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Interactive comment on "Climate-driven change in a Baltic Sea summer microplanktonic community – desalination play a more important role than ocean acidification" *by* Angela Wulff et al.

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Answer to the referee's comments Refereee #1

We thank referee #1 for the comments and suggestions to improve our paper. Indeed we agree that there are multiple ways of designing experimental approaches like ours. However, we believe that our approach, and the choices that are implicit in the design, adds one piece to the complex puzzle which was also stated by referee #1 "your study can add one piece to this complicated puzzle with no doubt". Please note that the changes we have made in the manuscript are highlighted in yellow.

*Referee comment. First, just as you have mentioned in the manuscript, to reach the

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target salinity, you chose to mix the seawater of higher salinity with Milli-Q water simply. We can see that not only the salinity but also AT and the amount of dissolved inorganic carbon will change. So it may confuse readers which factor the experiment outcomes actually result from. Although you have mentioned in the manuscript that “the reduced buffering capactity was not expected to affect the microorganisms in our experiment”, still, I suggest you have a supplementary experiment to distinguish the effect between salinity and alkalinity so you can illustrate clearly. After all, the carbonate system really concerns while taking ocean acidification study.

–> Author answer. Yes it is correct that we in this study chose to use Milli-Q water to reduce the salinity and consequently e.g. AT was initially lower. However, despite the reduced buffering capacity (AT) we did not find any significant effects of pCO2 and we believe that it strengthens our argument that the carbonate system was not of main importance in this study. The study was performed on a natural microbial community and it is not possible to repeat with the same community. However, in a yet to be submitted study on another Baltic microbial community, AT was reduced from approx. 1500 μmol/kg SW to 1000, with no effect on the microorganisms. Although we did have a control set-up with four replicate aquaria where we did not add nutrients, and another set-up with four "organism free" aquaria, one for each treatment (no replicates), in retrospect we should also have added "Milli-Q" controls. However, any experimental design implicates choices with potentially associated biases and we still believe our results are important enough to be shared with the research community.

* Referee comment. Second, the conclusion from your paper is that pCO2 showed only minor(no) effects. However, according to the description in the manuscript, it seems not convincible. To mimic the scenario by the end of this century, the target pCO2 is 960μatm but the actual pCO2 is far below this value (see Day 12 situation, eg. 833 μatm (SE 108) at salinity 6, and 579 μatm (SE 39) at salinity 3). In this case, the effect of high pCO2 may be inadequate to appear. Therefore, It’s better to adjust the flow rate and the position (depth) where ceramic air diffusers set or increase the amount of

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that so you can reach the target pCO2. In general, repeat the experiment again, if it's possible.

–> Author answer. Yes, it is correct that the pCO2 in the water after 12 days, and due to high CO2 uptake, differed from the targeted level of 960 μatm. But note that 960 μatm is the level projected for atmospheric CO2. Furthermore, we believe that it is more misleading to bubble the water intensively as this may promote an unrealistically high CO2 loss from the system. Forcing very acidic conditions to a system with high primary productivity will instead overestimate the effects of ocean acidification, as these blooming surface communities will never experience such high levels during the climate scenario that we have simulated (due to intense primary productivity). In our opinion, it is more realistic to start with high CO2 levels before the bloom and let it decrease as the bloom develops, just as in natural systems. The headspace will still represent simulated atmospheric levels of $\sim$960 μatm. Furthermore, we performed a pilot study in order to choose an appropriate flow rate (page 5, line 8–10) and the higher flow rate was chosen to somewhat compensate for the increased primary productivity over time. The increase in cell density is complicated to fully predict and compensate for, and also in a natural community an increased pCO2 concentration in the atmosphere will affect the carbon system differently depending on e.g. amount and composition of phytoplankton in the area. To illustrate the diurnal changes in the treatment aquaria and in situ we performed ca 30 hours measurements where samples were taken and analysed every second hour (Figs. 4 and 5).

* Referee comment. Besides, for the short term incubation like microcosms, why not take the batch culture into consideration as I wonder whether addition of nutrient in the halfway (like your experiment setup) will influence the final results. Maybe another choice is shorten the duration of incubation properly without nutrient supplement in the whole process. If it were me, I would do like this.

At last, I hope you could plot extra figures to show the change of growth rates and species diversity with time alone.

–> Author answer. We apologize, but we do not fully understand this comment. Nutrients were not added halfway but after sampling on days 2, 5, and 9 in order to maintain concentrations similar to the Baltic Sea's summer nutrient conditions (page 5, line 20–23). We wanted to avoid adding a factor such as "nutrient depletion" into this experiment. About experimental time – we aimed to ensure enough time for the organisms to be affected by the treatments but at the same time avoid "bottle effects". Please also note that referee #2 found the experimental period too short.

Furthermore, we did not use batch cultures but a natural microbial community so we do not really understand the comment about "batch culture"? However, as explained on page 5, line 22-23, "in an additional set of four aquaria manipulated with ambient levels, i.e. salinity 6 and 380 μatm CO2, no nutrients were added and used as a control for nutrient enrichment". If this is what referee #1 refers to as "batch culture" we can add more data than is shown in Table 1-3, perhaps in the supplementary material but we need guidance on what type of additional data we are expected to add. However, as explained on page 5, line 25-28, "because the aim of the experiment was to investigate potential combined effects of salinity and pCO2, samples from "nutrient controls" are not included in statistical analyses but results from inorganic and particulate organic nutrient analyses, carbon chemistry and chl a are shown in Table 1–3."

Referee #2

We thank referee #2 for the comments and suggestions to improve our paper. Indeed we agree that experiments including ours can be improved and it is particularly true for "freshening" experiments using natural communities and ambient seawater. However, we believe that despite indirect effects of dilution our results are important enough to be shared with the research community. We have tried to follow the recommendations by the referee and we answer point by point below. Please note that the changes we have made in the manuscript are highlighted in yellow.

*Referee comment. The main problem lies in the fact that salinity was adjusted with

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dilution of MilliQ, reducing salinity from 6 to 3 (50%). Firstly, this is problematic as the other treatment, increased CO2, kept the original water. There was addition of inorganic nutrients to the MilliQ to compensate for its loss by diluting, but otherwise the basic chemistry of the low salinity treatment was altered beyond the salinity effect. The authors are aware of this, e.g. pointing out that the alkalinity was different, but there might be more differences that currently is not taken into account, e.g. the DOC pool. A more elegant way would have been to dilute also the high CO2 treatment with artificial sea water (MilliQ with added sea salt) in a similar manner.

–> Author answer. In retrospect, yes, we do agree that the DOC pool was diluted in the low salinity treatments and a similar addition of "salted Milli-Q" to the high CO2 treatment had been a good option, but any experimental design implicates choices with potentially associated biases and we still believe our results are important enough to be shared with the research community. Despite the reduced buffering capacity we still did not find any treatment effects related to increased CO2, however, see our comments on the salinity reduction below.

* Referee comment. Another issue is the shock effect in this relatively short term experiment. The authors state the CO2/pH is quite variable in the study area, presumable affected e.g. by upwelling (high in CO2) and primary production (reducing CO2). As such the rapid change in the high CO2 treatment is probably something the plankton community may experience in a relatively short time frame.

–> Author answer. Yes we agree it is a relatively short experiment although referee #1 recommended an even shorter period. However, despite a possible "shock effect", 12 days allowed for ca 8-10 generations of diatoms and Dolichospermum spp. in the high CO2 treatments. Please see below for salinity issues.

* Referee comment. A drastic reduction in salinity (50%), however, might be more of a chock. Although the salinity change would be within the tolerable salinity window of the main species, I would expect an immediate effect of increased respiration,

causing reduced growth, due to acclimation to the new salinity (for example adjusting membranes and osmoregulation). These possible indirect effects of the low salinity treatment should at the very least be discussed. Although work with cultures is not directly comparable, acclimation period over several generations is normally used for determining a species salinity tolerance (e.g. one month used in Yamaguchi et al 1997, J . Plankton Res 19: 1167-1174), so drawing any long-term conclusion from this experiment is questionable.

–> Author answer. We agree that possible indirect effects of the low salinity treatments should be discussed and this has been inserted on page 14 line 1–6 "With our set-up, indirect effects of the lower salinity could not be ruled out. For example, the DOC pool was initially diluted in the low salinity treatments potentially reducing substrate access for heterotrophic bacteria. Indeed, bacterial production appeared higher initially in the high salinity treatments, but no significant differences remained after 12 days between the high and low salinity treatments, indicating that other factors than carbon availability limited bacterial growth."

Please note that the microbial community was inoculated in 0.2 μm filtered Baltic Sea surface water with either salinity 6 or 3 (page 4 line 27–28), that is, the water was diluted before the microbial community was added. We find a "shock effect" unlikely at these low salinities. Furthermore, when working with natural microorganisms, confinement itself represents a strong selective power – and should therefore be minimized. So in our setup we judged that the advantage of introducing an adaption period with gradual decreasing salinity would by far be counterbalanced by the selective powers induced by confinement itself. We agree that drawing long-term conclusions from our experiment should be made with caution and we have now emphasized this point in both Abstract and Discussion "However, long-term effects of the experimental treatments need to be further studied, and indirect effects of the lower salinity treatments could not be ruled out."

* Referee comment. Minor comments: P6, L21. Variable fluorescence is normally

denoted Fv (or you should use _F throughout). –> Author answer. Embarrassing, has been changed to Fv

* Referee comment. P9, L19-20, Testing the obvious defeats the purpose of statistics (if you dilute, of course there will be a treatment effect). –> Author answer. Well yes, but we think it is better to state the obvious and be consequent. Sometimes we think it is obvious and the statistics tell us a different story.

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