SPE-DOM is similar in apparent age and distribution to bulk DOM. Furthermore, Osterholz et al. (2015) showed that FT-ICR-MS is a valid method for characterizing the molecular evolution of DOM. It is, however, noteworthy that due to our analytical window small molecules (<150 Da) as well as colloidal material is lost. SPE-DOM is therefore the operationally defined fraction of marine DOM, which can be studied with the described methods and instrumentation. We will further discuss only SPE-DOC concentrations and the molecular composition of SPE-DOM obtained via FT-ICR-MS.

Two-source mixing model – The two-source mixing model is based on the hypothesis that marine DOM is a mixture of freshly produced microbial DOM (mesocosm) on top of a refractory background (NEqPIW DOM). For the mixing model, we took an integrated sample over the second year of the mesocosm experiment performed by Osterholz et al. (2015) assuming that due to our sampling time frame only semi-labile and semi-refractory DOM is present. NEqPIW is one of the oldest water masses on Earth (Bostock et al., 2010) and has long lost its contact to the productive surface layers. DOM in this water mass largely resisted microbial degradation (refractory DOM; Hansell et al., 2009). For model calculation and evaluation only peaks present in both endmember samples and with assigned molecular formulae were used (n = 3257). The molecular dissimilarity between the fresh and refractory endmember was 45 % on a Bray-Curtis scale indicating that 55 % of both DOM pools was either molecularly indistinguishable or the dissimilarities were not resolvable on the Bray-Curtis scale (Figure 3.2).

We used two different approaches to calculate the two-source mixing model. First, we mixed the methanol extracts of fresh DOM and refractory DOM in different ratios (50:50, 60:40, 70:30, 80:20 and 90:10 vol.-% fresh and refractory DOM, respectively) and analyzed them along with the pure endmembers via FT-ICR-MS. In order to obtain a finer resolution, we used in a second approach the mass spectra of the analyzed endmembers and mixed them arithmetically in one-percent steps from 99:1 to 1:99 % fresh and refractory DOM, respectively (Figure 3.4 B). The

molecular Bray-Curtis dissimilarity of the corresponding mixtures from both approaches was on average 3 %, which was within the rage of the detected analytical variation (Figure 3.2). We therefore conclude that both approaches are valid and we further use the finer resolution model.

The molecular differences between modeled fingerprints and Atlantic and Southern Ocean DOM were assessed via the Bray-Curtis dissimilarity. The modeled fingerprint having the lowest molecular dissimilarity to a DOM fingerprint from the Atlantic and Southern Ocean was defined as the optimum fit between model and marine DOM.

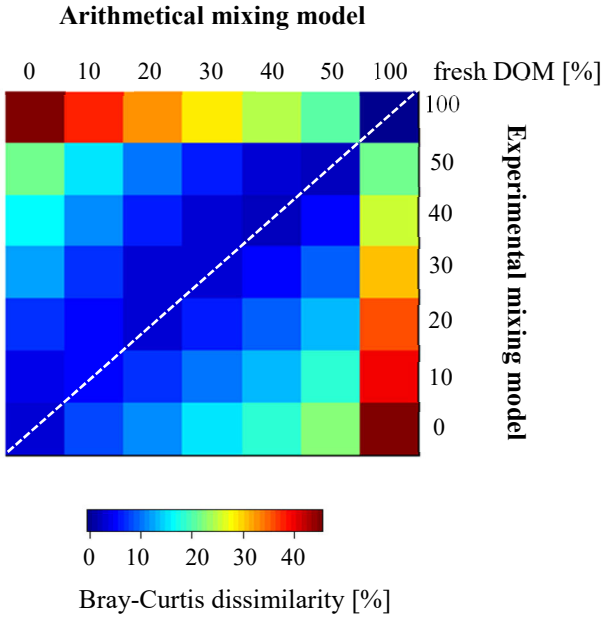

Figure 3.2 Evaluation of the different approaches to the two-source mixing model. Each square represents the direct comparison of molecular fingerprints constructed either arithmetically (mixing of the mass spectra of the endmembers; x-axis) or measured experimentally (mixing the extracts of the endmembers and analyzing the mixtures via FT-ICR-MS; y-axis). The color code corresponds to the molecular dissimilarity with blue being less dissimilar and red being more dissimilar. The white diagonal indicates the corresponding mixtures with the same amount of fresh DOM for both approaches.

3.4 Results

DOC concentration and DOM molecular composition – SPE-DOC concentrations in the deep Atlantic and Southern Ocean were uniformly low (20 – 30 µmol C l^{-1}) and higher in the surface mixed layer of the Atlantic (35 – 46 µmol C l^{-1}), where accumulation of DOM is possible due to the presence of a seasonal and permanent pycnocline (Figure 3.3 A). To test whether the concentration gradient is also reflected on the molecular level, the Bray-Curtis dissimilarity between our samples and NEqPIW DOM was calculated. This calculation provided an estimation to what extent the molecular DOM composition of our samples differed from the most refractory DOM in the ocean. In the surface mixed layer, DOM was most different from refractory DOM with Bray-Curtis dissimilarities of up to 14 % (Figure 3.3 B). However, the strong concentration gradient with double the concentration in the surface mixed layer compared to the underlying deeper water masses was not reflected on the molecular level. Surface mixed layer DOM appeared to be at least to 86 % molecularly indistinguishable from refractory DOM on a Bray-Curtis scale. The DOM in the Southern Ocean and the deeper water masses of the Atlantic were less dissimilar to NEqPIW DOM with dissimilarities of up to 8 %.

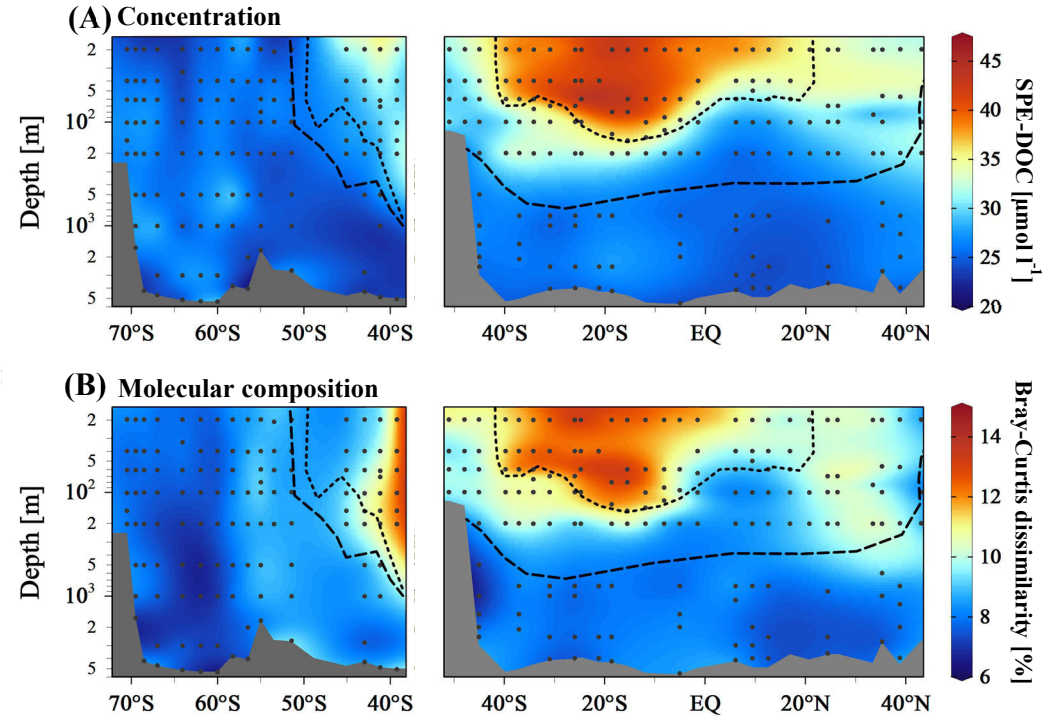

Figure 3.3 SPE-DOC concentration **(A)** and the molecular dissimilarity between samples from the Atlantic and Southern Ocean and NEqPIW DOM **(B)**. The left and right panels show sampling transects ANTXXVIII/2 and ANTXXVIII/5, respectively. The black dots correspond to sampling depths. Please note the different units and scaling of the color bar.

Optimum fit between model and Atlantic and Southern Ocean DOM – In order for the mixing model to explain any molecular variability in the ocean, a minimum in molecular dissimilarity (in other words: a maximum similarity) between Atlantic and Southern Ocean DOM and the modeled DOM compositions must exist (optimum fit). Furthermore, the optimum fit should correspond to more than one modeled fingerprint accounting for molecular dissimilarity patterns observed between our samples and refractory DOM from the NEqPIW (Figure 3.3 B). The optimum fit found between our samples and the model corresponded indeed to 27 different modeled fingerprints containing various amounts of fresh DOM. The water masses from the Atlantic and Southern Ocean can be grouped into three clusters (optimum fit clusters) according to their optimum model fit (Figure 3.4): The deep Atlantic and Southern Ocean (average optimum fit at 4 % fresh DOM, blue cluster in Figure 3.4), the subsurface water masses of the Atlantic (average optimum fit at 12 % fresh DOM, green cluster in Figure 3.4) and the surface water masses of the Atlantic

(average optimum fit at 20 % fresh DOM, orange cluster in Figure 3.4). For validation of the model we substituted our observed DOM compositions from the Atlantic and Southern Ocean with the 27 modeled optimum fit fingerprints and calculated again the molecular Bray-Curtis dissimilarity to refractory NEqPIW DOM (Figure 3.5 A). The resulting dissimilarity patterns showed a striking resemblance to the observed dissimilarity patterns of our samples to NEqPIW deep sea DOM, implying that 27 modeled molecular fingerprints with variable amounts of fresh DOM can explain the molecular geography of Atlantic and Southern Ocean DOM.

The residual molecular dissimilarity, which was not explained by the two-source mixing model is shown in Figure 3.5 B. However, the optimum fit reduced the molecular dissimilarity to almost the same level for all water masses ranging from 6 to 8 %. Hence, our model compensated the higher dissimilarities observed for the surface mixed layer and reduced its molecular dissimilarity to the same level Atlantic deep sea and Southern Ocean DOM showed to the refractory NEqPIW DOM.

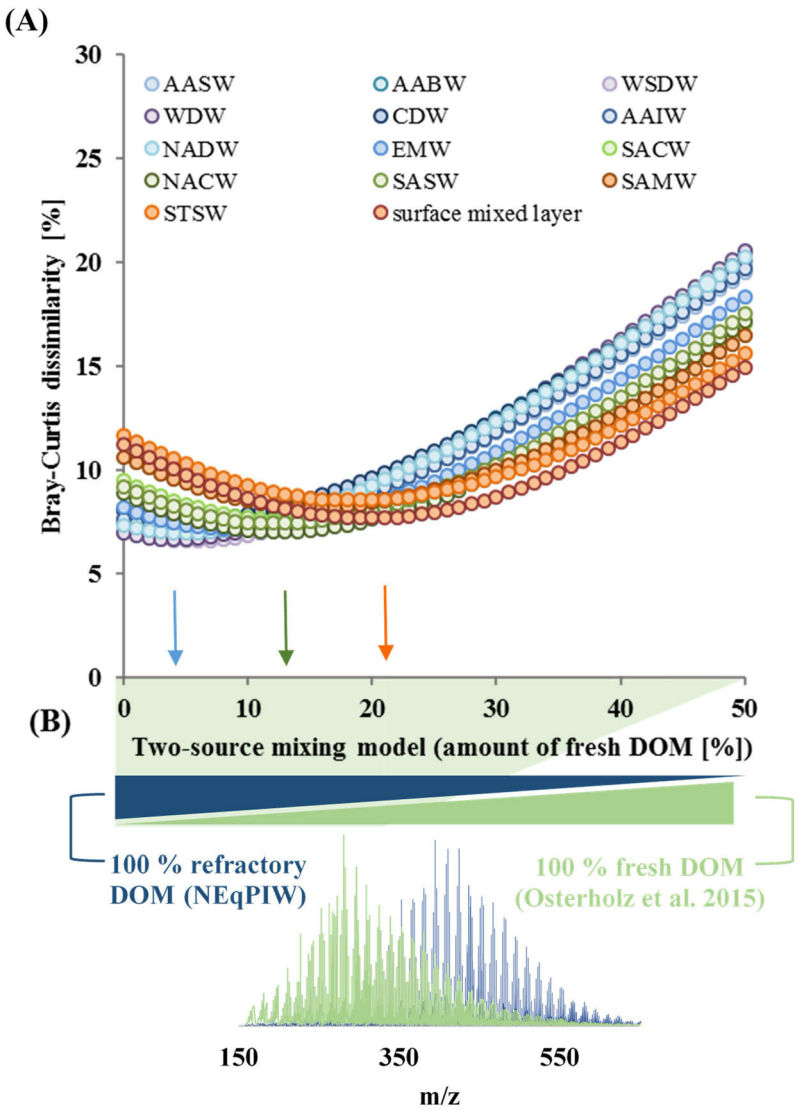

Figure 3.4 Molecular Bray-Curtis dissimilarity between two-source mixing model and Atlantic/Southern Ocean DOM samples (**A**) and concept of the two-source mixing model **(B)**. Similar colors in (A) correspond to water masses in the same optimum fit cluster (Atlantic deep sea/Southern Ocean in blue/purple, subsurface water masses of the Atlantic within the pycnocline in green and surface water masses of the Atlantic in orange). Arrows indicate the optimum fit between model and Atlantic/Southern Ocean DOM in the respective color. Averages of the molecular Bray-Curtis dissimilarity as well as the optimum fit for each water mass are shown. Standard deviations for the Bray-Curtis dissimilarity (y-axis) and the optimum fits (x-axis) are less than 2 % and 3 % (5 % for surface and subsurface water masses of the Atlantic) for each averaged water mass, respectively (error bars are not shown in graph). The two-source mixing model is constructed by mixing the mass spectra of fresh mesocosm DOM (Osterholz et al. 2015; green) and refractory NEqPIW DOM (blue) in 1-percent steps (B). The model displayed in percentage of fresh DOM (0 to 50 %) is shown on the x-axis of (A).

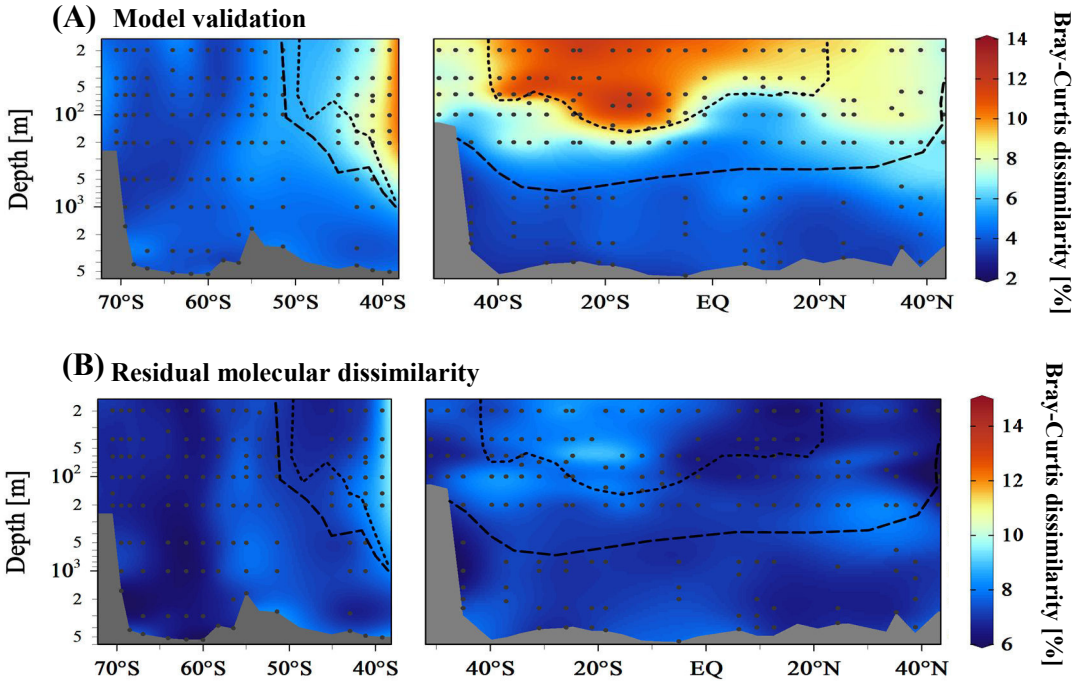

Figure 3.5 Validation of two-source mixing model **(A)** and residual molecular dissimilarity of Atlantic and Southern Ocean DOM, which is not explained by the two-source mixing model **(B)**. The model was validated by substituting Southern Ocean and Atlantic DOM samples with the modeled molecular signature of the optimum fits and calculating the Bray-Curtis dissimilarity to NEqPIW DOM. The dissimilarity patterns are strikingly similar to the dissimilarity patterns observed for Atlantic and Southern Ocean DOM and NEqPIW DOM as seen in Figure 3.3 B. The residual molecular dissimilarity for each sample in (B) corresponds to the Bray-Curtis dissimilarity of the optimum fits in Figure 3.4 A (y-axis).

The classification of DOM compounds into molecular categories provided information about the possible structures of the identified compounds and about the molecular characteristics of fresh and refractory DOM. With this information, we were able to identify the groups responsible for the observed the molecular dissimilarities and to draw conclusions concerning the cycling of fresh DOM in the ocean. The most abundant group of molecules in marine and mesocosm DOM were the highly unsaturated compounds followed by the unsaturated aliphatics, polyphenols, peptide-like molecular formulae and phenols (Figure 3.6 A – E). All compound categories were present in significantly higher abundances in freshly produced DOM (mesocosm DOM) except for the highly unsaturated compound group suggesting that unsaturation is a characteristic trait for more degraded forms of DOM. Oxygen-rich unsaturated compounds were significantly less abundant in mesocosm DOM indicating that the production of oxygen-rich compounds was not a major feature of microbial production. The molecular compositions of the optimum fits reflected

very well the respective proportions of the various compound groups in marine DOM except for the polyphenols, which were overall more abundant in model endmembers and optimum fits (Figure 3.6 C).

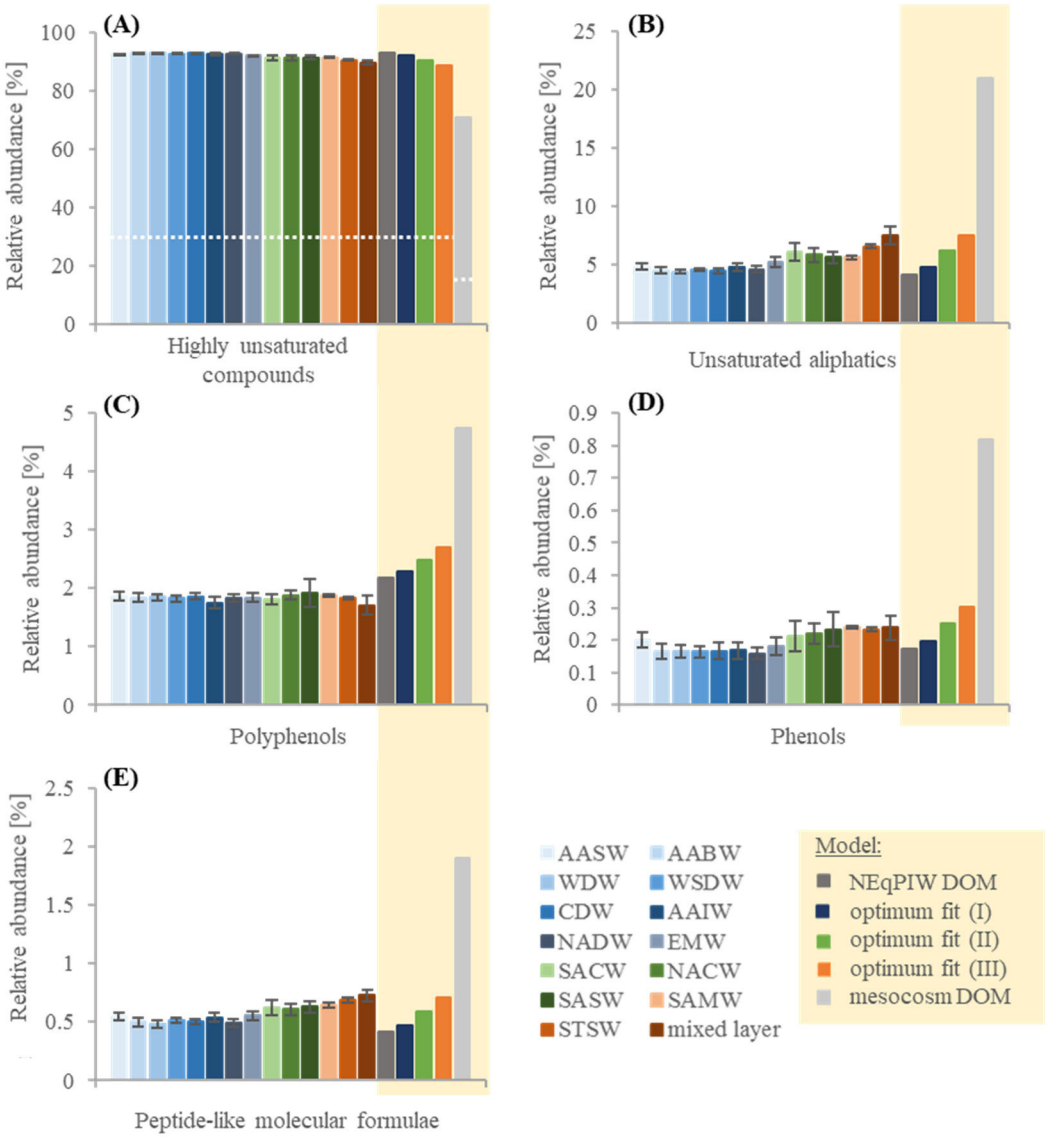

Figure 3.6 Average relative abundance of the molecular compound categories of highly unsaturated compounds **(A)**, unsaturated aliphatics **(B)**, polyphenols **(C)**, phenols **(D)** and peptide-like molecular formulae **(E)** for every water mass (observations) and the model (yellow boxes, NEqPIW and mesocosm DOM represent the fingerprints of the model endmembers; optimum fits I, II and III correspond to modeled fingerprints at the dissimilarity minimum of the two-source mixing model (blue, green and orange arrow, respectively, see Figure 3.4). The white dashed line in (A) indicates the relative contribution of oxygen-rich highly unsaturated compounds below white line (32 ± 1 % for Atlantic/Southern Ocean DOM, optimum fits I, II, III of the model and NEqPIW DOM; ~14 % for mesocosm DOM) and the oxygen-poor highly unsaturated compounds above white line (~ 59 ± 1 % for Atlantic/Southern Ocean DOM as well as for model).

3.5 Discussion

General molecular characteristics of DOM composition – Refractory DOM is found everywhere in the ocean. Due to its millennial stability (Hansell, 2013), it survives multiple cycles of the thermohaline circulation (1000 – 2000 years; Döös et al., 2012), explaining its presences at all depths. It is, however, still unknown whether its contribution to the total DOM pool is constant or whether it contributes variable amounts throughout the water column. The strong concentration gradient observed between the surface mixed layer and the deep sea was not reflected on the molecular level (double the concentration but only 6 % higher molecular variability in the surface mixed layer; Figure 3.3 A and B). The small, but observable molecular dissimilarities between surface and refractory DOM indicate, however, that substantially different processes in the euphotic layer shape the DOM composition and introduce a higher molecular variability. The results of our simple two-source mixing model strongly suggest that the higher molecular variability in the surface mixed layer is due to the addition of semi-labile and semi-refractory DOM on top of a refractory background. The DOM produced in the mesocosms is as diverse as refractory deep sea DOM on the molecular formulae level, but differs strongly in the relative abundance of compounds (Osterholz et al., 2015). More precisely, mesocosm DOM contained on average smaller molecules (~ 351 Da) than deep Atlantic and NEqPIW DOM (~ 424 Da and 426 Da, respectively, Figure 3.4 B). For the mixed layer we observed a smaller molecule size (~ 410 Da) than for all other DOM samples. An increase in average molecule size in the ocean goes along with molecular ageing (Flerus et al., 2012; Martínez-Pérez et al., 2017) and the molecule size in the mixed layer represents either the mixture of DOM pools with different ages (i.e. our two-source mixing model) or is the result of molecular aging. If the latter is true, molecular aging must be a rapid process acting on timescales of less than a year due to the seasonal mixing of the upper water layers. This, however, is contradictory to the year-long stability of semi-labile and semi-refractory DOM (Hansell, 2013). It is therefore unlikely that the observed molecular dissimilarities between the surface mixed layer and the underlying water masses are just the result of molecular ageing, but rather that, along with molecular aging, the mixing of DOM

signatures with different production and transformation histories is responsible for these patterns.

The classification of the molecular signatures into compound groups gives more valuable clues concerning the molecular composition of the samples and the model: Unsaturated aliphatics and peptide-like molecular formulae are indicative of bioactive processes shaping the DOM molecular composition. Their higher abundance in the mesocosms but also in the surface mixed layer of the Atlantic is therefore not surprising (Figure 3.6 B and E). It was shown that algal exudates are highly aliphatic (Sun et al., 1997), explaining the pattern observed in marine DOM composition. Phenolic and polyphenolic structures originate mostly from terrigenous sources (vascular plant material), but are also present in the open ocean (Figure 3.6 C and D). A study by Hernes and Benner (2006) shows a higher concentration of phenols in the North Atlantic compared to the Pacific, explaining the overestimation of phenols in our model. The residence time of 35 years in the Atlantic for phenols calculated by the same authors indicates their affiliation to the semi-labile and semi-refractory DOM pool and explains their lower abundance in the deep sea. Aromatic structures such as polyphenols are mostly photo-reactive (Stubbins and Dittmar, 2015) and their low contribution to the overall intensity (lowest in the surface mixed layer) in the Atlantic and Southern Ocean is not surprising. Due to the lack of water mass ventilation and the production of chromophoric DOM (CDOM) as byproduct of the oxidation of organic matter from sinking particles in the North Pacific (Nelson et al., 2010), NEqPIW DOM contains more polyphenolic substances than the deep Atlantic. These changes on the molecular level and also the decrease in DOM concentration due to microbial consumption (Hansell and Carlson, 1998) might account for the observable molecular dissimilarities between the deep Atlantic and North Pacific (Figure 3.3 B and Figure 3.5 B).

Mixing model – The optimum fits of DOM signatures between our model and Atlantic and Southern Ocean vary in the contribution of fresh DOM. Atlantic DOM is much younger than Pacific DOM as seen in the apparent radiocarbon age (Bauer et al., 1992) and the low but traceable amounts of fresh DOM in the deep Atlantic account for that younger age (4 % fresh DOM, blue arrow Figure 3.4). The highest contribution of fresh DOM (20 %, orange arrow Figure 3.4) was observed for the

surface water masses in the Atlantic Ocean reflecting very well the high biological activity in the euphotic zone. The optimum fit between model and the subsurface water masses within the pycnocline was at 12 % fresh DOM (green arrow Figure 3.4), indicating that within the pycnocline water from the surface mixed layer is mixed along isopycnal surfaces and exported via the winter-deepening of the seasonal thermocline (Carlson et al., 2010) into deeper layers resulting in a molecular composition resembling the modeled fingerprints of intermediate amounts of fresh DOM.

Our model provides molecular fingerprints with increasing amounts of laboratory-created DOM on top of a refractory background. The molecular dissimilarity between modeled fingerprints and Atlantic and Southern Ocean fingerprints decreased until their respective optimum fits and reduced the observed dissimilarity at most 6 % to a range of 5 – 10 % residual dissimilarity. Thus, the addition of semi-labile and semi-refractory DOM produced by heterotrophic microbes accounted for almost all observed molecular variability between surface and deep sea DOM (Figure 3.5 B). The residual molecular dissimilarity, which was observed between all Atlantic and Southern Ocean DOM and NEqPIW DOM, is probably due to processes not described in this study. Such process can include microbial consumption along the flow path of water masses (Hansell and Carlson, 1998), photodegradation (Stubbins and Dittmar, 2015), thermogenic transformation (Hawkes et al., 2015), or the removal of DOC due to aggregate formation (Chin et al., 1998). We tried to include photodegradation into our model by adding a third modeled dimension. We did that by constructing a second mixing model with the molecular fingerprints used for the optimum fits of the here presented model and the molecular fingerprints of a photodegradation experiment and checked whether the molecular dissimilarity would decrease any further. However, that was not the case. This does not mean that photodegradation has no influence on the molecular DOM composition, but rather that it is not captured with the chosen methods and the scope of this model. The Bray-Curtis dissimilarity accounts only for quantitative changes of relative peak abundances and provides no indication about qualitative changes on the molecular level, which is also a major feature of photodegradation.

Nevertheless, heterotrophic processing seems to be the major influencing factor on DOM composition in the surface ocean, although the surface layer is almost completely depleted in nutrients (Garcia et al., 2014) which are required in the microbial metabolism. However, Milici et al. (2016) found that although cell numbers are low in the gyres, bacterioplankton diversity at the surface is highest at mid-latitudinal regions of the Atlantic Ocean, indicating a closely interlinked biological and geochemical system capable of shaping the DOM pool in the oligotrophic surface water masses of the Atlantic. The high nutrient availability and production rates (Arrigo et al., 1998) in the Southern Ocean are not reflected on the molecular level. This is probably due to the well mixed water column, which does not allow accumulation of semi-labile and semi-refractory DOM above physical barriers, but reflects also the fast recycling and transient occurrence of labile DOM in the Southern Ocean (Kähler et al., 1997).

Another striking feature of the mixing model is that the molecular geography of the Atlantic and Southern Ocean can be reconstructed with only 27 mathematically created molecular fingerprints containing increasing amounts of fresh DOM on top of a refractory background. This is in two ways extraordinary: First, the DOM molecular variability in two major global oceans can be condensed to and explained by a surprisingly low number of different molecular compositions. Second, a distinct microbial community taken from the coastal North Sea is capable of producing DOM, which accounts for the dissimilarity patterns observed in the global ocean. Furthermore, they seem to transform initially heterogeneous DOM, which is during its production dependent on phytoplankton phylogeny (Becker et al., 2014), into a more homogenous composition detectable everywhere in the global ocean. The mesocosm experiment performed by Osterholz et al. (2015) showed that under laboratory conditions even a distinct bacterial community already possesses the metabolic capacity of producing DOM that is as diverse as open ocean DOM. This requires to some extent an independence between the producing community and the molecular DOM composition as indicated by several studies before (Landa et al., 2014; Mou et al., 2008; Sarmento and Gasol, 2012), a concept that our model suggests is also correct on a global scale.

The known contributions of fresh DOM to our modeled fingerprints and the knowledge of the optimum fit for every DOM fingerprint in the Atlantic and Southern Ocean allowed us for the first time to calculate the amount of the semi-labile and semi-refractory DOM fractions based on the molecular DOM composition. So far, reactivity fractions were determined based on concentration and physical properties of the water column (Figure 3.7 A; Hansell, 2013) The concentration of the semi-persistent DOM fractions (i.e. semi-labile and semi-refractory) corresponded to the amount of fresh DOM in the optimum model fit and can therefore be expressed as percentage of SPE-DOC for each sample. For example, the concentration of the semi-resistant fractions for a sample from the surface mixed layer with a SPE-DOC concentration of 40 µmol l^{-1} and an optimum model fit at 20 % fresh DOM would be 40 µmol l^{-1} * 0.2 = 8 µmol l^{-1}. This calculation yields concentrations of the semi-persistent fractions of 6 -12 µmol l^{-1} for the biological active surface layer and 0 – 4 µmol l^{-1} for the deep sea and the Southern Ocean (Figure 3.7 B). Consequently, the concentration of SPE-DOC, which is on the molecular level indistinguishable from deep sea DOM, is 23 – 40 µmol l^{-1} in the surface mixed layer. Whether it belongs to the truly refractory DOM pool, which persists in the ocean over the timescales of millennia or whether it is part of a molecularly identical fresher and therefore younger DOM pool, remains open to debate. It has been hypothesized that the long-term stability of DOM is independent from its intrinsic molecular structure and is rather driven by concentration (Arrieta et al., 2015). The results of our study show that a large fraction in excess of deep sea DOM concentrations has indeed an identical molecular composition as deep sea DOM on the compound level. This supports the dilution hypothesis of Arrieta et al. (2015). Additionally, Follett et al. (2014) showed that refractory DOM (radiocarbon depleted) co-cycles with fresh (radiocarbon enriched) DOM in the surface ocean, providing further evidence that bioavailability of DOM is not a matter of age or composition but rather the concentration in which compounds are present in the water column.

Hansman et al. (2015) showed that the molecular DOM composition in the deep North Atlantic is the result of conservative water mass mixing emphasizing the importance of this process on the molecular DOM composition. Our results revealed that one of the major drivers shaping DOM compositions in the Atlantic and Southern is the simple mixing of two molecularly distinct endmembers. This indicates

that the observed DOM molecular patterns in the ocean are not just the result of molecular aging, which is a very slow process taking place on the timescales of thousands of years, but is also the result of mixing waters. Especially the deepening of the thermocline in winter enables export of surface signatures into the mesopelagic, explaining the observed patterns in subsurface water masses of the Atlantic.

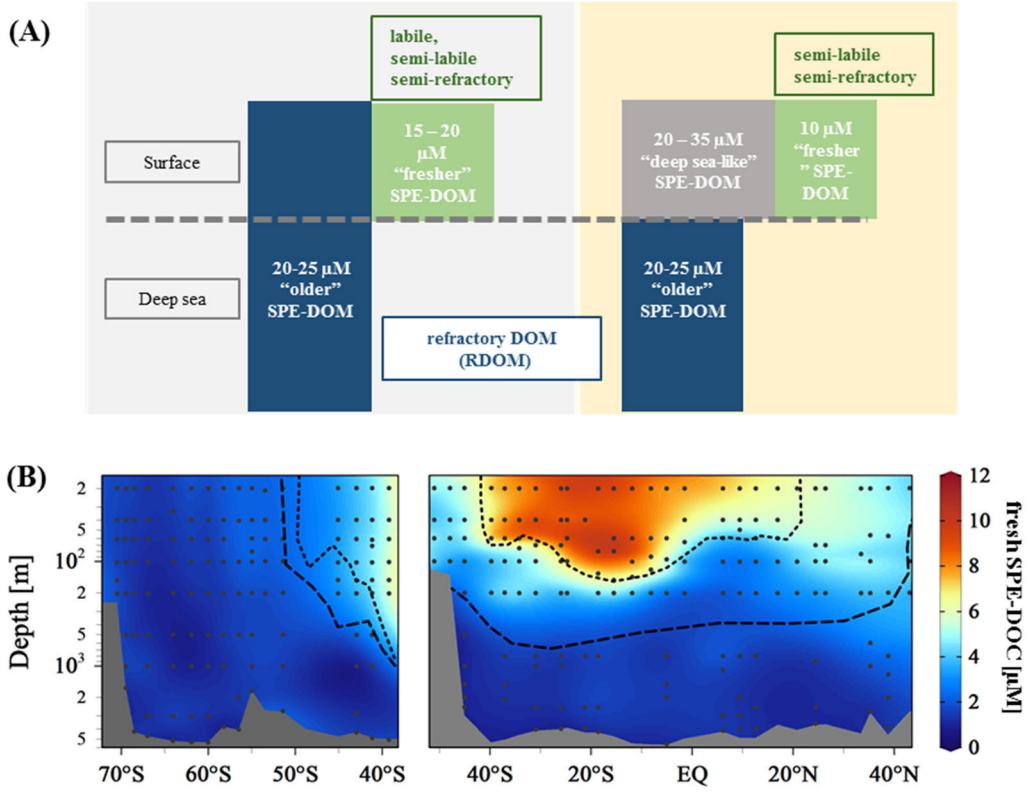

Figure 3.7 Conceptual illustration of the concentration of the DOM reactivity fractions determined via concentration (gray box, Hansell, 2013) and molecular composition (yellow box, this study) **(A)**. Concentration of fresh SPE-DOC along transects ANTXXVIII/2 (left) and ANTXXVIII/5 (right) determined via the two-source mixing model **(B)**. For calculation see text.

3.6 Conclusion

With this study, we traced the signal of microbial production in the global ocean and showed that the molecular geography of the Atlantic and Southern Ocean is indeed explainable by the simple mixing of two distinct molecular endmembers. We could follow the signal of fresh DOM into the abyssal ocean and conclude that

water mass mixing is probably one of the major processes facilitating the propagation of this signal. Furthermore, we are able to show that microbial production is by far the most important process shaping the molecular DOM composition in the open ocean and for the first time it is now possible to quantify the contribution of these semi-labile and semi-refractory fractions based on their molecular composition. These results offer new insights into the cycling the semi-persistent DOM fractions.

The chosen evaluation method of molecular dissimilarities (i.e. the Bray-Curtis dissimilarity) in this study provides a measure of the quantity of molecular differences between samples, but yields only limited information on the quality of the observed dissimilarities. Structural information of mesocosm and NEqPIW DOM revealed that organic acids prevail in both samples, although the overall molecular composition is different for fresh and truly refractory DOM (Osterholz et al., 2015). Behind each molecular formula detected in the marine DOM pool as many as 100,000 different isomers may be hidden, each present in picomolar concentrations (Zark et al., 2017), emphasizing the structural diversity of marine DOM. Elucidating the structural composition is a key component for understanding the cycling of DOM on the molecular level and ought to be considered in future studies.

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MANUSCRIPT III

Molecular geography of the Atlantic and Southern Ocean: Distribution patterns of organically bound dissolved nitrogen and biogeochemical implications

Maren Seibt[1], Thorsten Dittmar[1] and Jutta Niggemann[1]

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

Nitrogen is a key nutrient in marine ecosystems. The availability of nutrients in the euphotic water layer often limits the activity and the abundance of primary producers. Nitrogen is present in the ocean in inorganic and organic forms, both serving as energy source to microbes. In this chapter, the distribution and molecular composition of solid-phase extractable dissolved organic nitrogen (SPE-DON) of the Atlantic and Southern Ocean is reported and the associated biogeochemical implications explored.

All authors designed the study. Field work, sample preparation, ultrahigh resolution mass spectrometry analysis and data analysis was done by M. Seibt with input from all authors. M. Seibt wrote the manuscript with significant input from T. Dittmar and J. Niggemann.

The manuscript is prepared for submission to *Marine Chemistry* and presented with adjusted formatting according to the style of this thesis.

4.1 Abstract

Dissolved organic matter (DOM) contains nitrogen, sulfur and phosphorus in varying amounts. These elements influence the reactivity of organic molecules and are essential for sustaining microbial life. Thus, knowing their concentration, availability and global distribution is key to understanding global carbon and element cycles. In this study, we present detailed molecular-level information on DOM composition obtained via Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) from the Atlantic and Southern Ocean with special emphasis on the nitrogen containing DOM molecules. The distribution pattern of solid-phase extractable dissolved organic nitrogen (SPE-DON) showed unique features that can be related to biological activity and availability of inorganic nutrients. Significant negative correlation between the concentration of SPE-DON and inorganic nitrogen species (Pearson's $r = -0.77$, $p < 0.05$) indicated a close coupling and constant exchange between the two dissolved nitrogen pools. Based on a comprehensive data set of 383 DOM samples, with more than 4500 identified molecular formulae of which almost 2000 contained nitrogen, we revealed distinct SPE-DON distribution features. In the surface mixed layer of the Atlantic Ocean, two contrasting regimes were distinguished: The C/N ratio of SPE-DOM in the South Atlantic Gyre was significantly higher than in the North Atlantic Gyre, although inorganic nitrogen was limited across all Atlantic surface waters. We postulate that the lower C/N ratio of SPE-DOM in the North Atlantic Gyre is due to the occurrence of nitrogen-fixing cyanobacteria, which channel newly fixed nitrogen into the SPE-DON pool. In contrast, in the South Atlantic Gyre, the lack of inorganic nutrients leads to the more efficient use of organically bound nitrogen and consequently also to the production of N-depleted DOM, manifested in higher C/N ratios of the SPE-DOM pool. In the Southern Ocean, yet another regime can be defined: Inorganic nitrogen is not limited, therefore, organically bound nitrogen is less demanded as nutrient source and N-rich DOM is released, as reflected in lower C/N ratios in the Southern Ocean SPE-DOM. We show that global SPE-DON distribution patterns deviate from the bulk SPE-DOM distribution and that SPE-DON concentration and availability strongly depend on the prevailing nutrient regime and the capabilities of the prevailing microbial communities.

4.2 Introduction

DOM is one of the largest active pools of organic carbon on earth, but the mechanisms controlling its molecular composition and reasons for its millennial stability are still poorly understood (Dittmar, 2015; Ogawa and Tanoue, 2003). Photosynthesis performed by autotrophic organisms in the euphotic layer of the global ocean is the main source of marine DOM. Major constituents of marine DOM – as for any natural organic matter – are the elements carbon, hydrogen and oxygen, but heteroelements such as nitrogen, sulphur and phosphorous also comprise large proportions of marine DOM and form the pools of dissolved organic nitrogen (DON), phosphorous (DOP) and sulphur (DOS). DOM serves as the main carbon and nutrient source to heterotrophic microorganisms, thereby returning the energy gained via primary production to the marine food web (microbial loop; Azam et al., 1983; Pomeroy, 1974).

Nitrogen is a key nutrient for all organisms. The Redfield ratio (106C:16N:1P; Redfield, 1934) is commonly used as an indicator for the prevailing elemental stoichiometry within marine organisms as well as their nutritious needs (e.g. Klausmeier et al., 2004; Lenton and Watson, 2000; Sanudo-Wilhelmy et al., 2004). Inorganic nitrogen is often the primary limiting nutrient in the ocean (Falkowski, 1997), although low concentrations of more than one nutrient can co-limit phytoplankton growth. This is most commonly observed in oligotrophic oceanic regions (Arrigo, 2005). The concentration of biologically available forms of inorganic nitrogen (nitrate, nitrite and ammonium) is very low in the surface waters of the oligotrophic open ocean gyres (e.g. < 1 µmol l^{-1} of nitrate; Garcia et al., 2014). Major source of nitrate to the ocean surface layer is upwelling and diffusion of nitrate-rich deep water, where concentrations can reach > 20 µmol l^{-1} (Garcia et al., 2014). In upwelling regions, also found in the Southern Ocean, inorganic nitrogen concentrations are high throughout the whole water column and primary production is rather limited by the availability of iron (high nutrient, low chlorophyll regions; Martin, 1992). Sources of new nitrogen to the ocean are atmospheric deposition, microbial nitrogen fixation and terrestrial runoff. Major sinks for inorganic nitrogen in the

ocean are the microbially mediated nitrification, denitrification and anaerobic oxidation of ammonium (anammox) and to a lesser degree burial in the ocean sediments.

DON compounds vary greatly in size, complexity, turnover time and concentration (Benner et al., 1997; Letscher et al., 2013). Bulk DON concentrations at the surface and in the deep ocean range from 0.8 to 13 μmol l^{-1} and from 2 to 5 μmol l^{-1}, respectively (Bronk, 2002), with even higher concentrations in coastal areas. In the surface ocean, most of the dissolved nitrogen belongs to the DON pool (about 60 % of the total dissolved nitrogen (TDN); Bronk, 2002). In the deep sea, DON represents only about 10 % of the TDN pool, which is mostly composed of nitrate (Bronk, 2002). The most transient forms of DON are urea and individual free amino acids, which are readily taken up by bacteria and some primary producers (e.g. Amon et al., 2001; Eppley et al., 1977). More stable forms of the DON pool include amide N and have been proposed to be derived from degradation-resistant biomolecules (McCarthy et al., 1997) like bacterial cell wall components (McCarthy et al., 1998) or other biosynthetically derived substances (Aluwihare et al., 1997). Major sources of DON in the ocean are active and passive excretion by bacterio- and phytoplankton (Bronk and Ward, 1999; Ogawa et al., 2001), sloppy feeding (Ward and Bronk, 2001) and viral cell lysis (Fuhrman and Noble, 1995). Major sinks for DON in the ocean are bacterial and phytoplankton uptake (Bronk et al., 1994), photochemical degradation in the surface ocean (Bushaw-Newton and Moran, 1999) and the decomposition of organic matter and the mineralization of organically bound nitrogen to ammonium (Berman et al., 1999). About 41 % of the inorganic nitrogen (nitrate, ammonium) taken up by primary producers are released as DON (Bronk et al., 1994). Since most of the surface ocean is limited in biologically available nitrogen, DON plays an important role in global nutrient cycles.

A study from the ultra-oligotrophic South Pacific gyre identified a so far unknown heterotrophic diazotrophic community fixing atmospheric N_2, emphasizing the important role oligotrophic gyres play in the global nitrogen cycle (Halm et al., 2012). Due to the relatively stable concentration of DON in the ocean it was assumed that most DON molecules are resistant to microbial oxidation forming a refractory fraction of the total DOM pool (Bronk, 2002). Recent studies however suggest that

DON in the ocean is highly dynamic with well-balanced production and consumption rates (Bronk et al., 2007). Most studies target the concentration of bulk DON, providing no further information about the molecular composition of the DON pool. Various studies provide information about the most labile fractions of DON comprised of amino acids and amino sugars (e.g. McCarthy et al., 1997). However, knowledge on the biogeochemistry of other DON fractions is scarce. It is crucial to understand the DON molecular composition, because it directly influences its reactivity in biogeochemical processes. Applying ultra-high resolution mass spectrometry (FT-ICR-MS) provides detailed information on the molecular composition of a so far understudied fraction of DON.

In this study, we compiled a most comprehensive data set of 383 DOM samples from the Atlantic and Southern Ocean with samples spanning from the ocean surface to the deep sea and from various biogeographical regimes representing various environmental conditions and biological influencing factors. We present patterns of SPE-DON concentration, distribution and molecular composition in the Atlantic and Southern Ocean, providing the first detailed characterization of the molecular composition and distribution of DON in two major global oceans. We hypothesize that the prevailing microbial community influences the composition of SPE-DON on a molecular level. We further hypothesize that the SPE-DON composition in the North Atlantic Gyre differs significantly from the SPE-DON composition in the South Atlantic Gyre, due to differences in nutrient regimes and microbial community structure.

4.3 Material and methods

DOM samples were taken in the Atlantic and Southern Ocean during three RV Polarstern cruises (Figure 4.1): ANT-XXVIII/2 (Atlantic sector of the Southern Ocean; 39.2° S to 70.5° S), ANT-XXVIII/4 (Drake Passage and Antarctic Peninsula; 56.1° S to 62.4°S) and ANT-XXVIII/5 (Atlantic; 51° S to 47° N) in austral spring and summer (Dec 2011 – May 2012). All samples were directly filtered from the Niskin bottles of the CTD using pre-combusted (400 °C, 4h) 0.7 µm glass fibre filters (GF/F, Whatman, United Kingdom). Samples were taken in high depth resolution in the upper 200 m of the water column (20 m, 40 m, 60 m, 100 m, 200 m

and in the chlorophyll fluorescence maximum, if present). Samples from greater depths cover all major water masses (Figure 4.2).

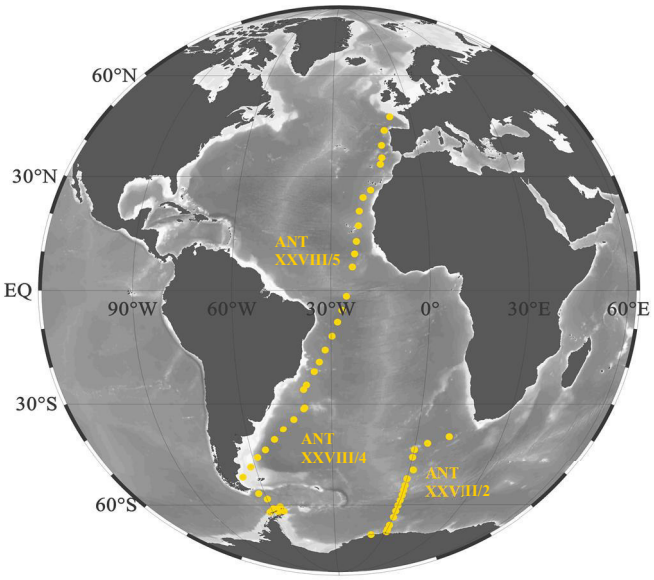

Figure 4.1 Sampling transects in the Atlantic and Southern Ocean (ANTXXVIII/2 from Cape Town/South Africa to Antarctica; ANTXXVIII/4 from Punta Arenas/Chile to the Antarctic Peninsula; ANTXXVIII/5 from Punta Arenas/Chile to Bremerhaven/Germany)

Samples for DOM analysis were extracted with a solid-phase extraction (SPE) method according to Dittmar et al. (2008). In brief, 4 l of filtered seawater were acidified to pH 2 (HCl, 25%, p.a., Carl Roth, Germany) and extracted via gravity flow using commercially pre-packed cartridges (1 g of sorbent, PPL, Agilent, USA). All cartridges were deionized with acidic ultrapure water (pH 2), dried with nitrogen gas and eluted with 6 ml methanol (HPLC-grade, Sigma-Aldrich, USA). Extracts were stored in the dark at -20°C until further analysis in the home laboratory.

SPE-DOC and SPE-DON concentrations were determined via the high temperature catalytic oxidation method (HTCO) on a Shimadzu TOC-VCPH/CPN Total Organic Carbon Analyzer equipped with an ASI-V autosampler and a TNM-1 module. Prior to analysis, the acidified samples were purged with synthetic air to remove inorganic carbon compounds. For DOC and TDN analysis of the DOM extracts, a SPE-DOM aliquot of 100 µl and 1 ml, respectively, was dried and re-dissolved in

10 ml of ultrapure water (pH 2; HCl, 25%, p.a., Carl Roth, Germany). For calibration, L-arginine solutions ranging from 12.5 to 500 µmol C l^{-1} and 10 to 333.3 µmol N l^{-1}, respectively, were used for calibration and Deep Atlantic Seawater reference material (DSR, D.A. Hansell, University of Miami, Florida, USA) as well as an in-house North Sea water reference were measured repeatedly during each run to control for instrument precision and accuracy. The resins used for DOM extraction only retain organic compounds; therefore, all nitrogen in the DOM extracts is organic and referred to as SPE-DON.

The carbon-based extraction efficiency was 53 ± 9 % for all Atlantic and Southern Ocean samples. The calculation of extraction efficiencies for DON is challenging, because DON cannot be directly measured, but is calculated by subtracting the inorganic nitrogen species from the total dissolved nitrogen (TDN). Because we do not have a complete data set of the inorganic nitrogen species covering our sampling transect, calculation of the bulk DON concentration is not possible. However, extraction efficiencies based on TDN and SPE-DON range between 2 – 24 %, with the lowest extraction efficiencies in the Southern Ocean and the Atlantic deep sea (highest dissolved inorganic nitrogen concentration). Broek et al. (2017) reported extraction efficiencies for DON between 30 – 39 % using the same extraction method as we did. Based on this published extraction efficiency and on reported DON concentration in the open ocean (Bronk, 2002), we assume that our DON extraction efficiencies were in the same range.

All DOM extracts were analyzed on a Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FT-ICR-MS, Bruker Daltonik GmbH, Bremen, Germany) equipped with a 15 Tesla superconducting magnet (Bruker Biospin, Wissembourg, France) and an electrospray ionization source (ESI; Apollo II ion source, Bruker Daltonik GmbH, Bremen, Germany). For analysis, all DOM extracts were mixed with ultrapure water and methanol (MS grade) to a final carbon concentration of 10 ppm and a water to methanol ratio of 1:1 (v/v). For analysis validation, an in-house reference sample (North Equatorial Pacific Intermediate Water (NEqPIW); Bostock et al., 2010), collected at the Natural Energy Laboratory of Hawaii Authority in 2009 (Green et al., 2014), was analyzed twice a day.

The samples were injected at a flow rate of 120 µl h^{-1} applying negative ion mode with a capillary voltage of 4000 V. Ion accumulation time in the hexapole was 0.25 s prior to transfer into the ICR cell. Mass spectra were recorded in a mass range of 152 - 2000 Da with 500 acquired scans per sample. All mass spectra were internally calibrated using a list of > 50 known molecular formula mass peaks from the in-house reference sample. All detected ions were singly charged and the mass error allowed for the internal calibration was less than 0.1 ppm. The overall intensity distribution was in the same order of magnitude for all samples. The mass to charge ratio, resolution and peak intensity were exported and processed using in-house Matlab routines. Molecular formulae were assigned to peaks with a minimum signal-to-noise ratio of 4 following the rules published by Koch et al. (2007) with following restrictions: $^{12}C_{1\text{-}130}{}^{1}H_{1\text{-}200}O_{1\text{-}50}{}^{14}N_{0\text{-}4}S_{0\text{-}2}P_{0\text{-}2}$. Only compounds with an assigned molecular formula were considered for further evaluation (~ 63 % of total peaks). These restrictions resulted in a data set comprising a total of 4695 molecular formulae of which 1925 contained one or more nitrogen atoms. All mass spectrometric data were normalized by dividing each peak intensity by the sum of all peak intensities with assigned molecular formulae of the respective sample.
Compositional differences on the molecular level were assessed by intensity weighted averages of the respective molecular feature for each sample. For example, the average number of nitrogen atoms per compound in each sample was calculated by multiplying the number of nitrogen atoms per compound with the respective peak intensity (intensity weighting), summing up the intensity weighted number of nitrogen atoms for the whole sample and divide it by the total sum of intensity of the respective sample (for an exemplary calculation refer to Table S4.1).

Following this routine for all molecular parameters gives valuable information about the overall molecular composition of a DOM sample. The statistical significance of differences in DOM concentration and composition for each water mass is shown in boxplots. All statistical analysis of DOM data was done with the software "R" (Version 3.2.0, package "vegan"; Oksanen et al., 2015).

4.4 Results

Water masses, SPE-DOC and SPE-DON – According to the sampling location, depth and hydrographic conditions (i.e. salinity and temperature) our samples were

taken within the following global water masses (Tomczak and Godfrey, 2001; numbers in brackets correspond to the numbers of samples per water mass): Antarctic Surface Water (AASW, n = 111), Circumpolar Deep Water (CDW, n = 26), Weddell Sea Warm Deep Water (WDW, n = 19), Weddell Sea Deep Water (WSDW, n = 8), Antarctic Bottom Water (AABW, n = 23), North Atlantic Deep Water (NADW, n = 22), Antarctic Intermediate Water (AAIW, n = 19), Eurafrican Mediterranean Water (EMW, n = 4), Subantarctic Surface Water (SASW, n = 35), Subtropical Surface Water (STSW, n = 11), South Atlantic Central Water (SACW, n = 30), North Atlantic Central Water (NACW, n = 23) and the surface mixed layer of the Atlantic (SML), subdivided into surface mixed layer of the South Atlantic Gyre (SML-SAG, n = 28) and the surface mixed layer of the North Atlantic Gyre (SML-NAG, n = 19; Figure 4.2 A).

SPE-DOC concentrations were generally low (~25 µmol l^{-1}) in the Southern Ocean and in the deep waters of the Atlantic Ocean (AASW; CDW, WDW, WSDW, AABW, NADW, AAIW, EMW). Intermediate concentrations (27 – 35 µmol l^{-1}) were found in the subsurface water masses of the Atlantic (SACW and NACW) and the surface water masses between the subtropical front and the polar front (STSW and SASW). The higher concentrations in these water masses are consistent with higher biological production at frontal zones (STSW and SASW; Laubscher et al., 1993) and downwelling and the associated export of carbon into the mesopelagic zone within the gyres (SACW and NACW; Carlson et al., 2010). Highest SPE-DOC concentrations (30 – 45 µmol l^{-1}) were found in the surface mixed layer of the Atlantic with significantly higher SPE-DOC concentrations in the SML-SAG than in the SML-NAG. The distribution of SPE-DOC follows the general DOC distribution patterns (Hansell et al., 2009), indicating that SPE-DOC comprises a representative and reproducible fraction of the total DOC pool (Figure 4.2 B).

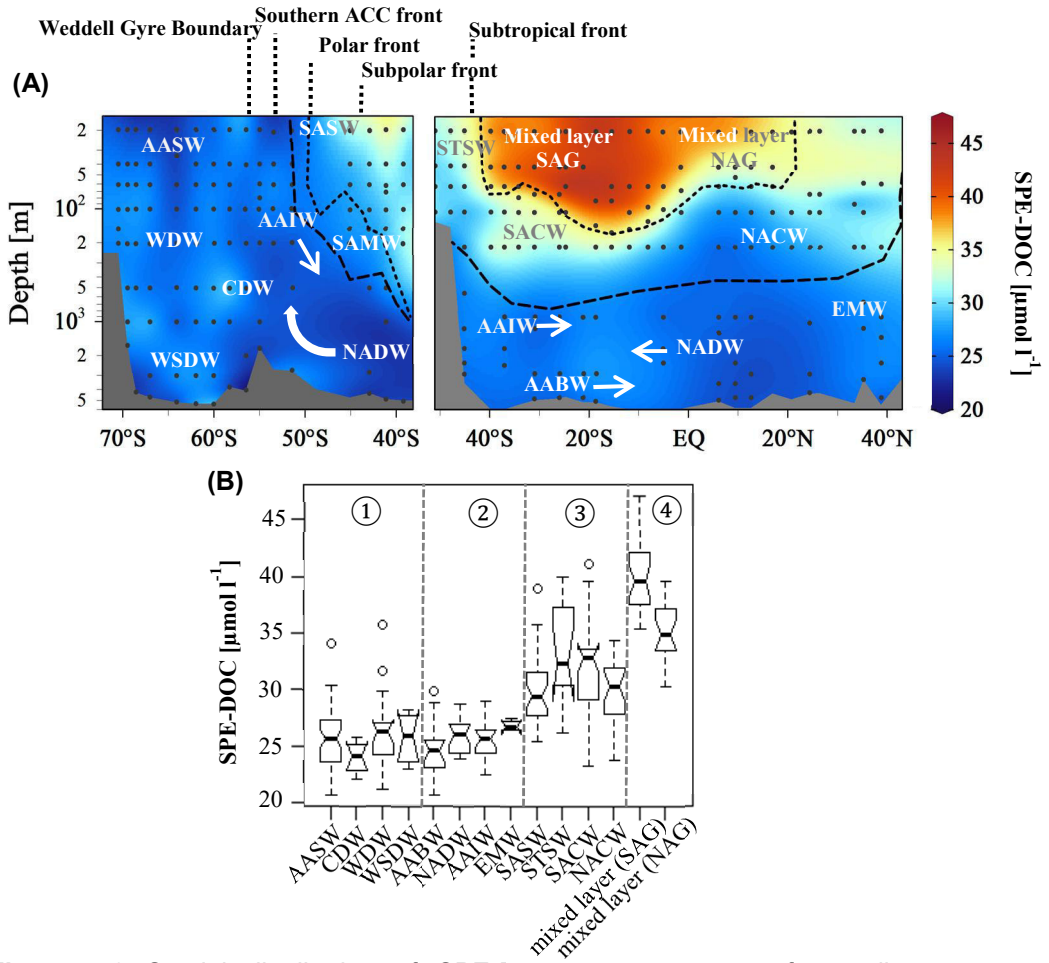

Figure 4.2 Spatial distribution of SPE-DOC concentrations of sampling transects ANTXXVIII/2 (left) and ANTXXVIII/5 (right) with major water masses and frontal systems **(A)** and boxplots of SPE-DOC concentration for the respective water masses **(B)**. Data of ANTXXVIII/4 corresponds well to data of ANTXXVIII/2 and are not shown, but included in boxplots. For number of samples and abbreviations of water masses refer to text. Arrows indicate the flow direction of the respective water mass. Black dots in (A) correspond to sampling depths and dashed lines indicate the upper and lower boundary of the pycnocline. The dashed lines in (B) group the water masses per sampling location and depth: group 1 includes all Southern Ocean water masses, group 2 includes the deep Atlantic water masses, group 3 includes the surface water masses of the South Atlantic between subtropical and polar front (STSW, SASW) and the subsurface water masses within the pycnocline (SACW, NACW) and group 4 includes the Atlantic surface mixed layer in both gyres. Notches in boxplots roughly indicate the 95 % confidence interval. If the notches do not overlap it is strong evidence that the medians differ significantly (McGill et al., 1978). All cross-sections are plotted with Ocean Data View (Schlitzer, 2015).

SPE-DON concentrations showed similar distribution patterns as SPE-DOC (Figure 4.3 A and B): Low and uniformly distributed concentrations in the Southern Ocean and the deep Atlantic Ocean (~0.9 µmol l^{-1}), intermediate concentrations (0.9 – 1.3 µmol l^{-1}) in the surface water masses between the subtropical front and the polar front and highest concentrations (1.2 – 1.4 µmol l^{-1}) in the surface mixed

layer. These trends follow the distribution patterns of the bulk DON pool (Bronk, 2002), indicating that SPE-DON comprises a reproducible representative fraction of the total DON pool. SPE-DON concentrations were negatively correlated with concentrations of inorganic nitrogen species (Pearson's r = -0.77, $p < 0.05$, data not shown), indicating a close coupling between both pools.

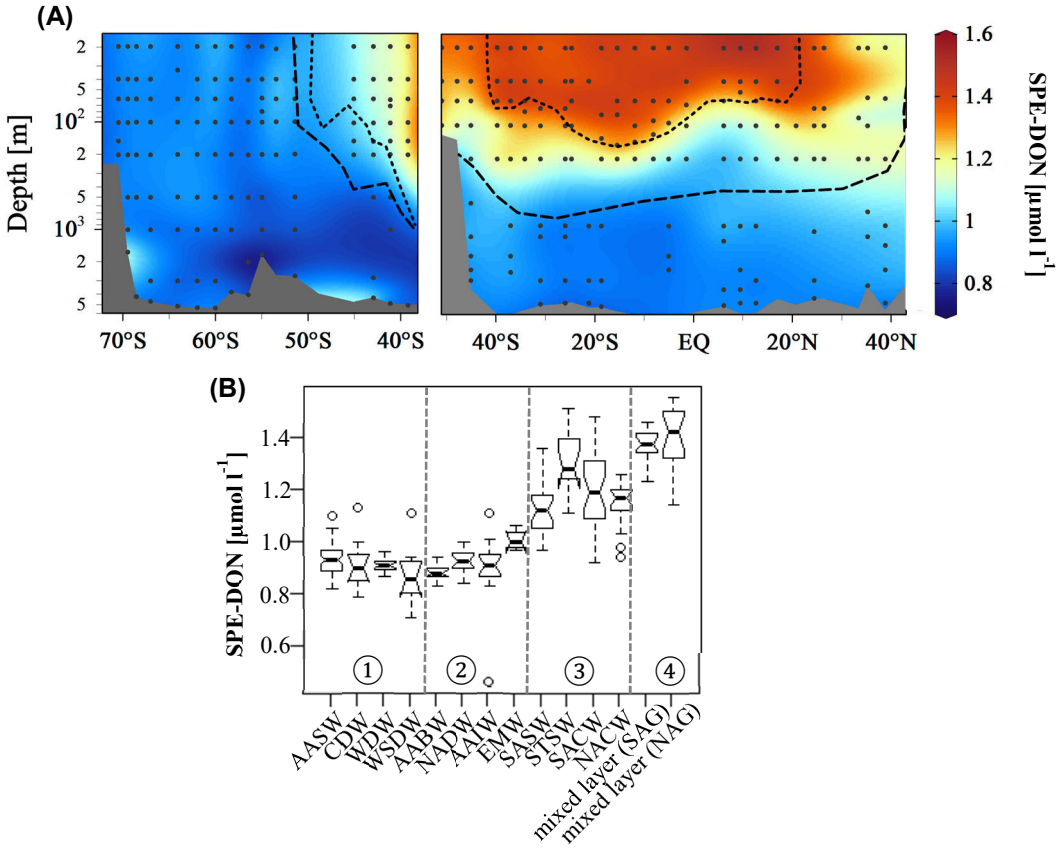

Figure 4.3 Spatial distribution of SPE-DON concentrations of sampling transects ANTXXVIII/2 (left) and ANTXXVIII/5 (right) **(A)** and boxplots of SPE-DON concentration for the respective water masses **(B)**. For water mass grouping and abbreviations of water masses in (B) refer to Figure 4.2 and text, respectively.

The SPE-DOC/SPE-DON (C/N_{SPE}) ratio revealed distinct patterns in the Atlantic and Southern Ocean. All C/N_{SPE} ratios were between 23 and 30, and therefore in the same range as previously reported C/N_{SPE} ratios of DOM extracted with the same method (Broek et al., 2017; Green et al., 2014). The C/N_{SPE} ratio showed distinct features in the surface and subsurface water masses and the mixed layer of the Atlantic: SPE-DON was similarly enriched in both, the South and the North Atlantic Gyre. SPE-DOC, however, was more enriched in the SAG compared to the

NAG, which resulted in a lower C/N_{SPE} ratio in the North Atlantic Gyre and a higher C/N_{SPE} ratio in the South Atlantic Gyre (Figure 4.4 A and B).

The C/N_{SPE} ratio is generally higher than DOC/DON (C/N_{bulk}) of marine DOM (Broek et al., 2017; Dittmar et al., 2008; Green et al., 2014). Dissolved organic leftovers in the ocean are significantly enriched in carbon relative to nitrogen with C/N_{bulk} ratios significantly higher than the Redfield ratio, ranging between 13 – 16 (Aminot and Kérouel, 2004) for the surface ocean and up to 22 in the deep sea (Hopkinson and Vallino, 2005). Although the higher C/N_{SPE} ratios indicate that the chosen extraction method (i.e. solid-phase extraction) discriminates against N-containing compounds, the general bulk SPE-DOC and SPE-DON trends show that the extracted fraction is representative for bulk DOM.

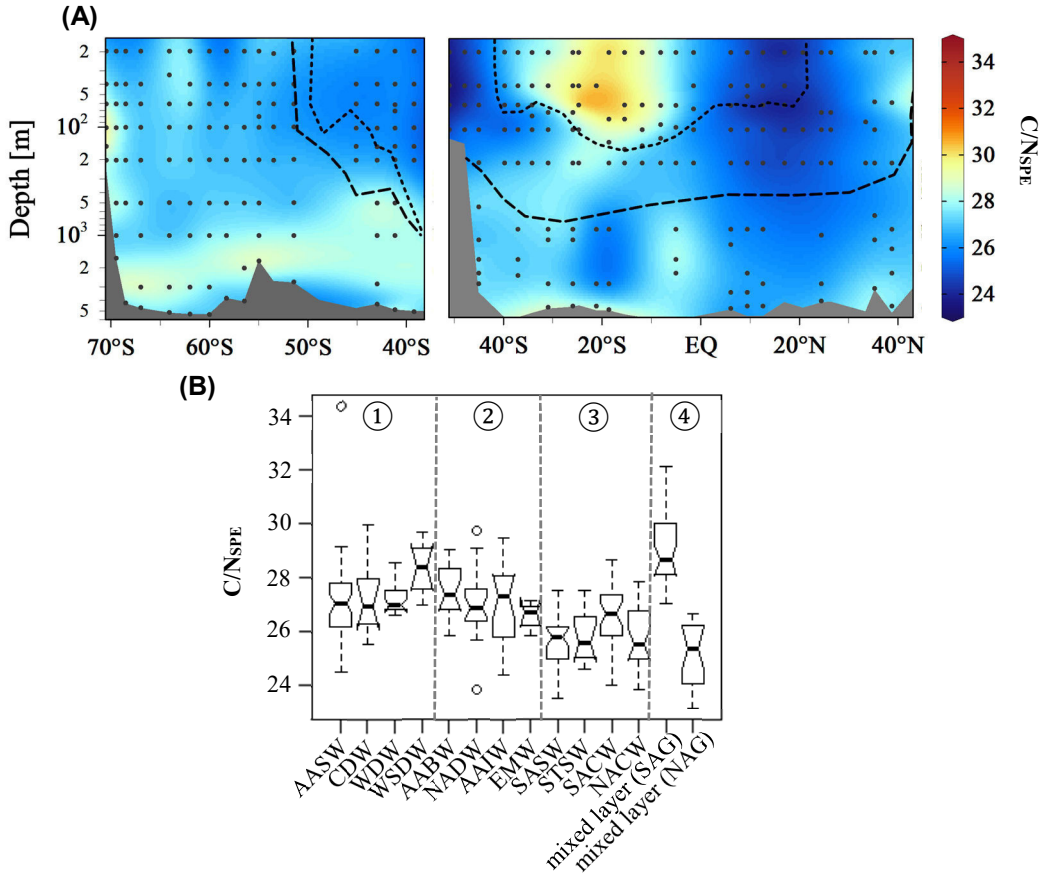

Figure 4.4 Spatial distribution of C/N_{SPE} ratios of sampling transects ANTXXVIII/2 (left) and ANTXXVIII/5 (right) **(A)** and boxplots of C/N_{SPE} ratios for the respective water masses **(B)**. For water mass grouping and abbreviations of water masses in (B) refer to Figure 4.2 and text, respectively.

Molecular composition – The average weight of DOM compounds was generally lower (415 ± 4 Da) in the surface and subsurface water masses of the Atlantic (SASW, STSW, SACW, NACW and SML) and higher in the deep sea and the Southern Ocean (422 ± 3 Da; Table 4.1), a feature also observed by Chen et al. (2014) and Flerus et al. (2012). The lower average molecular weight went along with a lower average number of carbon atoms per compound in the individual samples (Pearson's r = 0.9, p < 0.05; Figure 4.5 A and B).

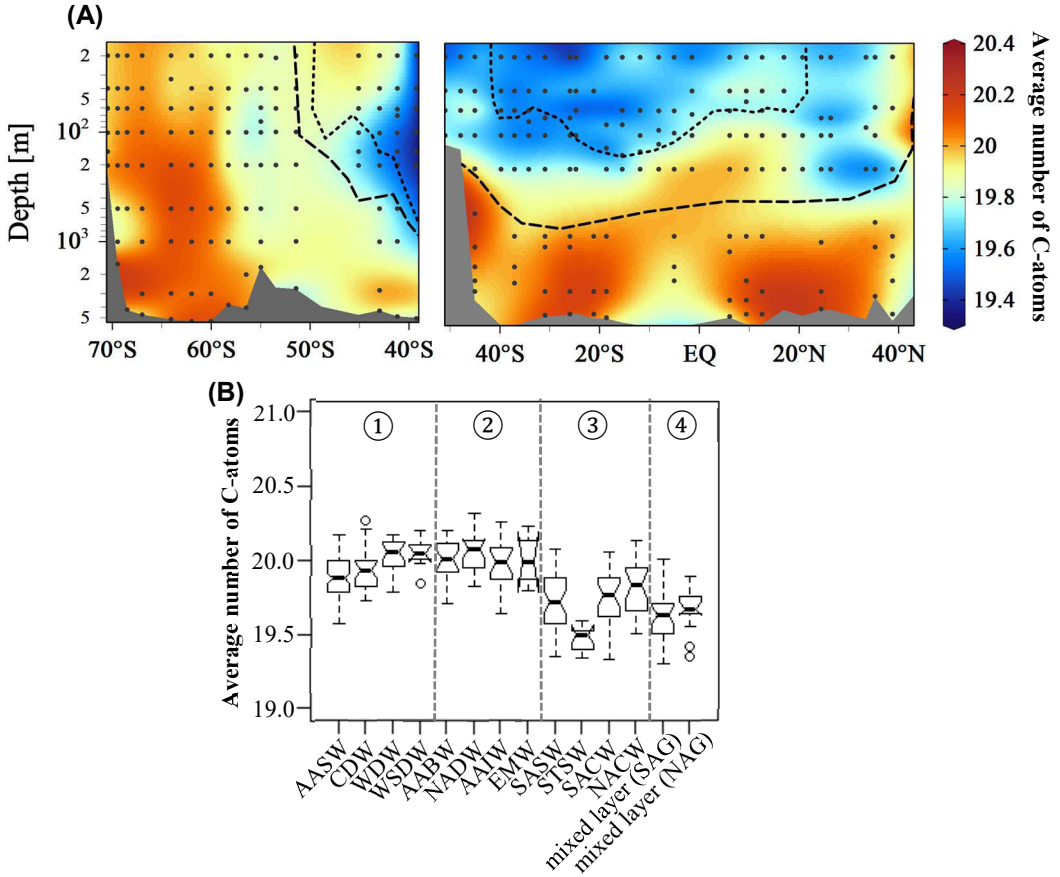

Figure 4.5 Spatial distribution of average number of carbon atoms per DOM compound in each sample of sampling transects ANTXXVIII/2 (left) and ANTXXVIII/5 (right) **(A)** and boxplots of average number of carbon atoms per DOM compound in each sample for the respective water masses **(B)**. For water mass grouping and abbreviations of water masses in (B) refer to Figure 4.2 and text, respectively.

Because of the lower average weight of DOM molecules in the surface water masses, the average number of nitrogen atoms per DOM compound is also lower (Figure 4.6 A and B) The correlation between the average weight and the average number of nitrogen per compound is, however, much weaker (Pearson's r = 0.43,

$p < 0.05$) than the correlation between weight and carbon atoms. The distinct distribution patterns of the average number of nitrogen atoms per DOM compound indicates that the distribution of DON is driven by independent processes (e.g. biological activity) and not simply a reflection of molecule size.

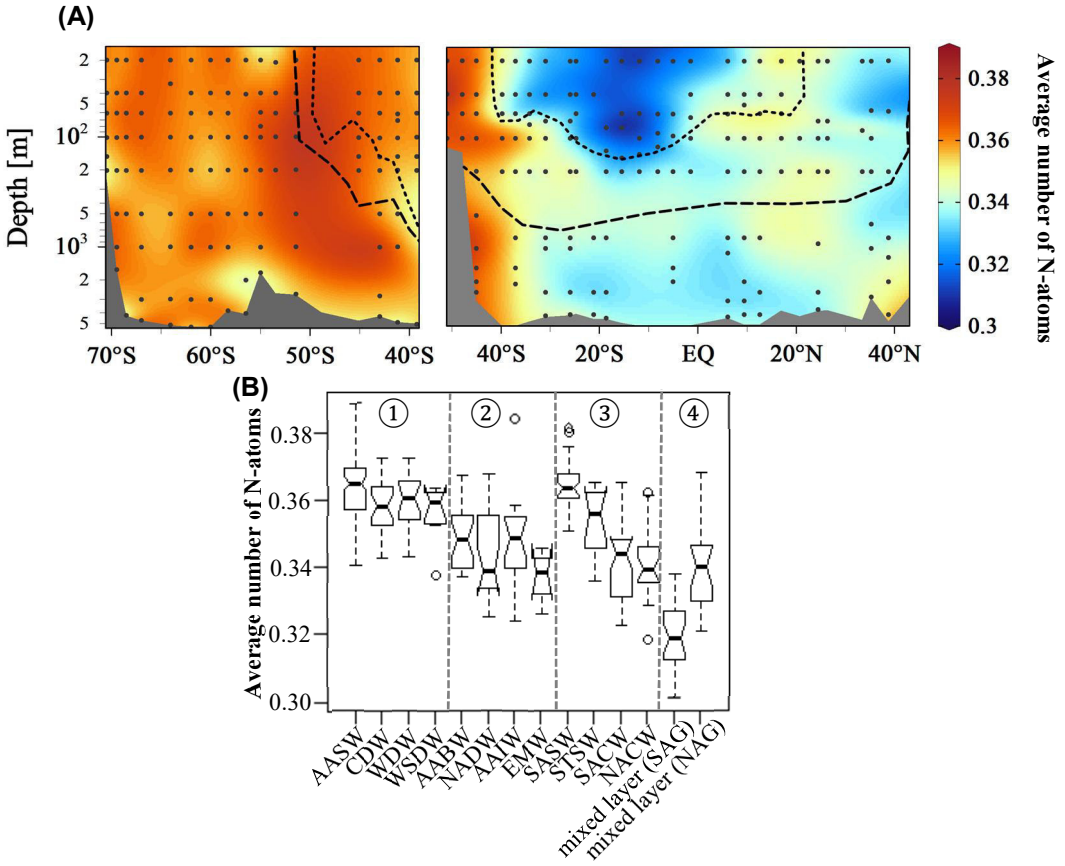

Figure 4.6 Spatial distribution of the average number of nitrogen atoms per DOM compound in each sample of sampling transects ANTXXVIII/2 (left) and ANTXXVIII/5 (right) **(A)** and boxplots of average number of nitrogen atoms per DOM compound in each sample for the respective water masses **(B)**. For water mass grouping and abbreviations of water masses in (B) refer to Figure 4.2 and text, respectively.

The ultrahigh resolution data of Atlantic and Southern Ocean molecular DOM composition showed distinct patterns for the molecular C/N ratios (C/N_{mol}; Figure 4.7 A ans B). In the water masses of the Southern Ocean (AASW, CDW, WDW, WSDW) C/N_{mol} ratios were low (N-rich) compared to the deep Atlantic Ocean (AABW, NADW, AAIW, EMW). The southern surface water masses of the Atlantic (SASW, STSW) had similar C/N_{mol} ratios as the Southern Ocean water masses, whereas the subsurface water masses of the Atlantic (SACW, NACW) were comparable to the Atlantic deep water masses. DOM in the SAG mixed layer had a high

C/N_{mol} ratio (N-depleted) compared to the NAG. The number of detected N-containing formulae was about the same for all samples as well as the C/N_{mol} ratio for the CHON molecular formulae, making it unlikely that the observed patterns of N-depletion were due to analytical artifacts, e.g. non-reproducible detection of low abundant compounds (Table 4.1).

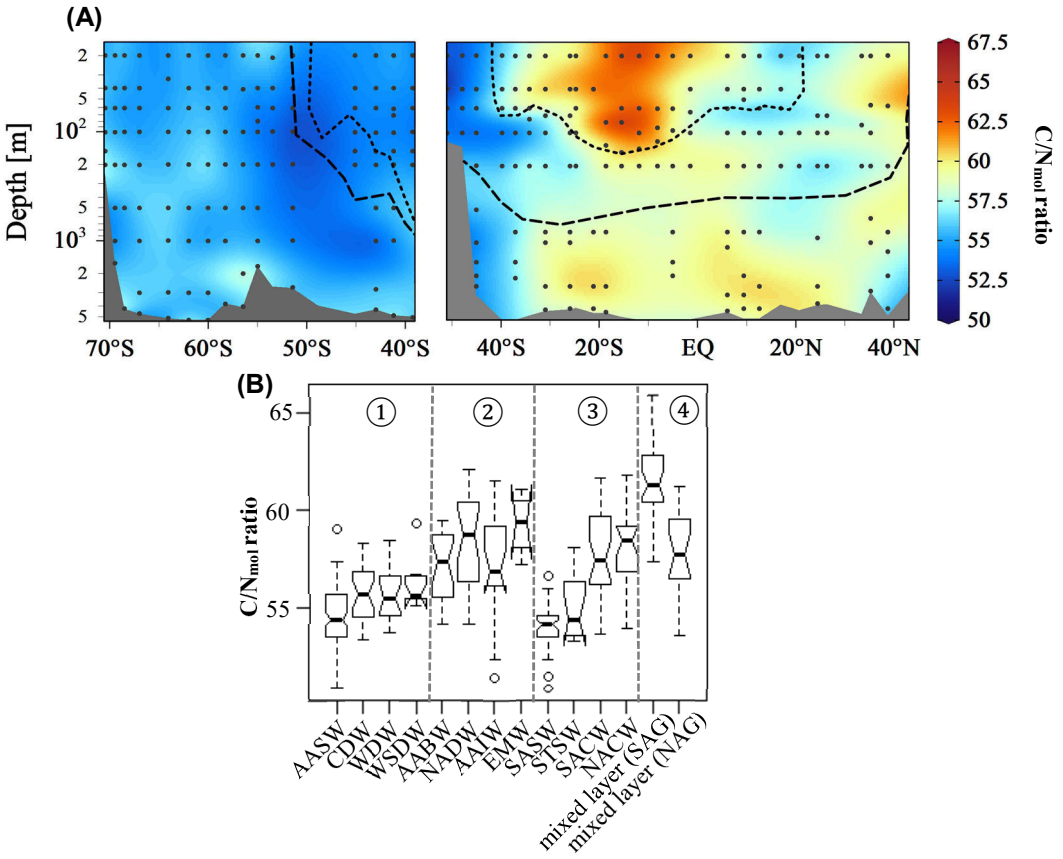

Figure 4.7 Spatial distribution of C/N_{mol} ratios of sampling transects ANTXXVIII/2 (left) and ANTXXVIII/5 (right) **(A)** and boxplots of C/N_{mol} ratios for the respective water masses **(B)**. For water mass grouping and abbreviations of water masses in (B) refer to Figure 4.2 and text, respectively.

In general, the bulk elemental composition (C/N_{SPE}) corresponded very well with the molecular data (C/N_{mol}). The ultrahigh resolution provided complementary information on elemental ratios, resolving the heterogeneity of the SPE-DON molecular composition in the Atlantic and Southern Ocean. Furthermore, the detailed molecular analysis allowed us to assign DOM compounds into specific compound groups. For that purpose, we followed the rules described in Romano et al. (2014). It is however noteworthy that molecular information obtained by FT-ICR-MS can represent millions of constitutional isomers (Hertkorn et al., 2007). Therefore, the

term “compound group” merely describes the possibility of a compound belonging to a specific group but this categorization is not unambiguous and alternative structures may exist for a given formula.

The by far largest DOM compound group was the group of highly unsaturated compounds, which were present in higher relative abundances in the Southern Ocean and the Atlantic deep sea and in lower relative abundances in the surface mixed layer. The opposite trend, albeit in much lower abundance, was visible for the unsaturated aliphatics, which were most abundant in the surface mixed layer (Table 4.1). The peptide-like molecular formulae had the highest abundance in the surface water masses of the Atlantic, most likely due to biological activity in the euphotic water layers. As evident by the classification of the CHON compounds, nitrogen was largely bound in polyphenolic and peptidic structures, besides the high abundance of highly unsaturated compounds. Differences between deep sea and surface waters were seen for peptide-like molecular formulae (more abundant at the surface) and highly unsaturated compounds (more abundant in the Atlantic deep sea and Southern Ocean). The group of CHO compounds (no heteroatoms), which was, based on number of detected compounds, as abundant as the CHON compounds, occupied more than two thirds of the total peak intensity. The affiliation to specific groups of the CHO compounds followed the same patterns as the total number of compounds with distinct differences between the Atlantic surface water masses and the Atlantic deep sea and Southern Ocean.

Table 4.1 Summary of number of assigned molecular formulae, sum of normalized peak intensity, average molecule weight (m/z), elemental composition and molecular compound groups for ultrahigh resolution data of total SPE-DOM (all) and the subgroups of SPE-DOM containing no hetero-elements (CHO) and nitrogen (CHON). Values are peak intensity weighted averages ± one standard deviation. ◊ and * correspond to deep Atlantic/Southern Ocean samples and subsurface and surface water masses of the Atlantic, respectively.

	all		**CHO**		**CHON**	
	◊	*	◊	*	◊	*
General						
Number of assigned molecular formulae	4040 ± 165	4040± 140	1848 ± 74	1859 ± 63	1737 ± 63	1716 ± 59
Sum of peak intensity	1000 ± 0	1000 ± 0	741 ± 8	746 ± 10	222 ± 7	216 ± 9
Average m/z of peaks	422 ± 3	415 ± 4	423 ± 3	416 ± 4	413 ± 3	408.13 ± 3
Elemental composition						
Average C	20.0 ± 0.2	19.7 ± 0.2	20.3 ± 0.1	20.0 ± 0.2	19.0 ± 0.2	18.7 ± 0.2
Average H	25.0 ± 0.2	25.0 ± 0.3	25.6 ± 0.2	25.5 ± 0.3	23.1 ± 0.2	23.1 ± 0.3
Average O	9.5 ± 0.1	9.3 ± 0.1	9.7 ± 0.1	9.5 ± 0.1	8.8 ± 0.1	8.7 ± 0.1
Average N	0.36 ± 0.01	0.34 ± 0.02			1.61 ± 0.01	1.59 ± 0.02
Average S	0.04 ± 0	0.04 ± 0				
Average P	0.01 ± 0	0.01 ± 0				
Average O/C	0.48 ± 0.01	0.47 ± 0.01	0.48 ± 0.01	0.47 ± 0.01	0.46 ± 0	0.46 ± 0
Average H/C	1.26 ± 0	1.27 ± 0.01	1.26 ± 0	1.28 ± 0.01	1.22 ± 0	1.23 ± 0.01
Average C/N_{mol}	55.8 ± 2.2	57.4 ± 3.1			11.8 ± 0.1	11.8 ± 0.2
Molecular categories [average % of total peak intensity]						
Polyphenols	2.4 ± 0.2	2.3 ± 0.2	1.2 ± 0.1	1.2 ± 0.1	5.7 ± 0.3	5.7 ± 0.5
Phenols	0.2 ± 0	0.3 ± 0.1	0.1 ± 0	0.2 ± 0.1	0.5 ± 0.1	0.6 ± 0.1
Highly unsaturated compounds	92.2 ± 0.5	90.2 ± 1.2	93.4 ± 0.4	91.1 ± 1.3	91.3 ± 0.4	90.5 ± 0.5
Unsaturated aliphatics	4.5 ± 0.3	6.4 ± 1.1	5.3 ± 0.4	7.5 ± 1.3		
Saturated fatty acids	0.1 ± 0.07	0.09 ± 0.07	0.01 ± 0.01	0.01 ± 0		
Carbohydrate-like formulae	0.06 ± 0.02	0.06 ± 0.02			0.19 ± 0.05	0.21 ± 0.06
Peptide-like formulae	0.52 ± 0.04	0.66 ± 0.07			2.36 ± 0.2	3.08 ± 0.43

4.5 Discussion

The molecular composition of the DON pool impacts the reactivity and biological availability of the nitrogen bound in DON compounds. The most transient forms of the DON pool (i.e. amino acids, amino sugars and urea) have been studied extensively (e.g. Biddanda and Benner, 1997; McCarthy et al., 1997). In this study, we present the molecular composition of a so far uncharacterized, presumably more semi-labile and refractory fraction of DON with residence times of months to years (Hansell, 2013).

4.5.1 Molecular geography of SPE-DON in the Atlantic and Southern Ocean

Spatial distribution of SPE-DON – The distribution of SPE-DON is similar to the distribution of SPE-DOC, with an accumulation above physical barriers (i.e. the pycnocline). However, higher SPE-DON concentrations reach deeper into the subsurface water masses within the pycnocline (Figure 4.3 A). The higher concentrations in these deeper layers suggest that a fraction of SPE-DON escapes mineralization in the surface layers (Knapp et al., 2005) and is exported to deeper layers during winter mixing or downwelling in the gyre interiors. It was shown before that the input of nitrate to the euphotic zone by upwelling of nutrient-rich deep waters in upwelling regions fuels accumulation of DON, which is transported into the subtropical gyres (Letscher et al., 2013). The same authors also showed that some of this DON is exported to the deeper euphotic zone and that the microbial community in the upper mesopelagic is capable of remineralizing surface DON much faster than the microbial community at the surface.

The traditional perception of the marine nitrogen cycle is that primary producers take up inorganic nitrogen and release it via a multitude of mechanisms as DON to the environment, where it is remineralized by heterotrophic bacteria. It has been shown, however, that bacteria can also utilize inorganic nitrogen (Kirchman and Wheeler, 1998) and may compete with primary producers for inorganic nitrogen. Furthermore, microbial activity does not only act as a sink for marine DON (remineralization), but is also thought to be a source of DON in the ocean (McCarthy et

al., 1998). Phytoplankton, on the other hand, can also assimilate small DON molecules such as amino acids (Palenik and Morel, 1990), thus act as a sink for DON compounds. These multiple interactions make it obvious that the cycling of DON in the ocean is very complex and the disentanglement of involved processes is a difficult undertaking. Along our sampling transects, the SPE-DON concentration was negatively correlated with inorganic nitrogen (Pearson's $r = -0.77$, $p < 0.05$, data not shown), indicating that both pools are closely coupled and that they may to some extent substitute each other as a nutrient source.

Based on our data, the distribution of SPE-DON is mainly driven by biological processes, whereas the distribution of SPE-DOC in the Atlantic and Southern Ocean is mostly driven by physicochemical processes such as the conservative mixing of water masses (Hansman et al., 2015) and accumulation above the pycnocline.

Molecular features of SPE-DON – The molecular-level information on SPE-DON allows us to characterize its composition not only based on its quantitative distribution, but also qualitatively. The trends observed on the concentration level are well represented on the molecular level (Figure 4.8). This remarkable consistency has also been reported for bulk and molecular carbon to sulfur ratios of DOM (Gomez-Saez et al., 2016), indicating that molecular analysis via FT-ICR-MS reproducibly targets a representative fraction of heteroatom-containing molecules in SPE-DOM.

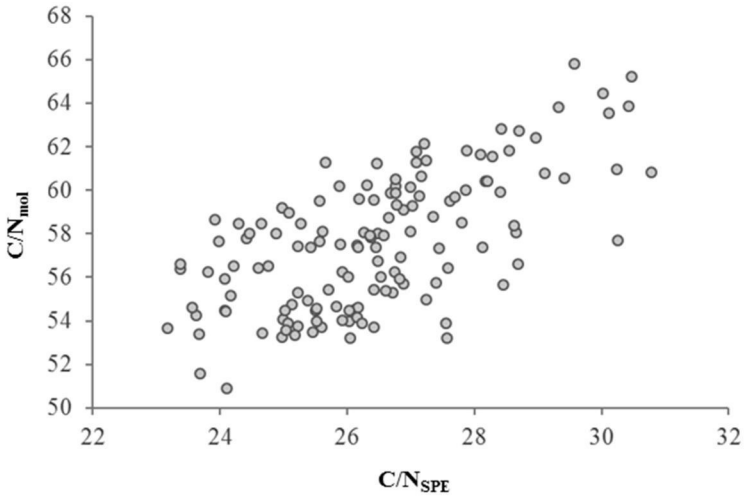

Figure 4.8 Linear correlation between C/N_{SPE} and C/N_{mol} (Pearson's $r = 0.64$, $p < 0.05$). Only data from the surface and subsurface water masses of the Atlantic is shown (mixed layer, STSW, SASW, NACW and SACW (group 3 and 4 of the boxplots; n = 141).

The use of FT-ICR-MS yields additional information, which is not resolved on the concentration level: The elemental analyzer quantifies the abundance of atoms, whereas the detailed molecular analysis yields relative abundances of molecules. On the molecular level, we observed that SPE-DOM molecules in the southern surface water masses of the Atlantic (SASW and STSW) and the whole Southern Ocean were enriched in nitrogen (Figure 4.6). In the surface mixed layer of the Atlantic, SPE-DOM molecules contained significant less nitrogen in the South Atlantic Gyre (also observed on the bulk level) and moderate levels in the North Atlantic Gyre. The C/N_{SPE} ratios in the North Atlantic Gyre were in the same range as respective ratios in the deep waters of the Atlantic Ocean.

Most of the SPE-DON molecules (CHON) belonged to the compound groups of highly unsaturated compounds, polyphenols and the peptide-like formulae (Table 4.1). Besides the highly unsaturated compounds, which were the by far the most abundant group in SPE-DOM (total SPE-DOM as well as SPE-DON), most of the nitrogen was bound in phenolic and peptidic structures. Most phenols are the molecular remnants of vascular plant material (lignins and tannins) transported to the ocean via rivers and terrestrial runoff (Opsahl and Benner, 1997). Nitrogen bound in phenolic structures was uniformly distributed with no specific trends between the surface and deep ocean (Table 4.1), reflecting the refractory nature of these compounds. The higher abundance of peptide-like formulae in the surface mixed layer is consistent with a high biological activity (Osterholz et al., 2016).

4.5.2 DON cycling under different nutrient regimes

DON cycling under excess N conditions in the Southern Ocean – Several water masses meet and mix in the Southern Ocean, forming new water masses, which are transported at deep and intermediate depths into all major global oceans. Due to this constant mixing and the physical properties, the water column in the Southern Ocean is not stratified and no pycnocline is formed. Furthermore, the Southern Ocean is rich in inorganic nutrients (high nutrient, low chlorophyll regions; Martin, 1992). Due to the lack of a stable stratification there is no accumulation of DOM above physical barriers such as the pycnocline. The high availability of inorganic nitrogen leads to the production of N-rich DOM. Furthermore, the exploitation of organically-bound nitrogen is often a costly process for marine microorganisms,

because it requires the production of substrate-specific enzymes, which break down DOM molecules making the nitrogen available for the organism (Boetius and Lochte, 1994). The production of N-rich DOM together with the fact that breaking down organic forms of nitrogen is an energetically unfavorable process favors the accumulation of DON in the Southern Ocean.

The signature of the N-enriched DOM produced in the Southern Ocean is exported via the AAIW and AABW, as reflected in low C/N_{mol} ratios (N-rich) for the Southern Ocean, including AAIW and AABW (Figure 4.7). The C/N_{mol} ratios in all deep Atlantic samples are higher (N-poor). The sequential loss of the Southern Ocean C/N_{mol} signature in the AAIW and AABW is probably the result of DON remineralization along the flow path of these deep-water masses. Hansman et al. (2015) showed that the molecular composition of deep sea DOM in the North Atlantic sampled on a transect spanning more than 5000 km is very similar on a Bray-Curtis scale (96 % similarity) and that the observed small differences are the result of conservative mixing of different water masses. Therefore, the molecular DOM signature of the formation site might also change due to water mass mixing along the flow path of Atlantic deep water masses.

DON cycling under N-depleted conditions in the subtropical gyres – SPE-DOC as well as SPE-DON are both enriched in the surface mixed layers. However, the C/N_{SPE} ratio reveals specific features in the DOM composition of the North and South Atlantic Subtropical Gyres. The transect of the Atlantic Ocean was sampled in April, therefore we encountered low chlorophyll-a concentrations in the South Atlantic Gyre and slightly higher chlorophyll-a concentrations in the North Atlantic Gyre (Signorini et al., 2015), which was also confirmed by our shipboard chlorophyll fluorescence data (data not shown). The subtropical gyres are nutrient limited ecosystems, with nitrogen being the primary limiting nutrient (Moore et al., 2013). Due to this limitation, DOM produced in these systems can be depleted in nitrogen, simply because its availability is extremely low (Biersmith and Benner, 1998; Kähler and Koeve, 2001). Furthermore, DON can serve as major nutrient source to organisms in these environments, facilitating an even greater depletion of nitrogen bound in DOM. Zweifel et al. (1993) showed that the addition of nutrients to samples from nutrient-depleted environments promoted DOC consumption, which

means at the same time that in addition to the physical barriers, i.e. the seasonal pycnocline, the lack of nutrients favors the accumulation of DOC. These processes might contribute to the higher concentrations of SPE-DOC and SPE-DON in the surface mixed layer of the Atlantic Ocean.

As revealed by our ultra-high resolution data set, the processes shaping the DON pool in the North Atlantic Gyre are significantly different from the South Atlantic Gyre. Whereas the DOM composition is mainly shaped by nutrient depletion in the South Atlantic Gyre, DOM in the North Atlantic Gyre contains significantly more nitrogen despite the similarly low levels of nitrate (Figure 4.7). Marine diazotrophic nitrogen fixation acts as a source of N-nutrients (Mahaffey et al., 2005) and supports primary production in otherwise nutrient-depleted systems (Capone et al., 2005). Mills et al. (2004) found that iron and phosphorous co-limit the nitrogen fixation in the tropical North Atlantic and that aeolian dust input from the Sahara promotes nitrogen fixation. In the Northeast Atlantic between 41 – 76 % of the fixed nitrogen is channeled into the DON pool (Benavides et al., 2011), providing a possible explanation for the low C/N ratio in the North Atlantic Gyre found in our data set.

4.6 Conclusion

Our study presents a unique data set providing in depth information on the concentration and molecular geography of SPE-DON in two major global oceans. Molecular-level information on DOM reveals distinct compositional patterns for biogeochemical provinces (North and South Atlantic Gyre and Southern Ocean) and relates them to processes driving the distribution of SPE-DON.

DON is an important nutrient source and plays a major role in the global nitrogen cycle. Understanding its molecular composition and the distribution of SPE-DON is of great importance for deciphering spatial characteristics of the nitrogen cycle and its implications for biological activity. Our data indicate that different nutrient regimes lead to different distributions of SPE-DON. Our study also shows how the availability of inorganic nitrogen can influence the composition of SPE-DON in the open ocean. The prevailing N species, however, determines its availability to marine organisms and is therefore key to biogeochemical processes.

Although the water masses in the surface Atlantic Ocean are similarly depleted in inorganic nutrients, the external input of iron and phosphorus via Saharan dust in the North Atlantic favors nitrogen fixation by diazotrophs, which influences the molecular composition and concentration of SPE-DON.

This study provides in depth information about the molecular composition of the DON pool persistent on longer time scales in marine ecosystems. The reactivity of DON is directly linked to its structural features like functional groups. Detailed structural characterization of the functionalities of the more persistent DON fractions is therefore a promising next step in completing the picture of the marine DON cycle.

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4.9 Supplementary materials

Table S4.1 shows an example of how the intensity-weighted averages of molecular parameters were calculated.

Table S4.1 Exemplary calculation of intensity weighting of the average number of N-atoms per sample. The average number of nitrogen atoms per compound in each sample is calculated by multiplying the number of nitrogen atoms per compound with the respective peak intensity (intensity weighting, [1]), summing up the intensity weighted number of nitrogen atoms for the whole sample [2] and dividing it by the total sum of intensity of the respective sample [3]. All shown peak intensities are examples and not real peak intensities measured in any sample.

Formula	#N-atoms	Peak intensities of Sample A	intensity-weighted #N-atoms of sample A [1]	Peak intensities of Sample B	intensity-weighted #N-atoms of sample B [1]
$C_9H_7NO_4$	1	7	7	5	5
$C_{10}H_{11}NO_3$	1	8	8	7	7
$C_{11}H_{15}NO_2$	1	8	8	4	4
$C_{10}H_{10}O_4$		8	0	4	0
$C_9H_{10}N_2O_3$	2	7	14	4	8
$C_{11}H_{14}O_3$		1	0	0	0
$C_{10}H_{14}N_2O_2$	2	1	2	6	12
$C_{12}H_{18}O_2$		6	0	5	0
$C_9H_9NO_4$	1	4	4	8	8
$C_{10}H_{13}NO_3$	1	0	0	7	7
Total intensity		50		50	
Sum of intensity-weighted #N-atoms [2]			43		51
Ratio of total intensity and sum of intensity-weighted #N-atoms (average number of nitrogen per compound in the respective samples) [3]		**0.86**		**1.02**	

5. CONCLUDING REMARKS AND PERSPECTIVES

This thesis presents the molecular geography of marine DOM in the Atlantic and Southern Ocean. The overall goal was to identify distinct patterns in the molecular composition of DOM in the ocean and to relate these patterns to biotic and abiotic processes. In the first study, two indices were established to assess the impact of bioproduction and photodegradation on the molecular composition of DOM, and successfully applied to samples from the Atlantic and Southern Ocean. In the second study, the molecular fingerprints from the Atlantic and Southern Ocean were compared to an arithmetically constructed two-source mixing model. It was shown that the molecular geography in the Atlantic and Southern Ocean can be explained as the result of mixing two specific endmembers, i.e. the molecular signatures of freshly produced and long-term stable DOM. In the third manuscript, the distribution of dissolved organic nitrogen (DON) in the Atlantic and Southern Ocean and the reasons behind the observed distribution were explored.

5.1 Concluding remarks

Here, the central hypotheses addressed in this thesis are summarized comprehensively and critically reviewed (see chapter 1.4).

1. Photodegradation and bioproduction leave characteristic imprints in the DOM molecular composition of the Atlantic and Southern Ocean.

The results of this thesis show that bioproduction and photodegradation leave characteristic imprints in the DOM molecular composition in the Atlantic and Southern Ocean. The impact of both processes was captured by two process-related indices ($I_{bioprod}$ and I_{photo}), which were introduced based on the results of two previously published laboratory experiments targeting exclusively photodegradation of deep sea DOM (Stubbins and Dittmar, 2015) and bioproduction (Osterholz et al., 2015). The indices facilitate the transfer of the process-related information from laboratory experiments to natural environments and identify the respective imprints in natural DOM samples. Both indices assess the relative extent of the respective process within a sample set. The maximum impact of bioproduction and photodegradation on DOM is observed in the subtropical and tropical euphotic layer of the global

ocean, where the solar energy input is highest, making the molecular composition highly variable in these waters (manuscript I).

DOM fractions in the ocean are stable on time scales varying from seconds to millennia (Hansell, 2013). The largest fraction appears biologically inert and is present at every water depth in the global ocean. The presence of semi-labile and semi-refractory DOM resulting from microbial production in the surface mixed layer of the Atlantic in addition to a refractory DOM background mainly explains molecular DOM compositional differences between the surface and deep ocean: The comparison of the molecular DOM composition from the Atlantic and Southern Ocean to an arithmetically constructed two-source mixing model with two distinct endmembers (i.e. truly refractory deep sea DOM and semi-labile and semi-refractory DOM produced during a controlled mesocosm experiment) showed that microbial production shapes the molecular composition in the surface layer of the Atlantic significantly and can still be traced in the deep sea (manuscript II).

As indicators of bioproduction and photodegradation, the $I_{bioprod}$ and the I_{photo} are valuable tools to assess the impact of both processes on the molecular DOM composition. The $I_{bioprod}$ calculated based on the modelled fingerprints (two-source mixing model, manuscript II) correlates significantly with the $I_{bioprod}$ calculated for natural DOM samples (Figure 5.1 A). The model, however, slightly overestimates the $I_{bioprod}$, which can be attributed to the extremely high $I_{bioprod}$ in pure mesocosm DOM ($I_{bioprod}$ of mesocosm DOM is 1.05). The I_{photo} does also correlate between modelled and natural fingerprints, but the calculated values differ from natural DOM (deviation from orange line in Figure 5.1 B). This is not surprising since the characteristic imprint of photodegradation on the molecular DOM composition was not included in the two-endmember mixing model. The inclusion of photodegradation into the model, however, did not further explain any of the observed molecular dissimilarities between modeled and observed composition of Atlantic and Southern Ocean DOM, indicating that the molecular signature of photodegradation is not propagated by water mass mixing like the molecular signature of microbial production is.

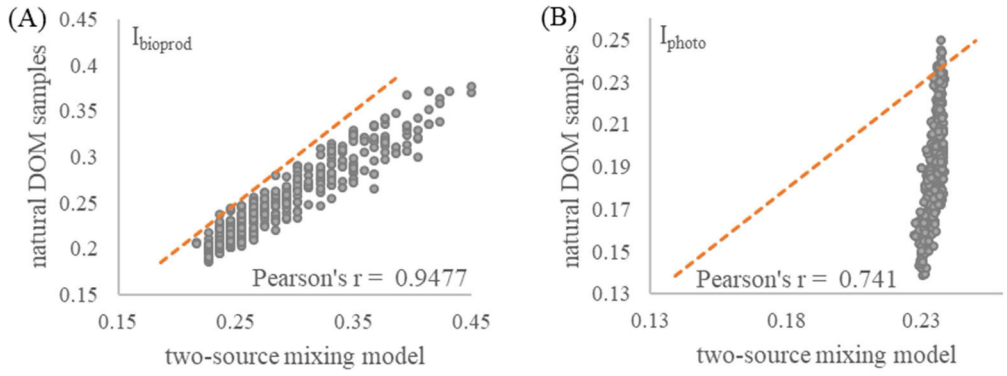

Figure 5.1 The $I_{bioprod}$ of natural DOM samples versus the $I_{bioprod}$ of the optimum fits of the two-source mixing model **(A)** and the I^{photo} of natural DOM samples versus the I_{photo} of the optimum fits of the two-source mixing model **(B)**. The orange line represents the line of equality between modeled and natural fingerprints.

Overall, the results of this thesis indicate that both bioproduction and photodegradation play important roles in shaping the molecular DOM composition. The extent and conservation of the molecular signatures, however, depends on additional factors, such as for example microbial community composition, nutrient availability and water mass mixing.

2. Water mass mixing drives the molecular geography of DOM in the Atlantic and Southern Ocean.

There are two major implications of the study presented in the third chapter: First, microbial production in the surface mixed layer largely explains the molecular DOM composition in the Atlantic and second, water mass mixing mainly drives the molecular geography in the Atlantic and Southern Ocean. The mixing of various amounts of freshly produced and refractory DOM accounted for almost all observed molecular variability between surface and deep sea DOM. A residual dissimilarity of ~ 5 to 10 % remains unexplained by water mass mixing, which is probably the result of other biotic and abiotic DOM transformation processes. For example, the discrepancy between observed and modeled H/C ratios in the deep sea (Figure 5.2 A; H/C of natural deep sea DOM < 1.27) may be the result of thermogenic transformation at hydrothermal vents. It has been shown that thermogenic processes produce DOM molecules with very low H/C ratios (Dittmar and Koch, 2006)

providing a possible explanation of lower H/C ratios in natural DOM than calculated with the modeled fingerprints. In the surface mixed layer, DOM is more saturated than the model suggests (Figure 5.2 A; H/C of natural surface DOM > 1.27). Photochemically produced molecules have a generally higher H/C ratio than photolabile compounds (Medeiros et al., 2015). In this case, photodegradation at the ocean's surface might change the overall DOM composition towards more saturated compounds, which is not reflected in the model. The degradation index (I_{deg}, Flerus et al., 2012) calculated with modeled fingerprints yields similar results: Mixing generally explains the distribution of I_{deg} very well, however, in the surface water masses of the Atlantic the observed degradation state is lower (DOM is "younger") than the two-source mixing model suggests (Figure 5.2 B; I_{deg} of natural DOM = 0.65 – 0.76). DOM removal mechanisms, such as photodegradation (Stubbins and Dittmar, 2015) or co-metabolism of fresh and young DOM in surface mixed layer (Cherrier et al., 1999) change the degradation signature of DOM. Both processes were not included in the model, explaining the mismatch of the I_{deg} between modeled and natural fingerprints.

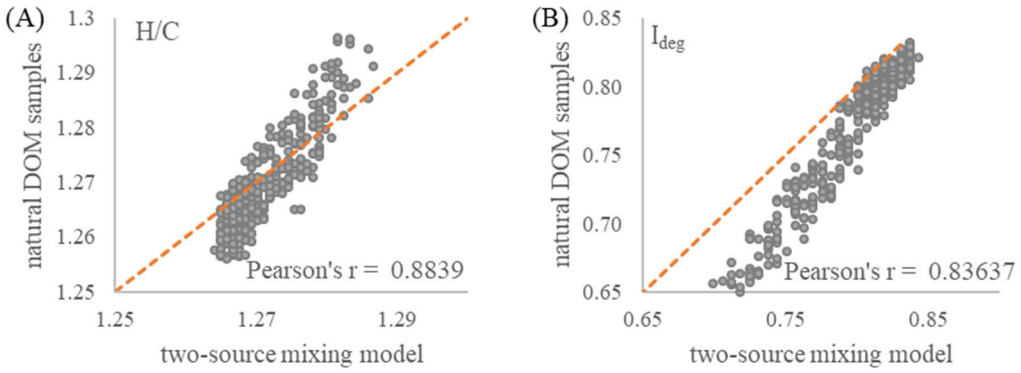

Figure 5.2 The H/C ratios of natural DOM samples versus the H/C ratios of the optimum fits of the two-source mixing model **(A)** and the I_{deg} of natural DOM samples versus the I_{deg} of the optimum fits of the two-source mixing model **(B)**. The orange line represents the line of equality between modeled and natural fingerprints.

The modelled molecular fingerprints with known contributions of fresh and refractory DOM helped to decipher the molecular geography in the Atlantic and Southern Ocean and identified the main processes driving it. In this context, it is remarkable

that a microbial community taken from the coastal North Sea is capable of producing DOM which is as diverse as open ocean DOM with a molecular signature also identifiable in DOM from the Atlantic and Southern Ocean. Until recently, semi-labile and semi-refractory DOM was quantified as excess concentration above the refractory background concentration (Hansell, 2013), but for the first time it is now possible to quantify the amount of the semi-persistent DOM fractions based on molecular information in a large variety of marine environments. The application of the two-source mixing model to other molecular data sets will help to trace the signature of microbial production in other marine environments and to understand the cycling of the semi-persistent DOM fractions.

3. The distribution of DON in the Atlantic and Southern Ocean mostly depends on nutrient availability and microbial community composition.

The study presented in the fourth chapter identifies the distribution patterns of solid-phase extractable (SPE)-DON in the Atlantic and Southern Ocean and proposes biotic and abiotic processes driving the distribution. Especially the nutrient regime and the presence of nitrogen fixing microbes seem to have a large impact on the distribution patterns of SPE-DON, as indicated for example by differences in the C/N ratios under different environmental conditions. Water mass mixing and microbial production, which mainly drive the molecular geography of bulk SPE-DOM, cannot explain the distribution of molecular C/N ratios in the Atlantic and Southern alone (Figure 5.3). Hence, the distribution of the nitrogen-containing molecules is to some degree independent from that of bulk DOM and mostly shaped by local processes.

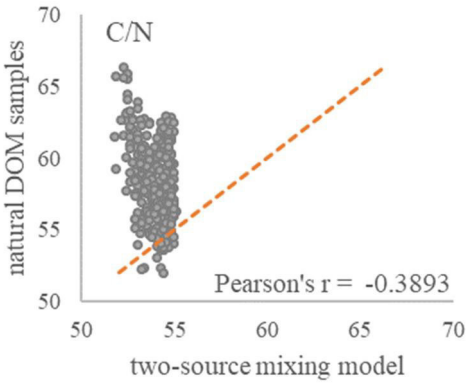

Figure 5.3 The C/N ratios of natural DOM samples versus the C/N ratios of the optimum fits of the two-source mixing model. The orange line represents the line of equality between modeled and natural fingerprints.

The described distribution patterns in chapter four provide novel insights into the cycling of SPE-DON in the Atlantic and Southern Ocean. The ultrahigh-resolution analysis via FT-ICR-MS makes it possible to molecularly characterize SPE-DON in unprecedented detail and to study its cycling not only on the bulk level but also on an individual compound level.

5.2 Future perspectives

The results of this thesis provide novel information about the cycling of DOM in the Atlantic and Southern Ocean and add to the growing knowledge to address fundamental questions that are still open to debate:

The millennial stability of DOM – Water mass mixing and microbial production mainly drive the molecular geography in the Atlantic and Southern Ocean, still the reasons why large amounts of DOM escape rapid turnover are not fully understood. In case of SPE-DON, availability of inorganic nutrients play a significant role in its distribution, indicating that abiotic factors summarized under the environmental stability hypothesis (Dittmar, 2015) are involved in stabilizing SPE-DON in the environment. Photodegradation impacts the molecular DOM composition, but apparently, the effects are only measurable locally and its characteristic signature is not transported via water mass mixing. The molecular signature of microbial production is equally distributed across the Atlantic surface water masses, without a

characteristic imprint of the prevailing microbial communities. This missing direct link is most likely due to the difference in observed time frames: The turnover of microbial cells is much shorter compared to the turnover of the semi-persistent DOM fractions. The longer turnover times of the semi-persistent DOM compounds also allows for their homogenously distribution by water mass mixing across a large spatial scale. The associations between DOM and the microbial communities across global water masses of the Atlantic and Southern Ocean are subject of a planned publication. DOM-microbial interactions will be studied via a novel combination of multivariate statistics as published by Osterholz et al. (2016b).

The complex interactions of various processes shaping the molecular composition in the ocean make it challenging to identify single mechanisms responsible for the millennial stability. The results of this thesis contribute new information but the overall picture is by no means complete.

DOM molecular geography in a changing global climate – Global climate change will impact many marine environments: The effect of ocean acidification on the DOM molecular composition was investigated (Zark et al., 2015), but many effects of a changing global climate on the marine carbon cycle are still under investigation and far from being completely understood (Moran et al., 2016; Sarmiento et al., 1998). Changes in ocean circulation will also impact the mixing of water masses and the long-term sequestration of organic carbon in the deep sea. Therefore, understanding the effects of global climate change on the DOM pool is one of the major challenges of future DOM research.

Structural elucidation of DOM – The ultrahigh-resolution analysis of DOM via FT-ICR-MS provides valuable insights into molecular DOM composition and distribution. Using tandem mass spectrometry, it is also possible to obtain structural information on the molecular DOM composition. Studies on the structural diversity of marine DOM indicate that behind a molecular formula many structural isomers may be hidden (Zark et al., 2017). Along a salinity gradient in a temperate estuary, the analysis via tandem mass spectrometry revealed that the structural composition of DOM did not vary significantly in the described system, irrespective of its origin from terrestrial or marine sources (Osterholz et al., 2016a). However, the molecular

signature of microbially produced DOM is structurally different from truly refractory DOM (Osterholz et al., 2015), but until now it is not clear whether the molecular structures of DOM compounds (intrinsic stability hypothesis; Dittmar, 2015) or the concentration of individual compounds (molecular diversity hypothesis; Dittmar, 2015) ultimately enable immediate processing or millennial sequestration in the pool of refractory DOM. Structural elucidation of marine DOM therefore remains one of the major challenges in future DOM research.

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6. CURRICULUM VITAE

Name: Anna Maren Seibt

Date of birth: August 21, 1984

Place of birth: Stuttgart, Germany

Education

Since 08/2011	Ph.D. student in the Research Group for Marine Geochemistry (ICBM-MPI Bridging Group), University of Oldenburg (Germany)
2009	Graduate student exchange (6 months), University of New England (Australia)
2008 – 2011	M.Sc. Marine Environmental Sciences, University of Oldenburg (Germany)
2005 – 2008	B.Sc. Environmental Sciences, University of Oldenburg (Germany)

Publications, peer-reviewed

Seibt, M., Dittmar, T. and Niggemann, J. Natural transformation of marine dissolved organic matter: Process-related molecular indices for biopro-duction and photodegradation. Limnology and Oceanography, in preparation.

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Conference contributions

Seibt, M., Niggemann, J., Osterholz, H. and Dittmar, T. The molecular geography of dissolved organic matter in the Atlantic can largely be explained by a simple two-source mixing model. Association for the Sciences of Limnology and Oceanography (ASLO), New Orleans (Lousiana), USA, February 2016 (Talk).

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(Maren Seibt)