

Anonymous Referee #2

Received and published: 18 June 2018

Title: Effect of elevated $p\text{CO}_2$ on trace gas production during an ocean acidification mesocosm experiment Author(s): Sheng-Hui Zhang et al. MS No.: bg-2018-148

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 acidification on gas production.

This is an okay idea. One major concern, however, is that the study was only five-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. 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. (2107) 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 figures.

Specific Comments

- 1) The abstract reads well.
- 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, falsifiable hypothesis. In this case, what do you hope to learn in a 5-week study? (This seems short term to me.)
- 3) The methods seem appropriate, to me.
- 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.
- 5) Than many correlations in the text could go in a table. This would make the text more readable.
- 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 CH_3I production rates that were similar to yours?

Technical Comments

- 1) Line 31 & 36: report the percentages as whole integers. It is nearly impossible to measure accurately to 0.1%.
- 2) Line 48: 'human activity' and 'anthropogenic' are the same. You do not need both in the sentence.

- 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.'
- 4) Line 78: perhaps start a new paragraph with 'halocarbons'
- 5) Line 189 to 192: delete. This is not an appropriate topic sentence, and it is from the introduction. No need to repeat here.
- 6) Line 192: delete the sentence and put (Fig. 3) in the following sentence.
- 7) Line 209: round '29.2%' to the '29%'.
- 8) Line 228: why Yu et al., unpublished data? Why not include the data here?
- 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?

Sorry but I cannot overlook the attempt to publish the same data in two papers. I realize that data from one paper can be used in another, but this needs to acknowledge the first paper and copywrite.

Response to Reviewer #2:

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 acidification on gas production.

This is an okay idea. One major concern, however, is that the study was only five-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 CO_2 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 CO_2 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 figures.

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 $310 \text{ cells mL}^{-1}$ according to the results of microscopic inspection. *Thalassiosira weissflogii* was found throughout the entire period in each bag, but the maximum concentration was $8,120 \text{ cells mL}^{-1}$, which was far less than the concentration of *Phaeodactylum tricornerutum* with a maximum density of about 1.5 million cells mL^{-1} (Liu et al., 2017).”

Specific 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, falsifiable 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 $p\text{CO}_2$ 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 CH_3I 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 $30 \text{ }^\circ\text{C}$ 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 0.20×10^6 and $0.16 \times 10^6 \text{ cells mL}^{-1}$. 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 ($11.65 \times 10^6 \text{ cells mL}^{-1}$) and 23 ($10.70 \times 10^6 \text{ cells mL}^{-1}$), while DMS concentrations in the LC and HC treatments peaked on days 25 ($112.1 \text{ nmol L}^{-1}$) and 30 ($101.9 \text{ nmol L}^{-1}$). 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 (11.65×10^6 cells mL^{-1}) and 23 (10.70×10^6 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 <i>a</i> (µg L ⁻¹)	CHBrCl ₂ (pmol l ⁻¹)	CH ₃ Br (pmol l ⁻¹)	CH ₂ Br ₂ (pmol l ⁻¹)	CH ₃ I (pmol l ⁻¹)	DCB (×10 ⁶ cells mL ⁻¹)	<i>T. weissflogii</i> (×10 ³ cells mL ⁻¹)	<i>P. tricornutum</i> (cells mL ⁻¹)
DMS	1									
DMSP	0.701**	1								
Chl <i>a</i>	0.597**	0.792**	1							
CHBrCl ₂	0.526	0.280	0.559	1						
CH ₃ Br	-0.413	-0.230	0.196	0.313	1					
CH ₂ Br ₂	0.310	0.180	0.001	-0.136	-0.308	1				
CH ₃ I	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		
<i>T. weissflogii</i>	0.410	0.617**	0.899**	0.301	0.322	0.028	0.680**	0.399	1	
<i>P. tricornutum</i>	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 <i>a</i> (µg L ⁻¹)	CHBrCl ₂ (pmol l ⁻¹)	CH ₃ Br (pmol l ⁻¹)	CH ₂ Br ₂ (pmol l ⁻¹)	CH ₃ I (pmol l ⁻¹)	DCB (×10 ⁶ cells mL ⁻¹)	<i>T. weissflogii</i> (×10 ³ cells mL ⁻¹)	<i>P. tricornutum</i> (cells mL ⁻¹)
DMS	1									
DMSP	0.752**	1								
Chl <i>a</i>	0.318*	0.738**	1							
CHBrCl ₂	0.324	0.094	0.326	1						
CH ₃ Br	-0.410	-0.349	0.065	0.076	1					
CH ₂ Br ₂	0.540*	0.352	0.142	0.233	-0.377	1				
CH ₃ I	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		
<i>T. weissflogii</i>	0.355	0.743**	0.930**	0.304	0.076	0.233	0.690**	0.567	1	
<i>P. tricornutum</i>	0.635**	0.954**	0.803**	0.143	-0.257	0.267	0.834**	0.559	0.820**	1

1

2 6) Much of the discussion on page 13 is literature rather than interpretation. Rather than merely
3 list other studies, compare results quantitatively. Did the other studies have CH₃I production rates
4 that were similar to yours?

5 We agree with reviewer's suggestion and have made the modification in the revised manuscript.

6 P13, L280-288 "The temporal dynamics of CH₃I in the HC and LC treatments are shown in Fig.
7 3-D. The CH₃I concentrations in the LC treatment varied from 0.38 to 12.61 pmol L⁻¹, with a
8 mean of 4.76 pmol L⁻¹. The CH₃I concentrations in the HC treatment ranged between 0.44 and
9 8.78 pmol L⁻¹, with a mean of 2.88 pmol L⁻¹. The maximum CH₃I concentrations in the HC and
10 LC treatments were both observed on day 23. The range of CH₃I concentrations during this
11 experiment was similar to that measured in the mesocosm experiment (< 1~10 pmol L⁻¹) in
12 Kongsfjorden conducted by Hopkins et al. (2013). In addition, the mean CH₃I concentration in the
13 LC treatment was similar to that measured in the East China Sea, with an average of 5.34 pmol L⁻¹
14 in winter and 5.74 pmol L⁻¹ in summer (Yuan et al., 2015)."

15 Technical Comments

16 1) Line 31 & 36: report the percentages as whole integers. It is nearly impossible to measure
17 accurately to 0.1%.

18 Thanks for the reviewer's suggestion. We have modified this in the revised manuscript.

19 P2, L36-43 "During the logarithmic growth phase (phase I), DMS concentrations in high pCO₂
20 mesocosms (HC, 1000 µatm) were 28% lower than those in low pCO₂ mesocosms (LC, 400 µatm).
21 Elevated pCO₂ led to a delay in DCB concentrations attached to *Thalassiosira weissflogii* and
22 *Phaeodactylum tricornutum* and finally resulted in the delay of DMS concentration in the HC
23 treatment. Unlike DMS, the elevated pCO₂ did not affect DMSP production ability of
24 *Thalassiosira weissflogii* or *Phaeodactylum tricornutum* throughout the 5 weeks culture. A
25 positive relationship was detected between CH₃I and *Thalassiosira weissflogii* and *Phaeodactylum*
26 *tricornutum* during the experiment, and there was a 40% reduction in mean CH₃I concentrations
27 in the HC mesocosms."

28 2) Line 48: 'human activity' and 'anthropogenic' are the same. You do not need both in the
29 sentence.

30 We agree with the reviewer's suggestion and have modified this in the revised manuscript.

31 P3, L53-56 "Anthropogenic emissions have increased the fugacity of atmospheric carbon dioxide
32 (pCO₂) from the pre-industrial value of 280 µatm to the present-day value of over 400 µatm, and
33 these values will further increase to 800–1000 µatm by the end of this century according to the
34 Intergovernmental Panel on Climate Change (Gattuso et al., 2015)."

35 3) Line 69: delete the sentence 'several studies have already, etc.' in the following sentence,
36 replace 'majority' with 'several studies have shown a negative impact, etc.'

37 We agree with the reviewer's suggestion and have made modification in the revised manuscript.
38 P4, L77-80 "Several studies have shown a negative impact of decreasing pH on DMS-production
39 capability (Hopkins et al., 2010; Avgoustidi et al., 2012; Archer et al., 2013; Webb et al., 2016),
40 while others have found either no effect or a positive effect (Vogt et al., 2008; Hopkins and
41 Archer, 2014)."

42 4) Line 78: perhaps start a new paragraph with 'halocarbons'
43 Thanks for the reviewer's suggestion. We have started a new paragraph with 'halocarbons' in the
44 revised manuscript..

45 5) Line 189 to 192: delete. This is not an appropriate topic sentence, and it is from the introduction.
46 No need to repeat here.
47 Thanks for the reviewer's suggestion. We have deleted this sentence in the revised manuscript.

48 6) Line 192: delete the sentence and put (Fig. 3) in the following sentence.
49 We agree with the reviewer's suggestion and have made the modification in the revised
50 manuscript.
51 P9, L207-P10, L209 "At the beginning of the experiment, the mean DMS, DMSP and DCB
52 concentrations were all low in both treatments due to the low concentrations of DMS, DMSP and
53 DCB in the original fjord water and possible loss during the filtration procedure (Fig. 2)."

54 7) Line 209: round '29.2%' to the '29%'.
55 We agree with the reviewer's suggestion and have made the modification in the revised
56 manuscript.
57 P11, L231-234 "However, a significant 29% reduction in DMS concentrations was detected in the
58 HC treatment compared with the LC treatment ($p = 0.016$), though no statistical difference for
59 DCB concentrations was found between the LC and HC treatments during phase I."

60 8) Line 228: why Yu et al., unpublished data? Why not include the data here?
61 We agree with the reviewer's suggestion. We have added the DCB data in the revised manuscript.

62 P8, L167-L175 "2.5 Enumeration of DMSP-consuming bacteria (DCB)
63 The number of DMSP-consuming bacteria (DCB) was estimated using the most probable number
64 (MPN) methodology. The MPN medium consisted of a mixture (1:1 v/v) of sterile artificial sea
65 water (ASW) and mineral medium (Visscher et al., 1991), 3 mL of which was dispensed in 6 mL
66 test tubes, which were closed off by an over-sized cap, allowing gas exchange. Triplicate dilution
67 series were set up. All test tubes contained 1 mmol L⁻¹ DMSP as the sole organic carbon source
68 and were kept at 30 °C in the dark. After 2 weeks, the presence/absence of bacteria in the tubes
69 was verified by DAPI staining (Porter and Feig, 1980). Three tubes containing 3 mL ASW
70 without substrate were used as controls."

71 P10, L217-L224 “Compared with DMSP, DMS and DCB concentrations showed similar trends
72 during the mesocosm experiment. DMS concentrations in the LC and HC treatments were 1.03
73 and 0.74 nmol L⁻¹, respectively, while DCB concentrations in the LC and HC treatments were
74 0.20×10^6 and 0.16×10^6 cells mL⁻¹. DMS and DCB concentrations did not increase significantly
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76 treatments peaked on days 21 (11.65×10^6 cells mL⁻¹) and 23 (10.70×10^6 cells mL⁻¹), while
77 DMS concentrations in the LC and HC treatments peaked on days 25 (112.1 nmol L⁻¹) and 30
78 (101.9 nmol L⁻¹). Both DMS and DCB concentrations began to decrease obviously during phase
79 III.”

80 P11, L231-L234 “However, a significant 29% reduction in DMS concentrations was detected in
81 the HC treatment compared with the LC treatment ($p = 0.016$), though no statistical difference for
82 DCB concentrations was found between the LC and HC treatments during phase I.”

83 P11, L244-L246 “In addition, a significant positive relationship was also observed between DMS
84 and DCB ($r = 0.643$, $p < 0.01$ in the LC treatment; $r = 0.544$, $p < 0.01$ in the HC treatment) during
85 this experiment.”

86 P12, L250-L253 “Moreover, DCB peaked on days 21 (11.65×10^6 cells mL⁻¹) and 23 (10.70×10^6
87 cells mL⁻¹) in the LC and HC treatments, respectively, as shown in Fig. 2-C. Similar to DMS,
88 DCB was also delayed in the HC mesocosm compared to that in the LC mesocosm.”

89 9) Line 258: the sentence does not make sense. Do you mean ‘attributed to biology’ rather than
90 ‘involve’. Also delete the quotes around ‘biogenic’. Why use quotes for an adjective?

91 We agree with the reviewer’s suggestion and have deleted this sentence in the revised manuscript.

92

93 **Effect of elevated $p\text{CO}_2$ on trace gas production during an**
94 **ocean acidification mesocosm experiment**

95 Sheng-Hui Zhang^{1,3§}, JuanYu^{1§}, Qiong-Yao Ding¹, Hong-Hai Zhang¹, Gui-Peng Yang^{1,2*}, Kun-
96 Shan Gao⁴, Da-Wei Pan³

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100 Marine Science and Technology, Qingdao 266237, China

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116 Author contributions

117 §Sheng-Hui Zhang and JuanYu contributed equally

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123 **Abstract**

124 A mesocosm experiment was conducted in Wuyuan Bay (Xiamen), China to investigate the effects of elevated
125 $p\text{CO}_2$ on phytoplankton species and production of dimethylsulfide (DMS), dimethylsulfoniopropionate (DMSP)
126 and DMSP-consuming bacteria (DCB) as well as four halocarbon compounds (CHBrCl_2 , CH_3Br , CH_2Br_2 , and
127 CH_3I). Over a period of 5 weeks, *Phaeodactylum tricornutum* outcompeted *Thalassiosira weissflogii* and
128 *Emiliana huxleyi*, comprising more than 99% of the final biomass. During the logarithmic growth phase (phase I),
129 DMS concentrations in high $p\text{CO}_2$ mesocosms (HC, 1000 μatm) were 28% lower than those in low $p\text{CO}_2$
130 mesocosms (LC, 400 μatm). Elevated $p\text{CO}_2$ led to a delay in DCB concentrations attached to *Thalassiosira*
131 *weissflogii* and *Phaeodactylum tricornutum* and finally resulted in the delay of DMS concentration in the HC
132 treatment. Unlike DMS, the elevated $p\text{CO}_2$ did not affect DMSP production ability of *Thalassiosira weissflogii* or
133 *Phaeodactylum tricornutum* throughout the 5 weeks culture. A positive relationship was detected between CH_3I
134 and *Thalassiosira weissflogii* and *Phaeodactylum tricornutum* during the experiment, and there was a 40%
135 reduction in mean CH_3I concentrations in the HC mesocosms. CHBrCl_2 , CH_3Br , and CH_2Br_2 concentrations did
136 not increase with elevated chlorophyll *a* (Chl *a*) concentrations compared with DMS(P) and CH_3I , and there were
137 no major peaks both in the HC or LC mesocosms. In addition, no effect of elevated $p\text{CO}_2$ was identified for any of
138 the three bromocarbons.

139 **Keywords:** ocean acidification, dimethylsulfide (DMS), dimethylsulfoniopropionate (DMSP), halocarbons,
140 phytoplankton, bacteria

141

142

143

144 **1. Introduction**

145 Anthropogenic emissions have increased the fugacity of atmospheric carbon dioxide ($p\text{CO}_2$) from
146 the pre-industrial value of 280 μatm to the present-day value of over 400 μatm , and these values
147 will further increase to 800–1000 μatm by the end of this century according to the
148 Intergovernmental Panel on Climate Change (Gattuso et al., 2015). The dissolution of this excess
149 CO_2 into the surface of the ocean directly affects the carbonate system and has lowered the pH by
150 0.1 units, from 8.21 to 8.10 over the last 250 years. Further decreases of 0.3–0.4 pH units are
151 predicted by the end of this century (Doney et al., 2009; Orr et al., 2005; Gattuso et al., 2015),
152 which is commonly referred to as ocean acidification (OA). The physiological and ecological
153 aspects of the phytoplankton response to this changing environment can potentially alter marine
154 phytoplankton community composition, community biomass, and feedback to biogeochemical
155 cycles (Boyd and Doney, 2002). These changes simultaneously have an impact on some volatile
156 organic compounds produced by marine phytoplankton (Liss et al., 2014; Liu et al., 2017),
157 including the climatically important trace gas dimethylsulfide (DMS) and a number of volatile
158 halocarbon compounds.

159 DMS is the most important volatile sulfur compound produced from
160 dimethylsulfoniopropionate (DMSP), which is ubiquitous in marine environments, mainly
161 synthesized by marine microalgae (Stefels et al., 2007), a few angiosperms, some corals (Raina et
162 al., 2016), and several heterotrophic bacteria (Curson et al., 2017) through complex biological
163 interactions in marine ecosystems. Although it remains controversial, DMS and its by-products,

164 such as methanesulfonic acid and non-sea-salt sulfate, are suspected to have a prominent part in
165 climate feedback (Charlson et al., 1987; Quinn and Bates, 2011). The conversion of DMSP to
166 DMS is facilitated by several enzymes, including DMSP-lyase and acyl CoA transferase
167 (Kirkwood et al., 2010; Todd et al., 2007); these enzymes are mainly found in phytoplankton,
168 macroalgae, *Symbiodinium*, bacteria and fungi (de Souza and Yoch, 1995; Stefels and Dijkhuizen,
169 1996; Steinke and Kirst, 1996; Bacic and Yoch, 1998; Yost and Mitchelmore, 2009). Several
170 studies have shown a negative impact of decreasing pH on DMS-production capability (Hopkins
171 et al., 2010; Avgoustidi et al., 2012; Archer et al., 2013; Webb et al., 2016), while others have
172 found either no effect or a positive effect (Vogt et al., 2008; Hopkins and Archer, 2014). Several
173 assumptions have been presented to explain these contrasting results and attributed the pH-
174 induced variation in DMS-production capability to altered physiology of the algae cells or of
175 bacterial DMSP degradation (Vogt et al., 2008; Hopkins et al., 2010, Avgoustidi et al., 2012;
176 Archer et al., 2013; Hopkins and Archer, 2014; Webb et al., 2015).

177 Halocarbons also play a significant role in the global climate because they are linked to
178 tropospheric and stratospheric ozone depletion and a synergistic effect of chlorine and bromine
179 species has been reported that they may account for approximately 20% of the polar stratospheric
180 ozone depletion (Roy et al., 2011). In addition, iodocarbons can release atomic iodine (I) quickly
181 through photolysis in the atmospheric boundary layer and I atoms are very efficient in the catalytic
182 removal of O₃, which governs the lifetime of many climate relevant gases including methane and
183 DMS (Jenkins et al., 1991). Compared with DMS, limited attention was received about the effect
184 of OA on halocarbon concentrations. Hopkins et al. (2010) and Webb et al. (2015) measured lower
185 concentrations of several iodocarbons, while bromocarbons were unaffected by elevated pCO₂

186 through two acidification experiments. In addition, an additional mesocosm study did not elicit
187 significant differences from any halocarbon compounds at up to 1,400 $\mu\text{atm } p\text{CO}_2$ (Hopkins et al.,
188 2013).

189 DMS and halocarbons play a significant role in the global climate and perhaps act a greater
190 extent in the future. Meanwhile, the combined picture arising from existing studies is that the
191 response of communities to OA is not predictable and further studies were required. Based on the
192 controversial results about OA on DMS and halocarbons production, a mesocosm experiment was
193 conducted in Wu Yuan Bay, Xiamen. The aim of this study was to investigate the influence of
194 elevated $p\text{CO}_2$ on diatoms and coccolithophores and to further understand how the productions of
195 DMS and halocarbons respond to OA.

196 2. Experimental method

197 2.1 General experimental device

198 The mesocosm experiments were carried out on a floating platform at the Facility for Ocean
199 Acidification Impacts Study of Xiamen University (FOANIC-XMU, 24.52 N, 117.18 E) (for full
200 technical details of the mesocosms, see Liu et al. 2017). Six cylindrical transparent thermoplastic
201 polyurethane bags with domes were deployed along the south side of the platform. The width and
202 depth of each mesocosm bag was 1.5 m and 3 m, respectively. Filtered (0.01 μm , achieved using
203 an ultrafiltration water purifier, MU801-4T, Midea, Guangdong, China) *in situ* seawater was
204 pumped into the six bags simultaneously within 24 h. A known amount of NaCl solution was
205 added to each bag to calculate the exact volume of seawater in the bags, according to a
206 comparison of the salinity before and after adding salt (Czerny et al., 2013). The initial *in situ*
207 $p\text{CO}_2$ was about 650 μatm . To set the low and high $p\text{CO}_2$ levels, we added Na_2CO_3 solution and

208 CO₂ saturated seawater to the mesocosm bags to alter total alkalinity and dissolved inorganic
209 carbon (Gattuso et al., 2010; Riebesell et al., 2013). Subsequently, during the whole experimental
210 process, air at the ambient (400 μatm) and elevated *p*CO₂ (1000 μatm) concentrations was
211 continuously bubbled into the mesocosm bags using a CO₂ Enricher (CE-100B, Wuhan Ruihua
212 Instrument & Equipment Ltd., Wuhan, China). Because the seawater in the mesocosm was filtered,
213 the algae in the coastal environment and their attached bacteria were removed and the trace gases
214 produced in the environment did not influence the mesocosm trace gas concentrations after the
215 bags were sealed.

216 2.2 Algal strains

217 *Emiliana huxleyi* (CS-369), *Phaeodactylum tricornutum* (CCMA 106), and *Thalassiosira*
218 *weissflogii* (CCMA 102) were inoculated into the mesocosm bags, with an initial
219 diatom/coccolithophorid cell ratio of 1:1. The initial concentrations of *Phaeodactylum*
220 *tricornutum*, *Thalassiosira weissflogii*, and *Emiliana huxleyi* inoculated into the mesocosm were
221 10, 10, and 20 cells mL⁻¹, respectively. *Phaeodactylum tricornutum* and *Thalassiosira*
222 *weissflogii* were obtained from the Center for Collections of Marine Bacteria and Phytoplankton
223 of the State Key Laboratory of Marine Environmental Science (Xiamen University).
224 *Phaeodactylum tricornutum* was originally isolated from the South China Sea in 2004 and
225 *Thalassiosira weissflogii* was isolated from Daya Bay in the coastal South China Sea. *Emiliana*
226 *huxleyi* was originally isolated in 1992 from the field station of the University of Bergen
227 (Raunefjorden; 60°18'N, 05°15'E). Before being introduced into the mesocosms, the three
228 phytoplankton species were cultured in autoclaved, pre-filtered seawater from Wuyuan Bay at
229 16 °C (similar to the in situ temperature of Wuyuan Bay) without any addition of nutrients.

230 Cultures were continuously aerated with filtered ambient air containing 400 μatm of CO_2 within
231 plant chambers (HP1000G-D, Wuhan Ruihua Instrument & Equipment, China) at a constant
232 bubbling rate of 300 mL min^{-1} . The culture medium was renewed every 24 h to maintain the cells
233 of each phytoplankton species in exponential growth. Meanwhile, no meaningful numbers of
234 bacteria were counted by flow cytometer in the pre-filtered seawater before the inoculations.

235 *2.3 Sampling for DMS(P) and halocarbons*

236 DMS(P) and halocarbons samples were generally obtained from six mesocosms at 9 a.m., then all
237 collected samples were transported into a dark cool box back to the laboratory onshore for
238 analysis within 1 h. For DMS analysis, 2 mL sample was gently filtered through a 25 mm GF/F
239 (glass fiber) filter and transferred to a purge and trap system linked to a Shimadzu GC-2014 gas
240 chromatograph (Tokyo, Japan) equipped with a glass column packed with 10% DEGS on
241 Chromosorb W-AW-DMCS (3 m \times 3 mm) and a flame photometric detector (FPD) (Zhang et al.,
242 2014). For total DMSP analysis, 10 mL water sample was fixed using 50 μL of 50 % H_2SO_4 and
243 sealed (Kiene and Slezak, 2006). After > 1 d preservation, DMSP samples were hydrolysed for 24
244 h with a pellet of KOH (final pH > 13) to fully convert DMSP to DMS. Then, 2 mL hydrolysed
245 sample was carefully transferred to the purge and trap system mentioned above for extraction of
246 DMS. For halocarbons, 100 mL sample was purged at 40 $^\circ\text{C}$ with pure nitrogen at a flow rate of
247 100 mL min^{-1} for 12 min using another purge and trap system coupled to an Agilent 6890 gas
248 chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with an electron capture
249 detector (ECD) as well as a 60 m DB-624 capillary column (0.53 mm ID; film thickness, 3 μm)
250 (Yang et al., 2010). The analytical precision for duplicate measurements of DMS(P) and
251 halocarbons was > 10%.

252 *2.4 Measurements of chlorophyll a*

253 Chlorophyll *a* (Chl *a*) was measured in water samples (200–1,000 mL) collected every 2 d at 9
254 a.m. by filtering onto Whatman GF/F filters (25 mm). The filters were placed in 5 mL 100%
255 methanol overnight at 4 °C and centrifuged at 5000 r min⁻¹ for 10 min. The absorbance of the
256 supernatant (2.5 mL) was measured from 250 to 800 nm using a scanning spectrophotometer (DU
257 800, Beckman Coulter Inc., Brea, CA, USA). Chl *a* concentration was calculated according to the
258 equation reported by Porra (2002).

259 *2.5 Enumeration of DMSP-consuming bacteria (DCB)*

260 The number of DMSP-consuming bacteria (DCB) was estimated using the most probable number
261 (MPN) methodology. The MPN medium consisted of a mixture (1:1 v/v) of sterile artificial sea
262 water (ASW) and mineral medium (Visscher et al., 1991), 3 mL of which was dispensed in 6 mL
263 test tubes, which were closed off by an over-sized cap, allowing gas exchange. Triplicate dilution
264 series were set up. All test tubes contained 1 mmol L⁻¹ DMSP as the sole organic carbon source
265 and were kept at 30 °C in the dark. After 2 weeks, the presence/absence of bacteria in the tubes
266 was verified by DAPI staining (Porter and Feig, 1980). Three tubes containing 3 mL ASW
267 without substrate were used as controls.

268 *2.6 Statistical analysis*

269 One-way analysis of variance (ANOVA), Tukey's test, and the two-sample *t*-test were carried out
270 to demonstrate the differences between treatments. A *p*-value < 0.05 was considered significant.
271 Relationships between DMS(P), halocarbons and a range of other parameters were detected using
272 Pearson's correlation analysis via SPSS 22.0 for Windows (SPSS Inc., Chicago, IL, USA).

273 **3. Results and Discussion**

274 3.1 Temporal changes in pH, Chl *a*, *Phaeodactylum tricornutum*, *Thalassiosira weissflogii*, and
275 *Emiliana huxleyi* during the experiment

276 During the experiment, the seawater in each mesocosm was well combined, and the temperature
277 and salinity were well controlled, with a mean of 16 °C and 29 in all mesocosms, respectively.
278 Meanwhile, we observed significant differences in pH levels between the two CO₂ treatments on
279 days 0–11, but the differences disappeared with subsequent phytoplankton growth (Fig. 1). The
280 phytoplankton growth process was divided into three phases in terms of variations in Chl *a*
281 concentrations in the mesocosm experiments as described in Liu et al. (2017): i) the logarithmic
282 growth phase (phase I, days 0–13), ii) a plateau phase (phase II, days 13–23, bloom period), and iii)
283 a secondary plateau phase (phase III, days 23–33) attained after a decline in biomass from a
284 maximum in phase II. The initial chemical parameters of the mesocosm experiment are shown in
285 Table 1. The initial mean dissolved nitrate (including NO₃⁻ and NO₂⁻), NH₄⁺, PO₄³⁻ and silicate
286 (SiO₃²⁻) concentrations were 54, 20, 2.6 and 41 μmol L⁻¹ for the low pCO₂ (LC) treatment and 52,
287 21, 2.4 and 38 μmol L⁻¹ for the high pCO₂ (HC) treatment, respectively. The nutrient
288 concentrations (NO₃⁻, NO₂⁻, NH₄⁺ and phosphate) during phase I were consumed rapidly and
289 their concentrations were below or close to the detection limit during phase II (Table 1). In
290 addition, although dissolved inorganic nitrogen (NH₄⁺, NO₃⁻, and NO₂⁻) and phosphate were
291 depleted, Chl *a* concentration in both treatments (biomass dominated by *Phaeodactylum*
292 *tricornutum*) remained constant over days 12–22, and then declined over subsequent days (Liu et
293 al., 2017). *Emiliana huxleyi* was only found in phase I and its maximal concentration reached 310
294 cells mL⁻¹ according to the results of microscopic inspection. *Thalassiosira weissflogii* was found
295 throughout the entire period in each bag, but the maximum concentration was 8,120 cells mL⁻¹,

296 which was far less than the concentration of *Phaeodactylum tricornutum* with a maximum density
297 of about 1.5 million cells mL⁻¹ (Liu et al., 2017).

298 3.2 Impact of elevated pCO₂ on DMS and DMSP production

299 At the beginning of the experiment, the mean DMS, DMSP and DCB concentrations were all low
300 in both treatments due to the low concentrations of DMS, DMSP and DCB in the original fjord
301 water and possible loss during the filtration procedure (Fig. 2). With the growth of phytoplankton,
302 DMS, DMSP and DCB showed slightly different trends during the mesocosm experiment. The
303 DMSP concentrations in the HC and LC treatments increased significantly along with the increase
304 of Chl *a* concentrations and algal cells, and stayed relatively constant over the following days. A
305 significant positive relationship was observed between DMSP and phytoplankton in the
306 experiment ($r = 0.961$, $p < 0.01$ for *Phaeodactylum tricornutum*, $r = 0.617$, $p < 0.01$ for
307 *Thalassiosira weissflogii* in the LC treatment, table 2; $r = 0.954$, $p < 0.01$ for *Phaeodactylum*
308 *tricornutum*, $r = 0.743$, $p < 0.01$ for *Thalassiosira weissflogii* in the HC treatment, table 3).
309 Compared with DMSP, DMS and DCB concentrations showed similar trends during the
310 mesocosm experiment. DMS concentrations in the LC and HC treatments were 1.03 and 0.74
311 nmol L⁻¹, respectively, while DCB concentrations in the LC and HC treatments were 0.20×10^6
312 and 0.16×10^6 cells mL⁻¹. DMS and DCB concentrations did not increase significantly during
313 phase I, but began to increase rapidly on day 15. DCB concentrations in the LC and HC treatments
314 peaked on days 21 (11.65×10^6 cells mL⁻¹) and 23 (10.70×10^6 cells mL⁻¹), while DMS
315 concentrations in the LC and HC treatments peaked on days 25 (112.1 nmol L⁻¹) and 30 (101.9
316 nmol L⁻¹). Both DMS and DCB concentrations began to decrease obviously during phase III.
317 Meanwhile, a significant positive relationship was also observed between DMS and

318 *Phaeodactylum tricornuntum* ($r = 0.560$, $p < 0.05$ in the LC treatment; $r = 0.635$, $p < 0.01$ in the
319 HC treatment), while no relationship was observed between DMS and *Thalassiosira weissflogii*
320 (table 2 and table 3) during the experiment.

321 In this study, no difference in mean DMSP concentrations was observed between the two
322 treatments, indicating that elevated $p\text{CO}_2$ had no significant influence on DMSP production in
323 *Phaeodactylum tricornuntum* and *Thalassiosira weissflogii* throughout this study. However, a
324 significant 29% reduction in DMS concentrations was detected in the HC treatment compared
325 with the LC treatment ($p = 0.016$), though no statistical difference for DCB concentrations was
326 found between the LC and HC treatments during phase I. This reduction in DMS concentrations
327 may be attributed to greater consumption of DMS and conversion to DMSO (Webb et al., 2015).
328 In addition, the peak DMS concentration in the HC treatment was delayed 5 days relative to that in
329 the LC treatment during phase II (Fig. 2-A). This result has been observed in previous mesocosm
330 experiments and it was attributed to small scale shifts in community composition and succession
331 that could not be identified with only a once-daily measurement regime (Vogt et al., 2008; Webb et
332 al., 2016). However, this phenomenon can be explained in another straightforward way during this
333 study. Previous studies have showed that marine bacteria play a key role in DMS production and the
334 efficiency of bacteria converting DMSP to DMS may vary from 2 to 100% depending on the
335 nutrient status of the bacteria and the quantity of dissolved organic matter (Simó et al., 2002, 2009;
336 Kiene et al., 1999, 2000). In addition, a significant positive relationship was also observed
337 between DMS and DCB ($r = 0.643$, $p < 0.01$ in the LC treatment; $r = 0.544$, $p < 0.01$ in the HC
338 treatment) during this experiment. All of these observations point to the importance of bacteria in
339 DMS and DMSP dynamics. During the present mesocosm experiment, DMSP concentrations in

340 the LC treatment decreased slightly on day 23, while the slight decrease appeared on day 29 in the
341 HC treatment (Fig. 2-B). In addition, the time that the DMSP concentration began to decrease was
342 very close to the time when the highest DMS concentration occurred in both treatments. Moreover,
343 DCB peaked on days 21 (11.65×10^6 cells mL⁻¹) and 23 (10.70×10^6 cells mL⁻¹) in the LC and
344 HC treatments, respectively, as shown in Fig. 2-C. Similar to DMS, DCB was also delayed in the
345 HC mesocosm compared to that in the LC mesocosm. Taken together, we inferred that the
346 elevated $p\text{CO}_2$ first delayed growth of DCB in the mesocosm, then the delayed DCB postponed
347 the DMSP degradation process, and eventually delayed the DMS concentration in the HC
348 treatment. In addition, considering that the algae and their attached bacteria were removed through
349 a filtering process before the experiment and the unattached bacteria were maintained in a
350 relatively constant concentration during this mesocosm experiment (Huang et al., 2018), we
351 further concluded that the elevated $p\text{CO}_2$ controlled DMS concentrations mainly by affecting DCB
352 attached to *Thalassiosira weissflogii* and *Phaeodactylum tricorunatum*.

353 3.3 Impact of elevated $p\text{CO}_2$ on halocarbon compounds

354 The temporal development in CHBrCl_2 , CH_3Br , and CH_2Br_2 concentrations is shown in Fig. 3 (A–
355 C) and the temporal changes of their concentrations were substantially different from those of
356 DMS, DMSP, *Phaeodactylum tricorunatum* and *Thalassiosira weissflogii*. The mean
357 concentrations of CHBrCl_2 , CH_3Br and CH_2Br_2 for the entire experiment were 8.58, 7.85, and 5.13
358 pmol L⁻¹ in the LC treatment and 8.81, 9.73, and 6.27 pmol L⁻¹ in the HC treatment. The
359 concentrations of CHBrCl_2 , CH_3Br , and CH_2Br_2 did not increase with the Chl *a* concentration
360 compared with those of DMS and DMSP, and no major peaks were detected in the mesocosms. In
361 addition, no effect of elevated $p\text{CO}_2$ was identified for any of the three bromocarbons, which

362 compared well with previous mesocosm findings (Hopkins et al., 2010, 2013; Webb, et al., 2016).
363 No clear correlation was observed between the three bromocarbons and any of the measured algal
364 groups (table 2 and table 3), indicating that *Phaeodactylum tricornutum* and *Thalassiosira*
365 *weissflogii* did not primarily release these three bromocarbons during the mesocosm experiment.
366 Previous studies have reported that large-size cyanobacteria, such as *Aphanizomenon flos-aquae*,
367 produce bromocarbons (Karlsson et al. 2008) and significant correlations between cyanobacterium
368 abundance and several bromocarbons have been reported in the Arabian Sea (Roy et al., 2011).
369 However, the filtration procedure led to the loss of cyanobacterium in the mesocosms and finally
370 resulted in low bromocarbon concentrations during the experiment, although *Phaeodactylum*
371 *tricornutum* and *Thalassiosira weissflogii* abundances were high.

372 The temporal dynamics of CH₃I in the HC and LC treatments are shown in Fig. 3-D. The CH₃I
373 concentrations in the LC treatment varied from 0.38 to 12.61 pmol L⁻¹, with a mean of 4.76 pmol
374 L⁻¹. The CH₃I concentrations in the HC treatment ranged between 0.44 and 8.78 pmol L⁻¹, with a
375 mean of 2.88 pmol L⁻¹. The maximum CH₃I concentrations in the HC and LC treatments were
376 both observed on day 23. The range of CH₃I concentrations during this experiment was similar to
377 that measured in the mesocosm experiment (< 1~10 pmol L⁻¹) in Kongsfjorden conducted by
378 Hopkins et al. (2013). In addition, the mean CH₃I concentration in the LC treatment was similar to
379 that measured in the East China Sea, with an average of 5.34 pmol L⁻¹ in winter and 5.74 pmol L⁻¹
380 in summer (Yuan et al., 2015). Meanwhile, a positive relationship was detected between CH₃I and
381 Chl *a*, *Phaeodactylum tricornutum* and *Thalassiosira weissflogii* ($r = 0.588$, $p < 0.01$ in the LC
382 treatment; $r = 0.834$, $p < 0.01$ in the LC treatment for *Phaeodactylum tricornutum*; $r = 0.680$ $p <$
383 0.01 in the LC treatment; $r = 0.690$, $p < 0.01$ in the HC treatment for *Thalassiosira weissflogii*; $r =$

384 0.717, $p < 0.01$ in the LC treatment; $r = 0.741$, $p < 0.01$ in the HC treatment for Chl *a*). This result
385 agrees with previous mesocosm (Hopkins et al., 2013) and laboratory experiments (Hughes et al.,
386 2013; Manley and De La Cuesta, 1997) identifying diatoms as significant producers of CH₃I.
387 Moreover, similar to DMS, the maximum CH₃I concentration also occurred after the maxima of
388 *Phaeodactylum tricornutum* and *Thalassiosira weissflogii*, at about 4 d (Fig. 3-D). This was
389 similar to iodocarbon gases measured in a Norway mesocosm conducted by Hopkins et al. (2010)
390 and chloriodomethane (CH₂CI) concentrations measured in another Norway mesocosm
391 conducted by Wingenter et al. (2007). Furthermore, the CH₃I concentrations measured in the HC
392 treatment were significantly lower than those measured in the LC treatment during the mesocosm,
393 which is in accord with the discoveries of Hopkins et al. (2010) and Webb et al. (2015) but in
394 contrast to the findings of Hopkins et al. (2013) and Webb et al. (2016). Throughout the mesocosm
395 experiment, there was a 40.2% reduction in the HC mesocosm compared to the LC mesocosm.
396 Considering that the phytoplankton species did not show significant differences in the HC and LC
397 treatments during the experiment, this reduction in the HC treatment was likely not caused by
398 phytoplankton. Apart from direct biological production via methyl transferase enzyme activity by
399 both phytoplankton and bacteria (Amachi et al., 2001), CH₃I is produced from the breakdown of
400 higher molecular weight iodine-containing organic matter (Fenical, 1982) through photochemical
401 reactions between organic matter and light (Richter and Wallace, 2004). Both bacterial methyl
402 transferase enzyme activity and a photochemical reaction may have reduced the CH₃I
403 concentrations in the HC treatment but further experiments are needed to verify this result.

404 **4. Conclusions**

405 In this study, the effects of increased levels of $p\text{CO}_2$ on marine DMS(P) and halocarbons release

406 were studied in a controlled mesocosm facility. A 28.2% reduction during the logarithmic growth
407 phase and a 5 d delay in DMS concentration was observed in the HC treatment due to the effect of
408 elevated $p\text{CO}_2$. Because the seawater in the mesocosm was filtered, the algae in the coastal
409 environment and their attached bacteria were removed and the trace gases produced in the
410 environment did not influence the mesocosm trace gas concentrations after the bags were sealed.
411 Therefore, we attribute this phenomenon to the DMSP-consuming bacteria attached to
412 *Phaeodactylum tricornutum* and *Thalassiosira weissflogii*. More attention should be paid to the
413 DMSP-consuming bacteria attached to algae under different pH values in future studies. Three
414 bromocarbons compounds were not correlated with a range of biological parameters, as they were
415 affected by the filtration procedure and elevated $p\text{CO}_2$ had no effect on any of the three
416 bromocarbons. The temporal dynamics of CH_3I , combined with strong correlations with biological
417 parameters, indicated biological control of the concentrations of this gas. In addition, the
418 production of CH_3I was sensitive to $p\text{CO}_2$, with a significant increase in CH_3I concentration at
419 higher $p\text{CO}_2$. However, without additional empirical measurements, it is unclear whether this
420 decrease was caused by bacterial methyl transferase enzyme activity or by photochemical
421 degradation at higher $p\text{CO}_2$.

422 Author contribution: Gui-Peng Yang and Kun-Shan Gao designed the experiments. Sheng-Hui
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Figure captions

570 Fig. 1 Temporal changes of pH in the HC (1,000 μatm , solid squares) and LC (400 μatm , white
571 squares) mesocosms (3,000 L). Data are mean \pm standard deviation, n = 3 (triplicate independent
572 mesocosm bags) (Origin 8.0).

573 Fig. 2 Temporal changes in DMS, DMSP and DCB concentrations in the HC (1,000 μatm , black
574 squares) and LC (400 μatm , white squares) mesocosms (3,000 L). Data are mean \pm standard
575 deviation, n = 3 (triplicate independent mesocosm bags).

576 Fig. 3 Temporal changes in CHBrCl_2 , CH_3Br , CH_2Br_2 and CH_3I concentrations in the HC (1,000
577 μatm , black squares) and LC (400 μatm , white squares) mesocosms (3,000 L). Data are mean \pm
578 standard deviation, n = 3 (triplicate independent mesocosm bags).

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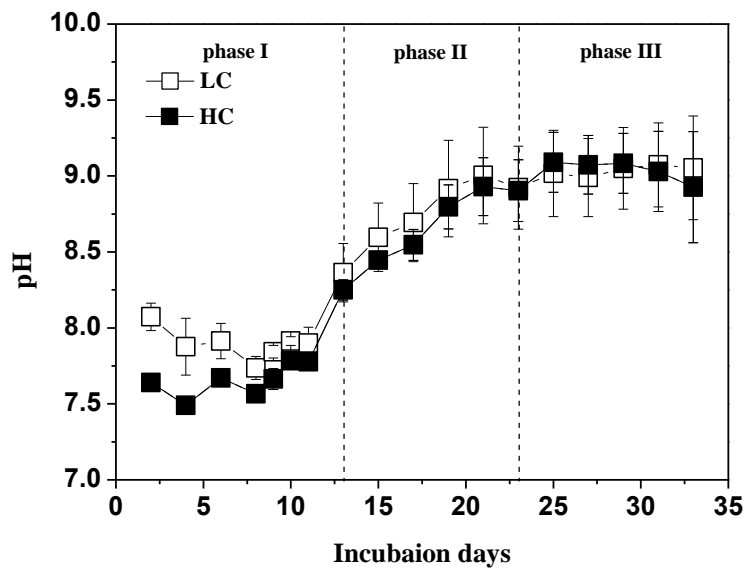
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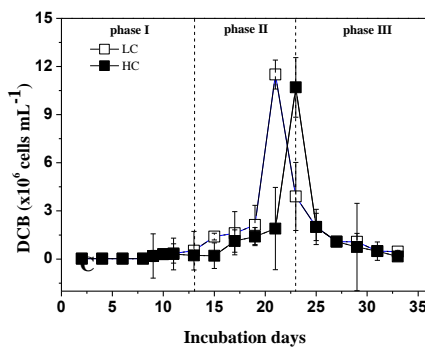
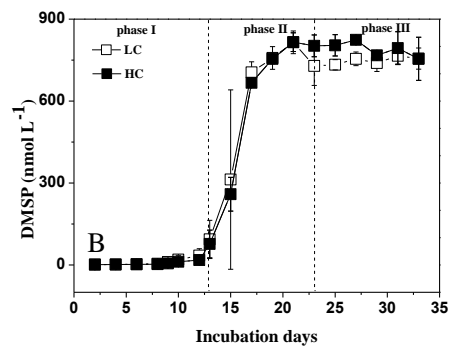
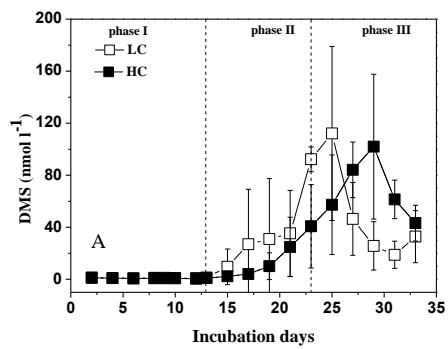
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588 **Fig. 1** Temporal changes of pH in the HC (1,000 μatm , solid squares) and LC (400 μatm , white squares)

589 mesocosms (3,000 L). Data are mean \pm standard deviation, $n = 3$ (triplicate independent mesocosm bags) (Origin

590 8.0).

591



592

593

594 **Fig. 2** Temporal changes in DMS (A), DMSP (B), DCB (C) concentrations in the HC (1,000 μatm , black squares)

595 and LC (400 μatm , white squares) mesocosms (3,000 L). Data are mean \pm standard deviation, $n = 3$ (triplicate

596 independent mesocosm bags) (Origin 8.0).

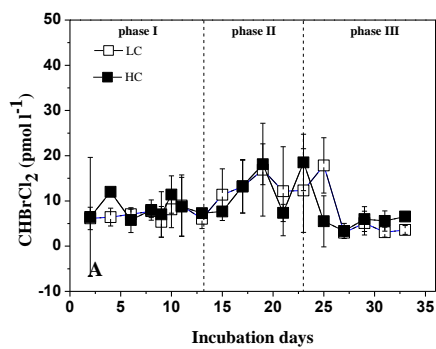
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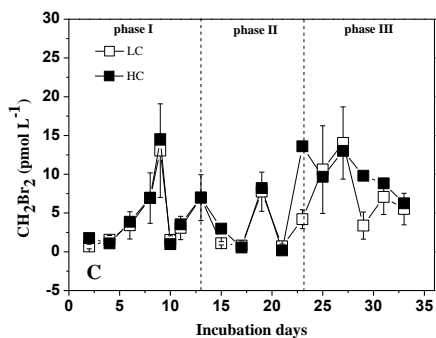
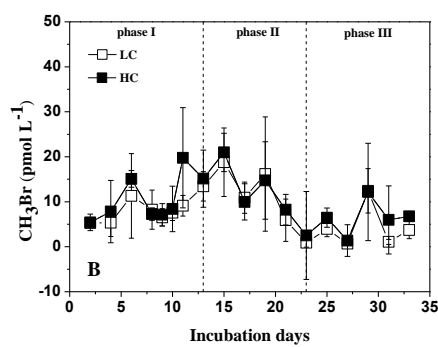
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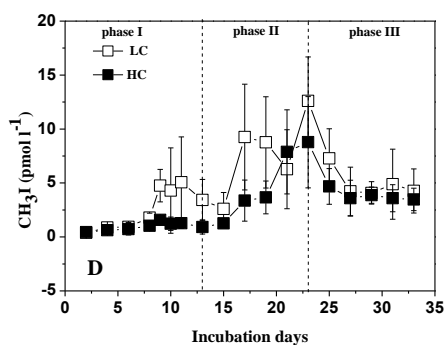
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604 **Fig. 3** Temporal changes in CHBrCl_2 (A), CH_3Br (B), CH_2Br_2 (C) and CH_3I (D) concentrations in the HC (1,000
 605 μatm , black squares) and LC (400 μatm , white squares) mesocosms (3,000 L). Data are mean \pm standard deviation,
 606 $n = 3$ (triplicate independent mesocosm bags) (Origin 8.0).

607

608 **Table 1.** The conditions of DIC, pH_T , pCO_2 and nutrient concentrations in the mesocosm experiments. “-” means
 609 that the values were below the detection limit.

		pH_T	DIC ($\mu\text{mol kg}^{-1}$)	pCO_2 (μatm)	$NO_3^-+NO_2^-$ ($\mu\text{mol L}^{-1}$)	NH_4^+ ($\mu\text{mol L}^{-1}$)	PO_4^{3-} ($\mu\text{mol L}^{-1}$)	SiO_3^{2-} ($\mu\text{mol L}^{-1}$)
day 0	LC	8.0 \pm 0.1	2181 \pm 29	1170~1284	52~56	19~23	2.6 \pm 0.2	38~40
	HC	7.5 \pm 0.1	2333 \pm 34	340~413	51~55	19~23	2.5 \pm 0.2	38~39
Phase I	LC	7.9~8.4	1825~2178	373~888	15~52	1.6~20	0.5~2.6	31~38
	HC	7.4~8.2	2029~2338	1295~1396	47~54	0.2~21	0.7~2.5	34~39
Phase II	LC	8.4~8.5	1706~1745	46~749	-- 15.9	-	0.1~0.5	10~24
	HC	8.4~8.6	1740~1891	59~1164	1.1~25	-	--0.1	29~30
Phase III	LC	8.5~8.8	1673~1706	30~43	-	-	-	10~16
	HC	8.6~8.7	1616~1740	34~110	-	-	--0.3	24~25

610

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 <i>a</i> (µg L ⁻¹)	CHBrCl ₂ (pmol l ⁻¹)	CH ₃ Br (pmol l ⁻¹)	CH ₂ Br ₂ (pmol l ⁻¹)	CH ₃ I (pmol l ⁻¹)	DCB (×10 ⁶ cells mL ⁻¹)	<i>T. weissflogii</i> (×10 ³ cells mL ⁻¹)	<i>P. tricornutum</i> (cells mL ⁻¹)
DMS	1									
DMSP	0.701**	1								
Chl <i>a</i>	0.597**	0.792**	1							
CHBrCl ₂	0.526	0.280	0.559	1						
CH ₃ Br	-0.413	-0.230	0.196	0.313	1					
CH ₂ Br ₂	0.310	0.180	0.001	-0.136	-0.308	1				
CH ₃ I	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		
<i>T. weissflogii</i>	0.410	0.617**	0.899**	0.301	0.322	0.028	0.680**	0.399	1	
<i>P. tricornutum</i>	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 <i>a</i> (µg L ⁻¹)	CHBrCl ₂ (pmol l ⁻¹)	CH ₃ Br (pmol l ⁻¹)	CH ₂ Br ₂ (pmol l ⁻¹)	CH ₃ I (pmol l ⁻¹)	DCB (×10 ⁶ cells mL ⁻¹)	<i>T. weissflogii</i> (×10 ³ cells mL ⁻¹)	<i>P. tricornutum</i> (cells mL ⁻¹)
DMS	1									
DMSP	0.752**	1								
Chl <i>a</i>	0.318*	0.738**	1							
CHBrCl ₂	0.324	0.094	0.326	1						
CH ₃ Br	-0.410	-0.349	0.065	0.076	1					
CH ₂ Br ₂	0.540*	0.352	0.142	0.233	-0.377	1				
CH ₃ I	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		
<i>T. weissflogii</i>	0.355	0.743**	0.930**	0.304	0.076	0.233	0.690**	0.567	1	
<i>P. tricornutum</i>	0.635**	0.954**	0.803**	0.143	-0.257	0.267	0.834**	0.559	0.820**	1