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

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**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) and dimethylsulfoniopropionate (DMSP) as well as four halocarbon compounds ($CHBrCl_2$, $CH_3Br$, $CH_2Br_2$, and $CH_3I$). Over a period of 5 weeks, *P. tricornutum* outcompeted *T. weissflogii* and *E. huxleyi*, comprising more than 99% of the final biomass. During the logarithmic growth phase (phase I), DMS concentrations in high $pCO_2$ mesocosms (1000 μatm) were 28.2% lower than those in low $pCO_2$ mesocosms (400 μatm). Elevated $pCO_2$ led to a delay in DMSP-consuming bacteria attached to *T. weissflogii* and *P. 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 *T. weissflogii* or *P. tricornutum* throughout the 5 week culture. A positive relationship was detected between $CH_3I$ and *T. weissflogii* and *P. tricornutum* during the experiment, and there was a 40.2% 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 peak 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), halocarbon, phytoplankton, bacteria

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## 1. Introduction

As a result of human activity, anthropogenic emissions has 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 (IPCC, 2014). 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 (Doneyet al., 2009; Orr et al., 2005), 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 the algal secondary metabolite dimethylsulfoniopropionate (DMSP) through complex biological interactions in marine ecosystems (Stefels et al., 2007). 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 already reported the sensitivity of DMS-production capability to ocean acidification. Majority of these experimental studies revealed 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 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 attribute 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, 2016). 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 ($CH_4$) 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 (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).

The combined picture arising from existing studies is that the response of communities to OA

is not predictable and requires further study. Here, we report a mesocosm experiment conducted to study the influence of elevated $pCO_2$ on the biogeochemical cycle of a laboratory-cultured artificial phytoplankton community of diatoms and coccolithophores that had been previously examined for the response to elevated $pCO_2$. Our objective was to assess how changes in the phytoplankton community driven by changes in $pCO_2$ impact dimethyl sulfur compounds and halocarbons (including $CH_3I$, $CHBrCl_2$, $CH_3Br$, and $CH_2Br_2$) release.

## 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) in Wu Yuan Bay, Xiamen (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*

Three phytoplankton strains were inoculated into the mesocosm bags, at $4 \times 10^4$ cells $L^{-1}$ each *P. tricornutum* (CCMA 106) and *T. weissflogii* (CCMA 102) were obtained from the Center for Collections of Marine Bacteria and Phytoplankton of the State Key Laboratory of Marine Environmental Science (Xiamen University). *P. tricornutum* was originally isolated from the South China Sea in 2004 and *T. weissflogii* was isolated from Daya Bay in the coastal South China Sea. *E. huxleyi* PML B92/11 was originally isolated in 1992 from the field station of the University of Bergen (Raunefjorden; 60°18'N, 05°15'E).

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

*2.5 Statistical analysis*

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

## 3. Results and Discussion

*3.1 Temporal changes in pH, Chl a, P. tricornutum, T. weissflogii, and E. huxleyi during the experiment*

Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-148
Manuscript under review for journal Biogeosciences
Discussion started: 16 May 2018




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 (Huang et al., 2018). Meanwhile, we observed significant differences in $pCO_2$ levels between the two $CO_2$ treatments on days 0–11, but the differences disappeared with subsequent phytoplankton growth (Fig. 1-A). The phytoplankton growth process was divided into three phases in terms of variations in Chl *a* concentrations (Fig. 1-B) in the mesocosm experiments: i) the logarithmic growth phase (phase I, days 0–12), ii) a plateau phase (phase II, days 12–22, bloom period), and iii) a secondary plateau phase (phase III, days 22–33) 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 μmol $L^{-1}$, 20 μmol $L^{-1}$, 2.6 μmol $L^{-1}$ and 41 μmol $L^{-1}$ for the LC treatment and 52 μmol $L^{-1}$, 21 μmol $L^{-1}$, 2.4 μmol $L^{-1}$ and 38 μmol $L^{-1}$ for the HC treatment, respectively. The nutrient concentrations ($NO_3^-$, $NO_2^-$, $NH_4^+$ and phosphate) during phase I were consumped rapidly and there concentrations were below or close to the detection limit during phase II (Table 1). Meanwhile, Chl *a* concentration increased rapidly and reached 109.9 and 108.6 mg $L^{-1}$ in the LC and HC treatments, respectively. In addition, although DIN ($NH_4^+$, $NO_3^-$, and $NO_2^-$) and phosphate were depleted, Chl *a* concentration in both treatments (biomass dominated by *P. tricornutum*) remained constant over days 12–22, and then declined over subsequent days as shown in Liu et al. 2017.

*E. huxleyi* was only found in phase I and its maximal concentration reached 310 cells $mL^{-1}$ according to the results of microscopic inspection (Fig. 2-C). *T. 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 *P. tricornutum* with a maximum cell density of about 1.5 million cells $mL^{-1}$ (Fig. 2-A and Fig. 2-B). *P. tricornutum* accounted for at least 99% of all of the biomass by the time the populations had entered the plateau phase (phase II). We did not detect any significant enhancement in elevated $pCO_2$ due to the large variation. However, significant differences between the two $pCO_2$ treatments were found on days 23 ($p = 0.006$) and 25 ($p = 0.007$) (Fig. 2-A), when the cell concentration declined. Although we did not observe any difference between the two $pCO_2$ treatments during the rapid growth period (days 8–15), a longer period of persistent cell growth and a slower pace during the decrease in population size in phase II were recorded under the HC condition compared to the LC condition (Fig. 2-A).

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

Several studies have already reported the sensitivity of DMS-production capability to decreases in seawater pH. However, these studies did not come to a unified conclusion (Vogt et al., 2008; Hopkins et al., 2010; Avgoustidi et al., 2012; Archer et al., 2013; Hopkins and Archer, 2014; Webb et al.,2016). Fig. 3 (A-B) shows the mean DMS and DMSP concentrations for the HC and LC treatments during the mesocosm experiment. At the beginning of the experiment, the mean DMS and DMSP concentrations were low in both treatments due to the low concentrations of DMS and DMSP in the original fjord water and possible loss during the filtration procedure. DMS and DMSP showed slightly different trends during growth in the mesocosm experiment. The DMSP concentrations in the HC and LC treatments increased significantly along with the increase in Chl *a* and cell concentrations, and stayed relatively constant over the following days. A significant positive relationship was observed between DMSP and phytoplankton in the experiment ($R^2 = 0.92$ $p < 0.01$ for *P. tricornutum*, $R^2 = 0.36$ $p < 0.01$ for *T. weissflogii* in LC treatment; $R^2 = 0.94$ $p$

< 0.01 for *P. tricornutum*, $R^2$ = 0.36 $p$ < 0.01 for *T. weissflogii* in HC treatment). Mean concentrations of DMS in the HC and LC treatments did not increase significantly (1.03 and 0.74 nmol $L^{-1}$ for the LC and HC treatments, respectively) during phase I, but began to increase rapidly beginning on day 15. The two treatments peaked on days 25 (112.1 nmol $L^{-1}$) and 30 (101.9 nmol $L^{-1}$), respectively, and then began to decrease during phase III. A significant positive relationship was observed between DMS and phytoplankton throughout the experiment ($R^2$ = 0.65 $p$ < 0.01 for *P. tricornutum*, $R^2$ = 0.80 $p$ < 0.01 for *T. weissflogii* in LC treatment; $R^2$ = 0.54 $p$ < 0.01 for *P. tricornutum*, $R^2$ = 0.73 $p$ < 0.01 for *T. weissflogii* in HC treatment).

A significant 28.2% reduction in DMS concentration was detected in the HC treatment compared with the LC treatment ($p$ = 0.016) during phase I and this reduction in DMS concentrations may be attributed to greater consumption of DMS and conversion to DMSO (Webb et al., 2015). In contrast, no difference in mean DMSP concentrations was observed between the two treatments, indicating that elevated $pCO_2$ had no significant influence on DMSP production in *P. tricornutum* and *T. weissflogii* during this study. In addition, the peak DMS concentration in the HC treatment was delayed 5 days relative to that in the LC treatment during phase II (Fig. 3-A). This result has been observed in previous mesocosm experiments and it was attributed to small scale shifts in community composition and succession that could not be identified with only a once-daily measurement regime (Vogt et al., 2008; Webb et al., 2016). However, this phenomenon can be explained in another straightforward way during this study. Previous studies have showed that marine bacteria play a key role in DMS production and the efficiency of bacteria converting DMSP to DMS may vary from 2 to 100% depending on the nutrient status of the bacteria and the quantity of dissolved organic matter (Simó et al., 2002, 2009; Kiene et al., 1999, 2000). All of these

Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-148
Manuscript under review for journal Biogeosciences
Discussion started: 16 May 2018




observations point to the importance of bacteria in DMS and DMSP dynamics. During the present mesocosm experiment, DMSP concentrations in the LC treatment decreased slightly on day 23, while the slight decrease appeared on day 29 in the HC treatment (Fig. 3-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, DMSP-consuming bacterial abundance peaked on days 19 and 21 in the LC and HC treatments, respectively, as shown in Fig. S1 (Yu et al., unpublished data). DMSP-consuming bacterial abundance 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 DMSP-consuming bacteria in the mesocosm, then the delayed DMSP-consuming bacteria abundance 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 DMSP-consuming bacteria attached to *T. weissflogii* and *P. tricornutum*. Moreover, the inhibition of elevated $pCO_2$ to DMSP-consuming bacteria might be another important reason for the reduction of DMS in the HC treatment during phase I.

*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 (C–E) and the temporal changes in their concentrations were substantially different from those of DMS, DMSP, *T. weissflogii*, and *P. tricornutum*. 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, 2016). No clear correlation was observed between the three bromocarbons and any of the measured algal groups, indicating that *T. weissflogii* and *P. tricornutum* did not primarily release these three bromocarbons during the mesocosm experiment. Previous studies have reported that large-size cyanobacteria, such as *Aphanizomenon flos*-aquae, produce bromocarbons (Karlsson et al. 2008) and significant correlations between cyanobacterium abundance and several bromocarbons have been reported in the Arabian Sea (Roy et al., 2011). However, the filtration procedure led to the loss of cyanobacterium in the mesocosms and finally resulted in low bromocarbon concentrations during the experiment, although *T. weissflogii* and *P. tricornutum* abundances were high.

$CH_3I$ prodution is usually involve to “biogenic”, as it is released directly by macroalgae and phytoplankton, and indirectly generated via a photochemical degradation with organic matter (Moore and Zafiriou, 1994; Archer et al., 2007; Laturnus, 1995). The $CH_3I$ concentrations in the HC and LC treatments are shown in Fig. 3-F. The maximum $CH_3I$ concentrations in the HC and LC treatments were both observed on day 23 (12.61 and 8.78 pmol $L^{-1}$ for the LC and HC treatments, respectively). A positive relationship was detected between $CH_3I$ and Chl *a* in both *T. weissflogii* and *P. tricornutum* ($R^2 = 0.35$ $p < 0.01$ in LC treatment; $R^2 = 0.76$ $p < 0.01$ in HC treatment for *P. tricornutum*; $R^2 = 0.48$ $p < 0.01$ in LC treatment; $R^2 = 0.48$ $p < 0.01$ in HC treatment for *T. weissflogii*; $R^2 = 0.54$ $p < 0.01$ in LC treatment; $R^2 = 0.53$ $p < 0.01$ in HC

267 treatment for Chl *a*). This result agrees with previous mesocosm (Hopkins et al., 2013) and
268 laboratory experiments (Hughes et al., 2013; Manley and De La Cuesta, 1997) identifying diatoms
269 as significant producers of $CH_3I$. Morevover, similar to DMS, the maximum $CH_3I$ concentration
270 also occurred after the maxima of *T. weissflogii* and *P. tricornutum*, at about 4 d (Fig. 3-F). This
271 was similar to iodocarbon gases measured in a Norway mesocosm conducted by Hopkins et al.
272 (2010) and chloroiodomethane ($CH_2ClI$) concentrations measured in another Norway mesocosm
273 conducted by Wingenter et al. (2007). Furthermore, the $CH_3I$ concentrations measured in the HC
274 treatment were significantly lower than those measured in the LC treatment during the mesocosm,
275 which is in accord with the discoveries of Hopkins et al. (2010) and Webb et al. (2015) but in
276 contrast to the findings of Hopkins et al. (2013) and Webb et al. (2016). Throughout the mesocosm
277 experiment, there was a 40.2% reduction in the HC mesocosm compared to the LC mesocosm.
278 Considering that the phytoplankton species did not show significant differences in the HC and LC
279 treatments during the experiment, this reduction in the HC treatment was likely not caused by
280 phytoplankton. Apart from direct biological production via methyl transferase enzyme activity by
281 both phytoplankton and bacteria (Amachi et al., 2001), $CH_3I$ is produced from the breakdown of
282 higher molecular weight iodine-containing organic matter (Fenical, 1982) through photochemical
283 reactions between organic matter and light (Richter and Wallace, 2004). Both bacterial methyl
284 transferase enzyme activity and a photochemical reaction may have reduced the $CH_3I$
285 concentrations in the HC treatment but further experiments are needed to verify this result.

286 **4. Conclusions**

287 In this study, the effects of increased levels of $pCO_2$ on marine DMS(P) and halocarbons release
288 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 elevated $pCO_2$. 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. Therefore, we attribute this phenomenon to the DMSP-consuming bacteria attached to *P. tricornutum* and *T. weissflogii*. More attention should be paid to the DMSP-consuming bacteria attached to algae under different pH values in future studies. Three bromocarbons compounds were not correlated with a range of biological parameters, as they were affected by the filtration procedure and elevated $pCO_2$ had not effect on any of the three bromocarbons. The temporal dynamics of $CH_3I$, combined with strong correlations with biological parameters, indicated biological control of the concentrations of this gas. In addition, the production of $CH_3I$ was sensitive to $pCO_2$, with a significant increase in $CH_3I$ concentration at higher $pCO_2$. However, without additional empirical measurements, it is unclear whether this decrease was caused by bacterial methyl transferase enzyme activity or by photochemical degradation at higher $pCO_2$.

Author contribution: Gui-peng Yang and Kun-shan Gao designed the experiments. Sheng-hui Zhang and Qiong-yao Ding carried out the experiments and prepared the manuscript. Hong-hai Zhang and Da-wei Pan revised the paper.

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446 **Figure captions**

447 Fig. 1. $CO_2$ partial pressure ($pCO_2$) and mean chlorophyll *a* (Chl *a*) concentrations in the HC
448 (1,000 µatm, solid squares) and LC (400 µatm, white squares) mesocosms (3,000 L).

449 Fig. 2. Temporal changes of *Thalassiosira weissflogii*, *Phaeodactylum tricornutum* and *Emiliania*
450 *huxleyi* cell concentrations in the HC (1,000 µatm, solid squares) and LC (400 µatm, white
451 squares) mesocosms (3,000 L).

452 Fig. 3 Temporal changes in DMS, DMSP, $CHBrCl_2$, $CH_3Br$, $CH_2Br_2$ and $CH_3I$ concentrations in
453 the HC (1,000 µatm, black squares) and LC (400 µatm, white squares) mesocosms (3,000 L).

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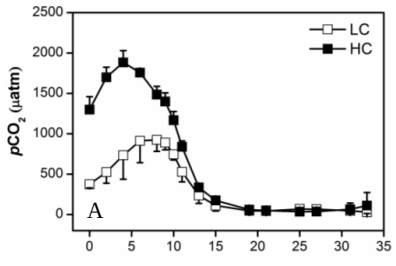


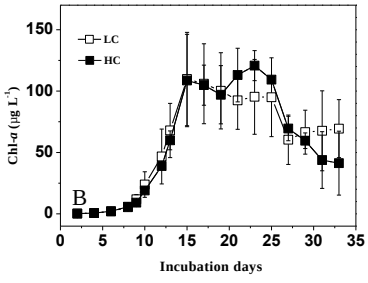


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457 **Fig. 1.** $CO_2$ partial pressure ($pCO_2$) and mean chlorophyll *a* (Chl *a*) concentrations in the HC (1,000 µatm, solid

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

459 (triplicate independent mesocosm bags) (Origin 8.0).

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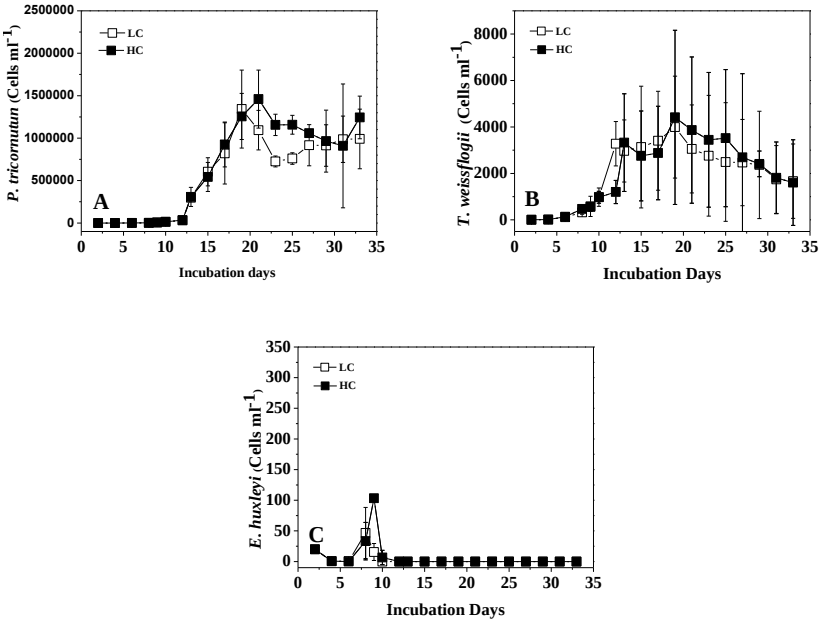


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465 **Fig. 2.** (A) *Thalassiosira weissflogii* cell concentrations; (B) *Phaeodactylum tricornutum* cell concentrations; (C)

466 *Emiliania huxleyi* cell concentrations. White squares represent the LC (400 µatm) treatment. Data are mean ±

467 standard deviation, n = 3 (triplicate independent mesocosm bags) (Origin 8.0).

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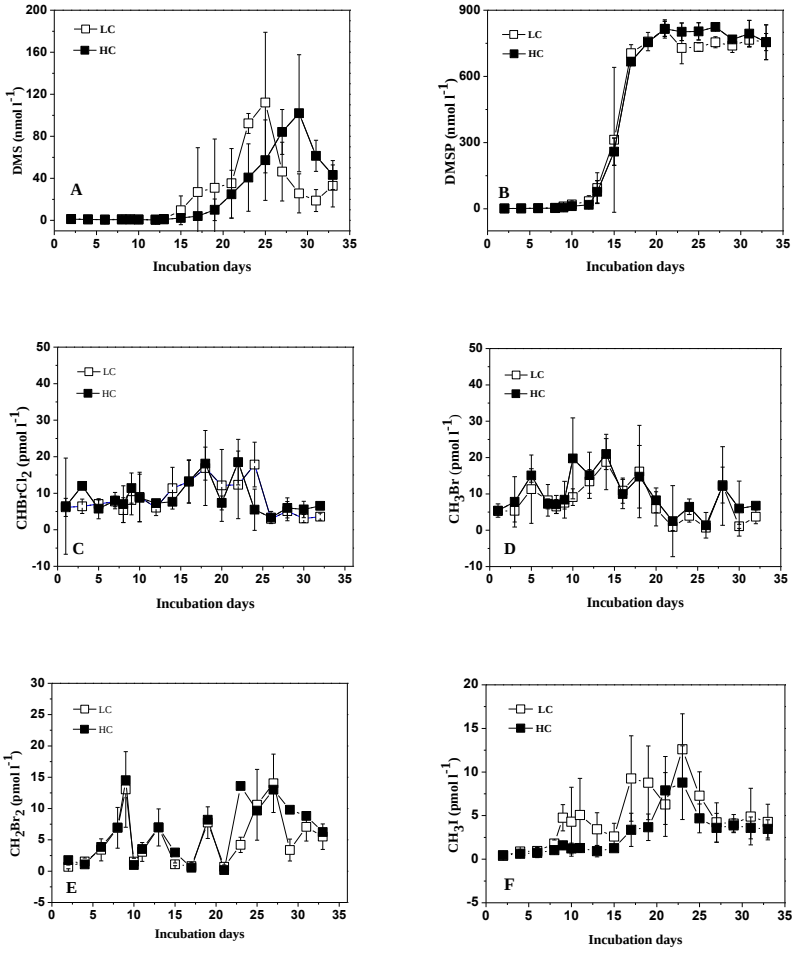


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473 **Fig. 3** Temporal changes in DMS, DMSP, $CHBrCl_2$, $CH_3Br$, $CH_2Br_2$ and $CH_3I$ concentrations in the HC (1,000

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

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

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477 **Table 1**. The conditions of DIC, $pH_T$, $pCO_2$ and nutrient concentrations in the mesocosm

478 experiments. "-" means 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 |

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