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Sources of iron and phosphate affect the distribution of diazotrophs in the North Atlantic

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ABSTRACT

Biological nitrogen fixation (BNF) supplies nutrient-depleted oceanic surface waters with new biologically available fixed nitrogen. Diazotrophs are the only organisms that can fix dinitrogen, but the factors controlling their distribution patterns in the ocean are not well understood. In this study, the relative abundances of eight diazotrophic phylotypes in the subtropical North Atlantic Ocean were determined by quantitative PCR (qPCR) of the *nifH* gene using TaqMan probes. A total of 152 samples were collected at 27 stations during two GEOTRACES cruises; Lisbon, Portugal to Mindelo, Cape Verde Islands (USGT10) and Woods Hole, MA, USA via the Bermuda Time Series (BATS) to Praia, Cape Verde Islands (USGT11). Seven of the eight diazotrophic phylotypes tested were detected. These included free-living and symbiotic cyanobacteria (unicellular groups (UCYN) A, B and C, *Trichodesmium*, the diatom-associated cyanobacteria *Rhizoselinia–Richelia* and *Hemiaulus–Richelia*) and a γ-proteobacterium (Gamma A, AY896371). The *nifH* gene abundances were analyzed in the context of a large set of hydrographic parameters, macronutrient and trace metal concentrations measured in parallel with DNA samples using the PRIMER-E software. The environmental variables that most influenced the abundances and distribution of the diazotrophic phylotypes were determined. We observed a geographic segregation of diazotrophic phylotypes between east and west, with UCYN A, UCYN B and UCYN C and the *Rhizosolenia–Richelia* symbiont associated with the eastern North Atlantic (east of 40°W), and *Trichodesmium* and Gamma A detected across the basin. *Hemiaulus–Richelia* symbionts were primarily found in temperate waters near the North American coast. The highest diazotrophic phylotype abundance and diversity were associated with temperatures greater than 22 °C in the surface mixed layer, a high supply of iron from North African aeolian mineral dust deposition and from remineralized nutrients upwelled at the edge of the oxygen minimum zone off the northwestern coast of Africa.

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1. Introduction

Biological nitrogen fixation (BNF) is an important source of biologically available nitrogen in the marine environment, as fixed forms of nitrogen are scarce in most open ocean surface waters. BNF is carried out by specific groups of bacteria and Archaea called diazotrophs. In areas such as the oligotrophic subtropical gyres they provide the most significant source of fixed nitrogen (Vitousek and Howarth, 1991; Karl et al., 2002; Duce et al., 2008). Over geological time scales, the magnitude of the global oceanic fixed nitrogen inventory has been determined by the balance between BNF and the combined nitrogen loss processes of denitrification and anaerobic ammonia oxidation (anammox) (Altabet, 2006; Codispoti, 2006).

Until a decade ago, it was believed that most of the BNF in the ocean was performed by the large, surface bloom-forming

Abbreviations: Al, aluminum; Anammox, anaerobic ammonium oxidation; ANOSIM, analysis of similarities; BATS, Bermuda Atlantic time series; BNF, biological nitrogen fixation; CVOO, Cape Verde Ocean Observatory; Fe, iron; Gamma A, *γ-proteobacteria A* AY896371; Het 1, *Rhizosolenia–Richelia* symbiont qPCR probe; Het 2, *Hemiaulus–Richelia* symbiont qPCR probe; MAR, mid-Atlantic ridge; OMZ, oxygen minimum zone; PCA, principle component analysis; qPCR, quantitative PCR; SIMPER, similarity percentages; SML, surface mixed layer; UCYN A, unicellular cyanobacteria group A (related to *Candidatus Atelocyanobacterium thalassa*); UCYN B, unicellular cyanobacteria group B (related to *Crocosphaera*); UCYN C, unicellular cyanobacteria group C (related to *Cyanothece*)

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Trichodesmium, a non-heterocystous, filamentous cyanobacterium, and by symbiotic associations between diatoms and the diazotroph *Richelia* sp. (Foster et al., 2007). Phylogenetic studies using *nifH*, the gene encoding for the iron protein subunit of the nitrogenase enzyme have revealed a much more diverse diazotrophic flora that includes unicellular and symbiotic cyanobacteria, heterotrophic bacteria and Archaea, all potentially contributing significantly to global oceanic BNF (Zehr et al., 1998; Langlois et al., 2005; Turk et al., 2011). High-throughput next generation sequencing studies have further enriched our knowledge of diazotroph phylogenetic diversity, and have identified the presence of unexplored groups of heterotrophic diazotrophs throughout the world's oceans (Farnelid et al., 2011).

Although the abundance of the diazotrophs *Trichodesmium* and *Richelia* can be determined by microscopy counts, many other diazotrophic unicellular cyanobacteria and heterotrophic bacteria in marine microbial communities cannot be visually identified with certainty by microscopy alone. Microscopic images of the elusive UCYN A, one of the most widely distributed diazotrophic cyanobacteria, have been obtained only recently (Krupke et al., 2013; Thompson and Zehr, 2013). To date, most oceanic heterotrophic diazotrophs are known only by their *nifH* sequences. To further complicate the matter, the abundance of diazotrophs is generally several orders of magnitude lower than the dominant phytoplankton and bacterioplankton (e.g. *Prochlorococcus* and *Pelagibacter*). This presents a challenge for detection and cultivation techniques. Quantitative-PCR (q-PCR) and TaqMan probes have been used to circumvent some of these difficulties (Langlois et al., 2008), allowing the quantitative detection of diverse phylogenetic clades defined by specific *nifH* sequences. This approach has already yielded valuable information on *nifH* phylotype distributions and abundances in the Pacific (Goebel et al., 2007; Church et al., 2008, Moisander et al., 2010) and Atlantic Oceans (Langlois et al., 2008; Turk et al., 2011).

Diazotroph distribution has been utilized to estimate areas of BNF and model the factors controlling BNF. However, the oceans remain vastly undersampled with respect to diazotroph abundance, distribution and community structure (Luo et al., 2012; Fernández et al., 2013), making it problematic to validate model-based predictions concerning the fate of BNF in a changing ocean (Goebel et al., 2007; Monteiro et al., 2010; Sohm et al., 2011). It is therefore important to collect additional data on the diazotroph distribution in regions that are currently undersampled in order to better constrain the factors controlling BNF.

Environmental parameters such as temperature, availability of phosphate, water column stability, upward diffusive fluxes of nutrients, light, and input of iron (Fe) via atmospheric mineral dust deposition have all been proposed as factors controlling the distribution of diazotrophs (Fernández et al., 2013). Although detected in almost every oceanic environment, diazotrophs are most abundant in the warm tropical and subtropical oceans where fixed nitrogen is depleted in surface waters (Langlois et al., 2008; Church et al., 2008; Stal, 2009; Moisander et al., 2010). In contrast to primary producers diazotrophs are not limited by fixed nitrogen availability, instead both phosphorus and dissolved Fe availability have been implicated in the control of the geographical distribution of diazotrophs and BNF (Falkowski, 1997; Karl et al., 2002; Mills et al., 2004; Moore et al., 2009). In the oligotrophic subtropical North Atlantic gyre, mineral dust deposition is the most significant source of dissolved Fe to the surface of the ocean (Gao et al., 2001; Jickells et al., 2005; Conway and John, 2014). In the eastern tropical Atlantic, between the Cape Verde Islands and the northwest African coast, upwelled regenerated nutrients from the sub-surface oxygen minimum zone (OMZ) are an additional potential source of macro- (N, P, Si) and micro-nutrients (e.g. Fe, Co) to the surface layers (Bergquist and Boyle, 2006; Noble et al., 2012; Rijkenberg et al., 2012; Fitzsimmons et al., 2013).

We used qPCR and eight phylotype-specific TaqMan probes and primer sets, representing the most commonly occurring marine diazotrophs in the surface Atlantic Ocean (Langlois et al., 2008) to estimate *nifH* abundances in an East–West transect across the subtropical North Atlantic Ocean. We compared the distribution and relative abundance of *nifH* phylotypes with hydrographic parameters, macronutrients and trace metal distributions from the surface to 400 m, as well as aerosol aluminum (Al) and Fe concentrations. This was possible through coordinated sampling of nucleic acids and a suite of trace metals dissolved in the water column and aerosols during the 2010 and 2011 US GEOTRACES research cruises.

2. Materials and methods

2.1. Cruise track and sample collection

Samples for measuring *nifH* gene abundances for qPCR were collected during two GEOTRACES cruises (USGT10 and USGT11) that took place in the subtropical North Atlantic Ocean from October 16th to November 2nd 2010 and from November 7th to December 10th 2011, respectively (Fig. 1). The cruise track (Fig. 1) included stations at the Bermuda Atlantic Time-series (BATS) site, Cape Verde Ocean Observatory (CVOO) site and the mid-Atlantic ridge (MAR). Seawater samples for *nifH* qPCR were collected from the conventional CTD/rosette at six depths per station ranging from 2 to 1000 m. Immediately after collection 1–2 L of seawater were vacuum filtered onto 0.22 μm Durapore filters (Millipore) to collect the natural microbial communities. The filters were stored at −80 °C until analysis in the laboratory. In total, 152 samples were collected from 27 stations with an average of 6 depths per station. Up to three samples were collected in the surface mixed layer (SML) at all of the stations sampled. A broad suite of trace metals and other macronutrients were sampled during these two US GEOTRACES cruises (Deep-Sea Research II special issue), enabling the analysis of the nucleic acid-derived *nifH* abundance measurements within the context of a large database of chemical and hydrographic parameters.

2.2. DNA extraction and qPCR

In the laboratory liquid nitrogen-frozen filters were crushed with plastic homogenizers and incubated for 5 min with a 5 mg mL^{-1} lysozyme in TE buffer solution. DNA was extracted using the AllPrep RNA/DNA Mini Kit (Qiagen) following the manufacturer's protocol, except that DNA was eluted twice with 40 μL TE buffer and incubated for 5 min before centrifuging. DNA was stored in small aliquots to avoid freeze/thaw cycles. DNA concentrations were determined using the Quant-iT PicoGreen dsDNA reagent (Molecular Probes, Life Sciences). The abundances of eight *nifH* phylotypes were determined by qPCR using the specific TaqMan probes and primers for Het 1 (*Rhizosolenia*–*Richelia* symbionts; Church et al., 2005) and Het 2 (*Hemiaulus*–*Richelia* symbiont; Foster et al., 2007), *Trichodesmium*, UCYN A (*Candidatus Atelocyanobacterium thalassa*), UCYN B (*Crocosphaera*), UCYN C (*Cyanothece*), Gamma A (*gamma-proteobacteria A*) and Cluster III (Langlois et al., 2008). Universal TaqMan master mix and concentrations of primers, probes and BSA were mixed as in Langlois et al. (2008) in a reaction volume of 25 μL, which included either 5 μL of plasmid standard, DNA sample or PCR water as template. Plasmid standards, samples and no-template controls were run in duplicate on the Roche LightCycler 480 using clear 384-well plates. Samples were amplified using the following program: 95 °C for 10 min, 45 cycles of [95 °C for 15 s, 60 °C for 1 min]. Data was collected at 60 °C. A ramp of 1.6 °C s^{-1} was used

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