

Final author comments on “Consistent increase in dimethyl sulphide (DMS) in response to high CO<sub>2</sub> in five shipboard bioassays from contrasting NW European waters” by F.E. Hopkins and S.D. Archer, manuscript number bg-2013-656

We thank both anonymous reviewers for their detailed, constructive, and positive reviews of our manuscript – we greatly appreciate the care and detail that has gone into its assessment, and as a result, our manuscript has greatly improved. Below we respond to their comments point by point. The referee comments are shown in italics, with our responses shown in bold.

#### Response to Anonymous Referee #1

##### 1. General comments

*This manuscript presents valuable data on CO<sub>2</sub> induced changes to DMSP and DMS cycling during bottle incubations of samples from NW European shelf waters and the Bay of Biscay. This area of research is highly topical and timely in view of recent findings on ocean acidification. The particular strength of this manuscript is that it provides a near synoptic view of DMS cycling as it covers rates of DMSP production as well as rates of DMS production and consumption. Much of the data are transparently shown, by and large clearly support the authors’ main conclusions, and apparently based on sound, up-to-date methodology. However, I do have concerns regarding various matters concerning context and presentation, and also regarding some aspects of data evaluation and interpretation.*

**We thank referee #1 for their positive view of our manuscript. We will endeavour to address all their concerns regarding context, presentation, and data evaluation and interpretation.**

*Firstly, the MS reports DMSP production rates obtained with the mass ratio progress method by Stefels et al 2009. In this method, a stable isotopic tracer is added to the sample, and subsequent changes in the ratio of labelled versus non-labelled product are monitored. As I understand, these data are then fitted to a linearised form of the logistic growth model to obtain a first order rate constant with the inverse of time as its unit (e.g. d<sup>-1</sup>). Unfortunately, the manuscript does not mention any of the underlying theory and assumptions, and merely points the reader to Stefels et al 2009.*

**A more comprehensive description of the method and theory used to determine DMSP production rates has been added to section 2.3, as follows:**

**“The specific growth rate of DMSP ( $\mu_{DMSP}$ ) was calculated assuming exponential growth from:**

$$\mu_t(\Delta t^{-1}) = \alpha_k \times \text{AVG} \left[ \ln \left( \frac{{}^{64}\text{MP}_{\text{eq}} - {}^{64}\text{MP}_{t-1}}{{}^{64}\text{MP}_{\text{eq}} - {}^{64}\text{MP}_t} \right), \ln \left( \frac{{}^{64}\text{MP}_{\text{eq}} - {}^{64}\text{MP}_t}{{}^{64}\text{MP}_{\text{eq}} - {}^{64}\text{MP}_{t+1}} \right) \right]$$

**(Stefels et al. 2009) where  ${}^{64}\text{MP}_t$ ,  ${}^{64}\text{MP}_{t-1}$ ,  ${}^{64}\text{MP}_{t+1}$  are the proportion of  $1 \times {}^{13}\text{C}$  labelled DMSP relative to total DMSP at time t, at the preceding time point (t-1) and at the subsequent time point (t+1), respectively. Values of  ${}^{64}\text{MP}$  were calculated from the protonated masses of DMS as:  $\text{mass } 64 / (\text{mass } 63 + \text{mass } 64 + \text{mass } 65)$ , determined by PTR-MS.  ${}^{64}\text{MP}_{\text{eq}}$  is the theoretical equilibrium proportion of  $1 \times {}^{13}\text{C}$  based on a binomial distribution and the proportion of tracer addition. An example of the change in proportion of mass ratio 64 ( ${}^{64}\text{MP}$ ) during incubations of water from experiment E05 at T48 for the different  $p\text{CO}_2$  treatments is shown in Figure S1 in the**

**supplementary material. An isotope fractionation factor  $\alpha_k$  of 1.06 is included, based on laboratory culture experiments using *Emiliania huxleyi* (Stefels et al. 2009)."**

*Furthermore, I could not find any example data illustrating the goodness of fit of observations to the logistic growth model used. I trust that some appropriate background and example data can be readily provided by the authors, perhaps complemented by some statistical measure of the goodness of fit.*

**A figure illustrating the progression of  $^{13}\text{C}$  into DMSP for each level of  $\text{CO}_2$  for one of the experiments (E05) has now been incorporated in Figure 4. As now explained in the text, exponential growth was assumed in determining the specific rate constants for DMSP synthesis, although the  $^{13}\text{C}$  incorporation is essentially linear over the time course of the experiments. The figure illustrates the level of precision underlying estimates of DMSP synthesis. Statistical comparison between treatments using ANCOVA is now included in the results section 3.4 and Figure 4.**

*I would also appreciate it if the authors could be more careful with their use of terminology in their manuscript: their ' $\mu_{\text{DMSP}}$ ' [d-1] is a rate constant, not a rate as stated e.g. at the top of section 3.4.*

**The text has been corrected to "rate constant" in all cases where  $\mu_{\text{DMSP}}$  ( $\text{d}^{-1}$ ) was referred to as a "rate".**

*Secondly, the manuscript reports biological consumption rates and on p 2276 lines 11 ff states that "Rates [...] were estimated from the slope of the linear decrease in  $^{13}\text{C}$ -DMS concentrations over the 10–12 h incubation period." While DMS consumption rates, i.e.  $\frac{d(\text{DMS})}{dt}$ , may be estimated from linear decreases over very short (yet not quite infinitesimal) time intervals with little error, I am less certain that this can be done over a 10-12 h period given that these processes are not linear with time. Perhaps the authors could demonstrate the validity of this approach by showing some example data that allow readers to assess if  $^{13}\text{C}$ -DMS decrease over time is approximately linear?*

**We found the consumption rate of  $^{13}\text{C}$ -DMS to be linear over a 10 – 12 h incubation period. We have added a plot to the supplementary material demonstrating this. We show data from the first kinetic experiment (KE1) which demonstrates linearity in loss rates over a 10.5 h period for a range of concentrations, from 3 – 74  $\text{nmol L}^{-1}$ . We refer the reader to this example data in the methodology text as follows:**

**"Rates of BC ( $\text{nmol L}^{-1} \text{d}^{-1}$ ) were estimated from the slope of the linear decrease in  $^{13}\text{C}$ -DMS concentrations over the 10 – 12 h incubation period (see Fig. S1 in the supplementary material for example data)".**

*Biological consumption rates are then corrected for changes in substrate concentrations from tracer additions (see equation 1). I entirely agree with this approach. However, application of equation 1 requires knowledge of the half saturation constant,  $K_s$ . Unfortunately, details on experimental determinations of  $K_s$  are tucked away in the paper's supplement with no critical discussion. On closer inspection, one can see that  $K_s$  were determined for stations (and possibly conditions) different to those used for the bottle incubation studies. This may warrant at least some discussion in the manuscript itself (how representative are these data?). The finding that values for  $K_s$  varied 5-fold should also be discussed briefly. I perfectly understand the experimental constraints which dictate corrections for changes in substrate levels, and I suspect that it was not possible to conduct bottle incubations and  $K_s$  determinations simultaneously. However, it is possible to provide at least some brief transparent discussion of the above together with an assessment of the uncertainties involved: how large were the applied corrections and how do they change with your choice of  $K_s$ ?*

The methodology for determination of  $K_s$  has now been moved to the methodology section of the main paper. The kinetic experiments were indeed performed at separate stations to the bioassays. Unfortunately, it just was not feasible to do the experiments in parallel. Therefore, three contrasting sites were chosen in order to give a good representation of the consumption kinetics likely to be encountered around NW European seas, and of course, within the bioassay experiments. Given the broad range in consumption kinetics that we observed, it seems feasible that our kinetic experiments give a reasonable representation of the consumption kinetics within the bioassay incubations.

In order to allow the reader to assess the uncertainties involved, and transparently see the size of the applied correction, we have added the following paragraph to the methodology section:

“The applied corrections resulted in decreases in the consumption rates of 8 – 51 %; the larger uncertainties are associated with relatively low *in situ* DMS concentrations, thus resulting in relatively high tracer additions. Applying the maximum (25.0 nmol L<sup>-1</sup>) and minimum (4.5 nmol L<sup>-1</sup>)  $K_s$  values to the correction give the following uncertainties on the loss rates: E01 13.8 – 24.9 %, E02 23.1 – 39.7 %, E03 7.0 – 9.1 %, E04 9.7 – 12.7 %, E05 1.1 – 7.7 %. Using the standard error of mean  $K_s$  to the correction (shown in Table S2) results in uncertainty on loss rates of: E01 3.8 – 6.9%, E02 6.3 – 9.5 %, E03 1.6 – 2.0 %, E04 1.9 – 2.3 %, E05 0.3 – 1.6 %”.

We now briefly discuss the variation in  $K_s$  values in the discussion section as follows (underlined):

“In addition, the results from three kinetic experiments revealed a large range in values of  $K_s$  and  $V_{max}$  in the study waters, implying contrasting levels of control of BC on surface ocean DMS concentrations in the study region (see supplementary information, Table S5). A broad range in these parameters is unsurprising given that the measured rates represent the activity of natural assemblages that will vary greatly in space and time in the dynamic shelf sea environment, rather than the activity of specific single enzymes or species. The three sites (KE1, KE2 and KE3) also encompassed a wide range of surface DMS concentrations of 1.0, 3.8 and 16.8 nmol L<sup>-1</sup>, respectively, and this was likely a reflection of the contrasting BC characteristics of the sites (Fig. S2)”.

And in section 4.2:

“It is important to reiterate that it was not feasible to perform the kinetic experiments in parallel to the bioassay incubations for rates of BC. Therefore, the three chosen sites for kinetic experiments are assumed to give a good representation of the consumption kinetics likely to be encountered around NW European seas, and of course, within the bioassay experiments, with the recognised caveat that they do not precisely represent the *in situ* kinetics for each bioassay experiment. The uncertainties associated with the use of mean  $K_s$  determined from the three kinetic experiments, and used to correct BC rates for tracer additions, are given in the methodology section. However, given the broad range of consumption kinetics observed and the large range in *in situ* DMS concentrations for each kinetic experiment (Table 2), it seems feasible that our kinetic experiments give a reasonable representation of the consumption kinetics within the bioassay incubations”.

*Thirdly, section 3.2 and figures 3A & B illustrate changes of DMS:DMS<sub>Pt</sub> ratios with hydrogen ion concentration. The figures show what looks like a polynomial fit. However, no fit model is described nor is there any rationale given for model choice. I believe the authors should either remove best fit lines and statistics or provide details and rationale in the accompanying text.*

**We accept the referee's comment, and we have now removed the best fit lines from the plots – the trend is clear enough for the reader to see without them.**

*Finally, the discussion section (4.1, p 2284 ff) refers to UV-induced responses from previous work without making it sufficiently clear that the author's experimental setup very likely excluded UV (see section 2.1.: use of LED panels and polycarbonate bottles which are known to cut off UV). It remains unclear to me how this relates to the authors' acidification experiments, unless they can provide some information (or even informed speculation) that there may be a reason to assume similarities between UV-stress responses and response to enhanced CO<sub>2</sub>.*

**The section in the discussion concerning UV-stress responses was intended to serve as an analogy to the CO<sub>2</sub> responses we observed during the bioassays – the intention was not to infer any role for UV-stress in the bioassays. In order to explain the large increases in DMS : DMSPt with increasing CO<sub>2</sub> we suggest that some kind of [H<sup>+</sup>]-driven sublethal/lethal stress may be playing a role. UV-stress has also been reported to result in sublethal/lethal cellular damage that leads to increases in DMS release. We do not suggest that this is driven by the same mechanism. We have re-worded the text in this section so as not to confuse the reader:**

**“This response may be compared to those observed when phytoplankton communities are subjected to other stressors. Gali et al. (2013) proposed that exposure to sub-lethal or lethal levels of ultra-violet radiation (UVR) could induce an increase in cell membrane permeability, eventually triggering apoptosis. This would lead to the release of intracellular DMSP, increasing its availability for catabolism by bacteria, and/or extracellular DLA. In the bioassays, DMSPt concentrations fell and DMS production was stimulated, suggesting that the induced changes to carbonate chemistry may have resulted in an increase in cellular permeability and lysis (Flynn et al. 2012, Richier et al. 2014), leading to increased DMS release from cells”.**

**And:**

**“Previous studies have demonstrated up to an order of magnitude increase in DMS production in phytoplankton exposed to UV stress (Sunda et al. 2002, Archer et al. 2010, Gali et al. 2013). The observed increases of DMS: DMSPt with increasing CO<sub>2</sub> suggest that elevated [H<sup>+</sup>] may drive an analogous response, stimulating increases in turnover of DMSP to DMS. This could be driven by the apparent susceptibility of smaller phytoplankton to changes in [H<sup>+</sup>] (Flynn et al. 2012, Richier et al. 2014).”**

2. Specific and editorial comments

*Abstract:*

*Some tangible information should be added to the abstract, for example study area, dates, what was measured and some quantitative information.*

**We have added the following information to the abstract:**

**“Experiments were performed in June/July 2011 using water collected from contrasting sites in NW European waters (Outer Hebrides, Irish Sea, Bay of Biscay, North Sea). Concentrations of DMS and DMSP, alongside rates of DMSP synthesis and DMS production and consumption, were determined during all experiments for ambient CO<sub>2</sub> and three high CO<sub>2</sub> treatments (500, 750, 1000 μatm)”.**

**“We observed consistent and marked increases in DMS concentrations relative to ambient controls (110 % (28 – 223 %) at 550  $\mu$ atm, 153 % (56 – 295 %) at 750  $\mu$ atm and 225 % (79 – 413 %) at 1000  $\mu$ atm), and decreases in DMSP concentrations (28 % (18 – 40 %) at 550  $\mu$ atm, 44 % (18 – 64 %) at 750  $\mu$ atm and 52 % (24 – 72 %) at 1000  $\mu$ atm)”.**

*Introduction:*

*Some references are missing or have incorrect in-text citations, e.g. page 2269 ff: Andreae 1990 (not in references), Kiene and Linn 2000 (should be Kiene et al 2000), Kim et al 201?. Please check references throughout.*

**All references have been checked and corrected.**

*Page 2270, “turn-of-the-century levels of CO<sub>2</sub>”: did you mean 2100? Please clarify.*

**Yes we did indeed mean year 2100. This has been clarified in the text as follows:**

**“Decreases in DMS have been observed under predicted turn-of-the-century (year 2100) levels of CO<sub>2</sub>, ranging from ~35 % (Archer et al. 2013) to ~60 % (Hopkins et al. 2010, Avgoustidi et al. 2012) relative to ambient controls”.**

*Materials and Methods:*

*Page 2276, lines 17 ff “[Gross production] was estimated as the difference between net DMS production and BC”. Is gross production not equal to the sum of net P plus BC? Please clarify.*

**The text has been altered and now reads:**

**“Gross production was estimated as the sum of net DMS production and BC”.**

*Also: no information is given on ancillary data, including pH, CO<sub>2</sub>, nutrients etc. How were these obtained? Can data sources be referenced? I also wondered why Table 1 gives 'TON' but not dissolved inorganic N (DIN) or nitrate, which would in my view be more useful for characterising the study sites. Is TON = DON + PON? If the authors have DON, which is obtained by subtracting DIN from TN, why is it not in Table 1?*

**Table 1 now report values for NO<sub>3</sub><sup>-</sup>, PO<sub>4</sub><sup>3-</sup> and Si(OH)<sub>4</sub>. We agree that this is more useful for characterising the study sites. Detailed methodologies for all ancillary parameters are given in Richier et al. (2014, this issue). The following has been added to the methods section:**

**“2.8 Ancillary parameters**

**A description of methodologies for all ancillary parameters described in Table 1 (carbonate chemistry, nutrients, total and size-fractionated chlorophyll *a*) is given in Richier et al. (2014).”**

*Results:*

*P 2278 line 26 ff. Please report coefficients of determination as per convention (values between 0 and 1) and not as percentages. See also Figure 3.*

**All changed.**

*P 2280 lines 8 ff: the authors state that variations in DMSP production rates are caused by physiological rather than taxonomic changes. Can this statement be made in the absence of any taxonomic data? Please clarify.*

**We accept the referee's comment. We have revised the section containing this statement accordingly, and also the section of the discussion that deals with this issue.**

*Discussion:*

*P 2283, line 11 ff: "As a result of this general reduction in phytoplankton biomass and productivity, ratios of DMSP : chl a were predominantly lower under high CO<sub>2</sub>." As far as I can see, the manuscript does only report cell number concentrations for phytoplankton below 10 μm, and does not report data on biomass changes during incubations. Also: if biomass and hence chl a decrease, then DMSP : chl a ratios should increase and not decrease. Please clarify.*

**We agree that "biomass" was not the correct terminology to use in this context. We have changed "biomass" to "abundance" in the aforementioned sentence.**

**The referee is right to highlight the confusing message regarding DMSP: chl a. A decrease in this ratio is likely to indicate either a change in the proportion of phytoplankton that produce DMSP (community level change), or a decrease in the proportion of C fixation invested in DMSP synthesis (physiological levels change). We have re-worded the section as follows:**

**"In all experiments, except E01, the abundance of small phytoplankton (<10 μm) was significantly lower under high CO<sub>2</sub> (Fig. 2 K – O, Table S4). For E03, E04 and E05, significantly lower [Chl a] with increasing CO<sub>2</sub> was also observed (see Richier et al. 2014, this issue). Furthermore, specific rates of DMSP synthesis (μDMSP) and DMSP production rates were either insensitive to high CO<sub>2</sub> (E03, E04) or showed marked declines (E02, E05) (Fig. 4 A - D). Thus the observed general reduction in phytoplankton abundance and productivity resulted in predominantly decreased ratios of DMSP: chl a under high CO<sub>2</sub> (Fig. S3), driven by either a change in the proportion of phytoplankton that produce DMSP, or a decrease in the proportion of C fixation invested in DMSP synthesis".**

*P 2286, line 26 ff: "... saturation of consumption kinetics was exceeded". Please rephrase; saturation cannot be exceeded.*

**The referee is correct – we have altered the text accordingly, and it now reads:**

**"In the two cases where DMS did exceed 10 nmol L<sup>-1</sup>, it is possible that consumption kinetics reached saturation".**

*P2287 line 8. Text refers to a missing Figure (Fig. 7). Please correct.*

**The text should have referred to Figure 6 – we have changed this in the text.**

*Section 4.3 Exploring the regional variability. This section arbitrarily discusses only data from stations E01 (Mingulay Reef) and E04 (SE North Sea) which are arguably the least representative for the shelf waters studied here. I recommend including all stations in this discussion.*

**Rather than "arbitrarily" discussing only E01 and E04, we stated in the manuscript that this decision was made in order to both prevent a long and complicated discussion, and to focus on**

each end of the DMS response spectrum i.e. the station that showed the weakest DMS response to high CO<sub>2</sub> (E04), and the station with the strongest response (E01). However, for completeness and at the request of both referees, we have now included all stations in this discussion.

#### Response to Anonymous Referee #2

##### General comments

*Overall this is a well written paper with relatively few minor errors. Plus points are the geographical coverage of the data set and the inclusion of data for DMSP production rates and DMS production and consumption rates. Negatives include a lack of clarity in aspects of the methodology and some unjustified or poorly justified assumptions. Revision of these aspects should lead to a good paper matching the publication criteria of Biogeosciences.*

**We thank referee #2 for their positive feedback. We will address all the negative aspects that they highlight.**

##### Specific comments

*1) The abstract is not very informative nor quantitative – please add more detail about where the study was done and how, what was measured and quote specific data.*

**We have added the following information to the abstract:**

**“Experiments were performed in June/July 2011 using water collected from contrasting sites in NW European waters (Outer Hebrides, Irish Sea, Bay of Biscay, North Sea). Concentrations of DMS and DMSP, alongside rates of DMSP synthesis and DMS production and consumption, were determined during all experiments for ambient CO<sub>2</sub> and three high CO<sub>2</sub> treatments (500, 750, 1000 μatm)”.**

**“We observed consistent and marked increases in DMS concentrations relative to ambient controls (110 % (28 – 223 %) at 550 μatm, 153 % (56 – 295 %) at 750 μatm and 225 % (79 – 413 %) at 1000 μatm), and decreases in DMSP concentrations (28 % (18 – 40 %) at 550 μatm, 44 % (18 – 64 %) at 750 μatm and 52 % (24 – 72 %) at 1000 μatm)”.**

*2) Much is made of the level of replication e.g. abstract line 5, 2272 line 23. However, this relates to the experiments conducted by all the scientists onboard the ship and it is an over-statement for the experiments presented in this manuscript which have a standard 3 replicates. Please correct.*

**We have removed the phrase “highly replicated” from the abstract. All further reference to replication in the text refers to the triplicate bottles used for the DMS/P studies, and we feel does not over-state the level of replication that we present.**

*3) Simulating the real world in an incubation experiment is challenging and all approaches have limitations. For ocean acidification the insurmountable issue is timescale; most natural waters will show a gradual shift in pH and CO<sub>2</sub> concentration over future decades rather than a sudden shift from ambient CO<sub>2</sub> to a higher level. The authors state their experiments assess only the short term response (2271 line 16), but it could be argued that this approach is equivalent to looking at a ‘shock response’ rather than an acclimation response –again it is a question of timescale. Please reword this section.*

We strongly agree with the referee that simulating OA in any experimental setting is problematic and far from the reality of OA predicted to occur on timescales of decades to centuries. However, comparing the timescale of all OA experiments that have been performed to date, the vast majority could be considered as the result of a “shock response” when compared to the predicted timescale for the onset of OA in the surface oceans.

During the cruise, the implications of a possible “shock response” to the acid/bicarbonate addition were investigated. A 2-day gradual increase of  $p\text{CO}_2$  was performed (See Richier et al. 2014), and compared to the response seen during routine experiments with a single dose of acid/bicarbonate injected into bioassay flasks. A similar response was seen in all bottles, suggesting our experimental technique had negligible/non-existent influence on the observed response. The section has been re-worded (shown below), and the sentence starting “Unlike past mesocosm studies,...” has been omitted.

**“In an attempt to bridge the gap between the complexity of interpreting processes in traditional mesocosm experiments and the limited applicability of unialgal culture experiments to natural systems, this study was designed to assess the ecophysiological response of a variety of natural microbial communities from a wide geographic area to high  $\text{CO}_2$ ”.**

*4) The authors argue that short term perturbation experiments are ‘superior’ to the 3-4 week enclosed mesocosm approach without any mention of the ‘bottle effect’ that was well documented in the literature more than a decade ago. This effect was amongst the strong reasons for adopting the mesocosm approach. The 2 references below are examples of molecular studies that document shifts in bacterial population composition on similar time-scales to those in the Hopkins and Archer experiments. Lebaron, P., Servais, P., Troussellier, M., Courties, C., Muyzer, G., Bernard, L., Schäfer, H., Pukall, P., Stackebrandt, E., Guindulain, T., and J. Vives-Rego. (2001) Microbial community dynamics in Mediterranean nutrient-enriched mesocosms: changes in abundances, activity, and composition., FEMS Microbiology Ecology, 34: 255 - 266 Schäfer H, Bernard L, Courties C, Lebaron P, Servais P, Pukall P, Stackebrandt E, Troussellier M, Guindulain T, Vives-Rego J, Muyzer G (2001) Microbial community dynamics in Mediterranean nutrient-enriched seawater mesocosms: changes in the genetic diversity of bacterial populations , FEMS Microbiology Ecology, 34: 243 – 253*

We are surprised that the referee suggests we argue that short term perturbation experiments are ‘superior’ to mesocosms. This was certainly not our intention, and we have been unable to identify any occasions where our discussions could be interpreted in this way. We consider the short term but highly replicated and geographically-wide experimental design complementary to, rather than superior to, previous OA studies that have been conducted with less replication and geographical coverage, but over longer timescales. For this study, priority was placed on experimental replication and wide geographical and environmental coverage – yet we still concede that this, and no other study, should be considered to robustly represent the changes in marine microbial communities that will occur with future OA. Richier et al. (2014, this issue) give a detailed analysis of the choice of experimental design used here in the context of other OA studies. Such an exhaustive discussion is outside of the scope of our manuscript, so in the final paragraph of the introduction we have now directed the reader to this as follows:

**“In an attempt to bridge the gap between the complexity of interpreting processes in traditional mesocosm experiments and the limited applicability of unialgal culture experiments to natural systems, this study was designed to assess the ecophysiological response of a variety of natural microbial communities from a wide geographic area to high  $\text{CO}_2$ . Detailed discussion of the choice of experimental design in the context of previous OA studies is given by Richier et al. (2014, this issue)”.**

The referee refers us to two papers: however, upon reading the papers in detail it is clear that there are some differences between this and the current study. The water was pre-filtered over a 200  $\mu\text{m}$  mesh and the authors concede that this likely led to trophic cascade effects (Schafer et al. 2006). They also note the possibility that confinement may cause changes to the microbial communities, relative to in situ. The experiments they describe also lasted up to 2 weeks – and they show that intense grazing effects on the microbial communities occurred after about 96 hours of incubation. Finally, they saw the most dramatic “bottle effects” in incubations that included addition of inorganic nutrients.

No screening of water was carried out during our study, but it is possible that confinement of the communities in bottles resulted in some changes to the microbial communities. There was also no manipulation of in situ nutrient concentrations. Our data show that changes to carbonate chemistry had a strong and unquestionable effect on the microbial communities, likely masking any possible confinement effects that may have occurred over the short 96 h incubation period. This could include community changes that may have resulted in intense grazing activity – if one is to compare to the mesocosm experiments above.

*5) The major conclusion that DMS concentration consistently increased when CO<sub>2</sub> was increased relative to the ambient controls (2268 line 10) whereas DMSpt decreased when CO<sub>2</sub> was increased. The biggest problem this dataset presents concerns the control data. The data for the time zero time-points were for water collected in a completely separate water collection cast to those for the subsequent 2 time points (top of page 2273). I can see the practicalities leading to this decision, but seawater is very heterogeneous in both space and time. This puts the validity of the all-important 1<sup>st</sup> data point in serious doubt. The 2nd cast was presumably taken at the same location but post-dawn and with analytical samples taken directly from the Niskin bottle and so not treated in an identical way in terms of handling, decanting into bottles etc. The authors need to convince the reader that this is an acceptable approach and optimally to prove it with data.*

We accept the referees concerns that there may be differences in the water masses sampled over the course of the 2 – 3 h between sampling for the bioassay 0 h samples and the subsequent CTD cast. Thus we are keen to clarify in the text our confidence in our 0 h measurement. Carbonate chemistry parameters (DIC, TA, pH, pCO<sub>2</sub>) were determined for both bioassay 0 h and for the 0 h CTD samples, and in fact, little variation was detected. The bioassay 0 h values fell within the range of values from triplicate samples from the 0 h CTD. This suggests there was little difference in water mass over the 2 h period between CTD casts. The table below has been added to the supporting information section summarising this data, and we have added the following text to the methods section:

“Carbonate chemistry parameters (dissolved inorganic carbon (DIC), total alkalinity (TA), pH, pCO<sub>2</sub>, see Richier et al. (2014) for details of methods) were determined from both 0 h bioassay bottles and the second CTD cast. Single values from bioassay 0 h bottles fell within the range of triplicate values from 0 h CTD casts (Table S2), suggesting little/no change in water mass between CTD casts”.

The referee refers to the time zero measurements as “all important”. However, we view the 48 h and 96 h time points as much more crucial to our findings – after all, it is at these time points that the CO<sub>2</sub> effects become apparent. If we were to remove the time zero values, it would not have any effect on our conclusions. Of course, we do not feel it necessary to remove these values, and we are confident that the 0 h CTD measurements are a valid representation of the 0 h bioassay samples.

Table S2. Comparison between 0 h bioassay samples and time zero CTD samples. The CTD cast for 0 h bioassay samples was performed at 0200 h for E01 – E04 and 0100 for E05, with the 0 h CTD cast following within 2 – 3 h.

Expt . I.D.	DIC ( $\mu\text{mol kg}^{-1}$ )		TA ( $\mu\text{mol kg}^{-1}$ )		pH (total)		pCO <sub>2</sub> ( $\mu\text{atm}$ )	
	Bioassay 0 h (n = 1)	CTD 0 h Mean(SD) (n = 3)	Bioassay 0 h (n = 1)	CTD 0 h Mean(SD) (n = 3)	Bioassay 0 h (n = 1)	CTD 0 h Mean(SD) (n = 3)	Bioassay 0 h (n = 1)	CTD 0 h Mean(SD) (n = 3)
E01	2091.9	2091.8 (0.9)	2310.9	2310.9 (2.3)	8.11	8.11 (0.01)	342.3	342.6 (4.2)
E02	n.d.	2094.6 (1.0)	n.d.	2322.2 (2.4)	n.d.	8.12 (0.01)	n.d.	333.7 (5.4)
E03	2084.1	2083.8 (0.6)	2343.5	2347.1 (3.6)	8.11	8.11 (0.01)	345.4	339.8 (6.0)
E04	2085.7	2085.5 (1.6)	2298.7	2295.6 (0.4)	8.05	8.05 (0.01)	395.4	400.6 (5.7)
E05	2084.9	2084.6 (1.5)	2307.9	2311.5 (3.6)	8.07	8.08 (0.01)	374.7	368.1 (4.4)

6) Also concerning the control/T0/initial conditions data, there is a mismatch between the E01 and E02 DMSpT data in Table 1 (59.6 and 25.9 nmol l<sup>-1</sup>) and Figure 2 (\_25 and 60). Perhaps the data in either table or figure have been switched?

**We thank the referee for highlighting this discrepancy – the data in the table has been switched around so the correct values are shown for E01 and E02.**

7) For detail of the method for measuring the DMSP synthesis and production rates the reader is referred to Archer et al 2013 (2274). I checked this and noticed that the isotope fractionation factor used is that derived from studies on cultures of *Emiliania huxleyi*. Here the data are for variable mixed populations and the assumptions behind this deserve the addition of a few lines in the discussion.

**The referee’s concerns on the lack of detail for measuring DMSP synthesis and production have now been addressed. See response to Referee #1 comment that begins: “Firstly, the MS reports DMSP production rates...”**

Likewise, the K<sub>s</sub> values determined were highly variable but a mean value was used (2277). As these were not measured at each site the implications need to be worked through better in the main paper discussion section.

**In order to allow the reader to assess the uncertainties involved in corrections using mean values of K<sub>s</sub>, and to transparently see the size of the applied correction, we have added the following paragraph to the methodology section:**

**“The applied corrections resulted in decreases in the consumption rates of 8 – 51 %; the larger uncertainties are associated with relatively low *in situ* DMS concentrations, thus resulting in relatively high tracer additions. Applying the maximum (25.0 nmol L<sup>-1</sup>) and minimum (4.5 nmol L<sup>-1</sup>) K<sub>s</sub> values to the correction give the following uncertainties on the loss rates: E01 13.8 – 24.9 %, E02 23.1 – 39.7 %, E03 7.0 – 9.1 %, E04 9.7 – 12.7 %, E05 1.1 – 7.7 %. Using the standard error of**

mean  $K_s$  to the correction (shown in Table S2), results in uncertainty on loss rates of: E01 3.8 – 6.9%, E02 6.3 – 9.5 %, E03 1.6 – 2.0 %, E04 1.9 – 2.3 %, E05 0.3 – 1.6 %”.

We now briefly discuss the variation in  $K_s$  values in the discussion section as follows (underlined):

“In addition, the results from three kinetic experiments revealed a large range in values of  $K_s$  and  $V_{max}$  in the study waters, implying contrasting levels of control of BC on surface ocean DMS concentrations in the study region (see supplementary information, Table S5). A broad range in these parameters is unsurprising given that the measured rates represent the activity of natural assemblages that will vary greatly in space and time in the dynamic shelf sea environment, rather than the activity of specific single enzymes or species. The three sites (KE1, KE2 and KE3) also encompassed a wide range of surface DMS concentrations of 1.0, 3.8 and 16.8 nmol L<sup>-1</sup>, respectively, and this was likely a reflection of the contrasting BC characteristics of the sites (Fig. S2)”.

And in section 4.2:

“It is important to reiterate that it was not feasible to perform the kinetic experiments in parallel to the bioassay incubations for rates of BC. Therefore, the three chosen sites for kinetic experiments are assumed to give a good representation of the consumption kinetics likely to be encountered around NW European seas, and of course, within the bioassay experiments, with the recognised caveat that they do not precisely represent the *in situ* kinetics for each bioassay experiment. The uncertainties associated with the use of mean  $K_s$  determined from the three kinetic experiments, and used to correct BC rates for tracer additions, are given in the methodology section. However, given the broad range of consumption kinetics observed and the large range in *in situ* DMS concentrations for each kinetic experiment (Table 2), it seems feasible that our kinetic experiments give a reasonable representation of the consumption kinetics within the bioassay incubations”.

8) *The light incubation conditions are poorly described. Please give more detail on the LED panels (2272 line 19). What wavelengths of light were covered? Give the in situ light conditions in Table 1 for comparison and say whether there any attempt to adjust the light to the ambient conditions (excepting UV)?*

Wavelength dependent light was not measured. The LED panels used provided simulated daylight. Integrated daily light dose within the experiments alongside the integrated mixed layer irradiance will be presented in Richier et al. (2014, this issue) following similar referee comments for that manuscript, and the reader will be directed to Richier et al. for this information to prevent replication. The integrated mixed layer irradiance is most relevant for comparison to *in situ* conditions. The daily light dose within experiments was considered to be as close to representative as possible of that experienced *in situ* by the microbial community.

The methodology text has been altered, and now reads:

“A constant light level (100  $\mu\text{E m}^{-2} \text{s}^{-1}$ ) incorporating an 18:6 light: dark cycle was provided by daylight simulation LED panels (Powerpax, UK). The daily light dose within the experimental bioassays was considered to be as close to representative as possible of that experienced *in situ* by the microbial community. Details of the integrated mixed layer irradiances at the time of sample collection for each experiment is given in Richier et al. (2014, this issue)”.

*The discussion section page 2284 is misleading given that polycarbonate bottles generally cut out UV light. Why should the response to elevated CO<sub>2</sub> be comparable to the response to UV?*

We did not intend to suggest that the CO<sub>2</sub> response is comparable to the response to UV – we apologise if it came across this way, and have ensured that all relevant text has been altered to remove this apparent supposition.

The reasoning behind the discussion involving UV was to highlight that increased [H<sup>+</sup>] may have resulted in sublethal/lethal cellular damage – a similar phenomenon has been reported in response to UV. This is likely via a different mechanism, and we have adjusted the text to make this clearer. We are not suggesting that the response to [H<sup>+</sup>] is any way related to a UV response; rather we wish to highlight that similar mechanisms may be at play i.e. UV stress results in increased cell lysis that leads to increased DMS:DMSP. Similarly therefore, elevated [H<sup>+</sup>] may result in sublethal/lethal cellular damage that results in increased cell lysis and increased DMS:DMSP. See response to similar comment by referee #1 for specifics of text changes.

*9) There is no discussion at all on grazing effects – which is surprising given that the 2nd author has published on this topic in the context of DMS and DMSP. This means there is an inherent but unwritten assumption that grazing is the same across all CO<sub>2</sub> treatments, but what evidence can the authors offer to corroborate this?*

The referee is correct - the bioassay experiments were conducted using unfiltered seawater, so our results represent the response of the whole community, including all grazers. Thus we recognise that grazing effects could certainly be influencing our results. However, grazing parameters were not quantified during this study, so no information is available to assess its possible role on the overall response to carbonate chemistry perturbations. We now include a comment on the potential influence of grazers on our results to clarify this.

*10) I agree that the data could be suggestive of algal processes, exudation and lyase activity being part of the responses seen in bulk DMS and DMSP concentration, but neither is proven here. The wording should be removed from the abstract and the wording in the main body of the paper needs to be toned down to a more suitable level to prevent others quoting this as an experimental finding. These parameters are very difficult to isolate experimentally and the authors should offer specific suggestions for future research concerning these processes.*

The abstract has been re-worded so as to remove this speculative conclusion. It now simply gives a summary of the findings for DMSP synthesis/production, and bacterial GP and BC as follows:

**“Significant decreases in DMSP synthesis rate constants ( $\mu\text{DMSP, d}^{-1}$ ) and DMSP production rates ( $\text{nmol d}^{-1}$ ) were observed in two experiments (7 – 90 % decrease), whilst the response under high CO<sub>2</sub> from the remaining experiments was generally indistinguishable from ambient controls. Rates of bacterial DMS gross consumption and production gave weak and inconsistent responses to high CO<sub>2</sub>”.**

We have also toned down the text in section 4.4, and offered some suggestions for future research, so it now reads:

**“Our data is suggestive of an increase in stress-induced algal processes (increased cell permeability resulting in increased DMSP release and cleavage to DMS and/or direct up-regulation of intracellular DLA and DMS release) induced by the changes to carbonate chemistry. However, this cannot be validated without direct measurements, so future studies could include better determination of algal-related processes, including measurements of dissolved DMSP concentrations to give an indication of loss of DMSP from phytoplankton cells, and direct measurements of DLA (Steinke et al. 2000)”.**

Other comments

*The following paper is relevant and should be mentioned: Lee, P.A. et al. 2009. Effects of increased pCO<sub>2</sub> and temperature on the North Atlantic spring bloom III Dimethylsulphoniopropionate. Marine Ecology Progress Series 388: 41-49 doi:10.3354/meps08135.*

**This paper has been added to the introduction as follows:**

**“Besides the mesocosm experiments, one past study reported the response of natural communities using a shipboard continuous culture system (Lee et al. 2009); here, elevated CO<sub>2</sub> had no effect on concentrations of DMSP”.**

*2269 line 3 – the Stefels et al 2000 paper concerns the overflow and not the antioxidant hypothesis.*

**This reference has now been omitted from this sentence.**

*2269 first full paragraph – the work of Todd et al on the wide variety of DMSPdependent DMS release pathways ('lyase') deserves a mention.*

**Todd et al. 2007 and Todd et al. 2009 are now cited in the text as follows:**

**“Indeed, most DMSP released from phytoplankton is either catabolised by bacteria to produce DMS (Todd et al. 2007, 2009), or demethylated/demethiolated to produce other key organosulphur compounds such as methanethiol (MeSH) (Kiene et al. 2000, Moran et al. 2012)”.**

*2271 line 9 – say how DMSP responded.*

**Sentence now reads:**

**“DMS decreased 2- to 10-fold in cultures of the coccolithophore *Emiliana huxleyi* under high CO<sub>2</sub> (Arnold et al. 2013, Avgoustidi et al. 2012), whereas intracellular DMSP responded differently, with both significant decreases (Avgoustidi et al. 2012) and increases (Arnold et al. 2013)”.**

*2272 – state whether water was 200 um filtered or not.*

**The water was not filtered. This has been clarified in the methodology text.**

*2272 was the pH maintained throughout the timecourse of the experiments. Add data to Figure 2.*

**pH was generally stable over the time course of experiments, the pH data has now been added to Figure 2.**

*2272 line 15 – give a full description of the septa lids.*

**The septa lids were constructed of high density polyethylene (HDPE) with a silicone/ Polytetrafluoroethylene (PTFE) septum. This has been added to the methods text.**

*2275 – States that system sensitivity and drift were monitored, but it isn't clear whether there was no drift or whether data were adjusted accordingly.*

We apologise for not including this detail in our methods. The text has been altered accordingly in the methods section:

**“The MSD was operated in single ion mode (SIM) and was programmed to detect the following ions: m/z 62, 61 and 47 for <sup>12</sup>C-DMS, m/z 64 for <sup>13</sup>C-DMS, and m/z 68 for deuterated DMS (CD<sub>3</sub>SCD), of which 100 uL of a 5 ppmv gas standard was injected into the purge gas stream for every sample in order to monitor and correct for system sensitivity and drift. By taking the ratio of m/z 62 or 64 to m/z 68, a greatly improved precision of analysis was attained”.**

*2280 line 11 – there is no taxonomic data so this can't be proven.*

**We accept the referee's comment. We have revised the section containing this statement accordingly, and also the section of the discussion that deals with this issue.**

*2286 line 27 do you mean consumption kinetics were saturated?*

**Yes – we do indeed. The text has been altered accordingly.**

*2287 line 8 do you mean Figure 6 there is no Figure 7.*

**Yes – it should have read “Figure 6” and the text has been altered accordingly.**

*2287 it is a pity to limit the discussion to E01 and E04!*

**For completeness and at the request of both referees, we have now included all stations in this discussion.**

*Table 1 please add nitrate and ammonium data as well as TON.*

**Based on the comments of referee #1, we have altered the data in Table 1 so it now shows NO<sub>3</sub><sup>-</sup>, PO<sub>4</sub><sup>3-</sup> and Si(OH)<sub>4</sub>. We believe this information is most useful for the reader to characterise the study sites.**

*Figure 2 the curves fitted to the data here are a bit misleading e.g. for site E03 DMSPt at ambient CO<sub>2</sub>, the concentrations at 48 and 96 h are very similar but the curve gives the impression of an increase.*

**Smoothed lines were used in the plots purely to guide the reader's eye to the clear trends, and were not intended to suggest any kind of extrapolation of the data in between time points. We feel that re-plotting all data with straight lines would not in any way alter the overall findings and conclusions, and so would be an unnecessary exercise. We have added the following sentence to the legends for figures 2, 4 and 5:**

**“Smoothed lines on plots do not represent extrapolation of data between time point measurements, but are used to highlight trends”.**

*Figure 5 legend mentions asterisks denoting a significant difference from ambient bioassays but none can be seen in the figure.*

**The missing asterisks have been added to the figure.**

Figure 6 legend mentions mean values open circles but there are none on the figure.

**The open circles had mistakenly been omitted – they have now been added to the figure.**

Figure S1 please provide a legend to indicate which profile is for which site.

**A legend has now been added to this figure.**

Figure S2 the kinetic curve is only provided for KE3, add the curves for the other 2 sites as well.

**All three kinetic curves are now shown in Figure S2.**

## References

- Archer, S.D., Ragni, M., Webster, R., Airs, R.L., Geider, R.J.: Dimethyl sulfoniopropionate and dimethyl sulfide production in response to photoinhibition in *Emiliana huxleyi*. *Limnol. Oceanogr.*, 55 (4), 1579 – 1589, 2010.
- Archer, S.D., Kimmance, S.A., Stephens, J.A., Hopkins, F.E., Bellerby, R.G.J., Schulz, K.G., Piontek, J., Engel, A.: Contrasting response of DMS and DMSP to ocean acidification in Arctic waters. *Biogeosciences*, 10, 1893 – 1908, 2013.
- Arnold, H.E., Kerrison, P., Steinke, M.: Interacting effects of ocean acidification and warming on growth and DMS-production in the haptophyte coccolithophore *Emiliana huxleyi*. *Glob. Change Bio.*, doi: 10.1111/gcb.12105, 2013.
- Avgoustidi, V., Nightingale, P.D., Joint, I., Steinke, M., Turner, S.M., Hopkins, F.E., Liss, P.S.: Decreased marine dimethyl sulfide production under elevated CO<sub>2</sub> levels in mesocosm and in vitro studies. *Environ. Chem.*, 9, 399 – 404, 2012.
- Flynn, K.J., Blackford, J.C., Baird, M.E., Raven, J.A., Clark, D.R., Beardall, J., Brownlee, C., Fabian, H., Wheeler, G.L.: Changes in pH at the exterior surface of plankton with ocean acidification. *Nature Clim. Change*, 2, 510 – 513, 2012.
- Galí, M., Ruiz-González, Lefort, T., Gasol, J.M., Cardelús, C., Romera-Castillo, C., Simó, R.: Spectral irradiance dependence of sunlight effects on plankton dimethylsulphide production. *Limnol. Oceanogr.* 58 (2), 489 – 504, 2013.
- Hopkins, F.E., Turner, S. M., Nightingale, P. D., Steinke, M., Liss, P.S.: Ocean acidification and marine biogenic trace gas production. *P. Natl. Acad. Sci. USA*, 107, 760-765, 2010.
- Stefels, J., Dacey, J.W.H., Elzenga, T.M.: In vivo DMSP-biosynthesis measurements using stable-isotope incorporation and proton-transfer-reaction mass spectrometry (PTR-MS). *Limnol. Oceanogr. Meth.*, 7, 595 – 611, 2009.
- Steinke, M., Malin, G., Turner, S., Liss, P.S.: Determinations of dimethylsulphoniopropionate (DMSP) lyase activity using headspace analysis of dimethyl sulphide (DMS). *J. Sea. Res.*, 43, 233 – 244, 2000.
- Todd, J.D., Rogers, R., Li, Y.G., Wexler, M., Bond, P.L., Sun, L., Curson, A.R.J., Malin, G., Steinke, M., Johnston, A.W.B.: Structural and regulatory genes required to make the gas dimethyl sulfide in bacteria. *Science*, 315, 666 – 669, 2007.

Todd, J.D., Curson, A.R.J., Dupont, C.L., Nicholson, P., Johnston, A.W.B.: The dddP gene, encoding a novel enzyme that converts dimethylsulfoniopropionate into dimethyl sulfide, is widespread in ocean metagenomes and marine bacteria and also occurs in some Ascomycete fungi. *Environ. Microb.* 11 (6), 1376 – 1385, 2009.

Richier, S., Achterberg, E.P., Dumousseaud, C., Poulton, A.J., Suggett, D.J., Tyrell, T., Moore, C.M.: Carbon cycling and phytoplankton responses within highly-replicated shipboard carbonate manipulation experiments around the Northwest European continental shelf. *Biogeosciences Discuss.*, this issue.