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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 were 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).

1. 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 are 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,

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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 in 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 a 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 as 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 define 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.

2. Methods and materials

2.1. 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 a Turner 10-AU fluorometer), and all cultures were harvested during the early stationary growth phase (first timepoint 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

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 ^{31}P-NMR) is 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[a]	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	$C_{108\pm 8}N_{19\pm 1}P_1$	$C_{59\pm 15}N_{9\pm 2}P_1$	$C_{26\pm 4}N_{5\pm 1}P_1$	$C_{86\pm 7}N_{8\pm 1}P_1$
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

[a] An equivalent aliquot of *Trichodesmium* POM was added to 'KILLED' treatments.