



# 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.** On the global scale, N<sub>2</sub> fixation provides the major external source of reactive nitrogen to the surface ocean, surpassing atmospheric and riverine inputs, and sustains ~ 50 % of new primary production in oligotrophic environments. The main goal of the VARIability of vertical and tropHic transfer of diazotroph derived N in the south wEst Pacific (VAHINE) project was to study the fate of nitrogen newly fixed by diazotrophs (or diazotroph-derived nitrogen) in oceanic food webs, and how it impacts heterotrophic bacteria, phytoplankton and zooplankton dynamics, stocks and fluxes of biogenic elements and particle export. Three large-volume (~ 50 m<sup>3</sup>) 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 changes. VAHINE was a multidisciplinary project involving close collaborations between biogeochemists, molecular ecologist, chemists, marine opticians and modellers. 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 main

hydrological and biogeochemical conditions of the study site before the mesocosm deployment and during the experiment itself 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 world (IPCC, 2013) 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<sub>2</sub>) 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<sub>2</sub> largely depends on the availability of fixed nitrogen (N) (Falkowski, 1997) in the surface ocean. In the vast nitrate (NO<sub>3</sub><sup>-</sup>)-limited

oligotrophic gyres, which cover  $\sim 60\%$  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), turning it into ammonium and organic N compounds. On the global scale, they provide the major external source of N for the ocean, surpassing atmospheric and riverine inputs (Gruber, 2004), and thus act as “natural fertilizers”, contributing to sustaining life and the BCP through the so called “ $N_2$ -primed prokaryotic C pump” (Karl et al., 2003, 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 2 decades. This includes the landmark discovery of unicellular diazotrophic organisms of pico- and nanoplanktonic size termed unicellular diazotrophic cyanobacteria (UCYN; see, e.g., Zehr et al., 2001) and new and unexpected ecological niches where diazotrophs are active, such as N-rich oxygen minimum zones (see, e.g., Dekaezemaeker 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 VARIability of vertical and troPHic transfer of diazotroph derived N in the south wEst Pacific (VAHINE) project proposes a scientific contribution to answer these questions, based on a combination of experimentation and modelling involving recently developed innovative techniques. The acronym VAHINE was chosen in order to refer to the Pacific culture where this experiment has been performed with the help of local people. 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 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.

Summarized conclusions of each article composing the special issue are provided in Sect. 4 of this manuscript (“Presentation of the special issue”). Additionally, a detailed literature review on knowledge regarding the fate of DDN in the ocean is provided in the synthesis article of the present issue (Bonnet et al., 2016a) together with a detailed description of

the experimental and modelling results obtained during the project that answer the above scientific questions.

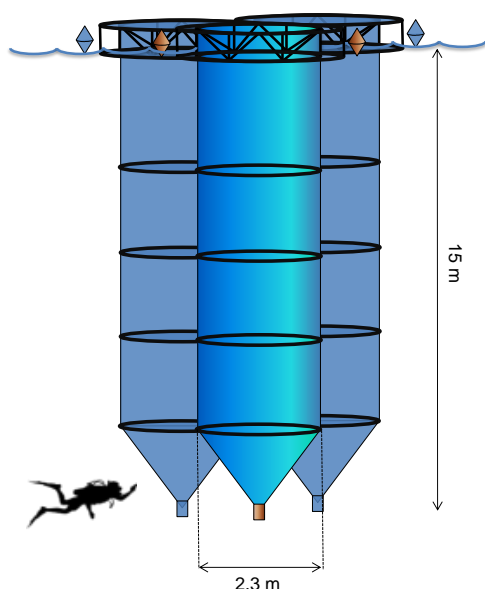
Below, we focus on the technical challenges and the methods developed to answer the scientific questions of the project. Studying the fate of DDN in the ocean is technically complex. First, it requires appropriate methodologies to trace the passage of DDN through the different components of the planktonic food web. During the VAHINE project, we made intensive use of high-resolution nanometre-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 presented in detail in Bonnet et al. (2016b) and in this special issue (Berthelot et al., 2016; Bonnet et al., 2015) and will not be detailed in this introduction.

Second, carrying out this research requires 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 follow plankton community changes, track the N transfer in the different compartments of the ecosystem (dissolved or particulate phases, small or 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 a few days (usually 24 to 72 h); the small volumes used (a few litres maximum) limit the number of parameters measured and they do not include 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 the isolation of water masses of several cubic metres from physical dispersion for several weeks, without disturbing temperature and light conditions, taking into account the biological complexity of the planktonic ecosystem; 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 mesocosm 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), the model chosen for this study (surface  $4.15\text{ m}^2$ , volume  $\sim 50\text{ m}^3$ ; Fig. 1) is sea-going mesocosms which are entirely transportable and which can be used under low to moderate wind and wave conditions (20–25 kn, 2.5 m wave height). They have been designed in the frame-

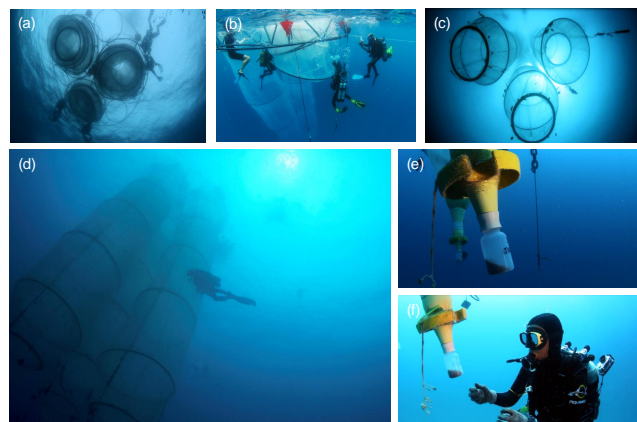


**Figure 1.** Drawing representing the main features of the large-volume mesocosm device.

work of the DUst experiment in a low Nutrient, low chlorophyll Ecosystem (DUNE) project (Guieu et al., 2010; Guieu et al., 2014) and consist of large transparent bags made of two 500  $\mu\text{m}$  thick films of polyethylene (PE) and ethylene-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 (Figs. 1, 2), a 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 replication and robustness of the data.

The mesocosms were made of three different parts (Figs. 1, 2): (i) the main cylinder, rigidified by five polyethylene rings maintaining the round shape of the bags and ending with two 8 cm wide PVC circles sandwiching the bags; (ii) the bottom cone (2.2 m height) also made of two 8 cm wide PVC circles, 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 by means of 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 ensuring the buoyancy of the system.

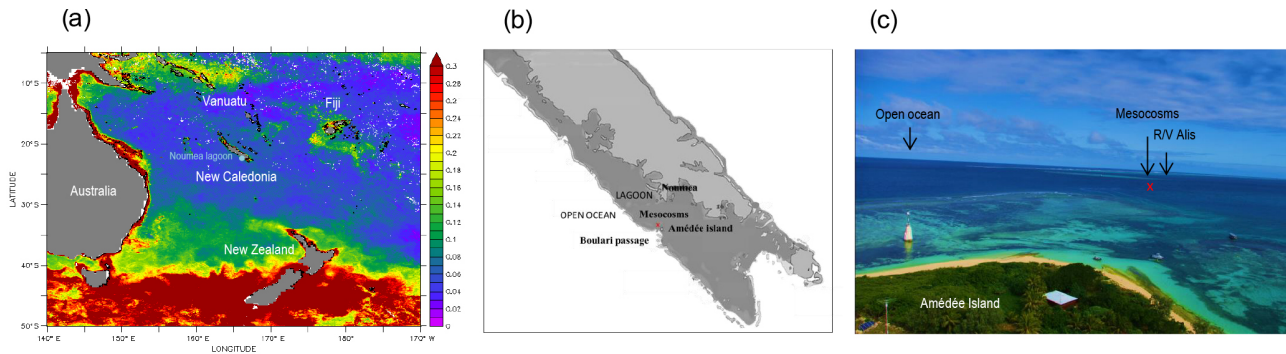
The mesocosms were moored using three screw anchors installed on the sea floor at 25 m depth. The three mesocosms were attached and moored with the anchors screwed in  $120^\circ$  from each other and connected to sub-surface buoys, which



**Figure 2.** View of the experiment from the side and the seafloor during (a–c) and after the deployment (d). Panels (e, f): collection of sediment traps by the scuba divers (photos: J. M. Boré and E. Folcher, IRD).

were themselves connected to surface buoys. The complete set-up 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,  $\text{N}_2$  fixation) and process studies under the same conditions as in the mesocosms. A fifth independent screw anchor was installed to hold two mobile plastic logistics platforms for instrumentation and the daily sampling by scientists.

The mesocosms were deployed on 13 January 2013 (day 0) with 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 sub-surface buoys tethered to 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 (day 1, 14 January), the divers closed the mesocosms by screwing together the main cylinder and the bottom cone using eight nylon screws and preventing further water exchange between inside and outside the mesocosms (Guieu et al., 2010). During the entire installation, the divers remained outside the bags to minimize disturbance and potential contamination of the water column.



**Figure 3.** Location of the study site of the VAHINE experiment. Panel (a): map showing surface chlorophyll a concentrations (Moderate Resolution Imaging Spectroradiometer, MODIS) in the south-western Pacific during the study period (January–February 2013); panel (b): map of the Noumea lagoon; panel (c): a view taken from Amédée Island showing the location of mesocosms and R/V *Alis*.

## 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).  $\text{NO}_3^-$  and chlorophyll a (Chl *a*) concentrations are typically  $< 0.04$  and around  $0.10$ – $0.15 \mu\text{g L}^{-1}$ , respectively, during the summer season (Fichez et al., 2010).
- (ii) Primary productivity is N-limited throughout the year (Torréton et al., 2010), giving  $\text{N}_2$ -fixing microorganisms a competitive advantage over non-diazotrophic organisms. New Caledonian waters support high  $\text{N}_2$  fixation rates ( $151$ – $703 \mu\text{mol N m}^{-2} \text{d}^{-1}$ ; Garcia et al., 2007) and high *Trichodesmium* spp. abundances (Dupouy et al., 2000; Rodier and Le Borgne, 2010, 2008) as well as 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 subject to tide currents and trade winds in the same way that New Caledonia is. In order to test the resistance of the mesocosms in a tropical ecosystem subject to trade winds (20–25 kn) and high tidal currents and to select the ideal location to deploy the mesocosms inside the lagoon, we performed a pilot study

in March 2012 (i.e. 1 year before the VAHINE project). Four potential study sites were tested, of which the Tabou Reef ( $22^\circ 29.073 \text{ S}$ – $166^\circ 26.905 \text{ E}$ ), located in close proximity to Bouлари Passage (Fig. 3b, c), was 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 1 nmi 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 an 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 ensure the scuba divers' security; (iv) the seafloor was mainly composed of sand, which is a prerequisite to implant to screw anchors in the substrate; and (v) it is seldom visited by amateur yachtsmen.

## 2.3 DIP fertilization

Dissolved inorganic phosphorus (DIP) availability has been reported to control  $\text{N}_2$  fixation in the south-west Pacific (Moutin et al., 2008, 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  $\sim 0.8 \mu\text{mol L}^{-1}$  of DIP on the evening of day 4 (16 January) of the experiment. Such concentrations have already been measured in the New Caledonian lagoon and were shown to be able to stimulate  $\text{N}_2$  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  $\text{N}_2$  fixation by  $0.8 \mu\text{mol L}^{-1}$  DIP in our experimental systems and an absence of stimulation without any DIP enrichment.

We diluted 5.66 g of  $\text{KH}_2\text{PO}_4$  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 then carefully introduced into each

mesocosm from the bottom to the surface through a braided PVC tubing (inner diameter = 9.5 mm) connected to a Teflon pump (St-Gobain Performance Plastics) gradually lifted up during the  $\text{KH}_2\text{PO}_4$  fertilization to ensure the homogenization of the solution.

When deployed, the mesocosms naturally trapped different volumes of seawater and the volume of each mesocosm had to be determined for biogeochemical budgets (Berthelot et al., 2015). As DIP concentrations were measured at three selected depths (1, 6, 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  $52\,790 \pm 490$  for M1,  $42\,620 \pm 430$  for M2 and  $50\,240 \pm 300$  L for M3, with the uncertainties calculated from the standard deviation of triplicate DIP measurements.

New Caledonian soils are very rich in metals. A third of its surface ( $5500\text{ km}^2$ ) 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 (<http://campagnes.flotteoceanographique.fr/series/85/>) from the Diapazon (DIAzotrophie PACifique ZONe) project around New Caledonia were higher than those reported in the subtropical 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 in the mesocosms.

## 2.4 Logistics and 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 set up on the R/V *Alis* (28.5 m), moored 0.5 nmi from the mesocosms, and on the Amédée sand island located 1 nmi from the mesocosms (Fig. 3b, c), on which we also set up a laboratory and accommodated scientists for the duration of the VAHINE experiment.

Sampling in the mesocosms started on 15 January (day 2). The experiment lasted for 23 days for logistical reasons (i.e. until 6 February) and sampling was performed daily at 07:00 LT from the sampling platform moored next to the mesocosms. Every day after collection, seawater samples were immediately taken to the R/V *Alis* and island Amédée for immediate processing.

Discrete samples were collected at three selected depths (1, 6, 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 and allowing the sampling of large volumes with the

least possible perturbation inside the mesocosms. For stock measurements, 50 L PE carboys were filled at each depth of each mesocosm and immediately transported onboard the R/V *Alis* for subsampling and samples treatments. For flux measurements (primary production, bacterial production,  $\text{N}_2$  fixation), samples were directly collected in incubation bottles and transported onboard to 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 was sampled every day from each mesocosms, corresponding to  $\sim 10\%$  of the total mesocosm volume sampled at the end of the 23-day experiment.

After seawater sampling, vertical conductivity–temperature–depth (CTD) profiles were performed (around 10.00 LT) using an SBE 19 plus Sea-Bird CTD in each mesocosm and outside the mesocosms to 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, f). They followed the same protocol every day: they gently tapped the cone of the mesocosms to dislodge sinking material retained on the walls, waited for 15 min and collected the 250 mL flasks screwed to the trap system of each mesocosm and immediately replaced it with a new one.

Vertical net hauls were performed every 4 days using a 30 cm diameter, 100 cm long,  $80\ \mu\text{m}$  mesh net fitted with a filtering cod end. On each sampling occasion, three vertical hauls were collected from each mesocosm and the lagoon waters, representing a total volume of  $2.13\text{ m}^3$ , i.e. 4 % of the total mesocosm volume. This sampling strategy was 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 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 making up this special issue). The CV ranged from 4 to 42 % depending on the parameter considered. It was lowest for total organic C (TOC) and dissolved organic N (DON) concentrations (4 and 9 %, respectively), which is very satisfactory as these CVs are close to the precision of

**Table 1.** Mean variation coefficients (CV = standard deviation  $\times$  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-day experiment. POP: particulate organic P. HNA stands for high nucleic acid bacteria, and LNA for low nucleic acid bacteria.

	Parameter measured	CV (%) between the three mesocosms
Standing stocks	NO <sub>3</sub> <sup>-</sup> concentrations	42
	DON concentrations	9
	DOP concentrations	21
	PON concentrations	21
	POP concentrations	26
	Chl <i>a</i> concentrations	26
	TOC concentrations	4
	TEP concentrations	24
Fluxes	Primary production	29
	Bacterial production	26
	N <sub>2</sub> fixation	34
Plankton abundances	<i>Prochlorococcus</i> abundances	30
	<i>Synechococcus</i> abundances	30
	Picoeukaryote abundances	31
	HNA abundances	22
	LNA abundances	11
	Average	24

the methods themselves, indicating a good reproducibility between mesocosms. It was highest for NO<sub>3</sub><sup>-</sup> concentrations (42 %), which is consistent with the fact that NO<sub>3</sub><sup>-</sup> concentrations were close to quantification limits of conventional methods ( $\sim 0.05 \mu\text{mol L}^{-1}$ ) during the 23-day experiment: when the mean value is close to 0, the CV approaches infinity and is therefore sensitive to small changes in the mean. For flux measurements of primary production (PP), bacterial production (BP) and N<sub>2</sub> fixation, the CVs were 29, 26 and 34 %, respectively, which is also satisfactory 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 spatial variability, well known in the natural environment for N<sub>2</sub> 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 is described in detail in several articles of the present issue and only some general features will be given here. For example, bulk N<sub>2</sub> fixation rates averaged  $18.5 \pm 1.1 \text{ nmol NL}^{-1} \text{ d}^{-1}$  (standard deviation was calculated on the average N<sub>2</sub> fixation rates of each mesocosm) over the 23 days of the experiment (all depths averaged together). N<sub>2</sub> fixation rates did not differ significantly among the three mesocosms ( $p < 0.05$ ; Kruskal–Wallis test; Berthelot et al., 2015). 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  $27.3 \pm 1.0 \text{ nmol NL}^{-1} \text{ d}^{-1}$ ). This to-

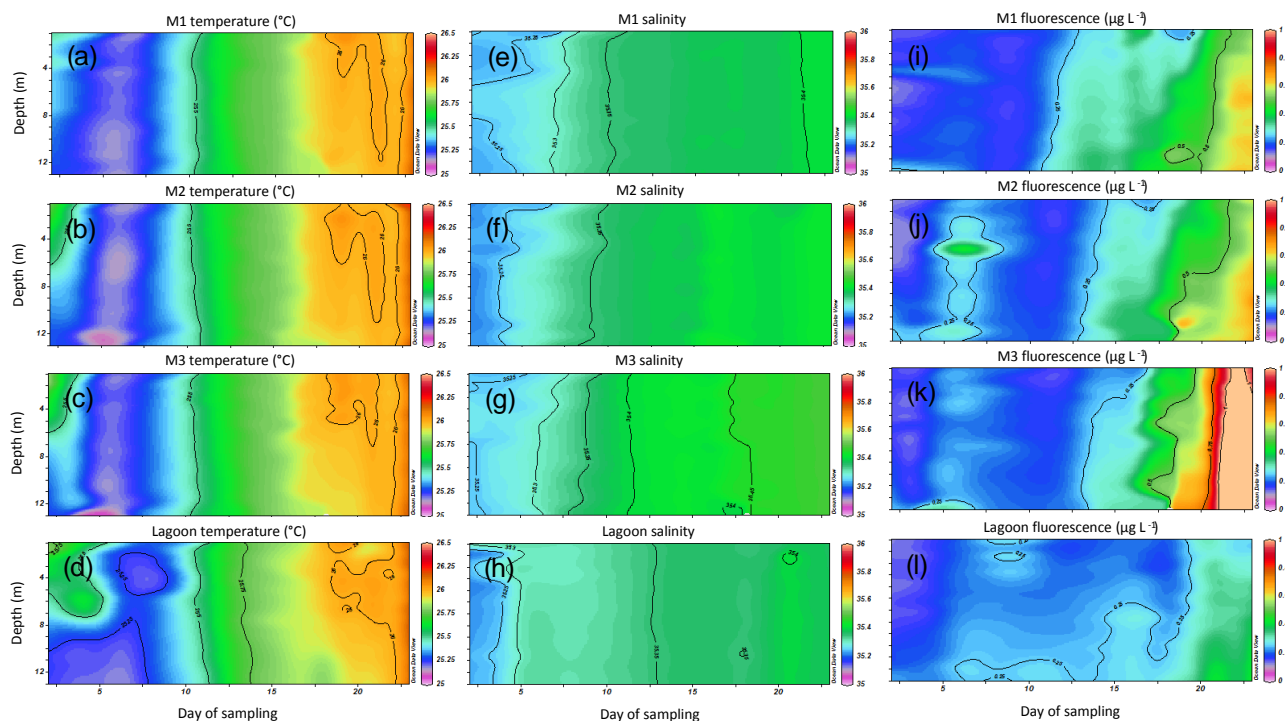
gether indicates good replicability between the mesocosms (Bonnet et al., 2015). Molecular data also report a shift in the diazotrophic community composition around day 15, with a bloom of UCYN-C consistently occurring in the three mesocosms (see Turk-Kubo et al., 2015). The same feature was observed for *Synechococcus* abundances, which increased by a factor of 2 from 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 beginning at  $\sim$  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 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 mesocosm deployment – 13 January, day 0) are summarized in Table 2. Seawater temperature was  $25.30^\circ\text{C}$ , which is slightly lower than the temperature reported in this season at the Amédée lighthouse station, while salinity (35.15) was typical for the season (Le Borgne et al., 2010). NO<sub>3</sub><sup>-</sup> and DIP concentrations were both reported to be  $0.04 \pm 0.01 \mu\text{mol L}^{-1}$ , and Chl *a* concentrations from fluorescence data ( $0.11 \mu\text{g L}^{-1}$ ) were typical of oligotrophic systems and in the range reported in the literature for this location (Fichez et al., 2010). DON and dissolved organic P (DOP) concentrations were  $4.65 \pm 0.46$  and  $0.100 \pm 0.002$ , and ambient N<sub>2</sub> fixation rates were  $8.70 \pm 1.70 \text{ nmol NL}^{-1} \text{ d}^{-1}$  before the mesocosm 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  $25.50^\circ\text{C}$  the day of the mesocosm closure (day 2) to  $26.24^\circ\text{C}$  on day 23. This warming is the typical trend observed in New Caledonia in the course of the summer season (Le Borgne et al., 2010). The water column was vertically homogeneous over the course of the experiment, except on 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-day experiment (35.2 to 35.4), suggesting a probably 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



**Figure 4.** Horizontal and vertical distributions of seawater temperature ( $^{\circ}\text{C}$ ), salinity and fluorescence ( $\mu\text{g L}^{-1}$ ) 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.

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

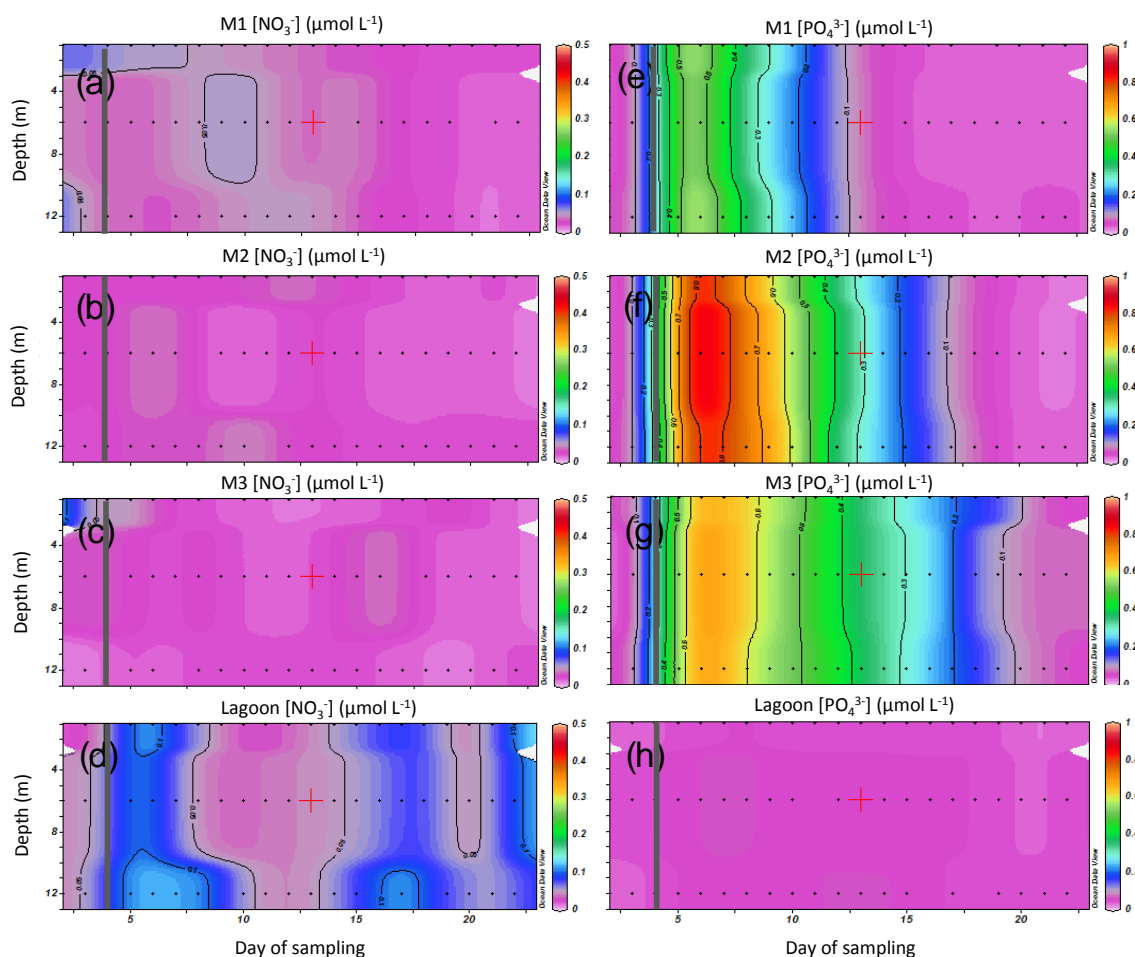
Temperature ( $^{\circ}\text{C}$ )	Salinity	$\text{NO}_3^-$ ( $\mu\text{mol L}^{-1}$ )	DIP ( $\mu\text{mol L}^{-1}$ )	Chl <i>a</i> fluo ( $\mu\text{g L}^{-1}$ )	DON ( $\mu\text{mol L}^{-1}$ )	DOP ( $\mu\text{mol L}^{-1}$ )	$\text{N}_2$ fixation ( $\text{nmol N L}^{-1} \text{d}^{-1}$ )
25.30	35.15	$0.04 \pm 0.01$	$0.04 \pm 0.01$	0.11	$4.65 \pm 0.46$	$0.10 \pm 0.02$	$8.70 \pm 1.70$

values measured in the Noumea lagoon (35.40) compared to inside (35.47) at the end of the experiment.

$\text{NO}_3^-$  concentrations (Fig. 5a–d) remained below  $0.1 \mu\text{mol L}^{-1}$  during the whole experiment in all mesocosms and in the lagoon waters. Average concentrations over the 23-day experiment and the three depths samples were close to detection limits of the method ( $0.01 \mu\text{mol L}^{-1}$ ) and are thus difficult to quantify accurately: they were  $0.04 \pm 0.02$ ,  $0.02 \pm 0.01$ ,  $0.02 \pm 0.02$  and  $0.06 \pm 0.04 \mu\text{mol L}^{-1}$  in M1, M2, M3 and in the lagoon waters, respectively. DIP concentrations (Fig. 5e–h) were also close to detection limits ( $0.005 \mu\text{mol L}^{-1}$ ) and on average  $0.04 \pm 0.01$ ,  $0.03 \pm 0.01$  and  $0.03 \pm 0.02 \mu\text{mol L}^{-1}$  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  $0.73 \pm 0.07$ ,  $0.98 \pm 0.01$  and  $0.77 \pm 0.03 \mu\text{mol L}^{-1}$  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). Subse-

quently DIP concentrations decreased steadily towards initial concentrations by the end of the experiment:  $0.03 \pm 0.01$ ,  $0.03 \pm 0.01$  and  $0.05 \pm 0.02 \mu\text{mol L}^{-1}$  in M1, M2 and M3, respectively (average of 23 days 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 homogeneous throughout the water column during the course of the experiment (Fig. 4i–l). Chl *a* slightly increased (by 0.1 to  $0.2 \mu\text{g L}^{-1}$ ) in the three mesocosms after DIP fertilization on days 5 and 6. After day 6, it consistently declined back to the initial (before fertilization) concentrations of  $0.12$ – $0.15 \mu\text{g L}^{-1}$ . On days 12, 13 and 14, Chl *a* concentrations re-increased dramatically to reach  $0.61$ ,  $0.65$  and  $1.02 \mu\text{g L}^{-1}$  in M1, M2 and M3 at day 23, respectively, indicating that the three mesocosms were relatively synchronized but the intensity of the phytoplankton



**Figure 5.** Horizontal and vertical distributions of  $\text{NO}_3^-$  and DIP ( $\mu\text{mol L}^{-1}$ ) 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.

bloom differed between the mesocosms, with a 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  $0.35 \mu\text{g L}^{-1}$  at day 23) but to a lower extent compared to that of the mesocosms.

#### 4 Presentation of the special issue

The goal of this special issue is to present the knowledge gained regarding the fate of DDN in an LNLC ecosystem based on the large data set acquired during the VAHINE mesocosm experiment. VAHINE was a multidisciplinary project involving close collaborations between biogeochemists, molecular ecologist, chemists, marine opticians and modellers. 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 ap-

proaches used to answer the main scientific questions of the VAHINE project is given in the synthesis paper Bonnet et al. (2016a).

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 complementary approaches and to investigate species successions in relation to hydrological parameters, biogeochemical stocks and fluxes during a diazotroph bloom in an LNLC ecosystem. By using polymerase chain reaction (PCR), which targeted 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 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



that a bloom of UCYN-C occurred during the second half (days 15 to 23, hereafter called P2), providing an unique opportunity to compare the DDN transfer and export efficiency associated with different diazotrophs. Complementary to this approach, Pfreundt et al. (2016b) used 16S tag sequencing to examine the temporal dynamics of the prokaryotic community and observed clear successions of prokaryotes during the experiment in relation to biogeochemical parameters. In a second study, Pfreundt et al. (2016a) also used metatranscriptomics to investigate the microbial gene expression dynamics from diazotrophic and non-diazotrophic taxa and highlighted specific patterns in the 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, concomitant with the UCYN-C bloom. 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 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 nanophytoeukaryotes during P2.

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. Berman-Frank et al. (2016) analysed the spatial and temporal dynamics of transparent exopolymeric particles (TEPs), which are sticky carbon-rich compounds that are formed, degraded and utilized in both biotic and abiotic processes, and measured a relatively stable TEP pool available as both a carbon source for plankton communities and facilitating aggregation and flux throughout the experiment.

The second point to be illustrated is that the bloom of diazotrophs (UCYN-C) obtained in the closed water body of the mesocosms following DIP fertilization offered the opportunity to track the fate of DDN in the ecosystem: Berthelot et al. (2015) describe the evolution of C, N and P pools and fluxes 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 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. Complementary to this approach, Knapp et al. (2015) reported the results of  $\delta^{15}N$  measurements on DON, particulate organic N (PON) and particles from sediment traps and further sub-

stantiated these results with a significantly ( $p < 0.05$ ) higher contribution of  $N_2$  fixation to export production during P2 ( $56 \pm 24\%$  and up to 80% at the end of the experiment) compared to P1 ( $47 \pm 6\%$ ). Bonnet et al. (2015) explored the fate of DDN on shorter timescales and revealed 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 particulate organic C (POC) exported at the height of the UCYN-C bloom. This export was mainly due to the aggregation of small ( $5.7 \pm 0.8 \mu m$ ) UCYN-C cells into large (100–500  $\mu m$ ) aggregates. Using a nanoSIMS approach, they also showed that  $21 \pm 4\%$  of the DDN was transferred to non-diazotrophic plankton, mainly picoplankton ( $18 \pm 4\%$ ) followed by diatoms ( $3 \pm 2\%$ ) during P2. 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*. 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 diazotroph blooms are more efficient at promoting non-diazotrophic production in N-depleted areas. In parallel, Hunt et al. (2016) estimated a mean  $\sim 30\%$  contribution of DDN to zooplankton biomass in the mesocosms based on natural  $^{15}N$  isotope measurements on zooplankton. They also provided evidence for direct ingestion and assimilation of UCYN-C-derived N by the zooplankton, results that were complemented by qPCR assays on several diazotroph phylotypes in zooplankton guts. Spungin et al. (2016) took advantage of the *Trichodesmium* bloom occurring outside the mesocosms 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.

The third point to be highlighted is that modelling was used at every stage of the project. Simulations performed with the 1D vertical biogeochemical mechanistic Eco3M-MED model were 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 in situ measurements and offered the opportunity to deconvolute the different interlinked biogeochemical processes occurring in the ecosystem to help understand the fate of DDN in oligotrophic ecosystems and the impact of  $N_2$  fixation on carbon export.

Finally, a synthesis study by Bonnet et al. (2016a) attempts to summarize our knowledge and the unresolved questions regarding the fate of DDN in the ocean to synthesize 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 methodological approaches used in this study to answer the scientific ques-

tions of the VAHINE project. After putting the different experimental findings in perspective, the modelling approach has also been used in the synthesis article as a tool to investigate the impact of N<sub>2</sub> fixation on marine productivity, export and food web composition by artificially removing N<sub>2</sub> fixation in the model.

*Author contributions.* Sophie Bonnet designed the experiments helped by Thierry Moutin and Jean-Michel Grisoni; Francis Louis designed the mesocosms; Jean-Michel Grisoni, Eric Folcher, Bertrand Bourgeois, Armelle Renaud and Jean-Michel Boré deployed the mesocosms and performed CTD and traps sampling; Martine Rodier analysed CTD data; Thierry Moutin was responsible for the nutrient analyses. Sophie Bonnet prepared the manuscript with contributions from all co-authors.

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