Experimental evidence of nitrogen control on $pCO_2$ in phosphorus-enriched humic and clear coastal lagoon waters

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INTRODUCTION

Carbon dioxide ($CO_2$) is one of the most important greenhouse gas in terms of global warming (IPCC, 2007; Royer et al., 2007; Solomon et al., 2008). The terrestrial biomass represents a relevant global stock of carbon (C), which is removed from the atmosphere by primary production (Gough et al., 2008). However, a significant part of this terrestrial organic matter is leached into aquatic ecosystems, where it may be buried in bottom sediments (Downing et al., 2008) or remineralized to $CO_2$ by aquatic biological decomposition (Aid Pik et al., 2001). In the watershed, most natural inland waters are relatively small, but their wide geographic distribution, high abundance, and common location at low altitudes make them a typical fate for the water inflow from broad areas, playing a crucial role on the global C cycle (Cole et al., 2007).

Coastal lagoons are ecosystems often altered by the human land use (Marotta et al., 2010b), which show intense C fluxes (Duarte et al., 2004; Marotta et al., 2010b). The terrestrial inputs from leaching and groundwater renew $CO_2$ in lakes by the contribution of inorganic C (Raymond et al., 1997; Marotta et al., 2010b), or organic substrates supporting the aquatic respiration (Cajigas et al., 1997; Jonsson et al., 2003). Photosynthesis and respiration are the major metabolic pathways determining whether what level of organic matter is produced and destroyed (Cole et al., 2000). Indeed, high terrestrial organic inputs may explain the positive general trend reported between dissolved organic carbon (DOC) and the partial pressure of $CO_2$ ($pCO_2$) in lake waters (Jonsson et al., 2003). Several studies have showed positive relationships in DOC and $pCO_2$ in lakes at high latitudes and even globally (Sobek et al., 2005) supporting the idea that lakes are an important source of $CO_2$ globally (Cajigas et al., 1994, 2007; Duarte and Prairie, 2005; Tranvik et al., 2009). Mean areal rates of $CO_2$ evasion from lakes are higher at low latitudes, probably by the potential positive effect of warmer conditions on the organic decomposition (Marotta et al., 2009, Kesten et al., 2010). In this way, the degradation of organic matter to $CO_2$ by bacteria shows important fluxes in the carbon cycling in natural aquatic ecosystems (Azam, 1998).

Additionally, the expansion of the human activities has intensified substantially the nitrogen (N) and phosphorus (P) input into ecosystems, often resulting in the eutrophication of natural waters (Vitousek and Mooney, 1997). These nutrients regulate aquatic primary production and respiration (Cole et al., 2000; Biddanda et al., 2001). Highly productive waters due to external inputs of inorganic nutrients tend to be net autotrophic, acting as a net sink for $CO_2$ (Duarte and Agusti, 1998), while, those waters are
highly enriched in organic substrates may show persistent CO₂ supersaturation (Carpenter et al., 2001; Marotta et al., 2012). Despite consistent evidence supporting the role of the limitation by either P (Schnidler et al., 2008) or N (Camacho et al., 2003), N and P co-limitation may be also crucial on the biological metabolism in natural waters (Conley et al., 2009; Paerl, 2003). The biological N fixation can contribute to reduce the role of N inputs to stimulate biological activity in P-enriched waters, although more evidences is still needed for a better understanding of N inputs to stimulate biological activity in P-enriched waters, where P is commonly enriched by domestic discharges.

In this study, we assessed the short-term effect of N additions on pCO₂ in P-enriched humic and clear coastal lagoon waters. We tested the hypotheses that lake pCO₂ is controlled by N availability in P-enriched waters.

MATERIALS AND METHODS

STUDY AREA

The experiment was conducted using surface waters from two tropical coastal lagoons situated at the same conservation area (Restinga de Jurubatiba National Park) in the north of Rio de Janeiro State (Brazil). Both coastal lagoons are elongated, with their main axis perpendicular to the shoreline (maximum depth <4.5 m; area ~6.5 km²), oligotrophic (nutrients and chlorophyll a in the Table 1) and relatively close to each other (distance 6.8 km). The mean daily temperature in this area ranges from 20.7°C in July to 26.2°C in February. Despite high inter-annual variability, the minimum and maximum monthly rainfall are typically observed in August (38 mm) and December (182 mm; INMET, 1992). The tropical climate reflects in warm coastal lagoon waters (>20°C).

Carapebus coastal lagoon (23°12’12.29”S and 41°36’53.22”W) has clear waters, while Comprida coastal lagoon (22°16’43.55”S and 41°39’24.76”W) has highly humic and dark waters. The dark color and high Color:DOC ratio in coastal lagoons waters of this region commonly reflects a higher contribution of terrestrial organic compounds from Restinga vegetation (Marotta et al., 2010a).

EXPERIMENTAL DESIGN

Surface waters from both coastal lagoons were incubated in open-air 3.0 l glass bottles (microcosms) directly exposed to sunlight and other weather changes next to the studied coastal lagoons in June 2003. Solar incidence was the same for all microcosms, as they were placed close to each other, representing common light conditions for surface waters near to the interface with the atmosphere. However, the light attenuation indicated by Secchi depth at the sampling time was different between both, almost threefold above in Comprida lagoon than in Carapebus lagoon (1.6 and 0.5 m, respectively). No rainfall had been recorded during the incubations and the water temperature inside the microcosms varied between 25 and 30°C during the experiment. The evaporation contributed to negligible water level reduction inside microcosms, which was compensated by adding filtered waters from the same lake during the experiment.

The experiment was carried out over 15 days in highly P-enriched treatments in which different amounts of N were added, and the control (i.e., no N addition) per coastal lagoon. Three replicates were used in each experimental treatment and the control totalizing 24 microcosms. 1.4 μM of P as KH₂PO₄ and K₂HPO₄ (1:1 mass ratio to attenuate changes in pH) and 2,8,28, and 120 μM of N as KNO₃ were daily added to +N+P, ++N+P, and +++N+P treatments, respectively. Nutrients were carefully added during the morning. Total additions were 20 μM P and 40, 400, and 1600 μM N in +N+P, ++N+P, and +++N+P treatments, respectively, during the experiment. These concentrations and the corresponding N:P ratio were chosen to simulate the nutrient levels typically observed in urban coastal lagoons at the same region outside the Restinga de Jurubatiba National Park. The control microcosms showed only the low nutrient levels observed in both environments (0.4 and 0.9 μM P and 38.1 and 45.2 μM N, respectively in Carapebus and Comprida lagoons). All measurements were performed by the end of the experimental time (day 15).

ANALYTICAL METHODS

pH was measured with a precision of 0.01 pH units using a Analion PM 608 pH meter and the total alkalinity following the Gran’s titration (APHA, 1992). Temperature and salinity were measured with a calibrated Thermosalinometer YSI-30. CO₂ concentrations in waters were determined using the pH-alkalinity method (Mackereth et al., 1978) with appropriate corrections for temperature, altitude, and ionic strength as Cole et al. (1994). pCO₂ was calculated from Henry’s law with appropriate corrections for temperature and salinity (Cole and Caraco, 1998) as in Marotta et al. (2003a).

Water samples for total P and N analyses were previously frozen. Total P concentrations were measured by the molybdenum blue method with pre-digestion and total N concentrations by the sum of Kjeldahl N and NO₃ forms (APHA, 1992). Chlorophyll a concentrations (a proxy for phytoplankton biomass) in water samples filtered through Whatman GF/F filters (0.7 μm pore size) were extracted with ethanol in the dark for 24 h before fluorometric determination, using an excitation wavelength of 435 nm and
an emission wavelength of 673 nm (Varian Eclipse). Total suspended solids (TSS) were analyzed by the difference of weight before and after filtering and drying GF/F filters. Water samples filtered in these Whatman GF/F filters were also analyzed for color at 430 nm (Strome and Miller, 1978) using a Beckman DU 80 spectrophotometer (Fullerton, CA, USA) in a 1-cm quartz cuvette, and acidified to pH < 2.0 to determine DOC by the high-temperature catalytic oxidation method using a TOC-5000 Shimadzu Analyzer. The bacterial production was estimated from the rate of incorporation of 3H-leucine (Smith and Azam, 1992), assuming a 3H-leucine dilution factor of 2 and a carbon:protein ratio of 0.86 (Wetzel and Likens, 1991). A volume of 1.3 ml of water from the microcosms and placed in an eppendorf (1.5 ml). In all tubes, respirodors were added 20 μl of 3H-leucine (5× diluted standard solution, 5 μCi μmol⁻¹, Amersham), reaching a final concentration of 20 nM and incubated for 45 min in the dark. After the incubation period, were added in respsiders, 98 μl of 180% trichloroacetic acid (TCA) stopping and starting the reaction extraction. Each tube was washed sequentially with 5% TCA and 80% ethanol and 500 μl of scintillation cocktail (Aquass and Dupont) was added to each tube and the radioactivity measured in a liquid scintillator. Bacterial production was calculated by assuming a dilution factor of intracellular leucine equal to 2, and a protein rate of carbon equal to 0.86 (Wetzel and Likens, 1991).

### RESULTS

Humic water microcosms from Comprida coastal lagoon showed average pCO2 values 10-fold higher than clear waters from Carapibus coastal lagoon in the controls and treatments +N+P and ++N+P (Figure 1). A comparison between control and the less N-enriched treatment (+N+P) showed no significant difference in pCO2 among them, both in clear and humic waters (one-way ANOVA, p > 0.05; Figure 1). In contrast, these pCO2 values in control and +N+P treatments were significantly higher (Tukey–Kramer, p < 0.05; Figure 1) than those respective humic or clear water with higher N-additions (+ +N+P and + ++N+P), which were not also significantly different from each other (one-way ANOVA, p > 0.05; Figure 1). CO2 supersaturation was persistent in all humic treatments but not in clear water microcosms. The clear water microcosms presented a shift from being a source of CO2 in the controls and +N+P treatment to becoming a sink in + ++N+P and + ++N+P treatments in relation to the atmosphere (Figure 1).

The humic water microcosms also showed no significant difference (one-way ANOVA, p > 0.05) for pelagic chlorophyll a and TSS comparing controls and +N+P. Additionally, these less N-enriched humic treatments (control and +N+P) showed chlorophyll a significantly lower than + ++N+P or + ++N+P, and TSS significantly lower only than + ++N+P (Tukey–Kramer, p < 0.05; Figures 2 and 3). However, the clear water microcosms showed no differences between treatments when chlorophyll a and TSS were all compared (one-way ANOVA, p > 0.05; Figures 2 and 3, respectively). Farther, humic water microcosms did not show any periphytic biomass on the microcosm wall, while a thick green periphytic biomass (non-pelagic microalgae) was observed at the edges of the + ++N+P and + ++N+P treatments microcosms.

Bacterial production increased with the amount of N added in both humic and clear water lake microcosms. However, this increase was significantly higher and more evident at the + ++N+P and + ++N+P humic lake water microcosms (Tukey–Kramer, p < 0.05; Figure 4).

### DISCUSSION

Overall, the humic waters from Comprida coastal lagoon showed a persistent CO2 supersaturation reaching higher pCO2 values than the controls or respective treatments with clear waters from Carapibus coastal lagoon. The humic nature of waters in Comprida coastal lagoon reflects the terrestrial DOC supply to heterotrophic bacteria in these ecosystems (Farjalla et al., 2009). Allochthonous organic resources contribute to high respiration rates and subsequently pCO2 within most lake waters (Duarte and Prairie, 2005; Cole et al., 2007). These results support the conclusion that, in that humic coastal lagoons waters have higher pCO2 values than the clear coastal lagoons, probably due to the more intense respiration of organic substrates (Marotta et al., 2010a).
Furthermore, P-enriched microcosms with higher N additions showed higher bacterial production rates and algal biomass (pelagic or periphytic chlorophyll a), suggesting that the N supply might limit the heterotrophic and autotrophic metabolic activity in P-enriched tropical coastal lagoon. Despite N2 fixation may be sufficient to allow biomass to continue to be produced even with extreme reductions in N inputs into lakes (Schindler et al., 2008; Smith and Schindler, 2009), our experimental evidences confirm that N might be a relevant control on eutrophication in coastal waters as previously pointed out (Conley et al., 2009; Paerl, 2009).

The CO2 balance was determined by higher N inputs, as higher N treatments showed strong net decreases in pCO2, supporting the potential role of aquatic primary producers on CO2 uptake (Carignan et al., 2000). Both heterotrophs and autotrophs are stimulated by the nutrient additions (Biddanda et al., 2001), although the net autotrophy may be favored in the balance, a general trend often reported for natural waters (Duarte and Agusti, 1998). Our results contrasted with the persistence of CO2 supersaturation in highly organic-enriched waters from whole-lake (Cole et al., 2000) or mesocosm studies (Marotta et al., 2012) also assessing the effects of experimental nutrient additions. One plausible explanation for this discrepancy would be the absence of the bottom sediment as an additional source of organic substrates to CO2 production within the microcosms.

Increases in the phytoplankton biomass (pelagic chlorophyll a) contributed to net CO2 decreases in highly N- and P-enriched microcosms with humic waters of the Comprida coastal lagoon, but not in those with clear waters of the Carapebus coastal lagoon, where no significant differences in pelagic chlorophyll a were reported among all experimental treatments or controls. Indeed, the CO2 decrease observed in more N- and P-enriched clear water microcosms was mainly related to the presence of periphyton biomass on the walls, which was absent in the humic water microcosms likely due to light attenuation in their dark waters (Thomaz et al., 2001). In humic waters, TSS increase might be related to the phytoplankton growth, as the pCO2 decreased without any periphyton growth on the microcosm walls. On the other hand, higher concentrations of non-algal solids in suspension (TSS not related to changes in chlorophyll a or any external particulate input) are a proxy for large-bodied zooplankton, which can be strongly stimulated under eutrophic conditions by the availability of algae (Cole et al., 2000). Despite the source of
experimental bias related to any extrapolation from the periphyton response on the microcosm walls to whole ecosystem scale, our results support a potential relevance of N control under P-enriched conditions on algae community. The strength of this zooplankton control on phytoplankton, but not on periphyton biomass in highly nutrient-enriched lake waters was previously reported using experimental mesocosms in another lake at the same studied region as in this work (Guariento et al., 2011). Thus, the absence of common grazers on zooplankton in tropical coastal lagoons, i.e., snails and fish (Guariento et al., 2013), might have contributed to the increase of the periphyton biomass in clear water nutrient-enriched microcosms.

In conclusion, our hypothesis was confirmed as N is an import driver on pCO2 in P-enriched coastal lagoons waters. Higher experimental N enrichments promoted a significant pCO2 decrease in both humic and clear coastal waters. The N inputs even under P-enriched conditions might lead to intense net decreases in CO2 in coastal lagoons waters. Both inorganic N and organic substrates inputs modulate the CO2 balance in freshwater and brackish coastal lagoons.

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