Final author comments on "Polar dimethylsulfide (DMS) production insensitive to ocean acidification during shipboard microcosm experiments: a meta-analysis of 18 experiments from temperate to polar waters." by F.E. Hopkins et al., manuscript number bg-2018-55.

Firstly, I would like to apologise for the delay in addressing the reviews to this manuscript. I have been on maternity leave for 12 months.

Secondly, I would particularly like to thank Reviewer #1 for providing a second review of our manuscript. We appreciate their time, effort and insight that have enabled us to improve the paper in many ways. Many thanks also to Reviewer #2 for their helpful, supportive and positive review of our manuscript.

The reviewers comments are shown *in italics*, with our responses shown **in bold**. For reference, our responses to the first round of reviews are shown ***in grey italics***. Line numbers in our response refer to the revised version. The marked-up version of the manuscript comes after the response (starting at pg 20), and line numbers refer to this version.

1. Response to Anonymous Referee #1

1.1: After reading manuscript and responses of the authors, I am disappointed with the revision. While the authors implemented many comments, most of my substantial criticism has either been rejected (e.g. 2.6, 2.16, 2.23, 2.24, 2.25, 2.30) or not accounted for appropriately, i.e. by just deleting part of the manuscript instead of discussing potential inconsistencies (e.g. 2.38). It seems to me that any criticism that questions the general interpretation of the dataset has not been included. Under these circumstances, I am not willing to provide a point by point reply to the response, but just highlight the most important points.

We regret that we disappointed the reviewer. After careful consideration of their comments, we have made some major changes to the manuscript which we will go through point-by-point below. We hope that we have more than adequately addressed their concerns.

1.2: One major problem with this dataset is that the experimental carbonate chemistry was not well controlled. For example, at the 1000µatm pCO2 level, T2 pCO2 levels vary between approx. 400 and 1000µatm (Table S2). Therefore, the data should be represented using the real carbonate chemistry instead of the assigned values. I understand that this implies replotting and reanalysing most of the data, but currently the levels that are tested against each other are actually not separated when it comes to carbonate chemistry.

We agree with the reviewer and have now rectified this issue, please see 1.9 below in which we fully address this concern.

In the next section (1.3 – 1.8), we have revisited the comments from the first review that the reviewer felt were not adequately addressed in our first response (2.6, 2.16, 2.23, 2.24, 2.25, 2.30, 2.38) and made changes to our manuscript.

1.3: 2.16 L229-231: I am wondering if it wouldn't make more sense to normalize DMSP concentrations to biomass? This is especially the case if you want to test for "stress-induced algal processes" (L135-136) rather than biomass-dependent effects.

OUR PREVIOUS RESPONSE: We feel this is not necessary, as we present specific rates of DMSP synthesis. In vivo DMSP synthesis is closely associated with photosynthesis within the cell, so

determination of the rate of this process gives an indication of the effects of stress-induced algal processes on DMSP production. This is a much more useful parameter than biomass-normalised DMSP standing stocks, as the DMSP pool is the net results of various and varying processes (see Stefels et al. 2009), with variable contributions to DMSP production by different groups of phytoplankton.

We believe that our previous response was a fair answer to a valid question, rather than a rejection of their criticism. We reiterate our point below. However, we have altered the text to create further clarity:

The reviewer refers to this line: "Gross DMSP production rates during the incubations (nmol L^{-1} h^{-1}) were calculated from μDMSP and the initial particulate DMSP (DMSPp) concentration of the incubations (shown in Figure 4)".

The line describes the accepted method for calculating gross DMSP production rates as previously published by, for example, Stefels et al. 2009, Archer et al. 2013 and Hopkins & Archer 2014, so we feel our previous response is fair. As we previously described, the ability to measure specific rates of DMSP synthesis using mass spectrometric techniques has greatly improved our ability to assess how the DMSP pool is regulated in response to physiological stress. This is a much more useful measurement than net changes in standing stocks of DMSP, whether normalised to biomass or not. For clarity, I have reworded the line and added references:

"In vivo DMSP gross production rates during the incubations (nmol L^{-1} h^{-1}) were calculated from μDMSP and the initial particulate DMSP (DMSPp) concentration of the incubations (Hopkins & Archer 2014, Stefels et al. 2009). These rates provide important information on how the physiological status of DMSP-producing cells may be affected by OA within the bioassays".

1.4: 2.23 L340-342: This in a strong indication for the importance of other drivers (nutrients, species composition, ...). You need to show these and check whether there are significant effects here.

OUR PREVIOUS RESPONSE: Whilst we agree it would be interesting to attempt to unravel what is driving any differences in DMSP production in polar waters, we feel that it is outside of the scope of this paper.

We apologise for our previous rather vague response. However, this was not intended as a rejection of criticism, rather we were not certain exactly what the reviewer was asking for. The reviewer refers to this line:

"Nevertheless, no consistent and significant effects of high CO_2 were observed for rates of *de novo* DMSP synthesis or DMSP production in polar waters".

Therefore, we assume they are referring to the lack of CO_2 response generally seen in μDMSP and DMSP production rates across all polar experiments. The data show little difference between any of the treatments, which suggests "other drivers (nutrients, species composition…)" do not play any importance either.

Alternatively, they may be referring to the apparent, but opposing, CO_2 response seen in these parameters at *Drake Passage* (time point 2, 96 h) and *Weddell Sea* (time point 2, 144 h). Here, it may be possible to unravel some more details of the observed response.

For the benefit of the reviewer, we have plotted the auxiliary measurements for *Drake Passage* (Figure R1) and *Weddell Sea* (Figure R2) below. The response we saw in DMSP production rates at *Drake Passage* (significantly higher DMSP production rates, Figure 6 F) corresponds to significantly higher nitrate concentrations for the *high+* treatment, relative to *ambient* (ANOVA $t = 7.913$, $p = 0.001$) (Figure R1 E). The remaining auxiliary measurements show no clear trends.

For *Weddell Sea*, the auxiliary data is quite noisy over the triplicate bottles, no trends are apparent (Figure R2), and no auxiliary measurements correspond to the significantly lowered DMSP production rates at time point 2, 144 h.

For both experiments, it is possible that there were changes in the composition of large phytoplankton (diatoms, dinoflagellates) that may have resulted in differences in DMSP production rates. However, this size fraction of phytoplankton was not quantified during these experiments.

We have edited the section of the manuscript (from L397) in question and added additional discussion (underlined) as follows:

"No consistent effects of high CO_2 were observed for either DMSP synthesis or production in polar waters, similar to findings for DMSP standing stocks. However, some notable but contrasting differences between CO_2 treatments were observed. There was a 36% and 37% increase in µDMSP and DMSP production respectively at 750 µatm for the *Drake Passage* after 96 h (Figure 5 E, F), and a 38% and 44% decrease in both at 750 µatm after 144 h for *Weddell Sea* (Figure 5 G, H). <u>For *Drake Passage*, the difference between treatments at 96 h coincided with significantly higher nitrate concentrations in the High CO_2 treatment (Nitrate/nitrite at 96 h: Ambient = 18.9 ± 0.2 µmol L^{-1}, +CO_2 = 20.2 ± 0.1 µmol L^{-1}, ANOVA $t = 7.913$, $p = 0.001$). However, it is uncertain whether the difference in nutrient availability between treatments (approximately 5 %) within this bioassay experiment would be significant enough to strongly influence the rate of DMSP production.</u>**

<u>**The differences in DMSP production rates did not correspond to any other measured parameter. It is possible that changes in phytoplankton community composition may have led to differences in DMSP production rates for *Drake Passage* and *Weddell Sea*, but no quantification of large cells (diatoms, dinoflagellates) was undertaken for these experiments**</u>**".**

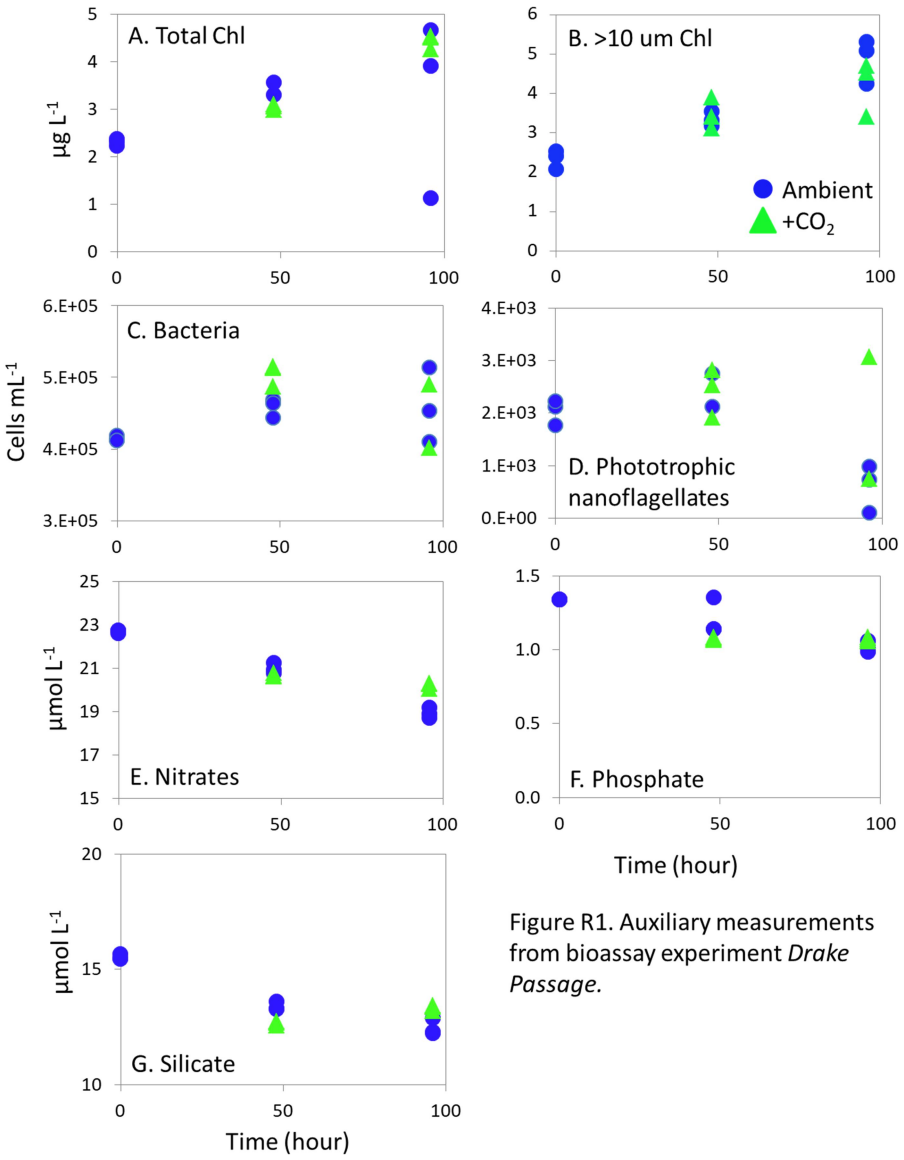

Figure R1. Auxiliary measurements from bioassay experiment *Drake Passage.*

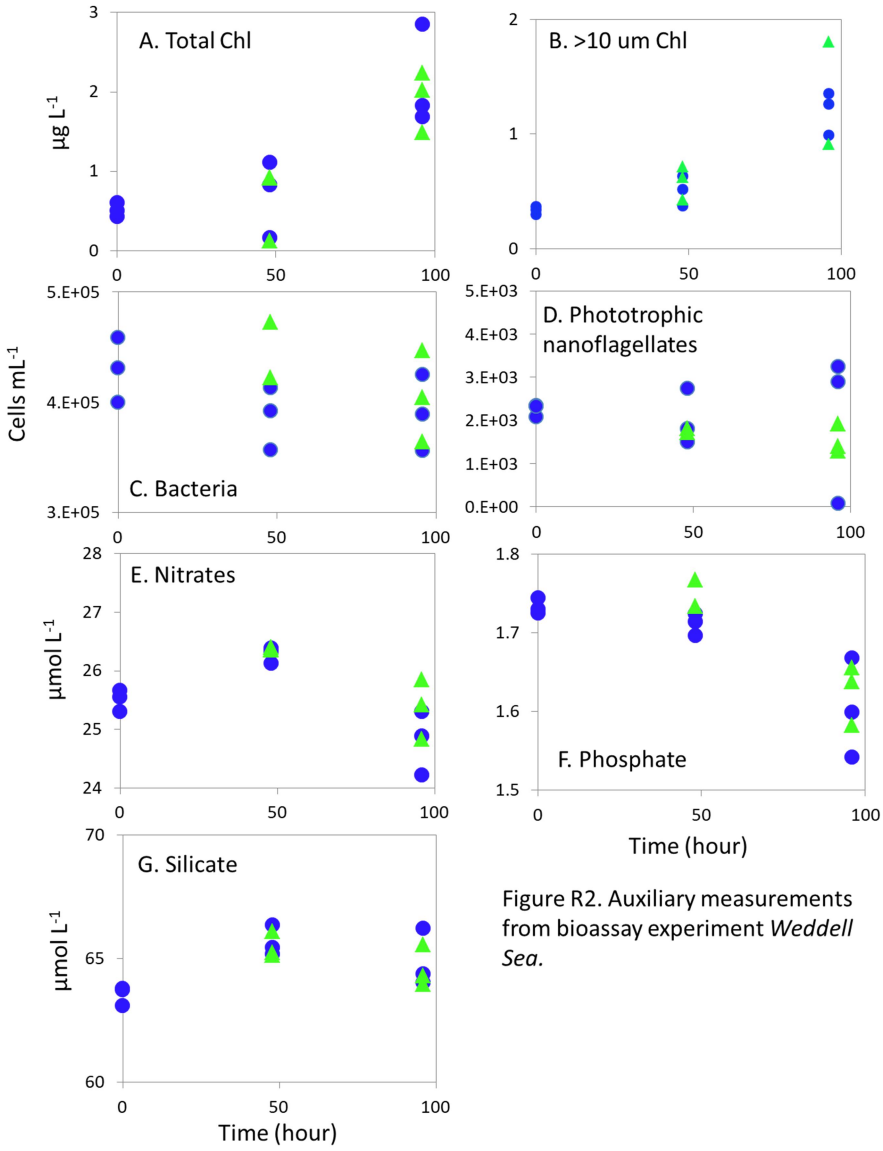

Figure R2. Auxiliary measurements from bioassay experiment *Weddell Sea.*

1.5: 2.24 L360ff: I really like this way of presenting the data. You should, however, also show the same plot with pCO2 instead of TA/DIC for comparison because I do not agree with you that this ratio gives a full overview of the in situ carbonate chemistry.

OUR PREVIOUS RESPONSE: We feel it would not be a useful exercise to replot all the data against pCO_2, based on single discrete measurements. The relevance of this value is unclear, as it can be so variable in space and time. We use DIC/Alk as it is the simplest way of representing the buffer capacity of the sampled waters. We could also have plotted against the Revelle factor of the sampled waters, and the relationship would have looked almost identical, as the Revelle factor is indeed a function of DIC/Alk, and quantified the ocean's sensitivity to an increase in CO_2. Therefore we believe that DIC/Alk is the simplest and more appropriate way of visualising our data in terms of its geographical location.

We agree this is a good way of visualising the data (Figures 7, 8, 9, revised numbering). However, we did not intend for this parameter (C_T/A_T) to give a full overview of the carbonate chemistry. Furthermore, the same plot with pCO_2 would not demonstrate the same phenomenon as this parameter varies so much in space and time in the surface ocean and does not correspond to

latitude. C_T/A_T was used because, similarly to the Revelle Factor (*R*), its value increases towards high latitudes (see Figure 3 in Sabine et al. (2004) Science, 305, 367 – 371). Therefore given the reviewer's concerns with regard to the use of C_T/A_T, we have decided to re-plot the figures using *R* for the sampled waters instead. *R* provides a measure of the total amount of CO_2 that can be dissolved in the mixed surface layer. Regions with high *R* are less-well buffered with respect to changing CO_2 input – such that an increase in atmospheric CO_2 would result in a greater increase in sea water pCO_2, compared to waters with low *R*. Cold polar waters have high Revelle Factors, primarily a result of the increased solubility of CO_2 in cold waters. Thus, these plots are a neat way of showing how the response of DMS and other parameters to OA varies between temperate and polar waters, and how the CO_2 sensitivity of different communities may relate to the in situ carbonate chemistry. This lowered buffering capacity in polar waters also results in a greater variability in carbonate chemistry experienced by surface ocean communities over a seasonal cycle. This is further impacted by other polar phenomena such as the seasonal build-up and retreat of sea ice. We discuss further details of this in our response to 1.10 below.

We have altered relevant text appropriately (From L433 onwards):

"The relative treatment effects ($[x]_{highCO2}/[x]_{ambientCO2}$) for DMS and DMSP (Figure 7), DMSP synthesis and production (Figure 8), and Chl *a* and phototrophic nanoflagellate abundance (Figure 9) are plotted against the Revelle Factor of the sampled waters. The Revelle Factor (*R*), calculated here with CO2Sys using measurements of carbonate chemistry parameters ($R = (\Delta pCO_2/\Delta TCO_2)/(pCO_2/TCO_2)$, Lewis and Wallace, 1998), describes how the partial pressure of CO_2 in seawater (PCO_2) changes for a given change in DIC (Revelle and Suess 1957; Sabine et al. 2004). Its magnitude varies latitudinally, with lower values (9 – 12) from the tropics to temperate waters, and the highest values in cold high latitude waters (13 – 15). Thus polar waters can be considered poorly buffered with respect to changes in DIC. An equivalent change in PCO_2 in temperate waters would result in smaller changes in pH or saturation states than would be observed in polar waters (Egleston et al. 2010). Furthermore, the seasonal sea ice cycle strongly influences carbonate chemistry, such that sea ice regions exhibit wide fluctuations in carbonate chemistry (Revelle and Suess, 1957; Sabine et al., 2004). Sampling stations with a *R* above ~12 represent the seven polar stations (right of red dashed line Fig. 6 and 7). The surface waters of the polar oceans have naturally higher levels of DIC and a reduced buffering capacity, driven by higher CO_2 solubility in colder waters (Sabine et al. 2004). Thus, the relationship between experimental response and *R* is a simple way of demonstrating the differences in response to OA between temperate and polar waters and provides some insight into how the CO_2 sensitivity of different surface ocean communities may relate to the *in situ* carbonate chemistry".

1.6: 2.25 L372 and throughout the entire manuscript: Report the time points in days or hours instead of T1, T2 etc. because this is not consistently the same time point as well as for better readability and consistency throughout the text.

OUR PREVIOUS RESPONSE: Reporting the time points in hours throughout the manuscript would make the results text clunky and confusing to read, as there is some variability. Therefore, we will keep the T_1/T_2 notation, but refer to the times broadly and refer the reader to Table 1 which outlines all the specific sampling times.
Added at L201: "(T_1, T_2, see Table 1 for specific times for each experiment)"..,
L410 now reads: "…was minimal at all sampling points…"
L413 now reads: "…particularly at T_1 (48 – 96 h)…"
Figure 7 legend text has been altered: "T_1 = 48 h, T2 = 96 h, except for Weddell Sea and South Georgia (72 h, 144 h)".
Figure 8 legend text has been altered: "T_1 = 48 h, T_2 = 96 h, except for Weddell Sea and South Georgia (72 h, 144 h) and South Sandwich (96 h, 168 h)".

We now agree and have now removed all use of T0, T1 and T2 notation and simply refer to exact times throughout the manuscript.

1.7: 2.30 L436-439: I do not agree that your data really shows this: Figure 9 indicates the Arctic Ocean carbonate chemistry to be actually more similar to the Atlantic than to the Southern Ocean.

OUR PREVIOUS RESPONSE: We disagree. The data show that the variability in carbonate chemistry in the polar oceans is much larger than in temperate waters – as described in the text the reviewer refers to.

Please see our detailed response to 1.10 below which we hope now thoroughly addresses these concerns.

1.8: 2.38 L543-550: I agree that it is an interesting finding that coastal DMS production seems to be more sensitive to OA than that from the open ocean. This finding does, however, really hint against the proposed mechanisms of insensitivity, because coastal systems are a lot more variable in carbonate chemistry compared to the open ocean (e.g. Thoisen et al. 2015). Thus, the interpretation of and conclusions from the dataset have to be reassessed.

OUR PREVIOUS RESPONSE: Given the reviewers comments on this issue, we believe that the comparison between 'coastal'and 'open ocean' waters complicates this part of our discussion, so we have removed mention of this comparison. We instead discuss the possibility that there is likely to be regional variability in the response of DMS to OA. The key point is that the DMS response to OA in polar regions is complex and likely to be influenced by a number of temporal and spatial factors. The main users of our data are climate modellers, and we wish to emphasise that when trying to model the future flux of DMS, it is important to take this variability into account. The section now reads (L623):
"Our findings contrast with two previous studies performed in Arctic waters (Archer et al. 2013, Hussherr et al. 2017) which showed significant decreases in DMS in response to OA. These discrepancies may be driven by differences in the sensitivity of microbial communities to changing carbonate chemistry between different areas, or by variability in the response to OA depending on the time of year, nutrient availability, and ambient levels of growth and productivity. This serves to highlight the complex spatial and temporal variability in DMS response to OA which warrants further investigation to improve model predictions".

Please see our detailed response to 1.11 below which we hope now thoroughly addresses these concerns.

1.9: Most importantly, I still not agree with using the nominal carbonate chemistry levels instead of the actual data. As already described in the first round of reviews, control and OA treatments diverge strongly in some of the experiments. As can be seen in table SI1, measured pCO2 levels varied strongly from the nominal values at all time points, and this discrepancy seems to be larger in the polar compared to the temperate experiments. Already at t0, actual values at 100 and 1400 µatm in both polar areas were in most cases several hundred µatm lower than assigned, i.e. about 20-40% lower. This trend is even more pronounced at T2, where measured pCO2 values are as low as 362 µatm in the 750 µatm, 421 µatm at the 1000 µatm, and 767 µatm in the 1400 µatm treatment. These pCO2 levels in the OA treatments significantly overlapped with control conditions. Thus, the presented carbonate chemistry data clearly does not meet the quality requirements of our scientific community, and therefore the nominal levels cannot be used for data analysis.

We now accept that using the nominal pCO_2 values had failed to provide a clear understanding of the data to the reader. All figures are now shown with the actual measured pCO_2 values, rather than 'nominal' values. Instead of referring to 'nominal' values, we have divided the treatments into Mid CO_2, High CO_2, High+ CO_2 and High++ CO_2, with the actual data shown in Figures 3 and 4 for each experimental time point. We hope this addresses the reviewer's concerns.

To explain this to the reader, the text in section 2.2 now reads:

"The carbonate chemistry within the experimental bottles was manipulated by addition of equimolar HCl and $NaHCO_3^-$ (1 mol L^{-1}) to achieve a range of CO_2 treatments: Mid CO_2 (Target: 550 µatm), High CO_2 (Target: 750 µatm), High+ CO_2 (Target: 1000 µatm) and High++ CO_2 (Target: 2000 µatm) (Gattuso et al., 2010). Three treatment levels were used during the sub-Arctic/Arctic microcosms (Mid, High, High+). For Southern Ocean experiments, two experiments (*Drake Passage* and *Weddell Sea*) underwent combined CO_2 and Fe additions (ambient, Fe (2 nM), High CO_2, Fe (2 nM) & High CO_2 (only high CO_2 treatments will be examined here; no response to Fe was detected in DMS or DMSP concentrations). Three CO_2 treatments (High, High+, High++) were tested in the last two experiments (*South Georgia* and *South Sandwich*)".

For all 18 experiments the actual attained pCO_2 values at t0 were on average 89% ± 12% (±1 SD) of target values. By T1 and T2 biological activity within the bottles resulted in a drawdown of DIC and a concomitant decrease in pCO_2 of around 25% by T1 and >50% by T2 (see Richier et al. 2018) – an unavoidable phenomenon within this experimental design. We hope that by clearly presenting all of the pCO_2 data for all experiments and all time points in Figures 3 and 4, this will now be clear to the reader.

1.10: Similarly, I still disagree with the statement that "a narrow range of values for all carbonate parameters was observed in the NW European shelf waters relative to the less well-buffered Arctic and Southern Ocean waters"(new L 490-492). As can be clearly seen in Figure 9, CT/AT ratios (which the authors use as a proxy for buffer capacity) were actually higher in the SO compared to the NW European shelf waters which had the lowest median. The "much larger" variability in polar waters (response letter 2.30) is in fact only observed for the Arctic. These facts clearly contradict the authors conclusion that "that populations from higher latitude, less well-buffered waters, already possess a certain degree of acclimative tolerance to variations in carbonate chemistry environment" (new L 646-648).

Having now revisited Figure 9, we agree with the reviewer, and concede that these data didn't really illustrate our point. The figure summarised the underway data along each cruise track so really only provided a snapshot of the variability within each region, whilst masking any detail. By grouping all the data together in this way, it also masked the regional differences within each cruise, making it impossible to appreciate the carbonate chemistry characteristics within each region. Any detail or evidence to illustrate our point was lost in the noise. Therefore, we have removed this figure from the paper.

Instead, we have supported our hypothesis with information from the paper by Tynan et al. (2016) which was intended to provide a carbonate chemistry context for all studies derived related to the polar cruises, and Rerolle et al. (2014) which provides underway pH measurements from the NW European shelf cruise. We discuss our findings in the context of the processes that influence carbonate chemistry variability in polar waters (strong biological drawdown, the influence of sea ice, organic matter respiration, water mass effects), and compare this to the variability observed in temperate waters.

The section in the manuscript now reads (from L581):

4.3 Adaptation to a variable carbonate chemistry environment

Given that DMS production by polar phytoplankton communities appeared to be insensitive to experimental OA compared to significant sensitivity in temperate communities, we hypothesise that polar communities are adapted to greater natural variability in carbonate chemistry over spatial and seasonal scales. The polar waters sampled during our study were characterised by pronounced gradients in carbonate chemistry over small spatial scales, such that surface ocean communities are more likely to have experienced fluctuations between high pH and low pH over short time scales (Tynan et al. 2016). For example, in underway samples taken along each cruise track, pH varied by 0.45 units (8.00 – 8.45) in the Arctic, and 0.40 units (8.30 - 7.90) in the Southern Ocean (Tynan et al. 2016). By comparison, pH varied by 0.2 units (8.22 - 8.02) in underway samples from the NW European shelf sea cruise (Rerolle et al. 2014).

The observed horizontal gradients in polar waters were driven by different physical and biogeochemical processes in each ocean. In the Arctic Ocean, this variability in carbonate chemistry was partly driven by physical processes that controlled water mass composition, temperate and salinity, particularly in areas such as the Fram Strait and Greenland Sea. Along the ice-edge and into the Barents Sea, biological processes exerted a strong control, as abundant iron resulted in high chlorophyll concentrations, low DIC and elevated pH. By contrast, variations in temperature and salinity had only a small influence on carbonate chemistry in the Southern Ocean in areas with iron limitation, and larger changes were driven by a combination of calcification, advection and upwelling. Where iron was replete, e.g. near South Georgia, biological DIC drawdown had a large impact on carbonate chemistry (Tynan et al. 2016). A further set of processes was in play in sea ice influenced regions. At the Arctic ice edge, abundant iron drove strong bloom development along the ice edge, whilst sea ice retreat in the Southern Ocean was not always accompanied by iron release (Tynan et al. 2016).

For comparison with Arctic stations, Hagens and Middelburg (2016) report a seasonal pH variability of up to 0.25 units from a single site in the open ocean surface waters in the Iceland Sea, whilst Kapsenberg et al. (2015) report an annual variability of 0.3 – 0.4 units in the McMurdo Sound, Antarctica. This implies that both open ocean and sea ice-influenced polar waters experience large variations in carbonate chemistry over seasonal cycles. By contrast, monthly averaged surface pCO_2 data collected from station L4 in the Western English Channel over the period 2007 – 2011 provides an example of typical carbonate chemistry dynamics in NW European shelf sea waters. Over this period, pH had an annual range of 0.15 units (8.05 – 8.20), accompanied by a range in pCO_2 of 302 – 412 µatm (Kitidis et al. 2012).

The sea ice environment in particular is characterised by strong spatial and seasonal variability in carbonate chemistry. Sea ice is inhabited by a specialised microbial community with a complex set of metabolic and physiological adaptations allowing these organisms to withstand wide fluctuations in pH up to as high as 9.9 in brine channels to as low as 7.5 in the under-ice water (Thomas and Dieckmann 2002; Rysgaard et al. 2012; Thoisen et al. 2015). The open waters associated with the ice edge also experience strong gradients in pH and other carbonate chemistry parameters. This can be attributed to two processes: 1. The strong seasonal drawdown of DIC due to rapid biological uptake by phytoplankton blooms at the productive ice edge which drives up pH. On the Arctic cruise, increases of up to 0.33 pH units were attributed to such processes in this region (Tynan et al. 2016). The effect was less dramatic in the Fe-limited and less productive Weddell Sea with gradients in pH ranging from 8.20 – 8.10 (Tynan et al. 2016). 2. The drawdown of DIC is countered by the release and accumulation of respired DIC under sea ice due to the degradation of organic matter. However, this accumulation occurs in subsurface/bottom waters, which are isolated from the productive surface mixed layer by strong physical stratification and

hence, of less relevance to the current study.
The influence of sea ice on carbonate chemistry combined with the strong biological drawdown of DIC in polar waters may have influenced the ability of some of the communities we sampled during our study to withstand the short term changes to carbonate chemistry they experienced within the bioassays. Two of our sampling stations were 'sea-ice influenced': *Greenland Ice Edge* and *Weddell Sea*. Both were in a state of sea ice retreat as our sampling occurred in the summer months. Sampling for the *Greenland Ice Edge* station was performed in open, deep water, near to an area of thick sea ice, with low fluorescence but reasonable numbers of diatoms (Leakey 2012). Similarly, the *Weddell Sea* station was located near the edge of thick pack ice but in an area of open water that allowed sampling to occur without hindrance by brash ice (Tarling 2013). At both stations we saw little or no response in DMS or DMSP to experimental acidification, which may imply that the *in situ* communities were more or less adapted to fluctuations in pH. Our experimental OA resulted in pH decreases of between 0.4 and 0.7 units. However, it is unclear whether the communities we sampled were able to withstand the artificial pH perturbation because they were adapted to living in sea ice, or whether they had adapted to cope with other fluctuations in carbonate chemistry that occur in polar waters.
In summary, this demonstrates the high variability in carbonate chemistry, including pH, which polar surface ocean communities may experience relative to their temperate counterparts. This may have resulted in adapted communities resilient to experimentally-induced OA. Of course, it is important to recognise that this data represent only a snapshot (4 – 6 weeks) of a year, and thus do not contain information on the range in variability over daily and seasonal cycles, timescales which might be considered most important in terms of the carbonate system variability experienced by the cells and how this drives CO_2 sensitivity (Flynn et al. 2012; Richier et al. 2018). Nevertheless, this inherent carbonate chemistry variability experienced by organisms living in polar waters may equip them with the resilience to cope with both experimental and future OA".

1.11: Lastly, I still find the question of coastal/open ocean vs. polar/temperate differences not resolved. The authors wrote in the first version of the manuscript: "We provide further evidence that, in contrast to temperate communities (Hopkins and Archer 2014), polar communities we sampled were relatively insensitive to variations in carbonate chemistry (Davidson et al. 2016; Richier et al. under review), manifested here as a minimal effect on net DMS production. Our findings contrast with two previous studies performed in coastal Arctic waters (Archer et al. 2013, Hussherr et al. 2017) which showed significant decreases in DMS in response to OA." After my comment that this contradicts their hypothesis of generally higher sensitivity of polar compared to temperate communities, the authors just omitted the description of the two cited studies as being "coastal" (response letter 2.38), instead of adapting their discussion to the fact these two coastal studies with higher natural carbonate chemistry variability contradict the hypothesis brought forward in this study. I the response letter, the authors furthermore argue that "The carbonate chemistry/pH variability may be as large in the open waters of the polar oceans as in coastal sea ice waters" (1.11) and they cite two studies to support this statement. It is a well-known and widely accepted fact, however, that coastal sites experience larger environmental variability that oceanic ones. For example, the study by Thoisen et al. (2015) that I referred to already in the first review, showed report a seasonal pH gradient as large as 0.8 units (7.5 -8.3) for a costal Arctic site. In conclusion, I am still convinced that the CO2 sensitivity observed in two previous coastal studies contradicts the hypothesis of this study. The attempt to just ignore instead of discuss this apparent contradiction is, in my view, not an acceptable scientific practise.

We absolutely agree that the results of the two previous studies (Archer et al. 2013, Hussherr et al. 2017) contradict the results of this study as we have made clear in the paper:
"Our findings contrast with two previous studies performed in Arctic waters (Archer et al. 2013, Hussherr et al. 2017) which showed significant decreases in DMS in response to OA. These

discrepancies may be driven by differences in the sensitivity of microbial communities to changing carbonate between different areas, or by variability in the response to OA depending on the time of year, nutrient availability, and ambient levels of growth and productivity. This serves to highlight the complex spatial and temporal variability in DMS response to OA which warrants further investigation to improve model predictions".

However, in the revised version, and given the reviewer's previous comments, we removed reference to 'coastal' as the Hussherr et al. (2017) study is not classified as coastal – this was our oversight and further reason that this argument did not stand. So we only discuss the discrepancies with the Hussherr et al. study in terms of differences in time of year and levels of productivity. This serves to highlight the complexities in the response of surface ocean communities to OA, and the difficulties encountered when attempting to compare results from experiments with vastly different designs.

However, we do accept that the discussion of the contradiction in findings could be handled more clearly in the paper. The reason we omitted some discussion following the first review was because we agreed with the reviewer that the coastal vs open ocean polar argument wasn't well handled and was creating confusion.

It seems that the issues arise in two sections of the paper: 4.3 Adaptation to a variable carbonate chemistry environment, and 4.4 Comparison to an Arctic mesocosm experiment.

We have re-worked the discussion in 4.3 (from L581). We no longer categorise results into coastal vs open ocean, as this does not do an adequate job of explaining our data. We have taken more consideration of the general polar environment, in particular the impact of sea ice and strong biological drawdown in carbonate chemistry. Two of our sampling stations were in sea ice zone, sampled in the period following recent ice melt. These two stations (Greenland ice edge and Weddell Sea) both showed the least response in DMS to OA out of all our experiments. Thus, we now argue that ice edge communities can withstand experimental OA, possibly due to the strong influence that sea ice formation and melt has on in situ carbonate chemistry and hence, acclimative tolerance of the associated communities. Our polar experiments in general agree with many previous studies that polar microbial/phytoplankton communities are able to resist experimentally-induced OA. We present the changes to section 4.3 in response 1.10 above.

We recognise that the results of Archer et al. (2013) and Hussherr et al. (2017) contradict our findings, so we have now re-visited section 4.4 with the reviewer's comments in mind and to make our point clearer. The reasons are not fully resolved but could be due to a number of factors:

Drawing comparisons between mesocosms and microcosms is challenging given the great differences in experimental design. Both address a different set of hypotheses, as already outlined in the discussion. Both have strengths and weaknesses. The EPOCA mesocosm experiment was located in a sheltered fjord on the west coast of Svalbard, heavily influenced by glacial meltwater, and during a year that had not experienced any winter sea ice. This environment is so different to the sampling stations for the microcosm experiments that it is perhaps unsurprising that the general response to OA was so different. In particular, the fjord was characterised by low in situ CO_2 concentrations of 185 µatm, whereas polar in situ CO_2 concentration in this study ranged from 273 µatm at *South Sandwich* to 510 µatm at *Weddell Sea*. The second half of the mesocosm experiment was nutrient-enriched, and it was following this perturbation that the greatest response in DMS and DMSP to OA was observed. The OA response became most evident when the nutrient-induced bloom led to the formation of a 'winners vs losers' dynamic – something not attained within the microcosms, which received no nutrient addition. Before the nutrient addition to the mesocosms, the response to OA in DMS and DMSP was detectable but minimal – and here I argue that perhaps this demonstrates some resilience within the fjord communities that could

result from adaptation to a coastally-influenced variability in carbonate chemistry. This resilience was nullified by the nutrient addition, as perhaps this allowed a shift in community composition to species with less tolerance to high CO_2.

The section beginning "The short duration of the microcosm experiments (4 – 7 d) allows…" may be creating confusion, although we are satisfied that our discussion is valid so have not made any changes here. In this section we compare the short term microcosm experiments (4 – 7 d) to the first 5 – 10 d of mesocosm experiments. This seems valid because one of the biggest discrepancies between microcosms and mesocosms is the duration of the experiments. We argue that for most mesocosms, little difference between treatments in terms of the DMS/P response is seen during this initial phase – so this could imply that the communities do possess some short-term tolerance to the induced OA. We then postulate that this may be because mesocosms are generally performed in coastal waters, wherein the communities may have naturally experienced a variable carbonate chemistry environment. It's only once a bloom-dynamic develops do we see strong responses in DMS/P to OA in mesocosm experiments, and this is where the data becomes less comparable to the microcosm experiments. Thus we go on to discuss the affect that nutrient addition has on the growth dynamics within the mesocosm, and how this could drive the observed response to CO2 addition.

1.12: Please also note that in my view, papers should be understandable and convincing by themselves. So while I did read Richier et al. (2018), I am providing my views on the current manuscript only.

We also agree that a paper should be able to stand alone, but some cross-referencing to a paper describing the same experiments from the same research cruises should be acceptable to the reader. This is useful to avoid over-complicating an interesting story about the response of DMS to OA and distracting from the main thrust of this manuscript.

2. Response to Anonymous Referee #2

2.1 The paper describes a series of high pCO2 incubation experiments performed on three cruises in temperate, Arctic and Southern Ocean waters during the summer period in each location. Studied in the incubations are the changes in DMS and DMSP over the short term (hours to days), and the changes in production rates of these compounds. The manuscript is clear and well written, and highlights the regional differences in the response to elevated pCO2, and attributes the differences to the variability in the carbonate system.

We appreciated these positive and supportive comments on our manuscript.

2.2 Given that two of the environments studied were in polar regions, discussion of the effect of sea ice on the carbonate chemistry and the existing response of the phytoplankton community to extreme environments was lacking within the manuscript, particularly a mention of the pH changes experienced by cells while living within the sea ice.

We agree and have now added the following section to the manuscript, which significantly improves our discussion and interpretation of the dataset (from L581):

4.3 Adaptation to a variable carbonate chemistry environment

Given that DMS production by polar phytoplankton communities appeared to be insensitive to experimental OA compared to significant sensitivity in temperate communities, we hypothesise that polar communities are adapted to greater natural variability in carbonate chemistry over spatial and seasonal scales. The polar waters sampled during our study were characterised by pronounced gradients in carbonate chemistry over small spatial scales, such that surface ocean communities are more likely to have experienced fluctuations between high pH and low pH over short time scales (Tynan et al. 2016). For example, in underway samples taken along each cruise track, pH varied by 0.45 units (8.00 – 8.45) in the Arctic, and 0.40 units (8.30 - 7.90) in the Southern Ocean (Tynan et al. 2016). By comparison, pH varied by 0.2 units (8.22 - 8.02) in underway samples from the NW European shelf sea cruise (Rerolle et al. 2014).

The observed horizontal gradients in polar waters were driven by different physical and biogeochemical processes in each ocean. In the Arctic Ocean, this variability in carbonate chemistry was partly driven by physical processes that controlled water mass composition, temperate and salinity, particularly in areas such as the Fram Strait and Greenland Sea. Along the ice-edge and into the Barents Sea, biological processes exerted a strong control, as abundant iron resulted in high chlorophyll concentrations, low DIC and elevated pH. By contrast, variations in temperature and salinity had only a small influence on carbonate chemistry in the Southern Ocean in areas with iron limitation, and larger changes were driven by a combination of calcification, advection and upwelling. Where iron was replete, e.g. near South Georgia, biological DIC drawdown had a large impact on carbonate chemistry (Tynan et al. 2016). A further set of processes was in play in sea ice influenced regions. At the Arctic ice edge, abundant iron drove strong bloom development along the ice edge, whilst sea ice retreat in the Southern Ocean was not always accompanied by iron release (Tynan et al. 2016).

For comparison with Arctic stations, Hagens and Middelburg (2016) report a seasonal pH variability of up to 0.25 units from a single site in the open ocean surface waters in the Iceland Sea, whilst Kapsenberg et al. (2015) report an annual variability of 0.3 – 0.4 units in the McMurdo Sound, Antarctica. This implies that both open ocean and sea ice-influenced polar waters experience large variations in carbonate chemistry over seasonal cycles. By contrast, monthly averaged surface pCO_2 data collected from station L4 in the Western English Channel over the period 2007 – 2011 provides an example of typical carbonate chemistry dynamics in NW European shelf sea waters. Over this period, pH had an annual range of 0.15 units (8.05 – 8.20), accompanied by a range in pCO_2 of 302 – 412 µatm (Kitidis et al. 2012).

The sea ice environment in particular is characterised by strong spatial and seasonal variability in carbonate chemistry. Sea ice is inhabited by a specialised microbial community with a complex set of metabolic and physiological adaptations allowing these organisms to withstand wide fluctuations in pH up to as high as 9.9 in brine channels to as low as 7.5 in the under-ice water (Thomas and Dieckmann 2002; Rysgaard et al. 2012; Thoisen et al. 2015). The open waters associated with the ice edge also experience strong gradients in pH and other carbonate chemistry parameters. This can be attributed to two processes: 1. The strong seasonal drawdown of DIC due to rapid biological uptake by phytoplankton blooms at the productive ice edge which drives up pH. On the Arctic cruise, increases of up to 0.33 pH units were attributed to such processes in this region (Tynan et al. 2016). The effect was less dramatic in the Fe-limited and less productive Weddell Sea with gradients in pH ranging from 8.20 – 8.10 (Tynan et al. 2016). 2. The drawdown of DIC is countered by the release and accumulation of respired DIC under sea ice due to the degradation of organic matter. However, this accumulation occurs in subsurface/bottom waters, which are isolated from the productive surface mixed layer by strong physical stratification and hence, of less relevance to the current study.

The influence of sea ice on carbonate chemistry combined with the strong biological drawdown of DIC in polar waters may have influenced the ability of some of the communities we sampled during our study to withstand the short term changes to carbonate chemistry they experienced within the bioassays. Two of our sampling stations were 'sea-ice influenced': *Greenland Ice Edge*

and *Weddell Sea*. Both were in a state of sea ice retreat as our sampling occurred in the summer months. Sampling for the *Greenland Ice Edge* station was performed in open, deep water, near to an area of thick sea ice, with low fluorescence but reasonable numbers of diatoms (Leakey 2012). Similarly, the *Weddell Sea* station was located near the edge of thick pack ice but in an area of open water that allowed sampling to occur without hindrance by brash ice (Tarling 2013). At both stations we saw little or no response in DMS or DMSP to experimental acidification, which may imply that the *in situ* communities were more or less adapted to fluctuations in pH. Our experimental OA resulted in pH decreases of between 0.4 and 0.7 units. However, it is unclear whether the communities we sampled were able to withstand the artificial pH perturbation because they were adapted to living in sea ice, or whether they had adapted to cope with other fluctuations in carbonate chemistry that occur in polar waters.
In summary, this demonstrates the high variability in carbonate chemistry, including pH, which polar surface ocean communities may experience relative to their temperate counterparts. This may have resulted in adapted communities resilient to experimentally-induced OA. Of course, it is important to recognise that this data represent only a snapshot (4 – 6 weeks) of a year, and thus do not contain information on the range in variability over daily and seasonal cycles, timescales which might be considered most important in terms of the carbonate system variability experienced by the cells and how this drives CO_2 sensitivity (Flynn et al. 2012; Richier et al. 2018). Nevertheless, this inherent carbonate chemistry variability experienced by organisms living in polar waters may equip them with the resilience to cope with both experimental and future OA".

2.3 A number of technical and specific comments arose on reading of the manuscript, and I recommend publication if these can be addressed.

Many thanks – we hope we have addressed all your comments appropriately.

2.4 Check pCO2 is in italics, as there were instances where it was not.

All checked and changed throughout.

2.5 L28. 'This implies that…' The previous sentence did not suggest this implication, so needs rewording. It seems like a sentence is missing here, which actually describes your findings, and is followed by the implication.

Sentence now reads: "If so, future temperate oceans could be more sensitive to OA resulting in a change in DMS emissions to the atmosphere, whilst perhaps surprisingly DMS emissions from the polar oceans may remain relatively unchanged".

2.6 L71 Please highlight that the 3.4 Tg S is from the whole southern ocean, as calculated by JT16.

Sentence now reads: "Around 3.4 Tg of sulfur is released from the Southern Ocean to the atmosphere between December and February, a flux that represents ~15 % of global annual emissions of DMS (Jarníková and Tortell 2016)".

2.7 L88. Is there a concurrent predicted decrease in the southern polar region pH as well?

Sentence now reads: "The greatest declines in pH are likely in the Arctic Ocean with a predicted fall of 0.45 units by 2100 (Steinacher et al. 2009), with a fall of ~0.3 units predicted for the Southern Ocean (McNeil and Matear 2008; Hauri et al. 2016)".

2.8 L96. During the introduction there is very little mention of DMSP, other than it is the precursor for DMS. Given that DMSP is one of your measured parameters in the experiments this requires further elaboration in the introduction, in particular the changes in DMSP as identified from the existing mesocosm experiments. This is important given that DMSP showed increases during the Arctic mesocosm.

We intentionally focused the introduction on DMS, as our main take home message from the paper is the DMS story which will be of interest to earth-system modellers. However, we agree that integrating some DMSP background may be useful – however, we have kept it brief to avoid lengthening what the reviewer considers an introduction which is 'already very long'.

Text now reads:

"Despite the imminent threat to polar ecosystems and the importance of DMS emissions to atmospheric processes, our knowledge of the response of polar DMS production to OA is limited to a single mesocosm experiment performed in a coastal fjord in Svalbard (Archer et al. 2013; Riebesell et al. 2013) and one shipboard microcosm experiment with seawater collected from Baffin Bay (Hussherr et al. 2017). Both studies reported significant reductions in DMS concentrations with increasing levels of pCO_2 during seasonal phytoplankton blooms. Hussherr et al. (2017) also saw reductions in total DMSP whilst Archer et al. (2013) observed a significant increase in this compound, driven by CO_2-induced increases in growth and abundance of dinoflagellates. However, these two single studies provide limited information on the wider response of the open Arctic or Southern Oceans".

2.9 L100-117. This section on mesocosms is interesting, but slightly irrelevant to the paper as a whole given that your studies are microcosm incubations. As the introduction is already very long, this section could be shortened to one or two sentences describing previous OA responses.

I have reduced the text down slightly but feel that the information that remains is useful for the later discussion comparing the results of microcosms with mesocosms.

Text now reads (from L104):

"Mesocosm experiments have been a critical tool for assessing OA effects on surface ocean communities (Engel et al. 2005; Kim et al. 2006; Engel et al. 2008; Schulz et al. 2008; Hopkins et al. 2010; Kim et al. 2010; Schulz et al. 2013; Webb et al. 2015; Crawfurd et al. 2016; Webb et al. 2016). The response of DMS to OA has been examined several times, predominantly at the same site in Norwegian coastal waters (Vogt et al. 2008; Hopkins et al. 2010; Avgoustidi et al. 2012; Webb et al. 2015), twice in Korean coastal waters (Kim et al. 2010; Park et al. 2014), and a single study in the coastal Arctic waters of Svalbard (Archer et al. 2013). Mesocosm enclosures, ranging in volume from ~11,000 – 50,000 L, allow the response of surface ocean communities to a range of CO_2 treatments to be monitored under near-natural light and temperature conditions over time scales (weeks - months) that allow a 'winners vs loser' dynamic to develop. The response of DMS cycling to elevated CO_2 is generally driven by changes to the microbial community structure (Engel et al. 2008; Hopkins et al. 2010; Archer et al. 2013; Brussaard et al. 2013). The size, construction and associated costs of mesocosms has limited their deployment to coastal/sheltered waters, resulting in minimal geographical coverage, and leaving large gaps in our understanding of the response of open ocean phytoplankton communities to OA."

2.10 L114 High cost is also a significant factor in why mesocosm experiments are so limited!

Agreed! Sentence now reads: "The size, construction and associated costs of the mesocosms has limited their deployment to coastal/sheltered waters, resulting in minimal geographical coverage, and leaving large gaps in our understanding of the response of open ocean phytoplankton communities to OA".

2.11 L120. Please clarify that the temperate experiments shown here are in addition to those in Hopkins and Archer 2014; ie this manuscript includes four previously unpublished temperate experiments, as well as those previously published.

Sentence now reads: "We build on the previous temperate NW European shelf studies of Hopkins & Archer (2014) by presenting data from four previously unpublished experiments from the NW European shelf cruise, and by extending our experimental approach to the Arctic and Southern Oceans".

2.12 L136, remove additional comma before reference.

Done.

2.13 L142 What are the main differences in the environment between temperate and polar environments? You have not mentioned the distinct seasonal cycle of sea ice formation and retreat, different at both poles, which will likely also alter the carbonate chemistry. Does the acclimation of polar phytoplankton to the physiological stress of survival through the polar winter give them an added advantage when it comes to acclimating to OA? Is DMSP produced by polar phytoplankton (i.e. osmoregulation during periods of extreme salinity shift) for a different reason than temperate phytoplankton?

We hope that our response to 2.2 above addresses most of these comments.

We have added some additional discussion with regard to the role of DMSP production by polar communities. We have kept it brief, as the data is not that conclusive, but agree with the reviewer that it is useful to include some discussion of this kind:

This section now reads (from L458):

"In contrast, at temperate stations, DMSP concentrations displayed a clear negative treatment effect, whilst at polar stations a positive effect was evident under high CO_2, and particularly at the first time point (48 – 96 h) (Fig. 7 C and D). *De novo* DMSP synthesis and DMSP production rates show a less consistent response in either environment (Fig. 8 A and B), although a significant suppression of DMSP production rates in temperate waters compared to polar waters was seen (Fig. 8B, Kruskal-Wallis One Way ANOVA $H = 8.711$, $df = 1$, $p = 0.003$). A similar but not significant response was seen for *de novo* DMSP synthesis (Fig. 8A).

This data suggests that DMSP concentrations in polar waters may be upregulated in response to OA compared to temperate waters. Given the potential photoprotective and antioxidant role that DMSP plays, and which may be particularly relevant in the highly variable polar sea-ice environment (e.g. irradiance, carbonate chemistry), these changes may reflect a physiological protective response to the experimental OA (Sunda et al. 2002; Galindo et al. 2016). An increase in DMSP concentrations could have either resulted from a physiological up-regulation of DMSP synthesis or a reduction in bacterial DMSP consumption processes. However, DMSP synthesis rates did not provide any conclusive evidence of upregulation in polar waters. Instead, we observed a suppression of rates in temperate waters which may reflect the adverse effects of rapid OA on DMSP producers (Richier et al. 2014, Hopkins and Archer 2014). In contrast, the lesser

response seen in polar waters may reflect a higher acclimative tolerance to rapid changes in carbonate chemistry amongst polar communities. Further experiments with polar communities would help to further unravel the potential importance of such mechanisms, and whether they facilitated the ability of polar phytoplankton communities to resist the high CO_2 treatments".

We have also simplified the text following on from this section to improve the clarity of the discussion (L516 onwards):

"The responses to OA observed for DMS and DMSP production are likely to be reflected in the dynamics of the DMSP-producing phytoplankton. In an assessment across all experiments, Richier et al. (2018) showed that the maximal response to OA of total Chl *a* and net growth rates of small phytoplankton (<10 µm) observed during each experiment, declined the most in relation to increased buffering capacity and temperature of the initial water. Generally, less significant relationships were found between the phytoplankton response and the other wide range of physical, chemical or biological variables that were examined (Richier et al. 2018).

In correspondence with the analyses carried out by Richier et al (2018), at 48 – 96 h (see Table 1), a statistically significant difference in response was seen between temperate and polar waters for Chl *a* (Kruskal-Wallis One Way ANOVA *H* = 20.577, *df* = 1, *p*<0.001). In general, at polar stations phytoplankton showed minimal response to elevated CO_2, in contrast to a strong negative response in temperate waters (Fig. 9A). By the second time point (96 – 144 h, see Table 1), no significant difference in response of Chl *a* between temperate and polar waters was apparent (Fig. 9B). As shown in Richier et al. (2014), phototrophic nanoflagellates responded to high CO_2 with large decreases in abundance in temperate waters and increases in abundance in polar waters (Fig. 9 C and D), with some exceptions: *North Sea* and *South Sandwich* gave the opposite response. The responses had lessened by the second time point (96 – 168 h, see Table 1).

In contrast, bacterial abundance did not show the same regional differences in response to high CO_2 (see Hopkins and Archer (2014) for temperate waters, and Figure S1, supplementary information, for polar waters). Bacterial abundance in temperate waters gave variable and inconsistent responses to high CO_2. For all Arctic stations, *Drake Passage* and *Weddell Sea*, no response to high CO_2 was observed. For *South Georgia* and *South Sandwich*, bacterial abundance increased at 1000 and 2000 µatm, with significant increases for *South Georgia* after 144 h of incubation (ANOVA *F* = 137.936, *p*<0.001). Additionally, at Arctic stations *Greenland Gyre* and *Greenland Ice-edge*, no overall effect of increased CO_2 on rates of DOC release, total carbon fixation or POC : DOC was observed (Poulton et al. 2016).

Overall, the observed differences in the regional response of DMSP and DMS to carbonate chemistry manipulation could not be attributed to any other measured factor that varied systematically between temperate and polar waters. These include ambient nutrient concentrations, which varied considerably but where direct manipulation had no influence on the response, and initial community structure, which was not a significant predictor of the phytoplankton response (Richier et al. 2018)".

2.14 L190 pCO2

Done.

2.15 L222. In the southern ocean, was this acidification fixing method used for the DMSP samples, given previous issues highlighted by del Valle et al 2011 in samples containing Phaeocystis antarctica?

We are grateful to the reviewer for pointing this out. Upon revisiting our methods section, we have altered the text accordingly to take account of this oversight. Text now reads (from L231):

"Methods for the determination of seawater concentrations of DMS and DMSP are identical to those described in Hopkins & Archer (2014) and will therefore be described in brief here. Seawater DMS concentrations were determined by cryogenic purge and trap, with gas chromatography and pulsed flame photometric detection (GC-PFPD) (Archer et al., 2013). DMSP concentrations were measured as DMS following alkaline hydrolysis. Samples for total DMSP concentrations from temperate waters were fixed by addition of 35 µl of 50 % H_2SO_4 to 7 mL of seawater (Kiene and Slezak 2006), and analysed following hydrolysis within 2 months of collection (Archer et al. 2013). Samples of DMSP that were collected in polar waters were hydrolysed within 1 h of sample collection and analysed 6 – 12 h later. The H_2SO_4 fixation method was not used for samples from polar waters given the likely occurrence of *Phaeocystis sp*. which can result in the overestimation of DMSP concentrations (del Valle et al. 2009). Similarly, concentrations of DMSPp were determined at each time point by gravity filtering 7 ml of sample onto a 25 mm GF/F filter and preserving the filter in 7 ml of 35 mM H_2SO_4 in MQ-water (temperate samples) or immediately hydrolysing (polar samples) and analysing by GC-PFPD. DMS calibrations were performed using alkaline cold-hydrolysis (1 M NaOH) of DMSP sequentially diluted three times in MilliQ water to give working standards in the range 0.03 – 3.3 ng S mL^{-1}. Five point calibrations were performed every 2 – 4 days throughout the cruise".

2.16 L253 spaces missing between mass 63 etc.

Sorted.

2.17 L281 composition used twice in the sentence.

Sentence now reads: "Small phytoplankton community composition was assessed by flow cytometry. For details of methodology, see Richier et al. (2014)".

2.18 L317. Please state the station where 1.5 µg L-1 was identified, as you have for the minimum.

Sentence now reads: "Chl a was also variable in polar waters, exceeding 4 µg L^{-1} at *South Sandwich* and 2 µg L^{-1} at *Greenland Ice-edge*, whilst the remaining stations ranged from 0.2 µg L^{-1} (*Weddell Sea*) to 1.5 µg L^{-1} (*Barents Sea*) (Figure 2)".

2.19 L318.'reflected in'. Please reword, firstly as reflected implies light reflectance (given the topic of irradiance), and secondly it reads oddly with two instances of 'in'.

Sentence now reads: "The high Chl *a* concentrations at *South Sandwich* correspond to low in-water irradiance levels at this station (Fig. 2 C)".

2.20 L320 – 328. Use of nM when the following paragraph uses nmol L-1

All changed to nmol L^{-1}.

2.21 L327. Could not understand the significance of the superscript 1, is it a typo?

Yes typo. Deleted.

2.22 L480. Should P be lower case?

Changed to lower case *p.*

2.23 L485. In this section I would like to see more discussion of sea ice and the extreme environment is imposes on the cells, which could account for some of their resilience to change. Many polar phytoplankton survive the extreme cold of the polar winter by living within the sea ice itself, in an extremely changeable habitat, and these cells seed the surface waters in summer time on melting of the sea ice. Cells are regularly exposed to hypersaline and highly nutrient variable environments, at temperatures below freezing, and in highly elevated pH environments (Thomas and Dieckmann 2002, Rysgaard et al 2012). Although your experiments were not associated directly with the polar sea ice and occurred during summer, the influence of the ice on the phytoplankton population will be dependent on the seed populations, and allow for greater tolerance of the incubation perturbation than in temperate communities. In the seasonal cycle of the Antarctic, the behaviour of the summer phytoplankton community development is dependent on the conditions experienced the previous winter (Venables et al 2013).

We have addressed this comment in 2.2 above.

2.24 L523. P in italics

Done.

2.25 L561. Hopkins reference is 2010b, but only 1 Hopkins 2010 ref is present.

The b has been deleted.

2.26 L609 italic p

Done.

2.27 L626, commas missing from references.

Commas inserted.

References: see manuscript.

Polar dimethylsulfide (DMS) production insensitive to ocean acidification during shipboard microcosm experiments ~~polar oceans may be insensitive to ocean acidification~~: a meta-analysis of 18 ~~microcosm~~ experiments from temperate to polar waters.

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Abstract. Emissions of dimethylsulfide (DMS) from the polar oceans play a key role in atmospheric processes and climate. Therefore, it is important to increase our understanding of how DMS production in these regions may respond to climate change. The polar oceans are particularly vulnerable to ocean acidification (OA). However, our understanding of the polar DMS response is limited to two studies conducted in Arctic waters, where in both cases DMS concentrations decreased with increasing acidity. Here, we report on our findings from seven summertime shipboard microcosm experiments undertaken in a variety of locations in the Arctic Ocean and Southern Ocean. These experiments reveal no significant effects of short term OA on the net production of DMS by planktonic communities. This is in contrast to similar experiments from temperate NW European shelf waters where surface ocean communities responded to OA with significant increases in dissolved DMS concentrations. A meta-analysis of the findings from both temperate and polar waters (n = 18 experiments) reveals clear regional differences in the DMS response to OA. Based on our findings, we hypothesise that the differences in DMS response between temperate and polar waters reflect the natural variability in carbonate chemistry to which the respective communities of each

region may already be adapted. ~~This implies that~~If so, future temperate oceans could be more sensitive to OA resulting in a change in DMS emissions to the atmosphere, whilst perhaps surprisingly DMS emissions from the polar oceans may remain relatively unchanged. By demonstrating that DMS emissions from geographically distinct regions may vary in their response to OA, our results may facilitate a better understanding of Earth's future climate. Our study suggests that the way in which processes that generate DMS respond to OA may be regionally distinct and this should be taken into account in predicting future DMS emissions and their influence on Earth's climate.

1 Introduction

The trace gas dimethylsulfide (DMS) is a key ingredient in a cocktail of gases that exchange between the ocean and atmosphere. Dissolved DMS is produced via the enzymatic breakdown of dimethylsulfoniopropionate (DMSP), a secondary algal metabolite implicated in a number of cellular roles, including the regulation of carbon and sulfur metabolism via an overflow mechanism (Stefels, 2000) and protection against oxidative stress (Sunda et al., 2002). Oceanic DMS emissions amount to 17 - 34 Tg S y^{-1}, representing 80 - 90% of all marine biogenic S emissions, and up to 50% of global biogenic emissions (Lana et al., 2011). DMS and its oxidation products play vital roles in atmospheric chemistry and climate processes. These processes include aerosol formation pathways that influence the concentration of cloud condensation nuclei (CCN) with implications for Earth's albedo and climate (Charlson et al., 1987; Korhonen et al., 2008a), and the atmospheric oxidation pathways of other key climate gases, including isoprene, ammonia and organohalogens (Chen and Jang, 2012; von Glasow and Crutzen, 2004; Johnson and Bell, 2008). Thus, our ability to predict the climate into the future requires an understanding of how marine DMS production may respond to global change (Carpenter et al., 2012; Woodhouse et al., 2013; Menzo et al., 2018).

The biologically-rich seas surrounding the Arctic pack ice are a strong source of DMS to the Arctic atmosphere (Levasseur, 2013). A seasonal cycle in CCN numbers can be related to seasonality in the Arctic DMS flux (Chang et al., 2011). Indeed, observations confirm that DMS oxidation products promote the growth of particles to produce aerosols that may influence cloud processes and atmospheric albedo (Bigg and Leck, 2001; Rempillo et al., 2011; Korhonen et al., 2008b; Chang et al., 2011). Arctic new particle formation events and peaks in aerosol optical depth (AOD) occur during summertime clean air periods (when levels of anthropogenic black carbon diminish), and have been linked to chlorophyll *a* maxima in surface waters and the presence of aerosols formed from DMS oxidation products such as methanesulfonate (MSA). The atmospheric oxidation products of DMS - SO_2 and H_2SO_4 - contribute to both the growth of existing particles and new particle formation (NPF) in the Arctic atmosphere (Leaitch et al., 2013; Gabric et al., 2014; Sharma et al., 2012). Thus, the ongoing and projected rapid loss of seasonal Arctic sea ice may influence the Arctic radiation budget via changes to both the DMS flux and the associated formation and growth of cloud-influencing particles (Sharma et al., 2012).

During its short but highly productive summer season, the Southern Ocean is a hotspot of DMS flux to the atmosphere, influenced by the prevalence of intense blooms of DMSP-rich *Phaeocystis antarctica* (Schoemann et al., 2005) and the presence of persistent high winds particularly in regions north of the sub-Antarctic front (Jarníková and Tortell, 2016). Around 3.4 Tg of sulfur is released from the Southern Ocean to the atmosphere between December and February, a flux that represents ~15 % of global annual emissions of DMS (Jarníková and Tortell, 2016). Elevated CCN numbers are seen in the most biologically active regions of the Southern Ocean, with a significant contribution from DMS-driven secondary aerosol formation processes (McCoy et al., 2015; Korhonen et al., 2008a). DMS-derived aerosols from this region are estimated to contribute 6 to 10 W m^{-2} to reflected short wavelength

radiation, similar to the influence of anthropogenic aerosols in the polluted Northern Hemisphere (McCoy et al., 2015). Given this important influence of polar DMS emissions on atmospheric processes and climate, it is vital we increase our understanding of the influence of future ocean acidification on DMS production.

The polar oceans are characterised by high dissolved inorganic carbon (C_T) concentrations and a low carbonate system buffering capacity, mainly due to the increased solubility of CO_2 in cold waters (Sabine et al., 2004; Orr et al., 2005). This makes these regions particularly susceptible to the impacts of ocean acidification (OA). For example, extensive carbonate mineral undersaturation is expected to occur in Arctic waters within the next 20 – 80 years (McNeil and Matear, 2008; Steinacher et al., 2009). OA has already led to a 0.1 unit decrease in global surface ocean pH, with a further fall of ~0.4 units expected by the end of the century (Orr et al., 2005). The greatest declines in pH are likely in the Arctic Ocean with a predicted fall of 0.45 units by 2100 (Steinacher et al., 2009), with a fall of ~0.3 units predicted for the Southern Ocean (McNeil and Matear, 2008; Hauri et al., 2016). OA is occurring at a rate not seen on Earth for 300 Ma, and so the potential effects on marine organisms, communities and ecosystems could be wide-ranging and severe (Raven et al., 2005; Hönisch et al., 2012). Despite the imminent threat to polar ecosystems and the importance of DMS emissions to atmospheric processes, our knowledge of the response of polar DMS production to OA is limited to a single mesocosm experiment performed in a coastal fjord in Svalbard (Riebesell et al., 2013; Archer et al., 2013) and one shipboard microcosm experiment with seawater collected from Baffin Bay (Hussherr et al., 2017). Both studies reported significant reductions in DMS concentrations with increasing levels of pCO_2 during seasonal phytoplankton blooms. Hussherr et al. (2017) also saw reductions in total DMSP whilst Archer et al. (2013) observed a significant increase in this compound, driven by CO_2-induced

increases in growth and abundance of dinoflagellates. However, these two single studies provide limited information on the wider response of the open Arctic or Southern Oceans.

Mesocosm experiments ~~are~~ have been a critical tool for assessing OA effects on surface ocean communities~~. Initial studies focused on the growth and decline of blooms with~~ (Engel et al., 2005; Engel et al., 2008; Schulz et al., 2008; Hopkins et al., 2010; Schulz et al., 2013; Webb et al., 2015; Kim et al., 2006; Kim et al., 2010; Crawfurd et al., 2016; Webb et al., 2016)~~, or without~~ (Webb et al., 2016; Crawfurd et al., 2016) ~~the addition of inorganic nutrients~~. The response of DMS to OA has been examined several times, predominantly at the same site in Norwegian coastal waters (Vogt et al., 2008; Hopkins et al., 2010; Webb et al., 2015; Avgoustidi et al., 2012)~~.~~, ~~There have also been two studies~~ twice in Korean coastal waters (Kim et al., 2010; Park et al., 2014), ~~as well as the~~and a single ~~mesocosm~~ study in the coastal ~~(sub)~~ Arctic waters of Svalbard (Archer et al., 2013). Mesocosm enclosures, ranging in volume from ~11,000 – 50,000 L, allow the response of surface ocean communities to a range of CO_2 treatments to be monitored under near-natural light and temperature conditions over time scales (weeks - months) that allow a 'winners vs loser' dynamic to develop. The response of DMS cycling to elevated CO_2 is generally driven by changes to the microbial community structure (Brussaard et al., 2013; Archer et al., 2013; Hopkins et al., 2010; Engel et al., 2008). The size~~,~~ ~~and~~ construction and associated costs of ~~the~~ mesocosms has limited their deployment to coastal/sheltered waters, resulting in minimal geographical coverage, and leaving large gaps in our understanding of the response of open ocean phytoplankton communities to OA.

Here, we adopt an alternative but complementary approach to explore the effects of OA on the cycling of DMS with the use of short-term shipboard microcosm experiments. We build on the previous temperate NW European shelf studies of Hopkins & Archer (2014) by presenting data from four previously unpublished experiments from the NW European shelf

cruise, and by extending our experimental approach to the Arctic and Southern Oceans. Vessel-based research enables multiple short term (days) near-identical incubations to be performed over extensive spatial scales, that encompass natural gradients in carbonate chemistry, temperature and nutrients (Richier et al., 2014; Richier et al., 2018). This allows an assessment to be made of how a range of surface ocean communities, adapted to a variety of environmental conditions, respond to the same driver. The focus is then on the effect of short-term CO_2 exposure on physiological processes, as well as the extent of the variability in acclimation between communities. The capacity of organisms to acclimate to changing environmental conditions contributes to the resilience of key ecosystem functions, such as DMS production. Therefore, do spatially-diverse communities respond differently to short term OA, and can this be explained by the range of environmental conditions to which each is presumably already adapted? The rapid CO_2 changes implemented in this study, and during mesocosm studies, are far from representative of the predicted rate of change to seawater chemistry over the coming decades. Nevertheless, our approach can provide insight into the physiological response and level of sensitivity to future OA of a variety of polar surface ocean communities adapted to different in situ carbonate chemistry environments~~,~~ (Stillman and Paganini, 2015), alongside the implications this may have for DMS production.

Communities of the NW European shelf consistently responded to acute OA with significant increases in net DMS production, likely a result of an increase in stress-induced algal processes (Hopkins and Archer, 2014). Do polar phytoplankton communities, which are potentially adapted to contrasting biogeochemical environments, respond in the same way? By expanding our approach to encompass both polar oceans, we can assess regional contrasts in response. To this end, we combine our findings for temperate waters with those for the polar oceans into a meta-analysis to advance our understanding of the regional variability and drivers in the DMS response to OA.

2 Material and Methods

2.1 Sampling stations

This study presents new data from two sets of field experiments carried out as a part of the UK Ocean Acidification Research Programme (UKOA) aboard the RRS James Clark Ross in the sub-Arctic and Arctic in June-July 2012 (JR271) and in the Southern Ocean in January-February 2013 (JR274). Data are combined with the results from an earlier study on board the RRS Discovery (D366) described in Hopkins & Archer (2014) performed in the temperate waters of the NW European shelf. Additionally, four previously unpublished experiments from D366 are also included (E02b, E04b, E05b, E06) as well as two temperate experiments from JR271 (NS and IB) (see Table 1). In total, 18 incubations were performed; 11 in temperate and sub-Arctic waters of the NW European shelf and North Atlantic, 3 in Arctic waters and 4 in the Southern Ocean. Figure 1 shows the cruise tracks, surface concentrations of DMS and total DMSP (DMSPt) at CTD sampling stations as well as the locations of sampling for shipboard microcosms (See Table 1 for further details).

2.2 Shipboard microcosm experiments

The general design and implementation of the experimental microcosms for JR271 and JR274 was essentially the same as for D366 and described in Richier et al. (2014), (2018) and Hopkins & Archer (2014), but with the additional adoption of trace metal clean sampling and incubation techniques in the low trace metal open ocean waters (see Richier et al. (2018)). At each station, pre-dawn vertical profiles of temperature, salinity, oxygen, fluorescence, turbidity and irradiance were used to choose and characterise the depth of experimental water collection. Subsequently, water was collected within the mixed layer from three successive separate casts of a trace-metal clean titanium CTD rosette comprising twenty-four 10 L Niskin bottles. Depth profiles of auxiliary measurements are shown in Figure 2. Each cast

was used to fill one of a triplicated set of experimental bottles (locations and sample depths, Table 1). Bottles were sampled within a class-100 filtered air environment within a trace metal clean container to avoid contamination during the set up. The water was directly transferred into acid-cleaned 4.5 L polycarbonate bottles using acid-cleaned silicon tubing, with no screening or filtration.

The carbonate chemistry within the experimental bottles was manipulated by addition of equimolar HCl and $NaHCO_3^-$ (1 mol L^{-1}) to achieve a range of ~~target~~ CO_2 treatments: Mid CO_2 (Target: 550 µatm), High CO_2 (Target: 750 µatm), High+ CO_2 (Target: 1000 µatm) and High++ CO_2 (Target: 2000 µatm) ~~values (550, 750, 1000, 2000 µatm)~~ (Gattuso et al., 2010). Three treatment levels were used during ~~For~~ the sub-Arctic/Arctic microcosms (Mid, High, High+)~~, additions were used to attain three target CO_2 levels (550 µatm, 750 µatm and 1000 µatm~~). For Southern Ocean experiments, two experiments (*Drake Passage* and *Weddell Sea*) underwent combined CO_2 and Fe additions (ambient, Fe (2 nM), ~~high~~ High ~~CO_2~~ CO_2~~(750 µatm)~~, Fe (2 nM) ~~+~~ & ~~h~~High CO_2 ~~(750µatm)~~ (only high CO_2 treatments will be examined here; no response to Fe was detected in DMS or DMSP concentrations). Three CO_2 treatments (High, High+, High++)~~750 µatm, 1000 µatm, 2000 µatm)~~ were tested in the last two experiments (*South Georgia* and *South Sandwich*). Full details of the carbonate chemistry manipulations can be found in Richier et al. (2014) and Richier et al. (2018). Broadly, achieved pCO_2 levels were well-matched to target values at ~~T$_0$~~the start of the experiments (0 h), although differences in pCO_2~~pCO_2~~ between target and initial values were greater in the higher pCO_2~~pCO_2~~ treatments, due to lowered carbonate system buffer capacity at higher pCO_2~~pCO_2~~. For all 18 experiments, actual ~~attained~~ pCO_2~~pCO_2~~ values at 0 h were on average around 89% ± 12% (± 1 SD) of target values. The attained pCO_2~~pCO_2~~ values, and pCO_2 at each experimental time point, are presented in Figures 3 and 4.~~Table S1 on the Supplementary Information. For simplicity, experimental data is presented against its target~~

~~('nominal') pCO_2 treatment throughout the paper.~~ After first ensuring the absence of bubbles or headspace, the bottles were sealed with high density polyethylene (HDPE) lids with silicone/ polytetrafluoroethylene (PTFE) septa and placed in the incubation container. Bottles were incubated inside a custom-designed temperature- and light-controlled shipping container, set to match (±<1°C) the *in situ* water temperature at the time of water collection (shown in Table 1) (see Richier et al. 2018). A constant light level (100 µE m^{-2} s^{-1}) was provided by daylight simulating LED panels (Powerpax, UK). The light period within the microcosms was representative of *in situ* conditions. For the sub-Arctic/Arctic Ocean stations, experimental bottles were subjected to continuous light representative of the 24 h daylight of the Arctic summer. For Southern Ocean and all temperate water stations, an 18:6 light: dark cycle was used. Each bottle belonged to a set of triplicates, and sacrificial sampling of bottles was performed at two time points (see Table 1 for ~~chosen time points~~exact times). Use of three sets of triplicates for each time point allowed for the sample requirements of the entire scientific party (3 x 3 bottles, x 2 time points (~~T_1, T_2,~~ see Table 1 for specific times for each experiment), x 4 CO_2 treatments = 72 bottles in total). Experiments were ~~generally~~ run for between 4 and 7 days (96 h – 168 h) (15 out of 18 experiments), with initial sampling proceeded by two further time points. For three temperate experiments (E02b, E04b, E05b, see Table 1) a shorter 2 day incubation was performed, with a single sampling point at the end. For E06 (see Table 1) high time frequency sampling was performed (0, 1, 4, 14, 24, 48, 72, 96 h) although only the data at 48 h and 96 h is considered in this analysis. Incubation times were extended for Southern Ocean stations *Weddell Sea*, *South Georgia* and *South Sandwich* (see Table 1) as minimal CO_2 response, attributed to slower microbial metabolism at low water temperatures, was observed for Arctic stations and the first Southern Ocean station *Drake Passage*. The magnitude of response was not related to incubation times, and expected differences in net growth rates (2- to 3-fold higher in

temperate compared to polar waters (Eppley, 1972)) did not account for the differences in response magnitude despite the increased incubation time in polar waters (see Richier et al. (2018) for detailed discussion). Samples for carbonate chemistry measurements were taken first, followed by sampling for DMS, DMSP and related parameters.

2.3 Standing stocks of DMS and DMSP

Methods for the determination of seawater concentrations of DMS and DMSP are identical to those described in Hopkins & Archer (2014) and will therefore be described in brief here. Seawater DMS concentrations were determined by cryogenic purge and trap, with gas chromatography and pulsed flame photometric detection (GC-PFPD) (Archer et al., 2013). DMSP concentrations were ~~subsequently~~ measured as DMS following alkaline hydrolysis. Samples for total DMSP concentrations from temperate waters were fixed by addition of 35 µl of 50 % H_2SO_4 to 7 mL of seawater (Kiene and Slezak, 2006), and analysed following hydrolysis within 2 months of collection (Archer et al., 2013). Samples of DMSP that were collected in polar waters were hydrolysed within 1 h of sample collection and analysed 6 – 12 h later. The H_2SO_4 fixation method was not used for samples from polar waters given the likely occurrence of *Phaeocystis sp.* which can result in the overestimation of DMSP concentrations (del Valle et al., 2009). Similarly, ~~C~~concentrations of DMSPp were determined at each time point by gravity filtering 7 ml of sample onto a 25 mm GF/F filter and preserving the filter in 7 ml of 35 mM H_2SO_4 in MQ-water (temperate samples) or immediately hydrolysing (polar samples) and analysing by GC-PFPD. ~~DMSP concentrations were subsequently measured as DMS following alkaline hydrolysis.~~ DMS calibrations were performed using alkaline cold-hydrolysis (1 M NaOH) of DMSP sequentially diluted three times in MilliQ water to give working standards in the range 0.03 – 3.3 ng S mL^{-1}. Five point calibrations were performed every 2 – 4 days throughout the cruise.

2.4 *De novo* DMSP synthesis

De novo DMSP synthesis and gross production rates were determined for all microcosm experiments, except *Barents Sea* and *South Sandwich*, at each experimental time point, using methods based on the approach of Stefels et al. (2009) and described in detail in Archer et al. (2013) and Hopkins and Archer (2014). Triplicate rate measurements were determined for each CO_2 level. For each rate measurement three x 500 mL polycarbonate bottles were filled by gently siphoning water from each replicate microcosm bottle. Trace amounts of $NaH^{13}CO_3$, equivalent to ~6 % of *in situ* dissolved inorganic carbon (C_T), were added to each 500 mL bottle. The bottles were incubated in the microcosm incubation container with temperature and light levels as described earlier. Samples were taken at 0 h, then at two further time points over a 6 - 9 h period. At each time point, 250 mL was gravity filtered in the dark through a 47 mm GF/F filter, the filter gently folded and placed in a 20 mL serum vial with 10 mL of Milli-Q and one NaOH pellet, and the vial was crimp-sealed. Samples were stored at -20°C until analysis by proton transfer reaction-mass spectrometer (PTR-MS) (Stefels et al. 2009).

The specific growth rate of DMSP (μDMSP) was calculated assuming exponential growth from:

$$\mu_t(\Delta t^{-1}) = \alpha_k \text{ x } AVG\left[\ln\left(\frac{^{64}MP_{eq} - {}^{64}MP_{t-1}}{^{64}MP_{eq} - {}^{64}MP_{t}}\right), \ln\left(\frac{^{64}MP_{eq} - {}^{64}MP_{t}}{^{64}MP_{eq} - {}^{64}MP_{t+1}}\right)\right] \quad (1)$$

(Stefels et al. 2009) where $^{64}MP_t$, $^{64}MP_{t-1}$, $^{64}MP_{t+1}$ are the proportion of 1 x ^{13}C labelled DMSP relative to total DMSP at time t, at the preceding time point (t-1) and at the subsequent time point (t+1), respectively. Values of ^{64}MP were calculated from the protonated masses of DMS as: mass 64/(mass63 + mass64 + mass65), determined by PTR-MS. $^{64}MP_{eq}$ is the

theoretical equilibrium proportion of 1 x ^{13}C based on a binomial distribution and the proportion of tracer addition. An isotope fractionation factor α_k of 1.06 is included, based on laboratory culture experiments using *Emiliania huxleyi* (Stefels et al. 2009). In vivo DMSP ~~Gross~~ grossDMSP production rates during the incubations (nmol L^{-1} h^{-1}) were calculated from µDMSP and the initial particulate DMSP (DMSPp) concentration of the incubations (Hopkins & Archer 2014, Stefels et al. 2009) ~~(shown in Figure 4~~). These rates provide important information on how the physiological status of DMSP-producing cells may be affected by OA within the bioassays.

2.5 Seawater carbonate chemistry analysis

The techniques and methods used to determine both the *in situ* and experimental carbonate chemistry parameters, and to manipulate seawater carbonate chemistry within the microcosms, are described in Richier et al. (2014) and will be only given in brief here. Experimental T_0 measurements were taken directly from CTD bottles, and immediately measured for total alkalinity (A_T) (Apollo SciTech AS-Alk2 Alkalinity Titrator) and dissolved inorganic carbon (C_T) (Apollo SciTech C_T analyser (AS-C3) with LICOR 7000). The CO2SYS programme (version 1.05) (Lewis and Wallace, 1998) was used to calculate the remaining carbonate chemistry parameters including pCO_2.

Measurements of T_A and C_T were made from each bottle at each experimental time point and again used to calculate the corresponding values for pCO_2 and pH_T. The carbonate chemistry data for each sampling time point for each experiment are summarised in Supplementary Table S1, S2 and S3 (Experimental starting conditions are given in Table 1).

2.6 Chlorophyll a (Chl *a*) determinations

Concentrations of Chl *a* were determined as described in Richier et al. (2014). Briefly, 100 mL aliquots of seawater from the incubation bottles were filtered through either 25 mm GF/F (Whatman, 0.7 µm pore size) or polycarbonate filters (Whatman, 10 µm pore size) to yield total and >10 µm size fractions, with the <10 µm fraction calculated by difference. Filters were extracted in 6 mL HPLC-grade acetone (90%) overnight in a dark refrigerator. Fluorescence was measured using a Turner Designs Trilogy fluorometer, which was regularly calibrated with dilutions of pure Chl *a* (Sigma, UK) in acetone (90%).

2.8 Community composition

~~Composition of s~~Small phytoplankton community composition was assessed by flow cytometry. For details of methodology, see Richier et al. (2014).

2.9 Data handling and statistical analyses

Permutational analysis of variance (PERMANOVA) was used to analyse the difference in response of DMS and DMSP concentrations to OA, both between and within the two polar cruises in this study. Both dependant variables were analysed separately using a nested factorial design with three factors; (i) Cruise Location: Arctic and Southern Ocean, (ii) Experiment location nested within Cruise location (see Table 1 for station IDs) and (iii) CO_2 level: 385, 550, 750, 1000 and 2000 µatm. Main effects and pairwise comparisons of the different factors were analysed through unrestricted permutations of raw data. If a low number of permutations were generated then the *p*-value was obtained through random sampling of the asymptotic permutation distribution, using Monte Carlo tests.

One-way analysis of variance was used to identify differences in ratio of >10 µm Chl *a* to total Chl *a* ($chl_{>10um}$: chl_{tot} , see Discussion). Initially, tests of normality were applied ($p<0.05$ = not normal), and if data failed to fit the assumptions of the test, linearity transformations of

the data were performed (logarithmic or square root), and the ANOVA proceeded from this point. The results of ANOVA are given as follows: F = ratio of mean squares, df = degrees of freedom, p = level of confidence. For those data still failing to display normality following transformation, a rank-based Kruskal-Wallis test was applied (H = test statistic, df = degrees of freedom, p = level of confidence).

3 Results

3.1 Sampling stations

At temperate sampling stations, sea surface temperatures ranged from 10.7°C for *Iceland Basin*, to 15.3°C for *Bay of Biscay*, with surface salinity in the range 34.1 – 35.2, with the exception of station E05b which had a relatively low salinity of 30.5 (Figure 2 and Table 1). Seawater temperatures at the polar microcosm sampling stations ranged from -1.5°C at sea-ice influenced stations (*Greenland Ice-edge* and *Weddell Sea*) up to 6.5°C for *Barents Sea* (Fig. 2 A). Salinity values at all the Southern Ocean stations were <34, whilst they were ~35 at all the Arctic stations with the exception of *Greenland Ice-edge* which had the lowest salinity of 32.5 (Fig. 2 B). Phototrophic nanoflagellate abundances were variable, with >3 x 10^4 cells mL^{-1} at *Greenland Gyre*, 1.5 x 10^4 cells mL^{-1} at *Barents Sea* and <3 x 10^3 cells mL^{-1} for all other stations (Fig. 2 D). Total bacterial abundances ranged from 3 x 10^5 cells mL^{-1} at *Greenland Ice-edge* up to 3 x 10^6 cells mL^{-1} at *Barents Sea* (Fig. 2 E).

Chl *a* concentrations in temperate waters ranged from 0.3 µg L^{-1} for two North Sea stations (*E05* and *North Sea*) up to 3.5 µg L^{-1} for *Irish Sea* (Figure 2 and Table 1). Chl a was also variable in polar waters, exceeding 4 µg L^{-1} at *South Sandwich* and 2 µg L^{-1} at *Greenland Ice-edge*, whilst the remaining stations ranged from 0.2 µg L^{-1} (*Weddell Sea*) to 1.5 µg L^{-1} (*Barents Sea*) (Figure 2). The high Chl *a* concentrations at *South Sandwich* ~~are reflected~~ ~~in~~correspond to low in-water irradiance levels at this station (Fig. 2 C).

In temperate waters, maximum DMS concentrations were generally seen in near surface measurements, ranging from 1.0 ~~nM~~ nmol L^{-1} for *E04* to 21.1 ~~nM~~ nmol L^{-1} for *E06*, with rapidly decreasing concentrations with depth (Figure 2 G). DMSP also generally peaked in the near surface waters, ranging from 12.0 ~~nM~~ nmol L^{-1} for E04 to 72.5 ~~nM~~ nmol L^{-1} for *E06*, but the maximum overall DMSP concentration of 89.8 ~~nM~~ nmol L^{-1} was observed at ~20 m for *E05b* (Figure 2 H). Surface DMS concentrations in polar waters were generally lower than temperate waters, ranging from 1 – 3 ~~nM~~nmol L^{-1}, with the exception of *South Sandwich* where concentrations of ~12 ~~nM~~ nmol L^{-1} were observed (Figure 2 G). DMSP generally ranged from 12 – 20 ~~nM~~ nmol L^{-1}, except *Barents Sea* where surface concentrations exceeded 60 ~~nM~~ nmol L^{-1} (Figure 2 H).

3.2 Response of DMS and DMSP to OA

The temporal trend in DMS concentrations showed a similar pattern for the three Arctic Ocean experiments. Initial concentrations of 1 – 2 nmol L^{-1} remained relatively constant over the first 48 h and then showed small increases of 1 - 4 nmol L^{-1} over the incubation period (Figure 3 ~~A – C~~). Increased variability between triplicate incubations became apparent in all three Arctic experiments by 96 h, but no significant effects of elevated CO_2 on DMS concentrations were observed. Initial DMSP concentrations were more variable, from 6 nmol L^{-1} at *Greenland Ice-edge* to 12 nmol L^{-1} at *Barents Sea*, and either decreased slightly (net loss 1 – 2 nmol L^{-1} GG), or increased slightly (net increase ~4 nmol L^{-1} *Greenland Ice-edge*, ~3 nmol L^{-1} *Barents Sea*) (Figure ~~4~~ 5 A – C). DMSP concentrations were found to decrease significantly in response to elevated CO_2 after 48 h for *Barents Sea* (Fig. 4 C, $t = 2.05$, $p = 0.025$), whist no significant differences were seen after 96 h. No other significant responses in DMSP were identified.

The range of initial DMS concentrations was greater at Southern Ocean sampling stations compared to the Arctic, from 1 nmol L^{-1} at *Drake Passage* up to 13 nmol L^{-1} at *South Sandwich* (Figure ~~43~~ 4~~D – G~~). DMS concentrations showed little change over the course of 96 – 168 h incubations and no effect of elevated CO_2, with the exception of *South Sandwich* (Fig. ~~3~~ 4 ~~G~~D). Here, concentrations decreased sharply after 96 h by between 3 and 11 nmol L^{-1}. Concentrations at 96 h were CO_2-treatment dependent, with significant decreases in DMS concentration occurring with increasing levels of CO_2 (PERMANOVA, $t = 2.61$, $p = 0.028$). Significant differences ceased to be detectable by the end of the incubations (168 h). Initial DMSP concentrations were higher at the Southern Ocean stations than for Arctic stations, ranging from 13 nmol L^{-1} for *Weddell Sea* to 40 nmol L^{-1} for *South Sandwich* (Figure ~~4~~ 5 D – G). Net increases in DMSP occurred throughout, except at South Georgia, and were on the order of between <10 nmol L^{-1} - >30 nmol L^{-1} over the course of the incubations. Concentrations were not generally pCO_2-treatment dependent with the exception of the final time point at *South Georgia* (144 h) when a significantly lower DMSP with increasing CO_2 was observed (PERMANOVA, $t = -5.685$, $p<0.001$).

Results from the previously unpublished experiments from temperate waters are in strong agreement with the five experiments presented in Hopkins and Archer (2014), with consistently decreased DMS concentrations and enhanced DMSP under elevated CO_2. The data is presented in the Supplementary Information, Table S4 and Figure S2, and included in the meta-analysis in section 4.1 of this paper.

3.3 Response of de novo DMSP synthesis and production to OA

Rates of *de novo* DMSP synthesis (µDMSP) at initial time points ~~(T_0)~~ ranged from 0.13 d^{-1} (*Weddell Sea*, Fig. ~~5~~ 6 G) to 0.23 d^{-1} (*Greenland Ice-edge*, Fig. ~~5~~ 6 C), whilst DMSP production ranged from 0.4 nmol L^{-1} d^{-1} (*Greenland Gyre*, Fig. ~~5~~ 6 B) to 2.27 nmol L^{-1} d^{-1}

(*Drake Passage*, Fig. ~~5~~ 6 F). Maximum rates of µDMSP of 0.37 -0.38 d^{-1} were observed at *Greenland Ice-edge* after 48 h of incubation in all CO_2 treatments (Fig. ~~5~~ 6 C). The highest rates of DMSP production were observed at *South Georgia* after 96 h of incubation, and ranged from 4.1 – 6.9 nmol L^{-1} d^{-1} across CO_2 treatments (Fig. ~~5~~ 6 J). Rates of DMSP synthesis and production were generally lower than those measured in temperate waters (Hopkins and Archer, 2014) (Initial rates: µDMSP 0.33 – 0.96 d^{-1}, 7.1 – 37.3 nmol L^{-1} d^{-1}), but were comparable to measurements made during an Arctic mesocosm experiment (Archer et al., 2013) (0.1 – 0.25 d^{-1}, 3 – 5 nmol L^{-1} d^{-1} in non-bloom conditions). The lower rates in cold polar waters likely reflect slower metabolic processes and are reflected by standing stock DMSP concentrations which were also lower than in temperate waters (5 – 40 nmol L^{-1} polar, 8 – 60 nmol L^{-1} temperate (Hopkins and Archer, 2014)). No consistent ~~evidence~~ of high CO_2 ~~sensitivity was seen~~were observed for ~~in~~ either DMSP synthesis or production in polar waters, similar to findings for DMSP standing stocks. However, ~~S~~some notable but ~~conflicting~~ contrasting differences between CO_2 treatments were observed. There was a 36% and 37% increase in µDMSP and DMSP production respectively at 750 µatm for the *Drake Passage* after 96 h (Figure ~~5~~ 6 E, F), and a 38% and 44% decrease in both at 750 µatm after 144 h for *Weddell Sea* (Figure 5 G, H). For *Drake Passage*, the difference between treatments at 96 h coincided with significantly higher nitrate concentrations in the High CO_2 treatment (Nitrate/nitrite at 96 h: Ambient = 18.9 ± 0.2 µmol L^{-1}, +CO_2 = 20.2 ± 0.1 µmol L^{-1}, ANOVA $F = 62.619$, $df = 1$, $p = 0.001$). However, it is uncertain whether the difference in nutrient availability between treatments (approximately 5 %) would be significant enough to strongly influence the rate of DMSP production.

The differences in DMSP production rates did not correspond to any other measured parameter. It is possible that changes in phytoplankton community composition may have led to differences in DMSP production rates for *Drake Passage* and *Weddell Sea*, but no

quantification of large cells (diatoms, dinoflagellates) was undertaken for these experiments. ~~Nevertheless, no consistent and significant effects of high CO_2 were observed for rates of *de novo* DMSP synthesis or DMSP production in polar waters.~~

4 Discussion

4.1 Regional differences in the response of DMS(P) to OA

We combine our findings from the polar oceans with those from temperate waters into a meta-analysis in order to assess the regional variability and drivers in the DMS(P) response to OA. Figures ~~6~~ 7 and ~~7~~ 8 provide an overview of the results discussed so far in this current study, together with the results from Hopkins & Archer (2014) as well as the results from 4 previously unpublished microcosm experiments from the NW European shelf cruise and a further 2 temperate water microcosm experiments from the Arctic cruise (*North Sea* and *Iceland Basin*, Table 1). This gives a total of 18 microcosm experiments, each with between 1 and 3 high CO_2 treatments.

Hopkins & Archer (2014) reported consistent and significant increases in DMS concentration in response to elevated CO_2 that were accompanied by significant decreases in DMSPt concentrations. Bacterially-mediated DMS processes appeared to be insensitive to OA, with no detectable effects on dark rates of DMS consumption and gross production, and no consistent response seen in bacterial abundance (Hopkins and Archer, 2014). In general, there were large short-term decreases in Chl *a* concentrations and phototrophic nanoflagellate abundance in response to elevated CO_2 in these experiments (Richier et al., 2014).

The relative treatment effects ($[x]_{highCO2}/[x]_{ambientCO2}$) for DMS and DMSP (Figure ~~6~~7), DMSP synthesis and production (Figure 8), and Chl *a* and phototrophic nanoflagellate abundance (Figure ~~8~~9) are plotted against the ~~ratio of C_T to A_T (C_T/A_T)~~Revelle Factor of the sampled

waters~~, in order to place our findings in context of the total experimental data set~~. The Revelle Factor (R), calculated here with CO2Sys using measurements of carbonate chemistry parameters ($R = (\Delta pCO_2/\Delta TCO_2)/(pCO_2/TCO_2)$, Lewis and Wallace, 1998)~~(Lewis and Wallace, 1998)~~, describes how the partial pressure of CO_2 in seawater (PCO_2) changes for a given change in DIC (Sabine et al., 2004; Revelle and Suess, 1957). Its magnitude varies latitudinally, with lower values (9 – 12) from the tropics to temperate waters, and the highest values in cold high latitude waters (13 – 15). Thus polar waters can be considered poorly buffered with respect to changes in DIC. Therefore, biologically-driven seasonal changes in seawater pCO_2 would result in larger changes in pH than would be experienced in temperate waters. (Egleston et al., 2010). Furthermore, the seasonal sea ice cycle strongly influences carbonate chemistry, such that sea ice regions exhibit wide fluctuations in carbonate chemistry (Revelle and Suess, 1957; Sabine et al., 2004)~~The value of C_T/A_T ranges from 0.84 – 0.95 within the mixed layer, and increases towards high latitude waters~~ (Sabine et al., 2004)~~. Thus,~~Sampling stations with ~~C_T/A_T~~a R above ~~0.91~~12 represent the seven polar stations (right of red dashed line Fig. 6 and 7). The surface waters of the polar oceans have naturally higher levels of DIC and a reduced buffering capacity, driven by~~ due to~~ higher CO_2 solubility in colder waters~~, and so are less resistant to local variations in C_T and A_T~~ (Sabine et al., 2004). Thus, the relationship between experimental response and ~~C_T/A_T~~R is a simple way of demonstrating the differences in response to OA between temperate and polar waters and provides some insight into how the CO_2 sensitivity of different surface ocean communities may relate~~s~~ to the *in situ* carbonate chemistry. The effect of elevated CO_2 on DMS concentrations at polar stations, relative to ambient controls, was minimal ~~at all~~both sampling points, and is in strong contrast to the results from experiments performed in waters with lower values of R on the NW European shelf. In contrast, ~~A~~at temperate stations, DMSP concentrations displayed a clear negative treatment effect, whilst at polar stations a positive

effect was evident under high CO_2, and particularly at the first time point~~T_1~~ (48 – 96 h) (Fig. ~~6~~ 7 C and D). *De novo* DMSP synthesis and DMSP production rates show ~~a similar~~a less consistent response in either environment ~~relationship with C_T/A_T~~ (Fig. ~~7~~ 8 A and B), although ~~with~~ a significant suppression of DMSP production rates in temperate waters compared to polar waters was seen (Fig. ~~7B~~8B, Kruskal-Wallis One Way ANOVA H = 8.711, df = 1, p = 0.003). ~~Although a~~ A similar but not significant ~~trend~~ response was seen for *de novo* DMSP synthesis~~, the difference between temperate and polar waters was not statistically significant~~ (Fig. ~~7A~~8A).

This data suggests that DMSP concentrations in polar waters may be upregulated in response to OA compared to temperate waters. Given the potential photoprotective and antioxidant role that DMSP plays, and which may be particularly relevant in the highly variable polar sea-ice environment (e.g. irradiance, carbonate chemistry), these changes may reflect a physiological protective response to the experimental OA (Sunda et al., 2002;Galindo et al., 2016). An increase in DMSP concentrations could have either resulted from a physiological up-regulation of DMSP synthesis or a reduction in bacterial DMSP consumption processes. However, DMSP synthesis rates did not provide any conclusive evidence of upregulation in polar waters. Instead, we observed a suppression of rates in temperate waters which may reflect the adverse effects of rapid OA on DMSP producers (Richier et al. 2014, Hopkins and Archer 2014). In contrast, the lesser response seen in polar waters may reflect a higher acclimative tolerance to rapid changes in carbonate chemistry amongst polar communities. Further experiments with polar communities would help to further unravel the potential importance of such mechanisms, and whether they facilitated the ability of polar phytoplankton communities to resist the high CO_2 treatments.

~~At T_1 (48 – 96 h, see Table 1), a statistically significant difference in response was seen between temperate and polar waters for Chl *a* (Kruskal-Wallis One Way ANOVA H =~~

20.577, *df* = 1, *p*<0.001), with minimal response to elevated CO_2 at polar stations, and in general a strong negative response was seen in temperate waters (Fig. 8A). By T_2 (96 – 144 h, see Table 1), no significant difference in response of Chl *a* between temperate and polar waters was detectable (Fig. 8B), although a slight positive response in Chl *a* was seen at some temperate stations, and polar stations showed a minimal response, with the exception of *Barents Sea* which saw strongly enhanced Chl *a* at T_2 (96 h) (Fig. 8 B).

In general, phototrophic nanoflagellates responded to high CO_2 with large decreases in abundance in temperate waters (Richier et al. 2014), and increases in abundance in polar waters (Fig. 8 C and D), with some exceptions: *North Sea* and *South Sandwich* gave the opposite response. The impacts had lessened by T_2 (96 – 168 h, see Table 1). In contrast, bacterial abundance did not show the same regional differences in response to high CO_2 (see Hopkins and Archer (2014) for temperate waters, and Figure S1, supplementary information, for polar waters). Bacterial abundance in temperate waters gave variable and inconsistent responses to high CO_2. For all Arctic stations, *Drake Passage* and *Weddell Sea*, no response to high CO_2 was observed. For *South Georgia* and *South Sandwich*, bacterial abundance increased at 1000 and 2000 µatm, with significant increases for *South Georgia* after 144 h of incubation (ANOVA $F = 137.936$, $p<0.001$). Additionally, at Arctic stations *Greenland Gyre* and *Greenland Ice-edge*, no overall effect of increased CO_2 on rates of DOC release, total carbon fixation or POC : DOC was observed (Poulton et al., 2016).

Across all experiments, the response of net total community Chl *a* and net growth rates of small phytoplankton (<10 µm) scaled with pCO_2 treatment, and strongly correlated with in situ carbonate chemistry, whilst no relationships were found with any of the other wide range of initial physical, chemical or biological variables (Richier et al. 2018). Overall, the observed differences in regional response to carbonate chemistry manipulation could not be attributed to any other measured factor that varied systematically between temperate and

~~polar waters. These include ambient nutrient concentrations, which varied considerably but where direct manipulation had no influence on the response, and initial community structure, which was not a significant predictor of the response (Richier et al. 2018).~~

~~In summary, the relative response in both DMS(P) and a range of biological parameters (Richier et al. 2018) to CO_2 treatment in polar waters follows a distinctly different pattern to experiments performed in temperate waters. In the following sections we explore the possible drivers of the regional variability in response to OA.~~

The responses to OA observed for DMS and DMSP production are likely to be reflected in the dynamics of the DMSP-producing phytoplankton. In an assessment across all experiments, Richier et al. (2018) showed that the maximal response to OA of total Chl *a* and net growth rates of small phytoplankton (<10 µm) observed during each experiment, declined the most in relation to increased buffering capacity and temperature of the initial water. Generally, less significant relationships were found between the phytoplankton response and the other wide range of physical, chemical or biological variables that were examined (Richier et al. 2018).

In correspondence with the analyses carried out by Richier et al (2018), at 48 – 96 h (see Table 1), a statistically significant difference in response was seen between temperate and polar waters for Chl *a* (Kruskal-Wallis One Way ANOVA $H = 20.577$, $df = 1$, $p<0.001$). In general, at polar stations phytoplankton showed minimal response to elevated CO_2, in contrast to a strong negative response in temperate waters (Fig. 9A). By the second time point (96 – 144 h, see Table 1), no significant difference in response of Chl *a* between temperate and polar waters was apparent (Fig. 9B). As shown in Richier et al. (2014), phototrophic nanoflagellates responded to high CO_2 with large decreases in abundance in temperate waters and increases in abundance in polar waters (Fig. 9 C and D), with some exceptions: *North*

Sea and *South Sandwich* gave the opposite response. The responses had lessened by the second time point (96 – 168 h, see Table 1).

In contrast, bacterial abundance did not show the same regional differences in response to high CO_2 (see Hopkins and Archer (2014) for temperate waters, and Figure S1, supplementary information, for polar waters). Bacterial abundance in temperate waters gave variable and inconsistent responses to high CO_2. For all Arctic stations, *Drake Passage* and *Weddell Sea*, no response to high CO_2 was observed. For *South Georgia* and *South Sandwich*, bacterial abundance increased at 1000 and 2000 µatm, with significant increases for *South Georgia* after 144 h of incubation (ANOVA $F = 137.936$, $p<0.001$). Additionally, at Arctic stations *Greenland Gyre* and *Greenland Ice-edge*, no overall effect of increased CO_2 on rates of DOC release, total carbon fixation or POC : DOC was observed (Poulton et al. 2016).

Overall, the observed differences in the regional response of DMSP and DMS to carbonate chemistry manipulation could not be attributed to any other measured factor that varied systematically between temperate and polar waters. These include ambient nutrient concentrations, which varied considerably but where direct manipulation had no influence on the response, and initial community structure, which was not a significant predictor of the phytoplankton response (Richier et al. 2018).

4.2 Influence of community cell-size composition on DMS response

It has been proposed that variability in the concentrations of carbonate species (e.g. pCO_2, HCO_3^-, CO_3^{2-}) experienced by phytoplankton is related to cell size, such that smaller-celled taxa (<10 µm) with a reduced diffusive boundary layer are naturally exposed to relatively less variability compared to larger cells (Flynn et al., 2012). Thus, short-term and rapid changes in carbonate chemistry, such as the kind imposed during our microcosm experiments, may have a disproportionate effect on the physiology and growth of smaller celled species. Larger cells

559 may be better able to cope with variability as normal cellular metabolism results in significant
560 cell surface changes in carbonate chemistry parameters (Richier et al., 2014). Indeed, the
561 marked response in DMS concentrations to short term OA in temperate waters has been
562 attributed to this enhanced sensitivity of small phytoplankton (Hopkins and Archer, 2014).
563 Was the lack of DMS response to OA in polar waters therefore a result of the target
564 communities being dominated by larger-celled, less carbonate-sensitive species?

565 Size-fractionated Chl *a* measurements give an indication of the relative contribution of large
566 and small phytoplankton cells to the community. For experiments in temperate waters, the
567 mean ratio of >10 µm Chl *a* to total Chl *a* (hereafter *>10 µm : total*) of 0.32 ± 0.08 was lower
568 than the ratio for polar stations of 0.54 ± 0.13 (Table 2). Although the difference was not
569 statistically significant, this might imply a tendency towards communities dominated by
570 larger cells in the polar oceans, which may partially explain the apparent lack of DMS
571 response to elevated CO_2. However, this is not a consistent explanation for the observed
572 responses. For example, the Arctic *Barents Sea* station had the lowest observed *>10 µm :*
573 *total* of 0.04 ± 0.01, suggesting a community comprised almost entirely of <10 µm cells; yet
574 the response to short term OA differed to the response seen in temperate waters. No
575 significant CO_2 effects on DMS or DMSP concentrations or production rates were observed
576 at this station, whilst total Chl *a* significantly increased under the highest CO_2 treatments
577 after 96 h (PERMANOVA $F = 33.239$, ~~P~~*p*<0.001). Thus, our cell size theory does not hold
578 for all polar waters, suggesting that regardless of the dominant cell size, polar communities
579 are more resilient to OA. In the following section, we explore the causes of this apparent
580 insensitivity to OA in terms of the environmental conditions to which the communities have
581 presumably adapted.

582 **4.3 Adaptation to a variable carbonate chemistry environment**

Given that DMS production by polar phytoplankton communities appeared to be insensitive to experimental OA compared to significant sensitivity in temperate communities, we hypothesise that polar communities are adapted to greater natural variability in carbonate chemistry over spatial and seasonal scales. ~~Given the increased solubility of CO_2 in colder waters, the poceansare characterised by higher Revelle factors (Sabine et al. 2004, Eglestone et al. 2010) and as such input parameters~~

~~The variation in *in situ* surface ocean carbonate chemistry parameters for all three cruises (see Tynan et al. 2016 for details), is summarised in Figure 9. These data demonstrate both the latitudinal differences in surface ocean carbonate chemistry between temperate and polar waters, as well as the within-region variability which is controlled by the respective buffer capacities. Thus, a narrow range of values for all carbonate parameters was observed in the NW European shelf waters relative to the less well-buffered Arctic and Southern Ocean waters.~~ The polar waters sampled during our study were characterised by pronounced gradients in carbonate chemistry over small spatial scales, such that surface ocean communities are more likely to have experienced fluctuations between high pH/~~$\Omega_{aragonite}$~~ and low pH/~~$\Omega_{aragonite}$~~ over short time scales (Tynan et al., 2016). ~~For example~~Overall For example, in underway samples taken along each cruise track, pH_T varied by ~~only 0.152 units (8.20 22 - 8.0502) in NW European shelf waters (Rerolle et al. 2014, compared to~~ 0.435 units (8.005 - 8.457.7) in the Arctic, and 0.40 units (8.3025 - 7.9085) in the Southern Ocean (Tynan et al. 2016). By comparison, pH varied by 0.2 units (8.22 - 8.02) in underway samples from the NW European shelf sea cruise (Rerolle et al. 2014).

The observed horizontal gradients in polar waters were driven by different physical and biogeochemical processes in each ocean. In the Arctic Ocean, this variability in carbonate chemistry was partly driven by physical processes that controlled water mass composition, temperate and salinity, particularly in areas such as the Fram Strait and Greenland Sea. Along

the ice-edge and into the Barents Sea, biological processes exertedhas a strong control, as abundant iron resulted in high chlorophyll concentrations, low DIC and elevated pH. By contrast, variations in temperature and salinity had only a small influence on carbonate chemistry in the Southern Ocean in areas with iron limitation, and larger changes were driven by a combination of calcification, advection and upwelling. Where iron was replete, e.g. near South Georgia, biological DIC drawdown had a large impact on carbonate chemistry (Tynan et al. 2016). A further set of processes was in play in sea ice influenced regions. At the Arctic ice edge, abundant iron drove strong bloom development along the ice edge, whilst sea ice retreat in the Southern Ocean was not always accompanied by iron release (Tynan et al. 2016). In summary, this demonstrates the high variability of pH and other carbonate chemistry parameters to which polar surface ocean communities may experience. This could drive the communities towards adaptation and resilience to experimentally-induced OA. Of course, it is important to recognise that this data represents only a snapshot (4 – 6 weeks) of a year, and thus does not contain information on the range in variability over daily and seasonal cycles, timescales which Although it might be expected considered most important in terms of the carbonate system variability experienced by the cells and how this drives CO_2 sensitivity that carbonate system variability on the level 'experienced' by the cells, i.e. ~daily cellular level variability, might be the most important factor driving sensitivity (Flynn et al. 2012; Richier et al. 2018)., our data represent only a snapshot (4 – 6 weeks) of a year, and thus do not contain information on the range in variability over seasonal cycles. Nevertheless, this inherent carbonate chemistry variability experienced by organisms living in polar waters may equip them with the resilience to cope with both experimental and future OA.

For comparison with Arctic stations, Hagens and Middelburg (2016) report a seasonal pH variability of up to 0.25 units from a single site in the open ocean surface waters in the Iceland Sea, whilst Kapsenberg et al. (2015) report an annual variability of 0.3 – 0.4 units in the McMurdo Sound, Antarctica. This implies that both ~~polar open ocean and coastal/~~open ocean and sea ice-influenced polar waters ~~locations~~ experience ~~equally~~ large variations in carbonate chemistry over seasonal cycles. By contrast, monthly averaged surface pCO_2 data collected from station L4 in the Western English Channel over the period 2007 – 2011 provides an example of typical carbonate chemistry dynamics in NW European shelf sea waters. Over this period, pH had an annual range of 0.15 units (8.05 – 8.20), accompanied by a range in pCO_2 of 302 – 412 µatm (Kitidis et al., 2012).

~~In open ocean waters this is driven by enhanced drawdown of C_T and CO_2 during the productive spring and summer months, countered by lower productivity and strong mixing in the winter . In coastal and sea-ice affected regions, seasonal pH variability may be enhanced further by tidal exchanges, and by dilution of C_T/A_T caused by sea-ice melt .~~ The sea ice environment in particular is characterised by strong spatial and seasonal variability in carbonate chemistry. Sea~~The~~ ice ~~itself~~ is inhabited by a specialised microbial community with a complex set of metabolic and physiological adapatations allowing these organisms to withstand wide fluctuations in pH up to as high as 9.9 in brine channels to as low as 7.5 in the under-ice water (Thomas and Dieckmann, 2002; Rysgaard et al., 2012; Thoisen et al., 2015). The open waters associated with the ice edge also experience strong gradients in pH and other carbonate chemistry parameters. This can be attributed to two processes: 1. The ~~S~~strong seasonal drawdown of DIC due to rapid biological uptake by phytoplankton blooms at the productive ice edge which drives up pH. On the Arctic cruise, increases of up to 0.33 pH units were attributed to such processes in this region (Tynan et al., 2016). The effect was less dramatic in the Fe-limited and less productive Weddell Sea with gradients in pH ranging

from 8.20 – 8.10 (Tynan et al., 2016). 2. The drawdown of DIC is countered by the release and accumulation of respired DIC under sea ice due to the degradation of organic matter. However, this accumulation occurs in subsurface/bottom waters, which are isolated from the productive surface mixed layer by strong physical stratification and hence, of less relevance to the current study.

~~However, t~~The influence of sea ice on carbonate chemistry combined with the strong biological drawdown of DIC in polar waters ~~The above factors~~ may have influenced the ability of some of the communities we sampled during our study to withstand the short term changes to carbonate chemistry they experienced within the bioassays. Two of our sampling stations were 'sea-ice influenced': *Greenland Ice Edge* and *Weddell Sea*. Both were in a state of sea ice retreat as our sampling occurred in the summer months. Sampling for the *Greenland Ice Edge* station was performed in open, deep water, near to an area of thick sea ice, with low fluorescence but reasonable numbers of diatoms (Leakey, 2012). Similarly, the *Weddell Sea* station was located near the edge of thick pack ice but in an area of open water that allowed sampling to occur without hindrance by brash ice (Tarling, 2013). At both stations we saw little or no response in DMS or DMSP to experimental acidification, which may imply that the *in situ* communities were more or less adapted to fluctuations in pH. ~~For comparison, o~~Our experimental OA resulted in pH decreases of between 0.4 and 0.7 units. However, it is unclear whether the communities we sampled were able to withstand the artificial pH perturbation because they were adapted to living in sea ice, or whether they had adapted to cope with other fluctuations in carbonate chemistry that occur in polar ~~ice-edge~~ waters.

In summary, this demonstrates the high variability in carbonate chemistry, including pH, which polar communities may experience relative to their temperate counterparts. This may have resulted in adapted communities resilient to experimentally-induced OA. Of course, it is

important to recognise that this data represent only a snapshot (4 – 6 weeks) of a year, and thus does not contain information on the range in variability over daily and seasonal cycles, timescales which might be considered most important in terms of the carbonate system variability experienced by the cells and how this drives CO_2 sensitivity (Flynn et al. 2012; Richier et al. 2018). Nevertheless, this inherent carbonate chemistry variability experienced by organisms living in polar waters may equip them with the resilience to cope with both experimental and future OA.

Adaptation to such natural variability may induce the ability to resist abrupt changes within the polar biological community (Kapsenberg et al., 2015). This is manifested here as negligible impacts on rates of *de novo* DMSP synthesis and net DMS production. A number of previous studies in polar waters have reported similar findings. Phytoplankton communities were able to tolerate a pCO_2 range of 84 – 643 µatm in ~12 d minicosm experiments (650 L) in Antarctic coastal waters, with no effects on nanophytoplankton abundance, and enhanced abundance of picophytoplankton and prokaryotes (Davidson et al., 2016; Thomson et al., 2016). In experiments under the Arctic ice, microbial communities demonstrated the capacity to respond either by selection or physiological plasticity to elevated CO_2 during short term experiments (Monier et al., 2014). Subarctic phytoplankton populations demonstrated a high level of resilience to OA in short term experiments, suggesting a high level of physiological plasticity that was attributed to the prevailing strong gradients in pCO_2 levels experienced in the sample region (Hoppe et al., 2017). Furthermore, a more recent study describing ten CO_2 manipulation experiments in Arctic waters found that primary production was largely insensitive to OA over a large range of light and temperature levels (Hoppe et al., 2018). This supports our hypothesis that, relative to temperate communities, polar microbial communities may have a high capacity to compensate for environmental variability (Hoppe et al., 2018), and are thus already adapted to, and are able

to tolerate, large variations in carbonate chemistry. Thus by performing multiple, replicated experiments over a broad geographic range, the findings of this study imply that the DMS response may be both a reflection of: (i) the level of sensitivity of the community to changes in the mean state of carbonate chemistry, and (ii) the ~~levels of~~ regional variability in carbonate chemistry experienced by different communities. This highlights the limitations associated with simple extrapolation of results from a small number of geographically-limited experiments e.g. Six et al. (2013). Such an approach lacks a mechanistic understanding that would allow a model to capture the regional variability in response that is apparent from the microcosms experiments presented here.

4.4 Comparison to an Arctic mesocosm experiment

Experimental data clearly provide useful information on the potential future DMS response to OA, but these data become most powerful when incorporated in Earth System Models (ESM) to facilitate predictions of future climate. To date, two modelling studies have used ESM to assess the potential climate feedback resulting from the DMS sensitivity to OA (Six et al., 2013;Schwinger et al., 2017), and both have used results from mesocosm experiments. However, the DMS responses to OA within our short term microcosm experiments contrast with the results of most previous mesocosm experiments, and of particular relevance to this study, an earlier Arctic mesocosm experiment (Archer et al., 2013). Whilst no response in DMS concentrations to OA was generally seen in the polar microcosm experiments discussed here, a significant decrease in DMS with increasing levels of CO_2 in the earlier mesocosm study was seen. Therefore, it is useful to consider how the differences in experimental design, and other factors, between microcosms and mesocosms may result in contrasting DMS responses to OA.

The short duration of the microcosm experiments (4 – 7 d) allows the physiological (phenotypic) capacity of the community to changes in carbonate chemistry to be assessed. In

732 other words, how well is the community adapted to variable carbonate chemistry and how
733 does this influence its ability to acclimate to change? Although the mesocosm experiment
734 considered a longer time period (4 weeks), the first few days can be compared to the
735 microcosms. No differences in DMS or DMSP concentrations were detected for the first
736 week of the mesocosm experiment, implying a certain level of insensitivity of DMS
737 production to the rapid changes in carbonate chemistry. In fact, when taking all previous
738 mesocosm experiments into consideration, differences in DMS concentrations have
739 consistently been undetectable during the first 5 – 10 days, implying there is a limited short-
740 term physiological response by the in situ communities (Hopkins et al., 2010; Avgoustidi et
741 al., 2012; Vogt et al., 2008; Kim et al., 2010; Park et al., 2014). This is in contrast to the
742 strong response in the temperate microcosms from the NW European shelf (Hopkins and
743 Archer, 2014). However, all earlier mesocosm experiments have been performed in coastal
744 waters, which like polar waters, can experience a large natural range in carbonate chemistry.
745 In the case of coastal waters this is driven to a large extent by the influence of riverine
746 discharge and biological activity (Fassbender et al., 2016). Thus coastal communities may
747 also possess a higher level of adaptation to variable carbonate chemistry compared to the
748 open ocean communities of the temperate microcosms (Fassbender et al., 2016).

749 The later stages of mesocosm experiments address a different set of hypotheses, and are less
750 comparable to the microcosms reported here. With time, an increase in number of generations
751 leads to community structure changes and taxonomic shifts, driven by selection on the
752 standing genetic variation in response to the altered conditions. Moreover, the coastal Arctic
753 mesocosms were enriched with nutrients after 10 days, affording relief from nutrient
754 limitation and allowing differences between pCO_2 treatments to be exposed, including a
755 strong DMS(P) response.(Archer et al., 2013; Schulz et al., 2013). During this period of
756 increased growth and productivity, CO_2 increases drove changes which reflected both the

757 physiological and genetic potential within the community, and resulted in taxonomic shifts.
758 The resultant population structure was changed, with an increase in abundance of
759 dinoflagellates, particularly *Heterocapsa rotundata*. Increases in DMSP concentrations and
760 DMSP synthesis rates were attributed to the population shift towards dinoflagellates. The
761 drivers of the reduced DMS concentrations were less clear, but may have been linked to
762 reduced DMSP-lyase capacity within the dominant phytoplankton, a reduction in bacterial
763 DMSP lysis, or an increase in bacterial DMS consumption rates (Archer et al., 2013). Again,
764 this is comparable to all other mesocosm experiments, wherein changes to DMS
765 concentrations can be associated with CO_2-driven shifts in community structure (Hopkins et
766 al., 2010; Avgoustidi et al., 2012; Vogt et al., 2008; Kim et al., 2010; Park et al., 2014; Webb
767 et al., 2015). However, given the lack of further experiments of a similar location, design and
768 duration to the Arctic mesocosm, it is unclear how representative the mesocosm result is of
769 the general community-driven response to OA in high latitude waters.

770 We did not generally see any broad-scale CO_2-effects on community structure in polar
771 waters. This can be demonstrated by a lack of significant differences in the mean ratio of >10
772 µm Chl *a* to total Chl *a* (*>10 µm : total*) between CO_2 treatments, implying there were no
773 broad changes in community composition (Table 2). *South Sandwich* was an exception to
774 this, where large and significant increases in the mean ratio of *>10 µm : total* were observed
775 at 750 µatm and 2000 µatm CO_2 relative to ambient CO_2 (ANOVA, $F = 207.144$, $p<0.001$, $df = 3$), demonstrated at even the short timescale of the microcosm experiments, it is possible
776 = 3), demonstrated at even the short timescale of the microcosm experiments, it is possible
777 for some changes to community composition to occur. Interestingly, this was also the only
778 polar station that exhibited any significant effects on DMS after 96 h of incubation (Figure
779 ~~3G~~4D). However, given the lack of similar response at 1000 µatm, it remains equivocal
780 whether this was driven by a CO_2-effect or some other factor. The results of our microcosm
781 experiments suggest insensitivity of *de novo* DMSP production and net DMS production in

782 the microbial communities of the polar open oceans to short term changes in carbonate
783 chemistry. This may be driven by a high level of adaptation within the targeted
784 phytoplankton communities to naturally varying carbonate chemistry.

785 In contrast to our findings, a recent single 9 day microcosm experiment (Hussherr et al.,
786 2017) performed in Baffin Bay (Canadian Arctic) saw a linear 80% decrease in DMS
787 concentrations during spring bloom-like conditions. It should be noted that this response was
788 seen over a range of pCO_2 from 500 - 3000 µatm, far beyond the levels used in the present
789 study. Nevertheless, this implies that polar DMS production may be sensitive to OA at certain
790 times of the year, such as during the highly productive spring bloom, but less sensitive during
791 periods of low and stable productivity, such as the summer months sampled during this study.
792 Furthermore, a number of other studies from both the Arctic e.g. (Coello-Camba et al., 2014;
793 Holding et al., 2015; Thoisen et al., 2015) and the Southern Ocean e.g. (Trimborn et al.,
794 2017; Tortell et al., 2008; Hoppe et al., 2013) suggest that polar phytoplankton communities
795 can demonstrate sensitivity to OA, in contrast to our findings. This emphasises the need to
796 gain a more detailed understanding of both the spatial and seasonal variability in the polar
797 phytoplankton community and associated DMS response to changing ocean acidity.

798 **5 Conclusions**

799 We have shown that net DMS production by summertime polar open ocean microbial
800 communities is insensitive to OA during multiple, highly replicated short term microcosm
801 experiments. We provide evidence that, in contrast to temperate communities (Hopkins and
802 Archer, 2014), the polar communities we sampled were relatively insensitive to variations in
803 carbonate chemistry (Richier et al., 2018), manifested here as a minimal effect on net DMS
804 production. Our findings contrast with two previous studies performed in Arctic waters
805 (Archer et al. 2013; Hussherr et al. 2017) which showed significant decreases in DMS in

response to OA. These discrepancies may be driven by differences in the sensitivity of microbial communities to changing carbonate chemistry between different areas, or by variability in the response to OA depending on the time of year, nutrient availability, and ambient levels of growth and productivity. This serves to highlight the complex spatial and temporal variability in DMS response to OA which warrants further investigation to improve model predictions.

Our results imply that the phytoplankton communities of the temperate microcosms initially responded to the rapid increase in pCO_2 via a stress-induced response, resulting in large and significant increases in DMS concentrations occurring over the shortest timescales (2 days), with a lessening of the treatment effect with an increase in incubation time (Hopkins and Archer 2014).

The dominance of short response timescales in well-buffered temperate waters may also indicate rapid acclimation of the phytoplankton populations following the initial stress response, which forced the small-sized phytoplankton beyond their range of acclimative tolerance and lead to increased DMS (Richier et al. 2018, Hopkins and Archer 2014). This supports the hypothesis that populations from higher latitude, less well-buffered waters, already possess a certain degree of acclimative tolerance to variations in carbonate chemistry environment. Although initial community size structure was not a significant predictor of the response to high CO_2, it is possible that a combination of both community composition and the natural range in variability in carbonate chemistry – as a function of buffer capacity – may influence the DMS/P response to OA over a range of timescales (Richier et al. 2018).

Our findings should be considered in the context of timescales of change (experimental vs real world OA) and the potential of microbial communities to adapt to a gradually changing environment. Microcosm experiments focus on the physiological response of microbial

communities to short term OA. Mesocosm experiments consider a timescale that allows the response to be driven by community composition shifts, but are not long enough in duration to incorporate an adaptive response. Neither approach is likely to accurately simulate the response to the gradual changes in surface ocean pH that will occur over the next 50 – 100 years, nor the resulting changes in microbial community structure and distribution. However, we hypothesise that the DMS response to OA should be considered not only in relation to experimental perturbations to carbonate chemistry, but also in relation to the magnitude of background variability in carbonate chemistry experienced by the DMS-producing organisms and communities. Our findings suggest a strong link between the DMS response to OA and background regional variability in the carbonate chemistry.

Models suggest the climate may be sensitive to changes in the spatial distribution of DMS emissions over global scales (Woodhouse et al., 2013; Menzo et al., 2018). Such changes could be driven by both physiological and adaptive responses to environmental change. Accepting the limitations of experimental approaches, our findings suggest that net DMS production from polar oceans may be resilient to OA in the context of its short term effects on microbial communities. The oceans face a multitude of CO_2-driven changes in the coming decades, including OA, warming, deoxygenation and loss of sea ice (Gattuso et al., 2015). Our study addresses only one aspect of these future ocean stressors, but contributes to our understanding of how DMS emissions from the polar oceans may alter, facilitating a better understanding of Earth's future climate.

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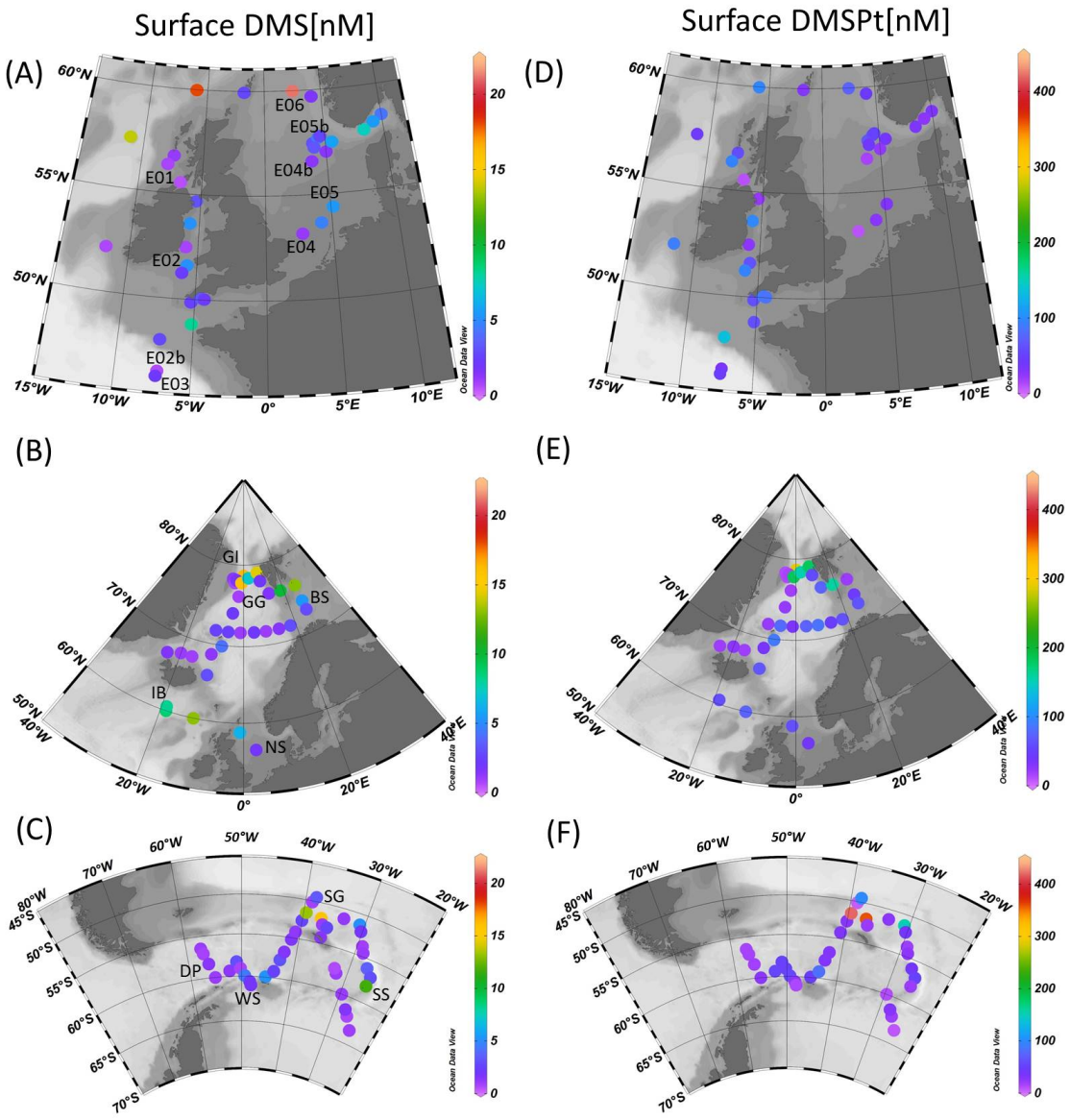
866

867 Table 1. Summary of the station locations and characteristic of the water sampled for the 18 microcosm experiments performed in temperate,
868 sub-polar and polar waters. All polar stations were sampled for JR271 and JR274, with the exception of NS and IB.

Cruise	Station ID	Location	Sampling location	Sampling date	Sampling depth (m)	SST (°C)	Salinity	Nitrate (uM)	Total Chl *a* ($\mu g\ L^{-1}$)	$chl_{>10\ \mu m}$: chl_{total}	pCO_2 (µatm) T_0	pH (total) T_0	Experimental timepoints T_1, T_2 (hours)	Reference
D366	E01	Mingulay Reef	56°47.688N 7°24.300W	8 June 2011	6	11.3	34.8	1.1	3.3	no data	334.9	8.1	48, 96	*Hopkins & Archer (2*
	E02	Irish Sea	52°28.237N 5°54.052W	14 June 2011	5	11.8	34.4	0.3	3.5	0.80 ± 0.03	329.3	8.1	48, 96	*Hopkins & Archer (2*
	E02b	Bay of Biscay	46°29.794N 7°12.355W	19 June 2011	5	14.5	35.6	0.9	1.8	no data	340.3	8.1	48	*This study*
	E03	Bay of Biscay	46°12.137N 7°13.253W	21 June 2011	10	15.3	35.8	0.6	0.8	0.43 ± 0.03	323.9	8.1	48, 96	*Hopkins & Archer (2*
	E04	Southern North Sea	52°59.661N 2°29.841E	26 June 2011	5	14.6	34.1	0.9	1.3	0.19 ± 0.02	399.8	8.0	48, 96	*Hopkins & Archer (2*
	E04b	Mid North Sea	57°45.729N 4°35.434E	29 June 2011	5	13.2	34.8	No data	0.5	0.14 ± 0.003	327.3	8.1	48	*This study*
	E05	Mid North Sea	56°30.293N 3°39.506E	2 July 2011	12	14.0	35.0	0.2	0.3	0.23 ± 0.01	360.2	8.1	48, 96	*Hopkins & Archer (2*
	E05b	Atlantic Ocean	59°40.721N 4°07.633E	3 July 2011	4	13.4	30.7	0.3	0.7	0.12 ± 0.01	310.7	8.1	48	*This study*
	E06	Atlantic Ocean	59°59.011N 2°30.896E	3 July 2011	4	12.5	34.9	0.4	1.1	0.14 ± 0.01	287.1	8.2	48	*This study*
JR271	NS	Mid North Sea	56°15.59N 2°37.59E	3 June 2012	15	10.8	35.1	0.04	0.3	0.52 ± 0.05	300.5	8.2	48, 96	*This study*
	IB	Iceland Basin	60°35.39N 18°51.23W	8 June 2012	7	10.7	35.2	5.0	1.8	0.27 ± 0.02	309.7	8.1	48, 96	*This study*
	GG-AO	Greenland Gyre	76°10.52 N 2°32.96 W	13 June 2012	5	1.7	34.9	9.3	1.0	0.34 ± 0.001	289.3	8.2	48, 96	*This study*
	GI-AO	Greenland ice edge	78°21.15 N 3°39.85 W	18 June 2012	5	-1.6	32.6	4.2	2.7	0.78 ± 0.03	304.7	8.1	48, 96	*This study*
	BS-AO	Barents Sea	72°53.49 N 26°00.09 W	24 June 2012	5	6.6	35.0	5.4	1.3	0.04 ± 0.01	304.3	8.1	48, 96	*This study*
JR274	DP-SO	Drake Passage	58°22.00 S 56°15.12 W	13 Jan 2013	8	1.9	33.2	22.0	2.4	1.00 ± 0.06	279.3	8.2	48, 96	*This study*
	WS-SO	Weddell Sea	60°58.55 S 48°05.19 W	18 Jan 2013	6	-1.4	33.6	24.9	0.6	0.67 ± 0.06	510.5	7.9	72, 144	*This study*
	SG-SO	South Georgia	52°41.36 S 36°37.28 W	25 Jan 2013	5	2.2	33.9	24.1	0.7	0.35 ± 0.04	342.6	8.1	72, 144	*This study*
	SS-SO	South Sandwich	58°05.13 S 25°55.55 W	1 Feb 2013	7	0.5	33.7	18.5	4.6	0.57 ± 0.02	272.6	8.2	96, 168	*This study*

869 Table 2. Mean (± SD) ratio of >10µm Chl *a* to total Chl *a* ($chl_{>10µm}$:chl_{total}) for polar
870 microcosm sampling stations. * indicates significant difference from the response to ambient
871 CO_2.

Station	Time	ambient	550 µatm	750 µatm	1000 µatm	2000 µatm
GG						
	48 h	0.3 ± 0.1	0.3 ± 0.03	0.4 ± 0.2	0.3 ± 0.1	N/A
	96 h	1.0 ± 0.02	0.9 ± 0.2	0.8 ± 0.1	0.7 ± 0.2	
GI						
	48 h	1.0 ± 0.1	1.0 ± 0.1	0.8 ± 0.1	1.0 ± 0.0	N/A
	96 h	1.0 ± 0.1	1.1 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	
BS						
	48 h	0.02 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	N/A
	96 h	0.04 ± 0.01	0.05 ± 0.04	0.05 ± 0.04	0.04 ± 0.04	
DP						
	48 h	1.0 ± 0.3	N/A	1.0 ± 0.1	N/A	N/A
	96 h	0.9 ± 0.1		1.0 ± 0.1		
WS						
	72 h	0.6 ± 0.1	N/A	0.7 ± 0.1	N/A	N/A
	144 h	0.7 ± 0.1		0.7 ± 0.1		
SG						
	72 h	0.3 ± 0.02	N/A	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.03
	144 h	0.5 ± 0.1		0.6 ± 0.04	0.5 ± 0.1	0.4 ± 0.03
SS						
	96 h	0.7 ± 0.04	N/A	1.5 ± 0.1*	0.7 ± 0.02	1.6 ± 0.1*
	168 h	0.9 ± 0.2		1.4 ± 0.02*	0.8 ± 0.004	1.4 ± 0.2*



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873 Figure 1. Surface (<5 m) concentrations (nM) of DMS (A-C) and total DMSP (D-F) for
874 cruises in the NW European shelf (D366) (A,D), the sub-Arctic and Arctic Ocean (JR271)
875 (B,E) and the Southern Ocean (JR274) (C,F). Locations of sampling stations for microcosm
876 experiments shown in letters/numbers. E01 – E05: see Hopkins & Archer 2014. NS = *North*
877 *Sea*, IB = *Iceland Basin*, GI = *Greenland Ice-edge*, GG = *Greenland Gyre*, BS = *Barents Sea*,
878 DP = *Drake Passage*, WS = *Weddell Sea*, SG = *South Georgia*, SS = *South Sandwich*.

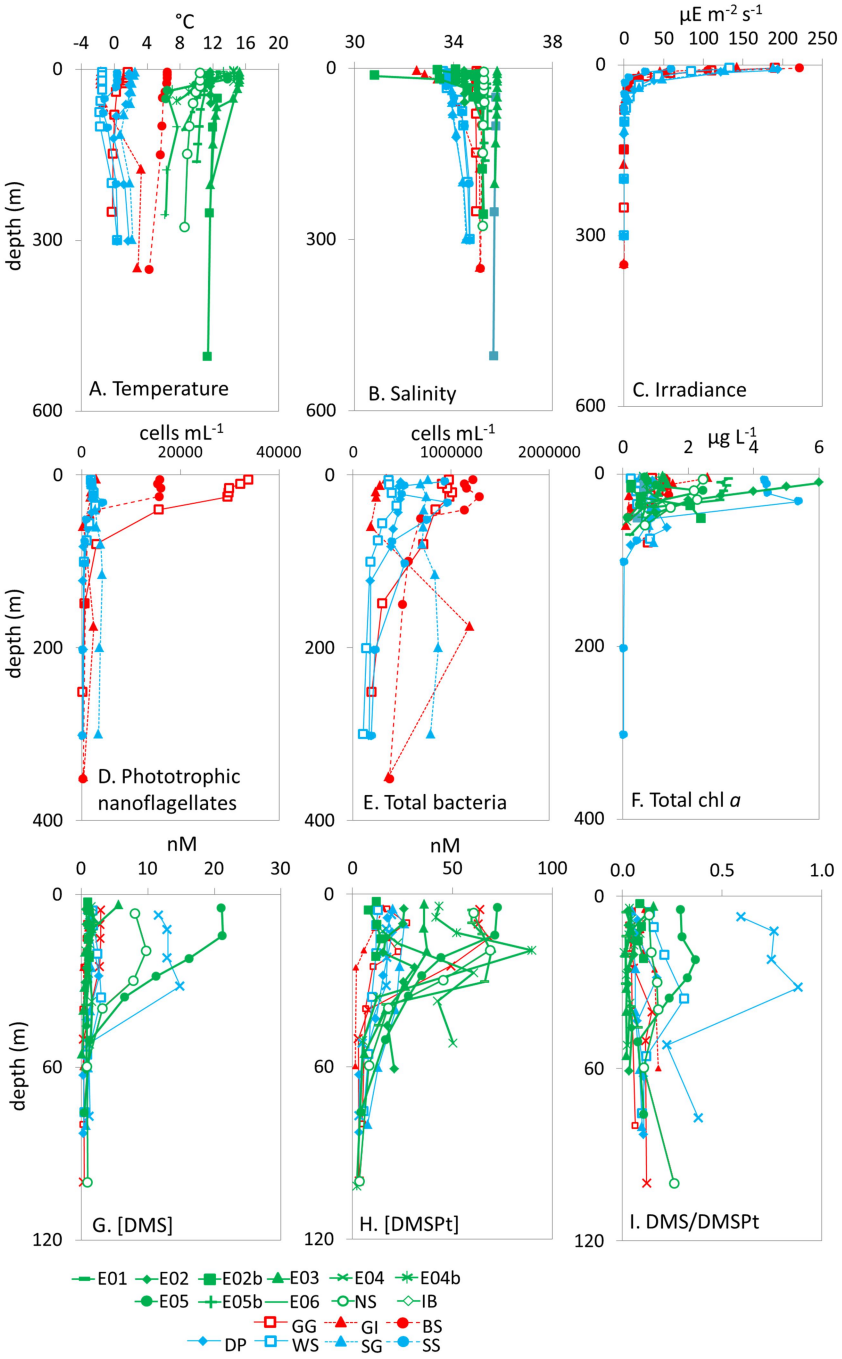
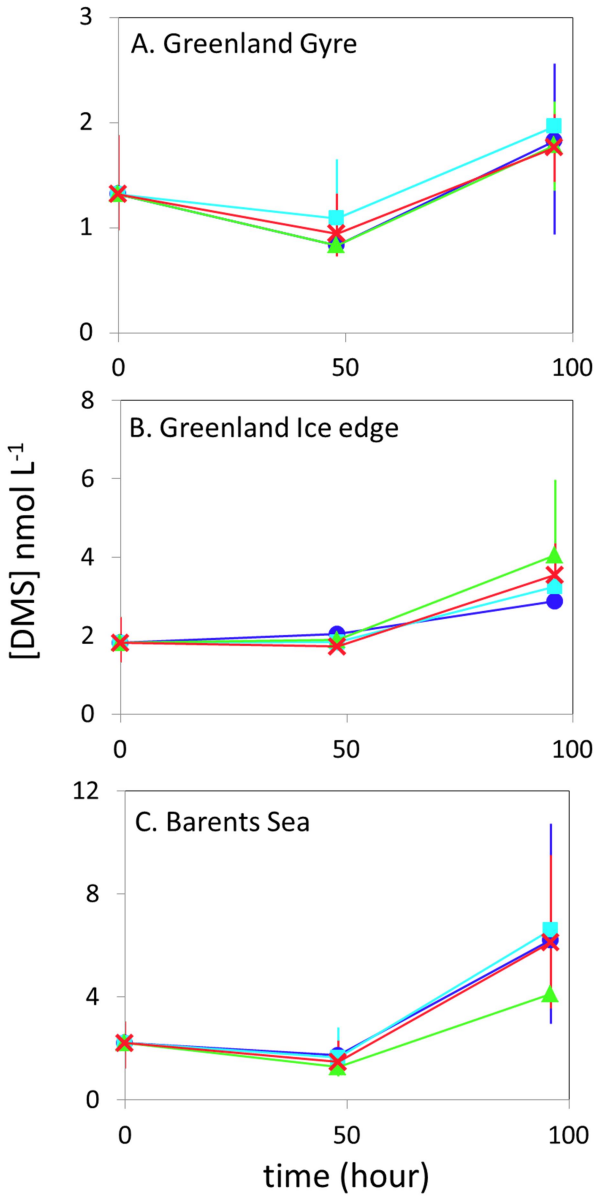


Figure 2. Depth profiles for all 18 sampling stations showing A. Temperature (°C), B. Salinity, C. Irradiance (µE m^{-2} s^{-1}), D. phototrophic nanoflagellate abundance (cells mL^{-1}), E. total bacteria abundance (cells mL^{-1}), F. total Chl a (µg L^{-1}), G. [DMS] (nM), H. total [DMSP] (nM) and I. DMS/DMSPt from CTD casts at sampling stations for microcosm experiments in temperate (green), Arctic (red) and Southern Ocean (blue) waters. See Table 1 for station details. Data for irrandiance, phototrophic nanoflagellates and total bacteria were not collected for temperate stations.

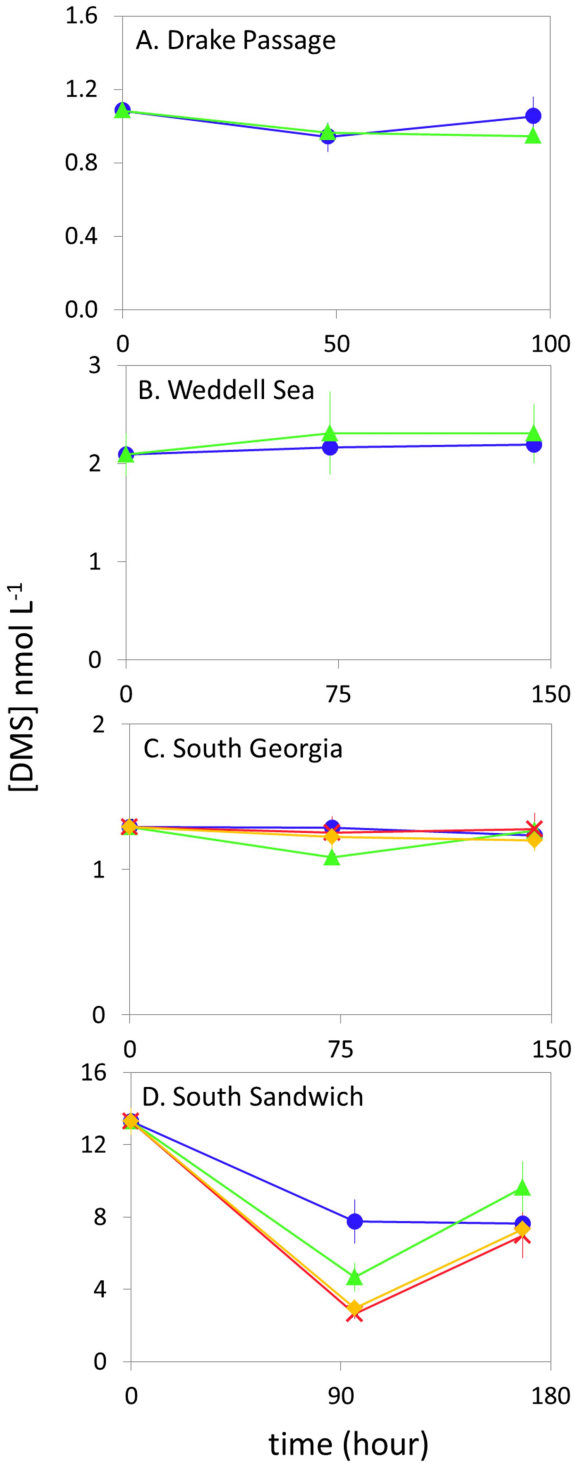


CO_2 treatment	pCO_2 (µatm) mean ± SD, n = 3		
	0 h	48 h	96 h
Ambient ●	326.8	No data	251.5 ± 5.1
Mid ■	565.2	493.8 ± 49.0	416.7 ±18.5
High ▲	741.8	607.6 ± 53.9	526.1 ± 18.1
High+ ×	1012.2	824.1 ± 99.5	668.6 ± 33.6

CO_2 treatment	pCO_2 (µatm) mean ± SD, n = 3		
	0 h	48 h	96 h
Ambient ●	312.2	281.1 ± 1.8	201.1 ± 3.4
Mid ■	583.9	444.2 ± 9.4	301.3 ± 8.4
High ▲	789.3	576.3 ± 35.2	412.6 ± 78.6
High+ ×	948.2	803.6 ± 10.5	483.8 ± 38.1

CO_2 treatment	pCO_2 (µatm) mean ± SD, n = 3		
	0 h	48 h	96 h
Ambient ●	310.6	291.8 ± 10.6	289.9 ± 1.5
Mid ■	535.1	482.8 ± 4.9	483.5 ± 18.6
High ▲	649.1	653.7 ± 3.6	547.0 ± 28.4
High+ ×	683.6	763.0 ± 12.4	673.5 ± 65.7

Figure 3. DMS concentrations (nmol L^{-1}) during experimental microcosms performed in Arctic waters ~~(A – C) and in Southern Ocean waters (D – G)~~. Data shown is mean of triplicate incubations, and error bars show standard error on the mean. Tables show measurements of pCO_2 (µatm) for each treatment at each sampling time point. Initial measurements (0 h) were from a single sample, whilst measurements at 48 h and 96 h show mean ± SD of triplicate experimental bottles. Locations of water collection for microcosms shown in Figure 1 C – F.



CO_2 treatment	pCO_2 (µatm) mean ± SD, n = 3		
	0 h	48 h	96 h
Ambient ●	287.0	237.8 ± 8.3	244.4 ± 11.2
High ▲	598.2	529.6 ± 80.3	491.6 ± 116.2

CO_2 treatment	pCO_2 (µatm) mean ± SD, n = 3		
	0 h	72 h	144 h
Ambient ●	275.1	554.2 ± 6.4	534.4 ± 30.5
High ▲	533.8	1280.8 ± 11.7	1177.6 ± 148.2

CO_2 treatment	pCO_2 (µatm) mean ± SD, n = 3		
	0 h	72 h	144 h
Ambient ●	342.6	342.4 ± 9.1	352.3 ± 34.7
High ▲	No data	639.9 ± 13.4	569.8 ± 36.5
High+ ×	823.4	809.1 ± 18.1	663.5 ± 11.5
High++ ◆	1410.4	1384.1 ± 82.7	1317.1 ± 54.0

CO_2 treatment	pCO_2 (µatm) mean ± SD, n = 3		
	0 h	96 h	168 h
Ambient ●	283.8	231.1 ± 3.3	187.2 ± 23.3
High ▲	No data	541.2 ± 73.2	362.4 ± 30.8
High+ ×	773.2	592.7 ± 62.5	421.4 ± 56.4
High++ ◆	1557.5	1122.3 ± 15.2	796.6 ± 45.0

Figure 4. DMS concentrations (nmol L^{-1}) during experimental microcosms performed in Southern Ocean waters. Data shown is mean of triplicate incubations, and error bars show standard error on the mean. Tables show measurements of pCO_2 (µatm) for each treatment at each sampling time point. Initial measurements (0 h) were from a single sample, whilst measurements at 48 h and 96 h show mean ± SD of triplicate experimental bottles. Locations of water collection for microcosms shown in Figure 1 C – F.

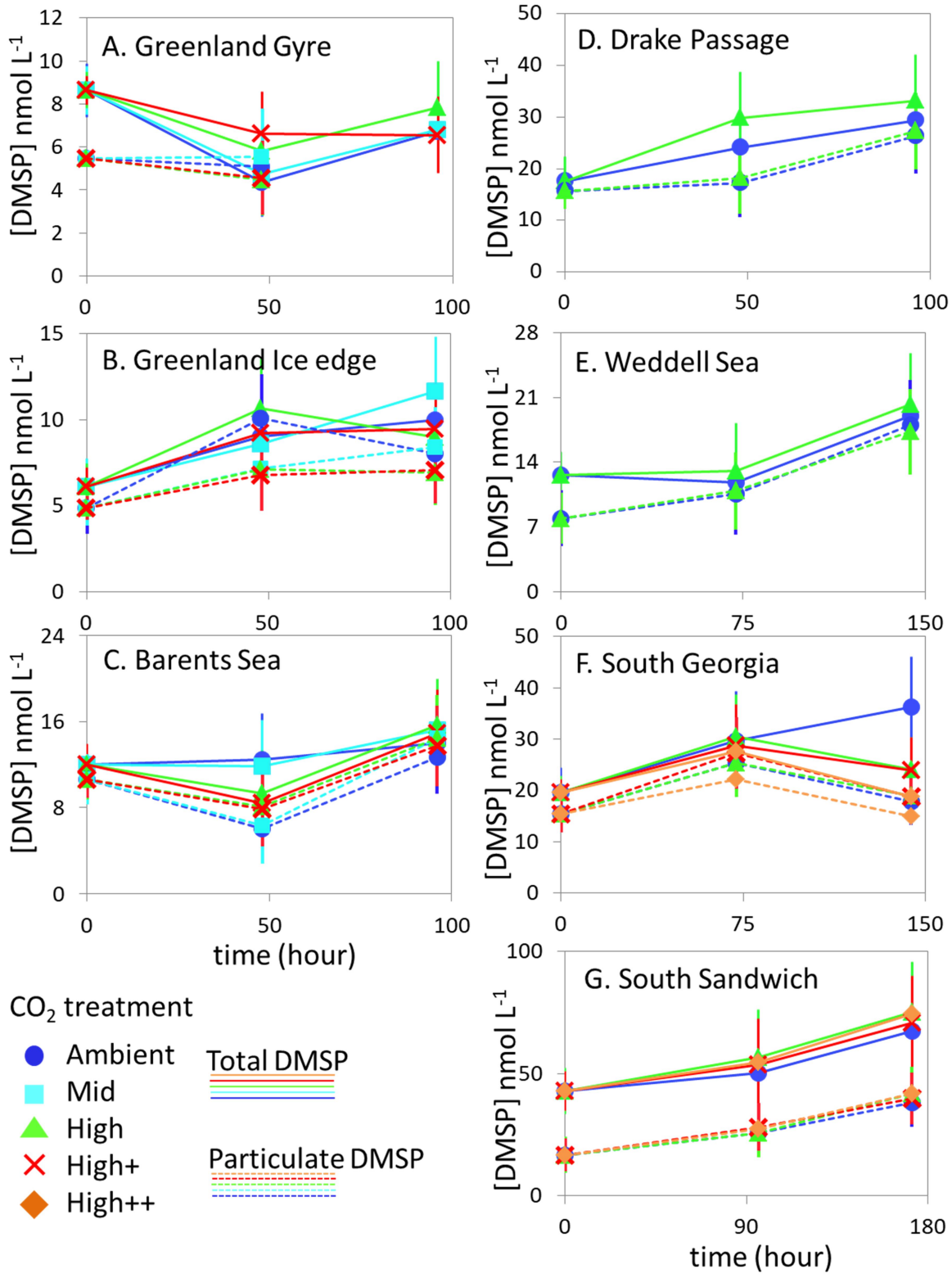


Figure 45. Total DMSP (solid lines) and particulate DMSP (dashed lines) concentrations (nmol L^{-1}) during experimental microcosms performed in Arctic waters (A - C) and in Southern Ocean waters (D – G). Data shown is mean of triplicate incubations, and error bars show standard error on the mean. Locations of water collection for microcosms shown in Figure 1 C – F. Particulate DMSP concentrations were used in calculations of DMSP production rates (Figure 6).

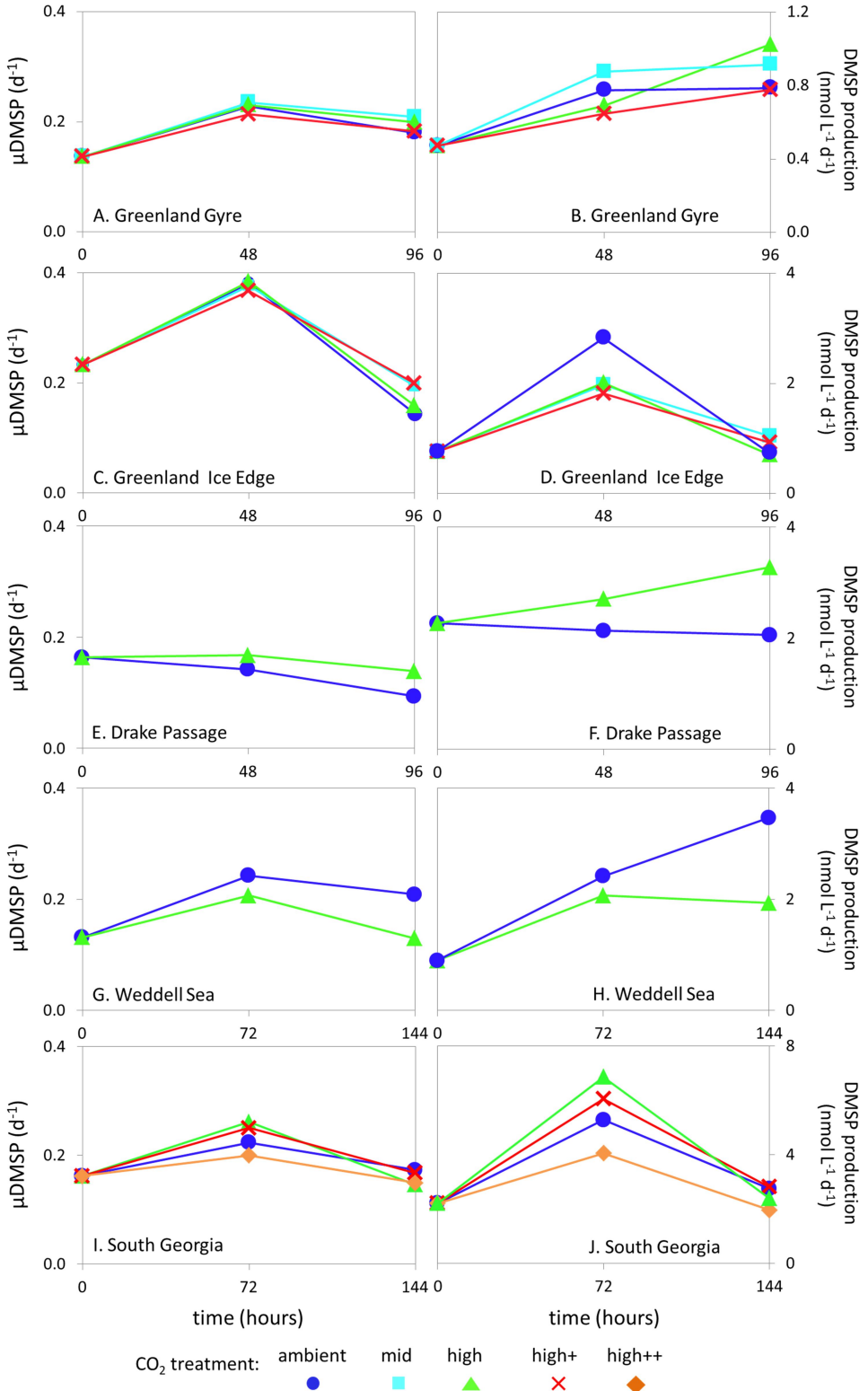


Figure ~~5~~6. De novo synthesis of DMSP (µDMSP, d^{-1}) (left column) and DMSP production rates (nmol L^{-1} d^{-1}) (right column) for Arctic Ocean stations *Greenland Gyre* (A,B), *Greenland Ice-edge* (C, D) and Southern Ocean stations *Drake Passage* (E, F), *Weddell Sea* (G, H) and *South Georgia* (I, J). No data is available for *Barents Sea* (Arctic Ocean) or *South Sandwich* (Southern Ocean).

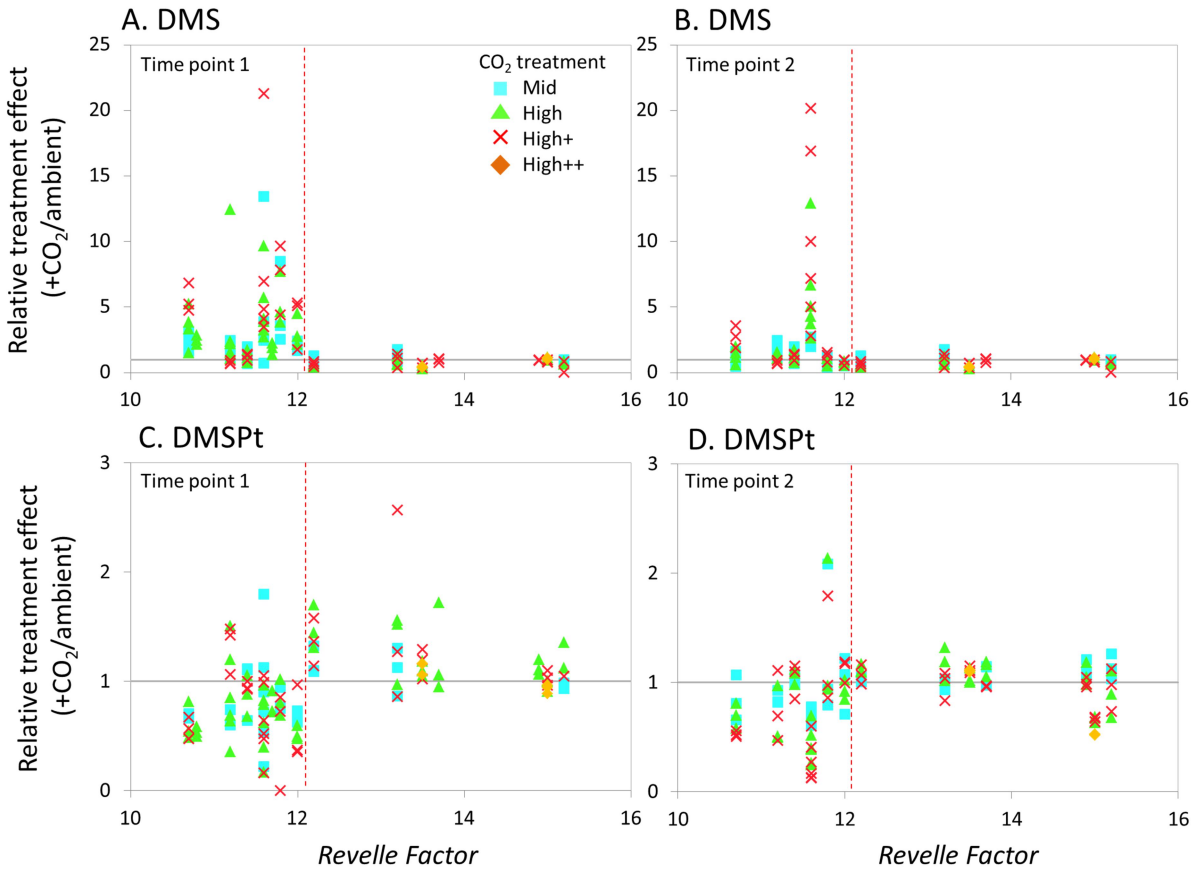


Figure ~~6~~7. Relationship between ~~the ratio of dissolved inorganic carbon C_T to total alkalinity (C_T/A_T)~~Revelle Factor of the sampled water and the relative CO_2 treatment effect at ($[x]_{highCO2}/[x]_{ambientCO2}$) for concentrations of DMS at T_1 (A) and T_2 (B), and for total DMSP concentrations at T_1 (C) and T_2 (D) for all microcosm experiments performed in NW European waters, sub-Arctic and Arctic waters, and the Southern Ocean. Grey solid line (= 1) indicates no effect of elevated CO_2. ~~C_T/A_T>0.91~~Revelle Factor > 12 = polar waters (indicated by red dashed line). T_1 = 48 h, except for WS and SG (72 h) and SS (96 h). For detailed analyses of the NW European shelf data, see Hopkins & Archer (2014).

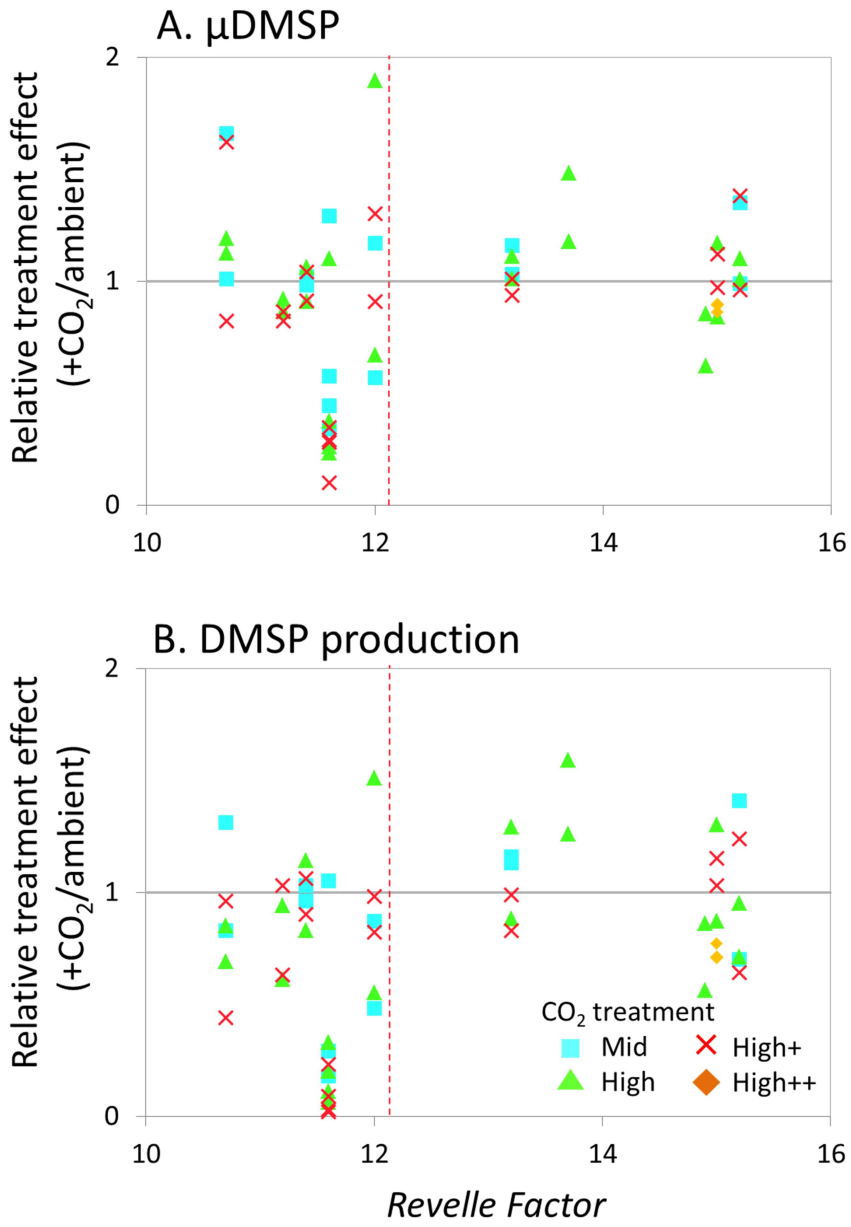


Figure ~~7~~8. Relationship between the ~~ratio of dissolved inorganic carbon C_T to alkalinity (C_T/A_T)~~Revelle Factor of the sampled water and the relative CO_2 treatment effect at ($[x]_{highCO2}/[x]_{ambientCO2}$) for de novo DMSP synthesis (µDMSp, d^{-1}) at T_1 (A) and T_2 (B), and DMSP production rate (nmol L^{-1} d^{-1}) at T_1 (C) and T_2 (D) for microcosm experiments performed in NW European waters, sub-Arctic and Arctic waters, and the Southern Ocean. Grey solid line (= 1) indicates no effect of elevated CO_2. ~~C_T/A_T>0.91~~Revelle Factor >12 = polar waters (indicated by red dashed line). T_1 = 48 h, T2 = 96 h, except for *Weddell Sea* and *South Georgia* (72 h, 144 h). For discussion of the NW European shelf data, see Hopkins & Archer (2014).

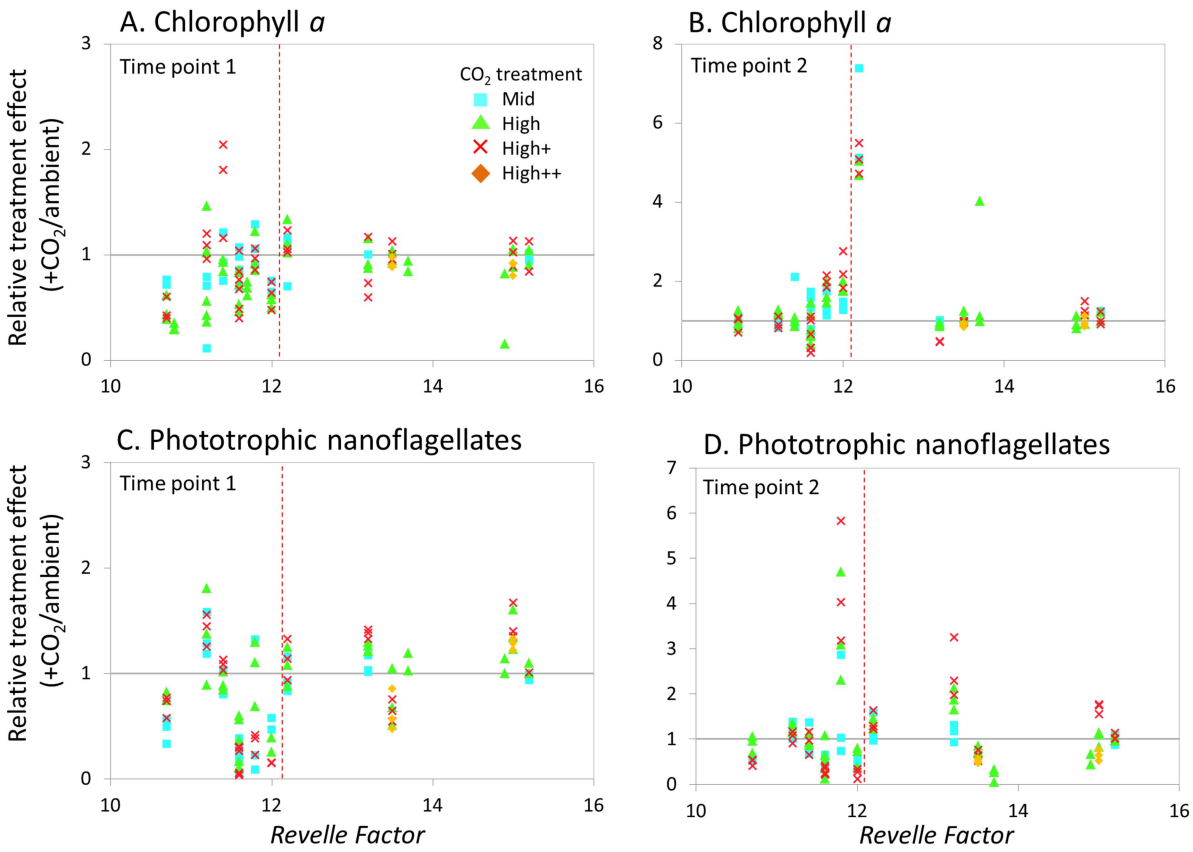


Figure ~~8~~9. Relationship between the ~~ratio of dissolved inorganic carbon (C_T) to total alkalinity (C_T/A_T)~~Revelle Factor of the sampled water and the relative CO_2 treatment effect ($[x]_{highCO2}/[x]_{ambientCO2}$) for chlorophyll *a* concentrations at T_1 (A) and T_2 (B) and phototrophic nanoflagellate abundance at T1 (C) and T2 (D) for all microcosm experiments performed in NW European waters, sub-Arctic and Arctic waters, and the Southern Ocean. Grey solid line (= 1) indicates no effect of elevated CO_2. ~~C_T/A_T>0.91~~Revelle Factor >12 = polar waters (indicated by red dashed line). T_1 = 48 h, T_2 = 96 h, except for *Weddell Sea* and *South Georgia* (72 h, 144 h) and *South Sandwich* (96 h, 168 h).