HAL open science

Transfer of diazotroph-derived nitrogen towards non-diazotrophic planktonic communities: a comparative study between Trichodesmium erythraeum, Crocosphaera watsonii and Cyanothece sp.

Hugo Berthelot, Sophie Bonnet, Olivier Grosso, Veronique Cornet, Aude Barani

To cite this version:

Hugo Berthelot, Sophie Bonnet, Olivier Grosso, Veronique Cornet, Aude Barani. Transfer of diazotroph-derived nitrogen towards non-diazotrophic planktonic communities: a comparative study between Trichodesmium erythraeum, Crocosphaera watsonii and Cyanothece sp.. Biogeosciences, 2016, 13 (13), pp.4005-4021. 10.5194/bg-13-4005-2016 . hal-01393443

HAL Id: hal-01393443

https://hal.science/hal-01393443

Submitted on 30 Oct 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution - NoDerivatives 4.0 International License

Biogeosciences, 13, 4005–4021, 2016
www.biogeosciences.net/13/4005/2016/
doi:10.5194/bg-13-4005-2016
© Author(s) 2016. CC Attribution 3.0 License.

Biogeosciences
Open Access

Transfer of diazotroph-derived nitrogen towards non-diazotrophic planktonic communities: a comparative study between *Trichodesmium erythraeum*, *Crocosphaera watsonii* and *Cyanothece* sp.

Hugo Berthelot[1], Sophie Bonnet[1,2], Olivier Grosso[1], Véronique Cornet[1], and Aude Barani[1]

[1]Aix Marseille Université, CNRS/INSU, Université de Toulon, IRD, Mediterranean Institute of Oceanography (MIO) UM 110, 13288, Marseille, France
[2]Institut de Recherche pour le Développement, CNRS/Aix-Marseille Université, Mediterranean Institute of Oceanography (MIO), 101 Promenade R. Laroque, BPA5, 98848, Noumea cedex, New Caledonia

Correspondence to: Hugo Berthelot (hugo.berthelot@gmail.com)

Received: 27 November 2015 – Published in Biogeosciences Discuss.: 15 January 2016
Revised: 17 May 2016 – Accepted: 24 May 2016 – Published: 13 July 2016

Abstract. Biological dinitrogen (N_2) fixation is the major source of new nitrogen (N) for the open ocean, and thus promotes marine productivity, in particular in the vast N-depleted regions of the surface ocean. Yet, the fate of the diazotroph-derived N (DDN) in marine ecosystems is poorly understood, and its transfer to auto- and heterotrophic surrounding plankton communities is rarely measured due to technical limitations. Moreover, the different diazotrophs involved in N_2 fixation (*Trichodesmium* spp. vs. UCYN) exhibit distinct patterns of N_2 fixation and inhabit different ecological niches, thus having potentially different fates in the marine food webs that remain to be explored. Here we used nanometer scale secondary ion mass spectrometry (nanoSIMS) coupled with $^{15}N_2$ isotopic labelling and flow cytometry cell sorting to examine the DDN transfer to specific groups of natural phytoplankton and bacteria during artificially induced diazotroph blooms in New Caledonia (southwestern Pacific). The fate of the DDN was compared according to the three diazotrophs: the filamentous and colony-forming *Trichodesmium erythraeum* (IMS101), and the unicellular strains *Crocosphaera watsonii* WH8501 and *Cyanothece* ATCC51142. After 48 h, 7–17 % of the N_2 fixed during the experiment was transferred to the dissolved pool and 6–12 % was transferred to non-diazotrophic plankton. The transfer was twice as high in the *T. erythraeum* bloom than in the *C. watsonii* and *Cyanothece* blooms, which shows that filamentous diazotrophs blooms are more efficient at promoting non-diazotrophic production in N-depleted areas. The amount of DDN released in the dissolved pool did not appear to be a good indicator of the DDN transfer efficiency towards the non-diazotrophic plankton. In contrast, the ^{15}N-enrichment of the extracellular ammonium (NH_4^+) pool was a good indicator of the DDN transfer efficiency: it was significantly higher in the *T. erythraeum* than in unicellular diazotroph blooms, leading to a DDN transfer twice as efficient. This suggests that NH_4^+ was the main pathway of the DDN transfer from diazotrophs to non-diazotrophs. The three simulated diazotroph blooms led to significant increases in non-diazotrophic plankton biomass. This increase in biomass was first associated with heterotrophic bacteria followed by phytoplankton, indicating that heterotrophs took the most advantage of the DDN in this oligotrophic ecosystem.

1 Introduction

The availability of nitrogen (N) is one of the key factors controlling primary productivity (PP) in the Ocean (Moore et al., 2013). By supplying new N to surface waters, biological N_2-fixation, mediated by some prokaryotes called diazotrophs, plays a critical role in sustaining PP in N-deprived waters such as subtropical gyres (Capone et al., 2005; Karl et al., 2002). The large filamentous bloom-forming cyanobacteria

Trichodesmium spp. and the diatoms-diazotrophs associations (DDAs) were first thought to be the main contributors to oceanic N_2-fixation (Capone et al., 1997; LaRoche and Breitbarth, 2005; Mague et al., 1974). However, the use of molecular tools has demonstrated that the diversity of diazotrophs was greater than previously thought, highlighting in particular the role of pico- and nano-planktonic unicellular cyanobacteria, termed UCYN (Needoba et al., 2007; Zehr et al., 1998, 2001). The latter are now considered to be of a major importance in the global N_2 fixation budget due to their broad distribution and high abundance in several oceanic basins (Luo et al., 2012; Moisander et al., 2010; Needoba et al., 2007). These observations are confirmed by the high contribution of N_2 fixation rates reported in the $< 10\,\mu m$ size fraction (Bonnet et al., 2009; Dore et al., 2002; Montoya et al., 2004).

While studies dealing with the diversity and the biogeographical distribution of diazotrophs in the ocean are on the increase, little is known regarding the fate of the fixed N_2 by the diazotrophs (hereafter called diazotroph-derived N, DDN) in the ocean. It remains unclear whether the DDN is preferentially directly exported out of the photic zone, recycled by the microbial loop, or transferred into larger organisms, subsequently enhancing indirect particle export. Some studies report low $\delta^{15}N$ signatures on zooplankton, evidencing the transfer of DDN towards higher trophic levels (Montoya et al., 2002). This transfer can be directly through the ingestion of diazotrophs (O'Neil et al., 1996; Wannicke et al., 2013), or indirect, i.e. mediated through the release of dissolved N by diazotrophs (Capone et al., 1994; Glibert and Bronk, 1994; Mulholland and Capone, 2001; Mulholland et al., 2004), which is taken up by heterotrophic and autotrophic plankton (Bonnet et al., 2016c), and is subsequently consumed by the zooplankton (e.g. O'Neil et al., 1996). Other studies performed in the tropical North Atlantic and Pacific Oceans report low $\delta^{15}N$ signatures on particles from sediment traps, suggesting that at least part of the DDN is ultimately exported out of the photic zone (Bourbonnais et al., 2009; Karl et al., 2002; Knapp et al., 2005). However, the export efficiency appears to depend on the diazotrophs involved in N_2 fixation in surface waters: while it has been demonstrated that DDAs directly contribute to particle export (Karl et al., 2012; Subramaniam et al., 2008; Yeung et al., 2012), *Trichodesmium* spp. is rarely found in sediment traps (Walsby, 1992) mainly due to its positive buoyancy, regulated by the production of carbohydrates (Romans et al., 1994). Data on the export efficiency of UCYN are scarce. During the VAHINE mesocom experiment designed to track the fate of DDN in the surface oligotrophic ocean, Berthelot et al. (2015b) showed that the production sustained by UCYN (mainly related to group C) resulted in a higher rate of particle export compared to the production sustained by DDAs. In this same special issue, Bonnet et al. (2016a) confirmed that UCYN-C significantly contribute to POC export (up to $22.4 \pm 5.5\,\%$ at the height of the UCYN-C bloom). However, most of the particle export associated with UCYN-C was probably mainly indirect through recycling processes and DDN transfer to surrounding planktonic communities (Bonnet et al., 2016a). However, such transfer of DDN to the surrounding planktonic communities and its potential impact on export production is poorly understood and rarely quantified.

The transfer of DDN to surrounding plankton is mediated through the dissolved pool as diazotrophs release a significant fraction of the fixed N (10–50 %) under the form of ammonium (NH_4^+) and dissolved organic N (DON; Benavides et al., 2013; Glibert and Bronk, 1994; Konno et al., 2010; Mulholland and Bernhardt, 2005; Mulholland et al., 2004). This release of DDN by diazotrophs has been linked to exogenous processes such as viral lysis (Hewson et al., 2004; Ohki, 1999), copepods sloppy feeding (O'Neil et al., 1996) or programmed cell death (Berman-Frank et al., 2004). Significant N release was also reported in axenic cultures, suggesting that it is also an endogenous process (Mulholland et al., 2004). Once released, fixed N compounds are potentially transferred to non-diazotrophic plankton communities, as suggested by massive developments of diatoms (Devassy et al., 1979; Dore et al., 2008; Lee Chen et al., 2011) and dinoflagellates (Lenes and Heil, 2010; Mulholland et al., 2006) during or following blooms of *Trichodesmium* spp. ^{15}N-enrichment measured in size-fractioned pico-plankton after $^{15}N_2$ incubations also supports the idea of a DDN transfer within the planktonic community; Bryceson and Fay, 1981; Garcia et al., 2007). However, this method probably overestimates the DDN transfer as it is not possible to discriminate between DDN that has been transferred to pico-plankton and N_2 fixation by pico-plankton itself. Bonnet et al. (2016c) recently measured the actual transfer of DDN from several *Trichodesmium* spp. blooms to different groups of autotrophic and heterotrophic plankton using single cell mass spectrometry analyses (nanoSIMS) coupled with cell sorting by flow cytometry after $^{15}N_2$ labelling, and showed that the DDN was predominantly transferred to diatoms and bacteria, and DDN was mainly converted to diatom biomass. This study was performed during naturally occurring *Trichodesmium* spp. blooms, but comparative studies on the transfer efficiency of DDN from different diazotrophs are lacking. *Trichodesmium* spp. and UCYN exhibit distinct patterns of N_2 fixation (the first fix during the day, while the second fix is during the night, e.g. Bergman et al., 2013; Dron et al., 2012) and inhabit different ecological niches (Luo et al., 2012), thus having potentially different fates in the marine food webs, that remains to be explored.

Here, we compared N_2 fixation rates, the quantity and the quality of DDN released in the dissolved pool and the transfer of DDN towards non-diazotrophic plankton from three distinct diazotrophic groups: *Trichodesmium erythraeum*, *Crocosphaera watsonii* and *Cyanothece* sp. For this purpose, we simulated blooms of these three diazotroph phylotypes by inoculating freshly sampled seawater containing

the natural planktonic assemblage with the three diazotrophic strains grown in culture mimicking the natural environment. NanoSIMS was used in combination with flow cytometry cell sorting and $^{15}N_2$ labelling to trace the passage of ^{15}N-labelled DDN into several groups of non-diazotrophic phytoplankton and bacteria to compare the DDN transfer efficiency from these three diazotroph groups.

2 Material and methods

2.1 Experimental setup

This experiment was carried in the New Caledonian lagoon (southwestern Pacific), which is a tropical low-nutrient low-chlorophyll (LNLC) system. The specific location at the entrance of the lagoon, 28 km off the coast (166.44° E, 22.48° S), was selected as this was the site where the 23 day VAHINE mesocosm experiment presented in this current issue was implemented in the austral summer of 2013. The VAHINE experiment was designed to track the fate of DDN in the ecosystem during a diazotroph bloom (Bonnet et al., 2016b). The present experiment performed in microcosms was designed to complement the mesocosm experiment and compare the fate of DDN originating from distinct groups of diazotrophs.

2.1.1 Cultures maintenance

Three unialgal cultures of diazotrophs abundant in the southwestern Pacific (e.g. Bonnet et al., 2015; Turk-Kubo et al., 2015) were used in this study to simulate blooms of the filamentous colony forming *Trichodesmium erythraeum* IMS101, and the UCYN strains *Crocosphaera watsonii* WH8501 and *Cyanothece* ATTC51142. They were grown in batch cultures under close to lagoon conditions, and maintained in exponentially growing phase under 120 photons m^{-2} irradiance on a 12 : 12 light : dark cycle at 27 °C. The culture medium was composed of 0.2 µm filtered and sterilized seawater collected in the New Caledonian lagoon (166.44° E, 22.48° S), at the study site where the DDN transfer experiment described below was performed. The collected seawater was characterized by lowF nitrate + nitrite (NO_x) concentrations ($< 0.1\,\mu mol\,L^{-1}$). It was amended with phosphate (PO_4^{3-}) and micronutrients according to the N-deplete YBCII medium recipe (Chen et al., 1996), except for PO_4^{3-} concentration, which was reduced to 10 instead of 50 µmol L^{-1} in the original medium. Cultures were acclimated to this medium for at least 10 generations before the experiment started. They were not axenic but manipulations under laminar flow hood and sterilization of the lab materials were performed in order to limit bacterial contamination. Before inoculation into natural seawater, and in order to control the biomass of diazotrophs added, cultures were monitored microscopically every 1–2 days on a Malassez counting cell for UCYN and on a 10 µm polycarbonate filter for *T. erythraeum*, using an epifluorescence microscope (Zeiss Axioplan, Jana, Germany) fitted with a green (510–560 nm) excitation filter.

2.1.2 DDN transfer experiment

Seawater containing the natural planktonic community was collected at the experimental study site on 2 February 2014 at 2 m depth, using an air-compressed Teflon pump (AstiPure™) connected to a polyethylene tubing. At the time of the sampling, the seawater temperature was 25.4 °C. Ambiant PO_4^{3-} and NO_x concentrations were $< 0.2\,\mu mol\,L^{-1}$. Seawater was transferred into 15 HCl-washed 4.5 L polycarbonate bottles equipped with septum caps and quickly brought back to the laboratory. Bottles were divided into five sets of three replicates. The first set was immediately amended with *Trichodesmium erythraeum* (hereafter referred to as "*T. erythraeum* treatment"), the second with the UCYN *Crocosphaera watsonii* (hereafter referred to as "*C. watsonii* treatment"), the third one with the UCYN *Cyanothece* spp. (hereafter referred to as "*Cyanothece* treatment"), the fourth set was left unamended and served as a control (hereafter referred to as "Control treatment"), and the last set was immediately processed as described below to characterize the initial conditions (T0). To simulate blooms of the different diazotrophs, we added 5.10^3 trichomes L^{-1} for *T. erythraeum* treatment and 1.10^6 cells L^{-1} for the UCYN treatments, to be representative of the diazotroph blooms observed in the southwestern Pacific region (Bonnet et al., 2015; Moisander et al., 2010; Rodier and Le Borgne, 2008; Shiozaki et al., 2014). Care was made to introduce a similar biomass of diazotrophs in each treatments in order to be able to compare the different treatments. The initial cultures were sufficiently concentrated in cells in such a way that the volume of culture added represented less than 1 % of the 4.5 L bottles volume, so nutrient concentrations, especially PO_4^{3-} concentrations were not significantly influenced by these additions, which represented $< 0.05\,\mu mol\,L^{-1}$ of added PO_4^{3-}.

Immediately after the diazotrophs inoculation, all 4.5 L bottles were amended with $NaH^{13}CO_3$ (EURISOTOP, 99 atom % ^{13}C, 5 g in 60 mL of deionized water) to obtain a ∼ 10 atom % ^{13}C-enrichment (1 mL in each 4.5 L bottles) and $^{15}N_2$ (98.9 atom % ^{15}N, Cambridge isotopes) enriched seawater, according to the protocol developed by Mohr et al. (2010) and fully described in Berthelot et al. (2015a). Briefly, $^{15}N_2$ enriched seawater was prepared by circulating 0.2 µm filtered seawater collected at the same site as described above through a degassing membrane (Membrana, Minimodule®, flow rate 450 mL min^{-1}) connected to a vacuum pump (< 850 mbar) for at least 1 h. The degassed seawater was transferred to a 2 L gas tight Tedlar® bag and amended with 1 mL of $^{15}N_2$ per 100 mL of seawater. The $^{15}N_2$ bubble was vigorously shaken for 5 to 10 min until its complete dissolution. The incubation bottles were then amended with 5 % vol : vol enriched seawater

and closed without headspace with septum caps. The final ^{15}N-enrichment of the N_2 pool in the incubation bottles was measured using a Membrane Inlet Mass Spectrometer (Kana et al., 1994) and was found to be 3.5 ± 0.2 atom % ($n = 9$). The potential contamination by $^{15}NO_x$ and $^{15}NH_3$ of the $^{15}N_2$ bottles, recently highlighted by Dabundo et al. (2014), was tested on one of our $^{15}N_2$ Cambridge Isotope batch. According to the model described in Dabundo et al. (2014), it appeared that the low level of contamination measured (1.4×10^{-8} mol of $^{15}NO_3$ mol^{-1} of $^{15}N_2$ and 1.1×10^{-8} mol NH_4^+ mol^{-1} of $^{15}N_2$) would only contribute to ~ 0.05 % of the DD^{15}N measured in our study and was thus neglected.

Except for the T0 set of bottles, all bottles were incubated for 48 h under in situ-simulated conditions in on-deck incubators at ~ 26.5 °C with continuous water flowing irradiances corresponding to the sampling depth using neutral screening. Bottles were gently mixed three times per day during the experiment to insure homogeneity. After incubation, the four sets of bottles (the three diazotrophs-amended treatments and the control treatment) were recovered and subsampled to analyze the following parameters: heterotrophic bacteria and phytoplankton abundances, N_2 fixation rates, DDN release, organic and inorganic nutrients concentrations and cellular ^{15}N- and ^{13}C-enrichment on diazotrophs and non-diazotrophic plankton groups (see below for detailed protocols). Unless otherwise stated, samples were taken individually in each bottle of each set, so each parameter was measured in triplicate in every treatment.

2.2 Plankton abundance determination

Samples for micro-phytoplankton were collected from the 4.5 L incubation bottles in 250 mL glass bottles and fixed with lugol (0.5 % final concentration). Diatoms, dinoflagellates and micro-zooplankton (ciliates) were identified and enumerated to the lowest possible taxonomic level from a 100 mL subsample following the Utermohl methodology (Hasle, 1978), using a Nikon Eclipse TE2000-E inverted microscope equipped with phase-contrast and a long distance condenser.

Pico-, nano-phytoplankton and bacterial abundances were determined using flow cytometry. For this purpose, samples were collected in 1.8 mL cryotubes, fixed with paraformaldehyde (final concentration 2 %), left at ambient temperature for 15 min in the dark, flash frozen in liquid N_2 and stored at −80 °C. Analyses were carried out at the PRECYM flow cytometry platform (https://precym.mio.univ-amu.fr/) using standard flow cytometry protocols (Marie et al., 1999) to enumerate phytoplankton and heterotrophe bacteria, using a FACSCalibur analyzer (BD Biosciences, San Jose, CA). Samples were thawed at room temperature and just before analyses, were added to each sample: 2 µm beads (Fluoresbrite YG, Polysciences), used as internal control (and to discriminate picoplankton < 2 µm $<$ nanoplankton populations), and Trucount beads (BD Biosciences), used to determine the volume analyzed. An estimation of the flow rate was calculated by weighing three tubes of samples before and after a 3 mn run of the cytometer. The cell concentration was determined from both Trucount beads and flow rate measurements. For picoplankton cells, the red fluorescence (670LP, related to chlorophyll *a* content) was used as trigger signal and cells were characterized by three other optical signals: forward scatter (FSC, related to cell size), side scatter (SSC, related to cell structure), and the orange fluorescence (580/30 nm, related to phycoerythrin content). Phytoplankton communities were clustered as *Synechococcus* spp. cell like (hereafter called *Synechococcus*), *Prochlorococcus* spp. cell like (hereafter called *Prochlorococcus*) and pico- and nano-eukaryotes (< 20 µm, hereafter called small eukaryotes). In addition, in the UCYN treatments, *C. watsonii* and *Cyanothece* clusters were determined. The resolution of these clusters was realized by comparing the UCYN treatments cytograms with the control one. The proportion of diazotrophic cells in these clusters (i.e. the proportion of the new counts in the UCYN treatments compared to the control treatment) was > 98 and > 90 % for *C. watsonii* and *Cyanothece*, respectively. For heterotrophic bacteria (hereafter called "bacteria") samples were stained with SYBR Green II (Molecular Probes, final conc. 0.05 % [v/v], for 15 min at room temperature in the dark), in order to stain nucleic acids; then cells were characterized by two main optical signals: side scatter (SSC, related to cell size and structure) and green fluorescence (530/40, related to SYBR Green fluorescence). For the calculation of heterotrophic prokaryotes abundances, phytoplankton cells, *Prochlorococcus* and *Synechococcus* particularly, were gated out on the basis of their chlorophyll *a* content (red fluorescence; Sieracki et al., 1995). All data were collected in log scale and stored in list mode using the CellQuest software (BD Biosciences). Data analysis was performed a posteriori using SUMMIT v4.3 software (Dako).

The abundance of *T. erythraeum* added to the natural planktonic assemblage was monitored microscopically: 300 mL from the 4.5 L bottles were filtered on a 10 µm polycarbonate filter in each triplicate bottle. The cells were fixed with paraformaldehyde (2 % final concentration) for at least 1 h at 4 °C and stored at −20 °C until counting using an epifluorescence microscope (Zeiss Axioplan, Jana, Germany) fitted with a green (510–560 nm) excitation filter.

2.3 N_2 fixation rates determination

For net N_2 fixation, 2 L from each 4.5 L bottle were filtered onto precombusted (450 °C, 4 h) GF/F filters. Filters were stored at −20 °C and dried at 60 °C for 24 h before analysis. The particulate organic N (PON) content and PON ^{15}N isotopic enrichment of each filter were measured by continuous-flow isotope ratio mass spectrometry coupled to an elemental analyser (EA-IRMS) using an Integra-CN mass spectrometer. The analytical precision associated with the mass deter-

mination averaged 2.8 % for PON. The analytical precision associated with ^{15}N was ±0.0010 atom % ^{15}N for a measured mass of 0.7 µmol N. The particulate inorganic N contribution was not taken into account. N_2 fixation rates were calculated according to Montoya et al. (1996). We considered the results to be significant when ^{15}N excess enrichment was higher than three times the standard deviation obtained with time zero samples ($n = 3$).

2.4 DDN released to the dissolved pool

300 mL of the filtrate obtained during N_2 fixation filtrations was recovered and stored in 500 mL SCHOTT glass flasks, poisoned with $HgCl_2$ (final concentration 10 µg L^{-1}) and stored at 4 °C for further measurement of the ^{15}N-enrichment of the dissolved pool. This was achieved using the two-step diffusion method extensively described in Berthelot et al. (2015a) and derived from Slawyk and Raimbault (1995). This method enables the differentiation of the NH_4^+ and DON pools and measures their respective ^{15}N-enrichment. It should be noted that in the DON recovery step, NO_x were also recovered. However, NO_x concentrations were very low during our experiments (< 0.2 µmol L^{-1}) with respect to DON concentrations (∼ 4.5 µmol L^{-1}). Furthermore, they were unlikely to be released by diazotrophs, thus unlikely ^{15}N-enriched. Nitrification, that converts NH_4^+ to NO_3^- at rates rising 5–10 nmol$L^{-1}d^{-1}$ in N-depleted surface waters (e.g. Yool et al., 2007) may have contributed to the underestimation of the transfer of $DD^{15}N$ in the NH_4^+ pool and to an overestimation of the $DD^{15}N$ in the DON pool. Nevertheless, in surface water, nitrification fluxes are found to be several orders of magnitude lower than NH_4^+ regeneration (Raimbault and Garcia, 2008) and were thus neglected in the interpretation of the results. Net DDN release rates were calculated according to Berthelot et al. (2015a).

2.5 Organic and inorganic nutrient analyses

Samples for NH_4^+ concentrations determination were collected in duplicate in 40 mL SHOTT flasks and NH_4^+ concentrations were measured according to Holmes et al. (1999) using a trilogy fluorometer (Turner Design, detection limit = 3 nmol L^{-1}). Samples for inorganic nutrients were collected in triplicate in 20 mL acid washed scintillation vials, poisoned with $HgCl_2$ (10 µg L^{-1} final concentration) and stored in the dark at 4 °C until analysis. NO_x and PO_4^{3-} concentrations were determined by standard colorimetric procedures (Aminot and Kérouel, 2007) on a segmented flow auto-analyzer. The quantification limit was 0.05 µmol L^{-1}. Samples for determination of DON concentrations were collected in 40 mL SHOTT flasks after filtration onto combusted GF/F filters (450 °C, 4 h) and stored at −20 °C until analysis. Concentrations were measured by wet oxidation according to Pujo-Pay and Raimbault (1994).

2.6 Cell sorting and sampling for nanoSIMS analyses

For flow cytometry cell sorting and subsequent analysis using nanoSIMS, samples were collected as follows to preconcentrate cells and facilitate cell sorting: for each treatment, 300 mL of each triplicate from the 4.5 L bottle were pooled and filtered onto 0.2 µm pore size 47 mm polycarbonate filters. Filters were quickly placed in a 5 mL cryotube® filled with 0.2 µm filtered seawater with PFA (2 % final concentration), for at least 1 h at room temperature in the dark. The cryovials were vortexed, for at least 10 s, in order to detach the cells from the filter and were stored at −80 °C until analysis. Cell sorting was performed on a Becton Dickinson Influx™ Mariner (BD Biosciences, Franklin Lakes, NJ) high speed cell sorter of the Regional Flow Cytometry Platform for Microbiology (PRECYM), hosted by the Mediterranean Institute of Oceanography, as described in Bonnet et al. (2016c). Planktonic groups were separated using the same clusters as for the phytoplankton abundance determination as described above. After sorting, the cells were recovered in Eppendorf tubes and immediately filtered onto a 0.2 µm pore size 25 mm filter. Particular care was taken to drop the cells on the surface as small as possible (∼ 5 mm in diameter) to ensure the highest cell density possible to facilitate further nanoSIMS analyses. In the UCYN treatments, additional "diazotroph" sort gates were defined. The gates were delimited around the new populations that appeared in the UCYN treatments, compared to the control.

Large phytoplanktonic cells (*T. erythraeum* and diatoms) were visible and easily recognized on the CCD camera of the nanoSIMS and thus did not require any cell sorting step. Thus, to recover these cells, 300 mL of each triplicate 4.5 L bottle were pooled together and filtered on 10 µm pore size 25 mm polycarbonate filters. The cells were fixed with PFA (2 % final concentration) for at least 1 h at ambient temperature. The filters were then stored at −20 °C until nanoSIMS analyses.

2.7 NanoSIMS analyses and data processing

NanoSIMS analyses were performed using a NanoSIMS N50 at the French National Ion MicroProbe Facility according to Musat et al. (2008) and Bonnet et al. (2016c). Briefly, a ∼ 1.3 pA Cesium (16 KeV) primary beam focused onto ∼ 100 nm spot diameter was scanned across a 256 × 256 or 512 × 512 pixel raster (depending on the image size) with a counting time of 1 ms per pixel. Samples were presputtered prior to analyses with a current of ∼10 pA for at least 2 min to achieve sputtering equilibrium and insure the analysis to be performed inside the cells by removing cell surface. Negative secondary ions ($^{12}C^-$, $^{13}C^-$, $^{12}C^{14}N^-$, $^{12}C^{15}N^-$ and $^{28}Si^-$) were collected by electron multiplier detectors, and secondary electrons were also imaged simultaneously. A total of 10–50 serial quantitative secondary ion images were generated, that were combined to create the fi-

nal image. Mass resolving power was ∼ 8000 in order to resolve isobaric interferences. From 20 to 100 planes were generated for each cells analyzed. NanoSIMS runs are time-intensive and not designed for routine analysis, but at least 20 cells from each community were analysed to assess the variability in isotopic composition under the same conditions. Thus, for diatoms only the three dominant species present in our experiment and previously counted microscopically were analysed. Data were processed using the LIMAGE and Look@NanoSIMS (Polerecky et al., 2012) software. Briefly, all scans were corrected for any drift of the beam and sample stage during acquisition. Isotope ratio images were created by adding the secondary ion counts for each recorded secondary ion for each pixel over all recorded planes and dividing the total counts by the total counts of a selected reference mass. Individual cells were easily identified in nanoSIMS ^{12}C, ^{14}N and ^{28}Si images that were used to define regions of interest (ROIs) around individual cells. For each ROI, the ^{15}N- and ^{13}C enrichment were calculated. In total, almost 1000 ROIs were used for this study.

2.8 Cell-specific biomass and DDN transfer calculations

The biomass of the added diazotrophs was measured at T0 by filtering an aliquot of each culture on a precombusted GF/F filter for PON determination as described above. The total biomass was divided by the number of cells determined microscopically to obtain the cell-specific biomass.

For diatoms, the biovolume of the three most abundant diatom taxa (*Chaetoceros* spp., *Bacteriastrum* spp. and *Thalassionema nitzschioides*) was estimated by measuring their cross, apical and transapical sections in order to calculate their biovolume according to Sun and Liu (2003). At least 50 measurements were performed for each diatom taxon. Biovolume was then converted to N cellular content according to Smayda et al. (1978) and using a C : N ratio of 6.6 : 1 (Redfield, 1934). These three taxa represented ∼ 75 % of the total diatom abundance in this experiment. The remaining 25 % was mainly composed of smaller diatoms (e.g. *Pseudo-Nitzschia* spp., *Cylindrotheca* spp. and *Leptocylindrus* spp.) that probably weakly contributed to the total diatom biomass.

For *Synechococcus*, the C content reported in Buitenhuis et al. (2012) was used (255 fg C cell^{-1}) and converted into N content according to the Redfield ratio of 6.6 : 1 leading to a value of 3.2 ± 0.9 fmol N cell^{-1}. For bacteria, the average N content of 0.15 ± 0.08 fmol N cell^{-1} (Fukuda et al., 1998) was assumed. For the small eukaryotes, the cellular N content of 9.2 ± 2.9 fmol cell^{-1} was used as reported in Gregori et al. (2001). The cellular N content of each group multiplied by their abundances allowed the calculation of the biomasses associated with each plankton group.

The DD^{15}N cell-specific N_2 fixation and transfer (in nmol L^{-1} 48 h^{-1}) that depict the amount of $^{15}N_2$ transferred from diazotrophs towards the non-diazotrophic plankton was calculated for each plankton group analysed as follows:

$$DD^{15}N = \frac{R_{cell}}{R_{N_2}} \times N_{con} \times A, \quad (1)$$

where R_{cell} is the mean ^{15}N-enrichment of individual cells (in atom %) after 48 h of incubation, R_{N_2} is the ^{15}N-enrichment of the $^{15}N_2$ in the dissolved pool (in atom %), N_{con} is the cellular N content (in nmol N cell^{-1}) and A is the plankton group specific abundance (in cell L^{-1}).

2.9 Statistical analyses

The effect of the diazotrophs treatments on the biomass associated with non-diazotrophs was tested using an Tukey HSD (honest significant difference) test. The differences in the ^{15}N-enrichment of cells between the different treatments and the natural abundance were tested using an unpaired non-parametric Mann–Whitney test, as the dispersion of values did not follow a normal distribution pattern. The statistical significance threshold was 5 % ($p < 0.05$). All the uncertainties associated with the parameters measured were taken into account and propagated over the different computations made.

3 Results

3.1 Plankton abundance and biomass

At the start of the experiment (T0), (i.e. ambient waters in which the DDN transfer experiment was performed), diatoms dominated the micro-phytoplanktonic community (89 % of the total abundance), mainly driven by the contribution of *Chaetoceros* spp. (6130 cells L^{-1}), *Thalassionema* spp. (5345 cells L^{-1}) and *Bacteriastrum* spp. (2391 cells L^{-1}), which together represented ∼ 75 % of the total diatom community (Table S1 in the Supplement). Dinoflagellates were an order of magnitude less abundant than diatoms and were mainly composed of *Gymnodinium* spp. and *Gyrodinium* spp. Few *Trichodesmium* spp. filaments were observed in the natural assemblage at abundances lower than 40 trichomes L^{-1}. Ciliate abundance was 430 cells L^{-1} including 40 to 100 tintinnids cells L^{-1}. The initial abundance of *Synechococcus*, *Prochlorococcus*, small eukaryotes and bacteria determined by flow cytometry was $5.4 \pm 1.1 \times 10^4$, $2.2 \pm 0.4 \times 10^4$, $1.4 \pm 0.1 \times 10^3$ and $5.9 \pm 1.5 \times 10^5$ cells mL^{-1}, respectively (Table S1).

Converted to biomass, *Synechococcus* dominated to phytoplanktonic biomass at T0 (120 ± 40 nmol N L^{-1}), followed by bacteria (90 ± 40 nmol N L^{-1}) and diatoms (40 ± 14 nmol N L^{-1}). The biomass associated with small eukaryotes and *Prochlorococcus* together represented less than 10 nmol L^{-1} (i.e. 3 % of the total biomass). The dinoflagellate and ciliate biomass values were 1–2 orders of magnitude lower than the diatom biomass, respectively, and were thus not considered in detail in this study.

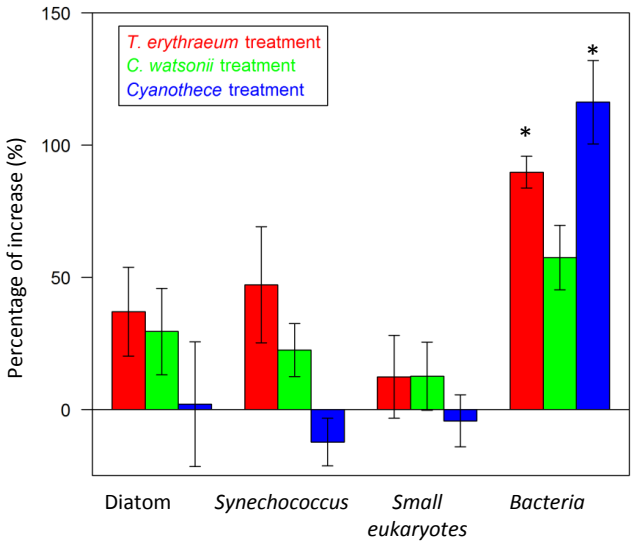

Figure 1. Relative increase of biomass associated with non-diazotrophic plankton groups considered in this study in the three diazotrophs-amended treatments relative to the control (%) after 48 h of incubation. Errors bar represent the standard deviations on triplicate incubations of both diazotrophs-amended treatments and control treatment. * Depict significant increase in biomass (unpaired Tukey HSD test, at 95 % levels of confidence).

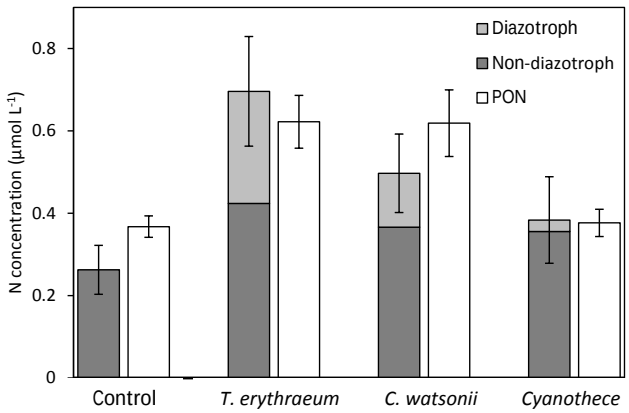

Figure 2. PON concentrations measured by mass spectrometry (EA-IRMS) and biomass associated with each plankton group in each treatment after 48 h of incubation (µmol L^{-1}). Errors bar represent the standard deviation on triplicate incubations.

In the control treatment after 48 h of incubation, the abundance of total diatoms and dinoflagellates increased by a factor of 2.3 and 1.9, respectively, while the abundances of bacteria remained stable and *Synechococcus* and *Prochlorococcus* abundances decreased by a factor of 1.4 and 1.3 respectively (Table S1). In the diazotrophs-amended treatments, the abundance of added diazotrophs decreased slightly in the *T. erythraeum* treatment (from 5×10^3 to $3.9 \pm 0.5 \times 10^3$ trichomes L^{-1}) and remained stable around 1×10^6 cells L^{-1} in the UCYN treatments (Table S1).

After 48 h of incubation, the biomass associated with non-diazotrophs increased in all the diazotrophs-amended treatments compared to the control (Fig. 1). The highest increase was observed in the *T. erythraeum* treatment (62 ± 39 %), mainly driven by a bacterial biomass increase of 90 ± 6 % and to a lesser extent by a *Synechococcus* (47 ± 22 %) and diatom (37 ± 17 %) biomass increase (Figs. 1 and 2). In the *C. watsonii* and *Cyanothece* treatments, the increase of biomass associated with non-diazotrophic plankton was 39 ± 39 and 35 ± 46 %, respectively. It was mainly driven by bacterial (58 ± 12 %), *Synechococcus* (23 ± 10 %) and diatom (30 ± 16 %) biomass increase in the *C. watsonii* treatment, and by bacterial biomass increase only (116 ± 16 %) in the *Cyanothece* treatment. The effect of diazotrophs on the biomass of small eukaryotes was less noticeable.

In all the treatments, the sum of the N biomass associated with every group of plankton was in good agreement with the actual PON concentrations measured by EA-IRMS after 48 h, indicating that the cellular N contents used in this study (described in Sect. 2) are realistic (Fig. 2).

3.2 N_2 fixation rates and DDN release

Net N_2 fixation rates determined by EA-IRMS in the control treatment were 1.5 ± 0.1 nmol L^{-1} 48 h^{-1} (Fig. 3). This N_2 fixation was attributed to the diazotrophs already present in the natural assemblage (probably *Trichodesmium* spp. that were found at low abundances in the control, data not shown). In the diazotroph-amended treatments, net N_2 fixation rates were 10 to 40 times higher than in the control, indicating the that diazotroph blooms artificially induced worked well: ∼ 60 nmol L^{-1} 48 h^{-1} in the *T. erythraeum* and *C. watsonii* treatments and 16 nmol L^{-1} 48 h^{-1} in the *Cyanothece* treatment (Fig. 3). The DDN released to the dissolved pool by diazotrophs represented 16.1 ± 6.7 % of the total N_2 fixation (where total N_2 fixation is defined as the sum of N_2 fixed recovered in the PON, DON and NH_4^+ pools) in the *T. erythraeum* treatment, 13.8 ± 1.9 % in the *C. watsonii* treatment, 30.5 ± 10.4 % in the *Cyanothece* treatment and 66.0 ± 21.9 % in the control treatment. In all cases, most of the ^{15}N released in the dissolved pool after 48 h of incubation was under the form of DON, which represented 77 to 81 % of the total N release in the diazotrophs-amended treatments without any differences between the treatments. The NH_4^+ release was below detection limit in the control treatment.

3.3 Cell-specific ^{15}N- and ^{13}C-enrichments and DD^{15}N transfer towards the non-diazotrophic plankton

NanoSIMS analyses revealed significant ^{15}N-enrichment in diazotrophic cells after 48 h of incubation compared to natural ^{15}N-enrichment (0.366 atom %; Figs. 4 and 5). Among the three diazotrophs added, *C. watsonii* and *Cyanothece* exhibited the highest ^{15}N-enrichments with 1.942 ±

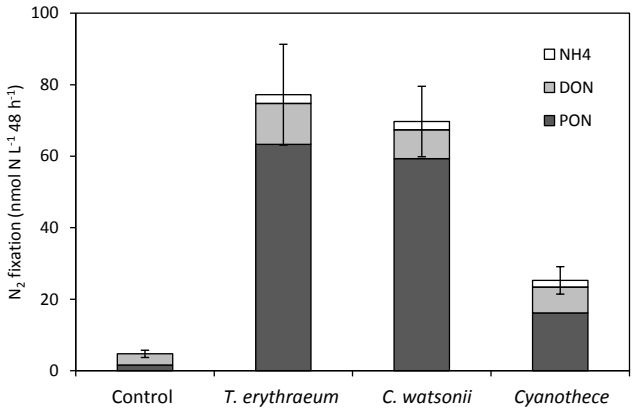

Figure 3. N_2 fixation rates (dark grey, nmol L^{-1} 48 h^{-1}), DDN release (nmol L^{-1} 48 h^{-1}) as DON (light grey) and NH_4^+ (white) in each treatment. Error bars represent the standard deviation of triplicate incubations and the propagated analytical error.

0.239 atom % ($n = 18$) and 2.501 ± 0.300 atom % ($n = 46$), respectively (Fig. 5). *T. erythraeum* ^{15}N-enrichment averaged 1.147 ± 0.233 atom % ($n = 68$). The ^{13}C-enrichment was similar for *T. erythraeum* (3.316 ± 0.634 atom %) and *C. watsonii* (3.124 ± 0.670 atom %) and higher for *Cyanothece* (4.612 ± 0.837 atom %). The correlation between ^{13}C-enrichment and ^{15}N-enrichment was significant for *T. erythraeum* ($r^2 = 0.50$, $p < 0.001$, $n = 68$), weaker but still significant for *C. watsonii* ($r^2 = 0.39$, $p = 0.005$, $n = 18$), and not significant for *Cyanothece* ($r^2 = 0.01$, $p = 0.500$, $n = 46$).

Cell specific N_2 fixation rates of diazotrophs were 140.8 ± 55.9 fmol N $cell^{-1}$ 48 h^{-1} (assuming 100 cell per trichomes), 50.3 ± 9.2 fmol N $cell^{-1}$ and 25.0 ± 3.5 fmol N $cell^{-1}$ 48 h^{-1} for *T. erythraeum*, *C. watsonii* and *Cyanothece* leading to N_2 fixation rates associated with the three groups of 54.8 ± 21.7, 54.5 ± 10.0 and 19.1 ± 2.7 nmol L^{-1} 48 h^{-1}, respectively.

NanoSIMS analyses performed on non-diazotrophic diatoms and cell-sorted *Synechococcus*, small eukaryotes, and bacteria also revealed ^{15}N-enrichments that were at times significantly higher than those measured in the control (Figs. 4 and 6). The ^{15}N-enrichment of non-diazotrophic plankton strongly depended on the treatment considered. When *T. erythraeum* provided the DD^{15}N, the ^{15}N-enrichment was significantly higher compared to the control for diatoms (0.468 ± 0.081 atom %, $n = 18$), *Synechococcus* (0.404 ± 0.090 atom %, $n = 105$) and bacteria (0.487 ± 0.071 atom %, $n = 45$; data are not available for small eukaryotes in *T. erythraeum* treatment). In the *C. watsonii* treatment, the ^{15}N-enrichment of non-diazotrophs was significantly higher compared to the control for *Synechococcus* (0.411 ± 0.079 atom %, $n = 134$) and bacteria (0.435 ± 0.05 atom %, $n = 34$) and not significantly different for diatoms (0.394 ± 0.077 atom %, $n = 23$) and small eukaryotes (0.383 ± 0.040 atom %, $n = 52$). In the *Cyanothece* treatment, the ^{15}N-enrichment of non-diazotrophs was significantly higher compared to the control for diatoms (0.446 ± 0.143 atom %, $n = 26$) and for *Synechococcus* (0.389 ± 0.080 atom %, $n = 25$), whereas no significant enrichments were observed for small eukaryotes (0.383 ± 0.030 atom %, $n = 88$) and bacteria (0.379 ± 0.027 atom %, $n = 38$). It should be noted that in the control, the ^{15}N-enrichment of all plankton groups (diatoms, *Synechococcus*, small eukaryotes and bacteria) was slightly higher (0.387 ± 0.048 atom %, $n = 301$) than the natural abundance (0.366 atom %) after 48 h of incubation (Fig. 6).

The amount of DD^{15}N transferred to non-diazotrophs corrected from N_2 fixation detected in the control treatment was higher in the *T. erythraeum* treatment (9.5 ± 4.9 nmol N L^{-1}) compared to the *C. watsonii* and *Cyanothece* treatments, where it was 4.1 ± 2.3 and 1.2 ± 0.9 nmol N L^{-1}, respectively. It represented 11.7 ± 4.4 % of total N_2 fixation in the *T. erythraeum* treatment and was significantly higher than in the *C. watsonii* (5.8 ± 2.7 %) and *Cyanothece* treatments (4.9 ± 2.4 %) (Table 1).

4 Discussion

The fate of DDN in the marine food web has been poorly studied, mainly due to technical limitations. Using ^{15}N and ^{13}C labeling coupled with cell sorting by flow cytometry and nanoSIMS analyses at the single cell level, we were able to trace the transfer of DD^{15}N from the diazotrophs to the dissolved pool and to the non-diazotrophic plankton, and compare the DD^{15}N transfer efficiency as a function of the diazotroph groups dominating the community.

4.1 Cell-specific photosynthesis and N_2 fixation

Cell-specific N_2 fixation rates measured using nanoSIMS are in the range of previous N_2 fixation rates measured in cultures using conventional N_2 fixation methods for the same strains of *T. erythraeum, C. watsonii* and *Cyanothece* (Berthelot et al., 2015a). This confirms the ability of nanoSIMS to accurately measure N_2 fixation rates, as previously shown in former studies (Finzi-Hart et al., 2009; Foster et al., 2013; Ploug et al., 2010). The high N_2 fixation rates induced by the inoculation of diazotrophs in the natural planktonic community (7–30 nmol N L^{-1} d^{-1}) are representative of those reported in the southwestern Pacific region under blooming conditions (Berthelot et al., 2015b; Bonnet et al., 2015; Garcia et al., 2007). Thus, the artificial diazotroph blooms induced for the purpose of this study provided realistic conditions to study the DDN transfer to non-diazotrophic plankton.

The significant correlation between ^{13}C- and ^{15}N-enrichments in *T. erythraeum* cells analyzed after 48 h of incubation argue that both PP and N_2 fixation occur simultaneously within the cells (Fig. 5). This appears in opposition

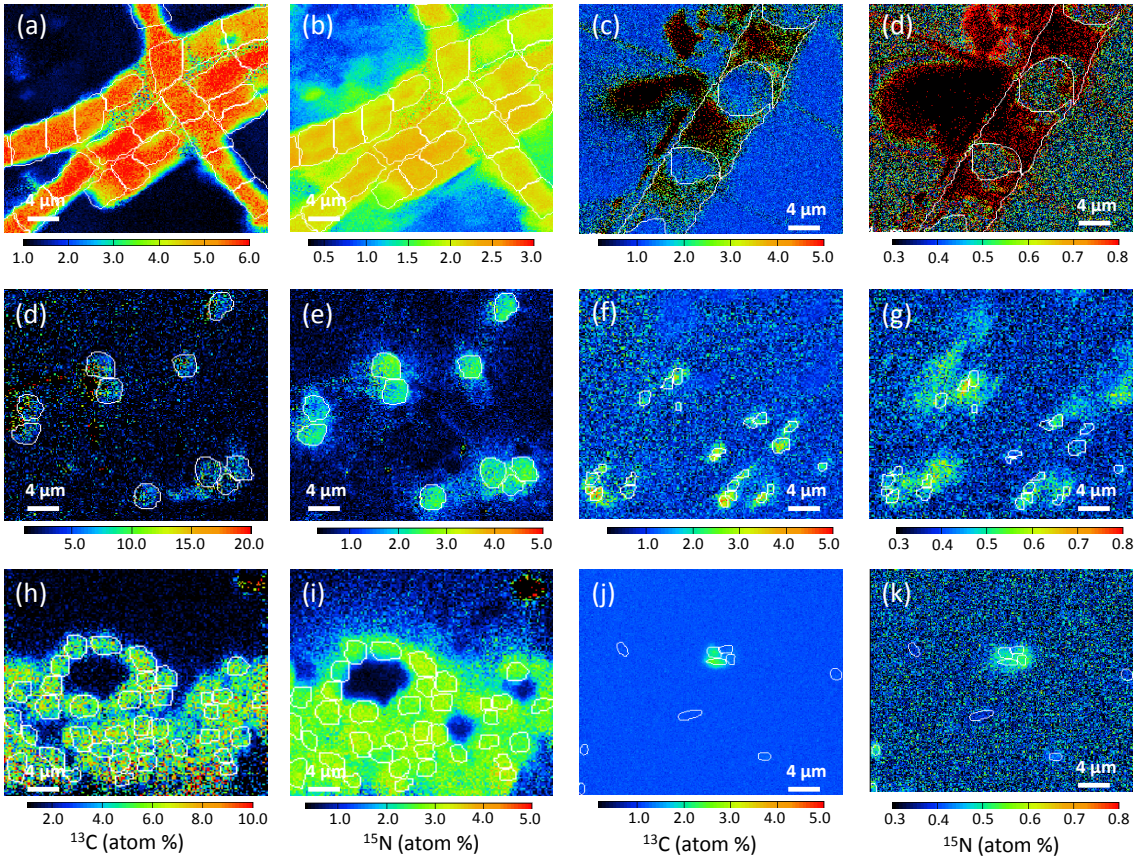

Figure 4. NanoSIMS images showing the isotopic enrichment found in cells after 48 h of incubation. ^{13}C- **(a, d, h, c, f, j)** and ^{15}N- **(b, e, i, d, g, k)** enrichments (atom %) are shown for *T. erythraeum* **(a, b)**, *C. watsonii* **(d, e)**, *Cyanothece* **(h, i)**, *Chaetoceros* sp. **(c, d)**, *Synechococcus* **(f, g)** and bacteria cells **(j, k)**. The white outlines define the regions of interest (ROIs), which were used to estimate the cells ^{13}C- and ^{15}N-enrichments.

with the idea of the cells specialization in N_2 fixation (called diazocytes) where high respiration rates and degradation of glycogen and gas vacuoles reduce the O_2 concentration enabling the expression of *nif* genes allowing daytime N_2 fixation (Bergman and Carpenter, 1991; Berman-Frank et al., 2001; Sandh et al., 2012). However, it has to be noticed that after 48 h of incubation with the tracers, it is highly probable that both ^{15}N and ^{13}C have been exchanged between cells, leading to a homogenization of the cells isotopic enrichments.

More surprisingly, the coupling between ^{13}C- and ^{15}N-enrichments for individual UCYN cells after 48 h of incubation is weaker than for *T. erythraeum* cells, in particular for *Cyanothece*. This appears counter-intuitive as UCYN are supposed to perform both N_2 fixation and photosynthesis within the same cell. This uncoupling suggests that UCYN cells might be at least partially specialized in photosynthesis or N_2 fixation, similarly to *Trichodesmium* spp. These results confirm the patterns already observed for *C. watsonii* (Foster et al., 2013). In addition, the weaker correlation between ^{13}C- and ^{15}N-enrichments in UCYN cells compared to *T. erythraeum* also suggests weaker extracellular fixed N and C exchanges between cells. These differences might be the result of the greater spatial proximity of *Trichodesmium* spp. cells within colonies and filaments compared to free living UCYN cells in the water column. According to this vision, the high production of extracellular polymeric substances observed in different *C. watsonii* strains (Sohm et al., 2011; Webb et al., 2009) might be a strategy to agglomerate the free living UCYN together to form colonies (Bonnet et al., 2016a; Foster et al., 2013), ensuring a spatial proximity and thus facilitating the exchange of metabolites between cells.

4.2 DDN release to the dissolved pool

The DD^{15}N released to the dissolved pool after 48 h accounted from 7 to 17 % of total N_2 fixation over the three diazotroph-amended treatments. These values are at the lower end of values (10–80 %) reported in *Trichodesmium* spp. blooms in the tropical Atlantic (Glibert and Bronk, 1994; Mulholland et al., 2006), southwestern Pacific (Bonnet et al., 2016c) or in mixed diazotroph assemblages of the North Pacific (Konno et al., 2010) and the Atlantic ocean

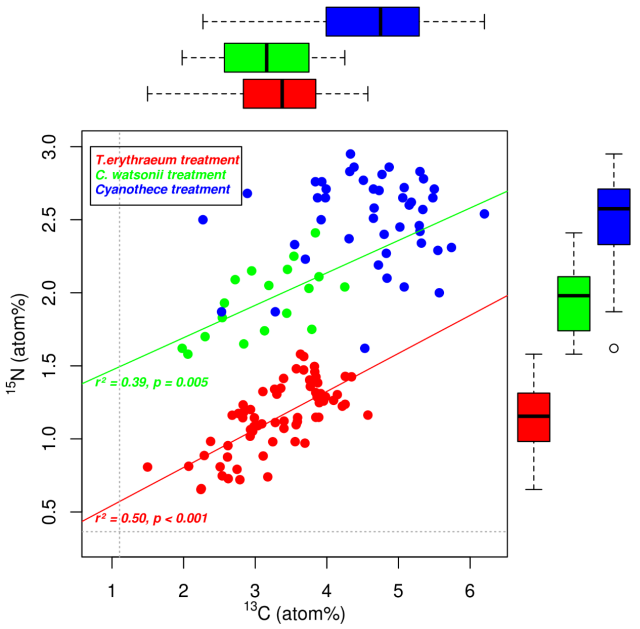

Figure 5. ^{15}N-enrichment (atom %) measured in *T. erythraeum* (red), *C. watsonii* (green) and *Cyanothece* (blue) cells relative to the ^{13}C-enrichment. The coloured line are the linear regressions for *T. erythraeum* (red), *C. watsonii* (green) shown with their respective r-squared and p-values. Regression is not significant for *Cyanothece* and thus not shown on the plot. Box plots of ^{13}C- and ^{15}N-enrichments are shown, following the same colour code, on horizontal and vertical axes, respectively.

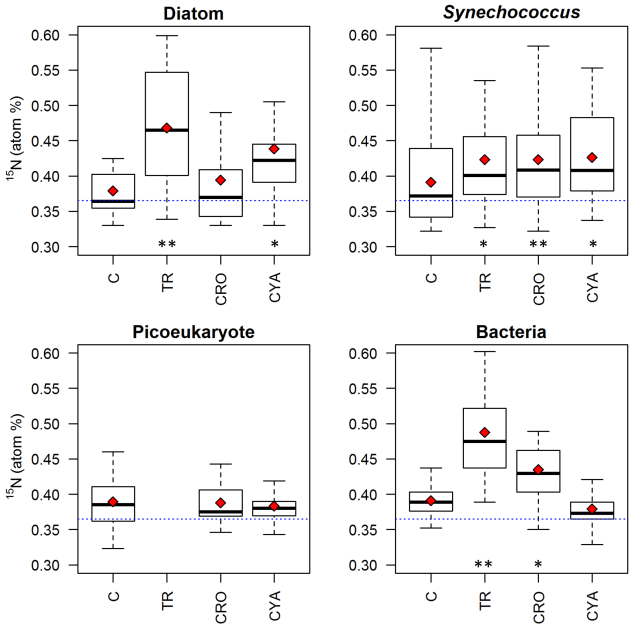

Figure 6. Box-plot of ^{15}N-enrichment measured in diatoms, *Synechococcus*, small eukaryotes and bacteria in the control (C), *T. erythraeum* (TR), *C. watsonii* (CRO) and *Cyanothece* (CYA) treatments. Red dots indicate the average values. Blue dotted lines depict the natural ^{15}N abundance. * and ** depict significant enrichments in diazotrophs treatments compared to the control treatment (unpaired Mann–Whitney–Wilcoxon test) at 95 and 99 % levels of confidences, respectively.

(Benavides et al., 2013). In contrast, these values of N release are at least 1 order of magnitude higher than those reported in unialgal cultures (< 2 %) for the same strains as those studied here (Berthelot et al., 2015a). It is probable that, in culture, the cells are maintained in optimal growth conditions (exponential growth phase, appropriate light, temperature and nutrient conditions) and optimize the N use, either through a low excretion rate of DDN or through an efficient uptake of DDN (Mulholland et al., 2001). Conversely, in the field, the sampling does not necessarily occur during the exponential growth phase, and exogenous factors may affect the release of DDN, such as viral lysis (Hewson et al., 2004; Ohki, 1999) and sloppy feeding (O'Neil et al., 1996). In this study, the diazotrophs added to natural seawater were healthy but may have been affected by exogenous factors after inoculation, leading to a moderate proportion (7–17 %) of DDN released in the dissolved pool. These results indicate that the proportion of N_2 fixed released in the dissolved compartment both depends on the cell status and on exogenous factors more than the type of diazotrophs involved in N_2 fixation, as previously stated by Berthelot et al. (2015a).

4.3 DDN transfer efficiency and pathways

T. erythraeum transferred ∼ 12 % of DD^{15}N towards the non-diazotrophic plankton. This is in good agreement with previous estimates by Bonnet et al. (2016c) using the same methodology, who report a DD^{15}N transfer of 7 to 12 % in naturally occurring *Trichodesmium* spp. blooms. These results confirm that *Trichodesmium* spp. enhances the development of non-diazotrophic plankton, as already suggested by the frequent observations of co-occurrence or succession of *Trichodesmium* spp. and non-diazotrophs, particularly in N-depleted environments (Devassy et al., 1979; Dore et al., 2008; Lee Chen et al., 2011; Lenes and Heil, 2010).

The DD^{15}N transfer was half as efficient (4–5 %) in the UCYN treatments compared to the *T. erythraeum* treatment (Table 1). This is in good agreement with the lower increase in plankton biomass associated with non diazotrophs in the UCYN treatments compared to the *T. erythraeum* treatment (Fig. 2). The ecology of UCYN is less characterized than that of *Trichodesmium* spp. and data on their co-occurrence with non-diazotrophic plankton in the ocean are scarce. In this issue, Bonnet et al. (2016a) used the single cell approach described here during a natural occurring bloom of UCYN-C (closely related to *Cyanothece* spp.) in the New Caledonian lagoon and measured a DD^{15}N transfer of 21 ± 4 % of

Table 1. Synthesis of the distribution of the recently fixed N_2 ($DD^{15}N$) in each of the planktonic groups analysed after 48 h of incubation (nmol L^{-1} 48 h^{-1}) and their respective proportion relative to the total fixed N_2 (%). n.a.: not analysed, n.c.: not calculated. Standard deviations are in parenthesis.

Planktonic group	$DD^{15}N$ (nmol L^{-1} 48 h^{-1})	% of total N_2 fixed
	Trichodesmium treatment	
Trichodesmium	63.05 (21.54)	78.0 (26.7)
Dissolved pool	8.30 (3.83)	10.3 (4.7)
Sum of non-diazotrophs	9.45 (3.54)	11.7 (4.4)
Bacteria	5.79 (3.41)	7.2 (4.2)
Diatom	1.99 (0.36)	2.5 (0.5)
Synechococcus	1.67 (0.88)	2.1 (1.1)
Small eukaryotes	n.a	n.c
	C. watsonii treatment	
C. watsonii	60.92 (10.06)	87.2 (14.4)
Dissolved pool	4.90 (14.10)	7.0 (3.0)
Sum of non-diazotrophs	4.08 (1.87)	5.8 (2.7)
Bacteria	2.36 (1.70)	3.4 (2.4)
Diatom	0.06 (0.05)	0.1 (0.1)
Synechococcus	1.63 (0.78)	2.3 (1.1)
Small eukaryotes	0.03 (0.00)	n.c.
	Cyanothece treatment	
Cyanothece	19.41 (5.28)	78.6 (21.4)
Dissolved pool	4.10 (9.85)	16.6 (5.0)
Sum of non-diazotrophs	1.19 (0.59)	4.9 (2.4)
Bacteria	−0.05 (0.42)	n.c.
Diatom	0.93 (0.28)	3.8 (1.1)
Synechococcus	0.29 (0.30)	1.2 (1.2)
Small eukaryotes	0.02 (0.00)	n.c.

the total N_2 fixation, mainly towards pico-planktonic communities. The $DD^{15}N$ transfer reported in the latter study is ∼3 times higher than in the present study. This discrepancy may result from the physiological differences between the UCYN-C ecotypes involved in both studied, and/or from the DDN release from diazotrophic cells that is potentially higher is natural communities compared to cultured cells as discussed above. The bloom reported in Bonnet et al. (2016a) study co-occurred with a doubling of *Synechococcus* and pico-eukaryotes abundances, as well as an increase of diatoms (Leblanc et al., 2016) and PP (Berthelot et al., 2015b). *Crocosphaera*-like cells observed in association with the diatom *Climacodium* sp. (Carpenter and Janson, 2000) have also been shown to transfer the recently fixed N_2 towards the host diatom cell (Foster et al., 2011). All these data confirm that UCYN are able to provide DDN to non-diazotrophic plankton and thus promote marine productivity in N-depleted areas.

The transfer of DDN towards phytoplankton or bacteria requires the release of N in the dissolved pool. Surprisingly, the total amount of $DD^{15}N$ recovered in the dissolved pool was not a good indicator of the $DD^{15}N$ transfer efficiency: the highest release of DDN was measured in the *Cyanothece* treatment (16.6 ± 4.9 % of the total N_2 fixation) and led to the lowest $DD^{15}N$ transfer efficiency (4.9 ± 2.4 % of the total N_2 fixation). In the *T. erythraeum* and in *C. watsonii* treatments, the proportion of $DD^{15}N$ recovered in the dissolved pool was lower (10.3 ± 4.7 and 7.0 ± 3.0 % of the total N_2 fixation, respectively) but led to higher $DD^{15}N$ transfer efficiencies (11.7 ± 4.4 and 5.8 ± 2.7 % of the total N_2 fixation, respectively). This suggests that the N compounds released by *Cyanothece* were less available for the surrounding plankton communities than the compounds released by *T. erythraeum* and *C. watsonii*.

On the opposite, the $^{15}NH_4^+$ enrichment appeared to be a relevant indicator of the DDN transfer efficiency: it was twice as high in the *T. erythraeum* treatment compared to the UCYN treatments (Table 2), leading to a $DD^{15}N$ transfer efficiency twice as high in the *T. erythraeum* treatments (Table 1). This coupling between $^{15}NH_4^+$ enrichment and transfer efficiency suggests that NH_4^+ is the major form of $DD^{15}N$ that is transferred to non-diazotrophic plankton, and that the DDN released under the form of DON is likely poorly available for the surrounding planktonic communities (Knapp et

Table 2. ^{15}N-enrichment (atom %) of diazotrophic cells, PON, NH_4^+ and DON pools and concentrations (µmol L^{-1}) of NH_4^+ and DON. In parenthesis are shown the standard deviations on triplicate incubations. n/a: not applicable, n.d.: not detected.

	Control	*T. erythraeum* treatment	*C. watsonii* treatment	*Cyanothece* treatment
	^{15}N-enrichment (atom %)			
Diazotrophic cells	n/a	1.15 (0.23)	1.94 (0.24)	2.50 (0.30)
PON	0.40 (0.01)	0.69 (0.13)	0.67 (0.01)	0.50 (0.01)
NH_4^+	n.d.	2.31 (0.81)	1.20 (0.15)	1.44 (0.44)
DON	0.37 (< 0.00)	0.37 (< 0.00)	0.37 (< 0.00)	0.38 (< 0.00)
	Concentrations (µmol L^{-1})			
NH_4^+	0.010 (0.002)	0.010 (0.003)	0.011 (0.004)	0.009 (0.003)
DON	4.05 (0.57)	3.99 (0.17)	4.54 (0.80)	4.18 (0.45)

al., 2005; Bourbonnais et al., 2009). This is in good agreement with the known higher bioavailability of NH_4^+ for phytoplankton compared to DON (e.g. Bradley et al., 2010; Collos and Berges, 2002). However, some DON compounds such as urea or amino acids can also be a significant source of N for planktonic communities, e.g. heterotrophic bacteria and mixotrophic plankton (Antia, 1991; Bronk, 2007). Unfortunately, the methodology used here can not asserts the importance of DON compared to NH_4^+ in the DDN transfer.

It should be noted that the increase of plankton biomass associated with non-diazotrophs in the present study cannot only be explained by the DD^{15}N provided by N_2 fixation within the time frame of the incubation (48 h). While the DD^{15}N transferred to non-diazotrophic plankton biomass ranged between 1 and 10 nmol N L^{-1} in the diazotrophs-amended treatments, the non-diazotrophic biomass increased from 90 to 160 nmol N L^{-1} in the diazotrophs-amended treatments. This suggests that production was also stimulated by DDN fixed prior the incubations, that was thus not ^{15}N labelled.

4.4 Plankton groups benefiting from the DDN

Bacterial biomass increased from 60 to 120 % after the addition of three diazotrophs; it was the plankton group which responded the most to the diazotrophs inoculations, whatever the treatment considered (Fig. 1 and Table S1). This is consistent with the high ^{15}N-enrichment of bacteria cells in *T. erythraeum* and *C. watsonii* treatments compared to the control treatment (Fig. 6). In contrast, the high bacterial biomass increase observed in the *Cyanothece* treatment contrasts with the relatively low ^{15}N-enrichment of bacterial individual cells measured in this treatment (Fig. 6). This suggests that, in the *T. erythraeum* and *C. watsonii* treatments, bacteria took advantage of the DD^{15}N released during the incubation, while in *Cyanothece* treatment, bacteria may have mainly relied on DDN fixed prior to the beginning of the incubation. This is consistent with the higher accumulation of DD^{15}N in the DON pool in the *Cyanothece* treatment compared to the two other treatments, indicating that the DON compounds released by *Cyanothece* are likely less bio-available for the planktonic community compared to those released by *T. erythraeum* and *C. watsonii*.

The presence of bacteria in *Trichodesmium* spp. colonies has been widely studied (Hewson et al., 2009; Hmelo et al., 2012; Nausch, 1996; Paerl et al., 1989; Rochelle-Newall et al., 2014; Sheridan et al., 2002). *Trichodesmium* spp. harbours high heterotrophic bacterial activity (Nausch, 1996; Tseng et al., 2005) and abundance is found to be at least 2 orders of magnitude higher in *Trichodesmium* spp. colonies than in surrounding waters (Sheridan et al., 2002). Associations between bacteria and UCYN are less documented. However, similarly to *Trichodesmium* spp., tight relationships may occur between UCYN and bacteria, as suggested by the significant increases of bacterial abundances in the UCYN treatments, but further investigations would be needed to understand the nature of their interactions.

Phytoplankton (diatoms, *Synechococcus* and small eukaryotes) was also stimulated by the diazotroph blooms, although to a lower extend compared to bacteria in all treatments (Fig. 1). However, the increase in phytoplankton biomass in the *T. erythraeum* and *C. watsonii* treatments together with the significant ^{15}N-enrichments in diatoms and *Synechococcus* (Fig. 6) argue that phytoplankton also took advantage of the DDN. This confirms the ability of diazotroph to promote non-diazotrophic primary producers as suggested by previous studies (e.g. Bonnet et al., 2016c; Devassy et al., 1979; Lee Chen et al., 2011; Lenes and Heil, 2010). The enhancement of large phytoplanktonic cells such as diatoms by DDN observed within the timespan of this study reveals the tight relationship that may occurs between the new production fuelled by diazotrophy and particle export in oligotrophic areas.

In the present study, the plankton community composition remained relatively stable in comparison to the Bonnet et al. (2016c) study, in which the authors observed sys-

tematic shifts from *Trichodesmium* spp. biomass towards diatom biomass. This difference is probably linked to the *Trichodesmium* cells status. In the Bonnet et al. (2016c) study, most of the analyzed *Trichodesmium* spp. cells were decaying, leading to the release of micromolar concentrations of NH_4^+ accumulating in the dissolved pool, whereas the main form of DDN released in the present study was DON. This rapid increase in NH_4^+ bio-availability was benefiting to diatoms, which are known to be highly competitive under high nutrient concentrations (Chavez and Smith, 1995; Kudela and Dugdale, 2000; Miller and Wheeler, 2012; Smetacek, 1998; Wilkerson et al., 2000). This synchronized destruction of the colonies has been shown to be possible within a few hours, mediated by programmed cell death (Berman-Frank et al., 2004). Furthermore, the dense bloom forming behaviour and maintenance of *Trichodesmium* spp. at the surface due to their positive buoyancy (Romans et al., 1994) may be an additional feature that helps the constitution of locally rich N layers promoting the diatom development. In the present study, the *T. erythraeum* colonies added to the incubation bottles were in exponential growing phase and the relative stability of their abundance throughout the experiment indicates no substantial cell breakages. The amount of DDN transfer in our study was thus mainly mediated by the release of NH_4^+ and DON during active N_2 fixation rather than the N release due to cell breakage and was thus much more limited than in the Bonnet et al. (2016c) study, leading to attenuated changes in the planktonic community composition.

5 Conclusions and ecological implications

This study reveals the various short term fates of DDN in the ocean and highlight the complex interactions between diazotrophs and their environment. First, it shows that the DDN released by diazotrophs in the dissolved pool as NH_4^+ is quickly transferred to non-diazotrophic plankton while the DDN released as DON is mostly accumulated in the dissolved pool. Second, the DDN transfer efficiency towards the non-diazotrophic plankton depends on the diazotrophs involved in N_2 fixation: it is twice as much for *T. erythraeum* compared to the DDN transfer associated with UCYN strains. This implies that *T. erythraeum* would be more efficient at promoting non-diazotrophic marine productivity in N-depleted areas than UCYN are. Finally, the results presented here suggest that diazotrophic activity first promotes heterotrophic plankton but also autotrophic plankton, albeit to a lower extent. Taken together, theses results show that the fates of DDN are diverse and would need further investigation, in particular in the vast open-ocean regions where primary productivity extensively depends on diazotrophy.

The Supplement related to this article is available online at doi:10.5194/bg-13-4005-2016-supplement.

Author contributions. S. Bonnet and H. Berthelot designed the experiments and S. Bonnet and H. Berthelot carried them out. All authors analyzed the samples. H. Berthelot prepared the manuscript, which was amended by S. Bonnet.

Acknowledgements. Funding for this research was provided by the Agence Nationale de la Recherche (ANR starting grant VAHINE ANR-13-JS06-0002). We thank François Robert, Adriana Gonzalez, Smail Mostefaoui and Rémi Duhamel from the French National Ion MicroProbe Facility hosted by the Museum National d'Histoire Naturelle (Paris) for providing nanoSIMS facilities and constant advice. We are grateful to Gerald Gregori from the Regional Flow Cytometry Platform for Microbiology (PRECYM) of the Mediterranean Institute of Oceanography (MIO) for the flow cytometry analyses support.

Edited by: E. Marañón

References

Aminot, A. and Kérouel, R.: Dosage Automatique des Nutriments dans les Eaux Marines, Ifremer, Plouzané, 2007.

Benavides, M., Bronk, D. A., Agawin, N. S. R., Pérez-Hernández, M. D., Hernández-Guerra, A., and Arístegui, J.: Longitudinal variability of size-fractionated N_2 fixation and DON release rates along 24.5° N in the subtropical North Atlantic, J. Geophys. Res.-Oceans, 118, 3406–3415, doi:10.1002/jgrc.20253, 2013.

Bergman, B. and Carpenter, E. J.: Nitrogenase confined to randomly distributed trichomes in the marine cyanobacterium *Trichodesmium thiebautii*, J. Phycol., 27, 158–165, doi:10.1111/j.0022-3646.1991.00158.x, 1991.

Bergman, B., Sandh, G., Lin, S., Larsson, J., and Carpenter, E. J.: *Trichodesmium* – a widespread marine cyanobacterium with unusual nitrogen fixation properties, FEMS Microbiol. Rev., 37, 286–302, doi:10.1111/j.1574-6976.2012.00352.x, 2013.

Berman-Frank, I., Lundgren, P., Chen, Y. B., Küpper, H., Kolber, Z., Bergman, B., and Falkowski, P.: Segregation of nitrogen fixation and oxygenic photosynthesis in the marine cyanobacterium *Trichodesmium*, Science, 294, 1534–1537, doi:10.1126/science.1064082, 2001.

Berman-Frank, I., Bidle, K. D., Haramaty, L., and Falkowski, P. G.: The demise of the marine cyanobacterium, *Trichodesmium* spp., via an autocatalyzed cell death pathway, Limnol. Oceanogr., 49, 997–1005, doi:10.4319/lo.2004.49.4.0997, 2004.

Berthelot, H., Bonnet, S., Camps, M., Grosso, O., and Moutin, T.: Assessment of the dinitrogen released as ammonium and dissolved organic nitrogen by unicellular and filamentous marine diazotrophic cyanobacteria grown in culture, Front. Mar. Sci., 2, 80, doi:10.3389/fmars.2015.00080, 2015a.

Berthelot, H., Moutin, T., L'Helguen, S., Leblanc, K., Hélias, S., Grosso, O., Leblond, N., Charrière, B., and Bonnet, S.: Dinitrogen fixation and dissolved organic nitrogen fueled primary production and particulate export during the VAHINE mesocosm experiment (New Caledonia lagoon), Biogeosciences, 12, 4099–4112, doi:10.5194/bg-12-4099-2015, 2015b.

Bonnet, S., Biegala, I. C., Dutrieux, P., Slemons, L. O., and Capone, D. G.: Nitrogen fixation in the western equatorial Pacific: rates, diazotrophic cyanobacterial size class distribution, and biogeochemical significance, Global Biogeochem. Cy., 23, 1–13, doi:10.1029/2008GB003439, 2009.

Bonnet, S., Rodier, M., Turk-Kubo, K. A., Germineaud, C., Menkes, C., Ganachaud, A., Cravatte, S., Raimbault, P., Campbell, E., Quéroué, F., Sarthou, G., Desnues, A., Maes, C., and Eldin, G.: Contrasted geographical distribution of N_2 fixation rates and nifH phylotypes in the Coral and Solomon Seas (South-Western Pacific) during austral winter conditions, Global Biogeochem. Cy., 29, 1874–1892, doi:10.1002/2015GB005117, 2015.

Bonnet, S., Berthelot, H., Turk-Kubo, K., Fawcett, S., Rahav, E., L'Helguen, S., and Berman-Frank, I.: Dynamics of N_2 fixation and fate of diazotroph-derived nitrogen in a low-nutrient, low-chlorophyll ecosystem: results from the VAHINE mesocosm experiment (New Caledonia), Biogeosciences, 13, 2653–2673, doi:10.5194/bg-13-2653-2016, 2016a.

Bonnet, S., Moutin, T., Rodier, M., Grisoni, J.-M., Louis, F., Folcher, E., Bourgeois, B., Boré, J.-M., and Renaud, A.: Introduction to the project VAHINE: VAriability of vertical and tropHIc transfer of diazotroph derived N in the south wEst Pacific, Biogeosciences, 13, 2803–2814, doi:10.5194/bg-13-2803-2016, 2016b.

Bonnet, S., Berthelot, H., Turk-Kubo, K., Cornet-Bartaux, V., Fawcett, S. E., Berman-Frank, I., Barani, A., Dekaezemacker, J., Benavides, M., Charriere, B., and Capone, D. G.: Diazotroph derived nitrogen supports diatoms growth in the South West Pacific: a quantitative study using nanoSIMS, Limnol. Oceanogr., in press, 2016c.

Bourbonnais, A., Lehmann, M. F, Waniek, J. J., and Schulz-Bull, D. E.: Nitrate isotope anomalies reflect N_2 fixation in the Azores Front region (subtropical NE Atlantic), J. Geophys. Res., 114, C03003, doi:10.1029/2007JC004617, 2009.

Bradley, P. B., Lomas, M. W., and Bronk, D. A.: Inorganic and organic nitrogen use by phytoplankton along Chesapeake Bay, measured using a flow cytometric sorting approach, Estuar. Coast., 33, 971–984, doi:10.1007/s12237-009-9252-y, 2010.

Bronk, D. A., See, J. H., Bradley, P., and Killberg, L.: DON as a source of bioavailable nitrogen for phytoplankton, Biogeosciences, 4, 283–296, doi:10.5194/bg-4-283-2007, 2007.

Bryceson, I. and Fay, P.: Nitrogen fixation in *Oscillatoria (Trichodesmium) erythraea* in relation to bundle formation and trichome differentiation, Mar. Biol., 61, 159–166, doi:10.1007/BF00386655, 1981.

Buitenhuis, E. T., Li, W. K. W., Vaulot, D., Lomas, M. W., Landry, M. R., Partensky, F., Karl, D. M., Ulloa, O., Campbell, L., Jacquet, S., Lantoine, F., Chavez, F., Macias, D., Gosselin, M., and McManus, G. B.: Picophytoplankton biomass distribution in the global ocean, Earth Syst. Sci. Data, 4, 37–46, doi:10.5194/essd-4-37-2012, 2012.

Capone, D. G., Ferrier, M. D., and Carpenter, E. J.: Amino acid cycling in colonies of the planktonic marine cyanobacterium *Trichodesmium thiebautii*, Appl. Environ. Microb., 60, 3989–3995, 1994.

Capone, D. G., Zehr, J. P., Paerl, H. W., Bergman, B., and Carpenter, E. J.: *Trichodesmium*, a globally significant marine cyanobacterium, Science, 276, 1221–1229, doi:10.1126/science.276.5316.1221, 1997.

Capone, D. G., Burns, J. A., Montoya, J. P., Subramaniam, A., Mahaffey, C., Gunderson, T., Michaels, A. F., and Carpenter, E. J.: Nitrogen fixation by *Trichodesmium* spp.: an important source of new nitrogen to the tropical and subtropical North Atlantic Ocean, Global Biogeochem. Cy., 19, GB2024, doi:10.1029/2004GB002331, 2005.

Carpenter, E. J. and Janson, S.: Intracellular cyanobacterial symbionts in the marine diatom *Climacodium frauenfeldianum* (Bacillariophyceae), J. Phycol., 36, 540–544, doi:10.1046/j.1529-8817.2000.99163.x, 2000.

Chavez, F. P. and Smith, S. L.: Biological and chemical consequences of open ocean upwelling, in: Upwelling in the Ocean: Modern Processes and Ancient Records, edited by: Summerhayes, C. P., Emeis, K.-C., and Angel, M. V., Wiley, 149–169, 1995.

Chen, Y.-B., Zehr, J. P., and Mellon, M.: Growth and nitrogen fixation of the diazotrophic filamentous nonheterocystous cyanobacterium *Trichodesmium* sp. IMS 101 in defined media: evidence for a circadian rhythm, J. Phycol., 32, 916–923, doi:10.1111/j.0022-3646.1996.00916.x, 1996.

Collos, Y. and Berges, J. A.: Nitrogen metabolism in phytoplankton, in: Marine Ecology, Encyclopedia of Life Support Systems (EOLSS), 1–18, 2002.

Dabundo, R., Lehmann, M. F., Treibergs, L., Tobias, C. R., Altabet, M. A., Moisander, P. H., and Granger, J.: The Contamination of Commercial $^{15}N_2$ Gas Stocks with ^{15}N Labeled Nitrate and Ammonium and Consequences for Nitrogen Fixation Measurements, PLoS ONE, 9, e110335, doi:10.1371/journal.pone.0110335, 2014.

Devassy, V. P., Bhattathiri, P. M. A., and Qasim, S. Z.: Succession of organisms following *Trichodesmium* phenomenon, Indian J. Mar. Sci., 8, 89–93, 1979.

Dore, J. E., Brum, J. R., Tupas, L. M., and Karl, D. M.: Seasonal and interannual variability in sources of nitrogen supporting export in the oligotrophic subtropical North Pacific Ocean, Limnol. Oceanogr., 47, 1595–1607, doi:10.4319/lo.2002.47.6.1595, 2002.

Dore, J. E., Letelier, R. M., Church, M. J., Lukas, R., and Karl, D. M.: Summer phytoplankton blooms in the oligotrophic North Pacific Subtropical Gyre: historical perspective and recent observations, Prog. Oceanogr., 76, 2–38, doi:10.1016/j.pocean.2007.10.002, 2008.

Dron, A., Rabouille, S., Claquin, P., Le Roy, B., Talec, A., and Sciandra, A.: Light-dark (12 : 12) cycle of carbon and nitrogen metabolism in *Crocosphaera watsonii* WH8501: relation to the cell cycle, Environ. Microbiol., 14, 967–981, doi:10.1111/j.1462-2920.2011.02675.x, 2012.

Finzi-Hart, J. A., Pett-Ridge, J., Weber, P. K., Popa, R., Fallon, S. J., Gunderson, T., Hutcheon, I. D., Nealson, K. H., and Capone, D. G.: Fixation and fate of C and N in the cyanobacterium *Trichodesmium* using nanometer-scale secondary ion

mass spectrometry, P. Natl. Acad. Sci. USA, 106, 6345–6350, doi:10.1073/pnas.0810547106, 2009.

Foster, R. A., Kuypers, M. M. M., Vagner, T., Paerl, R. W., Musat, N., and Zehr, J. P.: Nitrogen fixation and transfer in open ocean diatom-cyanobacterial symbioses, ISME J., 5, 1484–1493, doi:10.1038/ismej.2011.26, 2011.

Foster, R. A., Sztejrenszus, S., and Kuypers, M. M.: Measuring carbon and N_2 fixation in field populations of colonial and free-living unicellular cyanobacteria using nanometer-scale secondary ion mass spectrometry, edited by: Raven, J., J. Phycol., 49, 502–516, doi:10.1111/jpy.12057, 2013.

Fukuda, R., Ogawa, H., Nagata, T., and Koike, I. I.: Direct determination of carbon and nitrogen contents of natural bacterial assemblages in marine environments, Appl. Environ. Microb., 64, 3352–3358, 1998.

Garcia, N., Raimbault, P., and Sandroni, V.: Seasonal nitrogen fixation and primary production in the Southwest Pacific: nanoplankton diazotrophy and transfer of nitrogen to picoplankton organisms, Mar. Ecol.-Prog. Ser., 343, 25–33, doi:10.3354/meps06882, 2007.

Glibert, P. M. and Bronk, D. A.: Release of dissolved organic nitrogen by marine diazotrophic cyanobacteria *Trichodesmium* spp., Appl. Environ. Microb., 60, 3996–4000, 1994.

Grégori, G., Colosimo, A., and Denis, M.: Phytoplankton group dynamics in the Bay of Marseilles during a 2-year survey based on analytical flow cytometry, Cytometry, 44, 247–256, 2001.

Hasle, G. R.: The inverted microscope, in: Phytoplankton Manual, edited by: Sournia, A., Monographs on Oceanographic Methodology 6, UNESCO, Paris, 191–916, 1978.

Hewson, I., Govil, S., Capone, D., Carpenter, E., and Fuhrman, J.: Evidence of *Trichodesmium* viral lysis and potential significance for biogeochemical cycling in the oligotrophic ocean, Aquat. Microb. Ecol., 36, 1–8, doi:10.3354/ame036001, 2004.

Hewson, I., Poretsky, R. S., Beinart, R. A., White, A. E., Shi, T., Bench, S. R., Moisander, P. H., Paerl, R. W., Tripp, H. J., Montoya, J. P., Moran, M. A., and Zehr, J. P.: In situ transcriptomic analysis of the globally important keystone N_2-fixing taxon *Crocosphaera watsonii*, ISME J., 3, 618–631, doi:10.1038/ismej.2009.8, 2009.

Hmelo, L., Van Mooy, B., and Mincer, T.: Characterization of bacterial epibionts on the cyanobacterium *Trichodesmium*, Aquat. Microb. Ecol., 67, 1–14, doi:10.3354/ame01571, 2012.

Holmes, R. M., Aminot, A., Kérouel, R., Hooker, B. A., and Peterson, B. J.: A simple and precise method for measuring ammonium in marine and freshwater ecosystems, Can. J. Fish. Aquat. Sci., 56, 1801–1808, doi:10.1139/f99-128, 1999.

Kana, T. M., Darkangelo, C., Hunt, M. D., Oldham, J. B., Bennett, G. E., and Cornwell, J. C.: Membrane inlet mass spectrometer for rapid high-precision determination of N_2, O_2, and Ar in environmental water samples, Anal. Chem., 66, 4166–4170, doi:10.1021/ac00095a009, 1994.

Karl, D., Michaels, A., Bergman, B., Capone, D., Carpenter, E., Letelier, R., Lipschultz, F., Paerl, H., Sigman, D., and Stal, L.: Dinitrogen fixation in the world's oceans, Biogeochemistry, 57, 47–98, doi:10.1023/A:1015798105851, 2002.

Karl, D. M., Church, M. J., Dore, J. E., Letelier, R. M., and Mahaffey, C.: Predictable and efficient carbon sequestration in the North Pacific Ocean supported by symbiotic nitrogen fixation, P. Natl. Acad. Sci. USA, 109, 1842–1849, doi:10.1073/pnas.1120312109, 2012.

Knapp, A. N., Sigman, D. M., and Lipschultz, F.: N isotopic composition of dissolved organic nitrogen and nitrate at the Bermuda Atlantic Time-series Study site, Global Biogeochem. Cy., 19, GB1018, doi:10.1029/2004GB002320, 2005.

Konno, U., Tsunogai, U., Komatsu, D. D., Daita, S., Nakagawa, F., Tsuda, A., Matsui, T., Eum, Y.-J., and Suzuki, K.: Determination of total N_2 fixation rates in the ocean taking into account both the particulate and filtrate fractions, Biogeosciences, 7, 2369–2377, doi:10.5194/bg-7-2369-2010, 2010.

Kudela, R. M. and Dugdale, R. C.: Nutrient regulation of phytoplankton productivity in Monterey Bay, California, Deep-Sea Res. Pt. II, 47, 1023–1053, doi:10.1016/S0967-0645(99)00135-6, 2000.

Leblanc, K., Cornet, V., Caffin, M., Rodier, M., Desnues, A., Berthelot, H., Turk-Kubo, K., and Heliou, J.: Phytoplankton community structure in the VAHINE MESOCOSM experiment, Biogeosciences Discuss., doi:10.5194/bg-2015-605, in review, 2016.

LaRoche, J. and Breitbarth, E.: Importance of the diazotrophs as a source of new nitrogen in the ocean, J. Sea Res., 53, 67–91, doi:10.1016/j.seares.2004.05.005, 2005.

Lee Chen, Y., Tuo, S., and Chen, H.: Co-occurrence and transfer of fixed nitrogen from *Trichodesmium* spp. to diatoms in the low-latitude Kuroshio Current in the NW Pacific, Mar. Ecol.-Prog. Ser., 421, 25–38, doi:10.3354/meps08908, 2011.

Lenes, J. M. and Heil, C. A.: A historical analysis of the potential nutrient supply from the N_2 fixing marine cyanobacterium *Trichodesmium* spp. to *Karenia brevis* blooms in the eastern Gulf of Mexico, J. Plankton Res., 32, 1421–1431, doi:10.1093/plankt/fbq061, 2010.

Luo, Y.-W., Doney, S. C., Anderson, L. A., Benavides, M., Berman-Frank, I., Bode, A., Bonnet, S., Boström, K. H., Böttjer, D., Capone, D. G., Carpenter, E. J., Chen, Y. L., Church, M. J., Dore, J. E., Falcón, L. I., Fernández, A., Foster, R. A., Furuya, K., Gómez, F., Gundersen, K., Hynes, A. M., Karl, D. M., Kitajima, S., Langlois, R. J., LaRoche, J., Letelier, R. M., Marañón, E., McGillicuddy Jr., D. J., Moisander, P. H., Moore, C. M., Mouriño-Carballido, B., Mulholland, M. R., Needoba, J. A., Orcutt, K. M., Poulton, A. J., Rahav, E., Raimbault, P., Rees, A. P., Riemann, L., Shiozaki, T., Subramaniam, A., Tyrrell, T., Turk-Kubo, K. A., Varela, M., Villareal, T. A., Webb, E. A., White, A. E., Wu, J., and Zehr, J. P.: Database of diazotrophs in global ocean: abundance, biomass and nitrogen fixation rates, Earth Syst. Sci. Data, 4, 47–73, doi:10.5194/essd-4-47-2012, 2012.

Marie, D., Brussaard, C., Partensky, F., and Vaulot, D.: Flow cytometric analysis of phytoplankton, bacteria and viruses. In: Current Protocols in Cytometry, Hoboken, NJ, Wiley, 11.11.1–11.11.15., 1999.

Mague, T. H., Weare, N. M., and Holm-Hansen, O.: Nitrogen fixation in the North Pacific Ocean, Mar. Biol., 24, 109–119, doi:10.1007/BF00389344, 1974.

Miller, C. B. and Wheeler, P. A.: Habitat determinants of primary production in the sea. In: Biological Oceanography, Second Edition, John Wiley and Sons, Ltd., 2012.

Mohr, W., Großkopf, T., Wallace, D. W. R., LaRoche, J., and Grosskopf, T.: Methodological underestimation of oceanic nitrogen fixation rates, PLoS One, 5, e12583, doi:10.1371/journal.pone.0012583, 2010.

Moisander, P. H., Beinart, R. A., Hewson, I., White, A. E., Johnson, K. S., Carlson, C. A., Montoya, J. P., and Zehr, J. P.: Unicellular cyanobacterial distributions broaden the oceanic N_2 fixation domain, Science, 327, 1512–1514, doi:10.1126/science.1185468, 2010.

Montoya, J. P., Voss, M., Kahler, P., and Capone, D. G.: A simple, high-precision, high-sensitivity tracer assay for N_2 fixation, Appl. Environ. Microb., 62, 986–993, 1996.

Montoya, J. P., Carpenter, E. J., and Capone, D. G.: Nitrogen fixation and nitrogen isotope abundances in zooplankton of the oligotrophic North Atlantic, Limnol. Oceanogr., 47, 1617–1628, doi:10.4319/lo.2002.47.6.1617, 2002.

Montoya, J. P., Holl, C. M., Zehr, J. P., Hansen, A., Villareal, T. A., and Capone, D. G.: High rates of N_2 fixation by unicellular diazotrophs in the oligotrophic Pacific Ocean, Nature, 430, 1027–1032, doi:10.1038/nature02824, 2004.

Moore, J. K., Geider, R. J., Guieu, C., Jaccard, S. L., Jickells, T. D., LaRoche, J., Lenton, T. M., Mahowald, N. M., Marañón, E., Marinov, I., Nakatsuka, T., Oschlies, A., Saito, M. A., Thingstad, T. F., Tsuda, A., and Ulloa, O.: Processes and patterns of nutrient limitation, Nat. Geosci., 6, 1–10, doi:10.1038/NGEO1765, 2013.

Mulholland, M. R. and Bernhardt, P. W.: The effect of growth rate, phosphorus concentration, and temperature on N_2 fixation, carbon fixation, and nitrogen release in continuous cultures of *Trichodesmium* IMS101, Limnol. Oceanogr., 50, 839–849, doi:10.4319/lo.2005.50.3.0839, 2005.

Mulholland, M. R. and Capone, D. G.: Stoichiometry of nitrogen and carbon utilization in cultured populations of *Trichodesmium* IMS101: implications for growth, Limnol. Oceanogr., 46, 436–443, doi:10.4319/lo.2001.46.2.0436, 2001.

Mulholland, M. R., Ohki, K., and Capone, D. G.: Nutrient controls on nitrogen uptake and metabolism by natural populations and cultures of *Trichodesmium* (Cyanobacteria), J. Phycol., 37, 1001–1009, doi:10.1046/j.1529-8817.2001.00080.x, 2001.

Mulholland, M., Bronk, D., and Capone, D.: Dinitrogen fixation and release of ammonium and dissolved organic nitrogen by *Trichodesmium* IMS101, Aquat. Microb. Ecol., 37, 85–94, doi:10.3354/ame037085, 2004.

Mulholland, M. R., Bernhardt, P. W., Heil, C. A., Bronk, D. A., and O'Neil, J. M.: Nitrogen fixation and release of fixed nitrogen by *Trichodesmium* spp. in the Gulf of Mexico, Limnol. Oceanogr., 51, 1762–1776, doi:10.4319/lo.2006.51.4.1762, 2006.

Musat, N., Halm, H., Winterholler, B., Hoppe, P., Peduzzi, S., Hillion, F., Horreard, F., Amann, R., Jørgensen, B. B., and Kuypers, M. M. M.: A single-cell view on the ecophysiology of anaerobic phototrophic bacteria, P. Natl. Acad. Sci. USA, 105, 17861–17866, doi:10.1073/pnas.0809329105, 2008.

Nausch, M.: Microbial activities on *Trichodesmium* colonies, Mar. Ecol.-Prog. Ser., 141, 173–181, doi:10.3354/meps141173, 1996.

Needoba, J. A., Foster, R. A., Sakamoto, C., Zehr, J. P., and Johnson, K. S.: Nitrogen fixation by unicellular diazotrophic cyanobacteria in the temperate oligotrophic North Pacific Ocean, Limnol. Oceanogr., 52, 1317–1327, doi:10.4319/lo.2007.52.4.1317, 2007.

O'Neil, J. M., Metzler, P. M., and Glibert, P. M.: Ingestion of $^{15}N_2$-labelled *Trichodesmium* spp., and ammonium regeneration by the harpacticoid copepod *Macrosetella gracilis*, Mar. Biol., 125, 89–96, doi:10.1007/BF00350763, 1996.

Ohki, K.: Possible role of temperate phage in the regulation of *Trichodesmium* biomass: marine cyanobacteria, Bull. l'Institut océanographique, Monaco, Supplement, 287–291, 1999.

Paerl, H. W., Priscu, J. C., and Brawner, D. L.: Immunochemical localization of nitrogenase in marine *Trichodesmium* aggregates: relationship to N_2 fixation potential, Appl. Environ. Microb., 55, 2965–2975, 1989.

Ploug, H., Musat, N., Adam, B., Moraru, C. L., Lavik, G., Vagner, T., Bergman, B., and Kuypers, M. M. M.: Carbon and nitrogen fluxes associated with the cyanobacterium *Aphanizomenon* sp. in the Baltic Sea., ISME J., 4, 1215–1223, doi:10.1038/ismej.2010.53, 2010.

Polerecky, L., Adam, B., Milucka, J., Musat, N., Vagner, T., and Kuypers, M. M. M.: Look@NanoSIMS – a tool for the analysis of nanoSIMS data in environmental microbiology, Environ. Microbiol., 14, 1009–1023, doi:10.1111/j.1462-2920.2011.02681.x, 2012.

Pujo-Pay, M. and Raimbault, P.: improvement of the wet-oxidation procedure for simultaneous determination of particulate organic nitrogen and phosphorus collected on filters, Mar. Ecol.-Prog. Ser., 105, 203–207, doi10.3354/meps105203, 1994.

Raimbault, P. and Garcia, N.: Evidence for efficient regenerated production and dinitrogen fixation in nitrogen-deficient waters of the South Pacific Ocean: impact on new and export production estimates, Biogeosciences, 5, 323–338, doi:10.5194/bg-5-323-2008, 2008.

Redfield, A.: On the proportions of organic derivations in sea water and their relation to the composition of plankton, in: James Johnstone Memorial Volume, edited by: Daniel, R., University Press of Liverpool, 177–192, 1934.

Rochelle-Newall, E., Ridame, C., Dimier-Hugueney, C., and L'Helguen, S.: Impact of iron limitation on primary production (dissolved and particulate) and secondary production in cultured *Trichodesmium* sp., Aquat. Microb. Ecol., 72, 143–153, doi:10.3354/ame01690, 2014.

Rodier, M. and Le Borgne, R.: Population dynamics and environmental conditions affecting *Trichodesmium* spp. (filamentous cyanobacteria) blooms in the south–west lagoon of New Caledonia, J. Exp. Mar. Biol. Ecol., 358, 20–32, doi:10.1016/j.jembe.2008.01.016, 2008.

Romans, K. M., Carpenter, E. J., and Bergman, B.: Buoyancy regulation in the colonial diazotrophic cyanobacterium *Trichodesmium tenue*: ultrastructure and storage of carbohydrate, polyphosphate, and nitrogen, J. Phycol., 30, 935–942, doi:10.1111/j.0022-3646.1994.00935.x, 1994.

Sandh, G., Xu, L., and Bergman, B.: Diazocyte development in the marine diazotrophic cyanobacterium *Trichodesmium*, Microbiology, 158, 345–352, doi:10.1099/mic.0.051268-0, 2012.

Sheridan, C. C., Steinberg, D. K., and Kling, G. W.: The microbial and metazoan community associated with colonies of *Trichodesmium* spp.: a quantitative survey, J. Plankton Res., 24, 913–922, doi:10.1093/plankt/24.9.913, 2002.

Shiozaki, T., Kodama, T., and Furuya, K.: Large-scale impact of the island mass effect through nitrogen fixation in the western South Pacific Ocean, Geophys. Res. Lett., 41, 2907–2913, doi:10.1002/2014GL059835, 2014.

Sieracki, M. E., Haugen, E. M., and Cucci, T. L.: Overestimation of heterotrophic bacteria in the Sargasso Sea: direct evidence by

flow and imaging cytometry, Deep-Sea Res. Pt. I, 42, 1399–1409, doi:10.1016/0967-0637(95)00055-B, 1995.

Slawyk, G. and Raimbault, P.: Simple procedure for simultaneous recovery of dissolved inorganic and organic nitrogen in ^{15}N-tracer experiments and improving the isotopic mass balance, Mar. Ecol.-Prog. Ser., 124, 289–299, doi:10.3354/meps124289, 1995.

Smayda, T. J.: From phytoplankton to biomass, in: Phytoplankton Manual, edited by: Sournia, A., Monographs on Oceanographic Methodology 6, UNESCO, Paris, 273–279, 1978.

Smetacek, V.: Biological oceanography: diatoms and the silicate factor, Nature, 391, 224–225, doi:10.1038/34528, 1998.

Sohm, J. A., Edwards, B. R., Wilson, B. G., and Webb, E. A.: Constitutive extracellular eolysaccharide (EPS) production by specific isolates of *Crocosphaera watsonii*, Front. Microbiol., 2, 229, doi:10.3389/fmicb.2011.00229, 2011.

Subramaniam, A., Yager, P. L., Carpenter, E. J., Mahaffey, C., Björkman, K. M., Cooley, S., Kustka, A. B., Montoya, J. P., Sañudo-Wilhelmy, S. A., Shipe, R., and Capone, D. G.: Amazon River enhances diazotrophy and carbon sequestration in the tropical North Atlantic Ocean, P. Natl. Acad. Sci., 105, 10460–10465, doi:10.1073/pnas.0710279105, 2008.

Sun, J. and Liu, D.: Geometric models for calculating cell biovolume and surface area for phytoplankton, J. Plankton Res., 25, 1331–1346, doi:10.1093/plankt/fbg096, 2003.

Tseng, Y. F., Lin, F. J., Chiang, K. P., Kao, S. J., and Shiah, F. K.: Potential impacts of N_2-fixing *Trichodesmium* on heterotrophic bacterioplankton turnover rates and organic carbon transfer efficiency in the subtropical oligotrophic ocean system, Terr. Atmos. Ocean. Sci., 16, 361–376, 2005.

Turk-Kubo, K. A., Frank, I. E., Hogan, M. E., Desnues, A., Bonnet, S., and Zehr, J. P.: Diazotroph community succession during the VAHINE mesocosm experiment (New Caledonia lagoon), Biogeosciences, 12, 7435–7452, doi:10.5194/bg-12-7435-2015, 2015.

Walsby, A. E.: The gas vesicles and buoyancy of *Trichodesmium*, in: Marine Pelagic Cyanobacteria: *Trichodesmium* and other Diazotrophs, vol. 362, edited by: Carpenter, E. J., Capone, D. G., and Rueter, J. G., Springer, Dordrecht, the Netherlands, 141–161, 1992.

Wannicke, N., Korth, F., Liskow, I., and Voss, M.: Incorporation of diazotrophic fixed N_2 by mesozooplankton – case studies in the southern Baltic Sea, J. Marine Syst., 117, 1–13, doi:10.1016/j.jmarsys.2013.03.005, 2013.

Webb, E. A., Ehrenreich, I. M., Brown, S. L., Valois, F. W., and Waterbury, J. B.: Phenotypic and genotypic characterization of multiple strains of the diazotrophic cyanobacterium, *Crocosphaera watsonii*, isolated from the open ocean, Environ. Microbiol., 11, 338–348, doi:10.1111/j.1462-2920.2008.01771.x, 2009.

Wilkerson, F. P., Dugdale, R. C., Kudela, R. M., and Chavez, F. P.: Biomass and productivity in Monterey Bay, California: contribution of the large phytoplankton, Deep-Sea Res. Pt. II, 47, 1003–1022, doi:10.1016/S0967-0645(99)00134-4, 2000.

Yeung, L. Y., Berelson, W. M., Young, E. D., Prokopenko, M. G., Rollins, N., Coles, V. J., Montoya, J. P., Carpenter, E. J., Steinberg, D. K., Foster, R. A., Capone, D. G., and Yager, P. L.: Impact of diatom-diazotroph associations on carbon export in the Amazon River plume, Geophys. Res. Lett., 39, 1–6, doi:10.1029/2012GL053356, 2012.

Yool, A., Martin, A. P., Fernandez, C., and Clark, D. R.: The significance of nitrification for oceanic new production, Nature, 447, 999–1002, doi:10.1038/nature05885, 2007.

Zehr, J. P., Mellon, M. T., and Zani, S.: New nitrogen-fixing microorganisms detected in oligotrophic oceans by amplification of nitrogenase (*nif*H) genes, Appl. Environ. Microb., 64, 3444–3450, 1998.

Zehr, J. P., Waterbury, J. B., Turner, P. J., Montoya, J. P., Omoregie, E., Steward, G. F., Hansen, A., and Karl, D. M.: Unicellular cyanobacteria fix N_2 in the subtropical North Pacific Ocean, Nature, 412, 635–638, doi:10.1038/35088063, 2001.