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Biogeosciences Discussions

Interactive comment on "Primary production and respiration of hypersaline microbial mats as a response for high and low CO_2 availability" *by* L. Bento et al.

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Dear editor,

We greatly appreciate the valuable comments and suggestions received from anonymous referees #1 and #3, and Dr. Alexandra Rao. Our reactions to their recommendations and the actions that directly were taken to address them are described in detail below.

Actions taken in response to the comments by the anonymous referee #1: 1. Anonymous referee #1: "Light intensity. P12742L26. "Excess" is quite relative. What light level is actually excessive (even in the strict sense of 'supersaturating') depends on

the photoacclimation state of the sample. Without knowing the photosynthetic light response it is not possible to determine if a certain light level is excessive. As this determines the interpretation of the results regarding the exposure to different light intensities, it would be important to know the light-response of MPB photosynthesis, when it saturates, when is supersaturating etc." Comment: We agree. It was not an aim of our study to contribute to the complex question of microphytobenthos photoacclimation, a subject properly addressed by other studies (i.e. Serodio et al., 2008). We think that the most part of the problem correctly noted by anonymous referee #1 was induced by the use of the word "excess". Furthermore, the light level used was of the same magnitude of other experimental studies (Serodio et al., 2005; Blanchard & Gall, 1994; Lefebvre et al., 2011; Cruz & Serodio, 2008), and field conditions can be even 2-3 times higher (Perkins et al., 2001; Serodio et al., 2008; Chevalier et al., 2010). Action: Removed the expression "excess light" from the text.

2. Anonymous referee #1: Replication. All figures show measurements of a single profile under each experimental condition. In fact, it seems that the whole paper is based on observations of a single sample per treatment. Is this true, or are the figures showing only representative cases? Comment: We agree that clarification is necessary. Each round of experiments was conducted in one spot, where profiles were recorded repeatedly to show short-term effects while was blocked out. It was not our goal to describe the spacial heterogeneity of the microbial mat, which may require > 100 replicates for correct estimation (Spilmout et al., 2011). Action: We included in the text the time lapse to reach steady-states and explain why we chose the experimental design with successive microprofiles to accomplish our objective.

3. Anonymous referee #1: Biofilm community composition. This is a main aspect, that could condition many topics of the discussion, and which is not adequately addressed. The authors simply state that the samples are dominated by diatoms and cyanobacteria, but will would be important to know, at least 1) which of these groups is in fact dominant (and what is their proportional abundance) 2) main genera present, if all

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motile. Comment: We agree that more information on biofilm community composition could have supplemented the discussion, but the main, general conclusions are independent from the biofilm composition. The objective of this study was to demonstrate the short-term effect of atmospheric CO2 on the microbial mat and that was clearly accomplished. Action: In Discussion we now list several parameters, including community composition, which would be very interesting and important to include in deeper investigations of the role of CO2 limitation.

4. Anonymous referee #1: Microalgae biomass. This is also a crucial piece of information needed to adequately characterize the studied biofilms. Carbon depletion will ultimately depend on absolute photosynthetic rates, which naturally depend on the amount of photosynthetic organisms present. Comment: We agree that microalgae biomass rather than CO2 or light in some places will be the more limiting factor for production. For the present place we qualitative state that the phototrophs formed a dense mat, and the results confirmed that CO2 became the limiting factor within the top 0.3 mm while high rates of GOP were found down to about 1 mm depth.

5. Anonymous referee #1: Some results seem counter-intuitive. What is the explanation for: - the photosynthetic activity of the bottom layer of the sediment being stimulated by lower atmospheric CO2? (P12741 L11) - why a decrease in light intensity decreases respiration? (P12741L23-25) Comment: Evidently we have not explained all this very well and too many aspects with marginal connection to the data were addressed. Action: These discussions have been revised with emphasis on fewer and more straight forward interpretations, conclusions and suggestions. See also comment 12, Anonymous referee #1.

6. Anonymous referee #1: CO2 concentrations. Were the tested CO2 concentrations ecologically-relevant? Also, would be interesting if some information could be given regarding how long do changes in CO2 in the atmosphere take to be translated into changes within the biofilm. Comments and actions: All six different CO2 concentrations used in this study were ecologically or scientifically relevant. Five can be experienced

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by microalgae depending on their microhabitat. For instance in the lakes located in the studied area water pCO2 can vary from 3 to 20000 μatm (Marotta et al., 2010) and even higher values may occur in the sediment. In the revised text it is discussed how the results with the applied CO2 concentrations allow extrapolations to atmospheric changes in past and coming centuries. The effect of zero CO2 provided a dramatic and simple illustration of how CO2 limitation worked and is actually a favorite figure when explaining and teaching this subject. As now specified in the text we observed a time lapse of 30-60 minutes until CO2 changes were translated into new steady state oxygen profiles. It is also discussed how careful you must be in extrapolating these results to other situations, as water height and numerous other physical, chemical, and biological parameters are probably important and deserve further investigations.

7. Anonymous referee #1: P12738 L18-20. A scheme would be very useful. Comment: We agree. Action: A scheme is included as Figure 2.

8. Anonymous referee #1: P12739 L14. Halogen lamps are known to deliver a lot of infrared radiation. Did the halogen lamp heat the sample surface significantly? Comment and action: As described in P12740L13 our light source was a slide projector and the cooler from the slide projector kept the heat away from the sample. The distance to the mat (21.5 cm) and the recorded temperature of the water column (25-26 C) is specified in the text.

9. Anonymous referee #1: P12739 L27-. How accurate was this technique? Were the CO2 concentrations measured independently? Comment and action: The whole technique and the way by which the CO2 concentration was controlled and calculated is now explained in M&M (See also comment 7 from Alexandra Rao). The complete elimination of NPP at 0 ppm confirmed the successful elimination of CO2 from the air line and avoidance of any CO2 intrusion in tubes and chambers further downstream. In fact this improvised field-lab technology turned out to work better than most of the commercial gas-mixing systems we use in our main laboratories.

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10. Anonymous referee #1: P12742 L4. What does it mean exactly 'absolute carbon limitation'? This is presented as one of the key findings of this study, but the meaning of this expression is not clearly explained. Comment and action: We realize that "absolute carbon limitation" is a confusing expression and it is now eliminated. We now emphasize that decreasing atmospheric concentration translates directly into a growing zone with no NPP. It is well known that microphytes can use both CO2 and HCO3- as carbon source, but the actual carbonate speciation and transport in this very dynamic system is not resolved, and as our data have little to add there, we avoid a lengthy discussion.

11. Anonymous referee #1: P12742 L22. It is hard to accept that CO2 is the most important limiting factor for primary productivity – what about light? Also, wouldn't carbon limitation be dependent on microalgae biomass? Comment and action: Agree. In our efforts to draw attention to one overlooked and ignored factor we have ourselves been a little too narrow-minded. In the revised discussion we emphasize how many more interacting factors including light and abundance that have to be considered when evaluating primary productivity control at a specific place and time.

12. Anonymous referee #1: P12742 L23. Strickly speaking, a decrease in light intensity always causes an increase in photosynthetic efficiency. This is contradictory with the statement that elevated light intensities cause higher photosynthetic efficiencies (12743L11-12). Comment and action: This is a complex matter when dealing with a mixed community with a steep light gradient. As we did not study light adaption directly, most of this discussion has now been discarded. We do state that the observed CO2 limitation should lead to dual selection for the most light tolerant and the most light efficient forms. But without further experimental support we refrain from mentioning that this might in turn lead to the counter-intuitive conclusion that higher light intensities favors a more light efficient population in the lower part of the mat. This is yet another interesting aspect of CO2 limitation for future studies.

13. Anonymous referee #1: P12742 L24. Is photorespiration expected to occur in this particular communities? Another strong argument for knowing the composition of the

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biofilm. Comment: Photorespiration did occur at high rates in this community and the complete elimination of NPP in some zones indicated that intracellular storages of inorganic carbon were not important. Diatoms are indeed known to use photorespiration (e.g. Glud et al. (1992), but a role of the cyanobacteria cannot be excluded without much more thorough investigations of community composition, growth, stratification, etc. This, however, will not affect the major conclusions.

14. Anonymous referee #1: P12743 L26-27 Meaning "can become carbon limited" (in the context of elevated CO2)?? Comment: They can be carbon limited even with CO2 concentrations in atmosphere much higher than actual. These correlations should now be much clearer in the revised results and discussions.

15. Anonymous referee #1: P12739 L16,20: I think the IS units are umol m-2 s-1 P12742 L11 "Direct" instead of "Directly"? P12740 L8 Why not 50 micrometers? Comment: We agree and the changes were made.

Actions taken in response to the comments by Alexandra Rao: 1. Alexandra Rao: The authors often discuss "respiration", but they mean "aerobic respiration". It should be stated clearly somewhere that anaerobic respiration is not measured, so their estimates probably significantly underestimate total respiration in sediment (mat) samples. Comment and action: We just follow the conventional term used in oxygen microsensors literature since the first paper published by Niels Peter Revsbech (Revsbech et al., 1981) and other recent papers (Ulstrup et al., 2011; Wangpraseurt et al., 2012; Sommer et al., 2010; Pringault et al., 2009; Walker et al., 2011). In some places we now do discuss anaerobic respiration and aerobic, lithotrophic respiration which can be deduced from the data, and this is clearly distinguished from conventional, organotrophic aerobic respiration.

2. Alexandra Rao: and used frequently (abstract line 11; p. 12741 l. 2; p. 12741 l. 21; p. 12742 l. 7). This is consistent with poor use of the English language throughout the paper (see Technical Corrections below), but this is particularly bad because it sounds

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like respiration is responsible for part of the O2 produced by primary production, when of course, respiration and photosynthesis have opposite effects on O2 flux Comment: The English is hopefully better now. The wording “respiration represents XX% of GPP” was indeed incorrect and other expressions are now used when respiration is compared with GPP. For further clarification we have also renamed GPP to GOP (gross oxygen production) as CO2 limitation was found to decouple oxygen production from carbon fixation, which must be the real meaning of primary production.

3. Alexandra Rao: I understand the importance of high CO2, but I fail to see the point of testing the effect of low (0 and 100 ppm) atmospheric CO2 concentrations. Comment: See comment 6 to referee #1.

4. Alexandra Rao: Section 2.2. Measurements of pH and alkalinity are mentioned here, but not presented anywhere in the text. If the results are not presented, then the methods should not be mentioned. If the authors decide to present these results, then it’s also important to mention what kind of standards were used for pH and alkalinity measurements. The absence of this information is conspicuous. Comment: In situ pH and alkalinity are presented at table 1 and the methods are described in M&M. Alkalinity and in particular pH surely varied significantly in the mat and the overlying water during the experiments, but microsensors to record this were not available. When the chambers were opened after each of the two experimental rounds we used indicator strips to check pH. But realizing that these sparse data of poor quality are of virtually no use in the evaluation of the experiments, we have left them out in the new version. To our best knowledge no one has yet resolved the complex speciation and transport of inorganic carbon species in such a dynamic system, and this challenge is now put forward in Discussion.

5. Alexandra Rao: Section 2.2, line 20. How were the diatoms and cyanobacteria identified? Comment and action: They were identified by direct observation with dissection microscope and this is added to the text.

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6. Alexandra Rao: Section 2.3. How were NPP rates calculated? Yes, it's useful to know NPP was calculated using a particular kind of software, but it's also important to provide more detail about the calculations. Was it simply determined as the sum of upward and downward diffusive O2 fluxes in the euphotic surface layer of the mats? Comment and action: The approach and the use of the numerical simulation model is now explained much more detailed.

7. Alexandra Rao: Section 2.4. The sample chamber, covered with thin plastic film with an "opening" for the microsensor and no apparent CO2 measurements for verification, just doesn't seem very well controlled. Is there an outflow port for the air that's being pumped in to keep chamber pressure constant, or does pressure fluctuate? Given that flow rates change significantly with the aging of pump tubing, how well regulated is the peristaltic pump flow? Given all these uncertainties and no measurement verification, it's difficult to believe the CO2 levels calculated are at all precise! Comment: See response to comment 9 from Anonymous referee #1. Action: The technique to control CO2 is now more accurately described and a drawing of the experimental chamber is added.

8. Alexandra Rao: Section 2.4. The authors mention high and low CO2 levels, but precise concentrations should be listed in the Methods section. Comment and action: We agree and the concentration ranges are now specified in M&M .

9. Alexandra Rao: Section 2.4. Very little detail is provided about the microprofiling. Were profiles really measured "in the same spot" (p. 12740 l. 9)? If so, disturbances caused by microsensor insertion may well be responsible for the changes observed in microprofiles (Kühl and Revsbech 2001). Apparently, there is water overlying the microbial mats (p. 12740 l. 12), but there is no other mention of the overlying water – how deep is it? Was it stirred during measurements, to maintain a diffusive boundary layer (DBL)? How thick was the DBL? How was the sediment-water-interface discerned for flux measurements? How was the O2 sensor calibrated? Was there an offset in the calibration according to calculated concentrations in the mat overlying water? Was

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there a cross-calibration with Winkler titrations? How were sensors positioned at the mat-water-interface? How were the profiles aligned after measurement? How much waiting time was there at each depth before recording readings? How much time were the mats allowed to equilibrate after changing light and CO2 levels? Comment and action: We apologize for the insufficient description of the experimental set up. All the requested details are now described and explained in the revised M&M. We do not agree that profiling in the same spot could be responsible for changes observed in the microprofiles, and in M&M we now explain why repeated profiling in one spot is actually a way to avoid disturbances and irrelevant variations. See also response to comments 2, 6 and 9 from reviewer #1.

10. Alexandra Rao: Section 2.4, p. 12740, l. 12. The absence of changes in pH in the overlying water, despite dramatic changes in chamber CO2 levels (0 to 5000 ppm) is surprising, and suggests to me that there may be a big problem in the regulation of chamber CO2 levels. See comment 7 above. Comment: As a confirmation of the gas mixing system we observed similar oxygen profiles under normal atmospheric air as under a gas mix with the same prescribed CO2 concentration of 380 ppm.

11. Alexandra Rao: The terminology, "NPP participation in GPP" on p. 12741 line 24 is just another example of poor use of the English language, but this one really stands out. NPP doesn't "take part in" GPP in any way, although it could be said that NPP represents a fraction of GPP after accounting for respiration. Comment: We agree. As with comment 2 we have carefully inspected and corrected these phrases.

12. Alexandra Rao: What is meant by "absolute" carbon limitation, on p. 12742 l. 4? Comment: See answer to Anonymous referee #1 (comment 10).

13. Alexandra Rao: Section 4, 1st paragraph. The authors suggest that phototrophs are carbon limited when CO2 was removed from the chamber atmosphere, because respiration was equal to GPP, so there is a closed carbon cycle. This completely ignores anaerobic respiration, since the authors only measure O2 consumption and

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therefore can only consider aerobic respiration. This is a hypersaline lagoon, so I imagine anaerobic respiration is also important. Comment: The anaerobic processes are actually not hidden. The effect of anaerobic, organotrophic respiration on the oxygen balances depends on the fate of the reduced products; in this system primarily sulfur and iron species. If the reduced products are re-oxidized immediately, the net effect will be just as if it was aerobic, organotrophic respiration. If the reduced products pile up, i.e. as ironsulfide or pyrite, the excess CO2 generation relative to oxygen reduction will result in a net production of oxygen in the lower part of the oxic and photic zone. In the opposite case where reduced products are oxidized faster than they are generated, the lower zone will have a net consumption of oxygen. The latter is actually what was observed, in particular during the first round of experiments. This is now pointed out and discussed in the text. Notice that this has no impact on the evaluation of CO2 limitation in the upper production zone.

14. Alexandra Rao: The suggestion of a 2-layer system, in which the surface and deeper layers respond differently to changes in chamber CO2 levels (p. 12742 l. 12-17; p. 12741 l. 3-14; p.12743 l. 15-18) could have been affected by disturbances associated with repeated profiling in the same spot – see comment 9 above. For the sake of argument, assume repeated profiles are being measured in the same spot, each time under lower CO2 levels, from 5000 ppm to 380 ppm as in this study. Each time the sensor is inserted, porewater at this spot is mixed. It's entirely conceivable that this could explain the changes shown, e.g. in Fig. 2. Comment: We do not agree. As explained earlier (comment 9) artifacts from repeated profiling are assumed to be negligible on these samples (please check cited literature). Each graph at figure 2 represents the steady-state of each treatment. At least 6 profiles were performed for each treatment, in a time series. The steady-state represents at least 3 profiles that were considered stabilized since they had the same shape.

15. Alexandra Rao: Figure 1 – This 3-panel map is not very useful. In particular, the expansion from the 2nd panel to the 3rd panel provides no extra information about the

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relative dimensions of Visgueiro lagoon (depth, area) with respect to the neighboring lagoons. Either zoom in much more in the 3rd panel and provide more detail on Visgueiro and maybe a couple of surrounding lagoons, or eliminate the 3rd panel. Comment: We agree. Action: Figure 1 were taken out and the coordinates were provided.

16. Alexandra Rao: Figure 2 – I'm surprised the authors provide only 1 replicate profile at each CO2 level. I'm also surprised that it seems like all measurements were done on only 2 mat samples (1 for high CO2 measurements, 1 for low CO2 measurements, see Section 2.4). With no replication, it's difficult to have any confidence in the results. Comment: Each CO2 level represents a time series of profiles. Since we are describing a phenomenon that occurs in short temporal scale and measurements of gross primary production in a profile with the microsensor method takes at least 30-40 minutes to be made (Glud, 2008) this approach was chosen by the authors. It was not our goal to describe the spacial heterogeneity of the microbial mat, nor to use these measurements to illustrate a larger scale. See also comments to Anonymous referee #1, question 2. The phenomenon is confirmed since we have steady-state profiles of each treatment.

17. Alexandra Rao: If the issues mentioned above can be addressed, the manuscript also requires extensive language editing. Numerous spelling and grammatical mistakes, poor sentence structure and organization are evident throughout the text, making it difficult to understand in some cases Comment: We agree. Action: We revised closely the text to improve clarity

18. Alexandra Rao: The figures are not dicussed in order in the text. For example, in section 3.1, the text jumps from a reference to Fig. 2 to the following reference to Fig. 6. The order of the figures should be changed to match the order in which they are mentioned in the text. Comment: In the revised Result section the order of the figures fits more naturally. We could not avoid a few jumps as NPP is discussed somewhat separated from GOP, but the GOP profiles have to be shown close to their corresponding NPP profiles. Figure 7 integrates data from the previous 4 figures and

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is now introduced after them as requested.

Actions taken in response to the comments by anonymous referee #3: 1. Anonymous referee #3: The major problem I identify is replication. All figures show measurements of a single profile. Averages and standard deviations should be presented if, in fact, replicates were used. From reading Materials and Methods I get the feeling no replication was used. Comment: See response to comment 2 of anonymous referee #1 and comment 16 of Alexandra Rao.

2. Anonymous referee #3: The microphytobenthic community is very poorly described. The authors refer only diatoms and filamentous cyanobacteria. What was the relative contribution of each group? What were the main genera/species present? Comment: See response to similar comment 3 of anonymous referee #1 and comment 5 of Alexandra Rao.

3. Anonymous referee #3: The authors refer this manuscript as the "first account of carbon limitation in a microbial mat". Previous studies refer carbon limitation in microphytobenthic biofilms, for example Cook and Roy (2006) (this paper is in the reference list of the manuscript). Comment: We agree that our formulation was in inappropriate. It is now specified that the novelty of our study is the control of MPB production by CO2 in the air.

4. Anonymous referee #3: The English requires improvement. Comment: The text was carefully checked to improve clarity and language.

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