

Please see below the responses to the Anonymous Referee #1 and the actions taken regarding her/his concerns.

In the text below, the suggestions and comments of the Anonymous Referee #1 are in black and plain font and *our responses are in italics and blue font*.

Anonymous Referee #1

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This paper presents the bacterial and archaeal abundance and heterotrophic production in nine coastal wetlands. Based on Generalized linear models they conclude to switch from heterotrophic bacterial production towards heterotrophic archaeal production as salinity and virus abundance increased. This topic is very interesting in a context of global change. But in my opinion the conclusions are very speculative and based only on linear models between productivity and salinity or viral load. I am not a modeler, but the use of GLP must be justified and statistics must be provided. The experimental methods used are also to be discussed.

Material and Methods

Part In my opinion different methods are not very suitable:

1> Line 145: We obtained the heterotrophic prokaryotic abundance (HPA) by subtracting the cyanobacteria abundance (CyA) from prokaryotic abundance (PA). Cyanobacteria are not the only autotrophic organisms, there are also, for example, nitrifiers. What is the percentage of cyanobacteria? what is the objective in this paper to limit itself to present the HPA numbers? what is the experimental error in the quantification in comparison with the numbers of cyanobacteria? The authors probably wanted to link the number of heterotrophic organisms and their productivity. This is always tricky because the count concerns the total number of organisms without information on the active fraction.

We agree with the reviewer and thank this comment. In the new version, we have only considered the total prokaryotic abundance and the free, non-colonial cyanobacteria abundance. As the reviewer noted, nitrifiers are included in the autotrophic fraction. We have changed all the analysis that included the “heterotrophic prokaryotic abundance (HPA)” for the “prokaryotic abundance (PA)”.

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 are not included.

As the reviewer detected, we initially wanted to relate “heterotrophic prokaryotic abundance” with heterotrophic activity. Therefore, we subtracted the cyanobacteria cells from the total pool; however, we did not consider other autotrophic prokaryotes such as nitrifiers, and this calculation was not accurate. Now, we have presented only the total abundance of prokaryotes.

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. The main message of the MS is still the same.

2> line 146: Virus abundance With this protocol and depending on the cytometer used (not specified, but I imagine it is a Beckton Dickinson (BD)), the authors will only see particles >50nm in size and DNA virus. So they should not say in the text that they will have the actual abundance (since there are many smaller DNA viruses and also RNA viruses).

Indeed, the flow cytometer was a Beckton Dickinson FACSCalibur (Franklin Lakes, NJ, USA). Now, we have included this information in the methods.

We have now explicitly mentioned that these virus abundances represent minimum estimates of viral abundance in the methods section. Currently, there are no accepted approaches for direct counts of viruses containing RNA or double-stranded DNA for natural waters.

3> The use of erythromycin to discriminate bacterial versus archaeal production should be discussed. Erythromycin inhibits the growth of bacteria by interfering with protein biosynthesis. It binds with the 50S ribosomal subunit and thus prevents the translocation of peptides and the formation of polypeptides. The efficiency of EMY are related to medically relevant organisms (e.g. *Staphylococcus aureus*) and do not consider natural prokaryotic assemblages. It is important to note, however, that all other studies concerning the efficiency of EMY are related to medically relevant organisms (e.g. *Staphylococcus aureus*) and do not consider natural prokaryotic assemblages. Horizontal gene transfer and/or mutations of ribosomal binding sites might alter the susceptibility to EMY in archaeal and bacterial species. For Frank 2016, The addition of EMY reduced the bulk leucine incorporation by 77%. Evaluation of the inhibition efficiency of EMY on a cell-specific level showed no difference between Archaea ($76.0 \pm 14.2\% [SD]$) and Bacteria ($78.2 \pm 9.5\%$). Their results suggest that in complex open-ocean prokaryotic communities EMY is efficient as a domain-specific inhibitor. Line 160: "it appears to have better efficiencies (ca. 80%) in water of higher salinity and for specific functional groups as nitrifiers, particularly Firmicutes" I don't understand this sentence that needs to be rephrased, nitrifiers are not included in the firmicutes phylum. It is also necessary to qualify this statement because the authors also mention adaptation and resistance to EMY.

We agree with the reviewer. This sentence was misleading, and we thank this comment. The study of Du et al. (2016) showed that nitrifiers (i.e., ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB)) were susceptible to erythromycin, particularly the NOB. Moreover, other gram-positive bacteria that belong to the Firmicutes Phylum are also particularly sensitive. We have now rewritten this sentence to avoid this misunderstanding.

Results part

The results are presented in their entirety by integrating the entire dataset obtained from all 12 sites. For each site there is a strong salinity gradient and also a great heterogeneity in the bacterial numeration and production. Before integrating the whole dataset into a GLM model, the data could be presented and analyzed by station et compared.

In the new version, we have included simple regressions for each site in the supplementary information. The patterns were significant and consistent for the sites that included high numbers of ponds. We included this information about the site-specific relationships in the text (please see new results). However, we consider that the figures should appear only as supplementary information to make the paper more readable.

Line 215 : significantly , can you give a p-value?

We have now included in the text the p-value and R^2 .

Discussion

Do you have a hypothesis to explain from a physiological point of view the effect of TDN on the switch from heterotrophic bacterial to archaeal production?

Then the discussion turns to nitrification by archaea, I don't understand the connection since nitrifiers are autotrophic organisms.-

Line 270 : In our study, ammonia oxidation by archaea during nitrification likely is not a significant process due to the high concentrations of dissolved nitrogen in most wetlands: I don't understand this part of the discussion then nitrifiers are aerobic and except in atypical pathways they need oxygen to achieve nitrification.

We agree with the reviewer, and we have deleted this paragraph in the new version of the manuscript. We have mostly focused the discussion about the denitrification by archaea and heterotrophic activity across the salinity gradient.

More generally, there are only TDN data (including the concentrations of the different organic and inorganic nitrogen forms as well as nitrate, ammonium) and the discussion focuses on the transformation processes between the different oxidation states such as nitrification and denitrification. This seems to me very speculative the authors state in the final lines of the conclusion: Archaea appeared to be the main prokaryotes processing nitrogen in the most saline wetlands, I think this is based on a positive correlation between TDN and heterotrophic production by the archaea

We agree with the reviewer and have toned down the conclusions. We have deleted the paragraph about ammonia-oxidizing archaea since we only have data on heterotrophic archaeal activity.

Is there a cross-effect between DDT and salinity?

Assuming the reviewer means TDN instead of DDT. There is a correlation between TDN and salinity ($r= 0.33, p < 0.001$). This correlation is one of the reasons for using GLMs. The best model included TDN, instead salinity, as the main driver for the case of archaeal activity.