Click Here for Full Article

Physical forcing of nitrogen fixation and diazotroph community structure in the North Pacific subtropical gyre

Matthew J. Church,[1] Claire Mahaffey,[1,2] Ricardo M. Letelier,[3] Roger Lukas,[1] Jonathan P. Zehr,[4] and David M. Karl[1]

Received 1 November 2008; revised 13 March 2009; accepted 25 March 2009; published 20 June 2009.

[1] Dinitrogen (N_2) fixing microorganisms (termed diazotrophs) exert important control on the ocean carbon cycle. However, despite increased awareness on the roles of these microorganisms in ocean biogeochemistry and ecology, the processes controlling variability in diazotroph distributions, abundances, and activities remain largely unknown. In this study, we examine 3 years (2004–2007) of approximately monthly measurements of upper ocean diazotroph community structure and rates of N_2 fixation at Station ALOHA (22°45′N, 158°W), the field site for the Hawaii Ocean Time-series program in the central North Pacific subtropical gyre (NPSG). The structure of the N_2-fixing microorganism assemblage varied widely in time with unicellular N_2-fixing microorganisms frequently dominating diazotroph abundances in the late winter and early spring, while filamentous microorganisms (specifically various heterocyst-forming cyanobacteria and *Trichodesmium* spp.) fluctuated episodically during the summer. On average, a large fraction (~80%) of the daily N_2 fixation was partitioned into the biomass of <10 μm microorganisms. Rates of N_2 fixation were variable in time, with peak N_2 fixation frequently coinciding with periods when heterocystous N_2-fixing cyanobacteria were abundant. During the summer months when sea surface temperatures exceeded 25.2°C and concentrations of nitrate plus nitrite were at their annual minimum, rates of N_2 fixation often increased during periods of positive sea surface height anomalies, as reflected in satellite altimetry. Our results suggest mesoscale physical forcing may comprise an important control on variability in N_2 fixation and diazotroph community structure in the NPSG.

Citation: Church, M. J., C. Mahaffey, R. M. Letelier, R. Lukas, J. P. Zehr, and D. M. Karl (2009), Physical forcing of nitrogen fixation and diazotroph community structure in the North Pacific subtropical gyre, *Global Biogeochem. Cycles*, *23*, GB2020, doi:10.1029/2008GB003418.

1. Introduction

[2] The North Pacific subtropical gyre (NPSG) is one of the Earth's largest habitats and comprises the most expansive of the ocean gyres [*Sverdrup et al.*, 1942; *Karl*, 1999; *Karl et al.*, 2008]. Biomass and productivity in the NPSG are dominated by diverse microscopic plankton. To date however, the processes controlling the time and space variability in the activities and distributions of these marine microorganisms are not well understood, challenged in part by the sheer size and remoteness of this ecosystem together with the spatiotemporal complexity of the oceanic habitat.

[3] Since 1988, the Hawaii Ocean Time-series (HOT) program has conducted approximately monthly shipboard measurements on a suite of biogeochemical and physical properties at deep-water sampling sites in the subtropical North Pacific. The resulting ~20 years of observations provide unique insight into ecosystem dynamics occurring over time scales ranging from diurnal to interdecadal. Foremost among these observations has come recognition of the important role that N_2-fixing bacteria play in controlling bioelemental cycling and carbon export in subtropical ocean habitats. Geochemical and biological evidence suggests N_2 fixation may contribute more than half of the total nitrogen supporting new production in subtropical ocean ecosystems [*Michaels et al.*, 1996; *Dore et al.*, 2002; *Karl et al.*, 2002].

[4] Biological N_2 fixation is the exclusively prokaryote-mediated biochemical reduction of N_2 to ammonia; the reaction is catalyzed by the enzyme nitrogenase. In marine ecosystems, much of our understanding of N_2-fixing microorganism ecology and biogeochemistry derives from studies of filamentous cyanobacteria, including members of the genera *Trichodesmium* spp. and *Richelia* spp. These cyanobacteria are known to form large, near-surface ocean accu-

[1]Department of Oceanography, School of Ocean and Earth Science and Technology, University of Hawai'i at Mānoa, Honolulu, Hawaii, USA.
[2]Now at Department of Earth and Ocean Sciences, University of Liverpool, Liverpool, UK.
[3]College of Ocean and Atmospheric Sciences, Oregon State University, Corvallis, Oregon, USA.
[4]Ocean Sciences Department, University of California, Santa Cruz, California, USA.

Copyright 2009 by the American Geophysical Union.
0886-6236/09/2008GB003418$12.00

mulations [*Karl et al.*, 1992; *Carpenter et al.*, 1999; *Dore et al.*, 2008], that are often extensive enough to be detected by Earth-orbiting satellites [*Subramaniam et al.*, 2001; *Westberry and Siegel*, 2006; *Wilson and Qiu*, 2008]. The occurrence of such blooms appears highly variable and the processes underlying the formation of N_2-fixing blooms in the open ocean remain largely unknown [*White et al.*, 2007]. Although these filamentous, bloom-forming N_2-fixing organisms are known to be important to ocean biogeochemistry, application of molecular-based tools continues to broaden appreciation of the diversity, abundances, and activities of other, less conspicuous groups of diazotrophs in the oceans [*Zehr et al.*, 2001; *Mazard et al.*, 2004; *Langlois et al.*, 2005; *Church et al.*, 2008; *Moisander et al.*, 2008; *Zehr et al.*, 2008]. Surveys of planktonic *nifH* gene (the gene that encodes a component of nitrogenase) diversity and expression have identified several types of unicellular cyanobacteria as well as diverse members of the *Proteobacteria* that appear active in N_2 fixation in the sea [*Zehr et al.*, 1998; *Church et al.*, 2005b; *Langlois et al.*, 2005; *Fong et al.*, 2008]. To date however, the factors controlling the activities, diversity, and biomass of these different groups of marine diazotrophs remain largely unknown.

[5] In this study, we examined approximately 3 years (2004–2007) of monthly measurements on upper ocean (0–125 m) N_2 fixation with shipboard, satellite, and moored platform measurements of ocean physics and biogeochemistry at Station ALOHA (A Long-term Oligotrophic Habitat Assessment; 22°45′N, 158°W), the field site for the HOT program [*Karl and Lukas*, 1996]. Together, these time series measurements provided insight into the linkages between mesoscale physical dynamics and N_2-fixing microorganism community structure in the NPSG. By integrating these shipboard, mooring, and satellite-based time series measurements our results suggest that mesoscale physical processes play an important role in structuring diazotroph assemblages and controlling rates of N_2 fixation in this region.

2. Methods

2.1. Physical Habitat Measurements

[6] High-frequency determinations of seawater density and temperature at Station ALOHA were measured at two instrumented physical and biogeochemical bottom-moored platforms called HALE ALOHA (Hawaii Air-sea Logging Experiment A Long-term Oligotrophic Habitat Assessment) and WHOTS (Woods Hole Hawaii Ocean Time-series Station), respectively. HALE ALOHA operated from August 2004 to July 2007 and was deployed approximately 11 km west of center of the 11 km radius circle that defines Station ALOHA. The WHOTS mooring was initially deployed in August 2004 and continues to reside within the southeastern quadrant of the ALOHA circle. Both HALE ALOHA and WHOTS maintained a near-continuous presence during the study period (2004–2007), operating with a duty cycle of 3 to 6 months (HALE ALOHA) or ~1 year (WHOTS) with only brief breaks in data collection during mooring and instrument servicing. Among other instruments, the HALE ALOHA mooring held 8 to 12 self-contained, submersible thermistor data loggers (Richard Brancker Research, YSI46033 and TR-1050) and the WHOTS mooring contained 15 internally logging Sea-Bird SeaCATs distributed throughout the upper ocean (<155 m). Together these instruments provided high-frequency (10 min sampling interval) determinations of seawater temperature and conductivity. Daily averaged temperatures and potential density (σ_θ) were computed from these high-frequency records. In addition to these moored platform measurements, monthly ship-based vertical profiles of temperature, salinity, and pressure were obtained using a SeaBird conductivity-temperature-depth (CTD) sampler described by *Karl and Lukas* [1996].

2.2. Seawater Biogeochemistry

[7] Seawater samples for subsequent biogeochemical analyses were collected from discrete depths in the upper ocean using twenty four 10 L PVC sampling bottles attached to the CTD rosette sampling system. Water for determination of nutrient concentrations was subsampled into acid washed 125 ml or 500 ml polyethylene bottles and the bottles were capped and frozen upright. At the shore-based laboratory, high-sensitivity measurements of nitrate plus nitrite (N + N) and PO_4^{3-} were determined as described by *Dore and Karl* [1996] and *Karl and Tien* [1992], respectively. Concentrations of inorganic nutrients (N + N, PO_4^{3-}, and $Si(OH)_4$) in the subeuphotic zone waters were determined using either a Technicon Autoanalyzer II or a Bran and Luebbe Autoanalyzer III.

[8] Chlorophyll *a* concentrations were determined fluorometrically using a Turner AU-10 fluorometer as described by *Letelier et al.* [1996]. Determinations of particulate silica concentrations were based on a modified version of the *DeMaster* [1981] time course carbonate dissolution procedure as described by *Dore et al.* [2008].

2.3. Satellite-Derived Sea Surface Height Anomalies

[9] Weekly determinations of sea surface height anomalies (SSHA) in the vicinity of Station ALOHA (measured at 1/3° by 1/3° resolution) were obtained from the Archiving, Validation, and Interpretation of Satellite Oceanographic (AVISO) data server (http://las.aviso.oceanobs.com/). These near–real time satellite altimetry measurements represent the derived merged products of the TOPEX/Poseidon, ENVISAT, and JASON 1 satellite altimeters.

2.4. Rates of N_2 Fixation and Diazotroph Abundances

[10] Samples for subsequent measurements of diazotroph community structure and rates of N_2 fixation were collected on monthly HOT cruises to Station ALOHA. Seawater samples were obtained using the same CTD rosette sampling device previously described. Rates of N_2 fixation were measured from discrete water samples collected at 6 depths (5, 25, 45, 75, 100, 125 m) on a total of 31 cruises between November 2004 and September 2007, including selected measurements (November 2004, January 2005, July 2005) described elsewhere [*Fong et al.*, 2008; *Grabowski et al.*, 2008].

[11] Rates of N_2 fixation were determined on the basis of planktonic assimilation of $^{15}N_2$ [*Montoya et al.*, 1996]. Seawater was subsampled from the CTD rosette bottles into 25 L polyethylene carboys and four 4.5 L polycarbonate

bottles were filled, using care to minimize introduction of air bubbles; bottles were sealed with septum fitted caps, and spiked with 3 ml of 98% $^{15}N_2$ (Isotech Laboratories, Inc.). The 4.5 L bottles were attached to a surface-tethered, free drifting array, deployed before dawn, and incubated in situ for approximately 24 h. Upon recovery of the array, two bottles from each depth were pressure filtered onto precombusted 25 mm glass fiber filters for bulk seawater determinations of N_2 fixation. In addition, two bottles from each depth were sequentially size fractionated through 25 mm diameter, 10 μm polycarbonate filters followed by filtration onto 25 mm precombusted glass fiber filters to assess the partitioning of ^{15}N into microorganisms <10 μm in diameter. Glass fiber filters were dried for 24 h, pelleted, and the ^{15}N enrichment of particulate material captured on the filters was measured using an elemental analyzer-isotope ratio mass spectrometer (EA-IRMA Carlo Erba NC2500 coupled to a Thermo-Finnigan Delta S). Rates of $^{15}N_2$ fixation were calculated as described by *Capone and Montoya* [2001].

[12] Seawater samples for subsequent determination of cyanobacterial *nifH* gene abundances were collected from 8 depths in the upper ocean (5, 25, 45, 75, 100, 125, 150, 175 m) on a total of 34 cruises between October 2004 and October 2007. Water was subsampled from the CTD rosette bottles into acid washed 4.5 L polycarbonate bottles and between 1 and 2 L was filtered through 25 mm, 0.22 μm pore size Supor® (Gelman) or Durapore® filters (Millipore). Filters were stored in 2.0 ml microcentrifuge tubes containing 500 μl of Tris-EDTA (10 mM Tris, pH 7.4; and 1 mM EDTA) or 500 μl lysis buffer (20 mM Tris-HCL, pH 8.0; 2 mM EDTA, pH 8.0; 1.2% Triton X and 20 mg ml^{-1} lysozyme). Samples were flash frozen in liquid nitrogen and stored frozen at −80°C until extraction. DNA was extracted and purified using the Qiagen DNeasy kit following the manufacturer's recommended protocols.

[13] To evaluate temporal variations in the abundances of N_2-fixing microorganisms, we utilized the quantitative PCR (QPCR) assay described by *Short et al.* [2004] to amplify plankton *nifH* genes. For this study, we examined the dynamics of 6 *nifH* containing cyanobacteria frequently observed at Station ALOHA [*Zehr et al.*, 1998, 2001; *Church et al.*, 2005a]. These cyanobacteria included: *Trichodesmium* spp.; *Crocosphaera* spp.; three groups of uncultivated heterocystous cyanobacteria (termed Het-1, Het-2, and Het-3); and an uncultivated group of cyanobacteria termed "group A." Descriptions of the QPCR primers and probes used can be found in the work by *Church et al.* [2005a, 2008]. QPCR reactions were run in duplicate and the detection limit of these reactions corresponded to ~50 gene copies per liter of seawater.

3. Results

3.1. Seasonal and Episodic Variability in Environmental Forcing

[14] Consistent with the ~20 year climatology at Station ALOHA near-surface ocean temperatures, irradiance, and upper ocean concentrations of N + N all varied seasonally during the period of this study (Figure 1). Incident irradiance increased more than twofold in the summer (~50 mol quanta m^{-2} d^{-1}) relative to the wintertime minima (~15 mol quanta m^{-2} d^{-1}). Inventories (0–100 m) of N + N were highly variable, ranging between 0.075 to 10.3 mmol N m^{-2}, with peak concentrations coinciding with periods of wintertime cooling and increased mixing of upper ocean waters (Figure 1). In contrast, inventories of PO_4^{3-} were generally greater (ranging between 0.99 and 6.7 mmol P m^{-2}) and less seasonally variable than N + N (Figure 1). The resulting [N + N]:PO_4^{3-} ratios were elevated in the winter months and decreased during the summer, but the resulting ratios were consistently less than the Redfield ratio (Figure 1 and Table 1).

[15] Daily averaged measurements of upper ocean (<200 m) temperatures and σ_θ at the HALE ALOHA and WHOTS moorings were analyzed with weekly satellite-derived determinations of SSHA to evaluate the frequency and duration of episodic physical variability at Station ALOHA. Near-surface ocean (14 m) temperatures measured on the HALE ALOHA mooring fluctuated ~4°C over an annual cycle (typically peaking ~27.5°C in late August and declining to 23.2°C in February (Figure 2a)). Overlain on this seasonal variability were much weaker, but higher-frequency fluctuations in temperature and σ_θ that often persisted for several days (Figure 2a). Near-surface ocean (15 m) potential density fluctuated seasonally between ~22.5 kg m^{-3} and 24.2 kg m^{-3}, with seawater density increasing in the late winter and declining through the summer and fall (data not shown). In contrast to the upper ocean, neither temperature nor σ_θ in the lower euphotic zone (140 and 135 m, respectively) demonstrated clear seasonality (Figure 2b), with both properties displaying higher-frequency variability, often fluctuating over weekly to monthly time scales (Figure 2b).

[16] To identify the prominent time scales of upper ocean variability, the daily averaged HALE ALOHA temperature record from 14 m and 140 m were compared to the monthly and long-term (2004–2007) mean temperatures at these depths. Relative to the long-term mean temperatures in the upper euphotic zone (14 m) were dominated by seasonal forcing (Figure 2c), with weaker, day-to-day variations from the monthly mean persisting for periods of 1 to 3 weeks. In contrast, at 140 m daily temperature deviations from the monthly and long-term means were similar in magnitude with anomalously low or high temperatures occurring episodically and persisting for several weeks to months (Figure 2d). Temporal fluctuations in seawater temperature and σ_θ in the lower euphotic zone were synchronized with variations in satellite-derived SSHA (Figure 3). Over the period of observations in the present study (2004–2007), satellite SSHA varied between −12 cm to 15 cm. On average, a 1 cm variation in SSHA corresponded to ~0.07°C change in temperature in the lower euphotic zone (Figure 3a), while variations in σ_θ at 135 m were inversely correlated with variations in SSHA (Figure 3b). Variations in SSHA were also significantly correlated to vertical fluctuations in isopycnal surfaces in the lower euphotic zone. On average, a 1 cm variation in SSHA equated to a ~2.3 m displacement in the vertical position of the 24.6 kg m^{-3} isopycnal surface (typically coinciding with the vertical position of the deep

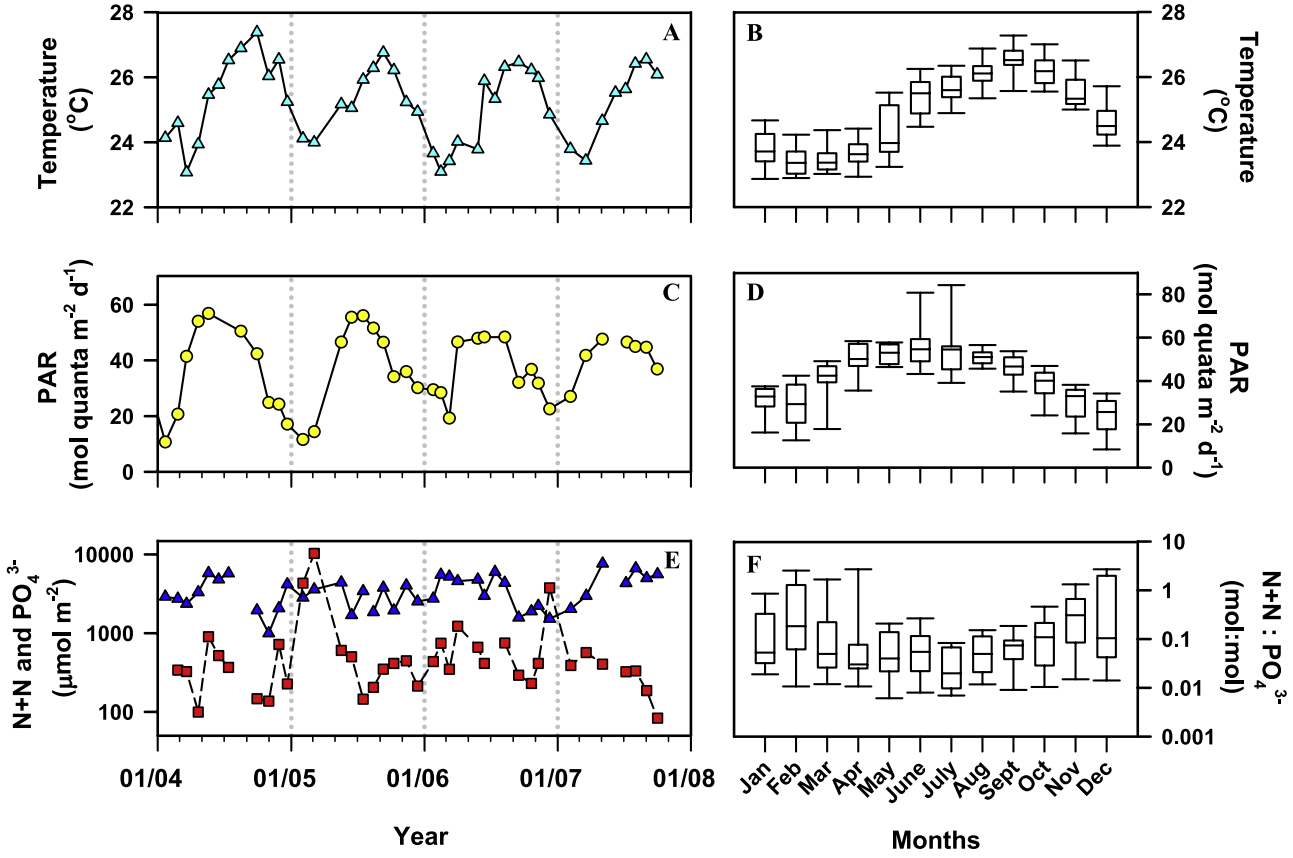


Figure 1. Temporal variability in temperature, irradiance (PAR), and nutrients at Station ALOHA. (a) Variations in near–sea surface temperatures measured at Station ALOHA during monthly HOT program cruises (2004–2007). (b) Box plots of monthly 20 year (1988–2007) climatology of near-surface ocean temperatures (5 m) at Station ALOHA; box boundaries define the 25th and 75th percentiles, lines inside the boxes represent median values, and the box whisker caps represent the 10th and 90th percentiles of the full time series temperatures. (c) Surface irradiance at Station ALOHA during this study. (d) Box plots depicting monthly 20 year climatology (1988–2007) surface PAR flux. (e) Depth-integrated (0–100 m) concentrations of N + N (squares) and PO_4^{3-} (triangles) at Station ALOHA during this study. (f) Box plots depicting monthly 20 year climatology (1988–2007) upper ocean (0–100 m) N + N:PO_4^{3-} ratios.

chlorophyll maxima) measured on the monthly HOT cruises (Figure 3c).

[17] Analyses of the SSHA record in the ALOHA region during the period of study revealed a total of 18 anomalous events where the SSHA deviated from the long-term (1992–2007) mean for this region (i.e., SSHA fluctuations greater than 1 standard deviation of the mean 15 year SSHA record for this region). In total, 10 of these 18 events were periods of strong positive SSHA and 8 corresponded with periods of strong negative SSHA. Monthly HOT cruises to Station ALOHA coincided with 8 of these positive (September 2005, December 2005, July 2006, September 2006, October 2006, November 2006, May 2007, and June 2007) and 6 negative (June 2005, February 2006, May 2006, June 2006, March 2007, August 2007) SSHA events.

3.2. Vertical Variability in N_2 Fixation and Diazotroph Abundances

[18] Vertical profiles of $^{15}N_2$ assimilation were measured at approximately monthly intervals from both whole and <10 μm postincubation size fractionated seawater samples over a 3 year period (October 2004 to September 2007). Rates of $^{15}N_2$ assimilation were elevated in the well-lit near-surface water ($\leq$25 m) where whole seawater rates of N_2 fixation ranged from 0.5 to 11 nmol N L^{-1} d^{-1} (averaging 2.2 ± 1.9 nmol N L^{-1} d^{-1} (Figure 4)), and <10 μm post-incubation size fractionated rates of N_2 fixation ranged from 0.1 to 1.5 nmol N L^{-1} d^{-1} (averaging 1.4 ± 1.0 nmol N L^{-1} d^{-1} (Figure 4)). Rates of N_2 fixation in both the whole seawater and <10 μm size fraction decreased with depth. On average, the proportion of assimilated $^{15}N_2$ partitioned into the biomass of microorganisms <10 μm averaged ~75% of the bulk seawater rate throughout the upper 25 m, and increased to ~100% deeper in the euphotic zone (Figure 4).

[19] Vertical profiles of diazotroph distributions based on the QPCR-derived gene abundances indicated that *nifH*-containing cyanobacteria were a highly variable component of the upper ocean plankton. The *nifH* phylotype abundances were generally greater in the well-lit, nutrient-depleted upper ocean and often fell to below detection (<10^2 *nifH* gene

Table 1. Upper Ocean Rates of N_2 Fixation, Primary Production, Inorganic Nutrient Concentrations, and *nifH* Gene Inventories at Station ALOHA Binned by Season and SSHA[a]

	N+N (μmol N m^{-2})	PO_4^{3-} (μmol P m^{-2})	[N+N]:PO_4^{3-} (mol:mol)	^{14}C PP (mmol C m^{-2} d^{-1})	Whole N_2 Fix (μmol N m^{-2} d^{-1})	<10 μm N_2 Fix (μmol N m^{-2} d^{-1})	<10 μm N_2 Fix (%)	Group A (*nifH* Copies per Square Meter)	*Crocosphaera* (*nifH* Copies per Square Meter)	*Trichodesmium* (*nifH* Copies per Square Meter)	Hets (*nifH* Copies per Square Meter)
Spring (n = 7)	635	4,346	0.17	44.4	121	119	95	6.5×10^{10}	3.7×10^{9}	2.3×10^{8}	2.4×10^{9}
Min	404	1,691	0.05	40.7	58	28	47	8.1×10^{9}	1.6×10^{7}	ND	2.6×10^{7}
Max	1,224	4,347	0.30	47.8	180	186	134	1.7×10^{11}	1.3×10^{10}	6.4×10^{8}	1.2×10^{10}
Summer (n = 10)	323	4,102	0.09	43.9	150	86	65	2.8×10^{10}	2.0×10^{10}	1.0×10^{10}	2.5×10^{10}
Min	145	1,576	0.04	26.4	74	52	17	1.1×10^{9}	2.7×10^{7}	6.5×10^{6}	ND
Max	751	6,670	0.19	50.7	307	130	103	1.5×10^{11}	1.3×10^{11}	4.4×10^{10}	1.6×10^{11}
Fall (n = 10)	628	2,696	0.37	34.3	76	50	67	1.4×10^{10}	9.8×10^{9}	5.6×10^{9}	2.5×10^{9}
Min	83	993	0.01	24.2	20	14	40	1.1×10^{8}	9.6×10^{7}	ND	7.8×10^{7}
Max	3,404	5,588	2.5	45.7	261	187	83	3.9×10^{10}	9.1×10^{10}	4.3×10^{10}	5.7×10^{9}
Winter (n = 7)	2,446	3,556	0.73	36.4	87	78	88	6.8×10^{10}	1.9×10^{8}	4.8×10^{8}	4.8×10^{9}
Min	348	2,035	0.07	30.7	54	31	57	1.2×10^{10}	ND	ND	3.1×10^{8}
Max	10,318	5,507	2.9	41.6	110	110	112	2.0×10^{11}	8.4×10^{8}	$2.3\ 10^{9}$	1.4×10^{10}
Positive SSHA (n = 9)	475	3,372	0.15	36.8	144	85	67	2.5×10^{10}	5.7×10^{8}	6.5×10^{8}	1.1×10^{10}
Min	213	1,576	0.08	24.1	39	27	17	2.7×10^{8}	2.6×10^{7}	ND	ND
Max	1,224	5,994	0.27	50.7	307	186	134	5.8×10^{10}	1.5×10^{9}	2.5×10^{9}	7.2×10^{10}
Negative SSHA (n = 4)	581	3,740	0.18	42.7	143	141	99	3.1×10^{10}	1.6×10^{9}	2.6×10^{8}	1.7×10^{9}
Min	414	1,691	0.13	39.6	106	110	89	8.1×10^{9}	1.6×10^{7}	ND	1.2×10^{8}
Max	746	5,507	0.30	47.8	180	186	103	4.8×10^{10}	6.0×10^{9}	5.0×10^{8}	5.7×10^{9}
Grand average[b]	961 (1,954)	3,588 (1,662)	0.34 (0.68)	39.3 (7.49)	111 (66)	83 (49)	77 (24)	4.0×10^{10} (5.0×10^{10})	9.5×10^{9} (2.7×10^{10})	5.3×10^{9} (1.3×10^{10})	9.6×10^{9} (2.9×10^{10})

[a]Mean depth integrated (0–100 m) rates of N_2 fixation, ^{14}C primary production (^{14}C PP), gene abundances, and concentrations of N+N and PO_4^{3-} are presented; seasons are defined as winter, January, February, and March; spring, April, May, and June; summer, July, August, and September; and Fall, October, November, and December. Positive and negative SSHA were characterized as periods where SSHA were >1 standard deviation from the mean merged AVISO SSHA (1992–2007) product for this region. Grand averages were calculated for study period (October 2004 to October 2007). ND indicates *nifH* gene abundances below detection.

[b]Values in parentheses are standard deviations.

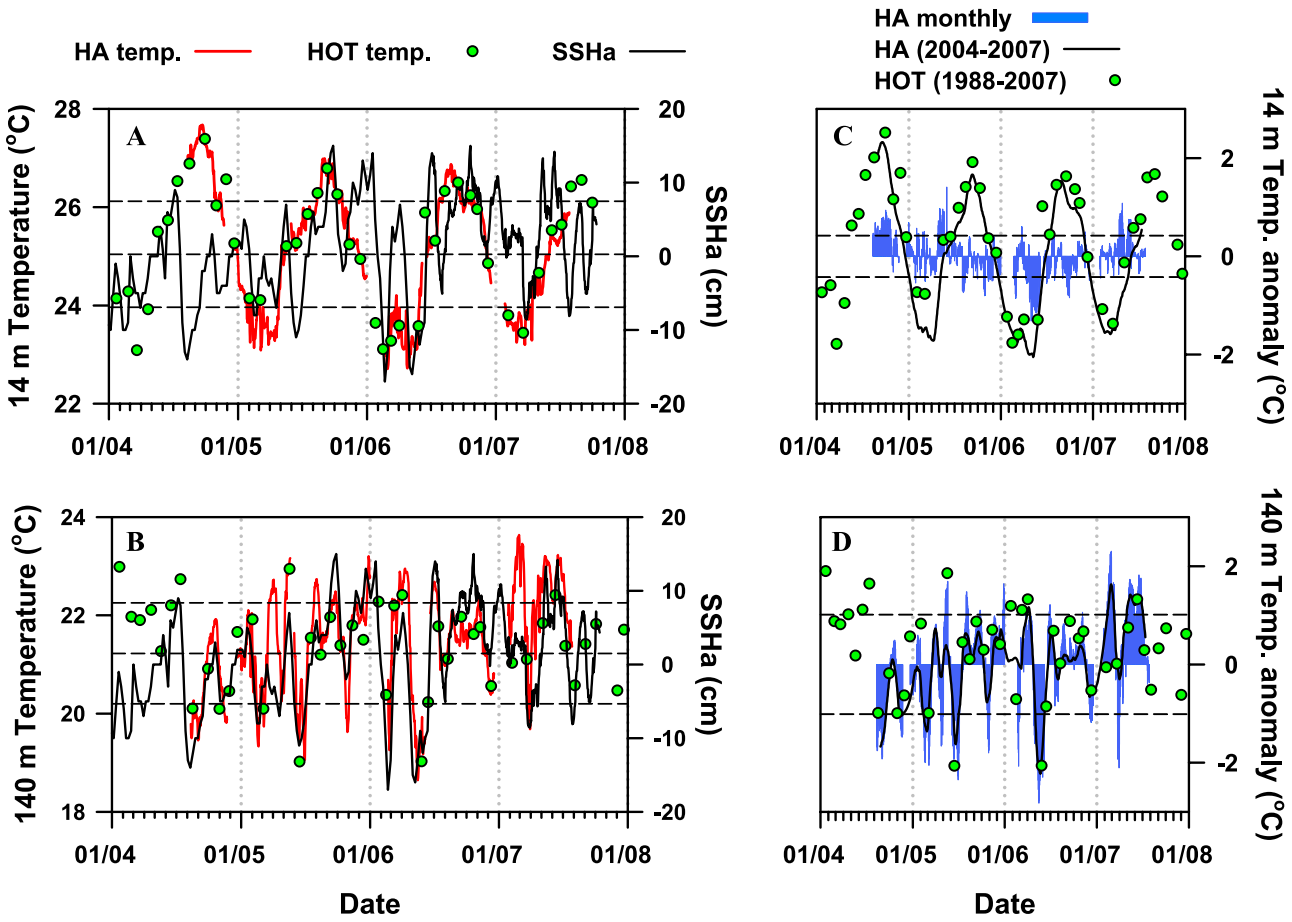


Figure 2. Time series of daily averaged HALE ALOHA temperatures (solid red line) and SSHA (solid black line) during this study (2004–2007). (a) Daily averaged near-surface ocean (14 m) temperatures measured on HALE ALOHA mooring (red line) and during monthly HOT cruises (green circles). Solid black line depicts merged AVISO SSHA product for region near ALOHA; also shown are mean SSHA (1992–2007) and ±1 standard deviation of the mean SSHA (dashed lines). (b) Daily averaged temperatures (140 m) measured on HALE ALOHA mooring (red line) and during monthly HOT cruises (green circles). Solid black and dashed lines are the same as those described for Figure 2a. (c) Temperature anomalies calculated from HALE ALOHA 14 m daily averaged temperatures. Thick solid line represents 30-point running mean of daily temperature deviation from the long-term (2004–2007) mean temperature at 14 m. Light blue bars are deviations in daily averaged 14 m temperatures from the monthly mean temperature at this depth; green circles are temperature anomalies during HOT cruises calculated relative to long-term (1988–2007) mean temperatures at Station ALOHA. Dashed lines depict ±1 standard deviation of the mean daily temperature deviation from monthly average. (d) Temperature anomalies calculated from HALE ALOHA 140 m daily averaged temperatures; lines and symbols are the same as described for Figure 2c.

copies per liter) in the lower regions of the euphotic zone (Figure 5). Among the six *nifH* phylotypes examined in this study the unicellular group A sequence type was often the most abundant. In the upper 75 m of the water, group A phylotype abundances averaged $\sim 10^5$ *nifH* copies per liter, decreasing to $<10^2$ copies per liter in the lower euphotic zone (Figure 5). When detectable, *nifH* abundances of *Trichodesmium* and *Crocosphaera* were greatest in the upper 75 m, varying between 10^3 to 10^4 *nifH* copies per liter, and frequently falling below the limits of detection ($<10^2$ *nifH* copies per liter) in the lower euphotic zone. The abundances of heterocystous *nifH* phylotypes were also highly variable; concentrations of these phylotypes were often below detection, particularly in the lower euphotic zone (Figure 5). Among the 3 heterocystous *nifH* phylotypes examined those targeted by the Het-2 primers and probes (attributed to the diatom endosymbiont *Richelia* [*Foster and Zehr*, 2006]) tended to be the most abundant, frequently accounting for ~60–100% of the three heterocystous cyanobacterial *nifH* genes examined.

3.3. Temporal Variability in N_2 Fixation and Diazotroph Abundances

[20] Depth-integrated (0–100 m) rates of N_2 fixation in both the whole seawater and <10 μm plankton size frac-

tionated samples were temporally variable, ranging more than an order of magnitude over the period of study (Figure 6). On average, there were no significant differences between rates of N_2 fixation in the whole and <10 μm seawater samples (Table 1 (one way ANOVA, P = 0.07)). Depth-integrated whole seawater N_2 fixation ranged between 20 and 310 μmol N m^{-2} d^{-1} (averaging 113 ± 66 μmol N m^{-2} d^{-1}), and <10 μm rates varied between ~14 and 189 μmol N m^{-2} d^{-1} (averaging ~86 ± 49 μmol m^{-2} d^{-1}). $^{15}N_2$ assimilation into <10 μm plankton biomass accounted for ~18 to >100% (averaging 76% (Table 1)) of the whole seawater N_2 fixation, suggesting assimilation of $^{15}N_2$ by microorganisms <10 μm accounted for a significant fraction of the total N_2 fixation. However, the large range of these data also indicates that $^{15}N_2$ assimilation by microorganisms >10 μm (difference between whole and <10 μm size fraction) was temporally variable, and at times comprised a major fraction of the total N_2 fixation. On selected occasions, rates of $^{15}N_2$ fixation in the <10 μm size fraction appeared greater than in the bulk seawater; however, the differences in these measured rates were not significant and likely derived from errors inherent to the size fractionation technique. Much of the temporal variability in the depth-integrated rates scaled on changes in the near-surface (<15 m) rates (Figure 6).

[21] Rates of N_2 fixation in the whole seawater samples tended to be elevated in the summer (July through September; averaging 150 μmol N m^{-2} d^{-1}) and lowest in the fall and winter (averaging 76 and 87 μmol N m^{-2} d^{-1}, respectively). However, this moderate seasonality was obscured by high interseasonal episodic variability, particularly during the summer months. As a result, there were no significant seasonal differences in whole seawater rates of N_2 fixation during the study (one-way ANOVA, P = 0.08). In contrast, rates of N_2 fixation in the <10 μm size fractionated samples were significantly greater in the spring (April through June) than at other times of the year (Table 1 (one-way ANOVA, P = 0.05)).

[22] Examination of the depth-integrated (0–100 m) *nifH* gene copies provided insight into temporal fluctuations in diazotroph abundances at Station ALOHA. Gene inventories of both unicellular (group A and *Crocosphaera* spp.) and filamentous (*Trichodesmium* spp. and heterocystous) cyanobacteria were highly variable, often fluctuating orders of magnitude between the monthly samplings (Figure 7). Over the 3 year period of this study, the group A phylotype abundances varied more than 3 orders of magnitude, with

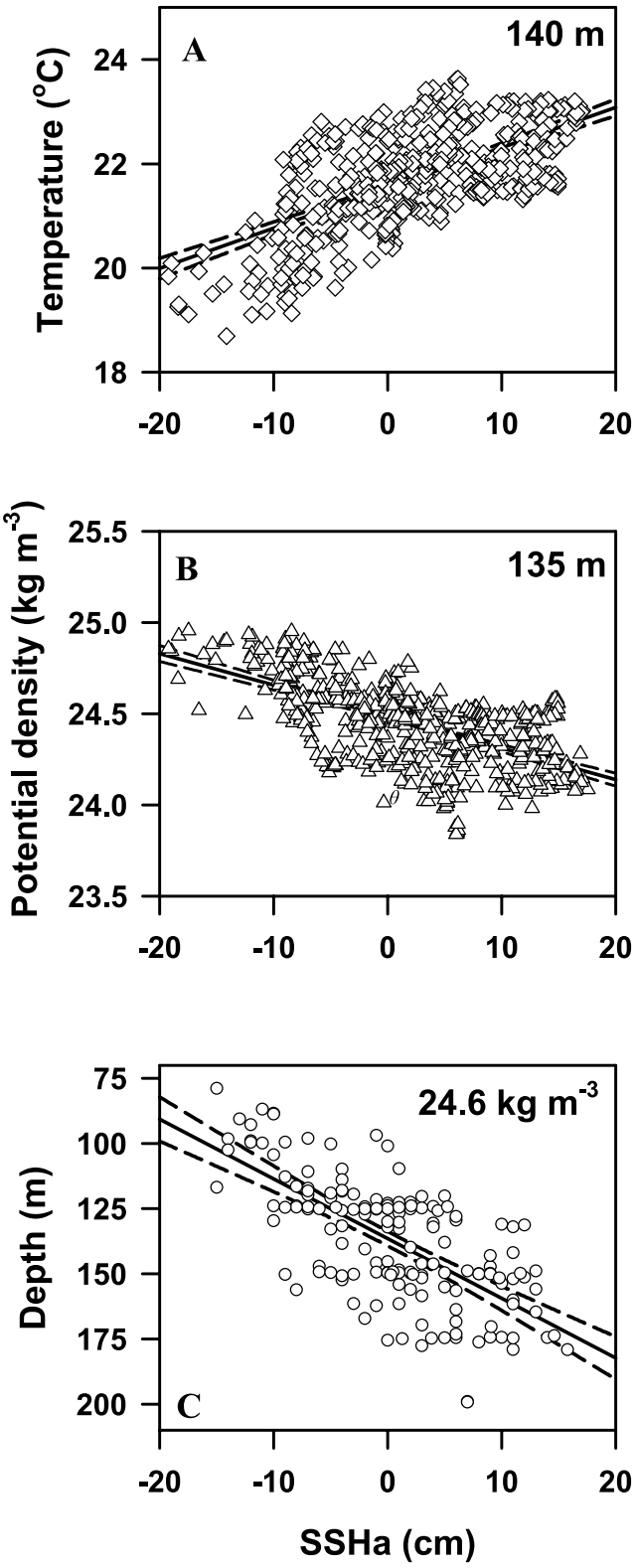


Figure 3. (a) Relationship between ocean temperatures measured at HALE ALOHA mooring (140 m) and satellite SSHA. Solid line is least squares linear regression with dashed lines indicating 95% confidence intervals of the regression; equation defining regression: temperature at 140 m is 0.08(SSHA) + 21.5; R^2 = 0.40, P < 0.0001. (b) Relationship between potential density (σ_θ) measured at WHOTS mooring (135 m) and satellite SSHA. Solid line is least squares linear regression with dashed lines indicating 95% confidence intervals of the regression; equation defining regression: σ_θ at 135 m is −0.02(SSHA) + 24.5; R^2 = 0.38, P < 0.0001. (c) Relationship between vertical position of 24.6 kg m^{-3} isopycnal surface at Station ALOHA determined on monthly HOT cruises and satellite SSHA (1992–2007). Solid line depicts least squares linear regression; equation describing regression: depth of 24.6 kg m^{-3} isopycnal surface is 2.3(SSHA) + 137; R^2 = 0.46, P < 0.0001.

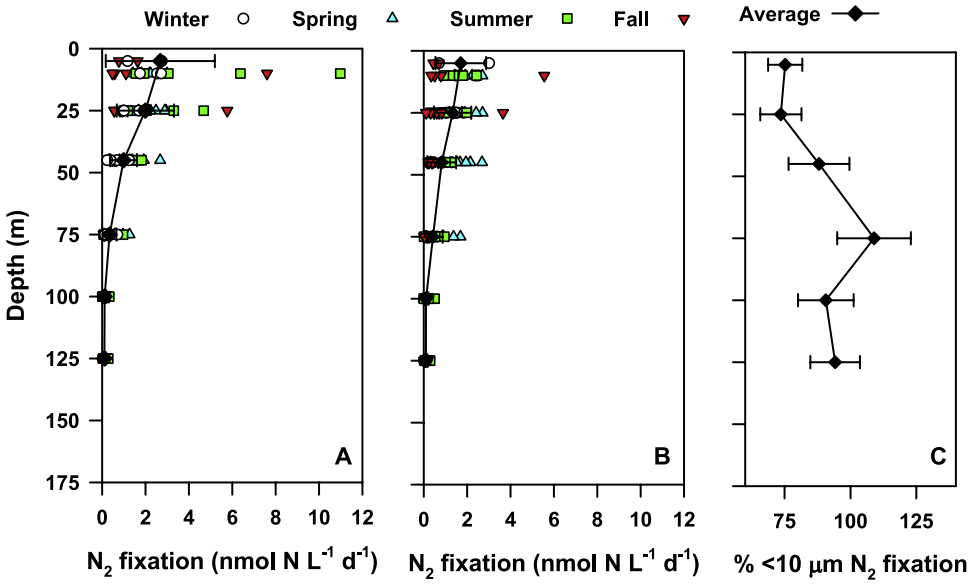


Figure 4. Depth profiles of daily $^{15}N_2$ fixation measured at Station ALOHA (2004–2007). (a) Daily rates of $^{15}N_2$ fixation in whole seawater samples. Symbols depict seasonal binning of measured rates: winter (open circles; January, February, and March), spring (triangles; April, May, and June); summer (squares; July, August, and September), and fall (inverted triangles; October, November, and December). Solid line with black diamonds depicts time-averaged mean rates of N_2 fixation. (b) Daily $^{15}N_2$ fixation in <10 μm, postincubation size fractionated samples (symbol designations are the same as in Figure 4a). (c) Time-averaged percent $^{15}N_2$ fixation partitioned into <10 μm particulate matter (<10 μm size fraction divided by whole seawater rates).

depth integrated gene abundances ranging from 1×10^8 to 2×10^{11} *nifH* copies per square meter (Table 1). Upper ocean (0–100 m) *nifH* gene inventories of *Crocosphaera* spp. ranged from below detection on 1 of the 33 samplings to $\sim 1 \times 10^{11}$ gene copies per square meter (Table 1). Gene inventories of the heterocystous phylotypes (dominated by the Het-2 phylotype) ranged from below detection to $>1 \times 10^{11}$ *nifH* copies per square meter. Depth integrated *Trichodesmium* phylotype abundances were also highly variable, ranging from below detection on 4 of the 33 samplings to $\sim 4 \times 10^{10}$ *nifH* copies per square meter.

[23] Gene copy abundances of the heterocystous cyanobacteria, *Trichodesmium*, and *Crocosphaera* were generally elevated in the summer months (July through September), while inventories of the unicellular group A phylotype tended to be greatest in the late winter and early spring (March through May (Figure 7)). None of the cyanobacterial phylotypes examined in this study demonstrated significant variability over seasonal time scales (one-way ANOVA, $P > 0.05$). Similar to the observed episodic variability in rates of N_2 fixation, the abundances of several of the *nifH* phylotypes also fluctuated markedly within seasons. In many cases, fluctuations in rates of N_2 fixation coincided with large changes in the abundances of specific *nifH* phylotypes. For example, in September 2005 whole seawater rates of N_2 fixation in the near-surface ocean increased more than threefold relative to the 3 year mean (Figure 8). These changes coincided with increased concentrations of chlorophyll *a* in the upper ocean (Figure 8) and enhanced concentrations of both particulate silica (a proxy for diatom biomass) and Het-2 phylotype abundances (Figure 8).

[24] Unicellular *nifH*-containing cyanobacteria (specifically the group A phylotype) were often the most abundant upper ocean diazotrophs, suggesting these microorganisms play an important role in N_2 fixation in this ecosystem. In March 2007, rates of N_2 fixation were not significantly different from the 3 year mean, but despite respectable rates of N_2 fixation, abundances of filamentous cyanobacteria (*Trichodesmium* and heterocystous phylotypes) were low or undetectable (Figure 8), while upper ocean concentrations of unicellular *nifH* cyanobacteria were elevated.

3.4. Mesoscale Forcing of N_2 Fixation and Diazotroph Community Structure

[25] On the basis of the HALE ALOHA temperature record and satellite-derived SSHA we examined whether episodic physical forcing of the upper ocean, presumably driven by mesoscale physical processes such as the passage of eddies or planetary waves, modified diazotroph dynamics at Station ALOHA. During this study, near-surface ocean rates of N_2 fixation in the whole seawater samples exceeded 6 nmol L^{-1} d^{-1} on 4 of the 28 total sampling occasions (July 2005, September 2005, July 2006, October 2006); near-surface ocean temperatures during these periods ranged between 25.2° and 26.8°C. Three of these 4 events coincided with periods of strong positive SSHA ($\geq$11 cm (Figure 9)); the one exception (July 2005) coincided the passage of a decaying anticyclonic eddy [*Fong et al.*, 2008]. In contrast to potential mesoscale sensitivities observed in

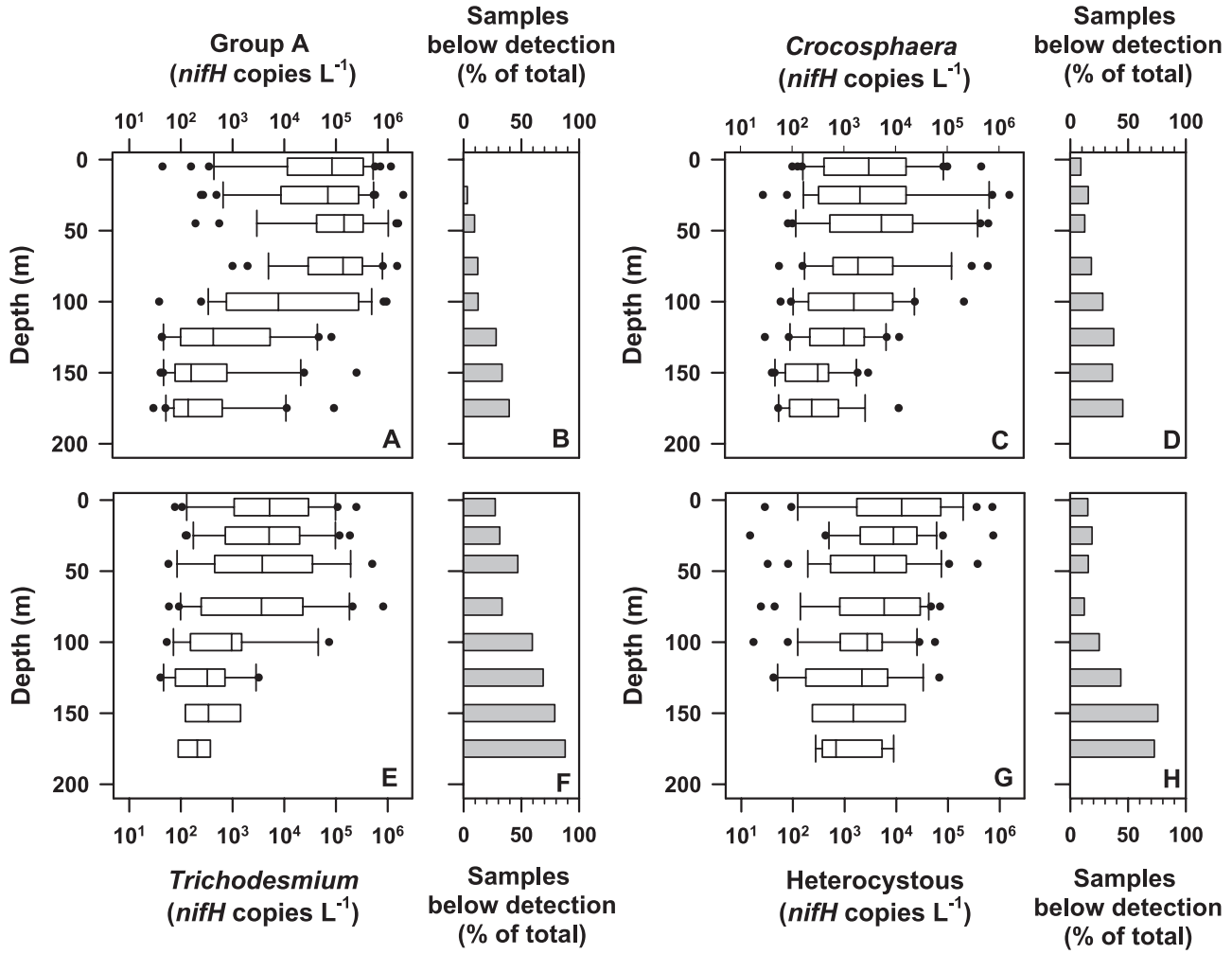


Figure 5. Vertical profiles demonstrating range in *nifH* gene abundances, when detectable, of the various cyanobacterial phylotypes measured at Station ALOHA (2004–2007). Percentile ranges of gene abundances shown with box plots, where outer boxes depict 25th and 75th percentiles, solid lines inside boxes are median *nifH* gene abundances, whisker bars are 10th and 90th percentiles, and filled circles represent *nifH* gene abundances lying outside the 10th and 90th percentiles. Bar graphs reflect percentage of total samples at indicated depths where *nifH* gene abundances were below the detection limits of the QPCR assay. (a, b) Group A phylotype, (c, d) *Crocosphaera* spp., (e, f) *Trichodesmium* spp., and (g, h) sum of various heterocystous cyanobacteria (Het-1, Het-2, and Het-3) sampled during this study.

the bulk community rates of N_2 fixation the <10 μm near-surface ocean rates demonstrated no clear relationship with variations in near-surface ocean temperatures or SSHA (Figure 9). Surface ocean rates of N_2 fixation in the <10 μm plankton size fraction never exceeded 6 nmol N L^{-1} d^{-1}; on one occasion (October 2006) rates increased to 5.5 nmol N L^{-1} d^{-1} and at the time of this sampling near-surface ocean temperatures and SSHA at Station ALOHA were 26.3°C and approximately 15 cm, respectively (Figure 9).

[26] Examination of temporal variations in diazotroph community structure (*nifH* abundances) relative to variations in upper ocean temperature and SSHA provided insight into the role of seasonal and mesoscale variability in controlling diazotroph community structure at ALOHA. Near-surface ocean *nifH* gene abundances of both unicellular diazotroph phylotypes (group A and *Crocosphaera*) demonstrated significant but opposing relationships with temperature (Figure 10a). Group A phylotype abundances were inversely correlated to near-surface ocean temperatures, with near-surface water abundances greatest when temperatures ranged between 23.4 and 25.6°C. Group A phylotype abundances demonstrated a sharp decline above temperatures of ~26.6°C. In contrast, *nifH* gene copy abundances of *Crocosphaera* demonstrated a significant positive relationship with temperature (Figure 10a). The abundances of the filamentous cyanobacteria (*Trichodesmium* and the various heterocystous cyanobacterial phylotypes) did not appear significantly correlated with changes in temperature. Moreover, only heterocystous cyanobacteria demonstrated a weak but significant dependence on variations in SSHA ($\log_{10}$ of gene abundance is 0.06(SSHA) + 4.1, R^2 = 0.21, P = 0.004). During periods when rates of N_2 fixation were elevated (and SSHA was

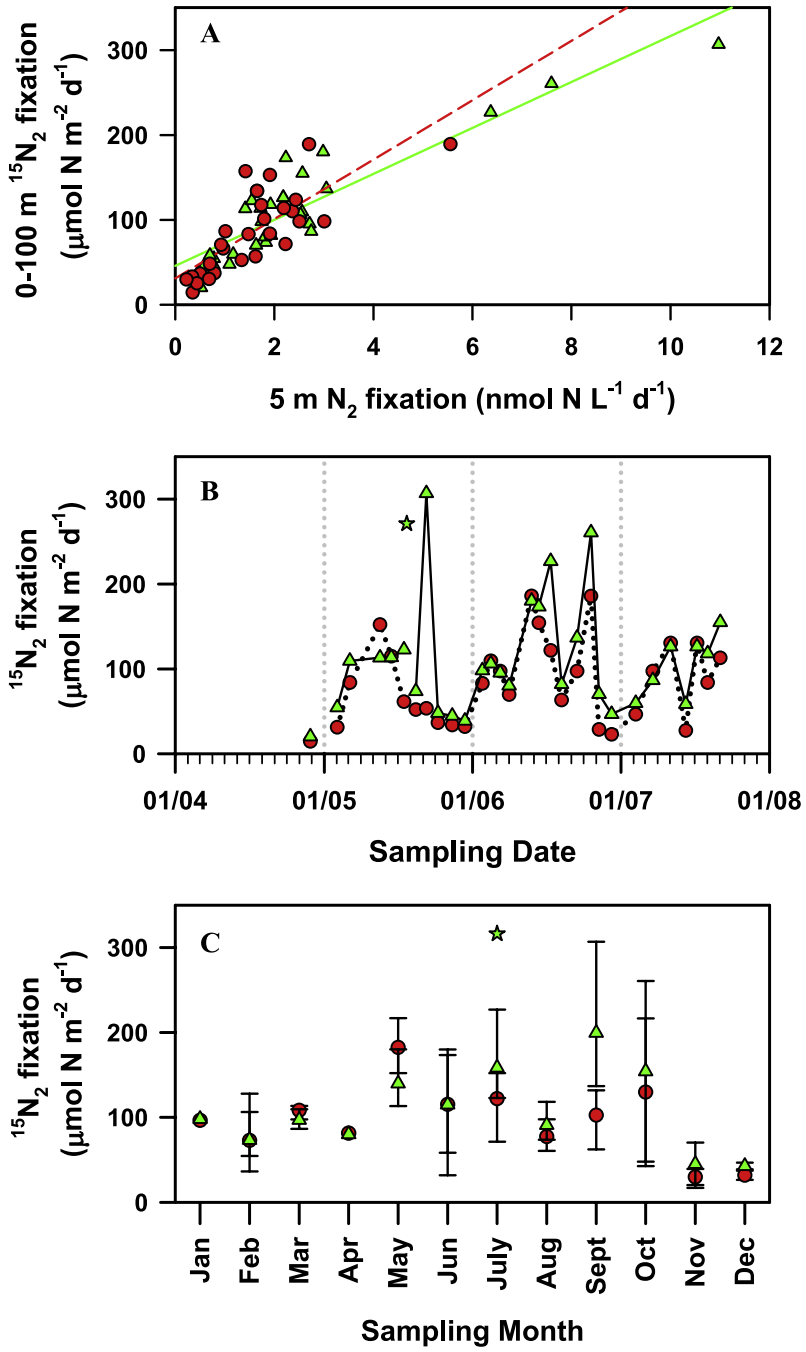


Figure 6. Depth-integrated (0–100 m) rates of upper ocean $^{15}N_2$ fixation at Station ALOHA. (a) Relationship between near-surface ocean (5 m) and depth-integrated rates of $^{15}N_2$ fixation in both whole seawater (triangles) and postincubation size fractionated (<10 μm) seawater (circles) samples. Solid line depicts least squares linear regression of whole seawater samples; whole seawater depth integrated rate is 27.2($^{15}N_{2surf}$) + 48.9; $R^2 = 0.89$, $P < 0.0001$. Dashed line depicts least squares linear regression of <10 μm size fractionated rates; equation defining depth integrated rate in <10 μm size fraction is 34($^{15}N_{2<10surf}$) + 31; $R^2 = 0.60$, $P < 0.0001$. (b) Time series of upper ocean (0–100 m) rates of $^{15}N_2$ fixation in whole seawater (triangles) and <10 μm size fractionated samples (circles). Star represents calculated rate of $^{15}N_2$ fixation (using relationship defined in Figure 6a) for whole seawater based on measured rate in near-surface waters (15 m) during eddy sampling cruise in July 2005. (c) Upper ocean (0–100 m) $^{15}N_2$ fixation binned by month. Triangles represent mean monthly rates of whole seawater $^{15}N_2$ fixation, and error bars depict minimum and maximum rates measured during study. Circles depict mean monthly rates of $^{15}N_2$ fixation in <10 μm size fractionated samples, and error bars reflect minimum and maximum rates from each month. Star depicts calculated rate (as in Figure 6b) for eddy sampling cruise in July 2005.

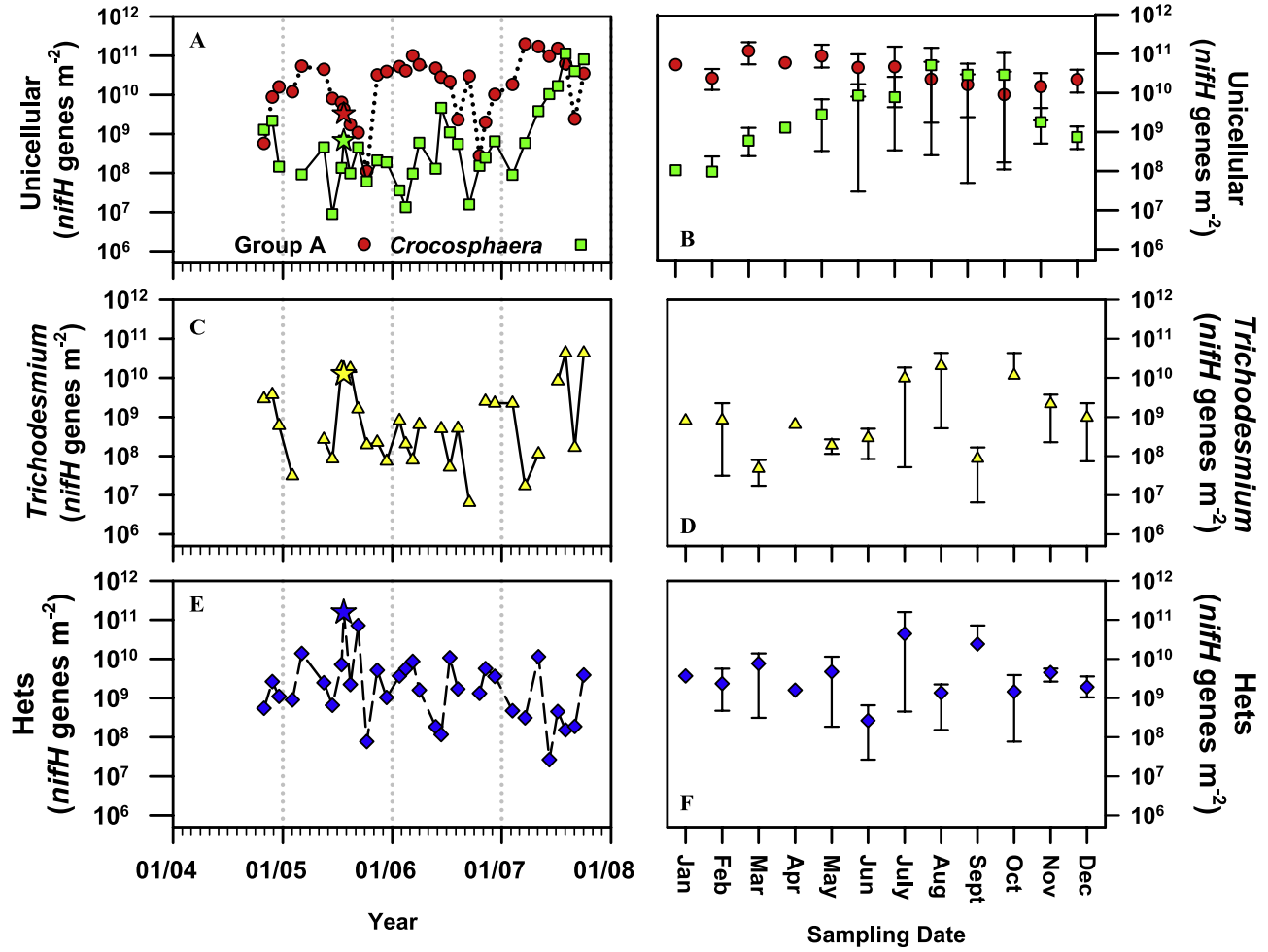


Figure 7. Time series of depth-integrated (0–100 m) *nifH* gene abundances at Station ALOHA. (a) Unicellular group A (circles) and *Crocosphaera* spp. (squares) *nifH* gene abundances; stars depict depth-integrated gene abundances measured during eddy sampling cruise in July 2005. (b) Monthly averaged unicellular *nifH* phylotype gene abundances; symbols represent mean *nifH* gene concentrations, and error bars are minimum and maximum detectable gene abundances. (c) Time series of *Trichodesmium* spp. phylotype *nifH* gene inventories. (d) Time series of *Trichodesmium* spp. phylotype *nifH* gene inventories binned by month; symbols and error bars are the same as in Figure 7b. (e) Time series of *nifH* gene inventories of the sum of the three groups of heterocystous cyanobacterial phylotypes (Het-1, Het-2, and Het-3) examined in this study. (f) Heterocystous *nifH* phylotype gene inventories binned by month; symbols and error bars are the same as in Figure 7b.

positive), heterocystous cyanobacteria (specifically the Het-2 phylotype) tended to comprise a dominant component of the diazotroph community, often accounting for more than 50% of the total *nifH* gene abundances when SSHA was $\geq$10 cm (Figure 10b).

[27] To identify possible mechanisms linking diazotroph activity to mesoscale dynamics we examined possible linkages between mesoscale forcing and the vertical distributions of various nutrients at Station ALOHA. Least squares linear regression analyses were used to evaluate the dependence of nutrient concentrations (N + N, PO_4^{3-}, and $Si(OH)_4$) at 5 depth horizons (25, 100, 150, 300, and 500 m) on variations in SSHA and fluctuations in σ_θ. These analyses revealed two important features regarding the coupling between nutrient concentrations and mesoscale forcing: (1) at the base of the euphotic zone and regions of the upper mesopelagic (150, 300, 500 m) nutrient concentrations were strongly related to fluctuations in σ_θ and nutrients generally demonstrated weak inverse relationships to SSHA and (2) at the two depths examined in the well-illuminated regions of the euphotic zone (25 and 100 m), nutrient concentrations were generally not significantly correlated with changes in SSHA, and only weakly related to variations in σ_θ (Table 2). Together, these data suggest mesoscale physical forcing plays an important role in vertically redistributing nutrients because of uplift or depression of isopycnal surfaces in the dimly lit regions water column, but the influences of such physical perturbations in the well-illuminated regions of the upper

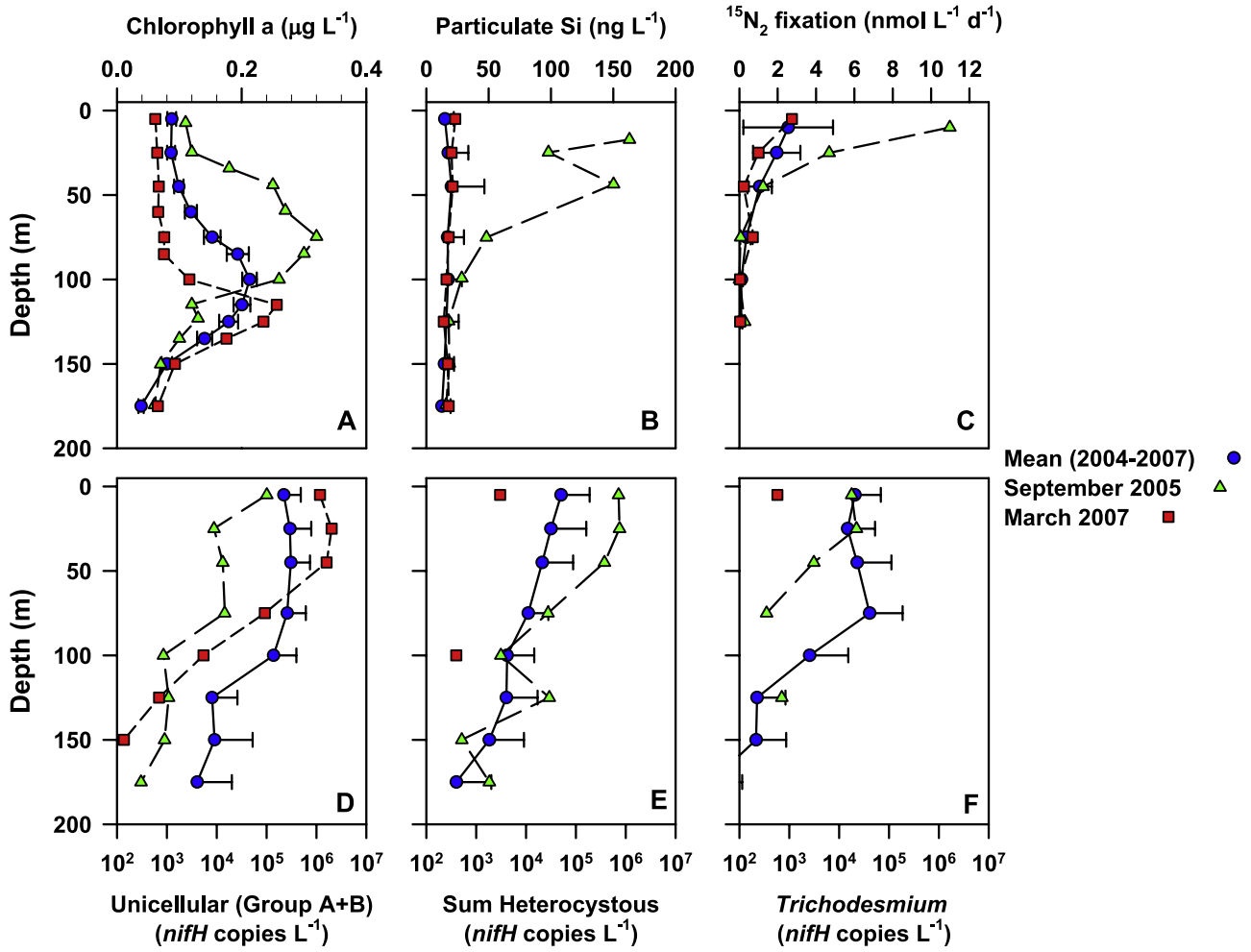


Figure 8. Depth profiles of (a) chlorophyll *a*, (b) particulate silica, (c) $^{15}N_2$ fixation (whole seawater), and (d, e, f) *nifH* phylotype abundances in September 2005 (triangles) and March 2007 (squares) relative to 3 year mean (2004–2007; circles). Note *nifH* gene copy abundances of heterocystous and *Trichodesmium* spp. phylotypes are often below detection in March 2007.

ocean are obscured by rapid biological assimilation and/or convective mixing.

4. Discussion

4.1. Mesoscale Variability in N_2 Fixation

[28] Over the past several decades, N_2-fixing microorganisms have increasingly become recognized as major biological controls on elemental cycling in the sea [*Capone and Carpenter*, 1982; *Karl et al.*, 2002; *Gruber*, 2005; *Gruber and Galloway*, 2008]. In this study, we utilized shipboard, ocean mooring, and satellite altimetry data to examine upper ocean habitat variability in the NPSG over daily to interannual time scales in an effort to define the processes controlling the temporal dynamics in the activities and abundances of N_2-fixing microorganisms. Together, these measurements provided insight into the time scales of physical variability at Station ALOHA, and shed insight into the potentially important role of mesoscale physical forcing in dictating diazotroph population structure and associated rates of N_2 fixation.

[29] Our results suggest alteration of the upper ocean habitat because of mesoscale physical dynamics favors the growth of selected groups of planktonic diazotrophs. Throughout the study, rates of N_2 fixation were consistently elevated in the near-surface waters that undergo strong seasonal forcing. However, during the summer when the upper ocean was warm, thermally stratified, and light flux was seasonally high, we observed abrupt changes in diazotroph community structure and elevated rates of N_2 fixation. These events often coincided with periods of positive SSHA. The mechanisms underlying the episodic variations in N_2-fixing microorganism activity and abundance remain unknown, but likely reflect imbalances in the growth and removal of selected groups of N_2-fixing organisms associated with mesoscale perturbations. Alternatively, such results may imply that mesoscale features transport waters enriched in N_2-fixing microorganism biomass into the ALOHA vicinity. From the results of this study, we hypothesize that when the near-surface ocean is warm, stratified, and depleted in N + N relative to PO_4^{3-} and Si, mesoscale

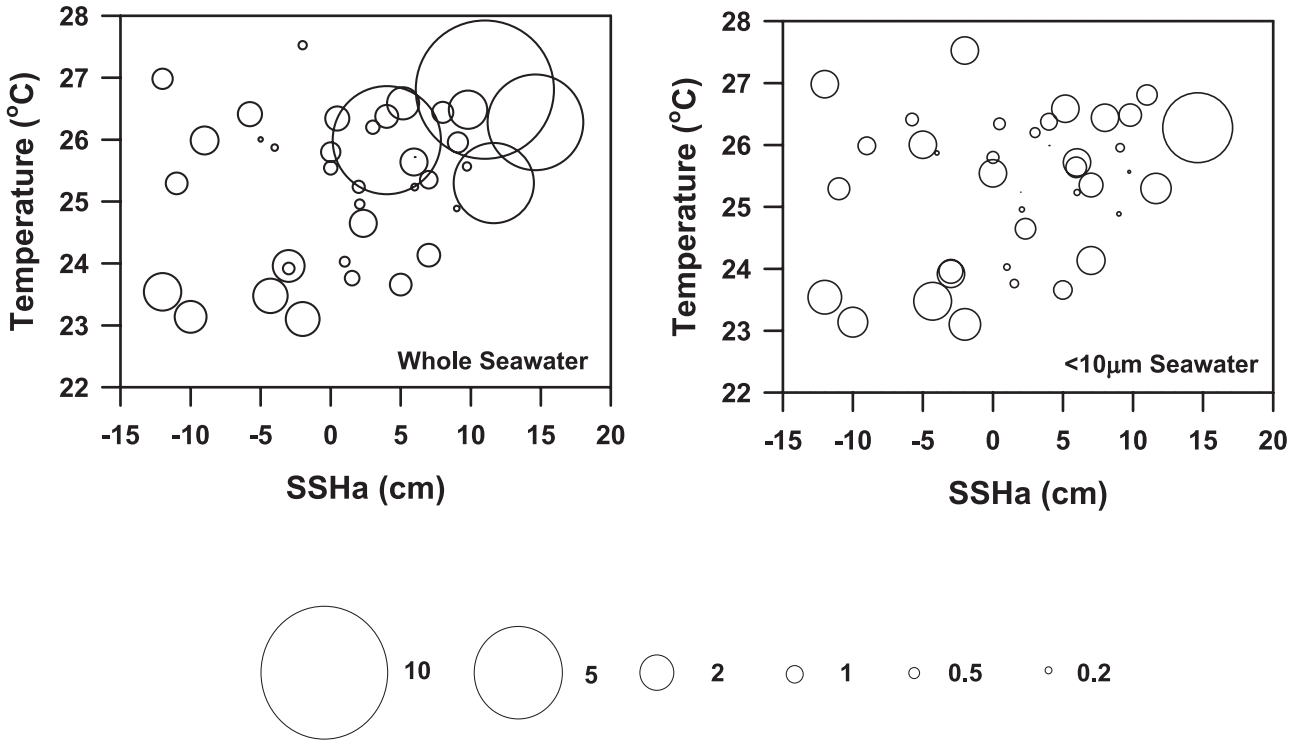


Figure 9. Bubble plots depicting relationships between near-surface ocean rates of $^{15}N_2$ fixation, temperature, and SSHA; (left) whole seawater $^{15}N_2$ fixation and (right) <10 μm size fraction. Bubble units are nmol N L^{-1} d^{-1}.

physical dynamics with associated submesoscale forcing can constitute important sources of nutrient input to the well-illuminated upper ocean. Our data suggest specific groups of diazotrophs respond differently to these physical perturbations, with larger, filamentous N_2-fixing cyanobacteria such as *Trichodesmium* and *Richelia* appearing most sensitive to these physical perturbations. Such results would suggest that the competitive successes of various groups of ocean diazotrophs may be partly determined by the strength of vertical nutrient input associated with mesoscale events.

[30] A number of previous studies have highlighted the important role of mesoscale physical events on plankton productivity and carbon export in oligotrophic oceans. Most often, such studies demonstrate that mesoscale physical processes enhance vertical fluxes of N + N to the well-lit upper ocean, thereby stimulating nitrate-supported new production [*McGillicuddy et al.*, 1998; *McNeil et al.*, 1999; *Letelier et al.*, 2000; *Sweeney et al.*, 2003; *Sakamoto et al.*, 2004; *Benitez-Nelson et al.*, 2007]. However, results from the current study suggest that the occurrence of mesoscale events (with attendant submesoscale dynamics) may also favor N_2 fixation supported new production in the oligotrophic ocean gyres. Such findings may not be restricted to the North Pacific Ocean; in a transatlantic survey of *Trichodesmium* spp. abundances, *Davis and McGillicuddy* [2006] observed elevated abundances of these diazotrophs associated with regions of positive SSHA (e.g., anticyclonic eddies). While the exact mechanisms that couple alterations in diazotroph community structure and N_2 fixation to such physical forcings remain unknown [*White et al.*, 2007], our results provide additional evidence of a potentially important but poorly understood role for mesoscale physical processes on ocean biogeochemistry and plankton ecology.

4.2. Temporal Variability in N_2 Fixation and Diazotroph Community Structure

[31] The coordinated measurements of *nifH* gene abundances and measurements of N_2 fixation in this study provided unique insight into the organisms controlling the dynamics of N_2 fixation at Station ALOHA. Consistent with previous studies in this region [*Dore et al.*, 2002; *Grabowski et al.*, 2008], rates of whole seawater N_2 fixation were generally elevated in the late spring to early fall when the upper ocean was warm, stratified, solar radiation was near its seasonal peak, and upper ocean [N + N]:PO_4^{3-} and [N + N]:$Si(OH)_4$ ratios decrease to their annual minimum. Moreover, abundances of several groups of diazotrophs, specifically *Trichodesmium* spp., heterocystous *nifH* containing cyanobacteria, and *Crocosphaera* spp., were frequently elevated during the summer. However, N_2 fixation and diazotroph abundances were also variable during the summer months; for example, rates of whole seawater $^{15}N_2$ fixation (0–100 m) ranged approximately fourfold (74 to 307 μmol N m^{-2} d^{-1}). In comparison, during the same period depth-integrated (0–100 m) summertime rates of ^{14}C primary production varied approximately twofold (26.4–50.7 mmol C m^{-2} d^{-1}).

[32] Consistent with previous studies in this region, time series measurements of N_2 fixation revealed that on average

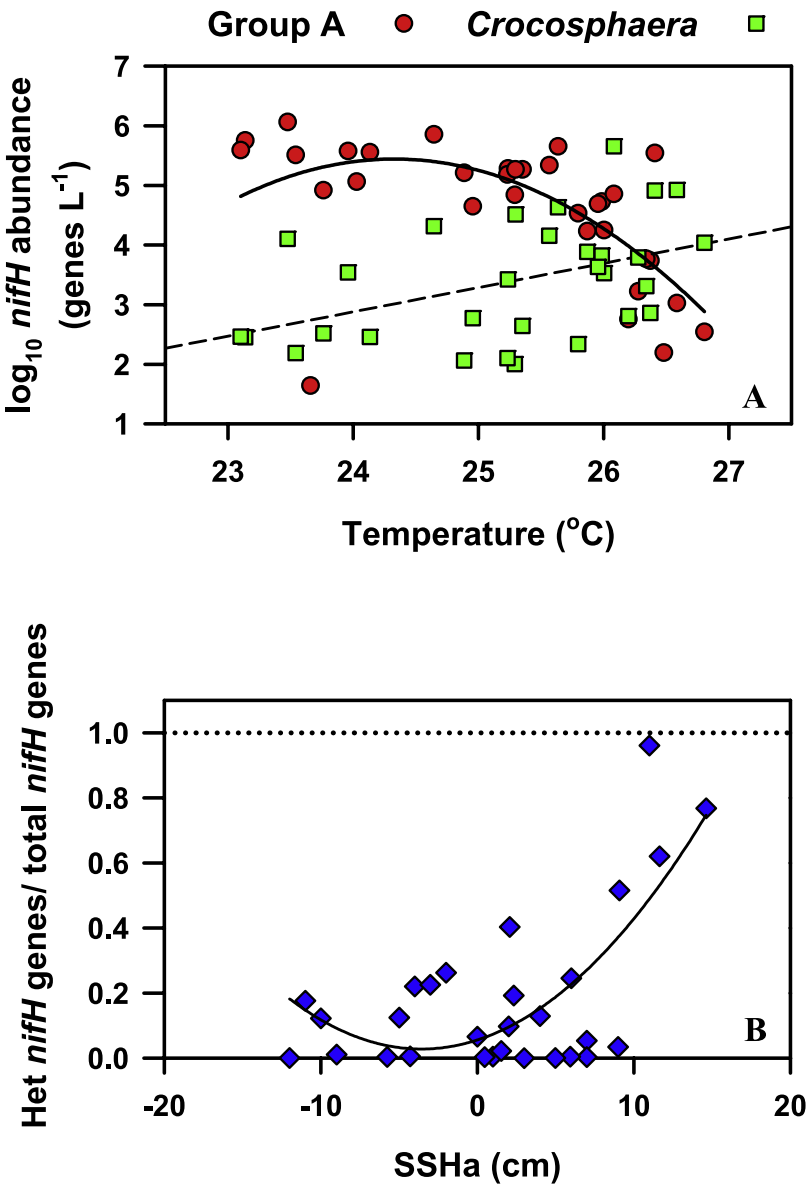


Figure 10. Relationships between near-surface ocean (5 m) *nifH* gene abundances, temperature, and SSHA. (a) Unicellular group A (circles) and *Crocosphaera* spp. (squares) *nifH* abundances; solid line is least squares regression of quadratic curve fit of $\log_{10}$ of group A abundances versus near-surface ocean temperatures ($\log_{10}$ of group A *nifH* gene copies is $20T - 0.42T^2 - 241$; $R^2 = 0.38$, $P = 0.004$). Dashed line is least squares linear regression of *Crocosphaera* spp. *nifH* abundances (when detected) versus near-surface ocean temperatures ($\log_{10}$ of *Crocosphaera nifH* gene copies is $0.4T - 6.9$; $R^2 = 0.20$, $P = 0.001$). (b) Contributions of heterocystous phylotype *nifH* gene abundances relative to total *nifH* abundances versus SSHA. Dotted line depicts least squares regression of quadratic curve fit (Het *nifH* gene copies divided by total *nifH* gene copies is $0.02(\text{SSHA}) + 0.002(\text{SSHA}^2) + 0.06$; $R^2 = 0.49$, $P = 0.0001$).

the majority of the daily $^{15}N_2$ assimilated into plankton biomass at ALOHA was partitioned into microorganisms <10 μm in diameter. Areal rates of $^{15}N_2$ assimilation associated with <10 μm plankton biomass averaged 84 ± 49 μmol N m^{-2} d^{-1} while rates of N_2 fixation in whole seawater samples averaged 113 ± 66 μmol N m^{-2} d^{-1}. On the basis of both indirect approaches (geochemical mass balances) and direct measurements a number of previous studies in the NPSG have estimated N_2 fixation accounts for 24 to 230 μmol N m^{-2} d^{-1} [*Letelier and Karl*, 1996; *Karl et al.*, 1997; *Deutsch et al.*, 2001; *Dore et al.*, 2002; *Montoya et al.*, 2004]. However, on selected occasions rates of N_2 fixation in the whole seawater samples were nearly six times greater than rates derived from <10 μm size fractionated biomass. Such results suggest episodic increases in large, filamentous diazotrophs play an important role in the annual new production of this ecosystem. The methods employed in the present study do not allow us to conclusively determine

Table 2. Least Squares Linear Regression Analyses Describing Temporal Variability in Nutrient Concentrations at Specified Depth Horizons as a Function of Potential Density and Sea Surface Height Anomalies[a]

Independent Variable	25 m	100 m	150 m	300 m	500 m
			Potential Density		
N + N (μM)	NS	0.15 σ_θ − 3.6 (R^2 = 0.06, P < 0.001)	1.8 σ_θ − 44.5 (R^2 = 0.43, P < 0.0001)	14.0 σ_θ − 352 (R^2 = 0.76, P< 0.0001)	36 σ_θ − 997 (R^2 = 0.91, P < 0.0001)
PO_4^{3-} (μM)	NS	0.02 σ_θ − 0.38 (R^2 = 0.03, P = 0.03)	0.11 σ_θ − 2.7 (R^2 = 0.22, P < 0.0001)	0.97 σ_θ − 24 (R^2 = 0.67, P < 0.0001)	2.9 σ_θ − 76 (R^2 = 0.91, P < 0.0001)
$Si(OH)_4$ (μM)	0.13 σ_θ − 1.9 (R^2 = 0.04, P = 0.01)	0.23 σ_θ − 4.0 (R^2 = 0.03, P = 0.05)	1.1 σ_θ − 25.7 (R^2 = 0.25, P < 0.0001)	15.3 σ_θ − 385 (R^2 = 0.79, P < 0.0001)	100 σ_θ − 2612 (R^2 = 0.96, P < 0.0001)
[N + N]:PO_4^{3-} (mol:mol)	NS	NS	7.1 σ_θ − 169 (R^2 = 0.14, P < 0.0001)	2.2 σ_θ − 44 (R^2 = 0.25, P < 0.0001)	−0.96 σ_θ + 39 (R^2 = 0.03, P = 0.007)
Si:N (mol:mol)	NS	NS	−5.9 σ_θ + 149 (R^2 = 0.03, P = 0.03)	0.11 σ_θ − 1.9 (R^2 = 0.08, P = 0.001)	1.4 σ_θ − 34.9 (R^2 = 0.73, P < 0.0001)
			Sea Surface Height Anomaly		
N + N (μM)	NS	NS	−0.05 (SSHA) + 0.98 (R^2 = 0.26, P < 0.0001)	−0.16 (SSHA) + 9.2 (R^2 = 0.29, P < 0.0001)	−0.07 (SSHA) + 28.1 (R^2 = 0.06, P = 0.0001)
PO_4^{3-} (μM)	NS	NS	−0.003 (SSHA) + 0.14 (R^2 = 0.14, P < 0.0001)	−0.01 (SSHA) + 0.69 (R^2 = 0.26, P < 0.0001)	−0.005 (SSHA) + 2.0 (R^2 = 0.04, P = 0.003)
$Si(OH)_4$ (μM)	NS	NS	−0.04 (SSHA) + 2.0 (R^2 = 0.19, P < 0.0001)	−0.18 (SSHA) + 9.0 (R^2 = 0.36, P < 0.0001)	−0.25 (SSHA) + 44.0 (R^2 = 0.12, P < 0.0001)
N + N:PO_4^{3-} (mol:mol)	NS	NS	−0.17 (SSHA) + 6.8 (R^2 = 0.07, P = 0.006)	−0.3 (SSHA) + 13 (R^2 = 0.08, P = 0.004)	NS
Si:N (mol:mol)	NS	NS	NS	−0.003 (SSHA) + 0.98 (R^2 = 0.18, P < 0.0001)	−0.005 (SSHA) + 1.6 (R^2 = 0.17, P < 0.0001)

[a]Equations describe regression of nutrient concentrations versus σ_θ and SSHA; coefficient of determination (R^2) and significance level (P) of the regression analyses are shown in parentheses. NS denotes linear regression analyses not significant (P > 0.05).

whether such results indicate that small (<10 μm) microorganisms typically dominate N_2 fixation at Station ALOHA, or whether such results reflect rapid food web recycling of ^{15}N labeled organic matter or ammonium [*Mulholland et al.*, 2004; *Mulholland*, 2007]. However, rates of N_2 fixation in the <10 μm size fraction were significantly greater in the spring than at other times of the year, a period that coincided with seasonal increases in the abundances of the uncultivated, unicellular group A phylotype. Metagenomic analyses indicate these microorganisms lack photosystem II (and therefore do not evolve O_2 during photosynthesis) but retain a functional photosystem I. Such findings suggest these microorganisms may utilize photoheterotrophy as a primary metabolism [*Zehr et al.*, 2008]. The apparent numerical dominance of the group A phylotype at Station ALOHA during the early spring when the upper ocean experiences relatively active mixing could reflect the competitive advantage photoheterotrophy provides these microorganisms over diazotrophs that depend exclusively on energy derived from sunlight. In a study conducted in the equatorial, subtropical, and high-latitude region of the North Atlantic, *Langlois et al.* [2008] also noted that the group A phylotype was often most prevalent in the subtropical waters when temperatures were relatively cool (22–23°C).

[33] The time series QPCR analyses of diazotroph community structure revealed that population abundances of several groups of diazotrophs, particularly filamentous *nifH* cyanobacterial phylotypes were highly dynamic. *Trichodesmium* and heterocystous cyanobacterial *nifH* gene abundances often fluctuated by as much as 3 or 4 orders of magnitude between the monthly cruises. This was in contrast to the approximately order of magnitude variability observed in rates of N_2 fixation, suggesting that ocean diazotrophs are capable of large changes in cellular activity, presumably driven in part by fluctuations in environmental conditions such as those controlling nutrient availability. The observed episodic variability in these filamentous diazotrophs was most pronounced during the warm summer months. One possible implication of our results is that although remote sensing has been successful in capturing episodic occurrences of these larger diazotrophs, such satellite ocean color remote sensing may overlook important population dynamics of abundant and active unicellular N_2-fixing microorganisms.

[34] Assuming actively growing, filamentous diazotrophs in the NPSG maintain biomass N:P ratios of between ~33:1 and 50:1 [*Sohm and Capone*, 2006; *White et al.*, 2006] and that rates of N_2 fixation are proportional to diazotroph growth, our measured rates of N_2 fixation suggest that between 0.3 and 8 μmol P m^{-2} d^{-1} were required to meet the growth demands of the diazotrophs at Station ALOHA. Previous studies suggest ~50% of the plankton P demand may be met through assimilation of organic P [*Björkman and Karl*, 2003]; thus, our measured N_2 fixation rates would require ~0.15 to 4 μmol P m^{-2} d^{-1} as PO_4^{3-}. Assuming PO_4^{3-} input balances particulate P export at Station ALOHA implies that P supply to the upper ocean ranges between 2 and 30 μmol P m^{-2} d^{-1}. Thus, during periods of enhanced N_2 fixation, a substantial fraction (~7 to 13%) of PO_4^{3-} introduced to the upper ocean may support the growth demands of N_2-fixing microorganisms.

4.3. Role of Mesoscale Physical Forcing on Ocean Biogeochemistry at ALOHA

[35] To better understand the time scales of physical forcing and its potential influence on N_2 fixation at Station ALOHA, we analyzed mooring and satellite-derived measurements of the near-surface temperatures and SSHA in the vicinity of Station ALOHA. On the basis of the average daily temperatures recorded at the HALE ALOHA mooring, seasonal-scale changes composed the dominant mode of physical forcing in the upper ocean where rates of N_2 fixation were elevated. However, in the less seasonally modified lower euphotic zone, temperatures fluctuated over weekly to monthly time scales and these time-dependent fluctuations were significantly correlated with satellite measurements of SSHA. These results indicate episodic forcing attributable to mesoscale physical processes appear to comprise a dominant component of weekly to monthly scale physical forcing in this ecosystem.

[36] During the approximately 3 years of monthly measurements conducted for this study, we encountered 4 occasions where near-surface ocean rates of N_2 fixation were more than one standard deviation above the mean rate derived from this study. All of these occasions coincided with periods where the upper ocean was warm, and during such periods peak rates of N_2 fixation appeared driven largely by microorganisms >10 μm in size. During 3 of the 4 occasions, abundances of the Het-2 phylotype were greater than 1 standard deviation above the 3 year average. Moreover, with one notable exception these events coincided with periods of strong (>11 cm) positive SSHA. The one exception to this general observation occurred when whole seawater rates of N_2 fixation in the near-surface ocean were more than twofold greater than the climatological mean, but SSHA was only weakly positive (4 cm); this sampling coincided with the passage of a decaying anticyclonic eddy through the ALOHA region [*Fong et al.*, 2008]. Together, these data suggest that larger filamentous diazotrophs out compete other members of the diazotroph assemblage during episodic periods when light fluxes are high, temperatures are warm, and mesoscale events perturb nutrient and light fields in the upper ocean.

[37] One of the more perplexing observations to emerge from this study was the episodic enhancement in N_2 fixation during summertime periods of positive SSHA. Numerous studies have demonstrated enhancement of both plankton biomass and growth associated with negative SSHA, such as those driven by cyclonic eddies or planetary waves [*McGillicuddy et al.*, 1998; *McNeil et al.*, 1999; *Letelier et al.*, 2000; *Sweeney et al.*, 2003; *Sakamoto et al.*, 2004; *Benitez-Nelson et al.*, 2007; *McGillicuddy et al.*, 2007]. Recently, *McGillicuddy et al.* [2007] reported the occurrence of a large diatom bloom associated with a mode water eddy in the Sargasso Sea; satellite detection of this feature identified a coherent region of positive SSHA consistent with downwelling of upper ocean isopycnal surfaces within the eddy. Other studies have observed enhanced nutrient fluxes and stimulation of biological activity associated with anticyclonic eddies [*Law et al.*, 2001; *Woodward and Rees*, 2001; *Peterson et al.*, 2005; *Fong et al.*, 2008]. Both models and observational data suggest interactions

between eddy flow fields and surface wind stress might increase vertical nutrient exchange within such anticyclones [*Martin and Richards*, 2001; *Woodward and Rees*, 2001; *McGillicuddy et al.*, 2007]. In addition, submesoscale dynamics associated with ocean eddies including nonlinear Ekman transport, frontogenesis, and ageostrophic circulation all appear capable of diapycnal nutrient exchange within anticyclones [*Mahadevan and Tandon*, 2006; *Capet et al.*, 2008; *Klein et al.*, 2008; *Mahadevan et al.*, 2008; *Nencioli et al.*, 2008].

[38] At Station ALOHA, stimulation of vertical nutrient exchange by mesoscale and/or accompanying submesoscale physical processes would enhance delivery of waters with low [N + N]:PO_4^{3-} and [N + N]:$Si(OH)_4$ ratios to the upper euphotic zone, creating an ideal habitat for growth of N_2-fixing microorganisms, including diatom-associated N_2-fixing symbionts [*Karl and Letelier*, 2008]. Such mesoscale and/or submesoscale delivery of P- and Si-laden waters to the near-surface ocean would be expected to promote the growth of larger filamentous N_2-fixing organisms such as *Trichodesmium* and heterocystous cyanobacteria that appear to have greater requirements for P than unicellular N_2-fixing cyanobacteria [*Sohm and Capone*, 2006]. The apparent stimulation of the heterocystous cyanobacteria that frequently occur as endosymbionts or episymbionts with diatoms further suggests that such mesoscale physical forcing may also enhance the growth of specific genera of ocean diatoms. During the passage of mesoscale events the growth of larger filamentous microorganisms would be expected to increase until the vertical flux of P-enriched waters falls below that threshold necessary to provide the filamentous diazotrophs with a competitive advantage over unicellular diazotrophs. Alternatively, tight top down (predation) control of the unicellular diazotroph populations may restrict these organisms from undergoing large shifts in population size during high-frequency nutrient inputs.

[39] Although peak rates of N_2 fixation frequently coincided with summertime periods of positive SSHA, neither ^{14}C primary production nor particle export fluxes increased significantly during these events. The measured rates of N_2 fixation from the present study were equivalent to between 10 and 121% (averaging 41%) of the contemporaneous particulate N export from the upper 150 m of the water (data not shown), increasing most substantially during periods of elevated N_2 fixation rather than during periods of low flux. *Dore et al.* [2002] estimated that measured rates of N_2 fixation accounted for 18–55% of the contemporaneous particulate nitrogen flux during four cruises to Station ALOHA between June 2000 and July 2001. Prior time series analyses of the $\delta^{15}N$ signature of sinking particulate matter and direct measurements of $^{15}N_2$ fixation at Station ALOHA indicate ~18–69% of new production in this system is supported by N_2 fixation [*Karl et al.*, 1997; *Dore et al.*, 2002; *Karl et al.*, 2002]. The results from the current study suggest that N_2 fixation supported production at Station ALOHA often forms an important component of new production in the NPSG, particularly in the summer coincident with positive SSHA. However, the lack of clear response in ^{14}C primary production to such mesoscale stimulation of N_2 fixation likely reflects the relatively small contribution of new nitrogen input to total primary production in this system. We suggest that the lack of a significant increase in particle export coincident with periods of elevated N_2 fixation likely reflects temporal offsets between microorganism assimilation of new nutrients and subsequent particle export from the upper ocean.

[40] If mesoscale and submesoscale introduction of nutrients underlies the observed episodic stimulation of N_2 fixation at Station ALOHA, we might anticipate that N_2 fixation would also be enhanced during periods of negative SSHA, such as those associated with the passage of cyclonic eddies where isopycnal uplift also introduces waters with low [N + N]:PO_4^{3-} and low [N + N]:$Si(OH)_4$ ratios. During this study, we sampled a total of 5 events where the SSHA was more than 1 standard deviation (<−7 cm) from the mean 15 year weekly SSHA (1992–2007) measured for this region. Three of these events occurred in the late spring (May and June), and N_2 fixation during these events was approximately equal to or exceeded the 3 year average depth-integrated (0–100 m) rates. However, during all of these events, rates of fixation were approximately equivalent in the whole seawater and <10 μm size fractionated water, indicating that most of the fixed nitrogen was partitioned into biomass of small microorganisms. It's conceivable that these cyclonic events passed through the ALOHA region too early in the optimal growing season for filamentous diazotrophs. Alternatively, mesoscale and submesoscale nutrient input that accompanies cyclonic features in this region may be less pronounced than those associated with anticyclones. Future efforts directed at modeling the interactions of mesoscale physics and microorganism growth may help elucidate the mechanisms that underlie the biogeochemical dynamics observed in this study.

5. Conclusions

[41] In conclusion, this study indicates N_2 fixation comprises an important but variable component of the ocean nitrogen cycle in the central NPSG. On average, unicellular N_2-fixing cyanobacteria dominated diazotroph abundances (on the basis of *nifH* gene concentrations); consistent with this finding, the majority of daily N_2 fixed at Station ALOHA was partitioned into the biomass of small (<10 μm) microorganisms. However, rates of N_2 fixation and the abundances of commonly observed diazotrophs at Station ALOHA were highly variable in time. Such results suggest episodic increases in larger, filamentous diazotrophs overlie a background population of smaller unicellular microorganisms. We suggest that mesoscale physical processes may fuel inputs of relatively P- and Si-enriched waters to the well-lit upper ocean, thereby altering the net growth of specific N_2-fixing organisms in this ecosystem.

[42] **Acknowledgments.** We are grateful to the scientists and staff that have contributed to making the HOT program successful; this study would not have been possible without their dedication and skill. We acknowledge the leadership of T. Dickey (University of California, Santa Barbara) for oversight of the HALE ALOHA mooring project and R. Weller (Woods Hole Oceanographic Institution) for his leadership of the WHOTS project. B. Watkins, L. Fujieki, P. Lethaby, J. Snyder, and F. Santiago-Mandujano facilitated collection of the mooring data. B. Qiu (University of Hawai'i) provided satellite altimetry data used for this study. This project

was supported by grants from the National Science Foundation, including OCE-0425363 to M.J.C. and J.P.Z., OCE-0326616 to D.M.K., OCE0326419 to R.M.L., and EF-0424599 to D.M.K. and J.P.Z. The WHOTS mooring is funded by NOAA (NA17RJ1223) to the Woods Hole Oceanographic Institution and by an NSF grant OCE-0327513 (R.L.). Additional support for this work derived from investigator grants from the Gordon and Betty Moore Foundation to D.M.K. and J.P.Z.

References

Benitez-Nelson, C. R., et al. (2007), Mesoscale eddies drive increased silica export in the subtropical Pacific Ocean, *Science*, *316*, 1017–1021, doi:10.1126/science.1136221.

Björkman, K. M., and D. M. Karl (2003), Bioavailability of dissolved organic phosphorus in the euphotic zone at Station ALOHA, North Pacific subtropical gyre, *Limnol. Oceanogr.*, *48*, 1049–1057.

Capet, X., J. C. McWilliams, M. J. Mokemaker, and A. F. Shchepetkin (2008), Mesoscale to submesoscale transition in the California current system. Part I: Flow structure, eddy flux, and observational tests, *J. Phys. Oceanogr.*, *38*, 29–43, doi:10.1175/2007JPO3671.1.

Capone, D. G., and E. J. Carpenter (1982), Nitrogen fixation in the marine environment, *Science*, *217*, 1140–1142, doi:10.1126/science.217.4565.1140.

Capone, D. G., and J. P. Montoya (2001), Nitrogen fixation and denitrification, in *Methods in Microbiology*, vol. 30, edited by J. H. Paul, pp. 501–515, Academic, San Diego, Calif.

Carpenter, E. J., J. P. Montoya, J. Burns, M. R. Mulholland, A. Subramaniam, and D. G. Capone (1999), Extensive bloom of a N_2-fixing diatom/cyanobacterial association in the tropical Atlantic Ocean, *Mar. Ecol. Prog. Ser.*, *185*, 273–283, doi:10.3354/meps185273.

Church, M. J., B. D. Jenkins, D. M. Karl, and J. P. Zehr (2005a), Vertical distributions of nitrogen-fixing phylotypes at Station ALOHA in the oligotrophic North Pacific Ocean, *Aquat. Microb. Ecol.*, *38*, 3–14, doi:10.3354/ame038003.

Church, M. J., C. M. Short, B. D. Jenkins, D. M. Karl, and J. P. Zehr (2005b), Temporal patterns of nitrogenase gene (*nifH*) expression in the oligotrophic North Pacific Ocean, *Appl. Environ. Microbiol.*, *71*, 5362–5370, doi:10.1128/AEM.71.9.5362-5370.2005.

Church, M. J., K. M. Björkman, M. A. Saito, D. M. Karl, and J. P. Zehr (2008), Regional distributions of nitrogen fixing bacteria in the Pacific Ocean, *Limnol. Oceanogr.*, *53*, 63–77.

Davis, C. S., and D. J. McGillicuddy (2006), Transatlantic abundance of the N_2-fixing colonial cyanobacterium *Trichodesmium*, *Science*, *312*, 1517–1520, doi:10.1126/science.1123570.

DeMaster, D. J. (1981), The supply and accumulation of silica in the marine environment, *Geochim. Cosmochim. Acta*, *45*, 1715–1732, doi:10.1016/0016-7037(81)90006-5.

Deutsch, C., N. Gruber, R. M. Key, J. L. Sarmiento, and A. Ganachaud (2001), Denitrification and N_2 fixation in the Pacific Ocean, *Global Biogeochem. Cycles*, *15*, 483–506, doi:10.1029/2000GB001291.

Dore, J. E., and D. M. Karl (1996), Nitrite distributions and dynamics at Station ALOHA, *Deep Sea Res., Part II*, *43*, 385–402, doi:10.1016/0967-0645(95)00105-0.

Dore, J. E., J. R. Brum, L. M. Tupas, and D. M. Karl (2002), Seasonal and interannual variability in sources of nitrogen supporting export in the oligotrophic subtropical North Pacific Ocean, *Limnol. Oceanogr.*, *47*, 1595–1607.

Dore, J. E., R. M. Letelier, M. J. Church, R. Lukas, and D. M. Karl (2008), Summer phytoplankton blooms in the oligotrophic North Pacific subtropical gyre: Historical perspective and recent observations, *Prog. Oceanogr.*, *76*, 2–38, doi:10.1016/j.pocean.2007.10.002.

Fong, A. A., D. M. Karl, R. Lukas, R. M. Letelier, J. P. Zehr, and M. J. Church (2008), Nitrogen fixation in an anticyclonic eddy in the oligotrophic North Pacific Ocean, *ISME J.*, *2*, 663–676, doi:10.1038/ismej.2008.22.

Foster, R. A., and J. P. Zehr (2006), Characterization of diatom-cyanobacteria symbioses on the basis of *nifH*, *hetR* and 16S rRNA sequences, *Environ. Microbiol.*, *8*, 1913–1925, doi:10.1111/j.1462-2920.2006.01068.x.

Grabowski, M. N. W., M. J. Church, and D. M. Karl (2008), Nitrogen fixation rates and controls at Station ALOHA, *Aquat. Microb. Ecol.*, *52*, 175–183, doi:10.3354/ame01209.

Gruber, N. (2005), Oceanography: A bigger nitrogen fix, *Nature*, *436*, 786–787, doi:10.1038/436786a.

Gruber, N., and J. N. Galloway (2008), An Earth-system perspective of the global nitrogen cycle, *Nature*, *451*, 293–296, doi:10.1038/nature06592.

Karl, D. M. (1999), A sea of change: Biogeochemical variability in the North Pacific subtropical gyre, *Ecosystems*, *2*, 181–214, doi:10.1007/s100219900068.

Karl, D. M., and R. M. Letelier (2008), Nitrogen fixation-enhanced carbon sequestration in low nitrate, low chlorophyll seascapes, *Mar. Ecol. Prog. Ser.*, *364*, 257–268, doi:10.3354/meps07547.

Karl, D. M., and R. Lukas (1996), The Hawaii Ocean Time-series (HOT) program: Background, rationale and field implementation, *Deep Sea Res., Part II*, *43*, 129–156, doi:10.1016/0967-0645(96)00005-7.

Karl, D. M., and G. Tien (1992), MAGIC: A sensitive and precise method for measuring dissolved phosphorus in aquatic environments, *Limnol. Oceanogr.*, *37*, 105–116.

Karl, D. M., R. M. Letelier, D. V. Hebel, D. F. Bird, and C. D. Winn (1992), Trichodesmium blooms and new nitrogen in the North Pacific gyre, in *Marine Pelagic Cyanobacteria*: Trichodesmium *and Other Diazotrophs*, edited by E. J. Carpenter, D. G. Capone, and J. G. Rueter, pp. 219–237, Kluwer Acad., Dordrecht, Netherlands.

Karl, D., R. Letelier, L. Tupas, J. Dore, J. Christian, and D. Hebel (1997), The role of nitrogen fixation in biogeochemical cycling in the subtropical North Pacific Ocean, *Nature*, *388*, 533–538, doi:10.1038/41474.

Karl, D., et al. (2002), Dinitrogen fixation in the world's oceans, *Biogeochemistry*, *57*, 47–98, doi:10.1023/A:1015798105851.

Karl, D. M., et al. (2008), The nitrogen cycle in the North Pacific trades biome: An evolving paradigm, in *Nitrogen in the Marine Environment*, edited by D. G. Capone et al., pp. 705–769, Elsevier, Amsterdam.

Klein, P., B. L. Hua, G. Lapeyre, X. Capet, S. Le Gentil, and H. Sasaki (2008), Upper ocean turbulence from high-resolution 3D simulations, *J. Phys. Oceanogr.*, *38*, 1748–1763, doi:10.1175/2007JPO3773.1.

Langlois, R. J., J. LaRoche, and P. A. Raab (2005), Diazotrophic diversity and distribution in the tropical and subtropical Atlantic Ocean, *Appl. Environ. Microbiol.*, *71*, 7910–7919, doi:10.1128/AEM.71.12.7910-7919.2005.

Langlois, R. J., D. Hümmer, and J. LaRoche (2008), Abundances and distributions of the dominant nifH phylotypes in the northern Atlantic Ocean, *Appl. Environ. Microbiol.*, *74*, 1922–1931, doi:10.1128/AEM.01720-07.

Law, C. S., A. P. Martin, M. I. Liddicoat, A. J. Watson, K. J. Richards, and E. M. S. Woodward (2001), A Lagrangian SF_6 tracer study of an anticyclonic eddy in the North Atlantic: Patch evolution, vertical mixing and nutrient supply to the mixed layer, *Deep Sea Res., Part II*, *48*, 705–724, doi:10.1016/S0967-0645(00)00112-0.

Letelier, R. M., and D. M. Karl (1996), Role of Trichodesmium spp. in the productivity of the subtropical North Pacific Ocean, *Mar. Ecol. Prog. Ser.*, *133*, 263–273, doi:10.3354/meps133263.

Letelier, R. M., J. E. Dore, C. D. Winn, and D. M. Karl (1996), Seasonal and interannual variations in photosynthetic carbon assimilation at Station ALOHA, *Deep Sea Res., Part II*, *43*, 467–490, doi:10.1016/0967-0645(96)00006-9.

Letelier, R. M., D. M. Karl, M. R. Abbott, P. Flament, M. Freilich, R. Lukas, and T. Strub (2000), Role of late winter mesoscale events in the biogeochemical variability of the upper water column of the North Pacific subtropical gyre, *J. Geophys. Res.*, *105*, 28,723–28,739, doi:10.1029/1999JC000306.

Mahadevan, A., and A. Tandon (2006), An analysis of mechanisms for submesoscale vertical motion at ocean fronts, *Ocean Modell.*, *14*, 241–256, doi:10.1016/j.ocemod.2006.05.006.

Mahadevan, A., L. N. Thomas, and A. Tandon (2008), Comment on "Eddy/wind interactions stimulate extraordinary mid-ocean plankton blooms" by D. J. McGillicuddy Jr. et al., *Science*, *320*, 448, doi:10.1126/science.1152111.

Martin, A. P., and K. J. Richards (2001), Mechanisms for vertical nutrient transport within a North Atlantic mesoscale eddy, *Deep Sea Res., Part II*, *48*, 757–773, doi:10.1016/S0967-0645(00)00096-5.

Mazard, S. L., N. J. Fuller, K. M. Orcutt, O. Bridle, and D. J. Scanlan (2004), PCR analysis of the distribution of unicellular cyanobacterial diazotrophs in the Arabian Sea, *Appl. Environ. Microbiol.*, *70*, 7355–7364, doi:10.1128/AEM.70.12.7355-7364.2004.

McGillicuddy, D. J., Jr., et al. (1998), Influence of mesoscale eddies on new production in the Sargasso Sea, *Nature*, *394*, 263–266, doi:10.1038/28367.

McGillicuddy, D. J., Jr., et al. (2007), Eddy/wind interactions stimulate extraordinary mid-ocean plankton blooms, *Science*, *316*, 1021–1026, doi:10.1126/science.1136256.

McNeil, J. D., H. W. Jannasch, T. Dickey, D. McGillicuddy, M. Brzezinski, and C. M. Sakamoto (1999), New chemical, bio-optical and physical observations of upper ocean response to the passage of a mesoscale eddy off Bermuda, *J. Geophys. Res.*, *104*, 15,537–15,548, doi:10.1029/1999JC900137.

Michaels, A. F., et al. (1996), Inputs, losses and transformations of nitrogen and phosphorus in the pelagic North Atlantic Ocean, *Biogeochemistry*, *35*, 181–226, doi:10.1007/BF02179827.

Moisander, P. H., R. A. Beinart, M. Voss, and J. P. Zehr (2008), Diversity and abundance of diazotrophic microorganisms in the South China Sea during intermonsoon, *ISME J.*, *2*, 954–967, doi:10.1038/ismej.2008.51.

Montoya, J. P., M. Voss, P. Kahler, and D. G. Capone (1996), A simple, high-precision, high-sensitivity tracer assay for N_2 fixation, *Appl. Environ. Microbiol.*, *62*, 986–993.

Montoya, J. P., C. M. Holl, J. P. Zehr, A. Hansen, T. A. Villareal, and D. G. Capone (2004), High rates of N_2 fixation by unicellular diazotrophs in the oligotrophic Pacific Ocean, *Nature*, *430*, 1027–1032, doi:10.1038/nature02824.

Mulholland, M. R. (2007), The fate of nitrogen fixed by diazotrophs in the ocean, *Biogeosciences*, *4*, 37–51.

Mulholland, M. R., D. A. Bronk, and D. G. Capone (2004), Dinitrogen fixation and release of ammonium and dissolved organic nitrogen by *Trichodesmium* IMS101, *Aquat. Microb. Ecol.*, *37*, 85–94, doi:10.3354/ame037085.

Nencioli, F., V. S. Kuwahara, T. D. Dickey, Y. M. Rii, and R. R. Bidigare (2008), Physical dynamics and biological implications of a mesoscale eddy in the lee of Hawai'i: Cyclone *Opal* observations during E-Flux III, *Deep Sea Res., Part II*, *55*, 1252–1274, doi:10.1016/j.dsr2.2008.02.003.

Peterson, T. D., F. A. Whitney, and P. J. Harrison (2005), Macronutrient dynamics in an anticyclonic mesoscale eddy in the Gulf of Alaska, *Deep Sea Res., Part II*, *52*, 909–932, doi:10.1016/j.dsr2.2005.02.004.

Sakamoto, C. M., D. M. Karl, H. W. Jannasch, R. R. Bidigare, R. M. Letelier, P. M. Walz, J. P. Ryan, P. S. Polito, and K. S. Johnson (2004), Influence of Rossby waves on nutrient dynamics and the plankton community structure in the North Pacific subtropical gyre, *J. Geophys. Res.*, *109*, C05032, doi:10.1029/2003JC001976.

Short, S. M., B. D. Jenkins, and J. P. Zehr (2004), The spatial and temporal distribution of two diazotrophic bacteria in the Chesapeake Bay, *Appl. Environ. Microbiol.*, *70*, 2186–2192, doi:10.1128/AEM.70.4.2186-2192.2004.

Sohm, J. A., and D. G. Capone (2006), Phosphorus dynamics of the tropical and subtropical North Atlantic: *Trichodesmium* spp. versus bulk plankton, *Mar. Ecol. Prog. Ser.*, *317*, 21–28, doi:10.3354/meps317021.

Subramaniam, A., C. W. Brown, R. R. Hood, E. J. Carpenter, and D. G. Capone (2001), Detecting *Trichodesmium* blooms in SeaWiFS imagery, *Deep Sea Res., Part II*, *49*, 107–121, doi:10.1016/S0967-0645(01)00096-0.

Sverdrup, H. U., M. W. Johnson, and R. H. Fleming (1942), *The Oceans*, Prentice-Hall, Englewood Cliffs, N. J.

Sweeney, E. N., D. J. McGillicuddy Jr., and K. O. Buesseler (2003), Biogeochemical impacts due to mesoscale eddy activity in the Sargasso Sea as measured at the Bermuda Atlantic Time-series Study (BATS), *Deep Sea Res., Part II*, *50*, 3017–3039, doi:10.1016/j.dsr2.2003.07.008.

Westberry, T. K., and D. A. Siegel (2006), Spatial and temporal distribution of Trichodesmium blooms in the world's oceans, *Global Biogeochem. Cycles*, *20*, GB4016, doi:10.1029/2005GB002673.

White, A. E., Y. H. Spitz, D. M. Karl, and R. M. Letelier (2006), Flexible elemental stoichiometry in *Trichodesmium* spp. and its ecological implications, *Limnol. Oceanogr.*, *51*, 1777–1790.

White, A. E., Y. H. Spitz, and R. M. Letelier (2007), What factors are driving summer phytoplankton blooms in the North Pacific subtropical gyre?, *J. Geophys. Res.*, *112*, C12006, doi:10.1029/2007JC004129.

Wilson, C., and X. Qiu (2008), Global distribution of summer chlorophyll blooms in the oligotrophic gyres, *Prog. Oceanogr.*, *78*, 107–134, doi:10.1016/j.pocean.2008.05.002.

Woodward, E. M. S., and A. P. Rees (2001), Nutrient distributions in an anticyclonic eddy in the northeast Atlantic Ocean, with reference to nanomolar ammonium concentrations, *Deep Sea Res., Part II*, *48*, 775–793, doi:10.1016/S0967-0645(00)00097-7.

Zehr, J. P., M. T. Mellon, and S. Zani (1998), New nitrogen-fixing microorganisms detected in oligotrophic oceans by amplification of nitrogenase (*nifH*) genes, *Appl. Environ. Microbiol.*, *64*, 3444–3450.

Zehr, J. P., et al. (2001), Unicellular cyanobacteria fix N_2 in the subtropical North Pacific Ocean, *Nature*, *412*, 635–638, doi:10.1038/35088063.

Zehr, J. P., et al. (2008), Globally distributed uncultivated oceanic N_2 fixing cyanobacteria lack oxygenic photosystem II, *Science*, *322*, 1110–1112, doi:10.1126/science.1165340.

M. J. Church, D. M. Karl, and R. Lukas, Department of Oceanography, School of Ocean and Earth Science and Technology, University of Hawai'i at Mānoa, Honolulu, HI 96822, USA. (mjchurch@hawaii.edu)

R. M. Letelier, College of Ocean and Atmospheric Sciences, Oregon State University, Corvallis, OR 97331, USA.

C. Mahaffey, Department of Earth and Ocean Sciences, University of Liverpool, Liverpool L69 3GP, UK.

J. P. Zehr, Ocean Sciences Department, University of California, Santa Cruz, CA 95064, USA.