

***Interactive comment on* “Eukaryotic community composition in the sea surface microlayer across an east-west transect in the Mediterranean Sea” by Birthe Zäncker et al.**

Anonymous Referee #3

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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. 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. Further, the results are compared between SML and UW as well as among basins, which adds another level of complexity. The

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

Specific comments: Abstract: Line 10-11: One understands that this main objective of the work, but it is not focus of the following sections. 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. Results: Line 150: Please refer to Fig. 5 in the text 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. Line 160: should be Fig. 5 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. 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. Line 194 and elsewhere: Please apply the term 'relative abundance' instead of 'abundance' or 'amount' (line 203) 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

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preparation, a more information is required here for an appropriate discussion. What is meant by 'previous to the research campaign'? A few days or weeks? Where is this shown in Fig. 3? Line 201: As mentioned above, this discussion does not refer to any data presented in this MS, and thus confusing. Line 202-203: It seems the authors contradict their statements above. Please clarify.

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