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Biogeosciences Discussions

Interactive comment on "Biochemical characteristics and bacterial community structure of the sea surface microlayer in the South Pacific Ocean" *by* I. Obernosterer et al.

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Response to Referee #1: 1) The light path used in the present study is indeed 2m long to increase the signal of the cDOM measurement. 2) We have replaced Eubacteria by Bacteria throughout the manuscript. 3) We now discuss in more detail the low EUB detection rate, including the possibility of an inefficient cell wall permeabilization (Section 3.3.) 4) We have added some discussion on this point in the last paragraph of the manuscript. We remain, however, careful with our conclusions on the impact of bacterial activity on air-sea exchange fluxes, as bacterial respiration was not determined in the present study. The development of novel techniques appropriate to measure biological fluxes in the sea surface microlayer seems very important to us for further studies.

Response to Referee#2

1) Low detection rate of the EUB probe. As suggested by Referee#2, we compared the detection rate of Bacteria by using either the probe EUB338I alone or a combination of the probes EUB338I, EUB II and EUB III. Our results, obtained from hybridizations on 2 independent filter pieces per station and depth indicated that the hybridization with a combination of the probes EUB338I, EUB II and EUB III did not enhance substantially the detection rate observed with EUB338I alone. The detection rate of Bacteria in SML samples amounted to 40ś12% (meanśSD, n=6) with the EUB338I probe alone and was 49ś7% (meanśSD, n=6) when using the probes EUB338I+EUB338II+EUB338III in combination. For samples collected at 5 m, the detection rates were 54ś9% and 51ś8% for EUB338I and EUB338I+EUB338II+EUB338III, respectively. In the revised version of the manuscript, we report now the results of EUB338I+II+III (Fig 5 and p. 14, lines 6-7). Our detection rate of Bacteria is indeed in the lower range of values reported in the literature (60-90%) and it is also lower than those we observe in the coastal and off-shore Mediterranean Sea and in the Southern Ocean (70-90%, unpublished data). We agree with Referee#2, the low metabolic activity and the small size of bacterial cells probably account for the low detection rate. As suggested by Referee#1, an inefficient cell-wall permeabilization could additionally be responsible for the low detection rate observed in the present study. Our re-investigation of the filters from the different stations and depths confirmed overall the results obtained and presented in the manuscript. An exception to this was Station MAR SML. The initial EUB338I detection rate was 19%, and it amounted to 47% (for EUB338I) and 48% (for EUB338I+II+III) following the hybridizations we did for the revision of the manuscript. These latter values are now reported in the manuscript. As described on p. 14, 2nd paragraph, the sum of the percent contribution of the 3 bacterial groups accounted for 82ś20% (meanśSD, n=3) in the surface microlayer and for 100ś10%)(meanśSD, n=3) in subsurface waters of the percent EUB. Only at Station GYR was the detection rate of the sum of the 3 groups slightly higher (by 13%) than that of the EUB probe. We agree, the relative contribution of Archaea to the prokaryotic community could be important, however, we

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did not specifically address this question in the present study. All these aspects are now considered in the text (Section 3.3.)

2) The observation that the sum of the percent contribution of the 3 bacterial groups is similar to that of EUB suggests that the probes we used did not miss a large fraction of bacteria belonging to these groups. This observation was stated in the previous version of the manuscript (Section 3.3) and it is now given in more detail in Section 3.3 (The sum of the relative contributions of Alpha-, and Gammaproteobacteria and Bacteroidetes amounted to 82ś20% (meanśSD, n=3) in the surface microlayer and equaled (meanśSD, 100ś10%) the percent EUB positive cells in subsurface waters).

3) We agree with Referee#2, the applied FISH-probes provide information on a low phylogenentic level, and the utilization of more specific FISH-probes could reveal differences in the relative abundance of specific phylotypes. To gain information on the bacterial groups that could be specific for the surface microlayer, more detailed information on the bacterial diversity would be required. The construction of clone libraries would allow to obtain this information, and to design more specific probes. We consider this approach very interesting, but it is beyond the scope of the present study. As described in Section 3.3. (1st paragraph), we did observe some differences in the SSCP-profiles between the surface microlayer and 5m, and we discuss these differences in the Discussion Section (p. 18-19). We also agree that the fingerprinting method CE-SSCP provides only limited information on the phylogenetic identity of the peaks detected. However, we think that CE-SSCP represents an appropriate tool to determine whether the bacterial community structure differs between the two layers. We systematically use an internal standard on each CE-SSCP run which enhances substantially the precision of the comparison of the profiles. This is described in Section 2.10.2. We are confident that the UPGMA dendrogram, based on the absence and presence of peaks, is a correct indicator of the similarity of the overall bacterial community structure. We cannot exclude the possibility that different phylotypes are represented by CE-SSCP-peaks with the same migration time in profiles from the two

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layers. However, during previous work on peak assignation we have never observed this feature. We are not convinced that the DNA to RNA ratio would provide straightforward information on the presence and activity of specific bacterial groups.

4) As discussed above, we entirely agree that the information obtained by the FISH-probes we have used in the present study is limited to a rather low phylogenetic level. We have included this aspect in the Discussion. For the interpretation of our MICRO-FISH data, we refer to observations from the literature reporting pronounced differences in the response of the major bacterial groups to specific environmental conditions (e.g. DOM source, nutrient concentrations). We agree, most likely not all members belonging to the major phylogenetic groups contribute to this response. We account for this by stating “these bacterial groups contain each a diverse assemblage of sub-groups” and at several instances that “members of the respective groups”.

5) We certainly agree with Referee#2, UV radiation is an important environmental factor in the surface microlayer, and we have stated this in the manuscript in the case of cDOM (p. 17, lines 14-17) and bacterial heterotrophic production (p. 17, lines 27- p.18, line 1). When preparing the manuscript, we did consider the possibility of linking the bacterial activity in the surface microlayer to the direct impact of UV radiation, but this interpretation is not straightforward. We do not have any measure of the duration of the surface microlayer, thus we do not know the exposure time of the bacterial community to UV radiation prior to sampling. If UV was a dominant factor for the inhibition of bacterial production in the surface microlayer on a short time scale, we could expect to observe the most pronounced inhibition in the late afternoon. This is not the case for the present data set. In our study, the inhibition in bacterial production in the surface microlayer relative to subsurface waters is independent of the time of the day. Interestingly, a very pronounced inhibition was observed in the morning (9:00h) at Station EGY. At this Station, calm wind conditions prevailed over a relatively long time period (18h). The observed inhibition could be due to an accumulated damage. We are, how-

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ever, unable to identify the factor that accounts for most of this inhibition. Besides UV radiation, drastic changes in temperature, salinity and pH most likely affect bacterial heterotrophic production. It is well known, that heterotrophic bacteria recover rapidly from UV-stress, and photochemical transformations of DOM can result in more labile or more refractory compounds, depending on the chemical composition of the DOM. All these processes render a firm conclusion on the importance of UV radiation on the observed inhibition of bacterial production difficult. We therefore decided to consider the different environmental parameters described above in an equal manner. Due to the lack of knowledge of the exposure time of bacteria to UV radiation and the different times of the day of surface microlayer collection, we consider it inappropriate to plot UV radiation against bacterial activity in the surface microlayer. The arguments given above hold also true for the bacterial community structure. We have no evidence that UV radiation has a stronger impact than any other environmental parameter on the presence or activity of specific phylotypes.

6) We have added some discussion on this point in the last paragraph of the manuscript. We remain, however, careful with our conclusions on the impact of bacterial activity on air-sea exchange fluxes, as bacterial respiration was not determined in the present study. The development of novel techniques appropriate to measure biological fluxes in the sea surface microlayer seems very important to us for further studies.

7) The contribution of bacterial biomass to the DOC pool accounts for roughly 1%. Thus even in unfiltered samples, the bacterial biomass has an extremely small contribution to DOC.

8) We refer at several instances to the paper by Agogue et al (FEMS, 2005). These authors report gram-positive bacteria in the surface microlayer of a coastal station off Barcelona harbor. By contrast, only very few gram-positive bacteria were detectable at their second sampling site, an oligotrophic station off Banyuls-sur-mer. We think it is unlikely that gram-positive bacteria were abundant in the South Pacific Ocean where

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the impact from continents is extremely low.

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