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Interactive comment on "Alternation of heterotrophic bacterial and archaeal production along nitrogen and salinity gradients in coastal wetlands" *by* Gema L. Batanero et al.

Anonymous Referee #2

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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

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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 spends 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.

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 ACI 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.

To quantify the fraction of different microbial classes, the author used fluorescence labeling follows by FACS counting. This is a powerful technique when applied to fresh samples. In this study, the authors feezed 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 aware that it might be impossible to repeat the cells counting; nevertheless, the authors should insert the

appropriate correction to the manuscript.

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.

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.

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.

Please provide the statistical support, including p-values and Rˆ2, for the data presented in figures 4, 5, and 6.

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