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CO_2 influence on oxygen dynamics and net primary production of the microphytobenthos: an experimental approach

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ABSTRACT:

Production of organic matter by phototrophs requires inorganic carbon, which in aquatic systems is taken up from the water column, sediment or atmosphere. Observations on a microphytobenthic mat overlaid with 2 mm of water and atmospheric air showed a tight balance between consumption and production of oxygen and, therefore, a bimodal pattern in the Net Primary Production (NPP). Enrichment of the air with CO_2 led to an enhancement of the NPP of a community, while the removal of all CO_2 from the air resulted in no NPP and a linear O_2 gradient from the overlying water to the lower part of the mat. The distribution and rates of gross photosynthetic oxygen production, measured as the oxygen decline within one to twos after light-dark shifts, showed little response to CO_2 depletion, suggesting that the photosynthetic electron flow was primarily redirected from CO_2 fixation to photorespiration. In nature, the observed control of NPP by atmospheric CO_2 concentration should be most pronounced in shallow-water and intertidal systems, and the productivity in these ecosystems may therefore be steadily increasing along with the increase in atmospheric CO_2 concentration.

Keywords:

Microbial mat, Carbon dioxide, Oxygen profile, Gross oxygen production, Net primary production.

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INTRODUCTION

Benthic microalgae and cyanobacteria appear in virtually all aquatic systems where sufficient light reaches the bottom, and they can be the major source of organic matter in many shallow water and intertidal ecosystems (Underwood and Kromkamp, 1999). Due to the high photosynthetic activity of the microphytobenthos, primary production might happen in limited carbon sources (Ludden, 1985; Rasmussen *et al*., 1983; Vieira *et al*., 2015). Experiments on the productivity of diatom populations in cultures (Admiraal *et al*., 1982) and natural mats (Jensen and Revsbech, 1989; Rothschild, 1994) have shown that photosynthetic activity is limited, besides light and nutrients by the supply of inorganic carbon and variations in pH (Cook and Roy, 2006).

Historically, concentrations of atmospheric CO_2 on multi-million-year timescales ranged since 278 ppm during the pre-industrial period, to 5300 ppm in estimates of Devonian and Triassic (Foster *et al*., 2015). In this sense, it is known that part of atmospheric CO_2 influenced in many primary producers and aquatic systems (Berner, 1997; Mc Elwain, 1998; Pagani *et al*., 2011). However, the perception of this large variation on ecophysiological and metabolic responses of microphytobenthos is poorly known and neglected in many ecosystem balances of gases (Duarte *et al*., 2004).

Although studies carried out *in situ* or near ambient conditions are limited, Krause-Jensen and Sand-Jensen (1998) suggested that inorganic carbon limitation is the major reason why maximum (integral) productivity is significantly lower in microphytobenthic mats than in dense macrophytes and phytoplankton systems. Underwood and Kromkamp (1999), stress that ecophysiological studies, which consider behavioral adaptations to carbon limitation are necessary. In this sense, experiments in which concentrations of Dissolved Inorganic Carbon (DIC) and pH have been manipulated demonstrate the advective relief of CO_2 limitation on microphytobenthic production in sandy sediments (Cook and Roy, 2006).

Additionally, organic carbon addition experiments have demonstrated the existence of CO_2 limitation in hypersaline microbial mats (Grotzschel *et al*., 2002). No studies, however, have directly tested the effect of changing atmospheric CO_2 concentration on the production of the microphytobenthos. This would be particularly important in shallow systems since the dissolved bicarbonate in the water column is not enough to buffer carbon fluxes. Brodie *et al*. (2014) argued that to make predictions related to the effect of atmospheric CO_2 concentration on benthic microalgae, a deeper understanding of how CO_2 concentrations influence this group is required. In this sense, light limitation may be an important factor that can directly interfere with carbon metabolism, affecting inorganic carbon transport activity and photosynthetic CO_2 affinity (Beardall and Raven, 2013).

Presence of microphytobenthos in hypersaline lagoon is the first photosynthetic communities proliferating in the shallow zone and they are related with all dynamic of carbon and oxygen dynamic (Duarte *et al*., 2004). Estimates have reported that primary production rates are around 5.5 mmolC/m2/d (Knoppers *et al*., 1996). In this sense, preliminary results showed that in the top few millimeters of an intact microphytobenthic

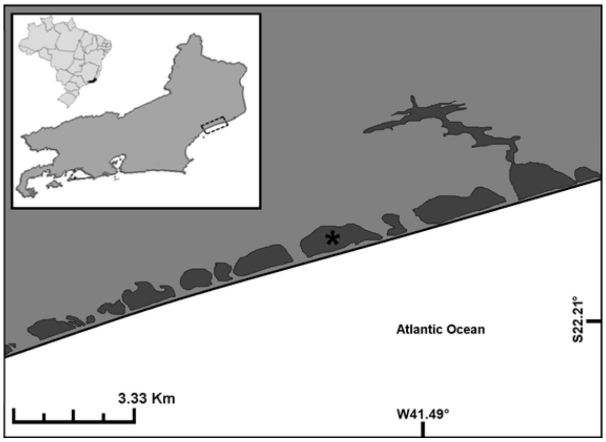

Figure 1. Geographic location of Visgueiro Lagoon (*) within the Jurubatiba National park (dashed line) in the State of Rio de Janeiro, Brazil

community incubated in a thin layer of water under ambient light, distinct flat-topped oxygen peaks are observed (Bento, unpublished data). Based on this information we hypothesize that oxygen pattern in sediments of hypersaline lagoons might be explained by CO_2 depletion, suppressing net primary production in most of the mat. The aim of the present study is, therefore, manipulating CO_2 concentrations in the air overlying a microphytobenthic mat, measuring the vertical distribution of O_2 and other metabolic parameters as net primary production, respiration and gross oxygen production.

MATERIAL AND METHODS

Sampling

Visgueiro Lagoon (22°11′S, 41°24′W) is located at the Jurubatiba National Park, northern Rio de Janeiro State, Brazil (Figure 1). This coastal hypersaline lagoon is oriented parallel to the coast. Its depth varies from 0-0.5 m, depending on the balance among ocean water exchange, evaporation and precipitation.

Water samples for measurement of pelagic parameters were collected with prewashed (HCl 0.5 M) plastic bottles (Table 1). pH was measured with an Analion PM 608 pH meter (Analion, Brazil) and total alkalinity was determined by the gran titration method. Temperature and salinity were measured with a YSI-30 thermo-salinometer (YSI, USA).

Table 1. Water column limnological parameters of Visgueiro Lagoon during the period of sampling

S. No	Visgueiro Lagoon	
1	Salinity	57.4 ± 0.6
2	Temperature (°C)	24.4± 0.9
3	Oxygen (mgL^{-1}/%)	4.3 ± 0.2
4	pH	8.94 ± 0.2
5	Alkalinity (μEqL^{-1})	2624 ± 0.6
6	Dissolved organic carbon (mgL^{-1})	73.7± 4.7
7	Water chlorophyll (μgL^{-1})	1.9 ± 0.5
8	Dissolved phosphorus ($\mu molL^{-1}$)	0.43± 0.1
9	Total phosphorus ($\mu molL^{-1}$)	2.22 ± 0.8
10	NH_4 ($\mu molL^{-1}$)	0.33± 0.2

Sediment samples were collected at 0.4 m depth with Plexiglas® cores (10 cm diam). The sample material was transported to the field lab and stabilized with lagoon water until the incubation process. A surface sample was inspected with a stereoscopic microscope for biological characterization. Benthic (pennate) diatoms and filamentous cyanobacteria of the genus *Microcoleus* were found to be the two dominant groups forming a one-mm thick mat on top of the sediment.

Experimental set up

A 5 mm layer of intact sediment from the top of the sample material was placed at the bottom of an experimental chamber, which was made from a sectioned 20 mL plastic vial (Figure 2). A thin layer of 2 mm of lagoon water was added to maintained the sediment in hydrated condition and to favor the CO_2 diffusion in the sediment. In addition, the chamber was closed with a thin, transparent piece of cling wrap with a slope that prevented condensed water from dripping into the sample. Air was blown in from the side of the experimental chamber through a needle pointing at the water surface, thereby keeping the water in circulation. An oxygen microsensor was inserted through a small hole also used as an air outlet.

The experimental CO_2 concentration was controlled by the mix of pure CO_2 with CO_2-free air as follows: pressurized air was passed through a wash bottle with alkaline NaOH solution to remove CO_2; the air was then humidified by passing through a wash bottle with pure water before being mixed with pure CO_2 and pumped into the chamber. The CO_2 line started in an exetainer, which was constantly and gently flushed with CO_2 from a tank. A small, controlled flow of CO_2 was drawn from the exetainer to the airline with an adjustable Ismatec IPC-24 V2.03 peristaltic pump (Kinesis, UK). The flow in the airline was measured by recording the time taken to fill an inverted and submerged flask with a known volume, while keeping the inside and outside water surfaces even to eliminate pressure differ-

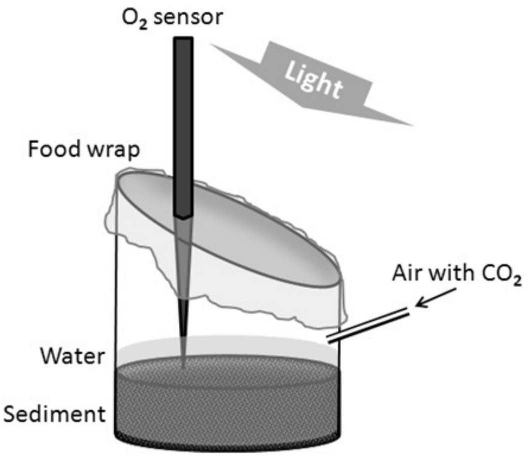

Figure 2. Set up for measuring profiles of O_2 and photosynthesis with controlled atmospheric CO_2 concentration. The phototrophic mat on the sediment is overlaid with 2 mm water and a restricted air phase with controlled gas

ences. The flow in the CO_2 line after the peristaltic pump was controlled by recording the travel speed of small amounts of water through a tube of known length and inner diameter. The concentration of CO_2 in the mixed line was calculated from the flow rates in the air and CO_2 lines. The values of CO_2 applied during the experiment were 5000, 2000, 550, 380, 100 and 0 ppm. These concentrations were chosen based on the literature records (Foster *et al*., 2015).

The microbial mat was positioned 21.5 cm from the light source, which was a slide projector with no front lenses (Halogen JC 24V 150W Base GY6.35, Light Express, Nards, Brazil). The Photo-synthetically Active Radiation (PAR) measured at the surface of the microbial mat before closing the vial with the use of cling wrap was estimated near 1.20 μWcm^{-2} (planar sensor) and 550 $\mu molm^{-2}s^{-1}$ (bulb sensor). Temperature in the overlying water was maintained at 25–26°C.

Oxygen profiles and production

Sediment oxygen profiles were measured with an oxygen microsensor with a tip diameter of 25 µm and 90% response time <4 s (Unisense, Denmark). The sensor was attached to a motor-driven micromanipulator and a controller (Encoder Mike Controller 18011, Unisense, Denmark). The microsensor measurements were carried out by a picoammeter (PA 2000, Unisense, Denmark) connected to an A/D converter, and calibrated against anoxic sediment and air saturated water according to Revsbech *et al*. (1981). Oxygen profiles were recorded by moving the sensor in steps of 50 µm, starting in the mixed water layer above the Diffusive Boundary Layer (DBL) of the sediment surface and ending in the anoxic zone 0.1–0.16 cm below the surface. After the measurements, profiles were aligned based on the DBL. The sampling time at each depth was '1 s' and conditions were considered steady state when at least three profiles showed the same shape.

The vertical distribution of Net Primary Production (NPP) was determined by searching for the minimum set of zones of constant consumption or production that could simulate the observed oxygen profile using the numerical model PROFILE (Berg *et al*., 1998). Gross Oxygen Production (GOP) at each position was determined from the linear decline in oxygen concentration within 1-2 second after temporary light shutoff (Revsbech and Jorgensen, 1983). An integrative view among GOP, respiration and NPP was carried out only for the upper production layer to avoid the influence of potential recycling of CO_2 from mineralization in the lower production layer. Since photorespiration is very important here, the more direct term GOP was preferred to the more conventional terms "Gross Primary Production" and "Apparent Gross Primary Production", which may be confusing or misleading.

In the first round of experiments, the CO_2 concentration was reduced stepwise from 5000 ppm to 380 ppm (the concentration at which the second experiment was conducted). In the second round, exploratory reductions from 380 ppm to 0 ppm were tested, as well as the effect of light influence (550 $\mu molm^{-2}s^{-1}$) on different CO_2 concentrations. Each experimental round was conducted in just one spot to eliminate confounding effects

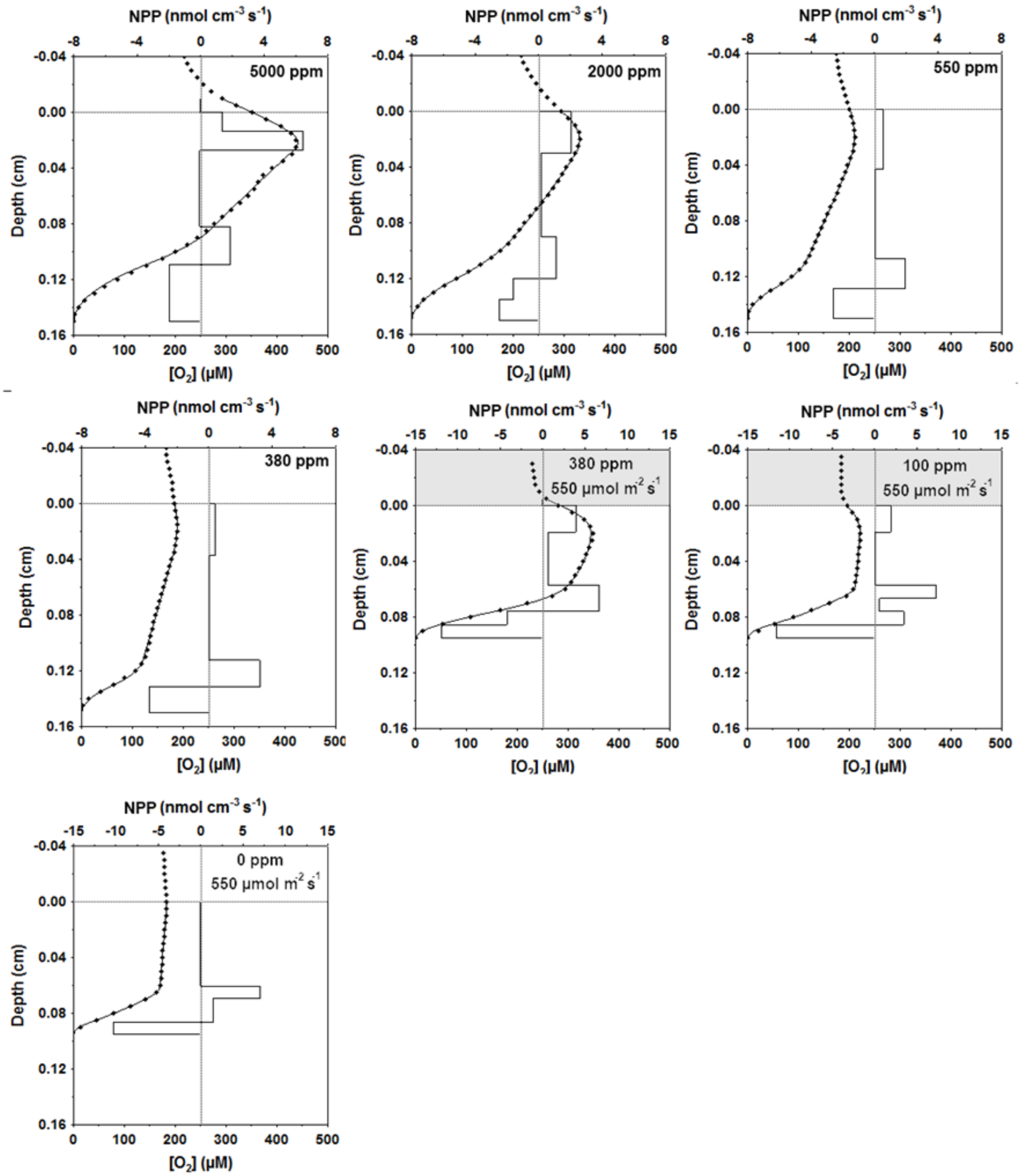

Figure 3. Measured oxygen concentration (dots), modeled oxygen profile (solid line near the dots) and modeled net primary production (NPP, square line) during the stepwise reduction of CO_2 concentrations. Gray header indicates treatment with additional light (2^{nd} experimental round)

of horizontal heterogeneity. Denis *et al*. (2012) compared successive microprofiles at the same spot with fluorescence data and concluded that high-frequency microprofiling is a reliable way to monitor short-term temporal changes in microphytobenthic primary production. In the present study, oxygen profiles were recorded repeatedly before and after changes in CO_2 or light. Steady-state profiles always developed within 30-60 min after GOP profiles were measured and before the next experimental round.

RESULTS

In both rounds of experiments, oxygen profiles changed markedly in maximum values and shape as the

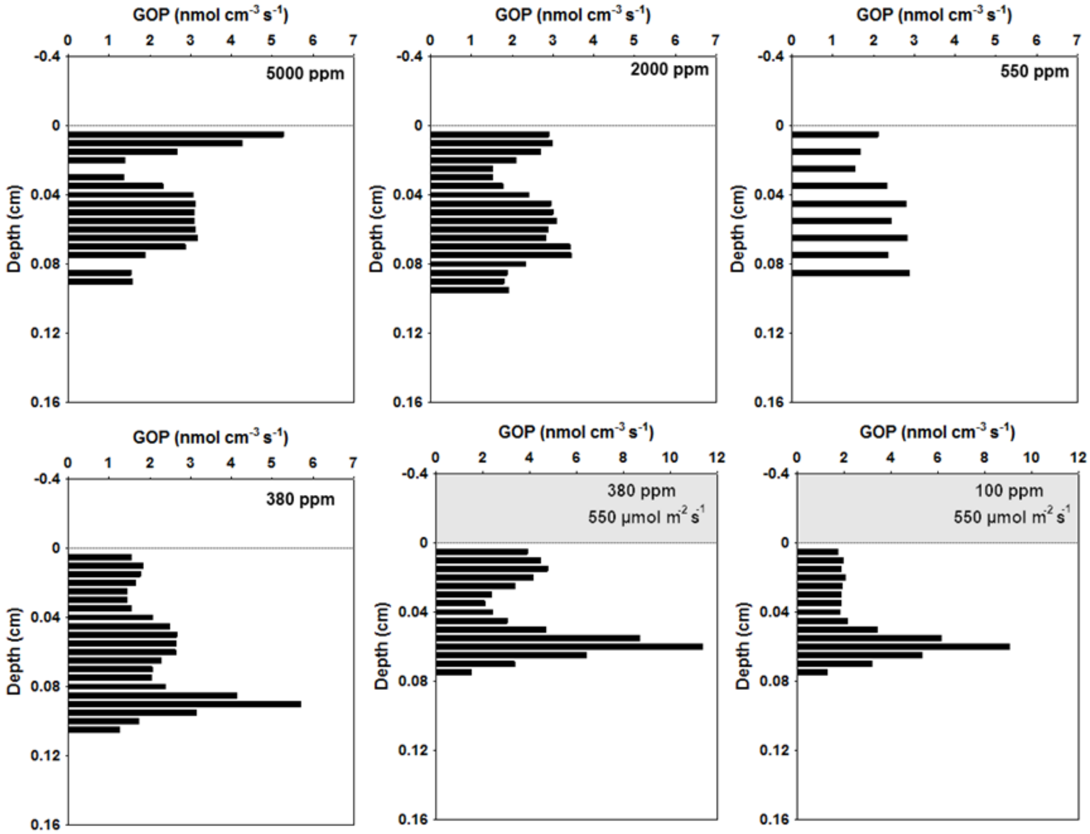

Figure 4. Vertical distribution of Gross Oxygen Production (GOP) recorded immediately after each of the O_2 profiles shown in Figure 3. Gray header indicates treatment with additional light (2nd experimental round)

CO_2 concentration of the overlying air decreased (Figures 3 and 4). The oxygen profiles, obtained in the first round of experiments, showed that the highest oxygen production occurred at 5000 ppm of CO_2 (≈430 µM), and the lowest one at 380 ppm of CO_2 (≈190 µM). In all profiles it was observed a bimodal pattern in the NPP, with two "production zones" situated above 0.04 cm and below 0.08 cm, as shown by the convex profiles (Figure 3). During the second experimental round, carried out under the effect of light, oxygen penetration was relatively low (0.08 cm) and the highest oxygen concentration was recorded at 380 ppm CO_2 (350 µM). As expected, the production of oxygen decreased with the reduction of CO_2 availability. The lowest value occurred at 0 ppm CO_2 (≈190 µM).

In all CO_2 concentrations, there were positive rates of GOP throughout the mat up to depths of 0.08–0.10 cm, including in the central zone with no detectable NPP (Figure 4). In general, treatments with CO_2 concentration below of 380 ppm showed peaks in the GOP at the deepest zone of oxygen production.

For the first round of experiments, an integrative view of NPP, respiration and GOP (Figure 5) showed that the total NPP was approximately five times higher at 5000 ppm than at 380 ppm at the upper production layer (0–0.04 cm depth). Respiration varied from approximately 70% of GOP at 380 ppm to only 3% at 5000 ppm. At 5000 ppm, NPP almost matched GOP. In contrast, the integrative view of the second round showed that GOP, in the upper production layer, were similar at 380 and zero ppm of CO_2 even with the highest respiration rate at zero CO_2 concentration (Figure 5).

DISCUSSION

Microphytobenthic mats exhibit high rates of primary production and can make a substantial contribu-

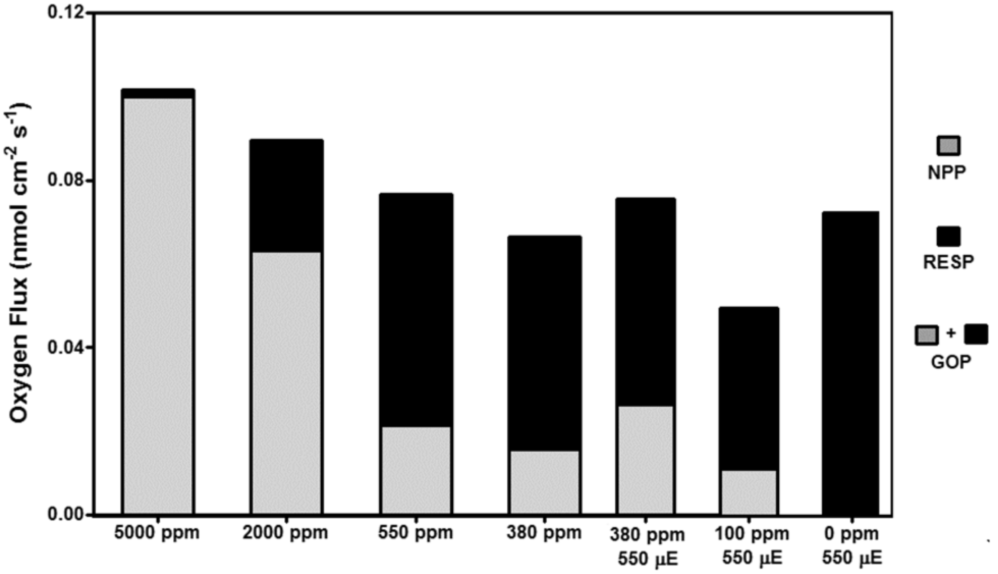

Figure 5. Depth of integrated Net Primary Production (NPP), respiration (RESP) and Gross Oxygen Production (GOP) in the upper 0.04 cm of the phototrophic mats

tion to the oxygen and carbon flows in aquatic environments. Specifically, the present study showed a 5-fold increase of NPP after the addition of a mix of pure CO_2 and CO_2 enrichment of the overlying air, suggesting that atmospheric CO_2 plays an important role in the regulation of oxygen dynamics NPP and respiration. This result also demonstrates the influence of this group of primary producers on intrinsic process among the surface, sediments and water column in hypersaline coastal lagoons.

As expected, the deepest oxygen penetration occurred in the richest CO_2 concentration treatments, probably because of the RUBISCO activity in the photosynthetic carbon reduction cycle in the deeper layers of the mat (Figures 3 and 4). This process is normally present in diatoms and cyanobacteria (Roberts *et al.* 2007; Shukla *et al.,* 2016) and is linked to the increase of CO_2 diffusion into the sediment due to the high concentration (Fenchel and Glud 2000; Gruca-Rokosz *et al.* 2011). On the other hand, the complete removal of CO_2 from the overlying air, even under conditions that favored primary production, resulted in strictly vertical oxygen gradients, with no net flux of O_2, despite the high rates of oxygen production and consumption. In this sense, Cook and Roy (2006) mentioned that microphytobenthic production is limited by advective transport of DIC, such that low values of DIC inhibit carbon fixation and the photosynthetic process. However, some studies have found that, for most groups of algae, including microbial mats, CO_2 concentration does not control the rate of photosynthesis if these organisms have mechanisms (CO_2-concentrating mechanisms) that supply the photosynthetic system at low CO_2 concentrations (Tortell *et al*., 1997; Raven *et al*., 2008). This system may not be efficient in algae at the boundary layer of the mat, but could explain the high values of GOP in the deeper layers at low CO_2 concentrations (Figure 5).

Corroborating the low influence of CO_2 on oxygen production, our data showed that with a GOP of approximately 3 nmol cm^{-3} s^{-1} and an oxygen concentration of 180 µm, the turnover time of oxygen in the "passive" zone was only ~3 min. Compared to NPP, GOP had little response to CO_2 changes, showing that, when no CO_2 was available for fixation, phototrophs maintained the activity of the photosynthetic and photorespiratory system (Figure 6). In addition, the tight control of NPP by CO_2 in the upper production zone implies that all CO_2 from aerobic and anaerobic carbon mineralization in the underlying sediment could be captured in the lower production layer (Brotas *et al.*, 2003). This would mean that old organic matter was simply exchanged for new biomass and that only NPP from the upper production zone enhanced the organic carbon pool of the system.

Bimodal distribution of GOP also supports the existence of different distribution patterns of phototrophs. More detailed studies incorporating species distribution, physiology, behaviour, nutrient limitations, grazing, mineralization, transport processes, and diurnal and tidal variations of light and water are required to fully delineate the roles of multiple limiting CO_2 sources in shaping benthic communities (Garcia-Pichel *et al.*, 1994; Bourgeois *et al.*, 2010; Cartaxana *et al.*, 2016). For this reason, more applied studies in this sense should be undertaken to clarify the roles of ecophysiological and behavioral mechanisms.

Simulating the natural conditions, we used 2 mm of water layer. The fact that a very thin layer was used strengthened the observed dependency on atmospheric CO_2. Larger water columns will retain more CO_2, facilitating the exchange of organic and inorganic carbon inside the systems. In this sense, the present approach can be applied to shallow lagoons and especially intertidal systems, since these systems are more prone to atmospheric control of production. Field studies in a tropical tidal salt flat showed that total NPP significantly declined with the development of typical “cut-off” oxygen profiles due to an exhaustion of the pore water inorganic carbon pool (Oliveira *et al.*, 2011).

Although the present study represents a microscale perspective, we can picture possible scenarios in the primary production of microphytobenthos in relation to the changes of atmospheric CO_2 concentrations. Presently, the CO_2 concentration of the Earth’s atmosphere is approximately 380 ppm, which is 36% higher than in the preindustrial era (280 ppm). Even greater concentrations are projected for the coming decades (IPCC, 2013). The observed direct effect of atmospheric CO_2 on NPP suggests, therefore, that many shallow and intertidal aquatic systems might experience a relatively rapid enhancement in productivity.

CONCLUSION

Changes in CO_2 concentrations of overlying air affect the oxygen dynamics of microbial mats from hypersaline coastal lagoons, influencing the depth of oxygen penetration in the sediment. In general, NPP of microphytobenthos is positively associated with the CO_2 addition. However, as the gaseous concentrations of CO_2 are reduced, low effect on the GOP is reported.

CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any personal, commercial or financial relationships that could be construed as potential conflicts of interest.

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AUTHOR CONTRIBUTIONS

LB, AP, and MK designed research and outlined experiments. LB conducted the experiments. All authors analyzed and interpreted the data. LB and VO wrote the manuscript. All authors read, critically revised and approved the final version of the manuscript.

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