

*Responses to Reviewer #1:*

Zabalegui et al present an analysis of surface microlayer seawater samples collected from Cape Verde. Extracted SML samples were analyzed via DART-MS approaches to characterize organic species present. The intent of the work is to relate seawater organic composition (both in the SML and underlying water) to the production of VOC that can go on and form secondary organic aerosol. To this end, the investigators conducted a parallel experiment where secondary organic aerosol was measured following the OH oxidation of VOC formed from illuminated SML surfaces. The paper describes a new application of DART-MS to SML characterization and novel measurements of SML in the ocean. The paper is likely publishable in ACP, following the authors attention to the following general and specific comments.

General Comments:

The direct link between SOA formation and seawater composition is not well established chemically. A few things might help in this discussion:

1) Describe in more detail what differentiates the SML samples that lead to SOA formation (e.g., where the collected at different times, do they have different organic/inorganic ratios, surface temperature, DMS). This begs the question why a more direct experiment wasn't done to link SML composition to SOA (like measuring the VOC).

*Marine trace gases such as dimethyl sulphide (DMS), VOCs and oxygenated (O)VOCs were measured in the frame of the field campaign conducted at the Cape Verde islands and discussed in the manuscript by van Pinxteren et al. that is available online in ACPD (van Pinxteren et al., in review, 2019). This manuscript describes the scientific content of the field campaign, the interconnection between the different facets of the MarParCloud and MARSU projects and the first findings to serve as an overview of each specific study such as the one described in the present work. This overview paper will be cited in the revised version of the manuscript to complement the information about the field campaign measurements and first results.*

*Lab-to-the-field experiments conducted to explore secondary aerosol formation potency were performed on a reduced number of SML samples (n=5) that were subsequently interrogated with the TM-DART-QTOF-MS-based untargeted metabolomics analytical strategy. Out of 5 tested samples, 2 led to SOA formation. Therefore, the number of analyzed samples is low to conclude on statistically significant differences from the values obtained from the measured parameters as suggested by the reviewer (sample collection date, time and temperature were presented in Table S1; and dissolved organic carbon values can be found in van Pinxteren et al., in review, 2019). However, the results obtained in the present study from both types of experiments provide a proof of concept that organic compounds may play a key role in aerosol formation processes at the water/air interface, 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).*

*All in all, we agree to the reviewer, but direct in-situ VOC measurements were not within the scope of the MARPARCLOUD field work.*

2) Figures such as F5 and S8 are confusing. Objectively, these appear to be the same figure, but yield different conclusions regarding the relationship between PC scores and SOA formation. I think a bit more discussion is needed to guide the reader through this relationship. For example, it is not clear what to take from text such as line 374: “results suggest that SML samples that led to particle formation were 375 enriched on boron-containing organic compounds and other unidentified molecules (Table S5)” In Table S5 there appears to be a slight increase in average intensity for the peaks listed, but these are likely only a very, very small subset of all the organic compounds present.

*Multivariate statistical techniques make use of all variables (compound features) simultaneously and deal with the relationship among them to reduce the data dimensionality, find underlying trends, and isolate those features relevant to class discrimination. Multivariate statistical methods can be supervised or unsupervised if class membership is provided or not, respectively.*

*Figure 5 shows a PCA scores plot of all SML samples analyzed by the TM-DART-QTOF-MS-based untargeted metabolomics strategy using the set of 51 features for averaged technical replicates. As indicated in the legend of Fig. 5, samples that were evaluated for particle formation during the Cape Verde field campaign were indicated with circles for those that led to SOA formation and rectangles for those that did not lead to SOA formation. Figure 5 shows that SML samples were not distinguished based on the collection method, i.e., GP or CAT (as it was clearly stated in line 366), and that those samples that were also evaluated for SOA formation during the field campaign and that led to aerosol formation were separated in the bidimensional scores map from those samples that did not yield to aerosol formation. Therefore, a further PCA model was built only with those samples (n=5) that were analyzed by both the lab-to-the-field approach and by TM-DART-QTOF-MS to explore sample clustering according to their feasibility of leading to particle formation using PCA, which is an unsupervised multivariate statistical method. Fig. S8A shows the PCA score plot of those 5 samples that were separated in different areas of the bidimensional map based on PC2 values according to their aerosol formation potency. Therefore, the loadings plot associated to PC2 was explored. Figures S8B showed that 7 out of 51 variables exhibited the largest weights for sample class separation based on a threshold applied to the PC2-associated loading values. Putative identification of these 7 features suggested the presence of boron-containing oxygenated organic compounds. By inspecting the relative levels of these features in both types of sample classes, an enrichment trend is observed for samples that led to SOA formation. Despite the limitation regarding the low number of samples (n=5) analyzed by both the lab-to-the field and the TM-DART-QTOF-MS approaches (discussed at the end of section 3.4 of the manuscript), and although compounds were only putatively annotated, the information reported in the work is considered to be a valuable result. Further investigation of this result is deserved in future studies considering that, 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)”.*

*To clarify a possible confusion suggested by the reviewer regarding PCA plots, the last paragraph of section 3.4 of the manuscript will be modified in the revised version of the manuscript as follows (inserted text shown in italics):*

“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. The PCA scores plot illustrated in Fig. 5 shows that SML samples were not distinguished based on the collection method, i.e., GP or CAT, and points out those SML samples that were also evaluated for SOA formation during the field campaign. As previously discussed, two of these SML samples (CAT8 and GP10) yielded SOA formation (Fig. 4). *Since CAT8 and GP10 were separated in the bidimensional scores map from the group formed of CAT3, CAT4 and CAT6; a further PCA model was built only with those samples (n=5) that were analyzed by both TM-DART-QTOF-MS and the lab-to-the-field approach (Fig. S8).* Figure S8A shows that PC2 clearly separates samples according to SOA formation. *Four out of 7 features that mainly contribute to sample class separation with largest absolute values in the loadings plot associated to PC2, and illustrated in Fig. S8B, were putatively identified as boron-containing organic compounds (Table S5).* Despite the limitations associated with the low number of samples used to perform statistical analysis, results suggest that SML samples that led to particle formation were enriched on boron-containing organic compounds and other unidentified molecules (Table S5). These results provide a proof of concept that organic compounds play a key role in aerosol formation process at the water/air interface.”

#### Specific Comments:

Line 53: I would suggest removing “secondary” as these processes influence the marine aerosol of both primary and secondary nature.

*We appreciate the reviewer’s suggestion. The word “secondary” will be removed from the statement in the revised version of the manuscript.*

Line 60: The work of Brüggemann did not prove that abiotic sources of VOCs are comparable to biological sources. This was a modeling study that scaled up laboratory experiments to the global scale.

*We agree with the reviewer’s remark. The statement will be modified in the revised version of the manuscript as follows:*

“On a global scale, interfacial photochemistry has recently been suggested to serve as an abiotic source of volatile organic compounds comparable to marine biological emissions (Brüggemann et al., 2018).”

Line 91: It would be helpful to include a short discussion here on the ionization process and the bias that it can introduce when attempting a holistic analysis. DART ionization proceeds in a very similar fashion to high pressure  $H_3O^+(H_2O)_n$  ion chemistry. As such, it is selective and dehydration reactions are common. It would be helpful to describe the advantages, but also the limitations when compared to ESI.

*Negative ionization DART follows negative ionization APCI-like mechanisms including electron capture, dissociative electron capture, proton abstraction, and anion adduction, which support the ionic species detected in the present study. Dehydration reactions are more commonly observed in positive ionization mode. Several*

*publications in the literature including 2 publications that were cited in the manuscript, discuss in detail DART mechanisms and the use of the negative ionization mode (McEwen and Larsen, 2009; Gross, 2014). The following additional reference will be included in the revised version of the manuscript to support the use of negative ionization DART-MS: Cody and Dane, 2013.*

*We agree with the reviewer that the ionization process introduces bias towards the fraction of the metabolome that can be interrogated with each mass spectrometry-based analytical platform. Indeed, different ionization techniques are able to cover different portions of the metabolome under study. Thus, different seawater fingerprints may be obtained with different ionization techniques providing complementary information. The strategy adopted in this work focused on minimum sample preparation and no sample desalinization by using a DART source that is less prone than ESI to ionization suppression by high salt contents as those expected in seawater samples. It is well known that seawater samples cannot be properly analyzed using an ESI source without a previous desalinization step.*

*The mechanisms operating in a DART ion source involve thermal desorption followed by plasma ionization. Therefore, a major limitation is that it requires analytes to be volatile or semi-volatile. In this regard, ESI offers the advantage of a covering more polar compounds than ionization sources operating under APCI-like mechanisms such as DART. The fraction of the marine metabolome that was covered with the implemented analytical strategy included lipophilic compounds extracted in acetonitrile and subsequently ionized under APCI-like mechanisms, limiting the analysis of more polar compounds. However, lipophilic compounds were proven to be involved in SOA chemistry and therefore consisted in an attractive fraction of the marine metabolome for interrogation.*

*Another limitation of the implemented analytical strategy is associated to compound identification and this was described in line 333 of the manuscript as follows:*

*“An expected limitation of TM-DART-QTOF-MS analysis was associated to spectral overlap; thus, in some cases the isotopic pattern was not considered for compound identification.”*

*Not all advantages of DART-MS were included in the manuscript. Compared to a direct infusion ESI-MS- or APCI-MS-based method, in DART-MS there is no need of rinsing any tubing used to infuse liquid into the ion source. This makes DART more resistant to memory effects, minimizing carryover, as all parts in contact with the sample are disposable, and allows high-throughput analysis, as there is no need for cleaning parts between sample runs (Monge and Fernández, 2014). Another advantage of DART compared to ESI is that it mostly produces singly charged ionic species, which facilitates metabolite identification. On the other hand, ESI sources allow coupling mass spectrometry with a different orthogonal separation technique such as liquid*

*chromatography, and hyphenated LC-MS systems provide the widest metabolome coverage with an additional dimension for compound identification, and are the most widely used analytical platforms in metabolomics (Kuehnbaum and Britz-McKibbin, 2013). In this regard, LC-ESI-HRMS-based methods provide the retention time as an additional orthogonal parameter to accurate mass and fragmentation pattern that would improve confidence in compound identification when compared to an authentic chemical standard, if possible. This would allow achieving the highest confidence (level 1) in compound identification as suggested by the Metabolomics Standards Initiative (Sumner et al., 2007).*

*Based on the reviewer's suggestion, the following statements will be added in the revised version of the manuscript to include ionization processes that occur in a DART source operated in negative ion mode, and limitations of DART compared to ESI for metabolome coverage and identification:*

*i) Modifications to be made in the introduction section (changes indicated in italics):*

*“Thermally-desorbed analytes having typically MW<1000, are ionized following atmospheric pressure chemical ionization-like pathways (Cody et al., 2005; Song et al., 2009a; Song et al., 2009b; McEwen and Larsen, 2009). Therefore, a major limitation is that it requires analytes to be volatile or semi-volatile, reducing the metabolome coverage. An important advantage of DART compared to electrospray ionization (ESI) for seawater analysis is that it is less affected by high salt levels (Kaylor et al., 2014; Tang et al., 2004), avoiding desalination processes that may lead to sample alteration. Conversely, ESI sources allow the coupling of MS to chromatographic systems that provide an additional parameter to improve confidence in compound identification when compared to an authentic chemical standard.”*

*ii) The end of section 3.1 will be modified as follows (changes indicated in italics):*

*“The selected OM extraction method with acetonitrile as extracting solvent favored the analysis of lipophilic compounds. In addition, to enhance the detection of organic acids, the analytical method was optimized operating the DART ion source in negative ionization mode, since it follows negative ionization APCI-like mechanisms including electron capture, dissociative electron capture, proton abstraction, and anion adduction (McEwen and Larsen, 2009; Cody and Dane, 2013; Gross, 2014).”*

Line 135: Were surface tension measurements made to more quantitatively make this comparison? Without this (or comparable information) the dilution conditions seem arbitrary? Could it also be done with IC measurements of [Cl-]?

*We appreciate the reviewer's comment, and do agree that such measurements would have provided valuable information. Unfortunately, these measurements suggested by the reviewer were not performed during the campaign before conducting the lab-to-the-field experiments.*

*As stated in the manuscript (line 135), sample centrifugation was conducted to isolate closer representations of the surface microlayer samples, considering the dilution factor inherent to the collection process, i.e., SML diluted with ULW contribution. Aerosol*

*particle formation was only detected after sample centrifugation and collection of 2 mL surface solution. That is, centrifugation was aimed at concentrating SML samples as a condition for aerosol formation.*

*The revised version of the manuscript will include the following statement to address this point:*

*“Centrifugation was aimed at concentrating SML samples as a condition for aerosol formation.”*

Line 175: What was the rationale for using negative ion mode with DART? Was positive ion mode also looked at? I was under the impression that most DART analysis was done in positive mode? Again, it would be very helpful to include some discussion of the ionization process and its selectivity.

*As stated above, negative ionization DART follows negative ionization APCI-like mechanisms including electron capture, dissociative electron capture, proton abstraction, and anion adduction. Examples that can be found in the literature that discuss in detail DART mechanisms and the use of the negative ionization mode include: McEwen and, Larsen, 2009; Cody and Dane, 2013; Gross, 2014. Two of these papers were cited in the manuscript and the publication by Cody and Dane, 2013 will be included in the revised version of the manuscript.*

*Negative ion mode was chosen to operate the DART ion source in order to detect lipophilic compounds, which were proven to be involved in SOA chemistry. Therefore, they consisted in an attractive fraction of the marine metabolome for interrogation and testing as discriminant compounds based on their relative levels between ULW and SML samples. During the analytical method development, both ionization modes were tested with different selectivity and sensitivity for the different type of compounds analyzed (amino acids, sugars and lipids indicated in Table S2); negative ion mode being more sensitive to the analysis of lipophilic compounds, including organic acids.*

*Regarding the inclusion of discussion on the ionization process, the manuscript already included the following statements:*

Line 91: “Thermally-desorbed analytes having typically MW<1000, are ionized following atmospheric pressure chemical ionization-like pathways (Cody et al., 2005; Song et al., 2009a; Song et al., 2009b; McEwen and Larsen, 2009).”

Line 261: “In addition, to enhance the detection of organic acids, the analytical method was optimized operating the DART ion source in negative ionization mode.”

Line 340: “Different types of species were generated for desorbed and ionized analytes (M) by the plasma-based source operated in negative mode, including [M-H]<sup>-</sup>, [M]<sup>-</sup> and [M]<sup>+</sup> ionic species.”

Line 345: “literature evidence suggests the production of radical anions based on electron capture mechanisms occurring in He-based plasma sources (Cody and Dane, 2016; Bridoux and Machuron-Mandard, 2013; Jorabchi et al., 2013).”

*Despite the inclusion of the previous statements described with citations in the manuscript regarding the ionization mechanisms that take place in a DART ion source operated in negative mode and following the reviewer's suggestion, the end of section 3.1 will be modified in the revised manuscript as follows (indicated in italics):*

*“The selected OM extraction method with acetonitrile as extracting solvent favored the analysis of lipophilic compounds. In addition, to enhance the detection of organic acids, the analytical method was optimized operating the DART ion source in negative ionization mode, since it follows negative ionization APCI-like mechanisms including electron capture, dissociative electron capture, proton abstraction, and anion adduction (McEwen and Larsen, 2009; Cody and Dane, 2013; Gross, 2014).”*

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