

5-12-2020

Molecular Properties Are a Primary Control on the Microbial Utilization of Dissolved Organic Matter in the Ocean

Yuan Shen

Ronald Benner

University of South Carolina - Columbia, benner@mailbox.sc.edu

Follow this and additional works at: https://scholarcommons.sc.edu/geol_facpub



Part of the [Biology Commons](#), and the [Oceanography and Atmospheric Sciences and Meteorology Commons](#)

Publication Info

Published in *Limnology and Oceanography*, Volume 65, 2020, pages 1061-1071.

This Article is brought to you by the Earth, Ocean and Environment, School of the at Scholar Commons. It has been accepted for inclusion in Faculty Publications by an authorized administrator of Scholar Commons. For more information, please contact digres@mailbox.sc.edu.

Molecular properties are a primary control on the microbial utilization of dissolved organic matter in the ocean

Yuan Shen^{1,2*}, Ronald Benner^{1,3*}

¹Marine Science Program, University of South Carolina, Columbia, South Carolina

²Ocean Sciences Department, University of California, Santa Cruz, California

³School of the Earth, Ocean and Environment, University of South Carolina, Columbia, South Carolina

Abstract

The global ocean sequesters a large amount of reduced carbon in dissolved organic molecules that can persist for centuries to millennia. The persistence of dissolved organic carbon (DOC) in the deep ocean has been attributed to inherently refractory molecules and to low concentrations of molecules, but the relative roles of molecular properties and molecular concentrations remain uncertain. We investigate both of these possibilities using bioassay experiments with unfiltered seawater collected from five depths (50–1500 m) at the Bermuda Atlantic Time-Series Study site. The microbial utilization of compositionally distinct forms of seawater DOC at in situ and elevated concentrations was determined. Microbial utilization of in situ organic carbon ranged from 6% to 7% in surface waters to 0% in deep water after 180 d. Additions of surface plankton-derived DOC ($\sim 18 \mu\text{mol L}^{-1}$), which was enriched in amino acids and carbohydrates, revealed substantial (50–75%) removal of the added DOC at all depths within 7 d. In sharp contrast, additions of C-18 isolated deep-sea DOC ($\sim 20 \mu\text{mol L}^{-1}$) showed insignificant or minimal utilization at all depths after 7 or 180 d, even when primed with labile substrates. These experiments demonstrate microbial communities from varying depths and environments in the ocean could rapidly utilize elevated concentrations of plankton-derived DOC, whereas these same microbes failed to utilize elevated concentrations of C-18 DOC. These results indicate molecular properties are the primary control on the microbial utilization of DOC in the ocean. Our findings imply a dynamic DOC reservoir with a flexible capacity for carbon sequestration in the global ocean.

The ocean stores a large quantity of nonliving organic carbon (670 Pg), and most of this carbon resides in small dissolved molecules (<1 kDa) that persist in the ocean for several thousand years (Williams and Druffel 1987; Ogawa and Tanoue 2003; Benner and Amon 2015). This large reservoir of long-lived dissolved organic carbon (DOC) plays an important role in carbon sequestration and the regulation of global climate, but the mechanisms controlling its persistence and fate remain enigmatic (Jiao et al. 2014; Carlson and Hansell 2015; Dittmar 2015). Based on early observations that concentrations of DOC are low and relatively constant throughout the deep ocean, two different, but not exclusive, ideas were conceived to explain the persistence of DOC in the ocean. Dissolved organic molecules could persist at threshold concentrations needed to support energy-efficient bacterial growth, a concept known as the dilution hypothesis (Jannasch 1967; Jannasch 1994). Barber (1968)

conducted the first test of the dilution hypothesis using seawater bioassay experiments (30–60 d) with elevated concentrations of deep ocean DOC. His observation that elevated concentrations of deep ocean DOC were resistant to microbial degradation led to the long-standing paradigm that the persistence of DOC in the deep ocean is due to the predominance of inherently refractory molecules.

The study by Arrieta et al. (2015) retested the dilution hypothesis using seawater bioassay experiments with additions of concentrated DOC extracted from deep ocean waters. Bacterial growth was observed in bioassay experiments (10–40 d) with added DOC, leading the authors to conclude that dilution is a primary control on the microbial utilization and storage of DOC in the deep ocean (Arrieta et al. 2015). This study challenges the paradigm that inherently refractory molecules dominate the large DOC reservoir in the deep ocean, and it has sparked debate about controls on the ocean carbon cycle (Jiao et al. 2015; Middelburg 2015; Wang et al. 2018). In contrast, a recent study using a different bioassay approach with carbon-free artificial seawater and concentrated DOC extracted from varying depths in the ocean found the concentrated DOC was not utilized (60–146 d) by native

*Correspondence: yuan187@gmail.com, (Y.S.); benner@mailbox.sc.edu (R.B.)

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

microbes from surface, mesopelagic, or deep waters (Shen and Benner 2018). The bioassay studies by Barber (1968), Arrieta et al. (2015), and Shen and Benner (2018) have some important differences in approach, but overall they suggest the coexistence of inherently refractory molecules in the ocean and molecules that are too dilute for microbial utilization. To what extent does each of these factors control the microbial utilization of DOC in the ocean?

The present study uses bioassay experiments with unfiltered seawater and different sources of concentrated DOC to further elucidate mechanisms controlling the microbial utilization of DOC in the ocean. Surface, mesopelagic, and deep waters were collected from the Bermuda Atlantic Time-Series Study (BATS) site to examine the utilization of organic carbon at natural and elevated concentrations by different microbial communities under a wide range of in situ nutrient conditions. The experiments were amended with plankton-derived DOC from surface water or C-18 extracted DOC from deep water to compare microbial responses to compositionally distinct forms of DOC. In addition, combined additions of plankton-derived DOC and C-18 extracted DOC were used to examine the role of priming in the microbial utilization of refractory DOC (Guenet et al. 2010). This study demonstrates the dominant role of molecular properties in controlling the microbial utilization of DOC in the ocean.

Materials and methods

Sample collection and onboard bioassay experiments

Seawater samples were collected on the R/V *Atlantic Explorer* in May 2016 at the BATS site. A rosette sampler equipped with 10-L Niskin bottles and a Conductivity-Temperature-Depth (CTD) instrument was used for water collection. Samples for depth profile analyses of total organic carbon (TOC) and total nitrogen (TN) were collected from 15 discrete depths (50, 100, 150, 200, 250, 300, 400, 500, 600, 800, 1000, 1350, 2000, 3250, and 4300 m). Unfiltered waters were drained directly from Niskin bottles into acid-cleaned 60 ml HDPE bottles and immediately frozen (-20°C) until analysis.

Additional unfiltered samples were collected for bioassay experiments from surface (50 m, 100 m), mesopelagic (300 m, 750 m), and deep (1500 m) waters. The microbial bioassay experiments were set up onboard within 30 min of seawater collection. Water samples from each depth were divided into an unamended control group and three amended groups that received $17\text{--}20\text{ }\mu\text{mol DOC L}^{-1}$ of plankton-derived dissolved organic matter (pDOM), $19\text{--}23\text{ }\mu\text{mol DOC L}^{-1}$ of C-18 isolated deep-sea DOM (C-18 DOM) and a combination of pDOM and C-18 DOM ($39\text{--}40\text{ }\mu\text{mol DOC L}^{-1}$), respectively. The amounts of pDOM or/and C-18 DOM added in each treatment were calculated as the net difference between total DOC concentrations determined prior to and after the substrate additions. All experiments were conducted in triplicate (40 mL amber glass bottles)

at room temperature ($21\text{--}24^{\circ}\text{C}$) in the dark for 180 d. Subsamples for DOC analysis were taken on days 0, 7, and 180.

The pDOM was collected previously by filtering (GF/F $0.7\text{ }\mu\text{m}$) the contents of a plankton tow ($50\text{-}\mu\text{m}$ mesh) in a diatom bloom. This filtration does not removal all bacteria, but the small volume additions ($\sim 100\text{ }\mu\text{L}$) of pDOM to incubations would have a minimal impact on microbial abundance. The filtered pDOM was stored frozen (-20°C) for future use. The pDOM was rich in amino acids and neutral sugars, and its rapid microbial utilization in previous bioassay experiments indicates the pDOM is a labile form of DOM (Shen et al. 2016b). The C-18 DOM was collected from a depth of 1200 m during a previous cruise in the north Atlantic Ocean, as described in Shen and Benner (2018). In brief, 80 L of seawater was filtered ($0.2\text{ }\mu\text{m}$ Nuclepore polycarbonate cartridge), acidified to pH 2.5, and passed through C-18 cartridges (Agilent Bond Elut; 10 g), followed by elution with methanol. The extracted DOM was dried under N_2 , redissolved in carbon-free artificial seawater, and filtered (GF/F $0.7\text{ }\mu\text{m}$) before storing at -20°C for future use. Previous microbial bioassay experiments indicated C-18 DOM is very resistant to microbial utilization and is considered refractory DOM (Shen and Benner 2018). Immediately prior to use, the frozen pDOM and C-18 DOM samples were thawed and added ($50\text{--}100\text{ }\mu\text{L}$) to the bioassay incubations.

The pDOM and C-18 DOM are representative of natural marine forms of labile and refractory DOM that differ in molecular properties. The concentrations of labile DOM in seawater are generally low and variable, with surface water concentrations typically accounting for $<5\text{ }\mu\text{mol L}^{-1}$ DOC and lower concentrations in deep water (Shen and Benner 2018). Therefore, the added pDOM ($\sim 18\text{ }\mu\text{mol DOC L}^{-1}$) increased the in situ concentrations of labile DOC by a factor of three or more in the bioassay experiments. The C-18 extraction recovered 30% ($14\text{ }\mu\text{mol DOC L}^{-1}$) of the DOC from deep water ($48\text{ }\mu\text{mol DOC L}^{-1}$; Shen and Benner 2018). Solid phase extraction methods, such as the silica-based C-18 extraction used herein, selectively concentrate molecules based on their molecular properties. Given a 30% extraction efficiency (Shen and Benner 2018), in situ concentrations of C-18 extractable DOC at BATS would range from $\sim 21\text{ }\mu\text{mol DOC L}^{-1}$ in the surface to $\sim 14\text{ }\mu\text{mol DOC L}^{-1}$ in the deep water. Therefore, the added C-18 DOM ($\sim 20\text{ }\mu\text{mol DOC L}^{-1}$) in our experiments approximately doubled the ambient concentrations of C-18 extractable molecules (i.e., refractory DOM) in the bioassay experiments.

Bulk chemical and molecular analyses

Water samples for measurements of total organic carbon (TOC) and total nitrogen (TN) were acidified to pH 2–3 with 2 mol L^{-1} hydrochloric acid (HCl) prior to analysis. Concentrations of TOC and TN were determined by high-temperature oxidation using a Shimadzu TOC-V analyzer equipped with an autosampler and a chemiluminescent nitrogen detector. Milli Q UV-Plus water and deep-sea reference standards were injected every sixth sample to monitor the blank and

performance of the TOC analysis (Benner and Strom 1993; Shen et al. 2016a). Instrumental blanks (Milli Q water) were negligible throughout the entire measurements of seawater samples. Precision of the analysis, represented by the coefficient of variation (CV; %) or standard deviation ($\mu\text{mol L}^{-1}$) of the concentrations of reference standards, was within 3.0% or $1.3 \mu\text{mol L}^{-1}$.

Aliquots of pDOM and C-18 DOM samples were analyzed for chromophoric DOM (CDOM) absorption and amino acids. The absorbance spectra (250–800 nm; A_λ) were measured using a Shimadzu ultraviolet-visible 1601 dual beam spectrophotometer and 1-cm quartz cuvettes. The absorbance was blank-corrected (subtracted by the average absorbance between 690 nm and 700 nm) and then converted to a Napierian absorption coefficient, a_λ (m^{-1}): $a_\lambda = 2.303 A_\lambda / 0.01$. Specific ultraviolet absorbance at 254 nm (SUVA_{254}) was calculated by dividing the absorbance measured at 254 nm in inverse meters (m^{-1}) by the concentration of DOC and were reported in units of $\text{m}^2 \text{gC}^{-1}$ or $\text{L mgC}^{-1} \text{m}^{-1}$ (Weishaar et al. 2003). The D- and L-enantiomers of amino acids were determined using an Agilent 1260 ultrahigh performance liquid chromatography system. Samples were hydrolyzed with a microwave-assisted vapor phase technique and derivatized with *o*-phthaldialdehyde and *N*-isobutyl-L-cysteine. Sample pretreatments and instrument conditions followed the procedures described in Kaiser and Benner (2005) and Shen et al. (2017). A total of 18 amino acids were determined in this

study and they were collectively termed total dissolved amino acids (TDAA). The DOC-normalized yields of TDAA (%DOC) were calculated as the percentages of total DOC measured as amino acid carbon. A commonly used degradation index (DI) was derived from multivariate analysis of the mole percentages of individual amino acids following the equation and revised parameters of Dauwe et al. (1999) and Kaiser and Benner (2009), respectively. In addition, the relative abundance (mol%) of major D-amino acids (Asx: asparagine + aspartic acid, Glx: glutamine + glutamic acid, Ser: serine, Ala: alanine) was calculated as: $(\text{D-Asx} + \text{D-Glx} + \text{D-Ser} + \text{D-Ala}) / [(\text{D-Asx} + \text{D-Glx} + \text{D-Ser} + \text{D-Ala}) + (\text{L-Asx} + \text{L-Glx} + \text{L-Ser} + \text{L-Ala})] \times 100$.

Statistical analysis

Significance of group comparison was tested using *T*-test (two-tailed, $\alpha = 0.05$) in IBM SPSS Statistics 23.

Results

Physical and chemical characteristics at BATS

Water samples for bioassay experiments were collected in late spring at BATS. This site is known to have strong seasonal patterns of water mixing, nutrient supply, and biological production (Michaels et al. 1994; Steinberg et al. 2001). A temperature profile during this study indicated relatively warm surface waters and a mixed layer of ~30 m (Fig. 1a).

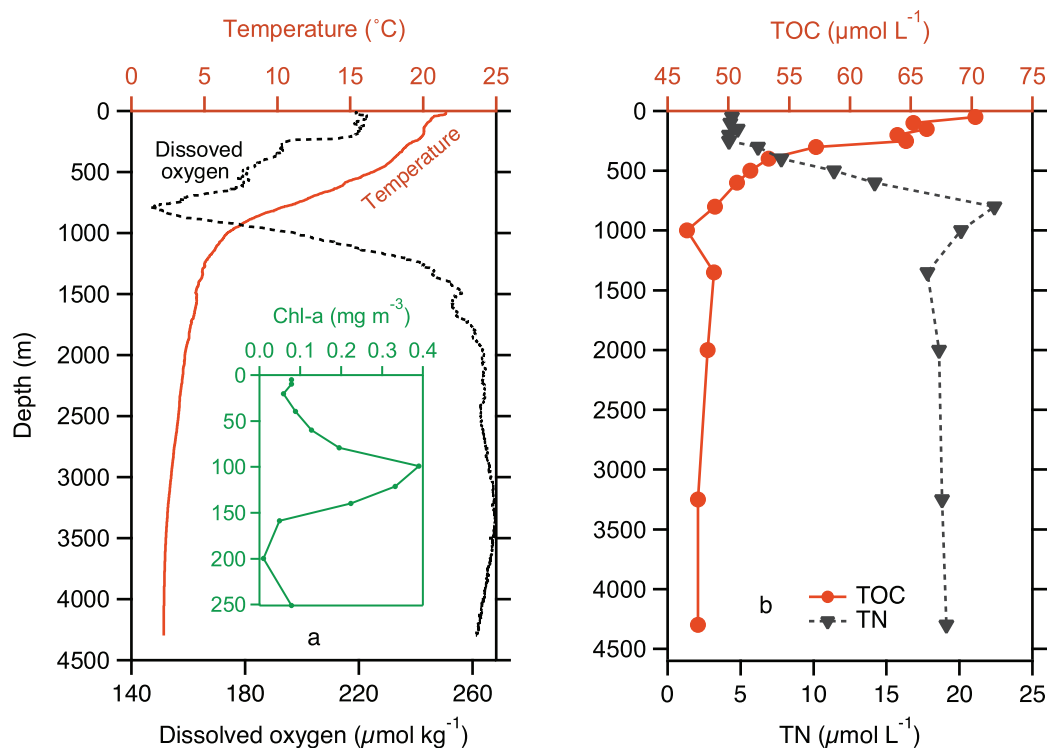


Fig. 1. (a) Profiles of temperature and dissolved oxygen at the Bermuda Atlantic Time-Series Study (BATS) site in May 2016. The inset shows the concentrations of chlorophyll-a (Chl-a) in the euphotic zone in May 2016. All data were obtained from <http://bats.bios.edu/bats-data/>. (b) Depth profiles of total organic carbon (TOC) and total nitrogen (TN) determined at 15 depths between 50 and 4300 m at BATS.

Chlorophyll-*a* in the upper water column showed a subsurface maximum of 0.4 mg m^{-3} at 100 m (Fig. 1a inset), indicating late spring bloom conditions. Dissolved oxygen (DO) decreased rapidly from the surface to mesopelagic waters, exhibiting a notable minimum of $\sim 150 \mu\text{mol kg}^{-1}$ at $\sim 800 \text{ m}$.

Concentrations of TOC decreased from $70 \mu\text{mol L}^{-1}$ at 50 m to $47 \mu\text{mol L}^{-1}$ at 4300 m (Fig. 1b). Most of the decrease in TOC concentrations occurred in the mesopelagic zone, with relatively stable concentrations below 1000 m. In comparison, concentrations of TN were low in surface water ($\sim 4.5 \mu\text{mol L}^{-1}$) and increased fivefold to a maximum of $22.5 \mu\text{mol L}^{-1}$ at 800 m depth. The maximal TN and minimal DO concentrations occurred at the same depth, indicating the remineralization of organic matter in mesopelagic waters. Concentrations of TN below 1000 m varied minimally in the range of $18\text{--}20 \mu\text{mol L}^{-1}$ (Fig. 1b). Overall, the profile measurements characterize strong physicochemical gradients across the surface, mesopelagic, and deep waters of the sampling region.

Chemical composition of plankton-derived DOM and C-18 isolated DOM

Optical properties and biochemical compositions of the two substrates used in this study (pDOM and C-18 DOM) were

determined and are compared in Fig. 2. The C-18 DOM had stronger absorption in the range of 250–265 nm compared to pDOM, with a higher SUVA_{254} value indicating greater aromaticity in the C-18 DOM (Fig. 2a). Absorption spectra of C-18 DOM were featureless and showed an exponential decline with increasing wavelength, similar to the shape of CDOM spectra observed previously in the open ocean and in C-18 DOM extracts (Andrew et al. 2013; Nelson and Siegel 2013). In contrast, the spectra of pDOM exhibited two notable “shoulders” with elevated absorption in the range of 270–290 nm and 300–350 nm (Fig. 2a). Previous culture experiments indicate oceanic plankton can release labile compounds, including aromatic amino acids (270–290 nm) and mycosporine-like amino acids (300–350 nm), that could contribute to the elevated absorption in these wavelength ranges (Strom et al. 1997; Shick and Dunlap 2002; Steinberg et al. 2004).

The reactivity of chromophores in pDOM was examined using short-term dark bioassay experiments with coastal seawater amended with pDOM (Fig. 2b). Changes in absorption coefficients were determined after 0 h, 12 h, 24 h, and 48 h of microbial degradation. Absorption in the range of 270–290 nm declined gradually, with about 30% of the absorption being removed after 48 h. In comparison,

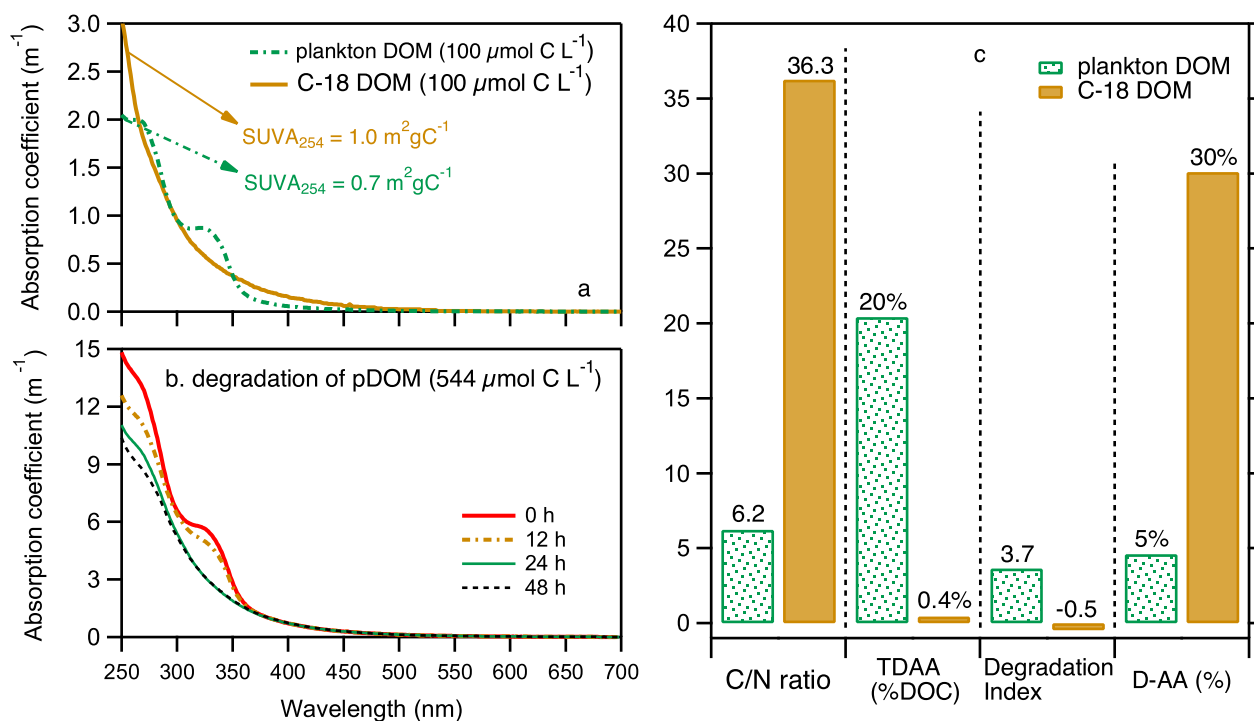
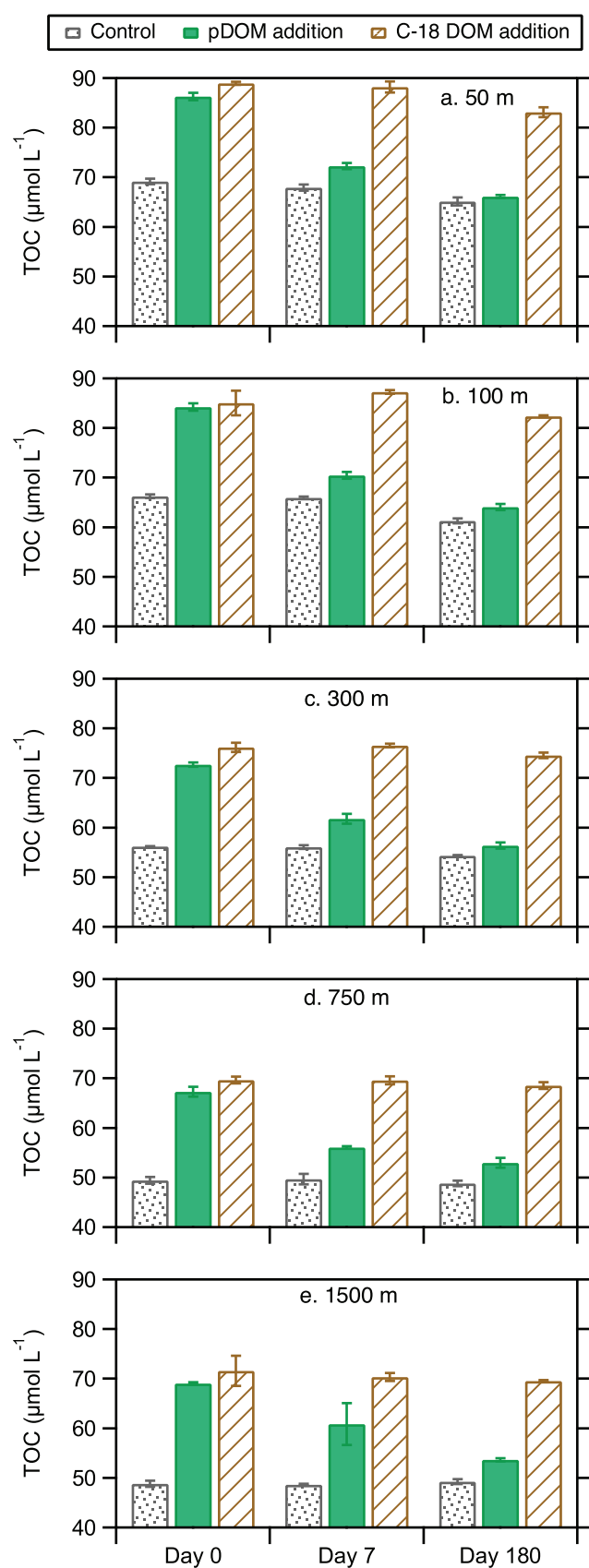


Fig. 2. (a) Comparison of the chromophoric properties of plankton-derived DOM (pDOM) and C-18 isolated DOM (C-18 DOM). Absorption coefficients are normalized to $100 \mu\text{mol C L}^{-1}$ for both substrates for a direct comparison of the spectra. Specific ultraviolet absorbance at 254 nm (SUVA_{254}) is calculated by dividing the absorbance measured at 254 nm by the concentration of DOC. (b) Changes in the absorption spectrum of pDOM during microbial degradation. The pDOM was added to coastal seawater (1:25, v/v) and incubated in the dark at room temperature for 48 h. (c) Comparison of the biochemical compositions of pDOM and C-18 DOM. C/N refers to the ratio of DOC to total dissolved nitrogen. TDAA (%DOC) refers to the percentage of DOC measured as total dissolved amino acids (TDAA). The degradation index (DI) is derived from a multivariate analysis of the mole percentage of individual amino acids. The mole percentage of major D-amino acids (D-AA) is calculated as: $(\text{D-Asx} + \text{D-Glx} + \text{D-Ser} + \text{D-Ala}) / [(\text{D-Asx} + \text{D-Glx} + \text{D-Ser} + \text{D-Ala}) + (\text{L-Asx} + \text{L-Glx} + \text{L-Ser} + \text{L-Ala})] \times 100$.



absorption removal in the range of 300–350 nm was more rapid with a 20–40% decline in 24 h. The 48 h of biodegradation of pDOM revealed the rapid evolution of a relatively featureless absorption spectrum that resembled those of C-18 DOM (Figs. 2a,b). The results demonstrate that chromophores contributing to the elevated absorption in the ultraviolet range are readily consumed by microbes and therefore labile.

Elemental and molecular analyses confirmed the optical observations and demonstrated distinct differences in the chemical composition between pDOM and C-18 DOM (Fig. 2c). The pDOM had a low C/N ratio (6.2) similar to the Redfield value, whereas the C-18 DOM exhibited a high C/N of 36.3 indicative of nitrogen depletion. A large fraction (20%) of the pDOC was identified as free and combined amino acids, in sharp contrast to that (0.4%) identified in the C-18 DOC. The 50-fold difference in amino acid abundance highlights the depletion of these labile molecules in the C-18 DOM. Consistent with these observations, the degradation index was 3.7 for pDOM and -0.5 for C-18 DOM, indicating the C-18 DOM is highly altered relative to the pDOM. The relative abundance of bacterial biomarkers (D-amino acids) was much higher in C-18 DOM (30%) than in pDOM (5%), further demonstrating more extensive bacterial alteration of the C-18 DOM. Overall, the optical and molecular properties reveal distinct compositional characteristics and potential bioreactivity between the freshly produced pDOM and extensively altered C-18 DOM.

Microbial degradation of marine organic matter

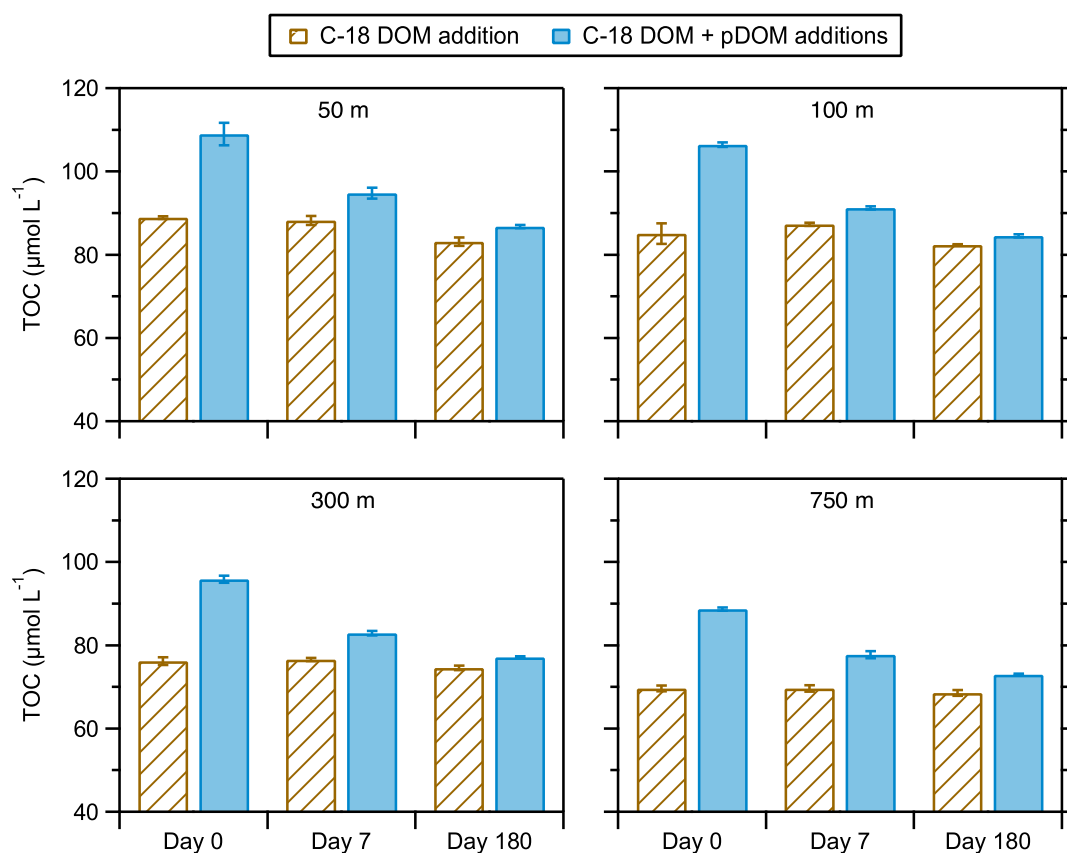
Microbial utilization of in situ DOM, pDOM, and C-18 DOM was determined and compared among surface (50 and 100 m; Figs. 3a,b), mesopelagic (300 and 750 m; Figs. 3c,d), and deep waters (1500 m; Fig. 3e). About 4–5 $\mu\text{mol L}^{-1}$ of in situ TOC in surface water was utilized by microbes within 180 d, accounting for 6–7% of the original TOC (Figs. 3a,b; Table 1). The addition of pDOM and C-18 DOM resulted in a net increase in TOC concentration by 17–18 $\mu\text{mol L}^{-1}$ and 19–20 $\mu\text{mol L}^{-1}$, respectively (Table 1). Over 75% of the added pDOM was utilized rapidly by surface microbes during the first 7 d of incubation, with another 10–20% being removed thereafter (Table 1). In contrast, no significant utilization of the added C-18 DOM was observed within 7 d at 50 m or 100 m (T -test, $p > 0.1$; Table 1). Minor removal of C-18 DOM occurred at 50 m after 180 d of incubation (by $\sim 2 \mu\text{mol DOC L}^{-1}$, T -test, $p < 0.05$), which was not detected at 100 m (T -test, $p > 0.1$). All amended incubations showed higher TOC concentrations relative to the controls at the end of the incubation (T -test,

Fig. 3. Changes in concentrations of total organic carbon (TOC) during bioassay experiments with unfiltered (a, b) surface (50 and 100 m), (c, d) mesopelagic (300 m and 750 m), and (e) deep (1500 m) waters. The plankton-derived DOM (pDOM) and C-18 isolated DOM (C-18 DOM) were added on day 0 at final concentrations of 17–20 $\mu\text{mol DOC L}^{-1}$ and 19–23 $\mu\text{mol DOC L}^{-1}$, respectively (see Table 1). The error bar represents the standard deviation of triplicate experiments.

Table 1. Concentrations of total organic carbon (TOC) during the bioassay experiments with unfiltered seawaters collected at the Bermuda Atlantic Time-Series Study site.

Depth	Day	Control	Amended with pDOM		Amended with C-18 DOM		Amended with pDOM and C-18 DOM	
			Bulk	pDOM	Bulk	C-18 DOM	Bulk	pDOM + C-18DOM
50 m	0	69.1±0.5	86.3±0.7	17.1±1.3	88.9±0.3	19.8±0.3	109.0±2.7	39.8±3.3
	7	67.9±0.6	72.2±0.6	4.3±0.5	88.2±1.1	20.3±0.5	94.8±1.4	26.9±0.8
	180	65.1±0.8	66.1±0.3	1.0±0.9	83.1±1.0	18.0±0.7	86.8±0.4	21.6±0.8
100 m	0	66.2±0.4	84.3±0.7	18.0±0.3	85.0±2.5	18.8±2.9	106.4±0.6	40.2±0.2
	7	65.9±0.2	70.5±0.7	4.5±0.6	87.3±0.4	21.4±0.2	91.2±0.4	25.3±0.4
	180	61.3±0.5	64.1±0.6	2.8±1.1	82.4±0.2	21.1±0.6	84.5±0.4	23.2±0.6
300 m	0	56.2±0.1	72.7±0.4	16.5±0.3	76.2±0.9	20.0±0.8	95.8±0.8	39.7±0.9
	7	56.0±0.3	61.8±1.0	5.7±1.2	76.5±0.4	20.5±0.6	82.9±0.5	26.9±0.9
	180	54.3±0.1	56.4±0.6	2.1±0.6	74.5±0.6	20.2±0.6	77.1±0.3	22.8±0.2
750 m	0	49.4±0.7	67.3±1.0	17.9±0.3	69.7±0.7	20.2±0.7	88.7±0.4	39.2±0.3
	7	49.7±1.0	56.1±0.2	6.4±0.8	69.6±0.8	20.0±0.4	77.7±0.8	28.1±1.3
	180	48.8±0.6	53.0±1.0	4.2±0.6	68.6±0.7	19.8±0.1	73.0±0.2	24.2±0.4
1500 m	0	48.8±0.6	69.0±0.2	20.0±0.4	71.6±3.0	22.8±3.0	n.d.	n.d.
	7	48.7±0.2	60.8±4.2	10.2±0.6	70.3±0.8	21.7±0.8	n.d.	n.d.
	180	49.3±0.5	53.7±0.3	4.8±0.3	69.5±0.2	20.3±0.5	n.d.	n.d.

pDOM = plankton-derived dissolved organic matter (DOM), C-18 DOM = C-18 isolated deep-sea DOM. The concentrations of pDOM or/and C-18 DOM at each time-point were calculated as the net difference in bulk TOC concentrations between amended groups and controls. Data are reported as average ± standard deviation (triplicate). n.d. = not determined.

**Fig. 4.** Changes in concentrations of total organic carbon (TOC) during bioassay experiments with unfiltered waters (50, 100, 300, and 750 m) amended with either C-18 DOM (19–23 μmol C L⁻¹) or with a combination of C-18 DOM and plankton-derived DOM (39–40 μmol C L⁻¹). The error bar represents the standard deviation of triplicate experiments.

$p < 0.01$), with an exception in the pDOM-amended group at 50 m (T -test, $p > 0.1$) (Figs. 3a,b).

Microbial utilization of TOC in mesopelagic waters (300 and 750 m) was minor compared to that in surface waters (Figs. 3c, d; Table 1). Total utilization of TOC in mesopelagic waters after 180 d decreased from $2 \mu\text{mol L}^{-1}$ at 300 m (T -test, $p < 0.01$) to $<1.0 \mu\text{mol L}^{-1}$ at 750 m (T -test, $p > 0.1$), which accounted for only 1–3% of the original TOC. In contrast, when pDOM was added to the incubations ($17\text{--}18 \mu\text{mol DOC L}^{-1}$), microbes at both depths rapidly utilized a large fraction (65%) of the added pDOM within 7 d and another 10–20% thereafter. Incubations amended with C-18 DOM ($\sim 20 \mu\text{mol DOC L}^{-1}$) showed no measurable net loss in TOC throughout the experiments at both depths (T -test, $p > 0.05$; Table 1). All amended incubations ended with higher concentrations of TOC compared to the controls (T -test, $p < 0.05$; Figs. 3c,d).

Microbial utilization of TOC in deep water was not measurable during the 180 d of incubation (T -test, $p > 0.1$; Fig. 3e; Table 1). Similar to microbes at other depths, the deep-sea microbes responded rapidly to the addition of pDOM but not to the addition of C-18 DOM. A total of 76% ($15 \mu\text{mol DOC L}^{-1}$) of the added pDOM was utilized (T -test, $p < 0.01$) and 65% of this utilization occurred within the first 7 d (T -test, $p < 0.01$; Fig. 3e). In contrast, removal of C-18 DOM was minimal and insignificant after 7 or 180 d of incubation (by $1\text{--}2 \mu\text{mol DOC L}^{-1}$; T -test, $p > 0.1$; Table 1). Final concentrations of TOC in the amended groups were higher than those in the controls (T -test, $p < 0.01$; Fig. 3e).

Incubations amended with a combination of C-18 DOM and pDOM were compared to the groups amended only with C-18 DOM to evaluate the priming effect (Fig. 4). The addition of pDOM resulted in $\sim 20 \mu\text{mol L}^{-1}$ increase in TOC concentration on day 0. Most of the added pDOM was utilized rapidly by microbes at all depths as observed previously (Fig. 3), suggesting that the presence of C-18 DOM did not limit the utilization of labile substrates. Final concentrations of TOC at all the depths examined were significantly higher in the double-amended groups (T -test, $p < 0.05$; Fig. 4), suggesting a lack of enhanced utilization of C-18 DOM with the additions of labile pDOM and therefore little or no priming effect.

The seawater samples collected for the bioassay experiments were not filtered to avoid the removal of any microorganisms and other potential alterations. Therefore we refer to TOC in all figures, Table 1 and the Results section. Nearly all of the organic carbon in the bioassay experiments is dissolved, so in the text we commonly refer to DOC in the Discussion section for consistency with similar bioassay studies (Barber 1968; Arrieta et al. 2015; Shen and Benner 2018).

Discussion

Most of the DOC in surface, mesopelagic, and deep waters at BATS was very resistant to microbial degradation and persisted during long-term incubations. About 6–7% of surface

water DOC was removed, 1–3% of mesopelagic DOC was removed, and no significant removal of DOC was observed in deep water. The observed resistance of seawater DOC to microbial degradation has led to its characterization as refractory DOC (Barber 1968; Ogura 1972; Hopkinson et al. 2002). Many factors can influence the microbial utilization of DOC in the ocean, including environmental conditions, microbial community structure, molecular properties (i.e., composition and structure), and the concentrations of dissolved molecules (Jiao et al. 2014; Carlson and Hansell 2015; Dittmar 2015). In the present study all incubations were conducted at the same temperature and atmospheric pressure. Water samples and microbial communities were collected from various depths, with the highest DOC and lowest nutrient concentrations in surface water and the lowest DOC and highest nutrient concentrations in deep water. The molecular properties of DOC also vary with depth, with declining concentrations of common biochemicals and bioreactivity with depth (Benner et al. 1992; Kaiser and Benner 2009). Given these confounding variables, the primary factors responsible for the decreasing bioreactivity of DOC with depth are uncertain. Therefore, we included bioassay experiments with additions of labile (pDOM) and refractory (C-18 DOM) forms of natural marine organic matter with contrasting molecular properties to gain further insights about the factors controlling the microbial utilization of DOC throughout the water column. The additions of pDOM ($\sim 18 \mu\text{M DOC}$) and C-18 DOM ($\sim 20 \mu\text{M DOC}$) increased the concentrations of these molecular forms of DOC by a factor of two or more, thereby facilitating their microbial utilization.

The microbial utilization of pDOM additions was rapid in water samples from all depths, with 50–75% removal of the pDOC within 7 d and 76–94% removal within 180 d. The pDOM is rich in free and combined amino acids and carbohydrates, which have been identified as major components of labile DOC (Cherrier et al. 1996; Amon et al. 2001; Shen et al. 2016b). The optical properties provided additional insights about the potential bioreactivity of chromophoric DOM. The broad absorption peaks at around 280 nm and 325 nm in the pDOM are indicative of aromatic amino acids and mycosporine-like amino acids (Shick and Dunlap 2002; Steinberg et al. 2004) and possibly other compounds. Molecules contributing to these optical properties were labile to microbial degradation. The lack of these diagnostic absorption patterns in the C-18 DOM indicates the limited abundance of these bioreactive chromophores. The overall extent of pDOC utilization was likely greater than observed because microbial utilization of labile substrates results in the production of refractory forms of DOC that persist during long-term incubations (Brophy and Carlson 1989; Ogawa et al. 2001; Koch et al. 2014).

The bioassay experiments demonstrate microbial communities in surface, mesopelagic, and deep waters rapidly and thoroughly utilized the pDOM and are poised to consume labile substrates as soon as they appear. In sharp contrast to

the rapid microbial utilization of pDOM, the C-18 DOM was very resistant to microbial degradation. No significant removal of C-18 DOC was observed in any of the incubations after 7 d, indicating the added C-18 DOC was not labile. Even after 180 d, the C-18 DOC persisted with no or minimal removal throughout the water column, verifying its refractory properties. The addition of C-18 extractable molecules more than doubled their original concentrations in deep water, so the elevated concentrations of these dissolved molecules were sufficient for substantial microbial utilization if the substrates were labile. These observations are consistent with those from a previous study with elevated (10-fold) concentrations of C-18 DOC as the sole carbon and energy source for microbial assemblages from surface, mesopelagic, and deep waters of the North Atlantic Ocean (Shen and Benner 2018). We further investigated the effects of priming on the removal of C-18 DOM by adding the labile pDOM to stimulate microbial growth and activity. The priming agent in these experiments, pDOM, was rapidly utilized, but a priming effect on the removal of C-18 DOM was not evident, further indicating the molecular properties of C-18 DOM shape its refractory nature. Overall, these results demonstrate the labile nature of pDOM and the refractory nature of C-18 DOM are primarily determined by molecular properties rather than concentrations in seawater. The C-18 DOC accounts for ~30% of the DOC in seawater (Shen and Benner 2018), indicating a substantial fraction of the DOC in the global ocean has refractory molecular properties and is very resistant to microbial degradation.

Bioassay experiments are the common approach used to determine the bioreactivity of substrates under defined environmental conditions (Ogura 1972; Søndergaard and Middelboe 1995; Lønborg et al. 2018). The term labile is used to characterize components that persist for minutes to weeks, whereas the term refractory is used to characterize components of DOM that persist in the ocean for centuries to millennia (Carlson 2002). Bioassay experiments are subject to bottle effects and are typically closed systems that are better suited for observing the bioreactivity of labile substrates than refractory substrates. Given the limited duration of these experiments, our characterization of C-18 DOM as refractory DOM can be considered speculative, but bioassay experiments clearly demonstrate these dissolved molecules are not labile, even in the presence of different microbial communities, labile substrates, and varying nutrient concentrations. Furthermore, the radiocarbon age of DOC in the deep ocean is often used as an indicator of its potential bioreactivity, with radiocarbon ages of several thousand years generally being indicative of refractory DOC (Druffel et al. 1992; Flerus et al. 2012). The radiocarbon ages of C-18 DOM collected from two deep water samples (1971 m, 2949 m) in the Arctic Ocean were 3770 and 3760 ybp (Benner et al. 2004). Deep water in the Arctic Ocean is derived from the North Atlantic, so these old ages of C-18 DOC provide independent evidence of the refractory properties of the C-18 isolated DOM.

Previous bioassay experiments with concentrated DOC extracted using styrene-divinylbenzene polymer (PPL) from deep ocean waters demonstrated the growth of microorganisms and removal of PPL-DOC during incubations lasting 10–40 d (Arrieta et al. 2015). These observations led the authors to speculate about the primary control on the microbial removal of DOC in the ocean. They proposed that deep ocean DOC is comprised of labile molecules that reside at concentrations too dilute for microbial utilization. However, their data also indicated a small fraction (mostly <6%) of the PPL-DOC was removed regardless of the concentration added to the bioassay experiments, indicating only a small fraction of the in situ DOC was labile and potentially too dilute for microbial utilization (Jiao et al. 2015). It is important to consider that the PPL sorbent concentrates DOC with a higher extraction efficiency ($\geq 40\%$) and somewhat different molecular properties (e.g., more polar molecules) than those concentrated with the C-18 sorbent, so it is possible the PPL sorbent concentrates some labile DOC whereas the C-18 sorbent concentrates predominantly refractory DOC (Shen and Benner 2018). Contrary to the observations of Arrieta et al. (2015), the results of the present study and our previous bioassay experiments with 10-fold concentrated C-18 DOC (Shen and Benner 2018) suggest the molecular properties of DOC are the primary control on the microbial utilization of DOC in the ocean. Two recent studies separately modeled the radiocarbon ages of deep-sea DOC and the removal of DOC based on the Arrieta et al. (2015) experimental results to assess the relative contributions of dilute labile and refractory molecules, and their results independently suggest a dominant presence of structurally resistant organic molecules in the deep ocean (Wilson and Arndt 2017; Wang et al. 2018).

It is challenging to define specific molecular properties that limit the microbial utilization of DOM (Zhang et al. 2018), but advances in the chemical characterization of DOM are leading to new insights. Solid phase extraction methods used for the concentration and isolation of marine DOM, such as C-18 used herein, commonly rely on a nonpolar stationary phase and hydrophobic interactions to preferentially adsorb mid- to nonpolar molecules from seawater (Benner 2002; Dittmar et al. 2008). The isolated DOM, often referred to as humic substances, is enriched in carbon relative to nitrogen ($C/N = 36\text{--}57$) and depleted in amino acids and carbohydrates (Druffel et al. 1992; Hedges et al. 1992; Koch et al. 2005; Shen and Benner 2018). Nuclear magnetic resonance spectroscopy and ultrahigh resolution mass spectrometry indicate the prevalence of carboxylated aliphatic molecules in marine humic substances (Hedges et al. 1992; Koch et al. 2005; Koprivnjak et al. 2009). More detailed characterizations of marine DOM reveal the occurrence of hundreds to thousands of carboxyl-rich alicyclic molecules (CRAM) with carboxyl-C:aliphatic-C ratios of 1:2 to 1:7 (Hertkorn et al. 2006; Hertkorn et al. 2013). Cyclic terpenoids, including sterols, hopanoids, and carotenoids, are thought to be important biochemical precursors of

CRAM (Hertkorn et al. 2006; Arakawa et al. 2017). These CRAM are abundant in seawater DOM (Hertkorn et al. 2006; Koprivnjak et al. 2009; Arakawa et al. 2017), and they appear to be relatively old and altered components of refractory DOC (Lechtenfeld et al. 2014).

Understanding the underlying mechanisms controlling DOC removal is critical because those mechanisms play a key role in regulating the size of the global ocean DOC reservoir. If most of the dissolved molecules in seawater are labile but too dilute for microbial utilization (i.e., dilution hypothesis), the size of the DOC reservoir would be constrained by the threshold concentrations and diversity of labile molecules. Recent studies indicate an amazing diversity of small molecules in seawater with estimates of more than 100,000 compounds (Hertkorn et al. 2013; Zark et al. 2017). Molecular diversity in seawater is already extremely high, indicating the DOC reservoir size (662 Pg C; Hansell 2013) is unlikely to change substantially over time if it is comprised of labile molecules. In contrast, if a large fraction of dissolved molecules in seawater persist due to their refractory molecular properties, as observed herein, there is greater potential for a more dynamic reservoir of DOC in the global ocean. The latter has been suggested to be the case in the ancient ocean where a dynamic and much larger oceanic DOC reservoir was thought to occur during the Neoproterozoic (1,000–541 million years ago) and Paleocene–Eocene epochs (~65–34 million years ago) (Rothman et al. 2003; Sexton et al. 2011). Our findings of the predominant occurrence of inherently refractory molecules in marine DOM indicates a flexible capacity of carbon storage in the modern global ocean.

References

- Amon, R. M. W., H. P. Fitznar, and R. Benner. 2001. Linkages among the bioreactivity, chemical composition, and diagenetic state of marine dissolved organic matter. *Limnol. Oceanogr.* **46**: 287–297. doi:10.4319/lo.2001.46.2.0287
- Andrew, A. A., R. Del Vecchio, A. Subramaniam, and N. V. Blough. 2013. Chromophoric dissolved organic matter (CDOM) in the Equatorial Atlantic Ocean: optical properties and their relation to CDOM structure and source. *Mar. Chem.* **148**: 33–43. doi:10.1016/j.marchem.2012.11.001
- Arakawa, N., L. I. Aluwihare, A. J. Simpson, R. Soong, B. M. Stephens, and D. Lane-Coplen. 2017. Carotenoids are the likely precursor of a significant fraction of marine dissolved organic matter. *Sci. Adv.* **3**: e1602976. doi:10.1126/sciadv.1602976
- Arrieta, J. M., E. Mayol, R. L. Hansman, G. J. Herndl, T. Dittmar, and C. M. Duarte. 2015. Dilution limits dissolved organic carbon utilization in the deep ocean. *Science* **348**: 331–333. doi:10.1126/science.1258955
- Barber, R. T. 1968. Dissolved organic carbon from deep waters resists microbial oxidation. *Nature* **220**: 274–275. doi:10.1038/220274a0
- Benner, R. 2002. Chemical composition and reactivity, p. 59–85. In D. A. Hansell and C. A. Carlson [eds.], *Biogeochemistry of marine dissolved organic matter*. Academic Press.
- Benner, R., and R. M. Amon. 2015. The size-reactivity continuum of major bioelements in the ocean. *Annu. Rev. Mar. Sci.* **7**: 185–205. doi:10.1146/annurev-marine-010213-135126
- Benner, R., and M. Strom. 1993. A critical evaluation of the analytical blank associated with DOC measurements by high-temperature catalytic oxidation. *Mar. Chem.* **41**: 153–160. doi:10.1016/0304-4203(93)90113-3
- Benner, R., J. D. Pakulski, M. McCarthy, J. I. Hedges, and P. G. Hatcher. 1992. Bulk chemical characteristics of dissolved organic matter in the ocean. *Science* **255**: 1561–1564. doi:10.1126/science.255.5051.1561
- Benner, R., B. Benitez-Nelson, K. Kaiser, and R. M. W. Amon. 2004. Export of young terrigenous dissolved organic carbon from rivers to the Arctic Ocean. *Geophys. Res. Lett.* **31**: L05305. doi:10.1029/2003GL019251
- Brophy, J. E., and D. J. Carlson. 1989. Production of biologically refractory dissolved organic carbon by natural seawater microbial populations. *Deep-Sea Res. Pt. I* **36**: 497–507. doi:10.1016/0198-0149(89)90002-2
- Carlson, C. 2002. Production and removal processes, p. 91–151. In D. A. Hansell and C. A. Carlson [eds.], *Biogeochemistry of marine dissolved organic matter*. Academic Press.
- Carlson, C. A., and D. A. Hansell. 2015. DOM sources, sinks, reactivity, and budgets, p. 65–126. In D. A. Hansell and C. A. Carlson [eds.], *Biogeochemistry of Marine Dissolved Organic Matter*. Academic Press.
- Cherrier, J., J. E. Bauer, and E. R. M. Druffel. 1996. Utilization and turnover of labile dissolved organic matter by bacterial heterotrophs in eastern north Pacific surface waters. *Mar. Ecol. Prog. Ser.* **139**: 267–279. doi:10.3354/meps139267
- Dauwe, B., J. Middelburg, P. Herman, and C. Heip. 1999. Linking diagenetic alteration of amino acids and bulk organic matter reactivity. *Limnol. Oceanogr.* **44**: 1809–1814. doi:10.4319/lo.1999.44.7.1809
- Dittmar, T. 2015. Reasons behind the long-term stability of dissolved organic matter, p. 369–388. In D. A. Hansell and C. A. Carlson [eds.], *Biogeochemistry of Marine Dissolved Organic Matter*. Academic Press.
- Dittmar, T., B. Koch, N. Hertkorn, and G. Kattner. 2008. A simple and efficient method for the solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater. *Limnol. Oceanogr. Meth.* **6**: 230–235. doi:10.4319/lom.2008.6.230
- Druffel, E. R. M., P. M. Williams, J. E. Bauer, and J. R. Ertel. 1992. Cycling of dissolved and particulate organic matter in the open ocean. *J. Geophys. Res.-Oceans* **97**: 15639–15659. doi:10.1029/92JC01511
- Flerus, R., O. J. Lechtenfeld, B. P. Koch, S. L. McCallister, P. Schmitt-Kopplin, R. Benner, K. Kaiser, and G. Kattner.

2012. A molecular perspective on the ageing of marine dissolved organic matter. *Biogeosciences* **9**: 1935–1955. doi:[10.5194/bg-9-1935-2012](https://doi.org/10.5194/bg-9-1935-2012)
- Guenet, B., M. Danger, L. Abbadie, and G. Lacroix. 2010. Priming effect: bridging the gap between terrestrial and aquatic ecology. *Ecology* **91**: 2850–2861. doi:[10.1890/09-1968.1](https://doi.org/10.1890/09-1968.1)
- Hansell, D. A. 2013. Recalcitrant dissolved organic carbon fractions. *Annu. Rev. Mar. Sci.* **5**: 421–445. doi:[10.1146/annurev-marine-120710-100757](https://doi.org/10.1146/annurev-marine-120710-100757)
- Hedges, J. I., P. G. Hatcher, J. R. Ertel, and K. J. Meyers-Schulte. 1992. A comparison of dissolved humic substances from seawater with Amazon River counterparts by ¹³C-NMR spectrometry. *Geochim. Cosmochim. Ac.* **56**: 1753–1757. doi:[10.1016/0016-7037\(92\)90241-A](https://doi.org/10.1016/0016-7037(92)90241-A)
- Hertkorn, N., R. Benner, M. Frommberger, P. Schmitt-Kopplin, M. Witt, K. Kaiser, A. Kettrup, and J. I. Hedges. 2006. Characterization of a major refractory component of marine dissolved organic matter. *Geochim. Cosmochim. Ac.* **70**: 2990–3010. doi:[10.1016/j.gca.2006.03.021](https://doi.org/10.1016/j.gca.2006.03.021)
- Hertkorn, N., M. Harir, B. Koch, B. Michalke, and P. Schmitt-Kopplin. 2013. High-field NMR spectroscopy and FTICR mass spectrometry: powerful discovery tools for the molecular level characterization of marine dissolved organic matter. *Biogeosciences* **10**: 1583–1624. doi:[10.5194/bg-10-1583-2013](https://doi.org/10.5194/bg-10-1583-2013)
- Hopkinson, C. S., J. J. Vallino, and A. Nolin. 2002. Decomposition of dissolved organic matter from the continental margin. *Deep-Sea Res. Part II-Top. Stud. Oceanogr.* **49**: 4461–4478. doi:[10.1016/S0967-0645\(02\)00125-X](https://doi.org/10.1016/S0967-0645(02)00125-X)
- Jannasch, H. W. 1967. Growth of marine bacteria at limiting concentrations of organic carbon in seawater. *Limnol. Oceanogr.* **12**: 264–271. doi:[10.4319/lo.1967.12.2.0264](https://doi.org/10.4319/lo.1967.12.2.0264)
- Jannasch, H. W. 1994. The microbial turnover of carbon in the deep-sea environment. *Glob. Planet. Change* **9**: 289–295. doi:[10.1016/0921-8181\(94\)90022-1](https://doi.org/10.1016/0921-8181(94)90022-1)
- Jiao, N., and others. 2014. Mechanisms of microbial carbon sequestration in the ocean—future research directions. *Biogeosciences* **11**: 5285–5306. doi:[10.5194/bg-11-5285-2014](https://doi.org/10.5194/bg-11-5285-2014)
- Jiao, N., and others. 2015. Comment on “Dilution limits dissolved organic carbon utilization in the deep ocean”. *Science* **350**: 1483–1483. doi:[10.1126/science.aab2713](https://doi.org/10.1126/science.aab2713)
- Kaiser, K., and R. Benner. 2005. Hydrolysis-induced racemization of amino acids. *Limnol. Oceanogr. Meth.* **3**: 318–325. doi:[10.4319/lom.2005.3.318](https://doi.org/10.4319/lom.2005.3.318)
- Kaiser, K., and R. Benner. 2009. Biochemical composition and size distribution of organic matter at the Pacific and Atlantic time-series stations. *Mar. Chem.* **113**: 63–77. doi:[10.1016/j.marchem.2008.12.004](https://doi.org/10.1016/j.marchem.2008.12.004)
- Koch, B. P., M. Witt, R. Engbrodt, T. Dittmar, and G. Kattner. 2005. Molecular formulae of marine and terrigenous dissolved organic matter detected by electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. *Geochim. Cosmochim. Ac.* **69**: 3299–3308. doi:[10.1016/j.gca.2005.02.027](https://doi.org/10.1016/j.gca.2005.02.027)
- Koch, B., G. Kattner, M. Witt, and U. Passow. 2014. Molecular insights into the microbial formation of marine dissolved organic matter: recalcitrant or labile? *Biogeosciences* **11**: 4173–4190. doi:[10.5194/bg-11-4173-2014](https://doi.org/10.5194/bg-11-4173-2014)
- Koprivnjak, J. F., and others. 2009. Chemical and spectroscopic characterization of marine dissolved organic matter isolated using coupled reverse osmosis–electrodialysis. *Geochim. Cosmochim. Ac.* **73**: 4215–4231. doi:[10.1016/j.gca.2009.04.010](https://doi.org/10.1016/j.gca.2009.04.010)
- Lechtenfeld, O. J., G. Kattner, R. Flerus, S. L. McCallister, P. Schmitt-Kopplin, and B. P. Koch. 2014. Molecular transformation and degradation of refractory dissolved organic matter in the Atlantic and Southern Ocean. *Geochim. Cosmochim. Ac.* **126**: 321–337. doi:[10.1016/j.gca.2013.11.009](https://doi.org/10.1016/j.gca.2013.11.009)
- Lønborg, C., X. A. Álvarez, S. D. Salgado, and C. Carreira. 2018. Organic matter bioavailability in tropical coastal waters: the Great Barrier Reef. *Limnol. Oceanogr.* **63**: 1015–1035. doi:[10.1002/lno.10717](https://doi.org/10.1002/lno.10717)
- Michaels, A. F., and others. 1994. Seasonal patterns of ocean biogeochemistry at the U.S. JGOFS Bermuda Atlantic time-series study site. *Deep-Sea Res.* **41**: 1013–1038. doi:[10.1016/0967-0637\(94\)90016-7](https://doi.org/10.1016/0967-0637(94)90016-7)
- Middelburg, J. J. 2015. Escape by dilution. *Science* **348**: 290–290. doi:[10.1126/science.aaa9852](https://doi.org/10.1126/science.aaa9852)
- Nelson, N. B., and D. A. Siegel. 2013. The global distribution and dynamics of chromophoric dissolved organic matter. *Annu. Rev. Mar. Sci.* **5**: 447–476. doi:[10.1146/annurev-marine-120710-100751](https://doi.org/10.1146/annurev-marine-120710-100751)
- Ogawa, H., and E. Tanoue. 2003. Dissolved organic matter in oceanic waters. *J. Oceanogr.* **59**: 129–147. doi:[10.1023/A:1025528919771](https://doi.org/10.1023/A:1025528919771)
- Ogawa, H., Y. Amagai, I. Koike, K. Kaiser, and R. Benner. 2001. Production of refractory dissolved organic matter by bacteria. *Science* **292**: 917–920. doi:[10.1126/science.1057627](https://doi.org/10.1126/science.1057627)
- Ogura, N. 1972. Rate and extent of decomposition of dissolved organic matter in surface seawater. *Mar. Biol.* **13**: 89–93. doi:[10.1007/BF00366559](https://doi.org/10.1007/BF00366559)
- Rothman, D. H., J. M. Hayes, and R. E. Summons. 2003. Dynamics of the Neoproterozoic carbon cycle. *Proc. Natl. Acad. Sci.* **100**: 8124–8129. doi:[10.1073/pnas.0832439100](https://doi.org/10.1073/pnas.0832439100)
- Sexton, P. F., R. D. Norris, P. A. Wilson, H. Pälike, T. Westerhold, U. Röhl, C. Bolton, and S. Gibbs. 2011. Eocene global warming events driven by ventilation of oceanic dissolved organic carbon. *Nature* **471**: 349–352. doi:[10.1038/nature09826](https://doi.org/10.1038/nature09826)
- Shen, Y., R. Benner, A. E. Murray, C. Gimpel, B. G. Mitchell, E. L. Weiss, and C. Reiss. 2017. Bioavailable dissolved organic matter and biological hot spots during austral winter in Antarctic waters. *J. Geophys. Res.-Oceans* **122**: 508–520. doi:[10.1002/2016JC012301](https://doi.org/10.1002/2016JC012301)
- Shen, Y., and R. Benner. 2018. Mixing it up in the ocean carbon cycle and the removal of refractory dissolved organic carbon. *Sci. Rep.* **8**: 2542. doi:[10.1038/s41598-018-20857-5](https://doi.org/10.1038/s41598-018-20857-5)
- Shen, Y., R. Benner, L. L. Robbins, and J. G. Wynn. 2016a. Sources, distributions, and dynamics of dissolved organic

- matter in the Canada and Makarov Basins. *Front. Mar. Sci.* **3**: 198. doi:[10.3389/fmars.2016.00198](https://doi.org/10.3389/fmars.2016.00198)
- Shen, Y., C. G. Fichot, S.-K. Liang, and R. Benner. 2016b. Biological hot spots and the accumulation of marine dissolved organic matter in a highly productive ocean margin. *Limnol. Oceanogr.* **61**: 1287–1300. doi:[10.1002/lno.10290](https://doi.org/10.1002/lno.10290)
- Shick, J. M., and W. C. Dunlap. 2002. Mycosporine-like amino acids and related gadusols: biosynthesis, accumulation, and UV-protective functions in aquatic organisms. *Annu. Rev. Physiol.* **64**: 223–262. doi:[10.1146/annurev.physiol.64.081501.155802](https://doi.org/10.1146/annurev.physiol.64.081501.155802)
- Søndergaard, M., and M. Middelboe. 1995. A cross-system analysis of labile dissolved organic carbon. *Mar. Ecol. Prog. Ser.* **118**: 283–294. doi:[10.3354/meps118283](https://doi.org/10.3354/meps118283)
- Steinberg, D. K., C. A. Carlson, N. R. Bates, R. J. Johnson, A. F. Michaels, and A. H. Knap. 2001. Overview of the US JGOFS Bermuda Atlantic Time-series Study (BATS): a decade-scale look at ocean biology and biogeochemistry. *Deep-Sea Res. Part II-Top. Stud. Oceanogr.* **48**: 1405–1447. doi:[10.1016/S0967-0645\(00\)00148-X](https://doi.org/10.1016/S0967-0645(00)00148-X)
- Steinberg, D. K., N. B. Nelson, C. A. Carlson, and A. Prusak. 2004. Production of chromophoric dissolved organic matter (CDOM) in the open ocean by zooplankton and the colonial cyanobacterium *Trichodesmium* spp. *Mar. Ecol. Prog. Ser.* **267**: 45–56. doi:[10.3354/meps267045](https://doi.org/10.3354/meps267045)
- Strom, S. L., R. Benner, S. Ziegler, and M. J. Dagg. 1997. Planktonic grazers are a potentially important source of marine dissolved organic carbon. *Limnol. Oceanogr.* **42**: 1364–1374. doi:[10.4319/lno.1997.42.6.1364](https://doi.org/10.4319/lno.1997.42.6.1364)
- Wang, N., Y.-W. Luo, L. Polimene, R. Zhang, Q. Zheng, R. Cai, and N. Jiao. 2018. Contribution of structural recalcitrance to the formation of the deep oceanic dissolved organic carbon reservoir. *Environ. Microbiol. Rep.* **10**: 711–717. doi:[10.1111/1758-2229.12697](https://doi.org/10.1111/1758-2229.12697)
- Weishaar, J. L., G. R. Aiken, B. A. Bergamaschi, M. S. Fram, R. Fujii, and K. Mopper. 2003. Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. *Environ. Sci. Technol.* **37**: 4702–4708. doi:[10.1021/es030360x](https://doi.org/10.1021/es030360x)
- Williams, P. M., and E. R. M. Druffel. 1987. Radiocarbon in dissolved organic matter in the central north Pacific Ocean. *Nature* **330**: 246–248. doi:[10.1038/330246a0](https://doi.org/10.1038/330246a0)
- Wilson, J. D., and S. Arndt. 2017. Modeling radiocarbon constraints on the dilution of dissolved organic carbon in the deep ocean. *Global Biogeochem. Cycles* **31**: 775–786. doi:[10.1002/2016GB005520](https://doi.org/10.1002/2016GB005520)
- Zark, M., J. Christoffers, and T. Dittmar. 2017. Molecular properties of deep-sea dissolved organic matter are predictable by the central limit theorem: Evidence from tandem FT-ICR-MS. *Mar. Chem.* **191**: 9–15. doi:[10.1126/sciadv.1500531](https://doi.org/10.1126/sciadv.1500531)
- Zhang, C., and others. 2018. Evolving paradigms in biological carbon cycling in the ocean. *Natl. Sci. Rev.* **5**: 481–499. doi:[10.1093/nsr/nwy074](https://doi.org/10.1093/nsr/nwy074)

Acknowledgments

We are grateful to Matthew McCarthy for providing berths on the BATS cruise, and to the captain and crew of the R/V *Atlantic Explorer* for providing sampling assistance. We thank Qinghui Huang for assistance with the CDOM degradation experiment with pDOM. This study was supported by the National Science Foundation (OCE-1233373 and PLR-1504137 to R.B.).

Conflict of Interest

None declared.

Submitted 22 May 2019

Revised 28 August 2019

Accepted 02 October 2019

Associate editor: Anya Waite