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Interactive comment on "Effects of wastewater treatment plant effluent inputs on planktonic metabolic rates and microbial community composition in the Baltic Sea" *by* Raquel Vaquer-Sunyer et al.

Anonymous Referee #2

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The study by Vaquer-Sunyer describes the effects of wastewater treatment plants effluent inputs on different microbial parameters. I find the topic interesting and relevant for publication in BG. However, the approaches used are not well explained and therefore it is hard to judge the results. Moreover, I miss precise definitions and statements in the discussion. Therefore the manuscript seems in the current status premature.

Introduction Line 50 ff. The introduction (and in the discussion) gives a very anthropogenic view about hypoxia. Various prokaryotic groups' life in oxygen minimum zones and often exists only at those conditions (read for example "Microbial ecology of ex-

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panding oxygen minimum zones" Jody J. Wright, Kishori M. Konwar & Steven J. Hallam). From a microbial point of view the biological diversity therefore increases by the presence of oxygen minimum zones. Moreover, oxygen minimum zones in the central Baltic Sea are connected to the presence of a halocline that prevents water mixing in spring and autumn and therefore a typical phenomenon in the Baltic Sea. The authors mean coastal water hypoxia (as in the cited Conley 2011 publication) that should become clearer in the introduction (and discussion).

Methods:

Line 105-105: what salt solution was used?

Line 131-133. Use space between unit and number.

Line 136: please give a short description who the calculation of the metabolic rates were performed. If I understand correctly, CR was estimated from change in oxygen over night and GPP from the sum of NCP and CR. Since DOC is produced during daytime the CR can differ between night and day. Typically incubations are performed at light and dark conditions in parallel to estimate CR and GPP. Please discuss that your approach gives comparable results as the parallel incubations.

Line 151: The 341-805r primers were designed for bacteria the protocols in Hugerth et al are for eukaryotes. If I understand correctly a two step PCR were performed, please describe the protocol in more detail, especially if the PCR contained several independent PCR cycles. This is important information since this can introduce a strong bias into the abundances estimates for bacteria.

Line 119: What happened to the data of the DPA measurements?

Result

Line 190 221 table 1 and 2: How was DOC and TOC measured (replicates, sensitivity,. . .)?

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Line 273: How was the change in the number of OTUs?

Discussion

Line 345: It is unclear what the abbreviation DOM stands for. Typically it is used for dissolved organic matter (which was not measured in this study), but in the manuscript it reads rather that N-rich dissolved organic matter is meant. Please clarify. The results suggest that certain concentrations of nutrients (Figure 2; figure 4, Figure 5 1:5; IN) cause a change in the GPP, CRR and BP compared to the control treatment. Moreover the statistics give often DOC and CHla as the variances explaining the variability (line 224, line 234, line2 44) and only for bacterial production a correlation with NO3. Therefore the statement: " OM significantly increased bacterial production, whereas it decreased gross and net primary production and community respiration rates" seems not justified.

Line 413. Alpha diversity is not only expressed in the Shannon index. I think you mean Shannon index here, but it would be interesting to see how the total number of OTUs (richness) change.

Please discuss also the artifacts that can arise from long term bottle incubations especially for t7.

P has also a strong impact on the eutrophication in the Baltic Sea since many Cyanobacteria are able to fix N (read for example "Andersson, A., Höglander, H., Karlsson, C., and Huseby, S. (2015). Key role of phosphorus and nitrogen in regulating cyanobacterial community composition in the northern Baltic Sea. Estuar. Coast. Shelf Sci. 164, 161–171."). Since P was also measured in the experiments, and found to have a strong influence on the BCC (Line 271), I wonder why P is rarely discussed.

Figures

The legend of figure 8 is almost not readable

The colors in the plot are not easy to distinguish the abbreviations should also be given

in the legend

Figure 2 and Figure 4: they are based on the same data, whereas one contains subtracted CR? Therefore I think one of them is sufficient.

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