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Interactive comment on "Ocean acidification changes the structure of an Antarctic coastal protistan community" *by* Alyce M. Hancock et al.

Anonymous Referee #1

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General comments: This study investigates the effect of elevated CO2 concentrations on prostistan community composition of Prydz Bay, East Antarctica. As the quantification of cell abundances at the species level is very labor intensive, often studies tend to neglect this very important aspect that has been considered in detail in the study by Hancock and co-authors. The data presented are interesting and I belief it should be published, but it needs a considerable revision to be acceptable for Biogeosciences. The authors need elaborate in more detail about the counting procedure, in particular for cells which were present only in low abundance as they often tend not to be evenly distributed in the Utermöhl chamber, causing easily wrong cell abundance estimates. For better readability of the manuscript, information on carbonate chemistry as well as on macronutrient concentrations over the course of the experiments is

needed. In particular, the onset of nutrient limitation on day 16 needs to be accounted for in the discussion of the development of the protistan community, which has been neglected so far. At the moment, the discussion mainly concentrates solely on the CO2 effects, which is fine until day 15, but not after this time point. This aspect needs to be addressed. For better and faster comparability of the figures of species-specific cell abundances, I recommend to use the unit 'cells per mL'. Furthermore, to strengthen the author's argument that growth of large-sized diatoms is more prone to high CO2 concentrations, a graph showing actually the different trends in total abundance of all small versus all large diatoms, similar to figure 3, is needed.

Introduction: P2, L5-6: This statement is not right as there are several studies that were already published on OA effects in various natural assemblages of Southern Ocean microbes (Tortell et al. 2008, Feng et al. 2010, Hoppe et al. 2013, McMinn et al. 2014, Young et al. 2015, Coad et al. 2016, Davidson et al. 2016, Thomson et al. 2016). Please rephrase. P2, L19-35: Considering that the authors already cited 8 papers that were published on CO2 effects, it is not really appropriate to write that "there have been relatively few studies". Please also cite the studies by Hoppe et al. 2013 PLOS One and Young et al. 2015 MEPS, which are currently missing. The latter two studies need also to be taken into account when summarizing the findings on CO2-dependent shifts in community composition in this paragraph. P2, L23-25: Please note that Feng et al. (2010) reported a shift from Cylindrotheca to Chaetoceros from 380 to 750 μatm pCO2, and not from Pseudo-nitzschia. Further Tortell et al. (2008) did not observe a CO2-triggered shift in Phaeocystis antarctica. It was reported that both summer and spring phytoplankton communities were dominated by P. antarctica and within the communities a shift among diatoms was observed.

Methods: P3, L17: Did the authors assess whether the gravity filtration procedure introduced cell damage and/or physiological fitness of the sampled microbial community? The latter could have affected the evolution of the community structure. P3, L25: To me is unclear why during the initial acclimation phase the community was exposed to

the extremely low light intensity of ∼1 μmol photons m-2 s-1. P3, L28-32: How was the light intensity adjusted? Were the minicosms not exposed to the natural irradiance cycle? Did the authors monitor daily in situ irradiances over the whole experiment? The manipulation of the light intensity remains unclear to me. P4, L2-12: I can understand that carbonate chemistry results are reported in detail in Deppeler et al. (submitted), but also for this manuscript there is the need to give information on the successful CO2 manipulation of each CO2 treatment at least in a table. For the interpretation and discussion on the results of the development of the community composition, it would be also helpful to give the information on carbonate chemistry (e.g. pH, fCO2) at the day of seawater sampling. P5, L2-11: The counting of particularly large diatoms can be problematic. To this end, it is recommended to count the whole Utermöhl chamber as species are not distributed evenly. In particular, chain-forming diatom species can be very patchy, making their quantification on the basis of 20 chosen fields of view difficult. How many cells did the authors count per species? How was the patchiness of species distribution within the chamber accounted for? Considering the low cell numbers, it is important to address this issue as otherwise easily wrong cell abundance estimates can be made.

I miss information on the development of the macronutrient (N, P, Si) concentrations over the duration of the experiment. This info needs to be provided either in a table or a figure. According to Deppeler et al. (submitted to Biogeosciences) N was depleted for most treatments at day 16, this means that in addition to the changes in fCO2 N also potentially acted as stressor at the end of the experiment, potentially influencing community composition at the end of the experiment. The latter information is not obvious when nutrient data are not shown in this manuscript and needs to be accounted for in the interpretation and discussion of the results. Hence, to assess the effect of increasing fCO2 levels on community composition, the authors should rather compare results at day 16 instead of day 18. For instance, the abundance of Fragilariopsis species < 20 μm of the 343, 506, and 634 μatm fCO2 treatments strongly dropped between day 16 and 18, coinciding with nitrate limitation at these specific fCO2 levels.

Results: P6, L21-22: It seems very unlikely that the high variation in protist abundance of the 635 μatm pCO2 treatment, accounting for ∼10000 cells per mL, comes from the increase in rare large cell diatom species, which were only present between 5 up to 200 cells per mL (Fig. 2-5). P6, L27-29: It is not really clear which figure underlines this statement. Also, it would be helpful to point out which diatoms were classified as 'large' and 'small'. For this statement, it would be good to have a graph showing actually the different trends in total abundance of all small versus all large diatoms, similar to figure 3. P6, L30: Do the authors now refer to Fig. 2b-d when they refer to discoid centric diatoms or is Fig. 2a meant, but then it does not make sense to refer to 'unidentified discoid centric'. Does the latter term refer to one single species or does it summarize all counts of unidentified discoid diatom species that were smaller than 2 μm? Another idea would be to add the cell volume of the species next to its name on the graph, making it easier to see the size differences at a glance. P7, L14: To what fCO2 treatment does the control refer to? 343 μatm? P7, L12-14: Looking at figure 3a, small Fragilariopsis cells of the 953 μatm reached highest cell abundances in comparison with all other fCO2 treatments at day 16 and 18. The authors, however, write "Abundances in the ÆŠCO2 treatments >953 μatm were lower but less than those in the control treatment. . ." How can this be? P7, L15-17: Why is a tolerance lower when cell abundance is higher? P7, L20-21: The species name is O. weissflogii and not weissfloggi. Also write 'pennate' instead of 'pennant'. Also, it is Pseudonitzschia and not Pseudonitzschia. Also change turgidulodies to turgiduloides.

Discussion: P9, L26-28: As in almost all figures cell abundances did not change between day 1 and 8, considering also that irradiance was very low, I am surprised about the statement that community composition changed. Did the authors characterize species composition of the initial community? As the information on the characterization of the initial community is missing this complicates the interpretation on subsequent species changes through the sampling. P9, L 32-33: Taking into account the very low irradiance between day 1 and 8 the cells were exposed to, it is not surprising the community showed a severe delay in growth among all treatments, a finding which

is not mentioned here. Apparently, the combination of very low irradiance and high fCO2 caused even stronger delay. This is worth to be mentioned. On which observation is the statement based that 'the protists required more than 8 days to acclimate to this high fCO2'? P10, L1-6: To underline the statement that community growth of the highest fCO2 treatment was lowest, why do the authors not calculate community growth rate? All data are there and this would strengthen their argumentation. P10, L9-15: I am not yet convinced about the statement that in 'diatoms the response was mainly size-related'. To underline this, a graph showing actually the different trends in total abundance of all small versus all large diatoms, similar to figure 3, is required. The authors even point out that 'a couple of species did not follow this trend'. P10, L9-19: The fact that nutrients became limiting either on day 16 or 18 needs to be elaborated in more detail. This aspect was fully neglected, only in L14 it is mentioned that 'Chaetoceros did not show a response to fCO2, but instead reflected the nutrient availability'. This aspect needs to be discussed also for the other species. P10, L19-20: The low tolerance to high pCO2 is also found and reported in Tortell et al. (2008) and Hoppe et al. (2013). P10, L20: 'Unlike diatom species, ... Phaeocystis dramatically declined ... at the three highest fCO2 levels'. It was, however, pointed out before that 'large diatoms showed ...a decrease at higher fCO2'. There is no controversy, please modify. P10, L23: Please specify the statement 'our study only finds this response in diatoms'. To which response is referred to? The increase in diatom abundance under high fCO2? But the opposite response for diatoms was claimed before. P10, L21: I disagree that there is a 'common consensus in other ocean acidification studies that pico- and nanoplankton abundance increases at high CO2 levels'. Like the dataset of the authors, there are several studies reporting the opposite for Southern Ocean communities (Tortell et al. 2008, Feng et al. 2010, Hoppe et al. 2013). Please rephrase more carefully. P10, L23-25: Repetition, please see L20. P10, L25-28: In line with the data by Hancock et al., in none of the cited studies Phaeocystis antarctica showed a positive growth response to high CO2, growth rather remained unaffected by CO2. Please also add Trimborn et al. 2017 Physiol. Plant, which is in line with the latter

observation. P10, L28-30: I disagree. The results from Feng et al. (2010) show no CO2 effect on the colonial Phaeocystis antarctica. Furthermore, it is not clear to me why the presence of the mucus could have any effect on the CO2 sensitivity of Phaeocystis. P11, L1: Please also cite Wu et al. 2014 that reported enhanced growth rates in response to high CO2 in large diatoms. P11, L3-6: As mentioned before, no CO2-dependent increase in Phaeocystis was reported in Tortell et al. (2008), Feng et al. (2010) or Trimborn et al. (2013). Please correct. Further Xu et al. did also not observe any CO2-dependent increase in Phaeocystis from the current to the 2060 scenario, but a significant decline from 2060 to 2100. Please note that in the latter study next to CO2, also temperature, light and Fe availability was changed, being therefore more difficult to compare with this data set here. P11, L13-15: Please also cite Trimborn et al. (2013) who actually investigated the CCM of Southern Ocean phytoplankton species, among them Phaeocystis antarctica. P11, L26-35: For better readability, please specify the direction of the observed responses of the different choanoflagellates, just saying 'there were differences' is not enough.

Discussion Part 4.4: In particular here, the onset in nutrient limitation at day 16 and 18 needs to be accounted for in the discussion of community-level responses as CO2 was not the only driver. The latter statement also applies for the overall discussion.

Figures: Fig. 1-7: For better and faster comparability between cell abundances of the different species, I would use the unit 'cells per mL' in all figures instead of using 'cells x 104 L-1' as in Fig. 2-4, 'cells x 105 L-1' as in Fig. 7 or 'cells x 107 L-1' as in Fig. 1 and 6. The latter makes it even more complicated as the Y-axis is also changing, hampering a fast comparison between cell numbers between graphs of different figures. In the legends of Fig. 1 to 7, it is referred to the pCO2 while in the M&M section it is referred to fCO2, please stick to one of them throughout the manuscript.

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