

## **Response to the Editor comments**

A paragraph on the variability within the mesocosms was added in the revised version at the beginning of the discussion section.

It is effectively common to plot sd or se in the figures in biological oceanography papers. Note that it is defined exactly what are the error bars in the corresponding legends, and that we use appropriate statistics when we want to make comparison between samples or means. (In this ms we used non parametric statistics).

1 **Heterotrophic bacterial production and metabolic balance**  
2 **during the VAHINE mesocosm experiment in the New**  
3 **Caledonia lagoon**

4  
5 **France Van Wambeke<sup>1</sup>, Ulrike Pfreundt<sup>2</sup>, Aude Barani<sup>1</sup>, Hugo Berthelot<sup>1</sup>,**  
6 **Thierry Moutin<sup>1</sup>, Martine Rodier<sup>3\*</sup>, Wofgang R. Hess<sup>2</sup> and Sophie Bonnet<sup>1,3</sup>**

7 [1]{Aix Marseille Université, CNRS/INSU, Université de Toulon, IRD, Mediterranean  
8 Institute of Oceanography (MIO) UM110, 13288, Marseille, France }

9 [2]{University of Freiburg, Faculty of Biology, Schaenzlestr. 1, D-79104 Freiburg, Germany }

10 [3]{Mediterranean Institute of Oceanography (MIO) – IRD/CNRS/Aix-Marseille University  
11 IRD Nouméa, 101 Promenade R. Laroque, BPA5, 98848, Nouméa CEDEX, New Caledonia }

12 [\*]{now at: IRD, Université de la Polynésie française - Institut Malardé - Ifremer, UMR 241  
13 Ecosystèmes Insulaires Océaniens (EIO), IRD Tahiti, PB 529, 98713 Papeete, Tahiti, French  
14 Polynesia }

15  
16 Correspondence to F. Van Wambeke (france.van-wambeke@mio.osupytheas.fr).

17

- Supprimé: .
- Supprimé: .
- Supprimé: .
- Supprimé: .
- Supprimé: .
- Supprimé: .
- Supprimé: .

## Abstract

Studies investigating the fate of diazotrophs through the microbial food web is lacking although  $N_2$  fixation can fuel up to 50% of new production in some oligotrophic oceans. In particular, the role played by heterotrophic prokaryotes in this transfer is largely unknown. In the frame of the VAHINE experiment, three replicate large-volume ( $\sim 50 \text{ m}^3$ ) mesocosms were deployed for 23 days in the new Caledonia lagoon and were intentionally fertilized on day 4 with dissolved inorganic phosphorus (DIP) to stimulate  $N_2$  fixation. We specifically examined relationships between heterotrophic bacterial production (BP) and  $N_2$  fixation, or primary production, determined bacterial growth efficiency and established carbon budgets. BP was statistically higher during the second phase of the experiment (P2: days 15-23), when chlorophyll biomass started to increase compared to the first phase (P1: days 5-14). Phosphatase alkaline activity increased drastically during the second phase of the experiment, showing adaptations of microbial populations after utilization of the DIP added. Notably, among autotrophs, *Synechococcus* abundances increased during P2, possibly related to its capacity to assimilate leucine and to produce alkaline phosphatase. Bacterial growth efficiency based on the carbon budget (27-43%), was notably higher than generally cited for oligotrophic environments and discussed in links with the presence of abundant species of Bacteria expressing proteorhodopsin. The main fate of gross primary production (particulate + dissolved) was respiration (67%), and export through sedimentation (17%). BP was highly correlated with particulate primary production and chlorophyll biomass during both phases of the experiment but slightly correlated, and only during P2 phase, with  $N_2$  fixation rates. Heterotrophic bacterial production was strongly stimulated after mineral N enrichment experiments, suggesting N-limitation of heterotrophic bacteria all over the experiment.  $N_2$  fixation rates corresponded to 17-37% of the nitrogen demand of heterotrophic bacteria. Our results suggest that most of the diazotroph derived nitrogen fuelled the heterotrophic bacterial community through indirect processes generating dissolved organic matter and detritus, like mortality, lysis and grazing of both diazotrophs and non-diazotrophs.

Supprimé: ¶

Mis en forme : Indice

Supprimé:  $N_2$  fixation fuels ~50% of new primary production in the oligotrophic South Pacific Ocean. The VAHINE mesocosm experiment designed to track the fate of diazotroph derived nitrogen (DDN) in the New Caledonia lagoon. Here, we examined the temporal dynamics of heterotrophic bacterial production during this experiment.

Supprimé: Three

Supprimé: We

Supprimé: rates

Supprimé: and primary production

Supprimé:

Supprimé: of the system from the DIP fertilization to the end of the experiment (days 5 to 23)

Supprimé: A

Supprimé: ,

Supprimé:

Supprimé: Heterotrophic bacterioplankton production (BP) and alkaline phosphatase activity (APA) were statistically higher during the second phase of the experiment (P2: days 15-23), when chlorophyll biomass started to increase compared to the first phase (P1: days 5-14).

Supprimé: (27-43%), possibly due to a high representation of

Supprimé: proteorhodopsin-containing organisms

Supprimé: within the picoplanktonic community

Supprimé: carbon budget showed that the

Supprimé: DDN reached

## 1 1 Introduction

2 In the South West Pacific ocean, the natural occurrence of abundant and diverse plankton taxa  
3 capable of dinitrogen ( $N_2$ ) fixation ( $N_2$ -fixing or diazotrophic organisms) (e.g., Moisander et  
4 al., 2010) can fuel ~50 % of new primary production. (Garcia et al., 2007; Bonnet et al.,  
5 2015c). However, little is known about the fate of the diazotroph-derived nitrogen (DDN) in  
6 this environment (Bonnet et al., 2015b). In particular, the role played by the microbial food  
7 web, and among them the heterotrophic bacteria in the transformation of DDN is largely  
8 unknown. In the central gyre of the South Pacific, where  $N_2$  fixation is lower than in the South  
9 West Pacific, nitrogen is the first element limiting growth of both phytoplankton and  
10 heterotrophic bacterioplankton as observed in short-term nutrient enrichment experiments  
11 (Bonnet et al., 2008; Van Wambeke et al., 2008a) or incubations with  $^{15}N$ - leucine or  $^{15}N$ -  
12  $NH_4^+$ , which significantly enhanced bicarbonate uptake (Halm et al., 2012). Such competition  
13 for nitrogen influences dissolved organic carbon accumulation in the surface layers and  
14 export. In the South West Pacific, however, the phytoplankton - heterotrophic bacterial  
15 coupling has mainly been investigated in the New Caledonia Lagoon. In this system,  
16 phytoplankton and bacterial production show seasonal patterns, with maxima in December-  
17 January and annual bacterial production representing 21 to 34 % of particulate primary  
18 production (Torreton et al, 2010), In the oligotrophic stations of the lagoon, based on a  
19 bacterial growth efficiency of 10% or less, dissolved phytoplankton release was not sufficient  
20 to sustain bacterial carbon demand (Rochelle-Newall et al., 2008). N-limitation of primary  
21 production is expected based on year-round dissolved inorganic nitrogen (DIN) to dissolved  
22 inorganic phosphorus (DIP) ratios and silicates to DIN ratios, respectively lower and higher  
23 than Redfield ratios (Torreton et al., 2010). However,  $N_2$  fixation is a recurrent feature in the  
24 lagoon (Garcia et al., 2007; Biegala and Raimbault, 2008), and no information is available on  
25 the potential role played by this process on the functioning of the microbial food web and how  
26 it could influence the factors limiting heterotrophic bacterial production. As blooms of  
27 diazotrophs are transient events, the production of varying sources and quality of organic  
28 matter is expected, which may influence biogeochemical fluxes, in particular heterotrophic  
29 bacterial production.

30 Through the VAHINE program (<http://mio.pytheas.univ-amu.fr/?VAHINE-Project>; Bonnet et  
31 al., 2015b), we experimentally investigated the fate of DDN in the planktonic food web and  
32 its potential impact on particle export. For this, we studied the development and the fate of a  
33 diazotroph bloom enhanced by intentional fertilization with DIP in large-volume (~50 m<sup>3</sup>)  
34 mesocosms deployed in the oligotrophic part of the New Caledonian lagoon, DIP being

Supprimé: shown to be

1 | considered to control the nitrogen input by dinitrogen fixation in the SW Pacific upper surface  
2 | waters (Moutin et al., 2005, 2008). The VAHINE experiment provided a unique opportunity  
3 | to study such phytoplankton-heterotrophic bacteria interactions by simultaneously using  
4 | biogeochemical techniques assessing stocks and fluxes in the same body of water for a period  
5 | of 3 weeks. In particular, our objectives were, i) to explore factors controlling heterotrophic  
6 | bacterial growth, ii) to examine the links between heterotrophic bacterial production and the  
7 | activity of N<sub>2</sub>-fixing organisms and primary producers and iii) to study the fate of carbon  
8 | inside mesocosms and the balance of autotrophy *versus* heterotrophy. The factors controlling  
9 | heterotrophic bacterioplankton were studied using short-term nutrient enrichment experiments  
10 | and measurements of alkaline phosphatase activity. In oligotrophic systems, assimilation of  
11 | organic nitrogen-containing molecules can also confer advantage for growth to some  
12 | cyanobacteria (Zubkov et al., 2004, Mary et al., 2008a). Thus we quantified fluxes of leucine  
13 | incorporation on a single cell basis, using flow sorting by cytometry (Talarmin et al., 2011).

## 15 | **2 Material and methods**

### 17 | **2.1 Mesocosm description and sampling strategy**

18 | Three large mesocosms (~50 m<sup>3</sup>) were deployed as open tubes with unfiltered, nutrient-poor,  
19 | waters of the Nouméa lagoon close to the Boulari passage (22°29.073 S - 166°26.205 E)  
20 | located 28 km of the coast from January 13 to February 4, 2013 (Fig. 1). After 2 days for  
21 | stabilizing mixing and verticality of mesocosms, they were closed at their bottom, which  
22 | constituted the starting day of the experiment, and a sediment trap was screwed at the basis of  
23 | the bottom cone of each mesocosm and changed every morning by SCUBA divers. The  
24 | mesocosms design is based on Guieu et al. (2010) and the choice of the site in the lagoon,  
25 | deployment and sampling strategy are described in details in Bonnet et al. (2015b). The three  
26 | triplicate mesocosms were supplemented with 0.8 μM KH<sub>2</sub>PO<sub>4</sub> between day 4 and day 5 of  
27 | the experiment to alleviate potential P limitation and induce a bloom of naturally present  
28 | communities of N<sub>2</sub> fixing organisms. All samples for the parameters described below were  
29 | collected every morning for 23 days using a clean Teflon pumping system from three selected  
30 | depths (1 m, 6 m, 12 m) in each mesocosm (M1, M2 and M3) and in surrounding waters (i.e.  
31 | outside the mesocosms, hereafter called Nouméa lagoon waters). Seawater from each  
32 | mesocosm was first filled in a polypropylene 50 L tank for stocks measurements, 4.5 L  
33 | polycarbonate bottles for rates measurements and 10 L carboys for diversity. All carboys were  
34 | immediately transferred onto the R/V Alis anchored close to the mesocosms to serve as a lab

1 platform to ensure a quick processing of the samples. Subsampling procedure and analysis for  
2 inorganic nutrients, chlorophyll *a* (Chl) and their associated phaeopigments, DIP turnover  
3 time and N<sub>2</sub> fixation rates are detailed in a companion paper (Berthelot et al., 2015). Primary  
4 production (PP) is determined from short term (~ 4 h) incubations around noon using <sup>14</sup>C  
5 labeling technique (see details in Berthelot et al., 2015) and a model of photosynthesis is then  
6 applied which reconstitute daily fluxes (Moutin et al., 1999). Primary production (PP) was  
7 determined from short term (~ 4 h) incubations around noon with H<sup>14</sup>CO<sub>3</sub> (see details in  
8 Berthelot et al., 2015) and a model of photosynthesis applied to calculate daily fluxes (Moutin  
9 et al., 1999). This model allows estimation of 24h-fluxes (dawn to dawn) from hourly rates,  
10 independent of starting time or duration of incubations, or of the geographic origin of the  
11 samples or of the time of the year (i.e. systems with varying day-light periods). This model  
12 avoids the general biases introduced by the large variety of incubation conditions used in the  
13 Steemann-Nielsen (1952) <sup>14</sup>C methodology (Regaudie-de-Gioux et al., 2014 and ref therein).  
14 Another advantage of this model is that it allows estimation of both PP (24h dawn-to-dawn)  
15 and Gross Primary Production (GPP). For 24 h incubations, GPP is 1.72 x PP as determined  
16 from the model (Figure 5 in Moutin et al., 1999). This constant is applicable as long as 24h-  
17 fluxes (dawn-to-dawn) are calculated using the same model.  
18  
19

## 20 **2.2 Heterotrophic bacterioplankton abundances**

21 Flow cytometry analyses were carried out at the PRECYM flow cytometry platform  
22 (<https://precym.mio.univ-amu.fr/>). Samples were analyzed using a FACSCalibur (BD  
23 Biosciences, San Jose, CA). For heterotrophic bacterial abundance (BA), 1.8 mL of seawater  
24 was fixed with formaldehyde (2 % final concentration, 15 minutes incubation at RT), frozen  
25 and stored in liquid N<sub>2</sub> until analysis in the laboratory. After thawing at room temperature, 0.3  
26 mL of each sample was incubated with SYBR Green II (Molecular Probes, final conc. 0.05 %  
27 [v/v]) for 15 minutes at room temperature in the dark) to stain nucleic acids (Marie et al.  
28 1997). Cells were characterized by 2 main optical signals collected from the 488 nm laser:  
29 side scatter (SSC, related to cell structure) and green fluorescence (530/40<sub>488 nm</sub>), related to  
30 nucleic acids staining. For the calculation of heterotrophic prokaryotes abundances,  
31 phytoplankton, in particular *Prochlorococcus* and *Synechococcus*, was gated out thanks to its  
32 red autofluorescence induced by the chlorophyll (Sieracki et al., 1995). We discriminated  
33 HNA (high nucleic acid) and LNA (low nucleic acid) cells and heterotrophic bacterial  
34 abundance (HBA) was calculated as the sum of both categories. TruCount beads (BD

1 Biosciences) and 2  $\mu\text{m}$  beads (Fluoresbrite YG, Polyscience) were added to the samples just  
2 before analysis. To determine the volume analyzed by the flow cytometer, the flow rate was  
3 estimated by weighing three tubes of samples before and after a 3 min run. The cell  
4 abundance was determined by dividing the number of cells by the volume analyzed  
5 determined both by the TruCount beads and flow rate. All data were collected in log scale and  
6 stored in list-mode using the CellQuest software (BD Biosciences). Data analysis was  
7 performed using the SUMMIT v4.3 software (Dako).

8

### 9 **2.3 Heterotrophic bacterial production**

10 Heterotrophic bacterial production (BP) was estimated daily using the  $^3\text{H}$ -leucine  
11 incorporation technique (Kirchman, 1993), adapted from the centrifuge method (Smith and  
12 Azam, 1992). For each sample, triplicate aliquots (1.5 mL) and one trichloroacetic acid (TCA)  
13 killed control were incubated with a mix of 6 nM hot leucine (L- $^3\text{H}$ ] leucine, Perkin Elmer®  
14 specific activity ranging 106 Ci  $\text{mmol}^{-1}$ ) and 14 nM cold leucine, at *in situ* surface  
15 temperature (on-deck incubators equipped with 50 % light intensity screen and cooled with  
16 circulating surface seawater), for 1 h. Linearity of leucine incorporation was checked  
17 regularly by time series experiments. The live incubations were terminated with 5 % TCA  
18 (final concentration). After three runs of centrifugation/aspiration of the supernatant (once  
19 with the fixed sea water sample, once with a 5 % TCA rinse, once with an 80 % ethanol  
20 rinse), the pellet was resuspended in Packard Ultima Gold MW Scintillation liquid®.  
21 Radioactivity was counted using a Liquid Scintillation Analyzer Packard® 1600TR and the  
22  $^3\text{H}$  counting efficiency was corrected for quenching. Concentration kinetic experiments  
23 showed that isotopic dilution factor ranged 1 to 1.56 and thus BP rates were calculated from  
24 leucine incorporation rates using conversion factor adjusted from 1.5 to 2.4  $\text{kg C mol}^{-1}$   
25 leucine. Daily rates were calculated assuming they are 24 times the hourly rate.

26

27

### 28 **2.4 Nutrient addition experiments**

29 The availability of phosphorus (P), nitrogen (N) and organic carbon (C) for heterotrophic  
30 bacteria was investigated by measuring changes in bacterial production following additions of  
31 DIP (0.25  $\mu\text{M}$  P),  $\text{NO}_3^-$  and  $\text{NH}_4^+$  (1  $\mu\text{M}$  each) or glucose (10  $\mu\text{M}$  C) (final concentrations).  
32 Two bioassays were realised, one right before (day 4) and one two weeks after (day 20) the  
33 DIP fertilization in M1 (samples from 1 m depth). Eight combinations were tested (P, N, C,  
34 PN, PC, NC and PNC) including the non-enriched control T. Each bioassay condition was

Supprimé: 5

1 tested in triplicate in 60 mL polycarbonate bottles incubated for 48 h under in situ-simulated  
2 conditions in the on-deck incubator (described in section 2.3). After incubation, each bottle  
3 was sub-sampled in order to measure BP using the leucine technique described in section 2.3.  
4

## 5 | **2.5 Alkaline phosphatase activity**

Supprimé: 6

6 Total alkaline phosphatase activity (APA) was measured at the three depths in M1, M2 and  
7 M3 and in Nouméa lagoon waters using the analog substrate methylumbelliferone phosphate  
8 (MUF-P, 1  $\mu$ M final concentration) (Hoppe, 1983). The linear increase in fluorescence of  
9 seawater with added MUF was measured over the incubation time (up to 8h), in the dark with  
10 a TKO 100 Hoefer DNA fluorometer (single-wavelength with excitation/emission fixed at  
11 365/460 nm but suitable for MUF). Concentration kinetics using a range from 25 to 2500 nM  
12 MUF-P were run on some occasions to check that the 1  $\mu$ M concentration used for routine  
13 measurements was sufficient to saturate enzyme activity. Blanks were run by adding the  
14 MUF-P to filtered boiled seawater and were shown to be insignificant. Calibration curves  
15 were made with MUF standards.  
16

## 17 | **2.6 Statistical analyses**

Supprimé: 7

18 Non parametric Mann-Whitney and Kruskal-Wallis tests were used to compare differences of  
19 each parameter studied between mesocosms, periods of time, or effect of various amendments  
20 on BP in the nutrient addition experiments. Model I linear regressions and Pearson correlation  
21 coefficient were used to study Log-log relationships between BP and Chl or PP; and evolution  
22 of DOC and POC with time.  
23

## 24 | **3 Results**

25 Salinity and temperature measurements show that the water column was not stratified over the  
26 course of the experiment, except the first two days, which were characterized by a slight  
27 stratification both inside and outside of the mesocosms (Bonnet et al., 2016). No vertical  
28 stratification was observed in the mesocosms for bacterial production or alkaline phosphatase  
29 activity (APA) (see exemplary data for M1 in Fig. S1) as for most of the parameters (Bonnet  
30 et al., 2015b; Turk-Kubo et al., 2015, Berthelot et al., 2015). For all description of  
31 biogeochemical stocks and fluxes, we thus used the average of the three depths to plot the  
32 temporal evolution within each mesocosm.  
33

Supprimé: Very

Supprimé:

Supprimé: little

Supprimé: exemplary

Supprimé: 3

### 34 | **3.1 Chlorophyll stocks and net primary production**



1 Based on the Chl and PP dynamics, two periods P1 (days 5-14) and P2 (days 15-23) were  
2 identified after DIP fertilization, which were also identified by Berthelot et al. (2015) based  
3 on biogeochemical characteristics and by Turk-Kubo et al. (2015) based on changes in  
4 abundances of targeted diazotrophs. Diatom heterocyst-forming symbionts associated with  
5 diatoms were abundant during P1 while a bloom of the unicellular N<sub>2</sub>-fixing cyanobacteria  
6 from Group C (UCYN-C) occurred P2 (Leblanc et al., 2015; Turk-Kubo et al. 2015). Chl  
7 stocks significantly increased during P2 compared to P1 in the three mesocosms (statistics are  
8 presented in Table 1). Chl concentrations during P2 in all three mesocosms were significantly  
9 higher than those in the Nouméa lagoon for the same period. PP showed the same trend as for  
10 Chl, being higher during P2 in all three mesocosms (Fig. 3, Table 1). However, the PP rates  
11 and Chl concentrations reached during P2 were not identical between the three mesocosms:  
12 M3 exhibited higher Chl concentrations during P2 ( $0.71 \pm 0.30 \mu\text{g L}^{-1}$ ) than M2 ( $0.49 \pm 0.18$   
13  $\mu\text{g L}^{-1}$ ) and M1 ( $0.42 \pm 0.14 \mu\text{g L}^{-1}$ ,  $p < 0.001$ ). This was equally true for PP rates ( $2.45 \pm$   
14  $0.76 \mu\text{mol C L}^{-1} \text{d}^{-1}$  in M3 compared to  $1.47 \pm 0.35 \mu\text{mol C L}^{-1} \text{d}^{-1}$  in M2 and  $1.09 \pm 0.22$   
15  $\mu\text{mol C L}^{-1} \text{d}^{-1}$  in M1,  $p < 0.001$ ). Significant increases of Chl and PP were also observed in  
16 lagoon waters (reaching in P2  $0.30 \pm 0.07 \mu\text{g Chl L}^{-1}$  and  $1.36 \pm 0.37 \mu\text{mol C L}^{-1} \text{d}^{-1}$ ,  
17 respectively, Table 1).

Supprimé: 4

### 19 3.2 Heterotrophic bacterioplankton abundance and production

20 Abundances of heterotrophic bacterioplankton (HBA) varied 10 fold, from 1.7 (day 9, M1, 1  
21 m) to  $12.8 \times 10^5 \text{ cells mL}^{-1}$  (M2, day 11, 6 m). Peaks of HBA were sporadic, like on day 11 in  
22 M1., but not repeated for the three depths sampled. They were possibly due to the presence of  
23 a patchy distribution of aggregates that could have biased some of the results. These peaks are  
24 occasional, and as they might reflect the reality of a patchy distribution, they were kept in the  
25 figures, statistics and estimates of means per day. Average HBA did not increase statistically  
26 between phase P1 and P2 in M1 and M2 and increased slightly ( $p < 0.05$ ) from ( $4.1 \pm 0.7$ ) to  
27 ( $5.0 \pm 1.4$ )  $\times 10^5 \text{ cells mL}^{-1}$  in M3 (Table 1, Fig. 3). Evolution of BP in the mesocosms was  
28 close to that in lagoon waters during P1, except for a peak only detected on day 4 (the  
29 morning before DIP fertilization) in M1 and M3, and at day 5 in M2. These peaks were not  
30 related to any HBA, Chl or PP increase but were related to a steep increase in  
31 *Rhodobacteraceae* 16S ribosomal RNA genes (Pfreundt et al., 2016b). Just like PP, BP  
32 significantly increased during P2 in all three mesocosms with higher values in M3 compared  
33 to M1 and M2 during P2 (Kruskal-Wallis test,  $p < 0.05$ ). This BP increase was also observed  
34 in lagoon waters but with lower amplitude (Table 1). In the three mesocosms, the log-log

Supprimé: However p

Supprimé: and a

Supprimé: 4

Supprimé: 2015b

1 relationship between BP and PP was significant only during P2 ( $r = 0.54$ ,  $p < 0.001$ ), whereas  
2 that between BP and Chl was significant during P1 and P2 ( $r = 0.4$ ,  $p < 0.001$  and  $r = 0.72$ ,  
3  $p < 0.001$ , respectively, Fig. 4). In lagoon waters, the BP/PP ratio slightly increased (but  
4 significantly) between P1 and P2 (0.33 to 0.39,  $p < 0.05$ , Table 1). The BP/PP ratio  
5 significantly increased during P2 in M1 (0.48 to 0.65,  $p < 0.001$ ), significantly decreased in  
6 M3 (0.50 to 0.35,  $p < 0.05$ ) and remained stable in M2 (Table 1). Such differences probably  
7 depended on varying maximal values of PP in different mesocosms and the exact days when  
8 PP or BP started to increase. Thus we examined the trend of BP/PP ratio with PP, keeping in  
9 mind the risk of autocorrelation. BP/PP ratio decreased when PP increased, with higher  
10 intensities in the mesocosms compared to lagoon waters, and consistent for P1 or P2 (Table 2).  
11 The log-log relationship between  $N_2$  fixation rates ( $nM d^{-1}$ ) and BP ( $mg C m^{-3} d^{-1}$ ) was  
12 insignificant during P1 and significant during P2 ( $\log(BP) = 0.13 * \log(N_2 \text{ fix rates}) + 0.73$ ,  $r$   
13  $= 0.21$ ,  $p = 0.04$ , data not shown).

Supprimé: 5

Supprimé:

### 15 3.3 Alkaline phosphatase activity

16 APA was homogeneous between the three depths sampled from the mesocosms (example for  
17 M1 on Fig. S1), but this was not the case in the Nouméa lagoon, where activity was often  
18 higher at 1 m depth compared to the two other depths (data not shown). A slight but very  
19 reproducible decrease of APA occurred on days 5 and 6 in all three mesocosms where DIP  
20 fertilization took place, and in lagoon waters only on day 5 (Fig. 3). DIP was consumed more  
21 rapidly in M1, mirrored by higher APA and lower TDIP between day 9 and 18 in this  
22 mesocosm (Fig. 3). APA then increased very rapidly in M1 and M2 after day 17, but only  
23 after day 21 in M3. Such delays were in agreement with the evolution of DIP, which was less  
24 rapidly consumed in M3 compared to M1 and M2 (Berthelot et al., 2015). Consequently,  
25 although mean APA increased significantly in all three mesocosms between P1 and P2 (Table  
26 1), it was lower in M3 compared to M1 and M2 during P2 (3.1 vs 7.5-7.9  $nmol MUF-P$   
27  $hydrolyzed L^{-1} h^{-1}$ , respectively,  $p < 0.01$ ). Finally, APA also increased significantly between  
28 P1 and P2 in the lagoon waters, albeit to a lower extent as in the mesocosms (from 3 to 5  
29  $nmol MUF-P hydrolyzed L^{-1} h^{-1}$ , Table 1). Noteworthy, APA in the lagoon waters exhibited  
30 the strongest increase between day 10 and 11 and stayed at this higher level until day 23, thus  
31 exhibiting different dynamics than in the mesocosms.

Supprimé: 3

Supprimé:

Supprimé: 4

Supprimé: 4

### 33 3.4 Enrichment experiments

1 In the two 48 h nutrient enrichment experiments performed on day 4 and on day 20, BP  
2 increased 3-fold after nitrogen addition ( $\text{NH}_4^+ + \text{NO}_3^-$ ) compared to the unamended controls.  
3 This significant increase was observed irrespectively of whether these N sources were added  
4 alone or in combination with DIP or glucose (N, NP, NC, NPC treatments, Fig. 5,  $p < 0.03$ ).  
5 Only PC and C addition on day 4 led to significant BP increase without N addition ( $p < 0.05$ )  
6 however, to a much lower extent than with all N combinations (factor 1.5 and 1.1,  
7 respectively). On day 20, only N amendments led to significant BP increases after 48 h  
8 incubations, compared to the unamended control (Fig. 5,  $p < 0.03$ ).  
9

Supprimé: NG

Supprimé: NPG

Supprimé: 6

Supprimé: PG

Supprimé: G

Supprimé: 6

### 10 3.5 Cell-specific leucine incorporation rates

11 Among the different groups sorted by flow cytometry, significant cell-specific leucine  
12 incorporation rates into macromolecules were obtained for heterotrophic bacterioplankton.  
13 LNA, HNA and hi-HNA cells had specific activities ranging from 4.6 to  $86 \times 10^{-21} \text{ mol cell}^{-1}$   
14  $\text{h}^{-1}$ . Overall, cell specific leucine incorporation rates for LNA cells were lower than or equal to  
15 rates for HNA cells, and rates for Hi-HNA cells were 1.2 - 4.8 times greater than rates for  
16 HNA cells (Table 3). All cell-specific rates increased when bulk activities increased. Among  
17 autotrophic groups, significant leucine incorporation was detected for *Prochlorococcus* cells  
18 (PRO) only on day 21 and day 23, due to the low volume available for sorting and a  
19 significant decrease of PRO abundances in the samples to be sorted, when compared to the  
20 abundances determined on samples analysed only three months after the experiment. We  
21 checked on fresh Mediterranean samples that *Prochlorococcus* cells were clearly detectable  
22 with the flow cytometer setting chosen, and could discard any instrument problem. We  
23 assumed that the PRO cells lower detection was due to the long storage period of  $^3\text{H}$ -leucine  
24 labelled samples until cell sorting (two years at  $-80^\circ\text{C}$ ) that could induce a loss of  
25 fluorescence or cell damages. We obtained a maximum of 1200 PRO cells sorted. Thus, even  
26 when the signal was significant, it was associated with a high standard deviation (40 % on day  
27 21, Table 3). On the opposite, *Synechococcus* cells (SYN) were well detected and their total  
28 abundance matched with the total counts determined on samples analysed three months after  
29 the experiment. Additional 651 nm and 355 nm laser excitations allowed us to distinguish two  
30 different sub-groups, not clearly distinguishable using only 488 nm laser excitation, separated  
31 mainly on the criterion of orange fluorescence (LO-SYN and HO-SYN) (Fig. 2), suggesting  
32 different relative amounts of accessory pigments (Neveux et al., 2010). Leucine incorporation  
33 was detected in both SYN groups for all samples analysed. For a given sampling date, cell

Supprimé: error

1 specific rates of both groups were almost equal, and increased on day 21 and 23 compared to  
2 days 15 and 19. They were lower than LNA cell-specific rates (from ~20 % of the LNA rates  
3 at day 15 to ~70 % at day 21). Cell-specific rates of LO-SYN and HO-SYN diverged only on  
4 day 23 (Table 3). At this date, cell specific rates for LO-SYN were twice as high as for LNA  
5 cells, reaching  $131 \times 10^{-21} \text{ mol cell}^{-1} \text{ h}^{-1}$ . Overall, the contribution of the two *Synechococcus*  
6 groups to the bulk activity was very low: it ranged 0.2 to 0.7 % for LO-SYN or HO-SYN (i.e.  
7 the contribution reached a max of 1.5 % for both SYN groups together), and 0.01-0.02 % for  
8 PRO (when detected), respectively. Contribution of LNA cells to the bulk activity was 4-12  
9 %. Thus, the most important contribution to the bulk leucine activity was due to HNA and Hi-  
10 HNA cells.

### 11 **3.6 Carbon budget**

12 We used the advantage of a day to day sampling in an enclosed system to compute a carbon  
13 budget that will allow to estimate the fate of phytoplankton-derived organic carbon and the  
14 metabolic balance. This carbon budget was calculated using time-integrated data, and thus  
15 considered the whole data set. First, each time point was averaged for the three sampling  
16 depths, and then time integration was calculated separately for each mesocosm assuming a  
17 linear trend between 2 successive days. A mesocosm average was calculated based on the  
18 time-integrated data obtained in each of the three mesocosms, with error bars representing the  
19 standard deviation (sd) among the three mesocosms (Fig. 6a). Gross primary production  
20 (GPP) is derived from PP assuming  $GPP=PP \times 1.72$  (Moutin et al., 1999) and represents the  
21 whole photosynthetic source of organic matter, including both particulate and extracellular  
22 release forms. The cumulated GPP at day 23 was  $38 \pm 11 \mu\text{M C}$  (Fig. 6b). Carbon exported by  
23 sedimentation into the traps ( $C_{exp}$ ) was corrected in  $\mu\text{M C}$  units based on a mean, constant  
24 water volume inside M1, M2, and M3 (see Berthelot et al., 2015 for details) and its cumulated  
25 value reached  $6.4 \pm 2.1 \mu\text{M C}$  on day 23. For POC and DOC, for which data were more  
26 irregular and showed outliers, we decided to calculate net variations of POC and DOC after a  
27 linear fit of the discrete data set between days 5 and 23 in each mesocosm (Table 4). POC  
28 increased linearly in M1 and M3 ( $0.12$  and  $0.48 \mu\text{mol C L}^{-1} \text{ d}^{-1}$ ,  $r = 0.32$   $p < 0.03$  and  $r = 0.70$   
29  $p < 0.001$ , respectively) and showed no trend in M2. A significant increase of DOC was only  
30 observed in M2 (Table 4). Due to the high sd resulting from variability in net variation of  
31 POC and DOC versus time between the three mesocosms, the average accumulation of DOC  
32 and POC estimated for the carbon budget was negligible (Fig. 6a), and the most important  
33 measured fate of GPP was  $C_{exp}$ , representing 17 % of GPP (Fig. 6b).  $GPP - (\text{net DOC} + \text{net}$   
34

1 POC + Cexp) can be considered as community respiration (CR). CR was calculated and  
2 reached  $27 \pm 11 \mu\text{M}$  cumulated from day 5 to 23, i.e. 71 % of GPP.

## 4 Discussion

### 4.1 Variability within the triplicate mesocosms

3  
4  
5  
6 Overall, M3 exhibited maximum peaks of chlorophyll biomass reached, PP and BP rates  
7 and these different responses were particularly seen during P2. A time lag of a few days in the  
8 succession of the different planktonic populations was noticed, particularly for nitrogen fixers  
9 (Turk Kubo et al. xxx), and DIP was consumed more rapidly in M1. However, slight  
10 divergence in biological and chemical evolution among different replicated mesocosms is not  
11 uncommon, particularly after the first week of enclosure (Martinez-Martinez et al., 2006;  
12 Pulido-Villena et al., 2014). Here, the divergence was probably resulting from a combination  
13 of bottom up (availability of DIP and nitrogen), and top down controls (grazing pressure and  
14 viral lysis). The initial conditions prevailing before the DIP enrichment could be also at the  
15 origin of the divergence. Indeed mesocosms were closed 3 days before the DIP addition, and  
16 many species of diazotrophs exhibit a patchy distribution (Bombar et al., 2015). In addition,  
17 Hunt et al. (2016) noticed larger amounts of zooplankton individuals in M3 at the beginning  
18 of the experiment, some of which, stressed by the mesocosms, might have died (some larger  
19 amounts of 'swimmers' were recovered in the traps in M3), contributing to supplementary  
20 sources of N in M3. Nevertheless, overall the replicability among mesocosms was considered  
21 sufficiently correct for most of the biogeochemical stocks, fluxes and abundances of  
22 phytoplankton groups (Bonnet et al., 2016) and thus our results are discussed based on  
23 averages.

### 4.2 N limitation and coupling between BP and $\text{N}_2$ fixation

24  
25  
26 BP was significantly enhanced on a short-term scale (1 - 2 days) by  $\text{NO}_3^- + \text{NH}_4^+$  but not DIP  
27 or glucose amendments, indicating that BP was directly N-limited, and/or indirectly after  
28 stimulation of N-limited phytoplankton (Fig. 4). In the New Caledonia lagoon, N-limitation  
29 has previously been suggested based on a one-year survey of nutrient ratios (Torréon et al.,  
30 2010). N-limitation is a recurrent feature observed in the ultra-oligotrophic South Eastern  
31 Pacific Gyre (Van Wambeke et al., 2008a; Halm et al., 2012), as assessed from short-term (1-  
32 3 days) enrichment experiments or incubations. As  $\text{N}_2$  fixation is assumed to be the only  
33 process providing a source of new nitrogen to the mesocosms in this experiment, we

Supprimé: 1

Supprimé: 1

Supprimé: 5

1 examined the potential links between N<sub>2</sub> fixation rates and BP. First, marine heterotrophic  
2 diazotrophs were detected at low abundances during the mesocosms experiment:  $\gamma$ -24774A11  
3 with ca 10<sup>2</sup>-10<sup>3</sup> *nifH* gene copies L<sup>-1</sup> (Turk-Kubo et al., 2015), and 16S tags corresponding to  
4 heterotrophic diazotrophs like *Bradyrhizobium* or *Mesorhizobium* were scarce (Pfreundt et al.,  
5 [2016b](#)). Therefore, N<sub>2</sub> fixation directly performed by heterotrophic bacteria probably  
6 accounted for a minor fraction of bulk N<sub>2</sub> fixation during the mesocosm experiment. Second,  
7 as the log-log relationship between N<sub>2</sub> fixation rates and bacterial production was not  
8 significant during P1, and only slightly significant during P2 (r = 0.21, p = 0.04), the  
9 excretion of DON and NH<sub>4</sub><sup>+</sup> by diazotrophs likely did not supply much nitrogen for  
10 heterotrophic prokaryotes directly, particularly during P1 when the main organisms  
11 responsible for diazotrophy were diatom-symbiotic (Turk-Kubo et al., 2015). Assuming a C/N  
12 molar ratio of around 6.8 for heterotrophic prokaryotic biomass (Fukuda et al., 1998), N<sub>2</sub>  
13 fixation might have provided 17 to 30 % of the nitrogen demand of heterotrophic prokaryotes,  
14 depending on the phase and the mesocosm considered (Table 1). This proportion increases to  
15 30 - 37 % if we consider a C/N ratio of 8.2, which seems more appropriate in the Pacific  
16 Ocean (Fukuda et al., 1998). Thus, N<sub>2</sub> fixation contributed to but was not sufficient to sustain  
17 100 % of the N requirements of heterotrophic bacteria during this study. Other potential  
18 sources were initial DON stocks, concentrations of which decreased slightly at the end of the  
19 experiment (Berthelot et al., 2015) and detritus. Indeed, there was a decay of larger  
20 phytoplankton cells after the closure of the mesocosms as discussed by Knapp et al. (2015)  
21 and Leblanc et al. (2015) following DIP availability (TDIP) as well as PP decreases (Berthelot  
22 et al., 2015) and *Synechococcus* 16S tags dropped substantially between day 2 and 4 (Pfreundt  
23 et al., [2016b](#)). Such detritus probably also contributed to sustain BP. NanoSIMS analyses were  
24 performed during a parallel experiment done at the height of a bloom of diazotrophic  
25 *Cyanothece*-like cyanobacteria (UCYN-C) on days 17-20 in M2 (Bonnet et al., 2015a). After  
26 24 h of <sup>15</sup>N<sub>2</sub>-incubations, these authors reported significant <sup>15</sup>N-enrichment in picoplanktonic  
27 cells (0.2-2  $\mu$ m fraction). This confirmed a rapid (one day) transfer of DDN (also <sup>15</sup>N-  
28 enriched) to picophytoplankton, and potentially heterotrophic bacteria. However, such  
29 transfer likely occurred indirectly through DON after mortality and grazing processes, as  
30 shown by model simulations run during the VAHINE project (Gimenez et al., 2015).

Supprimé: 2015b

Supprimé: much

Supprimé: 2015b

### 4.3 Alkaline phosphatase activity and P acquisition

A slight T<sub>DIP</sub> decrease was noticed in the mesocosms before the DIP spike but not in the lagoon, suggesting a lower P availability inside and not outside the mesocosms. Therefore, N<sub>2</sub>

1 fixers might benefit from continuous and variable inputs of DIP sources in the lagoon waters  
2 during that period. This is also confirmed by the low values of alkaline phosphatase activity in  
3 the lagoon at the start of the experiment. Whether these sources were coming from the  
4 benthos (Torréton et al., 2002), the atmosphere (soot emission can influence lagoon waters  
5 inside and outside the barrier reef, Mari et al., 2014) and/or currents (Fichez et al., 2010) is  
6 beyond the scope of this study. Inside the mesocosms, when the DIP added was consumed,  
7 the increase of APA observed could be due to i) a population switch towards phosphatase  
8 producers, which can be heterotrophic bacteria and phytoplankton, and ii) increases in  
9 specific activities due to enzymatic induction; or both. We used POP as a proxy of living  
10 biomass (Duhamel et al., 2007) to estimate specific activities (nmole MUF-P hydrolyzed per  
11 unit POP per unit time) and found the same trend for specific activities and for bulk APA (i.e.  
12 specific activity increased up to 10-fold). APA was produced by different phylogenetic groups  
13 of heterotrophic bacteria, but also by cyanobacteria, as shown on a metatranscriptomic study  
14 in the special issue (Pfreundt et al., 2016a), with the highest levels of alkaline phosphatase  
15 transcripts originating from *Synechococcus* on days 14 and 20. Our results and those of  
16 Pfreundt et al. (2016a) suggested a switch towards a microbial population that produced  
17 phosphatase to escape P depletion after a transient P-replete period. Although  $T_{DIP}$  decreased  
18 and APA increased up to values analogous to those observed in P-limited areas (Moutin et al.,  
19 2002; Van Wambeke et al., 2002, respectively), heterotrophic bacteria stayed continuously N-  
20 limited but not P-limited. As discussed in Pfreundt et al. (2016a) and Pfreundt et al. (2016b),  
21 some acquisition mechanisms of large P-containing organic molecules and reduction of  
22 cellular P quota also helped microbial communities to resist P depletion during P2 phase.

#### 24 **4.4. Cyanobacterial assimilation of leucine**

25 BP was used in this study as a strict proxy of heterotrophic bacterial production. As we  
26 incubated  $^3\text{H}$  leucine under light conditions, photoheterotrophic activity and the possibility  
27 that some photosynthetic cyanobacteria incorporate leucine could biases BP estimates.  
28 Whether light stimulation of bacterial production can be explained by direct (assimilation or  
29 organic molecules by autotrophs), or indirect effects (stimulation of BP through release of  
30 organic molecules or photo-labilization of organic matter), or both, is difficult to determine  
31 (Béjâ and Suzuki, 2008). Assimilation of methionine, leucine, and ATP was shown to be  
32 enhanced under light-incubation conditions in the North and South Atlantic Oceans and these  
33 increases are generally attributed to stimulation of *Prochlorococcus* and SAR11 (Evans et al.,  
34 2015), but the spectrum of organic molecules tested is low. In the New Caledonia lagoon,

Supprimé: 2

1 incubation of samples under different light regimes influences estimates of BP determined by  
2 the thymidine technique (Rochelle-Newall et al., 2008), but so far there is no information  
3 available on the light effect on leucine uptake around New Caledonia. The capacity of both  
4 marine *Prochlorococcus* and *Synechococcus* to assimilate some organic molecules is evident  
5 from culture-studies, as well as flow cytometry cell sorting and gene studies (Béjâ and  
6 Suzuki, 2008). Assimilation of leucine by cyanobacteria can also occur in the dark (Talarmin  
7 et al., 2011), but light clearly favours assimilation of leucine by cyanobacteria (Mary et al.,  
8 2008b). The polypropylene tubes used in this study to incubate BP attenuated the light  
9 intensity by 40 % without spectral distortion in the visible range (Richardson and Porter,  
10 2005). Under such conditions which were intermediary between simulated *in situ* light  
11 conditions and dark conditions, significant incorporation of leucine into macromolecules was  
12 seen by flow cytometry sorting of *Synechococcus* cells. We could not unambiguously verify  
13 leucine incorporation into *Prochlorococcus* cells due to technical reasons (low volumes and  
14 long storage limitations). To conclude, although the relative contribution of cyanobacteria  
15 (PRO + SYN) to the bulk (community) leucine assimilation into proteins was less than 2 %,  
16 and could not be responsible of a bias in BP estimates, we estimate significant potential for  
17 leucine to be assimilated by cyanobacteria. Note that we used a 10 nM leucine concentration  
18 for cell sorting, but *in situ* natural concentrations could be much lower. More studies are  
19 needed, investigating the potential use of other organic molecules in lower, close to *in-situ*  
20 concentrations. Mixotrophy may be the rule rather the exception in these experimental  
21 systems (Moore, 2013; Evans et al., 2015).

22

#### 23 **4.5 Phytoplankton-bacteria coupling and metabolic balance**

24 Torréton et al. (2010) report mean Chl concentration around  $0.3 \mu\text{g L}^{-1}$  over a seasonal cycle  
25 performed at an oligotrophic station in the New Caledonia lagoon. These concentrations are  
26 close to our reference conditions outside the mesocosms (lagoon waters), where means of Chl  
27 values were  $0.21$  and  $0.30 \mu\text{g L}^{-1}$  during P1 and P2 phases. Under these oligotrophic  
28 conditions, the seasonal variability of BP and PP in the Nouméa lagoon is much lower than in  
29 temperate waters, only about 3-fold with an average BP/PP ratio of 0.21, and some rare peaks  
30 reaching 0.6 (Torréton et al., 2010). This is in accordance with the range of BP/PP ratios  
31 encountered in the lagoon waters during our study, with average values ranging 0.33 to 0.39  
32 during phases P1 and P2, respectively. On the opposite, inside the mesocosms, average BP/PP  
33 ratios were generally higher than in the lagoon waters whatever the phase or the mesocosm  
34 considered. A negative trend of BP/PP with increasing PP was obtained, suggesting that a

Supprimé: 3



1 larger fraction of PP is channelled through the microbial food web when PP decreases, as  
2 observed in the oligotrophic Mediterranean Sea (Conan et al., 1999). This is also the trend  
3 when considering a large oceanic data set examined by Fouilland and Mostajir (2010). Indeed  
4 in their study, regression of  $\log(\text{BP})$  as a function of  $\log(\text{PP})$  resulted in a slope of 0.57,  
5 inferring that BP increases less rapidly than PP when PP increases. Contrarily to the weak  
6 (during P2) or absence of (during P1) correlation between BP and  $\text{N}_2$  fixation rates described  
7 above, strong relationships between BP and Chl, and between BP and PP were obtained  
8 during both phases (Fig. 4). This suggests that  $\text{N}_2$  fixation stimulated autotrophic communities  
9 during the VAHINE experiment, which may in turn have produced organic matter for  
10 heterotrophic prokaryotes. BP and PP are determined routinely, but to estimate the flux of PP  
11 channelled through heterotrophic bacteria, or to infer metabolic balance between autotrophy  
12 and heterotrophy, bacterial carbon demand (BCD) and gross primary production (GPP) must  
13 be also estimated. Such carbon fluxes are not directly estimated but are derived from PP and  
14 BP using additional measurements of bacterial growth efficiency (or bacterial respiration) and  
15 phytoplankton extracellular release of DOC. These parameters are less frequently acquired  
16 due to time-consuming and difficult technologies (del Giorgio and Cole, 1998; Nagata, 2000),  
17 which led to controversy on the metabolic balance in oligotrophic environments (Cole et al.,  
18 1988; Ducklow et al., 2002; Van Wambeke et al., 2008b; Fouilland and Mostajir, 2010;  
19 Moran and Alonso-Saez, 2010). BCD is derived from BP by the use of bacterial growth  
20 efficiency (BGE) or respiration rates which are not often measured concomitantly with PP and  
21 BP, and in many oligotrophic environments BCD/PP is higher than 1, or respiration exceeds  
22 PP (del Giorgio et al., 1997). To explain this, different arguments are generally proposed.  
23 First, other DOM sources than those deriving from phytoplankton (allochthonous sources) are  
24 used to sustain BCD. In [Pacific lagoons, excretion of mucus by coral has been proposed as a  
25 supplementary source of DOM for heterotrophic bacterioplankton \(Torréon et al., 2002, Wild  
26 et al., 2004\)](#). Second, phasing between BP and PP peaks during seasonal blooms and  
27 occasional presence of PP bursts (see for example Steinberg et al., 2001) are not always  
28 detected due to inappropriate sampling frequency for BP and PP measurements. Daily and  
29 parallel measurements of both BP and PP in the mesocosms avoided such problems in this  
30 study.  
31 Assuming negligible effect of [a biofilm development on the mesocosms walls \(Knapp et al.  
32 \(2015\)\)](#) on the plankton C budget, the main fate of photosynthetically fixed organic carbon  
33 during the experiment was respiration (71 % of GPP) then sedimentation (17 % of GPP). The  
34 different responses between the triplicate mesocosms led to a great propagation of errors and

Supprimé:

Supprimé: (

Supprimé:

Supprimé: 5

Supprimé: z

Supprimé: shallow

Supprimé: DOM released from benthos has been proposed as supplementary source of DOM for heterotrophic bacterioplankton (Torréon et al., 2002

Supprimé: We used the advantage of a day to day sampling in an enclosed system to compute a carbon budget that will allow to estimate the fate of phytoplankton-derived organic carbon and the metabolic balance. This carbon budget was calculated using time-integrated data, and thus considered the whole data set. First, each time point was averaged for the three sampling depths, and then time integration was calculated separately for each mesocosm assuming a linear trend between 2 successive days. A mesocosm average was calculated based on the time-integrated data obtained in each of the three mesocosms, with error bars representing the standard deviation (sd) among the three mesocosms (Fig. 7a). Gross primary production (GPP) is derived from PP assuming  $\text{GPP} = \text{PP} * 1.72$  (Moutin et al., 1999) and represents the whole photosynthetic source of organic matter, including both particulate and extracellular release forms. The cumulated GPP at day 23 was  $38 \pm 11 \mu\text{M C}$  (Fig. 7b). Carbon exported by sedimentation into the traps (Cexp) was corrected in  $\mu\text{M C}$  units based on a mean, constant water volume inside M1, M2, and M3 (see Berthelot et al., 2015 for details) and its cumulated value reached  $6.4 \pm 2.1 \mu\text{M C}$  on day 23. For POC and DOC, for which data were more irregular and showed outliers, we decided to calculate net variations of POC and DOC after a linear fit of the discrete data set between days 5 and 23 in each mesocosm (Table 4). POC increased linearly in M1 and M3 ( $0.12$  and  $0.48 \mu\text{mol C L}^{-1} \text{d}^{-1}$ ,  $r = 0.32$   $p < 0.03$  and  $r = 0.70$   $p < 0.001$ , respectively) and showed no trend in M2. A significant increase of DOC was only observed in M2 (Table 4). (...

Supprimé: this

Supprimé: (deduced from carbon budget)

1 thus on the variability on CR/GPP ratio ( $70 \pm 36 \%$ ). CR being lower than GPP, the biological  
2 system inside mesocosms was net autotrophic, with an upper error limit close to metabolic  
3 balance between autotrophy and heterotrophy.

4 In the lagoon, as close to Grande Rade Bay, long residence times favored local degradation,  
5 refractorisation of organic matter and not sedimentation (Mari et al., 2007). However, as these  
6 authors discussed, modification of phytoplankton community composition in Grande Rade  
7 Bay and the presence of metals could influence sticking properties of polymers. The  
8 confinement of the seawater inside the mesocosms probably favored to some extent the  
9 accumulation of UCYN-aggregates, as well as a possible reduction of grazing pressure (by a  
10 factor of 1.6) in the mesocosms compared to those in the lagoon waters (Turk-Kubo et al.,  
11 2015; Bonnet et al., 2015a; Hunt et al., 2016). However, UCYN-C formed large aggregates  
12 (100-500  $\mu\text{m}$ ) embedded in an organic matrix that included TEP, which were largely  
13 responsible for enhanced export flux through sedimentation observed during P2 (Berthelot et  
14 al., 2015; Berman-Frank et al., 2016; Knapp et al., 2015). TEP evolution with time, however,  
15 and the TEP-C to TOC ratio were similar in the lagoon waters, where wave turbulence and  
16 tidal effects were present, and in the enclosed mesocosms, where these hydrodynamics were  
17 reduced, and concentrations were similar (Berman-Frank et al., 2016). In an unconstrained  
18 ordination analysis, Pfreundt et al. (2016b) described significant differences in bacterial  
19 communities between M1 and the lagoon, but similar temporal dynamics. Direct comparisons  
20 of our export results with findings from open ocean studies should be made cautiously as our  
21 mesocosms were both shallower (15 m) than in typical oceanic export studies (> 100 m) and  
22 exhibited reduced turbulence,

Supprimé: ¶

23 In order to calculate the fraction of GPP that channeled, directly or indirectly, through the  
24 microbial food web, the bacterial carbon demand (BCD) must be estimated through additional  
25 estimates of bacterial respiration (BR) or bacterial growth efficiency (BGE). In an  
26 oligotrophic site inside the Nouméa lagoon, BGE was estimated at 10 % using incubated  
27 samples where oxygen changes were followed with time in the dark (Briand et al., 2004).  
28 However, as suggested by Aranguren-Gassis et al. (2012), using consistently low BGE  
29 derived from size fractionation experiments and long-duration incubations leads to probable  
30 BGE underestimation. In the lagoon, the use of a 10 % BGE would lead to BCD values higher  
31 than GPP (Rochelle-Newall et al., 2008). If we assume such low BGE in the mesocosms (10  
32 %), cumulated BR from day 5 to 23 would rise to  $93 \pm 6 \mu\text{M C}$ , which is not realistic  
33 compared to CR estimated from the carbon budget. A more appropriate BGE of  $27 \pm 9\%$ , to  
34  $43\% \pm 11\%$ , could be calculated, based on minimum and maximum ranges admitted for

Supprimé:

Supprimé:

1 BR/CR ratio (from 100 to 50 %, Lemée et al., 2002), and based on propagation of errors due  
2 to the variability within triplicate mesocosms (Table S1). For sensitivity analysis of BR and  
3 BGE calculation from the carbon budget, we examined whether the errors of different  
4 methodological assumptions (conversion factors, analytical errors) were higher than those  
5 arising from variability between triplicate mesocosms. We considered different errors based  
6 on literature data for all the parameters used in computation of BR and BGE (i.e. GPP, Cexp,  
7 DOC, POC, and BP). For GPP, we assumed  $GPP = 1.72 \times PP$ , i.e. PP/GPP ratio = 58 %. In  
8 the South Pacific Ocean, the mean average PP to GPP ratio (based on comparison between  
9 oxygen and  $^{14}C$  technique) was 47% (Van Wambeke et al., 2008b). If we consider that the  
10 sum of dissolved and particulate PP in the lagoon ( $^{14}C$  technique, Rochelle-Newall et al.,  
11 2008) is a good proxy of GPP, then an upper limit for this ratio is 65 % in the lagoon. We thus  
12 applied a 15 % variability to the PP/GPP ratio, leading to  $GPP=1.36 \times PP$  to  $2.32 \times PP$ , i.e.  
13 approximately a 30 % variability on the conversion factor. For BP, we assumed a 25 % daily  
14 variability of BP (Church et al., 2006, Van Wambeke et al., 2008c, Torréton et al., 2010). For  
15 Cexp, DOC and POC, we assumed analytical errors of 10 %. We then used propagation of  
16 errors to compute the error associated with BR and BGE (Table S1). For GPP, the errors  
17 resulting from triplicate mesocosms or resulting from conversion factor and analytical errors  
18 were the same. According to the propagation of errors, the error associated with GPP has the  
19 largest effect on estimates of BR. The uncertainty of DOC, POC, and Cexp arising from  
20 variability within the triplicate mesocosms is higher than the methodological error, whereas it  
21 is the opposite for BP. Overall, the uncertainty of BGE estimates arising from variability  
22 within triplicate mesocosms or methodology is similar ( $27 \% \pm 9 \%$  or  $27 \% \pm 10 \%$  for BGE  
23 based on  $BR=CR$ ,  $43 \% \pm 11 \%$  or  $43 \% \pm 12 \%$  for BGE based on  $BR=CR/2$ )  
24 The BGE values values determined from C budget could be potentially related to a beneficial  
25 effect of photoheterotrophy. Indeed, in a companion metatranscriptomic study performed in  
26 M1 (Pfreundt et al., 2016a), accumulation of proteorhodopsin transcripts was recurrently  
27 detected among varying groups of bacteria notably Pelagibacteraceae, and SAR86. These  
28 groups, belonging to the alpha- and gammaproteobacteria, respectively, were also abundant  
29 community members as observed through 16S sequencing (Pfreundt et al., 2016b). Aerobic  
30 anoxygenic phototrophic (AAP) bacterial abundances are reported to be particularly abundant  
31 in the South Pacific Ocean (Lami et al. 2007), but to date, AAP abundances are not available  
32 in the lagoon and they were not counted in this experiment. Nevertheless, Pfreundt et al.  
33 (2016a) detected expression of the *pufM* gene, encoding a photoreaction centre protein of  
34 AAP bacteria. Transcript abundances were an order of magnitude lower than for

**Supprimé:** Such

**Supprimé:** are more reliable than BR estimates based on Electron Transport System measurements (Aranguren-Gassis et al., 2012) and are in agreement with the

**Supprimé:** -

**Supprimé:**

**Supprimé:** Indeed, the BGE of a proteorhodopsin-containing strain (*Erythrobacter* sp.) was shown to increase during light periods in a continuous culture (Hauruseau et al., 2012).

**Supprimé:** I

**Supprimé:** SAR11

**Supprimé:** (Pfreundt et al., 2015a).

**Supprimé:** also played key roles in the microbial community as observed through 16S sequencing

**Supprimé:** 2015b

1 proteorhodopsin, and only observed for a group of *Rhodocyclaceae* on day 14 and much  
2 weaker for *Rhodobacteraceae* on day 18. This suggests that AAP bacteria did not play a  
3 major role in the investigated system and did not influence the above calculation to a large  
4 extent. *Dokdonia* sp. strain MED134, proteorhodopsin containing flavobacteria, were shown  
5 to increase the maximum number of cells reached when growing in the light compared to  
6 darkness. However, if DOM was added initially, light vs dark responses changed depending  
7 on DOM concentrations (Gomez-Consarnau et al., 2007). Other laboratory experiment, at the  
8 opposite, showed no difference in growth rates or maximum cell yields of *Pelagibacter*  
9 *ubique* cultures grown in natural seawater (in in a diurnal light regime or in complete darkness  
10 (Giovannoni et al., 2005). The BGE of a bacteriochlorophyll-containing strain (*Erythrobacter*  
11 sp.) was shown to increase during light periods in a continuous culture (Hauruseau and  
12 Koblížek, 2012). Thus the energy benefits of photoheterotrophy remain controversial, and  
13 related to the difficulty to have true oligotrophic conditions in pure culture. Based on an  
14 energy budget, Kirchman and Hanson (2013) suggested that the net energy gained by light is  
15 mostly sufficient to meet maintenance cost of AAP but is not enough to meet that of  
16 proteorhodopsin-based phototoheterotrophic bacteria. Heterotrophic bacteria are limited by N  
17 but also by energy in the South Pacific (Van Wambeke et al. 2008a); this could give an  
18 advantage to photoheterotrophic prokaryotes for growth and their success in this area.  
19 Assuming BGE values ranging from 27 to 43%, the BCD/GPP ratio would range from 63 to  
20 99 %. A large part of the GPP is thus channelled through the microbial food web pathway  
21 within 20 days. To examine potential links between phytoplankton release and BP, we  
22 estimated a extracellular release of 35 %, as determined previously inside the Nouméa lagoon  
23 (Rochelle-Newall et al., 2008). Such values are in agreement with higher percent extracellular  
24 release that are generally obtained in nutrient limited environments (Nagata, 2000). According  
25 to Rochelle-Newall et al. (2008), contemporaneous DOC excreted by phytoplankton was  
26 sufficient to meet BCD only in the coastal part of the lagoon, but not in the offshore  
27 oligotrophic part of the lagoon where the VAHINE experiment was performed, but these  
28 authors used a 10 % BGE. In the mesocosms, still based on a extracellular release  
29 representing 35 % of GPP, DOC release was estimated at 13  $\mu\text{M}$  C produced between 5-23  
30 days. This is not sufficient to satisfy BCD cumulated for the same period (calculated as 24-38  
31  $\mu\text{M}$  C) although we used BGE varying from 27 to 43 % as discussed above. Thus,  
32 heterotrophic bacteria in the mesocosms used additional, not contemporaneous, sources of  
33 organic matter derived from phytoplankton after transformation through the food web like  
34 enzymatic hydrolysis of detritus, viral lysis, and/or sloppy feeding.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34

## Conclusions

This study confirms that in the Nouméa lagoon, N<sub>2</sub> fixation is a relevant process to fuel the microbial food web and to sustain a biological system which is net autotrophic or close to metabolic balance. The relatively high BGE computed from the carbon budget (27-43 %) could be related to light-harvesting systems developed by abundant bacterial photoheterotrophs. The success of *Synechococcus* over *Prochlorococcus* described in companion papers (Leblanc et al., 2015; Pfreundt et al., 2016b) might be attributed to their ability to assimilate leucine, and possibly other amino acids, as well as reduction of their cellular P demand through sulfolipid synthesis. The relative importance of mixotrophy in these oligotrophic system implies that it is important to i) find alternative techniques to dark incubations to estimate bacterioplankton respiration, like with continuous measurements with oxygen microprobes during alternate light and dark periods (Pringault et al., 2007) and ii) to detect organisms responsible for the assimilation of a wide variety of organic molecules by cell sorting. From the carbon budget, a BCD to GPP ratio was estimated to range between 63 and 100 %, and thus a large part of the primary production is channelled through the microbial food web. Bacterial production was strongly coupled with Chl biomass and/or PP, rather than with N<sub>2</sub> fixation rates, suggesting that indirect routes through lysis, grazing and mortality of phytoplankton were substantial for providing labile organic matter for heterotrophic bacteria.

**Author contributions** SB was the chief scientist responsible of the VAHINE program, she designed and executed the experiment in mesocosms. FV sampled for and analyzed BP and APA, TM sampled for and analyzed T<sub>DIP</sub> and PP, AB performed the cell sorting, wrote the corresponding M&M section and made Fig. 2, HB contributed to the analyses of bacterial abundances by flow cytometry, MR sampled for and analyzed data, FV and UP equally wrote the manuscript and made the other figures. All the authors reviewed the manuscript.

**Acknowledgements** Funding for this research was provided by the Agence Nationale de la Recherche (ANR starting grant VAHINE ANR-13-JS06-0002), INSU-LEFE-CYBER program, GOPS, IRD and M.I.O. The participation of UP and WRH was supported by the German-Israeli Research Foundation (GIF), project number 1133-13.8/2011 and the MiSeq-based microbial community analysis by the EU project MaCuMBA (Marine Microorganisms: Cultivation Methods for Improving their Biotechnological Applications; grant agreement no:

Supprimé: 2015b

Supprimé: . Moreover, as indicated by the high expression of the sulfolipid biosynthesis gene *sqdB* (Pfreundt et al., 2015a), these cyanobacteria are able to

Supprimé: e

Mis en forme : Indice

1 311975) to WRH. The authors thank the captain and crew of the R/V Alis. We acknowledge  
2 the SEOH divers service from the IRD research center of Nouméa (E. Folcher, B. Bourgeois  
3 and A. Renaud) and from the Observatoire Océanologique de Villefranche-sur-mer (OOV,  
4 J.M. Grisoni) as well as the technical service of the IRD research center of Nouméa for their  
5 helpful technical support. C. Guieu, F. Louis and J.M. Grisoni from OOV are warmly thanked  
6 for the mesocosms design and their useful advice for deployment. We are grateful to the  
7 Regional Flow Cytometry Platform for Microbiology (PRECYM) of the Mediterranean  
8 Institute of Oceanography (MIO) for the flow cytometry analyses. We acknowledge Anne  
9 Desnues for help in sampling, Karine Leblanc, Bruno Charrière, Jules Héliou for analyzing  
10 TOC, POC and Chl data and four referees which helped to improve the ms.

Supprimé: and

## 1 References

2 [Béjà, O. and Suzuki, M.: Photoheterotrophic marine prokaryotes, in: Microbial Ecology of the](#)  
3 [Oceans, Second Edition, Kirchman, D. \(Ed.\), John Wiley & Sons, Inc, 131-157, 2008.](#)

4 [Berman-Frank, I., Spungin, D., Rahav, E., Van Wambeke, F., Berthelot, H., Turk-Kubo, K.,](#)  
5 [Bonnet, S. and Moutin T.: Dynamics of Transparent exopolymer particles \(TEP\) during a](#)  
6 [mesocosm experiment in the New Caledonia lagoon, Biogeosciences Discuss.,](#)  
7 [doi:10.5194/bg-2015-613, 2016,](#)

8 [Berthelot, H., Moutin, T., L'Helguen, S., Leblanc, K., Hélias, S., Grosso, O., Leblond, N.,](#)  
9 [Charrière, B., and Bonnet, S.: Dinitrogen fixation and dissolved organic nitrogen fueled](#)  
10 [primary production and particulate export during the VAHINE mesocosm experiment](#)  
11 [\(New Caledonia lagoon\), Biogeosciences, 12, 4099–4112, doi:10.5194/bg-12-4099-2015,](#)  
12 [2015,](#)

13 [Biegala, I., and Raimbault, P.: High abundance of diazotrophic pico-cyanobacteria \(< 3µm\)](#)  
14 [in a south-west Pacific coral lagoon, Aquat. Microb. Ecol., 51, 45–53, 2008.](#)

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

19 [Bonnet, S., Guieu, C., Bruyant, F., Prášil, O., Van Wambeke, F., Raimbault, P., Moutin, T.,](#)  
20 [Grob, C., Gorbunov, M. Y., Zehr, J. P., Masquelier, S. M., Garczarek, L., and Claustre, H.:](#)  
21 [Nutrient limitation of primary productivity in the Southeast Pacific \(BIOSOPE cruise\),](#)  
22 [Biogeosciences, 5, 215-225, doi:10.5194/bg-5-215-2008, 2008.](#)

23 [Bonnet, S., Berthelot, H., Turk-Kubo, K., Fawcett, S., Rahav, E., Berman-Frank, I., and](#)  
24 [l'Helguen, S.: Dynamics of N<sub>2</sub> fixation and fate of diazotroph-derived nitrogen during the](#)  
25 [VAHINE mesocosm experiment \(New Caledonia\), Biogeosciences Discuss., 12, 19579-](#)  
26 [19626, doi:10.5194/bgd-12-19579-2015, 2015a](#)

27 [Bonnet, S., Moutin, T., Rodier, M., Grisoni, J.M., Louis, F., Folcher, E., Bourgeois, B., Boré](#)  
28 [J.M., and Renaud, A.: Introduction to the VAHINE project: VARIability of vertical and](#)  
29 [tropHic transfer of fixed N<sub>2</sub> in the south wEst Pacific, Biogeosciences Discuss.,](#)  
30 [doi:10.5194/bg-2015-615, 2016,](#)

31 [Bonnet, S., Rodier, M., Turk-Kubo, K., Germineaud, C., Menkes, C., Ganachaud, A.,](#)  
32 [Cravatte, S., Raimbault, P., Campbell, E., Quéroué, F., Sarthou, G., Desnues, A., Maes, C.,](#)  
33 [and Eldin, G.: Contrasted geographical distribution of N<sub>2</sub> fixation rates and nifH](#)

**Supprimé:** Aranguren-Gassis, M., Teira, E., Serret, P., Martínez-García, M., and Fernández, E.: Potential overestimation of bacterial respiration rates in oligotrophic plankton communities, Mar. Ecol. Prog. Ser., 453, 1-10, 2012.¶

**Supprimé:** this issue (bg2015-612), 2015

**Supprimé:** 2015.

**Mis en forme :** Police :

**Supprimé:** Biogeosciences Discuss., this issue (bg2015-578), 2015a.

**Supprimé:**

**Supprimé:**

**Supprimé:**

**Supprimé:** this issue (bg2015-615), 2015b

1 phylotypes in the Coral and Solomon Seas (South-Western Pacific) during austral winter  
2 conditions, *Glob. biogeochem. Cycles*, 29, DOI: 10.1002/2015GB005117, 2015b.

3 Briand, E., Pringault, O., Jacquet, S., and Torrétion, J.-P.: The use of oxygen microprobes to  
4 measure bacterial respiration for determining bacterioplankton efficiency, *Limnol.*  
5 *Oceanogr.:Methods*, 2, 406-416, 2004.

6 Cole, J. J., Findlay, S., and Pace, M. L.: Bacterial production in fresh and saltwater  
7 ecosystems : a cross - system overview, *Mar. Ecol. Prog. Ser.*, 43, 1-10, 1988.

8 Conan, P., Turley, C., Stutt, E., Pujo-Pay, M., and Van Wambeke, F.: Relationship between  
9 phytoplankton efficiency and the proportion of bacterial production to primary production  
10 in the Mediterranean Sea, *Aquat. Microb. Ecol.*, 17, 131-144, 1999.

11 [Church, M. J., Ducklow, H. W., Letelier, R. M., and Karl, D. M.: Temporal and vertical](#)  
12 [dynamics in picoplankton photoheterotrophic production in the subtropical North Pacific](#)  
13 [Ocean. Aquat. Microb. Ecol.](#), 45, 41-53, 2006.

14 del Giorgio, P. A. and Cole, J. J.: Bacterial growth efficiency in natural aquatic systems., *Ann.*  
15 *Rev. Ecol. Syst.*, 29, 503-541, 1998.

16 del Giorgio, P., Cole, J. J., and Cimleris, A.: Respiration rates in bacteria exceeds  
17 phytoplankton production in unproductive aquatic systems, *Nature*, 385, 148-151, 1997.

18 Ducklow, H. W., Kirchman, D. L., and Anderson, T. R.: The magnitude of spring bacterial  
19 production in the North Atlantic Ocean, *Limnol. Oceanogr.*, 47, 1684-1693, 2002.

20 [Duhamel, S., Moutin, T., Van Wambeke, F., Van Mooy, B. A., Rimmelin, P., Raimbault, P.,](#)  
21 [and Claustre, H.: Growth and specific P-uptake rates of bacterial and phytoplanktonic](#)  
22 [communities in the Southeast Pacific \(BIOSOPE cruise\). Biogeosciences](#), 4, 941-956,  
23 [2007.](#)

24 Evans, C., Gómez-Pereira, P. R., Martin, A. P., Scanlan, D., and Zubkov, M. V.:  
25 Photoheterotrophy of bacterioplankton is ubiquitous in the surface oligotrophic ocean,  
26 *Prog. Oceanogr.*, 135, 139-145, 2015.

27 [Fichez, R., Chifflet, S., Douillet, P., Gérard, P., Gutierrez, F., Jouon, A., Ouillon, S., and](#)  
28 [Grenz, C.: Biogeochemical typology and temporal variability of lagoon waters in a coral](#)  
29 [reef ecosystem subject to terrigenous and anthropogenic inputs \(New Caledonia\), Mar.](#)  
30 [Poll. Bull.](#), 61, 309-322, 2010

31 Fouilland, E., and Mostajir, B.: Revisited phytoplanktonic carbon dependency of heterotrophic  
32 bacteria infreshwaters, transitional, coastal and oceanic waters, *FEMS Microbiol. Ecol.*,  
33 73, 419–429, 2010.

Supprimé: cycles

Supprimé: 2015c



- 1 Fukuda, R., Ogawa, H., Nagata, T., and Koike, I.: Direct determination of carbon and nitrogen  
2 contents of natural bacterial assemblages in marine environments., *Appl. Environ.*  
3 *Microbiol.*, 64, 3352-3358, 1998.
- 4 Garcia, N., Raimbault, P., and Sandroni, V.: Seasonal nitrogen fixation and primary  
5 production in the Southwest Pacific: nanoplankton diazotrophy and transfer of nitrogen to  
6 picoplankton organisms, *Mar. Ecol. Prog. Ser.*, 343, 25-33, 2007.
- 7 [Giovannoni, S. J., Bibbs, L., Cho, J. C., Stapels, M. D., Desiderio, R., Vergin, K. L., Michael](#)  
8 [S. Rappe, M. S., Laney, S., Wilhelm, L. J., Tripp, H. J., Mathur, E. J., and Barofsky, D. F.:](#)  
9 [Proteorhodopsin in the ubiquitous marine bacterium SAR11, \*Nature\*, 438, 82–85, 2005.](#)
- 10 [Gomez-Consarnau, L., Gonzalez, J. M., Coll-Llado, M., Gourdon, P., Pascher, T., Richard](#)  
11 [Neutze, R., Carlos Pedros-Alio, C., and Pinhassi, J.: Light stimulates growth of](#)  
12 [proteorhodopsin containing marine Flavobacteria, \*Nature Letters\*, 445, 2007.](#)
- 13 Gimenez, A., Baklouti, M., Bonnet, S., Moutin T.: Impact of a phosphate enrichment on  
14 biogeochemical fluxes and fate of diazotroph derived nitrogen: Modelling of the VAHINE  
15 mesocosms experiment, *Biogeosciences Discuss.*, [doi:10.5194/bg-2015-611, 2016](#),
- 16 Guieu, C., Dulac, F., Desboeufs, K., Wagener, T., Pulido-Villena, E., Grisoni, J.-M., Louis,  
17 F., Ridame, C., Blain, S., Brunet, C., Bon Nguyen, E., Tran, S., Labiadh, M., and  
18 Dominici, J.-M.: Large clean mesocosms and simulated dust deposition: a new  
19 methodology to investigate responses of marine oligotrophic ecosystems to atmospheric  
20 inputs, *Biogeosciences*, 7, 2765– 2784, 2010.
- 21 Halm, H., Lam, P., Ferdelman, T.G., Lavik, G., Dittmar, T., LaRoche, J., D'Hondt, S., and  
22 Kuypers, M.: Heterotrophic organisms dominate nitrogen fixation in the South Pacific  
23 Gyre, *The ISME Journal*, 6, 1238-1249, 2012.
- 24 Hauruseau, D., and Koblížek, M.: Influence of Light on Carbon Utilization in Aerobic  
25 Anoxygenic Phototrophs, *Appl. Environ. Microbiol.*, 78, 7414-7419, 2012.
- 26 Hoppe, H. G.: Significance of exoenzymatic activities in the ecology of brackish water:  
27 measurement by means of methylumbelliferyl-substrates, *Mar. Ecol. Prog. Ser.*, 11, 299-  
28 308, 1983.
- 29 [Hunt, B. P. V., Bonnet, S., Berthelot, H., Conroy, B. J., Foster, R. A., and Pagano, M.:](#)  
30 [Contribution and pathways of diazotroph derived nitrogen to zooplankton during the](#)  
31 [VAHINE mesocosm experiment in the oligotrophic New Caledonia lagoon,](#)  
32 [Biogeosciences Discuss.](#), [doi:10.5194/bg-2015-614, in review, 2016.](#)

Supprimé: this issue (bg2015-611),  
2015

- 1 Kirchman, D. L.: Leucine incorporation as a measure of biomass production by heterotrophic  
2 bacteria. In: Handbook of methods in aquatic microbial ecology, Kemp, P. F., Sherr, B. F.,  
3 Sherr, E. B., and Cole, J. J. (Eds.), Lewis, Boca Raton, 509-512, 1993.
- 4 [Kirchman, D. L. and Hanson, T. E.: Bioenergetics of photoheterotrophic bacteria in the](#)  
5 [oceans, \*Environmental microbiology reports\*, 5, 188-199, 2013.](#)
- 6 Knapp, A. N., Fawcett, S. E., Martinez-Garcia, A., Haug, G., Leblond, N., Moutin, T., and  
7 Bonnet, S.: Nitrogen isotopic evidence for a shift from nitrate- to diazotroph-fueled export  
8 production in VAHINE mesocosm experiments, [Biogeosciences Discuss.](#), 12, 19901-  
9 19939, doi:10.5194/bgd-12-19901-2015, 2015,
- 10 [Lami, F., Cottrell, M., Ras, J., Ulloa, O., Obernosterer, I., Claustre, H., Kirchman, D., and](#)  
11 [Lebaron, P.: High abundances of aerobic anoxygenic photosynthetic bacteria in the South](#)  
12 [Pacific ocean., \*Appl. Environ. Microbiol.\*, 73, 4198-4205, 2007.](#)
- 13 Leblanc, K., Cornet, V., Caffin, M., Rodier, M., Desnues, A., Berthelot, H., Heliou, J.,  
14 Bonnet, S.: Phytoplankton community structure in the VAHINE MESOCOSM experiment.  
15 *Biogeosciences Discuss*, doi:10.5194/bg-2015-605, 2016
- 16 Lemée, R., Rochelle-Newall, E., Van Wambeke, F., Pizay, M.-D., Rinaldi, P., and Gattuso,  
17 J.-P.: Seasonal variation of bacterial production, respiration and growth efficiency in the  
18 open NW Mediterranean Sea, *Aquat. Microb. Ecol.*, 29, 227-237, 2002.
- 19 [Mari X., Lefèvre J., Torrétón J-P., Bettarel Y., Pringault O., Rochelle-Newall E.,](#)  
20 [Marchesiello P., Menkes C., Rodier M., Migon C., Motegi C., Weinbauer M.G., Legendre](#)  
21 [L.: Effects of soot deposition on particle dynamics and microbial processes in marine](#)  
22 [surface waters. \*Glob. Biogeochem. Cycles\*, 2014GB004878, 2014](#)
- 23 [Mari, X., Rochelle-Newall, E., Torretón, J., Pringault, O., Jouon, A., and Migon, C.: Water](#)  
24 [residence time: A regulatory factor of the DOM to POM transfer efficiency., \*Limnol.\*](#)  
25 [Oceanogr.](#), 52, 808-819, 2007.
- 26 Marie, D., Partenski, F., Jaquet, S., and Vaultot, D.: Enumeration and cell cycle analysis of  
27 natural population of marine picoplankton by flow cytometry using the nucleic acid stain  
28 SYBR green I, *Appl. Environ. Microbiol.*, 63, 186-193, 1997.
- 29 [Martínez-Martínez, J., Norland, S., Thingstad T.F., Schroeder, D.C. Bratbak G., Wilson,](#)  
30 [W.H., Larsen, A.: Variability in microbial population dynamics between similarly](#)  
31 [perturbed mesocosms. \*J. Plankton Res.\*, 28, 8, 783-791, doi: 10.1093/plankt/fbl010, 2006.](#)
- 32 Mary, I., Garczarek, L., Tarran, G. A., Kolowrat, C., Terry, M. J., Scanlan, D. J., Burkill, P.  
33 H., and Zubkov, M. V.: Diel rhythmicity in amino acid uptake by *Prochlorococcus*,  
34 *Environ. Microb*, 10, 2124-2131, 2008a.

Supprimé: Biogeosciences  
Discussions, this issue (bg2015-576),  
2015

Supprimé: this issue (bg2015-605),

Supprimé: 5

- 1 Mary, I., Tarran, G. A., Warwick, P. E., Terry, M. J., Scanlan, D. J., Burkill, P. H., and  
2 Zubkov, M. V.: Light enhanced amino acid uptake by dominant bacterioplankton groups in  
3 surface waters of the Atlantic Ocean, *FEMS Microbiol. Ecol.*, 63, 36-45, 2008b.
- 4 Moisander, P. H., Beinart, R. A., Hewson, I., White, A. E., Johnson, K. S., Carlson, C. A.,  
5 Montoya, J. P., and Zehr, J. P.: Unicellular Cyanobacterial Distributions Broaden the  
6 Oceanic N<sub>2</sub> Fixation Domain, *Science*, 327, 1512-1514, 2010.
- 7 Moore, L. R.: More mixotrophy in the marine microbial mix, *Proc. Natl. Acad. Sci. USA*,  
8 110, 8323–8324, 2013.
- 9 Moran, X. A. and Alonso-Saez, L.: Independence of bacteria on phytoplankton? Insufficient  
10 support for Fouilland & Mostajir's (2010) suggested new concept, *FEMS Microb. Ecol.*,  
11 78, 203-205, 2011.
- 12 Moutin, T., Karl, D. M., Duhamel, S., Rimmelin, P., Raimbault, P., Van Mooy, B. A. S., and  
13 Claustre, H.: Phosphate availability and the ultimate control of new nitrogen input by  
14 nitrogen fixation in the tropical Pacific Ocean, *Biogeosciences*, 5, 95–109, doi:10.5194/bg-  
15 5-95-2008, 2008.
- 16 Moutin, T., Raimbault, P., and Poggiale, J. C.: Production primaire dans les eaux de surface  
17 de la Méditerranée occidentale : Calcul de la production journalière, *C. R. Acad. Sci. Paris*,  
18 *Sciences de la vie*, 322, 651–659, 1999.
- 19 [Moutin, T., Thingstad, T.F., Van Wambeke, F., Marie, D., Slawyk, G., Raimbault P., Claustre,](#)  
20 [H.: Does competition for nano-molar phosphate supply explain the predominance of the](#)  
21 [cyanobacterium \*Synechococcus\*? \*Limnol. Oceanogr.\*, 47, 1562-1567, 2002.](#)
- 22 Moutin, T., Van Den Broeck, N., Becker, B., Dupouy, C., Rimmelin, P., and Le Bouteiller,  
23 A.: Phosphate availability controls *Trichodesmium* spp. biomass in the SW Pacific Ocean,  
24 *Mar. Ecol. Prog. Ser.*, 297, 15–21, doi:10.3354/meps297015, 2005.
- 25 Nagata, T.: Production mechanisms of dissolved organic matter, in: *Microbial Ecology of the*  
26 *Oceans*, Kirchman, D. (Ed.), Wiley Liss, 121-152, 2000.
- 27 Neveux, J., Lefebvre, J-P., Le Gendre, R., Dupouy, C., Gallois, F., Courties, C., Gérard, P.,  
28 Fernandez, J-M., Ouillon, S.: Phytoplankton dynamics in the southern New Caledonian  
29 lagoon during a southeast trade winds event, *J. Mar. Syst.*, 82, 230–244., 2010.
- 30 Pfreundt, U., Spungin, D., Bonnet, S., Berman-Frank, I., and Hess, W.R.: Global analysis of  
31 gene expression dynamics within the marine microbial community during the VAHINE  
32 mesocosm experiment in the South West Pacific, *Biogeoscience Discuss.*, doi:10.5194/bg-  
33 [2015-564, 2016a](#),

Supprimé: -

Supprimé: this issue (bg2015-564),  
2015a.

- 1 Pfreundt, U. Van Wambeke, F., Bonnet S., and Hess, W.R.: Succession within the prokaryotic  
2 diversity during the VAHINE mesocosms experiment in the New Caledonia lagoon,  
3 [Biogeosciences, 13, 2319-2337, doi:10.5194/bg-13-2319-2016, 2016b](#),
- 4 [Pringault, O., Tassas, V., and Rochelle-Newall, E.: Consequences of respiration in the light on](#)  
5 [the determination of production in pelagic systems. Biogeosciences, 4, 105-114, 2007.](#)
- 6 [Pulido-Villena, E., Baudoux, A.-C., Obernosterer, I., Landa, M., Caparros, J., Catala, P.,](#)  
7 [Georges, C., Harmand, J., and Guieu, C.: Microbial food web dynamics in response to a](#)  
8 [Saharan dust event: results from a mesocosm study in the oligotrophic Mediterranean Sea,](#)  
9 [Biogeosciences, 11, 5607-5619, doi:10.5194/bg-11-5607-2014, 2014.](#)
- 10 [Regaudie-de-Gioux, A., Lasternas, S., Agustí, S., and Duarte, C. M.: Comparing marine](#)  
11 [primary production estimates through different methods and development of conversion](#)  
12 [equations, Frontiers in Marine Science, 1, article 19, doi: 10.3389/fmars.2014.00019, 2014.](#)
- 13 Richardson, T. B., and Porter, C. D.: Inactivation of murine leukaemia virus by exposure to  
14 visible light, *Virology*, 341, 321 – 329, 2005.
- 15 Rochelle-Newall, E. J., Torréron, J.-P., Mari, X., and Pringault, O.: Phytoplankton–  
16 bacterioplankton coupling in a subtropical South Pacific coral reef lagoon, *Aquat. Microb.*  
17 *Ecol.*, 50, 221-229, 2008,
- 18 Sieracki, M. E., Haugen, E. M., and Cucci, T. L.: Overestimation of heterotrophic bacteria in  
19 the Sargasso Sea: direct evidence by flow and imaging cytometry. *Deep Sea Res.*, I, 42,  
20 1399-1409, 1995.
- 21 Steinberg, D. K., Carlson, C. A., Bates, N. R., Johnson, R. J., Michaels, A. F., and Knap, A.  
22 H.: Overview of the US JGOFS Bermuda Atlantic Time-series Study (BATS): a decade-  
23 scale look at ocean biology and biogeochemistry, *Deep-Sea Res.* II, 48, 1405-1447, 2001.
- 24 Smith, D. C. and Azam, F.: A simple, economical method for measuring bacterial protein  
25 synthesis rates in sea water using <sup>3</sup>H-Leucine, *Mar. Microb. Food Webs*, 6, 107-114, 1992.
- 26 [Steeman-Nielsen, E.: The use of radioactive carbon \(14C\) for measuring production in the](#)  
27 [sea, J. Cons. Perm. Int. Explor. Mer, 18, 117–140, 1952.](#)
- 28 Talarmin, A., Van Wambeke, F., Catala, P., Courties, C., and Lebaron, P.: Flow cytometric  
29 assessment of specific leucine incorporation in the open Mediterranean, *Biogeosciences*, 8,  
30 253-265, 2011.
- 31 Torréron, J. P., Pages, J., and Talbot, V.: Relationships between bacterioplankton and  
32 phytoplankton biomass, production and turnover rate in Tuamotu atoll lagoons, *Aquat.*  
33 *Microb. Ecol.*, 28, 267-277, 2002.

Supprimé: Biogeoscience discuss.,  
this issue (bg2015-600), 2015b

Supprimé: b

Supprimé: T

1 Torréron, J.-P., Rochelle Newall, E., Pringault, O., Jacquet, S., Faure, V., and Brand, E.:  
 2 Variability of primary and bacterial production in a coral reef lagoon (New Caledonia),  
 3 *Mar. Poll. Bull.*, 61, 335-348, 2010.

4 Turk-Kubo, K. A., Berman-Frank, I., Hogan, M. E., Desnues, A., Bonnet, S., and Zehr, J. P.:  
 5 Diazotroph community composition during the VAHINE mesocosms experiment (New  
 6 Caledonia lagoon), *Biogeosciences*, 12, 7425-7452, doi:10.5194/bg-12-7435-2015, 2015.

7 Van Wambeke, F., Bonnet, S., Moutin, T., Raimbault, P., Alarçon, G., and Guieu, C.: Factors  
 8 limiting heterotrophic bacterial production in the southern Pacific Ocean, *Biogeosciences*,  
 9 5, 833-845, 2008a.

10 Van Wambeke, F., Obernosterer, I., Moutin, T., Duhamel, S., Ulloa, O., and Claustre, H.:  
 11 Heterotrophic bacterial production in the eastern South Pacific: longitudinal trends and  
 12 coupling with primary production, *Biogeosciences*, 5, 157-169, 2008b.

13 Van Wambeke, F., Tedetti, M., Duhamel, S., and Sempéré, R.: Diel variability of  
 14 heterotrophic bacterial production and underwater UV doses in the eastern South Pacific  
 15 *Mar. Ecol. Prog. Ser.*, 387, 97-108, 2008c.

16 Wild C., Rasheed M., Werner U., Franke U., Johnstone R., Huettel M.: Degradation and  
 17 mineralization of coral mucus in reef environments, *Mar. Ecol. Prog. Ser.*, 267, 159-171,  
 18 2004.

19 Zubkov, M. V., Tarran, G. A., and Fuchs, B. M.: Depth related amino acid uptake by  
 20 *Prochlorococcus cyanobacteria* in the southern Atlantic tropical gyre, *FEMS Microb. Ecol.*,  
 21 50, 153-161, 2004.

22

Supprimé: ution

Supprimé: etin

Supprimé: Discuss.

Supprimé: 9043-9079

Supprimé: 2015.

1 | Table 1 Averages  $\pm$  standard deviations of some parameters during phases P1 (from day 5 to  
2 | day 14) and P2 (from day 15 to day 23) in the three mesocosms M1, M2, M3 and in the  
3 | lagoon waters. HBA: heterotrophic prokaryotic abundances, BP: heterotrophic prokaryotic  
4 | production, AOA: alkaline phosphatase activity, TDIP: turnover time of DIP. N<sub>2</sub> fixation  
5 | contribution to BP (N<sub>2</sub>fix/BP ratio, in %) is based on a C/N of 6.8 for heterotrophic bacteria.  
6 | Mann - Whiney tests were performed to test significant differences between P1 and P2: \* p <  
7 | 0.05; \*\* 0.01 < p < 0.05; \*\*\* p < 0.001.

8

	M1 P1	M1 P2	<u>Lagoon</u> P1	<u>Jagoon</u> P2
Chl ( $\mu\text{g L}^{-1}$ )	0.19 $\pm$ 0.05 ***	0.42 $\pm$ 0.14	0.21 $\pm$ 0.03 ***	0.30 $\pm$ 0.07
% pheopigments	24 $\pm$ 3 ***	28 $\pm$ 5	23 $\pm$ 6	26 $\pm$ 3
HBA ( $\times 10^5$ cells $\text{mL}^{-1}$ )	3.9 $\pm$ 1.9	4.5 $\pm$ 1.7	<u>5.5 <math>\pm</math> 0.95</u>	<u>6.2 <math>\pm</math> 1.2</u>
PP ( $\mu\text{mol C L}^{-1} \text{d}^{-1}$ )	0.71 $\pm$ 0.27 ***	1.09 $\pm$ 0.22	0.85 $\pm$ 0.17 ***	1.36 $\pm$ 0.37
BP ( $\text{ng C L}^{-1} \text{h}^{-1}$ )	157 $\pm$ 49 ***	348 $\pm$ 42	135 $\pm$ 24 ***	256 $\pm$ 60
DOC $\mu\text{M C}$	59 $\pm$ 3	60 $\pm$ 2	60 $\pm$ 3	60 $\pm$ 2
POC $\mu\text{M C}$	8 $\pm$ 3 *	9 $\pm$ 1	6.6 $\pm$ 1.1 **	7.6 $\pm$ 1.3
APA ( $\text{nmole MUF-P hydr L}^{-1} \text{h}^{-1}$ )	1.5 $\pm$ 0.9 ***	8.0 $\pm$ 5.4	3.0 $\pm$ 2.3 **	5.0 $\pm$ 3.1
TDIP (days)	16 $\pm$ 15 ***	0.5 $\pm$ 0.3	2.0 $\pm$ 0.9 ***	0.9 $\pm$ 0.4
BP/PP ratio	0.48 $\pm$ 0.18 ***	0.65 $\pm$ 0.20	0.33 $\pm$ 0.11 *	0.39 $\pm$ 0.10
N <sub>2</sub> fix/BP ratio (%)	21 $\pm$ 11*	29 $\pm$ 16	22 $\pm$ 13 *	15 $\pm$ 8

Supprimé: (out)

Supprimé: out

Supprimé: out

Supprimé: na

Supprimé: na

	M2 P1	M2 P2	M3 P1	M3 P2
Chl ( $\mu\text{g L}^{-1}$ )	0.22 $\pm$ 0.03 ***	0.49 $\pm$ 0.18	0.20 $\pm$ 0.04 ***	0.71 $\pm$ 0.30
% pheopigments	23 $\pm$ 2 ***	28 $\pm$ 6	23 2	26 $\pm$ 15
HBA ( $\times 10^5$ cells $\text{mL}^{-1}$ )	2.2 $\pm$ 2.2	4.9 $\pm$ 1.8	4.1 $\pm$ 0.7 *	5.0 $\pm$ 1.4
PP ( $\mu\text{mol C L}^{-1} \text{d}^{-1}$ )	0.75 $\pm$ 0.15 ***	1.47 $\pm$ 0.35	0.73 $\pm$ 0.15 ***	2.45 $\pm$ 0.76
BP ( $\text{ng C L}^{-1} \text{h}^{-1}$ )	227 $\pm$ 114 ***	338 $\pm$ 116	168 $\pm$ 52 ***	422 $\pm$ 132
DOC $\mu\text{M C}$	58 $\pm$ 3 **	61 $\pm$ 1	61 $\pm$ 3	60 $\pm$ 2
POC $\mu\text{M C}$	10 $\pm$ 3	9 $\pm$ 1	9 $\pm$ 2 ***	13 $\pm$ 3
APA ( $\text{nmole MUF-P hydr L}^{-1} \text{h}^{-1}$ )	1.0 $\pm$ 0.8 ***	7.6 $\pm$ 7.6	0.6 $\pm$ 0.5 ***	3.18 $\pm$ 2.61
TDIP (days)	27 $\pm$ 19 ***	1.8 $\pm$ 2.0	25 $\pm$ 12 ***	3.0 $\pm$ 3.1
BP/PP ratio	0.65 $\pm$ 0.41	0.47 $\pm$ 0.16	0.50 $\pm$ 0.24*	0.35 $\pm$ 0.08
N <sub>2</sub> fix/BP ratio (%)	17 $\pm$ 16 ***	30 $\pm$ 18	25 $\pm$ 15	22 $\pm$ 11

9

1 Table 2. Log - Log relationships between BP/PP ratio and PP (expressed in mgC m<sup>-3</sup> d<sup>-1</sup>). In  
 2 mesocosms, phase P1 and P2 are separated for the regressions. r: Pearson correlation  
 3 coefficient, p: probability.

4

	equation	r	probability
phase P1	$\log(BP/PP) = -0,87 \log(PP) + 0,49$	0,59	< 0.001
phase P2	$\log(BP/PP) = -0,53 \log(PP) + 0,33$	0,6	< 0.001
lagoon waters	$\log(BP/PP) = -0,24 \log(PP) - 0,19$	0,28	< 0,01

5

6

Supprimé:

Supprimé:

Supprimé:

1 | Table 3 Specific leucine activities of main groups sorted. PRO : *Prochlorococcus*, LO-SYN  
 2 | (low orange fluorescence *Synechococcus*-like cells), HO-SYN (high orange fluorescence  
 3 | *Synechococcus*-like cells, PE (autotrophic pico-eukaryotes), LNA (low nucleic acid), HNA  
 4 | (high nucleic acid), Hi-HNA (high size and high nucleic acid) heterotrophic bacteria and bulk  
 5 | activities (total community leucine incorporation rates) corresponding to the same sample.  
 6 | When tests of reproducibility have been done, the corresponding data is indicated with its  
 7 | standard deviation. bdl: below detection limits, lag: lagoon waters

Supprimé: phytoplankton

Supprimé: error

Supprimé: .

Tableau mis en forme

8

	PRO	LO SYN	HO SYN	PE	<u>LNA</u>	<u>HNA</u>	<u>Hi-HNA</u>	bulk
	x 10 <sup>-21</sup> mole leu cell <sup>-1</sup> h <sup>-1</sup>							pmole leu L <sup>-1</sup> h <sup>-1</sup>
d15 M1	bdl	4.6 ± 1.9	3.5 ± 0.3	19 ± 5	<u>20.6 ± 0.2</u>	<u>67 ± 1</u>	<u>79</u>	97 ± 3
d19 M1	bdl	5,4	3,5	17	<u>27</u>	<u>16</u>	<u>80</u>	126 ± 3
d21 <u>lag</u>	69 ± 28	30 ± 4	25 ± 4	79 ± 12	<u>39</u>	<u>214</u>	<u>554</u>	186 ± 6
d23 M1	22	131	42	108	<u>56</u>	<u>113</u>	<u>356</u>	242 ± 7

Supprimé: out

9

10



1  
 2 Table 4. Linear regression fits on temporal trends of POC and DOC in M1, M2 and M3 from  
 3 days 5 to 23. DOC has been sampled only at 6 m depth in the 3 mesocosms. df: degree of  
 4 freedom, r: Pearson correlation coefficient, p: probability, ns: not significant. For POC trend,  
 5 some outliers have been suppressed from the regressions.

6

	Range $\mu\text{M}$	Outliers $\mu\text{M}$	slope	df	r	p
POC M1	4.7-12.4	19.3	0.12	35	0.32	0.02
POC M2	7.1-11.6	15.0, 15.0, 17.3	-0.009	28	0.03	ns
POC M3	6.5-18.9	no	0,47	36	0.70	< 0.001
DOC M1	54-64	no	0.071	13	0.15	ns
DOC M2	53-62	no	0.25	13	0.48	0.04
DOC M3	54-66	no	-0.12	14	0.22	ns

7

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33

## Figure Legends

**Figure 1.** Position of mesocosms implemented in the southwest lagoon of New Caledonia.

**Figure 2.** Example ([day 23 M3 1m](#)) of flow cytometry cytogram dot plot of: a) naturally non-fluorescent bacterioplankton groups discriminated by their DNA content (SYBR green-induced fluorescence in arbitrary units (a.u.) versus cell size (side scatter), after 488 nm laser excitation); b) phototrophic groups discriminated by their chlorophyll *a* content (related to the red fluorescence intensity (a.u.) versus phycoerythrin related to the orange fluorescence intensity (a.u.) after 488 nm laser excitation); c) low-orange (LO-SYN) and high-orange (HO-SYN) *Synechococcus*-like sub-groups separated by their chlorophyll *a* content (after 661 nm laser excitation) versus their phycoerythrin content (after 488 nm laser excitation).

**Figure 3.** Evolution of: a) chlorophyll *a* (Chl), b) heterotrophic bacterial abundance (HBA), c) primary production (PP), d) heterotrophic bacterial production (BP), e) alkaline phosphatase activity (APA) and f) DIP turnover time (TDIP) in the three mesocosms M1, M2, M3 and in the lagoon waters (lagoon). Each point is the mean of the three depths sampled, error bars are standard deviations. For lagoon HBA, only data from 1 m and 12 m depth are available occasionally and discrete data are presented instead.

**Figure 4.** Log-log relationships between heterotrophic bacterial production (BP) and a) primary production (PP) or b) chlorophyll *a* (Chl).

**Figure 5.** Response of heterotrophic bacterial production to the enrichment experiments conducted on days 2 and 20. Asterisks show significant responses in comparison to the unamended control (Co) after Mann Whitney test (\*:  $p < 0.05$ ).

**Figure 6.** Carbon budget of the mesocosms with time ( $\mu\text{M C}$ ). a) Evolution of time-integrated gross primary production (GPP). Cexp: C export in sediment traps (Cexp), time-integrated net POC and net DOC are calculated assuming linear fits of these variables between days 5 and 23 (see Table 3). b) Budget of time-integrated data on day 23. The difference  $\text{GPP} - (\text{Cexp} + \text{net DOC} + \text{net POC})$  was assumed to be community respiration (resp). The range of heterotrophic bacterial carbon demand (BCD) was calculated based on two hypotheses: BR =

Supprimé: 4

Supprimé: .

Supprimé: 5

Supprimé: 6

Supprimé: 7

1 | 100 % CR (~~BCD,1~~) or BR = 50 % CR (~~BCD,2~~). ~~Standard errors~~ are plotted from the sum of  
2 | each category, using propagation of errors.  
3 |

~~Supprimé:~~ DCB

~~Supprimé:~~ DCB

~~Supprimé:~~ Error bars

## Supplement of

F. Van Wambeke et al.

Correspondence to F. Van Wambeke ([france.van-wambeke@mio.osupytheas.fr](mailto:france.van-wambeke@mio.osupytheas.fr))

## Material and Methods

### Specific leucine assimilation assessment using cell sorting

Supprimé: 2.4.

On four occasions (day 15, M1, 6 m; day 19, M1, 6 m; day 21, lagoon waters, 1 m; day 23, M3, 1 m), additional seawater samples were incubated with  $^3\text{H}$  leucine for further cell sorting of phytoplankton and heterotrophic bacteria: five polypropylene (Nunc) tubes (3 live samples, 2 killed controls) were filled with 4.5 mL of seawater and incubated with pure  $^3\text{H}$  leucine (10 nM final conc.) for 1 h in the on-deck incubator as for bulk samples (section 2.3). Incubations were stopped by addition of 0.5 mL of 20 %, 0.2  $\mu\text{m}$ -filtered formalin, stored in the fridge for 15 minutes, and then stored in liquid  $\text{N}_2$  until sorting. Two additional non-incubated tubes (but fixed and stored in the same conditions) were used for total counts. Sorting of the radiolabeled samples were performed with on a BD Influx<sup>TM</sup> Mariner (BD Biosciences, San Jose, CA) high speed cell sorter equipped with three laser lines: 488 nm (Sapphire, Coherent), 561 nm (Jive, Cobolt) and 355 nm (Xcyte, JDSU). All data were collected in log scale, stored in list mode files and analyzed in real time for sorting using the BD FACSortware software (BD Biosciences), or analyzed a posteriori using FlowJo v7.6.5 software (Tree Star). A solution of 0.5x PBS, 0.2  $\mu\text{m}$  filtered, was used as sheath fluid. For heterotrophic prokaryote analyses and sorting, (using a drop purity sort mode), cells were stained with SYBR Green II as described above in section 2.2. Cells were characterized by two main optical signals collected from the 488 nm laser: side scatter (SSC) and green fluorescence (530/40<sub>488 nm</sub>), related to nucleic acid staining. Based on these criteria, low nucleic acid (LNA), high nucleic acid (HNA), and HNA with high SSC (Hi-HNA) groups were determined (Fig. 2) and sorted into different tubes. Like for determination of LNA and HNA abundances (section 2.2), phototrophic cells were excluded thanks to their red fluorescence. To analyze and sort the photosynthetic phytoplankton cells, the three laser lines were used. Red fluorescence (630LP<sub>488 nm</sub>) related to chlorophyll *a* content, was used as trigger signal. Phytoplankton cells were characterised by three other optical signals: forward scatter (FSC) related to cell size, side scatter (SSC), and the orange fluorescence (580/30<sub>488 nm</sub>) related to phycoerythrin. In addition, the chlorophyll *a* red fluorescence was collected from the 355 nm and 561 nm

1 excitation (630LP<sub>355nm</sub> and 630LP<sub>561nm</sub>). The cytogram red fluorescence (induced by the 561  
2 nm laser) vs orange fluorescence induced by the 488 nm laser evidenced two different  
3 subgroups of *Synechococcus* (one with Low and one with a High Orange fluorescence  
4 intensity, referred to as LO-SYN and HO-SYN, respectively, Fig. 2). Thus, four populations <  
5 2 µm were optically resolved and sorted simultaneously, directly into separate 2 mL  
6 Eppendorf centrifuge tubes, using the drop purity sort mode: *Prochlorococcus* (PRO), LO-  
7 SYN and HO-SYN, and pico-eukaryotes (PE).

8 Variable numbers of cells were sorted per sample depending on experiment and cell type, to  
9 achieve sufficient signal and a good compromise with the volume available. The  
10 phytoplankton collected cells ranged from 50 000-279 000 for the LO-SYN and HO-SYN  
11 groups, 16 000- 41 000 for the PE, to 1 600-10 000 for the PRO group. From the 1 mL SYBR  
12 Green II stained aliquot, the three heterotrophic prokaryote groups were simultaneously  
13 collected into separate 2 mL centrifuge plastic tubes, collecting a range of 100 000-250 000  
14 cells for LNA and HNA groups, and 15000-53000 cells for Hi HNA group. After sorting, 1.5  
15 mL of 5 % TCA was added into each tube and processed as for BP measurements. Bulk  
16 activities were realized in triplicate by subsampling directly 1.0 to 1.5 mL of samples from the  
17 5 mL tubes. In these tubes, 55 % TCA was added to give a final 5 % TCA concentration and  
18 the three series of centrifugations were run as for the BP measurements. Formalin-killed  
19 samples were also sorted in order to estimate blank values for each group. Dpm in the killed  
20 control of a given group were subtracted from dpm in the corresponding incubated sample.  
21 Blank values were independent of the number of sorted cells (on average  $27 \pm 9$  dpm). We  
22 checked that a linear increase of the dpm signal occurred with the number of sorted cells. The  
23 coefficient of variation between triplicate sorts ranged 1-5 % when dpm signal were > 1000  
24 dpm, but these values increased up to 30 % when the dpm signal were < 100 dpm due to  
25 methodological limitations (limited volume available or lower activities). For this reason we  
26 considered below detection limits all sorts where dpm values were less than twice the  
27 corresponding blank value. The radioactivity per cell was calculated and expressed in C units  
28 ( $10^{-21}$  mol leu cell<sup>-1</sup> h<sup>-1</sup>). It was multiplied by the abundance of cells mL<sup>-1</sup> within in sorted  
29 region to obtain the volumetric incorporation rate of each group, and the relative population  
30 activity was calculated as the population fraction of the bulk (i.e. total community) activity.

31 Considering a drop frequency set at 98500 per second and average sorting rates of 891 cells  
32 per second for heterotrophic bacteria, the probability of free bacteria being sorted  
33 simultaneously with an autotrophic cell in the same drop, leading to overestimation of leucine  
34 assimilation rate per cell for autotrophic cells, was 0.9 % and thus considered negligible. The

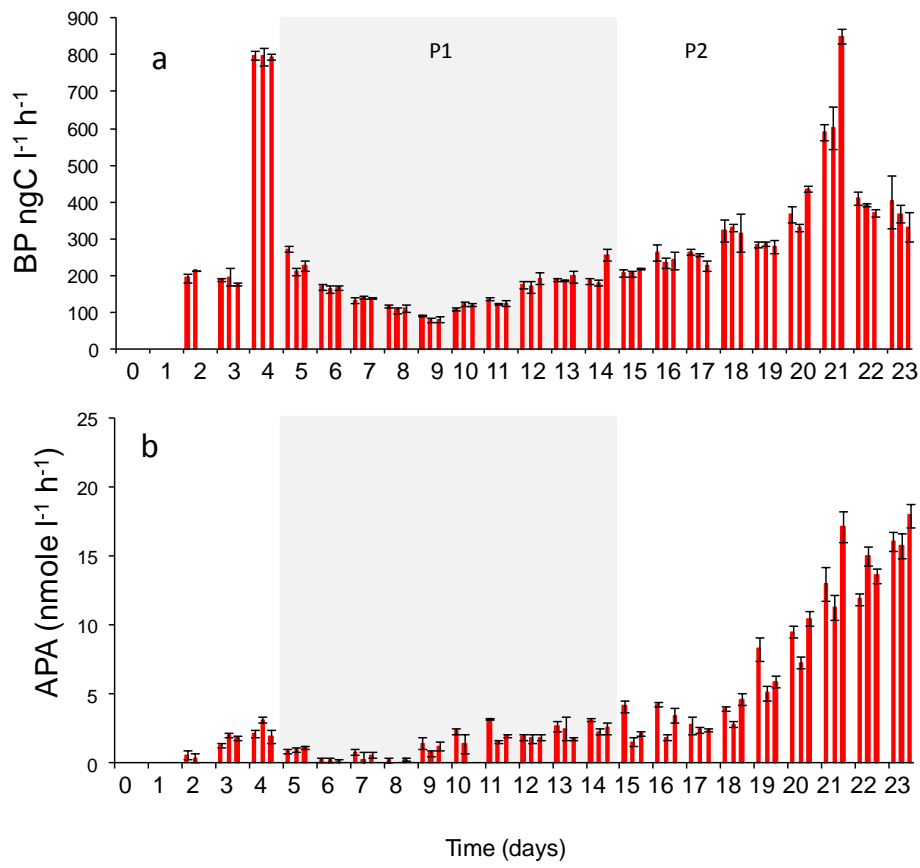
1 effect of a long storage of the samples before cell sorting was checked by counting abundance  
2 of cells mL<sup>-1</sup> directly on the Influx. For this, we used the additional non labeled tubes, ran  
3 using similar procedure (sample preparation with control beads, and flow rate determination)  
4 as described in section 2.2., except that we counted also Hi-HNA cells and the two categories  
5 of *Synechococcus*.

6  
7  
8  
9  
10  
11  
12  
13  
14

**Figures**

**Figure S1.** Evolution of: a) bacterial production and b) alkaline phosphatase in the mesocosm M1 at the three depths sampled. For each day 1 m, 6 m and 12 m are presented from left to right. For the BP plot error bars are standard deviations within triplicate measurements. For phosphatase activity, error bars are the standard errors of the slope of the linear regression MUF production as a function of time. The vertical bar between day 4 and 5 indicates DIP fertilization.

Supprimé: 3



1  
2

1  
2  
3  
4  
5  
6  
7  
8  
9

**Tables**

Table S1 : C budget. Time integrated measurements (average of the three mesocosms); sd-meso: standard deviation associated to the variability within the three mesocosms; sd-CF: standard deviation reflecting the range of conversion factors or analytical methods and assumptions. The errors considered for estimating sd-CF are discussed in the text: 30 % for GPP, 25 % for BP and 10 % for Cexp, DOC and POC. The sd in italics are computed assuming general laws of error propagation.

	<u>Time integrated measurements</u>	<u>sd - meso</u>	<u>sd -CF</u>
	<u>μMC</u>	<u>μMC</u>	<u>μMC</u>
<u>GPP</u>	<u>38.1</u>	<u>11.2</u>	<u>11.4</u>
<u>Cexp</u>	<u>6.4</u>	<u>2.1</u>	<u>0.6</u>
<u>POC</u>	<u>3.1</u>	<u>5.4</u>	<u>0.3</u>
<u>DOC</u>	<u>1.3</u>	<u>3.6</u>	<u>0.1</u>
<u>BP</u>	<u>10.4</u>	<u>0.6</u>	<u>2.6</u>
<u>resp 1=CR</u>	<u>27.3</u>	<u>11.6</u>	<u>11.5</u>
<u>resp 2=CR/2</u>	<u>13.7</u>	<u>5.8</u>	<u>5.7</u>
	<u>%</u>	<u>%</u>	<u>%</u>
<u>BGE1</u>	<u>27 %</u>	<u>9 %</u>	<u>10 %</u>
<u>BGE2</u>	<u>43 %</u>	<u>11 %</u>	<u>12 %</u>

10  
11



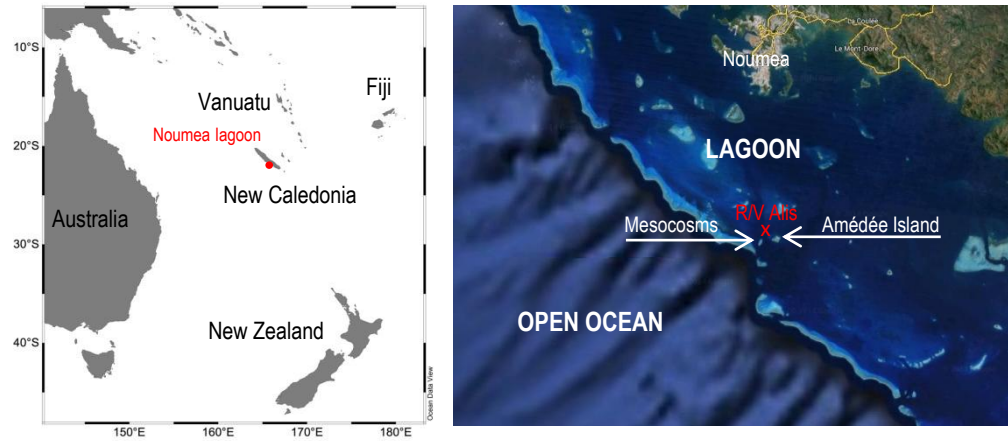


Fig. 1

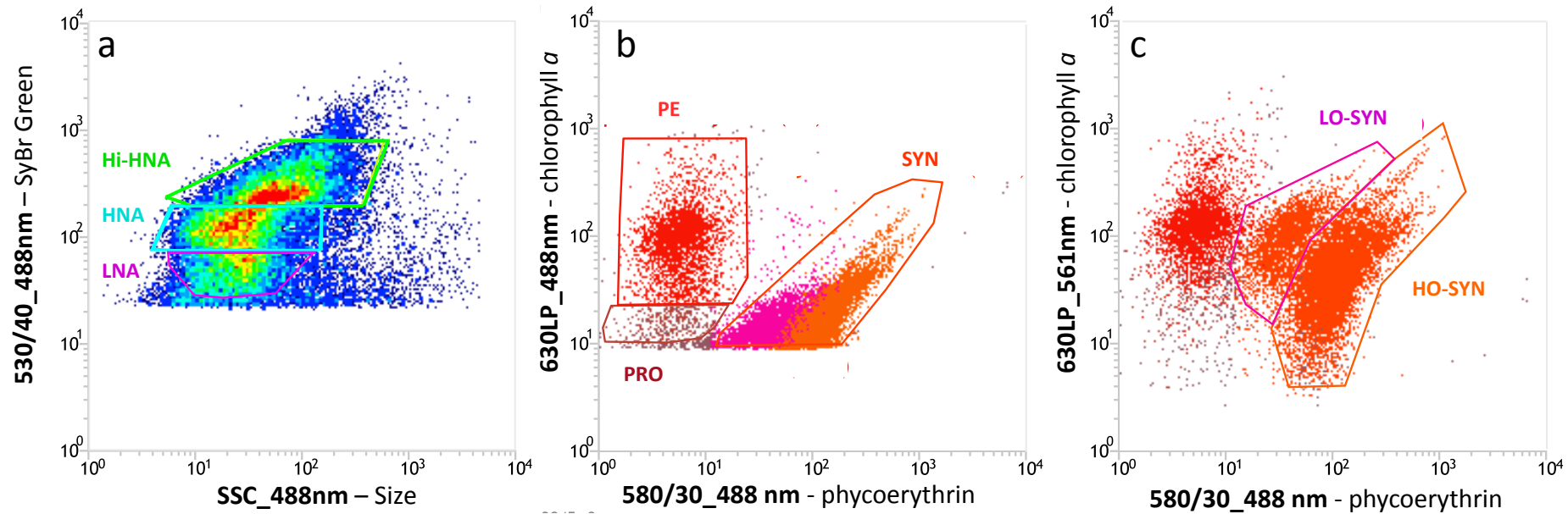


Fig. 2

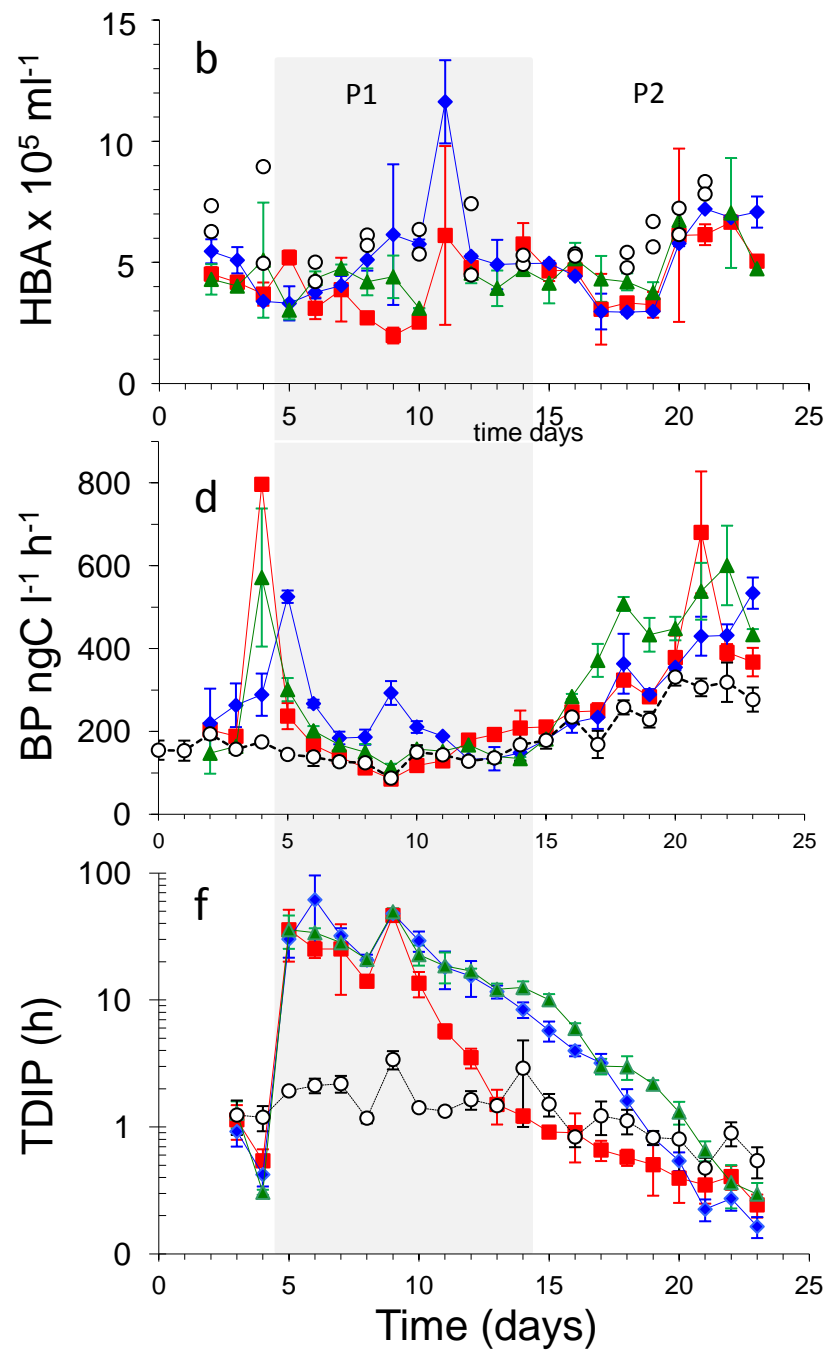
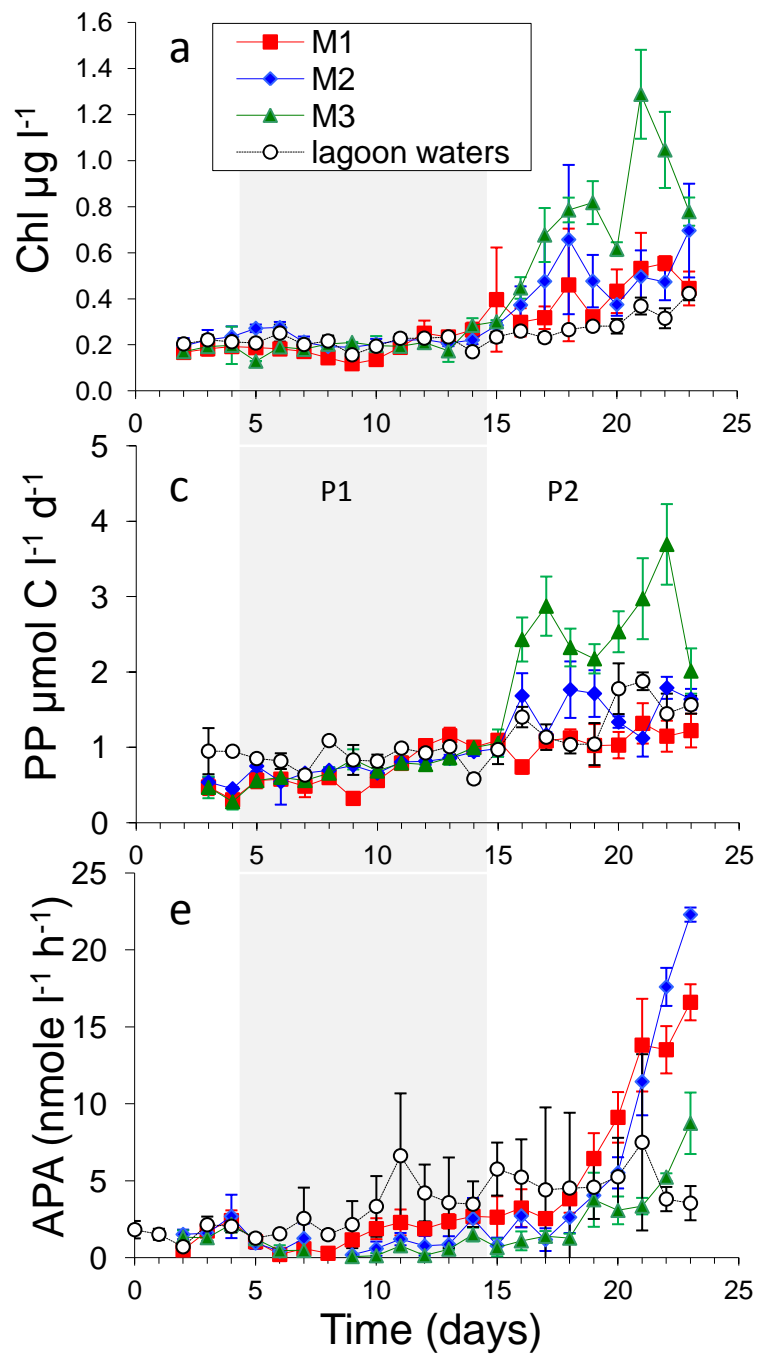


Fig. 3

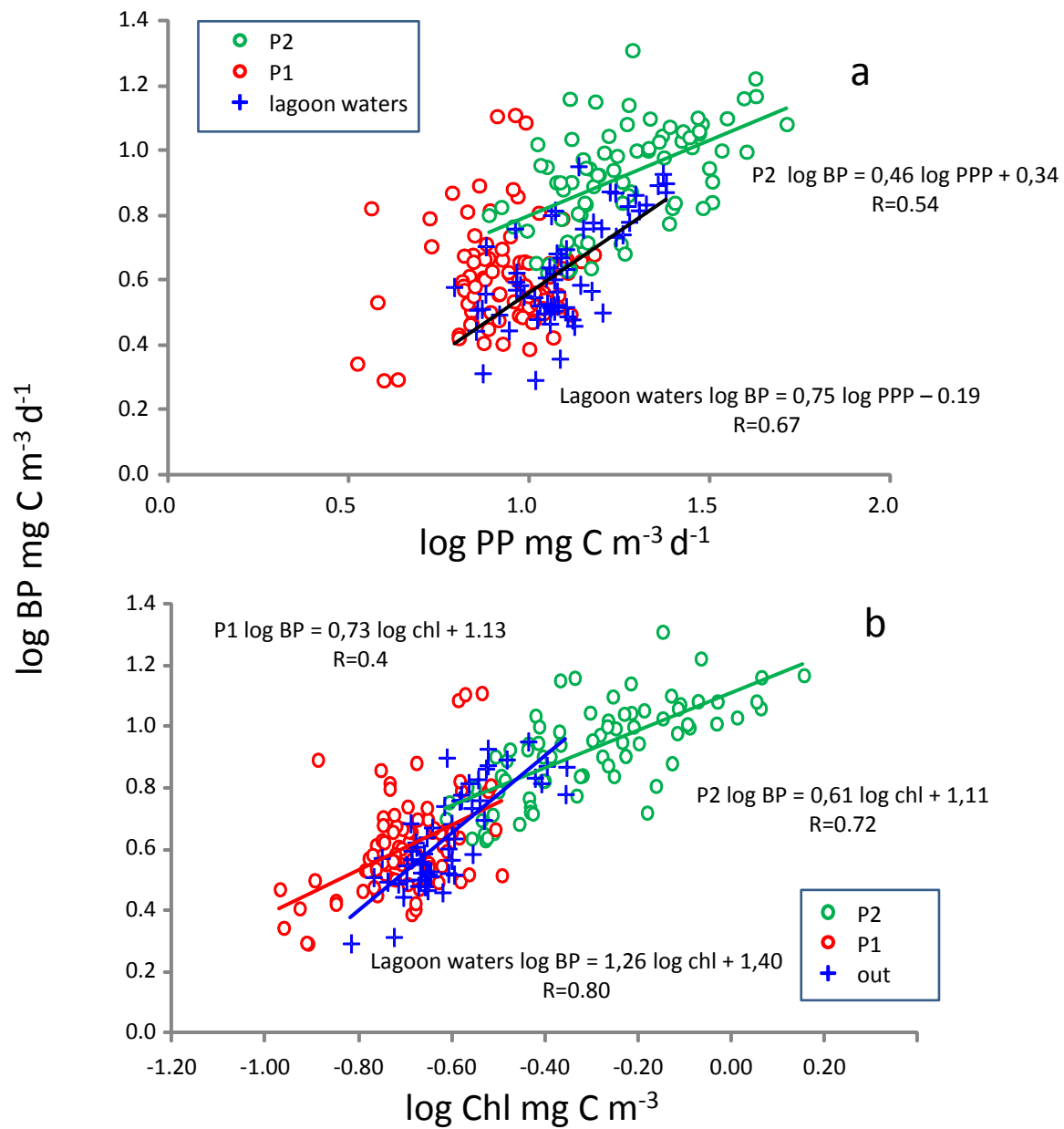


Fig. 4

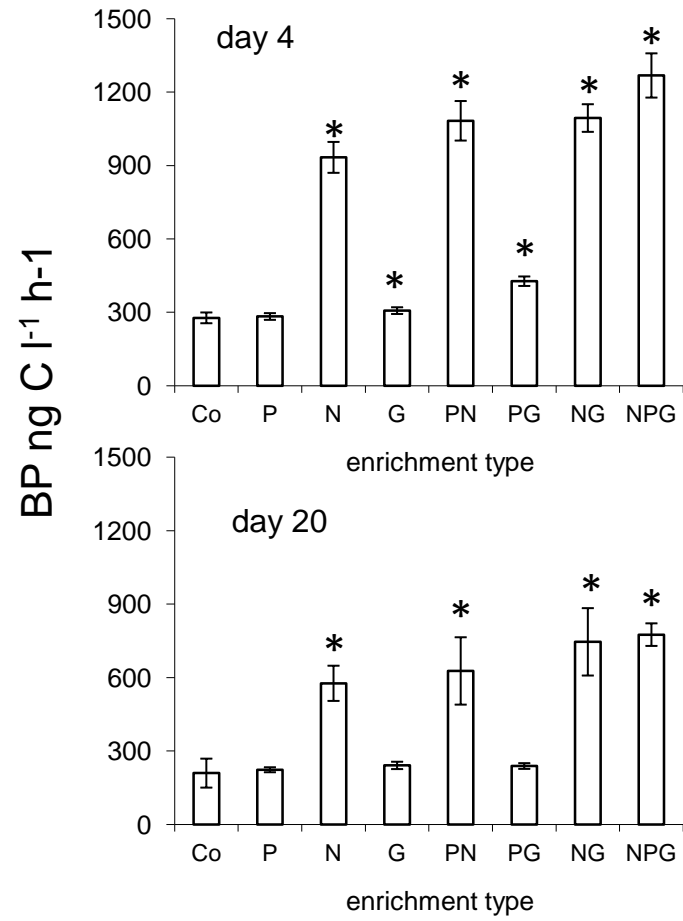


Fig. 5

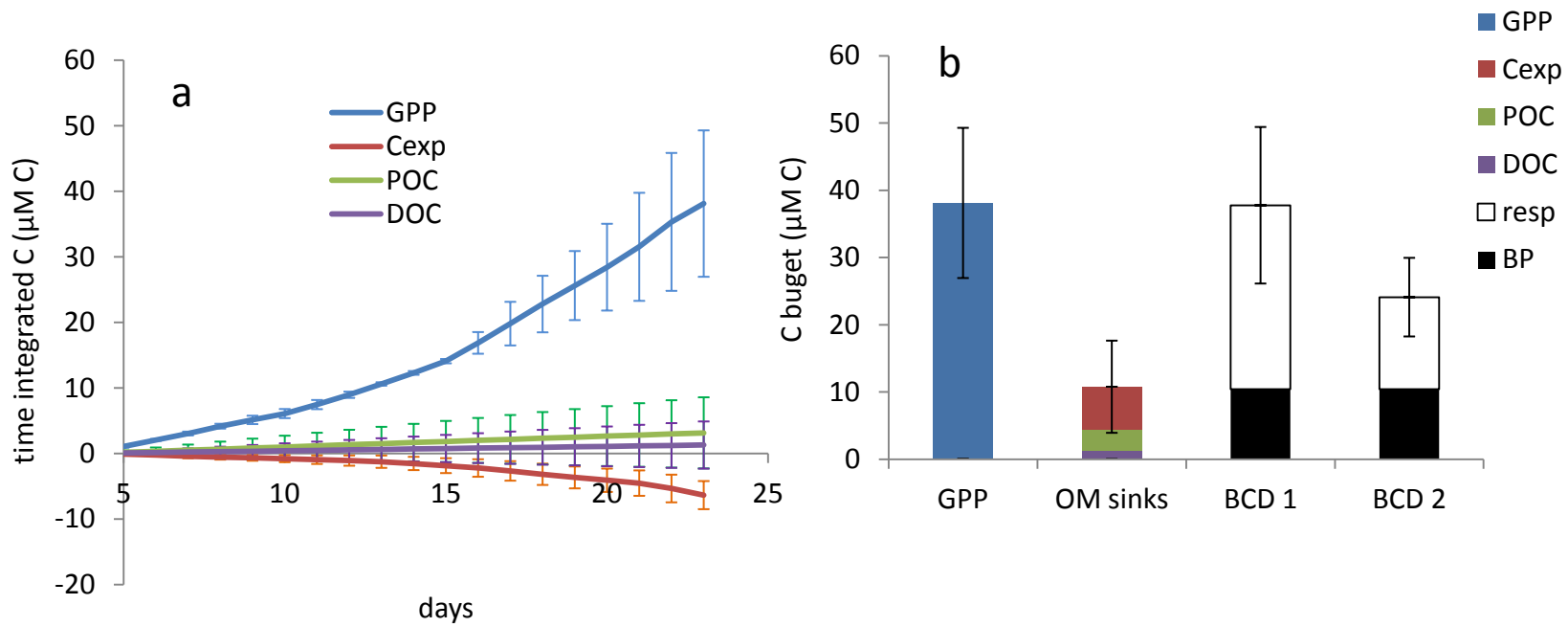


Fig. 6

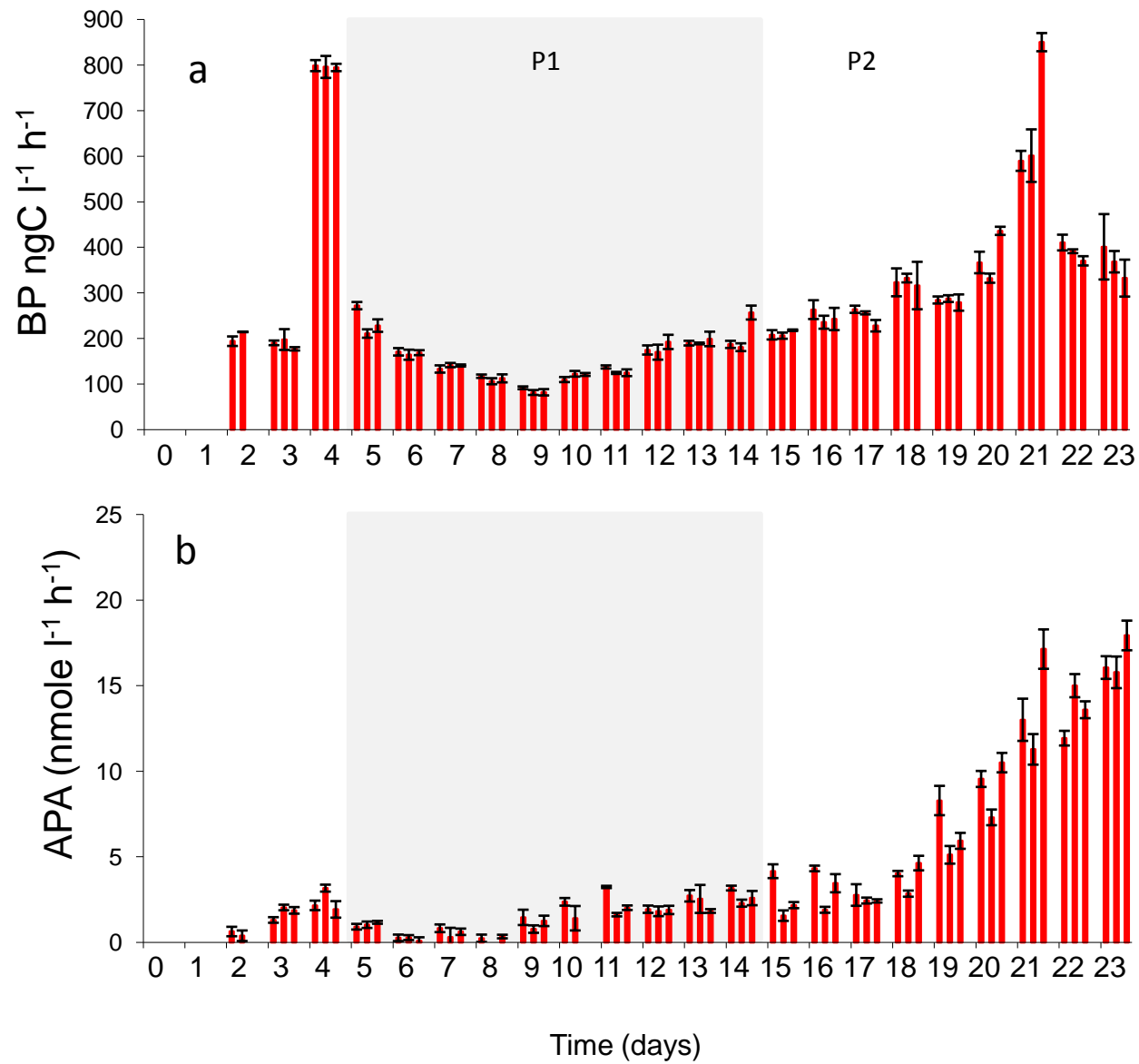


Fig. S1