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Understanding marine dissolved organic matter production: Compositional insights from axenic cultures of *Thalassiosira pseudonana*

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Abstract

Marine dissolved organic matter (DOM) is a key source of carbon and nutrients to microbial life in the oceans, but rapid biological utilization of labile DOM confounds its compositional characterization. In order to characterize potentially bioavailable DOM produced by phytoplankton, DOM from axenic cultures of *Thalassiosira pseudonana* cultivated in phosphorus (P) replete and low P conditions was extracted using high-recovery electrodialysis (ED) techniques, which resulted in an average dissolved organic carbon (DOC) recovery of 76% ± 7% from all cultures. Low P concentrations resulted in greater cell-normalized production of DOC relative to P replete culture controls at the same growth phase. Despite the different nutrient conditions, DOC composition and DOM molar ratios of carbon to nitrogen (C : N) were similar in all cultures. In contrast, low P concentrations influenced DOM molar carbon to phosphorus (C : P) ratios and dissolved organic phosphorus (DOP) composition. Under P replete and low P conditions, DOM C : P ratios were 130 (± 22) and 2446 (± 519), respectively. ^{31}P Nuclear Magnetic Resonance (NMR) spectroscopy identified P esters (> 90% of DOP) as the dominant P species in DOM produced under P replete conditions, with small or negligible contributions from phosphonates or glycerol P and polyphosphates. However, based on direct fluorometric analysis, DOP from low P cultures was greater than 8 times enriched in dissolved polyphosphate compared to DOP from replete cultures, which is consistent with the growing evidence that polyphosphate is a dynamic component of total P in low P ocean regions.

Dissolved organic matter (DOM) is a source of bioavailable carbon (C) and nutrients, including nitrogen (N) and phosphorus (P). In the ocean, DOM supports the growth of marine bacteria (Azam et al. 1983) and phytoplankton (Bronk et al. 2007; Duhamel et al. 2010), especially in oligotrophic ocean regions (Thomas 1971; Fogg 1983; Ducklow et al. 1995; Karl et al. 1998; Agusti and Duarte 2013). Furthermore, DOM composition influences marine microbial community structure (Neogi et al. 2011; Gomez-Consarnau et al. 2012; Nelson and Carlson 2012; Dinasquet et al. 2013) and ultimately carbon remineralization and export (Letscher and Moore 2015). Despite its critical role in ocean biogeochemistry, the composition of DOM remains poorly understood (Benner 2002).

DOM is produced via degradation of non-living organic matter and is also actively produced by living organisms (Carlson and Hansell 2014) including phytoplankton, which are considered a source of labile DOM (Cole et al. 1982; Norrman et al. 1995). Labile DOM is preferentially utilized, predominantly by marine heterotrophs (Jensen 1983; Kirchman et al. 1991; Reinthaler and Herndl 2005; Carlson and Hansell 2014), over timescales of minutes to weeks (Amon et al. 2001; Carlson 2002). However, ambient pools of marine DOM, which are typically characterized in compositional studies (Benner 2002), have radiocarbon ages in the range of thousands of years (Williams and Druffel 1987; Bauer et al. 1992; Loh et al. 2004; Karl and Bjorkman 2014;

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Druffel and Griffin 2015) and, thus, may not reflect freshly produced, labile DOM.

Ambient pools of marine DOM contain dissolved organic forms of C (DOC), P (DOP), and N (DON), including biomolecules and their derivatives (Repeta 2014). Of these forms, this study focuses on DOP in order to better understand intriguing compositional observations in the ocean. Diverse regions and depths of the ocean contain relatively consistent proportions of polyphosphates (molecules with at least three P atoms joined by P-O-P bonds), 8–13% of DOP; phosphonates (direct C-P bonds), 5–10%; and P esters (P-O-C bonds), 80–85% (Young and Ingall 2010). This uniform composition of DOP, which is distinct from that of marine organisms, has been attributed to microbial decomposition processes (Clark et al. 1998, 1999; Kolowith et al. 2001; Karl and Bjorkman 2014; Young and Ingall 2010). However, despite the critical role of these P forms as potential P sources (Martin et al. 2014; Van Mooy et al. 2015) and as possible drivers of climate (Diaz et al. 2008; Karl et al. 2008), the composition of DOP initially produced by microorganisms remains largely unexplored.

In order to characterize the DOM fraction initially supplied to marine systems, previous studies have examined DOM produced in controlled cultures of marine phytoplankton (Helebust 1965; Myklestad 1977; Obernosterer and Herndl 1995; Biddanda and Benner 1997; Biersmith and Benner 1998; Aluwihare and Repeta 1999; Granum et al. 2002). DOM compositional characterization in these studies focused on either specific compounds, such as polysaccharides, or bulk analysis of the high molecular weight fraction of DOM. Compositional differences between phytoplankton-derived DOM and ambient ocean DOM identified in these studies were attributed to selective utilization of certain compounds by bacteria (Biddanda and Benner 1997; Biersmith and Benner 1998; Aluwihare and Repeta 1999). In fact, the composition of phytoplankton-derived DOM can be altered by heterotrophic processing on timescales of days to weeks due to preferential consumption of not only specific compound classes, but also of specific molecules within compound classes (Amon et al. 2001).

Limited evidence based on analysis of specific compounds or bacterial utilization of DOM produced in culture studies suggests that nutrient availability influences the composition of phytoplankton-derived DOM (Myklestad and Haug 1972; Myklestad 1977; Obernosterer and Herndl 1995; Puddu et al. 2003). For example, phytoplankton growth under low P conditions can result in the production of DOM that supports a distinct microbial community compared to DOM produced under P replete conditions (Puddu et al. 2003), which may reflect bioavailability differences and, thus, compositional differences between DOM pools produced under different P regimes. Although N is often the limiting nutrient over vast marine areas, spatial and temporal increases in N abundance may shift systems to P limitation (Karl et al. 1995, 1997; Moore et al. 2013). Phytoplankton alter cellular P allocation in response to P scarcity, specifically through the substitution of sulfolipids for phospholipids (Van Mooy et al. 2009; Martin et al. 2011) and modulation of cellular phosphorus pools like polyphosphate compounds (Dyhrman et al. 2012; Martin et al. 2014; Diaz et al. 2016). Whether these P-dependent particulate dynamics translate to similar compositional shifts within the DOP pool remain unknown.

For all DOM studies, there are difficulties associated with obtaining a representative sample for chemical analysis (Hedges 1992; Benner 2002). Most chemical characterization techniques require naturally low concentrations of marine DOM ($\sim$ 1 ppm) to be isolated from the high salt content of seawater ($\sim$ 35,000 ppm) (Repeta 2014). Isolation difficulties traditionally limit DOM studies to the analysis of a small number of molecules that can be directly measured in a high salt matrix or to the characterization of the partial DOM fractions that can be recovered. For example, many studies have isolated DOM via ultrafiltration, which recovers the high molecular weight fraction of DOM ($>$ 1 kDa) (see references in Benner 2002). Thus, low molecular weight, potentially bioavailable molecules typically escape characterization (Kujawinski 2011). In fact, phytoplankton-derived DOM is dominated by low molecular weight molecules (Jensen 1983; Lancelot 1984), which have been suggested to have a composition distinct from molecules in the high molecular weight fraction (Benner et al. 1992; Biddanda and Benner 1997).

Recently developed electrodialysis (ED) extraction techniques have improved DOM recoveries by approximately three times compared to ultrafiltration (Koprivnjak 2009; Green et al. 2014). Higher recovery yields a final sample that is more representative of bulk DOM by diminishing the bias towards the high molecular weight DOM fraction recovered using ultrafiltration (Vetter et al. 2007; Koprivnjak et al. 2009). Thus, ED techniques offer the potential for new insights into the marine DOM composition. For example, the application of ED has recently revealed the presence of dissolved polyphosphates in diverse marine waters (Diaz et al. 2008; Young and Ingall 2010), which typically go undetected in ultrafiltration studies (e.g., Clark et al. 1998, 1999; Kolowith et al. 2001; Sannigrahi et al. 2006).

In previous culture studies, the low DOM extraction efficiencies of ultrafiltration required processing large volumes to obtain analytically relevant quantities for studies of bulk DOM composition (Biddanda and Benner 1997). Large volumes made it difficult to evaluate the composition of phytoplankton-derived DOM in the absence of bacteria and fungi (axenic conditions), which can consume the most bioavailable fractions on time scales of minutes to hours (Carlson 2002). The larger recoveries of ED permit the use of smaller culture volumes to obtain analytically useable DOM quantities for bulk compositional analyses. Additionally,

smaller culture volumes simplify the creation and maintenance of axenic conditions and allow for greater levels of replication and evaluation of key influences on DOM composition, such as nutrient availability. Thus, ED offers the opportunity to characterize labile DOM that is rapidly utilized. Here, ED techniques were used to extract DOM produced under P replete and low P conditions in triplicate, axenic cultures of the marine diatom, *Thalassiosira pseudonana*, in order to provide insight into the composition of freshly produced, potentially bioavailable DOM supplied to marine systems.

Methods

Cultures

Axenic *T. pseudonana* CCMP1335 was obtained from the National Center for Marine Algae and Microbiota (NCMA), Bigelow Laboratories, East Boothbay, ME. *T. pseudonana* was grown in f/2 medium (Guillard and Ryther 1962) prepared in an artificial seawater base (Kester et al. 1967). Artificial seawater without sodium biocarbonate was autoclaved before the addition of filter-sterilized (0.22 μm) sodium bicarbonate, nutrients, vitamins, and trace metals. For P replete cultures, the phosphate and nitrate concentrations were 36 μM and 880 μM, respectively, yielding a molar N : P ratio of 24. Cultures grown under the low P conditions were cultivated with a $\sim$ 30 fold reduction in inorganic P in culture media. For low P cultures, phosphate was reduced to 1 μM (N : P = 880). Cultures (2.5 L) for each nutrient regime were grown in triplicate at 22°C on a 12 h : 12 h light : dark cycle at 100 μE m^{-2} s^{-1} light intensity in 4 L borosilicate glass flasks. All cultures were inoculated with cells at the same growth phase grown under nutrient replete conditions. Growth of each replicate was monitored daily by measuring chlorophyll *a* (Chl *a*) fluorescence (excitation: 440 nm, emission: 680 nm; Shimadzu RF-5301PC Spectrofluorophotometer) (Lorenzen 1967). During early logarithmic phase, cell abundances were calculated using our empirically determined relationship between Chl *a* fluorescence and direct microscopic counts. In late logarithmic phase, cells were counted directly to reduce the uncertainty associated with using Chl *a* as a proxy for biomass under different nutrient regimes. In order to reduce the potential for differences in the rate of DOM release and DOM composition as a function of growth phase, DOM was harvested in the late logarithmic phase of growth for each nutrient condition (Fig. 1) (Myklestad et al. 1989; Urbani et al. 2005; Barofsky et al. 2009). Harvesting in the late logarithmic phase also limits the degree of DOM recycling and alteration by *T. pseudonana*, which would likely increase late into stationary phase as inorganic nutrients are depleted and DOP hydrolytic enzymes are induced (Dyhrman et al. 2012). Specifically, alkaline phosphatase activity increases in stationary phase cultures of *Emiliania huxleyi* (Dyhrman and Palenik 2003) and *Escherichia coli* (Chou et al. 2005), indicating a rise in the enzymatic hydrolysis of dissolved organic P, which is necessary to avoid in the current study. Harvesting DOM at this stage of growth should also mitigate additional DOC inputs due to cell lysis during stationary phase (Puddu et al. 2003). In order to consistently sample cultures, late logarithmic phase was defined to occur 24 h before the maximum cell density was reached for each nutrient condition. This experimental design was modeled after previous work focused on examining the physiological impact of low P on phytoplankton (Dyhrman and Palenik 1999, 2003; Wurch et al. 2014) including *T. pseudonana* (Dyhrman et al. 2012). Previous work has shown that *T. pseudonana*, grown in N : P > 800 and sampled in late logarithmic phase, exhibits a multifaceted physiological response to low P at the transcript, protein, and activity level (Dyhrman et al. 2012). Axenic conditions were confirmed for all cultures through plating on LB medium agar (DeBrouwer et al. 2002) and/or inoculating the culture into the heterotrophic K-medium and incubating in the dark. A *T. pseudonana* culture grown under non-sterile conditions was used as a positive control in our tests for axenic conditions.

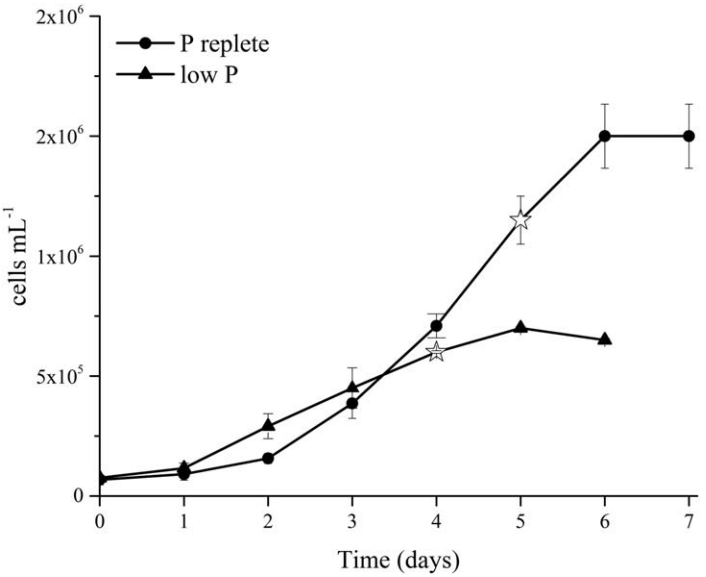

Fig. 1. Growth curves for P replete and low P *T. pseudonana* cultures. Stars indicate the time of harvest. During early logarithmic phase, cell abundances were calculated using our empirically determined relationship between Chl *a* fluorescence and direct microscopic counts. In late logarithmic phase, cells were counted directly. Average and standard deviation cell density measurements (cells mL^{-1}) of triplicate cultures for each nutrient condition at the time of harvest were as follows: P replete ($1 \times 10^6 \pm 1 \times 10^5$) and low P ($6 \times 10^5 \pm 5 \times 10^3$). Error bars indicate standard deviation of triplicate cultures for each condition.

Dissolved organic matter isolation

DOM produced by *T. pseudonana* was harvested by aseptically separating biomass from the media via centrifugation

(2.2×10^3 g, 7 min) and subsequent filtration (< 0.85 bars; Whatman GF/F, 0.7 μm nominal). This sample was then processed with ED within 24 h. This study used solely ED techniques to desalt DOM in contrast to previous studies that used ED in conjunction with reverse osmosis (Vetter et al. 2007; Koprivnjak et al. 2009; Green et al. 2014). With the system used in this study, the approximately 3 L starting volume is directly freeze-dried after ED processing, removing the need for water volume reduction by reverse osmosis. Thus, DOM losses that typically occur during reverse osmosis processing were avoided. ED was performed using Deukum electrodialysis stack (Deukum GmbH, Frickenhausen, Germany) coupled to a circulation system built in our lab. To condition the ED stack for sample processing, it was first rinsed with ultrapure water (18 MΩ cm), followed by sterile f/2 media. The electrodialysis stack consists of 10 cell pairs of alternating Neosepta anion AMX and cation CMX exchange membranes (Astom Corp., Tokyo, Japan) held between a cathode and anode situated at opposite ends of the stack. The electric potential across the stack was supplied by a 1.2 kW Sorensen DCS150-8EM1 power supply. Three pumps and associated valves control the flow and pressure of the sample, referred to as the diluate, the concentrate, which is the water reservoir that receives ions from the sample, and electrode rinse, which is a sodium sulfate solution that maintains ionic balance between the cathode and anode. During the ED process, anions are pulled through the AMX membranes toward the anode, while cations are pulled through CMX membranes toward the cathode. As the ions are pulled to their respective electrodes, they move from the diluate flow into the concentrate flow. The conductivity of the sample and the applied amperage were continuously monitored to keep the system below the limiting current density, which prevents large excursions in pH during sample processing (Vetter et al. 2007; Young and Ingall 2010). During ED processing, the concentrate was changed once when the conductivity of the sample was reduced to approximately 4.0 mS cm^{-1}. When changing the concentrate, the ED process was paused and 5/6 of the concentrate replaced with fresh, ultrapure water. This approach allows desalination to continue without causing a drastic salinity differential between the concentrate channel and the diluate channel, which improves DOM recoveries. Samples were processed from an initial conductivity $\sim 5 \times 10^4$ μS cm^{-1} to a conductivity of $\sim$ 150 μS cm^{-1}. The resulting desalinated sample was freeze-dried, homogenized using an agate mortar and pestle, and stored at −20°C until analysis.

Extraction and concentration of DOM using ED resulted in an average DOC recovery of 76% ± 7% from all cultures. These DOC recoveries are much greater than the highest recoveries obtained using the previously applied techniques including solid phase extraction, ultrafiltration, and ED coupled with reverse osmosis (Benner et al. 1992; McCarthy et al. 1993; Guo et al. 2000; Dittmar et al. 2008; Walker et al. 2011; Stubbins et al. 2014; Hertkorn et al. 2013; Green et al. 2014; Arrieta et al. 2015). The unrecovered DOC likely consists of surface-reactive molecules that are adsorbed to ED membranes. In other studies, such surface reactive molecules are desorbed from membranes using a strong base rinse of the system (Koprivnjak et al. 2011; Green et al. 2014). Because the use of strong base confounds characterization methods and likely alters DOM composition, it was avoided in this study. ED optimization experiments testing the recovery of specific compounds dissolved in artificial seawater achieved a 90.2% ± 2.1% recovery of glucose (molecular weight 180.2 g mol^{-1}), a 67.5% ± 9.9% recovery for ethylenediamine tetraacetic acid (EDTA, molecular weight 292.2 g mol^{-1}), and a 98.3% ± 1.6% recovery for vitamin B_{12} (molecular weight 1355.4 g mol^{-1}). The difference between glucose, EDTA, and vitamin B_{12} recoveries is consistent with the idea that charged molecules, like EDTA, are more likely to be sorbed to ED membranes relative to neutral molecules, like glucose and vitamin B_{12} (at the ED operating pH).

Dissolved phase analysis

Sample aliquots collected at the beginning and end of ED process were characterized with several methods to quantify the recoveries associated with the desalination process. DOC was measured using high temperature catalytic oxidation on a Shimadzu TOC-V (Benner and Strom 1993; Sharp et al. 2002). Dissolved inorganic carbon was measured with a flow injection analysis system by conductivity detection (Analytical Instruments Systems, Inc. LCC100 integrator) (Hall and Aller 1992). Dissolved inorganic nitrogen (nitrate and nitrite) was measured using anion chromatography (Metrohm A Supp 5 150.0/4.0 mm) coupled to ultraviolet detection (Beckman Coulter DU 720 detector) with a sodium chloride eluent (Beckler et al. 2014). Soluble reactive phosphorus was measured by spectrophotometry (Murphy and Riley 1962).

Solid phase analysis

Total carbon, nitrogen, and phosphorus were measured in freeze-dried DOM samples. Total phosphorus was measured by utilizing high temperature combustion (550°C for 2 h) followed by extraction in 1 N HCl agitated for 24 h (Solorzano and Sharp 1980) prior to spectrophotometric analysis (Murphy and Riley 1962). Carbon and nitrogen concentrations were measured using a CHNSO analyzer (Costech instruments) (Hedges and Stern 1984). Polyphosphate was quantified using a slightly modified version of the enzymatic extraction and fluorometric quantification method described in Martin and Van Mooy (2013). For these samples, nucleases were used to remove nucleic acid interference, as nucleic acids will also fluoresce in the presence of 4′,6′-diamidino-2-phenylindole (DAPI), the fluorometric stain used to quantify polyphosphate.

^{13}C NMR analysis

^{13}C cross polarized (CP) NMR spectra of freeze dried DOM samples were collected on a Bruker AVANCE III 400 spectrometer equipped with a Bruker double-channel 4-mm probe and operated at a ^{13}C frequency of 100.6 MHz. A variable-amplitude CP (VACP) sequence pulse was used with a 1.0 ms contact time. The samples were packed into a 4 mm diameter zirconia rotor with Kel-F Caps (Wilmad, New Jersey) and spun at 10 kHz. Chemical shifts for ^{13}C spectra were externally referenced using adamantane. The spectrum for each sample was phased, baseline-corrected, and integrated using Bruker's Topspin 3.0 software. The ^{13}C NMR spectral data were entered into a mixing model to estimate the molecular composition of the DOM in terms of non-specific amino acid, carbohydrate, and lipid content (Nelson and Baldock 2005; Sannigrahi et al. 2005). The relative intensity of seven chemical shift regions from the spectrum of each sample was fit and the C : N ratio of each sample was used as a constraint on compositional identifications. The fitting was performed using the average, upper, and lower limits of the C : N ratios representing the average and ± standard deviation of DOM C : N ratios from triplicate cultures.

^{31}P NMR analysis

Solid-state ^{31}P NMR spectra on solid samples were recorded on a Bruker AVANCE III-HD 500 spectrometer operating at 202.489 MHz using a 1.9 mm magic angle spinning (MAS) double resonance probe. SPINAL64 (McGeorge et al. 1999) proton dipolar decoupling with a field strength of 145 kHz was applied during acquisition, and a MAS speed of 20 kHz was used. Bloch decays of 50 ms were collected with a 200 parts per million (ppm) window after 30 degree excitation pulses. The number of transients collected varied, depending on the amount of material and ranged from 50,000 to 100,000 scans per sample (24–48 h).

Spectra were fit with up to 5 Lorentzian lines at 18 to 20 ppm (phosphonate or glycerol 1,2-cyclic phosphate), 0 to 2 ppm (orthophosphate), −10 to −12 ppm (diesters), and −22 to −24 ppm (polyphosphate) to determine percentage of each compound in total P. The NMR peak observed in the 18 to 20 ppm region of the NMR spectra has been traditionally attributed to the presence of phosphonates, however some ^{31}P NMR studies of *T. pseudonana* suggest that glycerol 1,2-cyclic phosphate, a biomolecule associated with phospholipid metabolism, is also found in this spectral region (Boyd et al. 1987; Tesson et al. 2008). Values reported have a technical error of ± 5%, similar to solid-state ^{31}P NMR studies in other systems (Diaz et al. 2008).

Results

Average cell-normalized concentrations of DOC (pg cell^{-1}) in the unprocessed culture media were twice as high under low P compared to P replete control conditions: P replete, 3 (± 1) and low P, 6 (± 1). Extraction and concentration of DOM using ED resulted in an average DOC recovery of 76 (± 7)% from all cultures. DOC recoveries for P replete controls and low P cultures were 76 (± 7)% and 75 (± 9)%, respectively. The unrecovered DOC likely consists of surface-reactive molecules that are adsorbed to ED membranes (see Methods). The exact composition of surface adsorbed molecules cannot be determined, but the consistency of recovery among nutrient regimes suggests that a quantitatively and compositionally similar fraction was lost from all nutrient regimes. ED effectively removed inorganic C (> 95%), which was consistently below the detection limit (150 μM) in all samples after ED processing. ED also removed inorganic N (99.79% ± 0.01%), and P (95.8% ± 0.9%) from all samples confirming that the material recovered by ED was dominantly composed of organic material.

Table 1. ^{13}C NMR speciation results of different biomolecules as a percent of total DOC

Biomolecule	P replete	Low P
Carbohydrates	30 ± 8	30 ± 3
Amino acids	70 ± 8	69 ± 5
Lipids	0 ± 0	<1 ± 2

Table 2. DOP and relative quantities of dissolved polyphosphate (polyP) content

Species	P replete	Low P
DOP (nmol mg^{-1})	13.0 ± 0.4	0.6 ± 0.4
polyP (peq mg^{-1})	548 ± 39	252 ± 27
polyP:DOP (eq mol^{-1})	4.2 ± 1.8	38 ± 8

DOM molar C : N ratios were similar for both the low P treatment and the control despite differences in growth (Fig. 1). Under P replete and low P conditions, DOM molar C : N ratios were 5.2 (± 0.6) and 5.2 (± 0.4), respectively. In contrast to the C : N ratios, DOM molar C : P ratios exhibited greater variability among nutrient conditions. Under P replete and low P conditions, DOM molar C : P ratios were 130 (± 22) and 2446 (± 519), respectively.

Under both nutrient conditions, ^{13}C NMR spectra revealed that amino acids were the dominant C-containing compound class in DOM (~ 70% of total C), followed by carbohydrates (~ 30%) and minor amounts of lipids (< 1%) (Table 1). With respect to P composition of P replete cultures, ^{31}P NMR spectroscopy identified P esters (90% ± 15%) as the dominant phosphorus species, followed by phosphonates or glycerol P (10% ± 15%) and polyphosphates (< 5%). In low P samples, all P compound classes were below detection under the conditions of our ^{31}P NMR analysis due to low total P content. The relative abundance of

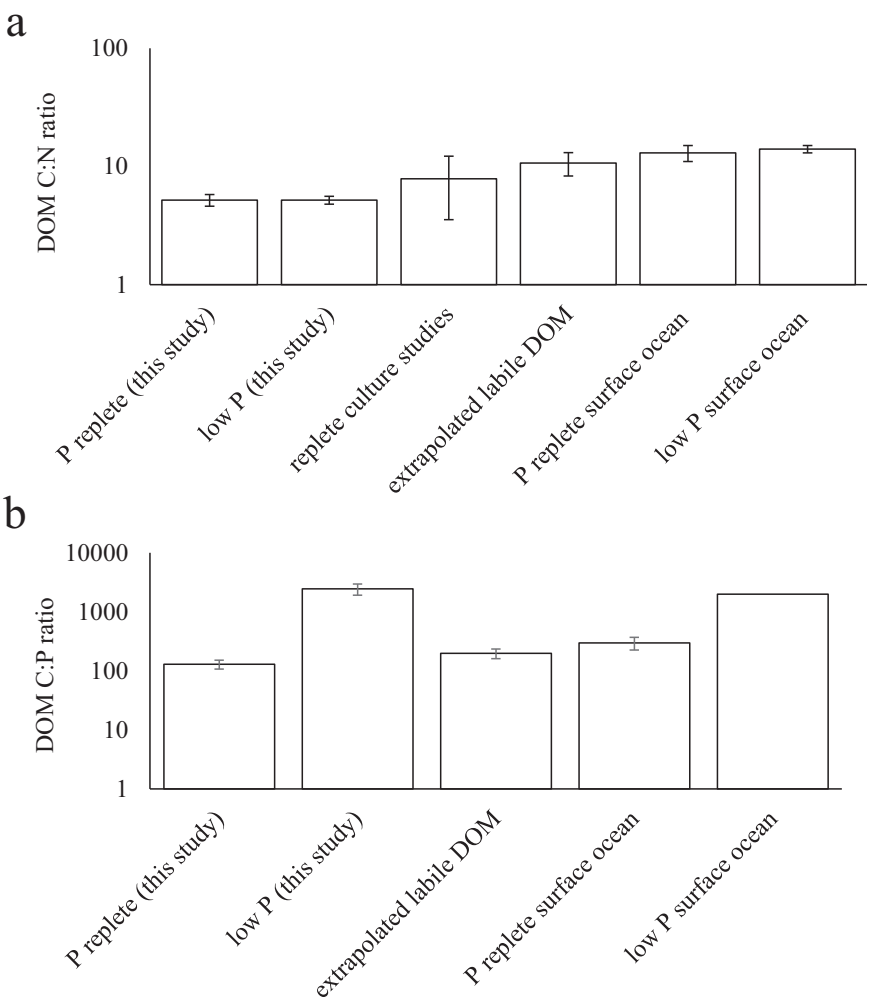

Fig. 2. Average C : N ratios (a) and C : P ratios (b) of cultured and ambient ocean DOM. Error bars represent standard deviation around average values. Biddanda and Benner (1997) report DOM C : N ratios from the marine species *Synechococcus bacillaris, Phaoecystis* sp., *Emiliania huxleyi*, and *Skeletonema costatum* grown under nutrient replete conditions. The extrapolated labile DOM C : N and C : P ratios are estimated by Hopkinson and Vallino (2005) from decomposition stoichiometry of DOM from four ocean regions. Average surface ocean DOM C : N ratios are reported from P replete (Hansell and Waterhouse 1997) and low P (Hansell and Carlson 2001) regions. Average surface ocean DOM C : P ratios are reported from P replete (Loh and Bauer 2000; Hopkinson and Vallino 2005) and low P regions (Lomas 2016).

polyphosphate, which was below detection with NMR, was quantified with direct fluorometric analysis. Because the fluorometric polyphosphate quantification method cannot measure absolute quantities (i.e., molar ratios of polyphosphate : total P > 1 are possible with this technique (Martin et al. 2014)), polyphosphate is reported in units of mole equivalents (eq), similar to previous studies that have used the same method (Martin et al. 2014; Diaz et al. 2016). In DOM collected from replete cultures, polyphosphate : DOP was < 5 eq mol^{-1}, but in low P samples, polyphosphates comprised approximately 38 eq mol^{-1} of DOP (Table 2). These results indicate that DOP was more than $\sim$ 8 times enriched in polyphosphate in low P compared to P replete cultures, even though total DOP content was much lower under low P conditions.

Discussion

In this study, ED techniques were applied in order to examine the bulk composition of freshly produced DOM in *T. pseudonana* cultures from a low P treatment relative to a control. DOM production in the ocean is certainly influenced by organisms other than *T. pseudonana*, and phytoplankton-derived DOM does exhibit some species-specific compositional differences (Myklestad 1974; Biddanda and Benner 1997). In addition to compositional differences of various phytoplankton species, other DOM sources, such as the decomposition of particulate matter, grazer mediated release, and release from prokaryotes (Carlson and Hansell 2014; Thornton 2014), may also play important roles in contributing to marine DOM composition. Nevertheless, *T. pseudonana*, a model diatom that is readily grown in culture,

provides an excellent starting point to examine the composition of freshly produced, phytoplankton-derived DOM.

The C : N ratios of DOM produced from axenic cultures of *T. pseudonana* were approximately 5.2 for both the low P treatment and the control. Biddanda and Benner (1997) characterized DOM C : N ratios averaged over an entire growth curve for non-axenic cultures grown under nutrient replete conditions of several common marine phytoplankton, *Synechococcus bacillaris* (4.1), *Phaoecystis* sp. (7.0), *Emiliania huxleyi* (6.3), and *Skeletonema costatum* (14.1), collected using ultrafiltration. C : N ratios of surface ocean DOM in diverse P replete ocean regions average 13 (± 2) (Hansell and Waterhouse 1997), while dissolved C : N in the low P Sargasso Sea is 14 (± 2) (Hansell and Carlson 2001) (Fig. 2a). Based on the decomposition stoichiometry of DOM from diverse ocean regions, Hopkinson and Vallino (2005) extrapolated a C : N ratio for labile DOM of 10.7 (± 2.4). The low DOM C : N ratios observed in axenic *T. pseudonana* cultures in this study are consistent with the lower ratio extrapolated for labile DOM relative to ambient surface ocean DOM. Additionally, a greater abundance of amino acids was observed in DOM produced by *T. pseudonana* (∼ 70%) relative to ambient surface ocean DOM (∼ 24%) (Sannigrahi et al. 2005). Enrichment in amino acids and associated low C : N ratios in DOM from our cultures relative to surface ocean DOM is consistent with the idea that N rich molecules are preferentially utilized in ocean surface waters (Williams et al. 1980; Hopkinson et al. 1997; Hopkinson et al. 2002). In fact, some freshly produced phytoplankton-derived proteins have turnover times of hours to days in marine systems, which suggests rapid utilization (Keil and Kirchman 1993). Similarly, amino acids have been observed to have a higher utilization rate relative to carbohydrates in phytoplankton-derived DOM in various surface ocean settings (Williams and Yentsch 1976; Ittekkot 1982; Cherrier and Bauer 2004).

Although C : N ratios and ^{13}C NMR DOC composition did not vary, C : P ratios and DOP composition varied between the low P treatment and control. DOM derived from *T. pseudonana* in the P replete control exhibited C : P ratios of 130 (± 22), which is lower than ratios observed in surface waters that are typically considered P replete, 299 (± 72) (Fig. 2b) (Loh and Bauer 2000; Hopkinson and Vallino 2005). Based on an extrapolation of the decomposition stoichiometry of DOM from diverse ocean regions, labile DOM is hypothesized to have a mean C:P ratio of 199 (± 37) (Hopkinson and Vallino 2005). The low DOM C : P ratios observed in axenic *T. pseudonana* cultures under P replete conditions in this study are consistent with the lower ratio extrapolated for labile DOM relative to ambient surface ocean DOM and reaffirm the suggestion that P is preferentially utilized during DOM decomposition (Loh and Bauer 2000; Kolowith et al. 2001; Hopkinson and Vallino 2005; Letscher and Moore 2015). Furthermore, under P replete conditions, P esters dominated P composition of *T. pseudonana*-derived DOM (∼ 90%) with smaller amounts of DOM with a chemical shift suggestive of phosphonates and/or glycerol P (∼ 10%) and polyphosphates (< 5%). Compositional dominance of P esters has been similarly observed in the high molecular weight fraction of DOM from non-axenic cultures of *Synechococcus bacillaris*, *Phaoecystis* sp., *Emiliania huxleyi*, and *Skeletonema costatum* grown under nutrient replete conditions (Clark et al. 1999). However, ambient ocean DOP contains a higher proportion of polyphosphates (8–13%) and phosphonates (5–10%) and a lower proportion of P esters (80–85%) relative to the DOP composition observed in phytoplankton cultures (Young and Ingall 2010). Thus, preferential utilization of dissolved P esters may produce the higher dissolved C : P ratios and higher relative proportions of dissolved polyphosphate and phosphonates observed in ambient marine DOM. This preferential utilization is consistent with a previously hypothesized mechanism to explain the composition of ambient ocean DOP (Clark et al. 1999; Kolowith et al. 2001; Young and Ingall 2010).

Low P concentrations had a strong effect on dissolved C : P ratios and DOP composition, which, along with reduced growth rates under low P conditions (Fig. 1), suggests that P concentrations resulted in a physiological response of *T. pseudonana*. Low P resulted in an average C : P ratio of 2446 (± 519), which is consistent with a DOM C : P ratio of approximately 2000 observed in the Sargasso Sea (Lomas 2016), a low P region (Martin et al. 2014) (Fig. 2b). Furthermore, low P conditions resulted in a distinct DOP composition. Based on direct fluorometric analysis, DOP contained ∼ 8 times more polyphosphate in the low P cultures compared to P replete cultures, even though total DOP content was much lower. As a metabolic response to low P, increases in phytoplankton polyphosphate content relative to other P containing biomolecules have been observed in the particulate fraction of *T. pseuodonana* (Dyhrman et al. 2012), *Trichodesmium* (Orchard et al. 2010), and *Synechococcus* (Martin et al. 2014). The relative increase in polyphosphates may reflect substitution of molecules, such as phospholipids, with non-P-containing forms like sulfolipids (Van Mooy et al. 2009). The high relative levels of dissolved polyphosphates in DOM observed under low P conditions in our cultures may be linked to the above previously reported metabolic responses of organisms to low P availability.

Conclusions

Nutrient ratios, DOC, and DOP composition of freshly produced, potentially bioavailable phytoplankton-derived DOM is compositionally distinct from ambient surface ocean DOM. The preferential utilization of N and P relative C during decomposition may lead to observed ambient surface ocean DOM C : N and C : P ratios. Furthermore, DOC and DOP compositional differences suggest preferential

utilization of amino acids and P esters relative to other C and P containing compounds. DOM production by *T. pseudonana* is strongly altered by low P concentrations resulting in increases in the ratio of dissolved polyphosphate to DOP. This increase is consistent with growing observations of polyphosphate enrichment in marine particulates (Martin et al. 2014) and increased polyphosphate gene abundance (Temperton et al. 2011) in low P ocean regions. Forecasted shifts towards P limitation in some ocean regions (e.g., the North Pacific (Kim et al. 2014)) may lead to higher dissolved C : P ratios and dissolved polyphosphate content, which may ultimately alter the species-specific bioavailability of DOP (Diaz et al. 2016) and microbial species composition.

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