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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.

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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

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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 We thank the reviewer for the suggestions and helpful comments which we have addressed below. âĂć 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. As mentioned in the results section 3.2, influence of irradiation was tested with the two stations with by far the lowest radiation representing the early morning sampling, but no significant correlations were found, making day/night sampling effects unlikely. âĂć 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. . . We added a section on the anti-estuarine circulation in the last paragraph of the introduction: The anti-estuarine circulations at the Strait of Gibraltar and the Straits of Sicily, transport low-nutrient surface waters into the basins, and deeper waters out of the basins, resulting in oligotrophic conditions in the western and ultra-oligotrophic conditions in the eastern Mediterranean basin (Krom et al., 2004; Tanhua et al., 2013). âĂć 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-

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phytoplankton cell abundances presented in Figure 5 (and corresponding text) are 1-2 orders of magnitude lower than usually reported in the Mediterranean Sea (∼104-105 cells/ml). The cell preservation method using fixation and freezing has been tested in our lab previously. In accordance with previous studies (e.g. Lepesteuer et al., 1993), we used this method and do not expect overproportional cell loss. It is true that the abundances are quite low at the surface. During the cruise, flow cytometry was also used down to 200 m depth to measure the phytoplankton abundance, showing that phytoplankton numbers were generally higher at 5-100 m than in the SML and ULW which makes it difficult to compare the general surface waters to the SML abundances. Since 5-100 m was beyond the scope of this paper, these numbers were not included in the manuscript. The numbers retrieved from the SML are in line with previous phytoplankton SML measurements (e.g. Joux et al., 2006). âĂć 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. We have added a table of EFs in the supplementary material (Table S1). The significance of the two different water layers is already described in section 3.2 âĂć 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. We added numbering to the panels and explained the dot's colour-code. The units used for TEP area and concentration is according to what is commonly used in literature. Information, including pictures on TEP area calculation, can be found in Engel, 2009 (Determination of marine gel particles). âĂć 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. A justification was added in the M&M part:

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400 ml of sample was pre-filtered through a mesh with 100 μm pore size in order to avoid zooplankton being captured on the filters and dominating the retrieved 18S sequences and subsequently filtered onto a Durapore membrane (Millipore, 47 mm, 0.2 μm) and immediately stored at -80°C. And the pre-filtration is also mentioned in the discussion: Even though diatoms most likely were not dominant in the samples, finding no diatom orders over 1 % in at least one of the samples might also indicate a bias of the primers used or of the pre-filtration removing larger cells and aggregates. âĂć I think the discussion should be elaborated. For example: ïĄűïĂăYou 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?). We have added to the existing discussion: Bacterial and microalgal numbers determined by flow cytometry decreased significantly from west to east, with bacteria showing the greatest decline. 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. ïĄűïĂăI 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? We have added a description on the ecological role of fungi in the marine environment in general and a more specific description of fungi in the SML. Fungi can either live a saprotrophic lifestyle, potentially competing for nutrients and substrate with phytoplankton or bacteria and adding to the recycling of organic matter, or they can live a parasitic lifestyle, directly attacking phytoplankton

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and potentially altering phytoplankton community composition through selective parasitism (Amend et al., 2019; Grossart et al., 2019). By parasitizing phytoplankton, fungi can render inedible phytoplankton accessible for zooplankton (Grossart et al., 2019). Coastal myconeuston in the English Channel was dominated by Ascomycota and Basidiomycota classes (Taylor and Cunliffe, 2014) compared to Ascomycota dominating the surface samples in the current study.

Minor comments/suggestions âĂć 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 âĹij0.5 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; âĹij17 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. . .). We added information on sampling time, duration and volume in the M&M and table 1: 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. As mentioned in the results section 3.2, influence of irradiation was tested, but no significant correlations were found, making day/night sampling effects unlikely. As described in the M&M, the sampling was carried out manually with a standard glass plate, counting the timing of the dips resulting in an average sampling rate of ∼17 cm s-1. We thank the reviewer for his remark and have updated the link to Cunliffe and Wurl, 2014. âĂć Line 57 Pourquoi pas? or Pourquoi pas The official name of the research vessel is Pourquoi pas? âĂć Line 68 “. . .The abundance and area of TEP and was measured microscopically. . .” (removed “and”) We removed the ‘and’ âĂć Line 77 (ditto line 83) Freezing seawater samples in -20 âŮęC without pre-freezing it in liquid nitrogen may

result in cell lose. The slow freezing at -20 âŮęC 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)? The cell preservation method using fixation and freezing has been tested in our lab previously. In accordance with previous studies (e.g. Lepesteuer et al., 1993), we used this method and do not expect overproportional cell loss. It is true that the abundances are quite low at the surface. During the cruise, flow cytometry was also used down to 200 m depth to measure the phytoplankton abundance, showing that phytoplankton numbers were generally higher at 5-100 m than in the SML and ULW which makes it difficult to compare the general surface waters to the SML abundances. Since 5-100 m was beyond the scope of this paper, these numbers were not included in the manuscript. The numbers retrieved from the SML are in line with previous phytoplankton SML measurements (e.g. Joux et al., 2006). The numbers represented in figure 5 show all algae, including cyanobacteria. âĂć 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 "picophytoplankton" 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)? Prochloroccocus were not detected in the flow cytometer. According to the suggestion, we changed the phytoplankton detected in the flow cytometer to picophytoplankton as the size of cells detected in the instrument is app. 0.2 – 20 μm. âĂć Paragraph in lines 42-49 I suggest to remove from the in-

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troduction (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. We added an explanation on why TEP are important in the SML in the introduction: TEP contain mainly polysaccharides (Mopper et al., 1995; Passow, 2002), occur ubiquitously in the ocean (Alldredge et al., 1993; Passow, 2002; Engel et al 2020), and are an important structural component of the SML (Wurl and Holmes, 2008). âĂć Line 50 "looked at the spatial distribution. . ." (add "the") Figure 5 âAËŸ c Please number the different ′ panels (A, B. . .) and revise the legends accordingly ("Abundance of bacteria (A), pico-phytoplankton (B), TEP area (C). . ."). âAËŸ c What's the difference between the ′ gray and black dots (different cruises?)? âAËŸ c 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. âAËŸ c 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). We thank the reviewer for the comment and have added panels in figure 5. TEP area in mm2 L-1 and TEP L-1 are commonly used in the literature to describe TEP which was measured microscopically, whereas μg GX L-1 is used for TEP measured colorimetrically (see also Engel et al., 2020). Please see below an example of TEP area measurement from Engel, 2004 a – All TEP are marked using a manual threshold in ImageJ, then the program automatically calculates the area that is taken up by TEP.

TEP L-1 is describing, how many particles are found per litre of seawater (using the above pictures, instead of taking the area into account, only counting how many particles were found per field of view). âĂć 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). A justification was added in the M&M part: 400 ml of sample was pre-filtered through a mesh with 100 μm pore

size in order to avoid zooplankton being captured on the filters and dominating the retrieved 18S sequences and subsequently filtered onto a Durapore membrane (Millipore, 47 mm, 0.2 μm) and immediately stored at -80°C. And the pre-filtration is also mentioned in the discussion: Even though diatoms most likely were not dominant in the samples, finding no diatom orders over 1 % in at least one of the samples might also indicate a bias of the primers used or of the pre-filtration removing larger cells and aggregates. Regarding the removal of TEP: While some very large TEP might have been removed, the vast majority of TEP that were found on the TEP filters (which were not pre-filtered) were less than 100 um in diameter. âĂć 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. We expanded the discussion: However, looking at the current study, no inhibition by UV radiation can be inferred from the data because phytoplankton were enriched despite high radiation values (e.g. stations S4 and 7) (Table 1). At the same time, TEP were significantly enriched during the sampling campaign while the phytoplankton community did not show significant differences. Previous studies suggested that TEP can protect phytoplankton and bacteria from UV radiation (Elasri and Miller, 1999; Ortega-Retuerta et al., 2009). Further studies would be needed to determine whether TEP production was higher in the SML due to phytoneuston UV protection or whether TEP formation rates were higher in the SML due to wind and wave shear at the surface (Carlson, 1993; Cunliffe et al., 2013). âĂć 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. Blank filters were not run daily, however, contamination of land plants seems unlikely since there were no plants in the lab or near the CTD sampling station onboard the RV. We thank the reviewer for suggesting the website. Please find below the output which suggests that the aerosols came from a terrestrial input. We have also included the be-

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low graph in the supplementary information and added an additional short explanation in the discussion: This is also corroborated by air mass trajectory backtracking using the HYSPLIT model (Fis. S3) which shows that aerosols likely were of terrestrial origin.

âĂć Line 217 “very oligotrophic” (instead of “veryoligotrophic”). BTW- I suggest saying ‘ultra-oligotrophic’. We changed the wording to ultra oligotrophic âĂć Lines 218-219 Please cite a reference to back up this statement. We apologise for the confusing phrasing, we were talking about our study and changed the sentence to: This is in good accordance with the present study in the ultra oligotrophic eastern Mediterranean Sea (Durrieu de Madron et al., 2011; Fogg, 1995; Wikner and Hagstrom, 1988) where low phytoplankton abundances, but high TEP enrichments of 1.1-17.3 were found in the present study.

Please also note the supplement to this comment:
https://bg.copernicus.org/preprints/bg-2020-249/bg-2020-249-AC1-supplement.pdf

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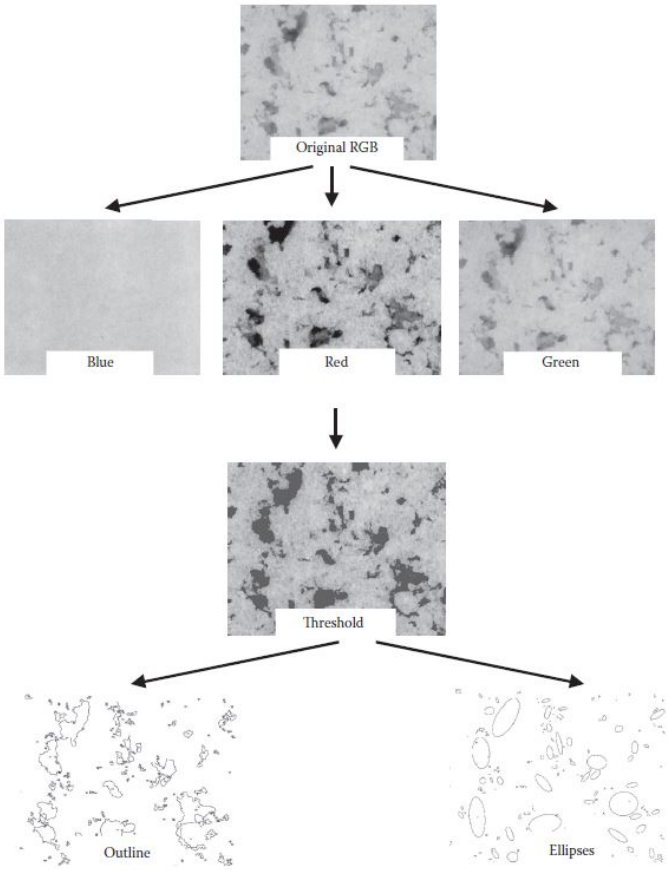

Fig. 1.

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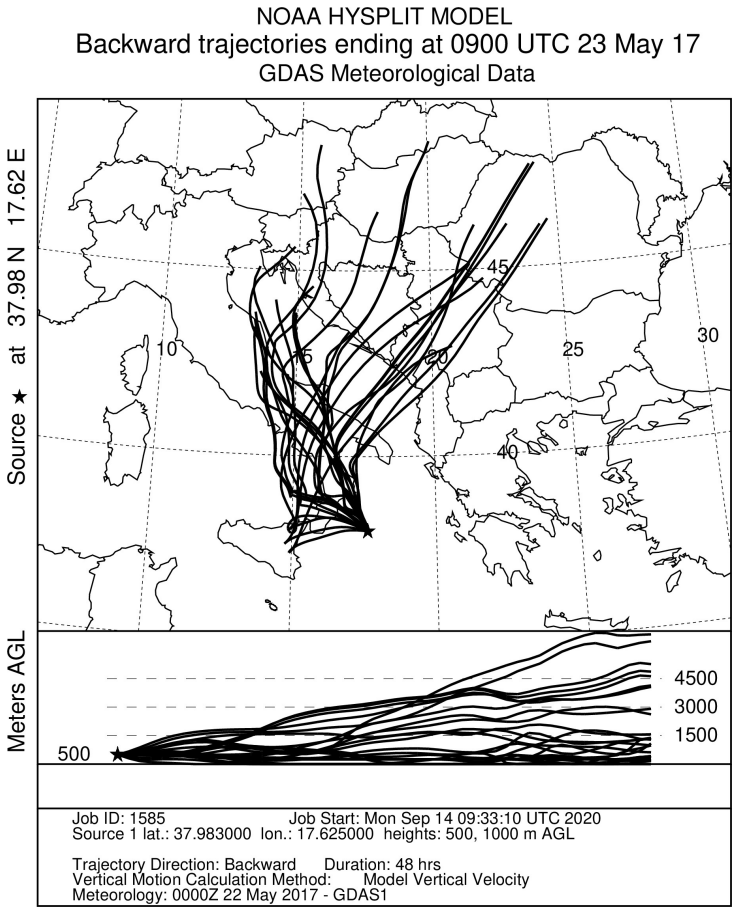
NOAA HYSPLIT MODEL
Backward trajectories ending at 0900 UTC 23 May 17
GDAS Meteorological Data
Source ★ at 37.98 N 17.62 E
10
15
20
25
30
40
45
Meters AGL
4500
3000
1500
500
Job ID: 1585 Job Start: Mon Sep 14 09:33:10 UTC 2020
Source 1 lat.: 37.983000 lon.: 17.625000 heights: 500, 1000 m AGL
Trajectory Direction: Backward Duration: 48 hrs
Vertical Motion Calculation Method: Model Vertical Velocity
Meteorology: 0000Z 22 May 2017 - GDAS1

Fig. 2.