Temperature-dependence of the relationship between pCO_2 and dissolved organic carbon in lakes

L. Pinho[1,2,3], C. M. Duarte[2,4,5], H. Marotta[6], A. Enrich-Prast[1,7]

1. Postgraduate Program in Ecology, Department of Ecology, Institute of Biology, Federal University of Rio de Janeiro, Rio de Janeiro. Av. Carlos Chagas Filho, 373 Cidade Universitária (Ilha do Fundão), P. O. Box 68020, Rio de Janeiro, RJ, Brazil.

2. Global Change Department, IMEDEA (CSIC-UIB), Mediterranean Institute for Advanced Studies. C. Miquel Marquès, 21, 07190, Esporles, I. Baleares, Spain.

3. Department of Chemical Oceanography, Rio de Janeiro State University, Rua São Francisco Xavier, 524 – Rio de Janeiro - RJ - CEP 20550-900, Pavilhão João Lyra Filho, 4º andar, sala 4018 Bloco E.

4. Faculty of Marine Sciences, King Abdulaziz University, P. O. Box 80207, 21589, Jeddah, Saudi Arabia.

5. The UWA Oceans Institute and School of Plant Biology, University of Western Australia, 35 Stirling Highway, 6009 Crawley, WA, Australia.

6. Department of Geography, Sedimentary Environmental Processes Laboratory (LAPSA/UFF), Federal Fluminense University. Av. Litorânea s/n, Campus Praia Vermelha, 24210 340, Niterói, RJ, Brazil.

7. Department of Environmental Changes, Linköping University, 581 83, Linköping, Sweden.

Corresponding author: luana.pinho@uerj.br

Abstract

The relationship between the partial pressure of carbon dioxide (pCO_2) and dissolved organic carbon (DOC) concentration in Brazilian lakes, encompassing 194 lakes across a wide latitudinal range in the tropics, was tested. Unlike the positive relationship reported for lake waters, which was largely based on temperate lakes, we found no significant relationship for low-latitude lakes (< 33°)**, despite very broad ranges in both pCO_2 and DOC. These results suggest substantial differences in carbon cycling** of low latitude **lakes, which must be considered when up scaling limnetic carbon cycling to global scales.**

Luana Queiroz Pinho 4/10/15 13:54
Eliminado: warm

Luana Queiroz Pinho 7/10/15 19:35
Eliminado: in

Luana Queiroz Pinho 4/10/15 13:55
Eliminado: warm

1.Introduction

Lakes cover less than 2% of the continent's surface [Downing et al., 2006; McDonald, 2012], but play a significant role in the global carbon (C) cycle [Cole et al., 1994; 2007; Tranvik et al., 2009], contributing significantly to C burial and emissions to the atmosphere [Cole et al., 2007; Downing et al., 2008 and Tranvik et al., 2009]. Dissolved organic carbon (DOC) represents a major C pool in lakes, with both autochthonous and allochthonous contributions [Duarte and Prairie, 2005; Cole et al., 2007; Prairie 2008; Tranvik et al., 2009], supporting heterotrophy [Sobek et al., 2007] and affecting key biological and physico-chemical processes involved in C cycling [Steinberg et al., 2006]. Large inputs of terrestrial organic C and its subsequent mineralization have been suggested to be a major driver of CO_2 supersaturation commonly encountered in lakes [Duarte and Prairie, 2005; Cole et al., 2007; Prairie 2008; Marotta et al., 2009].

The mechanistic connection between DOC and heterotrophic CO_2 production is believed to underpin the significant positive relationship between pCO_2 and DOC reported in comparative analyses [Houle, 1995; Sobek et al., 2005; Larsen et al., 2012]. However, recent analyses have revealed that the relationship between pCO_2 and DOC in lake waters is regionally variable and not universal [Lapiere and del Giorgio, 2012]. Hence, the relationship between pCO_2 and DOC reported in comparative analyses based on datasets dominated by temperate and high-latitude lakes (> 33°) may not be extrapolated for all types of lakes, mainly because the tropical low latitude lakes (< 33°) are generally underrepresented in global datasets [Raymond et al., 2013].

One priority of comparative studies is the latitudinal variance, where lake temperature, ice cover and mixing regime will differ and these climatically driven processes, in turn, should strongly influence OC cycling [Hanson et al., 2015]. At low latitudes, warm conditions over the whole year may increase the metabolic rates involved in the C cycling in terrestrial [Ometto e al., 2005] and aquatic [Marotta et al., 2009; Marotta et al., 2010] ecosystems on an annual basis compared to the high latitude lakes. High temperatures affect heterotrophic activity and the associated mineralization rates of organic matter in soils [Davidson et al., 2006], waters [López-Urrutia et al. 2007; Wohlers et al. 2008; Regaudie-de-Gioux and Duarte 2012] and aquatic sediments [Wadham et al., 2012; Gudasz et al., 2010, Marotta et al., 2014]. Enhanced heterotrophic activity in warm ecosystems would support high aquatic CO_2 production and subside high CO_2 evasion from global lake, to the atmosphere.

Luana Queiroz Pinho 24/9/15 11:35
Eliminado: Xenopoulos et al., 2003; ... [1]

Luana Queiroz Pinho 7/10/15 19:38
Eliminado:

Luana Queiroz Pinho 7/10/15 19:38
Eliminado: kinds …ypes of lakes, ma ... [2]

Luana Queiroz Pinho 4/10/15 13:56
Con formato: Sin Superíndice / Subíndice

Luana Queiroz Pinho 30/9/15 12:11
Con formato: EspacioPosterior: 10 pto, Control de líneas viudas y huérfanas, Adjust space between Latin and Asian text, Adjust space between Asian text and numbers

Luana Queiroz Pinho 24/9/15 11:48
Eliminado: of…priority ... [3]

Luana Queiroz Pinho 4/10/15 14:07
Eliminado: eading to CO_2 enrichmen ... [4]

Luana Queiroz Pinho 4/10/15 14:08
Con formato: Subíndice

The largest previous comparative analysis already published in the literature for global lake waters [Sobek et al., 2005] reported a significant positive relationship between DOC and pCO_2, and a non-significant variation of pCO_2 among lakes with changing temperature. However, both analyses were characterized by a paucity of low latitude data. A strong positive relationship between temperature and pCO_2 was observed when subtropical and tropical ecosystems were included in the data set [Marotta et al., 2009], likely caused by the potential increase in metabolic rates under warmer conditions [Brown et al., 2004, López-Urrutia et al., 2006]. Hence, the relationship between lake pCO_2 and DOC could also be temperature-dependent and, therefore, may differ between temperate and tropical lakes. The extensive low territory of Brazil, wich has a high density of lakes and ponds [Downing et al., 2006], is appropriate to examine general patterns in the tropics [e.g. Marotta et al., 2009, Kosten et al. 2010]. Here, we test the applicability of the relationship between pCO_2 and DOC, using inputs derived from high latitude dataset [Sobek et al., 2005] with added tropical and subtropical low latitude lakes from Brazil.

2.Methods

2.1.Study area and Lakes

Brazil extends from 5° 16' 20" North to 33° 44' 42" South, showing an area of approximately 8,547,000 km^2 constituting half of South America and encompasses a high diversity of low-latitude landscapes [Ab`Saber, 2003] that are predominantly located within tropical latitudes. We conducted a survey of pH, alkalinity and DOC between 2003 and 2011 in surface waters of 166 permanent lakes from 0 to 33° of south latitude across Brazil (Figure 1), yielding a total of 225 water samples. The lakes were sampled in representative biomes of Brazil: (1) Amazonia Forest (Amazonia Biome, n = 65), (2) Pantanal Floodplain (Pantanal Biome, n = 29) and the (3) Tropical (< 24^o of latitude) and (4) Subtropical (> 24^o and < 33^o of south latitude) Coasts, both in the Atlantic Forest Biome (n = 35 and n = 37 lakes, respectively; Figure 1). These biomes follow the classification of the Brazilian Institute of Geography and Statistics for biomes (IBGE 2004, ftp://geoftp.ibge.gov.br/mapas_tematicos/mapas_murais/biomas.pdf). Our dataset encompasses a broad inter-lake heterogeneity (n=166) for pH, alkalinity and DOC simultaneously sampled among Brazilian biomes and along the latitudinal gradient, independent of the year's season.

Luana Queiroz Pinho 5/10/15 00:30
Eliminado: A previous comparative analysis, characterized by a paucity of low latitude data, reported pCO_2 in lake waters to be independent of temperature [Sobek et al., 2005].

Luana Queiroz Pinho 5/10/15 00:32
Eliminado: However, a positive relationship between temperature and pCO_2 was observed when subtropical and tropical ecosystems were included in the analysis [Davidson et al., 2004; Marotta et al., 2009; Dillon et al., 2010].

Luana Queiroz Pinho 5/10/15 00:11
Eliminado: largely

Luana Queiroz Pinho 5/10/15 00:09
Eliminado: north-temperate- cold

Luana Queiroz Pinho 5/10/15 00:11
Eliminado: lakes

Luana Queiroz Pinho 7/10/15 19:43
Eliminado: Houle, 1995;

Luana Queiroz Pinho 7/10/15 19:43
Eliminado: ; Larsen et al., 2012

Luana Queiroz Pinho 7/10/15 19:43
Eliminado: ,

Luana Queiroz Pinho 5/10/15 00:11
Eliminado: to

Luana Queiroz Pinho 5/10/15 00:12
Eliminado: in

Luana Queiroz Pinho 5/10/15 00:08
Eliminado: Brazil has a large territory in the tropics, showing a high density of lakes and ponds [Downing et al., 2006], with a diversity of conditions that render them appropriate to examine general patterns [e.g. Marotta et al., 2009].

Luana Queiroz Pinho 7/10/15 19:44
Eliminado: that represents

Luana Queiroz Pinho 30/9/15 12:16
Eliminado: pCO_2

Luana Queiroz Pinho 30/9/15 12:16
Con formato: Fuente: Sin Cursiva

Luana Queiroz Pinho 30/9/15 12:16
Con formato: Fuente: Sin Cursiva

Luana Queiroz Pinho 7/10/15 19:45
Eliminado:

Luana Queiroz Pinho 30/9/15 12:16
Eliminado: pCO_2

The Amazonian Forest biome is formed by the most extensive hydrographic network of the globe; the Amazon River basin, wich occupies a total area about 6.11 million km² from its headwaters in the Peruvian Andes to the mouth in the Atlantic Ocean (ANA – www.ana.gov.br). The Amazon Forest is the Brazilian biome characterized by higher mean annual precipitation (approximately 2200 mm) and has warm mean air temperatures, approximately 25°C, high cloud coverage and high humidity with low fluctuations over the whole year [Chambers, 1999]. We sampled a wide variety of lakes, characteristic of different areas of the Amazonian Forest, encompassing "clear" (low DOC and suspended solids), "white" (low DOC and high suspended solids) and "dark" (high DOC and low suspended solids) lakes.

Luana Queiroz Pinho 7/10/15 19:46
Eliminado: ,

Luana Queiroz Pinho 7/10/15 19:46
Eliminado: that

Luana Queiroz Pinho 7/10/15 19:46
Eliminado: coupled to

Luana Queiroz Pinho 7/10/15 19:46
Eliminado: around

Luana Queiroz Pinho 7/10/15 19:47
Eliminado: and

The Pantanal Floodplain is the world's largest tropical freshwater wetland, extending across an area of about 150,000 km^2 between 16° and 20° S and 58° and 55° W [Por, 1995]. The annual averages temperature and precipitation are approximately 22°C and 1,000 mm, respectively [Mariot et al., 2007], with a strong seasonality and subsequent variation in the flooded area [Junk and Nunes da Cunha, 2005]. The high-water period occurs during the rainy summer (usually from September to December), and low waters typically during the dry winter (from March to July) [Hamilton, 2002].

Luana Queiroz Pinho 7/10/15 19:48
Eliminado: A

Luana Queiroz Pinho 7/10/15 19:48
Eliminado:

The Atlantic Forest biome extends along a broad latitudinal belt between 5° and 30° S from the subtropics to tropics and a narrow longitudinal section between 55° and 56° W and occupying an area of 1.11 million km^2 along the Brazilian coast (IBGE-www.ibge.gov.br). This biome is characterized by numerous shallow coastal lakes receiving high inputs of refractory organic matter [Farjalla et al., 2009] derived from the typical open xerophytic vegetation on sandy soils, where water retention is low [Scarano, 2002]. The mean air temperatures vary from 27° C in winter to 30°C in summer at the tropical coast [< 24° of latitude; Chellappa et al., 2009] and from 17 and 20° C at the subtropical coast [> 24° of latitude; Waechter, 1998]. The mean annual precipitation reaches 1,164 mm [Henriques et al., 1986] and 1,700 mm [Waechter, 1998] in the tropical and subtropical Brazilian coast, respectively. This biome is also characterized by strong seasonality, with rainy summers and dry winters [Chellappa et al., 2009].

Luana Queiroz Pinho 7/10/15 19:49
Eliminado: ,

Luana Queiroz Pinho 7/10/15 19:49
Eliminado:

2.2 Sampling Design and Analytical Methods

Our sampling design encompassed the most representative Brazilian biomes from tropical and subtropical coastal areas to tropical and subtropical forests (Amazon and Atlantic Forest) and inland wetlands (Pantanal), with the intra-lake heterogeneity and seasonal fluctuations randomly assessed and further integrated by means of each ecosystem. To analyze the relationship between pCO$_2$ and DOC in tropical lake waters ,we joined data of 194 lakes (< 33° of latitude) with both variables sampled at the same time, including 166 data from our own survey and 28 from the literature compilation. The values reported here represented, gathered in on an opportunistic manner, represent daily averages (N= 4 or 5 samples) for a given year´s season or/and one sampling time over different seasons, which were also both integrated by means of each lake. To test the globally importance of relationship between pCO$_2$ and DOC, we added our low latitude data (225) to the Sobek et al. [2005] dataset (4902 lakes) as this dataset had a paucity of tropical ecosystems (148 tropical lakes, but only one with pCO$_2$ and DOC sampled at the same time).

pH, salinity and temperature in waters were measured *in situ*. pH was determined using a pH meter (Digimed – DM2) with a precision of 0.01 calibrated with standard solutions (Mettler Toledo) of pH 4.01 and 7.00 units before each sampling hour. Temperature and salinity were measured using a Thermosalinometer (Mettler Toledo - SevenGo SG3) coupled to a probe inLab 737 previously calibrated with 0.01M KCl. Surface lake waters for total alkalinity and DOC analyses, taking care to avoid bubbles at about 0.5 m of depth using a 1L Van Dorn bottle. Alkalinity was determined in the field by the Gran´s titration with 0.0125 M HCl immediately after sampling [Stumm and Morgan, 1996]. Water samples for DOC were pre-filtered (0.7μm, Whatman GF/F) and preserved by acidification with H_3PO_4 85% to reach pH < 2.0 in sealed glass vials [Spyres et al., 2000]. In the lab, DOC was determined by high-temperature catalytic oxidation using a TOC-5000 Shimadzu Analyzer. pCO$_2$ concentrations in surface waters were calculated from pH and alkalinity following Weiss [1974], after corrections for temperature, altitude and ionic strength according to Cole et al. [1994].

We used additional corrections to address concerns about pCO$_2$ calculated from pH and TA (Gran titration) especially in low salinity or highly organic enriched DOC lake waters, even after corrections for temperature, altitude and ionic strength [Cole et al., 1994]. From the data set of Abril et al. [2015], we calculated fitted regression equations for median pH or DOC and respective % of pCO$_2$ corrections (Log pCO$_2$ correction (%) = - 0.9638 *pH + 7.755; R^2 = 0.9752, p < 0.005; Log pCO$_2$ correction (%) = - 0.9638 *pH +

Luana Queiroz Pinho 4/10/15 14:29
Eliminado: W

Luana Queiroz Pinho 4/10/15 14:30
Eliminado: plus

Luana Queiroz Pinho 4/10/15 14:28
Eliminado: published

Luana Queiroz Pinho 7/10/15 19:52
Eliminado: ,

Luana Queiroz Pinho 7/10/15 19:54
Eliminado: of pH

Luana Queiroz Pinho 7/10/15 19:55
Eliminado: were collected

Luana Queiroz Pinho 30/9/15 12:24
Eliminado: m

7.755; $R^2 = 0.9752$, $p < 0.005$). Statistical analyses were performed using raw and corrected data (Figure S2 and S3).

2.3. Statistical Analyses

The variables pCO_2 and DOC did not meet the assumptions of parametric tests even after logarithmic transformations [Zar, 1996], as data were not normally distributed (Kolmogorov-Smirnov, $p < 0.05$) and variances were heterogeneous (Bartlett, $p > 0.05$). Therefore, we used medians and non-parametric tests to compare these variables among biomes (Kruskall-Wallis followed by Dunn`s multiple comparison post hoc test, $p < 0.05$). The linear regression equations were fitted to compare our results with previous studies from Sobek et al., [2005]. Statistical analyses were performed using the software Graphpad Prism version 4.0 for Macintosh (GraphPad Software, San Diego, CA).

3.Results

The lake waters surveyed were warm across all biomes (median 25-75% interquartile range = 27.5° C, 25.2 – 30.1), but colder in subtropical coastal lakes (23.4° C, 20.0 – 26.2) compared to Pantanal and Amazonian lakes (29.5° C, 27.7 – 31.4 and 29.4° C, 27.6 – 31.0, respectively; Dunn's test, $p < 0.05$, Figure 2a). DOC concentrations were consistently high (6.3 mg C L^{-1}, 4.3 – 11.9) for all Brazilian biomes but significant lower in the Amazonian Forest (3.8 mg C L^{-1}, 2.7 – 5.8) than in the tropical coast (13.4 mg C L^{-1}, 6.1 – 32.8; Figure 2b; Dunn's test, $p < 0.05$). Most lakes (approximately 83% of raw data) showed surface waters supersaturated in CO_2 relative to atmospheric equilibrium (pCO_2 in atmospheric equilibrium of 403µatm, according Tans and Keeling 2014; data available in www.esrl.noaa.gov/gmd/ccgg/trends/global.html#global), with much higher pCO_2 values in Amazonian lakes (7,956 µatm, 3,033 – 11,346) than in subtropical coastal lakes (900 µatm, 391.3 – 3,212; Figure 2c; Dunn's test, $p < 0.05$).

The pCO_2 in surface waters of Brazilian lakes was independent of DOC concentrations (Linear regression for raw data, $p > 0.05$, Figure 3). After correcting our data and Sobek data with the contribution of organic acids on tatal alcalinity (TA) and subsequent pCO_2 data, using the fitted linear regression for the median values of the relative difference between calculated and measured pCO_2 with pH, both groups continuous with the same pattern observed before (not significant relationship for Tropical data ($p > 0.05$, $n = 194$) and positive relationship for Sobek

Luana Queiroz Pinho 5/10/15 00:41
Eliminado: Aware of the difficulties in determining the pCO_2 in lakes with high contribution of organic compounds [Wang et al., 2013] and also the possible overestimation due to the method of TA and pH rather than direct measurements of pCO_2 [Abril et al., 2015], we tested the relationship of pCO_2 and DOC considering the raw data and after correcting the contribution of organic acids on TA and subsequent pCO_2 data, using the fitted linear regression for the medians and averages values of the relative difference between calculated and measured pCO_2 with ranked pH and DOC values, according the data disposable of Abril et al., [2015] (Figure 3 and 4, more detail in support material).

Luana Queiroz Pinho 7/10/15 20:00
Eliminado: ,

Luana Queiroz Pinho 7/10/15 20:00
Eliminado: compared to

Luana Queiroz Pinho 7/10/15 20:00
Eliminado: compared to

Luana Queiroz Pinho 7/10/15 20:01
Eliminado: a

dataset (pCO_2 = 45,70 (± 1,84) x DOC + 623,7 (± 18,83), R^2= 0,12, p <0,0001, n = 4433), figure S2 a and b).

We did not find any positive relationship between pCO_2 and DOC for the Brazilian data (see more detail in support material), even after correcting our data and the Sobek data with the contribution of organic acids on TA and subsequent pCO_2 data; a fitted linear regression for the median values of the relative difference between calculated and measured pCO_2 with pH and median and average values with DOC was used. The range of pCO_2 for a similar DOC range in Brazilian lakes was larger than that reported by Sobek et al., [2005] for the dataset dominated by high-latitude cold lakes, despite the number of lakes in their dataset being much larger (see more detail in support material, figure S3).

We also calculated the DOC-pCO_2 relationship for two separate groups (DOC > and < 10 mg L^{-1}), the observed pattern was the same. We found non-significant relationships between DOC and pCO_2 using all data or only low latitude (<24°) lakes (linear regression, p>0.05), and significant positive linear regressions for those at high latitudes (>24°) in each DOC group, despite low R^2 values (R^2 = 0,08 and 0,03, p<0.05 for DOC > and < 10 mg L^{-1} respectively). Non-significant relationships in each Brazilian biome, with exception of Amazonia, also confirmed the DOC independence of pCO_2 in tropical lakes

4.Discussion

The Brazilian lakes sampled here were characterized by a prevalence of CO_2 supersaturation, consistent with general trends for lakes [e.g., Cole et al., 1994; Algesten et al., 2005] and previous reports for tropical lakes [Marotta et al., 2009]. The very high pCO_2 level observed here, with a median 900 and 8300 µatm for subtropical and Amazon lake waters, respectively, are consistent with those reported previously for the Amazon River and tributaries (2,000 - 12,000 µatm; Richey et al., [2002]), Amazon floodplain lakes (3,000 - 4,898 µatm; Rudorff et al., [2012]), Pantanal lakes and wetlands (2,732 - 10,620 µatm; Hamilton et al., [1995]), coastal lakes (768 - 9,866 µatm; Kosten et al., [2010]; 361 - 20,037 µatm; Marotta et al., [2010b]), and global values for tropical lakes (1,255 - 35,278 µatm; Marotta et al., [2009]), reservoirs (1,840 µatm; Aufdenkampe et al., [2011]) and wetlands (3,080 - 6,170 µatm; Aufdenkampe et al., [2011]).

The not significant or weakly negative relationship (Figure S2 and S3) between DOC and pCO_2 reported here for warm-low latitude lakes contrasted with significant positive relationships

Luana Queiroz Pinho 7/10/15 20:02
Eliminado: 4a and 4b

Luana Queiroz Pinho 7/10/15 20:03
Eliminado: Even after correcting our data and Sobek data with the contribution of organic acids on TA and subsequent pCO_2 data, using the fitted linear regression for the averages values of the relative difference between calculated and measured pCO_2 with pH and medians and average values with DOC, we continuous not observing a positive relationship between pCO_2 and DOC for Brazilian data (see more detail in support material). The range of pCO_2 for a similar DOC range in Brazilian lakes was larger than that reported by Sobek et al., [2005] for data set dominated by high-latitude cold lakes, despite the number of lakes in their data set being much larger.

Luana Queiroz Pinho 7/10/15 20:03
Con formato: Fuente: Sin Negrita

Luana Queiroz Pinho 7/10/15 20:04
Eliminado: ,

Luana Queiroz Pinho 7/10/15 20:04
Eliminado: earlier

Luana Queiroz Pinho 7/10/15 20:05
Eliminado: in

Luana Queiroz Pinho 7/10/15 20:05
Eliminado: (

Luana Queiroz Pinho 7/10/15 20:05
Eliminado: y)

Luana Queiroz Pinho 7/10/15 20:05
Eliminado: earlier

Luana Queiroz Pinho 7/10/15 20:06
Eliminado: (Figure 3a and 4a)

Luana Queiroz Pinho 7/10/15 20:06
Eliminado: (support material)

derived from previous datasets dominated by high-latitude lakes [Houle, 1995; Prairie et al., 2002; Jonsson et al., 2003; Sobek et al., 2005; Roehm et al., 2009; Lapiere and del Giorgio, 2012; Larsen et al., 2012]. The results presented show that warm-low latitude lakes range widely in pCO_2, reaching very high values, but tend to have comparatively more uniform DOC concentrations.

Tropical conditions based on higher annual temperatures and solar incidence typically increases the aquatic primary productivity activity [Paerl and Huisman 2008] that releases into waters the DOC produced by the CO_2 uptake of algae and submerged plants [Staehr and Sand Jansen 2007], which can withstand a negative variation in the pCO_2 with an increase in the DOC concentration [Marotta et al., 2010, Marotta et al, 2012, Hanson et al., 2015].The contrasting non-significant or weak negative relationship between $pCO2$ and DOC in warm Brazilian lakes here, with respect to that positive relationship for cold lake waters from the dataset of Sobek al. [2005], suggests a temperature dependence of the pCO_2 and DOC correlation in global lakes.

In conclusion, the finding that pCO_2 does not increase with DOC concentration in Brazilian tropical lakes rejects the hypothesis that DOC serves as a universal predictor for pCO_2 in lake waters [Larsen et al., 2012]. Even discounting a possible artifact of the method that could be causing an overestimation in the values of pCO_2, or considering the contribution of organic acids on the alkalinity, the pattern of no relationship between DOC and pCO_2 in the Tropical lakes was strongly confirmed. Despite limitations of our methodology, our work contributes data to the literature from tropical lakes that is frequently missing from global calculations.Therefore, our results suggest potentially important latitudinal differences from depositional aquatic environments, whose causes still need to be better addressed to improve accuracy of global C cycle models.

Authors Contribution

All authors contributed to the study design, data interpretation, and preparation or refinement of the manuscript. L. P. and H. M. performed the sampling and sample analyses.

Acknowledgments

This research is a contribution to projects from Brazilian research agencies (FAPERJ, CAPES

Luana Queiroz Pinho 7/10/15 20:07
Con formato: Fuente: Sin Negrita

Luana Queiroz Pinho 7/10/15 20:07
Con formato: Fuente: Sin Negrita

Luana Queiroz Pinho 5/10/15 03:37
Eliminado: Tropical conditions based on higher annual temperatures and solar incidence typically increases the aquatic primary productivity activity [Paerl and Huisman 2008] that releases into waters the DOC produced by the CO_2 uptake of algae and submerged plants [Staehr and Sand Jansen 2007], that can withstand a negative variation in the pCO_2 with an increase in the DOC concentration.

Luana Queiroz Pinho 7/10/15 20:08
Eliminado: t

Luana Queiroz Pinho 7/10/15 20:08
Eliminado:

Luana Queiroz Pinho 5/10/15 03:39
Eliminado: (

Luana Queiroz Pinho 5/10/15 03:39
Eliminado:),

Luana Queiroz Pinho 5/10/15 03:38
Con formato: Fuente: (Predeterminado) AdvPTimesB, 16 pt, Negrita, Portugués

Luana Queiroz Pinho 24/9/15 12:00
Eliminado: Despite limitations in the method of measuring the pCO_2, our work is important because it adds to the literature a data set about DOC and pCO_2 of tropical lakes so far not included in the global calculations until now.

Luana Queiroz Pinho 7/10/15 20:10
Eliminado: ed data

Luana Queiroz Pinho 7/10/15 20:10
Eliminado: wrote or

Luana Queiroz Pinho 7/10/15 20:10
Eliminado: commented

and CNPq). L. P. was supported by PhD scholarships from CAPES (period in Brazil) and FAPERJ (period in Spain). A. E-P. received postdoctoral and other CAPES and CNPq fellowships studies at the Linkoping University. H.M. was supported by a research fellowship from FAPERJ (Programa Jovem Cientista do Nosso Estado), and a research grant from CNPq

References

Abril, G., Bouillon, S., Darchambeau, F., Teodoru, C., Marwick, T., Tamooh, F., Ochieng Omengo, F., Geeraert, N., Deirmendjian, L., Polsenaere, P., and Borges, A.: Large overestimation of $p$$CO_2$ calculated from pH and alkalinity in acidic, organic-rich freshwaters. Biogeoscience, 12, 67-78, 2015.

Ab`Saber, A.: Os Domínios de Natureza no Brasil: Potencialidades paisagísticas, Ateliê editorial Brazil, 2003.

Algesten, G., Sobek, S., Bergstrom,A., Jonsson, A., Tranvik, L. and Jansson, M.: Contribution of sediment respiration to summer CO_2 emission from low productive boreal and subartic lakes, Microb. Ecol., 50, 529-535, 2005.

Aufdenkampe, A., Mayorga, E., Raymond, P., Melack, J. and Doney, S.: Riverine coupling of biogeochemical cycles between land, oceans, and atmosphere, Front. Ecol. Environ., 9, 1, 53-60, 2011.

Barreto, W.: Interpretation of Seasonal Variation of Metals and Abiotic Properties in a Tropical Lake Using Multivariate Analysis, Anal. Sci., 21 (3), 209-214, 2005.

Brow, J.,Gillooly, J., Allen, A., Savage, V., West, G.: Toward a metabolic theory of ecology, Ecology, 85 (7), 1771-1789, 2004.

Chambers, J., Higuchi, N., Schimel, J., Ferreira, L. and Melack, J.: Decomposition and carbon cycling of dead trees in tropical forests of the central Amazon, Oecologia, 122, 380-388, 1999.

Chellappa, N., Câmara, F. and Rocha, O.: Phytoplankton community: indicator of water quality in the Armando Ribeiro Gonçalves Reservoir and Pataxó Channel, Rio Grande do Norte, Brazil, Braz. J. Biol., 69, 241-251, 2009.

Cole, J. J., Caraco, N. F., Kling, G. W. and Kratz, T. K.: Carbon-Dioxide Supersaturation in the Surface Waters of Lakes, Science, 265, 1568-1570, 1994.

Cole, J. J., Prairie, Y. T., Caraco, N. F., McDowell, W. H., Tranvik, L. J., Striegl, R. G., Duarte, C. M., Kortelainen, P., Downing, J. A., Middelburg, J. J. and Melack, J.: Plumbing the global carbon cycle: Integrating inland waters into the terrestrial carbon budget, Ecosystems, 10, 171-184, 2007.

Davidson, E. and Janssens, I.: Temperature sensitivity of soil carbon decomposition and feedbacks to climate change, Nature, 440, doi:10.1038/nature04514, 2006.

Downing, J.A., Praire, Y.T., Cole, J.J., Duarte, C.M., Tranvik, L.J., Stiegl, R.G., McDowell, W.H., Kortelainen, P., Caraco, N.F., Melack, J.M. and Middleburg, J.: The global abundance and size distribution of lakes, ponds, and impoundments, Limnol. Oceanogr., 51, 2388-2397, 2006.

Downing, J. A., Cole, J. J., Middelburg, J. J., Striegl, R. G. and Duarte, C. M.: Sediment organic carbon burial in agriculturally eutrophic impoundments over the last century, Glob. Biogeochem. Cy., 22, GB1018, doi:10.1029/2006GB002854, 2008.

Duarte, C. M. and Prairie, Y. T.: Prevalence of heterotrophy and atmospheric CO_2 emissions from aquatic ecosystems, Ecosystems, 8, 862-870, 2005.

Farjalla, V. F., Amado, A. M., Suhett, A. L., and Meirelles-Pereira, F.: DOC removal paradigms in highly humic aquatic ecosystems, Environm. Sci. and Pol. Res. 16, 531-538, 2009.

Gudasz, C., Bastviken, D., Steger, K., Premke, K. and Sobek, S.: Temperature-controlled organic carbon mineralization in lake sediments, Nature, *466*, 478- 481, 2010.

Hamilton, S. K., Sippel, S.J. and Melack, J.M.: Oxygen depletion and carbon dioxide and methane production in waters of the Pantanal wetland of Brazil. Biogeochem. 30, 115-141, 1995.

Hamilton, S. K., Sippel S. J. and Melack, J. M.: Comparison of inundation patterns among major South American floodplains, Journ. of Geophysic. Res., 107, D20, 8038, doi:10.1029/2000JD000306, 2002.

Hanson, P., Pace, M., Carpenter, C., Cole, J. and Stanley, E.: Integrating Landscape Carbon Cycling: Research Needs for Resolving Organic Carbon Budgets of Lakes, Ecosystems, DOI: 10.1007/s10021-014-9826-9, 2015.

Henriques, R.P.B., Araújo, D.S.D. and Hay, J.D.: Descrição e classificação dos tipos de vegetação da restinga de Carapebús, Rio de Janeiro. Rev. Bras. Bot. 9: 173 – 189, 1986.

Houle, D., Carigan, R., Lachance, M. and DuPont, J.: Dissolved organic carbon and sulfur in southwestern Québec lakes: Relationships with catchment and lake properties, Limnol. Oceanogr., 40, 710-710, 1995.

Jonsson, A, Karlsson, J. and Jansson, M.: Sources of carbon dioxide supersaturation in clearwater and humic lakes in Northern Sweden, Ecosystems, 6, 224-235, 2003.

Junk, W. J. and Nunes de Cunha, C.: Pantanal: a large South American wetland at a crossroads, Ecol. Eng., 24, 391- 401, 2005.

Kosten, S., Roland, F., Da Motta Marques, D., Van Nes, E., Mazzeo, N., Sternberg, L., Scheffer, M. and Cole, J.: Climate-dependent CO_2 emissions from lakes, Glob. Biogeochem.Cy., 24, doi:10.1029/2009GB003618, 2010.

Lapierre, J. F. and del Giorgio, P. A.: Geographical and environmental drivers of regional differences in the lake $p$$CO_2$ versus DOC relationship across northern landscapes, Biogeosciences., 117, G03015, doi: 10.1029/2012JG001945, 2012.

Larsen, S., Andersen, T. and Hessen, D.: The $p$$CO_2$ in boreal lakes: Organic carbon as a universal predictor?, Glob. Biogeochem. Cy., 25, GB2012, doi:10.1029/2010GB003864, 2012.

López-Urrutia, Á. and Morán, X. A. G.: Resource limitation of bacterial production distorts the temperature dependence of oceanic carbon cycling, Ecology, 88, 817–822, 2007.

Mariot, M., Dudal, Y., Furian, S., Sakamoto, A., Valles, V., Valls, V., Fort, M. and Barbiero, L.: Dissolved organic matter fluorescence as a water-flow tracer in the tropical wetland of Pantanal of Nhecolândia, Brazil, Sci. Total Environm., 388, 184-193, 2007.

Marotta, H., Duarte, C. M., Sobek, S. and Enrich-Prast, A.: Large CO_2 disequilibria in tropical lakes, Glob. Biogeochem. Cy., 23, doi:10.1029/2008GB003434, 2009.

Marotta, H., Duarte, C. M., Pinho, L. and Enrich-Prast, A.: Rainfall leads to increased $p$$CO_2$ in Brazilian coastal lakes, Biogeosciences, 7, 1607-1614, 2010.

Marotta, H., Duarte, C.M., Guimarães-Souza, B. and Enrich-Prast, A.: 2012 Synergistic control of CO2 emissions by fish and nutrientes), Oecologia, 168, 3, 839-847, 2012.

Marotta, H., Pinho, L. Gudasz, C., Bastviken, D., Tranvik, L. and Enrich-Prast, A.: Greenhouse gas production in low-latitude lake sediments responds strongly to warming, Nat. Clim. Change, 4, 467-470, 2014.

McDonald, C., Rover, J., Stets, E. and Striegl, R.: The regional abundance and size distribution of lakes and reservoirs in the United States and implications for estimates of global lake extent, Limnol. Oceanogr., 57, 597-606, 2012.

Ometto, J. P. H. B., Nobre, A., Rocha, H., Artaxo, P. and Martinelli, L.: Amazonia and the modern carbon cycle: lessons learned, Oecologia, 143, 483-500, 2005.

Paerl, H. and Huisman, J., Blooms like it hot, Science, 320, 57-58, 2008.

Prairie, Y.: The summer metabolic balance in the epilimnion of southeastern Quebec lakes, Limnol. and Oceanogr., 47, 1, 316-321, 2002.

Prairie, Y. T.: Carbocentric limnology: looking back, looking forward, Canadian Journal of Fisheries and Aquatic Sci., 65(3), 543-548, 2008.

Raymond, P., Hartmann, J., Lauerwald, R., Sobek, S., McDonald, C., Hoover, M., Butman, D., Striegl, R., Mayorga, E., Humborg, C., Kortelainen, P., Durr, H., Meybeck, M., Ciais, P. and Guth, P.: Global carbon dioxide emissions from inland waters. Nature, 503, 355- 359, 2013.

Regaudie-de-Gioux A and Duarte C.M.: Temperature dependence of planktonic metabolism in the ocean, Glob. Biogeochem. Cycles, 26, GB1015, 2012.

Richey, J. E., Melack, J. M., Aufdenkampe, A. K., Ballester, V. M., and Hess, L. L.: Outgassing from Amazonian rivers and wetlands as a large tropical source of atmospheric CO_2, Nature, 416(6881), 617–620, 2002.

Roehm, C., Prairie, Y. T. and del Giorgio, P.: The $p$$CO_2$ dynamics in lakes in the boreal region of northern Québec, Canada, Global Biogeochem. Cy., 23, GB3013, doi:10.1029/2008GB00329, 2009.

Rudorff, C. M., Melack, J. M.; MacIntyre, S.; Barbosa, C. C. F.; and Novo, E. M. L. M.: Seasonal and spatial variability of CO_2 emission from a large floodplain lake in the lower Amazon, J. Geophys. Res., 117, G01002, doi: 10.1029/2011JG001699, 2012.

Scarano, F.: Structure, function and floristic relationships of plant communities in stressful habitats marginal to the Brazilian Atlantic rainforest, Annals of botan., 90, 4, 517-524., 2002.

Stumm, W. and Morgan, J. J.: Aquatic chemistry: chemical equilibria and rates in natural waters, vol 3. Wiley, New York, 1996.

Sobek, S., Tranvik, L. J. and Cole, J. J.: Temperature independence of carbon dioxide supersaturation in global lakes, Global Biogeochem. Cy., 19, GB2003, doi:10.1029/2004GB002264, 2005.

Spyres, G., Nimmo, M., Worsfold, P., Achterberg, E., Miller, A.: Determination of dissolved organic carbon in seawater using high temperature catalytic oxidation techniques, Trends in Analytic Chem., 19, 8, 498-506, 2000.

Staehr, P. and Sand-Jansen, K.: Temporal dynamics and regulation of lake metabolism, Limnol. and Oceangr. 52 (1), 108-110, 2007.

Steinberg, C. E. W., Kamara, S., Prokhotskaya, V., Manusadzianas, L., Karasyova, T., Timofeyev, M., Jie, Z. Paul, A., Meinelt, T., Farjalla, V., Matsuo, A., Burnison, B. and Menzel, R.: Humic substances in the environment with an emphasis on freshwater systems, Environm. Sci. Pollut. Res. Int., 15, 15-16, 2006.

Tans, P. and Keeling, R.: Trends in Atmospheric Carbon Dioxide. National Oceanic and Atmospheric Administration Earth System Research Laboratory Global Monitoring Division; Scripps Institution of Oceanography. http://www.esrl.noaa.gov/gmd/ccgg/trends/. (Accessed 19.05.15).

Tranvik, L.J., Downing, J.A., Cotner, J.B., Loiselle, S.A., Striegl, R.G., Ballatore, T.J., Dillon, P., Finlay, K., Fortino, K., Knoll, L.B., Kortelainen, P.L., Kutser, T., Larsen, S., Laurion, I., Leech, D.M., McCallister, S.L., McKnight, D.M., Melack, J.M., Overholt, E., Porter, J.A., Prairie, Y., Renwick, W.H., Roland, F., Sherman, B.S., Schindler, D.W., Sobek, S., Tremblay, A., Vanni, M.J., Verschoor, A.M., von Wachenfeldt, E. and Weyhenmeyer, G.A.: Lakes and reservoirs as regulators of carbon cycling and climate. Limnol. Oceanogr., 54, 2298-2314, 2009.

Wadham, J., Arndt, S., Tulaczyk, S., Stibal, M., M. Tranter, M., Telling, J., Lis, G., Lawson, E., Ridgwell, A., Dubnick, A., Sharp, M., Anesio, A. and Butler, C.: Potential methane reservoirs beneath Antarctica, Potential methane reservoirs beneath Antarctica, Nature, 488, 633-637, 2012.

Waechter, J. L.: Epiphyticorchids in eastern subtropical South America. Proc. 15th World Orchid Conference. Naturalia Publications, pp. 332-341, 1998.

Weiss, R. F.: Carbon Dioxide in water and seawater: The solubility of non-ideal gas, Mar. Chem., 2, 203-215, 1974.

Wohlers, J., Engel, A., Zollner, E., Breithaupt, P., Jurgens, K., Hoppe, H., Sommer, U. and Riebesell, U.: Changes in biogenic carbon flow in response to sea surface warming. PNAS, 106,17, 7067-7072, 2008.

Zar, J. H.: Biostatistical analysis, 3 ed., Prentice Hall., New Jersey, 1996.

Figures and subtitles

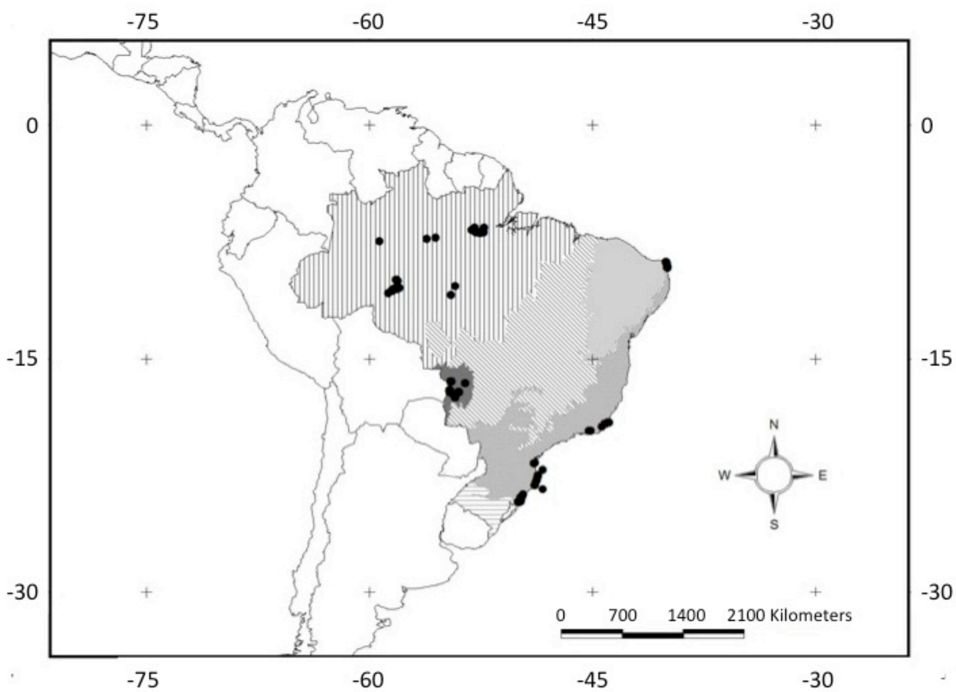

Figure 1. Geographic location of Brazilian lakes sampled in different biomes (IBGE 2004, available in ftp://geoftp.ibge.gov.br/mapas_tematicos/mapas_murais/biomas.pdf): Amazonia Forest (vertical lines), Pantanal Floodplain (dark gray), and Atlantic Forest (gray; Tropical and Subtropical costal lakes).

Unknown
Con formato: Fuente: (Predeterminado) Times New Roman

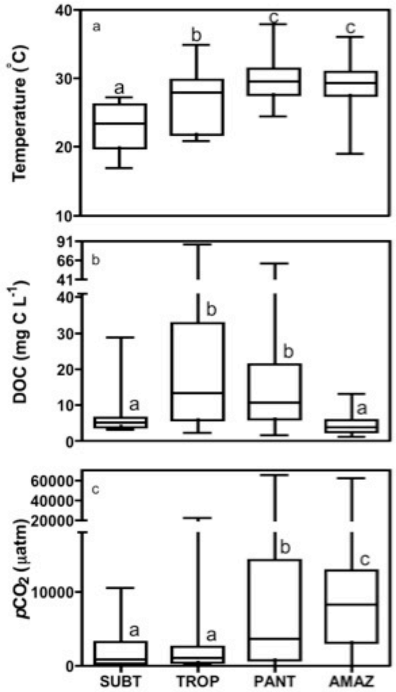

Figure 2. Values of (A) temperature (ºC), (B) DOC concentrations (mg C L^{-1}) and (C) $p\mathrm{CO_2}$ concentration (µatm) of Brazilian lakes sampled from different biomes, as defined by (SUBT) Subtropical Coastal lakes (n = 37), (TROP) Tropical coastal lake (n = 63), (PANT) Pantanal Floodplain (n = 58) and (AMAZ) Amazonia Forest (n = 67). The line depicts the median. The boxes show the quartiles, and the whiskers mark the 10th and 90th percentiles. Different lowercase letters near the boxplot indicate significant statistic differences between the groups (Kruskall-Wallis followed by Dunn`s multiple comparison post hoc test, $p < 0.05$).

Unknown
Con formato: Fuente: (Predeterminado) Times New Roman, Color de subrayado: Color personalizado(RGB(15;14;14)), Color de fuente: Color personalizado(RGB(15;14;14))

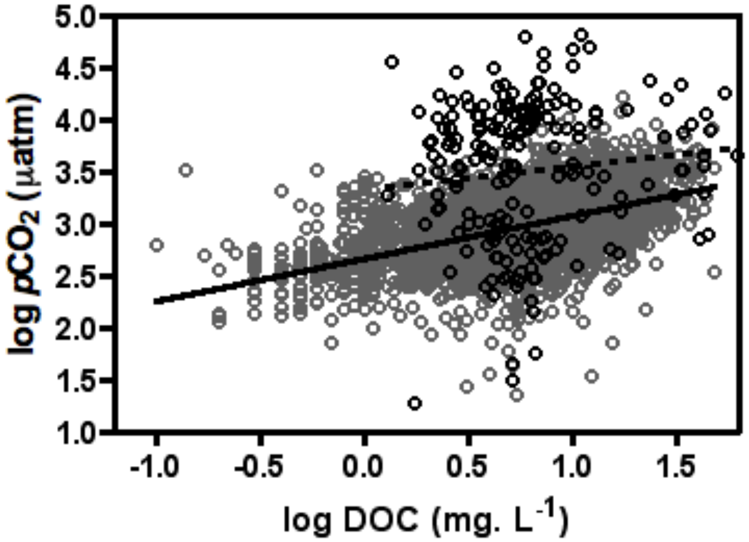

Figure 3. Comparisons of pCO_2 against DOC concentrations for lakes from this study (black circles) and from Sobek et al. [2005] (gray circles). Each point in the plot represents one measurement. The dashed line represents the linear regression for all Brazilian data points (not significant; $p > 0.05$), and the solid line represents the linear regression from Sobek et al. [2005] (log pCO_2 (µatm) = 2.67 + 0.414 log DOC (mg C L^{-1}); $R^2 = 0.26$; $p < 0.05$).

07th October 2015.

Dear Editor,

Attached, please find the second revised version of the manuscript “Temperature-dependence of the relationship between pCO_2 and dissolved organic carbon in lakes” to be reconsidered for publication in Biogeosciences. To address valuable criticisms from reviewers and the editor, we have included here a point-by-point response with details of the actions that have led to an improved manuscript. Finally, the English language has been thoroughly revised by a company specializing in English language review (with certification attached).

The main purpose of this manuscript was to report that the global relationship between pCO_2 and DOC in lake waters disappears once tropical ecosystems are included. This novel finding improves our knowledge of the C cycling of inland waters along the latitudinal gradient and provides new insights for further studies.

We thank the editor and both the reviewers for their very constructive suggestions.

Sincerely,

Luana Pinho on behalf of the co-authors

Interactive comment on “Temperature-dependence of the relationship between pCO_2 and dissolved organic carbon in lakes” by L. Pinho et al.

Response to Anonymous Referee #1

1) Comments from referee #1

General comments

The manuscript, "Temperature-dependence of the relationship between pCO_2 and dissolved organic carbon in lakes" by Pinho et al. has been improved substantially by the authors' revisions. It is my feeling that this is a timely study that will be well-cited, as it presents data on ecosystems often underrepresented in the carbon cycling literature. Methods are clear and appropriate, and the authors have done a good job of addressing potential shortcomings of this methodology. As in prior versions of the paper, it is concise and generally well written, though many of the new revisions contain grammatical errors that need attention. Specific comments are below.

Author´s response:
First, we thank referee #1 for confirming that our research topic could reveal potentially important unknown processes of C cycling at warmer tropical regions.

After all corrections, we sent the new version of the manuscript for review by a native English speaker to remove any grammatical errors.

Minor specific comments

1. ln 34: This point can be supported with fewer references.

1. Author´s response: We agree and reduced the number of references here.

The text now reads:

(Page 2, line 35) “Dissolved organic carbon (DOC) represents a major C pool in lakes, with both autochthonous and allochthonous contributions [Duarte and Prairie, 2005; Cole et al., 2007; Prairie 2008; Tranvik et al., 2009]”.

2. ln 36: the word “tactility” should be removed so that the sentence reads, “….supporting heterotrophy, …..”

2. Author´s response: We agree and deleted the word “tactility”.

The text now reads:

(Page 2, line 36) “supporting heterotrophy [Sobek et al., 2007] and affecting key biological and physico-chemical processes involved in C cycling [Steinberg et al., 2006].”

3. ln 39: Again, though I appreciate the thoroughness in updating references, this point can be supported with fewer citations.

3. Author´s response: We agree and also reduced the number of references here.

The text now reads:

(Page 2, line 38) "Large inputs of terrestrial organic C and its subsequent mineralization have been suggested to be a major driver of CO_2 supersaturation commonly encountered in lakes [Duarte and Prairie, 2005; Cole et al., 2007; Prairie 2008; Marotta et al., 2009]."

4. ln 49: Wording is unclear. I'm not sure what is meant by the "global caught".

4. Author´s response: We agree and replaced the word "caught" by "datasets".

The text now reads:

(Page 2, line 46) "Hence, the relationship between pCO_2 and DOC reported in comparative analyses based on datasets dominated by temperate and high-latitude lakes (>33^o) may not be extrapolated for all types of lakes, mainly because the tropical low latitude lakes (< 33^o) are generally underrepresented in global datasets".

5. ln 50: Should read, "One of the priorities of a comparative study…" or "One priority of a comparative study is…."

5. Author´s response: We agree and replaced the sentence with the suggested text.

The text now reads:

(Page 2, line 50) "One priority of comparative studies is the latitudinal variance, where lake temperature, ice cover and mixing regime will differ and these climatically driven processes, in turn, should strongly influence OC cycling [Hanson et al., 2015]."

6. ln 178: average, not averages
6. Author´s response: We removed this sentence.

7. ln 180: Change to "….we did not find…" rather than "…we continuous observing…"
7. Author´s response: We agree and changed the sentence as suggested.

The new text reads:
(Page 7, line 185) "we did not find any positive relationship between pCO_2 and DOC for Brazilian data".

8. ln 217: This sentence makes a very important point, but has very awkward construction. I would consider shortening it to something like, "Despite limitations of our methodology, our work contributes data to the literature from tropical lakes that is frequently missing from global calculations."

8. Author´s response: We agree and incorporated the suggested version of this sentence.

9. Figure 1: Enlarge axis labels.

9. Author´s response: We agree and addressed this change. The new figure is shown at the end of the comments section.

10. Figure 3: Use open circles, or if making plots in R (ggplot2 package), you could use alpha blending so that your trendline is visible. As presented, it is not visible.

10. Author´s response: We agree and changed figure 3 as suggested. The new figure is shown at the end of the comments section.

11. Figure 3: Edit the x-axis labels for the break between 30 and 40 so that they are farther apart. Alternately, you could just remove either the 30 or 40 from the axis labels.

11. Author´s response: We agree and changed figure 3 as suggested. The new figure is shown at the end of the comments section.

12. Figure 4: Same comment as above regarding trendline. Use the same format and axis scale for figures 3 and 4. I would suggest combining figures 3 and 4 into a block of 4 figures (a-d), so that the corrected plots are easily compared to the original.

12. Author´s response: We agree and combined both figures 3 and 4 as suggested. The new figure is shown at the end of the comments section.

Response to Anonymous Referee #3

1) Comments from referee #3

This paper reports the relationship of pCO$_2$ and DOC in Brazilian tropical lakes, indicating no or weak relationship was observed. These findings are intriguing and they are different from those derived from lower temperature temperate/boreal lakes. The authors' work adds new data to our collective body of knowledge and is important.

Author´s response: We thank the reviewer for recognizing the importance of our low-latitude dataset in a global context.

General comments:

1. As a whole, "introduction" is clearly addressed. The authors summarize the previous studies of the relationship of pCO$_2$ and DOC, indicating a lack of data in tropical lakes. Then the authors introduce the importance of temperature. However, logic transition is not smooth in some paragraphs and somewhat confusing. The specific comments are listed below:

a. Line 52, what do "low latitudes" mean? I think that the authors want to compare the differences between high latitudes and low latitudes. It might not be appropriate to use ONLY "temperate systems" and "WINTER" to represent high latitudes conditions.

1.a. Author´s response: In the text, we clarified that low latitudes mean < 33^o, including the parenthesis "(<33^o)" after the first reference of the expression "low latitudes" in the abstract (page 1, line 25) and introduction (page 2, line 48). We prefer to keep low and high latitudes, as the term "high latitudes" includes all those lakes situated at latitudes >33^o (e.g., subtropical, boreal or polar) as well as temperate lakes. Additionally, high latitude conditions do not represent only one season (like winter) but the entire year.

The text now reads:

(Page 2, line 52) "At low latitudes, warm conditions over the whole year may increase the metabolic rates involved in the C cycling in terrestrial [Ometto et al., 2005] and aquatic [Marotta et al., 2009; 2010] ecosystems on an annual basis compared to the high latitude lakes**."**

b. Line 59, "heterotrophic activity…support high fluxes of CO2, leading to CO2 enrichment". Here "fluxes" should be "production". In contrast, CO2 outgassing (high flux) from lakes to the atmosphere leads to a decrease in CO2 content in lake waters.

1.b. Author´s response: We replaced the word "fluxes" by "production" and clarified that the heterotrophic activity could support increases in CO2 outgassing from lakes to the atmosphere.

The text now reads:

(Page 3, line 58) "Enhanced heterotrophic activity in warm ecosystems would support high aquatic CO_2 production and subside high CO_2 evasion from global lake water to the atmosphere**."**

c. Line 62, the authors indicated that there was a paucity of low latitude data in Sobek et al. (2005). It is not correct. Sobek et al. (2005) included 148 tropical lakes. If the authors want to divide tropical lakes into high-temp and low-temp tropical regimes and indicate Sobek et al. (2005) doesn't have high temp data, they should address it clearly here.

1.c. Author´s response: We clarified that the larger dataset (4902 lakes) already published in the literature by Sobek et al. [2005] included 148 tropical lakes with pCO_2 data, but only one of these lakes had data for both pCO_2 and DOC values. In this way, our novel dataset included 225 points of pCO_2 and DOC data sampled at the same time, unlike that used by Sobek et al. [2005].

The text now reads:

(Page 5, line 124) "To analyze the relationship between pCO_2 and DOC in tropical lake waters, we joined data on 194 lakes (< 33^o of latitude) with both variables sampled at the same time, including 166 data samples from our own survey and 28 from the literature compilation."

(Page 5, line 130) "To test the global importance of the relationship between *p*CO_2 and DOC, we added our low latitude data (225) to the Sobek et al. [2005] dataset (4902 lakes) as this dataset had a paucity of tropical ecosystem data (148 tropical lakes, but only one with *p*CO_2 and DOC sampled at the same time)."

d. The last paragraph in Introduction: The authors should address that pCO2 and DOC are related but pCO2 is independent of temperature at high latitudes FIRST. Otherwise, it's confusing why temperature is related to pCO2 and DOC. However, here the relationship of pCO2 and DOC might be influenced "OR NOT" by temperature in tropical lakes.

1.d. Author´s response: We agree and rewrote this paragraph.

The text now reads:

(Page 3, line 61) "The largest previous comparative analysis already published in the literature for global lake waters [Sobek et al., 2005] reported a significant positive relationship between DOC and *p*CO_2 and a non-significant variation of *p*CO_2 among lakes with changing temperature. However, both analyses were characterized by a paucity of low latitude data. A strong positive relationship between temperature and *p*CO_2 was observed when subtropical and tropical ecosystems were included in the dataset [Marotta et al., 2009], likely caused by the potential increase in metabolic rates under warmer conditions [Brown et al., 2004, López-Urrutia et al., 2006]. Hence, the relationship between lake *p*CO_2 and DOC could also be temperature-dependent and, therefore, may differ between temperate and tropical lakes. The extensive low latitude territory of Brazil, which has a high density of lakes and ponds [Downing et al., 2006], is appropriate to examine general patterns in the tropics [e.g., Marotta et al., 2009, Kosten et al. 2010]. Here, we test the applicability of the relationship between *p*CO_2 and DOC using inputs derived from a high latitude dataset [Houle, 1995; Sobek et al., 2005; Larsen et al., 2012] with added tropical and subtropical low latitude lakes from Brazil."

2. There is no detailed description of methods. Detailed methods need to be addressed for a comparison of different datasets, including method precision/accuracy, progress of calculation, and unbiased data processing between different datasets. ONLY Based on these work, the authors can yield confident results. Some specific comments listed below:

a. Data source of published data? In line 122.

2.a. Author´s response: We described the dataset compiled from the literature in the support material. It is included at the end of the comments.

b. Line 126, pH is measured on which pH scale and is calibrated against what standards? If the standard has a precision of 0.1, how could we get pH results with a precision of 0.01 pH unit?

2.b. Author´s response: We agree and corrected the precision of standards.

The text now reads:

(Page 5, line 134) "pH, salinity and temperature were measured *in situ*. pH was determined using a pH meter (Digimed – DM2) with a precision of 0.01 calibrated with standard solutions (Mettler Toledo) of pH 4.01 and 7.00 units before each sampling hour."

c. Line 136-137, pCO2 calculated from pH and TA should use dissociation constants of carbonic acid. Weiss (1974) provides the equation of CO2 solubility. The authors should address which set of constants is used, and if these constants are valid for low salinity lake waters?

2.c. Author´s response: We agree and rewrote the sentence.

The text now reads:

(Page 5, line 147) "We used additional corrections to address concerns about pCO_2 calculated from pH and TA (Gran titration) especially in low salinity or highly organic enriched DOC lake waters, even after corrections for temperature, altitude and ionic strength (Cole et al., 1994). From the dataset of Abril et al. (2015), we calculated fitted regression equations for the median pH or DOC and respective % of pCO_2 corrections (Log pCO_2 correction (%) = - 0.9638 *pH + 7.755; R^2 = 0.9752, $p < 0.005$; Log pCO_2 correction (%) = - 0.9638 *pH + 7.755; R^2 = 0.9752, $p < 0.005$). Statistical analyses were performed using raw and corrected data (full details in the supporting information)."

d. Line 138, the authors might cite correction methods as G. W. Kling, G. W. Kipphut, M. C. Miller, Hydrobiologia, 240, 23 (1992).

2.d. Author´s response: See the author's response addressing comment 2.c for Reviewer 2.

e. Line 139-141. "aware of the difficulties in determining the pCO2…" It should be noted that there has no difficulty in determining (such as direct measurement) of pCO2. The problem in pCO2 calculation is raised from the existence of Organic-TA. As well, there is no problem in TA and pH methods. In addition, sentence construction in the following sentences in the paragraph should be revised to make it clear. Avoid using "TA" and "Alkalinity" interchangeably.

2.e. Author´s response: See the author's response addressing comment 2.c for Reviewer 2. In addition, we revised the whole manuscript to avoid confusion between both terms "TA" and "alkalinity."

f. The authors should address clearly how they remove the influence of TA in method section, or at least describe it concisely here and put details in the supplementary.

2.f. Author´s response: See the author's response addressing comment 2.c for Reviewer 2.

3. In the results part, the authors never address why they compare specific regions for temperature and DOC beyond a general description. It gives me a sense that the authors will

compare their differences and the magnitude of temperature and DOC should be related to somewhat.

3. Author´s response: Our aim here is to report DOC- *p*CO_2 relationships in lake waters along the latitudinal and temperature gradient at a broad spatial scale inside and outside of tropical borders (<33^o of latitude) and to avoid speculative discussions of possible differences among Brazilian biomes and their particularities. See the author's detailed response addressing comments 4 and 5 for Reviewer 2.

4. There is still no solid and strong evidence to support the primary conclusion of non-significant or weak negative relationship of pCO2 and DOC in Fig. 3a and 4a. What I see is a strong relationship for pCO2 and DOC at DOC<10 region with high pCO2. In contrast, there is no relationship for low pCO2 region. The authors should separate these regions to see if there is spatial difference which can be explained by different biomes.

4. Author´s response: This is not the purpose of our article. Our goal is only to provide evidence that the *p*CO_2 x DOC relationship cannot be considered universal for any type of lake. However, we made the correlations according your suggestions and found non-significant relationships between DOC and *p*CO_2 for all data or only low latitude (<33^o) lakes and significant relationships for those at high latitudes (>33^o) in each DOC group (DOC > and < 10 mg L^{-1}). With the exception of the Amazon only, we found non-significant relationships between DOC and *p*CO_2 for all biomes. Therefore, even with these results, our claim that the relationship *p*CO_2 x DOC is not universal for all types of lakes continues to be justified, thus supporting our conclusions.

We included the next paragraph in the manuscript:

> **"Even calculating the DOC-*p*CO_2 relationship for two separate groups (DOC > and < 10 mg L^{-1}), the observed pattern was the same. We found non-significant relationships between DOC and *p*CO_2 using all data or only low latitude (<24^o) lakes (linear regression, p>0.05), and significant positive linear regressions for those at high latitudes (>24^o) in each DOC group, despite low R^2 values (R^2 = 0.08 and 0.03, p<0.05 for DOC > and < 10 mg L^{-1} respectively). Non-significant relationships in each Brazilian biome, with exception of Amazonia, also confirmed the DOC independence of *p*CO_2 in tropical lakes."**

5. There is no evidence of temperature related issue for the relationship of pCO2 and DOC in tropical lakes to support authors' point. The authors do not need to remove fig. 3b in the previous manuscript. Using Ln(pCO2) vs. temperature can explain the physical control of pCO2 over temperature (see Takahashi et al. (1993) published in GBC). The authors also can check the relationship between DOC and temperature in tropical/temperature lakes. If there is no relationship between DOC and temperature, latitudinal difference is more convincible than temperature difference.

5. Author´s response: The control of temperature on *p*CO_2 is not only a physical-chemical issue but also an issue of biological concern. In addition, latitude and temperature are highly autocorrelated, and DOC could be from aquatic or terrestrial primary producers, supporting a net CO_2 sink or source. Because of these constraints and to determine the causes of fluctuation between DOC and *p*CO_2 from our study design, we preferred to avoid speculative discussions and kept the focus on reporting general patterns. Thus, the aim here was to report a novel pattern of non-universal DOC- *p*CO_2 behavior in global lake

waters after including tropical waters in the dataset, which reveals implications on the C cycling of inland waters along the latitudinal gradient.

The correlation of temperature and log of DOC and temperature and log of pCO_2 was showed in the figure 3 of paper disponible in the biogeoscience discussion, and removed after previous corrections.

6. Suitable interpretation of the mechanisms controlling the weak relationship of pCO2 and DOC and its broad impact are recommended in discussion.

6. Author´s response: We added more citations to address this but avoided unnecessary speculation.

The text now reads:

(Page 8, line 220) "Tropical conditions based on higher annual temperatures and solar incidence typically increase the aquatic primary productivity activity [Paerl and Huisman 2008] that releases into waters the DOC produced by the CO_2 uptake of algae and submerged plants [Staehr and Sand Jansen 2007], which can withstand a negative variation in the pCO_2 with an increase in the DOC concentration [Marotta et al., 2010; 2012; Hanson et al., 2015]."

Minor comments: Gramma/sentence construction/spelling need attention.

1. Line 48, "because of" should be "because".

1. Author´s response: We replaced "because of" with "because".

2. Line 79, "conducted a survey of pCO2…" should be "of pH, TA, and DOC". Here pCO2 is calculated from pH and TA. Same as in line 87.

2. Author´s response: We replaced "conducted a survey of pCO_2…" by "conducted a survey of pH, TA, and DOC …" in both cases.

3. Line 80, "0 to 33 °" should add "south", same as in Line 83.

3. Author´s response: We added the word "south" in both cases.

4. Line 86, link ftp://geoftp.ibge.gov.br/mapas_tematicos/mapas_murais/biomas is invalid, should be "biomas.pdf".

4. Author´s response: We added ".pdf" in the link.

5. Line 133, GF/F filter is 0.7 um, not mm.

5. Author´s response: We changed "m" to "µ".

6. Line 175, "(pCO2 = 45,70 ± 1,84 x DOC + 623,7 ± 18,83, R2= 0,12, p <0,0001, n = 4433)," I have no idea if it's "pCO2 = (45,70 ± 1,84) x DOC + (623,7 ± 18,83)???

6. Author´s response: We corrected the formula:"pCO2 = 45,70 (± 1,84) x DOC + 623,7 (± 18,83)"

7. The authors wrongly replace decimal point with comma in a lot of places. Such as line 175…

7. Author´s response: We revised the entire text.

8. Fig. 1, font of coordinates is too small. Link is invalid.

8. Author´s response: We changed the font and corrected the link to biome maps.

9. pCO_2 cannot be written as pCO2.

9. Author´s response: We agree.

10. Figure captions in support material are unclear.

10. Author´s response: We corrected all figures.

AJE AMERICAN JOURNAL EXPERTS

EDITORIAL CERTIFICATE

This document certifies that the manuscript listed below was edited for proper English language, grammar, punctuation, spelling, and overall style by one or more of the highly qualified native English speaking editors at American Journal Experts.

Manuscript title:

Temperature-dependence of the relationship between pCO2 and dissolved organic carbon in lakes

Authors:

L. Pinho, C. M. Duarte, H. Marotta, A. Enrich-Prast

Date Issued:

October 6, 2015

Certificate Verification Key:

333B-3821-39B5-4CF0-D37E

This certificate may be verified at www.aje.com/certificate. This document certifies that the manuscript listed above was edited for proper English language, grammar, punctuation, spelling, and overall style by one or more of the highly qualified native English speaking editors at American Journal Experts. Neither the research content nor the authors' intentions were altered in any way during the editing process. Documents receiving this certification should be English-ready for publication; however, the author has the ability to accept or reject our suggestions and changes. To verify the final AJE edited version, please visit our verification page. If you have any questions or concerns about this edited document, please contact American Journal Experts at support@aje.com.

Luana Queiroz Pinho 7/10/15 20:12
Eliminado: References ... [5]