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Dynamics of dimethylsulphoniopropionate and dimethylsulphide under different CO₂ concentrations during a mesocosm experiment

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Abstract. The potential impact of seawater acidification on the concentrations of dimethylsulphide (DMS) and dimethylsulphoniopropionate (DMSP), and the activity of the enzyme DMSP-lyase was investigated during a pelagic ecosystem CO₂ enrichment experiment (PeECE III) in spring 2005. Natural phytoplankton blooms were studied for 24 days under present, double and triple partial pressures of CO₂ (pCO₂; pH=8.3, 8.0, 7.8) in triplicate 25 m³ enclosures. The results indicate similar DMSP concentrations and DMSP-lyase activity (DLA) patterns for all treatments. Hence, DMSP and DLA do not seem to have been affected by the CO₂ treatment. In contrast, DMS concentrations showed small but statistically significant differences in the temporal development of the low versus the high CO₂ treatments. The low pCO₂ enclosures had higher DMS concentrations during the first 10 days, after which the levels decreased earlier and more rapidly than in the other treatments. Integrated over the whole study period, DMS concentrations were not significantly different from those of the double and triple pCO₂ treatments. Pigment and flow-cytometric data indicate that phytoplanktonic populations were generally similar between the treatments, suggesting a certain resilience of the marine ecosystem under study to the induced pH changes, which is reflected in DMSP and DLA. However, there were significant differences in bacterial community structure and the abundance of one group of viruses infecting nanoeukaryotic algae. The amount of DMS accumulated per total DMSP or chlorophyll-*a* differed significantly between the present and future scenarios, suggesting that the pathways for DMS production or bacterial DMS consumption were affected by sea-

water pH. A comparison with previous work (PeECE II) suggests that DMS concentrations do not respond consistently to pelagic ecosystem CO₂ enrichment experiments.

1 Introduction

Dimethylsulphide (DMS) is a volatile sulfur compound produced from the algal secondary metabolite dimethylsulphoniopropionate (DMSP) by complex biotic interactions in marine ecosystems (Stefels et al., 2007). DMS is the main natural source of sulfate aerosol to the atmosphere and the major route by which sulfur is recycled from the ocean to the continents. The particulate atmospheric oxidation products of DMS can act as cloud condensation nuclei and thereby affect the radiative properties of the atmosphere by reflecting solar radiation (Charlson et al., 1987).

The physiological roles of algal DMS and DMSP are not fully understood. DMSP is a compatible solute with multifunctional properties that is synthesized by marine phytoplankton for osmoregulation and cellular cryoprotection (Stefels, 2000). DMSP and its cleavage products DMS and acrylate have been suggested to serve as antioxidants under light or nutrient stress (Sunda et al., 2002), and to act as infochemicals (Nevitt, 1995; Zimmer-Faust et al., 1996; Wolfe, 2000; Steinke et al., 2006) or grazing deterrents (Wolfe et al., 1997; Strom et al., 2003).

The production of DMSP is strongly dependent on the species composition of the marine ecosystem under investigation. Some phytoplankton groups, such as the prymnesiophytes, are prolific producers of DMSP with high DMSP/cell ratios (Keller et al., 1989). The prymnesiophyte coccolithophore *Emiliania huxleyi* also contains DMSP-lyase



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isozymes (Steinke et al., 1998) and is able to enzymatically cleave DMSP to DMS. Other prymnesiophytes such as *Phaeocystis* and dinophytes also produce high concentrations of DMSP but many other algal taxa are poor DMSP-producers (Liss et al., 1994). Intracellular DMSP is released to the water during cell lysis caused by grazing (Dacey and Wakeham, 1986), or due to natural mortality and after viral infection (Malin et al., 1998). Once in solution, DMSP can be utilized by many bacteria as a sulfur, carbon or energy source via catabolic demethylation to 3-methylmercaptopropionate and 3-mercaptopropionate (Kiene and Linn, 2000; Howard et al., 2006). Bacteria have also been shown to enzymatically cleave DMSP to DMS and acrylate (Kiene, 1993; Ledyard and Dacey, 1996; Stefels and Dijkhuizen, 1996; Steinke and Kirst, 1996) and novel evidence suggests DMSP-dependent DMS-production without the release of acrylate (Todd et al., 2007). DMS can be used as a metabolite by bacteria (Vila-Costa et al., 2006), photochemically degraded at the sea surface (Brimblecombe and Shooter, 1986; Kieber et al., 1996), or transferred to the atmosphere (Liss and Slater, 1974). Since several biological components of the marine microbial food-web add to the physico-chemical processes that are involved in the production and consumption of DMSP and DMS, the concentrations of both may be affected by changes in environmental conditions. Thus, DMS could serve as a sensitive indicator to human-induced climate change.

Ocean acidification is one of the effects of increased anthropogenic CO₂. In the past 200 years, the oceans have absorbed approximately half of the CO₂ emitted by human activities such as fossil fuel burning and cement manufacturing (Sabine et al., 2004). This uptake of CO₂ has led to changes in the chemical equilibrium of the seawater and to a reduction of the pH of the ocean surface waters by 0.1 units. If emissions were to continue according to present trends, ocean surface pH could decrease by 0.3–0.5 units by the end of the 21st century. This is equivalent to a threefold increase of the concentration of H⁺ ions in the surface ocean (Caldeira and Wickett, 2005). The impacts of ocean acidification on marine organisms and ecosystems are still poorly understood. Laboratory experiments and field studies indicate that acidification will adversely affect calcification (Royal Society, 2005; Kleypas et al., 2006), a process by which marine organisms fabricate shells and plates from calcium and carbonate ions. Coccolithophores, such as *E. huxleyi*, are one of the phytoplanktonic groups expected to be strongly affected by ocean acidification (Riebesell et al., 2000). *E. huxleyi* is abundant in temperate oceans and is a prolific producer of DMS (Keller et al., 1989; Holligan et al., 1993; Malin et al., 1993). It is possible that the intracellular production of DMSP or its direct conversion to DMS by *E. huxleyi* DMSP-lyases is affected by ocean acidification. Additionally, as mentioned above, oceanic DMS production is a result of complex interactions within the marine food-web. Consequently, ocean acidification may affect DMS concentrations and fluxes by alter-

ing one or more of the various pathways or impacting some of the species involved. Ocean acidification may therefore affect the feedback of DMS on climate via aerosol formation, as described by the CLAW-hypothesis (Charlson et al., 1987). Previous studies (Avgoustidi, 2006¹; Avgoustidi et al., 2008²) showed reduced DMS concentrations under high CO₂ in both field and laboratory studies. If the results from these studies can be extrapolated to global scales, reduced DMS emissions could lead to a significant positive feedback on global warming.

Here, we present the concentrations of DMS, DMSP and DMSP-lyase activities (DLA) during a mesocosm study in a Norwegian Fjord in May and June 2005. Our goal was to investigate differences in DMS dynamics under elevated CO₂, to address factors that may result in altered DMS dynamics and to compare our findings to results from a previous field experiment (Avgoustidi et al., 2008²). Furthermore, we investigate the relevance of our results with respect to global climate change and its impact on global DMS fluxes.

2 Materials and methods

2.1 General experimental set-up

The experiment was conducted at the Espeland Marine Biological Station, University of Bergen (Norway) in May and June 2005. The set-up consisted of 9 polyethylene enclosures (ca. 25 m³, 9.5 m water depth) moored to a raft in the Raunefjord (60.3° N, 5.2° E): 3 bags with present day pCO₂, hereafter referred to as “1×CO₂” (350 ppmv partial pressure of CO₂), 3 bags with double pCO₂, referred to as “2×CO₂” treatments (700 ppmv) and 3 bags with triple pCO₂, referred to as “3×CO₂” treatments (1050 ppmv). These bags were simultaneously filled with unfiltered fjord water pumped from a depth of 12 m. Fresh water (0.6 m³) was mixed into the upper 5 m of the mesocosm bags to stratify the water column. The 2×CO₂ and 3×CO₂ bags were aerated with CO₂ enriched air, until the water pCO₂ reached the target values (day 0), the 1×CO₂ bags were aerated with ambient air. To allow biological processes to alter water pCO₂, no further adjustments were carried out after day 1. All mesocosm bags were covered with transparent hoods of ethylene tetrafluorethylene foil (Foiltec, Bremen, Germany), which allowed transmission of 95% of incoming light intensity for the complete solar spectrum. The headspaces underneath the hoods were kept at target pCO₂ by flushing them with CO₂-enriched air (23–35 L min⁻¹). A phytoplankton bloom was triggered via the addition of nutrients on day 0 (16 May 2005; 0.7 μmol L⁻¹ PO₄, 15 μmol L⁻¹ NO₃) and the bloom was studied over a

¹ Avgoustidi, V.: Dimethyl sulphide production in a double-CO₂ world, Ph.D. thesis, University of East Anglia, 2006.

² Avgoustidi, V., Joint, I., Nightingale, P. D., Steinke, M., Turner, S. M., and Liss, P. S.: Dimethyl sulphide production in a double-CO₂ world, in preparation, 2008.

period of 24 days. Throughout the study period, the upper 5 m of the water column were gently mixed by means of an airlift system. Further details of the set-up and procedures can be found elsewhere (Engel et al., 2005; Schulz et al., 2007).

2.2 Sampling for sulfur compounds

Samples from all nine mesocosms were taken daily at 10:30 h, simultaneous with other measurements conducted during PeECE III. Bubble-free sampling was carried out with nine 5 L polyethylene aspirators. Prior to sampling, all aspirators were thoroughly rinsed first with natural fjord water and then with water from the respective mesocosms. The mouths of the aspirators were covered with a 200 μm mesh in order to exclude mesozooplankton grazers and taps were left open to release air during sampling. The aspirators were then inverted and slowly immersed through the water surface to a depth of approximately 0.3 m. A minimum of 3 L of water was sampled before closing the taps, slowly turning over and capping off the aspirators and transporting them to a cold-room where the samples were stored at in situ water temperature (9–11.5°C) and in dim light. Sub-samples were taken using Teflon tubing and gas-tight syringes (20 mL) after slowly rotating the aspirators to re-suspend particulate matter.

2.3 Quantification of sulfur compounds

Particulate DMSP (DMSP_p): Slow syringe filtration was used to filter 5 to 20 mL of sample through 25 mm glass-fibre filters (Whatman GF/F). The filtrate was directly injected into a purge vessel for the analysis of DMS (see below). Thereafter, the filters were folded and placed into glass vials containing NaOH, using 3 mL of 500 mmol L⁻¹ NaOH in 4 mL screw-capped vials (days 1 to 4) or 13 mL of 500 mmol L⁻¹ NaOH in 20 mL crimp-sealed vials (days 5 to 24). The alkaline hydrolysis of DMSP resulted in equimolar quantities of DMS. Vials were sealed immediately with Teflon-coated septa, stored in the dark and transported to our laboratory at the University of East Anglia (UEA). The headspace analysis of DMS resulting from DMSP_p cleavage commenced with a 24 h incubation of the vials at a standard temperature of 30°C before manual injection of 50 to 200 μL of headspace for quantitative analysis of DMS using gas chromatography and flame-photometric detection (Shimadzu GC-2010 with 30 m \times 0.53 mm CP-Sil 5CB capillary column). DMS standards for calibration were prepared using commercial DMSP standard (Centre for Analysis, Spectroscopy and Synthesis (CASS), University of Groningen Laboratories, The Netherlands) at a final concentration of 0.3 to 3 $\mu\text{mol DMS L}^{-1}$ added to vials containing 3 or 13 mL 500 mmol L⁻¹ NaOH. The detection limit for a 20 mL sample was about 2 nmol L⁻¹ DMSP. The analytical error was less than 12%, as estimated from a comparison of replicate samples ($n=16$).

DMS: After filtration for DMSP_p, 5 to 18 mL of the filtrate was used for DMS analysis. The analytical volumes for the DMS measurements were adjusted during the course of the experiment to accommodate changes in concentration. DMS measurements were conducted within 2 h of sampling using the gas chromatographic system described above, in combination with a purge-and-trap system for cryogenic enrichment of DMS at -150°C (details in Vogt et al. (2008) and Turner et al. (1990)). Calibrations were carried out every 3–4 days with DMSP stock solution equivalent to 0.3 to 24.3 nmol L⁻¹ and addition of NaOH to more than 500 mmol L⁻¹. The detection limit of the above described gas chromatographic system was less than 0.3 nmol L⁻¹ DMS. The analytical error was 6%, as estimated from replicate calibration standards ($n=69$).

Dissolved DMSP (DMSP_d): After purging the water sample for DMS analysis was completed, 4 to 13 mL of purged, de-gassed sample was transferred into 20 mL vials and brought to a volume of 13 mL with MilliQ water for analysis of DMSP_d. Samples were adjusted to 500 mmol L⁻¹ NaOH by adding 684 μL of 10 mol L⁻¹ NaOH. Vials were immediately capped with Teflon-coated crimp seals and stored in the dark prior to analysis. Samples were incubated at 30°C for 24 h before manual injection of 200 μL of headspace for the analysis of DMS using the gas chromatographic system described above. DMS concentrations were quantified via the addition of DMSP standard to 13 mL 500 mmol L⁻¹ NaOH at a final concentration of 6 to 60 nmol L⁻¹. Detection limit in 13 mL of sample was about 1.3 nmol L⁻¹ DMSP_d.

Total DMSP (DMSP_t): Because of concerns about potential filtration artifacts (Kiene and Slezak, 2006) we also considered total DMSP (DMSP_t) concentrations for our analyses. DMSP_t was calculated as the sum of DMSP_d and DMSP_p concentrations.

DMSP-lyase activity (DLA): Measurements of DMSP-lyase activity were conducted using headspace measurements of DMS using the methods described in Steinke et al. (2000) and Steinke et al. (2007). In brief, 250 to 300 mL of seawater was filtered through polycarbonate filters of 47 mm diameter and 2 μm pore size (Whatman Nuclepore). The filters were folded twice and placed into cryo-vials before snap-freezing in liquid nitrogen and storage at -80°C. DLA samples were transported on dry ice to our laboratory at UEA. The DMSP-lyase was extracted using sonication on ice with a 3 mm sonotrode (5 bursts of 5 s at 5 W) into 1.8 mL of 300 mmol L⁻¹ sterile BTP buffer (1,3-bis[tris(hydroxymethyl)methylamino]propane) that was amended with 0.5 mol L⁻¹ NaCl at pH 8.2. Assays were conducted with 100 to 295 μL of the crude extract and linear production of DMS was quantified at 30°C for 15–45 min after the addition of buffer and 5 μL of 1.2 mol L⁻¹ DMSP stock ($t=0$) that was adjusted to pH 6.2 with NaOH to a total volume of 300 μL (final DMSP concentration was 20 mmol L⁻¹ and final pH was 8.2).

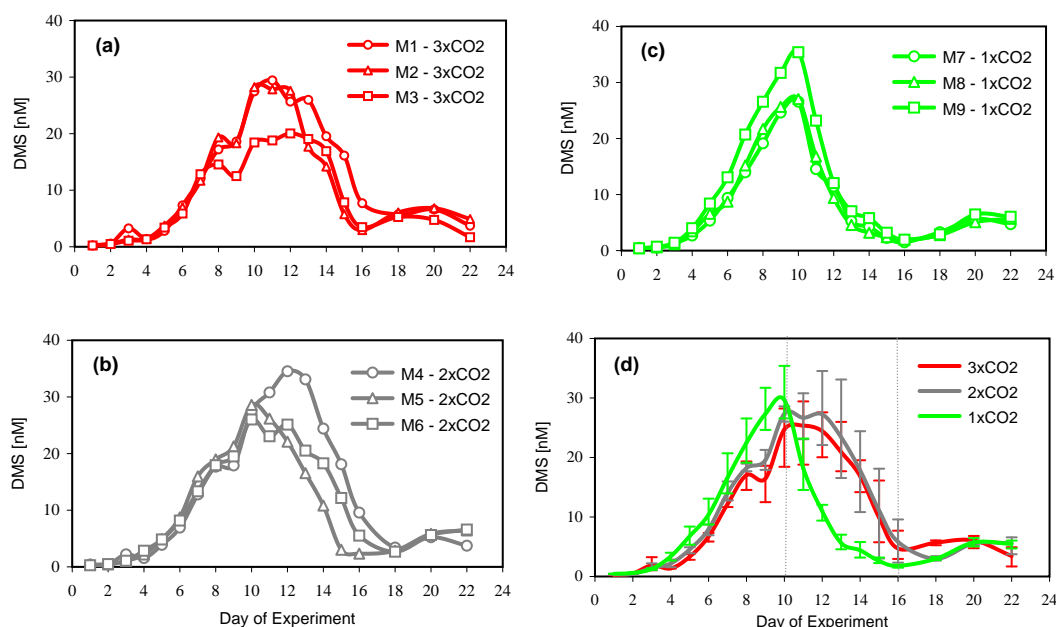


Fig. 1. DMS concentrations in nmol L^{-1} for the 3 enclosures of each treatment (a) $3\times\text{CO}_2$ (Mesocosms M1-M3) (b) $2\times\text{CO}_2$ (Mesocosms M4-M6) (c) $1\times\text{CO}_2$ (Mesocosms M7-M9) and (d) averages for all 3 treatments with range bars indicating the spread of the data. Green lines show present ($1\times\text{CO}_2$), grey lines $2\times\text{CO}_2$ and red lines depict $3\times\text{CO}_2$ treatments with $p\text{CO}_2$ of 350 ppmv, 700 ppmv and 1050 ppmv, respectively. Horizontal dotted lines indicate the separation between the 3 phases in DMS development (see text).

2.4 Additional measurements

Chlorophyll-*a* (chl-*a*) was determined in 250–500 mL sample filtered through 25 mm glass-fibre filters (Whatman GF/F). Diagnostic pigments were extracted according to Derenbach (1969). Pigment distributions were quantified using reverse-phase high-performance liquid chromatography (HPLC) analysis, using the method described in Barlow et al. (1997). CHEMTAX (Mackey et al., 1996) was used to derive the fraction of chl-*a* attributable to the dominant phytoplankton groups. For more information on chlorophyll-*a* measurements refer to Schulz et al. (2007) and Riebesell et al. (2007). While chlorophyll-*a* and pigments were not analysed in replicates during this study, the analytical error is estimated to lie within 10–15%, based on the results from previous analyses.

Counts of *Emiliania huxleyi* cells and other phyto-, bacterio- and virioplankton were conducted using a FAC-SCalibur flow cytometer (Becton-Dickinson) equipped with an air-cooled laser with an output power of 15 mW at 488 nm and a standard filter set-up. Phytoplankton counts were obtained from fresh samples at high flow rate (ca. $100 \mu\text{L min}^{-1}$). All samples were analysed during 300 s, and populations were discriminated based on dot plots of side scatter and red fluorescence. For details on the flow cytometric measurements, see Paulino et al. (2007). Heterotrophic bacteria and virus were detected and discriminated based on clusters observed in scatter plots of side scatter versus green

fluorescence, using SYBR Green I staining and following the method described in Larsen et al. (2007) and Paulino et al. (2007).

The partial pressure of CO₂ was quantified as described in Bellerby et al. (2007).

3 Results

3.1 DMS

DMS concentrations for the 3 enclosures of each treatment and the mean DMS concentrations for the 3 treatments are presented in Fig. 1. Three phases can be distinguished in the temporal development of DMS concentrations (Fig. 1d): From day 0 till day 10 (phase 1) DMS concentrations increased in all treatments. At the beginning of the experiment, DMS concentrations were low in all enclosures due to the low concentrations of DMS in the original fjord water and possible loss of DMS during the aeration procedure. After day 0, DMS concentrations increased in all treatments, with higher DMS concentrations in the $1\times\text{CO}_2$ than in the $2\times\text{CO}_2$ and $3\times\text{CO}_2$ treatments. On day 10 the maximum in DMS concentration was reached in $1\times\text{CO}_2$, with an average value of 29.5 nmol L^{-1} . Phase 2 (days 11–16) is characterized by a steep decline in DMS concentrations in $1\times\text{CO}_2$, and constant or declining DMS concentrations in $2\times\text{CO}_2$ and $3\times\text{CO}_2$ until day 16. The abrupt, steep decline in DMS concentrations was measured consistently in

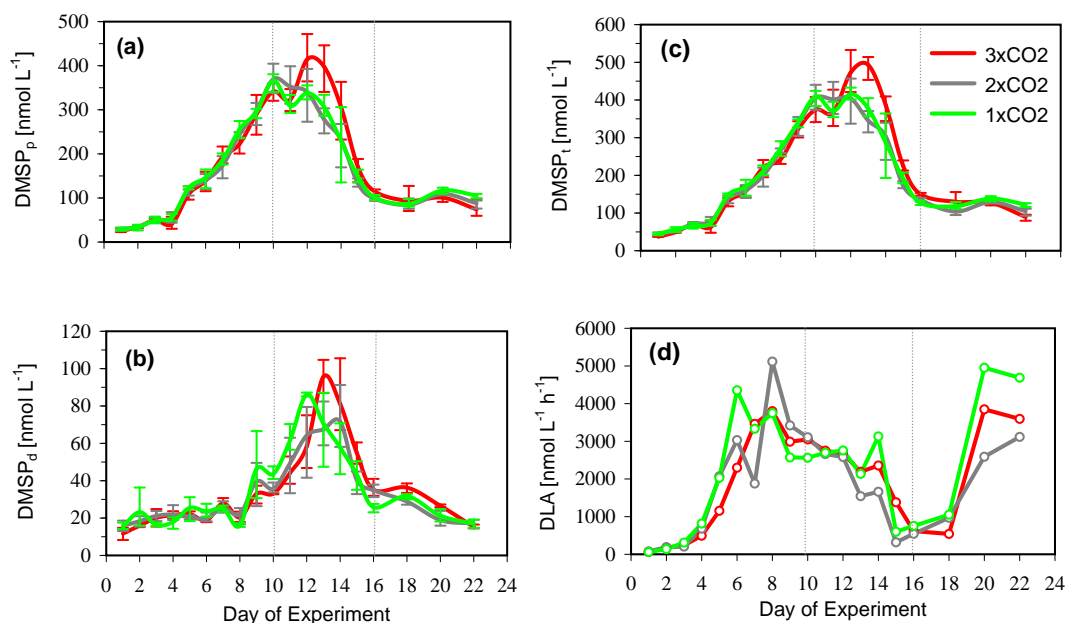


Fig. 2. Average (a) DMSP_p in nmol L^{-1} , (b) DMSP_d in nmol L^{-1} , (c) DMSP_t in nmol L^{-1} (d) DMSP -lyase activity (DLA) for selected bags 2 ($3\times\text{CO}_2$), 5 ($2\times\text{CO}_2$) and 8 ($1\times\text{CO}_2$) in $\text{nmol L}^{-1} \text{ h}^{-1}$. Green lines show $1\times\text{CO}_2$, grey lines $2\times\text{CO}_2$ and red lines depict $3\times\text{CO}_2$ treatments with $p\text{CO}_2$ of 350 ppmv, 700 ppmv and 1050 ppmv, respectively. The values shown are average values for 3 replicate bags. Vertical bars in (a–c) indicate the range of the data. Horizontal dotted lines indicate the separation between the 3 phases in DMS development (see text).

all $1\times\text{CO}_2$ enclosures. In the averages of the $2\times\text{CO}_2$ and $3\times\text{CO}_2$ treatments, DMS concentrations reached a plateau between day 10 and day 12, with maximum average concentrations of 27.4 nmol L^{-1} ($2\times\text{CO}_2$) and 25.3 nmol L^{-1} ($3\times\text{CO}_2$). In comparison to the $1\times\text{CO}_2$ treatments, the slope of the DMS decline was less steep in the $2\times\text{CO}_2$ and $3\times\text{CO}_2$ treatments. On day 16, DMS concentrations were below 6 nmol L^{-1} in all treatments. Phase 3 (days 17–22) is characterised by the onset of a smaller bloom of *Synechococcus* and dinoflagellates, which lead to a small increase in DMS concentrations in all treatments.

A two-way analysis of variance (ANOVA) with log transformed data for the 3 treatments showed that the temporal development of DMS between the 3 treatments was significantly different (Fig. 1d; $F=8.157$, $df=2$, $\sigma<0.001$, $p=0.05$)³. A posteriori testing with a Bonferroni test showed significant differences between the $1\times\text{CO}_2$ and $2\times\text{CO}_2$ treatments ($\sigma<0.001$, $p=0.05$). The significance of the difference between $1\times\text{CO}_2$ and $3\times\text{CO}_2$ ($\sigma=0.063$, $p=0.05$) proved inconclusive and the difference between $2\times\text{CO}_2$ and $3\times\text{CO}_2$ ($\sigma=0.192$, $p=0.05$) was not significant at the 95% confidence level.

The calculation of time integrated averages of DMS (days 0–22) showed that over the whole duration of the experiment, 25% more DMS was produced in $3\times\text{CO}_2$ and 14%

more DMS in $2\times\text{CO}_2$ than in $1\times\text{CO}_2$ in absolute terms. Using ANOVA, however, we did not find sufficient evidence to support that these differences in integrated DMS concentrations were significant ($F=1.799$, $df=2$, $\sigma=0.244$, $p=0.05$) at the 95% confidence level. This is due to the small sample size and a considerable spread in the means for each of the three treatments.

3.2 DMSP_p , DMSP_d , DMSP_t

The differences in DMS concentrations between present and enhanced $p\text{CO}_2$ treatments were not reflected in the particulate DMSP concentrations (Fig. 2a). This was also true for the dissolved fraction (Fig. 2b) and for total DMSP (Fig. 2c). As all enclosures showed very similar concentration patterns, we only show the mean concentrations for each treatment. At the beginning of the experiment, DMSP_p concentrations were below 50 nmol L^{-1} in all treatments. After day 4, DMSP_p rapidly increased in all treatments, and was maximal on day 10 in $1\times\text{CO}_2$ (366 nmol L^{-1}) and $2\times\text{CO}_2$ (370 nmol L^{-1}) and on day 12 in $3\times\text{CO}_2$ (415 nmol L^{-1}). Thereafter, DMSP_p declined in all treatments. DMSP_d concentrations remained constant at around 20 nmol L^{-1} until day 8 of the experiment, when it increased for all treatments. DMSP_d concentrations peaked on day 12 in $1\times\text{CO}_2$ (86 nmol L^{-1}), on day 14 in $2\times\text{CO}_2$ (72 nmol L^{-1}) and on day 13 in $3\times\text{CO}_2$ (96 nmol L^{-1}), whereafter DMSP_d decreased in all treatments. DMSP_t concentrations increased

³ F = ratio of mean squares, df = degrees of freedom, σ = significance of F-test and p = level of confidence.

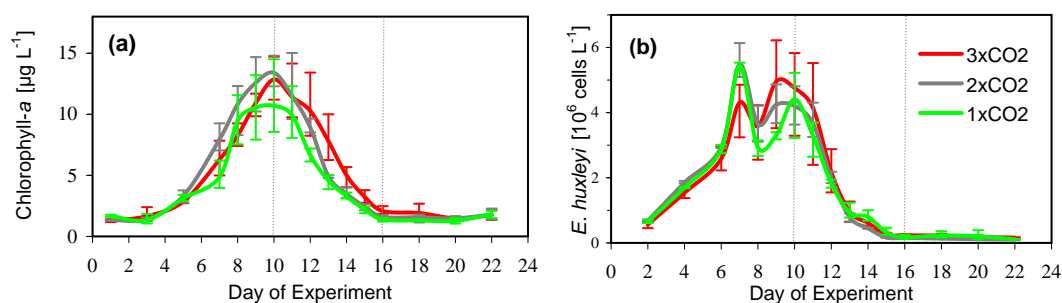


Fig. 3. (a) Chl-*a* in $\mu\text{g L}^{-1}$ and (b) *E. huxleyi* abundance in $10^6 \text{ cells L}^{-1}$ plotted as a function of time. Green lines show 1×CO₂, grey lines 2×CO₂ and red lines depict 3×CO₂ treatments with pCO₂ of 350 ppmv, 700 ppmv and 1050 ppmv, respectively. The values shown are average values for 3 replicate bags. Vertical bars indicate the range of the data. Horizontal dotted lines indicate the separation between the 3 phases in DMS development (see text).

steadily after day 4 and reached a first peak on day 10, with average DMSP_{*t*} concentrations of 374 nmol L⁻¹ in 1×CO₂, 405 nmol L⁻¹ in 2×CO₂ and 410 nmol L⁻¹ in 3×CO₂. DMSP_{*t*} concentrations in 1×CO₂ and 2×CO₂ declined after day 10 in a similar fashion. In the 3×CO₂ treatments, DMSP_{*t*} concentrations showed a brief increase and reached a maximal average concentration of 493 nmol L⁻¹ on day 13 before declining.

3.3 DMSP-lyase activity

The measured DMSP-lyase activity (DLA) was comprised of the activity of DMSP-lyase from algae and attached bacteria and has been analyzed without replication for each treatment (Fig. 2d). Due to our choice of filter (pore size of 2 μm), the potential contribution of many non-attached bacteria to DMSP-lyase activity was not included. We show data from mesocosm bags 2 (3×CO₂), 5 (2×CO₂), and 8 (1×CO₂), because most other measured parameters from collaborating groups are available for these bags. DLA peaked on day 6 for the present (4354 nmol L⁻¹ h⁻¹), and on day 8 for 2×CO₂ and 3×CO₂ treatments with values of 5116 and 3801 nmol L⁻¹ h⁻¹, respectively. After day 8, DLA decreased gradually in all treatments, until a minimum in activity was reached in all bags on day 15. After day 18, DLA increased rapidly in all treatments and reached a second maximum on day 20, with 4952 nmol L⁻¹ h⁻¹ for 1×CO₂, 2590 nmol L⁻¹ h⁻¹ for 2×CO₂ and 3849 nmol L⁻¹ h⁻¹ for 3×CO₂ treatments.

3.4 Ecosystem composition

All bags showed similar chl-*a* concentrations (Fig. 3a), with chl-*a* being slightly lower in 1×CO₂ than in 2×CO₂ and 3×CO₂. The maximum of average chl-*a* occurred on day 10 in all treatments. A succession of different phytoplankton taxa occurred during the course of the experiment (Riebesell et al., 2007). Between days 6 and 10, when most of the DMS was accumulated, the bloom was dominated by diatoms and

prymnesiophytes, including lithed *E. huxleyi* cells (Fig. 3b). During the whole study period, prasinophytes contributed up to 20% to total chl-*a*. Towards the end of the bloom, after day 18, dinoflagellate and *Synechococcus* species contributed significantly to total chlorophyll (Riebesell et al., 2007). A similar succession of species was observed in all treatments.

3.5 Contribution of the dominant phytoplankton groups to measured DMSP_{*p*}

We used HPLC pigment data (in $\mu\text{g chl-}a \text{ L}^{-1}$) and flow cytometry data (cells L⁻¹) in combination with literature values for the DMSP cell⁻¹ and chl-*a* cell⁻¹ or chl-*a* carbon⁻¹ of representative species of the dominant phytoplankton groups including prymnesiophytes, diatoms and dinoflagellates (Bucciarelli and Sunda, 2003; Buitenhuis et al., 1999; Geider et al., 1997; Keller et al., 1989; Steinke et al., 1998). This assessment provides a rough estimate of sources of DMSP in our experiment. Our findings suggest that over the duration of the experiment (days 0–22) approximately 11% of DMSP was produced by *E. huxleyi*, 20% by other prymnesiophytes, 22% by diatoms and 2% by dinoflagellates. This suggests that other taxa of prymnesiophytes may have contributed significantly to total DMSP_{*p*}. Prymnesiophytes and diatoms produced the majority of DMSP during phases 1 and 2, whereas dinoflagellates were important DMSP producers in phase 3. Because the uncertainty in the DMSP_{*p*} measurements was estimated to amount up to 12%, which is of the order of the detected DMS concentrations, it is unlikely that differences in DMS concentrations are reflected in our DMSP_{*t*} measurements. Thus, we cannot exclude small differences in the phytoplanktonic DMSP_{*p*} production to account for differences in DMS concentrations.

Table 1. Selected Spearman rank correlations (r_s) between DMS, DMSP_{*t*}, DLA and ecosystem parameters (days 0–22). Significance of correlations rejected at the 95% level (ns). Respective treatment (1×CO₂, 2×CO₂ and 3×CO₂) determined by the label in each row of the table.

| | DMS | DMSP _{<i>t</i>} | DLA |
|---|------|--------------------------|------|
| DMS (1×CO ₂) | 1.00 | 0.8 | 0.68 |
| DMS (2×CO ₂) | 1.00 | 0.98 | 0.8 |
| DMS (3×CO ₂) | 1.00 | 0.94 | 0.72 |
| DMSP _{<i>t</i>} (1×CO ₂) | 0.8 | 1.00 | 0.49 |
| DMSP _{<i>t</i>} (2×CO ₂) | 0.98 | 1.00 | 0.60 |
| DMSP _{<i>t</i>} (3×CO ₂) | 0.94 | 1.00 | 0.51 |
| DLA (1×CO ₂ , M8) | 0.68 | 0.49 | 1.00 |
| DLA (2×CO ₂ , M5) | 0.80 | 0.60 | 1.00 |
| DLA (3×CO ₂ , M2) | 0.72 | 0.51 | 1.00 |
| chl- <i>a</i> (1×CO ₂) | 0.82 | 0.84 | ns |
| chl- <i>a</i> (2×CO ₂) | 0.91 | 0.92 | 0.69 |
| chl- <i>a</i> (3×CO ₂) | 0.89 | 0.86 | ns |
| <i>E. huxleyi</i> (1×CO ₂) | 0.79 | 0.62 | ns |
| <i>E. huxleyi</i> (2×CO ₂) | 0.63 | 0.59 | ns |
| <i>E. huxleyi</i> (3×CO ₂) | 0.61 | 0.52 | ns |
| Total Prymnesiophytes (1×CO ₂) | 0.77 | 0.81 | ns |
| Total Prymnesiophytes (2×CO ₂) | 0.90 | 0.90 | ns |
| Total Prymnesiophytes (3×CO ₂) | 0.92 | 0.93 | ns |
| Total nanophytoplankton (1×CO ₂) | 0.77 | ns | ns |
| Total nanophytoplankton (2×CO ₂) | 0.56 | 0.56 | 0.58 |
| Total nanophytoplankton (3×CO ₂) | 0.72 | 0.64 | ns |

3.6 Relationships between DMS, DMSP_{*t*}, DLA and chlorophyll-*a*

We used Spearman rank correlation (r_s) to study the temporal correlation between DMS, DMSP_{*t*}, DLA and chl-*a* concentrations (Table 1). As a general trend, DMS, DMSP_{*t*} and chl-*a* tended to be more closely correlated in 2×CO₂ and 3×CO₂ than in 1×CO₂. DMSP_{*t*} and chl-*a* were temporally correlated in all 3 treatments and over the whole duration of the experiment ($n=16$; 1×CO₂: $r_s=0.84$, 2×CO₂: $r_s=0.92$, 3×CO₂: $r_s=0.86$). DMS and chl-*a* were temporally correlated in all treatments ($n=16$; 1×CO₂: $r_s=0.82$, 2×CO₂: $r_s=0.91$, 3×CO₂: $r_s=0.89$), as were DMS and DMSP_{*t*} ($n=19$; 1×CO₂: $r_s=0.80$, 2×CO₂: $r_s=0.98$, 3×CO₂: $r_s=0.94$). The lower correlations in 1×CO₂ in the latter two cases are due to the steep decline of DMS concentrations in 1×CO₂ after day 10. The high correlations of DMS, DMSP_{*t*} and chl-*a* point at a tight temporal coupling of these parameters; indeed there was only a small (1–2 days, 2×CO₂, 3×CO₂) or no phase lag (1×CO₂) between the peaks of these 3 compounds for all 3 treatments.

DLA was correlated with both DMS ($n=19$; 1×CO₂: $r_s=0.68$, 2×CO₂: $r_s=0.80$, 3×CO₂: $r_s=0.72$) and to a lesser extent with DMSP_{*t*} ($n=19$; 1×CO₂: $r_s=0.49$, 2×CO₂:

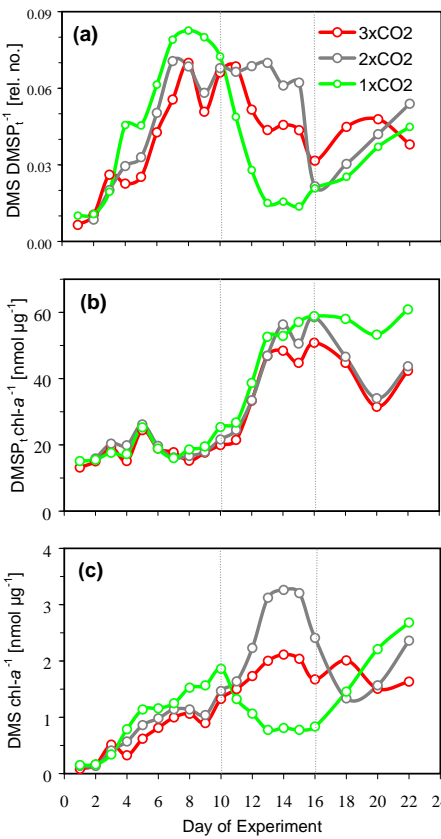


Fig. 4. Mean ratios of (a) DMS to DMSP_{*t*} (b) DMSP_{*t*} to chl-*a* in nmol µg^{−1} and (c) DMS to chl-*a* in nmol µg^{−1} for the 1×CO₂ (green lines), 2×CO₂ (grey lines) and 3×CO₂ (red lines) treatments. Horizontal dotted lines indicate the separation between the 3 phases in DMS development (see text).

$r_s=0.60$, 3×CO₂: $r_s=0.51$). However, only in 2×CO₂ did DLA correlated with chl-*a* ($r_s=0.69$).

Figure 4 shows the ratios of DMS, DMSP_{*t*} and chl-*a* against time. During phase 1, the ratio of DMS to DMSP_{*t*} followed a very similar trend for all treatments (Fig. 4a). From day 10–16 there was a phase lag between the peaks of DMS and DMSP_{*t*}, manifested in the divergence between the 2×CO₂, 3×CO₂ and 1×CO₂ curves. During the whole experiment, there were no significant differences between treatments in the ratio of DMSP_{*t*} to chl-*a* (Fig. 4b). This similar temporal development indicates that there were no major shifts in ecosystem composition that affected DMSP production and could have resulted in the differences in DMS concentrations between the 3 treatments. The ratio between DMS and chl-*a* shows significant differences between the treatments (Fig. 4c). In phase 1 of the experiment DMS and chl-*a* concentrations co-varied for all 3 treatments. During phase 2, significantly more DMS per chl-*a* was accumulated in the perturbed treatments, comparable to what was observed for DMS and DMSP_{*t*}.

3.7 Relationships between sulphur compounds and phytoplankton community composition

As above, we used Spearman rank correlation to study the dynamics of the sulphur compounds and ecosystem variables (Table 1). In all treatments, DMS concentrations were strongly correlated with total prymnesiophyte chl-*a* ($1\times\text{CO}_2$: $r_s=0.77$, $2\times\text{CO}_2$: $r_s=0.90$, $3\times\text{CO}_2$: $r_s=0.92$). DMS and *E. huxleyi* numbers were well correlated in $1\times\text{CO}_2$ ($r_s=0.79$) and slightly less in $2\times\text{CO}_2$ ($r_s=0.63$) and $3\times\text{CO}_2$ ($r_s=0.61$). DMS and *E. huxleyi* cell numbers showed a lag of ca. 2 days between their respective peaks for all 3 treatments. Furthermore, DMS correlated with total nanophytoplankton abundance (Table 1), which we defined to be the sum of *E. huxleyi*, and the abundance of two different nanophytoplankton groups, as determined by flow cytometry (Paulino et al., 2007).

During phase 1, DLA correlated well with dinoflagellate abundances, but correlations throughout the whole duration of the experiment were significant only in $1\times\text{CO}_2$ ($r_s=0.57$). During phase 3 DLA was linearly related to dinoflagellate abundances, with high R^2 values ($n=4$, $1\times\text{CO}_2$: $R^2=0.97$, $2\times\text{CO}_2$: $R^2=0.93$, $3\times\text{CO}_2$: $R^2=0.88$). Only in $2\times\text{CO}_2$ did DLA correlate significantly with the abundance of total bacteria (Allgaier et al., 2008). DLA did not correlate significantly with any of the biological rate measurements, such as primary production (Egge et al., 2007), bacterial protein production (BPP) or cell specific BPP (Allgaier et al., 2008).

4 Discussion

Several previous mesocosm studies conducted at the same facility in Bergen report DMSP, DMS and chl-*a* concentrations under present CO₂ (Levasseur et al., 1996; Williams and Egge, 1998; Wilson et al., 1998; Steinke et al., 2007). The DMSP and DMS concentrations we found are within the range of concentrations found in previous mesocosm studies, but concentrations vary with respect to the boundary conditions of the experiments, i.e. they depend on the organisms dominating the bloom and the manipulations under which the system was investigated. The species composition reported from this experiment is typical for waters in the investigated region and the time of the year. However, temperature and light intensities were unusually low for May, which could have influenced the bloom development and species succession (Schulz et al., 2007).

In contrast to a previous CO₂ enrichment study (Engel et al., 2005) conducted under very similar experimental conditions, only few biological parameters showed CO₂-related effects: Neither HPLC pigment analyses nor flow cytometry detected significant phytoplankton species shifts between treatments. The ecosystem composition, bacterial and phytoplankton abundances and productivity, grazing rates and total grazer abundance and reproduction were not significantly af-

fected by CO₂ induced effects (Riebesell et al., 2007; Riebesell et al., 2008; Egge et al., 2007; Paulino et al., 2007; Larsen et al., 2007; Suffrian et al., 2008; Carotenuto et al., 2007). This finding suggests that the system under study was surprisingly resilient to abrupt and large pH changes.

4.1 DMSP and DMS

The resilience of the system is well reflected in the suite of marine biogenic sulphur compounds. There were no differences in DMSP_p, DMSP_d, DMSP_t or DLA and only small differences in the temporal development of DMS. These differences in DMS concentrations may be due to several factors, as discussed below:

Prymnesiophytes such as *E. huxleyi* are high DMS producers and some have been found to be affected by ocean acidification (Riebesell, 2004). Furthermore, prymnesiophytes dominated the phytoplankton bloom in this experiment and possibly were important players in the production of DMSP and DMS during this experiment. DMS and DMSP correlated strongly with total prymnesiophyte chl-*a* in all treatments, in particular for the $2\times\text{CO}_2$ and $3\times\text{CO}_2$ treatments (Table 1). DMS and DMSP also correlated well with *E. huxleyi*, particularly in the $1\times\text{CO}_2$ treatment (Table 1). This finding, along with the fact that significantly more DMSP was produced by all prymnesiophytes than by *E. huxleyi* points at the presence of other, DMSP-producing prymnesiophyte species unidentified by HPLC analysis or flow cytometry during this experiment.

While there were no direct observations of prymnesiophytes other than *E. huxleyi* at the species level, indirect observations suggest the presence of at least one other prymnesiophyte: Larsen et al. (2007) found evidence for the presence of a virus identified as CeV, a virus infecting the prymnesiophyte *Chrysochromulina ericina*. The genus *Chrysochromulina* can produce DMSP, with a DMSP cell quota 4× as high as the one for *E. huxleyi* (Keller et al., 1989). In addition, *Chrysochromulina* species have been observed in the North Sea and in Norwegian coastal waters (e.g. Brussaard et al., 1996). Viral infection can lead to significant production of DMS (Malin et al., 1998) and viral infection is likely to have played an important role in terminating the bloom during this experiment. Furthermore, Larsen et al. (2007) found a CO₂ effect on the abundance of a group of high fluorescence viruses (HFV) identified by flow cytometry. HFV was suggested to be a composite group of several dsDNA viruses infecting nano-eukaryotic algae and is likely to have included CeV. From day 5, HFV was more abundant in $1\times\text{CO}_2$ than in the CO₂-enriched treatments. During days 15–22, HFV was ca. 1.7× more abundant in the $1\times\text{CO}_2$ treatments than in $2\times\text{CO}_2$ and ca. 2.4× more abundant in $1\times\text{CO}_2$ than $3\times\text{CO}_2$. *C. ericina* has been shown to grow optimally for a pH ranging from 7.5 to 8.4 (Rhodes and Burke, 1996) and may not have been affected as much as the calcifying *E. huxleyi* by the pH encountered during this experiment.

Differences in viral infection of *C. ericina*, however, could potentially explain parts of the observed differences in DMS concentration.

Observed small differences in *E. huxleyi* cell numbers (see Fig. 3b) could only partly account for the differences in DMS concentrations between the treatments. While flow cytometry determines the number of lithed *E. huxleyi* cells, unlithed *E. huxleyi* cells are measured as part of the other nanophytoplankton groups. Changes in the fraction of unlithed or “naked” *E. huxleyi* could account for changes in DMS. However, the fraction of unlithed cells is expected to be small and constant (A. Paulino, personal communication).

Even though we could exclude major shifts in ecosystem composition (Paulino et al., 2007; Larsen et al., 2007) to account for the differences in DMS, the effect of smaller shifts in species succession could not be studied with our measurements. Additionally, changes in algal physiology leading to altered DMS exudation rates or changes in DMSP cell quota of individual taxa were not studied.

During the course of the experiment a statistically significant difference in the community structure of free-living bacteria (0.2–5.0 μm fraction) was detected for the three different treatments (Allgaier et al., 2008). Denaturing Gradient Gel Electrophoresis (DGGE) band pattern analysis showed that while the populations of the 1 \times CO₂ and 2 \times CO₂ treatments were similar to the fjord population, the free-living bacterial communities of the 3 \times CO₂ treatments diverged much more from the original population. Despite these clear differences in bacterial community structure, the DMS concentration patterns of 3 \times CO₂ and 2 \times CO₂ were very similar. Currently, there is no quantitative evidence for an effect of pCO₂ on bacteria that degrade DMS or DMSP, but such an effect could lead to different DMSP or DMS consumption rates or to a different microbial DMS yield from DMSP, resulting in differences in DMS concentration patterns. The community structure of attached bacteria (>5.0 μm) did not exhibit statistical differences between the treatments.

Taken together, processes related to bacterial and viral activities may explain part of the difference in amount and temporal structure of DMS that we observed.

4.2 DLA

In general, DLA was considerably higher than previous measurements in *E. huxleyi* dominated waters in the North Atlantic and North Sea (Steinke et al., 2002a, b) and in a mesocosm experiment in 2003 (Steinke et al., 2007). No clear difference between the CO₂ treatments was observed. DLA correlated well with dinoflagellate and prymnesiophyte chl-*a* during phase 1. Hence, it is likely that phytoplanktonic DMSP-lyase contributed to DMS production during phase 1. Coccolithophores such as *E. huxleyi* contain the enzyme DMSP-lyase and they dominated the bloom during days 1–10. Except for the 2 \times CO₂ treatment during days 0–10 ($r_s=0.82$), we did not find significant temporal correlations

between DLA and *E. huxleyi* abundances. To our knowledge there is no published work investigating DMSP-lyase activity in *C. ericina*. In the beginning of the experiment, dinoflagellate chlorophyll levels were low, but at the end of the bloom (day 18 to day 22), a dinoflagellate bloom occurred in the mesocosms (Riebesell et al., 2007). Some dinoflagellates contain high amounts of DMSP_{*p*} per cell and can show high DMSP-lyase activity. The beginning of their bloom coincided well with the second increase in DLA after day 18. We found a significant linear correlation between DLA and dinoflagellates during phase 3 of the experiment, hence some of the DMSP-lyase activity detected in this phase of the bloom could be due to the increasing abundance of dinoflagellates. DLA did not correlate with any of the small phytoplankton groups, nor with most bacterial parameters. Only in 2 \times CO₂ did DLA (anti-)correlate with bacterial abundance.

Unfortunately, we cannot yet assess the importance of algal DLA for overall DMS accumulation in this study. DLA correlated much stronger and more consistently with DMS and DMSP_{*t*} than with any of the phyto- and bacterioplankton parameters.

4.3 Comparison with other DMS measurements during PeECE III

Several groups measured DMS during PeECE III. Air concentrations of DMS were in phase with our observed water measurements (Sinha et al., 2007; Wingenter et al., 2007) and there was a good general agreement between the water measurements (Vogt et al., 2008; Wingenter et al., 2007). While we find our absolute values for the integrated DMS mean concentrations to be very similar to those reported in Wingenter et al. (2007), we cannot confirm the conclusions of these authors that the differences were statistically significant. This discrepancy does not arise at the data level, but through the use of different statistical procedures for the interpretation of the results: Firstly, these authors report their differences to be statistically significant at the 80% and 90% confidence level, levels at which the significance of differences is generally rejected (Cowles and Davis, 1982 and references therein). Secondly, Wingenter et al. (2007) compare the means of 3 populations in pairs of 2 with respect to a fixed factor (CO₂) using a Student's *t*-test, which increases the probability of committing type I errors, i.e. the null hypothesis (no differences between populations) is rejected when in fact it is true (see e.g. Zar, 1999). We use One-way ANOVA, known to decrease the probability of type I errors and decided to adopt a more stringent significance criterion.

4.4 Comparison between PeECE II and PeECE III

In 2003, Avgoustidi et al. studied DMS dynamics during a mesocosm bloom (PeECE II) under present, pre-industrial and future CO₂ conditions (Avgoustidi et al., 2008²). The

experimental set-up and treatment of the mesocosms was similar in both Avgoustidi et al. and our study (Engel et al., 2005). Despite this, chl-*a* concentrations were approximately 3 times higher in the present study. Furthermore, our maximum DMSP_p values (data not shown) were approximately 2 times higher than those reported in Avgoustidi et al. Maximal DMS values were similar in both experiments, but the temporal development of the sulfur compounds was different. Whereas DMS, DMSP_p and chl-*a* were tightly coupled in the present study, the DMS peaks show a distinct lag behind the DMSP_p peaks in Avgoustidi et al. Hence, a major difference between the two experiments is the temporal yield of DMS from DMSP. Avgoustidi et al. found a significant decrease in DMS concentrations for treatments with elevated pCO₂. Despite the clear differences in the temporal dynamics of DMS concentrations, our results do not confirm the findings by Avgoustidi et al. when integrated over the whole duration of the experiment, but do when integrated up to day 12. This may partly be explained by differences in *E. huxleyi* cell number between the two experiments. While *E. huxleyi* cell numbers in our study were low (max. 5×10^6 cells mL⁻¹), cell numbers in Avgoustidi's experiment were considerably higher (up to 56×10^6 cells mL⁻¹). Both number densities are well within the natural range of cell numbers found for *E. huxleyi* in the open ocean (J. Egge, personal communication).

The behavior of DMS concentration patterns between perturbed and unperturbed treatments agreed for both studies during the exponential growth phase (days 0–10), but diverged for the post-bloom phase (days 11–22). Although poorly understood at present, changes in physiology could account for the reduced DMS production in 2×CO₂ and 3×CO₂ during the *E. huxleyi* bloom until day 10 in this study. Had the cell number been ten-fold, as in Avgoustidi et al., this effect might have been amplified and the results may have been more similar. Additional processes, including bacterial uptake and catabolism of DMSP, could have influenced the DMS dynamics after day 12 when the *E. huxleyi* bloom collapsed. DMS emissions by prymnesiophyte species other than *E. huxleyi* (such as *Chrysochromulina ericina*) may have contributed to the prolonged peak in the perturbed treatments. However, lack of data on the bacterial cycling of DMSP and DMS under various pCO₂ conditions precludes a full assessment of DMS dynamics during the second phase of the present experiment.

4.5 DMS and ocean acidification

The implications of our findings for the future global ocean and climate are still unclear. Firstly, the changes in pCO₂ studied here have been triggered abruptly from present values on day 0 to double and triple concentrations on day 2, without allowing the systems under study to fully acclimate or adapt. Future ocean acidification will proceed at a much slower rate and this temporal scale difference could potentially al-

leviate the consequences of ocean acidification. Secondly, blooms of the magnitude we observed in this mesocosm study in terms of chlorophyll-*a* are rare in the open ocean. DMSP_t concentrations of 300–500 nmol L⁻¹ and DMS concentrations of 40 nmol L⁻¹ are untypical in the open ocean, where the 95 percentile of all measured DMS concentration is below 5 nmol L⁻¹ (Kettle and Andreae, 2000). As the regions where DMS fluxes are most important are remote regions such as the Southern Ocean where chlorophyll is significantly lower, we cannot extrapolate our results to global scales at this point. Thirdly, mesocosms do not seem to respond in a consistent way to manipulations such as in CO₂ enrichment studies. We cannot confirm the finding of previous studies (Avgoustidi et al., 2008²) that DMS accumulation was significantly reduced under simulated seawater acidification. However, DMS concentrations varied between treatments in both studies. In particular, DMS proved to be one of the few measured parameters that had a clear response to the CO₂ perturbation in this mesocosm study.

5 Summary and conclusion

We studied DMS, DMSP_p and DMSP_d dynamics under 3 different pCO₂ conditions during a mesocosm experiment in Norway. There were no statistically significant differences in the temporal development of DMSP_t, DMSP_p and DMSP_d concentrations and in DLA, which hints at a certain resilience of the studied system to changes in pCO₂. However, we found differences in the temporal development of DMS concentrations. While DMS stayed elevated in the treatments with elevated pCO₂, we observed a steep decline in DMS concentration in the treatment with low pCO₂. As the ratio of DMS to DMSP varied strongly between treatments, but DMSP per chl-*a* did not, we hypothesize that the observed differences result from differences in DMS production or degradation mechanisms rather than from large shifts in community structure. Observed differences in bacterial community structure and viral abundances may play a role, but other mechanisms such as differences in exudation rates etc. cannot be excluded.

It is too early to draw conclusions regarding the importance of ocean acidification on the global sulphur cycle. This is only the third report that we are aware of that addresses changes in DMS dynamics under future CO₂ scenarios. As some marine trace gases appear to be sensitive to CO₂ enrichments (Wingenter et al., 2007) there is a need for further studies on the impact of ocean acidification on the production of climate-relevant gases such as DMS. Future studies should be conducted under open ocean conditions using for example free-floating mesocosms, should focus on rate measurements as well as concentrations, and must include estimations of bacterial DMSP consumption rates in combination with detailed analyses of the cellular DMSP quota of algal taxa present in the investigated habitat. Only then will

it be possible to separate physiological processes from the effect of trophic interactions on DMS dynamics and to assess possible implications for DMS fluxes under future climate change.

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