Science of the Total Environment 782 (2021) 146402

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Temporal dynamics of carbon sequestration in coastal North Atlantic fjord system as seen through dissolved organic matter characterisation

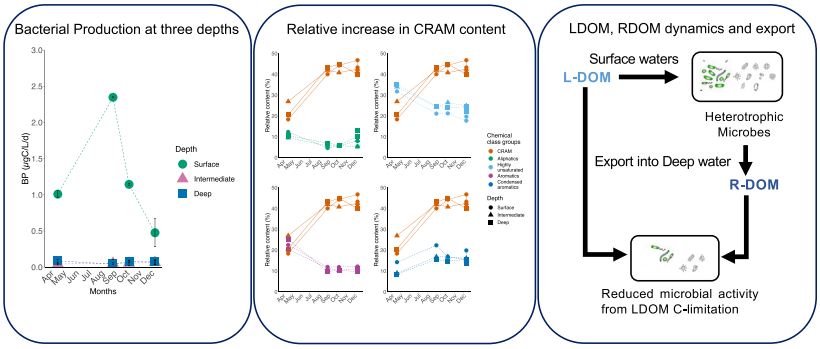
K. Avarachen Mathew [a,*], Murat Van Ardelan [b], Susana Villa Gonzalez [b], Olav Vadstein [c], Veena S. Vezhapparambu [d,1], Øystein Leiknes [a,2], Rahman Mankettikkara [e], Yngvar Olsen [a]

[a] Department of Biology, NTNU Norwegian University of Science and Technology, Norway
[b] Department of Chemistry, NTNU Norwegian University of Science and Technology, Norway
[c] Department of Biotechnology and Food Science, NTNU Norwegian University of Science and Technology, Norway
[d] Department of Petroleum Engineering, NTNU Norwegian University of Science and Technology, Norway
[e] Faculty of Biosciences, Fisheries and Economics, UiT The Arctic University of Norway, Norway

HIGHLIGHTS

- Microbial communities readily sequestered LDOM in surface water in all seasons.
- DOM composition varied significantly with different seasons in the fjord system.
- Bacterial production followed the chlorophyll *a* trend in the surface waters.
- Labile DOM Carbon limited bacterial activity in aphotic zone

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 25 September 2020
Received in revised form 3 March 2021
Accepted 5 March 2021
Available online 19 March 2021

Editor: José Virgílio Cruz

Keywords:
DOM chemical characterisation
CRAM
North Atlantic
Coastal waters

ABSTRACT

Fjord systems in higher latitudes are unique coastal water ecosystems that facilitate the study of dissolved organic matter (DOM) dynamics from surface to deeper waters. The current work was undertaken in the Trondheim fjord characterized by North Atlantic waters, and compared DOM fractions from three depths - surface (3 m), intermediate (225 m) and deep (440 m) in four seasons, from late spring to winter in 2017. The high-resolution mass spectrometry data showed that DOM composition varies significantly in different seasons rather than in different depths in the fjord systems. The bacterial community composition was comparable except at spring surface and summer intermediate depths. Bacterial production was minimal below the euphotic layer, even with sufficient availability of inorganic nutrients. The bacterial production rate in the surface waters was about 7 times and over 50 times higher than that of the aphotic zone in the winter and the summer seasons, respectively. The surface heterotrophic microbial communities might have rapidly consumed the available labile DOM, with the production of more refractory DOM limiting bacterial production in aphotic layers. The greater number of CRAM-like formulas determined in the surface waters compared to other depths supports our hypothesis. The

* Corresponding author.
E-mail addresses: mathew.avarachen@ntnu.no (K.A. Mathew), murat.v.ardelan@ntnu.no (M.V. Ardelan), susana.v.gonzalez@ntnu.no (S. Villa Gonzalez), olav.vadstein@ntnu.no (O. Vadstein), veenavezha@gmail.com (V.S. Vezhapparambu), Oystein.Leiknes@miljodir.no (Ø. Leiknes), rahman.mankettikkara@uit.no (R. Mankettikkara), yngvar.olsen@ntnu.no (Y. Olsen).
[1] Present Address: Amrita Center for Wireless Networks and Applications, Amrita Vishwa Vidyapeetham, Kollam, India.
[2] Present Address: Norwegian Environmental Agency, Trondheim.

https://doi.org/10.1016/j.scitotenv.2021.146402

Fjord
Bacterial production

refractory DOM sequestered in the water column may either be exported into sediments attached to particulate matter and marine gels, or may escape into the atmosphere as carbon dioxide/monoxide during the photochemical oxidation pathways, suggesting that it is involved in climate change scenarios.

1. Introduction

Dissolved organic matter (DOM) is the most abundant form of reduced carbon in the marine environment, adding up to some 700 Gt of carbon (D'Andrilli et al., 2010). This is almost as high as the atmospheric carbon pool, thus important to current research on climate change. In contrast, the amount of particulate organic matter (POM) is much less, comprising between 2 and 30 Gt of carbon, which includes all the living biomass that is mainly found in the upper euphotic layer, extending to about 200 m depth (He et al., 2016; Jiao et al., 2010; Stramska, 2009). DOM is a heterogeneous mixture with varying concentrations of organic molecules that differ temporally and spatially. A full characterisation of individual compounds in the DOM pool remains a challenge using state-of-the-art techniques, however, efforts are made to identify and classify these compounds either based on their elemental composition, or into chemical groups based on chemical formulas from mass spectrometric analyses.

DOM as a carbon source is first produced by phytoplankton, and later released into the environment directly from phytoplankton via exudation, during senescence and decay, or indirectly during predation and defecation by zooplankton. The chemical composition of the DOM varies depending on the phytoplankton species, its growth and nutritional stages, as well as on predation (Søndergaard and Middelboe, 1995). The average global net primary production is estimated to be between 45 and 60 GtC yr^{-1} (Longhurst et al., 1995; Taucher and Oschlies, 2011), and there is turnover of the phytoplankton biomass every 2–6 days (Behrenfeld and Falkowski, 1997; Behrenfeld et al., 2006; Kwiatkowski et al., 2017). The viral lysis of bacteria also releases DOM (Børsheim et al., 2005), and may be a major process relative to bacterial production (Vadstein et al., 2012).

A significant fraction of the DOM released from various sources contains chemical compounds that are rapidly used by heterotrophic microbes and are classified as labile DOM (LDOM). LDOM is estimated to be around 50% of dissolved organic matter produced photosynthetically (Biersmith and Benner, 1998). These compounds are used by heterotrophic microbes to build biomass, and its availability can limit microbial growth rate and production (Hansell, 2013; Hertkorn et al., 2006; Jiao et al., 2010; Ogawa et al., 2001). Extracellular and intracellular enzymatic conversion of LDOM can also result in the production of refractory or recalcitrant DOM (RDOM) (Arnosti, 2011; Wagner et al., 2020) that is more difficult for microbes to further degrade due to its chemical composition. RDOM can also be released via viral lysis and grazing including those from cell wall components (Jiao et al., 2010; Jiao and Zheng, 2011).

The process of carbon sequestration involves the biological capture and removal of carbon from biological cycles through the production of RDOM at all water depths (Jiao et al., 2014; Jiao and Zheng, 2011; Legendre et al., 2015). LDOM is taken up and removed by heterotrophic bacteria, yet RDOM from initial sources will remain, forming a larger fraction of the total DOM. Although heterotrophic bacteria utilise LDOM, the limitation of mineral nutrients may lead to the accumulation of LDOM in the environment (Thingstad et al., 1997). As a result, a mixture of LDOM and RDOM can be observed in a natural water sample. The natural DOM water sample represents a net balance of what remains after the production and consumption processes of DOM (Gruber et al., 2006). Fractions of the carbon sequestered in the form of RDOM may be stored in the deep ocean and surface sediment layers, preventing or slowing down the re-entry of carbon into the atmospheric carbon cycle for a few thousand years (Legendre et al., 2015; Miller et al., 1999). This is mainly because microbes are not able to efficiently metabolise RDOM, thereby withholding the release of carbon dioxide. Some studies have suggested that the sequestration of carbon takes place mainly in subsurface and deeper waters (Jørgensen et al., 2011; Omori et al., 2011; Yamashita and Tanoue, 2009). Few others reported that the highest bacterial abundance and activity which is linked to carbon sequestration is observed in surface waters (Garneau et al., 2008; Karl and Knauer, 1991; Tamburini et al., 2002). Thus, spatio-temporal studies prove be critical in order to elucidate the main dynamics in carbon sequestration. As the microbial community structure changes with depth (Bienhold et al., 2016; Brown et al., 2009; Treusch et al., 2009; Walsh et al., 2016; Wilson et al., 2017) and seasons (Cram et al., 2015; Gilbert et al., 2012; Suh et al., 2015), these studies offer implications for future climate conditions. Particularly, the microbial transformation is an important factor determining the DOM composition and carbon cycling (Jiao and Azam, 2011). Some operational taxonomic units (OTUs) grow faster with availability of specific growth substrates. The heterotrophic bacteria have differences in metabolic capacity, thus consumption and transformation of specific compounds of DOM would result in an increase in relative size of these bacteria. The resource partitioning and interactions between different bacterial groups leads to microbial successions relevant for the different metabolic pathways in biogeochemical carbon cycling (Landa et al., 2016; McCarren et al., 2010).

Carboxyl-rich alicyclic molecules (CRAM) are one of the most common forms of RDOM in the marine DOM pool and is reported to be of microbial origin (Hertkorn et al., 2006; Lechtenfeld et al., 2015). The production of these compounds influences the formation of larger particulate aggregates which increase export of organic matter to deeper waters (Hertkorn et al., 2006). The resulting aggregates, often referred to as marine colloidal gels, are in the particulate-dissolved organic matter continuum and are present in the interface between the operational classification of particulate organic carbon (POC) and dissolved organic carbon (DOC) (Chin et al., 1998; He et al., 2016). The formation of these marine gels favour either increased vertical fluxes or the export of the organic matter from the surface into deep waters (Verdugo et al., 2004).

Coastal regions are generally more productive systems per unit area than the pelagic regions of the ocean (Ryther, 1969). With high productivity, DOM production is also higher in coastal waters (Barrón and Duarte, 2015; Bauer and Druffel, 1998). Primary production varies on both temporal and spatial scales based on the availability of essential mineral nutrients, light and temperature conditions in coastal waters. Fjord systems form an integral part of coastal geography at higher latitudes in regions of Norway, Greenland, Chile, New Zealand and the Antarctic coast. The fjords often stretch out a few hundred kilometres, and their depths may vary from a few metres to over a thousand metres. The fjords are therefore excellent sites for coastal DOM studies in both surface and deep waters. The coastal fjord ecosystems are usually affected by both marine and freshwater conditions that vary regionally and with seasons. Increased river runoff replenishes the coastal waters with essential inorganic nutrients that, together with supply from deep-water, support primary production (Milzer et al., 2013). At higher latitudes, nutrient rich deep water favours characteristic spring blooms followed by peaks in zooplankton biomass, which are important in marine trophic food webs (Reigstad et al., 2002; Taylor and Ferrari, 2011). In a coastal fjord ecosystem, all these events and factors play a significant role in DOM production, mobilisation and dynamics. The spring bloom in temperate and Arctic regions, with maximal abundance of phytoplankton, produces a complex mixture of DOM in the surface

waters. The labile DOM derived from phytoplankton exudates and predation in the surface water supports the metabolic activities of heterotrophic bacteria for the microbial carbon pump (Olsen et al., 2011). The main objective of our study was to understand the temporal and spatial variation in carbon sequestration through RDOM production in coastal temperate fjord waters. The RDOM, represented as CRAM fractions, is affected by the DOM production and consumption phases in the marine environment. CRAM production is strongly determined by both the spatio-temporal factors affecting bacterial production, and microbial community composition, metabolising other labile DOM fractions.

2. Materials and methods

2.1. Sampling site

The samples were collected from the Trondheim fjord, one of the longest and deepest fjord systems in central Norway. The fjord is about 135 km long, extending over an area of about 1420 km^2, and with a depth of 617 m at the deepest point (Brooke and Järnegren, 2013). The Trondheim fjord receives freshwater influx from five main rivers; the Nidelva, the Gaula, the Stjørdalselva, the Orkla and the Verdalselva. The Trollet sampling station (63° 28.984 N 10° 17.998 E) is located in the open basin of the fjord (Supplementary Data Fig. 1), is far from the nearest river input and is therefore little affected by freshwater (Öztürk et al., 2002). The fjord system is strongly affected by the North Atlantic waters, and freshwater impact is usually limited to river mouths and very thin surface layers during heavy freshwater runoff. The sampling station is approximately 450 m deep, which makes it an ideal location for coastal temperate DOM studies of surface to deep water fjords.

2.2. Sample collection

The water samples from three depths; 3 m, 225 m and 440 m, were collected using a Niskin sampler attached to Seabird Electronics, SBE-19plus V2 CTD sensors during coastal cruises in the Trondheim fjord on RV Gunnerus. The 3 m and 440 m depths represented surface and deep waters respectively, and the intermediate sample collected at 225 m was half the depth of the station and below the euphotic zone. The samples were collected during late spring (20th April), late summer (1st September), autumn (10th October) and winter (12th December), 2017.

2.3. Inorganic nutrients analysis

The water samples were collected in clean vials and were immediately frozen at −20 °C until further analysis. The thawed water samples were filtered through sterile 0.2 cellulose acetate syringe filters (VWR, Trondheim) to remove any particulate matter. They were analysed for dissolved inorganic nitrogen (nitrate, nitrite and ammonium) and dissolved inorganic phosphate (Grasshoff et al., 2009) using an autoanalyser (O.I. Analytical Autosampler, TM-Autolab AS, Norway).

2.4. Bacterial production measurements

Bacterial production rates in the samples were measured using the tritiated methyl-(^{3}H) thymidine method (Bell, 1993). Samples of 10 mL volume and a final concentration of 20 nM with tritiated methyl-(^{3}H) thymidine were incubated for one hour. Incubation was stopped with the addition of formaldehyde, with a final concentration of 1%. The activity was measured using liquid scintillation counting (PerkinElmer Life and Analytical Sciences, Waltham, MA, USA) of the samples and control.

2.5. Fluorometric analysis for chlorophyll a

The water samples were filtered with Whatman GF/F filters (nominal pore size of 0.7 μm). The filters were pre-combusted at 450 °C for eight hours to remove any organic carbon contamination. The particulate matter on the GF/F filters was frozen at −20 °C until further fluorometric analysis. The chlorophyll *a* on the filters was extracted in methanol according to Strickland and Parsons (1972), and measured using a Turner Designs fluorometer (California, USA).

2.6. 16S rRNA gene amplicon sequencing and data processing

The method for amplicon sequencing followed that described in Navada et al. (2020). Briefly, samples of bacteria were collected using 0.2 μm sterile syringe tip filters from mixed cellulose esters with

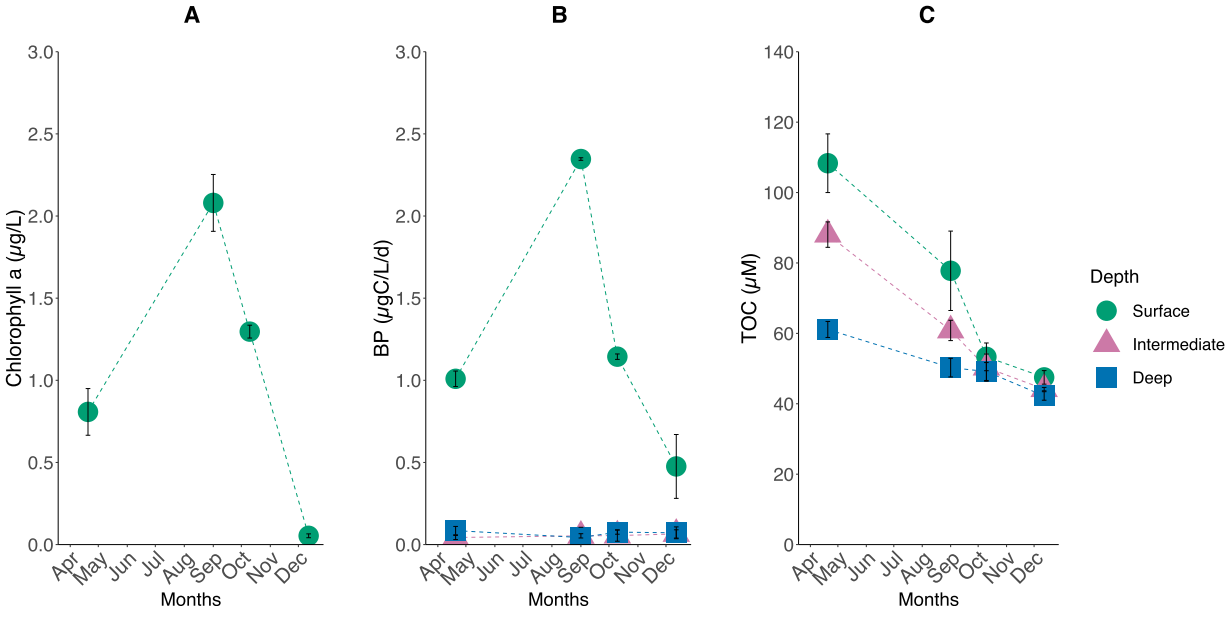


Fig. 1. (A) Chlorophyll *a* concentration in surface waters; (B) bacterial production rate (BP); and (C) dissolved total organic carbon concentration (TOC) from various depths in different seasons.

glycerine as filtration filter media (DynaGard™, Spectrum Laboratories, Inc., USA) and preserved at −20 °C. DNA was extracted using the DNeasy® PowerSoil Kit (Qiagen, Germany). The V3 and V4 regions of the 16S rRNA gene were targeted for sequencing, using broad range PCR primers (338F and 805R) with Illumina adapter sequences (the lower-case letters denote the adapter sequences 338F: 5′ cgtcggcagcgtcagatgtctataaga gacagnnnnCCTACGGGWGGCAGCAG-3′ and 805R: 5′-gtctcgtgggctcggagatgtgtataagagacagnnnn ACTACNVGGGTAT CTAAKCC-3′). The barcoded PCR products were pooled and sequenced on one MiSeq lane each (Illumina, USA) at the Norwegian Sequencing Centre in Oslo. The Illumina sequencing data was deposited and available at the European Nucleotide Archive (accession numbers ERS5514118 - ERS5514129). The data was processed using the USEARCH pipeline (version 11), and the merging of pair reads included primer sequences removal, quality filtering and demultiplexing, and the removal of chimera sequences and singletons. Operational taxonomic unit (OTU) clustering was performed at 97% similarity level using the UPARSE algorithm (Edgar, 2013), and taxonomic assignment was based on the Sintax command and the RDP reference dataset (Version 16). To remove bias due to differences in sequencing depth, all analyses were performed on an OTU table that had been normalised to 15,000 reads per sample (lowest number of reads in any sample). The bacterial richness, order one diversity (exponential. 1 Shannon index) and evenness were calculated.

2.7. Total organic carbon (TOC) analysis

The TOC water samples (35 mL) were collected in clean vials on board RV Gunnerus and frozen to −20 °C immediately after addition of 3 M HCl reducing to pH 2 to prevent any bacterial degradation under further analysis. The TOC, as non-purgeable organic carbon, was analysed using the high temperature catalytic oxidation method, with a Shimadzu TOC-L analyser at the GEOMAR Helmholtz Centre for Ocean Research Kiel, Germany. Olsen et al. (2006) reported from nutrient enrichment experiments conducted in the coastal Norwegian waters that DOC constituted 86–93% of TOC, even when POC was higher than natural conditions.

2.8. DOM characterisation

The water samples collected during the cruise were immediately transferred to the laboratory to make sure that the ambient temperature had a minimal effect during transport. The water samples were filtered through precombusted Whatman ™ GF/F filters. The DOM filtrate was treated with 5 M HCl to bring the pH down to 2, before preconcentration of the sample using Agilent Bond Elut ™ PPL cartridges (1 g, 6 mL; VWR Chemicals Trondheim, Norway). The acidification step prevents any further bacterial degradation of the DOM in the samples and increases the adsorptive efficiency of the PPL cartridges. The cartridges were preconditioned, and DOM sample collection were according to Dittmar et al. (2008), using 10 mL of ultrapure HPLC grade methanol (Alfa Aesar, Germany). In December 2017, duplicate DOM samples were collected for comparison of replicates.

The DOM samples stored at 4 °C were later analysed by direct infusion high resolution mass spectrometry with a Synapt G2-S mass spectrometer (Waters, Milford USA) using the electrospray ionisation (ESI) method for both positive and negative modes (ESI+ and ESI-). Leucine enkephalin with a concentration of 200 ng/mL was used for the Lockmass correction of exact mass measurements with a flow rate of 10 μl/min. The MS instrument used an electrospray ionisation source (both ESI- and ESI+), with capillary tip voltage set to 2.25 KV and sample cone voltage set at 30 V. The source temperature was kept at 120 °C and cone gas flow regulated to 100 L/h. The desolvation temperature was fixed at 350 °C with a gas flow rate of 800 L/h. The samples were acquired for 2 min each with a sample volume of 5 μl.

The Electrospray Ionisation (ESI) is a soft ionisation technique that ionises complex molecules, including proteins, with minimal or no fragmentation (Banerjee and Mazumdar, 2012; Smith et al., 1995). The ESI positive mode detects DOM with more basic functional groups, and the ESI negative mode detects DOM with acidic functional groups (Kujawinski, 2002; Ohno et al., 2016). Some DOM compounds showing amphoteric character with both acidic and basic functional groups can be detected in both the modes. The MS total ion chromatogram for each sample was processed and 40 scans were combined in the maximum peak area. Ultra-high resolution MS techniques such as FT-ICR-MS are adopted for better accuracy and precision in assigning DOM chemical formulas. High resolution MS techniques are widely used in the field of petroleomics for assigning chemical formulas into various heteroatom (nitrogen, oxygen, sulphur) combination groups with the help of PetroOrg (Corilo, 2014; Farenc et al., 2016; Grimm et al., 2017). PetroOrg was used in DOM studies also to assign molecular formulas as reported by Avneri-Katz et al. (2017), Letourneau and Medeiros (2019), and McDonough et al. (2020). The results were processed using PetroOrg SS software (18.0.3), which included the Kendrick Mass Defect for assigning elemental composition for carbon, hydrogen, oxygen, nitrogen, sulphur and phosphorus. The N1 class in the close shell was selected as reference setting as per PetroOrg recommendation. The calibration settings were selected to include a maximum error of 10 ppm and further, formulas with a maximum error of 3 ppm were only considered. The Kendrick Mass Defect helped to determine homologous formulas with the same degree of unsaturation, differing only in CH_2 units. The elemental composition ranges assigned for various elements were $C_{1-60}\,H_{1-210}\,O_{1-60}\,N_{0-4}\,S_{0-2}\,P_{0-1}$ (Simon et al., 2018). The mass spectra obtained after ESI mass spectrometric analysis gave ion abundance peaks along the mass/charge ratio (m/z) axis. The assigned chemical formulas were processed for singly charged atoms with m/z ranging from 150 to 700. The formulas with m/z less than 150 and more than 700 have higher degrees of uncertainty (Koch and Dittmar, 2006; Stubbins et al., 2010). Only formulas over 10,000 in relative ion abundances were considered for data processing. Signals below this limit were considered noise, as under this value it was difficult to differentiate ^{13}C isotopic patterns of the signals from the noise.

The double bound equivalence (DBE), which is a measure of the number of multiple bonds or ringed structures, was calculated as (Wagner et al., 2019):

$$DBE = 1 + 0.5(2C - H - N + P) \quad (1)$$

The modified aromaticity index (AI_{mod}) was calculated as (Koch and Dittmar, 2006):

$$AI_{mod} = (1 + C - 0.5O - S - 0.5(N + P + H))/(C - 0.5O - S - N - P) \quad (2)$$

The modified aromaticity index (AI_{mod}), DBE and ratios of elements were used to group the determined chemical formulas into “Aliphatic, Highly unsaturated, Aromatic, Condensed aromatic and CRAM-like groups” according to Shah Walter et al. (2018) and Wagner et al. (2019), also referring to classifications used in Hertkorn et al. (2006), Pracht et al. (2018), Šantl-Temkiv et al. (2013), and Stubbins et al. (2010). Formulas that could not be grouped into any of the aforementioned groups were classified as “Others” (Table 1). The formulas in the different groups may contain some compounds that are labile and some that are refractory, originating from different sources, including bacteria. The CRAM formulas are, however, predominantly refractory and of microbial origin (Koch and Dittmar, 2006; Šantl-Temkiv et al., 2013; Wagner et al., 2019).

2.9. Data analysis

Principal coordinate analysis (PCoA) of the DOM characterisation data was performed with all determined formulas between m/z 150 to 700 for both ESI negative and positive modes. The PCoA analysis with Bray-Curtis dissimilarity, calculated for the relative content of

Table 1
Chemical classification criteria for DOM used in the present study.

Classification of chemical class groups[a]	Classification Criteria	Known examples representative of few chemical class groups
Aliphatic	H/C > 1.5	Lipids, carbohydrates, peptides, sulfonic acids
Highly unsaturated	AI <0.5; H/C < 1.5; O/C < 0.9	Humic matter, phenolic compounds
Aromatic	0.5 ≤ AI <0.67	Polyphenols (lignin and tannin)
Condensed aromatic	AI ≥0.67	Poly cyclic aromatic compounds
CRAM	0.3 < DBE/C < 0.68; 0.2 < DBE/H < 0.95 0.77 < DBE/O < 1.75	Sterols, hopanoids, isoprenoids, terpenoids, crenarchaeols

[a] The compounds that did not qualify to be included in any of these five groups were put separately as "Others".

each chemical formula in the samples, helped to identify the temporal and spatial variation of the DOM composition in our study and reduces the problem with several zeros in the dataset (Legendre and Gallagher, 2001). The statistical analyses and plots were performed using R-studio (V.1.2.1335) using the tidyverse and ggplot2 packages (Wickham, 2016).

3. Results

3.1. Hydrographic data

Supplementary Data Fig. 2 shows the in situ temperature, salinity and density for December 2016, April, September and October 2017. In the late summer season (September), the surface temperature was around 14 °C and a strong thermocline was present at 50 m and 100 m where the temperature decreased from 13 °C to 8.5 °C. Towards autumn season, the average temperature was around 12 °C up to a depth of 50 m and observed a weak thermocline compared to September month. During April, there was a cooling in the surface temperature and it gradually increased from 6.3 °C in the surface to 8.1 °C at 50 m. The temperature below 140 m was 8 °C on average throughout the year (Supplementary Data Fig. 2A). The original CTD data form 2017 December was lost, the closest similar seasonal observation from the sampling station in December 2016 is provide as a reference. The salinity at surface 3 m ranged between 30 and 31.7 from spring to autumn. In December 2016, the salinity was 31. The salinity increased to 34.6 at 150 m depth and increased marginally to 34.8 until depth of 440 m. The salinity remained constant below 150 m depth during the different sampling periods (Supplementary Data Fig. 2B). The density at surface ranged between 22.2 and 24.1 in surface 3 m, lowest in summer and highest in autumn seasons respectively. The density was 27.6 kg/m^3 throughout the year at the depth 150 m. The density gradually increased with depth reaching 29.1 kg/m^3 at 440 m, irrespective of seasonal change (Supplementary Data Fig. 2C).

3.2. Nutrients

Nitrate was the main form of dissolved inorganic nitrogen, the concentrations of nitrite and ammonium were negligible (Supplementary Data Fig. 3A). Nitrate concentration in the surface waters ranged between 0.6 μM in late summer and 6.6 μM in winter, with a mean concentration and standard deviation (SD) of 3.6 ± 2.5 μM during the study period. Nitrate concentration decreased from late spring to late summer, and increased during subsequent seasons in surface waters, which was a result of nitrate uptake and depletion by phytoplankton. In the intermediate and deep waters, nitrate concentrations were equal and more than twice as high as in surface waters. It varied between 9.3 μM in deep water and 10.5 μM in intermediate water during late spring and winter seasons, respectively, and increased slightly temporally. The mean nitrate concentration of 9.9 ± 0.04 SD μM in the intermediate and deep waters was representative of North Atlantic coastal deep-water and remained similar through the study period.

The concentrations of phosphate showed a similar pattern of variation to nitrate concentration (Supplementary Data Fig. 3B). The concentrations ranged between 0.06 μM in late summer and 0.4 μM in winter in surface waters. The mean phosphate concentration in surface waters was 0.22 ± 0.15 SD μM, with the lowest values at the end of the summer season. The phosphate concentration in intermediate and deep waters ranged between 0.71 μM in late spring and 0.84 μM in winter seasons, with a mean concentration of 0.78 ± 0.05 SD μM, representative of North Atlantic coastal deep-water. The concentrations found in intermediate and deep waters were more than three times higher than the concentration in surface waters.

3.3. Surface chlorophyll a concentration and bacterial production

The surface chlorophyll *a* concentration at 3 m depth varied from late spring to winter seasons (Fig. 1A). The highest mean chlorophyll *a* concentration with standard error (SE) was measured in late summer, reaching 2.07 ± 0.17 μgL^{-1} and the lowest concentration of 0.05 ± 0.02 SE μgL^{-1} was found in the winter season. The mean surface chlorophyll *a* concentration during the study was 1.06 ± 0.85 SD μgL^{-1}. The late spring season was found to have a lower concentration than late summer and autumn seasons due to post-spring bloom conditions. The chlorophyll *a* concentration followed an inverse trend compared to the concentrations of nitrate and phosphate, as expected, which was a result of phytoplankton blooming and the use of inorganic nutrients for growth.

The pattern of variation in bacterial production (BP) for surface waters was similar to that found for the surface chlorophyll *a* concentration (Fig. 1B). The highest and lowest BP rates were measured in the late summer and winter seasons, respectively. The average BP in the surface waters during our study was 1.24 ± 0.79 SD μgCL^{-1}d^{-1}. A much lower BP was found in the intermediate and deep-water samples, showing a mean bacterial production rate of 0.06 ± 0.02 SD μgCL^{-1}d^{-1}, which was some twenty times less than in surface waters. The surface water bacterial production rate was approximately 7 times and over 50 times higher in the winter and the summer seasons respectively, and about 17 times higher in the other two seasons.

3.4. Total organic carbon

The total organic carbon (TOC) concentration in the surface waters ranged between 108 μM in late spring and 47.5 μM in winter seasons (Fig. 1C). The mean concentration measured was 71.7 ± 27.7 SD μM. The TOC concentration showed a gradual decrease from late spring to winter seasons. The TOC concentration in the autumn sample was almost half the concentration of late spring. As the particulate organic carbon concentrations were relatively low, DOC concentrations determined (TOC-POC) were within the error limits of TOC concentrations (Ogawa and Tanoue, 2003; Olsen et al., 2006).

The late spring TOC concentration was found to be highest for each depth, and the winter season values were lowest. The temporal difference in TOC concentrations was, however, reduced with depth. The TOC concentrations in the intermediate depth showed a decreasing trend from late spring to winter, similar to the surface waters, with a mean TOC concentration of 60.8 ± 19.4 SD μM. The mean TOC concentration for deep water samples during the study period was 50.7 ± 7.8 SD μM.

3.5. Bacterial community composition

The analysis of 16S rRNA gene sequences for the heterotrophic bacterial communities in the water samples of our study revealed that the

phylum *Proteobacteria* was dominant at all depths and all seasons (Fig. 2), and constituted between 60% and 78% of the sequences in any sample. At class level, *Alphaproteobacteria* (14–41%) and *Gammaproteobacteria* (21–48%) were the dominant taxa. Other prominent classes identified in the samples were *Flavobacteria* (2–13%), *Betaproteobacteria* (2–22%) and *Actinobacteria* (0.3–11%). Other bacterial classes that contributed less during the study, but were still significant (≥1%), were *Deltaproteobacteria, Bacilli, Sphingobacteriia, Epsilonproteobacteria, Planctomycetia, Verrucomicrobiae and Clostridia.*

The highest and lowest abundance of *Alphaproteobacteria* was observed in the summer deep (41%) and spring surface (14%) waters, respectively. The intermediate and deep samples in spring season had a similar abundance of 35%, which changed to 28% and 41% respectively in the summer season. The abundance of *Alphaproteobacteria* in summer surface sample was measured as 37%. In autumn, the abundance decreased along the depth gradient from 36% to 29%. Abundance throughout the winter season was relatively stable, at 38–37% in the water column.

The next most dominant class, *Gammaproteobacteria*, was least abundant in the spring intermediate sample (21%), and increased to 48% in the summer season. The abundance of surface and deep samples in the spring season was similar, and the surface sample in the summer season reached 28%. In autumn samples, the abundance of *Gammaproteobacteria* increased with depth from 23% to 32%, the opposite trend to that of *Alphaproteobacteria.* Although the surface and intermediate samples showed a similar abundance of 26% in the winter, the deep sample was less and similar in abundance to summer deep sample (22%).

Abundance of *Flavobacteria* was high in the intermediate sample, followed by the surface and deep samples in both spring (10–6%) and summer (12–4%) seasons. In autumn, the *Flavobacteria* abundance was highest in the surface sample (13%), and the intermediate and deep samples had similar abundances of 4% each. In the winter season, the abundance decreased from 4% to 2% from surface to deep. In the spring surface samples, *Betaproteobacteria* and *Actinobacteria* abundance were 22% and 11% respectively, while in other samples it was ≤6%.

In general, *Alphaproteobacteria* was more abundant than *Gammaproteobacteria*. The few exceptions when *Gammaproteobacteria* was more abundant than *Alphaproteobacteria* were the surface sample in late spring, intermediate sample during late summer and the deep sample during autumn. The late spring surface sample was very even, with *Betaproteobacteria*, *Actinobacteria* and *Flavobacteria* fractions contributing, in addition to the dominant class groups mentioned earlier. At the family taxonomic level, SAR11 *Alphaproteobacteria* was the most abundant family. Those bacterial sequences, consistently contributing less than 1% of the total abundance in all samples, was grouped together as “Others”. The sequences that could not be identified were grouped into an “Unassigned” class and ranged between 8% and 28%. In general, the bacterial community compositions were comparable between the different depths and seasons, except for surface waters in late spring and intermediate waters in late summer seasons.

Bacterial richness showed an increasing trend in the surface waters from late spring to winter and ranged between 63 and 929 OTUs (Fig. 3A). The intermediate waters increased relatively less in richness during and after the late summer season. The deep water closely followed the richness of intermediate waters in late spring and autumn seasons, however, the richness in deep water was lowest in the late summer and winter seasons.

The diversity of order one, Exp Shannon index, was lower in to intermediate and deep waters during the late spring and autumn seasons and ranged from 35 to 124 (Fig. 3B). The diversity number observed for deep waters reversed with respect to the surface waters in each sample, and ranged between 34 and 119, being highest in the late spring season. Intermediate waters showed the least change in the diversity number with seasons, with only a 1.8 times variation. The diversity numbers between intermediate and deep waters were more comparable than either were with surface waters.

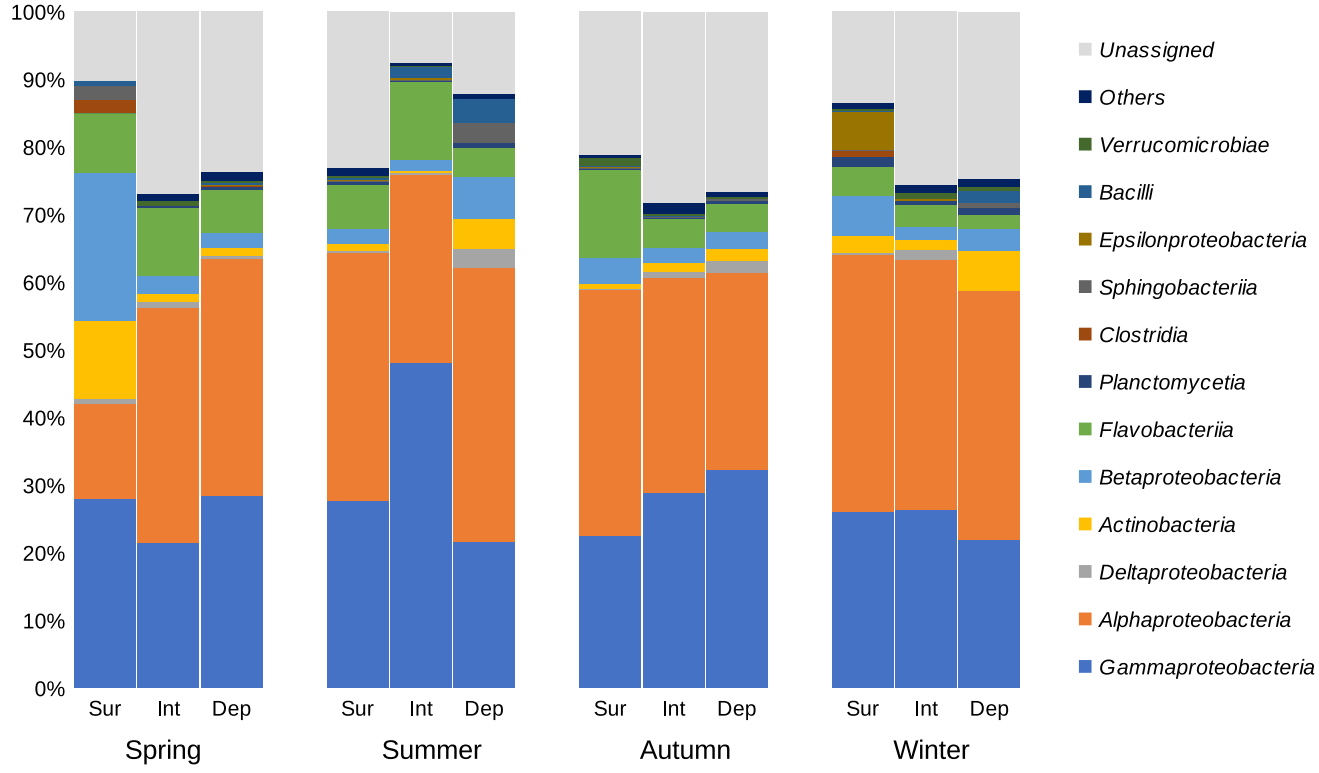


Fig. 2. Bacterial community composition assessment using 16S rRNA gene amplicon sequencing at three depths at class level (Sur-Surface; Int-Intermediate; Dep-Deep) during the study period.

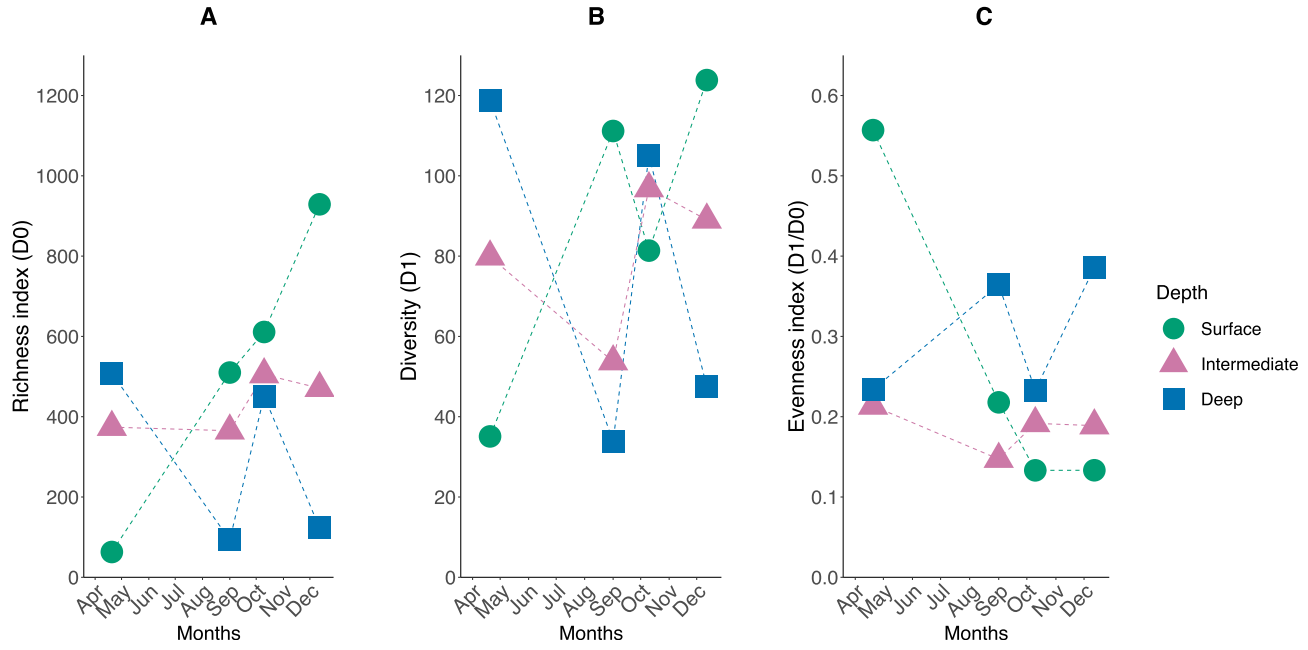


Fig. 3. Bacterial community composition during the study period across the different depths and seasons: (A) richness index; (B) diversity as exponential Shannon index; and (C) evenness index.

The evenness in microbial community distribution was lowest in intermediate waters and highest in surface water during the late spring season. The evenness ranged between 0.2 and 0.6 (Fig. 3C) and decreased in surface waters from late spring to winter. The evenness of the microbial community in surface and deep waters was comparable during late summer. The intermediate water showed marginal change in evenness according to temporal changes. Microbial evenness showed an increasing trend with depth during the autumn and winter seasons.

3.6. DOM characterisation

The processed mass spectrometric data from DOM samples for each season was plotted in the Van Krevelen diagram with the oxygen to carbon ratio on the X-axis and the hydrogen to carbon ratio on the Y-axis. Fig. 4A-D show Van Krevelen diagrams for each season with three depths (surface, intermediate and deep) for the individual chemical formulas in DOM negative mode from late spring to winter classified and plotted, based on relative intensities, depth and chemical class groups.

The total number of chemical formulas found in our study was higher in the late spring season than in the other seasons, both for negative and positive mode analysis of the DOM samples (Supplementary Data Tables 2 & 3). In general, the chemical formulas in the Aliphatic, Highly unsaturated, Aromatic and Condensed aromatic groups decreased with season from late spring to winter, however, the number of chemical formulas in the chemical class group of CRAM increased in the ESI negative mode with seasons from late spring to winter, and for ESI positive mode the number of chemical formulas spiked in late summer, after which it decreased. The number of chemical formulas determined in the CRAM-like group was higher in surface waters than intermediate and deep waters.

Even though the ESI mass spectrometric technique is not a fully quantitative technique, the total ion abundance found in the samples from mass spectrometric analysis was in accordance with the measured TOC concentrations, as similarly reported by Seitzinger et al. (2005). The relative ion abundance, calculated by dividing individual ion abundance by the total sum of all ion abundances in the mass spectra, was used to identify and determine quantitative differences between DOM samples (Bae et al., 2011; Persson et al., 2006). The relative ion abundances of the chemical class groups were calculated and expressed as percentage compositions.

The compounds in the different chemical class groups - Aliphatic, Highly unsaturated, Aromatic, Condensed aromatic and Others - are produced from various sources, including microbes, and contain both LDOM and RDOM (Table 1). It is therefore difficult to use the fraction of these chemical class groups as a potential indicator of microbial sequestration of LDOM. As in the previous section, formulas included in the chemical class group CRAM are predominantly of microbial origin and are a function of microbial sequestration producing RDOM. The relative increase in CRAM percentage is taken as an indication that the net yield of CRAM was higher as a result of microbial consumption of other DOM chemical class groups, which is explained below.

The CRAM fractions which were predominantly RDOM were plotted for comparison with each of the chemical class groups (Aliphatic, Highly unsaturated, Aromatic and Condensed aromatic) during different seasons. In ESI negative mode, the average CRAM content was about 22% of the total ion abundance in the late spring samples. Its relative content increased nearly two-fold in late summer and remained the same until winter (Fig. 5A-D). The relative content of CRAM in surface waters was lower than that in intermediate and deep waters during the late spring and late summer seasons, and it increased thereafter in the autumn and winter seasons.

The chemical class groups of Aliphatic, Highly unsaturated and Aromatic dissolved carbon components showed a decrease in their relative content compared to that of CRAM, included in all panels of Fig. 5A-C to facilitate comparison with other groups. The Aliphatic chemical class, constituting about 11% of total ion abundance in late spring, decreased to almost half in late summer, after which it again increased in winter samples (Fig. 5A). In the Highly unsaturated group, the late spring samples were reduced to about 23% from the late summer season onwards (Fig. 5B). The Aromatic group constituted about 23% of total ion abundance in the late spring sample and was reduced to almost half the content in all later seasonal samples (Fig. 5C). In the Condensed aromatic group, relative content increased to about 17% from late summer onwards (Fig. 5D).

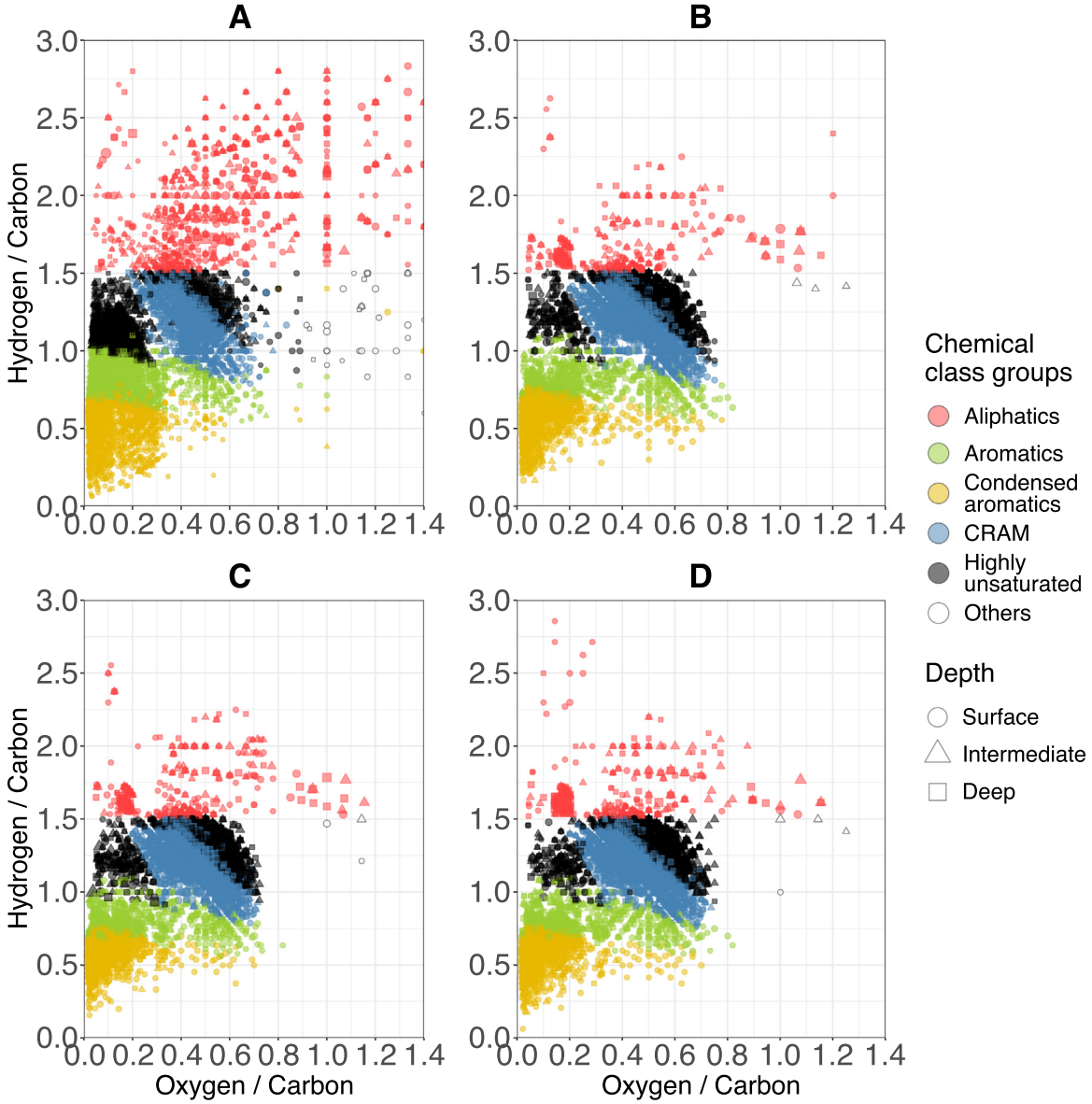


Fig. 4. Van Krevelen plots for DOM samples in the ESI negative mode showing the relative intensity of determined chemical formulas under various chemical class groups from three depths during late spring, late summer, autumn seasons and one winter sample, represented from A-D respectively.

The average atomic C/N ratio in late spring was similar for the three depth samples with an average ratio of 35 (Supplementary Data Fig. 4A). The value found in surface samples almost doubled towards the late summer and decreased slightly afterwards. The atomic C/N ratio for intermediate and deep-water samples after late spring was lower than in surface water samples, ranging between 57 and 52. The pattern of variation in atomic C/N ratios showed that the chemical formulas determined in the ESI negative mode were affected both by seasons and depths.

In ESI positive mode, the average relative CRAM content was around 13% of total abundance in the late spring season. It roughly tripled towards late summer, after which it decreased gradually towards the winter (Fig. 6A-D). The relative CRAM content in samples from surface and intermediate depths in the late summer and autumn seasons was higher than in the deep-water samples. During other seasons, the relative CRAM contents were similar at the three depths.

The Aliphatic and Condensed aromatic chemical components showed decreased fractions compared to CRAM over the study period (Fig. 6A & D). The relative content of the Aliphatic group components was reduced by more than half from late spring to about 11% in other seasons (Fig. 6A). Similarly, in the Condensed aromatic group, the relative content was reduced more than three-fold from the late spring season to fractions as low as 9% in the other seasons (Fig. 6D). The Highly unsaturated and Aromatic chemical class groups showed a slight increase in their relative content from late spring to winter (Fig. 6B & C).

The average atomic C/N ratio of surface samples were slightly higher throughout than that found in intermediate and deep waters (Supplementary Data Fig. 4B). The values ranged between 32 and 37 in surface waters, and between 28 and 32 for intermediate and deep-water samples.

3.7. Statistical analysis

The first two principal coordinates explained 64.5% of the variability (PCoA 1 53.3% and PCoA 2 11.2%) in the ESI negative mode data (Fig. 7A). The variations in late spring samples at three depths

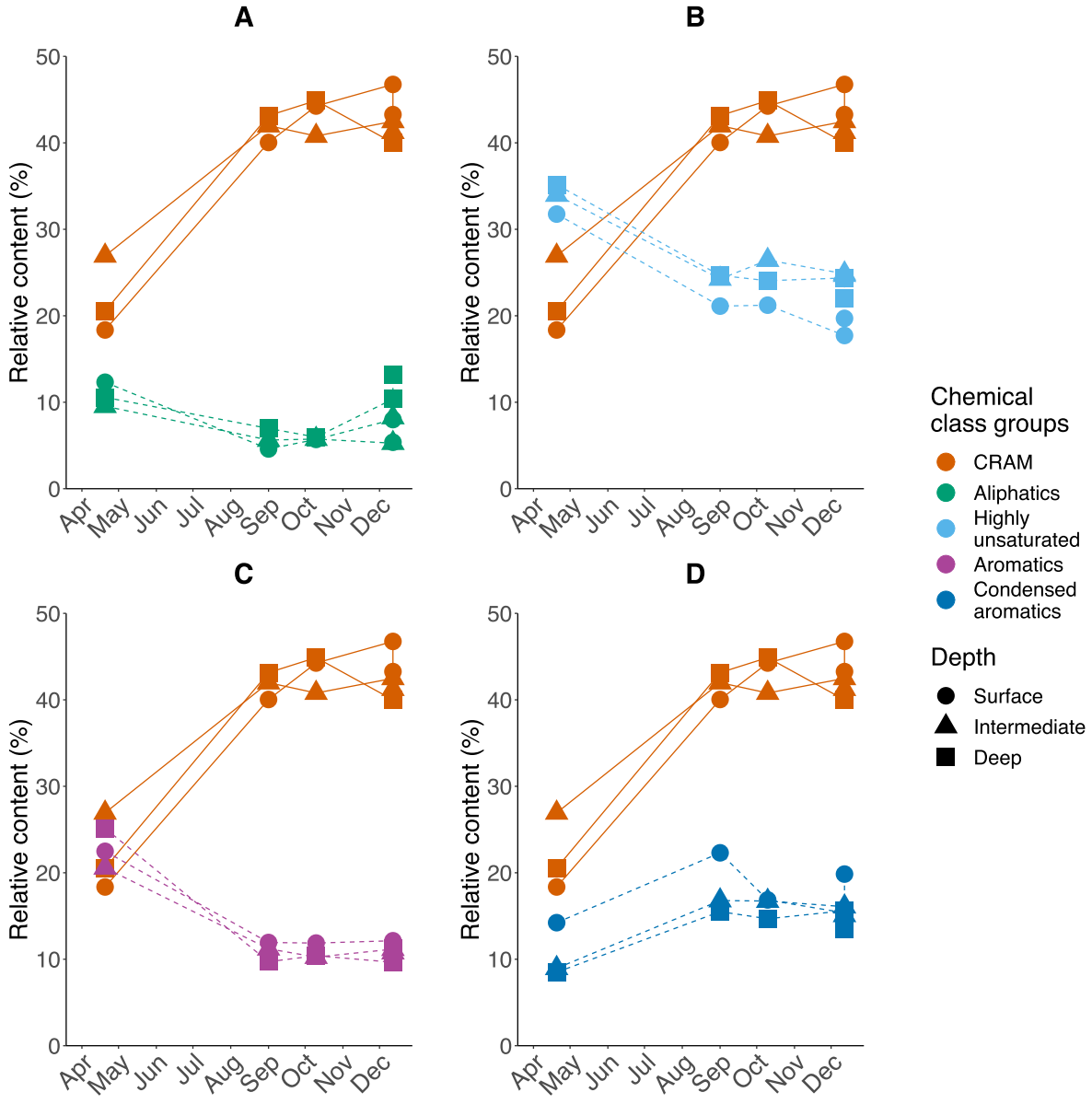


Fig. 5. Relative content in percentage for DOM chemical class groups in ESI negative mode from different sampling seasons and depths; the CRAM fraction is taken as the reference RDOM and other chemical class groups compared to it: (A) CRAM and aliphatic, (B) CRAM and highly unsaturated, (C) CRAM and aromatics and (D) CRAM and condensed aromatics.

contributed most to PCoA 1 values and were clustered together. All the other samples from late summer to winter seasons in all three depths made no substantial contribution to PCoA 1. PCoA 1 cannot explain the variability of the samples collected from different depths in late summer to winter seasons. PCoA 2 mainly explained the variability from depths within each season. The variability between surface and deeper waters was higher from late summer to winter seasons while in spring it was more similar. The intermediate and deep water DOM samples were clustered together in all seasons except spring with respect to PCoA 2. This is also the case for surface samples except the spring season. (Fig. 7A). PCoA for ESI negative mode DOM data were better explained by differences in seasons.

The PCoA explained 51.2% (PCoA 1 39.5% and PCoA 2 11.7%) of variability for ESI positive mode data (Fig. 7B). Similar to the ESI negative mode, the late spring samples were different from the other seasons and the variance was along PCoA 1. PCoA 1 could not explain variability due to the depth differences. The late spring samples did not show variations with depth and were clustered together with respect to both PCoA 1 and PCoA 2. The variation between surface and deeper samples within each season was better explained by PCoA 2 from late summer season onwards. While the intermediate and deep samples showed higher similarity, the surface samples differed from late summer to winter along PCoA 2. In ESI positive mode also, the variations in DOM chemical composition were better described by seasonal changes than differences in the fjord depth.

The effect of seasons and depth on DOM characterisation in our study was tested with two-way ANOVA with a significance level of 0.05 on arcsine-transformed relative abundance data. The variation for each of the different chemical class groups during the study period was significantly affected by seasons rather than depth (Supplementary Data Table 1). For ESI negative mode data, the Highly unsaturated, Aromatic and CRAM groups showed significant effect from seasonal succession. The depth variations showed a statistically important effect only in the case of Highly unsaturated fractions. The ESI positive mode results showed statistically relevant correlation with seasons for each of the five chemical class group fractions. There were no statistically

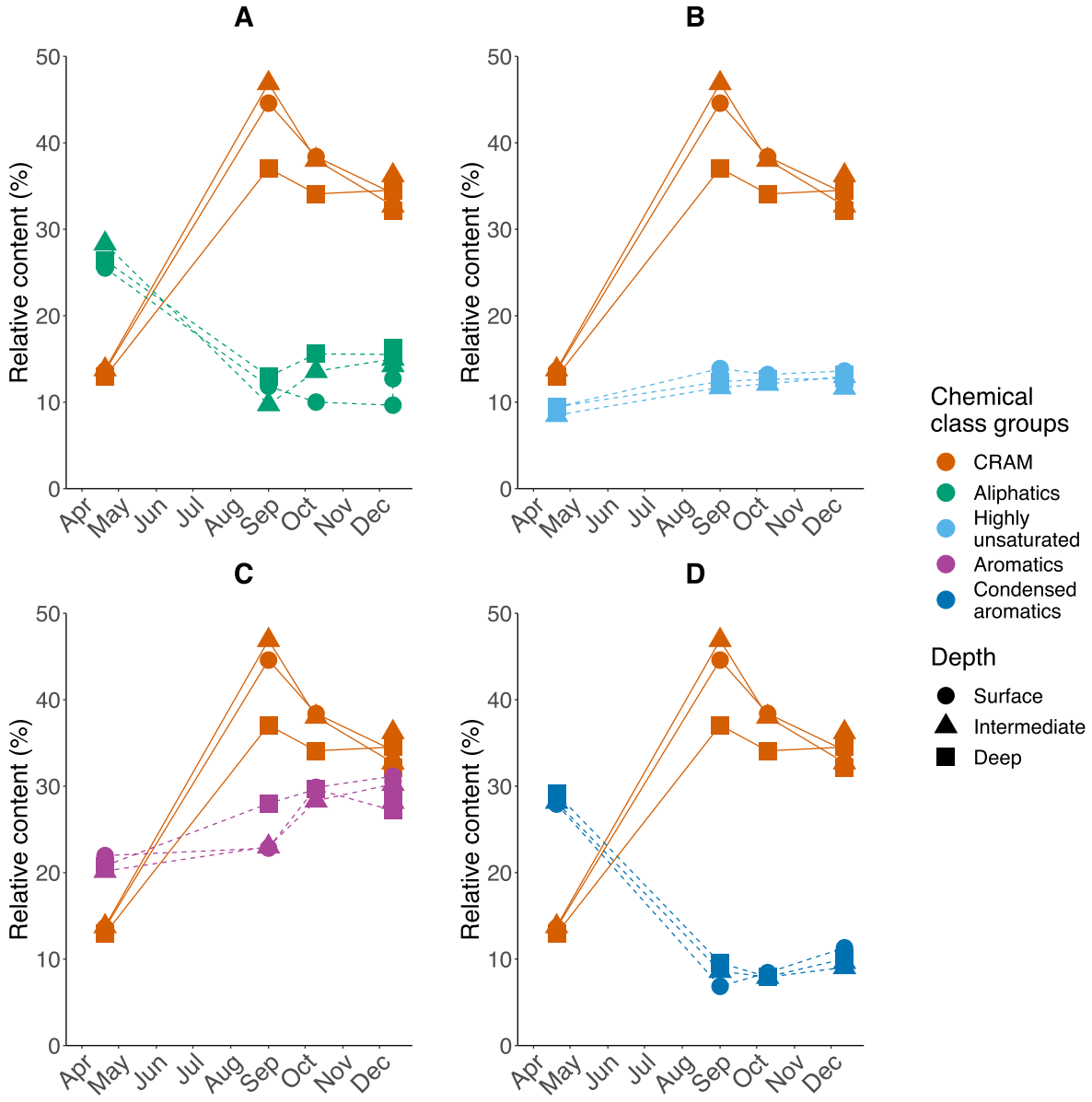


Fig. 6. Relative content in percentage for DOM chemical class groups in ESI positive mode from different sampling seasons and depths; the chemical class group fractions are compared to CRAM fractions: (A) CRAM and aliphatic, (B) CRAM and highly unsaturated, (C) CRAM and aromatics and (D) CRAM and condensed aromatics.

significant differences with depth for each of the chemical class group fractions. The combined effects of seasons and depth together were also not statistically valid for chemical class groups in our study for both positive and negative modes.

4. Discussion

Our study in the Trondheim fjord revealed the unique features of DOM dynamics in the temperate coastal fjord ecosystems with respect to DOM chemical class groups-Aliphatic, Highly unsaturated, Aromatic, Condensed aromatic and CRAM. We found that the variation in DOM chemical composition was significant with changes in the seasons rather than with different depth in the fjord system. The DOM produced from various biological components of the food web in the surface water were therefore apparently driven by seasonal succession cycles, and this difference of DOM composition in surface waters in turn affected the chemical class composition of DOM in intermediate and deep waters.

In our study, we observed a higher number of CRAM-like formulas, and higher bacterial production in surface waters relative to intermediate and deep waters. This may indicate that heterotrophic microbes in the surface waters metabolised labile DOM relatively quickly, and produced RDOM with lower carbon bioavailability for bacteria. A similar observation was reported by Amon (2016), who proposed that microbes sequester carbon as RDOM in the surface mixed layer. The relative CRAM content increase in the surface water samples and the increase in CRAM fractions indicated the microbial origin of RDOM, and supports previous findings reported by Jiao et al. (2018), Koch et al. (2014), and Lechtenfeld et al. (2015). The relative content of CRAM in the DOM samples increased from late spring to winter with the changing season, and were found to represent the major fraction of total isolated DOM (Figs. 5A-D and 6A-D).

The inorganic nitrate and phosphate concentrations followed a similar and expected pattern of variation for depths and seasons (Supplementary Data Fig. 3). The nutrient concentrations in the intermediate and deep waters were higher than in the surface waters, revealing

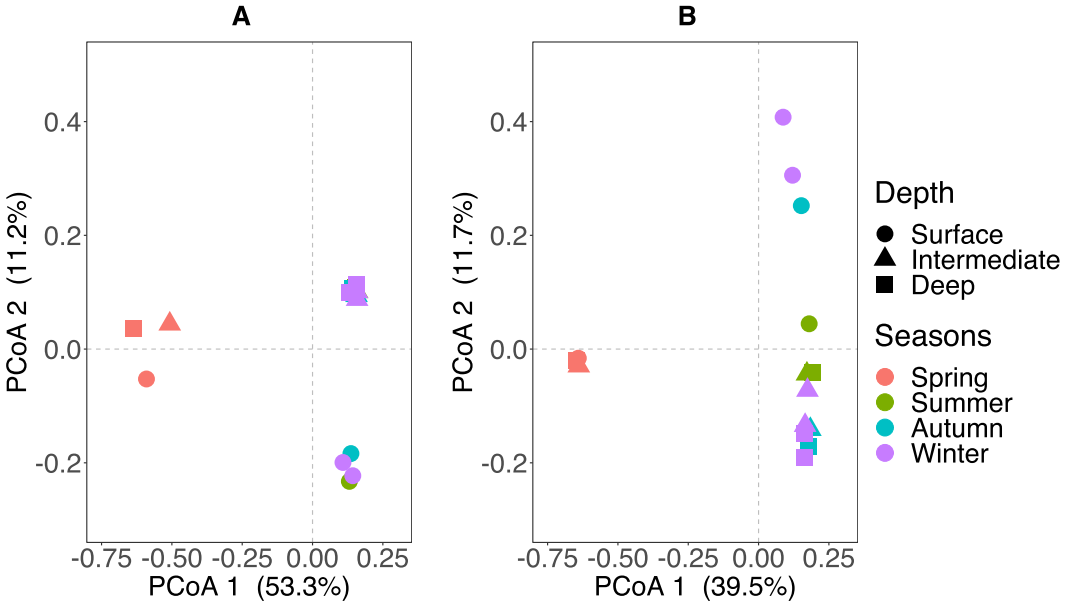


Fig. 7. PCoA plots of DOM samples in (A) ESI negative mode, (B) ESI positive mode.

nutrient saturating conditions for bacteria in deeper waters. The reduced nutrient concentration in surface waters is a result of the ubiquitous vegetation season characterised by high surface chlorophyll *a* concentration and phytoplankton primary production in temperate oceans (Fig. 1A). The blooming of phytoplankton accumulating inorganic nitrate and phosphate, and the changing phytoplankton communities in the surface waters according to season, contribute to the supply of dissolved organic matter (Fig. 1C). The production of labile DOM is mainly from phytoplankton, although other groups of organisms, such as zooplankton, may contribute with smaller amounts through grazing and defecation (Olsen et al., 2007). The labile DOM serves as the carbon source for heterotrophic bacteria, determining its growth and activity, together with inorganic nutrients. In a nutrient-limited situation, bacteria are believed to be better at scavenging inorganic nutrients competing with phytoplankton in the marine ecosystem (Olsen et al., 2011).

We found reduced bacterial production (BP) in deep water, even in the presence of high and biologically saturating inorganic nutrient concentrations, which could imply that BP was not limited by inorganic nutrients such as nitrate or phosphate. The TOC concentrations were higher in April (post-spring bloom condition) and was probably a result of higher organic matter production by the phytoplankton. The TOC concentrations in intermediate and deep waters were generally within the error limits of the surface waters concentrations. With the weakened thermocline and winter mixing towards autumn and winter months, the TOC concentrations across the water columns were similar (Supplementary Data Fig. 2A). This may suggest that majority of the total DOC in these aphotic depths was refractory DOC which limited bacterial production. This conclusion is supported by the observation that the bacterial production (BP) rate followed a very similar pattern of variation to that of the surface chlorophyll *a* concentration, showing a peak in the late summer season (Fig. 1B). Our findings agree with earlier results obtained in marine mesocosm experiments, which showed reduced bacterial production rates due to limitation by bioavailable carbon (Børsheim et al., 2005).

The bacterial activity in surface waters was found to be 6 to over 50 times higher than in the intermediate and deep waters (Fig. 1B), and was similar to that reported by Arístegui et al. (2009). This was in agreement with higher bacterial respiration reported in the photic layer by del Giorgio and Duarte (2002). Bacterial production may increase only 2–3 times for every 10 °C increase in temperature (Pomeroy and Wiebe, 2001). However, in our sampling site, the temperature of surface waters differed only up to 8 °C between the sampling times. Within a season, the difference in temperature was even lesser along the water column. Hence, it is likely that temperature had only minimal impact on the bacterial production compared to labile DOM. Kirchman et al. (2009) also concluded that a lack of labile DOM might be more important than temperature in affecting reduced bacterial production rates.

The bacterial richness, diversity and evenness showed variations between depths and different seasons (Fig. 4 A-C). The richness and diversity were relatively low in the surface waters in the late spring seasons compared to intermediate and deep waters. In the absence of the thermocline, the particulate matter from spring bloom senescence might have exported the surface microbial communities increasing the richness and diversity in the aphotic layer. Similarly, the salinity and density increase observed in April (Supplementary Data Fig. 2B-C) likely indicated the displacement of the less dense and less saline Norwegian Coastal Current (NCC) by the denser saline North Atlantic waters, a phenomenon common in spring and early summer seasons in the fjord. By late summer or early autumn, the NCC thickens in the fjord up to nearly 100 m of depth. This agreed with the findings reported by Ellingsen (2004). The export in spring season thus might be higher than other seasons in the fjord system (Wassmann, 2005). The differences between bacterial communities at class level are not statistically significant between depths or seasons. The changes in bacterial community composition, as observed in the richness, diversity and evenness, are probably driven by the less abundant OTUs, and the major groups that dominated the overall community composition were not changing significantly with seasons and depths. Even at the class level the group of "Unassigned" OTUs was 7 to 28%. This limits the possibility to deduce the function of specific OTUs or groups of OTUs in the conversion of DOM, suggesting the need for further research in this direction.

Lechtenfeld et al. (2015) proposed, based on bioassay experiments, that coastal bacterial communities quickly assimilated labile DOM and produced exometabolites that contain refractory molecules predominantly in the CRAM fractions. The Van Krevelen diagrams of the DOM samples showed that the chemical class of CRAM components was a prominent group found throughout the year at all depths (Fig. 4A-D). The late spring samples contained chemical compounds dispersed across the different chemical class groups (Fig. 4A) and the total number of chemical compounds reduced gradually with seasons. Even though

the total numbers of chemical compounds reduced with time during the season, both the relative content and the number of chemical compounds in the CRAM group increased in both the positive and negative modes (Supplementary Data Tables 2 & 3), (Figs. 4A-D; 5A-D; 6A-D). As shown in these figures, the increase in the number of chemical compounds and the relative content of CRAM was associated with a corresponding reduction in the other chemical class groups. An increase in relative CRAM content was observed from late spring to winter seasons for all depths. A simultaneous corresponding decrease in the relative content was seen for Aliphatic, Highly unsaturated and Aromatic chemical class groups in results obtained using the ESI negative mode method (Fig. 5A-C). A similar increasing trend in CRAM content was also observed in DOM positive mode compounds, however, a reduction in the DOM relative content was seen for both Aliphatic and Condensed aromatic compounds in the positive mode (Fig. 6A & C). The higher CRAM fractions correlate with higher bacteria production of RDOM, as concluded by Catalá et al. (2015) and Shen and Benner (2018). The principal coordinate analysis indicated that the samples from late spring can be uniquely identified from other seasons using PCoA 1 in both ESI negative and positive modes (Fig. 7A & B). The differences in depth could not explain the variability denoted by PCoA 1 for ESI negative and positive modes that were 53.3% and 39.3% respectively. While PCoA 2, explained the DOM variability from different depths in ESI negative mode, it represented the differences from both depth and seasons from late summer season onwards in ESI positive mode. Similarly, the two-way ANOVA suggested that the variation for each of the different chemical class groups during the study period was significantly affected by differences in seasons rather than the different depths (Supplementary Data Table 1).

In general, LDOM is nitrogen rich and has a lower C/N ratio than RDOM (Amon, 2016; Walker Brett et al., 2016). The late spring samples showed a relatively lower C/N ratio, and therefore also higher nitrogen contents than those taken in other seasons. Lønborg and Álvarez-Salgado (2012) and Davis et al. (2019) concluded that microbes preferentially remineralise P and N faster than C in DOM. The atomic C/N ratio of CRAM chemical groups ranged between 65 and 1994 for negative mode analysis (Supplementary Data Table 4). This ratio range was 36 to 361 for positive mode analysis (Supplementary Data Table 5). The C/N ratio was higher than other chemical class groups with few exceptions, and indicated that CRAM was more refractory in nature. Hopkinson and Vallino (2005) and Jiao et al. (2010) reported that the C:N:P ratio for LDOM was in the range 199:20:1, and that for RDOM was much higher and over 3511:202:1. The relatively higher C/N ratio found in the other seasons therefore agrees with a high fraction of RDOM that is represented by a high relative CRAM content. Even though the BP was reduced in the autumn and winter seasons, the relative content of RDOM represented by CRAM fractions remained similar to that in the late summer. The CRAM already produced during the late summer season was to a large extent resistant to microbial consumption and remained in the water column with weakened of thermocline that soon proceeded to winter mixing (Supplementary Data 2A).

Some studies including respiration and apparent oxygen utilisation measurements have concluded that microbial-derived refractory DOM production is more dominant in the deeper oceans below the surface waters (Yamashita and Tanoue, 2008). Most other studies suggest that RDOM formation and carbon sequestration takes place at all depths (Jiao et al., 2010; Legendre et al., 2015; Zhang et al., 2018). The relative fraction of each of the chemical class groups were similar in all the depths in each season. Meanwhile, there were higher C/N values for surface water than for intermediate and deep waters. A higher C/N ratio may suggest the overall higher refractory character of the surface DOM samples. This may suggest that the majority of the DOM in surface waters was already converted to RDOM by microbes, which is likely because the bacterial production was higher at the surface than in the deeper layers of waters, which showed very low production, close to zero. Similarly, the number of CRAM compounds determined were higher in the surface waters compared to intermediate and deep waters (Supplementary Data Tables 2 & 3). The results that indicated higher bacterial production, CRAM formulas and C/N ratios in the surface waters suggest that most of the LDOM fractions are sequestered in surface waters by microbes producing RDOM. This agrees with the findings of Kaiser and Benner (2008), that bacteria consumed labile DOM and produced RDOM. The LDOM that is not microbially degraded in surface waters is metabolised in deeper waters, supporting reduced microbial communities along the water column.

Sometimes, RDOM molecules may be excreted by phytoplankton or released from zooplankton grazing, but these flows of RDOM are generally believed to be lower (Kaiser and Benner, 2008; Kragh and Søndergaard, 2009), than those of bacteria. This may in a way limit the ability to characterise the RDOM from these sources in the natural conditions. Virus-mediated lysis of bacteria may also be a major source of RDOM (Vadstein et al., 2012) as compounds such as peptidoglycan released from bacterial cell walls are known to be dominant and biologically inert (Kaiser and Benner, 2008). The bacterial production clearly shows that much of the bacterial activity was limited to the surface waters where the majority of the LDOM is used by heterotrophic bacteria producing RDOM (Ogawa et al., 2001).

The CRAM content was found to be similar at different depths, but this cannot be explained by bacterial RDOM production at the respective depths because the DOC in intermediate and deep water did not support high bacterial production rates. This suggests that the RDOM produced in surface waters are mixed and exported into deeper waters. Davis and Benner (2007), recorded a similar rapid transport of labile DOM fractions in the western Arctic Ocean. Romera-Castillo et al. (2019) has proposed RDOM export from surface waters as a result of deep convection, turbulent mixing or overturning as a principal means of RDOM transport to deeper waters.

From a holistic viewpoint, RDOM will reach deeper waters through the aforementioned physical oceanographic forcing, however, in both shallow and deep coastal waters with a prominent thermocline, RDOM transport to deeper water is facilitated by a series of other chemical and biological processes. It is known that CRAM in bacterial exometabolites, phytoplankton derived polysaccharides rich in transparent exopolymer particles (TEP) and several other DOM molecules, facilitate the aggregation and formation of marine gels and fast sinking particles such as marine snow that increase the export processes of bacteria and phytoplankton debris (Fig. 8) (Decho and Gutierrez, 2017; Engel et al., 2004; Jiao et al., 2010). The rate at which these gels or particles sink ranged from less than 1 m to more than 1000 m per day according to their size (Arístegui et al., 2009). The sedimentation rates of particulate organic carbon in the comparable Norwegian fjords and coastal locations, with predicted values ranging between 74 and 107 g C m^{-2} yr^{-1}, suggest that DOM export is important and that comparable DOM-composition may be found in the three depths (Faust and Knies, 2019; Wassmann, 1984). The formed aggregates, existing as a dissolved-particulate matter continuum, may easily disintegrate in the deeper layers with the release of both RDOM and LDOM into the ambient waters. The aggregate formation and dynamics of DOM-POM continuum also pose a challenge that needs further studies. The LDOM released in the deeper waters may sustain some bacterial production, however, the amount of LDOM that reaches deeper waters and affects BP is relatively low (Kattner et al., 2011). The amount may become enhanced where there is strong diel vertical migration of zooplankton and mesopelagic fish (Calleja et al., 2018).

The RDOM accumulated in the deeper layers is either deposited into the sediments or remains as dissolved RDOM in the water column for a long time, depending on the depths (Hansell and Carlson, 2013; Watanabe and Kuwae, 2015). These processes, from photosynthesis to grazing and the release of LDOM and remineralisation to RDOM, represent the ultimate sink of CO_2 from the atmosphere to deep water and ultimately to marine sediments (Fig. 8) (Longhurst, 1991). The RDOM in surface waters undergoes photodegradation (photochemical oxidation)

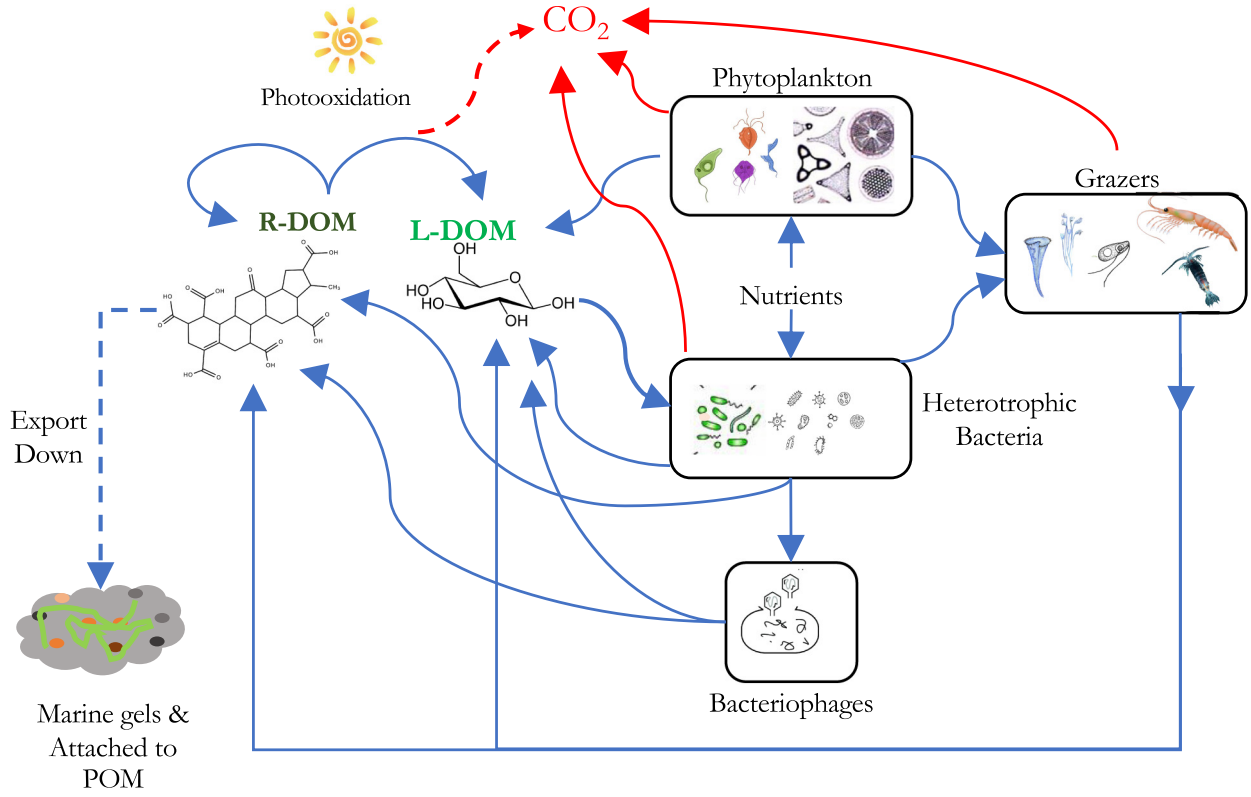


Fig. 8. Conceptual diagram showing LDOM and RDOM production, removal and export dynamics. Glucose is represented as LDOM and CRAM (Hertkorn et al., 2006) is represented as RDOM. The dashed lines denote the sink processes of carbon from RDOM.

and photoenhanced biodegradation processes as the spring seasons approaches, resulting in RDOM removal from the pool (Shen and Benner, 2018). The photochemical oxidation reactions are complex and probably produce other RDOM, LDOM, free radicals and toxic chemicals, acting as a CO_2 source for the atmosphere (Fig. 8).

5. Conclusion

Temperate coastal fjords are key ecosystems in DOM dynamics and in particular the formation of refractory DOM that may accumulate and reside for a long time in the sea, is largely mediated by heterotrophic microbes (Fig. 8). The DOM pool receives LDOM and RDOM from various production and consumption phases of phytoplankton, grazers, heterotrophic bacteria and bacteriophages. The DOM pool is significantly influenced by the seasons changes rather than differences in the water column depth. In sufficient nutrient concentrations, the surface bacterial communities rapidly sequester carbon as RDOM. The main sinks that regulate RDOM concentration may be export processes when adsorbed to particulate matter and marine gels, ending up in sediments, or photochemical oxidation in surface waters that may release carbon dioxide/monoxide back into atmosphere. The microbial communities in deep waters may metabolise any remaining traces and newly formed LDOM. With limited of LDOM as carbon source, the bacterial activity in deeper layers is reduced, compared to surface waters.

Considering the changing climate affecting marine environments, further research is needed for a better understanding of RDOM dynamics involving heterotrophic microbes and photo-oxidation, such as the action and importance of lysing activities by ubiquitous marine bacteriophages.

CRediT authorship contribution statement

K. Avarachen Mathew: Conceptualization, Methodology, Investigation, Formal analysis, Data analysis, Visualization, Writing - original draft preparation, Writing - Reviewing & Editing. **Murat Van Ardelan:** Conceptualization, Methodology, Investigation, Resources, Formal analysis, Supervision, Writing - Reviewing & Editing, Funding Acquisition. **Susana Villa Gonzalez:** Methodology, Formal analysis, Writing - Reviewing. **Olav Vadstein:** Conceptualization, Methodology, Investigation, Resources, Formal analysis, Supervision, Writing - Reviewing. **Vezhapparambu Veena S:** Data analysis, Visualization, Writing - Reviewing & Editing. **Øystein Leiknes:** Investigation, Formal analysis, Writing - Reviewing. **Rahman Mankettikkara:** Data analysis, Visualization, Writing - Reviewing & Editing. **Yngvar Olsen:** Conceptualization, Methodology, Investigation, Resources, Formal analysis, Supervision, Writing - Reviewing & Editing, Funding Acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The research project was supported by European Commission (Ocean Certain, FP7-ENV-2013-6.1-1; grant no: 603773) and Norwegian University of Science and Technology (NTNU). We thank the crew members from R/V Gunnerus for helping us in the sample collection, Kjersti Andresen and Siv Anina Etter, engineers in Department of Biology together with engineers in Department of Biotechnology and Food Science at NTNU for their help in sample analysis. We acknowledge the support from Professor Ingrid Bakken, Department of Biotechnology and Food Science at NTNU for helping in data submission to ENA. We thank Femi Anna Thomas, National Centre for Polar and Ocean Research, India for her helpful insights on the microbial community composition. We are grateful to Tale Skrove and Lone Sunniva Jevne and Tomasz Maciej Ciesielski from Department of Biology, NTNU for help in smooth

operations in R and making graphs and figures respectively. We are thankful to Maria Guadalupe Digernes, Department of Chemistry, NTNU for her support and suggestions for the work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.146402.

References

Amon, R.M.W., 2016. Ocean dissolved organics matter. Nat. Geosci. 9, 864–865.

Arístegui, J., Gasol, J.M., Duarte, C.M., Herndld, G.J., 2009. Microbial oceanography of the dark ocean's pelagic realm. Limnol. Oceanogr. 54, 1501–1529.

Arnosti, C., 2011. Microbial extracellular enzymes and the marine carbon cycle. Annu. Rev. Mar. Sci. 3, 401–425.

Avneri-Katz, S., Young, R.B., McKenna, A.M., Chen, H., Corilo, Y.E., Polubesova, T., Borch, T., Chefetz, B, 2017. Adsorptive fractionation of dissolved organic matter (DOM) by mineral soil: Macroscale approach and molecular insight. Org. Geochem. 103, 113–124. https://doi.org/10.1016/j.orggeochem.2016.11.004.

Bae, E., Yeo, I.J., Jeong, B., Shin, Y., Shin, K.-H., Kim, S., 2011. Study of double bond equivalents and the numbers of carbon and oxygen atom distribution of dissolved organic matter with negative-mode FT-ICR MS. Anal. Chem. 83, 4193–4199.

Banerjee, S., Mazumdar, S., 2012. Electrospray ionization mass spectrometry: a technique to access the information beyond the molecular weight of the analyte. International Journal of Analytical Chemistry 2012, 282574.

Barrón, C., Duarte, C.M., 2015. Dissolved organic carbon pools and export from the coastal ocean. Glob. Biogeochem. Cycles 29, 1725–1738.

Bauer, J.E., Druffel, E.R.M., 1998. Ocean margins as a significant source of organic matter to the deep open ocean. Nature 392, 482–485.

Behrenfeld, M.J., Falkowski, P.G., 1997. Photosynthetic rates derived from satellite-based chlorophyll concentration. Limnol. Oceanogr. 42, 1–20.

Behrenfeld, M.J., O'Malley, R.T., Siegel, D.A., McClain, C.R., Sarmiento, J.L., Feldman, G.C., Milligan, A.J., Falkowski, P.G., Letelier, R.M., Boss, E.S., 2006. Climate-driven trends in contemporary ocean productivity. Nature 444, 752–755.

Bell, R.T., 1993. Estimating production of heterotrophic bacterioplankton via incorporation of tritiated thymidine. Handbook of methods in aquatic microbial ecology 45 (6), 1709–1721.

Bienhold, C., Zinger, L., Boetius, A., Ramette, A., 2016. Diversity and biogeography of bathyal and abyssal seafloor bacteria. PLoS One 11, e0148016.

Biersmith, A., Benner, R., 1998. Carbohydrates in phytoplankton and freshly produced dissolved organic matter. Mar. Chem. 63, 131–144.

Børsheim, K.Y., Vadstein, O., Myklestad, S.M., Reinertsen, H., Kirkvold, S., Olsen, Y., 2005. Photosynthetic algal production, accumulation and release of phytoplankton storage carbohydrates and bacterial production in a gradient in daily nutrient supply. J. Plankton Res. 27, 743–755.

Brooke, S., Järnegren, J., 2013. Reproductive periodicity of the scleractinian coral Lophelia pertusa from the Trondheim Fjord, Norway. Mar. Biol. 160, 139–153.

Brown, M.V., Philip, G.K., Bunge, J.A., Smith, M.C., Bissett, A., Lauro, F.M., Fuhrman, J.A., Donachie, S.P., 2009. Microbial community structure in the North Pacific ocean. The ISME Journal 3, 1374–1386.

Calleja, M.L., Ansari, M.I., Røstad, A., Silva, L., Kaartvedt, S., Irigoien, X., Morán, X.A.G., 2018. The mesopelagic scattering layer: a hotspot for heterotrophic prokaryotes in the Red Sea twilight zone. Front. Mar. Sci. 5.

Catalá, T.S., Reche, I., Fuentes-Lema, A., Romera-Castillo, C., Nieto-Cid, M., Ortega-Retuerta, E., Calvo, E., Álvarez, M., Marrasé, C., Stedmon, C.A., Álvarez-Salgado, X.A., 2015. Turnover time of fluorescent dissolved organic matter in the dark global ocean. Nat. Commun. 6, 5986.

Chin, W.-C., Orellana, M.V., Verdugo, P., 1998. Spontaneous assembly of marine dissolved organic matter into polymer gels. Nature 391, 568–572.

Corilo Y. PetroOrg Software. Florida State University; All Rights reserved. http://www.petroorg.com 2014.

Cram, J.A., Chow, C.-E.T., Sachdeva, R., Needham, D.M., Parada, A.E., Steele, J.A., Fuhrman, J.A., 2015. Seasonal and interannual variability of the marine bacterioplankton community throughout the water column over ten years. The ISME Journal 9, 563–580.

D'Andrilli, J., Dittmar, T., Koch, B.P., Purcell, J.M., Marshall, A.G., Cooper, W.T., 2010. Comprehensive characterization of marine dissolved organic matter by Fourier transform ion cyclotron resonance mass spectrometry with electrospray and atmospheric pressure photoionization. Rapid Commun. Mass Spectrom. 24, 643–650.

Davis, J., Benner, R., 2007. Quantitative estimates of labile and semi-labile dissolved organic carbon in the western Arctic Ocean: a molecular approach. Limnol. Oceanogr. 52, 2434–2444.

Davis, C.E., Blackbird, S., Wolff, G., Woodward, M., Mahaffey, C., 2019. Seasonal organic matter dynamics in a temperate shelf sea. Prog. Oceanogr. 177, 101925.

Decho, A.W., Gutierrez, T., 2017. Microbial Extracellular Polymeric Substances (EPSs) in ocean systems. Front. Microbiol. 8.

Dittmar, T., Koch, B., Hertkorn, N., Kattner, G., 2008. A simple and efficient method for the solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater. Limnol. Oceanogr. Methods 6, 230–235.

Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat. Methods 10 (10), 996–998. https://doi.org/10.1038/nmeth.2604.

Ellingsen, I.H., 2004. Internal Tides and the Spread of River Plumes in the Trondheim Fjord.

Engel, A., Thoms, S., Riebesell, U., Rochelle-Newall, E., Zondervan, I., 2004. Polysaccharide aggregation as a potential sink of marine dissolved organic carbon. Nature 428, 929–932.

Farenc, M., Corilo, Y.E., Lalli, P.M., Riches, E., Rodgers, R.P., Afonso, C., Giusti, P., 2016. Comparison of atmospheric pressure ionization for the analysis of heavy petroleum fractions with ion mobility-mass spectrometry. Energy Fuel 30, 8896–8903.

Faust, J.C., Knies, J., 2019. Organic matter sources in North Atlantic Fjord sediments. Geochem. Geophys. Geosyst. 20, 2872–2885.

Garneau, M.-È., Roy, S., Lovejoy, C., Gratton, Y., Vincent, W.F., 2008. Seasonal dynamics of bacterial biomass and production in a coastal arctic ecosystem: Franklin Bay, western Canadian Arctic. Journal of Geophysical Research: Oceans 113.

Gilbert, J.A., Steele, J.A., Caporaso, J.G., Steinbrück, L., Reeder, J., Temperton, B., Huse, S., McHardy, A.C., Knight, R., Joint, I., Somerfield, P., Fuhrman, J.A., Field, D., 2012. Defining seasonal marine microbial community dynamics. The ISME Journal 6, 298–308.

del Giorgio, P.A., Duarte, C.M., 2002. Respiration in the open ocean. Nature 420, 379–384.

Grasshoff, K., Kremling, K., Ehrhardt, M., 2009. Methods of Seawater Analysis. John Wiley & Sons.

Grimm, F.A., Russell, W.K., Luo, Y.-S., Iwata, Y., Chiu, W.A., Roy, T., Boogaard, P.J., Ketelslegers, H.B., Rusyn, I., 2017. Grouping of petroleum substances as example UVCBs by ion mobility-mass spectrometry to enable chemical composition-based read-across. Environmental Science & Technology 51, 7197–7207.

Gruber, D.F., Simjouw, J.-P., Seitzinger, S.P., Taghon, G.L., 2006. Dynamics and characterization of refractory dissolved organic matter produced by a pure bacterial culture in an experimental predator-prey system. Appl. Environ. Microbiol. 72, 4184–4191.

Hansell, D.A., 2013. Recalcitrant dissolved organic carbon fractions. Annu. Rev. Mar. Sci. 5, 421–445.

Hansell, D.A., Carlson, C.A., 2013. Localized refractory dissolved organic carbon sinks in the deep ocean. Glob. Biogeochem. Cycles 27, 705–710.

He, W., Chen, M., Schlautman, M.A., Hur, J., 2016. Dynamic exchanges between DOM and POM pools in coastal and inland aquatic ecosystems: a review. Sci. Total Environ. 551-552, 415–428.

Hertkorn, N., Benner, R., Frommberger, M., Schmitt-Kopplin, P., Witt, M., Kaiser, K., Kettrup, A., Hedges, J.I., 2006. Characterization of a major refractory component of marine dissolved organic matter. Geochim. Cosmochim. Acta 70, 2990–3010.

Hopkinson, C.S., Vallino, J.J., 2005. Efficient export of carbon to the deep ocean through dissolved organic matter. Nature 433, 142–145.

Jiao, N., Azam, F., 2011. Microbial carbon pump and its significance for carbon sequestration in the ocean. Microbial Carbon Pump in the Ocean 43–45.

Jiao, N., Zheng, Q., 2011. The microbial carbon pump: from genes to ecosystems. Appl. Environ. Microbiol. 77, 7439–7444.

Jiao, N., Herndl, G.J., Hansell, D.A., Benner, R., Kattner, G., Wilhelm, S.W., Kirchman, D.L., Weinbauer, M.G., Luo, T., Chen, F., Azam, F., 2010. Microbial production of recalcitrant dissolved organic matter: long-term carbon storage in the global ocean. Nat. Rev. Microbiol. 8, 593.

Jiao, N., Robinson, C., Azam, F., Thomas, H., Baltar, F., Dang, H., Hardman-Mountford, N., Johnson, M., Kirchman, D., Koch, B., 2014. Mechanisms of microbial carbon sequestration in the ocean–future research directions. Biogeosciences 11, 5285–5306.

Jiao, N., Cai, R., Zheng, Q., Tang, K., Liu, J., Jiao, F., Wallace, D., Chen, F., Li, C., Amann, R., Benner, R., Azam, F., 2018. Unveiling the enigma of refractory carbon in the ocean. Natl. Sci. Rev. 5, 459–463.

Jørgensen, L., Stedmon, C.A., Kragh, T., Markager, S., Middelboe, M., Søndergaard, M., 2011. Global trends in the fluorescence characteristics and distribution of marine dissolved organic matter. Mar. Chem. 126, 139–148.

Kaiser, K., Benner, R., 2008. Major bacterial contribution to the ocean reservoir of detrital organic carbon and nitrogen. Limnol. Oceanogr. 53, 99–112.

Karl, D.M., Knauer, G.A., 1991. Microbial production and particle flux in the upper 350 m of the Black Sea. Deep Sea Research Part A. Oceanographic Research Papers 38, S921–S942.

Kattner, G., Simon, M., Koch, B., 2011. Molecular Characterization of Dissolved Organic Matter and Constraints for Prokaryotic Utilization. Science/AAAS, Washington, DC.

Kirchman, D.L., Morán, X.A.G., Ducklow, H., 2009. Microbial growth in the polar oceans—role of temperature and potential impact of climate change. Nat. Rev. Microbiol. 7, 451–459.

Koch, B.P., Dittmar, T., 2006. From mass to structure: an aromaticity index for high-resolution mass data of natural organic matter. Rapid Commun. Mass Spectrom. 20, 926–932.

Koch, B.P., Kattner, G., Witt, M., Passow, U., 2014. Molecular insights into the microbial formation of marine dissolved organic matter: recalcitrant or labile? Biogeosciences 11, 4173–4190.

Kragh, T., Søndergaard, M., 2009. Production and decomposition of new DOC by marine plankton communities: carbohydrates, refractory components and nutrient limitation. Biogeochemistry 96, 177–187.

Kujawinski, E.B., 2002. Electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI FT-ICR MS): characterization of complex environmental mixtures. Environ. Forensic 3, 207–216.

Kwiatkowski, L., Bopp, L., Aumont, O., Ciais, P., Cox, P.M., Laufkötter, C., Li, Y., Séférian, R., 2017. Emergent constraints on projections of declining primary production in the tropical oceans. Nat. Clim. Chang. 7, 355–358.

Landa, M., Blain, S., Christaki, U., Monchy, S., Obernosterer, I., 2016. Shifts in bacterial community composition associated with increased carbon cycling in a mosaic of phytoplankton blooms. The ISME Journal 10, 39–50.

Lechtenfeld, O.J., Hertkorn, N., Shen, Y., Witt, M., Benner, R., 2015. Marine sequestration of carbon in bacterial metabolites. Nat. Commun. 6, 6711.

Legendre, P., Gallagher, E.D., 2001. Ecologically meaningful transformations for ordination of species data. Oecologia 129, 271–280.

Legendre, L., Rivkin, R.B., Weinbauer, M.G., Guidi, L., Uitz, J., 2015. The microbial carbon pump concept: potential biogeochemical significance in the globally changing ocean. Prog. Oceanogr. 134, 432–450.
Letourneau, M.L., Medeiros, P.M., 2019. Dissolved organic matter composition in a marsh-dominated estuary: Response to seasonal forcing and to the passage of a hurricane. J. Geophys. Res. Biogeosci. 124 (6), 1545–1559. https://doi.org/10.1029/2018JG004982.
Lønborg, C., Álvarez-Salgado, X.A., 2012. Recycling versus export of bioavailable dissolved organic matter in the coastal ocean and efficiency of the continental shelf pump. Glob. Biogeochem. Cycles 26.
Longhurst, A.R., 1991. Role of the marine biosphere in the global carbon cycle. Limnol. Oceanogr. 36, 1507–1526.
Longhurst, A., Sathyendranath, S., Platt, T., Caverhill, C., 1995. An estimate of global primary production in the ocean from satellite radiometer data. J. Plankton Res. 17, 1245–1271.
McCarren, J., Becker, J.W., Repeta, D.J., Shi, Y., Young, C.R., Malmstrom, R.R., Chisholm, S.W., DeLong, E.F., 2010. Microbial community transcriptomes reveal microbes and metabolic pathways associated with dissolved organic matter turnover in the sea. Proc. Natl. Acad. Sci. 107, 16420.
McDonough, L.K., O'Carroll, D.M., Meredith, K., Andersen, M.S., Brügger, C., et al., 2020. Changes in groundwater dissolved organic matter character in a coastal sand aquifer due to rainfall recharge. Water Res. 169, 115201. https://doi.org/10.1016/j.watres.2019.115201.
Miller, L.A., Chierici, M., Johannessen, T., Noji, T.T., Rey, F., Skjelvan, I., 1999. Seasonal dissolved inorganic carbon variations in the Greenland Sea and implications for atmospheric CO2 exchange. Deep-Sea Res. II Top. Stud. Oceanogr. 46, 1473–1496.
Milzer, G., Giraudeau, J., Faust, J., Knies, J., Eynaud, F., Rühlemann, C., 2013. Spatial distribution of benthic foraminiferal stable isotopes and dinocyst assemblages in surface sediments of the Trondheimsfjord, central Norway. Biogeosciences 10, 4433–4448.
Navada, S., Sebastianpillai, M., Kolarevic, J., Fossmark, R.O., Tveten, A.-K., Gaumet, F., Mikkelsen, Ø., Vadstein, O., 2020. A salty start: brackish water start-up as a microbial management strategy for nitrifying bioreactors with variable salinity. Sci. Total Environ. 739, 139934.
Ogawa, H., Tanoue, E., 2003. Dissolved organic matter in oceanic waters. J. Oceanogr. 59, 129–147.
Ogawa, H., Amagai, Y., Koike, I., Kaiser, K., Benner, R., 2001. Production of refractory dissolved organic matter by bacteria. Science 292, 917–920.
Ohno, T., Sleighter, R.L., Hatcher, P.G., 2016. Comparative study of organic matter chemical characterization using negative and positive mode electrospray ionization ultrahigh-resolution mass spectrometry. Anal. Bioanal. Chem. 408, 2497–2504.
Olsen, Y., Agustí, S., Andersen, T., Duarte, C.M., Gasol, J.M., Gismervik, I., Heiskanen, A.-S., Hoell, E., Kuuppo, P., Lignell, R., Reinertsen, H., Sommer, U., Stibor, H., Tamminen, T., Vadstein, O., Vaqué, D., Vidal, M., 2006. A comparative study of responses in plankton food web structure and function in contrasting European coastal waters exposed to experimental nutrient addition. Limnol. Oceanogr. 51, 488–503.
Olsen, Y., Andersen, T., Gismervik, I., Vadstein, O., 2007. Protozoan and metazoan zooplankton-mediated carbon flows in nutrient-enriched coastal planktonic communities. Mar. Ecol. Prog. Ser. 331, 67–83.
Olsen, Y., Andersen, T., Gismervik, I., Vadstein, O., 2011. Marine heterotrophic bacteria, protozoan and metazoan zooplankton may experience protein N or mineral P limitation in coastal waters. Mar. Ecol. Prog. Ser. 436, 81–100.
Omori, Y., Hama, T., Ishii, M., Saito, S., 2011. Vertical change in the composition of marine humic-like fluorescent dissolved organic matter in the subtropical western North Pacific and its relation to photoreactivity. Mar. Chem. 124, 38–47.
Öztürk, M., Steinnes, E., Sakshaug, E., 2002. Iron speciation in the Trondheim Fjord from the perspective of iron limitation for phytoplankton. Estuar. Coast. Shelf Sci. 55, 197–212.
Persson, L., Alsberg, T., Ledin, A., Odham, G., 2006. Transformations of dissolved organic matter in a landfill leachate—a size exclusion chromatography/mass spectrometric approach. Chemosphere 64, 1093–1099.
Pomeroy, L.R., Wiebe, W.J., 2001. Temperature and substrates as interactive limiting factors for marine heterotrophic bacteria. Aquat. Microb. Ecol. 23, 187–204.
Pracht, L.E., Tfaily, M.M., Ardissono, R.J., Neumann, R.B., 2018. Molecular characterization of organic matter mobilized from Bangladeshi aquifer sediment: tracking carbon compositional change during microbial utilization. Biogeosciences 15, 1733–1747.
Reigstad, M., Wassmann, P., Wexels Riser, C., Øygarden, S., Rey, F., 2002. Variations in hydrography, nutrients and chlorophyll a in the marginal ice-zone and the central Barents Sea. J. Mar. Syst. 38, 9–29.
Romera-Castillo, C., Álvarez, M., Pelegrí, J.L., Hansell, D.A., Álvarez-Salgado, X.A., 2019. Net additions of recalcitrant dissolved organic carbon in the deep Atlantic Ocean. Glob. Biogeochem. Cycles 33, 1162–1173.
Ryther, J.H., 1969. Photosynthesis and fish production in the sea. Science 166, 72.
Šantl-Temkiv, T., Finster, K., Dittmar, T., Hansen, B.M., Thyrhaug, R., Nielsen, N.W., Karlson, U.G., 2013. Hailstones: a window into the microbial and chemical inventory of a storm cloud. PLoS One 8, e53550.
Seitzinger, S.P., Hartnett, H., Lauck, R., Mazurek, M., Minegishi, T., Spyres, G., Styles, R., 2005. Molecular-level chemical characterization and bioavailability of dissolved organic matter in stream water using electrospray-ionization mass spectrometry. Limnol. Oceanogr. 50, 1–12.
Shah Walter SR, Jaekel U, Osterholz H, Fisher AT, Huber JA, Pearson A, Dittmar T, Girguis PR. Microbial decomposition of marine dissolved organic matter in cool oceanic crust. Nature Geoscience 2018; 11: 334-339.
Shen Y, Benner R. Mixing it up in the ocean carbon cycle and the removal of refractory dissolved organic carbon. Scientific Reports 2018; 8: 2542.
Simon, C., Roth, V.-N., Dittmar, T., Gleixner, G., 2018. Molecular signals of heterogeneous terrestrial environments identified in dissolved organic matter: a comparative analysis of orbitrap and ion cyclotron resonance mass spectrometers. Front. Earth Sci. 6.
Smith, P.B., Snyder, A.P., Harden, C.S., 1995. Characterization of bacterial phospholipids by electrospray ionization tandem mass spectrometry. Anal. Chem. 67, 1824–1830.
Søndergaard, M., Middelboe, M., 1995. A cross-system analysis of labile dissolved organic carbon. Mar. Ecol. Prog. Ser. 118, 283–294.
Stramska, M., 2009. Particulate organic carbon in the global ocean derived from SeaWiFS ocean color. Deep-Sea Res. I Oceanogr. Res. Pap. 56, 1459–1470.
Strickland, J.D.H., Parsons, T.R., 1972. A Practical Handbook of Seawater Analysis.
Stubbins, A., Spencer, R.G.M., Chen, H., Hatcher, P.G., Mopper, K., Hernes, P.J., Mwamba, V.L., Mangangu, A.M., Wabakanghanzi, J.N., Six, J., 2010. Illuminated darkness: molecular signatures of Congo River dissolved organic matter and its photochemical alteration as revealed by ultrahigh precision mass spectrometry. Limnol. Oceanogr. 55, 1467–1477.
Suh, S.-S., Park, M., Hwang, J., Kil, E.-J., Jung, S.W., Lee, S., Lee, T.-K., 2015. Seasonal dynamics of marine microbial community in the south sea of Korea. PLoS One 10 (6), e.0131633. https://doi.org/10.1371/journal.pone.0131633.
Tamburini, C., Garcin, J., Ragot, M., Bianchi, A., 2002. Biopolymer hydrolysis and bacterial production under ambient hydrostatic pressure through a 2000m water column in the NW Mediterranean. Deep-Sea Res. II Top. Stud. Oceanogr. 49, 2109–2123.
Taucher, J., Oschlies, A., 2011. Can we predict the direction of marine primary production change under global warming? Geophys. Res. Lett. 38.
Taylor, J.R., Ferrari, R., 2011. Ocean fronts trigger high latitude phytoplankton blooms. Geophys. Res. Lett. 38.
Thingstad, T.F., HagstrÖm, Å., Rassoulzadegan, F., 1997. Accumulation of degradable DOC in surface waters: is it caused by a malfunctioning microbialloop? Limnol. Oceanogr. 42, 398–404.
Treusch, A.H., Vergin, K.L., Finlay, L.A., Donatz, M.G., Burton, R.M., Carlson, C.A., Giovannoni, S.J., 2009. Seasonality and vertical structure of microbial communities in an ocean gyre. The ISME Journal 3, 1148–1163.
Vadstein, O., Andersen, T., Reinertsen, H.R., Olsen, Y., 2012. Carbon, nitrogen and phosphorus resource supply and utilisation for coastal planktonic heterotrophic bacteria in a gradient of nutrient loading. Mar. Ecol. Prog. Ser. 447, 55–75.
Verdugo, P., Alldredge, A.L., Azam, F., Kirchman, D.L., Passow, U., Santschi, P.H., 2004. The oceanic gel phase: a bridge in the DOM–POM continuum. Mar. Chem. 92, 67–85.
Wagner, S., Fair, J.H., Matt, S., Hosen, J.D., Raymond, P., Saiers, J., Shanley, J.B., Dittmar, T., Stubbins, A., 2019. Molecular hysteresis: hydrologically driven changes in riverine dissolved organic matter chemistry during a storm event. Journal of Geophysical Research: Biogeosciences 124, 759–774.
Wagner S, Schubotz F, Kaiser K, Hallmann C, Waska H, Rossel PE, Hansman R, Elvert M, Middelburg JJ, Engel A, Blattmann TM, Catalá TS, Lennartz ST, Gomez-Saez GV, Pantoja-Gutiérrez S, Bao R, Galy V. Soothsaying DOM: A current perspective on the future of oceanic dissolved organic carbon. Front. Mar. Sci. 2020; 7.
Walker Brett, D., Beaupré Steven, R., Guilderson Thomas, P., McCarthy Matthew, D., Druffel Ellen, R.M., 2016. Pacific carbon cycling constrained by organic matter size, age and composition relationships. Nat. Geosci. 9, 888–891.
Walsh, E.A., Kirkpatrick, J.B., Rutherford, S.D., Smith, D.C., Sogin, M., D'Hondt, S., 2016. Bacterial diversity and community composition from seasurface to subseafloor. The ISME Journal 10, 979–989.
Wassmann, P., 1984. Sedimentation and benthic mineralization of organic detritus in a Norwegian fjord. Mar. Biol. 83, 83–94.
Wassmann P. Eutrophication, primary production and vertical export. In: P. Wassmann KOE, editor. Drainage Basin Nutrients and Eutrophycation: An Integrated Approach, University of Tromsø, Norway, 2005, pp. 126–138.
Watanabe, K., Kuwae, T., 2015. How organic carbon derived from multiple sources contributes to carbon sequestration processes in a shallow coastal system? Glob. Chang. Biol. 21, 2612–2623.
Wickham H. ggplot2: Elegant graphics for data analysis: Springer, 2016.
Wilson, B., Müller, O., Nordmann, E.-L., Seuthe, L., Bratbak, G., Øvreås, L., 2017. Changes in marine prokaryote composition with season and depth over an arctic polar year. Front. Mar. Sci. 4.
Yamashita, Y., Tanoue, E., 2008. Production of bio-refractory fluorescent dissolved organic matter in the ocean interior. Nat. Geosci. 1, 579–582.
Yamashita, Y., Tanoue, E., 2009. Basin scale distribution of chromophoric dissolved organic matter in the Pacific Ocean. Limnol. Oceanogr. 54, 598–609.
Zhang, C., Dang, H., Azam, F., Benner, R., Legendre, L., Passow, U., Polimene, L., Robinson, C., Suttle, C.A., Jiao, N., 2018. Evolving paradigms in biological carbon cycling in the ocean. Natl. Sci. Rev. 5, 481–499.