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Citation	Burkhardt, B. G., Watkins-Brandt, K. S., Defforey, D., Paytan, A., & White, A. E. (2014). Remineralization of phytoplankton-derived organic matter by natural populations of heterotrophic bacteria. Marine Chemistry, 163, 1-9. doi:10.1016/j.marchem.2014.03.007
DOI	10.1016/j.marchem.2014.03.007
Publisher	Elsevier
Version	Accepted Manuscript
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Remineralization of phytoplankton-derived organic matter by natural populations of heterotrophic bacteria

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Abstract

The relative lability, elemental stoichiometry, and remineralization rates of various particulate organic matter (POM) substrates by natural heterotrophic marine microorganisms was investigated. POM was harvested from laboratory cultures of a marine diazotroph (*Trichodesmium* IMS101), a cosmopolitan diatom (*Thalassiosira weissflogii*), a common marine cyanobacteria (*Prochlorococcus* MED4), and from surface waters off the Oregon coast. These POM resources were used as inoculants 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-frequency measurement of soluble phosphorus (P) and nitrogen (N) concentrations over a 6-d period. Rapid solubilization and near complete remineralization of particulate P (PP) occurred in all treatments where cultured POM was used, with lesser relative mobilization of P from a 'natural' POM sample isolated from surface seawater off the Oregon coast. Soluble P pools, likely consisting of surface-adsorbed inorganic P and inorganic P liberated from cells during harvesting of biomass accounted for 28% of natural PP pools and 80 ± 32% of cultured PP. ^{31}P nuclear magnetic resonance (NMR) confirmed that PP was predominately present as orthophosphate in all POM types. By the end of the incubation period, all added P from cultured material had been converted to dissolved inorganic P. This finding may be a caveat of our utilization of laboratory cultures and natural POM which has been exposed to high inorganic P concentrations (0.8-5.0 μmol L^{-1}), albeit it is consistent with previous reports of significant contributions of surface-adsorbed P to total particulate P. In contrast, over the course of these experiments, only 37-40% of added N had been remineralized to ammonium (NH_4^+). In general, N remineralization rates of cultured material increased with the amount of N added (per gram of dry material). The net yield of bacterial cells was also positively correlated to the initial amount of C and N added. Most notably, when corrected for non-biological turnover (i.e. removal of soluble pools), the N:P remineralization ratio of cultured material (8.5 ± 1.3) was independent of the N:P of added organic material (5-23).

Introduction

At the global scale, the mean nitrogen:phosphorus (N:P) ratio of marine particulate organic matter (POM) produced in the euphotic zone and the elemental stoichiometry of dissolved inorganic pools in the aphotic zone are both relatively well constrained at a mean value of $N_{16}:P_1$, i.e. the Redfield ratio (Redfield, 1958). At smaller spatial and temporal scales, however the elemental composition of marine plankton can vary widely (Fraga, 2001; Geider and Roche, 2002; Martiny et al., 2013), as does remineralization stoichiometry (Anderson and Sarmiento, 1994; Li and Peng, 2002). The N:P composition of suspended and sinking organic matter can influence the N:P ratio of remineralized nutrients that may then be resupplied to the surface ocean through vertical mixing. Accordingly, deviations from mean stoichiometry are important to understand as they have implications for the linkages between nutrient supply, surface productivity and carbon export via the so called biological pump.

The major organic constituents of life (e.g. proteins, carbohydrates, lipids, and nucleic acids) are each composed of different ratios of C:N:P. Accordingly, the C, N, and P content and hence the elemental stoichiometry of marine plankton is driven by the metabolic partitioning of elements among these different classes of molecules. Shifts in resource acquisition, growth and reproduction as well as taxonomic variability arising from evolutionary history, and physiological adaptation to the chemical environment all lead to variability of the molecular composition and C:N:P ratios of plankton (Geider and Roche, 2002; Klausmeier et al., 2004; Quigg et al., 2003; Sterner and Elser, 2002). For example, strains of the abundant marine cyanobacterium *Prochlorococcus* have a relatively small genome size (Bertilsson et al., 2003) and a capacity to substitute sulfur for P in cell-membrane lipids (Van Mooy et al., 2006). Both of these ecophysiological traits result in relatively low cellular P demand and high C:P and N:P ratios. Alternately, under conditions of surplus nutrient supply, certain classes of phytoplankton can store P in excess of their immediate growth requirements, increasing the cellular P quota and reducing N:P ratios (Diaz et al., 2008). Summarizing published data from 64 species of phytoplankton, Deutsch and Weber (2012) found that the cellular N:P ratios of eukaryotic phytoplankton (13.2 ± 7.03, n=53) and cyanobacterial isolates (22.1± 6.3, n=11) were on average 14.9 ±7.6. This average is not significantly different than the Redfield ratio (16) however the variability is on the order of 50%. If heterotrophic bacteria ‘are what they eat’, this variability

could impact remineralization processes: e.g. export of a P-rich cell may lead to excess remineralization and release of inorganic P relative to the Redfield ratio while remineralization of N rich cells such as those of diazotrophs will result in higher N release relative to P.

As phytoplankton die, sink, or are packaged into marine snow, organic matter is transported out of the euphotic zone to depth where it is decomposed by a remineralizing community of heterotrophic organisms. Globally, organic matter remineralization results in dissolved inorganic pools of N and P in near Redfield proportions in deep water. However, incubation studies with isolated heterotrophic populations (Chen and Wangersky, 1996; Gogou and Repeta, 2010; White et al., 2012) as well as diagnosis of remineralization rates via application of mixing models to nutrient profiles (Anderson and Sarmiento, 1994; Li and Peng, 2002) indicate that remineralization ratios can significantly deviate from Redfield stoichiometry. Much of this variability appears to be driven by the fact that dissolved organic P (DOP) is more reactive than dissolved organic C or N (Clark et al., 1998; Paytan et al., 2003). Accordingly, the residual dissolved organic matter (DOM) becomes rapidly P depleted and relatively C and N rich with depth; indicating 'preferential' remineralization of P (Clark et al., 1998). This apparent decoupling of P from C and N is key to both the degree of long-term C storage in recalcitrant DOM and to setting the dissolved inorganic N and P pools available for resupply to the euphotic zone (Jiao et al., 2010). This uncoupling can be driven not only by the lability of plankton-derived organic matter substrates but also by environmental constraints, enzymatic capacities, and nutrient status of remineralizing organisms (Hansell and Carlson, 2002). Despite this existing knowledge, it is not clear to what extent these individual factors impact the rates and stoichiometric ratios of organic matter remineralization.

The lability and elemental stoichiometry of POM and exuded DOM defines the availability of elements for the remineralizing communities. Phytoplankton cell size, growth rates, and nutritional status are all factors that can lead to differential investments in various classes of biomolecules and thus relative N and P content. Through decomposition, these different fractions are remineralized or altered at different rates (Harvey et al., 1995). In general, organic matter decomposition is thought to proceed in three stages: (1) a rapid (hours-days) turnover of highly reactive labile pools, typically high in N and P (e.g. nucleic acids) (2) slower turnover (days-weeks) of semi-labile pools and (3) long term (years) decomposition of more refractory pools

which are relatively rich in C and depleted in N and P (e.g. components of bacterial cell walls). At each stage, the elemental stoichiometry of remineralization is a function of the enzymatic capacity and relative nutritional demands of the remineralizing population. For example, P-limited microorganisms may retain a fraction of the P liberated, but release N (Harvey et al., 1995; Sterner and Elser, 2002) while C limited microorganisms will release more N and P relative to C (Nicholson et al., 2006). Overall, remineralization represents the integration of the elemental composition of suspended and sinking POM and stoichiometric needs of remineralizing communities.

To explore this relationship between POM source and remineralization rates and stoichiometry, we have conducted a suite of on-deck incubation experiments in the North Pacific Subtropical Gyre (NPSG) in March of 2011 near Station ALOHA (A Long-Term Oligotrophic Habitat Assessment; 22° 45'N, 158° 00'W). The aim of this work was to better quantify N and P remineralization as a function of the elemental composition of POM substrate and shed light on the relationship between variability in POM composition and the stoichiometry of nutrient regeneration.

Methods and Materials:

Preparation of particulate organic material

Large volume batch cultures (non-axenic) of three distinct and ecologically significant marine photoautotrophs were grown on a 12:12 light dark cycle at growth-saturating irradiances (150 μmol photons m^{-2} s^{-1}) at a constant temperature of 24°C. *Trichodesmium* (strain IMS101) was grown on YBCII media with no added N (Chen et al., 1996), *Thalassiosira weissflogii* was grown on F/2 media (Guillard, 1975; Guillard and Ryther, 1962) and *Prochlorococcus marinus* MED-4 was grown on the standard recipe for Pro99 media (Moore et al., 2007). All media had initial P concentrations of 5 μmol L^{-1}. YBCII media contain no added nitrogen (standard for culturing diazotrophs) and F/2 media contained the standard aliquot of nitrate. Growth was monitored by *in vivo* chlorophyll fluorescence (via either a Walz Water-PAM or Turner 10-AU fluorometer), and all cultures were harvested during the early stationary growth phase (first time-point following slope plateau). All cultured POM was isolated by gentle vacuum filtration (<100 mm Hg) onto a series of 25 mm diameter 2.0 or 5.0 μm Nucleopore filters (for *Prochlorococcus* and *Thalassiosira/Trichodesmium* respectively) to minimize cell breakage. A natural marine

POM sample was also collected from surface seawater. In order to collect sufficient organic material, this sample was taken from the productive upwelling system off the Oregon coast (Yaquina inlet, at time of collection phosphate = 0.81 μmol L^{-1}; nitrate = 1.45 μmol L^{-1}, chlorophyll = 0.35 μg L^{-1}) using an acid-cleaned bucket and filtered using a 0.2 μm filter. All filters were dried at 60°C, rinsed off with deionized water into petri dishes, dried again, transferred to clean polycarbonate centrifuge tubes, and stored at -20ºC. Each of the four POM samples (3 culture samples and one from off Oregon) was characterized for particulate C, N, and P content as well as for P compound composition using ^{31}P-NMR (analytical methods described below). These samples served as the different types of exogenous POM for decomposition experiments and their compositions are listed in Table 1.

Experimental Design

In March of 2011, 20-L aliquots of seawater were collected from the 75-m depth horizon at Station ALOHA in the NPSG. This depth horizon was targeted to capture a maximum abundance of active heterotrophic bacteria while minimizing the amount of ambient dissolved organic matter (determined from historical data available at http://hahana.soest.hawaii.edu/hot/). Immediately after collection, seawater was stored in the dark in an incubator continually flushed with surface seawater for ~72 hours to stop photoautotrophic growth while maintaining *in-situ* temperature. This 'aging' method was chosen over filtration for terminating photoautotrophic activity to better preserve the natural microbial community composition. This "aged" water was used for the decomposition experiments conducted in carboys capped with 3-port lids and internal tubing to permit airflow, minimize contamination, and allow for continuous sampling. Measured nutrient concentrations of this water at the time of collection as well as the climatological means for water collected at 75-m are noted in Table 2.

The dried POM material (cultured *Trichodesmium* IMS 101, "TRICHO", *Prochlorococcus* MED4, "PRO", *Thalassiosira weissflogii*, "DIATOM" and the natural POM from the Oregon coast, "OR-POM") was then added to the carboys with the aged Station ALOHA seawater (Table 1). Each treatment was prepared in duplicate except for the OR-POM due to the limited amount of particulate material obtained from the natural sample. In addition, a single CONTROL with no added POM was used to discern ambient remineralization, and a KILLED control (*Trichodesmium* + 50-mL saturated $HgCl_2$ solution) was used to quantify any passive or non-

biological release (in duplicate). For each treatment, dried biomass was added to the heterotrophic community in quantities equivalent to 1.0 μmol L^{-1} P (based on previous determination of C, N and P concentrations). This concentration was selected to ensure that as remineralization proceeds and soluble reactive P (SRP, considered to be equivalent to dissolved inorganic P) is released, concentrations were ample for detection via continuous flow-injection auto-analysis (Table 1). Concentrations of ammonium (NH_4) and SRP were obtained every 5 minutes for roughly the first half hour following POM addition to capture any solubilization trends (this time interval was selected to allow for iterative sampling of the control). This initial phase was followed by discrete sampling every 3 hours to obtain high-resolution remineralization rates and assess the N and P lability of the substrates. Samples were also collected for total dissolved N and P, nitrate + nitrite, and bacterial abundance (see methods below). Prior to all samplings, carboys were bubbled with high-purity air to ensure homogeneity.

Analytical Measurements

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 chlorophyll-a concentrations via the acidification method of Strickland and Parsons (1972). Samples were also collected for flow cytometry; daily triplicate 3-mL samples from each carboy were collected, fixed with 60-μL of 10% paraformaldehyde for 10 minutes in the dark, and stored at -80ºC for flow cytometric analysis of the abundance of heterotrophic bacteria. Bacterial abundances were measured on a Becton-Dickinson FACS-Caliber four-color flow cytometer with SYBR® Green stain as described by Sherr et al. (2001) and Marie et al. (1997).

Nutrients were analyzed using flow-through colorimetric methods on a Technicon Auto Analyzer II. SRP was analyzed using the phosphomolybdic acid reduction; ammonium (NH_4) was measured by the indophenol blue method (Gordon et al., 1993); and nitrate + nitrite (N+N) was analyzed using the cadmium reduction method of Armstrong et al. (1967). Detection limits were 55 nmol L^{-1} for SRP, 22 nmol L^{-1} for NH_4, and 8 nmol L^{-1} for N+N. Total dissolved P and N (TDP and TDN, respectively) 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 $NH_4^+ + NO_3^- + NO_2^-$, respectively.

Particulate C, N, and P content of each POM type was determined by collecting a subsample of the biomass onto combusted GFF filters, wrapping in foil, flash freezing, and storing at -80°C. The filters were then thawed and dried at 60°C overnight, folded into tin and silver boats, and run on a Carlo-Erba C/N Analyzer for particulate C (PC) and N (PN) content (Sharp (1974). For particulate P (PP) analyses 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. PP was then analyzed as SRP in a 1.0 cm cell at 880 nm following Strickland and Parsons (1972).

Molecular characterization of PP compounds was performed using subsamples of each POM type with ^{31}P nuclear magnetic resonance (NMR) spectral analysis as per Cade-Menun et al. (2005). Samples were freeze-dried, extracted with a 25-mL solution of 0.25M NaOH 0.05M Na_2EDTA for 4h, and then centrifuged. 1-mL aliquots of the supernatant and digested residue samples were analyzed for P concentrations via inductively coupled plasma optical emission spectroscopy (ICP-OES) to determine the extracted P and fraction that was not extracted. The remaining supernatant was analyzed for ^{31}P-NMR spectroscopy on a 600 MHz Varian Unity INOVA spectrometer equipped with a 10mm broadband probe at 20°C and a 90° pulse. Compounds were identified by their chemical shifts (ppm) relative to an external orthophosphoric acid standard. After standardizing the orthophosphate peak in all samples to 6 ppm, peak assignments were based on Tebby and Glonek (1991) Cade-Menun and Preston (1996) and Turner et al. (2003b,c). Peak areas were calculated by integration of spectra processed with a 5 Hz line broadening, using NUTS software (Acorn NMR Inc.) as described in Paytan et al., (2003). Finally, the relative contribution of surface-adsorbed P was assessed for remaining TRICHO and DIATOM POM samples via the oxalate rinse method described in Fu et al. (2005) ; not enough material remained from PRO and OR-POM for similar analyses.

Results

Water Samples and POM Characterization

Total P additions were similar for all experiments with cultured biomass (0.71-0.79 μmol P L^{-1}) albeit higher for the OR-POM experiment (0.96 μmol P L^{-1}). Given the different stoichiometries of the POM used in each experiment, C and N additions differed between treatments, with additions for TRICHO and OR-POM (77-82 μmol C L^{-1} and 8-14 μmol N L^{-1}, respectively,

Table 1) having higher C and N content compared to the PRO and DIATOM treatments (21-45 μmol C L^{-1} and 4-7 μmol N L^{-1}, respectively, Table 1). Particulate C, N, and P content for each POM sample are shown in Table 1. PN:PP and PC:PP values were below Redfield ratios (16:1 and 106:1, respectively) for all POM types, with the exception of *Trichodesmium*, which was relatively C and N rich and P poor (Table 1). These bulk stoichiometries reflect intracellular and surface-adsorbed elemental pools.

^{31}P-NMR molecular analysis revealed the majority (>72%) of PP was present as orthophosphate for all POM types (Table 1). TRICHO and DIATOM both contained monoesters, 13±2% and 5±5% respectively, and TRICHO also contained pyrophosphate (8±2%). Based on the P content extracted for the ^{31}P-NMR spectroscopy the P characterized (e.g. extraction yield) accounted for 95.7-96.4% of total P for the TRICHO samples, 79.4-83.1% of total P for DIATOM samples, 96.9% of total P for PRO and 56.1% of total P for OR-POM. Oxalate rinses applied to remove surface adsorbed P (Fu et al., 2005) from the TRICHO and DIATOM samples showed that 74 ± 12% of TRICHO PP and 72 ± 18% of DIATOM PP were extracellular. This treatment was not applied to PRO and OR-POM due to limited sample availability (Table 1).

Chemical characteristics of the seawater collected for the experiment were characteristic of the North Pacific subtropical gyre in spring (Table 2). Inorganic N:P ratios in the upper 100-m at Station ALOHA are typically <1.0 and both N and P concentrations are very low; TDN:TDP range from 16-25 (Karl et al., 2001). Following the 3-day "aging" period, nutrient concentrations in the seawater used for our degradation experiments were low and within the range observed in this region (0.08 ± 0.01 and 0.09 ± 0.07 μmol L^{-1} for NH_4 and SRP, respectively, Table 2). The ratio of inorganic N:P (NH_4^++NO_2^-+NO_3^-:SRP) was 0.9 ± 0.2, while TDN:TDP was 18 ± 8. Heterotrophic bacterial abundance was 4.4±2.4 x 10^5 cells mL^{-1}, also well within the range typically observed at 75-m at Station ALOHA (3.5-5.6 x 10^5 cells mL^{-1}).

N and P Reactivity

For all treatments, turnover rates were divided into two phases that we term soluble and labile. Soluble pools are defined here as the net increases of NH_4 or SRP occurring within 30 minutes following POM additions. In all treatments, soluble P pools were substantial (28-91% of total PP added), whereas soluble N (NH_4) accounted for only 1-3% of PN added. Following the initial P solubilization in the first 24 hours of experimentation, NH_4 began to accumulate and SRP

concentrations approached the total P added in all treatments except the OR-POM (Figure 1). We classify this as the labile phase of remineralization. Due to a ~4-6 hour gap between initial high-resolution flow-thru sampling and the start of the discrete sampling the exact timing that the labile remineralization began is not precisely determined. For simplicity, we term the fraction of added PP and PN that was not remineralized at the end of our experiment as other (e.g. not soluble or labile). N+N, DON, and DOP concentrations did not significantly change in any treatment, accordingly net losses of material to dissolved organic pools or net loss of NH_4 through ammonium-oxidation were not detectable. Note that the calculated rates of remineralization and the ratios of these rates refer to the net rates (e.g. rate of regeneration minus loss due to uptake by the bacteria or adsorption).

The relative contributions of the soluble, labile, and other fractions of the added organic matter for all experiments are shown in Figure 2. Remineralized N from culture-derived POM was largely within the fractions defined as labile (40 ± 24%, 37 ± 8%, and 39 ± 2%, for TRICHO, DIATOM, and PRO, respectively). Some release of N was seen also in the KILLED treatments (9 ± 4%) indicating non biogenic regeneration. N solubilization (e.g. remineralization within the first 30 minutes after POM addition) was minimal (<3% of added N). OR-POM was the least reactive material; 65% of added P and 93% of added N were not remineralized within the time frame of our experiment and hence classified as other. Of the remineralized pools in the OR-POM treatments, 7% of N and 9% of P was defined as labile with 26% of P defined here as soluble. In comparison, culture-derived organic material was significantly more reactive: 96-100% of added P and 41-45% of added N were soluble or labile, with P largely in the soluble pool (80 ± 15%, 79 ± 18%, and 82 ± 12% for TRICHO, DIATOM, and PRO treatments respectively). Notably, the KILLED ($HgCl_2$ + *Trichodesmium*) show similar results as the experiments with the fresh POM from cultures with respect to the SRP trends: 91± 9% of added P was in the soluble pool. However for the KILLED control NH_4 did not accumulate in the soluble or labile phases. This indicates that the soluble fraction of SRP as defined here (but not NH_4) was either surface-adsorbed or present as intracellular SRP that was liberated from the cells during harvesting and preparation of the POM, which may rupture the cell membranes (drying and freezing).

Rates and Ratios of Remineralization

Remineralization rates and ratios were calculated for the labile phase only – to avoid inclusion of abiotic P solubilization. Labile P remineralization was most rapid for the TRICHO and DIATOM treatments (0.06 μmol P L^{-1} d^{-1}) relative to PRO and OR-POM treatments (0.01 μmol P L^{-1} d^{-1}). Total N remineralization was proportional to N additions for cultured material with PRO having the smallest N additions and N turnover rates (0.2 μmol N L^{-1} d^{-1}), followed by the DIATOM and TRICHO treatments (0.2-0.74 μmol N L^{-1} d^{-1}, Table 3). With the exception of P solubilization in the killed treatment, there was no significant change in NH_4 or PO_4 concentrations in either the CONTROL or KILLED treatments.

The labile N:P remineralization rates were 9.8 ± 2.4, 7.1 ± 0.5, 8.8 ± 1.8, and 4.4 ± 1.6 for the TRICHO, DIATOM, PRO, and OR-POM treatments respectively (Figure 3, Table 3). Notably, differenced among the labile N:P remineralization ratios for cultured material were not significant (t-test, p values ranging from 0.37-0.63) despite the range of PN:PP added (5-19, Table 3). Remineralization ratios were not calculated for the CONTROL (remineralization was not observed) or for the KILLED treatments since N regeneration in these treatments was insignificant.

Bacterial dynamics

The abundance of heterotrophic bacteria was determined daily over the course of the experiment (Table 4, Figure 4). Initial bacterial cell abundance was 4.42 ± 2.43 × 10^5, and maximum abundances for each treatment were 2.9± 0.6 × 10^6, 2.5 ± 1.2 × 10^6,1.4 ± 0.1 × 10^6, 1.8 ± 0.7 × 10^6, for TRICHO, DIATOM, PRO, and OR-POM, respectively.

Bacterial abundances peaked between 1.5 and 3 days from the start of the experiment, with TRICHO increasing the most and OR-POM the least. TRICHO and DIATOM abundances peaked the earliest, followed by PRO, and finally OR-POM, which increased more gradually. In all treatments, bacterial cell numbers declined rapidly to values similar to those observed at the initiation of the experiment (Figure 4B). Maximum bacterial cell yields were positively correlated to the magnitude of initial PC added (r^2 = 0.95, F-test, p value = 0.026) and PN (r^2 = 0.95, F-test, p = 0.04) excluding the OR-POM treatment (Figure 4A). There was no significant correlation with PP added (r^2 = 0.49, F-test = 0.04, p = 0.3).

Discussion

Three major findings were derived from this study (1) the release and remineralization of P from particulate organic matter is much more rapid than that of N. Specifically, a substantial fraction of added P was solubilized soon after the addition of organic matter to seawater. This appears to be due to the fact that P in living cells is mostly present as SRP, as also seen in our ^{31}P-NMR spectra, and thus it is readily released. Being that media P concentrations were the same for all algae cultures, variability in surface adsorption and thus C:N:P of particulate matter may in part, reflect differences in cell surfaces ability to adsorb inorganic P. The lower value of soluble P for the OR-POM treatment may reflect the lower DIP values in the coastal seawater (~ 1 μmol L^{-1}) compared to those in the cultures (~ 5 μmol L^{-1}) – or larger contributions of detrital material to natural POM. In contrast, NH_4 is present in various organic compounds that must be "actively" degraded, such as amino acids and nucleic acids. (2) Following this fast solubilization step, the N:P remineralization stoichiometry of cultured POM material (8.5 ± 1.3) was independent of the N:P of added organic material (5-23) whereas N:P remineralization of the natural suspended particulate matter collected from coastal Oregon was notably lower (4.37), and (3) the cell yield of heterotrophic bacteria dependent on the magnitude of C and N added suggesting C and/or N limitation (not P) of the natural heterotrophic microbial communities at this location and time. Below we elaborate on the significance of these findings in relationship to the evolving understanding of nutrient cycling in the open ocean.

Preferential P Remineralization and P Utilization

Multiple methods of analysis provide clear and consistent evidence that the cultured and natural POM in this study contained a significant fraction of soluble reactive P. Specifically, rapid SRP release occurred in all POM treatments including KILLED controls, ^{31}P-NMR revealed that >72% of measurable P was orthophosphate, and oxalate wash of POM removed 74 ± 12% and 72 ± 18% of P from the TRICHO and DIATOM biomass, respectively. This is comparable to previously published NMR data of suspended particulate samples (Paytan et al. 2003). These SRP pools can only be associated with some combination of cell surface-adsorbed P and internal pools that were liberated from cells during POM processing (drying and freezing). While we must be cautious of applying results based on laboratory cultures grown in high P media to natural populations, there is nonetheless ample evidence in the literature that inorganic P (internal or surface adsorbed) can compose a large fraction of total cellular P pools. Sanudo-

Wilhelmy et al. (2004) and Fu et al. (2005) performed oxalate washes on a range of cultured species and natural assemblages and found surface P adsorption accounted for approximately 10-60% of PP. These same authors also report that surface adsorption can vary as a result of differences in growth phase, DIP concentration in the growth media, cell health and size, and the presence of metal hydrous oxides in solution (Sanudo-Wilhelmy et al. 2004). Loh & Bauer (1999) using a sequential extraction procedure also found up to 80% of suspended, sinking, and sedimentary PP samples in the North Pacific and Southern Ocean consisted of inorganic P. Yoshimura et al.(2007) used a common acid-extraction protocol to show particulate inorganic P (PIP) comprised ~20% of total PP; a strong positive correlation to chlorophyll indicated to the authors that this PIP was associated with phytoplankton cells. Finally, Miyata and Hattori (1986) found that orthophosphate pools accounted for 40-50% of total PP pools in suspended material collected from Tokyo Bay, where the phytoplankton community was dominated by the diatom *Skeletonema*. So while we must acknowledge that the very high contributions of inorganic soluble P to total PP found in our study (> 70%) likely overestimate surface-adsorbed and/or intracellular P contributions in natural populations, our findings is consistent with prevailing findings in the literature that inorganic P is a significant component of PP (10-90% of PP by various methods). This prevalence of inorganic P, whether intracellular or surface-adsorbed, may explain why P is more rapidly released and cycled relative to C and N.

The traditional understanding of organic matter degradation in the open ocean considers photosynthetically-produced POM to be the primary source of DOM. Organic molecules that make up DOM are then hydrolyzed by various enzymes and inorganic nutrients are liberated. These nutrients are either taken up directly into biomass (i.e. heterotrophic bacteria), or when in excess of bacterial nutritional needs, released into the environment. However, if P associated with POM is largely inorganic to begin with, it will not undergo microbially-mediated hydrolysis which can take time; instead, bioavailable DIP will be directly and rapidly released from particulate matter and the fraction passing through the DOP pool will be lower than expected based on C and N content. These findings may explain why “preferential P remineralization” has been suggested in previous studies (Jiao et al. 2010, Sannigrahi et al. 2006, Faul et al. 2005, Clark 1998). In fact the higher P release is not attributed to remineralization, if defined as the enzymatic breakdown on organic compound to inorganic nutrients, but rather P cycling may be driven in part by desorption and release of P from cells without a change in chemical form.

Remineralization Stoichiometry of Labile Organic Matter

Results from this experiment suggest that remineralization stoichiometry during the labile phase is not related to substrate stoichiometry. Specifically, the N:P remineralization ratio of cultured POM (8.5 ± 1.3, Fig. 3 and Table 3) is relatively low as compared to the Redfield stoichiometry and independent of the N:P of added POM (5-23). The N:P remineralization ratio (4.37) of suspended particulate matter collected off the coast of Oregon (PN:PP= 8.25) was also low. Even after removal of soluble P pools, this finding ratio is consistent with preferential P regeneration in the early stages of OM turnover. The lack of relationship between POM stoichiometry and remineralization stoichiometry suggests that, at least in the early stages of particle remineralization, the active degradation of organic matter is independent of the ratio of elements in the available substrate POM.

In all treatments where POM from cultures was used near complete solubilization and remineralization of P was achieved with lower N remineralization with a relatively fixed proportion to N content in the POM (40 ± 1%, Table 3). Consequently, total inorganic N:P released was proportional, albeit lower, to substrate N:P (r^2= 0.92, slope= 0.44, Table 3). Our results indicate that the dissolved inorganic pools reflect the net sum of abiotic and biotic processes: with dissolved inorganic P concentrations being driven largely by abiotic solubilization and N by microbial mediated remineralization, primarily reflecting the fact that much of the P is present in mobile inorganic forms associated with the cells. If nearly all PP in surface seawater more soluble/labile than PN, as in these experiments, one would expect that in nature N:P remineralization ratios (8.5 in our experiment) would be lower than bulk residual POM or DOM stoichiometry (e.g. the stoichiometry in the OM fraction that remains after remineralization). Indeed at Station ALOHA N:P ratios of suspended and dissolved OM become progressively more P deplete with depth (Karl et al., 2001): particulate N:P ratios below the euphotic zone (150-1500m) are on average 26.7 ± 9.2 (range = 10-50, n=441, data span 1989-2011) whereas DON:DOP ratios below 150m are 50.2 ± 36.9 (range = 7-277, n=1886, data span 1988-2001, all data from http://hahana.soest.hawaii.edu/hot/). A similar offset between remineralization stoichiometry and residual organic matter is derived also from modeling results. Anderson and Sarmiento (1994) applied a nonlinear inverse fit of observed nutrient profiles for the GEOSECS Pacific transects to a one- or two-end member mixing model and find a relatively

invariant N:P remineralization (12-16) in the upper 4000 m which is lower than observed ratios in POM and DOM. Finally, Li et al. (2002) also used a two-end member mixing model to diagnose remineralization ratios for Station ALOHA (data from 1994) and estimate remineralization N:P ratios of 13 ± 1. N:P remineralization ratios in this region are clearly less than the N:P stoichiometry of POM or DOM below the euphotic zone where particle remineralization should exceed particle production ratios; this is consistent with rapid P cycling relative to N in this oligotrophic setting and with our experiment results.

Karl et al. (2001) have shown that N:P ratios in POM steadily increased from near Redfield-ratios (~16) in 1989-1990 to ~25 in the late 1990's. This trend has persisted (Fig. 5) in the past decade, with suspended particulate N:P still significantly greater than Redfield stoichiometry. No time-series analyses have been conducted to assay changes in remineralization ratios over this period. However, if our results apply to longer timescales, we would expect concomitantly increasing N:P remineralization ratios in the upper water column of the NPSG (due to mass balance considerations). Further determination of N:P regeneration ratios via dissolved nutrients (as per Schneider et al. 2005) may well look into time-variant shifts in remineralization stoichiometry to investigate this hypothesis in more detail. Future research of this kind would also help to evaluate the importance of what Jiao et al.(2010) term the 'microbial carbon pump', where a portion of the C in sinking POM and DOM is composed of recalcitrant organic material. This relative recalcitrance and corresponding preferential remineralization of N and P can lead to more effective C export than would be anticipated from static Redfield-like remineralization stoichiometry.

Nutritional Status of Remineralizing Community

For this study, the growth rate and maximum abundance of heterotrophic bacteria appears to be determined by C supply (R^2= 0.95). While growth was also significantly correlated with particulate N additions (R^2= 0.92), the release of excess NH_4 and PO_4 into solution indicates N and P were not growth limiting. OR-POM additions induced the lowest growth rate and net increase in bacterial cells despite the relatively large C addition associated with this experiment, indicating that quality and not just quantity of C is likely a critical factor with respect to the rate and magnitude of bacterial growth and overall remineralization. With the exception of the DIATOM treatment, bacterial abundance did not maintain a stationary phase but declined

rapidly. These declines could have resulted from viral lysis, grazing, and/or nutrient limitation (i.e. trace elements). The continued N and P remineralization following the declines in bacterial growth could partly be driven by residual free exoenzymes; more likely however, this suggests that the remaining remineralizing community was hydrolyzing organic N and P compounds in order to obtain C to sustain growth (even without population net growth). This would indicate the bacterial community C demand may have exceeded the amount of readily available C supplied by the POM substrates (natural or from algal cultures), while N and P were provided in excess. There is evidence that labile C supply can limit organic matter remineralization in natural populations. For example, Kirchman et al. (1990) report labile C limitation of bacterial growth in the subarctic Pacific and concomitant low heterotrophic uptake rates of inorganic N and P. Van Wambeke et al. (2007) report that labile carbon (glucose) was the only factor to stimulate heterotrophic bacterial production in the Chilean upwelling zone and was a secondary factor (to nitrogen) in the South Pacific subtropical gyre. In oligotrophic environments, the elemental content of organic matter may limit (or co-limit) the productivity of heterotrophic bacteria. The factor or factors limiting production are surely not static in time not space in the ocean. A review of the factors that enhance bacterial productivity (see Table 5 of Van Wambeke et al. (2007)) indicate that P, C, N, and Fe have each been shown to stimulate the abundance and productivity of heterotrophic bacteria in various oceanic regions. Our results suggest the lability of C resources is a key determinant of remineralization rates in the NPSG, at least in the spring of 2011 for populations isolated from 75m. Further work could easily follow up on these findings by examining the depth profiles of bacterial remineralization and the factors that stimulate production over multiple seasons.

Conclusions

Experiments reported here were designed to shed light on the intricacies of early organic matter degradation and nutrient remineralization. We found the degradation of different POM types all resulted in the preferential release of P. This appears to result from two processes: (1) rapid P solubilization from internal stores or surface absorbed SRP, and (2) relatively higher rates of P remineralization compared to N. The N:P remineralization stoichiometry of fresh POM material from cultured phytoplankton (8.5 ± 1.3) appears to be independent of the N:P of added organic material (5-23), suggesting heterotrophs control the stoichiometry of nutrient supply during early

remineralization. Finally, correlations between C supply and cell yields, along with low heterotrophic NH_4 and PO_4 uptake suggests a deficiency of labile C relative to P and N following POM addition.

Acknowledgements

This research was supported by grants from the National Science Foundation (Division of Ocean Sciences- 0962362 to AW and 0961555 to AP) and funds from the NSF Science and Technology Center for Microbial Oceanography: Research and Education (C-MORE, NSF EF0424599 to David M. Karl, U. Hawaii). We would also like to thank the Captain and crew of the R/V Kilo Moana, as well as Dr. Barbara Cade-Menun for her help in processing the NMR spectra.

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Tables

Table 1. Characterization of initial dry POM added to each 20 L treatment via elemental analysis, NMR and oxalate wash. Molecular P characterization (determined from P-NMR.) are shown as relative percent. No POM was added to control treatments and a saturating solution of $HgCl_2$ was added to killed treatments along with *Trichodesmium* POM. NA indicates where sufficient dry material was not available for oxalate wash.

	TRICHO†	DIATOM	PRO	OR POM
Elemental Analysis:				
µmol C L^{-1}	77 +/- 6	45 +/- 11	21 +/- 4	82 +/- 7
µmol N L^{-1}	13.6 +/- 0.9	7.2 +/- 1.8	3.6 +/- 0.6	7.6 +/- 0.5
µmol P L^{-1}	0.71 +/- 0.02	0.77 +/- 0.09	0.79 +/- 0.1	0.96 +/- 0.05
C:N:P	$C_{108\pm8}N_{19\pm1}P_1$	$C_{59\pm15}N_{9\pm2}P_1$	$C_{26\pm4}N_{5\pm1}P_1$	$C_{86\pm7}N_{8\pm1}P_1$
NMR Results:				
% ortho-P	80±1	95±5	100	100
% monoester	13±2	5±5	0	0
% pyrophosphate	8±2	0	0	0
Oxalate Wash Results (TRICHO And DIATOM ONLY):				
% surface-absorbed	74±12	72±18	NA	NA

† An equivalent aliquot of *Trichodesmium* POM was added to 'KILLED' treatments

Table 2. Initial dissolved nutrient concentrations and heterotrophic bacterial abundance of whole seawater collected at 75m at St. ALOHA in March, 2011. For comparison the climatological March mean for the 75m sampling depth is reported (data from http://hahana.soest.hawaii.edu/hot/hot_jgofs.html). NH_4 is not measured by the HOT program (NA).

	March 2011 (This study)	**Climatological March Average at 75m for Station ALOHA**
NH_4 (µmol L^{-1})	0.08 ± 0.01	NA
PO4 (µmol L^{-1})	0.09 ± 0.07	0.07 ± 0.03
N+N (µmol L^{-1})	0.05 ± 0.07	0.02 ± 0.02
DOP (µmol L^{-1})	0.33 ± 0.20	0.22 ± 0.05
DON (µmol L^{-1})	7.56 ± 1.40	5.58 ± 0.60
bacterial abundance (cells mL^{-1})	$4.42 \pm 2.43 \times 10^5$	$4.13 \pm 1.38 \times 10^5$

Table 3. N:P ratios of added POM and remineralized pools. "Labile" refers to remineralization ratios based on data collected after day 1, e.g. after P solubilization and with the onset of NH_4 remineralization. N:P remineralization ratios were calculated as the linear slope of the regression line for NH_4 versus PO_4, using all values in the LABILE phase of remineralization. Error is calculated as the standard deviation of duplicate treatments (except for OR-POM which was a single treatment). There was no net remineralization of N or P in the CONTROL and only soluble P (not N) in KILLED treatments; values with no detectable change are designated 'NA'.

	TRICHO	DIATOM	PRO	OR POM	KILLED
Initial N:P of added POM	19 ± 1	9 ± 2	4.5 ± 0.8	8.2 ± 0.6	19 ± 1
Labile N:P Remineralization	9 ± 2	7.1 ± 0.5	8.8 ± 1.8	4.37	NA
Total N:P (Soluble +Remineralized)	8 ± 3	3.7 ± 0.9	1.8 ± 0.3	1.58	NA
P remineralization rate, μmol P L^{-1} d^{-1}	0.06 ± 0.03	0.06 ± 0.02	0.011 ± 0.007	0.01	NA
N remineralization, μmol N L^{-1} d^{-1}	0.7 ± 0.4	0.47 ± 0.05	0.20 ± 0.04	0.03	NA

Table 4. Initial, maximum, net increase and growth rates of heterotrophic bacteria in all treatments. The growth rate is calculated as the slope of the natural log of cell concentrations over the time period required to reach maximum cell yields. The time point at which cell maxima were observed is noted in parentheses in units of days, following the 'maximum'. No significant change was observed in the abundance of heterotrophic bacteria in KILLED or CONTROL treatments.

	Trichodesmium	*Thalassiosira*	*Prochlorococcus*	OR POM
Initial (bacteria mL^{-1}) $\times 10^6$	0.5 ± 0.3	0.5 ± 0.3	0.5 ± 0.3	0.5 ± 0.3
Maximum (bacteria mL^{-1}) $\times 10^6$	2.8 ± 0.6 (2)	2.5 ± 1.2 (2)	1.4 ± 0.6 (2)	1.8 ± 0.6 (3)
Net increase (bacteria mL^{-1}) $\times 10^6$	2.4 ± 0.6	2.0 ± 0.3	1.0 ± 0.3	0.6 ± 0.2
Growth rate (bacteria mL^{-1} day^{-1}) $\times 10^6$	0.94 ± 0.18	0.89 ± 0.33	0.59 ± 0.11	0.28 ± 0.06

Figure Legends

Figure 1. Time-series of NH_4^+ (A-E) and SRP (F-J) release in the first 30min after addition of POM and in the succeeding 6d. Replicate A-B for each treatment are shown as red squares or black circles respectively. The total added PN or PP is shown for each treatment as a dashed line with the mean value ± the standard deviation of replicate measures noted.

Figure 2. Percentage of total added PP (A) and PN (B) classified as soluble, labile, or other. The "Soluble" fraction is the amount of P released within the first 30 minutes following POM addition. "Labile" refers to the biologically remineralized fraction (over day 1-6) and "Other" refers to POM not remineralized. Error bars are the standard deviation of replicates.

Figure 3. Relationship between concentrations of phosphate (SRP) and $NH4^+$ over the labile phase of the remineralization (day 1-6). The first replicate 'A' is shown as a red symbol and the second replicate as a white symbol. A linear regression is fit to each replicate: TRICHO (triangles), DIATOM (circles), PRO (squares), and OR POM (plus signs). The mean slope of this regression and the standard deviation of replicates are noted in the legend, with the exception that the error for OR-POM is the standard error of the slope of the linear regression as this treatment was not replicated. Note that the different y-intercepts (P) correspond to the total concentration of P solubilized.

Figure 4. (A) Net bacterial growth (cells ml^{-1} $x10^6$) as a function of particulate carbon and nitrogen added from each POM source. There were significant, positive relationships between C and N added and the maximum yield of bacterial cells, when OR-POM is excluded. There was no significant relationship between maximum cell yield and P. Dark symbols represent carbon, light symbols represent nitrogen. Natural POM collected from Oregon (OR-POM) is shown in

triangles. (B) The concentration of heterotrophic bacteria over the course of the 6-day experiment.

Figure 5. Time-series of particulate suspended material in the upper 125 at Station ALOHA in the NPSG. Data span 1989-2011 and are calculated as the integrated PN divided by integrated PP in the upper 125 of the water column. The thick solid line is a 3-point running mean whereas the thin solid line is the linear regression ($y = 0.0028x - 14.3$ with units of days on the x-axis) which corresponds to an annual N:P increase of ~ 1 mol N:mol P over this time period.

Figures

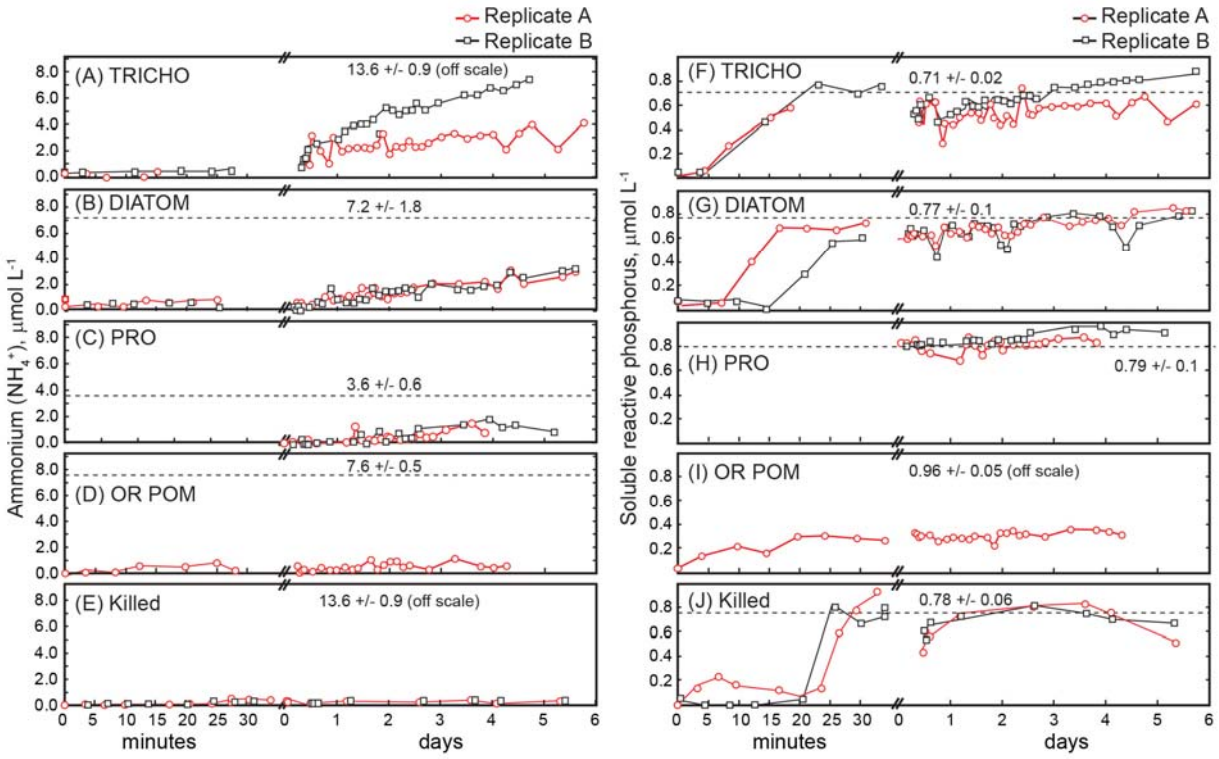

Figure 1

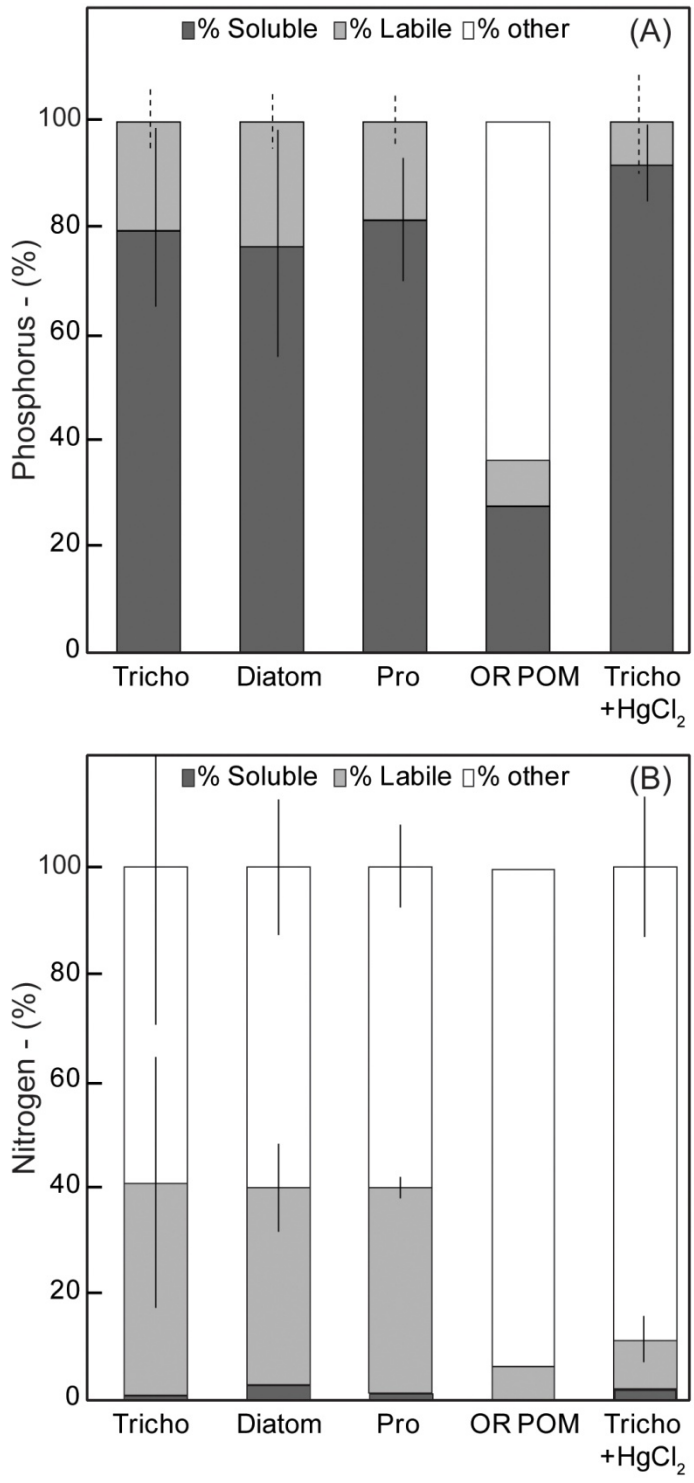
% Soluble
% Labile
% other
(A)
Phosphorus - (%)
100
80
60
40
20
0
Tricho
Diatom
Pro
OR POM
Tricho +HgCl2
% Soluble
% Labile
% other
(B)
Nitrogen - (%)
100
80
60
40
20
0
Tricho
Diatom
Pro
OR POM
Tricho +HgCl2

Figure 2

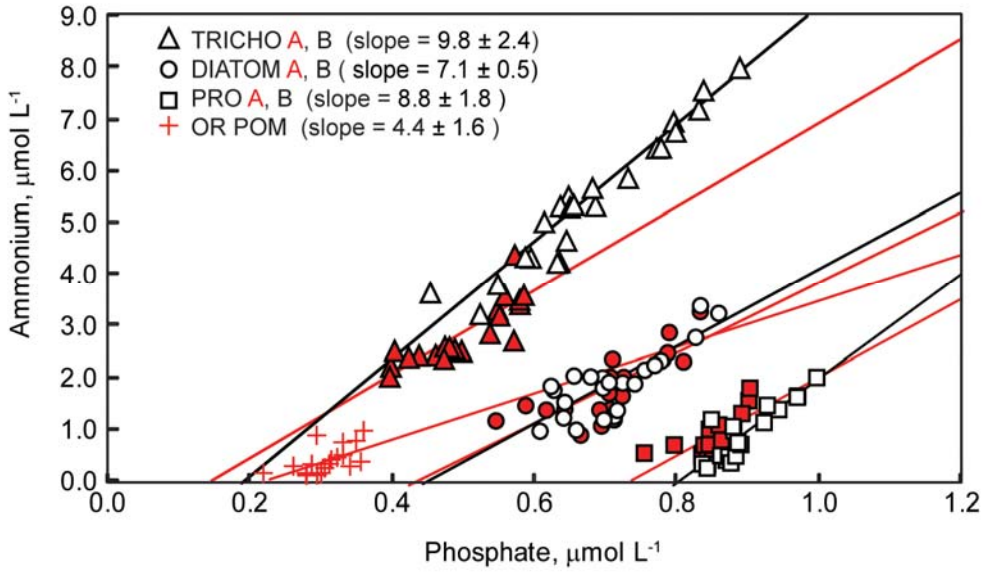
TRICHO A, B (slope = 9.8 ± 2.4)
DIATOM A, B (slope = 7.1 ± 0.5)
PRO A, B (slope = 8.8 ± 1.8)
OR POM (slope = 4.4 ± 1.6)
Ammonium, μmol L-1
Phosphate, μmol L-1
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
0.2
0.4
0.6
0.8
1.0
1.2

Figure 3

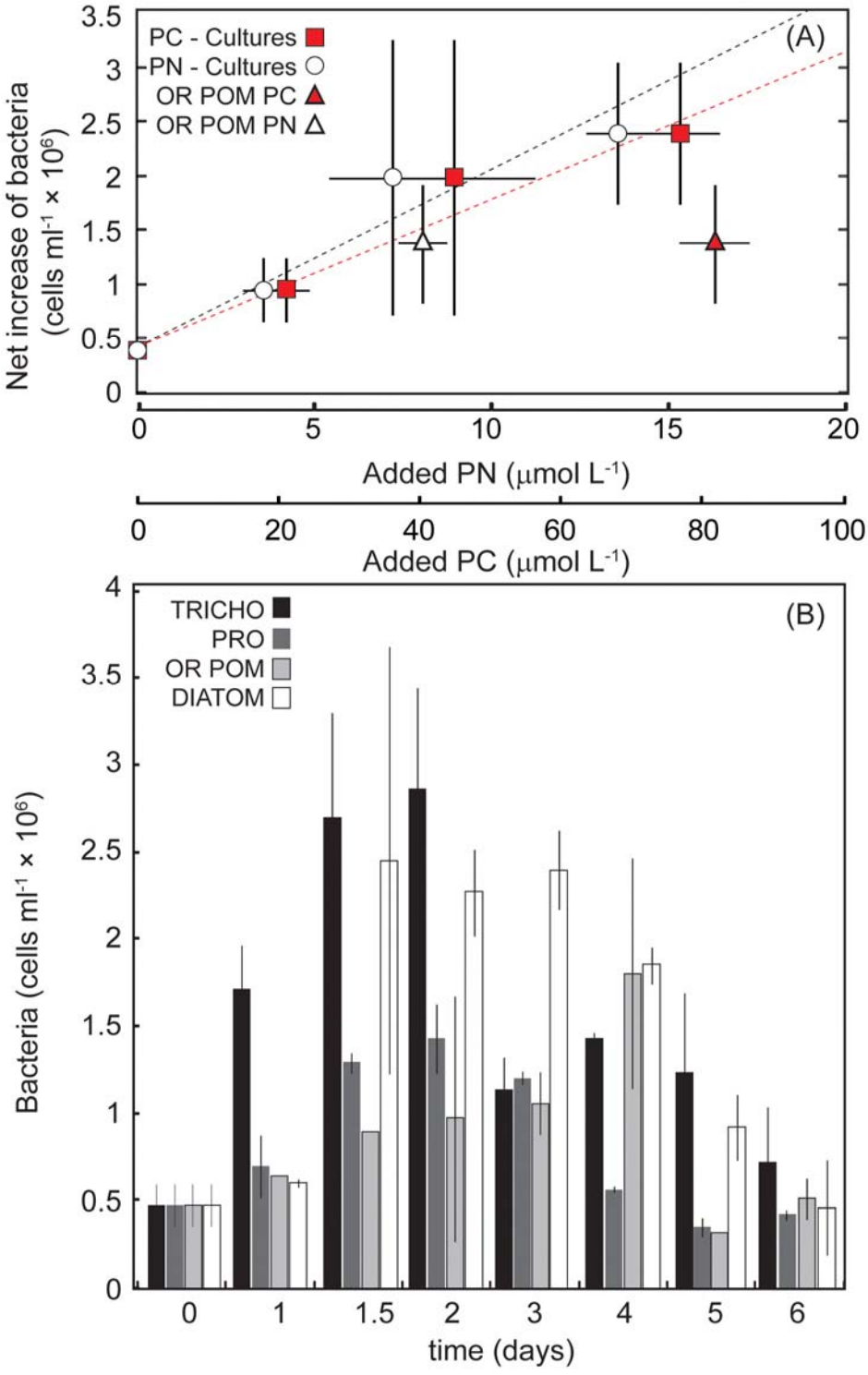
PC - Cultures
PN - Cultures
OR POM PC
OR POM PN
(A)
Net increase of bacteria (cells ml-1 × 10^6)
Added PN (μmol L-1)
Added PC (μmol L-1)
TRICHO
PRO
OR POM
DIATOM
(B)
Bacteria (cells ml-1 × 10^6)
time (days)

Figure 4

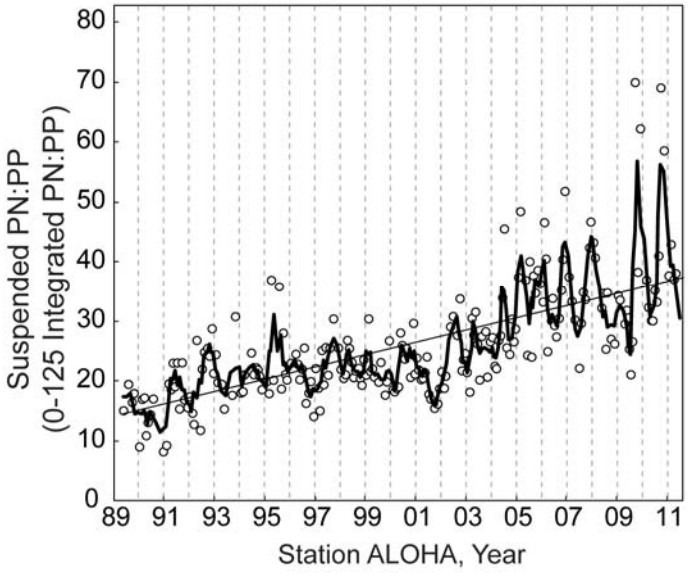
Suspended PN:PP
(0-125 Integrated PN:PP)
0
10
20
30
40
50
60
70
80
89
91
93
95
97
99
01
03
05
07
09
11
Station ALOHA, Year

Figure 5