

Review of “Eukaryotic community composition in the sea surface microlayer across an east-west transect in the Mediterranean Sea” This is an interesting work reporting original data on the abundance of eukaryotes and TEPs in a East-West transect in the Mediterranean. The data set seems of good quality, even though the depth of the discussion could be improved in some sections. I recommend the following modifications:

We thank the reviewer for the helpful comments. Please find below our reply to the specific comments.

-Line28. There are some reports on bacteria in the SML, also some recent work using 16S. I suggest this could be cited here.

Thank you for the suggestion, we added this in the introduction:

Despite the fact that microbes in the SML can directly and indirectly influence air-sea gas exchange, few studies have looked at the microbial community composition in the SML, mainly focussing on bacteria (Agogué et al., 2005; Joux et al., 2006; Obernosterer et al., 2008) and less on microbial eukaryotes (Taylor and Cunliffe, 2014).

- Lines 43-49. This paragraph is un-related with the rest of the introduction and the title of the manuscript. It could be removed from the introduction.

We have removed the paragraph.

- Line 53. It is not clear to me to which degree the organic matter in the SML is related to atmospheric inputs, it may be driven by partitioning of surfactant-like chemicals from underlying waters or exudates from microbes. In addition, later in the text it is said that the atmospheric influence is covered in another manuscript in preparation.

We have removed the reference to atmospheric inputs from the sentence:

The present study focuses on the organic matter (OM) and microbial eukaryotes distribution, focusing on the myconeuston community composition in the SML of the Mediterranean Sea using samples collected during the PEACETIME cruise in May and June 2017.

- Lines 63. I find it difficult to believe that such a control of this rate can be achieved, especially on a moving zodiac.

We have removed the specific speed:

The glass plate was immersed and withdrawn slowly and perpendicular to the sea surface.

- Line 65. How was it collected?

We expanded the explanation on the ULW collection:

The ULW samples were collected concurrently with two acid-cleaned and MilliQ rinsed glass bottles by immersing the closed bottles and opening them at app. 20 cm.

- Volume of SML and ULW collected?

We expanded the description of sample collection:

A total of app. 1.5 L of SML sample was collected in the course of 1 h. Sampling times are listed in table 1.

- Line 131. Is this the result of a spearman correlation?  $R=0.1$  means  $R^2=0.01$ . Even though  $p<0.05$ , I don't think this shows a high similarity. I think it needs an explanation of which data was used for this similarity measure.

We used ANOSIM to compare the eukaryotic communities in the SML and the ULW on ranked data based on Bray-Curtis dissimilarity. A R value close to 1 suggests dissimilarity between groups, whereas an R value close to 0 suggests no dissimilarity between groups, in our case no dissimilarity between the SML and the ULW.

- ASV is never defined in the manuscript, I guess it is "amplicon sequence variant", but a definition, and probably an explanation, is needed in methods or the first time it appears.

Yes, we meant amplicon sequence variant and have added the explanation to the first time ASV is appearing in the text.

- Were differences between SML and ULW tested with a paired test?

The differences between SML and ULW for flow cytometry counts and organic matter concentrations were determined with a t-test on the enrichment factors (EFs) over the different stations, thus there was no need for a paired test, since through the calculation of the EF the only respective SML and ULW samples were compared.

- Generally, the EF could be correlated with environmental variables such as wind speed.

That is a good point and we have correlated environmental variables with the EF, as described below for TEP:

While irradiation, water temperature and salinity did not correlate with TEP abundance or area, wind speed did have a significant negative correlation with TEP abundance in the SML ( $R^2 = -0.73$ ) and TEP area in the SML ( $R^2 = -0.75$ ) and the enrichment factor of TEP area ( $R^2 = -0.63$ ).

- Was DOC measured? This could influence the east-west differences, as well their composition.

While DOC could give interesting additional information, it was not measured in the SML throughout the cruise.

- Line 153. Rewrite...

We have rephrased the sentence to make it clearer:

Picophytoneuston abundance was on average  $3.3 \times 10^3 \pm 1.9 \times 10^3$  cells  $\text{ml}^{-1}$  in the SML and picophytoplankton abundance in the ULW was on average  $2.3 \times 10^3 \pm 1.7 \times 10^3$  cells  $\text{ml}^{-1}$  (range of  $1.4 \times 10^3 - 8.5 \times 10^3$  cells  $\text{ml}^{-1}$  in the SML,  $9.5 \times 10^2 - 7.1 \times 10^3$  cells  $\text{ml}^{-1}$  in the ULW).

- Line 176. Couldn't diatoms and other phytoplankton groups be very affected by UV radiation?, ok, this is commented later, but then the enrichment of phytoplankton can be derived by physical processes (buoyancy and fractionation at surface due to surface tension related issues), then this would be independent of radiation.

We included buoyancy of cells as a potential community shaping factor in the discussion:

In addition to buoyancy of cells, radiation, especially in the SML, where often high levels of UV-radiation occur, could potentially cause damage by photoinhibition.

- Note that the SML is generally enriched in hydrophobic and surfactant-like chemicals as many anthropogenic compounds. This has been described for PAHs, alkanes, PCBs, Perfluoralkyl substance, etc in the Mediterranean and elsewhere. This could also have an influence on the east-west differences, as concentrations of POPs in biota are higher in oligotrophic regions due to a complex interplay of factors (Berrojalbiz et al. 2011, Morales et al. 2015, González-Gaya et al. 2019). For example, for bacteria, it has been shown that the SML is especially enriched in those taxa having the potential to degrade pollutants (Martinez-Varela et al. 2020). Even though, organic pollutants were probably not measured in this cruise, it could be another factor to take into account or comment shortly. Fungi are known as being very efficient degrading persistent pollutants.

We thank the reviewer for this comment and have expanded the discussion to include the possible influence of chemicals enriched in the SML:

In addition, previous studies have shown that the Eastern Mediterranean Sea shows higher concentrations of organic pollutants (Berrojalbiz et al., 2011a, 2011b) and a *Cladosporium* strain has been observed to degrade polycyclic aromatic hydrocarbons (Birolli et al., 2018), highlighting another potential substrate for the fungi detected in the Ionian Sea.

- Line 231. Here and in other parts of the manuscripts. Bacteria was determined by bacterial counts (abundances), but this is a very limited information for this types of statements, as even bacterial abundance decrease, the abundance of some key taxa may increase.

We have added a sentence in the discussion on this limitation:

Even though overall bacterial numbers decrease, further molecular analyses would be needed to determine if the bacterial community is changing from west to east and if certain bacterial taxa can benefit from the ultra oligotrophic conditions.