

1 **Dynamics of N₂ fixation and fate of diazotroph-derived**
2 **nitrogen in a Low Nutrient Low Chlorophyll ecosystem:**
3 **results from the VAHINE mesocosm experiment (New**
4 **Caledonia)**

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1 **Abstract**

2 N₂ fixation rates were measured daily in large (~50 m³) mesocosms deployed in the tropical
3 South West Pacific coastal ocean (New Caledonia) to investigate the temporal variability in N₂
4 fixation rates in relation with environmental parameters and study the fate of diazotroph-derived
5 nitrogen (DDN) in a low nutrient, low chlorophyll ecosystem. The mesocosms were fertilized
6 with ~0.8 μM dissolved inorganic phosphorus (DIP) to stimulate diazotrophy. Bulk N₂ fixation
7 rates were replicable between the three mesocosms, averaged 18.5±1.1 nmol N L⁻¹ d⁻¹ over the 23
8 days, and increased by a factor of two during the second half of the experiment (days 15 to 23) to
9 reach 27.3±1.0 nmol N L⁻¹ d⁻¹. These later rates measured after the DIP fertilization are higher
10 than the upper range reported for the global ocean. During the 23-days of the experiment, N₂
11 fixation rates were positively correlated with seawater temperature, primary production, bacterial
12 production, standing stocks of particulate organic carbon (POC), nitrogen (PON) and phosphorus
13 (POP), and alkaline phosphatase activity, and negatively correlated with DIP concentrations, DIP
14 turnover time, nitrate, and dissolved organic nitrogen and phosphorus concentrations. The fate of
15 DDN was investigated during a bloom of the unicellular diazotroph UCYN-C that occurred
16 during the second half of the experiment. Quantification of diazotrophs in the sediment traps
17 indicates that ~10 % of UCYN-C from the water column was exported daily to the traps,
18 representing as much as 22.4±5.5 % of the total POC exported at the height of the UCYN-C
19 bloom. This export was mainly due to the aggregation of small (5.7±0.8 μm) UCYN-C cells into
20 large (100-500 μm) aggregates. During the same time period, a DDN transfer experiment based
21 on high-resolution nanometer scale secondary ion mass spectrometry (nanoSIMS) coupled with
22 ¹⁵N₂ isotopic labelling revealed that 16±6 % of the DDN was released to the dissolved pool and
23 21±4 % was transferred to non-diazotrophic plankton, mainly picoplankton (18±4 %) followed
24 by diatoms (3±2 %). This is consistent with the observed dramatic increase in picoplankton and
25 diatom abundances, primary production, bacterial production, and standing stocks of POC, PON,
26 and POP during the second half of the experiment. These results offer insights into the fate of
27 DDN during a bloom of UCYN-C in low nutrient, low chlorophyll ecosystems.

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1 **1 Introduction**

2 Next to light, nitrogen (N) is the major limiting factor for primary productivity in much of the
3 low-latitude surface ocean (Falkowski, 1997; Moore et al., 2013). Nitrate (NO_3^-) is the dominant
4 form of fixed nitrogen (N) in seawater and derives from the remineralization of sinking organic N
5 in the dark ocean. NO_3^- is supplied to photic waters by upward mixing and transport, and
6 constitutes the main source of fixed N for photosynthetic organisms in the temperate and high
7 latitude ocean. In the oligotrophic tropical and subtropical oceans, vertical mixing and transport
8 of NO_3^- is generally low and surface waters are often depleted in NO_3^- .

9 In these ocean deserts, specialized organisms termed N_2 -fixers (or diazotrophs) are able to use N
10 in its simplest and most abundant form on Earth and in seawater, namely dinitrogen (N_2).
11 Diazotrophs possess the nitrogenase enzyme, which cleaves the strong triple bond of the N_2
12 molecule to form bioavailable ammonium (NH_4^+) which is assimilated as amino acids enabling
13 biomass growth and division. N_2 fixation thus introduces a source of new bioavailable N to
14 surface waters, and is considered to be the most important external source of N to the ocean,
15 more significant than atmospheric and riverine inputs (Gruber, 2004).

16 The dynamics of microbial communities such as diazotrophs can change abruptly in the ocean in
17 response to small perturbations or environmental stressors. In particular, N_2 fixation has been
18 described as a very ‘patchy’ process in the ocean (Bombar et al., 2015). Many factors control the
19 distribution and activity of diazotrophs such as temperature (Bonnet et al., 2015; Moisaner et al.,
20 2010; Raveh et al., 2015; Staal et al., 2003), nutrient availability (mainly phosphate and iron)
21 (e.g., (Mills et al., 2004)), $p\text{CO}_2$ ((e.g. (Levitan et al., 2007))), ambient concentrations of fixed N
22 (NO_3^- and NH_4^+) (e.g., (Knapp et al., 2012), as well as physical forcing (e.g., (Fong et al., 2008)).
23 Most studies dedicated to understanding the controls on marine N_2 fixation have been undertaken
24 along large oceanic transects; these are particularly valuable and have recently led to the
25 compilation of a global ocean database of diazotrophy (Luo et al., 2012). Spatial variability in N_2
26 fixation is thus far better documented and understood than temporal variability, despite the
27 intimate connections between time and space scales in the ocean. Time-series stations with near-
28 monthly observations set up in the late 1980’s under the international JGOFS program in the
29 subtropical North Atlantic, Pacific, and Mediterranean Sea have provided valuable data regarding
30 the controls on N_2 fixation and its role in biogeochemical cycles on seasonal and inter-annual
31 timescales (Dore et al., 2008; Garcia et al., 2006; Grabowski et al., 2008; Karl et al., 2012; Knapp

1 et al., 2005; Orcutt et al., 2001), and have also revealed novel diazotrophic microorganisms (Zehr
2 et al., 2008) with unexpected metabolic strategies such as UCYN-A cyanobacteria that lack the
3 oxygen-producing photosystem II complex (Tripp et al., 2010). However, fairly little attention
4 has been paid to sub-seasonal variability in N₂ fixation and its biogeochemical drivers and
5 consequences.

6 In the framework of the VAHINE (VARIability of vertical and tropHic transfer of diazotroph
7 derived N in the south wEst Pacific) project, we deployed three large volume mesocosms (~50
8 m³, Fig. 1) in the tropical South West Pacific coastal ocean, a region known to support
9 diazotrophy during the austral summer (Dupouy et al., 2000; Rodier and Le Borgne, 2010, 2008).
10 Our goal was to study the high frequency temporal dynamics of N₂ fixation over short time scales
11 (sampling every day for 23 days), in relation to hydrological parameters, biogeochemical stocks
12 and fluxes, and the dynamics of phytoplanktonic and bacterial communities in the same water
13 mass.

14 The mesocosm approach allowed us to investigate the fate of the recently fixed N₂ and its transfer
15 from diazotrophs to non-diazotrophic organisms in this oligotrophic marine ecosystem.
16 Diazotrophs can typically release from 10 to 50 % of their recently fixed N₂ (or diazotroph
17 derived N, hereafter called DDN) as dissolved organic N (DON) and NH₄⁺ (Glibert and Bronk,
18 1994; Meador et al., 2007; Mulholland et al., 2006). This exudate is potentially available for
19 assimilation by the surrounding planktonic communities. However, such transfer of DDN to the
20 surrounding planktonic community and its potential impact on export production is poorly
21 understood and rarely quantified.

22 Over the course of this 23-day mesocosm experiment, diatom-diazotroph associations (DDAs)
23 were the most abundant N₂ fixers during the first half of the experiment (days 2 to 14), while a
24 bloom of the unicellular N₂-fixing cyanobacteria from Group C (UCYN-C) occurred during the
25 second half of the experiment (days 15 to 23) (Turk-Kubo et al., 2015). Berthelot et al. (2015b)
26 described the evolution of the C, N, and P pools and fluxes during the experiment and
27 investigated the contribution of N₂ fixation and DON uptake to primary production and particle
28 export. They also explored the fate of the freshly produced particulate organic N (PON), i.e.,
29 whether it was preferentially accumulated and recycled in the water column or exported out of
30 the system. Complementary to this approach, Knapp et al. (2015) report the results of a δ¹⁵N
31 budget performed in the mesocosms to assess the dominant source of N (i.e., NO₃⁻ versus N₂

1 fixation) fueling export production during the 23-day experiment. In the present study, we focus
2 specifically on the fate of DDN in the ecosystem during the UCYN-C bloom by studying i) the
3 direct export of diazotrophs into the sediment traps, and ii) the transfer of DDN to non-
4 diazotrophic plankton using high-resolution nanometer scale secondary ion mass spectrometry
5 (nanoSIMS) coupled with $^{15}\text{N}_2$ isotopic labelling during a 72 h-process experiment.

6 7 **2 Methods**

8 9 **2.1 Mesocosm description and sampling strategy**

10 Three replicate large-volume mesocosms (surface 4.15 m^2 , volume $\sim 50 \text{ m}^3$, Fig. 1) were
11 deployed in the oligotrophic New Caledonian lagoon, 28 km off the coast of Noumea (latitude:
12 $22^\circ 28,855' \text{ S}$; longitude: $166^\circ 26,724' \text{ E}$) from January 13th to February 6th 2013. They consisted
13 of large enclosures open to the air made of two $500 \text{ }\mu\text{m}$ -thick films of polyethylene (PE) and
14 vinyl acetate (EVA, 19 %), with nylon meshing in between to allow for maximum resistance and
15 light penetration (produced by HAIKONENE KY, Finland). The mesocosm bags were 2.3 m in
16 diameter and 15 m in height, and were equipped with removable sediment traps that enabled the
17 collection of sinking material once a day (Fig. 1b). To alleviate any potential phosphorus
18 limitation of diazotrophy in the mesocosms, the bags were intentionally fertilized with $\sim 0.8 \text{ }\mu\text{mol}$
19 L^{-1} of dissolved inorganic phosphorus (DIP) four days after the start of the experiment. A more
20 detailed description of the mesocosms setup, the selection of the study site, and the deployment
21 strategy can be found in Bonnet et al. (2016).

22 Vertical CTD profiles were performed every morning in each of the three mesocosms (hereafter
23 referred to as M1, M2, and M3) and in the surrounding waters (hereafter referred to as lagoon
24 waters) using a SBE Seabird CTD. All discrete samples for the parameters described below were
25 collected daily at 7 am at three depths (1, 6, and 12 m) in each mesocosm and in the lagoon
26 waters using braided PVC tubing (Holzelock-Tricoflex, inner diameter = 9.5 mm) connected to a
27 Teflon PFA pump (St-Gobain Performance Plastics) activated by pressurized air. Finally,
28 sediment trap samples were collected daily from each mesocosm by SCUBA divers.

1 2.2 Experimental procedures

2 2.2.1 N₂ fixation measurements within the mesocosms and methods 3 intercomparison

4 Seawater samples for N₂ fixation rate measurements were dispensed into HCl-washed 4.5 L
5 polycarbonate bottles that were sealed with septa and amended with ¹⁵N₂-enriched seawater
6 (Mohr et al., 2010; Wilson et al., 2012), hereafter called the ¹⁵N₂ dissolution method. Briefly, the
7 ¹⁵N₂-enriched seawater was prepared from 0.2 µm-filtered seawater (Sartobrand (Sartorius)
8 cartridges) collected from the same site in a 4.5 L polycarbonate HCl-washed bottle. Seawater
9 was first degassed through a degassing membrane (Membrana, Minimodule®, flow rate fixed at
10 450 mL min⁻¹) connected to a vacuum pump (<200 mbar) for at least 1 h. The bottle was then
11 closed with a septum cap and amended with 1 mL of ¹⁵N₂ (98.9 atom% ¹⁵N, Cambridge Isotopes
12 Laboratories, Inc) per 100 mL of seawater. The bottle was shaken vigorously to fragment the ¹⁵N₂
13 bubble, and incubated overnight at 20 m depth at the study site (3 bars) to promote ¹⁵N₂
14 dissolution. The experimental bottles were amended with 5 % vol:vol ¹⁵N₂ enriched seawater
15 (i.e., 225 mL), sealed without headspace with silicon septum caps, and incubated for 24 h on an
16 *in situ* mooring line located close to the mesocosms at the appropriate sampling depth. After 24 h,
17 12 mL of the incubated seawater were subsampled into Exetainers®. These were preserved
18 upside down in the dark at 4 °C and analyzed less than 6 months after the experiment using a
19 Membrane Inlet Mass Spectrometer (MIMS) (Kana et al., 1994) to quantify the ¹⁵N enrichment of
20 the N₂ pool in the incubation bottles. The MIMS analyses yielded an average atom% ¹⁵N for the
21 N₂ pool of 2.4±0.2 (n=10). After collection of the Exetainer® subsamples, 2.2 L from each
22 experiment bottle were filtered under low vacuum pressure (<100 mm Hg) onto a pre-combusted
23 (4 h at 450 °C) GF/F filter (25 mm diameter, 0.7 µm nominal porosity) for ‘bulk’ N₂ fixation rate
24 determination. The remaining volume (2.2 L) was pre-filtered through a 10 µm pore-size
25 polycarbonate filter, and collected on a pre-combusted GF/F filter for analysis of the pico- and
26 nanoplanktonic (<10 µm) N₂ fixation rates. Filters were stored at -20 °C until the end of the
27 VAHINE experiment, then dried for 24 h at 60 °C before mass spectrometric analysis (see
28 section ‘Mass spectrometry analyses’ below). Every day, an extra 2.2 L bottle was filled with
29 mesocosm surface water (from ~1 m), spiked with ¹⁵N₂, and immediately filtered to determine
30 the natural ¹⁵N enrichment of the PON, which is required for calculations of N₂ fixation rates.

1 In the present study, we decided to use the $^{15}\text{N}_2$ dissolution method to measure N_2 fixation rates
2 as several authors (Großkopf et al., 2012; Mohr et al., 2010; Rahav et al., 2013; Wilson et al.,
3 2012) have reported an underestimation of rates when using the bubble method (i.e., when the
4 $^{15}\text{N}_2$ gas is injected directly in the incubation bottle using a syringe, see below) due to incomplete
5 equilibration of the $^{15}\text{N}_2$ gas between the headspace and the seawater in the incubation bottles
6 compared to theoretical calculations. However, the differences observed between the two
7 methods appear to depend on the environmental conditions (Shiozaki et al., 2015). Here, we
8 performed an inter-comparison of both methods on day 11 in surface waters (from ~1 m)
9 collected from M1. Briefly, seawater samples from M1 were dispensed into twelve HCl-washed
10 4.5 L polycarbonate bottles as described above and closed with septum caps. Six bottles were
11 spiked with 4 mL $^{15}\text{N}_2$ (98.9 atom% ^{15}N , Cambridge isotopes Laboratories, Inc) via a gas-tight
12 syringe, hereafter called the bubble method. Each bottle was shaken 20 times to fragment the $^{15}\text{N}_2$
13 bubble and facilitate its dissolution. The six remaining bottles were treated as described above for
14 the dissolution method. All twelve bottles were then incubated for 24 h in an on-deck incubator at
15 irradiances corresponding to the sampling depth using screening, and cooled with circulating
16 surface seawater.

17 A recent study (Dabundo et al., 2014) reports potential contamination of some commercial $^{15}\text{N}_2$
18 gas stocks with ^{15}N -enriched NH_4^+ , NO_3^- and/or nitrite (NO_2^-), and nitrous oxide (N_2O). Dabundo
19 et al. (2014) analysed various brands of $^{15}\text{N}_2$ gas and found that the Cambridge Isotopes stock
20 (i.e., the one used in this study) contained low concentrations of ^{15}N contaminants, and the
21 potential overestimation of N_2 fixation rates modeled using this contamination level would range
22 from undetectable to $0.02 \text{ nmol N L}^{-1} \text{ d}^{-1}$. The rates measured in this study ranged from 0.5 to
23 $69.6 \text{ nmol N L}^{-1} \text{ d}^{-1}$ suggesting that, if present, stock contamination of the magnitude reported by
24 (Dabundo et al., 2014) would be too low to affect the results described here. To verify this, one of
25 our $^{15}\text{N}_2$ Cambridge Isotopes batches (18/061501) was checked for contamination following the
26 method described in Dabundo et al. (2014); it was $1.4 \times 10^{-8} \text{ mol of } ^{15}\text{NO}_3^- \text{ per mol of } ^{15}\text{N}_2$ and
27 $1.1 \times 10^{-8} \text{ mol NH}_4^+ \text{ per mol of } ^{15}\text{N}_2$. The application of this contamination level to our samples
28 using the model provided by Dabundo et al. (2014) indicates that our rates may only be
29 overestimated by ~0.05 %, confirming that our present results were unaffected by possible $^{15}\text{N}_2$
30 stock contamination.

2.2.2 Phenotypic characterization of UCYN in the water column and the sediment traps

To investigate the direct export of UCYN-C cells during the bloom of UCYN-C that occurred in the second half of the experiment, a detailed phenotypic characterization of UCYN-C was performed at the height of the bloom (days 17 and 19), both in the water column and in the sediment traps. In parallel, UCYN-C and other diazotroph phylotypes were quantified in the sediment traps on days 17 and 19 (analytical protocols are detailed below in section 2.3).

Seawater samples for microscopic analyses were collected every day from 1, 6, and 12 m in each mesocosm in 4.5 L polycarbonate bottles as described above. Samples were immediately filtered onto 2 μm 47 mm polycarbonate filters that were fixed with paraformaldehyde (4 % final concentration) and incubated for 15 minutes at room temperature, then stored at $-80\text{ }^{\circ}\text{C}$ until microscopic analysis. Formalin-fixed sediment trap samples were homogenized and 2 ml were filtered onto 2 μm polycarbonate filters for further microscopic analyses. To characterize the phenotype of UCYN (free living cells *versus* colonies) in the mesocosms as a function of depth, we performed a detailed microscopic analysis on days 17 and 19 in M2. Note that UCYN-A cannot be observed by standard epifluorescent microscopy. Filtered samples from each depth (1, 6, and 12 m) and from the sediment traps ($\sim 15\text{ m}$) were visualized using a Zeiss Axioplan (Zeiss, Jena, Germany) epifluorescence microscope fitted with a green (510-560 nm) excitation filter, which targeted the UCYN phycoerythrin-rich cells. For each filter, 47 photographs of various sections of the filter were taken at random. Each fluorescent particle was automatically delimited as a region of interest (ROI) using an in-house imageJ script. The photographs were scanned visually to remove ROIs that did not correspond to UCYN cells or UCYN aggregated cells. The area of each ROI was converted to equivalent volume assuming a spherical shape for all the aggregates. The volume of individual cells was determined from the average volume of the ROI represented by only one cell. The resultant cell volume was then used to compute the number of cells in each aggregate.

2.2.3 DDN transfer experiment

The fate of the fixed N_2 during the UCYN-C bloom (that occurred from days 15 to 23) was investigated on days 17 to 20 in M2 at 6 m. In addition to N_2 fixation measurements, seawater was sampled as described above into twelve additional 4.5 L HCl-washed polycarbonate bottles

1 equipped with septum caps. Full bottles were immediately amended with the dissolved $^{15}\text{N}_2$ gas
2 (98.9 atom% ^{15}N , Cambridge Isotopes Laboratories, Inc) as described above (dissolution
3 method), and with 1 mL of $80 \text{ g L}^{-1} \text{ NaH}^{13}\text{CO}_3$ solution (99 atom% ^{13}C , Cambridge Isotopes
4 Laboratories, Inc) and incubated *in situ* on the mooring line at 6 m-depth close to the mesocosms.
5 After 24 h, 36 h, and 72 h of incubation (hereafter referred to as T24 h, T36 h, and T72 h), three
6 replicate $^{15}\text{N}_2$ labelled bottles were recovered from the mooring line and subsampled for the
7 analysis of bulk N_2 fixation rates, DDN released to the dissolved pool, abundance of targeted
8 diazotrophs using qPCR, picophytoplankton and bacterial counts, and nanoSIMS analyses on
9 UCYN-C and non-diazotrophs (diatoms and the $0.2\text{-}2 \mu\text{m}$ fraction) to assess the DD^{15}N transfer
10 from diazotrophs to non-diazotrophs. All analytical protocols are detailed below in section 2.3.
11 Three 4.5 L bottles were kept as unamended controls (i.e., without $^{15}\text{N}_2$ addition) and were
12 immediately subsampled for the same parameters.

13

14 **2.3 Analytical protocols**

15 **2.3.1 Mass spectrometry analyses**

16 PON content and PON ^{15}N enrichment of samples collected for N_2 fixation rates determination
17 were measured using a Delta Plus Thermo Fisher Scientific isotope ratio mass spectrometer
18 (Bremen, Germany) coupled with an elemental analyzer (Flash EA, ThermoFisher Scientific). N_2
19 fixation rates were calculated according to the equations detailed in Montoya et al. (1996). Rates
20 were considered significant when the ^{15}N enrichment of the PON was higher than three times the
21 standard deviation obtained from T0 samples. The standard deviation was $0.004 \mu\text{mol L}^{-1}$ for
22 PON and 0.0001 atom% for the ^{15}N enrichment.

23

24 **2.3.2 Quantification of diazotrophs using qPCR in sediment traps and during the** 25 **DDN transfer experiment**

26 During the bloom of UCYN-C (days 17 and 19), immediately after sediment trap samples were
27 collected and prior to their fixation with formalin, trap material was homogenized and fresh
28 aliquots of 1 mL were subsampled from each jar (trap from M1, M2, and M3) and filtered onto
29 $0.2 \mu\text{m}$ Supor (Pall-Gelman) filters, flash frozen in liquid N_2 , and stored at $-80 \text{ }^\circ\text{C}$ until analysis.
30 For the DDN transfer experiment, after each incubation period, 2 L from each triplicate ^{13}C and
31 $^{15}\text{N}_2$ -labeled 4.5 L bottle were subsampled and filtered through $0.2 \mu\text{m}$ Supor (Pall-Gelman)

1 filters using gentle peristaltic pumping, and stored as described above. The abundance of eight
2 diazotrophic phylotypes was determined using Taqman® qPCR assays: unicellular
3 cyanobacterial groups A1 (UCYN-A1; (Church et al., 2005)), A2 (UCYN-A2; (Thompson et al.,
4 2014)), B (UCYN-B or *Crocospaera* spp.; (Moisander et al., 2010)), and C (UCYN-C; (Foster
5 et al., 2007)), the filamentous, colonial cyanobacteria *Trichodesmium* spp. (Church et al., 2005),
6 the two DDAs *Richelia* associated with both *Rhizosolenia* (het-1; (Church et al., 2005)) and
7 *Hemiaulus* (het-2; (Foster et al., 2007)) diatoms, *Calothrix* associated with *Chaetoceros* (het-3;
8 (Foster et al., 2007)), as well as a heterotrophic phylotype of gamma proteobacteria (γ -24474A11;
9 (Moisander et al., 2008)). All procedures are described extensively in the companion paper by
10 (Turk-Kubo et al., 2015). Briefly, DNA was extracted using a Qiagen DNeasy kit with
11 modifications to recover high quality genomic DNA from cyanobacteria including a freeze thaw
12 step, agitation and a proteinase K digestion. Extracts were tested for the presences of PCR
13 inhibitors, compounds sometimes present in DNA extracts from the environment or introduced in
14 the extraction process that reduce PCR efficiency, using either the UCYN-B or the UCYN-C
15 assay. If recovery of the spiked standard template in the sample extract was <98%, the sample
16 was considered inhibited, and diluted 1:10 with 5 kD filtered milliQ water. All extracts from the
17 sediment traps showed inhibition when undiluted, and no inhibition when diluted 1:10. DNA
18 extracts from the DDN transfer experiment showed no inhibition. All qPCR reactions were
19 carried out on diluted extracts as described in (Goebel et al., 2010). The limit of detection (LOD)
20 and limit of quantitation (LOQ) was 250 and 2000 *nifH* copies mL⁻¹, respectively, for the
21 sediment trap samples. The LOD and LOQ for DDN transfer experiment samples was 29 and 229
22 *nifH* copies L⁻¹, respectively.

23 24 **2.3.3 Quantification of the net release of DDN to the dissolved pool during the** 25 **DDN transfer experiment**

26 After each incubation period, 60 mL from each ¹⁵N₂-labeled 4.5 L bottle were subsampled and
27 filtered through pre-combusted (4 h, 450 °C) GF/F filters and immediately frozen for later
28 quantification of ¹⁵N release (i.e., DDN release) to the total dissolved N pool (TDN; i.e., the sum
29 of NO₂⁻, NO₃⁻, NH₄⁺, and DON). The dissolved N was oxidized to NO₃⁻ using the persulfate
30 oxidation method of Knapp et al. (2005) with the amendments of Fawcett et al. (2011). Briefly, 1
31 mL of potassium persulfate oxidizing reagent (POR) was added to duplicate 5 mL aliquots of

1 each subsample in 12 mL pre-combusted glass Wheaton vials, and to triplicate vials containing
2 varying quantities of two L-glutamic acid standards, USGS-40 and USGS-41 (Qi et al., 2003)
3 used to ensure complete oxidation and quantify the POR-associated N blank. The POR was made
4 by dissolving 6 g of sodium hydroxide and 6 g of four-times recrystallized, methanol-rinsed
5 potassium persulfate in 100 mL of ultra-high purity water (DIW). Sample vials were capped
6 tightly after POR addition, and autoclaved at 121°C for 55 minutes on a slow-vent setting. The
7 entire oxidation protocol was performed in duplicate (yielding a total of 4 oxidized aliquots for
8 each subsample).

9 The concentration of the resultant NO_3^- (i.e., TDN + the POR-associated N blank) was measured
10 by chemiluminescence (Braman and Hendrix, 1989), after which the TDN isotopic composition
11 was determined using the ‘denitrifier method’, wherein denitrifying bacteria that lack N_2O
12 reductase quantitatively convert sample NO_3^- to N_2O (Casciotti et al., 2002; Sigman et al., 2001).
13 The denitrifying bacteria (see below) are extremely sensitive to pH; care was thus taken to lower
14 sample pH to 7-8 after POR oxidation via the addition of 12N ACS grade HCl. The ^{15}N
15 enrichment of the N_2O was measured by GC-IRMS using a Delta V isotope ratio mass
16 spectrometer and custom-built on-line N_2O extraction and purification system. The international
17 reference materials, IAEA-N3, USGS-34, USGS-32, and an in-house N_2O standard were run in
18 parallel to monitor bacterial conversion and mass spectrometry, and each oxidized sample was
19 analyzed twice. The final TDN concentration and ^{15}N atom% were corrected for the N blank
20 associated with the POR. The DDN released to the TDN pool was calculated according to: ^{15}N
21 release ($\text{nmol L}^{-1} \text{d}^{-1}$) = $(^{15}\text{N}_{\text{ex}} \times \text{TDN}_{\text{con}}) / \text{N}_{\text{sr}}$, where $^{15}\text{N}_{\text{ex}}$ is the atom% excess of the TDN for a
22 given time point; the TDN_{con} is the TDN concentration measured at each time point, and N_{sr} is
23 the ^{15}N enrichment of the source pool (N_2) in the experimental bottles (i.e., 2.4 ± 0.2 atom% ^{15}N ;
24 see above).

25

26 **2.3.4 Picophytoplankton and bacteria counts during the DDN transfer experiment**

27 After each incubation period, 3.6 mL from each $^{15}\text{N}_2$ -labeled 4.5 L bottle were subsampled into
28 cryotubes, fixed with paraformaldehyde (2 % final concentration), flash frozen in liquid N_2 , and
29 stored at -80°C until analysis. Picoplankton analyses were carried out at the PRECYM flow
30 cytometry platform (<https://precym.mio.univ-amu.fr/>). Samples were analyzed using a
31 FACSCalibur (BD Biosciences, San Jose, CA). For heterotrophic bacterial abundance (BA), 1.8

1 mL of seawater was fixed with formaldehyde (2 % final concentration, 15 minutes incubation at room temperature in the dark), frozen and stored in liquid N₂ until analysis in the laboratory. After thawing, 0.3 mL of each samples was incubated with SYBR Green II (Molecular Probes, final conc. 0.05 % [v / v], for 15 minutes at room temperature in the dark), for the nucleic acid staining, according to Marie et al. (2000). Cells were characterized by 2 main optical signals: side scatter (SSC), related to cell size, and green fluorescence (530/40), related to nucleic acids staining. Based on these criteria, two subsets of bacteria (referred to low- and high nucleic acid-containing, or LNA and HNA, respectively) were optically resolved in all samples based on their green fluorescence intensity (Gasol et al., 1999). Just before analysis, 2 µm beads (Fluoresbrite YG, Polyscience), used as an internal control, and TruCount beads (BD Biosciences), used to determine the volume analyzed, were added to the samples. To assess autotrophic picoplankton abundances, the red fluorescence (670LP, related to chlorophyll *a* content) was used as trigger signal and phytoplankton cells were characterized by 3 other optical signals: forward scatter (FSC, related to cell size), side scatter (SSC, related to cell structure), and the orange fluorescence (580/30, related to phycoerythrin content). The 2 µm beads (Fluoresbrite YG, Polyscience) were also used to discriminate picoplankton (< 2 µm) from nanoplankton (> 2 µm) populations. The flow rate was estimated by weighing 3 tubes of samples before and after a 3 minutes run of the cytometer. The cells concentration was determined from both TruCount beads and flow rate measurements. 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). on a FACScalibur flow cytometer (BD Biosciences, Franklin Lakes, NJ) at the Regional Flow Cytometry Platform for Microbiology (PRECYM) (<https://precym.mio.univ-amu.fr/>). Standard protocols (Marie et al., 1999) were used to enumerate phytoplankton and heterotrophic prokaryotes. Samples were thawed at room temperature in the dark, homogenized by gentle shaking, and filtered through 20 µm strainers in order to avoid large aggregates clogging the instrument fluidics. Just before analysis, 1 mL of sample was transferred into a flow cytometry tube, and 10 µL of a 2 µm fluorosphere (Fluoresbryte™, Polysciences) solution were added. These beads were used both as an internal control, and to discriminate cell clusters. Flow cytometric analyses of heterotrophic prokaryotes required pre-staining with a fluorescent nucleic acid probe, SYBR® Green I (Sigma, Germany), at a 1:1000 v/v final dilution of the commercial solution (excitation 488 nm/emission 530 nm). Prior to analysis, samples were incubated with

1 SYBR® Green I for 15 minutes in the dark at room temperature. Side scatter (SSC) was used as
2 trigger signal and SYBR® Green I green fluorescence was collected in the green range of 510-
3 550 nm. Combining SYBR® Green I fluorescence and light scattering unambiguously
4 distinguishes cells from inorganic particles, detritus, and free DNA (Marie et al., 1999).

6 **2.3.5 Microscopic cell counts during the DDN transfer experiment**

7 *Microscopic cell counts.* In parallel with the picoplankton counts, diatoms, dinoflagellates, and
8 ciliates were enumerated from 100 mL subsamples collected from each mesocosm that were
9 preserved in Lugol's solution following the Utermöhl method (Hasle, 1978). Cells were counted
10 on a Nikon Eclipse TE2000-E inverted microscope equipped with phase-contrast and a long
11 distance condenser. All groups were quantified in each sample, and diatoms were identified to the
12 lowest possible taxonomic level to examine potential community composition changes and help
13 us to prioritize nanoSIMS analyses.

15 **2.3.6 NanoSIMS analyses and ¹³C and ¹⁵N assimilation rates during the DDN** 16 **transfer experiment**

17 *nanoSIMS analyses.* After each incubation period (24, 36 and 72 h), 250 mL from each labeled
18 4.5 L bottle were subsampled, fixed with 25 mL of paraformaldehyde (2 % final concentration)
19 and incubated for 24 h at 4 °C, then filtered successively through 25 mm diameter 10 µm, 2 µm,
20 and 0.2 µm pore size polycarbonate filters and rinsed with 0.2 µm filtered seawater. All filters
21 were then sputtered with gold and palladium to ensure conductivity prior to nanoSIMS analyses.
22 Diatoms and UCYN-C were analysed on the 10 µm filters, and the picoplanktonic (0.2-2 µm)
23 fraction was analysed on the 0.2 µm filters. Diatoms were easily recognized on the CCD (charge
24 coupled device) camera of the nanoSIMS, as were UCYN-C that formed large aggregates of
25 cells, facilitating their recognition for nanoSIMS targeted analyses. However, we cannot exclude
26 the possibility that some UCYN-B were analysed, despite being present at very low abundances,
27 i.e., almost two orders of magnitude less abundant than UCYN-C (Fig. 5) in the analysed
28 samples. Several analyses were performed for each group of cells of interest (an average of ~25
29 cells analysed for UCYN-C and diatoms, and between 62 and 140 cells analysed for the 0.2-2 µm
30 fraction per time point) to assess the variability of their isotopic composition. A total of ~400
31 individual cells were analysed by nanoSIMS in this experiment to ensure the robustness of the

1 data. NanoSIMS analyses were performed on a N50 (Cameca, Gennevilliers France) at the
2 French National Ion MicroProbe Facility according to methods previously described (Bonnet et
3 al., Accepted). A 1.3-3 pA 16 keV Cesium (Cs^+) primary beam focused onto a ~ 100 nm spot
4 diameter was scanned on a 256 x 256 or 512 x 512 pixel raster (depending on the raster areas,
5 which ranged from 15 μm x 15 μm to 50 μm x 50 μm) with a counting time of 1 ms per pixel.
6 Samples were implanted with Cs^+ prior to analysis to remove surface contaminants and increase
7 conductivity. For diatoms, the pre-implant was longer and with higher voltage (2-5 min, 17 pA)
8 to penetrate the silica shell. Negative secondary ions $^{12}\text{C}^-$, $^{13}\text{C}^-$, $^{12}\text{C}^{14}\text{N}^-$, $^{12}\text{C}^{15}\text{N}^-$, and $^{28}\text{Si}^-$ were
9 detected with electron multiplier detectors, and secondary electrons were imaged simultaneously.
10 Ten to fifty serial quantitative secondary ion mass planes were generated and accumulated in the
11 final image. Mass resolving power was ~ 8000 in order to resolve isobaric interferences. Data
12 were processed using the look@nanosims software package (Polerecky et al., 2012). All scans
13 were first corrected for any drift of the beam during acquisition, and C and N isotope ratio images
14 were created by adding the secondary ion counts for each recorded secondary ion for each pixel
15 over all recorded planes and dividing the total counts by the total counts of a selected reference
16 mass. Individual cells were easily identified in nanoSIMS secondary electron, $^{12}\text{C}^-$, $^{12}\text{C}^{14}\text{N}^-$, and
17 $^{28}\text{Si}^-$ images that were used to define regions of interest (ROI) around individual cells (^{28}Si data
18 are not presented here). For each ROI, the ^{15}N and ^{13}C enrichments were calculated.
19 ^{15}N assimilation rates were calculated for individual cells analysed by nanoSIMS. Our goal was
20 to determine the biological compartment to which the ^{15}N had been transferred. These were
21 performed after 24 h of incubation. Calculations were performed as follows (Foster et al., 2011;
22 Foster et al., 2013): Assimilation ($\text{mol N cell}^{-1} \text{d}^{-1}$) = $(^{15}\text{N}_{\text{ex}} \times \text{N}_{\text{con}}) / \text{N}_{\text{sr}}$, where $^{15}\text{N}_{\text{ex}}$ is the excess
23 atom% of the individual cells measured by nanoSIMS after 24 h of incubation; the N_{con} is the N
24 content of each cell determined as described below, and N_{sr} is the ^{15}N enrichment of the source
25 pool (N_2) in the experimental bottles (i.e. 2.4 ± 0.2 atom% ^{15}N in this experiment). The cell-
26 specific N assimilation rate was then multiplied by the cell number enumerated for each group of
27 phytoplankton and bacteria by microscopy and flow cytometry. Standard deviations were
28 calculated using the variability of ^{15}N enrichment measured by nanoSIMS on replicate cells and
29 the standard deviation of the estimated cellular N content (see below) of UCYN-C, non-
30 diazotrophic phytoplankton, and bacteria. Final standard deviations were calculated according to
31 propagation of errors laws.

1 To determine the N_{con} of diatoms, cell cross section, apical and transapical dimensions were
2 measured on the dominant diatom species present in the mesocosms and analysed by nanoSIMS
3 to calculate biovolumes. All dimensions were measured on at least 20 cells using a Nikon Eclipse
4 TE2000-E inverted microscope equipped with phase-contrast and a long distance condenser.
5 Dimensions were entered into the international diatom data base (Leblanc et al., 2012) in which
6 bio-volumes are calculated following the geometric model of each cell type as described in (Sun
7 and Liu, 2003). Carbon (C) content (C_{con}) was then calculated for the species of interest using the
8 equations of (Eppley et al., 1970) and (Smayda, 1978). For *Synechococcus* spp. and
9 picoeukaryotes, we used C_{con} data from Fu et al. (2007) (249 ± 21 fg C cell⁻¹) and Yentsch and
10 Phinney (1985) (2100 fg cell⁻¹), respectively. C_{con} was then converted to N_{con} using the Redfield
11 ratio of 6.6:1 (Redfield, 1934). For bacteria, an average N_{con} of 5.8 ± 1.5 fg N cell⁻¹ (Fukuda et al.,
12 1998) was used. For UCYN-C, cell dimensions were measured and the bio-volume was
13 calculated based on the equations reported in Sun and Liu (2003). C_{con} was then calculated using
14 the relationship between bio-volume and C_{con} (Verity et al., 1992) (22 pg cell⁻¹). C_{con} was then
15 converted to N_{con} (2.3 pg cell⁻¹) using a ratio of 8.5:1 (Berthelot et al., 2015a).

16

17 **2.4 Statistical analyses**

18 Spearman correlation coefficients were used to examine the relationships between N_2 fixation
19 rates, hydrological, biogeochemical, and biological variables in the mesocosms (n=57 to 61,
20 $\alpha=0.05$). The methods used to analyze the parameters reported in the correlation table are
21 described in detail in companion papers in this issue (Berthelot et al., 2015b; Bonnet et al., 2016;
22 Leblanc et al., 2016; Turk-Kubo et al., 2015).

23 A non-parametric Mann-Whitney test ($\alpha=0.05$) was used to compare the means of N_2 fixation
24 rates obtained using the dissolution and the bubble method, as well as to compare the means of
25 N_2 fixation between the different phases of the experiment, mean isotopic ratios between ¹⁵N₂-
26 enriched and natural abundance of N (0.366 atom%), and mean isotopic ratios between T24 h and
27 T72 h in the DDN transfer experiment.

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1 **3 Results**

3 **3.1 N₂ fixation rates in the mesocosms**

4 Bulk N₂ fixation rates averaged 18.5±1.1 nmol N L⁻¹ d⁻¹ throughout the 23 days of the experiment
5 in the three mesocosms (all depths averaged together) (Table 1). The variance between the three
6 mesocosms was low, and the temporal dynamics of the rates were similar (Fig. 2, Table 1),
7 indicating good replicability between the mesocosms. Based on our data on N₂ fixation
8 dynamics, we could identify three main periods during the experiments. These three periods were
9 also defined by Berthelot et al. (2015b) based on biogeochemical characteristics and by Turk-
10 Kubo et al. (2015) based on changes in abundances of targeted diazotrophs. During the first
11 period (P0; from day 2 to 4, i.e., prior to the DIP fertilization), the average bulk N₂ fixation rate
12 for the three mesocosms was 17.9±2.5 nmol N L⁻¹ d⁻¹ (Fig. 2a). These N₂ fixation rates decreased
13 significantly (p<0.05) by ~40 % from day 5 to ~15 (hereafter called P1) to 10.1±1.3 nmol N L⁻¹
14 d⁻¹, then increased significantly (p<0.05) from day 15 until the end of the experiment (day 15 to
15 23, hereafter called P2) to an average of 27.3±1.0 nmol N L⁻¹ d⁻¹ (Fig. 2a). Maximum rates were
16 reached during P2 (between days 18 and 21) with 69.7, 67.7 and 60.4 nmol N L⁻¹ d⁻¹ in M1 (12
17 m), M2 (6 m) and M3 (12 m), respectively. From day ~15 to 21, N₂ fixation rates were higher at
18 12 m depth than in the surface. The difference was significant in M2 and M3 (p<0.05), but not in
19 M1 (p>0.05). Size fractionation experiments indicate that 37±7 % of the measured N₂ fixation
20 was associated with the <10 μm size fraction (Fig. 2b), and N₂ fixation rates in this fraction
21 followed the same temporal trend as bulk N₂ fixation. These data indicate that for the experiment
22 as a whole, the majority (~63 %) of the N₂ fixation was associated with the >10 μm fraction. N₂
23 fixation rates measured in the lagoon waters were half those measured in the mesocosms, and
24 were on average 9.2±4.7 nmol N L⁻¹ d⁻¹ over the 23 days of the experiment.

25 The Spearman correlation matrix (Table 2) indicates that N₂ fixation was positively correlated
26 with seawater temperature in the mesocosms, which was not the case in lagoon waters, although
27 temperature was exactly the same inside and outside the mesocosms (from 25.4°C to 26.8°C)
28 (Bonnet et al., 2016). N₂ fixation in the mesocosms was also positively correlated with particulate
29 organic carbon (POC), PON, and particulate organic phosphorus (POP) (except in M2)
30 concentrations, Chl *a* concentrations, primary production, bacterial production, alkaline
31 phosphatase activity (APA), and *Synechococcus*, picoeukaryote and nanoeukaryote (except in

1 M2) abundances. N_2 fixation was negatively correlated with NO_3^- , DIP, DON, dissolved organic
2 phosphorus (DOP) (except in M2) concentrations and DIP turn-over time.

3 The intercomparison between the bubble and dissolution methods performed on day 11 in M2
4 indicates that rates determined for the 6 replicates were $7.2 \pm 0.8 \text{ nmol N L}^{-1} \text{ d}^{-1}$ and $6.4 \pm 2.0 \text{ nmol}$
5 $\text{N L}^{-1} \text{ d}^{-1}$ for the dissolution method and the bubble method, respectively, demonstrating that, at
6 least in this study, N_2 fixation rates were not significantly different ($p > 0.05$) between the two
7 methods.

8

9 **3.2 Phenotypic characterization of UCYN by microscopy**

10 The average size of the UCYN-C cells present in the mesocosms was $5.7 \pm 0.8 \text{ }\mu\text{m}$ ($n=17$). Both
11 free-living and aggregated UCYN-C cells were observed in the water columns of the mesocosms.
12 However, the detailed microscopic analysis performed on day 17 and day 19 in M2 (during the
13 bloom of UCYN-C) (Fig. 3) indicates that the proportion of free-living cells (ROI characterized
14 by one cell or two cells defined as dividing cells) was low ($< 1 \%$ on day 17 and $< 5 \%$ on day 19).
15 The average number of UCYN-C cells per aggregate increased with depth (Fig. 3a), with the size
16 of the aggregates reaching $50\text{-}100 \text{ }\mu\text{m}$ at 6 m and $100\text{-}500 \text{ }\mu\text{m}$ at 12 m depth. On day 17, the
17 number of cells per aggregate averaged 162, 74, and 1273 at 1, 6, and 12 m, respectively. On day
18 19, the aggregates were much smaller ($\sim 50 \text{ }\mu\text{m}$) with only 4, 11, and 19 cells per aggregate. The
19 sediment traps contained extremely high densities of UCYN-C cells with the average number of
20 cells per aggregate 60 to 50,000 times higher than that measured in the water column aggregates
21 (Fig. 3b-e).

22

23 **3.3 Quantification of diazotrophs in sediment traps**

24 qPCR analysis confirmed that UCYN-C was the most abundant diazotroph in the sediment traps
25 on days 17 and 19, with abundances reaching 2.7×10^8 to $4 \times 10^9 \text{ nifH copies L}^{-1}$ (Fig. 4a).
26 UCYN-C accounted for 97.4 to 99.2 % of the total *nifH* pool quantified in the traps. Abundances
27 were higher in M2 and M3 (1.8×10^9 in M2 and $3 \times 10^9 \text{ nifH copies L}^{-1}$ in M3) compared to M1
28 ($2.5 \times 10^8 \text{ nifH copies L}^{-1}$) on day 19. Het-1 and het-3 were always recovered in the sediment
29 traps, albeit at lower abundances (1.8 to $8.6 \times 10^6 \text{ nifH copies L}^{-1}$ for het-1 and 4.9×10^6 to $2.8 \times$
30 $10^7 \text{ nifH copies L}^{-1}$ for het-3) (Fig. 4b). They represented between 0.1 and 1.8 % of the targeted
31 *nifH* pool. UCYN-B was detected in all mesocosms on both days (except in M1 on day 19), and

1 UCYN-A2 and *Trichodesmium* were detected in M2 on day 17 but at low abundances (0.05 % of
2 the total *nifH* pool) compared to the other phylotypes. Het-2 was never detected in the traps, and
3 neither was γ 24774A11 or UCYN-A1.

4 Using the volume of each mesocosm (Bonnet et al., 2016) and the total *nifH* copies for each
5 diazotroph phylotype in the sedimenting material and in the water column the day before the
6 collection of the sediment traps (Turk-Kubo et al., 2015) (assuming a sinking velocity of the
7 exported material of $\sim 10 \text{ m day}^{-1}$, Gimenez et al. (2016)), we estimated the export efficiency for
8 each phylotype. For UCYN-C, 4.6 % and 6.5 % of the cells present in the water column were
9 exported to the traps per 24 h on day 17 and 19, respectively (assuming one *nifH* copy per cell).
10 For het-1, 0.3 and 0.4 % of cells were exported into the traps on day 17 and 19, for het-3, 15.5 %
11 and 10.5 % were exported, and for UCYN-B, 37.1 % and 15.5 % of UCYN-B were exported on
12 day 17 and 19, respectively.

13

14 **3.4. DDN transfer experiment performed on day 17**

15 Net $^{15}\text{N}_2$ uptake was $24.1 \pm 2.8 \text{ nmol N L}^{-1}$ during the first 24 h of the DDN transfer experiment
16 performed from days 17 to 20 (Fig. 5a). As expected, integrated $^{15}\text{N}_2$ uptake increased over the
17 course of the experiment to reach $28.8 \pm 4.3 \text{ nmol N L}^{-1}$ at T48 h and $126.8 \pm 35.5 \text{ nmol N L}^{-1}$ at
18 T72 h. The DDN quantified in the TDN pool ranged from $6.2 \pm 2.4 \text{ nmol N L}^{-1}$ at T24 h to 9.6 ± 1.6
19 nmol N L^{-1} at T72 h. Considering gross N_2 fixation as the sum of net N_2 fixation and DDN
20 release (Mulholland et al., 2004), the DDN released to the TDN pool accounted for 7.1 ± 1.2 to
21 20.6 ± 8.1 % of gross N_2 fixation.

22 During the 72 h targeted experiment (Fig. 5b) the diazotroph assemblage reflected that of the
23 mesocosms from which they were sampled: UCYN-C dominated the diazotrophic community,
24 comprising on average 62 % of the total *nifH* pool. The other most abundant phylotypes were
25 UCYN-A2 and het-2, which represented 18 and 13 % of the total *nifH* pool, respectively. UCYN-
26 A1, UCYN-B, het-1, het-3, and *Trichodesmium* were also detected but together they comprised
27 less than 8 % of the total targeted community. Phylotype abundances remained relatively stable
28 throughout the 72 h of the experiment.

29 NanoSIMS analyses performed on individual UCYN-C at 24 h (Fig. 6) revealed significant
30 ($p < 0.05$) ^{13}C ($1.477 \pm 0.542 \text{ atom\%}$, $n=35$) and ^{15}N ($1.515 \pm 0.370 \text{ atom\%}$, $n=35$) enrichments
31 relative to natural abundance, indicating that UCYN-C were actively photosynthesizing and

1 fixing N₂. The correlation between ¹³C enrichment and ¹⁵N enrichment was significant (r=0.85,
2 p<0.01, Fig. 6b). NanoSIMS analyses performed on diatoms and picoplankton (Fig. 5c) also
3 revealed significant (p<0.05) ¹⁵N enrichment of non-diazotrophic plankton, demonstrating a
4 transfer of DDN from the diazotrophs to other phytoplankton. Both diatoms and picoplanktonic
5 cells were significantly (p<0.05) more enriched at the end of the experiment (T72 h)
6 (0.489±0.137 atom%, n=12 for diatoms; 0.457±0.077 atom%, n=96 for picoplankton) than after
7 the first 24 h (0.408±0.052 atom%, n=23 for diatoms; 0.389±0.014 atom%, n=63 for
8 picoplankton). Finally, the ¹⁵N enrichment of picoplankton and diatoms was not significantly
9 different (p>0.05) during the DDN experiment.

10

11 **4 Discussion**

12

13 **4.1 The bubble vs. the dissolution method: an intercomparison experiment**

14 The inter-comparison experiment performed on day 11 reveals slightly lower, yet insignificantly
15 different (p>0.05) average N₂ fixation rates when using the bubble method compared to the
16 dissolution method. This result is in accordance with some comparisons made by Shiozaki et al.
17 (2015) in temperate waters of the North Pacific. However, a lower degree of dissolution of the
18 ¹⁵N₂ bubble may occur in warm tropical waters such as those near New Caledonia compared to
19 the cooler, temperate North Pacific waters. In calculating N₂ fixation rates using the dissolution
20 method, we used the value of 2.4±0.2 atom% for the ¹⁵N enrichment of the N₂ pool as measured
21 by MIMS. For the bubble method, we used the theoretical value of 8.4 atom% calculated for
22 seawater with a temperature of 25.5 °C and salinity of 35.3 (as was the case on day 11). If we
23 assume that equilibration was incomplete in our experiment using the bubble method, i.e., 75 %
24 instead of 100 % as shown by Mohr et al. (2010), we calculate higher, albeit still insignificantly
25 (p>0.05) N₂ fixation rates for the bubble method (8.3±2.8 nmol N L⁻¹ d⁻¹) compared to the
26 dissolution method (7.2±0.8 nmol N L⁻¹ d⁻¹), confirming that equivalent results are obtained with
27 both methods in this ecosystem.

28

29 **4.2 The temporal dynamics of N₂ fixation in the mesocosms**

30 Average N₂ fixation rates measured in the lagoon waters (outside the mesocosms, 9.2±4.7 nmol
31 N L⁻¹ d⁻¹, Table 1) are of the same order of magnitude as those reported for the Noumea lagoon

1 during austral summer conditions (Biegala and Raimbault, 2008). They are within the upper
2 range of rates reported in the global ocean database (Luo et al., 2012). Indeed, open ocean cruises
3 performed offshore of New Caledonia in the Coral and Solomon Seas (e.g., (Bonnet et al., 2015;
4 Garcia et al., 2007) also suggest that the South West Pacific Ocean is one of the areas with the
5 highest N₂ fixation rates in the global ocean.

6 Averaged over the 23 days of the experiment, N₂ fixation rates in the mesocosms were ~ 2 fold
7 higher (18.5±1.1 nmol N L⁻¹ d⁻¹) than those measured in lagoon waters (9.2±4.7 nmol N L⁻¹ d⁻¹).
8 The maximum observed rates of >60 nmol N L⁻¹ d⁻¹ from days 18-21 are among the highest
9 reported for marine waters (Luo et al., 2012). DIP concentration was the predominant difference
10 between the ambient lagoon waters and those of the mesocosms. The mesocosms were fertilized
11 with DIP on day 4, reaching ambient concentrations of ~0.8 µmol L⁻¹ compared to lagoon waters
12 in which DIP concentrations were typically <0.05 µmol L⁻¹. According to our experimental
13 assumption, diazotrophy would be promoted by high concentrations of DIP. Yet, in all three
14 mesocosms, N₂ fixation rates were negatively correlated with DIP concentrations and DIP
15 turnover time and positively correlated with APA (Table 2). Below, we describe the scenario that
16 likely occurred in the mesocosms, which likely explains these correlations.

17 During P0 (day 2 to 4), N₂ fixation rates were higher in the mesocosms than in the lagoon waters,
18 possibly due to the reduction of turbulence in the water column facilitated by the closing of the
19 mesocosms (Moisander et al., 1997) and/or to the reduction of the grazing pressure in the
20 mesocosms as total zooplankton abundances were slightly lower (by a factor of 1.6) in the
21 mesocosms compared to the lagoon waters (Hunt et al., 2016). The most abundant diazotrophs in
22 the mesocosms at P0 were *het-1* and *Trichodesmium*, which were probably the most competitive
23 groups under the initial conditions, i.e., NO₃⁻ depletion (concentrations were 0.04±0.02 µmol L⁻¹,
24 Table 3) and low DIP concentrations (0.03±0.01 µmol L⁻¹, Table 3). *Trichodesmium* is able to
25 use organic P substrates (DOP pool) under conditions of DIP deficiency (Dyhrman et al., 2006;
26 Sohm and Capone, 2006). 24 h after the DIP fertilization (day 5), N₂ fixation rates in the
27 mesocosms decreased by ~40 %, reaching rates comparable to those measured in lagoon waters
28 during P1 (day 5 to 14). Enhanced DIP availability likely enabled non-diazotrophic organisms
29 with lower energetic requirements and higher growth rates to outcompete the diazotrophs in the
30 mesocosms via utilization of recycled N derived from recent N₂ fixation. This is supported by the
31 observation that nano-eukaryotes and non-diazotrophic cyanobacteria such as *Prochlorococcus*

1 sp. increased in abundance during P1 (Leblanc et al., 2016) in the three mesocosms when N₂
2 fixation rates declined (Fig. 2).

3 During P2 (day 15 to 23), N₂ fixation rates increased dramatically in all three mesocosms. This
4 period was defined by a high abundance of UCYN-C, which were present in low numbers in the
5 lagoon and within the mesocosms during P0 and P1 (Turk-Kubo et al., 2015). The increase in
6 UCYN-C abundance was synchronous with a decrease in DIP concentrations in the mesocosms
7 (Turk-Kubo et al., 2015): UCYN-C abundance first increased in M1 (day 11), then in M2 (day
8 13), and finally in M3 (day 15). In all cases, the increase in UCYN-C abundance coincided with
9 low DIP turnover time, indicative of DIP deficiency (Berthelot et al., 2015b; Moutin et al., 2005).

10 Under NO₃⁻ depletion and low DIP availability, UCYN-C appeared to be the most competitive
11 diazotroph in the mesocosms, as they exhibited the highest maximum growth rates compared to
12 those calculated for the other diazotrophic phylotypes for the same period (Turk-Kubo et al.,
13 2015). Some *Cyanothece* strains possess the genes required for utilization of organic P substrates
14 such as phosphonates (Bandyopadhyay, 2011). Thus, UCYN-C, which were the major
15 contributors to N₂ fixation during P2 (see below), may have used DOP as a P source during this
16 period, consistent with the negative correlation observed between N₂ fixation rates and DOP
17 concentrations (except in M2, Table 2), and driving the significant decline in DOP concentrations
18 observed in all three mesocosms during P2 (Berthelot et al., 2015b; Moutin et al., 2005).

19 While temperature was not correlated with N₂ fixation in the lagoon, we observed a significant
20 positive correlation between these parameters in the mesocosms (Table 2), probably because
21 some diazotrophic phylotypes present in the mesocosms and absent in the lagoon waters were
22 particularly sensitive to seawater temperature. UCYN-C reached high abundances inside the
23 mesocosms, but was virtually absent in the lagoon waters outside the mesocosms. Turk-Kubo et
24 al. (2015) showed that UCYN-C abundance was positively correlated with seawater temperature,
25 suggesting that the optimal temperature for UCYN-C growth is above 25.6 °C. This result is
26 consistent with culture studies performed using three UCYN-C isolates from the Noumea lagoon
27 that are closely related to the UCYN-C observed here, indicating maximum growth rates at
28 around 30°C and no growth below 25 °C (Camps, Turk-Kubo, Bonnet, Pers. comm.).
29 Temperature above 25.6 °C and up to 26.7°C were reached on day 12 and were maintained
30 through to the end of the mesocosm experiment, possibly explaining why UCYN-C was not

1 observed during P0 (when temperature was 25.4°C) even though DIP turn-over time was low
2 (below ~1 d) (Berthelot et al., 2015b; Moutin et al., 2005).

3 If low DIP concentrations and seawater temperatures greater than 25.6 °C are prerequisites for
4 UCYN-C growth, an obvious question is why they did not thrive (despite being present at low
5 abundances) in the lagoon waters during P2 when similar conditions prevailed. We consider three
6 possible explanations that are discussed extensively in Turk-Kubo et al. (2015): first, it is
7 possible that UCYN-C are sensitive to turbulence, which was likely reduced in the mesocosms
8 compared to the lagoon waters that are susceptible to trade winds and tides. Second, grazing
9 pressures on UCYN-C may have been reduced as total zooplankton abundances were slightly
10 lower (by a factor of 1.6) in the mesocosms compared to those in the lagoon waters (Hunt et al.,
11 2016). Third, the water masses outside the mesocosms changed with tides and winds; thus, it is
12 possible that UCYN-C were absent from the water mass encountered outside the mesocosms
13 when we sampled for this experiment.

14 In the mesocosms, the cell specific $^{15}\text{N}_2$ fixation rate measured on day 17 (M2) for UCYN-C was
15 $6.3 \pm 2.0 \times 10^{-17}$ mol N cell $^{-1}$ d $^{-1}$. Multiplying this rate by the abundance of UCYN-C indicates that
16 UCYN-C accounted for 90 ± 29 % of bulk N_2 fixation during that period. This is consistent with
17 the positive correlation observed between N_2 fixation rates and UCYN-C abundances in M2
18 (Table 2). In M1 and M3, the correlation was also positive yet insignificant. This may have been
19 due to the low number of UCYN-C data points, thus decreasing the sensitivity of the statistical
20 test. Coupling between UCYN-C ^{13}C and ^{15}N incorporation was significant ($r=0.85$, $p<0.01$) (Fig.
21 6b) and contrasts with results reported by Berthelot et al. (2016) for UCYN-C, in which ^{13}C and
22 ^{15}N enrichment (and thus inorganic C and N_2 fixation) was uncoupled in the cells. Based on their
23 observations, these authors suggest that the heterogeneity in the ^{15}N and ^{13}C enrichments can be
24 explained by a specialization of some cells that induces variability in cell-specific ^{15}N -enrichment
25 e.g., diazocytes that contain the nitrogenase enzyme in the colonial filamentous *Trichodesmium*
26 sp. Spatial partitioning of N_2 and C fixation by colonial unicellular types was also evidenced for
27 diazocyte-like formation in colonial *Crocospaera.watsonii*-like (UCYN-B) cells (Foster et al.,
28 2013). Here, UCYN-C cells fixed both ^{13}C and ^{15}N proportionally, which suggests they did not
29 utilize diazocytes to separate diazotrophy from photosynthesis in our experiments.

30

31 **4.3 UCYN aggregation and export**

1 Throughout the 23 days of the experiment, the majority of N₂ fixation (63 %) occurred in the >10
2 μm size fraction, even during P2 when the small (5.7±0.8 μm) unicellular UCYN-C dominated
3 the mesocosm diazotrophic community. These findings can be explained by the aggregation of
4 UCYN-C cells into large (>10 μm) aggregates (Fig. 7) that were retained on 10 μm filters (Fig.
5 3). These large UCYN-C aggregates probably formed in part due to the presence of sticky TEP
6 (Berman-Frank et al., 2016) or other extracellularly-released proteins, and were characterized by
7 a high sinking velocity due to their large size (up to 500 μm in diameter) and a density greater
8 than that of seawater (Azam and Malfatti, 2007). Their aggregation and subsequent sinking
9 within the mesocosms likely explains why volumetric N₂ fixation rates were higher at 12 m than
10 at the surface during P2, as well as why the size of the aggregates increased with depth, and why
11 numerous large-size aggregates and extremely high abundances of UCYN-C were recovered in
12 the sediment traps. Aggregation processes may have been favored by the low turbulence in the
13 mesocosms and it would be necessary to confirm that such processes also occur in the open
14 ocean.

15 Colonial phenotypes of UCYN (UCYN-B) have been observed in the water column of the North
16 Tropical Pacific (ALOHA station) (Foster et al., 2013), but to our knowledge, this is the first time
17 that UCYN have been detected in sediment traps. Contrary to published data (e.g. (White et al.,
18 2012)), here we demonstrate a greater export efficiency of UCYN (~10 % exported to the traps
19 within 24 h) compared to the export of DDAs (efficiency of 0.24 to 4.7 %). Diatoms sink rapidly
20 and DDAs have been found in sediment traps at Station ALOHA (Karl et al., 2012; Karl et al.,
21 1997; Scharek et al., 1999a; Sharek et al., 1999b), in the Gulf of California (White et al., 2012),
22 and in the Amazon River plume (Subramaniam et al., 2008). In our study, we observed limited
23 export of het-1 (*Richelia* in association with *Rhizosolenia*) and het-3 (*Calothrix*) during P2, while
24 het-2 (*Richelia* associated with *Hemiaulus*) was never recovered in the sediment traps. This is
25 likely because *Hemiaulus* has a lower sinking rate than *Rhizosolenia* due to its smaller size, or
26 may be more easily grazed by zooplankton than *Rhizosolenia* or *Calothrix*, which are known to
27 be toxic to crustaceans (Höckelmann et al., 2009). We observed only rare occurrences of
28 *Trichodesmium* export in this study probably due to its extremely limited presence and low
29 growth rates in the mesocosms. Direct comparisons of our export results with findings from open
30 ocean studies should be made cautiously as our mesocosms were shallower (15 m) than typical

1 oceanic export studies (>100 m) and were also probably characterized by reduced turbulence
2 (Moisander et al., 1997).

3 We estimate that the direct export of UCYN-C accounted for 22.4 ± 5.5 % of the total POC
4 exported in each mesocosm at the height of the UCYN-C bloom (day 17) and decreased to
5 4.1 ± 0.8 % on day 19 (Fig. 4c, Fig. 7). This calculation is based on the total POC content
6 measured in the sediment traps (Berthelot et al., 2015b), our C_{con} for UCYN-C estimated as
7 described above, and published C_{con} for other diazotrophs. The corresponding export of het-1,
8 het-3, *Trichodesmium*, and UCYN-B on day 17 based on published C_{con} (Leblanc et al., 2012;
9 Luo et al., 2012), and using an average of three *Richelia* and *Calothrix* symbionts per diatom,
10 accounted for 6.8 ± 0.5 , 0.5 ± 0.02 , 0.3 ± 0.3 , and 0.1 ± 0.01 % of the POC export on day 17,
11 respectively, and for 4.2 ± 1.7 , 0.04 ± 0.03 of the POC export on day 19 (the contribution of
12 *Trichodesmium* and UCYN-B to POC export on day 19 was negligible). Thus, our data
13 emphasize that despite their small size relative to DDAs, UCYN-C are able to directly export
14 organic matter to depth by forming densely-populated aggregates that can rapidly sink. This
15 observation is further confirmed by the e ratio, which quantifies the efficiency of a system to
16 export POC relative to primary production (e ratio = POC export/PP) and was significantly
17 higher ($p<0.05$) during P2 (i.e., during the UCYN-C bloom; 39.7 ± 24.9 %) than during P1 (i.e.,
18 when DDAs dominated the diazotrophic community; 23.9 ± 20.2 %) (Berthelot et al., 2015b). It is
19 also consistent with the significantly ($p<0.05$) higher contribution of N_2 fixation to export
20 production during P2 (56 ± 24 %, and up to 80 % at the end of the experiment) compared to P1
21 (47 ± 6 %, and never exceeded 60 %) as estimated by Knapp et al. (2015) using a $\delta^{15}\text{N}$ budget for
22 the mesocosms. Our calculated contribution of N_2 fixation to export production is very high
23 compared to other tropical and subtropical regions where diazotrophs are present (10 to 25 %;
24 e.g., (Altabet, 1988; Knapp et al., 2005)). However, it is consistent with the high rates of N_2
25 fixation measured in the enclosed mesocosms compared to those from the lagoon and other
26 tropical pelagic studies (Luo et al., 2012). The direct export of UCYN-C and other diazotrophs
27 cannot solely explain the high e ratio estimated for P2. We thus hypothesize that a fraction of the
28 DDN export that occurred during P2 was transferred indirectly via primary utilization by non-
29 diazotrophic plankton cells that were eventually exported to the sediment traps (Fig. 7).

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31

1 **4.4 DDN transfer to non-diazotrophic phytoplankton and ecological** 2 **implications**

3 The amount of DDN measured in the TDN pool during the 72 h DDN transfer experiment is
4 higher than that reported for culture studies of *Cyanothece* populations (1.0 ± 0.3 to 1.3 ± 0.2 % of
5 gross N_2 fixation (Benavides et al., 2013; Berthelot et al., 2015a)). The DDN measured in the
6 TDN pool reflects the DDN release by diazotrophs during N_2 fixation and is likely
7 underestimated here as a fraction of this DDN has been taken up by surrounding planktonic
8 communities. In our experiment, other diazotrophs were present in addition to *Cyanothece*, and
9 they may have also contributed to the dissolved pool. Moreover, unlike in culture studies, field
10 experiments are also impacted by other exogenous factors such as viral lysis (Fuhrman, 1999)
11 and sloppy feeding (O'Neil and Roman, 1992; Vincent et al., 2007), which may enhance N
12 release.

13 This DDN release plays a critical role in the N transfer between diazotrophs and non-diazotrophs.
14 The cell-specific uptake rates of DDN during the DDN transfer experiment were calculated for
15 each cell analysed by nanoSIMS (diatoms and cells from the 0.2-2 μm fraction). By multiplying
16 cell-specific N uptake rates by the cellular abundance of each group on a particular day, we could
17 identify the specific pool (diazotrophs, dissolved pool, non-diazotrophs) into which the $DD^{15}N$
18 was transferred after 24 h, and the extent to which this $^{15}N_2$ accumulated. The results are
19 summarized in Fig. 7. After 24 h, 52 ± 17 % of the newly fixed $^{15}N_2$ remained in the UCYN-C
20 biomass, 16 ± 6 % had accumulated in the dissolved N pool, and 21 ± 4 % had been transferred to
21 non-diazotrophic plankton. In addition, 11 % of the newly fixed $^{15}N_2$ accumulated in a pool that
22 we refer to as 'others' (corresponding to diazotrophs other than UCYN-C and potential non-
23 diazotrophs to which $^{15}N_2$ was transferred; these cells were not analysed by nanoSIMS due to
24 their very low abundance). Uncertainties take into account both the variability of the ^{15}N
25 enrichment determined on ~ 25 cells per group by nanoSIMS, and the uncertainty in the N content
26 per cell measured or taken from the literature.

27 Within the fraction of DDN transferred to the non-diazotrophs after 24 h (21 %), we calculate
28 that 18 ± 4 % was transferred to picoplankton, and only 3 ± 2 % was transferred to diatoms (Fig. 7).
29 The ^{15}N enrichment of picoplankton and diatoms was not significantly different ($p>0.05$) in this
30 study, but as picoplankton dominated the planktonic community in the mesocosms at the time of
31 the DDN transfer experiment, they were the primary beneficiaries of the DDN. This is consistent

1 with the positive correlation between N₂ fixation rates, *Synechococcus*, and pico-eukaryote
2 abundances in the mesocosms (Table 2), as well as with the observed dramatic increase in
3 *Synechococcus* and pico-eukaryotes abundances (by a factor of >2 between P1 and P2) (Leblanc
4 et al., 2016). Diatom abundances also increased in the mesocosms by a factor of 2 between P1
5 and P2 (largely driven by *Cylindrotheca closterium*), but this increase occurred earlier than the
6 picoplankton increase, i.e., at the end of P1 (days 11-12). Maximum diatom abundances were
7 reached on day 15-16 at the very beginning of P2, and then declined by day 18 to reach
8 abundances similar to those observed during P1. These results suggest that diatoms were the
9 primary beneficiaries of DDN in the mesocosms at the start of P2, when N₂ fixation rates and
10 UCYN-C abundances increased dramatically. This is consistent with a previous DDN transfer
11 study performed in New Caledonia (Bonnet et al., Accepted) during which diatoms (mainly
12 *Cylindrotheca closterium*) advantageously competed and utilized DDN released during
13 *Trichodesmium* blooms. When the present DDN transfer experiment was performed (days 17 to
14 20), diatom abundances had already declined, likely due to DIP limitation (DIP turnover time
15 was low, i.e below 1d). We hypothesize that picoplankton were more competitive for DDN under
16 low DIP conditions as small cells with high surface to volume ratios are known to outcompete
17 larger cells for the available DIP (Moutin et al., 2002). Moreover, some prokaryotes from the 0.2-
18 2 µm size-fraction can utilize DOP compounds (Duhamel et al., 2012). In this study, we could
19 not discriminate the DDN transfer to pico-autotrophs from that to pico-heterotrophs, but it is
20 likely that both communities took advantage of the DDN, as both primary production (Berthelot
21 et al., 2015b) and bacterial production (Van Wambeke et al., 2015) were positively correlated
22 with N₂ fixation rates (Table 2) and increased dramatically following the increase in N₂ fixation
23 during P2. The standing stocks of POC, PON, and POP were also positively correlated with N₂
24 fixation rates, suggesting that DDN sustained productivity in the studied system.

25

26 **5 Conclusions**

27 While studies on the fate of DDN in the ocean are rare, the contribution of DDN to particle
28 export based on the δ¹⁵N signatures of exported material indicate that N₂ fixation can efficiently
29 contribute to export production in the oligotrophic ocean (Dore et al., 2008). The export of DDN
30 may either be direct, through the sinking of diazotrophs, or indirect, through the transfer of DDN
31 to non-diazotrophic plankton in the photic zone that are subsequently exported.

1 *Trichodesmium* is rarely recovered in sediment traps (Walsby, 1992) and most of the research
2 dedicated to the export of diazotrophs has focused on DDAs (Karl et al., 2012) due to their high
3 sinking velocity. Here, we demonstrate for the first time that UCYN can efficiently contribute to
4 POC export in oligotrophic systems, predominantly due to the aggregation of small ($5.7\pm 0.8\ \mu\text{m}$)
5 UCYN-C cells into large aggregates, which increase in size (up to $500\ \mu\text{m}$) with depth. Our
6 results suggest that these small (typically $3\text{-}7\ \mu\text{m}$) organisms should be considered in future
7 studies to confirm if processes observed in mesocosms are applicable to open ocean systems.
8 Moreover, the experimental and analytical approach used in this study allowed for the
9 quantification of the actual transfer of DDN to different groups of non-diazotrophic plankton in
10 the oligotrophic ocean. Our nanoSIMS results coupled with $^{15}\text{N}_2$ isotopic labelling revealed that a
11 significant fraction of DDN ($21\pm 4\ \%$) is quickly (within 24 h) transferred to non-diazotrophic
12 plankton, which increased in abundance simultaneously with N_2 fixation rates. A similar
13 nanoSIMS study performed during a *Trichodesmium* bloom (Bonnet et al., Accepted) revealed
14 that diatoms were the primary beneficiaries of DDN and developed extensively during and after
15 *Trichodesmium* spp. blooms. Diatoms are efficient exporters of organic matter to depth (Nelson
16 et al., 1995). These studies show that plankton grown on DDN in the oligotrophic ocean drive
17 indirect export of organic matter out of the photic zone, thus revealing a previously unaccounted
18 for conduit between N_2 fixation and the eventual export to depth of DDN from the photic zone.

19

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36

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3 and S. L'Helguen analyzed the samples. I. Berman-Frank took part in experimental planning,
4 preparation, and implementation of the project. S. Bonnet prepared the manuscript with
5 contributions from all co-authors.

6

7 **Figure captions**

8

9 **Figure 1.** (a) Mesocosms ($\sim 50 \text{ m}^3$) deployed in the framework of the VAHINE project. (b)
10 Sediment traps screwed onto the base of the mesocosms and were sampled daily by SCUBA
11 divers.

12

13 **Figure 2.** (a) Horizontal and vertical distributions of bulk N_2 fixation rates ($\text{nmol N L}^{-1} \text{ d}^{-1}$), and
14 (b) $<10 \mu\text{m}$ N_2 fixation rates ($\text{nmol N L}^{-1} \text{ d}^{-1}$) in M1, M2, M3, and lagoon waters. Note that N_2
15 fixation rates in the $<10 \mu\text{m}$ fraction were not measured (lower right panel). The grey bars
16 indicate the timing of the DIP addition on day 4.

17

18 **Figure 3.** (a) UCYN-C cells per aggregate in M2 on day 17 and 19. (b to e) Green excitation
19 (510-560 nm) epifluorescent replicate micrographs of UCYN-C on day 17 taken at 1 m depth
20 ($\times 40$) (b), 6 m depth ($\times 40$) (c), 12 m depth ($\times 40$) (d), and in the sediment traps ($\times 10$) (e). Scale bar
21 $20 \mu\text{m}$ (b to d) and $100 \mu\text{m}$ (e).

22

23 **Figure 4.** (a) Abundance of UCYN-C ($nifH$ copies L^{-1}) and (b) other $nifH$ phylotypes (UCYN-
24 A2, UCYN-B, *Trichodesmium*, het-1, het-3) ($nifH$ copies L^{-1}) recovered in the sediment trap on
25 day 17 and 19. (c) Proportion of POC export associated with diazotrophs in the sediment traps on
26 day 17 in M2 (height of UCYN-C bloom).

27

28 **Figure 5.** Results from the DDN transfer experiment performed from day 17 to 20 in M2. (a)
29 Temporal changes in $^{15}\text{N}_2$ uptake (white, nmol N L^{-1}) and quantification of DDN in the dissolved
30 pool (grey) over the course of the experiment. Error bars represent the standard deviation of three
31 independent replicate incubations. (b) Temporal changes in diazotroph abundance determined by
32 qPCR ($nifH$ gene copies L^{-1}) during the same experiment. Error bars represent the standard

1 deviation of triplicate incubations. (c) Summary of the nanoSIMS analyses. Measured ^{13}C and
2 ^{15}N atom% values of non-diazotrophic diatoms (white) and picoplankton (grey) as a function of
3 incubation time. The horizontal dashed line indicates the natural abundance of ^{15}N (0.366
4 atom%), and the error bars represent the standard deviation for the several cells analysed by
5 nanoSIMS.

6
7 **Figure 6.** (a) Green excitation (510-560 nm) epifluorescent micrographs of UCYN-C, (b) ^{13}C and
8 ^{15}N isotopic enrichment (atom%) in individual UCYN-C cells on day 17 in M2, (c, d) nanoSIMS
9 images showing the ^{13}C (c) and ^{15}N (d) enrichment of individual UCYN-C cells after 24 h of
10 incubation. The white outlines show regions of interest (ROIs), which were used to estimate the
11 $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ ratios.

12
13 **Figure 7.** Cartoon summary of the simplified pathways of N transfer in the first trophic level of
14 the food web and the potential impact on the sinking POC flux at the height of the UCYN-C
15 bloom in the VAHINE mesocosm experiment.

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1 **Table 1.** N₂ fixation rates (nmol N L⁻¹ d⁻¹) measured in the mesocosms and in lagoon waters.
 2 Table shows the range, median, mean, contribution of the <10 μm fraction to total rates (%), and
 3 the number of samples analysed (n).

	Range	Median	Mean	% <10 μm	n
M1	0.5-69.7	15.9	19.7	38	61
M2	3.0-67.7	15.1	18.1	43	57
M3	2.9-60.4	14.2	17.7	29	59
Average mesocosms	2.1-65.9	15	18.5	37	177
Lagoon waters	1.9-29.3	8.7	9.2	n.a	61

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1 **Table 2.** Spearman correlation matrix of N₂ fixation rates and hydrological parameters,
 2 biogeochemical stocks and fluxes, and planktonic communities (n=66). The significant
 3 correlations (p<0.05) are indicated in bold. n.a- not available.

	Parameter	M1	M2	M3	Lagoon waters
<i>Hydrological parameters</i>	Temperature	0.394	0.319	0.347	0.228
	Salinity	0.211	0.213	0.266	-0.122
<i>Biogeochemical stocks and fluxes</i>	NO ₃ ⁻	-0.539	-0.302	-0.341	0.145
	NH ₄ ⁺	0.152	0.103	0.006	0.197
	DIP	-0.613	-0.569	-0.482	-0.116
	DON	-0.329	-0.413	-0.235	-0.180
	DOP	-0.563	-0.157	-0.316	-0.243
	PON	0.575	0.293	0.494	0.077
	POP	0.514	0.001	0.439	0.036
	POC	0.399	0.352	0.356	-0.061
	Chl <i>a</i>	0.660	0.656	0.656	0.220
	Primary production	0.443	0.498	0.445	0.268
	Bacterial production	0.708	0.408	0.471	0.189
	T-DIP	-0.670	-0.603	-0.564	-0.190
	APA	0.575	0.568	0.273	-0.062
<i>Planktonic communities</i>	HNA	0.317	-0.043	0.458	n.a
	LNA	0.262	-0.021	0.000	n.a
	<i>Prochlorococcus</i>	0.429	-0.122	0.138	n.a
	<i>Synechococcus</i>	0.699	0.434	0.499	n.a
	Pico-eukaryotes	0.614	0.563	0.414	n.a
	Nano-eukaryotes	0.477	0.002	0.442	n.a
	Diatoms	-0.099	0.456	-0.200	n.a
	Dinoflagellates	0.242	-0.392	-0.321	n.a
	UCYN-A1	0.545	-0.521	-0.503	0.200
	UCYN-A2	0.127	-0.631	0.248	0.333
	UCYN-B	0.083	0.696	0.467	0.101
	UCYN-C	0.373	0.621	0.515	-0.167
	<i>Trichodesmium</i>	-0.145	0.147	0.285	-0.117
	DDAs	-0.036	-0.264	-0.527	0.262
γ-24774A11	0.327	0.497	-0.750	0.733	

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1 **Table 3.** Average NO_3^- , DIP, DON, and DOP concentrations ($\mu\text{mol L}^{-1}$) measured over the P0,
2 P1 and P2 periods. NO_3^- and DIP concentrations were determined using a segmented flow
3 analyzer according to (Aminot and Kerouel, 2007). The detection limit was 0.01 and 0.005 μmol
4 L^{-1} for NO_3^- and DIP, respectively. DON and DOP concentrations were determined according to
5 the wet oxidation procedure described in Pujo-Pay and Raimbault (1994) and Berthelot et al.
6 (2015b).

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	Average P0	Average P1	Average P2
NO_3^-	0.04±0.02	0.03±0.01	0.02±0.01
DIP	0.03±0.01	0.48±0.20	0.08±0.05
DON	5.19±0.37	5.22±0.54	4.73±0.49
DOP	0.14±0.01	0.16±0.03	0.12±0.02

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