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BACHELORARBEIT

Short-term dynamics of dissolved organic matter and bacterial communities in the open North Sea off Helgoland Island

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INHALTSVERZEICHNIS

I. VORWORT

Diese Arbeit wurde am Institut für Chemie und Biologie des Meeres der Carl von Ossietzky Universität in der Max-Plack-Forschungsgruppe Marine Geochemie angefertigt. Der Umfang dieses Projekts der Bachelorarbeit wurde durch die Kombination mit dem Kontaktpraktikum, das während des Bachelorstudiums der Umweltwissenschaften erbracht werden muss, erweitert. Das Praktikum wurde an der Biologischen Anstalt Helgoland des Alfred-Wegener-Instituts Helmholtz-Zentrum für Polar- und Meeresforschung in der Arbeitsgruppe Mikrobielle Ökologie absolviert.

Die Probenahme und ersten Schritte der Probenaufbereitung erfolgten im Sommer 2012 im Rahmen des Kontaktpraktikums auf der Nordseeinsel Helgoland. Dort wurden außerdem die molekularbiologischen Untersuchungen und die statistische Auswertung durchgeführt. Die geochemischen Untersuchungen und das Verfassen der Arbeit erfolgten in Oldenburg.

In Absprache mit meinen Gutachtern Prof. Dr. Thorsten Dittmar und Dr. Gunnar Gerdts wurde diese Arbeit in Form eines Manuskripts einer wissenschaftlichen Publikation angefertigt.

II. MANUSKRIPT

Short-term dynamics of dissolved organic matter and bacterial communities in the open North Sea off Helgoland Island

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1 Abstract

Dissolved organic matter (DOM) in the ocean is one of the largest active carbon pools on earth, similar in size to atmospheric CO_2 or all land plant biomass. Due to its richness in energy and nutrients it is fundamental for microbial life and for marine food webs. The microbial loop is an essential compartment in the global carbon cycle and is important for the transformation and recycling of organic matter and nutrients in the oceans. Microbial communities shape the molecular composition of DOM and vice versa. Earlier long-term studies have shown that seasonal dynamics in DOM composition and microbial communities exists. The aim of this study was to explore and characterize variations in composition of bacterial communities and DOM over short periods of time, ranging from hours to days. We hypothesize that variations in DOM composition are directly related to variations in the bacterial community and/ or environmental conditions. To test these hypotheses, water samples were taken daily over a time period of 20 days and hourly (over 24 hours) in the open North Sea off Helgoland Island. Sea water was analyzed for environmental variables, molecular DOM composition and the bacterial community structure. DOM was isolated from seawater by solid phase extraction and analyzed via ultrahigh resolution mass spectrometry (FT-ICR-MS, Fourier-Transform Ion Cyclotron Resonance Mass Spectrometry). To investigate bacterial community structure, Automated Ribosomal Intergenic Spacer Analysis (ARISA) fingerprinting was used. The current study did not reveal a direct relation between a bacterial community structure changes and variation in the composition of DOM, neither within daily sampling nor the 24 h time series. However both, bacterial community and DOM composition undergo a characteristic shift during the daily sampling, mainly driven by salinity. The 24 h sampling during this time captured much of the variation in salinity and the microbial community, accordingly. High variations of salinity during the sampling period indicate the presence of changes in different water masses that carry distinct molecular and microbial signatures. For the first time, these changes have been documented in such high temporal and analytical resolution.

2 Introduction

Dissolved organic matter (DOM) in the oceans represents one of the largest active carbon pools on earth (700 Gt), containing as much carbon as the Earth´s atmosphere or carbon stored in all land plant biomass (Hedges, 1992).

Hansell (2013) divided the marine DOM into two major classes with distinct reactivity and different functions in the carbon cycle: labile DOM and recalcitrant DOM. Labile DOM has a fleeting existence of minutes to days (Fuhrmann and Ferguson, 1986; Keil and Kirchmann, 1999), as it is rapidly consumed by heterotrophic bacteria. Hence, the freshly produced labile DOM is crucial for the bacterial community and can vary over short timescales. Recalcitrant DOM has turnover times of months to millennia (Bauer et al., 1992; Williams and Druffel, 1987) because it is resistant to rapid microbial degradation and therefore accumulates in the ocean. It serves as a reservoir of carbon until it is mineralized or removed by other mostly abiotic processes (e.g. Stubbins et al., 2012).

The microbial loop has been recognized as an essential part in the global carbon cycle (Azam, 1998). Most marine DOM originates from direct extracellular release during growth of phytoplankton, release and excretion during predation by grazing organisms, release via viral and bacterial cell lysis, solubilization of particles and bacterial transformation (Hansell and Carlson, 2002). The major process for the removal of marine DOM is consumption and remineralization by heterotrophic bacteria (Pomeroy, 1974). DOM that is assimilated into bacterial biomass is channeled via the bacteria to protozoa and to higher organisms (Azam, 1998). Thereby, the microbial loop transfers energy and nutrients to higher trophic levels and provides an important base for microbial life and marine food webs (Hansell et al., 2013).

In the frame of the 'Microbial Interactions in Marine Systems' project (MIMAS, http://mimas-project.de) the diversity and function of marine bacterioplankton in a world of global climate change is examined. In this context, a study on the island of Helgoland in the German Bight monitored DOM and microbial community composition over several years. This long term study revealed that changes in molecular composition of DOM and bacterial community exist (Niggemann et al., unpublished). A central aim of this study is to explore and characterize variations in composition of bacterial communities and DOM over much shorter periods of time, ranging from hours to days. The sampling station Helgoland Roads is well suited for this approach, as seasonal and long-term data for environmental variables (e.g. Raabe and Wiltshire, 2008; Wiltshire et al., 2008; Wiltshire et al., 2009) and microbial parameters are available (Gerdts et al., 2004; Sapp et al., 2007, Teeling et al., 2012).

So far, there are few time series studies available that focused on temporal dynamics of bacterial communities and DOM. Sintes et al. (2010) investigated a seasonal shift of DOC

concentrations and bacterial activity over an annual cycle. Even changes of bacterial community over one day could be shown in the experiment of McCarren et al. (2010) while adding high-molecular-weight DOM. In a study of Chauhan et al. (2009) tidal dynamics of DOC concentration and bacterial community was observed. But to our knowledge, there is a lack of studies combining bacterial community fingerprinting and molecular DOM composition in a time series, especially of short-terms observations. Short-term dynamics over days or hours may show just small changes and to investigate such small changes in molecular composition of DOM and bacterial community structure, ultrahigh resolution methods are necessary.

DOM has a highly complex composition consisting of millions of different molecules, most of them in very low concentration (pico- to femtomolar; Dittmar and Paeng, 2009). This makes the DOM composition a big challenge in analytical chemistry (Hedges et al., 2000) and knowledge about its molecular characterization is still scarce. Ultrahigh resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) is the only method so far that enables the molecular characterization of DOM composition. Coupled with electrospray ionization (ESI), intact polar molecules can be analyzed and the high mass accuracy allows determining the exact molecular formulae and elemental composition (Stenson et al., 2003). This technique has been successfully applied to marine DOM samples (e.g. Dittmar and Paeng, 2009; Flerus et al., 2012; Koch et al., 2008).

The detection of small but nonetheless relevant changes in the bacterial community structure is another analytical challenge. Automated Ribosomal Intergenic Spacer Analysis (ARISA) fingerprinting enables high resolution investigation of bacterial community structure. It is based on the length polymorphism of the intergenic spacer region. This species-specific region is located between the 16S and 23S genes on the ribosomal operon and it has been shown that it is appropriate for bacterial community fingerprinting (Barry et al., 1991). The resolution of ARISA was found to exceed other common fingerprinting methods like Denaturing Gradient Gel Electrophoresis (DGGE) or Terminal Restriction Fragment Length Polymorphism (T-RFLP; Danovaro et al., 2006; Okubo and Sugiyama, 2009) and has been successfully applied for samples from the marine environment (e.g. Krause et al., 2012; Needham et al., 2013; Sperling et al., 2013)

This study investigates short-term dynamics in composition of DOM and bacterial community over timescales of 20 days and 24 hours. We hypothesize that the bacterial community shapes the DOM composition and vice versa. Additionally, the study investigates the effect of environmental variables on the bacterial community and on the molecular composition of DOM.

3 Materials and Methods

3.1 Sampling procedure and sample preparation

Sampling took place at the Ferry Box site at the time series station Helgoland (54°18'3120N, 7°88'9750E) on Helgoland Island, which is located in the German Bight in the North Sea. Water samples were collected daily from August 6 to August 26, 2012, always at 13:00 h and hourly over 24 hours on August 13-14, 2012. Surface water was collected with a carefully rinsed bucket and transported in 1-liter glass bottles.

For dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) analysis, 20 ml of water was filtered through glass fiber filters (GMF and GFF with nominal pore size of 2.0 µm and 0.7 µm, respectively; Whatman, United Kingdom). A syringe (20 ml NORM-JECT®, latex free, Henke Sass Wolf, Germany) and polypropylene filter holder with a diameter of 47 mm (PP-47, Advantec®, Toyo Roshi Kaisha, Ltd., Japan) were used. The collected DOC and TDN samples were acidified to pH 2 (HCl 32% p.a., Roth, Germany) and stored at 4°C in the dark.

For solid phase extraction, two liters of seawater were collected by vacuum filtration through GMF and GFF filters using bottle top filters holder (reusable, Ø 47 mm, Nalgene®, USA) and transferred into 2 l Nalgene® bottles. All filtrates were adjusted to pH 2 with HCl (32% p.a., Roth, Germany) and stored at 4°C in the dark.

500 ml of seawater were vacuum filtered through 0.22 µm polycarbonate filters (GTTP, Ø 47 mm, Merck Millipore, USA) for DNA extraction. Filters were transferred to autoclaved Eppendorf caps (2 ml) and stored at -20°C.

All glassware and glass fiber filters used during sampling and sample treatment was pre-combusted for 4 hours at 400°C. All other materials were cleaned with acidified ultrapure water and rinsed with sample water before use.

For verification of possible contamination, procedural blanks were performed by repeating the sample preparation with ultrapure water.

3.2 Extraction of DOM

To study the molecular composition of DOM via FT-ICR-MS analysis, a highly concentrated salt-free organic sample is required. Therefore, the water samples were solid phase extracted following the method published by Dittmar et al. (2008) as this procedure has been shown to be the most efficient methods for extracting DOM for mass spectrometric analysis. For each sample a 1 g Varian Bond Elut PPL cartridge (Styrene divinyl benzene polymer sorbent; Agilent Technologies, USA) was used. These PPL cartridges have a pore size of 150 Å and retain a wide spectrum of highly polar to non-polar compounds (Dittmar et al., 2008) but

colloidal material and small ionic compounds might be selectively lost during the procedure. Before usage, the cartridges were rinsed with one cartridge volume of methanol (ULC/MS grade, Biosolve, Netherland) and refilled with methanol for conditioning overnight. Afterwards, the cartridges were rinsed with two cartridge volumes of ultrapure water, two cartridge volumes of methanol and two cartridge volumes of ultrapure water at pH 2. For extraction the filtered and acidified seawater (2 l) was gravity passed through each PPL cartridge. Subsequently, remaining salt was removed with two cartridge volumes of pH 2 ultrapure water. After drying with inert pure argon gas, DOM was eluted with 6 ml methanol (ULC/MS grade, Biosolve, Netherland) into 8 ml amber vials.

The extract volume was determined by weight. 100 µl of the methanol extracts were evaporated overnight and re-dissolved in 10 ml ultrapure water at pH 2 for DOC analysis. The extraction efficiency was calculated using the following equation:

(1) $$extraction\ efficiency\ [\%] = \frac{DOC\ amount\ of\ extract}{DOC\ amount\ of\ original\ sample} * 100$$

3.3 DOC and TDN quantification

Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) analyses of water samples and extracts were performed with a Shimadzu TOC-V_{CPH} analyzer equipped with a TNM-1 add-on and an ASI-V autosampler using the high-temperature catalytic oxidation (HTCO) method. By means of a platinum catalyst the sample was combusted at 720°C. The resulting CO_2 was measured quantitatively using a NDIR detector (non-dispersive infrared detector). According to Stubbins and Dittmar (2012) a calibration covering the expected concentrations range was applied. Thus the detection limit was 0.6-14.8 µm for DOC and 0.6-11.9 µm for TDN (Stubbins and Dittmar, 2012).The Deep Sea Reference Standard (DSR; Batch 13, Lot# 05-13, University of Miami) was used to determine the precision and accuracy of the measured concentrations in each run. Every sample was measured in duplicates.

3.4 Ultrahigh resolution DOM characterization

All samples were analyzed with a 15 Tesla Solarix® Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR-MS; Bruker Daltonik GmbH, Germany) to obtain information about the molecular composition of the DOM. For the FT-ICR-MS analysis, the methanol extracts were mixed with ultrapure water in a ratio of 1:1 and were diluted to a DOC concentration of 20 ppm. Electrospray ionization (ESI, Apollo II ion source; Bruker Daltonik GmbH, Germany) in negative ion mode was used to ionize the samples softly and keep the molecular ions intact (Dittmar and Koch, 2006; Koch et al., 2005). Samples were infused at 120 µl h^{-1}, capillary voltage was 4000 V, and an ion accumulation in the hexapole of 0.25 s

prior to transfer into the ICR cell was applied. A total of 500 scans were accumulated per run and mass spectra were evaluated in the range from m/z 150 to 2000 Da. The instrument was externally calibrated with arginine clusters and each mass spectrum was internally calibrated with a list of known molecular formulae mass peaks (Hawaii_DOM_20101029 (neg)). Exact masses, peak intensities and resolution of each sample were exported using the software DataAnalysis 4.0 SP 3 (Bruker Daltonik GmbH, Germany). Detected masses were matched over all samples and formulae were assigned to the detected masses using the following criteria: O ≥ 1; N ≤ 4; S ≤ 2; P ≤ 1; mass error<0.5 ppm. Detected masses were kept for analysis if the signal to noise ratio exceeded 4 and when they were detected in more than two samples. Masses that were present in less than 20% of the samples were allowed if the S/N ratio was >20. Additionally, formulas were deleted that contained following combinations: NSP, N_2S, N_3S, N_4S, N_2P, N_3P, N_4P, NS_2, N_2S_2, N_3S_2, N_4S_2, PS_2. 16 remaining double assignments were removed. After assignment, intensities were normalized to the sum of peak intensities of all masses with a S/N ratio >5. Original intensities were replaced by ratios of measured intensity divided by the peak sum and multiplied by 10000. Masses which are listed as known contaminations including their homologous series were eliminated and ^{13}C peaks were removed.

For identifying various types of organic compounds, certain formulae were visualized according to their H/C and O/C ratios in van Krevelen diagrams. The abundance of aromatic and condensed molecules was assessed by the double bond equivalents (DBE, equation 2) as a measure for the degree of unsaturation (Koch and Dittmar, 2006).

(2) $$DBE = 1 + \frac{1}{2}(2C - H + N + P)$$

Based on its molecular formula the aromaticity of each assigned compound described by applying the modified aromaticity index (AI_{mod}, equation 3), assessing the presence and extent of aromatic structures (Koch and Dittmar, 2006).

(3) $$AI_{mod} = \frac{1 + C - \frac{1}{2}O - S - \frac{1}{2}H}{C - \frac{1}{2}O - S - N - P}$$

Average of number of assigned formulae and intensity weighted averages of m/z of assigned formulae, AI_{mod}, DBE, the number of the atoms C, H, O, and the heteroatoms N, S, P and the molar ratios H/C and O/C were calculated.

3.5 Ferry Box data

Environmental data were obtained as part of the Helgoland Roads time series (Wiltshire et al., 2008). These data were measured with a Ferry Box installed on Helgoland (54°18’3120N, 7°88’9750E; Wiltshire, 2013) and are accessible via the open database PANGEA 2004

(http://www.pangaea.de). The data set used in this study was kindly provided by Karen H. Wiltshire (Biological Institute Helgoland; Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research).

3.6 DNA extraction, agarose gel electrophoresis and DNA quantification

The DNA extraction was performed as described in Sapp et al. (2007). Lysozyme (1 mg ml^{-1}) and sodium dodecyl sulfate (1%) were used for cell lysis. Genomic DNA was extracted with phenol-chloroform-isoamyl alcohol (25:24:1) and precipitated with isopropanol. The DNA extracts were eluted in 30 µl autoclaved ultrapure water and stored at -20°C until further processing.

Validation of extracted DNA was done by agarose gel electrophoresis, which was performed in a Tris(hydroxymethyl)-aminomethane (TRIS) acetate buffer containing 2 M TRIS, 1 M acetic acid, and 50 mM Ethylenediaminetetraacetic acid (EDTA; 0.5 M SL). Extracted DNA was diluted with autoclaved ultrapure water (1:9) and stoppmix (0.25% bromocresol purple, 50% glycerin (97%), 0.05 TRIS, pH 7.9) was added. Gels contained 0.8% agarose and electrophoreses were performed at 80 V and 2.00 A for 40 min. Finally, agarose gels were stained with GelRed (GelRed Nucleic Acid Stain 3x in water, Biotium, USA) for 30 min and photographed with a UV Transilluminator (ChemiDOC™ XRS Firma, Biorad, USA).

The success of Polymerase chain reaction (PCR) was also verified with agarose gel electrophoresis. First, amplified DNA was mixed with autoclaved ultrapure water in a ratio of 1:2. The diluted PCR products were mixed with the same volume ratio of stoppmix and were applied as described previously, except that gels contained 1.4% agarose.

DNA concentration and purity were determined by photometry using an Infinite M200 (Tecan Austria GmbH, Austria). DNA concentration was measured in duplicates.

3.7 Polymerase Chain Reaction

The intergenic spacer region in the rRNA operon was amplified in an Eppendorf MasterCycler (Eppendorf, Germany) using the forward primer L-D-Bact-132-a-A-18 (5'-CCGGGTTTCCCCATTCGG-3') and reverse primer S-D-Bact-1522-b-S-20 (5'-TGCGGCTGGATCCCCTCCTT-3'), the latter labeled with an infrared dye (Dy682; Ranjard et al., 2000). PCRs were performed in volumes of 25 µl containing about 2 ng template DNA (volume depends on DNA concentration). 2.5 µl Taq Buffer (10 x), 5 µl TaqMaster PCR Enhancer (5 x), 0.7 µl of each primer (20 µM), 0.75 µl deoxyribonucleotide triphosphates (dNTPs; 2.5 mM each) and 0.25 U Taq DNA polymerase (all reagents by 5 Prime, Germany). The amplification protocol was as follows: preliminary denaturation at 95°C for 3 min; 30 cycles of denaturation at 95°C for 1 min; 1 min annealing at 50°C and 1 min elongation at

68°C; an extension step at 68°C for 5 min and final cooling to 4°C until abortion of the run. Positive and negative controls were run as well.

3.8 Automated Ribosomal Intergenic Spacer Analysis (ARISA)

Depending on agarose gel band intensities, original or diluted PCR products were mixed with an equal volume of loading buffer. Together with the size standard IRDye® 700 with 50 bp to 1500 bp (Li-Cor, Germany), the PCR products were denatured at 95°C in a MasterCycler (Eppendorf, Germany) and subsequently cooled down on ice for 10 min. The PCR products were separated in a 5.5% polyacrylamide gel prepared following the manufacturer's protocol (LI-COR Biosciences, USA). The acrylamide (ready to use matrix by Li-Cor Biosciences) polymerized for 2 h after the addition of tetramethylethylendiamine (TEMED) and ammonium persulfate (APS). At least every ninth pocket of the comb was loaded with 0.5 µl of standard. ARISA was performed at 1500 V for 14 h on a LI-COR 4300 DNA Analyzer (LI-COR Bioscience, USA). A pre-run of 15 min at 45°C was carried out to precondition the gel and sequencer prior to loading the samples.

ARISA fingerprints were edited by BioNumerics Version 5.1 (Applied Maths NV, Belgium). The image was normalized according to the reference size standard and auto assignment was performed. Bands between 262 bp and 1500 bp length were analyzed. Binning to band classes was performed according to Kovacs et al. (2010).

3.9 Statistical analysis

For multivariate statistical analyses the software package PRIMER v.6 and the add-on PERMANOVA+ (both PRIMER-E, United Kingdom) were used. The analyses of molecular composition were performed on Bray-Curtis matrices, generated from square root transformed mass spectrometric data of each sample. The Jaccard index was applied to calculate the resemblance matrix for the bacterial community. Environmental variables (DOC and TDN concentrations, water level, water temperature, salinity, dissolved O_2 and CO_2 concentration, turbidity, pH, SiO_2, PO_4, NO_2, NO_3 and chlorophyll *a*) were log transformed prior to the analyses. For principal coordinates analysis (PCO) the environmental variables based on Euclidean distance were used. PCO analyses were performed to investigate inter-point dissimilarities of the samples referring to bacterial community, molecular composition of DOM, and environmental parameters discretely. The relationship between the molecular composition or the bacterial community and the environmental variables was investigated by distance-based linear models (DISTLM). The DISTLM model was built using stepwise selection, adjusted R^2, and applying 999 permutations at a significance level of $p<0.05$. The results were shown as marginal and sequential test. The marginal test revealed how much each variable

explains when taken alone, ignoring all other variables. Following the results of this test a sequential test was performed which examines whether the addition of that particular variable contributes significantly to the explained variation (Anderson et al., 2008). To visualize the results distance-based redundancy analysis (dbRDA) was used.

The relationship between molecular composition of DOM and bacterial community, as indicated by the respective Spearman rho-values was investigated with the RELATE subroutine. 999 permutations were applied at a significance level of $p<0.01$.

To test significant differences of two selected groups of samples regarding their DOM composition, bacterial community composition or environmental variables, permutational multivariate analysis of variance (PERMANOVA) was applied according to Anderson (2001). The PERMANOVA subroutine was performed with fixed factors, 999 permutations, and a significance level of $p<0.001$.

Univariate correlations (Pearson) between environmental parameters and the molecular DOM composition were calculated with Statistica 11 (StatSoft, USA) at a significance level of $p<0.05$.

4 Results

4.1 Oceanographic setting during the sampling campaign

During the sampling period, the water level at the Ferry Box varied between 1.4 m and 4.2 m due to the tidal cycle (Figure 1 B). The water temperature increased slightly from 17.1 to 18.5 °C over the 20 day period (Figure 1 A), but during the 24 h times series it remained nearly constant between 17.6 and 18.1 °C. Salinity shows relatively high variations over both, daily and hourly sampling period. During the first week the salinity decreased from 32.6 to 31.2 but it was rather constant in the second half of sampling period with a maximum of 32.3 (Figure 1 A). The hourly sampling took place during a strong increase in salinity and covers a range of 31.5 to 32.0 (Figure 1 B). The concentrations of O_2 and CO_2 in the water ranged from 7.1 to 9.2 mg l^{-1} and 285.3 to 382.2 µg l^{-1}, respectively. SiO_2 varied between 0 and 6.3 µm during daily sampling and 0 to 3.7 µm during hourly sampling. The nutrients PO_4, NO_2 and NO_3 ranged from 0 to 0.7 µM, 0.2 to 0.4 µm and 0.9 to 2.4 µM. SiO_2 and nutrients concentration below the detection limit are designated as 0. The pH decreased slightly from up to 8.38 in the first week to a minimum of 8.11 in the second week. Chlorophyll *a* ranged from 0.6 and 1.1 µg l^{-1} with a maximum on August 23. Slight variations of the DOC concentration in the range from 109 to 162 µm were found except for an outlier on August 24 (261 µM), TDN concentrations varied between 11.7 to 28.4 µM.

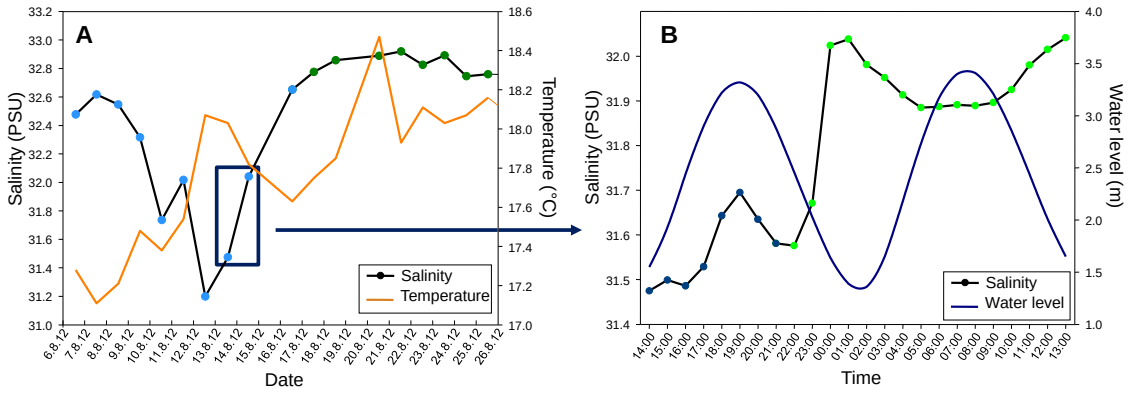

Figure 1 Salinity variation over daily (A) and hourly (B) sampling periods. Additionally, panel A depicts the water temperature and panel B the water level. Box and arrow indicate the timeframe of the 24 h series. Color code of dots (blue/ green) indicates different sample groups according to PCO analysis. Note the different scales of salinity in figure.

4.2 Molecular composition of DOM

The calculated extraction efficiencies were between 20% and 52%. Low extraction efficiencies were observed for samples with high original DOC concentration and might be reflect contamination of the respective water sample with organic solvents which were not extracted by the procedure. The sample of August 11 was eliminated from the data set

because of low extraction efficiency and high DOC and TDN concentration, which indicate contaminations.

To characterize the molecular composition of DOM, ultrahigh resolution mass spectrometry via FT-ICR-MS was applied. After removal of uncertain masses, a total of 4039 molecular formulae were assigned, ranging between 3662 and 3947 molecular formulae found per sample (average of all samples: 3877, Table 1). The identified peaks covered a mass range from 159 to 809 Da with weighted average masses between 363.6 and 385.4 Da (average of all samples: 374.9, Table 1).

The indices and values in Table 1 summarize information on the general characteristics of molecular structure of the DOM samples. From the low standard deviations, we infer that the samples were quite similar with respect to the molecular DOM composition.

Table 1 General molecular information on DOM composition and bulk of elemental composition based on FT-ICR-MS analysis summarizing all samples (n=42). Averages and standard deviation are given. All averages are weighted according to normalized peak intensity.

General		**Elemental composition**	
Number of assigned formulae	3877 (±54)	Average C	17.72 (±0.19)
Average m/z of assigned formulae	374.9 (±4.4)	Average H	21.99 (±0.23)
		Average O	8.32 (±0.14)
		Average N	0.35 (±0.01)
		Average S	0.10 (±0.02)
Molecular Indices		Average P	0.00 (±0.00)
Average AI_{mod}	0.27 (±0.01)	Average H/C	1.24 (±0.01)
Average DBE	7.90 (±0.12)	Average O/C	0.47 (±0.00)

From a first visual inspection, the mass spectra of samples obtained at different salinities (i.e. different water bodies) appeared very similar (Figure 2 A and C). By zooming into single nominal masses differences in presence and abundance of individual peaks became obvious. As an example, shown for the nominal mass of 319 Da, the sulfur-containing peak $C_{13}H_{20}O_7S$ was of higher intensity than the $C_{16}H_{16}O_7$ peak in the sample taken at lower salinity (Figure 2 B). In a higher salinity sample, this relationship was reversed (Figure 2 D).

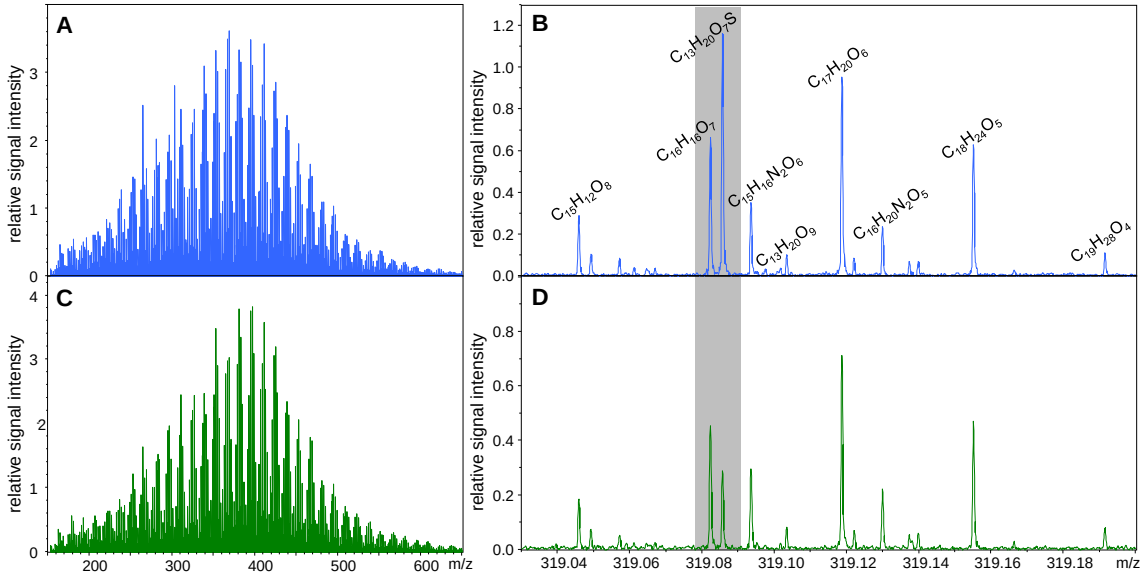

Figure 2 Examples of FT-ICR mass spectra of a sample taken at lower salinity (31.5 PSU; August 13, 2012; A and B) and a sample taken at higher salinity (32.7 PSU; August 24, 2012; C and D). Panel A and C show a mass range from 150 to 650 Da and panel B and D depict an expanded section of the respective mass spectrum at 319 Da with identified molecular formulae. The highlighted area in grey shows different relative intensities of molecules.

4.3 Linking microbial community, DOM composition and environmental variability

4.3.1 Environmental variables, bacterial community and DOM composition identify two characteristic regimes

To investigate inter-point dissimilarities between the samples according to environmental variables, bacterial community and DOM composition, PCO were applied.

PCO plots of environmental data, bacterial community and molecular composition of daily sampling are shown in Figure 3 A, C and E. Two different sample groups can be distinguished based on the environmental parameters (Figure 3 A). The PCO plots of bacterial community (Figure 3 C) and molecular composition (Figure 3 E) depict the same grouping. The two identified groups are in all three cases significantly different to each other (PERMANOVA, $p<0.001$). The first two axes of the PCO plots for environmental parameters described 47.5% of the total variation. The total variation of the first two axes amounts to 52.8% for bacterial community structure and to 45.4% for the DOM composition.

Figure 3 B, D and F depict the PCO plots of environmental variables, bacterial community and molecular DOM composition of the 24 h time series. From 21:00 h on the environmental data showed a clear shift indicating distinct groups (Figure 3 B). The same grouping could be distinguished by the PCO analysis of the bacterial community structure (Figure 3 D). In both cases PERMANOVA confirmed significant differences between these observed groups ($p<0.001$). Grouping of samples according to the molecular DOM composition was not found.

The first two axes of the PCO plot captured 61.9% of the variability of the environmental variables, 33.7% of the variability within the bacterial community structure and 41.4% of the DOM composition were explained.

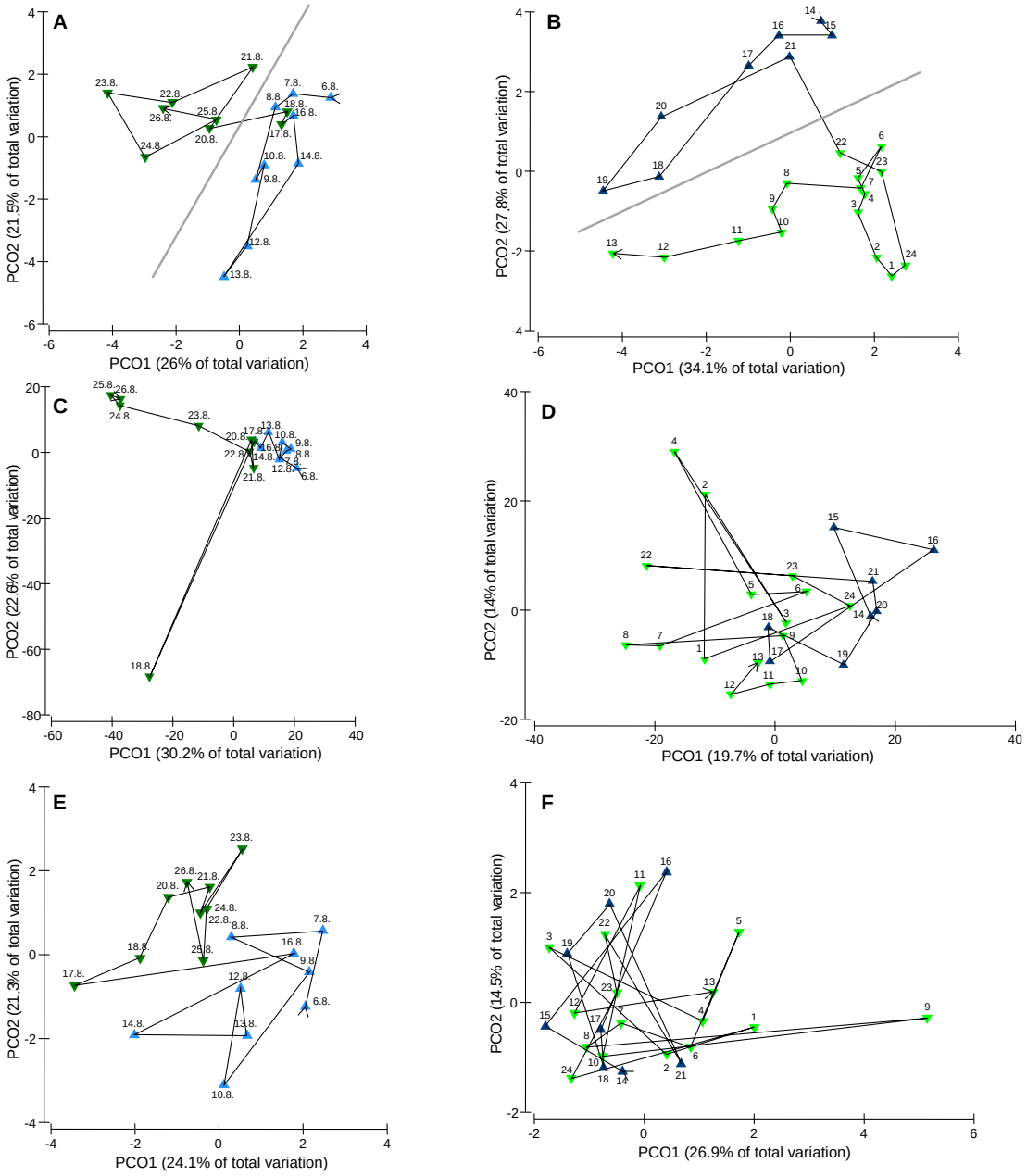

Figure 3 Principal coordinates analyses (PCO) of environmental variables based on Euclidean distance referring to daily (A) and hourly sampling (B), of bacterial community fingerprints based on Jaccard index referring to daily (C) and hourly sampling (D) and of molecular DOM composition based on Bray Curtis similarity referring to daily (E) and hourly sampling (F). Gray line separates and different colors indicate groups of samples.

4.3.2 Bacterial community and relation with the molecular DOM composition

The diversity of the bacterial community was examined using the ARISA fingerprinting technique. The number of ARISA band classes observed per sample ranged from 70 to 146 with an obvious outlier on August 18, 2012 which only showed 18 band classes.

To test the hypothesis that a relationship between bacterial community structure and DOM composition exists, the RELATE subroutine was used. This analysis however revealed that the bacterial community and molecular composition of DOM are not significantly related.

4.3.3 Relation of bacterial community or molecular DOM composition to environmental data

The relationship between bacterial community or DOM composition and environmental variables was analyzed with multiple regression analysis (DISTLM). The results are depicted in distance-based redundancy analyses biplots (dbRDA, Figure 4).

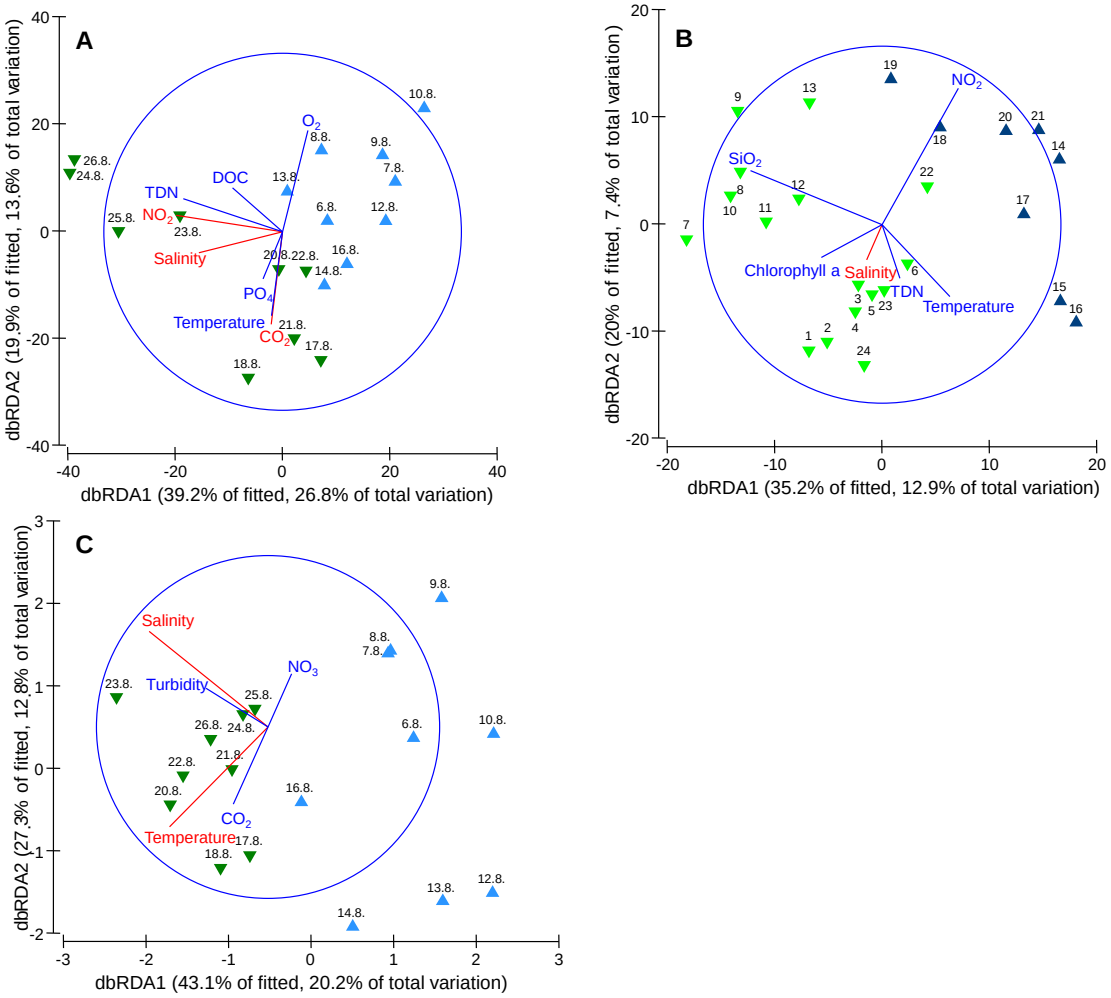

Figure 4 Distance-based redundancy analyses (dbRDA) of bacterial community fingerprints and environmental variables based on Jaccard index referring to daily (A; r^2: 0.683) and hourly sampling (B; r^2: 0.367) and of DOM composition and environmental variables based on Bray Curtis similarity referring to daily sampling (C; r^2: 0.468). Significant environmental variables of sequential test are depicted in red ($p<0.05$), others in blue.

The first two axes of the dbRDA plot of bacterial community of daily sampling explained 40.4% of the total and 59.1% of fitted variation. This indicated that most of the salient patterns in the fitted model are captured. The differences between bacterial communities were influenced by several environmental factors (Figure 4 A, Table 2). Marginal tests indicated TDN, temperature, salinity, pH and NO_2 to have a significant effect on the bacterial community structure. Among the investigated environmental variables, salinity CO_2 and NO_2 also exhibited a significant effect as revealed by a sequential test in the DISTLM model (Table 2). NO_2 contributes alone with 17.60%, CO_2 with 8.01% and salinity with 13.88% to the model (Table 2).

In case of samples taken hourly, bacterial community structure was significantly influenced by the individual effects of temperature, salinity, SiO_2, NO_2, NO_3 and chlorophyll *a* (Table 2). In sequential tests significant effects solely for salinity were confirmed, which contributes 10.51% to the model (Table 2). The first two axes of dbRDA explained 20.3% of the total and 55.2% of the fitted variation (Figure 4 B).

The results for the analysis of the DOM composition together with the environmental variables of the daily sampling are displayed in Figure 4 C. Here, 33% of the total and 77% of the fitted variation were covered. Temperature and salinity as well as water level, turbidity and pH were significant parameters as revealed by a marginal test in the DISTLM model (Table 3). Regarding the sequential tests solely salinity and temperature had significant effects on the molecular composition of the DOM (Table 3). Salinity contributed 16.64% and temperature 10.33% to the model.

In Table 3, the results of DISTLM of DOM composition referring to hourly sampling are listed. Neither marginal nor sequential tests revealed a significant contribution of environmental parameters (dbRDA not visualized).

All depicted dbRDAs showed the same grouping of samples consistent with the results from PCO analyses.

Table 2 Distance-based multivariate multiple regression (DISTLM; step wise, adjusted r^2) on the relation of environmental variables to the bacterial community structure (Jaccard) of daily (top) and hourly (bottom) sampling. Significant variables are highlighted in bold (p<0.05). Prop. is the proportion of variability explained by the respective variable.

Daily sampling

Marginal test				Sequential test			
Variable	Pseudo-F	P	Prop.	Variable	Pseudo-F	P	Prop.
DOC	1.7467	0.1096	0.098426	**NO_2**	3.4167	**0.0012**	0.17597
TDN	2.7312	**0.0102**	0.14581	**Salinity**	3.0378	**0.0022**	0.13878
Water level	1.2422	0.2622	0.072045	O_2	2.1015	0.0526	0.089438
Temperature	2.6561	**0.0088**	0.14237	**CO_2**	2.0205	**0.0367**	0.080146
Salinity	2.205	**0.0384**	0.12112	DOC	1.532	0.1037	0.058379
O_2	1.8895	0.0604	0.10562	PO_4	1.4981	0.1672	0.054814
Turbidity	1.1391	0.2399	0.066462	Temperature	1.2144	0.2617	0.043584
ph	2.9698	**0.0034**	0.15655	TDN	1.1848	0.3098	0.04175
SiO_2	0.99253	0.3664	0.05841				
PO_4	0.82315	0.597	0.048929				
NO_2	3.4167	**0.0011**	0.17597				
NO_3	0.82373	0.5901	0.048962				
Chlorophyll *a*	1.1425	0.2556	0.066649				
CO_2	1.533	0.1167	0.087436				

Hourly sampling

Marginal test				Sequential test			
Variable	Pseudo-F	P	Prop.	Variables	Pseudo-F	P	Prop.
DOC	1.0517	0.3862	0.045625	**Salinity**	2.583	**0.001**	0.10507
TDN	1.3434	0.1427	0.057551	NO_2	1.5621	0.0733	0.061961
Water level	1.0539	0.3821	0.045713	SiO_2	1.4582	0.1014	0.056605
Temperature	2.2598	**0.0034**	0.093151	Temperature	1.4471	0.1017	0.054947
Salinity	2.583	**0.0008**	0.10507	Chlorophyll *a*	1.2571	0.2062	0.047095
O_2	1.1956	0.2424	0.051545	TDN	1.1235	0.3217	0.041802
Turbidity	1.2362	0.217	0.053203				
ph	1.2932	0.1731	0.055518				
SiO_2	2.2519	**0.0032**	0.092855				
PO_4	1.4001	0.1241	0.059834				
NO_2	2.2667	**0.0035**	0.093406				
NO_3	1.6946	**0.0375**	0.07152				
Chlorophyll *a*	1.8566	**0.0219**	0.077825				
CO_2	1.1896	0.2501	0.051298				

Table 3 Distance-based multivariate multiple regression (DISTLM; step wise, adjusted R^2) on the relation of environmental variables to the molecular DOM composition (Bray-Courtis) of daily (top) and hourly (bottom) sampling. Significant variables are highlighted in bold (p<0.05). Prop. is the proportion of variability explained by the respective variable.

Daily sampling

Marginal test				Sequential test			
Variable	Pseudo-F	P	Prop.	Variable	Pseudo-F	P	Prop.
DOC	0.40556	0.9875	0.024721	**Salinity**	3.1938	**0.0004**	0.1664
TDN	0.9689	0.4631	0.057099	**Temperature**	2.1208	**0.0329**	0.10326
Water level	2.0789	**0.0243**	0.11499	CO_2	1.9447	0.0504	0.089075
Temperature	2.0731	**0.0254**	0.11471	Turbidity	1.3739	0.1965	0.061296
Salinity	3.1938	**0.0004**	0.1664	NO_3	1.086	0.3465	0.048133
O_2	1.6489	0.0877	0.093428				
Turbidity	1.7506	**0.0486**	0.098622				
ph	2.7993	**0.0019**	0.14891				
SiO_2	1.3644	0.1789	0.078574				
PO_4	0.64903	0.7942	0.038983				
NO_2	0.85943	0.5674	0.050976				
NO_3	0.83432	0.5964	0.049561				
Chlorophyll *a*	1.7385	0.0565	0.098008				
CO_2	1.0045	0.4223	0.059073				

Hourly sampling

Marginal test				Sequential test			
Variable	Pseudo-F	P	Prop.	Variable	Pseudo-F	P	Prop.
DOC	1.0879	0.3381	0.047118	Chlorophyll *a*	1.3622	0.1535	0.058307
TDN	0.9996	0.4134	0.043462	TDN	1.3598	0.1662	0.057267
Water level	0.92329	0.5004	0.040277	NO_2	1.3485	0.1697	0.055866
Temperature	1.3022	0.197	0.055884	Turbidity	1.5389	0.1076	0.062081
Salinity	1.3256	0.1753	0.056829	SiO_2	1.1754	0.2771	0.046983
O_2	0.65532	0.8271	0.028926				
Turbidity	0.94423	0.4556	0.041153				
ph	0.68363	0.7959	0.030138				
SiO_2	1.2263	0.2283	0.052799				
PO_4	0.86295	0.5723	0.037744				
NO_2	0.7621	0.7005	0.033481				
NO_3	0.85518	0.5708	0.037417				
Chlorophyll *a*	1.3622	0.161	0.058307				
CO_2	0.6702	0.8132	0.029563				

4.4 Relation of salinity and temperature to DOM composition

Distance-based redundancy analysis (dbRDA) of DOM composition and environmental variables of samples taken in daily intervals revealed that salinity and temperature were significantly related to the molecular DOM composition (Figure 4 C). To reveal the nature of these relationships, Pearson correlations between the two parameters and the relative intensity of each detected mass with molecular formula assignment of the daily sample set were calculated. In Figure 5 van Krevelen plots are shown, in which the element ratio H/C is plotted against the O/C ratio for each molecule that is significantly correlated ($p<0.05$) with salinity (Figure 5 A) or temperature (Figure 5 B).

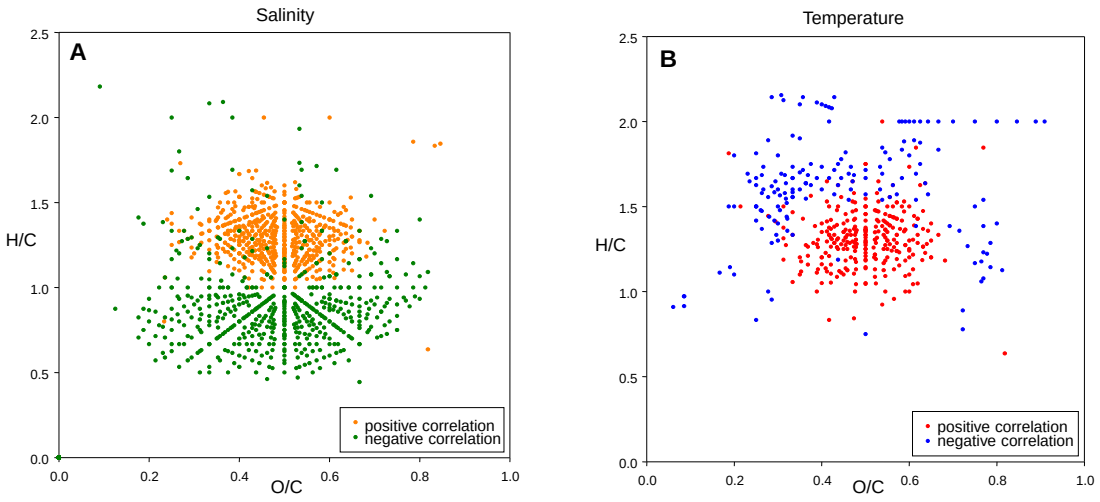

Figure 5 Van Krevelen plots for molecular formulae of DOM of daily sampling with relative intensity correlating significantly with salinity (A) and temperature (B). Molecules positively correlated with salinity are depicted in orange while negatively correlated molecules are shown in green. Positively correlated molecules with temperature are colored in red and in blue negatively correlated molecules are shown.

The molecules that are positively correlated with salinity occupy a distinct area of the van Krevelen diagram and exhibit higher H/C ratios (average H/C: 1.30) when compared to the molecules with a negative correlation to salinity (average H/C: 0.90). The molecules that are positively correlated with temperature are found in the center of the van Krevelen diagram (average H/C: 1.31, average O/C: 0.50), while the molecules with negative correlation to temperature are more scattered and reveal higher H/C ratios (average H/C: 1.60) and cover a broad range of O/C ratios.

5 Discussion

5.1 Relation between bacterial community and molecular DOM composition

The study revealed same variations in the bacterial community and the molecular DOM composition. However, these variations are not interdependently related. The first hypothesis that bacterial community and molecular composition of DOM affect each other on short time scales could not be confirmed in this study. The applied RELATE subroutine could not reveal a significant relationship between the bacterial community structure and the DOM composition. This was the case for both, daily and hourly sampling.

It has to be considered that there are several ways in which an existing relationship could be present but not be detected in this study. One possible explanation lies in the rapid consumption of the reactive molecules. Bacteria can deplete these molecules directly after their release by other organisms. Therefore it might be that no pool of labile DOM is available for measurement. This explanation is consistent with Amon and Benner (1996), Weiss and Simon (1999) and Kirchman et al. (1991) who found high turnover rates of labile DOM.

Another reason for the lack of evidence for a relation between the bacterial community structure and the DOM composition could be because the chosen analytical window is limited. During processing of FT-ICR-MS data for further analysis information on possibly important masses might have been eliminated due to the rather conservative approach. Furthermore data on masses that could not be assigned to formulae were ignored.

Furthermore a limitation of our method could be losses especially of freshly produced material during the solid phase extraction (SPE). A previous study of Flerus et al. (2012) has shown that SPE is a suitable method to distinguish between different ageing of marine oceanic DOM, it compared very similar water samples like the current study. However, colloidal material and low molecular weight DOM (< 250 Da) escape the analytical window that might be explain why no relation between bacterial community structure and DOM composition could be observed. Furthermore, it could also explain the low extraction efficiencies calculated in our analysis. Although the efficiencies were within the range described for marine samples by Dittmar et al. (2008), it is considerably lower than that of more recent studies (Osterholz et al., in review; Rossel et al., 2013; Seidel et al., in review).

5.2 Influences of environmental variables on bacterial community and molecular composition of DOM

The sampling period covered the range of typical summer conditions on Helgoland Roads (http://www.pangaea.de). Longtime studies observed medians of salinities ranging between 31 and 33 (Raabe and Wiltshire et al., 2008; Wiltshire et al., 2009). The salinity values show strong variation within few days. A shift in this order is exceptional for this observed timeframe. Dynamics of salinities at Helgoland Roads are controlled by hydrological and meteorological forces and by river discharges. Events with high salinity are mainly influenced by the transport of central North Sea water. Coastal water influx is related to the lower salinities (Wiltshire et al., 2009).

The daily sampling shows a distinct grouping of environmental variables, bacterial community, and DOM composition. The distribution of the samples hereby strongly suggests a relation of these two groups to salinity. For the environmental parameters and bacterial communities the same is true even for the 24 h time series. Multivariate statistics confirmed the hypothesis that variations in bacterial community and DOM composition are mainly driven by these salinity changes. While the statistical analyses also reveal a significant link between the variation of the bacterial community with CO_2 and NO_2, further examination is needed to explain this relationship. Further investigation might also explain the significant correlation of temperature and variation in DOM observed during the daily sampling. However, realistic ecological conditions involve many strongly correlating parameters. Therefore it has to be considered that the resulting significant parameters of the model could result from the multicollinearity (Graham, 2003).

For the contribution to changes in the DOM composition, the 24 h time series revealed no significant environmental parameters. The samples were too similar for observing any grouping via PCO analysis. If the DOM composition changed during this timeframe, these slight differences could not be discovered.

The frequent sampling in a narrow timeframe of only several days or 24 h was rarely applied in the field of microbial ecology and geochemistry of DOM before. Hence, little is known about the temporal dynamics on the short timescale this study focused on. Only few published reports using molecular fingerprinting for shorter timescales are available. Riemann and Middelboe (2002) as well as Hewson et al. (2006) showed that bacterial community composition was relatively stable over days and weeks. Studies of 24 h time series revealed also resilience or slight variation of bacterial communities (Fuhrmann et al.,1985; Needham, 2013; Rink et al., 2008; Shiah, 1999). Also there is a lack of knowledge of field studies focusing of FT-ICR-MS data over short timescales. Here we show that dynamics

in bacterial community and DOM composition can exist even over such comparably short timescales. While these changes might merely represent different water masses, the high resolution in time allows for a very detailed documentation of these fluctuations. Here the 24 h series is of special value as it increases the temporal resolution to more precisely analyze the turning point in the system caused by salinity changes.

5.3 Relation of salinity and temperature to DOM composition

The van Krevelen plot (Figure 5) shows that molecules, which are positively correlated with salinity, plot in the H/C and O/C range associated with marine DOM. Molecules negatively correlated with salinity pattern in the van Krevelen diagram are associated with terrigenous DOM (Kim et al., 2003; Koch et al., 2005; Sleighter and Hatcher, 2008). Marine DOM is more aliphatic and is sourced mainly from carbohydrates, amino acids and lipids, whereas terrestrial DOM is more aromatic and contains carboxyl and hydroxyl functionalities (Sleighter and Hatcher, 2008). Therefore it can be concluded that water with high salinity contained more of typical marine DOM and water with lower salinity contained more molecules which are typical for terrigenous components. This is in accordance with the expectations, as the high salinity represents marine water from the North Sea while the low salinities are due to coastal or riverine influences.

There is a positive trend in temperature during the sampling period, but no correlation with salinity existed (Pearson correlation coefficient, $r=0.12$, $p>0.6$). The van Krevelen plot of molecules correlated with temperature (Figure 5) shows another grouping. Most of the molecules that are negatively correlated with temperature have a higher H/C ratio than positively correlated molecules. Higher H/C ratio means the molecules are more saturated and might indicates recent production of labile DOM. Considering the temperature increased slightly during the sampling period these molecules that are negatively correlated decreased. A possible scenario could be that microbial activity had increased with the rising temperature. Because of the enhanced metabolism, the microbial community may consume more DOM (Pomeroy and Wiebe, 2001), especially the labile fraction of DOM. The increasing abundance of positively correlated molecules could be explained by the consumption of the labile DOM. As the labile molecules are being depleted the relative abundance of the positively correlated molecules increases. That means the positively correlated molecules increase in relative abundance.

6 Conclusion and Outlook

In this study, dynamics in microbial community and DOM composition over short time series were revealed. Bacterial community structure and DOM composition showed similar changes but a direct relation cannot be confirmed. The dynamics of bacterial community and molecular DOM composition are mainly driven by influences of different water masses. For the first time changes of microbial community and molecular DOM composition have been documented in such high temporal and analytical resolution.

Several modifications to the analysis could provide the ability to reveal more molecules which could have implications for our study. Modifying the criteria for molecular formulae assignment, to a less conservative state, could help to observe more changes in the DOM composition with increased sensitivity. Furthermore statistical analyses could include the masses without assigned formulae. If this analysis were to reveal several molecules which show significant correlations with the bacterial community the formulae could be assigned manually.

Due to the striking observation of changes in microbial community in this study the data set should be used for a deeper insight into the microbial ecology. Further information on bacterial community structure will be available through sequencing of bacterial DNA. Based on ARISA results samples will be selected for pyrosequencing of the bacterial 16S rDNA. The specific identification of bacterial species will be realized by 454-sequencing. This sequencing method offers new capabilities to investigate microbial community composition (Schuster, 2008) and may give some detailed indication of changes of bacterial community in our study focusing on a short time interval. Such detailed informations could even provide new insights in the relationship of bacterial community structure a molecular DOM composition.

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III. DANKSAGUNG

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EIDESSTATTLICHE ERKLÄRUNG

Hiermit versichere ich, dass ich diese Arbeit selbstständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe. Außerdem versichere ich, dass ich die allgemeinen Prinzipien wissenschaftlicher Arbeit und Veröffentlichung, wie sie in den Leitlinien guter wissenschaftlicher Praxis der Carl von Ossietzky Universität Oldenburg festgelegt sind, befolgt habe.

Irina Köster