

Interactive
Comment

***Interactive comment on “Dynamics of
dimethylsulphoniopropionate and
dimethylsulphide under different CO₂
concentrations during a mesocosm experiment”
by M. Vogt et al.***

M. Vogt et al.

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Response to referee #3 for manuscript bgd-4-3673-3699

General comments:

We thank the referee #3 for his thorough review of our manuscript and for his useful comments. In contrast to previous mesocosm studies, there were simply not enough varying parameters in order to explain DMS dynamics exclusively and unambiguously

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper



in this study. We studied correlations of most experimental parameters with the suite of our sulphur measurements and did not find any single process that could explain the difference in temporal behaviour between the control and the perturbed treatments. Hence, there is simply not one obvious explanation. By reporting the facts rather than describing several hypotheses, none of which were 100% convincing, we tried to avoid the danger of over-interpretation of this data. Process measurements would have been desirable, but both manpower and budget restrictions limited a comprehensive analysis of the entire sulphur cycle in a mesocosm study targeted at other aims.

In order to demonstrate this lack of conclusive evidence, we have repeated and completed a full set of correlation studies comprising of all correlations between the suite of sulphur compounds and phytoplanktonic and bacterial parameters. These results confirm again that there were several potential candidates to explain the differences in DMS (such as a strong correlation with *E. huxleyi* and DMS ($r_s= 0.79, 0.63, 0.61$ for $1\times\text{CO}_2$, $2\times\text{CO}_2$ and $3\times\text{CO}_2$, respectively) and slightly less viral attacks in the perturbed treatments) but then there was contradicting evidence for each and every of those hypotheses (such as no correlations at all between DLA and *E. huxleyi* or bacteria and not enough *E. huxleyi* to constitute the bulk of DMSP). We have added the results of selected additional correlation studies in the revised manuscript, according to the referee's suggestions in the specific comment below (see there for details) and tried to better constrain the possible causes for differences between the 3 treatments. However, we would like to stress again that these differences are small and will stay small, even by adding information. What we can learn from our study, which is one of the first studies to address the issue of changes in DMS under ocean acidification, is that DMS is probably not always sensitive to pH and we consider this important information.

This observed resilience of the DMS system to pH change was not observed in a previous study (V. Avgoustidi, in preparation), and we have added a paragraph

[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)[Discussion Paper](#)

comparing the current and the previous study to illustrate this difference. In the initial manuscript we omitted an extensive comparison with Avgoustidi et al., because their manuscript is not yet available to the public and the reader cannot verify the conclusions regarding such an inter-comparison. We now refer to Avgoustidi's PhD thesis in addition to the publication in preparation, the former being publicly available.

We also added a brief explanation for the difference between our and Wingenter et al.'s findings: Wingenter et al. (2007) report a difference in their integrated mean DMS concentrations, but this discrepancy between our respective results does not arise at the data level, but is due to the use of different statistical methods to interpret the data. Their use of the t-test for more than 2 populations is known to be associated with the introduction of Type I errors. We use ANOVA, which avoids this problem. In addition, these authors report their results at confidence levels of 90% and lower, whereas we use a more stringent confidence criterion (95% confidence level, or $p < 0.05$). Please see our detailed comment to the questions of referee #1 for further explanation. Sinha et al. (2007) do not report any differences between 1xCO₂, 2xCO₂ and 3xCO₂ and his air-measurements are entirely confined to the post-bloom phase (days 16-24), where we have very few measurements (5 sampling days), so that it is not possible to compare his and our results quantitatively.

We have reduced the speculative part of the "Discussion" section and have added information on DLA (see specific comments below).

Specific points:

1. P3682. L 19. The referee is right that we could use an *a posteriori* test to reveal the differences between the three groups in a pair-wise comparison. We have included

[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)[Discussion Paper](#)

information from a Bonferroni test in our analysis.

2. P3682. L 23. We have changed "time integrated averages of DMS production" to "time integrated averages of DMS concentrations".

3. P3683. In order to better integrate the DLA data, we have added results from our correlation studies between DLA, chl-a and *E. huxleyi*, $DMSP_t$ and DMS in the manuscript. We find significant correlations between DLA and dinoflagellates, but only few significant correlations with *E. huxleyi* abundances and prymnesiophytes. We have re-written the section on page P3689, including these new results. As DLA did not differ significantly between the 3 treatments, DLA is unlikely to explain differences in DMS concentrations. However, neither are $DMSP_t$ concentrations likely to explain DMS, and the referee does not suggest we remove all information about $DMSP_t$. One of our major finding is that DLA was much higher than previously reported in other field or laboratory experiments, which we now stress more in the discussion. Given that there are only few studies of DLA in natural, mixed populations (see Stefels et al., 2007) we consider this information of benefit for the scientific community.

4. P3685. L7. This sentence has been removed.

5. P3685 L25., P3687 L17, P3689 L5 The author is right that observed $DMSP_t$ cannot be explained by *E. huxleyi* alone. However, in the present manuscript we do not imply a role for *E. huxleyi* for the bulk of $DMSP_t$ concentrations, but we suggest a role for DMS concentrations. It is likely that a large fraction of $DMSP$ contained in phytoplankton other than *E. huxleyi* and was degraded by bacteria without production of DMS (see Allgaier et al. (this issue) concerning the role of bacteria for $DMSP$ degradation).

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper

Table 1 below shows our roughly estimated contributions of the dominant phytoplankton groups measured during PeECE III, assuming literature values for DMSP and chl-a per cell quota (see revised manuscript for details). We decided to distinguish between 3 phases in the development of DMS: During days 0-10 DMS increased in all 3 treatments, following bloom development. Days 11-16 are characterised by a decline in DMS in all treatments. Days 16-22 contain a second smaller peak in DMS levels, coincident with a secondary bloom of dinoflagellates and cyanobacteria. The *E. huxleyi* containing group of the prymnesiophytes clearly contributed most DMSP_p during days 0-16 (phase 1 and 2), when the bloom formed and highest DMS concentrations were measured.

We were examining a potential link between *E. huxleyi* and DMS, because it was one of the dominant phytoplankton groups during the mesocosm experiment and because of the known occurrence of DMSP-lyase in these species (Steinke et al., 2007; Stefels, 2007), which could potentially have affected DMS production upon algal lysis. We know now that other prymnesiophytes, such as *Chrysochromulina ericina* are also likely to have been present, information which was not available to us at the date of submission. These algae were not measured directly, but a group of viruses known to infect this group of algae (CeV) were detected during this experiment (Larsen et al., this issue). Several nanophytoplankton groups have been counted by flow cytometry, but their species distributions have not been determined. Cell counts (V. Martin, Universite Libre de Bruxelles) detected several nanophytonplankton groups of higher abundance, but their taxonomic position remained unspecified. To our knowledge, it has not yet been assessed whether *Chrysochromulina ericina* possesses DMSP-lyase.

[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)[Discussion Paper](#)

Estimated contribution to measured DMSP _p	Days 0-22	Days 0-10	Days 11-16	Days 16-22
Prymnesiophytes	31%	40%	42%	4%
<i>E. huxleyi</i>	11%	20%	5%	2%
Diatoms	22%	34%	9%	8%
Dinoflagellates	2%	2%	3%	11%

Table 1: Estimated contributions in % to total DMSP_p measured for the dominant phytoplankton groups during PeECE III. Average over all 3 treatments (1xCO₂, 2xCO₂, 3xCO₂), which showed very similar values.

We have added a paragraph illustrating the relative importance of *E. huxleyi*, other prymnesiophytes (such as *Chrysochromulina*) and the other dominant phytoplankton groups for DMSP_p concentrations and a sentence clarifying that a contribution to DMSP does not necessarily explain the differences in DMS. We decided against including such calculations in the original manuscript, because natural communities might show very different cell specific quota for DMSP than those reported in laboratory studies, and because we haven't determined individual DMSP cell quota on site to support our arguments. However, we stress that we include a qualitative rather than quantitative estimate of the contributions of the measured phytoplankton groups for DMSP and DMS in the revised manuscript.

Given the scarce knowledge about DMSP-lyases (see Stefels et al., 2007 for a review), we think that an analysis of cell-specific rates goes beyond the scope of this manuscript and would need to include measurements of substrate kinetics or of DMSP-lyase activity measurements in different size-classes. In addition, we have now verified our hypotheses regarding DLA using Spearman rank correlations and we only

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper



find significant correlations between DLA and *E. huxleyi* in the 2xCO₂ treatments (see our answer to comment #3), which makes this issue less central to the main argument.

6. P3688 L17+. Malin et al. (1994) find an increase in DMS production during viral infection. However, if bacterial abundances and activities also increase during the senescence phase of the bloom (Allgaier et al., this issue), this may also increase DMS sinks and hence does not necessarily need to stimulate DMS accumulation. When we submitted our manuscript, the results from our colleagues in Bergen were not yet available online - the group of viruses in question, HFV (see Larsen et al. 2007), were present already from the early days of the experiment and the trend in difference in abundance between treatments is already visible from day 10 on, with statistically significant differences after day 15. Hence, the abundance of this group of viruses can indeed have influenced DMS dynamics by removing the host populations more efficiently in 1xCO₂ than in 2xCO₂ and 3xCO₂, leading to DMS production later in 2xCO₂ and 3xCO₂ than in 1xCO₂.

References:

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