

## ***Interactive comment on “Biogeography of planktonic microbial communities across the whole Mediterranean Sea” by F. Mapelli et al.***

**Anonymous Referee #2**

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The ms. “Biogeography of planktonic microbial communities across the whole Mediterranean Sea” by Mapelli and co-authors is about the biodiversity of bacterioplankton communities in the Mediterranean Sea. This is an interesting topic, and the manuscript provides a potentially important contribution to this field of investigation. There are however many problems which needs to be addressed by the authors before it may become acceptable for Ocean Science.

Major comments i) The authors aims at discussing the distribution of bacterial communities according with different water masses. However, there is no discussion about which water masses were sampled. At P304 the authors discuss that the water masses distribution at the large scale investigated is too complex to be described. I do not agree with this statement. A deeper description of the main oceanographic charac-

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teristics is needed, and can allow the authors to group samples according to specific water masses, and to learn more about bacterial distribution. My suggestion is including this description in the first para (3.1) of the Results section, and to re-write the corresponding Discussion section.

ii) The ms. may benefit without doubt of a check of the english language. Possibly by a native speaker. There are many typos (a few examples: P292 L4 “marine microbiology investigation were”; P304 L24 “pattern were related”; Legend to Figure 2 “station name label are reported”, etc.)

iii) Many terms are often used improperly. Some examples are reported (the list is not exhaustive): P292 L8 “vertical transects” P292 L13 “ the boundary of the water column” P293 L5 “marine ecosystem hot-spot” P294 L16 “Mediterranean marine biomes” P296 L28 “the interaction among the environmental variables explaining biotic similarity” P298 L21-22 “according to the conditions that rule the subsurface and deep realms” P299 L13-14: “deep layer dwelling microbiome” P299 L23: “abyssal” P300 L21: “microbial stratification data” P301 L6: “microbial communities colonizing surface water” P303 L15: “the edge of the water profile” P304 L29: “bacterial zonation” Please check the entire ms for the use of the correct terms, and pay attention to a proper usage of terms for microbial oceanography and ecology.

iv) The title may be misleading. “Microbial” must be replaced with “bacterial”. I have also concerns on the use of the term “biogeography”. The authors used a low-resolution, fingerprinting technique (ARISA), which is better suited to infer diversity drivers and patterns than biogeographical features. NGS of the 16S rRNA gene would have been more useful in this sense.

v) Abstract, first sentence: a cruise itself is not an opportunity to justify a survey, or a scientific paper. Please remove the first sentence, and replace with another one introducing the scientific topic, and explaining why this study is important in this scientific context.

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- vi) why “sub-surface” and not “epipelagic”, as for the deeper layers?
- vii) Prokaryotic abundance: Figure 2 and Supplementary Table. Please provide standard deviation for each of the values presented.
- viii) P295 L19-25: how many replicates were analyzed for ARISA? If the analysis was limited to only one water sample per station or sampling depth, the results may be biased. Small scale variability may be high. Please provide clarifications about the use of replicates. If only one replicate was analyzed, this should be clearly underlined as a potential limitation of this study.
- ix) P296 L6-10: the authors say that ARISA fingerprints were obtained in two research institutes, and the two datasets were analyzed separately. This is good (there may be inter-laboratory variability), but there is no further mention about these two datasets in the Results, nor in the Discussion section.
- x) Figure 2 is hardly readable. I would suggest using a simpler and more common format.
- xi) P296 L14. please provide more information about the analysis of ARISA outputs. For instance, how peaks were binned?
- xii) P298 L 1-5: the authors are missing recent papers investigating the abundance and metabolism in deep water layers at the whole basin-scale (Luna et al. 2012 *Glob Biogeochem Cycles*; Zaccone et al. 2012 *Microb Ecol*), which are useful to compare the abundance data. In some cases (e.g. St. 297, 303, 319), data from the present study are around  $10^6$  cell L<sup>-1</sup>, which is one order of magnitude lower than reported (see the two papers above and also Yokokawa et al. 2010). How can the authors explain these differences?
- xiii) P298 L6 and L8: please be consistent with the term abundance. “Concentrations” and “cell numbers” are not appropriate terms.
- xiv) P298 L12-15: this sentence is unclear. Which statistics are the authors referring

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to?

- xv) P298 L18-20: this sentence is not needed. I suggest removing it.
- xvi) P299 L1: where are the ARISA data? A table displaying the number of OTUs per sample and Shannon Index should be provided. Please provide also another table showing the size and % of each OTUs found in the different samples. Both tables can be provided as Supplementary tables, if preferred.
- xvii) P299 L1-19 and below: please specify to which ARISA dataset the results are referring to (the one from University of Milan or that from the IEO?) I guess the results may have been slightly different, leading to different statistical outputs.
- xviii) P299 L17-19: this sentence is unclear. Similarity in community composition should be tested using SIMPER.
- xix) P301 L26 to P302 L15: the hypothesis of a longitudinal gradient in epipelagic bacterial communities, and its dependence upon the Suez influence, is fascinating to me, but sounds highly speculative. Microbes may behave differently to marine macro-organisms.
- xx) Analogously, the influence of latitude should be taken with caution. The Fuhrman et al. cited study covered a very large latitudinal gradient (from tropics to poles), while latitude in the Mediterranean Sea vary as little as a few degrees. Which hypothesis is behind the existence of a latitudinal gradient in the Mediterranean Sea?
- xxi) P303 L9: why distinguishing between upper and lower mesopelagic?
- xxii) P303 L17: sharply?
- xxiii) P303 L27: beta-diversity comes out from the blue. How was it calculated, and why only here?
- xxiv) The study by De Corte et al. 2009 was carried in the Med, not in another oceanic region.

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xxv) P305 L16: longitude is not a physico-chemical parameter

xxvi) The Conclusions section is too long.

xxvii) Table 2, 3 and 4: unclear what each number stands for (RDA1, RDA2, ecc.). Are numbers referring to specific environmental variables?

xxviii) Legend to Fig. 3, 4 and 5: Diversity of waters? And, I cannot see any biotic data in the PCA analysis. Please check carefully these legends.

Other comments:

i) P292 L15-17: “different physico-chemical factors were significantly related to microbial zonation both according to geographic position and across the water column in the whole Mediterranean Sea”. I think the opposite may be true, i.e. that microbial diversity was related with the different water masses.

ii) P293 L14-16: the Mediterranean Sea has indeed peculiar characteristics, but deep waters layers of all the oceans are hospitable for active microbial communities. Please reformulate this sentence.

iii) Legend to Figure 1: replace “bacterial abundance” with “prokaryotic abundance”. Stations are not “occupied”, but “sampled”. Why showing also stations where no microbiological investigations were carried out? No clear reasons to show them.

iv) Fig.1: if the map was drawn using the ODV Software, it should be specified by citing the relative bibliographic source (Schlitzer R., Ocean Data View, <http://odv.awi.de>, 2013).

v) Supplementary table: pressure is not needed.

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Interactive comment on Ocean Sci. Discuss., 10, 291, 2013.