Biogeosciences Discuss.,
https://doi.org/10.5194/bg-2018-148-AC2, 2018

Biogeosciences
Discussions

Interactive comment on "Effect of elevated pCO_2 on trace gas production during an ocean acidification mesocosm experiment" *by* Sheng-Hui Zhang et al.

Sheng-Hui Zhang et al.

gpyang@mail.ouc.edu.cn

Received and published: 29 July 2018

Dear Reviewer #2: We are grateful to your review of this paper and would like to express our thanks for the helpful and constructive comments. We have revised the manuscript and addressed all the comments. The main changes we made are as follows: General Comments The study examines production of volatile sulfur and halocarbon compounds in mesocosms of seawater with different dissolved carbon concentrations. The premise is to examine the impact of ocean acidiïňĄcation on gas production. This is an okay idea. One major concern, however, is that the study was only ïňĄve-weeks long, and there was no pretreatment of the phytoplankton. Thus, it

is not really a global change test, but rather it is a test of acid shock on phytoplankton. I suppose this is interesting. Thanks for the reviewer's suggestion and we also agree with that the mesocosm experiment is a test of acid shock on phytoplankton. In addition, simple pretreatment was conducted before the mesocosm experiment as described in Huang et al. (2018). Briefly, before being introduced into the mesocosms, the three phytoplankton species and their associated bacteria were cultured in autoclaved, pre-filtered seawater from Wuyuan Bay at 16°C (similar to the in situ temperature of Wuyuan Bay) without any addition of nutrients. Cultures were continuously aerated with filtered ambient air containing 400 μatm of CO2 within plant chambers (HP1000G-D, Wuhan Ruihua Instrument & Equipment, China) at a constant bubbling rate of 300 ml min–1. The culture medium was renewed every 24 h to maintain the cells of each phytoplankton species in exponential growth. We have added these pretreatment in the revised manuscript. P6, L135-P7, L141 "Before being introduced into the mesocosms, the three phytoplankton species were cultured in autoclaved, pre-filtered seawater from Wuyuan Bay at 16 °C (similar to the in situ temperature of Wuyuan Bay) without any addition of nutrients. Cultures were continuously aerated with filtered ambient air containing 400 μatm of CO2 within plant chambers (HP1000G-D, Wuhan Ruihua Instrument & Equipment, China) at a constant bubbling rate of 300 mL min–1. The culture medium was renewed every 24 h to maintain the cells of each phytoplankton species in exponential growth." Also, it appears to me that some of the data on temporal changes in chemistry and biology in the mesocosms have been published previously by Liu et al. (2017). Figure 1 is identical to Figure 1 and Figure 2 in Liu et al. (2017) and, at least, two panels in Figure 2 are in Figure 3 in Liu et al. (2017). Thus, only the data in Figure 3 are new. Unfortunately, you cannot publish the same data twice. Elsevier, the publisher of Marine Environmental Research, owns the copyright to those ïňĄgures. We agree with the reviewer's suggestion. In the revised manuscript, we deleted the conflicting figures and only described these results simply. P9, L187-L192"The phytoplankton growth process was divided into three phases in terms of variations in Chl a concentrations in the mesocosm experiments as described

in Liu et al. (2017): i) the logarithmic growth phase (phase I, days 0–13), ii) a plateau phase (phase II, days 13–23, bloom period), and iii) a secondary plateau phase (phase III, days 23–33) attained after a decline in biomass from a maximum in phase II." P9, L200-L204 "Emiliania huxleyi was only found in phase I and its maximal concentration reached 310 cells mLâĂŠ1 according to the results of microscopic inspection. Thalassiosira weissflogii was found throughout the entire period in each bag, but the maximum concentration was 8,120 cells mLâĂŠ1, which was far less than the concentration of Phaeodactylum tricornutum with a maximum density of about 1.5 million cells mLâĂŠ1 (Liu et al., 2017)." SpeciïňĄc Comments 1) The abstract reads well. Thanks for the reviewer's ratification. 2) The introduction is okay. However, it ends a bit abruptly. As written it is mostly a review of literature ending in an objective to do more research. Although a research objective is good, research should be question driven and present a testable, falsiïňĄable hypothesis. In this case, what do you hope to learn in a 5-week study? (This seems short term to me.) We agree with the reviewer's suggestion and have made modification in the revised manuscript. P5, L97-103 "DMS and halocarbons play a significant role in the global climate and perhaps act a greater extent in the future. Meanwhile, the combined picture arising from existing studies is that the response of communities to OA is not predictable and further studies were required. Based on the controversial results about OA on DMS and halocarbons production, a mesocosm experiment was conducted in Wu Yuan Bay, Xiamen. The aim of this study was to investigate the influence of elevated pCO2 on diatoms and coccolithophores and to further understand how the productions of DMS and halocarbons respond to OA." During this experiment, the nutrient concentrations (dissolved inorganic nitrogen (DIN) and phosphate) in phase II were below or close to the detection limit, though the Chl a concentration still maintained a relatively high concentration after 5 weeks incubation. We think that the stored nutrients in diatom cells might contribute to the biomass increase even after the depletion of nutrients in the surrounding seawater (Goldman et al., 1979; Sommer, 1989). Meanwhile, DMS, DCB and CH3I concentration decreased significantly after 5 weeks incubation. Therefore, 5 weeks incubation is

appropriate to this experiment. 3) The methods seem appropriate, to me. Thanks for the reviewer's ratification. 4) The results are okay. However, the discussion about the role of bacteria in DMSP dynamics, on page 10 and 11, seems like speculation to me. Where are the data on bacteria in the mesocosms? Speculation is okay, but data is better. We agree with the reviewer's suggestion. We have added the DCB data in the revised manuscript. P8, L167-L175 "2.5 Enumeration of DMSP-consuming bacteria (DCB) The number of DMSP-consuming bacteria (DCB) was estimated using the most probable number (MPN) methodology. The MPN medium consisted of a mixture (1:1 v/v) of sterile artificial sea water (ASW) and mineral medium (Visscher et al., 1991), 3 mL of which was dispensed in 6 mL test tubes, which were closed off by an over-sized cap, allowing gas exchange. Triplicate dilution series were set up. All test tubes contained 1 mmol L-1 DMSP as the sole organic carbon source and were kept at 30 °C in the dark. After 2 weeks, the presence/absence of bacteria in the tubes was verified by DAPI staining (Porter and Feig, 1980). Three tubes containing 3 mL ASW without substrate were used as controls." P10, L217-L224 "Compared with DMSP, DMS and DCB concentrations showed similar trends during the mesocosm experiment. DMS concentrations in the LC and HC treatments were 1.03 and 0.74 nmol L–1, respectively, while DCB concentrations in the LC and HC treatments were 0.20 × 106 and 0.16 × 106 cells mL–1. DMS and DCB concentrations did not increase significantly during phase I, but began to increase rapidly on day 15. DCB concentrations in the LC and HC treatments peaked on days 21 (11.65 × 106 cells mL–1) and 23 (10.70 × 106 cells mL–1), while DMS concentrations in the LC and HC treatments peaked on days 25 (112.1 nmol L–1) and 30 (101.9 nmol L–1). Both DMS and DCB concentrations began to decrease obviously during phase III." P11, L231-L234 "However, a significant 29% reduction in DMS concentrations was detected in the HC treatment compared with the LC treatment ($p = 0.016$), though no statistical difference for DCB concentrations was found between the LC and HC treatments during phase I." P11, L244-L246 "In addition, a significant positive relationship was also observed between DMS and DCB ($r = 0.643$, $p < 0.01$ in the LC treatment; $r = 0.544$, $p < 0.01$ in the HC treatment) during this

experiment." P12, L251-L253 "Moreover, DCB peaked on days 21 (11.65 $\times$ 106 cells mL-1) and 23 (10.70 $\times$ 106 cells mL-1) in the LC and HC treatments, respectively, as shown in Fig. 2-C. Similar to DMS, DCB was also delayed in the HC mesocosm compared to that in the LC mesocosm." 5) Than many correlations in the text could go in a table. This would make the text more readable. We agree with reviewer's suggestion and have add two tables in the revised manuscript.

Table 2. Relationships between DMS, DMSP, Chl a, CHBrCl2, CH3Br, CH2Br2, CH3I, DCB, Thalassiosira weissflogii (T. weissflogii) and Phaeodactylum tricornutum (P. tricornutum) concentrations in the LC treatments. DMS (nmol L-1) DMSP (nmol L-1) Chl a (μg L-1) CHBrCl2 (pmol l-1) CH3Br (pmol l-1) CH2Br2 (pmol l-1) CH3I (pmol l-1) DCB ($\times$106 cells mL-1) T. weissflogii ($\times$103 cells mL-1) P. tricornutum (cells mL-1) DMS 1 DMSP 0.701** 1 Chl a 0.597** 0.792** 1 CHBrCl2 0.526 0.280 0.559 1 CH3Br -0.413 -0.230 0.196 0.313 1 CH2Br2 0.310 0.180 0.001 -0.136 -0.308 1 CH3I 0.694** 0.654** 0.717** 0.596* -0.151 0.129 1 DCB 0.643** 0.520* 0.522* 0.394 -0.268 -0.038 0.762** 1 T. weissflogii 0.410 0.617** 0.899** 0.301 0.322 0.028 0.680** 0.399 1 P. tricornutum 0.560* 0.961** 0.821** 0.528 -0.032 0.162 0.588** 0.334 0.685** 1

Table 3. Relationships between DMS, DMSP, Chl a, CHBrCl2, CH3Br, CH2Br2, CH3I, DCB, Thalassiosira weissflogii (T. weissflogii) and Phaeodactylum tricornutum (P. tricornutum) concentrations in the HC treatments. DMS (nmol L-1) DMSP (nmol L-1) Chl a (μg L-1) CHBrCl2 (pmol l-1) CH3Br (pmol l-1) CH2Br2 (pmol l-1) CH3I (pmol l-1) DCB ($\times$106 cells mL-1) T. weissflogii ($\times$103 cells mL-1) P. tricornutum (cells mL-1) DMS 1 DMSP 0.752** 1 Chl a 0.318* 0.738** 1 CHBrCl2 0.324 0.094 0.326 1 CH3Br -0.410 -0.349 0.065 0.076 1 CH2Br2 0.540* 0.352 0.142 0.233 -0.377 1 CH3I 0.694** 0.816** 0.741** 0.690* -0.407 0.316 1 DCB 0.544* 0.522 0.549* 0.532 -0.311 0.368 0.851* 1 T. weissflogii 0.355 0.743** 0.930** 0.304 0.076 0.233 0.690** 0.567 1 P. tricornutum 0.635** 0.954** 0.803** 0.143 -0.257 0.267 0.834** 0.559 0.820** 1

6) Much of the discussion on page 13 is literature rather than interpretation. Rather than merely list other studies, compare results quantitatively. Did the other studies

have CH3I production rates that were similar to yours? We agree with reviewer's suggestion and have made the modification in the revised manuscript. P13, L280-288 "The temporal dynamics of CH3I in the HC and LC treatments are shown in Fig. 3-D. The CH3I concentrations in the LC treatment varied from 0.38 to 12.61 pmol L-1, with a mean of 4.76 pmol L-1. The CH3I concentrations in the HC treatment ranged between 0.44 and 8.78 pmol L-1, with a mean of 2.88 pmol L-1. The maximum CH3I concentrations in the HC and LC treatments were both observed on day 23. The range of CH3I concentrations during this experiment was similar to that measured in the mesocosm experiment (< 1$\sim$10 pmol L-1) in Kongsfjorden conducted by Hopkins et al. (2013). In addition, the mean CH3I concentration in the LC treatment was similar to that measured in the East China Sea, with an average of 5.34 pmol L-1 in winter and 5.74 pmol L-1 in summer (Yuan et al., 2015)." Technical Comments 1) Line 31 & 36: report the percentages as whole integers. It is nearly impossible to measure accurately to 0.1%. Thanks for the reviewer's suggestion. We have modified this in the revised manuscript. P2, L36-43 "During the logarithmic growth phase (phase I), DMS concentrations in high pCO2 mesocosms (HC, 1000 μatm) were 28% lower than those in low pCO2 mesocosms (LC, 400 μatm). Elevated pCO2 led to a delay in DCB concentrations attached to Thalassiosira weissflogii and Phaeodactylum tricornutum and finally resulted in the delay of DMS concentration in the HC treatment. Unlike DMS, the elevated pCO2 did not affect DMSP production ability of Thalassiosira weissflogii or Phaeodactylum tricornuntum throughout the 5 weeks culture. A positive relationship was detected between CH3I and Thalassiosira weissflogii and Phaeodactylum tricornuntum during the experiment, and there was a 40% reduction in mean CH3I concentrations in the HC mesocosms." 2) Line 48: 'human activity' and 'anthropogenic' are the same. You do not need both in the sentence. We agree with the reviewer's suggestion and have modified this in the revised manuscript. P3, L53-56 "Anthropogenic emissions have increased the fugacity of atmospheric carbon dioxide (pCO2) from the pre-industrial value of 280 μatm to the present-day value of over 400 μatm, and these values will further increase to 800–1000 μatm by the end

of this century according to the Intergovernmental Panel on Climate Change (Gattuso et al., 2015)." 3) Line 69: delete the sentence 'several studies have already, etc.' in the following sentence, replace 'majority' with 'several studies have shown a negative impact, etc.' We agree with the reviewer's suggestion and have made modification in the revised manuscript. P4, L77-80 "Several studies have shown a negative impact of decreasing pH on DMS-production capability (Hopkins et al., 2010; Avgoustidi et al., 2012; Archer et al., 2013; Webb et al., 2016), while others have found either no effect or a positive effect (Vogt et al., 2008; Hopkins and Archer, 2014)." 4) Line 78: perhaps start a new paragraph with 'halocarbons' Thanks for the reviewer's suggestion. We have started a new paragraph with 'halocarbons' in the revised manuscript.. 5) Line 189 to 192: delete. This is not an appropriate topic sentence, and it is from the introduction. No need to repeat here. Thanks for the reviewer's suggestion. We have deleted this sentence in the revised manuscript. 6) Line 192: delete the sentence and put (Fig. 3) in the following sentence. We agree with the reviewer's suggestion and have made the modification in the revised manuscript. P9, L207-P10, L209 "At the beginning of the experiment, the mean DMS, DMSP and DCB concentrations were all low in both treatments due to the low concentrations of DMS, DMSP and DCB in the original fjord water and possible loss during the filtration procedure (Fig. 2)." 7) Line 209: round '29.2%' to the '29%'. We agree with the reviewer's suggestion and have made the modification in the revised manuscript. P11, L231-234 "However, a significant 29% reduction in DMS concentrations was detected in the HC treatment compared with the LC treatment ($p = 0.016$), though no statistical difference for DCB concentrations was found between the LC and HC treatments during phase I." 8) Line 228: why Yu et al., unpublished data? Why not include the data here? We agree with the reviewer's suggestion. We have added the DCB data in the revised manuscript. P8, L167-L175 "2.5 Enumeration of DMSP-consuming bacteria (DCB) The number of DMSP-consuming bacteria (DCB) was estimated using the most probable number (MPN) methodology. The MPN medium consisted of a mixture (1:1 v/v) of sterile artificial sea water (ASW) and mineral medium (Visscher et al., 1991), 3 mL of which

was dispensed in 6 mL test tubes, which were closed off by an over-sized cap, allowing gas exchange. Triplicate dilution series were set up. All test tubes contained 1 mmol L-1 DMSP as the sole organic carbon source and were kept at 30 °C in the dark. After 2 weeks, the presence/absence of bacteria in the tubes was verified by DAPI staining (Porter and Feig, 1980). Three tubes containing 3 mL ASW without substrate were used as controls." P10, L217-L224 "Compared with DMSP, DMS and DCB concentrations showed similar trends during the mesocosm experiment. DMS concentrations in the LC and HC treatments were 1.03 and 0.74 nmol L–1, respectively, while DCB concentrations in the LC and HC treatments were 0.20 × 106 and 0.16 × 106 cells mL–1. DMS and DCB concentrations did not increase significantly during phase I, but began to increase rapidly on day 15. DCB concentrations in the LC and HC treatments peaked on days 21 (11.65 × 106 cells mL–1) and 23 (10.70 × 106 cells mL–1), while DMS concentrations in the LC and HC treatments peaked on days 25 (112.1 nmol L–1) and 30 (101.9 nmol L–1). Both DMS and DCB concentrations began to decrease obviously during phase III." P11, L231-L234 "However, a significant 29% reduction in DMS concentrations was detected in the HC treatment compared with the LC treatment ($p = 0.016$), though no statistical difference for DCB concentrations was found between the LC and HC treatments during phase I." P11, L244-L246 "In addition, a significant positive relationship was also observed between DMS and DCB ($r = 0.643$, $p < 0.01$ in the LC treatment; $r = 0.544$, $p < 0.01$ in the HC treatment) during this experiment." P12, L250-L253 "Moreover, DCB peaked on days 21 (11.65 × 106 cells mL-1) and 23 (10.70 × 106 cells mL-1) in the LC and HC treatments, respectively, as shown in Fig. 2-C. Similar to DMS, DCB was also delayed in the HC mesocosm compared to that in the LC mesocosm." 9) Line 258: the sentence does not make sense. Do you mean 'attributed to biology' rather than 'involve'. Also delete the quotes around 'biogenic'. Why use quotes for an adjective? We agree with the reviewer's suggestion and have deleted this sentence in the revised manuscript.

Please also note the supplement to this comment:

https://www.biogeosciences-discuss.net/bg-2018-148/bg-2018-148-AC2-supplement.pdf

Interactive comment on Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-148, 2018.