

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

4

5 **France Van Wambeke¹, Ulrike Pfreundt², Aude Barani¹, Hugo Berthelot¹, Thierry**
6 **Moutin¹, Martine Rodier^{3*}, Wolfgang R. Hess² and Sophie Bonnet^{1,3}**

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

1 **Abstract**

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

28

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 2015). However, little is known about the fate of the diazotroph-derived nitrogen (DDN) in
6 this environment (Bonnet et al., 2016b). 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 (Torréton 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., 2016b), 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 m³)
34 mesocosms deployed in the oligotrophic part of the New Caledonian lagoon, DIP being

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 N₂-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).

14

15 **2 Material and methods**

16

17 **2.1 Mesocosm description and sampling strategy**

18 Three large mesocosms (~50 m³) 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. (2016b). The three
26 triplicate mesocosms were supplemented with 0.8 µM KH₂PO₄ 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 N₂ 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 N₂ 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 ¹⁴C
5 labeling technique (see details in Berthelot et al., 2015) and a model of photosynthesis is then
6 applied which restitute daily fluxes (Moutin et al., 1999). Primary production (PP) was
7 determined from short term (~ 4 h) incubations around noon with H¹⁴CO₃ (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) ¹⁴C 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 N₂ 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_{488 nm}), 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 μ 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 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 H] leucine, Perkin Elmer®
14 specific activity ranging 106 Ci 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 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 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 μ M P), NO_3^- and NH_4^+ (1 μ M each) or glucose (10 μ 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

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**

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 methylumbellifluorone phosphate
8 (MUF-P, 1 μ 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 μ 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**

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., 2016b). 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., 2016b; 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

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 N₂-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 ± 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 ± 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).

18

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 ± 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

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 ($\log(BP) = 0.13 * \log(N_2\ fix\ rates) + 0.73$, r
13 = 0.21, $p = 0.04$, data not shown).

14

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.

32

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

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 86×10^{-21} mol cell $^{-1}$
14 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 ^3H -leucine
24 labelled samples until cell sorting (two years at -80°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

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 131×10^{-21} mol cell $^{-1}$ 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

12 **3.6 Carbon budget**

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

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.

3

4 Discussion

4.1 Variability within the triplicate mesocosms

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., 2016b) and thus our results are discussed based on
23 averages.

24

4.2 N limitation and coupling between BP and N₂ fixation

25 BP was significantly enhanced on a short-term scale (1 - 2 days) by $\text{NO}_3^- + \text{NH}_4^+$ but not DIP
26 or glucose amendments, indicating that BP was directly N-limited, and/or indirectly after
27 stimulation of N-limited phytoplankton (Fig. 4). In the New Caledonia lagoon, N-limitation
28 has previously been suggested based on a one-year survey of nutrient ratios (Torréton et al.,
29 2010). N-limitation is a recurrent feature observed in the ultra-oligotrophic South Eastern
30 Pacific Gyre (Van Wambeke et al., 2008a; Halm et al., 2012), as assessed from short-term (1-
31 3 days) enrichment experiments or incubations. As N₂ fixation is assumed to be the only
32 process providing a source of new nitrogen to the mesocosms in this experiment, we

1 examined the potential links between N_2 fixation rates and BP. First, marine heterotrophic
2 diazotrophs were detected at low abundances during the mesocosms experiment: γ -24774A11
3 with ca 10^2 - 10^3 *nifH* gene copies L^{-1} (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_2 fixation directly performed by heterotrophic bacteria probably
6 accounted for a minor fraction of bulk N_2 fixation during the mesocosm experiment. Second,
7 as the log-log relationship between N_2 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_4^+ 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_2
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_2 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., 2016a). After
26 24 h of $^{15}N_2$ -incubations, these authors reported significant ^{15}N -enrichment in picoplanktonic
27 cells (0.2-2 μm fraction). This confirmed a rapid (one day) transfer of DDN (also ^{15}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).

31
32 **4.3 Alkaline phosphatase activity and P acquisition**
33 A slight T_{DIP} decrease was noticed in the mesocosms before the DIP spike but not in the
34 lagoon, suggesting a lower P availability inside and not outside the mesocosms. Therefore, N_2

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.
23

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 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 (Béjà 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,

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 (Béjà 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

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(BP) as a function of log(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 N₂ 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 N₂ 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 (Torréton 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

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., 2016a; Hunt et al., 2016). However, UCYN-C formed large aggregates
12 (100-500 μm) 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 ($> 100\text{ m}$) and
22 exhibited reduced turbulence.

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 $93 \pm 6\text{ }\mu\text{M C}$, 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

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

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 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 phototrophic 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 μM C produced between 5-23
30 days. This is not sufficient to satisfy BCD cumulated for the same period (calculated as 24-38
31 μM 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 **Conclusions**

3 This study confirms that in the Nouméa lagoon, N_2 fixation is a relevant process to fuel the
4 microbial food web and to sustain a biological system which is net autotrophic or close to
5 metabolic balance. The relatively high BGE computed from the carbon budget (27-43 %)
6 could be related to light-harvesting systems developed by abundant bacterial
7 photoheterotrophs. The success of *Synechococcus* over *Prochlorococcus* described in
8 companion papers (Leblanc et al., 2015; Pfreundt et al., 2016b) might be attributed to their
9 ability to assimilate leucine, and possibly other amino acids, as well as reduction of their
10 cellular P demand through sulfolipid synthesis. The relative importance of mixotrophy in
11 these oligotrophic system implies that it is important to i) find alternative techniques to dark
12 incubations to estimate bacterioplankton respiration, like with continuous measurements with
13 oxygen microprobes during alternate light and dark periods (Pringault et al., 2007) and ii) to
14 detect organisms responsible for the assimilation of a wide variety of organic molecules by
15 cell sorting. From the carbon budget, a BCD to GPP ratio was estimated to range between 63
16 and 100 %, and thus a large part of the primary production is channelled through the
17 microbial food web. Bacterial production was strongly coupled with Chl biomass and/or PP,
18 rather than with N_2 fixation rates, suggesting that indirect routes through lysis, grazing and
19 mortality of phytoplankton were substantial for providing labile organic matter for
20 heterotrophic bacteria.

21

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

28

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19

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. N₂ fixation
 5 contribution to BP (N₂fix/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	Lagoon P1	lagoon 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 mL^{-1})	3.9 \pm 1.9	4.5 \pm 1.7	5.5 \pm 0.95	6.2 \pm 1.2
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 (nmole MUF-P hydr $\text{L}^{-1} \text{h}^{-1}$)	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 ₂ fix/BP ratio (%)	21 \pm 11 *	29 \pm 16	22 \pm 13 *	15 \pm 8

	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 \pm 2	26 \pm 15
HBA ($\times 10^5$ cells 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 (nmole MUF-P hydr $\text{L}^{-1} \text{h}^{-1}$)	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 ₂ 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 $\text{mgC m}^{-3} \text{ d}^{-1}$). 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	$\log (\text{BP/PP}) = - 0,87 \log(\text{PP}) + 0,49$	0,59	< 0.001
phase P2	$\log (\text{BP/PP}) = - 0,53 \log(\text{PP}) + 0,33$	0,6	< 0.001
lagoon waters	$\log (\text{BP/PP}) = - 0,24 \log(\text{PP}) - 0,19$	0,28	< 0,01

5

6

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

8

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

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 μM	Outliers μM	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 **Figure Legends**

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

4
5 **Figure 2.** Example (day 23 M3 1m) of flow cytometry cytogram dot plot of: a) naturally non-
6 fluorescent bacterioplankton groups discriminated by their DNA content (SYBR green-
7 induced fluorescence in arbitrary units (a.u.) versus cell size (side scatter), after 488 nm laser
8 excitation); b) phototrophic groups discriminated by their chlorophyll *a* content (related to the
9 red fluorescence intensity (a.u.) versus phycoerythrin related to the orange fluorescence
10 intensity (a.u.) after 488 nm laser excitation); c) low-orange (LO-SYN) and high-orange (HO-
11 SYN) *Synechococcus*-like sub-groups separated by their chlorophyll *a* content (after 661 nm
12 laser excitation) versus their phycoerythrin content (after 488 nm laser excitation).

13
14 **Figure 3.** Evolution of: a) chlorophyll *a* (Chl), b) heterotrophic bacterial abundance (HBA),
15 c) primary production (PP), d) heterotrophic bacterial production (BP), e) alkaline
16 phosphatase activity (APA) and f) DIP turnover time (TDIP) in the three mesocosms M1, M2,
17 M3 and in the lagoon waters (lagoon). Each point is the mean of the three depths sampled,
18 error bars are standard deviations. For lagoon HBA, only data from 1 m and 12 m depth are
19 available occasionally and discrete data are presented instead

20
21 **Figure 4.** Log-log relationships between heterotrophic bacterial production (BP) and a)
22 primary production (PP) or b) chlorophyll *a* (Chl).

23
24 **Figure 5.** Response of heterotrophic bacterial production to the enrichment experiments
25 conducted on days 2 and 20. Asterisks show significant responses in comparison to the
26 unamended control (Co) after Mann Whitney test (*: $p < 0.05$).

27
28 **Figure 6.** Carbon budget of the mesocosms with time ($\mu\text{M C}$). a) Evolution of time-integrated
29 gross primary production (GPP). Cexp: C export in sediment traps (Cexp), time-integrated net
30 POC and net DOC are calculated assuming linear fits of these variables between days 5 and
31 23 (see Table 3). b) Budget of time-integrated data on day 23. The difference GPP - (Cexp +
32 net DOC + net POC) was assumed to be community respiration (resp). The range of
33 heterotrophic bacterial carbon demand (BCD) was calculated based on two hypotheses: BR =

- 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.

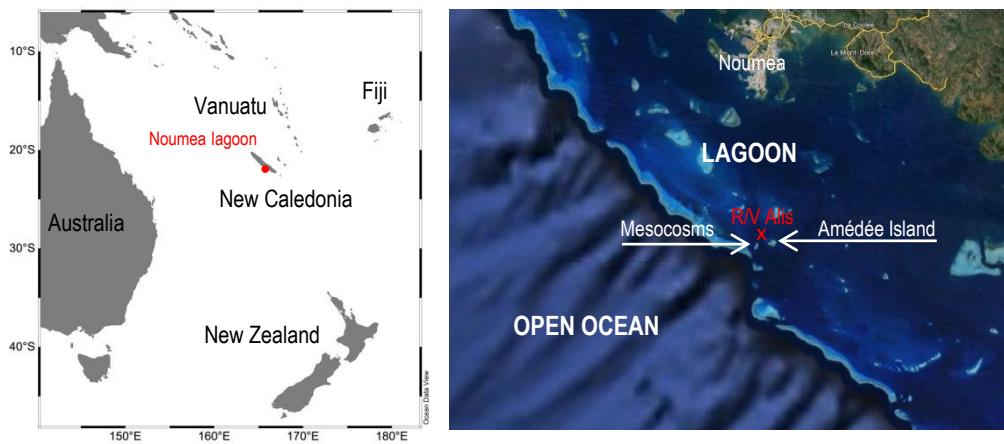


Fig. 1

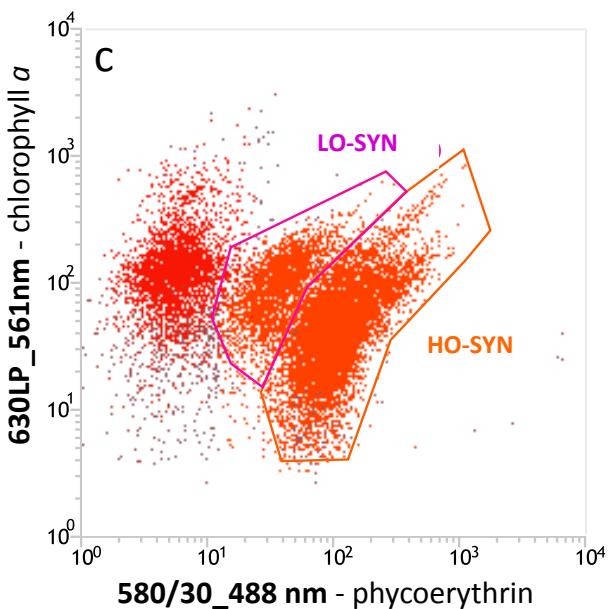
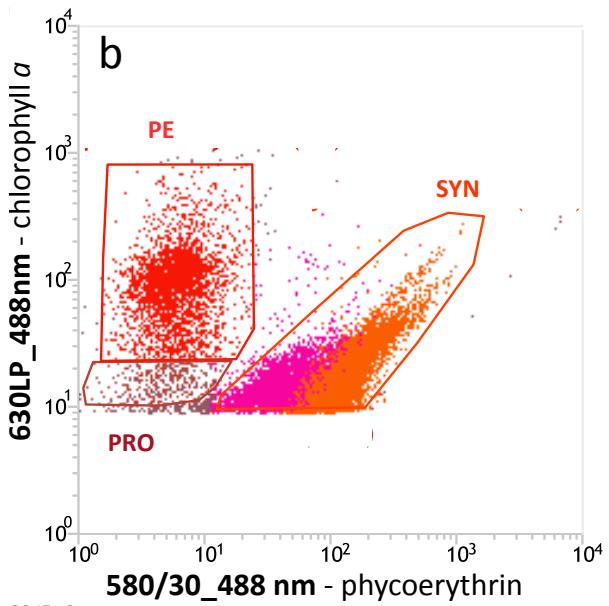
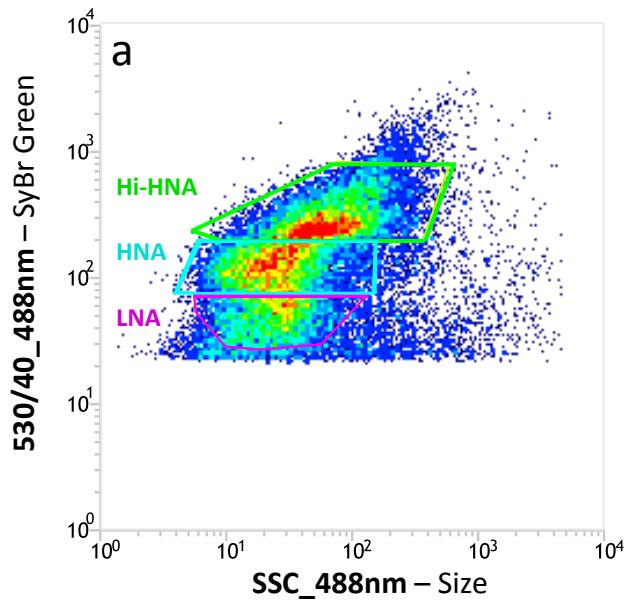


Fig. 2

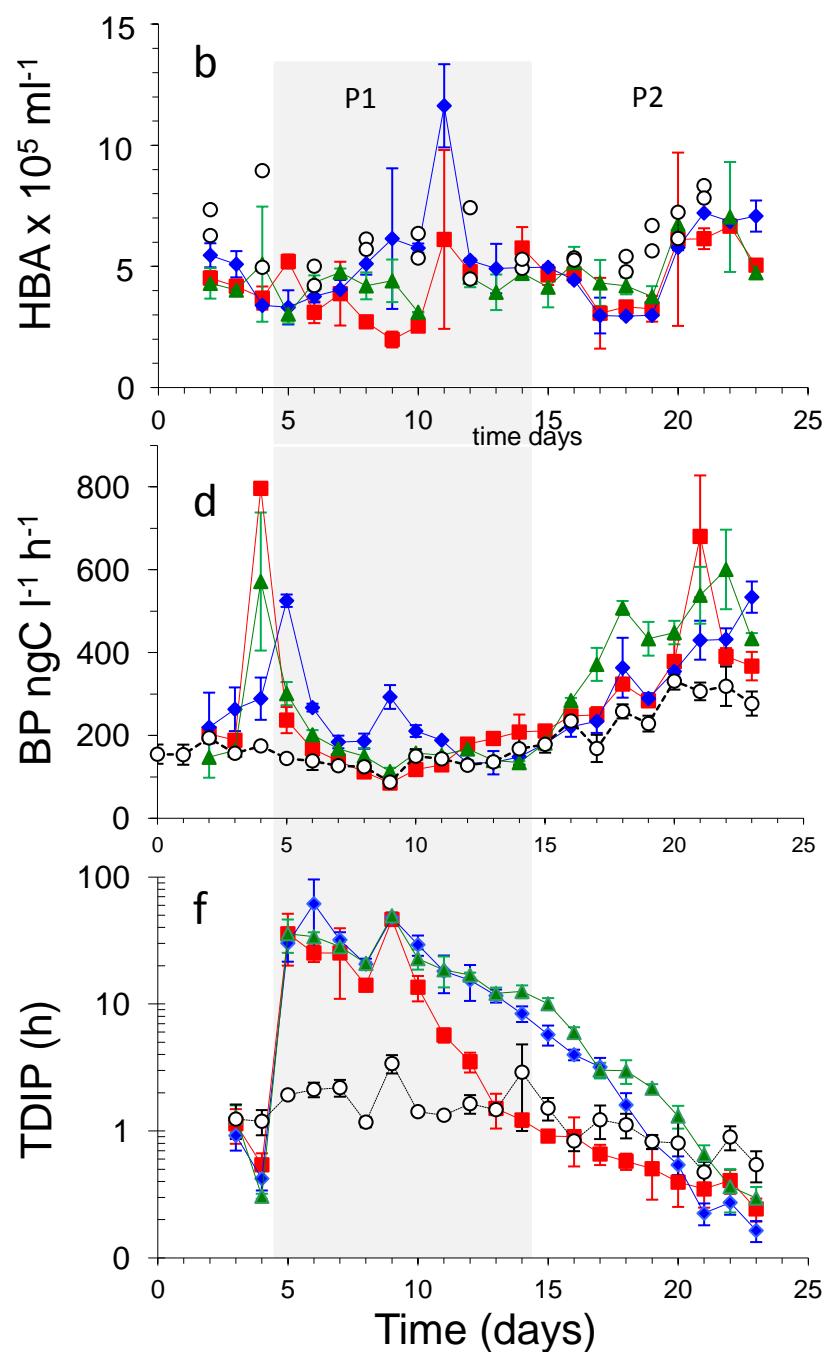
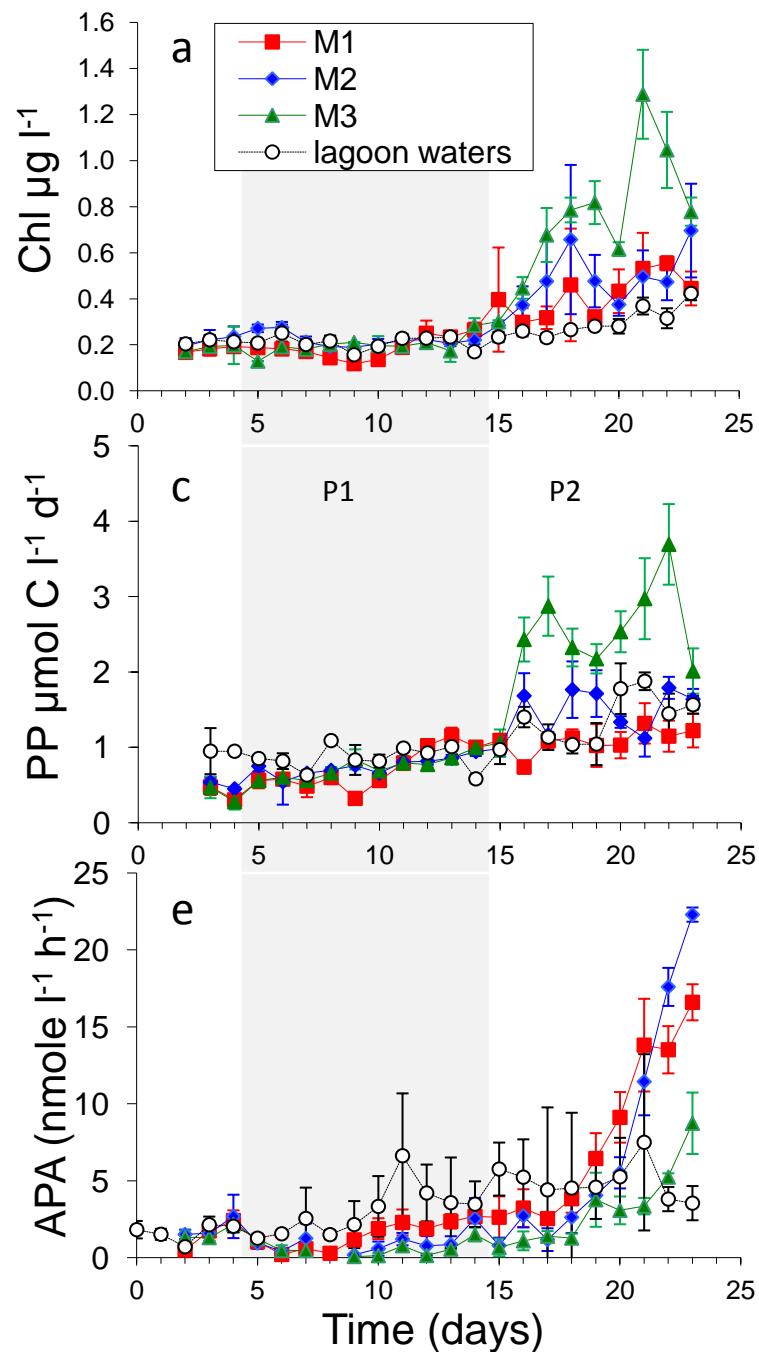


Fig. 3

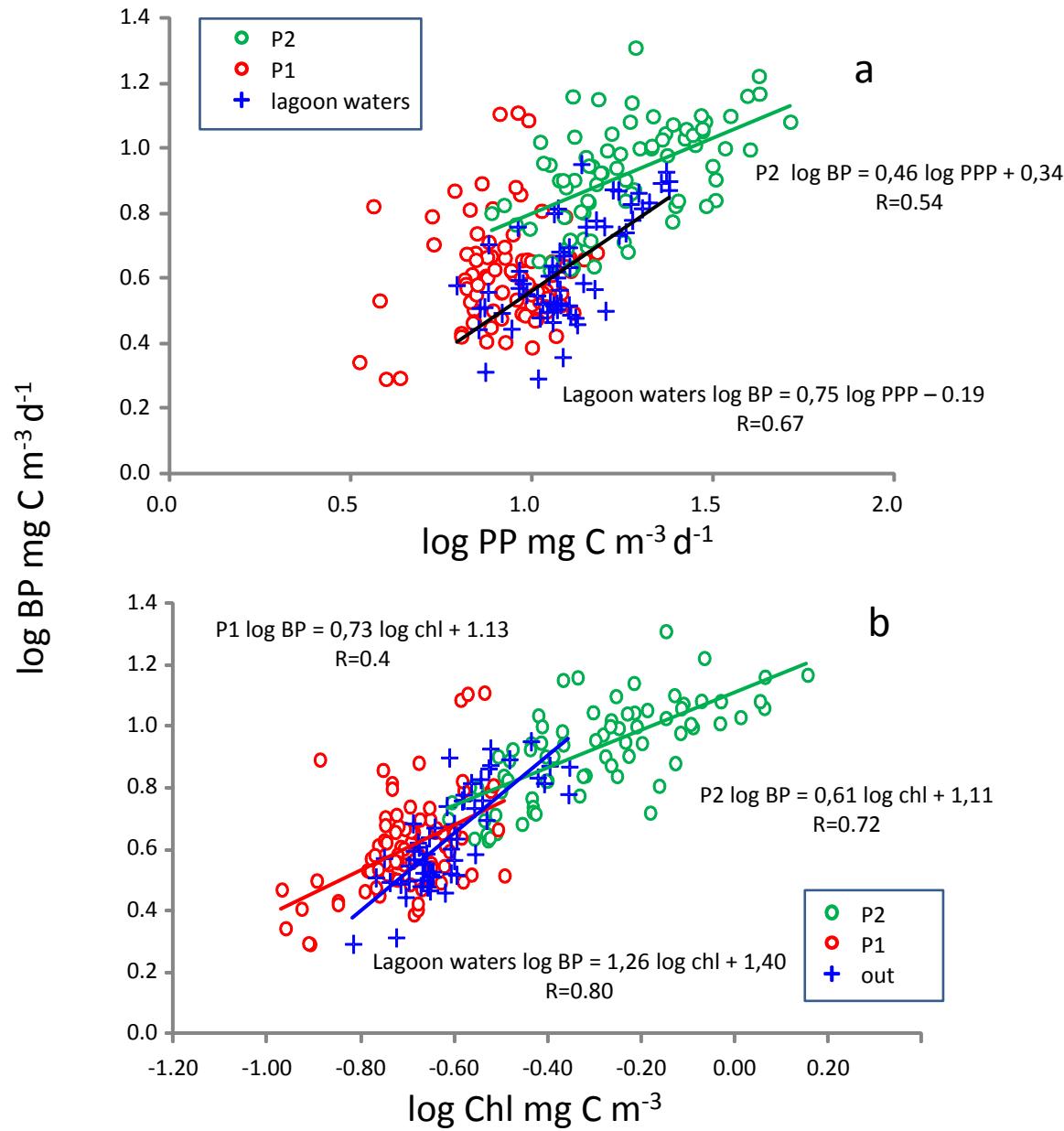


Fig. 4

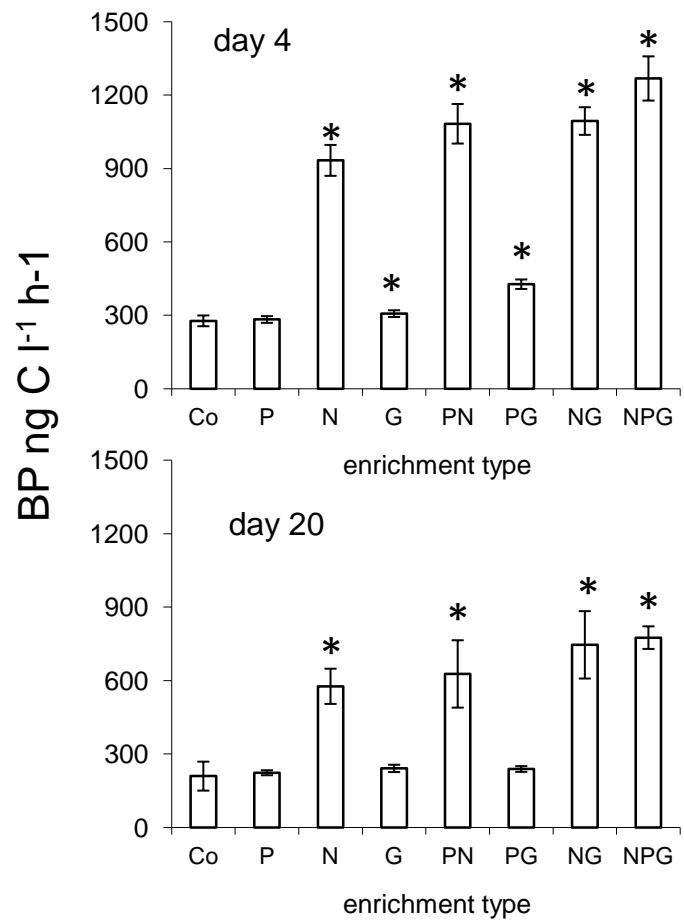


Fig. 5

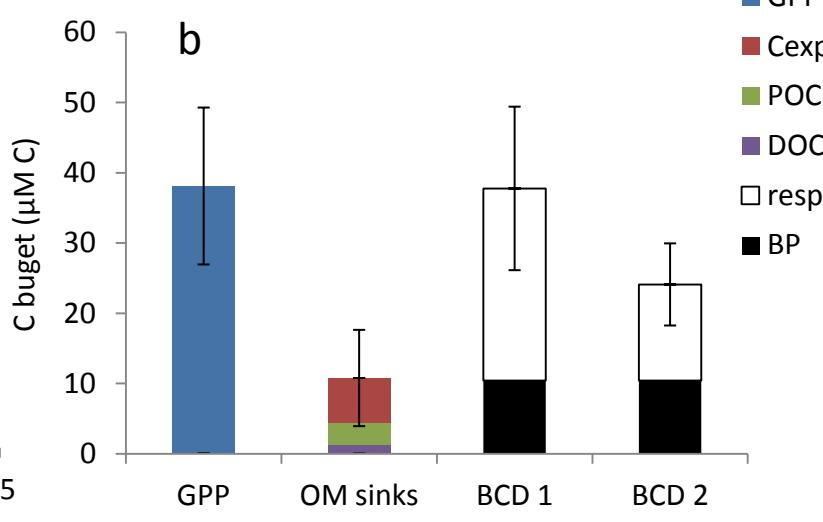
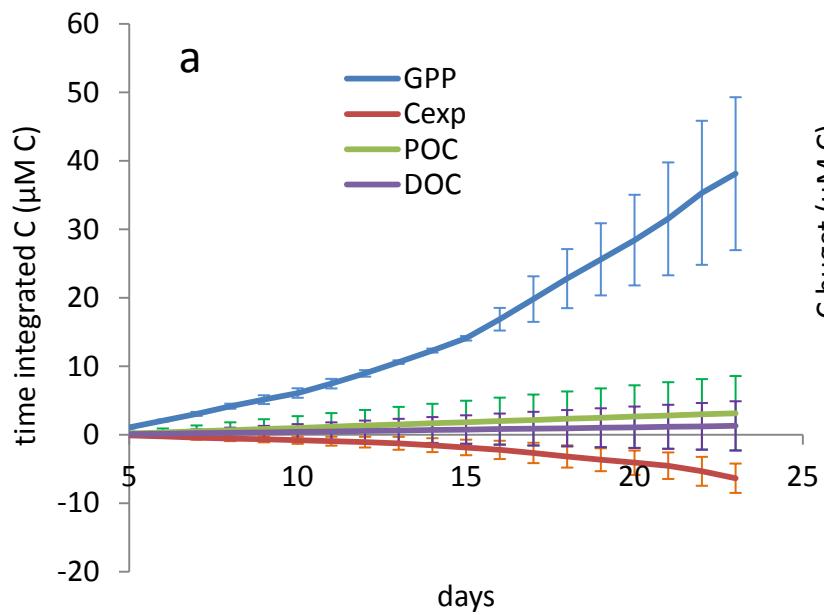


Fig. 6

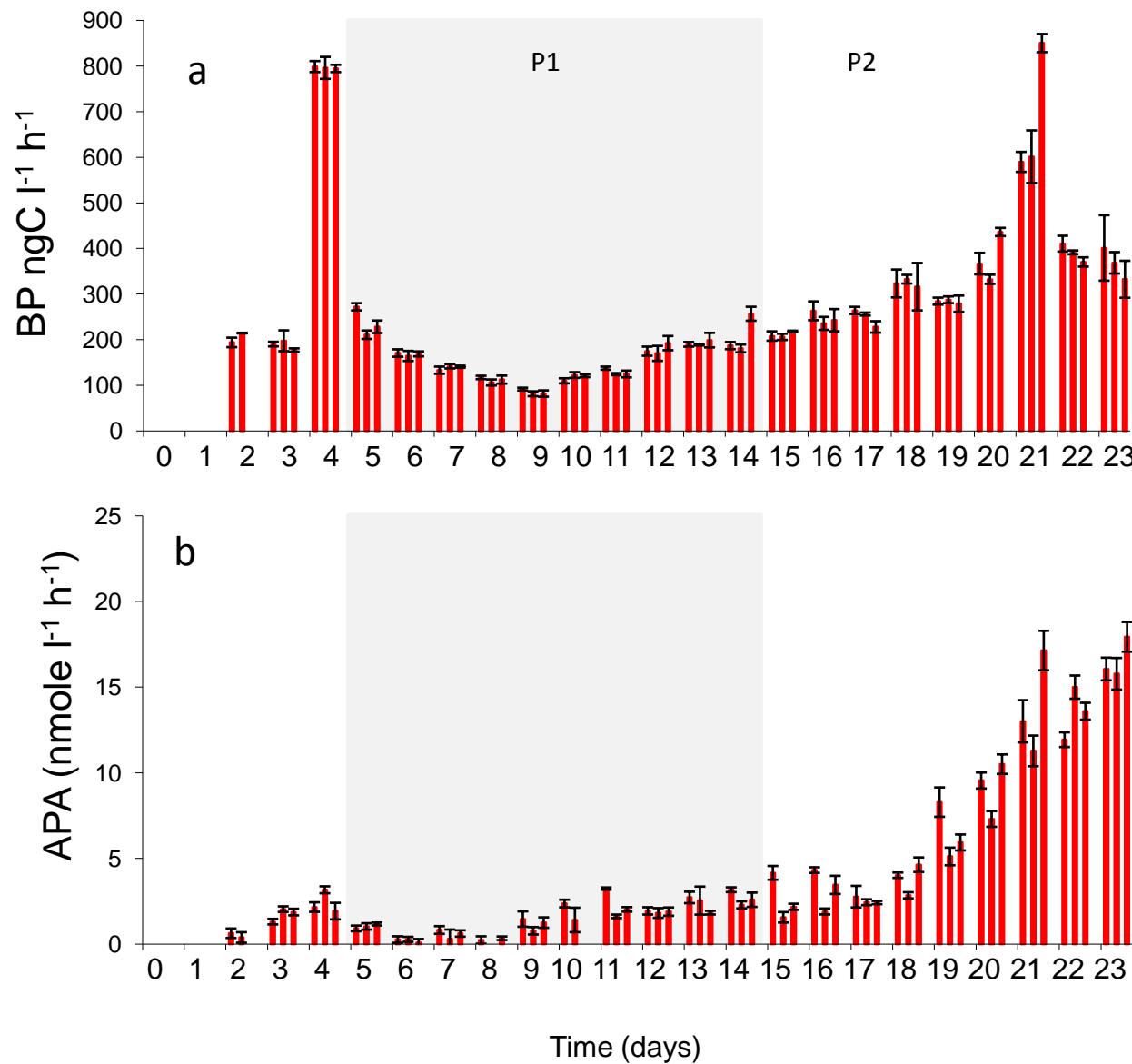


Fig. S1