NITROGEN FIXATION RATES AND CONTROLS AT STATION ALOHA

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By
Marcie N. W. Grabowski

Thesis Committee:

David Karl, Chairperson
Matthew Church
Michael Rappé

We certify that we have read this thesis and that, in our opinion, it is satisfactory in scope and quality as a thesis for the degree of Master of Science in Oceanography.

THESIS COMMITTEE

Chairperson

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ABSTRACT

Temporal and depth variability in rates of dinitrogen (N_2) fixation at Stn. ALOHA, an oligotrophic site in the North Pacific Subtropical Gyre (NPSG), were assessed with direct measurements and using an isotope mass balance model based on the particulate nitrogen (PN) export flux during the period April 2004 – March 2005. Measured N_2 fixation rates and the implied large fraction of new production supported by diazotrophy emphasize the importance of N_2 fixation in nutrient dynamics in the NPSG. Nearly all (95%) N_2 fixation in the euphotic zone (0 – 125 m) occurred in the upper 75 m and was mostly supported (57 – 76%) by diazotrophs less than 10 μm in diameter. Over the year long period of observation, N_2 fixation varied with measured soluble reactive phosphorus (SRP) inventories and previously documented seasonality of iron (Fe) delivery to the North Pacific. High surface diazotroph activity was observed in July – August 2004 (1.63 – 1.68 μmol N m^{-3} d^{-1}) and low N_2 fixation rates occurred September – November 2004 (0.38 – 0.68 μmol N m^{-3} d^{-1}). The dependence of diazotrophy on available Fe and phosphorus (P) was further explored using manipulation experiments which resulted in enhanced N_2 fixation rates upon addition of Fe and/or P. Occasional Fe and/or P limitation on diazotroph activity may result in a net decrease in N_2 fixation at Stn. ALOHA. The potential decreasing contribution of N_2 fixation to new production in the NPSG is suggested by the observed increase in $\delta^{15}N$ of exported PN during July 2001 – November 2004.

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INTRODUCTION

Marine Nitrogen Cycle

Oceans are responsible for one-half of the photosynthesis occurring on Earth (Field et al., 1998) and most of this production is supported in open-ocean environments such as subtropical gyres (Martin et al., 1987; Karl et al., 1996). Subtropical gyres comprise a significant portion, almost 40%, of the Earth's surface (Longhurst, 1998). What controls marine production, in the geologic past as well as currently, is an important and heavily debated question (Ryther and Dunstan, 1971; Schindler, 1977; Falkowski, 1997; Tyrrell, 1999).

Nitrogen (N) availability in the marine environment plays an important role in biological productivity. The N cycle involves an intricate set of primarily microbiologically-mediated processes. Over long time-scales (millennia), denitrification, which ultimately removes bioavailable N, and dinitrogen (N_2) fixation, which adds bioavailable N to the global ocean, are in overall balance (Karl et al., 2002; Gruber, 2004). Small perturbations to this balance impact the exchange of carbon dioxide (CO_2) between the ocean and atmosphere (Falkowski, 1997). Inorganic fixed N species, nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+) are in short supply (<30 nM) in the North Pacific Subtropical Gyre (NPSG) surface waters, while dissolved organic N is present at orders of magnitude greater concentration (5-6 μM) but may be only partially bioavailable. Dissolved N_2, however, is extremely abundant (400 μM; Emerson et al., 2002) throughout the water column and provides a nearly inexhaustible source of nitrogen for microbes capable of using it.

Evidence for and Importance of Nitrogen Fixation

In N limited systems, "new" production, in contrast to "regenerated" production, is fueled by allochthonous inputs of N such as upwelled NO_3^- originating below the *nitracline* or newly fixed N via N_2 fixation (Dugdale and Goering, 1967). In addition to enhancing productivity, N_2 fixation can impact the decoupling of carbon (C), N, and phosphorus (P) cycles, the C:N:P stoichiometry of exported organic matter, and net C sequestration to the deep sea. Evidence for the contribution of N_2 fixation in the global marine environment includes geochemical tracers such as natural ^{15}N isotope balance (Saino and Hattori, 1980; Saino and Hattori, 1987; Karl et al., 1997; Dore et al., 2002), anomalous N to P ratios (Michaels et al., 1996; Gruber and Sarmiento, 1997; Deutsch et al., 2001), dissolved inorganic carbon (DIC) drawdown in the absence of NO_3^- (Lee et al., 2002), and P drawdown in the NPSG throughout the past decade (Karl and Tien, 1997; Karl et al., 1997). Distribution and quantification of nitrogenase activity by *Trichodesmium*, a filamentous cyanobacterium, (Carpenter, 1973; Mague et al., 1974) and the discovery of widespread genetic potential for N_2 fixation (Zehr et al., 1998; Zehr et al., 2001) provide additional substantiation for the importance of N_2 fixation in the global marine N cycle.

The activity of diazotrophs has been shown to contribute new N comparable to that delivered via vertical advection of NO_3^- in oligotrophic oceans (Karl et al., 1992; Capone et al., 1997; Karl et al., 1997; Dore et al., 2002; Montoya et al., 2004). Using isotope mass balance and N:P stoichiometry, Karl et al. (1997) suggested that *Trichodesmium* supported one-quarter to one-half of the new production at Stn. ALOHA. Due to its significant impact on new production, *Trichodesmium* activity has important

implications for local C, N, and P transport and cycling (Letelier and Karl, 1998). With the discovery of new unicellular diazotrophs using molecular techniques, Zehr and others (1998) reinforced the value of quantifying rates and controls of whole community assemblages. As with *Trichodesmium*, N_2 fixation by small (<10 μm diameter), possibly unicellular organisms has proved to support a significant fraction of new production in the oligotrophic Pacific Ocean (Dore et al., 2002; Montoya et al., 2004). Cyanobacterial symbioses involving diatoms may also be a significant source of newly fixed N (Villareal, 1991; Karl et al., 2003).

Most information illustrating the significant spatial and temporal variability in global N_2 fixation has been gleaned from studies of *Trichodesmium*. For example, *Trichodesmium* abundance and activity generally appear highest in warm (>20°C), oligotrophic water that is highly stratified with low wind speed (<10 knots) (Carpenter and Romans, 1991). High surface concentrations of *Trichodesmium* have been detected remotely using Earth-orbiting satellites due to their specific reflectance properties resulting from possession of the accessory pigment, phycoerythrin, and gas vesicles (Subramaniam et al., 1999; Hood et al., 2002; Subramaniam et al., 2002; Capone and Subramaniam, 2005). In addition, considerable depth variation has been observed in measured N_2 fixation rates throughout the euphotic zone (Dore et al., 2002; Montoya et al., 2004). Although few studies have been able to substantially explain temporal variability of diazotroph activity, this variability may be linked to habitat seasonality or the aperiodic delivery of nutrients (iron (Fe) or P) (Karl et al., 1997).

Controls on Nitrogen Fixation Rates and Ecological Implications

In addition to quantifying N_2 fixation rates by various size classes of organisms, it is imperative to understand the rate controlling factors. Fe limitation of photosynthesis has been determined experimentally in high-nutrient, low-chlorophyll regions (Martin et al., 1994; Kolber et al., 1994; Coale et al., 1996). However, due to high Fe concentrations required by diazotrophs (Kustka et al., 2002; Kustka et al., 2003), Fe may play an even greater role in limiting N_2 fixation in low-nutrient, low-chlorophyll regions. Fe and P have been found to co-limit N_2 fixation in the Atlantic Ocean (Mills et al., 2004). In the NPSG, alternation between N and P (or P/Fe) limitation has been hypothesized to control production, export, and associated community structure and elemental stoichiometry (Karl, 2002). By determining the extent to which Fe and/or P control N_2 fixation at Station ALOHA, it may be possible to deconvolute the role of diazotrophy in controlling nutrient dynamics in the NPSG.

This study provides N_2 fixation rates during an annual cycle (April 2004 – March 2005) and an assessment of temporal surface variability and vertical structure of diazotroph activity at Stn. ALOHA. Potential rate limiting factors were identified using manipulation experiments involving P and Fe performed throughout the one-year study period. In addition, the relative contribution to new N from two sources, N_2 fixation and NO_3^-, was calculated using data on particulate N (PN) mass export and isotopic composition, and a model (Karl et al., 2002). This study provides evidence for the continued presence but possible decreasing contribution of N_2 fixation at Stn. ALOHA, a site representative of the oligotrophic NPSG.

METHODS

Station Location and Sampling Protocols

All sampling and measurements were performed in conjunction with the Hawaii Ocean Time-series (HOT) program at Stn. ALOHA (A Long-term Oligotrophic Habitat Assessment) in the NPSG. Stn. ALOHA (22°45'N, 158°W) is representative of the oligotrophic open ocean of the NPSG (Karl and Winn, 1991; Karl, 1995; Karl and Lukas, 1996). Since October 1988, monthly sampling of relevant biogeochemical and physical parameters has been performed. Water column sampling was conducted using a Sea-Bird CTD (conductivity, temperature, depth) sensor and 24 position pylon, aluminum-framed rosette sampler equipped with 12-liter PVC bottles. The mixed layer depth (MLD) is defined in this study as the depth at which potential density of the water exceeds that of the surface by 0.125 g cm^{-3}. Samples for this study were taken on HOT 158-159 (April – May 2004), HOT 161-165 (July – November 2004), and HOT 167-168 (February – March 2005 which cover one annual cycle from April 2004 to March 2005.

Nitrogen Fixation Rate Measurements and Manipulations

In this study, the ^{15}N isotopic tracer method (Montoya et al., 1996; Capone and Montoya, 2001) was used to measure N_2 fixation rates in the upper 125 m and the effects of P and Fe additions on N_2 fixation rates in surface water from Stn. ALOHA. Gross primary production at Stn. ALOHA can exceed net production by a factor of 2 – 3 (Juranek et al., 2002); if gross N_2 fixation scales with primary production, reported diazotrophy may underestimate gross N_2 fixation by a factor of 2 – 3. During the majority of cruises in this study, HOT 158-159 and HOT 161-165, whole water samples (~4 l, no pre-screening) were incubated in 4.7 liter acid-washed Tedlar® gas sampling

bags fitted with septa ports. Sampling bags were filled and manipulated so as to remove any trapped air and sealed with Teflon-backed septa. For HOT 167-168 4.5-l polycarbonate bottles were filled to overflowing with whole water samples and sealed using caps that included thick, silicone septa. Exactly 1 ml of $^{15}N_2$ gas (99%, Isotech) was injected into each sample bag or bottle using a gastight syringe with a fixed stainless steel needle. For depth profiles, samples were stored in shaded chambers with the appropriate temperature and light quality and quantity in a three-chamber on-deck incubator. Surface samples were incubated for 24 hours in an on-deck surface chamber with circulating seawater.

During May, July, August, October, November, January and March cruises manipulation experiments were performed with surface seawater to determine the effect of nutrient enrichment on N_2 fixation rates. Potassium phosphate (K_2HPO_4 source) was added to sample volumes at the start of ^{15}N-spiked incubations resulting in variable final dissolved P concentrations (170 – 361 nM) during all the above mentioned cruises. Single addition experiments were also performed with iron chloride ($FeCl_3$ source) (2 nM Fe addition) on the November, January and March cruises. Additions of Fe and P identical to those reported above were combined in a third set of incubations during these same three cruises. All manipulation experiments were carried out with parallel, unamended water as controls for comparison of N_2 fixation rates. To determine the diazotrophic response to longer-term incubation with amendments, nutrient additions were made at time zero to the March manipulation experiments and one bottle of each treatment was harvested after a 24-hour incubation with ^{15}N tracer for three consecutive days.

In an attempt to decipher diel variability of N_2 fixation in surface seawater at Stn. ALOHA (Church et al., 2005), a set of experiments was designed in which 12 water samples were injected with $^{15}N_2$ at the start of incubation and triplicate bottles were harvested every 12 hours over the course of two days.

With the exception of samples in the diel experiments, incubated samples were removed and filtered 24 hours after the $^{15}N_2$ addition. Post-incubation, samples from selected profiles and manipulation experiments were size fractionated to determine the contribution of greater than and less than 10 μm diameter diazotrophs to the net N_2 fixed during incubation. Due to the nature of sampling (i.e., rosette sampler and bottle incubations) large diazotrophs, such as *Trichodesmium* colonies or large, aggregate-forming diatoms with endosymbiotic N_2-fixing cyanobacteria, may be excluded. Known sample volumes were pressure filtered through pre-combusted GF/F filters, which were then stored frozen until preparation for analysis on the mass spectrometer.

Filters were dried at 60°C and pelleted for analysis using a Carlo-Erba Elemental Analyzer NC2500 interfaced with a Finnigan delta S ion ratio-monitoring mass spectrometer via a Finnigan ConFlow-II to determine particulate nitrogen (PN) mass and stable isotopic composition of the particulate nitrogen (δ^{15}N-PN). All nitrogen isotopic data are presented in standard delta notation.

$$\delta^{15}N_{sample} = \left\{ \left[\frac{(^{15}N/^{14}N)_{sample}}{(^{15}N/^{14}N)_{standard}} \right] - 1 \right\} * 1{,}000$$

Atmospheric N_2 gas is the reference standard ($^{15}N/^{14}N = 0.0036765$, $\delta^{15}N \equiv 0$ ‰). N_2 fixation rates were determined from PN mass and δ^{15}N-PN of incubated samples, the δ^{15}N-PN of unincubated samples, pre-existing dissolved N_2 concentration (Weiss, 1970),

and atom percent enrichment due to the addition of $^{15}N_2$ (Montoya et al., 1996). Unincubated samples represent natural abundance PN concentration and δ^{15}N-PN of the depth sampled. The detection limit of this assay with the reported volume used (4 – 4.5 l) is 0.03 nmol N l^{-1} d^{-1}. It is prudent to note that previously observed N_2 supersaturation of 0.3 – 2.6 % at Stn. ALOHA (Emerson et al., 2002), which is not accounted for in the presented rate measurements, would increase calculated N_2 fixation rates relative to the reported values by 2 – 3%.

All reported percentage increases are calculated as the increase in N_2 fixation rate of the treated samples above that of the control treatment as a percent of the control N_2 fixation rate. For example:

$$\% \text{ increase} = ((N_2 \text{ fixation})_{\text{treatment}} - (N_2 \text{ fixation})_{\text{control}}) / (N_2 \text{ fixation})_{\text{control}})$$

Sediment Trap Particulate Nitrogen Flux and ^{15}N Isotopic Composition

As one component of the core measurements, floating sediment traps (150 m) are deployed during each HOT cruise to obtain sinking PN mass flux and isotopic signature of the export production. The material collected in these traps is pre-screened through 333-μm Nitex mesh to exclude zooplankton "swimmers" prior to filtration onto a combusted GF/F. These sample filters were analyzed on the mass spectrometer as describe above.

Phosphorus Determination

Seawater was collected in acid-washed, high density polyethylene bottles from known depths using the CTD-rosette sampler. Samples were stored frozen until analyzed for soluble reactive phosphorus (SRP) using the MAGnesium Induced Coprecipitation (MAGIC) method (Karl and Tien, 1992) and measured

spectrophotometrically on a Beckman DU 640 (HOT protocols: http://hahana.soest.hawaii.edu). The MAGIC SRP measurement corrects for arsenate interference of the molybdenum blue colorimetric procedure (Johnson, 1971) and enhances both the sensitivity (detection limit ~ 1 nmol P l^{-1}) and the precision of the procedure.

Phycoerythrin

The concentration of the water-soluble accessory pigment phycoerythrin was determined in 3 size classes (>10 μm, 5.0 – 10 μm, and 0.4 – 5.0 μm) on each cruise. For these measurements, samples were pressure filtered sequentially through 10-μm Nitex® mesh followed by a 5.0 μm polycarbonate filter. A portion of the filtrate (1 l) was vacuum-filtered onto 0.4 μm polycarbonate filter. Each filter was placed in a 20 ml glass scintillation vial containing equal volumes of seawater and glycerol. Samples were placed on a shaker table to dislodge particles from the filter. Phycoerythrin fluorescence was measured on a Turner Designs TD700 fluorometer calibrated using commercial B-phycoerythrin standard (Cyanotech Corporation) with 544 nm (excitation) and 577 nm (emission) wavelength filters.

Microscopy

Samples collected for microscopy-based enumeration of phycoerythrin-containing cells were fixed with 2% formalin. Various sample volumes (100 – 300 ml) were vacuum-filtered onto black polycarbonate filters (0.22 μm) to achieve optimal cell density (5 – 20 cells per field enumerated). Two categories of phycoerythrin-containing cyanobacteria were enumerated (at 100 x) under an excitation wavelength of 510-560 nm: *Synechococcus* and *Crocosphaera*-like (3 – 8 μm diameter, coccoid cells).

Trichodesmium and free-living *Richelia* abundances were also recorded when observed. *Richelia*-diatom associations were not observed in any sample which may due to the low volume processed relative to cell density.

Statistics Used

Differences in the response to nutrient amendment, in whole water and the <10 μm size fraction, were analyzed using a one-way analysis of variance (ANOVA) with Tukey's post-hoc test. Linear regressions were used to assess correlations. All tests were performed using 95% probability. Statistical analyses were performed using either Excel (Microsoft, Inc.) or MINITAB Statistical Software (MINITAB, Inc.).

RESULTS

Depth Distribution and Temporal Variability of N_2 Fixation Measurements

Depth profiles of diazotroph activity from November 2004, February 2005 and March 2005 reveal significant variability within the euphotic zone. In each profile, highest activity was in the surface and decreased to below detection (detection limit = 0.03 μmol N m^{-3} d^{-1}) by about 75 m (Figure 1). The cumulative contribution of N_2 fixation as a function of depth also illustrates that euphotic zone diazotrophy was surface dominated (Figure 2). At any depth sampled within the upper 75 m, the small (<10 μm) diazotrophs were calculated to support between 31-100% (mean = 64%, SE = 5%) of the N_2 fixation (Table 1). Depth profiles indicate that, throughout the water column, the lowest N_2 fixation rates occurred in November 2004, and dramatically higher rates occurred in March 2005. In these depth profiles, depth trends of the N_2 fixation rates of small (<10 μm) diazotrophs do not parallel size-fractionated phycoerythrin concentrations (0.4 - 5.0 μm and 5.0 - 10.0 μm) or *Crocosphaera*-like cell counts (Figures 1 and 3). This observation suggests that 1) all diazotrophs may not contain phycoerythrin and 2) *Crocosphaera*-like cells may not be the dominant N_2 fixers.

Surface N_2 fixation rates were determined in whole water over the course of the sampling period (April 2004 – March 2005) (Figure 4). High diazotroph activity was observed in July and August of 2004 (1.63 - 1.68 μmol N m^{-3} d^{-1}) and the maximum rate measured occurred in March 2005, with an average rate of 2.56 μmol N m^{-3} d^{-1}. Consistently low N_2 fixation rates (0.38 - 0.68 μmol N m^{-3} d^{-1}) were observed during September – November 2004, with the lowest average rate in October. The most striking temporal increase in surface N_2 fixation rates occurred between February (0.99 μmol N

m^{-3} d^{-1}) and March (2.56 μmol N m^{-3} d^{-1}). Surface N_2 fixation rates during this 1-year study period do not correlate with any one size-fraction of phycoerythrin (PE) concentration (0.4 - 5.0 μm, 5.0 - 10.0 μm, or >10.0 μm) (Figure 5). These data indicate that 1) not all diazotrophs contain phycoerythrin, as mentioned previously, and 2) one size class of phycoerythrin-containing diazotrophs may not always dominate N_2 fixation rates.

Depth integrated (0 – 125 m) N_2 fixation rates reveal that more than 95% of N_2 fixation occurred in the upper 75 m for each time period sampled (Figure 2). Depth integrated N_2 fixation systematically increased 5.8 fold for the <10 μm diazotrophs (14.5 to 83.7 μmol N m^{-2} d^{-1}) and 3.8 fold for the >10 μm size class (6.9 to 26.3 μmol N m^{-2} d^{-1}) from November 2004 to March 2005 (integrated whole water rates increased from 20.2 to 109 μmol N m^{-2} d^{-1} over the same period) (Table 1). Depth integrated rates indicate that 57-76% of N_2 fixation is supported by the <10 um fraction with no systematic temporal increase in their contribution between the 3 profiles.

Geochemical Indicators of N_2 Fixation

Between April 2004 and March 2005, suspended δ^{15}N-PN from the surface varied from a minimum of -1.44 ‰ and a maximum of 2.10 ‰ (Figure 4). During August through October, the most depleted surface suspended δ^{15}N-PN values (-1.44 to -0.5 ‰) were observed. The heaviest δ^{15}N-PN (2.10‰) was observed in November.

Exported material is representative of new production under steady-state conditions (Eppley and Peterson, 1979) and therefore the isotopic characteristic of sinking PN should reflect the relative contributions of N_2 fixation and the upward diffusion of NO_3^-, two of the potential sources of new N to oligotrophic oceans.

Sediment trap data routinely obtained on HOT cruises overlaps in time N_2 fixation rates presented here through November 2004. Maximum and minimum PN flux was 390 μmol N m^{-2} d^{-1} and 206 μmol N m^{-2} d^{-1}, respectively (Figure 6). No consistent pattern was observed in this time-series with the two extrema occurring only two months apart, with the maximum in June followed by the August minimum. The stable N isotopic composition during this time varied between 2.45 ‰ (April 2004) and 4.7 ‰ (May 2004) with a decreasing trend ($r^2 = 0.45$) between PN mass flux and $\delta^{15}N$ of the sinking material. Since July 1989, the exported PN has displayed variable trends in the stable-N isotopic composition (Figure 7). During that time, exported PN $\delta^{15}N$ values fluctuated between -1.17 ‰ and 6.89 ‰. A decreasing trend in the isotopic composition of sinking PN was observed for the period of July 1989 – December 2002. This is in contrast to the subsequent period (December 2002 – November 2004), during which the $\delta^{15}N$ of exported PN sharply increased.

The comparative importance of N_2 fixation and NO_3^- to exported N can be approximated using the N isotopic signature of atmospheric N_2 ($\delta^{15}N = 0$ ‰), NO_3^- ($\delta^{15}N = 6.5$ ‰) (Karl et al., 1997), and sinking PN isotopic composition ($\delta^{15}N$-F_{PN}) and mass flux of sinking PN (F_{PN}) collected in sediment traps below the euphotic zone. Dore and others (2002) used the following two equations derived from Wada and Hattori (1991) to calculate the fraction of export PN (f) attributable to N_2 fixation ($F_{PN}[N_2]$)and NO_3^- ($F_{PN}[NO_3^-]$).

$$F_{PN}[N_2] = f * F_{PN} = F_{PN} * (1 - (\delta^{15}N - F_{PN}/6.5))$$

$$F_{PN}[NO_3^-] = (1-f) * F_{PN} = F_{PN} * (\delta^{15}N - F_{PN}/6.5)$$

Neither $F_{PN}[N_2]$ nor $F_{PN}[NO_3^-]$ displayed obvious seasonal patterns between April and November 2004 when separated in this manner (Figure 8). Little variability was observed in $F_{PN}[NO_3^-]$, with calculated values ranging from 109 to 174 umol m^{-2} d^{-1}. However, substantial variability, similar in magnitude to that exhibited in F_{PN}, was observed in $F_{PN}[N_2]$ (65 – 220 umol m^{-2} d^{-1}). The temporal changes in total PN flux seem to be more dependent on new N from diazotrophic activity than NO_3^- injection, because $F_{PN}[N_2]$ is highly and significantly correlated with F_{PN} (r^2=0.85, $p < 0.05$) whereas, $F_{PN}[NO_3^-]$ is not ($r^2 = 0.22$, $p > 0.05$). Monthly averages of $\delta^{15}N$- F_{PN} between July 2001 and November 2004 also showed irregular variability. In this 3.5-yr data set, N_2 fixation and NO_3^- input appeared to contribute equally to the temporal variability of F_{PN}, with $F_{PN}[N_2]$ and $F_{PN}[NO_3^-]$ both showing good correlation with total N flux ($r^2 = 0.73$, $p <$ 0.05; and $r^2 = 0.65$, $p < 0.05$ respectively) (Figure 9).

Soluble Reactive Phosphorus Variability

The temporal variability in surface SRP concentrations were similar to that observed in N_2 fixation rates between April 2004 and March 2005 (Figure 10). Highest recorded SRP concentrations during this time precede a two-month period of relatively high N_2 fixation rates in the surface water. The lowest surface SRP concentrations, occurring in October and November 2004 (6.1 – 9.9 nM), are concurrent with the lowest measured diazotroph activity. SRP turnover time during this 1-year study period varied from 2.1 – 14.8 days (Table 2). There is a moderate correlation between SRP turnover time and surface N_2 fixation rates ($r^2 = 0.39$, $p > 0.05$).

Nutrient Addition Experiments

To determine the effect of P addition on N_2 fixation rates, experiments were performed on seven of the nine cruises included in this study. The diazotroph response to P enrichment varied between cruises (Table 3). No increase in N_2 fixation rates relative to control treatments was observed with the addition of 157 – 277 nM P (representing a P increase of 140 – 1520 % relative to ambient) during May, July, August and November 2004, and March 2005. For November 2004 and March 2005 cruises, all samples were size-fractionated post-incubation. Neither whole water nor the <10 μm size-class showed enhanced N_2 fixation rates with amendment during those 2 sampling occasions. During October 2004, glucose was added in addition to P to observe the response of *Proteobacteria*, presumed heterotrophic, diazotrophs (Zehr et al., 2001; Church et al., 2005). While no response to the addition of glucose alone was measured, a positive response to the glucose and P (174 nM P added) treatment was observed (87% increase above control) suggesting the increase in N_2 fixation rate was due to P addition. Although this increase was not statistically significant at the 90% confidence interval ($z = 0.81$, $z_{crit\text{-}one\ tail} = 1.28$, $z_{crit\text{-}two\ tail} = 1.64$), a rate increase of 87% is substantial. During February 2005, P addition (160 nM) resulted in a large increase in diazotrophic activity relative to control treatments in both whole water (64% increase, not significant as tested by ANOVA) and the <10 μm diazotrophs (34% increase, not significant as tested by ANOVA) (Figure 11). A subsequent experiment (March 2005) in which variable concentrations of P were added (160 and 320 nM P) revealed an increase in N_2 fixation only in the higher P treatment in the whole water samples (by 30% above control) over the first day of incubation (Figure 12). In the <10 μm fraction, however, additions of 160

nM P and 320 nM P each resulted in the same relative increase of 21% during the first day. Due to the lack of replication at each time point, no statistical significance could be assigned. Over the course of this 3 day experiment, the average N_2 fixation rate at each time point decreased dramatically in whole water (3.1 to 0.5 μmol N m^{-3} d^{-1}) and size-fractionated (<10 μm) samples (2.7 to 0.5 μmol N m^{-3} d^{-1}) for all treatments (control, P (160 nM and 320 nM), Fe, and Fe/P), representing an average decrease of 85% (whole water) and 82% (<10 μm fraction) relative to respective initial measurements.

On the November, February and March cruises, Fe addition experiments were conducted. No significant increase in N_2 fixation rate relative to control incubations was observed in Fe (2.0 nM added) or combination Fe/P (2.0 nM Fe and 160 nM P added) treatments in whole water or the <10 μm size-fraction during November. However, identical manipulations in February revealed significantly enhanced N_2 fixation in whole water and the <10 μm size-class for both Fe and Fe/P treatments relative to control incubations (Figure 11). Whole water incubations amended with Fe showed significant increases in N_2 fixation rates, averaging 128% above control treatments (ANOVA, $p < 0.05$). Additions of Fe/P to whole water also resulted in statistically significant increases of 142% (ANOVA, $p < 0.05$). Diazotroph activity by small (<10 μm) organisms increased significantly 86% and 100% with addition of Fe and Fe/P, respectively (ANOVA, $p < 0.05$) . In the first 24 hours of the March enrichment experiment, whole community and <10 μm diazotrophs showed increased N_2 fixation rates with Fe addition (32% and 37%, respectively), and no increase with Fe/P addition (Figure 12). As mentioned previously, there is a striking decline in N_2 fixation in all treatments during this three-day time-course. Several possibilities exist for this observed decline, including

nutrient depletion to the detriment of N_2 fixation or potentially this lengthy incubation captured the crash of a bloom.

Diel Experiments

Temporal variation was observed in incubations sustained for two days and sampled every 12 hours during the November and February cruises. During the November experiment, N_2 fixation was constant for the first 24 hours, followed by small increases by 36 hours in both the whole water samples and the <10 um size-fraction (Figure 13). Whole water diazotroph activity was constant (~0.8 μmol N m^{-3} d^{-1}) for the duration of the incubation. N_2 fixation by small diazotrophs decreased by the end of day two (0.15 μmol N m^{-3} d^{-1}) relative to initial rate measurements (~0.5 μmol N m^{-3} d^{-1}). In contrast to the November results, parallel data from February showed a distinct pattern that can be linked to light-dark exposure times. In the latter experiment, N_2 fixation rates were initially low (0.34 μmol N m^{-3} d^{-1}) then reached their maxima of ~1.8 μmol N m^{-3} d^{-1} (whole water) and ~1.5 μmol N m^{-3} d^{-1} (<10 μm) within the first 24 hours (Figure 14). The subsequent two time points repeat this pattern: with greater exposure to dark, N_2 fixation rates are reduced compared to those samples with greater exposure to light.

DISCUSSION

N_2 Fixation, Fe and P Dynamics

In each depth profile, highest N_2 fixation is present at the surface and decreases to below the detection limit (detection limit = 0.03 μmol N m^{-3} d^{-1}) by about 75 m, with 95% of total diazotrophy taking place in the upper euphotic zone (< 75 m) (Figures 1 and 2). This depth dependence is consistent with the high energy requirement of diazotrophs and the increase of bioavailable N with depth which selects against diazotrophs. Given that diazotrophs demand 7-11 times more Fe than phytoplankton supported by ammonium (Kustka et al., 2003), the observed decrease in diazotrophy with depth is expected due to the decline in Fe concentration with depth within the euphotic zone at Stn. ALOHA (Boyle et al., 2005). The dependence of diazotrophy on Fe availability is highlighted in the February 2005 manipulation experiments in which N_2 fixation increases 128% and 142% for whole water and 86% and 100% above corresponding controls for small (<10 μm) organisms upon addition of Fe and Fe/P, respectively. N_2 fixation rates of the whole community and <10 μm diazotrophs also increased in March during a subsequent Fe enrichment experiment (32% and 37% above respective controls).

Although *Trichodesmium* has been viewed by some as the dominant diazotroph in the open ocean (Capone et al., 1997; Carpenter and Romans, 1991), at any depth sampled within the upper 75 m, size-fractionated N_2 fixation rates in this study show small (<10 μm) diazotrophs support 30-100% (mean = 64%, SE = 5%) of the whole water N_2 fixation (Table 1). This is a considerably larger fraction than the average N_2 fixation supported by <10 μm diazotrophs during 2000 – 2001 when the mean was 47% (SE = 8%) (Dore et al., 2002). Together these results suggest that smaller diazotrophs have

become more dominant or more active at Stn. ALOHA. Such changes in community structure have large implications for the N cycle and net C sequestration as small cells do not sink as readily as large cells. Reduced sinking would result in decreased strength of the N_2 fixation-fueled C pump and therefore decreased C sequestration. As hypothesized by Karl et al. (2001), a downward shift in the planktonic size structure would also lead to a reduction of C supply to higher trophic levels and a longer residence time for C, N and P in the euphotic zone due to increased dissolved organic matter (DOM) production. Indeed a significant increase in DOM inventory at Stn. ALOHA from 1989 to 1999 has been observed (Church et al., 2002). While a climate-forced increase in ocean stability may explain the population shift and organic matter accumulation, this "miniaturization" of diazotrophs may also be a response to nutrient (such as Fe or P) limitation. Insufficient data are available to determine the relation between PE concentration (>10 μm) and large diazotroph abundance. However, the data presented in this study suggest that PE concentrations may not directly reflect <10 μm N_2 fixation rates (Figures 1 and 3) because 1) diazotrophic activity may not scale with biomass and 2) not all N_2 fixers contain phycoerythrin.

N_2 fixation rates presented here agree well with previous observations. During November 2004 – March 2005, areal diazotroph activities in whole water and for small (<10 μm) organisms are similar to other published rates from the North Pacific (Table 4). N_2 fixation rates presented here from Stn. ALOHA (whole water and <10 μm) are also comparable to those observed in the tropical North Atlantic (Mahaffey et al., *in press*). In addition, the temporal trend of N_2 fixation at Stn. ALOHA for the period of April 2004 – March 2005 is consistent with previously documented seasonality in which higher N_2

fixation took place in the spring and summer months and lower activity was observed during winter (Dore et al., 2002). The natural variation in the abundance of the two N isotopes, ^{14}N (99.64% by atoms) and ^{15}N (0.36% by atoms), can also be used to examine temporal variability in processes involved in the marine N cycle. For example, N_2 fixation affects PN by adding largely ^{14}N (dissolved atmospheric N_2) and therefore "lightening" the subsequent particulate matter isotopic composition. Surface N_2 fixation was high in July and August 2004 (1.63 - 1.68 μmol N m^{-3} d^{-1}) followed by the most depleted surface suspended $\delta^{15}N$-PN (-1.44 to -0.5 ‰) from August through October 2004. Consistently low N_2 fixation rates were observed from September through November (0.38 - 0.68 μmol N m^{-3} d^{-1}) coincident with the heaviest recorded $\delta^{15}N$-PN of the surface suspended PN (2.10 ‰) (Figure 4). The temporal offset between N_2 fixation and suspended $\delta^{15}N$-PN is consistent with the addition of N_2-derived N into a dynamic pool of PN. Seasonality in suspended $\delta^{15}N$-PN (Figure 4B), which is not reflected in the temporal trend of exported $\delta^{15}N$-PN (Figure 6B), provides further evidence of the dynamic nature of the PN pool. This discrepancy may be a result of accumulation of N_2-derived dissolved organic nitrogen (DON) in the euphotic zone (Church et al., 2002). Anther complication to the PN pool dynamics is the consideration of zooplankton recycling and the subsequent effect on the isotopic composition of PN. Zooplankton will preferentially retain isotopically enriched N as biomass and excrete depleted ammonium (NH_4^+; Checkley and Miller, 1989). However, the light NH_4^+ may be reincorporated into particulate matter resulting in an unknown net effect on the isotopic character of the total PN pool.

N_2 fixation has been shown to contribute new N on the order of that delivered via advection of NO_3^- (Karl et al., 1992; Capone et al., 1997; Karl et al., 1997; Dore et al., 2002; Montoya et al., 2004). This can be assessed with a N budget based on measurements and estimates of N imports to and exports from the euphotic zone at Stn. ALOHA during April 2004 – March 2005 (Table 5). Given the calculated values from Table 5, N_2 fixation may contribute 10 – 46 % of new N to the euphotic zone at Stn. ALOHA. This range is probably a lower limit on the significance of N_2 fixation with respect to new N in the oligotrophic Pacific Ocean because N_2 fixation estimates calculated from 1) *Trichodesmium* N_2 fixation (Karl et al., 1992; Letelier and Karl, 1996) and 2) particulate N export isotope mass balance (this study; Karl et al., 1997) are equal to or greater than the above range. Issues related to undersampling also suggest that this range (10 – 46%) is an underestimate (see text below).

Experimental (Mills et al., 2004) and theoretical (Karl, 2002; Kustka et al., 2002; Kustka et al., 2003) evidence has been presented for the importance of Fe and P as controls on diazotrophy. Euphotic zone DIP declined over the past decade in the NPSG (Karl and Tien, 1997; Karl et al., 2001) and observed diazotroph limitation by P in the Atlantic Ocean (Sanudo-Wilhelmy et al., 2001) emphasize the potential for P control of diazotroph activity at Stn. ALOHA. In addition, Boyle et al. (2005) provide data indicating maximum (March – June) and minimum (July – January) dust delivery of Fe occurs concurrently with our measured N_2 fixation rate variability. Although total dissolved (<0.4 μm) Fe concentrations at Stn. ALOHA (0.2 - 0.8 nM) may not be considered limiting to primary production throughout most of the year (Boyle et al., 2005), the presence of colloidal (0.02 – 0.4 μm) Fe decreases the bioavailability of the

dissolved Fe pool (Wu et al., 2001) and may, therefore, exert control on N_2 fixation. Further, Wu et al. (2001) report that most "dissolved" Fe (80 – 90%) exists as much less bioavailable colloidal Fe rather than bioavailable soluble (<0.02 μm) organic ligand complexes in surface waters near Stn. ALOHA.

There are several lines of evidence that support diazotroph dependence on P and Fe at Stn. ALOHA. First, SRP dynamics indicate that P exerts control on N_2 fixation rates. SRP concentration and N_2 fixation rates are temporally coherent throughout the 1-year study period (Figure 10). Relatively high surface N_2 fixation rates (July and August 2004) occur after the highest observed SRP concentrations and the lowest surface SRP concentrations, occurring in October and November, are concurrent with the lowest measured diazotroph activity. Calculated SRP turnover time during April 2004 – March 2005 varies from 2.1 – 14.8 days (Table 2). The average SRP turnover time (8 d) during that same period is less than one-half of that calculated by Karl and Yanagi (1997) during September 1991 – March 1992 (17 d). Two subsequent studies determined P turnover times, via ^{32}P-uptake experiments, of 12 d during 1996 – 1997 (Björkman et al., 2000) and 6 d during 2000 – 2001 (Björkman and Karl, 2003). This progression to more rapid turnover times is consistent with the observed decrease in DIP at Stn. ALOHA between these study periods and the continued P requirement by diazotrophs. N_2 fixation limited by P is expected to result in faster turnover of the available SRP and enhanced use of dissolved organic phosphorus (DOP). Secondly, nutrient addition experiment results also provide information about the impact of P and Fe availability on diazotrophy. N_2 fixation rate responses to P and Fe addition varied between manipulation experiments (Table 3, Figures 11 and 12) which may be due to a combination or alternation of Fe and P

limitation at Stn. ALOHA. Manipulations performed in February 2005 indicated that N_2 fixation rates increased more with Fe amendment (significant increase, ANOVA, $p < 0.05$) than with P addition (increase not significant, ANOVA) for whole water and the <10 μm size class (Figure 11). However, a combination of Fe and P addition produced an increase on the same order as the Fe-only treatment suggesting that, while diazotroph activity was enhanced by P concentration, Fe availability may have had a stronger influence on the N_2 fixation rate. One month later (March 2005), a similar experiment showed slightly different dynamics. Whole water N_2 fixation rates responded positively, and approximately to the same magnitude, to P (only with addition of 320 nM) and Fe additions (30% and 37%, respectively) (Figure 12). However, when added together, P and Fe produced a depressed rate relative to the control treatment. The smaller size-class displayed increased N_2 fixation rates upon addition of P (same increase regardless of P concentration added: 160 nM or 320 nM) and Fe, suggesting the <10 μm diazotrophs may be more sensitive to Fe addition than P amendment. The most dramatic temporal increase in surface N_2 fixation rates occurred between February and March 2005 (0.99 - 2.56 μmol N m^{-3} d^{-1}) possibly due to a intense and sporadic dust event previously observed during this time of year (Johnson et al., 2003; Gong et al., 2003 summarized in Boyle et al., 2005) (Figure 4). Depth integrated (upper 125 m) N_2 fixation also dramatically increases from winter to spring (5.8 fold for the <10 μm diazotrophs and 3.8 fold for the >10 μm size class) (Table 1).

Two other factors that have proved important in previous N_2 fixation studies, mixed-layer depth (MLD) (Coles et al., 2004) and PE concentration (Dore et al., 2002), are not clearly connected to our observed diazotroph activity patterns. Unlike other

published results (Dore et al., 2002), our surface N_2 fixation rates (April 2004 – March 2005) do not correlate with any one size-fraction of PE concentration (0.4 - 5.0 μm, 5.0 - 10.0 μm, or >10.0 μm) (Figure 5). However, the period of relatively high surface N_2 fixation rates (July – August 2004) corresponds to highest observed PE in the largest size range (>10 μm) and the absolute maximum diazotrophy during our study period (March 2005) occurs during high PE concentration in the smallest size-class (0.4 – 5.0 μm). *Trichodesmium* abundance has been shown to be dependent on shallower MLD in coupled biological-physical models for the tropical north Atlantic (Coles et al., 2004) and subtropical North Pacific (Fennel et al., 2002). In contrast, surface N_2 fixation rates in April 2004 – March 2005 do not correlate with MLD ($r^2 = 0.02$, $p > 0.05$) at Stn. ALOHA.

A complex set of observations emerge from the three P/Fe manipulation experiments concerning water column history, including potential Fe supply and MLD, and diazotrophy. When MLD was the shallowest and any available Fe would be most concentrated in the euphotic zone and ambient N_2 fixation rate was fairly low (November 2004), there was no recorded response to Fe addition. During a period with a deep MLD, resulting in more dilute Fe concentration (February 2005), the most significant response to Fe addition was observed. On the contrary, a MLD nearly equal to that in November 2004 was present in March 2005 when there was a substantial increase in whole water and small diazotroph activity when Fe was added. This last experiment was performed during the highest measured ambient fixation. The manipulation experimental results and parallel trends in surface N_2 fixation rates and DIP inventories throughout our sampling year, suggest that diazotrophy is occasionally limited by bioavailable P and Fe at Stn.

ALOHA. Sporadic enhancement of N_2 fixation may be due to high Fe deposition events or variable P input or (more likely) a combination of these complex processes.

Export Production

Several assumptions have been made in the mass balance method to infer N_2 fixation-supported and NO_3^--supported export (see Dore et al., 2002 for detailed explanation of assumption validity). These include: (1) new N is balanced by exported N over time periods of months and there are no significant accumulations of N in surface waters, (2) inputs of new N (e.g., atmospheric deposition, or horizontal advection of NO_3^-) are negligible compared to N_2 fixation and vertical advection of NO_3^-, (3) $\delta^{15}N$-NO_3^- = 6.5 ‰ and $\delta^{15}N$-N_2 = 0 ‰ and are constant (the extrema of $\delta^{15}N$- F_{PN} for the whole time-series support the use of these end member $\delta^{15}N$ values), and (4) floating sediment traps collect material without an isotopic bias. However, floating sediment traps have been shown to undersample high-flux events (Benitez-Nelson et al., 2001) that are often associated with periods of increased N_2 fixation.

Temporal variability in F_{PN} during April-November 2004 is dominated by changes in $F_{PN}[N_2]$, whereas $F_{PN}[NO_3^-]$ displays relatively constant values (Figure 8). However, there is no correlation ($r^2 = 0.06$, $p > 0.05$) between $F_{PN}[N_2]$ and surface N_2 fixation rates. As a consideration of PN sinking rates, viewing surface N_2 fixation rates and $F_{PN}[N_2]$ with a one month time lag between measurements yields no correlation ($r^2 = 0.014$, $p > 0.05$). Similarly, the one sampling occasion (H165, November 2004) that includes both depth profile $^{15}N_2$-measured N_2 fixation rates and a $F_{PN}[N_2]$ calculation shows great disparity in areal N_2 fixation from direct depth-integrated rate measurement (20.2 μmol m^{-2} d^{-1}) and sediment trap-derived diazotroph activity (102 μmol m^{-2} d^{-1}).

There are several possible explanations for this difference. First, bottle incubations provide instantaneous rate measurements of the most abundant organisms. This is in contrast to depth- and community-integrated rates provided by sediment trap N mass and isotope data. Secondly, undersampling of large diazotrophs, such as *Trichodesmium* and *Hemiaulus* with endosymbiotic *Richelia*, and of high-frequency events may be more pronounced in bottle incubations. For example, previously reported rates of *Trichodesmium* N_2 fixation in the tropical Pacific (Mahaffey et al., *in press*) are substantially higher than the highest calculated diazotroph activity of 26.3 μmol m^{-2} d^{-1} presented here for >10 μm diazotrophs (Table 1). However, measured rates for whole water and <10 μm diazotrophs throughout the euphotic zone are similar to other published data (Table 5), suggesting that discrete N_2 fixation rate measurements underestimate diazotrophy due to undersampling. Another possibility is that specifically selecting *Trichodesmium* may overestimate large diazotroph activity. Surprisingly, from April to November 2004, $F_{PN}[N_2]$ is moderately positively correlated with MLD (r^2 = 0.54, $p > 0.05$) which suggests that deep MLD enhanced N_2 fixation during this period. The absence of correlation between directly measured surface N_2 fixation rate and MLD ($r^2 = 0.02$, $p > 0.05$) corroborates this claim. There is no relation with $F_{PN}[NO_3^-]$ and MLD ($r^2 = 0.0006$, $p > 0.05$).

Monthly averages of $F_{PN}[N_2]$ and $F_{PN}[NO_3^-]$ from July 2001 – November 2004, reveal several interesting conclusions. First, slightly higher calculated $F_{PN}[N_2]$ occurs each late spring during July 2001- November 2004 relative to the remainder of the year (Figure 9). However, this maximum is more irregular and much less pronounced than during spring- summer 1989-2001 (Dore et al., 2002). Second, during this later period

(2001 – 2004), monthly averages of $F_{PN}[N_2]$ and $F_{PN}[NO_3^-]$ show no correlation to averages of MLD ($r^2 = 0.11$, $p > 0.05$; and $r^2 = 0.08$, $p > 0.05$ respectively), contrary to previous observations. Finally, $\delta^{15}N\text{-}F_{PN}$ data during July 1989 – December 2002 reveal a significant decrease in $\delta^{15}N\text{-}F_{PN}$ (Figure 7). In contrast, a substantial increase in $\delta^{15}N\text{-}F_{PN}$ is observed during December 2002 - November 2004 (Figure 7). Coupled with the decade-long drawdown in DIP (Karl and Tien, 1997; Karl et al., 2001) and more rapid SRP turnover times, this shift may indicate that P has become limiting to *Trichodesmium* and other diazotrophs to an extent that they cannot compete with smaller phytoplankton for the bioavailable P. To exacerbate the competition, N_2 fixers may have provided DON (by exudation) (Gilbert and Bronk, 1994), thereby eliminating the advantage their diazotrophic capacity previously provided in an N-controlled ecosystem. Export production provides valuable information concerning nutrient dynamics, and this dramatic change in stable-N isotopes may imply decreasing contribution of N_2 fixation in the NPSG.

CONCLUSIONS

N_2 fixation continues to be important in nutrient dynamics in the NPSG as evidenced by (1) substantial diazotroph activity throughout the year in the upper euphotic zone, (2) the considerable fraction of export production supported by diazotrophy and (3) the temporal coherence between total downward PN flux and N_2-supported flux. This study has demonstrated the importance of P and Fe with respect to diazotroph distribution and activity at Stn. ALOHA. N_2 fixation rates at Stn. ALOHA display a temporal pattern that mimics surface SRP concentrations and seasonal Fe delivery and results from the nutrient addition experiments reveal increased N_2 fixation rates with P and/or Fe addition. The potential decreasing contribution of N_2 fixation in the NPSG, as indicated by increasing isotopic values of exported PN and N budget calculations, may be due to P limitation, a condition suggested by rapid SRP turnover times. Due to P drawdown during the past decade, diazotrophs may have lost the competitive advantage. The evidence presented here supports previous speculation (Dore et al., 2002; Hare and Mantua, 2000) concerning a potential climatic shift in the NPSG to a system less dependent on N_2 as a new N source.

While we do not yet have a predictive framework for determining changes in N_2 fixation, the data presented here help constrain the factors controlling diazotrophy in the NPSG. In addition, by understanding the role of P and Fe in diazotroph variability, we may be better able to assess changing biogeochemistry in the oligotrophic ocean.

Table 1. Depth distribution of N_2 fixation rate (μmol N m^{-2} d^{-1}) for whole, <10 μm, and >10 μm (calculated by difference), depth integrated rates (μmol N m^{-3} d^{-1}), and percent contribution by small (<10 μm) diazotrophs to total areal N_2 fixation rate. [1]data not available [2]measured rates are below assay detection limit (DL = 0.03 μmol N m^{-3} d^{-1}).

Cruise (Date)	Depth (m)	Whole Rate	<10 μm Rate	>10 μm Rate	Contribution of <10 μm
165	5	0.30 ± na[1]	0.35 ± na	<DL[2]	100
(Nov-04)	25	0.27 ± 0.01	0.13 ± 0.02	0.14 ± 0.02	48
	45	0.31 ± 0.04	0.19 ± 0.19	0.12 ± 0.19	61
	75	0.07 ± 0.02	0.04 ± 0.02	0.03 ± 0.03	57
	100	<DL	<DL	<DL	-
	125	0.05 ± 0.01	0.06 ± 0.03	<DL	100
	Integrated	20.2	14.5	6.9	72
167	5	0.78 ± 0.08	0.50 ± 0.01	0.28 ± 0.08	64
(Feb-05)	25	0.93 ± 0.33	0.46 ± 0.04	0.47 ± 0.33	49
	45	0.76 ± 0.07	0.52 ± 0.06	0.24 ± 0.09	68
	75	0.16 ± 0.04	0.05 ± 0.03	0.11 ± 0.05	31
	100	<DL	<DL	<DL	-
	125	0.06 ± 0.04	<DL	0.06 ± 0.04	0
	Integrated	54.5	31.1	23.4	57
168	5	2.66 ± 0.61	1.91 ± 0.46	0.75 ± 0.76	72
(Mar-05)	25	1.78 ± 0.27	1.48 ± 0.25	0.30 ± 0.37	83
	45	1.17 ± 0.05	0.90 ± 0.02	0.27 ± 0.05	77
	75	0.15 ± 0.08	0.08 ± 0.02	0.07 ± 0.08	53
	100	<DL	0.03 ± 0.01	<DL	100
	125	0.03 ± 0.03	<DL	0.03 ± 0.04	0
	Integrated	109.5	83.7	26.3	76

Table 2. Calculated SRP inventory (0-100 m) turnover time using P uptake estimated from depth integrated (0-100 m) primary production (calculated from 12-h in situ ^{14}C-uptake experiments). [1]range calculated assuming a C:P molar ratio of 100:1 (idealized phytoplankton; Karl and Yanagi, 1997) and 464:1 (P-limited *Prochlorococcus*; Bertilsson et al., 2003) [2]value is the monthly average of 2000-2004 primary production measurements at Stn. ALOHA for appropriate month.

Cruise Number	Date	SRP (mmol m^{-2})	Primary Production (mmol C m^{-2} d^{-1})	SRP Turnover Time (d) [1]
158	Apr-04	3.05	47.3	6.4 – 29.9
159	May-04	5.74	60.1	9.5 – 44.3
160	Jun-04	4.77	47.8	10.0 – 46.3
161	Jul-04	5.74	55.5[2]	10.3 – 48.0
162	Aug-04	3.68	48.9	7.5 – 34.9
163	Sept-04	1.93	39.0	5.0 – 23.0
164	Oct-04	0.98	45.6	2.1 – 10.0
165	Nov-04	2.03	27.3	7.4 – 34.5
166	Dec-04	4.22	28.6	14.8 – 68.6
167	Feb-05	2.90	33.6	8.6 – 40.0
168	Mar-05	3.68	41.7[2]	8.8 – 41.0

Table 3. Summary of whole water P addition experiments: ambient SRP, average P addition, total dissolved P (SRP + added), P addition as a percent of ambient P, and percent increase in N_2 fixation rates relative to control where increase was substantial. [1]data not available. [2]no statistically significant increase.

Date	Ambient SRP (nM)	Average Added P (nM)	Total P (nM)	% P added	Increase in rate
May 2004	na[1]	264	na	na	NS[2]
July 2004	71.4	168	239	140	NS
August 2004	37.1	157	194	320	NS
October 2004	6.1	174	180	2750	87%
November 2004	9.9	160	170	1520	NS
February 2005	21.4	160	181	650	64%
March 2005	40.5	160	201	300	NS
March 2005	40.5	320	361	690	30%

Table 4. Summary of direct areal N_2 fixation rate estimates in the Pacific Ocean as reported or calculated. [1]calculated areal rates integrated to 100 m, [2]acetylene reduction assay.

Areal Estimate ($\mu mol\ m^{-2}\ d^{-1}$)	Method	Reference
<10μm		
92	$^{15}N_2$	Zehr et al., 2001
23 ± 6[1]	$^{15}N_2$	Dore et al., 2002
66 ± 19	$^{15}N_2$	Montoya et al., 2004
43 ± 21	$^{15}N_2$	This Study
Bulk Water		
72 ± 13[1]	$^{15}N_2$	Dore et al., 2002
61 ± 26	$^{15}N_2$	This Study
Trichodesmium		
135	ARA[2]	Gunderson et al., 1976
33	ARA	Mague et al., 1977
85 – 140	ARA	Karl et al., 1997
53 – 143	ARA	Mahaffey et al., *in press*

Table 5. Annual N budget for Stn. ALOHA for April 2004 – March 2005. [1]range is 2 -3 times net primary production (Juranek et al., 2002), net production from direct in situ 12-h in situ ^{14}C-uptake experiments and assuming a C:N ratio of 6.6, July 2004 and March 2005 estimated from monthly average of 2000-2004 record; [2]calculated using an *f*-ratio of 0.06 (Eppley and Peterson, 1979) and annual estimate of gross primary production; [3]calculated using an *f*-ratio of 0.11 (Knauer et al., 1990) and annual estimate of gross primary production; [4]direct measurement of sediment trap (150 m) collected particulate N at Stn. ALOHA, July 2004 and December 2004 – March 2005 estimated from monthly averages of 2000-2004; [5]calculated from excreted dissolved N by migrant organisms in the central N. Pacific gyre (Longhurst and Harrison, 1988); [6]calculated by Karl et al. (1992) using nitricline gradients (0.03 – 0.07 mmol N m^{-4}; Chiswell et al., 1990) and an eddy diffusivity coefficient (3.7 x 10^{-5} m^2 sec^{-1}; Lewis et al., 1986); [7]calculated by Karl et al. (1992) using NO_3^- + NO_2^- concentration in the upper nitricline (1 – 2 mmol m^{-3}; Chiswell et al., 1990) and an upward vertical velocity (0.012 m d^{-1}; Munk, 1966); [8]measurements of wet and dry deposition in the central Pacific Ocean (Duce, 1986; Carrillo et al., 2002); [9]based on depth integrated (0-125 m) $^{15}N_2$-tracer measurements of N_2 fixation from November 2004, and February and March 2005, minimum value is based on low N_2 fixation (November 2004) dominating during the remainder of the year and the maximum is based on high N_2 fixation (March 2005) dominating the remainder of the year. Table after Karl et al. (1992).

Rate Process	N Flux (mmol N m^{-2} yr^{-1})
Accumulations	
Gross Primary Production[1]	4604 - 6906
Conventional Exports	
New Production[2]	138
New Production[3]	253
Particulate Export[4]	104
Migrant Zooplankton[5]	21-65
Conventional Imports	
Upward Eddy Diffusivity[6]	41-95
Upwelling[7]	4-9
Atmospheric Fallout[8]	8-26
Nonconventional Import	
N_2 Fixation[9]	11-35

Figure 1. Depth profiles of whole (A) and small (<10 μm) (B) diazotroph N_2 fixation rates during November 2004 (diamonds), February 2005 (squares), and March 2005 (triangles).

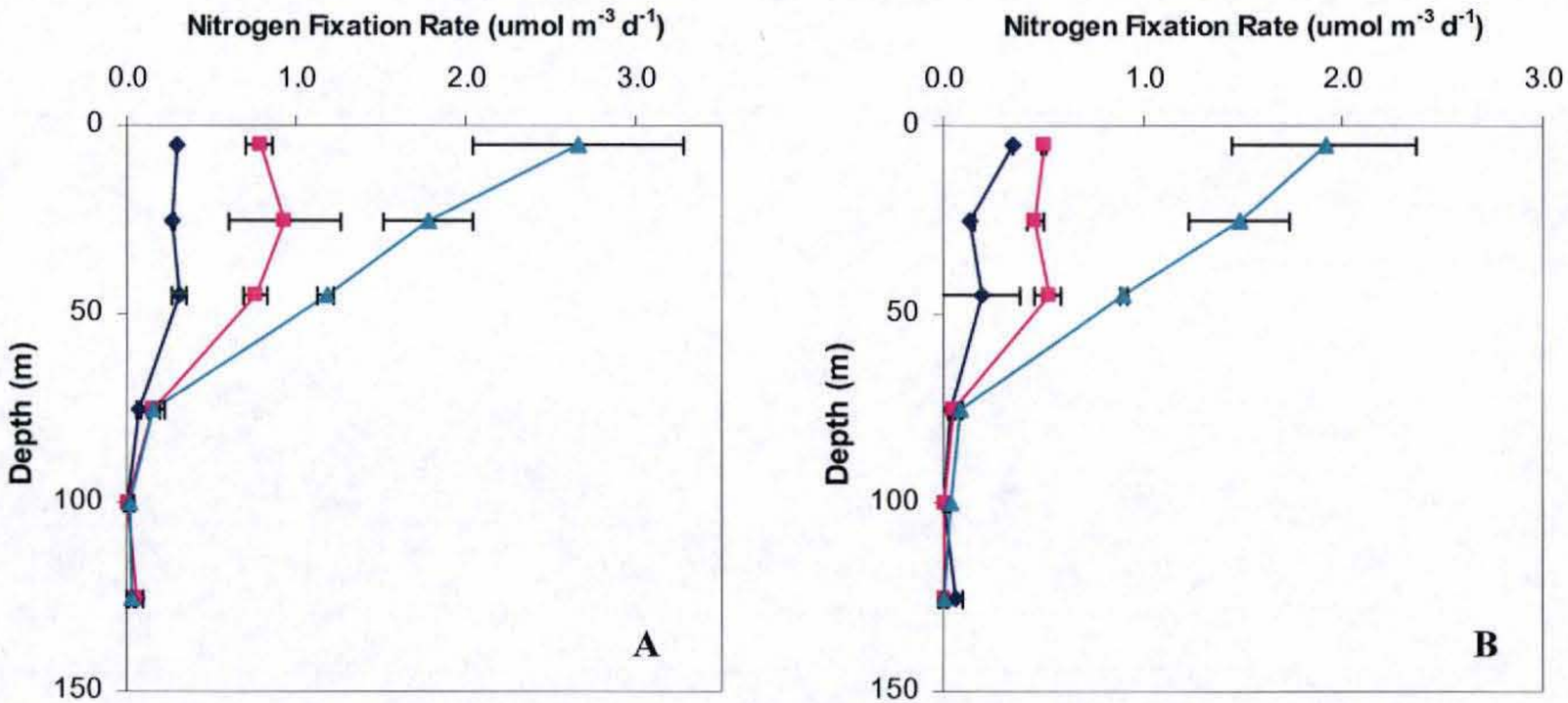


Figure 2. Cumulative contribution of whole (A) and small (<10 μm) (B) diazotroph N_2 fixation rates during November 2004 (diamonds), February 2005 (squares), and March 2005 (triangles).

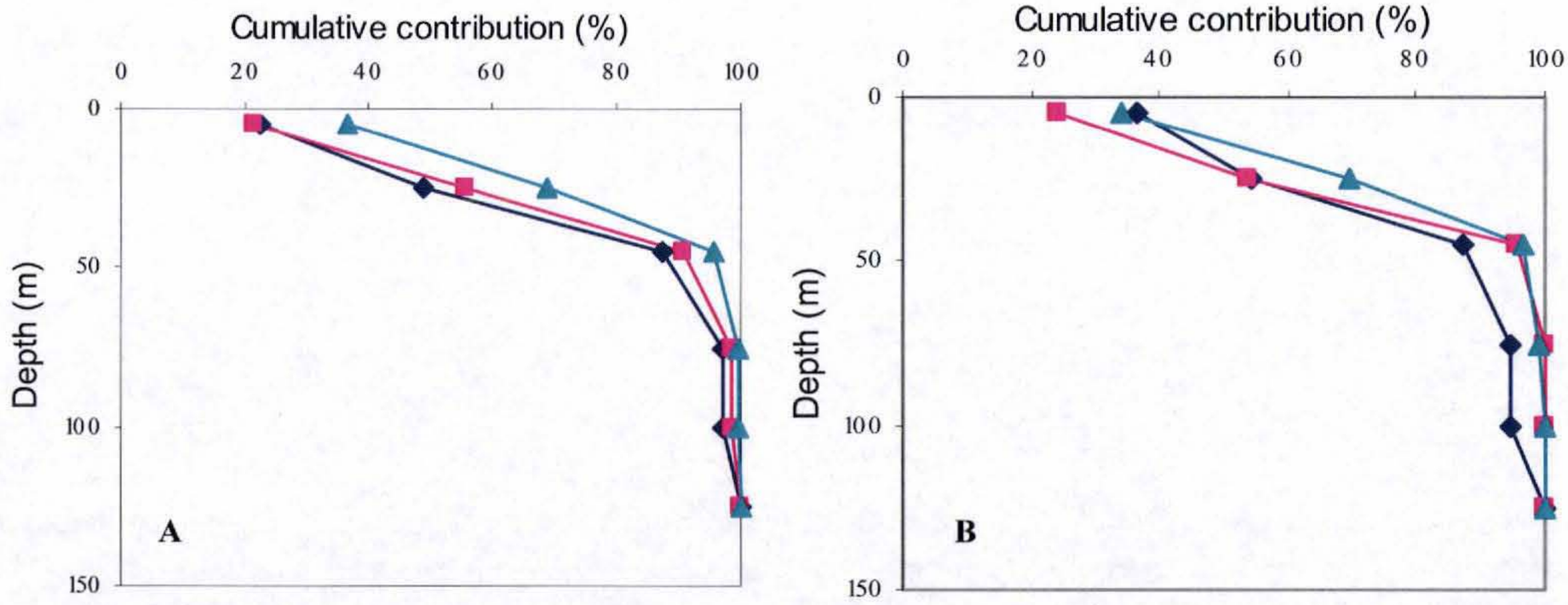
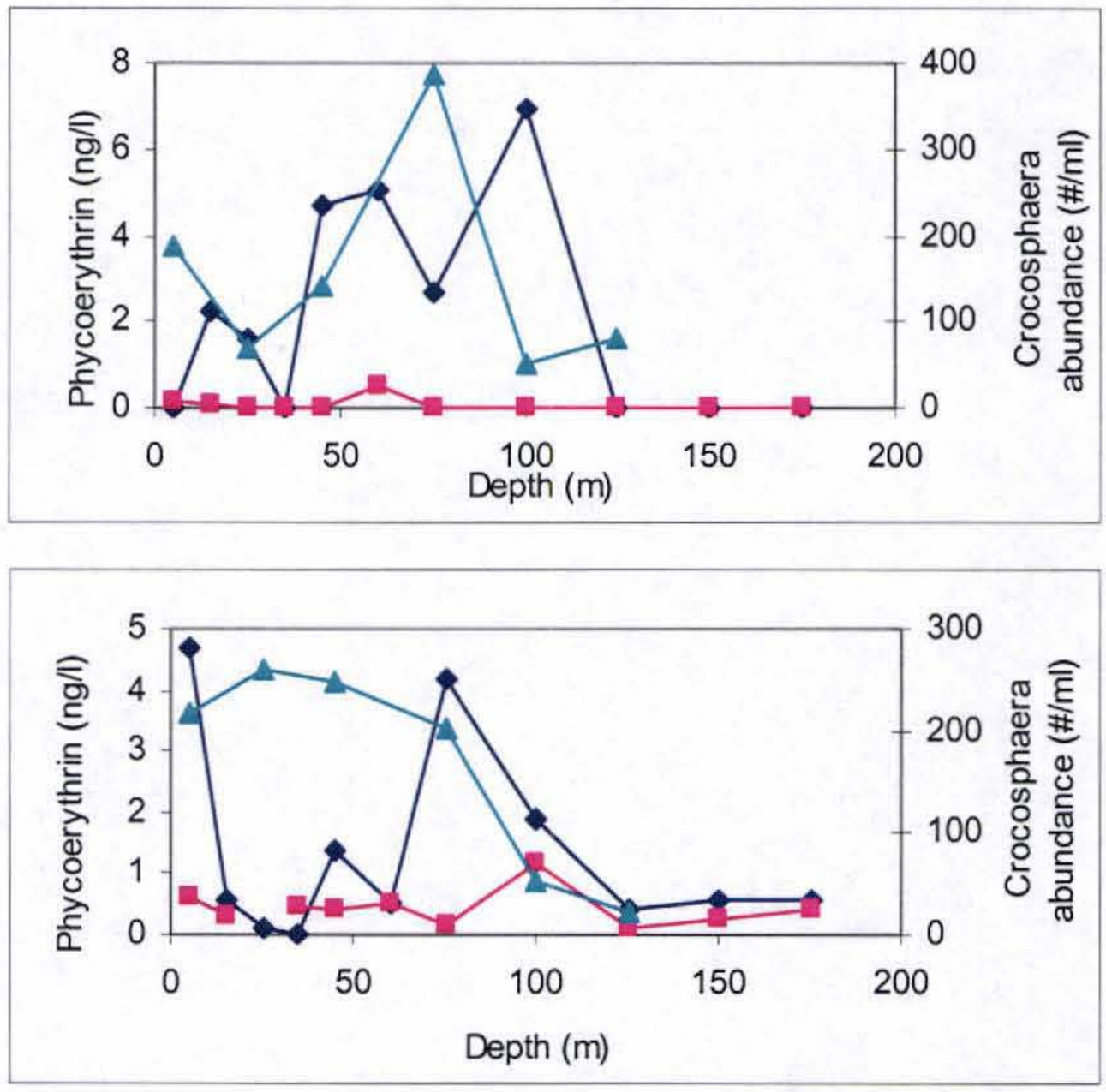


Figure 3. Depth profiles of phycoerythrin concentration (0.4 – 5.0 μm (diamonds) and 5.0 - 10.0 μm (squares)), and *Crocosphaera -like* cell (3 – 8 μm) abundance (triangles) from November 2004 (A), February 2005 (B) and March 2005 (C).



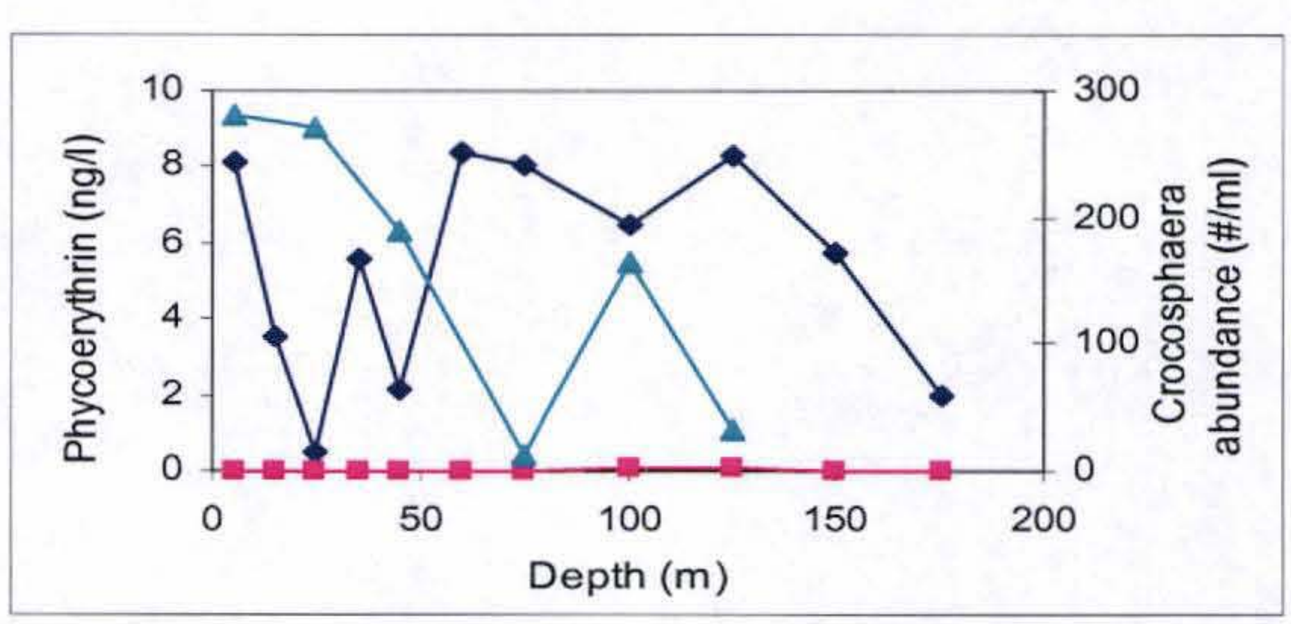


Figure 4. Surface N_2 fixation rates (A) and suspended (5 m) δ^{15}N-PN (B) in April 2004 – March 2005. Error bars are standard error of replicate samples. Dore et al. (2002) data, collected at Stn. ALOHA between July 2000 -June 2001, are provided for comparison.

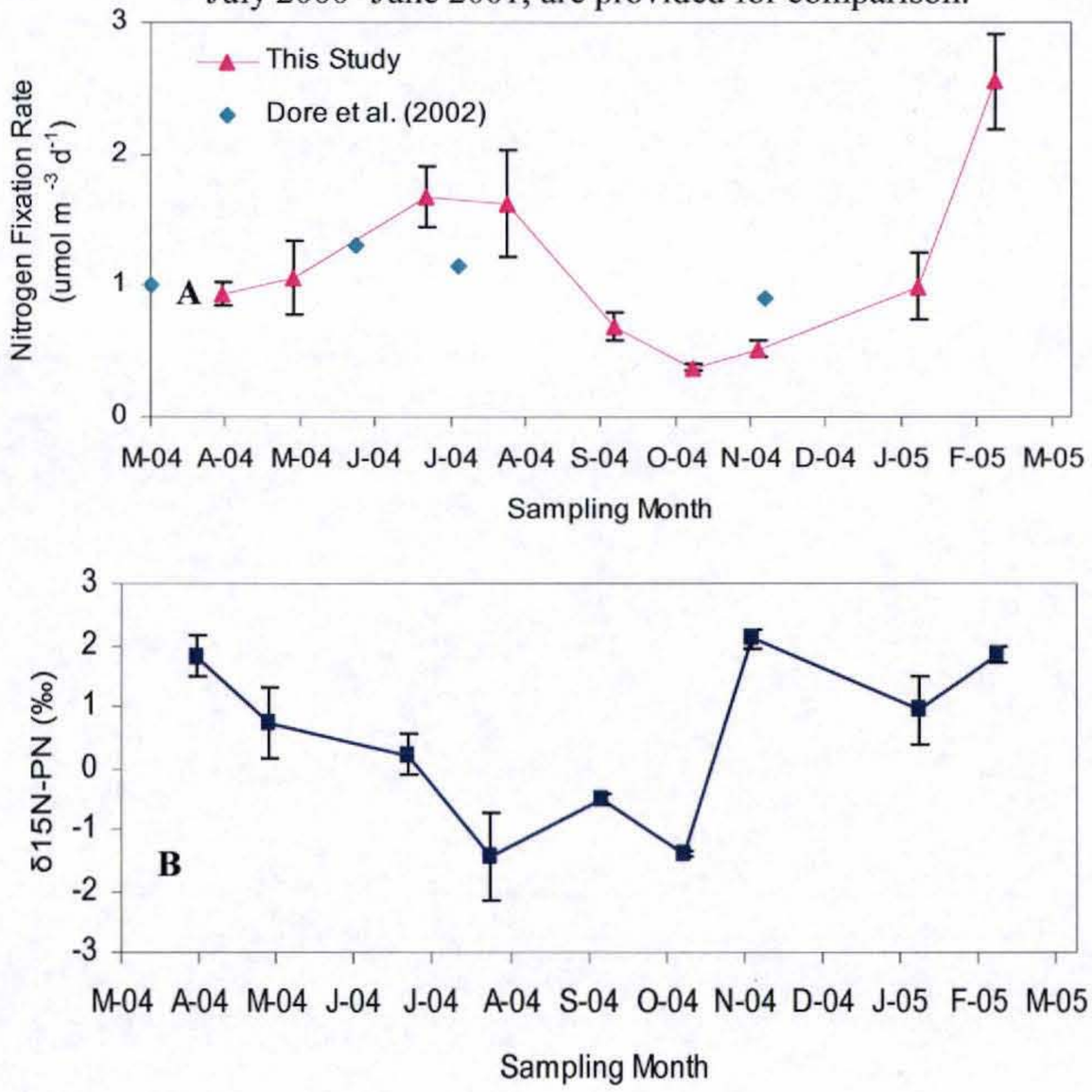


Figure 5. Surface N_2 fixation rates and size-fractionated phycoerythrin concentration April 2004 – March 2005.

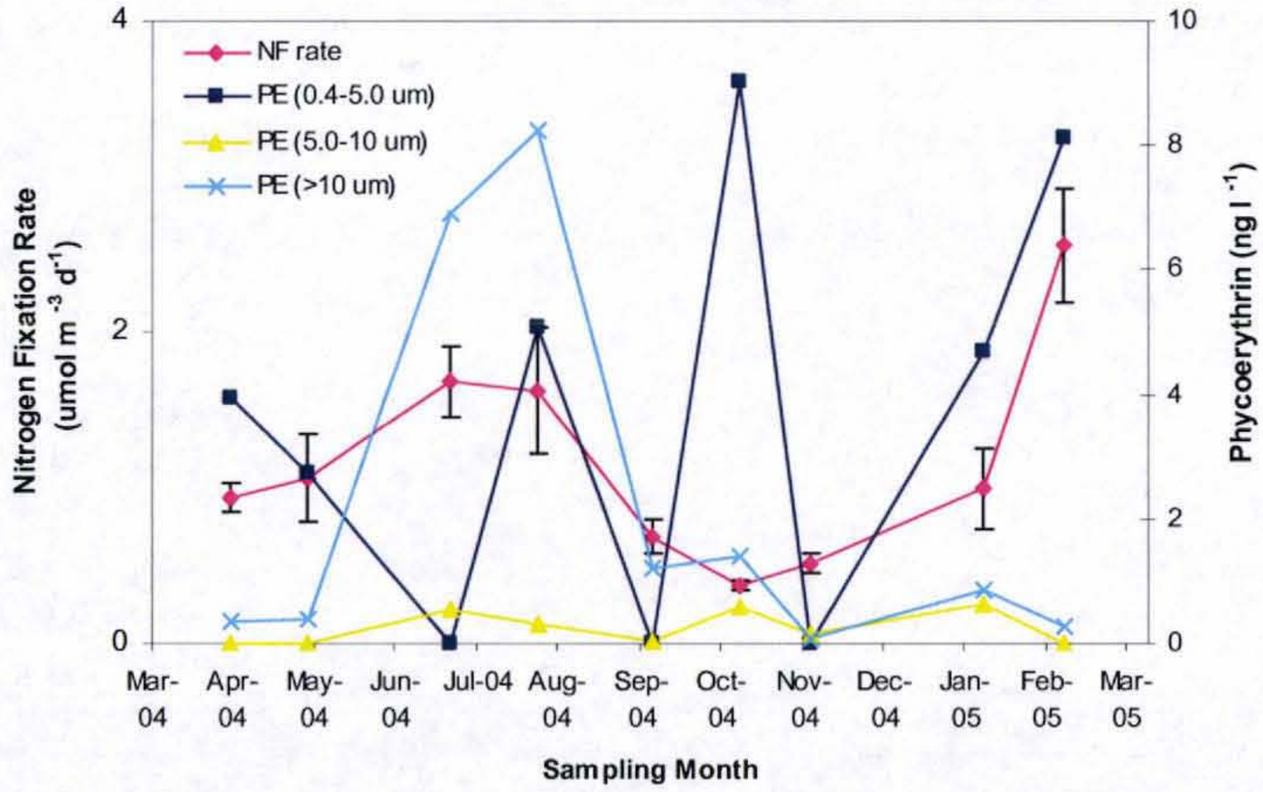
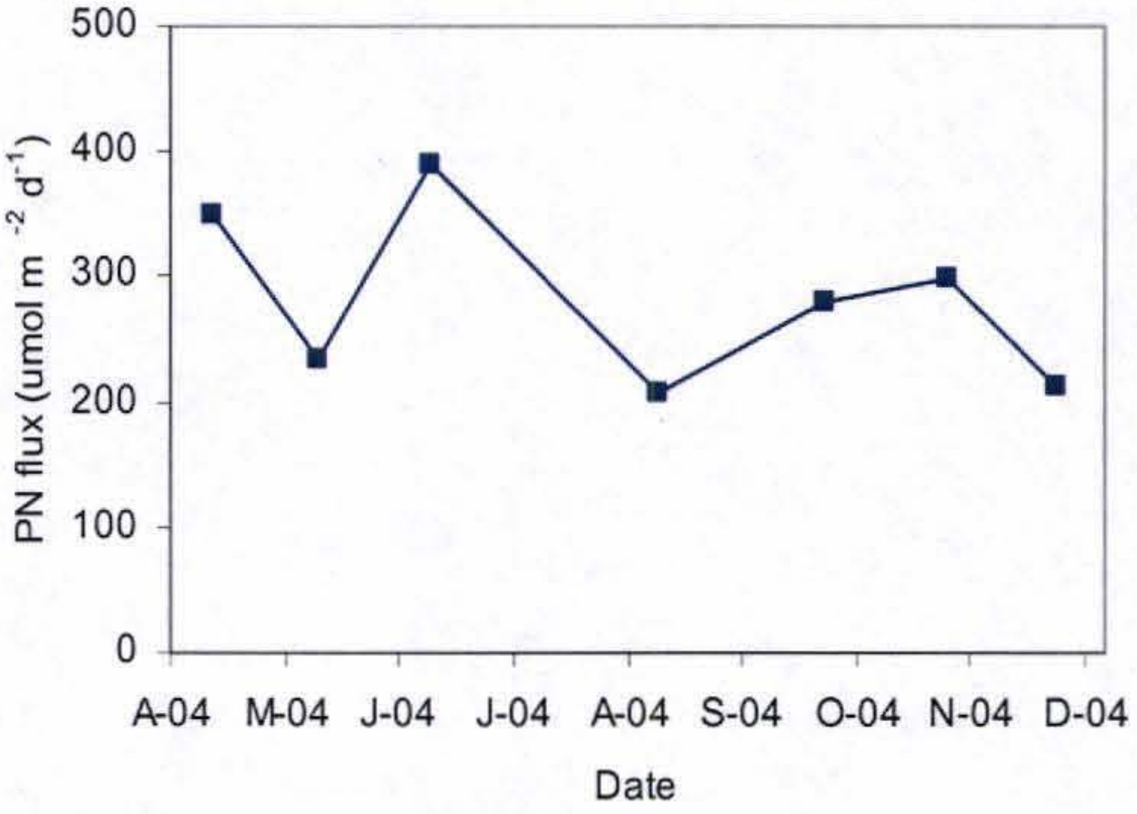


Figure 6. Mass (A) and δ^{15}N-PN (B) of export flux during April – November 2004.



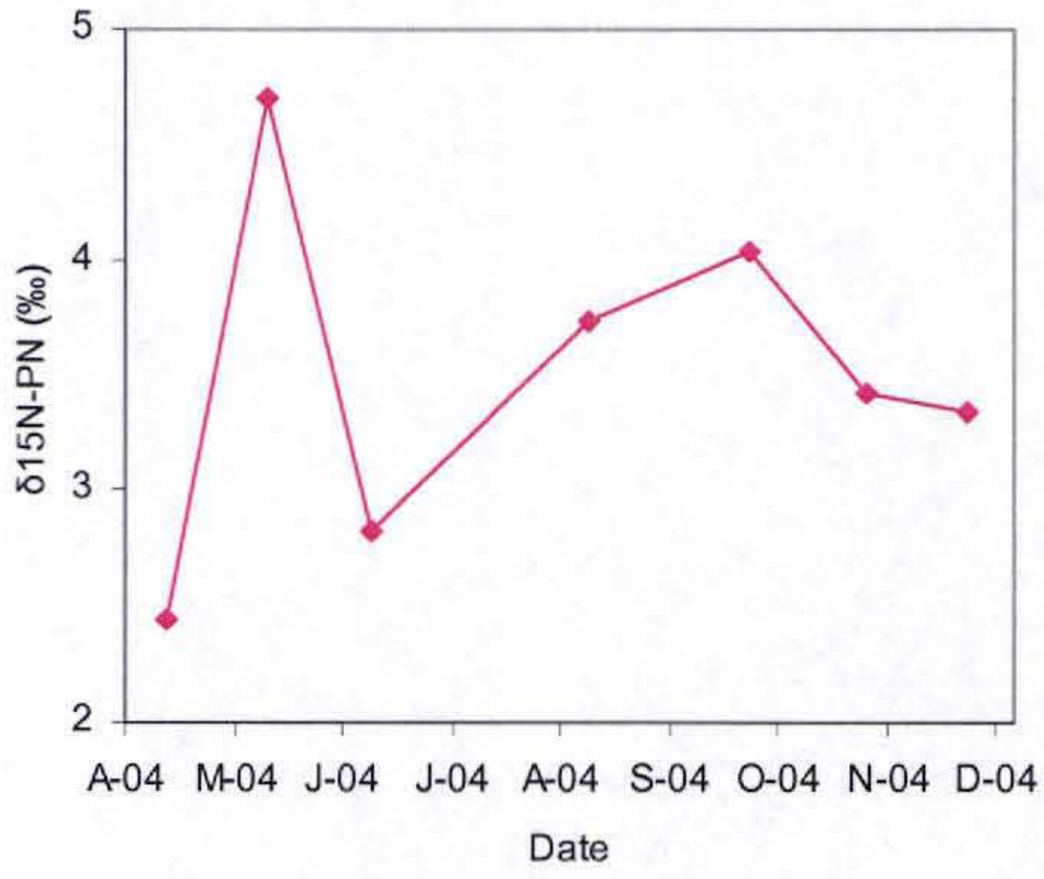


Figure 7. Time-series of export flux δ^{15}N-PN between July 1989 and November 2004. Equation for trendline between 1989 – December 2002: $y = -0.004x + 18.3$, $r^2 = 0.22$. Equation for trendline between December 2002 – 2004: $y = 0.0013x - 45.8$, $r^2 = 0.14$.

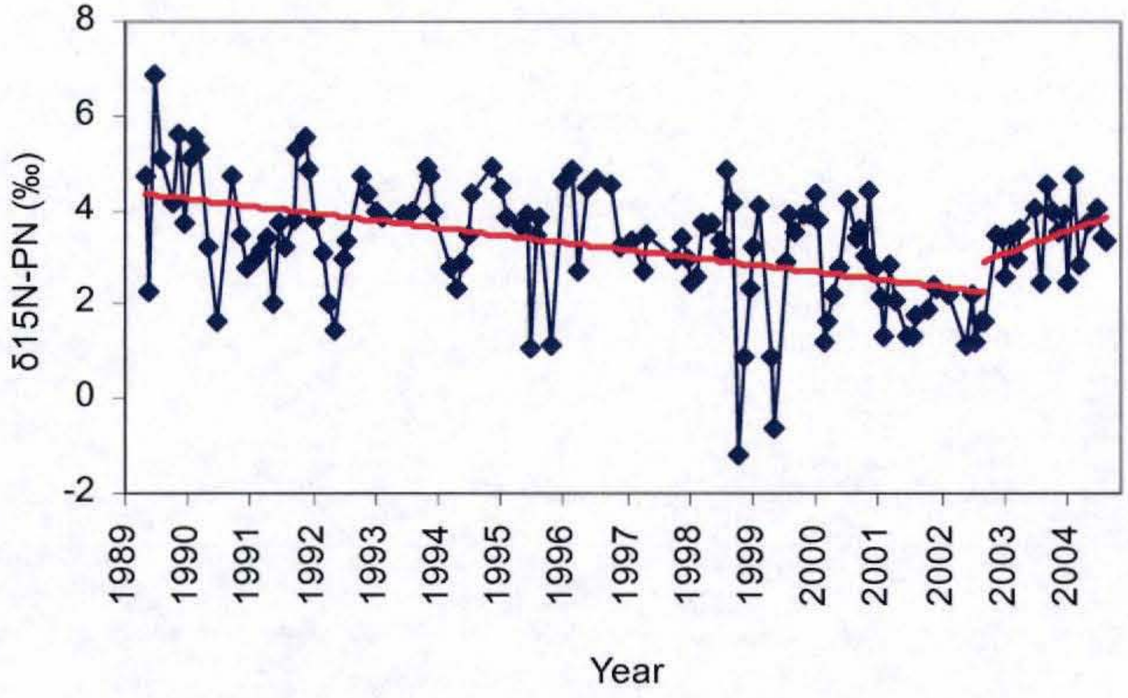


Figure 8. Total PN flux (diamonds), F_{PN}-[N_2] (squares) and F_{PN}-[NO_3] (triangles) during April – November 2004.

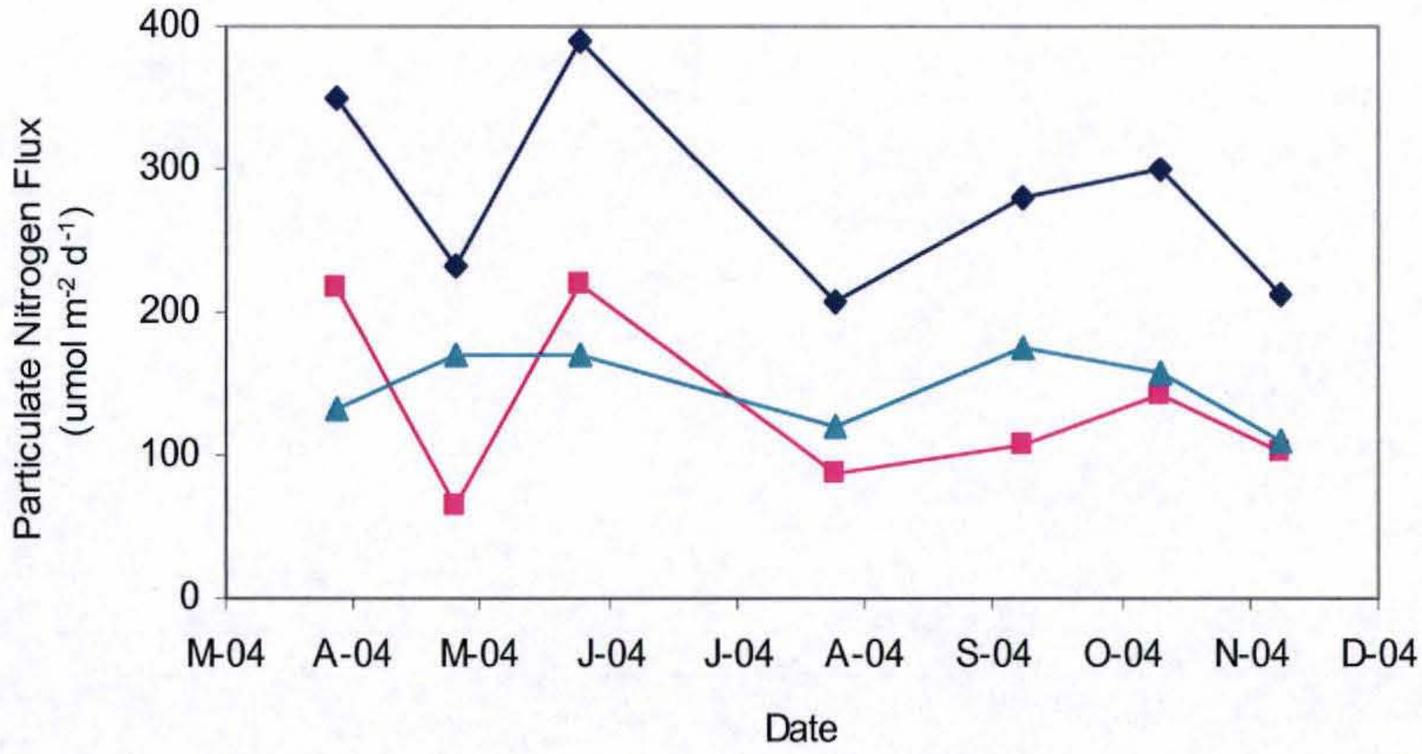


Figure 9. Seasonality of monthly averaged (July 2001 – November 2004) total PN export (diamonds), F_{PN}-[N_2] (squares) and F_{PN}-[NO_3] (triangles)

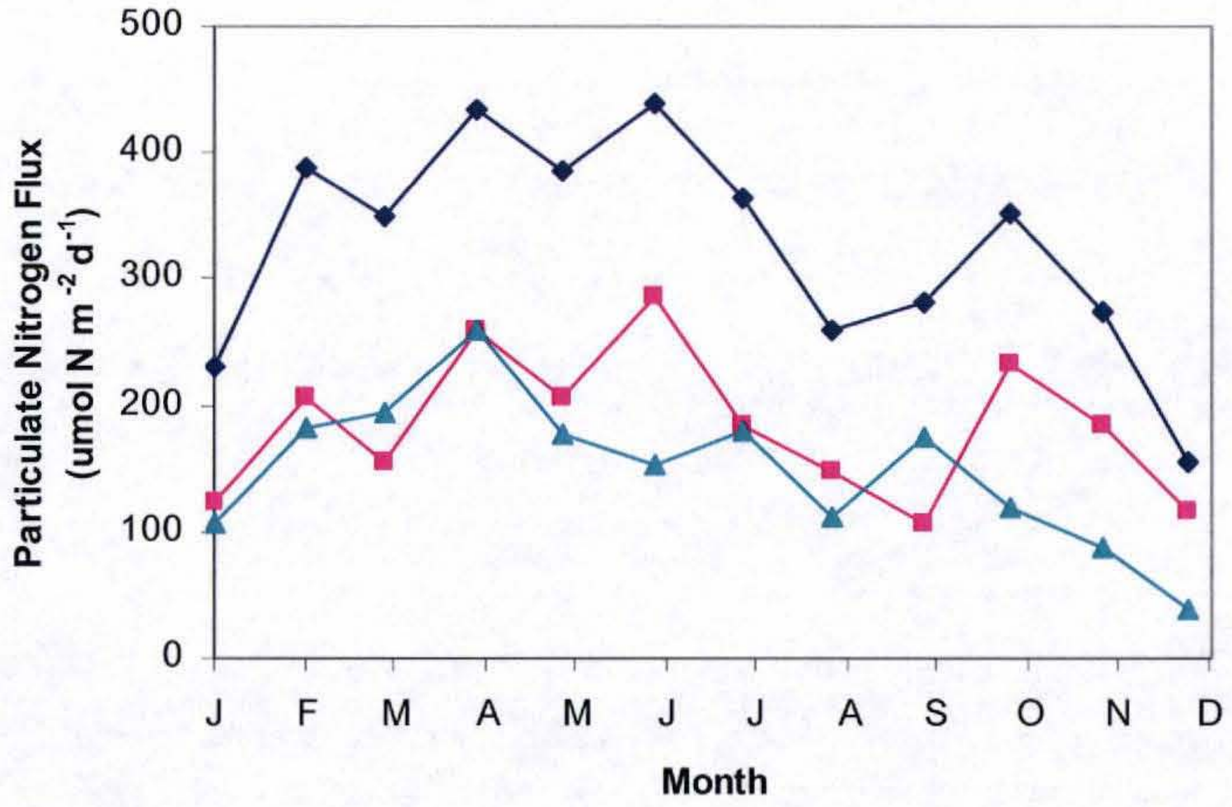


Figure 10. Surface N_2 fixation rates (diamonds) and SRP concentration (triangles) variability during April 2004 – March 2005.

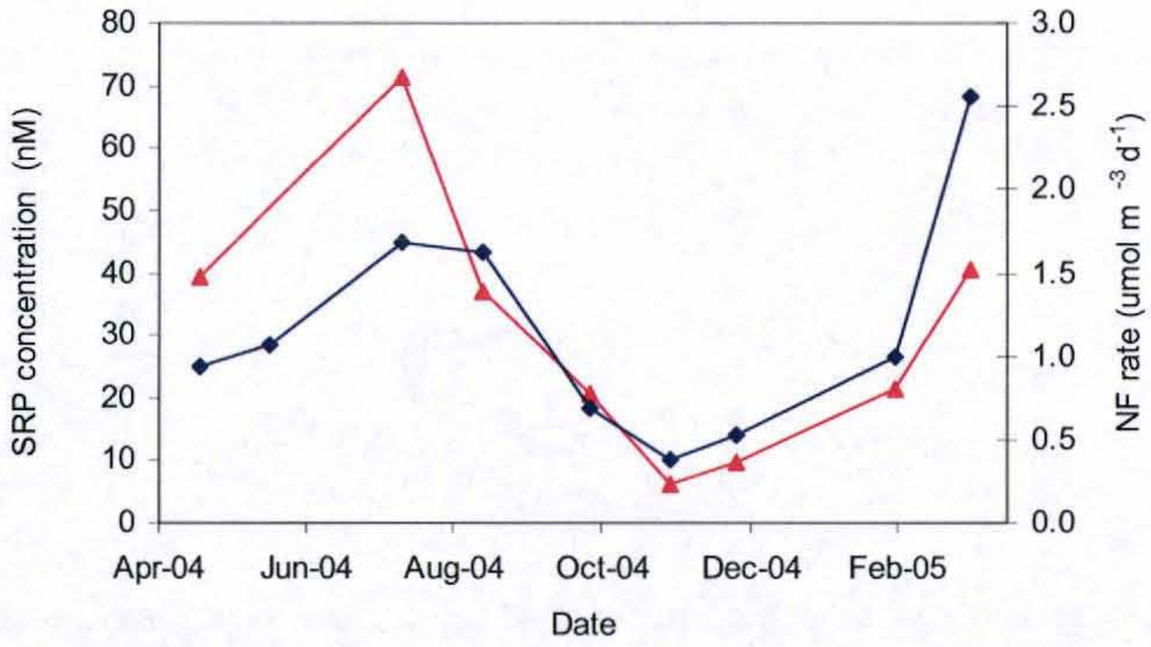


Figure 11: N_2 fixation rates in control (■), +P(■), +Fe (□) and +P/Fe (□) treatments for whole water and <10 μm diazotrophs from February 2005 manipulation experiment.

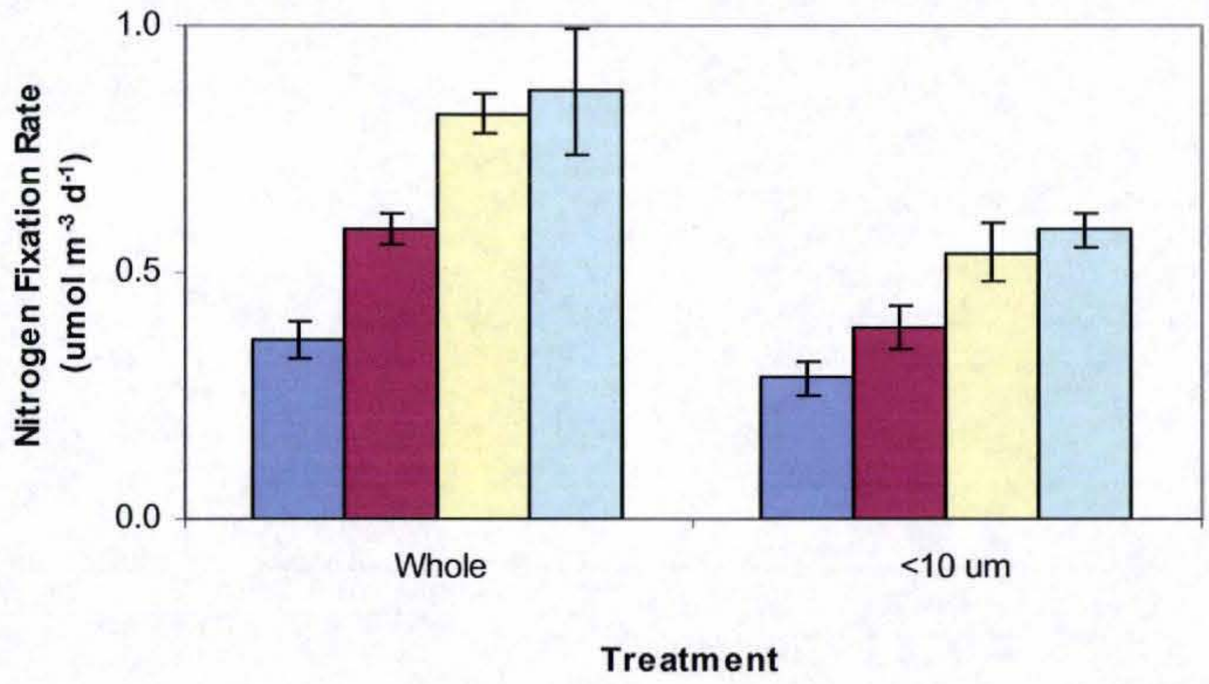


Figure 12: N_2 fixation rates in control (■), +P 160 nM (■), +P 320 nM (□), +Fe (□), and +P/Fe (■) treatments for whole water (A) and <10 μm diazotrophs (B) from March 2005 manipulation experiment.

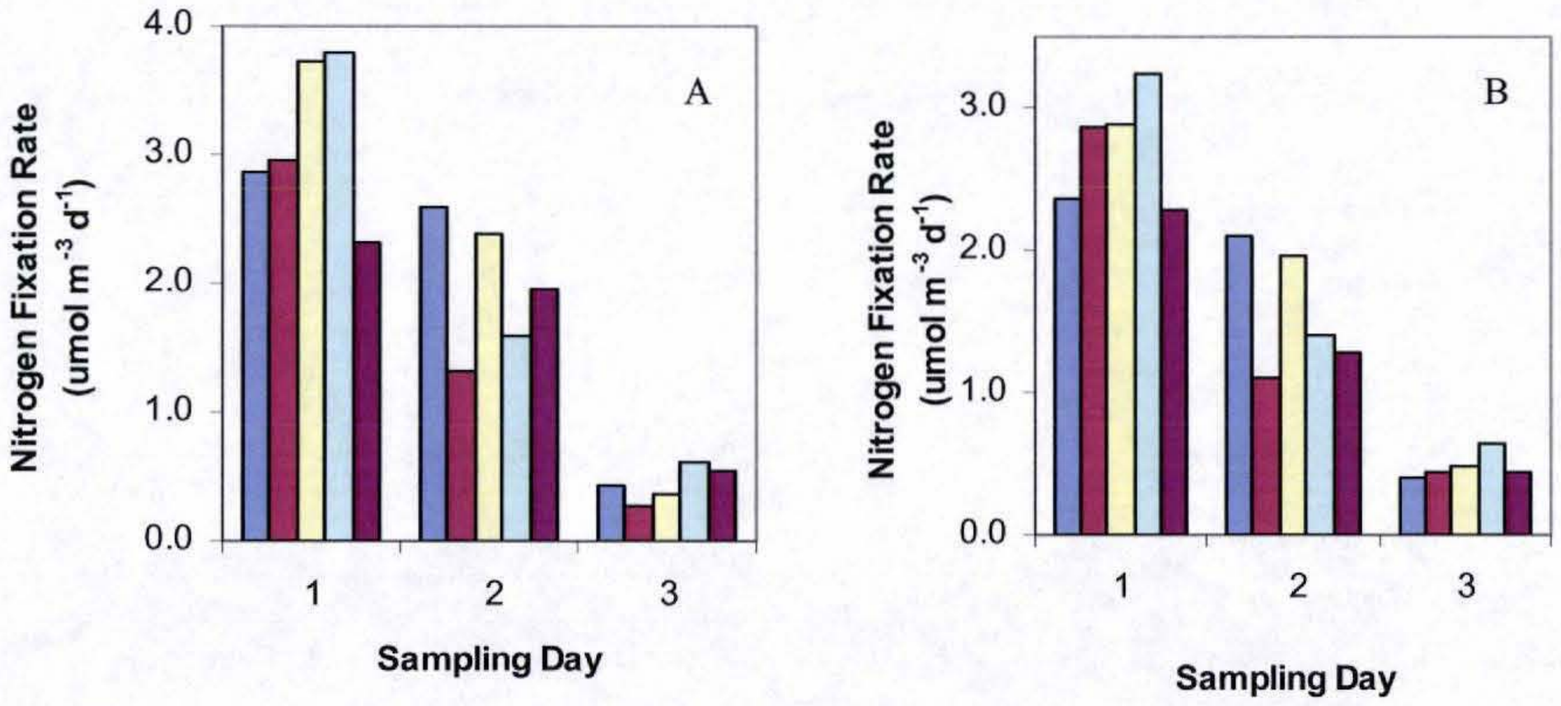


Figure 13. N_2 fixation rates of whole (diamonds) and <10 µm diazotrophs (squares) during a diel variability experiment in November 2004. Arrow represents timing of $^{15}N_2$ addition, i.e., start of incubation. Black bars represent dark hours.

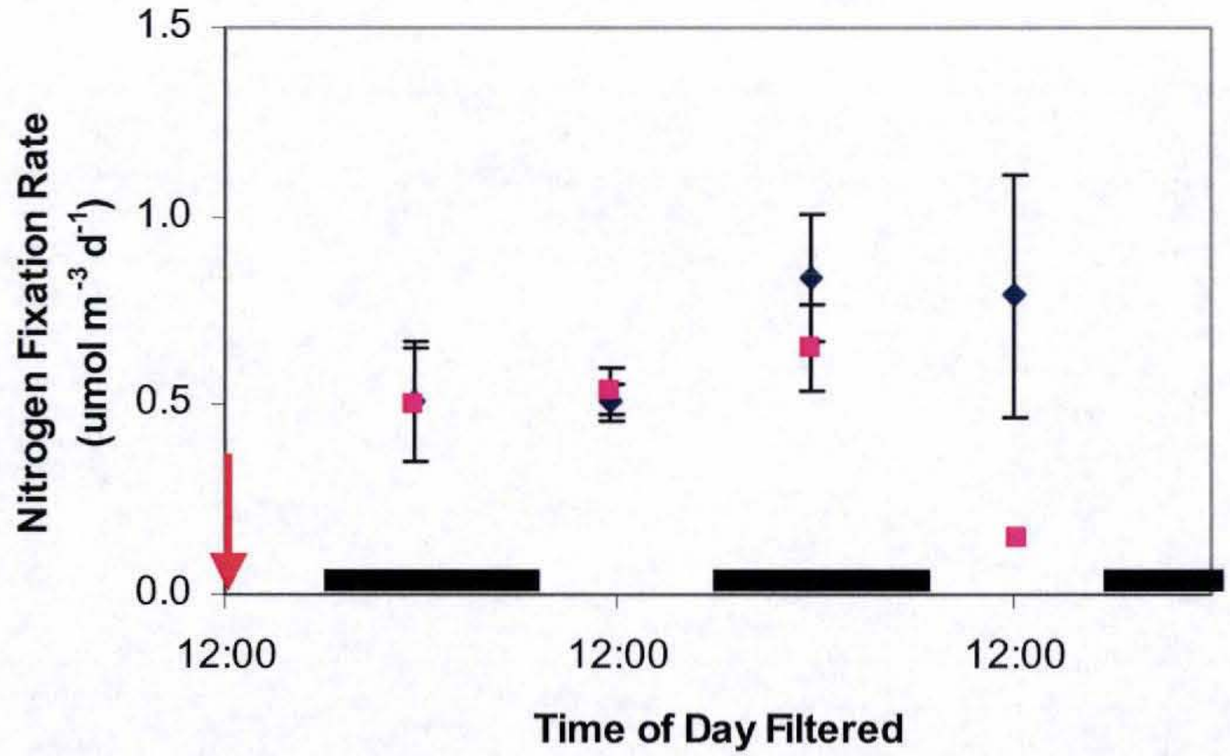
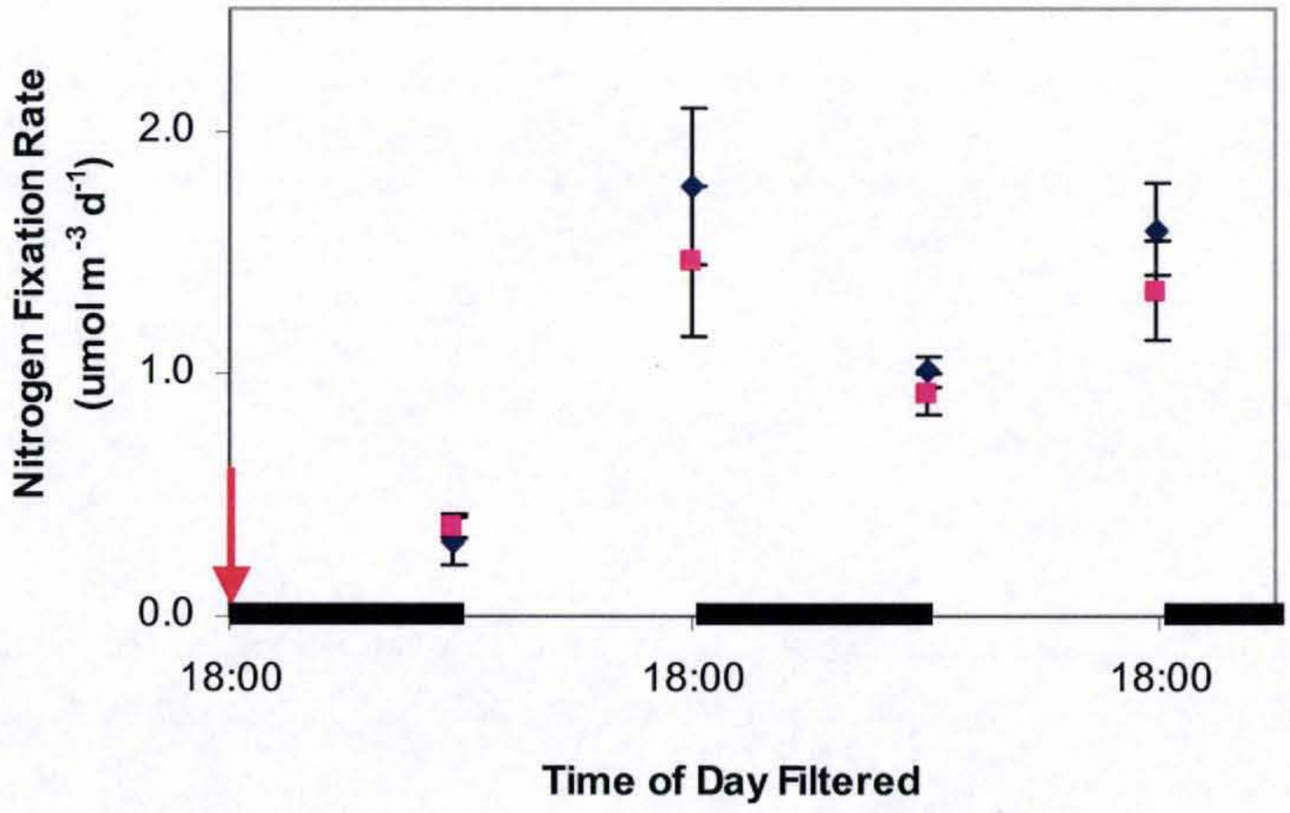


Figure 14. N_2 fixation rates of whole (diamonds) and <10 µm diazotrophs (squares) during a diel variability experiment in February 2005. Arrow represents timing of $^{15}N_2$ addition, i.e., start of incubation. Black bars represent dark hours.



REFERENCES

Benitez-Nelson C, Buesseler KO, Karl DM, Andrews J (2001) A time-series study of particulate matter export in the North Pacific subtropical gyre based on ^{234}Th:^{238}U disequilibrium. Deep-Sea Research I 48:2595-2611

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

Björkman KM, Karl DM (2003) Bioavailability of dissolved organic phosphorus in the euphotic zone at Station ALOHA, North Pacific Subtropical Gyre. Limnology and Oceanography 48:1049-1057

Björkman KM, Thomson-Bulldis AL, Karl DM (2000) Phosphorus dynamics in the North Pacific subtropical gyre. Aquatic Microbial Ecology 22:185-198

Boyle EA, Bergquist BA, Kayser RA, Mahowald N (2005) Iron, manganese, and lead at Hawaii Ocean Time-series station ALOHA: Temporal variability and an intermediate water hydrothermal plume. Geochimica et Cosmochimica Acta 69:933-952

Breitbarth E, Mills MM, Friedrichs G, LaRoche J (2004) The Bunsen gas solubility coefficient of ethylene as a function of temperature and salinity and its importance for nitrogen fixation assays. Limnology and Oceanography: Methods 2: 282-288

Capone DG (1993) Determination of nitrogenase activity in aquatic samples using the acetylene reduction procedure. In: Handbook of Methods in Aquatic Microbial Ecology (PF Kemp, BF Sherr, EB Sherr, and JJ Cole, Eds) 621-631. Lewis Press, Boca Raton, FL

Capone DG, Zehr JP, Paerl HW, Bergman B, Carpenter EJ (1997) *Trichodesmium*, a globally significant marine cyanobacterium. Science 276:1221-1229

Capone DG and Montoya JP (2001) Nitrogen fixation and denitrification. In: Methods in Microbiology (J Paul, Ed) pp. 501-515. Academic Press, NY

Carpenter EJ (1973) Nitrogen fixation by *Oscillatoria* (*Trichodesmium*) *thiebautii* in the southwestern Sargasso Sea. Deep-Sea Research 20:285-288

Carpenter EJ and Romans K (1991) Major role of the cyanobacterium *Trichodesmium* in nutrient cycling in the North Atlantic Ocean. Science 254: 1356-1358

Carrillo JH, Hasting MG, Sigman DM, Huebert BJ (2002) Atmospheric deposition of inorganic and organic nitrogen and base cations in Hawaii. Global Biogeochemical Cycles 16:1076-1091

Checkley DM Jr, Miller CA (1989) Nitrogen isotope fractionation by oceanic zooplankton. Deep-Sea Research 36:1449-1456

Chiswell S, Firing E, Karl DM, Lukas R, Winn C (1990) Hawaii Ocean Time-series data report 1, 1988 – 1989. SOEST Technical Report #1, University of Hawaii

Church MJ, Ducklow HW, Karl DM (2002) Multiyear increases in the dissolved organic matter inventories at Station ALOHA in the North Pacific Subtropical Gyre. Limnology and Oceanography 47:1-10

Church MJ, Short CM, Jenkins BD, Karl DM, Zehr JP (2005) Temporal patterns of nitrogenase gene (*nifH*) expression in the oligotrophic North Pacific Ocean. Applied and Environmental Microbiology 71:5362-5370

Coale KH, Johnson KS, Fitzwater SE et al. (1996) A massive phytoplankton bloom induced by an ecosystem-scale iron fertilization experiment in the equatorial Pacific Ocean. Nature 383:495-501

Coles VJ, Hood RR, Pascual M, Capone DG (2004) Modeling the impact of *Trichodesmium* and nitrogen fixation in the Atlantic Ocean. Journal of Geophysical Research 190, doi:10.1029/2002JC001754

Deutsch C, Gruber N, Key R, Sarmiento J (2001) Denitrification and N_2 fixation in the Pacific Ocean. Global Biogeochemical Cycles 15:483-506

Dore JE, Brum JR, Tupas LM, Karl DM (2002) Seasonal and interannual variability in sources of nitrogen supporting export in the oligotrophic subtropical North Pacific Ocean. Limnology and Oceanography 47: 1595-1607

Duce RA (1986) The impact of atmospheric nitrogen, phosphorus, and iron species on marine biological productivity. In P Buat-Menard (ed.) The role of air-sea exchange in geochemical cycling. D Reidel Publishing Company, 497-529

Dugdale, RC and Goering JJ (1967) Uptake of new and regenerated forms of nitrogen in primary productivity. Limnology and Oceanography 12:196-206

Emerson S, Stump C, Johnson B, Karl DM (2002) In situ determination of oxygen and nitrogen dynamics in the upper ocean. Deep-Sea Research I 49:941-952

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

Falkowski PG (1997) Evolution of the nitrogen cycle and its influence on the biological sequestration of CO_2 in the ocean. Nature 387: 272-275

Fennel K, Spitz YH, Letelier RM, Abbott MR, Karl DM (2002) A deterministic model for N_2 fixation at stn. ALOHA in the subtropical North Pacific Ocean. Deep-Sea Research II 49:149-174

Field CB, Behrenfeld MJ, Randerson JT, Falkowski PG (1998) Primary production of the biosphere: integrating terrestrial and oceanic components. Science 281: 237-240

Glibert PM and Bronk DA (1994) Release of dissolved organic nitrogen by marine diazotrophic cyanobacteria, *Trichodesmium* spp. Applied and Environmental Microbiology 60:3996-4000

Gong SL, Zhang XY, Zhao TL, McKendry IG, Jaffe DA, Lu NM (2003) Characterization of soil dust aerosol in China and its transport and distribution during 2001 ACE-Asia: 2. Model simulation and validation. Journal of Geophysical Research 108:4262, doi:10.1029/2002JD002633

Gruber N (2004) The dynamics of the marine nitrogen cycle and its influence on atmospheric CO_2 variation, *in* Follows M and Oguz T (eds.) Carbon Climate Interactions: New York, J Wiley and Sons

Gruber N and Sarmiento JL (1997) Global patterns of marine nitrogen fixation and denitrification. Global Biogeochemical Cycles 11: 235-266

Gundersen KR, Corbin JS, Hanson CL, Hanson ML, Hanson RB, Russell DJ, Stollar A, Yamada O (1976) Structure and biological dynamics of the oligotrophic ocean euphotic zone off the Hawaiian Islands. Pacific Science 30:45-68

Hood R, Subramaniam A, May L, Carpenter E, Capone D (2002) Remote estimation of nitrogen fixation by *Trichodesmium*. Deep-Sea Research II 49:123-147

Johnson DL (1971) Simultaneous determination of arsenate and phosphate in natural waters. Environmental Science and Technology 5: 411-414

Johnson KS, Elrod VA, Fitzwater SE, Plant JN, Chavez FP, Tanner SJ, Gordon RM, Westphal DL, Perry KD, Wu J, Karl DM (2003) Surface ocean-lower atmosphere interactions in the Northeast Pacific Ocean gyre: Aerosols, iron and the ecosystem response. Global Biogeochemical Cycles 17:1063 doi:10.1029/2002GB002004

Juranek LW, Quay PD, Karl DM (2002) Primary productivity rates at Station ALOHA determined by ^{18}O labeling and the triple isotope composition of dissolved oxygen. American Geophysical Union (Fall 2002 Meeting) Published Abstracts (#OS21B-0204)

Karl DM (1995) HOT and the North Pacific Gyre. Nature 378: 21-22

Karl DM (2002) Nutrient dynamics in the deep blue sea. Trends in Microbiology 10:410-418

Karl DM, Bates NR, Emerson S, Harrison PJ, Jeandel C, Llinas O, Liu K-K, Marty J-C, Michaels AF, Miquel JC, Neuer S, Nojiri Y, Wong CS (2003) Temporal studies of biogeochemical processes determined from ocean time-series observations during JGOFS era. In: Fasham MJR (ed.) Ocean Biogeochemistry: The role of the ocean carbon cycle in global climate change. Springer-Verlag, Berlin, 239-267

Karl DM, Bidigare RR, Letelier RM (2001) Long-term changes in plankton community structure and productivity in the North Pacific Subtropical Gyre: The domain shift hypothesis. Deep-Sea Research II 48:1449-1470

Karl DM, Christian JR, Dore JE, Hebel DV, Letelier RM, Tupas LM, Winn CD (1996) Seasonal and interannual variability in primary production and particle flux at Station ALOHA. Deep-Sea Research II 43:539-568

Karl DM, Letelier R, Hebel DV, Bird DF, Winn CD (1992) *Trichodesmium* blooms and new nitrogen in the North Pacific gyre. In: Carpenter EJ, Capone DG, Rueter JG (eds) Marine pelagic cyanobacteria: *Trichodesmium* and other diazotrophs. Kluwer Academic, Dordrecht, 219-237

Karl DM, Letelier R, Tupas L, Dore J, Christian J, Hebel D (1997) The role of nitrogen fixation in biogeochemical cycling in the subtropical North Pacific Ocean. Nature 388:533-538

Karl DM, Lukas R (1996) The Hawaii Ocean Time-series (HOT) Program: Background, rationale and field implementation. Deep-Sea Research II 43:129-156

Karl DM, Michaels A, Bergman B, Capone D, Carpenter E, Letelier R, Lipschultz F, Paerl H, Sigman D, Stal L (2002) Dinitrogen fixation in the world's oceans. Biogeochemistry 57/58:47-98

Karl DM, Tien G (1992) MAGIC: a sensitive and precise method for measuring dissolved phosphorus in aquatic environments. Limnology and Oceanography 37:105-116

Karl DM, Tien G (1997) Temporal variability in dissolved phosphorus concentrations in the subtropical North Pacific Ocean. Marine Chemistry 56:77-96

Karl DM, Winn CD (1991) A sea of change: monitoring the ocean's carbon cycle. Environmental Science and Technology 25:1976-1981

Karl DM, Yanagi (1997) Partial characterization of the dissolved organic phosphorus pool in the oligotrophic North Pacific Ocean. Limnology and Oceanography 42:1398-1405

Knauer GA, Redalje DG, Harrison WG, Karl DM (1990) New production at the VERTEX time-series site. Deep-Sea Research 37:1121-1134

Kolber ZS, Barber RT, Coale KH, Fitzwater SE, Greene RM, Johnson KS, Lindley S, Falkowski PG (1994) Iron limitation of phytoplankton photosynthesis in the equatorial Pacific Ocean. Nature 371: 145-149

Kustka A, Carpenter EJ, Sanudo-Wilhelmy SA (2002) Iron and marine nitrogen fixation : progress and future directions. Research in Microbiology 153:255-262

Kustka A, Sanudo-Wilhelmy S, Carpenter EJ, Capone DG, Raven JA (2003) A revised estimate of the iron use efficiency of nitrogen fixation, with special reference to the marine cyanobacterium *Trichodesmium* spp. (Cyanophyta). Journal of Phycology 39:12-25

Lee K, Karl DM, Wanninkhof R, Zhang J-Z (2002) Global estimates of net carbon production in the nitrate-depleted tropical and subtropical oceans. Geophysical Research Letters 29(19):1907, doi:10.1029/2001GL014198

Letelier RM, Karl DM (1996) Role of *Trichodesmium* spp. in the productivity of the subtropical North Pacific Ocean. Marine Ecology Progress Series 133: 263-273

Letelier RM, Karl DM (1998) *Trichodesmium* spp. sustained physiology and nutrient fluxes in the North Pacific subtropical gyre. Aquatic Microbial Ecology 15: 265-276

Lewis MR, Harrison WG, Oakey NS, Hebert D, Platt T (1986) Vertical nitrate fluxes in the oligotrophic ocean. Science 234:870-873

Longhurst A (1998) Ecological Geography of the Sea. Academic Press

Longhurst AR, Harrison WG (1988) Vertical nitrogen flux from the oceanic photic zone by diel migrant zooplankton and nekton. Deep-Sea Research 35:881-889

Mague TH, Mague FC, Holm-Hansen O (1977) Physiology and chemical composition of nitrogen-fixing phytoplankton in the central North Pacific Ocean. Marine Biology 41:213-227

Mague TH, Weare NM, and Holm-Hansen O (1974) Nitrogen fixation in the North Pacific Ocean. Marine Biology 24:109-119

Mahaffey C, Michaels AF, Capone DG (in press) The condundrum of marine N_2 fixation. American Journal of Science 305

Martin JH, Coale JH, Johnson KS and others (1994) Testing the iron hypothesis in ecosystems of the equatorial Pacific Ocean. Nature 371:123-129

Martin JH, Knauer GA, Karl DM, and Broenkow WW (1987) VERTEX: carbon cycling in the northeast Pacific. Deep-Sea Research 34:267-285

Michaels AF, Olson D, Sarmiento JL, Ammerman JW, Fanning K, Jahnke R, Knap AH, Lipschultz F and Prospero JM (1996) Inputs, losses and transformations of nitrogen and phosphorus in the pelagic North Atlantic Ocean. Biogeochemistry 35: 181-226

Mills MM, Ridame C, Davey M, LaRoche J, Geider RJ (2004) Iron and phosphorus co-limit nitrogen fixation in the eastern tropical North Atlantic. Nature 429:292-294

Montoya, JP, Holl CM, Zehr JP, Hansen A, Villareal TA, Capone DG (2004) High rates of N_2 fixation by unicellular diazotrophs in the oligotrophic Pacific Ocean. Nature 430:1027-1031

Montoya, JP, Voss M, Kahler P, Capone DG (1996) A simple, high-precision, high-sensitivity tracer assay for N_2 fixation. Applied and Environmental Microbiology 62:986-993

Ryther J G and Dunstan W M (1971) Nitrogen, phosphorus and eutrophication in the coastal marine environment. Science 171:1008-1013

Saino T and Hattori A (1980) ^{15}N natural abundance in oceanic suspended particulate matter. Nature 283:752-754

Saino T and Hattori A (1987) Geographical variation of the water column distribution of suspended particulate organic nitrogen and its ^{15}N natural abundance in the Pacific and its marginal seas. Deep-Sea Research 34: 807-827

Sanudo-Wilhelmy SA, Kustka AB, Gobler CJ, Hutchins DA, Yang M, Lwiza K, Burns J, Capone DG, Raven JA, Carpenter EJ (2001) Phosphorus limitation of nitrogen fixation by *Trichodesmium* in the central Atlantic Ocean. Nature 411:66-69

Schindler D W (1977) Evolution of phosphorus limitation in lakes. Science 195: 260-262

Subramaniam A, Brown CW, Hood RR, Carpenter EJ, Capone DG (2002) Detecting *Trichodesmium* blooms in SeaWiFS imagery. Deep-Sea Research Part II 49:107-121

Subramaniam A, Carpenter EJ, Karentz D, Falkowski PG (1999) Bio-optical properties of the marine diazotrophic cyanobacteria *Trichodesmium* spp. II – A reflectance model for remote sensing. Limnology and Oceanography 44:618-627

Tyrrell T (1999) The relative influences of nitrogen and phosphorus on oceanic primary production. Nature 400: 525-531

Villareal T A (1991) Nitrogen-fixation by the cyanobacterial symbiont of the diatom genus *Hemiaulus*. Marine Ecology Progress Series 76: 201-204

Wada E, Hattoi A (1976) Natural abundance of 15N in particulate organic matter in the North Pacific Ocean. Geochimica et Cosmochimica Acta 40:249-251

Weiss RF (1970) The solubility of nitrogen, oxygen and argon in water and seawater. Deep-Sea Research 17: 721-735

Wu J, Boyle E, Sunda W, Wen L-S (2001) Soluble and colloidal iron in the oligotrophic North Atlantic and North Pacific. Science 293:847-849

Zehr JP, Mellon MT, Zani S (1998) New nitrogen-fixing microorganisms detected in oligotrophic oceans by amplification of nitrogenase (*nifH*) genes. Applied and Environmental Microbiology 64:3444-3450

Zehr JP, Waterbury JB, Turner PJ, Montoya JP, Omoregie E, Steward GF, Hansen A, Karl DM (2001) Unicellular cyanobacteria fix N_2 in the subtropical North Pacific Ocean. Nature 412:635-638