

## ***Interactive comment on “Primary production and respiration of hypersaline microbial mats as a response for high and low CO<sub>2</sub> availability” by L. Bento et al.***

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**\*\*General Comments\*\***

This manuscript reports on calculations of primary production and respiration derived from O<sub>2</sub> microprofiles measured in microbial mats collected from the hypersaline Visgueiro lagoon in southern Brasil. Measurements are made at different CO<sub>2</sub> levels to investigate microbial carbon limitation. Measurements under high CO<sub>2</sub> (up to 5000 ppm) showed increasing photosynthetic activity (NPP and GPP) with increasing CO<sub>2</sub> levels in the surface of the mat, but decreasing NPP with increasing CO<sub>2</sub> levels at depth. Measurements at lower CO<sub>2</sub> levels (below 380 ppm) showed a decrease in

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NPP. A decrease in incident light resulted in a decrease in aerobic respiration, such that NPP represents a greater fraction of GPP.

While this study presents some interesting results, it does not merit publication in Biogeosciences due to 1) the lack of supporting ancillary data (e.g., measurements of chamber CO<sub>2</sub> levels, and overlying water or porewater pH), and 2) important flaws in measurement techniques, as listed below.

**\*\*Specific Comments\*\***

1. 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.

2. The wording “respiration represents XX% of GPP” is misleading 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 like respiration is responsible for part of the O<sub>2</sub> produced by primary production, when of course, respiration and photosynthesis have opposite effects on O<sub>2</sub> flux.

3. I understand the importance of high CO<sub>2</sub>, but I fail to see the point of testing the effect of low (0 and 100 ppm) atmospheric CO<sub>2</sub> concentrations.

4. 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.

5. Section 2.2, line 20. How were the diatoms and cyanobacteria identified?

6. Section 2.3. How were NPP rates calculated? Yes, it's useful to know NPP was

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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 O<sub>2</sub> fluxes in the euphotic surface layer of the mats?

7. Section 2.4. The sample chamber, covered with thin plastic film with an "opening" for the microsensor and no apparent CO<sub>2</sub> 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 CO<sub>2</sub> levels calculated are at all precise!

8. Section 2.4. The authors mention high and low CO<sub>2</sub> levels, but precise concentrations should be listed in the Methods section.

9. 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 O<sub>2</sub> sensor calibrated? Was there an offset in the calibration according to calculated concentrations in the mat overlying water? Was 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 CO<sub>2</sub> levels?

10. Section 2.4, p. 12740, l. 12. The absence of changes in pH in the overlying water, despite dramatic changes in chamber CO<sub>2</sub> levels (0 to 5000 ppm) is surprising, and

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suggests to me that there may be a big problem in the regulation of chamber CO<sub>2</sub> levels. See comment 7 above.

11. 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.

12. What is meant by "absolute" carbon limitation, on p. 12742 l. 4?

13. Section 4, 1st paragraph. The authors suggest that phototrophs are carbon limited when CO<sub>2</sub> 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 O<sub>2</sub> consumption and therefore can only consider aerobic respiration. This is a hypersaline lagoon, so I imagine anaerobic respiration is also important.

14. The suggestion of a 2-layer system, in which the surface and deeper layers respond differently to changes in chamber CO<sub>2</sub> 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 CO<sub>2</sub> 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.

15. 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 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.

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16. Figure 2 – I'm surprised the authors provide only 1 replicate profile at each CO<sub>2</sub> level. I'm also surprised that it seems like all measurements were done on only 2 mat samples (1 for high CO<sub>2</sub> measurements, 1 for low CO<sub>2</sub> measurements, see Section 2.4). With no replication, it's difficult to have any confidence in the results.

**\*\*Technical Corrections\*\***

1. 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. Below are some examples just in the abstract (this is by no means a comprehensive list):

- Line 3: "Different from most part of the literature" should be something like "Unlike previous studies" - Line 6: "in benthic gross and net primary production" should be "on benthic gross and net primary production" - Line 8: "Oxygen concentration profile" should be plural. - Line 10: "In this conditions" should be "Under these conditions" - Line 11: "Extreme close coupling" should be "Extremely close coupling" - Line 12: "can be even" should be "can even be" - Line 12: "environments with temporally no atmospheric CO<sub>2</sub> available" – what is the purpose of the word temporally here? Perhaps just remove it, since it doesn't mean anything here. - Lines 13 and 18: "submitted" should be "subjected" or "exposed"

2. The figures are not discussed 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.

**\*\*References\*\***

M.Kühl & N.P. Revsbech (2001). Biogeochemical Microsensors for Boundary Layer Studies, pp. 180-210. In: B.P. Boudreau & B.B. Jørgensen (eds.), *The Benthic Boundary Layer*, Oxford University Press, New York, 2001.

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