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Discussions

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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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What is the depth range of the subsurface chlorophyll maximum (SCM) layer?
The SCM depths were listed in a new table, Table 1, together with water temperatures. And the effect of incubation temperature was examined in results section, using previously reported Q_{10} values. It reveals that the incubation temperature did not affect the conclusion about the relationships between phosphatase activities or its kinetic parameters and SRP concentrations.

The final concentration used (1 μM) for the fluorescence substrates could be too low.
Actually the substrate concentration was low compared to other studies. 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. As noted in the first manuscript, this concentration was adopted to minimize the background fluorescence when detecting subtle changes in fluorescence.

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 V_{max} calculated from the 5 stations in which kinetics were done).
Moreover, there is generally a strong effect of the concentration range that is used for a kinetic assay and the kinetic parameters that are calculated. The apparent discrepancy between Fig. 3 between Fig. 3 and Table 1 (in the first manuscript) is because the highest activity was observed at station 7 of the KH-12-3 cruise, where the kinetics experiment was not conducted. Actually, the activity at the station was conspicuously high (the outlier in Fig. 2A). The chlorophyll-a specific MEA activity at the station 5 was 7.0 nmol μg^{-1} h^{-1}, which was close to V_m estimated from the kinetics experiment. We admit that this concentration used led to relatively wide ranges of the estimated kinetic parameters for DEA. However, we believe that this low concentration of substrates does not deteriorate the notion "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". This is because the concentration of potential substrates in the oligotrophic waters is at a nanomolar level, which is much lower than K_m values. Therefore, it is unnecessary to use V_m values for comparison of potential hydrolysis activities in natural waters. Moreover, taking into account the diester concentration comparable with that of monoesters (Suzumura et al., 1998; Monbet et al., 2009), it would be reasonable to compare the hydrolysis rate of the two substrates at the same concentrations.

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.
To correct for the second decomposition of diesters, we calculated the maximum hy-

drolysis 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, 16%. And this correction did not affect the overall pattern of DEA, in which it was highest at the surface of Stn. 9, and high from 15 to 35 °N. These examinations were briefly included in Materials and Methods.

Another issue that might be relevant is the use of Chl-a as an index of microbial biomass.
As the referee pointed out, it is more common to use bacterial cell concentration or carbon biomass 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 enzy-

matic 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.

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.
In Fig. 6, two data point with SPR concentration >200 nM, over the detection range of the present method, were removed. At this station, K_m was high and V_m was low, which suggests that the overall trend in Figs. 6A and B would be unchanged when these data points are added. Of course the effect of chlorophyll *a* concentration could not be excluded. Thus we added that notion in discussion.

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).
According to the comment, we added p-values and coefficients of determination to all the regression curves. And we also added on the discussion on the outlier in Fig. 2A.

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.
According to the comment, we added the list of stations with longitude and latitude.

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