Respond to RC1

RC1: 'Comment on egusphere-2025-5385', Anonymous Referee #1, 20 Dec 2025

This manuscript presents a comprehensive field study on the biomolecular composition of the sea surface microlayer (SML) in the Central Baltic Sea, with a particular focus on the role of cyanobacteria and phytoplankton size structure. The dataset is rich and combines multiple complementary approaches, including microscopy, flow cytometry, detailed biomolecular analyses, and surfactant measurements. The study has clear potential to advance our understanding of how phytoplankton community structure imprints on SML chemistry and potentially affects air–sea exchange processes.

We thank the referee for this positive and constructive assessment of our manuscript. We appreciate the recognition of the comprehensive dataset, the combination of complementary methodological approaches, and the potential relevance of our results for understanding how phytoplankton community structure influences SML chemistry and air–sea exchange processes. We believe that the referee's comments have helped to substantially improve the manuscript.

However, several issues need to be addressed before the manuscript can be considered for publication. Most importantly, the classification scheme based on HPA/LPA and phytoplankton size classes is conceptually confusing and, in its current form, hampers the interpretation of results and discussion. The conflation of size range and abundance category, as well as the use of cross-definitions (e.g. HPA>20 µm corresponding to LPA<20 µm), should be clarified or simplified, ideally by adopting more explicit ecological descriptors.

Thank you for highlighting this important point. We agree that the current classification scheme may be conceptually confusing, particularly due to the conflation of size classes and abundance categories and the use of cross-definitions (e.g., HPA>20 µm corresponding to LPA<20 µm). Both reviewers found the abbreviations difficult to understand and has caused confusion throughout the manuscript. We have decided to change the abbreviations to enable clearer classification and a clearer structure in the manuscript.

New abbreviations:

- **H-Micro/L-Micro**
  - Micro: includes microphytoplankton (larger 20µm) and refers to the net samples.
- **H-Nano /L-Nano**
  - Nano: includes pico- and nanophytoplankton (smaller and equal 20µm) and refers to the flow cytometry samples.

HPA>20µm and LPA>20µm will be changed to H-Micro and L-Micro. HPA<20µm and LPA<20µm will be changed to H-Nano and L-Nano in the whole manuscript (text and figures). We expect that these changes will substantially improve the clarity and interpretability of the results and discussion.

When presenting the results that describe the differences between the abundances of a size fraction, e.g., H-Micro vs. L-Micro (e.g. Fig. 5a), we will solely focus on these differences and refrain from explicitly reiterating the inverse relationship for the smaller size fraction. With this we will avoid cross-definitions.

The manuscript contains numerous punctuation and formatting inconsistencies throughout the text, which should be carefully checked and corrected by the authors.

We thank the reviewer for carefully reading the manuscript and for noticing the punctuation and formatting inconsistencies! We appreciate this detailed attention and will carefully correct these issues throughout the manuscript.

Overall, this is a potentially impactful contribution, but substantial revisions are required to improve conceptual clarity.

1. Specific scientific comment

Line100: The manuscript discusses surfactants, amino acids, and carbohydrates in the SML in relation to cyanobacteria. However, it remains unclear whether these compounds are produced directly by cyanobacteria or whether they primarily result from bacterial processing and degradation of cyanobacterial biomass and exudates. The relative roles of cyanobacteria versus heterotrophic bacteria in shaping the SML biomolecular composition require clearer clarification.

Based on the data presented, we cannot differentiate whether the detected biomolecular compounds originate directly from cyanobacterial production or from bacterial processing and degradation of cyanobacterial biomass and exudates. Our dataset does not allow us to disentangle these pathways mechanistically.

In the revised manuscript, we will clarify this limitation and change the respective interpretations. We will expand the Introduction and Discussion to more clearly acknowledge the potential contributions of both cyanobacteria and heterotrophic bacteria in shaping the biomolecular composition of the SML and to emphasize that our results reflect associations rather than direct source attribution.

Line103-105: The SML was sampled using a glass plate or Garrett Screen, whereas the ULW was sampled using nets and/or discrete water samples at ~1 m depth. Given the use of different sampling methods, it is unclear to what extent the observed molecular differences between the SML and ULW may be influenced by methodological biases, particularly with respect to particulate versus dissolved fractions. In addition, Table A1 suggests that ULW samples were collected using a Niskin bottle, which raises some ambiguity as to whether both net- and bottle-based approaches were used for ULW sampling. The authors should clarify the sampling strategy and discuss potential methodological effects on the SML–ULW comparison.

We thank the reviewer for this thoroughly comment! The underlying water (ULW) samples in this study were collected exclusively using a Niskin bottle at approximately 1 m depth for surfactant measurements. No net-based sampling was applied for ULW for surfactant studies, and the text will be revised to remove any ambiguity and to ensure consistency with Table A1. The use of a Garrett Screen (and, where conditions permitted, a glass plate) for sampling the sea surface microlayer (SML), together with Niskin bottle sampling for the underlying waters, represents a well-established and widely accepted methodological framework in SML research, as documented in the SCOR Working Group 141 guide (SCOR, 2014). These methods are considered standard for SML–ULW comparisons. Whenever meteorological conditions allowed, SML sampling was conducted directly from a small boat to ensure optimal sampling conditions, otherwise the Garrett Screen was used from the bow of the mother ship. Although different sampling approaches may preferentially capture particulate versus dissolved fractions, the applied methods follow community standards, and potential methodological effects are unlikely to fully explain the observed molecular differences between the SML and ULW.

We will address and discuss potential molecular differences that may arise from methodological or meteorological influences in the Supplement (Fig. S8). We will adjust Fig. S8 and to also show methodological differences in the total amino acid data.

In Section 2.2, net samples containing the >20 µm particulate fraction were diluted with filtered seawater at ratios ranging from 2:1 to 1:2 prior to subsampling. It is unclear whether this dilution procedure may have altered the structure of colloids or extracellular polymeric substances (EPS), potentially affecting aggregation state and surface activity measurements. The authors should briefly discuss whether dilution could influence EPS integrity and surfactant analyses.

We are grateful for this detailed comment. Surfactants were only taken from the SML and ULW samples, which were not diluted with filtered seawater. Only the net samples were diluted with filtered seawater due to very high biomass. We will clarify this in the method section, which collection method has been used to measure which parameter. We will also highlight, that net catches potentially enhance aggregation of formerly dissolved EPS into particulate forms. However, the contribution of

large phytoplankton cells to overall POC (~13%) in eutrophic systems is supported by the literature. Furthermore, we do not present EPS data in this manuscript.

In the microphytoplankton microscopy analysis, community composition and biomass estimates were restricted to the eight most dominant species. While these taxa likely dominate in abundance, it remains unclear whether less abundant species with potentially large cell volumes may still contribute disproportionately to biomass, EPS production, or surface-active compounds.

Thanks for this comment! Since we sampled during the main summer cyanobacterial bloom- time, we expected to find the phytoplankton community would be dominated by cyanobacteria in abundance and biomass. This expectation was based on several studies from previous years (HELCOM, 2025; Ploug, 2008; Zettler et al., 2024), which we also referenced. During the sampling period, cyanobacteria accounted for the majority of total microphytoplankton in terms of both abundance and volume, i.e. biomass. Nevertheless, we agree that rarer species may still play a role in shaping SML biogeochemistry. This potential limitation will be explicitly acknowledged, and a corresponding clarification will be added to the manuscript.

In Section 2.7, the manuscript states that triplicate 18 mL SML and ULW samples were prepared, but only one of the three replicate samples was analyzed. It is unclear why only a single replicate was measured and what the purpose of the remaining replicates was.

We thank the reviewer for spotting this inconsistency. We took triplicates for surfactant samples, of which two replicates were stored at 4 °C and one replicate at -20°C. Our intention was to measure the fresh surfactant samples directly after the cruise. However, due to logistical challenges this could not be realized. We decided to only include the surfactant replicate which was stored at -20°C and in alignment with the storage protocol of the amino acid and carbohydrate samples. We will clarify this in the method section: We will not mention that replicates were taken and stored at difference temperatures. We will revise accordingly and write: e.g. One sample of 18 mL SML and ULW was taken and stored at -20°C.

In Section 3.1, the classification of phytoplankton abundance is confusing. The low-abundance category of phytoplankton >20 µm is labeled as “LPA<20 µm”, which is identical to the low-abundance category of phytoplankton <20 µm. This notation conflates size class and abundance level and may mislead readers to interpret the low-abundance >20 µm group as belonging to a different size fraction. Low-abundance categories should retain the same size designation (i.e. >20 µm) and differ only in abundance. In addition, Aphanizomenon sp. is listed twice in the species list. Finally, the notation “300 x 10^3 cells mL^{-1}” does not follow standard scientific formatting and should be written as “300 × 10^3”.

We thank the reviewer for this detailed comment. We agree that the classification is confusing and that making mistake like writing <20µm, when >20µm is meant, is even more confusing. We decided to change the names of the categorization that is less confusion (see above: H-Micro, L-Micro, H-Nano, L-Nano) and we think that changing the names for the groups will improve readability and will lead to less confusion

Thank you for pointing out the formatting issue. We will revise the manuscript and change it to the correct scientific format.

In the second half of this section, the logic of the HPA/LPA classification becomes increasingly difficult to follow and begins to affect the interpretation of the results. Statements such as “under HPA>20 µm (which corresponds mostly with LPA<20 µm conditions)” and “LPA>20 µm conditions (which corresponds to HPA<20 µm conditions)” introduce cross-definitions that obscure which ecological states are actually being compared.

We thank the reviewer for this comment and agree that this cross-definition can be very confusing and may lead to misinterpretation. We will revise this in the manuscript. When comparing specific size fractions (e.g., H-Micro µm and L-Micro), we will focus strictly on the comparison between these two groups without implying direct equivalence. We believe that a clearer explanation of the relationship we observe between the Micro- (>20 µm) and Nano- (<20 µm) phytoplankton fractions will help reduce confusion. We will therefore describe in more detail that, as shown in Table A2, at 10 out of 15 stations we observe an inverse pattern: high abundance of >20 µm phytoplankton (Micro) is associated with

low abundance of <20 µm phytoplankton (Nano), and vice versa. However, this pattern is not observed at three stations (12-11, 14-01, and 21-02), and at two stations no >20 µm phytoplankton (Micro) were sampled, preventing classification into high or low >20 µm (Micro) abundance. We will explicitly point this out, but we will avoid statements such as “under HPA>20 µm (which corresponds mostly with LPA<20 µm conditions)” and “LPA>20 µm conditions (which corresponds to HPA<20 µm conditions).” Instead, we will refer only to the differences between the two groups as shown in the respective figures.

Please note: in this example, the abbreviations have not yet been changed so that the reviewer can still refer to the latest manuscript. The abbreviations will be changed in the next version of the manuscript.

In Section 3.2, there is an inconsistency between the statistical significance and the descriptive language used to interpret the results. For TAA, terms such as “tendency,” “slightly higher,” and “more pronounced” are repeatedly used, while the corresponding statistical tests are either not significant (e.g. p = 0.076) or not explicitly reported.

We agree with the reviewer that these terms are not suitable for the given statistical significance and are very often used. We will make appropriate changes in the manuscript for example write: The median value of *xyz* was higher than that of *xyz*, however, this observed difference was not statistically significant.

In Section 4.2, the manuscript repeatedly emphasizes that enrichment factors (EFs) are close to 1, while at the same time using relatively strong wording such as “significantly higher,” “substantially higher,” or “highest average EF” to describe differences. For example, the mean TAA EF of 1.2 ± 0.4 largely overlaps with the previously reported range of 0.8–1.2, raising the question of whether this truly represents enhanced enrichment in a statistical or process-based sense. It may be more appropriate to describe these values as being at the upper end of the historical range or as consistent with, but slightly elevated relative to, previous studies, rather than implying a clearly enhanced enrichment.

We will adjust this Section and use more suitable wording for describing the differences as suggested by the reviewer.

4.3 Section, In the discussion of POC composition, the authors state that cellular biomass accounts for only ~13.5% of POC>20 µm, with the remainder attributed to heterotrophic plankton, detritus, and extracellular material. This interpretation is conceptually sound and well supported by the literature. However, the subsequent statement that “POC>20 µm was related to cyanobacterial cellular biomass >20 µm (HPA>20 µm: 1.6 ± 1.7; LPA>20 µm: 0.7 ± 0.7%)” is unclear. It is not evident whether these percentages refer to correlation strength, explained variance, or relative contribution to POC. The authors should clarify the meaning of these values and how they were derived. In addition, “Synecococcus sp.” appears to be a typographical error and should be corrected to “Synechococcus sp.”.

We thank the reviewer for this this detailed comment. We will correct this section in the manuscript, as the percentage values refer to POC of TOC and were incorrect in the manuscript. We apologize for this error and will revise the manuscript accordingly.

Changes in text:

Line 587-588: […] this study, POC >20 µm accounted for an average of 1.2 ± 1.4% of TOC. In relation to the detected cyanobacterial cellular biomass, POC contributed 1.6 ± 1.7% to TOC under H-Micro conditions and 0.7 ± 0.7% under L-Micro conditions. Our results confirm the […]

We will correct the typographical error and change “Synecococcus sp.” to “Synechococcus sp.” In line 614.

1. Other comments and technical corrections

Line19: Please define the abbreviation “HPA” at its first occurrence.

Thank you for this. Since we have decided to change the abbreviations, we will write “high phytoplankton abundance” at this point in the manuscript.

Line36: Please add a space after the citation “(Engel et al., 2017)”.

A space will be added after the citation “(Engel et al., 2017)”.

Line53: The phrase “A large-scale oceanic in the Atlantic demonstrated” appears incomplete. A noun such as “study”, “survey”, or “investigation” seems to be missing.

We thank the reviewer for this comment. The sentence will be revised by adding the missing noun “study” to complete the phrase.

Line 121: In “>20µm”, a space is missing before “µm”. Please check and correct similar formatting issues throughout the manuscript.

Thank you for pointing out the formatting issue. A space will be added before “µm” in “>20 µm”, and similar issues will be corrected throughout the manuscript.

Line 123: “Garret” should be corrected to “Garrett”.

Thank you for catching this typo. “Garret” will be corrected to “Garrett”.

Line 130: The units “knt” and “knots” are used interchangeably. Please standardize the unit notation.

The unit notation will be standardized and used consistently throughout the manuscript. We decided to use “knots”.

Line 131: In “~14min”, please add a space before “min”.

Thank you for noting this. A space will be added before “min”.

Line 146: “within ≤2 h” is recommended to be revised to “within 2 h”.

The phrasing will be revised to “within 2 h”.

Line 153: “Bottle” should be “bottle”.

“Bottle” will be corrected to “bottle”.

Line 185: Please provide a reference for the chlorophyll *a* measurement method.

Thank you for this comment. A reference for the chlorophyll a measurement method will be added.

Line 215: “sample” should be corrected to “samples”.

“sample” will be corrected to “samples”.

Line 223-224: Thirteen amino acids are stated, but only twelve are listed. In addition, please revise the punctuation in the references according to journal style.

We thank the reviewer for this careful observation. The list of amino acids will be corrected to include all thirteen compounds (GABA was missing in the list), and the reference punctuation will be revised according to journal style.

Line 225: In “(Dittmar et al., 2009.; Engel & Händel, 2011)”, there is an extra punctuation mark after “2009”.

Thank you for pointing out the extra punctuation. This will be corrected.

Line 231: “Sample were measured” should be corrected to “Samples were measured”.

Thank you for noting this grammatical error. The sentence will be corrected to “Samples were measured”.

Line 236: “the samples ionic strength was standardized” should be revised to “the sample’s ionic strength was standardized”.

The sentence will be revised to “the sample’s ionic strength was standardized”.

Line 327, In the sentence “Cylindrotheca closterium and Chaetoceros sp. occurred at HPA>20 µm only with < 0.2 µg C L^{-1})”, there is an extra closing parenthesis.

Thank you for identifying this issue. The extra closing parenthesis will be removed.

Line 407: The term “later amino acids” is incorrect and should be replaced with “latter amino acids”.

Thank you for pointing this out. “later amino acids” will be replaced with “latter amino acids”.

Line 419: “glucosamin” is missing the final “e” and should be corrected to “glucosamine”.

Thank you for noting this typo. “glucosamin” will be corrected to “glucosamine”.

Line 435: The phrase “excluded … due to their low and abundance” is incomplete and should read “low abundance”.

Thank you for highlighting this. The phrase will be revised to “low abundance”.

Line 698: “Moring” should be corrected to “Morning”.

Thank you for catching this typo. “Moring” will be corrected to “Morning”