General comments: The MS by Zäncker and colleagues reports data on autotrophic and heterotrophic microbial cell abundances, TEP, carbohydrates, and 18S sequences from water collected in the sea surface microlayer (SML) and in underlying waters (ULW) in different basins in the Mediterranean Sea. The work presents a partial description of the biological and chemical characterization of the SML and ULW.

We thank reviewer #3 for reviewing the manuscript and the comments. Please find below a detailed answer to the raised questions and issues:

However, the way the data are presented and in particular discussed leaves the question on the authors' specific aim(s) open. The question that arises is 'What is the link between TEP, carbohydrates and microbial communities?' The authors provide no rationale for combining these specific results in one MS.

Carbohydrates are precursors for TEP (as stated in the introduction, line 42: Phytoplankton and phytoneuston can release precursors such as carbohydrates which can aggregate and form gelatinous particles such as transparent exopolymer particles (TEP). (Chin et al., 1998; Engel et al., 2004; Verdugo et al., 2004). TEP contain mainly polysaccharides (Mopper et al., 1995; Passow, 2002), occur ubiquitously in the ocean (Alldredge et al., 1993; Passow, 2002), and are an important structural component of the SML (Wurl and Holmes, 2008).), thus looking at TEP and carbohydrates combined provides a more complete picture than simply TEP alone. Since TEP are valuable for microbes as attachment site and food source and are structurally crucial for the SML, which is the target region of the present study, the authors concluded that all three components (carbohydrates, TEP, eukaryotes) are important components of the study and should thus be included. We have added this explanation also in the introduction:

The present study focuses on TEP as important structural components of the SML and their precursors, carbohydrates, as well as 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.
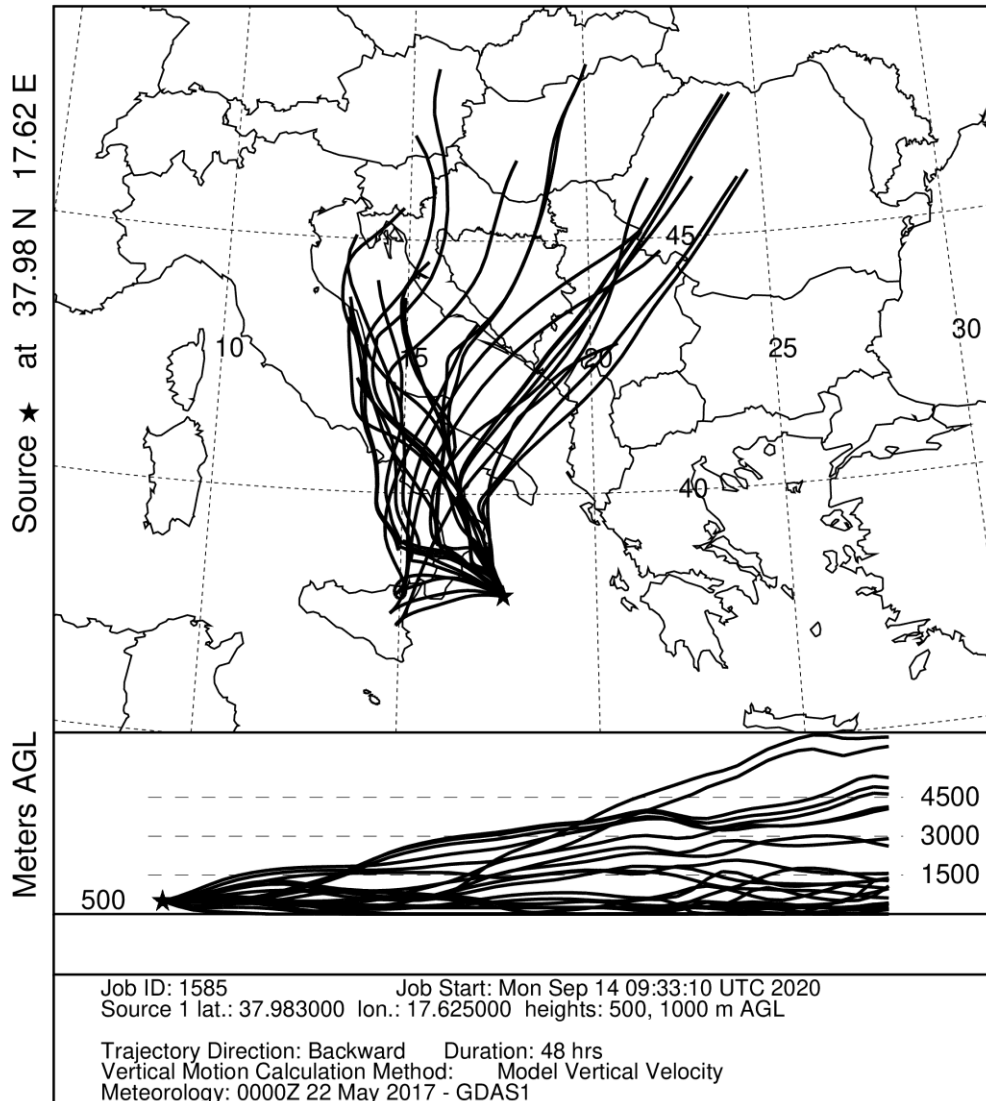
Further, the results are compared between SML and UW as well as among basins, which adds another level of complexity.

The authors acknowledge the added level of complexity, but given the major differences in not only eukaryotic community composition, but also trophic status and exchange with Atlantic waters in the different basins, when treating all basins together a lot of variability in the data would be lost.

The discussion of the data (in particular in the context of atmospheric deposition) is difficult to follow.

We have addressed more specific points on the atmospheric deposition below. In addition, we have used the NOAA HYSPLIT model to show the backwards trajectory of air masses 2 days prior to sampling in the Ionian Sea. The model results show that the air masses very likely originated above land, further corroborating the idea that the fungi found in the Ionian Sea, while thriving in this area of the Mediterranean Sea, have been introduced from terrestrial sources.

NOAA HYSPLIT MODEL
Backward trajectories ending at 0900 UTC 23 May 17
GDAS Meteorological Data

Source ★ at 37.98 N 17.62 E

Meters AGL

4500
3000
1500
500

Job ID: 1585   Job Start: Mon Sep 14 09:33:10 UTC 2020
Source 1 lat.: 37.983000 lon.: 17.625000 heights: 500, 1000 m AGL

Trajectory Direction: Backward   Duration: 48 hrs
Vertical Motion Calculation Method: Model Vertical Velocity
Meteorology: 0000Z 22 May 2017 - GDAS1

This MS is a contribution to the Special Issue of the PEACETIME project. The characteristics of the SML will certainly provide important insights to the overall project. I consider, however, that the MS cannot be accepted in its present form, but needs major revisions. I advice the authors to re-consider their main objective(s) and to present only the appropriate data. My further suggestion is to re-construct the discussion in a way that it focuses on the data presented in this MS. An original finding of the study is the high relative abundance of fungi sequences in the Thyrennian Sea, both in the SML and ULW. One possibility would be to focus the MS on eukaryotic diversity and fungi in particular.

As stated above, the authors have carefully considered which data to include in the manuscript and feel that including carbohydrates, TEP and microbes gives the best possible overview of SML dynamics.

We considered the suggestion of the reviewer to restructure the discussion and agree that the high relative abundance of fungi sequences in the Ionian Sea is an original and interesting finding. However, the discussion is already focussed on eukaryotic diversity (section 4.1 Eukaryotic diversity in the

surface of the Mediterranean Sea, lines 167-187) and fungi in particular (section 4.2 Fungi in the Ionian Sea, lines 189 – 231), and thus the authors feel like a restructuring of the discussion is not meaningful at this point.

Specific comments:

Abstract: Line 10-11: One understands that this main objective of the work, but it is not focus of the following sections.

Changed it to:

However, little is known about the distribution of microbial eukaryotes in the SML.

Methods: Line 89: If I understand correctly, the 20 mL samples collected onboard and frozen (-20∘) were not ultra filtered. The ultrafiltration step was done in the lab. I suggest to clarify this.

We clarified this:

In the home lab, high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) was applied on a Dionex ICS 3000 ion chromatography system (Engel and Händel 2011) for TCHO analysis.

Results: Line 150: Please refer to Fig. 5 in the text

We added a reference to figure 5.

Line 153-155 and legend of Fig. 5: Flow cytometry was used in the present study to determine phytoplankton abundance in the SML and in ULW. I suggest the authors clarify here the size fraction of the organisms that can be determined by flow cytometry (i.e. generally up to 20 µm). Any larger phytoplankton are not included in their counts.

We named the phytoplankton measured in the flow cytometer picophytoplankton and included the size range (0.2 – 20 µm) in the results section.

Line160: should be Fig. 5

Thanks for pointing this out, we have changed it accordingly.

Discussion: Line 173: Sequencing data provide information on the relative abundance of a given taxonomic unit, but no absolute values. I suggest re-writing the sentence accordingly.

We changed 'concentrations' to 'relative abundances'.

Line 191: In the previous paragraph the authors discuss the potential biases of sequencing data due to differences in gene copy numbers, and I totally agree. How would this impact their observations on fungi sequences? I suggest the authors include a short description of what is known on fungi copy numbers and whether this could have led to a potential overestimation in their data set.

We added a sentence on the impact on fungal sequences:

While fungi, like dinoflagellates and other eukaryotic groups, can have varying amounts of 18S rDNA gene copy numbers, the patchy distribution of fungi found in this study makes a consistent bias unlikely.

Line 194 and elsewhere: Please apply the term 'relative abundance' instead of 'abundance' or 'amount' (line 203)

We have made changes throughout the manuscript accordingly.

Line 198: What is the rationale for the conclusion that dust and rain lead to an increase in TEP and in unidentified dinoflagellates in the SML? Even if another MS on this issue is in preparation, a more information is required here for an appropriate discussion.

We have changed the wording of the sentence to 'coincided' instead of 'resulted' to not make claims that we don't discuss in the MS.

Station FAST_2 in the western basin was highly influenced by dust input in the area (Guieu et al., 2020; Tovar-Sánchez et al., 2020). This coincided not only with a high increase in TEP abundance in the SML, but also with a distinct increase in the relative abundance of unidentified dinoflagellates in the SML (Fig. 3).

What is meant by 'previous to the research campaign'? A few days or weeks? Where is this shown in Fig. 3?

Station FAST_2 is represented by the second bar in figure 3. We have changed the sentence to be more specific about the timing:

either deposited by dust or by rain days before this research campaign in the Ionian Sea or in other areas closeby.

Line 201: As mentioned above, this discussion does not refer to any data presented in this MS, and thus confusing.

As stated in the text, the community data is indeed presented in figure 4 and helps to rule out dust input as the main influencing factor of eukaryotic community composition. Thus, the authors feel like this section should be kept in the manuscript.

Line 202-203: It seems the authors contradict their statements above. Please clarify.

We have changed the sentence to make our point better understandable:

In addition, the highest relative abundance of fungi was found in the ULW and not the SML, making a simple atmospheric influence without any subsequent thriving of certain fungal taxa unlikely.