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Flexible elemental stoichiometry in *Trichodesmium* spp. and its ecological implications

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Abstract

We conducted laboratory experiments to assess the bioelemental plasticity of cultures of *Trichodesmium* IMS101 under phosphorus (P)-replete, P-restricted, and light-limited conditions. The results reveal a high degree of stoichiometric flexibility. Specifically, *Trichodesmium* IMS101 is capable of growth with carbon (C) : nitrogen (N) : P ratios of $C_{585\pm56} : N_{90\pm10} : P_1$, approximately six times higher than would be predicted by the Redfield reference ratio ($C_{106} : N_{16} : P_1$), thus signifying low cellular P quotas relative to C and N. Luxury consumption of P occurs rapidly after periods of prolonged P restriction, under both light and dark conditions, resulting in substantial increases in P quotas and reductions of C : N : P ratios ($C_{96\pm8} : N_{16\pm1} : P_1$). Comparisons of laboratory culture data to our field observations from the Northwest Atlantic and the North Pacific indicate that, while natural populations of *Trichodesmium* exhibit persistently low P content relative to C and N ($C_{290\pm15} : N_{53\pm3} : P_1$), the highest and lowest C : P and N : P ratios recorded in the laboratory are rarely observed in nature. We have also performed laboratory experiments intended to simulate the energetic and nutritional extremes that would occur as naturally migrating populations of *Trichodesmium* sink out of the euphotic zone into P-rich regions of the upper disphotic zone. The duration of dark survival for this isolate is on the order of 3–6 d, after which time cells are unable to recover from light deprivation. This finding provides a constraint on the temporal scale of vertical migration.

Species of the colony-forming marine cyanobacterial genus *Trichodesmium* have been well described as prominent dinitrogen (N_2) fixing organisms (diazotrophs) in the oligotrophic tropical and subtropical regions of the global ocean (Capone 2001; Karl et al. 2002). The notoriety of this genus has only increased in recent years as revisions of abundance estimates and N_2 fixation rates have emphasized *Trichodesmium* as a significant source of new nitrogen to otherwise nitrogen (N)-deficient ecosystems (LaRoche and Breitbarth 2005). More than just a delivery vehicle for reactive N (N_R), *Trichodesmium* spp. are also noted for their ability to episodically form large surface accumulations, thereby transiently dominating primary productivity and N cycling (Bowman and Lancaster 1965; Karl et al. 1992; Capone et al. 1998).

Trichodesmium is ecologically significant in oligotrophic oceanic regimes, such as the North Atlantic and North Pacific gyres, where the process of biological N_2 fixation has been documented to seasonally enhance the net transport of carbon (C) and nitrogen (N) out of the euphotic zone (Karl et al. 1997; Capone et al. 2005). Given that *Trichodesmium*-based productivity is by nature stochastic, a composite understanding of the role of this genus in elemental cycling will require characterization of physiological variability rather than just the biological averages. Specifically, if we can define the range of physiological and stoichiometric flexibility and begin to understand the underlying functionality driving deviations from average elemental composition, we will improve our capability to predict potential responses of oligotrophic marine ecosystems to the stresses imposed by a changing environment.

Assuming a fixed elemental stoichiometry for marine biota, e.g., the Redfield reference ratio (Redfield 1958), requires that the internal chemical content of cells is strictly regulated. From this perspective, variable stoichiometry must be the consequence of the modulation of the relative proportions or the governing transformation rates of intracellular pools of biomolecules. In the North Pacific Subtropical Gyre (NPSG), a fundamental characteristic of increased *Trichodesmium* productivity is a systematic alternation of the particulate and dissolved elemental pools relative to the canonical Redfield ratios ($C_{106} : N_{16} : P_1$) occurring as a primary result of the excess production of N_R through N_2 fixation (Karl et al. 1997; Dore et al. 2002). Particularly during bloom periods, the stoichiometric

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fluctuations of *Trichodesmium* spp. can be striking; for example, particulate matter collected from surface waters during an August 1989 *Trichodesmium* bloom in the NPSG had an average elemental composition of $C_{891} : N_{125} : P_1$ (Karl et al. 1992). These ratios indicate intense community level phosphorus (P) limitation relative to either C or N.

Phosphorus regulation of diazotrophic production, however, is not particularly well understood. While it is well known that cyanobacteria can grow with reduced cellular quotas of P (Karl et al. 2002; Bertilsson et al. 2003; Heldal et al. 2003), the minimal nutritional requirements needed to maintain active growth have not been constrained for *Trichodesmium* spp. Moreover, since both natural and cultured populations of *Trichodesmium* spp. have been shown to have a relatively low affinity for inorganic P ($k_m \cong$ 50–200 nmol P L^{-1} for North Pacific field populations, K. Björkman pers. comm.; 400 nmol P L^{-1} for *Trichodesmium* IMS101; Fu et al. 2005), it appears that this genus is a poor competitor for dissolved inorganic phosphorus (DIP) in its native oligotrophic habitats where ambient DIP can be less than 1 nmol P L^{-1} (Karl and Tien 1997). Conversely, uncoupling of growth and P-uptake rates in cultured populations suggests a capacity for the consumption of P in excess of metabolic requirements for growth (Fu et al. 2005). Given that *Trichodesmium* spp. can constitute a considerable fraction of total biomass in oligotrophic systems and can function as a significant year round source of N_R for the pelagic community, we need to define the bounds of elemental composition and the physiological mechanisms generating these changes in *Trichodesmium* spp. in order to better understand the extent to which this key oceanic diazotroph regulates the flow of elements within the ecosystem. The goal of the research presented below is to characterize the stoichiometric plasticity exhibited by cultured populations of *Trichodesmium* IMS101 in response to environmental fluctuations in energy and nutrient (P) availability in an experimental context. Empirical results are compared with data from in situ sampling of Northwest (NW) Atlantic and North Pacific *Trichodesmium* populations. This research focuses primarily on the physiological processes characteristic of *Trichodesmium* spp. that may generate significant deviations from mean stoichiometry, i.e., P sparing, luxurious P uptake, and vertical migration.

Materials and methods

Laboratory culture maintenance—Trichodesmium IMS101 was provided by M.R. Mulholland (Old Dominion University) in April of 2002 and has since been maintained at Oregon State University. This strain, originally isolated from coastal Atlantic waters (Prufert-Bebout et al. 1993), has been identified as *T. erythraeum* (Janson et al. 1999). Over the course of our experiments, all cultures were grown in batch mode in order to simulate the rapid growth and decline associated with natural *Trichodesmium* blooms. Cultured populations were maintained on an artificial seawater medium (YBCII) lacking combined nitrogen (N) sources as described by Chen et al. (1996). The typical initial DIP concentration of this medium is 50 μmol L^{-1}. Thus, this laboratory strain has long been adapted to DIP concentrations approximately 3–4 orders of magnitude higher than those experienced by natural oligotrophic populations. To acclimate laboratory cultures to lower DIP conditions, batch cultures were grown in YBCII medium with an initial DIP concentration of 0.5 μmol L^{-1} for three successive 14-d transfer periods. This initial DIP concentration was chosen as a result of preliminary experiments showing 0.5 μmol L^{-1} to be the lowest DIP level capable of sustaining extended batch culture growth. Over the course of the experiment, cultures were maintained in a recirculating 24°C water bath under computer-controlled, cool white fluorescent lights set on a 12 : 12 sinusoidal light : dark (LD) cycle with a noon maximum (E_{MAX}) of 500 μmol quanta m^{-2} s^{-1}, sufficiently higher than the typical half-saturation irradiance (E_K) for photosynthesis (300 μmol quanta m^{-2} s^{-1}; LaRoche and Breitbarth 2005). On 3-h intervals during the light cycle, air was gently bubbled from an aquarium pump, through sterile filters and autoclaved tubing, into each culture bottle through an opening in the cap of the culture bottle in order to redistribute the culture and ensure that CO_2 did not limit growth.

Parent batch cultures—Following acclimation to reduced DIP conditions, 200-mL subsamples of late-exponential growth phase *Trichodesmium* IMS101 were successively transferred to three 2.5-liter acid-washed and deionized water (DI)–rinsed polycarbonate culture bottles. Each replicate was diluted to a final volume of 2.3 liters with ultraviolet (UV)-sterilized, artificial YBCII media with an initial DIP concentration of 0.5 μmol L^{-1}. For further reference, these 2.5-liter replicates are termed the parent batch culture. Temperature and light were maintained at previously stated levels (12 : 12 LD, E_{max} = 500 μmol quanta m^{-2} s^{-1}, T = 24°C). Growth of the parent batch culture was monitored daily within the first hour of the light cycle by pulse amplitude fluorometry (PAM; model XE-PAM, Heinz Walz GmbH Instruments) so as to record increases in biomass as determined by maximal chlorophyll *a* fluorescence (Chl *a* F_M). At the initial point of transfer and at multiple points throughout the growth cycle, each parent batch culture was sampled in duplicate for particulate carbon (PC), particulate nitrogen (PN), particulate phosphorus (PP), and chlorophyll *a* (Chl *a*) concentrations by filtering aliquots of culture onto combusted (3 h at 450°C) 21-mm Whatman GF/F filters. Cultures were swirled prior to sampling to ensure homogeneity. Duplicate samples were also collected daily for microscopic enumeration of filament concentrations, determination of fluorescence parameters, and evaluation of heterotrophic bacterial contamination. All filtrations were performed under a low atmospheric pressure differential (maximum vacuum = 130 mm Hg) with acid-washed and DI-rinsed glassware. The filtrate from this sampling procedure was then collected for determination of inorganic nutrient concentrations (phosphate, ammonium, nitrate + nitrite). Following collection, Chl *a* samples were placed in liquid nitrogen and all other samples were stored at −20°C until time of analysis. Specific growth rates (μ) were independently calculated from the change of particulate elements, Chl *a*,

filament concentrations, and Chl *a* F_M over the exponential phase of growth.

Dark bottles—At three time points (days 5, 10, and 14) during the growth of the parent batch cultures, 650-mL subsamples of each of the three parent batch culture replicates were transferred to 1-liter acid-washed and DI-rinsed, polystyrene dark bottles. A 5.0 μmol L^{-1} spike of DIP was then added to each dark treatment. For reference, dark bottles (db) are termed db-A (t_i = day 5, n = 3), db-B (t_i = day 10, n = 3), and db-C (t_i = day 14, n = 3), where t_i refers to the day of batch culture growth that each db was initiated. On a daily basis, following transfer to dark bottles, duplicate samples were collected from each db replicate for Chl *a* content, dissolved inorganic nutrients, PC, PN, and PP composition, as previously described for the parent batch culture.

Recovery determination—At multiple time points throughout the experiment, 5-mL subsamples were taken from the parent batch cultures and db incubations in order to assess the response of the samples to external P additions (parent batch culture recovery samples) or reexposure to light (dark bottle recovery samples). The day of recovery initiation is denoted as t_{ri}. On days 11 and 14 of batch culture growth, subsamples were taken from the primary batch and amended with 0.5 μmol L^{-1} of DIP. Further DIP additions were not made to dark bottle recovery samples. All recovery samples were replaced in the incubator with the parent batch cultures (12 : 12 LD, E_{max} = 500 μmol quanta m^{-2} s^{-1}, T = 24°C). Growth of recovery samples was monitored daily via Chl *a* F_M. Also, selected samples were filtered and processed for Chl *a*, PP, PC, and PN determinations. A positive growth rate response to DIP additions was taken as initial confirmation of P limitation of the parent batch culture. Growth of db recovery samples was evaluated as a measure of the dark survival capacity of the cells.

Analytical procedures—Changes in biomass concentrations in each db were measured by three independent methods: Chl *a* concentrations, Chl *a* F_M, and microscopic enumeration of individual filaments. Chl *a* concentrations were determined fluorometrically, as described by Strickland and Parsons (1972) using a Turner 10-AU fluorometer (Turner Designs). Chl *a* F_M and the quantum yield of fluorescence (F_V F_M^{-1}) were measured using a PAM fluorometer employing blank corrections (Cullen and Davis 2003) with fresh YBC11 media. Use of Chl *a* F_M as a proxy for biomass is advantageous since it is noninvasive, requires relatively small volumes (≤4 mL), and fluorescence-derived parameters can be used as a general indicator of the physiological state of the culture (Campbell et al. 1998). Preliminary experiments with *Trichodesmium* IMS101 resulted in a significant correlation (r^2 = 0.96) between Chl *a* F_M and acetone extracted Chl *a* concentrations when cultures were sampled at equivalent light levels. Duplicate, 3-mL samples were fixed in 2.5% final concentration scanning electron microscopy (SEM) grade gluteraldehyde, stained with 5 μg 4′,6-diamidino-2-phenylindole, dihydrochloride (DAPI) mL^{-1}, and filtered onto black-stained 0.2-μm Nuclepore membrane filters. Slide mounted filters were kept frozen at −20°C until counts were performed. Entire slides were counted using UV-epifluorescence microscopy for enumeration of individual filaments. Selected slides were also examined for the presence of contaminating heterotrophic bacterial biomass following the protocol of Sherr et al. (2001).

Particulate C and N were analyzed on a Carlo Erba CHNS analyzer (model NA1500) using cystine (29.99% C and 11.66% N by weight) as the primary standard. Error terms for all elemental ratios were calculated using standard error (SE) propagation techniques (Bevington and Robinson 2003). Medium blanks processed during laboratory experiments had an average C and N content of 5.7 μg C $filter^{-1}$ (SE = 0.6, n = 14) and 0.51 μg N $filter^{-1}$ (SE = 0.16, n = 14), respectively (values for filtration of 25 mL medium). The magnitude of this blank correction corresponded to 14.3% ± 0.7% of the PN signal and 23.9% ± 0.9% of the PC signal for individual samples (n = 150). Filter blanks (n = 11) processed for field samples in the Atlantic accounted for 7.6% ± 0.6% of the PN signal and 18.4% ± 1.1% of the PC signal for individual samples (n = 52). For Pacific samples, filter blanks (n = 8) represented 12.2% ± 1.2% of the PN signal and 29.8% ± 2.7% of the PC signal for individual samples (n = 36).

Particulate P was analyzed according to current Hawaii Ocean Time-Series (HOT) program protocols. Filters were combusted in acid-washed, DI-rinsed glass test tubes for 4–5 h at 450°C in a muffle furnace. Samples were then cooled and immersed in 10 mL of 0.15 mol L^{-1} hydrochloric acid. Extracted samples were analyzed for P content using molybdenum blue spectrophotometry (Hebel and Karl 2001). Accuracy was assessed from the analysis of a known dry weight of certified reference material (National Institute of Standards, NIST 1515, orchard leaves, certified 0.159% P by weight). The measured P content of NIST 1515 reference material averaged 0.152% (SE = 0.003%, n = 16) for standards used relative to laboratory samples, 0.151% (SE = 0.004%, n = 8) for Atlantic reference samples, and 0.152% (SE = 0.016%, n = 6) for Pacific reference samples. Filter blanks used for laboratory experiments accounted for an average 13.8% (SE = 3.3%, n = 13) of the sample PP signal (n = 158). For field samples, filter blanks represented 11.6% (SE = 1.2%, n = 7) of the Atlantic PP signal (n = 24) and 14.1% (SE = 1.6%, n = 8) of the Pacific PP signal (n = 28).

A Technicon AutoAnalyzer II was used to measure phosphate and ammonium, while Alpkem RFA 300 components were used to measure nitrate plus nitrite concentrations from sample filtrates. Phosphate concentrations were determined following a modification of the molybdenum blue procedure of Bernhardt and Wilhelms (1967), where phosphate is measured as reduced phosphomolybdic acid employing hydrazine as the reductant. Ammonium concentrations were measured following an indophenol blue method modified from ALPKEM RFA methodology. The nitrate + nitrite analysis uses the basic method of Armstrong et al. (1967), with modifica-

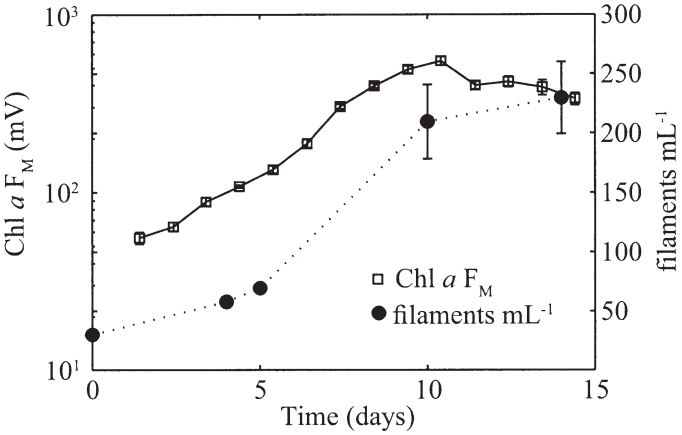

Fig. 1. Growth curve for batch culture of IMS101 based upon direct microscopic enumeration of filaments and Chl *a* F_M. Dark bottles were initiated on days 4, 10, and 14.

tions to improve the precision and ease of operation (Patton 1983).

Natural populations—Colonies of *Trichodesmium* were collected and analyzed for elemental stoichiometry during two separate cruises, the first in the tropical NW Atlantic (9°30′N–11°21′N, 50°05′W–56°31′W) aboard the R/V *Seward Johnson II* in April–May of 2003 and the second in the subtropical North Pacific (18°30′N–21°01′N, 154°50′–161°00′W) aboard the R/V *Roger Revelle* in July–August of 2003. Both cruises were components of the National Science Foundation (NSF) funded Marine Nitrogen fixation and Tropospheric Response to Aeolian inputs (MANTRA)/Potential Influences of Riverine and Aeolian inputs on Nitrogen fixation in the Atlantic (PIRANA) projects. *Trichodesmium* colonies were collected with either 202-μm mesh nets towed off ship drift near the surface or by oblique tows with 1-m diameter nets towed at depths of 5–30 m. Colonies were selected with a plastic bacteriological transfer loop and rinsed in filtered seawater. At each sampling point, at least 10 colonies per analysis were filtered onto precombusted GF/F filters for determination of particulate C, N, and P content. Following collection, all samples were stored at −20°C.

Results

Parent batch culture—The parent batch culture exhibited a typical pattern of lag and exponential growth phases. Over a 14-d period, the specific growth rate (μ) of the culture was equivalent to 0.13 d^{-1} when calculated from Chl *a* F_M and 0.15 d^{-1} when calculated from filament counts. Specific growth rates based upon particulate elements PC, PN, and PP were 0.19, 0.20, and 0.21 d^{-1}, respectively. This disparity in growth rates appears to be due to elongation of filaments over the course of batch culture growth (results not shown). Chl *a* F_M reached maximum values after 10 d, after which time Chl *a* F_M began to decline while filament density plateaued (Fig. 1). The decline in Chl *a* F_M coincided with a decline in the quantum yield of fluorescence ($F_V F_M^{-1}$) from an average of

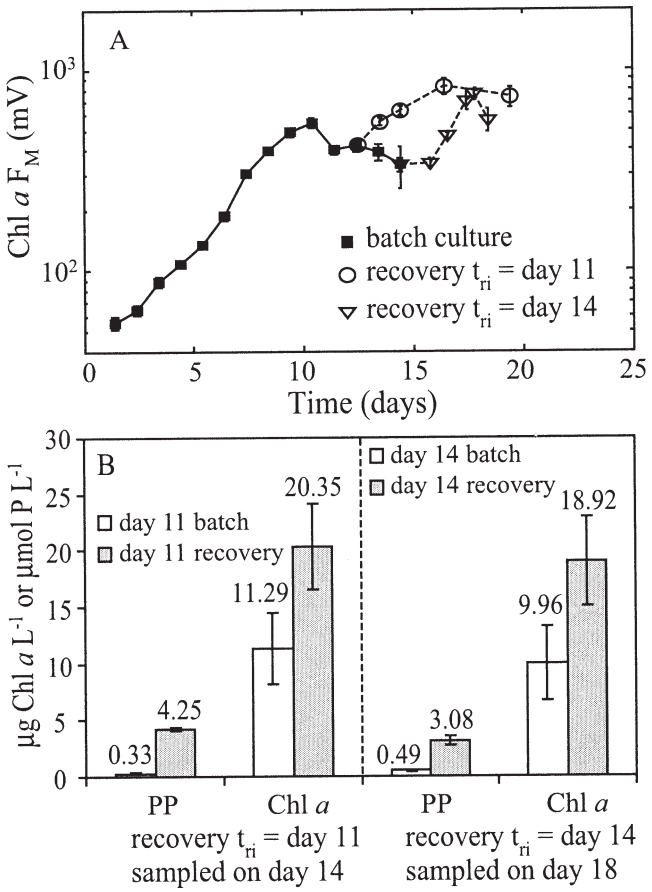

Fig. 2. (A) External P additions made on day 11 (t_{ri} = day 11) and then on day 14 (t_{ri} = day 14) resulted in an increase of Chl *a* F_M. (B) Comparison of PP and Chl *a* concentrations between recovery and batch culture samples also indicates a positive response to P additions. The first recovery assessment of PP and Chl *a* compares batch culture and recovery samples sampled on day 14, while the second recovery assessment refers to biomass sampled on day 18. Error bars represent the standard error.

0.35 to a minimal value of 0.24 (trend not shown) and was thus taken as an indicator of physiological stress (as in Berman-Frank et al. 2004). Following this decline, on days 11 and 14 of batch culture growth, the response of the batch culture to P additions was assessed. By all measures (Fig. 2), recovery samples responded positively to P additions. Positive growth of batch recovery samples was preliminary confirmation of P limitation of the parent batch culture beginning at day 11.

The temporal change of DIP, PP, and total P (DIP + PP) concentrations in the batch culture is shown in Fig. 3. PP concentrations increased steadily over the course of batch culture growth, whereas DIP was drawn down to a minimum concentration of 40 nmol L^{-1} (SE = 0.001 n = 6) after only 5 d (Fig. 3B). This concentration is typical of near surface values at Sta. ALOHA (Karl and Tien 1997). The balance of the batch culture P budget was assessed by examining deviations of total P from the total P measured at the initiation of the parent batch culture (TP_i). Figure 3A shows that DIP + PP decreased to a minimum

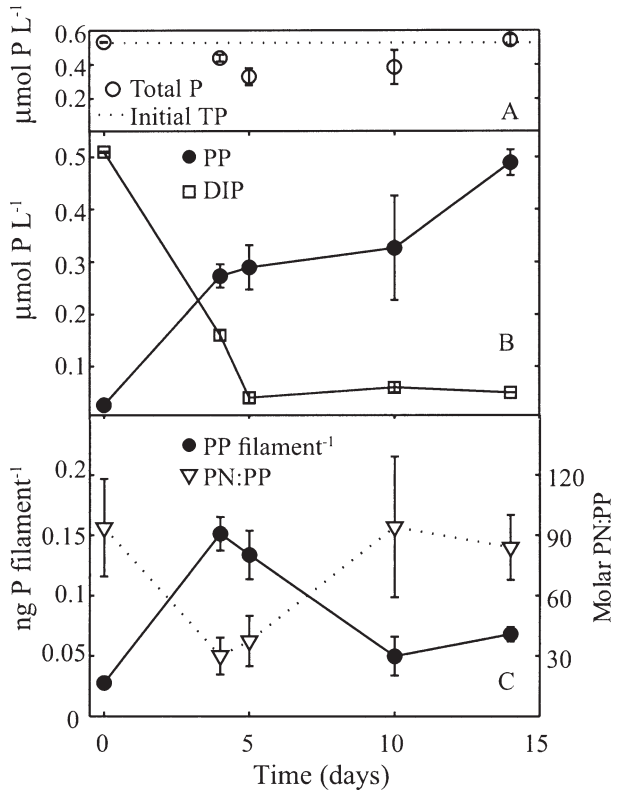

Fig. 3. P dynamics in the parent batch culture. (A) Total P (DIP + PP) compared with the initial measured total P (initial TP). (B) PP and DIP over 14 d of batch culture growth. (C) Change in measured PP content per filament and PN : PP ratios over the course of the batch culture. All error bars represent standard error.

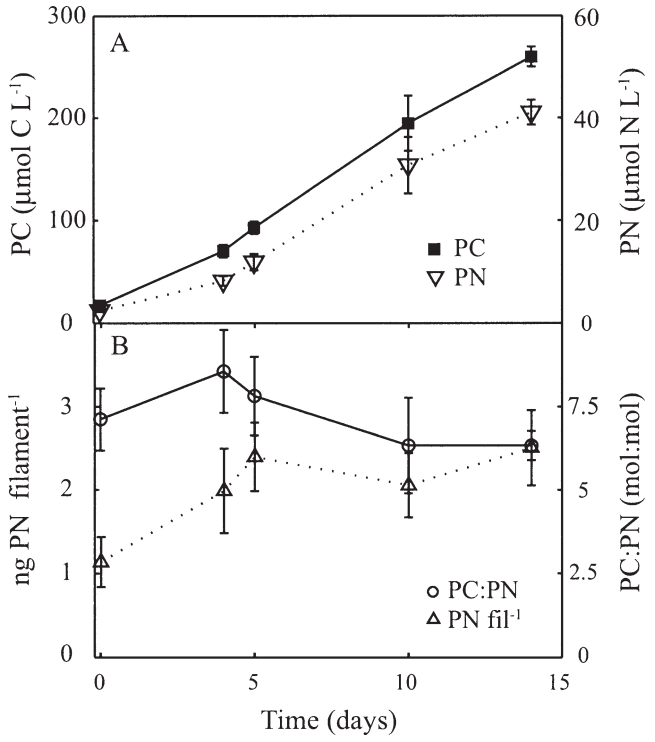

Fig. 4. (A) Batch culture PN and PC. (B) Batch culture PC : PN ratios and PN filament^{-1}. PC : PN ratios significantly differ from the Redfield reference ratio of 6.63 only on day 4.

at day 5 of the batch culture, indicating loss of P to some undetectable form. Batch culture PP content relative to filament number and PN peaked at day 4 (PP filament^{-1} = 0.15 ng P filament^{-1}, SE = 0.014, n = 6; PN : PP = 30.1 mol : mol, SE = 9.2, n = 6), after which time normalized PP content declined (Fig. 3C). Calculated over the period of maximum biomass increase (days 0–4), the specific growth rate of PP was 0.48 d^{-1}, while the specific uptake rate of PP filament^{-1} was 0.42 d^{-1}, corresponding to a turnover time of 1.4 to 1.6 d. The lowest normalized PP content was measured at time zero of batch culture growth (PN : PP = 93.8 mol : mol, SE = 24.2, n = 4; PP filament^{-1} = 0.03 ng P filament^{-1}, SE = 0.003, n = 4), consistent with preadaptation of cultures to P-restricted growth conditions.

Increases in batch PN and PC are shown in Fig. 4A. Batch culture PC : PN ratios (Fig. 4B) did not vary significantly during the course of growth (t-tests $p > 0.1$). However, samples taken on day 4 (PC : PN = 8.56, SE = 0.7, n = 6) were significantly greater than the Redfield reference ratio of 6.63 (t-test p = 0.02, df = 5). PN normalized to filament biomass reached a maximum after 5 d of growth, after which time PN filament^{-1} stabilized (Fig. 4B). PC quotas per filament peaked at day 5 (PC filament^{-1} = 16.1, SE = 1.2, n = 6) and then declined, reaching stable levels by day 10 (data not shown). Dissolved inorganic N ($NH_4^+ + NO_3^- + NO_2^-$) accumulated in the parent batch medium from an initial concentration of 0.08 μmol L^{-1} (SE = 0.004, n = 4) to a maximum concentration of 1.25 μmol L^{-1} (SE = 0.06, n = 6) at day 14, a net increase of 1.17 ± 0.02 μmol N L^{-1} or 3% of the total measured PN increase (38.7 μmol N L^{-1}).

Dark bottles: biomass decline—Subsamples of each parent batch replicate were transferred to dark bottles with added P on days 5 (db-A), 10 (db-B), and 14 (db-C). Figure 5 presents the decline in the number of intact filaments per volume in each dark treatment relative to the growth of the parent batch culture. Biomass change over time was measured by microscopic enumeration of filaments and in vivo Chl a F_M. Extracted Chl a concentrations were recorded for db-A only because of inadvertent loss of db-B and db-C samples. For this reason, only Chl a F_M and the number of filaments per volume are presented for db analyses. In all of the dark bottles, Chl a F_M (Fig. 6) and filament concentrations (Fig. 5) remained relatively stable for a period of 2–4 d of dark exposure, after which time biomass began to decline rapidly. The average rate of decline derived from filament counts was 15.6% d^{-1} for db-A, 12.8% d^{-1} for db-B, and 10.4% d^{-1} for db-C. The total percentage biomass lost over the course of dark incubations was 94%, 98%, and 83% for db-A, db-B, and db-C, respectively, based on filament counts. The total Chl a F_M loss and the rate of Chl a F_M losses closely parallel the decline of filament counts.

Dark bottles: recovery from nutrient and light stress—Samples taken from the early phases of db incubations

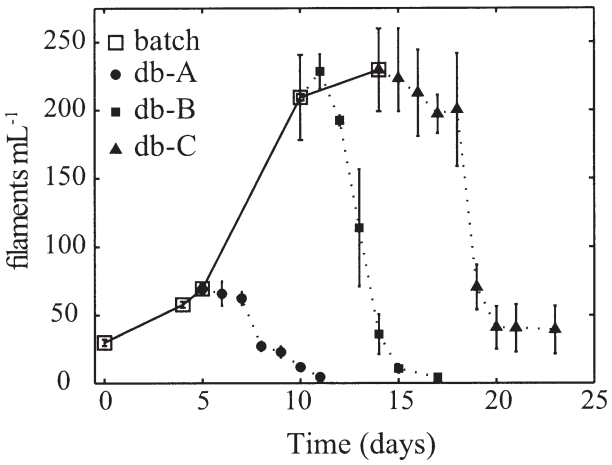

Fig. 5. Filament count per milliliter for batch culture and dark bottle samples. The total percentage filament biomass lost over the course of dark incubations was 95%, 89%, and 89%, for db-A, db-B, and db-C, respectively.

displayed positive growth rates when replaced in light conditions (Fig. 6), regardless of the nutritional state of the cells prior to dark bottle initiation. The average exponential phase specific growth rates of positive response recovery samples from db-A, db-B, and db-C were 0.27, 0.20, and 0.28 d^{-1}, respectively. Recovery samples taken from days 4–6 of db-A, days 5–7 of db-B, and days 7–8 of db-C did not increase in biomass in response to reintroduction to light conditions (Table 1).

Dark bottles: elemental concentrations and ratios—The elemental concentrations and ratios presented here represent a mixture of living and nonliving particulate material because there is no straightforward method for separating the two fractions either in cultures or in the field because of sampling constraints. The temporal change of PP, DIP, and total measured P (DIP + PP) concentrations are shown in Fig. 7A–C. The change in DIP concentrations explains 78%, 79%, and 92% of PP variation for db-A, db-B, and db-C, respectively (regressions not shown). All regressions are significant at the 95% confidence level. Conservation of measured P for dark incubations is addressed in the same manner as that described for the parent batch culture, by examining deviations of total P over time from the initial total measured P. In db-A, total P does not change significantly from the initial total P, indicating that P was conserved over the time course of this dark incubation. In contrast, a net loss of P is observed by the final day of the db-B incubation period and at three time points during the db-C incubation period. These losses indicate conversion of measurable P to a form undetectable by the analytical methods used in this study.

PC and PN concentrations for dark incubations are shown in Fig. 7D–F. In db-A, PN concentrations decreased by less than 10% (-0.9 μmol N L^{-1}) over the time course of the incubation, while PC concentrations dropped by 24%, a net loss of 22 μmol C L^{-1} (Fig. 7D). These decreases are reflected by a gradually declining PC : PN ratio for db-A (Fig. 8A). During the time course of db-B and db-C incubations, PN and PC concentrations increased over the first 1–2 d of the incubation period and then steadily returned to near or below initial concentrations (Fig. 7E–F). The net loss of PN recorded for db-B was 3.4 μmol N L^{-1} (11% of initial values), while PC concentrations fell by 37 μmol C L^{-1} (19% of initial PC), again reflected by a change in PC : PN ratios (Fig. 8B). With the exception of the initial time point, db-B and db-C PC : PN ratios were significantly lower than the Redfield PC : PN ratio of 6.63 (Fig. 8B–C).

Particulate C : P ratios for all db incubations indicate net P uptake relative to C (Fig. 8A–C) under dark conditions. PC : PP ratios in db-A reflect variation in PC (Fig. 7D) and a steady increase in bulk PP (Fig. 7A), resulting in PC : PP ratios ranging from an initial value of 293 (SE = 31, $n = 6$) to a minimum of 85 (SE = 5.5, $n = 6$). In db-B, PC : PP ratios reached a relatively stable minimum (days 1–7 average PC : PP = 99, SE = 5.5, $n = 36$) after a single day of darkness, whereas db-C required 4 d to reach a stable minimum (days 4–8 average PC : PP = 113, SE = 3.7, $n = 21$). PP per filament also increased over the course of all dark incubations (Fig. 8D–F). When evaluated over the time period for which cells were able to recover from the light period (days 0–3 for db-A, days 0–4 for db-B, and days 0–6 for db-C) (Table 1), specific P-uptake rates calculated from the net change in PP filament^{-1} are 0.50 $\pm$ 0.09 d^{-1} for db-A, 0.75 $\pm$ 0.13 d^{-1} for db-B, and 0.52 $\pm$ 0.09 d^{-1} for db-C. These correspond to P turnover times of 1.4 d (db-A), 0.9 d (db-B), and 1.3 d (db-C).

The net accumulation of dissolved inorganic fixed N ($NH_4^+ + NO_3^- + NO_2^-$) during the time course of dark bottle incubations was 0.09, 1.33, and 1.91 μmol L^{-1} for db-A, db-B, and db-C, respectively. These increases correspond to 0.8% (db-A), 4.3% (db-B), and 4.7% (db-C) of initial PN concentrations. Dark conditions did not result in the preferential loss of N from the particulate pool relative to C (Fig. 8A–C), Chl *a*, or filament concentration (data not shown); thus, the liberated N is likely the result of cell senescence and subsequent cell lysis.

Phosphorus budget considerations—Conservation of measured P in the parent batch and db incubations was addressed by calculating the difference between the concentration of total P (TP) and initial total measured P pools (TP_i) at each time point. This analysis allowed us to determine whether or not the cultures were ever contaminated with an outside source of P ($TP_i - TP > 0$), or, conversely, whether P was converted to a form of P that was not analyzed in this study (e.g., dissolved organic P [DOP] or intracellular polyphosphate) ($TP_i - TP < 0$). No P contamination was detected over the course of this experiment; however, P losses were observed. Negative values of ($TP_i - TP$) are termed missing P (P_M).

Given the analytical techniques used in this study, it is possible that the calculated P_M pool was comprised of intracellular polyphosphates and/or DOP. Polyphosphates are known to accumulate in most cyanobacteria (Grillo and Gibson 1979; Romans et al. 1994). If present and of high molecular weight, they would have not been detected by

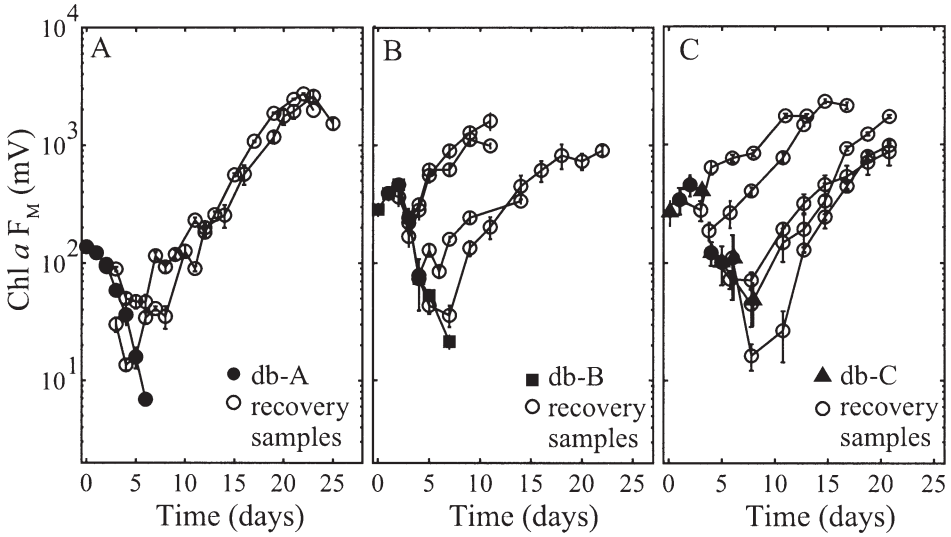

Fig. 6. Fluorescence based measure of biomass (Chl *a* F_M) for db samples and those recovery samples taken from (A) db-A, (B) db-B, and (C) db-C that responded positively to reintroduction to light conditions. Samples that did not recover are not shown. These occurrences are cited in Table 1.

our analytical methods, since high temperature acid hydrolysis is necessary to effectively retrieve the entire polyphosphate fraction of the particulate pool (Karl and Tien 1992). Our methodology would have only recovered low molecular weight polyphosphate. DOP concentrations were not measured.

Negative values of (TP_i − TP) were observed in the parent batch culture (Fig. 3A), indicating that a fraction of the P present at the initiation of the batch culture was converted to an undetectable P pool (P_M). In batch culture, calculated P_M concentrations peaked at 33% of total predicted PP on day 5, coinciding with minimal DIP levels (Fig. 3A). After day 5, the calculated P_M concentrations decreased, suggesting that cells began using intracellular polyphosphate pools or DOP to fuel growth once DIP was no longer available, a scenario consistent with the concept of luxury P consumption. The net increase in the concentration of PP over the entire 14-d batch culture, 0.463 μmol L^{-1} (SE = 0.025), is statistically balanced by the net drawdown of DIP over this same time period, 0.453 μmol L^{-1} (SE = 0.003). Negative values of (TP − TP_i) were also observed at select points during db-B and db-C incubations (Fig. 7B–C). In contrast to the calculated magnitude of the P_M pool observed in the parent batch culture, db P_M accumulations accounted for less than 13% of total predicted PP concentrations.

Table 1. Results of db subsample responses to light conditions. Samples that did not display a positive response are indicated by NR (no recovery). Growth rates (μ) were calculated from Chl *a* F_M. Db-A was terminated after sampling on day 6, db-B on day 7, and db-C on day 8, subsequent days are denoted by a dash. At the onset of db incubations, the filament specific P_Q was 0.136 ± 0.05, 0.054 ± 0.039, and 0.070 ± 0.014 ng P $filament^{-1}$ for db-A, db-B, and db-C, respectively.

Day	db-A	db-B	db-C
1	Not sampled	μ=0.23	μ=0.23
2	μ=0.29	μ=0.25	μ=0.36
3	μ=0.25	μ=0.17	Not sampled
4	NR	μ=0.22	μ=0.19
5	NR	NR	μ=0.30
6	NR	Not sampled	μ=0.32
7	—	NR	NR
8	—	—	NR

Field data—The oceanic regions from which our field samples were collected exhibit similar hydrographic properties. The average surface temperature and salinity of the nine stations sampled in the subtropical Pacific region were 26.8°C and 35.1‰, while the 19 stations sampled in the tropical NW Atlantic had a mean temperature of 27.3°C and mean salinity of 34.6‰. Annual mean surface phosphate concentrations for the Pacific region (~ 20°N, 158°W) are typically about 100 nmol L^{-1} (data from HOT climatology, 0–45 m mean phosphate), while the Atlantic region (~ 10°N, 53°W) ranges from 120 to 220 nmol L^{-1} (data from National Oceanographic Data Center World Ocean Atlas 2001). Particulate C : N : P stoichiometry for each sample group (Pacific and Atlantic) is presented in Fig. 9. The mean stoichiometry of the Pacific group was $C_{302\pm32} : N_{56\pm6} : P_1$, and $C_{285\pm33} : N_{49\pm3} : P_1$ for the Atlantic sample group. Results of *t*-tests assuming separate variance revealed no significant difference between the PC : PP (p = 0.59, df = 50), PN : PP (p = 0.19, df = 50), or PC : PN (p = 0.16, df = 86) ratios of samples collected from these oceanic regions.

Discussion

The composite data set presented here allows us to identify ecologically relevant sources of stoichiometric

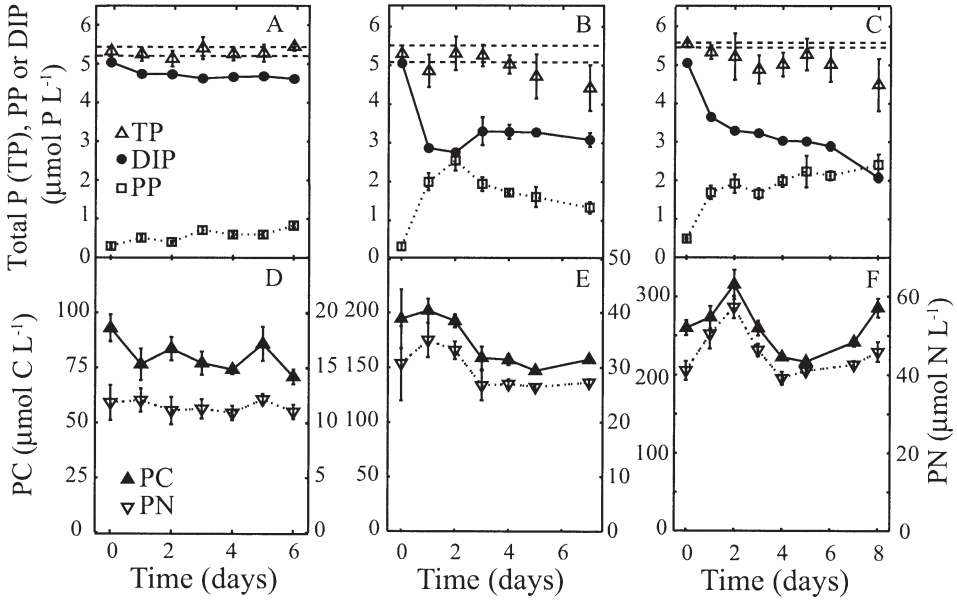

Fig. 7. (A–C) Temporal change in PP, DIP, and total measured P (TP = DIP + PP) for all dark bottle incubations. Dashed lines represent the standard error of initial total P for each dark bottle. TP is conserved in db-A, while net losses of P are observed by the final day of db-B and at multiple points during the db-C incubation period. (D–F) Temporal change in PC and PN concentrations for all dark bottle incubations.

variability, specific to *Trichodesmium* spp., which would permit this genus to alter the bulk elemental composition of dissolved and particulate pools, and thus regulate the flow of elements in otherwise nutrient-depleted marine environments. Specifically, results from this study suggest that *Trichodesmium* spp. have very low P requirements relative to C or N. Maintenance of diazotrophic growth with reduced P content would preferentially enhance both the C and N components of dissolved and particulate pools, thus favoring community scale P limitation. Conversely, cultured populations of *Trichodesmium* previously acclimated to low P conditions appear to have a high capacity for luxury consumption of P. Use of P in excess of the requirements for growth would be expected to alter C : N : P stoichiometry without a concomitant increase in growth rate (Sommer 1985). Lastly, this research addresses basic tenets of the P-mining vertical migration scenario: cell survival and P uptake in the absence of light.

Phosphorus sparing—Natural populations of *Trichodesmium* spp. occur in oceanic environments where dissolved phosphate concentrations are extremely low, typically at or near standard detection limits set by standard autoanalyzer technology (50 nmol P L^{-1}), thus leading to widespread speculation of regional scale P limitation of *Trichodesmium* growth (Karl et al. 1997; Sañudo-Wilhelmy et al. 2004; Fu et al. 2005). It has been suggested that *Trichodesmium* spp. may persist in a prolonged state of P limitation by decreasing internal P quotas, most likely through a reduction of P-rich cellular components such as RNA, an effect termed P sparing (Karl et al. 2002). Phosphorus-restricted laboratory populations, those cells harvested from media with the lowest measured DIP and PP filament^{-1} (batch days 0, 10, and 14; mean DIP = 0.054 μmol L^{-1}, SE = 0.004, n = 16), produced biomass with mean elemental stoichiometry of $C_{585\pm56} : N_{90\pm10} : P_1$, significantly higher than the Redfield reference ratio (*see Table 2 for range*). If we include P_M in this calculation of P-restricted stoichiometry, the mean ratio is not substantially altered ($C_{574} : N_{88} : P_1$). Furthermore, neither PN filament^{-1} (Fig. 4B) nor PC filament^{-1} were elevated during P restriction, reinforcing the conclusion that the high PC : PP and PN : PP measurements are a result of low P quotas rather than C or N storage. Figure 10 compares the full range of our empirical data (Fig. 10B) to the elemental composition of natural populations collected in the tropical NW Atlantic and the subtropical North Pacific (Fig. 10A). Most apparent is the observation that natural populations are consistently depleted in P relative to C or N, with 98% of the samples falling above the typical Redfield C : N : P stoichiometry of $C_{106} : N_{16} : P_1$.

In order to interpret this data set in terms of nutrient restriction, we must assume some critical N : P ratio indicating a transition point above which P limitation can be inferred. Based upon the physiologically feasible range of biomolecular composition for marine phytoplankton, Geider and LaRoche (2002) calculated that the critical N : P ratio for a given species is between 10 and 50 mol : mol. Genera with slow growth rates and high protein content such as *Trichodesmium* would have a critical N : P toward the higher end of this range. Similarly, Kustka et al. (2003) infer a critical N : P of 40 mol : mol from their own experimental data set. If we conservatively take the upper limit of 50 to represent the critical N : P ratio for *Trichodesmium*, our data suggest that 53% of field samples (64% of North Pacific samples; 38% of NW Atlantic samples) were P limited at the time of sampling.

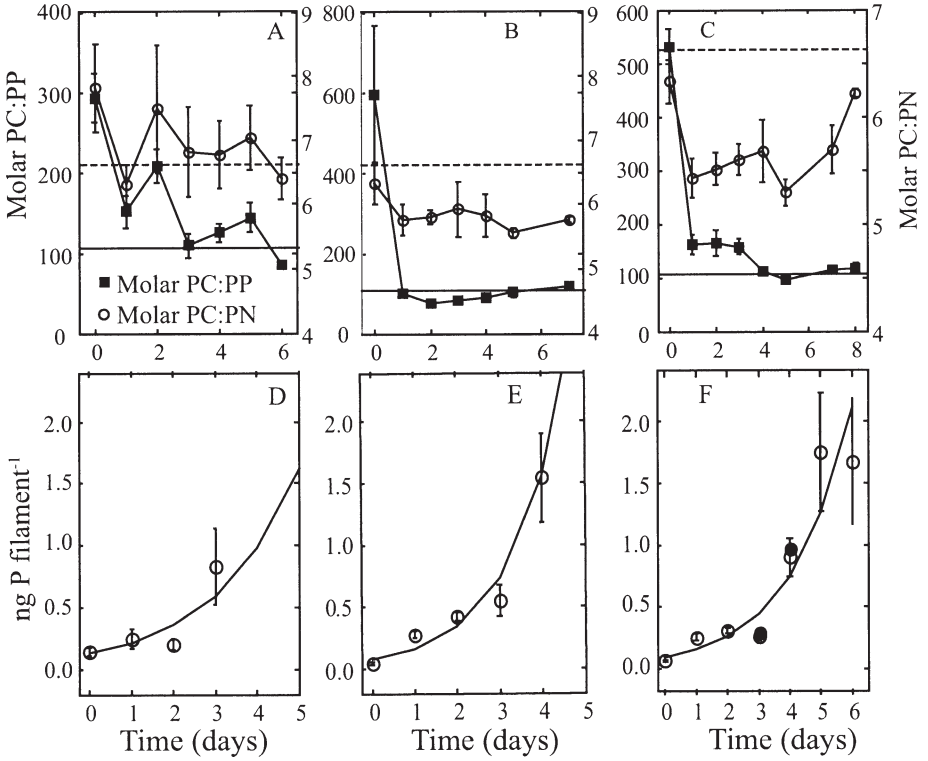

Fig. 8. (A–C) PC : PP and PC : PN ratios for each dark bottle incubation. In each subplot the solid line represents the Redfield PC : PP reference ratio of 106, while the dashed line represents the Redfield PC : PN reference ratio of 6.63. In all dark bottles, PC : PP ratios approach Redfield stoichiometry; however, the slope of this approach is notably more rapid in db-B and db-C. In db-A, PC : PN ratios differ from Redfield stoichiometry only at the initial time point. In contrast, the PC : PN stoichiometry of db-B and db-C are generally lower than the Redfield reference ratio. Note: scales differ for each dark bottle. All error bars represent standard error. (D–F) Phosphorus content per filament in dark incubations shown for the time period during which recovery analyses were positive. Filled circles indicate measured $P + P_M$ per filament.

When other field data are considered (Table 2) it is apparent that, for virtually every published observation, some fraction of the *Trichodesmium* population is P limited when a critical N : P of 50 mol : mol is used as the benchmark for P limitation. Thus, the composite range of field data is consistent with a paradigm of widespread P limitation of natural populations of *Trichodesmium*. Yet, if we assume that the stoichiometry of P-restricted laboratory cultures sampled during this study approximate a minimal P quota, it becomes evident that the extremes measured in the laboratory are rarely exhibited in nature. Specifically, only 8% of field samples from our own data set ($n = 4$, all from the NW Atlantic), a single observation of a NPSG bloom (Karl et al. 1992), and three observations from the data set of Sañudo-Wilhelmy et al. (2004) fall above the stoichiometric mean exhibited by P-restricted cultures from this study ($>C_{585} : N_{90} : P_1$).

An obvious question arising from this research is why the end member PC : PN : PP ratios observed in this study are not commonly observed in nature. We can speculate that the elemental composition of particulate matter in natural populations is more tightly constrained as a consequence of Liebig's law of the minimum, such that the low DIP concentrations characteristic of the oligotrophic environs inhabited by *Trichodesmium* spp. will only drive relatively moderate stoichiometric anomalies. Colimitation by another element, such as Fe, would preclude further stoichiometric deviation. In this research, the initial Fe concentrations in our media were on the order of 500 nmol L^{-1}, whereas Fe concentrations in the oligotrophic oceanic gyres are typically ~0.1 nmol L^{-1} (Wu et al. 2001; Boyle et al. 2005). Thus, in this study, the maintenance of such high levels of potentially colimiting elements ensured that P was the only limiting factor. In natural oligotrophic environments where multiple elements are in low abundance, this scenario is not likely. It is also possible that the extremes observed in the laboratory are more common in nature, albeit masked by the presence of surface adsorbed P. Recent work by Sañudo-Wilhelmy et al. (2004) indicates that 46–91% of total particulate P measured in natural samples of *Trichodesmium* collected from the North Atlantic existed in the form of surface adsorbed P, rather than intracellular P. Given that we did not process our samples in the same manner as these authors, we can only speculate that our values may represent a conservative estimate of intracellular stoichiometry.

Luxury consumption of phosphorus—In contrast to a reduction of nutrient quotas, deviations from mean stoichi-

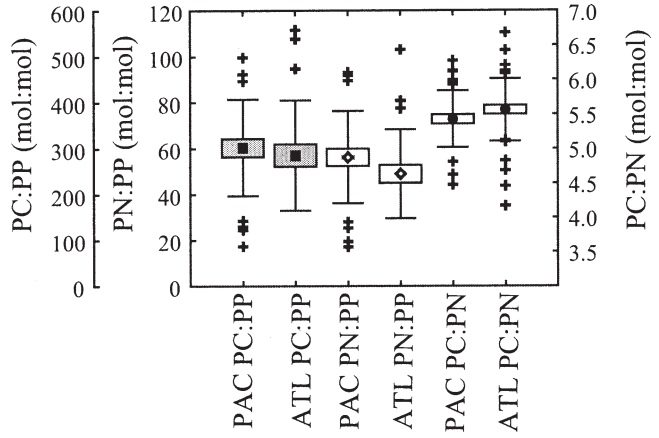

Fig. 9. Box and whisker plot for elemental stoichiometry of natural populations of *Trichodesmium* from this study. Boxes represent the mean ± standard error. Whiskers indicate standard deviation. Plus signs indicate extreme values, i.e., those data points falling outside of the upper or lower box value ± 6 SE.

ometry may also be driven by the consumption and subsequent storage of P in excess of the requirements for growth. In our culture experiments, P-restricted cells (P_Q = 0.03 ± 0.003 ng P filament^{-1}) transferred from medium with minimal DIP concentrations (70 ± 10 nmol P L^{-1}) increased their P content (P_Q = 0.15 ± 0.01 ng P filament^{-1}) fivefold in a span of 4 d after being exposed to fresh medium containing 500 nmol P L^{-1}. Over this time span, doubling times calculated from net increases in PP filament^{-1} (1.6 d) or PP concentrations alone (1.4 d) were shorter than the doubling times for biomass increase (Chl *a* F_M = 4.2 d, filament mL^{-1} = 4.2 d, PC = 2.0 d, PN = 2.3 d), indicating that P was consumed in excess of the demands for net balanced growth. Luxury consumption is also suggested by the time series of missing P pools observed in the parent batch culture: an unmeasured P pool (P_M) accumulated rapidly as P-limited cells were transferred to fresh media. After DIP was drawn down to minimum concentrations, PM began to be used. By the end of the batch culture incubation period, all P was accounted for. Finally, luxurious P consumption is also indicated by the enhancement of intracellular P quotas observed under dark, P-rich conditions ($C_{96\pm8} : N_{16\pm1} : P_1$). This finding is consistent with the research of Fu et al. (2005), who have shown that light is not a requirement for *Trichodesmium* P uptake.

Much like the extreme minima, such levels of P enrichment are not commonly sampled in the field, whereas only a single sample from our field data set and a few from other field observations (n = 1, Sañudo-Wilhelmy et al. 2001; n = 1 Kustka et al. 2003) conform to Redfield stoichiometry. Further examination of empirical data from other laboratory studies (Table 2) indicates that while *Trichodesmium* can in fact produce particulate matter similar to the typical Redfield ratio of $C_{106} : N_{16} : P$, this requires exposing cells to P concentrations (5–50 μmol P L^{-1}) orders of magnitude greater than what would be observed in the oligotrophic ocean. Following the work of Geider and LaRoche (2002), Kustka et al. (2003) suggest that *Trichodesmium* populations having PN : PP ratios less than 20 mol : mol would be indicative of populations engaging in luxurious P uptake and storage. Given the exceedingly high levels of DIP required to produce particulate matter with PN : PP ratios <20 in the laboratory, coupled with the low frequency of field measurements occurring below this threshold, it appears that either significant luxury consumption and P storage are not common in natural oligotrophic populations or that such P enrichments are infrequently sampled because they are short-lived physiological transients.

Vertical migration and phosphorus mining—The proposed scenario of P acquisition via buoyancy-mediated vertical migration (Villareal and Carpenter 1990) requires that natural populations transit from high-light, P-depleted surface waters to the depths of the phosphocline, acquire P in low-light conditions, and then respire dense cellular material (e.g., carbohydrate) at rates sufficient to allow a return to the euphotic zone where the cycle can begin anew. Such buoyancy-driven vertical migrations may allow this genus to fulfill elemental requirements with nutrients acquired at depth and potentially enhance elemental fluxes into the euphotic zone in the absence of strict physical control. These elemental inputs could become available to the remaining microbial assemblage, thereby inducing a successional pattern in the pelagic microbial community. While our data set does not address the rate of buoyant density change, we are able to address other basic assumptions of the P-mining scenario: dark period phosphorus uptake and long-term dark survival.

Evidence for active dark uptake of inorganic phosphorus by *Trichodesmium* is supported by increases in PP and PP filament^{-1} in dark incubations initiated at variable stages of growth. Measured doubling times of PP in the absence of light were between 1.7 and 2.8 d, while PP-based doubling times in light incubations were on the order of 1.4 to 3.3 d. Measurable dark uptake of P by *Trichodesmium* spp. is also corroborated by other research (Letelier and Karl 1998; Fu et al. 2005). In combination, these results suggest that encounters with the phosphocline could act to supply a substantial portion of the phosphorus required for growth assuming migrating colonies were able to return to the euphotic zone.

Net density loss rates must be sufficient to ensure upward migration in order for P assimilated at depth to be injected into surface waters. In this regard, in regions such as the NPSG, where the depth of the top of the phosphocline is typically greater than 90 m, the process of vertical migration may involve prolonged exposure to dark or low irradiance conditions. To our knowledge, no prior research has been conducted to assess the dark survival capacity of this genus. Our dark incubation experiments indicate that the IMS101 strain can survive in the absence of light for periods of 3–6 d, dependent upon the physiological state of the cell at the point of dark initiation. Populations transferred during early growth having relatively high intracellular P quotas retained the capacity to recover from light deprivation for a period of 3 d of dark exposure, after which time cells did not respond

Table 2. Elemental stoichiometry for cultured and natural populations of *Trichodesmium* spp. Data from batch culture measurements in this study cite the range of values for the minimum and maximum measured P quota (P_Q), where db values are derived from the time range over which cells were able to recover from light limitation (viable). Data from other sources are referenced.

Trichodesmium population/growth conditions	N : P	C : P	C : N	[ng P filament^{-1}]
Minimum P_Q (Batch culture: days 0, 10, and 14) $[PO_4]=0.05\pm0.004$ μmol L^{-1}	84 to 94	531 to 668	6.3 to 7.1	0.03 to 0.07
Maximum P_Q (Batch culture: days 4 and 5) $[PO_4]=0.10\pm0.03$ μmol L^{-1}	30 to 37	257 to 292	7.8 to 8.6	0.13 to 0.15
Maximum P_Q (db-ABC, viable) $[PO_4]=3.8\pm0.23$ μmol L^{-1}	14 to 18	82 to 109	5.3 to 6.8	0.55 to 1.7
IMS101: range of [DOP][1]	4.4 to 156	No data	No data	No data
IMS101: range of [Fe][2]	4.8 to 1.3	55 to 156	8.9 to 16	No data
IMS101 and GBRTRLI101: range of [DIP][3]	14 to 45	94 to 392	Not reported	No data
Lab range	4.4 to 156	55 to 668	5.3 to 16	0.03 to 1.7
NW Atlantic	22 to 103	133 to 558	4.2 to 6.7	No data
North Pacific	17 to 93	87 to 498	4.5 to 6.3	0.02 to 0.44*
Central Atlantic[4]	14 to 62	98 to 416	No data	0.04 to 0.34*
North Australia[2]	21 to 33	120 to 201	5.4 to 7.3	0.06 to 0.45*
North Australia[5]	44 to 87	No data	No data	No data
Gulf of Mexico[5]	65.6 to 66.3	No data	No data	No data
NPSG (Sta. ALOHA)[6]	42 to 52	269 to 330	6.1 to 6.4	0.39 to 0.48
1989 North Pacific Bloom[7]	125†	891†	7.1†	No data
North Australia[8]	14 to 52	79 to 310	No data	No data
NW Atlantic[9]	25 to 182	137 to 943	No data	0.02 to 0.2*
Field range	14 to 182	79 to 943	4.2 to 7.3	0.02 to 0.48

References: (1) Mulholland et al. (2002); (2) Berman-Frank et al. (2001); (3) Fu et al. (2005); (4) Sañudo-Wilhelmy et al. (2001); (5) Villareal and Carpenter (2003); (6) Letelier (1994); (7) Karl et al. (1992); (8) Kustka et al. (2003); (9) intracellular ratios from Sañudo-Wilhelmy et al. (2004).

*Using a conversion factor of 182 filaments per colony.

†Values for bulk particulate matter collected within a *Trichodesmium* bloom.

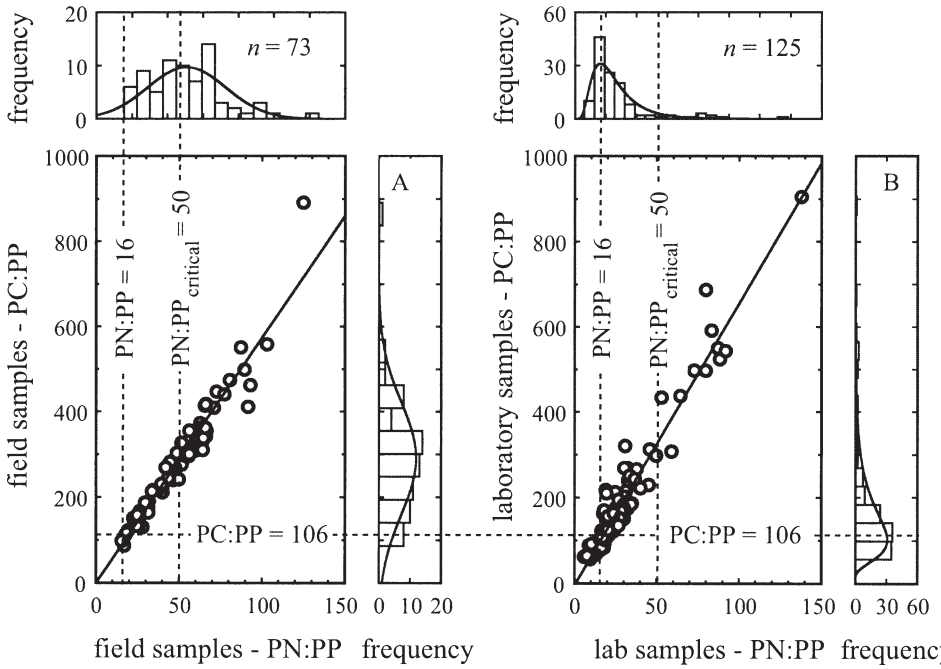

Fig. 10. PN : PP versus PC : PP for field and laboratory data sets from this study. Dotted lines show Redfield PC : PP (106), PN : PP (16) stoichiometry, and the critical PN : PP ratio ($PN : PP_{critical} = 50$). Histograms for each axis are also shown. Field data conform to a normal distribution [*p* values for the Kolomogorov-Smirnov and Lilliefors tests for normality (Zar 1999) are not significant at the 0.05 level]. Laboratory data adhere to a log normal distribution.

to light (Fig. 6A, Table 1). In contrast, samples isolated from P-restricted growth remained viable for 5–6 d (Fig. 6B–C, Table 1). If we speculate that this coastal isolate is representative of *Trichodesmium* in blue water, our results indicate that migrating populations would be able to persist for a minimum period of 3 to 6 d in total darkness and perhaps longer in dim light.

The ability to survive and consume P, in dark, nutrient-rich environments, at rates sufficient to support growth does not confirm vertical migration as a source of P to the euphotic zone, but rather implies that there is potential for this process to occur. The actual feasibility of phosphocline-scale migrations depends on whether the rate of *Trichodesmium* density change is sufficient to facilitate vertical migration to and from the depth of the phosphocline. Direct determination of colony density, via density centrifugation measurements or some comparable method, along with measures of parameters relevant to Stokes equation (e.g., colony size), would more closely constrain the actual range of vertical velocities achievable by natural populations of *Trichodesmium*. These measures will be crucial to a precise understanding of the potential for phosphocline-scale vertical migration in natural populations. Alternately, vertical migration is likely advantageous because movement alone, whether up or down, may enhance P contact and facilitate increased P uptake relative to nonmotile cells.

Ecological implications—In the oligotrophic biomes of the world's oceans, *Trichodesmium* spp. are ubiquitous and key for the addition of reactive N. For these reasons, and for their role in elemental cycling, there has been a substantial research effort devoted to understanding the ecology and physiology of this genus (*see* Karl et al. 2002 and references therein). Nonetheless, there are still many unanswered questions. In regions such as the NPSG where the system has been hypothesized to be in a period of N_R sequestration and phosphorus control of plankton rate processes (Karl et al. 2001), the physiological plasticity of *Trichodesmium* can have a profound impact on the composition of particulate and dissolved elemental pools and, furthermore, may play a regulatory role in net export of particulate matter from the upper ocean.

In the laboratory portion of this study, we show that *Trichodesmium* is able to grow with significantly reduced intracellular quotas of phosphorus. Conversely, when exposed to P following periods of P restriction, cultured populations appear to engage in luxury consumption of P. In sum, this organism is able to appreciably alter its biochemical composition in response to environmental fluctuations in light and nutrient availability. Comparison of these results to stoichiometric observations of natural populations indicates that the extremes of biochemical composition generated in the laboratory are rarely observed in nature. However, analysis of field data establishes *Trichodesmium* spp. as a genus typically possessing low proportions of intracellular P relative to either C or N. The ability to maintain net productivity with reduced P quotas is clearly an adaptation to the oligotrophic habitat. Given that natural populations only rarely appear to be pushed

toward their physiological limits with respect to any single nutrient, the additional P-sparing potential of this genus could further elevate the N component of dissolved and particulate elemental pools and ultimately intensify or establish ecosystem scale P limitation.

Another strategic feature of *Trichodesmium* physiology is the ability to regulate buoyancy and thereby affect bidirectional migrations in the water column. In order to achieve these migrations, *Trichodesmium* adjust their internal concentrations of carbohydrate reserves, and possibly other constituents. The intriguing potential of this process is whether or not these vertical migrations are of amplitudes sufficient to penetrate the phosphocline. If so, *Trichodesmium* would act as vectors for the upward transfer of phosphorus into the euphotic zone, thereby potentially offsetting some proportion of the P limitation incurred by their own diazotrophic production. Data presented in this study suggest that if these populations were to reach the phosphocline, P-uptake rates in the dark would be sufficient to support accumulation of P. Moreover, we present initial data detailing the dark survival capacity of this genus, indicating that migrating populations would be able to sustain light deprivation for periods of 3–6 d. It is acknowledged that these results address corollaries of the P-mining scenario. Confirmation of P mining should be verified by direct determination of the rate of cell density change in field populations.

Our research findings are in line with a growing body of literature illustrating deviations from static estimates of the elemental composition of marine particulate matter (Falkowski 2000; Sterner and Elser 2002; Bertilsson et al. 2003; and others). Assumptions of fixed elemental ratios such as the canonical Redfield ratio are widespread, since they allow us to conceptualize a rigid relationship between nutrient supply and the productivity of marine ecosystems. The perception of inflexible elemental stoichiometry, however, limits our ability to predict ecosystem responses to environmental variability, a key challenge in contemporary oceanography.

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