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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 #3

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General Comments:

The current study by Vaquer-Sunyer and colleagues describes the effects of wastewater treatment plant effluent inputs to the Baltic Sea on coastal planktonic microbial communities. The topic should be of interest to readers of Biogeosciences, and should be able to be made appropriate for publication after revision. The authors tested a number of relevant microbial parameters, and the experiments appear to be well-thought-out and executed, although some of the methods require some clarification. The main results showed an increase in bacterial production and decreases in primary produc-

tion and community respiration following amendment with wastewater, along with some changes in bacterial community composition. There is some confusion, especially during the discussion section, between discussion of metabolic activity vs. community composition - i.e. it seems that an increase in BP and decrease in PP is taken to indicate a shift in community type (autotrophic to heterotrophic), which was not tested or substantiated by the data in the paper. I find that the discussion section in particular becomes somewhat disjointed, and that some of the conclusions drawn from the study are overstated (i.e. presented with more certainty than the data allow). As such, the paper requires more thought and more careful presentation before it is ready for publication. I hope that specific comments below are helpful in this regard.

Specific Comments:

Introduction: Line 67-69: This statement regarding reduction of TN seems quite specific. Can a reference be provided?

Methods: Line 127: "in situ temperature" - are these the temperatures listed in Table 1? If so, please refer to Table 1 here.

Line 136: A description of the method to calculate metabolic rates (even if it is an abbreviated summary) should be provided here, not simply a reference to another paper that describes the method. I looked up the other paper, and it is not clear to me how all of the metabolic rates were derived from the data in the current paper.

Line 136: "water properties" - please either list all of the properties (maybe a better term would "physicochemical parameters") used in the statistical models, or refer to the table that contains them.

Line 154: What are the "biological replicates"? I did not get this from the description of the experimental/treatment design. Given this, I think that the treatment description (Line 97 onwards) needs to be improved - I see four experiments (one for each season) with five treatments each, but no replicates. Perhaps a list of everything that was tested for each treatment within each experiment should be included. It's not clear to me what exactly was measured on which sample.

Results: Line 186: Can you please clarify whether the nutrient determinations were done on the samples collected for each expieriment, following the filtration and freezing steps described in the Methods? I suggest making this clarification in the Methods so that the reader knows exactly where the reported data are coming from.

Line 192: The Methods section should be updated to include how the seawater samples for nutrient and chlorophyll analysis were collected and handled. I see a description of planktonic microbial community sample collection only. The description of how the samples were analyzed for nutrient and chl content, is complete, just not collection, filtration, storage, etc.

Line 203: "as a consequence of re-mineralization" is probably a good assumption to describe increasing nutrients, but because the source of the increased nutrients was not tested in the current study, this statement (and its degree of certainty) is not appropriate for the Results section.

Lines 204-209: I do not see a description in the "statistical methods" section of the Methods that could have been used to arrive at these conclusions regarding cal. The methods seem to cover metabolic rates and community structure, but no the relationships among physicochemical variables such as Chl and light. Please clarify this in the Methods.

Line 255: Rather than saying that BP 'depended on' DOC, it might be more useful to describe the direction of the relationship.

Line 267: "temperature significantly explained..." I question whether strong conclusions can be drawn regarding the influence of temperature. Given the range of temperatures (3,4,7, 18), it seems that the single high temperature (18) is an outlier and would exert extra influence on the correlations in the Mantel test. Can you address this in this

review response, since many of the relationships in the paper seem to revolve around temperature?

Line 273: “relatively similar” is unclear. Perhaps provide the range in alpha diversity across all experiments in parentheses and say “similar”.

Line 275-276: Can you clarify the wording please? I think what is meant is “a lower Shannon index was observed for all nutrient treatments compared to the controls”, but I am not certain based on current wording.

Line 281-282: Is the implication here that the Betaproteobacteria decreased in the control over time, rather than increasing in the treatments? That is interesting, and I suggest making clear what the conclusion related to this result is. Also, if there were changes in the control during the experiment, is there concern over bottle effects?

Line 284-285: "higher relative abundance" - Can you please add in parethenses how much higher the relative abundance was, compared to other treatments and controls (on average)? Also, is there statistical significance associated with this statement? It is fine if there is not, but I still suggest providing some numbers so that the reader can make the comparison more clearly.

Line 290: "increased in the control" Same question as above - With so many changes in the control, are we just seeing bottle effects over time? Can you comment on the validity of comparing these long incubations? Why would things be changing in the control?

Line 301: Can you please define what is meant by “finer phylogenetic scales”? i.e. at OTU level? Phylum level?

Line 301: “when communities responded to experimental treatments” I'm not sure what this means. Can you clarify whether you mean that you only looked at links between environmental and biological factors in experiments where there was a response to the treatment? Perhaps this needs to be split into more than one sentence to make the meaning clearer.

Line 302: "were positively correlated" Is this referring to the relative abundances of these groups? Can you please say what about these groups was correlated with temperature?

Line 309: It is not clear to me where the explanation of the variance is coming from here. Earlier in the paragraph, Pearson correlation is referred to, but I am not sure that makes sense here. Can you please specify?

Line 311: "8 major phyla" Are these 8 major phyla/classes listed somewhere in the paper? If not, please do so here.

Line 320: "strong correlation" Can you please say in parentheses what constitutes a "strong correlation"?

Line 321: Why "e.g."? Can you list all of the strong correlations, or only these few because there are too many?

Line 325: What is a substantial correlation? Please give a range, or an average, especially since the data is in the supplement. Listing something here allows the reader to better understand the relationship.

Discussion: Line 356: The type of modeling exercise described in this section is valuable, and can be used to support a hypothesis, but I would caution against using the term "validate" in this case. It implies a level of certainty that I do not think can be reached in the current study.

Line 359-360: Could you please provide the coefficients for each parameter in this model, so that the reader can get an idea of the rate of change in BGE associated with each variable? They could be listed as a rate in parentheses after each parameter, for example. Were all of the parameters "significant" in the model? How was the model selected?

Line 368: Bacterial carbon demand was not measured in this study, rather the authors assume it based on community respiration. This statement should be amended to reflect the level of certainty that can be supported by the data.

Line 369: The reduction in primary production does not lead to more carbon being used by the microbial loop. More carbon is used by the microbial loop because bacterial production (or respiration, which was not measured) increases.

Line 371: Could you provide a min-max range of the ratio of BP:NCP from your experiments to support this point (that the ecosystem moves towards heterotrophy)?

Line 372: Increasing carbon flow into the microbial loop should not result in reduction in the transfer of carbon to higher trophic levels. Organic matter entering the microbial loop through bacterial uptake should still be returned to higher trophic levels through coupling with the traditional food chain. The paper by Wohlers refers to a decrease in carbon fixed by primary production being transferred to higher trophic levels (not organic C uptake by heterotrophs), and (as far as I can tell), the Berglund paper simply suggests that increased runoff (and thereby nutrient inputs) and temperature should favor a heterotrophic bacteria based food web and decrease production. Either way, I can't see why the authors conclude that increasing carbon flow into the microbial loop alone should result in a reduction in C transfer to higher trophic levels.

Line 379-380: It's not clear to me how this is related to the current study or discussion.

Line 381-382: "A change in the planktonic community towards more heterotrophic communities" So far, this discussion has pointed out that rates of BP increased and the NCP decreased, with the addition of DOM. However, I don't think that there is evidence here that the community composition is shifting towards heterotrophy? Or, if there is evidence of this, it should be mentioned in the discussion here before lines 381-382.

Line 382: While it is true that a reduction in photosynthetic rates would decrease oxygen production, I do not see clear evidence from this study that a shift towards heterotrophic communities is occurring, or that any reduction in photosynthetic rates would be the result of such a shift. In short, Line 381-382 make some assumptions that should be revisited and substantiated with data, if it exists. If it does not, then this discussion point should be reworded so that it is supported by the data.

Line 390: "reducing the ecosystem capacity of removing nitrogen" Doesn't anoxia favor the removal of nitrogen (i.e. denitrification)?

Line 390 - 393: The final two sentences here (lines 390-393) do not flow from the previous discussion about anoxia and eutrophication. The text in this section should be revised to make clear points and conclusions, which are supported by the data.

Line 399: "disturbances" - Do you mean effluent inputs? what is meant by "disturbances"?

Line 401: Can temperature be de-convoluted from season or other parameters? The changes in temperature weren't really "experimental" changes, but matched the in situ conditions at the time of sample collection, correct? I guess I don't really understand what is meant by "changes in temperature". And I still have the concerns listed above regarding the range of temperatures and influence of outlying temperature.

Line 409 - 412: The sentence beginning "It is noteworthy" is not clear. The authors are suggesting that what changes in what relationships? Changes in the relationship between composition and function? What is the relationship between composition and function? I'm not sure where this sentence is going.

Line 410: the link between community composition and function should be susbtantiated with a reference.

Line 411-412: This is redundant with the discussion of theoretical BGE above.

Line 412-413: Lower diversity doesn't necessarily equate loss of function - aren't many functions redundant within a microbial community?

Line 422: "caused responses" What responses? It would be more correct to say that these certain populations responded to effluent inputs. Also, didn't the Verrucomicrobia increase in the control? So how are changes in Verrucomicrobia associated with effluent inputs?

Line 424: Nutrient inputs, or effluent inputs? The terminology used here is confusing, and I can't tell exactly what the authors are trying to conclude.

Line 430-431: "warming could increase cyanobacterial blooms" How did cyanos in this study respond to temperature?

Line 434-438: As written, this closing sentence (which should be used to drive home a major point of the current study) seems to focus on the results of a previous study instead. How does the current study and its findings support or add to the findings from the previous study? Also, the finding that "warming and effluent inputs increased planktonic respiration and bacterial production faster than primary production" is attributed to the previous study - I thought that this was a new conclusion of the current study? If not this, then what IS the new conclusion of the current study?

Line 443: The conclusion that this leads to an increase in BGE is stated with more certainty than can be derived from the current study. It assumes that the decrease in CR is also a decrease in BR, but that may not be the case. The conjecture is ok, but should not be stated as fact.

Line 448-449: If cyanos increased in summer, how is this be linked to effluent inputs and not temperature? Also, I assume that "abundance" is "relative abundance"?

Line 454: If cyanos are increasing due to effluent input, it is not clear to me how the conclusion that planktonic communities are shifting toward heterotrophic communities is made? Were the relative abundances of photo and heterotrophic organisms compared? Or is this based on rates of activity of the two groups? If the latter, this should be rephrased so that it does not lead the reader to conclude that the community structure is changing, and is responsible for a shift towards heterotrophy.

Line 460: Low BR (not CR) compared to BP leads to high BGE. Since BR was not measured in this study, the authors should be careful regarding the level of certainty they assign to these conclusions. While interesting, any conclusion related to BGE is theoretical and should be used to guide further research, not stated as fact.

Tables and Figures:

Table 1: As the table contains more information than only nutrient content, a more descriptive caption should be used. Perhaps “physicochemical parameters” would be more appropriate. The caption should also reflect the number of replicates used to arrive at the listed standard errors. Chemical symbols for nutrients should be listed with proper superscripts, subscripts, and charges (throughout the text as well). If all other chemical species are listed in molar concentrations, DOC should be too. It is best to keep these consistent.

Table 2: I notice here that the amount of P added is unknown for half of the treatments, which makes me question results related to changes in P. Can the authors address this please? Why is the carbon labeled as “TOC” if the samples were filtered as described in the Methods? How was C:N ratio calculated? Is it a ratio of DOC:DON? or DOC:DIN? Is it by mass, or moles?

Table 3: Some explanation of all factors tested and the model selection parameters should appear in the Methods section. How was the best model chosen? Were all factors tested initially (and what are all the potential factors)? The random factor for “experiment” is referred to as “season” in the Methods section, is it not? Please change one or the other so it is consistent.

Table 4: I notice a lack of correlation with organics - does this not imply that the shifts in composition are not related to effluent? "specific environmental variables" Which environmental variables were used? It seems that there should exist a table, similar

to tables 1 and 2, that gives the environmental parameters for each incubation (Tables 1 and 2 show environmental parameters at the collection site and in the effluent, respectively, correct?).

Figures 2, 4, and 5 should be of higher resolution. They appear blurry in the pdf.

Figure 3: What is a whole model plot? What is the model? Please clarify this caption. And if whole model refers to some sort of model selection, it should be described.

Figure 7: “A” is not labeled. It doesn’t seem to be possible to see all of the treatments in figure A. I think only showing Figure B would be more informative.

Figure 8: It would be easier to look at if we could see the controls first in the group for each time point and if the time points were separated somehow, perhaps by a small line

Figure 9: What were the highest and lowest correlations? Cutting it off at 0.4 seems like it would bin together a lot of data, unless there are no strong correlations. If there are no correlations >0.4, this should be made apparent by this figure and/or its legend. Cutting it off at 0.4 doesn’t tell me very much about what is going on here. I don’t think that “NOx” was used previously in the paper, so shoud be defined in the figure legend. Nutrients are incorrectly labeled again (missing charges etc). I would suggest just writing out the names if it is difficult to properly add superscripts etc. in the software used for the figures.

Minor comments: Line 151: change "was" to "were" Line 199: change "sunlight" to "solar" Line 323: "MWH-UniP1" Add “related” to the end of this OTU designation. Line 329: Use the correct designation for phosphate Line 349: Add (BR) after bacterial respiration to define the acronym. Line 375: Citations needed for “some studies”. Line 442: change "caused" to "was related to"

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