MICROBIAL ROLE IN THE CARBON CYCLE IN TROPICAL INLAND AQUATIC ECOSYSTEMS

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MICROBIAL ROLE IN THE CARBON CYCLE IN TROPICAL INLAND AQUATIC ECOSYSTEMS

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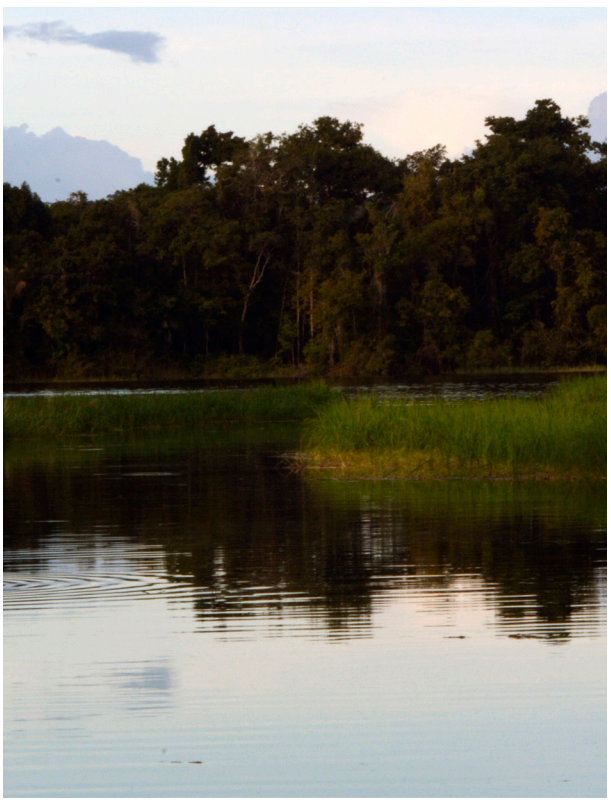

View of Lake Batata margin (Municipality of Oriximiná, Para State, Brazil - Brazilian Amazon) that shows the aquatic macrophytes and the rain forest and their reflection on the water. We propose that the reflected image represent the great importance of these carbon sources to the microbes in the water and to the aquatic ecosystems metabolism. Photo by Fábio Roland

Aquatic microorganisms are tidily related to the carbon cycle in aquatic systems, especially in respect to its accumulation and emission to atmosphere. In one hand, the autotrophs are responsible for the carbon input to the ecosystems and trophic chain. On the other hand, the

heterotrophs traditionally play a role in the carbon mineralization and, since microbial loop theory, may play a role to carbon flow through the organisms. However, it is not yet clear how the heterotrophs contribute to carbon retention and emission especially from tropical aquatic ecosystems.

Most of the studies evaluating the role of microbes to carbon cycle in inland waters were performed in high latitudes and only a few studies in the tropical area. In the prospective of global changes where the warm tropical lakes and rivers become even warmer, it is important to understand how microorganisms behave and interact with carbon cycle in the Earth region with highest temperature and light availability. This research topic documented microbial responses to natural latitudinal gradients, spatial within and between ecosystems gradients, temporal approaches and temperature and nutrient manipulations in the water and in the sediment.

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EDITORIAL
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Editorial: Microbial Role in the Carbon Cycle in Tropical Inland Aquatic Ecosystems

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Keywords: metabolism, lakes, lagoons, rivers, latitudinal gradients, microorganisms, plankton, microbial ecology

Editorial on the Research Topic

Microbial Role in the Carbon Cycle in Tropical Inland Aquatic Ecosystems

Microorganisms have been recognized as central to nutrient mineralization and recycling in aquatic ecosystems since Lindeman's groundbreaking work on the trophic-dynamic aspect of ecology (Lindeman, 1942). Since the seventies, the development of new analytical technologies led to important conceptual perspectives, such as the microbial loop and the microbial food web (as summarized elsewhere, e.g., Cotner and Biddanda, 2002; Weisse, 2004), which have been important to understanding connections between microbially-mediated allochthonous and autochthonous organic matter decomposition and carbon dioxide (CO_2) concentrations and fluxes to the atmosphere (e.g., Cole et al., 2007; Berggren et al., 2012; Fonte et al., 2013). Currently, one of the main foci of microbial ecologists is to open the "microbial playbill" so that we can better understand who is doing "what" and "when" in ecosystem "plays" (Logue et al., 2015).

It is well-documented how seasonal variation of temperature, light incidence, and precipitation affects microbial metabolism (e.g., Simon and Rosenstock, 1992; Berggren et al., 2010) in high latitude ecosystems. Considering that temperature and light incidence are less variable and remain high year-round in the tropics (Lewis, 1996), one could expect differences in metabolic processes among the latitudinal regions (Farjalla et al., 2009) and consequent effects on microbial respiration and carbon emissions (essentially CO_2 and methane) to the atmosphere. On the one hand, more intense metabolic processes are expected in lower latitudes. On the other hand, regional (e.g., flood pulse) or local (e.g., landscape characteristics) environmental conditions could be more relevant regulators of microbial metabolism than global factors (e.g., temperature, etc.). For instance, small rather than large planktonic organisms predominate at the base of microbial food webs of tropical aquatic ecosystems (i.e., pico- vs. nano-plankton) in comparison to temperate lakes, which typically means a higher flow of carbon through the microbes in the tropics than in temperate regions (Roland et al., 2010; Sarmento, 2012). Yet, certainly several new microbial ecology fundamentals will arise from asking questions that remain poorly understood, such as: (1) Does the current knowledge derived mostly from temperate ecosystems hold for tropical ecosystems? and (2) Can tropical ecosystems be good models to predict the changes in microbial metabolism and carbon cycling in temperate aquatic systems in light of climate warming scenarios?

The aim of this research topic—*Microbial role in the carbon cycle in tropical inland aquatic ecosystems*—was to provide a selection of studies that look at the wide variety of aspects of tropical microbial ecology including barely covered outlines (such as viruses–bacteria interactions or hydrodynamic events driven microbial communities) and their role to the carbon cycle. This research topic has documented 13 contributions that advance our knowledge on the microbial

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responses to natural latitudinal gradients, spatial and temporal patterns within and between ecosystems, and temperature and nutrient effects on microbial processes in the water and in the sediment. In order to better compile the information, the contributions were grouped in the following paragraphs as: (a) how physical and chemical variables (e.g., temperature and nutrients) affect carbon metabolism via microbes; (b) how latitudinal variation affects metabolism in the microbial food web components, and (c) how changes in environmental forces (such as flood pulse) affect microbial interactions.

Two meta-analysis studies focused on bacterial metabolism and top-down control and how they vary across latitudinal gradients. One study demonstrated that bacterial biomass production (BP) and respiration (BR) are higher while bacterial growth efficiency (BGE) is lower in the tropics (Amado et al.). Additionally, the other study demonstrated that bacterial and heterotrophic flagellate (HNF) abundance were lower in tropical than in temperate ecosystems (Segovia et al.). Furthermore, the authors still showed that the coupling between predator and prey did not differ between tropical and temperate zones, suggesting that other factors, such as the higher temperature or even HNF top-down control could be responsible for higher bacterial loss rates in the tropics.

One study that manipulated temperature and nutrients in tropical humic coastal lagoons (Scofield et al.) recorded increased BR and decreased BP and BGE with increasing temperature, confirming the results reported by Amado et al., and showing that temperature can be a valuable carbon metabolism predictor on a regional scale. Similarly, nutrient manipulations resulted in different metabolic responses and CO_2 saturation patterns among lagoons, indicating that temperature effects are modified by local ecosystem conditions (Peixoto et al.). Additionally, another study performed in an eutrophic reservoir (Almeida et al.), observed net heterotrophy, net CO_2 emission, and high ebullition and diffusive methane (CH_4) emissions. Despite high primary production rates, the shallow nature of the ecosystem, the high temperatures, and accumulated organic matter contributed to high decomposition rates. These three studies supported the idea that local factors are strong drivers of aquatic carbon metabolism at low latitudes.

The Amazon River basin is inserted in a low-latitude zone and it is recognized as a hotspot for freshwater CO_2 emissions globally (Raymond et al., 2013; Abril et al., 2014). The flood pulse is the major ecological force affecting carbon-related processes and organisms in this region (Amado et al., 2006; Melack and Coe, 2012). Indeed, the flood pulse was the main driver for bacterial metabolism and carbon mobilization in rivers due to changes in the organic matter origin and quality (Vidal et al.). Moreover, one study in floodplain lakes of a large Amazon tributary contained bacterial and viral abundances that increased with increasing distance from the Amazon River (Almeida et al.). The longitudinal gradient was attributed to a backwater effect caused by the Amazon River that increased water turbidity and decreased organic carbon quality (availability) and concentration in the floodplain lakes downstream, which might also reduce the carbon flow through to the microbial food web.

Three studies evidenced that there is a great deal of heterogeneity among coastal lakes in the subtropical region. One study using seasonal and diel approaches in a coastal lagoon in southern Brazil (Peri Lagoon, Santa Catarina state), showed that precipitation and temperature, as well as bacterial abundance were the main regulators of CO_2 concentration in the water (Schmitz Fontes et al.). On the other hand, the bacterial community and activity in another coastal subtropical lagoon (Laguna de Rocha, Uruguay), was carbon-limited and was mainly driven by organic matter inputs from the watershed or dilutions by the seawater (Alonso et al.). This resulted in shifts in the microbial community and the microbial food web through HNF and virus increase. In addition, the dominance of wetlands and aquatic macrophytes reduced microbial carbon metabolism and CO_2 concentration in the water when compared to the pelagic areas (i.e., higher depths and no macrophytes presence) creating heterogeneous conditions to carbon processing within the lake in a subtropical coastal lagoon in southern Brazil (Lake Mangueira, Rio Grande do Sul state; They et al.). Subtropical lakes are somewhat of a transition type of ecosystem, which consistently share regulation of microbial and carbon processes by local/regional environmental factors with tropical ecosystems, and by climate-driven seasonal environmental factors with temperate ecosystems.

Sediment metabolism has been extensively demonstrated to be relevant to the carbon balance in deep temperate lakes (e.g., Jonsson et al., 2001), but was poorly studied in tropical inland waters. Cardoso et al. observed high mineralization rates in the sediment of a mesotrophic tropical hydroelectric reservoir, which has driven the CO_2 variation in the water column and high internal spatial variation in the respiration. Also, Canterle et al. observed both spatial and temporal variation in the microbial CO_2 and CH_4 production and emission in the sediment of a subtropical shallow wetland ecosystem. Additionally, Liengaard et al. detected high emission of nitrous oxide (N_2O) in the largest wetland in the world (Pantanal, a pristine tropical wetland) providing up to 1.7% of global N_2O emissions, as result of high organic matter availability processed via anaerobic metabolism. These findings are especially relevant taking the climate warming scenario. The intensification of biological processes may increase greenhouse-gas emissions 2–4 times more in the tropics than in temperate sediments (Marotta et al., 2014).

The articles presented in this research topic investigate several aspects of microbial interaction with carbon cycling in tropical inland waters and produced some evidence that it can change across the latitudinal gradient as follows: (a) higher temperature in the tropics enhances bacterial carbon processing and reduces BGE; (b) the energy flow through the microbial food web in the tropics may be either reduced due to low BGE, or enhanced due to a possible high top-down control of HNF; and (c) as in temperate ecosystems it seems consistent that nutrient availability is also a key component of the aquatic metabolism and carbon processing in tropical ecosystems, but local or regional regulation may frequently be equally or even more relevant than globally controlled environmental factors. Nonetheless, several gaps still remain. For example, is absolutely pertinent a better understanding regarding the controls of trophic cascade relationships (e.g., top-down control of bacteria by HNF) in the microbial food

web and how landscape conditions (e.g., land use) affect carbon processing in aquatic ecosystems. Certainly, many opportunities exist for research focusing on the relationship of carbon and the microbial components of freshwater. As we commented before, we expect that these papers will stimulate new discussions and investigations to cover the great diversity of ecological processes that still need to be addressed and help formulate more robust general patterns for tropical freshwaters.

AUTHOR CONTRIBUTIONS

All authors listed, have made substantial, direct and intellectual contribution to the work, and approved it for publication.

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Tropical freshwater ecosystems have lower bacterial growth efficiency than temperate ones

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Current models and observations indicate that bacterial respiration should increase and growth efficiency (BGE) should decrease with increasing temperatures. However, these models and observations are mostly derived from data collected in temperate regions, and the tropics are under-represented. The aim of this work was to compare bacterial metabolism, namely bacterial production (BP) and respiration (BR), bacterial growth efficiency (BGE) and bacterial carbon demand (BCD) between tropical and temperate ecosystems via a literature review and using unpublished data. We hypothesized that (1) tropical ecosystems have higher metabolism than temperate ones and, (2) that BGE is lower in tropical relative to temperate ecosystems. We collected a total of 498 coupled BP and BR observations ($N_{total} = 498$; $N_{temperate} = 301$; $N_{tropical} = 197$), calculated BGE (BP/(BP+BR)) and BCD (BP+BR) for each case and examined patterns using a model II regression analysis and compared each parameter between the two regions using non-parametric Mann–Whitney U test. We observed a significant positive linear regression between BR and BP for the whole dataset, and also for tropical and temperate data separately. We found that BP, BR and BCD were higher in the tropics, but BGE was lower compared to temperate regions. Also, BR rates per BP unit were at least two fold higher in the tropics than in temperate ecosystems. We argue that higher temperature, nutrient limitation, and light exposure all contribute to lower BGE in the tropics, mediated through effects on thermodynamics, substrate stoichiometry, nutrient availability and interactions with photochemically produced compounds. More efforts are needed in this study area in the tropics, but our work indicates that bottom-up (nutrient availability and resource stoichiometry) and top-down (grazer pressure) processes, coupled with thermodynamic constraints, might contribute to the lower BGE in the tropics relative to temperate regions.

Keywords: microbial metabolism, BGE, carbon cycle, tropical, temperate

INTRODUCTION

Freshwater ecosystems are critical bioreactors in the global carbon cycle as they process a large fraction of the organic matter exported from terrestrial ecosystems (Cole et al., 2007; Tranvik et al., 2009). Heterotrophic bacteria play an important role in processing this organic matter and in releasing CO_2 and inorganic nutrients (Cole et al., 1994; Odum et al., 2004), and re-integrating dissolved organic matter (DOM) to the food web through the microbial loop (Azam et al., 1983). Thus, bacterial production (BP), and bacterial respiration (BR) are key processes in the carbon cycle of all aquatic systems. Two parameters derived from BP and BR are useful tools to understand the role of bacteria in ecosystem functioning: bacterial carbon demand (BCD), which represents the total amount of carbon processed by bacteria (BCD = BP + BR) and bacterial growth efficiency (BGE), which is the proportion of carbon taken up by bacteria that is converted into biomass (BGE = BP/[BP + BR]) (for review see Del Giorgio and Cole, 1998). Higher BGE leads to higher energy and organic matter availability to higher trophic levels, i.e., the microbial loop. On the other hand, lower in BGE may result in higher carbon mineralization rates through CO_2 production.

Several environmental factors may regulate bacterial metabolism and affect BGE and BCD. Water temperature is an important metabolic regulator and frequently correlates positively with BP, BR, and BCD, and negatively with BGE (Rivkin and Legendre, 2001; Biddanda and Cotner, 2002; López-Urrutia and Morán, 2007). Additionally, nutrient availability (such as N and P), DOM quality and stoichiometry, and bacterial predation pressure may also be important regulators of microbial activity (Farjalla et al., 2002, 2006; Hall and Cotner, 2007; Berggren et al., 2010; Vidal et al., 2011; Sarmento, 2012; Sarmento and Gasol, 2012). Considering that the tropical

region (between latitudes 23°26′S and 23°26′N) presents higher sunlight incidence and mean temperature (Lewis, 1987, 1996) and that additional trophic levels within the microbial food web can persist throughout the year (Sarmento, 2012), one might expect that many aquatic processes, such as BGE and BCD could present differences between tropical and temperate regions.

The metabolic theory of ecology predicts that organisms living in warmer conditions exhibit higher metabolic rates than organisms living at lower temperatures (Brown et al., 2004). On the other hand, the ATP paradox suggests that higher metabolic rates yield lower biomass with higher energy dissipation (lower growth efficiency; Pfeiffer et al., 2001; Pfeiffer and Bonhoeffer, 2002; MacLean, 2008). Thus, it could be deduced that at the higher temperatures in the tropics BGE should be reduced relative to the temperate zones. This relationship of decreased BGE with increasing temperatures has been demonstrated already mostly within temperate ecosystems (Hall and Cotner, 2007; López-Urrutia and Morán, 2007; Berggren et al., 2010). Another recent study comparing tropical humic ecosystems to temperate models observed that BR was higher and more variable in the humic tropical systems, and also that BP tends to stabilize, relative to BR (Farjalla et al., 2009). These findings contradict other studies in temperate ecosystems that observed higher BP variability, rather than BR (Del Giorgio and Cole, 1998; Roland and Cole, 1999). Thus, following the humic-tropical pattern, BR would increase proportionally more in relation to BP, and the average BGE in the tropical ecosystems would be lower than in temperate systems, implying a lower relevance of the microbial loop in terms of biomass, but higher relevance in terms of carbon mineralization in tropical systems. However, a recent review on the role of the microbial food webs in tropical lakes was not able to demonstrate that BP was higher in the tropics, compared to temperate systems in small dataset (Sarmento, 2012).

Therefore, assuming higher and more constant average temperatures in tropical aquatic ecosystems compared to temperate aquatic ecosystems, some important questions arise regarding bacterial function in these ecosystem: (1) Do bacteria grow less efficiently in the tropics than in temperate ecosystems? (2) Is the humic-rich tropical ecosystem pattern relevant in a larger context of tropical ecosystems types? (3) If bacteria do grow less efficiently in the tropics than in temperate regions, is temperature the most important factor driving these differences?

In this paper we hypothesize that (1) tropical inland aquatic ecosystems have higher metabolic rates (e.g., BCD) than temperate ecosystems and, (2) that BGE is lower due to high respiration rates tropics. This paper aims to address and discuss these hypotheses by re-analyzing freshwater BP, BR and BGE data from literature (mainly from the temperate region), and by adding published and unpublished data from the tropical region. This work is an effort to expand current paradigms of microbial biogeochemistry to the tropical inland aquatic ecosystems. We point out the importance of increasing data production on BP and BR in the tropics to achieve a more accurate understanding about carbon cycling and the role of bacteria in this vast and understudied part of the world.

METHODS

We pooled models and bacterial production (BP) and bacterial respiration (BR) data from the literature (**Table 1**) and unpublished data from Lake Superior and northern Minnesota lakes (Sampled by J. Cotner and A. Amado), Swedish lakes (Sampled by L. Vidal and W. Granéli) and Amazonian lakes and rivers (Sampled by L. Vidal, G. Abril, F. Artigas and F. Roland). Lake Superior samples were taken at six sampling stations over five cruises in the western arm of the lake, from May–October 2006. Northern Minnesota lakes were sampled in July 2006 (summer) and January 2007 (winter) in a central point of each lake. Nineteen Swedish lakes were sampled in July 2007 (summer) in the deepest part of each lake over a DOC gradient from 3.7 to 26.8 mg L^{-1}. Twenty Amazonian lakes and rivers were sampled in June and November (2009) at stations throughout the Amazon River basin (for details see **Table 1**). For the literature data we surveyed the papers used in the review by Del Giorgio and Cole (1998) and all papers available from 1998–2012 searching for "BGE" and "aquatic ecosystems" key-words in the ISI Web of Science (**Table 1**). We only used data from inland aquatic ecosystems where both BP and the correspondent BR rates were available. Literature data were extracted from tables and graphs and converted to microgram of carbon per liter per hour (μg C L^{-1} h^{-1}) and log-transformed to perform statistical analysis. When the data were only available in graphs, they were extracted from the graphs using the Digitizeit and GraphClick softwares.

Regarding the original data presented in this work, BP rates were measured using the method of Smith and Azam, (1992; [^{3}H]-leucine incorporation method). The incubation times varied between 0.5 and 5.0 h according to the local conditions (e.g., temperature, nutrients concentrations). BR rates were measured by dissolved oxygen consumption over a 24-h period. Oxygen concentrations were measured by different methods based on changes in dissolved oxygen concentrations over time. In samples from Lake Superior and from Minnesotan lakes we measured the changes in dissolved oxygen in the same sample over time (as initial and final) using a gold tip micro-probe connected to OXY-meter (Briand et al., 2004) controlled by the MicOx Software (Unisense©, Aarhus, Denmark). Samples from Amazonian ecosystems and Swedish lakes were measured in discrete samples at various time-points with sets of replicated flasks/vials using the Winkler technique and titrations with a potentiometric endpoint using a Mettler DL21 titrator (Granéli and Granéli, 1991). BR rates were transformed to carbon using a respiratory coefficient (RQ) of 1 (shown as being the most commonly used by Del Giorgio and Cole, 1998).

From the literature, we only considered BP data estimated by radioisotope incorporation (using tritium-labeled (^{3}H) leucine or thymidine incorporation). As all BP estimates were measured over similar time scales, we considered that both methods estimated equivalent rates (Kirchman, 1992) and, thus, we did not apply any conversion factor between the results from the two methods. We did not consider BP data estimated from bacterial biomass accumulation in batch cultures. Despite the fact that it has been previously suggested that both radioisotope incorporation and biomass accumulation in batch cultures methods yield

Table 1 | Database used in the analysis with the literature extracted and novel data.

References	*N*	Region	BR range (μgC L^{-1} h^{-1})	BP range (μgC L^{-1} h^{-1})	BGE range	BCD range (μgC L^{-1} h^{-1})	BR method	BP method
Anesio et al. (2005)	5	Temperate	7.30–8.80	1.7–4.1	0.18–0.32	9.30–12.90	DIC increase	Leucine
Benner et al. (1995)	17	Tropical	3.12–22.68	0.31–2.70	0.02–0.34	4.59–25.38	Oxygen Winkler	Leucine/ Thymidine
Berggren et al. (2007)	9	Temperate	2.78–4.09	0.54–2.36	0.12–0.44	3.84–6.15	DIC increase	Leucine
Berggren et al. (2009)	6	Temperate	2.88–7.96	0.63–2.63	0.18–0.26	3.50–10.58	DIC increase	Leucine
Berggren et al. (2010)	18	Temperate	0.32–4.05	0.27–1.48	0.24–0.56	0.58–5.33	DIC increase/Oxygen probe	Leucine
Biddanda and Cotner (2002)	14	Temperate	0.21–2.82	0.02–1.32	0.01–0.53	0.23–3.53	Oxygen Winkler	Leucine
Biddanda et al. (2001)	12	Temperate	0.23–3.66	0.02–0.82	0.06–0.39	0.28–4.04	Oxygen Winkler	Leucine
Cammack (2002)	28	Temperate	0.88–20.58	0.03–2.10	0.02–0.22	0.97–22.68	Oxygen Winkler	Leucine
Comte and Del Giorgio (2009)	6	Temperate	0.80–7.50	0.03–7.90	0.04–0.51	0.83–15.40	Oxygen MIMs	Leucine
Cotner and Amado Unpublished	30	Temperate	0.05–42.02	0.01–7.24	<0.01–0.50	0.11–49.25	Oxygen Microelectrodes	Leucine
Del Giorgio et al. (2006)	24	Temperate	2.53–11.56	1.00–3.96	0.14–0.38	3.89–14.95	Oxygen MIMs	Leucine
Farjalla et al. (2009)	24	Tropical	8.79–23.42	0.70–2.74	0.06–0.19	9.81–25.80	DIC increase	Leucine
Guillemette and Del Giorgio (2012)	11	Temperate	0.52–1.71	0.13–2.29	0.18–0.69	0.71–3.91	Oxygen MIMs	Leucine
Hall and Cotner (2007)	16	Temperate	0.37–12.82	0.11–2.14	0.04–0.62	0.48–13.31	Oxygen Winkler	Leucine
Kritzberg et al. (2005)	19	Temperate	0.68–16.91	0.05–1.01	0.01–0.34	0.85–17.08	Oxygen Winkler	Leucine
Lennon and Cottingham (2008)	5	Temperate	0.09–0.23	0.03–0.17	0.25–0.42	0.12–0.40	Oxygen Winkler	Leucine
Maranger et al. (2005)	34	Temperate	2.98–9.34	0.75–7.63	0.16–0.58	4.06–14.5	Oxygen Winkler	Leucine
Roland and Cole (1999)	19	Temperate	1.43–8.81	0.35–10.10	0.04–0.66	2.28–16.32	Oxygen Winkler	Leucine
Roland et al. (2011)	134	Tropical	0.92–67.98	0.07–9.71	0.01–0.40	0.99–71.34	Oxygen Winkler	Leucine
Schwaeter et al. (1988)	16	Temperate	6.31–38.94	3.12–8.46	0.21–0.45	10.15–38.94	Oxygen Winkler	Thymidine
Vidal and Granéli Unpublished	19	Temperate	5.01–83.23	0.02–0.48	<0.01–0.02	5.03–83.52	Oxygen Winkler	Leucine
Vidal et al. Unpublished	22	Tropical	1.56–51.53	0.03–3.45	<0.01–0.23	1.59–53.06	Oxygen Winkler	Leucine
Warkentin et al. (2011)	10	Temperate	6.57–190.00	0.13–14.40	0.01–0.26	6.70–195.40	Oxygen Probe	Leucine

BR, Bacterial respiration; BP, bacterial production; BGE, bacterial growth efficiency; BCD, bacterial carbon demand ranges display the minimum and maximum values from each study.

$N_{total} = 498$; $N_{tropical} = 197$; $N_{temperate} = 301$.

similar results (Del Giorgio and Cole, 1998), the latter method is employed at longer timescales and require grazers-free condition and the radioisotope incorporation methods represent conditions closer to those of the natural system.

We used literature BR data measured via three dissolved oxygen consumption methods [the two mentioned above plus by oxygen estimated by membrane inlet mass spectrometer (MIMS)] and through dissolved organic carbon (DIC) accumulation. The BR rates estimated as oxygen consumption were transformed to a carbon basis by respiratory coefficient (RQ) of 1, as in the description of the novel data above. BR rates are frequently estimated from filtered samples (over a very wide range of pore sizes, frequently from 0.45 to 3.0 μm), but also in unfiltered samples. In oligotrophic ecosystems BR accounts for up to 90% of plankton respiration (Biddanda et al., 2001) and, thus, it is acceptable that BR could even be measured in unfiltered water. For the purpose of this study, we used the corrections and assumptions of the authors in estimating BR from whole or filtered water measurements. As our aim was to compare tropical to temperate rates with the same criteria, we assumed that any discrepancies related to the filtration pore-size would be randomly distributed between the data from the two regions and would not affect the overall patterns.

The dataset presented here was constructed with data from inland aquatic ecosystems in both temperate and tropical regions ($N_{\text{total}} = 498$; $N_{\text{tropical}} = 197$; $N_{\text{temperate}} = 301$). Our dataset includes information mostly from lakes, rivers and reservoirs, excluding estuarine and marine data. To our knowledge, this is the largest dataset of BP and BR for freshwaters.

From BP and BR data, we calculated bacterial growth efficiency (BGE = BP/[BP + BR]) and bacterial carbon demand (BCD = BP + BR). Statistical analyses were performed in the R environment (www.r-project.org) using the "vegan" (Oksanen et al., 2008), "lmodel2" and "smart" packages. We performed model II linear regression using the major axis method (package "lmodel2" by Pierre Legendre) on log-transformed data between BP vs. BR, and BP vs. BGE from the whole data set and with tropical and temperate subsets. Slopes and intercepts for temperate and tropical subsets were compared using the "ma" function (package "smart" by David Warton) that tests hypotheses about slope or elevation ("elev.test") based on confidence intervals comparison. We performed non-parametric Mann–Whitney Rank Sum test to compare median values of bacterial metabolism parameters between tropical and temperate subsets. Figures were made on SigmaPlot v.12 software (Systat Softare Inc.).

Table 2 | Slope, intercept and confidence interval (c.i.) of Type II Linear Regressions between log transformed BP vs. BR, and BP vs. BGE for tropical, temperate and whole dataset.

	Slope	95% [c.i.]	Intercept	95% [c.i.]	*n*	r^2	*p*
Log BR vs. Log BP							
All data	0.78	[0.68: 0.90]	0.84	[0.83: 0.86]	498	0.27	<0.0001
Tropical	0.60	[0.47: 0.75]	1.04	[1.03: 1.04]	197	0.27	<0.0001
Temperate	0.66	[0.54: 0.80]	0.68	[0.66: 0.72]	301	0.25	<0.0001
Log BGE vs. Log BP							
All data	0.70	[0.62: 0.79]	−0.86	[−0.88: −0.85]	498	0.35	<0.0001
Tropical	0.73	[0.62: 0.85]	−1.09	[−1.09: −1.08]	197	0.46	<0.0001
Temperate	0.71	[0.62: 0.82]	−0.71	[−0.73: −0.69]	301	0.41	<0.0001

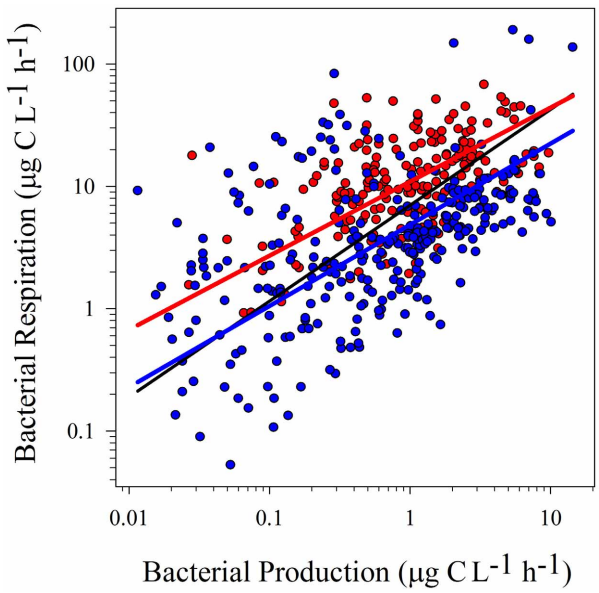

FIGURE 1 | Model II linear regressions between logBP and logBR from temperate (blue dots and blue line; $r^2 = 0.25$; $p < 0.0001$), tropical (red dots and red line; $r^2 = 0.27$; $p < 0.0001$) and the whole dataset (black line; $r^2 = 0.27$; $p < 0.0001$). See **Table 2** for confidence intervals.

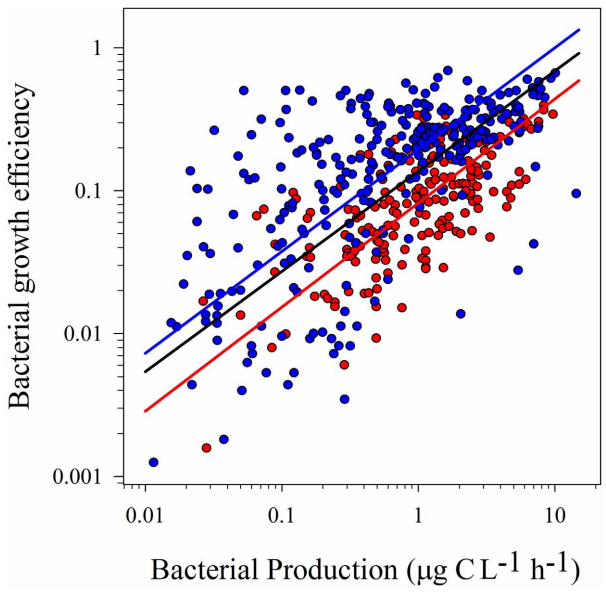

FIGURE 2 | Model II linear regressions between logBP and logBGE from temperate (blue dots and blue line; $r^2 = 0.41$; $p < 0.0001$) and tropical (red dots and red line; $r^2 = 0.46$; $p < 0.0001$) and the whole dataset (black line; $r^2 = 0.35$; $p < 0.0001$). See **Table 2** for confidence intervals.

RESULTS

Model II linear regressions on log-transformed BP and BR were significant, either considering the whole dataset or data from tropical and temperate regions separately (**Table 2**; **Figure 1**). Similarly, there was a significant positive relationship between log-transformed BP and BGE (**Table 2**; **Figure 2**).

Statistical tests on confidence intervals ("ma" function from "smart" package) showed no significant differences between slopes for temperate and tropical sub-sets, for both regressions (logBR vs. logBP and logBGE vs. logBP). However, the confidence interval for the intercepts was lower in the tropical subset (**Table 2**). These results were confirmed testing the elevation confidence intervals ("ma" function from "smart" package), which showed significant differences between the intercepts from temperate and tropical subsets (logBR vs. logBP: $p < 0.001$, Test statistic: $t = 14.73$ with 195 degrees of freedom, logBGE vs. logBP: $p < 0.001$, Test statistic: $t = -17.29$ with 195 degrees of freedom), indicating that, at similar BP levels, BGE was lower and BR was, at least, two-fold higher in tropical systems.

BP, BR and BCD presented higher median values in tropical than temperate subsets (logBR: $p < 0.001$, U Statistic = 13270.5; logBP: $p < 0.001$, U Statistic = 24013.5; LogBCD: $p < 0.001$, Mann–Whitney U Statistic = 15000.0; **Figure 3**), while the BGE median value was lower in the tropical subset (LogBGE: $p < 0.001$, U Statistic = 18352.0; **Figure 3**). It is worth noting that all parameters had a wider range of variation in temperate than the tropical subset (**Figure 3**).

DISCUSSION

This meta-analysis indicated that bacterial communities in tropical inland aquatic ecosystems had higher metabolic rates (BP, BR, and BCD) and lower BGE than temperate ecosystems. These

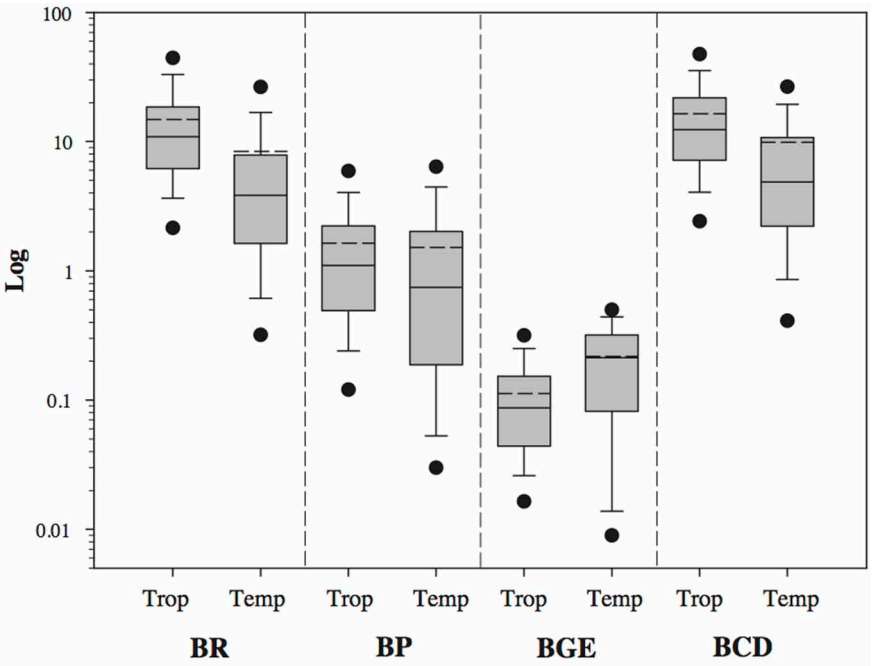

FIGURE 3 | Comparison of logBP, logBR, logBGE, and logBCD among temperate (Temp) and tropical (Trop) freshwater systems. The central full line indicates the median value, the dotted line indicates the arithmetic mean value, the boxes indicate the lower and upper quartiles, the vertical lines indicate the 10th and 90th percentiles, and the dots represent the 5th and 95th percentiles. Tropical and temperate data were significantly different (non-parametric Mann–Whitney Rank Sum test) in all the four variables (BP, BR, BGE and BCD with $p < 0.001$, see text for details).

results confirm the hypothesis presented here and the theoretical predictions from the literature (Lewis, 1987; Farjalla et al., 2009; Sarmento, 2012). We observed that BR rates were at least two fold higher in tropical ecosystems for a given BP, when compared to temperate ones. On one hand, heterotrophic bacteria process organic matter at faster rates in tropical ecosystems, regenerating inorganic nutrients more rapidly, but they do so while converting a smaller proportion of the organic matter into biomass. Even though we observed higher BP in the tropics, BR increased relatively more, resulting in lower BGE.

Higher BR rates in the tropics (**Figures 1**, **3**) indicate that, proportionally, more energy is required to maintain a similar bacterial biomass, when compared to temperate ecosystems. In other words, similar bacterial biomass could recycle a greater quantity of carbon in tropical ecosystems compared to temperate ones. Additionally, previous studies reported a comparatively lower bacterial abundance in tropical systems along a productivity gradient relative to temperate systems (Roland et al., 2010; Sarmento, 2012), reinforcing the idea of lower BGE in the tropics. Thus, regarding the allegedly important role of the microbial food web in energy and nutrient regeneration, (for review see Del Giorgio and Cole, 1998), we argue that remineralization is greatly emphasized in tropical inland aquatic ecosystems.

For the first time, we present here evidence of consistent differences in bacterial metabolic rates between tropical and temperate freshwater ecosystems, with a large and representative dataset. These observations were hypothesized in past tropical limnology literature (Lewis, 1987; Farjalla et al., 2009; Sarmento, 2012), but never demonstrated at large spatial scales. Lower BGE in the tropics is likely due in part to two factors that differ greatly between the tropics and the temperate regions: (1) higher average solar irradiance in the tropics and (2) fundamental differences in trophic structure. It is well established that the higher sunlight irradiance in aquatic ecosystems directly affects water temperature, substrate stoichiometry (e.g., higher C:N, C:P under higher light incidence; see Sterner et al., 1998) and nutrient availability (such as N and P). In turn, these factors are important regulators of bacterial activity in aquatic systems (Del Giorgio and Cole, 1998) and can have effects on metabolic efficiency (i.e., BGE) under different environmental conditions (Hall et al., 2009).

Temperature is considered a critical environmental factor affecting microbial metabolism and seems to play a major role in different aspects, from physiology to community structure and algal excretion rates. Recent studies have shown that temperature positively correlates with BP and BR, but negatively with BGE, especially in systems where nutrients (N and P) are not limiting (Rivkin and Legendre, 2001; López-Urrutia and Morán, 2007; Berggren et al., 2010). Usually, under nutrient limitation, BGE is already low (Berggren et al., 2010). Furthermore, it has been shown that the C:P ratio of heterotrophic bacterial biomass increases with increasing temperature even with no change in growth rates (Cotner et al., 2006). Moreover, bacteria with high biomass C:P ratio, as occur under strong P-limitation conditions present relatively lower BGE than when in low biomass C:P ratio (Phillips, 2012). Therefore, it seems reasonable to conclude

that the lower BGE in the tropics could result from higher average temperatures coupled with stronger nutrient, i.e., P limitation.

Previous studies predicted that nutrient recycling rates might be twice as high in tropical than in temperate ecosystems (Lewis, 1987). Our results corroborate this prediction showing that BR rates per BP unit was at least two fold higher in the tropics, consistent with higher temperatures and/or more nutrient-limited growth in the tropics. Although nutrient-limited growth has also been demonstrated in many temperate ecosystems as well, it is possible that the consistently higher BGE observed there could be due in part to less extreme nutrient limitation and perhaps stronger limitations by organic carbon availability (e.g., lower C:P ratios in primary producers). So, although nutrient regeneration rates may be high in warmer conditions, it is also likely that increasing growth demands and competition with autotrophs makes it more likely that bacteria in the tropics are limited by inorganic nutrients (Downing et al., 1999; Flecker et al., 2002).

Physical constraints of a thermodynamic nature should also be considered in order to explain the patterns observed. Considering that ATP production supports most biosynthetic assemblies (De Duve, 1991), a trade-off between rate and yield of ATP production might have important consequences to metabolism (Pfeiffer et al., 2001; Pfeiffer and Bonhoeffer, 2002; Schuster et al., 2008). The ATP paradox suggests that nature does not select for increased molar yield (detailed discussion in Schuster et al., 2008), because the highest thermodynamic efficiencies do not correlate to the highest growth rates (Westenhoff et al., 1983; Pfeiffer et al., 2001; Pfeiffer and Bonhoeffer, 2002) and this was confirmed in natural bacterial communities in aquatic ecosystems (Del Giorgio and Cole, 1998; Carlson et al., 2007). Thus, low metabolic rates lead to more efficient growth, while high metabolic rates (faster growth) results in low efficiency of biomass production (MacLean, 2008). For ecosystems, one of the implications is that one would expect cooler, more structured ecosystems where environmental changes are more predictable to have higher trophic efficiencies (Pfeiffer et al., 2001; Pfeiffer and Bonhoeffer, 2002); and lower efficiencies are expected in warmer, high metabolism ecosystems. Our observations are consistent with these predictions.

The tropics may also contrast with the temperate zone in terms of how sunlight interacts with the terrestrially-derived DOM. Assuming similar DOM export from terrestrial ecosystems and similar DOM concentrations in aquatic ecosystems at both tropical and temperate regions, greater photo-exposure in the tropics should increase production of reactive oxygen species (ROS) (Zepp and Cline, 1977; Cory et al., 2009). These compounds, such as singlet oxygen and hydrogen peroxide typically reduce microbial metabolism (Cory et al., 2010) by affecting cell structures activating repair mechanisms increasing the energy expenses (Madigan et al., 2010), and reducing BGE. ROS may also consume biologically available substrates, such as some amino acids, reducing bacterial growth and potentially BGE (Amado et al., 2007; Cory et al., 2010). Thus, the effects of higher DOM photochemical degradation and ROS production on microbial metabolism might be more pronounced in the tropics, also contributing to lower BGE (**Figures 1**, **3**) due to higher sunlight irradiance throughout the year.

In studying global phenomena, such as using meta-analysis, one needs to be cautious to avoid misinterpretation of data as a consequence of methodological artifacts. Regarding BR data, one could argue that using RQ equal to 1 to convert BR from oxygen consumption to carbon basis is not adequate to a large dataset with distinct ecosystems from different regions, because it has recently been shown that RQ can vary between 0.8 and 1.4 depending on the origin and oxidation state of the DOM (Berggren et al., 2011). However, without further information about each ecosystem and/or differences in RQs between the temperate and tropical regions, applying a global RQ of 1 seems to be the most logical and conservative solution. Nonetheless, the highest RQ values have been observed when bacteria use DOM compounds that are highly oxidized, such as photochemically degraded DOM (Berggren et al., 2011). Taking into account that tropical ecosystems are exposed to higher sunlight incidence (Lewis, 1987, 1996) and that DOM is more photochemically oxidized compared to temperate ecosystems (Farjalla et al., 2009), we should expect the highest RQ in the tropical bacterial communities. Higher RQ values would increase BR rates in tropical ecosystems even more, and lower BGE.

Finally, biotic interactions might also contribute to explain the differences observed in bacterial metabolism along the latitudinal gradient. The fact that tropical ecosystems have slightly higher BP and lower bacterial abundance (Roland et al., 2010; Sarmento, 2012) is a strong indication that bacterial loss factors (such as grazing by protists or virus-induced lysogeny) might be more relevant at low latitudes. Despite the few data available for tropical lakes, recent studies provided strong indications that grazing on bacteria by microzooplankton (mainly heterotrophic nanoflagellates) in tropical lakes should be relatively high (Tarbe et al., 2011; Sarmento, 2012). This expected high grazing pressure on bacteria would explain the lower bacterial abundances in the tropics along the productivity gradient (Roland et al., 2010; Sarmento, 2012). Alternatively, strong grazing control might likely maintain bacterial communities in a rapid growth condition (e.g., as in the exponential growth phase), resulting in high-energy metabolic expenditure (such as in the ATP paradox, see discussion above). Concerning viral infection, there is almost no information available on how viral infection varies with latitude or temperature, but it has been suggested that higher bacterial metabolic rates are usually associated with low viral induced lysogeny (Maurice et al., 2010).

CONCLUDING REMARKS

Including data from tropical freshwaters in a conventional (mainly temperate) bacterial metabolism database did not change radically the relationships among BP, BR and BGE previously reported. However, a comparative analysis of tropical against temperate data indicated that BP, BR, and BCD were higher in tropical than in temperate ecosystems. Moreover, comparing both regions, the difference was more pronounced in BR rates than in BP, and consequently BGE in tropical ecosystems was lower. Furthermore, BR rates per BP unit were at least two fold higher in the tropics than in temperate ecosystems indicating higher nutrients turnover rates. Higher annual temperatures, increased nutrient limitation and different food web configurations all help

to explain the higher energy dissipation in tropical regions. Lastly, higher sunlight exposure in the tropics contributes to higher photochemical DOM degradation and oxidation states and higher photochemical production of ROS. The current model of bacterial metabolism based on the relationship of BR and BP (Del Giorgio and Cole, 1998) is not adequate to predict the relationships between these two metabolic parameters efficiently at low latitudes. The addition of a large set of tropical data did not improve the general model, but highlighted some relevant factors that regulate microbial metabolism. Finally, we point out the need for intensive data collection on this topic, particularly in tropical regions in multiple biomes and ecosystem types.

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Coupling Between Heterotrophic Nanoflagellates and Bacteria in Fresh Waters: Does Latitude Make a Difference?

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Recent studies reported comparatively lower heterotrophic bacteria (HB) abundances in tropical regions, indicating that factors involved in bacterial losses could be more relevant in the tropics. Heterotrophic nanoflagellates (HNF) are considered the main predators of HB in aquatic ecosystems, and one should expect higher abundances in the tropics because of differences in the food web configuration (absence of large daphnids). However, there are no comprehensive studies comparing HB and HNF abundances in a latitudinal gradient. We hypothesized that HB abundance would be lower in the tropics because HNF abundance would be higher, resulting in a tighter HNF–HB coupling. To test this hypothesis, we compiled a large dataset of HB and HNF abundances from tropical and temperate freshwater environments. We found that both HB and HNF abundances were lower in the tropical region, and that HNF-HB coupling does not differ between temperate and tropical regions. The lower HNF abundance and lack of coupling may be explained by a strong top-down control on HNF and/or their herbivory preference. Besides, no relationship was found between bacterial specific growth rate and either chlorophyll-*a* and HB abundance, indicating that bacterial losses may have an important role in tropical freshwaters. Thus, we found that HNF is likely not the main controllers of HB abundance, and that grazing by ciliates and cladocerans, together with the physiological effects of higher temperatures, may explain the high bacterial loss rates in the tropics.

Keywords: bacterioplankton, cladocera, protist, predation, latitude

INTRODUCTION

Inland aquatic ecosystems play a relevant role in the global carbon cycle (Cole et al., 2007; Tranvik et al., 2009; Raymond et al., 2013). Low latitude freshwaters, particularly wetlands, represent a high percentage of global CO_2 evasion to the atmosphere compared to colder counterparts located in temperate regions (Marotta et al., 2009; Aufdenkampe et al., 2011; Barros et al., 2011;

Abril et al., 2014; Borges et al., 2015). The disproportional importance of tropical fresh waters in CO_2 net diffusion would be due to the high input of organic terrestrial carbon and further microbial heterotrophic respiration (Cole et al., 1994; del Giorgio et al., 1999), together with the higher temperatures (Kosten et al., 2010). In fact, bacterial biomass and production has been related to CO_2 lake concentrations (Tadonléké et al., 2012; Fontes et al., 2013), evidencing the importance of bacterioplankton in CO_2 emission dynamics. Thus, it is essential to identify the patterns and drivers of bacterial abundance, production and respiration across latitudinal gradients.

In this way, recent studies pointed out that, despite the slightly higher bacterial production in lower latitudes (Amado et al., 2013), the bacterial abundance found in those regions is lower, compared to temperate environments (Roland et al., 2010; Sarmento, 2012). This indicates that factors involved in bacterial loss would be more important in the tropics, since bacterial biomass does not seem to increase with increasing bacterial production in similar rates in both regions (Billen et al., 1990). The low bacteria:chlorophyll-*a* ratios found in warm waters suggest that grazing might be an important mechanism limiting bacterial abundance (Sarmento et al., 2008; Roland et al., 2010; Özen et al., 2013). These differences in HB abundance at different latitudes have been attributed, at least in part, to a higher top–down control of rotifers, ciliates, and nanoflagellates in warmer regions (Roland et al., 2010; Sarmento et al., 2010; Sarmento, 2012; Vázquez-Domínguez et al., 2012; Amado et al., 2013).

Because heterotrophic nanoflagellates (HNF) are considered the main responsible for channeling bacterial production to higher trophic levels (Fenchel, 1982; Sanders et al., 1989, 1992; Berninger et al., 1991), one should expect a higher top–down control on bacteria by the HNF in the tropics. Factors known to exert an influence on the predator–prey relationship between HNF and bacteria, such as temperature, bacterial, and HNF abundance (Peters, 1994; Vaqué et al., 1994; Gasol et al., 2002), vary widely with latitude. As temperature alters metabolic rates, it also influences all the other factors above cited, as predicted by the metabolic theory of ecology (MTE; Brown et al., 2004), which might also provide some insights on differences of microbial metabolic rates and trophic interactions between tropical and temperate regions.

The cornerstone of MTE is that metabolic rates, including grazing rates (Sarmento et al., 2010) and population growth rates (Savage et al., 2004), increase exponentially with temperature (Brown et al., 2004). For instance, bacterial abundance and production is thought to increase with increasing temperatures (White et al., 1991). However, the effects of temperature are not always straightforward, and increased temperatures may actually lead to a decrease in the abundance of the organisms, because the increased metabolic cost per individual means that a given supply of energy will support a smaller number of individuals (Brown et al., 2004; Savage et al., 2004; Sarmento et al., 2010). Yet, this assumption does not consider the effects of trophic interactions. For example, Jiang and Morin (2004) found that competition between the populations of two protists changed the outcome of temperature effects on their abundances, when compared with the isolated temperature effect on those populations. Also, Vasseur and McCann (2005) model states that temperature alone would not affect resource density in the absence of predators, implying that the effects of trophic interactions should also be taken into account.

Temperature has also been positively correlated with feeding rates, thereupon protist grazing rates on bacteria are expected to be higher with raised temperatures, since more food is required to fulfill their energy demand (Peters, 1994; Vaqué et al., 1994; Sarmento et al., 2010). Considering that tropical regions experience elevated temperatures throughout the year, bacteria might suffer a higher predation pressure, so that a larger proportion of bacterial production is taken by grazers (Sarmento et al., 2010), outbalancing bacterial growth stimulation by temperature. Indeed, in the few studies available for tropical region, HNF grazing on bacteria was found to be relatively high (Pirlot et al., 2007; Tarbe et al., 2011).

It is believed that HNF abundance in warm environments should be higher than in colder ones, owing to consistent differences in the food web structure along the latitudinal gradient (Sarmento, 2012; Özen et al., 2013). This is because in temperate environments there is a typical prevalence of large-bodied cladocerans, which are able to suppress the abundance of HNF (Gasol et al., 1995; Jürgens and Stolpe, 1995; Kalinowska et al., 2015). Actually, the predation pressure of *Daphnia* on HNF was found to result in a lack of coupling between HNF-bacteria in temperate systems, highlighting zooplankton as crucial regulators of bacterial abundance (Gasol and Vaqué, 1993; Jürgens et al., 1994). Meanwhile in the tropics, both temperature (Havens et al., 2015) and the high predation pressure exerted by the juvenile fishes, which are almost permanently present due to fish reproduction throughout the year (Fernando, 1994; Lazzaro, 1997; Iglesias et al., 2011), favor the development of small-bodied zooplankton. Those, in turn, would not be as efficient in reducing microbial abundances as their relatives of the temperate regions, thus the assumed greater HNF abundance would account for a tighter coupling between bacteria and HNF in tropical environments (Sarmento, 2012). Accordingly, elevated temperatures increasing microbial metabolism, along with the higher abundance of HNF and lower abundance of bacteria, all concur to the idea that HNF-bacteria coupling should differ across latitudinal gradients, being stronger in the tropics.

The aim of this study was to compare HNF and HB abundances in different latitudes (temperate vs tropical), as well as the HNF–HB coupling. Taking into account that HNF grazing pressure is thought to be the main explanation for lower bacterial abundance in tropical regions, and that the lack of HNF–bacterial coupling seems to be a widespread phenomenon in the temperate ones, we hypothesized that, in the tropics, (i) HB abundance would be lower, because (ii) HNF abundance would be higher, and consequently (iii) HNF–HB coupling would be stronger. We also investigated the importance of other predators and resources (i.e., chlorophyll-*a*) in explaining bacterial abundance in tropical environments. In order to test these hypotheses, we compiled a large dataset of HB and HNF abundances from tropical and temperate freshwater environments and compared

their abundances and the HNF-HB coupling, besides exploring other possible causes involved in bacterial losses in the tropics.

MATERIALS AND METHODS

Data Compilation

The dataset consists of 1047 observations of heterotrophic bacteria (HB) and HNF abundances from the literature in both tropical ($N_{trop} = 381$) and temperate ($N_{temp} = 666$) freshwater inland aquatic ecosystems. The data was gathered from some of the vast literature found for temperate environments as well as studies performed so far in the tropics, and encompasses a broad range of environment types, including shallow lakes, deep lakes, and reservoirs of various trophic status (**Table 1**). We also used abundance data of ciliates, rotifers, cladocerans, and copepods from tropical environments.

Data Analysis

HB and HNF Abundance and Relationship

To test whether HB and HNF abundances differ among tropical and temperate freshwater environments, we used non-parametric Mann–Whitney Rank Sum test. In addition, we also performed non-parametric Mann–Whitney Rank Sum test to compare median values of HB:HNF ratios between tropical and temperate environments. To examine the relationship between HNF and HB on tropical and temperate datasets, we performed model II linear regression using the major axis (MA) method (Legendre, 2014), and verified the normal distribution of the log-transformed data. We compared the slopes and intercepts for both regions using the "ma" function of the "smatr" package (Warton et al., 2012), that tests hypotheses about slope or elevation ("elev.test") based on confidence intervals comparison.

Bacterial Specific Growth Rate (SGR) Relationship with Chlorophyll-*a* and Bacterial Abundance

We used a dataset comprehending several tropical environments sampled in different seasons (Lobão et al., in preparation) to verify if bacterial SGR was more related to resources or predators. We estimated bacterial SGR using the equation proposed by Kirchman (2002): SGR = P/B, where P = bacterial production ($\mu gC\ L^{-1}\ h^{-1}$) and B = bacterial biomass ($\mu gC\ L^{-1}$). We performed linear regressions to test the relationship between SGR and HB abundance, which might provide some hints about the factors controlling their abundance. The rationale is that, considering the density-dependent logistic growth of bacteria, SGR is low when bacterial abundance is reaching the carrying capacity, meaning that they are limited by resource availability. Hence, a negative relationship between SGR and abundance indicates bottom-up control. Conversely, SGR is high when bacterial abundance is far from reaching the carrying capacity. Thus, the lack of relationship between SGR and abundance indicates top-down control, so that predators could be consuming bacteria at rates equal to or higher than their production (Wright and Coffin, 1984; Gasol et al., 2002).

Impact of Other Communities on HB and HNF Abundance

We examined the effects of potential predators on HB and HNF in the tropical region. We considered the abundances of HB and HNF as response variables separately, and performed multiple regressions for each one. For HB, we used the abundance of the predators HNF, ciliates, rotifers and cladocerans as explanatory variables, excluding copepods, which have a very low capture efficiency of picoplankton (Wilson, 1973; Finlay and Roff, 2004; Sommer and Sommer, 2006). For HNF, we used the abundance of the predators known to exploit them as food, such as ciliates, rotifers, cladocerans, and copepods.

Data was log-transformed and all analyses were performed in R Development Core Team (2013) using the libraries "vegan" (Oksanen et al., 2015), "lmodel2" (Legendre, 2014), and "smatr" (Warton et al., 2012). Figures were made on SigmaPlot v.12 software (Systat Softare Inc.).

RESULTS

At first, we considered all data we gathered from the literature in our analyses. However, some of the studies performed in highly eutrophic environments have found extreme values of HB and HNF abundance, never reported before on the literature (i.e., Fermani et al., 2013, 2015). As we did not found any equivalent conditions in the tropical dataset, and since we noticed that the data from those studies were outliers, we decided to disregard those values from all our analyses. In this way, we maintained a similar distribution of points among trophic states in the temperate (oligotrophic: 16%, mesotrophic: 50%, eutrophic: 34%) and tropical (oligotrophic: 19%, mesotrophic: 45%, eutrophic: 36%) regions. Nevertheless, we show them in the regression figure (**Figure 3**) for comparison purposes.

HB and HNF Abundance

Comparing HB and HNF abundance in tropical and temperate freshwater environments, we found higher values in the temperate region for both HB (logHB: $p < 0.001$, Mann–Whitney U Statistic = 218964.5; **Figure 1A**) and HNF (logHNF: $p < 0.001$, Mann–Whitney U Statistic = 189579; **Figure 1B**) communities.

HNF–HB Relationship

HB:HNF ratios were not significantly different between tropical and temperate environments (HB:HNF: $p = 0.3049$, Mann–Whitney U Statistic = 131703; **Figure 2**).

We found a significant positive relationship between HNF and HB for tropical and temperate regions. Comparing the regression models from both regions, we found no significant differences between the slopes, besides no differences in the confidence intervals for the intercepts (**Table 2**; **Figure 3**).

Factors Controlling HB and HNF Abundances

Linear regressions between SGR and HB abundance (**Figure 4**) were non-significant in most tropical systems

TABLE 1 | Database from each literature data in tropical and temperate environments used in all analysis. *To perform the analysis of the relationship between bacterial specific growth rates, chlorophyll-*a*, and bacterial abundance, we used a different dataset (see below).

Reference	*N*	HB abundance		HNF abundance	
		Minimum	Maximum	Minimum	Maximum
Tropical					
Domingues et al., submitted	46	5.93×10^5	6.17×10^6	1.80×10^3	2.75×10^4
Meira et al., in preparation	21	3.03×10^5	2.50×10^6	6.52×10^0	2.02×10^2
Morana et al., 2014	21	1.82×10^6	4.58×10^6	2.07×10^2	1.11×10^3
Velho et al., in preparation	36	1.46×10^5	7.54×10^5	1.10×10^2	2.35×10^3
Pereira et al., 2014	58	1.18×10^6	8.48×10^6	9.22×10^1	1.56×10^4
Pirlot et al., 2005	21	1.66×10^6	5.63×10^6	2.99×10^2	4.08×10^3
Segovia et al., 2014	72	1.46×10^5	1.26×10^6	1.09×10^2	1.21×10^4
Segovia et al., in preparation	106	4.18×10^4	2.33×10^6	1.78×10^1	1.53×10^3
Total	**381**				
Temperate					
Bennett et al., 1990	32	2.31×10^6	8.73×10^6	1.12×10^3	5.61×10^3
Berninger et al., 1993	81	7.33×10^6	2.21×10^7	8.07×10^3	7.14×10^4
Bird and Kalff, 1989	12	2.55×10^6	1.34×10^7	2.80×10^2	6.20×10^3
Bloem and Bär-Gilissen, 1989	34	4.00×10^6	1.00×10^7	2.00×10^2	3.40×10^4
Bloem et al., 1989	12	5.42×10^6	1.45×10^7	5.40×10^2	1.05×10^4
Christoffersen et al., 1990	10	4.13×10^6	5.91×10^6	8.75×10^1	1.08×10^3
Fermani et al., 2013*	41	2.34×10^7	1.08×10^8	9.40×10^3	1.12×10^5
Fermani et al., 2015*	36	1.39×10^6	2.87×10^8	1.47×10^2	3.89×10^5
Finlay et al., 1988	6	8.70×10^6	2.10×10^7	5.00×10^4	1.80×10^5
Güde, 1986	7	4.10×10^6	9.40×10^6	2.30×10^3	7.20×10^3
Güde, 1988	9	3.80×10^6	9.95×10^6	1.40×10^2	7.67×10^3
Jürgens and Güde, 1991	19	4.10×10^6	1.24×10^7	1.40×10^3	2.50×10^4
Jürgens and Jeppesen, 2000	10	4.76×10^6	1.56×10^7	2.29×10^3	1.29×10^4
Munawar and Weisse, 1989	72	3.90×10^5	3.35×10^6	4.40×10^2	5.79×10^3
Nakano et al., 1998	16	1.23×10^7	4.87×10^7	3.06×10^3	1.42×10^5
Pace et al., 1990	5	3.10×10^6	7.83×10^6	4.40×10^2	1.05×10^3
Pick and Caron, 1987	22	6.90×10^5	6.20×10^6	4.92×10^2	6.65×10^3
Šimek and Fuksa, 1989	12	1.98×10^6	4.89×10^6	9.20×10^1	1.39×10^3
Šimek et al., 1990	17	1.34×10^6	3.99×10^6	8.60×10^1	1.29×10^3
Šimek et al., 1997	32	2.05×10^6	4.60×10^6	1.35×10^3	4.45×10^3
Sommaruga, 1995	36	1.70×10^6	2.03×10^7	1.14×10^3	2.97×10^4
Vaqué and Pace, 1992	64	2.90×10^6	8.76×10^6	1.69×10^2	1.92×10^3
Weisse, 1990	24	5.69×10^5	6.56×10^6	5.40×10^2	8.11×10^3
Weisse, 1991	103	4.21×10^5	7.99×10^6	3.14×10^2	7.97×10^3
Wieltschnig et al., 2001	31	2.91×10^6	6.66×10^6	5.59×10^2	2.34×10^3
Total	**743**				

**Data from those references were considered outliers and were not used in our analysis.*

(six out of eight systems), pointing toward a regulation of bacterial numbers by predation for most systems. Taking all systems together, this relationship was not significant either.

We performed multiple regressions to evaluate the effects of potential predators on HB abundance. The regression model explained 28% of the variation in HB abundance of the tropical data and included the abundances of HNF, ciliates, rotifers and cladocerans (**Table 3**). The standardized regression coefficients of both HNF and rotifers were positive, thus an increase in HNF and rotifer abundance was associated with an increase in HB abundance, suggesting a bottom-up effect. As for the ciliates and cladocerans, we found a negative relationship, suggesting a top down effect, since an increase in ciliate and cladoceran abundance was associated with a reduction in HB abundance.

The best multiple regression model for HNF abundance included only ciliates and cladocerans and explained 32% of the HNF abundance variation. The standard regression coefficient of ciliates was positive, indicating a simultaneously increase in both variables. As for the cladocerans, we found a negative

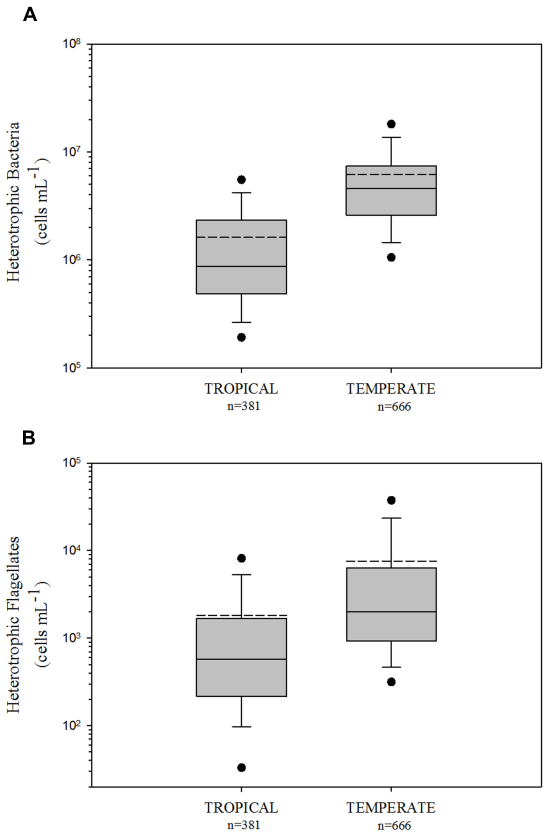

FIGURE 1 | Comparison of heterotrophic bacteria (A) and heterotrophic flagellates (B) among tropical and temperate freshwater environments. The central full line indicates the median value, the dotted line indicates the arithmetic mean value, the boxes indicate the lower and upper quartiles, the vertical lines indicate the 10th and 90th percentiles, and the dots represent the 5th and 95th percentiles. Tropical and temperate data were significantly different (non-parametric Mann-Whitney Rank Sum test) in the two variables (HB and HNF with $p < 0.001$, see text for details).

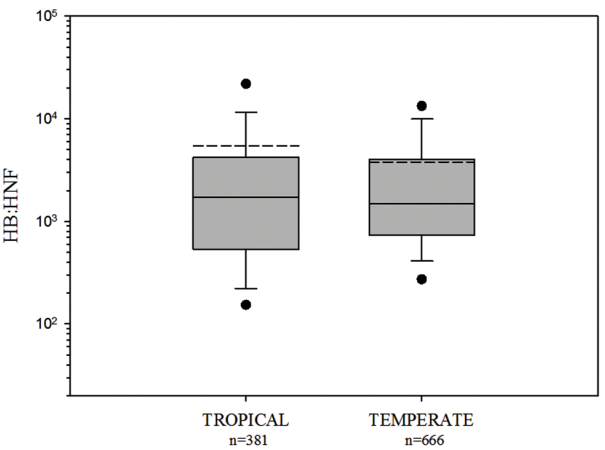

FIGURE 2 | Comparison of HB:HNF ratios among tropical and temperate freshwater environments. The central full line indicates the median value, the dotted line indicates the arithmetic mean value, the boxes indicate the lower and upper quartiles, the vertical lines indicate the 10th and 90th percentiles, and the dots represent the 5th and 95th percentiles. HB:HNF ratios were not significantly different between tropical and temperate environments (see text).

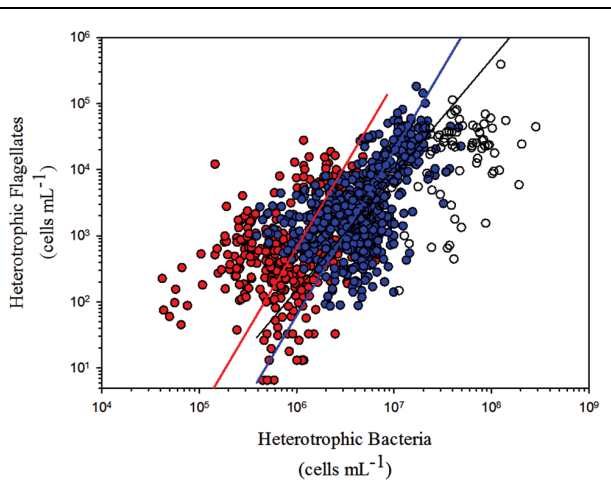

FIGURE 3 | Model II linear regressions between HNF and HB for tropical (red dots and red line; $r^2 = 0.14$; $p < 0.0001$) and temperate (blue dots and blue line; $r^2 = 0.30$; $p < 0.0001$) freshwater environments (see Table 2 for confidence intervals). Outliers disregarded from our analyses (blank dots) and model II linear regressions for temperate freshwater environments including those outliers (black line) are also shown (see Materials and Methods).

relationship, indicating a top–down effect of this group on HNF (**Table 3**).

DISCUSSION

We compiled for the first time a consistent HNF and HB abundance database for tropical freshwaters, and compared the abundances of those communities with the ones from the temperate environments, as well as explored probable causes of lower bacterial abundance in the tropics. We found that both HNF and HB abundances were lower in the tropics and that there is no difference in the HNF-HB coupling between those regions. Besides, HB abundances were apparently more regulated by predation, especially from ciliates and cladocerans.

TABLE 2 | Model II Linear Regression parameters between HNF and HB for tropical and temperate regions.

LogHNF vs. LogHB	Slope	95% (ci)	Intercept	95% (ci)	*n*	r^2	*p*
Tropical	2.49	(1.98:3.29)	−12.12	(−16.92:−9.04)	381	0.14	<0.0001
Temperate	2.48	(2.22:2.81)	−13.13	(−15.28:−11.38)	666	0.3	<0.0001

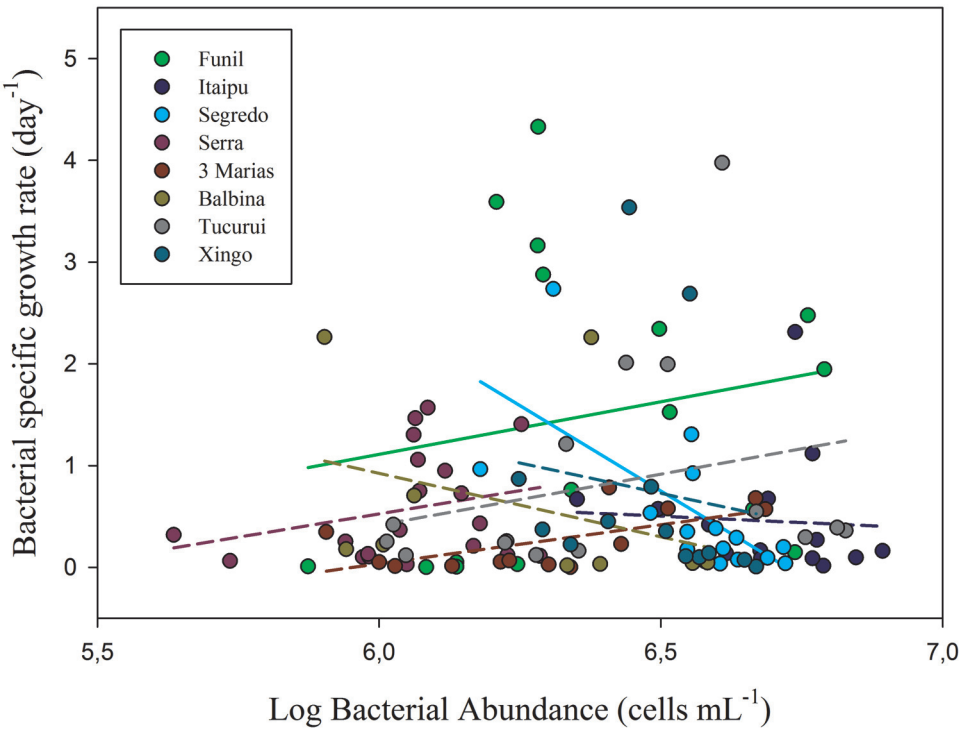

FIGURE 4 | Relationship between bacterial SGR and HB abundance in several tropical freshwater environments. Most linear regressions (six out of eight) were non-significant, suggesting regulation by predation. One regression was positive (β = 0.13; r^2 = 0.30) and only one was negative suggesting resource limitation, although not a very strong one (β = –0.18; r^2 = 0.49).

TABLE 3 | Regression analyses for HB and HNF abundance of the tropical region.

	Models		β (±SE)				
		r^2	HNF	Cili	Rot	Clad	Cop
HB	HNF, Cili, Rot, Clad	0.28	0.55 (±0.05)	**– 0.36** (±0.05)	0.31 (± 0.05)	**– 0.13** (±0.06)	–
HNF	Cili, Clad	0.32	–	0.60 (± 0.05)		**– 0.17** (±0.06)	–

β, standard regression coefficient; SE, standard error; HB, heterotrophic bacteria; HNF, heterotrophic nanoflagellates; Cili, ciliates; Rot, rotifers; Clad, cladocerans; Cop, copepods. Bold values are the negative β values.

Weak Evidence of Resource Limitation

Evidence found in the literature suggests that bacterial growth dependence on phytoplankton derived dissolved organic carbon (DOC) supply might not always be that relevant in low latitudes. Although there is evidence that phytoplankton derived DOC would be important for the bacterioplankton of large African tropical lakes (Stenuite et al., 2009; Morana et al., 2014), low HB:phytoplankton biomass ratios have been found (Sarmento et al., 2008). In a comparative analysis using different types of Brazilian freshwater ecosystems, Roland et al. (2010) found a much weaker HB:chlorophyll-*a* correlation in tropical when compared to the non-tropical environments. In this way, the bacteria–phytoplankton uncoupling seems to be a recurrent situation in south-American lowland lakes (e.g., Carvalho et al., 2003; Gocke et al., 2004; Rejas et al., 2005; Petrucio et al., 2006; Teixeira et al., 2011; Almeida et al., 2015), which are generally smaller and shallower, comparing to the East-African Great Lakes. White et al. (1991) reported a rather weak correlation between SGR and chlorophyll-*a* in freshwaters, and suggested that variations in the importance of grazing pressure may have contributed to this finding.

However, as allochthonous DOC may also constitute an important resource for HB (Tranvik, 1992), a regulation of HB by those carbon sources could also explain the weak dependency of bacteria on phytoplankton. Unfortunately, we do not have data concerning those variables, which would allow us to elucidate this point. Nonetheless, our results of non-significant relationships between bacterial SGR and HB abundance in most of the tropical systems analyzed (**Figure 4**), reinforce the idea that predation might be more relevant than resource limitation, whatever that resource could be. If HB abundance and SGR were not related, grazing was likely consuming HB at such a rate that it was limited by a small range of possible growth rates (Wright and Coffin, 1984; Gasol et al., 2002). Thus, we could infer that resource

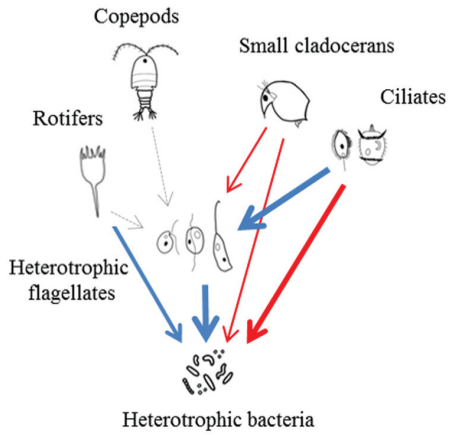

FIGURE 5 | Schematic representation showing possible impacts of predators and resources on HB and HNF in tropical regions. Dashed arrows indicate no relationship, blue arrows indicate positive and red arrows indicate negative relationships. The thickness of the arrows is proportional to the strength of the interaction.

limitation was not likely to restrain HB abundance in most tropical freshwater environments, and that a top-down control might prevail in these systems.

HNF Abundance is Also Lower in Tropical Environments

The assumption of a higher abundance of HNF in tropical, relative to temperate environments, was not corroborated in our study. Although large-bodied cladocerans are relatively low abundant in the tropics, the typical small bodied cladocerans, seem to exert a strong predation pressure on HNF, as evidenced by the negative standard coefficient multiple regression model (**Table 3**).

The impact of small-bodied cladocerans on HNF is somewhat unexpected, since in the tropics there is usually a smaller proportion of Daphniidae, which is replaced by Bosminids, Sidids, and Moinids (Dumont, 1994; Elmoor-Loureiro, 2000). However, the influence of cladocerans on the abundance of HNF was already verified in the bottom layer a tropical floodplain lake where those predators were more abundant, specially represented by *Bosmina hagmanni* and *Ceriodaphnia cornuta* (Segovia et al., 2014). In fact, the small-bodied cladocerans *Bosmina*, *Ceriodaphnia*, and *Diaphanosoma* were found to achieve higher weight-specific clearance rates on HNF than that of *Daphnia* species (Jürgens et al., 1996). Specifically, *Bosmina* have a particular foraging mode, different from filter-feeding, which allows certain selectivity and consequently more efficient removal of small flagellates compared to *Daphnia* (DeMott and Kerfoot, 1982), even at low food concentrations (DeMott, 1982). Thus, even though Daphnids are recognized as the main responsible for hampering the development of HNF in temperate ecosystems (Pace and Vaqué, 1994; Gasol et al., 1995; Jürgens and Stolpe, 1995), their low abundance in the tropics would not result in a weaker predation pressure of cladocerans on HNF, since other small-bodied cladocerans such as the Bosminids may replace *Daphnia*, in the sense that they would also be able to suppress HNF effectively. As for the ciliates, we found a positive relationship with HNF, indicating that both variables are increasing. It is possible that this could be the result of the control of both HNF and ciliates by variables related to their shared resources and predators (Auer et al., 2004; Segovia et al., 2014; Domingues et al., submitted).

HNF–HB coupling in the tropics does not seem to differ from that of the temperate regions. A top–down control by cladocerans on HNF may be keeping them from reaching the high abundances they presumably would have in the tropics, blurring their effects on bacteria (Gasol and Vaqué, 1993; Gasol, 1994; Wieltschnig et al., 2001; Segovia et al., 2014; Kalinowska et al., 2015). Another possible cause for the lack of HNF–HB coupling is the use of an alternative food resource by the HNF, such as the picophytoplankton (PPP). Herbivory preference by nanoflagellates, rather than bacterivory, was verified in the large tropical Lake Tanganyika (Tarbe et al., 2011). The preference of HNF for PPP was also found in shallow floodplain lakes in the tropical region (Meira et al., in preparation). In addition, the biomass of HNF was negatively related to PPP in tropical reservoirs of different trophic states, pointing out the importance of this interaction on these environments as well (Domingues et al., submitted). To sum up, the lower HNF abundance found, together with the similar HNF-HB coupling, suggests that HNF is probably not related to the lower HB abundance in the tropics.

Grazing by Ciliates and Cladocerans May Explain the Lower HB Abundance in the Tropics

The variables associated with HB abundance in the tropics were HNF, ciliates, rotifers, and cladocerans. HNF and rotifers were positively related with HB abundance, which means that they are likely feeding on bacteria but are not able to suppress their abundance. On the contrary, both ciliates and cladocerans showed a negative relationship, suggesting a top–down control on HB abundance. As stated before, resource limitation or predation by HNF are unlikely to be the reason why bacterial abundance is lower in the tropics. Thus, the negative effect of both ciliates and cladocerans could be part of the explanation for such a pattern.

Although there is a vast literature relating the prevalence of HNF as the major bacterivores (Fenchel, 1982; Sanders et al., 1989, 1992; Berninger et al., 1991), the relatively higher importance of ciliates as predators of bacteria was also documented. The dominance of ciliates as grazers of bacteria has been reported in occasions where HNF abundance is rather low (Kisand and Zingel, 2000; Tadonléké et al., 2005; Zingel et al., 2007). Also, ciliate community structure in the tropics may differ from that of the temperate regions. It is known that bacterivory is predominant among the small oligotrich ciliates (Stabell, 1996; Šimek et al., 2000), thus perhaps features such as ciliate community composition might be playing a role on the impact of ciliates in tropical environments, where there could be a larger proportion of those bacterivorous taxa. However, more studies

are necessary to draw such a conclusion. Another overlooked aspect would be the influence of temperature on the ciliate feeding rates. It has been shown that ciliate feeding rates increase considerably with the raise of temperature (Sherr et al., 1988; Rychert, 2011). Therefore, it is plausible to infer that the higher temperatures of the tropics may be a relevant factor.

Similarly to the impact on HNF, weight-specific filtering rates on bacteria were found to be higher for *Ceriodaphnia* and *Bosmina* than for the large *Daphnia magna* (Porter et al., 1983). Vaqué and Pace (1992) found that lakes dominated by large populations of *Bosmina longirostris* showed even slightly higher maximum values of grazing on bacteria (35×10^6 bacteria L^{-1} h^{-1}) than *Daphnia pulex* (30×10^6 bacteria L^{-1} h^{-1}), and concluded that, when in large numbers, populations of small cladocerans compensate for the lack of large Daphnids. Thus, if tropical environments are dominated by those small-bodied cladocerans, then their impact on bacterioplankton could be higher than in temperate environments. In addition, a positive relationship has been found between cladoceran filtering rate and temperature (Burns, 1969). For example, Mourelatos and Lacroix (1990) found that at a temperature of 20°C, a *Daphnia* of 0.5 mm size filtered as much as one twice its size but at a 10°C temperature, suggesting that at higher temperatures those small-bodied cladocerans should have an even greater impact. Moreover, a recent study found that pelagic cladocerans significantly explained the variation in bacterial community composition in tropical South American shallow lakes (Souffreau et al., 2015), demonstrating that the predation pressure of those microcrustaceans might also be responsible for changes in bacterial community structure.

Thus, ciliates and small cladocerans seem to play a central role in the pelagic food webs of tropical freshwater environments, and the fundamental differences in the food web structure of freshwater environments in temperate and tropical environments, together with the higher temperatures of the tropical ones, likely dictate the fate of bacterial production (**Figure 5**).

It is worth noting that virus lysis is also recognized as a major source of bacterial losses (Fuhrman and Noble, 1995), however, few studies concerning this topic were performed in the tropics. Low virus-to-bacterium ratios and frequency of visible infected cells were found in Amazonian floodplain lakes (Barros et al., 2010; Almeida et al., 2015) and African lakes (Bettarel et al., 2006). Barros et al. (2010) suggested that these low values could be related to the registered low bacterial abundances, which restrain the rates of encounter between the virus and the bacterial host cell, resulting in a low level of viral predation. As a corollary for this explanation, the comparable lower abundances of bacteria in the tropics should result in lower loss rates by viral attack than in the temperate systems. Nonetheless, relatively high values of virus-to-bacterium ratios were found in tropical reservoirs (Peduzzi and Schiemer, 2004) and in a tropical lake (Araújo and Godinho, 2009). Thus, bacterial mortality caused by virus should be taken into account when studying mechanisms controlling bacterial abundance in tropical freshwaters in the future to elucidate this issue.

CONCLUSION

Comparing tropical against temperate data reinforced the previous findings that bacterial abundance is lower in the tropics. Moreover, bacterial specific growth rate was not related to either chlorophyll-*a* and HB abundance, pointing to an important role of bacterial losses in the tropics. Besides, we found that HNF abundance is also lower in the tropics and that HNF-HB coupling is not different across latitudes. A top–down control on HNF and their herbivory preference may help explain the lack of HNF–HB coupling, and suggests that HNF is likely not the main cause for bacterial loss. It is possible that grazing by ciliates and cladocerans play a large role in controlling bacterial abundance in the warmer regions. However, this issue should be more investigated in future studies concerning tropical freshwater environments.

AUTHOR CONTRIBUTIONS

HS and BTS conceived and designed the study. CDD, BRM, FMLT, PF, FU and LFMV sampled and analyzed the abundance data of microbial communities from tropical/temperate freshwaters. LML and FR sampled and analyzed the bacterial specific growth rate data from tropical freshwaters. BTS wrote the manuscript with input from all coauthors.

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Potential changes in bacterial metabolism associated with increased water temperature and nutrient inputs in tropical humic lagoons

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Temperature and nutrient concentrations regulate aquatic bacterial metabolism. However, few studies have focused on the effect of the interaction between these factors on bacterial processes, and none have been performed in tropical aquatic ecosystems. We analyzed the main and interactive effects of changes in water temperature and N and P concentrations on bacterioplankton production (BP), bacterioplankton respiration (BR) and bacterial growth efficiency (BGE) in tropical coastal lagoons. We used a factorial design with three levels of water temperature (25, 30, and 35°C) and four levels of N and/or P additions (Control, N, P, and NP additions) in five tropical humic lagoons. When data for all lagoons were pooled together, a weak interaction was observed between the increase in water temperature and the addition of nutrients. Water temperature alone had the greatest impact on bacterial metabolism by increasing BR, decreasing BP, and decreasing BGE. An increase of 1°C lead to an increase of ∼4% in BR, a decrease of ∼0.9% in BP, and a decrease of ∼4% in BGE. When data were analyzed separately, lagoons responded differently to nutrient additions depending on Dissolved Organic Carbon (DOC) concentration. Lagoons with lowest DOC concentrations showed the strongest responses to nutrient additions: BP increased in response to N, P, and their interaction, BR increased in response to N and the interaction between N and P, and BGE was negatively affected, mainly by the interaction between N and P additions. Lagoons with the highest DOC concentrations showed almost no significant relationship with nutrient additions. Taken together, these results show that different environmental drivers impact bacterial processes at different scales. Changes of bacterial metabolism related to the increase of water temperature are consistent between lagoons, therefore their consequences can be predicted at a regional scale, while the effect of nutrient inputs is specific to different lagoons but seems to be related to the DOC concentration.

Keywords: environmental changes, bacterial metabolism, bacterial growth efficiency, tropical aquatic ecosystems, coastal lagoons, water temperature

Introduction

Changes in climate and biogeochemical cycles are among the most important effects of human alterations to ecosystems (Rockström et al., 2009). For instance, nitrogen deposition, and phosphorus loads related to sewage disposal are affecting natural aquatic ecosystems worldwide (Schindler and Vallentyne, 2008). Increases in air temperature and changes in rain patterns are also expected in most regions (IPCC, 2013). For the highly populated Brazilian Southeast in particular, an increase in the average daily temperature and dramatic changes in the intensity and periodicity of rainfall events are expected for the next century (Marengo et al., 2010; PBMC, 2013). These changes will impact the amount of energy and matter flowing into aquatic ecosystems by increasing water temperature and augmenting the input of nutrients from drainage areas, with consequences for the structure and functioning of aquatic ecosystems (Roland et al., 2012). Furthermore, in tropical aquatic ecosystems, synergy between climate-driven impacts and changes in the input of inorganic nutrients is expected, mainly in highly populated areas (Roland et al., 2012). Thus, studies integrating different environmental changes into the same experimental design are necessary to better understand the effects of synergistic interactions on the functioning of aquatic ecosystems.

Bacterioplankton play an essential function in the transfer of energy and materials in aquatic ecosystems. Bacterioplankton respiration (BR) mineralizes large amounts of organic carbon substrates, thereby contributing to CO_2 saturation in aquatic ecosystems, whereas bacterioplankton production (BP, i.e., secondary production) is an important source of energy for higher trophic levels through the microbial loop food chain (Fenchel, 2008). The ratio of carbon incorporated into bacterial biomass to total carbon assimilated by bacteria (bacterial growth efficiency, BGE) has been used to evaluate whether and where bacteria act as a carbon source or sink in aquatic ecosystems (for a review, see del Giorgio and Cole, 1998). Several environmental factors regulate BGE in aquatic ecosystems, and any changes in these environmental factors would alter bacterioplankton metabolism, which, in turn, would alter bacterioplankton function in aquatic ecosystems. For instance, low concentrations of N and P limit BP in aquatic ecosystems (e.g., Granéli et al., 2004; Smith and Prairie, 2004; Haubrich et al., 2009) but BR appears to be less affected in oligotrophic aquatic ecosystems. Low BGE is expected in aquatic ecosystems with high C:N and C:P ratios, and the input of inorganic nutrients in oligotrophic waters should increase BGE. Rising water temperature increases the permeability of the cell membrane to carbon substrates, stimulating both BP and BR (Van de Vossenberg et al., 1999; Mansilla et al., 2004). However, higher temperatures could also decrease some enzymatic reactions, increasing the metabolic cost of maintenance and cellular repair and consequently decreasing BGE (Hall and Cotner, 2007). The effect of temperature on BGE can also be mediated by nutrient concentrations, but this is not a general response pattern. For instance, some studies reported a negative relationship between BGE and temperature in nutrient limiting conditions (e.g., Kritzberg et al., 2010), others reported a negative relationship between BGE and temperature when nutrients were abundant (e.g., Hall and Cotner, 2007; Berggren et al., 2010), while others found little or no effect of temperature on BGE, and suggested that BGE was primarily regulated by the availability of nutrients (e.g., López-Urrutia and Morán, 2007; Lee et al., 2009). Most studies on the effects of temperature on bacterial metabolism were performed in temperate ecosystems, but those effects could be more dramatic in tropical systems, where organisms are already in their optimum temperature conditions (Deutsch et al., 2008). In addition, most of these studies failed to disentangle the effects of temperature and nutrients on bacterial metabolism; this article aims to fill this gap in the literature evaluating the main and interactive effects of water temperature and nutrient additions on bacterial metabolism in tropical aquatic ecosystems.

Tropical coastal lagoons of the Brazilian Southeast have several characteristics that make them suitable ecosystems for the evaluation of how changes in environmental condition affect bacterial metabolism. First, these lagoons provide a natural gradient in environmental conditions (for a review, see Esteves et al., 2008). Inorganic N and P concentrations are low, and oligotrophic and dystrophic conditions prevail, though some effects of cultural eutrophication on nutrient availability have also been observed in lagoons located near major cities (Esteves et al., 2008). Water temperature varies synchronously in these lagoons (Caliman et al., 2010), and daily changes of more than 10°C have been observed in the shallowest lagoons (depth <1 m; Farjalla et al., 2005). Second, climate- and human-driven impacts on these lagoons are predicted to increase during this century (Esteves et al., 2008; Roland et al., 2012; PBMC, 2013). For instance, local population triplicate in the last few years and should double in the next decade driven by the development of the offshore oil industry, and there is good a 1:1 relationship between population growth and cultural eutrophication of coastal aquatic ecosystems in the region (Esteves et al., 2008; Borges et al., 2009). Climate prediction indicates an increase of 0.5–1.0°C through 2040 and of 2–3°C between 2041 and 2070 (PBMC, 2013). Third, bacterioplankton metabolism has been extensively studied in these lagoons (e.g., Farjalla et al., 2002, 2005, 2009a). Previous studies have found that BP is usually limited by the availability of inorganic P, though N limitation and N and P co-limitation were also observed (Farjalla et al., 2002). The quality of bulk Dissolved Organic Carbon (DOC) favors BR over BP, and BGE is typically low in these lagoons (Farjalla et al., 2002, 2009a). Under high temperature conditions (above 40°C), the water temperature regulates bacterial metabolism (Farjalla et al., 2005).

In this study, we evaluated both the main and interactive effects of water temperature increases and inorganic nutrient additions on the bacterial metabolism of tropical coastal lagoons in the Brazilian Southeast. Based on previous results, we expected (i) an increase in BP and BGE after inorganic nutrient additions, (ii) a decrease in both BP and BR with higher temperatures, (iii) synergistic effects between temperature increase and inorganic nutrient additions on bacterial metabolism. We established

a full-factorial design in which water temperature and inorganic nutrients were manipulated and BP, BR, and BGE were evaluated following microcosm incubation. The increase in water temperature had a consistent effect on bacterial metabolism across all lagoons, increasing BR and decreasing both BP and BGE. The effects of nutrient additions varied among lagoons and seemed to be related to the concentration of DOC in the systems. We concluded that (i) different environmental stressors impact bacterial processes at different scales, and (ii) the temperature increase predicted for the next century would alter bacterioplankton function in these lagoons, diverting more carbon to bacterial catabolism and CO_2 production and/or less carbon to bacterial biomass and higher trophic level through the microbial loop.

Materials and Methods

Study Area

The study was conducted in the Cabiúnas, Carapebus, Comprida, Amarra-Boi, and Atoleiro shallow coastal lagoons, all located in Restinga de Jurubatiba National Park in the coastal region of the Brazilian Southeast (−22° 17′ 30″, −41° 41′ 30″). These lagoons constitute a natural gradient in DOC concentration varying from less than 12 to more than 110 mg L^{-1} (**Table 1**). More than 90% of the total DOC is composed of allochthonous humic substances mostly derived from an impermeable soil layer rich in organic matter (Farjalla et al., 2009a). Chlorophyll-a concentrations are low, as is the availability of nitrogen and phosphorus (**Table 1**). Despite being located on the coast, salinity is low as is the pH (**Table 1**), which reflects the major contribution of humic-rich freshwater to these systems. Other limnological features can be found in Caliman et al. (2010), and a detailed map of the region and the study lagoons can be found in Laque et al. (2010).

TABLE 1 | Abiotic conditions in the studied coastal lagoons during bacterial samplings in January/2009.

	pH	Salinity	DOC (mM)	DIN (μM)	DIP (μM)	Chlorophyll-a (μg L^{-1})
Cabiúnas	6.68	0.6	0.95	12.86	1.64	2.35
Carapebus	7.43	3.6	1.37	20.71	1.00	3.22
Comprida	4.07	0.1	4.18	37.86	1.54	2.16
Amarra-Boi	3.71	0.2	7.28	60.71	1.58	0.48
Atoleiro	3.43	0.2	9.18	43.57	1.85	0.92

DOC, Dissolved Organic Carbon; DIN, Dissolved Inorganic Nitrogen; DIP, Dissolved Inorganic Phosphorus. See below information about the analytical methods.
pH was obtained using a pH meter (Analion PM 608) and salinity was measured using a portable Thermosalinometer multi-functional probe (YSI-30). For DOC, DIN, and DIP analyses, water samples were filtered through 0.7-μm glass fiber filters (Φ = 25 mm, GF/F, Whatman). DOC concentration was determined using a Pt-catalyzed high-temperature combustion method with a TOC-5000 Shimadzu Carbon Analyzer. DIN concentrations were calculated as the sum of N-nitrate and N-ammonium concentrations, which were, in turn, analyzed using a Flow Injection Analysis System (FIA – Asia Ismatec; Zagatto et al., 1980) and spectrophotometry after applying blue indophenol (Koroleff, 1978), respectively. DIP concentrations were determined using the molybdenum blue reaction (Golterman et al., 1978). Chlorophyll-a concentrations were measured through ethanol extraction and spectrophotometry (Nusch and Palme, 1975).

Sampling and BP, BR, and BGE Evaluations

Water samples were collected on three alternate days from the central point of each lagoon in January/2009. Each sampling day was treated as a block in the ensuing analyses (see Statistical Analysis below). For sampling, we used 5-l polyethylene bottles previously washed with 10% HCl and rinsed with deionized water. The samples were taken to the laboratory and filtered through 0.7-μm glass fiber filters (Φ = 47 mm, Macherey-Nagel GF-3) to remove particles and larger organisms. All sampling and filtering procedures were performed in approximately 6 h. Filtered water samples were divided among 20-mL glass vials previously washed with 10% HCl and rinsed with Milli-Q water.

A full factorial design was established for each lagoon, with four levels of addition of two different nutrients (nutrient treatment) and three temperature manipulations (temperature treatment). The nutrient treatment consisted of N addition (addition of KNO_3, 50 μM final concentration), P addition (KH_2PO_4, 5 μM final concentration), N and P addition (additions of KNO_3, 50 μM final concentration, and KH_2PO_4, 5 μM final concentration) and a control with no nutrients added. Added nutrients represented an increase from ambient conditions (control treatments) of 82 to more than 300% for nitrogen and 270–500% for phosphorus. The temperature treatment included incubations at 25, 30, or 35°C in BOD incubation chambers. The average water temperature is 25°C in the studied systems (see Caliman et al., 2010 for review) but values up to 40°C were observed in sporadic drought events in summer months (Farjalla et al., 2005), which should occur more frequently according to projections for the region (Roland et al., 2012). Six replicates were performed for each treatment in each lagoon, yielding a total of 360 bacterial cultures. All incubations were performed in the dark for 48 h. Previous tests and other studies showed bacterial cultures established from water samples from these lagoons responded rapidly to nutrient additions or temperature changes. For instance, daily changes in bacterial production related to changes in the water temperature were observed in another lagoon located in the same area (Farjalla et al., 2005), while bacterial metabolism responded in 24–48 h after nutrient additions in Cabiúnas, Carapebus, and Comprida lagoons (Farjalla et al., 2002).

Bacterioplankton production was evaluated in each bacterial culture at the beginning and end of the 48-h incubation using the ^{3}H-leucine incorporation method (Kirchman et al., 1985) and trichloroacetic acid (TCA) protein extraction (Smith and Azam, 1992), as modified by Miranda et al. (2007). An intracellular isotopic dilution factor of two was used in the calculations (Simon and Azam, 1989). Incubations were performed in the dark for 45 min with 20 nM of ^{3}H-leucine (specific activity 150 Ci $mmol^{-1}$). The concentration of 20 nM of ^{3}H-leucine was previously established from saturation curves (unpublished data). We set up negative controls by adding 90 μL of TCA before starting the incubations. After the incubation, ^{3}H-leucine incorporation was stopped by the addition of 90 μL TCA. Bacterial protein was extracted by washing with 5% TCA and 80% ethanol. After protein extraction, we added a liquid scintillation cocktail [EcoLite(+)^{TM}] to each sample, and the samples were radio-assayed by liquid scintillation counting

(Beckman LS – 6500) after 2 days in the dark to reduce the chemiluminescence. Bacterial protein production was converted to BP (g C 1^{-1} h^{-1}) using a C:protein ratio of 0.86, according to Wetzel and Likens (1991). BP ratio unit was further converted to μM C h^{-1} to comparisons with other nutrient concentrations.

Bacterioplankton respiration was assessed by measuring oxygen consumption in each bacterial culture at the beginning and end of the 48-h incubation using an oxygen micro-sensor (Clark-type sensors, OX-N, Unisense) connected to a picoamperimeter (PA 2000, Unisense), following Briand et al. (2004). This approach is highly accurate, stable and has a low response time, and has been widely used to study bacterial metabolism (steering sensitivity <2%, response time <10 s; Briand et al., 2004). A respiratory quotient of 1.2 was used to convert oxygen measurements to carbon values (Berggren et al., 2012). Based on the results of BP and BR, we calculated BGE according to the formula BGE = BP/(BP+BR) (del Giorgio and Cole, 1998).

Statistical Analyses

The main and interactive effects of each factor on BP, BR, and BGE were assessed using linear models (LMs), linear mixed effect models (LMMs), or non-linear mixed effect models (NLMMs). First, to assess the global effects of treatments and their interactions we pooled together the data of all lagoons. We used LMM and NLMM with additions of N and P set as categorical explanatory factors, temperature as the continuous explanatory variable, sampling blocks nested within lagoons as the random categorical factor, and BP, BR, and BGE as the dependent variables. Because using NLMM had not improved the results (verified by using the Akaike's information criterion), we kept and showed only the LMM results. The Gaussian family (link = "identity") was used to fit the model. To meet test assumptions, BR and BGE were log-transformed. Because only the temperature treatment showed consistent effects on bacterial metabolism in this first analysis (see results below), we (i) ran LMs relating BP, BR, and BGE with the water temperature, and (ii) ran different LMMs for each lagoon to analyze the effects of N and P addition treatments and their interaction on each system separately, searching for common responses to nutrient additions among lagoons. In this case, N and P were set as categorical explanatory factors, sampling blocks nested within temperature treatments as the random categorical factor, and BP, BR, and BGE as dependent variables. Again BR and BGE data were log-transformed to meet test assumptions and the Gaussian family (link = "identity") was used to fit the model. Finally, as we observed two different groups of lagoons with similar types of bacterial responses to N and P additions (low and high DOC concentration ecosystems; low <1.5 μM C, high >4.0 μM C), we re-ran the latter analysis, pooling together the bacterial data for each group. We used contrast analysis to distinguish significant differences between the levels of N and P additions. Tests were performed using the "nlme" (Pinheiro et al., 2014) and "multcomp" (Hothorn et al., 2008) libraries in the R statistical software (R Development Core Team, 2014), and an α criterion level of 0.05 was used.

Results

Bacterioplankton respiration varied from 0.01 to 0.97 μM C h^{-1}, BP varied from less than 0.01–0.25 μM C h^{-1}, and BGE varied from values as low as 0.01 to values as high as 0.93 (Supplementary Table S1). The overall means were 0.36 μM C h^{-1} for BR, 0.10 μM C h^{-1} for BP and 0.15 for BGE. In general, Cabiúnas and Carapebus lagoons showed higher BP rates (means = 0.16 and 0.17 μM C h^{-1}, respectively) than Comprida, Amarra-Boi, and Atoleiro lagoons (means = 0.08, 0.07, and 0.03 μM C h^{-1}, respectively). The Carapebus lagoon showed higher BR rates (mean = 0.45 μM C h^{-1}) than the others (means = 0.37, 0.34, 0.27, and 0.30 μM C h^{-1} for Cabiúnas, Comprida, Amarra-Boi, and Atoleiro, respectively). BGE was highest in Cabiúnas lagoon (mean = 0.38), followed by Carapebus lagoon (mean = 0.29), Amarra-Boi and Comprida lagoons (means = 0.24 and 0.21, respectively), and Atoleiro lagoon (mean = 0.09; Supplementary Table S1).

In the first analysis, we pooled the data from all lagoons together to observe general patterns of response to nutrient additions and increase of water temperature. Water temperature alone had the greatest effect on bacterial metabolism (**Table 2**). BR increased with increasing temperatures while BP slightly decreased with increasing temperatures. Consequently BGE decreased with increasing temperatures mainly related to the highest BR in the highest incubation temperatures. Neither nutrient additions (N or P) nor their interaction (N and P) had consistent general effects on bacterial metabolism when data from all lagoons were pooled together (i.e., there was no general pattern of nutrient limitation among lagoons, **Table 2**). The only significant interaction between water temperature and nutrient addition was observed for bacterial respiration, in which N additions interacted with the increasing water temperatures, resulting in higher BR rates. Based on these results, we established simple LMs relating bacterial metabolism to water temperature for these lagoons (**Figure 1**). An increase of about 1°C in water temperature in the lagoons lead to an increase in BR rates of approximately 12.8 nM C h^{-1}, a slight decrease in BP rates of approximately 0.9 nM C h^{-1}, and a decrease in BGE of approximately 0.0116.

We next analyzed the data for each lagoon separately, searching for common responses to nutrient additions among lagoons. Lagoons with the lowest DOC concentrations (Cabiúnas and Carapebus) showed the strongest responses to nutrient additions: BP increased in response to N, P and the interaction between N and P, BR increased in response to N and the interaction between N and P additions, and BGE was negatively affected – mainly by the interaction between N and P additions (**Figure 2**). On the other hand, lagoons with the highest DOC concentrations (Comprida, Amarra-Boi, and Atoleiro) showed almost no significant relationship with nutrient additions: N and P additions slightly stimulated BR in Atoleiro bacterial cultures, the interaction between N and P had a strong positive effect on BR in Comprida bacterial cultures, and neither nutrient addition nor their interaction affected BP or BGE in any of these lagoons (**Figure 2**). We then pooled together

TABLE 2 | Results of linear mixed model (LMM) analysis for bacterial respiration, bacterial production and bacterial growth efficiency (BGE).

	Chi square value	*p* value
Bacterial respiration		
Intercept	0.38	0.706
N addition	1.52	0.128
P addition	0.21	0.832
NP additions	0.47	0.636
Temperature (+)	**2.76**	**0.006**
N addition:Temperature (+)	**2.12**	**0.034**
P addition:Temperature	0.51	0.604
NP additions:Temperature	0.17	0.864
Bacterial production		
Intercept	**3.58**	**<0.001**
N addition	1.13	0.258
P addition	0.13	0.895
NP additions	0.67	0.505
Temperature (–)	**2.11**	**0.035**
N addition:Temperature	1.95	0.053
P addition:Temperature	0.52	0.601
NP additions:Temperature	1.14	0.256
Bacterial growth efficiency		
Intercept	6.48	**<0.001**
N addition	0.19	0.852
P addition	0.26	0.797
NP additions	0.01	0.992
Temperature (–)	**4.23**	**<0.001**
N addition:Temperature	0.00	0.997
P addition:Temperature	0.45	0.651
NP additions:Temperature	0.62	0.537

We calculated the main and interactive effects of manipulated factors (nutrient additions and temperature) on bacterial parameters for the complete dataset (all lagoons are pooled together). The block and lagoon identity were inserted as a random factors in the models. Negative or positive signals indicate a negative or positive significant effect of the variable (or interaction) on the bacterial process. Significant effects are in bold.

the datasets for different lagoons in relation to DOC concentrations and observed that: (1) In the lagoons with the lowest DOC concentrations, BR was enhanced by the N addition and the interaction between N and P, BP was enhanced by N or P alone, and BGE decreased in response to the interaction between N and P additions, and (2) In the lagoons with the highest DOC concentrations, BR was enhanced by the N addition (**Table 3**).

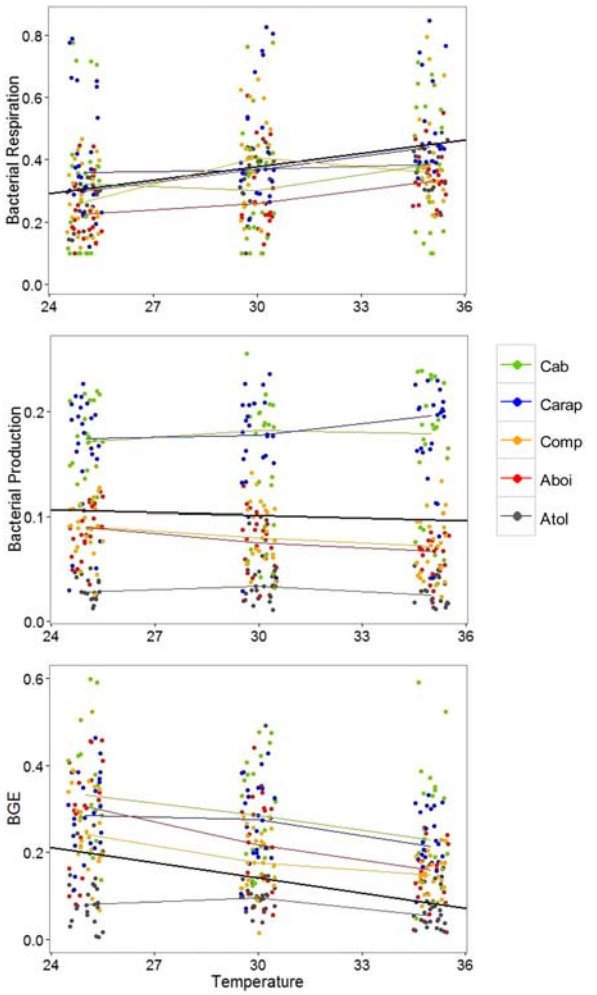

FIGURE 1 | Changes in bacterial respiration, bacterial production, and bacterial growth efficiency (BGE) in relation to the increase in water temperature in five humic tropical lagoons (Cabiunas – Cab, Carapebus – Carap, Comprida – Comp, Amarra-Boi – Aboi, and Atoleiro – Atol). For each temperature, all bacterial data related to different nutrient additions were pooled together because temperature effects were consistent for all lagoons and independent of nutrient additions (see **Table 2** for the analysis results). Colored thin lines link the medians of bacterial data for each lagoon at each incubation temperature. The black central line represents the best fitting line that predicts bacterial respiration (BR), production (BP), or growth efficiency (BGE) in response to water temperature. Intercepts and slopes of best fitting lines for BR, BP, or BGE are: −0.055 and 0.0144 (BR), 0.128, and −0.0009 (BP), and 0.488, slope = −0.0116 (BGE).

Discussion

The main goal of this study was to evaluate how bacterioplankton metabolism and its relative importance as a carbon link or sink is singly and/or synergistically influenced by the environmental changes predicted for tropical lagoons of the Brazilian Southeast. We verified that anabolic and catabolic bacterial processes have different trends in response to temperature and nutrient manipulation, and that the strength and generality of the response varies according to the process evaluated and the factor manipulated. For instance, the increase in water temperature increased BR, decreased BP and, consequently, decreased BGE. Furthermore, the addition of nutrients did not yield a consistent change in bacterial metabolism among all lagoons, but its impact was stronger for lagoons with lower DOC concentrations. Overall, we observed few interactions between water temperature and nutrient concentration, indicating these factors almost always impacted bacterial processes independently.

An increase in the average global temperature is a major environmental change expected for this century. The Brazilian

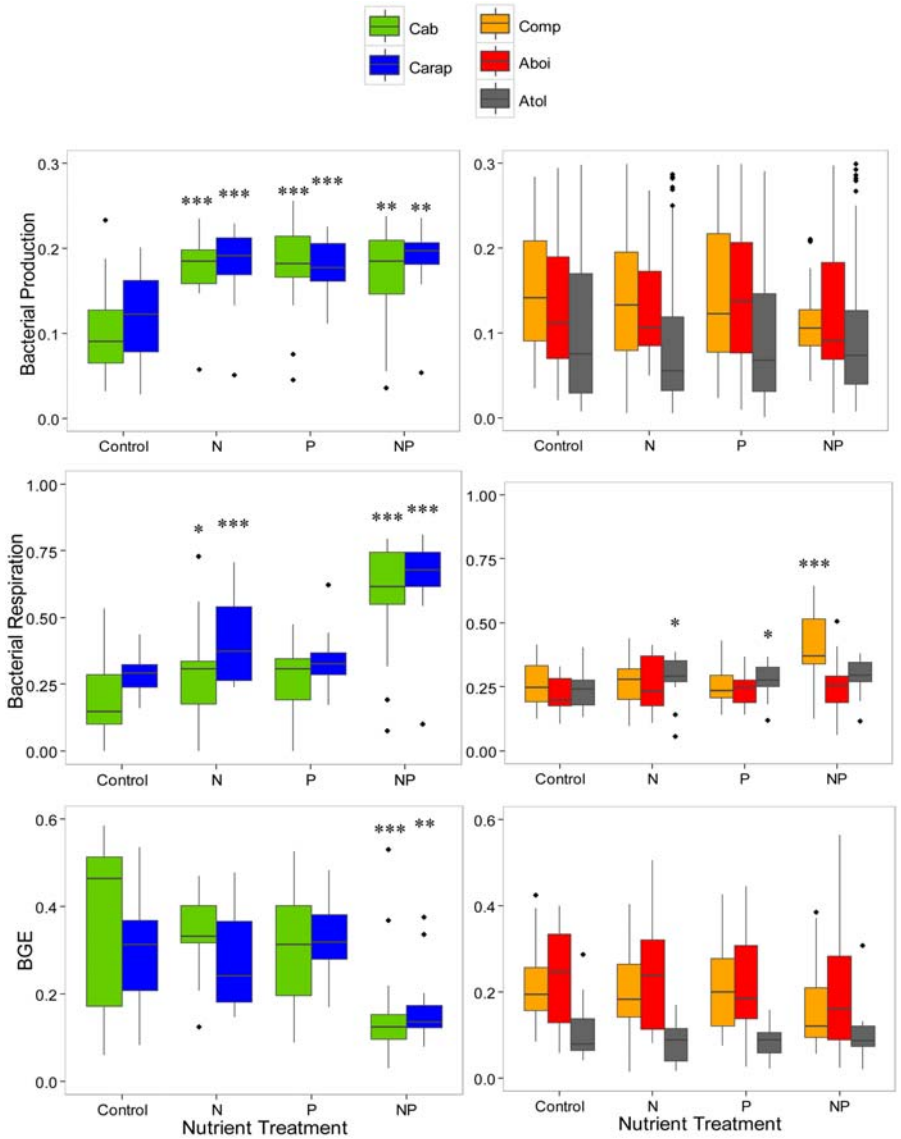

FIGURE 2 | Bacterial respiration (upper), bacterial production (middle), and BGE, lower) in relation to different nutrient additions in five humic tropical lagoons. Lagoons were grouped in different panels based on the bacterial responses to nutrient additions. Panels on the left show data from the lagoons with lower carbon concentrations (Cabiunas – Cab, Carapebus – Carap) while panels on the right show data from the lagoons with higher carbon concentrations (Comprida, Comp, Amarra-Boi – Aboi, Atoleiro – Atol). Control – no nutrient addtion, N – nitrogen addition, P – phosphorus addition, and NP – nitrogen and phosphorus additions. Asterisks indicates a significant difference in response of bacterial metabolism to nutrient additions compared with the control for each lagoon (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). Note that no comparison between lagoons is showed here.

Southeast is expected an increase of temperature of 0.5–1.0°C through 2040 and of 2–3°C between 2041 and 2070 (PBMC, 2013). In temperate systems, increases in temperature are associated with an overall increase in bacterial metabolism with a positive effect on BGE (Hall et al., 2008). This effect may be direct, via increased cellular activity, or indirect, via input of nutrients from the melting of drainage basins in aquatic systems. On the other hand, temperature is consistently higher in tropical systems where tropical organisms are living close to optimum thermal conditions (Deutsch et al., 2008). Even a small increase in water temperature in tropical ecosystems is expected to drive organisms beyond optimal growing conditions (Deutsch et al., 2008), increasing energetic requirements for cell maintenance as a consequence of changes in the membrane fluidity or in the functioning of the cellular enzymatic machinery (Nedwell, 1999; Pomeroy and Wiebe, 2001). The temperature manipulations in this experiment are in the upper temperature limit found in inland waters, and are inserted in the regular temperature range found in these lagoons (Caliman et al., 2010). Therefore, the observed increase in BR and decrease in BGE related to the increase in water temperature in this experiment were expected based on known changes in bacterial

TABLE 3 | Results of LMM analysis for bacterial respiration, bacterial production and BGE for two partial datasets pooled in relation to dissolved organic carbon (DOC) concentration in the lagoons.

	Low DOC lagoons	High DOC lagoons
Bacterial respiration		
Intercept	4.817***	8.081***
N addition	3.400**	2.262*
P addition	1.731	1.228
NP interaction	4.990***	0.971
Bacterial production		
Intercept	13.895***	12.528***
N addition	6.269***	1.157
P addition	5.989***	0.241
NP interaction	−0.238	0.279
Bacterial growth efficiency		
Intercept	9.437***	6.207***
N addition	1.307	0.272
P addition	1.174	−0.764
NP interaction	−4.503***	−0.434

*Cabiúnas and Carapebus lagoons were categorized as low DOC concentration lagoons, while Comprida, Amarra-Boi, and Atoleiro were categorized as high DOC concentration lagoons (see **Table 1** for DOC concentrations). We calculated the main and interactive effects of nutrient additions on bacterial parameters. Incubation temperature and lagoon identity were inserted as a random factors in the models. Negative or positive signals indicate a negative or positive significant effect of the variable (or interaction) on the bacterial process. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.*

physiology at high temperatures and based on the temperature range of our manipulations. In fact, higher BR rates and lower rates of carbon incorporation into bacterial biomass associated with higher temperatures were found in a survey of bacterial metabolism in tropical aquatic ecosystems (Amado et al., 2013).

As water temperature affected bacterial metabolism in all lagoons in a similar way, we established negative linear relationships between water temperature, BP and BGE, as well as a positive linear relationship between water temperature and BR. On one hand, the predictive slight negative effect of higher water temperatures on BP indicates that less carbon will be assimilated into bacterial biomass under future climate scenarios. Consequently, the microbial loop is expected to be slightly less influential as an alternative energy source for higher trophic levels in tropical aquatic ecosystems. On the other hand, the positive effect of higher temperatures on BR indicates a positive feedback between a climate change outcome (increase in average temperature) and a climate change driver (release of greenhouse gasses from natural systems). The positive and significant relationship between increasing temperature and BR has been observed previously in temperate regions, albeit, the magnitude of this effect on bacterial metabolism is relatively low compared to tropical aquatic ecosystems (Marotta et al., 2014). This marked effect of temperature in tropical inland waters is strongest in the 35°C treatment, where BR increased by 2.3–10.5-fold compared with BR in temperate aquatic ecosystems (Berggren et al., 2010; Kritzberg et al., 2010). Therefore, increases in water temperature may be more important for driving bacterial metabolism in tropical rather than temperate aquatic ecosystems, but this needs further investigation.

Bacterioplankton production is usually limited by the low P concentration in the water column of inland aquatic ecosystems (Granéli et al., 2004; Smith and Prairie, 2004; Bertoni et al., 2008), including the Carapebus and Comprida lagoons (Farjalla et al., 2002). Conversely, the nitrogen limitation of BP is far less common in inland aquatic ecosystems, yet has been observed in some Amazonian ecosystems (Rai and Hill, 1984), in the water accumulated in tank-bromeliads (Haubrich et al., 2009) and in the Cabiúnas lagoon (Farjalla et al., 2002), which is also studied here. Therefore, the observed lack of a clear change in BP in response to the addition of a specific nutrient may be associated with different limiting nutrients in different lakes. This explanation is partially corroborated by the results obtained after the dataset was divided based on DOC concentration in the lagoons: N or P stimulated BP in the lagoons with lowest DOC concentration while there was no clear pattern of change in the lagoons with highest DOC concentration. An alternative hypothesis is based on the co-limitation of BP by N and P in a given system, which may have occurred at Cabiúnas and Carapebus lagoons. However, this hypothesis is apparently contrary to the well-established Law of the Minimum (Liebig, 1843), which was first established to describe the patterns of growth limitation in individual plants but not natural communities. In natural communities, each individual or species can specifically respond to the availability of different nutrients, leading to a possible co-limitation by different nutrients of the entire community (for a review, see Harpole et al., 2011). In contrast to BP, BR was strongly influenced by N or NP additions in our study, which is an unexpected result because inorganic nutrients are more important to BP than to BR (e.g., Smith and Prairie, 2004). The nitrate added in the N and NP treatments could be anaerobically respired by denitrifying bacteria, though this scenario is unlikely because the microcosms remained under aerobic conditions throughout the experiment (data not shown) and this process would not be detected by our BR method.

The DOC concentration in the lagoons apparently influences the outcome of nutrient additions on bacterial metabolism. Ecosystems with high carbon concentrations usually show a large imbalance between nutrient availability in the system and nutrient demand by bacteria (Hessen, 1992; Jansson et al., 2006). Despite slightly higher nutrient concentration in the DOC-richest lagoons than in the DOC-poorest lagoons (**Table 1**), all lagoons showed relative greater carbon than nitrogen and phosphorus concentration (greater C:N and C:P ratios) in relation to bacterial demands (for bacterial elemental composition see Fagerbakke et al., 1996). Those ecosystems are usually characterized by strong nutrient limitation, low BP rates and low carbon conversion into CO_2 by respiration into bacterial cells (Farjalla et al., 2009a). In this experiment, lagoons with lower carbon concentrations had stronger responses to nutrient addition than lagoons with higher carbon concentrations, which was an unexpected result because of the largest imbalance between nutrient concentration and bacterial demands found in DOC-richest lagoons. We suggest

that the quality of DOC bulk for bacterial growth is inversely related to DOC concentration in these lagoons, and nutrient additions have a restricted influence in low quality DOC bulk ecosystems (Farjalla et al., 2002). Besides receiving highly humic DOC from the drainage basin, Cabiúnas and Carapebus lagoons contain extensive macrophyte stands, dominated by *Typha domingensis* and *Elecocharis interstincta*. These plants contribute high quality DOC to the water column by exuding excess organic carbon produced by photosynthesis or by leaching during the early stages of decomposition (Stepanauskas et al., 2000; Farjalla et al., 2009b). Periphytic primary production is severely limited by light availability in these coastal lagoons (Sanches et al., 2011) and light penetrates further down the water column of Cabiunas and Carapebus lagoons than in the highly humic lagoons. On the other hand, macrophyte stands are restricted or absent and autochthonous primary production is very low in Comprida, Amarra-Boi, and Atoleiro lagoons. The input of DOC is exclusively external via the drainage basin, and its composition is almost exclusively of highly refractory humic compounds. Therefore, the increase in bacterial metabolism after nutrient addition in the lowest DOC concentration lagoons was most likely related to the ease of consumption and assimilation of more labile autochthonous DOC compounds, while in the highest DOC concentration lagoons the refractory characteristics of the DOC bulk restricted the responses of bacterial metabolism after nutrient additions, at least over the duration of the experiment (48 h).

Some peculiarities of the experimental design may have influenced our results. For example, some delayed physiological and re-growth responses of bacterial communities related to the treatments could have occurred and been observed under longer incubation periods than those used in our experiment. Thus, our results represent only a snapshot of rapid responses of bacterial metabolism to changes in environmental conditions. Follow-up studies focused on longer incubation periods should be performed to determine whether these responses would be maintained for long time periods. Changes in the bacterial community composition during the experiment related to the different treatments may have occurred; however, as such changes were previously observed in longer experiments, we did not expect any significant effect here. The spatiotemporal representation of the study may have been compromised, as we sampled only once from each lagoon. Nonetheless, we have observed in previous studies that the greatest changes in bacterial processes occur between lagoons, not within the same lagoon over time or between sampling stations in the same lagoon (MacCord et al., 2013). The use of artificial microcosms was necessary for a thorough evaluation without the confounding effects of varying the concentrations of nutrients and water temperature on bacterial metabolism; regardless, extrapolation from studies in artificial microcosms to natural systems should be performed with caution. It is noteworthy that the nutrient concentrations used in this study were the same as those observed in the actual lagoons or in similar systems with domestic sewage input (Caliman et al., 2010). Finally, although an increase of 10°C is not predicted in any future climate scenario, this change in water temperature is usually found throughout a single day in summer months in shallow lagoons (Farjalla et al., 2005). The impact of these episodic events on long term microbial metabolism and carbon cycling in these ecosystems remain to be evaluated.

We conclude that increase in water temperature impacted microbial metabolism in the tropical coastal lagoons by increasing BR, slightly decreasing BP, and decreasing BGE. Nutrient inputs also changed the bacterial metabolism, but the magnitude of alterations varied among lagoons and seemed to be related to the availability and quality of carbon substrates in each system. Overall, both nutrient additions and increases in water temperature decreased BGE. This result points to important changes in the future carbon cycle of aquatic ecosystems related to the role of bacterioplankton. In the future, it is expected that the predicted environmental changes will favor processes related to bacterial catabolism, whereby relatively more carbon is converted into CO_2 than into bacterial biomass available to higher trophic levels through the microbial loop.

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Supplementary Material

The Supplementary Material for this article can be found online at: http://www.frontiersin.org/journal/10.3389/fmicb.2015.00310/abstract

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

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Natural and human-induced controls on carbon dioxide (CO_2) in tropical waters may be very dynamic (over time and among or within ecosystems) considering the potential role of warmer temperatures intensifying metabolic responses and playing a direct role on the balance between photosynthesis and respiration. The high magnitude of biological processes at low latitudes following eutrophication by nitrogen (N) and phosphorus (P) inputs into coastal lagoons waters may be a relevant component of the carbon cycle, showing controls on partial pressure of CO_2 (pCO_2) that are still poorly understood. Here we assessed the strength of N control on pCO_2 in P-enriched humic and clear coastal lagoons waters, using four experimental treatments in microcosms: control (no additional nutrients) and three levels of N additions coupled to P enrichments. In humic coastal lagoons waters, a persistent CO_2 supersaturation was reported in controls and all nutrient-enriched treatments, ranging from 24- to 4-fold the atmospheric equilibrium value. However, both humic and clear coastal lagoons waters only showed significant decreases in pCO_2 in relation to the controlled microcosms in the two treatments with higher N addition levels. Additionally, clear coastal lagoons water microcosms showed a shift from CO_2 sources to CO_2 sinks, in relation to the atmosphere. Only in the two more N-enriched treatments did pCO_2 substantially decrease, from 650 μatm in controls and less N-enriched treatments to 10 μatm in more N-enriched microcosms. Humic substrates and N inputs can modulate pCO_2 even in P-enriched coastal lagoons waters, thereby being important drivers on CO_2 outgassing from inland waters.

Keywords: eutrophication, pCO_2, nitrogen, humic coastal lagoons, clear water coastal lagoons

INTRODUCTION

Carbon dioxide (CO_2) is one of most important greenhouse gas in terms of global warming (IPCC, 2007; Royer et al., 2007; Solomon et al., 2010). The terrestrial biomass represents a relevant global stock of carbon (C), which is removed from the atmosphere by primary production (Gough et al., 2008). However, a significant part of this terrestrial organic matter leaches into aquatic ecosystems, where it may be buried in bottom sediments (Downing et al., 2008) or remineralized to CO_2 by aquatic biological decomposition (Aufdenkampe et al., 2011). In the watershed, most natural inland waters are relatively small, but their wide geographic distribution, high abundance, and common location at low altitudes make them a typical fate for the water inflow from broad areas, playing a crucial role on the global C cycle (Cole et al., 2007).

Coastal lagoons are ecosystems often altered by the human land use (Marotta et al., 2010b), which show intense C fluxes (Duarte et al., 2008; Marotta et al., 2010b) The terrestrial inputs from leaching and groundwaters enhance CO_2 in lakes by the contribution of inorganic C (Raymond et al., 1997; Marotta et al., 2010b), or organic substrates supporting the aquatic respiration (del Giorgio et al., 1997; Jonsson et al., 2003). Photosynthesis and respiration are the major metabolic pathways determining whether what level organic matter is produced and destroyed (Cole et al., 2000). Indeed, high terrestrial organic inputs may explain the positive general trend reported between dissolved organic carbon (DOC) and the partial pressure of CO_2 (pCO_2) in lake waters (Jonsson et al., 2003). Several studies have showed positive relationships in DOC and pCO_2 in lakes in high latitudes and even globally (Sobek et al., 2005) supporting the idea that lakes are an important source of CO_2 globally (Cole et al., 1994, 2007; Duarte and Prairie, 2005; Tranvik et al., 2009). Mean areal rates of CO_2 evasion from lakes are higher at low latitudes, probably by the potential positive effect of warmer conditions on the organic decomposition (Marotta et al., 2009; Kosten et al., 2010). In this way, the degradation of organic matter to CO_2 by bacteria shows important fluxes in the carbon cycling in natural aquatic ecosystems (Azam, 1998).

Additionally, the expansion of the human activities has intensified substantially the nitrogen (N) and phosphorus (P) input into ecosystems, often resulting in the eutrophication of natural waters (Vitousek and Mooney, 1997). These nutrients regulate aquatic primary production and respiration (Cole et al., 2000; Biddanda et al., 2001). Highly productive waters due to external inputs of inorganic nutrients tend to be net autotrophic, acting as a net sink for CO_2 (Duarte and Agusti, 1998), while, those waters are

highly enriched in organic substrates may show persistent CO_2 supersaturation (Carpenter et al., 2001; Marotta et al., 2012).

Despite consistent evidences supporting the role of the limitation by either P (Schindler et al., 2008) or N (Camacho et al., 2003), N and P co-limitation may be also crucial on the biological metabolism in natural waters (Conley et al., 2009; Paerl, 2009). The biological N fixation can contribute to reduce the role of N inputs to stimulate biological activity in P-enriched waters, although more evidences is still needed for a better understanding on N limitation in coastal lagoons waters, where P is commonly enriched by domestic discharges.

In this study, we assessed the short-term effect of N additions on pCO_2 in P-enriched humic and clear coastal lagoons waters. We tested the hypotheses that lake pCO_2 is controlled by N availability in P-enriched waters.

MATERIALS AND METHODS

STUDY AREA

The experiment was conducted using surface waters from two tropical coastal lagoons situated at the same conservation area (Restinga de Jurubatiba National Park) in the north of Rio de Janeiro State (Brazil). Both coastal lagoons are elongated, with their main axis perpendicular to the shoreline (maximum depth <4.5 m; area <6.5 km^2), oligotrophic (nutrients and chlorophyll *a* in the **Table 1**) and relatively close to each other (distant 6.8 km). The mean daily temperature in this area ranges from 20.7°C in July to 26.2°C in February. Despite high inter-annual variability, the minimum and maximum monthly rainfall are typically observed in August (38 mm) and December (182 mm; INMET, 1992). The tropical climate reflects in warm coastal lagoons waters (>20°C).

Carapebus coastal lagoon (22°13′21.29″S and 41°36′53.22″W) has clear waters, while Comprida coastal lagoon (22°16′44.55″S and 41°39′24.76″W) has highly humic and dark waters. The dark color and high Color:DOC ratio in coastal lagoons waters of this region commonly reflects a higher contribution of terrestrial organic compounds from Restinga vegetation (Marotta et al., 2010a).

EXPERIMENTAL DESIGN

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

The experiment was carried out over 15 days in highly P-enriched treatments in which different amounts of N were added, and the control (i.e., no N addition) per coastal lagoon. Three replicates were used in each experimental treatment and the control totalizing 24 microcosms. 1.4 μM of P as KH_2PO_4 and K_2HPO_4 (1:1 mass ratio to attenuate changes in pH) and 2.8, 28, and 120 μM of N as KNO_3 were daily added to +N+P, ++N+P and +++N+P treatments, respectively. Nutrients were carefully added during the morning. Total additions were 20 μM P and 40, 400, and 1600 μM N in +N+P, ++N+P and +++N+P treatments, respectively, during the experiment. These concentrations and the corresponding N:P ratio were chosen to simulate the nutrient levels typically observed in urban coastal lagoons at the same region outside the Restinga de Jurubatiba National Park. The control microcosms showed only the low nutrient levels observed in both environments (0.4 and 0.9 μM P and 58.1 and 45.2 μM N, respectively in Carapebus and Comprida lagoons). All measurements were performed by the end of the experimental time (day 15).

ANALYTICAL METHODS

pH was measured with a precision of 0.01 pH units using a Analion PM 608 pH meter and the total alkalinity following the Gran's titration (APHA, 1992). Temperature and salinity were measured with a calibrated Thermosalinometer YSI-30. CO_2 concentrations in waters were determined using the pH-alkalinity method (Mackereth et al., 1978) with appropriate corrections for temperature, altitude, and ionic strength as Cole et al. (1994). pCO_2 was calculated from Henry's law with appropriate corrections for temperature and salinity (Cole and Caraco, 1998) as in Marotta et al. (2010a).

Water samples for total P and N analyses were previously frozen. Total P concentrations were measured by the molybdenum blue method with pre-digestion and total N concentrations by the sum of Kjeldahl N and NOx forms (APHA, 1992). Chlorophyll *a* concentrations (a proxy for phytoplankton biomass) in water samples filtered through Whatman GF/F filters (0.7 μm pore size) were extracted with ethanol in the dark for 24 h before fluorimetric determination, using an excitation wavelength of 433 nm and

Table 1 | Nutrients, chlorophyll *a*, color, DOC, Color:DOC ratio, salinity (PSU – practical salinity unity), and pH in surface waters of Carapebus and Comprida coastal lagoons used in experimental microcosms. Values are means and units of each variable are described below.

Lagoon	Total N ($\mu mol\ l^{-1}$)	Total P ($\mu mol\ l^{-1}$)	Chlorophyll *a* ($\mu g\ l^{-1}$)	Color (430 nm)	DOC ($mg\ l^{-1}$)	Color:DOC ratio (abs at 430 nm:$mg\ l^{-1}$)	Salinity (PSU)	pH
Carapebus	45.3	1.0	13.8	0.014	9.84	0.0014	5.1	7.84
Comprida	58.1	0.4	2.5	0.102	17.43	0.0058	0.1	5.66

an emission wavelength of 673 nm (Varian Eclipse). Total suspended solids (TSS) were analyzed by the difference of weight before and after filtering and drying GF/F filters. Water samples filtered in these Whatman GF/F filters were also analyzed for color at 430 nm (Strome and Miller, 1978) using a Beckman DU 80 spectrophotometer (Fullerton, CA, USA) in a 1-cm quartz cuvette, and acidified to pH < 2.0 to determine DOC by the high-temperature catalytic oxidation method using a TOC-5000 Shimadzu Analyzer. The bacterial production was estimated from the rate of incorporation of ^{3}H-leucine (Smith and Azam, 1992), assuming a ^{3}H-leucine dilution factor of 2 and a carbon:protein ratio of 0.86 (Wetzel and Likens, 1991). A volume of 1.3 ml of water from the microcosms and placed in an eppendorf (1.5 ml). In all tubes, rejoinders were added 20 µl of 3H-leucine (5× diluted standard solution, 159 mCi mol^{-1}, Amersham), reaching a final concentration of 20 nM and incubated for 45 min in the dark. After the incubation period, were added in rejoinders, 90 µl of 100% trichloroacetic acid (TCA) stopping and starting the reaction extraction. Each tube was washed sequentially with 5% TCA and 80% ethanol and 500 µl of scintillation cocktail (Aquasol and Dupont) was added to each tube and the radioactivity measured in a liquid scintillator. Bacterial production was calculated by assuming a dilution factor of intracellular leucine equal to 2, and a protein rate of carbon equal to 0.86 (Wetzel and Likens, 1991).

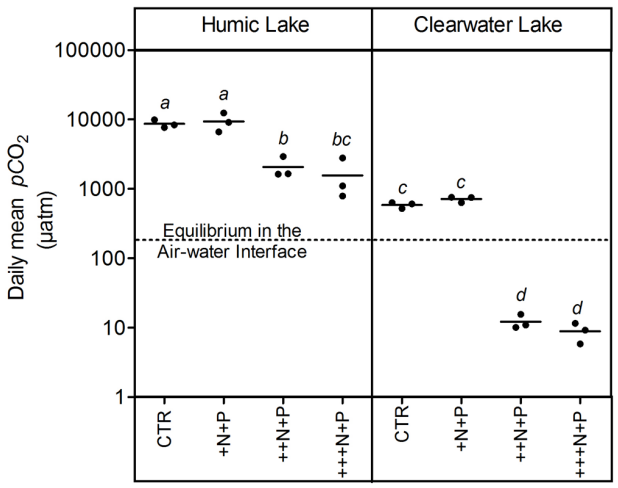

FIGURE 1 | Daily mean pCO_2 after different N additions for humic and clear waters, respectively from Lake Comprida and Lake Carapebus at the last day of the experiment. Each solid circle indicates one microcosm and the horizontal line the average. The four treatments are control (no additional nutrients) and three N levels (+N, ++N, and +++N, respectively 40, 400, and 1600 µM N – KNO_3) +P addition (+P 20 µM P – KH_2PO_4 and K_2HPO_4). No significant differences among treatments and lake waters were represented by equal lower case letters (Tukey–Kramer, $p > 0.05$). The dashed line represents the pCO_2 value at equilibrium with the overlying atmosphere (380 µatm). Note that values are in log scale.

STATISTICAL ANALYSIS

The data were log-transformed (except pH) to meet the assumptions of parametric tests, including significant Gaussian distribution (Kolmogorov–Smirnov, $p > 0.05$) and homogeneity of variances (Bartlett, $p > 0.05$). Hence, differences among experimental treatments and the control were tested with one-way ANOVA (significance $p < 0.05$) followed by the Tukey–Kramer *post hoc* test (significance $p < 0.05$). All statistics were performed using GraphPad Prism 5.01 for Windows.

RESULTS

Humic water microcosms from Comprida coastal lagoon showed average pCO_2 values 10-fold higher than clear waters from Carapebus coastal lagoon in the controls and treatments +N+P, ++N+P, +++N+P (Tukey–Kramer, $p < 0.05$; **Figure 1**). A comparison between control and the less N-enriched treatment (+N+P) showed no significant difference in pCO_2 among them, both in clear and humic waters (one-way ANOVA, $p > 0.05$; **Figure 1**). In contrast, these pCO_2 values in control and +N+P treatments were significantly higher (Tukey–Kramer, $p < 0.05$; **Figure 1**) than those respective humic or clear water with higher N-additions (++N+P and +++N+P), which were also not significantly different between them (one-way ANOVA, $p > 0.05$; **Figure 1**). CO_2 supersaturation was persistent in all humic treatments but not in clear water microcosms. The clear water microcosms presented a shift from being a source of CO_2 in the controls and +N+P treatment to becoming a sink in ++N+P and +++N+P treatments in relation to the atmosphere (**Figure 1**).

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

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

DISCUSSION

Overall, the humic waters from Comprida coastal lagoon showed a persistent CO_2 supersaturation reaching higher pCO_2 values than the controls or respective treatments with clear waters from Carapebus coastal lagoon. The humic nature of waters in Comprida coastal lagoon reflects the terrestrial DOC supply to heterotrophic bacteria in these ecosystems (Farjalla et al., 2009). Allochthonous organic resources contribute to high respiration rates and subsequently pCO_2 within most lake waters (Duarte and Prairie, 2005; Cole et al., 2007). These results support the conclusion that, in that humic coastal lagoons waters have higher pCO_2 values than the clear coastal lagoons, probably due to the more intense respiration of organic substrates (Marotta et al., 2010a).

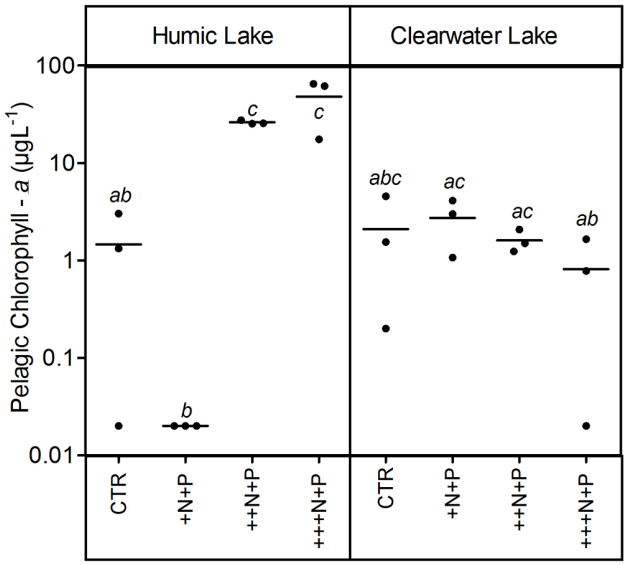

FIGURE 2 | Pelagic chlorophyll *a* after different N addition for humic and clear waters. Note that values are in log scale. Legend as described in **Figure 1**.

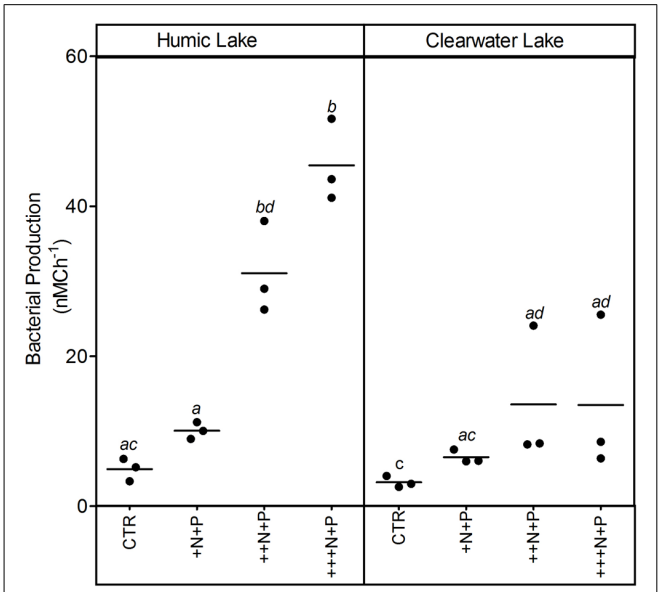

FIGURE 4 | Bacterial production after different N addition for humic and clear waters. Legend as described in **Figure 1**.

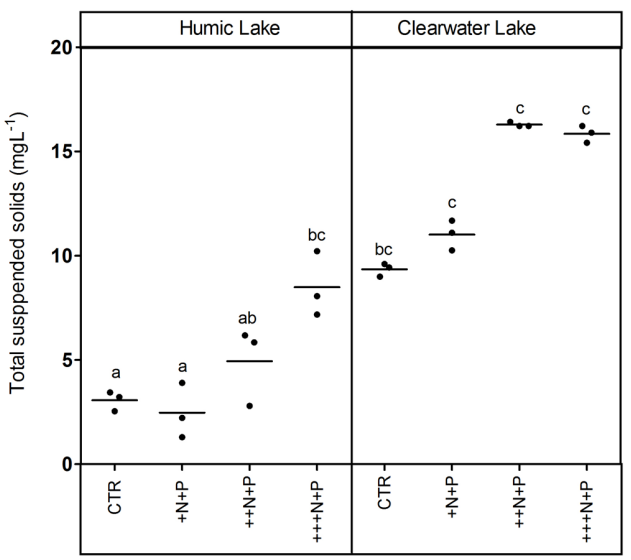

FIGURE 3 | Total suspended solids (TSS) after different N addition for humic and clear waters. Legend as described in **Figure 1**.

Furthermore, P-enriched microcosms with higher N additions showed higher bacterial production rates and algal biomass (pelagic or periphytic chlorophyll *a*), suggesting that the N supply might limit the heterotrophic and autotrophic metabolic activity in P-enriched tropical coastal lagoon. Despite N_2 fixation may be sufficient to allow biomass to continue to be produced even with extreme reductions in N inputs into lakes (Schindler et al., 2008; Smith and Schindler, 2009), our experimental evidences confirm that N might be a relevant control on eutrophication in coastal waters as previously pointed out (Conley et al., 2009; Paerl, 2009).

The CO_2 balance was determined by higher N inputs, as higher N treatments showed strong net decreases in pCO_2, supporting the potential role of aquatic primary producers on CO_2 uptake (Carignan et al., 2000). Both heterotrophs and autotrophs are stimulated by the nutrient additions (Biddanda et al., 2001), although the net autotrophy may be favored in the balance, a general trend often reported for natural waters (Duarte and Agusti, 1998). Our results contrasted with the persistence of CO_2 supersaturation in highly organic-enriched waters from whole-lake (Cole et al., 2000) or mesocosm studies (Marotta et al., 2012) also assessing the effects of experimental nutrient additions. One plausible explanation for this discrepancy would be the absence of the bottom sediment as an additional source of organic substrates to CO_2 production within the microcosms.

Increases in the phytoplankton biomass (pelagic chlorophyll *a*) contributed to net CO_2 decreases in highly N- and P-enriched microcosms with humic waters of the Comprida coastal lagoon, but not in those with clear waters of the Carapebus coastal lagoon, where no significant differences in pelagic chlorophyll *a* were reported among all experimental treatments or controls. Indeed, the CO_2 decrease observed in more N- and P-enriched clear water microcosms was mainly related to the presence of periphyton biomass on the walls, which was absent in the humic water microcosms likely due to light attenuation to primary production in their dark waters (Thomaz et al., 2001). In humic waters, TSS increase might be related to the phytoplankton growth, as the pCO_2 decreased without any periphyton growth on the microcosm walls. On the other hand, higher concentrations of non-algal solids in suspension (TSS not related to changes in chlorophyll *a* or any external particulate input) are a proxy for large-bodied zooplankton, which can be strongly stimulated under eutrophic conditions by the availability of algae (Cole et al., 2000). Despite the source of

experimental bias related to any extrapolation from the periphyton response on the microcosm walls to whole ecosystem scale, our results support a potential relevance of N control under P-enriched conditions on algae community. The strength of this zooplankton control on phytoplankton, but not on periphyton biomass in highly nutrient-enriched lake waters was previously reported using experimental mesocosms in another lake at the same studied region as in this work (Guariento et al., 2011). Thus, the absence of common grazers on zooplankton in tropical coastal lagoons, i.e., snails and fishes (Guariento et al., 2010), might have contributed to the increase of the periphyton biomass in clear water nutrient-enriched microcosms.

In conclusion, our hypothesis was confirmed as N is an important driver on pCO_2 in P-enriched coastal lagoons waters. Higher experimental N enrichments promoted a significant pCO_2 decrease in both humic and clear coastal lagoons waters. The N inputs even under P-enriched conditions might lead to intense net decreases in CO_2 in coastal lagoons waters. Both inorganic N and organic substrates inputs modulate the CO_2 balance in freshwater and brackish coastal lagoons.

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High Primary Production Contrasts with Intense Carbon Emission in a Eutrophic Tropical Reservoir

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Recent studies from temperate lakes indicate that eutrophic systems tend to emit less carbon dioxide (CO_2) and bury more organic carbon (OC) than oligotrophic ones, rendering them CO_2 sinks in some cases. However, the scarcity of data from tropical systems is critical for a complete understanding of the interplay between eutrophication and aquatic carbon (C) fluxes in warm waters. We test the hypothesis that a warm eutrophic system is a source of both CO_2 and CH_4 to the atmosphere, and that atmospheric emissions are larger than the burial of OC in sediments. This hypothesis was based on the following assumptions: (i) OC mineralization rates are high in warm water systems, so that water column CO_2 production overrides the high C uptake by primary producers, and (ii) increasing trophic status creates favorable conditions for CH_4 production. We measured water-air and sediment-water CO_2 fluxes, CH_4 diffusion, ebullition and oxidation, net ecosystem production (NEP) and sediment OC burial during the dry season in a eutrophic reservoir in the semiarid northeastern Brazil. The reservoir was stratified during daytime and mixed during nighttime. In spite of the high rates of primary production (4858 ± 934 mg C m^{-2} d^{-1}), net heterotrophy was prevalent due to high ecosystem respiration (5209 ± 992 mg C m^{-2} d^{-1}). Consequently, the reservoir was a source of atmospheric CO_2 (518 ± 182 mg C m^{-2} d^{-1}). In addition, the reservoir was a source of ebullitive (17 ± 10 mg C m^{-2} d^{-1}) and diffusive CH_4 (11 ± 6 mg C m^{-2} d^{-1}). OC sedimentation was high (1162 mg C m^{-2} d^{-1}), but our results suggest that

the majority of it is mineralized to CO_2 (722 ± 182 mg C m^{-2} d^{-1}) rather than buried as OC (440 mg C m^{-2} d^{-1}). Although temporally resolved data would render our findings more conclusive, our results suggest that despite being a primary production and OC burial hotspot, the tropical eutrophic system studied here was a stronger CO_2 and CH_4 source than a C sink, mainly because of high rates of OC mineralization in the water column and sediments.

Keywords: carbon dioxide, methane, organic carbon burial, net ecosystem production, semiarid, Caatinga

INTRODUCTION

Lakes and reservoirs occupy only a small fraction of the Earth surface, which contrasts with the large role they play in the global carbon (C) cycle (Cole et al., 2007; Tranvik et al., 2009). Because of large inputs of terrestrial inorganic and organic carbon (OC), lakes and reservoirs annually emit 0.1 and 0.3 Pg of C as methane (CH_4) and carbon dioxide (CO_2), respectively (Bastviken et al., 2011; Raymond et al., 2013). In artificial lakes, riverine emissions downstream of dams are also substantial (Guérin et al., 2006). Although lakes and reservoirs are globally important sources of C, they can simultaneously act as net C sinks since a great portion of the terrestrially derived OC is potentially buried in their sediments (Dean and Gorham, 1998; Downing et al., 2008). On a global scale, estimates suggest that lakes and reservoirs are 2–3 times larger C sources than sinks (Cole et al., 2007; Tranvik et al., 2009), yet this overall balance is still uncertain because studies on C burial are far more scarce than on C evasion (Mendonça et al., 2012).

Different factors contribute to the variability in C evasion from lakes and reservoirs. Globally, there is a significant latitudinal gradient in C fluxes from these systems (Kosten et al., 2010). Tropical systems are hotspots for C emissions and account for 34% of global inland water emissions (Raymond et al., 2013), and tropical reservoirs emit 3 and 5 times more CH_4 and CO_2 than non-tropical ones, respectively (Barros et al., 2011). In reservoirs, age is negatively correlated to C evasion (Abril et al., 2005; Barros et al., 2011). On a global scale, age together with latitude and OC inputs explains 40% of CO_2 and 54% of CH_4 emissions from hydroelectric reservoirs (Barros et al., 2011). For CO_2 in particular, dissolved inorganic C loading originating from soil respiration also contributes to evasion from lakes (Weyhenmeyer et al., 2015). External inorganic input may even support CO_2 emission from net autotrophic systems as demonstrated for several temperate systems (McDonald et al., 2013).

Size is also an important regulator of C cycling in lakes and reservoirs. The global area of lentic inland water ecosystems is dominated by millions of lakes and reservoirs smaller than 1 km^2 (Downing et al., 2006), and these systems play a large role in global freshwater C cycle (Bastviken et al., 2004; Downing, 2010; McDonald et al., 2012; Read and Rose, 2013). Small systems are generally characterized by higher water temperatures together with larger amounts of OC that increase C processing rates (Downing, 2010; Read and Rose, 2013). Moreover, oxygen concentrations tend to be lower in small lakes and reservoirs than in larger ones, enhancing greenhouse-gas emissions – particularly CH_4 – but also C burial (Downing, 2010). C sequestration in the sediments of these small systems can be as great as in terrestrial systems (forests and grasslands) and oceans (Downing, 2010). Thus, including small freshwater systems in global C budgets is an important challenge (Downing et al., 2006; Downing, 2010).

In addition to inter-lake variability, spatial variation within systems can also be substantial. This spatial variation is influenced by changes in river inflow and residence time, as well as by heterogeneity in primary production with, for instance, higher primary production in lacustrine zones of eutrophic systems (Pacheco et al., 2015). Ignoring spatial variation can result in up to 25% error in total system gas fluxes in large systems (Roland et al., 2010), and single-site measurements can overestimate OC burial rates by over 50% (Mackay et al., 2012; Mendonça et al., 2014).

Finally, the interaction between aquatic C fluxes and trophic state has been increasingly investigated. Although there is large variability in CO_2 water-atmosphere fluxes in eutrophic lakes (Kortelainen et al., 2006; Sand-Jensen and Staehr, 2009; Balmer and Downing, 2011), recent studies in temperate systems revealed that when eutrophication increases, lakes and reservoirs tend to have less intense CO_2 effluxes because of high CO_2 uptake by primary production (Gu et al., 2011; Trolle et al., 2012). One study carried out in 100s of US agriculturally eutrophic lakes indicated that 60% of them are CO_2-undersaturated (Balmer and Downing, 2011). Mass balances indicate that some lakes with extreme primary production become CO_2 sinks because CO_2 fixed by primary producers is buried in sediments as OC (Pacheco et al., 2013). Even though some temperate eutrophic systems may still function as atmospheric CO_2 sources (Sand-Jensen and Staehr, 2009; Balmer and Downing, 2011; Knoll et al., 2013), the high rates of OC burial in sediments frequently exceed CO_2 emissions (Knoll et al., 2013; Pacheco et al., 2013) and sometimes even the sum of CO_2 and CH_4 emissions (Sobek et al., 2012).

In tropical systems, the knowledge of the interplay between eutrophication and aquatic C fluxes is incipient and highly uncertain. Two studies from a Brazilian eutrophic reservoir indicate that it functions as a CO_2 source during the rainy season and a CO_2 sink during the dry season (Roland et al., 2010; Pacheco et al., 2015). The overall greenhouse-gas (GHG) balance of the system, however, remains unknown as OC burial and CH_4 emissions were not measured. In tropical sediments, OC mineralization rates are more substantial than in temperate sediments because of warmer temperatures (Gudasz et al., 2010; Cardoso et al., 2014), which leads to lower OC burial efficiencies

(Alin and Johnson, 2007; Mendonça et al., 2012) and a higher return of inorganic C to the water column. In addition, even if eutrophic systems are CO_2 sinks, they can still be overall GHG sources because CH_4 emissions might increase with increasing trophic status (Moss et al., 2011).

Here, we evaluated the carbon budget of a small eutrophic reservoir in Brazil's semiarid region through an intensive fieldwork that embraced measurements of a large set of carbon flux pathways. We hypothesized that in spite of being eutrophic this reservoir is a source of both CO_2 and CH_4 to the atmosphere and that the emissions to the atmosphere would be larger than the burial of OC in its sediments. This hypothesis was based on the following assumptions: (i) OC mineralization rates are high in warm water systems, so that water column CO_2 production rates override the high C uptake by primary producers, and (ii) increasing trophic status creates favorable conditions for CH_4 production.

MATERIALS AND METHODS

Study Site

This study was developed in a small (0.2 km^2; 160,000 m^3), 70-years-old eutrophic water supply reservoir located partly inside the Ecological Station of Seridó (ESEC; 6°34′49″S; 37°15′20″W), a conservation unit of the semiarid Caatinga biome in northeastern Brazil (BSh climate, Köppen's classification; **Figure 1**). The ESEC reservoir is part of the Piranhas-Assu watershed (44,600 km^2), and it is used for irrigation, recreation and water supply for humans and animals. The riparian zone is used for agriculture, pasture and domestic activities. The land cover is dominated by xerophitic vegetation typical of the Caatinga biome. The region is characterized by moderately drained shallow soils (Chromic Luvisol), with high-activity clay and high base saturation at the clay-enriched subsurface soil horizon (IUSS Working Group WRB, 2014).

Brazilian semiarid reservoirs are largely under-studied considering that they are numerous and crucial to water supply. With an area of almost 1,000,000 km^2, the Brazilian semiarid region is the most populated semiarid region on Earth (Barbosa et al., 2012). Accordingly, 100s of reservoirs have been constructed to compensate for the overall water deficit. In response to long water residence times, warm temperatures year-round and high loads of anthropogenic nutrients, Brazilian semiarid reservoirs are highly eutrophic (Lazzaro et al., 2003; Barbosa et al., 2012; Braga et al., 2015; Brasil et al., 2015). Eutrophication in these reservoirs is expected to become even more intense based on current climate change projections (Roland et al., 2012; Verspagen et al., 2014; Brasil et al., 2015). Our study was performed during the dry season, when eutrophication is generally maximum in Brazilian semiarid reservoirs because of high evaporation rates that make nutrients more concentrated (Barbosa et al., 2012; Braga et al., 2015).

The main source of water to the ESEC reservoir is precipitation. The regional climate is characterized as tropical semiarid, with low rainfall (∼700 mm per year) irregularly distributed throughout the year and an overall water deficit (Barbosa et al., 2012). Consequently, most rivers are temporary. Generally, the rainy season is concentrated in only 5 months (January to May), with practically no rainfall from July to November (**Figure 2**). The mean water depth during the sampling period (dry season) is 2 m, but the water column can be up to 4-m deep during the rainy season. During the dry season, the ESEC lake is highly eutrophic, with total phosphorus and chlorophyll-a concentrations of 98–104 and 31–64 $\mu g\ L^{-1}$, respectively (Costa et al., 2015).

Sampling and Analyses

Samples were taken during five consecutive days in July 2014, which characterizes the dry season (**Figure 2**). Dissolved oxygen (DO), water temperature, and radiation were measured every 15 min at 40 cm below surface at one location in the deepest part of the reservoir, with a luminescent sonde (LDO10115, Hach-Lange, Tiel, Netherlands), and a light meter, respectively (**Figure 3**).

Stratification and mixing patterns were assessed through temperature and DO vertical profiles performed using a luminescent sonde. Vertical profiles were measured in the morning and afternoon of July 15th, 16th, and 17th (with the exception of the morning of July 15th). Differences between the surface water temperature measured at 40 cm below the surface right before dawn and the bottom temperature measured during the day gave insight on the mixing pattern of the water column. Chlorophyll-a concentrations were measured at the surface and at the bottom at different sites between July 15th and 18th ($n = 48$) using a PHYTO-PAM (Heinz Walz GmbH, PHYTO-ED, Effelrich, Germany).

C Fluxes to the Atmosphere and CH_4 Oxidation

Water-air diffusive CO_2 and CH_4 fluxes were measured in triplicate using transparent floating chambers (base area = 660 cm^2; volume = 14,700 cm^3) connected to a portable GHG analyzer that uses laser absorption spectroscopy (PICARRO G2508 GHG analyzer; **Figure 3**). The partial pressure of CO_2, CH_4 and N_2O were measured continuously (every second) during 5-min intervals in the littoral ($n = 8$ for each gas) and pelagic zones ($n = 6$ for each gas), between 09:00 AM and 6:00 PM. The slope of the relationship between gas concentration and time was used to calculate the gas flux as follows:

$$F = \frac{V}{A} * slope * \frac{(P * F1 * F2)}{R * T}$$

Where, F is gas flux (mg C m^{-2} d^{-1}), V is chamber volume (m^3), A is chamber surface area (m^2), slope is the slope of the relationship between CH_4 or CO_2 and time (ppm/second); P is atmospheric pressure (kPa); F1 is the molecular weight for CO_2 (44) or CH_4 (16; g $mole^{-1}$); F2 is the conversion factor of seconds to days; R is gas constant (8.3144 J K^{-1} $mole^{-1}$); and T is temperature in Kelvin (K). Nitrous oxide (N_2O) fluxes were also measured but remained below the detection limit. When fluxes from littoral and pelagic zones were significantly different (i.e., t-test, $p < 0.05$), the average flux from the reservoir was presented as a weighted average, which considers the area of both zones.

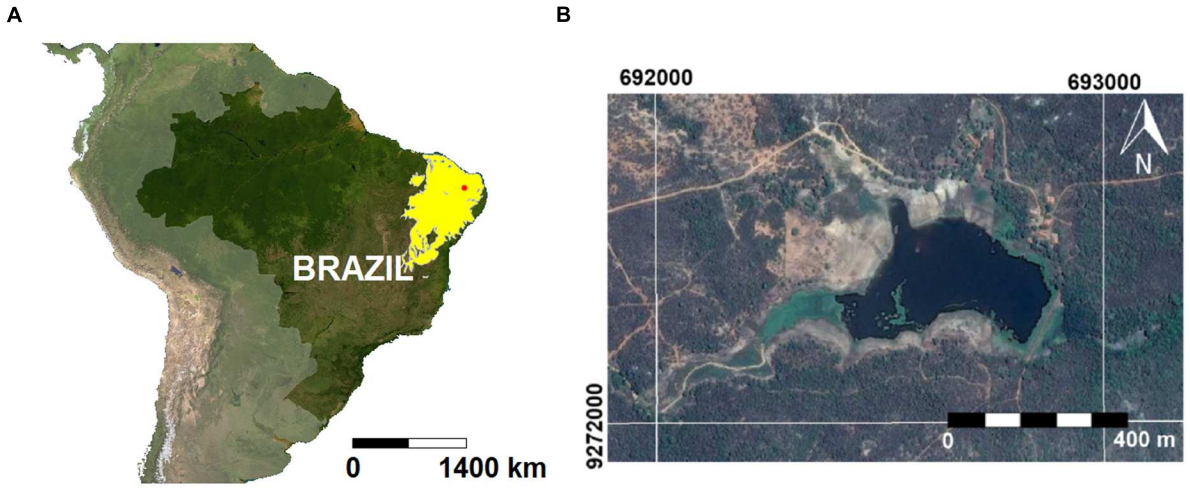

FIGURE 1 | (A) Map of Brazil with emphasis to the Caatinga biome (yellow area) and the location of the ESEC reservoir (red dot). **(B)** Satellite image of the ESEC reservoir.

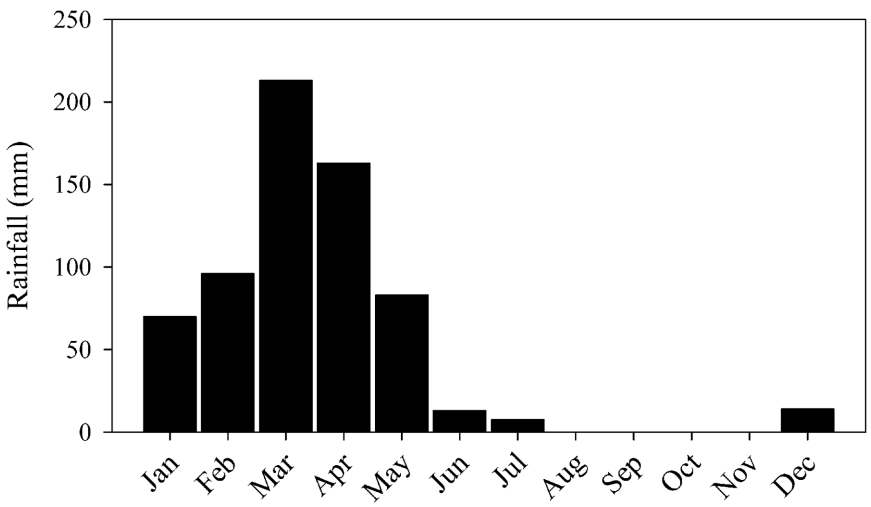

FIGURE 2 | Mean monthly rainfall between 1992 and 2014 in Serra Negra do Norte (Rio Grande do Norte, Brazil), near the ESEC reservoir. Source: Rio Grande do Norte Agricultural Research Company (EMPARN).

Water-air diffusive CO_2 fluxes were also estimated from pH and alkalinity (Stumm and Morgan, 1996). pH was measured with a pH-meter (HPH-1002) and alkalinity was assessed through titration with 0.02 N sulfuric acid ($n = 9$). CO_2 concentrations were then calculated using temperature-adjusted equilibrium constants. CO_2 fluxes were calculated based on CO_2 concentrations, CO_2 saturation with the atmosphere and gas transfer velocity ($k_{600} = 0.7$ m d^{-1}; Cole and Caraco, 1998), which was estimated based on wind records at the reservoir during the sampling days. Positive values denote CO_2 efflux and negative values denote CO_2 influx. Wind speed measurements were made 1.5 m above the water level and varied from 1.3 to 2.6 m s^{-1} (average = 1.8 m s^{-1}). These wind speeds were normalized to wind speed at 10 m above surface (Smith, 1985).

To estimate CH_4 ebullitive fluxes, we deployed bottom-moored triplicated funnels (base area = 800 cm^2; volume = 1,060 cm^3) connected to water-filled glass bottles at the pelagic zone of the reservoir during 24-h periods ($n = 3$; Rosa et al., 2003; **Figure 3**). Once ebullition occurs, the bubbles expel water from the bottles and form a headspace whose volume is equal to the bubble volume. Gas concentrations were determined in the Picarro G2508 GHG analyzer. To further calculate CH_4 flux through ebullition, the gas volume that accumulated inside the water-filled bottles was calculated by adding water to the bubble-formed headspace using a graduated pipette.

Methane oxidation was assessed as the CH_4 concentration decline over time in closed and flexible airtight medical blood bags (as in Bastviken et al., 2008; **Figure 3**). The 500 ml bags

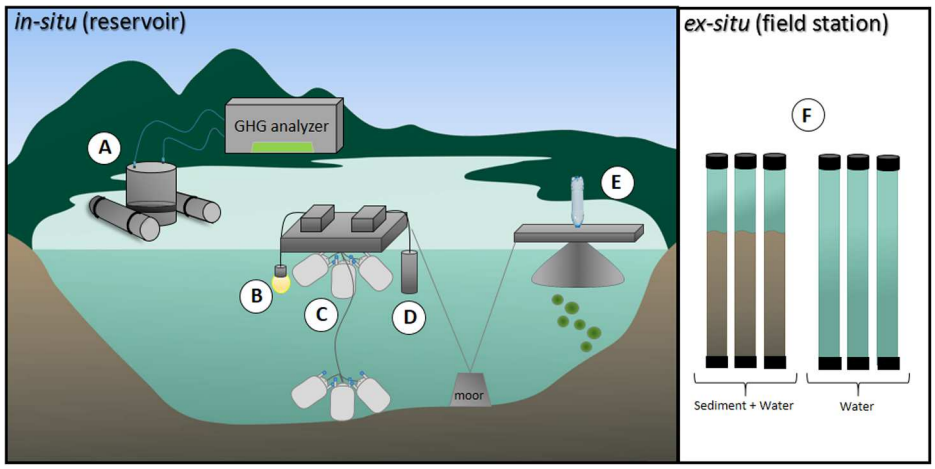

FIGURE 3 | Schematic representation of the field measurements made at the ESEC reservoir. (A) CO_2 and CH_4 diffusive fluxes were determined through a floating chamber connected to a GHG analyzer. **(B)** A light meter was utilized to measure radiation 40 cm below at 15-min intervals. **(C)** CH_4 oxidation was assessed as the CH_4 concentration decline over time in closed and flexible airtight medical blood bags incubated at surface and bottom. **(D)** A multi-parameter water quality sonde was utilized to measure water temperature and dissolved oxygen (DO) 40 cm below surface at 15-min intervals. **(E)** CH_4 ebullition was determined through bottom-moored funnels connected to water-filled glass bottles; the gas that accumulated in the bottles was measured in a GHG analyzer and CH_4 was then calculated. **(F)** Sediment organic carbon (OC) mineralization was determined *ex situ* via dark incubations of cores containing sediment plus water and only water. Detailed information on the methods are shown in Section "Materials and Methods."

were filled with water from the surface ($n = 9$) and from the bottom ($n = 8$). Care was taken to prevent gas bubble formation in the bags. Initial samples for CH_4 concentration (10 ml) were taken with a plastic syringe and transferred to evacuated and pre-capped 13-ml infusion vials. In order to preserve the samples, 25 μL of 2.45 M H_2SO_4 was added to the vials. The bags were incubated *in situ* at the same depth the water was sampled from (surface or near-bottom). Subsequent samples were taken from the bags after approximately 4 and 8 h. In the lab, 3 mL of atmospheric air was injected in each vial to create a headspace. After equilibration between the headspace and the water sample, the CH_4 concentration in the headspace was measured with the PICARRO G2508 GHG analyzer.

Net Ecosystem Production

The 15-min interval measurements of DO concentrations were used to calculate the lake system respiration, gross primary production (GPP) and net ecosystem production (hereafter NEP-system) according to Cole et al. (2000). The change in DO (ΔDO) in each 15-min interval is due to NEP and diffusive O_2 exchange (D) with the atmosphere (i.e., $\Delta DO = NEP + D$). Diffusion (D) can be calculated using the measured concentration in the water, the concentration in the water in equilibrium with the atmosphere (O_2-*sat*) and the gas transfer velocity (k), using the equation $D = k(O_2 - O_2\text{-}sat)$. Hence, the NEP of each time interval can be calculated based on DO change and calculated O_2 diffusion. Nighttime NEP (between 7 PM and 5 AM, which was the period the water column was mixed) was used to estimate R. One R was assigned to each day. Subsequently, we calculated GPP as the summation of NEP and R.

The vertical temperature and DO profiles showed that the DO sonde was continuously situated (at 40 cm below surface) in the upper mixing zone. Under mixed conditions, the NEP-system incorporates changes in DO caused by sediment respiration. By contrast, during stratification, oxygen-depleted waters resulting from hypolimnetic respiration accumulate in the bottom and are not diffused to the upper layers, so that change in DO is governed by planktonic metabolism. When the water column mixes at night, the sonde eventually captures sediment respiration that occurred during stratified periods. To obtain the pelagic NEP (hereafter NEP-pelagic, which results strictly from planktonic metabolism), we subtracted sediment respiration (i.e., sediment OC mineralization – see Materials and Methods, sub-heading "OC mineralization and burial in sediments") from the NEP-system. Sediment primary production was assumed to be non-existent, as there is no sunlight reaching the sediment. The assessment of both NEP-system and NEP-pelagic allowed us to infer the contribution of planktonic and sediment respiration to the system respiration. Conversions of oxygen fluxes to CO_2 fluxes were done assuming a respiratory quotient of 1:1.

OC Burial and Mineralization in Sediments

Organic carbon burial in sediments was assessed with sediment coring according to Mendonça et al. (2014). Sediment cores were sampled from nine different sites about evenly distributed using a gravity corer equipped with a hammer device (6 cm internal diameter and 120-cm long cores, UWITEC, Mondsee, Austria). The core was hammered into the sediment in order to retrieve cores containing the entire sediment layer at the sampling site, including some pre-flooding substrate. The transition between pre-flooding substrate and reservoir sediment was visually

identified in the field. Sediment cores from the post-flooding substratum were sub-sampled in 2–6 cm thick slices that were stored refrigerated in airtight plastic containers until laboratory analysis. Dry sediment mass of each sediment slice was measured gravimetrically. OC content of all slices was determined in a C analyzer (Shimadzu, TOC-V CPN) coupled to a solid sample module (SSM 5000A). Because OC content in the three analyzed cores showed low variability (5.6 ± 0.8, average ± SD), we used the average OC content to calculate burial in the other six cores.

The OC burial rate calculations were, analogous to Mendonça et al. (2014), based on OC mass results. OC mass (g C) in each sediment slice was measured as the product of OC content (g g^{-1}) and dry sediment mass (g). Total OC mass in each core was calculated as the sum of OC mass in all post-flooding sediment slices. Areal OC burial rates (g C m^{-2} $year^{-1}$) for each core were calculated from total OC mass (g C), core surface area (2.8 × 10^{-3} m^2) and the reservoir age (69 years in 2014). This results in a life-time average burial rate.

Integrated OC mineralization of the metabolically active layers of sediment was assessed as the change in DO resulting from aerobic processes in the sediment and the oxidation of anaerobically produced CH_4. Sediment cores (6 cm internal diameter and 60 cm long) containing approximately 20 cm of sediment (i.e., the metabolically active layer of sediments; Burdige, 2007) collected from different locations in the pelagic zone were promptly incubated on land in a dark room ($n = 5$), at *in situ* temperature (26°C). For each incubation triplicates of cores with sediment plus lake water and cores containing only lake water from the same site were incubated gastight without headspace (**Figure 3F**). The cores with only lake water were used to assess DO decrease due to respiratory processes in the water, excluding the sediments. DO concentrations were measured right upon incubation and after 4 h in all cores – the average DO concentration before incubation was 6.9 mg L^{-1}. The change in DO concentration through time multiplied by the water column height in each core resulted in DO consumption rates (i.e., respiration rates) per unit area. The areal respiration rates in the cores containing sediment subtracted by the areal respiration in cores containing only water reflected the OC mineralization in the sediment. DO concentrations did not reach hypoxia in any of the incubations (average final concentration was 6.1 mg L^{-1}). Also, during our sampling campaign, stratification was never long enough for hypolimnetic DO concentrations to reach values below 4 mg/L, justifying our aerobic incubations. OC mineralization is probably underestimated as we likely missed anaerobic mineralization (i.e., likely not all anaerobically produced CH_4 is oxidized in the overlying water in the core). Conversions of oxygen fluxes to CO_2 fluxes were done assuming a respiratory quotient of 1:1.

Organic carbon burial efficiency, defined as the percentage of the total OC reaching the sediments that remains in the sediment (i.e., that escapes mineralization), was calculated as the ratio between OC burial rate and OC gross sedimentation rate (both in g C m^{-2} $year^{-1}$). The OC gross sedimentation rate was calculated as the sum of OC burial and OC mineralization, considering that these are the two possible fates of OC that reaches the sediment.

RESULTS

Mixing Pattern and Chlorophyll-a

The temperature variation between surface and bottom waters was generally of 1–2°C as determined from vertical profiles, without any persistent thermocline. In the afternoon of the last day (July 17th), the water column stratified as indicated by both DO and temperature profiles (**Figure 4**). The vertical variation of DO accompanied the variation in temperature, with decreasing concentrations toward the bottom. Both temperature and DO profiles indicate that the water column fully mixes every night (**Figure 4**).

Chlorophyll-a concentrations showed little variability (51–69 μg L^{-1} considering surface and bottom samples together; average ± SD = 60 ± 5 μg L^{-1}; data not shown). The

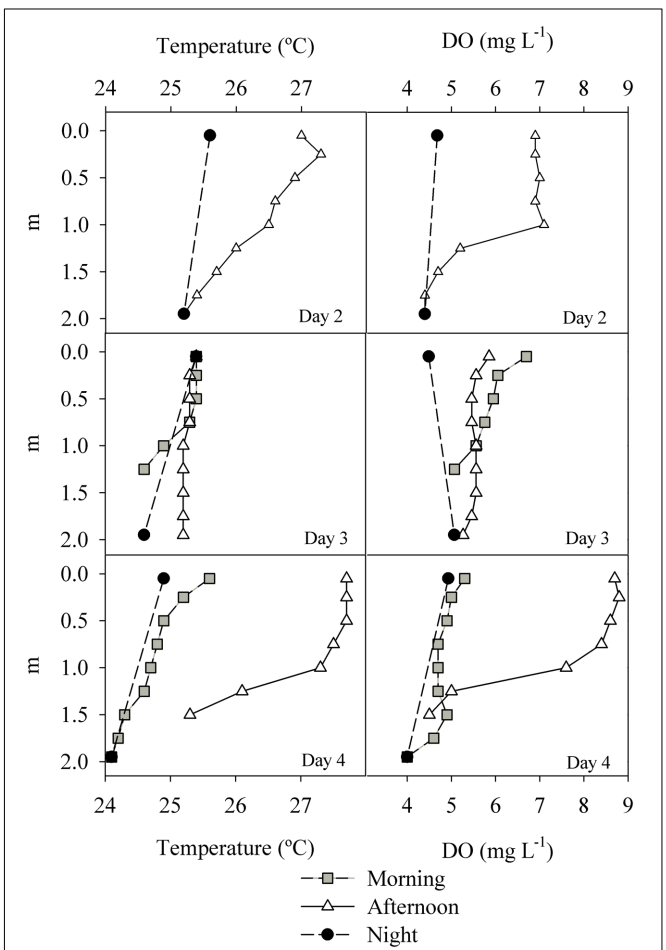

FIGURE 4 | Temperature and DO profiles measured in the ESEC reservoir between July 15th and 17th ("Day 2" and "Day 4"). Night profiles refer to measurements taken right before dawn.

concentrations of chlorophyll in illuminated epilimnetic waters were not significantly higher than in dark hypolimnetic waters (*t*-test; $p > 0.05$), reinforcing that the water column undergoes constant mixing.

C Fluxes to the Atmosphere and CH_4 Oxidation

The ESEC reservoir was a source of CO_2 to the atmosphere as indicated by CO_2 fluxes calculated directly via the floating chamber and indirectly through alkalinity and pH (**Figure 5**). The estimate from alkalinity and pH resulted in an average CO_2 efflux of 497 ± 213 (SD) mg C m^{-2} d^{-1}, whereas the direct measurement with the floating chamber resulted in an average flux of 518 ± 182 (SD) mg C m^{-2} d^{-1} (**Figure 5**). CO_2 fluxes calculated based on alkalinity and directly measured in floating chambers did not significantly differ (*t*-test, $p > 0.05$). Combining the results of both methods, the average CO_2 efflux from the ESEC reservoir was 510 mg C m^{-2} d^{-1}. There was no significant spatial variation in CO_2 efflux, with littoral and pelagic zones displaying similar fluxes (**Figure 6**).

The ESEC reservoir was also a source of CH_4, which evades through ebullition (∼60%) and diffusion (∼40%). The average CH_4 diffusive flux was 11 ± 6 mg C m^{-2} d^{-1} (average ± SD), whereas the CH_4 ebullitive flux averaged 17 ± 10 mg C m^{-2} d^{-1} (average ± SD; **Figure 5**). There was significant spatial variation in CH_4 diffusive fluxes, as the littoral zone diffused about two times more CH_4 than the pelagic zone (**Figure 6**). The average CH_4 diffusion, weighted by the area of the littoral and pelagic zones, was 11 mg C m^{-2} d^{-1}. The summation of average ebullitive and diffusive fluxes results in a total CH_4 efflux of 28 mg C m^{-2} d^{-1}. The oxidation of CH_4 averaged 31 ± 17 μg C m^{-2} d^{-1} at the surface and 18 ± 12 μg C m^{-2} d^{-1} at the bottom.

OC Mineralization and Burial in Sediments

Sediment OC mineralization rates as determined from *ex situ* experiments were 722 ± 182 mg C m^{-2} d^{-1} (average ± SD; **Figure 5**). The post-flooding sediment layer varied in thickness from 24 to 62 cm, which translated into a maximum sediment deposition rate of 0.9 cm $year^{-1}$ (average = 0.5 cm $year^{-1}$). The mean OC content of post-flooding sediment averaged 5.6 ± 0.8% (±SD). Thus, the sediment OC stock averaged 10.4 ± 4.2 kg C m^{-2} (±SD). Based on the OC content in the sediment cores, the core area and the reservoir age, the OC burial rates varied from 88 to 280 g C m^{-2} $year^{-1}$, averaging 161 g C m^{-2} $year^{-1}$ (440 mg C m^{-2} d^{-1}). Finally, considering the average rates of OC mineralization and burial, the OC sedimentation rates ranged from 307 to 499 g C m^{-2} $year^{-1}$, averaging 380 g C m^{-2} $year^{-1}$ (1040 mg C m^{-2} d^{-1}). The OC sedimentation and burial rates resulted in a burial efficiency of 29 ± 5% (average ± SD).

Net Ecosystem Production

The diel curves of volumetric NEP, GPP and respiration indicated substantial variation in NEP (**Figure 7A**). Integration of NEP, GPP and R over 24 h indicated that although daytime measurements indicated net autotrophy (**Figure 7A**), the 24-h integrated NEP indicated that respiration (5209 ± 992 mg C m^{-2} d^{-1}, average ± SD) exceeded GPP (4858 ± 934 mg C m^{-2} d^{-1}, average ± SD) on the 4 days (**Figure 7B**); thus, the system was net heterotrophic throughout the sampling period (**Figure 7C**). On the other hand, when sediment respiration (i.e., OC mineralization in sediments, shown in see OC Mineralization and Burial in Sediments) was discounted to calculate the NEP of the pelagic system, we observed net autotrophy on all days (**Figure 7C**). Even if the highest sediment respiration rate estimated over the 4 days is considered (error bars in

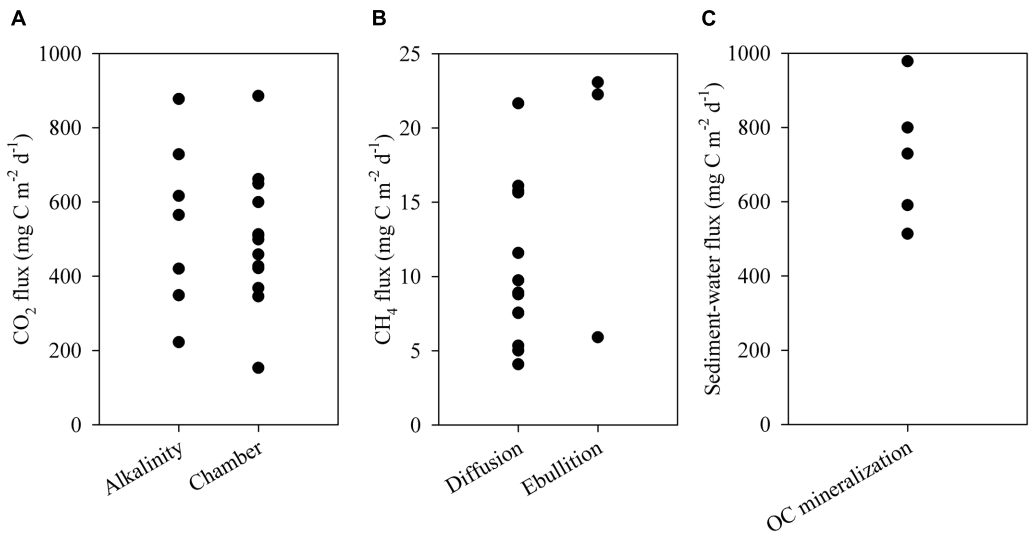

FIGURE 5 | (A) Water-atmosphere fluxes of CO_2 measured via alkalinity and floating chambers; **(B)** ebullitive and diffusive fluxes of CH_4 to the atmosphere; **(C)** sediment OC mineralization measured *ex situ*. Each data point represents one measurement.

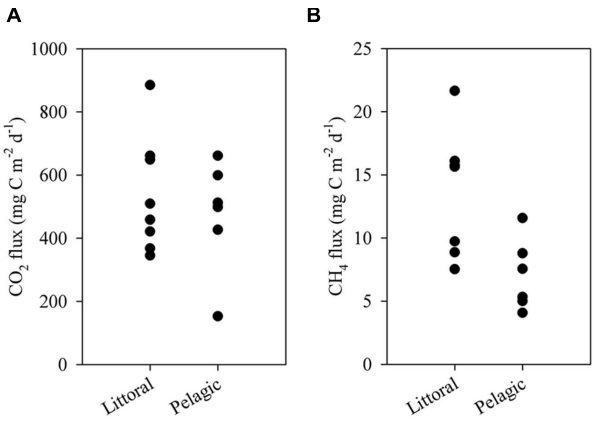

FIGURE 6 | Diffusive fluxes of CO_2 (A) and CH_4 (B) to the atmosphere in the littoral and pelagic zones of the ESEC reservoir. Each data point represents one measurement.

Figure 7C), the pelagic system still remains autotrophic, except on day 2. The average rates of NEP-system and NEP-pelagic were -350 ± 217 and 228 ± 217 mg C m^{-2} d^{-1}, respectively. The most heterotrophic days were days 2 and 3 (**Figure 7C**), when light intensity was lower (**Figure 7A**); conversely, the least heterotrophic day was day 1, when light intensity was highest.

DISCUSSION

Pelagic Metabolism and Water-Air C Fluxes

The strong variation in NEP (**Figure 7A**) is likely a reflection of the variability in irradiation and mixing of the water column, with a strong positive NEP in the early morning resulting from a combination of increased radiation and the onset of stratification. During stratification, the lower water layers become oxygen-depleted (**Figure 4**) which is reflected in the sonde-derived NEP estimates only when the water column mixes again (i.e., strong negative NEP after sunset; ∼18:00 h onward).

Overall, our findings indicate that the ESEC reservoir has an extremely high primary production (average GPP ∼4600 mg C m^{-2} d^{-1}), which is corroborated by high concentrations of chlorophyll-a (average ∼60 μg L^{-1}) and total phosphorus (∼100 μg L^{-1}; Costa et al., 2015) classifying it as a hypertrophic system (Wetzel, 2001). The strong CO_2 uptake due to the high GPP is counterbalanced by the extremely high ecosystem respiration. The measured respiration rates (∼5100 mg C m^{-2} d^{-1}) are higher than the respiration of over 35 global ecosystem types ranging from 3 mg C m^{-2} d^{-1} (Arctic desert) to 4178 mg C m^{-2} d^{-1} (tropical agriculture soil; Doering et al., 2011, and references therein). As a result, during the sampling period (representative of the dry season), we found prevalence of positive NEP-pelagic and negative NEP-system. This suggests that if sediment respiration is disregarded, as in the case of NEP-pelagic, the system is prevalently autotrophic; on the contrary, if sediment respiration is taken into consideration, the system is prevalently heterotrophic. The heterotrophic nature of the system is corroborated by the prevalence of strong atmospheric CO_2 efflux (**Figure 5A**).

Our estimate of the CO_2 efflux from the ESEC reservoir likely represents the lower end of the daily and yearly variation in CO_2 efflux, as our estimate is based on daytime effluxes measured during the dry season, a season that is likely to last longer and become drier over the coming years (Marengo et al., 2010). Aquatic primary production tends to peak during the dry season and loading of terrestrial C is minimal (Barbosa et al., 2012). Indeed, temporally resolved measurements in other Brazilian semiarid reservoirs (P. Junger, personal communication) and a hypertrophic tropical reservoir (Roland et al., 2010; Pacheco et al., 2015) suggest that the peak of heterotrophy occurs in the rainy season, when terrestrial C loading is high and primary production is lower.

Even with the likely underestimation, the CO_2 efflux from the ESEC reservoir is more than three times higher than the rates reported for other eutrophic systems worldwide (**Table 1**), which is likely due the high organic matter loading to the system in the wet period combined with high temperatures enhancing respiration rates (Gudasz et al., 2010; Scofield et al., 2015). Input of external inorganic C coming from soil respiration may also contribute (McDonald et al., 2013; Weyhenmeyer et al., 2015). The high CO_2 efflux is in accordance with the small size of the reservoir (Downing, 2010), but contradicts with the finding that old reservoirs (the ESEC reservoir is 70 years-old) tend to emit less CO_2 (Barros et al., 2011). Overall, the ESEC reservoir's CO_2 efflux falls within the range reported for (less eutrophic) tropical lakes and tropical non-Amazonian reservoirs, and it is higher than the rates reported for all other types of systems (**Table 1**).

The CH_4 efflux of the ESEC reservoir is similar to those reported for tropical non-Amazonian hydroelectric reservoirs, and higher than the average of temperate and boreal systems (**Table 1**). Ebullition was the most important CH_4 emission pathway (∼60%), which concurs with what has been found in temperate and boreal small lakes (Bastviken et al., 2004). As opposed to CO_2 fluxes, CH_4 diffusive fluxes showed significant spatial variation (**Figure 6**), with higher fluxes in the littoral zone that is dominated by rooted macrophytes. In shallow lakes, higher CH_4 emissions near the shore may be attributable to larger availability of plant-derived organic matter that indirectly fuels methanogenesis (Bastviken et al., 2010). Moreover, sediments in shallow zones are more exposed to wind-driven turbulence, which favors the release of CH_4.

Sediment OC Stock, Burial, and Mineralization

The C stock in the ESEC reservoir's sediment is high considering the C-impoverishment of the watershed. The Caatinga biome is characterized by shallow soils with xerophytic vegetation (Menezes et al., 2012). The C stock calculated for the ESEC

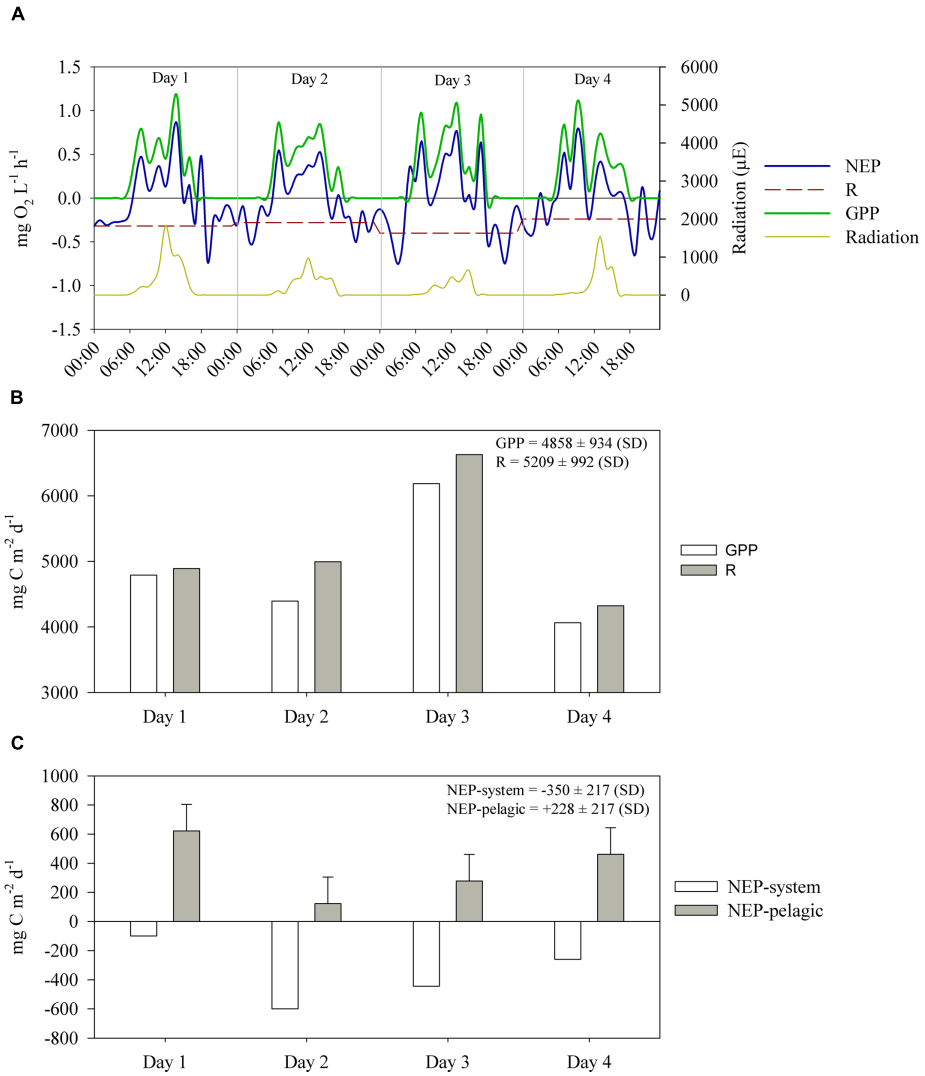

FIGURE 7 | (A) Hourly estimates, in oxygen units, of volumetric gross primary production (GPP), respiration (R) and net ecosystem production (NEP), between July 14 and 17. R is shown as an average of NEP during nighttime on each day. The average R over the 4 days was 0.31 ± 0.07 mg O_2 L^{-1} h^{-1}. **(B)** Daily estimates, in carbon units, of GPP and R. **(C)** Daily estimates, in carbon units, of areal NEP-system (i.e., considering sediment respiration) and NEP-pelagic (i.e., excluding sediment respiration, 722 ± 182 mg C m^{-2} d^{-1}) between July 14 and 17. The whiskers outside NEP-pelagic bars indicate the standard deviation of sediment respiration.

reservoir (average = 10.4 kg C m^{-2}) is about five times higher than the soil C stock for typical semiarid phytoecological units (2.0–3.1 kg C m^{-2}; Nóbrega, 2013) and similar to the C stock in tropical semiarid mangroves (Nóbrega et al., 2016). Mangroves are among the most important C burial hotspots worldwide (Howard et al., 2014), which suggests that the sediment of Brazilian semiarid reservoirs may act as important regional C sinks.

The OC burial in the ESEC reservoir (161 g m^{-2} $year^{-1}$) is higher than the values reported for temperate oligotrophic lakes (**Table 1**). Indeed, OC burial rates are commonly higher in eutrophic systems due to the fast deposition of highly organic sediments (Downing et al., 2008). The OC burial efficiency in the ESEC reservoir (average = 29%) fits the range of temperate lake sediments primarily composed by autochthonous OC (3–50%; Sobek et al., 2009). Data on OC burial efficiency in tropical systems that we know of is limited to one hydroelectric reservoir (Lake Kariba, 41%, Kunz et al., 2011) and one natural lake (Lake Kivu, ∼50%, Sobek et al., 2009), all showing higher efficiencies than the ESEC reservoir. The higher OC burial efficiencies in the hydroelectric reservoirs are due to the high sediment load from inflowing rivers while Lake Kivu sediments bury carbon efficiently because it is permanently anoxic.

The ESEC reservoir sediment OC mineralization is high and falls within the upper range of rates reported for worldwide lakes (Gudasz et al., 2010; Cardoso et al., 2014). This is probably because in the ESEC reservoir bottom waters are frequently oxygenated, temperatures are consistently

high and the water column is shallow (Sobek et al., 2009; Gudasz et al., 2010). Overall, about 70% of the OC reaching the ESEC reservoir's sediment is mineralized rather than buried. The typical labile properties of organic matter in eutrophic systems result in a low share of the sedimentary OC escaping mineralization (Sobek et al., 2009; Gudasz et al., 2010). In addition, high ambient temperatures also favor mineralization over burial (Gudasz et al., 2010; Marotta et al., 2014). The high percentage of OC mineralization is an important driver of the prevalent heterotrophic state of the reservoir and the subsequent atmospheric CO_2 efflux observed (Kortelainen et al., 2006; Cardoso et al., 2013).

CONCLUSION

Although our results demonstrate that the ESEC reservoir is a regional C burial hotspot, the sum of CO_2 (510 mg C m^{-2} d^{-1}) and CH_4 (27 mg C m^{-2} d^{-1}) emissions measured during the dry season outweighs the amount of OC burial in sediments (440 mg C m^{-2} d^{-1}) in terms of C units. This outweighing becomes much more imbalanced toward C emissions if it is considered that CH_4 is a GHG 34 times more potent than CO_2 in a 100-years time interval (Myhre et al., 2013). Accounting for the global warming potential of CH_4, we estimate that the ESEC reservoir emits about three times more CO_2-equivalents to the atmosphere than it buries as OC in the sediment. Assuming that the reservoir was less heterotrophic at the time of sampling (i.e., in the dry season) than during the wet season, our estimate likely represents the lower end of yearly variation in CO_2 efflux. Therefore, although temporally resolved data would render our findings more conclusive, our results suggest that the eutrophic ESEC reservoir is a much larger C source than it is a C sink. This is not in line with data from eutrophic and mesoeutrophic temperate lakes suggesting that they are larger C sinks than CO_2 (Knoll et al., 2013; Pacheco et al., 2013) and C (i.e., CO_2 plus CH_4) sources (Sobek et al., 2012). In the ESEC reservoir,

TABLE 1 | Comparison of carbon fluxes and organic carbon burial (OCB) in the ESEC reservoir with those found in other aquatic systems worldwide.

Climatic zone	System	Flux (mg C m^2 d^{-1})				OCB (mg C m^2 d^{-1})	Reference
		CO_2 Total	CH_4 Diffusion	CH_4 Ebullition	CH_4 Total		
Tropical	*Tropical semiarid eutrophic reservoir*	*510*	*11*	*17*	*28*	*440*	*This study*
	Brazilian oligotrophic hydroelectric reservoirs	151	16	–	–	116	Roland et al., 2010; Ometto et al., 2013; Mendonça et al., 2014
	Brazilian eutrophic hydroelectric reservoir	−1.2	36	–	–	–	Ometto et al., 2013; Pacheco et al., 2015
	Amazonian hydroelectric reservoirs	1096	–	–	137	–	Barros et al., 2011
	Tropical non-Amazonian hydroelectric reservoirs	685	–	–	41	–	Barros et al., 2011
Temperate	US (Ohio) eutrophic reservoir	31	–	–	–	841	Knoll et al., 2013
	US (Iowa) eutrophic reservoirs	−17.5	–	–	–	47–405	Downing et al., 2008; Pacheco et al., 2013
	Swiss mesoeutrophic reservoir	66	11	90	101	3049	DelSontro et al., 2010; Sobek et al., 2012
	Temperate lakes	271	4	10	14	–	Bastviken et al., 2004; Marotta et al., 2009
	Temperate hydroelectric reservoirs	290	–	–	2	–	Barros et al., 2011
	German eutrophic lakes	67–480	3–144	–	–	–	Casper et al., 2000, 2009
Boreal	Boreal hydroelectric reservoirs	206	–	–	6.8	–	Barros et al., 2011
	Boreal lakes	279	–	–	4.9	–	Phelps et al., 1998; Karlsson, 2001

Negative values indicate flux into the aquatic system.

CH_4 is responsible for the largest share of CO_2-equivalents emissions, but even if CH_4 emissions are disregarded, the system is still a slightly larger CO_2 source than a C sink. Hence, the idea of eutrophication shifting lakes into CO_2 sinks, as suggested for temperate eutrophic systems (Pacheco et al., 2013), may not be applicable to tropical eutrophic systems such as the semiarid, shallow ESEC reservoir. The underlying cause is probably the fact that temperate eutrophic systems bury a great deal of the C fixed via primary production, whereas warm eutrophic systems such as the ESEC reservoir respire a large share of this OC.

AUTHOR CONTRIBUTIONS

All authors listed participated in the fieldwork and have made substantial direct and intellectual contribution to the work, and approved it for publication.

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Hydrological pulse regulating the bacterial heterotrophic metabolism between Amazonian mainstems and floodplain lakes

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We evaluated *in situ* rates of bacterial carbon processing in Amazonian floodplain lakes and mainstems, during both high water (HW) and low water (LW) phases ($p < 0.05$). Our results showed that bacterial production (BP) was lower and more variable than bacterial respiration, determined as total respiration. Bacterial carbon demand was mostly accounted by BR and presented the same pattern that BR in both water phases. Bacterial growth efficiency (BGE) showed a wide range (0.2–23%) and low mean value of 3 and 6%, (in HW and LW, respectively) suggesting that dissolved organic carbon was mostly allocated to catabolic metabolism. However, BGE was regulated by BP in LW phase. Consequently, changes in BGE showed the same pattern that BP. In addition, the hydrological pulse effects on mainstems and floodplains lakes connectivity were found for BP and BGE in LW. Multiple correlation analyses revealed that indexes of organic matter (OM) quality (chlorophyll-a, N stable isotopes and C/N ratios) were the strongest seasonal drivers of bacterial carbon metabolism. Our work indicated that: (i) the bacterial metabolism was mostly driven by respiration in Amazonian aquatic ecosystems resulting in low BGE in either high or LW phase; (ii) the hydrological pulse regulated the bacterial heterotrophic metabolism between Amazonian mainstems and floodplain lakes mostly driven by OM quality.

Keywords: bacterial production, bacterial respiration, bacterial carbon demand, bacterial growth efficiency, hydrological pulse, Amazonian freshwater ecosystems

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Introduction

Bacterioplankton (generic term including heterotrophic aerobic Bacteria and Archaea) is considered the main agent for the removal of organic carbon (C) in aquatic systems (Williams, 2000). Bacterioplankton convert dissolved organic carbon (DOC) into biomass through bacterial production (BP) and into CO_2 through bacterial respiration (BR). The whole amount of carbon consumed by the bacterial metabolism on BP and BR has been referred as bacterial uptake or bacterial carbon demand (BCD), an important pathway in the global carbon cycle. The efficiency of bacterial carbon assimilation will determine if carbon is either passed to the next trophic level or converted into CO_2 (Del Giorgio et al., 1999). Bacterial communities in tropical inland aquatic ecosystems have higher metabolic rates (BP, BR, and BCD) and lower bacterial growth efficiency (BGE) than in temperate ecosystems (Amado et al., 2013).

In particular, BR measurements require the separation of bacteria from the rest of the plankton community. The filtration process can cause: the removal of the pressure by competitors and predators (Martínez-García et al., 2013); structure disruption of bacterial community (Del Giorgio and Cole, 1998; Del Giorgio et al., 2011); phytoplankton cells to suffer rupture; and the release of labile organic C to the filtered (Hopkinson et al., 1989) resulting overestimation of respiration measurements. On the other hand, the filtration process may cause an underestimation when particles are retained, which causes a significant percentage of adhered bacteria. Bacteria within a water column can reach high abundances in microhabitats such as aggregates (Grossart and Tang, 2010). This aspect is enhanced in Amazonian freshwater ecosystems due to the high levels of suspended materials. Amazonian aquatic ecosystems show high levels of turbidity reaching 151 nephelometric turbidity units (NTU) in mainstems and 128 NTU in floodplains lakes.

Large parts of the Amazon River are subjected to periodical floods in the surrounding central Amazon area, due mainly to rainfall in the headwaters (Junk, 1997). This creates large temporary wetlands called floodplain, which account with rivers for a total area of ca. 350,000 km^2 (Melack and Hess, 2011). The periodical connectivity of the rivers creates a great diversity of carbon sources across the mainstems and floodplains lakes (Mortillaro et al., 2011). The organic matter (OM) has been reported as refractory in the mainstem and it has been described as more labile in the Amazonian floodplains (Hedges et al., 1986; Moreira-Turcq et al., 2003; Aufdenkampe et al., 2007). Dissolved organic matter (DOM) originating from aquatic primary producers (planktonic algae and aquatic macrophytes) is usually more labile to bacterial growth (Amon and Benner, 1996; Farjalla et al., 2006). Composition and quality of OM in the Amazon Basin have been previously documented using stable isotopes, chlorophyll-a, elemental analysis, fatty acids (FAs), amino acids, and lignin phenols (Hedges et al., 1994; Bernardes et al., 2004; Mortillaro et al., 2011; Moreira-Turcq et al., 2013). In turn, these factors are important regulators of bacterial activity in aquatic systems (Del Giorgio and Cole, 1998) and can have effects on metabolic efficiency (i.e., BGE) under different environmental conditions (Hall and Cotner, 2007).

The aim of this study was to evaluate the changes in the BP, BR, BGE, and BCD in floodplain/mainstem Amazonian ecosystems.

Materials and Methods

Study Site

The Amazon River is the world's largest river with a drainage basin area of 6.1 $\times$ 10^6 km^2 covering about 40% of South America (Goulding et al., 2003). The mean annual discharge is 200 $\times$ 10^3 m^3 s^{-1} at Óbidos, the most downstream gauging station in the Amazon River (Callede et al., 2000). Due to the equatorial position (**Figures 1A–C**), temperature in the central Amazon basin is relatively constant around the year with a mean annual air temperature (MAAT) of ca. 27°C (New et al., 2002). Rivers within the Amazon drainage basin are traditionally classified according to water color, as well as physical and chemical parameters (Sioli, 1956): white water (e.g., Solimões and Madeira), black water (e.g., Negro), and clear water (e.g., Tapajós). Large parts of the central Amazon basin are subjected to periodical floods due mainly to spatial and temporal distribution of rainfall in the headwaters. This creates large temporary wetlands, i.e., seasonally flooded forests which cover a total area of ca. 350 $\times$ 10^3 km^2 (Junk, 1997; Melack and Hess, 2011). There is water exchange between flooded forests and the Amazon River which is highly influenced by the rising and falling of water during the rainy or dry seasons, respectively (Lesack and Melack, 1995). Five floodplain lakes were investigated in this study (**Figure 1C**): Cabaliana, Janauaca, Miriituba, Canaçari, and Curuai (**Figures 1D–F**), located in a gradient of decreasing

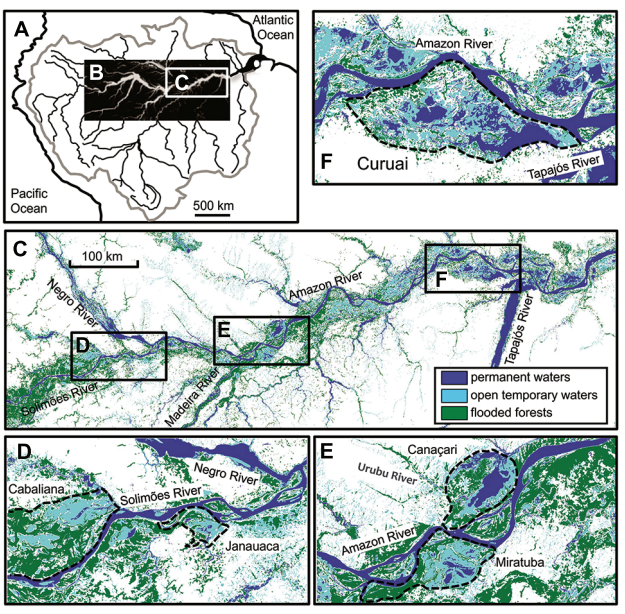

FIGURE 1 | Location of study areas across the Amazonian River ecosystems in a large (A–C) and small scale (D–F). Dashed black lines delineate the Cabaliana, Janauaca, Miratuba, Canaçari, and Curuai floodplains (Abril et al., 2014).

flooded forests and increasing open waters (Bourgoin et al., 2007). Cabaliana has a large area surrounded by flooded forests and two sub-regions. In the northern region, Piranha stream discharges black water while in the southern region white water, brought by the Solimões River, mixes with black water. Janauaca has a peculiar morphology with a ravine shape surrounded by flooded forests. Solimões water comes through the channel in the north and black water comes through the stream system in the south. Miriituba receives white water from the Madeira River and the Amazon River. It can be considered as a white water lake surrounded by flooded forests, with no significant contribution of black water streams. Canaçari has two well-defined sub-regions. In the northern region, the Urubu River discharges black water and in the southern region, the Amazon River discharges white water. Canaçari is surrounded by grass plains but is disconnected from flooded forests most of the year. Curuai is the largest lake in the central Amazon basin, mainly surrounded by grass plains and open waters. It receives white water from the Amazon River through small channels, apart from the main channel in the eastern side. There are no significant contributions of black water streams for Curuai Lake.

Sampling

The main channels of five mainstems were selected (Solimões, Negro, Madeira, Amazon, and Tapajós; **Figure 1**) as well as five floodplain lakes (Cabaliana, Janauacá, Canaçari, Miratuba, and Curuai; **Figure 1**). In total, three to six subsurface water samples were collected at the mainstem stations, and six to seven samples were collected at the floodplain lake stations. Samples were collected from an 800 km transect along the lower Amazon River basin from Manacapuru on the Solimões River to Santarém at the mouth of the Tapajós River. Two cruises were conducted, one in June 2009 during the high water (HW) season and October 2009, 1 month before the lowest water stage, referred to here as the low water (LW) season. In June, as the water level was the highest recorded in the last century, the study area was extensively inundated, enhancing exchange and mixing between the river mainstem, the flooded forest and the open floodplain lakes. In October, the water level was minimal, allowing little interaction with the main channel. The difference in water level at Óbidos between HW and LW was 6 m. The amplitude is generally 3–4 m upstream Manaus (Sioli, 1984).

Subsurface waters (1 m) were collected with a Van Dorn sampler from the ship on seven lakes in HW and LW. Subsamples for chlorophyll a, Tot-P, pH, turbidity, DOC, $^{13}\delta$C of the particulate organic carbon (POC), conductivity, O_2 and bacterial abundance (BA) were taken immediately after sampling. The samples for bacterial parameters were always collected between 7:00 and 11:00 a.m. into acid-washed plastic bottles.

Bacterial Analytical Methods

Bacterial production was measured in unfiltered samples right after sampling using the 4,5-^{3}H L-leucine [specific activity (SA), 50 Ci mmol^{-1}] incorporation based on the method of cold trichloroacetic acid (TCA) extraction (Simon and Azam, 1989; Wetzel and Likens, 2000). Ten millilitre of the sample were placed in a vial and leucine added at final concentration of 50 nM. Three replicate tubes plus two blanks were incubated in the dark for 30 min. After, the TCA was added to a final concentration of 20% and then incubated at 4°C. In addition, the samples were filtered through 0.22 μm Nuclepore membranes (Thomaz and Wetzel, 1995), being washed with TCA 5% and ethanol. The leucine incorporated into the bacterial biomass was measured in a Beckman LS 6500 scintillation counter. The protein production was converted to carbon production using a protein-to-carbon ratio of 0.86 (Wetzel and Likens, 2000).

Bacterial respiration was measured by following changes in dissolved oxygen during dark incubations. Boro-silicate glass bottles were carefully filled and three replicates were immediately fixed with Winkler reagents to determine the initial oxygen concentration. Three replicate bottles were incubated in the dark at *in situ* temperature and fixed with Winkler reagents after12 h. Dissolved oxygen measurements were made with an automatic titrator (DL50 Graphix, Mettler Toledo) based on potentiometric endpoint detection (Granéli and Granéli, 1991). The respiration rate was determined by the difference between final and initial samples measured at times zero and 12 h. To convert mg O_2 L^{-1} to mg C L^{-1}, we used a respiration quotient (RQ) = 1. In order to detect possible interferences of filtration on BR measurements, tests were conducted in June 2009 (HW) on filtered and unfiltered samples (total sample) from 13 stations. The BR was measured by following changes in dissolved oxygen during dark incubations of total and filtered water following the procedures described above. The filtered fraction was obtained from total samples filtered under low pressure with a vacuum pump through 1.2 μm (GF/C) Whatmann fiber filters right after sampling. The respiration measurements on the filtered fractions were significantly higher than the unfiltered samples ($p < 0.05$) for 100% of the stations (1.6 times higher). Therefore, the measurement obtained from the unfiltered water, and not the filtered fraction, was used as the estimate of BR in the present study.

Bacterioplankton abundance was estimated on subsamples collected in 40 ml sterile polyethylene flasks, preserved in borate-buffered 0.2 μm pre-filtered formalin (3% final concentration), and stored at 4°C. Subsamples were stained with DAPI (4 μg mL^{-1}) for 15 min (Porter and Feig, 1980), filtrated through 0.2 μm black polycarbonate membranes (Millipore© Isopore) previously mounted on GF/C Whatman fiber filters to optimize cell distribution, then mounted on slides with non-fluorescent oil (Olympus optical). Direct counts were performed at 1,250× magnification under an epifluorescence microscope (Leica Leitz DMR; 365 nm). In high-turbid waters, subsamples were pre-treated (before staining) by an addition of 150 μL of Tween, sonicated at 35 kHz for 5 min, and centrifuged at 3,000 *g* during 10 min at 4°C (Chevaldonné and Godfroy, 1997; Hubas et al., 2007). The abundance of free bacteria was estimated in untreated diluted samples and/or in subsamples pre-filtered through a 3-μm filter. The attached bacteria were deduced from total to free bacterial counts.

Bacterial Carbon Fluxes

Bacterial growth efficiency was calculated as BP/BCD (Del Giorgio and Cole, 1998). BCD was defined as the sum of BR and BP. BR, BP, and BCD were expressed as μg C L^{-1} h^{-1}.

Ancillary Parameters

Water temperature, conductivity, pH, O_2, and turbidity were measured with an YSI® multiprobe, calibrated every 10 days. Position was recorded with a global positioning system (GPS) at the same frequency of 1 min. During LW periods, most shallow and remote lakes were visited with the aid of a small boat and a 12V version of the complete measurement setup was used. DOC concentration was measured on water that was filtered through pre-combusted GF/F filters (4 h, 550°C), stored in pre-combusted glass bottles and acidified with ultrapure H_3PO_4. DOC concentrations were measured using a Shimadzu TOC-V_{CSH} analyzer. The detection limit for carbon was 4 μg/L. POC, total nitrogen (TN, i.e., organic and inorganic nitrogen), $\delta^{13}C$ and $\delta^{15}N$ isotope ratios were measured on the same sample aliquots by EA-IRMS (Carlo-Erba NA-1500 NC Elemental Analyser on line with a Fisons Optima Isotope Ratio Mass Spectrometer). The $\delta^{13}C$ and $\delta^{15}N$ of POC values are reported in per mil (‰) relative to Pee Dee Belemnite (PDB) standard and relative to air N_2, respectively. The analytical precision (as the standard deviation of repeated internal standard measurements) for the stable isotope measurements was 0.06 and 0.13% for $\delta^{13}C$ and $\delta^{15}N$, respectively (Moreira-Turcq et al., 2013). Chlorophyll-a concentrations (μg L^{-1}) were measured on GF/F filters, stored frozen before analysis, according to the method described by Yentsch and Menzel (1963), Holm-Hansen et al. (1965), and (Marker et al., 1980), using a 10-AU Turner Fluorometer.

Data Analyses

The differences between HW and LW were tested by Mann–Whitney Rank Sum Test because the data did not meet the normality and equal variance test criteria. Potential correlations between variables were determined through Spearman's correlation analysis without log-transformation of data. All statistical calculations were performed using SigmaPlot v12.5. For all statistical tests we assumed $p < 0.05$ as a threshold level for acceptance.

Results

Mainstem and Floodplain Environmental Variability

The subsurface water temperature did not differ between mainstems and floodplain lakes, although in the LW period the temperature was slightly higher (about 2°C) than in HW (**Table 1**). The DOC concentration range was similar in mainstems and floodplain lakes in HW and LW. The POC

TABLE 1 | Location and general features of the Amazon River subsystem (mainstem and floodplain lakes).

	Mainstem			Floodplain lakes		
	Min.	**Max.**	**Mean (± *SD*)**	**Min.**	**Max.**	**Mean (± *SD*)**
HW						
Water temperature	27.9	29.9	28.6 (± 0.02)	28.1	29.7	28.85 (± 0.02)
DOC	3.6	7.6	4.3 (± 0.43)	3.6	5.1	4.04 (± 0.15)
^{13}C-POC	–27.8	–34.55	30.21 (± 0.07)	–28.1	–30	29.18 (± 0.02)
C/N	6.19	11.43	8.25 (± 0.20)	6.87	8.86	8.15 (± 0.09)
pCO_2	3650	6850	4555.5 (± 0.43)	3000	8360	5448.5 (± 37)
Turbidity	3,5	151	41.83 (± 1.35)	9.5	61.2	28.9 (± 0.66)
Conductivity	12	56	31.33 (± 0.60)	43	74	49.7 (± 0.25)
pH	4,9	6.4	5.91 (± 0.11)	6.3	6.6	6.4 (± 0.02)
Chlorophyll-a	0.5	3.0	1.7 (± 0.92)	0.9	2.5	1.82 (± 0.40)
0_2	36.1	71.8	58.9 (± 0.39)	30.9	79.9	51.6 (± 0.34)
BA	0.8	1.3	1.06 (± 0.27)	0.3	1.4	0.97 (± 0.49)
LW						
Water Temperature	30.8	31.9	31.2 (± 0.02)	29.5	32.3	30.82 (± 0.04)
DOC	1.4	6.1	4.13 (± 0.59)	3.8	5.5	4.54 (± 0.18)
^{13}C-POC	–28	–29.5	28.9 (± 0.02)	–27.1	–29.7	28.46 (± 0.04)
C/N	8.65	11	9.82 (± 0.12)	5.82	8.28	6.68 (± 0.14)
pCO_2	750	4548	3032.7 (± 0.66)	298	7900	2985 (± 1.95)
Turbidity	6.4	56	23.7 (± 1.81)	17	128	59.6 (± 0.80)
Condensation	8	59	28.3 (± 0.95)	41	78	56.2 (± 0.26)
pH	4.7	6.9	6.1 (± 0.20)	6.6	7.7	7.1 (± 0.06)
Chlorophyll-a	1.7	9.8	4.83 (± 0.90)	9.3	73.7	32.74 (± 0.78)
0_2	73	100.4	83.9 (± 0.17)	63	110	86.4 (± 0.25)
BA	0.3	1.2	0.63 (± 0.78)	0.9	2.9	1.17 (± 0.29)

Water temperature (°C); dissolved organic carbon (DOC, mg L-1); stable isotope signal of POC (^{13}C-POC, ‰); partial pressure of the CO_2 (pCO_2, μatm); turbidity (NTU); conductivity (m s^{-1}); pH; Chlorophyll-a (μg L^{-1}); Oxygen (%) and bacterial abundance (BA; 10^6 cell mL^{-1}) and correspond to the respective year of study [High water (HW) and Low water (LW) phases] at each station.

isotopic signal differed only in mainstems in HW and LW. The chlorophyll-a range was similar in mainstems in HW and LW and increased in floodplain lakes in LW. The average BA was similar between mainstems and floodplain lakes in HW and in mainstems in HW and LW and increased in floodplain lakes in LW.

BP and BR

The BP rates varied from 0.02 to 0.36 μg C L^{-1} h^{-1} (0.16 ± 0.11) in the mainstems and from 0.02 to 0.60 μg C L^{-1} h^{-1} (0.36 ± 0.25) in the floodplain lakes in HW phase. BP ranged from 0.17 to 0.48 μg C L^{-1} h^{-1} (0.33 ± 0.22) in the mainstems and from 0.54 to 3.44 μg C L^{-1} h^{-1} (1.84 ± 0.96) in the floodplain lakes in LW (**Figure 2**). The levels of BP on floodplain lakes were significantly higher ($p < 0.05$) than on mainstems in LW. No difference was found between mainstems and floodplain lakes on HW. The BR rates varied from 1.54 to 12.15 μg C L^{-1} h^{-1} (6.11 ± 4.80) in the mainstems and from 5.43 to 17.82 μg C L^{-1} h^{-1} (9.41 ± 4.30) on floodplain lakes in HW (**Figure 3**). The levels of BR in LW varied from 9.43 to 14.28 μg C L^{-1} h^{-1} (11.85 ± 3.42) on mainstems and from 7.34 to 51.5 μg C L^{-1} h^{-1} (19.70 ± 16.16) on floodplain lakes. The BR did not show any significant difference between mainstems and floodplain lakes in HW and LW ($p > 0.05$).

BCD and BGE

The BCD varied from 1.58 to 12.36 μg C L^{-1} h^{-1} (6.27 ± 4.82) in mainstems and from 5.94 to 17.84 μg C L^{-1} h^{-1} (8.88 ± 5.33) in floodplain lakes in HW (**Figure 4**). In LW, BCD varied from 9.6 to 14.76 μg C L^{-1} h^{-1} (12.18 ± 3.65) in mainstems and from 9.57 to 19.62 μg C L^{-1} h^{-1} (15.98 ± 4.57) in floodplain lakes. The BCD did not show any significant difference between mainstems and floodplain lakes in HW and LW ($p > 0.05$). The lack of correlation between the simultaneous measurements of BP and BR in both sampling conditions resulted in a wide range of calculated BGE. BGE varied from 0.01 to 0.18 (0.05 ± 0.06) on mainstems and from 0.002 to 0.07 (0.05 ± 0.03) on floodplain lakes in HW, and from 0.02 to 0.03 (0.02 ± 0.007) in mainstems and from 0.03 to 0.23 (0.12 ± 0.08) in floodplain lakes in LW (**Figure 5**). The BGE in floodplain lakes were significantly higher ($p < 0.05$) than in mainstem in LW (**Figure 4**) and mainly driven by BP (**Tables 2** and **3**). During HWs, no significant difference was observed between the mainstem and the floodplain lakes ($p > 0.05$). No correlation was found between BP and BR in either sampling periods.

Bacterial Metabolism Correlation with Environmental Factors

Indexes of OM quality (chlorophyll-a, $\delta^{15}N$, and C/N ratios) were the strongest seasonal statistical predictors of bacterial carbon metabolism in the Amazonian subsurface waters. BP, BR, BCD, and BGE were not correlated to DOC or C^{13} (**Tables 2** and **3**). In contrast, the POC OM stoichiometry (C/N) showed a significant negative correlation to BGE ($r = -0.54$, $p < 0.05$) in HW. Besides, there was a strong tendency of increasing BA and BCD

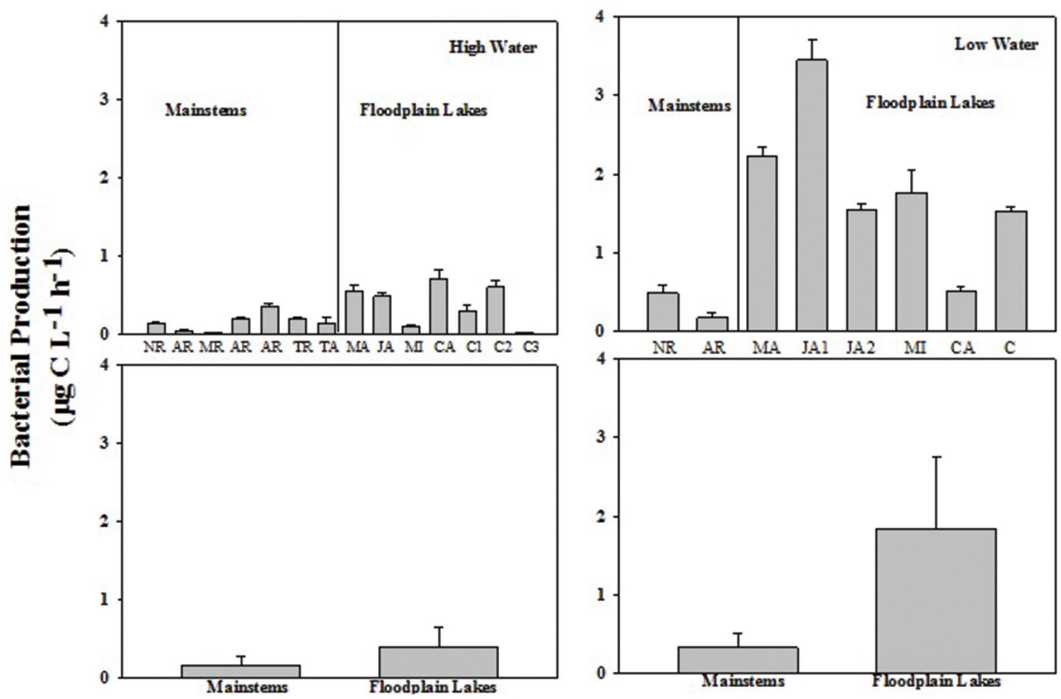

FIGURE 2 | Bacterial production (BP; μg C L^{-1} h^{-1}) by H^3-Leu incorporation across the Amazon River system in high water (HW; left upper panel) and low water (LW; right upper panel). CA, Cabaliana; JA, Janauacá; NR, Negro River; AR, Amazonas River; MR, Madeira River; MI, Miratuba; C, Curuai; TA, Tapajós River. Average and standard deviation for the mainstems and floodplain lakes in HW (left bottom panel) and LW (right bottom panel).

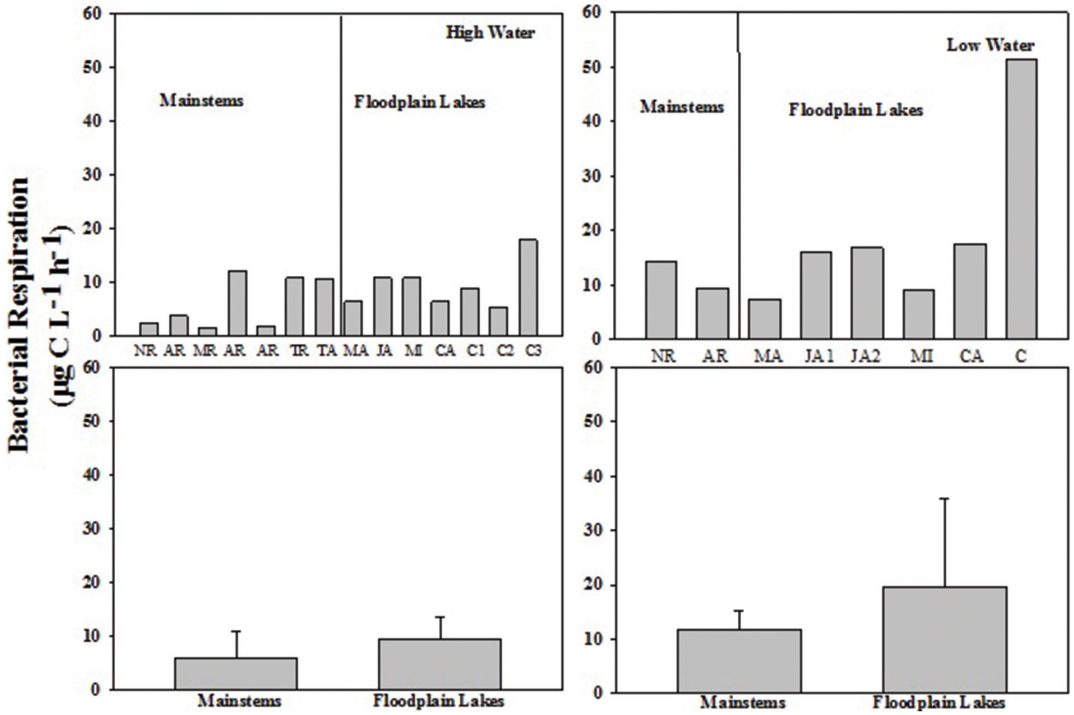

FIGURE 3 | Bacterial respiration (BR; μg C L^{-1} h^{-1}) across the Amazon River system in HW (left upper panel) and LW (right upper panel). CA, Cabaliana; JA, Janauacá; NR, Negro River; RAR, Amazonas River; MR, Madeira River; MI, Miratuba; C, Curuai; TA, Tapajós River. Average and standard deviation for the mainstems and floodplain lakes in HW (left bottom panel) and LW (right bottom panel).

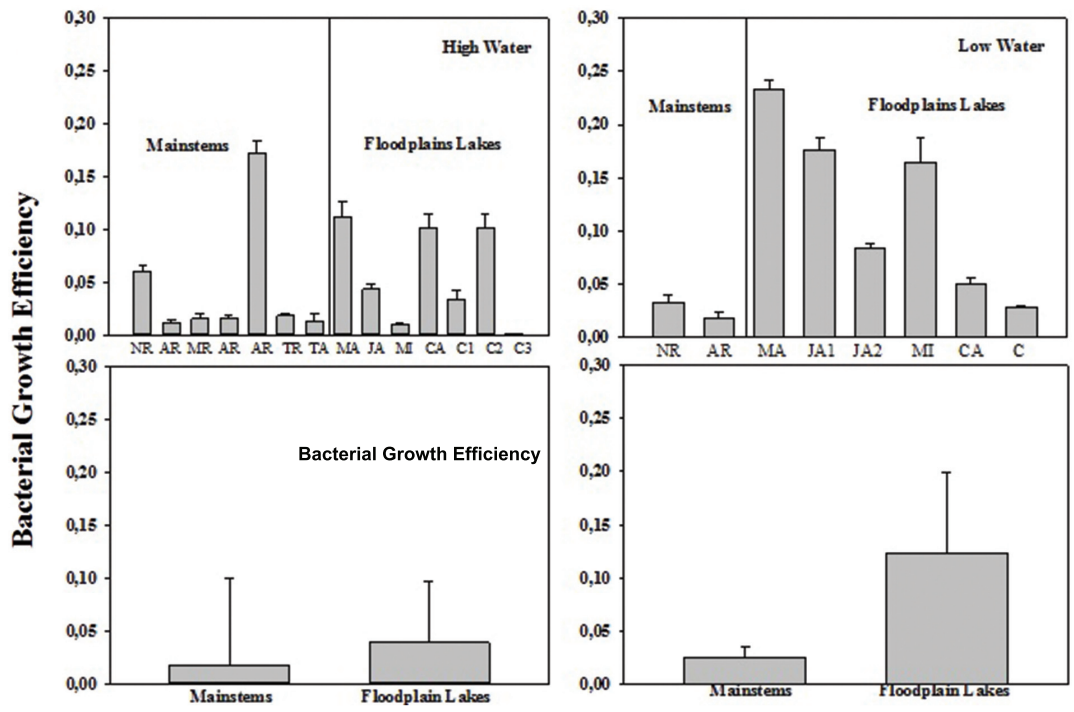

FIGURE 4 | Bacterial growth efficiency (BGE) across the Amazon River system in HW (left upper panel) and LW (right upper panel). CA, Cabaliana; JA, Janauacá; NR, Negro River; AR, Amazonas River; MR, Madeira River; MI, Miratuba; C, Curuai; TA, Tapajós River (upper panel). Average and standard deviation for the mainstems and floodplain lakes in HW (left bottom panel) and LW (right bottom panel).

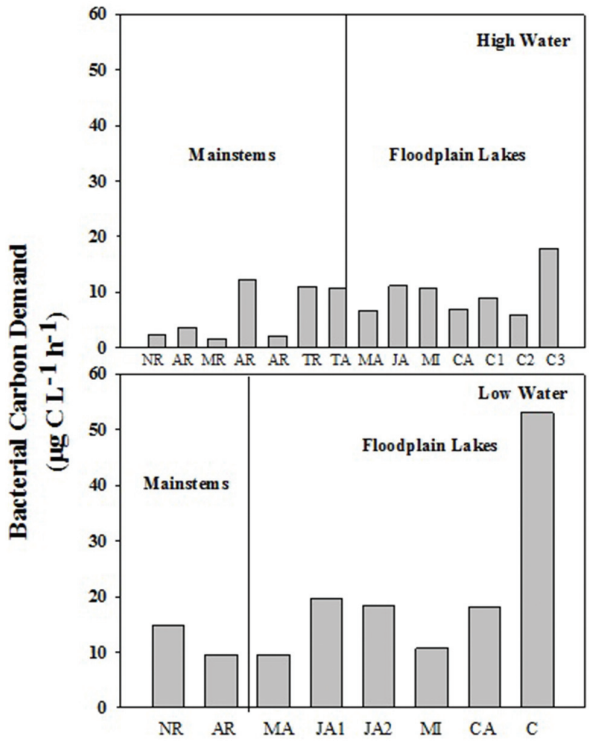

FIGURE 5 | Bacterial carbon demand (BCD) across the Amazon River system in HW (left upper panel) and LW (right upper panel). CA, Cabaliana; JA, Janauacá; NR, Negro River; AR, Amazonas River; MR, Madeira River; MI, Miratuba; C, Curuai; TA, Tapajós River (upper panel). Average and standard deviation for the mainstems and floodplain lakes in HW (left bottom panel) and LW (right bottom panel).

with chlorophyll-a concentration in LW ($r = 0.93$, $p < 0.0001$; $r = 0.68$, $p < 0.05$). The δ^{15}N of POC presented strong negative correlation to BP and BGE (r = -0.77 and –0.75, $p < 0.05$) in LW.

Discussion

The assessment of bacterial metabolic rates (BR, BP, BCD, and BGE) from Amazonian freshwater ecosystems is poorly understood. Most of studies considered only one or few freshwater ecosystems (Farjalla et al., 2002, 2006; Castillo et al., 2003; Amado et al., 2006). A study conducted by Benner et al. (1995) was the most comprehensive study considering samples taken from mainstems and tributaries. It showed that bacterial metabolism, represented by BP and BR, presented minimal spatial variability in Amazonian tributaries and mainstems but strong seasonal patterns of variability. Rivers are very dynamic and potentially subject to great spatial heterogeneity, making the identification of regulatory factors on bacterial community highly complex. Such heterogeneity suggests that a dynamic and variable microbial metabolism might be expected in rivers (Del Giorgio et al., 2011). Recently, Farjalla (2014) showed that BP increases in areas of mixing zones of Amazonian rivers. The Amazonian mainstems and floodplains studied in the present study reflect the wide variability of the world's largest river system (**Table 1**).

The consumption of OM by bacteria is driven by OM amount and composition (Kritzberg et al., 2005; Jansson et al., 2006; Vidal et al., 2011), therefore, the range of metabolic versatility of heterotrophic bacterial metabolism has often been assumed to occur as the response to large shifts in resources across major ecosystems or along extremely broad environmental gradients (Del Giorgio et al., 2011).

In Amazonian freshwater ecosystems, the carbon from the forest goes into the floodplain lakes and meets the in-lake OM production (i.e., phytoplankton and macrophytes), which is part processed before reaching the mainstem channel (Moreira-Turcq et al., 2003). Besides, the percentage of forest occupying the drainage basin in the study area showed a well-defined biogeographic gradient from flooded forests that are dominant upstream to open lakes and that are dominant downstream with temporal scale across the floodplain lakes (Abril et al., 2014). Consequently, the forest carbon litterfall entering the

TABLE 2 | Spearman correlation coeficients between measured variables in Amazonian systems to HW period; *$p < 0.05$, **$p < 0.01$, ***$p < 0.0001$.

	BR	BP	BCD	BGE	CHLOR-A	TURB	BA	DOC	C13	C/N	δ^{15}N
BR	–										
BP	ns	–									
BCD	0.98***	ns	–								
BGE	ns	0.85***	ns	–							
CHLOR-A	ns	ns	ns	ns	–						
TURB	ns	ns	ns	ns	–0.68**	–					
BA	0.50*	ns	ns	ns	ns	ns	–				
DOC	ns	ns	ns	ns	ns	ns	ns	–			
C13	ns	ns	ns	ns	–0.53**	0.81***	ns	ns	–		
C/N	ns	ns	ns	0.54**	ns	–0.64**	ns	ns	ns	–	
δ^{15}N	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	–

BR, bacterial respiration; BP, bacterial production; BCD, bacterial carbon demand; BGE, bacterial growth efficiency; CHLOR-A, chlorophyll-a; TURB, turbidity; BA, bacterial abundance; DOC, dissolved organic carbon; POC, stable isotopic of POC; C/N, carbon and nitrogen ratio; δ^{15}N, stable isotopic of nitrogen.

TABLE 3 | Spearman correlation coeficients between measured variables in Amazonian systems to LW period; *$p < 0.05$, **$p < 0.01$, ***$p < 0.0001$.

	BR	BP	BCD	BGE	CHLOR-A	TURB	BA	DOC	C13	C/N	$\delta^{15}N$
BR	–										
BP	ns	–									
BCD	0.88**	ns	–								
BGE	ns	0.93**	ns	–							
CHLOR-A	ns	ns	0.68**	ns	–						
TURB	ns	ns	ns	ns	ns	–					
BA	ns	ns	ns	ns	0.93***	ns	–				
DOC	ns	ns	ns	ns	ns	ns	ns	–			
C13	ns	ns	ns	ns	ns	ns	ns	ns	–		
C/N	ns	ns	ns	ns	–0.89**	ns	ns	0.77**	ns	–	
$\delta^{15}N$	ns	–0.77**	ns	–0.75**	ns	ns	ns	ns	ns	ns	–

BR, bacterial respiration; BP, bacterial production; BCD, bacterial carbon demand; BGE, bacterial growth efficiency; CHLOR-A, chlorophyll-a; TURB, turbidity; BA, bacterial abundance; DOC, dissolved organic carbon; C13, stable isotopic of POC; C/N, carbon and nitrogen ratio; $\delta^{15}N$, stable isotopic of nitrogen.

aquatic ecosystems may be also variable. The spatial variability of organic carbon across the Amazon floodplain basin where OM is spatially and seasonally structured may facilitates the evolution of a broad repertoire of functional attributes of bacteria to their environment (Comte and del Giorgio, 2010). In addition, floodplains represent a hotspot of primary production during LW and, consequently, a source of presumably fresh suspended particulate organic matter (SPOM) for much of the system, particularly from macrophytes. Seasonal water movements are a way to redistribute this fresh SPOM in the hydrological network via the transfer to the river main channel (Mortillaro et al., 2012).

Bacterial metabolism in Amazonian aquatic ecosystems is inversely related to the water level. The phytoplankton and macrophytes are the main forms of organic carbon at LW phase, and the allochthonous input of OM is a significant energy source to bacterial activity during HW level (Amado et al., 2006). It is also known that the synergy between low- and high-molecular-weight carbon sources and consequences for the bacterioplankton has recently been pointed out (Fonte et al., 2013). For instance, recent findings suggest that lignin and other terrestrially derived macromolecules contribute significantly to carbon dioxide outgassing from inland waters thorough microbial degradation in Amazon River systems (Ward et al., 2013). In the present study, it was possible to notice a clear difference on bacterial metabolism through BP and BGE between HW and LW phases and also between mainstems and floodplain lakes in LW (**Figures 2** and **4**). The range of bacterial metabolic processes found in the Amazonian freshwater ecosystems showed to be in part driven by the diversity of OM available across the Amazonian floodplain basin. Our results showed that indexes of OM quality (chlorophyll-a, N stable isotopes, and C/N ratios) were the strongest seasonal drivers of bacterial carbon metabolism. The effects of OM fate (origin and decomposition stage) on bacterial carbon consumption in the present study were suggested by the negative correlation of BGE and BP with C:N ratio in HW and by the strong negative coupling with $\delta^{15}N$ in LW. The increasing in stoichiometry of carbon may indicate carbon of terrestrial origin and N limitation, characteristic of periods of HW when the carbon from the forest is dominating and the nutrients are more diluted in the water. The higher $\delta^{15}N$ values attest for enhanced nitrogen recycling in the system (Ometto et al., 2006) in LW when there is an increasing in carbon from phytoplankton origin and nutrients availability. Indeed, positive relationships were evidenced between phytoplankton (expressed as chlorophyll-a concentration) and heterotrophic bacteria (expressed as abundance) and BCD in LW. The DOC quantity did not account for variations in BP and BR in the present study on both sampling times. Such lack of relationship between DOC and bacterial metabolism parameters may indicate that not the carbon concentration but the carbon quality is accounting for such variations. Recent studies in boreal and Amazonian freshwater ecosystems have shown that C alone is not enough to regulate bacterial metabolism; but instead, the stoichiometry of carbon must be considered. (Farjalla et al., 2002; Castillo et al., 2003; Vidal et al., 2011).

The BP rates were the responsible for most of BGE variability (Roland and Cole, 1999; Kritzberg et al., 2005) but not enough to increase BGE according to the present study. The higher respiration rates in relation to production showed that most of the BCD is converted into CO_2, which resulted in low BGE values in both sampling phases, (0.03 in HW and 0.06 in LW). These higher metabolic rates (e.g., BCD) and lower BGE values in tropical inland water ecosystems are lower than those observed in temperate ecosystems which is related to temperature when inserted in a global scenario, but also to intrinsic ecosystems aspects (Amado et al., 2013) like OM quality, as indicated in this study. Moreover, regarding BR rates, it is important to keep in mind that in the unfiltered water used during respiration incubations a range of protists and metazoans must have contributed directly to heterotrophic respiration resulting in BR and BCD overestimations and consequently BGE underestimations. In addition, trophic interactions may also have affected respiration rates as suggested by Biddanda et al. (2001), with bacteria accounting less with total respiration with increasing in system productivity. However, the respiration measurements between high and LW phase did not show any significant difference in the present study, which may indicate that increasing in phytoplankton production showed through

chlorophyll-a concentration in LW, did not resulted in changes in respiration rates. This was reinforced by the no correlation found between BR and Chlorophyll-a (**Tables 2** and **3**). Thus, we believe such possible bias of the measurements with no filtration would not affect the seasonal pattern found in the present study.

Concluding Remarks

Our results showed that the hydrologic connectivity, through carbon quality (chlorophyll-a, N stable isotopes, and C/N ratios), differentially drives bacterial carbon allocation across freshwater Amazonian ecosystems. In general, it showed higher BP in LW phase than in HW phase and higher BR rates than BP in both water phases. In addition, BR rates did not show any significant difference between HW and LW ($p > 0.05$). The same was registered to BCD. Average BGE was low in both seasons (0.03 and 0.06, HW and LW, respectively), suggesting that DOC was mostly allocated to catabolic bacterial cell processes besides BP increasing in LW. Consequently, changes in BP and BGE between the deep rivers and the adjacent shallow lakes showed the same pattern as BP. Multiple correlation analyses revealed that indexes of OM quality (chlorophyll-a, DOC and DOC, N stable isotopes, and C/N ratios) were the strongest seasonal drivers of bacterial carbon metabolism.

Our work indicated that: (i) the bacterial metabolism was mostly driven by respiration in Amazonian aquatic ecosystems resulting in low BGE in either HW or LW phase; (ii) the hydrological pulse regulated the bacterial heterotrophic metabolism between Amazonian mainstems and floodplain lakes, mostly driven by OM quality.

From the results presented in this study we could increase the discussion about bacterial carbon metabolism in Amazon floodplain ecosystems and we tried to fill the gap about *in situ* detailed knowledge of local factors regulating microbial metabolism.

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Viruses and bacteria in floodplain lakes along a major Amazon tributary respond to distance to the Amazon River

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In response to the massive volume of water along the Amazon River, the Amazon tributaries have their water backed up by 100s of kilometers upstream their mouth. This backwater effect is part of the complex hydrodynamics of Amazonian surface waters, which in turn drives the variation in concentrations of organic matter and nutrients, and also regulates planktonic communities such as viruses and bacteria. Viruses and bacteria are commonly tightly coupled to each other, and their ecological role in aquatic food webs has been increasingly recognized. Here, we surveyed viral and bacterial abundances (BAs) in 26 floodplain lakes along the Trombetas River, the largest clear-water tributary of the Amazon River's north margin. We correlated viral and BAs with temperature, pH, dissolved inorganic carbon, dissolved organic carbon (DOC), phosphorus, nitrogen, turbidity, water transparency, partial pressure of carbon dioxide (pCO_2), phytoplankton abundance, and distance from the lake mouth until the confluence of the Trombetas with the Amazon River. We hypothesized that both bacterial and viral abundances (VAs) would change along a latitudinal gradient, as the backwater effect becomes more intense with increased proximity to the Amazon River; different flood duration and intensity among lakes and waters with contrasting sources would cause spatial variation. Our measurements were performed during the low water period, when floodplain lakes are in their most lake-like conditions. Viral and BAs, DOC, pCO_2, and water transparency increased as distance to the Amazon River increased. Most viruses were bacteriophages, as viruses were strongly linked to bacteria, but not to phytoplankton. We suggest that BAs increase in response to DOC quantity and possibly quality, consequently leading to increased VAs. Our results highlight that hydrodynamics plays a key role in the regulation of planktonic viral and bacterial communities in Amazonian floodplain lakes.

Keywords: plankton, viruses, bacteria, Amazonian freshwater ecosystems, floodplain lakes, dissolved organic carbon, backwater effect

INTRODUCTION

Of the 10 largest tropical rivers in terms of discharge on Earth, four are in the Amazon basin, being the Amazon River itself the largest one (Latrubesse et al., 2005). In addition to a complexly arranged fluvial network, the Amazon floodplain is composed of extensive wetlands and about 9,000 floodplain lakes that cover nearly 70,000 km^2 (McClain, 2001), which are seasonally flooded by bordering rivers. The flood pulse is one of the most marked characteristics of Amazonian aquatic ecosystems (Junk et al., 1989), and it defines four distinct flood seasons: rising, high, falling, and low waters. Because peak discharges of the northern and southern tributaries of the Amazon River have different timings, the discharges of the Amazon River vary by a factor of 3, whereas its tributaries vary their discharges by a factor of 10 (Meade et al., 1991). As a result, even the largest tributaries have their water backed up by 100s of kilometers upstream of the mouth, with falling river stages being as much as 3 m higher than rising stages at a same discharge – the so-called backwater effect. The intricate hydrodynamics of Amazonian aquatic systems regulates the concentrations of organic matter and nutrients in Amazonian lakes (Forsberg et al., 1988), as well as a variety of aquatic communities, such as the planktonic ones, zoo-, phyto-, bacterio-, and virioplankton (Bozelli, 1994; Anesio et al., 1997; Huszar and Reynolds, 1997; Barros et al., 2010).

Viruses are ubiquitous in aquatic ecosystems, and increasing attention has been paid on their role in aquatic food webs since it was discovered that they are the most abundant aquatic components (Bergh et al., 1989). Viruses are not only abundant, but they also play an important biogeochemical function by releasing dissolved organic matter (DOM) and nutrients through host cell lysis (Fuhrman, 1999). In addition, viral activity can affect ecosystem respiration, primary production, bacterial and algal diversity, species distribution, and genetic transfer between microorganisms (Maranger and Bird, 1995; Fuhrman, 1999; Suttle, 2005). Likewise, bacteria are crucial players in aquatic ecosystems, processing large amounts of both autochthonously and allochthonously derived

organic carbon (Cotner and Biddanda, 2002). For this reason, bacteria and viruses are recognized as key alternative routes of organic matter and nutrient transfer to metazoan trophic levels, which was first introduced through the microbial loop concept (Pomeroy, 1974; Azam et al., 1983), and then by the viral loop (Fuhrman, 1999).

While temperature, nutrient, organic carbon, flood pulses, and light exposure are key bottom–up factors controlling bacterial dynamics in aquatic systems (Farjalla et al., 2002, 2006; Amado et al., 2013), the action of viruses is known to be an important top–down mechanism of bacterial regulation in aquatic ecosystems (Fuhrman and Noble, 1995). This viral control on bacteria is summarized in the "killing the winner" hypothesis: abundant prokaryotic types are exposed to strong viral pressure, because viral infection rate depends, among other things, on the abundance and type of prokaryotic host cells (Winter et al., 2010). Viruses impact directly on bacterial populations and indirectly on bacterial diversity by decreasing the density of dominant bacterial species (Maranger and Bird, 1995). Moreover, viruses can account for up to 40% of bacterial mortality in surface waters, which can be similar in magnitude to the effect caused by protistan grazing (Fuhrman and Noble, 1995).

Although there is a growing body of research on aquatic viral ecology, little is known about viral function in tropical environments (Peduzzi and Schiemer, 2004; Bettarel et al., 2006; Araújo and Godinho, 2009). This is particularly true for the Amazon, where to our knowledge only one study has investigated aquatic viruses to date (Barros et al., 2010). In Amazonian clear-water floodplain lakes, bacterial and viral abundances (VAs) are tightly coupled, and both of them are linked to the flood pulse and the concentration of suspended particles (Barros et al., 2010). This study was a key initial step toward a comprehensive understanding on the role of viruses in Amazonian aquatic ecosystems, but the variation of bacteria and virus between contrasting floodplain lakes is still unknown. Spatially explicit reports of virus–bacterium relationships have been documented for boreal, temperate and tropical African lakes (Maranger and Bird, 1995; Anesio et al., 2004; Bettarel et al., 2006), but no such study exists for the Neotropical region.

Here, we made an extensive survey of several floodplain lakes distributed along the margins of the Trombetas River, the second largest northern tributary of the Amazon River. Throughout the sampling stretch, the Trombetas River is permanently subject to a backwater effect caused by the Amazon River (Veiga Pires et al., 1988). The backwater effect becomes progressively more pronounced with increasing proximity to the confluence with the Amazon River (Meade et al., 1991). As a result, lower basin lakes are frequently flooded by the turbid waters of the Amazon River, whereas upper basin lakes are strictly flooded by the clear waters of the Trombetas River. In addition, the duration of floods may be longer in the lower basin. Thus, we hypothesized that both bacterial and VAs would change according to the distance to the Amazon River. This would occur because of different flood duration and intensity among lakes and waters with different sources. Contrasting flooding characteristics would eventually influence planktonic communities during low water, when lakes are more disconnected and dissimilar (Thomaz et al., 2007).

MATERIALS AND METHODS

SITE DESCRIPTION

The Trombetas River originates in the Guiana shield and is the largest northern clear-water tributary of the Amazon River, with a mean discharge of 2,555 m^3 s^{-1} (Moreira-Turcq et al., 2003). The total area of the Trombetas River basin is 120,000 km^2, 6% of which are covered by floodplain forests and lakes (Melack and Hess, 2010). These floodplain lakes exhibit large oscillation in water level over the year, with mean depths being as low as 1 m during low water periods and as high as 10 m during high water periods (Roland and Esteves, 1998).

The backwater effect of the Amazon River on its tributaries is a pattern well described in literature, and it gets gradually more pronounced following an upstream–downstream gradient (Meade et al., 1991). The Trombetas River, for instance, has been reported to be permanently subject to a backwater effect until Cachoeira Porteira, about 210 km upstream the mouth (Veiga Pires et al., 1988). Discharge and stage measurements from a gaging station 20 km upriver of our upper-most lake (Lake Macaco) confirm this (**Figure 1A**). At this gaging station, the river level is sometimes a few centimeters higher during falling stages than during rising stages at the same discharge, which is due to a time lag between the peak discharges of the Trombetas and Amazon rivers (**Figure 1B**).

We selected the peak of a low water period to perform our measurements, in order to sample the floodplain lakes in their most lake-like conditions. During low water, river-floodplain systems are more heterogeneous with respect to physical, chemical and biological variables, since the connectivity with the adjoining river is weakest (Thomaz et al., 2007). We sampled 26 floodplain lakes adjacent to the Trombetas River, following a north (upstream) to south (downstream) gradient (**Figure 2**). The northern-most lake is about 200-km distant from the confluence of the Trombetas with the Amazon River. We also sampled one site in the Trombetas River, located halfway from the confluence with the Amazon River until the northern-most lake. Satellite imagery from the free software Google Earth was used to measure the distance traveled through the Trombetas River main channel from the lake mouths until the confluence with the Amazon River.

WATER SAMPLING AND ANALYSIS

Water samples were taken from the upper 0.5 m at the center of each of the 26 lakes during a cruise along the Trombetas River between December 1st and 8th 2007 (low water period). In the field, subsamples were filtered through 0.7 μm glass microfiber filters (GF/F; Whatman) for further analysis of dissolved compounds. All water samples sent for laboratory analysis were kept refrigerated at ∼4°C and analyzed within 15 days after sampling. Dissolved oxygen (DO) concentrations and water temperature were measured at the subsurface with a portable oximeter (YSI-95) and a thermometer, respectively. Turbidity was measured using a turbidimeter La Motte 2008 Turbidity Meter, whereas samples for pH were analyzed using a pH meter (Micronal B474). The water transparency was measured using the Secchi disk. Preacidified (pH < 2) water samples for total nitrogen (TN) and total phosphorus (TP) were analyzed within 15 days using standard

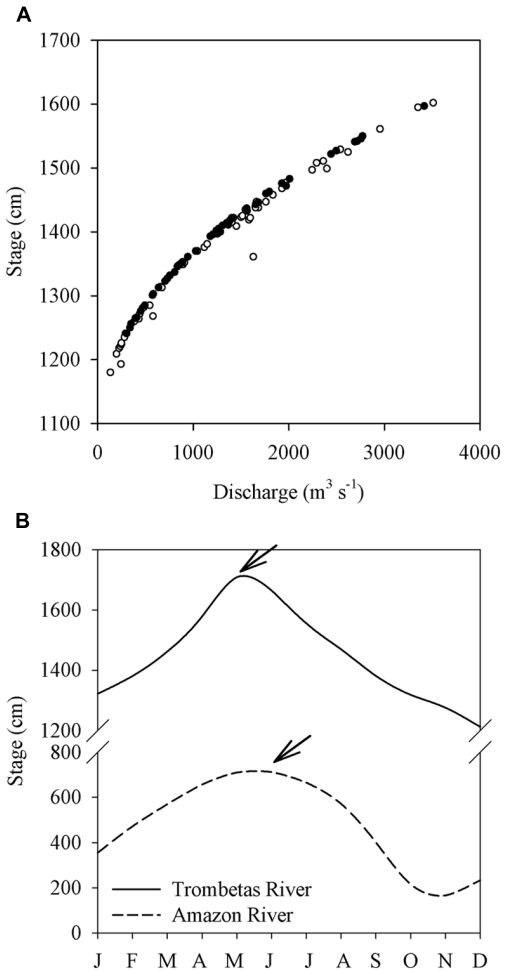

FIGURE 1 | (A) Stage-discharge relations in the Trombetas River at the Caramujo gaging station (1°3′54″S, 57°3′41″W) during the rising (open circles) and falling (solid circles) stages. **(B)** Mean stages of the Trombetas River at Caramujo between 1996 and 2013 (solid line) and the Amazon River at Óbidos (1° 55′9″S, 55°30′47″W) between 1968 and 2013 (dashed line); the X-axis indicates the months of the year. The arrows indicate the peak discharges of both rivers. Data were obtained downstream the confluence of both rivers. Discharges and stages data were obtained at the website of the Brazilian National Water Agency (http://hidroweb.ana.gov.br).

spectrophotometric techniques (Wetzel and Likens, 2000). Pre filtered samples of DOC and dissolved inorganic carbon (DIC) were analyzed on a Tekmar–Dohrmann Total Carbon Analyzer (model Phoenix 8000). DIC was analyzed following persulfate digestion, and pre-acidified (pH < 2) DOC samples were analyzed following high temperature oxidation with a UV lamp. The partial pressure of carbon dioxide (pCO_2) was calculated from DIC, pH, and water temperature according to Stumm and Morgan (1996).

VIRAL, BACTERIAL, AND PHYTOPLANKTON ABUNDANCES

Here, we consider bacteria as a generic term describing prokaryotic organisms (i.e., organisms lacking a nucleus, comprising the domains *Bacteria* and *Archaea*), since the method that we used (epifluorescence microscopy using SYBR stains) does not distinguish bacteria from Archaea. Additionally, free DNA and non-viral background fractions occasionally interferes the counting of viruses through epifluorescence miscroscopy using SYBR stains (Pollard, 2012). Samples for viral and bacterial abundances (BAs) were taken from the center of the lakes in triplicates. Immediately after sampling, the samples were fixed with glutaraldehyde solution (2% final concentration; pre-filtered on a 0.02 μM-pore-size filter). In the laboratory, on the same day of sampling, bacteria and viruses were stained with SYBR green (Molecular Probes, Eugene, OR, USA; Noble and Fuhrman, 1998), which is recommendable as immediate preparation of slides avoids viral decay that commonly occurs during storage of water samples. Two-milliliter samples were filtered on a 0.02 μm-pore-size Anodisc membrane filter (Whatman aluminum oxide) with a 0.45 μm-pore-size backing membrane filter. The filter was laid, sample side up, on a drop of SYBR green I solution (1:400) for 15 min in the dark. After being dried, the filter was placed on a glass slide and mounted with an antifade mounting solution (Patel et al., 2007), and kept frozen at −20°C until analysis within 15 days after sampling. For each filter, more than 200 viruses and 100 bacteria were directly counted in 20 fields. The fields were selected randomly. Analyses were performed under ×1,000 magnification with an epifluorescence microscope (Provis AX-70; Olympus, Melville, NY, USA) using light filters for blue excitation (450–490 nm wide bandpass).

Samples for phytoplankton enumeration were fixed in the field with acidic Lugol's solution at a final concentration of 1:100 (Soares et al., 2011). The samples were stored in dark glass-ware protected from light and analyzed within 15 days after sampling. In the laboratory phytoplankton abundances (PAs) were determined in an inverted microscope (Olympus IX 71) following the Utermöhl (1958) sedimentation method. At least one 100 specimens of the dominant species were enumerated (Lund et al., 1958) in random fields (Uhelinger, 1964).

STATISTICAL ANALYSIS

We used linear regressions to assess possible relationships between distance to the Amazon River and the limnological parameters and planktonic communities considered here. A linear regression was also used to verify the coupling between bacteria and viruses. We utilized $p < 0.05$ as a threshold level for the acceptance. All analyses were made on SigmaPlot version 11.0.

RESULTS

The distance from the lake mouths until the Amazon River varied between 31 km (Sapucuá Lake) and 192 km (Macaco Lake; **Table 1**). Water temperature was elevated and showed minor variation between lakes. Oxygen concentrations averaged 5.9 mg L^{-1} (range: 4.3–6.8 mg L^{-1}), and were about 80% of the oxygen saturation considering the water temperature and atmospheric pressure. The apparent oxygen deficit in the water was corroborated by CO_2 supersaturation (average pCO_2 = 2916 μatm; range 320–5856 μatm). Only one lake was below atmospheric equilibrium, here considered as 390 μatm (**Table 1**). The Trombetas River, sampled 90 km upstream the mouth, showed oxygen concentrations and pCO_2 similar to the average of the lakes, but it was less enriched in DOC, TN, and TP than most lakes. Turbidity was mostly below the detection limit in the upper basin lakes, as expected for clear-water systems. This is confirmed by the fact that the Secchi disk

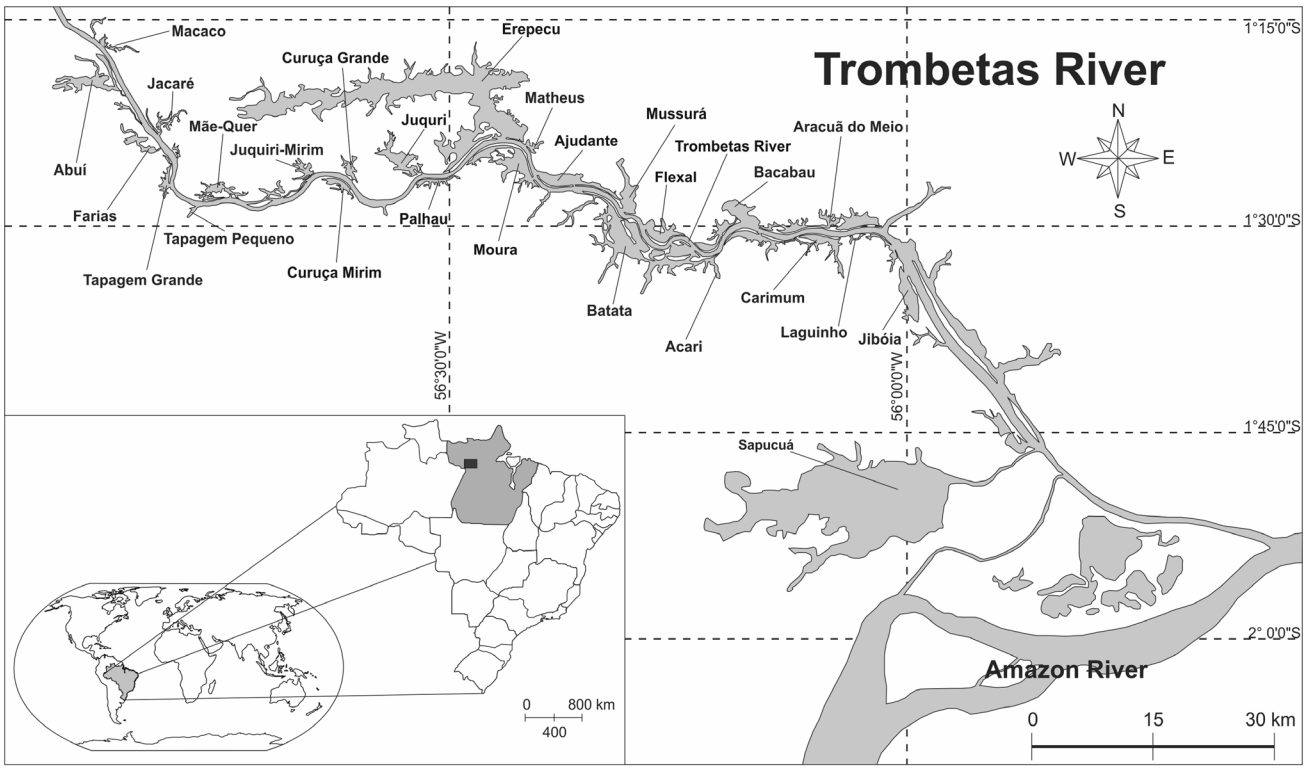

FIGURE 2 | Map of the study site, emphasizing the Trombetas River and its associated floodplain lakes.

transparency (SDT) had a positive relationship with distance to the Amazon River (**Table 2**). The distance to the Amazon River was also significantly and positively correlated to DOC concentrations and pCO_2 (**Table 2**).

Viral abundances were higher than BAs irrespective of the sampling site (**Figure 3A**), averaging 1.9×10^7 (± 0.7 SD) VLP mL^{-1} (range: 0.4–3.0 $\times 10^7$ VLP mL^{-1}); the VA of the Trombetas River (1.8×10^7 VLP mL^{-1}) was close to the average of the lakes. BAs varied by an order of magnitude, ranging from 0.6×10^6 cells mL^{-1} to 8.3×10^6 cells mL^{-1} (**Figure 3B**); the BA of the Trombetas River (1.9×10^6 cells mL^1) was in the lower range of the values found for the lakes. If on the one hand viral and BAs varied substantially between lakes, on the other hand, the virus-to-bacterium ratio (VBR) was less variable (**Figure 3C**), averaging 4.8 (± 1.46 SD; range: 2.2–9.1), and it was higher in the Trombetas River (9.8) than in all lakes. The PAs varied considerably (range: 318–22300 ind. mL^{-1}; **Figure 3D**), and the Trombetas River exhibited a very low PA (190 ind. mL^{-1}).

A simple linear regression analysis showed that BA has a positive relationship with VA ($r^2 = 0.69$; $p < 0.05$; **Figure 4**). On the other hand, no significant relationships were found when PAs were regressed against bacterial and VAs (**Table 2**). Both bacterial and VAs had a weak positive, but significant relationship with the distance from the lake mouths until the confluence of the Trombetas and Amazon rivers ($r^2 = 0.24$; $p < 0.05$ and $r^2 = 0.17$; $p < 0.05$, respectively; **Table 2**). DOC was significantly correlated to BAs ($r^2 = 0.16$; $p < 0.05$), but not to VAs (**Table 2**). Both bacterial and VAs showed positive and significant relationship with pCO2 ($r^2 = 0.23$; $p < 0.05$ and $r^2 = 0.20$; $p < 0.05$, respectively; **Table 2**). The VBR was not significantly related to any variable (**Table 2**).

DISCUSSION

HYDRODYNAMICS AFFECTING WATER CHEMISTRY, BACTERIA, AND VIRUSES

The results of our analysis indicated that viral and BAs, DOC, pCO_2, and water transparency of floodplain lakes adjacent to the Trombetas River increase as distance from the lake mouth until the Amazon River increases. We attribute this latitudinal gradient to a decreased intensity of the backwater effect of the Amazon River as one moves upriver. The intensity of the backwater effect in floodplain lakes bordering Amazon tributaries becomes progressively more pronounced with increasing proximity to the Amazon River (Meade et al., 1991). In other words, the backwater effect keeps the water level high during falling stages in lower basin lakes, which tend to present a higher proportion of river water with respect to local water at low water periods. The presence of riverine flood waters dilutes DOC, cells, and virus-like particles (Anesio et al., 1997; Farjalla et al., 2006; Barros et al., 2010), and increases allochthonous to autochthonous DOC ratio, as more terrestrially derived recalcitrant DOC enters the lakes (Farjalla et al., 2006). In addition, proximity to the Amazon River makes lakes subject to turbid flood waters, which is corroborated by the decreased SDT and increased turbidity in the lower basin lakes

Table 1 | Limnological characteristics of the 26 lakes and the Trombetas River.

Lakes	Coordinates	Distance to Amazon River (km)	Depht (m)	Temp (°C)	Secchi (m)	DO (mg L^{-1})	DOC (mg L^{-1})	DIC (mg L^{-1})	TP (μg L^{-1})	TN (μg L^{-1})	Turbitidy (NTU)	pH	pCO_2 (μatm)
Sapucuá	S 1°47′ 25″, W 55° 59′ 38″	31	2.2	28.8	0.4	5.5	4.6	2.7	87	577	31.8	7.7	320
Jibóia	S 1°38′ 05″, W 55° 59′ 30″	54	1.7	30.5	1.3	6.7	3.4	1.0	26	240	1.4	6.3	1575
Laguinho	S 1°31′ 43″, W 56° 04′ 07″	68	1.4	33.8	1.0	6.5	4.8	1.0	24	461	ND	5.0	2927
Aracuã do Meio	S 1° 30′ 48″, W 56° 07′ 37″	70	1.9	30.1	0.8	6.7	3.8	1.3	30	329	6.8	6.1	2323
Carimum	S 1° 31′ 37″, W 56° 06′ 06″	71	1.9	32.4	1.1	6.3	3.3	0.8	34	539	0.5	5.1	2312
Bacabau	S 1° 29′ 34″, W 56° 11′ 06″	82	1.9	30.5	1.2	5.7	4.6	1.5	28	363	0.7	5.5	3834
Acari	S 1° 33′ 06″, W 56° 13′ 11″	88	3.7	30.9	2.1	5.5	3.4	1.0	12	283	ND	5.1	2790
Flexal	S 1° 30′ 49″, W 56° 16′ 10″	95	2.4	30.7	0.9	5.4	4.3	1.1	31	503	4.6	5.3	2903
Batata	S 1° 31′ 56″, W 56° 18′ 31″	95	3.0	29.0	1.2	–	5.4	1.7	–	–	10.0	6.3	2430
Mussurá	S 1° 28′ 57″, W 56° 18′ 17″	100	2.4	30.1	0.5	5.5	3.9	1.8	27	469	18.6	6.4	2298
Ajudante	S 1° 27′ 21″, W 56° 22′ 45″	109	1.9	30.6	1.2	6.8	3.5	1.3	23	452	ND	5.9	2778
Moura	S 1° 25′ 36″, W 56° 25′ 4″	113	5.0	30.5	2.1	5.8	3.7	0.7	29	425	ND	5.4	1862
Matheus	S 1° 24′ 49″, W 56° 24′ 37″	115	1.6	32.6	1.6	6.5	5.6	0.8	30	568	ND	5.2	2373
Erepecu	S 1° 20′ 26″, W 56° 28′ 06″	119	4.2	31.5	1.3	6.1	4.3	1.0	27	426	ND	5.9	2249
Palhau	S 1° 26′ 46″, W 56° 31′ 14″	130	2.3	30.1	1.3	4.7	4.1	–	39	352	2.2	5.5	–
Juquiri-Grande	S 1° 25′ 02″, W 56° 34′ 06″	133	3.4	30.5	1.8	6.0	4.1	1.0	29	279	ND	5.4	2525
Curuçá-Mirim	S 1° 25′ 15″, W 56° 37′ 17″	145	2.9	31.7	1.9	5.9	4.2	1.1	37	504	ND	5.2	2943
Curuçá-Grande	S 1° 26′ 21″, W 56° 38′ 04″	145	2.1	32.4	1.7	6.0	5.5	1.1	24	510	ND	5.5	2908
Juquiri-Mirim	S 1° 24′ 55″, W 56° 40′ 13″	150	2.3	31.2	1.6	6.1	3.5	1.4	14	359	ND	5.2	3853
Mãe-Quer	S 1° 25′ 55″, W 56° 46′ 58″	162	2.8	31.2	2.0	5.6	4.5	1.4	50	608	ND	5.5	3520
Tapagem Pequeno	S 1° 25′ 59″, W 56° 51′ 26″	170	2.0	29.9	1.4	5.6	4.4	1.4	16	304	ND	5.4	3487
Tapagem Grande	S 1° 24′ 36″, W 56° 51′ 13″	172	4.0	29.2	1.7	5.7	4.7	1.5	18	364	0.9	5.4	3817
Farias	S 1° 21′ 45″, W 56° 53′ 12″	178	1.5	32.6	1.3	6.6	7.0	1.5	30	660	ND	4.6	4438
Jacaré	S 1° 20′ 32″, W 56° 51′ 01″	178	2.1	32.7	1.0	6.8	5.4	1.3	41	577	0.4	5.3	3521
Abuí	S 1° 16′ 17″, W 56° 56′ 56″	184	2.5	32.4	1.7	6.2	4.0	0.9	18	417	ND	5.5	2457
Macaco	S 1° 12′ 51″, W 56° 53′ 50″	192	2.3	29.9	1.6	4.3	6.6	2.5	37	609	0.6	5.7	5856
Trombetas River	S 1° 31′ 22″, W 56° 14′ 46″	90	2.5	30.0	1.8	5.8	3.6	1.4	17	259	ND	5.3	3509
Mean	–	–	*2.5*	*31.0*	*1.4*	*5.9*	*4.5*	*1.3*	*30*	*440*	*3.0*	*5.6*	*2916*
SD	–	–	*0.9*	*1.3*	*0.5*	*0.6*	*1.0*	*0.5*	*15*	*121*	*7.1*	*0.6*	*1040*

Temp., temperature; Secchi, Secchi disk transparency; DO, dissolved oxygen: DOC, dissolved organic carbon; DIC, dissolved inorganic carbon; TP, total phosphorus; TN, total nitrogen; pCO_2, partial pressure of carbon dioxide.

Table 2 | Simple linear regression relationships.

REGRESSION EQUATION	R^2	*p*-value	*n*
*SDT = 0.751 + (0.00514*D)*	*0.26*	*<0.05*	*26*
*pCO_2 = 903.0 + (16.470*D)*	*0.50*	*<0.05*	*25*
*logDOC = 0.537 + (0.000873*D)*	*0.20*	*<0.05*	*26*
*BA = 1.773 + (0.0205*D)*	*0.24*	*<0.05*	*26*
*VA = 1.051 + (0.00691*D)*	*0.17*	*<0.05*	*26*
logVBR = 0.757 − (0.000801*D)	0.08	0.17	26
logPA = 3.186 + (0.000515*D)	0.01	0.79	26
*BA = −1.315 + (8.668*logDOC)*	*0.16*	*<0.05*	*26*
VA = −0.121 + (3.127*logDOC)	0.13	0.07	26
BA = 3.062 + (0.367*logPA)	0.01	0.69	26
VA = 2.598 − (0.219*logPA)	0.02	0.56	26
*BA = −9.081 + (3.872*logpCO_2)*	*0.23*	*<0.05*	*26*
*VA = −3.062 + (1.435*logpCO_2)*	*0.20*	*<0.05*	*26*

D, distance traveled through the Trombetas River from the lake mouth until the Amazon River; SDT, Secchi disk transparency; pCO2, carbon dioxide partial pressure; DOC, dissolved organic carbon; BA, bacterial abundance; VA, viral abundance; VBR, virus-to-bacterium ratio; PA, phytoplankton abundance. The values were log transformed for DOC, VBR, and PA because the data failed the normality test (Shapiro–Wilk, $p < 0.05$). The significant relationships are shown in italics.

studied here. Also, we cannot discard the possibility that the higher turbidity in the lower basin lakes is partly anthropogenic, as this portion of the basin is closer to urban areas and human settlements. High turbidity implies that less labile autochthonous DOC is formed by primary producers. This is consistent with findings from Batata Lake, a clear-water floodplain lake heavily impacted by bauxite tailings where the turbid impacted sites exhibit lower DOC as well as bacterial and VAs than clear-water natural sites (Barros et al., 2010).

We suggest that a chain of events is triggered following the decreased intensity of the backwater effect as proximity to the Amazon River decreases: DOC increases (in quantity and likely in quality), leading to increased BAs, and ultimately VAs. It has been shown before that DOC stimulates bacterial growth, and that VAs respond to changes in BAs in a clear-water Amazonian lake (Farjalla et al., 2002; Barros et al., 2010). Finally, pCO_2 also increased with distance to the Amazon River, which is probably a result of increased bacterial respiration due to higher BA. Indeed, BA was positively correlated to pCO_2.

In addition to proximity to the Amazon River, it is likely that other factors also regulate bacteria and viruses in Amazonian floodplain lakes. At low water, the influence of parent rivers on floodplain lakes is substantially reduced, and some lakes become totally isolated from their associated rivers (Thomaz et al., 2007). The degree of dissociation with the parent river is, however, fairly variable among lakes, which results from differences in local inputs (Forsberg et al., 1988). Therefore, there are two factors that act simultaneously during low waters: (1) the backwater effect that tends to keep water level higher than expected by discharge, ultimately making lower basin less confined than upper basin ones; and (2) the rate of local inputs of water and associated chemical compounds.

The relative importance of local inputs depends on the hydraulic loading rate from the local drainage basin, which in turn depends on the drainage basin area to lake area ratio (BA:LA; Forsberg et al., 1988). Generally, lakes with a low BA:LA display a mixture of river and local water by the end of the low water period, whereas lakes with a high BA:LA are primarily characterized by the presence of local water. In Amazonian floodplain lakes, the BA:LA ratio can vary by up to two orders of magnitude from one lake to another (Forsberg et al., 1988) – and, in general, higher BA:LA leads to decreased nutrient availability because local water derived from forest runoff is usually less nutrient-enriched. The distance to the Amazon River (i.e., a proxy to the intensity of the backwater effect) had a significantly positive, but low explicability on bacteria and viruses. This low explicability is expected if one considers that a wide range of geological, hydrological, and environmental factors controls planktonic food webs and lake water chemistry. Therefore, we suggest that the BA:LA ratio is likely an important additional factor governing bacteria and viruses in the Trombetas floodplain lakes, as this ratio influences the availability of nutrients and DOM, as well as mixing and dilution of water.

RELATIONSHIPS OF VIRUSES WITH BACTERIA AND PHYTOPLANKTON

A strong virus–bacterium relationship plus a lack of relationship between virus and phytoplankton indicate that most viruses are bacteriophages (i.e., infect bacteria). The predominance of bacteriophages suggests that VAs increase with distance to the Amazon River because of increased BAs, as viral infection depends directly on the number of host cells (Brussaard, 2004). The predominance of bacteriophages in the lakes studied here is in line with the only existing report of virus–bacterium relationship in Amazonian aquatic ecosystems (Barros et al., 2010), which shows a strong correlation between bacterial and VAs, constant VBR and predominance of bacteriophages. Since the encounter between virus and host cell is mediated by random drift in the water column (Brussaard, 2004), it is expected that bacteriophages predominate in the oligotrophic lakes studied here. Indeed, VAs are usually more strongly correlated to BAs than to phytoplankton in surface waters (Cochlan et al., 1993; Fuhrman, 1999). Also, the relative importance of bacteria over phytoplankton increases in oligotrophic lakes (Cotner and Biddanda, 2002), which reinforces that a strong correlation between viral and BAs is likely to occur in clear-water Amazonian floodplain lakes. Finally, a lack of relationship between phytoplankton and bacteria is consistent with the fact that a low proportion of phytoplankton carbon is transformed into bacterial biomass in the tropics (Roland et al., 2010), probably because most of carbon utilized by tropical aquatic bacteria is potentially used to maintain their high respiration rates (Amado et al., 2013).

The VBR is highly variable in world lakes, with reported ratios ranging from 0.4 to over 100 (Maranger and Bird, 1995; Anesio et al., 2004; Clasen et al., 2008). In tropical systems, reported VBRs range from 4 to 22 (Peduzzi and Schiemer, 2004; Bettarel et al., 2006; Araújo and Godinho, 2009). Therefore, the VBRs of the lakes surveyed here are low (2.5−9.1; average = 4.7), fitting the lower range of values reported for tropical lakes. Nevertheless, our VBR

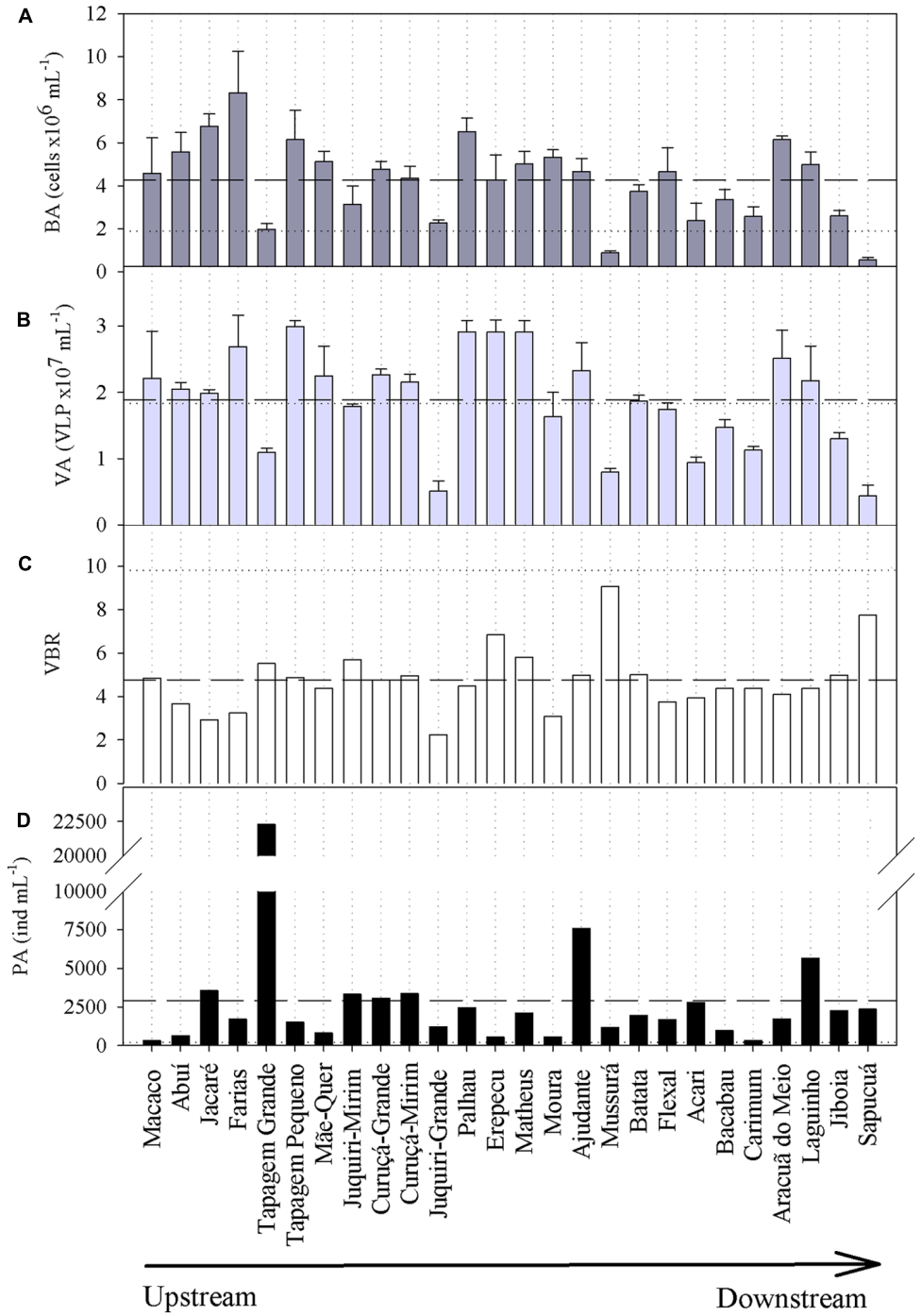

FIGURE 3 | (A) Bacterial abundances (BA), **(B)** viral abundances (VA), **(C)** virus-to-bacterium ratios (VBR), and **(D)** phytoplankton abundances (PA) in the floodplain lakes of the Trombetas River basin. Bars and traces in **(A,B)** represent mean and SD of the bacterial and viral counting, respectively. Dashed and dotted horizontal lines indicate the average of the lakes and the value of the Trombetas River, respectively.

is similar to the only existing description for clear-water floodplain lakes (4.3−6.1; Barros et al., 2010). In the Trombetas River main channel, we observed the highest VBR among all systems, as BA was within the lower range and VA was within the middle range of our dataset. A high abundance of viruses relative to bacteria in the Trombetas River is probably because the more turbulent riverine waters may facilitate the random encounter between viral and bacterial host cells. Finally, both bacterial and VAs were within the middle range of worldwide data (e.g., Maranger and Bird, 1995; Anesio et al., 2004; Bettarel et al., 2006; Clasen et al.,

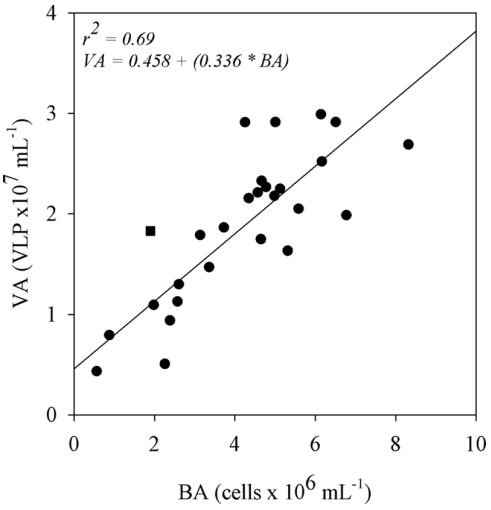

FIGURE 4 | Simple linear regression of BA with VA in the floodplain lakes. The Trombetas River (square) was not included in the regression and is shown as outer data. The relationship was significant ($p < 0.05$).

2008), which is in agreement with previous studies in Amazonian lakes (Anesio et al., 1997; Amado et al., 2006; Barros et al., 2010).

HYDRODYNAMICS AND THE PLANKTONIC VIRAL LOOP

The viral loop is a semi-closed loop connecting bacteria, viruses, and organic matter. It was initially idealized for marine systems (Fuhrman, 1999), in which the main external suppliers of DOM to the loop are grazers and primary producers. However, we propose that, in Amazonian floodplain lakes, there is a very relevant force that regulates the loop: hydrodynamics (**Figure 5**). A previous study showed that the flood pulse influences bacteria and viruses, with decreased abundances of both communities during floods (Barros et al., 2010); here, we show that viral and BAs increase in lakes less affected by the Amazon River backwater effect. Hydrodynamics also modulates viral communities in macrotidal estuaries, with VAs decreasing seaward because of dilution of viruses entering the estuary from the river (Auguet et al., 2005). Hence, our study builds on previous findings, underscoring the central role of hydrodynamics in shaping the viral loop. The action of hydrodynamics is not only through the regulation of the availability of DOM and nutrients, but also directly through water dilution and mixing of the microbial compartments. The schematic diagram that we propose underpins the role of viruses in the biogeochemistry of Amazonian aquatic ecosystems. Planktonic models indicate that bacterial respiration increases substantially in the presence of viral infection (Fuhrman, 1999). Hence, on the one hand, viral infection may contribute to CO_2 production – which is large in Amazonian surface waters (Richey et al., 2002;

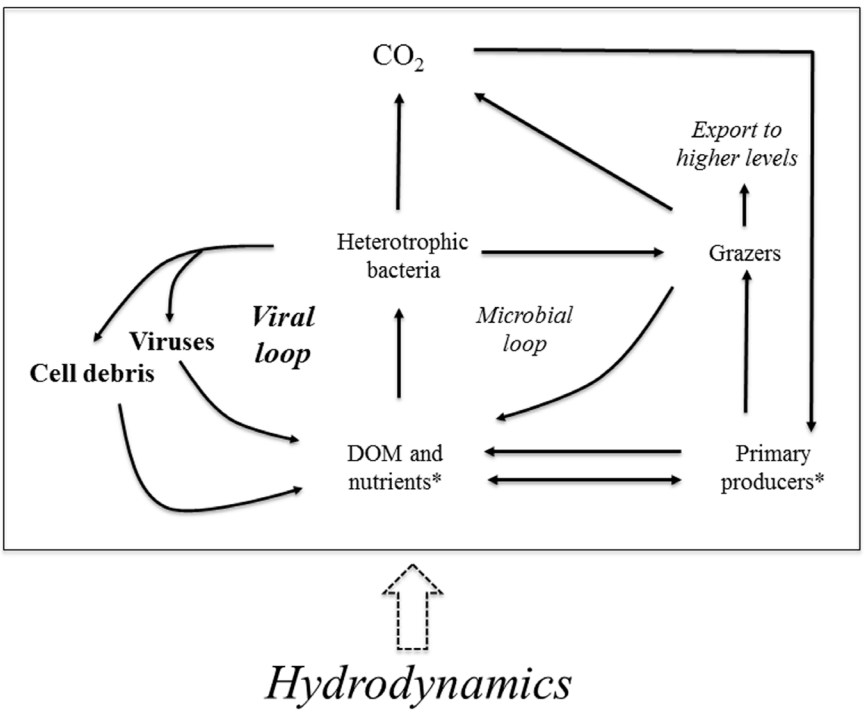

FIGURE 5 | Schematic diagram representing the viral loop, included within the microbial food web, in Amazonian lakes, emphasizing the importance of hydrodynamics. There is a semi-closed loop connecting bacteria, viruses, and dissolved organic matter (DOM). Primary producers and grazers are the two external suppliers of DOM to the loop. In Amazonian lakes, however, hydrodynamics is also a pivotal forcing influencing the loop, as it regulates the availability and quality of DOM. Additionally, hydrodynamics promotes water dilution and mixing that change the abundance of the different microbial compartments of the scheme. Modified from Fuhrman (1999). *there are two different arrows connecting "primary producers" with "DOM and nutrients" because primary producers uptake and release nutrients, but they only release DOM.

Abril et al., 2014), but on the other hand, this can possibly be counteracted by nutrient regeneration through viral lysis that ultimately favors the growth of primary producers (Shelford et al., 2012).

CONCLUSION

Although we do not have data for the high water period, existing literature data allow us to make some inferences. Data from 10 floodplain lakes in the Trombetas River basin indicate that there is a higher coefficient of variation for several limnological parameters – including water transparency and DOC – during low waters (Thomaz et al., 2007). This suggests that the lakes are more similar among themselves and with Trombetas River during floods, when they are connected. Thus, the inter-lake dissimilarity of bacterial and VAs that we found during low water is probably less significant during high water due to the increased connectivity. In summary, we found a latitudinal gradient in the characteristics of the floodplain lakes analyzed here. We attribute this spatiality to the backwater effect of the Amazon River on the Trombetas River, which tends to increase the ratio of river to local water in lower basin lakes. DOC enrichment, CO_2 supersaturation, water transparency, VAs and BAs significantly increase as distance to the Amazon River increases.

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Dynamics of planktonic prokaryotes and dissolved carbon in a subtropical coastal lake

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To understand the dynamics of planktonic prokaryotes in a subtropical lake and its relationship with carbon, we conducted water sampling through four 48-h periods in Peri Lake for 1 year. Planktonic prokaryotes were characterized by the abundance and biomass of heterotrophic bacteria (HB) and of cyanobacteria (coccoid and filamentous cells). During all samplings, we measured wind speed, water temperature (WT), pH, dissolved oxygen (DO), precipitation, dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), and carbon dioxide (CO_2). DOC was higher in the summer (average = 465 μM – WT = 27°C) and lower in the winter (average = 235 μM – WT = 17°C), with no significant variability throughout the daily cycles. CO_2 concentrations presented a different pattern, with a minimum in the warm waters of the summer period (8.31 μM) and a maximum in the spring (37.13 μM). Daily trends were observed for pH, DO, WT, and CO_2. At an annual scale, both biological and physical-chemical controls were important regulators of CO_2. HB abundance and biomass were higher in the winter sampling (5.60×10^9 cells L^{-1} and 20.83 μmol C L^{-1}) and lower in the summer (1.87×10^9 cells L^{-1} and 3.95 μmol C L^{-1}). Filamentous cyanobacteria (0.23×10^8–0.68×10^8 filaments L^{-1}) produced up to 167.16 μmol C L^{-1} as biomass (during the warmer period), whereas coccoid cyanobacteria contributed only 0.38 μmol C L^{-1}. Precipitation, temperature, and the biomass of HB were the main regulators of CO_2 concentrations. Temperature had a negative effect on the concentration of CO_2, which may be indirectly attributed to high heterotroph activity in the autumn and winter periods. DOC was positively correlated with the abundance of total cyanobacteria and negatively with HB. Thus, planktonic prokaryotes have played an important role in the dynamics of both dissolved inorganic and organic carbon in the lake.

Keywords: planktonic prokaryotes, carbon dioxide, subtropical lake, DOC, cyanobacteria

INTRODUCTION

Lakes are sentinels of global change to the extent that their storage and transformation of organic matter changes with global warming, with which there are many concerns (Cole et al., 2007; Adrian et al., 2009; Tranvik et al., 2009). Lakes generally function as sources of carbon dioxide (CO_2) to the atmosphere, mainly because of high inputs to and degradation through mineralization of terrestrial organic material in lakes (Cole et al., 1994, 2007; Kosten et al., 2010; Marotta et al., 2010a,b). However, CO_2 emissions among lakes vary, with tropical lakes responsible for a significant portion of CO_2 emissions compared to temperate lakes (Marotta et al., 2009a,b; Kosten et al., 2010). In addition, annual variability of these factors in lakes has also been reported (Trolle et al., 2012). Despite the studies on temporal and spatial variability of carbon fluxes among and within temperate and tropical lakes, little is known about the temporal dynamics in subtropical lakes and what factors drive carbon variability there.

The temperature dependence of community respiration and thus metabolic rates (Kosten et al., 2010; Yvon-Durocher et al., 2012), and of cyanobacteria dominance in shallow lakes (Kosten et al., 2012; Sarmento, 2012) suggest that in tropical and subtropical lakes, prokaryotes play a major role in carbon mineralization and production (via primary production) (Sarmento, 2012).

Studies encompassing the daily variability of planktonic prokaryotes and carbon are rare. A few authors have described bacterial abundance and daily shifts in respiration (Pringault et al., 2007, 2009; Sadro et al., 2011), but to our knowledge, none have investigated bacterial biomass variability and its relationship with dissolved carbon on daily and annual scales simultaneously.

Thus, our main objectives were to evaluate the dynamics of planktonic prokaryotes at two time scales (48 h and annual), including their relationship with dissolved inorganic and organic carbon in a subtropical lake.

MATERIALS AND METHODS

STUDY SITE

The study site is located in the littoral zone of the Peri coastal lake (**Figure 1**). The lake is surrounded by Atlantic Rain Forest and is separated from the sea by a vegetated sandbank, which isolates Peri Lake and prevents exchange of water with the adjacent ocean. Consequently, it is a freshwater system. Peri Lake is located in a protected area called "Parque Municipal da Lagoa do Peri (PMLP)" and supplies potable water for a significant percentage

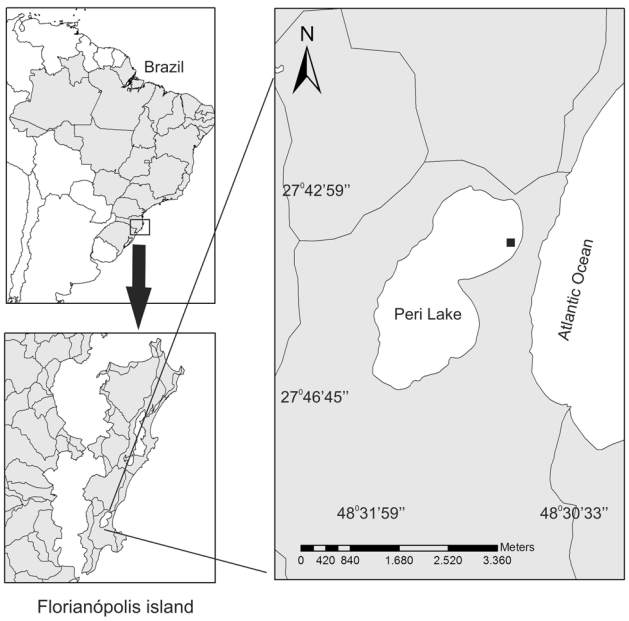

FIGURE 1 | Map of Peri Lake, showing the location of the sampling site (black dot) in the littoral zone.

of the population of Florianópolis. It has a surface area of 5.7 km^2, a maximum length of 4 km, an average width of 1.7 km, and average and maximum depths of 4.2 and 11 m, respectively. There are two main tributaries of the lake, which contain a volume of 21.2 million cubic meters of water. Peri Lake is classified as oligotrophic for nutrients and mesotrophic for chlorophyll-*a* (Chl-*a*) (Hennemann and Petrucio, 2011). The sampling site was located in the littoral zone of the lake (**Figure 1**), where the depth oscillates between 0.5 and 0.6 m deep, and the water column is continuously mixed.

METEOROLOGICAL VARIABLES

Wind velocity and precipitation were obtained over 9 days (7-days prior to and during the 48-h samplings) from EPAGRI/CIRAM (Centro de Informações de Recursos Ambientais e de Hidrometeorologia de Santa Catarina), which has a station near the lake.

EXPERIMENTAL DESCRIPTION

Aliquots of water were collected from the site at 3-h intervals during daylight and at 6-h intervals at night in October/2009, March/2010, May/2010, and August/2010 (representing austral spring, summer, autumn, and winter, respectively). In addition to data obtained from EPAGRI/CIRAM, wind velocity, temperature of the air and water, and dissolved oxygen (DO) were measured *in situ* during the study. Wind velocity and air temperature were measured with an Instrutherm TAD-500 anemometer, and water temperature (WT) and DO with a YSI 85 multi-parameter probe.

Water aliquots were taken to the laboratory (located at the park), where alkalinity (accuracy of 0.02) and pH (accuracy of 0.01) were measured. CO_2 concentrations were estimated from measurements of pH and alkalinity (Stumm and Morgan, 1996), with corrections for temperature, altitude, and ionic strength (Cole et al., 1994). Five-hundred milliliters of water was filtered with AP40 Millipore glass fiber filters prior to measuring Chl-*a* after its extraction with acetone (Lorenzen, 1967; Strickland and Parsons, 1972). Dissolved organic carbon (DOC) was analyzed from the filtrate fraction through oxidation under high temperature (680°C) using the Shimadzu TOC-5000 carbon analyzer (Sugimura and Suzuki, 1988).

PLANKTONIC PROKARYOTES

Fifteen milliliters water aliquots were fixed with a solution of 2% PFA (para-formaldehyde, final concentration) for further estimation of the abundance, biovolume and biomass of aerobic heterotrophic bacteria (HB) (hereafter HB), and cyanobacteria.

One milliliter aliquots were filtered in dark-polycarbonate membrane filters (θ 25 mm, 0.2 μm) (Millipore), stained with DAPI (4,6-diamidino-2-phenylindole) (1 μg mL^{-1}) (Porter and Feig, 1980), and mounted on microscope slides. Bacteria were counted using an epifluorescence microscope (Olympus BX-40) equipped with ultraviolet and green light filters. Ten fields of view were counted from each filter, where the length, width, and elongation of at least 200 heterotrophic bacterial cells were measured. For cyanobacteria, all coccoid cells and filaments were measured. Cell counting and measurements were performed with the "UTHSCSA Image Tool" freeware (University of Texas Health Science Center, San Antonio, TX, USA). Biovolume and biomass estimations were calculated using the algorithm from Norland (1993) and Massana et al. (1997).

STATISTICAL ANALYSES

Descriptive statistics were used to calculate the minimum, maximum, mean, and standard deviation of data. Log-transformed data showed significant Gaussian distribution (Kolmogorov–Smirnov, $p < 0.05$), homogeneity of variances (Bartlett, $p > 0.05$), and significant matching (F test, $p < 0.05$). We conducted one-way ANOVA tests with wind velocity, temperature, pH, DO, Chl-*a*, CO_2, DOC, bacterial abundance, and biomass for different periods of the day (morning, afternoon, and night), and for different months (October/2009, March, May, and August of 2010). The analyses were followed by *post hoc* testing of Tukey-HSD for multiple comparisons (significance level at $p < 0.05$) (Zar, 1996). Multiple regression models were used to identify the main drivers of CO_2 and DOC concentrations in the lake. Principal component analysis (PCA) was also conducted using all of the time interval data (daily and between months) and the abiotic and biotic variables (wind, WT, precipitation, DO, DOC, CO_2, and bacterial biomass – heterotrophic and cyanobacteria). All statistics were conducted using Statistica 7.0 software (Stat Soft Inc., USA).

RESULTS

ENVIRONMENTAL VARIABLES

Precipitation in the region of Peri Lake varied from 0 to 90 mm. The maximum value of 90 mm was measured in May, 3 days prior to the sampling (**Figure 2**). Wind speed was higher in August compared to the other months (ANOVA, Tukey-HSD, df = 48, $p < 0.05$), and significant daily variability was observed in the same period, with winds of 8.5 m s^{-1} in the morning. WT was

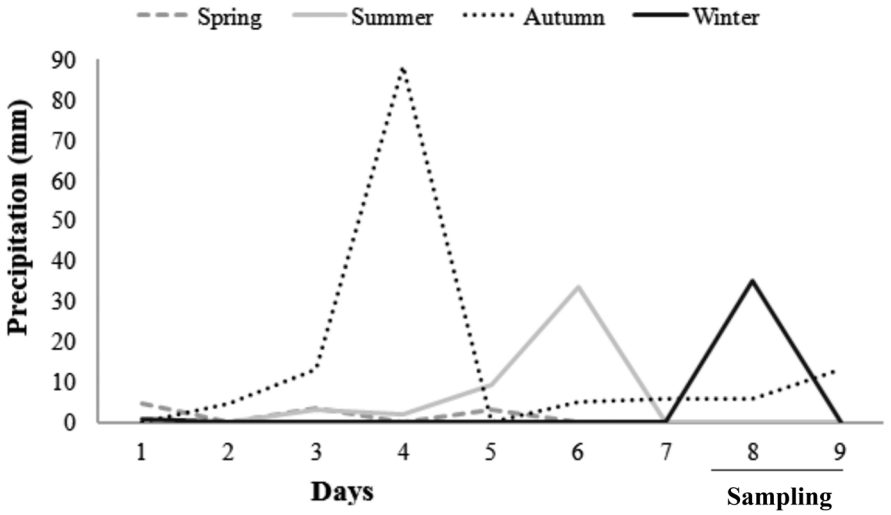

FIGURE 2 | Precipitation measurements (in mm) for the 7-day period prior to and during the 48-h samplings in October/2009, March/2010, May/2010, and August/2010 in Peri Lake.

Table 1 | Mean and standard deviation values of wind speed (WS) ($m\,s^{-1}$) measured in the field, water temperature (WT) (°C), pH, dissolved oxygen (DO) ($mg\,L^{-1}$), dissolved inorganic carbon (DIC) (μM), and chlorophyll *a* (Chl-*a*) ($\mu g\,L^{-1}$) during morning, afternoon, and night hours throughout the 48 h samplings in spring, summer, autumn, and winter.

Samplings	Period	WS $m\,s^{-1}$	WT	pH	DO $mg\,L^{-1}$	Chl-*a* $\mu g\,L^{-1}$
October 2009	Morning	2.7 ± 2.5	21.4 ± 1.1	6.3 ± 0.2	9.3 ± 0.6	5.8 ± 0.8
	Afternoon	2.5 ± 1.0	22.0 ± 0.1	6.4 ± 0.1	9.3 ± 0.2	6.3 ± 0.5
	Night	3.0 ± 2.4 b	20.0 ± 0.1* b*	6.3 ± 0.1 c	8.6 ± 0.3* a*	6.3 ± 1.2 b
March 2010	Morning	1.5 ± 0.2	27.4 ± 1.1	7.5 ± 0.2	7.6 ± 0.4	8.1 ± 0.5
	Afternoon	1.3 ± 0.8	27.7 ± 1.1	7.6 ± 0.2	8.0 ± 0.2	9.3 ± 1.4
	Night	1.0 ± 0.9 b	26.5 ± 0.5 a	7.5 ± 0.3 a	7.4 ± 0.5 c	8.9 ± 1.3 a
May 2010	Morning	1.5 ± 1.8	20.7 ± 0.5	7.1 ± 0.1	8.5 ± 0.3	4.9 ± 1.7
	Afternoon	1.9 ± 1.4	20.5 ± 0.7	7.3 ± 0.04*	8.9 ± 0.1	4.2 ± 1.7
	Night	0.7 ± 1.3 b	20.2 ± 0.4 b	7.1 ± 0.1 b*	8.3 ± 0.2 b	5.6 ± 2.6 b
August 2010	Morning	8.5 ± 1.8*	17.4 ± 1.4	7.1 ± 0.07	9.3 ± 0.2	8.9 ± 1.5
	Afternoon	4.5 ± 1.6	18.6 ± 1.6	7.2 ± 0.07	9.2 ± 0.2	8.7 ± 0.1
	Night	5.7 ± 0.6 a*	16.6 ± 1.3 c	7.1 ± 0.03 b	9.1 ± 0.2 a	7.9 ± 2.1 a

Lower-case letters indicate the result of ANOVA among samplings followed by the post hoc test of Tukey of normalized variables (N = 13). For each period of the day (morning, afternoon, and night) a N = 4 and N = 5 was used. The asterisks represent the different group after ANOVA of daily periods followed by the post hoc test of Tukey.

significantly higher in March, a summer month in the sampling (ANOVA, Tukey-HSD, df = 48, $p < 0.05$), with warmer waters in the afternoon (**Table 1**).

The highest pH was also measured in March, and the lowest occurred in October. Similar to temperature observations, pH tended to rise in the afternoon (**Table 1**). Dissolved oxygen (DO), on the other hand, was higher in August and October (ANOVA $p < 0.05$). There was a small daily shift in DO, with increasing values in the afternoon hours (**Table 1**).The daily shifts in CO_2 presented the opposite trend by comparison; whereas WT, DO, and pH increased in the afternoon, CO_2 decreased in the afternoon. Regarding the annual variability of CO_2 (between samplings or months), the highest values for both CO_2 and DO were detected in October and August (ANOVA, $p < 0.05$) (**Figure 3A**). Chl-*a*

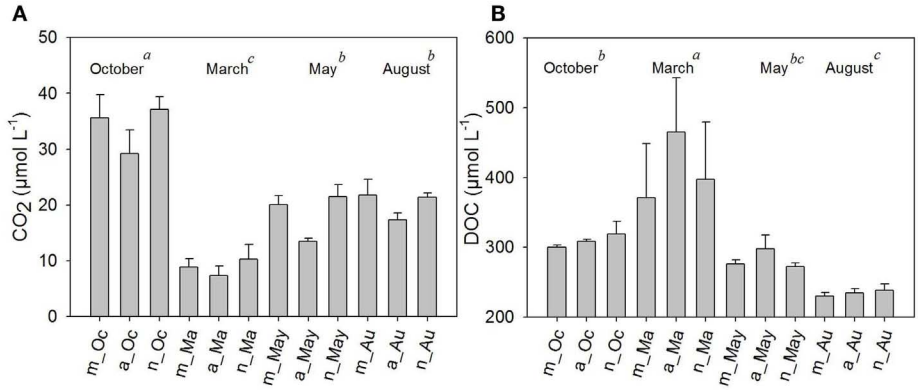

FIGURE 3 | Average values of carbon dioxide (CO_2) (A) and dissolved organic carbon (DOC) (B), in μM, measured in the morning, afternoon, and night hours in October/2009, March/2010, May/2010, and August/2010. Error bars represent the standard errors of each period of the day (m, morning; a, afternoon; n, night) in October (Oc), March (Ma), May (May), and August (Au). Lower-case letters represent the result of ANOVA testing for months followed by the *post hoc* Tukey test for homogenous groups ($p < 0.05$).

showed a daily variation only in March (summer sampling), with increasing Chl-*a* in the afternoon. Chl-*a* was higher during the extreme seasons: summer and winter (ANOVA $p < 0.05$) (**Table 1**), most likely due to turbulence caused by wind in August (implying the influence of the phytobenthos).

Dissolved organic carbon was approximately twofold higher in March compared to August (ANOVA, $p < 0.05$). No significant diel shifts occurred (**Figure 3B**); however, a tendency of increasing values in the afternoon compared to morning and night periods was seen in the summer and autumn months (**Figure 3B**).

PLANKTONIC PROKARYOTES

In October and March (spring and summer time, respectively), the abundance of cyanobacteria, of both coccus and filamentous cells, was higher in the afternoon than in the morning and night, whereas no increase occurred in May and August (**Figures 4B,C**). The abundance of the three bacterial groups was minimal near dawn on the first day of October and August, whereas in May, the lowest abundance was observed at dusk (6 p.m.) on the second day (**Figure 4**). The results show that the density of planktonic prokaryotes changes daily; however, these changes are dependent on day-to-day variability. Instead, the larger shifts in both abundance and biomass of bacteria were detected between samplings (**Figures 5** and **6**).

Heterotrophic bacteria peaked in the afternoon hours of August (average of 5.60×10^9 cells L^{-1}), whereas the lowest average (1.87×10^9 cells L^{-1}) was estimated in the morning hours of March, showing a significant variability between samplings (ANOVA, $p < 0.05$). No significant daily variation was observed (ANOVA, $p > 0.05$) (**Figure 5A**). Biomass varied from 3.95 (March) to 20.83 μmol C L^{-1} (August), with higher values observed in August and October (ANOVA, $p < 0.05$); no daily pattern was observed for the biomass of heterotrophs (**Figure 6B**).

Filamentous cyanobacteria (CCY*fil.*) present were composed mainly of *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya & Subba Raju (>90%); however, no toxicology study has been published yet to determine the presence of cyanotoxins. CCY*fil.* averages varied from 0.23×10^8 (August) to 0.68×10^8 filaments L^{-1} (March) (**Figure 5B**). The highest densities of these filamentous cells occurred in October and March, followed by May and August (ANOVA, $p < 0.05$). There was no daily shift in CCY*fil.* abundance (**Figure 5B**). The biomass of CCY*fil.* varied from 67.00 (May) to 167.16 μmol C L^{-1} (March), with no significant difference between samplings or days (**Figure 6A**). CCY*fil.* were responsible for the majority of total bacterial carbon (82.3% in the autumn sampling and 97.3% in the summer) in Peri Lake, confirming the importance of filamentous cyanobacteria in concentrating carbon in the form of filaments.

The averages of coccoid cyanobacteria (CCY*coc.*) abundance oscillated between 0.58×10^8 (May) and 1.48×10^8 cells L^{-1} (March) (**Figure 5B**). CCY*coc.* was higher in March and October, as observed for filamentous cyanobacteria, with no clear daily pattern. However, the biomass of coccoid cyanobacteria varied from 0.38 (March) to 1.07 μmol C L^{-1} (May), without significant variability between samplings (**Figure 6B**).

The abundance of CCY*coc.* was positively correlated with the abundance of CCY*fil.* ($r = 0.844$, $p < 0.001$), whereas the biomass of CCY*coc.* was positively correlated with the biomass of HB (CCY*coc.* × HB; $r = 0.427$, $p = 0.002$). In addition, there is an excess of DOC in Peri Lake, as carbon in the form of DOC is 18-fold and 100-fold higher than that in the biomass of HB in August and March, respectively (**Figures 3B** and **6B**).

MULTIVARIATE ANALYSES

The PCA depicted in **Figure 7** shows 60% of the data variability. The samplings performed in winter and spring were separated from the summer and autumn samplings by Axis 1, with DOC and temperature serving as important drivers for the separation of the summer sampling, and precipitation for the separation of the autumn one. CO_2, heterotrophic bacterial biomass, and wind velocity were important for the winter sampling, and biomass of CCY (filamentous and coccus) and DO had a greater influence in the spring (**Figure 7**).

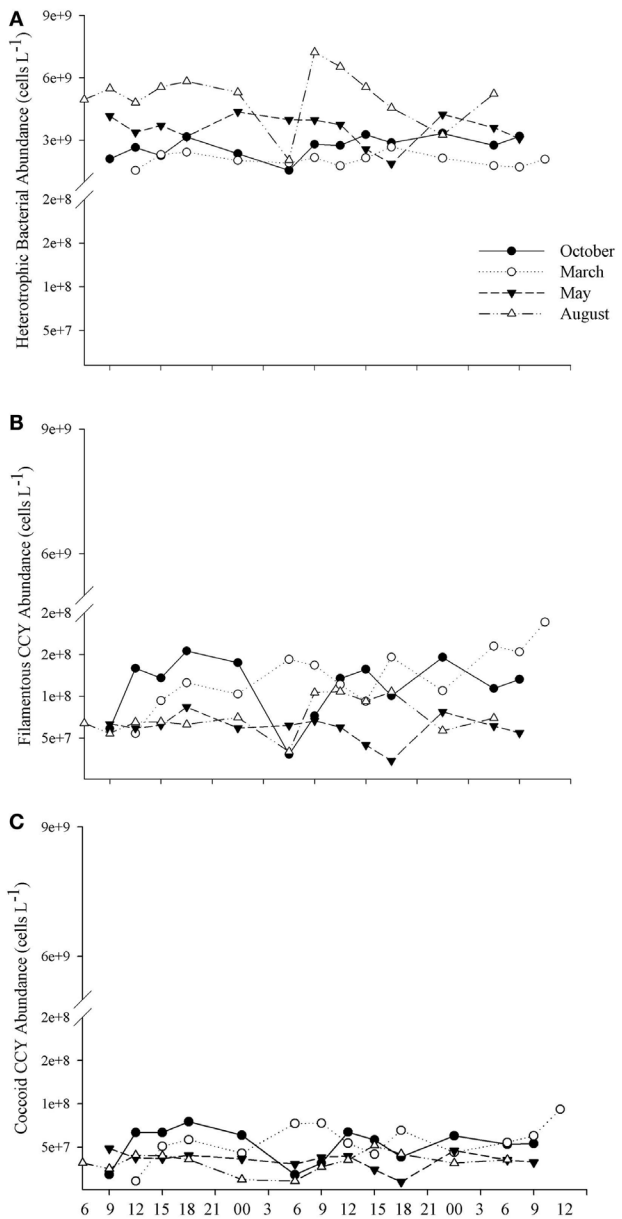

FIGURE 4 | The daily variability in the abundance of heterotrophic bacteria (A), coccoid cyanobacteria (B), and of filamentous cyanobacteria (C) for each 48 h sampling conducted in October/2009, March/2010, May/2010, and August/2010.

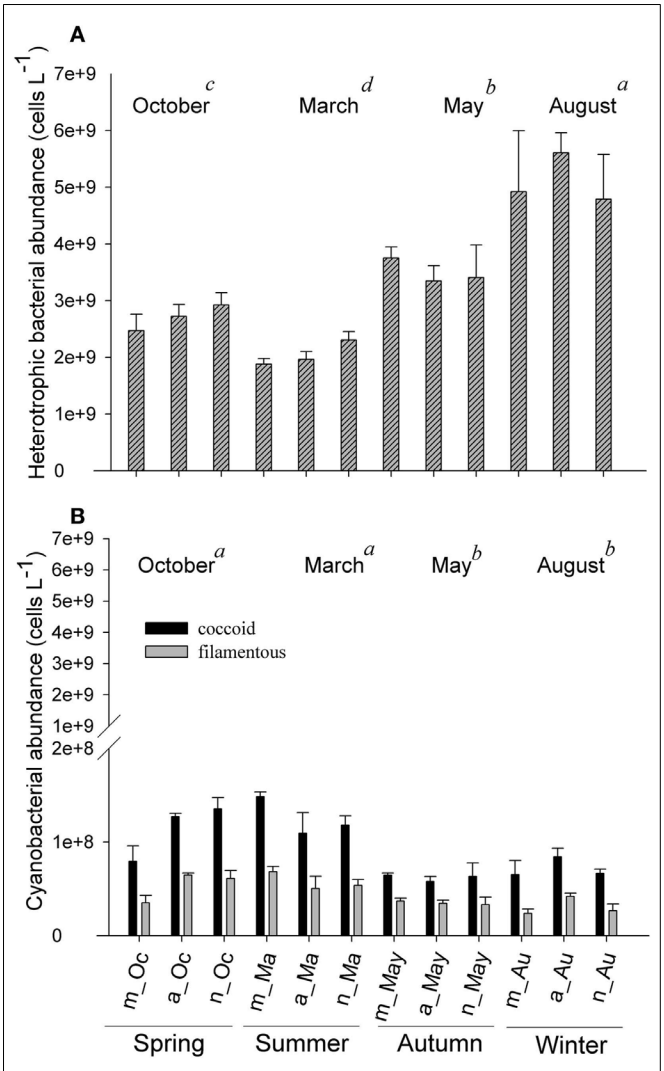

FIGURE 5 | Fluctuations in heterotrophic bacterial abundance (A) and cyanobacteria (filamentous and coccoid cells) (B), measured in the morning, afternoon, and night hours in October/2009, March/2010, May/2010, and August/2010. Error bars represent the standard errors of each period of the day (m, morning; a, afternoon; n, night) in October (Oc), March (Ma), May (May), and August (Au). Lower-case letters represent the result of ANOVA testing for months followed by the *post hoc* Tukey test for homogenous groups ($p < 0.05$). The areas shaded gray indicate the periods of lower photoautotrophic abundance.

Multiple regression models using DOC and CO_2 as dependent variables showed that 44% of the DOC variability was explained by the abundance of total cyanobacteria and of HB ($p < 0.05$) (**Table 2**). Precipitation, WT, and biomass of HB explained 61% of the CO_2 variability ($p < 0.05$) (**Table 3**).

DISCUSSION

The annual variability of the abundance and biomass of planktonic prokaryotes, both heterotrophs and cyanobacteria (filamentous and coccoid), was pronounced. Therefore, small daily patterns in the concentrations of DOC, CO_2, bacterial abundance, and biomass were observed. Day-to-day variability was reported for metabolic rates (net ecosystem production, gross primary production, and respiration), and changes in available incident irradiance was the main explanation for such variations (Staehr and Sand-Jensen, 2007).

Despite daily shifts in CO_2 not being significant, a trend of decreasing concentrations in the afternoon compared to morning and night was observed. This trend was contrary to temperature, pH, and DO patterns, which were not observed in the annual scale. These opposing findings suggest that biological constraints are

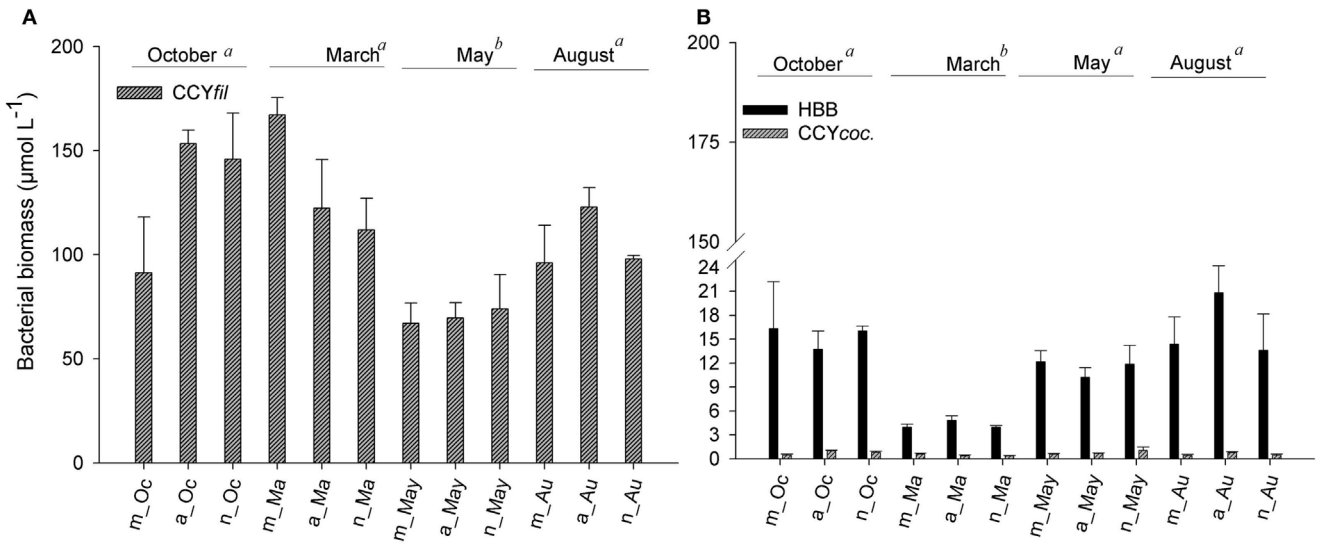

FIGURE 6 | Average values of biomass of filamentous cyanobacteria (A) and of heterotrophic bacteria and coccoid cyanobacteria (B) measured in the morning, afternoon, and night hours in October/2009, March/2010, May/2010, and August/2010. Error bars represent the standard errors of each period of the day (m, morning; a, afternoon; n, night) in October (Oc), March (Ma), May (May), and August (Au). Lower-case letters represent the result of ANOVA testing for months followed by the *post hoc* Tukey test for homogenous groups ($p < 0.05$).

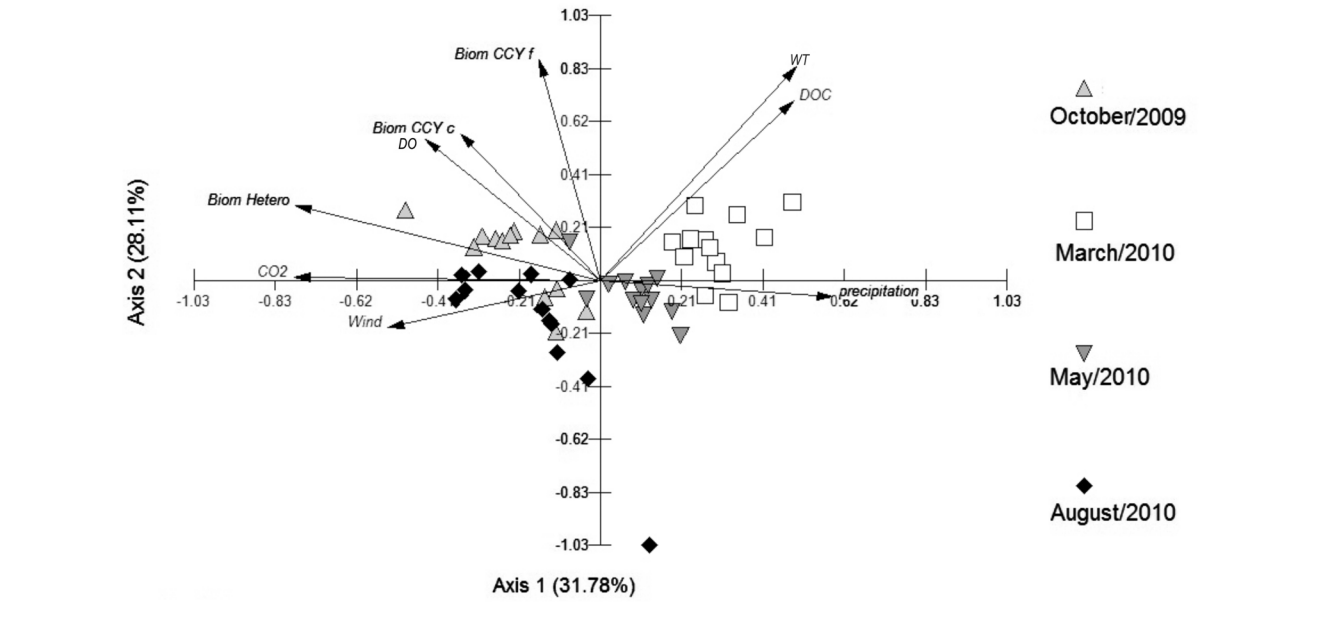

FIGURE 7 | Principal Component Analysis (PCA) with abiotic and biotic variables (wind, water temperature, precipitation, DO, DOC, CO_2, and bacterial biomass – heterotrophic and cyanobacteria).

more pronounced in smaller scales (at the diel scale as opposed to the annual scale). This is because oxygenic photosynthesis usually promotes a small rise in pH, as CO_2 is assimilated (thus, reducing the number of protons in the water). As oxygenic photosynthesis is stimulated under summer light intensities (Tonetta et al., submitted), the higher amount of solar radiation in the afternoon, when compared to morning hours (on a diel scale), could have stimulated primary production during this period of the day.

Additionally, the increasing abundance of cyanobacteria in the afternoon hours reinforced the suggestion that they downregulate CO_2 in the afternoon. At night and in the morning, CO_2 concentrations were higher. Accordingly, Sadro et al. (2011) reported a diel variability in planktonic community respiration in Emerald Lake, where higher aerobic respiration rates occurred at dusk and in the first part of the night. In Peri Lake, the higher concentrations of

Table 2 | Multiple regression model for dissolved organic carbon (dependent variable) using abiotic and biotic independent variables (precipitation, water temperature, dissolved oxygen, wind velocity, chlorophyll *a*, heterotrophic bacterial abundance and biomass, total cyanobacterial abundance and biomass).

	Beta	Std. Err.	B	Std. Err.	*t*(45)	*p*-Level
Intercept			2.193	1.765	1.243	0.220
DO	−0.242	0.129	−0.091	0.049	−1.867	0.068
Hetero Ab.	−0.375	0.122	−0.245	0.079	−3.080	**0.003**
Total CCY Ab.	0.554	0.220	0.316	0.126	2.519	**0.015**
Total CCY Biom.	−0.297	0.213	−0.143	0.104	−1.375	0.176

Data were log-transformed in order to achieve normality. N = 50, $R^2 = 0.4816$, adjusted $R^2 = 0.4356$, $F(4, 45) = 10.454$, $p < 0.001$. Significance of bold numbers, $p < 0.05$.

Table 3 | Multiple regression model for carbon dioxide (dependent variable) using abiotic and biotic independent variables (precipitation, water temperature, dissolved oxygen, dissolved organic carbon, wind velocity, chlorophyll *a*, heterotrophic bacterial abundance and biomass, total cyanobacterial abundance and biomass).

	Beta	Std. Err.	B	Std. Err.	*t*(42)	*p*-Level
Intercept			12.471	3.814	3.270	0.002
WT	−0.550	0.177	−0.098	0.032	−3.106	**0.003**
Hetero Biom.	0.314	0.147	2.731	1.283	2.129	**0.039**
Precipitation	−0.345	0.122	−0.041	0.015	−2.819	**0.007**
Wind	−0.261	0.145	−0.063	0.035	−1.803	0.078
DOC	−0.194	0.123	−0.478	0.303	−1.578	0.122
Chl-*a*	−0.129	0.121	−0.228	0.215	−1.062	0.294

Data were log-transformed in order to achieve normality. N = 50, $R^2 = 0.6620$, adjusted $R^2 = 0.6057$, $F(7, 42) = 11.752$, $p < 0.001$. Significance of bold numbers, $p < 0.05$.

CO_2 in the morning and at night were observed in all samplings.

Therefore, the variability of CO_2 between months of sampling was significant and much higher than the daily shifts. The PCA graph showed that temperature, precipitation, and DOC were the variables that were positively correlated with the summer month, whereas HB, wind, and CO_2 were higher in the winter. Thus, changes in the physical-chemical and biological factors are more pronounced annually than at the 48-h scale. In general, precipitation, temperature, and biomass of HB played the main role in the regulation of CO_2 dynamics (**Table 3**). However, precipitation and temperature were inversely correlated with CO_2. As higher temperature results in decreasing the solubility of gases dissolved in the water, loss of CO_2 would be expected in the summer months, when WT reached an average of 27°C, compared to 17°C in winter. However, this was not the case. Loss of CO_2 in temperate lakes is higher in winter and incorporation higher in summertime (Trolle et al., 2012). In Peri Lake, CO_2 fluxes between the atmosphere and the water were negative in the summer (creating an atmospheric CO_2 sink) and positive during other periods (Tonetta et al., in preparation). These observations point to the importance of biological factors in carbon dynamics within lakes.

In shallow coastal systems, wind plays a role in stimulating turbulent processes, which increase sediment resuspension and further release DOC and CO_2 from carbon-rich sediments and pore-water. However, in this study, the lack of a relationship between wind × CO_2 and wind × DOC, demonstrated by the multiple regression model, suggests either that the sediment is not an important source of carbon to the water column or that wind velocity was not strong enough to increase the upward release. Another explanation is the frequency of carbon measurements (within 48 h), as bacterioplankton abundance respond to turbulence after approximately 6–8 days of turbulence (Arin et al., 2002). Furthermore, DOC was higher in the calm weather periods, supporting the importance of cyanobacteria to organic carbon release.

Carbon dynamics in lakes is very difficult to model, as the terrestrial inputs and mineralization are difficult to measure (Hanson et al., 2011). The authors of that study suggested a few easily measurable parameters to estimate the fate of DOC in lakes, such as lake morphometry, residence time, and temperature, when the recalcitrance of DOC is known. As Peri Lake is a large, shallow lake, according to the Hanson et al. (2011) model, the residence time might exceed 1 year, and the majority of carbon may be lost through mineralization. This points to the importance of heterotrophic planktonic prokaryotes to the metabolism of the lake, which is corroborated by the fact the lake is net heterotrophic on an annual average (Tonetta in preparation).

Interestingly, DOC was higher in the summer and was dependent on cyanobacterial abundance, as shown in the multiple regression model. CCY, the dominant phytoplankton in Peri Lake (Hennemann and Petrucio, 2011), were positively correlated with DOC. This relationship indicates the autochthonous production of DOC by cyanobacteria, which was more pronounced in the summer. Cyanobacteria are, consequently, important producers of bacterial biomass and of DOC in Peri Lake, especially in the afternoon hours of the summer and spring months. Actively growing algae release substantial amounts of DOC via photosynthetic extracellular release (PER) (Baines and Pace, 1991). This exudation of organic material occurs when algal carbon fixation exceeds synthesis of new cell material during periods of sufficient irradiance (Panzenböck, 2007) and under nutrient-depleted conditions (Berman-Frank and Dubinsky, 1999). However, PER of organic matter by intact phytoplankton cells is not the principal pathway for DOC uptake by HB, but instead, byproducts of animal ingestion and digestion are (Jumars et al., 1989; Saba et al., 2011). Zooplankton feeding strategies may also be important to the production of DOC in Peri lake waters, but such were not evaluated in this study.

Terrestrial organic carbon subsiding CO_2 emissions through community respiration have been reported in other lakes around the world (Cole et al., 2006, 2007; Marotta et al., 2012). Consequently, the terrestrial dissolved organic carbon (tDOC) may account for up to 76% of pelagic bacterial demand (Cole et al., 2006). However, less than just 2% of the bacterial carbon was estimated to be transferred to zooplankton and, thus, up to the

food web (Cole et al., 2006). This process results in the sink of carbon in the lake.

Comparing 151 temperate lakes, Trolle et al. (2012) reported the highest efflux of CO_2 in winter and in those lakes with low Chl-*a* ($<11.2\,\mu g\,L^{-1}$), demonstrating a link with the trophic state of lakes and CO_2 flux. As Peri Lake had a Chl-*a* concentration of $7\,\mu g\,L^{-1}$ during this study, it might emit CO_2 to the atmosphere. This was already confirmed by previous studies regarding the CO_2 fluxes across two time scales: a 1- and a 5-year period (Tonetta et al., in preparation; Fontes et al., in preparation).

Heterotrophic bacterial abundance increased significantly in the winter in Peri Lake, most likely because HB are not regulated by availability of light as cyanobacteria are (Sarmento, 2012). In addition, the mixing processes caused by wind action over the surface of the lake improve the resuspension of organic material, indirectly stimulating HB. Irradiance has been reported to be the main factor shaping planktonic prokaryotes structure in a stratified subtropical lagoon, whereas the activity of cyanobacteria was stimulated in the bottom waters only given available light (Fontes and Abreu, 2009). Heterotrophic bacterial biomass was also higher in August (winter), indicating that heterotrophic bacterial production was stimulated. On the other hand, the abundance and biomass of cyanobacteria were higher in the warmer periods (spring and summer).

In Peri Lake, most of the bacterial carbon was stored in the filamentous cyanobacteria (mainly *Cylindrospermopsis raciborskii*), encompassing up to 98% of total bacterial carbon in March of 2010. This large reservoir of carbon is due to their larger cell size and filament forming strategy, which can function as a predation avoidance mechanism (Bouvy et al., 2001; Pernthaler et al., 2004). The abundance of coccoid cyanobacteria, or picophytoplankton, followed the filamentous bacteria pattern, as observed in the Conceição Lagoon (Fontes and Abreu, 2009), whereas their biomass followed the trends observed for the biomass of HB. However, DOC was produced by both groups of cyanobacteria, as shown in the increase in the abundance of total cyanobacteria. As *Cylindrospermopsis* is the dominant phylogenetic group of phytoplankton in Peri Lake, prokaryotic phytoplankton is important to production of particulate and dissolved carbon there as well.

With global warming, it is estimated that the levels of cyanobacteria will increase in shallow lakes (Kosten et al., 2012) and that more intensive rainfall events will occur, resulting in the intensification of lake CO_2 emissions (Cole et al., 2006; Dodds and Cole, 2007; Marotta et al., 2009a, 2010a,b). In addition, increasing organic material discharged into the water body might stimulate even greater respiration rates (Del Giorgio et al., 1997; Cole et al., 2006; Staehr et al., 2012). Our results indicated a direct relationship between CO_2 concentrations and the biomass of HB, pointing to HB as important players in lake community respiration. We showed that smaller and more abundant HB were more important in winter, whereas larger and less abundant filamentous cyanobacteria predominated in summer.

Changes in the community structure of planktonic prokaryotes over 48 h were small, but a general trend was observed in the spring and summer samplings, with an incremental increase in the abundance of coccoid and filamentous cyanobacteria in the afternoon. Significant changes in the abundance and biomass of planktonic prokaryotes and dissolved carbon (DOC and CO_2) were observed in the annual scale, when the CO_2 concentration and biomass of HB increased during colder and drier periods. Cyanobacteria, especially filamentous cyanobacteria, produced the majority of bacterial biomass and played an important role in releasing DOC into the water column, particularly in the summer. Thus, planktonic prokaryotes may play an important role in the dynamics of both dissolved inorganic and organic carbon in the lake.

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Environmental dynamics as a structuring factor for microbial carbon utilization in a subtropical coastal lagoon

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Laguna de Rocha belongs to a series of shallow coastal lagoons located along South America. It is periodically connected to the sea through a sand bar, exhibiting a hydrological cycle where physicochemical and biological gradients are rapidly established and destroyed. Its most frequent state is the separation of a Northern zone with low salinity, high turbidity and nutrient load, and extensive macrophyte growth, and a Southern zone with higher salinity and light penetration, and low nutrient content and macrophyte biomass. This zonation is reflected in microbial assemblages with contrasting abundance, activity, and community composition. The physicochemical conditions exerted a strong influence on community composition, and transplanted assemblages rapidly transformed to resembling the community of the recipient environment. Moreover, the major bacterial groups responded differently to their passage between the zones, being either stimulated or inhibited by the environmental changes, and exhibiting contrasting sensitivities to gradients. Addition of allochthonous carbon sources induced pronounced shifts in the bacterial communities, which in turn affected the microbial trophic web by stimulating heterotrophic flagellates and virus production. By contrast, addition of organic and inorganic nutrient sources (P or N) did not have significant effects. Altogether, our results suggest that (i) the planktonic microbial assemblage of this lagoon is predominantly carbon-limited, (ii) different bacterial groups cope differently with this constraint, and (iii) the hydrological cycle of the lagoon plays a key role for the alleviation or aggravation of bacterial carbon limitation. Based on these findings we propose a model of how hydrology affects the composition of bacterioplankton and of carbon processing in Laguna de Rocha. This might serve as a starting hypothesis for further studies about the microbial ecology of this lagoon, and of comparable transitional systems.

Keywords: bacterioplankton, carbon, estuary, Laguna de Rocha, hydrology

INTRODUCTION

Coastal lagoons are continental shallow and brackish water bodies partially or completely separated from the ocean by barriers of sand or coral (Woodroffe, 2002). From a hydrodynamic viewpoint their hydrology mixes characteristic features of shallow lakes, reservoirs, and rivers, which greatly determines ecosystem functioning (Mann, 2000). When coastal lagoons are connected to the ocean strong physicochemical gradients are generated especially in terms of salinity, light, and nutrient availability (Hauenstein and Ramírez, 1986). These transitional aquatic systems are extremely complex and characterized by high primary productivity, which in turn supports high secondary production (Viaroli et al., 1996). They are also sites of high biological diversity at all levels, from micro-organisms (Thompson et al., 2011) to birds (Aldabe et al., 2006), interacting through a complex food web with high adaptation potential and resilience capacity (Milessi et al., 2010). Consequently, coastal lagoons provide numerous ecosystem services such as water quality maintenance, fish production, carbon fixation and transformation, and protection from erosion (Gönenç and Wolflin, 2005).

In general, transitional coastal systems are particularly relevant for the global carbon cycle due to their disproportionally high productivity and participation in C turnover (compared to their size), and their linking of the terrestrial and marine phases of biogeochemical cycles (Schlesinger, 1997). Coastal lagoons, being shallow and strongly exposed to human activity, represent one of the ecosystem types most sensitive to external perturbations. Examples of current challenges for these systems include anthropogenic eutrophication, the alteration of the evaporation/precipitation balance due to climate change, wetland area loss, the local extinction of native species, and the introduction of exotic species (Meerhoff et al., 2012). Thus, studies on the ecological functioning of

coastal lagoons are essential to better understand the responses of these vulnerable aquatic systems to environmental change (Brito et al., 2012; Meerhoff et al., 2012).

Laguna de Rocha belongs to a series of coastal shallow lagoons located along the south-eastern line of South America. This large water body is periodically connected to the sea through a sand bar, exhibiting a hydrological cycle with three phases of rapid establishment and destruction of physicochemical and biological gradients (Conde et al., 2000). During the first phase, characterized by dominance of freshwater discharge, the lagoon exhibits homogeneous physicochemical characteristics: high loads of dissolved and particulate organic and inorganic components, high values of chl*a* and primary production, and brackish oligohaline conductivity values between 3 and 8 mS cm^{-1}. This phase usually lasts between 3 weeks and 3 months, until the increased water level leads to an overflow of the sand bar, and consequently to discharge into the Atlantic Ocean. In phase II, the ocean intrudes into the lagoon, usually under the influence of south-western winds. With the progression of the salinity gradient the Southern area of the lagoon shifts to a brackish meso- to polyhaline condition (10–45 mS cm^{-1}), characterized by low nutrient content, high UV penetration, low chl*a* and primary production, while the Northern zone remains brackish oligohaline (3–8 mS cm^{-1}). This phase is the most frequent state of the lagoon and typically lasts between 1 and 2 weeks and 4 months. Incessant south-western winds can then move saline waters further North (Phase III), so that most of the lagoon turns to brackish meso- to polyhaline condition and the associated characteristics. This phase is not very frequently attained, and typically lasts for a comparably short period (1–3 weeks; Conde et al., 2000).

The hydrological cycle in Laguna de Rocha exerts a strong effect on different planktonic communities (Bonilla et al., 2005; Calliari et al., 2009; Britos, 2010), including the bacterioplankton. The pelagic microbial assemblages of the lagoon exhibit high abundances, productivity and diversity (Piccini et al., 2006; Thompson et al., 2011), and all these parameters are affected by the changes in environmental conditions during the different phases of the hydrological cycle (Piccini et al., 2006). However, so far there is not sufficient information to postulate a model of how hydrology affects the composition and functioning of bacterioplankton in this lagoon, in particular focusing on the microbial carbon cycling.

The aim of this paper is to synoptically present and discuss results from three experiments about the interactions between bacterial communities, substrate and nutrient availability, and the hydrological dynamics of the lagoon. Specifically, we assessed the responses of bacterial assemblages to environmental change, and we examined aspects of bacterial carbon utilization and limitation patterns (growth, biomass production, and transfer to other trophic levels). Based on these observations, we propose a model for the functioning of the relationship between bacterioplankton and hydrology, which may serve as a framework for further studies both in the lagoon and in comparable transitional systems.

MATERIALS AND METHODS

STUDY SITE

Laguna de Rocha is a shallow, highly productive, and large brackish lagoon (mean depth, 0.6 m; area, 72 km^2), located in Uruguay, on the south-eastern coast of South America (34°33′S, 54°22′W) and part of a MaB/UNESCO Biosphere Reserve. This lagoon is influenced by freshwater from the drainage of three streams, Rocha stream (RS) being the most important one in terms of flow, and by periodic marine intrusions through a single mouth inlet from the Atlantic Ocean across a sand barrier.

This intercommunication of the lagoon with the ocean may occur several times per year and the water discharge of the lagoon during such events may reach up to 570 m^3 s^{-1} (Conde and Sommaruga, 1999). Aerial views of the closed and open situations can be found at http://zoology.uibk.ac.at/limno/images/rocha.html. Relevant limnological information about the system has been published elsewhere (Conde and Sommaruga, 1999; Conde et al., 1999).

EXPERIMENTS

Three independent sets of experiments were conducted to investigate the bacterial response to the hydrologic dynamics of the lagoon: A full transplant of bacterial communities between sites (Experiment 1), a gradual mixing of bacterial communities mimicking an environmental gradient (Experiment 2), and a manipulation of carbon and nutrient sources in a mesocosms (Experiment 3).

Experiment 1. Transplant of bacterial communities across environmental gradients

Sites for sampling and incubation of bacterial assemblages were selected based on their physicochemical characteristics at the time of the experiment (freshwater: Rocha stream [RS]; brackish water: Northern region of Laguna de Rocha [LN]). Conductivity was measured *in situ* with a portable meter (ES-12; Horiba Inc., Irvine, CA, USA). Water samples were analyzed for ammonium, nitrite, nitrate, and total nitrogen, soluble reactive phosphorus, total phosphorus, suspended solids, and organic matter content according to standard methods (APHA, 1995).

Experimental set up. Water samples from both chosen sites were collected in acid-washed 20 l plastic carboys in November 2004 (Southern springtime). Half of each sample was filtered through 0.8 μm pore-size filters to produce grazer-free (−G) treatments, whereas the original grazer community was maintained in the unfiltered second half of the samples (+G). The filtered and unfiltered water samples from both sites were placed in triplicate sets of pre-treated (acid-washed and deionized water rinsed) dialysis bags (diameter, 75 mm; molecular weight cut-off, 12,000–16,000 Da; Poly Labo, Switzerland), that were cut into pieces of 50 cm length to hold 2 l of water. A first set was filled with water (either for +G or −G treatments) from RS and incubated at RS ("freshwater incubation," RS–RS), another set was filled with water from LN and incubated at LN ("brackish water incubation," LN–LN). A third set bags was filled with water from RS and incubated at LN ("transplanted incubation," RS-LN) and a fourth set was filled with a 50:50 mix of water from RS and LN and incubated at LN ("mix incubation," Mix-LN). Samples were taken at 24, 48, and 72 h of incubation, and bacterial abundance, activity, and community composition were determined (see below). Conductivity (K) inside the dialysis bag was used as a proxy of changes in the main environmental characteristics (Conde et al., 1999).

Bacterial abundances. Portions of 3 ml were taken from each dialysis bag at each sampling, fixed with 2% (v/v) paraformaldehyde (PFA), incubated at room temperature in the dark for 1 h and then stored at −20°C until further analysis. The PFA-fixed samples were stained with 4,6′-diamino-2-phenylindole (DAPI; final concentration, 1 μg ml^{-1}), filtered onto polycarbonate filters (diameter 25 mm; pore-size, 0.2 μm; type GTTP; Millipore, and 1000 bacterial cells per sample were counted on an epifluorescence microscope (Axioplan II Imaging; Carl Zeiss, Jena, Germany).

Bacterial production. Bulk bacterial activity was estimated from the incorporation of [^{3}H]-L-leucine (Amersham, Little Chalfont, England) into bacterial biomass (Simon and Azam, 1989). Sample pools were made with triplicates, using *in situ* samples and samples taken from the dialysis bags after 72 h. Radiolabeled leucine (specific activity 63 Ci mmol^{-1}) was added at saturating concentrations (20 nM) to three sub-samples of each pool and to one formaldehyde-fixed control (final concentration, 3% v/v) from each water treatment (Piccini et al., 2006). These sub-samples were incubated in a water bath in the dark at *in situ* temperature (18.5°C) for 1 h and then fixed by the addition of formaldehyde. Macromolecule extraction was done with ice-cold TCA (5% w/v) and ethanol (80% v/v) as described previously (Simon and Azam, 1989). [^{3}H]-L-leucine incorporation was determined with a Beckman LS5000TD liquid scintillation counter (Beckman, Fullerton, CA, USA). Values were corrected for quenching (external standard method) and by subtraction of counts from the controls. Leucine incorporation was converted to bacterial protein and carbon production using published conversion factors (Simon and Azam, 1989).

Bacterial community composition. PFA-fixed bacteria from 3 ml of sample were filtered onto white 0.2 μm pore-size polycarbonate filters (diameter 47 mm). The filters were rinsed twice with 1× phosphate buffered saline (PBS) and once with distilled water and stored at −20°C. FISH with horseradish-peroxidase labeled probes and tyramide signal amplification (CARD-FISH) was performed as described previously (Piccini et al., 2006), see **Table A1** in Appendix for details on the targeted groups. After hybridization the samples were counterstained with DAPI (1 μg ml^{-1}). The relative abundance of each targeted group was determined by epifluorescence microscopy and semi-automated counting (Pernthaler et al., 2003).

Experiment 2. Mixing of bacterial communities (simulation of environmental gradient)

Water samples were obtained from two sites (LN and LS) selected according to their physicochemical characteristics at the time of the experiment (July 2007; Southern winter). Conductivity was measured *in situ* with a portable meter (ES-12; Horiba Inc., Irvine, CA, USA). Water samples were analyzed for ammonium, nitrite, nitrate, soluble reactive phosphorus, according to standard methods (APHA, 1995).

Experimental set up. Water samples from both sites were mixed in different proportions (% of water from LN to % of water from LS: 100:0, 90:10, 75:25, 50:50, 25:75, 10:90, 0:100) to a final volume of 500 ml. Each treatment was performed in triplicates. Immediately after mixing, triplicate 10 ml sub-samples plus one PFA pre-fixed control were taken from each variant. Subsequently, L-[4,5-^{3}H]Leucine (Amersham, specific activity 2.26 TBq mmol^{-1}) was added to these sub-samples at a final concentration of 10 nM. The incubations were run for 8 h in the dark at ambient water temperature (6°C) and stopped by addition of PFA (final concentration, 1%). Half of each sample was used to estimate bulk bacterial carbon production (BCP, applying the same protocol as for experiment 1), whereas the remaining volume served to trace the incorporation of the tracer by individual bacterial cells (see below).

Tracer incorporation by specific bacterial groups. After fixation all samples were filtered through polycarbonate filters (type GTTP, pore-size, 0.2 μm, diameter 25 mm, Millipore, Eschborn, Germany). The filters were rinsed twice with sterile phosphate buffered saline (PBS) and stored at −20°C until further analysis. To study the substrate uptake by specific bacterial groups we combined microautoradiography (MAR) and CARD-FISH as described previously (Alonso and Pernthaler, 2005). Triplicate samples of every treatment type were evaluated per bacterial group. Different MAR exposure times (4 h to 3 days) were tested to produce a maximum number of cells with silver grains (Alonso, 2012), and an optimal exposure time of 18 h was determined. All photochemicals were purchased from Kodak (Eastman Kodak, Rochester, NY, USA): autoradiography emulsion (type NTB-2), developer (type Dektol), and fixer. The development of the exposed slides followed the instructions of the manufacturer.

Bacterial community composition. The abundances of different bacterial populations were determined by CARD-FISH as described above (see **Table A1** in Appendix for details on the targeted groups). CARD-FISH and MAR-FISH preparations were embedded on microscopic slides in a previously described mounting medium containing 4,6′-diamidino-2-phenylindole (DAPI, final concentration, 1 μg ml^{-1}). Evaluation was carried out following the strategy outlined in Pernthaler et al. (2003) and Alonso and Pernthaler (2005) on a motorized microscopic system consisting of an epifluorescence microscope (AxioImager.Z1, Zeiss, Germany), a CCD Camera (Zeiss AxioCam MRm), and a motorized stage for eight microscopic slides (Zeiss WSB Piezodrive 05). Automation was achieved using the Visual Basic for Application module of the AxioVision software (Carl Zeiss) and comprised automated sample recognition and localization, multi-channel image acquisition, image processing, and cell counting routines for both FISH and MAR-FISH preparations (Zeder, 2010). A manual verification of the results of automated counting in a subset of preparations was assisted by the free counting software "ClickCounter" (http://www.technobiology.ch).

Experiment 3. Manipulation of carbon and nutrient sources in mesocosms

Two sampling sites were selected according to their physicochemical characteristics at the time of the experiment (December 2008, Southern late spring). Freshwater was obtained from Rocha stream (RS), whereas the brackish water site in the Northern part of

Laguna de Rocha (LN) served as the main location for sampling and incubations. Conductivity was measured *in situ* with a portable meter (ES-12; Horiba Inc., Irvine, CA, USA).

Experimental set up. Water samples from LN were distributed among six sets of triplicate mesocosms (PVC tubes, 4.5 l) that represented different treatments with respect to added carbon and nutrient sources. Nitrogen was added in the form of either NH_4Cl (Treatment 1), or urea (Treatment 2) at a final *N* concentration of 150 $\mu g\,l^{-1}$. Phosphorous was added to Treatment 3 as KH_2PO_4 at a final *P* concentration of 50 $\mu g\,l^{-1}$. To treatment 4 a macrophyte concentrate was added, which was prepared as follows: Five submerged stems of *S. californicus* (15–20 cm length) from the lagoon were homogenized using a lab blender and 500 ml of sterile Milli-Q water. Homogenates were pre-filtered through a 50 μm mesh to remove debris. The resulting crude extract was sterilized by filtration through 0.2 μm polycarbonate filters (Millipore) that had previously been soaked overnight in 10% HCl and rinsed with Milli-Q water. The plant extract was then added to a final concentration of 10% (v/v). Treatment 5 consisted of a 50:50 mix of LN and RS water, and Treatment 6 was a control of unmodified LN water.

The incubations were run for 42 h under *in situ* light and temperature conditions. Samples were taken at the beginning (t_0) and at regular intervals during the incubations (4, 8, 16, 24, 32, and 42 h) in order to follow (i) the biomass and composition of primary producers, (ii) bacterial abundance and the relative proportions of bacteria with different nucleic acid content, (iii) abundance of main bacterial groups, (iv) the abundances and predatory activity of bacterial grazers, and (v) viral abundances (see **Table A2** in Appendix for details on which samples were analyzed by which methods).

Biomass of primary producers. The relative amounts of phytoplankton chlorophyll-a (Chl*a*) and phycocyanin (PC) were estimated *in vivo* using an AquaFluor (Turner design) fluorometer.

Abundances of bacteria and picocyanobacteria. Bacterial and picocyanobacterial abundances were determined by flow cytometry. Samples for heterotrophic bacteria were stained with a dilution of dimethyl sulfoxide-Syto13 (Molecular Probes) at 2.5 mM, while picocyanobacterial abundance was determined from unstained portions of the same samples. All evaluations were done with a CyAn™ADP analyzer equipped with a 488 nm laser. Bacteria and picocyanobacteria were detected in cytometric biplots of 90° light scatter (SSC) vs. green (FL1) and red (FL3) fluorescence, respectively (Gasol et al., 1999). Three different populations of heterotrophic bacteria with contrasting content of nucleic acids (HNA, MNA, and LNA) were distinguished according to the intensity of the green fluorescence signal.

Abundance of main bacterial groups. Based on the data on bacterial abundance and proportions of HNA and LNA cells determined by flow cytometry, a subset of samples was selected for analyzing bacterial community composition by CARD-FISH and automated microscopy as described above (see **Table A1** in Appendix for details on the targeted groups).

Abundance and bacterivory of protistan grazers. Estimates of grazing rates by heterotrophic flagellates (HF) on bacteria were determined from the ingestion of fluorescently labeled bacteria (FLB; Sherr and Sherr, 1993). FLB were prepared as previously described (Sherr and Sherr, 1987) from a *Brevundimonas diminuta* (syn. *Pseudomonas diminuta*) strain obtained from the Spanish Type Culture Collection (Burjassot, València) and kept frozen (−20°C) until use. The size of FLB was assessed by epifluorescence microscopy and using image analysis (mean cell volume ± 1 SD: 0.083 ± 0.053 μm^3).

Grazing experiments were performed in sub-samples of 30 ml from each treatment at t0, t3, and t6. Tracers were added at about 20% of natural bacterial concentrations. Samples were fixed after 15 min of incubation with 4% cold glutaraldehyde (2%, final concentration), filtered through a 0.8 μm pore-size polycarbonate filter (Nucleopore, Whatman) and stored at −20°C until analyzed. Prior to the processing of filters by epifluorescence microscopy they were mounted on slides with a drop of Vectashield immersion fluid (Vecta Laboratories Inc.) mixed with DAPI. HF were enumerated at 10,040× magnification, and ingested FLB were counted at the same time. A mean of 80 HF were inspected on each filter, yielding about 30 ingested FLB. Ingestion rates (FLB $HF^{-1}\,h^{-1}$), clearance rates (nl $HF^{-1}\,h^{-1}$), and specific grazing rates (SGR, bacteria $HF^{-1}\,h^{-1}$) were estimated as described before (Unrein et al., 2007). Grazing impact on bacteria was estimated by multiplying SGR by the HF abundance (bacteria $ml^{-1}\,h^{-1}$). Bacterial turnover rates (% day^{-1}) were estimated by expressing the extrapolated daily grazing impact as a percentage of the corresponding bacterial abundance. Bacterial net growth rate (h^{-1}) was calculated by dividing the change in bacterial abundance (Ln transformed) between t6 and t0 by the incubation time. Net bacterial production (NBP; bact. $ml^{-1}\,h^{-1}$) was estimated by multiplying the net growth rate by the bacterial abundance at t0.

Viral abundance. Samples for virus counting were fixed with PFA at the final conc. of 1% and stored at −80°C until analyzed. Fixed samples were filtered onto 0.02 μm pore-size Anodisc filter and stained with SYBR Gold according to Chen et al. (2001). Viral counting was done using an inverted epifluorescence microscope (Olympus IX81), at least 1000 SYBR Gold-stained viral-sized particles were counted for each sample (Chen et al., 2001; Patel et al., 2007).

Statistical analyses. All statistical analyses were performed using GraphPad Prism version 5.0 for Mac OS X, GraphPad Software (San Diego, CA, USA). If required, data were log- or arcsine-transformed prior to analyses in order to approximate normality (Kolmogorov–Smirnov test); if this was not obtainable, parametric tests were replaced by their non-parametric alternatives such as Mann–Whitney U test for paired comparisons (M–W) and Kruskal–Wallis ANOVAs for multiple comparisons (K–W). *Post hoc* comparisons between samples after ANOVA were performed using Bonferroni tests. For experiment 1, a one-way ANOVA was performed to determine if differences in BCP between treatments were significant ($P \leq 0.05$). Differences in bacterial abundances between incubation times were analyzed by K–W followed by M–W for paired comparison. The differences between

the relative abundance of each bacterial group between incubation times and treatments were analyzed using two-way ANOVAs. For experiment 2, differences in bacterial abundance and carbon production between treatments were analyzed by one-way ANOVAs. The differences between treatments in the relative abundances of bacterial groups and of active bacteria were analyzed by two-way ANOVAs. For experiment 3, the differences in chl*a* concentration, cyanobacterial, and bacterial abundance among treatments and at different incubation times were analyzed by two-way ANOVAs.

RESULTS

EXPERIMENT 1. FULL TRANSPLANT OF BACTERIAL COMMUNITIES

Chemical characteristics of water at the study sites and in the dialysis bags

In parallel with higher conductivity, almost six times lower NO_3 concentrations were observed in LN than in RS (**Table A3** in Appendix), whereas total nitrogen was 2.6 times higher in the lagoon compartment than in the stream. All other parameters were similar at both sampling sites. A rapid convergence to the chemical conditions of the local environment was observed in the transplanted bags containing water from the RS site and incubated at the LN site: conductivity inside these bags rapidly increased, from freshwater levels (0.15 mS cm^{-1}) to the values registered in the lagoon *in situ* and inside the bags in the LN–LN incubations (9.3 mS cm^{-1}; **Figure A1** in Appendix).

Bacterial abundances and carbon production

Bacterial *in situ* abundances were slightly higher at the LN site (**Figure 1**). Of the unfiltered (+G) treatments only the incubations RS–RS, RS-LN, and Mix-LN showed significant increments in bacterial abundances during the experiment (**Figure 1**). In the filtered (−G) treatment of RS–RS, total bacterial abundance increased by more than fourfold, and was also significantly higher than in the corresponding +G treatment (**Figure 1**). Bacterial abundances in the −G treatment of RS-LN was also significantly higher after 48 h of incubation than *in situ*; however, there was no significant difference between +G and −G in the RS-LN treatment.

Lagoon samples showed higher *in situ* BCP than stream samples (0.15 ± 0.09 and 0.75 ± 0.62 $\mu gC l^{-1} h^{-1}$, respectively; **Figure 2**), but this difference was not statistically significant. BCP in RS–RS and RS-LN rose significantly after 72 h in both, +G and −G, treatments. The carbon production values were very similar for a given treatment in both environments. For the incubations containing lagoon water (LN–LN and Mix-LN) the increment in bacterial production was only significant in the −G treatments, where the highest values were observed (**Figure 2**).

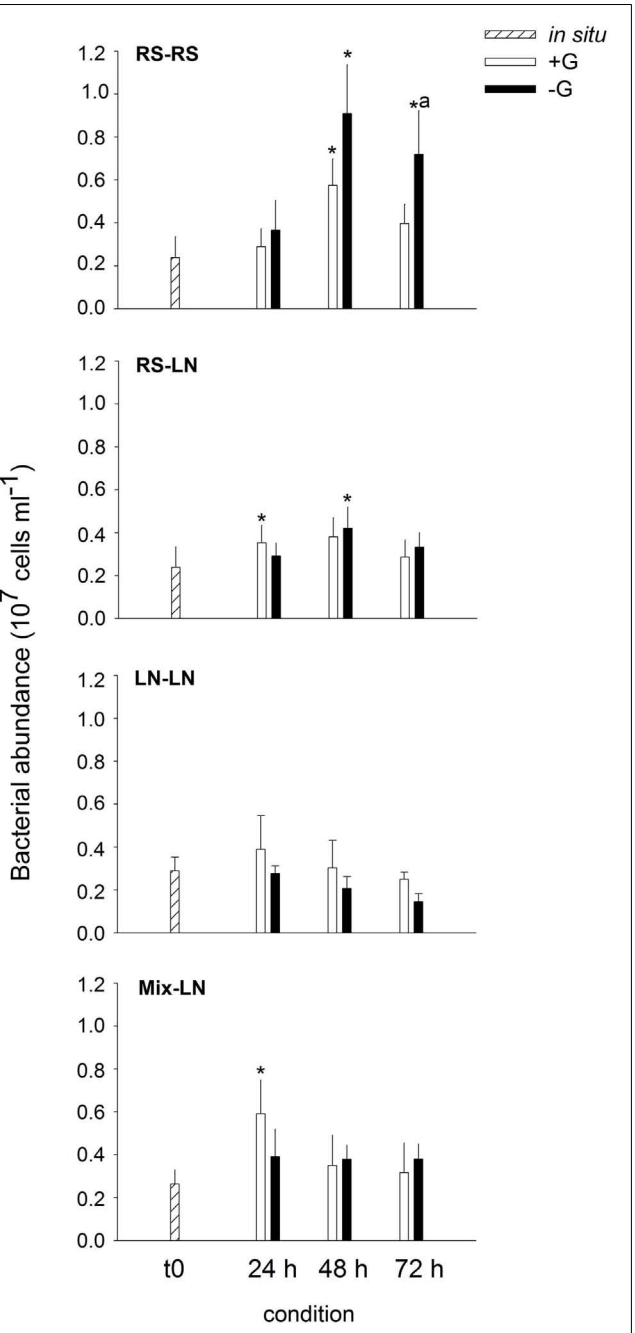

FIGURE 1 | Exp. 1, Evolution of bacterial abundance along the different incubations. * Means a significant difference with respect to t0, [a] means a significant difference between the corresponding +G and −G treatments.

Bacterial community composition and dynamics of the main bacterial groups

The two sampling sites differed in their *in situ* bacterial community composition. The lagoon was characterized by a higher proportion of *Alphaproteobacteria* and *Cytophaga-Flavobacteria*, while a higher proportion of *Betaproteobacteria* was found in the stream community (**Figure 3**). In all four treatments, after 72 h, the bacterial community composition of the unfiltered (+G) samples was more similar to the *in situ* community found at the incubation site on the corresponding sampling day than to the original community enclosed in the dialysis bags at the beginning of the incubation (**Figure 3**).

The main bacterial groups responded differently to the incubations (**Figure 4**). *Alphaproteobateria* from the RS community were similarly and moderately stimulated in RS–RS and RS-LN incubations, whereas this group was highly stimulated in the water from the LN site (LN–LN and Mix-LN) when incubated at the LN site (**Figure 4A**). *Betaproteobacteria* from the RS community

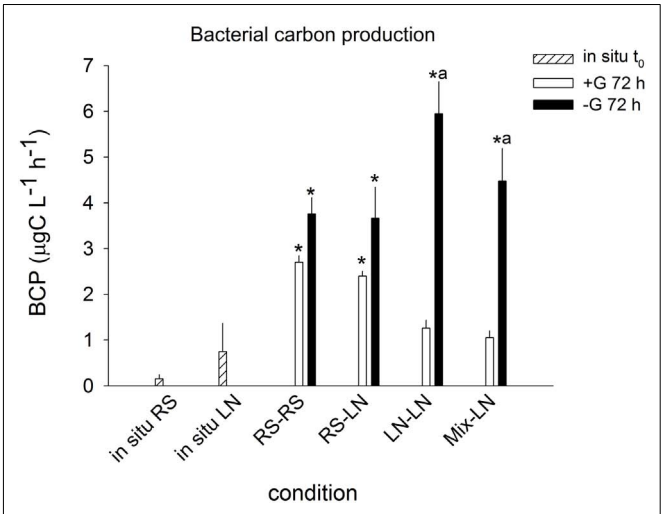

FIGURE 2 | Exp. 1, Comparison of bacterial carbon production *in situ* and after 72 h of incubation for the different treatments. * Means a significant difference with respect to t0, [a] means a significant difference between the corresponding +G and −G treatments.

showed a rapid and strong stimulation in both, the RS–RS and the RS-LN incubations, while their counterparts from the LN site were only belatedly and moderately stimulated in the LN–LN incubation (**Figure 4B**). *Gammaproteobacteria* were stimulated at all incubations. The most pronounced responses were observed when members from both, the RS site and the LN site, were incubated at their habitat of origin (RS–RS and LN–LN incubations, respectively; **Figure 4C**). *Cytophaga-Flavobacteria* was the group least stimulated by the treatments involving incubation in the habitat of origin. However, when members of this lineage from the RS compartment were incubated at the LN site their proportion in the community tripled with respect to the RS original community (**Figure 4D**).

When the relative abundances of the different bacterial groups were compared, they also showed different responses to the absence or presence of predators in the different incubation sites (**Figure 5**). With the exception of the LN–LN incubation, *Alphaproteobacteria* were generally more stimulated in the presence than in the absence of predators (**Figure 5A**). By contrast, *Gammaproteobacteria* were always much more stimulated in the absence of predators (**Figure 5C**). *Cytophaga-Flavobacteria* were also stimulated in the absence of predators, but only significantly so in the incubations where RS water was transferred to the LN site (**Figure 5D**). *Betaproteobacteria* of the RS community were stimulated more in the absence of predators, while they were preferentially stimulated in the presence of predators in the LN community (**Figure 5B**).

EXPERIMENT 2. GRADUAL MIXING OF BACTERIAL COMMUNITIES MIMICKING AN ENVIRONMENTAL GRADIENT

Since the sand bar was closed at the time of sampling the differences between both sampling points were relatively minor, conductivity being the most diverging variable (**Table A3** in Appendix).

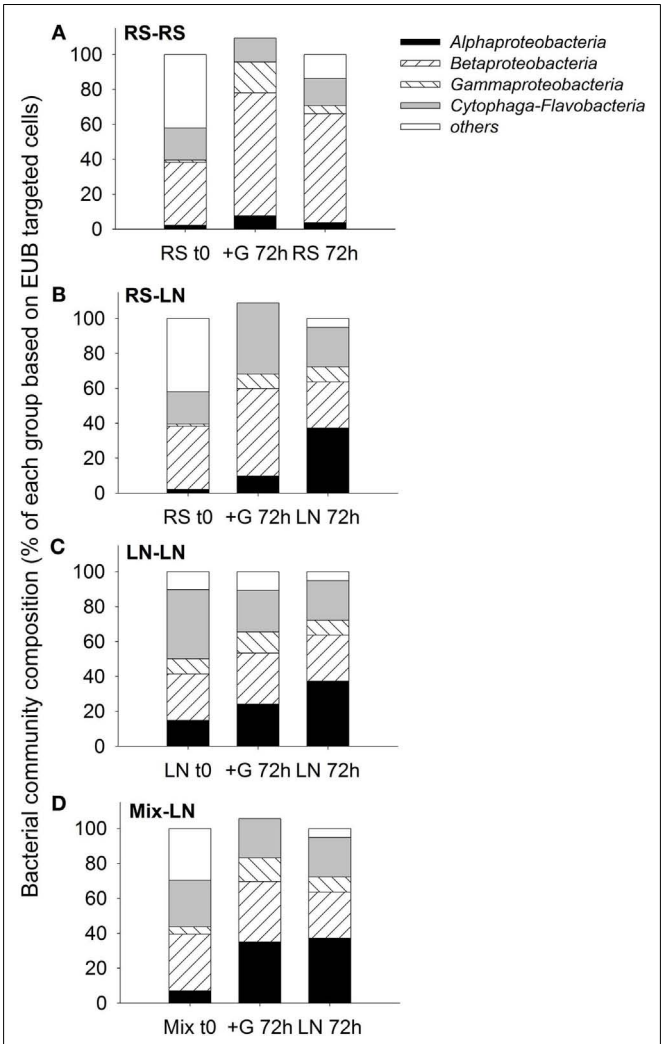

FIGURE 3 | Exp. 1, Comparison of bacterial community composition from the initial *in situ* (t_0) samples with the incubated unfiltered samples (treatment +G) after 72 h, and the *in situ* community of the corresponding incubation site after 72 h of the initial *in situ* sampling. (A) RS-RS incubation, **(B)** RS-LN incubation, **(C)** LN-LN incubation, and **(D)** Mix-LN incubation.

Bacterial abundance and BCP

Bacteria were about twice more abundant *in situ* at the LN site (100% LN); the maximal abundances was found in the treatment of 75% LN + 25% LS, and the minimal one in the treatment of 90% LS + 10% LN (**Figure 6A**). *In situ* BCP was also higher at the LN site; it was stimulated by mixing of water from LN with up to 25% of LS water (**Figure 6B**). For example, BCP in the 90% LN treatment was significantly higher than in both, the 100% LN and LS incubations, and in the 75% LN treatment it was significantly higher than in the 100% LS incubation.

Bacterial community composition and tracer incorporation at single cell level

Members of the *Alphaproteobacteria*, *Betaproteobacteria*, and *Cytophaga-Flavobacteria* constituted on average 66% of all cells

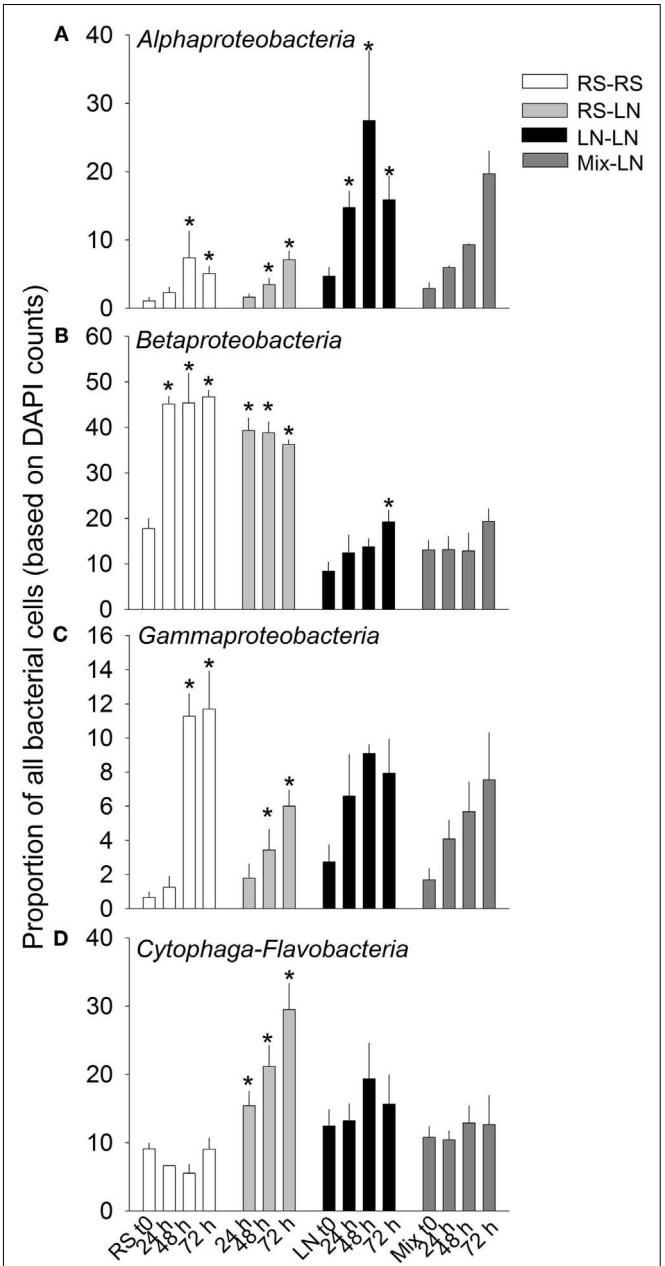

FIGURE 4 | Exp. 1, Abundance of the main bacterial groups during the incubation of the unfiltered (+G) treatments. *Means a significant difference with respect to t_0. **(A)** Alphaproteobacteria, **(B)** Betaproteobacteria, **(C)** Gammaproteobacteria, and **(D)** Cytophaga-Flavobacteria.

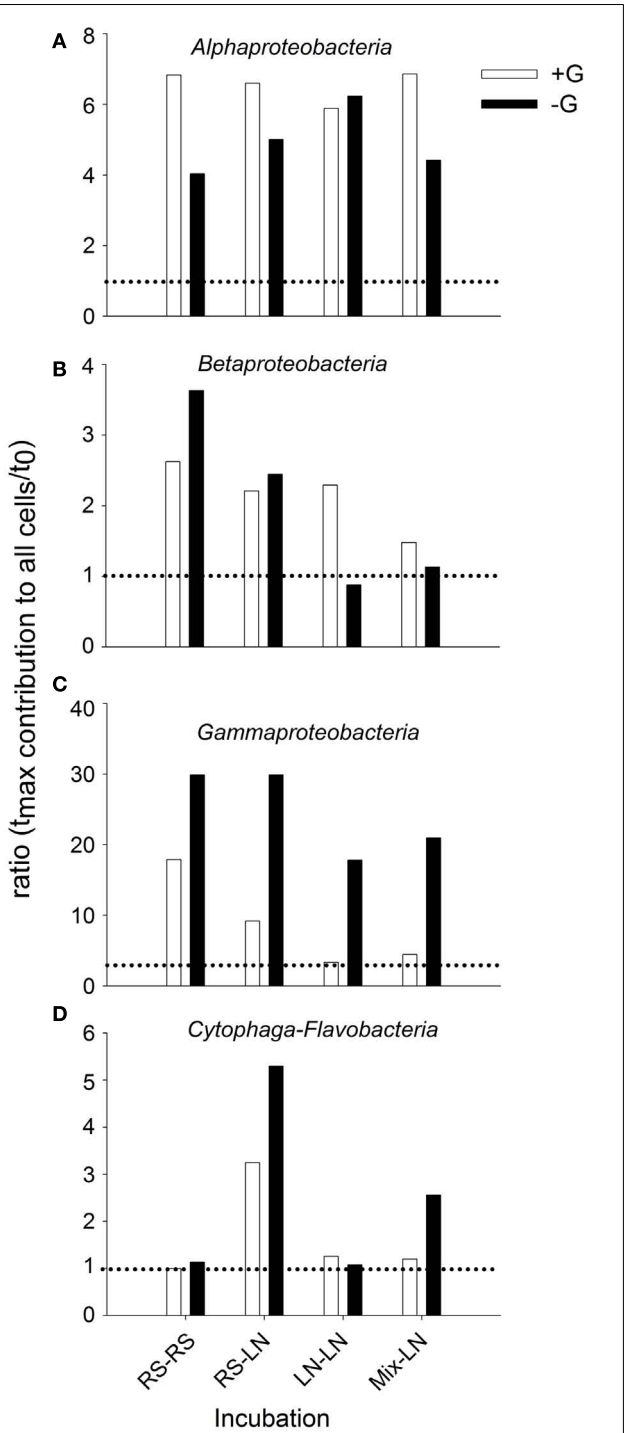

FIGURE 5 | Exp. 1, Ratios of maximal abundance over t_0 abundance for each of the main bacterial groups, comparing the changes observed for the unfiltered (+G) and filtered (−G) treatments. (A) Alphaproteobacteria, **(B)** Betaproteobacteria, **(C)** Gammaproteobacteria, and **(D)** Cytophaga-Flavobacteria.

hybridized with the general bacterial probe, without marked differences between both sampling sites (**Figure 6C**). However, treatment 75% LN diverged from the others due to an increased contribution of *Betaproteobacteria* (**Figure 6C**).

The gradual addition of LS water to LN water resulted in a stimulation of leucine incorporation by *Alpha-* and *Betaproteobacteria* (**Figure 7**). Both groups were particularly active in incubations containing up to 75% of LS water: in those treatments, the observed MAR+ cells were clearly above the level expected from the average activity in pure samples from both sites (100% treatments, **Figures 7A,B**). The lowest numbers of active cells in

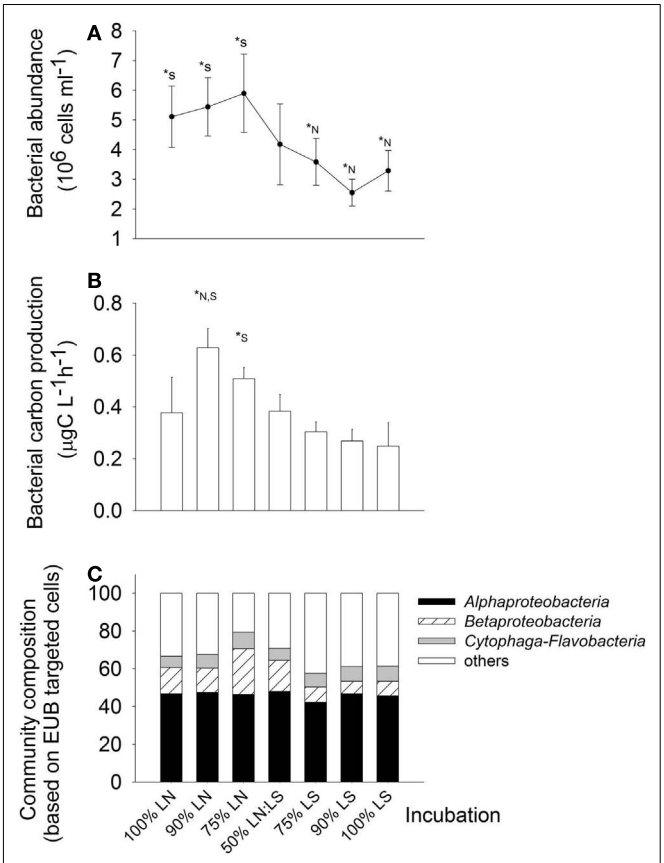

FIGURE 6 | Exp. 2, (A) Total bacterial abundance in the different treatments, (B) Bacterial carbon production for the different treatments, (C) bacterial community composition in the different treatments. * Indicate significant differences either to the 100% LN incubation (*N) or to the 100% LS incubation (*S).

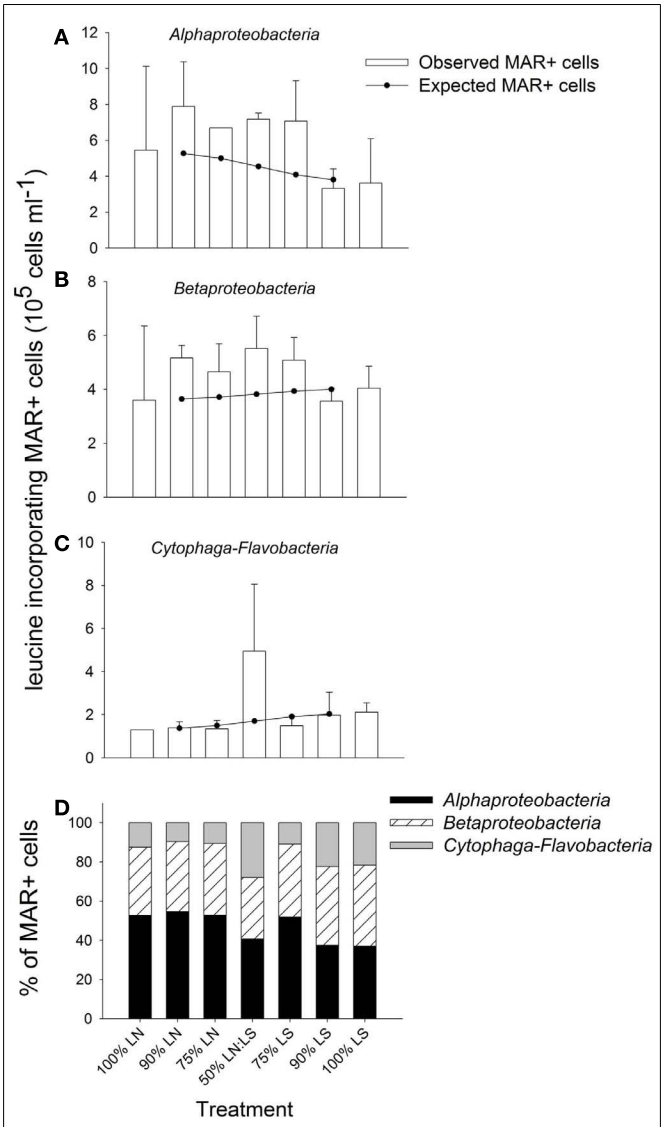

FIGURE 7 | Exp. 2, (A–C) Comparison of observed and expected numbers of MAR+ bacterial cells for each of the targeted groups and treatments. The expected numbers were calculated based on the results for the 100% LN and LS incubations, taking into account the percentage of each water sample in the different mix treatments. **(D)** Contribution of each group to all MAR+ cells per treatment. The total number of all MAR+ cells was calculated by summing the MAR+ cells of each of the targeted groups, as they constituted the great majority of all EUB cells.

these bacterial groups were found in incubations of 100% LS water. *Cytophaga-Flavobacteria* showed a high stimulation by the 50:50 mixing of water from LN and LS; the number of active cells from this lineage was clearly above the number expected from mere averaging in this treatment only (**Figure 7C**).

Alphaproteobacteria were the most important contributors to all leucine-incorporating (MAR+) cells in the incubations containing up to 75% LN water, while *Betaproteobacteria* was the most important group for incubations with a contribution of LS water >50% (**Figure 7D**). *Cytophaga-Flavobacteria* markedly increased their contribution in the 50:50 mixture, where the respective contributions of all three groups were most similar. *Cytophaga-Flavobacteria* exhibited highest changes in contribution with incubation conditions whereas *Betaproteobacteria* was the group with the most stable contribution in all treatments.

EXPERIMENT 3. MANIPULATION OF CARBON AND NUTRIENT SOURCES IN A MESOCOSMS

At the sampling time the RS and LN sites exhibited conductivities of 0.5 and 8.84 mS cm^{-1}, respectively. They also differed in the bacterial abundance (2.3×10^6 vs. 5.2×10^6, respectively).

Response of primary producers to the experimental manipulations

Chl*a* concentration followed a similar pattern over time in all treatments, reflecting the typical circadian cycle of chl*a* synthesis (**Figure A2A** in Appendix). Despite some differences in chl*a* concentration between treatments (i.e., the mix of LN and RS water exhibited the lowest chl*a* values and the treatment with addition of macrophyte extract the highest), none of the experimental manipulations lead to an excessive growth (i.e., a bloom) of primary producers. The picocyanobacterial abundance dropped by an average of ca. 40% (compared to the original abundance) over time

in all treatments, with the remarkable exceptions of treatment 5 (mix) in which even a moderate growth was observed (**Figure A2B** in Appendix).

Response of the bacterial community

Changes in abundance and community structure. Total bacterial number increased (average 6%) for all treatments at t1 (after 4 h; **Figure 8**). With the exception of treatment 4 (macrophyte conc. addition), this was the time point of highest bacterial numbers in the incubations. Maximal abundances in treatment 4 were found at t3 (16 h), being 15% higher than the respective abundance at t0 (**Figure 8**). A rapid decline on the bacterial abundance was observed already at t2 (8 h) in all variants except treatment 4, where the stimulation of bacterial growth was prolonged until t3 (**Figure 8**). At t6 (42 h, end of the experiment) the bacterial abundance was significantly reduced in all treatments, to less than half of the original value (**Figure 8**).

In addition, changes in phenotypic community structure were observed in terms of proportions of cells with high, medium, and low nucleic acid content (HNA, MNA, and LNA populations, respectively; **Figure 8**). The bacterial community at the end of the experiment was dominated by LNA cells in treatments 1 (ammonium), 2 (urea), and 3 (phosphate), and in the control treatment (**Figure 8**). By contrast, the dominant fraction in treatment 4 (macrophyte conc.) was constituted by HNA cells.

Dynamics of bacterial groups. The bacterial community *in situ* and in all treatments at t0 was dominated by *Alphaproteobacteria*, followed by *Actinobacteria*, *Cytophaga-Flavobacteria*, and *Betaproteobacteria*, and finally by *Gammaproteobacteria* (data not shown). The general decrease in the dominance *Alphaproteobacteria* during incubation was substantially attenuated in the treatment with macrophyte addition (**Figure 9A**). *Betaproteobacteria* at the maximal abundance time increased their abundance for the treatments with phosphate, macrophyte conc., and mix. At the decline time, the strongest drop in their abundance was observed for the treatment with macrophytes, and the most attenuated drop was encountered in the treatment with phosphate (**Figure 9B**). *Gammaproteobacteria* at the maximal abundance time showed an explosive growth in the macrophyte addition treatment, which they still maintained at the decline time (**Figure 9C**). A similar pattern was observed for *Actinobacteria* (**Figure 9D**). *Cytophaga-Flavobacteria* exhibited a constant decline in abundance which was also attenuated in the treatment with macrophyte addition, but especially in the treatments with ammonium or phosphate addition (**Figure 9E**).

Responses of heterotrophic flagellates and viruses. Heterotrophic flagellates abundances and grazing on the bacterial community were evaluated in selected samples. Although their numbers were very similar among treatments at t0 and t3, they

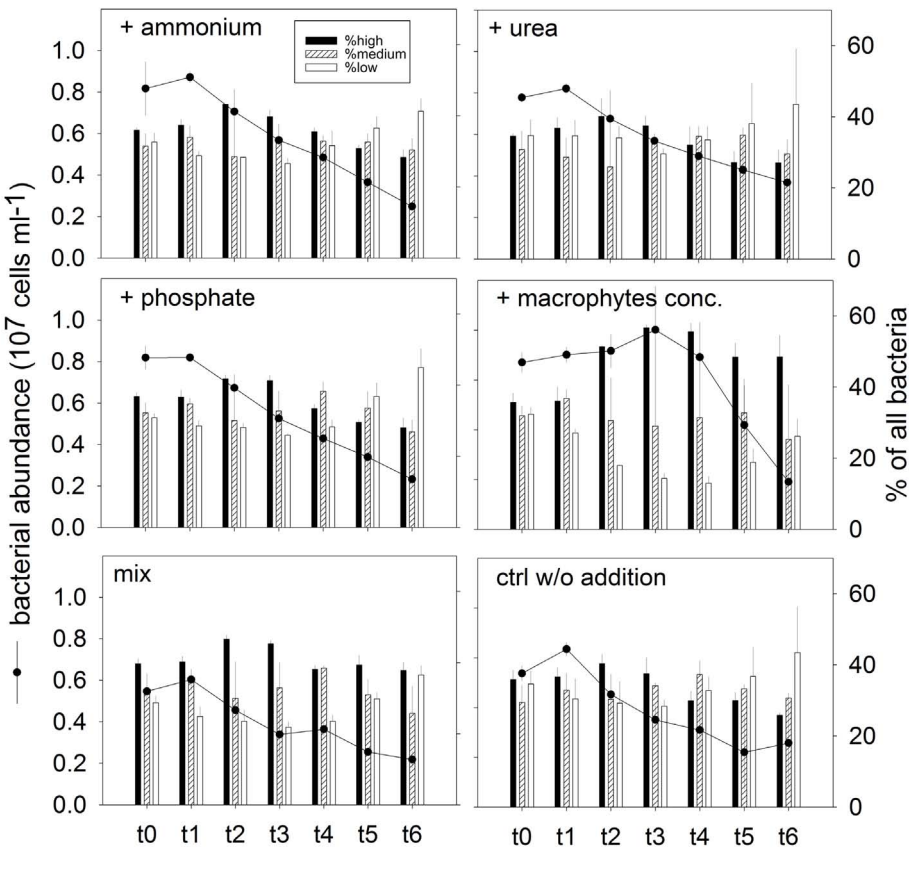

FIGURE 8 | Exp. 3, Total bacterial abundance (dots and lines) and community structure (bars), as determined by flow cytometry for the different treatments. The different populations represented by the bars were identified in the cytograms according to their nucleic acid content.

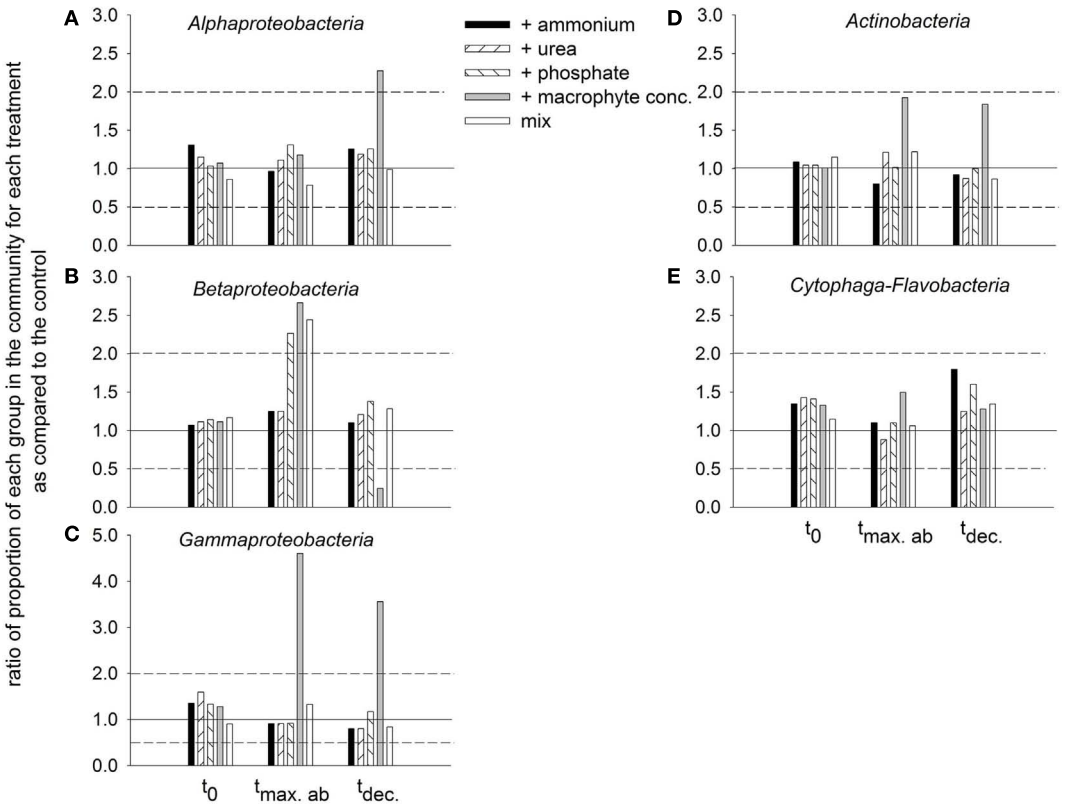

FIGURE 9 | Ratios of the proportion of the different groups in the bacterial community of each treatment in comparison to the control, at different incubation times (initial time = t_0, time of maximal bacterial abundance per treatment = $t_{max.ab}$, and time when bacterial abundance started to decline = t_{dec}.). The solid 1:1 line marks equality to the proportion observed at the control. The dashed lines mark twofold changes with respect to the control. **(A)** Alphaproteobacteria, **(B)** Betaproteobacteria, **(C)** Gammaproteobacteria, **(D)** Actinobacteria, and **(E)** Cytophaga-Flavobacteria.

exhibited explosive growth at the end of the experiment (t6) in the treatment with added macrophyte extract (**Figure 10A**). This pattern was reflected in the development of grazing rates and impact of the HF on the bacterial communities (**Figure 10B**). Toward the end of the experiment HF removed more than 200% of the bacterial standing stock per day in the treatment with macrophyte extract (**Table 1**). Bacterial abundance decreased in all treatments from t1 to t6, resulting in a negative NBP, i.e., a net loss of bacterial cell production along the experiment (**Figure 10B**). This loss was always higher than the HF grazing (**Figure 10B**), therefore HF grazing alone does not explain the drop in bacterial abundance, suggesting an alternative source of mortality for bacteria.

Viral abundances exhibited a twofold increase in the macrophyte conc. addition treatment toward the end of the experiment (t5) compared to t0, incrementing from 1.2×10^8 to 2.4×10^8 particles ml^{-1}. During the same period the viral abundance at the control treatment only rose from 1.3×10^8 to 1.7×10^8 particles ml^{-1}.

DISCUSSION

Although the study system did not exhibit its most extreme physicochemical zonation during our experiments, we nevertheless observed distinct differences in bacterial abundance, biomass production, and community composition between sites. Moreover, despite the low phylogenetic resolution of our community analysis (phyla, classes) specific response patterns of the different bacterial taxa to environmental changes (e.g., of hydrological conditions) could be clearly distinguished.

HYDROLOGY AFFECTING BACTERIAL COMMUNITIES AND CARBON TRANSFER TO HIGHER TROPHIC LEVELS

Sampling sites with contrasting salinities differed in bacterial community composition (Exp. 1, **Figures 3A–C**), with greater importance of *Betaproteobacteria* at lower conductivities, and of *Alphaproteobacteria*, *Cytophaga-Flavobacteria*, and *Actinobacteria* at the more brackish sites. This agrees with previous results from this system (Piccini et al., 2006) and from other estuarine environments (Kirchman et al., 2005; Alonso et al., 2010). The Northern site of the lagoon (LN) generally appears to be the zone of higher bacterial biomass production at oligohaline conditions, as compared to the meso-haline Southern sampling site (LS, Exp. 2, **Figure 6A**). This production is moreover seasonally dynamic, with higher values during summer (Exp. 1, **Figure 2**; Exp. 2, **Figure 6C**; Piccini et al., 2006). The difference between sites can mostly be explained by higher concentrations of nutrients and biomass of primary producers in the northern zone (Conde et al., 1999, 2000; Calliari et al., 2009), which in turn would fuel bacterial production (Cole et al., 1982).

In support of these *in situ* data, our experimental results also clearly indicated that bacterial abundances, community composition, and biomass production in Laguna de Rocha were highly dynamic, but tightly linked to hydrology: Microbial communities from the freshwater zone (RS) developed significantly different cell densities after 48 h of incubation at the brackish site (LN, Exp. 1, **Figure 1**), and the transplanted communities rapidly transformed to resemble the characteristic composition of the incubation zone (Exp. 1, **Figure 3**). Moreover, bacterial numbers, activity, and community composition changed even after only 8 h of incubation at particular degrees of mixing water from two brackish sites differing in salinity and nutrient levels (Exp. 2, **Figures 6A–C**; **Table A3** in Appendix).

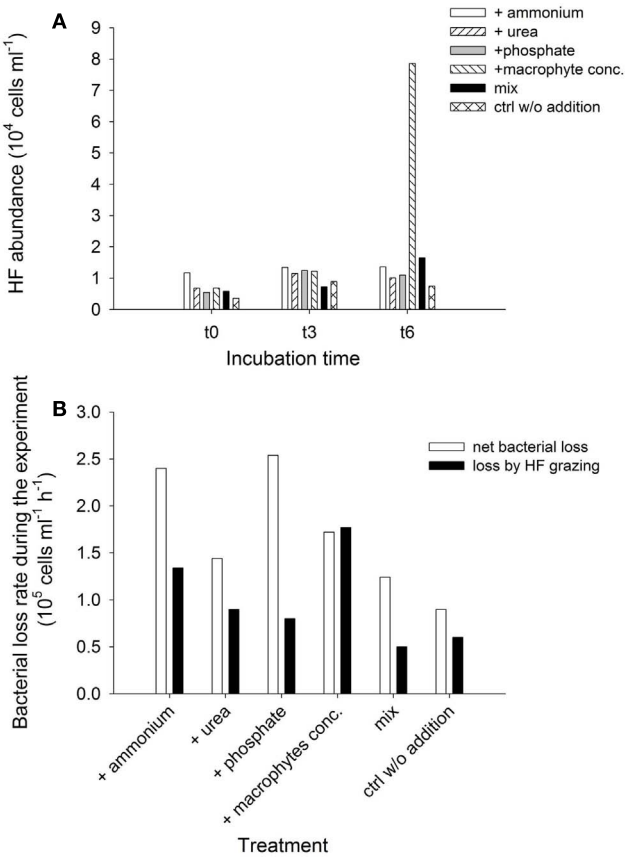

FIGURE 10 | Exp. 3, (A) Abundance of HF for selected times and treatments, (B) Comparison of net loss rate of bacterial abundance during the experiment and average loss rates due to HF grazing for each treatment.

While some freshwater microbes may also thrive under brackish conditions (Piwosz et al., in press), the studied bacterial taxa nevertheless differed in their sensitivity to environmental change: At more pronounced physicochemical differences between sites (Exp. 1) all three proteobacterial groups reached higher numbers if incubated in their original habitat (either freshwater or brackish; **Figures 4A–C**). Moreover, their activity was enhanced at a wide range of mixing ratios of water from two brackish sites (Exp. 2, **Figure 7**). By contrast, the abundances and activity of *Cytophaga-Flavobacteria* were particularly stimulated by strong environmental change, i.e., when the freshwater community was transplanted to the brackish site (Exp. 1, **Figure 4D**) or at a 50:50 mixing ratio of water from two brackish sites (Exp. 2, **Figures 7C,D**). A closer look into similar studies reveals parallels to some of our findings. When Gasol et al. (2002) transplanted freshwater communities between sites, the proteobacterial groups generally reached higher abundances when incubated in their original habitat, while *Cytophaga-Flavobacteria* were stimulated by habitat change, in particular if grazing pressure was concomitantly reduced (Gasol et al., 2002). Higher abundances of *Cytophaga-Flavobacteria* have also been found in the mixing fronts of estuarine systems (Bouvier and del Giorgio, 2002; Alonso et al., 2010). We extend these findings by showing that the contribution of *Cytophaga-Flavobacteria* to BCP may also increase at such conditions (**Figure 7C**).

Hydrology also seemed to influence the transfer of bacterial biomass to the upper trophic levels. Bacterial abundances of the freshwater community were highly stimulated if exposed at the freshwater site in the absence of predators, whereas this was not the case if assemblages were incubated at the oligohaline site (Exp. 1, **Figure 1**). However, although BCP was always significantly higher in the absence of predators, the differences between treatments with and without grazers were substantially more extreme in communities from the oligohaline site (**Figure 2**). BCP in the brackish community incubated without grazers was in fact comparable to that in highly eutrophic systems (Furtado et al., 2001). Our results thus suggest that protistan grazing controlled bacterial

Table 1 | HF grazing rates and impact on the bacterial abundance.

Treatment	Clearance rate (nl HF^{-1} h^{-1})			Specific grazing rate (Bact. HF^{-1} h^{-1})			Grazing impact (10^5 Bact/ml^{-1}h^{-1})			% of daily grazing impact (% Bact/day^{-1})		
	t0	t3	t6	t0	t3	t6	t0	t3	t6	t0	t3	t6
1	2.34	1.18	1.41	22.6	6.6	3.6	2.6	0.9	0.5	47	38	46
2	1.18	2.57	1.99	9.2	15.0	4.9	0.6	1.7	0.5	19	71	48
3	1.10	2.57	1.57	8.7	13.0	3.5	0.5	1.6	0.4	14	77	41
4	1.72	0.83	1.28	15.2	9.4	4.0	1.0	1.1	3.1	28	24	241
5	1.75	1.56	1.72	9.8	6.5	3.1	0.6	0.5	0.5	24	27	68
6	0.89	2.05	2.10	6.3	10.7	6.3	0.2	1	0.5	8	44	38

abundances at the freshwater site, while other factors, e.g., viral lysis or resource limitation, prevented a translation of biomass production into higher bacterial numbers in the brackish water assemblage.

Differences in grazing pressure along (freshwater) productivity gradients have been mainly attributed to differences in grazer abundances (Gasol et al., 2002), in particularly of HF (Jezbera et al., 2005), but may also be associated with qualitative changes in the grazer community. Rapid successions of HF populations with contrasting dietary preferences have been observed in parallel with salinity changes in an estuarine system (Piwosz and Pernthaler, 2010). Hydrology also played a role in controlling both, the abundances and community structure of micro-zooplankton ($>10\,\mu m$) in Laguna de Rocha (Britos, 2010). Changes in the hydrology, and therefore in salinity, might thus affect survival of protistan grazers, temporary releasing bacteria from grazing control. This in turn might eventually favor the growth of opportunistic, fast-growing bacteria (Eilers et al., 2000; Beardsley et al., 2003), as, e.g., suggested by the significant increase of *Gammaproteobacteria* in our bacterivory-free treatments (Exp. 1, **Figure 5**). These bacteria were most strongly affected by predation during incubations in both, freshwater and brackish environments (**Figure 5C**), particularly in the latter one. In fact, a release from top-down control, e.g., after marine intrusions, might be responsible for the extreme bloom of a single gammaproteobacterial species that has been observed in Laguna de Rocha (Piccini et al., 2006).

Interestingly, an apparent competition for resources between *Beta-* and *Gammaproteobacteria* in the brackish zone seemed to be modulated by grazers. At *in situ* conditions, i.e., presumably under resource shortage *Betaproteobacteria* clearly outperformed *Gammaproteobacteria* in the presence of predators, while the opposite was true in their absence (Exp. 1, **Figure A3** in Appendix). By contrast, under resource-rich conditions and high predation (Exp. 3, addition of macrophyte conc., **Figures 9B,C**) *Gammaproteobacteria* were favored over *Betaproteobacteria*. These findings indicate that members of both groups might share a similar ecological niche, and that the outcome of competition is determined by the interplay of resource availability and intensity of grazing pressure.

Actinobacteria in experiment 3 experienced the lowest overall decline in all treatments, indicating that they may prevail under carbon-limited conditions and/or sustained grazing pressure. These conclusions are supported by previous observations that *Actinobacteria* are able to grow on unusual carbon sources, and might thereby even profit from the degradation activity of other bacteria (Beier and Bertilsson, 2011; Eckert et al., in press). In addition, bacteria from this lineage are typically less affected by protistan grazers, e.g., they are underrepresented in the food vacuoles of heterotrophic nanoflagellates (Jezbera et al., 2006), possibly due to their minute cell size (Posch et al., 2007) and gram-positive cell wall (Tarao et al., 2009).

In conclusion, the studied phylogenetic groups of bacteria appeared to differ in their control by bottom-up or top-down mechanisms, which in turn were tightly dependent on the hydrology of the system (summarized in **Table 2**).

ENVIRONMENTAL FACTORS LINKED TO HYDROLOGICAL DYNAMICS THAT POSSIBLY AFFECT MICROBIAL ASSEMBLAGES

Conductivity was the most obviously changing factor between the different sampling sites; it represents a main structuring element of microbial assemblages in aquatic systems (Barberán and Casamayor, 2010), particularly of estuarine ones (Alonso et al., 2010). However the sites also differed in, e.g., nutrient concentrations (**Table A3** in Appendix). The mixing of water masses during marine intrusion moreover produces strong shifts in turbidity, as well as in chlorophyll-*a* and DOC concentrations (Conde et al., 2000), thus potentially affecting bacterial responses to habitat change beyond the direct and indirect effects of salinity.

Experiment 3 helped to further disentangle the respective influences of different factors that usually change during the hydrological cycle on the bacterial communities. As no bloom of primary producers developed at any experimental condition (**Figure A2** in Appendix), the responses of the bacterial community were likely direct consequences of the additions of the different nutrients and carbon sources. Although N and P have been shown to be limiting in Rocha Lagoon at specific hydrological conditions (Aubriot et al., 2004; Bonilla et al., 2005), neither nutrient significantly affected the – likely top-down induced – decline of bacterial abundances or

Table 2 | Summary of factors controlling the abundance and activity of the main bacterial groups in the lagoon, according to whether the response was observed for limnic or brackish samples.

	Control mechanism	α-*proteobacteria*	β-*proteobacteria*	γ-*proteobacteria*	*Cytophaga-flavobacteria*	*Actinobacteria*
Limnic	Top-down (predation) Bottom-up (physicochemical conditions)	Not affected Not affected	Strongly affected Low salinity High phosphate and nitrate	Strongly affected Low salinity High phosphate and nitrate	Slightly affected Sudden salinity change	Not assessed Not assessed
Brackish	Top-down Bottom-up	Slightly affected High salinity Allochthonous DOC	Not affected Allochthonous DOC Low salinity Phosphate	Very strongly affected Allochthonous DOC Low salinity Urea	Very strongly affected Allochthonous DOC Ammonium	Slightly affected Allochthonous DOC Urea

The bottom-up factors shown are the ones which characterized the treatment were a positive response was observed.

substantially altered microbial community composition (as compared to the control treatment, **Figures 8** and **9**). By contrast, the macrophyte concentrate (containing both, organic carbon and nutrients) stimulated the development of a presumably highly active population of HNA cells (**Figure 8**) and led to conspicuous taxonomic changes in the bacterial assemblages (**Figure 9**).

Our results thus point to carbon availability as a major driver of changes in the abundance and composition of these microbial communities. Bacterial carbon limitation in the lagoon is also suggested by the molar ratios of dissolved organic carbon, nitrogen, and phosphorous collected during several years and phases (Conde et al., 2000, 2002; Bonilla et al., 2005). The average C:N:P molar ratio observed over a wide range of conditions was 114:15:1, suggesting a carbon proportion well below values in other comparable systems (Farjalla et al., 2006). While this proportion is arguably higher than the typical stoichiometry of bacterial biomass (50:10:1; Fagerbakke et al., 1996), a large fraction of DOC in aquatic systems is refractory to bacterial degradation (del Giorgio and Davis, 2003). Moreover, bacterial carbon demand (BCD) greatly exceeds the need for biomass production, since a substantial portion of the consumed carbon is lost via respiration (del Giorgio and Cole, 1998). The high proportions of chromophoric DOM (an indication of refractory material) of total DOM in Laguna de Rocha (Conde et al., 2000; Piccini et al., 2009), and bacterial growth efficiencies of 0.33, as estimated from the current and earlier data sets according to del Giorgio and Cole (1998) and Lopez Urrutia and Morán (2007), also indicate that bacterial growth was indeed limited by DOC. An increase of BCP accompanied by changes in BCC was also observed in Laguna de Rocha upon experimental alteration of DOC composition via photodegradation (Piccini et al., 2009), which is an important mechanism to transform refractory DOM into forms more available for microbial utilization (Lindell et al., 1995; Wetzel et al., 1995; Moran and Zepp, 1997).

It is noteworthy that the stimulation of the bacterial community by an external carbon source in exp. 3 was accompanied by a markedly higher grazing impact on the microbial community (**Figure 10B**), and by strong increases of the abundances of both, HF (**Figure 10A**) and viruses (as compared to the control). This indicates that the positive effect of additional carbon on bacterial growth was indeed rapidly transferred to other components of the microbial trophic web (**Figure 10B**; **Table 1**).

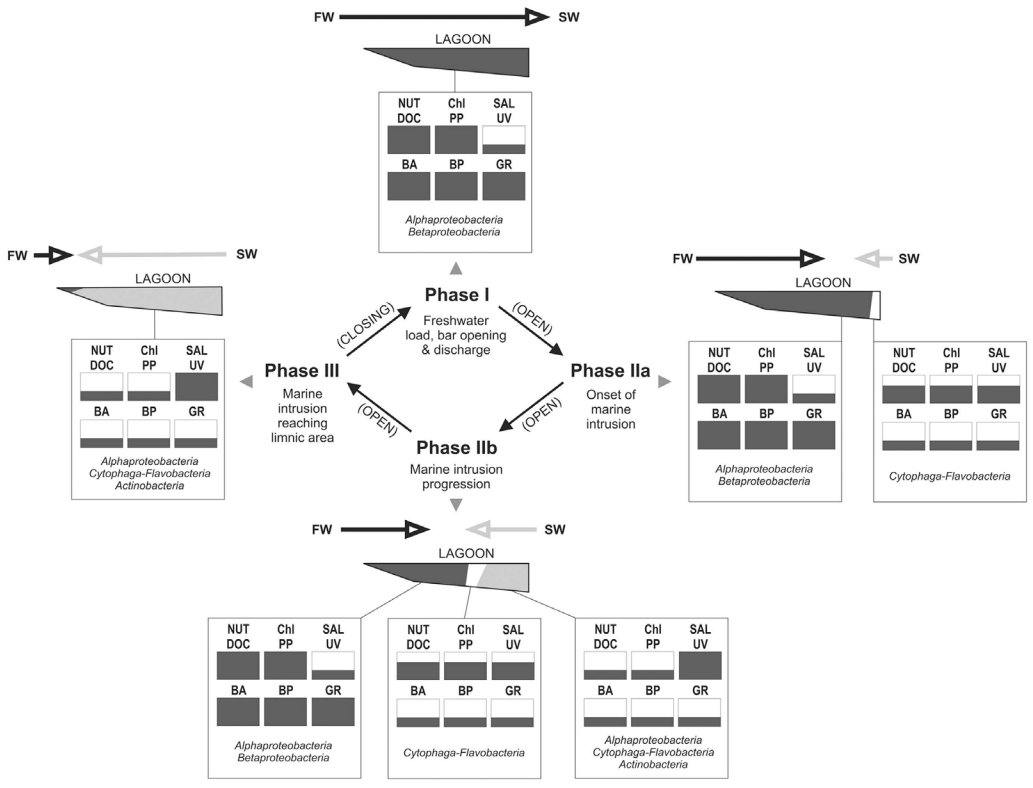

FIGURE 11 | Conceptualization of major hydrological phases occurring at Laguna de Rocha, with indication of changes in key abiotic variables and their consequences on the microbial community and processes, as derived from our data set. Each abiotic and microbial variable is indicated as being low, medium, or high (one third, to thirds or whole box in black, respectively). Most important hydrological processes are indicated for each phase. Main arrows indicate the extent and direction of the water movement (FW, freshwater input from the watershed; SW, seawater intrusion from the coastal zone into the lagoon; Nut, nutrients (N and P); DOC, dissolved organic carbon and chromophoric dissolved organic matter; Sal, salinity; UV, UV radiation; BA, bacterial abundance; BP, bacterial production; GRAZ, protist grazing on bacteria). The names of the bacterial groups indicate which would be the main components of the bacterial community in terms of abundance and carbon production at each depicted condition.

PROPOSED MODEL OF FUNCTIONING OF THE BACTERIAL GROUPS AND HYDROLOGY, FROM THE PERSPECTIVE OF CARBON PROCESSING

Based on the information from this and previous studies, we propose a model for the dynamics of the bacterial groups focusing on changes in microbial carbon processing along the hydrological cycle of the lagoon (**Figure 11**):

In Phase I (homogeneous oligohaline brackish situation) high phytoplankton production and terrestrial input are expected across the whole lagoon as discharge proceeds from the watershed (Conde et al., 1999, 2000; Calliari et al., 2009). This situation would imply high system-wide DOC concentrations, which in turn will be similarly affected by UV radiation, as the optical characteristics of the lagoon are homogeneous (Conde et al., 2000). During this phase we predict high bacterial abundances and production, and a strong grazing pressure, as observed for the oligohaline site in our experiments (Exp. 1, **Figures 1** and **2**; Exp. 2, **Figures 6A,C**). Microbial communities are expected to be dominated by *Alphaproteobacteria* and *Betaproteobacteria* (Exps. 1–3, **Figures 3**, **6** and **8**), which in turn would represent most of the cells contributing to BCP (Exp. 2, **Figure 7**).

During Phase II (marine intrusion) sudden environmental changes occur at the mixing front, derived from the mixing of water with low K, high chl*a*, high DOC, and high nutrients from the limnic zone with water with high K, low chl*a*, low DOC, and low nutrients from the marine intrusion (Conde et al., 2000). A transient stimulation of particular bacterial groups, especially of *Cytophaga-Flavobacteria*, both in abundance and contribution to BCP would be expected, as deduced from experiments 1 and 2 (**Figures 4** and **7**). In addition, a general relief of bacteria from grazing pressure is predicted (as observed in Exp. 1, **Figure 1**), with the exception of *Cytophaga-Flavobacteria* (possibly related to their higher per-cell activity; Exps. 1 and 2, **Figures 5** and **7**).

Once a zonation is established in Phase II, two very different habitats will form in the North and South of the lagoon (Conde et al., 2000). More terrestrial input and higher PP would be expected in the North (Calliari et al., 2009). Consequently more DOC would be available in this zone (Conde et al., 2000), albeit of a more recalcitrant quality (indicative of terrestrial origin, i.e., cDOM), which would not reach the Southern part of the lagoon (Conde et al., 2000). Due to progressive UV photodegradation (Piccini et al., 2009) this DOC is expected to increasingly stimulate carbon-limited bacterial growth (Exp. 3, **Figures 8** and **9**). Consequently, at this phase higher BP and bacterial abundance would be observed in the Northern oligohaline zone than in the Southern meso-polyhaline zone (as in Exp. 1 and 2, **Figures 1** and **6**). The community in the North would be dominated by *Alphaproteobacteria* and *Betaproteobacteria* in terms of abundance and also in terms of contribution to BCP (Exps. 1–3, **Figures 3** and **6–8**). In contrast, while the community would still be dominated by *Alphaproteobacteria* in the South, other important groups would be *Cytophaga-Flavobacteria* and possibly *Actinobacteria*. Although this latter group was only evaluated in the third experiment, a higher abundance of *Actinobacteria* in this zone has been previously observed after marine intrusions (Piccini et al., 2006). Moreover, there is also evidence from other aquatic systems that *Actinobacteria* adapt particularly well to strong UV radiation (Warnecke et al., 2005), a characteristic feature of the Southern zone in this phase (Conde et al., 2000). With respect to carbon transfer to upper trophic levels, a higher grazing pressure would be expected in the North (Exp. 1, **Figures 1** and **2**).

During phase III a homogeneous meso-polyhaline brackish lagoon is eventually established. Consequently, the whole system would develop the characteristics of the Southern zone in phase II (lower bacterial abundances and BCP, lower carbon transfer through grazing).

The best conditions for bacterial use of DOC, and carbon transfer to upper trophic levels would be expected during phase I, and during late phase II in the Northern zone. By contrast, the most pronounced carbon limitation of the bacterial community is expected to occur, during late Phase II in the South, and in the whole lagoon during the subsequent homogeneous polyhaline condition (Phase III). As the most frequent state of the lagoon is Phase II, carbon limitation would prevail in the Southern part of the lagoon during most of the hydrological cycle.

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APPENDIX

Table A1 | Oligonucleotide probes used in the different experiments.

Probe name	Target group	Reference	Used in experiment
ALF968	*Alphaproteobacteria*	Neef (1997)	1, 2, 3
BET42a	*Betaproteobacteria*	Manz et al. (1992)	1, 2, 3
GAM42a	*Gammaproteobacteria*	Manz et al. (1992)	1, 3
CF319a	*Bacteroidetes*	Manz et al. (1996)	1, 2, 3
HGC69a	*Actinobacteria*	Roller et al. (1994)	3
EUB I-III	Bacteria	Daims et al. (1999)	1, 2

Table A2 | Selected samples for the different evaluations of Exp. 3.

Treatment	Primary producers biomass and abundance of cyanobacteria	Bacterial abundance and community structure	Bacterial community composition	HNF abundance and grazing activity	Viral abundance
+Ammonium	t0, t1, t2, t3, t4, t5, t6	t0, t1, t2, t3, t4, t5, t6	t0, t1, t3	t0, t3, t6	
+Urea	t0, t1, t2, t3, t4, t5, t6	t0, t1, t2, t3, t4, t5, t6	t0, t1, t3	t0, t3, t6	
+Phosphate	t0, t1, t2, t3, t4, t5, t6	t0, t1, t2, t3, t4, t5, t6	t0, t1, t3	t0, t3, t6	
+Macrophyte concentrate	t0, t1, t2, t3, t4, t5, t6	t0, t1, t2, t3, t4, t5, t6	t0, t3, t5	t0, t3, t6	t1, t5
50:50Mix (RS:LN)	t0, t1, t2, t3, t4, t5, t6	t0, t1, t2, t3, t4, t5, t6	t0, t1, t3	t0, t3, t6	
Control without additions	t0, t1, t2, t3, t4, t5, t6	t0, t1, t2, t3, t4, t5, t6	t0, t1, t3	t0, t3, t6	t1, t5

Table A3 | Environmental characterization of the three sampling sites.

Sampling point[a]	Conductivity (mS cm^{-1})	SRP (μg l^{-1})	TP (μg l^{-1})	SiO_2 (μg l^{-1})	NH_4 (μg l^{-1})	NO_2 (μg l^{-1})	NO_3 (μg l^{-1})	TN (μg l^{-1})
RS (Exp. 1)	0.2	36.6 (4.8)	36.6 (2.2)	3389.2 (367.8)	7.4 (1.6)	1.2 (1.7)	39.0 (5.1)	52.9 (24.2)
LN (Exp. 1)	9.3	25.1 (1.3)	34.3 (2.5)	2283.3 (155.6)	11.2 (10.2)	1.1 (0.2)	6.7 (0.9)	138.3 (14.5)
LN (Exp. 2)	6.5	140.0	n.d	n.d	0	7.3	117.8	n.d
LS (Exp. 2)	11.9	194.7	n.d	n.d	0	2.6	131.1	n.d

Mean values and SD (between brackets) of the measured physicochemical characteristics at the different sampling points.

[a] *RS, Rocha stream (freshwater); LN, lagoon north (brackish water); LS, lagoon south (brackish water); TP, total phosphorous; TN, total nitrogen; SS, suspended solids; n.d, not determined.*

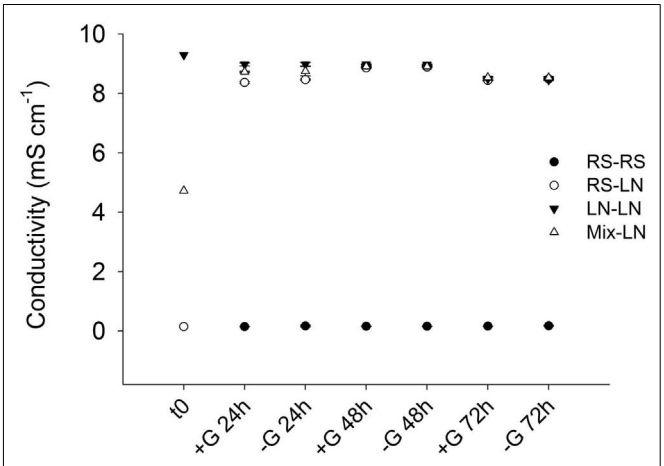

FIGURE A1 | Exp. 1, Evolution of conductivity inside the dialysis bags along incubation time.

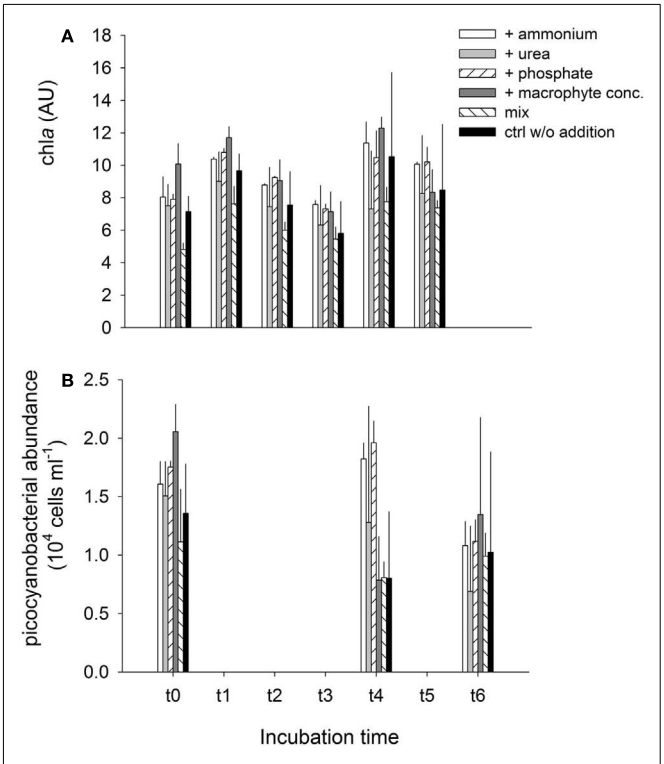

FIGURE A2 | Exp. 3, (A) Evolution of ch*la* concentration, and (B) Cyanobacterial abundance for the different treatments, during the experiment.

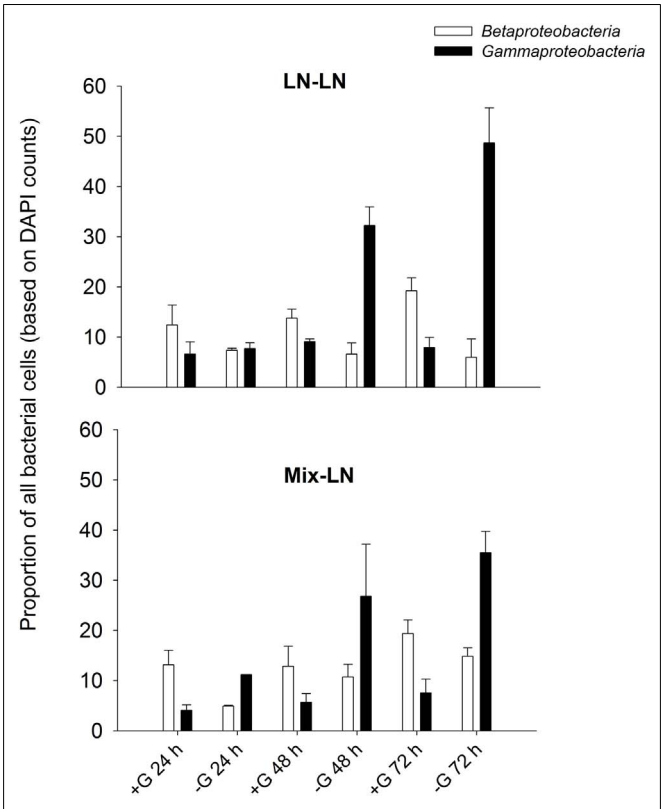

FIGURE A3 | Exp. 1, Percentages of *Beta-* and *Gammaproteobacteria* during incubations performed either with (+G) or without grazers (−G).

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Lower respiration in the littoral zone of a subtropical shallow lake

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Macrophytes are important sources of dissolved organic carbon (DOC) to littoral zones of lakes, but this DOC is believed to be mostly refractory to bacteria, leading to the hypothesis that bacterial metabolism is different in littoral and pelagic zones of a large subtropical shallow lake. We tested this hypothesis by three approaches: (I) dissolved inorganic carbon (DIC) accumulation in littoral and pelagic water; (II) O_2 consumption estimate for a cloud of points ($n = 47$) covering the entire lake; (III) measurement of O_2 consumption and CO_2 accumulation in dark bottles, pCO_2 in the water, lake-atmosphere fluxes of CO_2 (fCO_2) and a large set of limnological variables at 19 sampling points (littoral and pelagic zones) during seven extensive campaigns. For the first two approaches, DIC and O_2 consumption were consistently lower in the littoral zone, and O_2 consumption increased marginally with the distance to the nearest shore. For the third approach, we found in the littoral zone consistently lower DOC, total phosphorus (TP), and chlorophyll *a*, and a higher proportion of low-molecular-weight substances. Regression trees confirmed that high respiration (O_2 consumption and CO_2 production) was associated to lower concentration of low-molecular-weight substances, while pCO_2 was associated to DOC and TP, confirming that CO_2 supersaturation occurs in an attempt to balance phosphorus deficiency of macrophyte substrates. Littoral zone fCO_2 showed a tendency to be a CO_2 sink, whereas the pelagic zone showed a tendency to act as CO_2 source to the atmosphere. The high proportion of low-molecular-weight, unreactive substances, together with lower DOC and TP may impose lower rates of respiration in littoral zones. This effect of perennial stands of macrophytes may therefore have important, but not yet quantified implications for the global carbon metabolism of these lakes, but other issues still need to be carefully addressed before rejecting the general belief that macrophytes are always beneficial to bacteria.

Keywords: bacteria, macrophytes, phosphorus, chlorophyll *a*, humic substances, DOC, CO_2

INTRODUCTION

Subtropical shallow lakes may present an important and differentiated set of conditions that make them ecologically distinct from other types of lakes. Because of the shallow mean depths and the benign climate conditions, macrophytes can colonize extensive areas and grow continuously throughout the year. These plants are important sources of organic matter to the littoral zones; in many cases they contribute more carbon than do the algae (Wetzel, 1992; Lauster et al., 2006) and in some systems, they sustain most of the bacterial production (Stanley et al., 2003).

However, macrophyte-derived carbon is believed to be mostly in the form of high-molecular-weight polymer-like compounds (Bracchini et al., 2006), poor in nitrogen and phosphorus contents (Hessen, 1992), and therefore refractory to bacterial consumption. Studies finding that the presence of macrophytes can be detrimental to bacterioplankton are starting to accumulate. Wu et al. (2007) found lower bacterial diversity in submersed macrophyte-dominated areas than in areas with no macrophytes in the large Lake Taihu, China. Rooney and Kalff (2003) surveyed nine lakes with different percentages of macrophyte coverage, and found a significant decrease in the bacterial respiration rate with increasing macrophyte coverage. In southern Brazil, bacterial metabolism and biovolume were found to be lower in the littoral (covered with macrophytes) than in the pelagic zones of subtropical shallow lakes (They et al., 2010). If bacterial metabolism can be lower in the presence of macrophytes, there is a contradiction with the common belief that macrophyte-derived carbon always benefit bacteria, and it is reasonable to hypothesize that carbon cycling can be affected in the littoral zones of lakes that are extensively colonized by these plants.

Estimates of bacterial respiration are essential to assess the balance of a lake's metabolism because they provide a direct assessment of the fate of carbon within the system, reflecting its transfer from the organic to the inorganic pool (Jahnke and Craven, 1995). It is well established that plankton respiration increases with chlorophyll, phosphorus, and organic carbon concentrations in lakes (Pace and Prairie, 2005). The effect of primary production (in terms of chlorophyll *a*) on bacterial production has long

been recognized, even in systems dominated by terrestrial-carbon inputs (Kritzberg et al., 2005). Phosphorus can also play a role. High rates of respiration and CO_2 supersaturation have been associated with high humic content of the carbon pool, because they are an attempt to balance phosphorus deficiency in these low-nutrient-content substrates (Hessen, 1992). Moreover, the ratio of phosphorus to labile DOC can control partitioning between bacterial respiration and production (Cimbleris and Kalff, 1998; Smith and Prairie, 2004; del Giorgio and Newell, 2012). DOC concentration, in turn, is also positively associated with CO_2 supersaturation in lakes (Prairie et al., 2002).

In the present study, we tested the hypothesis that there is a difference in the general metabolism of carbon in the littoral versus pelagic zones in the large, subtropical shallow Lake Mangueira, through: (a) an experiment to measure dissolved inorganic carbon (DIC) accumulation in littoral and pelagic water; (b) measurement of respiration in a cloud of points covering littoral and pelagic zones; and (c) extensive temporal measurement of respiration, pCO_2, CO_2 fluxes, and limnological variables related to bacterial-carbon metabolism in a smaller cloud of points, also covering the whole lake.

MATERIALS AND METHODS

STUDY SITE

Lake Mangueira (80,800 ha, southern Brazil, state of Rio Grande do Sul) is a large, shallow, freshwater subtropical coastal lake. It is surrounded by extensive belts of wetlands, located primarily in the northern and southern areas. The DIC accumulation experiment (to test question a above) was done in the southernmost part of the lake, and the two clouds of points (to test questions b and c above) covered the entire lake (**Figure 1**).

DIC ACCUMULATION IN LITTORAL × PELAGIC WATER

Samples of pelagic and littoral water were collected and incubated for 14 days for determination of the cumulative DIC, in April–May 2007. We retrieved vials at days 0, 1, 3, 5, 7, 10, and 14, in triplicates ($n = 42$). The incubation vials (sterile 40 mL clear borosilicate vials, open top with silicone/PTFE septum and

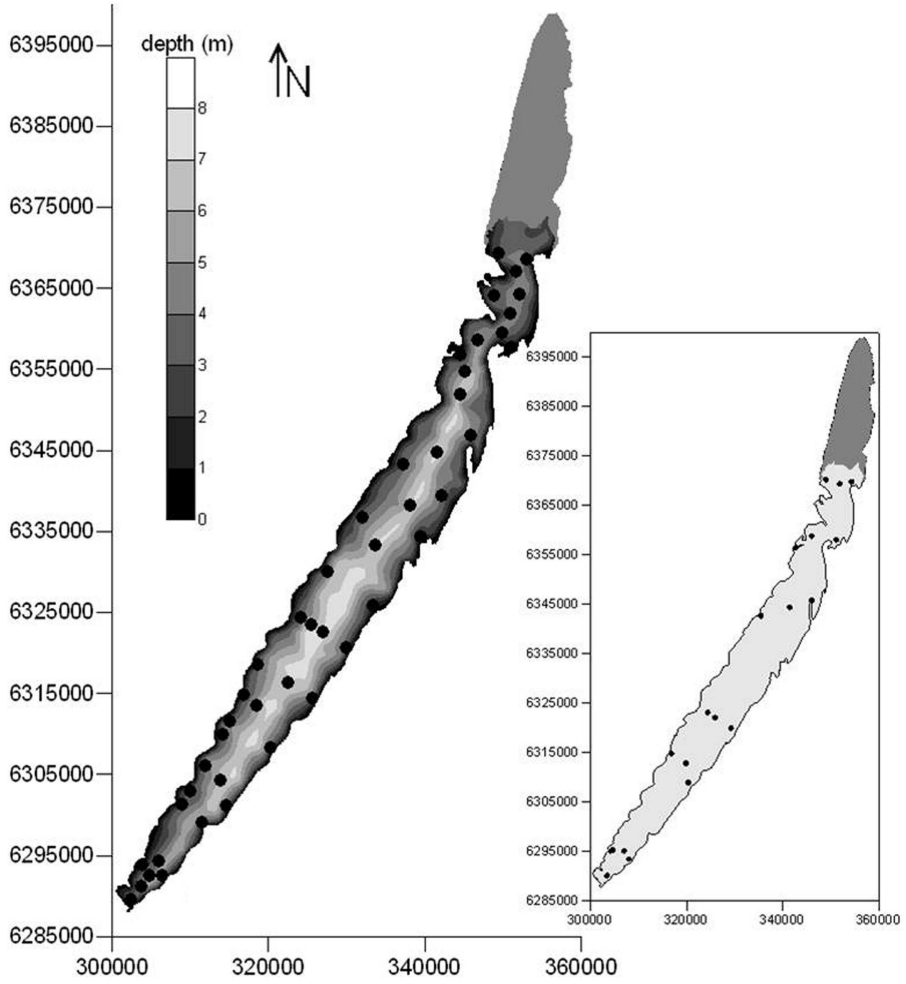

FIGURE 1 | Locations of sampling points for respiration rates sampled in March 2009 (main map), and extensive sampling campaigns of respiration rates and limnological variables carried out in 2010–2011 (inset) in Lake Mangueira, a large shallow lake in subtropical southern Brazil. Maps are georeferenced in UTM coordinate system.

teflon-lined screw caps) were carefully filled with no headspace (Farjalla et al., 2001) and incubated at room temperature (∼20°C) in the dark. DIC was analyzed in a Total Organic Carbon (TOC) Analyzer (Shimadzu VCPH). Bulk respiration rates were considered an approximation of bacterial respiration, since several pilot experiments with different water fractions showed no significant differences between the bulk and bacterial fractions after filtration in MN 640d Macherey–Nagel paper filters (2.0–4.0 μm mean retention size). This filtration has been extensively tested and successfully excludes ciliates, metazoans, and most flagellates.

RESPIRATION IN LITTORAL × PELAGIC ZONES

A large set of measurements of respiration rates, covering the entire Lake Mangueira, was taken in March 2009. The water was collected in the littoral and pelagic zones (littoral $n = 21$; pelagic $n = 26$) and the water was incubated in dark bottles, for 6 days in the laboratory (Wetzel and Likens, 2000). Dissolved oxygen was measured by a TOGA/SRI gas chromatograph (purge trap injector for water samples and TCD/HID detectors). Oxygen measurements were converted to carbon, assuming a molar conversion factor of 1.0 (del Giorgio et al., 1997). Results were expressed in $\mu g\, C\, L^{-1}\, h^{-1}$, assuming a constant respiratory rate.

Nineteen points in the littoral and pelagic zones (littoral $n = 12$; pelagic $n = 7$) covering the entire lake were collected for several limnological variables during seven campaigns: pH, Abs250, Abs365, Abs250:365, total phosphorus (TP), total nitrogen (TN), chlorophyll *a*, DOC, DIC, O_2 consumption, CO_2 accumulation, pCO_2 in the water, and CO_2 fluxes (fCO_2). With the exceptions of pCO_2 and CO_2 fluxes, all variables were collected in May, August, and November 2010 and March, May, August, and November 2011. pCO_2 and fCO_2 were estimated in February, May, August, and November 2010 and March, June, and August 2011.

Surface water was taken using a horizontal sampling bottle. pH was measured with a potentiometer (Wetzel and Likens, 2000). Abs250 and Abs365 refer to absorbance at 250 and 365 nm respectively, in 1 cm-optical path length quartz cuvettes. The former is proportional to the low-molecular-weight compounds, while the latter is proportional to the high-molecular-weight compounds present; and Abs250:365 is the ratio Abs250:Abs365 (Strome and Miller, 1978; Lindell et al., 1995). Samples for TN and TP were frozen in 1 L polyethylene bottles, and these nutrients were measured by colorimetry (Mackereth et al., 1989). Chlorophyll *a* was quantified through cold ethanol extraction (Jespersen and Christoffersen, 1987). DOC and DIC samples were collected in 30 mL precombusted (450°C for 1 h) amber glass bottles. DOC samples received a few drops of $H_3PO_4^{-3}$, and DIC and DOC were analyzed in the same TOC analyzer (Shimadzu VCPH).

Oxygen consumption and CO_2 accumulation were determined by incubation in glass serum bottles. The bottles were kept inside a thermal box (approximately 23°C) and taken to the laboratory, totaling 120 h of incubation. O_2 was measured in a TOGA/SRI gas chromatograph, and CO_2 indirectly from measurements of DIC and pH after correction of temperature and ionic strength (Stumm and Morgan, 1996). The measurements were made at the beginning and at the end of incubation. The ionic strength was estimated from the electrical conductivity of the water measured in the field, using the following conversion formula (Snoeyink and Jenkins, 1980):

$$IS \approx 1.6x10^{-5} \times \ Conductivity$$

where *IS* is the ionic strength and the water conductivity is measured in $\mu S\, cm^{-1}$.

The pCO_2 in the water was calculated based on the Law of Henry, with the following equation:

$$pCO_2^w = \frac{\alpha_0 DIC}{K_H}$$

where α_0 corresponds to the fraction of DIC as CO_2 and K_H is the dissolution constant of CO_2 expressed in moles $L^{-1}\, atm^{-1}$ corrected for temperature in kelvin (T_k) after the equation of Weiss (1974):

$$\ln K_H(CO_2) = -58.0931 + 90.5069\left(\frac{100}{T_k}\right) + 22.294\ln\left(\frac{T_k}{100}\right)$$

The flux of CO_2 (fCO_2, mmoles $C\, m^{-2}\, d^{-1}$) between atmosphere and lake was estimated with the following equation (MacIntyre et al., 1995):

$$fCO_2 = k_x(pCO_2^{atm} - pCO_2^w)$$

where pCO_2^{atm} is the partial pressure of CO_2 in the atmosphere (for 380 ppm), pCO_2^w is the partial pressure of CO_2 estimated for the surface layer of the lake, and k_x is the coefficient of mass transfer ($cm\, h^{-1}$), given by the following empirical relationship:

$$k_x = k_{600}\left(\frac{S_c}{600}\right)^{-x}$$

where x is equal to 0.66 for winds $<3.0\, m\, s^{-1}$ and equal to 0.5 for winds $>3.0\, m\, s^{-1}$, S_c is the number of Schmidt for CO_2, which is dependent on temperature (°C), according to the following relationship (Wanninkhof, 1992):

$$S_c\,(CO_2) = 1911.1 - 118.11T + 3.4527T^2 - 0.04132T^3$$

where k_{600} is estimated from wind velocity (Cole and Caraco, 1998):

$$k_{600} = 2.07 + \left(0.215\ U_{10}^{1.7}\right)$$

where U_{10} is the wind velocity ($m\, s^{-1}$).

Data from the Santa Vitória do Palmar meteorological station (www.inmet.gov.br), located 7.0 km from the lake, were used to obtain air temperature and wind velocity.

STATISTICAL TREATMENT

Differences between DIC accumulation in dark (littoral) and clear (pelagic) water samples and among different time periods for each type of water were tested with one-way non-parametric ANOVA (NPMANOVA in the univariate mode) test (Anderson, 2001). The dissimilarity measure used was Euclidean distance, with 9999 permutations. Software: PAST 2.14 (Hammer et al., 2001).

For the respiration data set, the mean rates of respiration in the littoral ($n = 21$) and pelagic zones ($n = 26$) were tested for difference with ANOVA after natural-log transformation (correction for normality). The association between rates of respiration (also transformed) of the cloud of points and the distances from the nearest margin was tested by linear regression (Software R 2.15.0, R Development Core Team, 2012).

The data from the O_2 consumption, CO_2 accumulation, pCO_2, CO_2 fluxes, and limnological variables obtained in the seven sampling campaigns were z-score standardized within each month and split into two categories, based on the distance from the nearest shore: littoral (<1000 m) and pelagic (≥1000 m). This threshold was based on the mean distance of the samples from the shore. Differences between the littoral and pelagic zones were tested for each variable individually (pH, Abs250, Abs365, Abs250:365, TP, TN, chl *a*, DOC, O_2, CO_2, fCO_2) by one-way NPMANOVA (Anderson, 2001). We used the software PAST v. 2.14 (Hammer et al., 2001) with Euclidean distance and 9999 permutations. *A posteriori* contrast tests used Bonferroni correction ($n \times p$, where n is the number of comparisons and p is the p value obtained by permutation) implemented in the software PAST 2.14 (Hammer et al., 2001).

The explanatory power of abiotic variables (pH, Abs250, Abs365, Abs250:365, TP, TN, chl *a*, and DOC) on O_2 consumption, CO_2 accumulation, pCO_2, and fCO_2 was assessed by binary recursive partitioning (regression tree) with the help of the R 2.15.0 (R Development Core Team, 2012) package {rpart} (Therneau et al., 2012) and following Crawley (2007). For O_2 consumption and CO_2 accumulation all months were included ($n = 133$); for CO_2 and fCO_2, given the unbalance in sampling months, February (2010) and June (2011) were excluded ($n = 95$).

RESULTS

DIC ACCUMULATION EXPERIMENT

The NPMANOVA test indicated that DIC was higher in the pelagic zone (23.24 ± 1.54 mg C L^{-1}) than in the littoral zone (16.82 ± 1.72 mg C L^{-1}), *pseudo-F* = 163.0, $p = 0.0001$. There was no change in DIC with time: Littoral zone: *pseudo-F* = 0.957, $p = 0.524$; pelagic zone: *pseudo-F* = 0.685, $p = 0.777$.

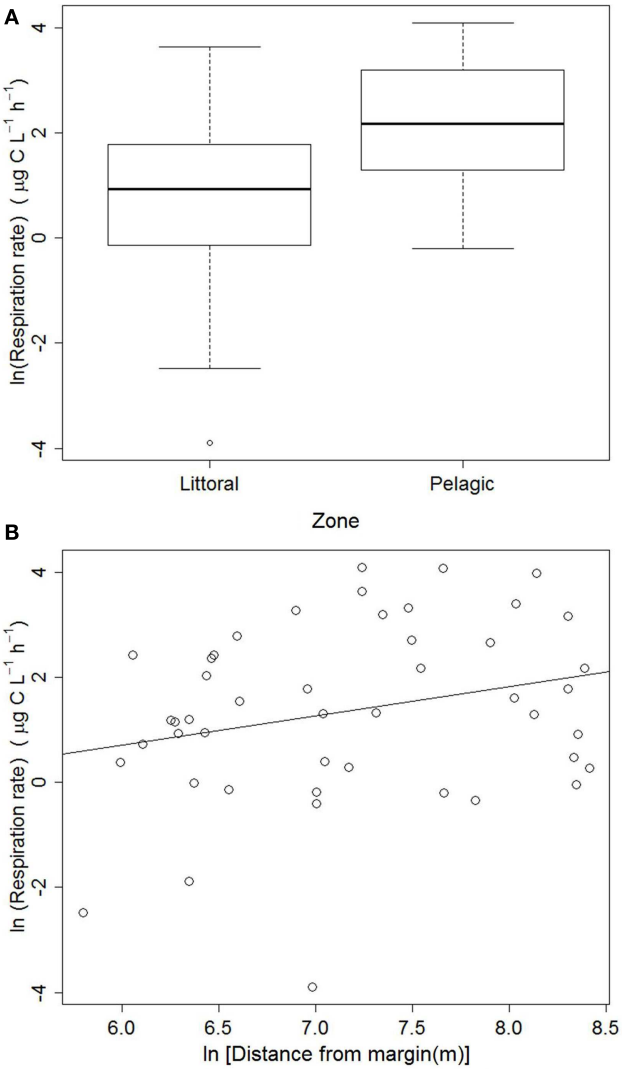

FIGURE 2 | Respiration rates in littoral ($n = 21$) and pelagic ($n = 26$) zones (A) and regression of respiration rates against the distance to the nearest shore (B) in Lake Mangueira, sampled in March 2009.

RESPIRATION CLOUDS OF POINTS

The respiration data collected from the entire lake revealed that respiration rates were significantly lower in the littoral zone: $F(1, 45) = 10.778$, $p < 0.002$ (**Figure 2A**). The regression between respiration rates and distance from the nearest shore was marginally significant: ln(Respiration) = −2.648 + 0.559[ln(Distance)], Adjusted $R^2 = 0.046$, $p = 0.079$ (**Figure 2B**).

The NPMANOVA with all months revealed a significantly higher Abs250:365 in the littoral zone and a significantly higher TP and chl *a* in the pelagic zone. DOC was marginally significantly higher in the pelagic zone (**Tables 1** and **2**).

The NPMANOVA by each month separately revealed that TP and chl *a* were frequently higher in the pelagic zones (May, August 2010 and March, August, and November 2011). Less frequent, but also consistent was higher Abs250 and Abs250:365 in littoral zones (May 2010 and May and August 2011). Punctually, littoral zones also showed higher pH (May 2010) and DIC (August 2011), while the pelagic zone showed higher CO_2 concentration (November 2010; **Tables 1** and **2**).

The regression trees revealed that the Abs250 was the major environmental variable influencing O_2 consumption, CO_2 production, and pCO_2, while DOC influenced most the fCO_2 (**Figures 3A,B**).

For O_2 consumption, the highest mean occurred at Abs250 < 0.064; at Abs250 ≥ 0.064, there was also influence of Abs365, chl *a*, DOC, and pH, with high O_2 consumption occurring at Abs365 ≥ 0.017 and DOC ≥ 2.54 mg L^{-1}. At lower concentrations of DOC (<2.54 mg L^{-1}), O_2 consumption was also high when the pH was ≥8.2. Lowest O_2 consumption occurred at Abs365 < 0.017 and chl *a* concentrations ≥4.02 µg L^{-1} (**Figure 3A**).

Table 1 | Mean ± standard deviation of individual environmental variables for all sampling campaigns and by month for the cloud of points assessed in Lake Mangueira from 2010 to 2011.

Variable		2010–2011	2010				2011			
		All months	February	May	August	November	March	May	August	November
pH	*L*	7.83 (±0.28)[a]	–	**7.94 (±0.27)[a]**	7.44 (±0.06)[a]	8.17 (±0.08)[a]	7.86 (±0.31)[a]	7.95 (±0.18)[a]	7.79 (±0.16)[a]	7.64 (±0.15)[a]
	P	7.84 (±0.27)[a]	–	**7.75 (±0.15)[b]**	7.44 (±0.04)[a]	8.16 (±0.15)[a]	7.98 (±0.26)[a]	7.85 (±0.14)[a]	7.89 (±0.10)[a]	7.83 (±0.29)[a]
Abs250	*L*	0.093 (±0.033)[a]	–	0.069 (±0.007)[a]	0.100 (±0.022)[a]	0.091 (±0.012)[a]	0.087 (±0.031)[a]	**0.102 (±0.055)[a]**	**0.112 (±0.054)[a]**	0.088 (±0.007)[a]
	P	0.086 (±0.021)[a]	–	0.074 (±0.018)[a]	0.106 (±0.022)[a]	0.103 (±0.021)[a]	0.084 (±0.016)[a]	**0.070 (±0.015)[b]**	**0.078 (±0.018)[b]**	0.087 (±0.010)[a]
Abs365	*L*	0.016 (±0.008)[a]	–	0.008 (±0.002)[a]	0.023 (±0.005)[a]	0.017 (±0.005)[a]	0.014 (±0.006)[a]	**0.017 (±0.014)[a]**	0.014 (±0.008)[a]	0.021 (±0.003)[a]
	P	0.017 (±0.008)[a]	–	0.010 (±0.003)[a]	0.026 (±0.010)[a]	0.022 (±0.009)[a]	0.015 (±0.005)[a]	**0.009 (±0.003)[b]**	0.014 (±0.004)[a]	0.020 (±0.002)[a]
Abs250:365	*L*	**6.49 (±2.57)[a]**	–	**9.11 (±1.18)[a]**	4.42 (±0.46)[a]	5.49 (±0.88)[a]	6.54 (±1.31)[a]	7.09 (±1.62)[a]	**8.48 (±4.59)[a]**	4.31 (±0.54)[a]
	P	**5.83 (±1.69)[b]**	–	**7.71 (±1.07)[b]**	4.24 (±0.77)[a]	4.96 (±0.89)[a]	5.72 (±0.89)[a]	8.08 (±1.45)[a]	**5.69 (±0.77)[b]**	4.44 (±0.72)[a]
TP (mg L^{-1})	*L*	**0.033 (±0.020)[a]**	–	**0.017 (±0.005)[a]**	0.020 (±0.006)[a]	0.025 (±0.007)[a]	**0.026 (±0.005)[a]**	0.027 (±0.006)[a]	0.042 (±0.015)[a]	**0.076 (±0.008)[a]**
	P	**0.037 (±0.018)[b]**	–	**0.025 (±0.007)[b]**	0.030 (±0.019)[a]	0.030 (±0.009)[a]	**0.031 (±0.008)[b]**	0.030 (±0.010)[a]	0.042 (±0.016)[a]	**0.069 (±0.004)[b]**
TN (mg L^{-1})	*L*	0.33 (±0.14)[a]	–	0.43 (±0.08)[a]	0.24 (±0.03)[a]	0.25 (±0.12)[a]	0.25 (±0.09)[a]	0.20 (±0.10)[a]	0.51 (±0.04)[a]	0.43 (±0.10)[a]
	P	0.35 (±0.16)[a]	–	0.43 (±0.07)[a]	0.26 (±0.04)[a]	0.20 (±0.14)[a]	0.29 (±0.07)[a]	0.23 (±0.09)[a]	0.55 (±0.15)[a]	0.48 (±0.07)[a]
chl *a* (μg L^{-1})	*L*	**3.03 (±1.09)[a]**	–	**2.76 (±1.18)[a]**	**3.43 (±1.01)[a]**	2.48 (±0.69)[a]	**2.82 (±0.88)[a]**	2.87 (±1.31)[a]	**3.04 (±0.71)[a]**	3.82 (±1.49)[a]
	P	**3.89 (±1.49)[b]**	–	**4.21 (±1.63)[b]**	**5.11 (±1.86)[b]**	3.14 (±1.23)[a]	**3.99 (±1.63)[b]**	3.41 (±1.25)[a]	**3.98 (±1.11)[b]**	3.38 (±0.93)[a]
DOC (mg L^{-1})	*L*	**1.89 (±1.23)[a]**	–	2.13 (±1.01)[a]	2.56 (±1.22)[a]	1.90 (±0.83)[a]	2.28 (±2.44)[a]	1.54 (±0.48)[a]	1.79 (±0.87)[a]	1.05 (±0.44)[a]
	P	**2.38 (±1.51)[b]**	–	2.86 (±0.94)[a]	2.57 (±0.79)[a]	1.92 (±0.40)[a]	4.02 (±2.24)[a]	2.45 (±1.64)[a]	1.80 (±1.23)[a]	1.05 (±0.83)[a]
DIC (mg L^{-1})	*L*	15.03 (±4.36)[a]	–	13.59 (±1.08)[a]	13.49 (±1.86)[a]	11.44 (±1.06)[a]	12.70 (±2.20)[a]	12.18 (±1.85)[a]	19.97 (±4.39)[a]	21.83 (±0.46)[a]
	P	14.41 (±3.99)[a]	–	13.73 (±1.09)[a]	12.30 (±1.41)[a]	11.02 (±0.78)[a]	12.25 (±2.38)[a]	12.72 (±0.95)[a]	16.48 (±3.13)[a]	22.35 (±0.56)[a]
O_2 (cons.; mg L^{-1})	*L*	2.57 (±1.64)[a]	–	2.98 (±1.57)[a]	3.11 (±1.46)[a]	1.95 (±2.22)[a]	1.53 (±0.56)[a]	4.24 (±2.22)[a]	1.94 (±0.48)[a]	2.21 (±0.60)[a]
	P	2.74 (±1.52)[a]	–	3.59 (±2.22)[a]	3.53 (±1.52)[a]	2.43 (±1.42)[a]	1.86 (±0.55)[a]	3.26 (±1.65)[a]	2.27 (±1.12)[a]	2.23 (±0.89)[a]
CO_2 (accum.; mg L^{-1})	*L*	5.81 (±4.14)[a]	–	6.36 (±6.32)[a]	4.21 (±4.90)[a]	**3.97 (±2.62)[a]**	4.78 (±2.49)[a]	6.26 (±2.86)[a]	7.50 (±2.80)[a]	7.61 (±5.34)[a]
	P	5.60 (±4.40)[a]	–	2.84 (±5.41)[a]	6.10 (±5.90)[a]	**6.92 (±3.90)[b]**	4.43 (±2.12)[a]	5.29 (±5.13)[a]	8.03 (±2.48)[a]	5.62 (±3.34)[a]
								June		
pCO_2 (μatm)	*L*	345 (±161)[a]	192 (±71)[a]	484 (±66)[a]	467 (±85)[a]	479 (±65)[a]	317 (±100)[a]	100 (±17)[a]	372 (±127)[a]	–
	P	368 (±181)[a]	192 (±38)[a]	453 (±53)[a]	531 (±99)[a]	566 (±134)[a]	337 (±64)[a]	98 (±10)[a]	401 (±120)[a]	–
fCO_2	*L*	−10.1 (±41.6)[a]	−71.0 (±24.6)[a]	34.4 (±22.5)[a]	10.1 (±10.4)[a]	26.9 (±18.5)[a]	−14.1 (±21.0)[a]	−54.5 (±3.5)[a]	−2.7 (±26.9)[a]	–
(mmol C m^2 $d^{(1)}$)	*P*	−5.6 (±45.2)[a]	−70.7 (±13.7)[a]	23.8 (±18.3)[a]	18.0 (±12.3)[a]	51.1 (±37.3)[a]	−9.7 (±13.3)[a]	−55.1 (±2.2)[a]	3.2 (±26.0)[a]	–

Different superscripts and bold indicate significant ($p < 0.05$) or marginal ($0.05 < p < 0.1$) differences between littoral and pelagic zones for each month (not among months), except for the global test with 2010–2011, where the difference of littoral and pelagic zones of all months was tested. Details of NPMANOVA in ***Table 2****. Groups coded according to the distance from the nearest shore: Littoral (L, < 1000 m) × Pelagic (P, ≥ 1000 m).*

Table 2 | Non-parametric MANOVA (NPMANOVA) analysis on individual environmental variables for all campaigns and by month for the cloud of points sampled in lake Mangueria from 2010 to 2011.

Year	Month	Variable	NPMANOVA	
			Output	Effect
2010–2011	All	Abs250:365	***F*** = 5.20, **p** = 0.023*	*L* > *P*
		TP	***F*** = 4.02, **p** = 0.044*	*P* > *L*
		Chl *a*	***F*** = 12.44, **p** < 0.001*	*P* > *L*
		DOC	*F* = 3.11, p = 0.083**	*P* > *L*
2010	May	pH	*F* = 3.95, p = 0.066**	*L* > *P*
		Abs250:365	***F*** = 7.02, **p** = 0.0172*	*L* > *P*
		TP	***F*** = 9.24, **p** = 0.010*	*P* > *L*
		Chl *a*	*F* = 4.15, p = 0.062**	*P* > *L*
	August	Chl *a*	***F*** = 4.78, **p** = 0.042*	*P* > *L*
	November	CO_2	*F* = 3.14, p = 0.093**	*P* > *L*
2011	March	TP	*F* = 2.91, p = 0.098**	*P* > *L*
		Chl *a*	*F* = 3.06, p = 0.090**	*P* > *L*
	May	Abs250	***F*** = 3.67, **p** = 0.042*	*L* > *P*
		Abs365	***F*** = 3.29, **p** = 0.044*	*L* > *P*
	August	Abs250	***F*** = 4.03, **p** = 0.045*	*L* > *P*
		Abs250:365	***F*** = 4.41, **p** = 0.013*	*L* > *P*
		Chl *a*	*F* = 3.97, p = 0.0597**	*P* > *L*
	November	TP	***F*** = 7.14, **p** = 0.015*	*L* > *P*

*Groups coded according to the distance from the nearest shore: littoral (L, < 1000 m) × Pelagic (P, × ≥ 1000 m). In bold: p < 0.05; *, 0.05 < p < 0.1). F*, pseudo-F.*

CO_2 production was also high at Abs250 < 0.064, but reached higher means at Abs250 ≥ 0.064), Abs250:365 < 7.35, DOC < 2.51 mg L^{-1}, pH ≥ 7.67, and high chl *a* concentrations (between 2.59 and 3.07 μg L^{-1}). At DOC concentrations <2.51 mg L^{-1} and low pH < 7.67, the mean was also high. The low values, however, occurred at Abs250 ≥ 0.064, and Abs250:365 ≥ 7.35 (**Figure 3B**).

pCO_2 showed high values at Abs250 ≥ 0.104. At lower Abs250 (<0.104), there was also influence of DOC, TP, pH, and Abs250, with lowest values occurring at DOC between 0.58 and 3.95 mg L^{-1}, TP ≥ 0.029 mg L^{-1}, and pH ≥ 7.70 (**Figure 4A**).

fCO_2 showed a strong relationship with DOC concentrations. At DOC ≥2.54 mg L^{-1}, and Abs250 ≥ 0.0853 it was found the highest positive value of fCO_2. A negative mean was found with values of DOC between 1.48 and 2.54 mg L^{-1} and at Abs250 ≥ 0.0846 (**Figure 4B**).

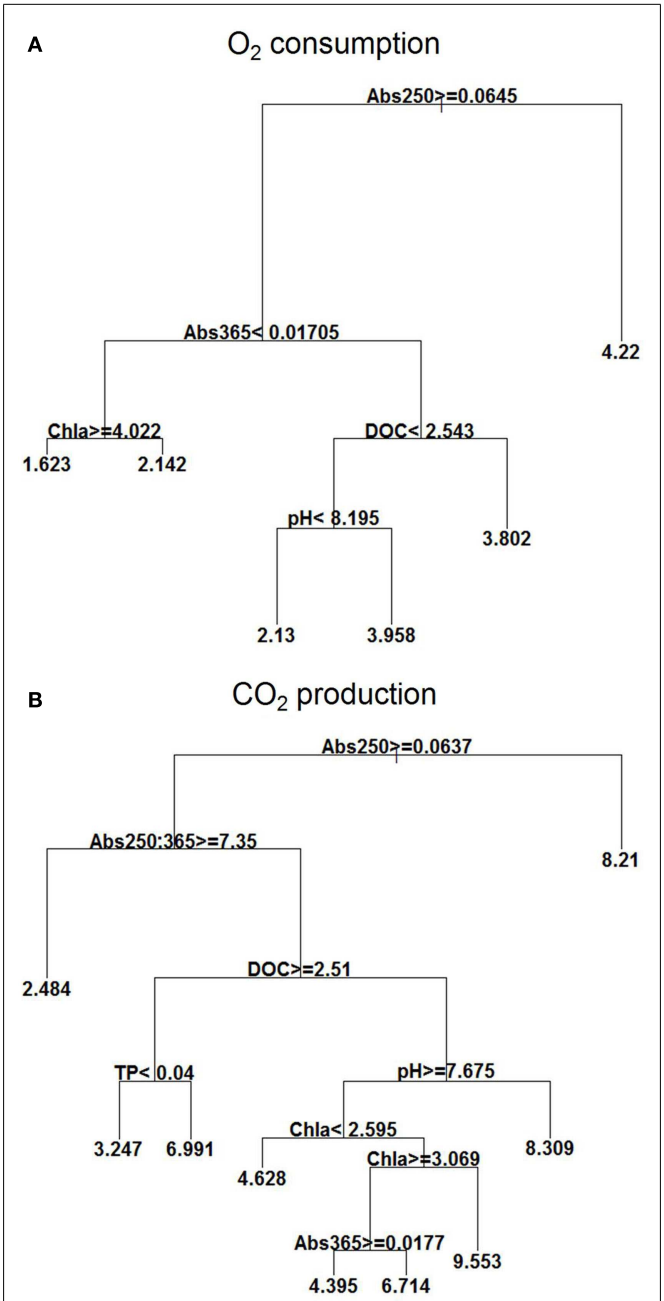

FIGURE 3 | Explanatory power of environmental variables assessed by regression tree on O_2 consumption (mg L^{-1}) (A) and CO_2 (mg L^{-1}) production (B) with the extensive campaigns data (*n* = 133). Means in terminal leafs. Notice that the partitioning criteria above nodes applies to the left branches (< or ≥), while the complement (≤ or >) applies to the right branches.

DISCUSSION

Our results showed that respiration can be lower in the littoral zone, based on the consistently lower DIC in water collected from this zone in incubation assays. In the first whole-lake survey, we found significantly lower respiration rates in these zones and a marginal ($p = 0.079$) increase of respiration rate with distance to the nearest shore. In the extensive sampling, this was not consistently found throughout all months. Marginally ($p = 0.093$) lower CO_2 accumulation was detectable in at least 1 month (November 2010) in the littoral zones. However, significantly higher DIC occurred in the littoral zones in August 2011. These differences are possibly associated with differences in environmental conditions between these zones, as shown by consistently higher chlorophyll *a* and TP, marginally higher DOC ($p = 0.083$) and significantly lower Abs250:365 in the pelagic zone, as shown by NPMANOVA analyses on the entire data set (all months). The regression tree analyses

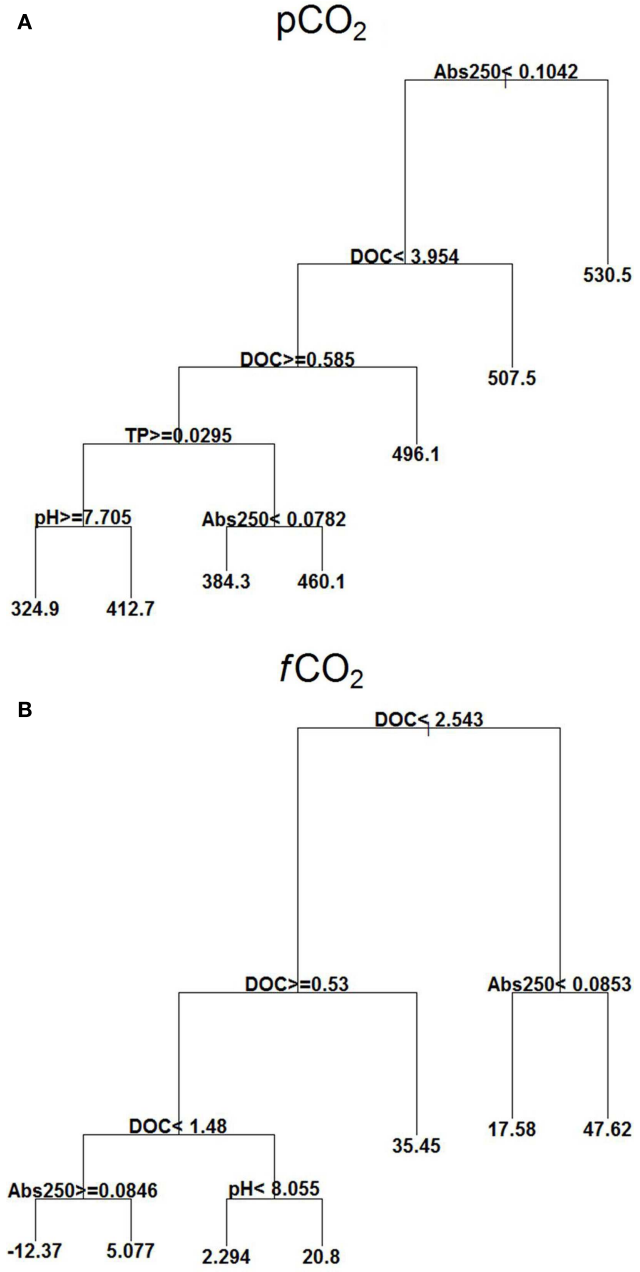

FIGURE 4 | Explanatory power of environmental variables assessed by regression tree on pCO_2 (μatm) (A) and fCO_2 (mmol C m^2 d^{-1}) (B) with extensive campaigns data ($n = 95$). Means in terminal leafs. Notice that the partitioning criteria above nodes applies to the left branches (< or ≥), while the complement (≤ or >) applies to the right branches.

confirmed that higher O_2 consumption and CO_2 production are associated to low Abs250, high DOC, and high Abs365. pCO_2 and fCO_2, at their turn, were higher at high Abs250, DOC, and TP.

Our results showed contrasting patterns. The DIC accumulation experiment and the extensive sampling of 2009 supported our hypothesis that respiration is lower in the littoral zone than in the pelagic zone. However, apart from the marginally higher CO_2 accumulation in November 2010 in the pelagic zone, the extensive sampling of 2010–2011 did not show such striking evidence in support of the hypothesis. This could be due to the smaller number of points sampled, but also the result of intensive mixing of Lake Mangueira by wind (Cardoso et al., 2012), which makes it difficult to detect abrupt differences in respiration in the field, as indicated by several marginal relationships found. This could have been crucial because of the smaller set of sampling points.

One hypothesis for the lack of detection of DIC accumulation is that in areas with an extensive coverage of macrophytes, like southern Mangueira, the macrophyte influence might reach pelagic zones as well. We unfortunately do not have enough data gathered yet to envisage how, why, and when this occurs. We nevertheless did observe that DIC was on average higher in the pelagic zone, likely due to very low accumulation rates (not statistically significant in <14 days). When we extend our perspective (spatially and temporally) to the whole lake and for different years and seasons, we see a different picture, where we do detect respiration (even though still low) enough to put in evidence differences between littoral and pelagic zones. Another important point to take into consideration is that lake Mangueira is of marine origin (former closed estuary) with significant accumulation of sea shell banks. This explains the high background levels of DIC in the lake, and could have masked measurements of bacterial respiration based on DIC. This also suggests that it is not the best method for this purpose, and may have been the cause for the disagreement between the patterns of DIC and O_2/CO_2 respiration rates observed.

As stated, in Lake Mangueira, rates of respiration are extremely low, and it is striking that it is impossible to detect respiration in dark bottles over a period shorter than 5 days. The mean respiration rates in the littoral (1.34 mmol O_2 m^{-3} h^{-1}) and pelagic zones (1.43 mmol O_2 m^{-3} h^{-1}) are closer to the lower range presented in the extensive compilation of plankton respiration rates (0.029 to 6.73 mmol O_2 m^{-3} h^{-1}) by Pace and Prairie (2005). This is also confirmed in a carbon-basis comparison, as the rates of respiration in this study (Littoral zone: Mean: 1.469 μg C L^{-1} h^{-1}, range: 0.008–6.241 μg C L^{-1} h^{-1}; Pelagic zone: Mean: 6.540 μg C L^{-1} h^{-1}, range: 0.250–23.718 μg C L^{-1} h^{-1}) are also close to the lower range reported in other systems: Delaware Bay and salt marshes (range: 2.53–13.14 μg C L^{-1} h^{-1}; del Giorgio and Newell, 2012) and 20 Quebec lakes (range: 0.168–2.138 μg C L^{-1} h^{-1}).

The differences in respiration in the littoral and pelagic zones of Lake Mangueira may reflect differences in the responses of bacterial metabolism to the following drivers: (I) quantity and bioavailability of DOC; and (II) phosphorus limitation. Our results supported both hypotheses, as indicated by the lower chl *a*, DOC, and TP, and the higher proportion of low-molecular-weight compounds (in terms of Abs250 and Abs250:365) in the littoral zone found in many cases.

The generally low DOC bioavailability in the form of humic substances (Münster and Chróst, 1990) indicates that a large proportion of dissolved carbon in Lake Mangueira is refractory to bacterial consumption. When compared to the values reported by Amado et al. (2006) for an Amazonian river and a stream, the content of humic substances in Lake Mangueira is 6 times higher. The humic content is at least 4 times higher than for values reported for the mesohumic Lake Sjättesjön (Lindell et al., 1995), or even as much as 12–17 times higher than reported for the Gutierrez River

(all comparisons on the basis of the Abs250:365 nm ratio; Pérez et al., 2003).

DOC is generally high in densely vegetated areas (Wetzel, 1992; Reitner et al., 1999); however, we found higher DOC in the pelagic zone, along with chl *a*, suggesting the importance of algal carbon to the system. This is in accordance with the long-recognized bacterial dependence on algal production (Cole, 1982), particularly because the more labile algal DOC sustains higher bacterial growth efficiency (Kritzberg et al., 2005). This was evident by the greater phytoplankton biomass concentrated (higher chlorophyll *a*) in the pelagic zone at the end of winter (August 2010), which was respired by bacteria with the rising temperatures in spring (highest CO_2 accumulation found in November 2010; **Table 1**).

The higher concentration of low-molecular-weight substrates in the littoral zone can also imply less availability of substrates (Amon and Benner, 1996). Contrariwise, these substrates are supposedly more easily taken up by bacteria, but they may be more refractory to consumption. Tranvik (1990) found higher bacterial production per unit of carbon in high-molecular-weight DOC than in low-molecular-weight DOC in lakes with different humic content in Sweden. In another study, Amon and Benner (1996) found higher bacterial production and respiration in high-molecular-weight (HMW, >1 KDa) than low-molecular-weight (LMW, <1 KDa) DOC. Based on these results, the authors proposed the size-reactivity continuum model, which predicts that the major path of degradation goes from large, highly reactive to small, highly recalcitrant molecules. If this hypothesis is broadly applicable to many ecosystems, it suggests that in Lake Mangueira the compounds lixiviated by the macrophytes may undergo some degradation, but accumulate as dissolved, unreactive LMW compounds with very low degradation rates in the littoral zones, in agreement with the higher Abs250:365 and lack of change in the DIC with time in the incubation experiment. Our results strongly support this hypothesis, as higher O_2 consumption, and CO_2 production were found for low Abs250 and high Abs365 or conversely lower O_2 consumption, and CO_2 production were found when the ratio Abs250:365 was too high.

The lower TP found in the littoral zone can be attributed to the generally low watershed load and also the great competitive advantage of macrophytes in taking up this nutrient. As noted by Vidal et al. (2011), not only carbon, but the carbon: phosphorus ratio controls bacterial production and respiration in lakes, and even a situation of labile DOC accumulation can occur under phosphorus limitation (Vadstein et al., 2003). In fact, increased bacterial density in the presence of macrophytes after phosphorus amendment has been described (Huss and Wehr, 2004; Joniak et al., 2007; Morozova et al., 2011). An interesting result was the lower TP in the pelagic zone in November 2011, which could be due to depletion of this nutrient in the pelagic zone during summer (**Table 1**); however, no similar depletion occurred in 2010, and cannot be assumed as a pattern based on our data. The regression trees indicated an effect of TP when DOC is high; low TP is associated to low CO_2 production, while the opposite situation also applies, supporting the hypothesis that low respiration can be associated to low TP. This is expected to generate lower pCO_2 in littoral zones where TP is lower; however a difference in pCO_2 between littoral and pelagic zones was not detected. The regression tree revealed that pCO_2 was low when TP was high in samples of high DOC. This is in agreement with low phosphorus leading to CO_2 supersaturation in order to balance this nutrient deficiency (Hessen, 1992), and suggests that this also occurs in lake Mangueira. As CO_2 concentration is only part of pCO_2, other factors affecting pCO_2 equilibrium need to be taken into account, and this may be the cause for the apparent contradiction between CO_2 production and pCO_2 predicted from TP.

Levels of pCO_2 were in general lower than in other lakes with similar or even lower TP content: mean around 660 μatm (range: 130–1010 μatm) for a range of Quebec lakes with variable trophic content; mean around 1 036 μatm (range: 1–20 249 μatm) for a comprehensive compilation of data for 1835 lakes distributed worldwide (Cole et al., 1994). These levels are far above those found in this study. This could be explained by the generally low DOC concentration in Lake Mangueira (mean around 2.2 mg L^{-1}), because it has been demonstrated that lakes are net heterotrophic (supersaturated with CO_2) above concentrations of 4–6 mg L^{-1} DOC (Prairie et al., 2002). This contrasts with the generally accepted view that most aquatic systems are net heterotrophic and function as net sources of CO_2 to the atmosphere (Cole et al., 1994; Duarte and Prairie, 2005), which makes Lake Mangueira (particularly the littoral zone) an exception to this general rule.

The analysis of the frequency of occurrence (histogram) of classes of fCO_2 between the lake surface and atmosphere in the littoral zone showed a higher frequency of positive (net influx of CO_2 to the lake), while the pelagic zone showed a higher frequency of negative values (net efflux of CO_2 from the lake) of fCO_2, even though there was no difference between the means in the littoral and pelagic zones. A closer inspection of the fluxes showed that they are very close to zero, and the lake functions as a sink of CO_2, especially during winter and spring (May to November), when the fCO_2 becomes close to zero or even positive (**Figure 5**). The regression tree showed that high, positive fCO_2 was associated to higher DOC and Abs250, contrary to the expectation that higher DOC is associated to higher CO_2 supersaturation and hence net efflux of CO_2 from the lake; negative fCO_2 was associated to intermediate values of DOC and high Abs250. However, more direct evidence needs to be gathered in order to draw more solid associations.

Besides main effects of DOC and nutrients, other possible explanations for low respiration in the littoral zone in Lake Mangueira have also been hypothesized: formation of hydrogen peroxide by UV radiation, release of allelochemicals by macrophytes, and limitation by micronutrients, e.g., iron and silicate. UV exposure of macrophytes products of degradation in Lake Mangueira has been demonstrated to decrease bacterial production, possibly by formation of H_2O_2 (They et al., submitted). The release of allelopathic substances by (mainly submersed) macrophytes is widely known (Gross et al., 2007; Mulderij et al., 2007), and has been considered as a possible explanation for lower bacterial diversity (Wu et al., 2007) and metabolism (They et al., 2010) in areas extensively colonized by macrophytes. We have data on Fe and silicate from the extensive campaigns that were not included in the results because they are out of objectives of this

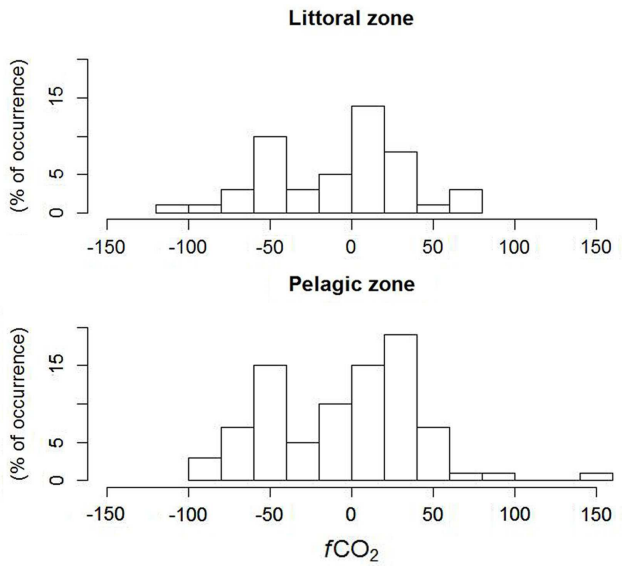

FIGURE 5 | Histogram of CO_2 fluxes between atmosphere and lake surface in littoral ($n = 12$) and pelagic ($n = 7$) zones in Lake Mangueira, sampled in 2010–2011.

study. Based on these data, there is no evidence of iron limitation, since the values found in the littoral zones (0.325 ± 0.253 mg L^{-1}) and pelagic zones (0.349 ± 0.248 mg L^{-1}) are far above concentrations reported to be limiting in other systems, like, e.g., open ocean ($<5.6 \times 10^{-6}$ mg L^{-1}; Oliver et al., 2004) or lake Erie (1.7×10^{-4}–1.09×10^{-2} mg L^{-1}; North et al., 2007). Silicate concentrations are far above those reported to be limiting (2 μm) for diatoms (Egge and Aksnes, 1992) in littoral (100.92 ± 23.99 μm) and pelagic zones (100.04 ± 23.83 μm), and thus there no evidence of possible impacts on bacterial supply of organic substrates derived from silicate limitation of phytoplankton.

One important and final consideration is that we found support for our hypotheses by different, independent methods: one-time DIC accumulation, extensive one-time sampling and extensive many-times sampling by O_2 consumption, and CO_2 production measurements. Each method has its own limitations, as we believed was crucial in the DIC experiment; had we employed a longer incubation period, we could have seen some detectable accumulation. Differences between littoral and pelagic zones has long been recognized, but what is generally believed is that macrophyte presence/carbon is always good to bacteria, a paradigm our data do not support.

The littoral zone of Lake Mangueira (especially in the north and south) is extensively colonized by emergent and submersed macrophytes, respectively, and these plants are expected to contribute large amounts of organic carbon to the system. However, our results showed that respiration in this zone can be lower than in the pelagic zone, at least during a part of the year. This may be the result of differences in DOC quality, mainly derived from macrophytes in the littoral zone and phytoplankton in the pelagic zone. The disappearance of these differences may be due to seasonality or to masking by mixing in this shallow, wind-dominated lake. The finding of lower respiration rates in the littoral zone means that DOC remains in the system, mostly in a low-molecular-weight, unreactive form. Hence, the general belief that macrophyte-derived carbon is always beneficial to bacteria is not supported. The littoral zone, therefore, shows a greater tendency to be a CO_2 sink, compared to the pelagic zone. If lower respiration in littoral zones is a common feature of subtropical shallow lakes dominated by macrophytes, there may be important and still unrecognized implications for their global carbon metabolism. However important these implications may seem, important issues (e.g., organic carbon molecular size and quality spectra, effect of taxonomic structure of macrophytes, bacterial taxonomic and functional diversity, the role of sediment bacteria, and more experimental and field evidence) need to be addressed for a better understanding of the many ways the interaction between macrophytes and bacteria can impact global carbon metabolism of lakes.

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Spatial variation of sediment mineralization supports differential CO_2 emissions from a tropical hydroelectric reservoir

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Substantial amounts of organic matter (OM) from terrestrial ecosystems are buried as sediments in inland waters. It is still unclear to what extent this OM constitutes a sink of carbon, and how much of it is returned to the atmosphere upon mineralization to carbon dioxide (CO_2). The construction of reservoirs affects the carbon cycle by increasing OM sedimentation at the regional scale. In this study we determine the OM mineralization in the sediment of three zones (river, transition, and dam) of a tropical hydroelectric reservoir in Brazil as well as identify the composition of the carbon pool available for mineralization. We measured sediment organic carbon mineralization rates and related them to the composition of the OM, bacterial abundance and pCO_2 of the surface water of the reservoir. Terrestrial OM was an important substrate for the mineralization. In the river and transition zones most of the OM was allochthonous (56 and 48%, respectively) while the dam zone had the lowest allochthonous contribution (7%). The highest mineralization rates were found in the transition zone (154.80 ± 33.50 mg C m^{-2} d^{-1}) and the lowest in the dam (51.60 ± 26.80 mg C m^{-2} d^{-1}). Moreover, mineralization rates were significantly related to bacterial abundance ($r^2 = 0.50$, $p < 0.001$) and pCO_2 in the surface water of the reservoir ($r^2 = 0.73$, $p < 0.001$). The results indicate that allochthonous OM has different contributions to sediment mineralization in the three zones of the reservoir. Further, the sediment mineralization, mediated by heterotrophic bacteria metabolism, significantly contributes to CO_2 supersaturation in the water column, resulting in higher pCO_2 in the river and transition zones in comparison with the dam zone, affecting greenhouse gas emission estimations from hydroelectric reservoirs.

Keywords: freshwater sediment, carbon mineralization, stable isotopes, pCO_2, heterotrophic bacteria, hydroelectric reservoir, Cerrado, Brazil

INTRODUCTION

Significant efforts have been made to understand the carbon fluxes into and out of inland waters. However, discerning the pathways of carbon flow, especially related to aquatic sediments, remains uncertain (Cole et al., 2007). For instance, the relationship between the allochthonous carbon loaded to aquatic ecosystems and the portion either mineralized or buried in sediments has yet to be better quantified (Battin et al., 2009; Sobek et al., 2011). Inland waters (e.g., lakes and reservoirs) receive large amounts of allochthonous organic matter (OM) transported from the surrounding watershed, part of which is sedimented. Once in the sediment, OM can have three major fates: (i) mineralization by aerobic or anaerobic bacteria and release back to water column as CO_2 and CH_4; (ii) re-suspension and mineralization in the water column; or (iii) burial in the sediment. The relative importance of each of these three processes is system-specific and influences the overall role of a system as net sink or source of carbon as greenhouse gases (GHG) to the atmosphere (Mendonça et al., 2012a). The limited knowledge of the lateral transport of carbon from soils, and its fates in lakes and reservoirs results in incomplete understanding of the terrestrial carbon balance (Tranvik et al., 2009).

Hydroelectric reservoirs are man-made freshwater ecosystems that can substantially alter regional and global carbon balance. During the first years after impounding, the mineralization of flooded vegetation and soils cause significant emissions of GHG to the atmosphere (Mendonça et al., 2012b). Growing demand for energy has motivated the construction of hydroelectric reservoirs worldwide. There are approximately 45,000 large hydroelectric reservoirs in operation in the world (World Commission on Dams [WCD], 2000), with a total surface area of about 350,000 km^2 (Barros et al., 2011). In Brazil, the increasing number of hydroelectric reservoirs in pristine areas (e.g., Amazon and Pantanal) has generated controversy among local people and among the scientific community worldwide (Fearnside, 2006; Sousa Júnior and Reid, 2010). One source of dispute is the effect of river damming on aquatic GHG emissions. In this context, estimating

inputs and outputs of OM in hydroelectric reservoirs is important in order to better understand the impacts of these systems on the environment and to support regional and global evaluations of the environmental costs and benefits of hydroelectric energy generation.

In tropical freshwater ecosystems, sediment OM is mineralized by aerobic and anaerobic respiration and fermentation resulting in the production of CO_2 or CH_4. The occurrence of aerobic or anaerobic processes in the sediment is regulated by the availability of electron acceptors (e.g., oxygen, nitrate, manganese, iron, and sulfate; Fenchel et al., 1998). Especially in the case of reservoirs, those processes are also regulated by mixing regimes, thermal structure of the water column and productivity (Kalff, 2002) that vary along a longitudinal gradient, resulting in the establishment of different zones. Those zones are mainly the area close to the river inflow, the transitional area between the river and the dam of the reservoir, and a lacustrine zone directly influenced by the dam of the reservoir (Thornton et al., 1990; De Junet et al., 2009). Additionally, the magnitude of the carbon degradation across reservoir zonation can be related to the amount and quality of organic carbon (OC) available (Conrad et al., 2010, 2011), plankton metabolism (Forbes et al., 2012), oxygen concentrations (Sobek et al., 2009) and the temperature of the water overlaying the sediment (Gudasz et al., 2010). Despite the mineralization processes in the sediment are gaining more attention in aquatic ecosystems (Algesten et al., 2005; Battin et al., 2009), studies considering the mineralization in the sediment of hydroelectric reservoirs are still rare, particularly in tropical areas.

In this study, our goal was to determine the OM mineralization in the sediment of three zones (river, transition, and dam) of a tropical hydroelectric reservoir in Brazil as well as characterize the source of the carbon pool available for mineralization. The results indicate that OM mineralization in the sediment vary along the different zones of the reservoir. It is mostly influenced by the allochthonous carbon pool and significantly contributes to CO_2 supersaturation in the water column affecting GHG emissions from the system.

MATERIALS AND METHODS

STUDY AREA DESCRIPTION

This study was conducted in the Manso Reservoir (14°52′ S, 55°46′ W) in January of 2009. The reservoir is located in southeastern Brazil, in the Cerrado (savannah-type) biome. It is a large (360 km^2) and deep (maximum depth 40 m) hydroelectric reservoir that was formed by damming the Manso River in 1999. The Manso River is a tributary of the Cuiabá River, one of the main drainages of the Pantanal, an important wetland in Brazil. Water residence time in the Manso Reservoir is approximately 2.5 years, and the turbine intake is located in the epilimnion (Roland et al., 2010). The reservoir is mesotrophic with total phosphorus (TP) concentrations ranges of 25–37 $\mu g L^{-1}$, total nitrogen (TN) concentrations ranges of 530–800 $\mu g L^{-1}$, and chlorophyll-a concentrations ranges of 2–6 $\mu g L^{-1}$ (Rangel et al., 2012). During the samplings the bottom water of the reservoir was oxygenated and neutral with dissolved oxygen (DO) concentrations varying from 4 (dam) to 7 (river and transition) mg L^{-1} and pH of 7 ± 0.3 (mean and standard deviation).

SAMPLING

Water samples for carbon stable isotope signature ($\delta^{13}C$) of the particulate organic matter (POM) were taken from the surface, middle and bottom of the water column in each of the different zones. Sediment cores were collected using a gravitational sediment sampler (Kajak, modified by Ambühl and Bührer, 1975) in the same three zones of one arm of the Manso Reservoir. These zones were river (14 m depth) – an area close to the inflow of the Manso River; transition (11 m depth) – an intermediate area between the river inflow and the dam, and dam (27 m depth) – a lacustrine area directly influenced by the dam of the reservoir (**Figure 1**). From each zone five sediment cores were taken. The upper 10 cm was transferred to incubation cores (54 mm inner diameter) without visible disturbance of the sediment structure. The incubation cores were then totally filled with water from the bottom of the sampling sites collected with a Van Dorn device. The incubation cores were equipped with internal magnetic stirring devices to allow mixing of the overlying water without disturbing the sediment surface. Aliquots of the overlying water were taken from each core to determine initial concentrations of dissolved inorganic carbon (DIC), pCO_2, pH, temperature and bacterial abundances. The cores were then sealed without headspace with an expandable polyvinyl chloride (PVC) stopper fitted with double o-rings and outlet tubing. The cores were maintained under *in situ* temperature and in the dark, to avoid primary production. Samples of water were taken after 12, 24, 72, and 96 h of incubation by pushing the PVC stopper downwards in the core and simultaneously filling plastic syringes. All cores were incubated in oxic conditions with initial DO concentration of 6.0 ± 0.3 mg L^{-1} and pH 7 ± 0.3. As the mineralization of OM in the sediment comprises different metabolic processes, including aerobic oxidation and different anaerobic oxidation that lead to the release of CO_2 in the water, we measured the net effect of all these processes, represented by the DIC production in the cores. Sediment samples from the top 2 cm, representing the layer immediately in contact with water, and 10 cm, the limit layer of significant bacterial activity in the sediment, where taken at the beginning (from extra cores) and at the end of the incubation (from cores used for the incubation) to analyze the concentrations of OM, total carbon (TC), TN, TP, and $\delta^{13}C$ isotope composition.

ANALYTICAL METHODS

Water samples from the cores were analyzed for pH using a pH meter (Micronal B474), DO concentrations with a Clark-type oxygen sensor coupled to a picoamperimeter (Unisense©, model PA 2000) and DIC following sodium persulfate digestion on a Tekmar-Dohrmann TC analyzer (model Phoenix 8000). The pCO_2 was measured both in the water overlying the cores and in the water surface of the reservoir using an infrared gas analyzer (IRGA- environmental gas monitor EGM-4; PP Systems). Measurements of pCO_2 were made directly using the headspace equilibrium method (Hesslein et al., 1991; Cole et al., 1994). Fifteen mL of atmospheric air was equilibrated with 20 mL of water by vigorous shaking for 1 min (Cole and Caraco, 1998). The headspace gas was transferred to a plastic syringe, and the concentration of CO_2 was immediately measured on the IRGA. Bacterial abundance from the overlying water of the sediment was estimated by direct counting using the

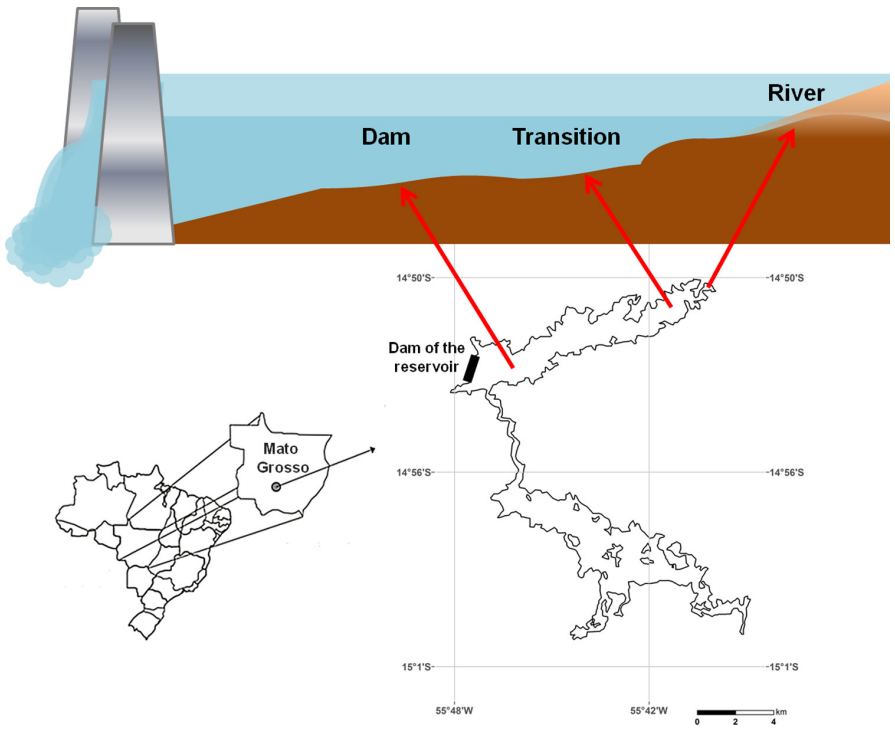

FIGURE 1 | Map of the study area showing the different sampling zones of the Manso reservoir: river (14 m depth) – an area close to the inflow of the Manso River; transition (11 m depth) – an intermediate area between the river inflow and the dam, and dam (27 m depth) – a lacustrine area directly influenced by the dam of the reservoir.

acridine orange method (Hobbie et al., 1977) under an Olympus BX60 fluorescence microscope.

Sediment samples of OM were analyzed by loss on ignition and TP by the colorimetric method according to Carmouze (1994). Concentrations of TC and TN in the sediment were analyzed according to Mackereth et al. (1978) using a PerkinElmer analyzer. Sediment particle sizes were quantified using a Malvern laser diffraction particle size analyzer. For the determination of water content and porosity, subsamples of fresh sediment were weighed in ceramic vessels and their weight loss recorded after heating for 4 days at 60°C (Dalsgaard et al., 2000).

The particulate organic carbon (POC) concentration and the stable isotope ratios of POC and sediment samples were analyzed using a gas isotope ratio mass spectrometer (Delta Plus, Finnigan Mat). One replicate sample and standards were analyzed after every set of 10 samples. Carbon isotopic ratios were expressed using delta notation as the ratio of the heavy to the light isotope ($\delta^{13}C$) over the ratio of the heavy to the light isotope of the international standards $\delta^{13}C$ Pee Dee Belemnite, respectively. Isotope ratios of the OM samples were used to determine the allochthonous contribution to the OM pool (Gu et al., 2011). We estimated the upper and lower limits for the contribution of each carbon source (autochthonous and allochthonous) to mineralization in the different zones of the reservoir by using a two-source isotope mixing model (Fry, 2006): terrestrial material and algae. Macrophytes, were excluded from the sources due to the low abundance in the sampled sites.

The end members (OM sources) for the two-source mixing model were determined as follows: during the sampling period there was a bloom of algae in the reservoir. POM samples were acquired by passing subsurface water samples through a glass fiber filter (0.7 μm porosity). Subsurface water samples were also used for phytoplankton analysis. The phytoplankton analysis showed dominance in abundance and composition (90%) of the cyanobacteria *Microcystis aeruginosa* in the POM samples. The $\delta^{13}C$ of these samples, −30.10‰, was used as the pelagic end member in the mixing model. During the samplings there was no visual confirmation of macrophytes along the reservoir. As the rivers are known to have a predominant contribution of allochthonous material in the surface waters (Kalff, 2002) we used POM samples from the surface water of this zone for isotope analysis. The $\delta^{13}C$ value of these samples (−19.26‰) was used as the allochthonous end member in the mixing model.

Differences in OC mineralization, pCO_2 in the incubation chambers and the surface waters, and bacterial abundance in the sediment among zones was tested using ANOVA, followed by Tukey's *post hoc* test performed in SigmaPlot 11.0.

RESULTS

The suspended POC stable isotope composition varied over depth and between the different zones (river, transition, and dam; **Figure 2**). In the river, $\delta^{13}C$ values decreased from −19.26 (surface) to −20.32 (middle), and to −21.51‰ (bottom). An opposite pattern was found for $\delta^{13}C$ values in the dam −30.10 (surface),

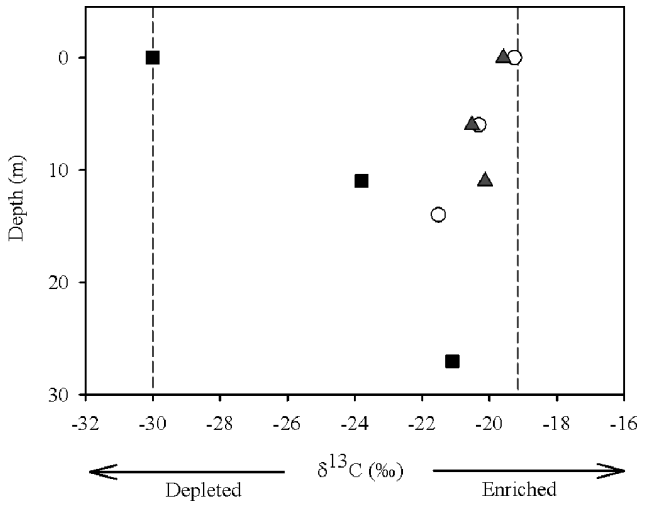

FIGURE 2 | Carbon isotope composition of the POM from the water sampled at different depths (surface, middle, and bottom) and in different zones (river = open circle, transition = gray triangle, and dam = black square) of the Manso Reservoir. Light gray lines represent the end members (allochthonous: $\delta^{13}C$ −19.26‰ and autochthonous: $\delta^{13}C$ −30.10‰).

−23.70 (middle), and −21.10‰ (bottom). The different zones of the reservoir had similar concentrations of OM and nutrients (TC, TN, and TP) in the first two centimeters of sediment (**Table 1**). Although the concentration of OM was similar, the composition of the pools differed (**Figure 3A**). The river zone sediment was mostly allochthonous, with allochthonous contributions of 56 (2 cm) and 48% (10 cm) and $\delta^{13}C$ of −25.50 and −26.20‰, respectively. In the transition zone, allochthonous contributions to the OM pool were 41% (2 cm) with $\delta^{13}C$ of −26.70‰ and 35% (10 cm) with $\delta^{13}C$ of −27.60‰. The dam zone was the only zone with distinct autochthonous composition. The 2 cm depth had a prominent phytoplankton component (−30.48‰) and the estimated allochthonous contribution was 7% to the OM pool. The 10 cm depth sediment was however, mostly allochthonous (60% of the OM pool, $\delta^{13}C$ was – 25.10‰). Field observations also revealed the presence of blooms of the cyanobacteria *M. aeruginosa* near the dam, which may have contributed to the aquatic signature of the OM pool in the first 2 cm of the sediment. In all zones the 2 cm sediment depth grain sizes were classified largely as fine (8–16 μm) and very fine silt (4–8 μm) classes. Porosity ranged from 0.70 ± 0.10 at the 2 cm depth to 0.52 ± 0.10 at the 10 cm for all zones.

The organic carbon mineralization was different among the three zones with the highest average values in the transition (154.80 ± 33.50 mg C m^{-2} d^{-1}), followed by the river (91.30 ± 28.70 mg C m^{-2} d^{-1}) and the dam (51.60 ± 26.80 mg C m^{-2} d^{-1}; **Figure 3B**). The bacterial abundance followed the same pattern as the mineralization rates as it was higher in the transition (5.10 ± 1.20 cells 10^6 mL^{-1}; **Figure 3C**) and the river (4.30 ± 1 cells 10^6 mL^{-1}). While no significant differences between bacterial abundance in the transition and river were found ($p = 0.44$), both zones were statistically different from the dam (3.30 ± 0.70; $p < 0.001$). The pCO_2 in the overlying water of the sediment was significantly different among the three zones ($p < 0.001$), gradually decreasing from the river to the dam, with highest values at the river (2370.60 ± 500 μatm) relative to the transition (2035.20 ± 10 μatm) and dam (1785.20 ± 100 μatm; **Figure 3D**). The pCO_2 in the surface water along the reservoir (data from Roland et al., 2010) also showed high values of pCO_2 on the river, but, with no statistical differences in the other zones ($p = 0.82$; **Figure 3D**). The organic carbon mineralization in the sediment was also positively related to the pCO_2 in the surface water of the reservoir ($p < 0.001$, $r^2 = 0.73$; **Figure 4**).

The bacterial abundance was positively related to OC mineralization ($p < 0.001$, $r^2 = 0.50$; **Figure 5**) in the overlaying water of the sediment cores.

DISCUSSION

The OC mineralization rates in the sediment indicated that the allochthonous OM supply was an important fuel to the metabolism in the sediment of different zones of the Manso Reservoir. The stable isotope analysis of POM from different water column depths suggested different pools of OC in the zones, with isotopic signatures more enriched (allochthonous) in the river and transition and more depleted (autochthonous) in the dam. The $\delta^{13}C$ of POM of the first 2 cm of sediment followed the same pattern of the POM sources in the water, and reflected recent processes showing more ^{13}C-enriched in the river and transition zones, probably a result of the terrestrial material coming from the littoral areas, and more depleted in the dam, where the effect of littoral areas is lower and there is higher contribution of primary

Table 1 | Organic matter (OM), total carbon (TC), total nitrogen (TN), ratio between TC and TN (C/N), total phosphorus (TP) concentrations at 2 and 10 cm of sediments sampled in the different zones of the Manso reservoir.

System	Depth (cm)	OM (% DW)	TC (% DW)	TN (% DW)	C/N (by atom)	TP (mg g DW $^{-1}$)
River	2	9.97	5.03	0.39	12.90	0.07
	10	14.64	2.83	0.17	16.65	0.08
Transition	2	10.73	2.25	0.18	12.50	0.07
	10	15.82	5.56	0.32	17.38	0.07
Dam	2	11.31	2.90	0.22	13.18	0.08
	10	2.35	0.59	0.04	14.75	0.04

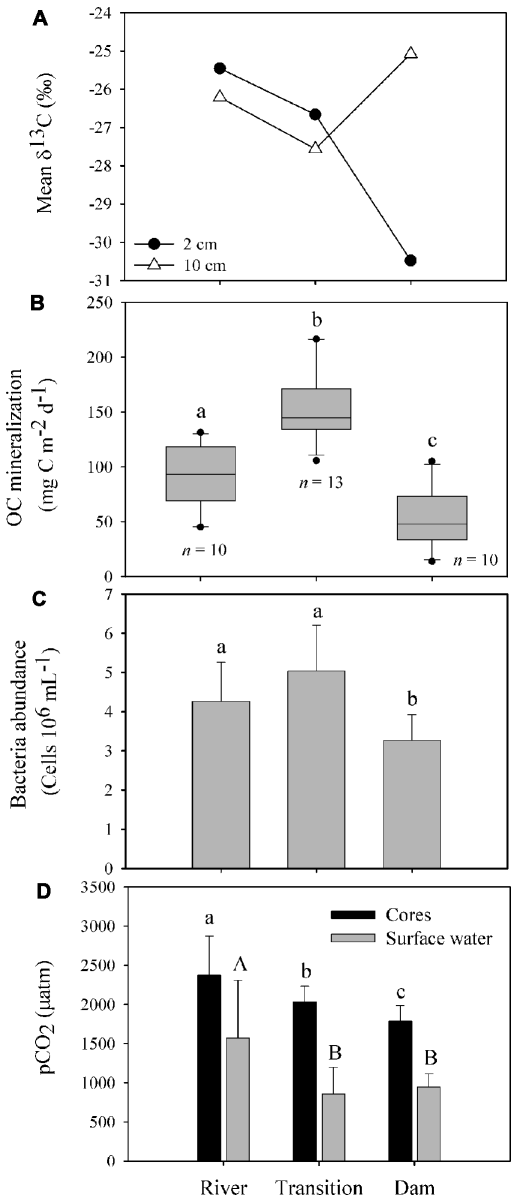

FIGURE 3 | Characteristics of OM composition, OC mineralization, bacteria abundance and partial pressure of CO_2 in the sediment of different zones of the Manso Reservoir. (A) Carbon isotope composition of the 2 and 10 cm of sediments sampled in different zones of the Manso Reservoir. **(B)** OC mineralization rates measured in the overlying water of sediment core incubations. The boundary of the box closest to zero indicates the 25th percentile, a line with the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Error bars above and below the box indicate the 90th and 10th percentiles and dark circles represent the outliers. **(C)** Mean and standard deviation of bacterioplankton abundance measured in the overlaying water of the sediment cores incubations. Small letters represent the differences between the pCO_2 from overlaying water of the sediment cores incubations (black bars) and capital letters represent the differences between the pCO_2 measure on the surface water of the reservoir (gray bars). **(D)** Means and standard deviations of partial pressure of CO_2 (pCO_2) from the overlaying water of the incubation cores (black bars) and in the surface water of the reservoir (gray bars; data from Roland et al., 2010). In all panels different letters represent significant differences (one-way ANOVA, Tukey's *post hoc*, $p < 0.001$).

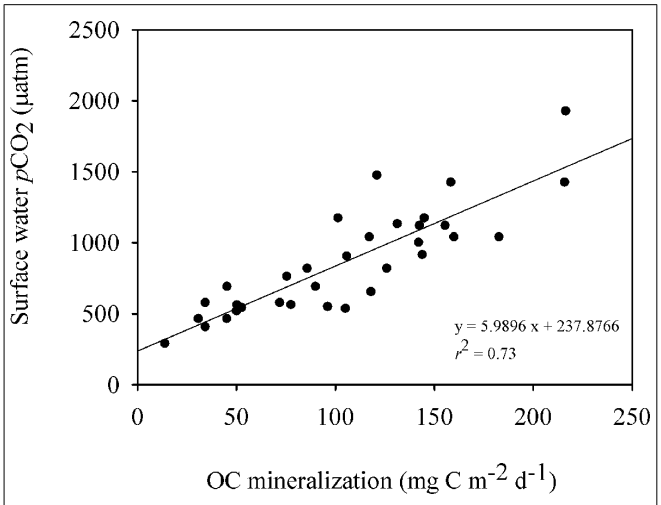

FIGURE 4 | Linear regression describing the relationship between OC mineralization rates in the sediment and the partial pressure of CO_2 (pCO_2) in the surface water of the reservoir ($n = 33$; $p < 0.001$).

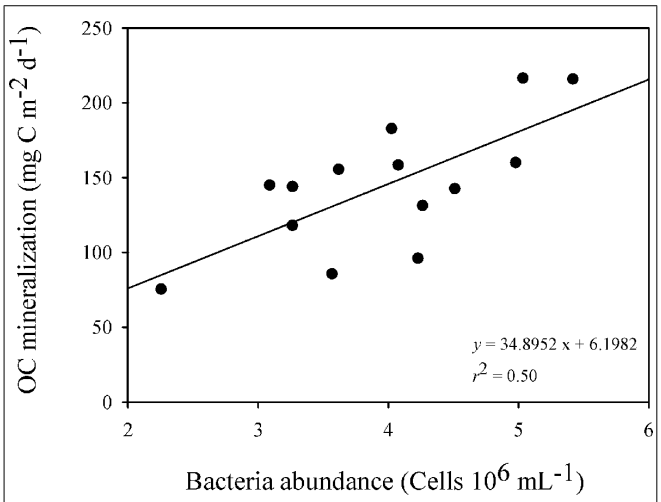

FIGURE 5 | Linear regression describing the relationship between bacteria abundance and OC mineralization rates from the overlying water of the incubation cores over time ($n = 18$; $p < 0.001$).

producers (e.g., phytoplankton) in relation to the other zones (Kalff, 2002; Tremblay et al., 2004). Degradation of POM during sinking along the water column and in surficial sediment layers may cause changes in the isotopic signature (Meyers and Eadie, 1993). Although, for a lake sediment of both autochthonous and allochthonous origin, the $\delta^{13}C$ has been reported to increase by 0.4–1.5‰ during the initial degradation, with only minor changes after that period (Gälman et al., 2009). This fractionation during initial degradation is comparatively small and will therefore only introduce a minor error in the calculated proportions of allochthonous POM. We observed a change in the POM signatures in the samples from the different water column depths of the dam zone, with more enriched signature in the bottom (27 m). However, the top 2 cm of sediment of the dam zone had a more

depleted signature. In this case, the depleted isotopic signature of the sediment may be related to past plankton blooms and previous mixing events that could have caused higher deposition of the phytoplankton from the water column. The 10 cm sediment layer was mostly allochthonous in the different zones, once the isotopically light phytoplankton debris may be preferentially degraded along the time. This layer can be seen as a reflection of previous conditions in the reservoir and is composed by older OM than the 2 cm.

The sediment sampled in 2 and 10 cm layers was mainly composed of fine and very fine silt, rich in carbon and nitrogen and poor in phosphorus compared to other water bodies (Lennon and Pfaff, 2005), what meets the mesotrophic condition of the system (Rangel et al., 2012). By the time of the survey, the Manso Reservoir had been in operation for 10 years and along its whole area, mainly in the dam, it was still possible to find a large number of dead trees remaining from the beginning of the impoundment. We believe the dead trees probably no longer contribute to mineralization in the sediment anymore, since they died more than 10 years ago and the remaining biomass was still there because of their very slow decay (Tremblay et al., 2004). Also, there was no visual contribution of aquatic macrophytes during the sampling, what indicates that phytoplankton and terrestrial material were, respectively, the most important autochthonous and allochthonous sources of carbon for the sediment mineralization during the sampling period.

A gradient of allochthonous OM contribution was found in the top 2 cm of sediment along the zones, with the greatest contribution in the river zone (56%), less in the transition zone (48%), and even less in the dam zone (7%). The OC mineralization followed the same pattern and increased with increasing inputs of allochthonous OM in the sediment. However, it is important to highlight that the origin and quality of the OM in the different zones may not be the same. For instance, the literature points out that allochthonous carbon reaching the rivers is mostly composed of terrestrial OM coming from the watershed, and this OM is more difficult to degrade and assimilate by aquatic bacteria (Kritzberg et al., 2005) and fungi (Gessner et al., 2007) communities, while the OM in the lacustrine area of the reservoir is more labile and is mostly released by primary producers in the water column (Tremblay et al., 2004; Forbes et al., 2012). However, in the river and in the transition zones, in spite of the OM be less degradable, there is higher OM deposition in comparison with the dam zone: as water flows become less turbulent upon entry of the river water into the reservoir, suspended particles will be rapidly deposited in the river zone and transition zone. We hypothesize that the higher amount of allochthonous OM deposition in the river and transition zones compensates for the low degradability of the allochthonous OM, because, with more OM available in the sediment, more OC is delivered to sediment bacteria and fungi, promoting higher OC mineralization rates in those zones. In addition, it is important to consider that OM sedimentation can also be influenced by the reservoir hydrodynamics, making it more difficult to trace OM source with a two source mixing model.

The Manso Reservoir is a typical tropical hydroelectric reservoir with a marked longitudinal gradient forming the river, the transition and the dam zones addressed in this study and first proposed by the reservoir zonation theory (see Thornton et al., 1990). The Manso Reservoir has complex hydrology, influenced by seasonal climatic conditions and by the river Manso dynamics. During the summer season (from late December to early March), when our sampling was performed, the Manso Reservoir is mostly stratified (Pacheco et al., 2011). However, sporadic heavy rain may occur in the region, mixing the entire water column of the reservoir. Studies made in the Manso reservoir during the same period of our sampling (Assireu et al., 2011; Pacheco et al., 2011) reported a plunge inflow of the river Manso in the reservoir, allocating the river at the hypolimnion level. This inflow of the Manso River in the reservoir highlights possible interactions between the river water and the POM pools in hypolimnion of the dam zone. In fact, it supports the similar $\delta^{13}C$ signatures of the POM found in the hypolimnion of dam (27 m) and the water samples of the river and the transition zones.

Heterotrophic bacteria were an important link between the allochthonous OM, the OC metabolism in the sediment and the release of carbon from sediment to water in the reservoir. In our survey we found a positive relationship between bacteria abundances and the OC mineralization rates (**Figure 5**). Similar relationships between bacterial abundance and OC mineralization in the sediment were found in a Chinese lake with different trophic conditions, where increasing contributions of allochthonous OC enhanced bacteria density, biomass, and diversity (Bai et al., 2012). Also, a positive relationship between OC mineralization in the sediments and bacteria biomass was found in a survey of eight boreal lakes (Gudasz et al., 2012). Those findings reinforce the importance of bacterial metabolic activities on the functioning of aquatic ecosystems (Pomeroy, 1974; Azam et al., 1983; Zehr, 2010) and call attention to the importance of biogeochemical processes developed by this community, such as respiration (Biddanda et al., 2001; Lennon and Pfaff, 2005) and the conversion of reduced forms of carbon into their biomass (Sherr and Sherr, 2002). Our findings highlighted that bacterial aerobic respiration was an important metabolic activity in the sediment of the reservoir and was fueled by the differential allochthonous OM delivery, as has been pointed out by the literature for lakes (Cole et al., 2000; Hanson et al., 2003; Kritzberg et al., 2004).

Evidence of allochthonous OC supporting metabolism in the water (Kritzberg et al., 2005; Cole et al., 2007; Karlsson et al., 2012) and in the sediment (Algesten et al., 2005; Kortelainen et al., 2006; Gudasz et al., 2010; Bai et al., 2012) of aquatic ecosystems has been reported in the literature, although the influence on OC mineralization in the sediments is still uncertain (Bai et al., 2012; Gudasz et al., 2012), especially for systems located in tropical areas and, in particular, man-made reservoirs (Battin et al., 2009; Tranvik et al., 2009). Furthermore, in carbon budget models the sediment is mainly seen as a site of carbon storage (Cole et al., 2007; Tranvik et al., 2009), and the amount of carbon that this compartment mineralizes back to the water and to the atmosphere is poorly considered. The carbon mineralized in the sediment, together with other sources of CO_2 from the water column, is a significant carbon source to the atmosphere; especially if we take in account the area occupied by reservoirs in the landscape is increasing worldwide (World Commission on Dams [WCD], 2000). For instance, in the three different zones of the Manso reservoir, we found an OC

mineralization average rate of roughly 100 mg C m^{-2} d^{-1}. If the entire area of the reservoir (360 km^2) is considered along 1 year, the carbon release from the sediment to the water is about 13 Gg C y^{-1}.

In this study we found a positive relationship between the OC mineralization in the incubation cores and the pCO_2 measurements made in the surface water of the Manso Reservoir ($r^2 = 0.73$), pointing toward sediment OC mineralization being an important source of the CO_2 emitted from the reservoir (**Figures 3D** and **4**), and toward a close link between sediment OC mineralization and the spatial variability in surface water pCO_2. It is important to point out that the pCO_2 measurements were made when the reservoir was not stratified, such that CO_2 production in the sediments can leave an imprint in the pCO_2 of the surface water; during water stratification periods, the carbon coming from the mineralization in the sediment may be trapped in the hypolimnion and may not contribute to the CO_2 flux from the water to the atmosphere. In addition, it is important to highlight that the relationship between the OC mineralization in the sediment and the pCO_2 in the surface water of the reservoir can also be regulated by other factors such as the CO_2 saturation in the water, the wind speed in the area and the depth of the reservoir (Guérin et al., 2006), which can affect the contribution of the OC mineralization to the amount of CO_2 emitted by the reservoir as a whole. Moreover, a study of five hydroelectric reservoirs in Brazil, including the Manso Reservoir (Roland et al., 2010), reported that pCO_2 and CO_2 emissions are also linked to spatial variations of the systems. For instance, their study shows that in general, considering all the reservoirs, the sites closest to the dam of the reservoir tend to have the lowest pCO_2 saturations compared to other zones such as transition and river. By considering the carbon flux reported by previous study (352 mg C m^{-2} d^{-1}; in Roland et al., 2010) and the average OC mineralization rate found in the sediments of the different zones of the Manso Reservoir (∼100 mg C m^{-2} d^{-1}), we estimate that the OC mineralization in the sediment can contribute up to 28% of the CO_2 emitted to the atmosphere. Those findings reinforce the importance of the relation between the OC mineralization in the sediment and the CO_2 emissions, but also show that this relation is shaped by other factors that need to be better understood in order to arrive at more accurate estimate.

In summary, we found that the spatial variability in OC mineralization rates in the sediment may be linked to differences in allochthonous OM supply to the sediments, and mirrored in both the spatial variability of surface water pCO_2 and bacterial abundances in the sediment. The OC mineralization rates in the sediment contribute significantly to the CO_2 emission from the water to the atmosphere. These results highlight that sediments should be considered in the assessment and management of carbon emissions from reservoirs to the atmosphere.

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Development of temporary subtropical wetlands induces higher gas production

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Temporary wetlands are short-term alternative ecosystems formed by flooding for irrigation of areas used for rice farming. The goal of this study is to describe the development cycle of rice fields as temporary wetlands in southern Brazil, evaluating how this process affect the gas production (CH_4 and CO_2) in soil with difference % carbon and organic matter content. Two areas adjacent to Lake Mangueira in southern Brazil were used during a rice-farming cycle. One area had soil containing 1.1% carbon and 2.4% organic matter, and the second area had soil with 2.4% carbon and 4.4% organic matter. The mean rates of gas production were 0.04 ± 0.02 mg CH_4 m^{-2} d^{-1} and 1.18 ± 0.30 mg CO_2 m^{-2} d^{-1} in the soil area with the lower carbon content, and 0.02 ± 0.03 mg CH_4 m^{-2} d^{-1} and 1.38 ± 0.41 mg CO_2 m^{-2} d^{-1} in the soil area with higher carbon content. Our results showed that mean rates of CO_2 production were higher than those of CH_4 in both areas. No statistically significant difference was observed for production of CH_4 considering different periods and sites. For carbon dioxide (CO_2), however, a *Two-Way* ANOVA showed statistically significant difference ($p = 0.05$) considering sampling time, but no difference between areas. The results obtained suggest that the carbon and organic matter contents in the soil of irrigated rice cultivation areas may have been used in different ways by soil microorganisms, leading to variations in CH_4 and CO_2 production.

Keywords: ecosystem development, % carbon, % organic matter, CH_4, CO_2

INTRODUCTION

Land use and land-use changes in agricultural and forest systems, industrial development, and urban expansion are among the sources of the current anthropogenic emissions of greenhouse gases (GHG) such as carbon dioxide (CO_2) and methane (CH_4). These activities have contributed to change the carbon and nitrogen cycles in terrestrial and aquatic ecosystems. Particularly, the contribution of agricultural soils to CO_2 and CH_4 emissions depends on biophysical processes and on the incorporation and decomposition of organic residues in the soil (Muñoz et al., 2010).

Wetlands, as well as rice paddies, contribute between 15 and 45% of global methane emissions (Segers, 1998). Organic matter tends to accumulate in the sediments of wetland soils. The carbon storage in soil organic matter is due to suppressed decomposition rates resulting from long-term soil saturation with water (Bohn et al., 2007). Soil organic matter is composed of a complex mixture of decayed plant and soil matter, with polymeric arrangements of these materials with other organic substrates (Wilson et al., 1983), whose rate and extent of degradation under either oxic and anoxic conditions are dependent on many biologic controls, including soil organic matter quality (Baldock and Skjemstad, 2000; Kristensen and Holmer, 2001; Blodau, 2002; Keller and Bridgham, 2007; Österreicher-Cunha et al., 2012).

The submerged conditions in rice paddies and the rice plants, especially their roots, supply the soil with organic carbon compounds which are mineralized by microorganisms (Hogberg and Read, 2006). The relationship between rice plants and microorganisms is important because the available substrates regulate many processes related to the emission or removal of gases (Insam and Wett, 2008).

Carbon gas production from wetland soil depends upon the rate of carbon deposition and the rate of mineralization (Smith et al., 2003). The rate of mineralization depends on a series of both biological and environmental factors and their interactions (Kristensen and Holmer, 2001). While the wetlands methane emissions result from anaerobic decomposition processes in deeper layers of wetland soil, CO_2 emissions are related to oxidation of methane in upper oxic soil layers and respiration (Bohn et al., 2007). The rice-paddy sediment provides the ideal conditions for methanogenesis (Roehm, 2005), because the sediment undergoes oxygen depletion due to high moisture and relatively high organic-substrate levels and the presence of a methane-producing subsurface anaerobic zone and an aerobic surface zone that oxidizes this gas (Whalen, 2005). Anaerobic mineralization of carbon is the principal regulator of methane production in the sediment (Segers and Kengen, 1998), where the activity of methanogenic microorganisms converts a relatively narrow layer of simple substrates to methane (Zinder, 1998). Hence, the methanogenic microbial community in rice field soil contributes about 13% to the global budget of CH_4 (Lelieveld et al., 1998). Other gases such as CO_2 are also related to microbial activity.

Gas emissions from wetlands normally show wide seasonal and temporal fluctuations, resulting from variations in environmental variables that regulate the microbial processes involved in

the flux (Liikanen et al., 2006). The main local controls of CO_2 productions from wetlands include the quality of soil organic substrates (Updegraff et al., 1995). Given the diverse variables that control the emission of methane and other gases from wetland environments (Yang and Chang, 1998; van der Nat and Middelburg, 2000; Conrad, 2002; Hirota et al., 2004; Whalen, 2005; Liikanen et al., 2006; Cheng et al., 2007; Welsch and Yavitt, 2007; Kao-Kniffin et al., 2010; Khosa et al., 2010; Inubushi et al., 2011), evaluating these processes and the effects of participating microorganisms is a complex task.

Rice fields are temporary wetlands formed by the extensive irrigation of areas for periods of approximately 90 days, with long postproduction drainage periods. Available data concerning these temporary wetlands at latitudes above 30° in the Southern hemisphere are sparse (Canterle et al., 2010; Rodrigues et al., 2011). Nevertheless, farming of irrigated rice represents the main crop for extensive lowland areas in southern Brazil, turning the soil of these areas in organic matter reservoirs, as the result of plant biomass accumulation at each production cycle. Considering that induced differences in the soil properties and microbiota characteristics may result in significant changes in the mineralization process, could rice fields, with different soil organic content and carbon, present different mineralization rates as measured by gas production? Therefore, the goal of this study is to describe the development cycle of rice fields as temporary wetlands, analyzing the dynamic of limnological variables and the mineralization process in the gas production (CH_4 and CO_2) in areas with different incorporated organic matter and carbon content in southern Brazil.

MATERIALS AND METHODS

STUDY SITE

The study was conducted during the 2005/2006 crop cycle in two areas of flooded-rice cultivation (BR-IRGA 410 cultivar). The water used to irrigate these areas was taken from Lake Mangueira (**Figure 1**) which, together with its contributing basin, form the Mangueira subsystem and cover an area of 1597 km^2. This subsystem belongs to the Taim Hydrological

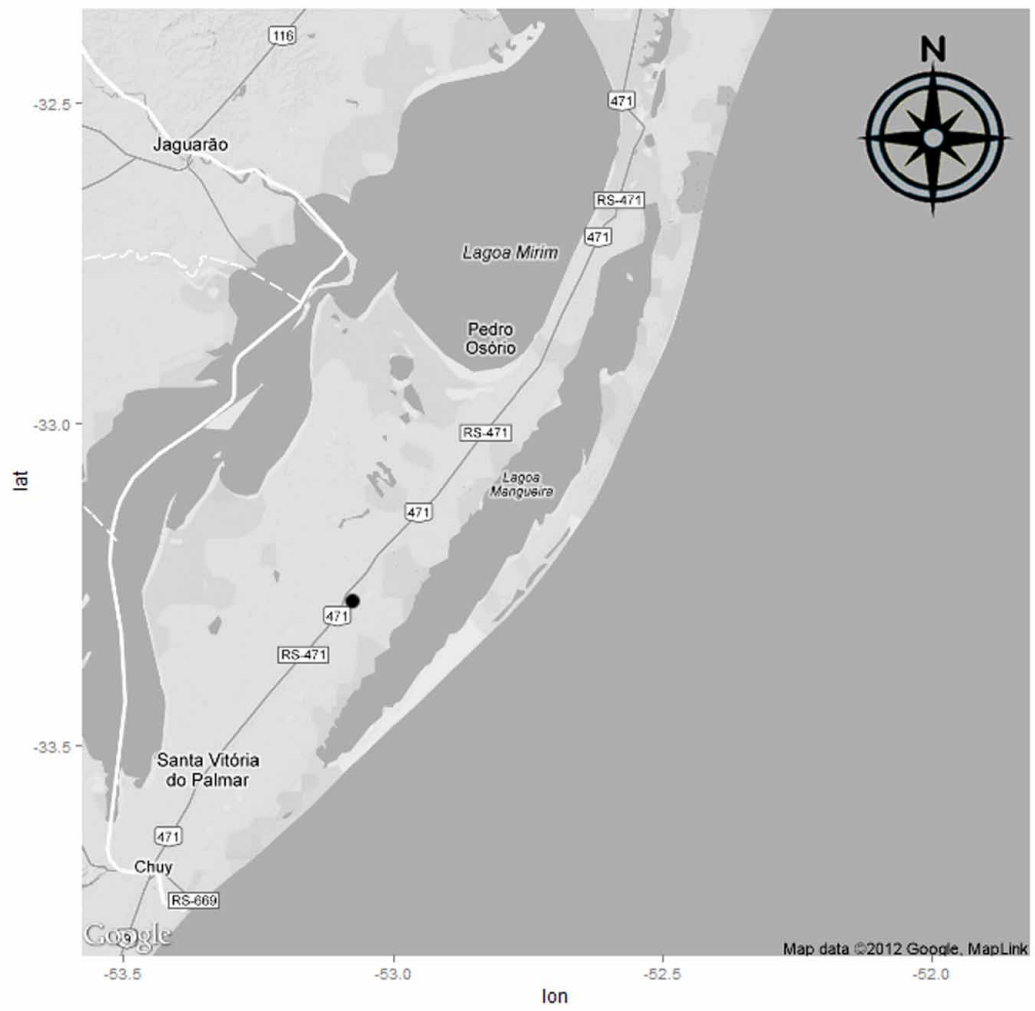

FIGURE 1 | Mangueira Lake, a large coastal lake in the state of Rio Grande do Sul, Southern Brazil, and localization of rice fields sampled (●).

System and is part of a gradient of floodplains characterized by the presence of freshwater wetlands and associated lakes, situated in Rio Grande do Sul State in southern Brazil (Motta-Marques et al., 2002). Part of the water used during irrigation of the cultivated areas returns to the lake via a drainage channel.

Each area has a soil with different organic-matter and carbon contents. Area 1 (S 33.288560°; W 53.092518°), 4 ha in extent, had soils with lower organic-matter and carbon contents. Area 2 (S 33.287297°; W 53.088699°), with an area of 1 ha, had higher organic-matter and carbon contents.

SAMPLING AND SAMPLE ANALYSIS

Sampling was conducted between 16/12/2005 and 16/03/2006, corresponding to the rice crop cycle between the period of flooding (first week of December) and harvest (last week of March). Five sample series for each area were taken in order to estimate the production of gases and bacterial abundance (BA), and four sample series (except in the last sampling) were taken for limnological analyses.

Water samples were collected from the water/sediment interface at each sampling site for the limnological analysis. Water temperature, dissolved oxygen and pH were determined using a multiparameter probe (Yellow Springs Instruments model YSI 6920). Ten 1-L water samples were collected for chemical analysis and chlorophyll a. The variables were determined as follows: total phosphorous (TP), soluble reactive phosphorus (SRP), total nitrogen (TN), and nitrate (NO3) were analyzed according to Mackereth et al. (1989). Total solids (TS) were estimated according to APHA (1999), and soluble reactive silicon (SRSi) was measured by the photometric method, using a commercial kit (Si Merck Spectroquant7 kit for silicate-sulfuric acid). Dissolved organic matter (DOM) was analyzed by the spectrophotometric method (Strome and Miller, 1978). Carbon (dissolved organic, DOC; and dissolved inorganic, DIC) was analyzed using a total organic carbon (TOC) analyzer (Shimadzu VCPH). Chlorophyll a was extracted from GF/F filters in 90% ethanol (Jespersen and Christoffersen, 1987) and measured by the spectrophotometric method (APHA, 1999).

Sediment samples from each area were collected during the first sampling (December/05) for chemical, element and macronutrient analyses as the following methods: percentage of organic matter was determinate by humid digestion, clay was determinate by densimeter method, K and P were determinates by Mehlich I method, S-SO_4 extracted with $CaHPO_4$ 500 mg L^{-1} of P, Zn and Cu were extracted with HCl 0,1 mol L^{-1}, B was extracted with hot water, Mn exchangeable extracted with KCl 1 mol L^{-1} and % soil carbon was analyzed using a TOC analyzer (Shimadzu VCPH).

For the CH_4 and CO_2 analysis, at five sites within each area, the surface layer (about 10 cm) of the sediment was collected in PVC tubes, placed in plastic bags and stored in a refrigerator for no more than 2 days until incubation. At the sediment sampling sites, water samples from the water/sediment interface were collected in 50-mL flasks and also stored in the refrigerator until incubation was performed. In the laboratory, each sediment sample was homogenized and an aliquot of 6 g was placed in a 25-mL glass flask and mixed with 5 mL of the water collected at the same site, forming five sludge slurry samples per area. Prior to incubation, gaseous nitrogen (N_2) was bubbled through the sludge slurry to create an anoxic environment (Minello, 2004). The flasks were closed with rubber septa, sealed with metal rings and incubated in the dark at a controlled temperature (20 ± 0.5°C). Gas production in the sediment was determined from the accumulation of CH_4 and CO_2 in the headspace of the incubation flasks (Casper, 1992). After a 5-d incubation period, an aliquot of the internal atmosphere of each flask was extracted to determine the CH_4 and CO_2 produced, using gas chromatography (Varian Star 3400, Varian) with a flame ionization detector, injector temperature of 120°C, a Chromosorb 102 column (12′ × 1/8″), (80/100) at 27°C, and using helium as the carrier gas. Gas production, measured by gas chromatography, was expressed as $g^{-1} \cdot L^{-1}$. The data conversions were carried out using the mass × sediment volume relation (obtained from the PVC cores used in the removal of sediment samples and volume of the sediment). We assumed a fixed sediment depth for gas production considering values observed in the literature. Data conversion to m^2 was done considering the core sampling area (28.27 cm^2) and assuming that most of the gas production was related to the upper 10 cm of the sediment layer (Casper, 1992).

For bacterioplankton abundance analysis, water samples from the water-sediment interface at each sampling point were fixed in 40% formaldehyde (final concentration in the sample = 4%) and prefiltered on quantitative paper (MN 640 d Macherey–Nagel; mean particle retention size 2.0–4.0 μm). Next, 2 ml of each sample was filtered and stained with acridine orange, and cells were concentrated onto 0.2-μm black polycarbonate membranes (GE), according to the modified protocol of Hobbie et al. (1977). BA, determined as cells per mL, was estimated using an inverted epifluorescence microscope (Olympus IX70).

DATA ANALYSIS

Data analysis was performed by using a *Two-Way* ANOVA to compare gas production, BA and limnological variables. With this approach the two sites were compared after extracting the temporal effect, at the same time as sampling period were compared with no effect of sampling site. Associations between the different variables (gases, limnological variables and BA at the water/sediment interface) were assessed through correlation analyses using Spearman correlation coefficients. Statistical analyses were done using SigmaPlot 10.0 software. A probability level of $\alpha = 0.05$ was adopted to determine statistical significance.

RESULTS

Analysis of soil from the sampling sites showed that the organic-matter, carbon and clay contents and pH were all higher in Area 2 than in Area 1 (**Table 1**). The percentages of organic matter and carbon in Area 1 sediment were approximately half those of Area 2. The mean pH values were also higher in Area 2. Regarding macronutrients, Area 1 showed a higher mean value for phosphorus, while higher mean values were obtained for potassium and manganese in Area 2.

The mean values for the limnological variables in both areas are presented in **Table 2**. During the study period, both rice fields

Table 1 | Results of chemical analyses of soil from the sampling sites.

Variable	Area 1	Area 2
Organic matter (%)	2.4 ± 0.2	4.4 ± 0.2
Carbon (%)	1.1 ± 0.2	2.4 ± 0.6
Clay (%)	12.3 ± 4.0	15.7 ± 3.1
K (%)	148.7 ± 60.4	150.5 ± 29.1
P (%)	25.3 ± 10.8	10.6 ± 2.1
Ca/Mg	3.1 ± 0.2	4.7 ± 2.0
Ca/K	6.6 ± 1.6	31 ± 13.2
Mg/K	2.1 ± 0.4	6.7 ± 1.5
S (%)	9.5 ± 2.7	9.8 ± 2.9
Zn (%)	2.3 ± 1.2	2.3 ± 0.5
Cu (%)	1.3 ± 0.3	0.7 ± 0.0
B (%)	0.4 ± 0.1	0.5 ± 0.1
Mn (%)	50.7 ± 22.0	6.3 ± 0.6
pH	5.6 ± 0.2	6.2 ± 0.1

presented similar temperature and pH. Mean concentrations of dissolved oxygen were higher in Area 1 than in Area 2. The concentrations of nutrients and chlorophyll *a* showed a tendency to decrease over the course of the rice-crop cycle.

No statistically significant difference was observed for limnological variables between the areas. However, *Two-Way* ANOVA showed significant difference considering different periods in sites for DOC ($p = 0.028$); TS ($p = 0.022$); SRSi ($p = 0.002$); organic matter (UV-DOC 254 nm) ($p = 0.024$); chlorophyll *a* ($p = 0.000$) and NO_3 ($p = 0.020$).

No statistically significant difference was observed for production of CH_4 considering different periods and sites. Methane production (**Figure 2**) showed a general decreasing gradient during the rice-crop cycle for the samples from Area 1; however, the samples from Area 2 showed irregular production levels, once that was observed alternating higher or lower values for methane production over time, although higher production values have been registered in all samples (see standard deviation). The highest

Table 2 | Results of analyses of water from the water/sediment interface samples.

Variable	Sampling 1		Sampling 2		Sampling 3		Sampling 4	
	Area 1	Area 2	Area 1	Area 2	Area 1	Area 2	Area 1	Area 2
DIC ($mg \cdot L^{-1}$)	19.66 ± 4.4	24.79 ± 4.5	21.82 ± 2.3	28.32 ± 3.5	25.47 ± 2.5	26.38 ± 1.1	27.74 ± 2.8	29.19 ± 3.2
DOC ($mg \cdot L^{-1}$)	15.11 ± 8.6	18.33 ± 10.6	12.52 ± 5.9	10.16 ± 2.7	7.12 ± 1.7	6.33 ± 0.8	8.30 ± 1.7	7.42 ± 1.9
Total Solids ($mg \cdot L^{-1}$)	310 ± 102	274 ± 96	238 ± 20	247 ± 12	221 ± 10	211 ± 11	201 ± 61	201 ± 17
SRSi ($mg \cdot L^{-1}$)	3.22 ± 0.77	3.47 ± 0.68	1.03 ± 0.90	1.56 ± 1.04	1.76 ± 0.84	2.14 ± 0.48	0.36 ± 0.33	0.27 ± 0.09
DOM—UV_{DOC} (254 nm)	0.44 ± 0.26	0.30 ± 0.13	0.03 ± 0.03	0.05 ± 0.03	0.14 ± 0.03	0.18 ± 0.03	0.02 ± 0.01	0.01 ± 0.01
Chlorophyll *a* ($\mu g \cdot L^{-1}$)	29.33 ± 21.85	33.19 ± 22.80	39.47 ± 17.28	42.73 ± 21.35	2.51 ± 1.38	2.52 ± 0.64	2.25 ± 1.20	1.64 ± 0.66
TP ($\mu g \cdot L^{-1}$)	5.9 ± 4.5	3.2 ± 1.9	2.3 ± 1.5	2.8 ± 1.7	1.0 ± 0.3	1.5 ± 0.2	0.1 ± 0.0	0.1 ± 0.0
SRP ($\mu g \cdot L^{-1}$)	2.82 ± 2.31	1.21 ± 0.88	0.30 ± 0.38	2.25 ± 2.40	0.14 ± 0.03	0.18 ± 0.03	0.03 ± 0.02	0.02 ± 0.01
TN ($\mu g \cdot L^{-1}$)	100 ± 20	170 ± 70	40 ± 20	40 ± 10	30 ± 20	20 ± 10	20 ± 10	10 ± 5
NO_3-N ($\mu g \cdot L^{-1}$)	50 ± 40	50 ± 20	20 ± 10	30 ± 20	10 ± 5	10 ± 5	NQ	NQ
Dissolved oxygen ($mg \cdot L^{-1}$)	7.6	5.9	10.6	6.8	9.9	7.7	5.9	5.5
Water temperature (°C)	22.1	21.2	27.2	26	23.8	22.8	26.3	28.4
pH	7.8 ± 0.2	7.4 ± 0.4	7.3 ± 0.3	7.2 ± 0.1	6.7 ± 0.4	6.9 ± 0.2	7.6 ± 0.4	7.3 ± 0.4

DOM, dissolved organic matter; DIC, dissolved inorganic carbon; DOC, dissolved organic carbon; SRSi, soluble reactive silicate; TP, total phosphorus; SRP, soluble reactive phosphorus; TN, total nitrogen; NO_3, nitrate.

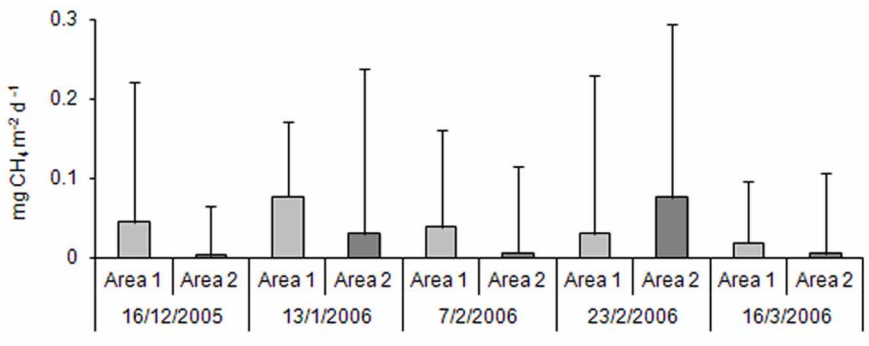

FIGURE 2 | Methane productions in the Area 1 and in the Area 2 throughout the rice crop cycle ($n = 3$).

values were obtained in Area 1 in the second sampling, after the application of urea; in Area 2, the effect of application of the inorganic fertilizer probably resulted in higher values only in the third sampling.

For CO_2, the highest values were observed in the third sampling, in both areas (**Figure 3**). A *Two-Way* ANOVA showed statistically significant difference ($p = 0.05$) considering sampling time, but no difference between areas.

Although the bacteria present in the sediment samples from the sampling sites, as well the attached bacteria have not been analyzed, unpublished data of the authors obtained in another study show an increase in density of methanogenic and methanotrophs microbial groups in experiments carried out with samples of the sediment-water interface. In addition, samples from the sediment-water interface site presents, normally, a consortium of microbes; it was added to the sludge slurry incubated in the experiment. These facts could be used to infer the effect of the microbial groups from the water/sediment interface samples in the gas production at the upper layer of sediment. Bacterioplankton abundance rates in the two areas showed similar trends as the values obtained for CH_4 and CO_2 production, with higher abundances in Area 1, which showed lower soil % organic matter and % carbon (**Figure 4**). The highest values for BA were observed in the second sampling, after the application of inorganic fertilizer in both areas. A *Two-Way* ANOVA showed no statistically significant difference was observed for BA considering different periods and sites.

The correlation analysis of CH_4 and CO_2 production showed that gases in Area 1 were positively correlated with BA from the water/sediment interface samplings (CH_4: $r = 0.54$; CO_2: $r = 0.56$; $p < 0.05$). On the other hand, in Area 2 the CH_4 production was not correlated with soil components or BA from the water/sediment interface samplings, while CO_2 production was negatively correlated ($r = -0.60$; $p < 0.05$) with BA from the water/sediment interface.

From the data obtained in this study, it is possible to estimate the contribution of temporary subtropical wetlands in the region, regarding the production of CH_4 and CO_2 gases. Considering the mean rates of gases production in the two areas ($n = 5$), mean methane production was 0.041 ± 0.02 mg m^{-2} d^{-1} in Area 1 and 0.024 ± 0.03 mg m^{-2} d^{-1} in Area 2, while the mean production rate for CO_2 production presented 1.178 ± 0.3 mg m^{-2} d^{-1} in Area 1 and 1.383 ± 0.41 mg m^{-2} d^{-1} in Area 2. The difference in mean values was not statistically significant.

When compared the values obtained for the gas production rates obtained in this study with the rates obtained in studies carried out in different environments and countries, can observe that our rates showed lower values (**Table 3**). According to the table, the lowest values of methane production were observed in studies

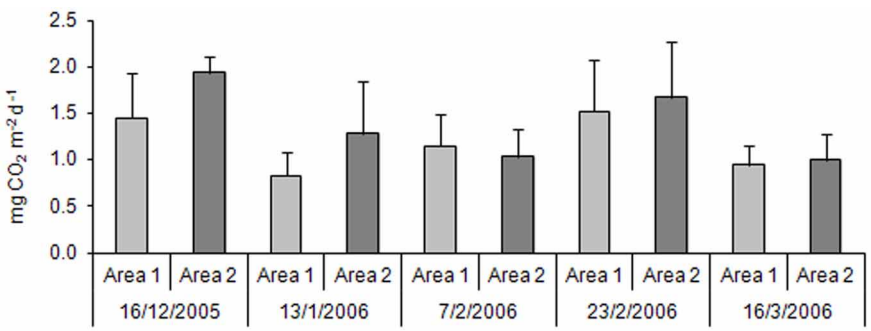

FIGURE 3 | Carbon dioxide productions in the Area 1 and in the Area 2 throughout the rice crop cycle ($n = 3$).

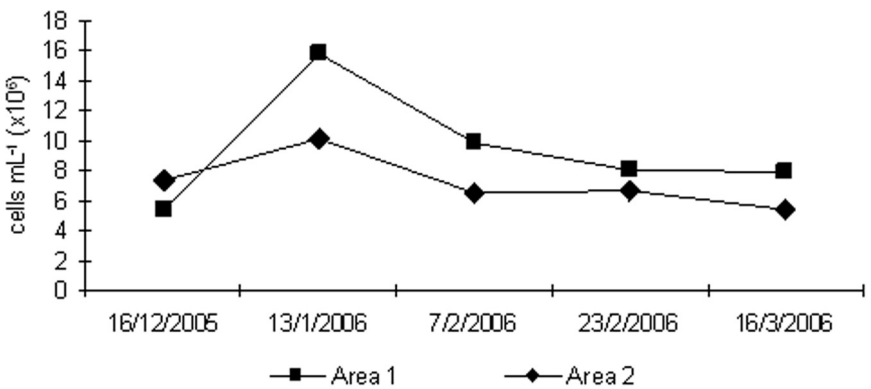

FIGURE 4 | Bacterioplankton abundance rates obtained from the water/sediment interface in both areas during rice cultivation.

Table 3 | Rates of CH_4 and CO_2 production in soils intermittent or permanently inundated.

Site/Country	CH_4 production (mg m^{-2} d^{-1})	CO_2 production (mg m^{-2} d^{-1})	Method	References
Rice field 1/Rio Grande do Sul, Brazil	0.04 ± 0.02	1.18 ± 0.3	Measured by accumulation of gas in the head space	This study
Rice field 2/Rio Grande do Sul, Brazil	0.02 ± 0.03	1.38 ± 0.41		
Rice field (bare soil)/Punjab, India	1.68 to 49.44	–	Measured by closed chamber technique	Khosa et al., 2010
Rice field (transplanting of rice crop)/Punjab, India	0.96 to 22.32	–		
Rice field 1/Gujarat, India	2536 to 17295	–	Measured by static chamber technique	Kumar and Viyol, 2009
Rice field 2/Gujarat, India	4838 to 10342	–		
Swamp/USA	83 to 155	–	Measured by chamber technique	Wilson et al., 1989
Marsh/USA	146 to 912	–	Measured by chamber technique	Alford et al., 1997
Permanently inundated wetland/USA	153.71	–	Measured by gas sampling chambers technique	Altor and Mitsch, 2006
Freshwater wetlands/USA				Mitsch and Gosselink, 1986
(a) Rice paddies	90.83	–	–	
(b) Michigan swamp	146.93	–	–	
(c) Dismal swamp/virgínia	1.33 to 20.03	–	–	
(d) Louisiana tidal freshwater marsh	587.75	–	–	
Lake dagow/Germany	1.40	–	Measured by accumulation of gas in the head space	Casper, 1992
Lake fuchskuhle/Germany	0.47	–		
Lake stechlin/Germany	0.32	–		

that use such methodology measurement of accumulation of gas in the head space.

DISCUSSION

In this study we found higher CO_2 production in Area 2 than Area 1, associated with higher carbon and organic-matter contents in the soil. Moreover, there was significant difference concerning sampling time, but no difference between areas. The overall CH_4 production as well as the BA obtained from water/sediment interface sampling did not differ significantly between the areas and sampling time.

Organic-carbon content parallels the rate of methane emission in rice fields (Kumar and Viyol, 2009). Differences in the organic chemistry of a specific soil type (Yavitt and Lang, 1990) and in the composition of the organic matter between environments, whether they are more labile or more refractory, influence mineralization (CO_2 and CH_4 formation) in the presence or absence of oxygen (Bastviken et al., 2003), resulting in variations in methane production rates between different wetland samples (Yavitt and Lang, 1990). Soil characteristics and microbiota, associated with other factors, and could result in different responses related to availability and carbon cycling (Österreicher-Cunha et al., 2012). The observed differences in the soil composition of the two areas used in this study, related to the % carbon and organic matter content, should be result in diverse responses to gas production in the samplings carried out over the cycle of rice crops.

The structure of the soil bacterial community, although it was not evaluated, was probably important for the gas emissions observed in this study. The numbers of methanogenic and methanotrophic bacteria as well as the type of methanotrophic bacteria affect CH_4 production (Wang et al., 2008). However, according to Krüger et al. (2005), observed variations in methanogenic processes are probably not caused by changes in the size of the methanogen community but in its activity. Observations on the pathway of CH_4 formation showed that substrate conditions most affect the methanogen community structure and function (Chin et al., 2004). The highest rates of methane and carbon-dioxide production, as well as microbial abundance from water-sediment interface samples occurred until the third sampling, before the rice plants had attained their maximum biomass (at the end of the culture cycle) in both areas. Although a number of environmental controls over bacterial respiration have been studied in wetlands (Segers, 1998), this fact implies that, probably, the differences in soil organic matter, rather than plant growth, drive most of the microbial activity, in concordance with results obtained by Welsch and Yavitt (2007). The different effects of organic matter may be closely related to the content of easily decomposable organic matter (Hou et al., 2000),

as well as the release of more-labile substrates that can be more easily assimilated by the microorganisms present in the water and sediment surface layers (Fonseca et al., 2004). Otherwise, the continuum of decomposability of the organic matter in soil can be altered by interactions with minerals within matrices capable of stabilizing potentially labile organic matter against biological oxidation (Baldock and Skjemstad, 2000), producing changes in the organic matter mineralization processes present in the soil of different areas. These variables probably influenced the methane production rates especially in Area l, even taking into account the differences in organic-matter concentrations.

The relationships between plants and soil microorganisms are important for bacterial activity (Hogberg and Read, 2006), and indirectly affect gas production. Methanotrophs are a diverse group of aerobic bacteria mainly found on the rhizosphere of aquatic plants, and their growth is favored by this aerobic microsite in the process of CH_4 consumption (Wang et al., 2008). Increased soil carbon contents often stimulate microbial growth and activity, which can increase the availability of soil nutrients and enhance plant growth (Weihong et al., 2000). The presence and the different species of macrophytes generally reduce GHG fluxes, due to a significant impact on oxygen dynamics, mainly via their capacity to increase redox potentials and dissolved oxygen concentration (Maltais-Landry et al., 2009). According to Bouchard et al. (2007), rapid root production and increased rooting depth enhance CH_4 oxidation to a relatively greater degree than methane production.

In this study, methane concentrations showed a decreasing gradient in successive samplings, especially in Area 1, in contrast to plant growth. The plant growth stage can affect the production of gases considering the development of underground biomass, and potential loss or GHG though leaves. The reduction in the methane concentrations observed as the cultivation cycle evolved could be an effect of plant and root development, substantially, or totally inhibiting methane production (Gilbert and Frenzel, 1998) and producing the irregular values obtained in this study. These results are in agreement with those of Picek et al. (2007), once that they found a gradual decline in both CH_4 and CO_2 toward the end of the growing season. The plants could attenuate methane emission, facilitating its oxidation by the transport and release of oxygen through the roots located in the aerobic surface zone of the sediment (Schrope et al., 1999; Whalen, 2005). However, measurements of growth of the rice were not performed throughout the cycle, which needs to be stated as a variable to be considered in the follow up works.

Permanently flooded areas produce more methane than those that are intermittently flooded (Kumar and Viyol, 2009), with or without the presence of emerging macrophytes (Altor and Mitsch, 2006). Variations in the water level can affect the emission of gases such as methane and CO_2 (Ratering and Conrad, 1998; Cheng et al., 2007; Hirota et al., 2007). In this study, the first sampling conducted in Area 2 resulted in the lowest methane production level of the entire cultivation cycle, when the areas reached minimal water layers. The drainage of the rice paddies resulted in decreased methane production, since this would increase the penetration of oxygen into the soil and suppress the production of this gas. On the other hand, the aeration process can result in an increase of CO_2 production, since that the degradation of organic matter is more efficient under oxidized conditions (Ratering and Conrad, 1998). Oxygen penetration into the soil permits oxidation of reduced sulfur to sulfate and ferrous iron to ferric iron. Sulfate and iron (III) favor the action of sulfur- and iron-reducing bacteria that use acetate and hydrogen substrates more efficiently than methanogenic bacteria (Ratering and Conrad, 1998). These processes lead to competition for the substrates, as observed by Minello (2004) in a study conducted on lagoon sediment, and, consequently, the hydrogen and acetate concentrations drop to levels that prohibit methane production (Ratering and Conrad, 1998; Conrad, 2002).

Under anaerobic conditions, the mineralization of organic carbon to CO_2 and CH_4 is carried out by a *consortium* of microbes, where the complex organic polymers are initially degraded by fermenting bacteria to yield a few simple products, which are subsequently used by methanogens to produce CH_4 (Conrad, 1989). The relative proportion in which CO_2 and methane gases are produced from organic material depends on the presence of sufficient inorganic oxidants, such as nitrate, manganese (IV), iron (III) and sulfate. When this is the case, the organic material is principally degraded to CO_2, increasing its production, and relatively little methane is produced (Yao and Conrad, 2000; Krüger et al., 2001). Our results showed that the mean rates of CO_2 production were higher than those of CH_4 in both areas. These results are also in agreement with those of Picek et al. (2007), who found that only 10% of the total carbon emissions from a constructed wetland were in the form of CH_4; and with Bastviken et al. (2003), who measured the mineralization of organic carbon in oxic and anoxic lake sediments and found that carbon-dioxide production dominated that of methane as a mineralization product. Also, the lower temperatures used in the incubations, closer to the value that is best suited for CO_2 production, as well as variables such as the products originating from the breakdown of organic matter, increase the likelihood of more efficient use of the exudates released by the organic matter throughout cultivation (Silvola et al., 1996) and explain the higher production of CO_2 than methane, as seen in the results obtained in the two cultivated areas.

The combination of low soil pH and potential methanotrophy, together with the addition of fertilizer, may have caused a low methane production in this temporary wetland. During the crop cycle of the rice paddies, two applications of urea fertilizer were made in both areas, during the initial phase of cultivation. Whereas Krüger and Frenzel (2003) observed little to no change in overall CH_4 emissions from rice fields in response to increased N-fertilization, other studies have found variable effects. The application of fertilizers could lead to a reduction rather than an increase in methane emission in wetlands formed by rice fields (Bodelier et al., 2000a). Urea increases soil pH and reduces methane production, especially when it is incorporated at some depth, since the majority of methane-producing organisms are neutrophilic and their production slows at pH values lower than 6.4 and higher than 7.8 (Wang et al., 1992). Also, the effect of fertilization stimulates the activity of methanotrophic organisms, controlling the emission of methane close to the plants, thus reducing its emission (Bodelier et al., 2000b).

The present study shows lower values compared with the data obtained in studies performed in other ecosystems, such as rice fields and wetlands of the Northern hemisphere. The lowest values of methane production were observed in studies that use such methodology measurement of accumulation of gas in the head space. However, comparison of rates of methane production from different studies is very difficult, since many variables such as environmental factors need to be considered in the analysis of gas production rates. In addition to these factors, different methods are used and the rates are dependent on both temperature and hydroperiod (Mitsch and Gosselink, 1986), which may result in variations in the data, hindering the analysis and interpretation of results.

The results obtained in this study suggest that the carbon and organic-matter contents in the soil of irrigated rice cultivation areas may have been used in different ways by soil microorganisms, leading to variations in CH_4 and CO_2 production. Identifying the conditions in which these processes occur and the types of organisms involved would help explain the variation observed in this and other studies. Our data suggest that differences between ecosystems, such as the carbon and organic matter content presents in temporary wetlands soil, could result in variations of CH_4 and CO_2 production rates even for limited periods of time.

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Extreme emission of N_2O from tropical wetland soil (Pantanal, South America)

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Nitrous oxide (N_2O) is an important greenhouse gas and ozone depleter, but the global budget of N_2O remains unbalanced. Currently, ~25% of the global N_2O emission is ascribed to uncultivated tropical soils, but the exact locations and controlling mechanisms are not clear. Here we present the first study of soil N_2O emission from the Pantanal indicating that this South American wetland may be a significant natural source of N_2O. At three sites, we repeatedly measured *in situ* fluxes of N_2O and sampled porewater nitrate (NO_3^-) during the low water season in 2008 and 2009. In 2010, 10 sites were screened for *in situ* fluxes of N_2O and soil NO_3^- content. The *in situ* fluxes of N_2O were comparable to fluxes from heavily fertilized forests or agricultural soils. An important parameter affecting N_2O emission rate was precipitation, inducing peak emissions of >3 mmol N_2O m^{-2} day^{-1}, while the mean daily flux was 0.43 ± 0.03 mmol N_2O m^{-2} day^{-1}. Over 170 days of the drained period, we estimated non-wetted drained soil to contribute 70.0 mmol N_2O m^{-2}, while rain-induced peak events contributed 9.2 mmol N_2O m^{-2}, resulting in a total N_2O emission of 79.2 mmol N_2O m^{-2}. At the sites of repeated sampling, the pool of porewater nitrate varied ($0.002 - 7.1$ μ mol NO_3^- g dW^{-1}) with higher concentrations of NO_3^- ($p < 0.05$) found in drained soil than in water-logged soil, indicating dynamic shifts between nitrification and denitrification. In the field, O_2 penetrated the upper 60 cm of drained soil, but was depleted in response to precipitation. Upon experimental wetting the soil showed rapid O_2 depletion followed by N_2O accumulation and a peak emission of N_2O (2.5 - 3.0 m mol N_2O m^{-2} day^{-1}). Assuming that the observed emission of N_2O from these wetland soils is generally representative to the Pantanal, we suggest that this undisturbed tropical wetland potentially contributes ~1.7% to the global N_2O emission budget, a significant single source of N_2O.

Keywords: tropical wetland, natural greenhouse gas source, microbial nitrogen cycling, nitrous oxide emission, soil oxic-anoxic transition

INTRODUCTION

The atmospheric concentration of nitrous oxide (N_2O) is increasing at an accelerating rate with anthropogenic sources estimated to account for ~38% of the current N_2O emission (IPCC, 2007). This is concerning because N_2O is a powerful greenhouse gas (IPCC, 2007) and the most important ozone depleter of the twenty-first century (Ravishankara et al., 2009). Modeling of the future global climate is dependent on our understanding of the mechanisms that control the atmospheric concentration of greenhouse gases (CO_2, CH_4, and N_2O) and our ability to obtain an accurate budget of anthropogenic and natural sources and sinks. Microbial processes play a major role in the global cycling of carbon and nitrogen (e.g., Gruber and Galloway, 2008) and while the cycling of both is closely linked (e.g., Schlesinger, 2010) most focus has been on carbon. Both CH_4 and N_2O are primarily biogenic (IPCC, 2007) and methanogens, nitrifiers, and denitrifiers are key players in regulating the global sources and sinks of CH_4 and N_2O. This underscores the need to better understand how environmental parameters and microorganisms interact to become sources or sinks of these greenhouse gases on a larger scale. In this study the focus is on tropical wetlands soils and we present *in situ* evidence that the world's largest freshwater wetland (Pantanal, Brazil) acts as significant as a source of N_2O.

Although our knowledge of sources and sinks of N_2O in different environments is increasing, the global N_2O budget remains unbalanced (Smith, 1997; IPCC, 2007). Currently, ~25% of the global N_2O emission is ascribed to uncultivated tropical soils, but the exact locations and controlling mechanisms, including the role of microbial processes, are not clear (D'Amelio et al., 2009). Furthermore, a recent study of the tropospheric distribution and variability of N_2O demonstrated that global sources of N_2O are concentrated in the tropics and suggests that South America has

an up to five times higher emission of N_2O than expected (Kort et al., 2011).

Several studies indicate that tropical forest ecosystems simultaneously accumulate, recycle, and loose nitrogen in far larger quantities than temperate ecosystems (Martinelli et al., 1999; Matson et al., 1999; Hedin et al., 2009). In spite of being rich in nitrogen, this recycling may include high bacterial N_2-fixation activity, a nitrogen paradox (Hedin et al., 2009) enabling tropical forest ecosystems to sustain large gaseous nitrogen losses (Houlton et al., 2006). Half of the world's wetland areas are found in the tropics (Neue et al., 1997); and if the nitrogen paradox is similarly applicable in these ecosystems, tropical wetlands may have a major and yet unresolved role in the global budget of atmospheric N_2O.

In both natural and impacted environments, the same microbial processes are responsible for the production of N_2O, i.e., nitrification ($NH_4^+ \rightarrow O_2 \rightarrow NO_3^-$) and denitrification ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow NO_2^- \rightarrow N_2$). Nitrifiers in soil can be stimulated to release N_2O at low O_2 availability (~5% air sat.; Bollmann and Conrad, 1998), soil moisture content of up to ~60% WFPS (Bateman and Baggs, 2005) and low pH (Mørkved et al., 2007). Denitrifying microorganisms can be stimulated to release N_2O by sudden onset of anoxia (Morley et al., 2008), high concentrations of NO_3^- (Blackmer and Bremner, 1978; Blicher-Mathiesen and Hoffmann, 1999), and low pH (Simek and Cooper, 2002). Both nitrification and denitrification can occur simultaneously in complex soil microsites with different access to O_2. This makes it difficult to associate a measured soil N_2O emission with a specific microbial process (Stevens et al., 1997). However, denitrification is often considered the main N_2O producing process in soils (Dobbie et al., 1999; Abbasi and Adams, 2000).

The continuous cycle of flooding and draining of wetlands affects important environmental soil parameters such as their O_2 content, pH, and redox potential and thereby modulates the biogeochemical processes involved in production and emission of N_2O (Baldwin and Mitchell, 2000). This hydrological pulse effect is well known in systems influenced by anthropogenic input of nitrogen, where hot spots or hot moments (McClain et al., 2003) of N_2O emission are induced by temporal or spatial oxic-anoxic transitions in for example riparian marshes (Hernandez and Mitsch, 2006), agricultural soil (Markfoged et al., 2011), and mangrove sediment (Allen et al., 2007). Similar flooding effects in tropical freshwater systems are much less explored.

Here we present the first study of the *in situ* fluxes of N_2O, the dynamics of soil nitrogen pools and soil O_2 content in Pantanal wetland soils at different times and water status. Three sites were sampled repeatedly over a period of 23–42 days in the beginning of the low water season in 2008 and 2009. Additionally, in the end of the low water season in 2010 10 sites were screened for *in situ* surface flux of N_2O and soil NO_3^- content.

MATERIALS AND METHODS

STUDY SITES

The Pantanal, a pristine tropical wetland in central South America, is shaped by the deposition of sediments into a tectonic depression in the Upper Paraguay River Basin, which formed during the last Andean compressive event (~2.5 Ma; Assine and Soares, 2004). The Pantanal supports a lush vegetation of floating and emersed herbs and is dominated by an annual flooding and precipitation cycle, alternately inundating and draining ~140,000 km^2 of soil (Swarts, 2000), thus representing the world's largest wetland (**Figure 1A**). Aquatic macrophytes and herbaceous plants colonize the entire gradient from permanently dry to permanently wet conditions. In the aquatic-terrestrial transition zone, the herbaceous plant communities die off when the water floods the area annually; this is often followed by an anoxic event in the river and flood water due to the massive decomposition of vegetation (Hamilton et al., 1997; Calheiros et al., 2000). During the flooded season, aquatic macrophytes like *Eichhornia crassipes* and *Salvinia auriculata* dominate the ecosystem, but decompose when left on the draining wetland soil as the water level decreases (Junk et al., 2006). The flooding pulse thus leads to a regular set-back of community development maintaining the system in an immature, but highly productive stage (Junk and Wantzen, 2004). The annual flooding cycle is driven by a distinct dry/wet season in the ~500,000 km^2 watershed, but due to a North-South slope of only 2–3 cm km^{-1}, there is a lag period of up to several months between precipitation in the watershed and the flooding of the Pantanal (Junk et al., 2006). This explains why the Pantanal receives the highest precipitation in the low water (drained) season (**Figure 2A**).

During three field campaigns in 2008 (42 days), 2009 (23 days), and 2010 (14 days), we investigated the *in situ* flux of N_2O and the soil nitrogen pools from wetland soil at representative sites, near the retreating edge of typical water bodies with temporary connection to the main water courses in the Pantanal (**Figure 1B**). In 2008 and 2009 a main site (site A: 19°01.16′S; 57°32.99′W) was chosen for repeated sampling. An additional site was chosen for repeated sampling in 2008 only (site B: 19°0.61′S; 57°33.51′W) and in 2009 only (site C: 18°59.28′S; 57°25.17′W). In 2010, 10 sites (A, B, D–K) representing different soil and habitat types were screened (**Figure 1B**; **Table 1**). All sampling was conducted during the drained season: In 2008 and 2009 sampling was conducted during the period of retreating water, while the screening in 2010 was conducted during the period of rising water level (**Figure 2A**).

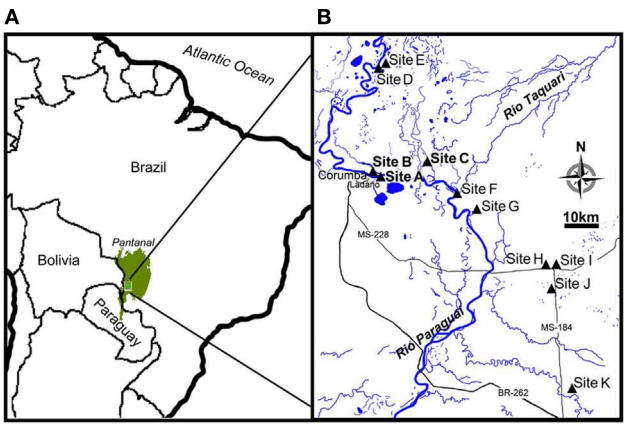

FIGURE 1 | Location of Pantanal and map insert of the study site. (A) Pantanal is shown as green area in the center of South America. **(B)** The course of Rio Paraguai flowing from north to south in the Pantanal and locations of the sampling sites (site A–K).

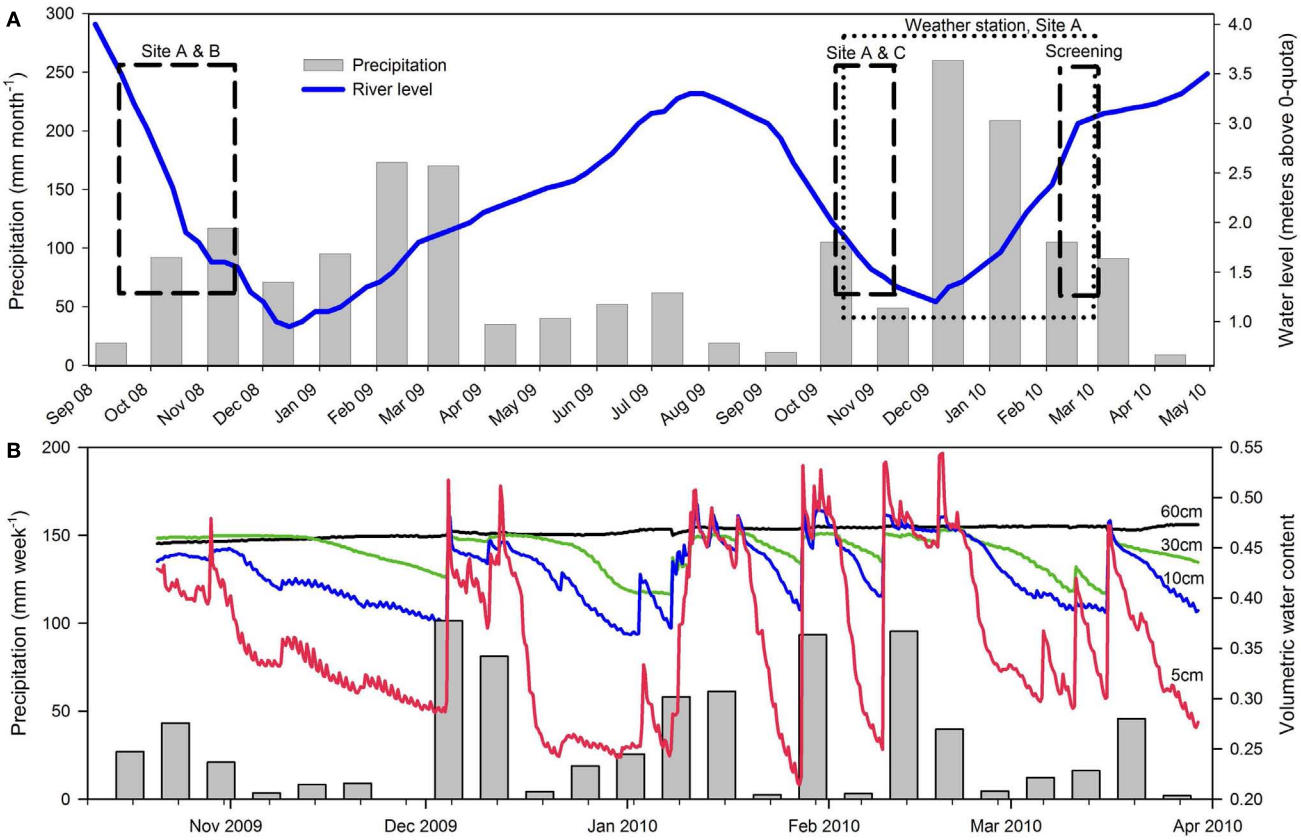

FIGURE 2 | Precipitation and the water level in Rio Paraguai during 2008–2010. (A) Monthly precipitation at Corumbá Airport is shown as gray bars (source: www.inmet.gov.br). Daily water level of Rio Paraguai at Ladário Naval Station is shown as blue line (source: www.cprm.gov.br). The period of each field campaign in 2008, 2009, and 2010 is indicated with black dashed boxes, while the dotted box indicates the period of precipitation, soil moisture, and soil temperature measurements at site A. **(B)** Precipitation and soil moisture content in 2009–2010 at site A, Level 2. Weekly precipitation is indicated as gray bars. Soil moisture at different depths below the soil surface is shown as lines; 5 cm (red), 10 cm (blue), 30 cm (green), and 60 cm (black) below the soil surface.

Precipitation and soil water content was measured for 170 days of the drained season 2009–2010 (**Figure 2B**).

Measurements and sampling were carried out along 10–25 m long transects ranging from the retreating water edge to ∼1 m above the initial water edge at sites A, B, and C. At the beginning of each field campaign in 2008 and 2009, the elevated end of transects had been drained for ∼1 month, while the lowermost end was initially water-logged, but drained during the field campaign. Three levels were selected along each transect: Level 1 at the lowest end, Level 2 in the center, and Level 3 at the highest end, thus representing both an increase in elevation and a gradual decrease in soil moisture content from Level 1 to Level 3. Due to an excessive amount of rain on site C during the 2009 field campaign, this site was water-logged during all measurements, while early flooding of site C in 2010 left it inaccessible.

IN SITU FLUX MEASUREMENTS OF N_2O

At each site, the *in situ* flux of N_2O was measured at the two lowest levels (Level 1 and 2) along the transect. Flux chambers ($n = 5$ at each level) made of PVC tubes ($\varnothing = 24$ cm, height = 20 cm) were inserted ∼15 cm into the soil. If any litter layer was present on the soil surface prior to inserting the chamber, it was replaced on the soil surface inside the chamber. *In situ* flux measurements were performed every 2–14 days by placing a lid on the tube and measuring the N_2O concentration for 30 min in each chamber with a photo-acoustic gas monitor (INNOVA 1312, LumaSense, Inc., Ballerup, Denmark). Flux chambers were lined with reflective material on the outside and were shaded during measurements to minimize temperature variations. The closed-chamber technique is known to create a bias by altering the diffusion gradient between soil and chamber headspace (Anthony et al., 1995). However, several studies have shown that this bias can be overcome by applying a non-linear regression method to describe the gas exchange (e.g., Kroon et al., 2008; Forbrich et al., 2010). In our case, the flux in each chamber at $t = 0$ was estimated by fitting the partial pressure increase to a three-parameter exponential function [$pp_t = pp_0 + a(1-e^{-bt})$] in Sigmaplot (Systat Software, Inc., Chicago, IL, USA), where pp_t is the partial pressure of the measured gas at time t, pp_0 is the initial partial pressure in the closed-chamber, t is time, and a and b are constants. Integrated emissions of N_2O were calculated for each level at each site, assuming linearity between subsequent measurements.

Table 1 | Screening of *in situ* N_2O flux, pH, and porewater NO_3^- in 2010 at 10 sites.

Location	Position (WGS 84)	Level	*In situ* N_2O flux ($mmol\,N_2O\,m^{-2}\,day^{-1}$)	pH	NO_3^- ($\mu mol\,NO_3^-\,cm^{-3}soil$)
Site A	19°01.16′S; 57°32.99′W	1	0.41 ± 0.05	5.32	0.19
		2	0.31 ± 0.02	4.96	0.91
		3	0.61 ± 0.29	4.43	2.69
Site B	19°00.61′S; 57°33.51′W	1	0.21 ± 0.03	4.37	0.16
		2	0.48 ± 0.12	4.13	3.45
Site D	18°43.56′S; 57°32.12′W	–	0.27 ± 0.02	4.50 ± 0.12	0.07 ± 0.04
Site E	18°44.08′S; 57°32.38′W	1	0.77 ± 0.18	5.08 ± 0.11	0.55 ± 0.08
		2	1.08 ± 0.17	4.75 ± 0.06	5.38 ± 2.94
Site F	19°04.26′S 57°20.08′W	1	0.22 ± 0.01	6.90 ± 0.06	0.03 ± 0.00
		2	0.41 ± 0.02	6.25 ± 0.03	0.05 ± 0.03
Site G	19°06.03′S; 57°16.85′W	–	0.18 ± 0.04	5.33 ± 0.06	0.79 ± 0.12
Site H	19°15.15′S; 57°04.83′W	–	0.20 ± 0.02	5.98 ± 0.10	0.02 ± 0.01
Site I	19°15.03′S; 57°04.04′W	–	0.45 ± 0.09	5.18 ± 0.08	0.07 ± 0.02
Site J	19°18.53′S; 57°03.29′W	–	0.09 ± 0.01	7.48 ± 0.08	0.29 ± 0.11
Site K	19°34.50′S; 57°01.22′W	1	0.30 ± 0.01	–	0.06
		2	0.30 ± 0.01	–	0.31

Mean ± SE (n = 5).

During the 2010 screening, the sites had to the best of our knowledge not received precipitation in the preceding days, and the *in situ* measurements are thus assumed to represent drained soil fluxes.

SOIL PARAMETERS

Porewater NO_3^-

Whole soil cores (∅ = 5.5 cm, length = 15 cm) were collected at all levels ($n = 3$ at each level) on every sample occasion at site A, site B, and site C (2008: three times at site A, three times at site B, 2009: five times at site A, four times at site C) and soil porewater immediately extracted *in situ* by inserting 0.2 µm Rhizon filters (Rhizosphere Research Products, Wageningen, Netherlands) into the side of the whole soil cores at 1.5, 3.5, and 6.5 cm below the soil surface. Samples of ∼0.5 ml of porewater were extracted at each depth by suction with a 60 ml syringe. Additional water samples were taken from rivers and water bodies and filtered (0.2 µm filter, Sartorius AG, Göttingen, Germany). Extracted porewater samples and water samples were immediately transferred to 1.5 ml tubes and stored on ice until return to the field laboratory, where they were stored at −20°C until further analysis. Nitrate analysis (sample size 5 µl) was performed using the vanadium chloride reduction method (Braman and Hendrix, 1989) in combination with a chemoluminescence detector (CLD 86, Eco Physics AG, Dürnten, Switzerland), calibrated ($r^2 = 1.00$) at nine different concentrations of NO_3^- (0, 25, 50, 75, 150, 300, 500, 750, 1500 µM). After extraction of soil porewater, the soil cores were sliced and the soil water content and soil dry weight was determined by weighing the soil slices before and after drying at ∼60°C for 48 h. Data from each soil NO_3^- profile was averaged (arithmetic mean) over the upper 6.5 cm soil column.

During the third field campaign (2010), soil NO_3^- was measured in a field laboratory with a NO_3^- biosensor (Unisense A/S, Aarhus, Denmark), calibrated ($r^2 = 0.99$) at six different concentrations of NO_3^- (0, 20, 40, 60, 260, 660 µM). Soil samples ($n = 1–3$) from the mixed upper ∼5 cm soil were collected by inserting a 50 ml screw cap centrifuge tube (Sarstedt AG, Nürmbrecht, Germany) directly into the soil. Soil samples were refrigerated up to 48 h until NO_3^- was measured in a 1% (wt/wt) NaCl solution in the field laboratory. The reason for adding NaCl was primarily a higher stability of the NO_3^- biosensor reading in a saline solution.

Means of porewater NO_3^- at site A, B, and C were analyzed with a GLM (two-way-ANOVA) with time and levels as factors. A Tukey's test was run for comparisons among means. Results were tested at a significance level of 95%. Analyses were performed using SAS 9.2 (SAS Institute, Inc., Cary, NC, USA).

Distribution of O_2 in soil

In 2009, the depth distribution of O_2 concentration was measured at site A and C at Level 2 (center of transect) with custom-built fiber-optic O_2 optodes (∅ = 2 mm; Rickelt et al., under review). The optical fibers were calibrated in an O_2-free solution (0.2 M ascorbate, pH 12) and in water equilibrated with atmospheric air prior to installation in the soil at 13 fixed depths (2.5, 5, 10, 15, and 20–100 cm with 10 cm intervals). The optodes were connected to a four-channel fiber-optic O_2 detector system (OXY-4, Presens GmbH, Regensburg, Germany) at each visit to the sites (site A: $n = 22$, site C, $n = 9$).

Soil moisture and precipitation

Precipitation, soil temperature, and seasonal changes in volumetric soil water content were measured for a period of 170 days (2009–2010) at site A, Level 2. The volumetric soil water content was logged using soil moisture sensors (Theta-Probes ML2x, Delta-T Devices, Ltd., Cambridge, UK) installed in four depths; 5, 10, 30, and 60 cm below the soil surface in one profile. Each probe was calibrated in the laboratory using depth-specific soil

samples from the site. Precipitation was measured using a "Tipping Bucket" rain gage. Soil temperature and precipitation sensors were logged at 10 min intervals, while the Theta-probes were logged every 6 h (CR10X Datalogger, Campbell Scientific, Ltd., Loughborough, UK).

N_2O DYNAMICS IN SOIL WETTED EITHER EXPERIMENTALLY OR BY NATURAL PRECIPITATION

Dry soil cores (∅ = 5.5 cm, length = 15 cm) for experimental wetting were collected from level 3 at site A and site B. Further soil cores were collected immediately after a natural, moderate precipitation event of short duration (<15 min). Optical O_2 microoptodes were constructed according to Klimant et al. (1995) and mounted in hypodermic needles and connected to a fiber-optic O_2 meter (Microx TX3, Presens GmbH, Regensburg, Germany). Electrochemical N_2O microsensors were constructed according to Andersen et al. (2001) with a fortified outer casing to avoid breaking the sensor in the coarse wetland soil (Markfoged et al., 2011) and connected to a picoammeter (PA2000, Unisense A/S, Aarhus, Denmark). Both types of sensors had tip diameters of ∼100 µm and a detection limit of ∼5 Pa. The O_2 sensors were linearly calibrated from a two-point calibration in O_2-free solution (20% ascorbic acid, pH 11), and in water equilibrated with atmospheric air. The N_2O sensors were linearly calibrated from a two-point calibration in a 0 and 1% solution of N_2O produced by mixing 0.5 ml of saturated N_2O water into 49.5 ml water. The sensors were mounted on a motorized micromanipulator and both sensor position and data collection were controlled by a PC running SensorTrace Pro software (Unisense A/S, Denmark). Retrieved dry soil cores were wetted from below with aerated river water and concentration profiles (n = 20–30) of O_2 and N_2O were obtained over the following 56–72 h. Additional profile measurements were done in a soil core retrieved after wetting by a natural rain event. The N_2O flux, J, was calculated from the concentration gradient in the water layer on the soil surface using Fick's first law ($J = -D\,\delta C/\delta x$), where D is the N_2O diffusivity in water at experimental temperature (2.41×10^{-5} cm^2 s^{-1}), C is the N_2O concentration in µmol l^{-1} calculated from the measured partial pressure and the experimental temperature according to Weiss and Price (1980), and x is the vertical distance in cm.

RESULTS

IN SITU FLUX MEASUREMENTS OF N_2O

The *in situ* flux of N_2O at the sites of repeated sampling varied considerably over time in both 2008 (**Figure 3A**) and 2009 (**Figure 3B**) the mean daily flux of N_2O varying between 0.04 and 1.37 mmol N_2O m^{-2} day^{-1} at site C, Level 1 and site A, Level 2 respectively (**Table 2**). *In situ* fluxes of N_2O during the 2010 screening (**Figure 3C**) varied between 0.09 mmol N_2O m^{-2} day^{-1} (site J) and 1.08 mmol N_2O m^{-2} day^{-1} (site E; **Table 1**).

Peak events of *in situ* N_2O flux were apparently closely associated with sudden and heavy precipitation causing increasing soil water content in the upper 10 cm soil layer which was followed by an increased flux of N_2O measureable 6–12 h later (**Figure 4**).

SOIL PARAMETERS

Porewater NO_3^-

At site A (2008) the porewater NO_3^- content increased significantly ($p < 0.05$), going from wet soil at Level 1 to drained soil at Level 3 (**Figure 5A**). In addition, over time the porewater NO_3^- content increased significantly ($p < 0.05$) at Level 1 as the soil drained, while a significant decrease ($p < 0.05$) was observed at Level 2 and 3 (**Figure 5A**). In 2009 the porewater NO_3^- content at site A similarly increased significantly ($p < 0.05$) from wet soil at Level 1 to drained soil at Level 3 (**Figure 5A**). In addition, a significant increase ($p < 0.05$) over time in porewater NO_3^- content was observed at Level 1. The same trend was observed between levels

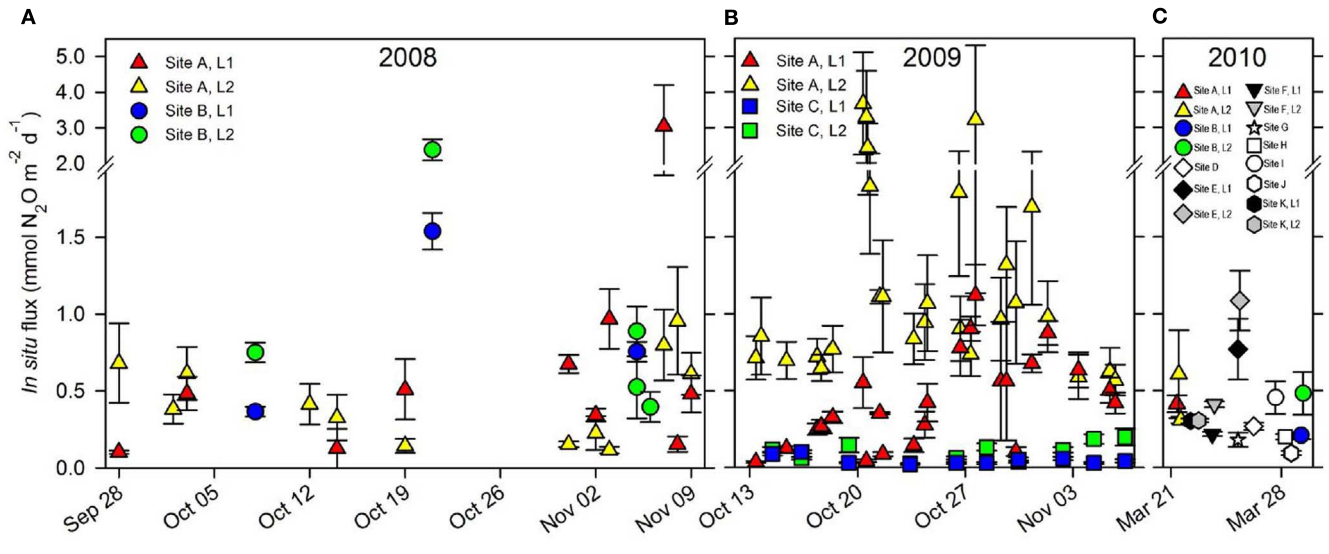

FIGURE 3 | *In situ* flux of N_2O (mean ± S. E., n = 5) from all sites 2008–2010. (A) *In situ* flux of N_2O in 2008 from site A, Level 1 (▲) and Level 2 (▲) and from site B, Level 1 (●) and Level 2 (●). **(B)** *In situ* flux of N_2O in 2009 from site A, Level 1 (▲) and Level 2 (▲) and from site C, Level 1 (■) and Level 2 (■). **(C)** *In situ* flux of N_2O in 2010 from site A, B, and site D–K. Note scale break on y-axis.

Table 2 | Integrated flux and mean daily flux of N_2O at each Level at the sites of repeated sampling.

Site	Level	days	Integrated N_2O flux mmol N_2O m^{-2}	Mean N_2O flux mmol N_2O m^{-2} day^{-1}
A	1†	42	22.0 ± 5.6	0.52 ± 0.13
	2†	42	14.0 ± 3.4	0.33 ± 0.08
	1‡	23	12.9 ± 1.7	0.55 ± 0.07
	2‡	23	32.0 ± 5.0	1.37 ± 0.21
B	1	29	17.8 ± 1.8	0.64 ± 0.06
	2	29	26.7 ± 4.0	0.92 ± 0.14
C	1	23	1.0 ± 0.1	0.04 ± 0.00
	2	23	2.1 ± 0.4	0.09 ± 0.02

Mean ± SE (n = 5).

N.B. site C was completely water-logged during the entire field campaign.

† 2008, ‡ 2009.

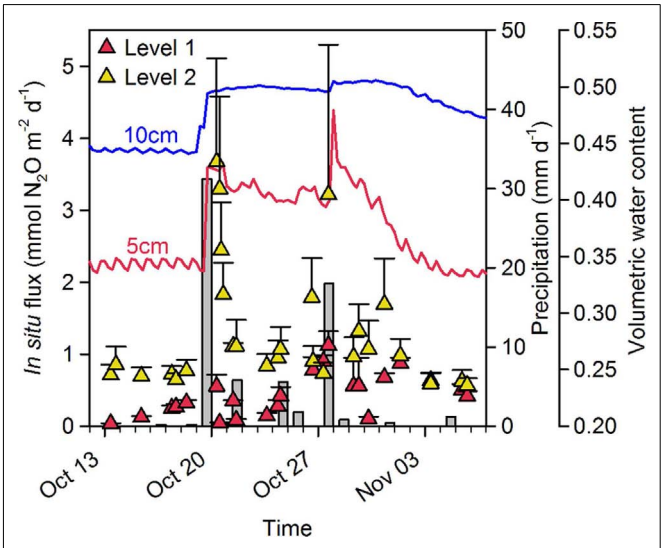

FIGURE 4 | *In situ* N_2O flux, precipitation and soil moisture content at site A in 2009. Precipitation (gray bars: daily precipitation) caused a sudden increase in soil water content (5 cm: red line, 10 cm: blue line) in the top 10 cm soil layer and triggered an increase in the *in situ* flux of N_2O [Mean + SE ($n = 5$)] at Level 1 (▲) and Level 2 (▲).

at site A in 2010, with porewater NO_3^- content increasing from Level 0 to Level 3 (**Figure 5A**).

At site B in 2008 (**Figure 5B**) the porewater NO_3^- content at Level 3 was significantly higher ($p < 0.05$) than at Level 1 and Level 2. In addition, a significant increase ($p < 0.05$) over time in porewater NO_3^- content was observed at Level 1 and Level 3 (**Figure 5B**). The same trend was observed between levels at site B in 2010, with porewater NO_3^- content increasing from Level 0 to Level 2 (**Figure 5B**).

In 2008 site C (**Figure 5C**) soil samples ($n = 3$) collected on a single occasion showed a soil NO_3^- content of 0.73 ± 0.09 μmol NO_3^- g dW^{-1}. However, in 2009 (**Figure 5C**) the overall porewater NO_3^- content at site C was much lower than at site A or B, presumably due to the water-logging of the soil. Comparing between levels at site C in 2009 (**Figure 5C**, small insert) the porewater NO_3^- content at Level 1 was significantly lower ($p < 0.05$) than at Level 2, but not compared to Level 3.

The soil characteristics from the 2010 screening of 10 sites are shown in **Table 1**. Soil NO_3^- content varied between 0.06 μmol cm^{-3} soil (site K) and 5.38 μmol cm^{-3} soil (site E).

The NH_4^+ concentration in river waters ranged between 0.6 ± 0.5 and 10.6 ± 4.8 μmol NH_4^+ l^{-1} (**Figure 6A**) and ranged between 1.0 ± 0.1 and 8.7 ± 2.6 μmol NH_4^+ l^{-1} in the other water bodies (**Figure 6B**). The NO_3^- concentration in rivers ranged between 0.1 and 12.6 ± 0.8 μmol NO_3^- l^{-1} (**Figure 6A**) and ranged between 0.1 and 3.4 ± 1.2 μmol NO_3^- l^{-1} in the other investigated water bodies (**Figure 6B**).

Depth distribution of O_2 in soil

At site A, Level 2, O_2 penetrated to a depth of ∼60 cm (37–97% air sat.) but fluctuated throughout the field campaign in response to precipitation (**Figure 7A**). At site C, Level 2, O_2 was not detected in the soil at Level 2 except the first measurement (**Figure 7B**) in consistency with the soil being water-logged during the field campaign.

Soil moisture and precipitation

Precipitation measured at Level 2 at site A resulted in an increase in soil moisture. This was most pronounced near the soil surface (5 cm) and less in the deeper soil layers. A subsequent decrease in soil moisture occurred after each rain event, most rapidly at the surface due to evaporation and drainage (**Figure 2B**).

MICROSENSOR MEASUREMENTS IN WETTED SOILS

Upon wetting of drained soil cores collected from Level 3 at site A and site B, porewater O_2 depletion occurred within a few hours (**Figures 8A–D**), followed by a rapid accumulation of N_2O. The accumulation of N_2O within the soil persisted for 2–3 days (**Figures 9A,B**). During this period, N_2O diffused into the overlying air causing peak emissions of 3.02 mmol N_2O m^{-2} day^{-1} from the Site A core (**Figure 9C**) and 2.53 mmol N_2O m^{-2} day^{-1} from the Site B core (**Figure 9D**), i.e., peak events similar in size and timing to the emission peaks found by *in situ* flux measurements.

The integrated emission from the experimentally flooded soil of 3.7 mmol N_2O m^{-2} over 3 days (soil core from site A) and 2.92 mmol N_2O m^{-2} over 2.3 days (soil core from site B) represented only a small fraction (35–38%) of the net production of N_2O in the upper 4 cm of the soil matrix, the difference being consumed within the water-logged soil 2–3 days after the wetting event (**Figures 9E,F**).

DISCUSSION

Our study represents the first study of soil N_2O emission and the dynamics of the porewater nitrate in the Pantanal and revealed a large and hitherto unknown source of N_2O in the largest wetland of the world. In this discussion, we relate our observed *in situ* N_2O fluxes to the soil porewater nitrate and soil moisture and precipitation data and compare these results with those found for other tropical systems. Thereafter, we discuss our findings of N_2O production, accumulation, and emission in experimentally wetted soil cores in relation to studies of parameters influencing the reduction

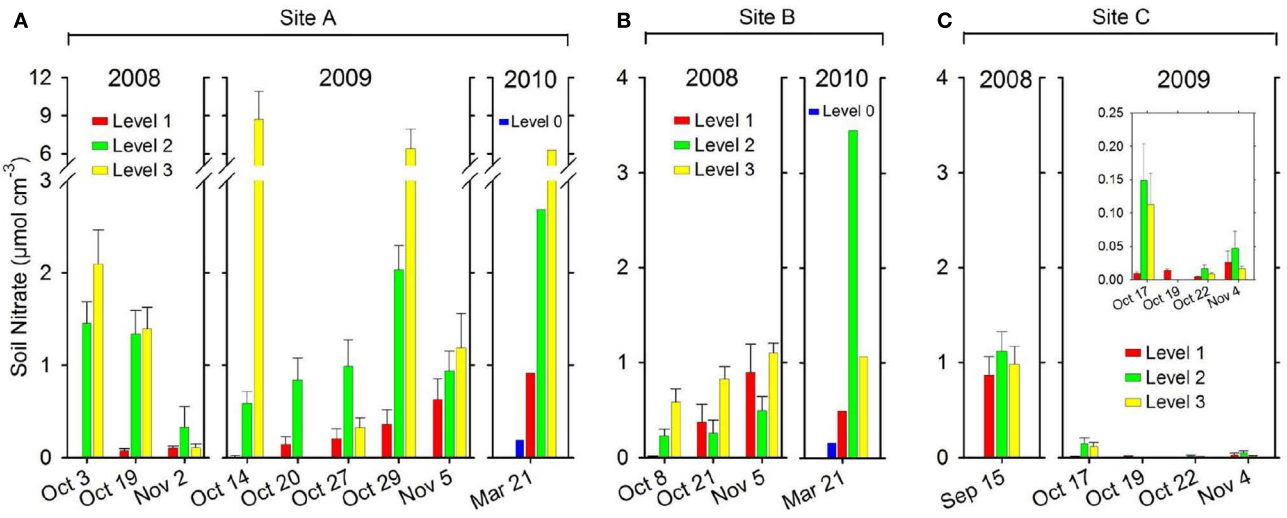

FIGURE 5 | Soil porewater NO_3^- per soil volume (µmol N cm^{-3} soil) measured along a transect from Level 1 to Level 3 at each site. (A) site A, **(B)** site B, and **(C)** site C and site D–K (one or two levels only) in 2008, 2009, and 2010. Mean + SE ($n = 9$). At site A and site B a Level 0 was introduced in 2010 because the water level was much lower by the end of the drained season. Level 0 was situated close to the water edge and therefore equivalent to Level 1 with regard to distance to the water edge in 2008 and 2009. Please note scale break on *y*-axis on (A and D) and insert figure with different scale on **(C)**.

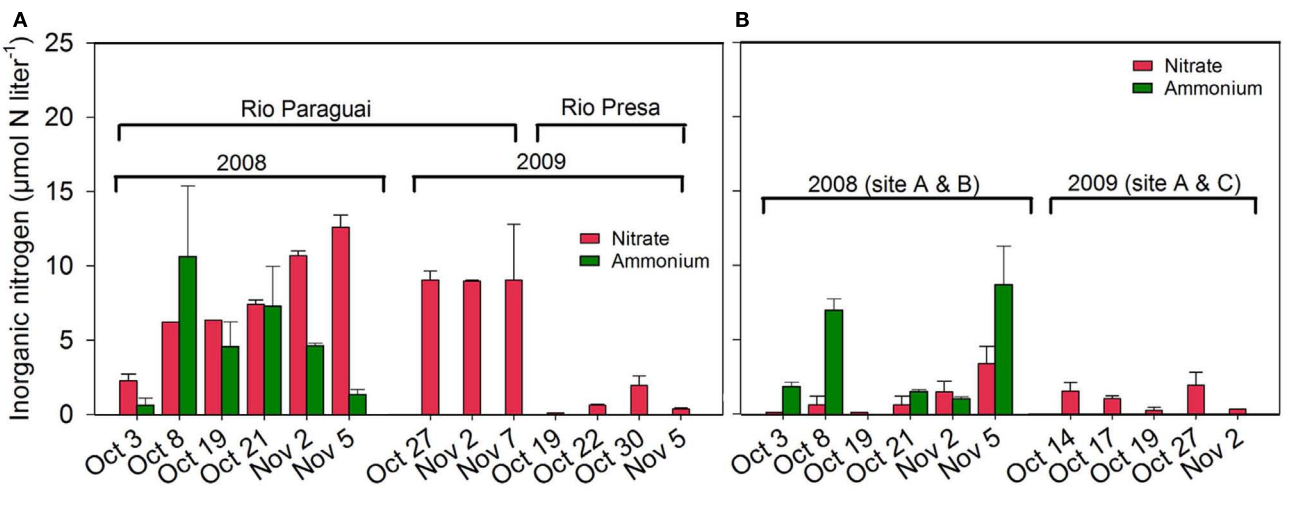

FIGURE 6 | Inorganic nitrogen (NH_4^+ and NO_3^-) in the water phase in (A) Rio Paraguai and Rio Presa and in (B) water bodies near Site A, B, and C.

of N_2O in soil. Finally we speculate on how the observed emission of N_2O could be supported in a natural system like the Pantanal and how this might link into the cycling of carbon.

IN SITU FLUX OF N_2O

The measured fluxes of N_2O from Pantanal wetland soils had a high temporal variability (**Figure 3**) and were generally high (**Tables 1** and **2**). The average N_2O emission from the Pantanal wetland soils was 10–390 times higher when compared to other unfertilized tropical systems (Matson and Vitousek, 1987), and 4–6 times higher when compared to fertilizer-induced N_2O emission peaks in tropical forest soils (Hall and Matson, 1999). The fluxes of N_2O from the Pantanal wetland soil were thus comparable to fluxes reported from heavily fertilized forest or agricultural soils receiving regular inputs of nitrogen (e.g., Hall and Matson, 1999; Ruser et al., 2006). In soil fertilized with high NO_3^- concentrations the primary end product of denitrification upon wetting is often N_2O (Ruser et al., 2006), and although not fertilized, the drained Pantanal wetland soils similarly contained high concentrations of NO_3^-. The high soil NO_3^- content thus explains the high emission of N_2O from the drained Pantanal wetland soils.

Disturbance of tropical soil (e.g., by conversion from forest to pasture or cyclic flooding) has also been shown to increase the emission of N_2O (Keller et al., 1993; Kern et al., 1996; Veldkamp

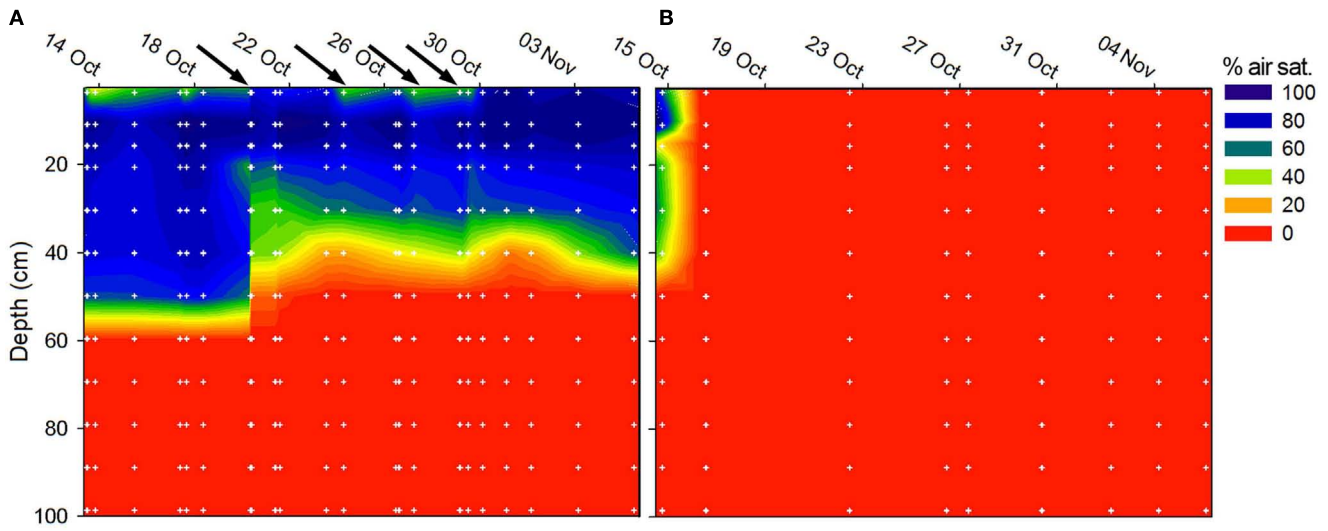

FIGURE 7 | Soil O_2 concentration at (A) site A, Level 2, and (B) site C, Level 2 during field work in 2009. The O_2 concentration maps were compiled from individual oxygen profiles ($n = 22$ at site A, $n = 9$ at site C) composed of measurements at 12 depth (white dots). The black arrows on the time scale in subfig **(A)** indicate precipitation events as measured at site A, Level 2.

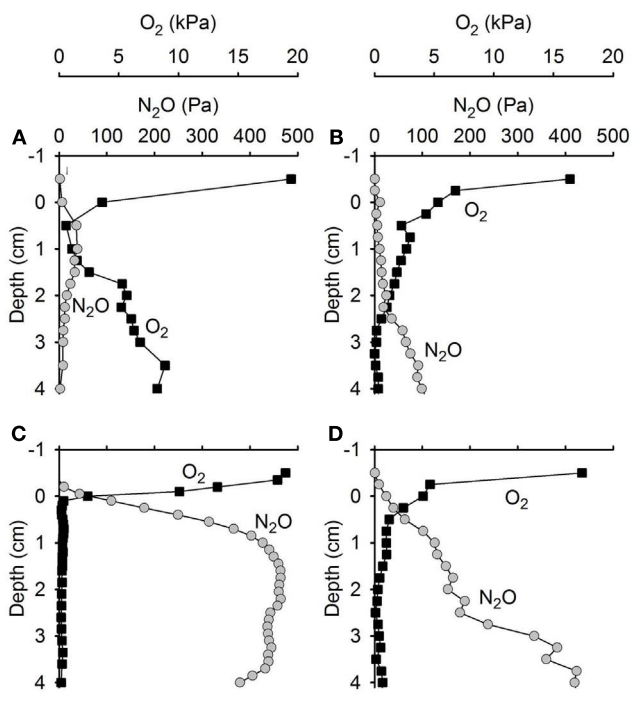

FIGURE 8 | Concentration profiles of N_2O and O_2 in soil cores from (A,C) site A and (B,D) site B experimentally wetted to simulate precipitation. N_2O and O_2 concentrations change rapidly after wetting of the soil. **(A,B)** Four hours after wetting. **(C,D)** Ten hours after wetting.

et al., 1999). In the Pantanal the development of the plant community is continuously disturbed by the alternating flooding and draining of the soil converting the almost exclusively terrestric system to an almost exclusively aquatic system (Junk and Wantzen, 2004). Such disturbance by seasonal flooding, combined with a cyclic high biomass input and massive microbial decomposition, inevitably influences the transformation and storage of nitrogen compounds in the soil.

DYNAMICS OF POREWATER NITRATE

The concentration of soil porewater NO_3^- in our study shifted dramatically from being undetectable in water-logged soil to $> 1000\,\mu mol\,NO_3^-\,l^{-1}$ in drained soil indicating dynamic shifts between nitrate production and consumption in the soil. Extreme *in situ* concentrations of 10 - 30mmol $NO_3^-\,l^{-1}$ observed in some samples might be ascribed to high evaporation and capillary forcing drawing nitrate-rich water up from deeper layers of soil and resulting condensation of nitrate near the surface (Wetselaar, 1960). At the beginning of the drained season at the sites of repeated sampling, we found that still water-logged soil contained no NO_3^-, but after 3–6 weeks of draining NO_3^- could be found and increased further, presumably due to nitrification. This is supported by the O_2 profiles (**Figure 7**) showing that drained soil was aerated to a depth of 20–50 cm interrupted only by short anoxic spells caused by a precipitation-induced increase in soil moisture.

Periodically flooded soils in the Amazon have similarly been found to be rich in inorganic nitrogen (Koschorreck, 2005). However, most of the inorganic nitrogen was removed during the first weeks of drying due to coupled nitrification-denitrification (Koschorreck, 2005), whereas decaying plant material may have supplied a continuous input of inorganic nitrogen to the Pantanal wetland soil.

As the wetland soil was draining at site A and B, only sparse plant growth (typically *Panicum maximum*) was observed, and 2–3 months after the end of the flooded season most of the soil surface at site A, B, and C was still covered with decaying aquatic macrophytes (largely *E. crassipes*). In the absence of inorganic

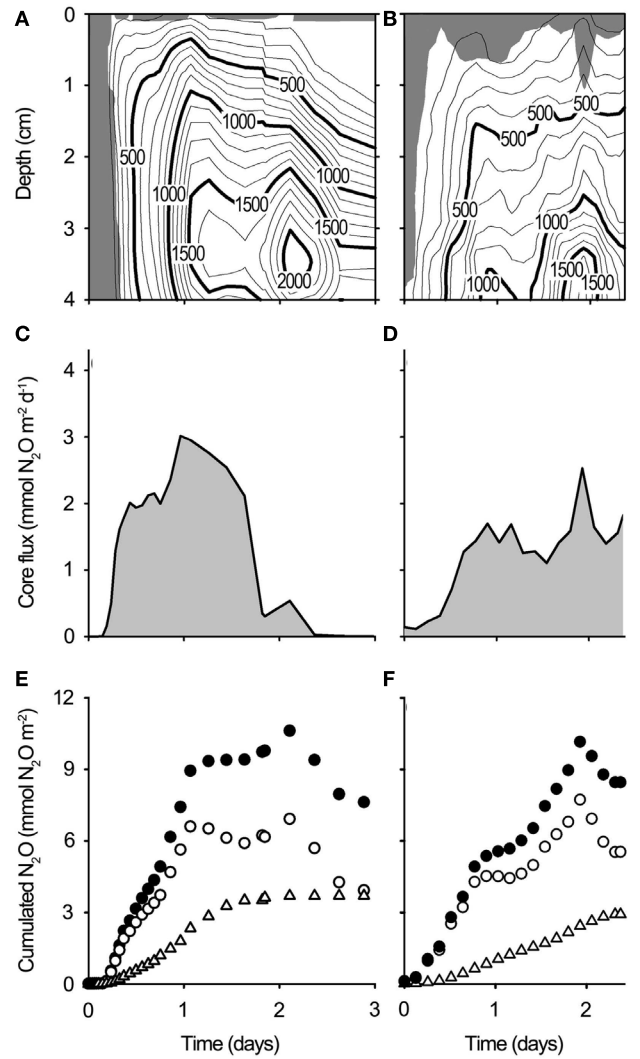

FIGURE 9 | N_2O dynamics in whole soil cores from Site A and Site B experimentally re-wetted to simulate precipitation. (A,B) Contour plots showing the N_2O concentration across time and depth in re-wetted soil cores [**(A)**: Site A, **(B)**: Site B]. Units are pressure equivalents (Pa). The soil surface is 0 cm. The shaded area indicates the oxygenated zone (>1 kPa O_2). **(C,D)** N_2O emission from re-wetted soil cores [**(C):** Site A, **(D)**: Site B] as calculated from the N_2O gradient in the water layer above the soil. **(E,F)**, Cumulated N_2O net production (●), cumulated N_2O emission (△), and cumulated soil N_2O (○) in re-wetted soil cores [**(E)**: Site A, **(F)**: Site (B)].

nitrogen uptake by plant growth, we suggest that the large pool of nitrogen released from mineralization of the decaying plant residues was largely available for microbial nitrification and denitrification throughout the entire drained period. Such a continued input of nitrogen may explain the high nitrate content found in the Pantanal wetland soil even months after draining.

The water level during the 2009 flood was lower than average (**Figure 2A**) and areas that had not been flooded during the 2009 flooded season were covered by thick plant growth (e.g., *Costus spiralis, P. maximum*) by the end of the drained period in early 2010. In such non-flooded areas with high plant activity, plant-microbe competition for inorganic nitrogen would likely decrease the availability of inorganic nitrogen for soil microbial N transformations and gaseous N loss.

N_2O PRODUCTION AND REDUCTION PROCESSES IN THE SOIL

It is well known that sudden onset of anoxia (Bollmann and Conrad, 1998) and high concentrations of NO_3^- (Blackmer and Bremner, 1978) can increase the emission of N_2O. The four step reduction pathway of NO_3^- to N_2 is governed by four specific enzymes. The genes encoding each enzyme has multiple transcriptional promoters that are activated by different environmental parameters, and each enzyme has different substrate requirements and inhibitors (Zumft, 1997). This leads to temporary differences in the production and consumption rates of each intermediate causing a temporary accumulation of these intermediates (Frunzke and Zumft, 1986; Cervantes et al., 1998; Zhou et al., 2008). The high NO_3^- content of the drained Pantanal wetland soil combined with sudden anoxia may have delayed or partly inhibited the reduction of N_2O, stimulating a temporary high accumulation of N_2O as seen in the experimentally flooded soil cores (**Figures 8** and **9**). The emitted N_2O represented 35–38% of the registered cumulated net production (cumulated soil + emitted N_2O, **Figure 9**) the rest being retained and reduced to N_2 within the soil. Any additional N_2O production balanced by simultaneous reduction to N_2 would not be registered in this experimental set up and therefore it is unknown how much larger the N_2 production was.

Microsensor measurements of O_2 and N_2O in soil cores sampled immediately after a natural, moderate rain event (**Figure 10**) revealed a heterogeneous distribution of O_2 in the soil, reflecting a complex soil matrix with a combination of water-filled pores and gas-filled cracks, root channels, and other macro pores. During this partial water-logging of the soil the transport of gas was dominated by a mix of diffusion in water phase and gas phase, thereby allowing the fast escape of N_2O produced several cm below the soil surface rather than being temporarily accumulated as a transient pool of N_2O until being finally reduced to N_2. Partial wetting of the soil by a moderate rain shower may thus favor hot moments (McClain et al., 2003) of shorter duration, but with higher N_2O emissions than during complete waterlogging after heavy rain. Such a mixed situation of water-filled and gas-filled pore space has previously been associated with high N_2O fluxes, even facilitating the efficient transport of N_2O to the atmosphere (Markfoged et al., 2011).

At site A, we observed O_2 penetration to a depth of 50–60 cm at Level 2, while oxidized iron was observed in soil layers at 80–90 cm depth in holes dug at Level 3. These observations, together with the observed increase in soil NO_3^- content over time, suggest that nitrifiers could contribute to the production of N_2O throughout the drained soil. Anaerobic microsites in the generally oxic layers may, however, cause locally intense denitrification (Smith, 1980). A relative increase of the anoxic volume in the soil, e.g., by temporary increased soil moisture as observed in the soil after a rain shower, would further favor denitrification temporarily.

Peak events of N_2O flux from the drained Pantanal wetland soil were apparently closely coupled to precipitation events and variations in water content in the soil matrix (**Figure 4**). Increased soil moisture by a rain shower did not result in a complete depletion of O_2 in the soil (**Figure 7A**), but rather increased the anoxic soil

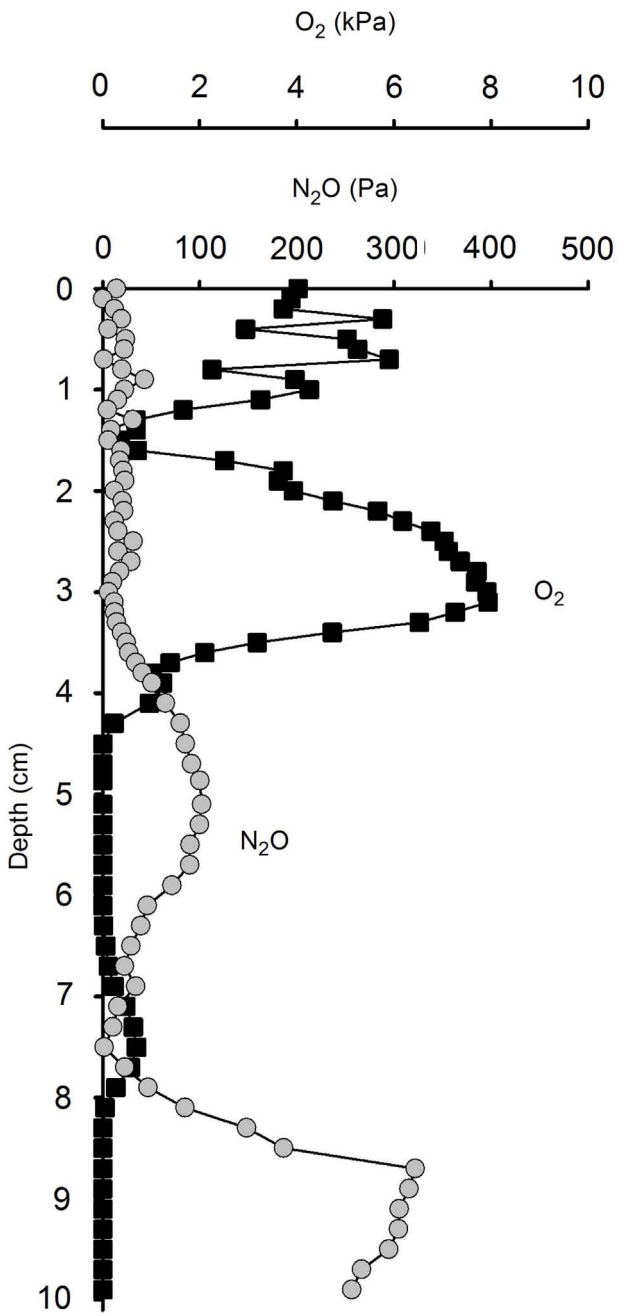

FIGURE 10 | Concentration profiles of N_2O and O_2 in a naturally re-wetted soil core. The pores in this rain-wetted soil are partially gas-filled and partially water-filled. This creates steep gradients of N_2O and O_2 because of the much slower diffusion and lower solubility in water as compared to gas.

volume thus temporarily increasing the heterotrophic turnover of organic matter *via* denitrification. Fluctuations in the anoxic soil volume and fluctuating gas transport parameters thus, controlled the relative contribution of nitrification and denitrification to the total emission of N_2O from the Pantanal wetland soil.

SUGGESTED N_2O SOURCE STRENGTH OF THE PANTANAL

As the drained wetland soil was rich in NO_3^- at both the beginning and end of the drained season we suggest that the observed N_2O emission continued throughout drained season. To calculate an estimate of the seasonal N_2O flux we therefore classified each flux measurement as either a drained soil flux or a precipitation-triggered peak event flux. Considering the 10 sites as pseudo-replicates a total of 116 *in situ* flux measurements (each representing a mean of five chambers) were performed from 2008 to 2010. We classified 94 measurements as *drained soil fluxes* (mean $= 0.43 \pm 0.03$ mmol N_2O m^{-2} day^{-1}), while 22 measurements were considered *precipitation-triggered peak events* (mean $= 1.54 \pm 0.24$ mmol N_2O m^{-2} day^{-1}). The experimental flooding of soils (**Figure 8**) and the *in situ* N_2O flux measurements (**Figure 3**) suggest that a typical peak event lasted ∼1 day. Therefore, cumulative emissions were calculated assuming linear changes between subsequent measurements of drained soil fluxes, while precipitation-triggered peak event fluxes were assumed to last 1 day.

Precipitation and soil moisture data at site A showed that, during 170 days of the drained season 2009–2010, there were at least six events of heavy precipitation and increased soil moisture that likely triggered a peak N_2O emission event. We therefore assume that during the 170 day period precipitation-triggered peak events contributed 9.2 mmol N_2O m^{-2}, while non-wetted drained soil flux contributed 70.0 mmol N_2O m^{-2} to the total emission of N_2O. Consequently, we suggest that the cumulated N_2O emission from the wetland soil during 170 days of the drained season was 79.3 mmol N_2O m^{-2}, with precipitation-triggered peak events contributing ∼12% of the total N_2O emission. In contrast, wetting events in forest soil in Rondônia, Brazil were estimated by (Garcia-Montiel et al., 2003) to contribute <2% of the annual emissions.

For the purpose of estimating the N_2O source strength of all the seasonally flooded soils in the Pantanal during the drained season, we calculated that the N_2O flux from drained soil over a period of 170 days would be 0.30 Tg N (79.3 mmol N_2O m^{-2} × 28 g N mol^{-1} × 1.4×10^{11} m^{-2} of seasonally flooded soil (Swarts, 2000). With an estimated global N_2O source strength of 17.7 Tg N $year^{-1}$ (IPCC, 2007) the Pantanal would thus contribute 1.7% to the global N_2O emission budget, a significant single source of N_2O.

Obviously, our calculations rely on extrapolation from a relatively small data set and need further confirmation by measurements over larger spatio-temporal scales. However, our findings are strongly supported by a recent analysis of the tropospheric distribution and variability of N_2O which showed that N_2O emissions are concentrated in the tropics and that South America has an up to five times higher emission of N_2O than expected (Kort et al., 2011). In addition, the analysis by Kort et al. (2011) demonstrated that global N_2O sources are concentrated in the tropics in November and January, thus coinciding with the drained season in the Pantanal and our findings of high N_2O emission.

SOURCE OF NITROGEN

During 170 days of the drained season the loss of nitrogen from the soil *via* emission of N_2O alone would be 158.5 mmol N m^{-2},

requiring an annual input of at least 158.5 mmol N m^{-2} or 22.2 kg N ha^{-1} to balance this loss assuming that the system is in steady-state. So where does this nitrogen come from?

Several reports indicate that natural tropical systems may export very large quantities of nitrogen (e.g., Martinelli et al., 1999; Matson et al., 1999), deemed the "*tropical nitrogen paradox*", because input of nitrogen, presumably by N_2-fixation, would have to occur in a nitrogen rich environment (Hedin et al., 2009). A spatial decoupling of the N_2-fixation and the nitrogen rich soil due to epiphytic N_2-fixers has been proposed to solve this paradox for tropical forest systems (Hedin et al., 2009). Could the seasonal production of aquatic macrophytes in the Pantanal be the natural nitrogen source driving nitrification and incomplete denitrification and N_2O emission (**Figure 11**)?

Floating mats of *E. crassipes* have a seasonal biomass production of 10–20 t dry weight ha^{-1} with an estimated nitrogen content of 30–50 kg N t dry $weight^{-1}$ and cover 5–100% of water bodies when the Pantanal is flooded (de Neiff et al., 2006). Such a decaying mat would supply the soil with 300–1,000 kg N ha^{-1} $year^{-1}$ and ∼10 times as much carbon (Abdo and Da Silva, 2002; Xie et al., 2004), which eventually must be mineralized. As the water phase in the Pantanal was generally poor in inorganic nitrogen (**Figure 6**), we suggest that N_2-fixing bacteria associated with the aquatic macrophytes (Iswaran et al., 1973; Purchase, 1977) are a major source of nitrogen to the system. Carignan and Neiff (1992) measured a total N_2-fixation of 2.88 mmol N m^{-2} day^{-1} in a floating *E. crassipes* mat suggesting an input of 65–85 kg N ha^{-1} $year^{-1}$. Our calculated loss of nitrogen of 22.2 kg N ha^{-1} from the Pantanal wetland soil *via* N_2O would thus be in the range of 2–34% of the above estimated input of nitrogen to the soil *via* N_2-fixation and aquatic macrophytes. This estimate is comparable to managed tropical soil where up to 28% of the applied N was lost as N_2O (Veldkamp et al., 1998), but represents a much higher fraction than known from temperate soils. Fertilizer-induced N_2O emission from temperate agricultural soils are generally in the range of 0.3–7% of the applied nitrogen fertilizer (Bouwman, 1996; Velthof et al., 2009), roughly a fivefold lower fraction than from the Pantanal wetland soil. The seasonal input of nitrogen by aquatic macrophytes, like the regular application of fertilizer, may therefore be the major cause of the high emission of N_2O from the Pantanal wetland soil.

The carbon and nitrogen cycles are closely interlinked in wetlands and the large biomass input suggested above would imply a large input of carbon to the Pantanal wetlands. As the wetland soils of the Pantanal are not peat soils and have a C:N ratio of ∼10–20 (data not shown), this input of carbon must be mineralized. Evidence for such mineralization can be found in studies of CH_4 production and emission from lakes and flooded areas during the flooded season (Marani and Alvala, 2007) and the low water

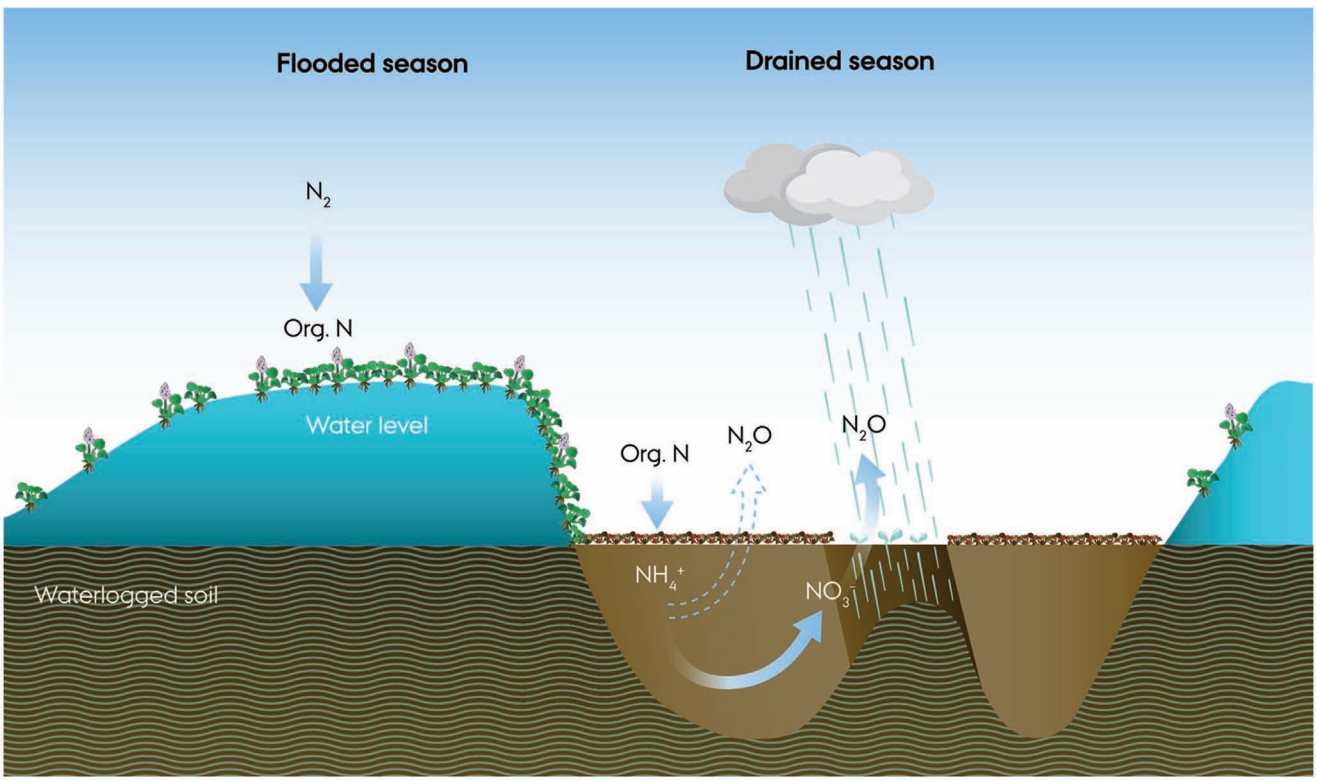

FIGURE 11 | A conceptual drawing of microbial nitrogen cycling and N_2O emission during a 1 year flood cycle of the Pantanal. During flooding, intense nitrogen fixation accompanies the growth of floating meadows dominated by water hyacinths (*Eichhornia crassipes*). As the water retreats, the dense, decaying mats release ammonium, and obscure light, preventing growth of other plants. As the soil is drained and aerated, O_2 becomes available for intense nitrification in the soil while rain showers frequently deplete the O_2 and elicit denitrification with bursts of N_2O until the drained season ends with re-flooding of the soil.

period (Bastviken et al., 2010) suggesting an annual loss of CH_4 to the atmosphere of 450–500 kg C ha^{-1} $year^{-1}$, this also makes the Pantanal a significant source of the greenhouse gas CH_4.

CONCLUSION

Studies of nitrogen dynamics and N_2O emissions from tropical freshwater wetlands are noticeably scarce. Furthermore, the contribution of N_2O from tropical freshwater wetlands has largely been considered negligible (Matson and Vitousek, 1990). The six major tropical freshwater wetlands in South America are estimated to cover an area of 500,000 km^2 that is flooded annually (Hamilton et al., 2002), while globally tropical wetlands are estimated to cover 5,000,000 km^2 (Neue et al., 1997). Based on our measurements of the N_2O flux from wetland soil we suggest that the Pantanal may be contributing 1.7% to the annual global N_2O emission budget during the drained season; this is a significant hitherto ignored single source of N_2O.

It is currently unknown to what extent the Pantanal wetland system with its dynamic cycling of nitrogen can be compared to other wetlands. The global N_2O budget is not balanced (IPCC, 2007), which has been attributed to either a major unknown source or uncertainties in the quantification of one or more known sources (Smith, 1997). Kort et al. (2011) measured atmospheric concentrations of N_2O suggesting that South America has a much higher emission of N_2O than expected, supporting our observation of the Pantanal as a significant, but hitherto unknown source of N_2O.

Our study underscores the direct and indirect importance of flooding and precipitation patterns in tropical watersheds and wetlands, where sudden natural wetting events can cause significant N_2O emission comparable to heavily fertilized agricultural soils. This first study of the dynamics of soil nitrogen pools and emission of N_2O from the world's largest wetland thus emphasizes the current lack of knowledge about nitrogen cycling in undisturbed wetlands, and about how such systems may alter in response to a changing global climate. Lastly, it underscores the paramount importance of varying environmental boundary conditions modulating microbial mineralization processes in the carbon and nitrogen cycle of wetland soils.

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