Responses to referees 1, 2 3 and 4

We thank the four referees for their constructive comments. We briefly resume here main modifications of the ms:

- The abstract was re-written
- A supplementary information was created, in which we put M & M section on cell sorting, a new table (S1) and the former Figure 3.
-M & M section 2.1 has been implemented with more details regarding estimates of PP and GPP based on the Moutin et al. (1999) methodology.
- We moved part of description of the carbon budget in a new Results section (3.6).
- The discussion section has been implemented
    - with a sensitivity test on the carbon budget,
    - with a paragraph on turbulence,
    - with a paragraph on alkaline phosphatase activity
    - with a more extended section on the photoheterotrophy topic, including anoxygenic phototrophic bacteria.

The references added in the responses to the referees, but not included in the previous submitted version of the ms are listed at the end.

Response to the referee 1

1) I have been wondering about controls for the impact of the mesocosm incubation. Perhaps this is addressed in one of the companion papers but it would still be nice to have some comment on it here. Indeed, while the mesocosms used here are of very large volume, the fact that water is contained within these large, vertical "socks" still means that wind, wave and tidal action is reduced. Mari et al. 2007 showed that the residence time in this system played a strong role in determining carbon export and perhaps the authors could comment on this aspect, particularly as regards their partitioning estimates in the later part of the discussion.

We added the following paragraph in the discussion section 'Phytoplankton-bacteria coupling and metabolic balance':

'In the lagoon, as close to Grande Rade Bay, long residence times favored local degradation, refractorisation of organic matter and not sedimentation (Mari et al., 2007). However, as these authors discussed, modification of phytoplankton community composition in Grande Rade Bay and the presence of metals could influence sticking properties of polymers. The confinement of the seawater inside the mesocosms probably favored to some extent the accumulation of UCYN-aggregates, as well as a possible reduction of grazing pressure (by a factor of 1.6) in the mesocosms compared to those in the lagoon waters (Turk-Kubo et al., 2015; Bonnet et al., 2015a; Hunt et al., 2016). However, UCYN-C formed large aggregates (100-500 µm) embedded in an organic matrix that included TEP, which were largely responsible for enhanced export flux through sedimentation observed during P2 (Berthelot et al., 2015; Berman-Frank et al., 2016; Knapp et al., 2015). TEP evolution with time, however, and the TEP-C to TOC ratio were similar in the lagoon waters, where wave turbulence and tidal effects were present, and in the enclosed mesocosms, where these hydrodynamics were reduced, and concentrations were similar (Berman-Frank et al., 2016). In an unconstrained ordination analysis, Pfreundt et al. (2015) described significant differences in bacterial communities between M1 and the lagoon, but similar temporal dynamics. Direct comparisons of our export results with findings from open ocean studies

should be made cautiously as our mesocosms were both shallower (15 m) than in typical oceanic export studies (> 100 m) and exhibited reduced turbulence.'

2) In this work the authors looked at heterotrophic bacteria. Did the authors separate the free-living and attached fractions? Do the authors have any thoughts on how the attachment of bacteria to particles (I am thinking of those that are tightly associated (or attached) to the autotrophs in the mesocosms) might alter the fluxes? I am thinking specifically about the determinations of uptake of leucine into the Prochlorococcus and Synechococcus cell sorted fractions. Does the cell sorting separate off the associated bacteria? What about other small phytoplankton cells?

We did not separate free and attached bacteria when estimating BP. Given the amounts of aggregates embedded with TEP and UCYN-C cells, particularly during the second phase, it is clear that attached bacteria participated also to a large extent our BP rates. For cell sorting, the purity sort mode was used, which excluded cells with fluorescence different from the chosen window, so that other not targeted phytoplankton cells were excluded. This purity sort mode also excluded aggregates with more than one fluorescing cell. Heterotrophic bacteria were not naturally fluorescent and those adsorbed to *Synechococcus* and *Prochlorococcus* could potentially be sorted with cyanobacterial cells. We analyzed some sorted samples by epifluorescence microscopy after staining with DAPI and found no attached cells on the phytoplankton groups sorted; probably attached bacteria were more associated to detritus or small aggregates which were not sorted. With the settings used on the Influx flow cytometer, the drop frequency was 98500 per second. The frequency of free heterotrophic bacteria sorting 44, 336 and 511 sort per second for Hi-HNA, HNA and LNA cells, respectively. Thus the probability to have a drop including an heterotrophic cell in the phytoplankton sort was (44+336+511)/98500, i.e. less than 1%. It is thus unlikely that co-occurrence of free bacteria within autotrophic cells sorted would influence the leucine per cell activity determined for autotrophs.

The following sentence was added in supplementary section describing M&M for cell sorting:

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

3) The bacterial production estimates were all conducted in the light and in the discussion the authors provide an interesting discussion of the implications of the work. However, for the export calculations and for the BCD and BGE calculations, the authors determine daily rates of production by multiplying by 24h the rates. Did the authors check for differences between light and dark uptake of leucine? If N2 uptake occurs in the light (as with primary production), and if we suppose that the highest rates of DDN release occur during N2 fixation, then one could suppose that BP would be higher during the light due to the supply of readily available N, which would in turn give higher BGE estimates. Given the link between PP and BP that was observed here, it appears that this is at least true for primary production, but is this the case for N2 fixation? Can the authors comment on this aspect and on how it would alter their calculations? Indeed, it is rather intriguing that the heterotrophic bacterial production does not seem to be tightly coupled with DDN, despite the N limitation observed.

A large set of direct and indirect mechanisms could influence diel cycle of in situ production of heterotrophic prokaryotes. Among direct effects light variability (PAR radiation, UV radiation) influence heterotrophic bacteria, leucine assimilation by mixotrophic

cyanobacteria, photoheterotrophy. Among indirect effects, changes in the rythm of release of different qualities of organic matter can occur, due to the diel ryhtm of primary producers, viral lysis or heterotrophic flagellate grazing, as well as the photooxidation processes. Like the referee 1 suggested, it is also possible that a varying diel supply of N derived from diazotrophs would impose a diel cycling on heterotrophic prokaryotes, if processes are tightly coupled. Inside the mesocosms, different species of diazotrophs were present and exhibited successions (Turk-Kubo et al., 2015). Some of them are known to fix N_2 at night (UCYN-B and C), and others during the day (*Trichodesmium*, diatoms-diazotroph associations (DDA), UCYN-A). When DDAs dominated (during P1), fixation of N_2 was probably enhanced during the day and, during P2, when UCYN-C were dominating the diazotrophic community, fixation was probably enhanced at night. This work did not include high frequency sampling to investigate such hypotheses and their potential consequences on a diel rhythmicity of BP. However, some daily trends of BP have been determined by other authors in the lagoon, but using thymidine instead of leucine. Torréton et al. (2010) found a daily variability of BP-based thymidine of ~20 % when meteorological conditions were stable (i.e. no wind, no rain). Church et al. (2006) investigated the effect of light in samples from station ALOHA in the North Pacific and obtained no effects with thymidine, but described some effects with leucine. They attributed this discrepancy to the incapacity of cyanobacteria to assimilate thymidine. In the western Pacific close to the Marquises Islands, we determined diel cycles of BP using leucine and found a variation of 24 % (Van Wambeke et al., 2008c), with values of BP increasing during the afternoon–sunset period within surface layers and decreasing the rest of the diel period. We attributed these variations to changes in UVA and UVB doses, but penetration of UV was reduced in the mesocosms as the plastic walls stop lateral UV penetration. In order to estimate potential effects of a diel variability of BP, a sensitivity test was added in the discussion to test the consequences of a 25 % diel change in BP on the carbon budget (see below).

The following paragraph was added in the ms:

'For BP, we assumed a 25 % daily variability of BP based on studies from Torréton et al. (2010), Church et al. (2006) and Van Wambeke et al. (2008c).'

*4) The authors have used the relation of GPP= PP * 1.72. If I understand correctly this means that over 40% of gross primary production is lost to cellular respiration and the release of dissolved production. I saw that the article has a French reference, can the authors perhaps add a little explanation of how this value is determined and where it was determined. Given the importance of this calculation for the rest that follows (Fig. 7 and the calculations of respiration, etc), it is critical that more information is provided one how this is determined to allow the reader to see how the calculations in Fig. 7 and the associated text were made. Indeed, even small differences in this value will have an effect on the determination of the other factors, if I follow correctly the text. Perhaps a sensitivity calculation would be interesting here to show how robust the calculation is?*

In Moutin et al. (1999), an abridged section of the paper is written in English. The paper is available there in pdf format: http://mio.pytheas.univ-amu.fr/~moutin/IMG/pdf/ArticlesPDF/Moutin_et_al_99.pdf. In this paper, a model was developed to reconstitute the daily trend of primary production from experimental data based on Steemann-Nielsen (1952) ^{14}C methodology. The authors incubated waters from the western Mediterranean Sea for different periods of time during the day in different seasons. The resulting model allows to estimate 24h-fluxes (dawn to dawn) from hourly fluxes, independent of starting time, duration, or geographic origin of the samples and whatever the time of the year (i.e. systems with varying day-light periods). The use of this

model removes the general biases introduced by the large variety of incubation conditions used in the Steemann-Nielsen (1952) ^{14}C methodology (Regaudie-de-Gioux et al., 2014 and ref therein). A validation of the model with samples from the Atlantic Ocean at different seasons was presented at the 50 birthday of Steemann-Nielsen work in Bangor (2002). The other advantage of this model is that it allows to estimate both PP and GPP. For 24 h incubations, GPP is 1.72 x PP as determined from the model (Figure 5 in Moutin et al., 1999), and this value is also applicable anywhere as long as 24h-PP fluxes (dawn-to-dawn) are calculated using the same model (see for example Loisel et al., 2011). GPP in the Moutin et al. (1999) model represents the whole photosynthetic production of organic matter, as previously written in lines 16-19 p 19880 of the submitted MS. Loss terms correspond to all ^{14}C material not recovered on the filters, including extracellular release (excretion, lysis, sloppy feeding) and subsequent heterotrophic respiration after heterotrophic bacterial assimilation of ^{14}C-derived organic matter. However, the model is unable to differentiate between these different loss terms, mainly due to a non equilibrium state between ^{14}C and ^{12}C in the different phases.

The end of M&M section 2.1 on that topic was complemented as follows:

'Primary production (PP) was determined from short term (~ 4 h) incubations around noon with $H^{14}CO_3$ (see details in Berthelot et al., 2015) and a model of photosynthesis applied to calculate daily fluxes (Moutin et al., 1999). This model allows estimation of 24h-fluxes (dawn to dawn) from hourly rates, independent of starting time or duration of incubations, or of the geographic origin of the samples or of the time of the year (i.e. systems with varying day-light periods). This model avoids the general biases introduced by the large variety of incubation conditions used in the Steemann-Nielsen (1952) ^{14}C methodology (Regaudie-de-Gioux et al., 2014 and ref therein). Another advantage of this model is that it allows estimation of both PP (24h dawn-to-dawn) and Gross Primary Production (GPP). For 24 h incubations, GPP is 1.72 x PP as determined from the model (Figure 5 in Moutin et al., 1999). This constant is applicable as long as 24h-fluxes (dawn-to-dawn) are calculated using the same model.

About the sensitivity analysis, the following paragraph was added to the discussion section and a new table (S1) was added in the supplementary section.

'.... A more appropriate BGE of 27 ± 9% to 43% ± 11% could be calculated, based on minimum and maximum ranges admitted for the BR/CR ratio (from 100 to 50 %, Lemée et al., 2002), and based on propagation of errors due to the variability within triplicate mesocosms (Table S1). For sensitivity analysis of BR and BGE calculation from the carbon budget, we examined whether the errors of different methodological assumptions (conversion factors, analytical errors) were higher than those arising from variability between triplicate mesocosms. We considered different errors based on literature data for all the parameters used in computation of BR and BGE (i.e. GPP, Cexp, DOC, POC, and BP). For GPP, we assumed GPP = 1.72 x PP, i.e. PP/GPP ratio = 58 %. In the South Pacific Ocean, the mean average PP to GPP ratio (based on comparison between oxygen and ^{14}C technique) was 47% (Van Wambeke et al., 2008b). If we consider that the sum of dissolved and particulate PP in the lagoon (^{14}C technique, Rochelle-Newall et al., 2008) is a good proxy of GPP, then an upper limit for this ratio is 65 % in the lagoon. We thus applied a 15 % variability to the PP/GPP ratio, leading to GPP=1.36 x PP to 2.32 x PP, i.e. approximately a 30 % variability on the conversion factor. For BP, we assumed a 25 % daily variability of BP (Church et al., 2006, Van Wambeke et al., 2008c, Torréton et al., 2010). For Cexp, DOC and POC, we assumed analytical errors of 10 %. We then used propagation of errors to compute the error associated with BR and BGE (Table S1). For GPP, the errors resulting from triplicate mesocosms or resulting

from conversion factor and analytical errors were the same. According to the propagation of errors, the error associated with GPP has the largest effect on estimates of BR. The uncertainty of DOC, POC, and Cexp arising from variability within the triplicate mesocosms is higher than the methodological error, whereas it is the opposite for BP. Overall, the uncertainty of BGE estimates arising from variability within triplicate mesocosms or methodology is similar (27 % ± 9 % or 27 % ± 10 % for BGE based on BR= CR, 43 % ± 11 % or 43 % ± 12 % for BGE based on BR=CR/2).

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.

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

Specific comments:

19871, line 18: No vertical structure was observed in the water column in the mesocosm. Was this also the case in the water outside of the mesocosms?

We added the following sentence to this paragraph:

'Salinity and temperature measurements show that the water column was not stratified over the course of the experiment, except the first two days, which were characterized by a slight stratification both inside and outside of the mesocosms (Bonnet et al. 2016).'

Why is M3 so different?

A complete section of the introducting paper of the special issue (section 2.5 page 9 in Bonnet et al., 2016) is devoted to replicability among mesocosms, which was considered good for many biogeochemical stocks, fluxes and abundances of phytoplankton groups. Slight divergence in biological and chemical evolution among different replicated mesocosms is not uncommon, particularly after the first week of enclosure (Martinez-Martinez et al., 2006; Pulido-Villena et al., 2014). Here, M1 is also slightly divergent from the two other mesocosms considering the lower maximum PP and BP rates. The succession of the different planktonic populations exhibited a time lag in the three mesocosms, probably due to a combination of bottom up (availability of DIP and nitrogen), and top down controls (grazing pressure and viral lysis). The initial conditions prevailing before the DIP enrichment could be

also at the origin of slight divergence. Indeed mesocosms were closed 3 days before the DIP addition, and many species of diazotrophs exhibit a patchy distribution (Bombar et al., 2015). For instance, Hunt et al. (2016) noticed larger amounts of zooplankton individuals in M3 at the beginning of the experiment, some of which, stressed by the mesocosms, might have died (some larger amounts of 'swimmers' were recovered in the traps in M3), contributing to supplementary sources of N in M3.

19878, section 4.3: I am wondering if the authors measured the dissolved fraction of primary production during the PP measurement? The complete method is cited as being in an associated article, but it would be nice to have it noted in the text.

No, the dissolved fraction of primary production was not determined during this VAHINE mesocoms experiment. We referred to data published in the oligotrophic sector of the Nouméa lagoon by Rochelle-Newall et al. (2008).

19880, line 3: Coral mucus can also be an important source of organic matter in coral reef systems (Wild et al.)

I used the term benthos as a general term including corals. Note that Torréton et al. (2002) described possible excretion of mucus by coral. To be more explicit, the sentence is now modified as: 'excretion of mucus by coral has been proposed as a supplementary source of DOM for heterotrophic bacterioplankton in Pacific lagoons (Torréton et al., 2002, Wild et al., 2004).'

19878, line 25: change to "and" from "ad"

This has been corrected.

19879, line 9: maybe change the format of the () around log BP and log PP.

This has been corrected. We now write log (BP) and log (PP)

The authors do not really discuss the AP activity although it is present in 2 figures (3 and 4). Perhaps they can add a short interpretation of these results to the discussion. Do they have any thoughts on why it may have decreased in the lagoon on Day 5? Could there have been an input of P? From what source? Atmospheric deposition? This apparently can be an important factor in stimulating production and sinking flux in this system (Mari et al. 2014).

We added the following paragraph to discuss APA:

'A slight T_{DIP} 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_2 fixers might benefit from continuous and variable inputs of DIP sources in the lagoon waters during that period. This is also confirmed by the low values of alkaline phosphatase activity in the lagoon at the start of the experiment. Whether these sources were coming from the benthos (Torréton et al., 2002), the atmosphere (soot emission can influence lagoon waters inside and outside the barrier reef, Mari et al., 2014) and/or currents (Fichez et al., 2010) is beyond the scope of this study. Inside the mesocosms, when the DIP added was consumed, the increase of APA observed could be due to i) a population switch towards phosphatase producers, which can be heterotrophic bacteria and phytoplankton, and ii) increases in specific activities due to enzymatic induction; or both. We used POP as a proxy of living biomass (Duhamel et al., 2007) to estimate specific activities (nmole MUF-P hydrolyzed per unit POP per unit time) and found the same trend for specific activities and for bulk APA (i.e. specific activity increased up to 10-fold). APA was produced by different phylogenetic groups of heterotrophic bacteria, but also by cyanobacteria, as shown on a metatranscriptomic study in the special issue (Pfreundt et al., 2016), with the highest levels of alkaline

phosphatase transcripts originating from *Synechococcus* on days 14 and 20. Our results and those of Pfreundt et al. (2016) suggested a switch towards a microbial population that produced phosphatase to escape P depletion after a transient P-replete period. Although T_{DIP} decreased and APA increased up to values analogous to those observed in P-limited areas (Moutin et al., 2002; Van Wambeke et al., 2002, respectively), heterotrophic bacteria stayed continuously N-limited but not P-limited. As discussed in Pfreundt et al. (2016) and Pfreundt et al. (2015), some acquisition mechanisms of large P-containing organic molecules and reduction of cellular P quota also helped microbial communities to resist P depletion during P2 phase.'

Concerning Fig. 3, I am wondering if it is necessary that it is in the main body of the text. Perhaps it could be in the Supp. Mats section. At present there are quite a lot of Figs and Tables and as it shows almost the same information as is in some of panels in Fig. 4, perhaps it could be moved to the Supp mats.

We agree and moved Fig 3 as well as section 3.5 of M & M 'Cell-specific leucine incorporation rates' to the Supplementary Material. Numbering of figures has been changed accordingly.

Similarly, perhaps Table 2 can be removed as if I understand correctly the same information is presented in Fig. 5.

In fact, this does not show the same information. Table 2 presents log(BP/PP) = f (log(PP)), whereas Figure 5 (now Fig 4) shows log (BP) = f(log (PP)) and log BP= f(log (Chl)), so we would rather keep both, Table 2 and Figure 4, in the main body of the MS.

Can the authors add some lines to the graphs to show when P1 and P2 occurred in the Figs?

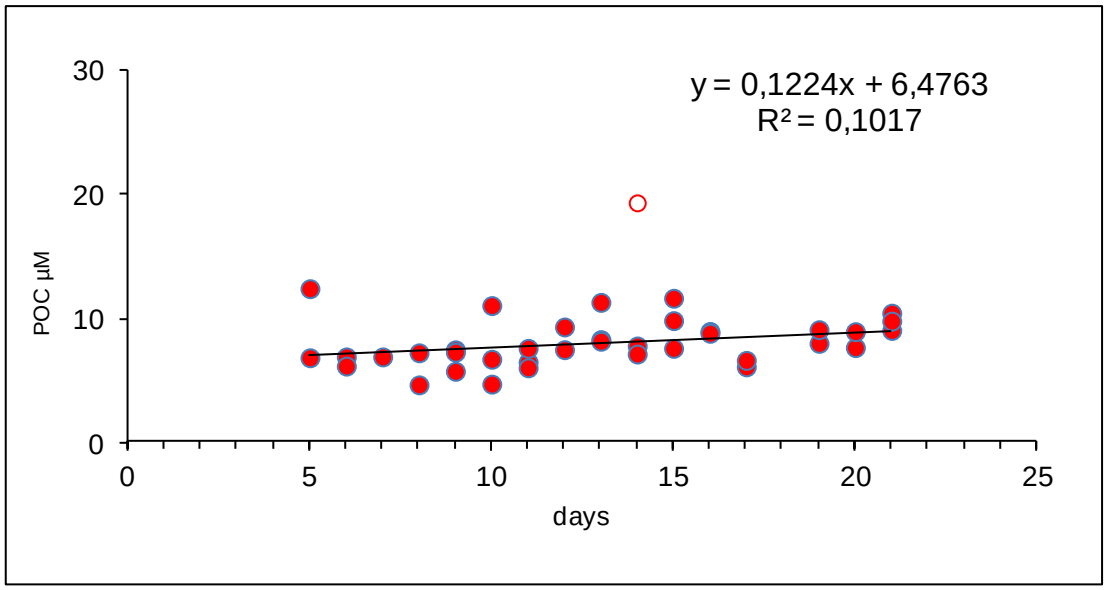
Phases P1 and P2 are now visible in the figures.

Which sample(s) are shown in Fig. 2?

This figure shows samples from day 23 in mesocosm M3. This information is now added to the legend of the figure. We also corrected the reference of the last sample in Table 3 accordingly, as it was badly referenced, we apologize for this error (it is day 23 M3 instead of day 23 M1)

Concerning Table 4, I appreciate the honesty of the authors to say that some outliers were removed and they provide the values. What do these outliers correspond to (manipulation error? Contamination? Resuspension?) How was it determined that they were outliers?

It was impossible to distinguish between a contamination during sampling or a patchy distribution of aggregates in the volume filtered for POC measurements, as only one filter per sample was available. The samples considered as outliers pointed out from the POC trend with time and/or one of the three depth samples showed a value very different from the two other ones. See for example the plot below describing the POC trend in M2:



In the conclusions, the authors also refer to the need for techniques that are adapted to the measurement of bacterial respiration in the light. Have the authors looked at the work of Pringault et al. from the Southwest Lagoon of New Caledonia?

Yes, we know this work, which suggested that respiration is higher in the light. However, this paper does not discuss photoheterotrophy and thus we just cite it in our conclusion as an example of alternative technique as follows:

'The relative importance of mixotrophy in these oligotrophic systems 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.'

Response to the Referee 2

The paper by Van Wambeke et al. is one of a series of papers describing the complex mesocosm experiment VAHINE organized by S.Bonnet in New Caledonia. This paper deals with the coupling between N2 fixation and bacterial production in the mesocosms. I have to admit I am not an expert on quantitative dynamics of heterotrophic bacterial communities and I do not follow this field in detail. I always felt that the quantitative estimates of turnovers and efficiencies of bacterial stocks involve too many assumptions that question the validity of the results. Still, I found that this paper uses some interesting and for me new approaches that I am happy I had a chance to read the paper.

To give some constructive criticism, I feel that there are some unnecessary overlaps with other papers in the VAHINE series. For example, the abstract states that in this paper the authors examined relationships between N2 fixation rates and primary production. However, this was done in detail in the accompanying paper by Berthelot et al. Also, some data from Berthelot et al. paper are repeated here.

We apologize for the error in the abstract. The sentence is corrected: 'We specifically examined relationships between N_2 fixation rates and heterotrophic bacterial production'

When writing for publication in a special issue we must find an equilibrium between redundancy and the necessity to write as much as possible a self-explaining article. For this reason, we decided to plot figures describing time evolution of PP and Chlorophyll because we largely developed BP-PP and BP-CHL relationships in this MS. T_{DIP} data are also described in the paper by Berthelot et al. (2015) but we also recurrently refer to T_{DIP} as a good indicator of DIP availability and compared it to our estimates of APA. Note that Berthelot et al. (2015) plotted 3 days moving averages whereas we used depth averages.

Text p.19874, l 20- I found confusing the description of data for cell-specific rates of leucine incorporation. Where are the data for heterotrophic bacterial phytoplankton LNA, HNA and hi-HNA shown? Table 3 provides data for autotrophic phytoplankton cells. Since this constitutes important part of the paper, I strongly recommend to clear this issue in the text and in the Table 3.

We added values of cell specific incorporation of leucine for the heterotrophic bacterioplankton groups LNA, HNA and hi-HNA in Table 3.

As far as I can see, the authors mix bacteriochlorophyll and rhodopsin. The paper by Hauruseu and Koblizek (2012) studied photoheterotrophic AAP bacterium Erythrobacter that contains bacteriochlorophyll, not rhodopsin. The contribution of photoheterotrophy to overall energy budget of AAP bacteria is different from flavobacteria containing proteorhodopsin like SAR11. So I suggest the authors correct this in the discussion text on p. 19881 and in the abstract as well.

It is a pity the experiment did not look for the response of bchl containing AAPs to the nutrient enrichment. Also, at the time of the review I could not locate the paper by Pfreundt , Spungin, Bonnet, Berman-Frank and Hess that should be already on the BGS web.

We agree with the referee and apologize for the error in the citation of Hauruseau and Koblížek (2012). The paper by Pfreundt et al. (2016) is now available on the BG site. The discussion has been changed as follows:

'... A more appropriate BGE of 27 to 43 % could be calculated, based on minimum and maximum ranges admitted for BR/CR ratio (from 100 to 50 %, Lemée et al., 2002). Such values could potentially be related to a beneficial effect of photoheterotrophy. Indeed, in a

companion metatranscriptomic study performed in M1 (Pfreundt et al., 2016), accumulation of proteorhodopsin transcripts was recurrently detected among varying groups of bacteria notably Pelagibacteraceae and SAR86. These groups, belonging to the alpha- and gammaproteobacteria, respectively, were also abundant community members as observed through 16S sequencing (Pfreundt et al., 2015). Aerobic anoxygenic phototrophic (AAP) bacterial abundances are reported to be particularly abundant in the South Pacific Ocean (Lami et al. 2007), but to date, AAP abundances are not available in the lagoon and they were not counted in this experiment. Nevertheless, Pfreundt et al. (2016) detected expression of the *pufM* gene, encoding a photoreaction centre protein of AAP bacteria. Transcript abundances were an order of magnitude lower than for proteorhodopsin, and only observed for a group of *Rhodocyclaceae* on day 14 and much weaker for *Rhodobacteraceae* on day 18. This suggests that AAP bacteria did not play a major role in the investigated system and did not influence the above calculation to a large extent. *Dokdoni*a sp. strain MED134, proteorhodopsin containing flavobacteria, were shown to increase the maximum number of cells reached when growing in the light compared to darkness. However, if DOM was added initially, light vs dark responses changed depending on DOM concentrations (Gomez-Consarnau et al., 2007). Other laboratory experiment, at the opposite, showed no difference in growth rates or maximum cell yields of *Pelagibacter ubique* cultures grown in natural seawater (in in a diurnal light regime or in complete darkness (Giovannoni et al., 2005). The BGE of a bacteriochlorophyll-containing strain (*Erythrobacter* sp.) was shown to increase during light periods in a continuous culture (Hauruseau and Koblížek, 2012). Thus the energy benefits of photoheterotrophy remain controversial, and related to the difficulty to have true oligotrophic conditions in pure culture. Based on an energy budget, Kirchman and Hanson (2013) suggested that the net energy gained by light is mostly sufficient to meet maintenance cost of AAP but is not enough to meet that of proteorhodopsin-based phototoheterotrophic bacteria. Heterotrophic bacteria are limited by N but also by energy in the South Pacific (Van Wambeke et al., 2008a); this could give an advantage to photoheterotrophic prokaryotes for growth and their success in this area.'

The abstract has been completely rewritten

I am missing some discussion / explanation for the data that are out of the trend, for example, what happened on day 11 with HBA abundances?

This sentence was added in section 3.2: 'Peaks of HBA were sporadic, like on day 11 in M1, but not repeated for the three depths sampled. They were possibly due to the presence of a patchy distribution of aggregates that could have biased some of the results. These peaks are occasional, and as they might reflect the reality of a patchy distribution, they were kept in the figures, statistics and estimates of means per day.'

Why was the mesocosm M3 so different?

Please see our response to referee 1 on that topic.

Regarding the model calculations for net autotrophy shown in Fig.7 – I found it highly speculative. The overall balance depends on the assumption that GPP= PP x 1.72. But is this valid for the situation in the NC lagoon? The relation between GPP and PP (better NPP) given as NPP/GPP can range anywhere from 0.2-0.6...

Note that if GPP=PPx 1.72 then PP/GPP=1/1.72=0.58, i.e; 58%.

I agree that NPP should be a more appropriate term. However, PP is the term used in the other papers of the special issue, and we need to be consistent. Please see our response to referee 1 on that topic.

Minor comments:

p.19866, l.27: "located 28km off the coast"
This has been corrected.

p.19872,l.7: "occurred during P2"
This has been corrected.

In the Legend to Fig.7, the Bacterial Carbon Demand is wrongly abbreviated as DCB
This has been corrected.

Response to the Referee 3

General comments

The study is well designed but everybody knows that experiments are far from reality although are needed for understanding how communities behave when are submitted to driving changes. However, what it bothers me is the high variability among these three replicates. M3 presented higher values than the other two mesocosms for chl a and PP, while for APA showed lower values. There is any evident reason for this lack of replication? Of course part of this variability disappeared when averages are taken into account and results are clear and conclusive.

Please see our response to referee 1 on that topic.

Authors, please, clarify whether the results derived from this experiment, are explaining the importance of N2 fixation in the South Pacific. In other words what is the advance of the knowledge in this topic after applying this study?

This study confirmed that in the Nouméa lagoon, N_2 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. Bacterial production was strongly coupled with Chl biomass and/or PP, rather than with N_2 fixation rates, suggesting that indirect routes through lysis, grazing and mortality of non-diazotrophic phytoplankton were substantial for providing labile organic matter for heterotrophic bacteria. These sentences are presented in the conclusion.

Specific comments

Abstract

Perhaps authors should start the abstract with a more explicative sentence instead of this statement, because it is not clear the reasoning of the text.

We reorganized the abstract

In line 2 an "is " is lacking before "designed"

This correction has been made.

Line 4: picoplanktonic instead of picoplanctonic.

This sentence has been corrected.

Line 24: . . . processes, like mortality, lysis and grazing are from phytoplankton?

Yes. The sentence has been corrected.

Introduction

Page 19865 Are you sure that waters of the New Caledonian lagoon are really representative that what happen in the surface waters of SW Pacific?

The offshore oligotrophic part of the lagoon can be influenced for some periods by coastal, benthos input or by atmospheric deposition of nutrients and organic matter. The lagoon is semi-enclosed and thus also highly influenced by oceanic waters. The lagoon waters are known to be primarily N-limited and present, low nutrient, low chlorophyll biomass and intermittent blooms of N_2 nitrogen fixers, which were sufficient conditions to use the offshore oligotrophic part of the lagoon as a reference N-limited site for our experiment. The introduction section was re-written completely.

Results

Authors said that very little stratification is observed in the mesocosms, and in the lagoon?

We added the following sentence to this paragraph:

'Salinity and temperature measurements show that the water column was not stratified over the course of the experiment, except the first two days, which were characterized by a slight stratification both inside and outside of the mesocosms (Bonnet et al. 2016)'

Page 19874 Authors said: APA in the lagoon waters exhibited the strongest increase between day 10 and 11 and stayed at this higher level until day 23. Question: it this means that the community is highly P limited?

Bacterial production was primarily N limited as deduced form enrichment experiments. A discussion on APA has been added in the MS, see response to referee 1.

Discussion

Page 19876, line 20, delete first "much"

This has been corected.

Page 19879, line 20, is Alonso-Saez

This has been corrected.

Figure 4b is difficult to follow the lagoon waters

We did our best to see dots better. Maybe a problem of pdf format.

Response to the Referee 4

Detailed comments: The authors should address better in the discussion the differences between the three mesocosms. Considering the large variation between M3 and the other mesocosms.

Please see our response to referee 1 on that topic

The authors should address the alkaline phosphatase results in the discussion or remove from the manuscript if not.

We added a paragraph of discussion on APA (see response to referee 1). Note that links between APA and taxonomic succession and AP gene expression are also presented in Pfreundt et al. (2016) and Pfreundt et al. (2015).

The authors should discuss briefly that they only analyzed the free living fraction of the bacterioplankton but that particle associated bacteria may have a significant role in the system.

With the centrifuge technique 1.5 ml is analyzed which includes both free and attached bacteria. We recognize that activity associated to big aggregates which are not sufficiently represented in a 1.5 ml sample should have been omitted from our BP estimates.

I find the abstract misleading and I think that results from other papers presented in the special issue should not be introduced here but kept to the discussion.

The abstract was completely reorganized.

Technical comments:

P 19863- Line 2: The VAHINE mesocosm experiment "was" designed (...) L 13-15: rephrase this sentence

This sentence is removed in the new version of the abstract.

P 19864- L 16-17: is 27-43% BGE what you find or what is found in oligotrophic environment? Rephrase accordingly

These values are BGE that we found. The sentence was rephrased in the new version of the abstract.

L20: to not be sufficient

The sentence was modified as 'dissolved phytoplankton release was not sufficient to sustain bacterial carbon demand'.

In the nutrient addition experiment the treatments are not named the same way between methods and discussion. Be consistent and use C or G for the glucose addition.

We corrected for 'C' everywhere.

Part of the C budget paragraph (method and results) could be moved to the results section. It is a bit heavy on the discussion section.

This has been done.

Fig. 3 and 4: Show the addition of DIP and P1 and P2 phases.

Grey areas have been added to distinguish P1 and P2 phases.

Fig. 4: Chl.a axis, use point not comma.

This has been done.

What about the bacterial abundance in the lagoon waters?

We analyzed some of the frozen samples recently (one every other day) and added corresponding data on the Figure.

Fig. 5: use the same legend for both graphs, in general use out or lagoon water throughout the figures and tables

This has been done.

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