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

B. Qu (Referee)
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Increases of anthropogenic emissions of $CO_2$ since the Industrial Revolution are known to have influenced organisms and the delivery of oceanic ecosystem services at a global scale. This is an interesting piece of work that shows the effect of elevated $pCO_2$ on trace gases production including DMS and four halocarbon compounds through a mesocosm experiment. The study is based on the development of a bloom created by the addition of three different species of cultured phytoplankton to nutrient enriched coastal water enclosed in mesocosms. Considering that the impact of ocean acidification on DMS and halocarbons remains controversial, it is necessary to conductfurther study about this aspect. Overall, this paper is well written and the major points are discussed with clarity. I recommend this article to be published in Biogeosciences after modification. My major criticism to the manuscript is that the authors point the algae and their attached bacteria in the coastal environment were removed through filtration process, have you measured the bacterial abundance in the mesocosm before the three different species of algae inoculated? In addition, this manuscript lacks the initial concentrations of Phaeodactylum tricornuntum, Thalassiosira weissflogii, and Emiliania huxleyi inoculated into the mesocosm. There are also some minor thinks that I list below:

P3, L54 "Further decreases of 0.3–0.4 pH units are predicted by the end of this century (Doneyet al., 2009; Orr et al., 2005), which is commonly referred to as ocean acidification (OA)." Please update the latest references in this section.

P3, L61 "DMS is the most important volatile sulfur compound produced from the algal secondary metabolite dimethylsulfoniopropionate (DMSP) through complex biological in teractions in marine ecosystems (Stefels et al., 2007)." DMSP is not only produced by algae, but also by terrestrial plants and marine bacteria. Please re-word this section.

P4, L75 Replace "attribute" by "attributed".

P8, L167-L168 What is "LC" and "HC", low $CO_2$ and high $CO_2$? Please use the full name for the first time in the manuscript.

P8, L172 The unit of chl a is not unified with Fig. 1, please check.

P9, L192 Replace "for" by "of"

P9, L196 delete "growth in"

P9, L197-198 Replace "increase in Chl *a* and cell concentrations" by "increase in Chl *a* concentrations and algal cells"

Response to Reviewer #1:

Dear Reviewer #1:

We are grateful to your review of this paper and would like to express our thanks for your helpful and constructive comments. We have revised the manuscript and addressed all the comments point by point. The main changes we made are as follows:

Increases of anthropogenic emissions of $CO_2$ since the Industrial Revolution are known to have influenced organisms and the delivery of oceanic ecosystem services at a global scale. This is an interesting piece of work that shows the effect of elevated $pCO_2$ on trace gases production including DMS and four halocarbon compounds through a mesocosm experiment. The study is based on the development of a bloom created by the addition of three different species of cultured phytoplankton to nutrient enriched coastal water enclosed in mesocosms. Considering that the impact of ocean acidification on DMS and halocarbons remains controversial, it is necessary to conductfurther study about this aspect. Overall, this paper is well written and the major points are discussed with clarity. I recommend this article to be published in Biogeosciences after modification. My major criticism to the manuscript is that the authors point the algae and their attached bacteria in the coastal environment were removed through filtration process, have you measured the bacterial abundance in the mesocosm before the three different species of algae inoculated? In addition, this manuscript lacks the initial concentrations of *Phaeodactylum tricornuntum*, *Thalassiosira weissflogii*, and *Emiliania huxleyi* inoculated into the mesocosm.

Thanks for the reviewer's suggestion and we have added some details about this mesocosm experiment in the revised manuscript.

P6, L125-129 "*Emiliania huxleyi* (CS-369), *Phaeodactylum tricornuntum* (CCMA 106), and *Thalassiosira weissflogii* (CCMA 102) were inoculated into the mesocosm bags, with initial diatom/coccolithophorid cell ratio was 1:1. The initial concentrations of *Phaeodactylum tricornuntum*, *Thalassiosira weissflogii*, and *Emiliania huxleyi* inoculated into the mesocosm were 10, 10, and 20 cells $mL^{-1}$, respectively."

P7, L141-142 "Meanwhile, no meaningful numbers of bacteria were counted by flow cytometer in the pre-filtered seawater before the inoculations."

There are also some minor thinks that I list below:

P3, L54 "Further decreases of 0.3–0.4 pH units are predicted by the end of this century (Doneyet al., 2009; Orr et al., 2005), which is commonly referred to as ocean acidification (OA)." Please update the latest references in this section.

Thanks for the reviewer's suggestion and we have updated the latest references in the revised manuscript.

P3, L58-60 "Further decreases of 0.3–0.4 pH units are predicted by the end of this century (Doney et al., 2009; Orr et al., 2005; Gattuso et al., 2015), which is commonly referred to as ocean acidification (OA)"

"Gattuso, J. P., Magnan, A., Bille, R., Cheung, W. W. L., Howes, E. L., Joos, F., Allemand, D., Bopp, L., Cooley, S. R., Eakin, C. M., Hoegh-Guldberg, O., Kelly, R. P., Portner, H. O.,

Rogers, A. D., Baxter, J. M., Laffoley, D., Osborn, D., Rankovic, A., Rochette, J., Sumaila, U.R., Treyer, S., Turley, C.: Contrasting futures for ocean and society from different anthropogenic $CO_2$ emissions scenarios. Science, 349 (6243), aac4722, 2015."

P3, L61 "DMS is the most important volatile sulfur compound produced from the algal secondary metabolite dimethylsulfoniopropionate (DMSP) through complex biological in teractions in marine ecosystems (Stefels et al., 2007)." DMSP is not only produced by algae, but also by terrestrial plants and marine bacteria. Please re-word this section.

Thanks for the reviewer's suggestion and we have reworded this section in the revised manuscript.

P3, L67-71 "DMS is the most important volatile sulfur compound produced from dimethylsulfoniopropionate (DMSP), which is ubiquitous in marine environments, mainly synthesized by marine microalgae (Stefels et al., 2007), a few angiosperms, some corals (Raina et al., 2016), and several heterotrophic bacteria (Curson et al., 2017) through complex biological interactions in marine ecosystems."

"Raina, J. B., Tapiolas, D., Motti, C. A., Foret, S., Seemann, T., Tebben, J.: Isolation of an antimicrobial compound produced by bacteria associated with reef-building corals. PeerJ, 4, e2275, 2016"

"Curson, A. R., Liu, J., Bermejo Martinez, A., Green, R., Chan, Y., Carrion, O.: Dimethylsulfoniopropionate biosynthesis in marine bacteria and identification of the key gene in this process. Nat. Microbiol., 2, 17009, 2017."

P4, L75 Replace "attribute" by "attributed".

Thanks for the reviewer's suggestion and we have reworded this section in the revised manuscript.

P4, L80-84 "Several assumptions have been presented to explain these contrasting results and attributed the pH-induced variation in DMS-production capability to altered physiology of the algae cells or of bacterial DMSP degradation (Vogt et al., 2008; Hopkins et al., 2010, Avgoustidi et al., 2012; Archer et al., 2013; Hopkins and Archer, 2014; Webb et al., 2015)"

P8, L167-L168 What is "LC" and "HC", low $CO_2$ and high $CO_2$? Please use the full name for the first time in the manuscript.

Thanks for the reviewer's suggestion and we have used the full name for the first time in the revised manuscript.

P9, L192-195 "The initial chemical parameters of the mesocosm experiment are shown in Table 1. The initial mean dissolved nitrate (including $NO_3^-$ and $NO_2^-$), $NH_4^+$, $PO_4^{3-}$ and silicate ($SiO_3^{2-}$) concentrations were 54, 20, 2.6 and 41 μmol $L^{-1}$ for the low $pCO_2$ (LC) treatment and 52, 21, 2.4 and 38 μmol $L^{-1}$ for the high $pCO_2$ (HC) treatment, respectively."

P8, L172 The unit of chl *a* is not unified with Fig. 1, please check.

According to the opinion of reviewer 2#, Fig. 1 was replaced.

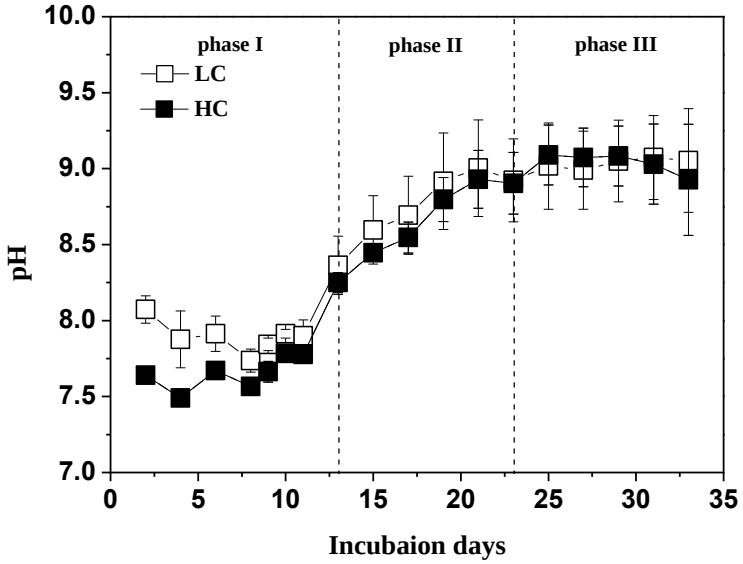


**Fig. 1.** Temporal changes of pH in the HC (1,000 µatm, solid squares) and LC (400 µatm, white squares) mesocosms (3,000 L). Data are mean ± standard deviation, n = 3 (triplicate independent mesocosm bags) (Origin 8.0).

P9, L192 Replace "for" by "of"

Thanks for the reviewer's suggestion and we have reworded in the revised manuscript according all reviewers' suggestion.

P10, L207-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)."

P9, L196 delete "growth in"

Thanks for the reviewer's suggestion and we have modified in the revised manuscript.

P10, L217-218 "Compared with DMSP, DMS and DCB concentrations showed similar trends during the mesocosm experiment."

P9, L197-198 Replace "increase in Chl *a* and cell concentrations" by "increase in Chl *a* concentrations and algal cells"

Thanks for the reviewer's suggestion and we have modified in the revised manuscript.

P10, L210-212 "The DMSP concentrations in the HC and LC treatments increased significantly along with the increase of Chl *a* concentrations and algal cells, and stayed relatively constant over the following days."

# Effect of elevated $pCO_2$ on trace gas production during an ocean acidification mesocosm experiment

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Author contributions

[§]Sheng-Hui Zhang and JuanYu contributed equally

**Abstract**

A mesocosm experiment was conducted in Wuyuan Bay (Xiamen), China to investigate the effects of elevated $pCO_2$ on phytoplankton species and production of dimethylsulfide (DMS), dimethylsulfoniopropionate (DMSP) and DMSP-consuming bacteria (DCB) as well as four halocarbon compounds ($CHBrCl_2$, $CH_3Br$, $CH_2Br_2$, and $CH_3I$). Over a period of 5 weeks, *Phaeodactylum tricornuntum* outcompeted *Thalassiosira weissflogii* and *Emiliania huxleyi*, comprising more than 99% of the final biomass. During the logarithmic growth phase (phase I), DMS concentrations in high $pCO_2$ mesocosms (HC, 1000 μatm) were 28% lower than those in low $pCO_2$ mesocosms (LC, 400 μatm). Elevated $pCO_2$ 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 $pCO_2$ did not affect DMSP production ability of *Thalassiosira weissflogii* or *Phaeodactylum tricornuntum* throughout the 5 weeks culture. A positive relationship was detected between $CH_3I$ and *Thalassiosira weissflogii* and *Phaeodactylum tricornuntum* during the experiment, and there was a 40% reduction in mean $CH_3I$ concentrations in the HC mesocosms. $CHBrCl_2$, $CH_3Br$, and $CH_2Br_2$ concentrations did not increase with elevated chlorophyll *a* (Chl *a*) concentrations compared with DMS(P) and $CH_3I$, and there were no major peaks both in the HC or LC mesocosms. In addition, no effect of elevated $pCO_2$ was identified for any of the three bromocarbons.

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

## 1. Introduction

Anthropogenic emissions have increased the fugacity of atmospheric carbon dioxide ($pCO_2$) 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). The dissolution of this excess $CO_2$ into the surface of the ocean directly affects the carbonate system and has lowered the pH by 0.1 units, from 8.21 to 8.10 over the last 250 years. Further decreases of 0.3–0.4 pH units are predicted by the end of this century (Doney et al., 2009; Orr et al., 2005; Gattuso et al., 2015), which is commonly referred to as ocean acidification (OA). The physiological and ecological aspects of the phytoplankton response to this changing environment can potentially alter marine phytoplankton community composition, community biomass, and feedback to biogeochemical cycles (Boyd and Doney, 2002). These changes simultaneously have an impact on some volatile organic compounds produced by marine phytoplankton (Liss et al., 2014; Liu et al., 2017), including the climatically important trace gas dimethylsulfide (DMS) and a number of volatile halocarbon compounds.

DMS is the most important volatile sulfur compound produced from dimethylsulfoniopropionate (DMSP), which is ubiquitous in marine environments, mainly synthesized by marine microalgae (Stefels et al., 2007), a few angiosperms, some corals (Raina et al., 2016), and several heterotrophic bacteria (Curson et al., 2017) through complex biological interactions in marine ecosystems. Although it remains controversial, DMS and its by-products, such as methanesulfonic acid and non-sea-salt sulfate, are suspected to have a prominent part in climate feedback (Charlson et al., 1987; Quinn and Bates, 2011). The conversion of DMSP to

DMS is facilitated by several enzymes, including DMSP-lyase and acyl CoA transferase (Kirkwood et al., 2010; Todd et al., 2007); these enzymes are mainly found in phytoplankton, macroalgae, *Symbiodinium*, bacteria and fungi (de Souza and Yoch, 1995; Stefels and Dijkhuizen, 1996; Steinke and Kirst, 1996; Bacic and Yoch, 1998; Yost and Mitchelmore, 2009). 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). Several assumptions have been presented to explain these contrasting results and attributed the pH-induced variation in DMS-production capability to altered physiology of the algae cells or of bacterial DMSP degradation (Vogt et al., 2008; Hopkins et al., 2010, Avgoustidi et al., 2012; Archer et al., 2013; Hopkins and Archer, 2014; Webb et al., 2015).

Halocarbons also play a significant role in the global climate because they are linked to tropospheric and stratospheric ozone depletion and a synergistic effect of chlorine and bromine species has been reported that they may account for approximately 20% of the polar stratospheric ozone depletion (Roy et al., 2011). In addition, iodocarbons can release atomic iodine (I) quickly through photolysis in the atmospheric boundary layer and I atoms are very efficient in the catalytic removal of $O_3$, which governs the lifetime of many climate relevant gases including methane and DMS (Jenkins et al., 1991). Compared with DMS, limited attention was received about the effect of OA on halocarbon concentrations. Hopkins et al. (2010) and Webb et al. (2015) measured lower concentrations of several iodocarbons, while bromocarbons were unaffected by elevated $pCO_2$ through two acidification experiments. In addition, an additional mesocosm study did not elicit significant differences from any halocarbon compounds at up to 1,400 µatm $pCO_2$ (Hopkins et al.,

2013).

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 $pCO_2$ on diatoms and coccolithophores and to further understand how the productions of DMS and halocarbons respond to OA.

## 2. Experimental method

### *2.1 General experimental device*

The mesocosm experiments were carried out on a floating platform at the Facility for Ocean Acidification Impacts Study of Xiamen University (FOANIC-XMU, 24.52 °N, 117.18 °E) (for full technical details of the mesocosms, see Liu et al. 2017). Six cylindrical transparent thermoplastic polyurethane bags with domes were deployed along the south side of the platform. The width and depth of each mesocosm bag was 1.5 m and 3 m, respectively. Filtered (0.01 μm, achieved using an ultrafiltration water purifier, MU801-4T, Midea, Guangdong, China) *in situ* seawater was pumped into the six bags simultaneously within 24 h. A known amount of NaCl solution was added to each bag to calculate the exact volume of seawater in the bags, according to a comparison of the salinity before and after adding salt (Czerny et al., 2013). The initial *in situ* $pCO_2$ was about 650 μatm. To set the low and high $pCO_2$ levels, we added $Na_2CO_3$ solution and $CO_2$ saturated seawater to the mesocosm bags to alter total alkalinity and dissolved inorganic carbon (Gattuso et al., 2010; Riebesell et al., 2013). Subsequently, during the whole experimental

process, air at the ambient (400 μatm) and elevated $pCO_2$ (1000 μatm) concentrations was continuously bubbled into the mesocosm bags using a $CO_2$ Enricher (CE-100B, Wuhan Ruihua Instrument & Equipment Ltd., Wuhan, China). Because the seawater in the mesocosm was filtered, the algae in the coastal environment and their attached bacteria were removed and the trace gases produced in the environment did not influence the mesocosm trace gas concentrations after the bags were sealed.

*2.2 Algal strains*

*Emiliania huxleyi* (CS-369), *Phaeodactylum tricornuntum* (CCMA 106), and *Thalassiosira weissflogii* (CCMA 102) were inoculated into the mesocosm bags, with an initial diatom/coccolithophorid cell ratio of 1:1. The initial concentrations of *Phaeodactylum tricornuntum*, *Thalassiosira weissflogii*, and *Emiliania huxleyi* inoculated into the mesocosm were 10, 10, and 20 cells $mL^{-1}$, respectively. *Phaeodactylum tricornuntum* and *Thalassiosira weissflogii* were obtained from the Center for Collections of Marine Bacteria and Phytoplankton of the State Key Laboratory of Marine Environmental Science (Xiamen University). *Phaeodactylum tricornuntum* was originally isolated from the South China Sea in 2004 and *Thalassiosira weissflogii* was isolated from Daya Bay in the coastal South China Sea. *Emiliania huxleyi* was originally isolated in 1992 from the field station of the University of Bergen (Raunefjorden; 60°18'N, 05°15'E). Before being introduced into the mesocosms, the three phytoplankton species were cultured in autoclaved, pre-filtered seawater from Wuyuan Bay at 16 ℃ (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. Meanwhile, no meaningful numbers of bacteria were counted by flow cytometer in the pre-filtered seawater before the inoculations.

*2.3 Sampling for DMS(P) and halocarbons*

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

*2.4 Measurements of chlorophyll a*

Chlorophyll *a* (Chl *a*) was measured in water samples (200–1,000 mL) collected every 2 d at 9

293 a.m. by filtering onto Whatman GF/F filters (25 mm). The filters were placed in 5 mL 100%
294 methanol overnight at 4 ℃ and centrifuged at 5000 r $min^{-1}$ for 10 min. The absorbance of the
295 supernatant (2.5 mL) was measured from 250 to 800 nm using a scanning spectrophotometer (DU
296 800, Beckman Coulter Inc., Brea, CA, USA). Chl *a* concentration was calculated according to the
297 equation reported by Porra (2002).

298 2.5 *Enumeration of DMSP-consuming bacteria (DCB)*

299 The number of DMSP-consuming bacteria (DCB) was estimated using the most probable number
300 (MPN) methodology. The MPN medium consisted of a mixture (1:1 v/v) of sterile artificial sea
301 water (ASW) and mineral medium (Visscher et al., 1991), 3 mL of which was dispensed in 6 mL
302 test tubes, which were closed off by an over-sized cap, allowing gas exchange. Triplicate dilution
303 series were set up. All test tubes contained 1 mmol $L^{-1}$ DMSP as the sole organic carbon source
304 and were kept at 30 ℃ in the dark. After 2 weeks, the presence/absence of bacteria in the tubes
305 was verified by DAPI staining (Porter and Feig, 1980). Three tubes containing 3 mL ASW
306 without substrate were used as controls.

307 *2.6 Statistical analysis*

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

312 **3. Results and Discussion**

313 *3.1 Temporal changes in pH, Chl a, Phaeodactylum tricornuntum, Thalassiosira weissflogii, and*
314 *Emiliania huxleyi during the experiment*

During the experiment, the seawater in each mesocosm was well combined, and the temperature and salinity were well controlled, with a mean of 16 $^{o}$C and 29 in all mesocosms, respectively. Meanwhile, we observed significant differences in pH levels between the two $CO_2$ treatments on days 0–11, but the differences disappeared with subsequent phytoplankton growth (Fig. 1). 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. The initial chemical parameters of the mesocosm experiment are shown in Table 1. The initial mean dissolved nitrate (including $NO_3^-$ and $NO_2^-$), $NH_4^+$, $PO_4^{3-}$ and silicate ($SiO_3^{2-}$) concentrations were 54, 20, 2.6 and 41 μmol $L^{-1}$ for the low $pCO_2$ (LC) treatment and 52, 21, 2.4 and 38 μmol $L^{-1}$ for the high $pCO_2$ (HC) treatment, respectively. The nutrient concentrations ($NO_3^-$, $NO_2^-$, $NH_4^+$ and phosphate) during phase I were consumped rapidly and their concentrations were below or close to the detection limit during phase II (Table 1). In addition, although dissolved inorganic nitrogen ($NH_4^+$, $NO_3^-$, and $NO_2^-$) and phosphate were depleted, Chl *a* concentration in both treatments (biomass dominated by *Phaeodactylum tricornuntum*) remained constant over days 12–22, and then declined over subsequent days (Liu et al., 2017). *Emiliania huxleyi* was only found in phase I and its maximal concentration reached 310 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 cells $mL^{-1}$, which was far less than the concentration of *Phaeodactylum tricornutum* with a maximum density of about 1.5 million cells $mL^{-1}$ (Liu et al., 2017).

337 *3.2 Impact of elevated $pCO_2$ on DMS and DMSP production*

338 At the beginning of the experiment, the mean DMS, DMSP and DCB concentrations were all low
339 in both treatments due to the low concentrations of DMS, DMSP and DCB in the original fjord
340 water and possible loss during the filtration procedure (Fig. 2). With the growth of phytoplankton,
341 DMS, DMSP and DCB showed slightly different trends during the mesocosm experiment. The
342 DMSP concentrations in the HC and LC treatments increased significantly along with the increase
343 of Chl *a* concentrations and algal cells, and stayed relatively constant over the following days. A
344 significant positive relationship was observed between DMSP and phytoplankton in the
345 experiment (r = 0.961, $p < 0.01$ for *Phaeodactylum tricornuntum*, r = 0.617, $p < 0.01$ for
346 *Thalassiosira weissflogii* in the LC treatment, table 2; r = 0.954, $p < 0.01$ for *Phaeodactylum*
347 *tricornuntum*, r = 0.743, $p < 0.01$ for *Thalassiosira weissflogii* in the HC treatment, table 3).
348 Compared with DMSP, DMS and DCB concentrations showed similar trends during the
349 mesocosm experiment. DMS concentrations in the LC and HC treatments were 1.03 and 0.74
350 nmol $L^{-1}$, respectively, while DCB concentrations in the LC and HC treatments were 0.20 × $10^6$
351 and 0.16 × $10^6$ cells $mL^{-1}$. DMS and DCB concentrations did not increase significantly during
352 phase I, but began to increase rapidly on day 15. DCB concentrations in the LC and HC treatments
353 peaked on days 21 (11.65 × $10^6$ cells $mL^{-1}$) and 23 (10.70 × $10^6$ cells $mL^{-1}$), while DMS
354 concentrations in the LC and HC treatments peaked on days 25 (112.1 nmol $L^{-1}$) and 30 (101.9
355 nmol $L^{-1}$). Both DMS and DCB concentrations began to decrease obviously during phase III.
356 Meanwhile, a significant positive relationship was also observed between DMS and
357 *Phaeodactylum tricornuntum* (r = 0.560, $p < 0.05$ in the LC treatment; r = 0.635, $p < 0.01$ in the
358 HC treatment), while no relationship was observed between DMS and *Thalassiosira weissflogii*

359 (table 2 and table 3) during the experiment.

360 In this study, no difference in mean DMSP concentrations was observed between the two
361 treatments, indicating that elevated $pCO_2$ had no significant influence on DMSP production in
362 *Phaeodactylum tricornuntum* and *Thalassiosira weissflogii* throughout this study. However, a
363 significant 29% reduction in DMS concentrations was detected in the HC treatment compared
364 with the LC treatment ($p$ = 0.016), though no statistical difference for DCB concentrations was
365 found between the LC and HC treatments during phase I. This reduction in DMS concentrations
366 may be attributed to greater consumption of DMS and conversion to DMSO (Webb et al., 2015).
367 In addition, the peak DMS concentration in the HC treatment was delayed 5 days relative to that in
368 the LC treatment during phase II (Fig. 2-A). This result has been observed in previous mesocosm
369 experiments and it was attributed to small scale shifts in community composition and succession
370 that could not be identified with only a once-daily measurement regime (Vogt et al., 2008; Webb et
371 al., 2016). However, this phenomenon can be explained in another straightforward way during this
372 study. Previous studies have showed that marine bacteria play a key role in DMS production and the
373 efficiency of bacteria converting DMSP to DMS may vary from 2 to 100% depending on the
374 nutrient status of the bacteria and the quantity of dissolved organic matter (Simó et al., 2002, 2009;
375 Kiene et al., 1999, 2000). In addition, a significant positive relationship was also observed
376 between DMS and DCB (r = 0.643, $p$ < 0.01 in the LC treatment; r = 0.544, $p$ < 0.01 in the HC
377 treatment) during this experiment. All of these observations point to the importance of bacteria in
378 DMS and DMSP dynamics. During the present mesocosm experiment, DMSP concentrations in
379 the LC treatment decreased slightly on day 23, while the slight decrease appeared on day 29 in the
380 HC treatment (Fig. 2-B). In addition, the time that the DMSP concentration began to decrease was

very close to the time when the highest DMS concentration occurred in both treatments. 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. Taken together, we inferred that the elevated $pCO_2$ first delayed growth of DCB in the mesocosm, then the delayed DCB postponed the DMSP degradation process, and eventually delayed the DMS concentration in the HC treatment. In addition, considering that the algae and their attached bacteria were removed through a filtering process before the experiment and the unattached bacteria were maintained in a relatively constant concentration during this mesocosm experiment (Huang et al., 2018), we further concluded that the elevated $pCO_2$ controlled DMS concentrations mainly by affecting DCB attached to *Thalassiosira weissflogii* and *Phaeodactylum tricornuntum*.

*3.3 Impact of elevated $pCO_2$ on halocarbon compounds*

The temporal development in $CHBrCl_2$, $CH_3Br$, and $CH_2Br_2$ concentrations is shown in Fig. 3 (A–C) and the temporal changes of their concentrations were substantially different from those of DMS, DMSP, *Phaeodactylum tricornuntum* and *Thalassiosira weissflogii*. The mean concentrations of $CHBrCl_2$, $CH_3Br$ and $CH_2Br_2$ for the entire experiment were 8.58, 7.85, and 5.13 pmol $L^{-1}$ in the LC treatment and 8.81, 9.73, and 6.27 pmol $L^{-1}$ in the HC treatment. The concentrations of $CHBrCl_2$, $CH_3Br$, and $CH_2Br_2$ did not increase with the Chl *a* concentration compared with those of DMS and DMSP, and no major peaks were detected in the mesocosms. In addition, no effect of elevated $pCO_2$ was identified for any of the three bromocarbons, which compared well with previous mesocosm findings (Hopkins et al., 2010, 2013; Webb, et al., 2016). No clear correlation was observed between the three bromocarbons and any of the measured algal

403 groups (table 2 and table 3), indicating that *Phaeodactylum tricornuntum* and *Thalassiosira*
404 *weissflogii* did not primarily release these three bromocarbons during the mesocosm experiment.
405 Previous studies have reported that large-size cyanobacteria, such as *Aphanizomenon flos*-aquae,
406 produce bromocarbons (Karlsson et al. 2008) and significant correlations between cyanobacterium
407 abundance and several bromocarbons have been reported in the Arabian Sea (Roy et al., 2011).
408 However, the filtration procedure led to the loss of cyanobacterium in the mesocosms and finally
409 resulted in low bromocarbon concentrations during the experiment, although *Phaeodactylum*
410 *tricornuntum* and *Thalassiosira weissflogii* abundances were high.
411 The temporal dynamics of $CH_3I$ in the HC and LC treatments are shown in Fig. 3-D. The $CH_3I$
412 concentrations in the LC treatment varied from 0.38 to 12.61 pmol $L^{-1}$, with a mean of 4.76 pmol
413 $L^{-1}$. The $CH_3I$ concentrations in the HC treatment ranged between 0.44 and 8.78 pmol $L^{-1}$, with a
414 mean of 2.88 pmol $L^{-1}$. The maximum $CH_3I$ concentrations in the HC and LC treatments were
415 both observed on day 23. The range of $CH_3I$ concentrations during this experiment was similar to
416 that measured in the mesocosm experiment (< 1~10 pmol $L^{-1}$) in Kongsfjorden conducted by
417 Hopkins et al. (2013). In addition, the mean $CH_3I$ concentration in the LC treatment was similar to
418 that measured in the East China Sea, with an average of 5.34 pmol $L^{-1}$ in winter and 5.74 pmol $L^{-1}$
419 in summer (Yuan et al., 2015). Meanwhile, a positive relationship was detected between $CH_3I$ and
420 Chl *a*, *Phaeodactylum tricornuntum* and *Thalassiosira weissflogii* ($r = 0.588$, $p < 0.01$ in the LC
421 treatment; $r = 0.834$, $p < 0.01$ in the LC treatment for *Phaeodactylum tricornuntum*; $r = 0.680$ $p <$
422 0.01 in the LC treatment; $r = 0.690$, $p < 0.01$ in the HC treatment for *Thalassiosira weissflogii*; $r =$
423 0.717, $p < 0.01$ in the LC treatment; $r = 0.741$, $p < 0.01$ in the HC treatment for Chl *a*). This result
424 agrees with previous mesocosm (Hopkins et al., 2013) and laboratory experiments (Hughes et al.,

2013; Manley and De La Cuesta, 1997) identifying diatoms as significant producers of $CH_3I$. Moreover, similar to DMS, the maximum $CH_3I$ concentration also occurred after the maxima of *Phaeodactylum tricornuntum* and *Thalassiosira weissflogii*, at about 4 d (Fig. 3-D). This was similar to iodocarbon gases measured in a Norway mesocosm conducted by Hopkins et al. (2010) and chloroiodomethane ($CH_2ClI$) concentrations measured in another Norway mesocosm conducted by Wingenter et al. (2007). Furthermore, the $CH_3I$ concentrations measured in the HC treatment were significantly lower than those measured in the LC treatment during the mesocosm, which is in accord with the discoveries of Hopkins et al. (2010) and Webb et al. (2015) but in contrast to the findings of Hopkins et al. (2013) and Webb et al. (2016). Throughout the mesocosm experiment, there was a 40.2% reduction in the HC mesocosm compared to the LC mesocosm. Considering that the phytoplankton species did not show significant differences in the HC and LC treatments during the experiment, this reduction in the HC treatment was likely not caused by phytoplankton. Apart from direct biological production via methyl transferase enzyme activity by both phytoplankton and bacteria (Amachi et al., 2001), $CH_3I$ is produced from the breakdown of higher molecular weight iodine-containing organic matter (Fenical, 1982) through photochemical reactions between organic matter and light (Richter and Wallace, 2004). Both bacterial methyl transferase enzyme activity and a photochemical reaction may have reduced the $CH_3I$ concentrations in the HC treatment but further experiments are needed to verify this result.

## 4. Conclusions

In this study, the effects of increased levels of $pCO_2$ on marine DMS(P) and halocarbons release were studied in a controlled mesocosm facility. A 28.2% reduction during the logarithmic growth phase and a 5 d delay in DMS concentration was observed in the HC treatment due to the effect of

447 elevated $pCO_2$. Because the seawater in the mesocosm was filtered, the algae in the coastal
448 environment and their attached bacteria were removed and the trace gases produced in the
449 environment did not influence the mesocosm trace gas concentrations after the bags were sealed.
450 Therefore, we attribute this phenomenon to the DMSP-consuming bacteria attached to
451 *Phaeodactylum tricornuntum* and *Thalassiosira weissflogii*. More attention should be paid to the
452 DMSP-consuming bacteria attached to algae under different pH values in future studies. Three
453 bromocarbons compounds were not correlated with a range of biological parameters, as they were
454 affected by the filtration procedure and elevated $pCO_2$ had no effect on any of the three
455 bromocarbons. The temporal dynamics of $CH_3I$, combined with strong correlations with biological
456 parameters, indicated biological control of the concentrations of this gas. In addition, the
457 production of $CH_3I$ was sensitive to $pCO_2$, with a significant increase in $CH_3I$ concentration at
458 higher $pCO_2$. However, without additional empirical measurements, it is unclear whether this
459 decrease was caused by bacterial methyl transferase enzyme activity or by photochemical
460 degradation at higher $pCO_2$.

461 Author contribution: Gui-Peng Yang and Kun-Shan Gao designed the experiments. Sheng-Hui
462 Zhang, Juan Yu and Qiong-Yao Ding carried out the experiments and prepared the manuscript.
463 Hong-Hai Zhang and Da-Wei Pan revised the paper.

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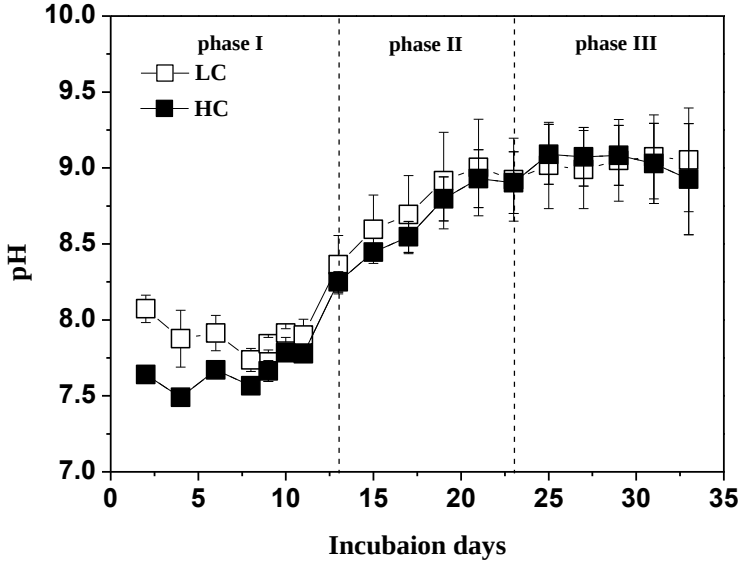
## Figure captions

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

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

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




626

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

628 mesocosms (3,000 L). Data are mean ± standard deviation, n = 3 (triplicate independent mesocosm bags) (Origin

629 8.0).

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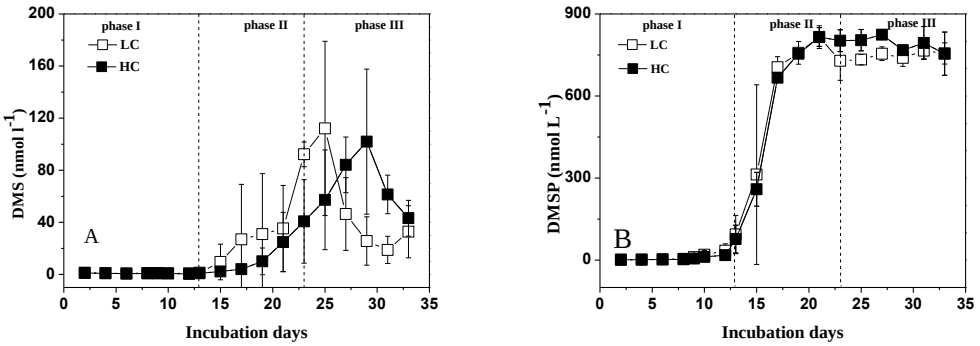


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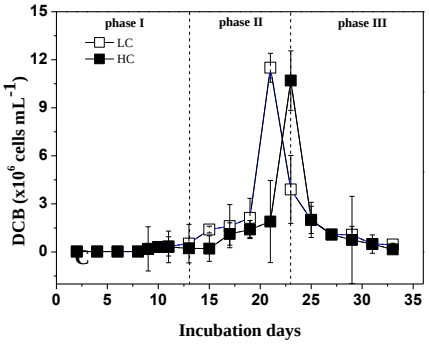


633 **Fig. 2** Temporal changes in DMS (A), DMSP (B), DCB (C) concentrations in the HC (1,000 μatm, black squares)

634 and LC (400 μatm, white squares) mesocosms (3,000 L). Data are mean ± standard deviation, n = 3 (triplicate

635 independent mesocosm bags) (Origin 8.0).

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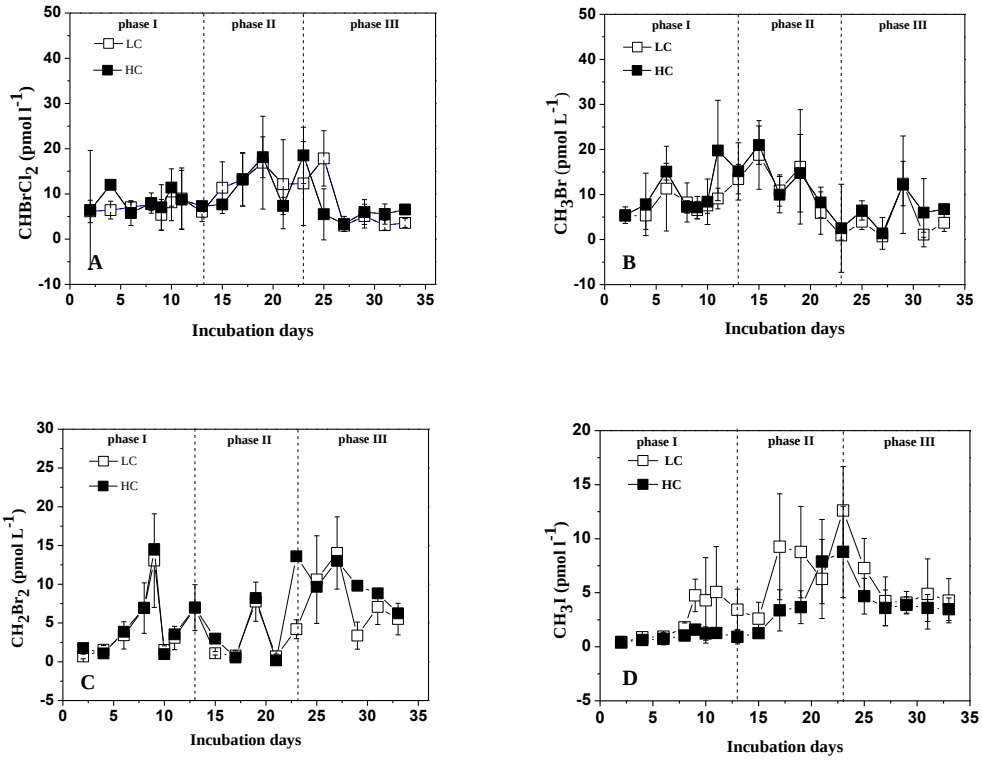


642

643 **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

644 μatm, black squares) and LC (400 μatm, white squares) mesocosms (3,000 L). Data are mean ± standard deviation,

645 n = 3 (triplicate independent mesocosm bags) (Origin 8.0).

646

647 **Table 1**. The conditions of DIC, $pH_T$, $pCO_2$ and nutrient concentrations in the mesocosm experiments. "-" means

648 that the values were below the detection limit.

| | | $pH_T$ | DIC ($\mu mol\ kg^{-1}$) | $pCO_2$ ($\mu atm$) | $NO_3^-+NO_2^-$ ($\mu mol\ L^{-1}$) | $NH_4^+$ ($\mu mol\ L^{-1}$) | $PO_4^{3-}$ ($\mu mol\ L^{-1}$) | $SiO_3^{2-}$ ($\mu mol\ L^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| day 0 | LC | 8.0±0.1 | 2181±29 | 1170~1284 | 52~56 | 19~23 | 2.6±0.2 | 38~40 |
| | HC | 7.5±0.1 | 2333±34 | 340~413 | 51~55 | 19~23 | 2.5±0.2 | 38~39 |
| PhaseI | 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 |

649

**Table 2**. Relationships between DMS, DMSP, Chl *a*, $CHBrCl_2$, $CH_3Br$, $CH_2Br_2$, $CH_3I$, 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 $L^{-1}$) | $CHBrCl_2$ (pmol $l^{-1}$) | $CH_3Br$ (pmol $l^{-1}$) | $CH_2Br_2$ (pmol $l^{-1}$) | $CH_3I$ (pmol $l^{-1}$) | DCB (×$10^6$ cells $mL^{-1}$) | *T. weissflogii* (×$10^3$ cells $mL^{-1}$) | *P. tricornutum* (cells $mL^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| DMS | 1 | | | | | | | | | |
| DMSP | 0.701** | 1 | | | | | | | | |
| Chl *a* | 0.597** | 0.792** | 1 | | | | | | | |
| $CHBrCl_2$ | 0.526 | 0.280 | 0.559 | 1 | | | | | | |
| $CH_3Br$ | -0.413 | -0.230 | 0.196 | 0.313 | 1 | | | | | |
| $CH_2Br_2$ | 0.310 | 0.180 | 0.001 | -0.136 | -0.308 | 1 | | | | |
| $CH_3I$ | 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*, $CHBrCl_2$, $CH_3Br$, $CH_2Br_2$, $CH_3I$, 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 $L^{-1}$) | $CHBrCl_2$ (pmol $l^{-1}$) | $CH_3Br$ (pmol $l^{-1}$) | $CH_2Br_2$ (pmol $l^{-1}$) | $CH_3I$ (pmol $l^{-1}$) | DCB ($\times 10^6$ cells $mL^{-1}$) | *T. weissflogii* ($\times 10^3$ cells $mL^{-1}$) | *P. tricornutum* (cells $mL^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| DMS | 1 | | | | | | | | | |
| DMSP | 0.752** | 1 | | | | | | | | |
| Chl *a* | 0.318* | 0.738** | 1 | | | | | | | |
| $CHBrCl_2$ | 0.324 | 0.094 | 0.326 | 1 | | | | | | |
| $CH_3Br$ | -0.410 | -0.349 | 0.065 | 0.076 | 1 | | | | | |
| $CH_2Br_2$ | 0.540* | 0.352 | 0.142 | 0.233 | -0.377 | 1 | | | | |
| $CH_3I$ | 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 |