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Biogenic Composition of the Sea-Surface Microlayer in Response to a Changing Environment

Dissertation
zur Erklarung des akademischen Grades
eines Doktors der Naturwissenschaften
- Dr. rer. nat. -
der Mathematischen-Naturwissenschaftlichen Fakultät
der Christian-Albrechts-Universität zu Kiel

Vorgelegt von
Luisa Galgani
Kiel, 2013

GEOMAR
Helmholtz Centre for Ocean Research Kiel

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1. Gutachter: Prof. Dr. Anja Engel, GEOMAR, Kiel

2. Gutachter: Prof. Dr. Hermann W. Bange, GEOMAR, Kiel

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In memoria del Professor Enzo Tiezzi,

per avermi insegnato che la più bella storia del mondo

è proprio quella dell' "arancia blu"

TABLE OF CONTENTS

SUMMARY

The sea-surface microlayer (SML) is the oceanic uppermost boundary in contact to the atmosphere, a sort of biofilm matrix where microorganisms and organic material largely contribute to its physical and chemical structure. By accumulating organic material, this surface film damps capillary waves and influences air-sea gas exchange across the ocean's surface. Furthermore, organic compounds in the SML contribute to organic enrichment of bursting bubbles at the sea-surface, creating droplets that later dry out in the air as organic marine aerosols. Given their extension, the oceans are the largest source of atmospheric aerosols susceptible to affect regional and global climate through radiation, precipitation, and cloud formation. At periods of high biological productivity, sea-spray droplets are largely constituted of water insoluble organic particles, mainly as marine microgels, that may derive from the SML. Marine gels originate in the size-continuum aggregation of dissolved organic matter (DOM) precursors from the colloidal size (< 1 μm) that assemble to larger particles reaching several millimeters. Gels are hotspots for intense microbial activity, as they are rich in nutrients and act as a physical substrate for cells to grow upon. Microbial processes, like DOM exudation and degradation, contribute to the continuous recycling of this gelatinous material.

The present thesis investigated how organic compounds and gel particles in the SML vary as a function of biological activity in the surface ocean impacted by anthropogenic climate change. The work has been articulated in three parts, comprising a laboratory study, a sea-going mesocosm experiment, and a sampling campaign to the Central Arctic.

The first study conducted in the laboratory with the marine diatom *Thalassiosira weissflogii* highlighted the enhanced presence of proteinaceous marine gels in the SML, determined as results of solely phytoplankton and bacterial metabolism. While polysaccharidic marine gels could also form from dispersed colloidal material in sterile seawater, proteinaceous particles in the SML might have derived from bacterial contribution to the biofilm matrix through cell lysis or direct release of exudates.

The SML response to ocean acidification was studied during the mesocosm experiment conducted in Southern Norway, in the late spring of 2011. Results from this experiment showed enhanced proteinaceous characteristics of the gelatinous SML components which covered a larger surface area, whereas polysaccharidic particles were smaller but more

abundant. In the SML, the effects of rising pCO_2 levels led to increased bacterial abundance, changing concentrations of carbohydrates and amino acids, and compositional shift from proteinaceous to more polysaccharidic marine gels.

In the Arctic region, both ocean acidification and global warming are forcing rapid changes in the environment. During the sea ice minimum in summer 2012, the SML was sampled in first open spots in the melting sea ice (melt ponds), open leads at the ice edge and in the open ocean. Our results confirmed that proteinaceous gels are larger and cover an extended area in the SML, contributing to a higher fraction to the gelatinous structure, while polysaccharidic gels are smaller and in a lesser extent. Moreover, the diagenetic state of DOM as indicator of its lability suggested an important bacterial contribution to the DOM turnover in the SML.

It can be concluded that the composition of the SML mirrors biological dynamics in the water column like phytoplankton bloom, DOM exudation and degradation. However, the fact of being an interface adds complexity to the SML system through the emerging of new processes. The gelatinous properties of the SML might be mainly due to larger proteinaceous compounds, while small particles important for aerosol formation are most abundant in the polysaccharidic class. The different partitioning of polysaccharidic/proteinaceous marine gels is suggested to be associated to microbial activity of bacterial SML communities. The presence of a large continuous film is an important issue for air-sea gas exchange, whereas the abundance of small gels is determinant for POA formation. Ocean acidification and surface warming, by influencing biological dynamics, affect the composition of the SML and the turnover of DOM in the surface ocean. This external anthropogenic forcing altering microbial SML processes might raise additional feedbacks on global air-sea gas exchange and POA emission. The direction of these changes is yet hard to predict. Since SML dynamics are likely globally valid, further studies are urgently required to increase our knowledge about the role of surface films in air-sea gas exchange before man-made impacts on earth's climate become irreversible.

ZUSAMMENFASSUNG

Der ozeanische Oberflächenfilm ist die oberste Grenzschicht des Ozeans zur Atmosphäre, eine Art Biofilm-Matrix, deren physikalische und chemische Struktur weitgehend durch Mikroorganismen und organisches Material geprägt sind. Reichert sich organisches Material an der Oberfläche an, werden Kapillarwellen gedämpft und der Gasaustausch über die Oberfläche des Ozeans beeinflusst. Aufsteigende Luftbläschen, die an ihrer Oberfläche mit organischem Material angereichert sind, zerplatzen an der Meeresoberfläche und schleudern so organische Verbindungen in die Luft, die dann dort zu kleinen marinen Aerosol-Partikeln eintrocknen. Angesichts ihrer Größe, sind die Ozeane die größte Quelle atmosphärischer Aerosole, die wiederum das regionale und globale Klima im Zusammenhang mit Strahlung, Niederschlag und Wolkenbildung beeinflussen können.

Zu Zeiten hoher biologischer Produktivität bestehen diese marinen Aerosol-Partikel hauptsächlich aus wasserunlöslichen organischen Partikeln, zumeist marinen Mikrogelen, des ozeanischen Oberflächenfilms. Marine Gele entstehen durch die Aggregation gelöster organischer Kolloide (DOM, < 1µm), die sich zu mehrere Millimeter großen Partikeln zusammenlagern. Marine Gele zeichnen sich durch hohe mikrobielle Aktivität aus, da sie reich an Nährstoffen sind und den Zellen eine Oberflächen zum Wachsen bieten. Mikrobielle Prozesse, wie Exsudation und Abbau von DOM, sorgen für ein kontinuierliches Recycling dieses gelartigen Materials.

Die vorliegende Arbeit untersucht, wie organische Verbindungen und Gelpartikel im Oberflächenfilm durch die biologische Aktivität im oberen Ozean beeinflusst werden. Im speziellen werden mögliche Änderungen in der biologischen Aktivität durch den anthropogenen Klimawandel berücksichtigt. Die Arbeit ist in drei Teile gegliedert und umfasst eine Labor-Studie, ein Mesokosmos-Experiment und eine Probenahme-Kampagne in der zentralen Arktis.

Die erste Studie, die im Labor mit der marinen Kieselalge *Thalassiosira weissflogii* durchgeführt wurde, zeigte das verstärkte Vorkommen von proteinhaltigen marine Gele im Oberflächenfilm, was sich ausschließlich aus dem Stoffwechsel von Phytoplankton und Bakterien ergab. Während polysaccharidhaltigen Gele sich auch aus kolloiddispersem Material in sterilem Seewasser bilden können, können proteinhaltige Partikel im Oberflächenfilm auch durch Bakterien im Biofilm entstanden sein.

Die Auswirkungen von Ozeanversauerung auf den ozeanischen Oberflächenfilm wurden während des Mesokosmos-Experiments in Südnorwegen im Frühjahr 2011 untersucht. Die Ergebnisse zeigten den großen Anteil proteinhaltiger Gele im Oberflächenfilm, die gleichzeitig eine größere Fläche einnahmen als die kleineren aber häufigeren polysaccharidhaltigen Partikel. Die erhöhten CO_2-Konzentrationen in den Mesokosmen führten zu höheren Bakterienzellzahlen und veränderten Zucker- und Aminosäure-Konzentrationen im Oberflächenfilm. Außerdem änderte sich die Zusammensetzung der marinen Gele von proteinhaltigen zu mehr polysaccharidhaltigen Gelen.

Ozeanversauerung und -erwärmung führen zu schnellen Veränderungen der Umwelt in der Arktis. Während des Meereis-Minimums im Sommer 2012 wurde der Oberflächenfilm in Schmelztümpeln des Meereises, in Wasserflächen zwischen den Eisschollen und im offenen Ozean beprobt. Die Ergebnisse bestätigten, dass proteinhaltige Gele größer sind und den größeren Anteil der Gele im Oberflächenfilm stellen, während die polysaccharidhaltigen Gele kleiner und in geringerem Umfang zu finden waren. Der diagenetische Zustand des DOM deutete darauf hin, dass Bakterien eine wichtige Rolle im Umsatz des organischen Materials im Oberflächenfilm spielten.

Zusammenfassend komme ich zu dem Schluss, dass die Zusammensetzung des ozeanischen Oberflächenfilms die biologischen Prozesse in der Wassersäule wie beispielsweise eine Phytoplanktonblüte oder Exsudation und Abbau von DOM widerspiegeln. Da es sich um eine Grenzfläche handelt, sind allerdings weitere komplexe Prozesse von Bedeutung. Die gelartigen Eigenschaften des Oberflächenfilms kommen vor allem durch proteinhaltige Verbindungen zustande, während die kleineren Partikel, die für die Aerosolbildung wichtig sind, hauptsächlich aus Polysacchariden zu bestehen scheinen. Die unterschiedliche Partitionierung von polysaccharid- und proteinhaltigen Gelen scheint hauptsächlich mit der Aktivität der Bakterien im Oberflächenfilm in Verbindung zu stehen. Das Vorhandensein eines ausgedehnten kontinuierlichen Oberflächenfilms ist ein wichtiger Aspekt für den Gasaustausch zwischen Atmosphäre und Ozean, während die Anzahl kleiner Gelpartikel ein bestimmender Faktor der Aerosolbildung ist. Ozeanversauerung und -erwärmung könnten, durch Beeinflussung der biologischen Prozesse im oberen Ozean, die Zusammensetzung des Oberflächenfilms und seiner mikrobiellen Gemeinschaft sowie den Umsatz von DOM verändern. Durch diesen anthropogenen Einfluss auf die mikrobiellen Prozesse an der Meeresoberfläche könnten sich auch Rückkopplungen auf den Gasaustausch zwischen

Atmosphäre und Ozean sowie in der Aerosolbildung ergeben. Die Richtung dieser Veränderungen ist schwer vorherzusagen. Da die Prozesse innerhalb des ozeanischen Oberflächenfilms wahrscheinlich von globaler Bedeutung sind, werden weitere Studien dringend benötigt um die Rolle von Oberflächenfilmen für den Gasaustausch zwischen Ozean und Atmosphäre besser zu verstehen bevor der menschliche Einfluss auf das globale Klima irreversibel wird.

GENERAL INTRODUCTION

THE SEA-SURFACE MICROLAYER: FROM PIONEER STUDIES TO CONTEMPORARY CONCEPTS

On sunny low-wind days with calm waters, the surface of the sea appears like a smooth film that glitters by reflecting sunlight. In the presence of moderate waves, this surface becomes a little rough and tiny ripples move following the wind direction. The sea-surface microlayer (SML) is also known as the "hair of the water" in some countries, due to it being the thickness of a human hair. This interface at the border between the sea and the air is an organic layer (Hardy, 1982) up to 1 mm thick, (e.g., Williams, 1967, Sieburth, 1983, Wurl et al., 2011b) where natural and anthropogenic surfactants accumulate with respect to the water below (Hardy, 1982, Hardy et al., 1985,Liss and Duce, 2005).

Interfaces are "where two qualitatively different entities meet and affect each other"[1]. The interaction of two separate systems has thrilled scientists and aroused interest for a long time, since interfaces are connection areas where properties of the two compartments in contact get mutual influences. Ships' oil spills on the sea-surface stimulated the curiosity of Benjamin Franklin enough to conduct an experiment and write his observations on oil slick-formation as early as 1773, as referred by Sieburth (1983). Some years later, Pockels (1891) and Langmuir (1917) conducted experiments on the effects of organic films on surface tension and wave damping. It was this early work on the compressibility of monolayers and water surface tension that encouraged the development of the "Langmuir Trough". In aquatic ecosystems, the first reports on naturally occurring organic surface films and their influence on water viscosity are due to observations by H.D. Thoreau in 1854 followed by a subsequent scientific description by Reynolds in 1881, as cited by McDowell and McCutchen (1971).

It was not until the 1960s that biological surface films were observed in the Sargasso Sea (Sieburth and Conover, 1965) where *Trichodesmium* blooms formed visible organic surface slicks covering large areas up to 25 km. Whether the slicks might be visible to the naked eye or not, films occur on all water surfaces and in all oceans of the world. The traditional concept of a marine biologically-derived layer consisted of a multi-layer structure of "dry"

[1] As defined in "Advanced Learner's dictionary", Oxford.

(lipid layer) and "wet" (protein-polysaccharide layer) surfactants (Hardy, 1982, Sieburth, 1983). This has now been replaced by the model of a hydrated loose gel of carbohydrates, proteins and lipids, that from dissolved colloids adsorbed at the sea-air interface rearranges to form a gel-like organic matrix (Sieburth, 1983)(figure 1).

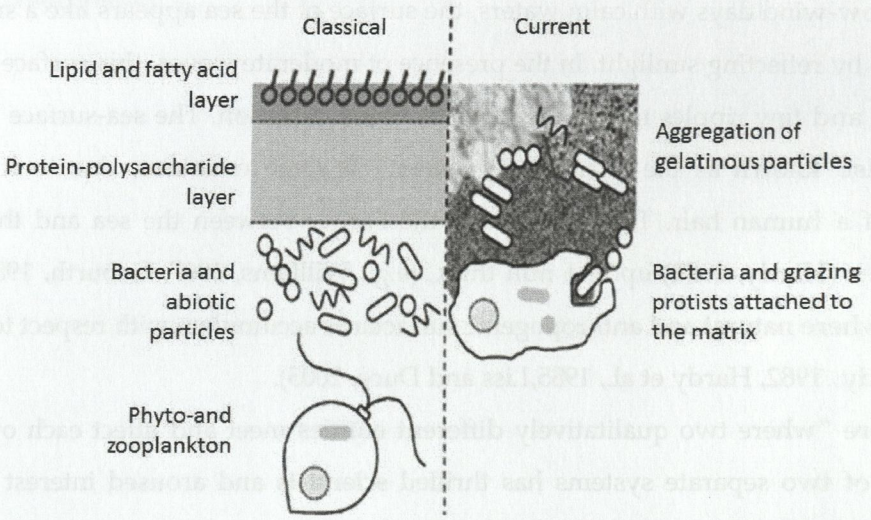

Figure 1. Simple model of the air-water interface according to the classical view until 1982, and the current concept introduced by Sieburth in 1983. The classical model shows a multilayer stratified structure with upper 'dry surfactants' (lipid layer) and a lower 'wet surfactants' (protein–polysaccharide layer). Bacteria and abiotic particles are closely attached to the protein-polysaccharide layer. This model has been revised and the interface is now expected to be a heterogeneous matrix of dissolved colloids that rearrange to form a gelatinous layer (Adapted from Cunliffe 2011).

The formation of the SML relies on compounds ascending through the water column to the surface because of their surface-active properties, but it is also known as a simultaneous source and repository for airborne particles (Williams, 1967, Hardy, 1982).

On the waterside, the extent to which compounds accumulate in the SML is described as enrichment factor (EF), calculated as follows

$$EF = [X]_{SML} / [X]_{ULW(or\ BW)} \quad (1)$$

Where $[X]_{SML}$ is the concentration of a given compound $[X]$ in the SML and $[X]_{ULW(or\ BW)}$ is the concentration of the same compound in the underlying water (ULW or bulk water, BW)(Liss and Duce, 2005).

The early hypothesis of the SML structure advanced by Sieburth in 1983 was based on evidences of dissolved organic compounds like carbohydrate, protein and lipid enrichment in the upper 150 µm of the surface (Garrett, 1967, Sieburth et al., 1976, Kattner and Brockmann, 1978). At that time particulate compounds had been observed in great amounts and probably higher enrichment than dissolved material, with the effect of minimized surface tension and enhanced formation of a continuous film (Kattner et al., 1983). Particles were believed to originate from living organisms (Sieburth et al., 1976), parts of dead cells, or other film-like material originating from plankton bloom remains of unknown composition (Harvey, 1966).

From these early observations on natural surface films the gelatinous nature of the SML is no longer a hypothesis: film-like particles observed by Harvey (1966) might have been marine gels that, according to more recent studies, may represent major components of the ocean-air boundary layer (Wurl and Holmes, 2008, Cunliffe and Murrell, 2009, Cunliffe et al., 2009).

DISSOLVED ORGANIC MATTER (DOM), MARINE GELS, AND HETEROTROPHIC ACTIVITY IN THE SML

Before further discussing the gelatinous SML according to more recent developments, it is now important to step back to the surface matrix of dissolved organic colloids (carbohydrates, proteins, lipids) to introduce the role of dissolved organic matter (DOM) in the ocean and, more specifically, in marine gel formation.

Oceans are the largest source of the Earth's reduced organic carbon, most of which resides in the so-called "dissolved" phase of organic matter - DOM (Benner et al., 1992, Benner, 2002). DOM in the ocean exists through a continuum of sizes and the partitioning between particulate and dissolved OM is basically an operational definition; it is common use to define constituents of water samples as "dissolved" if passing through filters of 0.2 - 1.0 µm pore size, and thereafter colloids fall in the dissolved phase (Benner et al., 1992, Wells, 2002). According to size, DOM can be further distinguished into high molecular weight (HMW) and low molecular weight (LMW) DOM based on the possibility for compounds to pass through (LMW DOM) or be retained by (HMW DOM) a 1000 Dalton cut off membrane (Benner et al., 1992, Benner, 2002).

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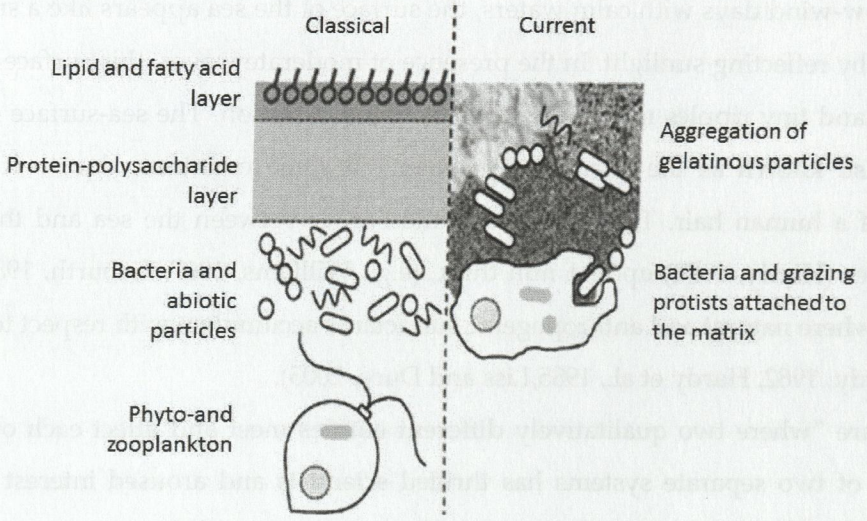

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Based on its reactivity, the DOM pool is in a succession of biological liability, from refractory material that turns over on long time scales (centuries to millennia) to semi-labile DOM, utilized by microbes and remineralized in a time frame of months to years, to labile DOM with a turnover time from minutes to days (Carlson, 2002). Fresh DOM production occurs in the surface euphotic ocean that accounts for a higher concentration of more reactive and labile (i.e. less diagenetically altered) HMW DOM with respect to a more refractory LMW DOM (Amon and Benner, 1994). Although most of the DOM is uncharacterized at the molecular level, carbohydrates are the largest class of biomolecules so far identified, followed by amino acids with these two groups representing 10-20% of ocean's dissolved organic carbon (DOC) (Benner et al., 1992, Benner, 2002).

Most DOM that is produced is either rapidly consumed by heterotrophic bacteria (Amon and Benner, 1994, Amon and Benner, 1996) or assembled into the particulate fraction (POM) (Chin et al., 1998, Engel et al., 2004, Verdugo et al., 2004), whereas most DOM remaining in the ocean as LMW DOM is resistant to biological utilization (Amon and Benner, 1996, Ogawa and Tanoue, 2003) . Bacteria can also contribute to DOM production through active release or cell lysis (Ogawa et al., 2001). Identifying DOM sources and phase transition processes between DOM and POM pools is essential while assessing the role of oceanic OM in the global carbon cycle (Benner, 2002, Verdugo, 2012). Marine colloids, which have physical characteristics of polymer gels (Alldredge et al., 1993), constitute a very large fraction of DOM and their spontaneous aggregation is an important step in the transformation process of DOM into POM (Chin et al., 1998, Verdugo and Santschi, 2010). Free-dispersed colloids in seawater coagulate to form larger hydrated three-dimensional jelly structures that link the marine dissolved and particulate organic matter pools (Verdugo et al., 2004, Verdugo and Santschi, 2010, Verdugo, 2012).

Dissolved colloids released by phyto-and bacterioplankton during their metabolic processes undergo physico-chemical mechanisms of polymer gels: these precursors organize in three-dimensional supramolecular structures, held together by physical or chemical cross-links between the polymers of the network (Chin et al., 1998, Verdugo and Santschi, 2010). Seawater embedded in this network prevents the three-dimensional structure from collapsing, and at the same time the network holds the solvent in a thermodynamic equilibrium with the surroundings (Alldredge et al., 1993). Depending on their

concentration, polymers in solution can readily entangle with each other forming a random network connected by low-energy physical bonds (figure 2).

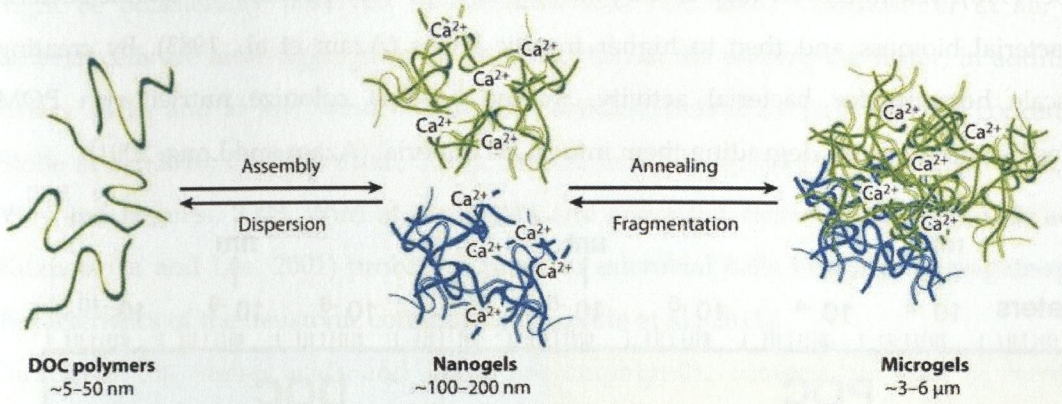

Figure 2. Phase transition of dispersed colloids in seawater from DOC polymers to microgels (From Verdugo, 2012).

The cross-links connections between polymers are reversible and depend on the number of bonds, and ultimately, on the polymer length (Verdugo, 2012). The network of polymer gels, predominantly polyanionic (Verdugo and Santschi, 2010), is stabilized by Calcium (Ca^{2+}) bonds; therefore, specific functional groups (like the carboxyl group of acidic polysaccharides, -RCOOH) of the monomers constituting the polymer chain determine their growth and reactivity to specific elements, like trace metals (Engel et al., 2004). Phase transitions for microgels depend on environmental factors like pH, temperature, pressure and ion density (Passow, 2002); from the abiotic assemblage of dissolved precursors released by phytoplankton (< 0.2 µm) into polymer gels (Chin et al., 1998) the formation of larger particles (> 0.4 µm) relies on a further cascade of aggregation processes converting dissolved polysaccharides into particulate organic carbon in the form of macrogels (Engel et al., 2004) (figure 3).

According to methodological procedures, marine gels can be distinguished into Transparent Exopolymer Particles (TEP), of mainly polysaccharidic, and Coomassie Stainable Particles (CSP), of proteinaceous composition (Engel, 2009). In the ocean, TEP and CSP are found closely associated so that it has been suggested that these two classes of marine gels might represent only subunits of the same particle (Engel, 2009).

The interaction of marine gels with heterotrophic bacteria, besides providing a physical structure, makes them an essential element of the microbial loop in the ocean (Verdugo,

2012), since these organic particles represent nutrient-rich substrates for bacterial uptake and remineralization of organic matter (Azam et al., 1993, Long and Azam, 1996, Mari and Kiørboe, 1996). The microbial loop is the pathway of DOC incorporation by marine bacteria into bacterial biomass and then to higher trophic levels (Azam et al., 1983). By creating microscale hotspots for bacterial activity, marine bacteria colonize nutrient-rich POM particles like marine gels, degrading them into DOM material (Azam and Long, 2001).

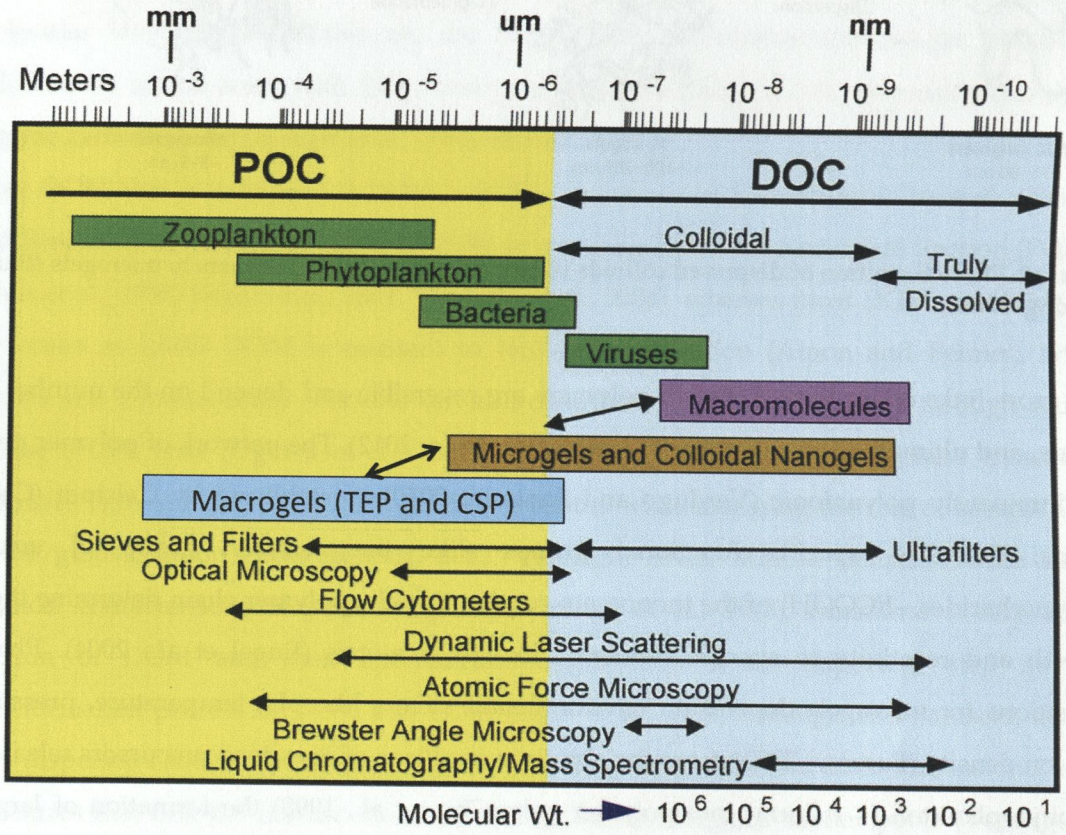

Figure 3. Size aggregation continuum of DOC into the colloidal phase to the POC pool (Adapted from Verdugo et al. 2004). Also shown are the measuring methods for detecting each particle size range.

Microorganisms inhabiting the water-air interface are known as *neuston*, from an early definition by Naumann in 1917 (Sieburth, 1983). Bacterioneuston – bacterial communities of the SML - can be highly different from the communities of the water below although originating in scavenging processes and being affected by spatial and meteorological heterogeneity (Stolle et al., 2010). High levels of UV radiation, temperature changes and organic anthropogenic pollutants (Wurl and Obbard, 2004, Agogué et al., 2005) do not

encourage bacterial development in the SML (Cunliffe et al., 2009, Stolle et al., 2009, Cunliffe et al., 2011) however, enhanced activity supported by high DOM and POM concentrations might be occasionally observed (Kuznetsova and Lee, 2001, Obernosterer et al., 2005). Bacterial cells are more aggregated in the SML than in the underlying water; in addition, in surface slicks and at low wind conditions particle-attached bacteria may be concentrated (Stolle et al., 2010, Cunliffe et al., 2011). Enrichment in aggregates (Keith Bigg et al., 2004, Wurl and Holmes, 2008, Wurl et al., 2011b) and enhanced extracellular enzymatic activity (Kuznetsova and Lee, 2001) probably confer to microbial cells buoyant, aggregate-specific characteristics of the neustonic community (Cunliffe et al., 2011).

Carbohydrates, amino acids and lipids are amphiphilic compounds, that is, have both hydrophobic and hydrophilic moieties that enhance their surface-active properties facilitating their adsorption at the water-air interface (Henrichs and Williams, 1985). Additionally, water surface-tension can stimulate collision between dissolved precursors resulting in the formation and growth of polysaccharidic marine gels (Wurl et al., 2011b). The formation of larger particles contributes, in turn, to lower surface water tension thus rendering the SML a stable environment even under wind speeds up to 8.7 m s^{-1} (Wurl et al., 2011a).

Since marine gels can ascend the water column (Azetsu and Passow, 2004) and fuel the gelatinous SML (Mari, 2008), the heterotrophic degradation of these nutrient-rich macromolecules converting POM into DOM (Verdugo, 2012) might represent an additional source of dissolved carbohydrates and amino acids at the water-air interface (Cunliffe et al., 2013).

AIR-SEA GAS EXCHANGE AND PRIMARY ORGANIC MARINE AEROSOLS (POA)

The oceans cover 71% of the world's surface, and so does the sea-surface microlayer (MacIntyre, 1974) and besides being a highly dynamic biofilm it is a physically stable environment due to strong surface-tension forces (Hardy, 1982). Breaking waves in the ocean may disrupt it for a temporary period, but these kinds of waves only represent 3-4% of the ocean (MacIntyre, 1974) and even at higher wind speeds the SML is still present (Wurl et al., 2011b).

Surface tension is a force expressed in Newton per meter, which tends to reduce the area of free surfaces or interfaces, affecting the shape of sea-spray droplets. In seawater, surface tension is greater than in fresh water at the same temperature and can be expressed as

$$\gamma = (75.63 - 0.144T + 0.221S) \times 10^{-3} \text{ [N m}^{-1}\text{]} \quad (2)$$

Where temperature (T) is expressed in °C and salinity (S) in ppt (Massel, 2007).

Due to its continuity on the ocean's surface, the SML is a critical component of air-sea gas exchange processes across the water-air interface (Liss and Duce, 2005, Frew and Liss, 2005). Turbulent and molecular diffusion drive gases and matter fluxes between the ocean and the atmosphere: the first process is mainly found in the water right below the SML and in the air in close contact to it, while the second one occurs at the level of the SML itself where capillary waves are suppressed (Upstill-Goddard, 2006) implying effects on aerosols emission (Laß et al., 2013).

Upstill-Goddard (2006) has proposed a conceptual model for the air-sea interface at steady state (figure 4). Across the interface, gas transfer velocities in air and water define the transport rates, which depend on the depth of the sub-diffusive layers and the physicochemical and biological structure of the SML (Cunliffe et al., 2013). Different layers are connected in a continuous way; however, the resistance to the gas transfer from one layer to the other is variable and the thinnest diffusive sub-layers are the ones providing the highest resistance (Massel, 2007).

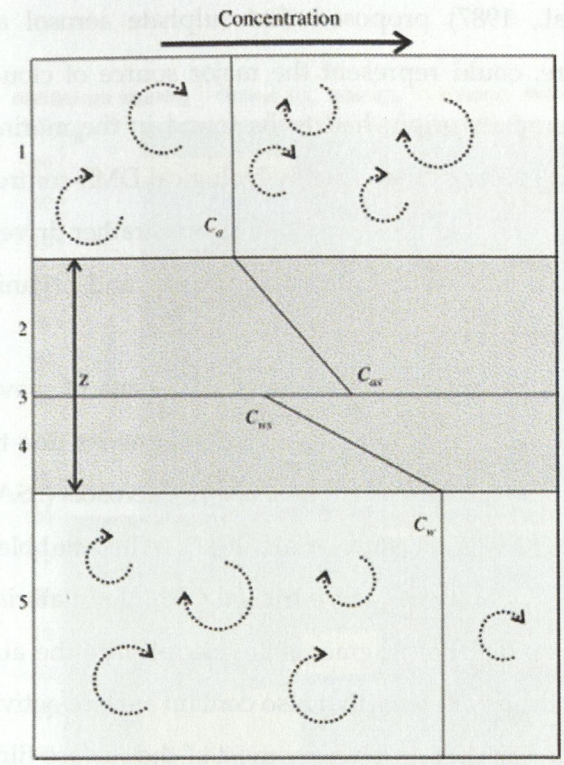

Figure 4. Conceptual model of air-water interface at steady state, where compartments 1 and 5 represent boundary layers where turbulent diffusion dominates with gas concentrations C_a (in the air) and C_w (in the water). Regions 2-4 represent diffusive sub-layers and region 3 is the air-water interface. C_{as} and C_{ws} are gases concentrations at the air-side and water-side respectively. (From Upstill-Goddard, 2006).

The concentration difference in turbulent layers or diffusive sub-layers does not occur for all gases: methane (CH_4), a less soluble gas, shows a discontinuous concentration at the water air-interface (figure 4) while carbon dioxide (CO_2), more soluble, does not (Upstill-Goddard, 2006): CO_2 transfer rate can be increased four times or more by small ripples that thin the layer across which the gas has to pass through molecular diffusion and additionally by large waves (MacIntyre, 1974).

Methane is a long-lasting greenhouse gas and oceanic emissions are one of the largest contributors of methane to the atmosphere (Bange et al., 1994). Additionally, its distribution patterns in the turbulent and diffusive layers and the subsequent diffusivity across the air-water interface is mediated by bacterioneuston, a possible sink (Upstill-Goddard et al., 2003) and source (Cunliffe et al., 2013) of this climate-relevant gas.

Furthermore, the gas transfer across the ocean surface is of particular importance as many gases might be involved in atmospheric photochemical reactions that transform particulate matter and modify aerosol composition (Matrai et al., 2008). On the ocean surface, dimethyl sulphide (DMS) dominates as volatile sulphur compounds representing a major biological contribution to gaseous atmospheric sulphur (Bates et al., 1992). More than twenty-five years

ago, the CLAW hypothesis (Charlson et al., 1987) proposed that sulphate aerosol as oxidation product of DMS in the atmosphere, could represent the major source of cloud condensation nuclei (CCN) over the oceans and its origin had to be found in the marine boundary layer. Years of study have provided evidence that a solely biological DMS-control over CCN population probably does not occur, and that the aerosol emission is rather driven by the bursting of bubbles at the ocean surface introducing marine inorganic and organic compounds to the atmosphere (Quinn and Bates, 2011).

The presence of a surface organic film that dampens capillary waves by means of wave energy dissipation is relevant: all compounds this film accumulates and compresses due to surface water tension (Liss and Duce, 2005) can be further ejected as sea-spray aerosols (SSA) through bursting bubbles and white cap episodes (de Leeuw et al., 2011). When bubbles form and ascend the water column, the bubbles' membrane gets enriched with film material from the SML and as soon as the bubble bursts the film fragments are ejected into the air. The bursting of the bubbles is followed by jet drops of water that also contain surface-active material (Cunliffe et al., 2013) and probably the smallest drops carry most of the surface film (Blanchard, 1964).

A great fraction of SSA with $r_{80} < 1$ μm (the radius of the particle as a relative humidity of 80%) has an organic composition (de Leeuw et al., 2011) especially during periods of high primary productivity (O'Dowd et al., 2004). These organic particles directly emitted are known as primary organic aerosols (POA) and differentiate from secondary organic aerosols (SOA), which, through atmospheric chemical reactions, are converted into less volatile species and later distributed to the particulate phase (Aiken et al., 2008).

During phytoplankton blooms, submicron POA dominates total aerosol mass distribution over the ocean and is mostly composed of water insoluble colloids (figure 5) (O'Dowd et al., 2004, Facchini et al., 2008).

Aerosols are involved in climate radiative effects, either directly (radiative properties based on aerosol optical thickness) or indirectly (formation and properties of clouds) (Andreae and Rosenfeld, 2008). Aerosols' effects on climate ultimately influence the marine atmosphere and Earth's albedo, either by aerosol concentration alone which affects light scattering, or by acting as cloud condensation nuclei (CCN), on which water vapor condenses leading to the formation of clouds (Novakov and Penner, 1993, Andreae and Rosenfeld, 2008, de Leeuw et al., 2011).

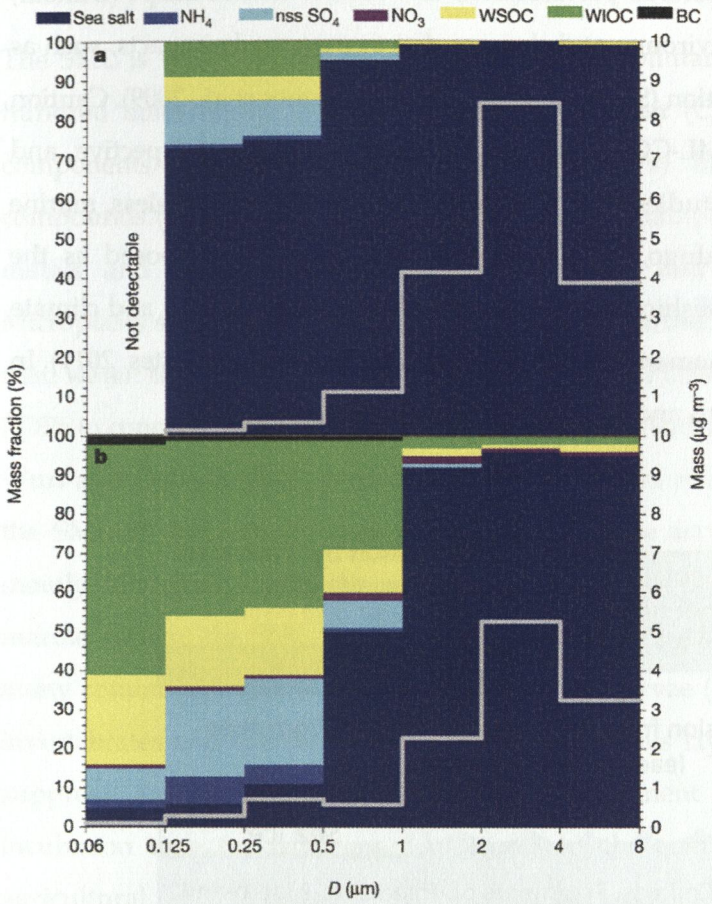

Figure 5. Chemical and mass size distributions for North Atlantic marine aerosol during periods of low (a) and high (b) biological productivity. WSOC = Water Soluble Organic Carbon, WIOC = water Insoluble Organic Carbon, BC = Black Carbon.

(From O'Dowd et al., 2004).

Although aerosol-induced cloud modifications are still poorly understood (Solomon et al., 2007), it has been suggested that POA originating in the SML have a direct determination on CCN concentration and therefore clouds formation and properties (Quinn and Bates, 2011). Since the SML might be a gelatinous layer, most water insoluble POA is potentially composed of marine gels. Due to the importance of this aerosol-cloud interaction, a wide range of studies have been performed in the Arctic to study the gelatinous structure of the SML and the composition of marine aerosols in this region, where low-level clouds (below the height of 2000 m) play a regulating role for climate by controlling radiation balance (Bigg and Leck, 2001, Leck and Bigg, 2005b, Leck and Bigg, 2007, Bigg and Leck, 2008, Gao et al., 2012). Carbohydrate-like submicron particles (Russell et al., 2010) originating in the SML (Leck et al., 2002, Keith Bigg et al., 2004, Leck and Bigg, 2005a, Matrai et al., 2008), are a suspected significant CCN source able to influence low-level arctic clouds properties during

the summer (Orellana et al., 2011). The Arctic is a special, fragile region. Fragile in the sense that it is extremely vulnerable to serious perturbation, to which it cannot adapt (Dunbar, 1973), and is experiencing rapid environmental changes due to man-made impacts, such as global warming and ocean acidification (Solomon et al., 2007, Steinacher et al., 2009). Caution is needed in speculations about SML-CCN-cloud interactions on a wider perspective, and implemented field and laboratory studies should address this question; nonetheless, marine gels are ubiquitous particles (Verdugo, 2012), and the SML might be proposed as the primary source of CCN hence establishing a link between surface ocean biology and climate dynamics, likely to be a global phenomenon (Leck and Bigg, 2007, Quinn and Bates, 2011). In figure 6, all processes implied in POA and CCN formation are displayed.

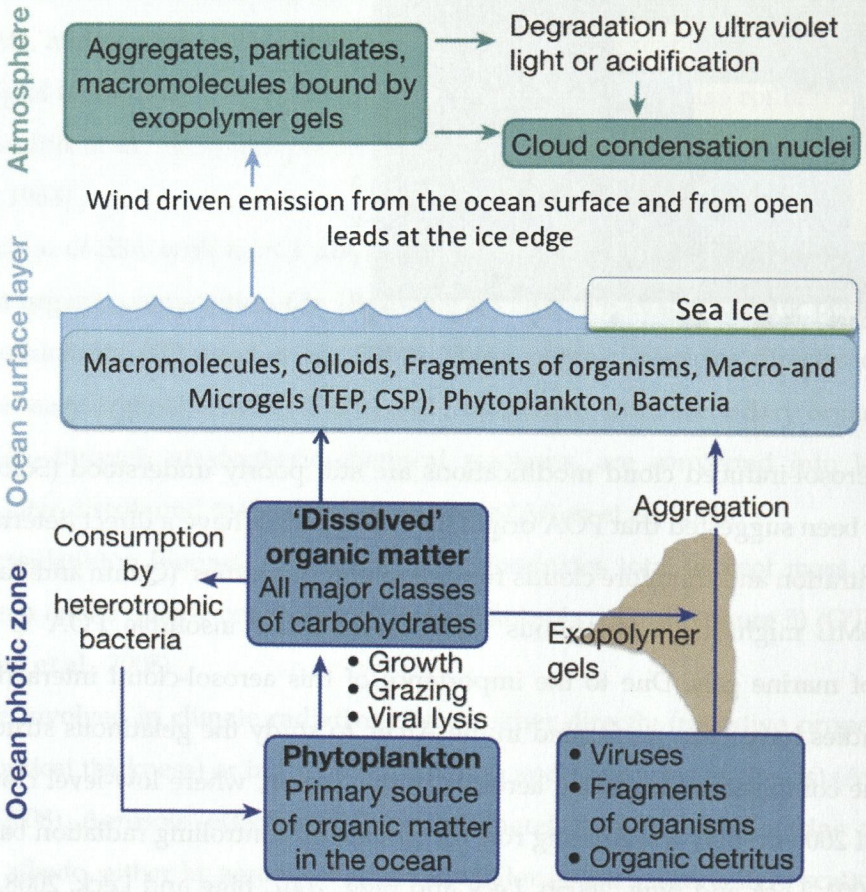

Figure 6. Ocean processes of organic compounds from the photic water column to the ocean surface layer, comprising the SML and Sea ice, to the atmosphere, determining CCN population (Adapted from Quinn and Bates, 2011).

POLLUTANTS IN THE SML

The SML is also a repository of anthropogenic pollutants which can accumulate up to a hundred times higher than in the underlying water (Cunliffe et al., 2013). Among these components, persistent organic pollutants (POPs) like hydrocarbons, organochlorine compounds (PCBs, polychlorinated biphenyls for example) and pesticides, as well as heavy metals and microplastics can be found (Wurl and Obbard, 2004, Andrady, 2011). Microplastics are plastic compounds resistant to further degradation and often less dense than water: they can float on the surface and absorb POPs, representing an additional way of POPs to concentrate in the SML (Teuten et al., 2007, Andrady, 2011).

Wurl and Obbard (2004) provided a very exhaustive review on POPs and heavy metals in the SML. In their study, they pointed out that the accumulation of these compounds of mainly terrestrial origin can imply serious ecotoxicological consequences for the whole marine system: the SML is a unique micro habitat for a variety of organisms: fish eggs of many commercial species, (i.e., atlantic cod) and larvae (echinoderm, clam) bacteria, micro-invertebrates and marine algae. Contaminants in this layer are likely to increase mortality, suppress growth rates and encourage development of abnormalities and prolonged incubation time for fish eggs. Unfortunately, the combination of wastewater discharge, agricultural and industrial run-off, shipping activities and atmospheric deposition of combustion residues makes coastal areas, bays and harbors highly enriched in these pollutants compared to open ocean (Wurl and Obbard, 2004), when these areas are also widely utilized as spawning grounds for a wide range of different species.

It has been suggested that the accumulation of pollutants in these areas goes along with high enrichment of organic matter in the SML: the reduced surface tension already caused by high density of particulate OM provides a substrate for pollutants enrichment leading to formation of visible slicks (Garabetian et al., 1993). Studies have shown that DOM and POM can control the concentration and fate of heavy metals in the SML (Wurl and Obbard, 2004): metal-complexing moieties in the colloidal OM phase are known (Wells et al., 1998) and the DOC pool also contains polyanionic polysaccharides that could bind metal cations in solution (Benner et al., 1992, Benner, 2002).

Little is yet known about pollutant transfer across the water-air interface from both direction and the export either to the atmosphere or to the pelagic ocean; moreover, the implications of

the latter dynamics for global processes are also uncertain and need further investigation (Wurl and Obbard, 2004, Cunliffe et al., 2013).

SAMPLING TECHNIQUES

There are different techniques for sampling the SML, with the need of establishing a univocally recognized best practice that allows for the measurement of SML thickness as well (Cunliffe et al., 2013). According to Zhang and colleagues (Zhang et al., 1998, Zhang, 2003), the thickness of the SML is 50±10 μm referred to as "the layer of sudden physical changes" that was measured in laboratory studies. However, the thickness of the SML greatly depends on sampling techniques and remains to be determined (Cunliffe et al., 2011). Many methods have been tested and main differences rely on the affinity of SML compounds to either one or the other material of the sampling device (e.g., Van Vleet and Williams, 1980, Agogué et al., 2004, Stolle et al., 2009).

Historically, one of the mainly recognized practices was first introduced by Garrett (1965). It consists of a 16-mesh screen of 0.18 mm diameter wire, withdrawn from beneath the surface across the water-air interface while maintaining it horizontal to the sea-surface (Intergovernmental Oceanographic Commission, 1985). This method allows for the collection of a 150-400 μm – thick SML, with large volume for sample analysis but that might be a mixture of SML and underlying water (Cunliffe et al., 2011).

Another technique is the rotating drum described by Harvey (1966): a surface collector, like a battery-operated catamaran vessel of approximately 2.0 m length x 1.4 m width x 0.5 m height (Knulst et al., 2003) equipped with a smooth rotating cylinder having a hydrophilic surface like ceramics or Teflon, is placed on the water, and the sample collected on the surface of the cylinder is removed as the cylinder rotates via a wiper closely attached. This device can remove a very thin microlayer < 100 μm (Matrai et al., 2008) and can also provide large sample volumes (Harvey, 1966) but because of its dimensions, its applicability might be restricted to field samples in open sea areas during calm conditions.

Ultimately, a simple and quite common method is the glass plate (Harvey and Burzell, 1972). It consists of usual glass, 4-5 mm thick, inserted into the water perpendicular to the surface and withdrawn at a controlled rate (~20 cm sec^{-1}). The sample is retained through surface tension and can collect a layer of approximate thickness 60 to 150 μm, which is removed by

the use of a wiper. The disadvantage of this method is as with the mesh screen, that the sampling operation is difficult to standardize (Cunliffe et al., 2011).
The glass plate method was chosen during this study for its easy-to-handle characteristic, the applicability in every study site, from laboratory experiments to field campaign, and the possibility of comparing our results with colleagues involved in our research experiments.

CLIMATE CHANGE AND THE SML

While climate variability is the result of natural causes for occasional climate variation, climate change is attributed "directly and indirectly to human activity that alters the composition of global atmosphere in addition to natural variability observed over comparable time periods" (UNFCCC, 1994). Such human activities, mainly fossil fuel burning, change in land use, agricultural and industrial practices lead to the increased abundance of greenhouse gases and aerosols altering the energy balance of the Earth (Solomon et al., 2007).
Latest measurements on atmospheric CO_2 report a concentration of 398.09 ppm (July 2013, Mauna Loa Observatory, NOAA). Rising atmospheric CO_2 cannot be counterbalanced by primary production at the same rate as it is emitted by anthropogenic activities. The increased partial pressure of CO_2 in the atmosphere leads to an increased dissolution of the gas into the ocean. CO_2 reacts rapidly with water forming carbonic acid (H_2CO_3), a weak acid that dissociates fast to bicarbonate (HCO_3^-), carbonate (CO_3^{2-}) and hydrogen (H^+) ions. Through photosynthetic carbon fixation and calcium carbonate formation ($CaCO_3$), marine biological processes play an important role in the global carbon cycle and in the control of atmospheric CO_2. $CaCO_3$ in seawater acts as a buffer for increasing CO_2, and its saturation state is a function of the carbonate ion concentration (Zeebe and Wolf-Gladrow, 2001). However, the equilibration and dissolution of the excess anthropogenic CO_2 into marine systems leads to a progressive decline in surface ocean pH and CO_3^{2-} concentration as a result of increased H^+ concentration; a process which is referred to as ocean acidification (Caldeira and Wickett, 2003).
Whereas 50% of anthropogenic CO_2 exists in the upper first 400 m of the water column, the highest concentration is found in the immediate subsurface waters because of gas-exchange

processes across the air-sea interface (Sabine et al., 2004). Seawater pH is expected to drop by up to 0.5 units in the next 100 years (Caldeira and Wickett, 2003).

Besides the gradual dissolution of CO_2 by oceanic waters, an indirect effect of climate change is the increase in sea-surface temperature. According to the IV IPCC report (Solomon et al., 2007), an expected rise in surface air temperature by 1.1 to 6.4°C by the year 2100 will lead to a further global average sea-surface warming, which has already increased by 0.74°C during the last century. Influences of ocean acidification and sea-surface warming have been widely investigated for marine calcifying organisms (Riebesell et al., 2000, Borchard et al., 2011, Bach et al., 2012, Borchard and Engel, 2012), for phyto- and bacterioplankton metabolic activities and ecosystem structures (Riebesell et al., 2007, Engel et al., 2008, Piontek et al., 2010, Endres et al., 2013, Engel et al., 2013, Piontek et al., 2013) and for the recycling of organic matter in the ocean (Engel, 2002, Engel et al., 2010).

Marine bacteria are the greatest consumers and contributors to the DOM pool in the ocean (Benner, 2002) and under elevated CO_2 microbial degradation of organic matter seems to increase (Piontek et al., 2010, Endres, 2013). It has been proposed that, rising oceanic CO_2 will facilitate an enhanced upward flux of marine gels to the SML reducing their stickiness and promoting swollen, less dense structures (Mari, 2008) hence stimulating intense bacterioneuston activity (Cunliffe et al., 2011).

Effects of climate change, like ocean acidification, are most dramatically observed in the Arctic Ocean, yet how the Arctic will withstand these new conditions is hard to predict because changes happen so fast that long-term responses are difficult to foresee. The Arctic Monitoring and Assessment Programme (AMAP, 2013) has recently released a summary for policymakers with major key findings concerning marine biota as well as social-economic implications of Arctic Ocean acidification. Cold waters hold more carbon dioxide but at the same time, sea-surface warming and reduced sea-ice cover lead to a freshening of arctic waters.

All these combined effects decrease the concentration of ions able to buffer the increased uptake of CO_2, and enhance the undersaturation of biologically produced $CaCO_3$ with the result, that ocean acidification effects occur in a rather shorter timescale in the Arctic than elsewhere (Yamamoto-Kawai et al., 2009, Steinacher et al., 2009).

Ocean acidification must be contextualized together with all processes that act simultaneously accelerating the effects of global change. Rapid changes occur for a variety of

factors: the loss of sea ice and sea ice albedo, due to surface warming, accelerates the further extending of open water (dark body) which absorbs even more radiation creating a positive feedback. Furthermore, the stable stratification of the arctic atmosphere reduces the exchange of air between the surface and the troposphere above, thus inducing the quick warming of a layer close to the surface during the summer (Hov et al., 2007, Serreze et al., 2007). In turn, arctic primary productivity enhanced by surface warming and sea ice loss (Arrigo et al., 2008, Arrigo et al., 2012, Boetius et al., 2013) is suggested to affect the organic composition of arctic surface waters and SML, with implications for aerosol composition and CCN activation influencing Earth's albedo and radiative budget (Orellana et al., 2011). Such positive feedbacks in the Arctic are referred to as "tipping points", meaning the moment when internal dynamics triggered by global climate warming start promoting a change previously driven by external forces (Walker, 2006).

Onto this backdrop of alterations like ocean acidification and temperature rise, comes the question of how the SML will react, and why these studies will be important. All biological responses of the ocean to changes in atmospheric temperature, atmospheric contaminants and greenhouse gas concentration are suggested to affect the composition and reactivity of the SML where ocean-air interactions take place. During the last decades many studies have addressed the composition of the SML itself and its role in air-sea gas exchange and aerosol formation. Nowadays it becomes of enormous importance to understand how climate change affects the SML, and how this is reflected in global processes involving atmospheric dynamics and, ultimately, aerosol and cloud feedbacks on climate.

MacIntyre (1974) suggested plotting, on a logarithmic scale, a cross section of the ocean from the size of a water molecule lying on the surface to a maximum depth of 10 kilometers (figure 7). Taking the dimension of the water molecule of 1-2 angstroms, corresponding to 10^{-10} meters, the top millimeter in the logarithmic scale corresponds to the top half of the ocean. This way of viewing the "surface" of course does not account for water masses but it is helpful to visualize the importance of the very top of the ocean, in terms of processes. In his figure, MacIntyre still refers to the old view of dry and wet surfactants that structure the SML in a sort of multi-layer environment, but nevertheless his idea has the advantage to make us think on how complex the ocean's surface is, and how many interactions take place there.

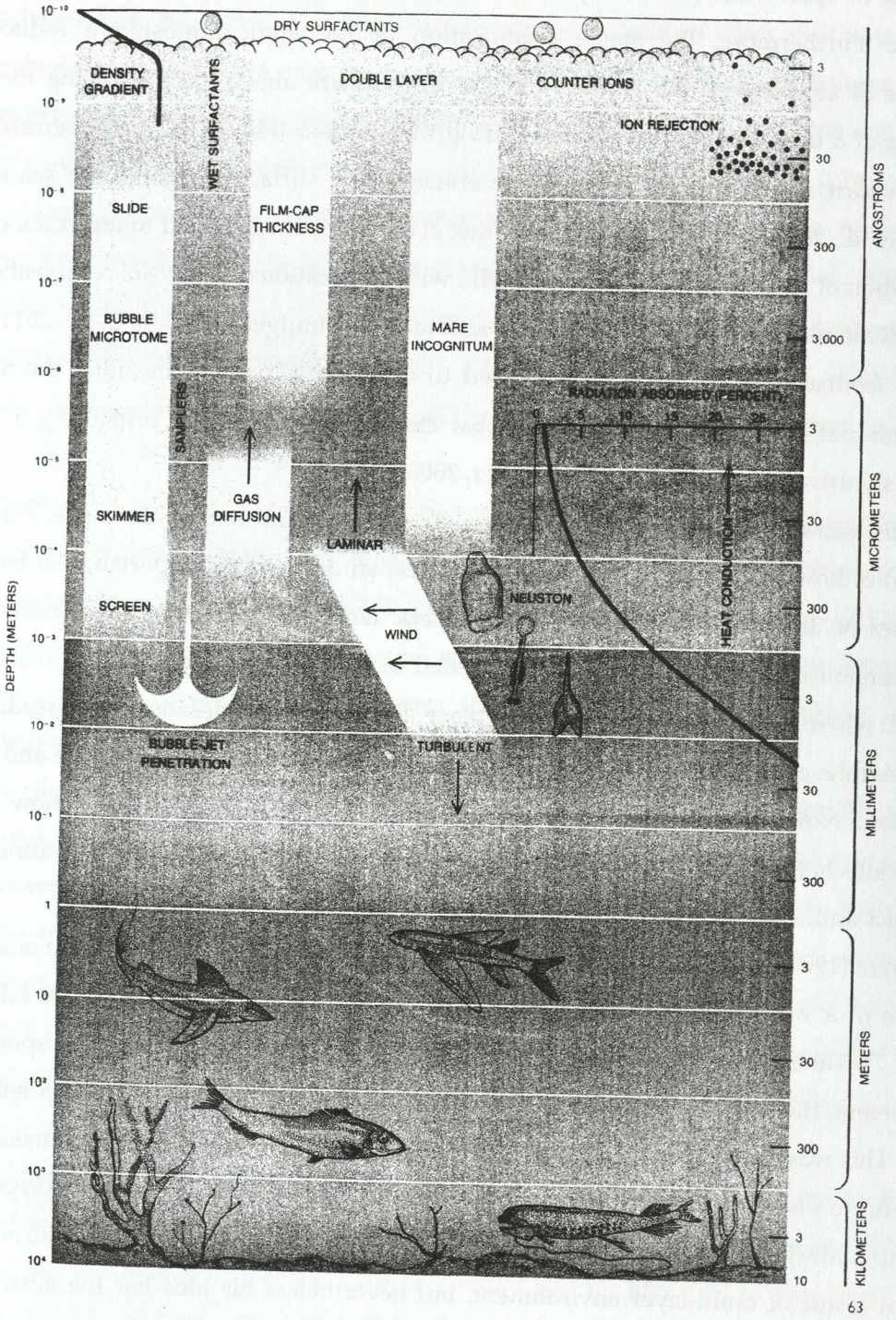

Figure 7. Cross section of the ocean on a logarithmic scale, starting from 10^{-10} m, the dimension of a water molecule lying on the surface (1-2 angstroms) to a maximum depth of 10 kilometers (From MacIntyre. 1974).

OUTLINE OF THE THESIS

All ocean-atmosphere interactions have to pass through the SML, the ocean's skin. This boundary layer between the marine and the atmospheric systems is recognized being a very special and independent environment susceptible of mediating air-sea gas exchange and the emission of aerosols across the water-air interface.

As a continuation of previous studies on the composition of the SML, e.g. (Matrai et al., 2008, Wurl and Holmes, 2008, Cunliffe and Murrell, 2009, Stolle et al., 2010, Orellana et al., 2011, Gao et al., 2012, van Pinxteren et al., 2012), this thesis focused on gel particles like TEP and CSP because of their general importance in mediating vertical carbon transport, either to the sea-surface or to the deep ocean. The coupling of these macromolecules to bacterial activity in the SML might highlight processes relevant for gas exchange properties of the surface ocean and the emission of POA from marine systems. This project was aimed at understanding how climate change can impact the composition and emission of marine aerosols to the atmosphere affecting the properties of the sea-surface microlayer.

From a laboratory experiment intended to elucidate the biological origin of the gelatinous SML, the present thesis further develops in a mesocosm study simulating future ocean scenarios of increasing CO_2. To the best of the author's knowledge, there are no previous investigations on ocean acidification effects on the SML. Ultimately, results from a field campaign in the Central Arctic conducted during the summer of the 2012 sea ice minimum are described. Special emphasis is given to gel particles like TEP and CSP in the SML, with the purpose to investigate how DOM turnover and SML composition can be altered by climate-change related processes. Significance and possible implications for POA emission, and, at long last, for climate relevant atmospheric dynamics likely to be globally valid are also discussed.

The thesis has been formatted into three manuscripts, of which the outline is given here.

Manuscript I reports on the gelatinous composition of the sea-surface microlayer and the accumulation of gel particles as a result of biological activity during a laboratory experiment. Results on marine gels (TEP and CSP), bacterial abundance, total nitrogen and total combined carbohydrates are compared between the SML and the underlying water of three sampling tanks where a diatom culture was added, and one control (blank) reference tank of

solely seawater. The diatom culture of the three tanks was grown at three different pCO_2 in chemostats and the temporal differences in the microlayer components are also discussed.

Manuscript II deals with CO_2-mediated changes of the SML composition during a mesocosm study. Results on marine gels, bacterial abundance and activity, total combined carbohydrates and total amino acids are compared and discussed. The size-distribution of marine gels in the SML at different pCO_2 is presented along with possible implications for POA dynamics.

Manuscript III presents data from the ARK27-3 cruise to the central Arctic on board of RV Polarstern in the summer of 2012, where SML and underlying water samples from melt ponds, open leads at the ice edge and open sea were taken. Results on DOM dynamics and marine gels in the SML are discussed in order to understand how the loss of sea-ice (and ultimately climate change) influences surface water biology and the emission and composition of POA in the central Arctic, where low level clouds play a regulating role for climate.

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MANUSCRIPTS

MANUSCRIPT I:

Luisa Galgani and Anja Engel (2013)

Accumulation of Gel Particles in the Sea-Surface Microlayer during and Experimental Study with the Diatom *Thalassiosira weissflogii*

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Luisa Galgani, Christian Stolle, Sonja Endres, Kai G. Schulz, Klaus Jürgens and Anja Engel (2013)

The Sea-Surface Microlayer is susceptible to Ocean Acidification

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MANUSCRIPT III:

Luisa Galgani, Judith Piontek and Anja Engel (2013)

The Composition of the Sea-Surface Microlayer in the Central Arctic under enhanced Sea Ice Melting Conditions

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DECLARATION OF CONTRIBUTION TO EACH MANUSCRIPT

MANUSCRIPT I:

Data acquisition: Luisa Galgani sampled and analyzed marine gel particles, total combined carbohydrates, bacterial abundance and total nitrogen.
Data interpretation and preparation of the manuscript: Luisa Galgani with comments from Anja Engel.

MANUSCRIPT II:

Data acquisition: Luisa Galgani sampled and analyzed marine gels, amino acids and carbohydrates in the sea-surface microlayer with help from Christian Stolle, Sonja Endres and other participants of the mesocosm experiment (mesocosms logistics and sampling). Christian Stolle provided data on bacterial abundance and activity. Kai G. Schulz provided pH and Chlorophyll *a* data.
Data interpretation and preparation of the manuscript: Luisa Galgani with comments from co-authors.

MANUSCRIPT III:

Data acquisition: Luisa Galgani sampled the melt ponds with support from Judith Piontek and other cruise participants and analyzed marine gels, total and dissolved organic carbon and amino acids. Judith Piontek provided data on bacterial abundance and activity.
Data interpretation and preparation of the manuscript: Luisa Galgani with comments from co-authors.

ACCUMULATION OF GEL PARTICLES IN THE SEA-SURFACE MICROLAYER DURING AN EXPERIMENTAL STUDY WITH THE DIATOM *THALASSIOSIRA WEISSFLOGII*

Luisa Galgani[1,2]

Anja Engel[1]

[1] GEOMAR Helmholtz-Zentrum für Ozeanforschung Kiel

Düsternbrooker Weg 20

24105 Kiel, Germany

[2]Alfred-Wegener-Institut Helmholtz-Zentrum für Polar- und Meeresforschung

Am Handelshafen 12

27570 Bremerhaven, Germany

ABSTRACT

Since the early 80's, the sea-surface microlayer (SML) has been hypothesized as being a gelatinous film. Recent studies have confirmed this characteristic, which confers properties that mediate mass and energy fluxes between ocean and atmosphere, including the emission of primary organic aerosols from marine systems. We investigated SML thickness and composition in five replicate indoor experiments between September and December 2010. During each experiment, the SML and underlying seawater were sampled from four seawater tanks: one served as control, and three were inoculated with *Thalassiosira weissflogii* grown in chemostats at 180, 380 and 780 ppm pCO_2. We examined organic material enrichment factors in each tank, paying particular attention to gel particles accumulation such as polysaccharidic Transparent Exopolymer Particles (TEP) and the proteinaceous Coomassie Stainable Particles (CSP). While previous studies have observed carbohydrates and TEP enrichment in the microlayer, little is yet known about proteinaceous gel particles in the SML. Our experiments show that CSP dominate the gelatinous composition of the SML. We believe that the enrichment in CSP points to the importance of bacterial activity in the microlayer. Bacteria may play a pivotal role in mediating processes at the air-sea interface thanks to their exudates and protein content that can be released through cell disruption.

Keywords: Sea-Surface Microlayer; Extracellular Polymeric Substances; Organic Matter; Bacterial Abundance; Polysaccharides; TEP; CSP.

1. INTRODUCTION

The sea-surface microlayer (SML) is a specific and dynamic ecosystem at the water-air interface. The SML is susceptible to modification by photochemical reactions, wind-driven atmospheric deposition, water circulation and biological activity: it can be a simultaneous sink and source of natural and anthropogenic compounds [1]. It includes a complex matrix of organic material and microorganisms that at times may form slicks, thus lowering seawater surface tension and influencing air-sea gas and energy exchange [2-4].

It has been widely recognized that physical, chemical and biological processes are very different in the SML compared to the underlying water. However, the determination of its thickness raises some controversy: Zhang [5-7] earlier defined the SML as the uppermost 50±10 μm in situ and in laboratory experiments, while current estimates strongly depend on sampling techniques [8, 9]. Based on scientific literature, the SML can be operationally defined as a several-layer structured microhabitat between 1 and 1000 μm thick: it has been proposed to study physicochemical characteristics in the upper 60 μm, and species dependent biological and ecological features over the upper 1000 μm [1].

Gel particles are three-dimensional networks of polymers penetrated by seawater that can range from 1 nm to several millimeters [10-12] and contribute to the microlayer structure. Transparent exopolymer particles (TEP) are a special group of gel particles composed of polysaccharides that contain acidic sugars [11, 13]. Their sticky properties facilitate aggregate formation and colonization by bacteria [14, 15]. A gelatinous-type composition of the surface film, i.e. a structured hydrated layer of carbohydrates, proteins and lipids was hypothesized early by Sieburth [16] and highlighted in more recent studies focused on polysaccharidic microgels [10, 12, 17].

According to past studies, gel particles can form spontaneously from free polymeric dissolved organic matter (DOM) [18-20] and the growth of these polymers might be enhanced in the SML because of its enrichment in surface-active polysaccharides [10]. Polysaccharides arise mostly from phytoplankton exudates and also represent a considerable fraction of high molecular weight (HMW; >1kDa) DOM in the surface ocean [21]. In the SML, polysaccharides account for about 30% of dissolved organic matter while proteins constitute approximately 16% [16]. Proteins are included in another important class of gel particles known as Coomassie Stainable Particles (CSP) which can be stained using

Coomassie Brilliant Blue, a protein-binding dye [11, 22]. CSP may serve as substrate for particle-associated microbes because pelagic bacteria use proteins as major source of reactive nitrogen [22]. While the abundance and enrichment of protein-like material in the SML has been pointed out by various studies [2, 23-25], to our knowledge the evidence of CSP enrichment has been recorded only once [26], suggesting that TEP and CSP observed might have represented the same particle with a mixed proteinaceous/carbohydrate nature.

Our aim in this study was to obtain a more comprehensive picture of the gelatinous composition of the surface microlayer with respect to its polysaccharidic and proteinaceous components. We chose to explore the dynamics of the microlayer that arise from polymeric components released by phyto- and bacterioplankton. For this purpose we used filtered (< 0.2 µm) North Sea water as medium and as control, and a non-axenic strain of the diatom *Thalassiosira weissflogii* as source of fresh organic matter. *T. weissflogii* was grown at 180, 380 and 780 ppm pCO_2 to further examine if increasing CO_2 scenario may impact surface ocean composition in terms of organic matter and SML formation.

2. METHODS

2.1. EXPERIMENTAL SET UP

Five replicate indoor experiments were performed from October to December 2010. In each experiment, three polyethylene tanks (61 x 36 x 31 cm, sampling surface of 2196 cm^2) were filled with 60 L of North Sea Water (NSW) previously filtered through cellulose acetate cartridges (Sartobran P, 0.2 µm capsule, Sartorius). 10 L of a non-axenic culture of the diatom *Thalassiosira weissflogii* was then added to each tank. The diatom culture was grown for approximately 30 days in three chemostats at D = 0.3 d^{-1}, at an irradiance of 170-180 µmol photons $cm^{-2}s^{-1}$, with a light:dark cycle of 12 h:12 h, and a temperature of 15°C. The chemostats were aerated with 180, 380 and 780 ppm pCO_2, respectively at a gas flow rate of approximately 60 ccm each, similar to the set-up described in Borchard *et al.* [27]. Cell abundance in the chemostats was on average 17266 cells mL^{-1} at 180 ppm, 26503 cells mL^{-1} at 380 ppm and 24414 cells mL^{-1} at 780 ppm pCO_2. One extra tank was filled with 70 L of < 0.2 µm filtered NSW solely and served as a control tank. Before each use, tanks were cleaned with HCl 10% and intensively rinsed with deionized water. The purpose of this setup consisted in getting SML and bulk water samples from simple defined conditions: a

reference of "sterile" filtered seawater, and seawater added with phytoplankton culture which had released distinct exudates according to different pCO_2 growth exposure.

2.2. SEA-SURFACE MICROLAYER SAMPLING

The glass plate approach was chosen to sample the sea-surface microlayer [5, 17, 28, 29]. First introduced by Harvey [30], it is based on the principle that a hydrophilic surface, immersed in the water and withdrawn at a controlled rate can retain a layer of approximately 60 - 100 μm thickness through viscous retention [2]. A glass plate of dimensions 500 x 200 x 4 mm with an effective sampling area of 2000 cm^2 (including both sides of the plate), was pushed vertically into the surface and pulled out at about 20 cm sec^{-1}: at each dip we were able to collect approximately 5 to 7 mL of sample from the glass plate, and we choose to repeat the procedure three times in order to get the first 100 - 150 μm of the surface. The thickness of the surface film was calculated dividing the volume collected by the area of the tank. The glass plate was wiped through Teflon blades and the sample was collected into sterilized glass bottles according to Stolle *et al.* [8]. Prior to use, both the glass plate and the Teflon blades were cleaned with ethanol 70% first and thoroughly rinsed with Milli-Q water thereafter. The bulk water was collected at about 20 cm below the surface from an opening in the tank to avoid any introduction of contamination to the tank itself. Results from the SML samples were compared to those of bulk water and expressed as enrichment factors (EF), defined as:

$$EF = [x]_{SML} / [x]_{BW} \quad (1)$$

Where $[x]$ is the concentration of a given parameter in the SML or in the bulk water (BW) [2]. From the four tanks, samples were taken from SML and from bulk water within one hour from the culture addition (t_0), and then after 24 and 48 hours (t_{24} and t_{48}), mixing the water after each sampling. Five experiments were performed, yielding a total of $n = 15$ samples for the SML in control and $n = 45$ samples for treatments.

2.3. CHEMICAL AND BIOLOGICAL PARAMETERS

Determined parameters included total nitrogen (TN), total combined carbohydrates with a molecular weight > 1 kDa (TCCHO), bacterial abundance, Transparent Exopolymer Particles (TEP) and Coomassie Stainable Particles (CSP). The experiment was replicated five times. TN and TCCHO were determined for three replicates, CSP for four replicates, TEP and bacterial abundance were determined for five replicates. However, it was not possible to perform the analysis for all the previous mentioned parameters over the total 60 samples collected.

TCCHO and TN samples from the SML and bulk water were diluted with Milli-Q water in a ratio of 1:20 for sample analysis. In the experiments I and II, only SML samples were diluted. Therefore, when calculating enrichment factors of these parameters, data from experiments I and II were excluded. No dilution was necessary for bacterial cell number, TEP and CSP.

For TN, 20 mL were filled into pre-combusted (8 h, 500°C) glass ampoules, preserved with 80 µl phosphoric acid (H_3PO_4) 85% and stored at 0°C until analysis. Analysis was performed in four replicates with a Shimadzu TOC - VCSH analyzer with Total Nitrogen (TNM-1) unit, using potassium nitrate (KNO_3) calibration standard over the measurement range 0 - 57 µmolN L^{-1}, and Deep Sea Water reference from Hansell laboratory, University of Miami, Florida.

For bacterial cell number, 750 µl of sample were fixed with 37.5 µL glutaraldehyde (25%) and stored at -20°C until enumeration no later than six months from collection.

Abundance was determined after staining with SYBR Green in DMSO (2%) and analysis with a Flow Cytometer FACSCalibur 4CA (Becton Dickinson).

Microscopical analysis was applied to TEP and CSP. One mL of sample was filtered through polycarbonate filters (Nuclepore) of 0.4 µm pore size (Whatmann) in two replicates, and immediately stained with Alcian Blue (AB) solution for TEP and with Coomassie Brilliant Blue G (CBBG) for CSP following the method described in Engel (2009), and the CytoClear slide technique [31]. TEP and CSP samples were stored at -20°C until microscopy. For each sample two filters were stored, and thirty images were taken per filter area at x200 magnification with a Zeiss microscope. The equivalent spherical diameter (ESD) of individual particles was calculated by measuring its cross-sectional area with an image-

analysis software (ImageJ, U.S. National Institutes of Health), and counts were combined and classified into 66 logarithmic size classes from 1 to 33.5 µm [32, 33].

For total combined polysaccharides > 1 kDa (TCCHO), 15 mL of sample were filled into combusted glass vials (8 h, 500°C) using 25 mL disposable syringes. Samples were frozen immediately and kept at -20°C until analysis. Right before analysis samples were thawed and desalination was performed by membrane dialysis (1 kDa MWCO, Spectra Por) for 5 h at 0°C. To yield monomeric CHO, acid hydrolysis of desalinated samples was conducted with 0.8 M HCl final concentration for 20 h at 100°C followed by neutralization through acid evaporation (N_2) for 5 h at 50°C. After neutralization Milli-Q water was added to the dry residue and analysis was conducted by Ion Chromatography in two replicates on a Dionex ICS 3000 system following the protocol by Engel and Händel (2011)[34].

2.4. DATA TREATMENT

Our experiment was intended to address certain aspects of SML composition and dynamics. In particular, we investigated if there is an equal occurrence of components in both SML and bulk water, or if some of these components are enriched in either one or the other compartment. We focused on organic matter like polysaccharidic and proteinaceous gel particles. When referring to polysaccharides, we aimed at understanding whether carbohydrate composition of SML and bulk water is comparable, or if we find selective enrichment of some sugars.

Moreover, changes in SML composition were studied, with respect to the addition of fresh organic material > 0.2 µm derived from a phytoplankton culture, and with respect to organic matter produced at different CO_2 concentrations.

Assuming a SML thickness of about 100 µm, it was sufficient to sample three times with the glass plate to completely remove the microlayer of the tanks. *i.e.*, for a sampling surface area of 2196 cm^2 and a 100 µm thickness, the volume of the microlayer ideally would be 21.96 mL, approximately the same obtained after three dips of the plate. Therefore it is assumed that for every time point of sampling (t_0, t_{24} and t_{48}), a new microlayer was formed and sampled. Nevertheless, to assess variations among repetitive samplings, concentrations in SML and bulk water for each parameter have been tested with a one-way Repeated Measure ANOVA on normal distributed data with factor being the time of sampling.

Enrichment factors (EF) were calculated based on observations of both SML and bulk water. The number of observations varies between the different analyses. A complete dataset, like for bacterial abundance and TEP, consists of 15 samples for each CO_2 treatment. A dataset for CSP consists in 12 samples for each CO_2 treatment, while for TN and TCCHO the dataset comprises nine observations for each CO_2 treatment. The Friedman Repeated Measures ANOVA on Ranks test was applied on non-normal distributed data (e.g. EF) to assess significant differences between pCO_2 treatments with factor being pCO_2. Spearman Rank correlation coefficients were calculated to determine significant correlations between enrichment factors and SML thickness.

Statistical significance was accepted for $p < 0.05$, and all tests were run with SigmaPlot 12.0 (Systat).

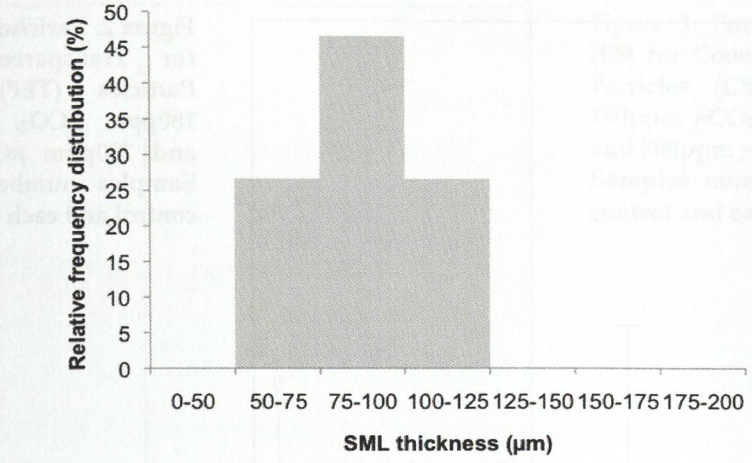
3. RESULTS

3.1. SML THICKNESS

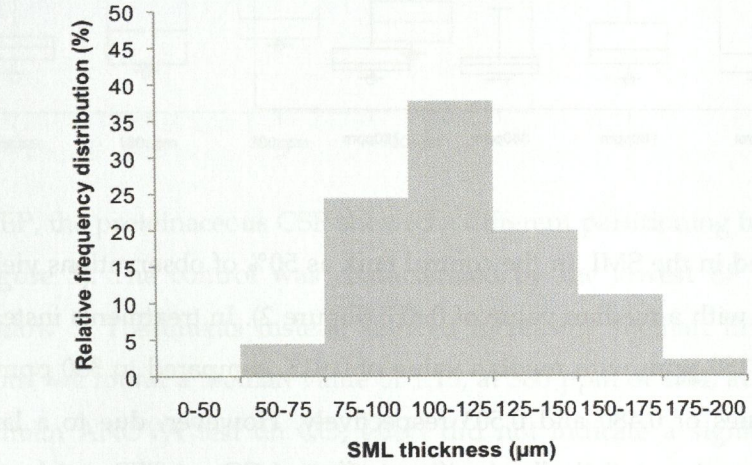
The SML thickness was calculated as the quotient between the volumes obtained with three dips of the glass plate and the surface area of the tank, as resumed in the equation:

$$d_{SML}\ (cm) = V\ (cm^3) / A\ (cm^2) \quad (2)$$

As showed in Figure 1(a), in 47% of the observations SML thickness was comprised between 75 and 100 µm in the control tank. Smaller percentages (27%) relate to thickness of 50 - 75 µm and 100 - 125, being overall in the range between 50 and 125 µm (43%). Most of the observations for treatments (Figure 1 (b), 82%) were comprised between 75 and 150 µm, with 38% in the range 100 - 125 µm, 24% between 75 and 100 µm and 20% between 125 and 150 µm. Smaller percentages were also recorded, below 75 µm (4%) and above 150 µm (13%). Thus, our reference microlayer for this study was represented by the upper 150 µm of the surface.



(a)



(b)

Figure 1. Relative frequency distributions of SML thickness in the five experiments in control ((a), n = 15) and in treatments ((b), n = 45).

3.2. GEL PARTICLES: TEP AND CSP

TEP data proceed from all five experiments, while CSP data were collected in experiments II, III, IV and V.

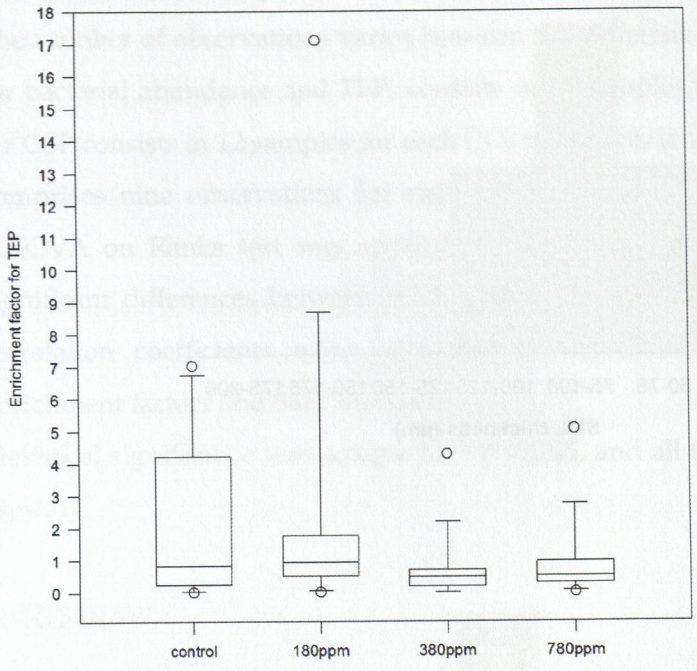


Figure 2. Enrichment factors (EF) for Transparent Exopolymer Particles (TEP) in control, 180ppm pCO_2, 380ppm pCO_2 and 780ppm pCO_2 treatments. Samples number n = 15 for control and each treatment.

TEP were enriched in the SML in the control tank as 50% of observations yielded EF values between 0 and 4, with a median value of 0.817 (Figure 2). In treatments instead, the highest EF was found at 180 ppm with median value of 0.913, compared to 380 ppm and 780 ppm with median values of 0.480 and 0.503 respectively. However, due to a large variability within each treatment, a statistically significant effect of CO_2 on TEP enrichment in the SML was not confirmed (Friedman ANOVA test, $p = 0.158$, $n = 15$).

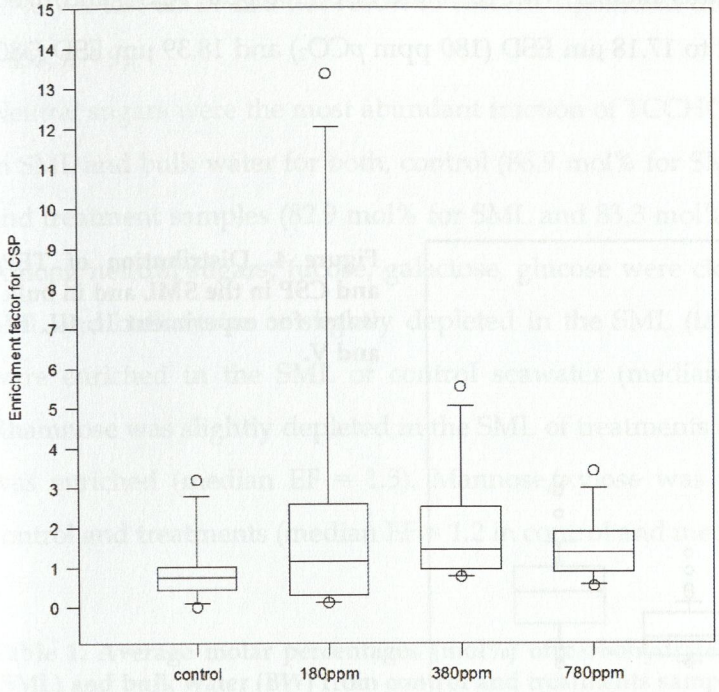


Figure 3. Enrichment factors (EF) for Coomassie Stainable Particles (CSP) in control, 180ppm pCO_2, 380ppm pCO_2 and 780ppm pCO_2 treatments. Samples number n = 12 for control and each treatment.

Compared to TEP, the proteinaceous CSP showed a different partitioning between SML and bulk water (Figure 3). The control was characterized by the lowest EF with 50% of the observations below 1. Treatments instead showed increasing median EF with increasing pCO_2: at 180 ppm, we found a median value of 1.15, at 380 ppm of 1.44, at 780 ppm of 1.34. Although Friedman ANOVA test on CO_2 effect did not indicate a significant trend (p = 0.126), it is interesting to notice how the variability of the values seemed to be distributed over a narrower range with increasing pCO_2. 50% of EF ranged from below 1 to ~2.7 at 180 ppm, from 1 to 2.5 at 380 ppm, and from 1 to ~1.9 at 780 ppm.

CSP dominated gel particles abundance (Figure 4), particularly in the SML. Here median TEP area was 0.2 x 10^6 μm^2 mL^{-1} while median CSP area was 1 x 10^6 μm^2 mL^{-1}, five times higher. In the bulk water median TEP area was 0.4 x 10^6 μm^2 mL^{-1}, double than in SML; median CSP area was the same as in the SML but in the latter higher abundances were also observed. As well shown in the box plot, TEP abundances in the bulk water were higher than in the SML, while CSP abundances in the bulk water on the contrary were lower than in the SML. The equivalent spherical diameter (ESD) is a proxy for particles size. For TEP in the control, the median ESD in the SML was 14.08 μm and larger than in bulk water with 8.42 μm. Similar observations were made for the 780 ppm pCO_2 treatment, yielding 18.97 and 16.31 μm ESD in SML and bulk water, respectively. At low and medium pCO_2 conditions

instead, average TEP size in SML was smaller with 16.60 µm ESD (180 ppm pCO_2) and 16.80 µm ESD 380 ppm pCO_2 compared to 17.18 µm ESD (180 ppm pCO_2) and 18.39 µm ESD (380 ppm pCO_2) in the bulk water.

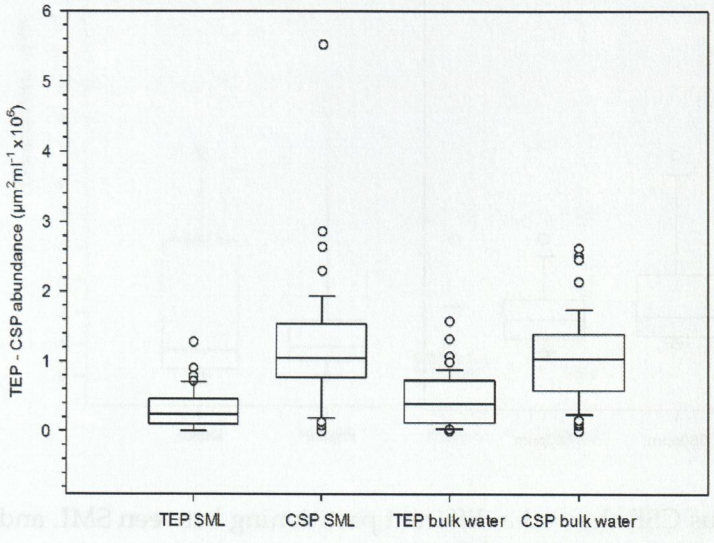


Figure 4. Distribution of TEP and CSP in the SML and in bulk water for experiment II, III, IV and V.

CSP generally included bigger particles than TEP, with values being higher in the SML than in the bulk water. The largest difference was observed at 380 ppm pCO_2 with a median size of 32.06 µm ESD in SML compared to 21.31 µm ESD in the bulk water. In the control, CSP median size was 25.31 µm ESD in SML and 25.92 µm ESD in bulk water; at 180 ppm pCO_2 median SML CSP size was 25.45 µm ESD compared to 23.37 µm ESD in bulk water, while in the highest pCO_2 scenario, 780 ppm, CSP median size in SML was 29.13 µm ESD against 23.45 µm ESD in bulk water.

3.3. TOTAL COMBINED CARBOHYDRATES (TCCHO)

Total combined carbohydrates (> 1kDa; TCCHO) varied between 0.2 and 5.1 µM L^{-1} in SML and between 0.7 and 4.2 µM L^{-1} for bulk water (data not shown). Highest enrichment of TCCHO was observed in the control (median EF = 1.22) (Figure 5). Median EF in treatments were 0.87, 0.87 and 1.09 for 180 ppm, 380 ppm and 780 ppm pCO_2 respectively. Friedman

ANOVA test on EF did not result to discern significant differences with pCO_2 gradient (p = 0.435, n = 9).

Neutral sugars were the most abundant fraction of TCCHO, and yielded similar percentages in SML and bulk water for both, control (86.9 mol% for SML and 87.3 mol% for bulk water) and treatment samples (82.9 mol% for SML and 83.3 mol% for bulk water) (Tables 1 and 2). Among neutral sugars, fucose, galactose, glucose were close to equal distribution between SML and bulk water or slightly depleted in the SML (table 1a). Rhamnose and arabinose were enriched in the SML of control seawater (median EF = 4.5 and 1.5 respectively). Rhamnose was slightly depleted in the SML of treatments (median EF = 0.9) while arabinose was enriched (median EF = 1.5). Mannose/xylose was more abundant in both, SML in control and treatments (median EF = 1.2 in control and median EF = 1.4 in treatments).

Table 1. Average molar percentages (mol%) of carbohydrates in TCCHO > 1kDa of microlayer (SML) and bulk water (BW) from control and treatments samples. In order of listing: fucose (Fuc), rhamnose (Rha), galactosamine (GalN), arabinose (Ara), glucosamine (GlcN), galactose (Gal), glucose (Glc), mannose/xylose (Man/Xyl), gluconic acid (GlucAc), muramic acid (MurAc), galacturonic acid (GalURA) and glucuronic acid (GlcURA).

mol%	Fuc	Rha	GalN	Ara	GlcN	Gal	Glc	Man + Xyl	GlucAc	MurAc	GalURA	GlcURA
control SML	1.3	5.4	10.4	4.2	1.9	1.4	55.6	18.9	0.0	0.0	0.6	0.4
control BW	2.6	1.7	9.3	3.3	1.9	2.9	60.4	16.4	0.0	0.0	1.1	0.4
treatments SML	2.7	2.1	10.4	4.0	2.5	2.2	50.0	21.7	0.3	1.5	0.5	1.9
treatments BW	2.8	2.6	11.2	4.6	2.6	2.3	53.6	17.3	0.1	1.5	0.3	0.9

Table 2. Average molar percentages (mol%) and standard deviations of carbohydrates in TCCHO > 1 kDa in control and treatments, for microlayer (SML) and bulk water (BW), merged into three main classes: neutral sugars (Fuc, Rha, Ara, Gal, Glc, Man/Xyl), amino sugars (GalN, GlcN and MurAc) and acidic sugars (GlucAc, GalURA and GlcURA).

mol%	Neutral sugars (%)	Amino sugars (%)	Acidic sugars (%)
control SML	86.9 (±52.6)	12.2 (±20.2)	0.9 (±1.9)
control BW	87.3 (±45.2)	11.2 (±17.2)	1.5 (±3.8)
treatments SML	82.9 (±41.2)	14.5 (±28.1)	2.7 (±7.4)
treatments BW	83.3 (±58.6)	15.4 (±26.1)	1.3 (±4.5)

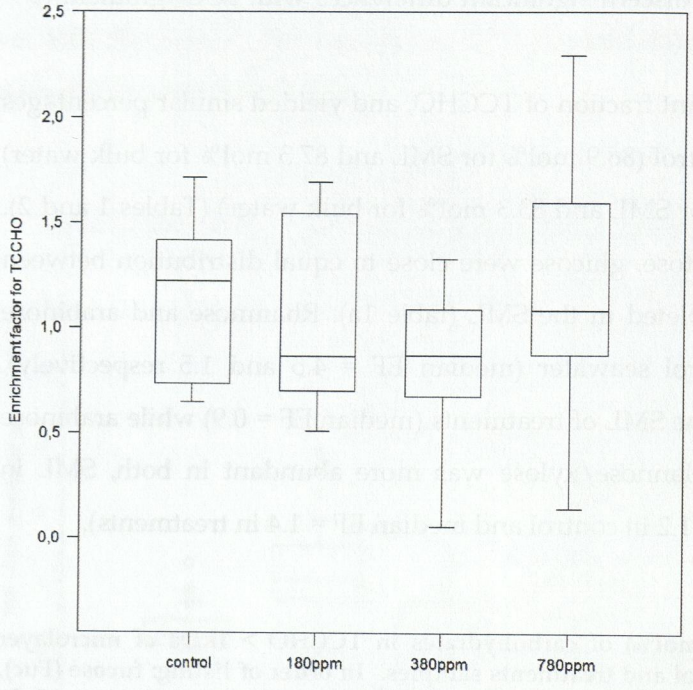


Figure 5. Enrichment factors (EF) for Total Combined Carbohydrates (TCCHO) determined in control, 180ppm pCO_2, 380ppm pCO_2 and 780ppm pCO_2 treatments. For control and each treatment n = 9 samples.

Glucose dominated the composition of polysaccharides in all experiments, with values up to 85 mol% in control and 80 mol% in treatments (Figure 6). Mannose/Xylose were the second most abundant sugars with percentages of up to 40 mol% in both control and treatments. Fucose represented up to approximately 20 mol% of TCCHO in both control and treatments (Figure 6). Amino sugars were observed in relatively high percentages, especially in treatment samples (Table 2). With a range of 9 - 11 mol%, Galactosamine was the most abundant of amino sugars in control as well as treatment samples (Table 1). Contribution of uronic acids, including galacturonic acid and glucuronic acid, to TCCHO > 1kDa was low in general, yielding 0.9 ± 1.9 mol% for SML and 1.5 ± 3.8 mol% for bulk water in control (Table 2). Gluconic acid was detected in low percentages in treatments, where acidic sugars represented 2.7 ± 7.4 mol% of total polysaccharides in SML and 1.3 ± 4.5 mol% in bulk water (Tables 1 and 2).

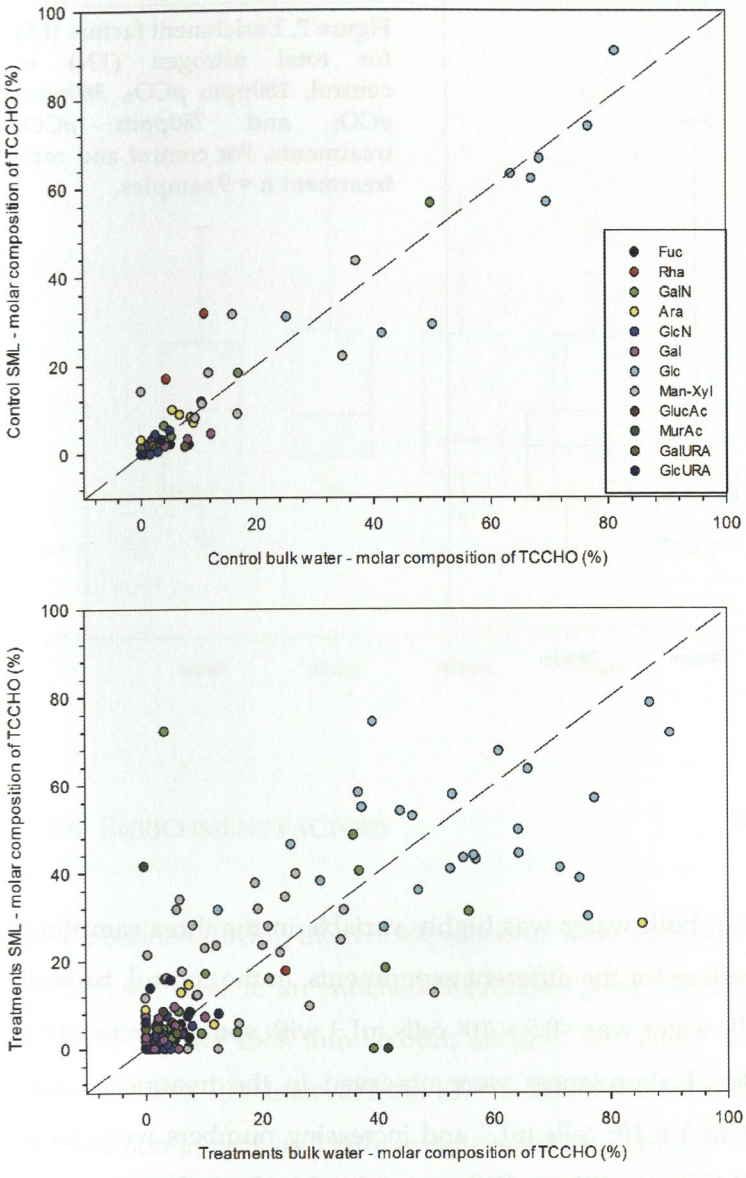


Figure 6. Molar fractions (mol%) of neutral sugars (Fuc, Rha, Ara, Gal, Glc, Man/Xyl), amino sugars (GalN, GlcN and MurAc) and acidic sugars (GlucAc, GalURA and GlcURA).

3.4. TOTAL NITROGEN (TN)

Total nitrogen was determined in the experiments III, IV and V. In general, enrichment factors for TN were highly variable (Figure 7), and did not significantly depend on pCO_2 (Friedman ANOVA test on ranks, $p = 0.352$, $n = 9$). In the control, median EF was 1.01, while in the treatments it increased from 1.24 at 180 ppm to 1.38 at 780 ppm. At 380 ppm median EF was 0.71.

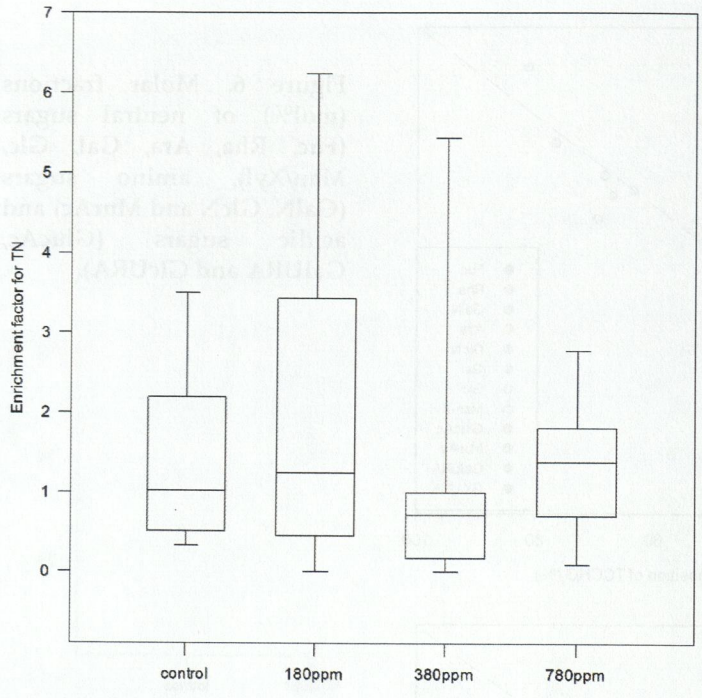


Figure 7. Enrichment factors (EF) for total nitrogen (TN) in control, 180ppm pCO_2, 380ppm pCO_2 and 780ppm pCO_2 treatments. For control and each treatment n = 9 samples.

3.5. BACTERIAL CELL NUMBER

Bacterial abundance in SML and in bulk water was highly variable in the three samplings, both over time (t_0, t_{24} and t_{48}) as well as for the different experiments. In the control, bacterial abundance for both SML and bulk water was $<0.5 \times 10^6$ cells mL^{-1} with some enrichment in the SML. Generally higher bacterial abundances were observed in the treatment tanks, ranging from 0.5×10^6 cells mL^{-1} to 3×10^6 cells mL^{-1} and increasing numbers were found after 48 hours of sampling for all CO_2 conditions. Differences in abundance between t_0, t_{24} and t_{48} were statistically significant for all treatments and controls in the five experiments (One Way Repeated Measure ANOVA on normal distributed data, $p < 0.01$ and $n = 20$ for SML and $p < 0.01$, $n = 20$ for bulk water). In most observations, SML was enriched in bacteria with respect to bulk water. Bacterial EF was calculated for each tank and treatment, and suggested that median EF tended to decrease with pCO_2 increase (Figure 8). However, a CO_2 effect was not significant ($p = 0.93$, $n = 15$, Friedman ANOVA). For 50% of the treatments observations, EF for bacteria ranged between 0.8 and 1.7, with outliers up to 3.

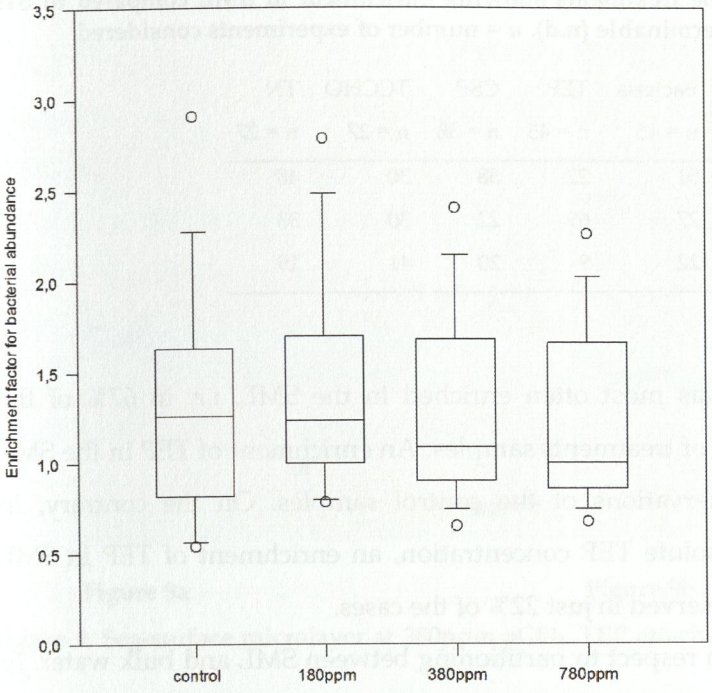


Figure 8. Enrichment factors for bacterial abundance in control, 180ppm pCO_2, 380ppm pCO_2 and 780ppm pCO_2 treatments. For control and each treatment n = 15 samples.

3.6. ENRICHMENT FACTORS

All EF obtained during the five experiments were included in a meta-analysis (Tables 3 and 4). When we refer to an enriched microlayer (yes) we considered all EF > 1.1, while for a depletion (no) we took into account all EF < 0.9. These reference enrichment factors were chosen within a 10% interval from EF = 1, which we consider as uncertainty range; *i.e.* equal concentration in SML and bulk water cannot be excluded. Enrichment or depletion based on factors between 0.9 and 1.1 was hence assumed as being not unambiguously determinable (n.d.).

Table 3. Percentage of samples in the control showing enrichment in SML compared to BW (yes), no enrichment (no) and not determinable (n.d). n = number of experiments considered.

Control %	Bacteria	TEP	CSP	TCCHO	TN
	n = 15	n = 15	n = 12	n = 9	n = 9
yes (enrichment)	67	40	17	56	44
no (depletion)	27	53	67	44	44
n.d.	6	7	16	0	12

Table 4. Percentage of samples in the treatments showing enrichment in SML compared to BW (yes), no enrichment (no) and not determinable (n.d). *n* = number of experiments considered.

Treatments %	Bacteria n = 45	TEP n = 45	CSP n = 36	TCCHO n = 27	TN n = 27
yes (enrichment)	51	22	58	30	48
no (depletion)	27	69	22	30	33
n.d.	22	9	20	41	19

In general, bacterial abundance was most often enriched in the SML, *i.e.* in 67% of the observations of control and in 51% of treatments samples. An enrichment of TEP in the SML was observed in 40% of the observations of the control samples. On the contrary, in treatment tanks having higher absolute TEP concentration, an enrichment of TEP in SML compared to the bulk water was observed in just 22% of the cases.

CSP differed clearly from TEP with respect to partitioning between SML and bulk water. In the control tank, CSP were mostly depleted in the SML, *i.e.* only 17% of observations account for enrichment. In contrast, 58% of treatment tanks showed enrichment in the SML. For total combined carbohydrates (TCCHO), most observations from control tank suggested an enriched microlayer (56%). Instead, for treatment samples an equal amount of observations showed enriched or depleted SML, and in a high percentage of treatment samples enrichment was not clearly determinable (41%). For total nitrogen (TN), no clear pattern of distribution between SML and bulk water was found in control tank, as the same percentages account for enrichment and depletion, and 12% of samples suggested equal distribution. In treatments, TN was enriched in the SML in more experiments (48%), while 33% of the cases manifested depletion. Still, 19% of samples showed similar concentrations in SML and bulk water.

In principle, any component in the SML may become diluted during sampling with the glass plate due to co-sampling of bulk water. This would result in larger sampling volume of SML and thus in greater thickness of SML, and consequently in an underestimation of EF. In order to account for potential bias in EF due to the amount of bulk water collected simultaneously, we related all enrichment factors to SML thickness. Spearman Rank correlation coefficients resulted to be not significant ($p > 0.05$) in any case contemplated in our study, indicating that the sampling volume was not susceptible to interfere with results for EF.

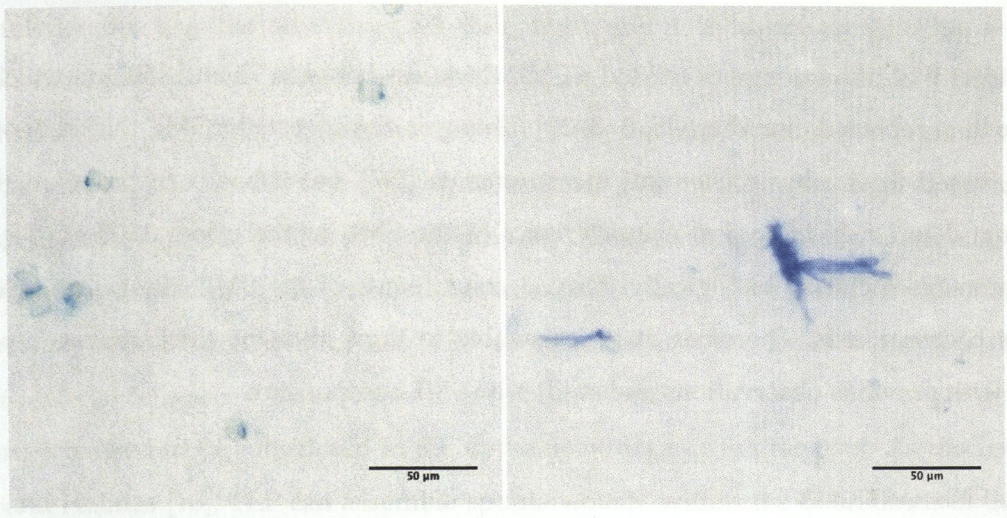


Figure 9a Figure 9b

Figure 9. Sea-surface microlayer at 380ppm pCO_2. TEP attached to diatom cells (a), and "free" CSP (b).

4. DISCUSSION

The aim of this study was to obtain a better understanding of the chemical and biological composition of the SML. Principally we were interested in SML formation as a result of phyto- and bacterioplankton exudation. Consequently, we examined if SML composition would change because of increasing pCO_2 applied to the phytoplankton culture, source of fresh organic material. The phytoplankton culture was grown at controlled conditions of pCO_2 and temperature, and then transferred into tanks where we tested for microlayer formation. Effect of time on accumulation of bacterial cells and gel particles in the SML was investigated sampling the tanks at three different time points. Each pCO_2 treatment was tested against a control tank solely containing filtered seawater, and the whole set up of the experiment was replicated five times.

4.1. THICKNESS OF THE SML

The majority of measurements yielded a SML thickness between 75 and 150 µm, which was greater than reported elsewhere [5, 6, 8, 29]. Zhang *et al.* suggested a SML thickness of 50 ± 10 µm, based upon physicochemical measurements [5-7], but it has been proposed that is meaningful to study biological characteristics of the SML in the upper 1000 µm [1]. Our measurements included biologically derived constituents of the SML like gel particles, as well as bacterial cells. Therefore, it is reasonable to think that the thickness we reported agrees with previous observations and studies on SML composition.

4.2. GEL PARTICLES

Gel particles indicate a biogenic gelatinous nature of the surface film [10, 26, 35], with TEP being the hitherto abundant gels reported in the SML. TEP can facilitate the formation of aggregates in seawater [36] and SML [12]. Our meta-analysis (Tables 3 and 4) suggested that accumulation of TEP and CSP in the SML might be different. While TEP abundance was more pronounced in the SML of control tank compared to the treatment tanks, CSP were mostly enriched in the SML of treatments, but depleted in the control. Examining gel particle distribution in the SML in more detail (Figure 4) showed that CSP were always more abundant than TEP across the five experiments. The presence of TEP > 0.4 µm in the control can be explained by its characteristic to form abiotically from dissolved material smaller than 0.2 µm [18, 19, 37, 38]. On the other hand, TEP have often been observed in diatom aggregates [13, 36, 39-41]. Attachment to diatom cells and subsequent sinking to the bottom of the tanks are hence potential explanations for reduced TEP presence in the surface microlayer, as reflected by the larger average particle size in bulk water and smaller in the microlayer (Figures 9(a) and 9(b)). In natural environments, the formation of ocean bubbles scavenges surface-active material from the pelagic water column and transports it to the surface thus contributing to SML enrichment in organic matter. This situation was not considered in our set up, because bubble size and bubbling intensity could hardly be controlled well enough to allow for comparison between pCO_2 treatments. However, TEP formation has been shown to be enhanced with bubbling and breaking waves [42] and the lack of continuous bubbling in the incubation tanks might be addressed as another factor

limiting TEP production. Wurl *et al.* (2011) investigated a possible bias in TEP SML accumulation due to adhesion to the glass plate, but found no evidence for this [38]. *Thalassiosira weissflogii* may release both small [39] and copious amounts [43] of TEP. A more recent study suggested that TEP production in *T. weissflogii* may be closely related to specific bacteria strains [40]. We did not investigate bacteria community composition during this study, but lack of specific strains may explain low TEP abundance in this experiment. At the same time, the degradation of particulate organic matter (like TEP and CSP) by heterotrophic bacteria might be responsible for low TEP concentration in the SML of our experiments, where bacterial cells accumulated more than in bulk water.

Two aspects need to be considered in the dynamic of organic matter pool. As discussed by Wurl and Holmes [10], TEP can assemble spontaneously within the boundaries of the SML thanks to precursors from bacterial exudates. Additionally, colloidal and particulate organic matter forms in the water column and is vertically transported to the surface where heterotrophic bacteria find an optimal environment [44]. To what extent CSP can form within the SML is still unclear. CSP are protein-containing marine gel particles, but it is uncertain whether or not TEP and CSP represent different chemical subunit of the same gel particle or are separate particles. It has been suggested that CSP are closely associated to TEP [11], even though the strong CSP enrichment of our treatments was not recorded for TEP as well (Figure 4). As extracellular polymeric substances, their abundance, as discussed for TEP, is presumably dependent on the algae-bacteria interaction as well as on the changing composition of the bacterial community [45].

The observation of CSP enrichment may be corroborated by earlier reports of enhanced presence of proteins and higher rates of extracellular enzymatic peptide hydrolysis in the SML [23, 25]. An enrichment of SML with dissolved amino acids was recently reported for the subtropical Atlantic in a transect from the Mauritanian upwelling area to the oligotrophic gyre and for the western Mediterranean Sea [46]. The amino acid pool might increase in the SML as a consequence of organic matter leaching from bacterial damaged cells because of viruses or other stress factors typical of that environment [2]. Enrichment in the SML has been well documented for dissolved free amino acids (DFAA) and for particulate amino acids [2, 47]. Proteinaceous matter is a significant source of organic nitrogen in surface ocean [26] and microbial communities of the SML rapidly metabolize DFAA [48, 49]. Total hydrolysable amino acids can be found as dissolved material, colloids or submicron particles

[25] that migrate to the surface rather than being produced in situ within the SML [23]. Organisms and particularly bacteria take up free carbohydrates and amino acids for their metabolism, resulting in a loss of dissolved organic matter (DOM). When there is a high production of DOM, for example during phytoplankton blooms, another pathway of converting dissolved into particulate organic carbon (POC) is aggregation into particles, as demonstrated for TEP [19]. We suggest that spontaneous assembly of gel-like particles from DOM may account for CSP also, since CSP were observed in control and treatment tanks as well.

4.3. TOTAL COMBINED CARBOHYDRATES ANALYSIS

Early studies suggested that total carbohydrates could be less strongly associated with the SML [47]. During this study, little enrichment of TCCHO and TEP in SML was observed (Tables 3 and 4). Our experiments were connoted by the predominance of neutral sugars and in particular by glucose as primary product of photosynthesis. Glucose is possibly the most abundant sugar in seawater as early studies reported [50-54]. Acidic polysaccharides are a characteristic fraction of TEP [13, 54-56] and their low abundance in our experiment reflected the lower TEP concentration.

Amino sugars like galactosamine and glucosamine were found in relatively high mol% compared to recent field studies [54, 55]. Peptides and amino acids are common algal products but usually represent a small fraction of the total algal extracellular production [57], even if there is also evidence of high abundance and frequency of amino sugars in oceanic samples [58]. Galactosamine, glucosamine and muramic acid contribute to the structure of peptidoglycan, dominant cell wall polymer and therefore are important constituents of marine carbon and nitrogen cycles [58]. Bacterial protein content supports the amino acid pool and may provide precursors for proteinaceous gel particle assemblage, such as observed in the SML of this study.

4.4. BACTERIAL ABUNDANCE

The abundance of bacterial specific organic components and of proteinaceous gel particles together with an enrichment of bacteria in the SML suggests a major role of bacteria in

determining the gelatinous character of SML during this study. Bacteria associated to the SML might result from a migration of bacterial cells from the bulk water to the surface, where they benefit from the accumulation of organic and inorganic substrates [8, 59]. The observed increase in bacterial numbers in the SML over time, confirms this idea. Recent studies suggested that because of the stressful environment, a specifically adapted SML bacterial community is unlikely [8]. However a specific bacterioneuston community originating from the underlying waters might concentrate at the surface through upward passive transport [60]. Nevertheless the observed tendency of bacterial abundance to be higher in the SML, suggests that bacteria not only find in gel particles optimal energy source to be degraded, but they contribute to the continuous gel matrix formation through the release of exudates.

4.5. IMPLICATIONS FOR OCEAN'S SURFACE PROCESSES IN FUTURE CLIMATE SCENARIOS

One aspect of our study was to investigate CO_2 effects on SML dynamics, because increasing pCO_2 and decreasing pH may impact biological production of organic matter in marine systems [61] and thus SML formation and composition. Pre-industrial (180ppm), current (380ppm) and predicted future ocean pCO_2 by the year 2100 (780ppm) were applied to phytoplankton cultures in this experiment.

A direct relationship between TEP and CO_2 uptake has been demonstrated before, pointing to the importance of exopolymers released by phytoplankton as sink for CO_2 [62]. In surface waters, coupled acidification and rising sea-surface temperature might enhance this process, thus implying changes in the microbial food web [55] and consequently in the recycling of dissolved organic matter in the ocean. Heterotrophic bacteria are the main consumers of organic carbon in the ocean. Therefore, changes in bacterial activity thus could highly affect the oceanic carbon budget and the cycling of nutrients, in particular nitrogen, which limits primary productivity in many regions [63]. The enrichment of proteinaceous exudates in the SML found in our study suggests that bacterial activity might crucially affect surface ocean dynamics. The results that have been shown do not ascertain a particular CO_2 effect on microlayer gelatinous composition, but because of SML complexity this should be studied in natural environments and is an aspect for future investigations. We expect that an enhanced

release of exopolymers by phyto- and bacterioplankton due to anthropogenic changes in climate may impact surface ocean processes and among them, air-sea gas fluxes.

5. CONCLUSION

This study investigated the upper 150 µm of the water column in order to understand the composition of the SML with respect to bulk water as a result of phytoplankton and bacterial activity. Enrichment of the microlayer was found in the particulate organic matter fraction to which bacterial cells and gel particles contribute. This study suggests that the gelatinous nature of the sea-surface microlayer is not solely determined by polysaccharides but also by proteinaceous material, which we found more abundant in the form of CSP. Among gel particles, TEP showed microlayer enrichment related to low bacterial abundance (control tank) confirming TEP formation from abiotic sources, which conversely does not occur for CSP. Therefore we reason that to the protein-like gelatinous matrix, bacteria are a determining factor because of their amino acidic exudates and probably to a greater extent than phytoplankton. In natural systems the mutual association between bacteria and gel particles renders the microlayer a unique environment subject to many processes and inputs from bulk water and from the atmosphere. The surface matrix can be enriched and depleted of its components, and the metabolic activity of the SML community might influence exchange of gases between ocean and atmosphere on a global scale, as pointed out previously [46]. The ocean's breathable skin therefore should not be intended as a "waste" region that collects all the material scavenged from the bulk water, but as a dynamic and living habitat that continuously changes in an independent way.

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THE SEA-SURFACE MICROLAYER IS SUSCEPTIBLE TO OCEAN ACIDIFICATION

Luisa Galgani*[1, 2]

Christian Stolle[3]

Sonja Endres[1]

Kai G. Schulz[1, 4]

Klaus Jürgens[3]

Anja Engel[1]

[1] GEOMAR Helmholtz-Zentrum für Ozeanforschung Kiel

Düsternbrooker Weg 20

24105 Kiel, Germany

[2]Alfred-Wegener-Institut Helmholtz-Zentrum für Polar- und Meeresforschung

Am Handelshafen 12

27570 Bremerhaven, Germany

[3]Leibniz Institute for Baltic Sea Research,

Seestrasse 15

18119 Rostock, Germany

[4]Centre for Coastal Biogeochemistry

School of Environmental Science and Management

Southern Cross University

P.O. Box 157 Lismore, NSW 2480, Australia

MAIN TEXT

The sea-surface microlayer (SML) is the ocean's uppermost boundary to the atmosphere and in control of climate relevant processes like gas exchange or emission of marine primary organic aerosols (POA) (Liss and Duce, 2005). The SML includes surface active organic compounds like polysaccharides, proteins and lipids, and represents a complex surface film with gel particles and microbial communities as major constituents (Wurl and Holmes, 2008, Cunliffe and Murrell, 2009). Organic components and gels in the SML originate largely from biological production (Cunliffe and Murrell, 2009). The ocean is known to act as a net sink for atmospheric carbon dioxide (CO_2) (Sabine et al., 2004). Therefore, the continuous increase in anthropogenic CO_2 concentration leads to a progressive decline in ocean's pH (Caldeira and Wickett, 2003, Sabine et al., 2004). This is known as ocean acidification (Caldeira and Wickett, 2003), with potential consequences for marine microbial activity (Piontek et al., 2010, Engel et al., 2013, Piontek et al., 2013).

Despite the relevance of SML in ocean-atmosphere interaction processes, we have little knowledge about the SML structural characteristics and its sensitivity to external drivers like pH. Here, we present data from an extensive mesocosm sampling campaign, indicating that ocean acidification affects the amount and composition of organic matter as well as the abundance and activity of microorganisms accumulating in the SML at times of phytoplankton blooms. Our results reveal a potential coupling between anthropogenic CO_2 and biogenic properties of the SML, and point to a hitherto disregarded feedback process between ocean and climate change.

Recent studies have emphasized that the composition of the SML is characterized by high abundance of marine gel particles (Wurl and Holmes, 2008), hydrated organic supramolecular structures vitally important for microbial processes and carbon cycling in the ocean (Passow, 2002). Hydrogels originate from high molecular weight polymers like polysaccharides and peptides released by phyto- and bacterioplankton cells during growth and decay(Chin et al., 1998, Engel et al., 2004). In an initial step, these polymers assemble to water insoluble colloidal nano- and microgels (Chin et al., 1998, Verdugo et al., 2004), and further aggregate to larger particles of several millimeters size (Engel et al., 2004, Verdugo, 2012). Polysaccharidic gels in the ocean were attributed mainly to phytoplankton exudation

(Passow, 2002), while the production of protein-containing gels has been related to cell lysis, death, and absorption of proteins onto non-proteinaceous particles (Long and Azam, 1996). The properties of gel particles to ascend the water-column might be due to their density (Azetsu and Passow, 2004). Under natural conditions the adsorption of gels to rising bubbles is likely to be the dominant mechanism accumulating organic material in the SML (Azetsu and Passow, 2004), where the action of waves and turbulent shear further facilitate their collision and aggregation (Wurl and Holmes, 2008, Kuznetsova et al., 2005). Polysaccharidic and proteinaceous gels may be closely associated, but are operationally confined into two distinct classes according to analytical techniques (Engel, 2009). In the ocean, and particularly within the SML, gels represent substrates for marine phyto-and bacterioplankton to attach and grow upon, facilitating the formation of an active biofilm (Long and Azam, 1996, Passow, 2002, Flemming and Wingender, 2010, Cunliffe et al., 2011). In addition to gels ascending from the water-column, *de novo* production of gels can occur at the ocean-atmosphere interface due to compression of dissolved organic matter (DOM) during surface wave action (Wurl et al., 2011), or due to photochemical or bacterial breakdown of particles. Microcolloidal organic particles in the SML, such as marine gels, might provide a new source for submicron POA with the emission of sea spray to the lower atmosphere (Leck and Bigg, 2005). Water insoluble organic particles dominate total submicron marine aerosol mass during bloom periods (O'Dowd et al., 2004). These particles originating in bubble bursting events have a polysaccharidic composition (Russell et al., 2010) and are suggested to act as cloud condensation nuclei (CCN) in the high Arctic region, where low-level clouds play a climate-regulating role by reflecting incoming solar radiation (Leck and Bigg, 2005, Orellana et al., 2011). However, so far little is known about the origin and fate of proteinaceous gels in the SML. It has been suggested, however, that amino acids and proteinaceous gels may become enriched in SML and sea-spray aerosols (Kuznetsova et al., 2005). While still poorly understood, the marine POA-cloud feedback is an emerging issue in present day and future scenarios of surface ocean-lower atmosphere interactions, due to its high potential of controlling earth's radiation budget and energy fluxes (Solomon et al., 2007).

In the ocean, rising uptake of anthropogenic CO_2 may enhance autotrophic carbon fixation and increase the release of organic polymers from phytoplankton (Hein and Sand-Jensen, 1997, Engel et al., 2013). Thereby, a high production of extracellular polymers supports the

accumulation of gel particles (Engel, 2002) that may contribute to a surface biofilm formation. However, enhanced organic matter production might be counteracted by higher heterotrophic activity (Engel et al., 2013),
and thus, changing the balance of autotrophy versus heterotrophy (Cunliffe et al., 2011) with possible positive feedbacks on rising atmospheric CO_2 (del Giorgio and Duarte, 2002).
The aim of this study was to examine the impact of a phytoplankton bloom on the composition and microbial dynamics of the SML in response to CO_2 enrichment as expected for future ocean acidification scenarios (Caldeira and Wickett, 2003, Sabine et al., 2004). We participated in a large-scale mesocosm experiment in late spring of 2011, when nine floating Kiel Off Shore Mesocosms for Ocean Simulation (KOSMOS) were deployed in Raunefjord, Norway. These flexible (25 m long) structures contained a water volume of approximately 50 - 75 m^3 and allowed for pH manipulation of the enclosed water masses by the stepwise addition of CO_2 saturated seawater (Riebesell et al., 2013). Over a period of 31 days, we sampled six mesocosms every other day: two with a field pCO_2 of about 300 µatm as control without CO_2 addition (2 and 4), and four mesocosms with target pCO_2 levels of 600 (8), 900 (1), 1300 (5), and 2000 µatm (7) obtained by the progressive addition of CO_2-rich seawater. On experimental day 14, nutrients were added to all mesocosms (5 µmol L^{-1} nitrate and 0.1 µmol L^{-1} phosphate) to stimulate a phytoplankton bloom. Median pH values of the whole period according to *in situ* temperature ranged from 8.100 in control (2 and 4) to 7.558 at maximum pCO_2 level (7). Water temperature ranged from 6.8°C at the beginning of the experiment, to 10.0°C towards the end. SML samples were collected wiping off the surfaces of a glass plate vertically inserted into the mesocosms, repeating the procedure three times. The surface area within the mesocosms allowed for a limited volume of SML. Therefore, similar pCO_2 levels were combined by pooling following samples: 2 and 4, as control, 1 and 8, as medium pCO_2, and 5 and 7 as high pCO_2 for chemical analyses.
Two phytoplankton blooms were observed in the water column of the mesocosms, as derived from increases in chlorophyll *a* concentrations. The first bloom occurred around day 3, while the second one around day 19 was likely induced by the addition of nutrients (figures 1 **a** and s4). In general, bloom development influenced the organic composition of the SML, although some of the variability in the data might be inferred to the continuous disturbance while sampling the SML. The increase in phytoplankton biomass in the mesocosms was significantly accompanied by an enhanced formation of polysaccharidic gels

(figures 1 b, C = 0.55, $p < 0.05$, $n = 16$ and table s1) and accumulation of total amino acids (THAA) (figures 1 c, s6, C = 0.67, $p < 0.01$, $n = 16$ and table s1) in the SML. Total combined carbohydrates (TCCHO) concentrations did not change significantly in response to the phytoplankton bloom. Both, THAA and TCCHO concentrations were in the range of previous studies on the SML (Kuznetsova et al., 2005, Wurl and Holmes, 2008).

Proteinaceous gels in the SML covered a larger area ($\mu m^2 L^{-1}$) than polysaccharidic gels (figures 1 b and s5 b), with greater extension observed four days after the first phytoplankton bloom ($p < 0.05$, cross-correlation analysis, C = 0.54, $n = 14$), and significantly correlated to cell-specific and total bacterial activity ($p < 0.05$, C = 0.70, $n = 13$ for leucine uptake rate and table s1). Sea-surface organic films are suggested to affect molecular diffusion of gases (Liss and Duce, 2005). Therefore, proteinaceous gels with associated bacterial activity might strengthen the importance of the SML in mediating air-sea gas exchange through microbial metabolism (Cunliffe et al., 2011). While phytoplankton exudation may represent a specific source of polysaccharidic gels (Passow, 2002) thus explaining the observed temporal coupling with chlorophyll *a* concentration, the temporal shift of proteinaceous gels is consistent with the idea of new particle formation from dissolved precursors with enhanced proteinaceous characteristics in the SML (Kuznetsova et al., 2005). SML bacterial abundance and activity increased steeply after nutrients addition, showing a clear peak on day 19 concomitant to the second phytoplankton bloom (figures 1 d and s5 c, d). Albeit the strong effect of nutrients addition, bacterial abundance was positively correlated to THAA concentrations ($p < 0.05$, C = 0.64, $n = 12$ and table s1) suggesting a contribution of bacterial biomass to the amino acids pool by 11% - 22% (supplementary materials).

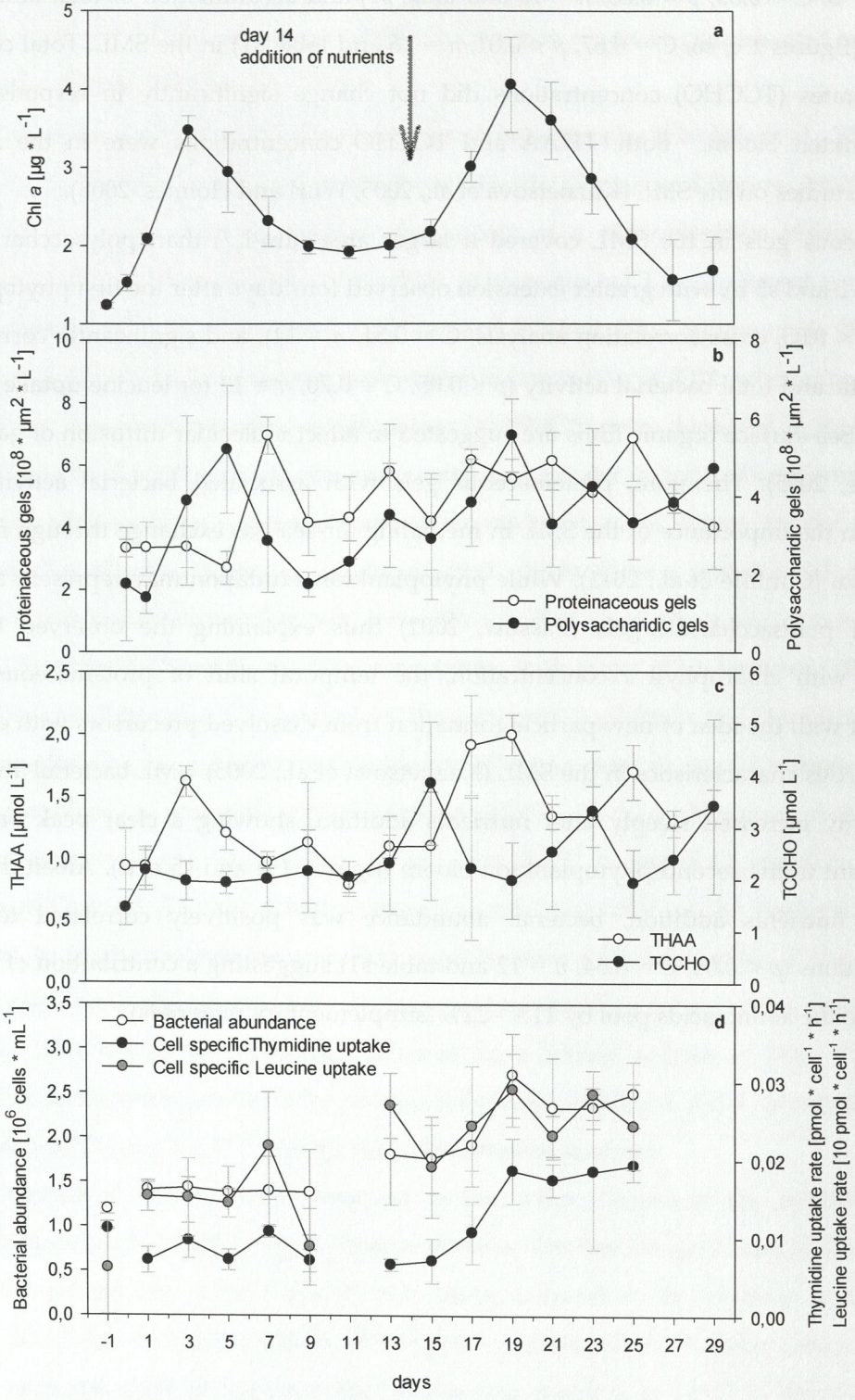

Figure 1. Chlorophyll *a* concentrations measured in the water column (a) and SML components, all expressed as daily averages of all treatments: marine gels area (b), total hydrolysable amino acids (THAA) and total combined carbohydrates (TCCHO) (c), bacterial abundance and activity as cell specific thymidine and leucine uptake rate (d). The CO_2 gradient was established on day 5 and on day 14 nutrients were added to the enclosed water in all mesocosms.

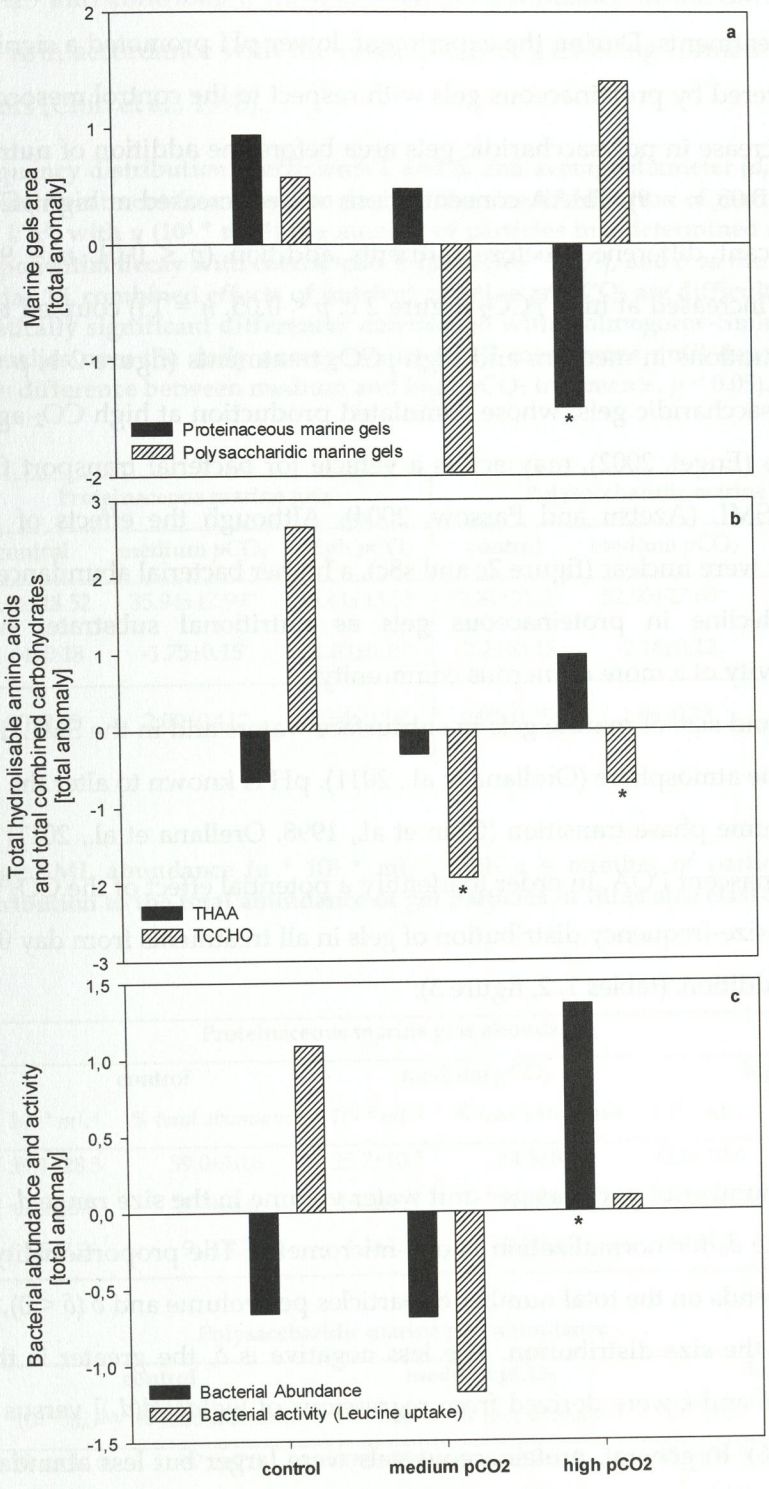

Figure 2. Sum of daily anomalies over the whole experimental period per each treatment for: marine gels area (a), total hydrolysable amino acids (THAA) and total combined carbohydrates (TCCHO) (b), bacterial abundance and total activity (Leucine uptake rate) (c) normalized over the daily average value of all mesocosms. Stars (*) below the graphs bars indicate statistically significant differences based on Kolmogorov-Smirnov tests on normal distributed daily anomalies (y_{ij}) in the whole period (* = different from control, $p < 0.05$).

Among the mesocosms, the variability of organic SML components was partly related to the different CO_2 treatments. During the experiment, lower pH promoted a significant decrease of total area covered by proteinaceous gels with respect to the control mesocosm ($p < 0.05$, $n = 16$) and an increase in polysaccharidic gels area before the addition of nutrients (figure s5 a, b and s8, $p < 0.05$, $n = 9$). THAA concentrations were increased at high pCO_2 (figures 2 b, s6) with significant differences before nutrients addition ($p < 0.01$, $n = 9$, s8). Bacterial abundance was increased at high pCO_2 (figure 2 c, $p < 0.05$, $n = 13$) coupled to a decrease in TCCHO concentrations in medium and high pCO_2 treatments (figure 2 b, $p < 0.05$, $n = 16$). Ascending polysaccharidic gels, whose stimulated production at high CO_2 agrees well with previous studies (Engel, 2002), may act as a vehicle for bacterial transport from the water column to the SML (Azetsu and Passow, 2004). Although the effects of pCO_2 on total bacterial activity were unclear (figure 2c and s8c), a higher bacterial abundance could explain the observed decline in proteinaceous gels as nutritional substrate, supporting the degradation activity of a more numerous community.

The abundance and size of marine gels in subsurface waters and in the SML determine their fate as CCN in the atmosphere (Orellana et al., 2011). pH is known to alter the size of gels by promoting a volume phase transition (Chin et al., 1998, Orellana et al., 2011) thus affecting the dynamics of nascent POA. In order to identify a potential effect of the CO_2 manipulation, we analyzed the size-frequency distribution of gels in all treatments from day 0 to day 15, i.e. before nutrient addition, (tables 1, 2, figure 3):

$$dN/d(d_p) = kd_p^{\delta} \quad (1)$$

where dN is the number of particles per unit water volume in the size range d_p to $[d_p + d(d_p)]$. Our reference size d_0 for normalization is one micrometer. The proportionality factor k is a constant that depends on the total number of particles per volume and δ ($\delta < 0$), describes the spectral slope of the size distribution. The less negative is δ, the greater is the fraction of larger gels. Both δ and k were derived from regressions of $\log[dN/d(d_p)]$ versus $\log[d_p]$ (Mari and Kiørboe, 1996). In general, proteinaceous gels were larger but less abundant compared to polysaccharidic ones (table 1, 2, figure 3). However, high pCO_2 promoted a significant increase in abundance (figure 3, table 1, $p < 0.05$, $n = 9$) and a significant decrease in size, d_p (table 1, $p < 0.01$, $n = 9$). For both proteinaceous and polysaccharidic gels, the smaller size

fraction (0.4 - 1.25 µm) contributed most to total gel abundance in the SML, up to 68.5 % (table 2), which is in accordance with the assumption of gels being formed by assembly of smaller precursors (Chin et al., 1998).

Table 1. Size frequency distribution coefficients k and δ, and average diameter (d_p, µm) for marine gel particles for each treatment from day 0 to day 15. The size distribution of marine gels followed the equation $y = k^*x^{\delta}$, with y (10^3 * mL^{-1}) the number of particles in a determined size class x (µm), describing an exponential decay with coefficients k (particles * mL^{-1}), and δ as the spectral slope of the curve. After day 15, combined effects of nutrient addition and CO_2 are difficult to discern. Stars (*) indicate statistically significant differences determined with Kolmogorov-Smirnov tests run on normalized anomalies over the daily average value of all mesocosms until day 15 (* = different from control, ** = difference between medium and high pCO_2 treatments, $p < 0.05$).

		Proteinaceous marine gels			Polysaccharidic marine gels		
$y = k * x^{\delta}$		control	medium pCO_2	high pCO_2	control	medium pCO_2	high pCO_2
Average	k	34.06±18.52	35.94±17.99**	45.41±13.02*	72.81±31.23	52.90±27.60**	84.92±45.88
	δ	-1.64±0.18	-1.75±0.15*	-1.83±0.19*	-2.23±0.15	-2.18±0.12	-2.18±0.18
Average d_p *(µm)*		2.00±0.49	2.00±0.41**	1.54±0.16*	0.98±0.25	1.06±0.33	1.08±0.31*

Table 2. Average SML abundance (n * 10^3 * mL^{-1}, with n = number of particles) and relative percentage contribution to the total abundance of gel particles in three size classes until day 15 for each treatment.

Proteinaceous marine gels abundance						
	control		medium pCO_2		high pCO_2	
	10^3 * mL^{-1}	% total abundance	10^3 * mL^{-1}	% total abundance	10^3 * mL^{-1}	% total abundance
0.4 - 1.25 µm	39.1±28.3	59.0±10.6	25.7±10.7	54.8±8.2	42.8±10.4	62.9±6.5
1.25 - 5 µm	15.6±5.7	29.9±8.6	15.2±5.3	32.8±6.1	19.4±5.4	28.8±5.6
> 5 µm	4.3±1.3	9.1±4.7	4.8±2.1	10.3±3.7	4.1±1.3	6.2±1.7

Polysaccharidic marine gels abundance						
	control		medium pCO_2		high pCO_2	
	10^3 * mL^{-1}	% total abundance	10^3 * mL^{-1}	% total abundance	10^3 * mL^{-1}	% total abundance
0.4 - 1.25 µm	59.0±32.0	67.2±7.3	39.9±18.1	62.7±9.8	55.6±26.5	64.5±4.1
1.25 - 5 µm	22.5±6.4	27.4±4.3	19.0±10.0	29.9±5.1	27.3±12.9	31.6±3.0
> 5 µm	2.7±0.8	3.4±1.1	2.1±1.3	3.2±0.8	3.5±2.0	3.9±1.3

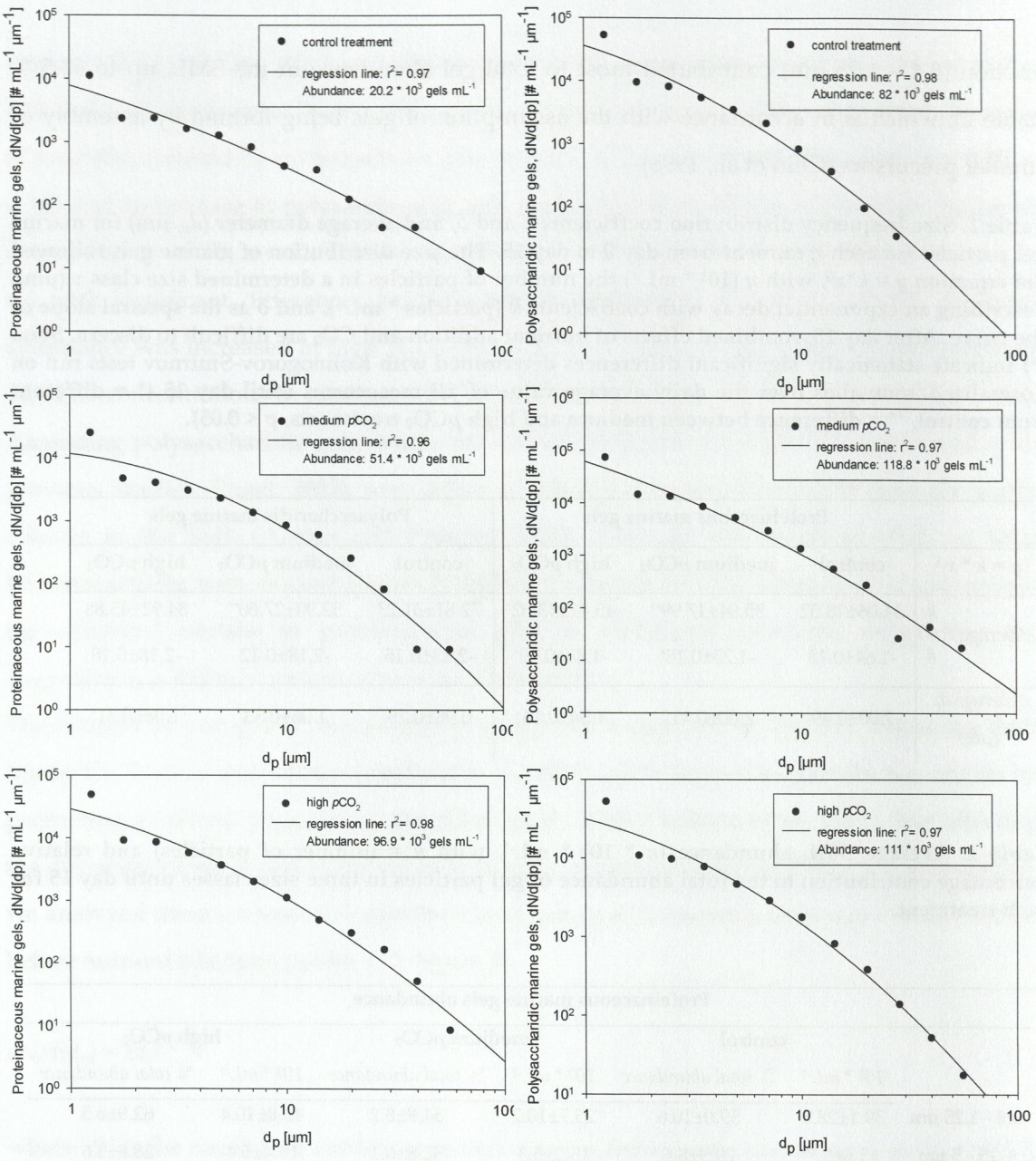

Figure 3. Logarithmic distribution of the size frequency spectra and abundance of proteinaceous and polysaccharidic marine gel particles according to the different pCO_2 treatments on day 5, when the pCO_2 gradient was established.

During biologically productive periods, marine POA reveal a high fraction of water insoluble organic particles (O'Dowd et al., 2004), of polysaccharidic gel-like composition (Russell et al., 2010, Orellana et al., 2011). In the ocean, high abundance of polysaccharidic gels has been related to phytoplankton blooms (Mari and Kiørboe, 1996, Passow, 2002). This study shows that the appearance of polysaccharidic gels in the SML was closely coupled to

phytoplankton development. Proteinaceous gels contributed even more to the SML composition, in accordance with the enrichment of proteinaceous material found in natural SML samples and in sea-spray aerosols (Kuznetsova et al., 2005). In addition, bacterial cells, or DOM released during their lysis might contribute to a biofilm matrix (Flemming and Wingender, 2010) as suggested for the protein-like SML of our study.

According to our experiment, acidification of seawater may affect the composition of organic matter, and in particular the extension and abundance of proteinaceous gel particles in the SML, reflecting the sensitivity of marine microorganisms to environmental change. These characteristics of the SML might be relevant for air-sea gas exchange, by acting on capillary wave damping and molecular diffusion of gases (Liss and Duce, 2005). An active bacterial community in the SML might be responsible for marine gels dynamics and DOM turnover, thus influencing the composition and the CCN activation potential of nascent marine aerosols (Prather et al., 2013). Atmospheric CCN density, together with greenhouse gases, control the earth's radiative budget (Solomon et al., 2007). These two main driving forces might be tightly connected to the properties of the marine air-water interface and subsurface waters. We suggest that the coupling of SML gelatinous components and microbial communities makes the top millimeter of the ocean a sensitive environment, whose structure and processes might help understanding ocean-atmosphere dynamics in a future high CO_2 world.

SUPPLEMENTARY MATERIAL

The chlorophyll *a* development in the water column showed a first bloom at about day 3 with an average concentration of 3.24±0.25 µg L^{-1} (control), 3.59±0.37 µg L^{-1} (medium pCO_2) and 3.58±0.22 µg L^{-1} (high pCO_2) followed by an amplified second bloom on day 19, with concentrations of 4.14±0.19 µg L^{-1} (control), 4.62±0.04 µg L^{-1} (medium pCO_2) and 3.41±0.40 µg L^{-1} (high pCO_2) induced by the addition of nutrients on day 14 (figure s4). In the first phase of the experiment, before nutrients addition, chlorophyll *a* average concentrations were higher in the control mesocoms, with a reversed development in the second phase, after the nutrients were added (figure s4). Significant correlations between chlorophyll *a* in the water column and SML components according to the different CO_2 treatments are shown in table s1. Correlations coefficients were computed based on Pearson or Spearman tests.

Polysaccharidic gel particles accounted for a smaller total area than proteinaceous gels, on average 3.37±1.35 x 10^8 μm^2 L^{-1} in control, 3.08±1.63 x 10^8 μm^2 L^{-1} at medium pCO_2 and 3.73±1.71 x 10^8 μm^2 L^{-1} at high pCO_2, following chlorophyll *a* development more clearly before the addition of nutrients on day 14 (figure s5a). The total area of proteinaceous gels was on average of the whole period 5.13±1.77 x 10^8 μm^2 L^{-1} in control, 4.88±1.37 x 10^8 μm^2 L^{-1} at medium pCO_2 and 4.34±1.57 x 10^8 μm^2 L^{-1} at high pCO_2. Highest values for proteinaceous particles' area in the three treatments were observed within a four-day delay from the first phytoplankton bloom in the water column (figure s5b).

Maximum bacterioneuston abundances were 3.24 x 10^6 cells mL^{-1} (day 19, control), 2.83 x 10^6 cells mL^{-1} (day 25, medium pCO_2) and 2.55 x 10^6 cells mL^{-1} (day 25, high pCO_2) (figure s5c, d). In control, total thymidine uptake rates ranged between 8.99 (pre-nutrients addition, day 5) to 59.50 (day 19) pmol L^{-1} h^{-1} while leucine uptake ranged between initial values of 59.31 up to 915.35 pmol L^{-1} on day 19. In the medium pCO_2 treatment, initial rates were 12.85 pmol L^{-1} for thymidine and 7.19 pmol L^{-1} for leucine with maximum values of 58.82 pmol L^{-1} (day 25, thymidine) and 824.17 pmol L^{-1} (day 19, leucine). At high pCO_2, uptake rates varied between 13.84 pmol L^{-1} for thymidine (day -1) and 156.12 pmol L^{-1} for leucine (day -1). In the pre-addition phase in particular, averages rates for both measurements were higher at high pCO_2 (thymidine: 10.90±3.42 pmol L^{-1}, 11.09±2.53 pmol L^{-1}, and 13.41±3.65 pmol L^{-1} in control, medium pCO_2 and high pCO_2 respectively, and leucine: 229.4±128.pmol L^{-1}, 192.6±124.5 pmol L^{-1}, and 249.2±161.8 pmol L^{-1} in control, medium pCO_2 and high pCO_2 respectively).

Considering the different CO_2 manipulations in details, total hydrolysable amino acids (THAA) in the SML followed the chlorophyll *a* pattern of the water column (figure s6) and maximum THAA concentrations were found during the phytoplankton bloom events. On day 3 for example, THAA concentration in control mesocosms was 1.54±0.15 µmol L^{-1}, in medium pCO_2 1.56±0.16 µmol L^{-1} and in high pCO_2 1.76±0.17 µmol L^{-1}. On day 19, 2.05±0.20 µmol L^{-1} was the concentration of THAA for control, 1.81±0.18 µmol L^{-1} in medium pCO_2 and 2.11±0.21 µmol L^{-1} was found in high pCO_2. The average concentration during the whole experiment was 1.24 ± 0.37 µmol L^{-1} in control, 1.27 ± 0.37 µmol L^{-1} in medium pCO_2 treatment and 1.38 ± 0.46 µmol L^{-1} in high pCO_2 treatment. THAA concentrations were higher at high pCO_2 in both phases (pre- and post-nutrients addition).

Bacterial contribution to nitrogen and carbon content of amino acids (THAA-N and THAA-C, respectively), as N_{bac} and C_{bac}, were estimated according to Fagerbakke and colleagues

(1996), who determined N_{bac} and C_{bac} in the same Raunefjord in June being 2.2±0.3 fg N and 9±1 fg C. N_{bac} and C_{bac} were calculated multiplying bacterial total abundance (cells mL^{-1}) by the N and C content estimated by Fagerbakke et al. (fg), and the result given in µM according to the atomic mass of N and C. Both N_{bac} and C_{bac} significantly correlated to THAA-N and THAA-C ($p < 0.05$, $n = 12$). The average percentages of bacterial N and C contribution to THAA-N and THAA-C were 11.3±2.3 (%-N_{bac}) and 22.1±4.6 (%-C_{bac}).

Total combined carbohydrates (TCCHO, figure s7) had an irregular development in the SML with an average concentration during the whole experiment of 2.77±1.10 µmol L^{-1} in control, 2.05±0.82 µmol L^{-1} in medium pCO_2 and 2.23±0.82 µmol L^{-1} in high pCO_2, which, on all mesocosms, was not significantly related to the chlorophyll *a* development.

Since the addition of nutrients on day 14 might have incremented the variability of the system, CO_2 effects in the pre-nutrient phase (until day 15) for bacterial abundance and activity, for gel particles, and for THAA and TCCHO were determined separately according to the analysis of anomalies expressed as percentages of deviations (figure s8). According to this analysis, proteinaceous marine gels area decreased at high pCO_2, while polysaccharidic gels area significantly increased (figure s8 a). Interestingly, TCCHO concentrations significantly decreased before nutrient addition (figure s8 b), which indicates that they were probably removed from the SML by an enhanced formation of polysaccharidic gels. The increase in THAA at high pCO_2 and bacterial abundance (figure s8 c) might be related to bacterial biomass contributing to the amino acids pool.

Table s1. Significant correlations per each treatment based on Pearson Correlation Coefficient and Spearman Correlation Coefficient, C. Only correlations where C > 0.05 and $p < 0.05$ are shown. n = 16 for Chlorophyll a (Chl a) measured in the water column, and Proteinaceous gels, Polysaccahridic Gels, Total Hydrolisable Amino Acids (THAA) measured in the SML. n = 12 for Bacterial Abundance, total Thymidine uptake rate (TdR) and Leucine uptake rate (Leu), measured in the SML. Blue dots = control, grey dots = medium pCO_2 and red dots = high pCO_2.

	Proteinaceous Gels	Polysaccaridic Gels	THAA	Bacterial abundance	TdR	Leu
Chl *a*		● ●	●	● ●	●	● ●
Proteinaceous Gels	—	●	●	●	●	●
Polysaccharidic Gels		—	● ●	●	● ●	● ●
THAA			—	●	● ●	● ●
Bacterial abundance				—	● ●	● ● ●
TdR					—	● ●
Leu						—

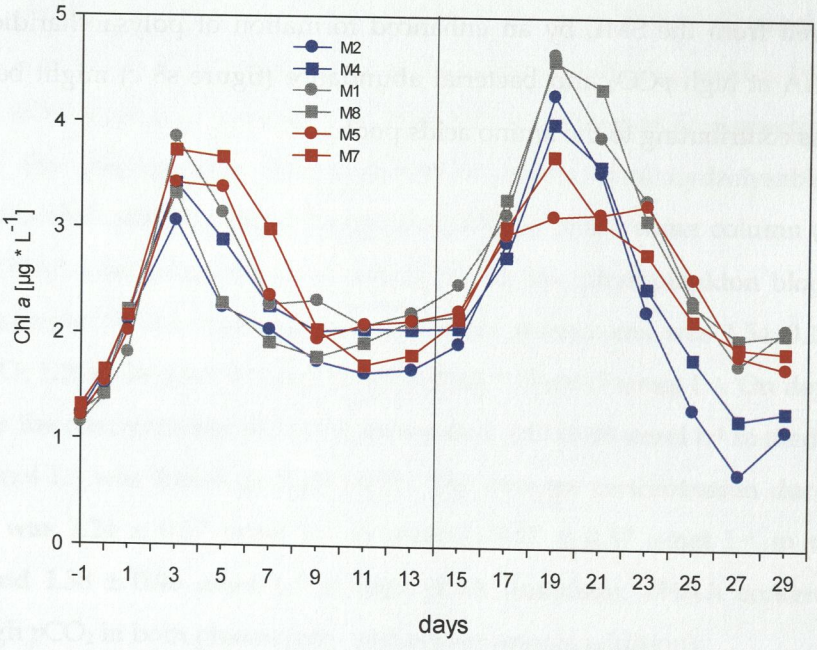

Figure s4. Temporal development of Chlorophyll *a* in the water column of control mesocosms (M2 and M4), medium pCO_2 (M1 and M8) and high pCO_2 treatment (M5 and M7), from day - 1 to day 29, end of the experiment. The black line on day 14 indicates the nutrients addition.

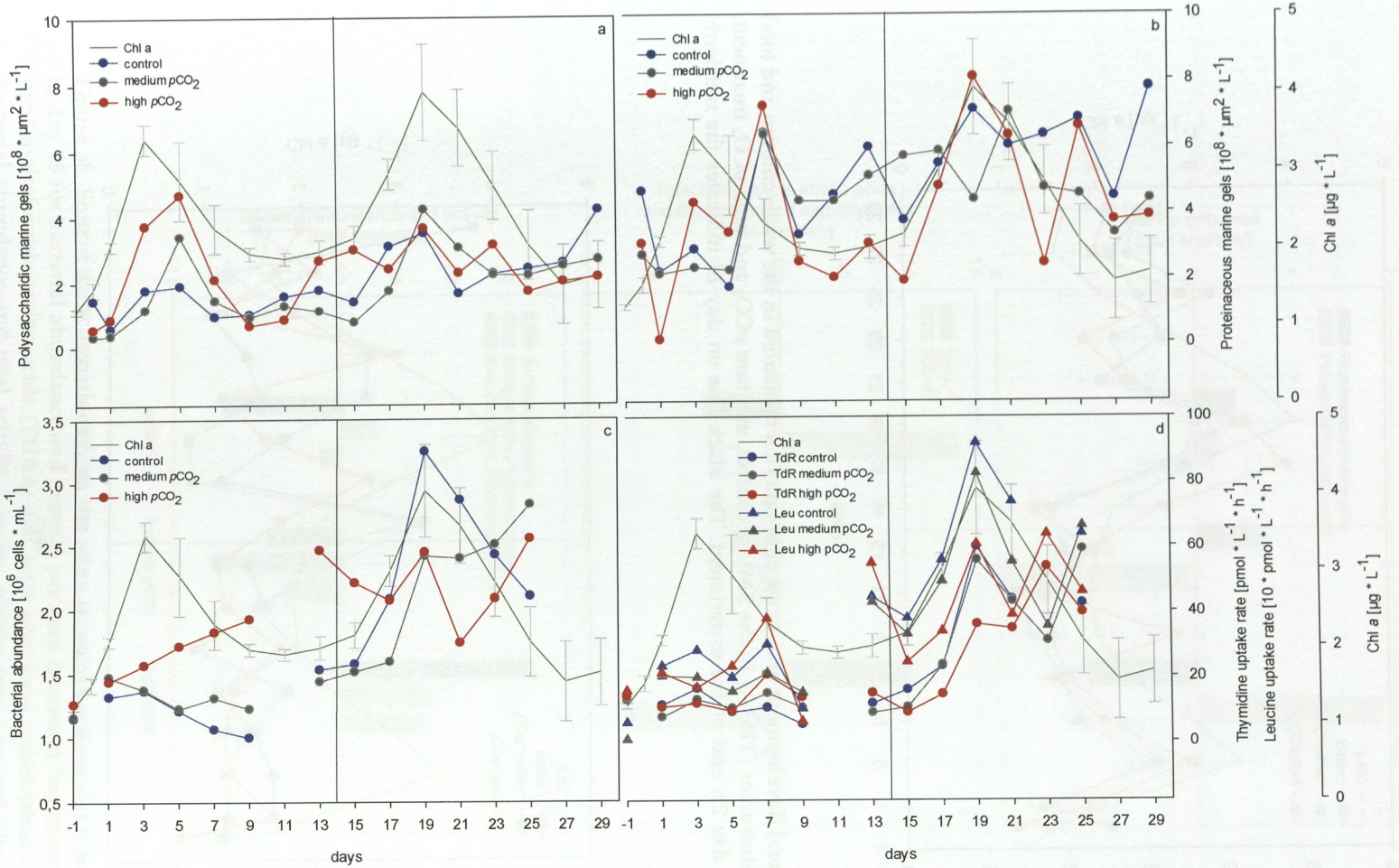

Figure s5. Average Chlorophyll a concentrations of all mesocosms measured in the water column (green line) and SML components: on the top panels, marine gels area (polysaccharidic gels, a, proteinaceous gels, b) per each treatment from the beginning of the experiment (day -1, but for marine gels day 0) until the end of the experiment, and on the lower panels, bacterial abundance (c) and total activity (Leucine and Thymidine incorporation rates, d) per each treatment from day -1 to day 25. The CO_2 gradient was established on day 5, and the black line on day 14 indicates the addition of nutrients.

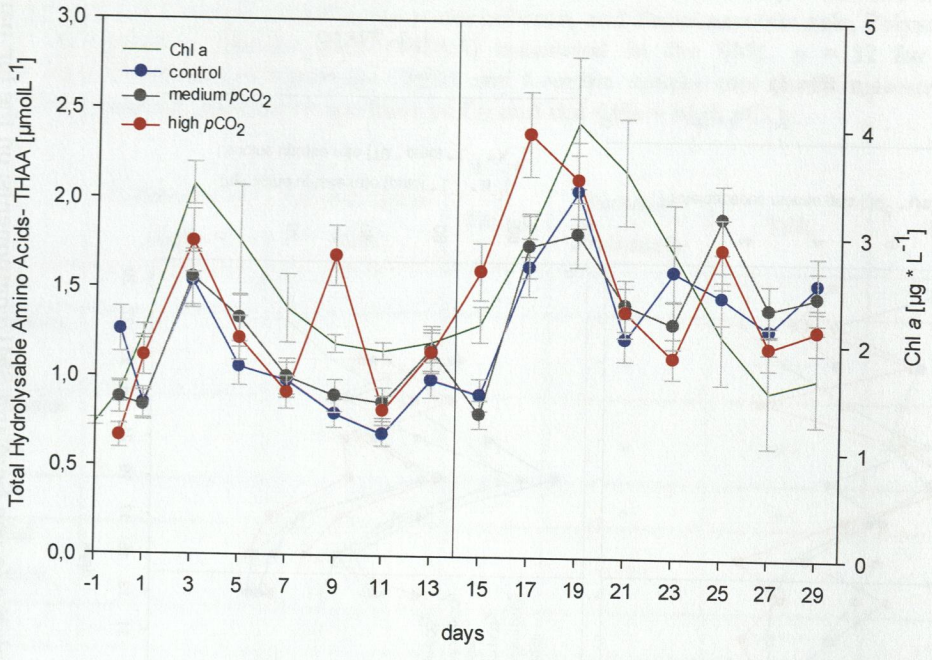

Figure s6. Temporal development of average chlorophyll *a* measured in the water column and total hydrolysable amino acids (THAA) in the SML of control, medium pCO_2 and high pCO_2 treatment, from day - 1 to day 29, end of the experiment. The black line on day 14 indicates the nutrients addition.

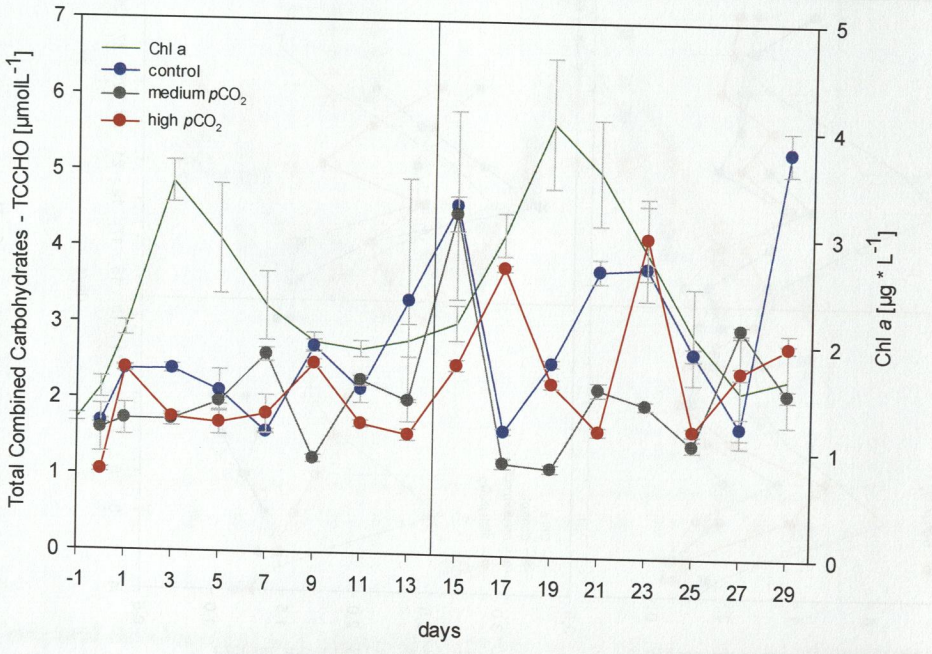

Figure s7. Temporal development of average chlorophyll *a* measured in the water column and total combined carbohydrates (TCCHO) in the SML of control, medium pCO_2 and high pCO_2 treatment, from day - 1 to day 29, end of the experiment. The black line on day 14 indicates the nutrients addition.

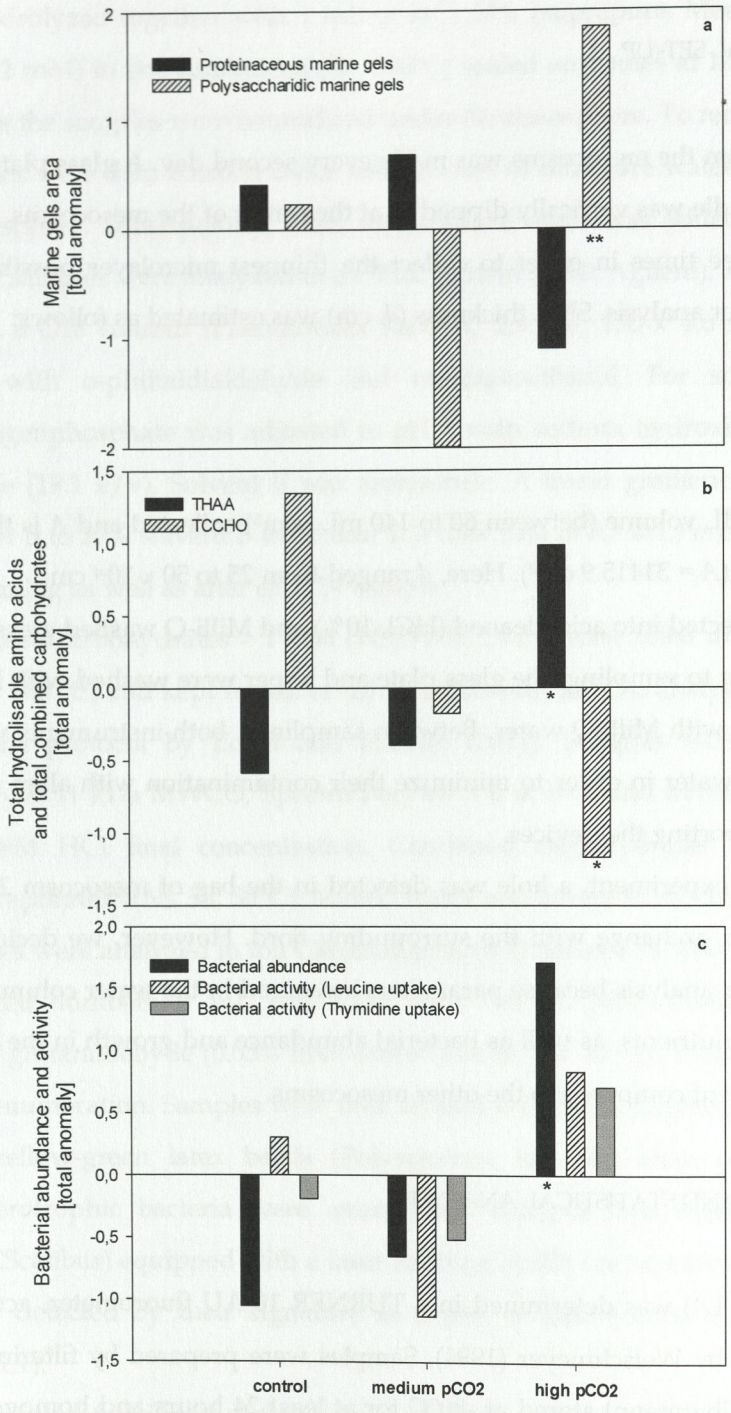

Figure s8. Sum of daily deviations from the mean (anomalies) from day 0 to day 15 (day -1 to day 15 for bacterial abundance and growth) per each treatment for: marine gels area (a), total hydrolysable amino acids (THAA) and total combined carbohydrates (TCCHO) (b), bacterial abundance and total activity (Leucine uptake rate, Leu and Thymidine uptake, TdR) (c) normalized to the daily average value of all mesocosms. Stars (*) below the graphs bars indicate statistically significant differences (* = different from control, ** = difference between medium and high pCO_2 treatments, $p < 0.05$).

METHODS

EXPERIMENTAL SET-UP

SML sampling from the mesocosms was made every second day. A glass plate of 40 x 70 cm with a central handle was vertically dipped in at the center of the mesocosms. The procedure was repeated three times in order to collect the thinnest microlayer possible but enough volume to allow for analysis. SML thickness (d, cm) was estimated as follows:

$$d = V / A \quad (2)$$

Where V is the SML volume (between 60 to 140 mL - cm^3) collected and A is the surface area of the mesocosms (A = 31415.9 cm^2). Here, d ranged from 25 to 50 x 10^{-4} cm.

Samples were collected into acid cleaned (HCl, 10%) and Milli-Q washed glass bottles with a Teflon wiper. Prior to sampling, the glass plate and wiper were washed with HCl (10%) and intensively rinsed with Milli-Q water. Between samplings, both instruments were copiously rinsed with fjord water in order to minimize their contamination with alien material while handling or transporting the devices.

At the end of the experiment, a hole was detected in the bag of mesocosm 2, which might have caused water exchange with the surrounding fjord. However, we decided to include mesocosm 2 in our analysis because parameters measured in the water column, such as pH, chlorophyll *a* and nutrients, as well as bacterial abundance and growth in the SML were not significantly different compared to the other mesocosms.

PARAMETERS AND STATISTICAL ANALYSIS

Chlorophyll *a* (µg L^{-1}) was determined in a TURNER 10-AU fluorometer, according to the method described by Welschmeyer (1994). Samples were prepared by filtering 250-500 mL on GF/F filters (Whatmann) stored at -80°C for at least 24 hours and homogenized in 90% acetone using glass beads (2 and 4 mm) and a cell mill (Welschmeyer, 1994).

For total hydrolysable amino acids analysis (THAA), 5 mL of sample were filled into pre-combusted glass vials (8 h, 500°C) and stored at -20°C until analysis. Analysis was

performed according to the methods described by Lindroth & Mopper (1979). 1 mL of sample was hydrolyzed together with 1 mL of HCl 30% (suprapure, Merck) and 10 µL of ascorbic acid (11 mM) in pre-combusted (8h, 500°C) sealed ampoules at 100°C for 20 hours. After hydrolysis the samples were neutralized under N_2 atmosphere. To remove traces of the acid, the samples were then washed twice with 0.5 mL of ultrapure water and dried down under N_2 atmosphere. Subsequently, 1 mL of ultrapure water was added to the final dry residue and the samples were analyzed in a HPLC system (1260, Agilent). Amino acids were separated with a C18 column (Phenomenex Kinetex, 2.6 µm, 150 x 4.6 mm) after in-line derivatization with o-phthaldialdehyde and mercaptoethanol. For solvent A 0.1 M sodiumdihydrogenphosphate was adjusted to pH 7 with sodium hydroxide and premixed with acetonitrile (19:1 v/v). Solvent B was acetonitrile. A linear gradient was run starting from 6% solvent B to 27% solvent B in 40 min at a flow rate of 0.8 mL/min. Standards were run in the beginning as well as after each 5th sample.

For total combined carbohydrates > 1 kDa (TCCHO), 15 mL were filled into pre-combusted glass vials (8 h, 500 °C) and kept frozen at -20 °C until analysis. The analysis was conducted according to the protocol by Engel and Händel (2011). Samples were desalinated by membrane dialysis (1 kDa MWCO, Spectra Por) for 5 h at 6°C, and hydrolyzed for 20 h at 100°C with 0.8M HCl final concentration. Combined carbohydrates were hydrolyzed through acid evaporation (N_2, 5h, 50°C). Milli-Q water was added to the dry residue and two replicate samples were analyzed in Ion Chromatography (Dionex ICS 3000).

For bacterial cell numbers, 1 mL was fixed with 100 µL paraformaldehyde (1% final concentration)/glutaraldehyde (0.05% final concentration) for 30 min in the dark and stored at -80°C until enumeration. Samples were then stained with SYBR Green (2.5 µM, Molecular Probes) and yellow-green latex beads (Polysciences, 0.5 µm) were added as internal standard. Heterotrophic bacteria were enumerated using a flow cytometer (Becton & Dickinson FACScalibur) equipped with a laser emitting at 488 nm at a constant flow rate (35 µL min^{-1}) and detected by their signature in a plot of side scatter (SSC) versus green fluorescence (FL1).

The incorporation of ^{3}H-methyl-thymidine (^{3}H-TdR, 60.1 Ci mmol^{-1}, 50 nM final concentration, Hartmann Analytics) and ^{14}C-leucine (261 mCi mmol^{-1}, 50 nM final concentration, Hartmann Analytics) was measured to estimate heterotrophic bacterial biomass production in 2.5 mL water samples according to the method of Chin-Leo and

Kirchman (1988). Duplicate samples were incubated for 60 - 90 min at the *in situ* temperature in the dark. Incorporation was stopped by addition of formaldehyde (10% v/w) and fixation in the dark at 5°C. A third sample, serving as a blank, was fixed for at least 10 min prior to the addition of substrates. All samples were filtered on 0.22 µm polycarbonate filters (Millipore). Four mL of scintillation cocktail were added to the filters, after which the incorporated substrates were counted in a scintillation counter (Packard). Bacterial parameters as abundance and growth were combined according to control (M2 and M4), medium pCO_2 (M1 and M8), high pCO_2 (M5 and M7).

Total area, particles number and equivalent spherical diameter (d_p) of gel particles were determined by microscopy after Engel (2009). Therefore, 20 to 30 mL were filtered through 0.4 µm Nuclepore membranes (Whatmann) and stained with 1ml Alcian Blue solution for polysaccharidic marine gels and 1 mL Coomassie Brilliant Blue G (CBBG) working solution for proteinaceous gels, mounted onto Cytoclear© slides at -20°C until microscopy analysis.

To determine effects of rising pCO_2 on SML composition, we considered every treatment as an independent data set of replicate samples. To achieve this, and exclude the temporal variability in the system, we calculated first the daily overall mean of all mesocosms as follows:

$$\bar{y}_i = \frac{1}{3}\sum_{j}^{3}(x_j)_i \tag{3}$$

where i = days and j = treatment.

Then we calculated the daily anomalies (y_{ij}) per each treatment j normalized to $\bar{y}_i$:

$$y_{ij} = \frac{x_{ij} - \bar{y}_i}{\bar{y}_i} \tag{4}$$

The total deviation (TD_j) of each treatment was determined in the following way:

$$TD_j = \sum_{i=1}^{N} y_{ij} \tag{5}$$

Statistical tests were performed with SigmaPlot package (Systat Software Inc) and Cross Correlation function was determined with Free Statistics Software by Wessa P., (2012) (Office for Research Development and Education, http://www.wessa.net/rwasp_cross.wasp). Statistical significance was accepted for $p < 0.05$. Pearson Correlation Coefficients and Spearman Correlation Coefficients (C) were determined for normal and non-normal distributed data, respectively, on the averages of all mesocosms as shown in figure 1. Detailed correlations according to the different treatments are given in the supplementary material (table s1). Statistical significance for the CO_2 effect as shown in figure 2 was determined with Kolmogorov-Smirnov tests on daily anomalies (y_{ij}) given the data being normal distributed.

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THE COMPOSITION OF THE SEA-SURFACE MICROLAYER IN THE CENTRAL ARCTIC UNDER ENHANCED SEA ICE MELTING CONDITIONS

Luisa Galgani[1,2]

Judith Piontek[1]

Anja Engel[1]

[1] GEOMAR Helmholtz-Zentrum für Ozeanforschung Kiel

Düsternbrooker Weg 20

24105 Kiel, Germany

[2]Alfred-Wegener-Institut Helmholtz-Zentrum für Polar- und Meeresforschung

Am Handelshafen 12

27570 Bremerhaven, Germany

ABSTRACT

Organic compounds exuded by phytoplankton accumulate in the surface microlayer (SML) of ice-free waters of the Central Arctic, whether meltponds or open water spots; the SML is the layer at the immediate air-water interface, susceptible of mediating the emission of primary organic aerosols (POA) to the atmosphere. During the summer of 2012, when the sea ice declined to a historical minimum, the chemical composition of arctic surface films was investigated in terms of the diagenetic state of organic matter and potential enrichment in the microlayer, from first stages of sea ice melting to open sea samples. Results on the diagenetic composition of dissolved organic matter (DOM) revealed a higher percentage of freshly produced semi-labile DOM in the first phase of sea ice melting, based on the carbon-normalized yields of dissolved amino acids (DAA-%OC). In the higher saline environments, the presence of polysaccharidic marine gels was accentuated. The small size fraction of gel particles (0.4 - 1.25 μm) that is highly relevant for POA emission represented ~80% of total volume concentration (ppm) in the SML. Our results suggest that DOM composition in arctic surface microlayers reflects microbial processes likely to change as the environment is modified by the retreat of sea ice. These changes are expected to influence POA composition, therefore establishing additional feedbacks on global carbon cycling and climate in the future Arctic Ocean.

INTRODUCTION

The Arctic is experiencing fast environmental changes due to global warming, which resulted in the greatest loss of sea ice cover ever recorded with the minimum of 3.41 million square kilometers in the summer of 2012 (National Snow and Ice Data Center, Colorado, USA). Many factors are responsible for the loss of arctic sea ice: increased atmospheric concentration of greenhouse gases as well as change in aerosol abundance and land use alter the radiative budget of the Earth, leading to a net global warming of the atmosphere (Solomon et al., 2007), the main reason for sea ice loss. This climate change also triggers a complex mechanism whose secondary effects are as powerful as the main driving forces. Besides rising temperatures, there is an increased advection of warm waters into the Arctic region due to global oceanic currents (Comiso et al., 2008, Chylek et al., 2009, Screen and Simmonds, 2010, Spielhagen et al., 2011) as well as atmospheric circulation favouring ice moving out of the Arctic Ocean through the Fram Strait (Maslanik et al., 2007, Arrigo et al., 2008, Rabe et al., 2013). Moreover, arctic sea ice is not only decreasing in volume but also getting younger, since multiyear ice (MYI) has been extensively replaced by thinner first year ice (FYI), which shows increasing ease of melting (Arrigo et al., 2008, Comiso, 2011, Maslanik et al., 2011). The decrease of sea ice extent reduces surface albedo and contributes to enhance the heat content of the ocean consequently accelerating sea ice melting processes in spring and summer (Serreze et al., 2007, Screen and Simmonds, 2010, Sedlácek et al., 2011).

The first step of ice melting is the formation of visible pools, referred to as melt ponds, where meltwater is collected during late spring and summer (Hohenegger et al., 2012). Meltponds contribute to the lowering of the sea ice albedo by absorption of incident solar radiation (Polashenski et al., 2012), thereby increasing the light availability for under-ice primary production (Nicolaus et al., 2013). A greater extension of open water and a longer growing season due to sea ice loss are expected to enhance primary productivity in the Arctic Ocean, leading to an increased production of organic matter in the upper water column (Arrigo et al., 2008). However, because of a thinner sea ice cover and a higher light transmittance, widespread under-ice phytoplankton blooms have been observed with major implications for the entire pelagic ecosystem and biomass export to the deep sea floor (Mundy et al., 2009, Arrigo et al., 2012, Boetius et al., 2013). Changes on sea ice cover and enhanced organic

matter production in surface waters do not only affect downward fluxes to the ocean interior, but will have relevant effects on ocean-atmosphere interactions as well.

Among other factors such as warm oceanic currents, the freezing or melting of sea ice is regulated by surface energy fluxes influenced by low-level arctic clouds coverage (Serreze et al., 2000, Liu et al., 2008, Liu et al., 2009). Small aerosol particles on which water vapor condenses are known as cloud condensation nuclei (CCN), and their concentration and density determine the radiative forcing and albedo, that is, the capacity of clouds to reflect incoming solar radiation (Novakov and Penner, 1993), thus influencing melting or freezing of sea ice. These properties of clouds are of enormous importance in the arctic environment where sea ice albedo is decreasing fast and more radiation is stored in open water. However, processes involving aerosols in modifications of clouds' properties are still poorly understood (Solomon et al., 2007).

North of 80°N, a major source of CCN during the arctic summer relies on nano- and micro gels of polysaccharides and other organic compounds exuded by surface water phytoplankton (Orellana et al., 2011). Primary organic aerosols (POA) can account for ~63% of total submicron aerosol mass during phytoplankton bloom periods, ~45% of which is represented by water insoluble colloids derived from organic matter enriched bubbles bursting at the sea-surface (O'Dowd et al., 2004). In the arctic seas, these water insoluble compounds dominate 15 - 45% of total submicron aerosol mass and have polysaccharidic characteristics similar to those of dissolved organic components in the sea-surface microlayer (Russell et al., 2010).

The sea-surface microlayer (SML) is the marine layer at the immediate air-sea interface, covering 70% of earth's surface and susceptible of mediating air-sea gas exchange and POA emission to the atmosphere (Liss and Duce, 2005, Cunliffe et al., 2013). Since the SML has the characteristics of a gelatinous film (Cunliffe and Murrell, 2009, Wurl and Holmes, 2008), marine gels in particular are believed to be an important source for atmospheric aerosols (Leck and Bigg, 2005, Orellana et al., 2011). Marine gels are water insoluble polymers derived from dissolved precursors of polysaccharidic (Transparent Exopolymer Particles, TEP) or proteinaceous composition (Coomassie Stainable Particles, CSP), exuded by marine microorganisms during metabolic processes and distinguished according to analytical procedures (Alldredge et al., 1993, Long and Azam, 1996, Passow, 2002a, Engel, 2009).

The enhanced primary productivity in the Arctic due to sea ice loss is envisaged to intensify the release of dissolved organic matter (DOM) by biological activity in surface waters. DOM and marine gels will likely imply structural changes of the SML, with potential implications for POA emission and hence, the composition of arctic clouds. In the Central Arctic, recent publications reported on the composition of the SML, and the potential of marine gels to act as precursors for airborne particles (Matrai et al., 2008, Orellana et al., 2011, Gao et al., 2012, Karl et al., 2013). In these studies, the SML was sampled in open leads at the ice edge. Since the ice melting process leads to a variety of conditions of physical and biological characteristics of the pack ice (Hohenegger et al., 2012), during the ARK 27-3 expedition to the Central Arctic, the SML was sampled from different sites: melt ponds, open leads in the pack ice and open sea across 7 different ice stations (figures 1, 2). The cruise took place in the eastern-central ice covered basins between 82° to 89°N and 30° to 130°E between 2nd of August and 8th of October, 2012. In the summer of the 2012 sea ice minimum, the area visited by *RV Polarstern* was characterized by a domination of FYI (> 95%) and melt pond coverage of approximately 30 - 40% (Boetius et al., 2013).

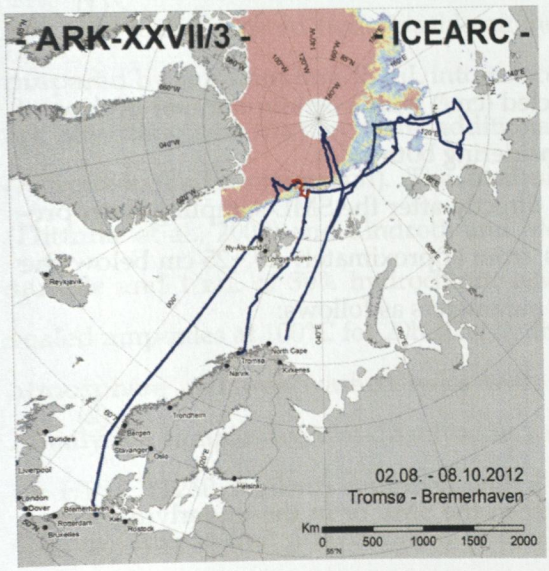

Figure 1. Cruise track of RV Polarstern during cruise leg ARK 27-3 (IceArc). Map courtesy of Sebastian Albrecht.

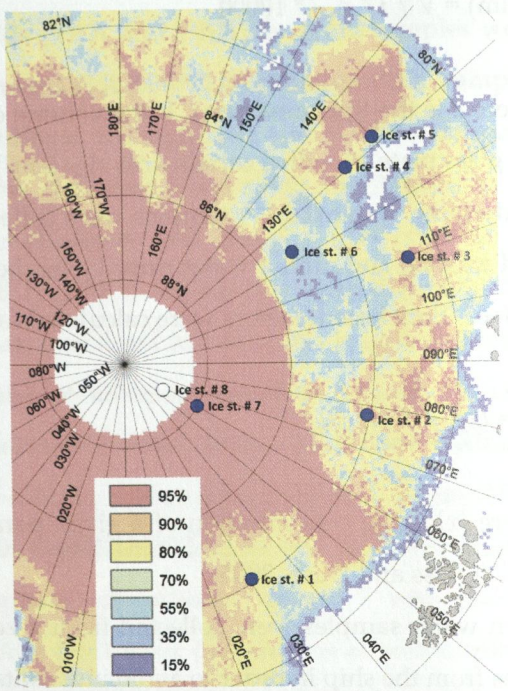

Figure 2. Ice cover in August 2012 in percentages (in the legend box percentages of ice concentration are shown) and stations sampled during ARK 27-3. On ice station #8, no samples for SML were collected because of the frozen surface.

METHODS

SAMPLING SITE AND PROCEDURE

During the cruise, 21 open water spots were sampled across seven different ice stations. (Figures 1, 2) These spots of open water were categorized in four different types (figure 3): 1) freshwater melt ponds mostly of melted snow (t1), and very shallow (~50 cm depth), 2) intermediate melt ponds (t2) deeper and partly connected with the ocean with an accentuated ice melting process, 3) open leads in the pack ice (t3) and 4) open sea samples (t4). The surface microlayer was sampled from all different locations with a glass plate of 250 x 500 x 4 mm dimensions with an effective sampling area of 2000 cm^2 (Stolle et al., 2009, Galgani and Engel, 2013). The sample was wiped out with Teflon blades, which allowed the water to be collected into glass bottles previously pre-acid washed (HCl 10%) and thoroughly rinsed with Milli-Q water. The thickness of the sea-surface microlayer collected was on average 54.8±4.8 µm, and was calculated according to

$$d\ (\mu m) = V / t * A_{GP} * 10000 \quad (1)$$

where d is the thickness (µm), V the volume collected (cm^3) with a certain number of dips of the glass plate (t) with a certain area A_{GP} (cm^2), considering both sides.

Underlying water (ULW) samples were collected directly after the SML-sampling with pre-acid washed and milli-q water rinsed glass bottles, from approximately 20 ~25 cm below the surface. Enrichment factors were calculated for all parameters as follows:

$$EF = [x]_{SML} / [x]_{ULW} \quad (2)$$

where $[x]$ is the concentration of a given parameter in the SML or in the underlying water (*ULW*) (Liss and Duce, 2005).

Open water samples were collected from a zodiac between the ice floes at about 4 nautical miles from the ship in order to avoid any contamination.

TOTAL AND DISSOLVED ORGANIC CARBON

Samples for total organic carbon (TOC) were prepared filling 20 mL into pre-combusted (8 h, 500°C) glass ampoules and acidified with 80 µL of 85% phosphoric acid (H_3PO_4). Samples for DOC were prepared with the same procedure but prior to acidification they were additionally filtered through pre-combusted (8 h, 500°C) GF/F filters (Whatman). TOC and DOC samples were stored for three months at 0 - 2°C until analysis. TOC and DOC concentrations were determined by high temperature catalytic oxidation with a Shimadzu TOC-VCSH analyzer and as the mean of quadruplicate measurements. The calibration was made with the TOC standard solution (1000 mg L^{-1} from Merck-Certipur® ref. 109017) over the measurement range of 0 - 600 µM C, and Deep Sea Water reference (Batch 10 – 2010 Lot #05-10) from Hansell laboratory, University of Miami, Florida. Particulate Organic Carbon (POC) values were retrieved subtracting DOC from TOC concentrations.

TOTAL AND DISSOLVED AMINO ACIDS

For total and dissolved hydrolysable amino acids (TAA and DAA) analysis, samples were prepared by sub-sampling 15 mL into pre-combusted (8 h, 500°C) scintillation vials. Samples for the dissolved fraction were additionally filtered through 0.45 µm GHP membranes (Acrodisk, Pall Corporation). The analysis was performed according to Dittmar et al. (Dittmar et al., 2009) and Lindroth and Mopper (Lindroth and Mopper, 1979). One mL of sample and 1 mL of 30% hydrochloric acid (HCl) (Merck, suprapure) were hydrolyzed in sealed ampoules at 100°C for 20h. The hydrolysate was dried in a microwave under nitrogen atmosphere and was washed twice with 0.5 mL of ultrapure water to remove the HCl. Finally the samples were re-dissolved in 1 mL ultrapure water. Amino acids were separated by HPLC (1260, Agilent) equipped with a C18 column (Phenomenex Kinetex, 2.6µm, 150 x 4.6mm) after in-line derivatization (2 min) with o-phthaldialdehyde and mercaptoethanol. For solvent A 0.1 M sodiumdihydrogenphosphate was adjusted to pH 7 with sodium hydroxide (NaOH) and premixed with acetonitrile (19:1 v/v). Solvent B was acetonitrile. A linear gradient was run starting from 6% solvent B to 27% solvent B in 40 min at a flow rate of 0.8 mL/min. Standards were run in the beginning as well as after each 5th sample. The carbon content of DAA and TAA (DAA-C and TAA-C) was normalized to the amount of

dissolved organic carbon and reported as DAA-%OC and TAA-%OC according to previous studies (Amon and Fitznar, 2001, Benner, 2002, Kaiser and Benner, 2009). Particulate amino acids (PAA) were determined subtracting DAA from TAA concentrations.

BACTERIAL ABUNDANCE AND ACTIVITY

For bacterial abundance, 4 mL of sample were fixed with 200 µL glutaraldehyde (25%) and stored at -20°C until enumeration within six months from collection. Abundance was determined after staining with SYBR Green in DMSO (2%) and the analysis performed with a Flow Cytometer FACSCalibur 4CA (Becton Dickinson). Bacterial cell numbers were estimated by manual gating of the bacterial subpopulation in the cytogram of side scatter vs. green fluorescence. Yellow-green fluorescent latex beads (Polyscience) and TruCount beads (Becton Dickinson) were used to normalize the counted events to volume (Del Giorgio and Gasol, 2000). Bacterial activity as protein production was estimated from the uptake of ^{3}H-leucine (Specific Activity 100 Cimmol^{-1}) added to 1.5 mL of sample at a final concentration of 20 nmol L^{-1}. Duplicate incubations were conducted in the dark at 0°C. The addition of trichloracetic acid (TCA) at a final concentration of 5% terminated the incubations after incubation times of 1 - 20 hours, depending on the activity. All samples were processed by the microcentrifuge method in a scintillation counter as described by Smith and Azam (Smith and Azam, 1992).

MARINE GEL PARTICLES: TEP AND CSP

Marine gel particles were determined microscopically with the CytoClear slide technique according to Engel (Engel, 2009) and Logan et al. (Logan et al., 1994). Twenty to 80 mL of sample were filtered through polycarbonate filters (Nucleopore) of 0.4 µm pore size (Whatmann) in two replicates, and immediately stained with Alcian Blue (AB) solution for TEP and with Coomassie Brilliant Blue G (CBBG) for CSP and the CytoClear slide technique (Logan et al., 1994). CytoClear slides were stored at -20°C until microscopy. For each slide, thirty images were taken randomly per filter cross section at 200x magnification with a light microscope equipped with a digital AxioCam HRc camera (Zeiss). The analysis of the cross-sectional area of marine gels was performed with an image analysis software (ImageJ, U.S.

National Institutes of Health) allowing the calculation of the equivalent spherical diameter (ESD) of individual particles, particles number and total area.

The size frequency distribution of marine gel particles was determined according to their ESD, described with a power function of the type:

$$dN/d(d_p) = k_p^{\delta} \quad (3)$$

where dN is the number of particles per unit volume in the size range d_p to $[d_p + d(d_p)]$, k is the constant which depends on the concentrations of particles, and δ is the spectral slope ($\delta < 0$) describing the size distribution. A less negative δ implies an increase in the fraction of larger marine gels. k and δ were both derived from regressions of $\log[dN/d(d_p)]$ versus $\log[d_p]$ (Mari and Burd, 1998, Mari and Kiørboe, 1996, Harlay et al., 2009).

The volume concentration of TEP and CSP refers to the mean volume of the particles in a certain size class; changes in this parameter indicate particles dynamics such as aggregation/disaggregation processes. Since TEP are fractal aggregates, the volume and the carbon content of these marine gel particles are assumed to be proportional to r^D, with r being the equivalent spherical radius (µm) and D the fractal scaling dimension associated with the size-distribution of marine gels (Mari and Kiørboe, 1996, Mari, 1999, Engel, 2009). We considered these assumptions to be valid for CSP as well, since there is no general consensus whether TEP and CSP are different particles or subunits of the same particle. Therefore, TEP and CSP carbon content (TEP-C_{micro} and CSP-C_{micro}, expressed in µM), was determined from marine gel size spectra according to Mari (Mari, 1999, Engel, 2009)

$$\text{TEP-}C_{micro} \text{ (or CSP-}C_{micro}) \text{ [µg L}^{-1}] = 0.25 \times 10^{-6}\, r^D \quad (4)$$

with D = - 2.55.

STATISTICAL ANALYSIS

Statistical tests as Pearson and Spearman Rank Order correlations were performed with SigmaPlot (Systat Software Inc.) package. Statistical significance was accepted for $p < 0.05$.

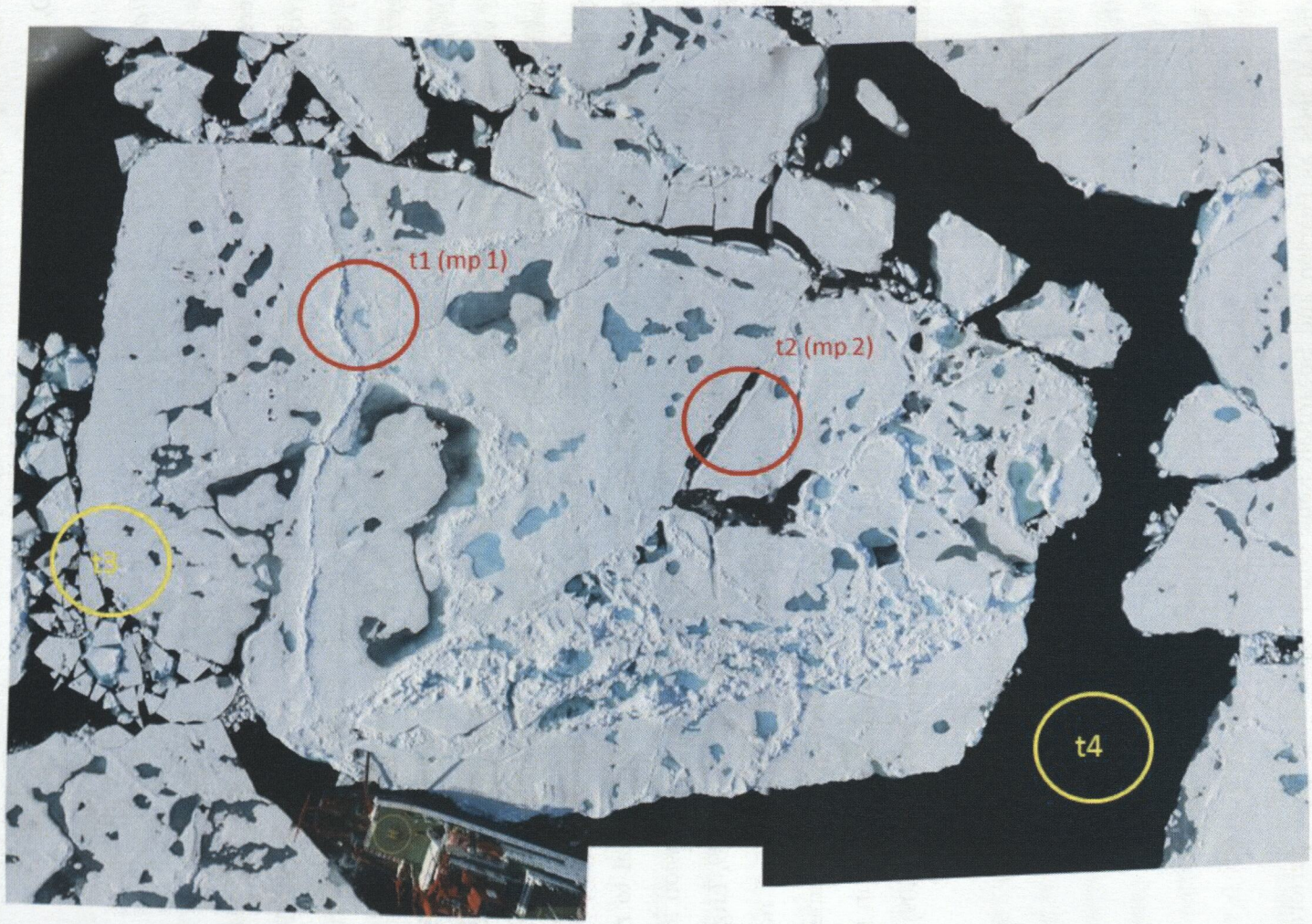

Figure 3. Aerial image of the first ice floe with the four different typologies of sampling locations. t1 (mp 1, freshwater melt pond) and t2 (mp 2, intermediate melt pond) are actual sampled melt ponds, while locations t3 and t4 are examples for open leads in the pack ice (t3) and open sea (t4). Aerial image courtesy of Stefan Hendricks, Sea Ice Physics, Alfred Wegener Institute.

RESULTS

SURFACE MICROLAYER COMPOSITION AND ENRICHMENT FACTORS

The different typologies of sampling locations showed distinct features of SML composition. Total and dissolved organic carbon (TOC and DOC) concentrations increased in the transition from freshwater (t1) to intermediate melt ponds (t2), open leads in the pack ice (t3) and open sea (t4). For simplicity, we will refer hereafter to each sampling location according to its typology number as shown in figure 3. In particular, average TOC concentrations with 36.6±22.0 μM (t1), 61.1±24.5 μM (t2), 150.9±32.6 μM (t3) and 190.4±36.8 μM (t4) were substantially different across sampling sites. DOC followed the same pattern, showing lowest values and highest variability in t1 samples. Average DOC concentrations were 25.3±21.2 μM (t1), 43.9±22.7 μM (t2), 130.1±25.7 μM (t3) and 106.6±34.1 μM (t4). While average DOC concentrations represented 65±22% (t1), 68±14% (t2) and 87±6% (t3) of TOC, respectively, in open sea samples (t4) DOC represented only 58±21% of TOC with high values in the particulate fraction (figure 4). Enrichment factors for TOC, DOC and POC were diverse (figure 4). In t1 samples, TOC and DOC were clearly enriched in the microlayer (enrichment factor, EF > 1) while no consistent enrichment of POC was found. In t2 samples, which showed higher heterogeneity due to different salinities, depths, and amounts of microalgal biomass, the median EF for POC was > 1 while for DOC was < 1, pointing to changing organic matter composition and size as the ice further melts and deeper holes connect the ponds with the ocean below. In t3, DOC represented the larger fraction of TOC and in the vast majority of samples was enriched in the SML. In t4 samples POC represented a high percentage of TOC (42±21%), contributing to the organic matter enrichment in the SML mostly present in the particulate fraction.

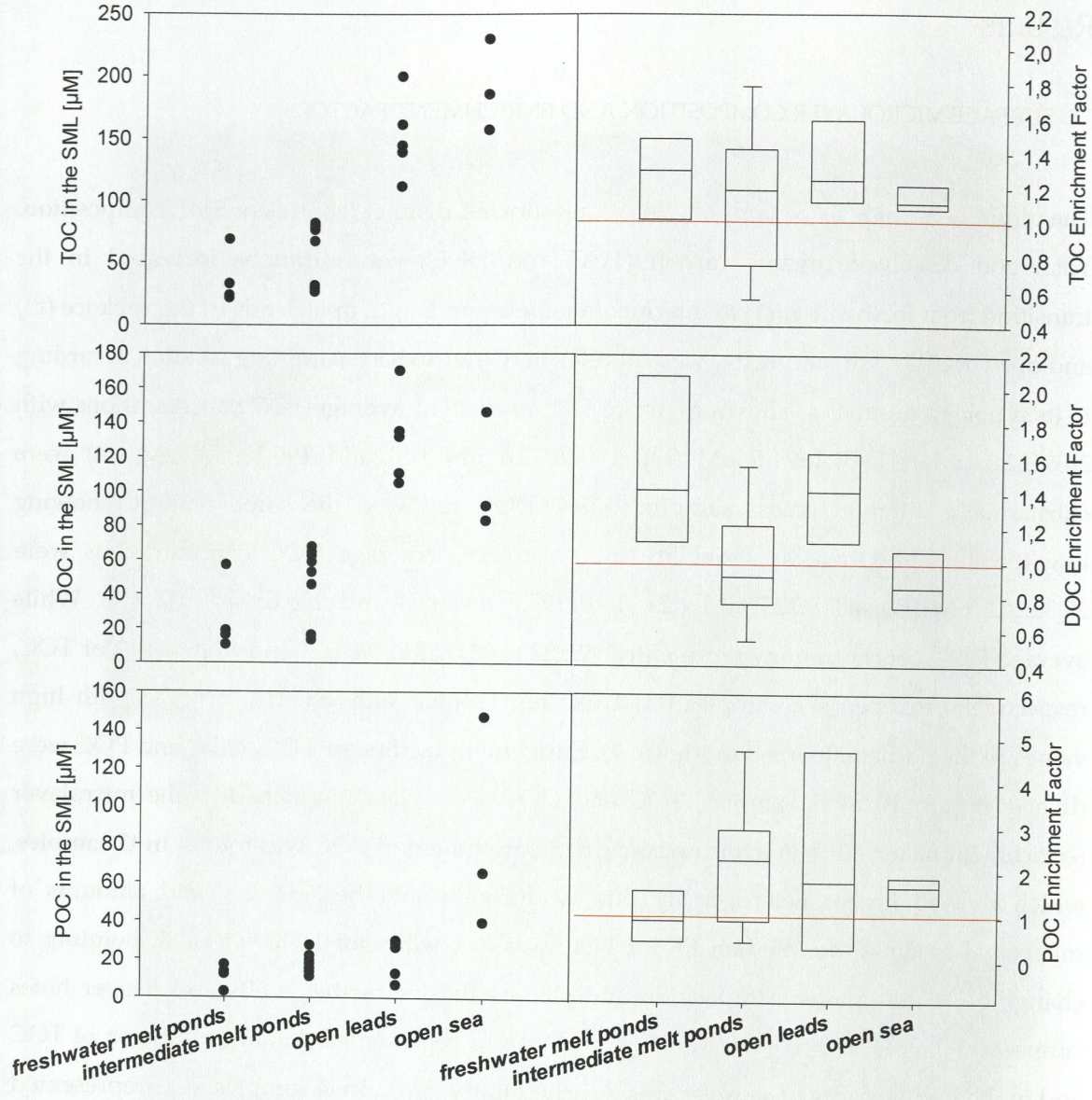

Figure 4. On the left side, concentrations for total, dissolved and particulate organic carbon (TOC, DOC and POC respectively) in the sea-surface microlayer of different sampling locations. On the right side, box plots showing enrichment factors for TOC, DOC and POC according to the different sampling sites. The horizontal red line indicates enrichment factor = 1 (no enrichment).

Total hydrolysable amino acids (TAA) concentrations were on average 588.5±374.6 nM (t1), 779.6±444.8 nM (t2), 851.8±526.0 nM (t3) and 560.3±62.7 nM (t4) (figure 5). Concentrations varied considerably between the different sampling sites, and were, in most cases enriched in the SML (EF > 1, figure 4), except for intermediate melt ponds (t2). Dissolved amino acids

(DAA) contributed more than 50% to total amino acids in t1 (60.7±24.2%), t2 (55.4±19.3%) and t4 sites (59.3±9.7%), while percentages in t3 (44.6±8.4%) were lower. DAA were always enriched in the SML of t1, t3 and t4 (EF > 1), but not consistently in t2 samples (figure 5). DAA concentrations were on average similar in all typologies of sampling sites, with 379.9±291.3 nM (t1), 393.9±220.9 nM (t2), 377.1±266.3 nM (t3) and 331.9±59.9 nM (t4). Particulate amino acids (PAA) concentrations reached 208.6±141.9 nM (t1), 385.7±265.0 nM (t2), 474.7±273.2 nM (t3) and 228.4±59.1 nM (t4). PAA were enriched in the SML of open leads (t3) but not always in t1, t2 and t4 samples even if median EF values were > 1.

Bacterial abundance in the SML of melt ponds (both freshwater and intermediate) was in general low in comparison to abundances frequently found in seawater, sometimes below the detection limit of the instrument. Abundances ranged from 0.47×10^5 cells mL^{-1} to 2×10^5 cells mL^{-1} in t1 and t2 samples. In open leads and open sea SML, the abundances were higher, ranging from 4.4×10^5 cells mL^{-1} to 6.6×10^5 cells mL^{-1}, and 5.8×10^5 cells mL^{-1} to 6.1×10^5 cells mL^{-1} in t3 and t4 respectively. EF for bacterial abundance were mostly below 1 in t1, t2 and t4 samples (figure 6) while in open leads (t3) bacterial cells seemed to be in most cases enriched in the SML. Enrichment factors for bacterial activity were always below 1 (figure 6), except for one sample in an open lead in the pack ice which showed EF = 1.

Marine gel particles total area and enrichment factors are shown in figure 7. TEP and CSP in general increased in the SML in the transition from melt ponds to open sea samples. TEP area was on average $0.17\pm0.05 \times 10^8$ μm^2 L^{-1} (t1), $0.35\pm0.19 \times 10^8$ μm^2 L^{-1} (t2), $0.83\pm0.61 \times 10^8$ μm^2 L^{-1} (t3) and $1.03\pm0.90 \times 10^8$ μm^2 L^{-1} (t4). CSP area was larger than TEP area, with average values of $0.58\pm0.35 \times 10^8$ μm^2 L^{-1} (t1), $1.20\pm0.82 \times 10^8$ μm^2 L^{-1} (t2), $0.91\pm0.30 \times 10^8$ μm^2 L^{-1} (t3) and $2.08\pm1.18 \times 10^8$ μm^2 L^{-1} (t4). TEP enrichment factors were quite diverse, being median EF > 1 only in t2 and t4 samples. EF for CSP was always > 1 in t3 and t4 samples and median EF > 1 in t1 and t2 (figure 7).

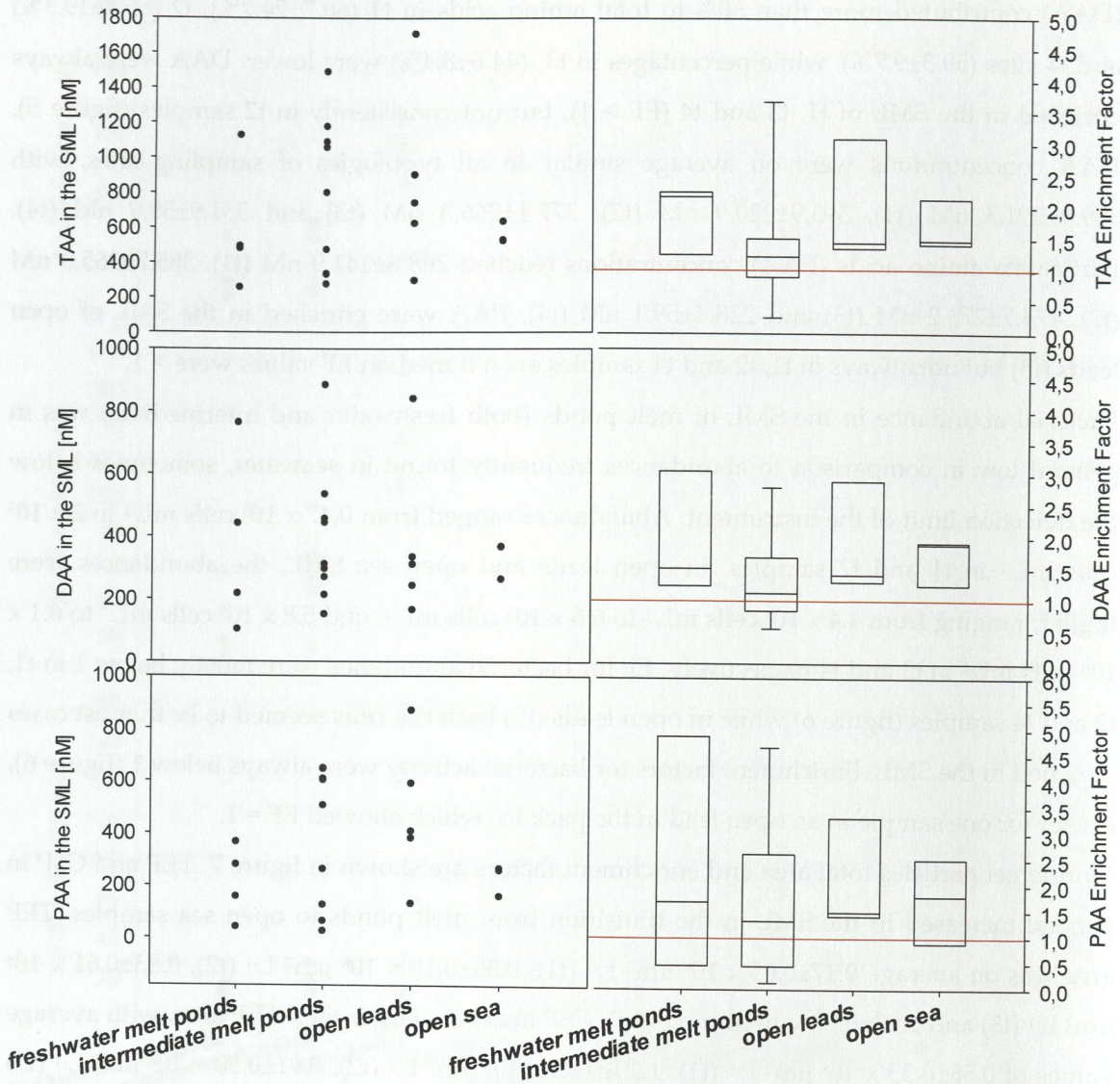

Figure 5. On the left side, concentrations for total, dissolved and particulate amino acids (TAA, DAA and PAA respectively) in the sea-surface microlayer of different sampling locations. On the right side, box plots show enrichment factors for TAA, DAA and PAA according to the different sampling sites. The horizontal red line indicates enrichment factor = 1 (no enrichment).

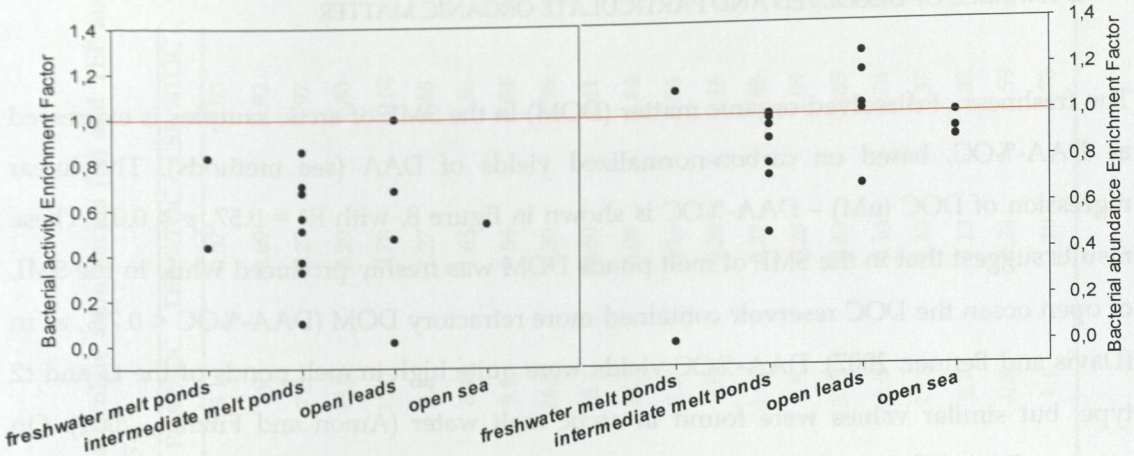

Figure 6. On the left side, enrichment factors for bacterial activity and on the right side, enrichment factors for bacterial abundances in different sampling locations.

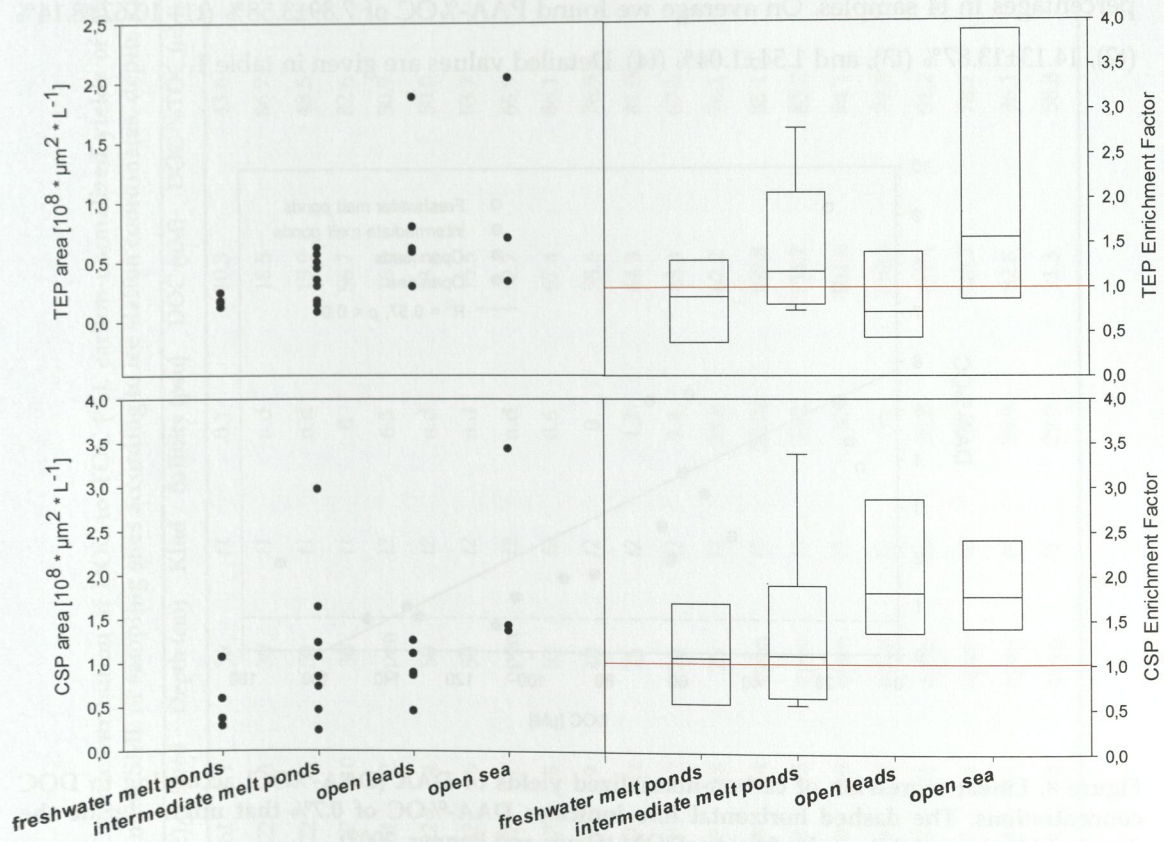

Figure 7. On the left side, total area covered by TEP and CSP in the sea-surface microlayer of different sampling locations. On the right side, box plots show enrichment factors for TEP and CSP according to the different sampling sites. The horizontal red line indicates enrichment factor = 1 (no enrichment).

DYNAMICS OF DISSOLVED AND PARTICULATE ORGANIC MATTER

The freshness of dissolved organic matter (DOM) in the SML of arctic samples is expressed as DAA-%OC, based on carbon-normalized yields of DAA (see methods). The linear regression of DOC (µM) - DAA-%OC is shown in figure 8, with $R^2 = 0.57$, $p < 0.01$. These results suggest that in the SML of melt ponds DOM was freshly produced while in the SML of open ocean the DOC reservoir contained more refractory DOM (DAA-%OC < 0.7%, as in (Davis and Benner, 2007). DAA-%OC yields were quite high in melt ponds of the t1 and t2 type, but similar values were found in arctic melt water (Amon and Fitznar, 2001). On average, DAA-%OC was 5.85±2.3% (t1), 4±1.76% (t2) 1.01±0.5% (t3), and 1.25±0.52% (t4). We calculated the PAA-%OC the same way. PAA contribution to POC was very variable and quite high in melt ponds (t1 and t2) and open leads samples (t3), accounting for lower percentages in t4 samples. On average we found PAA-%OC of 7.89±3.58% (t1) 10.67±8.14% (t2), 14.13±13.87% (t3), and 1.54±1.04% (t4). Detailed values are given in table 1.

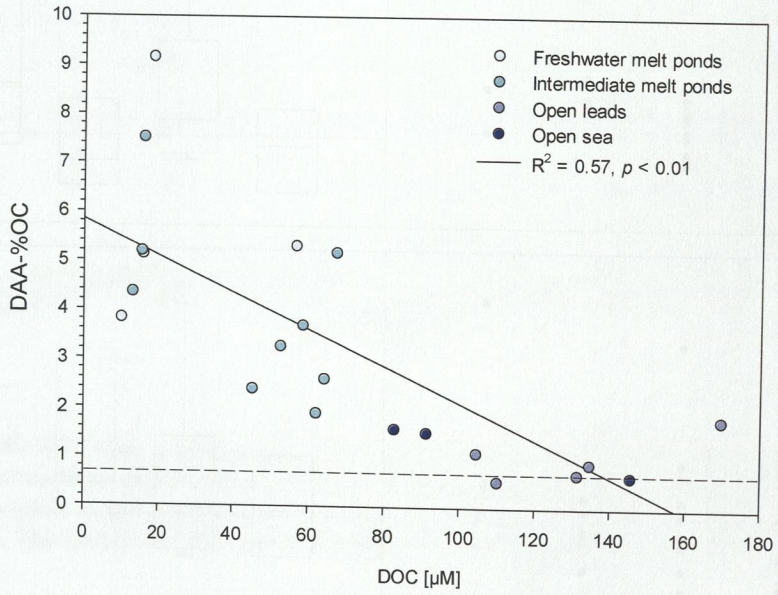

Figure 8. Linear regression of carbon-normalized yields of DAA (DAA-%OC) according to DOC concentrations. The dashed horizontal line indicates DAA-%OC of 0.7% that might define the threshold between labile and refractory DOM (Davis and Benner, 2007).

Table 1. Values for DOC and contribution of DOC to TOC (%), carbon-normalized yields of dissolved and particulate amino acids (%) and TEP and CSP contribution to TOC (%) in the SML of sampling sites according to ice station coordinates, depth, kind and salinity.

Lat.(N)	Lon.(E)	Site#	Depth (cm)	Kind	Salinity (psu)	DOC (µM)	DOC-%TOC (µM)	DAA-%OC	PAA-%OC	TEP-%TOC	CSP-%TOC
84.05583	31.22361	1	30	*t1*	0.1	10.3	43.6	3.8	5.1	0.57	2.50
84.02056	76.11222	3	30	*t1*	n.d	18.5	86.7	9.1	5.9	0.69	2.92
84.02056	76.11222	5	50	*t1*	n.d	15.9	48.5	5.1	7.5	0.27	1.97
82.81333	109.6917	10	30	*t1*	0	56.7	82.6	5.3	13.0	0.29	1.80
84.05583	31.22361	2	open	*t2*	6.3	16.0	50.9	7.5	15.0	0.78	10.62
84.02056	76.11222	4	50	*t2*	n.d	13.2	50.0	4.3	3.8	1.27	5.46
84.02056	76.11222	6	50	*t2*	n.d	15.5	53.7	5.2	8.1	1.65	3.56
82.81333	109.6917	7	open	*t2*	n.d	52.7	65.9	3.3	9.2	0.56	2.04
82.81333	109.6917	8	53	*t2*	0.6	67.4	86.1	5.2	24.5	0.39	2.95
82.81333	109.6917	9	40	*t2*	0	58.6	76.9	3.7	15.1	0.60	1.41
83.05139	129.9967	11	25	*t2*	1.7	64.3	81.0	2.6	18.2	0.09	1.84
83.05139	129.9967	12	20	*t2*	1.4	45.3	67.6	2.4	1.2	0.16	0.55
83.05139	129.9967	13	25	*t2*	28.8	62.2	76.1	1.9	0.9	0.20	1.16
82.09	131.1308	14	open	*t3*	30.02	169.8	85.1	1.8	11.7	0.27	0.85
82.09	131.1308	15	open	*t3*	30.2	134.7	83.7	0.9	6.5	0.28	1.09
87.92972	61.12889	19	open	*t3*	~30	104.6	94.1	1.1	37.7	0.43	0.85
87.92972	61.12889	20	open	*t3*	~30	110.2	79.5	0.5	2.0	0.30	0.74
87.92972	61.12889	21	open	*t3*	30.6	131.4	91.2	0.7	12.8	1.33	0.37
85.23583	123.0417	16	open	*t4*	30	145.7	78.7	0.6	2.7	0.13	0.82
85.25139	122.9244	17	open	*t4*	29.9	82.8	36.1	1.6	0.8	0.26	0.59
85.27833	122.8014	18	open	*t4*	29.9	91.3	58.3	1.5	1.1	1.10	2.73

Melt ponds, open leads and open sea differed mostly in salinity. In table 2, correlation coefficients of salinity to other parameters are given, according to Spearman Rank Order Correlation. As shown in table 2, there is a direct relationship between salinity and TEP (as area and C-content), bacterial abundance and DOC concentrations, but an inverse correlation of salinity to DAA-%OC. Salinity instead did not significantly affect POC, PAA-%OC, CSP (area and C-content) and amino acids concentrations (as dissolved and particulate fractions).

Table 2. Spearman Rank Order Correlation between salinity and TEP area, carbon content (C-content), bacterial abundance, DOC and DAA-%OC in the SML of all samples. *C* = correlation coefficient.

Salinity	TEP area	TEP-C content	Bacterial abundance	DOC	DAA-%OC
C	0.60	0.53	0.68	0.80	-0.83
significance	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.01$	$p < 0.01$

Bacterial abundance in the SML was inversely correlated to DAA-%OC (Spearman Rank Order Correlation, $C = -0.70$, $p < 0.01$) but directly correlated to TEP (area and C-content, Spearman Rank Order Correlation, $C = 0.70$, $p < 0.01$ and $C = 0.68$, $p < 0.01$ respectively).
We found a significant positive correlation between CSP particles' area with TOC (Spearman Rank Order Correlation, $C = 0.43$, $p < 0.05$), TAA (Spearman Rank Order Correlation, $C = 0.47$, $p < 0.05$) and CSP-C content to both DAA and PAA (Spearman Rank Order Correlation, $C = 0.46$, $p < 0.05$ and $C = 0.48$, $p < 0.05$).

MARINE GEL PARTICLES ABUNDANCE AND SIZE-FREQUENCY DISTRIBUTION

Marine gel abundance and concentration were quite diverse. In general, CSP particles were found in higher numbers and accounted for a larger volume than TEP particles. TEP abundance ranged from 1.86 to 24.43 x 10^3 particles mL^{-1} and TEP volume concentration from 0.02 to 1.11 ppm. CSP abundance instead ranged from 5.26 to 30.9 x 10^3 particles mL^{-1} and CSP volume concentration from 0.19 to 3 ppm. CSP contributed more to TOC (%) than TEP (table 1). Table 3 resumes averages abundances, volume concentrations, carbon content and percentages of marine gels to total organic carbon in the SML according to the different types of sampling sites.

Table 3. Averages TEP and CSP particles abundance (10^3 particles mL^{-1}), contribution of small size fraction (0.4 - 1.25 µm) to total particles abundance, volume concentration (ppm), carbon content (C-content) and contribution to total TOC (%) in the SML of the different typologies of sampling locations.

	TEP abundance	TEP (0.4 - 1.25 µm) % of total abundance	TEP volume concentration	TEP-C content	TEP-%TOC
Site type	[10^3 particles mL^{-1}]	[%]	[ppm]	[µM]	[%]
t1	4.2±2.3	82.0±6.1	0.06±0.02	0.14±0.04	0.5±0.2
t2	10.2±4.7	85.4±4.1	0.13±0.08	0.29±0.15	0.6±0.5
t3	13.5±6.9	77.1±3.7	0.41±0.39	0.76±0.65	0.5±0.5
t4	15.3±8.9	75.5±4.2	0.41±0.40	0.85±0.78	0.5±0.5

	CSP abundance	CSP (0.4 - 1.25 µm) % of total abundance	CSP volume concentration	CSP-C content	CSP-%TOC
Site type	[10^3 particles mL^{-1}]	[%]	[ppm]	[µM]	[%]
t1	13.5±5.8	80.5±4.7	0.39±0.15	0.77±0.31	2.3±0.5
t2	13.0±5.8	74.4±8.2	0.90±0.58	1.51±0.87	3.3±3.1
t3	18.2±3.2	80.8±4.7	0.71±0.41	1.19±0.52	0.8±0.3
t4	19.8±9.6	75.0±0.7	1.51±1.30	2.38±1.64	1.4±1.2

The amount of particles in the smallest size fraction (0.4 - 1.25 µm), which can be of great importance for POA emission, represented the highest percentage of marine gels abundance with significant differences between TEP and CSP (t-test, $n = 21$, $p < 0.05$).

The size-frequency distribution of gel particles allowed calculating the spectral slope value δ as descriptor of the size-distribution function (see methods). A less negative spectral slope implies that there is a higher fraction of marine gels that accounts for larger particles. Spectral slope values were not different for TEP and CSP according to typology of sampling site (figure 9). However, average TEP-δ was -3.18±0.2 and average CSP-δ was -2.91±0.12, indicating that in general, CSP marine gels were characterized by higher numbers of larger particles with respect to TEP. Taken all together, data on marine gel size frequency indicated that in the SML of arctic sampling sites, the abundance of gel particles was higher in the proteinaceous fraction (CSP), especially in open sea samples (t4). However, CSP particles were in general larger and in the small size fraction (0.4 - 1.25 µm) they accounted for a

significantly smaller percentage (on average 77.2±6.6%, t-test with $n = 21$, $p < 0.05$) on total gels abundance than their polysaccharidic counterpart (TEP, with on average 81.4±5.8%).

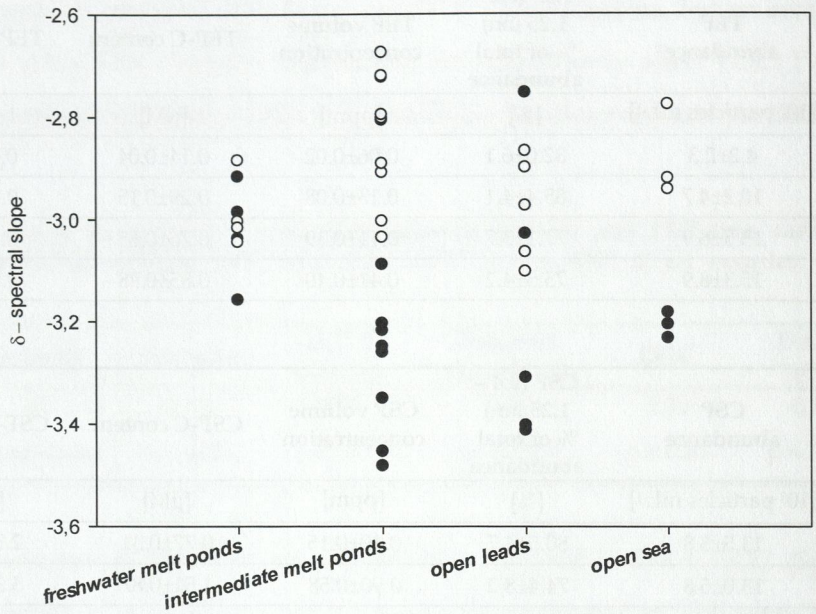

Figure 9. Spectral slope values for TEP and CSP in the SML of different sampling sites.

DISCUSSION

SEA-SURFACE MICROLAYER COMPOSITION AND DOM DYNAMICS

From the bulk composition of the SML, enrichment factors and diagenetic state of DOM as DAA-%OC, it is evident that melt ponds and open leads in the pack ice can be highly heterogeneous compared to open sea samples. Nevertheless, across different ice stations and, therefore, variable sea ice conditions, the SML of freshwater melt ponds and open leads in the pack ice was always enriched in DOC and DAA, while intermediate melt ponds and open sea accounted for higher amounts of particulate organic matter (POM) in terms of POC and PAA (figures 4, 5).

Freshwater melt ponds accounted for most of the labile OM in comparison to more saline environments, like some intermediate melt ponds, open leads at the ice edge and open sea

samples (figure 8). Lower DOC concentrations, the high DAA-%OC yields and the overall enrichment in DOC and DAA in the SML of these sites might hint to predominant primary production processes over bacterial degradation, as also expected by the low bacterial abundances and activities in the SML, and overall depletion in every sampling site compared to the underlying water.

In freshwater melt ponds, areas of TEP and CSP were lower than at other sampling sites, while gel particle area in intermediate melt ponds, open leads and open sea samples was quite similar to values previously reported for FYI ice cores and melt water below the ice floes (Krembs and Engel, 2001). In arctic ice cores, extracellular polymeric substances (such as TEP and CSP) released by the diatom *Melosira arctica* were found to change porosity of sea ice and increase salt retention (Krembs et al., 2011). The extracellular substances able to modify sea-ice properties and ice algae habitat likely include ice binding proteins that can also be found in ice-adapted bacteria (Raymond, 2011). In our samples salinity was significantly correlated to DOC, freshness of DOM (as DAA-%OC), bacterial abundance, TEP area and TEP carbon content (TEP-C). One hypothesis behind this observation could be the fact that freshwater melt ponds are new habitats for ice algae and bacteria that are apt to live in the saline brines inside the pack ice (Horner et al., 1992), so even primary production of DOM might be lower in these new ponds. However, it is still possible that autotrophic DOM production oversteps heterotrophic DOM degradation, resulting in a higher DAA-%OC as more fresh material is released and accumulated. The presence of marine gels, like TEP and CSP, even if in lower extent than in other sampling sites (figure 7) might result from the microbial formation of a superficial gel-like matrix of extracellular polymeric substances as a protection against UV radiation, which in freshwater melt ponds may be higher due to the lower depth and the absence of mixing (Hader et al., 2007, Flemming and Wingender, 2010). Furthermore, UV light might promote the photochemical breakdown of POM into DOM (photodissolution) (Mayer et al., 2006), therefore increasing the concentration of DOM that might be available for assemblage into larger polymers where bacterial DOM uptake is inhibited. DOC precursors that lead to the formation of marine gels are polyelectrolyte polymers in the colloidal size fraction (< 1 µm)(Verdugo et al., 2004). The formation and stability of larger polymeric structures like marine gels, depend on the length of the polymeric chains, on the polyelectrolytes in DOC, and on the presence of counterions, such as Ca^{2+}, inducing fast reversible phase transition due to electrostatic and ionic bonds

(Verdugo, 2012). This feature further suggests that the increased salinity, and therefore the higher concentration of counterions able to stabilize their structure might explain increasing areas of polysaccharidic gels from freshwater melt ponds to open water at the ice edge and open ocean (figure 7). Higher concentrations of DOM and the aggregation of DOM precursors into TEP (Engel et al., 2004) might stimulate a bacterial community in the SML that profit from these compounds as a nutrient-rich source (Passow, 2002b). The inverse correlation of salinity to DAA-%OC might suggest that the turnover of DOM by heterotrophic metabolism is higher in more saline environments where bacterial communities are better adapted to live, rather than in freshwater ponds of recent formation and fast refreezing processes.

Intermediate melt ponds are marine environments similar to the surrounding ocean, connected to it but without the disturbance of waves and usually with lower surface salinity (Lee et al., 2011). In late summer days as temperatures drop fast below zero, the ponds refreeze, incorporating algal aggregates of *M. arctica* and other phytoplankton species into the soft iced surfaces, producing an effect that is not occurring in freshwater melt ponds because of lower salinity and faster freezing process (Lee et al., 2011). We observed these algal aggregates and slowly refreezing in soft surface ice in intermediate melt ponds and open leads sites. Intermediate melt ponds were also characterized by a SML enriched in POC (median EF > 2) as well as TEP and CSP (median EF > 1) suggesting an accumulation of extracellular substances released by primary productivity and bacterial metabolism. The increased salinity probably favored the assemblage of small dissolved components into polymer gels (Engel et al., 2004) and their accumulation in the SML since EF for TEP were higher than freshwater meltponds and open leads (figure 7). A second layer of ice was observed forming around the bottom of many intermediate melt ponds, a common feature of this kind of sites which limits mixing in the system and supports further algal growth (Lee et al., 2011).

Open leads in the pack ice differ from melt ponds and open sea samples. These sites are specific environment where bubbling processes mediate the atmospheric emission of SML DOM, such as marine gels, at low wind speeds and in the absence of breaking waves (Norris et al., 2011). The SML composition open leads was highly variable and in terms of POM, substantially different from open sea samples. The higher contribution of DOC to the total OC pool (figure 4) may be attributed to free polymeric dissolved precursors (as DAA), while

PAA concentrations and EF (figure 5) might instead explain CSP enrichment (figure 7). In open leads of the Central Arctic, TOC and bacteria-enriched surface microlayers have been observed (Keith Bigg et al., 2004, Matrai et al., 2008, Gao et al., 2012), suggesting that bacteria might contribute to the higher PAA concentrations (figure 5). A reduced TEP production and enrichment might also be explained by limited wave action that can facilitate abiotic aggregation processes (Wurl et al., 2011). Open sea samples are characterized instead by higher POC concentrations to which probably CSP mostly contributed (figures 4, 5, 7). The Arctic Ocean is a large reservoir for organic carbon. In open sea sites, winds, waves and bubbling processes that scavenge DOM from the water below to the SML are enhanced. All these factors contribute to the aggregation of DOM into larger polymers, thereafter thoroughly remineralized by surface bacterial communities as indicated by the DAA-%OC yields (figure 8).

MARINE GELS ABUNDANCE AND IMPLICATIONS FOR POA

The debate whether TEP and CSP might be the same particle or different subunits of the same particle is still open (Engel, 2009), and CSP in the SML have been determined only in few studies so far (Kuznetsova et al., 2005, Galgani and Engel, 2013). In our samples, CSP were found in higher abundance and concentration with respect to TEP and this has been recently observed in completely different study conditions and environments (Galgani and Engel, 2013, Galgani et al., in prep.). In the high Arctic, the major source of CCN has been proposed to have a polysaccharidic composition (Russell et al., 2010, Orellana et al., 2011) but so far there is no evidence limiting the possibility of surface microlayers to provide proteinaceous material for POA. Instead, there have been results of amino acids in arctic aerosols that might have a biological origin in the marine surface layer (Scalabrin et al., 2012). Submicron CSP (0.4 - 1.25 μm) contributed less (77.2±6.6%) than TEP (81.4±5.8%) in the same size fraction to total gels abundance in the SML, being characterized by an increased fraction of large particles (figure 9). Still CSP total abundance was higher than TEP, the proportions being relatively similar despite the different sampling locations.

We suggest that in the Central Arctic Ocean, as sea ice retreats, the release of dissolved polymer precursor is enhanced by higher primary productivity; their aggregation into larger

particles such as TEP is facilitated by increasing salinity stabilizing the polymeric structure of marine gels through electrostatic interactions and ionic bonds.

Marine gels are hotspots for intense microbial activity, and we might expect that the higher gel abundance and area coverage may suppose increased microbial heterotrophic metabolism as reflected in less labile, or more degraded, DOM from first open spots in thinning sea ice. CSP particles might represent the result of bacterial contribution to the gelatinous surface matrix, which, through degradation and exudation of DOM, modifies the structure of marine gels originating from polysaccharidic content. Bacteria can be found incorporated into microgels in arctic surface microlayers or aerosols (Keith Bigg et al., 2004) contributing to the TOC/TAA pool and to CSP if cells lyse because of extreme UV radiation. Particle-associated bacteria should preferentially degrade nitrogen-rich compounds faster with respect to carbon (Orellana et al., 2007), but the higher carbon content of CSP as compared to TEP (table 3) suggests that these two compounds might be sub-fractions of the same particle, as we did not see a selective decrease of CSP. The lower area found in freshwater melt ponds for both types of marine gels might reflect UV radiation either inhibiting the aggregation of dispersed polymers in seawater into larger particles or accentuating their photolysis (Orellana and Verdugo, 2003, Ortega-Retuerta et al., 2009).

We conclude that surface ocean biology and DOM turnover in the Arctic Ocean impacted by global warming is expected to influence atmospheric processes like CCN emission and cloud composition. These effects are likely to derive from structural changes of POA precursors in the gelatinous arctic surface layers mediated by bacterial DOM turnover and DOM photo-degradation. A gel-like SML (Wurl and Holmes, 2008) might be ubiquitously present, although in the Arctic Ocean its dynamics fluctuate much as a result of physical (sea-ice melting or freezing) and biological (primary and secondary production) events. The loss of MYI in favor of FYI is not only reflected in an enhanced total sea ice loss but also enhanced primary productivity as the ice retreats. Melt pond percentage cover is likely to change, as well as melt ponds characteristics in favor of shallower, wider freshwater melt ponds (Lüthje et al., 2006). SML dynamics, like organic matter production and degradation, are probably more complex in the Arctic than elsewhere due to the fast changing habitat. A combined approach of physico-chemical and biological studies is needed to investigate the coupling of surface-ocean/atmospheric processes in the Arctic crucial for global carbon cycle and climate regulation.

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GENERAL DISCUSSION

This doctoral thesis focused on three main aspects of the sea-surface microlayer (SML) that can be outlined in three words: composition, response, transition.
The first manuscript deals with the composition of the SML as a result of phytoplankton and bacterial activity with emphasis on polysaccharidic and proteinaceous gel particles. The second investigates, through a large-scale mesocosm experiment simulating future ocean conditions of increasing pCO_2, how the SML responds to ocean acidification and ultimately, to climate change. Last, the third manuscript describes the transition of the SML throughout different environments encountered in the Arctic Ocean, itself subject to rapid environmental changes likely to initiate atmospheric significant feedbacks for global climate dynamics.

THE GELATINOUS COMPOSITION OF THE SEA-SURFACE MICROLAYER AND PROBABLE DYNAMICS

The SML has gelatinous characteristics, and has been imagined as a gel-like matrix of organic particles mostly composed by polysaccharides and proteins and embedded neustonic communities (Sieburth, 1983, Wurl and Holmes, 2008, Cunliffe and Murrell, 2009, Cunliffe et al., 2011). This hypothesis has been rather founded on field studies and required testing through reproducible laboratory experiments, focusing on the two classes of gel particles, polysaccharidic TEP and proteinaceous CSP (manuscript I). During this study, in a control tank containing sterile (0.2 µm filtered) seawater, TEP particles in the SML were found to exceed the concentration of underlying water most of the times while CSP, in the same tank, were rather depleted in the SML. The opposite situation - CSP enrichment and TEP depletion in the SML - was observed in treatment tanks, differing from the control by being supplemented with cultures of the diatom *Thalassiosira weissflogii*, grown in chemostats at different pCO_2 conditions. Whereas CO_2 did not seem to have an effect on marine gels in the SML, highlight of this study was the enhanced presence of CSP particles with respect to TEP, a major finding of all research works in the course of this thesis (manuscripts I, II and III).
The second study dealt with ocean acidification effects on SML composition (manuscript II). The results of the mesocosm campaign conducted in Bergen revealed that the composition of the SML reflected the development of a phytoplankton bloom, inferring that the SML is

tightly linked to biological processes of the underlying euphotic ocean and in particular the exudation of organic matter. Moreover, the study determined how ocean acidification altered the gelatinous components of the SML, suggesting that this might have occurred most probably by affecting the bacterioneuston community. While the area covered by polysaccharidic marine gels (TEP) in the SML increased along with bacterial abundance, proteinaceous marine gel area (CSP) decreased with increasing pCO_2.

Through the size-frequency distribution analysis of gels in the SML, we tried to link the SML composition to possible dynamics involved in primary organic aerosol (POA) formation.

In general, CSP area was larger than TEP area and the CSP pool was characterized by larger particles. TEP were smaller but more abundant, especially in the size fraction (0.4 - 1.25 µm) most relevant for POA formation. Overall, the results showed that rising pCO_2 affects the polysaccharidic/proteinaceous characteristics of the gelatinous SML, pointing to the hypothesis that these compositional changes might be mediated to microbial processes. By altering the gelatinous SML, ocean acidification might trigger secondary feedbacks on air-sea gas exchange and POA emission, subsequently affecting Earth's radiative budget.

In the third study of this thesis, the SML was sampled across a range of water surfaces in the Central Arctic Ocean during the summer of the 2012 sea ice minimum. Results from this campaign showed that the composition and reactivity of dissolved organic matter (DOM) in the SML changed with the melt progression of the ice, from the first meltwater pools on the ice surface, to open leads, followed by the open sea. In melt ponds, primary production was presumed responsible for the more labile DOM (expressed as carbon-normalized yields of amino acids, DAA-%OC), while bacterial activity and DOM degradation might have been inhibited by strong UV radiation. The presence of TEP went along with increasing bacterial abundance, probably reflecting a higher rate of DOM turnover. The larger extension (as area) that CSP showed in the SML might be the result of DOM exudation by bacteria embedded in a proteinaceus matrix to which they directly contributed (Flemming and Wingender, 2010). Results on marine gel size frequency indicated that in the SML of arctic sampling sites the abundance of gel particles was higher in the proteinaceous fraction (CSP), especially in open sea samples. However, CSP particles were in general larger and in the small size fraction (0.4 - 1.25 µm) they accounted for a smaller percentage on total gel abundance than their polysaccharidic counterpart (TEP), similarly to what observed during the laboratory and the mesocosm study (manuscripts I and II). CSP particles - to the best of the author's

knowledge- have been determined in the SML only in one study so far (Kuznetsova et al., 2005), revealing that CSP had comparable sizes to TEP and were generally concentrated in the SML. As it remains unclear whether TEP and CSP are the same or different particles (Passow, 2002, Engel, 2009), in the following, TEP and CSP will be described as marine gels of polysaccharidic and proteinaceous composition.

This thesis points to the idea that the SML can be partly a gelatinous film where bacteria and marine gels are closely associated and concentrated with respect to the water below (manuscript I). Following this hypothesis, the SML reflects biological processes occurring in the water column and gets enriched in organic material proceeding from below by buoyant particles and rising bubbles (manuscript II). However, the coupling of gels and bacteria accumulated in the SML might be responsible for the composition and turnover of SML organic matter; changes in the organic matter pool might have implications for atmospheric emissions (manuscript III).

The SML reflects the dynamics the whole marine system experiences in a warmer and high CO_2 ocean. By affecting bacterial metabolism, ocean acidification is suspected to induce additional changes in the gelatinous structure of the SML promoting either the degradation or release of gels (manuscript II). In a system dominated by phytoplankton exudates rather than heterotrophic activity (manuscript I), the effect of CO_2 might have not been clear because of a limited bacterial metabolism.

In the ocean, the direct effect of rising temperature on biological processes might diverge between autotrophic and heterotrophic communities, with resulting altered carbon fluxes additionally affected by a thermal stratification that delays vertical carbon export (Riebesell et al., 2009). In a warmer ocean, carbon respiration might dominate over autotrophic fixation (Wohlers et al., 2009), and although the release of extracellular carbohydrates by phytoplankton might increase (Engel et al., 2010), the probability of aggregates formation and their degradation by bacterial metabolism is also likely to be promoted (Piontek et al., 2009). These dynamics might be also visible in the SML, as the melting of sea ice due to increasing temperatures was accompanied a shift in the composition of organic matter (manuscript III).

The observations during the course of this work suggest that the bacterioneuston community has an important role in altering the gelatinous matrix in the SML. This might be achieved by being attached to gel particles as nutrient-rich substrates (Passow, 2002), and modifying

these particles by either degradation of organic components or direct release of exudates (through cell disruption, active release of extracellular enzymes, viral lysis or even photochemical degradation), or both (Azam et al., 1993, Jiao et al., 2010). The spontaneous aggregation of free DOM polymers into marine gels (as discusses for the control tank of manuscript I) as the result of physical interactions of specific moieties in the polymeric chain (Chin et al., 1998) might also occur for proteinaceous dissolved precursors but probably not to the same extent as polysaccharides. Some HMW DOM isolated from seawater resists rapid microbial degradation and have sufficiently long lifetimes to accumulate in the surface and be recruited in aggregation processes (Aluwihare et al., 1997, Repeta and Aluwihare, 2006); hydrolysable sugars might be part of this HMW DOM pool (Gogou and Repeta, 2010). Dissolved free amino acids are rapidly removed from the water column by bacterial uptake (Carlson, 2002), while dissolved combined amino acids (DCAA) might condense to carbohydrates through glycosylation mediated by specific enzymes (up to 50% of the DCAAs pool) and thus be slowly assimilated (Keil and Kirchman, 1993), leading to organic polymers of a mixed polysaccharidic and proteinaceous composition.

Exudation of DOM by phytoplankton has been identified at every life stage the life cycle as HMW and LMW DOM compounds like carbohydrates, proteins and lipids (Bjørnsen, 1988, Myklestad, 1995, Carlson, 2002). During a phytoplankton bloom, the release of dissolved colloidal compounds might be enhanced (Kepkay et al., 1997) and this fraction of organic carbon includes a high polysaccharide content that might be rapidly metabolized by heterotrophic activity (Benner et al., 1992). The presence of high concentration of DOM stimulates the activity of marine bacteria, which rapidly consume LMW DOM first (Amon and Benner, 1996). Bacterial activity might result in an increased uptake of amino acids for the nitrogen content, and therefore removal from the water column. Despite their low concentrations, dissolved free amino acids and neutral sugars have high turnover rates inferring a high flux of these compounds in the ocean (Amon and Benner, 1994, Carlson, 2002).

It is proposed here that there are predominantly two pathways leading to the formation of a gelatinous SML (figure 1). First, DOM precursors might ascend the water column to the SML where due to surface-active characteristics and to seawater surface tension, new particles can be formed, facilitated by the stable conditions of a surface film (Kuznetsova et al., 2005, Wurl et al., 2009). In the second path, DOM precursors freely dispersed throughout the water first

assemble into gel polymers through abiotic processes (Chin et al., 1998). During subsequent high phytoplankton activity, the so-formed marine gels further aggregate into larger macromolecular establishing the link between the DOM and the POM pools (Engel et al., 2004). These particles are a vehicle for oceanic carbon to be exported either to the deep sea floor or to the ocean surface and are intensively colonized by marine bacteria (Passow et al., 2001, Passow, 2002, Azetsu and Passow, 2004, Engel, 2009).

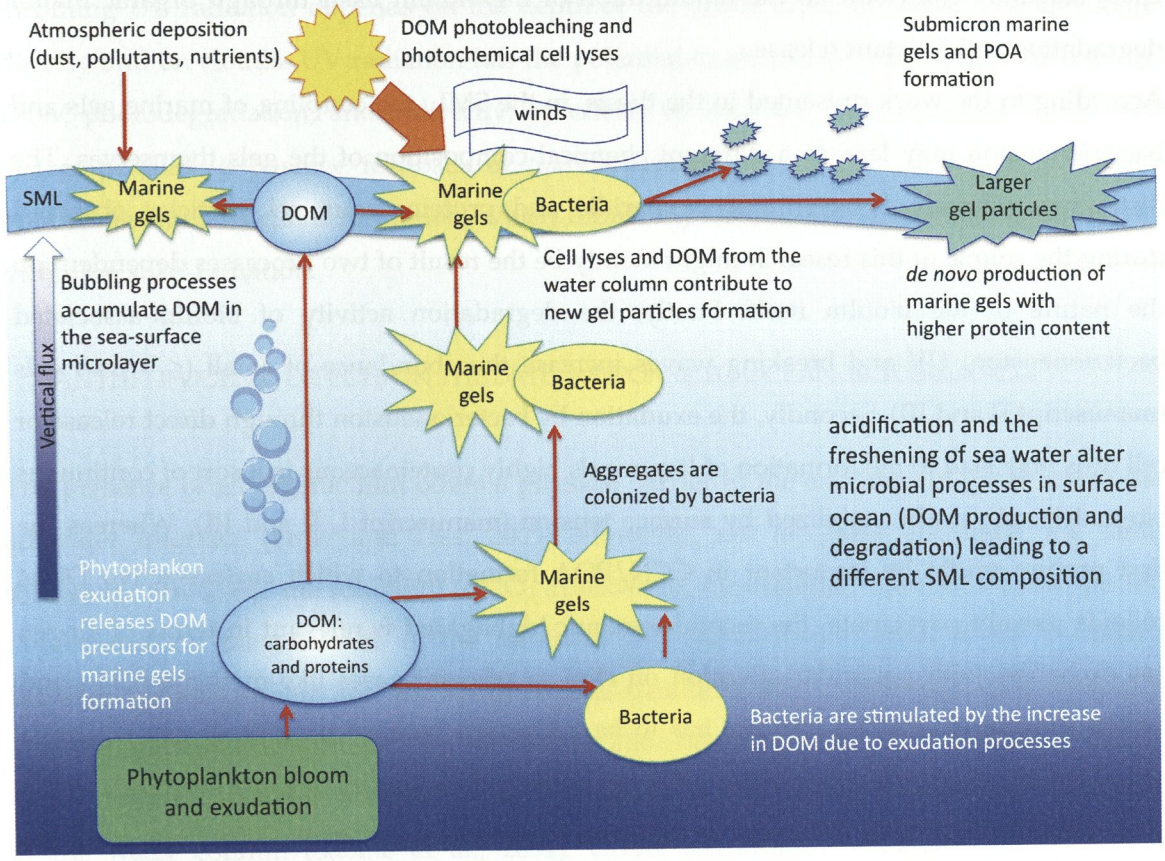

Figure 1. Conceptual model of the interactions between various components in the DOM cycle that lead to the gelatinous composition of the SML.

Several factors determine the community structure of the bacterioneuston that concentrate in the SML by the upward rise of cells attached to buoyant particles (Joux et al., 2006, Stolle et al., 2011) or rising bubbles (Carlucci and Bezdek, 1972).

Bacterioneuston in the SML receives high doses of UV radiation compared to bacterioplankton. In general, bacteria might react to UV by forming biofilms (Hader et al., 2007, Ruiz Gonzalez et al., 2013), thus protecting themselves in a demanding habitat that

does not promote the adaptation of a specific community (Stolle et al., 2009). Biofilms are one of the most successful life strategies on earth: a biofilm is formed by the release of extracellular polymers by microorganisms (that might be carbohydrates, proteins, and lipids) keeping the cells together, promoting their interaction, and retaining extracellular enzymes that allow for a sort of external digestive system (Flemming and Wingender, 2010). In this respect, the SML might be regarded as a biofilm where bacterioneuston activity can be higher compared to the underlying water (Kuznetsova and Lee, 2001, Obernosterer et al., 2005) and may contribute to the modification of the biofilm itself through organic matter degradation and constant release.

According to the work presented in the thesis, in the SML, the coupling of marine gels and bacterioneuston may lead to a different chemical composition of the gels themselves. The distinct partitioning between polysaccharidic and proteinaceous gel particles observed during the course of this research might mainly be the result of two processes dependent on the nature of the biofilm itself. Firstly, the degradation activity of biofilm-associated bacterioneuston, UV and breaking waves increase the abundance of small (< 1 μm) gels (manuscript II and III). Secondly, the exudation by bacterioneuston through direct release or cell lysis may lead to the formation of larger gels highly proteinaceous in a sort of continuous bio-matrix, physically stabilized by surface tension (manuscript I, II and III). Whereas the first process might be important in CCN/POA formation to which surface-active DOM colloids already participate, the second dynamic highlighted is relevant in terms of air-sea gas exchange, with particular attention on climate relevant gases like methane (CH_4) and carbon dioxide (CO_2). This process has to be addressed because the rate of gas exchange across the air-sea interface depends on the thickness and continuity of a layer through which gases pass by the process of molecular diffusion (Upstill-Goddard et al., 2003, Frew and Liss, 2005, Cunliffe et al., 2013). Although these two bacteria-mediated processes might seem controversial, their balance depends on many complex interactions between internal and external inputs to the SML, and anthropogenic impacts will likely drive to one or another direction difficult to predict.

UV radiation is an important factor influencing microbial degradation of POM/DOM in the SML. While UV-C (40 - 280 nm) is strongly absorbed by the atmosphere and UV-A (320 - 400 nm) causes minor damages to living organisms, UV-B (280 - 320 nm) has the highest energy per photon. UV-B might enhance bacterial cell lysis (Hader et al., 2007), inhibit bacterial

activity in the SML (Herndl et al., 1993) or promote a preferential uptake of carbohydrates (Santos et al., 2012). Additionally, photolysis of DOM produces small molecules readily available for bacterial uptake (Reinthaler et al., 2008) and impedes the assemblage of DOM colloids into larger polymers, whose stability relies in the length of the polymeric chains (Orellana and Verdugo, 2003). In the SML, strong UV-B radiation might also inhibit autotrophs, resulting in favored DOM degradation processes (Obernosterer et al., 2005). UV effects are additional co-factors that might modify the structure of the SML. The extent of incoming UV radiation depends on the depth of the ozone layer, on aerosol composition, density and on clouds. UV radiation has the potential to alter SML composition directly by DOM photodegradation, and indirectly, by effects on bacterioneuston activity. These two factors produce a positive feedback of marine systems to CCN/POA emission, aerosol optical density and cloud formation, which ultimately control the surface energy fluxes of incoming solar radiation.

ANTHROPOGENIC EFFECTS ON THE SML: RESPONSE TO OCEAN ACIDIFICATION

The presence of an organic film offers a physical barrier to the exchange of gases across the air-water interface (Frew and Liss, 2005). However, not all gases encounter the same diffusivity obstacles: the solubility of CO_2 in seawater, for example, does not exhibit specific dependence on the thickness of the turbulent and sub-diffusive layers at the boundary between the atmosphere and the ocean (Upstill-Goddard, 2006). Therefore, CO_2 is rapidly diffused into marine systems. Its increasing atmospheric pressure provokes the excess anthropogenic CO_2 to be absorbed by the ocean and highly concentrate in the first 400 meters of the water column (Sabine et al., 2004). Ocean acidification enhances phytoplankton exudation and the formation of marine gels in the euphotic zone (Engel, 2002, Borchard and Engel, 2012), stimulates bacterial abundance and microbial degradation of organic matter (Piontek et al., 2010, Endres, 2013) and promotes a vertical upward flux of swollen, larger marine gels (Mari, 2008) that contribute to the buildup of the SML. While bacterioneuston abundance and activity increased along with enhanced polysaccharidic marine gels (manuscript II), proteinaceous marine gels decreased at high pCO_2 thus inferring to the hypothesis of polysaccharides/proteins partitioning in the gelatinous SML controlled by bacterioneuston DOM and POM degradation.

Given the global extension of the SML (MacIntyre, 1974, Wurl et al., 2011), enhanced bacterioneuston activity in comparison to the underlying water might be an additional driving force for air-sea gas exchange controlling the fluxes of climate relevant gases like CO_2 (Calleja et al., 2005, Reinthaler et al., 2008) and CH_4 (Upstill-Goddard et al., 2003).

PRESENT AND FUTURE OCEAN: THE THREAT OF THE ARCTIC

As one of the aspects of climate change is ocean acidification, another effect is surface ocean warming (Meehl et al., 2007). Rising atmospheric CO_2 concentration leads to an increase of surface warming, and at high latitudes this process is enhanced by the sea ice/snow albedo temperature feedback (Chylek et al., 2009). Additionally, oceanic circulation might bring warm waters into the Arctic Ocean, thus enhancing the further melting of sea ice (Comiso et al., 2008). The freshening of arctic waters due to the massive loss of multi-year ice (MYI) (Comiso, 2011) is likely to decrease the buffer capacity of the Arctic Ocean to increased water CO_2 levels, with the effects of amplified acidification of arctic seas (Steinacher et al., 2009). Primary productivity is enhanced by increasing temperature and sea ice melting because more light is available for under-ice primary production (Arrigo et al., 2008, Arrigo et al., 2012) with implications for massive carbon export to the deep waters (Boetius et al., 2013). Bacterial degradation of organic matter is also stimulated by higher temperatures (Piontek et al., 2009). In the SML, this effect might lead to an increased biological turnover of DOM, resulting in the breakdown of larger aggregates belonging to the POM pool into smaller microgels (< 1 μm), interfering with processes leading to POA and subsequent CCN formation (Orellana et al., 2011). Furthermore, increased bacterioneuston activity has the potential to enhance the production and turnover of trace gases such as DMS adding a further positive feedback to short-term increasing atmospheric greenhouse gases concentrations (del Giorgio and Duarte, 2002, Upstill-Goddard et al., 2003).

SML composition and bacterioneuston-driven SML alteration might be seen as climate relevant processes due to their influence on air-sea interfacial dynamics. As ocean acidification directly affects the SML and its microbial life, this will likely involve feedbacks with climate relevant consequences.

These consequences might be quickly observed in sensitive ecosystems like high latitude regions. In the Arctic Ocean, the effects of climate change happen so fast that the system is

not able to adapt, and we are not able to fully understand the system dynamics before they have already changed.

WHAT REMAINS TO BE CLARIFIED

Earlier studies on the organic composition of the SML have focused on lipids material because of their surface-active properties and their insolubility in seawater (Garrett, 1967, Kattner and Brockmann, 1978, Kattner et al., 1983). Lipids are important constituents of marine organisms, ubiquitous and relatively stable (Frka et al., 2011). Despite their low concentrations in seawater, lipids significantly contribute to physical properties of surface films, are important as specific biological markers (Brinis et al., 2004, Liss and Duce, 2005, Gašparović et al., 2007) and as indicators of freshness of organic matter (Goutx et al., 2003). Lipids and fatty acids are abundant components of surface slicks, generally defined as monolayers at the nanometric scale, away from the sampling capacity of usual SML samplers (Cunliffe et al., 2013), and which, by their expansion, can cause substantial capillary wave damping and influence air-sea gas transfer (Garrett, 1967). In the SML, phytoplankton contribution to the lipids class, together with abiotic or bacterial-driven lipids degradation, show a seasonal trend (Frka et al., 2011). The establishment of a surface monolayer slick might not be dependent on phytoplankton abundance, but rather on photochemical and biochemical transformations of organic substances accumulating at the surface (Laß et al., 2013). This thesis, being rather focused on the gel-like structure of the SML, did not include these factors, nonetheless of great relevance. Although the role of lipids in the composition of the SML has been reconsidered (Sieburth, 1983, Cunliffe et al., 2011), they might still represent essential structural components of the very first nanolayer, as well as "glue" for the entangled biological network of gels, organic compounds and neustonic organisms. A comprehensive analysis of lipids, marine gels, microorganisms coupled to monolayers sampling is imaginable to provide new ideas on sources, processes and fate of organic matter in the SML and its role on air-sea gas exchange.

"HISTORICAL TIMES, BIOLOGICAL TIMES" AND FUTURE PERSPECTIVES

This journey into the very skin of the ocean has rather opened many more perspectives than expected at the beginning. The gelatinous composition of the SML, its response to climate change, and its possible significance in the Arctic Ocean for atmospheric dynamics have been investigated, focusing on part of the oceanic source of CCN and POA instead of real atmospheric measurements.

There is so much to do, still. The renewed attention on the SML in marine research highlights the fact that most of its processes are still unknown; what is certain is that these processes are relevant in the scenario of changing climate. During the Arctic campaign we collected aerosol samples and ice cores that will help better understanding many processes related to DOM/POM turnover and POA emission in a changing environment. However, future investigations should be conducted in many other directions.

One aspect to be clarified is to what extent POA might comprise proteinaceous microgels, given the high abundance of these particles found in laboratory and field SML samples, and what this might imply in global climate dynamics. Even in remote environments it is now quite difficult to unravel the difference between anthropogenic and marine natural contribution to aerosol fluxes. Recently, the development of a new laboratory facility for sea spray production - the ocean-atmosphere wave flume at the Scripps Institution of Oceanography - has provided interesting results towards understanding the coupling of surface ocean biology and CCN emission, showing that bacteria and phytoplankton have the effect of reducing the hygroscopicity of CCN, that is, the capacity of aerosol particles to activate as cloud droplets (Prather et al., 2013).

Another emerging question is the role of pollutants in the SML, because the toxicity of these compounds on the whole neuston community (both autotrophs and heterotrophs) (Wurl and Obbard, 2004) is another aspect to be considered to gain a more comprehensive understanding of microbial processes and DOM turnover in the SML. Efforts should be directed to investigate the interaction of marine gel particles to heavy metals, POPs, and microplastics, which are likely to accumulate at the surface and be transported for large distances.

The role of the SML in air-sea gas exchange is another issue given the widening of oxygen minimum zones in the ocean and the release of climate relevant gases like carbon dioxide,

methane and nitrous oxide, an ozone depleting gas (N_2O) (Ravishankara et al., 2009). A recent cruise campaign to the upwelling region off Peruvian coast has been conducted (Cruise Leg M91, SOPRAN/SOLAS Project) where the SML and underlying water have been sampled from 40 stations in order to assess the influence or the organic surface film in air-sea gas exchange. Results from this campaign will provide new insights into the role of SML and its mediation of gas fluxes across the ocean-air interface.

During the cruise it would have been interesting to sample trace gases such as DMS and methane directly within the SML. However, limitations related to the SML sampling technique entail immediate loss to the atmosphere. A useful tool would be the employment of microsensors that directly measure oxygen, CO_2, and other gases *in situ* within the SML.

With the most common sampling procedures nowadays applied, sampling requires some time and the sample gets in contact with ambient air.

Each SML sampling method has its advantages and disadvantages, which may be related to costs, availability, handling facility and mostly differing in the SML thickness that can be collected (Cunliffe et al., 2011). There are some issues concerning the affinity of SML components to one or other device: amino acids, for example, might adhere more on the sides of a glass plate, and in general a standardized method is desirable (Cunliffe et al., 2013). However, as no standardized technique has yet been agreed upon, the preferential use of one or other device depends on the study conditions and volume required for sample analysis. Since the attention of the scientific community on the SML is at present very high, it is expected that a "best practice" for handling SML samples will soon be needed.

Critical emerging issues in a future ocean will be pursued by new research directions of the international Surface Ocean – Lower Atmosphere Study (SOLAS) strategies (Law et al., 2013). This PhD project was included in the German contribution to SOLAS, as SOPRAN project (Surface Ocean Processes in the Anthropocene).

In the era of anthropogenic climate change, we need to fully understand the complex interactions that take place in the tiny surface layer that links the ocean and the atmosphere.

Natural systems have a long, biological history of adaptation that deviates from the short-scale historical times of mankind. Any anthropogenic perturbation in the system decreases its stability and these processes – biological processes, unlikely mechanical processes – are not reversible. Time has one direction and it is our legacy to individuate complex responses of complex interactions soon before man-made impacts might become irrevocable.

Interesting phenomena occur when two or more rhythmic patterns are combined, and these phenomena illustrate very aptly the enrichment of information that occurs when one description is combined with another.

Gregory Bateson, Mind and Nature (1979)

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EIDESSTATTLICHE ERKLÄRUNG

Hiermit bestätige ich, dass die vorliegende Arbeit mit dem Titel:

Biogenic Composition of the Sea-Surface Microlayer in Response to a Changing Environment

Von mir selbständig verfasst worden ist und keine weiteren Quellen und Hilfsmittek als die angegebenen verwendet wurden. Die vorliegende Arbeit ist unter Einhaltung der Regeln guter wissenschafltlicher Praxis der Deutschen Forschungsgemeinschaft entstanden und wurde nicht im Rahmen eines Prüfungsverfahrens an anderer Stelle vorgelegt. Veröffentlichte oder zur Veröffentlichung eingereichte Manuskripte wurden kenntlich gemacht. Ich erkläre mich einverstanden, dass diese Arbeit an die Bibliothek des GEOMAR und die Universitätsbibliothek der CAU weitergeleitet wird.

Kiel, September 2013

Luisa Galgani

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Cover image

K. Hokusai „The Great Wave off Kanagawa" (1829-32)

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