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

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In the introduction the authors wrote, "datasets on phosphorus cycling across different ocean basins are still lacking".
As pointed out by the referee, "still lacking" was inappropriate. We changed the phrase to "scarce".

The next sentence is not true either: ". . . no reports on cycling or utilization of phosphate diesters. . .": see for example the work of Bjorkman and co-authors at Station ALOHA in the North Pacific subtropical gyre.
According to the comment, we added the citation to Bjorkman et al. (2000).

The sampling location is poorly described.

We added the list of sampling stations as Table 1. This includes the location of sampling sites, depth of SCM, and temperature and chlorophyll a concentration at 10 m and SCM. SCM was determined from the profile of chlorophyll fluorescence. When the peak of the fluorescence was not obvious, the bottom of the surface mixing layer was adopted as SCM, as described in Materials and Methods.

As I said earlier I find the enzymatic assays far from straightforward, particularly for diesterase.
To correct for the second decomposition of diesters, we calculated the maximum hydrolysis rate of the monoester formed by the hydrolysis of the diester substrate using kinetic parameters of MEA at five stations. This is based on the assumption that the increase in the fluorescence intensity resulted from the cleavage of fluorescent moieties from the diester substrate. The calculated maximum contribution of the second hydrolysis was $<$3% of total DEA at stations other than Stn. 9. At Stn. 9, the contribution was relatively high, $\sim$16%. We also corrected for the effect of incubation temperatures using Q_{10} values of 1.5 and 2.0, and confirmed that the general trend of both MEA and DEA activities and the relationship between the activities or kinetic parameters and SRP concentrations. Actually, as pointed out by the referee, substrate concentration was lower than the saturating concentration. It may be insufficient to measure maximum hydrolysis rate at some stations. Therefore, the expression was changed and I describe that it was somewhat an underestimate as an index of maximum hydrolysis rate.
Kinetic parameters were calculated using four data points, including blank measurements (origin), which are mathematically sufficient for estimation of two parameters. The graph (fig. 1) is a data plot of DEA kinetics at 10 m of Stn. 17. We can see that the enzymatic activity was detectable at the lowest substrate concentration, and fitting was successful.
High-sensitive measurement of LDOP was described in detail in Hashihama et al. (2013), as cited in the manuscript. The detection limit of the method is sufficiently low compared to the substrate concentration added (50 nM). Since several zero values

of LDOP were inappropriate, the values below the detection limit were regarded as 3 nM. Of course there is a problem resultant from the specificity of commercially available enzymes. However, it would be more accurate than the estimation based on the assumption that a constant proportion of total DOP is enzymatically labile (e.g. Duhamel et al., 2011).
As the referee pointed out, there have been more studies in which bacterial cell concentration or carbon biomass was used to normalize enzymatic activities in the ocean. In the present study, we used chlorophyll *a* concentration, an index of autotrophic biomass, instead. This is because we had a more extensive data set of chlorophyll *a* than that of bacterial cell concentration, which has missing figures at some sampling stations. First, as cited in the first manuscript, the trans-Atlantic study by Mather et al. (2008) revealed that the data pattern of APA activity did not change significantly whether normalized by chlorophyll *a* concentration, bacterial cell concentration, or sum of autotrophic and heterotrophic biomass. Moreover, some other field studies used a chlorophyll *a* concentration as a denominator of APA activity (Dyhrman and Ruttenberg, 2006, Limnol Oceanogr; Villareal et al., 2007, Deep-Sea Res I) and showed a temporal or spatial variation of APA. As shown in Table 1 of the revised manuscript, chlorophyll *a* concentration within the subtropical gyre varied within a relatively small range (by 2 times). Therefore the change in specific MEA and DEA activities in these areas are considered to reflect real changes in enzymatic activities rather than phytoplankton biomass. High concentration of chlorophyll *a* was observed in the equatorial upwelling area or the transition area to the subtropical gyre, where the surface SRP concentration was higher than 200 nM. These data points are removed from the regression analysis in Figs. 5 and 6. From these, we considered that chlorophyll *a* concentration can be used to normalize enzymatic activity in the subtropical waters as well as bacterial biomass. Of course we do not exclude the possibility that a different conclusion would be extracted when using bacterial biomass and it would be the next step of our study, in which major players of hydrolysis of phosphate esters are elucidated. What we must emphasize here is that non-normalized enzyme activity

did show spatial variation in response to SRP concentration, but to a smaller degree than normalized activity, and that some correlations between enzymatic activities and environmental parameters were statistically insignificant when not normalized.

I am surprised that the ratio of dissolved to total enzyme activity is constant throughout the entire dataset considering the wide range of environment sampled.
As for the proportion of the activity in the dissolved fraction, we must emphasize that the figure was the slope of the regression line with an interval of confidence, instead of the average value of all the proportions with a standard deviation. Actually it fluctuated among the stations: the range of the value was 13-49% and 13-68% for MEA and DEA, respectively. Even after removing the outlier or all the data $>$0.5 nM h^{-1}, the slope was not significantly different (0.27 $\pm$ 0.019 and 0.30 $\pm$ 0.027, respectively) and it did not affect the conclusion.

The authors argue that dissolved MEA was lower at SCM than at 10m which is in contradiction with another study showing increase in dissolved activity with depth higher than 100 m (Baltar et al. 2010).
We argue in this paragraph about "percentage" of the activities in the dissolved fraction, not the absolute volumetric activity. In Duhamel et al. (2011), the vertical variation of percentage of the dissolved activity is not explicitly discussed. But Fig. 2 in their paper suggests that the percentage did not show apparent trend (or slightly lower percentage at deeper layers). Anyway, according to the comment, we rearranged the manuscript to make more conservative discussion here.

Discussion page10108 line 10-18: why would the authors expect MEA and DEA to be controlled by DOP since phosphatase activity is controlled by SRP availability?
As in the comment, DOP concentration does not directly control alkaline phosphatase activities. However, if some fraction of DOP is bioavailable, it can affect the activity of the enzymes. Similar analyses were made in other previous studies (Duhamel et al., 2011, Limnol Oceanogr; Suzumura et al., 2012; Front Microbiol).

Table 1.: why SRP values >200 nM?
SRP concentrations at Stn. 1 were too high to be detected by a highly-sensitive method. Therefore, we arbitrarily regarded the concentrations as >200 nM. Although the data obtained by conventional methods are also available, they were not used for consistency.

Fig. 3 is hard to read.
According to the comment, Fig. 3 was rearranged.

Interactive comment on Biogeosciences Discuss., 10, 10095, 2013.

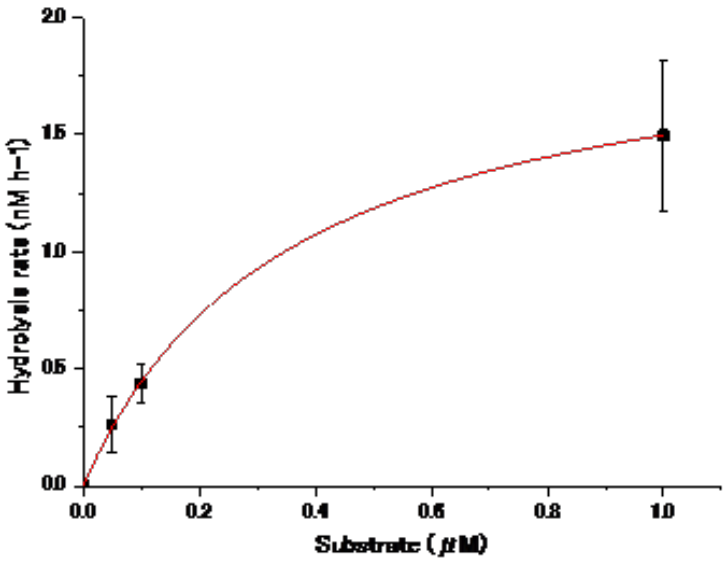

Fig. 1.