

## ***Interactive comment on “Ocean acidification of a coastal Antarctic marine microbial community reveals a critical threshold for CO<sub>2</sub> tolerance in phytoplankton productivity” by Stacy Deppeler et al.***

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Response to Reviewer 1

General comments: In this work, Deppeler et al. installed six minicosm to study how ocean acidification will affect coastal microbial communities, including photoautotrophs and heterotrophs. This kind of field work is rather difficult to conduct, because it requires large amount of resources, participation of different groups and limited by meteorological condition and logistical support. They stated that there existed a tipping

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point for CO<sub>2</sub> effects, ocean acidification with CO<sub>2</sub>>1140 uatm would decrease primary production of phytoplankton, while no consistent effects on bacteria. This is an interesting finding, however, the data analysis is inadequate, especially for the threshold, the author should present a fig, the x-axis is pCO<sub>2</sub>, and y-axis could be GPP, FV/FM or other parameters, to clearly show there is a tipping point. Overall, this manuscript is well structured, while there are some flaws need to be fixed.

Response: We present figures of production over time in each CO<sub>2</sub> treatment and statistically compare these response surfaces to identify CO<sub>2</sub>-induced differences among treatments. To address the issue raised by the reviewer we drafted graphs showing the rate of productivity against fCO<sub>2</sub> at each time with the intention of adding a threshold value (Fig. 1). We thank the reviewer for their suggestion as plotting our data versus fCO<sub>2</sub> allowed us to better discriminate the trends in rates of Chl a accumulation and 14C-GPP among the CO<sub>2</sub> treatments. This visualisation of the data showed that the downturn in these parameters occurred between 634 and 953 uatm fCO<sub>2</sub> and could be discerned following  $\geq 12$  days incubation. In addition, we acknowledge that nutrient limitation confounds our ability to determine an fCO<sub>2</sub> threshold on the final day of the experiment (day 18). We will include a figure in the manuscript and provide further consideration of these results in the text.

Specific comments: Introduction: This section is well written, reflected the background of this study Method: I recommend the author to present a picture of whole scene of the minicosm, that will be much easier for the reader to follow the method.

Response: We have added in a figure showing a photograph of several minicosm tanks to aid our description of the minicosm setup.

P4 Line24, the seawater was transferred from another location by helicopter, my impression is that the community structure might be different with the local seawater where the experiment done. The major concern is that seawater in minicosm might contact with local seawater during the manipulation, is the contamination even for all

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minicosms? Because you don't have replication for each CO<sub>2</sub>, even the contamination happened differently for minicosm, while the statistics cannot tell you.

Response: We believe this comment is borne of a misinterpretation of our methods, which should be clarified by our inclusion of the photograph above. The only time local seawater (from immediately offshore) was added to the contents of the minicom tanks was in the daily additions of <1 l of 0.22 µm filtered and CO<sub>2</sub>-saturated seawater that was added to the contents of each tank to return the fCO<sub>2</sub> back to the target value. We had omitted stating that this seawater was 0.22 µm filtered before CO<sub>2</sub> enrichment and subsequent addition to the minicosms and have added this to the Methods.

P4 Line 33 Why you use blue filter? Are the transmission spectra available?

Response: A quarter CT blue filter was used to convert the tungsten lighting to a daylight spectral distribution. This is achieved by attenuating wavelengths <500 nm by ~20% and >550 nm by ~40%. The transmission spectra of the 150W HQI-TS/NDL metal halide lamps is available online at [www.osram.com.au/media/resource/hires/335357/powerstar-hqi-ts-excellence-70-w-and-150-w-the-latest-innovation-in-quartz-tec.pdf](http://www.osram.com.au/media/resource/hires/335357/powerstar-hqi-ts-excellence-70-w-and-150-w-the-latest-innovation-in-quartz-tec.pdf). We have included this information in the manuscript.

P6 Line 17, Why ammonium was not measured? It is actually an important nutrient for phytoplankton.

Response: We agree that ammonium is an important nutrient for phytoplankton growth and we did measure the concentration of this nutrient in our tanks. However, we omitted showing this data because it rapidly fell below detection limits (by day 12) and showed no CO<sub>2</sub> treatment-related differences. We have updated the manuscript to include ammonium and our justification for omitting it from analysis in our results.

P9 Line 5, Are AZ and EZ directly dissolved in milliQ water? I remember these two reagents are quite difficult to dissolve in pure water. Here is just a reminder.

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Response: It is true that many studies use DMSO or Acetone to dissolve EZ and AZ, but there are also some papers that have used MilliQ water, (e.g. Young et al., 2001). We acknowledge the reviewer's comment, however, we encountered no problems with the solubility of these reagents in MilliQ water.

P10 Line 7, I understand that it is impossible to run 6 CO<sub>2</sub> with replicates, however, I think the author should do more job on statistics instead of simple comparison with ANOVA. They could try to do some curve fitting, e.g. exponential rising for POC, PON, Chla, decay of nutrients etc, to extract some valuable numbers for comparison.

Response: The statistical analysis did include curve fitting using quadratic regression models. The ANOVA analysis was performed to test for significant differences among these curves. We recognise that this may not have been clear in the manuscript and have updated our results and the table captions in the Supplementary file.

Results: This section is well written Discussion: This section is somewhat redundant, the author talked too much about CCM. CCMs are quite complicated and involved by many proteins, enzymes, and ion channels. The present data is obtained only using two CA inhibitors, so to what extent these data can reflect the activity of CCM? Moreover, you only measured chlorophyll fluorescence, which is direct measurement of light reaction, however, CA only participates in CO<sub>2</sub> acquisition for dark reaction, so the measured parameters further limit the interpretation of data for CCM. I suggest the author to compress CCM related paragraph.

Response: We are grateful for the reviewer's comment and have condensed the CCM discussion. We have also included a sentence to highlight the limitation to our interpretation, having only measured light reactions.

P14 Line 22 "photosynthetic . . . process", this is a very short sentence, please rephrased.

Response: We have reviewed this sentence and rephrased it as the reviewer has re-

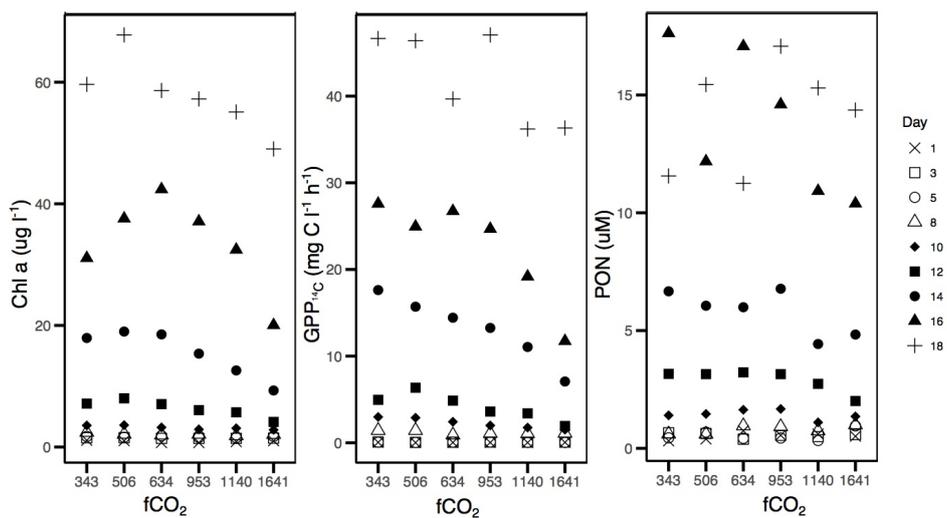
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quested.

References: Young E, Beardall J, Giordano M (2001) Inorganic carbon acquisition by *Dunaliella tertiolecta* (Chlorophyta) involves external carbonic anhydrase and direct  $\text{HCO}_3^-$  utilization insensitive to the anion exchange inhibitor DIDS. *European Journal of Phycology* 36 (1):81-88

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**Fig. 1.**  $\text{CO}_2$  threshold analysis for chlorophyll a accumulation,  $^{14}\text{C}$ -gross primary productivity rate, and accumulation of particulate organic nitrogen

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