General answer Stoltenberg et al.:

We thank the referees for their constructive and insightful comments. We have carefully addressed all suggestions and revised the manuscript accordingly, which has improved its clarity, transparency, and overall quality. Key revisions include clarifying the methodological framework and assumptions underlying the N_2O flux and photochemodenitrification estimates, expanding essential methodological descriptions for context, and refining the interpretation and wording of the results, to better acknowledge uncertainties and limitations. We believe that these changes have substantially strengthened the manuscript.

Reviewer 1:

This study quantifies nitrous oxide (N2O) concentrations in the sea surface microlayer (SML, the upper 1 mm of the water column) and the underlying water (ULW) during a phytoplankton bloom in a mesocosm experiment. The authors also estimate N2O fluxes and discuss potential pathways for N2O production in the SML, including microbial nitrification, release from phytoplankton, and photochemodenitrification. Overall, this work addresses an important and understudied topic. It provides valuable data, as no previous research has measured N2O concentrations in the SML. Understanding this layer is critical because it may play a key role in regulating fluxes of this potent greenhouse gas.

My primary concern is the statistical analysis, which currently appears insufficient to fully address the research questions. A more rigorous examination of the dataset is necessary to reveal potential dynamics in N2O concentrations. The manuscript states that there are no temporal trends in N2O within the ULW and SML (line 201), that mean concentrations in both layers do not differ significantly, and that no diurnal patterns were detected (lines 201–210). However, beyond a t-test, the methods used to assess these trends are not described in detail. A simple comparison of averages is not adequate to rule out differences or identify underlying patterns. I strongly recommend that the authors apply more robust statistical approaches to explore these relationships and potential drivers among all measured variables. For example, Figure 1 shows notable changes in temperature and salinity during the experiment, could these influence N2O concentrations? Similarly, what about chlorophyll a or other parameters, such as surfactants? That might indicate the potential role of phytoplankton.

Temporal trends (including diel trends) may not be visually apparent but could emerge when covariates are incorporated into the analysis. Besides, “samples to measure N2O concentration at the SML were taken every three days alternating either 30 minutes past sunrise or 10 hours past sunrise.”. Whether the sample was taken 30 minutes past sunrise or 10 hours past sunrise could be relevant for the temporal trend. Strengthening the statistical framework would significantly enhance the manuscript’s contribution and provide deeper insights into the processes governing N2O dynamics at the sea surface.

We thank the reviewer for this thoughtful and constructive comment and for highlighting the importance of a rigorous statistical evaluation of potential drivers of N_2O variability. We agree in principle that multivariate and more advanced statistical approaches can be valuable tools to disentangle complex relationships among physical, chemical, and biological parameters.

In the present study, however, our decision to limit the statistical analysis was based on the characteristics of the dataset and the observed behaviour of N_2O concentrations. As shown clearly in Figure 3 and the associated time series, N_2O concentrations in both the ULW and SML remain remarkably constant throughout the entire experimental period and show no significant difference over time (we now added information on the sampling time in the figure caption). These constant N2O concentrations persists despite pronounced temporal variability in other measured parameters, including light availability, temperature, salinity, chlorophyll *a*, and the progression of the phytoplankton bloom. No significant or systematic changes in N_2O concenrations are apparent with diel cycling, increasing salinity, or biological development, nor do the data suggest layer-specific differences. To further proof this we now applied a generalized additive mixed model (GAMM) to the N_2O concentration data and we also added a statistics part into the method section (for more details on the application and outcome see methods line 187 to 201 and results lines 245 to 260).
Given the absence of visible trends, gradients, or co-variability in the N_2O time series, we consider that the application of more complex statistical methods (e.g., correlation analyses or multivariate models) would not provide additional mechanistic insight beyond what is already evident from the data. In particular, statistical relationships would be difficult to interpret meaningfully in the absence of detectable N_2O variability and could give a misleading impression of underlying controls where none are supported by the observations.
To clarify this rationale, we have revised the manuscript to more explicitly state that the lack of observed temporal, diel, or vertical variability in N_2O—despite substantial changes in environmental and biological parameters—is a key result of this study, and that this motivated our conservative statistical approach. We also now better justify why simple comparative statistics were deemed sufficient in this specific context (line 240 ff).
We appreciate the reviewer's suggestion and hope that this clarification adequately explains our reasoning.

My second concern relates to the Methods section. While I understand that many details of the incubation setup are described in Bibi et al. (2025), the current manuscript still lacks essential information needed to fully understand the experimental design. Readers should not have to rely entirely on another source to grasp the methodology. For example, the depth at which ULW samples were collected and the sampling procedure should be clearly stated. A brief explanation of the glass plate method for SML sampling may be beneficial, too. A brief description of the mesocosm facility is also necessary at the beginning, including the total volume of the setup, and if the setup has a mixing system.

In line 82, the authors mention that nutrients were added to trigger a phytoplankton bloom; the exact amounts or concentrations should be provided here rather than referring to the previous article. Additionally, the manuscript notes that "Jade Bay water was replenished with 4.5 L per day to replace the water removed by sampling." The potential impact of this replenishment on N_2O concentrations should be discussed, as it could influence the interpretation of the results. I wonder if the addition of water could cause mesocosm mixing or if it may add some N_2O or dilute it.

We thank the reviewer for this comment and for emphasizing the importance of clearly describing the experimental design. As stated the mesocosm experiment was conducted within the framework of the DFG-funded BASS project and involved multiple research groups

addressing different aspects of the sea surface microlayer. To avoid extensive repetition of identical methodological descriptions across the resulting manuscripts, a dedicated overview paper (Bibi et al., 2025) was written, which provides a comprehensive and detailed description of e.g. the mesocosm setup, experimental timeline, nutrient additions, salinity development, and bloom dynamics. This overview paper is published in the same special issue to which we submitted this manuscript and to which all other manuscripts with further results were submitted.

In the present manuscript, we therefore deliberately focus on the aspects of the experimental design that are directly relevant for the interpretation of the N_2O measurements, while referring to Bibi et al. (2025) for full technical and procedural details. Nevertheless, we have ensured that the key elements necessary to follow the study and its conclusions are summarized in the Methods section (e.g. the mixing system is described in line 71, sampling depth of ULW is mentioned in line 96 and further details concerning the sampling procedures are stated in paragraph 1.2 N2O Sampling). However, we have now carefully re-checked the manuscript and clarified several points to make these descriptions more explicit. E.g. concerning the daily water addition from Jade Bay we now added the total volume of the mesocosm basin (13,600 L) in line 68 ff., to highlight that the 4.5 L of daily Jade Bay water correspond to only 0.033% of the original volume and therefore are not considered to have any effect on the N_2O concentrations nor on any other parameter in the basin.

We hope this approach balances clarity and conciseness and allows readers to access additional methodological detail where needed without unnecessary duplication.

Since the estimated rates of photochemodenitrification are derived from nitrite concentrations, the authors should provide a description of the analytical method used to measure nitrite, including its detection limit and sensitivity. Additionally, it would be important to discuss whether the nitrite detection limit could constrain the ability to identify diel variations in photochemodenitrification rates.

We thank the reviewer for this suggestion. While the nitrite measurements were conducted by another project partner and are described in detail in the BASS overview paper (Bibi et al., 2025), we agree that a brief summary of the analytical approach and detection limits is useful for context. We have therefore added a concise description of nitrite sampling, analysis, and detection limits to the Methods section. We now also briefly discussed the detection limit of nitrite and its relevance for the identification of diel variations in lines 329 ff.

SML sampling method: The use of the glass plate technique for collecting N_2O samples from the sea surface microlayer is not ideal, as it may underestimate N_2O concentration. However, the discussion provided in Section 3.1 is valuable and helps address these concerns.

We thank the reviewer for this positive feedback.

Some minor comments below:

Please check the section numbering.

The section numbering was revised and should now be correct.

Figures may need some work. See the asterisks on the axis titles, carefully check figure captions, and the dimensioning issue in Fig 2. It might be a problem during the preprint editing, but in my version of the manuscript, I see ULW samples as yellow circles rather than open circles.

Thank you for highlighting the issues. We carefully checked all the figures and figure captions again. The dimensioning issue in figure 2 is now resolved, the asterisks are removed and the caption of figure 2 was corrected.

The detailed explanation provided in sections 1.3 – 1.6, which includes equations that are often omitted in manuscripts, is excellent. The community will appreciate that the authors included this information.

We appreciate the kind feedback.

Did the authors check for outliers in the dataset? If the maximum concentration of 16.6 nM is an outlier, this should be explicitly stated rather than repeatedly highlighted throughout the manuscript (e.g., lines 202, 207, …).

Thank the reviewer for the suggestion, the part was now rephrased to clarify state that we excluded the data point as an outlier (Line 242).

I recommend that the authors verify the N2O gas exchange calculations and, if possible, compare the approach used (based on Liss and Merlivat, 1986) with alternative parameterizations. The estimated flux values appear unusually high (up to 4.8 nmol N2O L^{-1} h^{-1}).

We thank the reviewer for this comment. The N_2O gas exchange calculations were carefully checked and are based on the parameterization of Liss and Merlivat (1986), as described in the Methods section. This approach was deliberately chosen because it is largely derived from wind–water tunnel experiments and thus better reflects the limited fetch and small-scale wind and wave conditions of the mesocosm setup. In contrast, commonly used open-ocean parameterizations are based on conditions that are not applicable to land-based mesocosms and would therefore not be appropriate here. We have now clarified this rationale and the interpretation of the comparatively high flux values in the discussion.

Table 1. I suggest authors specify if the values from McLeod et al 2021 were cultures exposed to natural sunlight or if they were UV irradiated.

We appreciate the suggestion and added the information into the table and the text (line 281).

Note that lines 307 and 308 are missing in the preprint pdf.

We apologize for the missing part and completed the sentence as follows: “This could have led to the establishment of a steady state during the day time which persisted during night time because sources and the sink were not active during the night.”