Radiative forcing of methane fluxes offsets net carbon dioxide uptake for a tropical flooded forest

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Abstract
Wetlands are important sources of methane (CH₄) and sinks of carbon dioxide (CO₂). However, little is known about CH₄ and CO₂ fluxes and dynamics of seasonally flooded tropical forests of South America in relation to local carbon (C) balances and atmospheric exchange. We measured net ecosystem fluxes of CH₄ and CO₂ in the Pantanal over 2014–2017 using tower-based eddy covariance along with C measurements in soil, biomass and water. Our data indicate that seasonally flooded tropical forests are potentially large sinks for CO₂ but strong sources of CH₄, particularly during inundation when reducing conditions in soils increase CH₄ production and limit CO₂ release. During inundation when soils were anaerobic, the flooded forest emitted 0.11 ± 0.002 g CH₄-C m⁻² d⁻¹ and absorbed 1.6 ± 0.2 g CO₂-C m⁻² d⁻¹ (mean ± 95% confidence interval for the entire study period). Following the recession of floodwaters, soils rapidly became aerobic and CH₄ emissions decreased significantly (0.002 ± 0.001 g CH₄-C m⁻² d⁻¹) but remained a net source, while the net CO₂ flux flipped from being a net sink during anaerobic periods to acting as a source during aerobic periods. CH₄ fluxes were 50 times higher in the wet season; DOC was a minor component in the net ecosystem carbon balance. Daily fluxes of CO₂ and CH₄ were similar in all years for each season, but annual net fluxes varied primarily in relation to flood duration. While the ecosystem was a net C sink on an annual basis (absorbing 218 g C m⁻² (as CH₄-C + CO₂-C) in anaerobic phases and emitting 76 g C m⁻² in aerobic phases), high CH₄ effluxes during the anaerobic flooded phase and modest CH₄ effluxes during the aerobic phase indicate that seasonally flooded tropical forests can be a net source of radiative forcings on an annual basis, thus acting as an amplifying feedback on global warming.

KEYWORDS
CH₄ and CO₂ fluxes, Eddy-covariance, global change, greenhouse gas balance, hyperseasonal savanna, net ecosystem carbon balance, tropical wetlands

1 | INTRODUCTION

Wetlands occupy 4%–6% of the earth’s surface (Junk et al., 2014; Kuehn, Steiner, & Gessner, 2004), and play an important role in regulating the global carbon (C) cycle (Barbosa et al., 2016; Sjögersten et al., 2014). In fact, wetlands are the primary natural sources of atmospheric CH₄ (Turetsky et al., 2014), accounting for about 1/3 of total global emissions (Kirschke et al., 2013; Whalen, 2005). However,
wetlands have great potential for C sequestration because anaerobic conditions resulting from permanent or seasonal soil saturation inhibit microbial respiration (Mitra, Wassmann, & Vlek, 2005). Wetlands appear to be the prominent driver of the interannual variations in the rate of growth of atmospheric CH$_4$ (Bousquet et al., 2006; Nisbet, Dlugokencky, & Bousquet, 2014), and whether wetlands are C sources or sinks depends strongly on the processes by which hydrologic variations affect both CH$_4$ and CO$_2$ fluxes (Deutscher, Griffith, Paton-Walsh, & Borah, 2010; Petrescu et al., 2015).

The current and future contribution of wetlands to the global greenhouse gas (GHG) budget is still uncertain due to limited knowledge of the combined and synergistic responses of CH$_4$ and CO$_2$ to climatic variability (Petrescu et al., 2015; Sturtevant & Oechel, 2013). The uncertainty of CO$_2$/CH$_4$ stoichiometry of wetlands is due to a variety of sources, such as the lack of reliable estimates of global wetland area (Melton et al., 2013), difficulties in the parameterization of terrestrial anaerobic sources and oxidative sinks (Melton et al., 2013; Poulter et al., 2017), and the scarcity of CH$_4$ flux measurements, especially from tropical wetlands, which are thought to be responsible for up to a third of the global wetland CH$_4$ emissions (Kirschke et al., 2013; Riley et al., 2011). To date, most of the CH$_4$ flux measurements took place in boreal and temperate regions of the Northern Hemisphere (Blais, Lorrain, & Tremblay, 2005; Petrescu et al., 2015; Turetsky et al., 2014). Since an estimated 30% of global wetlands are in the tropics (Mitsch et al., 2010), the lack of tropical CH$_4$ flux estimates leads to large uncertainties in the global wetland C balance (Petrescu et al., 2015).

The Pantanal of South America is the largest continuous wetland in the world, covering a total flood area of 160,000 km$^2$ (Junk, 2013). The hydrology of the Pantanal is highly seasonal, with a low water season usually spanning from September to December and a high water season occurring between January and as late as August (Junk et al., 2014). Peak flooding typically occurs in March (northern Pantanal) and at the end of July (southern Pantanal) (Bastviken et al., 2010), and the north–south flood level gradient creates a complex mosaic of habitats such as seasonally flooded forests and grasslands, and open water environments such as lakes and river channels (Padovani, 2010). During the flood period, physical, chemical, and biological processes severely deplete oxygen content in the soil through reduced oxygen diffusion, oxidation-reduction reactions and microbiological activity (Pezeshki & DeLaune, 2012). The drop in the oxygen content of the soil under acidic conditions (soil pH of 4.5) is accompanied by a reduction in the soil oxidation-reduction potential (ORP) ($E_r$) with several alterations in the ionic content of the soil. For example, reduction of manganese, iron, and sulfate ions and this sequence of chemical reactions results in negative $E_r$ and may lead to a reduction of CO$_2$ to CH$_4$ (Dalmagro et al., 2016; Vepraskas & Faulkner, 2000).

The different Pantanal aquatic habitats are important sources of atmospheric CH$_4$ and CO$_2$, but due to the size and heterogeneity of the region, there is considerable spatial and temporal variability in CH$_4$ and CO$_2$ fluxes (Bastviken et al., 2010; Bergier et al., 2015; Dalmagro et al., 2018; Hamilton, Sippel, & Melack, 1995; Marani & Alvala, 2007). Prior studies have described fluxes from lakes or permanently flooded wetlands (Bastviken et al., 2010; Bergier et al., 2015; Hamilton et al., 1995; Marani & Alvala, 2007), but rates of CO$_2$ and CH$_4$ fluxes from seasonally flooded forests, which represent over 30% (~48,000 km$^2$) of the Pantanal area (Junk et al., 2006), have not yet been quantified. Of the total area of the Pantanal (160,000 km$^2$), 12% (~19,000 km$^2$) can be flooded, that is, larger flood area than the seasonally flooded areas in the interior of the Okavango Delta of Botswana in Africa (Mitsch et al., 2013). Pantanal forests are also increasingly under pressure from climatic and land use change (Junk, 2013), underscoring the importance of understanding current and future C fluxes.

Given the large hydrologic variation, our objective was to provide a continuous and ecosystem level quantification of simultaneous CO$_2$ and CH$_4$ fluxes from a seasonally flooded forest of the Pantanal using eddy covariance coupled with ancillary measurements of environmental conditions within soils and flood waters. We hypothesized that this flooded forest would be a strong annual sink for CO$_2$ but a large annual source for CH$_4$. We further hypothesized that the positive radiative forcing associated with the CH$_4$ loss would be large enough to counteract the negative forcing associated with the net CO$_2$ uptake, causing the flooded forest to be a net positive (i.e., amplifying) feedback on radiative forcing. This study represents the first ecosystem-scale and multi-year data for flooded forests in the Pantanal, which is crucial for understanding the role of tropical wetlands in the global C balance and net radiative forcing.

## 2 | MATERIALS AND METHODS

### 2.1 Site description

The research site is in the northern Pantanal (16.498 S, 56.412 W, 120 m altitude) (Figure 1). The site is part of a research station managed by the Federal University of Mato Grosso (UFMT) within a national reserve managed by the Brazilian Social Service of Commerce (SESC Pantanal). The study site is seasonally flooded with pronounced wet (November to April) and dry (May to October) periods. Flooding occurs by local rainfall and the overflow of the Cuiaá river (Girard, Fantin-Cruz, Oliveira, & Hamilton, 2010) that is located approximately 2 km away from the research site. The vegetation of the site is typical of successional forests in the region, with a mean leaf area of 7.4 m$^2$ m$^{-2}$ and a mean height of 6 m (Prado, 2015), dominated by *Combretum lanceolatum* (*Combretaceae*), a common species found on the river banks of the Pantanal region (Santos, Cunha, Tomás, Abreu, & Arieira, 2006). Vascular or semi-aquatic plants such as *Thalia geniculata* and *Nymphaea sp.* occur in more open areas (Pott & Pott, 1994). Flooding at the research site typically occurs between December and June with a maximum flood depth less than 1 m (https://youtu.be/0sEnR6vUIY8). Flood waters are turbid at the beginning of the flood period, due to suspended sediment, but become transparent after a few months, later changing to a dark brown color. During the flood period, the mean dissolved oxygen (O$_2$) and ORP ($E_r$) of the water column were between 1.3-1.4 mg/L.
and 466–630 mV, respectively. In addition, dissolved organic carbon in water (DOC) had a mean concentration of 7.0 mg/L comprised primarily of humic and fulvic acids (Dalmagro et al., 2018).

The mean annual temperature at the site was 24.9°C for the 2012–2017 period, with maximum daily temperatures of 31.1°C (September/2015) and daily minimum values of 11.3°C (July/2013). Precipitation averaged 1,486 mm/year, with more than 80% falling during the wet season. These conditions are consistent with the 30-year average for the region (Vourlitis & da Rocha, 2011). The soil type in the region is classified as Dystric Gleysol (Couto, Klinger, Jacomine, Nunes Da Cunha, & Vechiatto, 2002) with an average concentration of 429 g/kg of sand, 254 g/kg of silt and 317 g/kg of clay, mean soil organic matter (SOM) (0–0.10 m depth) of 17 g/kg and a soil pH of 4.7 (Vourlitis, Dalmagro et al., 2015; Vourlitis, Lobo et al., 2015).

2.2 Environmental variables

Soil oxygen content and ORP measurements were obtained using sensors installed at a depth of 0.10 m. The oxygen concentration (O₂, %, accuracy <0.02% O₂ drift per day) and temperature (Tₐₕₒᵢₗ, o°C, ±1°C) of the soil were measured with an OS-100 (Apogee Instruments, Logan, UT), while ORP (mV, ±0.1%) was measured using a CSIM11 probe (Campbell Scientific, Logan, UT). The direct readings of the ORP sensors were internally referenced to a saturated K/KCl electrode, requiring a correction based on the soil temperature to derive the corrected ORP $E_h$ (mV) (Vepraskas & Faulkner, 2000):

$$E_h = ORP - 0.6743T_{soil} + 273.76.$$  

Micrometeorological variables were measured at 20 m height, close to the eddy covariance sensors. Air temperature ($T_{air}$, °C, ±0.2°C) and relative humidity (RH, %, ±2%) were measured using a thermohygrometer (HMP45AC, Vaisala Inc., Woburn, MA). The net radiation ($R_n$, W/m², ±10% for daily totals) and incident solar radiation ($R_s$, W/m², ±10% for daily totals) were measured using a net radiometer (NR-LITE-L25, Kipp & Zonen, Delft, The Netherlands) and a pyranometer (LI-100X, LI-COR Biosciences, Lincoln, NE, USA, absolute error in natural daylight is ±5% maximum; ±3% typical), respectively. Precipitation was measured using a micrometeorological station WXT520 (Vaisala Inc., Helsinki, Finland, ±5%) installed near the base of the tower, 2 m above the ground, in an open area so that precipitation was not intercepted by the tree canopy. All environmental variables were stored and processed using a data logger (CR1000, Campbell Scientific, Inc., Logan, UT), which recorded a half-hourly average of measurements taken every 30 s.
2.3 | CO₂ and CH₄ flux measurements

An eddy covariance system was mounted on a 20 m high research tower along with environmental sensors (Figure 1). The eddy covariance system consisted of a 3D sonic anemometer used to measure three-dimensional, orthogonal components of velocity (u, v, w) and determine high-frequency sonic temperature fluctuations, as well as two infrared gas analyzers to measure CO₂ and CH₄ concentrations. Between 1 January 2014 and 30 June 2014, we used a WindMaster 3D anemometer (Gill Instruments Ltd., Lymington, UK, accuracy ±0.05 m/s), and between 1 January 2015 and 30 July 2017, we used a RM-Young 3D anemometer (Model 81000, R.M. Young Company, Traverse City, MI, ±0.05 m/s). For CO₂ concentration measurements, we used an open path infrared gas analyzer LI-7500A (LI-COR Biosciences, Lincoln, NE, 1% accuracy). We used an open path LI-7700 (LI-COR Biosciences, Lincoln, NE, 1% accuracy) to measure CH₄ concentrations. The LI-7700 uses a mirror system that was regularly cleaned with purified water through an automatic pumping system, which was actuated once a day or when the CH₄ signal strength was below 50%. Both LI-7500A and LI-7700 systems were calibrated every 6 months using ultra high-purity nitrogen as the zero-point gas for CO₂ and CH₄, and reference gases with concentrations of 401 ppm CO₂ in air (certified grade ±1 ppm) and two standard gas concentrations for CH₄ (1 ppm and 5 ppm CH₄ certified grade ±0.1 ppm) (White Martins Inc., Cuiabá, MT, Brazil). Measurements were performed at 10 Hz with half-hour averages computed from the raw data. In this study, net fluxes of CO₂ and CH₄ are presented as negative values when C is accumulating in the terrestrial ecosystem, and as positive values when the terrestrial ecosystem is a net source to the atmosphere. Therefore, negative values for FCO₂ and FCH₄ represent net CO₂ and CH₄ uptake, respectively.

2.4 | Eddy covariance raw data processing

The raw eddy covariance data were processed using EddyPro® software (v.6.1.0) (LI-COR Biosciences, Lincoln, Nebraska) at 30-min intervals and quality control flags of 2 described by Foken et al. (2004) were removed from the time series. We used the EddyPro® software to perform the following corrections: (1) Webb-Pearman-Leuning air density fluctuations (Detto & Katul, 2007; Webb, Pearman, & Leuning, 1980), (2) spectral corrections (Moncrieff, Clement, Finnigan, & Meyers, 2004), (3) humidity correction of the sonic temperature (Van Dijk, Moene, & Bruin, 2004), and (4) the correction of the incorporated frequency response (Lee, Massman, & Law, 2004; Massman, 2000). In addition, a data tracking procedure was used to remove spurious values due to sensor failures that were outside the physical and/or biologically plausible ranges, especially during rainy events. Values below a signal strength of 90% for CO₂ and 20% for CH₄ sensor were removed, which occurred in response to precipitation or accumulation of particulates on the reflecting mirror. Values collected with a friction velocity (u*) <0.11 m/s (obtained using the online tool from the Max Planck Institute for Biogeochemistry, see below), were also removed due to insufficient turbulence. Following this data removal procedure, CH₄ and CO₂ flux data gaps represented 30.3% of all data recorded between 2013 and 2017. Analysis of the tower footprint, calculated using the online tool created by Kljun, Calanca, Rotach, and Schimid (2004), indicated that the maximum contribution to the measured flux was 34 m upwind of the tower, with 90% of all fluxes originating within 250 m of tower (Figure 1).

Data gaps were filled using the marginal distribution sampling (MDS) method, standardized using REddyProc. REddyProc employs an algorithm based on the methods proposed by Falge et al. (2001), but considers both the covariation of the fluxes with meteorological variables and the temporal autocorrelation of fluxes as described by Reichstein et al. (2005). Although this method is widely used for filling CO₂ data gaps, the MDS technique can be applied to CH₄ fluxes as well (Alberto et al., 2014; Matthes, Sturtevant, Verfaillie, Knox, & Baldocchi, 2014). More information on the data procedure was previously described by Zanella De Arruda et al. (2016). Energy balance closure (Figure 2) of our system showed a relationship of L_e + H = 0.71 (R² = 0.79; n = 20,910 observations) and is within the ranges reported by Biudes et al. (2015).

Net GHG flux in terms of CO₂ equivalents (CO₂eq) was calculated as the sum of CO₂ and CH₄ fluxes, expressed in CO₂eq units, using global warming potentials (GWP) for CH₄ of 34 and 86 for 100- and 20-year timeframes, respectively, corresponding to 1 unit of CO₂ for both timeframes (Myhre, Shindell, & Bréon, 2013). These GWP factors include climate-carbon feedback considerations (Myhre et al., 2013).

2.5 | Statistical analyses

Data were collected from 1 January 2013 to 29 June 2017, with interruptions during the 2014 dry season due to failure of the sonic anemometer, October 2016 due to problems with the LI-7700 sensor, and July 2016 due to E_h sensor failure. Based on values of soil O₂ concentration and E_h, we separated the data into anaerobic phase (flooded soil: when O₂ values were close to zero and E_h was <0 mV) and aerobic phase (non-flooded soil: when O₂ values were approximately 15% and E_h was >0 mV). In addition, to better assess the dynamics of the main C exchange processes, we separated our FCO₂ and FCH₄ data into nighttime and daytime fluxes, based on incident solar radiation. For the purposes of comparisons between phases, we used the bootstrap technique that calculated a 95% confidence interval (CI) by constructing 1,000 sets of sample data starting by random sampling (with substitution) of the observed daily series. Statistically significant differences in the mean values of each phase were determined by the degree of overlap in ±95% bootstrapped CI (Efron & Tibshirani, 1994).

Non-parametric covariance analysis (ANCOVA) was performed to test significant differences in the average lines observed with the simple linear regression models between the two phases (aerobic and anaerobic), after robust linear regressions were applied (Conover & Conover, 1980). For these analyses, the data between the phases were withdrawn (i.e. transition periods from positive to negative E_h and O₂ from close to zero to values close to 15%).
RESULTS

3.1 Environmental variables

Precipitation varied substantially over the 3-year study period (1551, 1599 and 978 mm/year for 2014, 2015, and 2016, respectively) as the study coincided with El Niño (2015–2016) and La Niña (2016–2017) phases (Figure 3a). However, flooding at the research site occurs primarily from overbanking of the Cuiabá River, which is linked with precipitation occurring in the headwaters of the basin, so local flooding can be decoupled from
local precipitation. Interannual variations in precipitation of the region resulted in different durations of soil saturation and standing water, marked here as anaerobic and aerobic phases (Table 1). In general, precipitation during the aerobic phase was insufficient to compensate for evapotranspiration, while precipitation during the end of the aerobic phase, and throughout the anaerobic phase, was sufficient to maintain saturation and soil O₂ concentration close to zero (Figure 2b).

The soil ORP (E_h) for 2014, 2015 and 2016 differed significantly between the anaerobic and aerobic phases (Table 1; Figure 2c). The transition between the two phases was rapid, going from average values of +472 mV to −189 mV over a period of days (Figure 2c). The anaerobic phase of 2016 presented the lowest values of E_h (−268 ± 0.8 mV), even though precipitation was lowest that year during the anaerobic phase, while 2015 had the highest value of E_h (−138 mV) during the aerobic phase (Table 1). Soil O₂ concentrations were consistently ~15% during the aerobic phases, and 0.1% during the anaerobic phases (Table 1, Figure 2c).

3.2 CH₄ and CO₂ fluxes and carbon balance

Seasonal flooding operated like an on-off switch for CH₄ emissions (Figure 4a). Although the duration of the anaerobic period varied by over 80 days, CH₄ fluxes (F(CH₄)) were remarkably consistent among periods (c. 0.10–0.12 g CH₄·C·m⁻²·d⁻¹, Table 1) and exhibited similar seasonal trends (Figure 4a). For example, CH₄ emissions increased rapidly as soon as anaerobic conditions were established, and immediately reached a peak early during the anaerobic phase (Figure 4a). The highest values of F(CH₄) were always observed when soil E_h values were less than −100 mV (Figures 3c and 4a). According to the covariance analysis (ANCOVA), all covariables and phases had significant interactions (Table 2). In addition, differences in slopes were found for both E_h and O₂ for both phases and T_soil during the anaerobic phase (Table 3, Figure 5). These results reinforce the idea that this ecosystem emits CH₄ during the anaerobic phase as the system becomes more oxy-reductive, and neutral during the aerobic phase. On average (±95% CI), F(CH₄) ranged between 0.113 ± 0.002 and 0.002 ± 0.001 g CH₄·C·m⁻²·d⁻¹ for the anaerobic and aerobic periods, respectively (Figure 6a,b, Table 1). Differences between the means of the night and daytime CH₄ fluxes were observed only during the anaerobic period, with the daytime fluxes being almost two times higher than nighttime fluxes (0.14 ± 0.002 and 0.075 ± 0.009 g CH₄·C·m⁻²·d⁻¹, respectively) (Figure 4a, Table 3).

In contrast, net CO₂ fluxes were strongly negative during the anaerobic phases, resulting in overall net CO₂ uptake by the forest, with mean values of −1.6 ± 0.2 g CO₂·C·m⁻²·d⁻¹ and 0.3 ± 0.1 g CO₂·C·m⁻²·d⁻¹ during the anaerobic and aerobic periods, respectively (Figure 6a,b; Table 1). As with F(CH₄), F(CO₂) during the anaerobic period was strikingly consistent among periods (Figure 4b), ranging from −1.2 g CO₂·C·m⁻²·d⁻¹ in 2016, which had the lowest precipitation of any anaerobic period, to −1.6 g CO₂·C·m⁻²·d⁻¹ which was the wettest anaerobic period (Table 1). In general, net CO₂ uptake was the highest immediately after flooding and declined over time during the flooded period such that F(CO₂) was either in balance, or slightly positive, at the end of the flood cycle (Figure 4b). During the aerobic phases, the forest became a net C source 0.8 g CO₂·C·m⁻²·d⁻¹ in 2015 but was approximately in balance in 2016 (Table 1) because of a large precipitation event that occurred at the end of the 2016 dry season (Figures 3a and 4a,b). The mean nighttime CO₂ fluxes (respiration) did not differ between the anaerobic and aerobic phases (3.6 ± 0.8 and 3.5 ± 1.2 g CO₂·C·m⁻²·d⁻¹, respectively), while for daytime CO₂ fluxes, the aerobic phase absorbed on average 3 g CO₂·C·m⁻²·d⁻¹ less than the anaerobic phase (Figure 4b, Table 2).

The C balance (CO₂+C + CH₄+C) in the anaerobic phase was practically identical in 2014 and 2015 (−250 and −259 g C m⁻², respectively), but was lower in 2016 (−145 g C m⁻²) caused mainly by the shorter duration of anaerobic soil conditions (Table 1). On average, our data showed that the forests absorbed −218 g C m⁻² (as CO₂+C + CH₄+C) from the atmosphere during the anaerobic phase, and emitted +76 g C m⁻² during the aerobic phase, resulting in an annual net C accumulation of −141.2 g C m⁻² year⁻¹ over the study period after accounting for the net DOC flux (Figure 6; Table 1).

3.3 CO₂ equivalent exchange

We assessed the potential influence of CH₄ and CO₂ fluxes on radiative forcing by summing up fluxes in CO₂ equivalent (CO₂eq) terms based on the 20-year and 100-year radiative forcings of CH₄ and CO₂, where the global warming potential (GWP) of CH₄ is 86 times that of CO₂ in CO₂eq terms on a 20-year basis, and equivalent to 34 CO₂eq on a 100-year basis including climate–carbon feedbacks (Myhre et al., 2013). On a 20-year basis, we found that the study area acted as an overall positive net forcing (i.e., net CO₂eq source to the atmosphere) during both aerobic and anaerobic periods. Net CO₂eq fluxes were more than five times greater in anaerobic periods (7.1 ± 0.8 g CO₂eq·m⁻²·d⁻¹) compared to aerobic periods (1.3 ± 0.4 g CO₂eq·m⁻²·d⁻¹), respectively (Table 1, Figure 6c,d). When considering 100-year GWP values, aerobic periods remained a small CO₂eq source (1.2 ± 0.1 g CO₂eq·m⁻²·d⁻¹), while anaerobic periods were on average a small net CO₂eq sink (−0.7 ± 0.2 g CO₂eq·m⁻²·d⁻¹) (Table 1, Figure 6c,d). Annual net CO₂eq values for the ecosystem were positive (i.e. net source of CO₂eq to the atmosphere) for both 20-year and 100-year time frames.

4 DISCUSSION

Our data indicate that seasonally flooded tropical forests of the Pantanal are potentially large C sinks, but strong sources of CH₄ when anaerobic soil conditions concomitantly increase CH₄ production and limit CO₂ production, but in general, the seasonally flooded forests of the Pantanal are large sinks of C. The interannual variations in the CO₂ and CH₄ fluxes, and C balance, were driven by the seasonal dynamics of precipitation and surface flooding that
<table>
<thead>
<tr>
<th>Season</th>
<th>2014</th>
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<tr>
<td></td>
<td>Anaerobic</td>
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<td>Length (days)</td>
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<td>Mean O₂ (%)</td>
<td>0.2 ± 0.01</td>
<td>15.1 ± 0.1</td>
<td>0.2 ± 0.01</td>
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<tr>
<td>Mean Eₖ (mV)</td>
<td>−162 ± 4</td>
<td>536 ± 2</td>
<td>−138 ± 4</td>
<td>409 ± 2</td>
<td>−268 ± 0.8</td>
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<tr>
<td>Mean F₃₂₀ (g CO₂-C m⁻² d⁻¹)</td>
<td>−1.6 ± 0.2</td>
<td>0.12 ± 0.01</td>
<td>0.003 ± 0.001</td>
<td>0.116 ± 0.003</td>
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<td>Mean F₊₄ (g CH₄-C m⁻² d⁻¹)</td>
<td>0.10 ± 0.002</td>
<td>0.12 ± 0.01</td>
<td>0.003 ± 0.001</td>
<td>0.116 ± 0.003</td>
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<td>Net CO₂eq flux, 20-year basis (g CO₂eq m⁻² year⁻¹)</td>
<td>1533 ± 163</td>
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affected the duration of the anaerobic and aerobic phases of the soil. Within the C balance, CH$_4$ emissions were relatively low in relation to CO$_2$, suggesting that only a small component of C losses resulted from the release of CH$_4$. However, given its greater potential for global warming compared to CO$_2$ (Meehl et al., 2007), CH$_4$ emissions from tropical wetlands are still important from the point of view of radiative forcing (Sjögersten et al., 2014), especially during the anaerobic phase.

Seasonally flooded tropical wetlands may be a stronger net source of radiative forcing in the near term (e.g. 20 years) than perhaps previously recognized. While our system was a significant net C sink during anaerobic conditions (~218 g C m$^{-2}$ on average as CO$_2$-C + CH$_4$-C), anaerobic seasons were a significant net source of CO$_2$ on a 20-year time horizon, and a minor net source in CO$_2$ terms for a 100-year time horizon (Table 1). Thus, the spatial extent and duration of seasonal flooding in tropical wetlands, and related CO$_2$/CH$_4$ stoichiometry, are key variables for understanding the complex role of these spatially and temporally heterogeneous landscapes in the global climate system.

Net CO$_2$eq can also be calculated without consideration of climate–carbon feedbacks, resulting in GWP factors for CH$_4$ of 28 and 84 for 100- and 20-year timeframes, respectively (Myhre et al., 2013). In this case, the net CO$_2$eq flux on a 20-year basis would be reduced from 1,533 g CO$_2$eq m$^{-2}$ year$^{-1}$ with climate–carbon feedbacks to 1,493 g CO$_2$eq m$^{-2}$ year$^{-1}$ without climate–carbon feedbacks, a difference of only 2.6%. On a 100-year basis, however, the net annual CO$_2$eq flux changes from a net source of 91 g CO$_2$eq m$^{-2}$ year$^{-1}$ with climate–carbon feedbacks to a net sink of 82 g CO$_2$eq m$^{-2}$ year$^{-1}$ without climate–carbon feedbacks. However, climate feedbacks from natural wetlands are substantial and must be considered in strategies to limit global warming (Comyn-Platt et al., 2018). For this reason, we opted to use GWP values with climate–carbon feedback.
Furthermore, our results do not consider N₂O fluxes, which reportedly have episodically high emission rates during soil rewetting in the Pantanal (Lienegaard et al., 2014). An estimated mean annual N₂O flux of 138.4 mmol N₂O m⁻² (Lienegaard et al., 2014) is equivalent to 1,632 g CO₂eq m⁻² year⁻¹ on a 20-year time horizon (GWP for N₂O = 268 on a 20-year basis, Myhre et al., (2013)), which would approximately double the net radiative forcing on a 20-year time horizon for our study area to a total of 3,165 CO₂eq m⁻² year⁻¹. For a 100-year time horizon, N₂O represents 95% of the net radiative forcing, augmenting the 91 CO₂eq of CO₂ + CH₄ (Table 1) by an additional 1,815 g CO₂eq (using a GWP for N₂O = 298 on a 100-year basis, Myhre et al., 2013).

The tight correspondence between F₄ and soil E₄, soil temperature, and microbial substrate availability (expressed as the organic acid concentration in peat water) suggests that hydrological variations will strongly affect the annual dynamics of CH₄ flux (Christensen et al., 2003; Poulter et al., 2017). While this result may be expected, it has important implications for the role of flooded tropical forests in the global C cycle and climate change. For example, El Niño Southern Oscillation (ENSO) gives rise to significant differences in surface precipitation and temperature between El Niño and La Niña years, particularly in the tropics. As a result, ENSO has the potential to play a significant role in determining the inter-annual variability of wetland emissions and the global atmospheric CH₄ growth rate (Fiona, Gedney, & Collins, 2016; Hodson, Poulter, Zimmermann, Prigent, & Kaplan, 2011; Pandey et al., 2017). Climate change in tropical wetlands of the Pantanal is anticipated to cause an increase in temperature and a change in precipitation patterns by increasing the duration of the dry season but also increasing the intensity of rainfall events (Bergier et al., 2018; Junk, 2013). Under this scenario, the growing season can be significantly reduced, with water-limiting conditions leading to lower C accumulation by vegetation (da Silva Sallo, Sanches, Morais Dias, Silva, & Souza, 2017). Given these predictions, the current and future C balance of seasonally flooded tropical areas is uncertain, since an increase in the aerobic phase would reduce both net CO₂ uptake and CH₄ emissions. Given the relatively small CH₄ efflux in relation to the net CO₂ uptake (Figure 4a,b), presumably an increase in the intensity and/or duration of the dry season would cause seasonally flooded forests to become net C sources to atmosphere.

The accumulation of C in tropical wetlands is due to the dynamic balance of photosynthetic and respiratory processes that regulate the net C accumulation in biomass and soil (Petrescu et al., 2015), which is dependent on environmental conditions (Reddy & DeLaune, 2008). Previous studies of gas exchange of Pantanal forest species have reported high rates of potential photosynthesis during both aerobic and anaerobic phases (Dalmagro et al., 2013; Prado, 2015). Pantanal plant communities can thus have high physiological performance employing different strategies under a wide range of soil O₂ concentrations and E₄ (Dalmagro et al., 2016). However, net CO₂ uptake declined over time during the anaerobic phase, switching from a strong net sink during the early anaerobic phase to a net CO₂ source during the end of the anaerobic phase (Figure 4b). Thus,

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<th>Year</th>
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**Table 3** Anaerobic and aerobic season start dates and lengths, along with mean (±95% confidence interval (CI)) night and daytime CO₂ flux (F₄), daytime CH₄ flux (F₄,night) and daytime CH₄ flux (F₄,day).
although forest tree species had similar values of potential photosynthesis between the aerobic and anaerobic phases, the decline in net CO$_2$ uptake indicates that other processes, including leaf senescence, litter decomposition, and/or soil CO$_2$ efflux (Johnson et al., 2013; Dalmolin et al., 2015; Vouritis, Hentz, Pinto, Carneiro, & Souza, 2017; Lathuillière et al., 2017), may become more important as flooding subsides.

Biomass growth may represent a large C flux in tropical wetlands and represents a major gap in current understanding to assess the role of tropical wetlands in the global C cycle, since wood growth represents a large net C input and storage pool (Sjögersten et al., 2014). Biometric measurements of flooded tree trunks of the Pantanal, and from woodlands and forests in the footprint of the flux tower, estimated that the aboveground woody C stock varied between 8 and 100 Mg C ha$^{-1}$ for young trees (<67 years) and woodlands, respectively, and 100–110 Mg C ha$^{-1}$ for older stands (Schöngart, Arieira, Felfili Fortes, Cezarine De Arruda, & Nunes Da Cunha, 2011).

Studies of a riparian forest dominated by *Vochysia Divergens*, a common species in floodplains of the Pantanal Basin, indicate a high rate of increase in trunk radius during the period when the soil was flooded (da Silva Sallo et al., 2017). Within the footprint of the tower, data collected from 160 trees of different species between 2013 and 2017 showed an average rate of accumulation of $\sim$186 g aboveground biomass C m$^{-2}$ year$^{-1}$ (Figure 6a,b). These results show that Pantanal tree species are resilient to both drought and flood (Dalmagro et al., 2016), helping contribute to the large C sink. However, recent research suggests that CH$_4$ emissions from tree stems is the dominant source of regional CH$_4$ emissions for flooded tropical forest environments (Pangala et al., 2017). It should be noted that the flux tower measurements in this study integrate CH$_4$ emissions from tree stems within bulk emissions that also include soil fluxes, ebullition and diffusion from the water surface, and other sources.

Rapid changes in soil water status during the aerobic phase mainly alter CO$_2$ fluxes (Figure 4). Long periods without precipitation affect vegetation phenology (Dalmagro et al., 2013; Dalmolin et al., 2015) by increasing litter production, and thus, soil respiration (Sanches et al., 2008). Studies conducted in an upland forest and a separate flooded forest near the research site show that during the dry season (aerobic phase), the vegetation presented a higher
leaf senescence with peaks in litter production during August and October with an annual production of 2.5 Mg C ha\textsuperscript{-1} for upland forest and 3.3 Mg C ha\textsuperscript{-1} for flooded forest (Pinto junior et al., 2018). Although differences in litter production and decomposition rates (k) of the two forests were not significantly different, the values of k for these forests (0.3) suggest that up to 30% is being lost each year (Pinto junior et al., 2018), while the rest may be accumulating in the soil or lost by lateral flow (e.g., DOC export by water). In turn, litter accumulation during the dry season contributes to an increase in CO\textsubscript{2} and CH\textsubscript{4} flux during the onset of the aerobic phase (Dalmagro et al., 2018). When precipitation occurs during the aerobic phase, an increase in CO\textsubscript{2} emission fluxes is observed, partly due to the increase in CO\textsubscript{2} efflux from the soil. Recent studies of CO\textsubscript{2} efflux from Pantanal tree island forests showed mean values of 4.71 g CO\textsubscript{2}-C m\textsuperscript{-2} d\textsuperscript{-1} (Lathuillière et al., 2017) that were correlated with soil type, and litterfall production, decomposition, and quality, as well as the duration of soil water saturation (Pinto junior et al., 2018). On the other hand, the increase in soil water content during the aerobic phase triggers the emergence of new leaves and branches, which maximizes the C gain by increasing the rates of C assimilation by plants at the beginning of the rainy season (Dalmolin et al., 2015). This result demonstrates the complexity and heterogeneity in the C balance of seasonally flooded tropical forests during the aerobic phase.

In the anaerobic (flooded) phase, recent studies have shown that part of the respiratory C comes from dissolved organic carbon (DOC) (Cole, 2013; Dalmago et al., 2018; Vourlitis et al., 2017; Ward et al., 2017). This DOC is derived mainly from material transported from upland forests (Dalmago et al., 2017), and is likely an important C source for methanogenesis during the anaerobic phase (Gondwe & Masamba, 2014). The high rate of F\textsubscript{CH} that was observed immediately after flooding and the onset of soil anoxia was presumably fed by this relatively large pool of labile DOC. Once produced, however, CH\textsubscript{4} fluxes are affected by a combination of processes that include oxidation by methanotrophic bacteria in the soil (Petrescu et al., 2015), and water column, as well as transport through plant

**FIGURE 6** Net fluxes of CH\textsubscript{4}-C and CO\textsubscript{2}-C during aerobic (a) and anaerobic (b) phases, with arrows indicating direction of net fluxes. Net radiative forcing of the ecosystem expressed in CO\textsubscript{2} equivalent (CO\textsubscript{2eq}) fluxes are presented using 20-year and 100-year global warming potentials (GWP\textsubscript{s}) for CO\textsubscript{2} and CH\textsubscript{4} for aerobic (c) and anaerobic (d) phases for the flooded Pantanal forest ecosystem. The net ecosystem carbon balance (NECB) presented between panels (a) and (b) is the sum of fluxes derived from eddy covariance (net CO\textsubscript{2}-C and net CH\textsubscript{4}-C) from both study phases and the net DOC flux. Net CO\textsubscript{2} fluxes represent gross primary productivity minus ecosystem respiration, and hence account for C stored in biomass. Accumulation of C in aboveground biomass (i.e. wood C) represents an average from 160 trees in the footprint of the flux tower that were measured annually between 2013 and 2017 (unpublished data). The lateral flow of DOC was calculated using the equation DOC = (PPT – ET + ΔS) * [DOC], where: PPT: precipitation, ET: evapotranspiration and ΔS: water level variation, accounting for inputs of DOC to the ecosystem through flooding and DOC exports through lateral drainage, resulting in a net export flux of 0.8 g C m\textsuperscript{-2} year\textsuperscript{-1}. For the DOC flux, data published by Dalmagro et al. (2018) were used.
structures known as "aerenchyma" tissue (Bhullar, Iravani, Edwards, & Venterink, 2013; Whiting, Chanton, Bartlett, & Happell, 1991), and ebullition (Poulter et al., 2017). In addition to serving as a channel for transporting CH₄, wetland plants can also provide C substrates for methanogenesis through the production of soil organic matter and exudation of DOC (Whiting et al., 1991). The increase in CO₂ fixation during the aerobic phase in our study may favor the production of CH₄ by increasing labile C for methanogenesis (Song et al., 2015).

In conclusion, our data show an increase in CO₂ emissions during the aerobic phase and CO₂ absorption during the anaerobic phase. We found only minor CH₄ emissions in the aerobic phase, but when the soil becomes anoxic, CH₄ emissions increased rapidly. Thus, the long-term C balance of these ecosystems ultimately depends on hydrological conditions and the duration of flooding which can change under future climatic conditions altering the composition of the vegetation and consequently the fluxes of CO₂ and CH₄. Further, when considering GWP, the system was a significant net source of CO₂eq fluxes to the atmosphere on an annual basis when expressed using 20-year time-frames for GWP factors, and a minor source of net annual CO₂eq fluxes when expressed on a 100-year basis. For the 20-year time frame, anaerobic periods exhibited strong net CO₂eq fluxes to the atmosphere for the study area, with aerobic (dry) periods maintaining smaller net CO₂eq fluxes to the atmosphere. This differed from reported CO₂eq fluxes for a seasonally flooded wetland in the temperate zone, which was found to be a CO₂eq sink on a 20-year GWP basis during the flooded phase, a CO₂eq source during the drier stage, and a net CO₂eq source on an annual basis (Lee et al., 2017).

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