

Global Biogeochemical Cycles



RESEARCH ARTICLE

10.1029/2018GB006130

Key Points:

- High rates of N₂ fixation were observed in temperate, western North Atlantic coastal waters
- Diverse diazotrophic groups were identified from samples collected in western North Atlantic coastal waters
- Global estimates of new N inputs from N₂ fixation should be revised to include coastal waters

Supporting Information:

- Supporting Information S1

Correspondence to:

M. R. Mulholland,
mmulholl@odu.edu

Citation:

Mulholland, M. R., Bernhardt, P. W., Widner, B. N., Selden, C. R., Chappell, P. D., Clayton, S., et al. (2019). High rates of N₂ fixation in temperate, western North Atlantic coastal waters expand the realm of marine diazotrophy. *Global Biogeochemical Cycles*, 33, 826–840. <https://doi.org/10.1029/2018GB006130>

Received 12 NOV 2018

Accepted 3 JUN 2019

Accepted article online 10 JUN 2019

Published online 6 JUL 2019

High Rates of N₂ Fixation in Temperate, Western North Atlantic Coastal Waters Expand the Realm of Marine Diazotrophy

M. R. Mulholland¹ , P. W. Bernhardt¹ , B. N. Widner^{1,2} , C. R. Selden¹ , P. D. Chappell¹ , S. Clayton¹ , A. Mannino³ , and K. Hyde⁴

¹Department of Ocean, Earth, and Atmospheric Sciences, Old Dominion University, Norfolk, VA, USA, ²Now at Woods Hole Oceanographic Institute, Woods Hole, MA, USA, ³Goddard Space Flight Center, National Aeronautics and Space Administration, Greenbelt, MD, USA, ⁴Northeast Fisheries Science Center, National Oceanic and Atmospheric Administration, Narragansett, RI, USA

Abstract Dinitrogen (N₂) fixation can alleviate N limitation of primary productivity by introducing fixed nitrogen (N) to the world's oceans. Although measurements of pelagic marine N₂ fixation are predominantly from oligotrophic oceanic regions, where N limitation is thought to favor growth of diazotrophic microbes, here we report high rates of N₂ fixation from seven cruises spanning four seasons in temperate, western North Atlantic coastal waters along the North American continental shelf between Cape Hatteras and Nova Scotia, an area representing 6.4% of the North Atlantic continental shelf area. Integrating average areal rates of N₂ fixation during each season and for each domain in the study area, the estimated N input from N₂ fixation to this temperate shelf system is 0.02 Tmol N/year, an amount equivalent to that previously estimated for the entire North Atlantic continental shelf. Unicellular group A cyanobacteria (UCYN-A) were most often the dominant diazotrophic group expressing *nifH*, a gene encoding the nitrogenase enzyme, throughout the study area during all seasons. This expands the domain of these diazotrophs to include coastal waters where dissolved N concentrations are not always depleted. Further, the high rates of N₂ fixation and diazotroph diversity along the western North Atlantic continental shelf underscore the need to reexamine the biogeography and the activity of diazotrophs along continental margins. Accounting for this substantial but previously overlooked source of new N to marine systems necessitates revisions to global marine N budgets.

Plain Language Summary Measurements suggest that at present, the marine nitrogen (N) budget is not balanced, and that rates of N losses exceed rates of N inputs in the world's oceans. Identifying quantitatively significant sources of new N inputs via marine dinitrogen (N₂) fixation could potentially offset this imbalance. Here we provide an unprecedentedly large data set showing high rates of seasonally and interannually averaged N₂ fixation rates over a large swath of western North Atlantic Ocean coastal waters along the continental shelf of North America, an area where N₂ fixation was previously thought to be negligible. If marine N₂ fixation has also been seriously underestimated in other coastal systems, global estimates of N inputs from N₂ fixation need to be revised upward, offsetting the current marine N budget imbalance.

1. Introduction

Biological dinitrogen (N₂) fixation is an important input component for marine nitrogen (N) budgets that can alleviate N limitation of primary productivity in oligotrophic ocean regions (Capone et al., 2005; Carpenter & Capone, 2008; Montoya et al., 2004; Zehr & Paerl, 2008). However, biogeography and activity of diazotrophs in coastal regions, where high concentrations of fixed N are thought to inhibit N₂ fixation, has not been broadly examined. Estimates suggest that 21–30% of primary productivity in the ocean occurs on the continental shelf (Jahnke, 2007); however, geochemical and climatological models are poorly resolved there because of their spatial and temporal heterogeneity (Deutsch et al., 2007; Gruber & Sarmiento, 1997). Geochemical models suggest that marine N₂ fixation rates have been underestimated and so identifying additional realms where biological N₂ fixation occurs may help balance global N budgets (Gruber & Sarmiento, 1997; Galloway et al., 2004; Codispoti, 2006; Deutsch et al., 2007; Landolfi et al., 2018).

©2019. The Authors.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

Until recently, planktonic N_2 fixation was thought to be restricted to oligotrophic tropical and subtropical systems where certain cyanobacterial diazotrophs are known to thrive (Carpenter et al., 1999; Carpenter & Capone, 2008; Church et al., 2005; Langlois et al., 2005; Moisander et al., 2010; Needoba et al., 2007). We now know that marine diazotrophs are diverse and include unicellular cyanobacteria as well as noncyanobacterial diazotrophs that occupy a wider range of marine habitats than previously thought (Berthelot et al., 2017; Bombar et al., 2016; Messer et al., 2016; Moisander et al., 2010; Rees et al., 2009; Zehr & Turner, 2001), but we know little about their physiological capabilities and environmental controls on their biogeography. N_2 fixation was thought to be trivial in coastal waters, including the North American continental shelf in the western North Atlantic Ocean, because higher dissolved N inputs and concentrations were thought to inhibit diazotrophic growth and activity (Conley et al., 2009; Howarth et al., 1988; Marino et al., 2002; Nixon et al., 1996; Zehr & Paerl, 2008). Based on their phylogenetic affiliations and the lack of quantifiable N_2 fixation rates, the high *nifH* gene diversity and presence of diverse bacterial diazotroph groups in coastal systems were attributed to microorganisms being transported there from terrestrial systems or sediments, rather than to autochthonous populations of active planktonic diazotrophs (Jenkins et al., 2004; Zehr et al., 2003). Until recently, few rate measurements were available from coastal waters (Conley et al., 2009; Howarth et al., 1988). High N_2 fixation rates have now been measured during summer in temperate northwestern Atlantic and Pacific coastal waters when dissolved N concentrations in surface water were seasonally depleted (Mulholland et al., 2012; Shiozaki et al., 2015), in temperate and tropical coastal systems (Cassar et al., 2018; Chen et al., 2014; Grosse et al., 2010; Larsson et al., 2001; Moisander et al., 2010; Mulholland et al., 2012; Rees et al., 2009; Shiozaki et al., 2015; Voss et al., 2006; Zhang et al., 2012), in nutrient-rich coastal upwelling systems (Voss et al., 2004; Wen et al., 2017), and in coastal Arctic Seas (Blais et al., 2012; Harding et al., 2018; Sipler et al., 2017), broadening the latitudinal range and the diversity of habitats supporting diazotrophy. Active N_2 fixation and expression of *nifH* (the gene encoding the iron protein in the N_2 -fixing nitrogenase enzyme) have been measured in nitrate (NO_3^-)-replete ($\sim 10 \mu M$) upwelling waters in the eastern tropical Atlantic Ocean (Voss et al., 2004; Sohm et al., 2011), in surface waters with elevated nitrate (NO_3^-) concentrations in the Pacific Ocean (Moisander et al., 2010), and in coastal waters influenced by the Mekong River plume (Bombar et al., 2011; Grosse et al., 2010; Voss et al., 2006). Even within the tropical North Atlantic basin, where high rates of N_2 fixation have long been associated with *Trichodesmium* blooms (e.g. Capone et al., 2005), it now appears that the unicellular symbiotic diazotroph UCYN-A contributes substantially to new N inputs (Martínez-Pérez et al. 2016).

Globally, the continental shelf comprises just 8% of the world's oceans at present, but these regions contribute disproportionately to primary productivity and carbon (C) sequestration relative to oceanic realms (Jahnke, 2007; Muller-Karger et al., 2005) thereby influencing global C budgets. Along riverine and estuarine influenced continental shelf regions such as the western North Atlantic continental shelf, inputs of terrestrial and fresh water microbes can be augmented by tropical and subtropical diazotrophs introduced into coastal waters from mixing with oligotrophic Gulf Stream waters where N_2 fixation is known to occur (Capone et al., 2005; Carpenter & Capone, 2008; Mulholland et al., 2012). Previously, we found high rates of N_2 fixation rates and abundant *nifH* gene copies during summer in coastal waters influenced by the Chesapeake and Delaware Bay plumes and in coastal waters between Cape Hatteras and the Gulf of Maine; and the presence of gene copies from tropical diazotrophs was detected in mid-Atlantic shelf waters north of Cape Hatteras and along Georges Bank (Mulholland et al., 2012). In the present study, N_2 fixation rates and diazotroph abundance are reported from seven cruises in the western North Atlantic Ocean along the North American continental shelf between Cape Hatteras to the Gulf of Maine, spanning 10° of latitude and longitude (Figure 1). Cruises were conducted over a 4-year period and were undertaken in all four seasons: two each in spring, summer, and fall, and another in winter. This represents the most comprehensive assessment of planktonic N_2 fixation rates from spatially and temporally heterogeneous coastal waters where N_2 fixation rates were previously thought to be negligible.

2. Materials and Methods

N_2 fixation rates were measured relative to nutrient concentrations and hydrographic properties during seven of the National Oceanic and Atmospheric Administration's (NOAA) Ecosystem Monitoring Program's ichthyoplankton surveys along the North American continental shelf in the western North

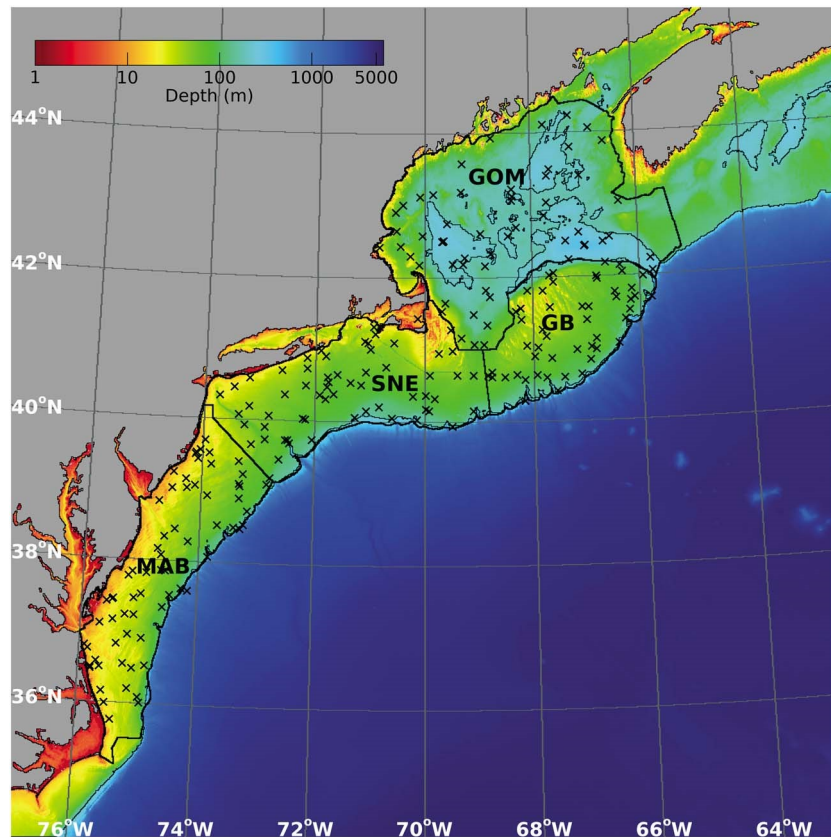


Figure 1. Bathymetric map of the study region. The X symbols represent an example of the 120 stations sampled during a quarterly NOAA NMFS Ecosystem Monitoring cruise. MAB = Middle Atlantic Bight; GB = George's Bank; GOM = Gulf of Maine; SNE = Southern New England Shelf; CH = Cape Hatteras; NOAA = National Oceanic and Atmospheric Administration; NMFS = National Marine Fisheries Service.

Atlantic Ocean from Cape Hatteras to Nova Scotia between 35.7 and 44.1°N and -75.9 to -65.7°W. Five cruises were aboard the NOAA vessel *Delaware II* between 17–28 August 2009, 3–19 November 2009, 2–17 February 2010, 26 May to 9 June 2010, and 6–21 November 2010, and two were aboard the NOAA vessel *Henry Bigelow* between 3–15 June 2011 and 8–23 August 2012. The study area comprises four ecoregions: the Mid-Atlantic Bight (MAB), between Cape Hatteras and 39.1°N; the Southern New England Shelf (SNE), between 39.1°N and 41.5°N but west of -70°W; Georges Bank (GB) the shelf area east of -70°W; and the Gulf of Maine (GOM, Figure 1). During each of the seven NOAA ichthyoplankton surveys, a randomly stratified set of stations as well as three fixed positions were occupied within the designated survey area.

Temperature, salinity, chlorophyll *a* fluorescence, and photosynthetically active radiation (PAR) profiles were measured to a maximum depth of 500 m during each cruise using a Seabird conductivity, temperature, and depth sensor and PAR sensor mounted to a 12-Niskin bottle sampling rosette. Water samples were collected from 2 to 4 depths at each station, depending on the water depth, using Niskin bottles mounted to the conductivity, temperature, and depth rosette. Samples were collected from the upper 6 m and near the bottom at shallow stations where the water column was well mixed. When the water column was stratified, samples were collected from the surface mixed layer and at the depth of the fluorescence maximum at shallow stations. At deeper stations, water samples were collected from the surface mixed layer and the depth of the fluorescence maximum as well as additional intermediary water depths. Water from Niskin bottles was drained into acid-cleaned and copiously rinsed carboys to homogenize water for N₂ fixation rate measurements. Water from Niskin bottles was gently pumped through 0.2- μ m Supor cartridge filters or gravity filtered through Millipore filters to collect samples directly into rinsed duplicate sterile polypropylene conical tubes for nutrient analyses. Samples for analysis of particulate N and carbon (C) and chlorophyll *a*

were collected onto precombusted (450 °C for 2 hr) GF/F filters (nominal pore size of 0.7 μm). Filters and filtrate were frozen in sterile tubes until analysis. Nutrient concentrations were measured colorimetrically using an Astoria Pacific nutrient analyzer according to manufacturer specifications. Ammonium concentrations were measured manually using the phenol-hypochlorite method (Solarzano, 1969). Detection limits for nitrate, nitrite, ammonium, and phosphate were 70, 70, 40, and 30 nM, respectively. Chlorophyll *a* concentrations were measured fluorometrically using the nonacidification method after extraction in acetone (Welschmeyer, 1994). Euphotic depths were calculated as the depth at which PAR was 1% of surface irradiance.

For uptake experiments, whole water from each depth was dispensed from carboys into acid-cleaned and Milli-Q water rinsed incubation bottles. Tracer additions (< 10%) of highly enriched (99%) $^{15}\text{N}_2$ (Cambridge Isotopes) were added to gas tight bottles using an opening and closing syringe (Montoya et al., 1996). Incubation bottles were then transferred to deck incubators plumbed with flow-through surface seawater to maintain near-ambient water temperatures and covered with neutral density screen to reproduce light levels at the depth of water collection. Incubations were terminated after 24 hr by filtration through precombusted (450 °C for 2 hr) GF/F filters and frozen until analysis. Samples were dried and pelleted into tin discs for isotopic analysis using a Europa 20/20 mass spectrometer with an automated nitrogen and carbon analyzer preparation module. Rates of uptake were calculated using a mixing model, and error was propagated as described previously (Gradoville et al., 2017; Montoya et al., 1996). Because of the incomplete equilibration of $^{15}\text{N}_2$ tracer when it is introduced as a gas bubble (Großkopf et al., 2012; Mohr et al., 2010; Mulholland et al., 2012; Wilson et al., 2012), the bubble addition method used in this study may have underestimated rates of N_2 fixation by a factor of 1.4 or more (Großkopf et al., 2012; Mohr et al., 2010; Mulholland et al., 2012). However, seawater equilibration of $^{15}\text{N}_2$ gas in site water was impractical during cruises because of the randomized cruise track through hydrographically and biogeochemically variable coastal waters, and the bubble removal technique (Jayakumar et al., 2017) had not yet been perfected in 2009 when the first cruises were undertaken. We calculate that if bubble equilibration took place over the first 8 hr of our incubations and reached a maximum of 88% dissolution in the incubation bottles (which were gently rolling in incubators over the incubation period) over that time period, our rates of N_2 fixation could have been underestimated by a factor of about 1.8, similar to what we calculated previously (1.4; Mulholland et al., 2012) and what has been estimated in direct comparisons of bubble addition and bubble equilibration methods (2; Großkopf et al., 2012). We realize this calculation is imperfect given the variability in gas solubility with respect to sample agitation and temperature, both of which varied over the course of cruises and incubation experiments. Further, a recent meta-analysis suggests that the underestimation of N_2 fixation rates made using the bubble method may be negligible for 12- to 24-hr incubations, such as those employed here (Wannicke et al., 2018). Therefore, rates presented here should be considered *minimum* rates of in situ N_2 fixation.

To compare rates of N_2 fixation with environmental variables including dissolved inorganic N (DIN), dissolved inorganic phosphorus (DIP), the DIN:DIP ratio, and temperature, we made property-property plots for the pooled surface data, data sorted by region, and data sorted by cruise and season. Because we found no significant linear relationships between rates of N_2 fixation and any of those variables, we binned our surface rates into increments of 2 °C temperature and 1-μM DIN to compare median N_2 fixation rates with temperature and DIN concentrations as well as temperature and the DIN:DIP ratio.

Areal rates of N_2 fixation in the euphotic zone were calculated by integrating volumetric rates over the euphotic zone. The euphotic zone was defined as the depth at which PAR was 1% of the surface PAR and was measured directly during daylight hours. Because diazotrophs include autotrophic and heterotrophic groups, we multiplied surface rate measurements by the depth of the upper mixed layer and the deeper rate measurements by the difference between the depth strata from which they were collected and either the base of the upper mixed layer or the depth of the nearest overlying rate measurement. We then added the depth-integrated rates together over the entire water column (for shallow stations and when the water column was well mixed), to the euphotic depth (for samples collected during the day), or to the deepest sampling depth or upper 50 m (when samples were collected during the dark). The upper mixed layer depth was calculated using the Levitus sigma-t criterion of 0.125 change from surface. Sea surface temperature (SST) maps on which areal rates are superimposed were constructed using Group for High Resolution Sea Surface

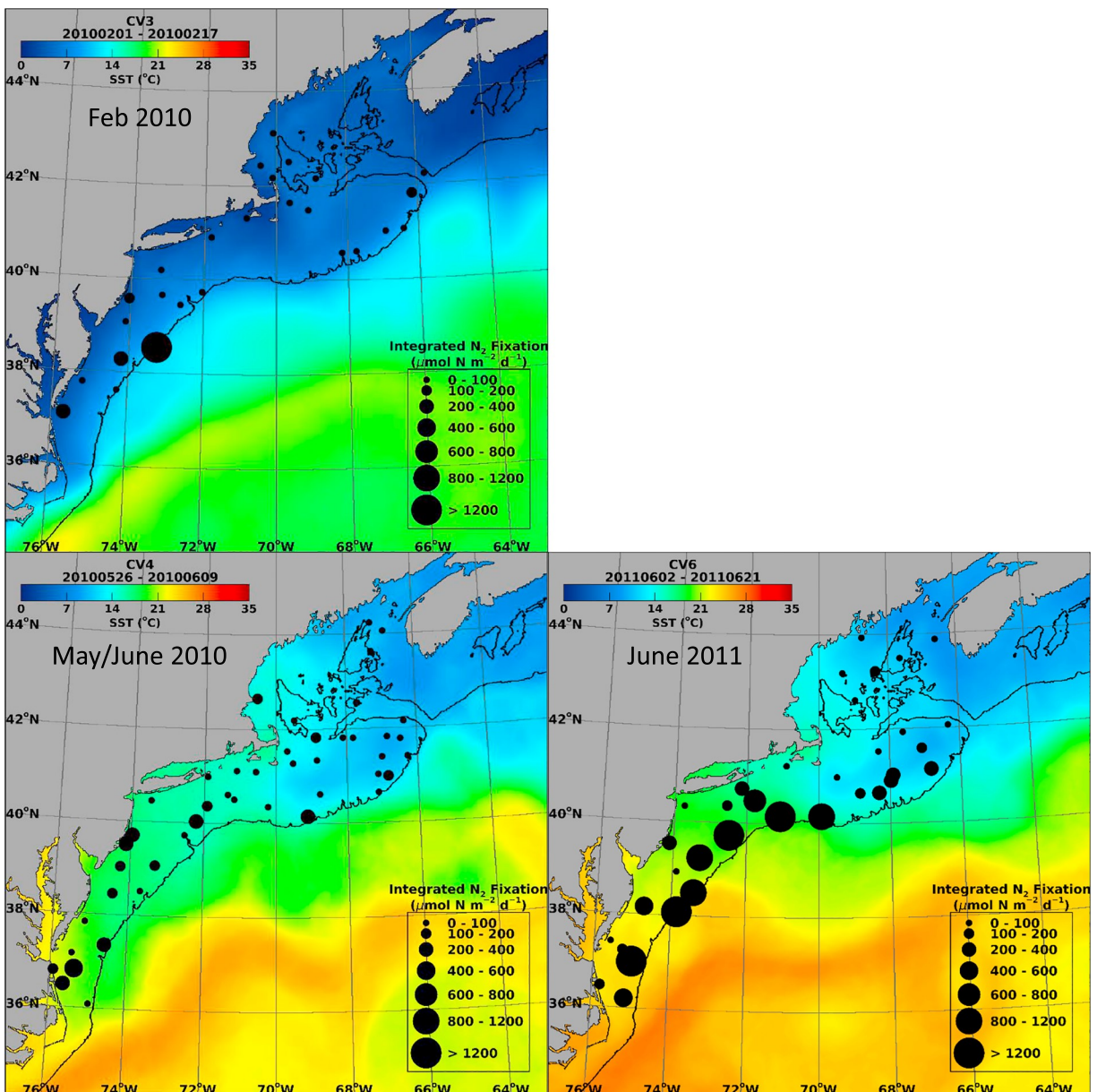


Figure 2. Areal rates of N_2 fixation ($\mu\text{mol N}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$) during cruises along the western North Atlantic continental shelf during February 2010 (top panel) and May/June 2010 and June 2011 (bottom row). Depth-integrated rates are superimposed on satellite observations of the average surface water temperature during the cruise period.

Temperature Level 4 MUR (v4.1) satellite data averaged over the cruise period (Jet Propulsion Laboratory MUR MEaSUREs Project, 2015).

Samples were collected onto $0.2\text{-}\mu\text{m}$ Sterivex filters for molecular analyses and immediately frozen and transported to Old Dominion University, where they were transferred to -80°C freezers. Select samples were chosen for molecular analysis based on N_2 fixation rates. RNA and DNA were coextracted from filters using the AllPrep RNA/DNA minikit (Qiagen) with minor adjustments including a bead-beating step and a QIAshredder spin-column step. RNA was treated with amplification-grade DNase I (Invitrogen) and converted to cDNA using SuperScript III first-strand synthesis primed with the *nifH3* primer (Zehr & Turner, 2001). Diversity of the active diazotroph community was investigated by amplifying a partial *nifH* fragment from cDNA using degenerate primers and a nested polymerase chain reaction (PCR) protocol (Zehr & Turner, 2001) with the adjustment that, in the second round of PCR,

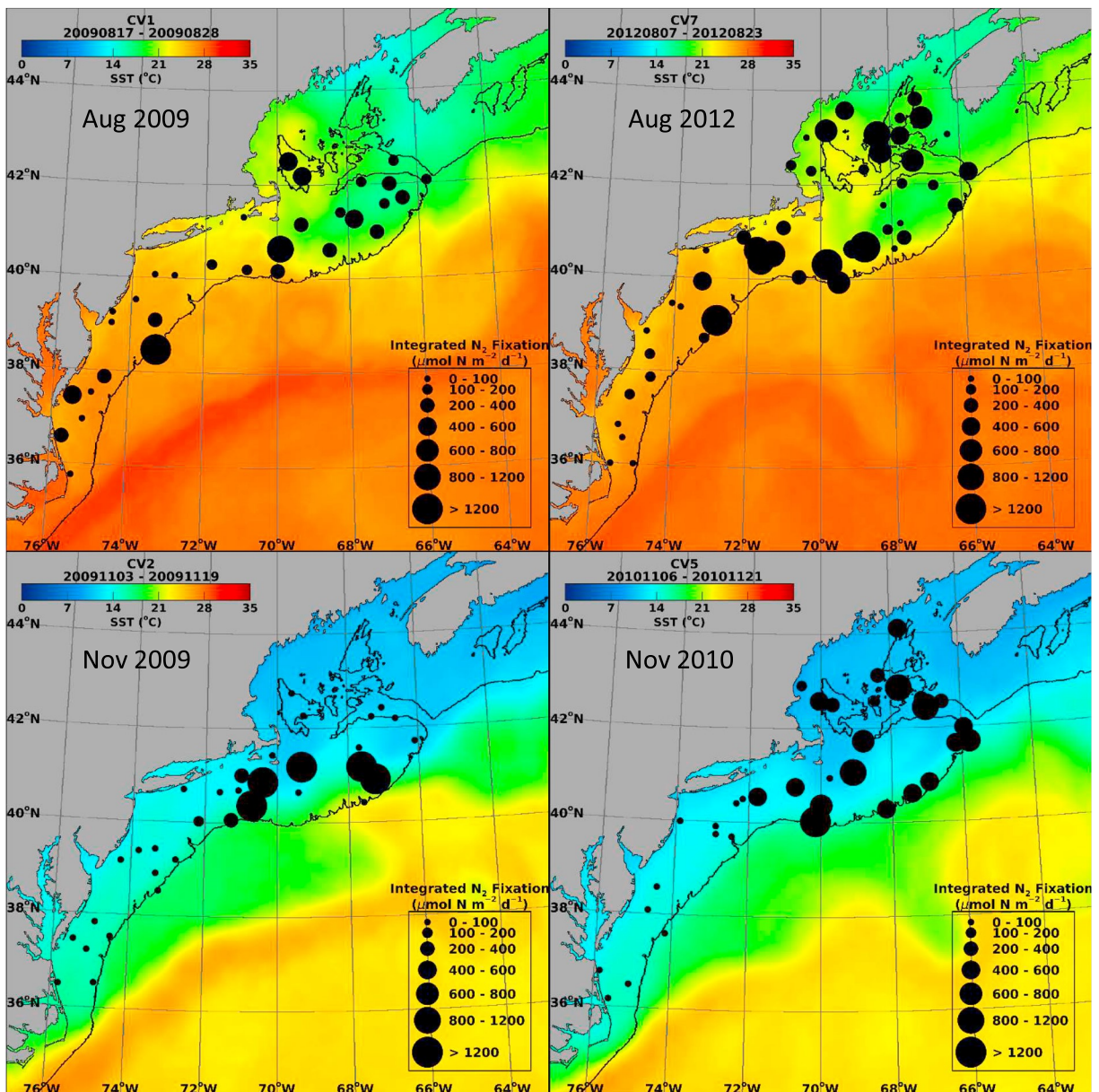


Figure 3. Areal rates of N_2 fixation ($\mu\text{mol N}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$) during cruises along the western North Atlantic continental shelf during August 2009 and 2012 (top row) and November 2009 and 2010 (bottom row). Depth-integrated rates are superimposed on satellite observations of the average surface water temperature during the cruise period.

primers were modified to include the Illumina overhang adapter sequences for two step amplicon sequencing (http://www.illumina.com/content/dam/illumina-20support/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf). Initial PCR products were gel purified and continued through index PCR (http://www.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf) and sequencing on an Illumina MiSeq sequencer using a 2×300 -bp kit. Sequences were demultiplexed and imported into the CLC Genomics Workbench (Qiagen, Germany). Reads were imported in pairs, trimmed, and merged in CLC before being exported in fasta format. As the number of reads per sample was not uniform, a random sampling of 20,000 reads was extracted from each set of sample reads to normalize the data sets prior to analysis. *NifH* community composition was determined using the minimum entropy decomposition pipeline (Eren et al., 2015). Representative nodes (what minimum entropy

Table 1
Seasonal Mean and Median N_2 Fixation Rates by Study Region

	Seasonal range ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$)	Seasonal average ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$)	Seasonal median ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$)	Study area annual mean ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{year}^{-1}$)	# of stations sampled	Study area annual median ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{year}^{-1}$)
GB						
Fall	0–843.1	230.9	226.6	61,173	$n = 13$	50,340
Spring	0–393.6	88.2	73.6		$n = 13$	
Summer	36.4–1911	225.8	125.9		$n = 18$	
Winter	125.5	125.5	125.5		$n = 1$	
GOM						
Fall	0–676	254.2	157.4	42,869	$n = 16$	29,793
Spring	0–67.8	28.0	25.3		$n = 9$	
Summer	23–514.6	165.2	121.4		$n = 19$	
Winter	22.43	22.4	22.4		$n = 1$	
MAB						
Fall	0–111	29.5	20.3	46,985	$n = 18$	23,050
Spring	0–1694	289.1	120.6		$n = 26$	
Summer	4.6–600	138.3	75.1		$n = 23$	
Winter	0–162	58.0	36.6		$n = 6$	
SNE						
Fall	0–4106	352.1	16.1	83,804	$n = 17$	30,295
Spring	0–1735	210.1	44.7		$n = 20$	
Summer	27–873	313.2	225.2		$n = 18$	
Winter	0–81.3	43.0	46.0		$n = 6$	

Note. MAB = Mid-Atlantic Bight; SNE = Southern New England Shelf; GOM = Gulf of Maine; GB = Georges Bank.

decomposition calls operational taxonomic units) were classified by nucleotide BLAST (Altschul et al., 1990) against an in-house database of *nifH* sequences. We recognize the recent concern regarding the propensity for amplicon sequencing to miss certain taxonomic groups of diazotrophs, in particular the genus *Richelia*, the endosymbiont to the diatom *Rhizosolenia*.

To assess which single or combination of environmental variable(s) best explained the observed dissimilarity of expressed *nifH* sequences between sites, a Bray-Curtis dissimilarity matrix was first generated in PRIMER (v.6; Clarke & Gorley, 2006) from expressed sequence relative abundance and funneled into the BEST analysis (Clarke & Ainsworth, 1993). Using the BIO-ENV algorithm (Spearman rank correlation method), the Bray-Curtis dissimilarity matrix (Bray & Curtis, 1957) was compared to Euclidean distance matrices generated from varied combinations of measurements (sample depth, bottom depth, temperature, salinity, PAR, chlorophyll *a* concentration, nitrate plus nitrite concentration, phosphate concentration, net primary productivity, and N_2 fixation rate). A permutation test (10,000 randomizations) was used to determine significance.

3. Results and Discussion

Areal rates of N_2 fixation ranged from below detection to $4,106 \mu\text{mol N}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ during the seven cruises (Figures 2 and 3, Tables S1–S7 in the supporting information). These rates are within or higher than the ranges observed in tropical and subtropical oceanic regions ($3.7\text{--}703 \mu\text{mol N}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$; Capone et al., 2005; Carpenter & Capone, 2008), and temperate oceanic waters (Mulholland et al., 2012; Rees et al., 2009). Volumetric N_2 fixation rates were usually highest in surface waters where they ranged from below detection to $130 \text{ nmol N}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ (Tables S1–S7) with many on the high end of those reported previously (Luo et al., 2012 and references therein). N_2 fixation rates at the depth of the chlorophyll maximum were usually lower than those measured in surface waters, ranging from below detection to $68.9 \text{ nmol N}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ over all seven cruises. Using seasonally averaged areal N_2 fixation rates for each of the four regions (MAB, SNE, GB, and GOM) and integrating over a year, we estimate that the total planktonic N input from N_2 fixation in shelf

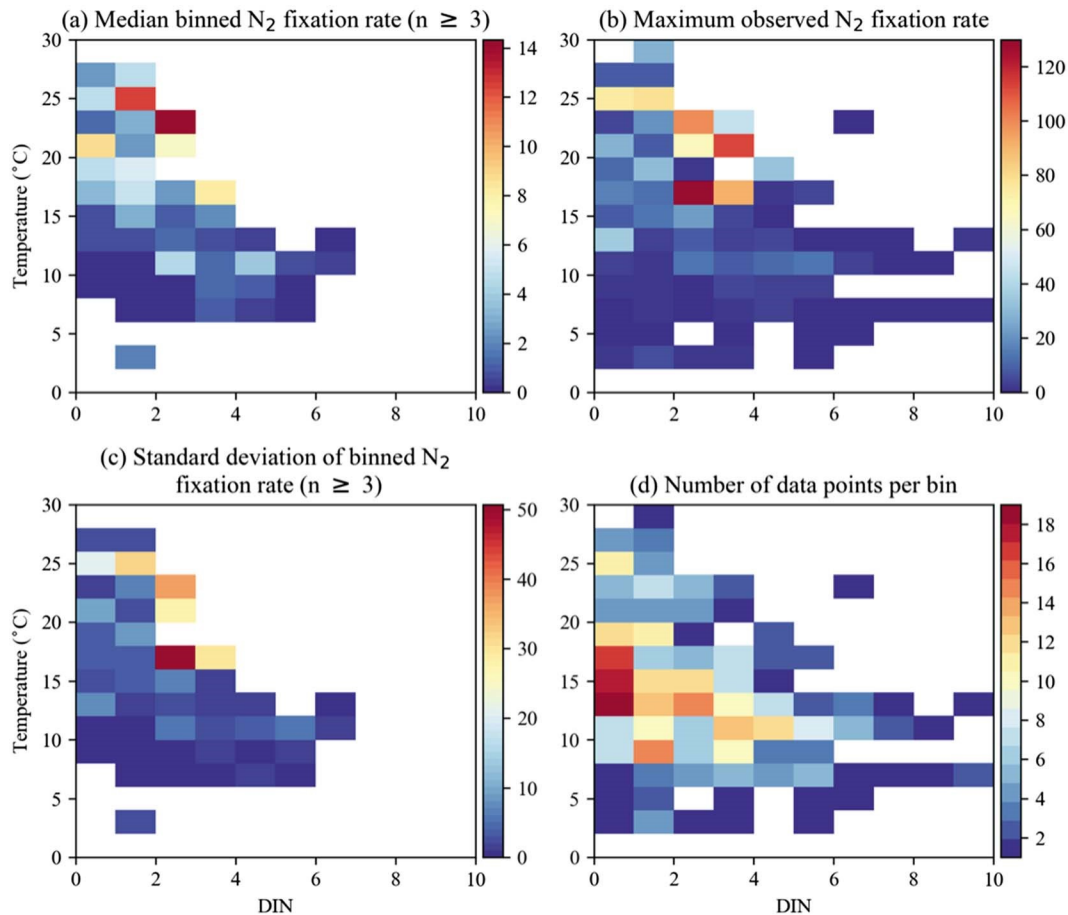


Figure 4. Median (a) and maximum (b) volumetric N₂ fixation rates (nmol N·L⁻¹·d⁻¹) binned by temperature and dissolved inorganic nitrogen (DIN) concentration (μM), along with the standard deviations of N₂ fixation rates (c) and number of data points (d) within each bin. Statistics in Figures 4a and 4c were only calculated for bins with a minimum of three data points.

waters between Cape Hatteras and Nova Scotia (35–45°N latitude) is about 0.02 Tmol N/year, (Table 1). Even though the study region accounted for just 6.4% of the total North Atlantic continental shelf area, we calculated that the annual N inputs from N₂ fixation were comparable to those estimated for the entire North Atlantic continental shelf area (0.02 Tmol N/year; Nixon et al., 1996) and up to 7% of the estimated basin-wide N inputs from N₂ fixation (Capone et al., 2005; Carpenter & Capone, 2008; Mahaffey et al., 2005).

To better understand the biogeography of active N₂ fixation and diazotroph groups, hydrographic properties and nutrient concentrations were also measured. Although high temperatures and low concentrations of DIN, typical of the oligotrophic tropical and subtropical gyres, and eutrophic fresh and brackish water environments enriched in DIP relative to DIN, have been implicated as sites hospitable for diazotrophy (Conley et al., 2009; Howarth et al., 1988; Zehr & Paerl, 2008), N₂ fixation was detected throughout the study area even when DIN concentrations were measurable (range was below analytical detection to 10.9 μmol N/L, Figure 4) and when the DIN:DIP ratio was in excess of 16 (Figure 5), the average ratio of these elements in marine primary producers (see also Tables S1–S7). Although there were no linear relationships between N₂ fixation rates and DIN:DIP ratios ($R^2 = 0.01$) or DIN concentrations ($R^2 = 0.02$) during any of the cruises (data not shown), in general, N₂ fixation rates were higher when DIN concentrations were less than 4 μM (Figure 4) and DIN:DIP ratios less than 16 (Figure 5). However, the maximum observed N₂ fixation rates for each bin did not always comply with this general pattern and data density was low at high DIN concentrations (Figure 4d) and DIN:DIP ratios (Figure 5d). The majority of our rate measurements were made in waters where DIN concentrations were <5 μM. Active N₂ fixation was previously observed in NO₃⁻-replete (~10 μM) upwelled waters in the eastern tropical North and South Atlantic Oceans (Sohm et al., 2011; Voss

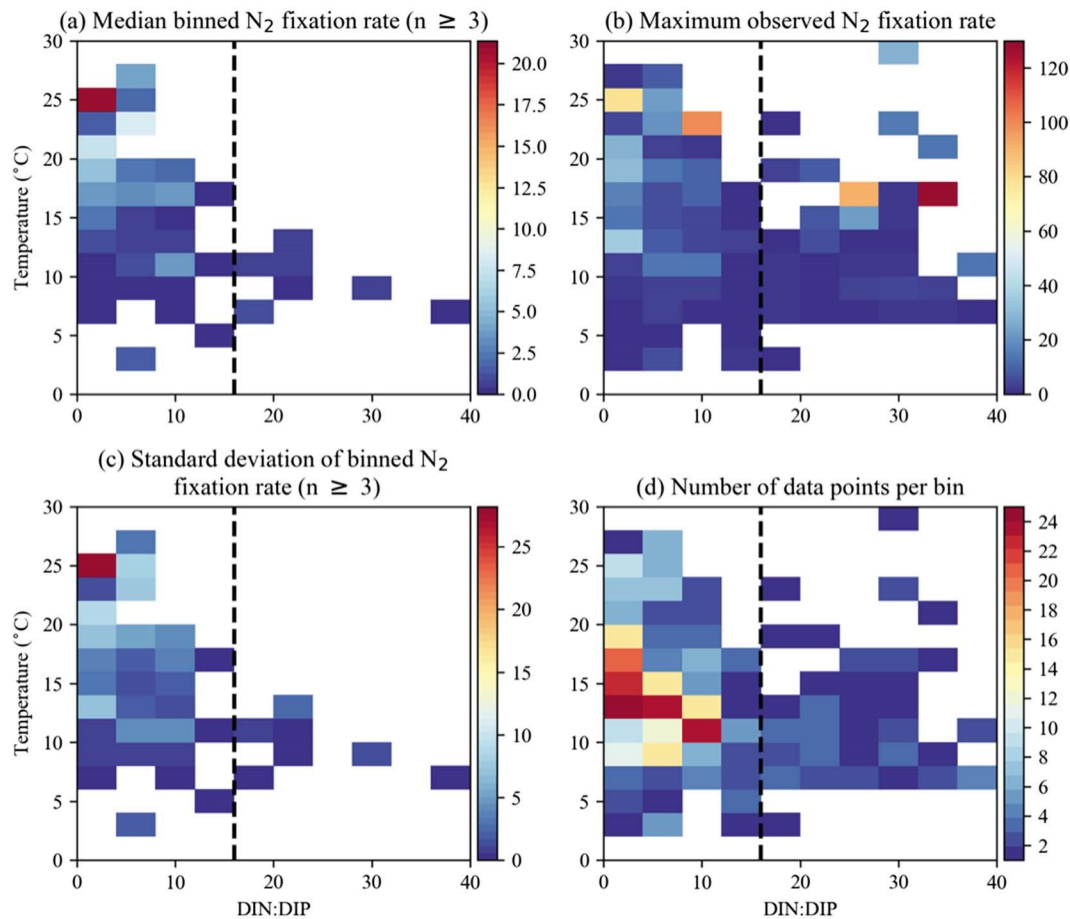


Figure 5. Median (a) and maximum (b) volumetric N_2 fixation rates ($\text{nmol N}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$) binned by temperature and dissolved inorganic nitrogen (DIN):dissolved inorganic phosphorus (DIP) ratios, along with the standard deviations of N_2 fixation rates (c) and number of data points (d) within each bin. Statistics in Figures 5a and 5c were only calculated for bins with a minimum of three data points. The 16:1 DIN:DIP ratio is indicated with the broken black line.

et al., 2004), in NO_3^- -enriched surface waters in the Pacific Ocean (Moisander et al., 2010), and in N-replete culture systems (Knapp et al., 2012; Mulholland et al., 2001).

In this study, N_2 fixation rates were generally, but not always, higher in the well-lit surface mixed layer ranging from below detection to $130 \text{ nmol N}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ (Tables S1–S7) where diazotrophic cyanobacteria thrive. Planktonic N_2 fixation rates by some marine cyanobacteria are thought to be limited by temperature (Carpenter & Capone, 2008). Indeed, higher rates of N_2 fixation were measured during cruises between June and November, when surface water temperatures were warmer. However, linear regression analyses suggest no relationship between water temperature and N_2 fixation rates either in the pooled data ($R^2 = 0.10$) or in surface waters ($R^2 = 0.08$). Binned data show that while rates of N_2 fixation were generally higher when water temperatures were higher, maximum N_2 fixation rates were often observed at lower temperatures (Figures 4 and 5). Further, the majority of our rate measurements were made when water temperatures were between 12 and 20 °C. N_2 fixation by cyanobacteria was previously thought to be constrained to surface waters with temperatures >20 °C (e.g., Carpenter & Capone, 2008), but more recently, active N_2 fixation has been detected in higher-latitude temperate regions (Cassar et al., 2018; Moisander et al., 2010) and even coastal Arctic Seas (Blais et al., 2012; Harding et al., 2018; Sipler et al., 2017). The high rates of coastal N_2 fixation reported here are comparable to or higher than those measured in the temperate English Channel (Rees et al., 2009) and coastal waters in the northwestern Pacific (Shiozaki et al., 2015) and Atlantic (Cassar et al., 2018) Oceans; however, measurements from coastal systems are still sparse.

We note that the highest depth-integrated N_2 fixation rates were often associated with frontal features, characterized by strong SST gradients during the late fall (November 2009 and 2010) and summer (June 2011 and

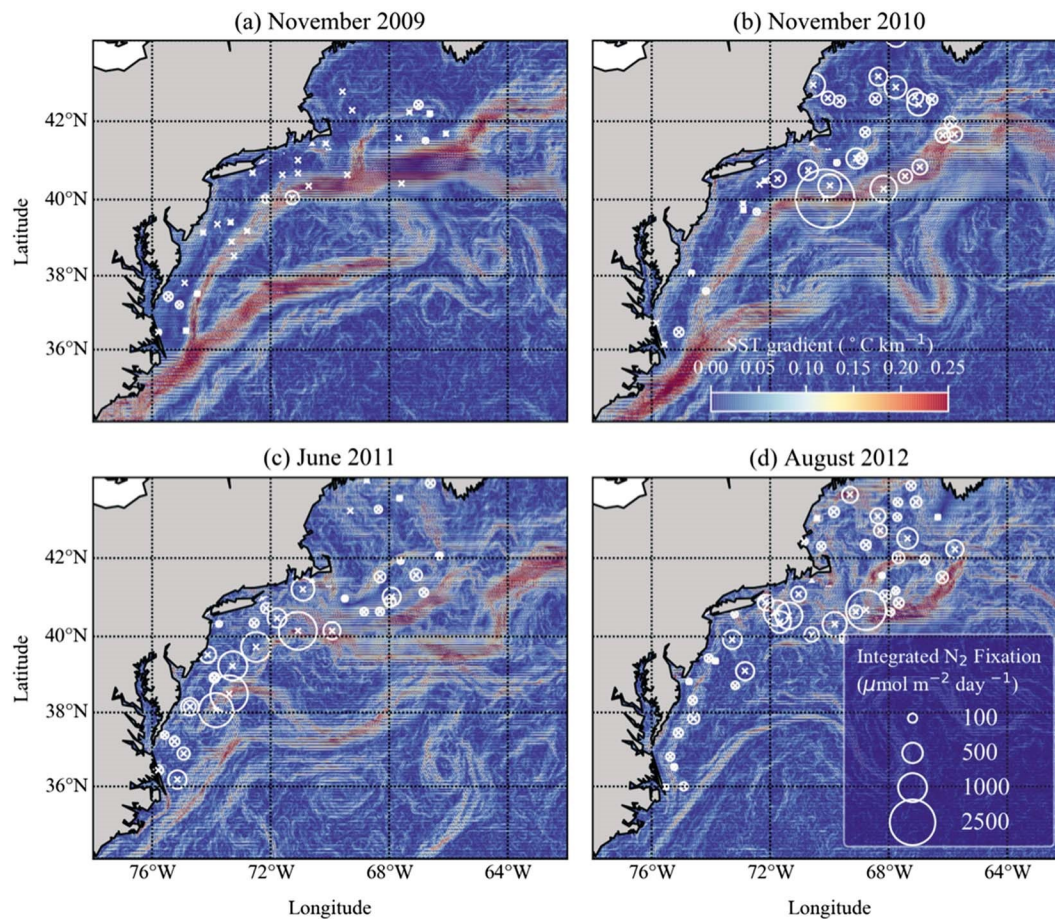


Figure 6. Integrated N_2 fixation ($\mu\text{mol N}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$) for the November 2009 (a), November 2010 (b), June 2011 (c), and August 2012 (d) cruises plotted over the absolute sea surface temperature (SST) gradient ($^{\circ}\text{C}/\text{km}$) for the study region, calculated from the SST fields used to generate Figures 2 and 3. The SST gradient is a proxy for the location of large-scale coastal open ocean front found along the shelf break of the northeastern coast of the United States.

August 2012) cruises (Figure 6). The patterns were less clear in spring and summer as the sampling during those cruises either did not extend far enough offshore to reach the shelf break front (February and May 2010), or because few stations were sampled in the frontal region to visualize patterns (August 2009). Although this result is only based on data from four cruises, it suggests the intriguing possibility that N_2 fixation might be locally enhanced at the shelf break front along the northeast coast of the United States. This enhancement may be due to a range of factors, including enhanced local vertical nutrient supply at the front, the relief of temperature limitation at the front, or the mingling of diverse diazotrophic populations from coastal and open ocean communities.

Based on analyses of select samples, unicellular Group A cyanobacteria (UCYN-A) dominated the expressed *nifH* sequences throughout the study area during all four seasons (Figure 7 and Table 2), consistent with previous observations of its distribution (Moisander et al., 2010). Relative abundances of UCYN-A were high at stations on Georges Bank (latitudes $>40^{\circ}\text{N}$), concurrent with observations that this organism thrives in cooler water than tropical and subtropical cyanobacterial diazotrophs (Langlois et al., 2008; Moisander et al., 2010). *Trichodesmium* was detected only at a station along the shelf break front where hydrography was modulated by the Gulf Stream (Figure 6). At one nearshore station, *Pseudomonas* was the dominant group expressing *nifH*.

BEST analysis (Clarke & Ainsworth, 1993) in PRIMER (v.6; Clarke & Gorley, 2006) was used to determine what combination of environmental parameters (depth, temperature, salinity, PAR, chlorophyll *a*, nitrate plus nitrite [NO_x] concentrations, phosphate [PO_4^{3-}] concentrations, net primary productivity, and N_2 fixation rate) best explained diazotrophic community composition across the study region. As opposed to a

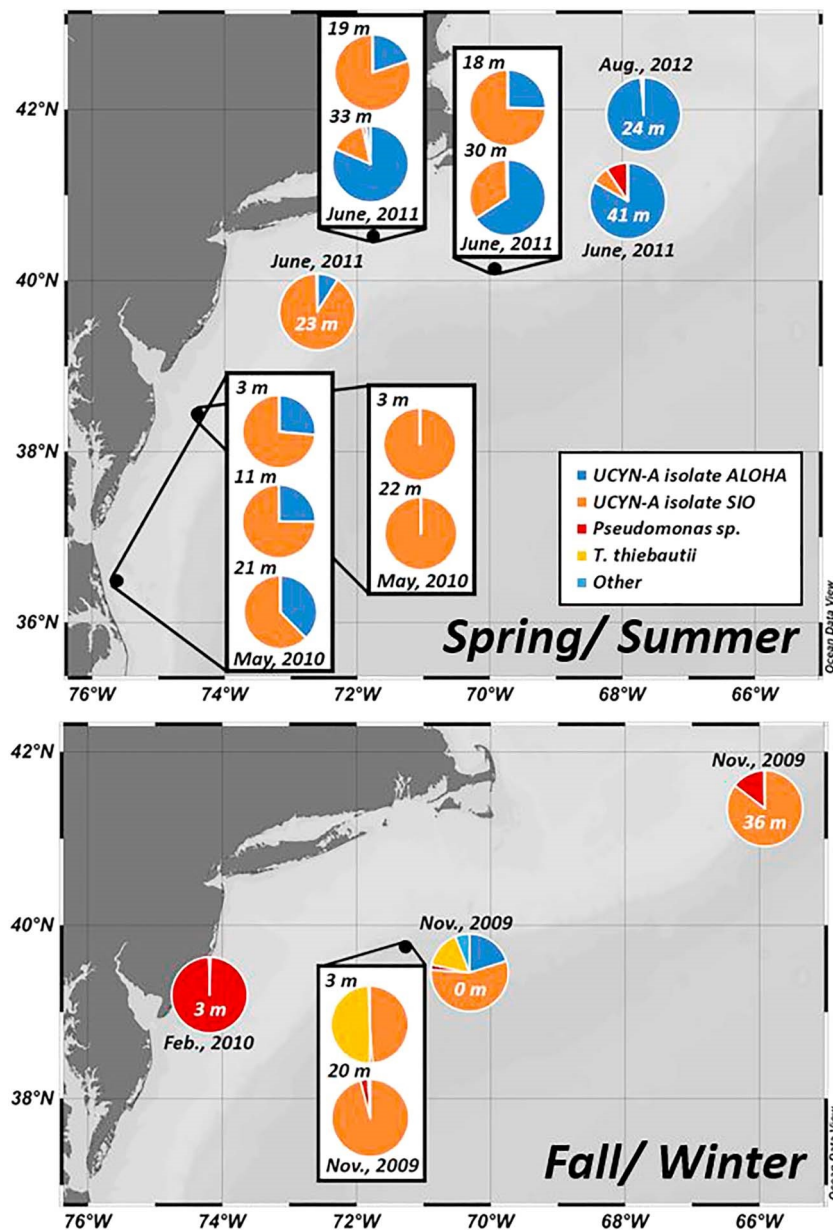


Figure 7. Relative abundance of *nifH* genes expressed by dominant diazotrophic groups during spring and summer cruises (top panel) and fall and winter cruises (bottom panel). UCYN-A isolates are from Station Aloha in the tropical North Pacific (ALOHA) and from the eastern North Pacific in coastal waters near Scripps Institute of Oceanography (SIO) in San Diego, CA, USA.

canonical correlation, this approach compares rank similarity matrices of community structure and environmental parameters. The strongest correlation to Bray-Curtis community dissimilarity (Bray & Curtis, 1957) occurred through the combination of temperature, chlorophyll *a*, [NO_x], and net primary productivity (Spearman $\rho_s = 0.549$, $p = 0.012$; Table 2). When chlorophyll *a* concentrations were excluded from the analysis, the combination of temperature, [NO_x], and net primary productivity still offered the strongest correlation (Spearman $\rho_s = 0.535$, $p = 0.022$), suggesting that these factors are closely linked to diazotroph community composition.

Temperature, [NO_x], and primary productivity can all vary between water masses as well as vacillate seasonally within water masses (Tables S1–S7). The correlation between diazotroph community composition and these combined factors may thus represent regional and seasonal variations in diazotroph community

Table 2
Diazotroph Diversity at Select Stations and Depths Along the Mid-Atlantic Continental Shelf During Seasonal Cruises Between November (Nov) 2009 and August (Aug) 2012

Long (°W)	Lat (°N)	Location	Date	Depth (m)	Temp. (°C)	Shannon diversity index (<i>H</i>)	Shannon's equitability (<i>E_H</i>)	N ₂ fixation rate (nmol N·L ⁻¹ ·d ⁻¹)	Relative abundance of group (%)				
									UCYN-A isolate ALOHA	UCYN-A isolate SIO	Pseudomonas sp.	T. thiebautii	Other
-69.929	40.150	SNE	June 2011	17.2	11.3	0.610	0.340	13.4 (1.4)	25.27	74.71		0.01	0.01
-74.045	39.563	MAB	Feb 2010	1.83	2.4	0.062	0.030	2.04 (3.2)	0.17	0.43	99.09	0.06	0.26
-75.632	36.483	MAB	May 2010	1.93	19.1	0.596	0.287	17.8 (6.1)	26.49	73.43	0.07		0.02
-75.632	36.483	MAB	May 2010	9.73	14.0	0.578	0.278	1.34 (2.8)	25.20	74.73		0.01	0.06
-75.632	36.483	MAB	May 2010	19.8	13.3	0.698	0.336	1.77 (2.5)	37.60	62.28	0.07	0.01	0.05
-65.775	41.680	GB	Nov 2010	34.7	10.5	0.441	0.227	6.36 (2.9)	0.09	85.31	14.26		0.34
-67.667	42.019	GB	Aug 2012	22.7	18.0	0.075	0.042	1.97 (1.0)	98.79	1.17	0.03		0.01
-71.785	40.476	SNE	June 2011	18.1	12.7	0.810	0.307	6.00 (1.1)	19.95	79.80	0.09	0.04	0.12
-71.785	40.476	SNE	June 2011	32.1	8.0	0.608	0.253	0.87 (0.4)	81.54	15.15	1.49		1.82
-69.929	40.150	SNE	June 2011	30.4	12.0	0.910	0.438	BDL	65.82	33.52	0.60		0.07
-67.915	41.019	SNE	June 2011	40.5	10.3	0.571	0.274	9.12 (1.3)	83.32	7.42	9.22	0.02	0.01
-72.495	39.725	SNE	June 2011	22.5	15.2	0.402	0.250	6.96 (0.8)	8.93	90.69	0.38	0.01	
-71.274	40.051	SNE	Nov 2009	2.43	14.8	1.135	0.473	4.50 (2.1)	0.07	48.69	1.25	49.52	0.48
-71.274	40.051	SNE	Nov 2009	19.1	14.8	0.209	0.079	2.08 (1.9)	0.05	95.87	3.28	0.03	0.77
-74.445	38.443	MAB	May 2010	2.43	15.7	0.711	0.342	5.56 (2.0)	0.04	99.76	0.06		0.14
-74.445	38.443	MAB	May 2010	21.4	6.6	0.493	0.214	0.80 (1.7)	0.05	99.86	0.01	0.01	0.08
-70.146	40.026	SNE	Nov 2010	0		1.312	0.424	82.1 (27.0)	20.16	56.01	2.16	15.67	5.99

Note. MAB = Mid-Atlantic Bight; SNE = Southern New England Shelf; GB = Georges Bank.

composition with respect to water masses and their interactions. Temperature, however, is also known to influence rates of enzymatic activity and microbial growth rates. Consequently, temperature is typically a major driving force in defining the realized niches of many marine microbes, including some diazotrophs (Carpenter & Capone, 2008). For example, filamentous cyanobacterial diazotrophs such as *Trichodesmium* generally inhabit warmer waters (Breitbart et al., 2007; Capone et al., 2005) while UCYN-A and some proteobacterial diazotrophs are active under a much broader range of temperatures (Harding et al., 2018; Martinez-Perez et al., 2016; Moisaner et al., 2010).

Concentrations of NO_x are also thought to be fundamental to delimiting the range of certain diazotrophs as N₂ fixation by many groups are thought to be inhibited by the presence of fixed N (Carpenter & Capone, 2008). However, it now appears that some diazotrophic groups may be more sensitive to this effect than others, thereby influencing the composition of diazotroph assemblages in waters bearing significant NO_x concentrations or subject to NO_x inputs (Foster et al., 2007; Moisaner et al., 2012; Mulholland et al., 2001; Voss et al., 2006). For example, certain symbiotic diazotrophs appear to lack genes necessary to transport and assimilate some forms of dissolved inorganic N (Caputo et al., 2018) and may thus be less responsive to changes in ambient N concentrations than other diazotrophs. Both temperature and NO_x concentrations

may thus play a direct role in shaping diazotroph biogeography; however, determining the extent of this influence in the study region is beyond the scope of this study.

The relationship between primary productivity and diazotroph community composition is more enigmatic. Cyanobacterial diazotrophs may contribute to primary productivity directly (Capone et al., 2005; Montoya et al., 2004) or participate in symbioses with other photoautotrophic phytoplankton (Carpenter et al., 1999; Foster et al., 2007; Harding et al., 2018), while heterotrophic diazotrophs may rely on primary producers for a supply of organic carbon (Moisander et al., 2012). UCYN-A, which is abundant in this study, is known to form symbioses with a haptophyte (Thompson et al., 2012). While the limited scope of the molecular data presented here precludes a more detailed assessment of the environmental controls on diazotroph biogeography, the observed correlations between diazotroph community dissimilarity and temperature, primary productivity, and NO_x support the hypothesis that these factors are important in diazotroph niche separation.

4. Conclusions

This is the most comprehensive interannual assessment to date of planktonic N_2 fixation rates from neritic waters, regions where N_2 fixation were previously thought to be negligible. Results presented here indicate that N_2 fixation rates along the temperate western North Atlantic continental shelf between Cape Hatteras and the Gulf of Maine are comparable to, or higher than, those observed in most oceanic systems (Figures 1 and 2 and Tables S1–S7) suggesting that coastal N_2 fixation rates have been seriously underestimated. We calculate that N_2 fixation along this small fraction of North Atlantic continental shelf contributed an amount of new N previously estimated for the entire North Atlantic continental shelf area. Further, many of highest N_2 fixation rates were observed at frontal regions where water masses mix and exchange nutrients and microorganisms. This begs not only for a reassessment of coastal N_2 fixation worldwide but also for a reexamination of the biogeography of diazotrophic groups and their physiological capacities and limitations in coastal environments where the physical and chemical environments are highly variable on short temporal and spatial scales. This understanding is paramount as the growth and activity of cyanobacterial diazotrophs is projected to increase in the future as a result of increases in pCO_2 and sea surface temperatures (Hutchins et al., 2009; Paerl & Huisman, 2009; Paerl & Otten, 2013). Diazotrophs, like other marine microbes, appear to have a range of physiological tolerances and responses to DIN suggesting that water temperature and DIN concentrations alone do not control diazotroph distribution and activity in the marine environment (Mulholland et al., 2001, 2012; Knapp, 2012). It is more likely that the biogeography of diazotrophic groups is controlled by a complex suite of environmental parameters that we do not yet fully understand and that the realized niches of diazotrophic groups are shaped by competitive interactions as well as environmental conditions. To better estimate oceanic N inputs via planktonic N_2 fixation, we require a better understanding of the biogeography, activity, and physiological capacities of diazotrophic groups with respect to environmental and hydrographic variability.

Acknowledgments

Data presented in the body and supporting information of this manuscript have been deposited in the National Aeronautics and Space Administration (NASA) repository, SeaBASS and is publicly available at the following DOI address: 10.5067/SeaBASS/CLIVEC/DATA 001. This work was supported by a grant from NASA to M. R. M., A. M., and K. H.; a grant from NSF to P. D. C.; and the Jacques S. Zaneveld and Neil and Susan Kelley Endowed Scholarships to C. S. We thank NOAA for ship time and the captain and crew of NOAA vessels *Delaware II* and *Henry Bigelow* for assistance during field sampling. Data have been submitted to SeaBASS (<https://seabass.gsfc.nasa.gov/>), NASA's preferred archival repository.

References

- Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, 215(3), 403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)
- Berthelot, H., Benavides, M., Moisander, P. H., Grosso, O., & Bonnet, S. (2017). High-nitrogen fixation rates in the particulate and dissolved pools in the western tropical Pacific (Solomon and Bismarck Seas). *Geophysical Research Letters*, 44, 8414–8423. <https://doi.org/10.1002/2017GL073856>
- Blais, M., Tremblay, J.-É., Jungblut, A. D., Gagnon, J., Martin, J., Thaler, M., & Lovejoy, C. (2012). Nitrogen fixation and identification of potential diazotrophs in the Canadian Arctic. *Global Biogeochemical Cycles*, 26, GB3022. <https://doi.org/10.1029/2011GB004096>
- Bombar, D., Moisander, P. H., Dippner, J. W., Foster, R. A., Voss, M., Karfeld, B., & Zehr, J. P. (2011). Distribution of diazotrophic microorganisms and *nifH* gene expression in the Mekong River plume during intermonsoon. *Marine Ecology Progress Series*, 424, 39–52.
- Bombar, D., Paerl, R. W., & Riemann, L. (2016). Marine non-cyanobacterial diazotrophs: Moving beyond molecular detection. *Trends in Microbiology*, 24(11), 916–927.
- Bray, J. R., & Curtis, J. T. (1957). An ordination of the upland forest communities of southern Wisconsin. *Ecological Monographs*, 27, 325–349.
- Breitbarth, E., Oschlies, A., & LaRoche, J. (2007). Physiological constraints on the global distribution of *Trichodesmium*—Effect of temperature on diazotrophy. *Biogeosciences*, 4(1), 53–61. <https://doi.org/10.5194/bg-4-53-2007>
- Capone, D. G., Burns, J. A., Montoya, J. P., Subramaniam, A., Mahaffey, C., Gunderson, T., et al. (2005). Nitrogen fixation by *Trichodesmium* spp.: An important source of new nitrogen to the tropical and subtropical North Atlantic Ocean. *Global Biogeochemical Cycles*, 19, GB2024. <https://doi.org/10.1029/2004GB002331>

- Caputo, A., Stenegren, M., Pernice, M. C., & Foster, R. A. (2018). A short comparison of two marine planktonic diazotrophic symbiose-shighlights an un-quantified disparity. *Frontiers in Marine Science*, 5–2. <https://doi.org/10.3389/fmars.2018.00002>
- Carpenter, E. J., & Capone, D. G. (2008). Dinitrogen fixation. In D. G. Capone, D. A. Bronk, M. R. Mulholland, & E. J. Carpenter (Eds.), *Nitrogen in the marine environment*, (pp. 141–198). Burlington, MA: Elsevier.
- Carpenter, E. J., Montoya, J. P., Burns, J., Mulholland, M. R., Subramaniam, A., & Capone, D. G. (1999). Extensive bloom of a N₂-fixing diatom/cyanobacterial association in the tropical Atlantic Ocean. *Marine Ecology Progress Series*, 185, 273–283. <https://doi.org/10.3354/meps185273>
- Cassar, N., Tang, W., Gabathuler, H., & Huang, K. (2018). Method for high frequency underway N₂ fixation measurements: Flow-through incubation acetylene reduction assays by cavity ring down laser absorption spectroscopy (FARACAS). *Analytical Chemistry*, 90(4), 2839–2851. <https://doi.org/10.1021/acs.analchem.7b04977>
- Chen, Y. L. L., Chen, H. Y., Lin, Y. H., Yong, T. C., Taniuchi, Y., & Tuo, S. (2014). The relative contributions of unicellular and filamentous diazotrophs to N₂ fixation in the South China Sea and the upstream Kuroshio. *Deep Sea Research, Part I*, 85, 56–71. <https://doi.org/10.1016/j.dsr.2013.11.006>
- Church, M. J., Short, C. M., Jenkins, B. D., Karl, D. M., & Zehr, J. P. (2005). Temporal patterns of nitrogenase gene (*nifH*) expression in the oligotrophic North Pacific Ocean. *Applied and Environmental Microbiology*, 71, 5362–5370.
- Clarke, K. R., & Ainsworth, M. (1993). A method of linking multivariate community structure to environmental variables. *Marine Ecology Progress Series*, 92, 205–219. <https://doi.org/10.3354/meps092205>
- Clarke, K. R., & Gorley, R. N. (2006). PRIMER v.6 User Manual/Tutorial. PRIMER-E, Plymouth.
- Codispoti, L. A. (2006). An oceanic fixed nitrogen sink exceeding 400 Tg N a⁻¹ vs the concept of homeostasis in the fixed-nitrogen inventory. *Biogeosciences*, 3(4), 1203–1246. <https://doi.org/10.5194/bgd-3-1203-2006>
- Conley, D. J., Paeli, H. W., Howarth, R. W., Boesch, D. F., Seitzinger, S. P., Havens, K. E., et al. (2009). Controlling eutrophication: Nitrogen and phosphorus. *Science*, 323(5917), 1014–1015. <https://doi.org/10.1126/science.1167755>
- Deutsch, C., Sarmiento, J. L., Sigman, D. M., Gruber, N., & Dunne, J. P. (2007). Spatial coupling of nitrogen inputs and losses in the ocean. *Nature*, 445(7124), 163–167. <https://doi.org/10.1038/nature05392>
- Eren, A. M., Morrison, H. G., Lescault, P. J., Reveillaud, J., Vineis, J. H., & Sogin, M. L. (2015). Minimum entropy decomposition: Unsupervised oligotyping for sensitive partitioning of high-throughput marker gene sequences. *ISME Journal*, 9, 968–979.
- Foster, R. A., Subramaniam, A., Mahaffey, C., Carpenter, E. J., Capone, D. G., & Zehr, J. P. (2007). Influence of the Amazon River plume on distributions of free-living and symbiotic cyanobacteria in the western tropical north Atlantic Ocean. *Limnology and Oceanography*, 52, 517–532.
- Galloway, J. N., Dentener, F. J., Capone, D. G., Boyer, E. W., Howarth, R. W., Seitzinger, S. P., et al. (2004). Nitrogen cycles: Past, present, and future. *Biogeochemistry*, 70(2), 153–226. <https://doi.org/10.1007/s10533-004-0370-0>
- Gradoville, M. R., Bombar, D., Crump, B. C., Letelier, R. M., Zehr, J. P., & White, A. E. (2017). Diversity and activity of nitrogen-fixing communities across ocean basins. *Limnology and Oceanography*, 62(5), 1895–1909. <https://doi.org/10.1002/lno.10542>
- Grosse, J., Bombar, D., Doan, H. N., Nguyen, L. N., & Voss, M. (2010). The Mekong River plume fuels nitrogen fixation and determines phytoplankton species distribution in the South China Sea during low- and high-discharge season. *Limnology and Oceanography*, 55(4), 1668–1680. <https://doi.org/10.4319/lno.2010.55.4.1668>
- Großkopf, T., Mohr, W., Baustian, T., Schunck, H., Gill, D., Kuypers, M. M. M., et al. (2012). Doubling of marine dinitrogen-fixation rates based on direct measurements. *Nature*, 488(7411), 361–364. <https://doi.org/10.1038/nature11338>
- Gruber, N., & Sarmiento, J. L. (1997). Global patterns of marine nitrogen fixation and denitrification. *Global Biogeochemical Cycles*, 11(2), 235–266. <https://doi.org/10.1029/97GB00077>
- Harding, K., Turk-Kubo, K. A., Sipler, R. E., Mills, M. M., Bronk, D. A., & Zehr, J. P. (2018). Symbiotic unicellular cyanobacteria fix nitrogen in the Arctic Ocean. *Proceedings of the National Academy of Sciences USA*, 115(52), 13371–13375. <https://doi.org/10.1073/pnas.1813658115>
- Howarth, R. W., Marino, R., Lane, J., & Cole, J. J. (1988). Nitrogen fixation in freshwater, estuarine, and marine ecosystems. 1. Rates and importance. *Limnology and Oceanography*, 33, 669–687.
- Hutchins, D. A., Mulholland, M. R., & Fu, F.-X. (2009). Nutrient cycles and marine microbes in a CO₂-enriched ocean. *Oceanography*, 22, 88–105.
- Jahnke, R. A. (2007). Global Synthesis. In K. K. Liu, L. Atkinson, R. Quinones, & L. Talaue-McManus (Eds.), *Carbon and nutrient fluxes in continental margins: A global synthesis*, (pp. 597–616). San Diego, CA: Springer-Verlag.
- Jayakumar, A., Chang, B. X., Widner, B., Bernhardt, P. W., Mulholland, M. R., & Ward, B. B. (2017). Biological nitrogen fixation in the oxygen minimum region of the eastern tropical North Pacific Ocean. *ISME Journal*, 11(10), 2356–2367. <https://doi.org/10.1038/ismej.2017.97>
- Jenkins, B. D., Steward, G. F., Short, S. M., Ward, B. B., & Zehr, J. P. (2004). Fingerprinting diazotroph communities in the Chesapeake Bay by using a DNA microarray. *Applied and Environmental Microbiology*, 70(3), 1767–1776. <https://doi.org/10.1128/AEM.70.3.1767-1776.2004>
- Jet Propulsion Laboratory MUR MEaSURES Project (2015). GHRSSST level 4 MUR global foundation sea surface temperature analysis (v4.1). Ver. 4.1. PO.DAAC, CA, USA. Dataset accessed [YYYY-MM-DD] at <https://doi.org/10.5067/GHGM-4FJ04>.
- Knapp, A. N. (2012). The sensitivity of marine N₂ fixation to dissolved inorganic nitrogen. *Frontiers in Microbiology*, 3, 374–374.
- Knapp, A. N., Dekaezemaeker, J., Bonnet, S., Sohm, J. A., & Capone, D. G. (2012). Sensitivity of *Trichodesmium erythraeum* and *Crocospaera watsonii* abundance and N₂ fixation rates to varying NO₃⁻ and PO₄³⁻ concentrations in batch cultures. *Aquatic Microbial Ecology*, 66(3), 223–236. <https://doi.org/10.3354/ame01577>
- Landolfi, A., Kähler, P., Koeve, W., & Oschlies, A. (2018). Global marine N₂ fixation estimates: From observations to models. <https://doi.org/10.3389/fmicb.2018.02112>
- Langlois, R. J., Hümmel, D., & LaRoche, J. (2008). Abundances and distributions of the dominant nifH phylotypes in the Northern Atlantic Ocean. *Applied and Environmental Microbiology*, 74(6), 1922–1931. <https://doi.org/10.1128/AEM.01720-07>
- Langlois, R. J., LaRoche, J., & Raab, P. A. (2005). Diazotrophic diversity and distribution in the tropical and subtropical Atlantic Ocean. *Applied and Environmental Microbiology*, 71(12), 7910–7919. <https://doi.org/10.1128/AEM.71.12.7910-7919.2005>
- Larsson, U., Hajdu, S., Walve, J., & Elmgren, R. (2001). Baltic Sea nitrogen fixation estimated from the summer increase in upper mixed layer total nitrogen. *Limnology and Oceanography*, 46(4), 811–820. <https://doi.org/10.4319/lno.2001.46.4.0811>
- Luo, Y.-W., Doney, S. C., Anderson, L. A., Benavides, M., Berman-Frank, I., Bode, A., et al. (2012). Database of diazotrophs in global ocean: Abundance, biomass and nitrogen fixation rates. *Earth System Science Data*, 4(1), 47–73. <https://doi.org/10.5194/essd-4-47-2012>

- Mahaffey, C., Michaels, A. F., & Capone, D. G. (2005). The conundrum of marine N₂ fixation. *American Journal of Science*, 305(6-8), 546–595. <https://doi.org/10.2475/ajs.305.6-8.546>
- Marino, M., Chan, F., Howarth, R. W., Pace, M., & Likens, G. E. (2002). Ecological and biogeochemical interactions constrain planktonic nitrogen fixation in estuaries. *Ecosystems*, 5(7), 719–725. <https://doi.org/10.1007/s10021-002-0176-7>
- Martinez-Perez, C., Mohr, W., Loscher, C. R., Dekaezemacker, J., Littmann, S., Yilmaz, P., et al. (2016). The small unicellular diazotrophic symbiont, UCYN-A, is a key player in the marine nitrogen cycle. *Nature Microbiology*, 1(11), 16163. <https://doi.org/10.1038/NMICROBIOL.2016.163>
- Messer, L. F., Mahaffey, C., Robinson, C. M., Jeffries, T. C., Baker, K. G., Bibiloni Isaksson, J., et al. (2016). High levels of heterogeneity in diazotroph diversity and activity within a putative hotspot for marine nitrogen fixation. *ISME Journal*, 10(6), 1499–1513. <https://doi.org/10.1038/ismej.2015.205>
- Mohr, W., Großkopf, T., Wallace, D. W. R., & LaRoche, J. (2010). Methodological underestimation of oceanic nitrogen fixation rates. *PLoS ONE*, 5(9), e12583. <https://doi.org/10.1371/journal.pone.0012583>
- Moisander, P. H., Beinart, R. A., Hewson, I., White, A. E., Johnson, K. S., Carlson, C. A., et al. (2010). Unicellular cyanobacterial distributions broaden the oceanic N₂ fixation domain. *Science*, 327(5972), 1512–1514. <https://doi.org/10.1126/science.1185468>
- Moisander, P. H., Zhang, R., Boyle, E. A., Hewson, I., Montoya, J. P., & Zehr, J. P. (2012). Analogous nutrient limitations in unicellular diazotrophs and *Prochlorococcus* in the South Pacific Ocean. *ISME Journal*, 6(4), 733–744. <https://doi.org/10.1038/ismej.2011.152>
- Montoya, J. P., Holl, C. M., Zehr, J. P., Hansen, A., Villareal, T. A., & Capone, D. G. (2004). High rates of N₂ fixation by unicellular diazotrophs in the oligotrophic Pacific Ocean. *Nature*, 430(7003), 1027–1031. <https://doi.org/10.1038/nature02824>
- Montoya, J. P., Voss, M., Kaehler, P., & Capone, D. G. (1996). A simple, high precision tracer assay for dinitrogen fixation. *Applied and Environmental Microbiology*, 62(3), 986–993.
- Mulholland, M. R., Bernhardt, P. W., Blanco-Garcia, J. L., Mannino, A., Hyde, K., Mondragon, E., et al. (2012). Rates of dinitrogen fixation and the abundance of diazotrophs in North American coastal waters between Cape Hatteras and Georges Bank. *Limnology and Oceanography*, 57(4), 1067–1083. <https://doi.org/10.4319/lo.2012.57.4.1067>
- Mulholland, M. R., Ohki, K., & Capone, D. G. (2001). Nutrient controls on nitrogen uptake and metabolism by natural populations and cultures of *Trichodesmium* (Cyanobacteria). *Journal of Phycology*, 37(6), 1001–1009. <https://doi.org/10.1046/j.1529-8817.2001.00080.x>
- Muller-Karger, F. E., Varela, R., Thunell, R., Luerssen, R., Hu, C., & Walsh, J. J. (2005). The importance of continental margins in the global carbon cycle. *Geophysical Research Letters*, 32, L01602. <https://doi.org/10.1029/2004GL021346>
- Needoba, J. A., Foster, R. A., Sakamoto, C., Zehr, J. P., & Johnson, K. S. (2007). Nitrogen fixation by unicellular diazotrophic cyanobacteria in the temperate oligotrophic North Pacific Ocean. *Limnology and Oceanography*, 52(4), 1317–1327. <https://doi.org/10.4319/lo.2007.52.4.1317>
- Nixon, S. W., Ammerman, J. W., Atkinson, L. P., Berounsky, V. M., Billin, G., Boicourt, W. C., et al. (1996). The fate of nitrogen and phosphorus at the land-sea margin of the North Atlantic. *Biogeochemistry*, 35(1), 141–180. <https://doi.org/10.1007/BF02179826>
- Paerl, H. W., & Huisman, J. (2009). Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. *Environmental Microbiology Reports*, 1(1), 27–37. <https://doi.org/10.1111/j.1758-2229.2008.00004.x>
- Paerl, H. W., & Otten, T. G. (2013). Harmful cyanobacterial blooms: Causes, consequences, and controls. *Environmental Microbiology*, 65(4), 995–1010. <https://doi.org/10.1007/s00248-012-0159-y>
- Rees, A. P., Gilbert, J. A., & Kelly-Gerreyn, B. A. (2009). Nitrogen fixation in the western English Channel (NE Atlantic Ocean). *Marine Ecology Progress Series*, 374, 7–12. <https://doi.org/10.3354/meps07771>
- Shiozaki, T., Nagata, T., Ijichi, M., & Furuya, K. (2015). Nitrogen fixation and the diazotroph community in the temperate coastal region of the northwestern North Pacific. *Biogeosciences*, 12(15), 4751–4764. <https://doi.org/10.5194/bg-12-4751-2015>
- Sipler, R. E., Gong, D., Baer, S. E., Sanderson, M. P., Roberts, Q. N., Mulholland, M. R., & Bronk, D. A. (2017). Preliminary estimates of the contribution of Arctic nitrogen fixation to the global nitrogen budget. *Limnology and Oceanography Letters*, 2(5), 159–166. <https://doi.org/10.1002/lo2.10046>
- Sohm, J. A., Hilton, J. A., Noble, A. E., Zehr, J. P., Saito, M. A., & Webb, E. A. (2011). Nitrogen fixation in the South Atlantic Gyre and the Benguela upwelling system. *Geophysical Research Letters*, 38, L16608. <https://doi.org/10.1029/2011GL048315>
- Solarzano, L. (1969). Determination of ammonia in natural waters by the phenylhypochlorite method. *Limnology and Oceanography*, 14, 16–23.
- Thompson, A. W., Foster, R. A., Krupke, A., Carter, B. J., Musat, N., Valot, D., et al. (2012). Unicellular cyanobacterium symbiotic with a single-celled eukaryotic alga. *Science*, 337(6101), 1546–1550. <https://doi.org/10.1126/science.1222700>
- Voss, M., Bombar, D., Loick, N., & Dippner, J. W. (2006). Riverine influence on nitrogen fixation in the upwelling region off Vietnam, South China Sea. *Geophysical Research Letters*, 33, L07604. <https://doi.org/10.1029/2005GL025569>
- Voss, M., Croot, P., Lochte, K., Mills, M., & Peeken, I. (2004). Patterns of nitrogen fixation along 10°N in the tropical Atlantic. *Geophysical Research Letters*, 31, L23S09. <https://doi.org/10.1029/2004GL020127>
- Wannicke, N., Benavides, M., Dalsgaard, T., Dippner, J. W., Montoya, J. P., & Voss, M. (2018). New perspectives on nitrogen fixation measurements using ¹⁵N₂ gas. *Frontiers in Marine Science*, 5. <https://doi.org/10.3389/fmars.2018.00120>
- Welschmeyer, N. A. (1994). Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and pheopigments. *Limnology and Oceanography*, 39(8), 1985–1992. <https://doi.org/10.4319/lo.1994.39.8.1985>
- Wen, Z., Lin, W., Shen, R., Hong, H., Kao, S.-J., & Shi, D. (2017). Nitrogen fixation in two coastal upwelling regions of the Taiwan Strait. *Scientific Reports*, 7(1), 17601. <https://doi.org/10.1038/s41598-017-18006-5>
- Wilson, S. T., Boettjer, D., Church, M. J., & Karl, D. M. (2012). Comparative assessment of nitrogen fixation methodologies, conducted in the oligotrophic North Pacific Ocean. *Applied and Environmental Microbiology*, 78, 6516–6523.
- Zehr, J. P., Jenkins, B. D., Short, S. M., & Steward, G. F. (2003). Nitrogenase gene diversity and microbial community structure: A cross-system comparison. *Environmental Microbiology*, 5(7), 539–554. <https://doi.org/10.1046/j.1462-2920.2003.00451.x>
- Zehr, J. P., & Paerl, H. W. (2008). Biological nitrogen fixation in the marine environment. In D. L. Kirchman (Ed.), *Microbial ecology of the oceans*, (pp. 481–525). Hoboken, NJ: Wiley-Liss, Inc. <https://doi.org/10.1002/9780470281840.ch13>
- Zehr, J. P., & Turner, P. J. (2001). Nitrogen fixation: Nitrogenase genes and gene expression. In J. H. Paul (Ed.), *Methods in microbiology*, (pp. 271–286). London, UK: Academic Press.
- Zhang, R., Chen, M., Cao, J., Ma, Q., Yang, J., & Oiu, Y. (2012). Nitrogen fixation in the East China Sea and southern Yellow Sea during summer 2006. *Marine Ecology Progress Series*, 447, 77–86. <https://doi.org/10.3354/meps09509>