DIAZOTROPHS_CO2 dinitrogen fixation and nifH analytical methods.

Rates of $^{15}N_2$ fixation were assessed using the ^{15}N isotopic tracer technique. Whole seawater samples from six discrete depths (5, 25, 45, 75, 100, and 125 m) were withdrawn into acid-washed duplicate 4.3 L polycarbonate bottles and sealed with caps fitted with a silicone septa. Three mL of $^{15}N_2$ gas (98 %; Isotech Laboratories ©) was injected into each polycarbonate bottle through the silicone septum using a gas-tight syringe fitted with a stainless steel needle. The bottles were attached to a free drifting array, deployed before dawn and incubated at *in situ* light and temperature for ~24 h. After recovery of the array, the entire volume of each bottle was filtered onto pre-combusted glass microfiber (GF/F) filters (25 mm diameter) for whole seawater N_2-fixation estimates. All GF/F filters were placed on combusted foil pieces in Petri dishes and stored frozen at -20 °C for transport. At the shore based laboratory, GF/F filters were dried for 24 h at 60°C, pelleted and ^{15}N assimilation on each filter was analyzed with an elemental analyzer-isotope ratio mass spectrometer (Carlo-Ebra EA NC2500).

For the determination of cyanobacterial *nifH* gene dynamics, whole seawater samples were collected from the same six discrete depths where N_2 fixation was measured into clean, acid-washed 4 L polycarbonate bottles and ~2 L was filtered through 25 mm diameter 0.22 µm Durapore® filters (Millipore) for DNA. These filters were stored in microcentrifuge tubes containing 500 µL DNA lysis buffer (20 mM Tris-HCL, pH 8.0; 2 mM EDTA, pH 8.0; 1.2% Triton X and 20 mg mL^{-1} lysozyme), quickly frozen in liquid nitrogen and kept at -80˚C until extraction. DNA samples were extracted using the DNeasy kit (Quiagen®) and RNA samples using the RNeasy Mini Kit (Quiagen®) following the manufacturer's protocol. Determinations of diazotroph gene abundances (gene copies L^{-1}) were assessed by the quantitative polymerase chain reaction (QPCR) technique. Six different *nifH* genes (*Trichodesmium* spp., the uncultivated cyanobacteria termed "Group A", *Crocosphaera* sp. and three uncultivated heterocyctous cyanobacteria termed "Het-1, Het-2 and Het-3") were amplified in duplicate reactions using a TaqMan 5'-fluorogenic exonuclease qPCR assay approach. Each 25 µL TaqMan PCR reaction was carried out using an Applied Biosystems 7300 real-time thermocycler with each sample containing 12.5 µL TaqMan 2x Universal PCR MasterMix (Applied

Biosystems), 7.25 μL nuclease-free, molecular-biology grade water (Sigma), 1.0 μL each of 10 μM forward and reverse phylotype-specific *nifH* primers (Church et al. 2005a,b), 0.5 μL of 10 μM fluorogenic probe, 0.75 μL of 100x Bovine Serum Albumin (New England Biolabs), and 2 μL of DNA extracted with the methods described previously. A six point (ranging 10^4 to 10^9 genes per reaction) standard curve was created by serial dilutions of plasmids containing the *nifH* gene inserts with a 10-fold dilution.