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Limited utilization of extracted dissolved organic matter by prokaryotic communities from the subtropical North Atlantic

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The ocean contains a large reservoir of dissolved organic matter (DOM) that persists for millennia. Both the very dilute concentrations of individual DOM molecules and intrinsic recalcitrance to microbial decay imparted by molecular structure are suggested mechanisms for this long residence time. Here, we report an experiment comparing the responses of surface and deep prokaryotes to DOM isolated and enriched by solid-phase extraction from surface and deep waters of the subtropical North Atlantic Ocean. Extracts from both depths were qualitatively characterized as biologically recalcitrant given their similarly high C : N ratios of 26. Surface prokaryotes measurably drew down extracted dissolved organic carbon (DOC) concentrations, but the drawdown was only 4% of the initial enriched DOC concentration regardless of enrichment level or depth. Deep microbes, in contrast, did not cause observable changes in DOC concentrations. Surface and deep prokaryotes had similar temperature-normalized growth responses to extracts from each depth. Biological indicators (e.g., kinetics) suggest that prokaryotes were less efficient at catalyzing surface than deep DOM (catalytic efficiencies of 0.003–0.005 vs. 0.02–0.03 h^{-1}, respectively). These values indicate qualitative differences in extracted DOM from the two depths, perhaps suggesting a variable nature of the refractory DOC depending on depth. Moreover, only a small portion of the extracted DOM was biologically utilizable, regardless of concentration factor or depth, and essentially only a small fraction of it was incorporated into biomass. Microbial selection against substrates that meet modest energy but no growth demands may be a factor contributing to the long-term stability of marine DOM.

Dissolved organic matter (DOM) is the largest reservoir of reduced carbon in the ocean (Hedges 2002). Most DOM is synthesized in the surface ocean via primary production, where it is biologically labile and remineralized to CO_2 by heterotrophic processes (Hansell 2013). However, a small portion of DOM resists degradation and can therefore be transported to great depths, where it displays long life times (Druffel et al. 1992), constituting the vast majority of the marine DOM inventory (Hansell et al. 2009).

Although open ocean dissolved organic carbon (DOC) concentrations are relatively low (40 μM) at depth as compared to surface waters (60–80 μM), a substantially lower concentration (nM) of bioavailable substrate (e.g., glucose) is enough to stimulate microbial growth (Azam and Hodson 1981). However, DOM comprises a highly diverse pool with thousands of compounds, making the concentration of any one particular substrate in the bulk DOM pool presumably very low (Dittmar 2015). Consequently, prokaryotic abundance and productivity in the deep ocean are primarily supported by substrates introduced with particulate organic matter exported to depths from the surface ocean (Nagata et al. 2000; Hansell and Ducklow 2003; Yokokawa et al. 2013).

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Additional Supporting Information may be found in the online version of this article.

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There are several factors that could contribute to the old age and low reactivity of DOM at depth, including natural environmental conditions, molecular composition of DOM, polymer size and the low concentration of individual substrate molecules (Dittmar 2015). One hypothesis for how DOM can be held biologically inaccessible to microbial communities is through transformation by the microbial carbon pump (Jiao et al. 2010), where prokaryotic heterotrophs (bacteria and archaea) metabolize labile DOM into a more recalcitrant form, thus expanding the reservoir for carbon storage in the deep ocean. Certain compositional factors, such as the C : N ratio, provide information on the bioavailability of DOM. As DOM becomes less bioavailable, its individual molecules become smaller and the C : N ratio becomes greater (Benner et al. 1997; Walker et al. 2016). Amino acids and peptides are exceptions to this general trend, however, as they are small yet bioavailable molecules with low C : N ratios (Amon and Benner 1996). Most oceanic DOM has C : N ratios of 10–25, with the more recalcitrant fractions exhibiting the higher values (Benner et al. 1997; Ogawa et al. 2001). Microbially available organic material typically exhibits lower C : N ratios (5–7; Ogawa et al. 2001), illustrating the importance of nitrogen-bearing DOM as a substrate.

Another theory suggests that concentrations of individual structural components of DOM in the deep ocean are too dilute to act as a viable substrate for the resident microbial communities (Dittmar 2015). Arrieta et al. (2015) presented experimental evidence that prokaryotic growth increased with concentration of extracted DOM. However, prokaryotic abundances and communities differ with depth (Jing et al. 2013), water mass (Reinthaler et al. 2010; Ghiglione et al. 2012), latitude (De Corte et al. 2016), and season (Carlson et al. 2004) in the marine environment. Different prokaryotic communities respond differently to DOM of distinct composition and origin (Carlson et al. 2004; Shen and Benner 2018). For instance, Letscher et al. (2015) showed that prokaryotic communities from the subsurface ocean grown on bulk surface DOM exhibited a more efficient drawdown of DOC and ammonium (NH_4^+) than surface prokaryotic communities on the same substrate, suggesting that biological recalcitrance in one environment may be more biologically labile to a different heterotrophic community. Moreover, carbon demands and growth efficiencies of prokaryotic communities are dependent on the quality of DOM present (Apple and del Giorgio 2007; Shen and Benner 2020). Phytoplankton-derived DOM has a faster microbial response than even elevated concentrations of C18-extracted DOM (Shen and Benner 2020). As surface DOM is typically considered more labile than deep DOM, there are also likely different growth efficiencies in deep versus surface prokaryotic communities.

In this study, we conducted incubation experiments to assess the prokaryotic response to solid-phase-extracted DOM. Solid-phase extraction (SPE) is a simple and useful technique to concentrate DOM from marine environments (Dittmar et al. 2008). Extraction is not complete, however, as the SPE process does not retain small, polar molecules as well as large carbohydrates (Swenson et al. 2014). The DOM extracted in this study is therefore likely missing those components. Our goal was to test whether the resident microbial communities displayed distinctive capabilities in mineralizing concentrated extracted DOM from both surface and mesopelagic waters of the oligotrophic North Atlantic.

Methods

Sample collection area

Seawater samples from both surface (20 m) and mesopelagic (800 m) depths were collected from Niskin bottles mounted to a rosette frame with a conductivity, temperature and depth sensor package on cruises in the Strait of Florida at 27°00′N, 79°39′W aboard the R.V. *Walton Smith* in June and September 2016. DOM extraction was conducted with waters collected in June, while the inoculation (i.e., collection of prokaryotes) was conducted in September.

SPE of surface and deep DOM

Both surface (32 liters) and deep (64 liters) waters were filtered through 0.2-μm pore-size Supor© filters (Pall) to remove ambient microbial communities. Before extraction, aliquots were collected to measure initial DOC concentrations. The filtered seawater was then acidified to pH 2 and the marine DOM was extracted using priority pollutant (PPL) SPE cartridges (Varian Bond Elut, 1 g), following Dittmar et al. (2008). The SPE cartridges were activated by passing one cartridge-full (4 mL) of methanol and subsequently 4 mL of acidified Milli-Q® water (pH = 2). Surface and deep waters were passed through the SPE cartridges via gravity filtration, with 4 liters of sample per cartridge. After extraction, cartridges were rinsed with acidified Milli-Q® and dried with N_2 gas. The DOM was eluted from each cartridge with methanol, which was subsequently removed by evaporation with N_2 gas. These DOM extracts were then placed in a drying oven at 25°C for 24 h and subsequently stored at −20°C until the incubation experiments. To ensure the extracted DOM was indeed dissolved and that it had not aggregated into particulate material, we subsampled the resuspended DOM, determining the DOC concentration before and after filtering through a combusted 0.7-μm Whatman GF/F filter; 100% of the extract passed through the filter.

Incubation experimental setup

Waters for initiating the incubations were collected from surface (20 m) and mesopelagic waters (800 m); the latter has been out of contact with the atmosphere for centuries, as it is dominantly Antarctic Intermediate Water, originating from the Southern Ocean (Schmitz 1995).

Five liters of deep and surface waters were collected directly from Niskin bottles aboard the *R.V. Walton Smith*. Four liters of deep and surface waters were filtered (0.2-μm Supor© 47 mm filters) into separate combusted glass jugs (Fig. 1).

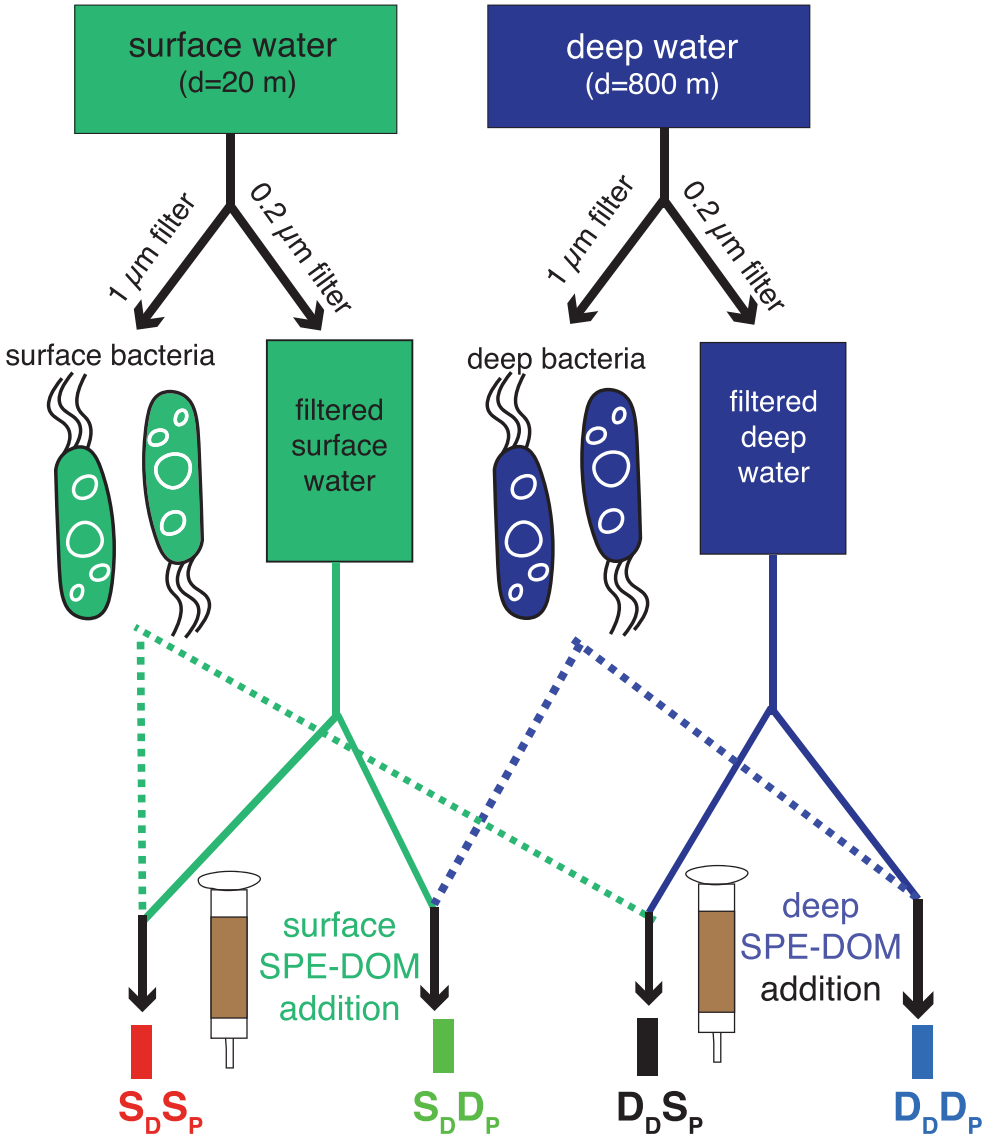

Fig 1. Scheme of experimental design. The schematic in green (left) represents surface water collected (depth = 20 m), while that in dark blue represents deep water (depth = 800 m). Surface and deep waters were separated based on filtration into inoculum (1 μm GF/F filtrate) and filtered background water (0.2 μm Supor filtrate). These deep and surface inoculums and deep and surface background waters were then combined at a 1 : 5 ratio in different combinations: D_DD_P is deep DOM incubated with deep prokaryotes, D_DS_P is deep DOM with surface prokaryotes, S_DD_P is surface DOM incubated with deep prokaryotes, and S_DS_P is surface DOM incubated with surface prokaryotes. Then, each of the four combinations was provided with different enrichments of DOM from waters of the same depth as the background water, generating a total of 16 incubations (enrichment values and factors are in Supporting Information Table S1).

Then, one liter of either whole surface or deep water was filtered with 1-μm pore-size precombusted glass fiber filters (Millipore) into combusted glass jugs to remove grazers; this water holds the inoculum of surface and deep prokaryotes from their respective locations. Incubations were initiated at the ratio of 4 liters of filtrate (0.2 μm filtered) to 1 liter of inoculum (1 μm filtered) (Fig. 1). Briefly, deep or surface prokaryotes were mixed with either surface or deep water, generating four mixing scenarios (Fig. 1): surface water incubated with surface prokaryotes, deep water incubated with surface prokaryotes, surface water incubated with deep prokaryotes, and deep water incubated with deep prokaryotes. The total volume for each mixture was 2.5 liters.

Each mixture was separated into five and three 500-mL incubations of deep and surface waters, respectively. They were then enriched with aliquots of extracted DOM in a concentration series, where the depth origin of the extracted DOM (collected in July) was the same as the water employed for the incubation (collected in September). This series ranged from no enrichment to a maximum DOC concentration of

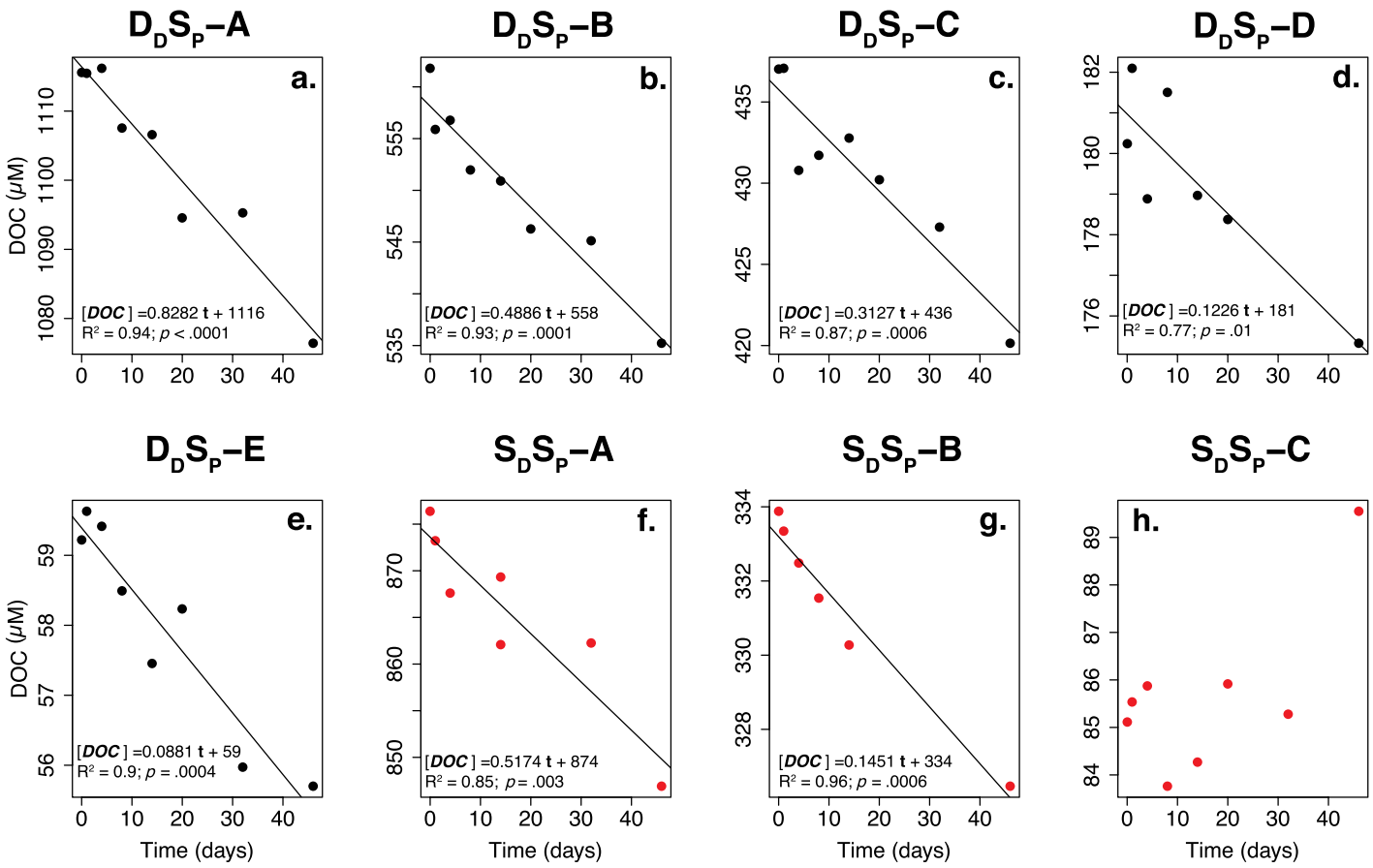

Fig 2. Drawdown of deep (a-e) and surface (f-h) DOC by surface prokaryotes. The different starting concentrations are designated A through E for D_DS_P and A through C for S_DS_P, where A is the most concentrated for each experiment, E is the control for D_DS_P and C is the control for S_DS_P (more details are described Supporting Information Table S1). D_DS_P is deep DOM and S_DS_P is surface DOM, both incubated with surface prokaryotes.

1600 μM (Supporting Information Fig. S1; Fig 2; Supporting Information Table S1). To resuspend the dried DOM, 5 mL of 0.2-μm filtered ambient seawater was added to the dried DOM and shaken vigorously to ensure DOM dissolution. Each incubation was conducted in the dark and subsamples for tracking ammonium, DOC, total dissolved nitrogen, and prokaryotic abundance were collected regularly over the 46-d incubations. The incubations with deep and surface prokaryotes were at 4°C and 25°C, respectively, to keep the communities at their ambient temperatures.

Ammonium measurements

Ammonium concentrations were determined using a fluorescence method with an orthophthaldialdehyde mixed reagent (Holmes et al. 1999). Subsamples were analyzed immediately after collection to reduce potential contamination. Samples were reacted with the orthophthaldialdehyde mixed reagent at room temperature for 2 h. Subsequently, fluorescence was measured on a Turner fluorometer at an excitation/emission wavelength of 350/410–600 nm. Samples were standardized using ammonium chloride. The precision for ammonium measurements was 0.01 μM, or a coefficient of variation of 8%.

Nitrate measurements

Nitrate concentrations are estimated as the sum of nitrate and nitrite $\left(NO_3^- + NO_2^-\right)$. Concentrations were determined via a heated reduction of all NO_x species to nitric oxide (NO) with acidic vanadium ($V(III)$) and analyzed by chemiluminescence (Braman and Hendrix 1989). Samples were standardized using potassium nitrate; the precision of nitrate measurements is $\pm 2\,\mu$M, with a coefficient of variation of 7%.

DOC and total dissolved nitrogen measurements

DOC and total dissolved nitrogen were measured by high temperature combustion using a Shimadzu TOC-L with autoinjection (precision of 2%) (Dickson et al. 2007). Samples of DOC were standardized using potassium hydrogen phthalate and all measurements were quality controlled using Consensus Reference Material (Hansell 2013; Hansell Organic Biogeochemistry Laboratory, University of Miami). The precision for DOC measurements is 1 μM, or a coefficient of

variation of 2–3%, while the precision for total dissolved nitrogen is 0.5 μM, or a coefficient of variation of 5–10%. Dissolved organic nitrogen was then calculated by subtracting the inorganic dissolved nitrogen $\left(NO_3^- \text{and} NH_4^+\right)$ concentrations from total dissolved nitrogen. The propagated uncertainty associated with the dissolved organic nitrogen measurement is $\pm 2\,\mu$M, or a coefficient of variation of 14%.

Prokaryotic abundance measurements, growth rates, and Michaelis–Menten kinetics

To enumerate prokaryotes by flow cytometry, 2 mL samples were fixed with glutaraldehyde (0.5% final concentration), held at 4°C for 10 min, and frozen at −80°C until analysis. Samples were thawed to room temperature and 0.5 mL subsamples were stained with SYBR Green I and placed in the dark for 10 min. Prokaryotes were enumerated on an Attune flow cytometer in a plot of green fluorescence vs. side scatter (Del Giorgio et al. 1996).

Growth rate constants were calculated as follows (Madigan et al. 2015):

$$k = \left(\left(\log_{10}N - \log_{10}N_0\right) * 2.303\right)/t\,) * 24, \quad (1)$$

where k is the specific growth rate in cells h^{-1}, N is the final number of cells in the exponential phase, N_0 is the number of cells at the start of the incubation and t is time (days) of the exponential phase. The equation is converted to log base 2, as the growth constant is assessing the doubling of a cell, and divided by 24 to convert from days to hours.

Temperature plays an important role in the metabolic activity of a cell. To account for change in activity associated with temperature, the rate constants for the incubations with deep prokaryotes were adjusted using Q10, the factor by which the reaction rate increases when the temperature has been raised by 10°C. Here, a Q10 was implemented to account for the relative change in metabolic rate due to the different temperatures of the incubations (25°C and 4°C) The adjusted growth rates that account for temperature are as follows (Raven and Geider 1988):

$$Q_{10} = \left(\frac{k_1}{k_2}\right)^{\frac{10}{T_2 - T_1}}, \quad (2)$$

where k_1 and k_2 are the rate constants at temperatures T_2 and T_1 (4°C and 25°C, respectively).

The quality of DOM as a substrate to heterotrophs was assessed with Michaelis–Menten kinetics (Wright and Hobbie 1966).

$$V_o = V_{max}\left(\frac{[DOC]}{[DOC] + K_m}\right), \quad (3)$$

where V_o is the initial velocity of the reaction, V_{max} is the maximum velocity of the reaction, [DOC] is substrate concentration, and K_m is the Michaelis–Menten constant (the concentration of substrate when the reaction velocity is one half of V_{max}). To calculate K_m, the Michaelis–Menten curve was plotted with the "drm" package in R, using the growth rate constants as the reaction rates for each DOC concentration. The catalytic efficiency (CE) was calculated with the formula:

$$CE = \frac{V_{max}}{K_m}. \quad (4)$$

Finally, the prokaryotic growth efficiency (PGE) assesses substrate incorporated into prokaryotic growth versus total carbon consumption. This term can be expressed as follows:

$$PGE\,(\%) = 100 \times \frac{PP}{TC}, \quad (5)$$

where PP is prokaryotic production at the end of the exponential phase in terms of carbon (assuming one cell is 10^{-15} fg C; Nagata et al. 2000) and TC is the total carbon removed (at t_2; Supporting Information Table S3).

Results and discussion

Preliminary SPE results

The extraction efficiency of DOC was 48% ± 12% for all SPE samples, similar to recoveries reported previously for seawater (Dittmar et al. 2008; Coppola et al. 2015). We extracted 1331 μmol C of DOM from 64 liters of deep water (45% ± 12%, original [DOC] = 46 μM) and 1208 μmol C of DOM from 32 liters of surface water (53% ± 17%, original [DOC] = 71 μM). The justification for these high amounts of extracted organic carbon was to create an environment with much higher substrate concentrations than in nature; our reasoning was that enrichment would improve accessibility to the prokaryotic communities.

Initial conditions of the incubations

Initial values for each variable are given in Supporting Information Table S1. The initial concentrations of DOC and dissolved organic nitrogen varied linearly with DOM enrichment; the more enriched the DOC, the more enriched the dissolved organic nitrogen (Supporting Information Fig. S1A; R^2 = 0.98). This effect was consistent for DOM whether extracted from the surface or deep waters. The C : N ratio of the extracted DOM (calculated as the inverse of the slope in Supporting Information Fig. S1A) was 26, suggesting that it was a fraction of DOM common to both surface and deep waters being extracted. Typically, refractory DOM is rich in carbon relative to nitrogen (C : N ratios from 10 to 25; Benner et al. 1997; Ogawa et al. 1999), while more bioavailable DOM has lower C : N ratios (Ogawa et al. 2001). The C : N ratios of 26 suggest that this isolated DOM fraction was biologically recalcitrant in both depth realms.

Changes in DOC concentration over the incubation period

Over the 46-d incubations, there was no observable drawdown of DOC, whether enriched or ambient, in incubations inoculated with deep prokaryotic communities (Supporting Information Table S2). In contrast, prokaryote communities from the surface exhibited linear drawdowns of DOC (Fig. 2; Supporting Information Table S2) throughout the experiment (i.e., there was no observable change in the shape of the curve, suggesting no change in the bioavailability of the DOM throughout the incubation).

In all incubations with surface prokaryotes, the DOC drawdown fraction was proportionate to the initial DOC concentration (Fig. 3; Supporting Information Table S1). The maximum DOC enrichment (1117 μM C) had a drawdown of 40 μM C, 3.7% of the total DOC (Fig. 3). This fractional drawdown was consistent at all enrichments and combinations (Fig. 3 slope of 4%, R^2 = 0.93); that is, total DOC removed by surface prokaryotes was proportional (4%) to initial DOC concentrations, regardless of depth or concentration factor.

Even when the DOC was concentrated to values that greatly exceed the natural system (1600 μM), only 4% of the DOC pool was available to the microbes (Fig. 3); the other 96% of the DOC remained unavailable. It appears either that concentrating the DOC to these levels was not sufficient to make the majority of it accessible to microbes, or there is another control on the availability of this fraction of organic matter.

Differential microbial response to extracted DOM

Even though the drawdown of DOC was consistently 4%, regardless of concentration enrichment factor or DOM origin, distinctions were observed in the microbial response to the two types of DOM. Microbial response was assessed with changes in prokaryotic abundance, growth rates, and enzyme kinetics. Prokaryotic abundance increased from 2- to 36-fold (Supporting Information Figs. S2, S3). In all experiments, growth rates increased with the initial DOC concentrations, suggesting that the more substrate provided, the more prokaryotes grew, and at faster growth rates (Fig. 4). The deep and surface prokaryotes had different responses to deep and surface DOM, with the surface prokaryotes having higher growth rates than the deep prokaryotes when provided deep DOM (Fig. 4). This offset in growth rate between the surface and deep prokaryotes can be explained by temperature; the deep prokaryotes were incubated at the colder 4°C to reflect their natural environment. The temperature-adjusted rate constants (Eq. 2) suggest that if the deep prokaryotes were incubated at 25°C, they would be at least as efficient at metabolizing deep DOM as the surface prokaryotes (Fig. 4). The inverse is true for the surface prokaryotes; if incubated at 4°C, their metabolic rates would be slower than those metabolic rates for the deep prokaryotes (Fig. 4).

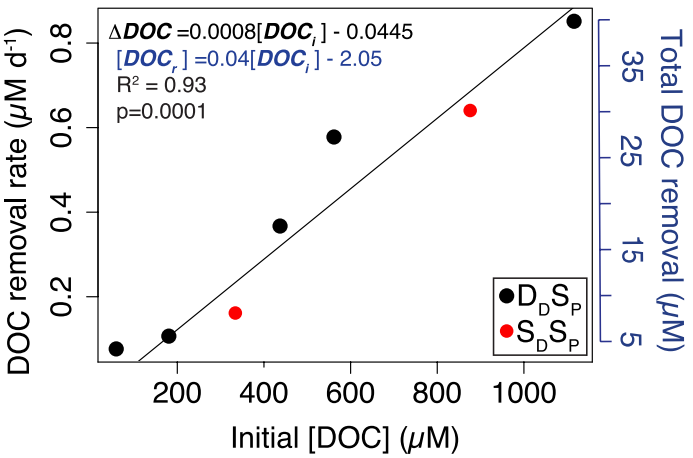

Fig 3. The rate of DOC removal (ΔDOC [μM d^{-1}], black *y*-axis) and the total DOC removed (μM; blue *y*-axis) by surface prokaryotes vs. initial DOC concentration (μM) after addition of deep DOM (D_DS_P, black points) and surface DOM (S_DS_P, red points). The linear equations for each trend are listed in black for the rate of DOC removal and blue for total DOC removal. Note that the removal rate and total removal are directly related (removal rate is divided by total number of days [46]) to establish total DOC removal), so the statistics of each trend are identical.

Typically, Q10 values in biological systems are 2; however, there is a wide range of Q10 values associated with marine DOM consumption. For example, Løngborg et al. (2018) estimated a Q10 of 1.7 for labile material and 8 in the mesopelagic. Busmann (1999) reported Q10 values ranging from 2.9 to 4.8 for humic substances (extracted with an XAD resin) in the Arctic Ocean. Furthermore, Mazuecos et al. (2015) estimated that mesopelagic bacteria in the Indian and Atlantic Oceans have Q10 values of 3.7. A more accurate Q10 relative to this study would thus be between 3 and 8, to represent the range between humic substances and the mesopelagic. With a Q10 of 8, the estimated growth rate of deep bacteria is approximately 450 h^{-1}, which is substantially larger than any of the other growth rates. A Q10 value of 3 is used here to show at a minimum how deep prokaryotes provided DOM would respond at higher temperatures.

When provided deep DOM as substrate, surface prokaryotes grew exponentially, plateauing after 8 d (Supporting Information Fig. S2), at the fastest growth rate for all experiments (Supporting Information Table S3). The sample with the greatest and most rapid growth contained 1117 μM C of deep DOM incubated with surface prokaryotes (Supporting Information Fig. S2); the final number of cells was 36 times greater than the original. The experiments with deep prokaryotes also had exponential growth phases proportional to DOC enrichment (Supporting Information Table S2), where the most enriched incubations had the fastest growth rates (to 8 d), while the growth phase of the control lasted for the entire experiment.

For both surface and deep prokaryotes, incubations with surface DOM as substrate had slower exponential growth phases (Fig. 4); the minimum exponential growth phase for all three incubations was 20 d (Supporting Information Table S3).

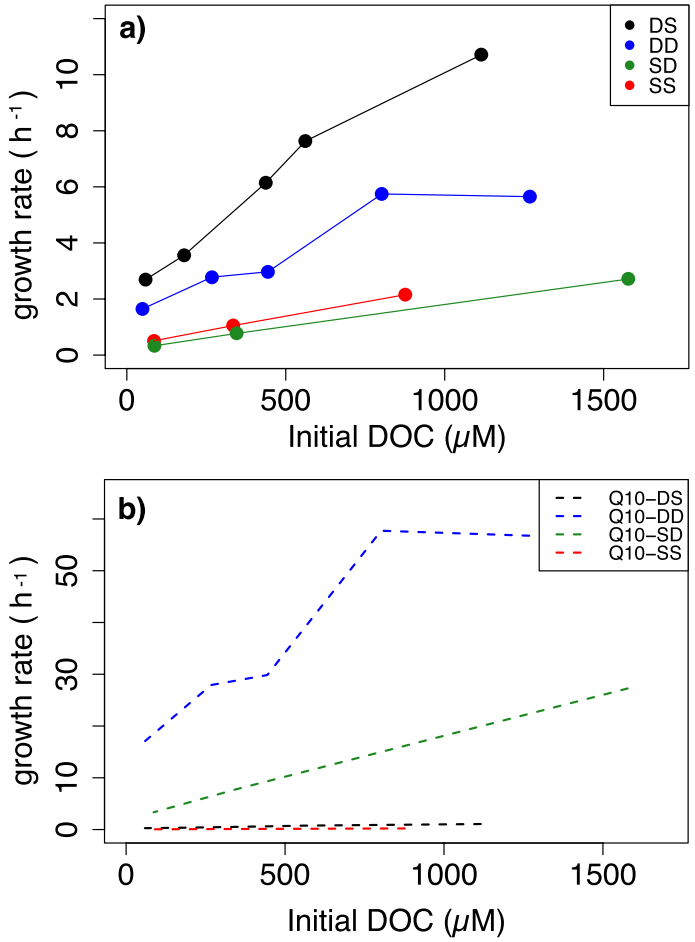

Fig 4. (**a**) Growth rate (k, h^{-1}) vs. initial DOC concentration for each experiment and (**b**) adjusted growth rates (using Q10 = 3; Eq. 2) if deep prokaryotes were incubated at 25°C and surface prokaryotes at 4°C, assuming a Q10 of 3. D_DD_P is deep DOM incubated with deep prokaryotes, D_DS_P is deep DOM with surface prokaryotes, S_DD_P is surface DOM incubated with deep prokaryotes, and S_DS_P is surface DOM incubated with surface prokaryotes.

The incubations with deep DOM as substrate had the highest growth rates for both surface and deep prokaryotes, while those incubations with surface DOM had the lowest growth rates (Eq. 1; Fig. 4; Supporting Information Table S3). These results imply a qualitative difference between the surface and deep DOM substrate.

The K_m (Eq. 3) values for those samples with deep DOM enrichments are 532 and 640 μM for deep and surface prokaryotes, respectively (Fig. 5; Supporting Information Table S3). The catalytic efficiencies (Eq. 4) associated with these K_m values are 0.016 and 0.026 h^{-1}, respectively. The K_m values for those samples with surface DOM enrichments are 2987 and 1027 μM for deep and surface prokaryotes, respectively; their associated catalytic efficiencies are 0.003 and 0.005 h^{-1}, respectively (Fig. 5; Table S3). The surface and deep K_m values are high in all incubations (Fig. 5A), especially when compared to the low concentrations of DOC in the natural environment, and the low catalytic efficiencies suggest that the DOM is difficult to catabolize. Nevertheless, the catalytic efficiencies for those incubations with deep DOM are an order of magnitude higher than those with surface DOM (Fig. 5B). The K_m and CE values (Eqs. 3, 4) suggest that both surface and deep prokaryotes have a lower affinity for surface DOM.

An incubation experiment conducted using waters from the same locale as in this experiment (Florida Strait) found measurable drawdown of bulk surface DOM (i.e., not SPE) by surface and mesopelagic microbial communities when incubated at room temperature (21°C; Letscher et al. 2013). Bulk DOM produced in the surface ocean includes labile, polar organic compounds that facilitate a rapid growth response (Søndergaard and Middelboe 1995; Carlson and Ducklow 1996). However, DOM extracted with the PPL cartridge includes hydrophobic low molecular weight material and some semipolar components such as proteins (Dittmar et al. 2008); while polar, labile compounds such as carbohydrates and acetates are present at higher concentrations at the surface, these molecules would likely not be retained by SPE and thus would be largely absent in surface DOM. Extracted DOM has a more depleted $\Delta^{14}C$ value than bulk surface DOM (Coppola et al. 2015). Therefore, if bulk DOM from the surface were to be used in here instead of DOM, past studies suggest that we would observe a different response than observed in this study. Letscher et al. (2013) observed differences in microbial response to bulk surface and deep DOM. However, as we used extracted DOM in our study, there was a similar DOC drawdown to the deep and surface fractions.

The results of this experiment show that the drawdown of carbon is proportional to DOM enrichment (Fig. 2). However, this drawdown greatly exceeds the amount of carbon (10–15 fg per bacterial cell [1 fg = 10^{-15} g], Nagata et al. 2000; Reinthaler et al. 2006) required to be incorporated into prokaryotic biomass in support of their growth. Given that each cell is approximately 10 fg C (8.3×10^{-16} mol C), the concentration of carbon associated with cell growth at the end of the exponential phase ranged from 0.01 to 0.2 μM C in all incubations where DOC drawdown was observed. The resulting prokaryotic growth efficiencies (Eq. 5; Supporting Information Table S3) indicate that ≤ 1% of all carbon consumed was assimilated, apart from when > 1000 μM deep DOC was incubated with surface prokaryotes, which held a prokaryotic growth efficiency of 2.5% (Supporting Information Table S3). Considering these low growth efficiencies and high respiration rates, the majority of DOM consumed by the prokaryotes was directly respired; its primary role was the gain of energy.

Past studies in oligotrophic environments similar to the waters described here report prokaryotic growth efficiencies ranging from > 1% to 2% (Del Giorgio and Cole 1998; Baltar et al. 2010). The prokaryotic growth efficiencies from this experiment are either similar or lower, regardless of concentration factor, suggesting that extracted DOM is not as readily incorporated into biomass for growth when compared to bulk

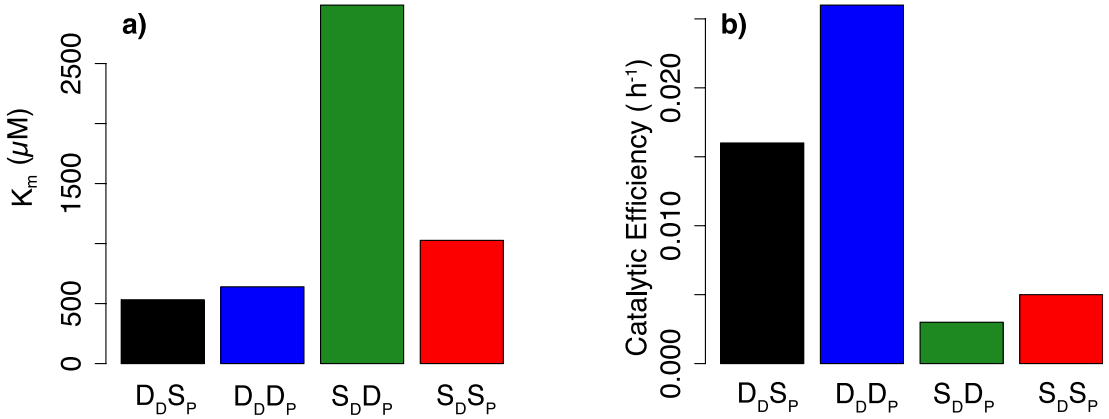

Fig 5. (**a**) Michaelis–Menten constants (K_m; μM) and (**b**) catalytic efficiencies (h^{-1}) for each incubation, where D_DD_P is deep DOM incubated with deep prokaryotes, D_DS_P is deep DOM with surface prokaryotes, S_DD_P is surface DOM incubated with deep prokaryotes, and S_DS_P is surface DOM incubated with surface prokaryotes.

DOM, even in oligotrophic environments. One reason for this high respiratory fate of the utilized DOM could be nitrogen limitation; for every μmol of DOC that was consumed in this experiment, less than 0.01 μmol of dissolved organic nitrogen were made accessible to microbes (calculated based on the linear equation in Supporting Information Fig. S1). Considering that microbe biomass typically has low C : N ratios (5–7; Ogawa et al. 2001), prokaryotes would require substantially more nitrogen for every unit of carbon consumed in order to convert the substrate to biomass. Past studies have suggested that the C : N ratio plays an important factor as to whether a substrate will be assimilated into biomass (Del Giorgio and Cole 1998). Amino acids, for example, with their low C : N ratios, enhance prokaryotic growth rates and efficiencies (Del Giorgio and Cole 1998; Amon and Benner 1996). The C : N ratios of the DOM extracted in this study were high, so the microbes were not provided an adequate supply of N.

Generally, DOM from the deep would more closely resemble bulk DOM from the deep, as there is less labile, high-molecular-weight material there. Surface prokaryotes are accustomed to a diet of labile DOM that can easily be assimilated into their biomass (Carlson and Hansell 2015). Here, the surface prokaryotes were provided with high concentrations of substrate in the form of extracted DOM, which instead would consist of a higher proportion of humics and lipids that would be of a lower quality for assimilation than molecules such as amino acids and carbohydrates, which can be directly assimilated into a cell. As such, when exposed to DOM, the respiration rate, rather than the assimilation rate, is much higher because the DOM is less able to be incorporated for growth, likely due to its unfavorable C : N ratio. The extracted DOM also requires a high amount of energy to break down, as evident in the high K_m and CE values.

Discrepancy between surface- and deep-extracted DOM

The offset in surface- and deep-extracted DOM as an incorporable substrate for both surface and deep prokaryotes questions the classic two-component model of DOM, in which there is a labile fraction overlaying a recalcitrant fraction. In this classic view, recalcitrant DOM is largely homogenously distributed throughout the water column, so the key difference between the deep and the surface is that the surface layer also includes labile and semilabile, photosynthetically derived DOM (Hansell 2013). The similar C : N ratios in both the surface and deep DOM in this experiment support the homogeneity of the recalcitrant fraction of DOM. However, the distinct microbial responses to the two depths, regardless of concentration, suggest a qualitative difference in the bioavailability of recalcitrant DOM from different depth realms.

Surface- and deep-extracted DOM have different molecular compositions; the deep has highly aromatic compounds and polycyclic aromatics while the surface DOM includes highly unsaturated aliphatic compounds due to the addition of photodegradation products (Medeiros et al. 2015). The light-absorbing aromatic components of deep DOM are produced in situ by bacteria in the deep ocean (Yamashita and Tanoue 2009). However, once deep waters reach the surface in upwelling regions, DOM in those waters is exposed to sunlight and can phototransform (Mopper et al. 1991). In fact, a study testing the photochemical transformations of deep DOM found that it became more chemically stable and similar in molecular composition to surface-extracted DOM when exposed to sunlight (Medeiros et al. 2015). The photodegradation products would be present in the surface DOM of this experiment and therefore would potentially be more resistant to chemical attack than the DOM from the deep, which would theoretically contain less of this transformed material.

Deep microbial processing could also play a role in providing a bioavailable portion of deep DOM. Sinking particles containing fresh organic material solubilize at depth and undergo microbial processing, generating semilabile or semirefractory material (Jiao et al. 2010; Hansell 2013). Furthermore,

bioavailable DOM produced from chemoautotrophy and subsequent microbial processing could play a role in the deep ocean (Reinthaler et al. 2010). Autotrophic fixation of DOM in the deep supports free living prokaryotic communities (Hansman et al. 2009); processes such as chemoautotrophy and localized microbial transformations of sinking material could provide a bioavailable source of deep DOM absent in the surface DOM.

Another reason the deep DOM appears more bioavailable than its surface counterpart could be due to a nutrient limitation in the incubations with surface waters. The SPE cartridges do not concentrate inorganic nutrients, so the nutrients present in the incubation were there only from the background waters of the experiment. Oligotrophic surface waters are depleted in nutrients such as nitrate and phosphate; this low-nutrient character would have been present in our surface incubations as well. The incubations conducted in deep water would have higher concentrations of nitrate (31 μM), phosphate and iron, which could have removed the nutrient limitation of the prokaryotes, thus increasing the catalytic efficiencies for those incubations with deep DOM. As the only substrate we provided the prokaryotes was recalcitrant DOM, the addition of nutrients associated with deep waters could have enhanced the biological uptake of DOM from the deep as opposed to the surface. Enhanced bacterial production in incubations amended with nutrients has been reported previously. For example, DOM accumulates in the Mediterranean due to its low phosphate content (Thingstad et al. 1998), yet in an incubation of Mediterranean waters amended with additional phosphate, bacterial production increased (Thingstad et al. 2005). Nitrate and phosphate amendments added to incubations with labile DOM caused an increase in bacterial production (Carlson et al. 2002). Nutrient limitation could therefore play a key role in why we observe differences in the prokaryotic response to surface and deep DOM.

Implications for DOM stability in the ocean: The microbial side of the paradox

The consistent 4% drawdown of DOC by surface microbes, regardless of concentration factor, suggests that only a small and specific portion of DOM was made accessible to prokaryotes in this experiment. One explanation is that the inaccessible molecules (those not microbially utilized) in DOM did not meet their "concentration threshold", requiring even more concentrating to become accessible to the prokaryotic community. In this scenario, the linear drawdown of DOC would turn into a nonlinear decline as more molecules met their threshold and became bioavailable with concentrating. However, as we never reached the point in this experiment where the drawdown was nonlinear, we cannot determine at which DOC concentration more than 4% of the molecules within DOM would become available. Considering the high molecular diversity in DOM, the concentration threshold to exceed limitations due to dilution could be very high.

These results are similar to what was observed in previous work; while there was an increase in microbial abundance and specific growth rate when microbes were provided enhanced concentrations of DOC (Arrieta et al. 2015), less than 6% of the accessible DOC in that experiment was consumed (Jiao et al. 2015). We found likewise an increase in microbial abundance and specific growth rates (Fig. 4), yet only 4% of DOC were accessible to the microbial communities and consumed, regardless of concentration factor or depth origin.

Alternatively, the intrinsic stability hypothesis states that factors such as chemical resistance to microbial breakdown govern the bioavailability of DOM in the ocean (Dittmar 2015). In that case, DOC drawdown would always remain 4% (i.e., the same 4% remains accessible), even if the substrate was concentrated to extremely high concentrations. In a series of bioassay studies, C18-extracted DOC provided to microbes was not consumed at all (Shen and Benner 2018, 2020), and only when also subjected to other conditions (photodegradation, priming, different regions), were there observable changes in DOC consumption.

These mechanistic explanations have focused on the nature of the DOM being its limiting factor: it is either too low in concentration or its molecular structure is intrinsically not available. But the biological indicators in this study suggest that deep DOM does not support microbial growth (i.e., low prokaryotic growth efficiency). Even though there is clear remineralization of the DOC (Fig. 2), low prokaryotic growth efficiencies suggest that very little of it is assimilated into biomass; therefore most of the carbon was respired. Would microbes select against substrates that do not support growth?

Just because microbes are not carbon limited does not mean they are not energy limited. For example, microbial growth on oxidized substrates (acetate, glycolate, and even glucose) is energy limited. These compounds are incorporated into microbial biomass with low efficiency, even in favorable environments, such as an excess of inorganic nutrients (Del Giorgio and Cole 1998). Deep DOM is considered to be highly oxidized, and the low energetic yield of degrading these highly oxygenated compounds is likely not sufficient to gain metabolic energy for growth. An additional consumption of substrate with higher energetic yield may therefore be required in order to gain biomass (Kattner et al. 2011).

In this experiment, the K_m and CE values suggest that a lot of energy is needed to break down the DOM. As such, if a substrate is limited in quality, the vast majority of it would be used primarily for maintenance energy rather than growth (Russell and Cook 1995; Del Giorgio and Cole 1998). Likewise in this study, microbes from both the surface and deep were able to catabolize a portion of the extracted DOM in order to meet their minimum energy requirements. However, in order to efficiently grow, perhaps microbes would rely on a different substrate, particularly one that provides more energy and nitrogen.

Sinking biogenic particles introduce readily bioavailable DOM, which supports growth, so selection would favor that substrate source. Past studies have illustrated the importance of sinking particles as a bioavailable substrate for deep bacteria (Nagata et al. 2000; Hansell and Ducklow 2003; Yokokawa et al. 2013). Bacteria turnover in the bathypelagic is very slow (up to 30 years; Nagata et al. 2000, 2010; Reinthaler et al. 2006), implying that deep microbes there have little opportunity to grow. While microbes may have modest access to recalcitrant DOM to maintain their minimum catabolic energy required to survive, it is energetically costly to break down and thus would likely not be selected were there another available substrate. Sinking particles deliver a more energetic and N-enriched form of DOM everywhere in the ocean; perhaps the access to those molecules precludes further use of recalcitrant DOM.

Regardless of whether extracted DOM is highly molecularly diverse or too intrinsically stable, it does not appear to be the primary source of growth for marine microbes. While a marine microbe may respire small amounts of recalcitrant DOM to survive, it likely relies on periodic events of sinking biogenic particles for substantial growth. Recalcitrant DOM, therefore, remains largely untouched, contributing to its persistence in the ocean for millennia.

Summary

In waters from the subtropical surface and mesopelagic North Atlantic, prokaryotes from the surface ocean provided with concentrated DOM up to 1600 μM C were able to measurably draw down up to 40 μM of DOC, although most of it was not assimilated into biomass. Regardless of concentration factor, only 4% of the extracted DOM was accessible to the prokaryotes in the experiment. Biological indicators such as k_m and catalytic efficiencies suggest that the deep DOM was easier to catalyze than surface DOM for both surface and deep prokaryotes, yet both types of DOM yielded low prokaryotic growth efficiencies, indicating that most of the carbon was respired rather than incorporated into biomass. Overall, the results of this study suggest that there likely exist multiple controls on the microbial utilization of recalcitrant DOM.

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Conflict of Interest

None declared.

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