1 11th January 2016.

3 Dear Editor,

5	Attached please find the revised version of the manuscript "Temperature-dependence of
6	the relationship between pCO_2 and dissolved organic carbon in lakes" to be reconsidered for
7	publication in Biogeosciences. To address valuable criticisms from the Reviewer 4, we have
8	included here a point-by-point response with details of the actions.
9	Our main purpose remains to show that the significant global relationship between pCO_2
10	and DOC previously reported in lake waters is not found after including tropical ecosystems.
11	This novel finding improves our knowledge of the C cycling in inland waters, highlighting the
12	role of the latitudinal temperature gradient to increase predictability of pCO_2 under different
13	DOC in lakes.
14	We thank the reviewer 1 to accept the article as fit for publication, the reviewer 4 for his
15	suggestions and the editor for managing all revision process. We believe that all reviewers have
16	led to an improved manuscript, which could be suitable to be published in <i>Biogeosciences</i> .
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18	
19	Sincerely,
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21	
22	Luana Pinho on behalf of the co-authors
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30 Interactive comment on "Temperature-dependence of the relationship between pCO₂ and dissolved organic carbon in lakes" by L. Pinho et al. 31 32 33 Response to Anonymous Referee #4 34 35 36 1) Comments from referee #4 37 38 General comments 39 40 The manuscript by Pinho et al set out to examine the effect of temperature on the relationship between pCO2 and dissolved organic carbon (DOC) in lake waters. pCO2 data were collected 41 from 194 Brazilian lakes in low-latitude and tropical regions in South American. The authors 42 found no significant correlation between pCO2 and DOC in low-latitude lakes, which is different 43 from those observed in temperate regions. They then concluded the temperature dependence of 44 the pCO2 and DOC relation. However, this work is solely based on the comparison with those 45 reported by Sobek et al. (2005). No further discussion was made in the manuscript. 46 47 48 Author's response: 49 First, we would like to thank the referee #4 for the very constructive comments. 50 51 In the larger dataset already published in the literature, Sobek et al. [2005] analyzed 4902 52 lakes globally distributed (CO₂ data), but only one warm tropical lake with pCO₂ and DOC data. Our aim was to add new data and insights after including warm-low latitude tropical 53 lakes in global compilations. 54 55 Now, we have better discussed our results with those reported in other regional reviews on 56 high-latitude lake waters (e.g. Larsen et al 2010, Lapierre and Del Giorgio 2012). 57 58 Lines 213 to 219: 59 60 The text now reads: In this way, the inclusion of warm tropical data in our study revealed novel 61 increases in the variability of the DOC- pCO_2 relationship in lakes over the latitudinal 62 gradient. One explanation for this pattern is that even similar DOC concentrations, 63 representing the total pool of DOC, may show different mixtures between origins from 64 65 aquatic primary producers and terrestrial sources [Kritzberg et al., 2006]. The autochthonous DOC (i.e. produced in the lake) is related to the net CO₂ uptake [Staehr and 66 67 Sand Jansen 2007], while the allochthonous DOC (i.e. produced in the catchment) is 68 resource to the net CO₂ release in lake waters [Sobek et al., 2007]." 69

70

- 72 Depending on the sources of DOC, pCO2 could be positively or negatively correlated with DOC
- 73 when DOC is mostly derived from terrestrial input or mostly produced in situ. Secondly,

First of all, DOC is not the only parameter regulating the pCO2 abundance in the water column.

temperature could be a master parameter affecting pCO2 abundance only when all other environmental settings, biological, chemical and physical parameters, remain the same. Now, the question is then "so what" and why pCO2 has to be correlated with DOC or why temperature has to be a factor affecting this correlation?

79 Author's response:

81 Our study design does not allow testing causes of the non-significant relationship found 82 between DOC and *p*CO₂. However, we agree with suggestions from the reviewer to 83 improve discussion, and included the following sentences:

- 85 Lines 220 to 225:
- 8687 The text now reads:

- 93 the DOC-*p*CO₂ relationship in global lakes"
- 94 95

80

84

96 Most importantly, instead of using DIC, their pCO2 data were derived from total alkalinity,
97 which includes additional contributions from dissolved organic ligands (DOC), but was not even
98 mentioned or corrected. Overestimation of TA values cannot be ignored when DOC
99 concentration is high especially in lake waters (up to >100 ppm in some samples, see Fig. 2).
100 Therefore, pCO2 reported in this manuscript could be questionable.

102 Overall, I suggest that the authors exploit their data set further, teasing out different factors that103 could regulate pCO2, and produce additional meaningful conclusions.

- 104105 Author's response:
- 106

107 We agree and had corrected the potential contribution of DOC to total alkalinity (TA),
108 using data reported in Abril et al (2015). This point was clarified in the main text and
109 supplementary information.

- 110111 The text now reads:
- 112 Lines 151 to 156:

113 "In order to address the potential contribution of DOC to TA, which is especially

- 114 important in DOC-enriched acid freshwaters, we used the data set from Abril et al., [2015]
- 115 to correct pCO_2 values calculated from pH and TA after the corrections for temperature,
- altitude and ionic strength [Cole et al., 1994]. Full details on fitted regression equations to

117 correct pCO₂ in function of the DOC and pH are described in the supplementary
118 information section. (Figure S3)."

119 120

121 Lines 179 to 190:

122 "The same absence of positive significance pattern was found comparing at corrected data. Negative (Linear regression, p < 0.05, $R^2 = 0.02$, $pCO_2 = -95.96$ (± 39.89) x 123 DOC + 6526 (\pm 640.7) or non significant (Linear regression, p > 0.05) DOC-pCO₂ 124 125 relationship for tropical lakes (N = 194, DOC and pH corrected, respectively (figure S3a 126 and c) contrasting with a significant positive relationship for those at other latitudes (N =4,433) (Linear regression, p < 0.05, $R^2 = 0.20$, $pCO_2 = 80.24 (\pm 2.36) \times DOC + 721.6 (\pm 24.10)$ 127 for DOC corrected data and $R^2 = 0.12$, $pCO_2 = 45.70 (\pm 1.84) \times DOC + 623.7 (\pm 18.83)$ for 128 129 pH corrected data, respectively) (Figure S3b and d, full details on corrections in the 130 supplementary information)."

131

132 Supplementary information: "In order to correct the potential contribution of organic 133 acids on pCO_2 values, we used the linear relationship between DOC concentrations and the 134 log of difference of calculated and measured pCO_2 from Abril et al. 2015; (for both 135 variables were used medians, Supplementary Figure 1). The same procedure was 136 performed between pH and the log of difference of calculated and measured pCO_2 137 (Supplementary Figure 2). Using both equations, we corrected each pCO_2 value used in 138 Figure S3"

139

140 Figure S3: "The relationship between pCO2 values and DOC concentrations for surface 141 lake waters after correcting the contribution of organic acids on TA and 142 subsequent pCO2 data for the low latitude lakes (a and c) and Sobek lakes (b and d). The 143 fitted linear regression of the relative difference between calculated and measured pCO2144 with ranked DOC (a and b) and pH (c and d) according Abril et al. 2015. A non-significant 145 linear regression was observed for pH corrected data of low-latitude lakes (figure S3c, p > 146 0.05, n = 194). The solid line represents the fitted linear regression for negative relationship 147 between pCO2 values and DOC concentrations for DOC corrected data of lowlatitude lakes (Linear regression, p < 0.05, $R^2 = 0.03$, n = 194, $pCO_2 = -98.76$ (± 39.92) x 148

- 149 DOC + 6529 (\pm 641.1), Figure S3a) and positive relationship between pCO2 values and
- 150 DOC concentrations for other latitudes showed in Sobek's data (Linear regression, p <
- 151 0.05, $R^2 = 0.20$, $pCO_2 = 64.43 (\pm 2.04) \times DOC + 625.1 (\pm 20.87)$ and $R^2 = 0.12$, $pCO_2 = 45.70 (\pm 10.16) \times 10^{-10}$
- 152 1.84) x DOC + 623.8 (± 18.83)) for DOC corrected data and pH corrected data (S3c and
- 153 **S3d**, respectively, N = 4,433 for both)."
- 154
- 155
- 156
- 157 Specific comments:
- 158 Methods: The authors should add more detailed information on the methods, such as QA&QC
- 159 for DOC and total alkalinity measurements, including the use of certified reference materials and 160 the diluted HCl for titration.
- 161

173

162 Author's response: We agree and change the sentence.

- 163164 The text now reads:
- 165 Lines 136 to 138:
- 166 "pH was determined using a pH meter (Digimed DM2) with reference standards certified 167 by Mettler Telede (4.00 ± 0.01 and 7.00 ± 0.01 units) before each compling hour "
- by Mettler Toledo (4.00 ± 0.01 and 7.00 ± 0.01 units) before each sampling hour."
- 169 Lines 146 to 148:

170 "In the lab, DOC was determined by high-temperature catalytic oxidation using a TOC171 5000 Shimadzu Analyzer, quality control was checked with calibration curve made with 172 potassium hydrogen phthalate before each sample battery analisys."

- 174 2. Line 152-153: Delete the repeated equation.
- 175 2.Author's response: We agree and corrected the equation.
- 176177 The text now reads:

178 Lines 179 to 190: "The same absence of positive significance pattern was found comparing 179 at corrected data. Negative (Linear regression, p < 0.05, $R^2 = 0.02$, $pCO_2 = -95.96 (\pm 39.89) x$ 180 DOC + 6526 (± 640.7) or non significant (Linear regression, p > 0.05) DOC-pCO₂ 181 relationship for tropical lakes (N = 194, DOC and pH corrected, respectively (figure S1a 182 and c) contrasting with a significant positive relationship for those at other latitudes (N =4,433) (Linear regression, p < 0.05, $R^2 = 0.20$, $pCO_2 = 80.24$ (± 2.36) x DOC + 721.6 (± 24.10) 183 for DOC corrected data and $R^2 = 0.12$, $pCO_2 = 45.70 (\pm 1.84) \times DOC + 623.7 (\pm 18.83)$ for 184 pH corrected data, respectively) (Figure S1 b and d, full details on corrections in the 185 supplementary information). The range of pCO_2 for a similar DOC range in Brazilian lakes 186 187 was larger than that reported by Sobek et al., [2005] for the dataset dominated by high-188 latitude cold lakes, despite the number of lakes in their dataset being much larger (more 189 details in supplementary information section, figure S1)." 190

191

192 3. Line 152-153: Is this equation applicable to all DOC concentrations or for a specific range of193 DOC concentrations?

3.Author's response: We clarified the sentence and rewrote the paragraph as cited above.

4. Line 220-224: High temperature and solar radiation may increase primary production and the
uptake of CO2, resulting in a negative correlation between pCO2 and DOC assuming DOC is
positively related to biomass or Chl-a. However, if the degradation of terrestrial DOC prevails in
lake waters under high temperature/solar radiation, could pCO2 be positively correlated to
terrestrial DOC?

4.Author's response:

204 We rewrote the sentence.

The text now reads:

Lines 213 to 225: "In this way, the inclusion of warm tropical data in our study revealed novel increases in the variability of the DOC-pCO₂ relationship in lakes over the latitudinal gradient. One explanation for this pattern is that even similar DOC concentrations, representing the total pool of DOC, may show different mixtures between origins from aquatic primary producers and terrestrial sources [Kritzberg et al., 2006]. The autochthonous DOC (i.e. produced in the lake) is related to the net CO₂ uptake [Staehr and Sand Jansen 2007], while the allochthonous DOC (i.e. produced in the catchment) is resource to the net CO₂ release in lake waters [Sobek et al., 2007]. The increased DOC release from aquatic primary producers into waters under tropical conditions, especially warmer annual conditions and higher solar incidence, can offset any positive relationship between pCO₂ and the terrestrial DOC that subsides the net aquatic heterotrophy [Marotta et al., 2010; 2012]. This contributes to explain non-significant relationships reported here (Figure 3), suggesting a temperature dependence of the DOC-pCO₂ relationship in global lakes."

227	Temperature-de	pendence of	the relationship	between	pCO_2 and	dissolved	organic	carbon in

228	lakes	
229	L. Pinho ^{1,2,3} , C. M. Duarte ^{2,4} , H. Marotta ^{5,6} , A. Enrich-Prast ^{1,7}	Luana Queiroz Pinho 11/1/16 14:14
230 231 232	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.	Eliminado: ⁵ Luana Queiroz Pinho 11/1/16 14:14 Eliminado: ⁶
233 234	 Global Change Department, IMEDEA (CSIC-UIB), Mediterranean Institute for Advanced Studies. C. Miquel Marquès, 21, 07190, Esporles, I. Baleares, Spain. 	
235 236 237	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.	
238 239	4. King Abdullah University of Science and Technology (KAUST), Red Sea Research Center (RSRC), Thuwal, 23955-6900, Saudi Arabia	
240 241 242	5. Research Center on Biomass and Water Management (NAB/UFF), Sedimentary Environmental Processes Laboratory (LAPSA/UFF), International Laboratory of Global Change (LINCGlobal), Federal Fluminense University. Av. Litorânea s/n, Campus Praia Vermelha, 24210 340, Niterói, RJ, Brazil.	
243 244	6. Postgraduate Program in Geography, Postgraduate Program in Geosciences (Geochemistry), Federal Fluminense University, 24220-900, Niterói, RJ, Brazil.	
245 246	 7. Department of Environmental Changes, Linköping University, 581 83, Linköping, Sweden. Corresponding author: luana.pinho@uerj.br 	Luana Queiroz Pinho 11/1/16 14:14 Eliminado: 4. Faculty of Marine Sciences, Abdulaziz University, P. O. Box 80207, 215
247		Jeddah, Saudi Arabia

248 Abstract

The relationship between the partial pressure of carbon dioxide (pCO₂) and dissolved 249 250 organic carbon (DOC) concentration in Brazilian lakes, encompassing 194 lakes across a 251 wide latitudinal range in the tropics, was tested. Unlike the positive relationship reported 252 for lake waters, which was largely based on temperate lakes, we found no significant Eliminado: 4. Faculty of Marine Sciences, King Abdulaziz University, P. O. Box 80207, 21589, Jeddah, Saudi Arabia. ...[1]

relationship for low-latitude lakes (< 33°), despite very broad ranges in both pCO_2 and DOC levels. These results suggest substantial differences in the carbon cycling of low latitude lakes, which must be considered when up scaling limnetic carbon cycling to global scales.

263

264 1.Introduction

265 Lakes cover less than 2% of the continent's surface [Downing et al., 2006; McDonald, 266 2012] but play a significant role in the global carbon (C) cycle [Cole et al., 1994; 2007; Tranvik 267 et al., 2009], contributing significantly to C burial and emissions to the atmosphere [Cole et al., 268 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 269 270 [Duarte and Prairie, 2005; Cole et al., 2007; Prairie 2008; Tranvik et al., 2009], supporting 271 heterotrophy [Sobek et al., 2007] and affecting key biological and physico-chemical processes 272 involved in C cycling [Steinberg et al., 2006]. Large inputs of terrestrial organic C and its 273 subsequent mineralization have been suggested to be a major driver of CO₂ supersaturation 274 commonly encountered in lakes [Duarte and Prairie, 2005; Cole et al., 2007; Prairie 2008; 275 Marotta et al., 2009].

276 The mechanistic connection between DOC and heterotrophic CO_2 production is believed to 277 underpin the significant positive relationship between pCO_2 and DOC reported in comparative 278 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 279 280 not universal [Lapiere and del Giorgio, 2012]. Hence, the relationship between pCO_2 and DOC 281 reported in comparative analyses based on datasets dominated by temperate and high-latitude 282 lakes $(> 33^{\circ})$ may not be extrapolated for all types of lakes, mainly because the tropical low 283 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 et al., 2005] and aquatic [Marotta et al., 2009; 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 CO₂ production and subside high CO₂ evasion
from global lake water to the atmosphere.

295 The largest previous comparative analysis already published in the literature for global lake 296 waters [Sobek et al., 2005] reported a significant positive relationship between DOC and pCO_2 297 and a non-significant variation of pCO_2 among lakes with changing temperature. However, both 298 analyses were characterized by a paucity of low latitude data. A strong positive relationship 299 between temperature and pCO_2 was observed when subtropical and tropical ecosystems were 300 included in the dataset [Marotta et al., 2009], likely caused by the potential increase in metabolic 301 rates under warmer conditions [Brown et al., 2004: López-Urrutia et al., 2006]. Hence, the 302 relationship between lake pCO_2 and DOC could also be temperature-dependent and, therefore, 303 may differ between temperate and tropical lakes. The extensive low latitude territory of Brazil, 304 which has a high density of lakes and ponds [Downing et al., 2006], is appropriate to examine 305 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 a high 306 307 latitude dataset [Sobek et al., 2005] with added tropical and subtropical data of low latitude lakes 308 from Brazil.

309

- 310 2.Methods
- 311 2.1.Study area and Lakes

Brazil extends from 5° 16' 20" North to 33° 44' 42" South, showing an area of 312 313 approximately 8,547,000 km², constituting half of South America and encompasses a high diversity of low-latitude landscapes [Ab'Saber, 2003] that are predominantly located within 314 315 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), 316 317 yielding a total of 225 water samples. The lakes were sampled in representative biomes of Brazil: 318 (1) the Amazonia Forest (Amazonia Biome, n = 65), (2) the Pantanal Floodplain (Pantanal 319 Biome, n = 29) and (3) the Tropical (< 24° of latitude) and (4) Subtropical (> 24° and < 33° of 320 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.

326 The Amazonian Forest biome is formed by the most extensive hydrographic network on the 327 globe: the Amazon River basin, which occupies a total area of approximately 6.11 million km² 328 from its headwaters in the Peruvian Andes to its mouth in the Atlantic Ocean (ANA -329 www.ana.gov.br). The Amazon Forest is the Brazilian biome with the highest mean annual 330 precipitation (approximately 2200 mm) and has warm mean air temperatures, approximately 331 25°C, high cloud coverage and high humidity with low fluctuations over the whole year 332 [Chambers, 1999]. We sampled a wide variety of lakes, characteristic of different areas of the 333 Amazonian Forest, encompassing "clear" (low DOC and suspended solids), "white" (low DOC 334 and high suspended solids) and "dark" (high DOC and low suspended solids) lakes.

The Pantanal Floodplain is the world's largest tropical freshwater wetland, extending across an area of approximately 150,000 km² between 16° and 20° S and 58° and 55° W [Por, 1995]. The annual average 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 occur during the dry winter (from March to July) [Hamilton, 2002].

The Atlantic Forest biome extends along a broad latitudinal belt, between 5° and 30° S 342 343 from the subtropics to tropics and a narrow longitudinal section between 55° and 56° W, and occupies an area of 1.11 million km² along the Brazilian coast (IBGE-www.ibge.gov.br). This 344 345 biome is characterized by numerous shallow coastal lakes receiving high inputs of refractory 346 organic matter [Farjalla et al., 2009] derived from the typical open xerophytic vegetation on 347 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 [$< 24^{\circ}$ of latitude; Chellappa et al., 2009] 348 and from 17 and 20°C at the subtropical coast [> 24° of latitude; Waechter, 1998]. The mean 349 350 annual precipitation reaches 1,164 mm [Henriques et al., 1986] and 1,700 mm [Waechter, 1998] 351 in the tropical and subtropical Brazilian coast, respectively. This biome is also characterized by 352 strong seasonality, with rainy summers and dry winters [Chellappa et al., 2009].

354 2.2 Sampling Design and Analytical Methods

355 Our sampling design encompassed the most representative Brazilian biomes from tropical 356 and subtropical coastal areas to tropical and subtropical forests (Amazon and Atlantic Forest) 357 and inland wetlands (Pantanal), with the intra-lake heterogeneity and seasonal fluctuations 358 randomly assessed and further integrated by means of each ecosystem. To analyze the 359 relationship between pCO_2 and DOC in tropical lake waters, we joined data on 194 lakes (< 33° 360 of latitude) with both variables sampled at the same time, including 166 data samples from our 361 own survey and 28 from the literature compilation (Table S1). The values reported here 362 represented, gathered in an opportunistic manner, represent daily averages (N=4 or 5 samples) 363 for a given year's season or/and one sampling time over different seasons, which were also both 364 integrated by means of each lake. To test the global importance of the relationship between pCO_2 365 and DOC, we added our low latitude data (225) to the Sobek et al. [2005] dataset (4902 lakes) as 366 this dataset had a paucity of tropical ecosystem data (148 tropical lakes, but only one with pCO_2 367 and DOC sampled at the same time).

368 pH, salinity and temperature were measured in situ. pH was determined using a pH meter 369 (Digimed – DM2) with reference standards certified by Mettler Toledo (4.00 ± 0.01 and 7.00 ± 370 0.01 units) before each sampling hour. Temperature and salinity were measured using a 371 Thermosalinometer (Mettler Toledo - SevenGo SG3) coupled to a probe in Lab 737 previously 372 calibrated with 0.01 M KCl. Surface lake water was collected for total alkalinity and DOC 373 analyses, taking care to avoid bubbles at approximately 0.5 m of depth using a 1 L Van Dorn 374 bottle. Total alkalinity (TA) was determined in the field by the Gran's titration method with 375 0.0125 M HCl immediately after sampling [Stumm and Morgan, 1996]. Water samples for DOC 376 were pre-filtered (0.7 µm, Whatman GF/F) and preserved by acidification with 85% H₃PO₄ to 377 reach a pH \leq 2.0 in sealed glass vials [Spyres et al., 2000]. In the lab, DOC was determined by 378 high-temperature catalytic oxidation using a TOC-5000 Shimadzu Analyzer, checked with a 379 calibration curve made with xxx before each analysis. pCO_2 concentrations in surface waters 380 were calculated from pH and alkalinity following Weiss [1974], after corrections for 381 temperature, altitude and ionic strength according to Cole et al. [1994].

Luana Queiroz Pinho 7/1/16 17:21 Eliminado: a precision of 0.01 calibrated with standard solutions (Mettler Toledo) of pH 4.01 and 7.00 units

Luana Queiroz Pinho 6/1/16 14:02 Eliminado: A

386	In order to address the potential contribution of DOC to TA, which is especially
387	important in DOC-enriched acid freshwaters, we used the data set from Abril et al., [2015]
388	to correct pCO ₂ values calculated from pH and TA after the corrections for temperature,
389	altitude and ionic strength [Cole et al., 1994]. Full details on fitted regression equations to
390	correct pCO ₂ in function of the DOC and pH are described in the supplementary
391	information section, (Figure S <u>3</u>).

392 2.3. Statistical Analyses

393 The variables pCO_2 and DOC did not meet the assumptions of parametric tests even after 394 logarithmic transformations [Zar, 1996] as the data were not normally distributed (Kolmogorov-395 Smirnov, p < 0.05) and the variances were heterogeneous (Bartlett, p > 0.05). Therefore, we used 396 medians and non-parametric tests to compare these variables among biomes (Kruskall-Wallis 397 followed by Dunn's multiple comparison post hoc test, p < 0.05). The linear regression equations 398 were fitted to compare our results with those of previous studies from Sobek et al., [2005]. 399 Statistical analyses were performed using the software Graphpad Prism version 4.0 for 400 Macintosh (GraphPad Software, San Diego, CA).

401 3.Results

402 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) than in 403 Pantanal and Amazonian lakes (29.5° C, 27.7 - 31.4 and 29.4° C, 27.6 - 31.0, respectively; 404 Dunn's test, p < 0.05, Figure 2a). DOC concentrations were consistently high (6.3 mg C L⁻¹, 4.3 405 - 11.9) for all Brazilian biomes but significant lower in the Amazonian Forest (3.8 mg C L⁻¹, 2.7 406 -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). 407 Most lakes (approximately 83% of raw data) showed surface waters supersaturated in CO2 408 409 relative to the atmospheric equilibrium (pCO_2 in atmospheric equilibrium is 400.83 µatm, 2015 410 annual mean; data available in www.esrl.noaa.gov/gmd/ccgg/trends), with much higher pCO_2 411 values in Amazonian lakes (7,956 µatm, 3,033 – 11,346) than in subtropical coastal lakes (900 412 μ atm, 391.3 – 3,212; Figure 2c; Dunn's test, p < 0.05).

413 The pCO_2 in the surface waters of Brazilian lakes was independent of DOC 414 concentrations (Linear regression for raw data, p > 0.05, Figure 3). The same absence of 415 positive significance pattern was found comparing at corrected data. Negative Linear

Luana Queiroz Pinho 5/1/16 15:12

Eliminado: 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

roz Pinho 5/1/16 15:

Eliminado: 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; R² = 0.9752, p < 0.005; Log pCO_2 correction (%) = -0.9638 *pH + 7.755; R² = 0.9752, p < 0.005). Statistical analyses were performed using raw and

corrected data Luana Queiroz Pinho 7/1/16 16:36

Eliminado: 2 and S3

Luana Queiroz Pinho 6/1/16 11:05 Eliminado: according to Tans and Keeling 2014

432	regression, $p < 0.05$, $R^2 = 0.03$, $n = 194$, $pCO_2 = -98.76 (\pm 39.92) \times DOC + 6529 (\pm 641.1)$ or non	
433	<u>significant (Linear regression, p > 0.05) DOC-pCO_2 relationship for tropical lakes (N = 194,</u>	
434	DOC and pH corrected, respectively (figure S3a and c) contrasting with a significant	
435	positive relationship for those at other latitudes (N = 4,433) (Linear regression, $p < 0.05$, $R^2 =$	
436	<u>0.20</u> , $pCO_2 = 64.43 (\pm 2.04) \times DOC + 625.1 (\pm 20.87)$) for DOC corrected data and $R^2 = 0.12$,	
437	<u>$pCO_2 = 45.70 (\pm 1.84) \times DOC + 623.8 (\pm 18.83)$</u> for pH corrected data, respectively) (Figure	
438	S3 b and d, full details on corrections in the supplementary information). The range of pCO_2	
439	for a similar DOC range in Brazilian lakes was larger than that reported by Sobek et al., [2005]	
440	for the dataset dominated by high-latitude cold lakes, despite the number of lakes in their dataset	
441	being much larger (more details in supplementary information section, figure S3).	
442		\wedge

443 4.Discussion

444 The Brazilian lakes sampled here were characterized by a prevalence of CO₂ 445 supersaturation, consistent with general trends previously reported for global lakes [e.g., Raymond et al, 2013; Cole et al., 1994; 2007] including those at tropical latitudes [Marotta et al., 446 447 2009]. The very high pCO_2 levels observed here, with a median of 900 and 8,300 µatm for 448 subtropical and Amazon lake waters, respectively, are consistent with those reported previously 449 for the Amazon River and tributaries (2,000-12,000 µatm; Richey et al., [2002]), Amazon 450 floodplain lakes (3,000 - 4,898 µatm; Rudorff et al., [2012]), Pantanal lakes and wetlands (2,732-451 10,620 µatm; Hamilton et al., [1995]), and coastal lakes (768 - 9,866 µatm; Kosten et al., [2010]; 361-20,037 µatm; Marotta et al., [2010]) and for global values for tropical lakes (1,255-35,278 452 453 µatm; Marotta et al., [2009]), reservoirs (1,840 µatm; Aufdenkampe et al., [2011]) and wetlands 454 (3,080-6,170 µatm; Aufdenkampe et al., [2011]). 455 The non-significant or weakly negative relationship (Figure S3) between DOC and pCO_2

reported here for warm low-latitude lakes contrasted with significant positive relationships 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 and low values, but tend to have comparatively more uniform DOC

461 concentrations (Figure 3). More intense metabolic processes that uptake and release CO₂ in lake

462 waters, respectively autotrophy and heterotrophy, could determine an enhanced variability in lake

463 <u>*p*CO₂</u> with decreasing latitude [Marotta et al., 2009].

Luana Queiroz Pinho 7/1/16 16:12

Eliminado: After correcting our data and the Sobek data with the contribution of organic acids on total alkalinity (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 continued with the same pattern observed before (not a significant relationship for Tropical data (p > 0.05, n = 194) and a positive relationship for the Sobek dataset ($pCO_2 = 45,70 (\pm 1,84) \times DOC + 623,7 (\pm 18,83), R^2 = 0.12, p < 0,0001, n = 4433$), figure S2 a and b).

We also calculate the DOC > 2 and < 10 mg L⁻¹), and the observed pattern was the same. We found nonsignificant relationships between DOC and pCO₂ using all data or only the data from low latitude (< 33°) lakes (linear regression, p > 0.05), and we found significant positive linear regressions for those at high latitudes (> 33°) in each DOC group, despite low R² values (R² = 0.08 and 0.03, p<...[2] Luana Queiroz Pinho 8/1/16 13:53

Eliminado: see

Luana Queiroz Pinho 7/1/16 16:27 Eliminado: support material Luana Queiroz Pinho 7/1/16 16:27 Eliminado: 3 ana Queiroz Pinho 7/1/16 17:01 Eliminado: We also calculated the DOC-pCO2 two separate groups (DOC > and < 10 mg L^{-1} [3] Luana Queiroz Pinho 8/1/16 13:54 Eliminado: 1994; Algesten et al., 2005 Luana Queiroz Pinho 8/1/16 13:55 Eliminado: and previous reports for Luana Queiroz Pinho 8/1/16 13:56 Eliminado: kes Luana Queiroz Pinho 8/1/16 13:56 Eliminado: Luana Queiroz Pinho 8/1/16 13:57 Eliminado: Luana Queiroz Pinho 7/1/16 17:09

13

Eliminado: 2 and S3

544	In this way, the inclusion of warm tropical data in our study revealed novel increases in
545	the variability of the DOC-pCO2 relationship in lakes over the latitudinal gradient. One
546	explanation for this pattern is that even similar DOC concentrations, representing the total pool
547	of DOC, may show different mixtures between origins from aquatic primary producers and
548	terrestrial sources [Kritzberg et al., 2006]. The autochthonous DOC (i.e. produced in the lake) is
549	related to the net CO2 uptake [Staehr and Sand Jansen 2007], while the allochthonous DOC (i.e.
550	produced in the catchment) is resource to the net CO ₂ release in lake waters [Sobek et al., 2007].
551	The increased DOC release from aquatic primary producers into waters under tropical
552	conditions, especially warmer annual conditions and higher solar incidence, can offset any
553	positive relationship between pCO_2 and the terrestrial DOC that subsides the net aquatic
554	heterotrophy [Marotta et al., 2010; 2012]. This contributes to explain non-significant
555	relationships reported here (Figure 3), suggesting a temperature dependence of the DOC-pCO2
556	relationship in global lakes,

557 In conclusion, the finding that pCO_2 does not increase with DOC concentration in 558 Brazilian tropical lakes rejects the hypothesis that DOC serves as a universal predictor for pCO_2 559 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 560 561 acids on the alkalinity, the pattern of no relationship between DOC and pCO_2 in the Tropical lakes was strongly confirmed (Figure S3). Therefore, our results contributing to fill the tropical 562 563 gap suggest potentially important latitudinal differences for depositional aquatic environments, 564 whose causes still need to be better addressed to improve accuracy of global C cycle models.

565

566 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.

569

570 Acknowledgments

571 This research is a contribution to projects from Brazilian research agencies (FAPERJ, CAPES
572 and CNPq). L. P. was supported by PhD scholarships from CAPES (period in Brazil) and
573 FAPERJ (period in Spain). A. E-P. received postdoctoral and other CAPES and CNPq
574 fellowships during studies at Linkoping University. H.M. was supported by a research fellowship
14

Luana Queiroz Pinho 8/1/16 14:05 Eliminado: Tropical conditions based on higher annual temperatures and solar incidence typically

Luana Queiroz Pinho 5/1/16 14:50

Eliminado: increase the aquatic primary productivity activity [Paerl and Huisman 2008] that releases into waters the DOC produced by the CO₂ uptake of algae and submerged plants [Staehr and Sand Jansen 2007], which can withstand a negative variation in the *p*CO₂ with an increase in the DOC concentration [Marotta et al., 2010; 2012; Hanson et al., 2015]. The contrasting non-significant or weak negative relationship between *p*CO₂ and DOC in warm Brazilian lakes observed here, with respect to the positive relationship for cold lake waters from the dataset of Sobek al. [2005], suggests a temperature dependence of the *p*CO₂ and DOC correlation in global lakes.

Luana Queiroz Pinho 8/1/16 14:13

Eliminado: Despite the limitations of our methodology, our work contributes data to the literature from tropical lakes that is frequently missing from global calculations.

595 from FAPERJ (Programa Jovem Cientista do Nosso Estado), and a research grant from CNPq

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- 754 Figures and subtitles

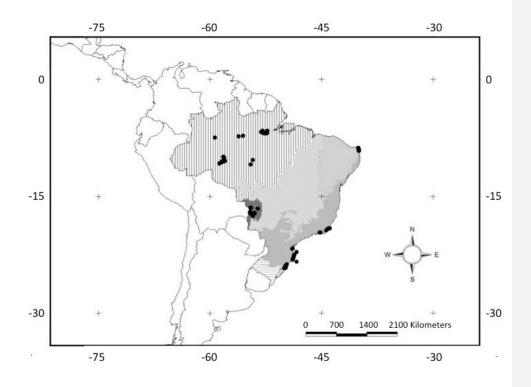




Figure 1. Geographic location of Brazilian lakes sampled in different biomes (IBGE 2004,
available in ftp://geoftp.ibge.gov.br/mapas_tematicos/mapas_murais/biomas.pdf): Amazonia
Forest (vertical lines), Pantanal Floodplain (dark gray), and Atlantic Forest (gray; Tropical and
Subtropical costal lakes).

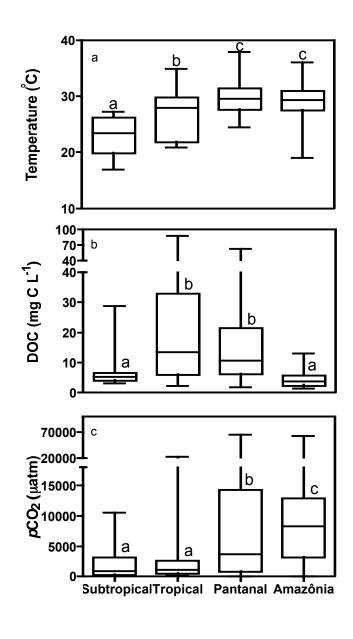
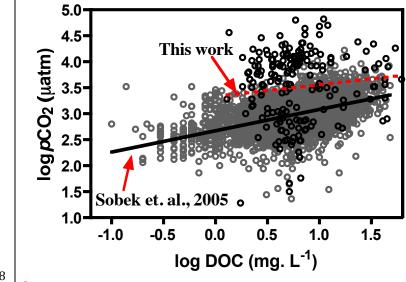


Figure 2. Values of (A) temperature (°C), (B) DOC concentrations (mg C L⁻¹) and (C) pCO₂
concentration (µatm) of Brazilian lakes sampled from different biomes, as defined by (SUBT)
Subtropical Coastal lakes (n = 37), (TROP) Tropical coastal lake (n = 63), (PANT) Pantanal

Floodplain (n = 58) and (AMAZ) Amazonia Forest (n = 67). The line depicts the median. The boxes show the quartiles, and the whiskers mark the 10^{th} and 90^{th} 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).



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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] $(p < 0.05, R^2 = 0.26, \log pCO_2 (\mu atm) = 2.67 + 0.414 \log DOC (mg C L⁻¹)).$

Luana Queiroz Pinho 7/1/16 15:06 Eliminado: ; $R^2 = 0.26$; p < 0.05