Revision notes on the revised manuscript "Unraveling the physiological responses of morphologically distinct corals to low oxygen" (#2024:01:95392:0:1:REVIEW) by Long et al., resubmitted for publication in PeerJ. We sincerely thank the editor and reviewers for their thorough reading of the manuscript and their helpful remarks that can help us to improve the manuscript. We have carefully considered and addressed all the suggestions. Both annotated (with track changes) and clean versions are provided along with this submission. Herein, we explain how and where each point of suggestion has been addressed in this manuscript (in blue) and the "Line" referred to in this response is the line number appears in manuscript without track change.

Reviewer 1:

Line 114 – Was the HOBO data logger placed near any photosynthetic organisms (seagrass meadows, coral reef etc.) or where there any algal blooms observed near the HOBO location during the duration of the experiment? It could be useful to include this information as these factors could influence the recorded DO.

Response: A HOBO logger was deployed in a reef surrounded by coral colonies, without seagrass or macroalgae nearby (Lines 115-117). Although some local observations suggested algal bloom events, we opted not to include them in this study due to the lack of precise data. Nevertheless, we are dedicated to exploring dissolved oxygen levels in the Kham Island area further to understand the origins and impacts of low oxygen conditions in the reef ecosystem.

Line 147 – The authors state the O2 concentration in the stock seawater was manipulated via N2 gas. Was there any CO2 added to the treated seawater atier this step? Passing N2 gas through the water column to remove O2 will also have the effect of removing CO2, and thereby reducing the amount of available dissolved inorganic C (HCO3-) which could have a negative impact on coral productivity (Roberty et al 2020) and subsequent calcification rate.

Response: Thank you for highlighting this aspect, which we thoroughly addressed in our experimental procedures. Initially, we sought to introduce CO_2 gas into our preliminary experiment but encountered challenges in pH control. Nevertheless, we closely monitored pH and total alkalinity throughout, ensuring a pH difference within approximately 0.1 across treatments. For further details, please consult the provided raw data.

Line 149 – Was there any stirring/agitation of the water atier the chambers were sealed with parafilm? The diffusive boundary layer surrounding marine organisms can affect photosynthesis/photorespiration by inhibiting O2 diffusion away from the surface (Larkum, Koch & Kuhl 2003).

Response: Due to equipment constraints, continuous water flow in the chamber system wasn't feasible. However, to counteract stagnant conditions, we manually stirred the water every three hours. Additionally, we compared physiological attributes (maximum quantum yield, zooxanthellae density, and chlorophyll concentration) between the initial and final stages of the ambient treatment (controls) and no

Commented [CN1]: Please make sure this information is phrased better and added to the main text and specify the line numbers where added

Commented [CN2]: Please make sure this information is phrased better and added to the main text and specify the line numbers where added

significant differences were found. This suggests that the lack of constant stirring did not have negative effects. This enables confident utilization of this data in our analysis. We acknowledge the study's limitations in the discussion section (Lines 510-515).

Commented [CN3]: Please make sure this information is phrased better and added to the main text and specify the line numbers where added

Result: Line 307 – It may be helpful to state that the respiration rate is decreasing in hypoxia and anoxia conditions relative to the control as, at a glance, the figure seems to show an increasing rate in respiration (in P. acuta, av respiration rate of -1.5 in control vs ~-0.1 and ~0.1 in hypoxia and anoxia conditions respectively)
Response: We have updated this information in Lines 317-321.

Discussion: The authors found some evidence of increased photosynthesis under hypoxia and anoxia conditions which could be explained by reduced rates of photorespiration in hypoxia and anoxia conditions relative to the control. This would be contrary to the findings of Osinga et al (2017), whereby net photosynthesis in Galaxea fascicularis was not affected by either hyperoxia or hypoxia. Further, some corals (Porites spp and Pocillopora damicornis) can concentrate dissolved inorganic carbon at the site of calcification (Allison et al 2014). As such, a brief discussion on the impact photorespiration may or may not have on the corals in this study could be an important addition.
Response: It has been added to Lines 438-440.

Reviewer 2:
In line 97, it is necessary to spell out the abbreviated species names for clarity. Additionally, a citation is needed to support the assertion that "physiological oxygen requirements for many organisms increase." This citation would provide scientific backing to the claim, enhancing the credibility of the statement and ensuring that the information is properly sourced.
Response: The abbreviated species name has been updated in Lines 98, and the citation has been updated in Line 65.

To enhance the justification for selecting the three species, it would be beneficial to provide additional details on the species utilized, their prevalence and population density at the specific site, as well as the rationale behind their selection. Incorporating this information into the methodology section would bolster the rationale behind the choice of these species.
Response: The species chosen for this study were selected based on the 2019 survey report by the Department of Marine and Coastal Resources, Thailand (DMCR) (Lines 110-112), which identified them as dominant species in the Kham Island area. However, the report did not include information on population density. Additionally, we have updated the information provided in Line 129 to clarify that the three experimental species represent different coral morphologies.

Commented [CN4]: Please make sure this information is phrased better and added to the main text and specify the line numbers where added

A statement that the corals recovered from the fragmenting would be helpful to determine that a week of acclimation was long enough to see the tissue regeneration at the site of fracture.
Response: We conducted daily measurements of MQY to assess coral recovery and health following fragmenting. Additionally, we observed tissue regeneration in the incised coral fragments, confirming their successful recovery.

Commented [CN5]: Please make sure this information is phrased better and added to the main text and specify the line numbers where added

Include the units for the dissolved oxygen measurements, pH measurements (NSB, total, seawater scale?) and total alkalinity.
Response: The units were updated in Lines 163-165 for all mentioned measurements.

I am happy to see that the authors included the specific parameters used for the PAM. This is extremely helpful with comparisons across studies.

Can you provide evidence of the artificial seawater solution that was used (3.2% NaCl).
Response: We apologize for the mistake regarding the salinity levels in the holding tank. In fact, we ensured that all tanks were set up with a salinity of 32 PSU, as indicated by the field records. Text was corrected in Lines 138 and 188.

Additional details regarding the experimental procedures, particularly concerning the chambers and incubations, are necessary for clarity. For instance, crucial information such as the size and volume of the chambers is absent. It is unclear whether 72 individual chambers were employed for each coral fragment and if mixing occurred within these chambers. Furthermore, the methods for monitoring conditions within each chamber and whether the chambers were fully closed need clarification. While the volume of the chamber is integral to the equation for calculating Net Primary Productivity (NPP), it is not explicitly stated in the methods section. Additionally, the biological mass-to-volume ratio holds significant importance for determining respiration and photosynthetic rates, as highlighted in Svendesen et al. (2016). Lastly, it is unclear whether NPP, respiration (R), and Gross Primary Productivity (GPP) were standardized to coral surface area or mass.
o Svendsen, M. B. S., Bushnell, P. G., & Steffensen, J. F. (2016). Design and setup of intermittent flow respirometry system for aquatic organisms. Journal of fish biology, 88(1), 26-50.
Response: We apologize for any confusion caused to the reviewer. In our study, we initially collected a total of 96 coral nubbins by cutting them from source colonies. After acclimation, we selected 24 nubbins (8 per species) to assess their initial physiological status through Symbiodiniaceae density and chlorophyll concentration analysis. The remaining 72 nubbins (24 fragments per species) were then subjected to treatment conditions, with each treatment or species having eight replicates (n=8). This ensured coverage across eight distinct genetic colonies. Please see figure R1 below for the clarification.

Commented [CN6]: Please additional state number of nubbins per colony

Commented [CN7]: Please additionally state number of nubbins per treatment per colony

Commented [CN8]: Please amend Figure R1 with details on the oxygen concentrations used in the treatments and use to replace the top half of Figure 2, with additional details on the coral species.

To maintain experimental integrity, each nubbin was individually housed within a closed chamber with a volume of 710 cm³. We have updated the description provided in Lines 145–151 accordingly. We acknowledge the reviewers' concerns regarding the limitations posed by the size of the closed system, and we have addressed these limitations in the discussion section (Lines 510-515).

Commented [CN9]: The Volume used in the calculation of NPP should be the volume of water, not the volume of the chamber. The volume of water is estimated by subtracting the volume of the nubbin from the volume of the chamber. The volume of the nubbin can be estimated from surface area. Please fix in your methods.

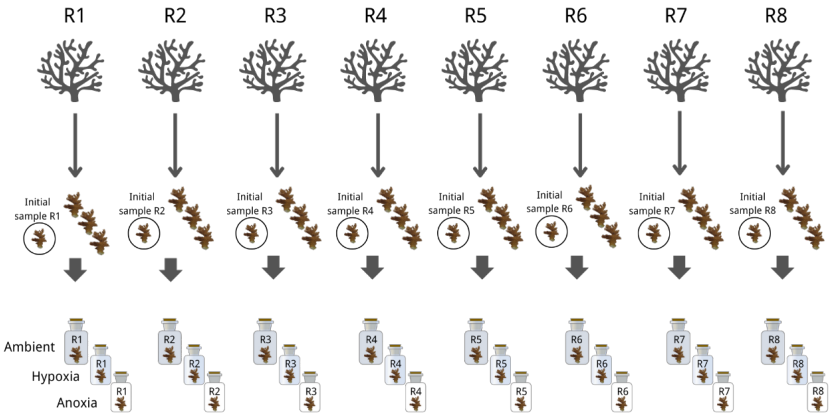

Figure R1. Diagram of colonies separation of species *Pocillopora acut*a (all three species separate the same).

To enhance clarity, it is essential to address several key questions regarding the experimental procedure. Firstly, the duration of coral incubation needs clarification. Additionally, it's crucial to specify the time variable utilized in the calculation of calcification rates. Moreover, information regarding the volume of water used in the incubation process is lacking. If the corals are being incubated for 12 hours in the same volume of water, it raises concerns about potential issues such as the accumulation of waste materials, which could adversely impact the corals. This accumulation may confound the experimental treatment, leading to misleading results. Therefore, adjusting the duration or volume of incubation water may be necessary to mitigate these potential confounding factors and ensure the accuracy of the experimental outcomes.

Response: We acknowledge the reviewer's concerns regarding the chamber size and the duration of incubation, as addressed in Lines 151 and 162 of our manuscript. We fully recognize the limitations associated with these factors and have included a thorough discussion in Lines 510-515 of the manuscript. Additionally, we carefully re-examined the data from all ambient replicates and found no significant effects, reaffirming the validity of the data we have presented.

Commented [CN10]: This is not a good response to the reviewers concerns. Instead add to your results a short paragraph addressing these issues. For example, mention that your ambient corals exhibited values of chlorophyll, MQY, NPP, and R consistent with other studies of corals in chambers by using references with similar values/rates. Additionally, present the statistical test of the stability of the ambient corals through time for all of the repeated measurements as an argument for no negative impact of the chamber on coral health.

Experimental design: Was every genet or genotype of each species adequately represented within each treatment group

Response: Yes, each treatment or species had eight replicates (n=8), ensuring representation across distinct genetic colonies. Further details are provided in Lines 145–151 and illustrated in Figure R1 (above diagram).

Commented [CN11]: Again, per my above comments, please make clear in the methods that every colony (genet) was represented in every treatment and timepoints.

Regarding the oxygen sensor, could the authors provide additional details concerning the specific type of oxygen sensor utilized? Moreover, clarification on the frequency of dissolved oxygen (DO) measurements, along with whether they were conducted in the bulk seawater or in close proximity to the coral surface, would be beneficial.

Response: In our article, we discussed three pieces of equipment used for dissolved oxygen (DO) measurement, all of which were optical oxygen sensors. The HOBO® U26-001 data logger (Onset, USA) was employed to record DO levels in the reef area, where 0.5 meters from the nearest colony. Additionally, the AAQ-RINKO 176 multiprobe (JFE Advantech Co. Ltd., Hyogo, Japan) was utilized to record various parameters, including DO monthly. Finally, DO levels in each chamber were measured using a multiparameter benchtop meter (inoLab® Multi 9630 IDS, Xylem Analytics, Oberbayern, Germany) during each measurement session.

The statistical approach requires further clarification for better understanding. It remains unclear which factors were included in the two-way ANOVA analysis. Additionally, additional information regarding the factor "day" is necessary. If "day" represents the time of exposure and the measurements are taken repeatedly from the same individuals over time, a repeated measures design should be incorporated into the statistical approach to address the non-independent nature of these measurements. Consequently, more explicit elucidation on the statistical methodology is warranted, including details on how potential sources of variability were addressed, such as the repeated measures aspect.

Response: We have revised the content in Lines 238-240 and conducted a repeated ANOVA test to validate our findings (please refer to supplementary data Table S2, S6, S7, S8, S9 for details).

Commented [CN12]: Repeated measures ANOVA is not simply a 2-way ANOVA (ie time X treatment) as you describe in Lines 238-240. You need to incorporate the fact that you made repeated measurements on the same nubbin to track colony-level effects. This is done by adding a random effect of "nubbinID" or just "colonyID" to your models. Typically this is done with lmer mixed models in R as MQY~time*treat + (1|colonyID)

It is difficult to determine the validity of the research findings without more information regarding the experiment setup, specifically the size of the chambers and length of incubation

Response: We apologize for any confusion caused. The updated information regarding the chamber is now included in Lines 150-151 and Lines 159-161.

In situ measurements of dissolved oxygen levels offer valuable background information regarding the typical conditions to which the corals are exposed. However, it remains unclear whether the corals were all collected from the same area where the loggers were deployed. Clarification on this point would enhance our understanding of the environmental context in which the corals were studied.

Response: Thank you for your observation. To clarify, the DO sensor was installed at

the same sites from which the corals were sampled. We have provided additional information in Line 117 to address this.

Figure 2 indicates that chlorophyll and symbiont measurements were taken at 0 hours and 72 hours, while Figure 5 presents data labeled as "initial" and the various treatments. However, it remains ambiguous whether "initial" refers to measurements taken before exposure in each treatment or if it represents ambient conditions. To enhance clarity, it would be beneficial to specify whether "initial" denotes the pre-exposure measurements within each treatment. Additionally, considering the inherent biological variation among fragments, genotypes, and species, it might be advantageous to analyze the percent change before and after treatment. Standardizing each individual to its initial measurement could effectively control for biological variation and provide a clearer understanding of treatment effects.
Response: We apologize for any confusion caused by our previous explanations. To clarify, the term "initial" in our figures refers to measurements taken before the exposure of samples to different treatments. We have updated Figure 2 to accurately reflect the sampling for Symbiodiniaceae and chlorophyll levels prior to treatment exposure. We recognize the importance of analyzing percent changes to effectively account for biological variation among fragments, genotypes, and species. Additionally, we have addressed this by standardizing each individual to its initial measurement, which allows for a clearer understanding of the treatment effects. Further details on the consistency among replicated treatments from the same colony, which show minimal inherent biological variation, are provided in Lines 145–151.

Commented [CN13]: I think that the bottom visual of Figure 2 is awkward and can be condensed and synthesized when the new visual from R1 is added to it so that this is all clearer.

The field-based data clearly indicates that these corals frequently experience hypoxic conditions, suggesting that such conditions may serve as a selective pressure at the field site.
Response: Thank you for pointing this out. Indeed, we observed low oxygen levels in the sampling area, suggesting that such conditions may serve as a selective pressure at the field site, thereby enhancing the ecological relevance of our experiment. It is important to note, however, that our sampling did not occur during episodes of low oxygen. Additionally, we took great care to select healthy colonies to ensure the integrity of our experimental data.

Commented [CN14]: Please make sure this information is phrased better and added to the main text and specify the line numbers where added

In the discussion section, the authors address the variation in responses potentially attributed to morphological factors. However, the study does not explicitly mention morphological differences between the species until this point. Although the examined species exhibit distinct morphologies, other significant differences such as skeletal morphology, symbiont types, and tissue thickness are also likely to contribute to hypoxia sensitivity and merit discussion. This is particularly relevant given that the main finding and argument presented by the authors revolve around early impairment of the photosystems. Therefore, a comprehensive examination of all pertinent morphological and physiological differences among the species would enrich the discussion and provide a more nuanced understanding of the observed responses to

hypoxia.
Response: We have added more discussion about morphologies difference in Lines 470-485.

Reviewer3:
Line 37, Is this statement also referring to anoxia or hypoxia?
Response: Yes, we corrected the description in Lines 37-38.

Line 50, Clarify why you present a range of up to 3.5mg/L, and whether you are referring to hypoxic water conditions or hypoxia intratissue condition.
Response: We mentioned the hypoxic water conditions. It has been corrected to 2 mg L^{-1} (Line 51).

Line 56, For clarity, change '-' to 'and' so readers can follow which RCP results in which %
Response: It has been changed from '-' to 'and' (Line 57).

Line 61, Specify heat source and whether this is referring to ocean warming or atmospheric warming.
Response: This is referring to atmospheric warming, it has been added in Line 62.

Line 84, Provide an alternative reference as this reference does not support this statement.
Response: It has been updated in Lines 84-85.

Line 86, Update this reference to the correct publication date of 2021.
Response: Reference was updated in Line 85 and 87.

Line 87, Provide reference for this sentence.
Response: It has been updated (Lines 87-88).

Line 95, Change to 'previous work'
Response: It has been updated, please see in Line 95.

Line 97, Provide full names when first mentioned.
Response: It has been updated in Line 98.

Line 111, in terms of? Specify how the condition of the reef was determined.
Response: The reef condition mentioned here is based on the survey conducted by the Department of Marine and Coastal Resources. They used the Line Intercept Transect method to observe various aspects of the reef. According to their findings, the survey reported that 66.7% of the coral was dead, while 20.1% had a live coral to dead coral ratio of at least 3:1 (LC:DC≥3:1) (DMCR, 2019).

Line 115, was the logger attached to a coral or? this is important information to specify for interpreting the recorded data.
Response: The logger was set up at a sea base, which is located in a reef area next to coral colonies, more information was indicated in Lines 115–117.

Line 116, reformat to 22nd June, correct throughout paper.
Response: It has been corrected, please see Line118-119.

Line 118, specify how often measurements were taken in main text.
Response: The measurements were done monthly. This information has been added to Line 122.

Line 135, Light levels seem very low, please provide a justification.
Response: Kham Island is quite turbid water, and the light level is referred to in the monthly recorded AAQ. Please see the information in Table S1.

Line 138, Were the corals tested for any health measurements e.g. photosynthetic efficiency prior to experiment?
Response: During the acclimatization period, we closely monitored the tissue regeneration process and assessed the health condition of the Nubbins by measuring the Maximum Quantum Yield (MQY) every day.

Commented [CN15]: Please make sure this information is phrased better and added to the main text and specify the line numbers where added

Line 148, Was pH adjusted or measured after nitrogen bubbling?
Response: Yes, we monitored pH levels to ensure that differences between treatments remained within a narrow range of approximately 0.1. For more detailed information, please refer to the raw data provided.

Commented [CN16]: Please make sure this information is phrased better and added to the main text and specify the line numbers where added

Line 156, what size were the chambers?
Response: The chamber size is 710 cm^3 and it has been updated to Line 151.

Line 158, What was the extent of oxygen draw down? Provide the rate in order to help identify whether the water volume to sample size was appropriate for measurements.
Response: The size of the chamber and its water volume have been detailed in Line 151 for clarification. Furthermore, we addressed the limitation regarding the chamber size and expanded upon this aspect in Lines 510-515 to provide a more comprehensive discussion. These additions aim to enhance the understanding of the experimental setup and the associated limitations.

Line 160, change to 'start and end'.
Response: It has been updated in Line 166.

Line 170, change to 'acclimation'.
Response: It has been updated to Line 177.

Line 173, how were these settings determined, provide reference where possible.
Response: The reference is updated in Line 182.

Line 182, clarify whether the supernatant or pellet was used for the cell counts. How many technical replicates were used? Report in main text rather than only supplementary data.
Response: The suspension was used for cell counting, and we had three technical replicates. We indicated in Lines 188–190.

Line 185, was 'remaining slurry' the algal pellet again? please specify.
Response: We used the remaining slurry include algal pellet for chlorophyll analysis, the text was revised in Line 192.

Line 210, is GPP not calculated by adding respiration to NPP? Please check, cite appropriate reference and correct throughout results/paper.
Response: Thank you for your clarification. Indeed, Net Primary Production (NPP) represents the productivity remaining after deducting Respiration (R) from Gross Primary Production (GPP). Typically, GPP is calculated by adding Respiration to NPP. In our dataset, respiration is represented as negative values to denote oxygen consumption. However, we converted these negative values to positive ones when using them in our calculations of GPP. The equation in our text has been corrected accordingly, as seen in Line 218.

Line 232, Not sure what is meant by this sentence? do you mean one-way anova was used to test for statistical significance of measurements prior to the death of this species?
Response: In our experimental setup, we considered two factors of treatments and days. However, due to the mortality of *P. acuta* within a single day, we only had one factor for this species, which was treatment. Consequently, we conducted a one-way ANOVA analysis for *P. acuta*. To clarify, we revised this paragraph in Lines 243-245.

Line 243, previously mentioned that a few months were not captured? please clarify.
Response: It has been revised in Line 251.

Line 244, justify why 12:00 and 24:00 sample timings were used.
Response: The logger was set up in the field for a year, the measurement of DO at 12:00 and 24:00 represent the DO conditions at light and dark, respectively.

Line 252, Was hypoxia identified during the 12:00 or 24:00 (or both) measurement?
Response: The hypoxia was identified during both 12:00 and 24:00 measurement, as they represent light and dark condition, respectively.

Line 266, were the reductions in hypoxia and anoxia relatively the same for the same

species? i.e. could the hypoxia and anoxia response be ranked similarly for each species?
Response: Anoxia had a more pronounced effect compared to hypoxia within the same species, as indicated by the statistical data (Fig. 4). Specifically, the most significant effect from anoxia was observed in *P. acuta*.

Line 267, assuming this was the case for only Porites?
Response: We have revised the text in Lines 276-279 to ensure clarity regarding the effects observed across different species, not limited to Porites.

Line 277, in terms of all coral species? or only T. mesenterina?
Response: Lines 286-287 refers to all coral species, not just *T. mesenterina*. We have updated the description in Lines 289-291 to reflect this.

Line 290, Is 'density' missing from the sentence? Symbiont density?
Response: Yes, it was an error, we revised in the main text, please see Line 299.

Line 293, change to 'resulting in overall coral holobiont tissue loss'
Response: The text has been updated in Line 302-303.

Line 310, specify what was compared for the interactions.
Response: We have removed the ambiguous sentence from the text.

Line 364, This is great to hear - will the monitoring continue? Please provide the duration of the hypoxia events in your dataset.
Response: Thank you for your inquiry. We do plan to continue monitoring dissolved oxygen levels in the area. The duration of the hypoxia events in our dataset has been updated in Lines 376-379 of the text.

Line 375, do you mean 'most common. within this region'?
Response: Yes, it is. For clarification, we added the description in Lines 387-388.

Line 377, integrity or just efficiency?
Response: We have corrected it, please see Line 389.

Line 383, change to 'is typically associated' as you have not exactly shown this detail in this study.
Response: We have corrected it in Lines 395-396.

Line 399, Arguably, an impact on the function of symbiotic algal photosynthetic efficiency will disrupt the symbiosis as the coral largely depends on photosynthates. Consider removing this statement.
Response: We have removed this sentence as a suggestion.

Line 403, Provide more of an explanation on why Chl a fluorescence could serve as a biomarker and explain how other environmental conditions impact chl fluorescence.
Response: We have added the description in Lines 410-413.

Line 427, This is a good point to make
Response: Thank you for your suggestion.

Line 429, Please clarify or remove this statement as it is not clear what you mean.
Response: We apologize for making the reviewer confused, and we have removed this ambiguous sentence.

Line 436, What about heterotrophic feeding by corals, please address this aspect.
Response: We have added the importance of heterotrophic feeding on calcification in Lines 448-452.

Line 451, Was there a significant difference in calcification rates between species? How was the calcification rates of Pocillopora in this study?
Response: In our study, we did not directly compare the actual calcification rates among species. Instead, we focused on observing the differences in response to treatments among species. However, we did note that the calcification rate of *Pocillopora* significantly decreased compared to ambient conditions (Fig. 9).

Line 463, General stress or hypoxia? please specify.
Response: It is the low oxygen stress, we have specified this point in the main text, please see Line 491.

Line 464, If these species are most sensitive to stress, surely it would be better if these species were targeted for stress priming programs. Please provide more specific detail than 'conservation plans'.
Response: We have updated the specific detail about the management as “Given the susceptibility of *P. acuta* and similar branching species, it is crucial that management efforts prioritize these corals when developing conservation plans and strategies. Additionally, regional areas with high branching coral coverage should be closely monitored. Strict control measures should be implemented to mitigate factors that may cause algal blooms, and continuous monitoring of water body health and coral conditions is essential. This holistic approach will help ensure the long-term resilience and sustainability of coral reef ecosystems”. Please see Lines 493-497.

Line 481, Justify the use of structural integrity as this study does not cover this metric but rather photosynthetic efficiency.
Response: We have corrected it in Line 519.