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Biogeosciences Discussions

Interactive comment on "Sulphur compounds, methane, and phytoplankton: interactions along a north-south transit in the western Pacific Ocean" *by* C. Zindler et al.

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Response to referee #1 for manuscript bgd-9-C5919-2012

We thank the referee for working on our ms. The comments and advice were given in a precise manner and were really helpful. They gave us a framework for improving our submission. We changed the methods and discussion sections based on the suggestions by the referee. We also toned down our conclusion statements.

R1: Abstract: The last sentence here is a pretty bold statement. I'm not sure that the (relatively weak) correlation of CH4 with various S compounds justifies these conclusions. I'd suggest toning down the language here.

Authors: We have changed the sentence to weaken the statement: "We conclude that both DMSP and DMSO and/or their degradation products might serve as potential substrates for CH4 production in the oxic surface layer western Pacific Ocean." Of course, further investigations are necessary to support this finding.

R1: Methods: In general, I think more details are needed here, as I was unsure about a number of things. For example, the S analyses (other than DMSO) were apparently run immediately, but it's not clear exactly what the analysis sequence was. I presume that the authors collected water, ran a DMS analysis, then added NaOH and ran a follow up DMSP analysis and then stored the high pH samples for subsequent laboratory DMSO analysis. Is this correct?

Author: Yes, this is correct. We described the analytical procedure in Zindler et. al (2012) and modified this ms, including a more thorough description.

R1: What about filtrations? How were these conducted? Did the authors use gentle gravity filtration to address the issue of cell lysis during filtration as discussed by Kiene?

Authors: As we described in detail in Zindler et al. (2012), we gently filtered the samples through a glass fibre filter attached to a syringe. We were aware of the easy cell lysis during this filtration technique. However, we worked gently to avoid pressure on the cells and we minimized the DMS lost due to the contact with the atmosphere by using this technique. We added a short sentence mentioning the filtration procedure and giving the original reference.

R1: How were the calibrations done? Were these gas phase calibrations of the GC system (e.g. using a permeation tube with dilution gas), or did the authors produce liquid calibration standards to calibrate the entire analytical system (i:e: both purge and trap and detector components)?

Authors: We used liquid standards prepared according to Kiene (1993) and calibrate the entire system. The calibration procedure is described in Zindler et al. (2012). It

was noted in the text that liquid standards were used.

R1: What do the error terms ±represent? Std. dev, std. err? Are these mean values derived from triplicate analyses?

Authors: We added the missing information to the text: The mean analytical errors are given in standard deviations calculated according to David (1951). We added that the standard deviations are from the triplicate measurements mentioned in the first sentence (page: 15015 line: 10).

R1: There is no mention of poisoning the CH4 samples prior to storage. I presume that samples were indeed poisoned and the details should be given.

Authors: Yes, we poisoned the samples with HgCl2. We added this information in the text.

R1: Can you please add a reference for the quantitative filter pad technique.

Authors: We used the (Tassan and Ferrari, 1995) technique as described and modified in Taylor et al. 2011. Now this modified technique has been published (Rottgers and Gehnke, 2012). We added this information in the ms.

R1: I am not familiar with the pigment analysis methods used by the authors to extract quantitative information on phytoplankton taxonomic composition. Is there a reason why they chose not to use the CHEMTAX program which has been widely used across the oceanographic community?

Authors: Yes, there is a reason: For the entire transect no CHEMTAX matrix was available, only for the tropical parts within +/10° (Higgins and Mackey 2000). Therefore we used, as described, the widely accepted method by Vidussi et al. 2001, modified by Uitz et al. 2006 and Hirata et al. 2011, to calculate major phytoplankton groups from eight marker pigments which is a global method. _Further, we also calculated the results with the above mentioned CHEMTAX matrix and the results looked very much alike the Vidussi approach for the region within 10° as you can see from the correlation

coefficients (r2) between the two data sets for six groups were all above 0.68 (see table below). The group of phrochlorophytes was not derived with the method by Higgins and Mackey 2000 because the matrix (and their pigment data base) did not include div-a. As the marker pigment alloxanthin was not detected within these latitudes (but it was outside this region) for our Transbrom Sonne data set, no cryptophytes were detected. Table (see Fig1): Comparison of Transbrom Sonne pigment data between sampled between 10°N and 10°S (n=34) calculated with the CHEMTAX matrix of Higgins and Mackey 2000 and with Vidussi et al. 2001, modified by Uitz et al. 2006 and Hirata et al. 2011

R1: It's not clear how the different size classes were determined – using size fractionated filtration? I don't see how this could be done on the basis of pigment concentrations alone sincethere are, for example, dinoflagellates and diatoms with very different sizes.

Authors: As described in the ms page 15016 line 23 and and following pages.: "Table 2 in Taylor et al. (2011) summarizes the pigments analysed in this study and provides the information about which pigments have been allocated as marker pigments for the different phytoplankton groups. According to a procedure proposed by Vidussi et al. (2001) which was modified by Uitz et al. (2006) and most recently by Hirata et al. (2011), we estimated the contributions of three phytoplankton size classes (i.e. micro-, nano- and phytoplankton representing the size classes of 20–200 μm, 2–20 μm and < 2 μm, respectively) and seven phytoplankton groups based on the measured concentrations of seven diagnostic pigments (DP) to the biomass. The DP, the calculation procedure of the weighted relationships of these marker pigments and the determination of their biomasses are described in the Supplement." It is true that this method is not accounting for the fact that you can find small diatoms and dinoflagellates in the nanoplankton range and large coccolithophres in the microplankton, but it works in the general view and is widely accepted.

R1: A transformation is mentioned for non-Gaussian data, but there are no details on

what that transformation actually is.

Authors: The referee is right. We did not mention that we log-transformed the data. We added it in the text.

R1: It seems to me that the authors should use a Type II regression since both the x and y variables are measured with error.

Authors: The reviewer is right. We looked into whether Type I or Type II should be used and we see the reviewer's point. Because so many regressions were done we decided to compute the Type II regression on a handful of individual cases to test the outcome. We see that the r2 value for the regression either did not change or became slightly better. For example, the Type II regression of methane against the marker pigment 19′-butanoyloxyfucoxanthin was higher, changing from 0.605 in the Type I regression to 0.630 in the Type II. This represents about a 4% improvement. In the future we will surely use the Type II regression as indicated by the reviewer, but for this manuscript the overall conclusions are not changed and the r2 values are only slightly elevated.

Results and Discussion: R1: p. 15019 – I'm not sure that I agree that the clusters reflect Longhurst's provinces. It seems to me that clusters 2, 3 and 4 are all present across the two main provinces sampled. I think only cluster 1 shows a distribution that is linked to one of the biogeographic provinces.

Authors: The referee is right. We changed the statement in the text: The spatial distributions of cluster 1 reflect the biogeographic province Kuroshio Current (KURO) as defined by Longhurst (1998) while cluster 2 to 4 are distributed throughout the three main provinces North Pacific Tropical Gyre (West) (NPTW), West Pacific Warm Pool (WARM) and Archipelagic Deep Basins (ARCH) (Fig. 4).

R1: Top of p. 15020. I think it could be made a little clearer that the author's are comparing their observations with climatological predictions from Lana.

Authors: We referred on page 15019, line 25 and page 15020, line 2 and 4 to the

climatology of Lana et al. (2011). However, we mention again in the text that we used the climatology data to compare it with our in-situ data to make it clearer: "For the Longhurst provinces KURO, ARCH and AUSE (see Fig. 4) the mean October concentrations of DMS from the climatological predictions are given as $\sim$ 1 nmol L-1, $\sim$ 5 nmol L-1 and $\sim$ 4 nmol L-1, respectively (Lana et al. 2011). The differences between the climatological data and the data from our cruise might be caused by interannual variability and a general mismatch between climatological means and in-situ data."

R1: p. 15020. I'm not sure how relevant it is to compare the W. Pacific sulfur data to measurements of the E. China Sea. Would we expect the numbers to be similar? If so, why is the comparison valid?

Authors: We deleted this part of the discussion.

R1: Section 3.4 I don't think it makes that much sense to compute an overall mean for each expedition. There is very good reason to believe that different regions of the cruise track represent different systems, so why lump them all together. It would make a lot more sense to me to compile the data into (for example) 1 degree temperature bins. In the same section, I'd be a little cautious about over-interpreting the 'positive trend' in DMSP:DMSO with temperature at SST <5 C. This is really based only on a couple of low points. Perhaps the temperature binning approach would provide more data points to help fill in the plot.

Authors: We treated our data according to Simó and Vila-Costa's (2006) computation in order to compare our data set with the data sets listed in their paper. The referee is right that the data presented in our study were collected in a heterogenic region. We binned the data like the referee suggested. However, the resolution of the measurements in the colder waters (<20°C) was too low to compute reasonable averages (only one or two data points per temperature degree). Only in the higher temperature regions were there enough data points per temperature degree. The DMSP:DMSO ratio between the temperature range of 21 to 29°C was between 0.1 and 0.5, similar to the average

range of the entire data set (0.22). Thus, to combine the data in 1 degree temperature bins did not change the outcome of the results. We think that we cannot ignore the five data points in the cold region between 0 and 10° because they represent five previously published cruise dataset (i.e. not our data). Thus, we think it deserves mentioning but the interpretation is not overstated in the ms.

R1: Bottom p. 15021. I don't really follow the logic of the coccolithophore argument.

Authors: We tried to find an explanation for the high DMSP:DMSO ratio in the temperature range between 5 and 10°C. It is unlikely that temperature directly influences the DMSP:DMSO ratio. Thus, another parameter presumably seems to be responsible for the trend in the DMSP:DMSO ratio. We think that coccolithophorids, which are important DMSP producers, might affect the ratio by producing more DMSP compared to DMSO. And because of their preference to live in boreal regions with water temperature of around 9°C they might be the missing link between the colder temperature and the high DMSP:DMSO ratio.

R1: Overall, I found that the section describing various correlations was rather convoluted. It seemed that the authors took the approach of correlating everything to everything else. While this approach did yield some significant correlations, which are discussed in further detail, the significant results were, in my view, somewhat 'diluted' by the large number of correlations that were presented. Moreover, it seems to me that the authors could have included a number of other variables that could have significant explanatory power. For example, why not include Chl a in the multiple regression as opposed to running a separate analysis. Also, what about other physical variables such as surface PAR, mixed layer depth, wind-speed etc.. Might it be possible to construct a more general step-wise regression attempting to product the best empirical description of S compound distributions? I think it would be ok to include some of the pair-wise correlations (e:g: DMSO and DMSP) if they are used to highlight a specific significant result.

Authors: As we mentioned in the manuscript we did not include Chl a for the regression models because this pigment occurs in all phytoplankton groups. We selected marker pigments for the models which are mainly included in one algae group to identify the influence of specific algae groups on the sulphur compounds. Additionally, the predictors, in our case the pigments, need to be independent in the models for a reasonable statistical output, meaning that the pigments should only be found in one algae group. The referee's suggestion about including additional variables is valid. However, we tested all the mentioned parameters and could not find any significant influences of these parameters on the sulphur compounds. By including too many parameters in the calculation of the linear regression models the statistical robustness is diminished and it can lead to misinterpretation. Step-wise regressions are based on the same mathematical fundamentals as the MLRM. For thoroughness we also calculated some step-wise regressions and found similar results as the MLRM. We did shorten the sections describing the correlations for clarity.

R1: Related to the point above, I think the authors sometimes overstep their interpretation of causality based on correlative evidence. I would suggest a slight change of wording in a number of places where conclusions are drawn based on the regression results.

Authors: We have added a paragraph addressing the correlation causation problem at the beginning of section 3.3. We acknowledge that correlation does not mean causation and have tried to read through the text and tone down overstated conclusive statements.

R1: p. 15028, first para: The discussion is focused on DMSO and DMSP, but then seems to 'backtrack' somewhat to revisit arguments already presented for DMS. Towards the end of the paragraph, it seems that photo-oxidation could be mentioned.

Authors: We deleted the parts presenting DMS again in this section. An overall conclusion is given in the summary section. The referee made a good suggestion to mention

photo-oxidation again. However, we think that this enlarged the section too much and might give the impression of repetition (which we are trying to cut out per the suggestions of all reviewers). We think that we mentioned sufficiently the UV stress as possible explanation in several parts of the ms. We also tried to reword the conclusions we draw from the correlations toward more suggestive rather than concrete.

Section 3.6.4. R1: I think it would be useful to present % saturation values for methane in addition to concentrations.

Authors: We agree, CH4 saturations have been added as well as a sentence in the method section which explains the calculation of the CH4 saturations.

R1: Fig. 7. It seems to me that the regression of CH4 vs. Chl a is heavily weighted by two points at high Chl a.

Authors: We tested all regressions with the Cook's distance to identify if certain data points have an overwhelming influence on the regression. This was not the case for all regressions we have presented in the manuscript.

R1:Bottom p. 15029. The idea that DMSO and DMSP serve as substrates for methanogenesis is certainly consistent with the correlations observed, but I think more direct evidence would be needed to draw the kind of firm conclusion presented in the text. I'd suggest toning down the language a bit.

Authors: We changed the sentence to: "Therefore, we conclude that algae derived DMSP and DMSO might be considered as possible precursors for CH4 production in the western Pacific Ocean. However, further direct evidences are necessary to support this suggestion."

Tables: R1: Table 1: Errors ($\pm$) are only given for some variables. Why? What do the errors represent – std. dev., std. err.?

Authors: We could only calculate the standard deviation according to David (1951) for the sulphur compounds because these compounds were measured in triplicates. We

added the missing errors for the sulphur compounds in the individual clusters. The errors for the TChl-a data were approximately 2%, we added this in the ms.

R1: Table 2: I found the layout of this very hard to read. In particular, the placement of letters and of individual outputs seemed rather random. The first letter to appear is 'a', then 'd', then 'i' etc. What about b,c,e,f,g, etc. Also, wouldn't it make more sense to group all of the full data set results together at the top, then group the cluster 2 results and finally the cluster 4 results?

Authors: Tables 2 and 3 are extracts from tables 1 and 2 in the supplement, respectively. In the tables in the supplement the letters are given in sequence and some are not included in the main body of ms for clarity. Thus, the letters seemed to be random in the tables in the ms. However, we want to keep the placement of the letters for a better comparison between the tables of the ms and the supplement. We grouped the results in the tables as they were discussed in the text of the ms. We decided to take the order: DMS entire data set, DMS cluster 2, DMS cluster 4; DMSPd entire data set, DMSPd cluster 2, DMSPd cluster 4; and so on for DMSPp, DMSOd and DMSOp. We think this grouping is better than grouping by clusters because we want to be consistent with text for clarity (i.e. we grouped the discussion by chemical species and not by region).

R1: Table 3. I would make the same comment with respect to organization of entries in the table. I think the results could be presented in a more logical arrangement. Note also that one r2 value is missing (model I).

Authors: We added the missing R2 value. Please see the comments above for table 2 regarding the organization.

Figures R1: Fig. 1, I presume that the position of the lines on the figure correspond to the positions of the colorbars (i:e: leftmost data corresponds to leftmost colorbar). This is not explicitly stated and should be. The font size seems very small to me.

Authors: The referee is right that the position of the lines and the colorbars are consistent. We added this information in the caption of figure 1. We also enlarged the font sizes of the figure.

R1: Fig. 2. I found this plot very hard to read and basically useless. I think the presentation would be more effective as line plot with symbols. If necessary, you could have a series of sub-plots to avoid crowding the figure too much.

Authors: please, see answer to Fig. 3

R1: Fig. 3. I found details hard to see because most of the data were compressed at the bottom of the axis. Perhaps you can have a two panel figure showing the total chl data on top and the other group specific info in the bottom panel. Such a figure would likely make Fig. 2 redundant.

Authors: We now merged the information of Fig.2 and Fig.3 in one figure (new Figure 2) with two panels as recommended by the reviewers. The upper panel shows the total chl concentration in correspondence to the latitude sampled, the lower panel shows the ratio of phytoplankton group divided by the total chl-a concentration in correspondence to the latitude sampled.

R1: Fig. 4 The color scheme was a bit hard to see here. In particular, the blue color for cluster 3 could not be readily distinguished from the green of cluster 2. Perhaps the use of different symbol types would help.

Authors: We now renewed Fig. 4 (new Fig. 3) again with introducing different symbols for different clusters but still kept the colors because also the two figures in supplement refer to those.

R1: Fig. 7. I think it's worth emphasizing in the figure legend that there is a second y axis for the 19-but data.

Authors: We added a side note in the figure caption that the 19-but data are shown in the upper x-axis.

References: Kiene, R. P.: Measurement of dimethylsulfid (DMS) and dimethylsulfoniopropionate (DMSP) in seawater and estimation of DMS turnover rates, in: Handbook of Methods in Aquatic microbial Ecology, Lewis Publishers, 601–610, 1993. David, H. A.: Further applications of range to analysis of variance, Biometrika, 38, 393–409, 1951. Longhurst, A.: Ecological geography of the sea, Academic Press, San Diego, 1998. Rottgers, R., and Gehnke, S.: Measurement of light absorption by aquatic particles: Improvement of the quantitative filter technique by use of an integrating sphere approach, Applied Optics, 51, 1336-1351, 2012. Tassan, S., and Ferrari, G. M.: An alternative approach to absorption measurements of aquatic particles retained on filters, Limnology and Oceanography, 40, 1358-1368, 1995.

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Group	Haptophytes	Chrysophytes	All Cyanobacteria	Chlorophytes	Diatoms	Dinoflagellates
r^2	0.91	0.91	0.88	0.8	0.72	0.68

Fig. 1. Table for referee #1