Responses to Reviewer #3:

Synopsis:

This study describes the application of a new method (TM-DART-QTOF-MS) to the study of dissolved organic components of seawater. A major advantage of the approach is its relative insensitivity to salt, making desalination of the samples unnecessary. Data reduction techniques are used to distinguish and characterize the samples. Special attention is given to an attempt to distinguish surface microlayer (SML) composition from underlying water (ULW) composition. Finally, experiments are performed on a subset of the samples to test their ability to generate secondary organic aerosol in a photochemical reactor.

Just for clarification, lab-to-the-field experiments were performed during the field campaign at Cape Verde islands, whereas the untargeted chemical screening by TM-DART-QTOF-MS was performed after the campaign.

Broad impressions:

This manuscript is mostly easy to read, and it describes the analytical methods applied in a high level of detail. I agree with the authors that there is great potential in the presented approach, and I appreciate their thorough characterization of the analytical method. However, not enough attention has been given to the actual biogeochemical system being studied, both in terms of the sampling methods and the interpretation. As a result, I do not think the conclusions pertaining to the SOA-forming chemistry of the surface microlayer are supported by the work presented. I think that either the scope of the work needs to be reduced and the conclusions about actual marine chemistry cut back, or the analysis and interpretation need to be expanded significantly. I recommend the former. This analytical approach shows promise; it's a proof of concept for a strategy to distinguish seawater compositional types and their potential for reactive chemistry. But this manuscript has not shown in a compelling way what actually distinguishes SML from ULW, or SOA-forming organics from non-SOA-forming organics.

We acknowledge the reviewer's observations to the work described in the submitted manuscript. To clarify some aspects of the comments provided by the reviewer, we describe the objectives of the work:
i) to develop an ambient mass spectrometry-based untargeted metabolomics method that would allow a comprehensive screening of seawater samples with no need of desalination using a DART ionization source operated in negative mode and coupled to a high resolution mass spectrometer;
ii) to isolate by means of multivariate statistical methods a panel of ionic species that were present in both SML and ULW samples but based on their relative levels they differentiated seawater samples according to their collection depth (i.e., SML or ULW);
iii) to provide putative identification of these discriminant ionic species (based on the ionic species detected according to the ionization mechanisms operating in a negative mode DART ion source, based on accurate mass values and on isotopic patterns) to understand their possible implication in secondary organic aerosol (SOA) formation

processes at the water/air interface based on their functional groups and chemical families;
iv) to develop a lab-to-the-field approach to evaluate the SOA formation potency of SML samples;
v) to apply multivariate statistical methods to analyze the data acquired by TM-DART-QTOF-MS from the subset of SML samples that were also analyzed in-site by a lab-to-the-field approach;
vi) to isolate by means of Principal Component Analysis, which is a non-supervised method, those features (ionic species) with largest weight in differentiating samples that lead to particle formation from those that did not lead to particle formation according to the results from field experiments;
vii) to provide putative identification to those discriminant features;
viii) to connect the results obtained from both type of experiments, i.e., the seaomics and the lab-to-the-field approaches, which consist in two different and complementary strategies.

As clearly stated in the whole manuscript and summarized in the abstract, a panel of 11 ionic species detected in all seawater samples (SML and ULW) allowed sample class discrimination by means of supervised multivariate statistical models. Tentative identification of these species suggest that saturated fatty acids, peptides, fatty alcohols, halogenated compounds, and oxygenated boron-containing organic compounds may be involved in water-air transfer processes and in photochemical reactions at the water-air interface of the ocean. Results from the lab-to-the-field experiments conducted to explore secondary aerosol formation potency of a reduced number of samples (n=5), which were also subsequently interrogated with the TM-DART-QTOF-MS-based untargeted metabolomics analytical strategy, provide a proof of concept that organic compounds may play a key role in aerosol formation processes at the water/air interface. We consider that these results do contribute to the chemical characterization of the sea surface microlayer composition through the implementation of a developed mass spectrometry-based untargeted metabolomics analytical method utilizing an ambient ionization source. We also consider that experiments conducted in-site to evaluate SOA formation potency of SML samples add value to the untargeted chemical analysis. Therefore, seaomics results were discussed in terms of their implications on SOA formation. To our understanding, the novel analytical method and the results described in the manuscript may be interesting for the scientific community and should therefore be considered for publication in ACP.

More studies have been and are planned to be conducted on the same seawater samples for a deeper and complementary characterization of SML and ULW samples, and to understand to what extent is seawater a source of marine organic matter on aerosol particles and cloud water, as described in the following overview paper: van Pinxteren et al., in review, 2019. This paper provides an introduction to the MarParCloud (Marine biological production, organic aerosol Particles and marine Clouds: a process chain) campaign at the Cape Verde islands and the MARSU project, describing the scientific content of the field campaign, the interconnection between the different facets of the project and the first findings to serve as an overview of each specific study. A detailed description of water sampling methods for the different studies conducted in the frame of the campaign can be found in the cited manuscript. This paper will be cited in the revised version of the manuscript.

In line with the previous discussion, and to address the reviewer's suggestions, the following changes will be done on the revised manuscript (edits indicated in italics):

i) Abstract:

"Results from these experiments and the analytical seaomics strategy provide a proof of concept that organic compounds *may* play a key role in aerosol formation processes at the water/air interface."

ii) Section 3.4, Line 375:

"These results provide a proof of concept that organic compounds *may* play a key role in aerosol formation process at the water/air interface."

iii) Conclusions:

"Combined results from TM-DART-QTOF-MS and on-site SOA formation testing experiments on SML samples, suggest that organic compounds enriched at the water/air interface *may* play a key role in aerosol formation process."

Major comment 1:

The samples were frozen upon collection without any filtration. They were then thawed for analysis and centrifuged to remove large particles and colloids. This processing will result in the lysis of intact cells present in the samples, releasing dissolved compounds that will not be removed by centrifugation and will be included in the mass spectrometric analysis. One of the main reasons to perform this analysis is to understand the reactivity of the surface microlayer, and the inclusion of chemical species that were likely not available for photochemistry in the ambient environment makes this analysis difficult to interpret in that context. That is an issue that can certainly be addressed in a future study, but at the very least it needs to be discussed in this manuscript, and unless the authors can convincingly argue otherwise, it seriously limits their ability to make claims about SML reactivity. The SML can have much higher concentrations of particles (e.g. cells) than bulk water, so the impact of this effect could be very large.

We agree with the reviewer that the fact that samples were not filtered immediately after collection suggest that the analytical method was utilized for a comprehensive analysis of the marine metabolome including both the endo and exometabolome. Actually, this information was stated in line 361 of the manuscript as follows:

"It is worth noting that organic compounds identified in the discriminant panel may have derived both from the secreted (exometabolome) and/or intracellular metabolites (endometabolome) of biological organisms such as algal species and microorganisms present in seawater."

Since all samples were identically treated, the results are useful to illustrate relative differences in metabolites that are present in all samples collected at different depths (i.e., ULW and SML). As indicated in Table S4, fold changes were calculated for *discriminant features detected in all samples (both SML and ULW). Within the 11 discriminant features detected in all samples, 3 were statistically enriched in SML samples and 2 were statistically enriched in ULW samples with 7 out of 11 exhibiting positive fold changes towards SML samples.*

The manuscript clearly indicates that results obtained with the seaomics and the lab-to-the-field approaches provide a proof of concept that organic compounds may play a key role in aerosol formation process at the water/air interface. In addition, results from the atmospheric simulation experiments conducted on SML samples were in agreement with previous laboratory studies that demonstrated air-sea interfacial driven chemistry as a source of marine secondary aerosol (Roveretto et al., 2019; Ciuraru et al., 2015; Fu et al., 2015).

In agreement with the reviewer's remark, a different study may be conducted in the future with a different aim and design, based on the results obtained in the present study.

Further analyses of these seawater samples have been and will be conducted with additional analytical platforms to provide a complementary characterization of SML and ULW samples as detailed in van Pinxteren et al., in review, 2019. For example, enrichment factors obtained for bacterial abundance in SML samples ranged between 0.88 and 1.21 (van Pinxteren et al., in review, 2019) despite the expected larger concentrations, as suggested by the reviewer.

Regarding the experiments performed in-site during the field campaign with the lab-to-the-field approach to evaluate the feasibility of SML samples to lead to SOA formation, the strategy was to use seawater samples with as little modification as possible. Centrifugation was mainly conducted to concentrate the surface microlayer from the seawater. As expected, this step partly removed large particulate matter and colloids. But, centrifugation was aimed only at concentrating SML samples as a condition for aerosol formation.

The revised version of the manuscript will include the following statement for clarification:

"Centrifugation was aimed at concentrating SML samples as a condition for aerosol formation."

Major comment 2:

How can we infer that the handful of species that were identified as the best discriminants of SML vs. ULW are the species that are participating in important SML photochemistry and air-sea exchange? That is extremely speculative, even if the cell lysis issue discussed above is resolvable. Is there reason to think that boron-containing oxygenated organics are good SOA formers? Aren't there probably thousands of other potentially reactive compounds that covary with the SML-determining features that are identified here? There is a serious lack of discussion of these issues in this manuscript.

The best discriminant feature panel for SML vs. ULW samples comprises species that may participate in SML photochemistry and air-sea exchange based on the functional *groups provided by the putatively identified ionic species in agreement with previous evidence reported in the literature cited in the manuscript. The following statements addressed in the original version of the manuscript the potential role of putatively identified discriminant compounds in SOA formation processes:*

Line 38: "A panel of 11 ionic species detected in all samples allowed sample class discrimination by means of supervised multivariate statistical models. Tentative identification of these species suggests that saturated fatty acids, peptides, fatty alcohols, halogenated compounds, and oxygenated boron-containing organic compounds may be involved in water-air transfer processes and in photochemical reactions at the water-air interface of the ocean."

Line 359: "Possible sources of halogenated compounds in SML samples are photochemical reactions occurring at the water/air interface (Roveretto et al., 2019; Donaldson and George, 2012)."

Line 364: "Putative identification of the discriminant panel capable of differentiating SML from ULW samples provides further evidence to support secondary organic aerosol (SOA) formation detected with the lab-to-the-field approach during the campaign."

Line 381: "Tentative identification of the discriminant metabolite panel suggests that halogenated compounds, fatty alcohols, and oxygenated boron-containing organic compounds may be involved in water-air transfer processes and in photochemical reactions at the water-air interface of the ocean."

We also clearly stated at the end of section 3.4 (Discriminant Compound Identification & Role in Aerosol Particle Formation) the limitation associated to the low number of samples (n=5) that were able to be analyzed by both the lab-to-the field approach and the TM-DART-QTOF-MS approach. We still consider an interesting result the fact that by implementing a non-supervised multivariate statistical method such as PCA, samples were able to be grouped according to their feasibility of leading to particle formation as identified with the lab-to-the-field approach. The manuscript does not claim that boron-containing oxygenated organics are good SOA formers. The putative identification of features with the largest weight in the loadings plot associated to PC2 in Figure S8 suggest the presence of boron-containing oxygenated organic compounds. The manuscript clearly indicates in Table S5 that boron-containing oxygenated organic compounds are 4 putatively identified compounds based on accurate mass and isotopic pattern analysis out of 7 discriminat features selected based on a threshold applied to the PC2-associated loading values. These compounds were only putatively annotated but still the information is considered to be a valuable result that deserves further investigation in future studies since, as stated in the manuscript, "boron-containing compounds are known to be ubiquitous in vascular plants, marine algal species, and microorganisms (Dembitsky et al., 2002)".

Minor comments:

It is not clear to this reviewer that it is necessary to coin the term "seaomics" in order to adequately describe the analysis presented.

We believe that "seaomics" is a simplified but straightforward term that summarizes the untargeted metabolomics strategy utilized to interrogate seawater samples.

Page 5, section 2.3: last sentence of first paragraph is hard to understand.

We agree with the reviewer's remark. The sentence will be modified in the revised manuscript as follows:

"Subsequently, 2 mL of surface solution was collected from each centrifugal vessel to isolate closer representations of SML samples considering the dilution factor inherent to the collection process, i.e., SML diluted with ULW contribution, and leading to a total sample volume of 24 mL for subsequent experiments."

References

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