Reply to the Referee 1 comments.

Firstly, we want to thank the reviewer for his/her productive suggestions. Our replies are reported in italics below each of the Referee comments.

The manuscript by Mapelli et al. is an interesting work looking at the biogeography of bacterioplankton across the entire Mediterranean Sea. Overall, I believe that the quality of the data set is good, providing a synoptic view of the distribution of bacterioplankton communities in the Mediterranean Sea, and trying to link it to changes in environmental parameters. The authors have also done a good job when synthesizing and presenting the data. However, I believe that some particular issues should need to be further clarified before this manuscript can be accepted for publication in Ocean Science.

The authors use several times the word "diversity", but they used ARISA as the molecular fingerprinting technique of choice. ARISA is a good tool for analyzing differences in community structure but it is not sensitive enough for analyzing the full richness and diversity of a natural community (as shown by the last generation sequencing techniques). Therefore I suggest being cautious with the use of those words in the manuscript.

According to the referee comment we replaced the term "diversity" throughout the manuscript with different terms such as bacterial community composition (or structure) or bacterial assemblages.

In the Results and Discussion section (particularly in 3.4) they write about the differences between the eastern and western basin in terms of bacterioplankton community composition, and they try to link it to variations in water masses. However, they did not characterize the specific water masses and their distribution. Therefore, it is difficult to clearly establish a link between water masses and the bacterioplankton community structure.

We agree with the referee that we cannot relate bacterioplankton community composition with different water masses since the latter were not identified for each sample. Nonetheless the Figure A included in this file (please see also our reply to your last comment) shows the T/S diagrams illustrating that indeed different water masses were sampled across the water column of the eight stations selected for the finer vertical scale investigation. To clarify the differences between the different seawater samples we also revised the section 3.1 improving the discussion of those physico-chemical parameters that are used for water masses identification. Moreover, to provide a framework of the characteristics of the sampled water we added the Supplementary Figure 1 showing a section of salinity in the Mediterranean Sea recorded during the Meteor cruise M84/3, where the samples collected and analyzed for bacterial community structure and/or abundance are indicated.
However, the main topic of the paper was a microbiological study that did not aspire to provide an exhaustive oceanographic survey. That said, we tried to highlight the hypothetical nature of the correlation between bacterial community structure and water masses, the former being statistically related to those physical parameters (i.e. temperature, salinity) generally used to identify different water masses.

Another possible explanation for the differences found in bacterioplankton communities between both basins could be related to the variations found in phytoplankton communities between both basins. There are numerous reports about the strong link that exists between phytoplankton and bacterioplankton communities, where changes in phytoplankton abundance or community structure provokes changes in bacterioplankton community

structure, many times due to changes in the DOM quality/quantity provoked by those changes in phytoplankton.

We thank the referee for sharing his/her consideration about the possible influence of phytoplankton community and related changes of DOM quality/quantity on the in bacterioplankton communities. We implemented the data discussion (section 3.4) on the light of this remark.

A clearer explanation of the stations covered is required. There are stations in the Supplementary Table that were not included in Fig.1. Moreover, the legend of Fig. 1 is not clear to me: "Stations where both bacterial abundance and community composition (ARISA fingerprinting) were determined are indicated by encircle dots and station numbers. Underlined stations numbers indicate those stations were only the bacterial abundance was measured". But, what are the single dots? I would remove the stations that were not sampled for parameters used in this manuscript, if that is the case. I would also try to mark clearly in that Fig.1 the 8 stations that were used for the finer vertical scale profiling of bacterioplankton communities.

We have simplified the Figure 1 according to the referee suggestion. The single dots, previously representing the sampled stations where any microbiological analysis was performed, have been removed from the revised Figure. The revised Figure 1 includes now only and all the stations where the prokaryotic abundance and/or bacterial community structure were determined. The Supplementary Table 1 includes additional information showing which of the stations were analyzed for i) only prokaryotic abundance, ii) bacterial community structure by ARISA fingerprinting, iii) both measurements. Furthermore, we clearly marked by encircled dots the 8 stations that were sampled at the finer vertical scale profiling.

I am missing the rationale about why those 8 stations were selected for the finer scale profiling. Authors should include some words about it if they have not done so already.

The Meteor M84/3 cruise represented a great and rare opportunity to realize a multidisciplinary survey on the whole Mediterranean Sea. In this context, we had the possibility to collect water samples from the subsurface and bathypelagic layers from a high number of stations. However, due to time and water availability restrictions, only eight stations were selected to study the vertical gradient in the bacterial community composition, aiming to cover the whole longitudinal transect in the Mediterranean Sea (about 3500 km long). By choosing these eight stations we attempted to sample the different known water masses in the Mediterranean Sea namely the Atlantic Surface Water, the subsurface salinity maximum associated with Levantine Intermediate Water and the deep waters in the eastern, western, Adriatic and Aegean Sea. As suggested by the referee, we added to the section 2.1 a sentence to explain the rationale for the selection of these stations. The result and discussion section 3.1 has been also implemented to clarify the differences, in terms of physico-chemical parameters, characterizing the sampled seawater.
In addition, the Figure A included to the present file (please, see below) shows that across the water column of the station selected for the finer vertical profiling we sampled different water masses. Figure A is a T-S plot for each of the stations, where salinity is plotted as a function of temperature, and dots indicate the sampling depths.

A clean rewrite by a good editor would help throughout.

The English has been revised by a native English speaker throughout the manuscript in order to improve its clarity.

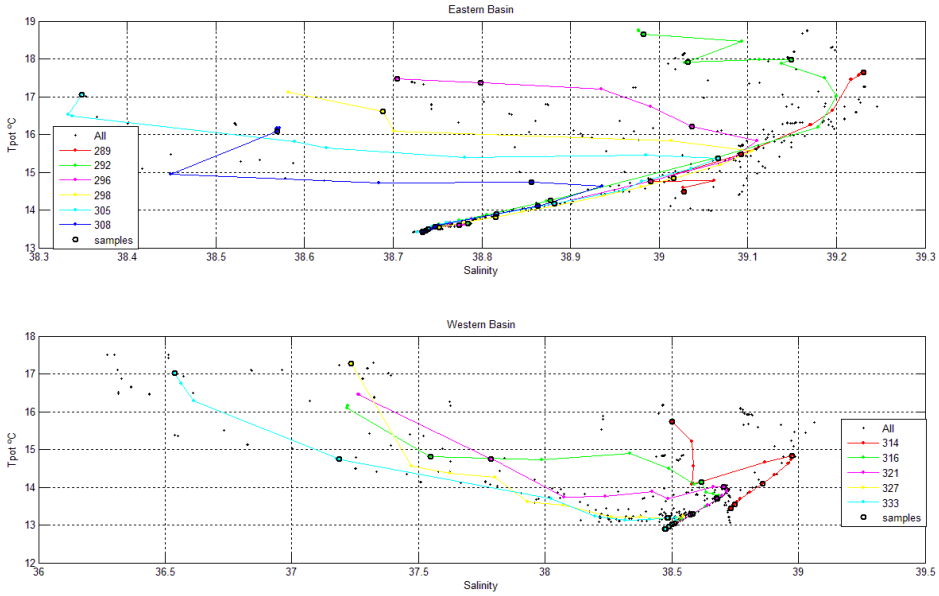

Figure A: T-S diagrams of the stations sampled at finer vertical scale for the depiction of prokaryotic abundance and/or bacterial community structure across the water column.