Review of manuscript by Zäncker et al. entitled "*Eukaryotic community composition in the sea surface microlayer across an east-west transect in the Mediterranean Sea*" (bg-2020-249).

The study by Zäncker et al. present the spatial distribution of eukaryotic phytoplankton and fungi species in the surface microlayer and the underlying water across different sub-basins at the Mediterranean Sea during summertime. Data show that the SML is a hotspot for different fungi which govern, to some extent, organic matter degradation. Besides, the differences between the SML and the ULW are negligible, and phytoplankton/bacteria show the typical E-W oligotrophic gradient previously reported in numerous studies.

Overall, the paper is nicely written, however I think it can be greatly improved. Moreover, I found a few critical points that warrant clarifications; mostly in the sample's collection (e.g., DNA extraction and different collection hours) and preservation (e.g., flow-cytometry analyses). To conclude, I think the paper should undergo a major revision before I can recommend its publication in *Biogeoscience*.

General comments/suggestions

- Comparison of the SML (and ULW) properties between basins/station may be problematic as it seems that the samples were collected in different hours of the day (e.g., station S7 vs. S6). Different collection hours may affect the phytoplankton composition through top-down interactions (i.e., daily migration of zooplankton). The authors should discuss this possible bias.
- I suggest adding a short paragraph in the introduction describing the Mediterranean's general west to east anti-estuarine circulation and the trophic gradient it generates (i.e., easternmost stations are 'more oligotrophic' than the western stations, etc.). There is also a N-S trophic gradient that may be relevant to this study (and is not discussed at all in the results or discussion sections). This is the rational for taking samples in different basins across the Mediterranean, representing different oligotrophic characteristics…
- Phytoplankton abundance measurements may be underestimated due to wrong preservation of the samples. Freezing the seawater samples in -20 °C rather than in liquid nitrogen and then -80 °C slowly generates ice crystals that may break some of the cells, and thus result in underestimation of the actual counts. Indeed, the pico-phytoplankton cell abundances presented in Figure 5 (and corresponding text) are 1-2 orders of magnitude lower than usually reported in the Mediterranean Sea (~10^4-10^5 cells/ml).
- In section 2.7 you describe how you calculated the 'enrichment factors' between the SML and ULW, however this data is not presented in the manuscript (but only used as a correlation variable). I suggest adding a table with the EF values (and whether the

differences were significant or not). It will greatly help the reader to understand the differences between the two water layers.

- Figure 5 should be revised. Briefly, there's seems to be problems in the units used for TEP (area and concentration), the panels are not numbered which makes the reading more difficult to follow, the dot's color-code is unclear etc. Please see more details in the table below. Further, I suggest adding to the supporting information a few microscopic images showing example TEP area.
- By pre-filtering the seawater onto 100 µm mesh (line 96), you may have removed some fungi and large-size diatoms/dinoflagellates (as indicated in lines 175-177). Please justify this pre-filtration step.
- I think the discussion should be elaborated. For example:
  - ❖ You should discuss why you don't see any differences in the eukaryotic diversity between the SML and underlying water in all sites (it's not the organic matter…). Given the lack of (spatial) correlation between phytoplankton and microgels/ TCHO may infer that these organic matter may be refractory in the eastern basin compared to the western basin, or that phytoplankton/bacteria are outcompeted for these 'goods' (perhaps the fungi?).
  - ❖ I suggest you discuss how fungi may interact with phytoplankton and bacteria in marine LNLC environments. Do they utilize the same nutrients (thereby competing with the microbes)? Did you find any toxic fungi in the different layers? Can you say anything about the role of fungi in the SML and ULW's food web?
  - ❖ Please provide information on fungi biomass/ activity and diversity in other LNLC regions. Do you expect that fungi be more important in oligotrophic vs. meso-/eutrophic marine environments? Does your findings comparable to these other sites?

<u>Minor comments/suggestions</u>

Section 2.1	It is unclear how much water were collected in each station, what were the collection hours (day vs. night…), and how much time it took to collect it. Seems that for all analyses the authors needed ~0.5 L from the SML, which is a lot when using the glass plate approach (Harvey, 1966)… More importantly, if samples were collected in different hours of the day (e.g., S7 vs. S3 based on irradiance presented in Table 1), this might affect the microbial communities in the SML and ULW through daily migration of zooplankton and thus gazing. This issue can affect the abundance/diversity of the eukaryotic microbes in both

	water layers. Please provide the information and discussion where appropriate. Did you use a mechanic instrument (that can also control the sampling rate; ~17 cm s^{-1})? If so, an image showing this instrumentation may be a nice addition (especially given that the link you provided of Cunliffe and Wurl, 2014 does not work…).
Line 57	*Pourquoi pas?* or *Pourquoi pas*
Line 68	"…The abundance and area of TEP ~~**and**~~ was measured microscopically…" (removed "and")
Line 77 (ditto line 83)	Freezing seawater samples in -20 °C without pre-freezing it in liquid nitrogen may result in cell lose. The slow freezing at -20 °C creates ice crystals which results in cell breakdown, thus leading to underestimation of pico-phytoplankton/bacterial abundances. Indeed – your pico-phytoplankton cell abundance (e.g., Figure 5) is low by 1-2 orders of magnitude relative to previous studies from the Mediterranean surface water. Do the numbers presented in Figure 5 only show the eukaryotic algae (i.e., without the cyanobacteria)?
Abstract, Line 82 and throughout	Your flow-cytometry analyses enabled you to enumerate pico/nano-phytoplankton and not the total phytoplankton fraction which is also comprised of larger algal communities (large diatoms, dinoflagellates etc.). What is the cell-size range of the flow-cytometer you used? Usually, to get total "phytoplankton" you should have measured chlorophyll.*a* and /or run complimentary microscope analyses. This is especially important given that the SML is rich in large-size phytoplankton (Hardyet al., 1988). I suggest changing the term "phytoplankton" to "pico-phytoplankton" throughout. BTW – Did you see any differences (SML vs. underlying water and between basins) in the pico-phytoplankton communities (e.g., *Prochlorococcus*:*Synechococcus* ratio, prokaryotes:eukaryotes ratio)?
Paragraph in lines 42-49	I suggest to remove from the introduction (and maybe move it to the discussion?). I'm aware you tried to describe what is TEP, but it has little connection the way it's written with the

	SML's background. Maybe adding a sentence saying that TEP prevalent the SML… etc.
Line 50	"looked at **the** spatial distribution…" (add "the")
Figure 5	• Please number the different panels (A, B…) and revise the legends accordingly ("Abundance of bacteria (A), pico-phytoplankton (B), TEP area (C)…"). • What's the difference between the gray and black dots (different cruises?)? • TEP area – I don't understand the units. What is mm L^{-1} (ditto in the text)? I suggest adding a figure in the SI (or atl least in your reply) explaining this. • TEP concentration – I don't understand the units. what is TEP per L^{-1} (ditto in the text)? Do you mean µg GX L^{-1} (if so, there's something off with the numbers).
Line 96 and lines 175-177	Please justify why you used pre-filtration for the DNA extractions. By doing so, you may have taken out fungi's mycelium as well as large-size diatoms/dinoflagellates (that are often found in the western basin water as indicated in lines 175-177). You may have also taken out TEP with its rich microbiome (algae, bacteria and fungi).
Section 4.1	You should discuss why you didn't see any differences between the SML and underlying water in all sites, while chemically-wise (total carbohydrates and TEP) you found significant differences. Currently, the discussion in this section is a bit weak.
Lines 195-197	There's also a possibility it's a contamination… Did you run blank filters? You can also look at the air-mass backward trajectories (https://ready.arl.noaa.gov/HYSPLIT_traj.php) and see where aerosols came from the day before you sampled there, namely if you received any terrestrial origin particles.
Line 217	"very oligotrophic" (instead of "veryoligotrophic"). BTW- I suggest saying 'ultra-oligotrophic'.
Lines 218-219	Please cite a reference to back up this statement.