Response to Anonymous Referee #1

The authors wish to acknowledge thank Referee #1 for the detailed suggestions. We have taken all of these comments into consideration and are confident that the improvements made will improve the manuscript and make it suitable for publication in Biogeosciences.

Our responses follow the referee's comments below in bold text.

GENERAL COMMENT.
Meng et al. propose here to further our knowledge of the negative effects of Ocean Acidification on marine calcifiers, i.e. reduced calcification, by characterizing various properties of shells of oyster Crassostrea angulata. This study describes the effects of experimentally induced OA on the shell surface, structure, crystallographic composition, crystallographic orientation, mechanical strength and density of C. angulata exposed to four different pH treatments (including the control treatment). This multimodal characterization and imaging approach adds to the scientific understanding of the effects of OA of the shell structure of a commercially important species of oyster.

The science presented here is sound, as are the statistical analyses associated to the findings. The main issues here reside with the redaction of the manuscript itself, the wording and terminology. Parts of the Methods, Results and Discussion sections are confused, and I do have a couple of questions regarding the methods (e.g. control treatments, and testing under hydrated conditions) that could expand the discussion further.

This study warrants publications but the text needs to be reworked to avoid confusion and some references need to be added. It took some time to carefully annotate the pdf file to help with wording and English but the authors should be more careful in future. The confused English distracts from the data and information presented. I hope this helps.

REPLY: We thank Referee #1 for the appreciation of our work and the carefully annotated pdf file. The manuscript has been carefully checked accordingly for any imprecise wording, terminology and references, and corrected throughout.

SPECIFIC COMMENTS
1. Title: consider changing title to "Ocean acidification affects mechanical and structural properties of Portuguese oyster shells (Crassostrea angulata)".

REPLY: Referee #1 suggests changing the title and so does Referee #2 although their suggested revised titles are different. We consider that it is more informative to state the way in which mechanical properties are altered so we have adopted the more specific suggestion of Referee #2 and propose the following title "Ocean acidification reduces hardness and

stiffness of the Portuguese oyster shell with impaired microstructure: a hierarchical analysis".

2.Wording and vocabulary:
- "corrode/corrosion": this relates to metal not carbonates, "dissolution" is more adapted to carbonate calcification;

REPLY: We have replaced the word "corrode/corrosion" with "dissolve/dissolution".

- "loose": my understanding is that this work is used for structural studies in engineering, not in crystallography. If the authors insist on using this word, I think it should be defined clearly in the ms;

REPLY: We have replaced "loosened" by "porous" referring to the suggestion in the PDF file.

- "microstructure": this word refers to the structure of the crystals themselves, not the structure of the shell. So if you are talking about crystal orientation or shell porosity you are talking about structure not microstructure. Please review the ms and change the terminology accordingly;

REPLY: We appreciate this suggestion for the replacement of "microstructure". However, "microstructure" is a term commonly used in biomineralization for oyster shell structural characterization when referring to the shell structure at micrometer scale, for example MacDonald *et al*., (2010). We have added this reference accordingly for clarification.

The reference:
MacDonald, J., Freer, A., and Cusack, M.: Alignment of crystallographic *c*-axis throughout the four distinct microstructural layers of the oyster *Crassostrea gigas*, Cryst. Growth Des., 10, 1243-1246, 10.1021/cg901263p, 2010.

- "down-sifting": can't you just say decrease (?) why make it complicated;

REPLY: We have replaced the word "down-sifting" with "decrease".

- "bottom-up": this is more of an ecological (i.e. food chain interactions) or physical oceanography (i.e. seawater mixing) term. I would just delete this term from the ms totally

REPLY: We have deleted the word "bottom-up".

- "erode/erosion": this is a geological term, use "dissolution" instead.

REPLY: Noted, we have replaced the word "erode/erosion" throughout with "dissolution".

3. Methods and Discussion: I was wondering whether the authors considered the fact that certain carbonate materials produced by marine calcifiers have increased strength when hydrated. For example, pearl oysters are very solid underwater but very brittle once dried. Using ethanol to preserve the samples is the easiest way but could it have affected the shell strength by extreme dehydration?

REPLY: The authors agree that this is a very interesting point. However, our work focuses on the comparing the effect of ocean acidification on the biomineralized structures. Since all samples from control and treatments were preserved and examined with identical methods, this ensures that the results and conclusions are appropriate for this objective. Therefore, the hydration of the shell in this study would not be considered as one of the compounding factors of this comparative study. We do not consider it necessary to discuss the use of ethanol as a preservation method here because it is commonly used in studies that investigate the effect of ocean acidification on biomineralized shells, for example Chan et al., (2012). We have added this reference accordingly to justify our choice of preservation method.

The reference:
Chan, V. B., Li, C., Lane, A. C., Wang, Y., Lu, X., Shih, K., Zhang, T., and Thiyagarajan, V.: CO_2-driven ocean acidification alters and weakens integrity of the calcareous tubes produced by the serpulid tubeworm, *Hydroides elegans*, PloS ONE, 7, e42718, 10.1371/journal.pone.0042718, 2012.

Is micro-CT and nano-indentation doable in a medium that would preserve the shell (i.e. neutral)?

REPLY: Both micro-CT and nanoindentation measurements were carried out in ambient air conditions so we can reassure the referee that the analytical conditions are neutral. This methodology was consistently used in the control and treated samples and allowed us to make conclusions about the impacts of ocean acidification.

Please discuss Technical corrections See pdf document

Please also note the supplement to this comment:
https://www.biogeosciences-discuss.net/bg-2018-204/bg-2018-204-RC1- supplement.pdf

REPLY: All suggestions on phrasing for clarity and corrections of typos in PDF documents have been carefully considerated and revised.

In particular:

Line 47 technically speaking, seawater is not getting more acidic but less basic (i.e. less alkaline). The pH scale is such the seawater pH predicted for the near future are not acidic because acidic pHs are found below ~6. "Ocean acidification" is a generally accepted "layman's term" to describe the seawater getting less basic but you can't really say the seawater is getting acidic (yet). I suggest you reword this sentence.

REPLY: We have reworded the sentence here "…Oceans currently absorb about a third of anthropogenic CO_2, which dissolves in seawater forming carbonic acid and increases the concentration of hydrogen ion, this chemical process is popularly known as ocean acidification (OA)."

Line 48: Define the term "pCO_2"

REPLY: We have defined "pCO_2". "… is highly vulnerable to high carbon dioxide partial pressure (pCO_2; µatm) …."

Line 84 you need to specify which one is considered the control.

REPLY: We have specified the control in the method section "Four environmentally and climatically relevant pH levels (the control: pH 8.1; the low pH treatments: pH 7.8, 7.5, and 7.2) were selected as proxies to investigate the effect of CO_2-driven OA on oyster shells." In addition, we have added a schematic of the experimental system in the method for clarity.

Line 120 consider replacing with "lip"

REPLY: We have replaced "bill" by "edge", a term defined by Galtsoff (1964) for the description of edible oyster shells. We have added the reference in the revised version.

The reference:
Galtsoff, P. S.: The American oyster, *Crassostrea virginica* Gmelin, Fish. Bull., 64, 1-480, 1964.

Line 121 Is this due to dissolution? Other reasons??

REPLY: The referee asked why the edge of shell is fragile. It is because the edge region is newly formed and naturally thin which can refer to the scanning electron micrograph (SEM) of the full shell cross-sectional surface in Fig. 2a. We have also reworded the sentence to improve the clarity.

Line 127-128 Please describe you standardization and the thresholds used for the images. Depending on exposure, thresholds can be very variable...

REPLY: The porosity thresholding was calculated using the non-diffracted regions of SEM images produced by backscattered electrons. Therefore, a change in contrast, or focus position of the SEM would not affect the ability to distinguish between brightly well diffracted calcite and the porous space in between. In addition, all specimens were examined with same settings of SEM, so we considered the results would be comparative in this study. To improve the clarity, we revised the sentence in the method Line 127 "…The cross-sectional porosity of foliated laminated structure was calculated using ImageJ software by standardizing and converting an SEM image to thresholding where the non-diffracted regions of SEM images were defined as pores."

Line 163 Which ones? If they are all in resine, which shells did you use?

REPLY: The referee asked about the specimens used in Micro-CT scanning. Those were the complete individuals directly scanned by Micro-CT without being embedded in resin. They were randomly selected from the treatment (n = 3) which we have raised in Line 167.

Line 178 It would be great to have a 3D video/file of the micro-CT density results for one shell of each pH level as supplement material

REPLY: The authors thanks the suggestion on the micro-CT density results. We presented the 3D shell density maps in Figure 5a-d which showed the overall decrease of shell density with decreasing pH. We have considered this figure sufficient to support the points we concluded.

Line 194 What does this means in terms of orientation? Be clear. Why should we care about colour variations? Use the correct terminology.

REPLY: The "color variation" represented the changes of crystallographic orientation corresponding to the color key showed in Fig 4, which is a term commonly used to describe the results obtained by EBSD when referring to the crystallographic orientation map or the pole figures, for example Fitzer et al., (2014). To improve the clarity, we have added the reference and sentences into line 194 "…The crystallographic orientation maps (Fig. 3.i) showed changes in crystallographic orientation from the control (pH 8.1) to low pH conditions (pH 7.8, 7.5 and 7.2) as represented by color change corresponding to the color key. The spread of data points in pole figures (Fig. 3.ii) highlighted the variation in crystallographic orientation between the juvenile oysters under the low pH and the control conditions. …"

The reference:
Fitzer, S. C., Cusack, M., Phoenix, V. R., and Kamenos, N. A.: Ocean acidification reduces the crystallographic control in juvenile mussel shells, J. Struct. Biol., 188, 39-45, 10.1016/j.jsb.2014.08.007, 2014.

Line 207 Do you not have more to say here? This section seems very short. The methods section talk about hardness (H) and stiffness (S), could you give some values for these variables maybe?

REPLY: We apologize for the inconsistency of using the terminology - "Young's modulus" which is the measurement of the stiffness. We have revised the result section accordingly.

Line 212 – 221
This entire section needs work. It is very unclear. You need explain better how you got the density/volume in order for us to understand what you are measuring: shell density/volume or mineral density volume?? I'm really confused right now.

REPLY: We measured "Volume ratio (%)" of the corresponding partial density range and utilized linear regressions to determine the relationships between "Volume ratio (%)" and "density (g/cm^3)" which we have raised in the method Line 172. For clarity, we have revised sentences in line 212. ".. A similar decrease is visible in the linear regressions (Volume ratio (%) = b × density (g/cm^3) +a) in Fig. 5. f…"

Line 244 Consider changing to: "Oyster shells mechanical properties under OA"

REPLY: In order to be consistent and informative with the later subheading in discussion, we have kept the subheading "4.1 Effect of ocean acidification on shell mechanical features: a hierarchical analysis".

Line 286-289 Unclear. What are you trying to say?

REPLY: We were discussing the potential explanation of the porous foliated layer based on the calcification mechanism of mollusk. This section has been revised and added new reference. "…Marine invertebrate's calcification has highly controlled mechanisms and remained to be explored by further studies. Animals are capable in actively increasing the site of calcification by pumping proton out of the calcification site, thereby enabling calcium carbonate precipitation (Toyofuku et al., 2017). Supersaturated calcite conditions of oysters were found restricted to the shell edge including the outer mantle and the first intracellular nucleation site (Mount et al., 2004). Undersaturated calcite conditions may be maintained elsewhere in contact with the inner shell surface (Addadi et al., 2006; Thomsen et al., 2010). Therefore, in low pH conditions due to OA, these inner areas of newly formed minerals, which are precipitated as structural building blocks for the prismatic and foliated layers, may still be prone to dissolution. When the shell dissolution rate is faster than the mineralization rate, organisms tend to produce thinner and lighter (less dense) shells resulting in impaired shell microstructure. This may explain the multiple negative effects of reduced pH in our results, including porous and less dense foliated layers…."

The new reference:
Toyofuku, T., Matsuo, M. Y., de Nooijer, L. J., Nagai, Y., Kawada, S., Fujita, K., Reichart, G.-J., Nomaki, H., Tsuchiya, M., Sakaguchi, H., and Kitazato, H.: Proton pumping accompanies calcification in foraminifera, Nat. Commun., 8, 14145, 10.1038/ncomms14145, 2017.

Line 320 Why are you not talking about the commercial implications like aquaculture and so on?

REPLY: The authors agreed with the referee. We have added the commercial implications in the revised discussion. "….This biological effect of OA on shell structures and mechanical features should be incorporated to the coastal oceanographic biophysical models to accurately project the survival of oysters in near-future coastal oceans which is vital for

commercial shellfisheries to plan for sustainable growth under climate change induced acidification."