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Biogeosciences Discussions

Interactive comment on "Nutrients limitation of primary productivity in the Southeast Pacific (BIOSOPE cruise)" *by* S. Bonnet et al.

S. Bonnet et al.

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Referee 2.

Comments on the manuscript Nutrient limitation of primary productivity in the Southeast Pacific (BIOSOPE cruise) Authors: Sophie Bonnet, Cécile Guieu, Flavienne Bruyant, Ondrej Prasil, France Van Wambeke, Patrick Raimbault, Carolina Grob, Thierry Moutin, Max Y. Gorbunov, Jonathan P. Zehr, Sylvie M. Masquelier, Laurence Garczarek, Hervé Claustre

Dear reviewer,

We are pleased to provide below answers to your comments, as well as a revised version of our manuscript. We made our best to take into consideration your different comments have been very constructive for us.

General comments.

Methods.

More details have been given on the techniques the less described in the first version of the manuscript:

- For primary production measurements, the following paragraph has been added: After each time point, 250 ml of each microcosm has been subsampled for primary production determination. Primary production has been quantified according to the experimental protocol detailed in Moutin Raimbault (2002). Each sample (Three 250-ml polycarbonate bottles) was inoculated with 0.37 MBq of NaH14CO3 (Amersham CFA3) and incubated in a deck incubator equipped with a Nickel screen (50

- For nutrient analysis, the following paragraph has been added: Ambient nutrient concentrations have been measured at each of the three stations before the incubation experiments; they have also been measured during the incubations. Nitrate, nitrite and phosphate concentrations have been analysed using a Technicon Autoanalyser II (Treguer and Le Corre, 1975). The measurements in the nanomolar range (lower detection limit = 3 nmoles l-1) were obtained from a sensitive method according to Raimbault et al. (1990). Nitrate at submicromolar levels (detection limit 0.05 μmoles l-1) and phosphate (detection limit 0.02 μmoles l-1) were measured according to Armstrong et al. (1967). Ammonium concentrations were measured using the sensitive method of Holmes et al. (1999) having a detection limit of 5 nmoles l-1. Silicate concentrations were determined on land on poisoned samples (mercuric chloride 1 μg ml-1) four months after sampling.

- For the experimental setup in general, more details have been given on the different treatments performed at each station, the starting time and the duration of the experiments, the parallel incubations for rate measurements and the light conditions. This paragraph has been transformed as follows: All experimental setups were performed under strict trace metal clean conditions inside a clean container. Seawater

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was collected at 30 m depth using a trace metal-clean Teflon pump system and was dispensed into acid-washed 4.5-liter transparent polycarbonate bottles. Under a laminar flow hood, nutrients or dust were added either alone or and in combination: +Fe, +NPSi and +FeNPSi at station HNL, +Fe, +N, +P, +FeN, +FeNP and +dust at stations GYR and EGY. The final concentrations were 1 μmol l-1 NH4+, 2 μmol l-1 NaNO3-, 0.3 μmol l-1 NaH2PO4, 2 nmol l-1 FeCl3 and 0.25mg l-1 of dust. Despite that Saharan event was unlikely to occur in the Southeast Pacific, the dust used in this experiment was the Saharan soils collected and characterized by Guieu et al. (2002) in order to allow a comparison with earlier efforts (Mills et al., 2004; Blain et al., 2004, Bonnet et al., 2005). Each fertilization was performed in triplicate. The bottles were immediately capped with parafilm, sealed with PVC tape, and incubated for 48h in an on-deck incubator with circulating surface seawater at appropriated irradiance (50

- Small volumes used for N2 fixation measurements. During the BIOSOPE cruise, uptake of 3 forms of nitrogen was performed simultaneously: nitrate, ammonium and dinitrogen. And we were not able to work with large water samples. But we have defined an analytical protocol and a detection limit to take into account this problem. All the procedure is explain in a companion paper (Raimbault and Garcia, this issue). See below: The dual isotopic enrichment analysis were performed on a Integra-CN mass spectrometer calibrated with glycin references every batch of 10-15 samples, with a very low detection limit of 3 μg N (0.2 μmoles N, corresponding to this given by the manufacturer) See figure below

The accuracy of our analytical system was also regularly verified using reference materials from the International Atomic Energy Agency (AIEA, Analytical Quality Control Services).

REFERENCE results obtained with our mass spectrometer IAEA 310A Urée delta15N = 478240; 47.3 ś 0.27 8240; IAEA 310B Urée delta15N = 2448240; 243 ś 0.46 8240; IAEA 309A Glucose delta13C = 93.98240; 94.3 ś 0.66 8240; IAEA 309B Glucose delta13C = 5358240; 532 ś 2.0 8240;

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The mean delta15N does not vary between 0.2 and 10 μmoles N. Then, the low background of the system allows to analyse safely samples containing low nitrogen concentrations (0.2 μmole = 2.8 μg N), values often observed in surface oligotrophic waters.

Finally, tThe 15N isotope enrichment of a sample is reported in term of atom Therefore, value of time zero enrichment is necessary and determined with samples (same volume as incubated sample) which are filtered immediately after isotope addition. For N2 experiments, time zero value, established with 8 samples, was 0.3676 ś 0.007Due to natural variation of delta15N (-5 to 158240;), We considered as significant, results with 15N excess enrichments higher than 0.014 Finally, according to the experimental conditions, the detection limit for nitrogen fixation, calculated from significant enrichment (0.014

- The volume filtered for nifH analysis is 3L. This information has been added in the Methods section in the version of the manuscript.

Results.

- A statistical test has been done to prove that the FeN treatment at station EGY is different from both the Fe and N treatments. This test has been done for every treatment and every station and different letters have been placed above the bars for treatments showing different responses, as in Mills et al., (2004). - The only treatments for which we have measured the DFe concentrations in the incubated samples are the ones amended with dust at station GYR, in order to determine the percentage of Fe dissolved. As indicated in the text, the dissolution of Fe is extremely low in the SE Pacific compared to the values we found in the Mediterranean Sea (at the same dust concentration). We interpreted this difference by the difference in organic ligands concentrations between the Pacific and the Mediterranean waters (Bonnet, 2005; Mendez et al., 2007). The DFe measured at T24 and T48 in the dust treatments at GYR are consistent and indicate a good reproducibility at low values, indicating the absence of any unintended contamination during the course of the experiment. Note also that the

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3 replicates of the control at the 3 stations and the 2 time points always indicate good reproducibility for any of the parameters measured. If any unintended contamination had occurred, it would have given random and inconsistent results. Please note also that all the handling have been performed using trace metal clean techniques in a clean container, all the bottles were capped with cleaned parafilm and were sacrificed after each incubation time in order to avoid contamination in the bottles. The same type of experiment has been performed in the Mediterranean Sea and in the Atlantic Ocean by the same people (Guieu, Blain, Bonnet...) without any contamination.

Discussion.

- We totally agree with the comment related to the interpretation of Fv/Fm. It has been shown using continuous cultures mimicking the conditions of a stable natural environment, that Fv/Fm does show constant high values over a wide range of growth rates including cultures in nutrient limited conditions (Fig. 5 in Cullen et al. 1992). Further phytoplankton culture work has since then show how Fv/Fm was not a very good indicator of nutrient stress (Parkhill et al. 2001). In summary high values of Fv/Fm (between 0.5 to 0.63) are a good indication of nutrient replete status OR nutrient limited phytoplankton population in balanced growth (this being obtained using semi-continuous or continuous cultures to obtain steady-state); while low values of Fv/Fm (below 0.5) are a good indication of a phytoplankton population being in UN-balanced growth AND nutrient stressed (Macintyre et al. 1997; Parkhill et al. 2001).

- The fact that Raimbault et al. have measured during the cruise significant N2 fixation rates while they are below detection limit in our experiments may have different explanations:

(Note that the 15N experiments as well as the analytical procedure were the same during the regular stations and our incubation experiments 8211; all the samples have been analyzed by Patrick Raimbault).

o As pointed out by the reviewer, our incubation experiments were performed at 30m

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depth and do not include information on vertical variability of N2 fixation. In the gyre (stations GYR and EGY), the significant N2 fixation rates measured by Patrick Raimbault are in surface waters (5m). At 30m depth, the rates he has measured are extremely low and close to the detection limit (like the values he measured in our incubations) o Nitrogen fixation in the ocean is characterized by quite heterogeneous distribution. The N2 fixation data reported by Patrick Raimbault available for stations HNL, GYR and EGY were not performed at the same day than the measurements he performed in our experiments. These 3 stations represented actually long stations during which we stayed from 3 to 5 days on site. It is not possible that N2 fixation were patchy enough so he could measure rate just above the detection limit in his samples and just below the detection limit in our samples at 30m-depth.

- The nifH abundances data on table 2 of our manuscrips indicate the quasi absence of nitrogen fixing cyanobacteria in our portion of interest (station HNL to station EGY). However, it has to be noted that the molecular data indicate large numbers of diazotrophic cyanobacteria at the beginning (Marquesas) and at the end (Chilean upwelling) of the transect, where Patrick Raimbault measured the highest N2 fixation rates. o Marquesas: 342 copies per liter of groupeB, 45 copies per liter of Trichodesmium and 178 copies per liter of Cheat/Calothrix. o Stations 20 and 21: 108 copies per liter of groupe B and 20 copies per liter of Trichodesmium (These data are not included in the present manuscripts because these areas are out of our area of interest) In between these two extremes, molecular techniques indicate only very low or undetectable diazotrophic cyanobacteria. However, these techniques allowed to detect heterotrophic diazotrophic bacteria, that could be responsible of the N2 fixation rates measured by Patrick Raimbault (these rates being low in the middle of the transect). o Station HNL: sequences related to Vibrio diazotrophicus o Station GYR: alpha and gamma Protebacteria o Station EGY: sequences related to Vibrio diazotrophicus These data have been added in the Results section of the manuscript.

The result section related to the abundance of nitrogen fixers has been changed as

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follows:

3.3.3 Abundance of nitrogen fixers Water samples from 13 stations situated between HNL and EGY (Figure 1a) were examined for presence of N2-fixing microorganisms by amplification of the nifH gene. After amplification, cloning and sequencing the nitrogenase genes, our results indicate the absence of the filamentous cyanobacteria Trichodesmium, or any large (3-7 61549;m) unicellular putative nitrogen fixing cyanobacteria (Group B). The results suggest however the presence of low numbers of Group A cyanobacterial phylotypes at two stations (less than 200 copies l-1, Table 2), and low numbers of non-cyanobacterial nifH sequences (Vibrio diazotrophicus and proteobacteria), that must explain the significant dinitrogen fixation rates measured by Raimbault et al., (2007) during the transect. It has to be noted that the molecular data for both extremities of the transect (data not related to these incubations) indicate the presence of larger number of cyanobacteria close to the Marquesas archipelago (up to 342 copies l-1 Groupe B, 45 copies l-1 Trichodesmium and 178 copies l-1 Cheat/Calothrix) and close to the Chilean upwelling (108 copies l-1 Groupe B and 20 copies l-1 Trichodesmium). These higher densities of diazotrophs are consistent with the higher dinitrogen fixation rates measured by Raimbault et al., (2007) at both these extremities of the transect.

A sentence has also been added in the discussion section: At the other stations located between stations HNL and EGY, diazotrophic heterotrophic bacteria detected by molecular tools must be responsible of the dinitrogen fixation rates measured at regular stations by Raimbault et al., (this issue). - Discrepancy between our nitrogen fixation results and recent models predictions (Deutsch et al., 2007): we are in contact with Curtis Deutsch since his paper came out in January 2007. His paper is based on a geochemical approach at global scale, considering N and P inventories. As Curtis Deutsch says himself, his maps of inferred N2 fixation should not be interpreted pixel-by-pixel but rather for the basin scale patterns. For example in the South Pacific, the hot-spot that appears off Peru does not appear nearly as strong under different model

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circulation/mixing schemes, whereas the large-scale disappearance of excess PO4 in surface waters traversing the tropical South Pacific is a robust signal no matter what circulation model is used. That doesnt mean that N2 fixation is happening in the gyre, which is the furthest away from nutrient sources of all types (P, Fe, etc.). However, it has to be noted that our study took place in the late spring and we cannot exclude higher nitrogen fixation rates later in the season (summer).

Minor comments.

- The title has been changed as suggested: Nutrient limitation of primary productivity in the Southeast Pacific (BIOSOPE cruise)

- The terms minimum threshold concentration has been deleted. The new sentence is The nutrients requirements also vary among different phytoplanktonic species.

- The reference Claustre et al has been added in the list

- The + between NH4+ and 2 μmol l-1 NaNO3- has been replaced by a comma

- Page 2744, line 13: the sentence has been replaced by even after dust, Fe, and FeP additions (Table 2). - Of course, mg14C mgChl-1 has been replaced by mgC mgChl-1 on figure 2. - Legend of Table 2: nif has been replaced by nifH. The superscript a has been explained as well as the asterisks in Table 3.

Thank you very much for your attention to our study,

Sincerely,

Sophie Bonnet

Interactive comment on Biogeosciences Discuss., 4, 2733, 2007.