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Interactive comment on "Dynamics of phytoplankton community structure in the South China Sea in response to the East Asian aerosol input" by C. Guo et al.

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We thank the reviewer for her/his constructive suggestions for our paper. Please see our detailed response to reviewer's comments below.

"- The leaching procedure is a key to design this kind of experiments. No information was given by the authors regarding source of the seawater used for the leaching, time length of the leaching, pH of the leachate, method for storage of the leachate, and background nutrients and trace-metals concentrations in the original seawater. If the authors used natural seawater, concentration and characteristic of metal-binding organic ligands may affect biological availability of trace-metals in the leachate. Freezing

C4320

of the aerosols before leaching may change the solubility of elements in the particles. Based on the data shown in Fig. 2, trace metals and nutrients concentrations in the leachate (4000 ml/5 ml = 800 times of the concentration shown in Fig. 2) could be micro-molar to sub-milli-molar levels. Such high concentrations in seawater could results in formation of metal (iron, etc.) colloidal particles and thus biological availability may change through adsorption and precipitation processes in the leachate. So application of the observed phytoplankton responses in the microcosm to natural atmospheric deposition event could be problematic."

The reviewer commented on issues related to the procedure of making aerosol solution and raised concerns on various steps. We agree that there are many factors that can change the solubility and chemical properties of the aerosol, including the formation of metal-organic ligand complex and colloidal particles. However, the initial objective of this research is to look at atmospheric nitrogen input on phytoplankton community dynamics. We have no intention to solve all the puzzles of the chemical behavior of the aerosol during the leachate preparation. We also realize that it needs to be very careful when dealing with trace metals because it requires ultraclean facility throughout the whole experimental and analytical processes due to their extremely low concentration. Therefore we have never intended to focus our research on the trace metals in aerosol. When you read the whole paper, you will find that we only touched the topic of trace metal when our results cannot be explained by N or P enrichment. There is no doubt that trace metals in aerosol play a very important role in microbial processes and biogeochemical cycling in the South China Sea, but that was not the aim of this study.

Having said that, we have tried our best to reduce the artificial effects and followed the practices of other studies (e.g., Martin et al. 1991; Hsu et al. 2010). Since the PM2.5 aerosol was pumped on a filter, they closely attached on the filter. It is difficult to scrape the small particles off the filter, so adding the aerosol directly to the seawater is not feasible. The second way is adding the aerosol filter to the seawater, but we worried that the quartz filters would become fibers suspended in the seawater, affecting the

physical and chemical environment in the microcosm. So we choose the method of making the aerosol leachate first. Pre-filtered natural seawater in the South China Sea was used to make the leachate. We choose the leaching time of about 1 h according to previous studies (Martin et al. 1991; Aguilar-Islas et al. 2010, Hsu et al. 2010), and the leachate was stored in dark under 4 degree. Some other studies also used aerosol leachate to do the enrichment experiments (Martin et al. 1991; Herut et al. 2005; Hill et al. 2010).

It is true that the ligand concentration can limit the solubility of iron. But we already simulated the entire process by using the natural seawater of South China Sea to dissolve the filter. The formation of metal (iron, etc.) colloidal particles in the leachate may result in reduced nutrient and trace metal enrichment, but the biological effect can still be tested, which supported our view that the East Asian aerosol have strong effect on phytoplankton community structure in the South China Sea.

We know that many factors affect the aerosol ion solubility, including aerosol size, aerosol type, leaching time, and leaching solution chemistry (see refs in Aguilar-Islas et al. 2010, and Hsu et al. 2010). But using different leaching protocols (e.g., using different leaching solution) have been proved to produce much less variability in aerosol ions solubility than using different types of aerosol according to previous study (Aguilar-Islas et al. 2010). Considering that the scale and source of atmospheric event in the South China Sea also vary, our emphasis of this study is not to mimic strictly the chemical processes of the deposition and dissolution of aerosol, but to demonstrate a trend of biological effect of the aerosol in waters of different trophic conditions. So we think the variation during leaching process is not the key issue of our study.

- Trace-metal clean techniques should be applied to test the effect of trace-metals in the leachate. The authors used acid (10% HCl)-cleaned bottles for their incubation experiments, but it is not clear that they used trace-metal clean techniques through the experiments; from the collection of surface seawater and sample treatments, to the sub-sampling and parameter measurement. The authors should present initial and final

C4322

concentrations of trace-metals in the control bottles to confirm that there was no serious contamination throughout the experiments, because discussions on iron limitation are included in this paper. Stock solutions of inorganic nutrients used for the experiment 3 should be purified to remove trace-metal impurities.

As our initial intention of this study is to look at the N input, but not trace metal. So we did not measure the trace metal concentrations in the microcosm during the experiment, but only measured trace mental composition of the aerosol for reference. We tried our best to avoid any contamination during experiment. Because our aerosol leachate has high trace metal concentration, thousands times higher than in natural seawater as the reviewer points out, the contamination issue that may have occurred during sampling and experimental setup is likely not a big concern. Besides, because we are aware that our approach is not trace metal free, we do not focus our research on trace metal. If all our bottles or sampling procedures are somewhat contaminated, despite our best effort, then the difference between aerosol enrichment and N and P enrichment observed in Exp. 3 may still be attributed to the unknown substance (although we speculate metals) in the aerosol.

- The corresponding amount of aerosols added in the experiments was 0.02 and 0.2 μ g/L ((70 mg/450 ml)x(0.5 or 5 ml/4000 ml)) for the low- and high-treatments. The authors should explain validity of these additions by comparing these values with the observed aerosol deposition fluxes in the South China Sea. Total amounts of added leachate were different between Exp. 1 (0.5 or 5 ml) and Exp. 2 (0.2 or 2 ml x4= 0.8 or 8 ml), and it is difficult to compare the observed results directly.

The approximate amount of aerosol added to the micocosm was 20 and 200 μ g/L (not 0.02 and 0.2 μ g/L), which are in the range of the real aerosol input in the South China Sea. The estimated mean atmospheric flux of dust over the South China Sea is 43 g m-2 yr-1 for the coastal region and 10 g m-2 yr-1 for the off-shore region (Gao et al. 1997). If we calculate the average mixing layer depth as 50 m, the average daily dust flux to the China Sea is about 2400 μ g L-1 d-1 in the coastal region and 550 μ g L-1 d-1

((43 or 10 g m-2 yr-1/365 d)/50m). Zhang et al. (1993) also obtained a similar value of 53.7 g m-2 yr-1 by calculating the annual wind-dust flux to the China Sea.

Our addition dose is lower than that, because we only added the PM2.5 fraction, which contain more nutrient content relative to the weight. Furthermore, the East Asian aerosol flux over China Sea is highly variable among different season and different year (e. g., Zhang et al. 1993, Cohen et al. 2004, Zhang et al. 2007). For example, the aerosol concentrations in winter could be a factor of 2-3 to that in summer; and the daily atmospheric flux can ranged from less than 5 mg m-2 yr-1 to more than 500 mg m-2 yr-1. So the amount of the addition is realistic, and can reflect the true condition.

Exp.1 and 2 are designed to simulate different scenaries, i.e., single pulse vs. continuous deposition. Because the flux during a single storm event is usually higher than sustained period, therefore our design is reasonable.

- Although the authors measured picoplankton abundance and Fv/Fm every 24 hours, only the Fv/Fm values for PM7 and S412 experiments were shown. Without knowing daily changes in phytoplankton abundance and nutrient concentrations during the experiments, evaluation based only on the results obtained at the end of the experiments (Day 3 or 4) could be misleading. It is possible that the phytoplankton in the low-treatments responded within 24 hours and then became nutrient-limitation again after Day 2.

Agree. We have the daily change of picoplankton abundance. The reason they were not included is simply because we try not to make our paper too long. The relative patterns among control and 2 treatments in each day were similar, that's why we only show the data of one day. But we can add those data to the paper. It is possible that the phytoplankton could become nutrient limited again quickly. But when we compared the treatments with control, it is evident that the decrease of Chl a in the Low treatment could still be attributed to some other factors besides nutrient limitation.

We measured inorganic nutrient concentrations in the initial and final time point of each

C4324

experiment. However, a lot of data points, especially the N concentration, fall below the detection limit of the instrument. So we did not use the data.

The initial Fv/Fm values measured at station C3a and SEATs were higher than 0.9, which we believe is impossible in the South China Sea. We think it is possibly because of the low biomass at these two stations. We observed Fv/Fm curves in the region of noise even the FIRe is running at its highest possible gaining (2600). Because we are not confident on those data, we did not show them in the paper.

P.6641; L.10 The "high-aerosol, low Chl" condition may suggests that the atmospheric nutrient supply is not enough for phytoplankton to grow or the hydrographic condition such as light-limitation by deep mixing during winter playing an important role on the control of phytoplankton production.

Agree. It has been also suggested that the Fe solubility in the South China Sea is low due to a lack of Fe-binding organic ligands in the surface water, resulting the "high-aerosol, low Chl" (Wu et al. 2003)

P.6642; L.12 It is not clear why the authors used aerosol particles only smaller than 2.5 μ m. Most of the dust particles may be in the fraction larger than 2.5 μ m, and most of the aerosol samples would be anthropogenic origins.

Because fine particle (PM2.5) was inferred to be more important in East Asia, due to its anthropogenic origin and high nutrient and trace metal content (Cohen et al. 2004, Hsu et al. 2010). Also, the long distance transportation from landmass to the South China Sea favors small size particles over larger particles (Duce et al. 1991). It has been reported that the fine particle (PM2.5) dominate the coarse particle (PM10) over the Northern South China Sea (Zhang et al. 2007). Therefore, we choose the PM2.5 fraction.

P.6643; L.3 Material and cleaning procedure of the screen should be described.

We have changed it as suggested. Thanks.

P.6643; L.6-7 Please show the temperature range during the incubations and reasons for selecting 40% light intensity for the incubation.

The sea surface temperature at four stations was showed in Table 2. Light intensity of 40% is used to simulate the in situ sunlight at the sea surface.

P.6643; L.19-21 The concentrations of added inorganic nutrients were different from those supplied from the addition of the aerosol leachate, so that direct comparison of the yield of phytoplankton biomass is inappropriate.

Agree. This was resulted from the measurement variations of aerosol leachate before and after cruise. We did a preliminary nutrient release test by spectrophotometer before cruise and find that ~200 μ g aerosol can release DIN for ~1 μ M, so we decided to add DIN at final concentration of 1 μ M. Unfortunately, we got higher DIN concentrations in the aerosol leachate measured by Skalar autoanalyzer after cruise (2.3 μ M). We will delete the inappropriate comparison.

P.6646; L.9 Soaking in DMF at -20âĎČ for 2 hour might not be enough to extract plant pigments effectively.

The method for HPLC analysis was according to Furuya et al. 1998. DMF is an efficient solvent for HPLC analysis of phytoplankton pigments, because of its strong extractability, which expedites the extraction process. And long time extraction may cause degradation of phytoplankton pigments.

P.6647; L.17 Based on the data in Fig. 2, DIN concentration is about 2.5 μ M and phosphate is about 0.01 μ M, so that N/P ratio should be >200.

Yes. We did a wrong calculation here. The ratio should be 235.

P. 6647; L.19- In the section 3.2, please explain possible reasons for the observed decrease in Chl concentration in controls bottles of the experiments S412 and A1.

The decrease may be due to either nutrient limitation or grazing or both in the control

C4326

bottle. We have focused on the relative change in treatments as compared to the controls. It will require other type of experiments to figure out what was going on in the control bottles.

P.6649; L.10-11 An increase in cell abundance in the low-treatments can only be seen in Synechococcus at station PM7 and S412.

The increase was also showed at C3a, though the increase is not significant. We will add words "at most stations".

P.6649; L.17-19 An increase in abundance of picoeukaryotes is significant only in SEATs and S412 experiments.

Yes. That is why we did not use the word "significant". We just report that there is a cross-board increase of average value of picoeukaryote abundance.

P.6650; L.2-4 Abundance increased only in the high-treatments.

Yes. We have changed it as suggested.

P.6650; L.9-11 It is not clear whether the authors counted dinoflagellates cell number only for autotrophic species or both autotrophic and heterotrophic species. Explanation for inconsistency between the decreasing trend in the dinoflagellates cell number and the increasing trend in the peridinin pigment concentration is needed.

We counted dinoflagellated cell number for both. Because the dinoflagellates abundance was very low, counting by microscope using only 10-30 ml sample results to large variations. Actually, based on Fig. 6, significant decline of dinoflagellate abundance only occurred in High treatment at C3a. The discrepancy between the microscopic counting and pigment analysis can be due to 1) the large variation in microscopic counting as mentioned above, 2) there was an increase in autotrophic dinoflagellates although the total dinoflagellates decreased, and 3) there is an increase of pigment contents in dinoflagellate although the number remained unchanged or even declined slightly. Due to the fact that dinoflagellate abundance was two orders of magnitude lower than diatoms, we may not need to include the rather highly variable data in Fig. 6.

P.6650; L.12 Initial abundance of the protist grazers are needed to confirm the increasing trend.

Agree. The abundance of protist grazers increased during the experiments. We have added the initial data in the revised paper.

P.6650; L.23-25 The magnitudes of variability in Fv/Fm driven by changes in phytoplankton community structure often exceed that induced by nutrient limitation (Suggett et al. 2009, Mar Ecol Prog Ser, 376:1-19), and so the observed shift in dominant phytoplankton from pico-cyanobacteria to diatoms should be considered before interpretations of physiological status.

We agree that the Fv/Fm could also contain taxonomic information besides physiological status. Therefore, after aerosol addition, two factors drove the increase of Fv/Fm: first, relieve of nutrient starvation in the phytoplankton communities; second, the nutrient induced phytoplankton taxonomic shift from picophytoplankton to microphytoplankton. And both of the 2 factors resulted from to the addition of aerosol-derived nutrient. We have modified the paper accordingly.

P.6651; L.2 Cellular Chl a and carbon contents are lower than what? Side scatter signal (Fig. 8-k, m and n) and red fluorescence (Fig. 9-k and n) showing significant increases in some experiments.

We have changed the sentence.

P.6652; L.5-6 In the experiment PM7, the observed increase in chl-a was 0.35 μ g for the addition of 0.2 μ g/L aerosol, and so the Chl/aerosol ratio should be much higher.

The aerosol concentration in the High treatment is 200 μ g/L, but not 0.2 μ g/L. Our calculation is right.

C4328

P.6652; L.13-15 Total amount of added DIN is different between the high-treatments and N+P/N+Si, and thus direct comparison of the final biomass is wrong. Discussion on iron-limitation does not have profound meaning without data on iron addition treatments and in situ dissolved iron concentrations. The reported dissolved iron concentration (0.2-0.3 nM) seems to be enough for oceanic phytoplankton species to grow with atmospheric N supplies.

We have explained DIN issue in previous comments. According to previous studies, a positive response to Fe addition in seawater with ambient concentrations of DFe of a few hundred picomoles has been observed in the sub-Antarctic region of the Southern Ocean (Sedwick et al. 2002). And phytoplankton growth can be stimulated by the addition of Fe even though ambient concentrations of DFe were relatively high (0.40–0.45 nM) in the northeast Atlantic Ocean (Blain et al. 2004). Therefore, it is possible that phytoplankton growth was stimulated by Fe addition with ambient Fe concentration of 0.2-0.3 nM in the South China Sea.

P.6652-3; section 4.2 No statistically significant decrease in chl-a relative to the controls was observed in this study, instead significant increase in abundance of picophytoplankton was observed in some of the low treatments by FCM measurements. FCM data on cellular red fluorescence suggesting that decrease in cellular chl-a content of pico-eukaryotes was one of the reason for a slight decrease in total chl-a concentration.

The reviewer is right to point out that the decrease in cellular chl a of picoeukaryotes was one of the reason for the slight decrease in total chl a concentration. We have incorporated this point in our revised paper.

P.6654; L.5-6 Reference is needed for this suggestion.

In fact, we wrote this sentence based on our observation in this study, but we made a mistake by writing "it has been suggested". We have corrected the sentence accordingly.

P.6654; L.9 It seems to be useful to calculate growth rates of phytoplankton species based on the microscopic enumeration data to examine the degree of growth enhancement by the aerosol leachate.

Yes, we can calculate the growth rates of phytoplankton species based on the initial and final microscopic data. But the calculated growth rate is the net growth rate, which is the balance of both growth and grazing. And we think showing the abundance data of the final time point can serve the same purpose.

P.6654; L.11-24 Again, discussion on iron-limitation does not have profound meaning without data on iron addition treatments and in situ dissolved iron concentrations. The FCM data on cellular red fluorescence and side scatter signal shows that both Prochlorococcus and Synechococcus enhanced physiologically in the high-treatments.

Here we simply cited other researchers' study to show the effect that may be induced by atmospheric deposition on large phytoplankton cells. We will consider reducing the content talking about iron limitation.

We agree that it is not appropriate to say that Prochlorococcus and Synechococcus did not benefit from high aerosol addition. We have changed the sentence.

P.6655; L.2 Trace-metal toxicity depend on their chemical speciation and the information about organic metal-binding ligands are key to evaluate the metal toxicity in the SCS.

Agree. We simply speculate that the toxicity is a possible reason that caused cyanobacteria abundance decrease.

P.6655; L.3 It is not clear what kind of the observed negative effect the authors are talking here.

It means the decrease of Prochlorococcus and Synechococcus abundances caused by aerosol. We have modified the sentence.

C4330

P.6655; L.4-7 Is the difference in abundance between the high-aerosol and the N+P treatments is statistically significant? From Fig. 2, DIN concentration in the high aerosol treatment (about 2.5 μ M) seems to be higher than that of the N+P treatment (1 μ M). FCM data on cellular red fluorescence showing physiological status in the high treatment was better than the controls in the experiment 3. How about the effect of grazing on the Prochlorococcus abundance.

Yes, the difference is significant. We assume that the grazing rate in aerosol and N+P treatment is similar.

P.6655; L.23-27 The selective grazing should be happen in the control bottles, but there was no significant change in the cellular red fluorescence and side scatter signal in the control.

We are not clear what is the reviewer's point here. We simply explained some possible reasons for the small decrease of cellular carbon and ChI a content of picoeukaryotes comparing with control. The reviewer is right that the selective grazing may also occurred in the control. Compared with initial, the cellular carbon and ChI a content of picoeukaryote in control also increased, but the different nutritional value may induce different degree of selective grazing in the treatments. Besides, in the paper, we also mentioned other reasons for the decrease, for example, the change of picoeukaryote community structure.

P.6662; L.14 The manuscript in preparation should be deleted from the references.

According to the Biogeoscience website, works "submitted to", "in preparation", "in review", or only available as preprint, should also be included in the reference list.

Figs. 4-9 Initial values should be included in the figures.

We have changed the figures as suggested.

Fig. 4 Please show the date of the sampling for each measurement. Data for PM7 is missing.

We have added this information in the revised paper. We did not collect HPLC samples at PM7. We have added the information to the method part 2.3.7.

Figs. 5, 7, 9 and 10 It is not clear which panel showing the data obtained at 48 hour. Why the authors showing the data only for 48 and 96 hours?

We have the daily change of picoplankton abundance. The reason they were not included is simply because we try not to make our paper too long. The relative patterns among control and 2 treatments in each day were similar, that's why we only show the data of one day. We have made appropriate changes on the figures in the revised paper.

Fig. 6 Is it true that all the data obtained at 96 hours? Data for A1 is missing.

Yes. We did not collect microscopic samples at A1. We have added the information to the method part 2.3.5.

Fig. 7 Why ciliate data for PM7 and A1 is missing? No measurements or abundance was low?

No measurement. No samples were collected at A1, and samples collected at PM7 were used to count microphytoplankton only. Besides, since using 10-30 ml samples for ciliate counting may induce large statistic variation, we have decided to delete this figure.

Fig. 10 Please show the data for C3a, SEATs and A1. According to the text, Figs 8 and 9 should be Figs 9 and 10, and Fig. 10 should be Fig. 8.

We have explained the reason of not showing data at C3a and SEATs. Fv/Fm value at A1 was not measured because of the limitation of manpower.

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C4332

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Interactive comment on Biogeosciences Discuss., 8, 6637, 2011.

C4334