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Nitrogen fixation rates and controls at Stn ALOHA

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ABSTRACT: Rates of dinitrogen (N_2) fixation were measured at Stn ALOHA in the North Pacific Subtropical Gyre (NPSG) on 9 cruises during the period April 2004 to March 2005. On each cruise, a near-surface (5 m) seawater sample was incubated with ^{15}N-labeled N_2 under simulated *in situ* conditions for 24 h prior to filtration of either whole water or <10 µm filtrate on microfine (0.7 µm nominal porosity) glass fiber filters; on 3 cruises, surface to 125 m depth profiles of size-fractionated N_2 fixation rates were also obtained. Nearly all (on average 95%) of the net N_2 fixation in the euphotic zone occurred in the upper 75 m, and was mostly (64 ± 5 [SE]%) contained in the <10 µm size fraction following a 24 h incubation period. Highest surface diazotroph activity at this site was observed in July to August (1.63 to 1.68 µmol N m^{-3} d^{-1}) and lowest N_2 fixation rates occurred in September to November (0.38 to 0.68 µmol N m^{-3} d^{-1}). Vertically integrated rates of whole community N_2 fixation (measured in November, February and March) varied 5-fold (20.2 to 109 µmol N m^{-2} d^{-1}). The short-term response of the microbial community to the addition of iron (Fe) and/or phosphorus (P) was variable, suggesting that contemporaneous N_2 fixation at Stn ALOHA may be controlled by the population dynamics of the various diazotroph species rather than by instantaneous resource limitation.

KEY WORDS: Nitrogen fixation · Diazotroph · Stn ALOHA · North Pacific gyre

INTRODUCTION

In nitrogen (N)-limited ecosystems, 'new' production is fueled by allochthonous inputs of N including upward eddy diffusion of nitrate (NO_3^-) from beneath the euphotic zone and dinitrogen (N_2) fixation within the euphotic zone (Dugdale & Goering 1967). In contrast, 'regenerated' production is supported by locally recycled N including the products of excretion/exudation, grazing, viral lysis, death/autolysis, organic matter decomposition and nitrification. In most open ocean ecosystems, new production is a relatively minor portion (≤10 to 15%) of total production and until recently was thought to be supported exclusively by NO_3^- (Eppley & Peterson 1979). This paradigm, especially the quantitative significance of N_2 fixation in new production, is beginning to change (Karl et al. 1997, Capone et al. 2005). Evidence for the contribution of N_2 fixation in the global marine environment derives from geochemical tracers that integrate over long timescales and large spatial scales, as well as from biological measurements that track contemporary, local processes. Geochemical tracers include natural ^{15}N isotope distributions and mass balances (Saino & Hattori 1980, Karl et al. 1997, Dore et al. 2002), anomalous N to phosphorus (P) ratios of subeuphotic-zone inorganic nutrient pools (Michaels et al. 1996, Deutsch et al. 2007), dissolved inorganic carbon (DIC) drawdown in the absence of NO_3^- (Michaels et al. 1994) and temporal dynamics of soluble reactive P pools (Karl & Tien 1997, Karl 2007). Biological tracers include enumeration of putative N_2-fixing microorganisms (Capone et al. 1997, Zehr et al. 2001), field measurements of N_2 fixation using either the acetylene reduction technique or ^{15}N-labeled N_2 (Dore et al. 2002, Montoya et al. 2004), and measurements of the genetic potential for N_2 fixation (Zehr et al. 1998, Church et al. 2005). Collectively, these data sets provide a robust substantiation for the importance of N_2 fixation in the global marine N cycle. In addition to enhancing productivity, N_2 fixation can impact the decoupling of carbon (C), N and P cycles, the C:N:P stoichiometry of exported organic matter and net C sequestration to the deep sea. Many of these data sets were collected during the Hawaii Ocean

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Time-series (HOT) program at its deep-ocean Stn ALOHA (A Long-term Oligotrophic Habitat Assessment (22° 45′ N, 158° W) in the North Pacific Subtropical Gyre (NPSG) where systematic changes in physical and biogeochemical habitat variability can be observed despite natural ecosystem variability.

Herein we report near-surface water N_2 fixation rates during an annual cycle (April 2004 to March 2005) and provide an assessment of the vertical structure of diazotroph activity (November, February, March) at Stn ALOHA. This research clarifies the importance of quantifying diazotrophic activity of whole microbial community assemblages. Using isotope mass balance and N:P stoichiometry, Karl et al. (1997) suggested that *Trichodesmium* spp. supported one-quarter to one-half of the new production at Stn ALOHA. The discovery of small (<10 µm) diazotrophs (Zehr et al. 1998) and subsequent measurement of N_2 fixation in this size class (Dore et al. 2002, Montoya et al. 2004, Holl et al. 2007) illustrates the need to assess the variable rates and metabolic responses of large and small diazotrophs. Potential controls on N_2 fixation were also investigated using nutrient (P, Fe) manipulation incubation experiments.

MATERIALS AND METHODS

Station location and sampling protocols. All sampling and measurements were performed in conjunction with the HOT program at Stn ALOHA. Water column sampling was conducted using a Sea-Bird CTD sensor package (conductivity, temperature, depth, fluorescence, dissolved oxygen) that was integrated with a 24-position pylon, aluminum-framed rosette sampler equipped with 12 l PVC water sampling bottles. Samples were taken on HOT cruises 158 to 159 (April to May 2004), 161 to 165 (July to November 2004) and 167 to 168 (February to March 2005), which covers 1 annual cycle from April 2004 to March 2005.

Nitrogen fixation rate measurements and experimental manipulations. A ^{15}N isotopic tracer method (Montoya et al. 1996, Capone & Montoya 2001) was used to measure N_2 fixation rates. During the majority of cruises in the present study, whole water samples (~4 l, no pre-screening) were incubated in 4.7 l acid-washed Tedlar gas sampling bags fitted with gas-tight Teflon-backed septa. For HOT cruises 167 and 168, 4.5 l polycarbonate bottles were filled to overflowing with whole water samples and sealed using caps that were fitted with thick, silicone septa. Exactly 1 ml of $^{15}N_2$ gas (99%, Isotech) was injected into each sample bag or bottle using a gas-tight syringe and stainless steel needle. For each sampling occasion and at each depth sampled, a parallel, unincubated sample was processed, as is required to calculate N_2 fixation rates. For depth profiles, samples were incubated in a 3-chambered deck incubator designed to control both temperature and light quality and quantity. For the 3 cruises during which depth profiles were obtained (November, February and March), the temperatures in the upper 125 m were 20.6 to 26.6°C, 21.6 to 24.0°C and 20.9 to 24.2°C, and the incubation temperatures were set at 21 to 26°C, 21 to 26°C and 22 to 24°C, with an average difference between ambient and incubated temperature of 0.7°C. Water samples were grouped to best match the temperatures and light levels of their depth of origin.

During several cruises, nutrient addition experiments were performed with surface seawater to determine the effect of P and Fe on N_2 fixation rates. P (K_2HPO_4) and Fe ($FeCl_2$) were added to final concentrations ranging from 170 to 360 nM and 2 nM, respectively, either as single nutrient enrichments or combinations. Because stringent trace metal clean procedures were not employed, this study is only able to assess the response of diazotrophy relative to a control treatment, with each treatment having presumably equal, but unquanitified, Fe contamination.

Size fractionation. To determine the size distribution of the assimilation of $^{15}N_2$, samples from selected profiles and nutrient perturbation experiments (all samples from November, February and March) were size-fractionated following a 24 h whole water incubation. Two treatments were prepared: (1) whole water filtered onto a combusted GFF filter (Total) and (2) a 10 µm Nitex mesh filtrate also filtered onto a GFF filter (<10 µm). N_2 fixation in the large (>10 µm) size fraction was calculated as the difference between these 2 measurements (>10 µm = Total – (<10 µm)). The GFF filters were stored frozen (–20°C) until preparation for analysis by mass spectrometry.

Sample processing and data reporting. 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 PN ($\delta^{15}N$-PN). All nitrogen isotopic data are presented in standard delta notation:

$$\delta^{15}N_{sample} = \left\{ \left[\frac{(^{15}N/^{14}N)_{sample}}{(^{15}N/^{14}N)_{standard}} \right] - 1 \right\} \times 1000 \quad (1)$$

Atmospheric N_2 gas is the reference standard ($^{15}N/^{14}N = 0.0036765$, $\delta^{15}N = 0$‰). At each depth sampled, N_2 fixation rates were determined from PN mass and $\delta^{15}N$-PN of incubated samples, the $\delta^{15}N$-PN of unincubated samples (T_0 samples), ambient dissolved N_2 concentration, 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 respect to the analytical capabilities of the instruments used is 0.06 µmol N m^{-3} d^{-1}. This value was calculated for the smallest PN masses sampled, using twice the conservative upper estimate of instrument precision (twice 0.8‰). Additionally, the detection limit of this assay calculated from twice the average standard error of triplicate T_0 values (twice 0.32‰) and an average PN mass sampled is 0.03 µmol N m^{-3} d^{-1}.

The results from the nutrient addition experiments are reported as percent changes calculated as the N_2 fixation rate of the nutrient-treated samples relative to that of the control treatment expressed as percent change:

$$\% \text{ change} = \{[(N_2 \text{ fixation})_{\text{treatment}} - (N_2 \text{ fixation})_{\text{control}}] / (N_2 \text{ fixation})_{\text{control}}\} \times 100 \quad (2)$$

Differences in the response to nutrient amendments were analyzed using a 1-way ANOVA with Tukey's post hoc test. Model 1 linear regression analysis was used to assess correlation. All statistical tests were performed using $p < 0.05$ as a criterion for significance. Statistical analyses were performed using either Excel (Microsoft) for simple *t*-tests and regression analyses or MINITAB Statistical Software for ANOVA with Tukey's test.

Ancillary measurements. Soluble reactive phosphorus (SRP) was routinely measured using standard HOT program protocols (for complete HOT protocols, see http://hahana.soest.hawaii.edu/hot/methods/results.html). Seawater was collected in acid-washed, high-density polyethylene bottles from known depths using the CTD-rosette sampler. Samples were stored frozen until analyzed using the magnesium-induced coprecipitation (MAGIC) method (Karl & Tien 1992). SRP inventory turnover time was calculated using P uptake estimated from depth integrated (0 to 100 m) primary production (calculated from 12 h *in situ* ^{14}C uptake experiments) and assuming a C:P molar ratio of 100:1 (idealized plankton; Karl & Yanagi 1997).

Phycoerythrin, an accessory pigment found in cyanobacteria, was determined in 3 size classes (>10 µm, 5.0 to 10 µm, and 0.4 to 5.0 µm) using the *in vivo* glycerol-uncoupling method on each cruise (Dore et al. 2002). With many marine diazotrophs being cyanobacteria (although not all cyanobacteria are diazotrophs), phycoerythrin was used as a relative indicator of cyanobacteria abundance in the larger size fractions (>5.0 µm). *Trichodesmium* spp., unicellular diazotrophic cyanobacteria such as *Crocosphaera* spp., and endosymbiotic nitrogen fixers possess this accessory pigment. 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 a 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 cells from the filter. Phycoerythrin fluorescence was measured on a Turner Designs TD700 fluorometer calibrated using commercial β-phycoerythrin standard (Cyanotech) with 544 nm (excitation) and 577 nm (emission) wavelength filters.

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

RESULTS

Depth distribution and temporal variability of N_2 fixation measurements

Depth profiles of N_2 fixation from November, February and March revealed significant variability within the euphotic zone. In each profile, highest activity was near the surface and rates decreased with increasing water depth to below detection (≤0.03 µmol N m^{-3} d^{-1}) at approximately 75 m (Fig. 1). The cumulative contribution of N_2 fixation as a function of depth also illustrates that euphotic zone diazotrophy was near-surface-dominated (Fig. 2). At all depths sampled, the majority (mean 64 ± 5 [SE] %, range 31 to 100%) of net N_2 fixed was found in the <10 µm fraction (Table 1). Depth-integrated rates (0 to 125 m) indicated that the lowest N_2 fixation rates occurred in November (20.2 µmol m^{-2} d^{-1}, whole water) and dramatically higher rates occurred in March (109 µmol m^{-2} d^{-1}, whole water). In these profiles, depth trends of the size distribution of N_2 fixed do not parallel size-fractionated phycoerythrin concentrations (0.4 to 5.0 µm and 5.0 to 10.0 µm) or microscopy counts of *Crocosphaera*-like cells (data not shown). Surface N_2 fixation rates were determined in whole water over the course of the sampling period (April to March) (Fig. 3). Highest diazotroph activity was observed in July and August (1.63 to 1.68 µmol N m^{-3} d^{-1}) and the maximum rate measured occurred in March. Consistently lower N_2 fixation rates (0.38 to 0.68 µmol N m^{-3} d^{-1}) were observed

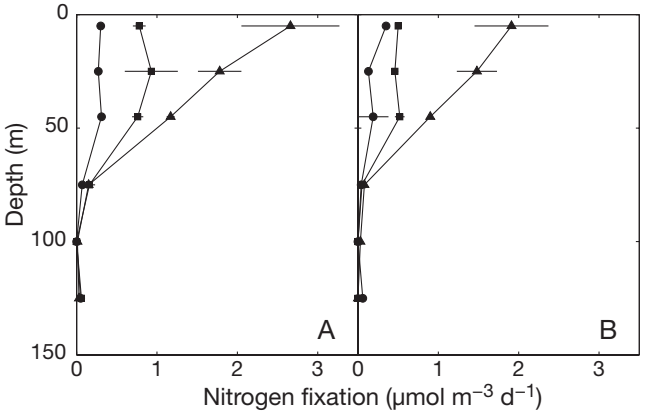

Fig. 1. Depth profiles of (A) whole-water and (B) size-fractionated (<10 µm) N_2 fixation rates during November 2004 (●), February 2005 (■) and March 2005 (▲). Error bars are SE (n = 3, where samples replicated)

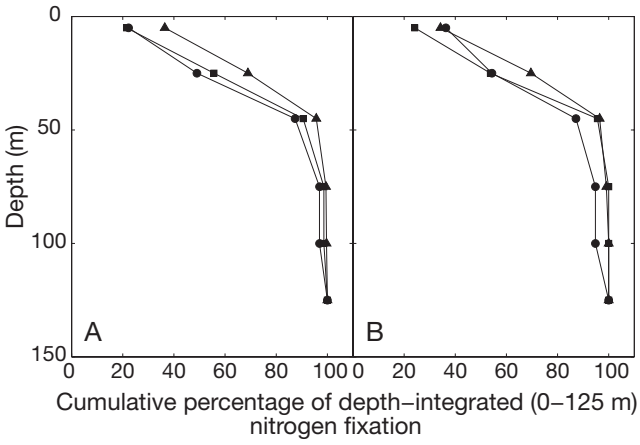

Fig. 2. Cumulative contribution of (A) whole-water and (B) size-fractionated (<10 µm) N_2 fixation rates during November 2004 (●), February 2005 (■) and March 2005 (▲)

during September to November, with the lowest rate in October.

The background ^{15}N isotopic abundance of the suspended particulate matter collected at 5 m showed a systematic seasonal trend with isotopically enriched values ($\delta^{15}N \geq 2$‰) in the winter when N_2 fixation rates are at their minimum, to maximum depletions ($\delta^{15}N$ = –1 to –2‰) in summer. These observations are consistent with an enhanced summertime supply of N via N_2 fixation (Fig. 3).

Table 1. N_2 fixation rates (mean ± SE, n = 3 [when samples replicated]; µmol N m^{-3} d^{-1}) in the upper portion of the water column (0 to 75 m) at Stn ALOHA. Rates were measured using $^{15}N_2$ tracer, and size-fractionated following a 24 h incubation. DL = detection limit of 0.03 µmol m^{-3} d^{-1}

Depth (m)	Whole water	Size fraction <10 µm	Size fraction >10 µm	Contribution of <10 µm size fraction (%)
HOT-165 (Nov 2004)				
5	0.30	0.35	<DL	100
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
Depth -integrated rate	20.2	14.5	6.9	
HOT-167 (Feb 2005)				
5	0.78 ± 0.08	0.50 ± 0.01	0.28 ± 0.08	64
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
Depth-integrated rate	54.4	31.1	23.4	
HOT-168 (Mar 2005)				
5	2.66 ± 0.61	1.91 ± 0.46	0.75 ± 0.76	72
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 ± 0.01	0.07 ± 0.08	53
Depth-integrated rate	109	83.7	26.3	

SRP variability

The temporal variability in surface SRP concentrations was similar to that observed in N_2 fixation rates between April and March (Fig. 3). Highest recorded SRP concentrations during this time precede a 2 mo period of relatively high N_2 fixation rates in the surface water. The lowest surface SRP concentrations, occurring in October and November (6.1 to 9.9 nM), are concurrent with the lowest measured diazotroph activity. SRP turnover time during this 1 yr study period varied from 2.1 to 14.8 d. There is a substantial, positive but non-significant correlation between SRP turnover time and surface N_2 fixation rates ($r^2 = 0.39$, $p = 0.07$).

Nutrient amendment experiments

To determine the effect of P addition on N_2 fixation rates, experiments were performed on 7 of the 9 cruises included in the present study. Variable concentrations of P (160 to 320 nM PO_4) were added during these experiments. The diazotroph response to P enrichment varied between cruises (Table 2). Although substantial increases in N_2 fixation rates were occasionally observed upon addition of P (October, November, February, March), no response was statistically significant relative to control treatments based on *t*-tests ($p < 0.05$) and ANOVA.

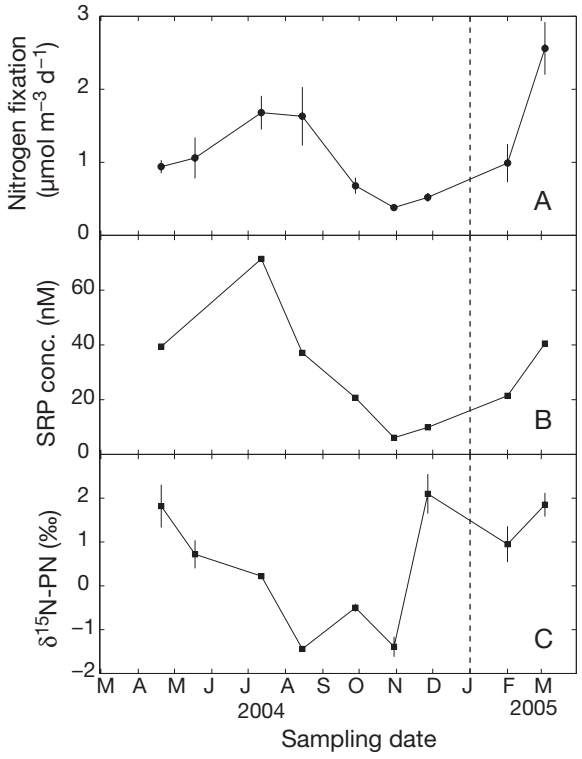

Fig. 3. (A) Surface (5 m) N_2 fixation rates, (B) soluble reactive phosphorus (SRP) concentration and (C) suspended δ^{15}N-PN (particulate nitrogen) from April 2004 to March 2005. Error bars in (A) and (C) are SE (n = 3). The vertical dashed line marks the end of 2004

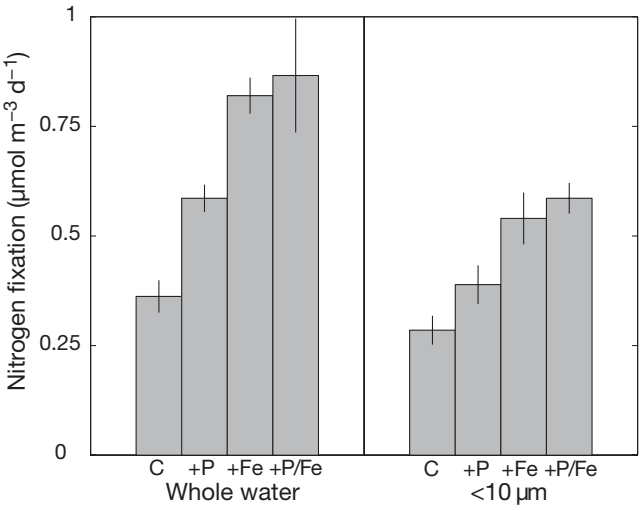

Fig. 4. N_2 fixation rates in control, +P, +Fe, and +P/Fe treatments for whole-water and <10 µm size fraction diazotrophs from the February 2005 manipulation experiment. Error bars are SE (n = 3)

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 combined Fe and P (2.0 nM Fe and 160 nM P added) treatments in whole water or in the <10 µm size fraction in November. However, identical manipulations in February revealed significantly enhanced N_2 fixation in whole water and in the <10 µm size fraction for both Fe and Fe + P treatments, relative to control incubations (Fig. 4). 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 and P to whole water also resulted in significant increases of 142% (ANOVA, $p < 0.05$). Incorporation of ^{15}N into the <10 µm size fraction increased 86 and 100% with addition of Fe and Fe + P, respectively (both increases significant, ANOVA, $p < 0.05$). In the March enrichment experiment, whole water and <10 µm size fraction both showed increased N_2 fixation rates with Fe addition (32 and 37%, respectively), and no increase with Fe and P addition (Fig. 5). Statistical significance of these increases (March experiment) could not be assessed due to lack of replication.

Table 2. Summary of whole-water P-addition experiments. Where ±SE is shown, n = 3. SRP = soluble reactive phosphorus, NF = nitrogen fixation, na = not available

Date	Ambient SRP (nM)	Average added P (nM)	Total P (nM)	% P added	Control NF rate (µmol m^{-3} d^{-1})	+PO_4 NF rate (µmol m^{-3} d^{-1})
May 2004	na	264	na	na	0.43 ± 0.12	0.48 ± 0.03
Jul 2004	71.4	168	239	140	1.68	1.56
Aug 2004	37.1	157	194	320	1.63 ± 0.40	1.72 ± 0.22
Oct 2004	6.1	174	180	2750	0.38 ± 0.03	0.71
Nov 2004	9.9	160	170	1520	0.60 ± 0.11	1.07
Feb 2005	21.4	160	181	650	0.36 ± 0.04	0.59 ± 0.03
Mar 2005	40.5	160	201	300	2.87	2.96
Mar 2005	40.5	320	361	690	2.87	3.73

DISCUSSION

In N-limited oceanic regions like the NPSG, N_2-fixing microorganisms would have a selective ecological advantage provided they have a source of energy and an available supply of other essential nutrients, especially P and Fe. At Stn ALOHA, the total N_2 fixation rate is the product of the biomass of N_2-fixing microbes and the biomass-specific rate of N_2 fixation, each controlled by (solar) energy availability and nutrient availability. In each depth profile, highest N_2 fixa-

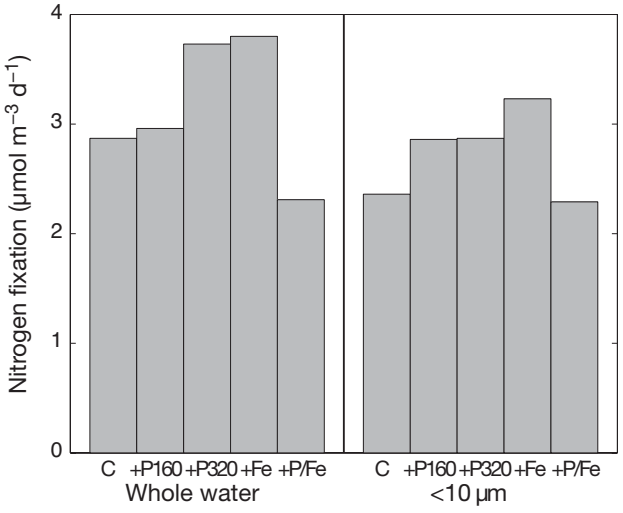

Fig. 5. N_2 fixation rates in control (C), +P 160 nM, +P 320 nM, +Fe, and +P/Fe treatments for whole-water and <10 µm size fraction diazotrophs from the March 2005 manipulation experiment

tion is present near the surface and the rates decrease to below the detection limit (detection limit = 0.03 µmol N m^{-3} d^{-1}) at approximately 75 m; an average of 95% of total diazotrophy takes place in the upper euphotic zone (0 to 75 m; Figs. 1 & 2). This depth dependence of N_2 fixation is consistent with the high energy requirement of diazotrophs (Karl et al. 2002). Solar energy photon fluxes at the 75 m depth horizon vary from approximately 1 mol quanta m^{-2} d^{-1} in winter to >2 mol quanta m^{-2} d^{-1} in summer (Letelier et al. 2004). At greater depths, the systematic increase in fixed N, particularly nitrate, would also select against N acquisition via N_2 fixation. Depth trends of the size distribution of N_2 fixed do not parallel size-fractionated phycoerythrin concentrations (0.4 to 5.0 µm and 5.0 to 10.0 µm, respectively) or microscopy counts of *Crocosphaera*-like cells (data not shown) suggesting that (1) not all diazotrophs contain phycoerythrin, (2) *Crocosphaera*-like cells may not be the dominant N_2 fixers, (3) cell numbers or biomass cannot be used to estimate rates (i.e. variable N_2 fixation rate per unit biomass) and/or (4) the ^{15}N in the <10 µm size fraction may not be solely attributed to N_2 fixation (i.e. it could be a consequence of 'secondary' labeling via excretion of $^{15}NH_4^+$ or ^{15}N-labeled dissolved organic nitrogen [^{15}N-DON]). Because diazotrophs require nearly an order of magnitude more Fe than photolithoautotrophs growing on ammonium (Kustka et al. 2003), the biomass of diazotrophs and therefore total rates of N_2 fixation may be controlled by competition for Fe and P. The potential dependence of diazotrophy on Fe availability is highlighted in the February manipulation experiments in which N_2 fixation increased 128 and 142% for whole water and 86 and 100% for the small (<10 µm) size fraction above corresponding controls following addition of Fe and Fe + P, respectively (Fig. 4). However, the effect of the addition of P or Fe (or both) appeared to be variable perhaps due to the design of the field experiments or the complex dynamics of P and Fe or the availability of other essential nutrients. Consequently, further investigation is required to determine proximate and ultimate controls on diazotrophy at Stn ALOHA.

Although *Trichodesmium* spp. has been viewed as a dominant diazotroph in the open ocean (Capone et al. 1997), the post-incubation ^{15}N incorporation in the present study indicates that labeling of the <10 µm size fraction is generally much greater than in the >10 µm fraction (mean 64 ± 5 [SE]%; range 30 to 100%). As mentioned previously, the labeling of <10 µm particulate matter with ^{15}N is controlled by assimilation of $^{15}N_2$ (i.e. N_2 fixation) as well as uptake of recently released $^{15}NH_4^+$ or ^{15}N-DON from diazotrophs, which could affect the inferred size distribution of N_2 fixation. The potential role of small, perhaps unicellular, diazotrophs at Stn ALOHA (also see Dore et al. 2002) presents a new paradigm for open ocean systems. Although other interpretations are also possible, a shift in the size structure of diazotrophy has large implications for the N cycle and for net C sequestration, as small cells may not sink as readily as large cells, such as diatoms that harbor diazotrophic cyanobacteria. 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.

N_2 fixation rates presented here agree well with previous observations. From November to March, areal diazotroph activities in whole water and for the <10 µm size fraction are similar to other published rates from the North Pacific (Table 3). 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. 2005). In addition, the temporal trend of N_2 fixation at Stn ALOHA for the period of April to March is consistent with previously documented seasonality in which higher N_2 fixation took place in the spring and summer months and lower

Table 3. Summary of euphotic zone N_2 fixation rate estimates in the North Pacific Ocean. ARA = acetylene reduction assay

Areal estimate (μmol N m^{-2} d^{-1})	Method	Source
<10 μm size fraction		
92	$^{15}N_2$	Zehr et al. (2001)
23 ± 6	$^{15}N_2$	Dore et al. (2002)
66 ± 19	$^{15}N_2$	Montoya et al. (2004)
43 ± 21	$^{15}N_2$	Present study
Whole water		
72 ± 13	$^{15}N_2$	Dore et al. (2002)
61 ± 26	$^{15}N_2$	Present study
***Trichodesmium* spp.**		
135	ARA	Gundersen et al. (1976)
33	ARA	Mague et al. (1977)
85–140	ARA	Karl et al. (1997)
53–143	ARA	Mahaffey et al. (2005)

activity was observed during winter (Dore et al. 2002). The natural variation in the abundance of the 2 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 depleting ^{15}N in the newly formed particulate organic matter. Surface N_2 fixation was high in July and August (1.63 to 1.68 μmol N m^{-3} d^{-1}) followed by the most depleted surface-suspended δ^{15}N-PN (–1.44 to –0.5‰) from August through October. Consistently low N_2 fixation rates were observed from September through November (0.38 to 0.68 μmol N m^{-3} d^{-1}), coincident with the heaviest recorded δ^{15}N-PN of the surface-suspended PN (2.10‰; Fig. 3). The observed trends in N_2 fixation and δ^{15}N-PN are consistent with a contribution from N_2 fixation. The temporal offset between N_2 fixation and suspended δ^{15}N-PN illustrates that N_2 fixation contributes to large and dynamic N pools and that suspended δ^{15}N-PN can also be affected by other processes such as recycling of N in the surface waters at Stn ALOHA (Checkley & Miller 1989). N_2 fixation has been shown to contribute new N on the order of that delivered via advection of NO_3^- from beneath the euphotic zone (Capone et al. 1997, Karl et al. 1997, Dore et al. 2002, Montoya et al. 2004). The rates measured in the present study, and in most other ^{15}N-uptake experiments, are probably minimum estimates because they do not track gross N_2 fixation (Bronk et al. 1994). Any $^{15}N_2$ reduced and subsequently added to the dissolved inorganic or dissolved organic pools will not be accounted for with the methods employed. There are also potential problems related to undersampling both in time and in space, because oceanic N_2 fixation has been suggested to be an ephemeral phenomenon in open ocean ecosystems (Dore et al. 2008). Furthermore, if N_2 fixation is supported by large organisms such as *Trichodesmium* spp. or endosymbiont-containing diatoms, then the conventional water collection methods used in the present study may have selected against them.

Experimental (Mills et al. 2004) and theoretical (Karl 2002, Kustka et al. 2003) evidence has been presented for the importance of Fe and P as controls on diazotrophy. Euphotic zone SRP decline of nearly 80% over the past decade at Stn ALOHA (Karl 2007) and observed diazotroph limitation by P in the Atlantic Ocean (Sañudo-Wilhelmy et al. 2001) emphasize the potential for P control of diazotroph activity. In addition, Boyle et al. (2005) provide data indicating that maximum (March to June) and minimum (July to January) dust delivery of Fe at Stn ALOHA occurs concurrently with our measured N_2 fixation rate variability. Total dissolved (<0.4 μm) Fe concentrations at Stn ALOHA (0.2 to 0.8 nM) may not be considered limiting to primary production throughout most of the year (Boyle et al. 2005). However, the presence of colloidal (0.02 to 0.4 μm) Fe suggests that not all 'total dissolved Fe' may be available for metabolism (Wu et al. 2001), which could lead to concentrations of available Fe that limit diazotroph activity.

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 were temporally coherent throughout the 1 yr study period (Fig. 3). Relatively high surface N_2 fixation rates (July and August) occurred after the highest observed SRP concentrations; in contrast, the lowest surface SRP concentrations occurred with the lowest rates of N_2 fixation. P-stressed plankton may cause more rapid turnover times of SRP inventories. Calculated SRP turnover time during April 2004 to March 2005 varied from 2.1 to 14.8 d. The average SRP turnover time (8 d) during that same period is less than one-half of that calculated by Karl & Yanagi (1997) during September 1991 to 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 & Karl 2003). This overall progression to more rapid turnover times is consistent with the observed decrease in SRP at Stn ALOHA between these study periods, and the continued P requirement by diazotrophs and all other microbes. In an environment with increased competition for SRP, diazotrophs may likewise accelerate their turnover of SRP or enhance their use of dissolved organic phosphorus (DOP) which represents nearly 90% of the total dissolved P pool in the near-surface waters at Stn ALOHA (Karl & Björkman 2002). Experi-

ments conducted in the present study occasionally revealed substantial increases in N_2 fixation upon P amendment. The variability in response relative to ambient SRP concentration (i.e. response to amendment during low SRP in October and November and during higher SRP in March) indicates that P alone does not continuously control diazotroph activity. Furthermore, treatments amended with Fe showed substantial and significant increases in N_2 fixation. These observations support the hypothesis that, along with SRP dynamics, Fe availability plays a critical role in diazotroph activity at Stn ALOHA.

CONCLUSIONS

N_2 fixation is important in the nutrient dynamics and productivity of the NPSG as evidenced by the substantial diazotroph activity throughout the year in the upper euphotic zone. Rates of N_2 fixation at Stn ALOHA are controlled both by the biomass of N_2-fixing microorganisms and the activity per unit biomass. Diazotroph biomass and biomass-specific rate are determined by nutrient dynamics, competition, natural selection and light levels. N_2 fixation rates at Stn ALOHA display a seasonal pattern that mimics surface SRP concentrations and seasonal Fe delivery from the atmosphere, which impacts the biomass of N_2-fixing microorganisms and ultimately the rates of N_2 fixation. While we do not yet have a predictive framework for determining changes in N_2 fixation, the data presented here help to delimit the number of 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.

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