

## **Final author comments (AC):**

I would like to express my sincere thanks to the reviewers of this article for their careful reading, the improvements and corrections they have made, and their highly pertinent questions, including those regarding the limitations of this study and, in particular, the difficulty of linking atmospheric measurements to marine biology, which lies at the heart of the approach presented. A response to each of these questions is provided in the document below, and I would like to assure the reviewers that their suggestions will be taken into account in the next version of the article, particularly regarding the statistical analysis of the results presented, the discussion of these results and the changes in the figures.

## **Reviewer 1 comments (RC1):**

The manuscript „DMS, MeSH and nanoparticles in semi-controlled deck-borne experiments using Antarctic seawater: on the effect of UV light” from Chamba et al., reports on fluxes of MeSH and DMS during on-deck incubations in so called Air-sea Interaction Tanks (ASITs) and their relation to particle formation. The authors found differences in fluxes using Antarctic waters dependent on UV light availability and furthermore could show that significant new particle formation was only visible when no UV light was available. Seawater concentrations of MeSH and DMS have been calculated from air mixing ratios and are related to biological parameters in order to explain the variability of those gases. The data shows that nanophytoplankton cell counts could better explain DMS variabilities compared to the widely used proxy chl-a.

The presented work shows a clear research idea in order to improve our understanding on the influence of biologically produced trace gases and their air-sea exchange on particle formation in the atmosphere. The manuscript is well structured and most of the time well presented. However, before publication, I would like to address following points.

General comments:

Calculation from atmospheric mixing ratios to oceanic water concentrations.

Could the authors elaborate a bit on the factor 2.6 (deviation from equilibrium) used in eq(3). They refer to data from Rocco et al. (2025a), but it is still unclear where the factor 2.6 is derived from. I understand that the authors derived the factor for MeSH from data shown in Fig.S7 to match the calculated water concentrations from the ASIT with the seawater measurements at the UW outlet from Wohl & Williams et al., (2026). Given that

this factor accounts for the deviation from equilibrium I was wondering why MeSH has an almost 3 times stronger factor than DMS?

Response: The factor 2.6 is the deviation to the equilibrium for DMS, derived from the air and sea-water measurements in Rocco et al, (2025a). The factor for DMS was also calculated with measurements from Wohl and Williams, (2026) and is equivalent to the one measured in Rocco et al, (2025a). For simplicity and clarification, we modified the text as:

“Seawater concentrations of DMS in the ASIT (DMS<sub>w</sub>) were calculated from headspace concentrations by using an average departure to the thermodynamical equilibrium. This departure to the thermodynamical equilibrium is based on the excellent correlation between the theoretical seawater concentrations calculated from the equilibrium with the headspace concentrations in the run-trough mode of the control ASIT, and the measured concentrations in the underway seawater by Wohl & Williams. (2026). The slope of the measured versus calculated seawater concentrations was found to be 2.6 for DMS and 7.2 for MeSH. The slope found in the present study for DMS is very similar to the one used in Rocco et al. (2025a) that used the same experimental procedure (MeSH<sub>w</sub> concentration was not measured in this previous study).”

Our hypothesis to explain that this equilibrium factor is stronger for MeSH than for DMS is that MeSH is more reactive than DMS. This would induce a larger deviation to the Henri's law equilibrium.

Links between dissolved gases and microorganisms during experiments.

The authors try very well to explain the links between dissolved gases and microorganisms. However, there are no direct measurement of dissolved trace gases within the ASITs during the experiments. DMS and MeSH are solely calculated based on measured atmospheric mixing ratios. Calculated fluxes (and finally water concentrations) are always in misbalance between water and atmosphere. This is partly accounted for in the calculation of dissolved aqueous concentrations, however, fast changing atmospheric mixing ratios throughout an experiment could overestimate the calculated variability in the aqueous phase. This can particularly be seen in Fig.S7. On Feb 25<sup>th</sup> DMS air mixing ratios significantly decrease (Fig.3), so do the calculated DMS water concentrations. However, direct measurements from Wohl & Williams et al. (2026) at the underway outlet at the same time show significantly higher concentrations. A similar offset (but vice versa) is visible on March 7<sup>th</sup>. Furthermore, calculated DMS and MeSH concentrations at the start of ExpB are significantly higher compared to measured water values at the same time. If this discrepancy is potentially induced only through calculation from air-side to water-side values it does not reflect the actual trace gas variability in the water phase of ExpB. Therefore, drawing conclusions from correlating

measured biological parameters with calculated trace gas concentrations should be undertaken with care. Moreover, the authors state that they found no strong correlation between aqueous DMS and MeSH and biological parameters during continuous flow which already points to my previous point (when having two continuous flows (water and air) instead of just one).

Response: This point is valid and represents one of the challenges of the study; however, we do not believe that we have systematically overestimated the concentrations in the aqueous phase, particularly because Experiment B begins on 8 March at 12:00, when the aqueous concentrations of DMS and MeSH are at their peak, according to our latest continuous measurements and those of Wohl and Williams. (2026), and so the water type kept inside the ASIT are particularly favouring DMS and MeSH emissions. Unfortunately, we do not have any PTR-MS measurements from the start of Experiment B. In fact, it is expected for the measurements in ASITs to differ slightly from those in the UW, due to the 6-hours average residence time of the seawater in the run-through mode.

Specific comments:

Figure 1: It is not clear to me what the points of the cruise track exactly mean. As the distance between the points changes from time to time, I was thinking that each point might be a discrete (or averaged continuous) measurement point. But as no data is shown before Feb 22<sup>nd</sup> and after March 11<sup>th</sup> I might be wrong. Please clarify in the figure caption.

Response: Each point is an hourly point (the distance between two points changes according to the speed of the ship) from the beginning to the end of the campaign. This is now indicated in the caption.

l.125. Which kind of seawater pump was used for the water supply? A centrifugal pump e.g. might damage phytoplankton and causes cell lysis which would significantly influence e.g. DMS and MeSH concentrations and subsequent fluxes when monitored in static flow mode in the experiments (e.g. dissolved DMSP conversion to DMS/MeSH). At least the community in the ASITs would not be “natural” anymore.

Response: The water intake for UW measurement and ASITs supply is located 4-5 m below sea level, and consists of an epoxide-free silicone pipe connected to a centrifugal pump (BKM KC-10.11, Tecnum) in which the parts in contact with the fluid are made of polypropylene and glass. The water is pumped to the laboratories through epoxide-free silicone pipes. The pumping system is designed to be gentle and non-toxic to microorganisms and provide an uncontaminated continuous source of near-surface

seawater for underway measurements, including a thermosalinograph and a chl-a fluorometer.

Simultaneous measurements of chl-a and DMS concentrations from the underway pumping system and a dedicated Go-Flo launched overboard, or the uppermost bottle from a CTD cast have been compared during a previous cruise, and are in very good agreement (Royer et al., 2014).

We added these details to the material and methods section.

Royer, S.-J., Galí, M., Saltzman, E. S., McCormick, C. A., Bell, T. G., and Simó, R.: Development and validation of a shipboard system for measuring high-resolution vertical profiles of aqueous dimethylsulfide concentrations using chemical ionisation mass spectrometry, *Environmental Chemistry*, 11, 309–317, <https://doi.org/10.1071/EN13203>, 2014.

l.258ff.: All mentioned compounds which show no enrichment in the ASIT headspace are (much) less soluble in seawater compared to MeSH and DMS. Therefore, proposed cold seawater and absence of wind friction should have a stronger influence (dampening) on the gas exchange on MeSH and DMS than on the other gases. Very low oceanic concentrations might be the dominant factor, however, is there any possibility to prove such low concentration with the help of data availability from Wohl & Williams (2026)? With the biological data provided in the manuscript together with a concentration range of seawater DMS of 1-3nM, isoprene (direct product by phytoplankton) should be supersaturated in the water phase which would lead to elevated atmospheric values (at least during the experiments).

Response: The mean ambient atmospheric concentrations were around 50 ppt and mean oceanic concentrations (UW) were 0.07 nM according to the measurement made during the campaign that are presented in Crabeck & Rocchi (in press). They also present concentrations reaching 1.5nM in top sea-ice, this could therefore be one of the sources of isoprene in the atmosphere that was identified as missing in the study by Ferracci et al. 2024. These works could explain the absence of isoprene emissions in the ASITs with high isoprene air concentrations and relatively low concentrations in seawater. This discussion is now added in the manuscript (l.290).

Figure 3: Did the authors also measure DMS and MeSH in the ambient line as they did for ozone? As there appears to be a (known) loss of ozone in the long sampling lines (and in the ASIT itself) it would be helpful to rule that out for the other gases. If there are losses as well e.g. in the ASIT this could potentially explain why the authors did not see an increase in atm mixing ratios of isoprene, BTEX, etc. Flux from the water to the air and

loss in the ASIT itself could cancel each other out. Furthermore, information of kind of particle filters is missing in the manuscript. Might those filters also influence ozone levels downstream if particles (already loaded on filters after some time) react with ozone?

Response: As for ozone, all VOCs from PTR-MS were measured in ambient air, flushing line and ASITs meaning that we know the losses in the long line for all of these compounds. Except for ozone, none of them showed a marked difference between ambient air and flushing line. As the length of the flushing air line is significantly longer than the length of the ambient air line (25m vs 3.5m), we can rule out significant losses in the line going from the ASIT'S headspace (2.5m). However, we cannot rule out the possibility of losses in the ASIT's headspace that might mask emissions of BTEX or isoprene, but that would mean that emissions of these compounds are particularly low given the measurements taken during a previous campaign using the same set-up (Rocco et al., 2021; Rocco et al., 2025). This discussion is added to the manuscript (l.294).

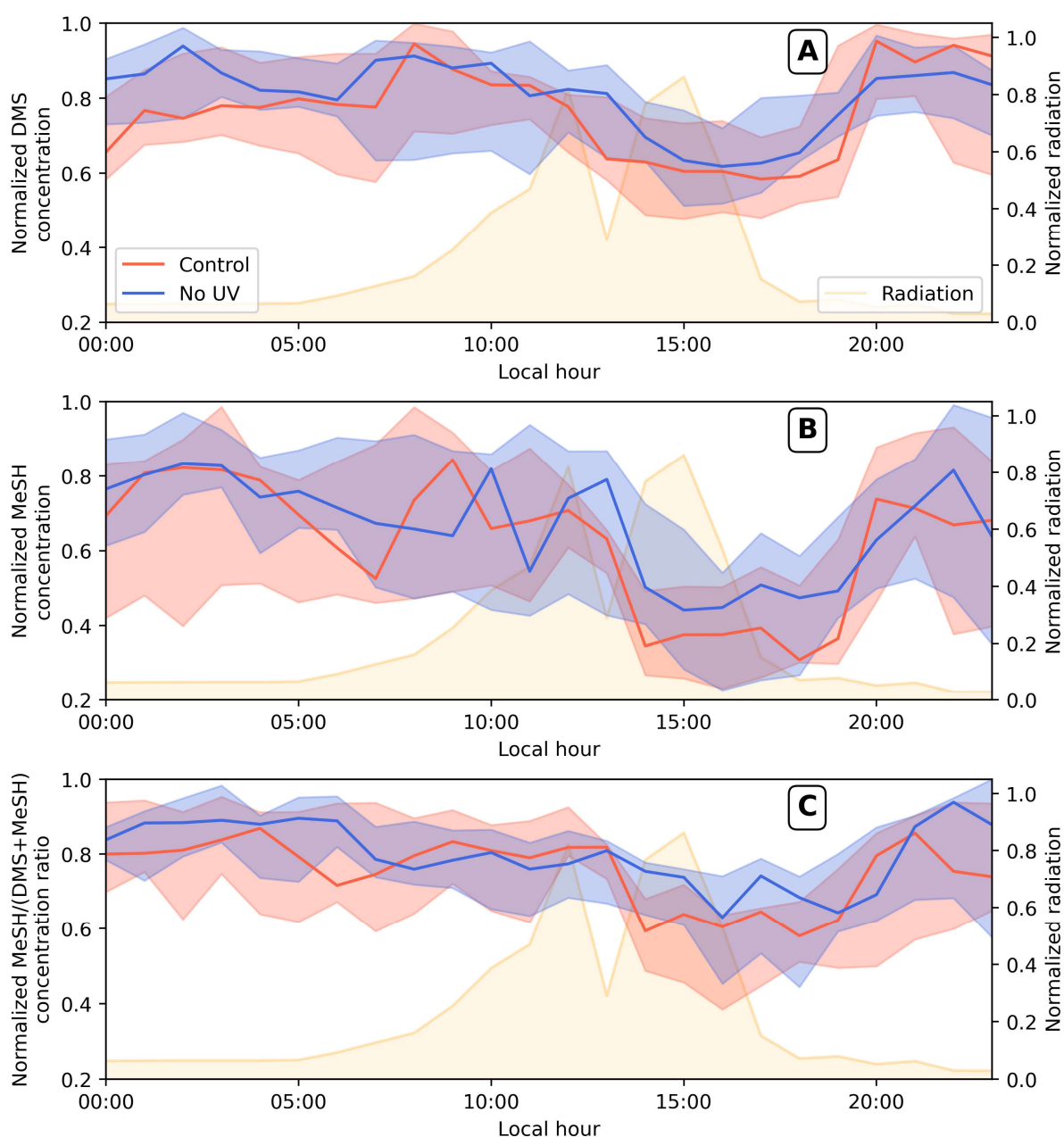
Figure 5: For the fluxes in the noUV Exp A (green crosses) there seems to be a more exponential relation (than linear) which is not visible in the Control ASIT. Did the authors check if this might be a significant difference? This feature is not visible in the relation between atm mixing ratios. Wouldn't this imply that under no UV conditions atmospheric MeSH reacts faster than DMS whereas with UV the loss rates in the atmosphere are similar?

Response: We should be careful not to over-interpret our results given the uncertainties in our measurements, and interpretation should be helped with modelling tool that takes into account the flow ASITs dynamics, preexisting concentrations in the flushing air and known reactivities, as was performed by Salignat et al. for the Sea2Cloud measurements.

Without the help of a model, we find it difficult to draw reliable conclusions.

Figure 7, Figure S3: Diel variations. Did the authors try to normalize the atmospheric mixing ratios (e.g. to the maximum or mean value per day) when checking diel variations? As discussed earlier in the manuscript the absolute concentrations changed significantly dependent on regions (e.g. coastal vs open ocean). This would probably reduce the standard deviation and a potential dial cycle might be more pronounced (visually but also statistically). This technique could also be applied to the radiation data as of now it seems that data is skewed to stronger irradiances in early afternoon.

Response: We would like to thank the reviewer for this good suggestion, which does indeed allow for a better observation of the shape of the daily variations. However, the normalization procedure prevents from observing differences between the two ASITs (as shown in the figure below), which is a key finding for the discussion on the effect of UV light. The figure and caption of the normalized concentrations are now added to the supplementary (Fig.S5) and mentioned in the text, which was changed accordingly to the details observed in this new figure.



I.398ff/Figure 9: Do the authors have any explanation why the particle numbers in the noUV-ASIT are lower during each experiment compared to the particle data of the respective region (coastal vs ExpA and open ocean vs ExpB)?

Response: It might be linked to the fact that both Exp A and Exp B are run with seawater that has the lowest "potential" to form new particles when looking at the time evolution of particles formed under the run-through mode (figure 8) but we don't have much more elements to discuss these differences.

l.408ff.: It is obvious that chl-a values at the beginning of ExpA are significantly lower ( $0.5\mu\text{g/L}$ ) than following concentrations ( $2.0\mu\text{g/L}$ ) throughout the experiment. Has this sample also been taken from the tap of one of the ASITs or directly at the UW pump outlet? If so, could the location of sampling bias the data? Nanoplankton 15-20 $\mu\text{m}$  data show a similar trend (also for ExpB). Furthermore, are chl-a datapoints hidden behind the legend of ExpA or is there no data available for 6am and 12pm?

Response: The first sample (green cross) show a point taken directly at the UW pump outlet and so can have a bias compared to the sample taken from the tap of the ASIT. For Chl-a, the concentrations were also quite variable in the UW around the beginning of the experiment A. For Exp B it can appear even more different considering that we are missing the first sample of the experiment. Therefore, we do not overinterpret the change in Chla at the start of the experiments, but rather the evolution of biological parameters in the following data points.

We also are missing the last chl-a samples for exp A.

Fv/Fm ratio: Could there also be mechanistic stress (e.g. pumping shear stress) showing up in the Fv/Fm ratio? It is quite obvious that Fv/Fm is much lower in the beginning of the experiments compared to later stages. According to Fig. 11 UW Fv/Fm ratios are generally lower compared to ASIT ratios. Do the authors also have Fv/Fm data from CTD measurements? This could give valuable insight if microbial community in ASIT is either just less stressed compared to being pumped through hoses, or if microbes are in general less stressed compared to the "real ocean". If light stress would be the only issue for Fv/Fm ratios, the difference between UW and ASITs should be vice versa due to the different water depths (UW: 4m, ASIT:  $\sim 1\text{m}$ ).

Response: As mentioned in a previous comment, chl-a and DMS concentrations were compared after UW with pumping system to CTD measurements but unfortunately, we do not have a similar comparison for Fv/Fm measurements. We can add to this comment that, as mentioned earlier, the pumping system to bring water in the ASITs in the same as for UW sampling, but the long residence time during experiments after being pumped may increase Fv/Fm ratio.

l.456ff. Aqueous-phase reactivity of DMS. I like the discussion about the potential loss to explain differences in DMS water concentrations in between both ASITs. How about DMS to DMSO oxidation as a potential loss mechanism? Additionally, I was wondering if the authors also considered a potential stronger DMS production in the noUV ASIT compared to the control ASIT. As the authors already pointed out, the difference in between both treatments is strongest during the two experiments. Is there anything known about phytoplankton types around Antarctica with inhibited DMSP production under UV stress?

Response: We relied on the study by Salignat et al. who concluded that DMS concentration differences between ASITs (with different levels of headspace ozone) were fully explained by aqueous phase reactivity, but yes in the case of UV-light induced stress a different DMS production could be possible. We added this possibility in a sentence here (l.520).

The study by Salignat which is under review, waiting for the editor final decision, is added to the present document.

## **Final author comments (AC):**

I would like to express my sincere thanks to the reviewers of this article for their careful reading, the improvements and corrections they have made, and their highly pertinent questions, including those regarding the limitations of this study and, in particular, the difficulty of linking atmospheric measurements to marine biology, which lies at the heart of the approach presented. A response to each of these questions is provided in the document below, and I would like to assure the reviewers that their suggestions will be taken into account in the next version of the article, particularly regarding the statistical analysis of the results presented, the discussion of these results and the changes in the figures.

## **Reviewer 2 comments (RC2):**

Antarctic seawater: on the effect of UV light”, presents some interesting and novel results applying a new technique to study this topic in the Southern Ocean. Overall, the article is interesting, and the authors present some intriguing results in an article that read well and it is easy to follow. However, without a more rigorous statistical treatment of the data it is very hard to assess if the differences presented by the authors are significant or not, which hinders the ability of the article to convey a more compelling argument.

For example, the authors report a very interesting result on the influence of UV light absence on particle formation, but in the article, there is no statistical test to compare the results (a Wilcoxon pairwise test will suffice). Besides in figures 9B and 9C have different Y axis so it is very hard to assess if the differences are significant or not, which clearly limits the ability of the manuscript to convey a strong message with significant results. Therefore, my main concern is with the statistical analysis, or the lack of it for most of the article. My general suggestion for the authors is to include a statistical analysis section in the methods where they explain in detail how they tested for the significance of the differences they mention throughout the article. Following the example in lines 369-371, when the authors compare DMS fluxes, will greatly improve the quality of the article and will give more confidence in the findings and conclusions of the present work.

**Response:** Thank you for your general comment on the significance of these results. We have included statistical tests wherever we felt it was necessary (see the comments below). In the case of Figures 9B and 9C, this was a deliberate choice, as the differences are too great for the boxplots to be clearly visible on the same axis as the Control-ASIT. We, however, added this to the legend (l.403).

In fact, in some parts of the manuscript (lines 538-540) the authors refer to cryptophytes abundance as closely related to DMS and MeSH sweater concentrations, which does not correspond with the results the authors present in figure S8A where there is no significant relationship between cryptophytes and DMS/MeSH sweater concentrations. Results in figure 11 look like they are not significant and therefore should not be considered as such, that is why the authors should show the results from some statistical test that supports the differences found by them because as the article is it looks like there are no differences. Therefore, a general review of the statistics and results interpretation in light of the new statistical analysis is needed to improve the quality of the article. There are more examples in the pdf enclosed including the statistical problems of using a linear regression forced through the origin (figure 13) which are not addressed in the text.

#### Response:

The reviewer is right concerning the relationship to cryptophytes: a relationship was found with older values of DMS and MeSH that were corrected since. We modified this whole part and checked for statistical verification for all other relationships.

#### Specific comments

##### Role of phytoplankton in dissolved gases concentrations

Diatoms in Antarctica are also capable of producing DMSP (see details in pdf enclosed) and since they may represent a large fraction of the phytoplankton community in some parts of the study area, their contribution towards the DMS and MeSH concentrations may be not negligible. I do not know if the authors have some data/samples to study microphytoplankton, including diatoms, but so far, they have present none. If that is the case it is impossible to include the information now, but at least the authors should discuss this situation in the introduction, methods and discussion sections so they fully address the potential limitations and problems derived for not considering this large group within the phytoplankton community.

Response: The taxonomic analysis of the micro- and nanophytoplankton communities in the PolarChange cruise was presented by Rocchi et al. 2025 (Figures 3 and S5; and Table S4). The microplankton community was characterised using the Utermöhl method on 125mL neutral Lugol fixed seawater (Edler and Elbrächter 2010), hetero and autotrophic flagellates by epifluorescence microscopy and the pico- and nanophytoplankton abundances by flow cytometry. Furthermore, the contribution of the main groups to biomass in terms of carbon was estimated from cell volume estimations using the carbon-to-volume relationships estimated by Menden-Deuer and Lessard (2000) on diatoms and dinoflagellates. At the time of the cruise, diatoms accounted for less than 40% on average while dinoflagellates, which are main producers of DMSP, were particularly dominant, accounting for more than the 60% of the

microphytoplanktonic biomass. Likely, the study took place during a diatom postbloom and dinoflagellates were the main contributors to DMS and MeSH in the ASITs experiments.

This discussion is now added in the methods section (l.249).

Rocchi, A., Fitzsimons, M. F., Akenga, P., Sotomayor, A., Sà, E. L., Güell-Bujons, Q., Vila, M., Castillo, Y. M., Dall'Osto, M., Vaqué, D., Wohl, C., Simó, R., and Berdalet, E.: Distribution of alkylamines in surface waters around the Antarctic Peninsula and Weddell Sea, <https://doi.org/10.5194/egusphere-2025-407>, 12 February 2025.

Edler, L. and Elbrächter, M.: The Utermöhl method for quantitative phytoplankton analysis, in: Microscopic and Molecular Methods for Quantitative Phytoplankton Analysis, edited by: Karlson, B., Cusack, C., and Bresnan, E., IOC Manuals and Guides No. 55, UNESCO Publishing, Paris, 110 pp., <https://doi.org/10.25607/OBP-1371>, 2010.

Menden-Deuer, S. and Lessard, E. J.: Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton, *Limnol. Oceanogr.*, 45, 569–579, <https://doi.org/10.4319/lo.2000.45.3.0569>, 2000.

Finally, all the relationships between microbial abundances and the seawater concentrations of DMS and MeSH are related to the experiments where the DMS and MeSH seawater concentrations were not measured directly, but back calculated from atmospheric measurements where mixing ratios and the fast transformation of the DMS into SO<sub>2</sub>/H<sub>2</sub>SO<sub>4</sub>/MSA may likely affect the back calculation of seawater concentrations. This calls for some nuance and care when analyzing the patterns observed, specially when the authors do not report such patterns for the continuous flow observations, which is always difficult observed since both (sea and air) are two moving fluids.

Response: For the PolarChange and also for previous ASIT campaigns, we did not detect any SO<sub>2</sub>/H<sub>2</sub>SO<sub>4</sub>/MSA produced within the ASITs headspace, which is expected given the low reactivity of DMS in the atmosphere, and was confirmed in the Salignat et al. study. The excellent linear relationship between headspace DMS concentrations and measured dissolved DMS concentrations when they are available is also a confirmation that we can rely on atmospheric concentrations to calculate seawater dissolved concentrations. Sea and air are indeed two moving fluids, but the residence time of both is such that the thermodynamic equilibrium is nearly reached, while air is sufficiently quickly renewed for chemical reaction to be negligible (for DMS).

More specific comments are available in the pdf enclosed.

Thank you very much for your comments on the figures, text and references; We will take them into account to improve the clarity of the manuscript. I have copied comments including questions or asking for details from the PDF here so that I can answer them:

L.125: Did the pumping and the flushing take place at the same time or was this sequence (pump-flush-pum-flush)?

Response: Pumping and flushing took place continuously, at the same time. This is now detailed in the manuscript (L.125).

L.205: The viruses in the Southern Ocean are larger than in other marine ecosystems and they seem to be quite endemic, so I wonder how does this impact the viral abundance assessment if you are using general settings for other areas of the global ocean.

See for example

Alarcón-Schumacher, T., Guajardo-Leiva, S., Martinez-Garcia, M., & Díez, B. (2021). Ecogenomics and adaptation strategies of Southern Ocean viral communities. *Msystems*, 6(4), 10-1128.

Zhai, C., Kennedy, F., Liang, Y., Wang, M., & McMinn, A. (2025). Antarctic Marine Viruses: A Review and Future Perspectives. *Ocean-Land-Atmosphere Research*, 4, 0101.

It may be worth mentioning how the authors dealt with this situation.

Response: We thank the referee for highlighting this important point. It is well established that Antarctic waters often host a relatively high proportion of nucleocytoplasmic large dsDNA viruses (NCLDV, or “giant viruses”), particularly during blooms of phytoplankton such as haptophytes (e.g., *Phaeocystis*, *Coccolithophoridae*), Prasinophytes (e.g., *Mamiellophyceae*, *Pyramimonas*), and Cryptophytes (e.g., *Cryptophyceae*). In contrast, diatoms are predominantly infected by smaller ssDNA and ssRNA viruses (<20 nm).

Importantly, the cytometric settings applied in our study followed widely used protocols for marine viral enumeration and are consistent with previous studies conducted in the Southern Ocean (Evans et al. 2009). These settings are designed to capture the full spectrum of viral subpopulations, including both small and large viruses. It should be noted that viral “size” in flow cytometry does not directly correlate with side scatter, but rather with fluorescence intensity. Larger viruses, which generally possess larger genomes, exhibit stronger fluorescence signals due to increased nucleic acid staining (Brussaard 2004; Evans et al. 2009). Therefore, these standard settings are expected to adequately account for the presence of larger viral groups in the Southern Ocean and are unlikely to result in a systematic underestimation of viral abundance.

In flow cytometric analyses, these larger viruses are typically detected within the V3 viral subpopulation due to their higher nucleic acid content. Under conditions of especially high abundance of large phytoplankton hosts (e.g., *Phaeocystis* blooms), an additional high-fluorescence viral cluster (sometimes referred to as V4) may be observed (Vaqué et al. 2025). However, such a subpopulation was not detected in our samples. Notably, bacteriophages, which typically dominate marine viral communities in terms of abundance, are generally associated with lower fluorescence signals and are predominantly encompassed within the V1 and V2 subpopulations (Biggs et al. 2021).

In agreement with this interpretation, Alarcón-Schumacher et al. report that viral genome sizes are substantially larger in giant viruses that infect eukaryotic hosts.

We included this discussion in the material and method section (l.223).

Biggs TE, Huisman J, Brussaard CPD (2021) Viral lysis modifies seasonal phytoplankton dynamics and carbon flow in the Southern Ocean. *ISME J* 15:3615–3622. <https://doi.org/10.1038/s41396-021-01033-6>

Brussaard CPD (2004) Optimization of procedures for counting viruses by flow cytometry. *Appl Environ Microbiol* 70:1506–1513. <https://doi.org/10.1128/AEM.70.3.1506-1513.2004>

Evans C, Pearce I, Brussaard CPD (2009) Viral-mediated lysis of microbes and carbon release in the sub-Antarctic and Polar Frontal zones of the Australian Southern Ocean. *Environ Microbiol* 11:2924–2934. <https://doi.org/10.1111/j.1462-2920.2009.02050.x>

Vaqué D, Berdalet E, Sotomayor-Garcia A, Estrada M, Cabrera-Brufau M, Masdeu-Navarro M, Rocchi A, López-Alforja X, Vila M, Marrasé C, Simó R, Dall’Osto M, Sala MM. (2025). Assessing the relationship between viruses and protists and their role in dimethylsulphoniopropionate release in Antarctic surface microlayers. *Antarctic Science*. doi: 10.1017/S0954102025100205

l.270: it will be good if the authors can say which option is more plausible so readers have a more informed opinion. Including references to support the statement will also be a nice addition.

Response: It is very difficult to distinguish between the two possibilities, particularly as they vary over time depending on the compounds emitted in ASITs, humidity, temperature, and concentrations of NO<sub>x</sub> and ozone. In the Salignat et al. study, a model is used to evaluate ozone losses due to reactivity (within the limits of the known VOC present...). and we found that reactivity and deposition accounted for about half of the losses each. We added this to the manuscript (l.301).

l.275 : Does this mean air temperature or seawater temperature? I assume it is the former, but it may be better to use air or seawater so it is 100% clear to which ambient does the temperature correspond to.

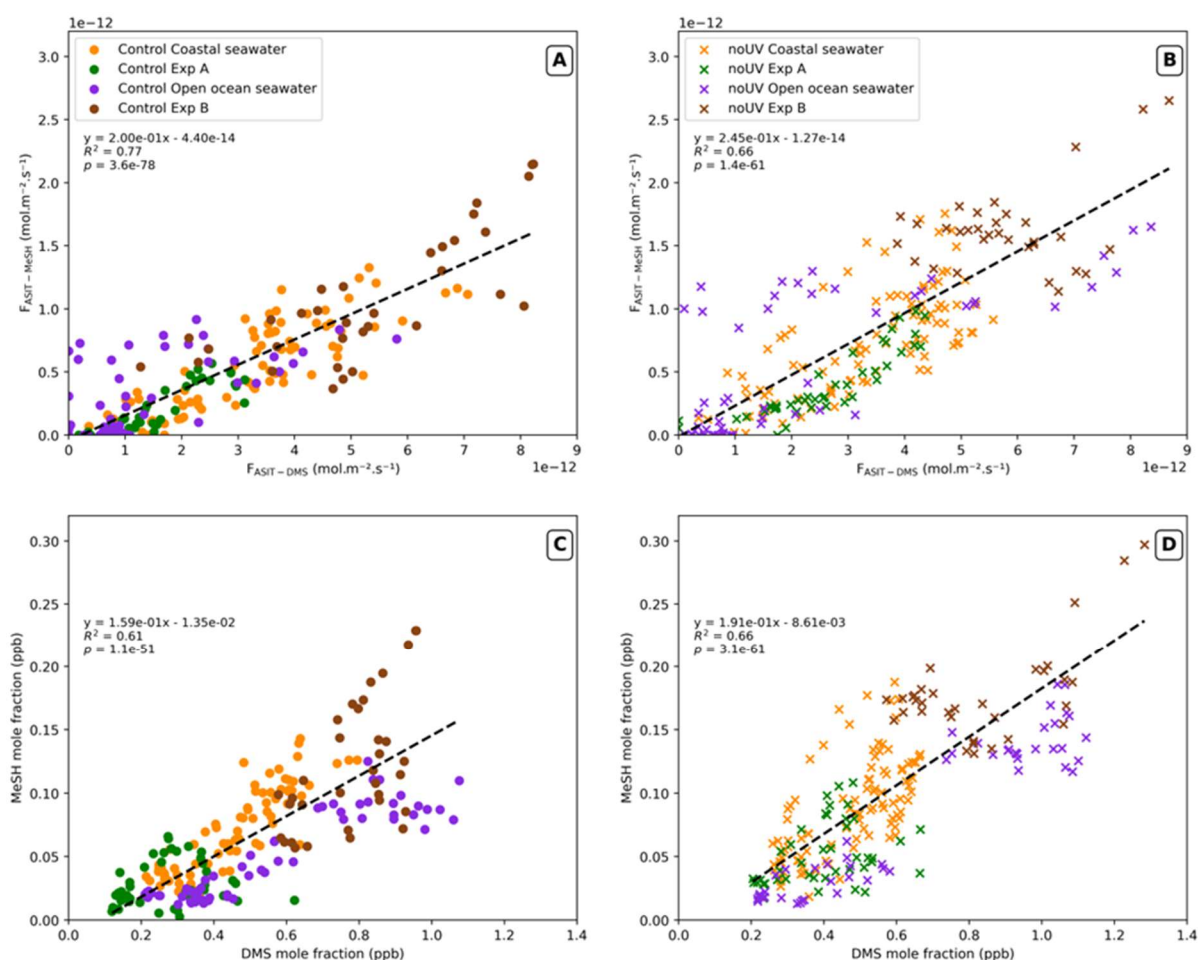
Response: Yes it is mean ambient air temperature, we precised it in the text (l.303).

l.288: I suggest the authors to remove the term ambient from the manuscript and use just air instead since it is clearer what it means.

Response: We keep the term ambient air concentration to mark the difference to ASIT air concentrations.

l.314: It seems like the authors have a nice linear relationship here so I suggest them to include the linear model fit in the figures and also the statistical information (formula,  $R^2$  and p value) so readers can assess if such apparent relationship is significant or not. The formula may be very useful for other researchers working in the area to assess indirectly on these parameters when they do not have it.

Response:  $R^2$ , formula and p value are included in the 4 plots of the figures for all measurements combined as in the following figure:



l.324: Ok, but are such differences significant statistically speaking? Some basic statistics like ANOVA, kruskal wallis or wilcoxon test are missing here.

Response: Differences between each water-type and experiment are significant (test Mann-Whitney U). This is now added in the text.

l.331: The authors should use a test like a wilcoxon pair-wise test to see if the differences between both groups are significant or not.

Response: The median value is decreasing through the day after radiation start to increase and increase in the hours following the sunset which makes it not clearly anti-correlated to radiation. So, the Mann-Whitney U test between daytime and nighttime (followed by radiation measurements) is significant ( $p=0.048$ ,  $r=0.152$ ) but limited while we don't have enough full day and night median values to perform a significant non-paired Wilcoxon test. We changed the sentence in the text.

l.370: Why is this information not included for all the other comparisons made in the manuscript? This kind of information should always be together with such statements even if the differences are not significant.

Since no information about statistical test is given in the methods, please include here within the brackets how the test was performed. Using R or MatLab, which version, function and so on please. It is critical information for the reproducibility of the study.

**Response:** This information will be included for all other comparison and the details of the test used will be included in the code availability section: Statistical analyses were performed using Python (version 3.11) with the SciPy library (version 1.16.2; Virtanen et al., 2020) at a significance level of  $\alpha = 0.05$ . Normality of paired differences was assessed with the Shapiro-Wilk test; as normality was rejected ( $p < 0.05$ ), the Wilcoxon signed-rank test was applied to compare the two experimental conditions, with effect size quantified by the rank-biserial correlation coefficient  $r$ .

l.379: Statistics ?

**Response:** Now included in the text : The median value of the FASIT-MeSH/(FASIT-DMS+FASIT-MeSH) ratio obtained for all data combined was slightly lower in the Control-ASIT (Median = 0.15; Q1 = 0.11; Q3 = 0.19) than in the noUV-ASIT (Median = 0.17; Q1 = 0.13; Q3 = 0.22) with a significant difference ( $W = 1025$ ,  $p\text{-value} = 1.17 \times 10^{-19}$ ,  $r = 0.835$ ). This was also observed for the air concentrations ratios and calculated aqueous concentrations ratios with median values of aqueous concentration ratios of 0.27 (Q1 = 0.20; Q3 = 0.33) in the Control-ASIT and 0.32 (Q1 = 0.26; Q3 = 0.38) in the noUV-ASIT (significant:  $W = 1495$ ,  $p\text{-value} = 4.38 \times 10^{-24}$ ,  $r = 0.840$ ).

l.392: Authors should only use this word if they are referring to the results of a statistical test, which is not the case in this situation. So my suggestion will be to include here some statistical support for the comparison.

**Response:** We changed the sentence to include the statistical test for  $n_1$  particles concentrations between the noUV and Control ASIT : Filtering out UV light had a large impact on the formation of new particles, which were observed in significantly higher concentrations in the absence of UV-light as shown in Figure 8 (Wilcoxon signed-rank test between both ASITs,  $W = 44$ ,  $p = 1.20 \times 10^{-27}$ ,  $r = 0.993$ ).

l.406: The problem here is that the authors did not consider any microphytoplankton groups such as dinoflagellates or diatoms. Which in some parts of this Antarctic region may represent the largest fraction of the phytoplankton community. Therefore the authors

should change this to reflect what they have studied (e.g. pico-nanophytoplankton) and include some sentences about this caveat or limitation in this study in the discussion and methods sections.

Response: The answer to this comment follows the answer in the specific comment section. In fact, measurements of diatoms were made in the UW samples and not in the ASITs sample therefore we decided to not include it in this manuscript. But the reviewer is right and we should add the specification on pico-nanophytoplankton on top of the information now added in the methods section.

l.565: This is true, but not for virus V2 so the authors should explain this situation. Why the relationship is positive and significant for V1 and V3 viruses, but not for V2.

It is worth mentioning that a similar strong but negative relationship is present for 2 classes of phytoplankton, and that seems counterintuitive since those groups are producers of DMSP the precursor for DMS and MeSH. The authors should mention this since it is rather interesting.

The same happens with chlorophyll concentrations. Therefore the authors should explain this inverse relationship between DMSP producers (phytoplankton) and DMS/MeSH. Specially since in this area of Antarctica and others it is known that chlorophyll and DMSP tend to show a positive relationship that not always is translated to DMS.

See for example

Petrou, K., Raina, J. B., Fernandez, E., Ostrowski, M., O'Brien, J., Haydon, T. D., ... & Nielsen, D. A. (2026). Dimethylsulfoniopropionate and marine microbial associations along an Antarctic glacial–open ocean interface. *Limnology and Oceanography*, 71(1), e70290.

Li, C. X., Chen, K., Sun, X., Liu, L., Xin, M., Liu, X. L., & Wang, B. D. (2024). Summer sea ice melting enhances phytoplankton and dimethyl sulfide production. *Limnology and Oceanography*, 69(10), 2453-2472.

Response: The (positive, significant) correlations found among DMS and MeSH in the seawater and the different microorganisms including viruses should not be interpreted as a direct cause-effect relationship, or a short-term response occurring in the ASITs. The correlations should be taken individually, as indicators of the diverse, dynamic and complex biological processes undergoing in the ASIT experiments. For this reason, we cannot provide specific explanations for each one of the estimated correlations.

Me modified the text:

#### 4.2 Links between dissolved gases and microorganisms during experiments

We explored the potential links among the different biological elements and chemical compounds measured in the ASIT experiments by considering the temporal patterns and the correlations between the different parameters (Figures 12 and S.8). Note that significant correlations should not always be interpreted as a direct cause-effect relationship, nor a short-term response occurring in the ASITs. Furthermore, the correlations should be taken individually, as indicators of the diverse, dynamic and complex biological processes undergoing in the ASIT experiments, somewhat apparently contradictory in some cases.

L586: Furthermore, a significant positive relationship between estimated aqueous concentrations of MeSH and DMS and HNA bacterial abundances was observed in both ASITs ( $R^2 = 0.43$  and  $0.49$  in Control-ASIT;  $R^2 = 0.57$  and  $0.63$  for noUV-ASIT, respectively for MeSH and DMS). This relationship was particularly noticeable in open ocean waters (Fig. 12). A relationship of sulfur compounds with V1 and V3 virus abundances was also observed in both ASITs during experiments, but not with V2 virus (Fig. S.8). As explained above, dynamic and diverse processes occurred in the ASITs experiments which differently affected the particular biogeochemical elements at play, in this case targeting the virus groups.

L595 : When a linear regression is forced through the origin (intercept is  $x=0, y=0$ ) the  $R^2$  value tends to be inflated and it is not so representative (it can even be negative) so it should not be used here. The p value is still a good indicator if the relationship is significant or not, but here the p value is not represented so we do not know if the regression is significant.

There should be very good reason for using a linear regression forced through the origin, that should go beyond that being able to compare it with data done in another part of the globe that is far away. If the authors forced the regression through the origin is like saying that if there were no nanophytoplankton there will be no DMS in seawater which we know is not true since other plankton groups are able to produce DMS directly or indirectly.

The authors should do a better case to defend this statistical approach instead a regular linear regression and in the revised version or the response I hope at least they show the results of both regressions including their p values so we can see if this relationships are significant or not.

Response: The p-value of the linear regression once forced through the origin are  $p=1.7 \times 10^{-13}$  for the PolarChange data and  $p=5 \times 10^{-12}$  for the Sea2Cloud data which, we agree, are not a self-sufficient argument to force the regression through the origin but simply means that the regressions presented are significant. The reviewer is right in pointing to the fact that forcing the correlation through the origin induces a scientific

bias. As the p value shows that the correlation is insignificant when not forcing through the origin, we decided to remove this subsection (l.641).