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Title: Remineralization of Marine Particulate Organic Matter

Abstract approved:

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Marine microorganisms play a significant role in the cycling of nutrients in the open ocean through production, consumption, and degradation of organic matter (OM). Carbon (C), nitrogen (N), and phosphorus (P) are essential ingredients in every known recipe for life. However, the cycling of each of these elements proceeds at different rates such that the ratio of C:N:P can vary widely between particulate, dissolved, organic, and inorganic pools. To better understand the mechanisms controlling these transformations, this study investigated the bacterial remineralization of photosynthetically-derived organic matter derived from cultures of *Trichodesmium* IMS101, *Thalassiosira weissflogii*, *Prochlorococcus* MED4, and particulate material collected from the surface waters of an upwelling regime. Experiments were conducted at sea for a short duration (<6d) and in the laboratory for longer periods (<150 days). In all treatments, across experiments, we observed rapid and selective P remineralization independent of the type of organic material added. Full solubilization and remineralization of P typically occurred within a week. Conversely, N remineralization was slower, with only 39-45% of particulate N (PN) remineralized in shorter (6d) experiments and 55-75% of PN remineralized in <150d experiments. Nitrification was observed after 70-98 days depending on the remineralizing bacteria (isolated from either the Oregon coastal upwelling regime or the North Pacific Subtropical Gyre (NPSG). Notably, these events did not transform the full

complement of ammonium to nitrate. This differential lability between N and P led to rapid changes in the N:P ratio of inorganic pools as organic matter was depolymerized by varying bacterial populations. The variable input of potentially limiting elements could have consequences for primary productivity and particle export. Finally, we observed that in short-term experiments with heterotrophic bacteria collected from the NPSG, the N:P ratio of remineralization (11 ± 2.2) was independent of the N:P of added organic material (5-23). This uniformity of inorganic ratios implies differential lability and N:P composition of residual semi-labile and refractory organic matter. Formation of refractory C and N rich organic matter, often termed the microbial pump, is a significant pathway for the transport and sequestration of elements in the aphotic zone of the ocean interior. The experimental results reported here suggest that differential supply of POM leads to rapid and preferential P remineralization, N:P remineralization independent of the N:P of added substrates, and variable N:P of residual organic matter. These findings help constrain our knowledge of elemental cycling in the marine environment.

Remineralization of Marine Particulate Organic Matter

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Brian Gary Burkhardt, Author

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CONTRIBUTION OF AUTHORS

Angelicque White and Adina Paytan contributed intellectually to the experimental design and interpretation of data for Chapters 2 and 3. Angelicque White, Yvette Spitz, and Fred Prahl contributed to the editing process for both chapters. Katie-Watkins-Brandt assisted with sample analysis and experiments for Chapters 2-3.

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1 INTRODUCTION

1.1 Background

1.1.1 Marine Biogeochemical Cycles

Carbon (C), nitrogen (N) and phosphorus (P), are essential elements for all living organisms, and collectively make up the framework for cellular growth, structure, and metabolism. At the molecular level, these elements are bound into organic matter (OM) as a range of compounds that comprise proteins (amino acids), membranes (phospholipids), and genetic information (nucleic acids). In the sun-lit surface ocean, OM is synthesized by phytoplankton through photosynthesis. Sinking, grazing and flocculation lead to export of this OM from the surface to the aphotic ocean interior where it is respired by heterotrophic organisms. As this material is broken down through heterotrophic activity, C, N, and P are cycled between particulate, dissolved, organic and inorganic pools. Upwelling of these waters then resupplies inorganic nutrients to the surface ocean where they once again fuel photosynthetic growth. In this manner, elemental cycling in the ocean is characterized by tight coupling between the uptake and decomposition of inorganic and organic resources. These processes are regulated by microbial activity.

1.1.2 Microbial Mediated Remineralization of C, N, and P

Microbes, including bacteria, viruses, and protists, make up the dominant form of life in the ocean and account for more biomass, diversity, and energy transfer than the sum of all multicellular organisms (Pomeroy et al., 2007). In the photic layer, phytoplankton account for nearly half of global photosynthesis, fixing 35-65 Gt C per year (Arrigo, 2004; Field et al., 1998; Longhurst et al., 1995). A majority of this OM is respired within the water column by heterotrophic microorganisms, with a small residual fraction reaching the seafloor (Lee et al., 2004). The transformation and recycling of marine OM largely occurs within an intricate food web that was first referred to as the "microbial loop" by Azam et al. (1983). While research in the last few decades has enhanced our understanding of marine microbial ecology, many underlying processes of this microbial web are still not well understood.

The process of OM remineralization by heterotrophic communities is an essential cycle within the microbial loop, controlling the recycling and supply or preservation of essential nutrients. Considering 75% of marine photosynthesis occurs in oligotrophic ocean regions where dissolved inorganic N (DIN) and P (DIP) are often below levels of detection, the supply rates of N, P, and/or iron (Fe) are arguably the major factors limiting primary production (del Giorgio and Duarte, 2002; Karl et al., 2001; Kirchman et al., 2000). The extent that N and P are resupplied is a balance of the elemental stoichiometries and lability of OM sources and heterotrophic nutritional requirements. While heterotrophic bacteria typically have low C:N and C:P relative to most phytoplankton, bacteria have relatively high C demands as a source for respiration as well as biomass synthesis (Kirchman, 1994). In addition, it is understood that a relatively large fraction of dissolved organic C (DOC), relative to N (DON) and P (DOP), is inaccessible to bacteria. This preferential remineralization of N and P is indicated by C rich DOM in deep waters (Church et al., 2002; Charles S Hopkinson and Vallino, 2005). Consequently, bacterial respiration of OM, often results in the liberation of excess N and P. In this way, the decoupling of elemental compositions contributes to the supply ratio of limiting nutrients, as well as to C sequestration in the form of refractory DOM. Thus process of production and preservation of organic C in the ocean has become known as the microbial pump, and plays an essential role in balancing global C fluxes (Peterson, 1979).

1.1.3 Controls and Rates of Remineralization: POM and Elemental Ratios

In 1958, Alfred Redfield published observations of the striking coherence between the stoichiometry of dissolved elements in deep water and the composition of suspended particles in what is now known as the Redfield ratio (106C:16N:1P). In addition, he hypothesized that the rates of nutrient recycling may ultimately control production, as these timescales determine the ratio and concentration of nutrients in the absence of external inputs. Elemental rates and ratios have been thoroughly studied, quantified, and used as a reference for nutrient limitation and modeling ever since (Geider and Roche, 2002; Charles S. Hopkinson and Vallino, 2005; Redfield, 1958).

The Redfield ratio is apt as a global mean, however it reflects the net effects of microbial activities over long space and time scales (Arrigo, 2004; Klausmeier et al., 2004; Redfield, 1958). Deviations from this mean are widespread. In the laboratory, phytoplankton cultures grown under a range of nutritional regimes exhibit particulate N:P (PN:PP) ratios ranging from <5 to >100. Under nutrient replete conditions however, PN:PP typically ranges from 5-19, but the critical N:P, conveying the transition from N to P limitation is typically much higher, ranging from 20-50 (Geider and Roche, 2002). Whether these large swings in elemental stoichiometry also reflect changes in lability (the potential that organically bound N and P can be remineralized) has not been well quantified.

At the cellular level, N and P comprise approximately 10% and 2-4% of marine microbial cells by mass, respectively (Karl and Bjorkman, 2000). Proteins and amino acids make up the largest source of N within the cell, typically exceeding 60% of total N. Nucleic acids (DNA+RNA), though more labile, account for only 2-15% of cellular N, but can account for a major fraction of cellular P. Under certain conditions, nucleic acids can account for >95% of P within certain species (Geider and Roche, 2002). In the oligotrophic ocean, primary producers typically contain relatively more N-rich cellular components (protein and pigments) for acquiring resources (Arrigo, 2004). Additionally, species that dominate these regions (ie. *Prochlorococcus* and *Synechococcus*) have some of the smallest known genomes, reducing their P demand for nucleic acid production. Similarly, diazotrophs, capable of N_2-fixation, can have N:P ratios that exceed 40 due to an abundance of low-P components essential for light harvesting (Arrigo, 2004; LaRoche and Breitbarth, 2005). As a result, exported POM in these regions would be expected to have high N:P ratios; and in fact that is often what is observed. The N:P ratio of exported particles in the North Pacific Subtropical Gyre range from ~20-40 (Karl, 1999). In contrast, coastal species, in upwelling margins experience rapid nutrient inputs. These species are capable of rapid growth, are more rich in RNA and DNA, and consequently have relatively low cellular N:P (Arrigo, 2004).

In addition to molecular structure and relative lability of OM substrates, various studies have investigated how physical (e.g. temperature, pH, stratification upwelling)

and biological (e.g. the abundance, community structure, enzymatic capacity, and nutritional requirements of heterotrophic populations) factors contribute to the rates and ratios of remineralization (Wetz and Wheeler, 2004; Wohlers et al., 2009). Quantification of these processes determines the biochemical and spatial distributions of these elements, as well as potential shifts in N and P ratios and microbial productivity over space and time (Karl et al., 1996; Redfield, 1958).

1.1.4 Thesis Objectives

In summary, the relative coupling of C, N, and P during OM degradation by heterotrophic populations is influenced by multiple environmental and ecological factors. In order to effectively quantify and model important global scales nutrient cycling, it is imperative to enhance our current knowledge of the factors controlling how these essential elements are recycled and distributed.

The main objectives of this thesis are focused on enhancing the existing literature of marine organic matter remineralization by natural heterotrophic communities, with regards to relative rates and extents of N and P turnover. Chapter two will focus on the relationship between the N:P ratio of particulate organic matter formed by a different photosynthetic organisms and the N:P ratio of heterotrophic remineralization in short term (6d) incubations. Chapter three expands on these results and examines the remineralization rates and ratios over for an oligotrophic and coastal system over long term (<150d) incubations.

1.2 Literature Cited

Arrigo, K. R. (2004), Marine microorganisms and global nutrient cycles, *Nature*, *437*(7057), 349-355.

Azam, F. (1983), The Ecological Role of Water-Column Microbes in the Sea, *Mar. Ecol. Prog. Ser*, *1*, 257-263.

Church, M. J., H. W. Ducklow, and D. M. Karl (2002), Multiyear increases in dissolved organic matter inventories at Station ALOHA in the North Pacific Subtropical Gyre, *Limnology and Oceanography*, *47*(1), 1-10.

del Giorgio, P. A., and C. M. Duarte (2002), Respiration in the open ocean, *Nature*, *420*(6914), 379-384.

Field, C. B., M. J. Behrenfeld, J. T. Randerson, and P. Falkowski (1998), Primary production of the biosphere: integrating terrestrial and oceanic components, *Science*, *281*(5374), 237-240.

Geider, R. J., and J. L. Roche (2002), Redfield revisited: variability of C [ratio] N [ratio] P in marine microalgae and its biochemical basis, *European Journal of Phycology*, *37*(01), 1-17.

Hopkinson, C. S., and J. J. Vallino (2005), Efficient export of carbon to the deep ocean through dissolved organic matter, *Nature*, *433*(7022), 142-145.

Karl, D. (1999), Minireviews: A Sea of Change: Biogeochemical Variability in the North Pacific Subtropical Gyre, *Ecosystems*, *2*(3), 181-214.

Karl, D., J. Christian, J. Dore, D. Hebel, R. Letelier, L. Tupas, and C. Winn (1996), Seasonal and interannual variability in primary production and particle flux at Station ALOHA, *Deep Sea Research Part II: Topical Studies in Oceanography*, *43*(2), 539-568.

Karl, D. M., and K. M. Bjorkman (2000), Phosphorus, the staff of life, *Nature*, *406*, 31-32.

Karl, D. M., K. M. Bjorkman, J. E. Dore, L. Fujieki, D. V. Hebel, T. Houlihan, R. M. Letelier, and L. M. Tupas (2001), Ecological nitrogen-to-phosphorus stoichiometry at station ALOHA, *Deep Sea Research Part II: Topical Studies in Oceanography*, *48*(8), 1529-1566.

Kirchman, D. (1994), The uptake of inorganic nutrients by heterotrophic bacteria, *Microbial Ecology*, *28*(2), 255-271.

Kirchman, D. L., B. Meon, M. T. Cottrell, D. A. Hutchins, D. Weeks, and K. W. Bruland (2000), Carbon versus iron limitation of bacterial growth in the California upwelling regime, *Limnology and Oceanography*, 1681-1688.

Klausmeier, C. A., E. Litchman, T. Daufresne, and S. A. Levin (2004), Optimal nitrogen-to-phosphorus stoichiometry of phytoplankton, *Nature*, *429*(6988), 171-174.

LaRoche, J., and E. Breitbarth (2005), Importance of the diazotrophs as a source of new nitrogen in the ocean, *Journal of Sea Research*, *53*(1), 67-91.

Lee, C., S. Wakeham, and C. Arnosti (2004), Particulate organic matter in the sea: the composition conundrum, *AMBIO: A Journal of the Human Environment*, *33*(8), 565-575.

Longhurst, A., S. Sathyendranath, T. Platt, and C. Caverhill (1995), An estimate of global primary production in the ocean from satellite radiometer data, *Journal of Plankton Research*, *17*(6), 1245-1271.

Peterson, B. J. (1979), Particulate organic matter flux and planktonic new production in the deep ocean, *Nature*, *282*, 677-680.

Pomeroy, L. R., P. J. l. Williams, F. Azam, and J. E. Hobbie (2007), The microbial loop, *Oceanography*, *20*(2), 28.

Redfield, A. C. (1958), The biological control of chemical factors in the environment, *American Scientist*, *46*(3).

Wetz, M. S., and P. A. Wheeler (2004), Response of bacteria to simulated upwelling phytoplankton blooms.

Wohlers, J., A. Engel, E. Zallner, P. Breithaupt, K. Jorgens, H.-G. Hoppe, U. Sommer, and U. Riebesell (2009), Changes in biogenic carbon flow in response to sea surface warming, *Proceedings of the National Academy of Sciences*, *106*(17), 7067-7072.

Stoichiometric remineralization of nitrogen and phosphorus from various particulate organic matter sources

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2 Stoichiometric remineralization of nitrogen and phosphorus from various particulate organic matter sources

2.1 Abstract

Herein, we report on the relative lability, elemental stoichiometry, and remineralization ratios of various particulate organic matter (POM) by a natural marine heterotrophic population. POM was harvested from laboratory cultures of a marine diazotroph (*Trichodesmium* IMS101), a cosmopolitan diatom (*Thalassiosira* weissflogii), a common marine cyanobacteria (*Prochlorococcus* MED4), and natural POM collected off the Oregon coast. In a field experiment conducted at the Hawaii Ocean Time-series Station ALOHA in the North Pacific Subtropical Gyre, POM from these various sources was added to seawater collected from below the surface mixed layer, incubated in the dark, and remineralization rates were quantified via high-resolution monitoring of phosphorus (P) and nitrogen (N) turnover over a 6-d period.

Rapid and near complete solubilization and remineralization of particulate P (PP) occurred in all cultured POM treatments, with lesser mobilization of P from the natural POM. Soluble P pools, assumed to be either surface-adsorbed inorganic P or inorganic P reserves liberated from cells during harvesting of biomass accounted for 26% of natural PP pools and 56-95% of cultured PP. In contrast, over the course of these experiments, only 39-45% of particulate N (PN) was remineralized to ammonium (NH_4^+). POM isolated from cultures of *Trichodesmium* was enriched in C and N (per gram of dry material) and associated with the highest rates of N and P remineralization and heterotrophic bacterial growth. Most notably, when corrected for non-biological turnover (ie. no soluble pools), the N:P remineralization ratio of cultured material (11 ± 2.2) was independent of the N:P of added organic material (5-23).

2.2 Introduction

Carbon (C), nitrogen (N), and phosphorus (P) are essential nutrients required for all life. Through biosynthesis, these elements are forged into a range of organic compounds that make up the framework for cellular growth, structure, and metabolism. These structural and functional macromolecules, including genetic material (nucleic acids), membranes (phospholipids), carbohydrates, and proteins (amino acids), are unique in elemental composition and abundance within cells. In this way, the elemental stoichiometry of an organism, and ultimately the organic matter (OM) they produce, are reflective of phylogenic and environmental variability.

At the global scale, the mean C:N:P ratio of marine particulate organic matter (POM) produced in the surface and dissolved inorganic pools at depth are relatively well constrained at a value of 106C:16N:1P, known as the Redfield ratio (Redfield, 1958). This coupling is presumed to result from the cycling of C, N, and P through the production and degradation of OM. However, when examining regional data or vertical profiles, it is apparent that the timescales of C, N, and P remineralization are not coupled, as ratios of particulate and dissolved, organic and inorganic pools vary over space and time (Karl et al., 2001, Martiny et al., 2013). In general, OM is divided into a rapidly cycled labile fraction, typically high in N and P, a moderate semi-labile fraction, and a refractory component relatively rich in C and depleted in N and P. This "preferential" remineralization of N and P supports productivity in the surface, while determining the extent of C preservation (Jiao et al., 2010).

On finer scales, N and P remineralization are also decoupled, such that OM typically becomes rapidly P depleted and relatively C and N rich (Anderson and Sarmiento, 1994; Clark et al., 1998; Paytan et al., 2003). While a number of studies have documented preferential P remineralization relative to N or C, the time and depth scales have not been well quantified (Anderson and Sarmiento, 1994; Clark et al., 1998; Paytan et al., 2003). At the Hawaii Ocean Time-Series (HOT) in the oligotrophic North Pacific, the P nutricline is typically shallower than N, with reversals of this trend on inter-annual timescales (Karl et al., 2001). Possible causes for these shifts are not well understood, but are likely attributed to factors involved with the type of POM sinking or the

remineralizing community. Additionally, the particulate and dissolved organic pools at this same site are not mirrored spatially and temporally as would be expected through our current understanding of organic C, N, and P decomposition (Sannigrahi et al., 2006). Consequently, these cases of nonconformity, in addition to regional dynamics have prevented the development of accurate models of nutrient recycling. With a clearer understanding of the driving factors of these trends on a regional and phylogenic basis these models may be improved.

Remineralization trends of C, N, and P can vary as a result of many factors, including the production (elemental stoichiometry and molecular composition) of organic matter substrates, as well as environmental constraints, enzymatic capacities, and nutrient status of the remineralizing organisms (Hansell and Carlson, 2002). On the production side, a large and growing canon of research has documented cause for shifts in elemental stoichiometry from molecular to regional scales (Anderson and Sarmiento, 1994; Hedges et al., 2002; Paytan et al., 2003; Sannigrahi et al., 2006). The traditional Redfield ratio has a standard deviation more than 50%, however recent findings suggest the range of PN:PP ratios of both cultured and natural populations is generally driven by P (Bertilsson et al., 2003; Sañudo-Wilhelmy et al., 2004; White et al., 2006). RNA production as well as genome size may play a role in PN:PP ratios, as nucleic acids can account for a significant portion of cellular P. For example, the most abundant phytoplankton (*Prochlorococcus* and *Synechococcus*) are cyanobacteria with small genomes and a relatively low P demand. However, environmental adaptations can cause shifts in cellular machinery, changing N content as well. In the oligotrophic ocean, species typically contain relatively more N-rich components (protein and pigments) for harvesting vital resources, which may also contribute to high cellular N:P ratios. Diazotrophs capable of N_2-fixation can have N:P ratios that exceed 40, due to ample N access and low-P components (Arrigo, 2004; LaRoche and Breitbarth, 2005). Contrarily, coastal species accustomed to upwelling events are typically built for rapid growth. As a result, these species are more rich in nucleic acids (RNA + DNA), and typically lower N:P (Arrigo, 2004). Furthermore, ecologically significant taxa can vary in elemental stoichiometry in a manner that impacts OM lability, albeit this potential is not well characterized.

In this study, experiments were designed to quantify the rate and elemental stoichiometry of OM remineralization by a natural heterotrophic population in the North Pacific Subtropical Gyre through a high-resolution sampling regimen. The overarching objective was to constrain potential differences of N and P remineralization that may be related to taxonomic variation of POM substrates. Findings will add to the existing knowledge regarding the factors controlling nutrient regeneration in the sea.

2.3 Methods and Materials:

2.3.1 POM preparation:

Large volume batch cultures were grown on a 12:12 light dark cycle at saturating growth irradiances (150 μmol photons m^{-2} s^{-1}) at a constant temperature of 24°C. Cultures were non-axenic. *Trichodesmium* (strain IMS101) was grown on YBCII media (Chen et al., 1996), modified for an initial DIP addition of 5μmol P L^{-1}. *Thalassiosira weissflogii* was grown on F/2 media, again modified for initial DIP levels of 5μmol P L^{-1} *Prochlorococcus* MED-4 was grown on the standard recipe for Pro99 media (Moore et al., 2007). Growth of all cultures was monitored by *in vivo* chlorophyll fluorescence (via either a Walz Water-PAM or Turner 10-AU fluorometer). All cultures were harvested during the early stationary growth phase. In addition, natural marine POM was collected from surface water from the upwelling regime off the Oregon coast (OR POM). Biomass was isolated by gentle vacuum filtration (<100 mmHg) onto a series of 25mm diameter 2.0-5.0 μm Nucleopore filters (depending on the culture) to minimize cell breakage and remove ambient bacteria. Filters were dried at 60°C in a drying oven and material was transferred to clean polycarbonate centrifuge tubes and stored at -20ºC. Each of the four POM isolates were sub-sampled and characterized for particulate C, N, and P composition (analytical methods described below).

2.3.2 Experimental Design:

In March of 2011, a suite of on-deck incubation experiments were conducted in the North Pacific Subtropical Gyre (NPSG) near Station ALOHA (A Long-Term Oligotrophic Habitat Assessment; 22° 45'N, 158° 00'W) to quantify the rates and trends of N and P remineralization with various POM substrates. Seawater was collected in 20-L acid-washed, autoclaved polycarbonate carboys from the euphotic zone (75m) at Station

ALOHA and stored in the dark for ~72 hours to discourage photoautotrophic growth and preserve the heterotrophic community. Carboys were capped with vented 3-port lids and internal tubing for sampling. This depth horizon was targeted to capture a maximize abundance of active heterotrophic bacteria while minimizing the amount of natural organic matter. All carboys were stored in an on-deck incubator maintained at *in situ* temperature (~24ºC) via surface seawater circulation. Four types of POM, including cultures of three distinct and ecologically significant marine photoautotrophs (*Trichodesmium* IMS 101, *Prochlorococcus* MED4, and *Thalassiosira weissflogii*) and natural POM (OR POM from here on) were isolated, dried, and characterized. POM was then added to carboys at levels targeted for a 1.0 µmol L^{-1} P addition so as to mimic an export pulse and ensure detection of P pools for accurate rate quantification.

Consequently, the amount of C, N, and total mass varied between treatments. Treatments included (+*Trichodesmium*, +*Prochlorococcus*, +*Thalassiosira*, +OR-POM, and a killed control (+*Trichodesmium* +$HgCl_2$) with 50-mL added of saturated mercuric chloride. The mass of POM added for duplicate 20-L treatments were 0.5g *Trichodesmium*, 1.0g *Prochlorococcus*, 1.0g *Thalassiosira*, and one replicate of 0.6g Oregon POM (Table 2-2). For each treatment, ammonium (NH_4^+) and soluble reactive phosphate (SRP, considered to be equivalent to dissolved inorganic phosphate, DIP) were measured continuously via flow-injection auto-analysis (methods below) for a period of hours to capture any rapid initial nutrient solubilization, and collected at 3-hr intervals thereafter. In addition discrete samples were collected for total dissolved N and P, nitrate + nitrite (N+N) and bacterial abundance (methods below). Prior to all samplings, treatments were bubbled with high-purity air to ensure homogeneity.

2.3.3 Additional sampling and analytical methods

Biomass (extracted chlorophyll and heterotrophic bacteria):

During the dark holding period and at daily intervals over the course of the experiment, 5-ml samples were collected from each treatment for measurement of extracted chlorophyll-a concentrations via the acidification method of Strickland and Parsons (1972). Again at daily intervals, triplicate 3mL samples from each replicate were

collected, fixed with 60 µL of 10% paraformaldehyde, and stored at -80ºC for flow cytometric analysis of bacterial concentrations,

Post-cruise, bacterial abundance was measured by flow cytometry on a Becton-Dickinson FACS-Caliber four-color flow cytometer with SYBR® Green stain as described by Sherr et al. (2001). Samples were stained for 10 min in the dark with a 10^{-4} diluted stock of SYBR ® Green as in Marie et al. (1997). Heterotrophic bacteria, including High and Low Nucleic Acid clusters (HNA, LNA) were plotted and gated using fluorescence (515-545 nm), Side Scatter (SSC), and Forward Scatter (FSC) on log scales. Bead counts were used to determine sample volume with 1.0 µm Fluoresbrite® microspheres with a known concentration calculated from calibration with True Count® polystyrene standardization beads.

Particulate and dissolved elements:

Nutrients were analyzed using a flow-through colorimetric method on a Technicon Auto Analyzer II. Soluble reactive phosphorus (SRP) was analyzed by the phospho-molybdic acid reduction, employing a 5-cm pathlength flow cell at 880nm. Analysis of ammonium (NH_4^+) was performed by the indophenol blue method modified from ALPKEM RFA (ALPKEM, 1984) using a 5 cm flow cell with detection at 640 nm. Nitrite + Nitrate (N+N) was analyzed using the cadmium reduction method of Armstrong et al. (1967), utilizing a 10-turn cadmium coil and 0.05 mol L^{-1} imidazole buffer. The detection limits, calculated as the (mean blank value + three standard deviations of the blank) were 55 nmol L^{-1} for SRP, 22 nmol L^{-1} for NH_4^+, and 8 nmol L^{-1} for N+N. Standards where prepared in a 3% NaCl Milli-Q matrix. Standard curves for each nutrient were made before each run, and blanks where run before and after to correct for any baseline shifts.

Totals for P and N were determined by the alkaline persulfate oxidation method (Valderrama, 1981) using a 1:10 oxidant to sample ratio. Dissolved organic P (DOP) and N (DON) were calculated as the difference of TDP and SRP and TDN less the sum of reduced N pools ($NH_4^+ + NO_3^- + NO_2^-$) respectively.

Particulate samples were collected onto combusted GFFs, wrapped in combusted foil, flash frozen, and stored at -80°C. For particulate C/N analysis, thawed filters were dried overnight, balled in Sn boats, and run on a Carlo-Erba C/N Analyzer. Particulate P samples were thawed and combusted at 450°C for 4.5 hours, then extracted with 0.15 M HCl for 1 hour at 60 °C. A 1.0 cm cell with a Cary UV-Vis double beam spectrophotometer was utilized for analysis at 880 nm following a 15 min NH_4-molybdate/ascorbic acid reaction.

2.4 RESULTS and DISCUSSION

2.4.1 Initial Water and POM Characterization

Initial water characterization samples were taken after a 3 day 'aging' period in the dark and at *in situ* temperature. At this location (Station ALOHA) inorganic concentration are typically low with values ranging from 10-100 nmol SRP L^{-1} and 20-100 nmol N+N L^{-1} in the upper 100m (Karl et al., 2001). In our experiments, concentrations were near instrument detection limits with 0.05±0.07 µmol N+N L^{-1}, 0.09±0.07 µmol SRP L^{-1}, and 0.08±0.01 µmol NH_4^+ L^{-1}. Initial DOP concentrations, 0.05±0.08 µmol L^{-1} made up 36% of the TDP pool. DON concentrations (6.2 ±0.2 µmol L^{-1}) composed 98% of the TDN pool. Inorganic N:P ratios in the upper 100m at Station ALOHA are typically <1.0 as a result of extreme N and P deficiency, while TDN:TDP range from 16-25 (Karl et al., 2001). In this study, initial water measurements of the inorganic N:P (NH_4^++NO_2^-+NO_3^-:SRP) ratio was 0.9±0.2, while TDN:TDP was 45±35. Initial heterotrophic bacterial concentrations were 4.4±2.4x10^5 cells ml^{-1}, and within the range typically observed at 75m at Station ALOHA (3.5-5.6x10^5 cells ml^{-1}) (Table 2-1).

Characterization of particulate C, N, and P content for each POM type are shown in Table 2-2. *Trichodesmium* was relatively C and N rich (3072±227 µmol C g^{-1}, 544±35 µmol N g^{-1}), with 7-8 times the C and N content per dry mass, and ~ 5 times as much P per gram as *Prochlorococcus*. *Thalassiosira* contained about twice as much C, N, and P per gram as *Prochlorococcus*. Oregon POM was the most P rich with 30.81±1.72 µmol P g^{-1}. Particulate C:N (PC:PN) ratios for *Trichodesmium*, *Thalassiosira*, and

Prochlorococcus were 5.7±0.2, 6.25±0.05, and 5.9±0.6 respectively. Oregon POM had a significantly higher C:N ratio of 10.4±0.2 when compared to all three cultures (t-test, all p values <0.001). PN:PP and PC:PP values were below the benchmark Redfield ratios for all POM types, with the exception of *Trichodesmium* (PN:PP = 23±2, PC:PP = 127±12). PN:PP ratios were 9.6±2.4, 5.5±1.3, and 7.3±2.1 for *Thalassiosira*, *Prochlorococcus*, and OR POM respectively. Molar PC:PP values were 60±15, 32±10, and 76±22 for *Thalassiosira*, *Prochlorococcus*, and OR POM respectively.

All treatments contained a soluble inorganic P pool that was detected immediately after the addition of biomass, seen in the SRP and NH_4^+ time series (Figure 2-1). There was no detectable soluble NH_4^+ or N+N measured after POM additions. Soluble P release was also observed in the "Killed" ($HgCl_2$ + *Trichodesmium*) treatment, indicating that this P was either surface-adsorbed or intracellular SRP liberated by the culture harvesting process. While both have been observed in natural settings, the following results are presented with and without this soluble fraction (calculated as the net SRP increase <5 min after POM addition) to isolate the biologically-mediated remineralization.

Comparisons of the initial PC:PP, and PN:PP ratios with and without soluble pools were significantly different for all treatments (t-test, all p values <0.04). PC:PP ratios less soluble P pools were 234±29, 203±84, 427±395, and 103±31 for *Trichodesmium*, *Thalassiosira*, *Prochlorococcus*, and OR POM respectively, and PN:PP ratios less soluble P were 46±7, 30±13, 72±52, and 10±3 for *Trichodesmium*, *Thalassiosira*, *Prochlorococcus*, and OR POM respectively.

2.4.2 PN and PP lability

Dissolved N and P pools were measured continuously for ~1hr after the addition of POM and then at 3 hour intervals over a 6-d period to track the stoichiometric transformation of POM to dissolved inorganic pools (SRP, NH_4^+). Figure 2-1(a-d) shows the temporal change in SRP and NH_4^+ concentrations in these duplicate treatments over time. In general, heterotrophic bacteria abundances tracked net NH_4^+ release (Figure 2-1, e-h). For all cultured POM treatments, bacterial growth was positively related to the concentration of PC (R^2=0.90) and PN (R^2=0.84) added, but insignificant and negative

relationship with PP added (R^2=0.02). The addition of *Trichodesmium* POM yielded the highest net bacterial abundance (max-initial = 2.4±0.6 x10^6 cells mL^{-1}) and fastest growth rates (1.5±0.2 x10^6 cells mL^{-1} day^{-1}), and this relationship held when treatments were normalized to the mass of POM added. Net bacterial yields for *Thalassiosira*, *Prochlorococcus*, and OR POM were 2.0±0.4 x10^6, 9.5±0.3 x10^5, 1.3±0.7 x10^6 cells mL^{-1} respectively (Table 2-3).

For all POM treatments, turnover was distinctly divided into three phases that we characterize as "soluble", "labile", and "other". As noted above, all treatments contained a soluble inorganic P pool that was detected immediately after the addition of biomass. Within the first 24 hours following the initial P solubilization, SRP concentrations briefly decreased (mean drawdown of 0.083 ± 0.019 µmol L^{-1}across treatments). This drawdown was not associated with any changes in the remineralization trends for N. Consequently, "labile" P has been quantified as the biological turnover of P following soluble release and subsequent drawdown. "Labile" N turnover is simply the net increase after POM addition. "Other" is the fraction of added PP and PN that did not result in a net increase in dissolved inorganic pools within the experimental timeframe.

Lability fractions are presented as percent in Figure 2-2. All cultured POM treatments resulted in 96-100% P turnover, with 56-95% as soluble P. *Prochlorococcus* contained the largest fraction of soluble P (95%). Freshly collected natural POM (OR POM) contained less soluble P (27%) and less P was remineralized (3%) over the incubation period. For all treatments, percent N turnover was low relative to P. Only 39-45% of N from cultured POM, and 4% of OR POM was converted to DIN. For *Trichodesmium*, *Thalassiosira*, and *Prochlorococcus*, the percent of N turnover relative to P within 6 days was 41%, 44%, and 45% respectively. Consequently, N:P ratios of labile pools were proportionally smaller relative to initial PN:PP of organic substrates. Additionally, killed-control treatments (*Trichodesmium* +$HgCl_2$) resulted in 83% P turnover, suggesting a significant fraction of *Trichodesmium* PP may be non-biological remineralization.

2.4.3 Rates and Ratios of Remineralization

Rates of N and P remineralization were calculated as the linear regression of the "labile" fraction and are presented as simple rates of change in μmol L^{-1} d^{-1} (Table 2-4). Absolute rates of N and P remineralization were highest for *Trichodesmium* treatments (0.11±0.06 μmol P L^{-1} day^{-1}, 1.4±0.84 μmol N L^{-1} day^{-1}) and lowest for the OR POM treatment (0.031 μmol P L^{-1} day^{-1}, 0.141 μmol N L^{-1} day^{-1}). Rates of remineralization as percent added were 8.2%, 4.7%, 4.6%, and 2.0% of PP day^{-1}, and 5.3%, 6.7%, 9.9%, and 1.1% of PN $day^{-1}$for *Trichodesmium*, *Thalassiosira*, *Prochlorococcus*, and OR POM respectively. Notably, the N:P remineralization ratios did not vary significantly (t-test, p values ranging from 0.45-0.91) despite a wide range of N:P content in added POM (5-23). Specifically, the N:P remineralization ratios (slope of N:P) were 10.2±3.0, 9.9±1.3, and 12.7±2.2 for *Trichodesmium*, *Thalassiosira*, and *Prochlorococcus* respectively (Figure 2-3), with error being the standard deviation of replicates. In contrast, the N:P ratio of remineralization was 2.2 for OR POM. Despite this coherence in net stoichiometric turnover over the entire 6 days, trends of remineralization (less soluble fraction) were distinctly different between cultures. The timescales of N and P turnover varied throughout experimentation. For *Prochlorococcus*, *Thalassiosira*, and OR POM, inorganic N:P ratios increased over the incubation period. In contrast, N:P ratios in *Trichodesmium* treatments increased to 42 ± 19 after 1d and then steadily decreased to a final N:P of 16± 16, suggesting *Trichodesmium* may contain a pool of highly labile N relative to other treatments.

If we examine the elemental composition of the POM not solubilized or remineralized (classified here as 'other'), N:P values exceeded 500 for *Trichodesmium* and *Prochlorococcus,* 165 for Thalassiosira and 11 for OR-POM. These are severely N depleted and approach or exceed the DON:DOP values (> 50N:P) observed in deep waters of Station ALOHA (Karl et al., 2001). Uptake into heterotrophic biomass may also makeup a fraction of the 'other' pool. However, after maximum bacterial abundances were reached (1.5-3.0 days), cell numbers decreased close to initial levels, suggesting this likely did not function as a net sink. However, as bacterial number increased, a net SRP drawdown occurred in live treatments, suggesting rates of P uptake were exceeding rates of DOP remineralization during this period.

2.5 Conclusion

All POM treatments in this study resulted in preferential remineralization of particulate P relative to N. In the early remineralization phase, a significant portion of PP, as internal or externally adsorbed soluble inorganic P may bypass the dissolved organic pool, while PN is decomposed to NH_4 at a relatively slower rate. Rapidly released soluble P accounted for a significant fraction of POM, resulting in dissolved inorganic N:P ratios to remain below 1.0 within the first hours of remineralization. After solubilization, the rate of N remineralization exceeded P (μmol L^{-1} day^{-1}), but in proportions less than Redfield trajectories (16N:1P), resulting in increasing dissolved inorganic N:P ratios over time, as seen in the mesopelagic at Station ALOHA (Karl et al., 2001; Karl and Bjorkman, 2000). When corrected for non-biological turnover (ie. no soluble pools), the N:P ratio of remineralization over 6-d did not differ between cultured treatments (range: 8.3 - 14.3, mean ± standard deviation = 11.0 ± 2.2) despite a wide PN:PP of cultured POM added (range: 5-23). This suggests that the residual OM, the semi-labile to refractory OM, must vary as a function of the stoichiometry of added POM. For comparison, N:P values beneath the photic layer at Station ALOHA are consistently 14±1 (Karl et al., 2001). Figure 2-3 includes comparisons of N:P remineralization ratios at this site with an inverse model by Sarmiento & Anderson (14:1), and a mixing model (12.4) by Li et al. (2000).

The 'plasticity' of cellular P content has been well documented in several studies of cultured and natural populations of marine photoautotrophs (Bertilsson et al., 2003; Sterner and Elser, 2002; White et al., 2006). A fraction of this P variability has been suggested to be a function of persistent yet variable pools of cell surface-adsorbed P. Sanudo-Wilhemy et al. (2004) applied an oxalate wash to remove surface-bound P from cultured and natural populations. These authors as well as other groups have found the surface-bound P can account for as much as 15-50% of PP and varies as a function of cell health, ambient DIP concentrations, cell size, growth phase, and presence of metal hydrous oxides (Fu et al., 2005). In our study, cultures were grown on highly enriched media (≥5μmol P) and harvested during exponential growth phase, therefore PP values may represent partial fraction of surface adsorbed P. Additionally, material was harvested

and dried so we cannot rule out the possibility that the soluble P pools correspond to internal DIP pools, which can also comprise a significant fraction of PP (Miyata and Hattori, 1986). Nonetheless, "killed" control measurements with *Trichodesmium*+$HgCl_2$ suggest up to 83% of PP and 9% of PN turnover in these degradation experiments was non-biological.

More than 96% P turnover occurred in less than 6 days for cultured POM, while less than half of PN was remineralized for all treatments. Correlations between net bacterial growth with total added C and N, in addition to the trends of NH_4^+ release and bacterial growth, suggest that productivity in the early remineralization phase may be controlled by DON lability. Hopkinson et al. (2002) showed similar results with 180-day DOM addition incubations, where C:N ratios of initial DOM and remineralized fractions were relatively consistent (12 and 11 respectively). These findings further suggest that heterotrophic growth efficiency and biological remineralization in surface waters is more intimately linked with C and N cycling and than P.

Preferential P remineralization appears to result from both biological remineralization as well as the release of internal or externally adsorbed soluble inorganic P. While daily trends of biological N:P remineralization were different over a 6 day period, overall N:P rates did not vary between different initial PN:PP of cultured substrates. These findings provide evidence for preferential P remineralization and variable N:P turnover on shorter timescales as a result of non-biological release as well as heterotrophic turnover.

2.6 Literature Cited

Anderson L.A., Sarmiento J.L. (1994) Redfield ratios of remineralization determined by nutrient data analysis. Global Biogeochemical Cycles 8:65-80.

Armstrong F.A., Stearns J.R., Strickland J.H. (1967) The measurement of upwelling and subsequent biological processes by means of the Technicon AutoAnalyzer and associated equipment. Deep Sea Research I 14:381-389.

Arrigo K.R. (2004) Marine microorganisms and global nutrient cycles. Nature 437:349-355.

Bertilsson S., Berglund O., Karl D.M., Chisholm S.W. (2003) Elemental composition of marine *Prochlorococcus* and *Synechococcus*: Implications for the ecological stoichiometry of the sea. Limnology and Oceanography 48:1721-1731.

Chen Y.-B., Zehr J.P., Mellon M. (1996) Growth and nitrogen fixation of the diazotrophic filamentous nonheterocystous cyanobacterium *Trichodesmium* sp. IMS 101 in defined media: Evidence for a circadian rhythm. Journal of Phycology 32:916-923.

Clark L.L., Ingall E.D., Benner R. (1998) Marine phosphorus is selectively remineralized. Nature 393:426-426.

Fu F.-X., Zhang Y., Leblanc K., Sanudo-Wilhelmy S.A., Hutchins D.A. (2005) The biological and biogeochemical consequences of phosphate scavenging onto phytoplankton cell surfaces. Limnology and oceanography:1459-1472.

Guillard R.R. (1975) Culture of phytoplankton for feeding marine invertebrates, Culture of marine invertebrate animals, Springer. pp. 29-60.

Hansell D., Carlson C. (2002) Biogeochemistry of marine dissolved organic matter. Academic Press, London.

Hedges J., Baldock J., Gelinas Y., Lee C., Peterson M., Wakeham S. (2002) The biochemical and elemental compositions of marine plankton: A NMR perspective. Marine Chemistry 78:47-63.

Hopkinson C.S., Vallino J.J., Nolin A. (2002) Decomposition of dissolved organic matter from the continental margin. Deep Sea Research Part II: Topical Studies in Oceanography 49:4461-4478.

Jiao N., Herndl G.J., Hansell D.A., Benner R., Kattner G., Wilhelm S.W., Kirchman D.L., Weinbauer M.G., Luo T., Chen F. (2010) Microbial production of recalcitrant dissolved organic matter: long-term carbon storage in the global ocean. Nature Reviews Microbiology 8:593-599.

Karl D., Björkman K., Dore J., Fujieki L., Hebel D., Houlihan T., Letelier R., Tupas L. (2001a) Ecological nitrogen-to-phosphorus stoichiometry at station ALOHA. Deep-Sea Research Part II 48:1529-1566.

Karl D.M., Bjorkman K.M., Dore J.E., Fujieki L., Hebel D.V., Houlihan T., Letelier R.M., Tupas L.M. (2001b) Ecological nitrogen-to-phosphorus stoichiometry at station ALOHA. Deep Sea Research Part II: Topical Studies in Oceanography 48:1529-1566.

LaRoche J., Breitbarth E. (2005) Importance of the diazotrophs as a source of new nitrogen in the ocean. Journal of Sea Research 53:67-91.

Li Y.-H., Karl D.M., Winn C.D., Mackenzie F.T., Gans K. (2000) Remineralization ratios in the subtropical North Pacific gyre. Aquatic Geochemistry 6:65-85.

Marie D., Partensky F., Jacquet S., Vaulot D. (1997) Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYBR Green I. Applied and Environmental Microbiology 63:186-193.

Martiny A.C., Pham C.T., Primeau F.W., Vrugt J.A., Moore J.K., Levin S.A., Lomas M.W. (2013) Strong latitudinal patterns in the elemental ratios of marine plankton and organic matter. Nature Geoscience 6:279-283.

Miyata K., Hattori A. (1986) A simple fractionation method for determination of phosphorus components in phytoplankton: Application to natural populations of phytoplankton in summer surface waters of Tokyo Bay. Journal of Oceanography 42:255-265.

Moore L.R., Coe A., Zinser E.R., Saito M.A., Sullivan M.B., Lindell D., Frois-Moniz K., Waterbury J., Chisholm S.W. (2007) Culturing the marine cyanobacterium *Prochlorococcus*. Limnol. Oceanogr. Methods 5:353-362.

Paytan A., Cade-Menun B.J., McLaughlin K., Faul K.L. (2003) Selective phosphorus regeneration of sinking marine particles: evidence from ^{31}P-NMR. Marine Chemistry 82:55-70.

Redfield A.C. (1958) The biological control of chemical factors in the environment. American Scientist 46:205-221.

Sannigrahi P., Ingall E., Benner R. (2006) Nature and dynamics of phosphorus-containing components of marine dissolved and particulate organic matter. Geochimica et Cosmochimica Acta 70:5868-5882.

Sañudo-Wilhelmy S.A., Tovar-Sanchez A., Fu F., Capone D.G., Carpenter E.J., Hutchins D.A. (2004) The impact of surface-adsorbed phosphorus on phytoplankton Redfield stoichiometry. Nature 432:897-901.

Sherr B.F., Sherr E.B., del Giorgio P. (2001) Enumeration of total and highly active bacteria, in: J. Paul (Ed.), Methods in microbiology, marine microbiology, Academic Press. pp. 129-159.

Sterner R., Elser J. (2002) Ecological Stoichiometry: The biology of elements from molecules to the biosphere. 1st ed. Princeton University.

Strickland J.D.H., Parsons T.R. (1972) A Practical Handbook of Seawater Analysis. Second Edition ed. Fisheries Research Board of Canada, Ottawa.

Valderrama J.C. (1981) The simultaneous analysis of total nitrogen and total phosphorus in natural waters. Marine Chemistry 10:109-122.

White A.E., Spitz Y.H., Karl D.M., Letelier R.M. (2006) Flexible elemental stoichiometry in *Trichodesmium* spp. and its ecological implications. Limnology and Oceanography 51:1777-1790.

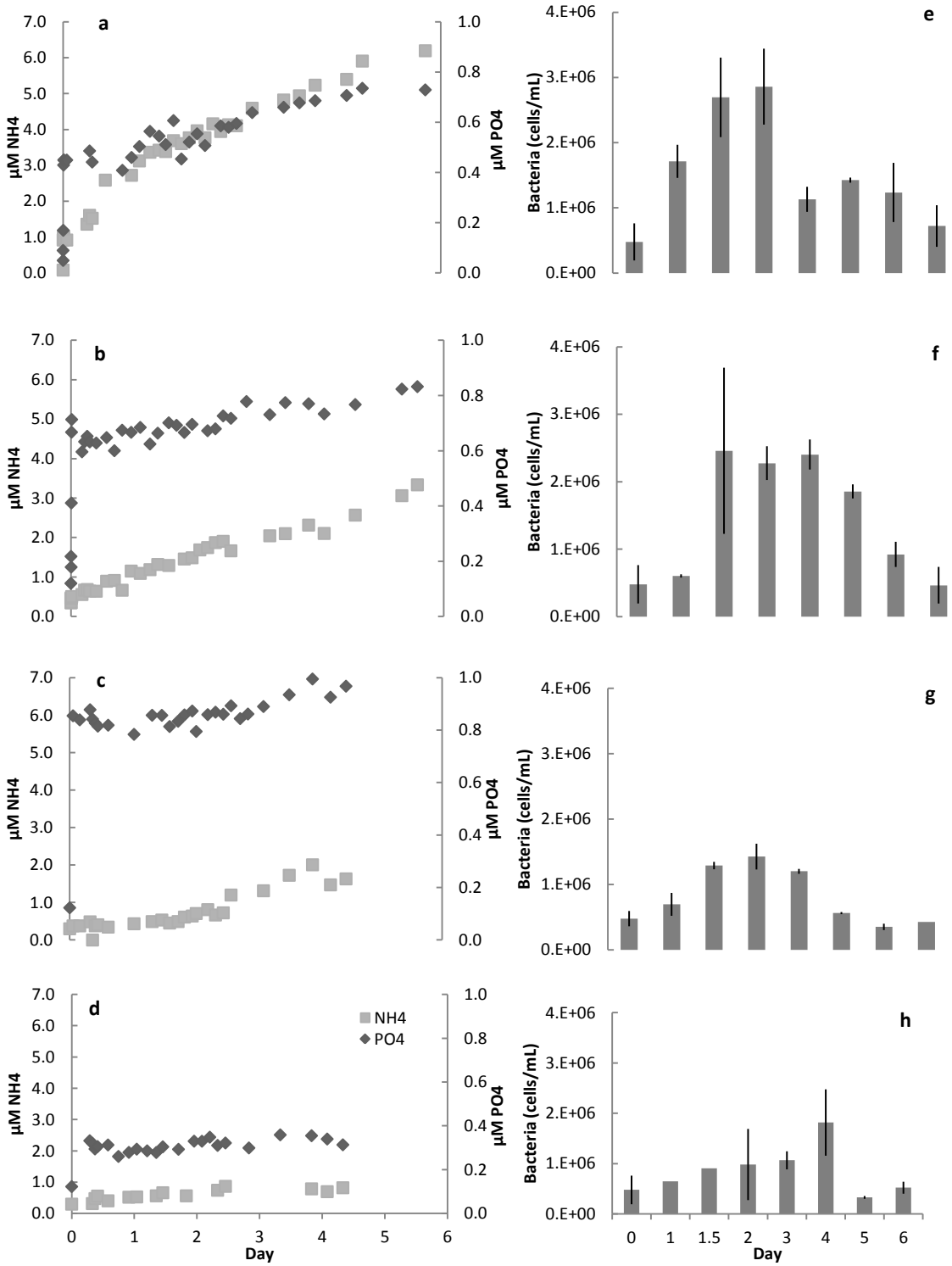
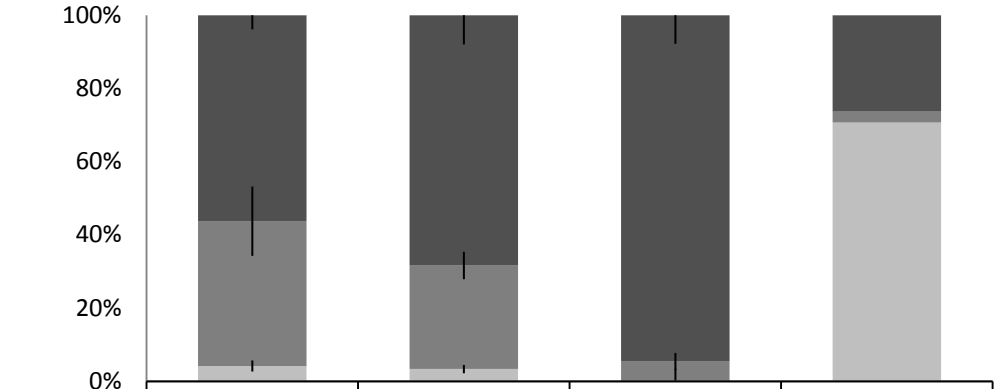


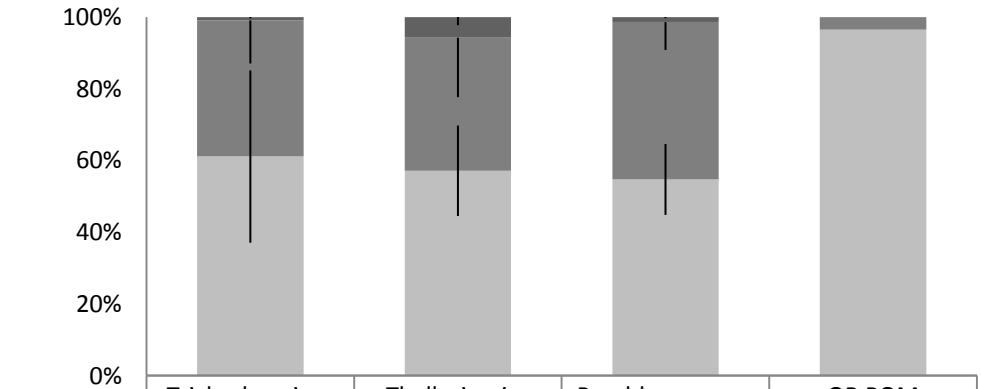
Figure 2-1 SRP (dark diamonds) and NH_4^+ (grey squares) released over time following POM addition for (a) 0.5g *Trichodesmium* (b) 1.0g *Thalassiosira* (c) 1.0g *Prochlorococcus* (d) and 0.6g OR POM (d). Corresponding heterotrophic bacterial abundances over time are shown to the right for each treatment (e-h). Error bars represent the standard deviation of duplicate samples from each timepoint.

P



	Trichodesmium	Thallasiossira	Prochlorococcus	OR POM
% Soluble	56%	68%	95%	26%
% Labile	40%	28%	5%	3%
% other	4%	3%	0%	71%

N



	Trichodesmium	Thallasiossira	Prochlorococcus	OR POM
% Soluble	1%	6%	1%	0%
% Labile	38%	37%	44%	4%
% other	61%	57%	55%	96%

Figure 2-2. Percentage of total added PP (P) and PN (N), converted to SRP/NH_4^+. "Soluble" fraction is the amount of instantaneous increase following POM addition. "Labile" refers to the biologically remineralized fraction (through 130 hours). "Other" refers to the fraction that did not result in a net increase in SRP or NH_4^+. Error bars represent the propagated errors calculated from duplicate treatments; these are not included for 'OR POM' as we only had sufficient material for a single replicate.

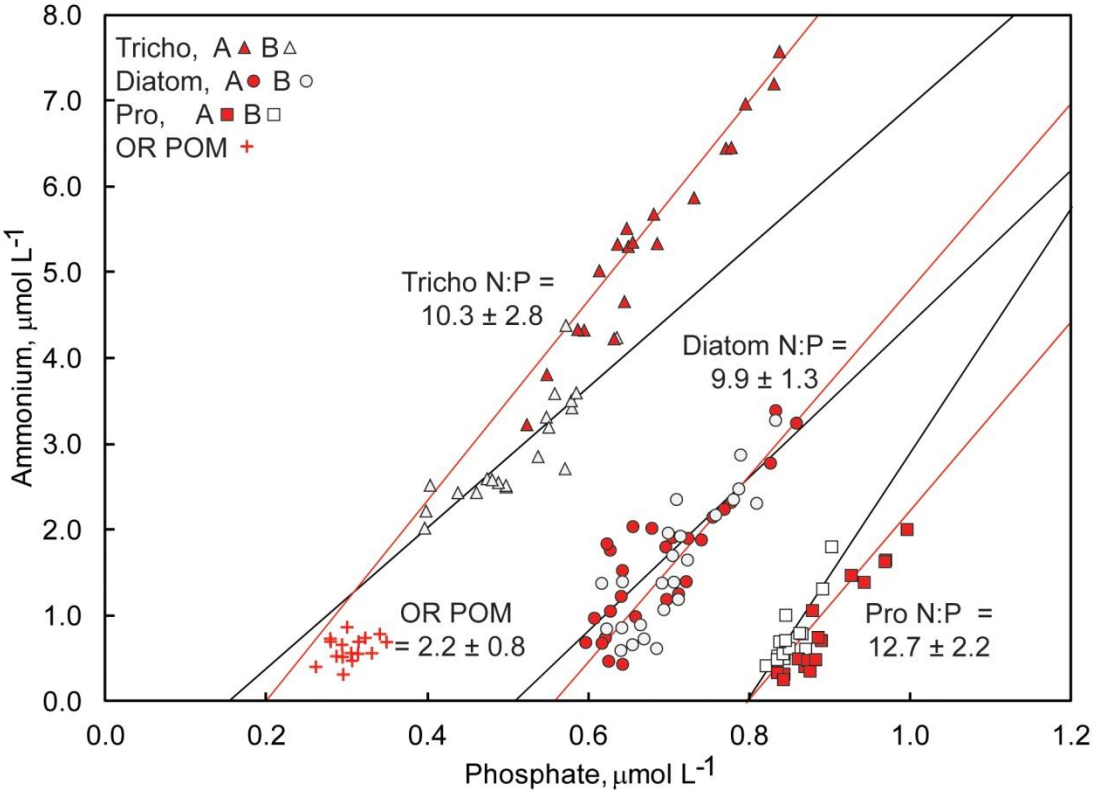


Figure 2-3. Relationship between inorganic N and P pools over the full course of the experiment following P solubilization. The first replicate 'A' is shown as a red symbol and the second replicate as a white symbol. Replicate treatments for *Trichodesmium* (triangles), the diatom *Thalassiosira* (circles), *Prochlorococcus* (squares), and OR POM (plus signs) are each fit to individual regressions to calculate the N:P remineralization ratio. The mean and standard deviation for each treatment is denoted. Note that the different intercepts (P) correspond to the amount of P solubilized. Also the slope of replicate treatments does not differ from the remineralization ratio (12.3, t-test values ranging from 0.45-0.91) calculated for Station ALOHA by Li et al. (2000).

Table 2-1 Initial dissolved nutrient concentrations and heterotrophic bacterial abundance collected from St. ALOHA at 75m measured in this study and compared to those reported by Karl et al. (2001).

	this study		**range at ALOHA (0-100m) (Karl et al. 2001, HOT)**
NH4 (µmol L^{-1})	0.08	(0.01)	
PO4 (µmol L^{-1})	0.09	(0.07)	<.01 - .10
N+N (µmol L^{-1})	0.05	(0.07)	<.02 - .10
DOP (µmol L^{-1})	0.05	(0.08)	.10 - .30
DON (µmol L^{-1})	6.17	(1.96)	4.5 - 6.5
bacterial abundance (cells mL^{-1})	4.42 E+05	(2.43 E+05)	3.5 - 5.6 E+05

Table 2-2 Initial mass of dry POM and concentrations of particulate C, N, and P added to each 20 L treatment.

	Trichodesmium		*Thalassiosira*		*Prochlorococcus*		Oregon POM	
	mean	*stdev*	*mean*	*stdev*	*mean*	*stdev*	*mean*	*stdev*
dry POM (g)	0.50		1.00		1.00		0.60	
µmol C L^{-1}	76.79	5.67	44.98	9.19	24.99	4.22	69.95	19.19
µmol N L^{-1}	13.60	0.87	7.19	1.44	4.27	0.70	6.75	1.80
µmol P L^{-1}	0.69	0.02	0.75	0.09	0.73	0.11	0.92	0.05

Table 2-3. N:P ratios for initial particulate "POM" presented with and without contributions from soluble P and N fractions ("POM -soluble"); "soluble", fraction instantly released; "labile", calculated as the net increase in SRP and NH_4^+ (not including soluble release). "Other" refers to the amount of PN and PP added that was not converted to SRP or NH_4^+ within the timeframe of experimentation. N:P "turnover rates" were calculated via linear regressions of N and P with the error representing the standard deviation of replicate samples.

	Trichodesmium	*Thalassiosira*	*Prochlorococcus*	*Oregon POM*
POM	23 (2)	10 (2)	5 (1)	7 (2)
Labile	19 (5)	13 (3)	39 (17)	10
Soluble	0.3 (0.1)	0.8 (0.3)	<0.1	<0.1
Turnover Total (Soluble+Labile)	8.0 (0.3)	4.2 (0.4)	2.2 (0.2)	1.0
other	>500	165	>500	11.3
POM (-soluble)	46 (7)	30 (13)	72 (52)	10 (3)

Table 2-4. Net increase and growth rates of heterotrophic bacteria.

	Trichodesmium	*Thalassiosira*	*Prochlorococcus*	OR POM
Net increase (bacteria mL^{-1})	2.4±0.9 E+06	1.9±0.6 E+06	9.5±0.5 E+06	1.3±0.7 E+06
Growth rate (cells mL^{-1} day^{-1})	1.5±0.2 E+06	1.3±0.4 E+06	5.4±0.1 E+05	3.3±0.7 E+05

Long term remineralization trends of particulate organic matter for an open ocean and coastal heterotrophic community

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3 Long term remineralization trends of particulate organic matter for an open ocean and coastal heterotrophic community

3.1 Abstract

Here, we report results from two separate experiments examining the relative lability and elemental stoichiometry of particulate organic matter remineralization (POM) by disparate natural populations of marine heterotrophs over a period of ~150 days. Seawater was collected from the oligotrophic North Pacific Subtropical Gyre (HI) and the upwelling regime off the Oregon coast (OR), and stored in the dark to discourage photoautotrophic growth and isolate the heterotrophic community. POM was harvested from laboratory cultures of ecologically significant taxa from each respective region, a diazotroph (*Trichodesmium* IMS101) (HI) and the diatom (*Thalassiosira weissflogii)* (OR). Following exogenous POM addition, inorganic phosphorus (P) and nitrogen (N) regeneration was monitored to quantify the approximate timescales of N:P remineralization for these regions.

During an initial high resolution sampling period (within a day after POM additions), we observed rapid and complete solubilization and remineralization of particulate P (PP) in all live treatments, with incomplete turnover (55% and 75% for HI+*Trichodesmium* and OR+*Thalassiosira* respectively) of particulate N (PN) into ammonium (NH_4^+). In addition, a nitrification event occurred in both experiments converting NH_4^+ to nitrate (N+N) after 63d (HI) and 98d (OR). Both POM sources contained a rapidly released soluble P pools, assumed to be either surface-adsorbed inorganic P or inorganic P reserves liberated from cells during harvesting of biomass, accounted for 42% and 85% of total PP. The ratio of N:P remineralization varied over time for the *Trichodesmium*+HI experiment, and less so with OR+*Thalassiosira*, suggesting a greater decoupling of N and P may occur in this open ocean region.

3.2 Introduction

Within the marine environment, rates and ratios of C, N, and P remineralization are reflected through the elemental stoichiometry and molecular makeup of OM substrates produced within the photic layer, balanced with the nutritional requirements and enzymatic capacities of the heterotrophic community at depth (Lee et al., 2004). While global means of elemental ratios and OM respiration are typically utilized for modeling nutrient cycles and determining nutrient limitation, regional and temporal deviations regarding the timescales of C, N, and P remineralization have often been overlooked in these processes (Anderson and Sarmiento, 1994; Li et al., 2000; Redfield, 1958). The coastal and open oceans are distinctly different in nutrient supply, elemental composition, physical processes, and community structure. With regard to the extent and periodicity of nutrient supply, coastal waters typically have strong seasonal upwelling and terrestrial inputs of nutrients (Wetz et al., 2008). Dissolved inorganic concentrations off the Oregon coast can range from 0.1 to >10 μmol N L^{-1} N and 0.1 to >1.0 μmol P L^{-1} (Peterson, 1979). In contrast, in the open ocean it is not unusual for inorganic N and P concentrations to fall below instrument detection limits (< ~30 nmol L^{-1}), and inorganic N:P to be <1.0 (Karl and Bjorkman, 2000). Additionally, temperature ranges vary throughout the ocean resulting in different enzymatic rates of organic matter hydrolysis (White et al., 2012). Moreover, these regions sustain differences in the abundance, community structure, enzymatic capacity, and nutritional requirements of the heterotrophic populations.

Many previous OM degradation studies in these regions have focused on cycling of a single element (N for example), and ignored or glossed over the interdependence and coupling of macroelements for the purposes of productivity and decomposition. Furthermore, the relative trends and timescales of N and P remineralization by different heterotrophic communities have not been well quantified. As the temporal decoupling between C, N, and P remineralization determines the spatial distribution and supply of bioavailable nutrients, the quantification of these processes is imperative to understanding the controls of ocean productivity. In addition, understanding taxonomic impacts on the lability and stoichiometry of OM and OM remineralization is necessary to

constrain this variability of elemental cycling in a changing acidifying and warming ocean (Hopkinson and Vallino, 2005).

To further the current scientific knowledge regarding the factors controlling C, N, and P distributions, POM degradation experiments were performed to quantify the relative trends of N and P remineralization over a 100-150d period. Two parallel, but not comparative experiments were conducted. Heterotrophic populations were collected from a coastal (Oregon) and open ocean (Station ALOHA, HI) site and POM derived from ecologically significant cultures respective to each region was added: this consisted of a diazotrophic cyanobacteria (*Trichodesmium* IMS 101) and a common diatom (*Thalassiosira weissflogii*) for HI and OR, respectively. N and P remineralization and heterotrophic community composition was be monitored in high resolution for 1d (continuous) and at moderate to low resolution for another ~ 150d (daily to weekly).

3.3 Methods and Materials:

3.3.1 POM preparation:

Large volume batch cultures were grown on a 12:12 light dark cycle at saturating growth irradiances (150 μmol photons m^{-2} s^{-1}) at a constant temperature of 24^{o}C, with all efforts made to maintain axenic cultures. *Trichodesmium* (strain IMS101) was grown on YBCII media (Chen et al., 1996), modified for an initial DIP addition of 5μmol P L^{-1}. *Thalassiosira weissflogii* was grown on F/2 media (Guillard, 1975). Growth of all cultures was monitored by *in vivo* chlorophyll fluorescence (via either a Walz Water-PAM or Turner 10-AU fluorometer). All cultures were harvested during the early stationary growth phase, and concentrated into a slurry. Different biomass isolation methods were performed as *Thalassiosira* cells were concentrated by sequential centrifuging using a Marathon® centrifuge, the buoyancy of *Trichodesmium* prevents pellet formation. Therefore *Trichodesmium* cells were isolated by gentle filtration (<100 mmHg) onto a series of 25mm 5.0 μm polycarbonate filters to minimize cell breakage (Bertilsson et al., 2003). All particulate organic matter (POM) was flash frozen in liquid nitrogen and stored at -80°C in 50 mL Falcon tubes. *Trichodesmium* filters were thawed, and re-suspended with seawater (same whole water used for experiment to prevent mixing or impurities). The concentrated slurries were homogenized by brief vortexing

before all additions and sub sampling for characterized of particulate C, N, and P composition.

3.3.2 Experimental Design:

Large volumes of whole seawater were collected from the euphotic zone at two distinct and frequently monitored locations to compare the remineralizing capacities of different microbial communities. Station ALOHA (A Long-Term Oligotrophic Habitat Assessment; 22° 45'N, 158° 00'W), the open ocean time-series in the oligotrophic North Pacific Subtropical Gyre (NPSG), and NH-10 (44° 39'N, 124° 17'W), ten miles off the upwelling Oregon coast along the Newport Hydrographic line. NPSG water (HI) was collected in September of 2011 from 75m with CTD niskins (*in situ* temperature 24°C), and NH-10 (OR) was collected in December 2011 from <5m depth with a clean sampling bucket (in situ temperature 8.5°C). Each water sample was homogenized into clean 200 gallon drums and stored in the dark at room temperature (~22°C) for 15 days and 33 days, for HI and OR respectively. Once chlorophyll *a* measurements diminished to filter blank levels, samples were divided into 20 L polycarbonate carboys and capped with vented 3-port lids and internal tubing for sampling. All materials were acid-washed and autoclaved before use. Treatments were transferred to a dark incubator maintained at respective *in situ* temperatures to maintain accurate enzymatic and metabolic activities. Chlorophyll *a* and bacterial abundances were monitored throughout 'ageing' storage and experimentation. Initial samples were qualitatively spot-checked for bacteria, grazers, and viruses.

Following POM and seawater preparation, biomass was added volumetrically to treatments: duplicates of HI+*Trichodesmium*, OR+ *Thalassiosira*, single replicates of "killed" controls with saturated $HgCl_2$ (HI+*Trichodesmium*+$HgCl_2$ and OR+ *Thalassiosira*+$HgCl_2$) and control (OR and HI + no biomass). Biomass was added in amounts targeted for a ~1.0 µmol P addition to simulate an export event within ample range of instrument detection. Remineralization was tracked by net transfer to dissolved inorganic N and P (DIN and DIP respectively), measured as NH_4^+ and N+N, and SRP (proxy for DIP). Nutrient measurements were paralleled to changes in bacterial and viral

abundance. Continuous nutrient data was collected through the first hour after addition, daily for the initial week, and weekly thereafter.

3.3.3 Analytical Measurements:

Particulate and Dissolved elements:

Nutrients were analyzed using a flow-through colorimetric method on a Technicon Auto Analyzer II. . Soluble reactive phosphorus (SRP) and ammonium (NH_4^+) analysis was performed following methods from ALPKEM RFA (ALPKEM, 1984). Nitrite + Nitrate (N+N) was analyzed using the cadmium reduction method of Wood et al. (1967). Standards where prepared in a 3% NaCl Milli-Q matrix. A LNSW blank was additionally run, as NH_4^+ concentrations are typically high in deionized water. A 5-point standard curve was made before each run, and blanks where run before and after to correct for any baseline shifts. Total dissolved P (TDP) and N (TDN) were determined by the alkaline persulfate oxidation method (Valderrama, 1981).

Biomass (extracted chlorophyll, heterotrophic identification and abundance):

During the dark holding period and at daily intervals over the course of the experiment, 5-ml samples were collected from each treatment for measurement of extracted chlorophyll-a concentrations via the acidification method of Strickland and Parsons(1972). Again at daily intervals, triplicate 3mL samples from each replicate were collected, fixed with 60 µL of 10% paraformaldehyde, and stored at -80ºC for flow cytometric analysis of bacterial concentrations.

Bacterial abundance was measured by flow cytometry on a Becton-Dickinson FACS-Caliber four-color flow cytometer with SYBR® Green stain as described by Sherr et al. (2001). Samples were stained for 10 min in the dark with a 10^{-4} diluted stock of SYBR ® Green as in Marie et al. (1997). Heterotrophic bacteria, including High and Low Nucleic Acid clusters (HNA, LNA) were plotted and gated using fluorescence (515-545 nm), Side Scatter (SSC), and Forward Scatter (FSC) on log scales.

Viral enumeration was performed with an epifluorescence microscope and SYBR® Green stain as in Suttle and Fuhrman (2010). A fresh

phenylenediamine/Glycerol/PBS antifade solution was prepared for the preservation of slides. All work occurred under low lights with autoclaved Milli-Q and 0.02 µm-filtered reagents to prevent stain fading and introduction of exogenous viral particles.

Samples of 100-200 mL were collected and preserved with 1% (final concentration) paraformaldehyde or 30% Formalin for quantitative CARD-FISH microscopy analysis (Pernthaler et al., 2002). Fluorescent probes were utilized for a known nitrifying bacterium off the OR coast, crenarchea (proxy for nitrifying archea), as well as a general 16S probe (all prokaryotes). All filtered samples were stained with DAPI (a general DNA stain) to matchup and confirm cellular integrity of fluorescent counts.

3.4 RESULTS and DISCUSSION

3.4.1 Initial Water Characterization

In water concentrations for HI were 0.08±0.07 µmol N+N L^{-1}, 0.02±0.01 µmol SRP L^{-1}, and 0.08±0.04 µmol NH_4^+ L^-, while coastal values were 4.6±0.2 µmol N+N L^{-1}, 1.02±0.01 µmol SRP L^{-1}, and 0.9±0.1 µmol NH_4^+ L^-. For HI, dissolved organic pools made up 96% and 85% of TDN and TDP respectively. Concentrations were 7.4±0.5 µmol DON L^{-1} and 0.12 ±0.04 µmol L^{-1} DOP. DON values at the coastal site were 7.2±0.1 µmol DON L^{-1}, and made up 56% of TDN; DOP levels were below detection. Initial heterotrophic bacterial concentrations were 3.46±0.07x10^5 cells mL^{-1} for HI, and 7.7±0.9x10^5 cells mL^{-1} for OR.

3.4.2 PN and PP lability

Initial particulate C:N:P ratios of added biomass were 95:21:1 for *Trichodesmium*, and 60:9:1 for *Thalassiosira* (Table 3-2). For all treatments, POM contained a soluble P fraction quantified by an instant release of SRP (85% of *Trichodesmium* and 42% of *Thalassiosira)*, suggesting a significant portion of PP turnover may not be biologically mediated. For both experiments, 100% of PP added was turned over to SRP, for live and $HgCl_2$ "killed" treatments. However, it should be noted that while no net bacterial growth occurred in $HgCl_2$ treatments, flow cytometric abundances showed populations may have not completely diminished. Meanwhile, only 55% and 75% of PN was

converted to DIN within the timeframe of these incubations for *Trichodesmium* and *Thalassiosira* respectively (Figure 3-2). Consequently, N:P ratios of labile pools were lower relative to initial PN:PP added (7.4±0.4 and 5.7±0.8 for *Trichodesmium* and *Thalassiosira* respectively) (Table 3-3), and suggests there may be a significant decoupling of N and P turnover with *Trichodesmium*+HI.

3.4.3 Rates of Remineralization

Rapidly released soluble P accounted for a significant fraction of both POM sources, and presumably resulted from internal stores or externally adsorbed soluble inorganic P. In the following, "labile" remineralization has been quantified as the net biological turnover, excluding any soluble fraction and drawdown, while 'other" is the fraction of added PP and PN that did not result in a net transfer to the dissolved inorganic pools within the experimental timeframe.

Without soluble pools, rates of P turnover (as % of initial PP) in the first 5 days were 2.6±0.6 and 3.2±0.2% day^{-1} for HI and OR respectively. Rates of N turnover in the first 5 days were 13±1% day^{-1} for HI+ *Trichodesmium* and 3.4±0.4% day^{-1} for OR+*Thalassiosira*. For HI, 90% of the total net turnover occurred within the first 4 days for N and 6 days for P. In contrast, the OR experiment took 38 days for N, and 53 days for P. A nitrification event, determined by a near or complete drawdown of NH_4^+ with simultaneous, but not equivalent release of N+N, initiated at 70 days in the HI experiment and 105 days in the OR experiment. Differences in net NH_4^+ drawdown and N+N increase were 3.1±0.1 for HI and 4.1±3.0 for OR. Nitrification did not occur in any Killed treatments. Quantitative CARD-FISH microscopy for the OR experiment discerned nitrification was predominantly by Crenarchaeota.

Over the first 5 days of decomposition, the N:P of remineralization rates (calculated using the linear regressions of NH_4^+ and SRP turnover in µmol N L^{-1} day^{-1} /µmol P L^{-1} day^{-1}) were 71±8 and 8.0±0.3 for HI+ *Trichodesmium* and OR+*Thalassiosira* respectively. Through 60 days however, N:P turnover rates were 13±2 for HI+ *Trichodesmium*, and 9.2±0.3 for OR+*Thalassiosira*. These findings suggest a relatively greater decoupling of N:P may occur with *Trichodesmium* biomass and the HI heterotrophic community.

In live POM treatments, a net draw down of SRP occurred that coordinated with increasing bacterial abundances. A drawdown of 0.05± 0.01 µmol L^{-1} occurred within the first day for HI at 24°C and a relatively greater net decrease of 0.17± 0.08 extending through 48 hours for OR at 9°C. Assuming net SRP drawdown is exclusively due to bacterial uptake, calculated cellular P values would be approximately 1.3±0.3 fg P $cell^{-1}$ for HI, and 1.2 ±0.6 fg P $cell^{-1}$, would account for 8±2% for *Trichodesmium* and 10±5% for *Thalassiosira* taken up and remineralized through bacterial biomass.

Trends in heterotrophic bacterial and viral abundances showed maximum net growth within the first week of both experiments. To determine bacterial growth limitation, glucose was added to subsamples of HI treatments. Near complete drawdown of DIN and SRP pools following glucose additions suggests these populations were C limited following an initial period of remineralization.

3.5 Conclusions

In these regional experiments, preferential P remineralization was observed over short time scales with rapid and total turnover of P due to internal or externally adsorbed soluble inorganic P. This decoupling of N:P remineralization extended through the <150d duration of experimentation as a significant fraction of PN was not remineralized. Total PN turnover ranged from 55-75% for both regional experiments, resulting in lower N:P values of final dissolved pools relative to initial PN:PP of OM substrates. This was seen to a greater extent in the HI+*Trichodesmium* experiment. Nitrification (ammonium oxidation) occurred over timescales ranging from 70d for HI to 105 for OR, and nitrifying populations in OR surface waters were dominated by Crenarchaeota.

3.6 Literature Cited

Anderson L.A., Sarmiento J.L. (1994) Redfield ratios of remineralization determined by nutrient data analysis. Global Biogeochemical Cycles 8:65-80.

Bertilsson S., Berglund O., Karl D.M., Chisholm S.W. (2003) Elemental Composition of Marine Prochlorococcus and Synechococcus: Lmplications for the Ecological Stoichiometry of the Sea. Limnology and oceanography:1721-1731.

Chen Y.-B., Zehr J.P., Mellon M. (1996) Growth and nitrogen fixation of the diazotrophic filamentous nonheterocystous cyanobacterium *Trichodesmium* sp. IMS 101 in defined media: Evidence for a circadian rhythm. Journal of Phycology 32:916-923.

Guillard R.R. (1975) Culture of phytoplankton for feeding marine invertebrates, Culture of marine invertebrate animals, Springer. pp. 29-60.

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

Karl D.M., Bjorkman K.M. (2000) Phosphorus, the staff of life. Nature 406:31-32.

Lee C., Wakeham S., Arnosti C. (2004) Particulate organic matter in the sea: the composition conundrum. AMBIO: A Journal of the Human Environment 33:565-575.

Li Y.-H., Karl D.M., Winn C.D., Mackenzie F.T., Gans K. (2000) Remineralization ratios in the subtropical North Pacific gyre. Aquatic Geochemistry 6:65-85.

Marie D., Partensky F., Jacquet S., Vaulot D. (1997) Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYBR Green I. Applied and Environmental Microbiology 63:186-193.

Pernthaler A., Pernthaler J., Amann R. (2002) Fluorescence in situ hybridization and catalyzed reporter deposition for the identification of marine bacteria. Applied and Environmental Microbiology 68:3094-3101.

Peterson B.J. (1979) Particulate organic matter flux and planktonic new production in the deep ocean. Nature 282:677-680.

Redfield A.C. (1958) The biological control of chemical factors in the environment. American Scientist 46.

Sherr B.F., Sherr E.B., del Giorgio P. (2001) Enumeration of total and highly active bacteria, in: J. Paul (Ed.), Methods in microbiology, marine microbiology, Academic Press. pp. 129-159.

Strickland J.D.H., Parsons T.R. (1972) A Practical Handbook of Seawater Analysis. Second Edition ed. Fisheries Research Board of Canada, Ottawa.

Suttle C.A., Fuhrman J.A. (2010) Enumeration of virus particles in aquatic or sediment samples by epifluorescence microscopy. Manual of Aquatic Viral Ecology. ASLO:145-153.

Valderrama J.C. (1981) The simultaneous analysis of total nitrogen and total phosphorus in natural waters. Marine Chemistry 10:109-122.

Wetz M.S., Hales B., Wheeler P.A. (2008) Degradation of phytoplankton-derived organic matter: Implications for carbon and nitrogen biogeochemistry in coastal ecosystems. Estuarine, Coastal and Shelf Science 77:422-432.

White A.E., Watkins-Brandt K.S., Engle M.A., Burkhardt B., Paytan A. (2012) Characterization of the rate and temperature sensitivities of bacterial remineralization of dissolved organic phosphorus compounds by natural populations. Frontiers in Microbiology 3.

Wood E., Armstrong F., Richards F. (1967) Determination of nitrate in sea water by cadmium-copper reduction to nitrite. J. mar. biol. Assoc. UK 47:23-31.

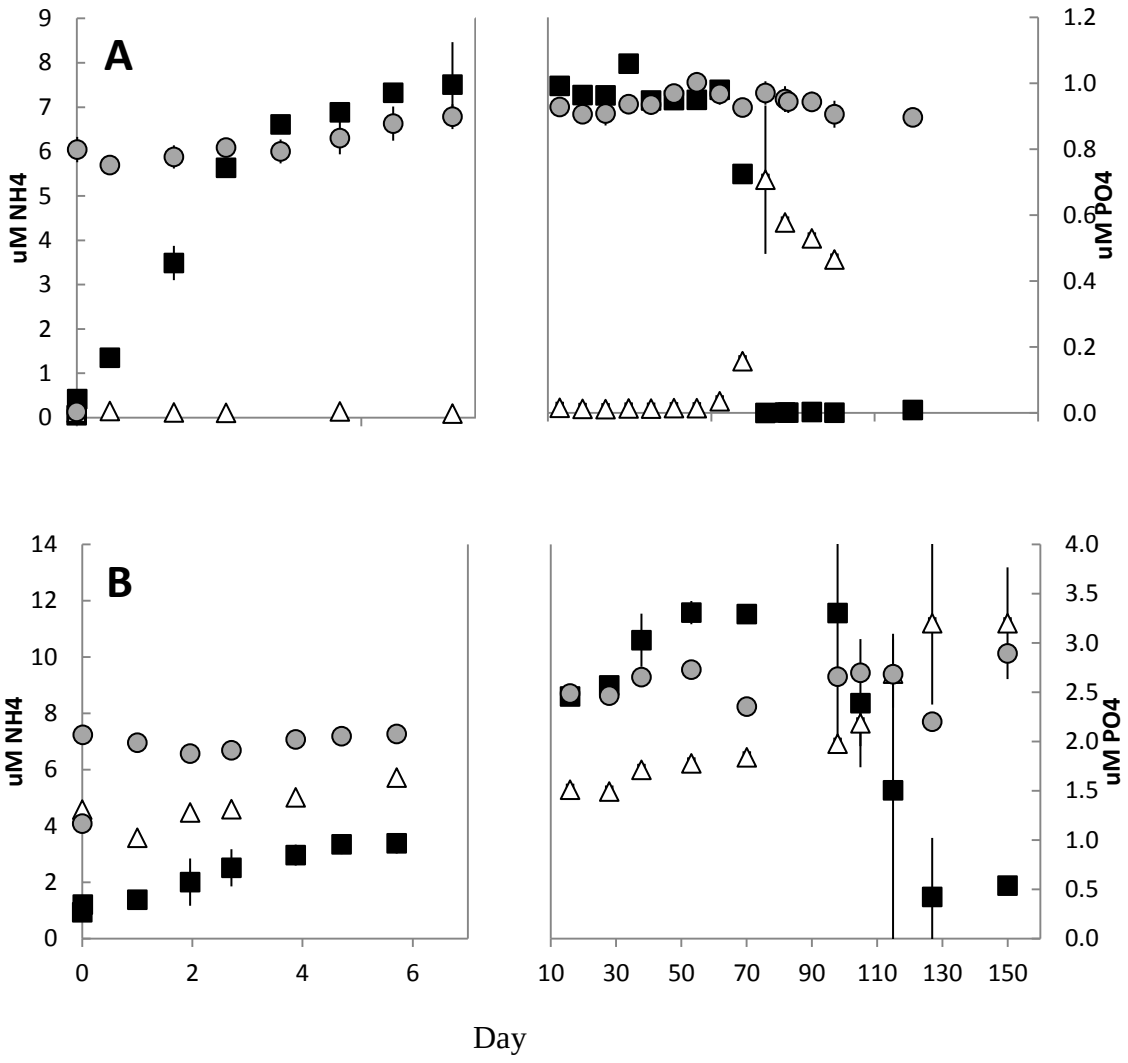

Figure 3-1 SRP (grey circles), NH_4^+ (dark squares), and NO_2 + NO_3 (triangles) released over time following POM addition for HI+*Trichodesmium* at 24°C (A), and *Thalassiosira*+OR water at 9°C (B).

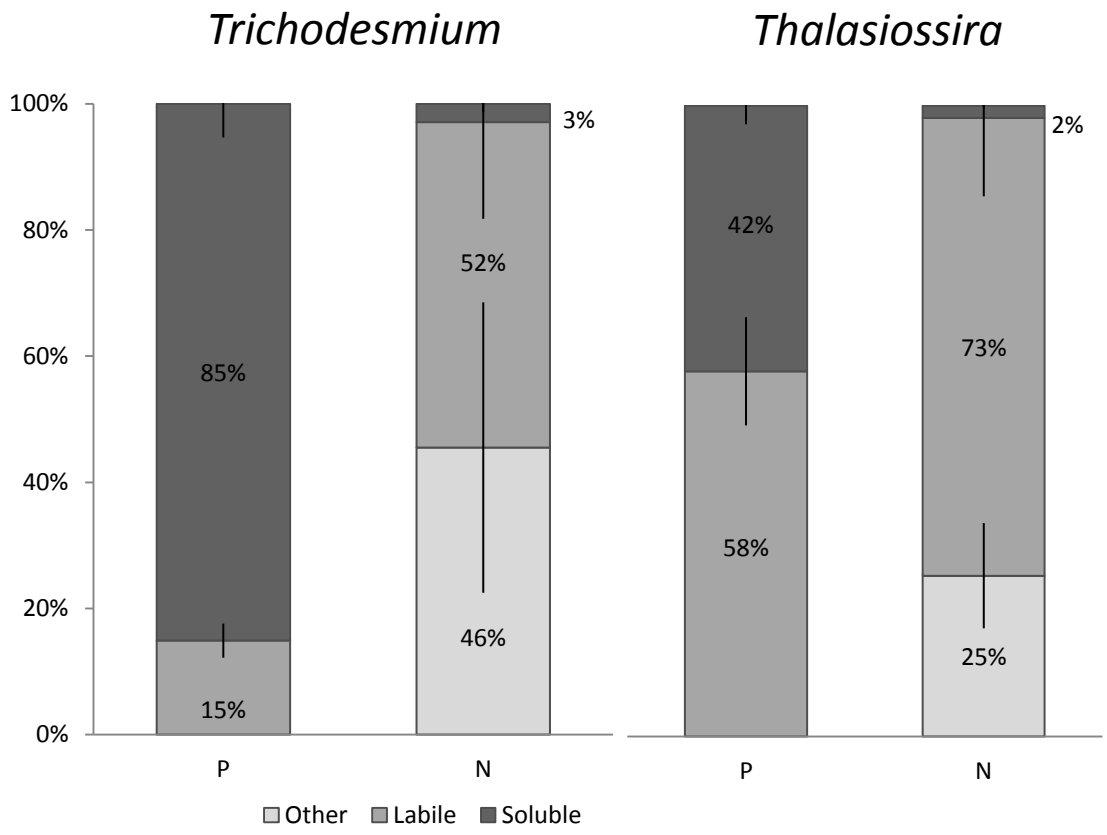

Figure 3-2. Percentage of total added PP and PN transferred to DIP and DIN pools for *Trichodesmium* and *Thalassiosira*. "Soluble" fraction is the amount of instantaneous increase following POM addition. "Labile" refers to the biologically remineralized fraction, calculated as the net transfer to inorganic pools less soluble fraction. "Other" refers to the fraction added not resulting in a net increase in DIP or DIN.

Table 3-1 Initial dissolved nutrient concentrations and heterotrophic bacterial abundance collected for each collection site.

	ALOHA	+/-	NH-10	+/-
NH4 (µmol L^{-1})	0.08	0.01	0.94	0.05
PO4 (µmol L^{-1})	0.09	0.07	1.02	0.01
N+N (µmol L^{-1})	0.05	0.07	0.46	0.1
DOP (µmol L^{-1})	0.05	0.08	n/d	
DON (µmol L^{-1})	6.17	1.96	7.1	1.7
bacterial abundance (cells mL^{-1})	4.42 E+05	2.43 E+05	7.73 E+05	(0.9 E +05)

Table 3-2. Initial concentrations of added PC, PN, and PP for each treatment, and ratios of initial POM.

	Trichodesmium	***Thalassiosira***
µmol C/L	58.05	99.38
µmol P/L	0.85	1.87
µmol N/L	12.53	14.28
C:N	4.6	7.0
C:P	68.1	53.1
N:P	14.7	7.6

Table 3-3. N:P ratios of measured pools. "POM" is presented with and without soluble fraction "POM (-soluble)". "Soluble" is the non-biologically remineralized inorganic fraction instantly released with addition. "Labile" is the non-soluble fraction of POM added resulting in a net increase in SRP and NH_4^+. "Other" refers to amount of N and P added that was not converted to SRP or NH_4^+ within the timeframe of experimentation.

	Trichodesmium	+/-	***Thalassiosira***	+/-
POM	21	7	9	1
POM (-soluble)	88	39	13	1
Soluble	0.5	0.2	0.35	0.05
Labile	47	20	10	1
Labile+Soluble	7	3	5.68	0.75

4 Conclusions

All POM treatments in this study resulted in preferential remineralization of particulate P relative to N. Though a number of studies have documented preferential P remineralization relative to N or C (Anderson and Sarmiento, 1994; Clark et al., 1998; Paytan et al., 2003), to our knowledge, this is the first to quantify fine scale fractions and high-resolution rates. Rapidly released soluble P accounted for a significant fraction of POM, resulting in low initial dissolved inorganic N:P ratios. After release of soluble P, the rate of biological N remineralization exceeded P (μmol L^{-1} day^{-1}), in proportions less than Redfield trajectories (16N:1P), but within range of existing models by Sarmiento & Anderson (14:1), and Li et al. (2000) (12.4:1). Remineralization rates of N:P (less soluble pool) with populations collected from two difference seasons Station ALOHA ranged from 9.9±1.3 to 13.7±3.0 for all cultured POM treatments over short and long timescales. These values fall near or within the range of deep water (N+N:SRP) measurements (14±1) by Karl et al. (2001), suggesting relatively fixed elemental requirements of the remineralizing community that almost exclusively occurs within the first week.

Given that both P adsorption and large internal DIP pools are known facets of phytoplankton P physiology this might explain the mechanisms behind the concept of selective P remineralization. In these studies, cultures were grown on highly enriched media (≥5μmol P) and harvested during exponential growth phase, therefore PP values may represent partial fraction of surface adsorbed P. Additionally, material was harvested and dried and so we cannot rule out the possibility that the soluble P pools measured corresponded to internal DIP pools, which can also comprise a significant fraction of PP (Miyata and Hattori, 1986). Additionally, lower percentages of total PN turnover into NH_4^+ was seen in all POM treatments with both coastal (OR) and open ocean (HI) heterotrophic communities, suggesting ammonification of OM may be a significant rate limiting step in N remineralization.

Despite regional differences, bacterial growth tracked rates of N release and SRP drawdown for both populations, suggesting that while N was not limiting bacterial growth, the acquisition of C from organic N compounds may play an important role in

bacterial productivity and N remineralization while DIP is more rapidly available and taken up.

In summary, these experimental results suggest that differential supply of POM leads to rapid and preferential P remineralization that is partially non-biological, and is observed with natural OM substrates and remineralizing communities. The ratio of N:P remineralization is independent of the N:P of added OM substrates. This constancy of N:P remineralization by the heterotrophic populations observed in this study, suggests variable N:P of OM substrates will result in proportionally variable residual organic matter. These findings help constrain our knowledge of the elemental cycling in the marine environment.

5 Bibliography

Anderson L.A., Sarmiento J.L. (1994) Redfield ratios of remineralization determined by nutrient data analysis. Global Biogeochemical Cycles 8:65-80.

Armstrong F.A., Stearns J.R., Strickland J.H. (1967) The measurement of upwelling and subsequent biological processes by means of the Technicon AutoAnalyzer and associated equipment. Deep Sea Research I 14:381-389.

Arrigo K.R. (2004) Marine microorganisms and global nutrient cycles. Nature 437:349-355.

Azam L. (1983) 1 The Ecological Role oi Water-Column Microbes in. Mar. Ecol. Prog. Ser 1:257-263.

Bertilsson S., Berglund O., Karl D.M., Chisholm S.W. (2003a) Elemental Composition of Marine Prochlorococcus and Synechococcus: Lmplications for the Ecological Stoichiometry of the Sea. Limnology and oceanography:1721-1731.

Bertilsson S., Berglund O., Karl D.M., Chisholm S.W. (2003b) Elemental composition of marine *Prochlorococcus* and *Synechococcus*: Implications for the ecological stoichiometry of the sea. Limnology and Oceanography 48:1721-1731.

Chen Y.-B., Zehr J.P., Mellon M. (1996) Growth and nitrogen fixation of the diazotrophic filamentous nonheterocystous cyanobacterium *Trichodesmium* sp. IMS 101 in defined media: Evidence for a circadian rhythm. Journal of Phycology 32:916-923.

Church M.J., Ducklow H.W., Karl D.M. (2002) Multiyear increases in dissolved organic matter inventories at Station ALOHA in the North Pacific Subtropical Gyre. Limnology and oceanography 47:1-10.

Clark L.L., Ingall E.D., Benner R. (1998) Marine phosphorus is selectively remineralized. Nature 393:426-426.

del Giorgio P.A., Duarte C.M. (2002) Respiration in the open ocean. Nature 420:379-384.

Field C.B., Behrenfeld M.J., Randerson J.T., Falkowski P. (1998) Primary production of the biosphere: integrating terrestrial and oceanic components. Science 281:237-240.

Fu F.-X., Zhang Y., Leblanc K., Sanudo-Wilhelmy S.A., Hutchins D.A. (2005) The biological and biogeochemical consequences of phosphate scavenging onto phytoplankton cell surfaces. Limnology and oceanography:1459-1472.

Geider R.J., Roche J.L. (2002) Redfield revisited: variability of C [ratio] N [ratio] P in marine microalgae and its biochemical basis. European Journal of Phycology 37:1-17.

Guillard R.R. (1975) Culture of phytoplankton for feeding marine invertebrates, Culture of marine invertebrate animals, Springer. pp. 29-60.

Hansell D., Carlson C. (2002) Biogeochemistry of marine dissolved organic matter. Academic Press, London.

Hedges J., Baldock J., Gelinas Y., Lee C., Peterson M., Wakeham S. (2002) The biochemical and elemental compositions of marine plankton: A NMR perspective. Marine Chemistry 78:47-63.

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

Hopkinson C.S., Vallino J.J., Nolin A. (2002) Decomposition of dissolved organic matter from the continental margin. Deep Sea Research Part II: Topical Studies in Oceanography 49:4461-4478.

Jiao N., Herndl G.J., Hansell D.A., Benner R., Kattner G., Wilhelm S.W., Kirchman D.L., Weinbauer M.G., Luo T., Chen F. (2010) Microbial production of recalcitrant dissolved organic matter: long-term carbon storage in the global ocean. Nature Reviews Microbiology 8:593-599.

Karl D., Christian J., Dore J., Hebel D., Letelier R., Tupas L., Winn C. (1996) Seasonal and interannual variability in primary production and particle flux at Station ALOHA. Deep Sea Research Part II: Topical Studies in Oceanography 43:539-568.

Karl D.M., Bjorkman K.M. (2000) Phosphorus, the staff of life. Nature 406:31-32.

Karl D.M., Bjorkman K.M., Dore J.E., Fujieki L., Hebel D.V., Houlihan T., Letelier R.M., Tupas L.M. (2001) Ecological nitrogen-to-phosphorus stoichiometry at station ALOHA. Deep Sea Research Part II: Topical Studies in Oceanography 48:1529-1566.

Kirchman D. (1994) The uptake of inorganic nutrients by heterotrophic bacteria. Microbial Ecology 28:255-271.

Kirchman D.L., Meon B., Cottrell M.T., Hutchins D.A., Weeks D., Bruland K.W. (2000) Carbon versus iron limitation of bacterial growth in the California upwelling regime. Limnology and oceanography:1681-1688.

Klausmeier C.A., Litchman E., Daufresne T., Levin S.A. (2004) Optimal nitrogen-to-phosphorus stoichiometry of phytoplankton. Nature 429:171-174.

LaRoche J., Breitbarth E. (2005) Importance of the diazotrophs as a source of new nitrogen in the ocean. Journal of Sea Research 53:67-91.

Lee C., Wakeham S., Arnosti C. (2004) Particulate organic matter in the sea: the composition conundrum. AMBIO: A Journal of the Human Environment 33:565-575.

Li Y.-H., Karl D.M., Winn C.D., Mackenzie F.T., Gans K. (2000) Remineralization ratios in the subtropical North Pacific gyre. Aquatic Geochemistry 6:65-85.

Longhurst A., Sathyendranath S., Platt T., Caverhill C. (1995) An estimate of global primary production in the ocean from satellite radiometer data. Journal of Plankton Research 17:1245-1271.

Marie D., Partensky F., Jacquet S., Vaulot D. (1997) Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYBR Green I. Applied and Environmental Microbiology 63:186-193.

Martiny A.C., Pham C.T., Primeau F.W., Vrugt J.A., Moore J.K., Levin S.A., Lomas M.W. (2013) Strong latitudinal patterns in the elemental ratios of marine plankton and organic matter. Nature Geoscience 6:279-283.

Miyata K., Hattori A. (1986) A simple fractionation method for determination of phosphorus components in phytoplankton: Application to natural populations of phytoplankton in summer surface waters of Tokyo Bay. Journal of Oceanography 42:255-265.

Moore L.R., Coe A., Zinser E.R., Saito M.A., Sullivan M.B., Lindell D., Frois-Moniz K., Waterbury J., Chisholm S.W. (2007) Culturing the marine cyanobacterium *Prochlorococcus*. Limnol. Oceanogr. Methods 5:353-362.

Paytan A., Cade-Menun B.J., McLaughlin K., Faul K.L. (2003) Selective phosphorus regeneration of sinking marine particles: evidence from ^{31}P-NMR. Marine Chemistry 82:55-70.

Pernthaler A., Pernthaler J., Amann R. (2002) Fluorescence in situ hybridization and catalyzed reporter deposition for the identification of marine bacteria. Applied and Environmental Microbiology 68:3094-3101.

Peterson B.J. (1979) Particulate organic matter flux and planktonic new production in the deep ocean. Nature 282:677-680.

Pomeroy L.R., Williams P.J.l., Azam F., Hobbie J.E. (2007) The microbial loop. Oceanography 20:28.

Redfield A.C. (1958) The biological control of chemical factors in the environment. American Scientist 46:205-221.

Sannigrahi P., Ingall E., Benner R. (2006) Nature and dynamics of phosphorus-containing components of marine dissolved and particulate organic matter. Geochimica et Cosmochimica Acta 70:5868-5882.

Sañudo-Wilhelmy S.A., Tovar-Sanchez A., Fu F., Capone D.G., Carpenter E.J., Hutchins D.A. (2004) The impact of surface-adsorbed phosphorus on phytoplankton Redfield stoichiometry. Nature 432:897-901.

Sherr B.F., Sherr E.B., del Giorgio P. (2001) Enumeration of total and highly active bacteria, in: J. Paul (Ed.), Methods in microbiology, marine microbiology, Academic Press. pp. 129-159.

Sterner R., Elser J. (2002) Ecological Stoichiometry: The biology of elements from molecules to the biosphere. 1st ed. Princeton University.

Strickland J.D.H., Parsons T.R. (1972) A Practical Handbook of Seawater Analysis. Second Edition ed. Fisheries Research Board of Canada, Ottawa.

Suttle C.A., Fuhrman J.A. (2010) Enumeration of virus particles in aquatic or sediment samples by epifluorescence microscopy. Manual of Aquatic Viral Ecology. ASLO:145-153.

Valderrama J.C. (1981a) The simultaneous analysis of total nitrogen and total phosphorus in natural waters. Marine Chemistry 10:109-122.

Wetz M.S., Wheeler P.A. (2004) Response of bacteria to simulated upwelling phytoplankton blooms.

Wetz M.S., Hales B., Wheeler P.A. (2008) Degradation of phytoplankton-derived organic matter: Implications for carbon and nitrogen biogeochemistry in coastal ecosystems. Estuarine, Coastal and Shelf Science 77:422-432.

White A.E., Spitz Y.H., Karl D.M., Letelier R.M. (2006) Flexible elemental stoichiometry in *Trichodesmium* spp. and its ecological implications. Limnology and Oceanography 51:1777-1790.

White A.E., Watkins-Brandt K.S., Engle M.A., Burkhardt B., Paytan A. (2012) Characterization of the rate and temperature sensitivities of bacterial remineralization of dissolved organic phosphorus compounds by natural populations. Frontiers in Microbiology 3.

Wohlers J., Engel A., ZÃ¶llner E., Breithaupt P., JÃ¼rgens K., Hoppe H.-G., Sommer U., Riebesell U. (2009) Changes in biogenic carbon flow in response to sea surface warming. Proceedings of the National Academy of Sciences 106:7067-7072.

Wood E., Armstrong F., Richards F. (1967) Determination of nitrate in sea water by cadmium-copper reduction to nitrite. J. mar. biol. Assoc. UK 47:23-31.

Appendix: Supplemental Tables and Figures

Table 2-4 Percentages and rates of remineralization (µmol L^{-1} day^{-1}) normalized per gram added. "Soluble" is the fraction measured by instantaneous release of SRP/NH_4^+. "Labile" is the fraction calculated as the net increase in SRP and NH_4^+ after soluble release. "Other" refers to amount of N and P added that was not converted to SRP or NH_4^+ within the timeframe of experimentation. "Turnover rates" are calculated as the linear regression of the "Labile" fraction. Ratio of turnover rates is the rate of N turnover divided by the rate of P turnover.

	Trichodesmium		*Thallasiossira*		*Prochloroccocus*		OR POM
PO_4^+		+/-		+/-		+/-	
% Soluble	59%	4%	68%	8%	95%	8%	26%
Turnover Rate (µmol L^{-1} day^{-1})	0.108	0.063	0.036	0.009	0.034	0.002	0.031
% Labile	41%	10%	28%	4%	5%	2%	3%
% other	0%	0%	3%	1%	0%	3%	71%
NH_4^-							
% Soluble	1%	0%	6%	2%	1%	0%	0%
Turnover Rate (µmol L^{-1} day^{-1})	1.448	0.840	0.481	0.019	0.351	0.046	0.141
% Labile	38%	12%	37%	17%	44%	8%	4%
% other	61%	24%	57%	13%	55%	10%	96%
Ratio of Turnover Rate (NH_4^+/PC	13.46	0.14	13.55	3.02	10.25	1.93	4.55

Table 2-5. Comparisons of POM C:N, C:P, and N:P ratios with other studies.

	C:N	+/-	C:P	+/-	N:P	+/-	Notes	Reference
Prochlorococcus (MED-4)								
total	5.9	0.6	32	10	5.5	1.3	media N:P =	this study
soluble corrected	5.9	0.5	427	396	72.4	52.3		
P-replete	5.7	0.7	121	17	21.2	4.5	media N:P = 800	Bertilsson et al. 2003
P-limited	7.4	0.2	464	28	62.3	14.1	media N:P = 16	
Trichodesmium spp.								
total (IMS101)	5.6	0.1	127	12	22.5	1.8	YBC-II (50µmol P)	this study
soluble corrected (IMS101)	5.7	0.1	316	43	65.0	12.5		
total								Sanudo-Wilhelmy et al. 2004
intracellular								
P-replete (IMS101)	6.0		96	8	16	1	YBC-II (50µmol P)	White et al. 2006
P-restricted (IMS101)	6.5		585	56	90	10		
field collected	5.5		290	15	53	3		
Thalassiosira weissflogii								
total	6.2	0.0	60	15	9.6	2.4		this study
soluble corrected	6.8	0.1	203	84	29.9	12.8		
Oregon POM								
total	10.4	0.1	76	22	7.3	2.1		this study
soluble corrected	10.4	0.1	103	31	10.0	2.9		