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Interactive comment on "Acidification counteracts negative effects of warming on diatom silicification" *by* Alexandra Coello-Camba and Susana Agustí

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General comments: This manuscript presents data on the effects of temperature and CO2 on cell size, valve thickness, sinking rate and silica incorporation rate of in situ diatom communities. It's interesting to see that increased CO2 mitigates the negative effects of warming on silicification. However, I found several serious problems in the study: 1) my main concern is the replicates in the experiment, no detailed information can be found in the manuscript. From figure 1, there is only one data point for one temperature treatment. 2) The carbonate system parameters are missing to further constrain carbonate chemistry. 3) I think it's not proper to classify species according to cell size. Cell size can vary a lot even for the same species. The dominate species in-

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formation should be provided. 4) Why the silica incorporation rate normalized to volume rather than biomass? If the biomass in different treatments were distinct, the rates can say nothing. - Authors' response: 1) More information on the number of replicates has been added (lines 37-38, page 2): " For the 2009 experiments we used two replicate bottles for each treatment, and three replicates for treatment in the 2010 experiment.". 2) More information on the carbonate parameters can be found on Coello-Camba et al. (2014) in the revised manuscript we added the following paragraph (lines 10-11, page 3: "Total hydrogen ion concentrations (i.e., pH) and total alkalinity (TA) were measured daily along the experiment (see Coello-Camba et al. 2014)"). 3) According to the reviewer's comment, we identified the diatom groups observed here to genus level (lines 22-26, page 6): " Valve measurements were performed on the centric diatoms most abundantly observed in our samples after a process of cell cleaning (Fig. 1A); these were identified to genus level as Coscinodiscus sp. (21.4 0.38 μm initial cell diameter) from the 2009 open sea community experiment, and Thalassiosira sp. population 1 (7.4 0.04 μm initial cell diameter) from the 2009 fjord community experiment), and population 2 (6.6 0.04 μm initial cell diameter) from the 2010 experiment". 4) The method followed here (Leblanc and Hutchins (2005) and Shimizu et al. (2011)) allows the calculation of the incorporation rates of biogenic silica in μmol BSi L-1 d-1units, as it is referred to the concentration of PDMPO incorporated in a specific volume of sample (250 mL) during one day of incubation. To determine the silica incorporation rate we followed the standard procedure described in Leblanc and Hutchins (2005) and Shimizu et al. (2011). This measurement is an incorporation rate, a time-related parameter. We estimated the silica incorporation rates per unit of diatom biomass in the revised manuscript (values shown in lines 5-6 page 8), and observed that this ratio did not show any significant relationship with increased temperature or pCO2. Silicification is performed by active cells, although the measurement of biomass is not related to the state of the cells and includes no actively growing cells and a component of detritical biomass. Probably, the presence of non active cell biomass influenced the incorporation rate vs. biomass ratio and prevented us for finding clear responses of the ratio

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with increased temperature or pCO2. The incorporation rates showed here reflecting the silicification process help us to identify the overall silicification responses of the communities and thus the consequences for the biogeochemical cycles.

Specific comments: - Page 1 line 1: I don't think this title is appropriate for this paper. In two of three experiments, the authors only focus on the effects of temperature. Moreover, the authors discuss a lot on effects of temperature, rather than interactions of OA and temperature. - Authors' response: We have modified the title to include the negative effect of increased temperature, highlighting the main finding during our study: "Acidification counteracts negative effects of increased temperature on diatom silicification"

-Page 2 line 20: "stressors"? Increased CO2 mitigates the negative effects of increased temperature. So can you call CO2 "stressor"? - Authors' response: As the term stressor results confusing in this sentence, we have changed it for the word "factors" in the reviewed version of the manuscript (lines 21-23, page 2).

-Page 2 line 26: The information of dominate species in these communities should be added. - Authors' response: As suggested by the reviewer, the main groups studied here have been identified to a genus level (lines 22-26, page 6: " Valve measurements were performed on the centric diatoms most abundantly observed in our samples after a process of cell cleaning (Fig. 1A); these were identified to genus level as Coscinodiscus sp. (21.4 0.38 μm initial cell diameter) from the 2009 open sea community experiment, and Thalassiosira sp. population 1 (7.4 0.04 μm initial cell diameter) from the 2009 fjord community experiment), and population 2 (6.6 0.04 μm initial cell diameter) from the 2010 experiment".

-Page 2 line 31: Two pCO2 treatments? In Figure 2, you showed three pCO2 levels. Moreover, the pCO2 values are self-contradictory in method and results parts. - Authors' response: In agreement with the reviewer's observation we realize that this information was not well described. We referred to the planned treatments, although

we should instead refer to the actual treatments. The methods were well described in Coello-Camba et al. (2014). According to this, we improved the description in the Methods section (lines 33-34, page 2): "Seven temperature treatments were set for the 2009 experiments and three temperature treatments in 2010; in this last experiment temperatures were combined with three pCO2 treatments (Table 1)", and (lines 13-14, page 6): "The CO2 values actually measured along the experiment resulted in 217.7 (37), 780.8 (46) and 1652(72) ppm respectively".

-Page 2 line 35: How many replicates in the experiment? In fig. 1, only one data point for per treatment. Does this mean that there is only one bottle for per treatment? - Authors' response: More information on the number of replicates has been added (lines 37-38, page 2): " For the 2009 experiments we used two replicate bottles for each treatment, and three replicates for treatment in the 2010 experiment.". Also, Figure 1 has been completed by adding the error bars to the plots.

-Page 3 line 6: Were the bottles aerated throughout the experiment or stopped when target pH was achieved? - Authors' response: The bottles were constantly aerated throughout all the incubation time. This information has been added to lines 3-7, page 3:" Throughout the 2010 experiment the target pCO2 level was achieved by fitting each experimental 20 L bottle with a bubbling system connected to CO2 bottles and air mixture bottles. The gas mixture was continuously provided by mass flow controllers (model GFC17, Aalborg Instruments and Controls, Inc.), setting a flow rate of 0-10 L min-1 for air mixture and 0-10 mL min-1 for CO2".

-Page 3 line 8: The light tubes on the top or side of bottles? Did the author measure light in bottles? - Authors' response: The light tubes were located at the top of the incubation chambers. We had previously measured the light transmitted through polycarbonate bottles, and observed that their walls filter UVB radiation and reduce a 10% of PAR.

Page 3 line 9: The carbonate system parameters are missing to further constrain car-

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bonate chemistry. - Authors' response: We added more information on the carbonate parameters in the revised manuscript (lines 10-11, page 3: "Total hydrogen ion concentrations (i.e., pH) and total alkalinity (TA) were measured daily along the experiment (see Coello-Camba et al. 2014)").

Page 3 line 12: The information of filtration pressure should be added. - Authors' response: For all our filtrations we used low vacuum pressures. We specified in the revised manuscript that we used gentle filtration to process our samples avoiding cell damage (line 20, page 3 and line 18, page 4).

Page 3 line 14-21: It's better to add some references for this method. - Authors' response: As suggested by the reviewer, this information has been added to the revised manuscript (lines 22-25, page 3).

Page 3 line 27: How many samples measured for one treatment? Again, how many replicates for per treatment? - Authors' response: Each treatment has been sampled for cell measurements at the end of incubation, two replicates per treatment (lines 13-14, page 4: " Initial, intermediate and final samples (2 replicates of each) were taken to determine the rate at which newly synthesized silica was being incorporated into the valves of diatoms".

Page 3 line 38: When did the author measure the rate of incorporation of silica? At the end of experiments? Samples were incubated under light or darkness? - Authors' response: As indicated above, in lines 13-14, page 4 we indicate the timing of the samplings for the measurement of silica incorporation rates. The bottles for measuring this parameter were incubated under the same conditions than the correspondent treatment (lines 16-17, page 4): " 250 mL of sample were incubated with PDMPO (to a final concentration of 0.125 μM) for 24 h under the corresponding light, temperature and pCO2 conditions for each treatment".

Page 4 line 1: The information of filtration pressure should be added. - Authors' response: See above.

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Page 4 line 22: From my perspective, this model is useless for the discussion. You can analyze the interaction of these two factors from fig. 3A and C. - Authors' response: By using this model we can define the presence or absence of interaction by comparison between observed (3C) and predicted effects (3B). We have added in the revised manuscript a better explanation of the usefulness of the model applied (lines 1-6, page 5). "In order to determine mathematically the existence of synergy or antagonism in the effects of increased temperature and pCO2 in silica incorporation rates of diatoms, we used the independent action (IA) model described by Payne et al. (2001). This model has been recommended for the prediction of the joint effects of dissimilarly acting factors (stressors that influence independently the regulation of a life-history trait by different mechanisms). It assumes additivity and denotes non-additivity by deviations of the measured from the predicted (reference) responses (Coors and De Meester, 2008)". The use of simple and often intuitively applied effect summation is only appropriate under the condition of a linear relationship between the intensity of the single stress factors and their effects (Coors and De Meester, 2008). Thus, effect summation could not be applied here as increased temperature and pCO2 typically show non-linear dose-response curves.

Page 5 line 9: Median values of density of the cytoplasm and cell wall density were used for calculation the sinking rate. However, I think these parameters may be species-specific and influenced by treatment, such as temperature. - Authors' response: We improved the description in the revised manuscript. The lack of cytoplasm density measurements in the literature compels us to use a constant value for living cells (Miklasz and Denny (2010). We used the same assumption in the formula to estimate the potential sinking rates of living diatoms. In the revised version of the manuscript, in lines 36-37, page 5, we added the following information: " We have assumed here a constant value for cytoplasm and valve densities in living diatoms as there are very few literature data on this topic (Miklasz and Denny, 2010)" and it is not clear yet if the cytoplasm density could be species-specific (Miklasz and Denny, 2010). We used the equation described in Miklasz and Denny (2010) to estimate the changes

in the potential sinking speed of a diatom due to variations in valve size and thickness.

Page 5 line 23: More detailed data analysis information should be provided. - Authors' response: This information has been added to line 8, page 6: "Student's t-tests were run to perform statistical analysis of data using JMP software"

Page 5 line 29: These values were mean of each pCO2 treatment? Please add the standard deviation. In the method, you said there were two pCO2 levels. - Authors' response: Yes; the methods section has been improved, and the suggested information has been added to the manuscript in lines 13-14, page 6: " The average CO2 values actually measured along the experiment resulted in 217.7 (37), 780.8 (46) and 1652 (72) ppm respectively".

Page 6 line 2: Can you tell whether the test cells belonged to one species or one genus according to their valves? - Authors' response: We could identify the diatom groups observed here to genus level. We added to the revised manuscript the identification to genus level of the diatom groups studied here (lines 23-26, page 6), as Coscinodiscus sp. (21.4 0.38 μm initial cell diameter) from the 2009 open sea community experiment, and Thalassiosira sp. population 1 (7.4 0.04 μm initial cell diameter) from the 2009 fjord community experiment), and population 2 (6.6 0.04 μm initial cell diameter) from the 2010 experiment.

Page 6 line 3-5: I think it's not proper to classify species according to cell size. Cell size can vary a lot even for the same species. The dominate species information should be provided. - Authors' response: See above.

Page 7 line18-21: These sentences are repetition of the method section. - Authors' response: As the reviewer stated here, the referred sentence was repeated from the Methods section and has been removed in the revised version of the manuscript.

Page 9 line 8: Cautions should be taken to draw this conclusion: you only test the interaction of pCO2 and temperature for the third experiment. What will happen for

the second one? The in situ temperature for the second experiment is 6.2 C. Will the increased pCO2 counteracts negative effects of warming when temperature increases by 4C or more for diatoms in these waters? Base on the third experiment (at 10.3, increased pCO2 acted synergistically with temperature), the answer may be " no". The author should add some discussion about this. - Authors' response: We have modified the referred paragraph in the new version of the manuscript (lines 2-10, page 10) "Our results demonstrate that the effects of increased temperature and pCO2 on the silicification process in the diatoms studied here are interactive rather than additive, showing a temperature dependent capacity of increased pCO2 to buffer the negative effects of warming. Therefore, as long as the increase in temperature does not surpass the buffering capacity of pCO2 (expected threshold above 6C (Holding et al., 2015) the increase of this latter factor will help diatoms to retain their sinking properties, preserving their role in the biogeochemical cycles of key elements, such as silica and carbon". Our results demonstrate that the effects of increased temperature and pCO2 on the silicification process in diatoms are interactive rather than additive, showing a temperature dependent capacity of increased pCO2 to buffer the negative effects of warming. We observed that at about 6C the effect of increased pCO2 is interactive, with synergy counteracting the effect of temperature. But further increases in temperature would be too strong to be balanced by pCO2, and their interaction would then be synergistic, leading to stronger decreases in the silica incorporation rates than those predicted by simple additivity.

Page 9 line 12: I suggest to change "stressor" to "factor". - Authors' response: As suggested by the reviewer, in this sentence in the reviewed version of the manuscript we changed the word "stressor" to "factor" (line 8, page 10).

Page 16, table 3: Can the microscopic method test the minimal variation of valve thickness (7 nm for temperature increasing 10C)? - Authors' response: Although the theoretical maximum resolving power in optical microscopy is 0.2 μm at 1000x magnification, this parameter is quite dependent on the detection mode used. Digital imaging

systems allow image enhancement and perform considerably better contrast than the nonlinear human eye, so the standard resolution criteria do not apply when these image analysis softwares are used (Hajjar et al., 1999). This way, the method we followed here used 1600x magnification allowing a maximum resolving power of 0.125 μm, plus image analysis system, had an adequate resolution (0.05 μm approx.) to perform the measurements of valve thicknesses. Besides this, as we indicated in the text, we did not observe significant variations in the valve thickness measurements with temperature or pCO2 (lines 34-35, page 6). We added the methodological information in the revised manuscript lines 36-39, page 3.

Page 17 figure 1: Why only one data point for one temperature treatment? How many replicates in the experiment? - Authors' response: The referred figure has been modified by adding the error bars to the points; as indicated above, (lines 37-38, page 2): " For the 2009 experiments we used two replicate bottles for each treatment, and three replicates for treatment in the 2010 experiment.".

Page 17 figure 2: For panel A, what's the pCO2 treatment for every temperature column? Mean value of three pCO2 treatments. Same for panel B, what's the temperature treatment for every pCO2 column? Why the rate normalized to volume rather than biomass? If the biomass in different treatments were distinct, the rates can say nothing. - Authors' response: In figure 2A all pCO2 treatments have been considered when analyzing the effect of temperature, and in figure 2B all temperature treatments have also been considered when analyzing the effect of pCO2. - The method followed here (Leblanc and Hutchins (2005) and Shimizu et al. (2011)) allows the calculation of the incorporation rates of biogenic silica in μmol BSi L-1 d-1units, as it is referred to the concentration of PDMPO incorporated in a specific volume of sample (250 mL) during one day of incubation. - To determine the silica incorporation rate we followed the standard procedure described in Leblanc and Hutchins (2005) and Shimizu et al. (2011). This measurement is an incorporation rate, a time-related parameter. We estimated the silica incorporation rates per unit of diatom biomass in the revised manuscript (values

shown in lines 5-6, page 8), and observed that this ratio did not show any significant relationship with increased temperature or pCO2. Silicification is performed by active cells, although the measurement of biomass is not related to the state of the cells and includes no actively growing cells and a component of detritical biomass. Probably, the presence of non active cell biomass influenced the incorporation rate vs. biomass ratio and prevented us for finding clear responses of the ratio with increased temperature or pCO2. The incorporation rates showed here reflecting the silicification process help us to identify the overall silicification responses of the communities and thus the consequences for the biogeochemical cycles.

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Figure 1

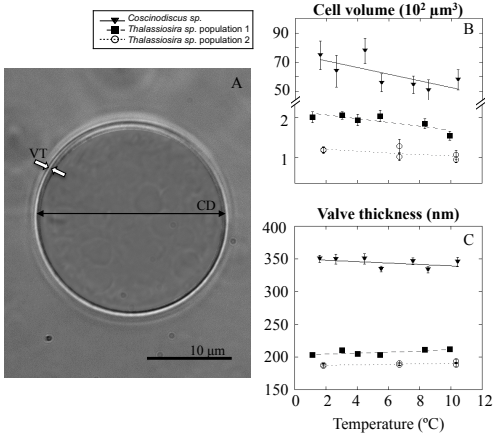

Fig. 1. New Figure 1

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