Please find in blue text below our response to all three reviewers.

Besides the answers given previously to the reviewers, we have complied with the editors requests for major revisions and have included biogeochemical parameters in the method section, included the diazotroph community data as part of the main results and added the nifH gene copies analyses in the method section as well. Furthermore, all comments below have been answered point by point. Please refer to the docx version with track changes to see additions.

Anonymous Referee #1
Received and published: 23 February 2016
Leblanc and colleagues present floristic results from a LNLC mesocosm experiment in Noumea designed to stimulate diazotrophy and follow the transfer of newly fixed N through the ecosystem. Specifically, they present data on chlorophyll a and phycoerythrin pigments and the abundances of pico- and nano-phytoplankton, diatoms and dinoflagellates. Following a lag period, the DIP-treated mesocosms responded with increased pigments overall, and a notable increase in Synechococcus and a decrease in diatoms. Diatom species composition was also affected. The pigment and phytoplankton data in this manuscript represent a tremendous amount of careful work and should be published in some form. However, I am having difficulty reviewing this as a standalone work. It reads more like a collection of results or a data report than a cohesive paper. Because this manuscript appears to be part of a special volume, it may be that the importance of these measurements in the overall context of the mesocosm study would become clearer when the whole volume is considered. However, I cannot review it as such. The separation of material into individual papers appears to have been done in a rather awkward fashion. For example, this paper on phytoplankton carefully excludes diazotroph abundances – why? The whole point was to stimulate diazotrophs, and they may have become an important part of the phytoplankton community – indeed, the phycoerythrin results suggest so. In addition, data from other papers are inserted here without explanation: e.g., measurements of N2 fixation rates, nutrient concentrations and nifH gene copy numbers in Figs. 11, 12 and S3 that were never described in the Methods section. Also, much of the Discussion section focuses on explaining results that do not appear in the present manuscript.

We understand the reviewer's point of view about the separation of results in this special issue, and this paper is likely suffering from the intent not to repeat too many results presented in the other papers. The separation between results for this special issue was a fruit of long discussions between potential co-authors, and the focus of the experiment being the study of diazotrophs and the fate of derived DDN, several other papers already presented in lengthy details the diazotroph community, using very different techniques based on qPCR (Turk-Kubo et al.), 16S tag sequencing (Pfreundt et al.,a) and metatranscriptomic to investigate the microbial gene expression dynamics from diazotrophic and non-diazotrophic taxa (Pfreundt et al.,b). These techniques need to be described at some length and we felt that the inclusion of all other taxonomic and pigment information there would have resulted in too large papers that would have lost focus. This is the reason we felt that all other taxonomic data except for diazotrophs (that we yet chose to show in the supplementary material as average boxplots for the three periods in order not to show results similarly to these other papers) could warrant for another "community structure" paper that would complement the main information provided in Turk-Kubo et al. and Pfreundt et al. If the reviewer feels that these data can not stand alone despite the fact that they are clearly included in a special issue and complementary to other papers, we propose to rewrite part of the paper as to include the diazotrophs group as a whole, maybe with some distinction between "total diazotrophs","total UCYN" and "total filamentous" as the fine description of the succession within

Technically, the paper is clearly written and the figures are nicely constructed. Although, I do not see the value of the contour plots (Figs. 2, 4-7), especially since there do not appear to be any clear depth-dependent patterns that I can see nor any discussion of depth effects on any of the measured parameters. Each 4-panel contour figure could be presented more effectively as a line plot like Fig. 3 with depth-averaged values, or, alternatively, as a box plots as in Fig. 13.

We agree that the ODV contour plots could be easily replaced by line plots, we chose this graphic output because many other ODV plots were presented in the companion papers and felt that it provided some homogeneity in the special issue, but line plots can easily be redrawn if needed.

In short, I do not think this is a complete manuscript on its own, especially if diazotroph abundances are not included and other data sets are pulled from other manuscripts without explanation. I recommend that the authors reconsider how they divide up the experimental results between manuscripts. The data presented here may be best included within a more cohesive work.

We agree to include diazotroph data, and also better describe in the methods section the different biogeochemical data used. Again, these are described in full in the companion papers (Berthelot et al., Bonnet et al.a, b) and the aim of the paper was not to redescribe the entire biogeochemical environments in the results, but rather use the main fluxes average to explain / caracterize the different planktonic succession phase. If needed, we can add sections to the material and methods to describe how nutrient stocks, primary production and N_2 fixation fluxes were obtained for instance.

Anonymous Referee #2
Received and published: 14 April 2016
This manuscript provides results of the changes in phytoplankton community composition during a mesocosm experiment in Low Nutrient, Low Chlorophyll waters in the Southwest Pacific. The primary objective was to stimulate a bloom of N2-fixing cyanobacteria through PO4 addition and track the resulting particulate carbon fluxes resulting either directly for the N2-fixing organisms (including those associated with diatoms) or indirectly through the diazotrophs providing a source of fixed, reduced nitrogen to the enclosed system. The manuscript by Leblanc et al. provides a thorough overview of the changes in non-diazotroph phytoplankton communities within three replicate mesocosms during three phases of development. Phase one of the analysis follows a spike of PO4 to each mesocosm that was meant to stimulate N2-fixation. The second phase corresponded with a transition from N2-fixing cyanobacteria to non-N2-fixers, following the depletion of the PO4 spike. From the data presented, there appears to by some rather complex dynamics of the phytoplankton communities not only throughout the duration of the experiment but among the mesocosms. For example, it is apparent that mesocosm 3 achieved higher amounts of phytoplankton biomass during phase 2 of the experiment associated with increases in pico- and nano-eukaryotes that were not replicated in the other experiments. In addition, the substantial increase in phycoerythrin in mesocosm 3 that was somewhat replicated in the control sampling but not found in the other incubations is somewhat perplexing.

It is true that there is some degree of variability between mesocosms, which can hardly be avoided. Replicability for such large volume experiments and over >3 weeks time is always difficult to obtain. From the literature, slight divergence in biological and chemical evolution among different replicated mesocosms is not uncommon, particularly after the first week of enclosure (Martinez-Martinez et al., 2006; Pulido-Villena et al., 2014). However we feel that this variability is not so important that it undermines the main results of the VAHINE experiment, which successfully triggered a diazotroph bloom, and allowed to follow the fate of DDN through the food web and in the downward export flux. A paragraph (section 2.5, together with Table 1) in Bonnet et al's introductory paper deals with mesocosms variability. We reproduce some of it below, and argue that Bonnet et al successfully make the case that the degree of variability observed is acceptable and that globally the three mesocosms were well reproduced in their main patterns.

"For example, bulk N_2 fixation rates averaged 18.5 ± 1.1 nmol NL_{-1} d$_{-1}$ (standard deviation was calculated on the average N_2 fixation rates of each mesocosm) over the 23 days of the experiment (all depths averaged together). N_2 fixation rates did not differ significantly among the three mesocosms ($p < 0:05$; Kruskall–Wallis test; Berthelot et al., 2015). Moreover, we consistently observed the same temporal dynamics over the three mesocosms, such as the dramatic increase of rates from days 15 to 23 (during which they reached 27.3 ± 1.0 nmol N L$_{-1}$ d$_{-1}$. This together indicates good replicability between the mesocosms (Bonnet et al., 2015). Molecular data also report a shift in the diazotrophic community composition around day 15, with a bloom of UCYN-C consistently occurring in the three mesocosms (see Turk-Kubo et al., 2015). The same feature was observed for *Synechococcus* abundances, which increased by a factor of 2 from day 15 to day 23 in every mesocosm (Leblanc et al., 2016). Finally, the diatom community, which was very diverse during the first half of the experiment, suddenly shifted beginning at day 10, and *Cylindrotheca closterium* consistently became the dominant diatoms in the three mesocosms (Leblanc et al., 2016). These observations, together with the CV reported above, indicate that biogeochemical and biological conditions were comparable between the three mesocosms."

In addition to this, the initial conditions prevailing before the DIP enrichment could be also at the origin of slight divergence. Indeed mesocosms were closed 3 days before the DIP addition, and many species of diazotrophs exhibit a patchy distribution (Bombar et al., 2015). Hunt et al. (2016) also noticed larger amounts of zooplankton individuals in M3 at the beginning of the experiment, some of which, stressed by the mesocosms, might have died (some larger amounts of 'swimmers' were recovered in the traps in M3), contributing to supplementary sources of N in M3. This might explain why M3 was more different than the other 2 mesocosms.

I found the article well-written and for the most part, the methods used seem applicable for the general objectives of the paper in detailing the phytoplankton taxonomy transitions throughout the bloom. The manuscript is more of a descriptive account of the phytoplankton successions rather than being able to provide definitive reasoning as to why certain groups of phytoplankton changed in abundance when they did. Noticeably absent are the changes in abundance in N2-fixers at the same resolution as what is presented for the non-diazotroph phytoplankton groups apart from phase averages in N2-fixation rates in figure 11 and gene expression data in the supplemental. Although I gather this is due to another paper that will be part of the same special issue describing these results (Turk-Kubo et al. 2015).

Indeed diazotroph succession data is already presented in two other papers (Turk-Kubo et al. this 2015) and (Pfreundt et al, this issue). Kendra Turk-Kubo agrees to add these data again here in the main result section. We can argue that they would be presented here in a very synthetic way (boxplot figures of P1 and P2 – the figure in supplement would be promoted up to results and

discussed more in link with the other data to get a sense of the entire phytoplanktonic community. Some brief description of methods could be added too.

In addition, it is also surprising that chlorophyll concentrations do not necessarily provide much insight into the phytoplankton composition dynamics. In fact, during the peak abundance of the C. closterium around days 15 and 16, chlorophyll concentrations remained quite low. Chlorophyll concentrations seem to be better correlated with pico and nano-eukaryotes that bloomed near the end of phase 2 of the observation period. In addition, the conversion to carbon biomass presented in Figure 10 was rather unsatisfactory. It would have been interesting to compare these biomass estimates with those that were exported to determine whether similar proportions exist between the two or if there is a preferential export of certain groups (e.g. diatoms etc.).

We agree that biomass estimates for the entire community is always a difficult exercise. But we feel that Figure 10 allowed to quickly assess in very visual the actual contribution of each group, which is biased by the very wide abundance ranges presented in the previous figures. The relative contributions to PROC and SYN for instance are much clearer here, with PROC contributing very little to biomass, where as they are the second most abundant organism, of course due to their small sizes.

As explained below to your specific comment below, this paper was not dedicated to comparison of the community succession and impact on export, as it is at length described in Bonnet et al.this issue and Knapp et al.this issue Also it is not possible to disentangle from trap POC which group contributes more to biomass, except for N_2-fixers which was done using molecular biology techniques. See below for more detail on POC export calculations.

Thus in some regards, I find the results presented to be somewhat incomplete. However, as part of a special issue dedicated to the VAHINE mesocosm experiment, this manuscript does constitute an important contribution to describing the overall outcomes. Although further support and discussion on principle factors governing the transitions observed in phytoplankton group successions would benefit the manuscript immensely.

Specific Comments:

Line 62 – Given the primary outcome was to determine whether a diazotroph bloom would increase C export fluxes to depth, I am curious to know if this was the case. Although I can imagine this will be presented in other papers (possibly lead by Bonnet?), perhaps a short discussion as to whether this was the case would add value to this manuscript. Clearly, the resulting changes in non-diazotroph abundances would have also contributed to the overall outcome in influencing C export potential.

Export of diazotroph and non diazotroph is described in Bonnet et al. (see Fig 4 below) as well as the export efficiency for each group, and also discussed in Knapp et al (this issue). Some insertion in the discussion can be added, but again we might get comments back that POC export measurements need to be presented and detailed in the method / results section, but is is already presented in two other papers in the special issue. We feel again that this paper was not meant to be a synthesis paper of the VAHINE experiment, already successfully published in this issue by Bonnet et al in both an introductory and result papers, and also in Berthelot et al., but merely a description of the rest of the community along side the very focused papers on N_2 fixation fluxes and diazotrophic communities. See below for what is already described in Bonnet's paper, maybe a short reference to these results in the discussion would suffice. The calculations say that according to qPCR quantification of diazotrophs in the sediment traps and in the water column, ~10 % of UCYN-C from the water column was exported to the traps daily. Based on microscopic

observations, UCYN-C abundances counted in traps material was converted in carbon biomass based on cell to carbon conversion factors and represented as much as 22.4 ± 5.5 % of the total POC exported at the height of the UCYN-C bloom. So a 10 % average export of UCYN-C relative to POC to the traps and a maximum value of 22 % and up to 7 % for the DDAs. The similar calculations can not be made for other non N_2-fixing groups, as they were not enumerated in the traps (in fecal pellets, aggregates etc…).
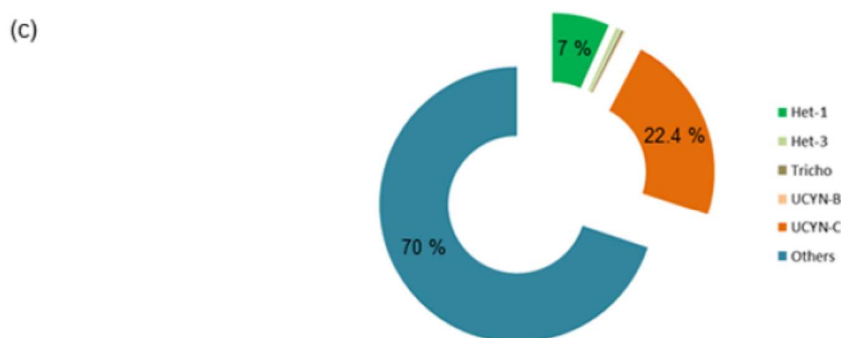

Figure 4. (a) Abundance of UCYN-C (nifH copies L^{-1}) and **(b)** other nifH phylotypes (UCYN-A2, UCYN-B, Trichodesmium, het-1, het-3) (nifH copies L^{-1}) recovered in the sediment trap on day 17 and 19. **(c)** Proportion of POC export associated with diazotrophs in the sediment traps on day 17 in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

Figure from Bonnet et al. this issue : "Thus, our data emphasize that, despite their small size relative to DDAs, UCYN-C are able to directly export organic matter to depth by forming densely populated aggregates that can rapidly sink. This observation is further confirmed by the e ratio, which quantifies the efficiency of a system to export POC relative to primary production (e ratio=POC export/PP) and was significantly higher (p < 0.05) during P2 (i.e., during the UCYN-C bloom; 39.7 ± 24.9 %) than during P1 (i.e., when DDAs dominated the diazotrophic community; 23.9 ± 20.2 %) (Berthelot et al., 2015b)."

Line 79 – So do the authors think that the transition from DDAs to cyanobacterial N2-fixers may have influenced the phytoplankton composition somehow?
It is very difficult to nail which factor influenced each group succession as we believe is a result of a complex interplay between abiotic factors and biological interactions between groups. Clearly though, we think DDAs receded because the DIP addition allowed for other groups to dominate and for unicellular diazotroph to increase after a certain lag time, but looking at the non diazotroph biomass plot (fig 10), only the SYN and diatom biomass showed significant evolution over time.

Line 161 – Include the volumes settled for micro-phytoplankton enumeration.
The following line will be added: "Sedimented volume was on average 140 ml but ranged between 100 and 180 ml depending on cell density"

Line 170 – It is unfortunate that cell volume estimates of diatoms counted were not measured as this can vary substantially for a given species. It should therefore be noted that these C biomass estimates be taken with extreme caution.

The following line will be modified as such: "Unfortunately the cellular sizes were not measured during diatoms cell counts, thus diatoms were converted to C using average size data compiled for each species from a global 171 ocean database (Leblanc et al., 2012). Results should therefore only meant to present relative evolution of diatoms during the main phases of the experiment and should be interpreted with caution".

Line 218 – : : :were comprised of between: : :
I believe this typo was corrected in the proof reading, it does no longer appear.

Line 347 – It is surprising there is little discussion of Si limitation of diatom growth. Clearly with Si concentrations below 2 uM, this would favor very lightly silicified species (such as C. closterium) or non-diatom phytoplankton groups. I would guess that after PO4 addition, Si is a major regulator of diatom growth (as well as possibly Fe).
Line 400 – Is there evidence to support this hypothesis within the scientific literature? Why would *C. closterium* have such a higher NH4 affinity. It's likely more related to their low Si requirements relative to other diatoms.
My answer to both comments : It is true DSi was on average 1.5 ± 0.4 µM which is typical of some tropical low Si waters, and indeed we found the characteristic assemblages of warm water lightly silicified species. I believe that this is overall the case in these waters, and that it is not the DSi concentrations, relatively stable during the experiment that triggered the change towards lightly silicified species, they were all here from the start. However, *C. closterium* is particular in that it is often observed associated to diazotrophs (which was again the case of a recent cruise carried out last year by the same group between Nouméa and Tahiti). Either their nutrient kinetic constants make them best suited to exploit any release of N from diazotrohs, or they have a natural biological association with this group. DFe in the lagoon waters were not measured and could not be handled in trace metal clean way during this experiment, we believe it can hardly constitute a limiting factor for growth inside the mesocosms and that close to the islands.

Line 409 – It's also very likely that these dinoflagelates were mixotrophic. Gyrodinium/ Gymnodinium are well known to exhibit heterotrophy within low nutrient environments when this mode is more favorable.
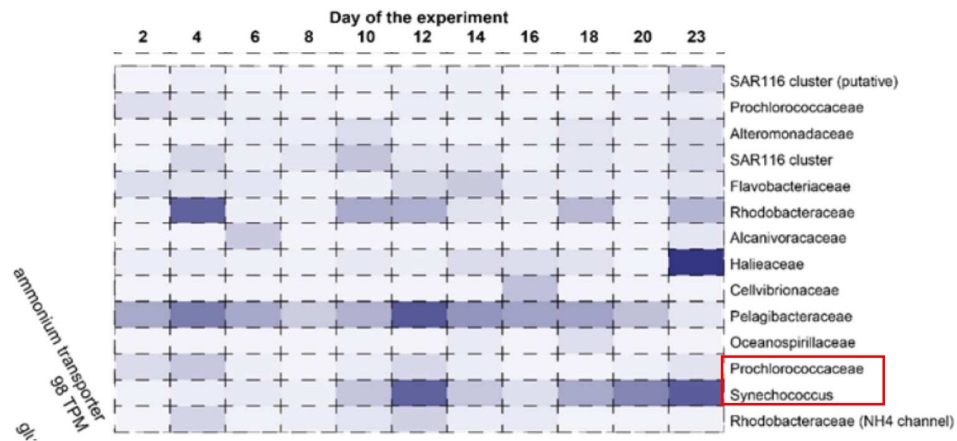True. We can modifiy this sentence "It is however possible that dinoflagellates growth may have been stimulated by DDN, but that their biomass was kept unchanged by subsequent grazing." by "It is however possible that dinoflagellates growth may have been stimulated by DDN, but that their biomass was kept unchanged by subsequent grazing, or that their mixotrophic regime allowed them to exploit changes in the dissolved organic pool or go over to phagocytosis (Jeong et al. 2010)"

Jeong, Hae Jin et al. 2010 Growth, feeding and ecological roles of mixotrophic and heterotrophic dinoflagellates in marine planktonic food webs http://dx.doi.org/10.1007/s12601-010-0007-2

Line 463 – Indeed, this might be the case for a number of phytoplankton groups and not just Synechococcus.
Maybe, but the gene expression analyses for the prokaryotic community in Pfreundt et al, this issue-second paper – figure 6, clearly shows that SYN is increasing significantly gene expression for NH$_4$ transporter, while PRO is not at all. But yes other non targeted group could as well, but we have no data to document it either way. Similarly, in this transcriptomic study an increase of sulfolipid gene expression was observed for SYN, and much less for others groups, which again

argues for an an adaptative advantage of SYN which decreased its cellular P quota and thus, P demand.

Line 515 – remove "to "in benefited to the entire: : :
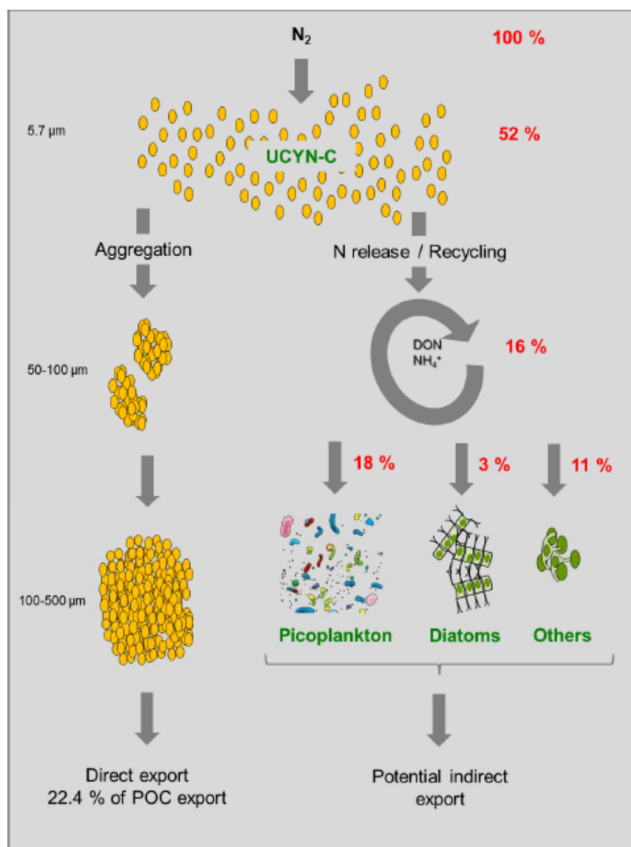OK

Line 517 – observed by, not on. OK

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

Line 518 – More precisely, what are these clear implications for the efficiency of C export by DDN? What groups were exported?

This synthetic figure from Bonnet et al this issue combines trap data, nifH analyses, ^{15}N incubations and nanosims data and illustrates the main results. She shows that the labeled ^{15}N$_2$ triggers the UCYN-C bloom, which contributes directly up to 22% of POC in traps (nifH data) and that DDAs contribute up to 7 % of trap POC but also that the ^{15}N is tracked up the food chain with the use of Nanosims during parallel incubations in smaller volumes, and stimulates picopk, diatoms and others (zooplankton as well). But unfortunately a clear final relative % of each group in the sediment traps can not be measured in this experiment, which is why she mentions "Potential indirect export" beneath the non diazotroph groups. I find however delicate to change the last sentence to indicate the 30% of POC export was related to UCYN-C (22%) and DDAs (7%) while 70% was from "other groups", since these data are not presented in our paper. But some better indication of this can be added in the discussion section, with reference to Bonnet et al.

Figure 5 – Missing M labels of panels to be consistent with other figures. Noted

Anonymous Referee #3
Received and published: 17 April 2016
This papers describes part of a very interesting and exhausitve study about phytoplankton succession and phenology during a mesocosm experiment in LNLC waters. The presented data on floristic identification and pigment analysis is extremely well presented and documented. However, being part of a more exhaustive study containing important complementary data (nutrients, biological processes as production, molecular biology, other diazotroph identifications, TEP concentrations, sediment traps...), it seems very difficult to have an good view of the overall work and results from this study. For instance, there is a clear gap between the "results" section (phytoplankton and pigment results) and the "discussion" section where all other data is combined but not always presented. Most of these other results are described in parallel papers.
It seems to me that this work clearly deserves to be published. However, results shoud be re-structured differently in order to give a broad and complete view and understanding of phytoplankton /ecosystem functionning. I therefore suggest the autors to review the structure of the paper by presenting the required database and information on complementary and essential measured data.

We understand all reviewer's remarks regarding division of data for this specific paper. This paper was one of the last finished, and most papers had a clear focus, set of questions and specific methodologies used ($^{15}N_2$ incubations, Nanosims labelling, qPCR, gene expressions etc…). The diazotrophic community being the main focus, these organisms were already described in two other papers using molecular techniques, with large methodological sections devoted to description of these techniques. We felt among co-authors that it was important the non diazotrophic community was described as well to help understand the evolution of the main biogeochemical stocks and fluxes not necessarily dominated by the diazotrophs themselves. Knapp et al (this issue) show that not all exported N originates from $^{15}N_2$ fixation, and Van Wambeke et al. (this issue) further states that N_2 fixation fluxes can only support a small part of the bacterial production, so other sources of N are to be considered.

The main issue here with including the diazotroph community in this paper, apart from being redundant with other papers, was that there are no direct cell counts of these groups. Data entirely relies on the qPCR data and nifH gene expression. I agree it is very unfortunate these organisms were not enumerated directly, but I suspect techniques for doing this are quite difficult to use and that molecular data was quicker to obtain and sufficed to answer the main objectives of the project. This being said, we agree among co-authors, and if the editors agree, to move up the diazotroph community data from supplement to results section, and add some short methodological section referring to Turk-Kubo et al's paper. However this data set is based on nifH gene abundance and not on cell number, so in any case they will NOT be directly comparable to the other groups data presented here which are all in cells L^{-1}. Conversion from nifH copies to cell number is at present not reliable as number of nifH copies per cell can vary from 1 to 32 for instance just taking the number of *Richelia* symbionts found in the diatom *Rhizosolenia clevei*. If needed, we can also add short sections in M&M referring to nutrient, primary production, and $^{15}N_2$ fixation fluxes with reference to the other papers where these data are lengthily presented, to avoid this gap between M&M / results / and discussion. My main concern which prevented me from adding diazotrophs and other biogeochemical fluxes was that it was all already presented elsewhere, but again, if editors agree, we can easily harmonize M&M and results with our discussion. Our results are also presented in a very synthetic way, as boxplots for the main periods, so the data would not be presented in the same graphical way than in the other papers.

Phytoplankton community structure in the VAHINE MESOCOSM experiment

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Abstract

The VAHINE mesocosm experiment was designed to trigger a diazotroph bloom and to follow the subsequent transfer of diazotroph derived nitrogen (DDN) in the rest of the foodweb. Three mesocosms (50 m^3) located inside the Nouméa lagoon (New Caledonia, South West Pacific) were enriched with dissolved inorganic phosphate (DIP) in order to promote N_2 fixation in these Low Nutrient Low Chlorophyll (LNLC) waters. Initial diazotrophic community were dominated by diatom diazotroph associations (DDAs), mainly by *Rhizosolenia/Richelia intracellularis*, and by *Trichodesmium* which fueled enough DDN to sustain the growth of other diverse diatom species and *Synechococcus* populations, that were well adapted to limiting DIP-levels. After DIP fertilization (1 μM) on day 4, an initial lag time of 10 days was necessary for the mesocosm ecosystems to start building up biomass. Yet changes in community structure were already observed during this first period, with a significant drop of both *Synechococcus* and diatom populations, while *Prochlorococcus* benefited from DIP-addition. At the end of this first period, corresponding to when most added DIP was consumed, the diazotroph community changed drastically and became dominated by *Cyanothece*-like (UCYN-C) populations, which were accompanied by a monospecific bloom of the diatom *Cylindrotheca closterium*. During the second period, biomass increased sharply together with primary production and N_2 fixation fluxes near tripled. Diatom populations, as well as *Synechococcus* and nano-phytoeukaryotes showed a re-increase towards the end of the experiment, showing efficient transfer of DDN to non diazotrophic phytoplankton.

1. Introduction

Atmospheric dinitrogen (N_2) fixation by marine planktonic diazotrophic organisms is the major source of new N to the ocean, and this process is particularly important in sustaining primary productivity in oligotrophic N-limited environments at low latitudes (Capone et al., 2005). On a global scale, N_2-fixation estimates converge around 140 ± 50 Tg N y^{-1} (Gruber, 2004). The increase in primary productivity through diazotroph derived nitrogen (DDN) has been shown to increase carbon (C) export to depth (White et al., 2013). Diazotrophs have also been seen to contribute directly to C export (Subramaniam et al., 2008; Karl et al., 2012) and together these processes are capable of significantly impacting the biological C pump (Dore et al., 2008; Karl et al., 2012). A wide variety of autotrophic organisms are able to fix atmospheric N_2, from picoplanktonic and nanoplanktonic sized unicellular cyanobacteria (termed UCYN) to the heterocyst diazotroph in symbiotic association with diatoms (DDAs) and to the larger filamentous colonies of *Trichodesmium*. Each group possesses different growth and N_2-fixation potential uptake rates and responds differently to environmental factors, depending on their ecological niches.

If N_2-fixation rates are routinely measured in the oligotrophic ocean, much less is known about which organisms contribute to this process as well as the fate of this newly fixed N_2 in the planktonic community. The VAHINE (VAriability of vertical and tropHIc transfer of fixed N2 in the south wEst Pacific) mesocosms experiment was designed to address this particular issue, and to determine the primary routes of transfer of DDN along the planktonic food web. This project aimed at following the dynamics of a diazotroph bloom and investigate the evolution of the rest of the planktonic community (heterotrophic prokaryotes, pico-, nano-micro-phytoplankton and zooplankton) during this bloom event in order to determine whether the DDN rather benefited the classical food web or the microbial loop, as well as following the evolution of fluxes and stocks of biogenic elements. Finally, the VAHINE experiment was designed to determine whether a diazotroph bloom would increase the C export fluxes to depth.

Due to inherent logistical difficulties in answering these questions, that is to follow a naturally occurring diazotroph bloom in the open ocean and quantify the fate of DDN as well as C transfer to depth, a new approach involving mesocosms deployment was carried out in this project. A set of three replicate large-volume (ca. 50 m^3) mesocosms equipped with sediment traps at their bottom end were deployed in a protected area of the Nouméa lagoon in New Caledonia (South West Pacific), a site known for its warm oligotrophic waters favorable to

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70 recurrent *Trichodesmium* blooms (Rodier and Le Borgne, 2010) and characterized by high N_2-fixation rates (Bonnet et al., *under rev*.).

Lagoon waters in Nouméa are known to be primarily N-limited (Jacquet et al., 2006; Torréton et al., 2010), which would favor the growth of diazotrophic organisms, but DIP availability was also suggested to exert the ultimate control on N-input by N_2 fixation in the
75 western side of the South Pacific Ocean (Moutin et al., 2005; 2008). After isolation of the water column inside the mesocosms, DIP was added to each mesocosm in order to stimulate a diazotroph bloom event. The VAHINE experiment successfully allowed to follow a 2-phase diazotroph succession, associated to some of the highest N_2-fixation rates measured in the South West Pacific and composed of a succession of various diazotrophic organisms: DDAs were
80 abundant during the first half (P1) of the experiment (up to day 14), while unicellular N_2-fixing cyanobacteria from Group C (UCYN-C) dominated the diazotroph community during the second half (P2) of the experiment (days 15 to 23) (described in details in Turk-Kubo et al., 2015). In support of the other main results presented in this VAHINE special issue, this paper presents the evolution of the phytoplanktonic community structure during this experiment.
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2. Material and Methods

2.1. Mesocosms

Three large volume mesocosms were deployed in an LNLC ecosystem at the entrance
90 of the Nouméa lagoon (New Caledonia) located 28 km off the coast (22°29.1'S– 166°26.9'E) in 25 m deep waters (Fig. 1). This system is under the influence of oceanic waters coming from the South through the open shelf, which then exit the lagoon, pushed by trade winds and tidal currents through various openings of the barrier reef (Ouillon et al., 2010). The mesocosms consisted of three enclosed polyethylene and vinyl acetate bags equipped with sediment traps
95 at bottom. The mesocosms approximate height was 15 m, with an opening of 4.15 m^2 and a total volume of ca. 50 m^3 (see Guieu et al., 2010 and Bonnet et al., 2016 for full technical description of the mesocosms). Mesocosms were deployed on January 12th 2013 by scuba divers and left opened to stabilize the in-bag water column for 24h. Mesocosms were enclosed the following day (day 1), and the experiment was carried out between January 13th and
100 February 4th for 23 days. In order to alleviate potential DIP-limitation of diazotrophic organisms, the three mesocosms were homogeneously fertilized with 0.8 µM DIP on the evening of day 4 (see Bonnet et al., 2016 for details), marking the start of P1 (P0 corresponding

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to the period prior to fertilization between day 1 and 4).

Sampling occurred every day at 7 am at three selected depths (1, 6 and 12 m) in each mesocosm (hereafter called M1, M2 and M3) from a platform moored next to them and water was collected in large 50 L carboys using a Teflon pump connected to PVC tubing. To ensure quick processing of samples, the carboys were immediately transferred to the R/V *Alis* moored 0.5 nautical mile from the mesocosms or to the inland laboratory setup for this occasion on the Amédée Island located 1 nautical mile off the mesocosms. The seawater surrounding the mesocosms (hereafter called lagoon waters) was sampled every day for the same parameters at the same three depths.

2.2. Sample collection and analyses methods

2.2.1. Determination of chlorophyll a (Chl *a*) concentrations

Chlorophyll a (Chl *a*) concentrations were determined from 0.55 L water samples filtered onto 25 mm GF/F Whatman filters in the three mesocosms and outside at all sampling depths. *In situ* Chl *a* concentrations were determined by fluorometry after methanol extraction (Herbland et al., 1985), using a Turner Design fluorometer (module # 7200-040, Chl *a* extracted-acidification) calibrated with pure Chl *a* standard (Sigma).

2.2.2. Determination of phycoerythrin (PE) concentrations

Water samples (4.5 L) were filtered onto 0.4 µm Nucleopore polycarbonate membrane filters (47 mm diameter) and immediately frozen in liquid nitrogen until analysis. In the laboratory, phycoerythrin (PE) was extracted in a 4 mL glycerol-phosphate mixture (50/50) after vigorous shaking for resuspension of particles (Wyman, 1992) and analyzed by spectrofluorometry according to methods described in Neveux et al. (2009). The PE fluorescence excitation spectra were recorded between 450 and 580 nm (emission fixed at 605 nm), using a Perkin Elmer LS55 spectrofluorometer and emission and excitation slit widths adjusted to 5 and 10 nm, respectively. Quantitative estimates of phycoerythrin were obtained from the area below the fluorescence excitation curve, after filter blank subtraction. PE analyses were made only at 6 m-depth in the three mesocosms and in lagoon waters.

2.2.3. Pico- and nano-phytoplankton enumeration by flow cytometry

Samples for flow cytometry were collected from each carboys corresponding to each mesocosms and lagoon waters at the three depths in 1.8 mL cryotubes, fixed with 200 µL of

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135   paraformaldehyde solution (2 % final concentration), flash frozen in liquid nitrogen, and stored at -80 °C. Flow cytometry analyses were carried out at the PRECYM flow cytometry platform (https://precym.mio.univ-amu.fr/) using standard flow cytometry protocols (Marie et al., 1999) to enumerate phytoplankton. Samples were analyzed using a FACSCalibur (BD Biosciences, San Jose, CA). Briefly, samples were thawed at room temperature in the dark and homogenized.

140   Just before analyses, 2 µm beads (Fluoresbrite YG, Polyscience) used as internal control to discriminate pico-plankton (< 2 µm) and nano-plankton (> 2 µm) populations, and Trucount^{TM} beads (BD Biosciences) used to determine the volume analyzed, were added to each sample. An estimation of the flow rate was calculated, weighing 3 tubes of samples before and after a 3 min run of the cytometer. The cell concentration was determined from both Trucount^{TM} beads

145   and flow rate measurements. The red fluorescence (670LP, related to Chl *a* content) was used as trigger signal and phytoplankton cells were characterized by 3 other optical signals: forward scatter (FSC, related to cell size), side scatter (SSC, related to cell structure), and the orange fluorescence (580/30 nm, related to phycoerythrin content). Several clusters were resolved, *i.e. Synechococcus*, *Prochlorococcus*, pico- and nano-phytoeukaryotes. Another cluster appeared

150   in the nano-planktonic class-size as a stretched cloud of highly dispersed red and orange fluorescence. Its positioning on the cytogram suggests that this cluster corresponded to a gradient of particules comprised between 2 and 20 µm, including a mix of live and dead cells embedded in aggregates, mucus and maybe pollens (data not shown). All data were collected in log scale and stored in list mode using the CellQuest software (BD Biosciences). Data

155   analysis was performed *a posteriori* using SUMMIT v4.3 software (Dako).

2.2.4. Micro-phytoplankton enumeration by microscopy

    Samples for micro-phytoplankton enumeration and identification were collected in each mesocosms at mid-depth (6 m) in 250 mL amber glass bottles and fixed with 5 mL neutralized

160   formalin. Samples were stored in the dark and at 4°C until analysis. Diatoms and dinoflagellates were identified and counted in an Utermöhl chamber on a TE-2000 Nikon inverted microscope following Utermöhl, (1931). Sedimented volume was on average 140 ml but ranged between 100 and 180 ml depending on cell density.

2.2.5. Biomass conversions

165   The different groups were converted to carbon biomass in order to present an estimated overview of the relative dynamics of each group. Pico-phytoplankton C was computed using

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average values compiled from a global ocean database, i.e. 60 fg C per *Prochlorococcus* cell, 255 fg C per *Synechococcus* cell and 1319 fg C per pico-phytoeukaryote cell (Buitenhuis et al., 2012). Nano-phytoeukaryotes were assimilated to a sphere of 4 µm diameter and converted to C using Verity et al. (1992), which was equivalent to 10 pg C per nano-phytoeukaryote cell. Unfortunately the cellular sizes were not measured during diatoms cell counts, thus diatoms were converted to C using average size data compiled for each species from a global ocean database (Leblanc et al., 2012). Results are therefore only meant to present relative evolution of diatoms during the main phases of the experiment and should be interpreted with caution. Finally, dinoflagellates were converted by assimilating them to a 30 µm sphere (which corresponds roughly to observations) and using Menden-Deuer and Lessard (2000).

2.2.6. Diazotroph abundances and targeted diazotroph community succession

The abundances of specific diazotrophic phylotypes was determined using quantitative polymerase chain reaction (qPCR) targeting a component of the nitrogenase gene (*nifH*) associated to nine diazotrophic phylotypes. Briefly, 50 L water samples were collected at each depth each day and filtered through 25 mm 0.2 µm Supor® filters (Millipore, Billarica, CA), using gentle peristaltic pumping. Filters were flash-frozen in liquid nitrogen, then stored at -80°C and shipped on dry ice back to laboratory. Diazotroph phylotypes targeted were regrouped for this study into the unicellular groups A (UCYN-A1+UCYN-A2), B (UCYN-B) and C (UCYN-C), the gamma proteobacteria (γ-247744A11), the colonial filamentous non heterocyst-forming cyanobacteria (*Trichodesmium*) and last the heterocyst-forming diazotrophic symbionts associated with diatoms (DDAs). These include the symbionts *Richelia* sp. associated to *Rhizosolenia* species (het-1), *Hemiaulus* species (het-2) and the symbionts *Calothrix* sp. associated to *Chaetoceros* species (het-3). Results are presented in number of *nifH* copies L^{-1} for each phylotype and cannot easily be converted to cellular abundance because there is very little information about the number of *nifH* copies per genome for each diazotroph target. For a full description of the methods used, refer to Turk-Kubo et al. (2015).

2.2.7. Nutrients

Samples for dissolved inorganic phosphorus (DIP), nitrate (NO_3^-) and total nitrogen (TN) concentrations were collected in 40 mL glass bottles and stored at -20°C before analysis. Concentrations were determined using a segmented flow analyzer according to

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Aminot and Kérouel (2007). The detection limit was 0.01 µM for NO_3^- and 0.005 µM for DIP respectively. Dissolved Organic Nitrogen (DON) was derived after substracting PON measurements from TN measurements, with a precision of 0.5 µM. PON samples were collected by filtering 1.2 L on precombusted (450°C 4h) and acid washed (HCl 10%) GF/F filters and analyzed according to Pujo-Pay and Raimbault (1994), with a detection limit of 0.06 µM for PON. Samples for NH_4^+ were collected in 40 mL glass bottles and analyzed by the fluorescence method according to Holmes et al. (1999) on a trilogy fluorometer. The detection limit was 0.01 µM.

2.2.8. Nitrogen fixation rates

Samples for nitrogen fixation measurements were collected in 4.5 L polycarbonate bottles and amended with $^{15}N_2$-enriched water according to Mohr et al. (2010). After 24h, samples were filtered on combusted (450°C, 4h) GF/F filters and stored at -20°C. Filters were then dried at 60°C for 24h prior to analysis using a Delta plus mass spectrometer (Thermo Fisher Scientific) coupled with an elemental analyzer (Flash EA, Thermo Fisher Scientific) for PON concentrations and PON 15N enrichment determinations. Fluxes were calculated according to the equation given in Montoya et al. (1996). For a full description of the method used, refer to Berthelot et al. (2015b).

2.2.9. Primary production rates

Samples for primary production were collected in 60 mL bottles and amended with ^{14}C and incubated for 3 to 4h on a mooring line close to the mesocosm at the same sampling depth. Samples were filtered on 0.2 µm polycarbonate filters and placed into scintillation vials with 250 µL of HCl 0.5 M. After 12h, 5 mL of ULTIMA Gold MV scintillation cocktail were added to each vial before counting on a Packard Tri-Carb ® 2100 TR scintillation counter. Primary productivity was calculated according to Moutin et al. (2002).

1.3. Results

3.1. Pigment distribution

Chlorophyll *a* (Chl *a*) remained low (close to 0.2 µg L^{-1}) during the first 14 days of the experiment in all three mesocosms (Fig. 2) and similar to the lagoon waters. A significant increase, which was not observed outside of mesocosms was observed by day 15 in all 3 mesocosms, which characterizes the beginning of the second phase (P2). M1 and M2 behaved

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more closely with similar doubling in average concentrations to around 0.4 µg L^{-1}, but with a few peaks at higher concentrations (up to 0.7 µg L^{-1} in M1, and up to 1.0 µg L^{-1} in M2 on day 18). M3 showed a similar trend but with a higher increase of Chl a, with an average concentration of 0.7 µg L^{-1} during P2, and a higher peak value of 1.4 µg L^{-1} on day 21.

Following the DIP-addition, PE remained close to initial values in all three mesocosms (close to 0.1 µg L^{-1}) and lower than in lagoon waters until day 11 (Fig. 3). PE concentrations then increased to an average of 0.2 µg L^{-1} in M1 and M2, with daily variations, but increased up to a higher average concentration (0.5 µg L^{-1}) in M3 during P2, with a peak value of 1.0 µg L^{-1} on day 19. Lagoon concentrations remained lower than in M3, but slightly above M1 and M2 during the first 15 days (0.2 µg L^{-1}), and increased to parallel M3 concentrations between day 20 and 22.

3.2. Pico- & Nano-phytoplankton distribution

The numerically dominant organism in the phytoplankton community during the experiment was *Synechococcus* (Fig. 4), which abundances ranged between 16 000 and 285 000 cells mL^{-1} (min and max values for all mesocosms). During P0, abundances were initially high but decreased steadily right after mesocosm enclosure, in order to increase again only several days after DIP addition. The average concentrations during P1 (day 5-14) in all three mesocosms was 54 000 cells mL^{-1} and it nearly doubled during P2 (day 15-23) to 116 500 cells mL^{-1}. *Synechoccocus* increased more strongly after day 15, but the largest increase was observed in M3 with a peak value on day 19 close to 285 000 cells ml^{-1}.

Prochlorococcus (Fig. 5) showed intermediate abundance values (<50 000 cells mL^{-1}). Contrary to *Synechococcus*, they were initially low during P0 (close to 10 000 cells mL^{-1}), but increased strongly right after DIP addition in the three mesocosms. Apart from this similar initial response, the evolution of this group was less reproducible between mesocosms, with different patterns observed. A net decrease was observed by day 10 in M1, while abundances peaked on this same day in M2 and were intermediate in M3. Overall abundances were almost twice as low in M1 (4 600 to 23 400 cells mL^{-1}) than in M2 and M3 (8 400-10 000 to 42 900-43 500 cells mL^{-1}). *Prochlorococcus* were more abundant towards the end of the experiment in all mesocosms (>20 000 cells mL^{-1}), but with a much higher number in M3 on day 21 (>40 000 cells mL^{-1}).

Next in order of abundance, pico-phytoeukaryotes ranged between 500 and 7 500 cells mL^{-1} on average (Fig. 6). They were present during P0 with abundances > 2 000 cells mL^{-1} but

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decreased right after DIP addition. They remained in low abundances mainly until day 18, where they increased in all mesocosms (up to > 3 000 cells mL^{-1}), but with twice as many cells (> 7 000 cells mL^{-1}) in M3 than in M1 and M2. Pico-phytoplankton showed more contrasted responses in the three mesocosms in the transition period between day 10 and 15, with an increase in abundance in M1, stable values in M2 and a decrease in M3.

Finally nano-phytoeukaryotes abundances were comprised between 400 and 3 700 cells mL^{-1} (Fig. 7). They were generally lower during P0 (<1 000 cells mL^{-1}) and seemed to respond to DIP addition with a small increase in numbers following day 4. No clear pattern can be derived from their distribution during the experiment but for a general increase over the last few days (after day 20) and higher abundances in M3 (> 3 000 cells mL^{-1}), similarly to what was already observed for *Synechococcus* and pico-phytoeukaryotes.

3.3. Diatom community structure

The dominant micro-phytoplanktonic organisms during the experiment were diatoms (Fig. 8), which abundances ranged from 5 700 to 108 000 cells L^{-1} in all mesocosms. They were initially high during P0, despite large variations between mesocosms already on day 2. They seemed to increase slightly right after DIP addition on day 5 (except in M1) and then decreased during P1 in all mesocosms until day 10-11, when they again started to grow, building up to bloom values (100 000 cells L^{-1}) around day 15-16 in the three mesocosms (on average twice as large in M1 than in M2 and M3).

The diatom community was composed of a diverse assemblage, which changed significantly over the course of the experiment. From day 2 to day 12, the diatom community structure was diverse but very reproducible between mesocosms, despite near triple differences in abundances. Diatoms were initially numerically dominated by *Chaetoceros* spp. (*Hyalochaete* and *Phaeoceros*), which together accounted for 25 to 36 % of the total diatom abundances (Fig. S1). *Chaetoceros* spp. remained the most abundant group a couple of days longer in M3, until day 14. In this first period, *Leptocylindrus* sp. was the second next most abundant genera contributing to 21 to 33 % to total diatoms in average over the 9 first days, and decreased to 16% from day 10 to 12, and then remained below 10 % until the end of the experiment. *Cerataulina* spp.'s abundance was third next in M1, with 15 and 12 % contribution in the first 5 days, but was below 5 % in the other 2 mesocosms. *Bacteriastrum* spp.'s abundance was third next in M3 with 12% over the first 7 days, while it remained below 5 % in the two other mesocosms. Finally *Thalassionema* spp.'s contribution was close to 10 % over the first 7 days in all three mesocosms, and decreased strongly after day 7.

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295   From day 10 to day 18-19, *Cylindrotheca closterium,* which was inferior to 2 % of diatoms in the first few days, increased dramatically in all three mesocosms and represented between 33 and 86 % of the diatom abundances, even reaching between days 15 and 17 > 95 % of total abundance. After days 18-19, their contribution decreased again in favor of *Navicula* spp., *Chaetoceros* spp., *Leptocylindrus* spp. and *Guinardia* spp.

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3.4. Dinoflagellate distribution

Dinoflagellates average abundance over the experiment was ca. 3 000 cells L^{-1}, an order of magnitude inferior to diatoms. Dinoflagellates varied from 1 000 to 11 700 cells L^{-1} (min and max values for all mesocosms) and increased slightly (by a factor of 1.3 on average over 305   the three mesocosms) between P1 and P2, but this increase was more pronounced in M3 on days 16-17. Average dinoflagellate abundance was also 3 to 4 times lower in M1 (1400 cells L^{-1}) compared to M2 (3400 cells L^{-1}) and M3 (4 400 cells L^{-1}). The numerically dominant species were from the *Gymnodinium*/*Gyrodinium* group.

3.5. Biomass distribution of the phytoplanktonic community

310   The main phytoplanktonic groups were converted to C biomass and averaged for each day of sampling for all mesocosms and all depths (Fig. 10). Given the assumption used for C conversion (see methods section), these figures are only meant to give a rough estimate of C allocation between groups, yet it has the merit to immediately convey the weight contribution of each group, otherwise difficult to infer from abundance numbers.
315   Diatoms were the main contributors to phytoplankton C biomass (66 %) during P0 (day 2), while *Synechococcus* was the second largest contributor (19 %), followed by nano-phytoeukaryotes (9 %). Nano-phytoeukaryotes relative biomass showed the strongest increase during P1 (from 9 to 17 %), followed by *Synechococcus* (from 19 to 23 %) and dinoflagellates (from 2 to 8 %) while diatoms decreased (from 66 to 47 %). During P2, *Synechococcus* 320   continued to increase (to 28 %) and diatoms to decrease (to 40 %) while all other groups remained fairly stable. The evolution of *Prochlorococcus* contribution to biomass was negligible over the course of the experiment and remained below 2 % during the 3 periods.

3.6. Diazotroph community distribution

325   The evolution of the targeted diazotrophic community is presented as the number of *nifH* copies L^{-1} and as averages over the two main periods for a general overview of their

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relative dynamics (Fig. 11), since the evolution of the diazotrophic community on a daily basis during the experiment is described at length in the companion paper of Turk-Kubo et al. (2015). Important differences were observed between P1 and P2. The diazotroph community was initially dominated by the Het-1 group (*Richelia/Rhizosolenia* association) with $1.3x10^5$ *nifH* gene copies L^{-1}, followed by *Trichodesmium* with $2.0x10^4$ *nifH* copies L^{-1} and by UCYN-A with $1.7 x10^4$ *nifH* copies L^{-1}. The Het-2 group (*Richelia/Hemiaulus* association) was less abundant ($9.5x10^3$ *nifH* copies L^{-1}), while the other groups (UCYN-C, γ24774A11, UCYN-B, Het-3 in decreasing order) were negligible in abundance (0 to maximum1 300 *nifH* copies L^{-1}). During P2, the four dominant groups (Het-1, *Trichodesmium*, UCYN-A, Het-2) all decreased in abundance by a factor X3.7, X1.5, X1.6 and X1.7 respectively. The two least abundant groups UCYN-B, Het-3) during P1 increased only by a few hundred *nifH* copies L^{-1} (with maximum values of 600 *nifH* copies L^{-1}). On the other hand the UCYN-C group showed a drastic increase (more than 10-fold) from $1.3x10^3$ *nifH* copies L^{-1} (P1) to $1.2x10^5$ *nifH* copies L^{-1} (P2).

3.4.   Discussion

Following the DIP addition on the evening of day 4, the VAHINE experiment was characterized by two distinct phases regarding nutrient availability, primary and heterotrophic bacterial production fluxes (Berthelot et al., 2015b; Van Wambeke et al., 2016) and the dynamics of the diazotrophs community as identified by quantitative polymerase chain reaction (qPCR) in Turk-Kubo et al. (2015). This experiment successfully triggered the development of a large diazotroph community, evidenced by the measured N_2-fixation fluxes which were among the highest ever reported (Bonnet et al., 2016). The first 10 days following the DIP fertilization (P1) were dominated mainly by DDAs, coupled with average N_2 fixation rates over the three mesocosms of 10.1 ± 1.3 nmol N L^{-1} d^{-1}, while the following 9 days (P2) were dominated by the unicellular N_2-fixing cyanobacteria from group C (UCYN-C) which resulted in a near tripling of average N_2-fixation rates (27.3 ± 1.0 nmol N L^{-1} d^{-1}) and a more moderate increase in primary productivity, which increased from 0.9 to 1.5 µmol C L^{-1} d^{-1} between P1 and P2 (Berthelot et al., 2015b) (Fig. 12). A concomitant strong increase in average Chl *a* concentrations was observed, which nearly tripled from 0.20 to 0.54 µg L^{-1}. Similar primary production and N_2-fixation rates as well as Chl *a* concentrations were observed in the lagoon waters and the mesocosms during P1, but all parameters clearly increased during the second

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period inside the mesocosm experiment (Fig. 12), reflecting a delayed effect (~10 days) on the planktonic community, presumably affected by the combination of both DIP addition and turbulence reduction due to the entrapment of the water column. Results are discussed

365   following this two-phase characterization of the evolution of the biological compartment over the course of the experiment.

~~3.1.~~4.1.   Initial phytoplankton community composition during P0 (day 0-4)

The experiment started in LNLC waters, characterized by low (< 50 nM) DIN and DIP

370   concentrations (Fig. 13), moderate DSi (1.4 µM) and low Chl a (0.2 µg L^{-1}) (Berthelot et al., 2015b). Primary production was on average low (0.4 µmol C L^{-1} d^{-1}) while nitrogen fixation was elevated (17 nmol N L^{-1} d^{-1}).

Diatoms were an important part (> 50%) of the phytoplanktonic biomass over the first few days (Fig.10, 14). This was surprising given the highly oligotrophic nature of the water mass,

375   but can be explained by the presence of microplanktonic diazotrophs which could have stimulated the growth of other diatoms by indirect transfer of DDN. The diazotroph community analyses indicate that DDAs (in particular the het-1 *Rhizosolenia bergonii*/*Richelia* association) were dominant at the beginning of the experiment, and that other diazotrophs such as *Trichodesmium* and UCYN-A were also present.

380   Within the pico-phytoplankton size-class, *Synechococcus* was the dominant organism, representing 85 % of the C biomass, while *Prochlorococcus* and pico-phytoeukaryotes represented 5 and 10 % respectively. This relative allocation of biomass between these three groups remained stable throughout the experiment with very little variations (SD < 4% on all groups). A previous study conducted in the Nouméa Lagoon waters, showed that

385   *Synechococcus* was dominating over *Prochlorococcus* over most of the DIN range and that pico-phytoplankton remained a negligible component of this size-class, which is consistent with our findings (Jacquet et al., 2006). *Synechococcus* was the most abundant group initially and 16S data showed that these high abundances were maintained in the Nouméa lagoon but that they crashed in M1 after mesocosm closure and further DIP addition (Pfreundt et al., 2016a).

390   This was the case in all three mesocosms (Fig. 6). In our system, both DIN and DIP were low, and the competitive advantage held by *Synechococcus* could derive from their ability to replace phospho-lipids in their cell membrane by sulfolipids during P-limitation (Van Mooy et al., 2009). Even if other groups such as *Prochlorococcus*, UCYN-B (*Crocosphaera*),

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Trichodesmium and some diatom species are also able to perform the same replacement of membrane lipids to save on cellular P demand (Van Mooy et al., 2009), it seems that *Synechococcus* was the most efficient organism using this substitution metabolism to resist P-stress in our initial conditions (Pfreundt et al., 2016b). This group was probably also benefiting from DDN to circumvent *in situ* DIN limitation.

3.2.4.2. Phytoplankton community composition during P1 (day 5 - 14)

In the period following DIP addition, production fluxes remained very close to lagoon waters (Fig. 12) similarly to nutrient stocks (Fig. 13). Average primary production increased from 0.4 to 0.9 µmol C L^{-1} d^{-1} while N_2 fixation actually decreased slightly (from 17 to 10 nmol N L^{-1} d^{-1}). DIP addition however impacted phytoplankton community of both diazotrophs and non diazotrophs which started to depart from initial conditions as described below.

The DIP addition did not seem to immediately alter the main diatom species distribution, which remained fairly stable from day 2 to day 9 (Figs. 8, S1). However, it seems that diatom concentrations, after a rapid surge on day 5 in M2 and M3 corresponding to higher DIP levels in these mesocosms compared to M1, decreased significantly until day 9 in all mesocosms (Fig. 8, 10). As a potential mechanism, the DIP addition could have stimulated diatom growth initially, which would have then pushed diatoms into N-limitation if DDN was not sufficient to sustain this sudden increase in growth, and could have resulted in this initial decline in cell numbers. Another hypothesis could be that the water column enclosure, by reducing turbulence or by increasing the predator-prey encounter occurrences, could have been detrimental to the accumulation of diatoms during the first few days.

Although DDAs dominated the diazotroph community during P1, they however did not dominate the diatom community as a whole. *Rhizosolenia bergonii* (associated to *R. intracellularis*) represented less than 2 % of the diatom biomass initially, i.e. before DIP addition and increased to only around 8 % of the diatom biomass during P1, which was otherwise dominated by the very large *Pseudosolenia calcar-avis* (10 to 90 µm diameter, 200 to 800 µm length), which has a disproportionate impact on biomass, despite very low cellular abundance. This diatom is known as an "S-strategist" (Reynolds, 2006) i.e. it is a large, slow growing species adapted to high nutrient stress and high light level and is usually found in very small mixed layer depths and in very low nitrate waters. The rest of the dominating diatom flora during the first 9 days of P1 was also comprised of species known to thrive in warm nutrient

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poor waters such as *Cerataulina*, *Guinardia* and *Hemiaulus* genera, while the numerically dominant *Chaetoceros* and *Leptocylindrus* species were more ubiquitous and fast growing species (Brun et al., 2015). The relative high abundance of diatoms other than DDAs and S-strategists in these nutrient depleted waters at the beginning of the experiment could have been fueled by secondary release DDN (Mulholland et al., 2004; Benavides et al., 2013; Berthelot et al., 2015a). During this first period, the majority (over 50 %) of N_2-fixation was associated to the $> 10\,\mu m$ size-fraction (Bonnet et al., 2015) and was most likely the product of both *Richelia* and *Trichodesmium* but it cannot be determined which of these groups contributed most to the nitrogen uptake flux and subsequent DDN release. Only one diatom cell count is available for the lagoon waters on day 16, but it confirms that diatom community structure outside the mesocosms remained similar to our initial assemblage, composed of *Chaetoceros*, *Leptocylindrus* and *Guinardia* as well as *Pseudosolenia calcar-avis*.

A significant shift was observed within the diatom community after a few days during the second half of P1. The numerically dominant group of *Chaetoceros* spp. was gradually replaced by the small pennate diatom *Cylindrotheca closterium*, initially present in all mesocosms but in low abundance. Despite this dramatic increase in cell numbers leading to a near monospecific bloom at the transition period between P1 and P2, the overall diatom biomass yet decreased due to the small size of this pennate species. Interestingly, the climax of *C. closterium* was synchronous with an increase in UCYN-C populations that was not observed in the lagoon waters at any time and where the diazotroph community remained characterized by an increasing amount of DDAs and decreasing UCYN-A populations (Turk-Kubo et al., 2015). Both UCYN-C and *C. closterium* populations closely followed the staggered decrease in DIP as well as a small increase in temperature in the three mesocosms (Berthelot et al., 2015) hinting to bottom-up control of these groups. This solitary pennate diatom is found worldwide in both pelagic and benthic environments. It is likely that its dominance occurred through a better adaptation to the shift in abiotic factors occurring in the mesocosms from day 5 and on, i.e. much higher DIP level, decreased turbulence, as well as small increases in both temperature and salinity around day 9 in all mesocosms, which were accentuated during P2 (Bonnet et al., 2015). In a previous study involving perturbation experiments in small volume microcosms conducted in high latitude HNLC (High Nutrient Low Chlorophyll) waters in the Bering Sea and in New Zealand, it was also shown that a simple Zn addition was able to induce a very rapid shift from *Pseudo-nitzschia* spp. to *Cylindrotheca closterium* community through subtle interplays in both their affinity for this trace metal (Leblanc et al., 2005). It is likely that this

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rather small and lightly silicified species can be considered as an opportunist species with high growth rates, allowing it to rapidly outcompete other diatoms when abiotic conditions become favorable. In support of this hypothesis, massive developments of *C. closterium* have previously been observed during *Trichodesmium* blooms in the South West pacific as well as in the near shore waters of Goa in western India (Devassy et al., 1978; Bonnet et al., *under rev*.). One hypothesis for this recurrent co-occurrence of *C. closterium* with various diazotrophic groups would be that this diatom species has a better immediate affinity for DDN, probably in the form of NH_4^+, than other diatoms.

Several studies have previously demonstrated the development of diatoms as well as dinoflagellates following N release by *Trichodesmium* spp. (Devassy et al., 1978; Dore et al., 2008; Lenes and Heil, 2010; Chen et al., 2011; Bonnet et al., *under rev.*). In contrast, dinoflagellates here only showed a moderate increase towards the middle of the experiment (days 16-17) in M3 and an increase in the last few days in M2 but no clear trend could otherwise be detected (Fig. 9), and their biomass remained overall stable over the course of the experiment (Fig. 10). It is however possible that dinoflagellates growth may have been stimulated by DDN, but that their biomass was kept unchanged by subsequent grazing, or that their mixotrophic regime allowed them to exploit changes in the dissolved organic pool or go over to phagocytosis (Jeong et al. 2010).

In the pico-phytoplankton community, *Synechococcus* and pico-phytoeukaryotes exhibited very similar dynamics, with a distinct drop after DIP-addition and a re-increase with a higher degree of variability between the three mesocosms from the middle of P1 approximately (Figs. 4, 6, 13). A likely explanation would be that they started to benefit from DDN and increased growth rates again only once the UCYN-C population started to increase. On the other hand, *Prochlorococcus* clearly benefited from the DIP-addition (Fig. 5), with a strong increase in cell numbers in the beginning of P1, which yet only results in a relative increase of 1 % to phytoplankton biomass (Fig. 10). Nano-phytoeukaryotes, which were low initially increased right after DIP addition and continued to increase towards the end of P1 (Fig. 7) probably also thriving on DDN.

4.3. Phytoplankton community composition during P2 (day 15 to 23)

The second period of this mesocosm experiment showed major changes compared to P1. The introduced DIP was rapidly consumed during P1 (Fig. 13) allowing a strong build-up

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490   of biomass (Chl *a* and PE) together with a near tripling of N_2-fixation rates (27 nmol N L^{-1} d^{-1}) which were significantly superior to lagoon values, which also increased but more moderately (Fig. 12). This evolution is clearly due to the stimulation of a different diazotroph community inside the mesocosm, with higher N_2-fixation rates, which in turn increased DDN release and resulted in a larger consumption of all inorganic nutrients compared to outside waters (Fig. 13).

495   This second phase, corresponding approximately to the moment when DIP was completely consumed (to less than 0.1 µM), was characterized by an important shift in the diazotroph community. Clear differences between the mesocosms and lagoon waters were evidenced, the first being dominated by UCYN-C (*Cyanothece*), followed by het-1 (more abundant in M1) and *Trichodesmium* (more abundant in M3) while the latter were still

500   dominated by DDAs, *Trichodesmium* and UCYN-A (Turk-Kubo et al., 2015). The UCYN-C cells (around 6 µm) grew in the mesocosms and rapidly achieved the highest *nifH* gene copies values for all diazotrophs during P2, while most other groups diminished (Fig. 11), most notably het-1 (Turk-Kubo et al., 2015).

At the transition between P1 and P2, the development of UCYN-C was paralleled by a
505   drastic change in diatom community structure, which became almost monospecifically dominated by *C. closterium*. It seems however that this stimulating effect was not durable, as this *C. closterium* bloom started to crash rapidly (most significantly in M1), from day 19-20, which was accompanied by a shift in species distribution, with the return of the *Chaetoceros* spp. and the appearance of *Navicula* spp. One hypothesis regarding this sharp decline of *C.*
510   *closterium* towards the end of the experiment could be top-down control by grazers, leading to a shift towards less palatable diatom species.

In the mesocosms, UCYN-C rapidly aggregated in the form of large aggregates (from 100-500 µm) and Berman-Frank et al. (2016) showed that UCYN-C abundances were positively correlated to transparent exopolymer particles (TEP) concentrations, which could hint to a
515   direct production by these organisms. Moreover, *C. closterium* has also been associated to large mucilage aggregate formations in the Mediterranean (Najdek et al., 2005) and it is known to produce TEP under nutrient stress (Alcoverro et al., 2000). Thus, both the dominating diatom and the UCYN-C could have produced the TEP and/or TEP precursors leading to the formation of these large aggregates in the mesocosms, which resulted in an important contribution (22%
520   of POC) of UCYN-C to export in the mesocosm traps during the second phase (Bonnet et al., 2015).

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Interestingly, *Synechococcus* increased again strongly during P2 (Fig. 14), showing its greater competitive advantage over other pico-phytoplankton groups in the P-limited and DDN rich environment by reducing its P cellular demand and use up the newly available DDN. This hypothesis was supported by gene expression dynamics from metatranscriptomic analysis which showed that *Synechococcus* (but not *Prochlorococcus*) was expressing genes for sulfo-lipid biosynthesis proteins over the course of the experiment whenever it was abundant, and also increased transcript accumulation for NH_4 transporters towards the end of the experiment (Pfreundt et al., 2016b). Another competitive advantage is its mixotrophic character, as *Synechococcus* cells are also able to assimilate amino acids (Van Wambeke et al., 2016). Based on its genome, Palenik et al. (2003) have also shown that *Synechococcus* is clearly more nutritionally versatile and a 'generalist' compared with its *Prochlorococcus* relatives, likely explaining its success in this experiment.

In the last few days, the evolution of populations in M3 departed strongly from the other mesocosms, with higher primary productivity, N_2-fixing fluxes and biomass accumulation, originating from the larger development of *Synechococcus,* pico-phytoeukaryotes*,* but also *Trichodesmium* populations, which may have been favored by the slower DIP decrease and the slightly higher salinities measured in this mesocosm compared to the other two. The PE signal showed a strong increase only in M3, and was likely mainly driven by this increase in *Synechococcus* and *Trichodesmium*, which were in much higher abundance in this mesocosm. It is likely that the PE accumulation was not so much correlated to the increase in UCYN-C, as related *Cyanothece* strains did not show any PE signal in culture (comm. pers. Rodier) and because their contribution to biomass was rather small.

The evolution of dinoflagellates, overall dominated by cells < 50 µm belonging to the *Gymnodinium*/*Gyrodinium* spp. mix, showed no distinct patterns between P1 and P2 (Figs. 9, 10, 14) and no reproducible trends between mesocosms, as detailed previously. Dinoflagellates are comprised of autotrophs, heterotrophs as well as mixotrophs, which makes it difficult to relate their dynamics to bottom-up control factors, and is more likely reflecting the result of biological interactions with other groups.

5. Conclusion

The VAHINE mesocosm enclosure experiment and subsequent DIP-addition in coastal LNLC waters inside the Nouméa lagoon successfully triggered a succession in the diazotroph

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community that stimulated both primary production and exceptionally high N_2-fixation rates after a lag time of approximately 10 days compared to fluxes observed in the surrounding lagoon waters. A distinctly different planktonic community developed inside the mesocosms, which were generally well replicated despite slight timing and concentration variations of the different groups observed. A diverse diatom community was initially (P0) dominant in these nutrient limited waters, and was most likely fueled by DDN release by present DDAs (namely *Rhizosolenia/Richelia*), *Trichodesmium* and UCYN-A. *Synechococcus* was the other main component of phytoplankton and is known to hold a competitive advantage at limiting P levels with its ability to replace phospho-lipids by sulfo-lipids as well as use NH_4^+ from DDN.

After DIP addition, the average Chl *a* concentrations did not show any increase for another 10 days, yet shifts in the community structure were observed during this first period (P1). Both *Synechococcus* and pico-phytoeukaryotes populations dropped while *Prochlorococcus* clearly benefited from the sudden P availability. Diatoms, after an initial surge on the day following P addition without changes in community structure rapidly decreased and started to re-increase only after a week. Between day 11 and 15, a monospecific bloom of *C. closterium* developed, closely coupled to the apparition of UCYN-C populations, both following the staggered decrease in P-availability in the three mesocosms. The association of *C. closterium* blooms during other diazotroph bloom events has already been recorded in previous studies and indicates that this diatom species could be very efficient in using up DDN while P levels are still sufficient.

The second period (P2), when DIP was again depleted was defined by an important increase in Chl *a*, associated to increases in primary production and near tripled N_2-fixation rates. These changes were coupled to important shifts in the diazotroph community, which became dominated by UCYN-C, which rapidly aggregated. *Synechococcus*, diatoms and nano-phytoeukaryotes abundances re-increased towards the end of the experiment, revealing an efficient transfer of DDN to these groups, this time fueled by UCYN-C rather than by DDAs and *Trichodesmium.*

In conclusion, we show that the elevated N_2-fixation rates, stimulated by a DIP-fertilization in enclosed mesocosms in LNLC waters benefited the entire planktonic community with clear stimulation of both diazotrophic and non-diazotrophic groups mainly observed by *Synechococcus* and diatom species other than DDAs, which has clear implications for the efficiency C export fueled by DDN.

Figure legend:

Fig. 1: Location map of mesocosms deployment off Nouméa in New Caledonia.

Fig. 2: Total Chl *a* in µg L^{-1} at each of the three depths (1, 6 and 12m) inside each mesocosm (M1, M2 and M3) and outside of mesocosms (OUT).

Fig. 3: Total phycoerythrin in µg L^{-1} at the intermediate depth (6 m) inside each mesocosm and in the control area outside of mesocosms.

Fig. 4: *Synechococcus* in cells mL^{-1} at each of the three depths (1, 6 and 12m) inside each mesocosm (M1, M2 and M3).

Fig. 5: *Prochlorococcus* in cells mL^{-1} at each of the three depths (1, 6 and 12m) inside each mesocosm (M1, M2 and M3).

Fig. 6: Pico-eukaryotes in cells mL^{-1} at each of the three depths (1, 6 and 12m) inside each mesocosm (M1, M2 and M3).

Fig. 7: Nano-eukaryotes in cells mL^{-1} at each of the three depths (1, 6 and 12m) inside each mesocosm (M1, M2 and M3).

Fig. 8: Diatom genera/species abundance in cells L^{-1} at the intermediate depth (6 m) in each mesocosm.

Fig. 9: Total dinoflagellate abundance (in cells L^{-1}) at the intermediate depth (6 m) inside each mesocosm.

Fig. 10 : Dynamics of the biomass of the main groups constituting phytoplankton communities in biomass (diazotrophs not included) over the course of the experiment for *Prochlorococcus*

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(PROC), *Synechococcus* (SYN), pico-phytoeukaryotes (PICO), nano-phytoeukaryotes (NANO), diatoms (DIAT) and dinoflagellates (DINO).

Fig. 11: Boxplots of targeted diazotrophs groups in nifH gene copies L^{-1} in the three mesocosms during the two periods P1 and P2.

625 Fig. 12: Boxplots of primary production (in μmol C L^{-1} d^{-1}), N_2-fixation rates (in nmol N L^{-1} d^{-1}) and Chl *a* concentrations (in μg L^{-1}) in the three mesocosms (top panels) and in the lagoon waters (bottom panels) during the two periods P1 and P2.

Fig. 13: Boxplots of nutrients, DIP, NO_3, DON in μM and NH_4^+ (in nM) in the three mesocosms (top panels) and in the lagoon waters (bottom panels) during the two periods P1 and P2.

630 Fig. 14: Boxplots of the main phytoplanktonic groups in cells L^{-1} in the three mesocosms during the two periods P1 and P2.

Supplementary Figures:

Fig. S1: Main diatom genera/species composition in % contribution at the intermediate depth (6 m) in each mesocosm.

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Fig. S2: Average ($\pm$SD) contribution to C biomass of the main groups constituting phytoplankton communities (diazotrophs not included) over the course of the experiment following the three periods P0, P1 and P2 for *Prochlorococcus* (PROC), *Synechococcus* (SYN), pico-phytoeukaryotes (PICO), nano-phytoeukaryotes (NANO), diatoms (DIAT) and

640 dinoflagellates (DINO).

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