

Please see below the responses to the Anonymous Referee #2 and the actions taken regarding her/his concerns.

In the text below, the suggestions and comments of the Anonymous Referee #2 are in black and plain font and *our responses are in italics and blue font*.

This manuscript presents an analysis of the salinization effect on the microbial communities' composition and activities. In this study, microbial communities from 112 ponds across the western Mediterranean coast were analyzed based on 13 biotic and abiotic parameters. The salinization effect of the coastal wetland is an important outcome of the sea-level rise and has been directly linked to the ongoing global climate change. Thus, a better understanding of the microbial community response to sea-level rise is an essential step forward in the development of holistic eco-economic models of climate change consequences. The authors concluded that the concentration of Total Dissolved Nitrogen (TDN) positively correlated with the abundances of heterotrophic prokaryotes, but negatively affected the heterotrophic bacterial activity. Additionally, the authors suggested that a decline in the heterotrophic bacterial activity is due to elevated salinity and higher viral titer. Although these findings are interesting and important to decode the ecosystem response to environmental perturbation, a few methodological and statistical justifications might strengthen the manuscript. One of the primary authors' conclusion is that heterotrophic bacterial activity is negatively affected by virus titer and salinity. Nevertheless, based on the info in Table 1, in 36.1% (39/108) of the samples, the authors failed to detect virus abundances. On the other hand, the salinity of these samples spans 4 orders of magnitude ranging from 0.2 to 238.8 ppt. Thus, the authors might want to address this disagreement between the major conclusion and the presented data.

*We are sorry about this misunderstanding. In Table 1, "n. d." means "not determined" but not "not detected". Unfortunately, we could not count the samples of viruses from the Camargue, Sardinia, and Tunisia wetlands due to preservation problems during the travels. Now, we have included the meaning of "n.d." in the legend of Table 1 to avoid this misunderstanding among the readers. That is, there is not a disagreement between the conclusions and the data. It was a pitfall that we did not include the meaning of "n.d." in the previous version of this MS.*

The authors used GLM to determine the main drivers of the microbial patterns. One of the primary advantages of the GLM does not need to transform the data to meet the linear model assumptions. Instead, GLM analysis allows modifying the model assumptions, thus that it is not clear why the authors applied data transformations (line 177-180). Additionally, the model selection based AIC may be problematic or even inaccurate when compare the models of transformed/modified data and original datasets.

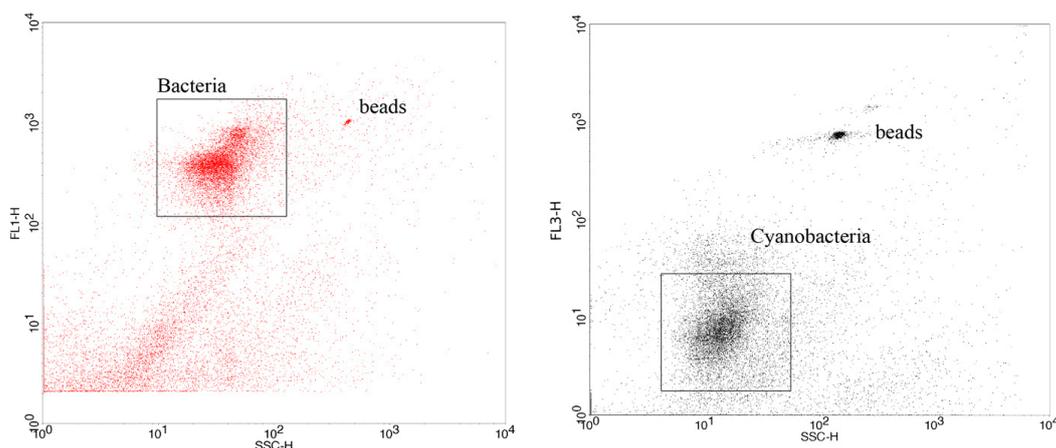
Finally, to increase the readability and reproductivity of the data analysis, the author might include the chosen model assumptions in the method section.

*We used an identity link and a normal error distribution, which we will now specify in the text. We respectfully disagree with the other statements. GLMs are more flexible than traditional regression methods, but they still will produce spurious results unless the precise nature of the data, the link function, and the error function are selected with care. Just by conducting a GLM there is no guarantee that the models will be reliable. In our case, the nature of the data mean that use of a normal error distribution and inspection of model residuals to identify the*

*transformation that removes heteroscedasticity is a valid approach. Using these kind of GLMs without transformations produces strong heteroscedasticity, so the results are entirely unreliable with a very strong influence of samples that are outliers. Using AIC for model selection does not solve that problem, and similarly the P values would not be meaningful. We selected those transformations that were required to remove heteroscedasticity, as already explained in the methods of the manuscript.*

To quantify the fraction of different microbial classes, the author used fluorescence labeling followed by FACS counting. This is a powerful technique when applied to fresh samples. In this study, the authors froze the sample in liquid nitrogen and stored at -80C until analysis (lines 134-137). Frequently, freezing the bacteria cell leads to cell disruption and DNA release, unless the protective reagents such glycerol were introduced prior to freezing. The molecular probe, Cyber Green I, which was used in this study, frequently fails to distinguish between environmental and cellular DNA. Moreover, Cyber Green I, equally labels eukaryotic, prokaryotic, and environmental cells, which may introduce biases into data interpretation. I am aware that it might be impossible to repeat the cells counting; nevertheless, the authors should insert the appropriate correction to the manuscript.

*We have used well-established flow cytometry procedures for counting prokaryotes and viruses (i.e., Gasol & del Giorgio 2000; Brussard et al. 2010), trying to be extremely careful. We are confident about the performance of these analyses and the data obtained. Usually, the side scatter (SSC) is different for each size group. Viruses, prokaryotes, and eukaryotes have different SSC windows. We selected different SSC windows at the beginning of the counting process for viruses and prokaryotes that we kept them all the time. Free-living, non-colonial cyanobacteria can be discriminated of the prokaryotes pool by their content in chlorophyll a (red fluorescence) in the FL3. Please see the figure below. Usually, environmental DNA is out of these SSC windows, in the noisy region. We have now included these details in the method section.*



The authors calculated the abundances of the heterotrophic prokaryotes by subtracting cyanobacterial abundance from prokaryotic abundance (lines 145-146). The authors might extend the discussion about this approach since cyanobacteria are not the only ones with autotrophic capacities in the system, other non-photosynthetic autotrophs are involved in sulfur, iron, and nitrogen transformation might play an essential role in the coastal ecosystem. Moreover, many cyanobacterial strains exhibit a multicellular lifestyle, growing as

filaments that can be hundreds of cells long and endowed with intercellular communication. Thus, it is crucial to clarify how exactly cyanobacteria were counted.

*We agree with the reviewer that there are more autotrophic prokaryotes besides cyanobacteria, such as nitrifiers and other groups that we did not consider in the previous version. Therefore, the calculation of the “heterotrophic prokaryotic abundance (HPA)” was not correct. In the new version, we have included only the total abundance of prokaryotes. Please see also the reply to reviewer #1 on this point.*

*Cyanobacteria cells were counted using a different sample and with different cytometer conditions (please see the method section). They only represent free-living cells; colonial filaments or aggregates were not included.*

*We have deleted all the statistical analyses, figures and tables that included the “heterotrophic prokaryotic abundance (HPA)”. Now, we have included similar analyses but using the total abundance of prokaryotes (i.e., without cyanobacteria subtraction). The main message of the MS is still the same.*

Throughout the manuscript, the authors use the term “production”; I find this term misleading. The biological production usually refers to primary productivity; in this study, the authors applied leucine incorporation assay to measure protein synthesis or community activity. To increase the manuscript readability, the authors might want to replace the “production” to “activity” as it was written inline 277.

*Bacterial production and bacterial activity (protein synthesis) can be both found in the scientific literature (see, for instance, Cole et al. 1988; Kirchman et al. 1985). However, we have followed the reviewer suggestion and changed “production” by “activity” to make the MS more readable.*

To distinguish between bacterial and archaeal activities, the authors applied erythromycin, which binds to the 23S rRNA component of the 50S ribosome and interferes with the assembly of 50S subunits. Although usage of erythromycin is a common practice to limit bacterial protein synthesis, however many bacteria have natural erythromycin resistance. Moreover, since erythromycin blocks mainly bacterial protein synthesis and has a limited effect on eukaryotic activities, based on the presented data, I not sure for what extend the signal recorded in this study is a result of bacterial, archaeal, or eukaryotic protein synthesis. Thus, the authors might want to clarify the methodological limitation of erythromycin usage in this study.

*We have included more details about erythromycin susceptibility of specific bacteria functional groups related to the nitrogen cycle (i.e., ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB)) or other Phyla such as Firmicutes.*

Please provide the statistical support, including p-values and  $R^2$ , for the data presented in figures 4, 5, and 6.

*We have now included the statistical support (p-value and  $R^2$ ) for the data presented in the figures 4, 5 and 6.*