# Biogeochemical and biological impacts of diazotroph blooms in a Low Nutrient Low Chlorophyll ecosystem: synthesis from the VAHINE mesocosm experiment (New Caledonia)

**Sophie Bonnet[1,2], Melika Baklouti[1], Audrey Gimenez[1], Hugo Berthelot[1], Ilana, Berman-Frank[3]**

[1] {IRD, Aix Marseille Université, CNRS/INSU, Université de Toulon, Mediterranean Institute of Oceanography (MIO) UM 110, 13288, Marseille-Noumea, France, New Caledonia}

[2] {Institut de Recherche pour le Développement, AMU/ NRS/INSU, Université de Toulon, Mediterranean Institute of Oceanography (MIO) UM110, 98848, Noumea, New Caledonia}

[3] {Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat Gan, Israel}

Correspondence to: S. Bonnet (sophie.bonnet@ird.fr)

## Abstract

In marine ecosystems, $N_2$ fixation provides the predominant external source of nitrogen (N) (140±50 Tg N $yr^{-1}$), contributing more than atmospheric and riverine inputs to the N supply. Yet the fate and magnitude of the newly-fixed N, or diazotroph-derived N (hereafter named DDN) in marine ecosystems is poorly understood. Moreover, whether the DDN is preferentially and directly exported out of the photic zone, recycled by the microbial loop, and/or transferred into larger organisms remains unclear. These questions were investigated in the framework of the VAHINE (VAriability of vertical and tropHIc transfer of diazotroph derived N in the south wEst Pacific) project. Triplicate large volume (~ 50 $m^3$) mesocosms were deployed in the tropical South West Pacific coastal ocean (New Caledonia). The mesocosms were intentionally fertilized with ~0.8 μM dissolved inorganic phosphorus (DIP) at the start of the experiment to stimulate diazotrophy. A total of 47 stocks, fluxes, enzymatic activities and diversity parameters were measured daily inside and outside the mesocosms by the 40 scientists involved in the project. The experiment lasted for 23 days and was characterized by two distinct and successive diazotroph blooms: a dominance of diatom-diazotroph associations (DDAs) during the first half of the experiment (days 2-14) followed by a bloom of UCYN-C during the second half of the experiment (days 15-23). These conditions provided a unique opportunity to compare the DDN transfer and export efficiency associated with different diazotrophs. Here we summarize the major experimental and modelling results obtained during the project and described in the VAHINE Special issue, in particular those regarding the evolution of the main standing stocks, fluxes and biological characteristics over the 23-days experiment, the contribution of $N_2$ fixation to export fluxes, the DDN released to dissolved pool and its transfer to the planktonic food web (bacteria, phytoplankton, zooplankton). We then apply our Eco3M modelling platform to further infer the fate of DDN in the ecosystem and role of $N_2$ fixation on productivity, food web structure and carbon export. Recommendations for future work are finally provided in the conclusion section.

## 1 Introduction

Atmospheric dinitrogen ($N_2$) is the largest pool of nitrogen (N) on earth yet it is unavailable for most organisms that require N for growth. Biological fixation of $N_2$ (or diazotrophy) is catalyzed by the nitrogenase enzyme (encoded by the *nifH* genes) that converts the inert triple-bond $N_2$ into bioavailable ammonium ($NH_4^+$). This process has long been studied in terrestrial agriculture as it increases the yield of crops associated with diazotrophs. In the ocean, diazotrophy provides the predominant external source of N (140±50 Tg N $yr^{-1}$) contributing more than atmospheric and riverine inputs (Gruber, 2004). Moreover, $N_2$ fixation acts as a natural fertilizer adding a source of new N that is available for non-diazotrophic primary producers and bacterioplankton especially in Low Nutrient, Low Chlorophyll (LNLC) ecosystems, where N is the proximal limiting nutrient (Moore et al., 2013). LNLC ecosystems include the vast oligotrophic subtropical gyres and represent more than 60 % of the global ocean area. $N_2$-fixing organisms have a competitive advantage and sustain a large percentage (~50 %) of new primary production (PP) e.g. (Karl et al., 2002) in these vast ecosystems.

The non-heterocystous filamentous cyanobacterium *Trichodesmium* spp. remains the most studied marine diazotroph. Based on direct rate measurements, *Trichodesmium* accounts for a quarter to half of geochemically-derived estimates of marine $N_2$ fixation at the global scale (Mahaffey et al., 2005). Diverse cyanobacteria and bacteria also fix $N_2$ in marine waters. These include: (1) the heterocystous cyanobacteria frequently found in association with diatoms (diatom-diazotroph associations, hereafter referred to as DDAs (Foster and O'Mullan, 2008)) efficient at exporting organic matter out of the photic zone (Karl et al., 2012), (2) unicellular cyanobacterial lineages (UCYN-A, B, and C) with a size range from 1 to 6 μm (Moisander et al., 2010), which are key oceanic diazotrophs (Luo et al., 2012) accounting for the predominant fraction of $N_2$ fixation in many tropical oceans (Bonnet et al., 2009; Montoya et al., 2004), and (3) non-cyanobacterial $N_2$-fixing bacteria and archaea that are still poorly characterized yet recent studies show they are abundant and active across the world's oceans (Bonnet et al., 2013; Farnelid et al., 2011; Farnelid and Riemann, 2008; Moisander et al., 2014).

While the role and contribution of marine $N_2$ fixation on biogeochemical cycles has been intensely investigated, a critical question that remains poorly studied is the fate of newly-fixed N, or diazotroph-derived N (hereafter named DDN) in LNLC ecosystems (Mulholland, 2007). It remains unclear whether the DDN is preferentially exported directly out of the photic zone,

recycled by the microbial loop, and/or transferred into larger organisms, subsequently enhancing indirect particle export.

This question was investigated in the framework of the VAHINE (VAriability of vertical and tropHIc transfer of diazotroph derived N in the south wEst Pacific) project. Here we summarize the major results described in the VAHINE Special issue and integrate them to obtain general conclusions from the experiment. In this introduction, we first summarize some of our knowledge regarding the fate of DDN in the ocean, describe the ongoing technical challenges to study this question, and the specific scientific objectives of the VAHINE project.

## 1.1 Current knowledge on the fate of DDN in the ocean

### 1.1.1 DDN release to the dissolved pool

Diazotrophs release some of the recently fixed $N_2$ as dissolved organic N (DON) and $NH_4^+$ to the surrounding waters (Glibert and Bronk, 1994; Meador et al., 2007; Mulholland et al., 2006). Several studies have reported elevated DON and $NH_4^+$ concentrations during and immediately after *Trichodesmium* spp. blooms in the Indian (Devassy et al., 1979; Devassy et al., 1978; Glibert and O'Neil, 1999), Pacific (Karl et al., 1992; Karl et al., 1997b), and Atlantic (Lenes et al., 2001) oceans. Subsequent culture (Hutchins et al., 2007; Karl et al., 1992; Karl et al., 1997a) and field studies (Benavides et al., 2013b; Konno et al., 2010; Mulholland and Bernhardt, 2005) have quantified that diazotrophs release ~50 % of the total fixed $N_2$ to the dissolved pool. Most of these studies were performed on the conspicuous *Trichodesmium* spp. and were based on the difference between gross $N_2$ fixation (measured by acetylene reduction assays) and net $N_2$ fixation (Mulholland et al., 2004) measured using the $^{15}N_2$ labelling technique (Montoya et al., 1996). The recent modification of the $^{15}N_2$ labelling method (Mohr et al., 2010) led to higher net $N_2$ fixation rates and potentially reduced the gap between gross and net $N_2$ fixation. Applying the new $N_2$ fixation method and the direct measurement of the $^{15}N$ signature on the released DON and $NH_4^+$ demonstrated low release rates from *Trichodesmium* spp. and from three strains of UCYN-B and C (<1 % of total $N_2$ fixation) (Berthelot et al., 2015a). Similar experiments (examining the direct $^{15}N$ measurement on released molecules) showed low release by UCYN-C (~1 %, (Benavides et al., 2013a)). Culture studies probably represent lower end estimates of DDN release, as in the field, exogenous factors such as viral lysis (Hewson et al., 2004; Ohki, 1999) and sloppy feeding (O'Neil et al., 1996) may enhance the leakage of DDN by UCYN, yet such field studies on these organisms are rare.

### 1.1.2 Transfer of DDN to the trophic chain and impact on plankton community composition

The transfer of DDN towards the first levels of the food chain (phytoplankton, bacteria) is mainly achieved through the dissolved pool. Devassy et al. (1979) first observed that as blooms of *Trichodesmium* spp. decayed in the Indian ocean, diatom populations increased (mainly *Chaetoceros* sp.), followed by a succession of cladocerans, dinoflagellates, green algae, and finally copepods. In the Atlantic, a high abundance of non-diazotrophic diatoms and dinoflagellates succeeded blooms of *Trichodesmium* spp. (Devassy et al., 1978; Furnas and Mitchell, 1996; Lenes et al., 2001), while in the pelagic waters of the Kuroshio current, *Trichodesmium* spp. and diatom abundance were positively correlated (Chen et al., 2011). These studies suggest a potential transfer of DDN from diazotrophic to non-diazotrophic phytoplankton. Actual calculations of DDN transfer were first performed by Bronk et al. (2004), Lenes and Heil (2010) and Sipler et al. (2013), who demonstrated how the DDN released by *Trichodesmium* spp. affected the bloom dynamics of the toxic dinoflagellate *Karenia brevis* in the Gulf of Mexico. Results from size-fractionation of picoplankton after $^{15}N_2$ incubations also supported the idea of a DDN transfer towards non-diazotrophic plankton (Bryceson and Fay, 1981; Olendieck et al., 2007; Garcia et al., 2007). Yet, this method could not discriminate the DDN transfer towards non-diazotrophic picoplankton from $N_2$ fixation by picoplankton itself and thus likely overestimated the DDN transfer.

Thus, the actual transfer of DDN towards non-diazotrophic phytoplankton and bacteria remains poorly quantified and challenging due mainly to technical limitations as it requires appropriate methodologies to track the passage of DDN through the different components of microbial food web. Moreover, the planktonic groups (autotrophic *versus* heterotrophic, small *versus* large phytoplankton) that benefit the most from this DDN and develop during/after diazotroph blooms have not been identified so far despite their potential to differentially influence the structure of the trophic chain and eventually the mode of carbon (C) export from the photic zone.

Regarding higher trophic levels, low $\delta^{15}N$ signatures measured on zooplankton indicate that DDN is transferred towards secondary producers (Montoya et al., 2002b). This transfer can be direct through the ingestion of diazotrophs (O'Neil et al., 1996; Wannicke et al., 2013a), or indirect, i.e. mediated by the dissolved N released by diazotrophs (Capone et al., 1994; Glibert and Bronk, 1994; Mulholland et al., 2004). The dissolved N (both DIN and DON) is taken up by heterotrophic and autotrophic plankton and then potentially grazed on by zooplankton, yet these pathways remain poorly explored.

The transfer of DDN to zooplankton may possibly depend on the diazotroph community composition in the water column. Toxicity of *Trichodesmium* spp. (Kerbrat et al., 2010) combined with poor nutritional quality reduce grazing pressure by copepods other than several harpacticoïds including *Macrosetella gracilis* (O'Neil, 1999; O'Neil and Roman, 1992). Stable isotope measurements performed on zooplankton suggest higher DDN uptake when the diazotroph community is dominated by DDAs rather than *Trichodesmium* spp. (Montoya et al., 2002a). Grazing experiments on UCYN have not been conducted so far and the potential of UCYN as a conduit of DDN into marine food webs remains unexplored.

### 1.1.3 Export of DDN out of the photic zone

Low $\delta^{15}N$ signatures in particles from sediment traps in the tropical North Pacific suggest that at least part of the DDN is ultimately exported out of the photic zone (Karl et al., 2012; Karl et al., 1997b; Scharek et al., 1999a; Sharek et al., 1999b). The export of DDN may either be direct through sinking of diazotrophs, or indirect, through the transfer of DDN to non-diazotrophic plankton in the photic zone, that is subsequently exported. While DDAs can directly contribute to particle export (Karl et al., 2012; Subramaniam et al., 2008; Yeung et al., 2012), the DDN export efficiency appears to depend on the diazotroph community composition present in surface waters.

The positive buoyancy of *Trichodesmium* spp. probably prevents its downward flux and settling in sediment traps (Capone et al., 1997; Walsby, 1992), although programmed cell death (PCD) causing bloom demise can cause rapid export of *Trichodesmium* biomass to depth (Bar-Zeev et al., 2013; Berman-Frank et al., 2004; Spungin et al., In review, 2016). In the Eastern Tropical North Pacific, when the diazotrophic community was dominated by UCYN-A and *Trichodesmium* spp., $N_2$ fixation contributed ~10 % of the export (White et al., 2012); when DDAs dominated the diazotrophic community they contributed ~44 % of export production, thereby suggesting that DDAs have a higher export efficiency compared to *Trichodesmium* spp. and UCYN-A. Despite their recent recognition as key oceanic diazotrophs (Luo et al., 2012), the export efficiency of UCYN from other lineages (UCYN-B and UCYN-C) is currently undetermined as no published studies of natural UCYN-B andC blooms and their fate in the ocean are available to date.

The determination of direct *versus* indirect export requires diazotroph quantification in both the water column and in sediment traps in addition to clarifying the actual transfer of DDN to the different groups of autotrophic and heterotrophic plankton. Few studies have thus focused on the direct coupling between $N_2$ fixation and particulate export in general (see references

above). Ideally such studies require the successful encounter of an oceanic diazotroph bloom, deployment of sediment traps, and long-term (several weeks) monitoring of the biogeochemical characteristics of the water body influenced by the bloom, which are rarely accomplished. The patchy distribution of diazotrophs in the surface ocean (Bombar et al., 2015), the temporal lag between production and export, and the hydrodynamic features that may decouple production in surface and export below the photic zone (Buesseler et al., 2007) also make these studies very challenging.

## 1.2 Scientific objectives of the VAHINE project

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.

To achieve these goals and concurrently determine $N_2$ fixation and particle export, we isolated large water masses containing ambient planktonic communities by deploying three large-volume (~50 $m^3$) mesocosms (Bonnet et al., 2016b) thereby maintaining a unique water-mass with minimal disturbance of the *in-situ* light and temperature conditions (Guieu et al., 2016). The experimental location in the southwestern Pacific region was chosen as in this area some of the highest rates of oceanic $N_2$ fixation occur (Bonnet et al., 2015; Messer et al., 2015). Additionally, to enhance $N_2$ fixation, the mesocosms were intentionally fertilized with dissolved inorganic phosphorus (DIP). The experiment lasted 23 days and was characterized by a dominance of DDAs during the first half of the experiment (days 2-14) and a bloom of UCYN-C during the second half of the experiment (days 15-23), providing a unique opportunity to compare the DDN transfer and export efficiency associated with specific diazotrophs in this experimental system. Some additional process experiments performed on *Trichodesmium* spp. which bloomed outside the mesocosms on the last two days are also presented here.

Below, we summarize the scientific strategy used in this study, as well as some of the major results obtained during this project and propose some scientific perspectives for the future.

## 2 Scientific strategy

### 2.1 Brief description of the mesocosms and study site

The large-volume (~50 $m^3$) mesocosm experiment was undertaken in New Caledonia, located 1500 km east of Australia in the Coral Sea (southwestern tropical Pacific, Fig. 1). Three replicate polyethylene and vinyl acetate mesocosms (diameter 2.3 m, height 15 m, volume ~50 $m^3$, Fig. 2) were deployed 28 km off the coast of New Caledonia at the entrance to the Noumea coral lagoon (22°29.073 S - 166°26.905 E) for 23 days from January 13$^{th}$ to February 6$^{th}$ (austral summer). The New Caledonian lagoon was chosen as it is a well-studied environment (Special issue Marine Pollution Bulletin 2010 (Grenz and LeBorgne, 2010)) submitted to high oceanic influence (Ouillon et al., 2010) and exhibiting typical LNLC conditions during the summer season ($NO_3^-$ concentrations <0.04 µmol $L^{-1}$ and chlorophyll a (Chl *a*) ~0.10-0.15 µg $L^{-1}$ (Fichez et al., 2010). Primary productivity is N-limited throughout the year (Torréton et al., 2010), giving diazotrophs a competitive advantage. New Caledonian waters support high $N_2$ fixation rates (151-703 µmol N $m^{-2}$ $d^{-1}$, (Garcia et al., 2007)), as well as high *Trichodesmium* spp. (Dupouy et al., 2000; Rodier and Le Borgne, 2010, 2008), and UCYN abundances (Biegala and Raimbault, 2008), therefore representing an ideal location to implement the VAHINE project and study the fate of DDN in the marine ecosystem.

DIP availability can control $N_2$ fixation in the southwestern Pacific (Moutin et al., 2008; Moutin et al., 2005), hence the mesocosms were intentionally fertilized with ~0.8 µM DIP ($KH_2PO_4$) on the evening of day 4 to alleviate any potential DIP limitation and promote $N_2$ fixation and even diazotroph blooms for the purpose of the project.

The mesocosms used for this study are well suited for conducting replicated process studies on the first levels of the pelagic food web (Bonnet et al., 2016b; Guieu et al., 2010; Guieu et al., 2014). They are equipped with sediment traps allowing the collection of sinking material. Due to the height of the mesocosms (15 m), they do not represent processes occurring in the full photic layer but allow studying the dynamics of C, N, P pools/fluxes and export associated with the plankton diversity in the same water mass, and comparing these dynamics. before/after the DIP fertilization, and under contrasted conditions regarding the diazotroph community composition (cf below). Detailed surveys performed in LNLC environments revealed that temperature and light conditions are not affected by the presence of the mesocosms compared to surrounding waters (Bonnet et al., 2016b; Guieu et al., 2010; Guieu

et al., 2014). These studies also revealed a good replicability and low variability between stocks, fluxes and plankton diversity measurements among the replicate mesocosms. Hence, the discussion below will consider the average between the three mesocosms deployed in this study.

### 2.2 Sampling strategy and logistics

A complete description of the mesocosms design and deployment strategy is given in the introductory article (Bonnet et al., 2016b). In total, over 47 stocks, fluxes, enzymatic activities and diversity parameters were measured daily by the 40 scientists involved in the project. Protocols for each measured parameter are detailed in the specific contributions to this special issue and will not be described here. Modelling has also accompanied all steps of the project (see Gimenez et al. (In review, 2016) and section 5 below).

Sampling for stocks, fluxes and plankton diversity measurements was performed daily at 7 am in each of the three mesocosms (M1, M2 and M3) and in surrounding waters (hereafter called 'lagoon waters') from day 2 (January 15$^{th}$, the day of the mesocosms closure) to day 23 (February 6$^{th}$) at three selected depths (1, 6 and 12 m) to study the vertical variability within mesocosms and in lagoon waters. For flux measurements, bottles were incubated on an in situ mooring line at the appropriate sampling depth set up close to the mesocosms. Vertical CTD profiles were then performed daily at 10 am in every mesocosm and in lagoon waters using a SBE 19 plus Seabird CTD to obtain the vertical profiles of temperature, salinity and fluorescence. Finally, sediment traps were collected daily by SCUBA divers at 10:30 am, see details in Bonnet et al. (2016b).

## 3 Evolution of the main standing stocks, fluxes and biological characteristics during the VAHINE experiment

Initial hydrological and biogeochemical conditions (i.e. conditions in ambient waters the day of mesocosms deployment - January 13$^{th}$, day 0) were typical of those encountered in the oligotrophic Noumea lagoon during austral summer conditions (Fichez et al., 2010; Le Borgne et al., 2010), with seawater temperature of 25.5°C, surface salinity of 35.15, $NO_3^-$-depleted waters (0.04±0.01 µmol $L^{-1}$), low DIP concentrations (0.04±0.01 µmol $L^{-1}$), and Chl *a* concentrations of 0.20 µg $L^{-1}$. $N_2$ fixation rates were 8.70±1.70 nmol N $L^{-1}$ $d^{-1}$ and the diazotroph community was dominated by DDAs (het-1 3.1 x $10^4$ *nifH* copies $L^{-1}$ and het-2 1.2 x$10^4$ *nifH* copies $L^{-1}$) as well as UCYN-A2 (1.5 x $10^4$ *nifH* copies $L^{-1}$) and UCYN-A1 (5.6 x

$10^3$ *nifH* copies $L^{-1}$), which together accounted for 95 % of the total *nifH* pool in the lagoon waters prior to the mesocosms closure (Turk-Kubo et al., 2015).

During the 23-days VAHINE mesocosm experiment, three major periods could be defined based on the main C, N, P stocks and fluxes (Berthelot et al., 2015b) and on the identity of the most abundant diazotrophs that developed in the mesocosms (Turk-Kubo et al., 2015): **P0** from days 2 to 4 (i.e. prior to the DIP fertilization that occurred on the evening of day 4), **P1** from days 5 to 14, and **P2** from days 15 to 23 (Figs. 3 and 4). Figure 3 reports the main hydrological and biogeochemical parameters during the experiment. Figure 4 provides a synoptic view of the main changes (positive, negative, neutral) in the major stocks, fluxes, and plankton community composition measured during P1 and P2 respectively.

Seawater temperature (Fig. 3) gradually increased both inside and outside the mesocosms over the 23-days of the experiment from 25.5°C to 26.2°C on day 23, which is the general trend observed during austral summer conditions (Le Borgne et al., 2010). The water column was well homogenized inside the mesocosms throughout the experiment (Bonnet et al., 2016b). $NO_3^-$ concentrations remained close to detection limit of conventional micromolar methods (0.02 µmol $L^{-1}$) both inside and outside the mesocosms throughout the 23 days of the experiment (Fig. 3). The low (0.04 µmol $L^{-1}$) DIP concentrations measured during P0 increased in the mesocosms right after the fertilization up to ~0.8 µmol $L^{-1}$, then decreased quickly to reach values close to initial DIP concentrations (~0.04 µmol $L^{-1}$) at the end of the experiment.

A major objective of the experiment was to study the development of diazotroph blooms and the fate of DDN. Thus, our investigation of the biological response focused on diazotrophs and their subsequent influence on biological and biogeochemical signatures. $N_2$ fixation rates tripled between P1 and P2, to reach extremely high rates during P2 (27.3±1.0 nmol N $L^{-1}$ $d^{-1}$ on average and up to 70 nmol N $L^{-1}$ $d^{-1}$ (Bonnet et al., 2016a)) (Fig. 3), ranking among the highest rates reported in marine waters (Luo et al., 2012). DDAs dominated the diazotroph community composition during P1, and a bloom of UCYN-C occurred during P2 (Fig. 4). Standing stocks of Chl *a* and particulate organic N (PON) increased by a factor of 3 and 1.5 between P1 and P2 and subsequently, export of PON dramatically increased (by a factor of 5) in the mesocosms during P2 (Fig. 3). These results emphasize that the experimental mesocosm setup provided ideal conditions to study the fate of DDN associated with different diazotroph communities (DDAs *versus* UCYN-C).

The synoptic view of the mesocosm dynamics (Fig. 4) indicates that after the DIP fertilization, DIP concentrations and DIP turn-over time increased significantly during P1, and

alleviated P-limitation in the microbial communities as reflected in the significant decline in alkaline phosphatase activity (APA). The major biomass-indicative standing stock parameters (Chl *a*, PON, particulate organic C (POC) and P (POP)) did not increase immediately after the DIP fertilization (P1) but during P2 (see below). Only PP increased significantly by a factor of 2 during P1, associated with a significant increase in $N_2$-fixing DDAs and *Prochlorococcus* abundances. During P1, enhanced DIP availability enabled non-diazotrophic organisms with lower energetic requirements and higher growth rates such as *Prochlorococcus* to outcompete the diazotrophs in the mesocosms via utilization of recycled N derived from $N_2$ fixation (Bonnet et al., 2016a). Thus, while PP increased, $N_2$ fixation rates decreased significantly after the DIP spike.

During P2, diazotrophy was characterized by the significant increase in UCYN-C abundances that reached up to 7 x $10^5$ *nifH* copies $L^{-1}$, concomitant with the utilization of DIP and the significant decline in DIP concentrations, DIP turn-over time, and a parallel increase of total APA. In all three mesocosms, the increase in UCYN-C abundances coincided with the day at which the DIP turnover time declined below 1 d, indicative of DIP limitation (Berthelot et al., 2015b; Moutin et al., 2005). UCYN-C may have also utilized dissolved organic phosphorus (DOP) as a P source (Bandyopadhyay, 2011), driving the significant decline in DOP concentrations observed during P2 ((Berthelot et al., 2015b), Fig. 4). The mesocosm approach also enabled the calculation of *in situ* growth rates for UCYN-C. These reached~2 $d^{-1}$ during P2, i.e. higher than growth rates of any other diazotrophic phylotypes during P2 (Turk-Kubo et al., 2015), and indicating that, under $NO_3^-$ depletion and low DIP availability, UCYN-C was the most competitive diazotroph in the mesocosms.

Under the high $N_2$ fixation conditions encountered during P2 (27.3±1.0 nmol N $L^{-1}$ $d^{-1}$), all standing stocks (Chl *a*, POC, PON, POP) increased in the mesocosms, together with PP and BP (Fig. 4). The corresponding $NO_3^-$, DIP, DON and DOP stocks for P2 decreased, indicating active consumption by the planktonic communities. As no external supply of $NO_3^-$ was provided to the enclosed mesocosms, we calculated that the consumption of the $NO_3^-$ stock initially present in the mesocosms (0.04 µmol $L^{-1}$) represented less than 11 % of the integrated $N_2$ fixation rates. Therefore, $N_2$ fixation supplied nearly all of the new production during the experiment. Our results demonstrate that in oligotrophic N-depleted systems, as long as DIP does not limit $N_2$ fixation (Berthelot et al., 2015b), diazotrophs can provide enough new N to sustain high PP rates (exceeding 2 µmol C $L^{-1}$ $d^{-1}$) and high biomass (~ 10 µmol $L^{-1}$ of POC

and 0.7 µg $L^{-1}$ of Chl *a*). Furthermore, during P2, DON provided an additional N source for non-diazotrophic phytoplankton and bacteria (Berthelot et al., 2015).

Concurrent with the development of diazotrophic (UCYN-C) populations, the abundance of *Synechococcus*, pico-eukaryote, and nano-eukaryote primary producers also increased at the end of P2 (i.e. around day 16) (Leblanc et al., In review, 2016). The non-diazotrophic diatoms responded rapidly (i.e. around day 10-11) and increased to bloom values (100,000 cells $L^{-1}$) simultaneously with the UCYN-C bloom on days 15-16 and prior to the increases in the pico- and nanophytoplankton (Pfreundt et al., 2016; Van Wambeke et al., Accepted). A drastic change in the diatom community structure paralleled the UCYN-C bloom with an almost monspecific bloom dominated by *Cylindrotheca closterium.* Despite the significant increase in BP during P2 and enrichments in the 16S transcripts of specific bacterial groups (Pfreundt et al., In review, 2016), the total abundance of heterotrophic bacteria did not change (Van Wambeke et al., Accepted), probably due to grazing. Finally, no consistent temporal pattern in zooplankton biomass was detected over the course of the experiment (Hunt et al., Accepted), although changes were observed regarding the contribution of DDN to zooplankton biomass (see below).

## 4. Tracking the fate of $N_2$ fixation

### 4.1. Contribution of $N_2$ fixation to export fluxes

We specifically utilized the mesocosm approach to determine whether the composition of the diazotroph community influenced the subsequent export of particulate matter, and if so, how this was manifested. During P1, DDAs dominated the diazotroph community. For this time period, the biomass indices (Chl *a*, POC, PON, POP) were stable within the mesocosms (Fig. 3, 4), suggesting that the DDN associated with DDAs remained within the symbiotic associations (i.e. was poorly transferred to the rest of the planktonic community). Moreover, the amount of recently fixed $N_2$ equaled that of exported PON, suggesting that the recently fixed $N_2$ by DDAs was rapidly exported (Fig. 5a) as was also observed for DDAs in the tropical North Pacific at Station ALOHA (Karl et al., 2012). DDAs such as het-1 (*Richelia* in association with the diatom *Rhizosolenia* spp.), which dominated the DDA community during P1 in the mesocosms (Turk-Kubo et al., 2015) have indeed been shown to sink at high rates in the ocean (Scharek et al., 1999a).

During P2 and the UCYN-C bloom, the increases in Chl *a*, POC, PON, and POP concentrations in the mesocosms suggest that a fraction of the recently produced biomass sustained by $N_2$ fixation remained in the water column. The mesocosms enabled us to

403 determine whether export associated with diazotrophs was direct (through the sinking of
404 diazotrophic cells) or indirect (through the transfer of DDN to non-diazotrophic plankton that
405 is subsequently exported). The direct export of UCYN has rarely been studied (White et al.,
406 2012). Yet, UCYN contribution to vertical flux and export was assumed to be lower than the
407 contribution of DDAs due to their small size of (1 to 6 µm) and low sinking rates compared to
408 DDAs (up to 500 µm comprised of dense silica shells). qPCR quantification of diazotrophs in
409 the sediment traps revealed that ~10 % of UCYN-C from the water column was exported to
410 the traps daily, representing as much as 22.4±5.5 % of the total POC exported at the height of
411 the UCYN-C bloom (Bonnet et al., 2016a). Mechanistically, the vertical downward flux was
412 enabled by the aggregation of the small (5.7±0.8 µm) UCYN-C cells into large (100-500 µm)
413 aggregates, the size of which increased with depth (Fib. 5b) possibly due to a sticky matrix
414 composed also of transparent exopolymeric particles (TEP). TEP concentrations increased
415 during P2 (Fig. 4) providing both a nutrient source and aggregation enhancing substrate
416 (Berman-Frank et al., 2016). These data, reported for the first time from the VAHINE
417 experiment (Bonnet et al., 2016a), emphasize that despite their small size relative to DDAs,
418 UCYN-C are able to directly export organic matter to depth, indicating that these small
419 organisms should be considered in future biogeochemical studies.
420 The direct export of UCYN-C and other diazotrophs could not solely explain the very high
421 exported matter observed during P2 (Bonnet et al., 2016a), suggesting another pathway of
422 export during that period. An experiment performed during the UCYNC bloom using
423 nanoSIMS (nanoscale Secondary Ion Mass Spectroscopy) as described in Bonnet et al.,
424 (2016) demonstrated that a significant fraction of DDN (21±4 %) was quickly (within 24 h)
425 transferred to non-diazotrophic plankton, revealing that $N_2$ fixation was fuelling non-
426 diazotrophic plankton growth in the water column (Fig. 5b), suggesting an indirect export
427 pathway in addition to the direct export of UCYN-C. The fact that UCYN-C fuelled non-
428 diazotrophic plankton during P2 is consistent with the increase in biomass indicators as well
429 as the increase in non-diazotrophic phytoplankton abundances (diatom and picoplankton)
430 simultaneously with or after the UCYN-C bloom during P2.
431 The high export efficiency associated with the UCYN-C bloom compared to that associated
432 with the DDAs during VAHINE was also indicated by *e*-ratio calculations (e-ratio =
433 POCexport/PP), which quantify the efficiency of a system to export particulate C relative to
434 the C fixed by PP. During P2, the *e*-ratio was significantly ($p<0.05$) higher (i.e., during the
435 UCYN-C bloom; 39.7±24.9 %) than during P1 (i.e., when DDAs dominated the diazotrophic
436 community; 23.9±20.2 %) (Berthelot et al., 2015b). $\delta^{15}N$ measurements on DON, PON and

particles from sediment traps 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 %) (Knapp et al., 2015). The contribution of $N_2$ fixation to export (up to 80 %) was very high in our study compared with reports from other tropical and subtropical regions where active $N_2$ fixation contribute 10 to 25 % to export production (e.g. (Altabet, 1988; Knapp et al., 2005)). This is consistent with the extremely high $N_2$ fixation rates measured in the mesocosms (up to 70 nmol N $L^{-1}$ $d^{-1}$) and compared with those measured from other regions (Luo et al., 2012).

Export associated with *Trichodesmium* spp. was not studied in the present mesocosm experiment as only limited numbers of *Trichodesmium* spp. were counted in the mesocosms (Turk-Kubo et al. 2015). Its potential for export is discussed below based on parallel studies from the region and intensive short-term experiments on surface blooms of *Trichodesmium* that appeared outside the mesocosms on days 22-23 (Spungin et al., In review, 2016).

## 4.2. DDN release and transfer to the food web

### 4.2.1 DDN release and transfer to non-diazotrophic phytoplankton and bacteria

Within VAHINE we also assessed the quantity of DDN entering the planktonic food web as a function of the dominant diazotroph players, and examined which planktonic communities benefited the most from the DDN (i.e. small *versus* large phytoplankton or microbial food web).

Diazotrophs transfer DDN to phytoplankton and heterotrophic prokaryotes via the dissolved N pool (DON and $NH_4^+$). During the maximal abundance of UCYN-C, these were responsible for 90±29 % of total $N_2$ fixation rates in the mesocosms (Bonnet et al., 2016a). During this period, the DDN released to the dissolved pool accounted for 7.1±1.2 to 20.6±8.1 % of gross $N_2$ fixation (Bonnet et al., 2016a) (based on the direct measurement of the isotopic signature ($^{15}N$) of the total dissolved N according to the denitrifying method (Knapp et al., 2005)). This proportion is higher than that reported for UCYN-C in monospecific cultures using an equivalent method (1.0±0.3 to 1.3±0.2 % of gross $N_2$ fixation (Benavides et al., 2013a; Berthelot et al., 2015a). At the same time as UCYN-C bloomed, the diverse diazotroph community present in the mesocosms (Turk-Kubo et al., 2015) also contributed to the DDN release. . Additionally, exogenous factors such as viral lysis (Fuhrman, 1999) and sloppy feeding (O'Neil and Roman, 1992) occur in natural populations and could enhance N release compared to the mono-culture studies. Here, we demonstrate that natural UCYN blooms may result in substantial DDN release to the marine environment.

The physiological state of cells probably plays a critical role in the quantity and availability of DDN to the microbial communities as demonstrated in a study (applying identical methodology) from two naturally-occurring blooms of *Trichodesmium* spp. in the same area (New Caledonian lagoon) (Bonnet et al., Accepted). DDN release from these blooms was slightly higher (bloom 1: 20±5 to 48±5 % and bloom 2: 13±2 to 28±6 % of gross $N_2$ fixation) compared to UCYN-C (Bonnet et al., Accepted). A decaying *Trichodesmium* spp. bloom (Bloom 1)lead to high DDN release rates and high $NH_4^+$ accumulation (up to 3.4 µM) in the dissolved pool, while we did not observe this in exponentially growing *Trichodesmium* (Bloom 2). . The importance of physiological status rather than specific diazotroph types was further substantiated in earlier *Trichodesmium* culture studies (Mulholland et al., 2004; Mulholland and Capone, 2000) and similar DDN release between *Trichodesmium* spp. and three strains of UCYN-B and C were found by Berthelot et al. (2015a).

Previous comparisons between gross and net $N_2$ fixation rates indicated high DDN release rates for oceanic populations of *Trichodesmium* spp. (40-50 % of gross $N_2$ fixation on average, and up to 97 %, (Mulholland, 2007) and references therein). The physiological status of these populations may have influenced the fluxes. Furthermore, the values could reflect a methodological overestimation due to the use of the $^{15}N_2$ bubble method (Großkopf et al., 2012; Montoya et al., 1996) that may lead to greater differences between gross and net $N_2$ fixation (see introduction). Currently, direct measurement of the $^{15}N$ signature of the dissolved N pool itself (either the TDN pool through the Knapp et al. (2005) method or both the $NH_4^+$ and the DON using the Slawyk and Raimbault (1995) method) appears the preferred method to accurately quantify the amount of DDN released by diazotrophs in the dissolved pool (Berthelot et al., 2015a).

Once released in the form of $NH_4^+$ and/or DON, DDN can be taken up by surrounding planktonic communities. Experimental evidence from nanoSIMS experiments during VAHINE indicate that 21±4 % of the $^{15}N_2$ fixed during the UCYN-C bloom was transferred to the non-diazotrophic plankton after 24 h of incubation (Bonnet et al., 2016a). Among these 21±4 %, 18±3 % was transferred to picoplankton (including both pico-phytoplankton and heterotrophic prokaryotes) and 3 % to diatoms (Fig. 5b), suggesting that picoplankton would be more competitive than diatoms using DDN, which is consistent with the increase in *Synechococcus* and pico-eukaryote abundances by a factor of two following the UCYN-C bloom (Leblanc et al., In review, 2016; Pfreundt et al., 2016). The short-term nanoSIMS experiment was performed on day 17, when pico- and nanoplankton dominated the

phytoplankonic biomass and diatom abundances declined probably due to DIP limitation (Leblanc et al., In review, 2016). Picoplankton can efficiently utilize low DIP concentrations (Moutin et al., 2002) and/or can use alternative DOP sources (Benitez-Nelson and Buesseler, 1999). This may explain why picoplankton were the first beneficiaries of the DDN from UCYN-C specifically from days 17-23, although we cannot exclude that diatoms had also benefited from the DDN from UCYN-C earlier in the experiment (between days 10-11 and days 15-16 when they reached bloom values of ~100 000 cells $L^{-1}$). A significant increase of both PP and BP during P2 (Fig. 2) suggests that both autotrophic and heterotrophic communities benefited from the DDN (Bonnet et al., 2016a). Calculations based on C:N molar ratios show that $N_2$ fixation may have provided ~30 % of the N demand of the N-limited bacteria during P2 (compared to ~20 % during P1), the rest provided by detritus and DON (Van Wambeke et al., Accepted), which concentrations decreased during the 23 days (Berthelot et al., 2015b). Throughout VAHINE, the biological system inside the mesocosms was net autotrophic with an upper error limit close to the metabolic balance between autotrophy and heterotrophy (Van Wambeke et al., Accepted). The relationships between BP and $N_2$ fixation rates were weak (during P2) or absent (during P1) yet tightly coupled between BP and Chl *a* concentrations, and between BP and PP. This suggests that $N_2$ fixation stimulated autotrophic communities and these subsequently fueled heterotrophic prokaryotes through the production and release of dissolved organic matter including C (DOC) (Van Wambeke et al., Accepted).

In a recent study performed at the VAHINE study site, (Berthelot et al., In review, 2016) compared the DDN transfer efficiency to several groups of non-diazotrophic plankton as a function of the diazotroph groups dominating the community (*Trichodesmium* spp. *versus* UCYN-B *versus* UCYN-C). Simulated blooms of *Trichodesmium* spp., UCYN-B and UCYN-C grown in culture added to ambient lagoon communities reveal that the primary route of transfer of DDN towards non-diazotrophs is $NH_4^+$, and DON mainly accumulates in the dissolved pool, whatever the diazotroph considered. In all cases, the presence of diazotrophs stimulated biomass production of non-diazotrophs, with heterotrophic prokaryotes the main DDN beneficiaries followed by diatoms and picophytoplankton. NanoSIMS analyses revealed that heterotrophic prokaryotes were highly $^{15}N$-enriched, confirming they can directly benefit from the DDN (Berthelot et al., In review, 2016). Further studies are needed to study the indirect stimulation of heterotrophic prokaryotes through the release of DOC by diazotrophs and non-diazotrophic phytoplankton that were stimulated by the DDN.

Similar experiments ($^{15}N_2$ labelling, flow cytometry cell sorting and nanoSIMS) performed on three naturally-occurring *Trichodesmium* spp. blooms in the southwestern Pacific illustrated that DDN was predominantly transferred to diatoms (Bonnet et al., Accepted). These results indicate that the extensive oceanic blooms of *Trichodesmium* spp. can contribute to a large indirect downward flux of organic matter by promoting large cells (e.g., diatoms and dinoflagellates) characterized by efficient export rates (Nelson et al., 1995, Bonnet et al., Accepted; Devassy et al., 1979; Lenes et al., 2001).

Direct export flux of *Trichodesmium* spp. blooms may also occur in cases where rapid (< 2 d) bloom mortality occurs via a programmed cell death (PCD) (Berman-Frank et al., 2004; Berman-Frank et al., 2007). PCD in *Trichodesmium* spp. is characterized by the loss of buoyancy (collapse of gas vesicles) and increased production of TEP and aggregation leading to enhanced and massive vertical flux (Bar-Zeev et al., 2013). A *Trichodesmium* spp. bloom that occurred outside the VAHINE mesocosms on days 23-24 displayed mechanistic features of PCD including mass mortality within 24 h, loss of gas vesicles, and high production of TEP (Spungin et al., In review, 2016). While we could not directly quantify the export flux as no sediment traps were deployed in the lagoon water outside the mesocosms, the characteristics of the bloom, lack of grazer influence and the demise of biomass suggests this would lead to high rates of export (Spungin et al., In review, 2016) as demonstrated in culture simulations (Bar-Zeev et al., 2013) (Fig 5c).

### 4.2.2 DDN transfer to zooplankton

DDN transfer to zooplankton may either be direct through the ingestion of diazotrophs, or indirect, i.e. mediated through the release of dissolved DDN by diazotrophs taken up by heterotrophic and autotrophic plankton and subsequently grazed by zooplankton. During the VAHINE experiment, the percent contribution of DDN to zooplankton biomass averaged 30 % (range = 15 to 70 %) (Hunt et al., Accepted), which is in upper range of values reported from high $N_2$ fixation areas such as the subtropical north Atlantic (Landrum et al., 2011; Mompean et al., 2013; Montoya et al., 2002a), the Baltic Sea (Sommer et al., 2006; Wannicke et al., 2013b), and the pelagic waters off the New Caledonian shelf (Hunt et al., 2015).

During VAHINE all four of the qPCR targeted diazotrophs (*Trichodesmium* spp., het-1, het-2, UCYN-C) were found in zooplankton guts indicating a direct grazing of these four phylotypes (Hunt et al., Accepted). Overall, the most frequently detected targets were het-1 (during P1; 17 to 180 *nifH* copies copepod$^{-1}$) and UCYN-C (during P2; 7 to 50 *nifH* copies copepod$^{-1}$), i.e. the most abundant phylotypes encountered in the mesocosms during P1 and P2, respectively.

572 However, *Trichodesmium* spp. and het-2 were also detected at relatively high abundances in
573 copepod guts (~280 *nifH* copies copepod$^{-1}$) despite their low abundance in the mesocosms,
574 suggesting selective feeding and a possible top down control through zooplankton grazing for
575 these two phylotypes.
576 Direct and efficient zooplankton grazing on UCYN-C was further substantiated by targeted
577 grazing experiments during VAHINE which consisted of $^{15}N_2$-labeled bottle incubations of
578 freshly collected zooplankton in the presence of natural phytoplankton assemblages. The $^{15}N_2$
579 label was taken up by the diazotroph in the incubation bottles and used as a marker of
580 zooplankton diazotroph ingestion and/or ingestion of non-diazotrophic plankton grown on
581 DDN. Zooplankton were highly $^{15}N$ enriched after 72 h of incubation during the UCYN-C
582 bloom (P2), slightly enriched during P1 when DDAs dominated to diazotrophic community,
583 and not enriched at all when a *Trichodesmium* spp. bloom was encountered outside the
584 mesocosms during P2 (Hunt et al., Accepted). This was a surprising finding given that het-1,
585 and to a lesser extent *Trichodesmium* spp. were detected in copepod guts, and would suggest
586 that UCYN-C are much more efficiently transferred to zooplankton compared to DDAs and
587 *Trichodesmium* spp. While we demonstrated direct grazing of zooplankton on *Trichodesmium*
588 spp., DDAs and UCYN-C, further studies are required to quantify a more general contribution
589 of direct and indirect transfer of DDN to zooplankton.
590

591 ## 5 Modelling as a tool to infer the fate of DDN and the role of $N_2$ fixation on
592 ## productivity, food web structure and C export

593 Modelling has accompanied every stage of the VAHINE project. Mesocosm 1D-vertical
594 simulations with the biogeochemical mechanistic Eco3M-MED model (Alekseenko et al.,
595 2014), enriched with diazotrophs for the present study, and embedded in the Eco3M
596 modelling platform (Baklouti et al., 2006), were utilized prior to the *in situ* experiments to aid
597 in the scientific design of the experiment and in understanding the need and the optimal
598 timing of the DIP enrichment. The biogeochemical model was first assessed using *in situ* data
599 from the mesocosms and then applied to study the fate of DDN in the ecosystem (Gimenez et
600 al., In review, 2016). Finally, one of the main strengths of the modelling tool lies in the
601 opportunity that it offers to separate the different processes that are deeply interlinked. Here
602 we employed this to infer the role of $N_2$ fixation on productivity, food web structure, and C
603 export. The simulation of the mesocosm experiment (including DIP enrichment) reported in
604 Gimenez et al. (In review, 2016) hereafter referred to as the 'REF' simulation, and its main
605 results relative to the fate of the DDN are summarized below.

606 At the end of the REF simulation (set at 25 days in the model), 33 % of the DDN was found
607 in the diazotrophs, 43 % in the non-diazotroph organisms, 16 % in the DON pool, 3 % in the
608 particulate detrital organic pool and 5 % in the traps, indicating that $N_2$ fixation efficiently
609 benefited non-diazotrophic organisms and contributed to particle export. The model results
610 substantiated the mass balance of N (Berthelot et al., 2015b) demonstrating that during the
611 first 10 days of the experiment, planktonic organisms did not significantly benefit from the
612 DDN and that DDN did not accumulate in the water column (was not transferred to non-
613 diazotrophic plankton). After day 10, the DDN proportion increased in all the non-
614 diazotrophic plankton groups, and simultaneously decreased in the non-living pools, although
615 DON concentrations lagged decreasing only from day 13. This decrease in DDN proportion in
616 the abiotic N pools is due both to the assimilation of mineral and organic nutrients by
617 phytoplankton and heterotrophic prokaryotes, as well as to the sinking of the produced
618 organic matter through aggregation processes.
619 The model results further showed that the fraction of DDN in the exported particulate matter
620 increased from day 10 until the end of the simulation, consistent with the high *e*-ratio
621 (Berthelot et al., 2015b) during P2 (see above) and with the $\delta^{15}N$-budget ( Knapp et al.
622 (submitted)), emphasizing the higher contribution of $N_2$ fixation to export production during
623 P2 compared to P1 (Gimenez et al., In review, 2016).
624 In the model, diazotrophs were assumed to release equal amounts of $NH_4^+$ and DON at a rate
625 which increases non-linearly with the absolute and relative N contents of diazotrophs
626 (Gimenez et al., In review, 2016). During P1, DDN accumulated in the DON pool (nearly up
627 to 40 % of the DDN generated from the beginning of the experiment is found in DON on day
628 13), whereas the proportion of DDN associated with $NH_4^+$ decreased rapidly from day 5 as
629 $NH_4^+$ was immediately used by heterotrophic bacteria and phytoplankton. The proportion of
630 DDN associated with DON decreased later (i.e. during P2) when the inorganic N pool was
631 depleted. The model results are consistent with the $^{15}N$ measurements from the $NH_4^+$ and
632 DON pools, indicating that $NH_4^+$ was preferentially transferred to non-diazotrophic plankton
633 compared to DON, which accumulated in the dissolved pool (Berthelot et al., In review,
634 2016).
635 The model results were further validated in the distribution of the DDN among the biotic
636 compartments. Small-size (pico- and nano-) phytoplankton, heterotrophic prokaryotes,
637 heterotrophic nanoflagellates and ciliates were the main beneficiaries of DDN, as observed by
638 the nanoSIMS studies (Berthelot et al., In review, 2016; Bonnet et al., 2016a). Small-size
639 phytoplankton and heterotrophic prokaryotes were indeed the main consumers of $NH_4^+$ and

640 labile DON (the model excludes DON uptake by large-size phytoplankton), and heterotrophic
641 nanoflagellates and ciliates respectively feed on heterotrophic prokaryotes and small-size
642 phytoplankton. These results therefore indicate that DDN was transferred predominantly
643 through pico-, nanophytoplankton, and the microbial loop during the VAHINE experiment.
644
645 Both the *in situ* and modelling work summarized in the previous sections demonstrate the
646 important contribution and role of the diazotrophic communities to PP (non-diazotrophic) and
647 BP, to zooplankton feeding, and eventually to C export.
648 To further assess the role of $N_2$ fixation within the ecosystem, we used the REF simulation
649 from Gimenez et al. (In review, 2016) and compared it to a new simulation in which we
650 removed the $N_2$ fixation capability of diazotrophs (hereafter named 'NOFIX simulation'). The
651 NOFIX simulation also included the following changes compared to the REF simulation to be
652 consistent with the new environmental conditions: (i) the initial relative N quotas of
653 diazotrophs have been set to 25 % (instead of 100 % in the reference simulation, i.e. same
654 value as the one used for non-diazotrophs). As the initial total N was identical to the one of
655 the REF simulation, the N content of diazotrophs has been allocated to the detrital N
656 compartment; (ii) all along the NOFIX simulation, only the detrital particulate compartment is
657 allowed to sink at a constant rate of 0.7 m $d^{-1}$ (see Gimenez et al. (In review, 2016)), whereas
658 in the REF simulation, this was also the case only until day 10 beyond which all the
659 compartments were allowed to sink at a rate increasing with time, in order to mimic the
660 observed increase in the particulate sinking flux due to TEP release and aggregation .
661 When comparing the REF and NOFIX simulations (Fig. 6), we note that the shapes of the PP
662 and BP curves remain the same, showing an increase in PP and PB during P2 in both
663 simulations. However, in the NOFIX simulation, the magnitude of PP and BP is reduced by
664 2.5 and 1.5-fold respectively. Furthermore, according to the model, $N_2$ fixation fueled 43.5 %
665 of PP and 8 % of BP during the 23 days of the simulated experiment. This does not
666 necessarily mean that non-diazotrophic autotrophs benefit more from the DDN compared to
667 heterotrophs as the DDN was nearly equally distributed between autotrophs and heterotrophs
668 (and slightly higher in heterotrophs) (Gimenez et al., In review, 2016). This higher effect on
669 PP than on BP is derived from the fact that the diazotrophs themselves (and therefore a part of
670 PP since only autotrophic diazotrophs were considered in the model) were strongly affected
671 by their inability to fix $N_2$ as suggested by the far lower abundance of UCYN-C in the NOFIX
672 simulation compared to the REF one (Fig. 6). This also explains why removing $N_2$ fixation
673 first affected PP (~ day 10) and only later influenced BP (~ day 15).

We further assumed that, apart from diazotrophs, the organisms primarily influenced by the lack of $N_2$ fixation (in the simulation) should be the organisms that benefited the most from the DDN (i.e. in which the highest percentages of DDN have been calculated by the model (see Fig. 6 in Gimenez et al. (In review, 2016)). These organisms include small (< 10 µm) phytoplankton, heterotrophic prokaryotes, heterotrophic nanoflagellates, and ciliates. Small phytoplankton and heterotrophic bacteria were indeed influenced (Fig. 7), and to a lesser extent and later heterotrophic nanoflagellates and ciliate abundance, but only until day 16. After day 16, ciliate abundance was slightly higher (<5 % between day 16 and 23) in the NOFIX simulation compared to the REF one, resulting predominantly from a top-down effect due to increased copepod predation in the NOFIX simulation from day 10 to day 23 (results not shown).

Our model did not include DDAs and did not allow the uptake of DON by large phytoplankton (i.e. diatoms). Thus, the DDN content in diatoms, and therefore in mesozooplankton, was probably slightly underestimated by the model in the REF simulation (Gimenez et al., In review, 2016) compared to *in situ* data (Hunt et al., Accepted). As a result, large phytoplankton and mesozooplankton abundances were nearly similar in the REF and NOFIX simulations (not shown). Hence, apart from ciliates (whose mortality also fuels the detrital particulate compartment as large phytoplankton and mesozooplankton), the organisms that mostly benefited from the DDN were the small organisms, which mortality fuels the dissolved organic pool.

How does $N_2$ fixation impact C export? Absence of $N_2$ fixation (NOFIX simulation) reduced export by 30 % on day 23 compared to the REF simulation (Fig. 8). This difference in C export reaches 50 % when the simulation duration is extended until day 35 (not shown). These results indicate that $N_2$ fixation and the subsequent new production promotes C export to depth as the experimental VAHINE results demonstrated (Berthelot et al., 2015b; Knapp et al., 2015).

It is likely that during the experiment, TEP release favored aggregation and accumulation of particles and subsequently enhanced vertical flux from the different compartments in the water column. To represent the latter phenomenon, we considered in the model that 10 % of the living and non-living compartments were allowed to sink after day 10 (see Gimenez et al. (2016) for more details). Since this extra aggregation is mainly attributable to diazotrophs, it was not represented in the NOFIX simulation. However, we ran a third simulation (not shown) to further analyze the excess of C export in the REF simulation as compared to the

NOFIX one (Fig. 8). This third simulation is intermediate between the REF and the NOFIX simulations in that sense that only the $N_2$ fixation capability by diazotrophs is removed (but aggregation processes are still represented). This simulation indicated that C export is nearly equal to that of the REF simulation after 25 days (they differ by only 2.9 %), thereby suggesting that during the 25 first days, the suppression of $N_2$ fixation does not significantly impact carbon export fluxes. This further suggests that the higher C export in the REF simulation during P2 (Fig.8) is mainly due to aggregation processes mediated by diazotrophs-derived TEP release and the subsequent export of diazotrophs (Berman-Frank et al., 2016; Bonnet et al., 2015a). However, beyond day 25, the difference in C export between the REF and the third simulation increases up to 25% on day 35. In other words, the $N_2$ fixation process per se (by supporting PP and BP fluxes) contributes more and more to the enhanced C export as $N_2$ fixation fluxes increase. Hence, on day 30, $N_2$ fixation supports ~50 % of the excess C export observed between the REF and the NOFIX simulations, the remaining still being attributed to aggregation processes.

To conclude, $N_2$ fixation has a significant impact on both direct and indirect C export via diazotroph fueling of non-diazotrophic plankton as well as via aggregation processes. The model provides a lower limit of the major role played by $N_2$ fixation on C export due to an underestimate of the DDN content in diatoms, and in mesozooplankton. Finally, this study also points the need of further investigation on aggregation processes in relation with TEP release and its representation in models since its influence on C export may be of the same order of magnitude as the $N_2$ fixation process per se.

## 6 Conclusions and future work

The VAHINE project provided unique opportunities to study and compare the fate of $N_2$ fixation associated with different diazotrophs in the marine environment. The results showed that when the diazotroph community was dominated by DDAs, the DDN remained within the symbiotic associations, was poorly transferred to the non-diazotrophic phytoplankton and heterotrophic prokaryotes, yet could be transferred directly to zooplankton through grazing. The project results further substantiated previous data showing rapid export to depth of the recently fixed $N_2$ by DDAs (Karl et al., 2012). An opportune bloom of UCYN-C during the VAHINE project demonstrated that when UCYN-C dominated the diazotroph community, ~ 25 % of the DDN was quickly (24 h) transferred to the planktonic food web through the release of DON and $NH_4^+$ to the dissolved pool. These additional N sources were subsequently transferred to zooplankton, both directly (through the grazing of UCYN-C) and

indirectly through the grazing of plankton grown on DDN from UCYN-C. Moreover, the VAHINE data explicitly revealed that when UCYN-C dominated the diazotroph community, the efficiency of the system to export POC relative to PP (*e*-ratio) was higher than when DDAs dominated. This export is both direct, through the sinking of small (5.7±0.8 µm) UCYN-C cells aggregated into large (100-500 µm) particles having high sinking rates, and indirect through the sinking of plankton benefitting from the enriched source of DDN. Future projects should extend the investigation of DDN export below the photic layer in the open ocean (~70-150 m in the oligotrophic ocean) to confirm the process study obtained during VAHINE in mesocosms in an experimental 15 m-depth water column. In particular, are the aggregation processes of UCYN also observed in the open ocean? Although technically and logistically challenging, this feat may be accomplished through locating a research vessel in a 1D structure (cyclonic eddy harboring high UCYN abundances for example) where horizontal advection is reduced and sediment traps are deployed to study the biological and biogeochemical characteristics of the photic zone for one to two weeks.

The VAHINE project also provided a unique opportunity to compare the transfer efficiency of DDN from UCYN and *Trichodesmium* spp. to the different compartments of the planktonic food web, and revealed that the main beneficiaries of the DDN depend on both the physiological status (e.g. nutritionally balanced, stationary or decline phase) and the type of diazotroph. When *Trichodesmium* spp. bloom decay they release large amounts of $NH_4^+$ and mainly support diatom growth, indicating a large potential of indirect organic matter export during/after *Trichodesmium* spp. blooms. This is further substantiated by the study of PCD indicating a rapid direct export of *Trichodesmium* spp. itself but further studies are needed in open ocean *Trichodesmium* spp. blooms to extrapolate our results to the field.

$NH_4^+$ appears to be the main form of DDN transferred to non-diazotrophic plankton. In future studies, it would be necessary to refine the chemical composition of DON released by different diazotrophs to assess its lability as a function of the diazotrophs involved in $N_2$ fixation and the stage of the bloom. It would also be informative to explore the amount and chemical composition of released DOC and better study the potential of diazotrophs to stimulate heterotrophs and their subsequent impact on the ocean metabolic balance.

Finally, in the future ocean, some diazotrophs such as *Trichodesmium* spp. (Hutchins et al., 2007; Levitan et al., 2007) and UCYN-B (Fu et al., 2008) (no study is available on UCYN-C) may develop extensively under high temperature and $pCO_2$ conditions (Dutkiewicz et al., 2015), while others such as UCYN-A would not be affected (Law et al., 2012). The results from the VAHINE project revealed that the diazotroph community composition can impact

776 the planktonic food web structure and composition in the surface ocean, and also affects the
777 efficiency of particulate matter export to depth. Thus, current and predicted global changes
778 require further knowledge and understanding of the fate and implications of changing
779 scenarios of $N_2$ fixation in the future oceans.
780
781
782 ## Acknowledgements
783 Funding for this research was provided by the Agence Nationale de la Recherche (ANR
784 starting grant VAHINE ANR-13-JS06-0002), the INSU-LEFE-CYBER program, GOPS and
785 IRD. The authors thank the captain and crew of the R/V *Alis*. We acknowledge the SEOH
786 diver service from Noumea, as well as the technical service of the IRD research center of
787 Noumea for their helpful technical support together with C. Guieu, J.-M. Grisoni and F. Louis
788 for the mesocosm design and the useful advice. Partial funding to IBF was provided through a
789 collaborative grant with SB from the Israel Ministry of Science and Technology (MOST) and
790 the High Council for Science and Technology (HCST)-France, and a German-Israeli
791 Foundation for Scientific Research and Development (GIF) grant No. 1133-13.8/2011.
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812

**Figure legends.**

**Figure 1.** Study site of the VAHINE experiment. Location map of New Caledonia in the Southwestern Pacific (a), Map of the Noumea lagoon showing the location of mesocosms at the entrance of the lagoon, 28 km off the coast (b).

**Figure 2.** View of the mesocosms from above (a), from the seafloor (b) and view of the sediment traps that collect sinking particles (c) (Photos credits: J.M. Boré and E. Folcher, IRD).

**Figure 3.** Evolution of sea surface temperature (°C) (a), $NO_3^-$ (µmol $L^{-1}$) (b), DIP (µmol $L^{-1}$) (c), Chl a (µg $L^{-1}$) (d), $N_2$ fixation rates (nmol N $L^{-1}$ $d^{-1}$) (e), PON concentrations (µmol $L^{-1}$) (f), DON concentrations (µmol $L^{-1}$) (g) and PON export (µmol $d^{-1}$) (h) over the 23 days of the VAHINE mesocosm experiment. Lines represent the average of the three mesocoms and shaded areas represent the measured min and max values.

**Figure 4.** Upper panel: Diazotroph community composition in the VAHINE mesocosm experiment during the experimental period. *nifH*-based abundances were summed for each sampling day to determine the percent contribution to the total diazotroph community from each major phylotype (data from Turk-Kubo et al. (2015)). Bottom panel: simplified evolution of the major standing stocks, rates and plankton abundances measured during P1 (days 5 to 14) and P2 (days 15 to 23). Protocols for each parameter measurements are described in Berthelot et al. (2015), Bonnet et al. (2016a,b), Van Wambeke et al., (2016), Berman-Frank et al., (2016), Leblanc et al. (2016), Turk-Kubo et al., (2015) and Hunt et al., (2016). Squares are represented in green when a significant ($p<0.05$) increase was observed between each period (i.e. between P0 and P1 or between P1 and P2, Kruskall-Wallis test, $\alpha=0.05$), in red when a significant ($p<0.05$) decrease was observed and in grey when no significant change was observed between the different periods.

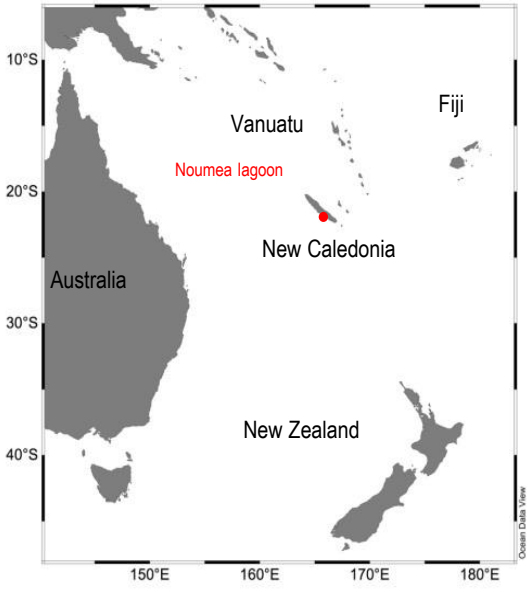
**Figure 5.** Summary of the simplified pathways of the potential DDN transfer in the first trophic level of the food web and potential of direct *versus* indirect export of particulate matter for DDAs (a), UCYN-C (b) and *Trichodesmium* (c). DDN transfer data from (Bonnet et al., Accepted; Bonnet et al., 2016a)

**Figure 6.** Evolution of PP (µmol C $L^{-1}$ $d^{-1}$) (a) and bacterial production (ng C $L^{-1}$ $h^{-1}$) in the REF simulation (blue line) and the NOFIX simulation (black line) (i.e. when the $N_2$ fixation process is removed).

**Figure 7.** Evolution of plankton abundances (cells $L^{-1}$) in the REF simulation (blue line) and the NOFIX simulation (black line) (i.e. when the $N_2$ fixation process is removed). TRI: *Trichodesmium* spp., UCYN: UCYN-C, BAC: heterotrophic bacteria, PHYS: small phytoplankton, HNF: heterotrophic nanoflagellates.

**Figure 8.** Evolution of C content collected in the mesocosm particle traps (mmol C) in the REF simulation (blue line) and the NOFIX simulation (black line) (i.e. when the $N_2$ fixation process is removed).

a)


10°S
20°S
30°S
40°S
150°E
160°E
170°E
180°E
Vanuatu
Fiji
Noumea lagoon
New Caledonia
Australia
New Zealand
Ocean Data View

b)


Noumea
Le Mont-Dore
LAGOON
R/V Alis
Mesocosms
Amédée Island
OPEN OCEAN

Figure 1.

a)

b)

c)

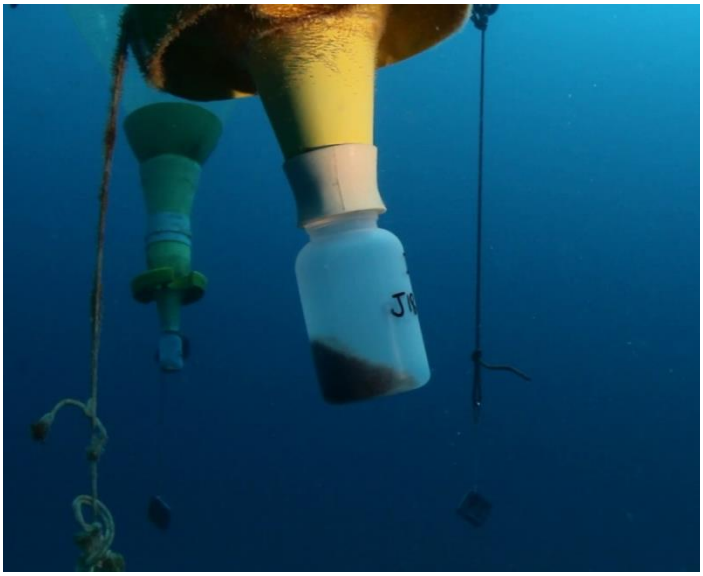


Figure 2.

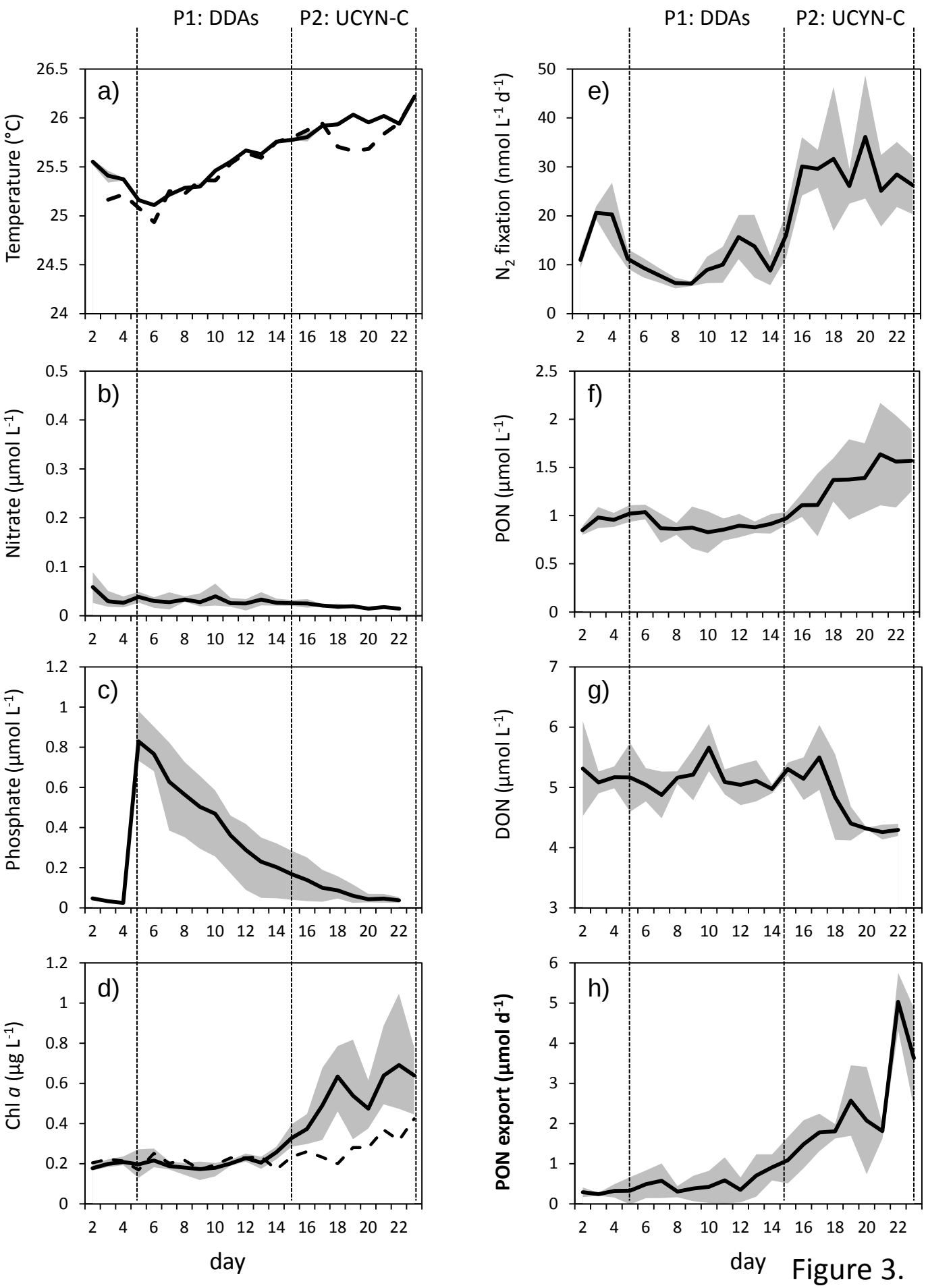
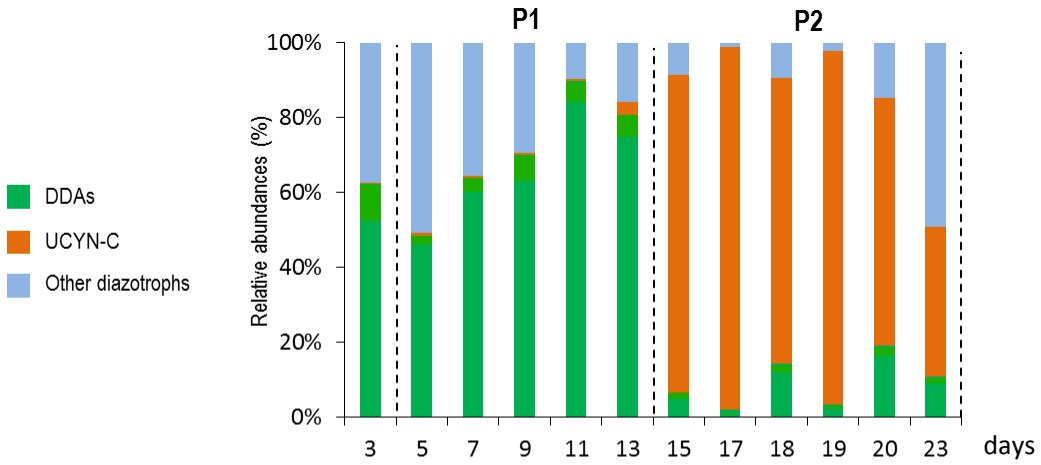

P1: DDAs
P2: UCYN-C
a)
Temperature (°C)
b)
Nitrate (µmol L-1)
c)
Phosphate (µmol L-1)
d)
Chl a (µg L-1)
e)
N2 fixation (nmol L-1 d-1)
f)
PON (µmol L-1)
g)
DON (µmol L-1)
h)
PON export (µmol d-1)
day

Figure 3.




| | | | |
|---|---|---|---|
| **Standing stocks** | $NO_3^-$ | | - - - |
| | DIP | + + + | - - - |
| | DON | | - - - |
| | DOP | | - - - |
| | PON | | + + + |
| | POP | | + + + |
| | POC | | + + + |
| | Chl *a* | | + + + |
| | TEP | - - - | + + + |
| **Rates** | $N_2$ fixation | - - - | + + + |
| | Primary Production | + + + | + + + |
| | Bacterial Production | | + + + |
| | PON, POC, POP export | | + + + |
| | APA | - - - | + + + |
| | T-DIP | | - - - |
| **Plankton composition** | Diatoms | | |
| | Dinoflagellates | | |
| | *Prochlorococcus* | + + + | |
| | *Synechococcus* | | + + + |
| | Pico-eukaryotes | | + + + |
| | Nano-eukaryotes | | + + + |
| | Bacteria | - - - | |
| | UCYN-A | | |
| | UCYN-B | | + + + |
| | UCYN-C | | + + + |
| | DDAs | + + + | - - - |
| | *Trichodesmium* | | |
| | gamma-24774A11 | | |
| | Zooplankton biomass | | |

Figure 4.

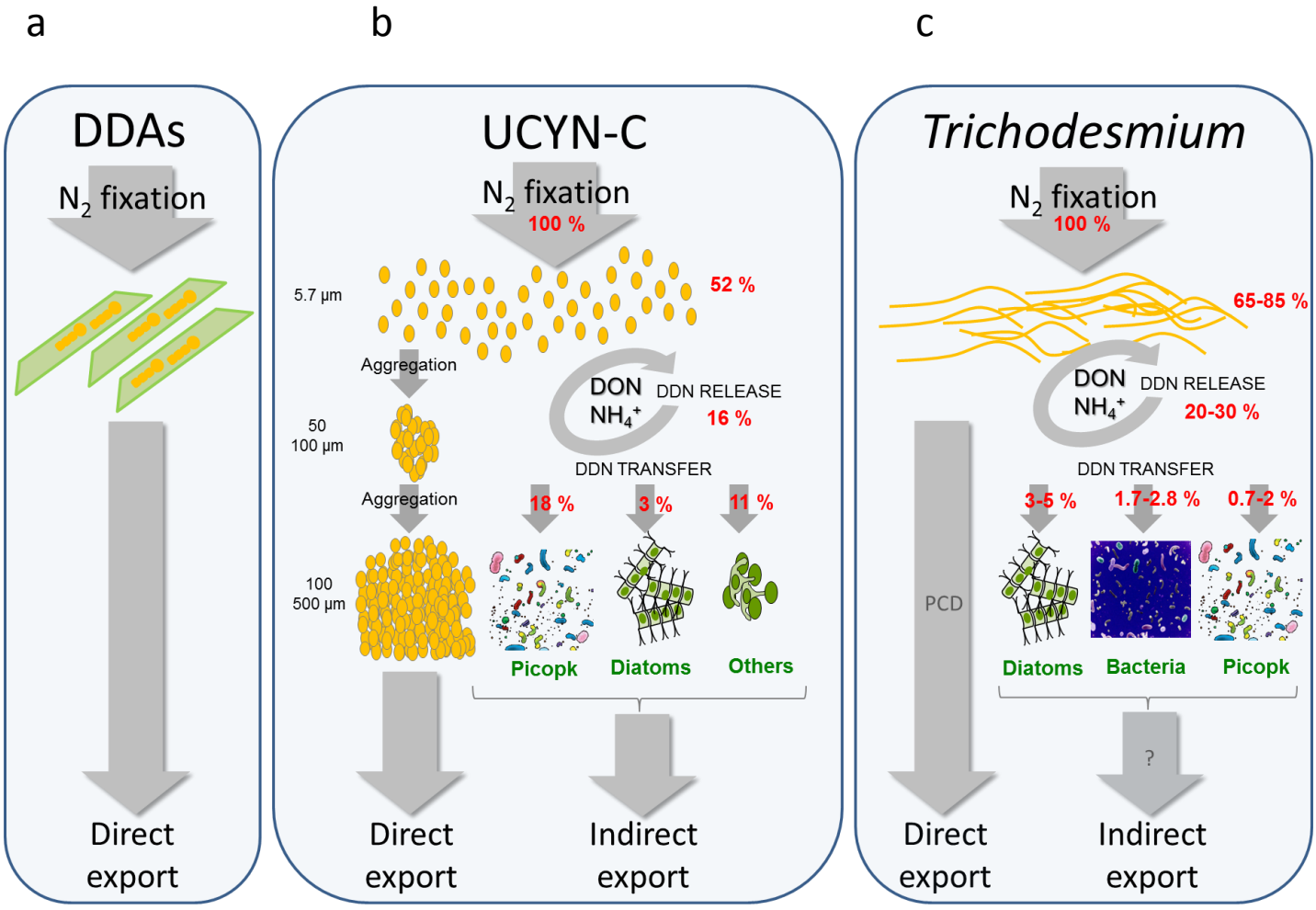

a
b
c
DDAs
N2 fixation
Direct export
UCYN-C
N2 fixation
100 %
5.7 µm
52 %
Aggregation
DON
NH4+
DDN RELEASE
16 %
50
100 µm
DDN TRANSFER
Aggregation
18 %
3 %
11 %
100
500 µm
Picopk
Diatoms
Others
Direct export
Indirect export
Trichodesmium
N2 fixation
100 %
65-85 %
DON
NH4+
DDN RELEASE
20-30 %
DDN TRANSFER
3-5 %
1.7-2.8 %
0.7-2 %
PCD
Diatoms
Bacteria
Picopk
?
Direct export
Indirect export

Figure 5.

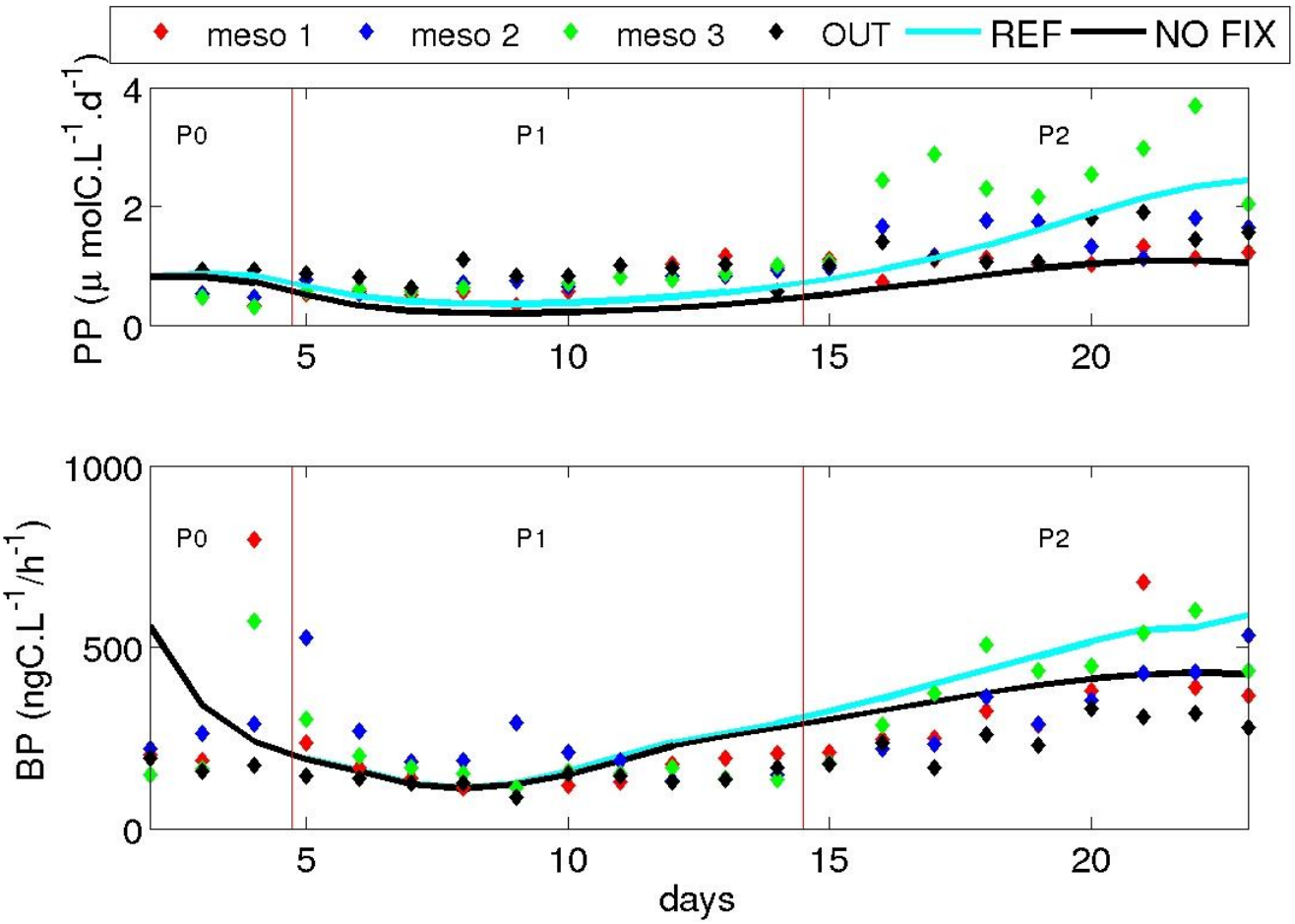

meso 1
meso 2
meso 3
OUT
REF
NO FIX
PP (µ molC.L-1.d-1)
BP (ngC.L-1/h-1)
P0
P1
P2
0
2
4
500
1000
5
10
15
20
days

Figure 6.

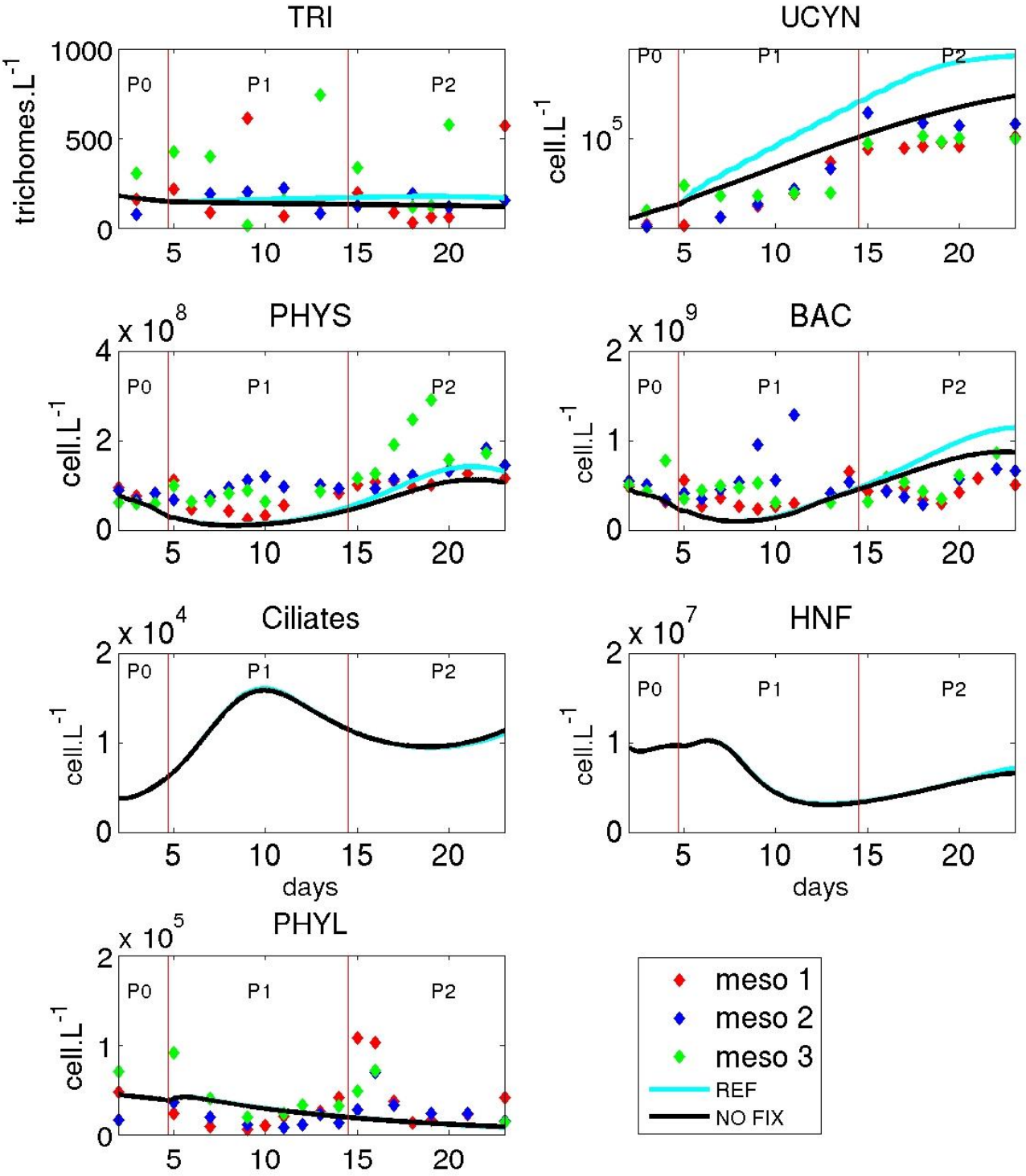

TRI
trichomes.L-1
1000
500
0
P0
P1
P2
5
10
15
20
UCYN
cell.L-1
x 10^5
PHYS
x 10^8
4
2
BAC
x 10^9
Ciliates
x 10^4
HNF
x 10^7
PHYL
x 10^5
days
meso 1
meso 2
meso 3
REF
NO FIX

Figure 7.

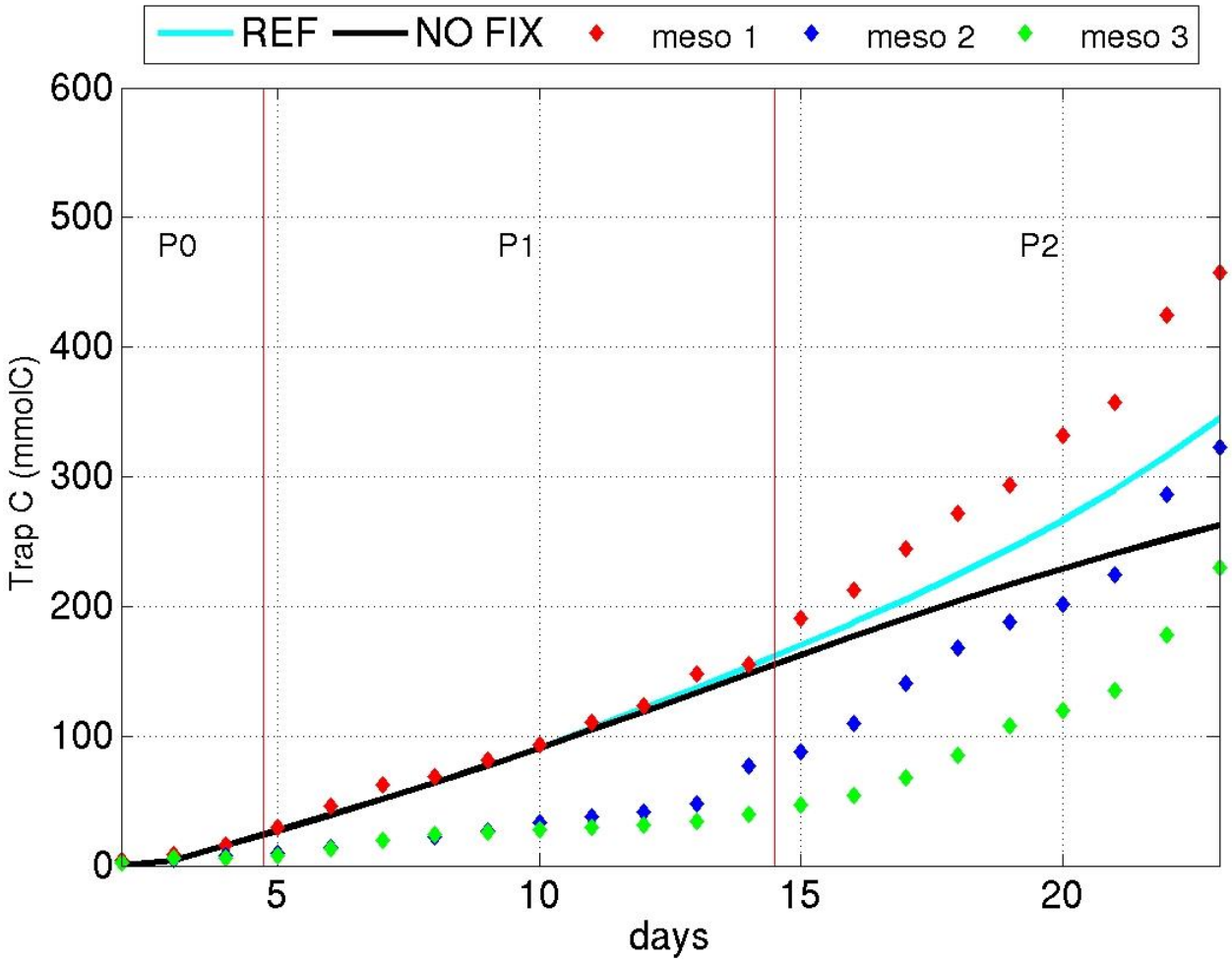

REF
NO FIX
meso 1
meso 2
meso 3
P0
P1
P2
Trap C (mmolC)
days
600
500
400
300
200
100
0
5
10
15
20

Figure 8.

## References cited


Alekseenko, E., Raybaud, V., Espinasse, B., Carlotti, F., Queguiner, B., Thouvenin, B., Garreau, P., and Baklouti, M.: Seasonal dynamics and stoichiometry of the planktonic community in the NW Mediterranean Sea; a 3D modeling approach, Ocean Dynamics, 64, 179–207, 2014.

Altabet, M. A.: Variations in Nitrogen Isotopic Composition between Sinking and Suspended Particles - Implications for Nitrogen Cycling and Particle Transformation in the Open Ocean, Deep Sea Research, 35, 535-554, 1988.

Baklouti, M., Faure, V., Pawlowski, L., and Sciandra, A.: Investigation and sensitivity analysis 665 of a mechanistic phytoplankton model implemented in a new modular numerical tool (Eco3M) dedicated to biogeochemical modelling, Progress in Oceanography, 71, 34–58, 2006.

Bandyopadhyay, A., Elvitigala, T., Welsh, E., Stöckel, J., Liberton, M., Min, H., Sherman, L. A. and Pakrasi, H. B.: Novel metabolic attributes of the genus cyanothece, comprising a group of unicellular nitrogen-fixing Cyanothece, MBio, 2, 2011.

Bar-Zeev, E., Avishay, I., Bidle, K. D., and Berman-Frank, I.: Programmed cell death in the marine cyanobacterium Trichodesmium mediates carbon and nitrogen export, The ISME journal, 7, 2340-2348, 2013.

Benavides, M., Agawin, N., Arístegui, J., Peene, J., and Stal, L.: Dissolved organic nitrogen and carbon release by a marine unicellular diazotrophic cyanobacterium, Aquatic microbial ecology, 69, 69-80, 2013a.

Benavides, M., Bronk, D. A., Agawin, N. S. R., Pérez-Hernández, M. D., Hernández-Guerra, A., and Arístegui, J.: Longitudinal variability of size-fractionated N2 fixation and DON release rates along 24.5°N in the subtropical North Atlantic, Journal of Geophysical Research, 118, 3406-3415, 2013b.

Benitez-Nelson, C. R. and Buesseler, K. O.: Variability of inorganic and organic phosphorus turnover rates in the coastal ocean, Nature, 398, 502-505, 1999.

Berman-Frank, I., Bidle, K. D., Haramaty, L., and Falkowski, P. G.: The demise of the marine cyanobacterium, Trichodemsium spp., via an autocatalyzed cell death pathway, Limnology and Oceanography, 49, 997-1005, 2004.

Berman-Frank, I., Rosenberg, G., Levitan, O., Haramaty, L., and X., M.: Coupling between autocatalytic cell death and transparent exopolymeric particle production in the marine cyanobacterium *Trichodesmium*, Environmental microbiology, 9, 1415-1422, 2007.

Berman-Frank, I., Spungin, D., Rahav, E., F., V. W., Turk-Kubo, K., and Moutin, T.: Dynamics of transparent exopolymer particles (TEP) during the VAHINE mesocosm experiment in the New Caledonia lagoon, Biogeosciences Discussions, doi: doi:10.5194/bg-2015-612, 2016. 2016.

Berthelot, H., Bonnet, S., Camps, M., Grosso, O., and Moutin, T.: Assessment of the dinitrogen released as ammonium and dissolved organic nitrogen by unicellular and filamentous marine diazotrophic cyanobacteria grown in culture, Frontiers in Marine Science, 2, 2015a.

Berthelot, H., Bonnet, S., Grosso, O., Cornet, V., and Barani, A.: Transfer of diazotroph derived nitrogen towards non-diazotrophic planktonic communities: a comparative study between *Trichodesmium erythraeum*, *Crocosphaera watsonii* and *Cyanothece* sp., Biogeosciences Discussions, doi: doi:10.5194/bg-2015-607, In review, 2016. In review, 2016.

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

Biegala, I. C. and Raimbault, P.: High abundance of diazotrophic picocyanobacteria (< 3 μm) in a Southwest Pacific coral lagoon, Aquatic Microbial Ecology, 51, 45-53, 2008.

Bombar, D., Taylor, C. D., Wilson, S. T., Robidart, J. C., Rabines, A., Turk-Kubo, K. A., Kemp, J. N., Karl, D. M., and Zehr, J. P.: Measurements of nitrogen fixation in the oligotrophic North Pacific Subtropical Gyre using a free-drifting submersible incubation device, Journal of Plankton Research, 37, 727–739, 2015.

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

Bonnet, S., Berthelot, H., Turk-Kubo, K., Fawcett, S. E., Rahav, E., L'Helguen, S., and Berman-Frank, I.: Dynamics of N2 fixation and fate of diazotroph-derived nitrogen in a low nutrient low chlorophyll ecosystem: results from the VAHINE mesocosm experiment (New Caledonia) Biogeosciences, 13, 2653-2673 2016a.

Bonnet, S., Biegala, I. C., Dutrieux, P., Slemons, L. O., and Capone, D. G.: Nitrogen fixation in the western equatorial Pacific: Rates, diazotrophic cyanobacterial size class distribution, and biogeochemical significance, Global Biogeochemical Cycles, 23, 1-13, 2009.

Bonnet, S., Dekaezemacker, J., Turk-Kubo, K. A., Moutin, T., Hamersley, R. M., Grosso, O., Zehr, J. P., and Capone, D. G.: Aphotic $N_2$ fixation in the Eastern Tropical South Pacific Ocean, PloS one, 8, e81265, 2013.

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

Bonnet, S., Rodier, M., Turk, K., K., Germineaud, C., Menkes, C., Ganachaud, A., Cravatte, S., Raimbault, P., Campbell, E., Quéroué, F., Sarthou, G., Desnues, A., Maes, C., and Eldin, G.: Contrasted geographical distribution of N2 fixation rates and nifH phylotypes in the Coral and Solomon Seas (South-Western Pacific) during austral winter conditions, Global Biogeochemical Cycles, 29, 2015.

Bronk, D. A., Sanderson, M. P., Mulholland, M. R., Heil, C. A., and O'Neil, J. M.: Organic and inorganic nitrogen uptake kinetics in field populations dominated by Karenia brevis. In: Harmful Algae, Steidinger K, V. G., Heil CA (Ed.), Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography and Intergovernmental Oceanographic Commission of UNESCO, St. Petersburg, FL, 2004.

Buesseler, K. O., Antia, A. N., Chen, M., Fowler, S. W., Gardner, W. D., Gustafsson, Ö., Harada, K., Michaels, A. F., V.D., R., Loeff, M., Sarin, M., Steinberg, D. K., and Trull, T.: An assessment of the use of sediment traps for estimating upper ocean particle fluxes, Journal of Marine Research, 65, 345-416 2007.

Capone, D. G., Ferrier, M. D., and Carpenter, E. J.: Amino Acid Cycling in Colonies of the Planktonic Marine Cyanobacterium *Trichodesmium thiebautii*, Applied and Environmental Microbiology, 60, 3989-3995, 1994.

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

Chen, Y. L., Tuo, S., and Chen, H. Y.: Co-occurrence and transfer of fixed nitrogen from *Trichodesmium* spp. to diatoms in the low-latitude Kuroshio Current in the North West Pacific., Marine Ecology Progress Series, 421, 25-38, 2011.

Devassy, V. P., Bhattathiri, P. M. A., and Qasim, S. Z.: Succession of organisms following *Trichodesmium* phenomenon, Indian Journal of Marine Sciences, 8, 89-93, 1979.

Devassy, V. P., Bhattathiri, P. M. A., and Qasim, S. Z.: *Trichodesmium* phenomenon, Indian Journal of Marine Sciences, 7, 168-186, 1978.

Dupouy, C., Neveux, J., Subramaniam, A., Mulholland, M. R., Montoya, J. P., Campbell, L., Carpenter, E. J., and Capone, D. G.: Satellite captures trichodesmium blooms in the southwestern tropical Pacific, EOS, 81, 13-16, 2000.

Dutkiewicz, S., Morris, J. J., Follows, M. J., Scott, J., Levitan, O., Dyhrman, S. T., and Berman-Frank, I.: Impact of ocean acidification on the structure of future phytoplankton communities, Nature Clim. Change, 5, 1002-1006, 2015.

Farnelid, H., Andersson, A. F., Bertilsson, S., Al-Soud, W. A., Hansen, L. H., Sørensen, S., Steward, G. F., Hagström, A., and Riemann, L.: Nitrogenase gene amplicons from global marine surface waters are dominated by genes of non-cyanobacteria, PloS one, 6. e19223. , 2011.

Farnelid, H. and Riemann, L.: Heterotrophic $N_2$-fixing bacteria: overlooked in the marine nitrogen cycle? In: Nitrogen Fixation Research Progress, Nova Science Publishers, New York, 2008.
Fichez, R., Chifflet, S., Douillet, P., Gérard, P., Gutierrez, F., Jouon, A., Ouillon, S., and Grenz, C.: Biogeochemical typology and temporal variability of lagoon waters in a coral reef ecosystem subject to terrigeneous and anthropogenic inputs (New Caledonia), Marine Pollution Bulletin, 61, 309-322, 2010.
Foster, R. A. and O'Mullan, G. D.: Nitrogen-Fixing and Nitrifying Symbioses in the Marine Environment. In: Nitrogen in the Marine Environment, G., C. D., Bronk, D. A., Mulholland, M., and Carpenter, E. J. (Eds.), Elsevier Science, 2008.
Fu, F. X., Mulholland, M. R., N., G., Beck, A., Bernhardt, P., Warner, M., Sañudo-Wilhelmy, S., and Hutchins, D. A.: Interactions between changing $pCO_2$, $N_2$ fixation, and Fe limitation in the marine unicellular cyanobacterium *Crocosphaera*, Limnology and Oceanography, 53, 2008.
Furnas, M. J. and Mitchell, A. W.: Pelagic primary production in the Coral and southern Solomon Seas, Marine and Freshwater Research, 47, 395-705, 1996.
Garcia, N., Raimbault, P., and Sandroni, V.: Seasonal nitrogen fixation and primary production in the Southwest Pacific: nanoplankton diazotrophy and transfer of nitrogen to picoplankton organisms, Marine Ecology Progress Series, 343, 25-33, 2007.
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, In review, 2016. In review, 2016.
Glibert, P. M. and Bronk, D.: Release of dissolved organic nitrogen by marine diazotrophic cyanobacteria, *Trichodesmium* spp., Applied and Environmental Microbiology, 60, 3996-4000, 1994.
Glibert, P. M. and O'Neil, J. M.: Dissolved organic nitrogen release and amino-acid oxidase activity by *Trichodesmium*. In: Marine cyanobacteria, ORSTOM (Ed.), Bulletin de l'Institut Océanographoque, L. Charpy and T. Larkum, Paris, 1999.
Grenz, C. and LeBorgne, R.: New Caledonia tropical lagoons: an overview of multidisciplinary investigations, Marine Pollution Bulletin, 61, 2010.
Großkopf, T., Mohr, W., Baustian, T., Schunck, H., Gill, D., Kuypers, M. M. M., Lavik, G., Schmitz, R. A., Wallace, G. W. R., and LaRoche, J.: Doubling of marine dinitrogen-fixation rates based on direct measurements, Nature, 488, 361-363, 2012.
Gruber, N.: The dynamics of the marine nitrogen cycle and its influence on atmospheric $CO_2$. In: The ocean carbon cycle and climate., Follows, M. and Oguz, T. (Eds.), Kluwer Academic, Dordrecht, 2004.
Guieu, C., Dulac, F., Desboeufs, K., Wagener, T., Pulido-Villena, E., Grisoni, J.-M., Louis, F., Ridame, C., Blain, S., Brunet, C., Bon Nguyen, E., Tran, S., Labiadh, M., and Dominici, J.-M.: Large clean mesocosms and simulated dust deposition: a new methodology to investigate responses of marine oligotrophic ecosystems to atmospheric inputs, Biogeosciences, 7, 2765-2784, 2010.
Guieu, C., Dulac, F., Ridame, C., and Pondaven, P.: Introduction to project DUNE, a DUst experiment in a low Nutrient, low chlorophyll Ecosystem, Biogeosciences, 11, 425-442, 2014.
Hunt, B. P. V., Allain, V., Menkes, C., Lorrain, A., Graham, B., Rodier, M., Pagano, M., and Carlotti, F.: A coupled stable isotope-size spectrum approach to understanding pelagic food-web dynamics: A case study from the southwest sub-tropical Pacific, Deep Sea Research Part II: Topical Studies in Oceanography, 113, 208-224, 2015.
Hunt, B. P. V., Bonnet, S., Berthelot, H., Conroy, B. J., Foster, R., and Pagano, M.: Contribution and pathways of diazotroph derived nitrogen to zooplankton during the VAHINE mesocosm experiment in the oligotrophic New Caledonia lagoon, Biogeosciences, doi: doi:10.5194/bg-2015-614, Accepted. Accepted.
Hutchins, D. A., Fu, F. X., Zhang, Y., Warner, M. E., Feng, Y., Portune, K., Bernhardt, P. W., and Mulholland, M. R.: $CO_2$ control of Trichodesmium $N_2$ fixation, photosynthesis, growth rates, and elemental ratios: Implications for past, present, and future ocean biogeochemistry, Limnology and Oceanography, 52, 1293-1304, 2007.

Karl, D., Michaels, A., Bergman, B., Capone, D. G., Carpenter, E. J., and Letelier, R.: Dinitrogen fixation in the world's oceans, Biogeochemistry, 57/58, 47-98, 2002.
Karl, D. M., Church, M. J., Dore, J. E., Letelier, R., and Mahaffey, C.: Predictable and efficient carbon sequestration in the North Pacific Ocean supported by symbiotic nitrogen fixation, Proceedings of the National Academy of Sciences, 109, 1842–1849, 2012.
Karl, D. M., Letelier, R., Hebel, D. V., Bird, D. F., and Winn, C. D.: *Trichodesmium* blooms and new nitrogen in the North Pacific Gyre, Kluwer Academic Publishers, Dordrecht, 1992.
Karl, D. M., Letelier, R., Tupas, L., Dore, J., Christian, J., and Hebel, D.: The role of nitrogen fixation in biogeochemical cycling in the subtropical North Pacific Ocean, Nature, 388, 533-538, 1997a.
Karl, D. M., Letelier, R. M., Tupas, R., Dore, J., Christian, J., and Hebel, D. V.: The role of nitrogen fixation in biogeochemical cycling in the subtropical North Pacific Ocean, Nature, 388, 533-538, 1997b.
Kerbrat, A. S., Darius, H. T., Pauillac, S., Chinain, M., and Laurent, D.: Detection of ciguatoxin-like and paralysing toxins in *Trichodesmium* spp. from New Caledonia lagoon, Marine Pollution Bulletin, 61, 360-366, 2010.
Knapp, A. N., Fawcett, S. E., Martinez-Garcia, A., Leblond, N., Moutin, T., and Bonnet, S.: Nitrogen isotopic evidence for a shift from nitrate- to diazotroph-fueled export production in VAHINE mesocosm experiments, Biogeosciences Discussions, 12, 19901-19939, 2015.
Knapp, A. N., Sigman, D. M., and Lipschultz, F.: N isotopic composition of dissolved organic nitrogen and nitrate at the Bermuda Atlantic Time-series Study site, Global Biogeochemical Cycles, 19, 1-15, 2005.
Konno, U., Tsunogai, U., Komatsu, D. D., Daita, S., Nakagawa, F., Tsuda, A., Matsui, T., Eum, Y. J., and Suzuki, K.: Determination of total $N_2$ fixation rates in the ocean taking into account both the particulate and filtrate fractions, Biogeosciences, 7, 2369–2377, 2010.
Landrum, J. P., Altabet, M. A., and Montoya, J. P.: Basin-scale distributions of stable nitrogen isotopes in the subtropical North Atlantic Ocean: Contribution of diazotroph nitrogen to particulate organic matter and mesozooplankton, Deep Sea Research Part I: Oceanographic Research Papers, 58, 615-625, 2011.
Law, C. S., Breitbarth, E., Hoffmann, L. J., McGraw, C. M., Langlois, R. J., LaRoche, J., Marriner, A., and Safi, K. A.: No stimulation of nitrogen fixation by non-filamentous diazotrophs under elevated $CO_2$ in the South Pacific, Global Change Biology 18, 3004-3014, 2012.
Le Borgne, R., Douillet, P., Fichez, R., and Torréton, J. P.: Hydrography and plankton temporal variabilities at different time scales in the southwest lagoon of New Caledonia: A review, Marine Pollution Bulletin, 61, 297-308, 2010.
Leblanc, K., Cornet-Barthaux, V., Caffin, M., Rodier, M., Desnues, A., Berthelot, H., Turk-Kubo, K., and Héliou, J.: Phytoplankton community structure in the VAHINE MESOCOSM experiment, Biogeosciences Discussions, doi: doi:10.5194/bg-2015-605, In review, 2016. In review, 2016.
Lenes, J. M., Darrow, B. P., Catrall, C., Heil, C. A., Callahan, L., Vargo, G. A., Byrne, R. H., Prospero, J. M., Bates, D. E., Fanning, K. A., and Walsh, J. J.: Iron fertilization and the *Trichodesmium* response on the West Florida shelf, Limnology and Oceanography, 46, 1261-1277, 2001.
Lenes, J. M. and Heil, C. A.: A historical analysis of the potential nutrient supply from the $N_2$ fixing marine cyanobacterium *Trichodesmium* spp. to *Karenia brevis* blooms in the eastern Gulf of Mexico, Journal of Plankton Research, 32, 1421-1431, 2010.
Levitan, O., Rosenberg, G., Šetlík, I., Šetlíkova, E., Gtigel, J., Klepetar, J., Prášil, O., and Berman-Frank, I.: Elevated CO2 enhances nitrogen fixation and growth in the marine cyanobacterium Trichodesmium, Global Change Biology, 13, 1-8, 2007.
Luo, Y. W., Doney, S. C., Anderson, L. A., Benavides, M., Bode, A., Bonnet, S., Boström, K. H., Böttjer, D., Capone, D. G., Carpenter, E. J., Chen, Y. L., Church, M. J., Dore, J. E., Falcón, L. I., Fernández, A., Foster, R. A., Furuya, K., Gómez, F., Gundersen, K., Hynes, A. M., Karl, D. M., Kitajima, S., Langlois, R. J., LaRoche, J., Letelier, R. M., Maranón, E., McGillicuddy Jr, D. J., Moisander, P. H., Moore, C. M., Mourino-Carballido, B., Mulholland, M. R., Needoba, J. A.,

Orcutt, K. M., Poulton, A. J., Raimbault, P., Rees, A. P., Riemann, L., Shiozaki, T., Subramaniam, A., Tyrrell, T., Turk-Kubo, K. A., Varela, M., Villareal, T. A., Webb, E. A., White, A. E., Wu, J., and Zehr, J. P.: Database of diazotrophs in global ocean: abundances, biomass and nitrogen fixation rates, Earth System Science Data 5, 47-106, 2012.

Mahaffey, C., Michaels, A. F., and Capone, D. G.: The conundrum of marine $N_2$ fixation, American Journal of Science, 305, 546-595, 2005.

Meador, T. B., Aluwihare, L. I., and Mahaffey, C.: Isotopic heterogeneity and cycling of organic nitrogen in the oligotrophic ocean, Limnology and Oceanography, 52, 934-947, 2007.

Messer, L. F., Mahaffey, C., M Robinson, C., Jeffries, T. C., Baker, K. G., Bibiloni Isaksson, J., Ostrowski, M., Doblin, M. A., Brown, M. V., and Seymour, J. R.: High levels of heterogeneity in diazotroph diversity and activity within a putative hotspot for marine nitrogen fixation, The ISME journal, doi: 10.1038/ismej.2015.205, 2015. 2015.

Mohr, W., Grosskopf, T., Wallace, D. R. W., and LaRoche, J.: Methodological underestimation of oceanic nitrogen fixation rates, PloS one, 9, 1-7, 2010.

Moisander, A. M., Serros, T., Pearl, R. W., Beinart, A., and Zehr, J. P.: Gammaproteobacterial diazotrophs and *nif*H gene expression in surface waters of the South Pacific Ocean, The ISME journal, doi: doi: 10.1038/ismej.2014.49, 2014. 1-12, 2014.

Moisander, P. H., Beinart, R. A., Hewson, I., White, A. E., Johnson, K. S., Carlson, C. A., Montoya, J. P., and Zehr, J. P.: Unicellular Cyanobacterial Distributions Broaden the Oceanic $N^2$ Fixation Domain, Science, 327, 1512-1514, 2010.

Mompean, C., Bode, A., Benitez-Barrios, V. M., Dominguez-Yanes, J. F., Escanez, J., and Fraile-Nuez, E.: Spatial patterns of plankton biomass and stable isotopes reflect the influence of the nitrogen-fixer *Trichodesmium* along the subtropical North Atlantic, Journal of Plankton Research, 35, 513-525, 2013.

Montoya, J. P., Carpenter, E. J., and Capone, D. G.: Nitrogen fixation and nitrogen isotope abundances in zooplankton of the oligotrophic North Atlantic, Limnology and Oceanography, 47, 1617-1628, 2002a.

Montoya, J. P., Carpenter, E. J., and Capone, D. G.: Nitrogen fixation and nitrogen isotope abundances in zooplankton of the oligotrophic North Atlantic Ocean, Limnology and Oceanography, 47, 1617-1628, 2002b.

Montoya, J. P., Holl, C. M., Zehr, J. P., Hansen, A., Villareal, T. A., and Capone, D. G.: High rates of $N_2$ fixation by unicellular diazotrophs in the oligotrophic Pacific Ocean, Nature, 430, 1027-1031, 2004.

Montoya, J. P., Voss, M., Kahler, P., and Capone, D. G.: A simple, high-precision, high-sensitivity tracer assay for $N_2$ fixation, Applied and Environmental Microbiology, 62, 986-993, 1996.

Moore, C. M., Mills, M. M. M., Arrigo, K. R., Berman-Frank, I., Bopp, L., Boyd, P. W., Galbraith, E. D., Geider, R. J., Guieu, C., Jaccard, S. L., Jickells, T. D., La Roche, J., Lenton, T. M., Mahowald, N. M., Maranon, E., Marinov, I., Moore, J. K., Nakatsuka, T., Oschlies, A., Saito, M. A., Thingstad, T. F., Tsuda, A., and Ulloa, O.: Processes and patterns of oceanic nutrient limitation, Nature Geoscience, 6, 701–710, 2013.

Moutin, T., Karl, D. M., Duhamel, S., Rimmelin, P., Raimbault, P., Van Mooy, B. A. S., and Claustre, H.: Phosphate availability and the ultimate control of new nitrogen input by nitrogen fixation in the tropical Pacific Ocean, Biogeosciences, 5, 95-109, 2008.

Moutin, T., Thingstad, T. F., Van Wambeke, F., Marie, D., Slawyk, G., Raimbault, P., and Claustre, H.: Does competition for nanomolar phosphate supply explain the predominance of the cyanobacterium *Synechococcus*?, Limnology and Oceanography, 47, 1562-1567, 2002.

Moutin, T., Van Den Broeck, N., Beker, B., Dupouy, C., Rimmelin, P., and LeBouteiller, A.: Phosphate availability controls Trichodesmium spp. biomass in the SW Pacific ocean, Marine Ecology-Progress Series, 297, 15-21, 2005.

Mulholland, M. R.: The fate of nitrogen fixed by diazotrophs in the ocean, Biogeosciences, 4, 37-51, 2007.

Mulholland, M. R. and Bernhardt, P. W.: The effect of growth rate, phosphorus concentration, and temperature on N2 fixation, carbon fixation, and nitrogen release in continuous cultures of Trichodesmium IMS101, Limnology & Oceanography, 50, 839-849, 2005.
Mulholland, M. R., Bernhardt, P. W., Heil, C. A., Bronk, D. A., and O'Neil, J. M.: Nitrogen fixation and regeneration in the Gulf of Mexico, Limnology and Oceanography, 51, 176-177, 2006.
Mulholland, M. R., Bronk, D. A., and Capone, D. G.: $N_2$ fixation and regeneration of $NH_4^+$ and dissolved organic N by *Trichodesmium* IMS101, Aquatic Microbial Ecology, 37, 85-94, 2004.
Mulholland, M. R. and Capone, D. G.: The nitrogen physiology of the marine N-2-fixing cyanobacteria Trichodesmium spp., Trends in Plant Science, 5, 148-153, 2000.
O'Neil, J. M.: Grazer interactions with nitrogen-fixing marine Cyanobacteria: adaptation for N-acquisition?, Bull. Inst. Oceanogr. Monaco, 19, 293-317, 1999.
O'Neil, J. M., Metzler, P., and Glibert, P. M.: Ingestion of $^{15}N_2$-labelled *Trichodesmium*, and ammonium regeneration by the pelagic harpacticoid copepod *Macrosetella gracilis*, Marine Biology, 125, 89-96, 1996.
O'Neil, J. and Roman, M. R.: Grazers and Associated Organisms of *Trichodesmium*. In: Marine Pelagic Cyanobacteria: *Trichodesmium* and other Diazotrophs, Carpenter, E. J., Capone, D.G., and Rueter, J.G. (Ed.), NATO ASI Series, Springer Netherlands, 1992.
Ouillon, S., Douillet, P., Lefebvre, J. P., Le Gendre, R., Jouon, A., Bonneton, P., Fernandez, J. M., Chevillon, C., Magand, O., Lefèvre, J., Le Hir, P., Laganier, R., Dumas, F., Marchesiello, P., Bel Madani, A., Andréfouët, S., Panché, J. Y., and Fichez, R.: Circulation and suspended sediment transport in a coral reef lagoon: The south-west lagoon of New Caledonia, Marine Pollution Bulletin, 61, 269-276, 2010.
Pfreundt, U., Spungin, D., Berman-Frank, I., Bonnet, S., and Hess, W. R.: Global analysis of gene expression dynamics within the marine microbial community during the VAHINE mesocosm experiment in the South West Pacific, Biogeosciences Discussions, doi: doi:10.5194/bg-2015-564, In review, 2016. In review, 2016.
Pfreundt, U., Van Wambeke, F., Caffin, M., Bonnet, S., and Hess, W. R.: Succession within the prokaryotic communities during the VAHINE mesocosms experiment in the New Caledonia lagoon, Biogeosciences, 13, 2319-2337, 2016.
Rodier, M. and Le Borgne, R.: Population and trophic dynamics of Trichodesmium thiebautii in the SE lagoon of New Caledonia. Comparison with T. erythraeum in the SW lagoon, Marine Pollution Bulletin, 61, 349-359, 2010.
Rodier, M. and Le Borgne, R.: Population dynamics and environmental conditions affecting Trichodesmium spp. (filamentous cyanobacteria) blooms in the south-west lagoon of New Caledonia, Journal of Experimental Marine Biology and Ecology, 358, 20-32, 2008.
Scharek, R., Latasa, M., Karl, D. M., and Bidigare, R. R.: Temporal variations in diatom abundance and downward vertical fux in the oligotrophic North Pacific gyre, Deep Sea Research Part I, 46, 1051-1075, 1999a.
Sharek, R. M., Tupas, L. M., and Karl, D. M.: Diatom fluxes to the deep sea in the oligotrophic North Pacific gyre at Station ALOHA, Marine and Ecological Progress Series, 82, 55-67, 1999b.
Sipler, R. A., Bronk, D. A., Seitzinger, S. P., Lauck, R. J., McGuinness, L. R., Kirkpatrick, G. J., Heil, C. A., Kerkhof, L. J., and Schofield, O. M.: *Trichodesmium*-derived dissolved organic matter is a source of nitrogen capable of supporting the growth of toxic red tide *Karenia brevis*, Marine and Ecological Progress Series, 483, 31-45, 2013.
Slawyk, G. and Raimbault, P.: Simple procedure for simultaneous recovery of dissolved inorganic and organic nitrogen in $^{15}$N-tracer experiments and improving the isotopic mass balance, Marine and Ecological Progress Series, doi: doi:10.3354/meps124289, 1995. 1995.
Sommer, S., Hansen, T., and Sommer, U.: Transfer of diazotrophic nitrogen to mesozooplankton in Kiel Fjord, Western Baltic Sea: a mesocosm study, Marine Ecology Progress Series, 324, 105-112, 2006.
Spungin, D., Pfreundt, U., Berthelot, H., Bonnet, S., AlRoumi, D., Natale, F., Hess, H. R., Bidle, K. D., and Berman-Frank, I.: Mechanisms of Trichodesmium bloom demise within the New Caledonia

Lagoon during the VAHINE mesocosm experiment, doi: doi:10.5194/bg-2015-613, In review, 2016. In review, 2016.
Torréton, J.-P., Rochelle-Newall, E., Pringault, O., Jacquet, S., Faure, V., and Briand, E.: Variability of primary and bacterial production in a coral reef lagoon (New Caledonia), Marine Pollution Bulletin, 61, 335, 2010.
Turk-Kubo, K. A., Frank, I. E., Hogan, M. E., Desnues, A., Bonnet, S., and Zehr, J. P.: Diazotroph community succession during the VAHINE mesocosms experiment (New Caledonia Lagoon), Biogeosciences, 12, 7435-7452, 2015.
Van Wambeke, F., Pfreundt, U., Barani, A., Berthelot, H., Moutin, T., Rodier, M., Hess, W., and Bonnet, S.: Heterotrophic bacterial production and metabolic balance during the VAHINE mesocosm experiment in the New Caledonia lagoon Biogeosciences, 12, 19861-19900, Accepted.
Walsby, A. E.: The gas vesicles and buoyancy of *Trichodesmium*, Marine Pelagic Cyanobacteria: Trichodesmium and other Diazotrophs, 1992. 141-161, 1992.
Wannicke, N., Korth, F., Liskow, I., and Voss, M.: Incorporation of diazotrophic fixed $N_2$ by mesozooplankton - Case studies in the southern Baltic Sea, Journal of Marine Systems, 117-118, 1-13, 2013a.
Wannicke, N., Korth, F., Liskow, I., and Voss, M.: Incorporation of diazotrophic fixed $N_2$ by mesozooplankton - Case studies in the southern Baltic Sea, Journal of Marine Systems, 117-118, 1-13, 2013b.
White, A. E., Foster, R. A., Benitez-Nelson, C. R., Masqué, P., Verdeny, E., Popp, B. N., Arthur, K. E., and Prahl, F. G.: Nitrogen fixation in the Gulf of California and the Eastern Tropical North Pacific, Progess in Oceanography, 109, 1-17, 2012.