Noumea, February 8th 2016; Marseille, February 11th 2016

Revisions [Manuscript bg-2015-615]

We thank both reviewers for very useful suggestions. We have addressed all of their concerns below, and in a revised manuscript. A point by point response to reviewers is below (comments are in italics with our replies below), and all new text in the corresponding manuscript is track change mode.

Reviewer 1.

While the article is generally well written and structured, several grammatical errors and instances of improper use of the English language (e.g. past perfect instead of simple past) detract from its quality. I have taken the liberty of going through the manuscript using "track changes" to provide suggested rewrites of these uncomfortable passages.
We are very grateful to the reviewer for providing such suggestions. They all have been taken into account in the revised version of the manuscript.

After what appears to have taken substantial exercise of the imagination, the authors came up with the acronym "VAHINE" choosing the appropriate lettering from the title (see above). It is puzzling then that the authors have not seen fit to explain, justify or even acknowledge this rather unusual acronym which, to my limited understanding of the Polynesian language, means "woman". Perhaps a sentence to that effect might be in order.
We agree that the acronym VAHINE is quite unusual. We have added a sentence page 3 line 30 to explain this choice: 'The acronym VAHINE (VAriability of vertical and tropHIc transfer of diazotroph derived N in the south wEst Pacific) was chosen in order to take reference to the Pacific culture where this experiment has been performed with the help of local people'. The project is also leaded by a woman and some readers might see a fully assumed feminist Act.

While it is understandable that collaborating authors will want to set forth their results in detail in their individual articles, the reader of this manuscript is left largely in the dark as to results of the experiment: the fate the N fixed through P stimulation. Once again, a brief sentence or two describing the major findings appears to be in order.
We decided not to provide one or two sentences of the major results after each scientific question, as it would require many explanations. We rather extended the section 4 of the manuscript 'Special issue presentation' and summarized the major results of each contributing paper to the special issue to provide the reader the main findings of this study. Section 4 has thus been totally modified.

Reviewer 2.

Page 3 line 32-34, Page 4, line 1-7 The four stated research questions could be better formulated. I would recommend removing the three subquestions or making them independent. Have you considered structuring them to indicate any priority in research? At the moment it sounds like you wanted to measure everything.

The three subquestions have been removed and the questions formulated as research priorities as follows: 'The main scientific research priorities of the project were:

i) To quantify the DDN which enters the planktonic food web,

ii) To investigate how the development of diazotrophs influences the subsequent diversity, gene expression, and production of primary producers, heterotrophic bacterioplankton, and subsequently the zooplankton abundance,

iii) To examine whether different functional types of diazotrophs significantly modify the stocks and fluxes of the major biogenic elements (C, N, P),

iv) To elucidate whether the efficiency of particulate matter export depends on the development of different functional types of diazotrophs.

You might also want to consider including a single sentence next to each of the research questions highlighting the results from the project to provide the reader with an immediate answer as to what was found.

Please see response to Reviewer 1: We decided not to provide one or two sentences of the major results after each scientific question, as it would require many explanations. We rather extended the section 4 of the manuscript 'Special issue presentation' and summarized the major results of each contributing paper to the special issue to provide the reader the main findings of this study. Section 4 has thus been totally modified.

Page 5 Line 7. I have not read the DUNE project work, but I recommend including some additional references to the use of mesocosms in ecological studies.

An additional sentence and aadditional references have been provided, among which a review paper on mesocosms (Stewart et al., 2013) page 5 line 15: 'Mesocosms are now widely used in ecological studies (Riebesell et al., 2013; Stewart et al., 2013) and enable isolation of water masses of several cubic meters from physical dispersion for several weeks…'.

The Stewart et al. reference has also been added page 5 line 25: 'Among the different types of mesocosms available (Stewart et al., 2013), the model of mesocosms chosen for this study (surface 4.15 m^2, volume ~50 m^3, Fig. 1) are sea-going mesocosms entirely transportable that can be used under low to moderate wind/wave conditions (20-25 knots/2.5 wave height). They have been designed in the framework of the DUNE project (Guieu et al., 2010; Guieu et al., 2014) and consist in large transparent bags made…'

Page 16 Line 18 A black and white map would be equally useful as the color images shown demarking the position of the mesoscom experiment. I realize its bland but can be effective.

Figure 3b has been replaced by a black and white map.

Page 7 Line 21 How was the concentration of DIP decided and what would have happened if you added more or less?

We decided to add 0.8 µM of phosphate because such concentrations have already been measured in the New Caledonian lagoon and pre-experiment modelling studies and were shown to be able to stimulate N_2 fixation. We knew that phosphate availability was the ultimate control of nitrogen inputs by N_2 fixation there (Moutin et al., 2005, 2008). Finally, simulated (thanks to the ECO3M model, see Gimenez et al., 2016, This issue) and experimental responses obtained after a 0.8 µM phosphate enrichment are close, and simulated response without phosphate enrichment show a very different and low response of the plankton community inside the mesocosms (Gimenez et al., 2016). The addition of more phosphate might not change so much the short term (20 days) response of the system because the time of the response is mainly controlled by the lag time between change in growth rate at the cell level and change in growth rate at the population level (Gimenez et al., 2016). The

following sentence has been added to the text: 'Such concentrations have already been measured in the New Caledonian lagoon and were shown to be able to stimulate N_2 fixation. The amount of DIP added was also chosen based on the modelling work performed by Gimenez et al. (2016), confirming a clear stimulation of N_2 fixation by 0.8 µmol L^{-1} DIP in our experimental systems, and an absence of stimulation without any DIP enrichment.

Did you consider also adding iron or something micro-nutrient?
New Caledonian soils are very rich in metals. A third of its surface (5500 km^2) is covered by soils originating from ultramafic rocks (peridotites and serpentinites) which have exceptionally high levels of metals such as Fe, Ni, Cr, Co, and Mn. Lagoon waters are thus rich is metals as well and Fe in particular is not limiting for phytoplankton growth. We thus decided not to supplement the mesoscosm with trace metals. The following sentence has been added page 8 line 31: 'New Caledonian soils are very rich in metals. A third of its surface (5500 km^2) is covered by soils originating from ultramafic rocks which have exceptionally high levels of metals such as Fe, Ni, Cr, Co, and Mn (Jaffré, 1980). Consequently, dissolved trace metals are particularly abundant in the Noumea lagoon (Migon et al., 2007). Iron concentrations measured during the Diapalis cruises around New Caledonia were higher than those reported in the sub-tropical North Pacific and the high iron inputs in this region are hypothesized to drive the South West Pacific towards a DIP depletion (Van Den Broeck et al., 2004). Metals were thus not supplemented to the mesocosms'.

Page 8 Line 30 Why did you stop sampling after 23 days when you had the maximum fluorescence? I realize this probably relates to logistics, but clearly there were still changes occurring inside the bags that would have been good to capture.
We totally agree with this comment, it would have been very interesting to sample after day 23 as we reached the maxima of fluorescence at that period. The R/V Alis was assisting the project and for logistical reasons (time ship allocated to the project), it was planned in advance that the experiment could only last for 23 days. The Eco3M model platform (companion paper from Gimenez et al., 2016) was used to simulate carbon export up to day 35 and shows that C export greatly increased after day 23.

Figures: I am not sure that Figure 5a-c is necessary. I am not a big fan of the ocean data view color palette. For example it is difficult to interpret changes in fluorescence.
Nitrate concentrations were close to quantification limits during the course of the experiment, indicating that other sources of nitrogen (among which N_2 fixation) were providing the major sources of nitrogen to the system. We believe it is important to keep the nitrate plots in Figure 5 to inform the reader of the nitrate deficiency in the system with respect to phosphate.

Literature cited

Gimenez, A., Baklouti, M., Bonnet, S., and Moutin, T.: Biogeochemical fluxes and fate of diazotroph derived nitrogen in the food web after a phosphate enrichment: Modeling of the VAHINE mesocosms experiment, Biogeosciences Discussions, doi: doi:10.5194/bg-2015-611, 2016. 2016.

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Introduction to the project VAHINE: VAriability of vertical and tropHIc transfer of diazotroph derived N in the south wEst Pacific

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Abstract

At the global scale, N_2 fixations provides the major external source of reactive nitrogen to the surface ocean, surpassing ~~before~~ atmospheric and riverine inputs, and sustains ~50 % of new primary production in oligotrophic environments. The main goal of the VAHINE project was to study the fate of nitrogen newly fixed by diazotrophs (or diazotroph-derived nitrogen) in oceanic food webs, how it impact heterotrophic bacteria, phytoplankton and zooplankton dynamics, stocks and fluxes of biogenic elements and particle export. Three large-volume (~50 m3) mesocosms were deployed in a tropical oligotrophic ecosystem (the New Caledonia lagoon, south-eastern Pacific) and intentionally fertilized with ~0.8 µM of dissolved inorganic phosphorus (DIP) to stimulate diazotrophy and follow subsequent ecosystem and fluxes changes. VAHINE was a multidisciplinary project involving close collaborations between biogeochemists, molecular ecologist, chemists, marine opticians and modelers. This introductory paper describes in detail the scientific objectives of the project as well as the implementation plan: the mesocosm description and deployment, the selection of the study site (New Caledonian lagoon) and the logistical and sampling strategy. The ~~description of the~~ main hydrological and biogeochemical conditions of the study site before the mesocosms deployment and during the experiment itself ~~is then detailed~~are described, and a general overview of the papers published in this special issue is presented.

1 General context and objectives of the VAHINE project

Climate change is now widely recognized as the major environmental problem facing the globe (IPCC, 2014) and is at the heart of human, environmental and economical issues. On a global scale, the oceanic biological carbon pump (BCP) influences climate trends: it consists of the photosynthetic fixation of carbon dioxide (CO_2) by oceanic algae (phytoplankton) in the upper illuminated ocean, followed by the downward flux of some of this material mainly due to gravitational settling. The BCP transfers approximately 5-15 GT of carbon (C) from the surface ocean to the oceans interior every year (Henson et al., 2011).

The efficiency of our oceans to take up excess CO_2 largely depends on the availability of fixed nitrogen (N) (Falkowski, 1997) in the surface ocean. In the vast nitrate (NO_3^-)-limited oligotrophic gyres, which cover ~60 % of the global ocean surface, fixed N is principally provided through the biological fixation of atmospheric dinitrogen (N_2) by N_2-fixing (or diazotrophic) organisms (Karl et al., 2002). Diazotrophs fix N_2 gas dissolved in seawater (the largest reservoir of N on Earth) into ammonium and organic N compounds. At the global scale, they provide the major external source of N for the ocean, surpassing ~~before~~ atmospheric and riverine inputs (Gruber, 2004), and act thus as 'natural fertilizers', contributing to sustain life and the BCP through the so called 'N_2-primed prokaryotic C pump' (Karl et al., 2003; Karl et al., 2012).

Important progress on the magnitude and the ecological role of marine N_2 fixation in biogeochemical cycles has been made by the international oceanographic community over the last two decades. They include the landmark discovery of unicellular diazotrophic organisms of pico- and nanoplanktonic size termed UCYN, e.g. (Zehr et al., 2001), and new and unexpected ecological niches where diazotrophs are active, such as N-rich oxygen minimum zones, e.g. (Dekaezemacker et al., 2013; Fernandez et al., 2011). Thus, we have gained a much better understanding of this process. However, a critical question that remains poorly studied is the fate of N newly fixed by diazotrophs (or diazotroph derived N, hereafter referred to as DDN) in oceanic food webs, and its impact on CO_2 uptake and export (BCP) (Mulholland, 2007). The VAHINE project proposes a scientific contribution to answer these questions, based on a combination of experimentation and model~~l~~ing involving recently developed innovative techniques. The acronym VAHINE (VAriability of vertical and tropHIc transfer of diazotroph derived N in the south wEst Pacific) was chosen in order to take reference to the Pacific culture where this experiment has been performed with the help of local people. The main scientific research priorities~~questions~~ of the ~~VAHINE~~ project were:

i) To quantify the DDN which enters the planktonic food web,

ii) To investigate how the development of diazotrophs influences the subsequent diversity, gene expression, and production of primary producers, heterotrophic bacterioplankton, and subsequently zooplankton abundance,

iii) To examine whether different functional types of diazotrophs significantly modify the stocks and fluxes of the major biogenic elements (C, N, P),

iv) To elucidate whether the efficiency of particulate matter export depends on the development of different functional types of diazotrophs.

~~i) What is the primary route of transfer of DDN through the planktonic food web, i.e. is DDN preferably transferred to large size (e.g. diatoms), small size (pico-, nanophytoplankton) phytoplankton, or to the microbial food web? How much DDN is transferred to zooplankton?~~
~~ii) Does the development of diazotrophs influence auto- and heterotrophic plankton diversity and gene expression dynamics, as well as pico-, nano-, and microphytoplankton abundances? Do they influence zooplankton dynamics?~~
~~iii) Does the development of diazotrophs significantly modify the stocks, fluxes, ratios of the major biogenic elements (C, N, P)?~~
~~iv) Does the development of diazotrophs influences the efficiency of carbon export? Is this export direct or indirect?~~

Summarized conclusions of each article composing the special issue are provided in section 4 of this manuscript (Special issue presentation). Additionally, ~~A~~a detailed literature review on ~~our~~ knowledge regarding the fate of DDN in the ocean is provided in the synthesis article of the present issue (Bonnet et al., Submitted) together with a detailed description of the experimental and modelling results obtained during the project that answer the above scientific questions.

Below,~~-~~ ~~Here~~ we ~~will~~ focus on the technical challenges and the methods developed to answer the scientific questions of the project. ~~.~~

Studying the fate of DDN in the ocean is technically complex. First, it requires appropriate methodologies to trace the passage of DDN through the different components of planktonic food web. During the VAHINE project, we intensively used high-resolution nanometer scale secondary ion mass spectrometry (nanoSIMS) in combination with flow cytometry cell sorting and $^{15}N_2$ labelling to trace the passage of ^{15}N-labelled DDN into several groups of non-diazotrophic phytoplankton and bacteria. This technique and results are extensively presented in Bonnet et al. (Accepted) and in thise special issue (Berthelot et al., 2016; Bonnet et al., 2015) and will not be detailed here.

Second, it requires ~~to~~ the monitoring of the chemical, biological and biogeochemical characteristics of a water body affected by a diazotroph bloom for a long period of time (15-30 days) to be able to ~~track~~ follow plankton community changes, track the N transfer in the different compartments of the ecosystem (dissolved/particulate phases, small/large plankton, export material) and elaborate biogeochemical budgets. Small-scale laboratory microcosm experiments have been frequently used in ocean biogeochemical studies, but their limited realism can make extrapolations to natural systems difficult to justify. They limit the duration of experiments to few days (usually 24 to 72 h), the small volumes used (few liters maximum) limit the number of parameters measured and they do not include ~~the~~ export terms. To overcome these difficulties, we decided to use the technology of large-volume mesocosms. Mesocosms are now widely used in ecological studies (Riebesell et al., 2013; Stewart et al., 2013) and enable ~~to~~ isolation of~~e~~ water masses of several cubic meters from physical dispersion for several weeks, without disturbing temperature and light conditions, taking into account the biological complexity of the planktonic ecosystem at large scales~~, and~~; they thus provide a powerful approach to maintain natural planktonic communities under close-to-natural self-sustaining conditions for several weeks. Moreover, the responses obtained from mesocosms studies (isolated from hydrodynamics) provide useful parameterizations for ecosystem and biogeochemical models.

2 Implementation of the VAHINE project

2.1 Mesocosms description and deployment

Among the different types of mesocosms available (Stewart et al., 2013), ~~T~~the model ~~mesocosms (surface 4.15 m^2, volume ~50 m^3, Fig. 1)~~ chosen for this study (surface 4.15 m^2, volume ~50 m^3, Fig. 1) are sea-going mesocosms entirely transportable that can be used under low to moderate wind/wave conditions (20-25 knots/2.5 wave height). They have been designed in the framework of the DUNE project (Guieu et al., 2010; Guieu et al., 2014) and~~.~~

They consist in large transparent bags made of two 500 µm thick films of polyethylene (PE) and vinyl acetate (EVA, 19 %), with nylon meshing in between to allow maximum resistance and light penetration (produced by HAIKONENE KY, Finland) (Fig. 2). They are 2.3 m in diameter and 15 m in height and are equipped with removable sediment traps for sinking material collection (Fig. 1, 2), which wasa prerequisite to answering some of the questions of the project. In the framework of VAHINE, we deployed three mesocosms (hereafter named M1, M2 and M3) to ensure a replication and robustness of the data.

The mesocosms were made of three different parts (Fig. 1, 2): i) the main cylinder, rigidified by five polyethylene rings maintaining the round shape of the bags and ending with two 8 cm width PVC circles sandwiching the bags ii) the bottom cone (2.2 m height) also made of two 8 cm width PVC circles. It was equipped with the sediment trap system, on which is screwed a 250 mL flask collecting sinking material, allowing an easy daily collection and replacement by SCUBA divers, iii) the PE flotation frame supporting the bags and attached at three points my means ofthanks to specific PVC cylindrical structures at the level of the upper ring and at the level of the ring just below the sea-surface. The structure was equipped with six buoys insuring the buoyancy of the system.

The mesocosms were moored using three screw anchors installed on the sea floor at (25 m depth). The three mesocosms were attached together and moored with the anchors screwed 120° from each other and connected to sub-surface buoys, which were themselves connected to surface buoys. The complete setup was a solid mooring capable of absorbing the sea swell while maintaining a supple and strong structure and ensuring that no tension was applied directly to the bags. An *in situ* mooring line was installed on an independent screw anchor to incubate subsamples collected from the mesocosms for production measurements (primary production, N_2 fixation) and process studies under the same conditions as in the mesocosms.

A fifth independent screw anchor was installed to hold the two mobile plastic logistics platforms for necessary to welcome the scientists and instrumentation for and the daily sampling by scientists.

The mesocosms were deployed on January 13th 2013 (day 0) thanks withto the assistance of four professional SCUBA divers. The group of three main cylinders was first deployed and the initial operations were performed on a coral shoal near the deployment site. The bags, cinched by three small elastic ropes, were placed inside and fixed to the flotation frame at three places using the designed PVC pieces. Once fixed, the system was transported to the deployment site, and attached to the subsurface buoys tethered located at the vertical ofto the screw anchors. Small ballast weights were set up at the base of the bags and the elastic ropes

released, allowing the main cylinders to gently deploy vertically with the assistance of the SCUBA divers (Fig. 2e,f). Once deployed, the main cylinders were left opened for 24 h to stabilize the water column inside. The following day ~~after~~ (day 1, January 14th), the divers closed the mesocosms by screwing together the main cylinder and the bottom cone using eight nylon screws preventing ~~any~~ further water exchange between inside and outside the mesocosms (Guieu et al., 2010). During the entire installation, the divers ~~followed instructions to~~ remained outside the bags to minimize disturbance and potential contamination of the water column.

2.2 Selection of the study site

The mesocosms were deployed during austral summer conditions (January-February 2013) in the oligotrophic New Caledonian coral lagoon (Noumea lagoon). New Caledonia is located in the South West Pacific ocean, 1500 km east of Australia in the Coral Sea (Fig. 3a), and hosts one of the three largest reef systems worldwide. It still displays intact ecosystems and its ecological and patrimonial value has been recognized through its registration as a UNESCO world heritage site. This site has been chosen for several reasons: i) it is a tropical low-nutrient low-chlorophyll (LNLC) ecosystem strongly influenced by oceanic oligotrophic waters inflowing from outside the lagoon (Ouillon et al., 2010). NO_3^- and chlorophyll a (Chl a) concentrations are typically < 0.04 µmol L^{-1} and around 0.10-0.15 µg L^{-1}, respectively, during the summer season (Fichez et al., 2010). ii) Primary productivity is N-limited throughout the year (Torréton et al., 2010)~~,~~ giving N_2-fixing microorganisms a competitive advantage over non-diazotrophic organisms. New Caledonian waters support high N_2 fixation rates (151-703 µmol N m^{-2} d^{-1}, Garcia et al., 2007), high *Trichodesmium* spp. abundances (Dupouy et al., 2000; Rodier and Le Borgne, 2010, 2008) as well as unicellular diazotrophic cyanobacteria (UCYN) (Biegala and Raimbault, 2008). The New Caledonian lagoon therefore represented an ideal location to track the fate of DDN in the ecosystem and implement the VAHINE project.

Before the VAHINE project, the mesocosms chosen for this study had only been deployed in protected bays of the temperate Mediterranean Sea, which is not submitted to tide currents and trade winds as New Caledonia is. In order to test the resistance of the mesocosms in a tropical ecosystem submitted to trade winds (20-25 knots) and high tidal currents, and to select the ideal location to deploy the mesoscosms inside the lagoon, we performed a pilot study in March 2012 (i.e. one year before the VAHINE project). Four potential study sites ~~have been~~were tested of which~~and~~ the Tabou Reef (22°29.073 S - 166°26.905 E) located in

close proximity to Boulari passage (Fig. 3b, c) has been selected as the ideal location to implement the project as it met the following specifications required for the technical deployment and sustainability of the mesocosms: i) the site was protected from the dominant trade winds by the submerged reef located less than one nautical mile from the study site, ii) it was located 28 km from the New Caledonian coast at the exit of the lagoon and was strongly influenced by oceanic waters, typical of a LNLC environment (see below, initial conditions), iii) it was 25 m-deep, which is in the range required (17-25 m) to deploy 15 m high mesocosms and insure the SCUBA divers security, iv) the seafloor was mainly composed of sand, which is a prerequisite to implant to screw anchors in the substrate, v) it is seldom ~~was low~~ visited by amateur yatchmen.

2.3 DIP fertilization

Dissolved inorganic phosphorus (DIP) availability has been reported to control N_2 fixation in the southwest Pacific (Moutin et al., 2008; Moutin et al., 2005). To alleviate any potential DIP limitation in the mesocosms and enhance a bloom of diazotrophs for the purpose of this study, the mesocosms were intentionally fertilized with ~0.8 µmol L^{-1} of DIP on the evening of day 4 (January 16th) of the experiment. Such concentrations have already been measured in the New Caledonian lagoon and were shown to be able to stimulate N_2 fixation. The amount of DIP added was also chosen based on the modelling work performed by Gimenez et al. (2016), confirming a clear stimulation of N_2 fixation by 0.8 µmol L^{-1} DIP in our experimental systems, and an absence of stimulation without any DIP enrichment.

We diluted 5.66 g of KH_2PO_4 in three 20-L carboys filled with filtered surface seawater collected close to the mesocosms. The carboy contents were homogenized and 20 L of each solution were~~have~~ then been carefully introduced in each mesocosm from the bottom to the surface ~~thanks~~ through a braided PVC tubing (inner diameter = 9.5 mm) connected to a Teflon pump (St-Gobain Performance Plastics) gradually lifted up during the KH_2PO_4 fertilization to insure homogenization of the solution.

When deployed, the mesocosms naturally trapped different volumes of seawater and the volume of each mesocosms had to be determined for biogeochemical budgets (Berthelot et al., 2015). As DIP concentrations were measured at three selected depths (1 m, 6 m, 12 m) before (evening of day 4) and after (morning of day 5) the fertilization, the delta DIP was used to calculate the volume of each mesocosm based on the assumption that no DIP was consumed during the night between day 4 and day 5. The DIP concentrations were homogeneous over depth on day 5 and the mesocosm volumes were calculated as 52,790±490

L for M1, 42,620±430 L for M2 and 50,240±300 L for M3, with the uncertainties calculated from standard deviation of triplicate DIP measurements.

New Caledonian soils are very rich in metals. A third of its surface (5500 km^2) is covered by soils originating from ultramafic rocks which have exceptionally high levels of metals such as Fe, Ni, Cr, Co, and Mn (Jaffré, 1980). Consequently, dissolved trace metals are particularly abundant in the Noumea lagoon (Migon et al., 2007). Iron concentrations measured during the Diapalis cruises around New Caledonia were higher than those reported in the sub-tropical North Pacific and the high iron inputs in this region are hypothesized to drive the South West Pacific towards a DIP depletion. Metals were thus not supplemented to the mesocosms.

2.4 Logistics, sampling strategy

As the mesocosms were moored 28 km off the coast, all the experimental work had to be performed on site: scientific laboratories were setup on the R/V Alis (28.5 m) moored 0.5 nautical mile from the mesocosms, and on the Amédée sand island located one nautical mile from the mesocosms (Fig. 3b, c), on which we set up a laboratory and accommodated scientists for the duration of the VAHINE experiment.

Sampling in the mesocosms started on January 15th (day 2). The experiment lasted for 23 days for logistical reasons (i.e. ~~It was performed daily for 23 days~~ until February 6th) and sampling was performed daily at 7 am from the sampling platform moored next to the mesocosms. Every day after collection, seawater samples were immediately carried out to the R/V Alis and the Amédée for immediate processing.

Discrete samples were collected at three selected depths (1 m, 6 m, 12 m) in each mesocosm and outside (hereafter termed 'lagoon waters') using a braided PVC tubing connected to the Teflon PFA pump activated by pressurized air from diving tanks, allowing ~~to~~ samplinge of large volumes with the least possible perturbation inside the mesocosms. For stock~~s~~ measurements, 50-L PE carboys were filled at each depth of each mesocosm, immediately transported onboard the R/V Alis for subsampling and samples treatments. For flux~~es~~ measurements (primary production, bacterial production, N_2 fixation), samples were directly collected in incubation bottles and transported onboard to ~~skip~~ avoid the subsampling step and minimize the time between collection, tracer spikes and incubation. For prokaryotic diversity and gene expression measurements, 10-L carboys were filled (from M1 only) and carried out to the Amédée laboratory for immediate processing. A total of 220 L were sampled every day from each mesocosms, corresponding to ~10 % of the total mesocosms volume sampled at the end of the 23-days experiment.

After seawater sampling, vertical CTD profiles were performed (around 10 am) using a SBE 19 plus Seabird CTD in each mesocosm and outside the mesocosms to ~~obtain~~ document the vertical structure of temperature, salinity and fluorescence. The CTD *in situ* fluorescence data were fitted to the Chl *a* data from fluorometry measurements using a linear least squares regression.

Sediment traps were then collected daily from each mesocosm by two SCUBA divers (Fig. 2e, f1). They followed the same protocol everyday: they ~~carefully~~ gently tapped~~hit~~ the cone of the mesocosms to dislodge ~~in case some~~ sinking material ~~was~~ retained on the walls, waited for 15 minutes, and collected the 250 mL flasks screwed to the trap system of each mesocosm and immediately replaced it ~~by~~ with a new one.

Vertical net hauls were performed every four days using a 30 cm diameter, 100 cm long, 80 µm mesh net fitted with a filtering cod end. On each sampling occasion, three vertical hauls were collected from each mesocosm and lagoon waters, representing a total volume of 2.13 m^3, i.e. 4 % of the total mesocosm volume. This sampling strategy was~~has been~~ chosen to minimize the effect of zooplankton catches on the plankton abundance and composition in the mesocosms.

2.5 Replicability among the mesocosms

Guieu et al. (2010) and Guieu et al. (2014) have performed several mesocosm experiments in the Mediterranean Sea~~,~~ and demonstrated that the type of mesocosms used in the present study is well adapted to conduct replicated process studies on the first levels of the pelagic food web in LNLC environments. In order to evaluate the reproducibility among the three ~~deployed~~ mesocosms deployed during VAHINE, we calculated the coefficient of variation (CV, %) of the main stocks and fluxes measured every day for 23 days for every sampling depth (Table 1, the methods are described in detail in the publications composing this special issue). The CV ranged from 4 to 42 % depending on the parameter considered. It was ~~the~~ lowest for TOC and DON concentrations (4 and 9 %, respectively), which is very satisfying as these CV are close to the precision of the methods themselves, indicating a good reproducibility between mesocosms. It was ~~the~~ highest for NO_3^- concentrations (42 %), which is consistent with the fact that NO_3^- concentrations were close to quantification limits of conventional methods (~0.05 µmol L^{-1}) during the 23-days experiment: when the mean value is close to zero, the CV approaches infinity and is therefore sensitive to small changes in the mean. For flux measurements of~~such as~~ PP, BP and N_2 fixation, the CV's were~~as~~ 29, 26 and 34%, respectively, which is also satisfying given the natural spatial heterogeneity of plankton

in the environment due to aggregation, (Seebah et al., 2014), or to the buoyancy of some diazotrophs such as *Trichodesmium* (Capone et al., 1997), which introduces ~~some~~ spatial variability, well known in the natural environment for N_2 fixation (Bombar et al., 2015). Another criterion to evaluate the consistency between mesocosms is to compare the evolution of the biogeochemical conditions and the plankton community composition between mesocosms. This approach ~~It~~ is described in details in several articles of the present issue and only some general features will be given here. As an example, bulk N_2 fixation rates averaged 18.5±1.1 nmol N L^{-1} d^{-1} (standard deviation was calculated on the average N_2 fixation rates of each mesocosm) over the 23 days of the experiment ~~in the three mesocosms~~ (all depths averaged together). ~~. The variance between the three mesocosms was low, and~~ N_2 fixation rates did not differ significantly ~~from~~ among the three mesocosms ($p<0.05$, Kruskall-Wallis test, (Berthelot et al., 2015). ~~and w~~Moreover, we consistently observed the same temporal dynamics over the three mesocosms, such as the dramatic increase of rates from days 15 to 23 (during which they reached 27.3±1.0 nmol N L^{-1} d^{-1}). This together indicates good replicability between the mesocosms (Bonnet et al., 2015). Molecular data also report a shift in the diazotrophic community composition around day 15, with a bloom of UCYN-C consistently occurring in the three mesocsoms, see (Turk-Kubo et al., 2015). The same feature was observed for *Synechococcus* abundances, which increased by a factor of two since day 15 to day 23 in every mesocosm (Leblanc et al., 2016). Finally, the diatom community which was very diverse during the first half of the experiment suddenly shifted ~~since~~ beginning ~day 10 and *Cylindrotheca closterium* consistently became the dominant diatoms in the three mesocosms (Leblanc et al., 2016). These observations, together with the CV reported above indicate that ~~the~~ biogeochemical and biological conditions were comparable between the three mesocosms.

3 Initial conditions and evolution of the core parameters during the experiment

Initial hydrological and biogeochemical conditions (i.e. conditions in ambient waters the day of mesocosms deployment - January 13th, day 0) are summarized in Table 2. Seawater temperature was 25.30°C, which is slightly lower than the ~~classical~~ temperature reported at this season at the Amédée lighthouse station ~~(Le Borgne et al., 2010).~~ while s~~S~~alinity (35.15) was ~~35.15~~, ~~a classical~~ typical ~~alue~~ for ~~salinity measured at this~~ the season ~~at the Amédée lighthouse station~~ (Le Borgne et al., 2010). NO_3^- and DIP concentrations were both reported at 0.04±0.01 µmol L^{-1} ~~for both~~, and Chl *a* concentrations from fluorescence data (0.11 µg L^{-1})

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were typical of oligotrophic systems and ~~are~~ in the range reported in the literature for this location (Fichez et al., 2010). Dissolved organic N (DON) and P (DOP) concentrations were 4.65±0.46 and 0.100±0.002 and ambient N_2 fixation rates 8.70±1.70 nmol N L^{-1} day^{-1} before the mesocosms deployment.

Seawater temperature measured daily by vertical CTD profiles inside the mesocosms and in the lagoon waters (Fig. 4a-d) gradually increased over the 23-days of the experiment from 25.50°C the day of the mesocosms closure (day 2) to 26.24°C on day 23. This warming is the ~~classical~~ typical trend observed in New Caledonia along the summer season (Le Borgne et al., 2010). The water column was vertically homogeneous ~~not stratified~~ over the course of the experiment, except the two first days, which were characterized by a slight stratification inside and outside the mesocosms. Data indicate therefore a good reproducibility between the three mesocosms and between the mesocosms and the Noumea lagoon waters.

Salinity data (Fig. 4e-h) indicate a small and gradual increase in the three mesocosms during the 23-days experiment (35.2 to 35.4) ~~indicating~~ suggesting a probable higher level of evaporation in the mesocosms compared to the Noumea lagoon. Moreover, lagoon waters constantly receive some low salinity waters from the coast due to rainfall advected by tide currents, which may also explain the slightly lower salinity values measured in the Noumea lagoon (35.40) compared to inside (35.47) at the end of the experiment.

NO_3^- concentrations (Fig. 5a-d) remained below 0.1 µmol L^{-1} during the whole experiment in all mesocosms and in the lagoon waters. Average concentrations over the 23-days experiment and the three depths samples were close to detection limits of the method (0.01 µmol L^{-1}) and are thus difficult to quantify accurately: they were 0.04±0.02 µmol L^{-1}, 0.02 ±0.01 µmol L^{-1}, 0.02±0.02 µmol L^{-1}, and 0.06±0.04 µmol L^{-1} in M1, M2, M3 and in the lagoon waters, respectively. DIP concentrations (Fig. 5e-h) were also close to detection limits (0.005 µmol L^{-1}) and on average 0.04±0.01, 0.03±0.01 and 0.03±0.02 µmol L^{-1} before the DIP fertilization (days 2 to 4, hereafter called P0) in M1, M2 and M3 (average over the three depths). They increased after the fertilization on day 5 to 0.73±0.07, 0.98±0.01, 0.77±0.03 µmol L^{-1} in M1, M2 and M3. The intensity of the DIP fertilization differed slightly among the mesocosms, likely reflecting the different volume of the mesocosms (see above). Subsequently ~~the~~ DIP concentrations decreased steadily towards initial concentrations by the end of the experiment: 0.03±0.01, 0.03±0.01 and 0.05±0.02 µmol L^{-1} in M1, M2 and M3, respectively (average of days 23 over the three depths). However, the DIP pool was first exhausted in M1 (day 14), then M2 (day 19) and finally M3 (day 23). A more detailed description of the evolution of

stocks and fluxes of biogenic elements during the experiment can be found in (Berthelot et al., 2015).

Chl *a* fluorescence was homogenous ~~over~~ throughout the water column during the course of the experiment (Fig. 4i-l). Chl *a* slightly increased (by 0.1 to 0.2 µg L^{-1}) in the three mesocosms after ~~the~~ DIP fertilization on days 5 and 6. After day 6, they consistently ~~decreased~~ declined back to the initial (before fertilization) concentrations of 0.12-0.15 µg L^{-1}. On days 12, 13 and 14, Chl *a* concentrations re-increased dramatically to reach 0.61, 0.65 and 1.02 µg L^{-1} in M1, M2 and M3 at day 23, respectively, indicating that the three mesocosms were relatively synchronized but the intensity of the phytoplankton bloom differed between the mesocosms, with a ~~higher~~ greater increase observed in M3 compared to M2 and M1. In the lagoon waters, Chl *a* concentrations also gradually increased over the experiment (concentrations reached 0.35 µg L^{-1} at day 23) but to a lower extend compared to that of the mesocosms.

4 Special issue presentation

The goal of this special issue is to present the knowledge gained regarding the fate of DDN in a LNLC ecosystem based on the large dataset acquired during the VAHINE mesocosm experiment. VAHINE was a multidisciplinary project involving close collaborations between biogeochemists, molecular ecologist, chemists, marine opticians and modelers. Most of the contributions to this special issue have benefited from this collective and collaborative effort. The philosophies and summarized results of the different papers composing the special issue are presented briefly hereafter and a synthesis paper of all the multidisciplinary approaches used to answer the main scientific questions of the VAHINE project is proposed at the end of the issue.

First, thanks to the high frequency (daily) sampling of the same water body for 23 days, this project provided a unique opportunity to characterize the diversity of the planktonic assemblage using several ~~and~~ complementary approaches, and investigate species successions in relation to hydrological parameters, biogeochemical stocks and fluxes during a diazotroph bloom in a LNLC ecosystem. By using PCR targeting a component of the nitrogenase gene (*nifH*), sequencing and qPCR assays, Turk-Kubo et al. (2015) fully characterized the diazotroph community composition within the mesocosms and the New Caledonian (Noumea) lagoon and calculated *in situ* growth and mortality rates for natural populations of diazotrophs, which is rarely accomplished. They revealed that the diazotroph community was

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dominated by Diatom-Diazotroph Associations (DDAs) during the first period of the experiment after the DIP fertilization (days 5 to 14; hereafter called P1), and a bloom of UCYN-C occurred during the second half (days 15 to 23, hereafter called P2), providing ~~the~~an unique opportunity to compare the DDN transfer and export efficiency associated with different diazotrophs. ~~This study provided the first growth rates for the uncultivated UCYN-A2 and the UCYN-C phylotypes, and the first opportunity to study an *in situ* bloom of UCYN-C.~~ Complementary to this approach, Pfreundt et al. (2015) used 16S tag sequencing to examine the temporal dynamics of the prokaryotic community and observed clear successions of prokaryotes during the experiment, in relation with biogeochemical parameters. ~~to examine heterotrophic bacterial diversity and successions during the experiment and whether they evolved concurrently to that of diazotrophic and non-diazotrophic phytoplankton groups.~~ In a second study, Pfreundt et al. (Submitted) also used metatranscriptomics to investigate the microbial gene expression dynamics from diazotrophic and non-diazotrophic taxa and highlighted specific patterns of expression of genes involved in N, DIP, iron and light utilization along the different phases of the experiment. ~~(~~Van Wambeke et al., (2015) revealed that heterotrophic bacterioplankton production and alkaline phosphatase activity were statistically higher during P2. Their results suggest that most of the DDN reached the heterotrophic bacterial community through indirect processes, like mortality, lysis and grazing. In parallel, Leblanc et al. (2016) focused on the phytoplankton assemblages and dynamics ~~along the experiment~~ from pigment signatures, flow cytometry and taxonomy analyses and revealed a monospecific bloom of the diatom *Cylindrotheca closterium* and an 2-fold increase in *Synechococcus* and nano-phytoeukaryotes during P2, concomitant with the UCYN-C bloom. ~~In parallel, {Van Wambeke, 2015 #922}~~

Tedetti et al. (2015) used bio-optical techniques to describe the spectral characteristics and the variability of dissolved and particulate chromophoric materials according to the phytoplankton community composition and revealed a coupling between the dynamics of the N_2 fixation and that of chromophoric material in the South West Pacific through *Synechococcus* bloom. ~~along the experiment~~. Berman-Frank et al. (2016) analyzed the spatial and temporal dynamics of transparent exopolymeric particles (TEP), which are sticky carbon rich compounds that are formed, degraded, and utilized in both biotic and abiotic processes, and ~~evaluated~~ measured a relatively stable TEP pool available as both a carbon source for plankton communities and facilitating aggregation and flux throughout the experiment~~their role as an energy source for the auto- and heterotrophic communities.~~

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Second, the bloom of diazotrophs (UCYN-C) obtained in the closed water body of the mesocosms following ~~thanks to the~~ DIP fertilization offered the opportunity to track the fate of DDN in the ecosystem: Berthelot et al. (2015) described the evolution of C, N, P pools and fluxes ~~along~~ during the course of the experiment and report a 3-fold increase in Chl *a* concentrations and N_2 fixation rates and a 5-fold increase in C export during the second half of the experiment (UCYN-C bloom). They also reveal that the *e*-~~-~~ratio that quantifies the efficiency of a system to export particulate organic C ~~compared to PP~~ was significantly higher ($p < 0.05$) during P2 than during P1, indicating that the production sustained by UCYN-C was more efficient at promoting C export than the production sustained by DDAs. ~~investigated the contribution of N_2 fixation and DON use to primary production and particle export. They also explored the fate of the freshly produced particulate organic N, i.e. whether it was preferentially accumulated and recycled in the water column or exported out of the system.~~ Complementary to this approach Knapp et al. (2015) reported the results of $\delta^{15}N$ measurements on DON, PON and particles from sediment traps and further substantiated these results with a significantly ($p<0.05$) higher contribution of N_2 fixation to export production during P2 (56±24 % and up to 80 % at the end of the experiment) compared to P1 (47±6 %)~~a $\delta^{15}N$ budget performed in the manipulative mesocosms to assess the dominant source of N (from NO_3^- and/or N_2 fixation) fueling export production along the 23 days experiment, and discuss how the measured geochemical signals correspond to concurrent shifts in diazotroph and phytoplankton community composition~~. Bonnet et al. (2015) explored the fate of DDN at shorter time scales ~~during the height of the UCYN-C bloom~~ and revealed that ~ 10 % of UCYN-C from the water column were exported daily to the traps, representing as much as 22.4 ± 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±0.8 µm) UCYN-C cells into large (100–500 µm) aggregates. They also showed using a nanoSIMS approach that 21±4 % of the DDN was transferred to non-diazotrophic plankton, mainly picoplankton (18 ± 4 %) followed by diatoms (3 ± 2 %) during P2. ~~investigated the relative contribution of each diazotroph phylotype to direct C export. They also quantified the DDN released in the dissolved pool and its subsequent transfer to different groups of plankton (picoplankton, diatoms) by using nanoSIMS coupled with $^{15}N_2$ isotopic labelling.~~ The same nanoSIMS approach was used by Berthelot et al. (2016) in a parallel experimental study to compare the DDN transfer efficiency into non-diazotrophic plankton, whether it comes from UCYN-C, UCYN-B or *Trichodesmium*~~, UCYN-C or UCYN-B~~. They showed that the transfer was twice as high during a *Trichodesmium* bloom than during a UCYN-B or UCYN-C bloom, arguing that

filamentous diazotrophs blooms are more efficient at promoting non-diazotrophic production in N depleted areas. In parallel, Hunt et al. (2016) estimated a mean ~ 30 % contribution of DDN to zooplankton biomass ~~the contribution of DDN to zooplankton biomass in the mesoscosms~~ in the mesocosms based on naturael ^{15}N isotope ~~values~~ measurements on zooplankton. They also provided evidence for direct ingestion and assimilation of UCYN-C-derived N by the zooplankton~~studied the transfer of $^{15}N_2$ labelled phytoplankton to zooplankton under contrasting situations (UCYN versus *Trichodesmium* versus Diatom-Diazotrophs associations (DDAs) dominance)~~, results that were complemented by qPCR assays on several diazotroph phylotypes in zooplankton guts. Spungin et al. (2016) took advantage of the *Trichodesmium* bloom occuring outside the mesoscoms to specifically investigate its decline and understand changes in genetic underpinning and features that could elucidate varying stressors or causes of mortality of *Trichodesmium* in the natural environment.

Third, modelling was used at every stage of the project. Simulations performed with the 1D-vertical biogeochemical mechanistic Eco3M-MED model ~~Eco3M-MED model~~ have been used prior to the VAHINE experiment to help in the scientific implementation of the project (timing and quantification of the DIP fertilization). Gimenez et al. (2016) validated the model using the *in situ* data measured during the whole experiment, and provided additional information such as stoichiometry of planktonic organisms that could not be inferred from~~through~~ *in situ* measurements and offered the opportunity to deconvolute the different interlinked biogeochemical processes occurring in the ecosystem to help understanding the fate of DDN in oligotrophic ecosystems and the ~~its~~ impact of N_2 fixation on carbon export. Finally, a synthesis study by Bonnet et al. (Submitted) attempt~~sed~~ to summarize our knowledge and the unresolved questions regarding the fate of DDN in the ocean, synthetize and link the major experimental and modelling results obtained during the project and described in the VAHINE Special issue. It reconciles the diverse and complementary ~~valuable~~ methodological approaches used in this study to answer the scientific questions of the VAHINE project. After putting in perspective the different experimental findings, the modelling approach has also been used in the synthesis article as a tool ~~here~~ to investigate the impact of N_2 fixation on marine productivity, export and food web composition by artificially removing N_2 fixation in the model.

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Author contribution: S. B. designed the experiments helped by T.M. J.M.G., F.L. designed the mesocosms, J.M.G., E.F., B.B., A.R. and J.M.B. deployed the mesocosms and performed CTD and traps sampling, M.R. analyzed CTD data, T.M was responsible for the nutrient analyses. S. Bonnet prepared the manuscript with contributions from all co-authors.

Figure legends.

Figure 1. Drawing representing the main features of the large-volume mesocosm device.

Figure 2. View of the experiment from the side and the seafloor during (a-c) and after the deployment (d). e-f collect of sediment traps by the SCUBA divers (Photos: J.M. Boré and E. Folcher, IRD).

Figure 3. Location of the study site of the VAHINE experiment. Map showing surface chlorophyll a concentrations (MODIS) in the Southwestern Pacific during the study period (January-February 2013), b) Map of the Noumea lagoon, c) a view taken from the Amédée Island showing the location of mesocosms and R/V Alis.

Figure 4. Horizontal and vertical distributions of seawater temperature (°C), salinity and fluorescence (µg L^{-1}) in M1 (a,e,i), M2 (b,f,j), M3 (c,g,k), and lagoon waters (d,h,l). The grey bars indicate the timing of the DIP spike on day 4.

Figure 5. Horizontal and vertical distributions of $NO_3^-{}_x$ and DIP (µmol L^{-1}) in M1 (a,e), M2 (b,f), M3 (c,g), and lagoon waters (d,h). The grey bars indicate the timing of the DIP spike on day 4.

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Table 1. Mean variation coefficients (CV = standard deviation x 100 / mean, %) calculated for samples collected at the same time and the same depth in the three mesocosms. The CV derived from these calculations was averaged over the 23-days experiment.

	Parameter measured	CV (%) between the three mesocosms
Standing stocks	NO_3^- concentrations	42
	DON concentrations	9
	DOP concentrations	21
	PON concentrations	21
	POPconcentrations	26
	Chl *a* concentrations	26
	TOC concentrations	4
	TEP concentrations	24
Fluxes	Primary production	29
	Bacterial production	26
	N_2 fixation	34
Plankton abundances	*Prochlorococcus* abundances	30
	Synechococcus abundances	30
	Pico-eukaryote abundances	31
	HNA abundances	22
	LNA abundances	11
	Average	24

Table 2. Initial conditions (hydrological and biogeochemical parameters) recorded at 6 m-depth just before the mesocosm deployment (January 13th).

Temperature (°C)	Salinity	[NO_3] (µmol L^{-1})	[DIP] (µmol L^{-1})	[Chl *a* fluo] (µg L^{-1})	[DON] (µmol L^{-1})	[DOP] (µmol L^{-1})	N_2 fixation (nmol N L^{-1} d^{-1})
25.30	35.15	0.04±0.01	0.04±0.01	0.11	4.65±0.46	0.10±0.02	8.70±1.70