Supporting Information

Methane emissions from Pantanal, South America, during the low water season: Towards more comprehensive sampling

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This supporting information contains seven pages, two tables, and three figures.

Methods

Methane flux measurements

Measurements of CH_4 flux at each site were performed during a full 24 h period using floating chambers as described by Bastviken et al. (*1*). The chambers were made of 7 L plastic buckets which were equipped with styrofoam floats to float with the edges reaching 4 cm below the water surface. Each chamber covered an area of 0.043 m^2. A hole was drilled at the top of the chamber and an 11 mm rubber stopper was put in the hole. A piece of PVC tubing (25 cm long, 5 mm outer diameter, 3 mm inner diameter) was inserted through the stopper in one end and attached to a luer-lock syringe valve at the other end. The tubing facilitated sampling from the chambers by syringe with minimal disturbance of the chamber position. The chambers were covered with aluminum foil to reflect the sunlight and minimize internal heating. Each chamber was attached to a separate styrofoam float by a 1 m line. This additional float was connected to a stone with another line to anchor the chamber. When measuring in or next to macrophyte stands, chambers were sometimes attached to plants above the water level to avoid drifting.

For the 24 h measurements (8 to 34 chambers per lake), only initial and final samples were taken. A 50 ml polypropylene plastic syringe (Becton Dickinson) equipped with a polycarbonate stopcock was attached to the tubing leading to the chambers. The syringe was pumped three times to mix the chamber content before withdrawing 50 ml of gas from the chamber and closing the stopcock. Due to the remote location quick

analysis was not possible so the samples were transferred to storage vials. Vials were prepared prior to sampling and consisted of 20 ml glass vials filled completely with saturated NaCl and capped with a 10 mm thick butyl rubber stopper (Apodan, Denmark) with an aluminum crimp seal. The NaCl brine in the vial was displaced through a second needle when the sample was injected into the vial from the syringe. Previous tests confirmed that CH_4 samples can be stored extended time periods in such vials. The massive stoppers are thick enough to prevent leakage and the high concentration of NaCl prevented both dissolution and oxidation of CH_4 in the remaining brine in the vials with samples.

Analyzes

Concentrations of CH_4 in the storage vials were measured in the laboratory with a GC-FID (Shimadzu 8A) equipped with a 500 μL injection loop and a 2 m x 3.2 mm stainless steel column packed with HayeSep Q 80/100. The column oven temperature was 40ºC and the N_2 carrier gas pressure setting was 80 kPa which resulted in a column flow of about 10 mL min^{-1}. Certified 99.9 ± 2 ppm, 1000 ± 20 ppm, and 1.95 ± 0.02 %, CH_4 standards (Air Liquide) were used for calibration.

Lake water CH_4 concentrations samples were collected with a 50 ml plastic syringe with the stopcock submerged a few centimeters below the water surface. After rinsing with some water to remove air bubbles along the walls slightly more than 40 ml of water was drawn into the syringe. A headspace equilibration method was used to extract the CH_4 from the water (*2*). Water volume in the syringe was adjusted to 40 ml

and 20 ml of air was introduced into the syringe before closing the valve. Care was taken to avoid breathing into the syringe. The syringe was shaken vigorously for 1 minute which was tested to be enough to equilibrate the water with the headspace, and the headspace was then transferred to a storage vial as described above. Air samples taken at the same time were also transferred to storage vials to correct the measured headspace concentration for the ambient CH_4 levels. The corrected headspace concentration was used to account for the amount of CH_4 that remained dissolved in the sample syringe using Henry's Law adjusted for temperature according to Wiesenburg and Guinasso (*3*). The total amount of CH_4 in the sample syringe was calculated and this amount was then divided by the water volume to obtain the surface water CH_4 concentration.

Flux calculations

The flux into the diffusion chambers was calculated as described in Bastviken et al. (2004). The diffusive flux across the water surface into the floating chamber can be described by the equation

$$F = k(C_w - C_{fc}), \qquad (1)$$

where F is the flux (moles m^{-2} d^{-1}), k is the piston velocity (m d^{-1}), and C_w is the measured CH_4 concentration in the water (moles m^{-3}), and C_{fc} is the CH_4 concentration in the water given equilibrium with the CH_4 partial pressure in the floating chamber (*4*). This equation implies that the flux is driven by the concentration difference which will decrease with time in the chambers. A simple calculation of the total amount of CH_4 that

entered the chambers divided with the time of measurement will underestimate the instantaneous flux rate. To better estimate the instantaneous flux we solved for *k*. Equation 1 was rewritten as

$$\frac{(P_t - P_0)\cdot V}{R\cdot T\cdot A} = k\cdot(P_w\cdot K_h - P_0\cdot K_h), \qquad (2)$$

where P_t, and P_0 is the partial pressure of methane in the chamber (Pa) at times *t* and 0, respectively, *V* is the chamber volume (m^{-3}), *R* is the gas constant (8.314 m^3 Pa K^{-1} mol^{-1}), *T* is the temperature (K), A is the bottom area of the chamber (m^2), P_w is the partial pressure of CH_4 in the chamber at equilibrium with C_w (Pa), and K_h is the Henry's Law constant for CH_4 (moles m^{-3} Pa^{-1}). Thereby,

$$\frac{dP}{dt} = K\cdot(P_w - P), \text{ where} \qquad (3)$$

$$K = k\cdot\frac{K_h\cdot R\cdot T\cdot A}{V}. \qquad (4)$$

The solution for Equation 3 is

$$(P_w - P) = C\cdot e^{(K\cdot t)}, \qquad (5)$$

where C is a constant determined by setting $t = 0$. After solving for k, the instantaneous flux was calculated using Equation 1. The temperature dependence of K_h was calculated from the Bunsen coefficients (*3*).

Ebullition was determined by subtracting the diffusive flux from the Diffusion chambers from the total flux into the chambers without bubble shields. In many cases the ebullition was extensive enough to result in chamber concentrations higher than the equilibrium with CH_4 in the water. In such cases simple flux calculations (accumulated CH_4 divided by chamber area and time) would underestimate fluxes due to diffusion of CH_4 from the chamber into the water once the equilibrium concentration in the chamber was passed. To compensate for this we made a simple mass balance model accounting for diffusive flux (in any direction) over time in the chamber and estimating ebullition rates by fitting the modelled chamber concentration to the measured final chamber concentration. The average of the calculated piston velocity values (gas transfer coefficient, k; see Supporting Information) from the diffusion chambers on the same lake and the water and chamber concentrations of CH_4 were used to calculate the diffusive flux according the equation 1 in the supporting information. The average ebullition rate during the whole measurement period was represented by a given constant rate for all time steps in the model throughout the chamber deployment (since no data regarding temporal variability between time steps were available). This assumption was supported by the short term flux measurements indicating continuous ebullition (see results section). If ebullition occurred in a non-continuous way our model would be biased for the individual chambers. This would result in underestimated fluxes when most ebullition

occurred early during the measurement period, and overestimated fluxes for chambers if ebullition occurred late in the deployment, but given random timing of the ebullition events throughout the incubation periods the errors in the average ebullition estimate for the whole lake should be much smaller. The ebullition calculated from the model was 10 ± 7 % (average ± 1 SD) higher than the uncorrected ebullition estimates.

References

1. Bastviken, D.; Cole, J.; Pace, M.; Tranvik, L. Methane emissions from lakes: Dependence of lake characteristics, two regional assessments, and a global estimate. *Global Biogeochemical Cycles* **2004,** *18* doi:10.1029/2004GB002238.
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4. Cole, J. J.; Caraco, N. F. Atmospheric exchange of carbon dioxide in a low-wind oligotrophic lake measured by the addition of SF_6. *Limnol. Oceanogr.* **1998,** *43*, 647-656.

Figure legends

Figure S1. Map of the Pantanal showing the locations where measurements were made. Some locations were very close to each other and are not separated with different symbols at this scale. We thank Mr. Luiz Alberto Pellegrin for contributing the map.

Figure S2. Methane fluxes at different times in lake L1 based on short term measurements (repeated sampling every 10th minutes for 40 minutes) in chambers partly covering floating macrophytes (A) and over nearby open water just outside the macrophyte belt (B). Measurements were made the same day and the time delay between measurements in the two panels are due to practical constraints handling six replicate chambers with frequent sampling. See Table S1 for lake information.

Figure S3. Total CH_4 flux versus maximum depth of the studied lakes. The line denotes a non-linear regression ($\log10(F) = -1.509 + 4.908z - 2.838z^2 + 0.4341z^3$; F is flux and z depth, adjusted $R^2 = 0.40$). The dashed lines gives the 95% confidence interval.

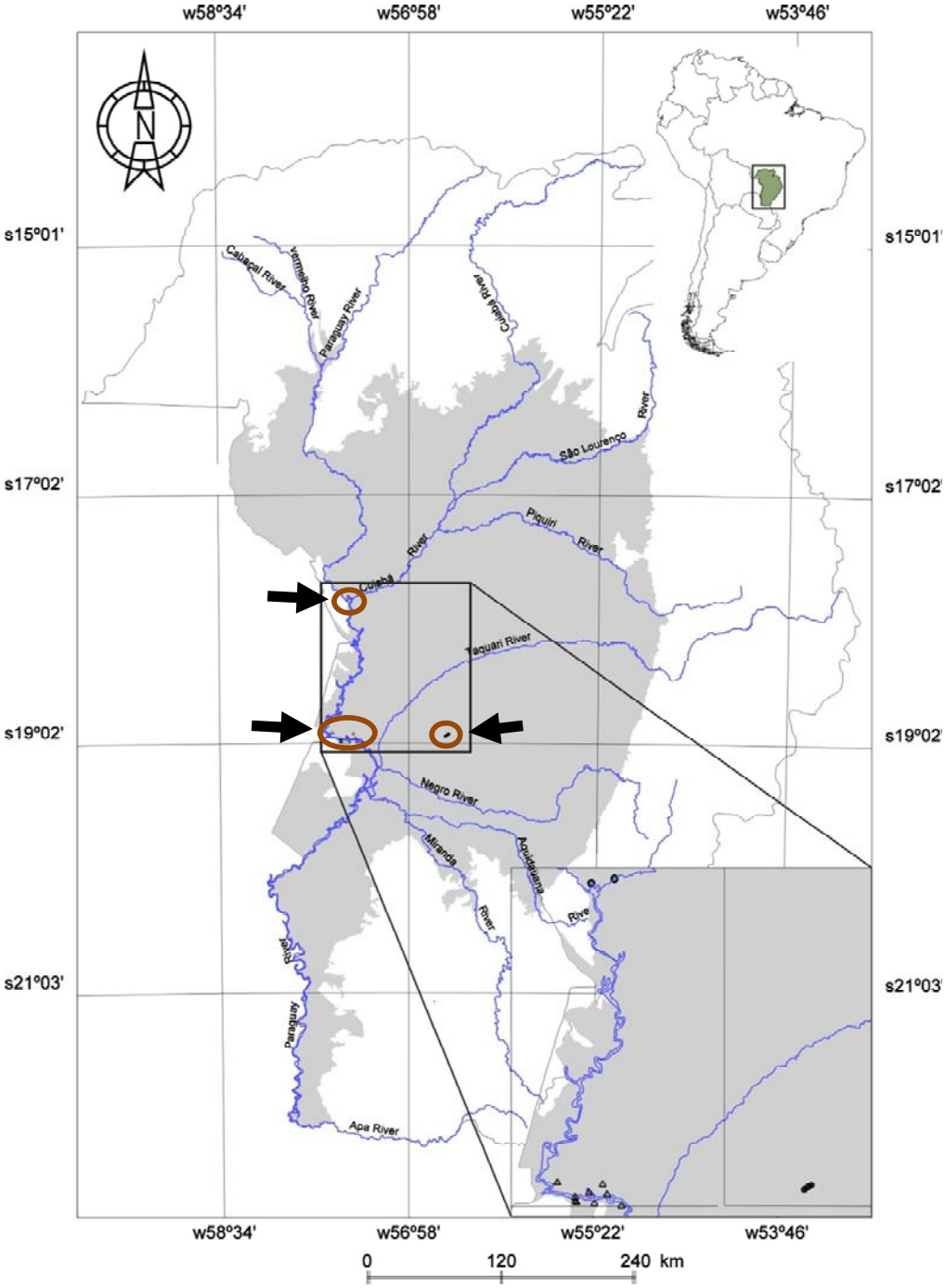
Table S1. Overview of the studied lakes and some physicochemical characteristics including position, area during low water period (ALWP), max depth (z_{max}), pH, alkalinity, sediment water content, organic carbon and organic nitrogen content, and C/N ratio. Mean values are reported. FW and DW denote fresh weight and dry weight, respectively, and nd indicates that no data are available.

						Water chemistry		Sediment chemistry			
Lake ID	**Location**	**Position**		**ALWP**	**z_{max}**	**pH**	**Alkalinity**	**Water content**	**Org. C**	**Org. N**	**C/N ratio**
				km^2	m		mekv L^{-1}	% of FW	% of DW	% of DW	
Belém	Ladario	57°29'40"W	18°59'26"S	nd	2.6	6.5	0.39	nd	nd	nd	nd
Presa	Ladario	57°25'27"W	18°59'49"S	nd	2.7	6.6	0.50	32.0	2.85	0.24	12.0
Bracinho	Ladario	57°32'63"W	19°00'25"S	nd	2.5	6.7	0.38	nd	nd	nd	nd
Lobo	Ladario	57°36'90"W	18°57'11"S	nd	1.9	6.5	0.44	74.8	6.45	0.64	10.0
Tereza	Ladario	57°26'28"W	18°57'38"S	nd	3.4	6.7	0.41	59.7	1.30	0.10	13.4
L1	Ladario	57°32'53"W	19°01'17"S	0.06	2	5.9	0.56	92.0	10.88	0.91	12.0
L2	Ladario	57°32'34"W	19°01'34"S	0.12	1.5	6.0	0.80	65.5	3.58	0.31	11.7
L3	Ladario	57°28'23"W	19°01'50"S	0.35	2	6.3	0.44	50	1.52	0.14	11.0
L4	Ladario	57°22'07"W	19°02'21"S	0.10	1.5	6.6	0.64	86.4	9.73	0.88	11.0
TR	National Park	57°28'54"W	17°51'12"S	71.4	2.5	6.0	0.41	nd	0.75	0.07	10.7
BB	National Park	57°23'33"W	17°50'15"S	36.3	1.2	6.2	0.33	nd	0.74	0.08	9.4
N6b[1]	Nhumirim	56°37'57"W	18°58'01"S	0.12	nd	8.1	5.55	78.3	14.05	1.13	12.5
N7a[1]	Nhumirim	56°38'46"W	18°58'25"S	0.10	0.5	10.7	16.94	33.3	5.76	0.48	12.1
N8a[1]	Nhumirim	56°39'36"W	18°58'59"S	0.16	0.5	9.0	6.37	41.4	3.82	0.36	10.6
N14	Nhumirim	56º39'22"W	18º59'41"S	nd	0.8	5.7	1.05	69.6	8.03	0.72	11.1
N19a	Nhumirim	56º37'54"W	19º00'14"S	nd	0.8	5.3	0.42	65.2	8.80	0.78	11.3

[1]Lake N6b was very rich in organic matter as indicated by a dark brown water color. There were no macrophytes in this lake. Lakes N7a and N8a had very high carbonate levels and a green water color from highly abundant cyanobacteria. Macrophytes were absent.

Table S2. Overview over the results for each lake, including sampling date, number of flux measurements (n), average water temperature (T), methane concentration in the surface water ($[CH_4]_{aq}$), piston velocity (k_{CH4}), diffusive flux, total flux, and percentage of total flux being ebullition. Note that k_{CH4} is the apparent piston velocity for methane (see eg. *4* for how to calculate *k* for other gases from this value).

Lake	**Date**	**n**	**T** °C	**$[CH4]_{aq}$** µM	**k_{CH4}** m d^{-1}	**Diffusive flux** mmol m^{-2} d^{-1}	**Total flux** mmol m^{-2} d^{-1}	**Ebullition** %
Belém	15-Sep-2008	12	26	0.39	0.52	0.20	0.64	69
Presa	15-Sep-2008	11	27	0.5	1.09	0.86	1.69	49
Bracinho	16-Sep-2008	12	26	3	1.36	1.81	15.53	88
Lobo	16-Sep-2008	12	24	1.28	0.46	1.78	5.62	68
Tereza	17-Sep-2008	12	24	0.36	0.52	0.18	0.25	28
L1	5-Dec-2006	34	30	1.35	0.70	0.81	16.39	95
L2	7-Dec-2006	13	30	1.84	0.40	0.70	7.39	91
L3	7-Dec-2006	13	30	0.28	1.44	0.72	2.09	66
L4	7-Dec-2006	13	30	0.51	1.72	0.87	21.99	96
TR	26-Nov-2006	26	31	0.44	1.49	0.65	5.74	89
BB	26-Nov-2006	15	30.5	0.38	1.25	0.50	5.63	91
N6b	29-Nov-2006	10	27	1.32	0.36	0.48	12.45	96
N7a	29-Nov-2006	10	34	0.17	0.36	0.66	1.42	54
N8a	29-Nov-2006	12	29.5	0.83	0.83	0.69	5.17	87
N14	29-Nov-2006	8	29.5	0.58	0.67	0.39	20.68	98
N19a	29-Nov-2006	8	30	1.75	0.67	0.73	11.71	94
All		**221**				**0.74**	**8.79**	**91**



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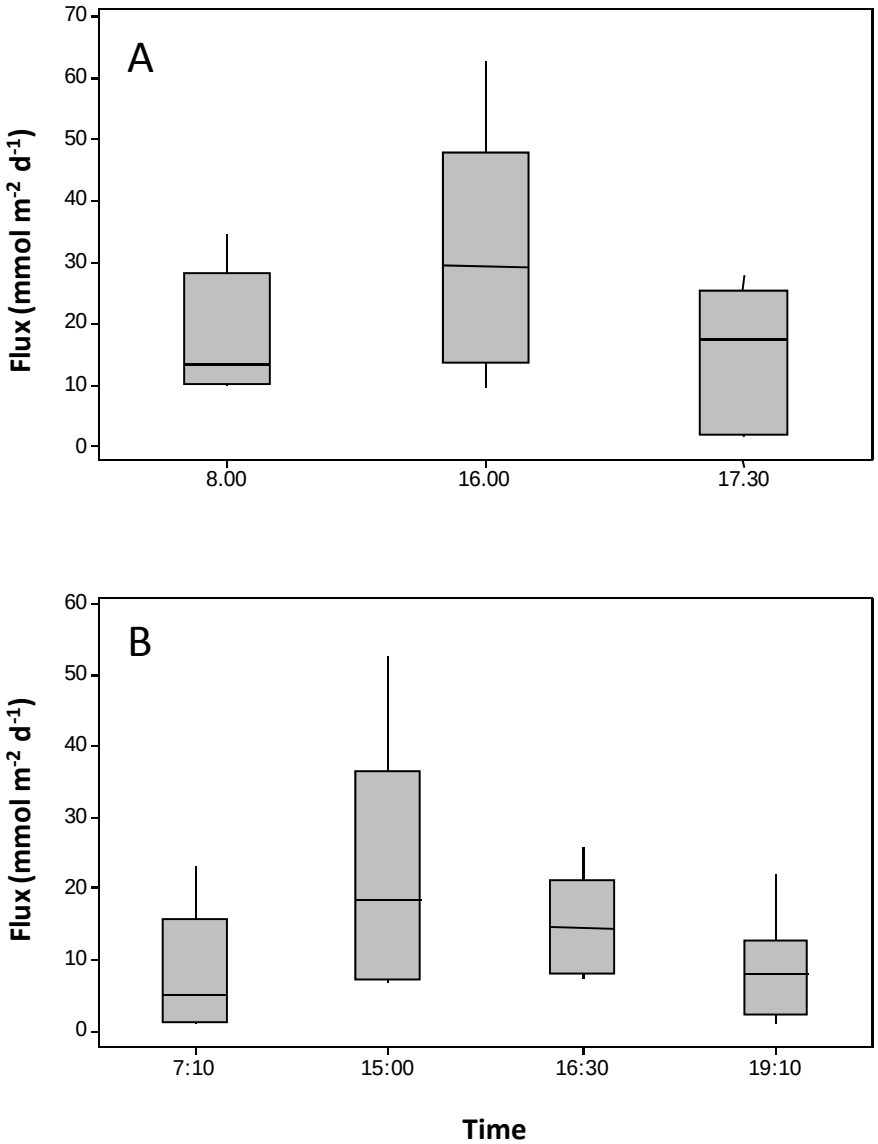

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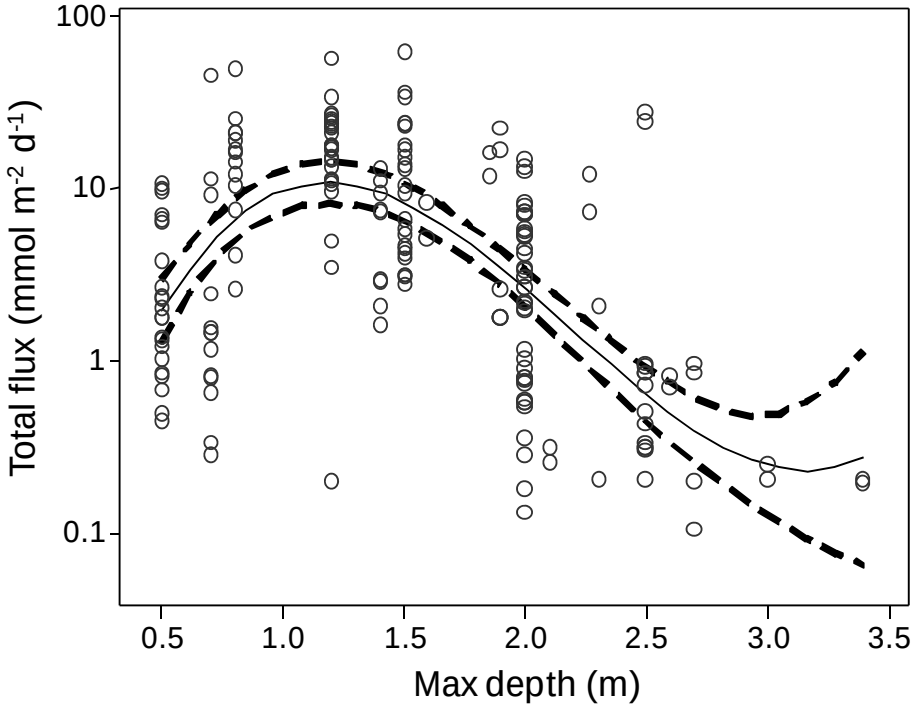

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