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Dynamics of N₂ fixation and fate of diazotroph-derived nitrogen in a low nutrient low chlorophyll ecosystem: results from the VAHINE mesocosm experiment (New Caledonia)

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12, 19579–19626, 2015

Results from the VAHINE mesocosm experiment

S. Bonnet et al.

[Title Page](#)

[Abstract](#)

[Introduction](#)

[Conclusions](#)

[References](#)

[Tables](#)

[Figures](#)



[Back](#)

[Close](#)

[Full Screen / Esc](#)

[Printer-friendly Version](#)

[Interactive Discussion](#)



Abstract

N_2 fixation rates were measured daily in large ($\sim 50\text{ m}^3$) mesocosms deployed in the tropical South West Pacific coastal ocean (New Caledonia) to investigate the spatial and temporal dynamics of diazotrophy and the fate of diazotroph-derived nitrogen (DDN) in a low nutrient, low chlorophyll ecosystem. The mesocosms were intentionally fertilized with $\sim 0.8\ \mu\text{M}$ dissolved inorganic phosphorus (DIP) to stimulate diazotrophy. Bulk N_2 fixation rates were replicable between the three mesocosms, averaged $18.5 \pm 1.1\ \text{nmol N L}^{-1}\ \text{d}^{-1}$ over the 23 days, and increased by a factor of two during the second half of the experiment (days 15 to 23) to reach $27.3 \pm 1.0\ \text{nmol N L}^{-1}\ \text{d}^{-1}$. These rates are higher than the upper range reported for the global ocean, indicating that the waters surrounding New Caledonia are particularly favourable for N_2 fixation. During the 23 days of the experiment, N_2 fixation rates were positively correlated with seawater temperature, primary production, bacterial production, standing stocks of particulate organic carbon, nitrogen and phosphorus, and alkaline phosphatase activity, and negatively correlated with DIP concentrations, DIP turnover time, nitrate, and dissolved organic nitrogen and phosphorus concentrations. The fate of DDN was investigated during the bloom of the unicellular diazotroph, UCYN-C, that occurred during the second half of the experiment. Quantification of diazotrophs in the sediment traps indicates that $\sim 10\%$ of UCYN-C from the water column were exported daily to the traps, representing as much as $22.4 \pm 5.5\%$ of the total POC exported at the height of the UCYN-C bloom. This export was mainly due to the aggregation of small ($5.7 \pm 0.8\ \mu\text{m}$) UCYN-C cells into large ($100\text{--}500\ \mu\text{m}$) aggregates. During the same time period, a DDN transfer experiment based on high-resolution nanometer scale secondary ion mass spectrometry (nanoSIMS) coupled with $^{15}\text{N}_2$ isotopic labelling revealed that $16 \pm 6\%$ of the DDN was released to the dissolved pool and $21 \pm 4\%$ was transferred to non-diazotrophic plankton, mainly picoplankton ($18 \pm 4\%$) followed by diatoms ($3 \pm 2\%$) within 24 h of incubation. This is consistent with the observed dramatic increase in picoplankton and diatom abundances, primary production, bacterial production and standing stocks of

Results from the VAHINE mesocosm experiment

S. Bonnet et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



particulate organic carbon, nitrogen and phosphorus during the second half of the experiment in the mesocosms. These results offer insights into the fate of DDN during a bloom of UCYN-C in low nutrient, low chlorophyll ecosystems.

1 Introduction

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

In these ocean deserts, specialized organisms termed N_2 -fixers (or diazotrophs) are able to use N in its simplest and most abundant form on Earth and in seawater, namely dinitrogen (N_2). These diazotrophs possess the nitrogenase enzyme, which cleaves the strong triple bond of the N_2 molecule to form bioavailable ammonium (NH_4^+). N_2 fixation thus introduces a source of new bioavailable N to surface waters, and is considered to be the most important external source of N for the ocean, before atmospheric and riverine inputs (Gruber, 2004).

15 The dynamics of microbial communities such as diazotrophs can change abruptly in the ocean in response to small perturbations or environmental stressors. In particular, N_2 fixation has been described as a very “patchy” process in the ocean (Bombar et al., 2015). Many factors control the distribution and activity of diazotrophs such as temperature (Raveh et al., 2015; Staal et al., 2003), nutrient (mainly phosphate and iron) availability (e.g., Mills et al., 2004), $p\text{CO}_2$ (e.g. Levitan et al., 2007), ambient concentrations of fixed N (NO_3^- and NH_4^+) (e.g. Knapp et al., 2012), as well as physical forcing
25 (e.g. Fong et al., 2008). Most studies dedicated to understanding the controls on marine

Results from the VAHINE mesocosm experiment

S. Bonnet et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



N_2 fixation have been undertaken along large oceanic transects; these are particularly valuable and have recently led to the compilation of a global ocean database of diazotrophy (Luo et al., 2012). Spatial variability in N_2 fixation is thus far better documented and understood than temporal variability, despite the intimate connections between time and space scales in the ocean. Time-series stations with near-monthly observations set up in the late 1980's under the international JGOFS program in the subtropical North Atlantic, Pacific, and Mediterranean Sea have provided valuable data regarding the controls on N_2 fixation and its role in biogeochemical cycles on seasonal and inter-annual timescales (Dore et al., 2008; Garcia et al., 2006; Grabowski et al., 2008; Karl et al., 2012; Knapp et al., 2005; Orcutt et al., 2001), and have also revealed novel diazotrophic microorganisms (Zehr et al., 2008) with unexpected metabolic strategies such as UCYN-A cyanobacteria that lack the oxygen-producing photosystem II complex (Tripp et al., 2010).

In the framework of the VAHINE project, we deployed three large volume mesocosms ($\sim 50\text{ m}^3$, Fig. 1) in the tropical South West Pacific coastal ocean, a region known to support diazotrophy during the austral summer (Dupouy et al., 2000; Rodier and Le Borgne, 2010, 2008) in order to study the temporal dynamics of N_2 fixation at high frequency (every day for 23 days) in relation to hydrological parameters, biogeochemical stocks and fluxes, and the dynamics of phytoplanktonic and bacterial communities in the same water mass.

The mesocosm approach allowed us to investigate the fate of the recently fixed N_2 and its transfer from diazotrophs to non-diazotrophic organisms in this oligotrophic marine ecosystem. Diazotrophs can typically release from 10 to 50 % of their recently fixed N_2 (or diazotroph derived N, hereafter called DDN) as dissolved organic N (DON) and ammonium (NH_4^+) (Glibert and Bronk, 1994; Meador et al., 2007; Mulholland et al., 2006). This exudate is potentially available for assimilation by the surrounding planktonic communities. However, such transfer of DDN to the surrounding planktonic community and its potential impact on export production is poorly understood and rarely quantified.

Results from the VAHINE mesocosm experiment

S. Bonnet et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

**Results from the
VAHINE mesocosm
experiment**

S. Bonnet et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

Over the course of this 23 day mesocosm experiment, diatom-diazotroph associations (DDAs) were the most abundant N_2 fixers during the first half of the experiment (days 2 to 14), while a bloom of the unicellular N_2 -fixing cyanobacteria from Group C (UCYN-C) occurred during the second half of the experiment (days 15 to 23) (Turk-Kubo et al., 2015). The contribution of N_2 fixation to primary production and export was investigated during the two phases of the experiment in a companion paper by Berthelot et al. (2015b). Here, we focus on the short-term fate of DDN in the mesocosms during the UCYN-C bloom by studying (i) the direct export of diazotrophs into the sediment traps, and (ii) the transfer of DDN to non-diazotrophic plankton using high-resolution nanometer scale secondary ion mass spectrometry (nanoSIMS) coupled with $^{15}N_2$ isotopic labelling during a 72 h-process experiment.

2 Methods

2.1 Mesocosm description and sampling strategy

Three replicate large-volume mesocosms (volume $\sim 50 m^3$, surface $4.15 m^2$, Fig. 1) were deployed in the oligotrophic New Caledonian lagoon, 28 km off the coast of Noumea (latitude: $22^{\circ}28,855 S$; longitude: $166^{\circ}26,724 E$) from 13 January to 6 February 2013 (austral summer). They consisted of large bags made of two $500 \mu m$ -thick films of polyethylene (PE) and vinyl acetate (EVA, 19%), with nylon meshing in between to allow for maximum resistance and light penetration (produced by HAIKONENE KY, Finland). The mesocosm bags were 2.3 m in diameter and 15 m in height, and were equipped with removable sediment traps that enabled the collection of sinking material once a day (Fig. 1b). To alleviate any potential phosphorus limitation of diazotrophy in the mesocosms, the bags were intentionally fertilized with $\sim 0.8 \mu mol L^{-1}$ of dissolved inorganic phosphorus (DIP) four days after the start of the experiment. A more detailed description of the mesocosms setup, the selection of the study site, and the deployment strategy can be found in Bonnet et al. (2015a).

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

2.2 Sampling, N₂ fixation measurements, and method intercomparison

Seawater samples for N₂ fixation rate measurements were dispensed into HCl-washed 4.5 L polycarbonate bottles that were sealed with septa and amended with ¹⁵N₂-enriched seawater (Mohr et al., 2010; Wilson et al., 2012), hereafter called the ¹⁵N₂ dissolution method. Briefly, the ¹⁵N₂-enriched seawater was prepared from 0.2 μm-filtered seawater (Sartobrand (Sartorius) cartridges) collected from the same site in a 4.5 L polycarbonate HCl-washed bottle. Seawater was first degassed through a degassing membrane (Membrana, Minimodule[®], flow rate fixed at 450 mL min⁻¹) connected to a vacuum pump (< 200 mbar) for at least 1 h. The bottle was then closed with a septum cap and amended with 1 mL of ¹⁵N₂ (98.9 atom% ¹⁵N, Cambridge Isotopes Laboratories, Inc) per 100 mL of seawater. The bottle was shaken vigorously to fragment the ¹⁵N₂ bubble, and incubated overnight at 20 m depth at the study site (3 bars) to promote ¹⁵N₂ dissolution. The experiment bottles were amended with 5 % vol:vol ¹⁵N₂ enriched seawater (i.e. 225 mL), sealed without headspace with silicon septum caps, and incubated for 24 h on an in situ mooring line located close to the mesocosms at the appropriate sampling depth. After 24 h, 12 mL of the incubated seawater were subsampled into Exetainers[®]. These were preserved upside down in the dark at 4 °C and analyzed less than 6 months after the experiment using a Membrane Inlet Mass Spectrometer (MIMS) (Kana et al., 1994) to quantify the ¹⁵N enrichment of the N₂ pool

were spiked with 4 mL $^{15}\text{N}_2$ (98.9 atom% ^{15}N , Cambridge isotopes Laboratories, Inc) via a gas-tight syringe, hereafter called the bubble method. Each bottle was shaken 20 times to fragment the $^{15}\text{N}_2$ bubble and facilitate its dissolution. The six remaining bottles were treated as described above for the dissolution method. The twelve bottles were then incubated for 24 h in an on-deck incubator at irradiances corresponding to the sampling depth using screening, and cooled with circulating surface seawater.

A recent study (Dabundo et al., 2014) reports potential contamination of some commercial $^{15}\text{N}_2$ gas stocks with ^{15}N -enriched NH_4^+ , NO_3^- and/or nitrite (NO_2^-), and nitrous oxide (N_2O). Dabundo et al. (2014) analysed various brands of $^{15}\text{N}_2$ gas and found that the Cambridge Isotopes stock (i.e., the one used in this study) contained low concentrations of ^{15}N contaminants, and the potential overestimation of N_2 fixation rates modeled using this contamination level would range from undetectable to $0.02 \text{ nmolNL}^{-1} \text{ d}^{-1}$. The rates measured in this study ranged from 0.5 to $69.6 \text{ nmolNL}^{-1} \text{ d}^{-1}$ suggesting that, if present, stock contamination of the magnitude reported by (Dabundo et al., 2014) would be too low to affect the results described here. To verify this, we analyzed one of our Cambridge Isotopes batches and found that the contamination of the $^{15}\text{N}_2$ gas stock was $1.4 \times 10^{-8} \text{ mol of } ^{15}\text{NO}_3^- \text{ mol}^{-1} \text{ of } ^{15}\text{N}_2$, and $1.1 \times 10^{-8} \text{ mol NH}_4^+ \text{ mol}^{-1} \text{ of } ^{15}\text{N}_2$. The application of this contamination level to our samples using the model described in Dabundo et al. (2014) indicates that our rates may only be overestimated by $\sim 0.05\%$ and confirmed that our present results were unaffected by possible $^{15}\text{N}_2$ stock contamination.

2.3 Phenotypic characterization of UCYN by microscopy

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 $2 \mu\text{m}$ 47 mm polycarbonate filters that were fixed with paraformaldehyde (2% final concentration) and incubated for 15 min at room temperature, then stored at -80°C until microscopic analysis. Formalin-fixed sediment trap

BGD

12, 19579–19626, 2015

Results from the VAHINE mesocosm experiment

S. Bonnet et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



**Results from the
VAHINE mesocosm
experiment**

S. Bonnet et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

5 samples were homogenized and 2 mL were filtered onto 2 μm polycarbonate filters. To
characterize the phenotype of UCYN (free living cells vs. colonies) in the mesocosms
as a function of depth, we performed a detailed microscopic analysis on days 17 and 19
in M2 (during the bloom of UCYN-C and during the DDN transfer experiment described
below). Filtered samples from each depth (1, 6, and 12 m) and from the sediment traps
(~ 15 m) were visualized using a Zeiss Axioplan (Zeiss, Jena, Germany) epifluores-
cence 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
10 as a region of interest (ROI) using an in-house imageJ script. The photographs were
then 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 deter-
mined from the average volume of the ROI represented by only one cell. The resultant
15 cell volume was then used to compute the number of cells in each aggregate.

2.4 Quantification of diazotrophs using qPCR in sediment traps and during the DDN transfer experiment

During the bloom of UCYN-C (days 17 and 19), immediately after sediment trap sam-
ples were collected and prior to their fixation with formalin, trap material was homoge-
nized and fresh aliquots of 1 mL were subsampled from each jar (trap from M1, M2, and
M3) and filtered onto 0.2 μm Supor (Pall-Gelman) filters, flash frozen in liquid N_2 , and
stored at -80°C until analysis. For the DDN experiment (see below), after each incuba-
tion period, 2 L from each triplicate ^{13}C and $^{15}\text{N}_2$ -labeled 4.5 L bottle were subsampled
and filtered through 0.2 μm Supor (Pall-Gelman) filters using gentle peristaltic pump-
ing, and stored as described above. The abundance of eight diazotrophic phylotypes
25 was determined using Taqman[®] qPCR assays: unicellular cyanobacterial groups A1
(UCYN-A1, Church et al., 2005), A2 (UCYN-A2, Thompson et al., 2014), B (UCYN-B or
Crocospaera spp., Moisaner et al., 2010), and C (UCYN-C, Foster et al., 2007), the

**Results from the
VAHINE mesocosm
experiment**

S. Bonnet et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

filamentous, colonial cyanobacteria *Trichodesmium* spp. (Church et al., 2005), *Richelia* associated with both *Rhizosolenia* (het-1, Church et al., 2005) and *Hemiaulus* (het-2, Foster et al., 2007) diatoms, *Calothrix* associated with *Chaetoceros* (het-3, Foster et al., 2007), as well as a heterotrophic phylotype of gamma proteobacteria (γ -24474A11, Moisaner et al., 2008). All procedures are described extensively in the companion paper by (Turk-Kubo et al., 2015). Briefly, DNA was extracted using a Qiagen DNeasy kit with modifications to recover high quality genomic DNA from cyanobacteria including a freeze thaw step, agitation and a proteinase K digestion. Extracts were tested for the presence of inhibitors using either the UCYN-B or UCYN-C qPCR assay, and if recovery of the spiked qPCR standard was $< 98\%$, the sample was considered inhibited, and diluted 1 : 10 with 5kD filtered milliQ water. All extracts from the sediment traps showed inhibition when undiluted, and no inhibition when diluted 1 : 10. DNA extracts from the DDN transfer experiment showed no inhibition. All qPCR reactions were carried out on diluted extracts as described in (Goebel et al., 2010). The limit of detection (LOD) and limit of quantitation (LOQ) was 250 and 2000 *nifH* copies mL⁻¹, respectively, for the sediment trap samples. The LOD and LOQ for DDN transfer experiment samples was 29 and 229 *nifH* copies L⁻¹, respectively.

2.5 DDN transfer experiment and nanoSIMS analyses

The fate of the fixed N₂ during the UCYN-C bloom was investigated on days 17 to 20 in M2 at 6 m. In addition to N₂ fixation measurements, seawater was sampled as described above into twelve additional 4.5 L HCl-washed polycarbonate bottles equipped with septum caps. Full bottles were immediately amended with the dissolved ¹⁵N₂ gas (98.9 atom% ¹⁵N, Cambridge Isotopes Laboratories, Inc) as described above (dissolution method), and with 1 mL of 80 g L⁻¹ NaH¹³CO₃ solution (99 atom% ¹³C, Cambridge Isotopes Laboratories, Inc) and incubated in situ on the mooring line at the appropriate sampling depth close to the mesocosms. After 24, 36, and 72 h of incubation (hereafter referred to as T24, T36, and T72 h), three replicate ¹⁵N₂ labelled bottles were recovered from the mooring line and subsampled for the analysis of bulk N₂ fixation rates,

DDN released in the dissolved pool, abundance of targeted diazotrophs using qPCR as described above, picophytoplankton and bacterial counts, and nanoSIMS analyses on UCYN-C and non-diazotrophs (diatoms and the 0.2–2 μm fraction) to assess the DD¹⁵N transfer from diazotrophs to non-diazotrophs. Three 4.5 L bottles were kept as unamended controls (i.e., without ¹⁵N₂ addition) and were immediately subsampled for the same parameters.

Net release of DDN to the N dissolved pool. After each incubation period, 60 mL from each ¹⁵N₂-labeled 4.5 L bottle were subsampled and filtered through pre-combusted (4 h, 450 °C) GF/F filters and immediately frozen for later quantification of ¹⁵N release (i.e., DDN release) to the total dissolved N (TDN; i.e., the sum of NO₂⁻, NO₃⁻, NH₄⁺, and DON) pool. The dissolved N was oxidized to NO₃⁻ using the persulfate oxidation method (Knapp et al., 2005) with amendments of Fawcett et al. (2011). Briefly, 1 mL of potassium persulfate oxidizing reagent (POR) was added to duplicate 5 mL aliquots of each subsample in 12 mL pre-combusted glass Wheaton vials, and to triplicate vials containing varying quantities of two L-glutamic acid standards, USGS-40 and USGS-41 (Qi et al., 2003) used to ensure complete oxidation and quantify the POR-associated N blank. The POR was made by dissolving 6 g of sodium hydroxide and 6 g of four-times recrystallized, methanol-rinsed potassium persulfate in 100 mL of ultra-high purity water (DIW). Sample vials were capped tightly after POR addition, and autoclaved at 121 °C for 55 min on a slow-vent setting. The entire oxidation protocol was performed in duplicate (yielding a total of 4 oxidized aliquots for each subsample).

The denitrifying bacteria (see below) are extremely sensitive to pH; care was thus taken to lower sample pH to 7–8 after oxidation via the addition of 12N ACS grade HCl. The concentration of the resultant NO₃⁻ (i.e., TDN + the POR-associated N blank) was measured via chemiluminescent analysis (Braman and Hendrix, 1989), after which the TDN isotopic composition was determined using the “denitrifier method”, wherein denitrifying bacteria that lack N₂O reductase quantitatively convert sample NO₃⁻ to N₂O (Casciotti et al., 2002; Sigman et al., 2001). The ¹⁵N enrichment of the N₂O was measured by GC-IRMS using a Delta V isotope ratio mass spectrometer and custom-built

BGD

12, 19579–19626, 2015

Results from the VAHINE mesocosm experiment

S. Bonnet et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



on-line N₂O extraction and purification system. The international reference materials, IAEA-N3, USGS-34, USGS-32, and an in-house N₂O standard were run in parallel to monitor bacterial conversion and mass spectrometry, and each oxidized sample was analyzed twice. The final TDN concentration and ¹⁵N atom% were corrected for the N blank associated with the POR. The DDN released to the TDN pool was calculated according to: ¹⁵N release (nmolL⁻¹ d⁻¹) = (¹⁵N_{ex} × TDN_{con})/N_{sr}, where ¹⁵N_{ex} is the atom% excess of the TDN for a given time point; the TDN_{con} is the TDN concentration measured at each time point, and N_{sr} is the ¹⁵N enrichment of the source pool (N₂) in the experimental bottles (i.e., 2.4 ± 0.2 atom% ¹⁵N; see above).

Picophytoplankton and bacteria counts

After each incubation period, 3.6 mL from each ¹⁵N₂-labeled 4.5 L bottle were subsampled into cryotubes, fixed with paraformaldehyde (2% final concentration), flash frozen in liquid N₂, and stored at -80 °C until analysis. Flow cytometry analyses were carried out at the PRECYM flow cytometry platform (<https://precy.mio.univ-amu.fr/>). Samples were analyzed using a FACSCalibur (BD Biosciences, San Jose, CA). For heterotrophic bacterial abundance (BA), 1.8 mL of seawater was fixed with formaldehyde (2% final concentration, 15 min 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 min 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 (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

BGD

12, 19579–19626, 2015

Results from the VAHINE mesocosm experiment

S. Bonnet et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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 ($< 2 \mu\text{m}$) from nanoplankton ($> 2 \mu\text{m}$) populations. The flow rate was estimated by weighing 3 tubes of samples before and after a 3 min 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).

Microscopic cell counts

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

nanoSIMS analyses

After each incubation period (24, 36 and 72 h), 250 mL from each labeled 4.5 L bottle were subsampled, fixed with 25 mL of paraformaldehyde (2% final concentration) and incubated for 24 h at 4 °C, then filtered successively through 25 mm diameter 10, 2, and 0.2 μm pore size polycarbonate filters and rinsed with 0.2 μm filtered seawater. All filters were then sputtered with gold and palladium to ensure conductivity prior to nanoSIMS analyses. Diatoms and UCYN-C were analysed on the 10 μm filters, and the picoplanktonic (0.2–2 μm) fraction was analysed on the 0.2 μm filters. Several analyses

BGD

12, 19579–19626, 2015

Results from the VAHINE mesocosm experiment

S. Bonnet et al.

[Title Page](#)

[Abstract](#)

[Introduction](#)

[Conclusions](#)

[References](#)

[Tables](#)

[Figures](#)

[⏪](#)

[⏩](#)

[◀](#)

[▶](#)

[Back](#)

[Close](#)

[Full Screen / Esc](#)

[Printer-friendly Version](#)

[Interactive Discussion](#)



Results from the VAHINE mesocosm experiment

S. Bonnet et al.

[Title Page](#)

[Abstract](#)

[Introduction](#)

[Conclusions](#)

[References](#)

[Tables](#)

[Figures](#)



[Back](#)

[Close](#)

[Full Screen / Esc](#)

[Printer-friendly Version](#)

[Interactive Discussion](#)



ter et al., 2011, 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 atom% of the individual cells measured by nanoSIMS after 24 h of incubation; the N_{con} is the N content of each cell determined as described below, and N_{sr} is the ^{15}N enrichment of the source pool (N_2) in the experimental bottles (i.e. 2.4 ± 0.2 atom% ^{15}N in this experiment). The cell-specific N assimilation rate was then multiplied by the cell number enumerated for each group of phytoplankton and bacteria by microscopy and flow cytometry. Standard deviations were calculated using the variability of ^{15}N enrichment measured by nanoSIMS on replicate cells and the standard deviation of the estimated cellular N content (see below) of UCYN-C, non-diazotrophic phytoplankton, and bacteria. Final standard deviations were calculated according to propagation of errors laws.

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

2.6 Statistical analyses

Spearman correlation coefficients were used to examine the relationships between N₂ fixation rates, hydrological, biogeochemical, and biological variables in the mesocosms ($n = 57$ to 61 , $\alpha = 0.05$). The methods used to analyze the parameters reported in the correlation table are described in detail in companion papers in this issue (Berthelot et al., 2015b; Bonnet et al., 2015a; Leblanc et al., 2015; Turk-Kubo et al., 2015).

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

3 Results

3.1 N₂ fixation rates in the mesocosms

Bulk N₂ fixation rates averaged 18.5 ± 1.1 nmolNL⁻¹ d⁻¹ over the 23 days of the experiment in the three mesocosms (all depths averaged together) (Table 1). The variance between the three mesocosms was low, and the temporal dynamics of the rates were similar (Fig. 2, Table 1), indicating good replicability between the mesocosms. Three main periods were identified based on the N₂ fixation dynamics; these were also identified by Berthelot et al. (2015b) based on biogeochemical characteristics and by Turk-Kubo et al. (2015) based on changes in abundances of targeted diazotrophs. During the first period (P0; from day 2 to 4, i.e., prior to the DIP fertilization), the average bulk N₂ fixation rate for the three mesocosms was 17.9 ± 2.5 nmolNL⁻¹ d⁻¹ (Fig. 2a). These N₂ fixation rates decreased significantly ($p < 0.05$) by ~ 40 % from day 5 to ~ 15 (hereafter called P1) to 10.1 ± 1.3 nmolNL⁻¹ d⁻¹, then increased significantly ($p < 0.05$) from day

BGD

12, 19579–19626, 2015

Results from the VAHINE mesocosm experiment

S. Bonnet et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



15 until the end of the experiment (day 15 to 23, hereafter called P2) to an average of $27.3 \pm 1.0 \text{ nmolNL}^{-1} \text{ d}^{-1}$ (Fig. 2a). Maximum rates were reached during P2 (between days 18 and 21) with 69.7, 67.7 and $60.4 \text{ nmolNL}^{-1} \text{ d}^{-1}$ in M1 (12 m), M2 (6 m) and M3 (12 m), respectively. From day ~ 15 to 21, N_2 fixation rates were higher at 12 m depth than in the surface. The difference was significant in M2 and M3 ($p < 0.05$), but not in M1 ($p > 0.05$). Size fractionation experiments indicate that $37 \pm 7\%$ of the measured N_2 fixation was associated with the $< 10 \mu\text{m}$ size fraction (Fig. 2b), and N_2 fixation rates in this fraction followed the same temporal trend as bulk N_2 fixation. These data indicate that for the experiment as a whole, the majority ($\sim 63\%$) of the N_2 fixation was associated with the $> 10 \mu\text{m}$ fraction. N_2 fixation rates measured in the lagoon waters were half those measured in the mesocosms, and were on average $9.2 \pm 4.7 \text{ nmolNL}^{-1} \text{ d}^{-1}$ over the 23 days of the experiment.

The Spearman correlation matrix (Table 2) indicates that N_2 fixation was positively correlated with seawater temperature in the mesocosms, which was not the case in lagoon waters, although temperature was exactly the same inside and outside the mesocosms (from 25.4°C to 26.8°C) (Bonnet et al., 2015a). N_2 fixation in the mesocosms was also positively correlated with particulate organic carbon (POC), particulate organic nitrogen (PON), and particulate organic phosphorus (POP) (except in M2) concentrations, Chl *a* concentrations, primary production, bacterial production, alkaline phosphatase activity (APA), and *Synechococcus*, picoeukaryote and nanoeukaryote (except in M2) abundances. N_2 fixation was negatively correlated with NO_3^- , DIP, DON, dissolved organic phosphorus (DOP) (except in M2) concentrations, and DIP turn-over time.

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

BGD

12, 19579–19626, 2015

Results from the VAHINE mesocosm experiment

S. Bonnet et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



3.2 Phenotypic characterization of UCYN by microscopy

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

3.3 Quantification of diazotrophs in sediment traps

qPCR analysis confirmed that UCYN-C was the most abundant diazotroph in the sediment traps on days 17 and 19, with abundances reaching 2.7×10^8 to 4×10^9 *nifH* copies L^{-1} (Fig. 4a). UCYN-C accounted for 97.4 to 99.2 % of the total *nifH* pool quantified in the traps. Abundances were higher in M2 and M3 (1.8×10^9 in M2 and 3×10^9 *nifH* copies L^{-1} in M3) compared to M1 (2.5×10^8 *nifH* copies L^{-1}) on day 19. Het-1 and het-3 were always recovered in the sediment traps, albeit at lower abundances (1.8 to 8.6×10^6 *nifH* copies L^{-1} for het-1 and 4.9×10^6 to 2.8×10^7 *nifH* copies L^{-1} for het-3) (Fig. 4b). They represented between 0.1 and 1.8 % of the targeted *nifH* pool. UCYN-B was often detected (except in M1 day 19), and UCYN-A2 and *Trichodesmium* were detected in M2 on day 17 but at low abundances (0.05 % of the total *nifH* pool) compared to the other phylotypes. Het-2 was never detected in the traps, and neither was $\gamma 24774\text{A}11$ or UCYN-A1.

Results from the VAHINE mesocosm experiment

S. Bonnet et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Using the volume of each mesocosm (Bonnet et al., 2015a) and the total *nifH* copies for each diazotroph phylotype in the water column (Turk-Kubo et al., 2015) and in the sediments, we were able to calculate the export efficiency for each phylotype: for UCYN-C, 10.0 and 9.5 % of the cells present in the water column were exported per 24 h in the traps on day 17 and 19, respectively (assuming one *nifH* copy per cell). For het-1, 0.2 % of cells were exported into the traps on both days, for het-3, 3.2 and 4.7 % were exported, and for UCYN-B, 3.7 and 45.6 % of UCYN-B were exported on day 17 and 19, respectively. These results indicate that UCYN were more efficiently exported than DDAs in this experiment.

3.4 DDN transfer experiment performed on day 17

Net N₂ fixation rates were $24.1 \pm 2.8 \text{ nmolNL}^{-1} \text{ d}^{-1}$ during the first 24 h of the DDN transfer experiment performed from days 17 to 20 (Fig. 5a). Rates decreased at 48 h to $19.2 \pm 2.8 \text{ nmolNL}^{-1} \text{ d}^{-1}$ and increased to $42.2 \pm 11.8 \text{ nmolNL}^{-1} \text{ d}^{-1}$ at T72 h. The DDN released to the TDN pool ranged from $6.2 \pm 2.4 \text{ nmolNL}^{-1} \text{ d}^{-1}$ at T24 h to $3.2 \pm 0.5 \text{ nmolNL}^{-1} \text{ d}^{-1}$ at T72 h. Considering gross N₂ fixation as the sum of net N₂ fixation and DDN release (Mulholland et al., 2004), the DDN released to the TDN pool accounted for 7.1 ± 1.2 to 20.6 ± 8.1 % of gross N₂ fixation.

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

NanoSIMS analyses performed on individual UCYN-C at 24 h (Fig. 6) revealed significant ($p < 0.05$) ¹³C (1.477 ± 0.542 atom%, $n = 35$) and ¹⁵N (1.515 ± 0.370 atom%, $n = 35$) enrichments relative to natural abundance, indicating that UCYN-C were ac-

BGD

12, 19579–19626, 2015

Results from the VAHINE mesocosm experiment

S. Bonnet et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



tively photosynthesizing and fixing N_2 . The correlation between ^{13}C enrichment and ^{15}N enrichment was significant ($r = 0.85$, $p < 0.01$, Fig. 6b). NanoSIMS analyses performed on diatoms and picoplankton (Fig. 5c) also revealed significant ($p < 0.05$) ^{15}N enrichment of non-diazotrophic plankton, demonstrating a transfer of DDN from the diatoms to other phytoplankton. Both diatoms and picoplanktonic cells were significantly ($p < 0.05$) more enriched at the end of the experiment (T72 h) (0.489 ± 0.137 atom%, $n = 12$ for diatoms; 0.457 ± 0.077 atom%, $n = 96$ for picoplankton) than after the first 24 h (0.408 ± 0.052 atom%, $n = 23$ for diatoms; 0.389 ± 0.014 atom%, $n = 63$ for picoplankton). Finally, the ^{15}N enrichment of picoplankton and diatoms was not significantly different ($p > 0.05$) during the DDN experiment.

4 Discussion

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

The inter-comparison experiment performed on day 11 reveals slightly lower, yet insignificantly different ($p > 0.05$), average N_2 fixation rates when using the bubble method compared to the dissolution method. This result is in accordance with some comparisons made by Shiozaki et al. (2015) in temperate waters of the North Pacific. However, one might expect a lower degree of dissolution of the $^{15}N_2$ bubble in warm tropical waters such as those near New Caledonia compared to the cooler, temperate North Pacific waters. In calculating N_2 fixation rates using the dissolution method, we used the value of 2.4 ± 0.2 atom% for the ^{15}N enrichment of the N_2 pool as measured by MIMS. For the bubble method, we used the theoretical value of 8.4 atom% calculated for seawater with a temperature of $25.5^\circ C$ and salinity of 35.3 (as was the case on day 11). If we assume that equilibration was incomplete in our experiment using the bubble method, i.e., 75 % instead of 100 % as shown by Mohr et al. (2010), we calculate higher N_2 fixation rates for the bubble method (8.3 ± 2.8 nmolNL $^{-1}$ d $^{-1}$) compared

BGD

12, 19579–19626, 2015

Results from the VAHINE mesocosm experiment

S. Bonnet et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



to the dissolution method ($7.2 \pm 0.8 \text{ nmolNL}^{-1} \text{ d}^{-1}$), although the difference is still not significant ($p > 0.05$).

4.2 The temporal dynamics of N_2 fixation in the mesocosms

Average N_2 fixation rates measured in the lagoon waters (outside the mesocosms, $9.2 \pm 4.7 \text{ nmolNL}^{-1} \text{ d}^{-1}$, Table 1) are of the same order of magnitude as those reported for the Noumea lagoon during austral summer conditions (Biegala and Raimbault, 2008). They are within the upper range of rates reported in the global ocean database (Luo et al., 2012). Indeed, open ocean cruises performed offshore of New Caledonia in the Coral and Solomon Seas (e.g., Bonnet et al., 2015b; Garcia et al., 2007) also suggest that the South West Pacific Ocean is one of the areas with the highest N_2 fixation rates in the global ocean.

In the mesocosms, rates were on average twice as high ($18.5 \pm 1.1 \text{ nmolNL}^{-1} \text{ d}^{-1}$) as those measured in lagoon waters ($9.2 \pm 4.7 \text{ nmolNL}^{-1} \text{ d}^{-1}$), with the maximum observed rates of $> 60 \text{ nmolNL}^{-1} \text{ d}^{-1}$ ranking among the highest reported for marine waters (Luo et al., 2012). The predominant difference between the ambient lagoon waters and those of the mesocosms were the modified DIP concentrations. The mesocosms were fertilized with DIP on day 4, reaching ambient concentrations of $\sim 0.8 \mu\text{molL}^{-1}$ compared to lagoon waters in which DIP concentrations were typically $< 0.05 \mu\text{molL}^{-1}$. According to our experimental assumption, diazotrophy would be promoted by high concentrations of DIP. Yet, in all three mesocosms, N_2 fixation rates were negatively correlated with DIP concentrations and DIP turnover time and positively correlated with APA, suggesting that DIP deficiency may have induced favorable conditions for N_2 fixation (Table 2). Below, we describe the scenario that likely occurred in the mesocosms, which explains these unexpected negative correlations.

During P0 (day 2 to 4), N_2 fixation rates were higher in the mesocosms than in the lagoon waters, possibly due to the reduction of turbulence in the water column facilitated by the closing of the mesocosms (Moisander et al., 1997). The most abun-

BGD

12, 19579–19626, 2015

Results from the VAHINE mesocosm experiment

S. Bonnet et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Table 2), and driving the significant decline in DOP concentrations observed in all three mesocosms during P2 (Berthelot et al., 2015b; Moutin et al., 2005).

While temperature was not correlated with N_2 fixation in the lagoon, in the mesocosms we observed a significant positive correlation between these parameters (Table 2), probably because some diazotrophic phylotypes present in the mesocosms and absent in the lagoon waters were particularly sensitive to seawater temperature. UCYN-C reached high abundances inside the mesocosms, but was virtually absent in the lagoon waters outside the mesocosms. Turk-Kubo et al. (2015) showed that UCYN-C abundance was positively correlated with seawater temperature, suggesting that the optimal temperature for UCYN-C growth is above 25.6 °C. This result is consistent with culture studies performed using three UCYN-C isolates from the Noumea lagoon that are closely related to the UCYN-C observed here, indicating maximum growth rates at around 30 °C and no growth below 25 °C (Camps, Turk-Kubo, Bonnet, personal communication, 2015). Temperature above 25.6 °C and up to 26.7 °C were reached since day 12 up to the end in the mesocosm experiment, possibly explaining why UCYN-C was not evident during P0 (when temperature 25.4 °C) even though DIP turn-over time was ~ 1d (Berthelot et al., 2015b; Moutin et al., 2005).

If low DIP concentrations (turn-over time less than 1 d) and seawater temperatures greater than 25.6 °C are prerequisites for UCYN-C growth, an obvious question is why they did not thrive (despite being present at low abundances) in the lagoon waters during P2 when similar conditions prevailed. Below, we consider three possible explanations that are discussed extensively in Turk-Kubo et al. (2015): first, it is possible that UCYN-C are sensitive to turbulence, which was likely reduced in the mesocosms compared to the lagoon waters that are susceptible to trade winds and tides. Second, grazing pressures on UCYN-C may have been reduced as total zooplankton abundances were slightly lower (by a factor of 1.6) in the mesocosms compared to those in the lagoon waters (Hunt et al., 2015). Third, the water masses outside the mesocosms changed with tides and winds, so it is possible that UCYN-C were absent from the water mass encountered outside the mesocosms when we sampled for this experiment.

Results from the
VAHINE mesocosm
experiment

S. Bonnet et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



and Malfatti, 2007). Their aggregation and subsequent sinking in the mesocosms likely explains why volumetric N_2 fixation rates were higher at 12 m than at the surface during P2, as well as why the size of the aggregates increased with depth, and why numerous large-size aggregates and extremely high abundances of UCYN-C were recovered in the sediment traps.

Colonial phenotypes of UCYN (UCYN-B) have been observed in the water column of the North Tropical Pacific (ALOHA station) (Foster et al., 2013), but to our knowledge, this is the first time that UCYN have been detected in sediment traps. Contrary to published data (e.g. White et al., 2012 we demonstrate a greater export efficiency of UCYN ($\sim 10\%$ exported to the traps within 24 h) compared to the export of DDAs (efficiency of 0.24 to 4.7%). Diatoms sink rapidly and DDAs have been found in sediment traps at Station ALOHA (Karl et al., 2012, 1997; Scharek et al., 1999a; Sharek et al., 1999b), in the Gulf of California (White et al., 2012), and in the Amazon River plume (Subramaniam et al., 2008). In our study, we observed limited export of het-1 (*Richelia* in association with *Rhizosolenia*) and het-3 (*Calothrix*) during P2, while het-2 (*Richelia* associated with *Hemiaulus*) was never recovered in the sediment traps. This is likely because *Hemiaulus* has a lower sinking rate than *Rhizosolenia* due to its smaller size, or may be more easily grazed by zooplankton than *Rhizosolenia* or *Calothrix*, which are known to be toxic to crustaceans (Höckelmann et al., 2009). *Trichodesmium* was very rarely exported in this study, probably due to its extremely limited presence and low growth rates in the mesocosms. Direct comparisons of our export results with findings from open ocean studies should be made cautiously as our mesocosms were both shallower (15 m) than typical oceanic export studies (> 100 m) and probably characterized by reduced turbulence (Moisander et al., 1997).

We estimate in M2 that the direct export of UCYN-C accounted for $22.4 \pm 5.5\%$ of the total POC exported in each mesocosm at the height of the UCYN-C bloom (day 17) and decreased to $4.1 \pm 0.8\%$ on day 19 (Fig. 4c, Fig. 7). This calculation is based on the total particulate organic C (POC) content measured in the sediment traps (Berthelot et al., 2015b), our C_{con} for UCYN-C estimated as described above, and published

BGD

12, 19579–19626, 2015

Results from the VAHINE mesocosm experiment

S. Bonnet et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



1.3 ± 0.2 % of gross N₂ fixation; Benavides et al., 2013; Berthelot et al., 2015a). In our experiment, other diazotrophs were present in addition to *Cyanothece*, and they may also have contributed to the dissolved pool in contrast to pure culture studies. Moreover, unlike culture studies, field experiments, are also impacted by other exogenous factors such as viral lysis (Fuhrman, 1999) and sloppy feeding (O'Neil and Roman, 1992; Vincent et al., 2007), which may enhance N release.

This DDN release plays a critical role in the N transfer between diazotrophs and non-diazotrophs. The cell-specific uptake rates of DDN during the DDN transfer experiment were calculated for each cell analysed by nanoSIMS (diatoms and cells from the 0.2–2 µm fraction). By multiplying cell-specific N uptake rates by the cellular abundance of each group on a particular day, we could identify the specific pool (diazotrophs, dissolved pool, non-diazotrophs) into which the DD¹⁵N was transferred after 24 h, and the extent to which this ¹⁵N₂ accumulated. The results are summarized in Fig. 7. After 24 h, 52 ± 17 % of the newly fixed ¹⁵N₂ remained in the UCYN-C biomass, 16 ± 6 % had accumulated in the dissolved N pool, and 21 ± 4 % had been transferred to non-diazotrophic plankton. In addition, 11 % of the newly fixed ¹⁵N₂ accumulated in a pool that we refer to as “others” (corresponding to diazotrophs other than UCYN-C and potential non-diazotrophs to which ¹⁵N₂ was transferred; these cells were not analysed by nanoSIMS due to their very low abundance). Uncertainties take into account both the variability of the ¹⁵N enrichment determined on ~ 25 cells per group by nanoSIMS, and the uncertainty in the N content per cell measured or taken from the literature.

Within the fraction of DDN transferred to the non-diazotrophs after 24 h (21 %), we calculated that 18 ± 4 % was transferred to picoplankton, and only 3 ± 2 % was transferred to diatoms (Fig. 7). The ¹⁵N enrichment of picoplankton and diatoms was not significantly different ($p > 0.05$) in this study, but as picoplankton dominated the planktonic community in the mesocosms at the time of the DDN transfer experiment, they were the primary beneficiaries of the DDN. This is consistent with the positive correlation between N₂ fixation rates, *Synechococcus* and pico-eukaryote abundances in the mesocosms (Table 2), as well as with the observed dramatic increase in *Synechococ-*

BGD

12, 19579–19626, 2015

Results from the VAHINE mesocosm experiment

S. Bonnet et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



2008). *Trichodesmium* is rarely recovered in sediment traps and most of the research dedicated to the export of diazotrophs has focused on DDAs (Karl et al., 2012) due to their high sinking velocity. Here, we demonstrate for the first time that UCYN can efficiently contribute (up to $22.4 \pm 5.5\%$ at the height of the bloom) to POC export in oligotrophic systems, indicating that these small (typically 3–7 μm) organisms should be considered in future studies. This export was predominantly due to the aggregation of small ($5.7 \pm 0.8 \mu\text{m}$) UCYN-C cells into large aggregates, increasing in size (up to 500 μm) with depth. Moreover, the experimental and analytical approach used in this study allowed for the quantification of the actual transfer of DDN to different groups of non-diazotrophic plankton in the oligotrophic ocean, and therefore reveals another level of complexity to the processes that occur between N_2 fixation and the eventual export of organic matter. A study based on high-resolution nanometer scale secondary ion mass spectrometry (nanoSIMS) coupled with $^{15}\text{N}_2$ isotopic labelling revealed that a significant fraction of DDN ($21 \pm 4\%$) is quickly (within 24 h) transferred to non-diazotrophic plankton, which increased in abundance simultaneously with N_2 fixation rates, a part of it having potentially contributed, in turn to export production.

Author contributions. S. Bonnet designed the experiments and S. Bonnet and H. Berthelot carried them out. S. Bonnet, H. Berthelot, K. A. Turk-Kubo, S. Fawcett and S. L'Helguen analyzed the samples. I. Berman-Frank took part in experimental planning, preparation, and implementation of the project. S. Bonnet prepared the manuscript with contributions from all co-authors.

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Results from the VAHINE mesocosm experiment

S. Bonnet et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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BGD

12, 19579–19626, 2015

Results from the VAHINE mesocosm experiment

S. Bonnet et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



**Results from the
VAHINE mesocosm
experiment**S. Bonnet et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

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BGD

12, 19579–19626, 2015

Results from the VAHINE mesocosm experiment

S. Bonnet et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Results from the VAHINE mesocosm experiment

S. Bonnet et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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Results from the VAHINE mesocosm experiment

S. Bonnet et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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Results from the VAHINE mesocosm experiment

S. Bonnet et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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Results from the VAHINE mesocosm experiment

S. Bonnet et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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Results from the VAHINE mesocosm experiment

S. Bonnet et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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Results from the VAHINE mesocosm experiment

S. Bonnet et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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Table 2. Spearman correlation matrix of N₂ fixation rates and hydrological parameters, biogeochemical stocks and fluxes, and planktonic communities ($n = 66$). The significant 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		

Results from the VAHINE mesocosm experiment

S. Bonnet et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion





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

BGD

12, 19579–19626, 2015

Results from the VAHINE mesocosm experiment

S. Bonnet et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Results from the
VAHINE mesocosm
experiment

S. Bonnet et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

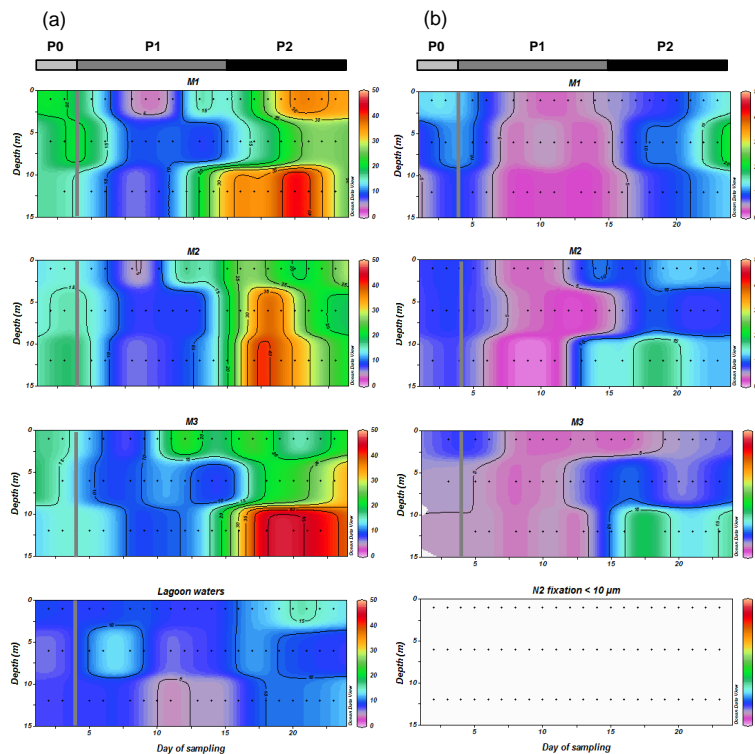


Figure 2. (a) Horizontal and vertical distributions of bulk N_2 fixation rates ($\text{nmol N L}^{-1} \text{d}^{-1}$), and (b) $< 10 \mu\text{m}$ N_2 fixation rates ($\text{nmol N L}^{-1} \text{d}^{-1}$) in M1, M2, M3, and lagoon waters. Note that N_2 fixation rates in the $< 10 \mu\text{m}$ fraction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

Results from the VAHINE mesocosm experiment

S. Bonnet et al.

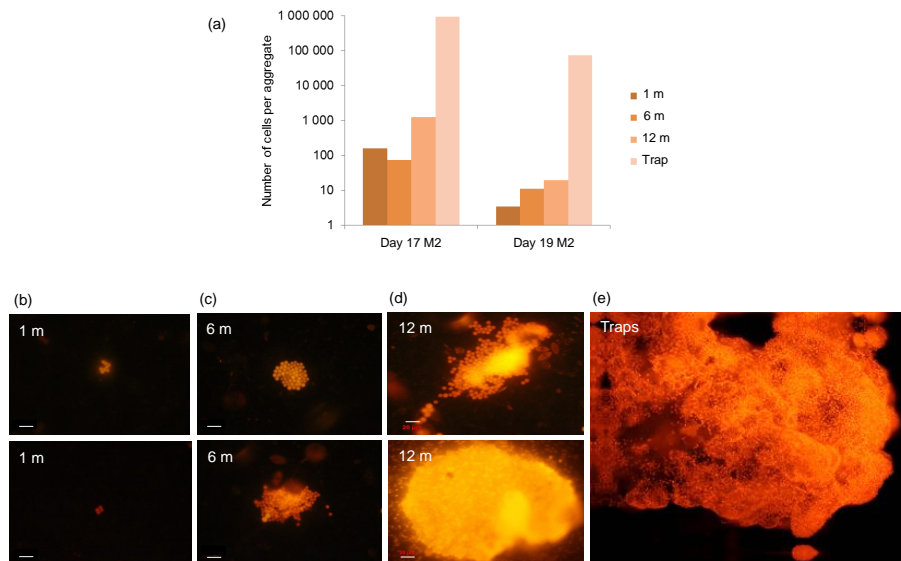


Figure 3. (a) UCYN-C cells per aggregate in M2 on day 17 and 19. (b to e) Green excitation (510–560 nm) epifluorescent replicate micrographs of UCYN-C on day 17 taken at 1 m depth (x40) (b), 6 m depth (x40) (c), 12 m depth (x40) (d), and in the sediment traps (x10) (e). Scale bar 20 μm (b to d) and 100 μm (e).

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Results from the
VAHINE mesocosm
experiment

S. Bonnet et al.

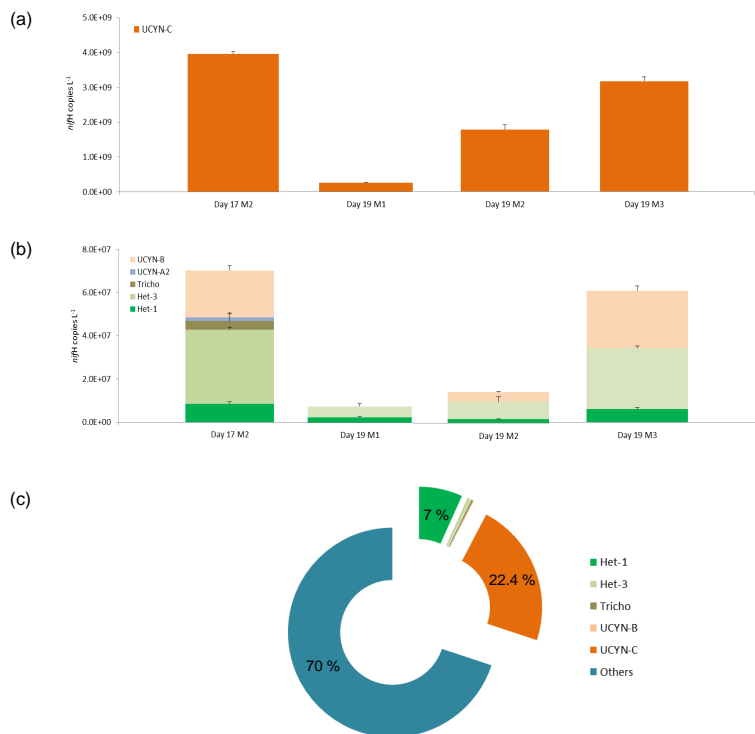


Figure 4. (a) Abundance of UCYN-C (*nifH* copies L⁻¹) and (b) other *nifH* phylotypes (UCYN-A2, UCYN-B, *Trichodesmium*, het-1, het-3) (*nifH* copies L⁻¹) recovered in the sediment trap on day 17 and 19. (c) Proportion of POC exported associated with diazotrophs in the sediment traps on day 17 in M2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

Results from the
VAHINE mesocosm
experiment

S. Bonnet et al.

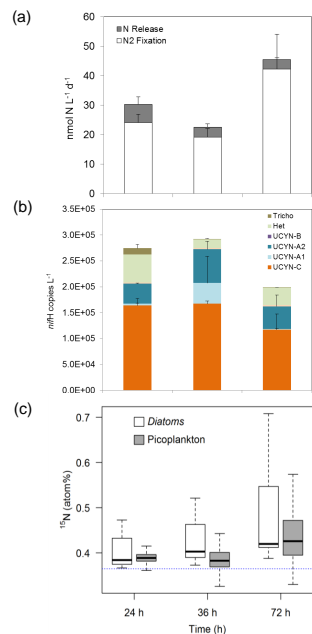


Figure 5. Results from the DDN transfer experiment performed from day 17 to 20 in M2. **(a)** Temporal changes in the rate ($\text{nmol N L}^{-1} \text{Nd}^{-1}$) of N_2 fixation (white) and DDN release (grey) over the course of the experiment. Error bars represent the standard deviation of three independent replicate incubations. **(b)** Temporal changes in diazotroph abundance determined by qPCR (*niifH* gene copies L^{-1}) during the same experiment. Error bars represent the standard deviation of triplicate incubations. **(c)** Summary of the nanoSIMS analyses. Measured ^{13}C and ^{15}N atom% values of non-diazotrophic diatoms (white) and picoplankton (grey) as a function of incubation time. The horizontal dashed line indicates the natural abundance of ^{15}N (0.366 atom%), and the error bars represent the standard deviation for the several cells analysed by nanoSIMS.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

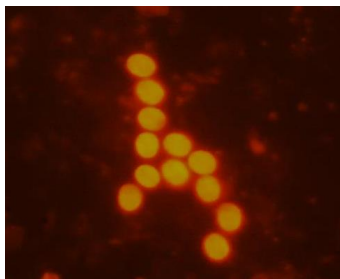
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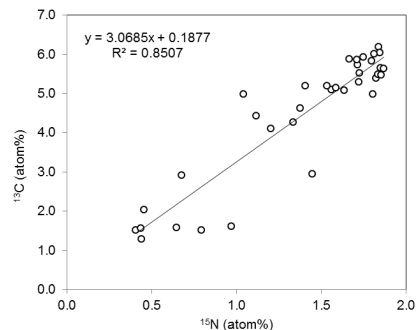
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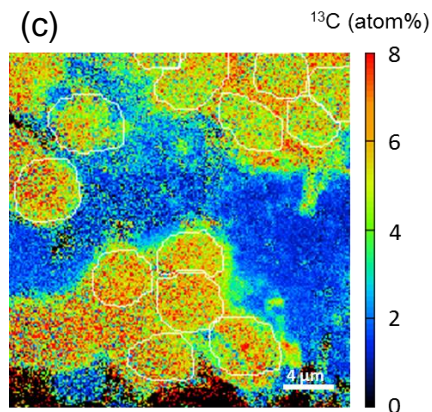
(a)



(b)



(c)



(d)

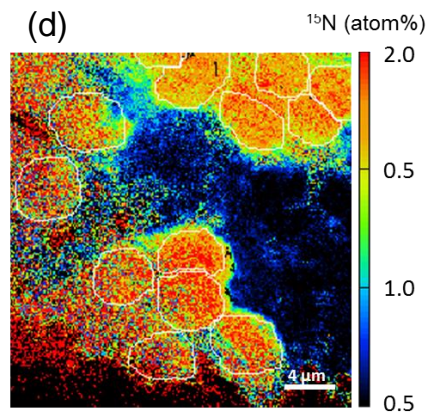


Figure 6. (a) Green excitation (510–560 nm) epifluorescent micrographs of UCYN-C, (b) ^{13}C and ^{15}N isotopic enrichment (atom%) in individual UCYN-C cells at day 17 in M2, (c, d) nanoSIMS images showing the ^{13}C (c) and ^{15}N (d) enrichment of individual UCYN-C cells after 24 h of incubation.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

Results from the
VAHINE mesocosm
experiment

S. Bonnet et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



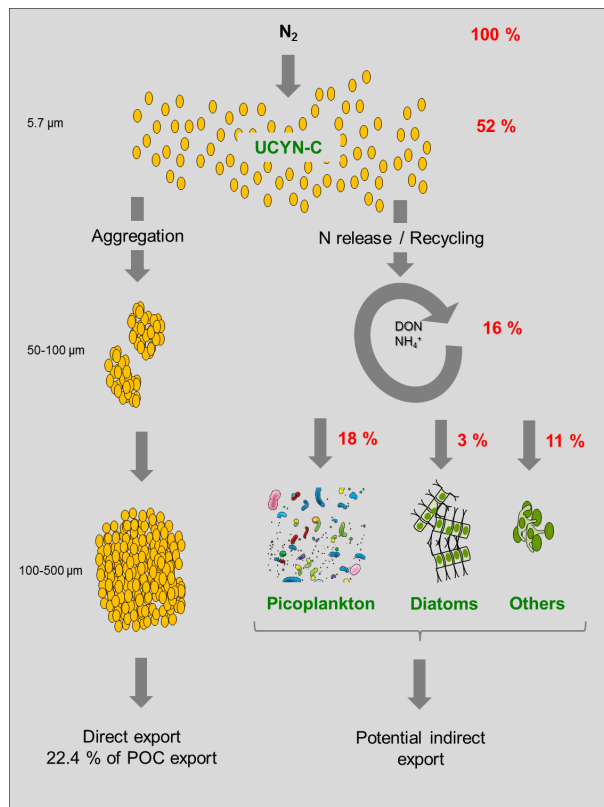


Figure 7. Cartoon summary of the simplified pathways of N transfer in the first trophic level of the food web and the potential impact on the sinking POC flux at the height of the UCYN-C bloom in the VAHINE mesocosm experiment.