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Interactive comment on "Phosphate monoesterase and diesterase activities in the North and South Pacific Ocean" *by* M. Sato et al.

Anonymous Referee #1

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The idea behind the manuscript by Sato et al. is an interesting one, looking at the distribution of hydrolytic activities of phosphate monnoesterase and diesterase across the open Pacific Ocean. However, I believe that some particular issues should need to be further clarified before this manuscript is accepted for publication in Biogeoscience.

What is the depth range of the subsurface chlorophyll maximum (SCM) layer? It would be important to know how big was this range since the study covers a wide area, where significant changes in the SCM depth between stations could occur. This could be relevant assuming that all the incubations were done at the 10 m depth temperature; therefore, if there was a strong variability in depth among the SCM samples, a differential effect of temperature incubation could have happened, maybe explaining why the pattern obtained at 10 m was not so clearly observed at the SCM.

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The final concentration used (1 μM) for the fluorescence substrates could be too low. In fact, the authors say that this concentration is in agreement to previous studies, but they do not mention that there are other reports in the same region using considerably higher concentration. Just to give an example, Koike & Nagata (1997 Deep-Sea Research II, Vol. 44. pp. 2283-2294) used 150-200 μM. Moreover, even in the Suzumura et al. (2012) (one of the three examples they provide) measurements were taken on all samples to determine Vmax with an excess concentration of the substrate (200 μM), even though when Suzumura et al. were using 50, 100, 200, 500, and 1000 nM MUF-P concentrations too determine the other kinetic parameters.

Another thing that requires more explanation is the strong discrepancy observed between the data shown in Fig. 3 (i.e., the MEA rates directly obtained) and the data from Table 1 (the Vmax calculated from the 5 stations in which kinetics were done). In theory those values should be more or less in a similar range. However, in Fig. 3A,C rates were up to 50 nmol μg-1 h-1 (according to the scale bar of the figure) whereas in the kinetics (Table 1) the Vmax obtained were much lower (ranging from 0.36-8.29 nmol μg-1 h-1). This might suggest a problem in the calculation of the kinetic parameters or maybe an error in the final concentrations of substrate used.

Even more critical is the fact that the method behind the main novelty of this manuscript, the distribution of open ocean diesterase activity, does not seem to be very reliable. The authors already mention in the methods section that the Bis-MUP can overstimate the DEA, since one molecule of Bis-MUP can release two molecules of 4-MUF and being afterwards catalyzed by monoesterases (and not by diesterase). I think this is quite relevant, and the authors should have included a empirical validation of this method, quantifying these processes in order to better constrain the real DEA rates. Moreover, the Km obtained for the DEA (Table 1) further suggests that the 1 μM (maximum final concentration) used was too low, since Km were most of the time higher that 1 μM (in fact, Km reached up to 7.73 μM). Moreover, there is generally a strong effect of the concentration range that is used for a kinetic assay and the kinetic parameters that are

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calculated; meaning that if higher concentrations that 1 μM would have been used for the kinetics the resulting Km would probably had been even higher. This has strong implications for the manuscript. For example, the authors wrote that "MEA was more than three times as high (3.1 to 19.4 times at 10 m, 4.5 to 18.2 times at SCM) as DEA at all the stations during the KH-12-3 cruise (Fig. 4), suggesting that the phosphate monoester was a much more important phosphorus source for microbes in the surface waters than the diester"(p. 10103). However, DEA was probably higher since the DEA assay was done with not saturating concentrations.

Another issue that might be relevant is the use of Chl-a as an index of microbial biomass. Since the study covered such a wide area, in which phytoplankton and heterotrophic bacteria are known to change so much, I wonder what the effect of that assumption may have in the results obtained. In fact, heterotrophic bacteria are know to be key users of alkaline phosphatase in the ocean, and a strong change in the Chl-a concentration does not necessary imply a sudden change in the biomass of heterotrphic bacteria. Moreover, the authors found that "In general, since the areas with high phosphate esterase specific activities are characterized by a low chlorophyll a concentration, absolute values of MEA and DEA (not normalized by chlorophyll a concentration) showed smaller horizontal variations (data not shown). Similarly, differences in volumetric esterase activities between 10 m and SCM were relatively small compared with those in specific activities". This raises the question of whether the differences reported by the authors are reflecting real changes in the enzymatic activities or just changes in the phytoplankton biomass.

In Fig. 6 (showing the relation between MEA, DEA and SRP) there are just 8 data points, whereas in Table 1 (where the data for this plot were originally obtained from) there are 10 points. I wonder what happened to the two missing data points, and how including these two points would affect the function parameters. Moreover, the fact that "when Vm was not normalized to the chlorophyll a concentration, the relationship was insignificant ($p > 0.05$, data not shown)", raises the question of whether the relation

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between SRP and enzymatic activities is more due to a strong effect of the use of Chl-a as an index of microbial biomass than to changes in enzymatic activities at all.

The statistical support (p-value) is missing in most of the graphical comparisons shown (Fig. 2, 5, 6 and sometimes also the R2 is not provided). Moreover, in Fig. 2A, where the proportion of dissolved relative to total MEA is obtained, there is one point that is probably affecting the slope obtained (and therefore the calculated proportion of dissolved MEA). I wonder if that fitting line is significant, and how would the slope of that line change if that point was discarded.

Finally, when discussing Fig. 3 in the text the authors talk about latitude and longitude to refer to the stations, but not latitude or longitude data is provided in that Fig. 3.

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