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Molecular evidence for rapid dissolved organic matter turnover in Arctic fjords

Helena Osterholz *, Thorsten Dittmar, Jutta Niggemann

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

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ABSTRACT

Dissolved organic matter (DOM) in the ocean comprises one of the largest active carbon pools on earth. Deep water formation at high latitudes carries DOM from the active surface layers to the deep ocean. However, information on sources and fate of DOM in the Arctic Ocean is limited. To reveal the relevance of autochthonous DOM production and transformation in Arctic fjord systems to the global deep ocean DOM pool, we performed a comprehensive study on the molecular composition of DOM and the composition of the associated microbial communities in four selected fjords of Svalbard. At various water depths, a total of 34 samples were taken in fall 2010 for the determination of bulk concentrations of dissolved organic carbon (DOC) and total dissolved nitrogen (TDN), for the molecular characterization of solid-phase extractable DOM as well as for microbial community fingerprinting.

While TDN concentration and the composition of the microbial community showed a clear distinction between surface and bottom water samples, bulk DOC (~60 μmol C L^{-1}) and dissolved black carbon (~1.8% of DOC) as a marker for terrestrial input were uniformly distributed. In-depth molecular-level analyses of the DOM composition using ultrahigh resolution mass spectrometry via Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) revealed insignificant variation of the relative abundance of 11630 molecular masses that were detected in the water samples.

From these findings we conclude that DOM produced during the spring/summer bloom is rapidly transformed within the short, but productive warm season by the specialized resident microbial community. Thus, in fall the DOM pool mainly consists of semi-refractory and refractory material, most of which has been introduced from Arctic Ocean water inflow. Assuming that our findings are representative for high latitude marine systems in general, the contribution of autochthonous seasonal DOC production in plankton bloom situations to the DOC pool in regions of deep water formation might be marginal.

1. Introduction

Marine dissolved organic matter (DOM) comprises one of the largest active carbon pools on earth, similar in size to atmospheric CO_2 or all land plant biomass (Hedges, 1992). The global oceanic net primary production is estimated at 50 Gt C per year (Hedges, 1992; Williams and Druffel, 1987). A significant fraction of the fixed carbon is transferred to the DOM pool, where it is readily remineralized by microorganisms within the microbial loop (Azam, 1998; Jiao et al., 2010; Pomeroy, 1974) or channeled into the deep ocean, where it resides for hundreds to thousands of years.

Hansell (2013) divided the oceanic DOM pool into five fractions with distinct lifetimes along a continuum of reactivity starting with labile DOM that is removed within hours to days and thus does not accumulate in seawater. The observable, recalcitrant DOC fractions are further distinguished as semi-labile DOM with turnover times between months and years, which can be followed as seasonal DOC variation in the surface ocean, semi-refractory DOM with a model lifetime of about 20 years, refractory DOM with a model lifetime of 16,000 years, and the ultra-refractory DOM with a lifetime of around 40,000 years. Most DOC is retained in the refractory DOM pool in the deep ocean, comprising ~630 Gt carbon. The reasons why so much DOM escapes the microbial respiration even though it constitutes the major carbon and energy resource to support bacterial life are not yet well understood (Dittmar and Paeng, 2009; Jiao, 2011).

For a long time, focus in DOM research has been on the analysis of selected compound classes in DOM, e.g. amino acids, carbohydrates (Amon et al., 2001), lignin (Opsahl et al., 1999) or dissolved black carbon (DBC, Dittmar, 2008). Together these groups account for only ~5% of the total DOM, thus ignoring the major share. Only recent advances in technology, especially of ultrahigh resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), have made it possible to characterize the compounds making up this huge DOM reservoir on a molecular level. Combined with soft electrospray ionization (ESI), intact polar molecules can be analyzed, and the high

* Corresponding author. Tel.: +49 441 798 3380; fax: +49 441 798 3358.
E-mail address: helena.osterholz@uni-oldenburg.de (H. Osterholz).

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mass accuracy allows for the assignment of molecular formulae to >70% of the tens of thousands of detected peaks per sample (Stenson et al., 2003). Previous research on the molecular composition of DOM by FT-ICR-MS revealed a universal fingerprint consisting of apparently biologically refractory compounds that is found all over the world's oceans (Dittmar and Paeng, 2009; Flerus et al., 2012). Other examples of successful FT-ICR-MS applications include the differentiation of terrigenous and marine DOM (Koch et al., 2005), the characterization of biodegradable DOM in rivers (Kim et al., 2006), the identification of possible source markers for photodegradation and bacterial alteration (Kujawinski et al., 2009), and the tracing of the age of bulk marine DOM by combining FT-ICR-MS measurements with radiocarbon age determination (Flerus et al., 2012).

The Arctic is, besides the Southern Ocean, a major site of ocean deep water formation (Rudels and Quadfasel, 1991). Thus, Arctic waters are a major source of DOM to the deep ocean (Amon et al., 2003), but reports on DOM concentration and especially composition in the Arctic Ocean are scarce. Previous studies focused on input and distribution of terrigenous DOM by the major Russian and Canadian rivers (e.g. McClelland et al., 2012). Here, in order to reveal the relevance of autochthonous DOM production and transformation in Arctic fjord systems, we performed a comprehensive study on the molecular composition of DOM and the composition of the simultaneously sampled microbial community in four fjords of Svalbard. The archipelago of Svalbard is located in the high Arctic between 76 and 81° north latitude and 10 to 30° east longitude (Fig. 1). The fjords on the western coast are influenced by Atlantic and Arctic water masses and the glacial meltwater inflow at their heads (Hop et al., 2002). Among them, the Kongsfjord ecosystem is the best-studied area with respect to phytoplankton dynamics, microbial communities and oceanography (Cottier et al., 2005; Hop et al., 2002; Iversen and Seuthe, 2011; Svendsen et al., 2002).

Anthropogenic influence on the remote ecosystem of Svalbard is marginal, and the fjord water masses are well stratified (Svendsen et al., 2002). Thus, Svalbard fjords are ideal model systems to study the differences between fresher surface water that has recently experienced primary production and the older bottom waters. We hypothesized that glacial meltwater input and phytoplankton blooms affect surface water DOM composition and the resident microbial community, and that this imprint is clearly distinguishable when compared to bottom waters. In order to characterize the molecular composition of the fjord DOM, we applied ultrahigh resolution mass spectrometry via FT-ICR-MS. In addition, dissolved black carbon (DBC) was quantified as a molecular marker to assess land-derived DOM, which has been shown to carry a universal thermogenic imprint worldwide (Jaffé et al., 2013). Surface and bottom water microbial communities were characterized by total bacterial cell counts using flow cytometry and fingerprinting via denaturing gradient gel electrophoresis (DGGE).

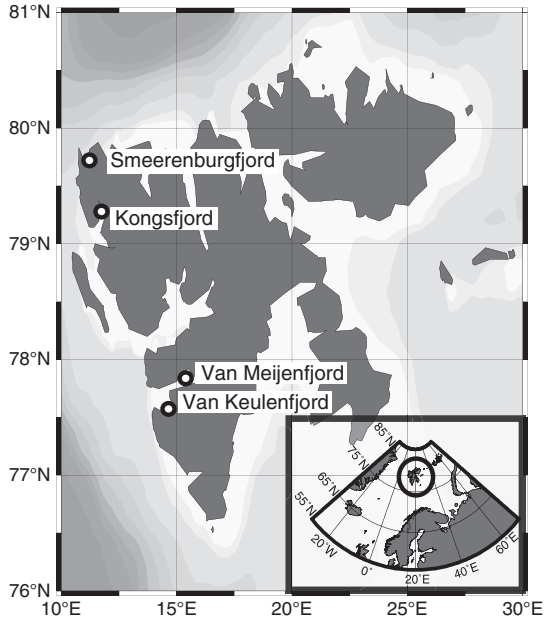

Fig. 1. Map of Svalbard, showing locations of sampled fjords. The rectangular inlay shows the location of the Svalbard archipelago (circled) north of the European mainland. Map created using Ocean Data View (R. Schlitzer, http://odv.awi.de).

2. Materials & methods

2.1. Site description and sampling

During the field campaign (August 28 to September 5, 2010), the water masses of four fjords on the west coast of Svalbard, Norway, were sampled (Table 1, Fig. 1). Vertical profiles of temperature and salinity were recorded by a CTD (Seabird MicroCAT-SBE-37) for three stations per fjord located on transects from fjord head towards the ocean. Three to four water depths were chosen at each station based on the CTD profiles: surface water was taken with a bucket, intermediate water samples from the pycnocline and deep water samples close to the bottom were retrieved with a 4 L Niskin bottle. Subsamples for total cell counts were preserved with glutaraldehyde (25%, Carl Roth, Germany) at a final concentration of 1% and stored at −20 °C. Subsamples for molecular microbiological analysis were filtered on board and stored at −20 °C. The water samples for chemical DOM analysis were stored at ambient temperature (~0 °C) in the dark and processed on board or upon arrival at Kings Bay Marine Laboratory, Ny Ålesund, within 48 h.

A small meltwater stream and a piece of floating iceberg (cleaned by repeated rinsing with ultrapure water and melted in the laboratory) were sampled in the vicinity of Ny Ålesund to assess the input of DOC, TDN and DBC by land-derived freshwater input.

2.2. Bacterial cell counts (flow cytometry)

The fixed samples were thawed and stained with SybrGreen I (1:10,000 dilution of commercial stock, Molecular Probes, United Kingdom) for 30 min at room temperature. After the addition of a defined amount of a polychromatic bead suspension (0.5 μm Fluoresbrite Microspheres, Polysciences Inc., USA), the determination of cell numbers was conducted with a FACScalibur flow cytometer (Becton Dickinson, USA). The flow rate was determined using the suspension of polychromatic beads before and after analysis (Marie et al., 1997). Data acquisition and analysis were performed with the Cell Quest Pro software (Becton Dickinson, USA).

2.3. Microbial community analysis (DGGE)

Samples taken in Smeerenburgfjord and in van Keulenfjord were prepared for microbial community analysis. 500 mL of seawater were filtered through 3 μm polycarbonate (PC) filters (Millipore, USA) to retain the particle-associated bacteria. Of this filtrate, 250 mL were passed through 0.2 μm PC filters (Millipore, USA) to retain the free-living microbial community. The filters were stored frozen at −20 °C. The DNA extraction procedure was modified after Zhou et al. (1996). The bacterial primer set 907R (5′ – CCG TCA ATT CM TTT GAG TTT – 3′) and GM5F (5′ – (CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC CCG CCC G)–CC TAC GGG AGG CAG CAG – 3′, both MWG Biotech, Germany) was used for amplification of 16S rRNA gene fragments in a touchdown PCR program modified after Muyzer et al. (1993). DGGE was performed on an Ingeny phor U 2 × 2 (Ingeny International BV, Netherlands) system with a gradient from 40 to 70% urea/formamide at a constant voltage of 100 V for 20 h in TAE buffer (40 mmol L^{-1} Tris-acetate, 1 mmol L^{-1} EDTA, pH 7.4). Cluster analysis of DGGE band pattern using densitometric