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Anomalously high abundance of *Crocosphaera* in the South Pacific Gyre

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Abstract :

The unicellular diazotrophic cyanobacterium Crocosphaera contributes significantly to fixed nitrogen inputs in the oligotrophic ocean. In the western tropical South Pacific Ocean (WTSP), these diazotrophs abound thanks to the phosphorus-rich waters provided by the South Equatorial Current, and iron provided aeolian and subsurface volcanic activity. East of the WTSP, the South Pacific Gyre (SPG) harbors the most oligotrophic and transparent waters of the world's oceans, where only heterotrophic diazotrophs have been reported before. Here in the SPG we detected unexpected accumulation of Crocosphaera at 50 m with peak abundances of 5.26×105nifH gene copies L–1. The abundance of Crocosphaera at 50 m was in the same order of magnitude as those detected westwards in the WTSP and represented 100% of volumetric N2 fixation rates. This accumulation at 50 m was likely due to a deeper penetration of UV light in the clear waters of the SPG being detrimental for Crocosphaera growth and N2 fixation activity. Nutrient and trace metal addition experiments did not induce any significant changes in N2 fixation or Crocosphaera abundance, indicating that this population was not limited by the resources tested and could develop in high numbers despite the oligotrophic conditions. Our findings indicate that the distribution of Crocosphaera can extend into subtropical gyres and further understanding of their controlling factors is needed.

Introduction

The Western Tropical South Pacific (WTSP) is a recognized hotspot of N_2 fixation activity with an estimated contribution of ~21% to the global fixed nitrogen input (Bonnet *et al.* 2017; Tang *et al.* 2019). In this region, the filamentous and colony-forming *Trichodesmium* and unicellular *Crocosphaera* thrive thanks to year-round surface seawater temperatures above 25°C, provision of phosphorus-rich waters transported westwards with the South Equatorial Current, and iron (Fe) inputs are predicted from island weathering and hydrothermal inputs along the Tonga-Kermadec volcanic arc (Bonnet *et al.* 2018; Caffin *et al.* 2018; Guieu *et al.* 2018; Messié *et al.* 2020).

Crocosphaera is a unicellular diazotrophic cyanobacterium widespread in the open ocean (Moisander *et al.* 2010). It can be present as different phenotypes, including the small cell type (<4 µm) and the large cell type (>4 µm), which are known to have different extracellular polysaccharide excretion capabilities (Bench *et al.* 2013) and geographical distributions (Bench *et al.* 2016). While *Crocosphaera* and *Trichodesmium* often share the same niche in the open ocean (Stenegren *et al.* 2018), *Crocosphaera* differ from *Trichodesmium* in its N_2 fixation diel cycle, where *Crocosphaera* fixes N_2 at night and *Trichodesmium* during the day (Zehr and Capone 2020). Also these species have different strategies to cope with iron limitation, *Crocosphaera* being advantaged by its low size as well as iron reconditioning between day and night times (Saito *et al.* 2011). These diazotrophs also differ in their organic phosphorus uptake capabilities, with *Trichodesmium* being able of using both phosphonates (P-C bonds) and phosphoesters (P-O-C bonds) (Dyhrman *et al.* 2006), while *Crocosphaera* only has genes to process the latter (Dyhrman and Haley 2006).

At the boundary between the WTSP and the South Pacific Gyre (SPG) (~170°W longitude) *Trichodesmium* and *Crocosphaera* populations decline abruptly due to the lack of sufficient Fe to sustain their growth and N_2 fixation activity, and are outcompeted by heterotrophic diazotrophs such as Gamma A and the unicellular non-photosynthetic cyanobacterial symbiont, UCYN-A (Bonnet *et al.* 2008; Halm *et al.* 2011; Shiozaki *et al.* 2018; Stenegren *et al.* 2018). During the OUTPACE cruise (Oligotrophic to UlTra oligotrophic PACific Experiment, doi: 10.17600/15000900), crossing the WTSP and the western flank of the SPG, we unexpectedly detected high abundances of *Crocosphaera* in the ultraoligotrophic waters of the SPG. Intrigued by this accumulation, here we

inspect further the vertical distribution of *Crocosphaera,* and the cell-specific N_2 fixation rate and abundance response to nutrient and trace metal additions in bottle incubations.

Materials and methods

Sampling, hydrographic and chemical variables

Samples were collected between March 23 and 27, 2015 at a station located in the oligotrophic SPG (18.42°S 166.06°W), during the OUTPACE cruise (Moutin *et al.* 2017). At this station, six consecutive casts were carried out to study the vertical distribution of the abundance, N_2 fixation activity and organic/inorganic nutrient limitation of *Crocosphaera* (Table S1).

Seawater was obtained from thirteen depths in the photic layer (6, 17, 30, 40, 50, 60, 70, 90, 120, 150 and 180 m) using either a regular or trace metal clean (TMC) rosette frame (Table S1) holding 24 hydrographic bottles (12 L) and sensors for temperature, salinity (SBE 911plus), chlorophyll fluorescence (Wetlabs ECO-AFL/FL, Philomath, Oregon, USA). Inorganic nutrient concentrations (nitrate plus nitrite —NO_x, and phosphate—here referred to as dissolved inorganic phosphorus or DIP) were measured by colorimetric methods with a detection limit of 0.05 µM (Aminot and Kérouel 2007). Dissolved organic carbon (DOC) concentrations were measured by high-temperature catalytic oxidation as described in Panagiotopoulos et al. (Panagiotopoulos *et al.* 2019). Dissolved organic phosphorus (DOP) was measured by a wet oxidation procedure (Pujo-Pay and Raimbault 1994). Dissolved iron (dFe) concentrations were obtained by flow injection analyses as described in Bonnet and Guieu (Bonnet and Guieu 2006), and are available from the supplementary material of Guieu et al. (Guieu *et al.* 2018).

Abundance of diazotrophs

Crocosphaera cells were enumerated and measured from 2.3 L seawater samples filtered on 2 µm pore size 25 mm diameter polycarbonate membranes and fixed with paraformaldehyde diluted in filtered seawater (2% final concentration). The samples were stored at -80°C until enumeration and size measurement using an epifluorescence microscope (Zeiss Axioplan, Jena, Germany) fitted with a green (510–560 nm) excitation filter. They were counted on 40 fields (1.3 mm^2 per field; 0-2800 *Crocosphaera*-like cells per field) scanned and analyzed with the ImageJ1 software. *nifH*

gene copy numbers of UCYN-B (whose closest cultured relative is *Crocosphaera watsonii*), UCYN-A1, UCYN-A2, *Trichodesmium* and the *Richelia* symbionts of diatoms (diatom-diazotroph associations, DDAs): het1, het2 and het3 were enumerated by TaqMan quantitative PCR (qPCR) assays as described in Stenegren et al. (Stenegren *et al.* 2018). In nutrient amendment experiments (described below), *Crocosphaera* cells were counted by flow cytometry as described in Berthelot et al., (Berthelot *et al.* 2016) (Fig. S1).

Bulk N_2 fixation rates

Bulk N_2 fixation rates were measured from samples collected in four consecutive casts while on station (Table S1). For each profile, triplicate seawater samples were transferred to transparent polycarbonate 4.3 L bottles, closed gas-tight with silicon septa screwcaps, and spiked with 5 mL of $^{15}N_2$ (98.9% atom%, Cambridge isotopes). The $^{15}N_2$ gas batch used did not contain significant concentrations of other nitrogenous compounds labeled with ^{15}N, as previously confirmed (Dabundo *et al.* 2014; Benavides *et al.* 2015). The samples were incubated in situ for 24 h on a surface-tethered line at the same depths as samplings. Only at the four depths where the abundance of *Crocosphaera* was maximal (i.e., 30, 40, 50 and 60 m), samples were incubated in shaded on-deck incubators at the irradiance corresponding to each of these depths using neutral density screening adapted to mimic the light intensity at each corresponding sampling depth. Incubations were stopped by vacuum filtration of the samples onto pre-combusted 25 mm GF/F filters and stored at -20°C until analysis. A subsample of 1 L was filtered onto 0.2 µm polycarbonate filters for subsequent single-cell N_2 fixation analyses (see below). Surface and DCM seawater samples were also filtered at time zero (not labelled or incubated) to determine the natural isotopic signature of ambient particulate nitrogen. Bulk particulate nitrogen concentrations and $^{15}N/^{14}N$ ratios were determined with an elemental analyzer coupled to an isotope ratio mass spectrometer (EA-IRMS, Integra 2, SerCon Ltd). The accuracy of the instrument was verified every ten samples using international reference materials IAEA-N-1 and IAEA-310A. The analytical precision associated with the mass determination averaged 2.8% for particulate organic nitrogen. The analytical precision associated with ^{15}N was 0.0010 atom%^{15}N for a measured mass of 0.7 µmol N. N_2 fixation rates were calculated with the equations in Montoya et al. (Montoya *et al.* 1996). The detection limit was 0.035 nmol N L^{-1} d^{-1}. To avoid an overestimation of the $^{15}N_2$ gas equilibration time and consequent underestimation of N_2 fixation rates (White *et al.*

2020), we measured the dissolved ^{15}N enrichment in incubation bottles using membrane inlet mass spectrometry analyses as previously described (Kana *et al.* 1994; Bonnet *et al.* 2018).

Cell-specific N_2 fixation rates

Single-cell rates of *Crocosphaera*-like N_2 fixation rates were determined at 30, 40, 50 and 60 m by nanoscale secondary ion mass spectrometry (nanoSIMS 50L). To maximize the number of cells analyzed in each nanoSIMS image, *Crocosphaera*-like cells were sorted with a Becton Dickinson Influx Mariner flow cytometer (BD Biosciences, Franklin Lakes, NJ) prior to nanoSIMS analyses as described in Berthelot et al. (Berthelot *et al.* 2016). After determining the *Crocosphaera*-like cluster, the cells were sorted on a 13 mm diameter 0.2 µm pore size polycarbonate membrane and prepared for nanoSIMS analyses as previously described (Berthelot *et al.* 2016; Bonnet *et al.* 2016). Samples were sputtered prior to analyses with a current of ~10 pA for at least 2 min. Subsequently, a cesium primary beam of ~1.3 pA cesium (16 KeV) focused onto a ~100 nm spot diameter region was scanned across a 256 x 256 or 512 x 512 pixel raster (depending on the image size), with a counting time of 1 ms per pixel. Mass resolving power was ~8000 to resolved isobaric interferences. Negative secondary ions ($^{12}C^{14}N^-$, $^{12}C^{15}N^-$) were collected by electron multiplier detectors, and secondary electrons were also imaged simultaneously. A total of 10 to 50 serial quantitative secondary ion images were generated and were combined to create the final image. Between 20 and 100 planes were generated for each cell analyzed. The number of *Crocosphaera*-like cells analyzed with the nanoSIMS ranged between 40 and 80 per sample.

The ^{15}N enrichment of target *Crocosphaera*-like cells was used to calculate single-cell N_2 fixation rates as previously described (Foster, Sztejrenszus and Kuypers 2013; Bonnet *et al.* 2016). The cellular nitrogen content of *Crocosphaera* cells (fmol N $cell^{-1}$) was calculated on the basis of their diameter, converted to biovolume assuming a spherical shape and converted to carbon (Verity *et al.* 2012). A C:N ratio of 8.5:1 was used to transform cellular carbon content to cellular nitrogen content based on sorted *Crocosphaera* cells analyzed by EA-IRMS (see above). The cellular nitrogen content was multiplied by the abundance of *Crocosphaera* cells (determined by microscopy counts) to assess the biomass of the whole group present in our samples.

Whole community nutrient amendment experiment

Amendment experiments were performed inside a shipboard clean-air (Class 100) laboratory container, under strict trace metal clean conditions according to the GEOTRACES guidelines (www.geotraces.org/images/Cookbook.pdf). Seawater was sampled from 50 m (where the abundance of *Crocosphaera* was maximal) using a trace metal clean Teflon (PTFE) diaphragm pump connected to plastic tubing and dispensed into detergent and trace metal grade acid washed 4.3 L transparent polycarbonate bottles.

Sets of triplicate bottles received amendments of different nutrients known to limit N_2 fixation which included iron ("Fe", solution of ferric citrate at 10 nM final concentration), nickel ("Ni" nickel sulfate at 10 nM final concentration), dissolved inorganic phosphorus ("DIP", suprapur sodium dihydrogen phosphate at 1 µM final concentration), dissolved organic phosphorus ("DOP", a mix of methylphosphonic acid, 2-aminoethylphosphonic acid and fructose 1,6-biphosphate at 1 µM final concentration) and volcanic ashes from the archipelago of Vanuatu (Ash), and aerosols ("dust") collected on board during the OUTPACE cruise (as described in (Guieu *et al.* 2018). Incubations were run for 48 h. The response of bulk N_2 fixation rates and *Crocosphaera* abundance to these additions were tested by mass spectrometry and qPCR assays, respectively, as described above. Primary production rates were measured using 10% ^{13}C-bicarbonate labeling and 24 h incubations as previously described (Berthelot *et al.* 2017). Heterotrophic bacterial production rates were obtained from tritiated leucine incubations as detailed in Van Wambeke et al. (Van Wambeke *et al.* 2018).

Results

Hydrological and biogeochemical environment

Hydrological conditions at our sampling stations remained fairly stable along the six consecutive casts carried out at our sampling station (Fig. 1). Temperature was >29°C from the surface down to 30 m at the thermocline, decreasing gradually to 19°C at 200 m (Fig. 1a). Salinity was 35.2 from the surface to 30 m and increased to ~36 at 200 m, showing more variability among casts than temperature (Fig. 1b). The daytime cast (158) showed slightly higher oxygen and fluorescence levels than night time casts (190 and 018). Oxygen levels were ~187 mL L^{-1} between the surface and 30 m, increasing to 200-210 mL L^{-1} between 40 and 100 m, then decreasing to ~170 mL L^{-1} at 200 m (Fig.

1c). Fluorescence profiles depicted a sharp deep chlorophyll maximum (DCM) at 140 m (Fig. 1d). PAR profiles reflect that the conditions were cloudy during the second cast (cast160). At the surface, PAR levels reached 250 µmol photons m^{-2} s^{-1}, decreasing exponentially with depth (Fig. 1e). The 1% PAR level was located at 175 m.

The concentrations of NO_X were <0.05 µM between the surface and 120 m, then increasing linearly with depth down to 3.5 µM at 175 m (Fig. 1f). The concentrations of DIP were 0.28 µM at the surface, stable at 0.13 - 0.16 µM between 16 and 92 m, gradually increasing back to ~0.3 µM at 150 m (Fig. 1g). DOC and DOP concentrations showed stable levels at 74 µM and 0.15 µM, respectively, between the surface and 60 m, decreasing gradually with depth (Fig. 1h-i). A decrease in DOP concentrations between 0.15 and 0.13 µM was observed at 60 m (Fig. 1i). The concentration of dFe was 0.4 nM at the surface and decreasing to ~0.16 -0.2 nM between 50 and 150 m (Fig. 1j).

Bulk and cell-specific N_2 fixation rates

Bulk N_2 fixation rates ranged between 0.2 and 0.5 ± 0.1 nmol N L^{-1} d^{-1} from the surface down to 30 m, increased to 1.3 nmol N L^{-1} d^{-1} at 40 m, subsequently peaking at 50 m and reaching a maximum of 2 nmol N L^{-1} d^{-1}, then decreasing back to 1 nmol N L^{-1} d^{-1} at 60 m (Fig. 2a). Cell-specific *Crocosphaera*-like N_2 fixation rates were measured at 30, 40, 50 and 60 m depth. Converting cell-specific to volumetric N_2 fixation rates demonstrated that *Crocosphaera* was the major active diazotroph, contributing 54 to 100% of bulk N_2 fixation rates. Cell-specific rates were 0.08 ± 0.02 fmol N $cell^{-1}$ h^{-1} at the surface and peaked at 2 ± 0.2 fmol N $cell^{-1}$ h^{-1} at 50 m (Fig. 2b).

Abundance of diazotrophs

The depth-integrated (down to 120 m) abundance of *Crocosphaera* in the SPG station was two to three orders of magnitude higher than observed in previous studies in the South Pacific (Fig. S2). At the SPG station sampled, the average volumetric abundance of UCYN-B cells was 8.9 ± 0.2 x 10^4 *nifH* gene copies L^{-1}, their maximum abundance being measured at 50 m with 5.3 ± 0.2 x 10^5 *nifH* gene copies L^{-1}. Epifluorescence microscopy counts showed lower abundances at the surface and 20 m (1.1 - 2.5 x 10^2 cells L^{-1}), but agreed with qPCR at finding a peak of 2.5 x 10^5 cells L^{-1} at 50 m (Fig. 2c). The average cell diameter of the *Crocosphaera*-like cells observed under the microscope and subsequently analyzed by nanoSIMS was 3.5 µm (Fig. S3). Other diazotroph groups were

enumerated using qPCR. *Trichodesmium*, UCYN-A1 and UCYN-A2 were only detected at 60 m with $3.7 \times 10^{2} \pm 2.2 \times 10^{2}$, $2.9 \pm 0.2 \times 10^{4}$ and $3.6 \pm 0.2 \times 10^{4}$ *nifH* gene copies L^{-1}, respectively (data not shown). DDA groups Het-1, Het-2 and Het-3 were not detected.

Microbial community response to nutrients and trace metal additions

The response of the whole microbial community from 50 m to DIP, DOP, dFe, Ni, ash, or dust addition is shown in Fig. 3a. N_2 fixation and primary production rates were not significantly enhanced by any of the amendments provided (Fig. 3a, one-way ANOVA $p>0.05$). Heterotrophic bacterial production rates were significantly enhanced by DOP additions (Fig. 3a, 0.9-fold increase, one-way ANOVA Tukey test $p = 0.04$).

The abundance of UCYN-A1 increased significantly in response to DIP, DOP and ash additions (one-way ANOVA Tukey test $p = 0.05$, 0.03 and 0.02, respectively), but responded negatively to additions of Ni (Fig. 3a). The abundance of *Crocosphaera* did not respond to DIP, Fe or Ni amendments, and decreased in DOP, ash and dust amendments. However, none of these differences were statistically significant (Fig. 3a, one-way ANOVA $p = 0.09$). Finally, *Trichodesmium* was only stimulated by DOP additions (1-fold, one-way ANOVA Tukey test $p = 0.02$).

Discussion

Previous studies have reported *Crocosphaera* abundances up to 10^{7} *nifH* gene copies L^{-1} in the North Pacific Subtropical Gyre (Church *et al.* 2005; Bench *et al.* 2016; Wilson *et al.* 2017) and the WTSP (Moisander *et al.* 2010; Bonnet *et al.* 2015). However, previous studies in different parts of the SPG have reported low to undetectable *Crocosphaera* (Bonnet *et al.* 2008; Halm *et al.* 2011; Shiozaki *et al.* 2018). Here we report on an unexpected high abundance of *Crocosphaera* peaking at 50 m in the ultraoligotrophic waters of the SPG.

The vertical distribution of *Crocosphaera* at our station in the SPG differed from that observed in the North Pacific in previous studies. In the North Pacific Subtropical Gyre, Church et al. (Church *et al.* 2005) reported *Crocosphaera* abundances of ~10^{3}-10^{4} *nifH* gene copies L^{-1} from the surface to the mixed layer depth, which was located at ~80 m during their study in the boreal winter. Also at station ALOHA but during the boreal summer season, Wilson et al. (Wilson *et al.* 2017) found a

similar vertical distribution but with abundances equal or higher than 10^6 *nifH* gene copies L^{-1}. Similarly, Bench et al. and Shiozaki et al. (Bench *et al.* 2016; Shiozaki *et al.* 2018) reported abundances of 10^5 - 10^7 *nifH* gene copies L^{-1} in the upper mixed layer at various sites in the North Pacific Subtropical Gyre and the WTSP, with a sharp decrease below 50 m. This typical vertical distribution differs from that observed here, where low abundances of 10^2 *nifH* gene copies L^{-1} were measured at the surface, followed by a peak of 2.56 x 10^5 *nifH* gene copies L^{-1} at 50 m and a sharp decline down to the base of the photic zone (Fig. 2a). *Crocosphaera* peaked at 50 m and not at the subsurface as seen in previous studies cited above. The high irradiance measured in the upper water column layer (>200 µmol m^{-2} s^{-1}; Fig. 1e) typical of the SPG, recognized as the clearest waters in the world's oceans (Twardowski *et al.* 2007) is well above the optimum at which N_2 fixation saturates in *Crocosphaera* (i.e. ~100 µmol m^{-2} s^{-1}; (Inomura *et al.* 2019) explaining their accumulation at deeper levels in the water column as compared to other regions of the ocean. However, the cell-specific N_2 fixation peak observed here at 50 m corresponds to ~80 µmol m^{-2} s^{-1} PAR (Fig. 1e), which would be suboptimal for *Crocosphaera* (Inomura et al. 2019) and does not explain their accumulation at this depth. Growth, N_2 and carbon fixation are negatively affected by UV radiation in cultures of *Crocosphaera* (Zhu et al. 2020). While no UV data is available for our cruise, previous studies have found a deeper penetration of UV in the clear waters of the SPG as compared to other ocean regions (Morel et al., 2007). This could explain why *Crocosphaera* accumulated deeper in the water column during our study (Fig. 2) as compared to shallower previous observations in other open ocean regions (Church et al. 2005; Wilson et al. 2017; Stenegren et al. 2018; Moisander et al. 2010).

Previous studies have detected two subpopulations of *Crocosphaera* in the South Pacific: large type (>4 µm) and small type (<4 µm) (Bench *et al.* 2016). Genome comparisons of several *Crocosphaera* strains have shown that while being very similar at the genome level, strains differ widely in their phenotype and strategies to cope with nutrient and trace metal stress (Webb *et al.* 2009). The large type harbors more DOP scavenging and iron stress response genes than the small type (Dyhrman and Haley 2006; Bench *et al.* 2013). The *Crocosphaera* cells observed here belonged to the small type (~3.5 µm) (Fig. S3) and peaked at 50 m, coinciding with the lowest dFe concentrations measured during our cruise (0.16 nM, Fig. 1j) (Guieu *et al.* 2018). While these dFe concentrations are limiting for phytoplankton growth in the SPG (Behrenfeld and Kolber 1999), recent *Crocosphaera* cell-specific dFe uptake rates measured in the same region (0.46 - 1.21 amol Fe $cell^{-1}$ d^{-1}) confirm that this diazotroph is well adapted to thrive in dFe-limited environments (Lory et al. in rev.).

Alternatively, the ~2-3°C temperature increase between 50 m and the on-deck incubators may have alleviated Fe limitation in *Crocosphaera* (Yang *et al.* 2021).

The peak of *Crocosphaera* at 50 m also coincided with a decrease in DIP concentrations at 50 - 60 m (Fig. 1g), while DOP concentrations did not seem to be variable (Fig. 1h) as compared to the surface. This could be expected in a small *Crocosphaera* cell type population, less genetically equipped to scavenge DOP than the large cell type (Bench *et al.* 2013). The locally available concentrations of dFe and DIP seemed to be sufficient to fulfill the needs of this *Crocosphaera* population, since responses to either dFe or DIP additions were not observed (Fig. 3b).

West of the SPG along the OUTPACE cruise transect, *Crocosphaera* abundances peaked at 10^4 - 10^5 *nifH* gene copies L^{-1} at 40 m, in the region where dFe concentrations were highest, i.e. between 175°E and ~175°W (Guieu *et al.* 2018). In this region, specific *Crocosphaera*-like N_2 fixation rates were 0.5 - 2.5 fmol N $cell^{-1}$ h^{-1}, only contributing 6 to - 10% to bulk N_2 fixation rates (Bonnet *et al.* 2018). At the SPG station, *Crocosphaera* cell-specific N_2 fixation ranged between 0.7 and 1.4 fmol N $cell^{-1}$ h^{-1}, representing 100% of bulk N_2 fixation rates at all depths except at 30 m (Fig. 2c). These rates are in good agreement with previous *Crocosphaera*-like cell-specific N_2 fixation measurements in the North Pacific Subtropical Gyre (between 0.1 and 1.1 fmol N $cell^{-1}$ h^{-1}; (Foster, Sztejrenszus and Kuypers 2013)) and the WTSP (2.1 fmol N $cell^{-1}$ h^{-1}; (Berthelot *et al.* 2016)). Similar cell-specific rates have been reported for other widespread diazotrophs such as *Trichodesmium* (2.3 fmol N $cell^{-1}$ h^{-1}; (Berthelot *et al.* 2016)) and UCYN-A1 (~1 fmol N $cell^{-1}$ h^{-1}; (Martínez-Pérez *et al.* 2016)), reinforcing the good health and important diazotrophic activity of *Crocosphaera* in the SPG during this study.

Overall, the high abundance, N_2 fixation activity and lack of response to any nutrient or trace metal additions of *Crocosphaera* observed in the SPG station indicate that this population was healthy, active, and largely contributing to fixed nitrogen inputs in this ultraoligotrophic environment. In light of the predicted expansion of subtropical gyres (Polovina, Howell and Abecassis 2008), our results suggest that the possibly overlooked abundance and distribution of low-nutrient adapted *Crocosphaera* in the SPG deserves further study.

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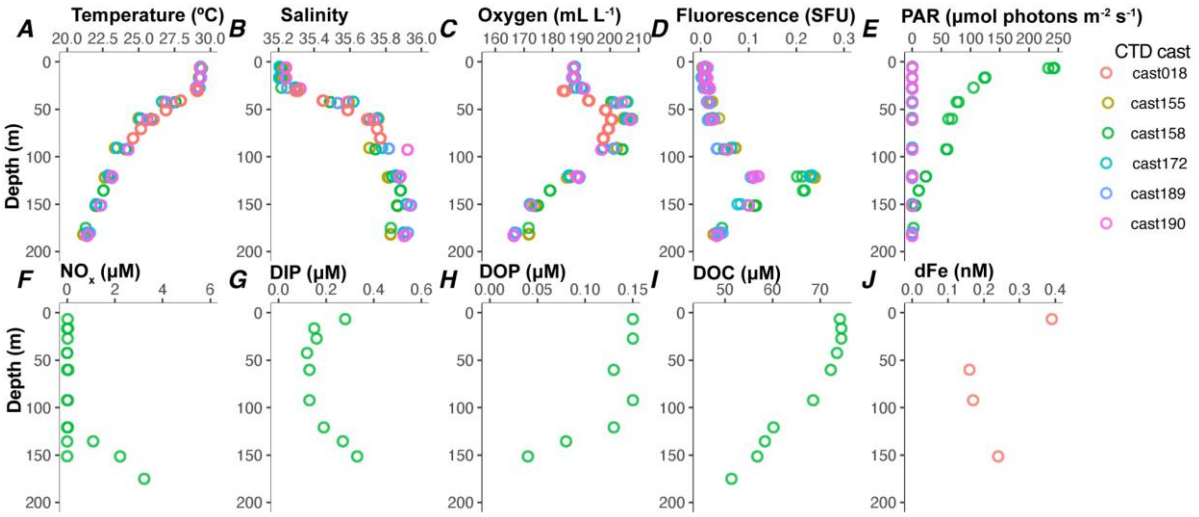

Fig.1: Profiles of hydrographic and chemical variables measured at the SPG station. Along four consecutive casts (cast018, cast158, cast160 and cast190) the following hydrographic variables were recorded: a) temperature, b) salinity, c) dissolved oxygen, d) fluorescence (arbitrary values), e) photosynthetically active radiation -PAR-. On cast018 chemical variables were recorded as follows: f) nitrate + nitrite (NO_x) concentrations, g) dissolved inorganic phosphorus (DIP) concentrations, h) dissolved organic phosphorus (DOP) concentrations, i) dissolved organic carbon (DOC) concentrations, and j) dissolved iron (dFe) concentrations (the latter published in Guieu et al. (Guieu *et al.* 2018)).

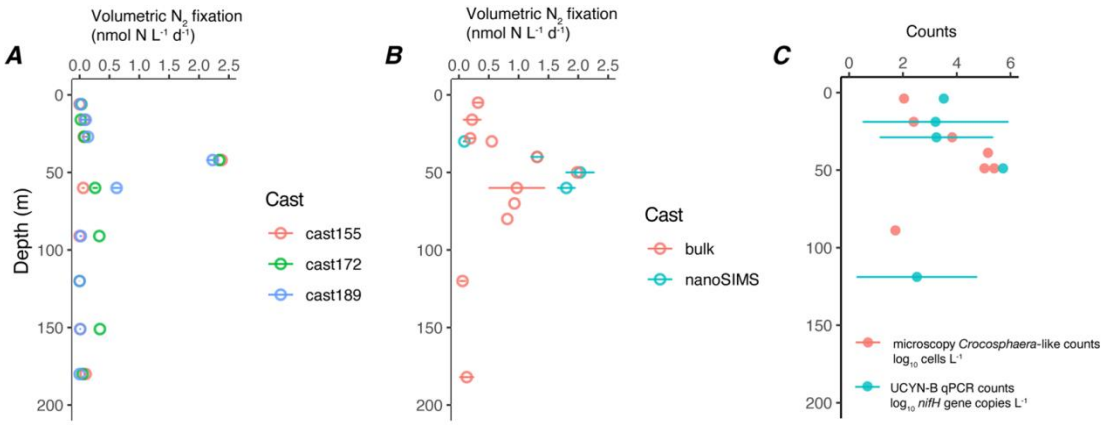

Fig. 2: Average bulk community and *Crocosphaera*-specific N_2 fixation rates. a) Volumetric N_2 fixation rates from bulk seawater samples collected during consecutive casts 155, 172 and 189; b) volumetric N_2 fixation rates from cast 190 including bulk seawater and *Crocosphaera*-specific N_2 fixation rates converted to volumetric rates considering cell abundance (blue dots); c) Vertical variability of *Crocosphaera* abundance quantified by epifluorescence microscopy and qPCR assays with a primer-probe set targeting UCYN-B (see Materials and methods). Error bars indicate the standard deviation of the mean. Note that replicates were not available for microscopy counts.

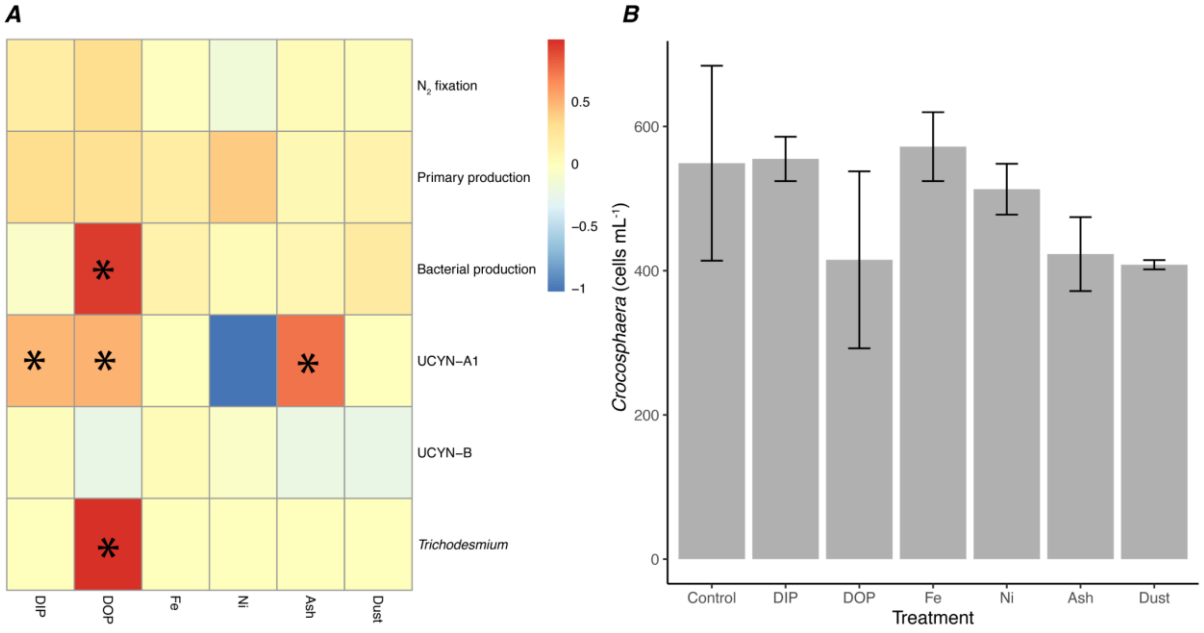

Fig. 3: Microbial activity and abundance responses to nutrient and trace metal additions at 50 m in the SPG station. a) Fold-change responses with respect to the control of N_2 fixation, primary production and heterotrophic bacterial production rates, and abundance of UCYN-A1, UCYN-B and *Trichodesmium* as estimated by qPCR assays. b) UCYN-B changes in abundance as a response to nutrient and trace metal additions. Error bars indicate the standard deviation of the mean. Asterisks indicate statistical significance (see Results section).