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

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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

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Citation:

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 oceanic 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 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).
Until recently, planktonic N₂ 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. N₂ 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 N₂ 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 N₂ 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 N₂ fixation and expression of nifH (the gene encoding the iron protein in the N₂-fixing nitrogenase enzyme) have been measured in nitrate (NO₃⁻)-replete (~10 μM) upwelling waters in the eastern tropical Atlantic Ocean (Voss et al., 2004. Sohm et al., 2011), in surface waters with elevated nitrate (NO₃⁻) 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 N₂ 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 N₂ fixation is known to occur (Capone et al., 2005; Carpenter & Capone, 2008; Mulholland et al., 2012). Previously, we found high rates of N₂ 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, N₂ 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 N₂ fixation rates from spatially and temporally heterogeneous coastal waters where N₂ fixation rates were previously thought to be negligible.

2. Materials and Methods

N₂ 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
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-μm 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*

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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. MMiddle Atlantic Bight; GB = George’s Bank; GoM = Gulf of Maine; SNE = Southern New England Shelf; CH = Cape Hatteras; NONational Oceanic and Atmospheric Administration; NMFS = National Marine Fisheries Service.
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%) 15N2 (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 pelletized 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 15N2 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 N2 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 15N2 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 N2 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 N2 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 N2 fixation.

To compare rates of N2 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 N2 fixation and any of those variables, we binned our surface rates into increments of 2 °C temperature and 1-μM DIN to compare median N2 fixation rates with temperature and DIN concentrations as well as temperature and the DIN:DIP ratio.

Areal rates of N2 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
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-μm Sterivex filters for molecular analyses and immediately frozen and stored in a liquid nitrogen dewar and transported to Old Dominion University, where they were transferred to −80 °C freezers. Select samples were chosen for molecular analysis based on N₂ 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,
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

**Figure 3.** Areal rates of N$_2$ fixation ($\mu$mol N·m$^{-2}$·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.
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 N₂ fixation rate). A permutation test (10,000 randomizations) was used to determine significance.

3. Results and Discussion

Areal rates of N₂ fixation ranged from below detection to 4,106 μmol N·m⁻²·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 (3.7–703 μmol N·m⁻²·d⁻¹; Capone et al., 2005; Carpenter & Capone, 2008), and temperate oceanic waters (Mulholland et al., 2012; Rees et al., 2009). Volumetric N₂ fixation rates were usually highest in surface waters where they ranged from below detection to 130 nmol N·L⁻¹·d⁻¹ (Tables S1–S7) with many on the high end of those reported previously (Luo et al., 2012 and references therein). N₂ 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 nmol N·L⁻¹·d⁻¹ over all seven cruises. Using seasonally averaged areal N₂ 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 N₂ fixation in shelf waters...
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 μM, 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 (R2 = 0.01) or DIN concentrations (R2 = 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 et al., 2004), in NO₃⁻-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, N₂ fixation rates were generally, but not always, higher in the well-lit surface mixed layer ranging from below detection to 130 nmol N·L⁻¹·d⁻¹ (Tables S1–S7) where diazotrophic cyanobacteria thrive. Planktonic N₂ fixation rates by some marine cyanobacteria are thought to be limited by temperature (Carpenter & Capone, 2008). Indeed, higher rates of N₂ 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 N₂ fixation rates either in the pooled data (R² = 0.10) or in surface waters (R² = 0.08). Binned data show that while rates of N₂ fixation were generally higher when water temperatures were higher, maximum N₂ 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. N₂ 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 N₂ 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 N₂ 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.

Figure 5. Median (a) and maximum (b) volumetric N₂ fixation rates (nmol N·L⁻¹·d⁻¹) binned by temperature and dissolved inorganic nitrogen (DIN):dissolved inorganic phosphorus (DIP) ratios, along with the standard deviations of N₂ 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.
We note that the highest depth-integrated N\textsubscript{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 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\textsubscript{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 $>40^\circ$N), concurrent with observations that this organism thrives in cooler water than tropical and subtropical cyanobacterial diazotrophs (Langlois et al., 2008; Moisander et al., 2008). 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$_3^-$] 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 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$_3^-$], 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$_3^-$], 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$_3^-$], 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 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

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.
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 (Breitbarth 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; Moisander et al., 2010).

Concentrations of NO\textsubscript{x} are also thought to be fundamental to delimiting the range of
certain diazotrophs as N\textsubscript{2} 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\textsubscript{x} concentrations or subject to NO\textsubscript{x} inputs
(Foster et al., 2007; Moisander 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\textsubscript{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.

### 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**

<table>
<thead>
<tr>
<th>Long  (°W)</th>
<th>Lat  (°N)</th>
<th>Location</th>
<th>Date</th>
<th>Depth (m)</th>
<th>Temp. (°C)</th>
<th>Shannon diversity index (H)</th>
<th>Shannon’s equitability (E\textsubscript{41})</th>
<th>N\textsubscript{2} fixation rate (nmol N L\textsuperscript{-1} d\textsuperscript{-1})</th>
<th>UCYN-A isolate ALOHA</th>
<th>UCYN-A isolate SIO</th>
<th>Pseudomonas sp.</th>
<th>T. thiebautii</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>−69.929</td>
<td>40.150</td>
<td>SNE</td>
<td>June 2011</td>
<td>17.2</td>
<td>11.3</td>
<td>0.610</td>
<td>0.340</td>
<td>13.4 (1.4)</td>
<td>25.27</td>
<td>74.71</td>
<td>0.01</td>
<td>0.01</td>
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<tr>
<td>−74.045</td>
<td>39.563</td>
<td>MAB</td>
<td>Feb 2010</td>
<td>1.83</td>
<td>2.4</td>
<td>0.062</td>
<td>0.030</td>
<td>2.04 (3.2)</td>
<td>0.17</td>
<td>0.43</td>
<td>99.09</td>
<td>0.06</td>
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Note. MAB = Mid-Atlantic Bight; SNE = Southern New England Shelf; GB = Georges Bank.

Concentrations of NO\textsubscript{x} are also thought to be fundamental to delimiting the range of
certain diazotrophs as N\textsubscript{2} 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\textsubscript{x} concentrations or subject to NO\textsubscript{x} inputs (Foster et al., 2007; Moisander 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\textsubscript{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 (Moirander 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 NO₂ 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 N₂ fixation rates from neritic waters, regions where N₂ fixation were previously thought to be negligible. Results presented here indicate that N₂ 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 N₂ fixation rates have been seriously underestimated. We calculate that N₂ 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 N₂ 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 N₂ 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 pCO₂ and sea surface temperatures (Hutchins 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 N₂ 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


