



# Extreme emission of N<sub>2</sub>O from tropical wetland soil (Pantanal, South America)

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Nitrous oxide (N<sub>2</sub>O) is an important greenhouse gas and ozone depleter, but the global budget of N<sub>2</sub>O remains unbalanced. Currently, ~25% of the global N<sub>2</sub>O 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<sub>2</sub>O emission from the Pantanal indicating that this South American wetland may be a significant natural source of N<sub>2</sub>O. At three sites, we repeatedly measured *in situ* fluxes of N<sub>2</sub>O and sampled porewater nitrate (NO<sub>3</sub><sup>-</sup>) during the low water season in 2008 and 2009. In 2010, 10 sites were screened for *in situ* fluxes of N<sub>2</sub>O and soil NO<sub>3</sub><sup>-</sup> content. The *in situ* fluxes of N<sub>2</sub>O were comparable to fluxes from heavily fertilized forests or agricultural soils. An important parameter affecting N<sub>2</sub>O emission rate was precipitation, inducing peak emissions of >3 mmol N<sub>2</sub>O m<sup>-2</sup> day<sup>-1</sup>, while the mean daily flux was 0.43 ± 0.03 mmol N<sub>2</sub>O m<sup>-2</sup> day<sup>-1</sup>. Over 170 days of the drained period, we estimated non-wetted drained soil to contribute 70.0 mmol N<sub>2</sub>O m<sup>-2</sup>, while rain-induced peak events contributed 9.2 mmol N<sub>2</sub>O m<sup>-2</sup>, resulting in a total N<sub>2</sub>O emission of 79.2 mmol N<sub>2</sub>O m<sup>-2</sup>. At the sites of repeated sampling, the pool of porewater nitrate varied (0.002 – 7.1 μmol NO<sub>3</sub><sup>-</sup> g dW<sup>-1</sup>) with higher concentrations of NO<sub>3</sub><sup>-</sup> (*p* < 0.05) found in drained soil than in water-logged soil, indicating dynamic shifts between nitrification and denitrification. In the field, O<sub>2</sub> penetrated the upper 60 cm of drained soil, but was depleted in response to precipitation. Upon experimental wetting the soil showed rapid O<sub>2</sub> depletion followed by N<sub>2</sub>O accumulation and a peak emission of N<sub>2</sub>O (2.5 – 3.0 mmol N<sub>2</sub>O m<sup>-2</sup> day<sup>-1</sup>). Assuming that the observed emission of N<sub>2</sub>O 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<sub>2</sub>O emission budget, a significant single source of N<sub>2</sub>O.

**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<sub>2</sub>O) is increasing at an accelerating rate with anthropogenic sources estimated to account for ~38% of the current N<sub>2</sub>O emission (IPCC, 2007). This is concerning because N<sub>2</sub>O 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<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O) 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<sub>4</sub> and N<sub>2</sub>O are primarily biogenic (IPCC, 2007) and methanogens, nitrifiers, and denitrifiers are key players

in regulating the global sources and sinks of CH<sub>4</sub> and N<sub>2</sub>O. 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<sub>2</sub>O.

Although our knowledge of sources and sinks of N<sub>2</sub>O in different environments is increasing, the global N<sub>2</sub>O budget remains unbalanced (Smith, 1997; IPCC, 2007). Currently, ~25% of the global N<sub>2</sub>O 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<sub>2</sub>O demonstrated that global sources of N<sub>2</sub>O are concentrated in the tropics and suggests that South America has

an up to five times higher emission of N<sub>2</sub>O than expected (Kort et al., 2011).

Several studies indicate that tropical forest ecosystems simultaneously accumulate, recycle, and lose 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<sub>2</sub>-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<sub>2</sub>O.

In both natural and impacted environments, the same microbial processes are responsible for the production of N<sub>2</sub>O, i.e., nitrification (NH<sub>4</sub><sup>+</sup> → O<sub>2</sub> → NO<sub>3</sub><sup>-</sup>) and denitrification (NO<sub>3</sub><sup>-</sup> → NO<sub>2</sub><sup>-</sup> → NO → NO<sub>2</sub><sup>-</sup> → N<sub>2</sub>). Nitrifiers in soil can be stimulated to release N<sub>2</sub>O at low O<sub>2</sub> 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<sub>2</sub>O by sudden onset of anoxia (Morley et al., 2008), high concentrations of NO<sub>3</sub><sup>-</sup> (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<sub>2</sub>. This makes it difficult to associate a measured soil N<sub>2</sub>O emission with a specific microbial process (Stevens et al., 1997). However, denitrification is often considered the main N<sub>2</sub>O 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<sub>2</sub> content, pH, and redox potential and thereby modulates the biogeochemical processes involved in production and emission of N<sub>2</sub>O (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<sub>2</sub>O 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<sub>2</sub>O, the dynamics of soil nitrogen pools and soil O<sub>2</sub> 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<sub>2</sub>O and soil NO<sub>3</sub><sup>-</sup> content.

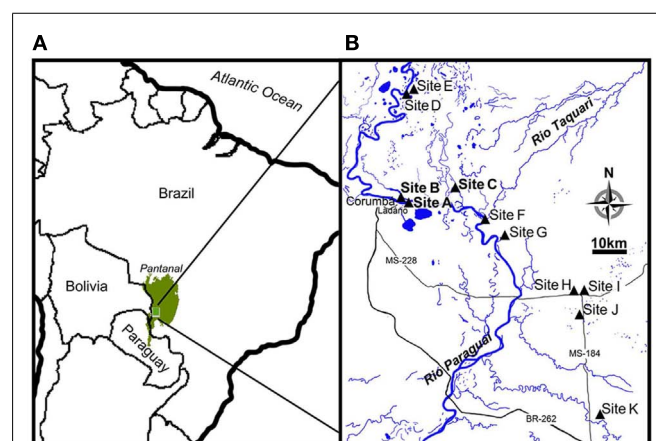
## MATERIALS AND METHODS

### STUDY SITES

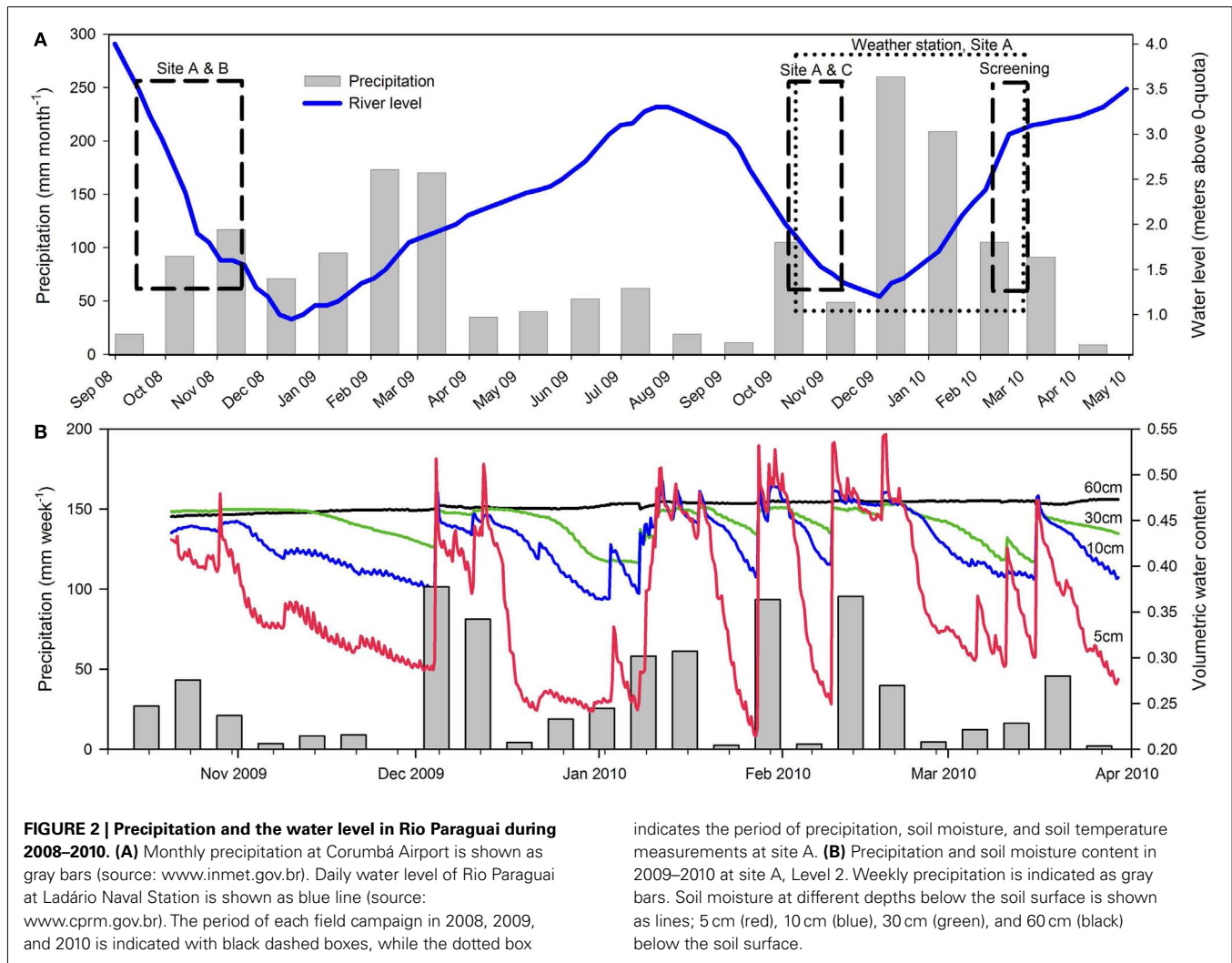
The Pantanal, a pristine tropical wetland in central South America, is shaped by the deposition of sediments into a tectonic depression in the Upper Paraguay River Basin, which formed during the last Andean compressive event (~2.5 Ma; Assine and Soares, 2004). The Pantanal supports a lush vegetation of floating and

emerged herbs and is dominated by an annual flooding and precipitation cycle, alternately inundating and draining ~140,000 km<sup>2</sup> 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<sup>2</sup> watershed, but due to a North-South slope of only 2–3 cm km<sup>-1</sup>, 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<sub>2</sub>O 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).



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<sub>2</sub>O

At each site, the *in situ* flux of N<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O were calculated for each level at each site, assuming linearity between subsequent measurements.

**Table 1 | Screening of *in situ* N<sub>2</sub>O flux, pH, and porewater NO<sub>3</sub><sup>-</sup> in 2010 at 10 sites.**

Location	Position (WGS 84)	Level	<i>In situ</i> N <sub>2</sub> O flux (mmol N <sub>2</sub> O m <sup>-2</sup> day <sup>-1</sup> )	pH	NO <sub>3</sub> <sup>-</sup> (μmol NO <sub>3</sub> <sup>-</sup> cm <sup>-3</sup> 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<sub>3</sub><sup>-</sup>

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<sup>2</sup> = 1.00) at nine different concentrations of NO<sub>3</sub><sup>-</sup> (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<sub>3</sub><sup>-</sup> profile was averaged (arithmetic mean) over the upper 6.5 cm soil column.

During the third field campaign (2010), soil NO<sub>3</sub><sup>-</sup> was measured in a field laboratory with a NO<sub>3</sub><sup>-</sup> biosensor (Unisense A/S, Aarhus,

Denmark), calibrated (r<sup>2</sup> = 0.99) at six different concentrations of NO<sub>3</sub><sup>-</sup> (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<sub>3</sub><sup>-</sup> 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<sub>3</sub><sup>-</sup> biosensor reading in a saline solution.

Means of porewater NO<sub>3</sub><sup>-</sup> 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<sub>2</sub> in soil

In 2009, the depth distribution of O<sub>2</sub> concentration was measured at site A and C at Level 2 (center of transect) with custom-built fiber-optic O<sub>2</sub> optodes (∅ = 2 mm; Rickelt et al., under review). The optical fibers were calibrated in an O<sub>2</sub>-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<sub>2</sub> 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<sub>2</sub>O DYNAMICS IN SOIL WETTED EITHER EXPERIMENTALLY OR BY NATURAL PRECIPITATION

Dry soil cores ( $\varnothing = 5.5$  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<sub>2</sub> microoptodes were constructed according to Klimant et al. (1995) and mounted in hypodermic needles and connected to a fiber-optic O<sub>2</sub> meter (Microx TX3, Presens GmbH, Regensburg, Germany). Electrochemical N<sub>2</sub>O 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  $\sim 100$   $\mu\text{m}$  and a detection limit of  $\sim 5$  Pa. The O<sub>2</sub> sensors were linearly calibrated from a two-point calibration in O<sub>2</sub>-free solution (20% ascorbic acid, pH 11), and in water equilibrated with atmospheric air. The N<sub>2</sub>O sensors were linearly calibrated from a two-point calibration in a 0 and 1% solution of N<sub>2</sub>O produced by mixing 0.5 ml of saturated N<sub>2</sub>O 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 ( $n = 20\text{--}30$ ) of O<sub>2</sub> and N<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub>O diffusivity in water at experimental temperature ( $2.41 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ ),  $C$  is the N<sub>2</sub>O concentration in  $\mu\text{mol l}^{-1}$  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<sub>2</sub>O

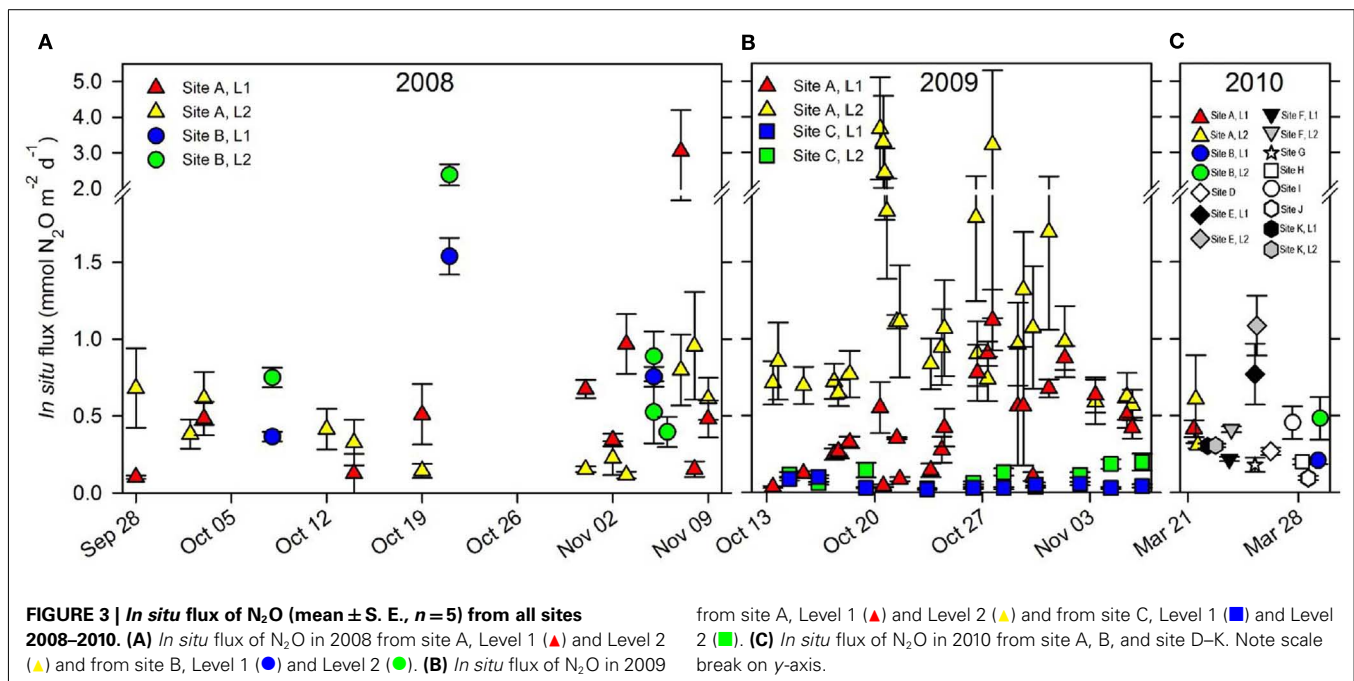
The *in situ* flux of N<sub>2</sub>O at the sites of repeated sampling varied considerably over time in both 2008 (Figure 3A) and 2009 (Figure 3B) the mean daily flux of N<sub>2</sub>O varying between 0.04 and 1.37 mmol N<sub>2</sub>O m<sup>-2</sup> day<sup>-1</sup> at site C, Level 1 and site A, Level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

Peak events of *in situ* N<sub>2</sub>O 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<sub>2</sub>O measurable 6–12 h later (Figure 4).

### SOIL PARAMETERS

#### Porewater NO<sub>3</sub><sup>-</sup>

At site A (2008) the porewater NO<sub>3</sub><sup>-</sup> 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<sub>3</sub><sup>-</sup> 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<sub>3</sub><sup>-</sup> 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<sub>3</sub><sup>-</sup> content was observed at Level 1. The same trend was observed between levels

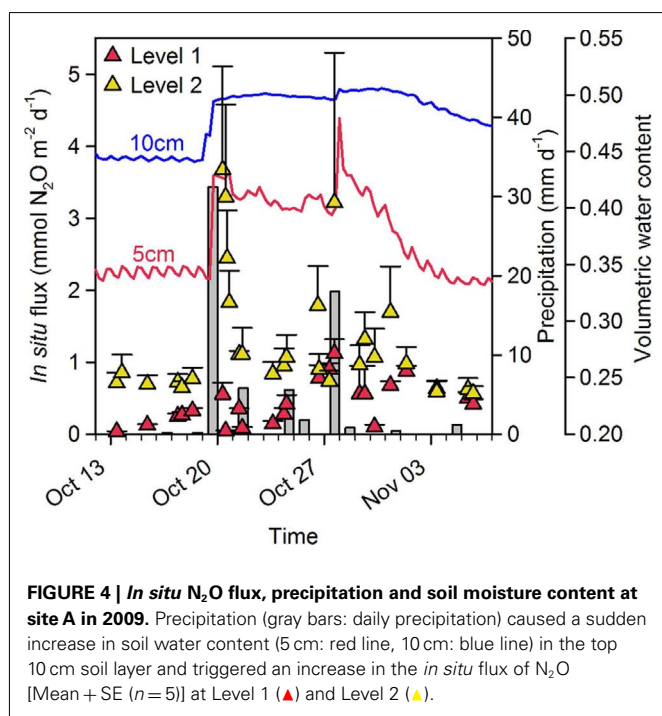


**Table 2 | Integrated flux and mean daily flux of N<sub>2</sub>O at each Level at the sites of repeated sampling.**

Site	Level	days	Integrated N <sub>2</sub> O flux mmol N <sub>2</sub> O m <sup>-2</sup>	Mean N <sub>2</sub> O flux mmol N <sub>2</sub> O m <sup>-2</sup> day <sup>-1</sup>
A	1 <sup>†</sup>	42	22.0 ± 5.6	0.52 ± 0.13
	2 <sup>†</sup>	42	14.0 ± 3.4	0.33 ± 0.08
	1 <sup>‡</sup>	23	12.9 ± 1.7	0.55 ± 0.07
	2 <sup>‡</sup>	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.  
<sup>†</sup> 2008, <sup>‡</sup> 2009.



at site A in 2010, with porewater NO<sub>3</sub><sup>-</sup> content increasing from Level 0 to Level 3 (Figure 5A).

At site B in 2008 (Figure 5B) the porewater NO<sub>3</sub><sup>-</sup> 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<sub>3</sub><sup>-</sup> 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<sub>3</sub><sup>-</sup> 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<sub>3</sub><sup>-</sup> content of  $0.73 \pm 0.09 \mu\text{mol NO}_3^- \text{g DW}^{-1}$ . However, in 2009 (Figure 5C) the overall porewater NO<sub>3</sub><sup>-</sup> 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<sub>3</sub><sup>-</sup> 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<sub>3</sub><sup>-</sup> content varied between  $0.06 \mu\text{mol cm}^{-3}$  soil (site K) and  $5.38 \mu\text{mol cm}^{-3}$  soil (site E).

The NH<sub>4</sub><sup>+</sup> concentration in river waters ranged between  $0.6 \pm 0.5$  and  $10.6 \pm 4.8 \mu\text{mol NH}_4^+ \text{l}^{-1}$  (Figure 6A) and ranged between  $1.0 \pm 0.1$  and  $8.7 \pm 2.6 \mu\text{mol NH}_4^+ \text{l}^{-1}$  in the other water bodies (Figure 6B). The NO<sub>3</sub><sup>-</sup> concentration in rivers ranged between 0.1 and  $12.6 \pm 0.8 \mu\text{mol NO}_3^- \text{l}^{-1}$  (Figure 6A) and ranged between 0.1 and  $3.4 \pm 1.2 \mu\text{mol NO}_3^- \text{l}^{-1}$  in the other investigated water bodies (Figure 6B).

#### Depth distribution of O<sub>2</sub> in soil

At site A, Level 2, O<sub>2</sub> 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<sub>2</sub> 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).

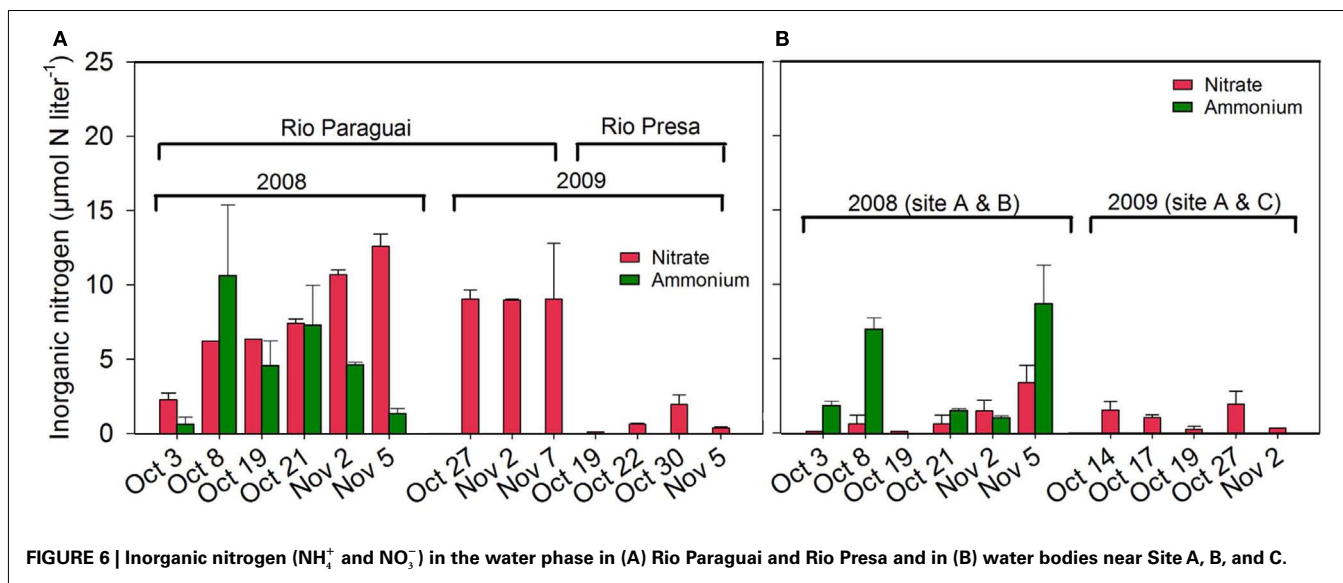
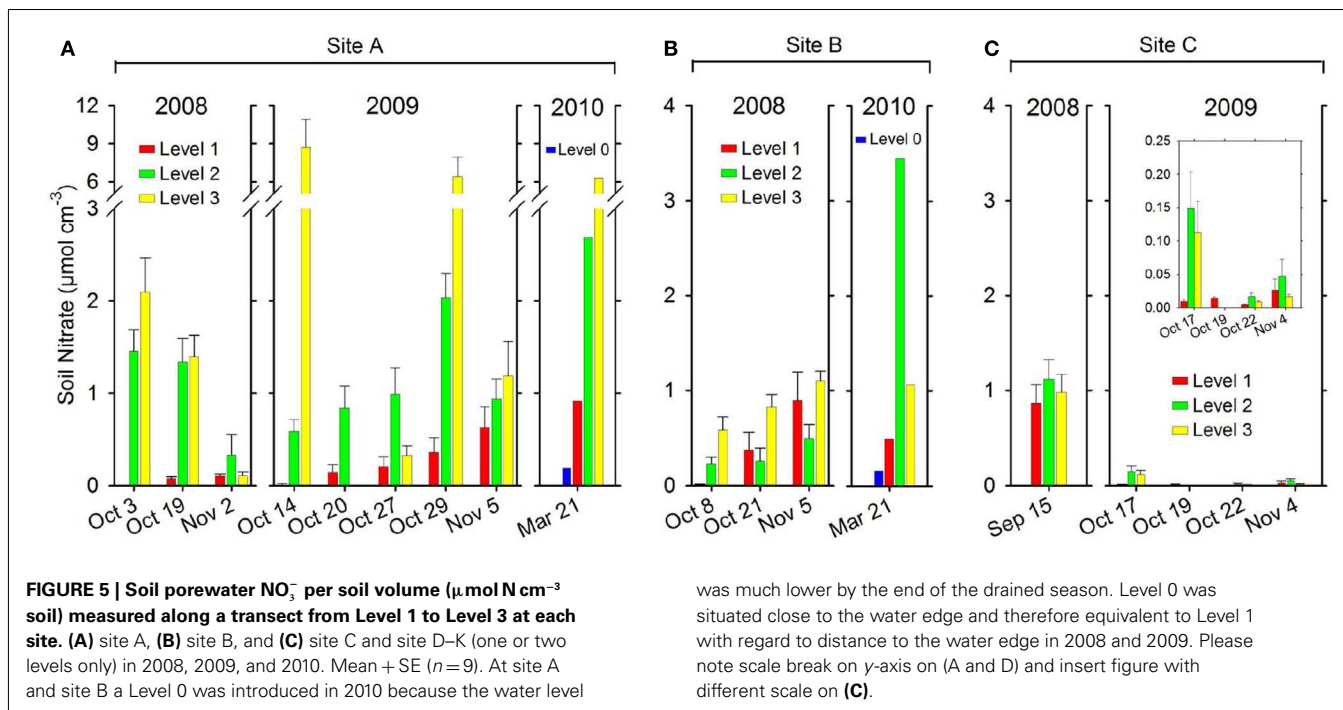
#### MICROSENSOR MEASUREMENTS IN WETTED SOILS

Upon wetting of drained soil cores collected from Level 3 at site A and site B, porewater O<sub>2</sub> depletion occurred within a few hours (Figures 8A–D), followed by a rapid accumulation of N<sub>2</sub>O. The accumulation of N<sub>2</sub>O within the soil persisted for 2–3 days (Figures 9A,B). During this period, N<sub>2</sub>O diffused into the overlying air causing peak emissions of  $3.02 \text{ mmol N}_2\text{O m}^{-2} \text{ day}^{-1}$  from the Site A core (Figure 9C) and  $2.53 \text{ mmol N}_2\text{O m}^{-2} \text{ day}^{-1}$  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  $3.7 \text{ mmol N}_2\text{O m}^{-2}$  over 3 days (soil core from site A) and  $2.92 \text{ mmol N}_2\text{O m}^{-2}$  over 2.3 days (soil core from site B) represented only a small fraction (35–38%) of the net production of N<sub>2</sub>O 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<sub>2</sub>O emission and the dynamics of the porewater nitrate in the Pantanal and revealed a large and hitherto unknown source of N<sub>2</sub>O in the largest wetland of the world. In this discussion, we relate our observed *in situ* N<sub>2</sub>O 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<sub>2</sub>O production, accumulation, and emission in experimentally wetted soil cores in relation to studies of parameters influencing the reduction



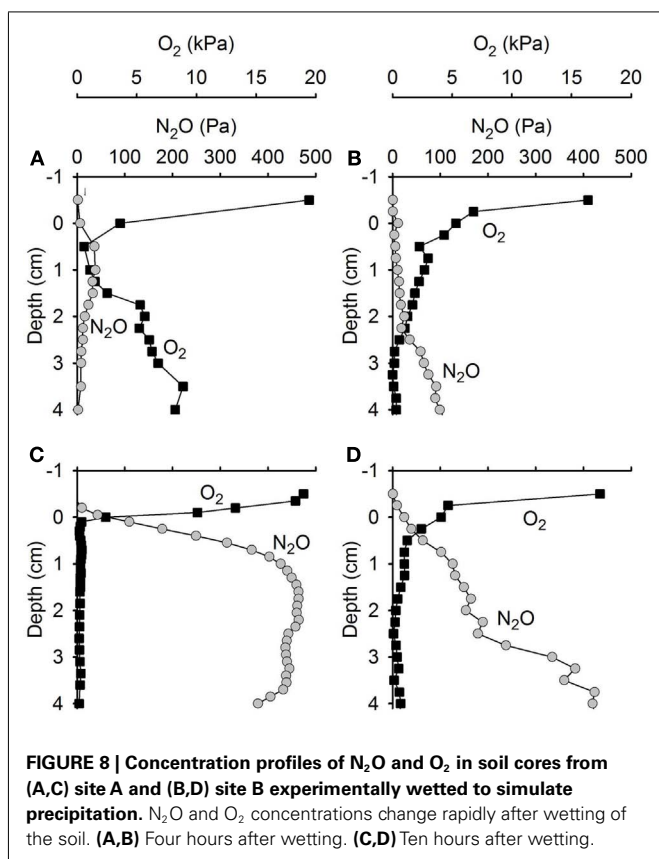
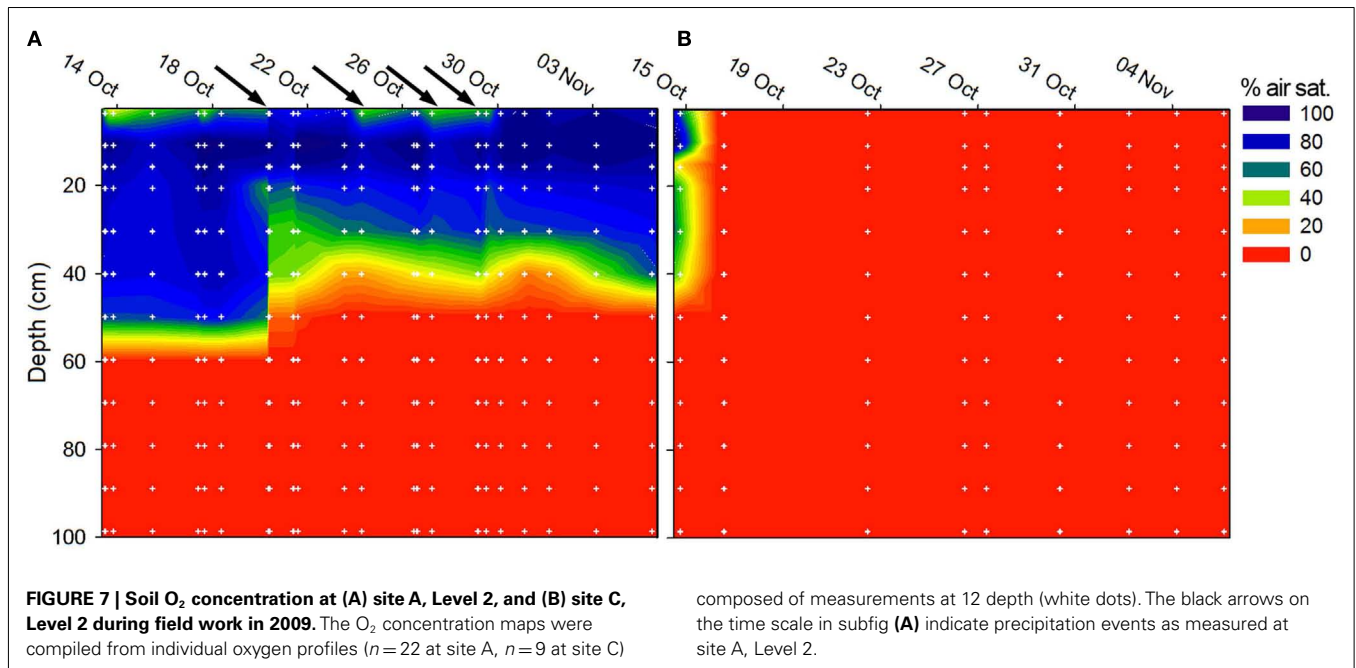
of N<sub>2</sub>O in soil. Finally we speculate on how the observed emission of N<sub>2</sub>O could be supported in a natural system like the Pantanal and how this might link into the cycling of carbon.

**IN SITU FLUX OF N<sub>2</sub>O**

The measured fluxes of N<sub>2</sub>O from Pantanal wetland soils had a high temporal variability (Figure 3) and were generally high (Tables 1 and 2). The average N<sub>2</sub>O 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<sub>2</sub>O emission peaks in tropical forest soils (Hall and Matson, 1999). The fluxes

of N<sub>2</sub>O 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<sub>3</sub><sup>-</sup> concentrations the primary end product of denitrification upon wetting is often N<sub>2</sub>O (Ruser et al., 2006), and although not fertilized, the drained Pantanal wetland soils similarly contained high concentrations of NO<sub>3</sub><sup>-</sup>. The high soil NO<sub>3</sub><sup>-</sup> content thus explains the high emission of N<sub>2</sub>O 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<sub>2</sub>O (Keller et al., 1993; Kern et al., 1996; Veldkamp



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 terrestrial 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.

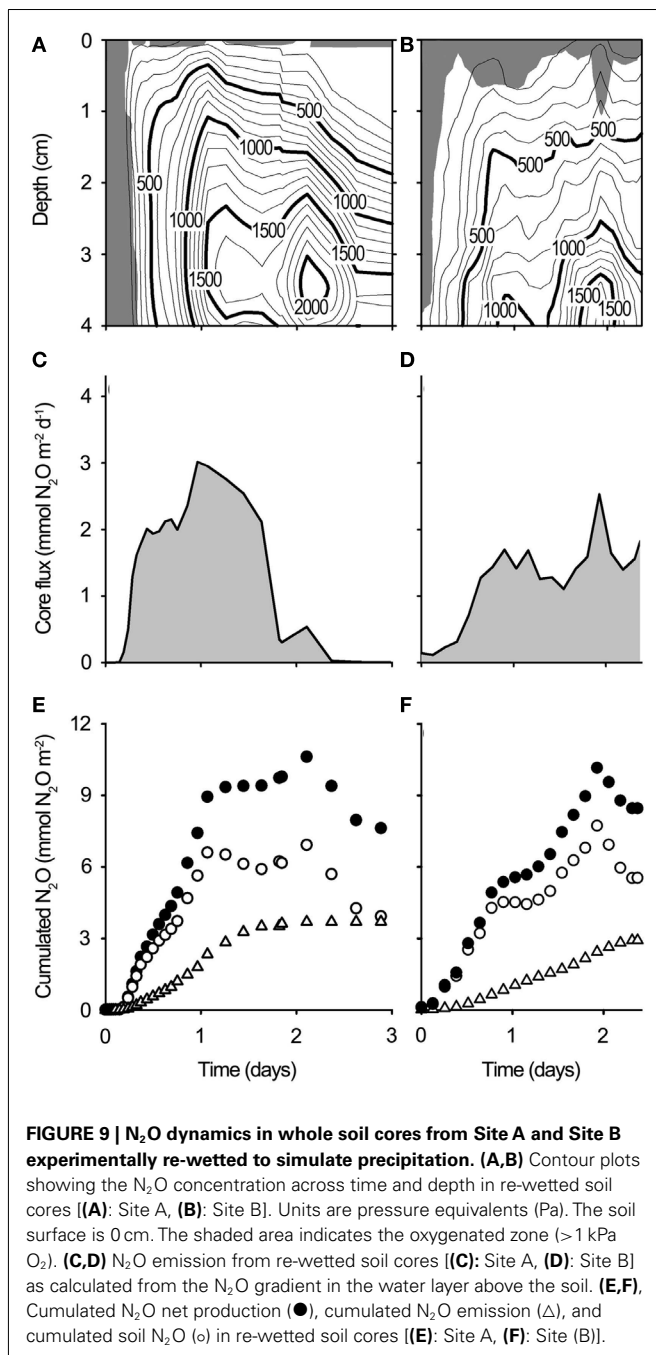
#### DYNAMICS OF POREWATER NITRATE

The concentration of soil porewater NO<sub>3</sub><sup>-</sup> in our study shifted dramatically from being undetectable in water-logged soil to > 1000 μmol NO<sub>3</sub><sup>-</sup> l<sup>-1</sup> in drained soil indicating dynamic shifts between nitrate production and consumption in the soil. Extreme *in situ* concentrations of 10 - 30 mmol NO<sub>3</sub><sup>-</sup> l<sup>-1</sup> 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 (Wetzel, 1960). At the beginning of the drained season at the sites of repeated sampling, we found that still water-logged soil contained no NO<sub>3</sub><sup>-</sup>, but after 3–6 weeks of draining NO<sub>3</sub><sup>-</sup> could be found and increased further, presumably due to nitrification. This is supported by the O<sub>2</sub> 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





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.

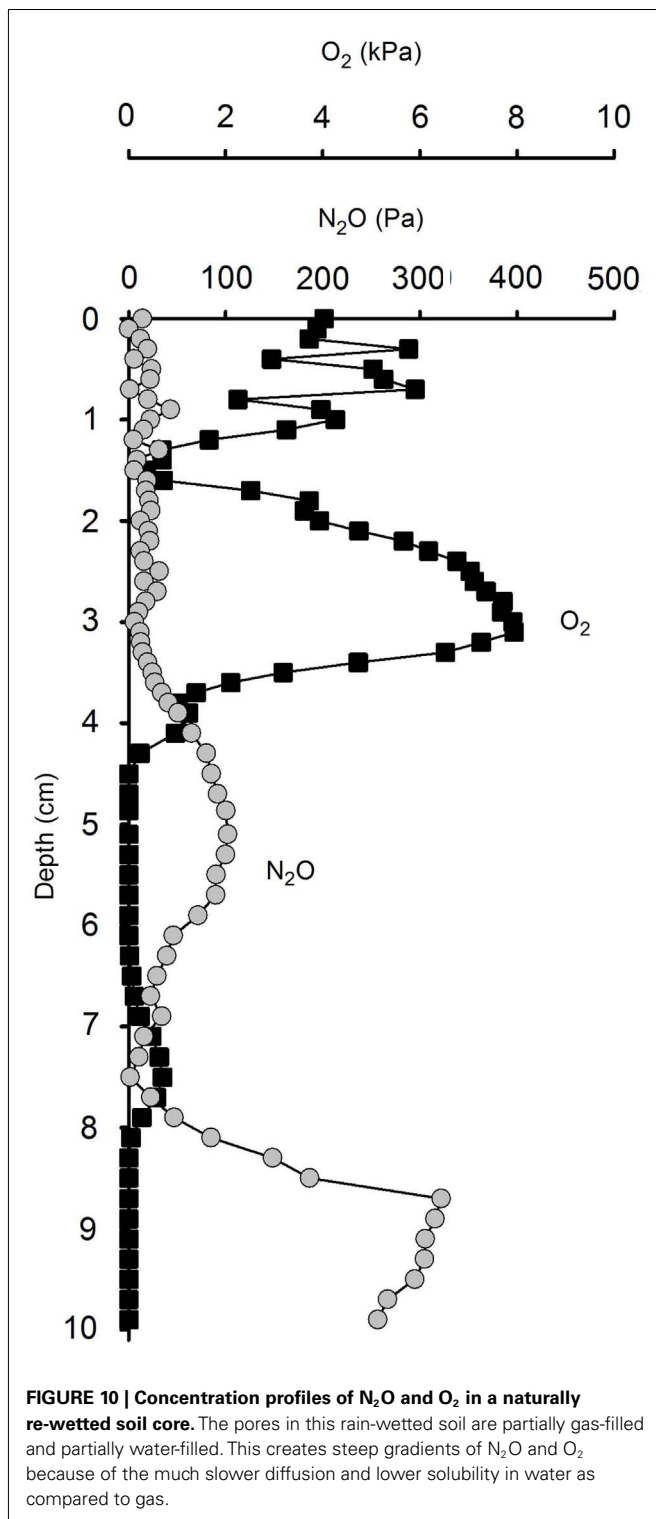
#### N<sub>2</sub>O PRODUCTION AND REDUCTION PROCESSES IN THE SOIL

It is well known that sudden onset of anoxia (Bollmann and Conrad, 1998) and high concentrations of NO<sub>3</sub><sup>-</sup> (Blackmer and Bremner, 1978) can increase the emission of N<sub>2</sub>O. The four step reduction pathway of NO<sub>3</sub><sup>-</sup> to N<sub>2</sub> 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<sub>3</sub><sup>-</sup> content of the drained Pantanal wetland soil combined with sudden anoxia may have delayed or partly inhibited the reduction of N<sub>2</sub>O, stimulating a temporary high accumulation of N<sub>2</sub>O as seen in the experimentally flooded soil cores (Figures 8 and 9). The emitted N<sub>2</sub>O represented 35–38% of the registered cumulated net production (cumulated soil + emitted N<sub>2</sub>O, Figure 9) the rest being retained and reduced to N<sub>2</sub> within the soil. Any additional N<sub>2</sub>O production balanced by simultaneous reduction to N<sub>2</sub> would not be registered in this experimental set up and therefore it is unknown how much larger the N<sub>2</sub> production was.

Microsensor measurements of O<sub>2</sub> and N<sub>2</sub>O in soil cores sampled immediately after a natural, moderate rain event (Figure 10) revealed a heterogeneous distribution of O<sub>2</sub> 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<sub>2</sub>O produced several cm below the soil surface rather than being temporarily accumulated as a transient pool of N<sub>2</sub>O until being finally reduced to N<sub>2</sub>. 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<sub>2</sub>O 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<sub>2</sub>O fluxes, even facilitating the efficient transport of N<sub>2</sub>O to the atmosphere (Markföged et al., 2011).

At site A, we observed O<sub>2</sub> 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<sub>3</sub><sup>-</sup> content over time, suggest that nitrifiers could contribute to the production of N<sub>2</sub>O 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<sub>2</sub>O 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<sub>2</sub> in the soil (Figure 7A), but rather increased the anoxic soil



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<sub>2</sub>O from the Pantanal wetland soil.

### SUGGESTED N<sub>2</sub>O SOURCE STRENGTH OF THE PANTANAL

As the drained wetland soil was rich in NO<sub>3</sub><sup>-</sup> at both the beginning and end of the drained season we suggest that the observed N<sub>2</sub>O emission continued throughout drained season. To calculate an estimate of the seasonal N<sub>2</sub>O 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 ± 0.03 mmol N<sub>2</sub>O m<sup>-2</sup> day<sup>-1</sup>), while 22 measurements were considered *precipitation-triggered peak events* (mean = 1.54 ± 0.24 mmol N<sub>2</sub>O m<sup>-2</sup> day<sup>-1</sup>). The experimental flooding of soils (Figure 8) and the *in situ* N<sub>2</sub>O 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<sub>2</sub>O emission event. We therefore assume that during the 170 day period precipitation-triggered peak events contributed 9.2 mmol N<sub>2</sub>O m<sup>-2</sup>, while non-wetted drained soil flux contributed 70.0 mmol N<sub>2</sub>O m<sup>-2</sup> to the total emission of N<sub>2</sub>O. Consequently, we suggest that the cumulated N<sub>2</sub>O emission from the wetland soil during 170 days of the drained season was 79.3 mmol N<sub>2</sub>O m<sup>-2</sup>, with precipitation-triggered peak events contributing ~12% of the total N<sub>2</sub>O 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<sub>2</sub>O source strength of all the seasonally flooded soils in the Pantanal during the drained season, we calculated that the N<sub>2</sub>O flux from drained soil over a period of 170 days would be 0.30 Tg N (79.3 mmol N<sub>2</sub>O m<sup>-2</sup> × 28 g N mol<sup>-1</sup> × 1.4 × 10<sup>11</sup> m<sup>-2</sup> of seasonally flooded soil (Swarts, 2000). With an estimated global N<sub>2</sub>O source strength of 17.7 Tg N year<sup>-1</sup> (IPCC, 2007) the Pantanal would thus contribute 1.7% to the global N<sub>2</sub>O emission budget, a significant single source of N<sub>2</sub>O.

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<sub>2</sub>O which showed that N<sub>2</sub>O emissions are concentrated in the tropics and that South America has an up to five times higher emission of N<sub>2</sub>O than expected (Kort et al., 2011). In addition, the analysis by Kort et al. (2011) demonstrated that global N<sub>2</sub>O 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<sub>2</sub>O emission.

### SOURCE OF NITROGEN

During 170 days of the drained season the loss of nitrogen from the soil *via* emission of N<sub>2</sub>O alone would be 158.5 mmol N m<sup>-2</sup>,

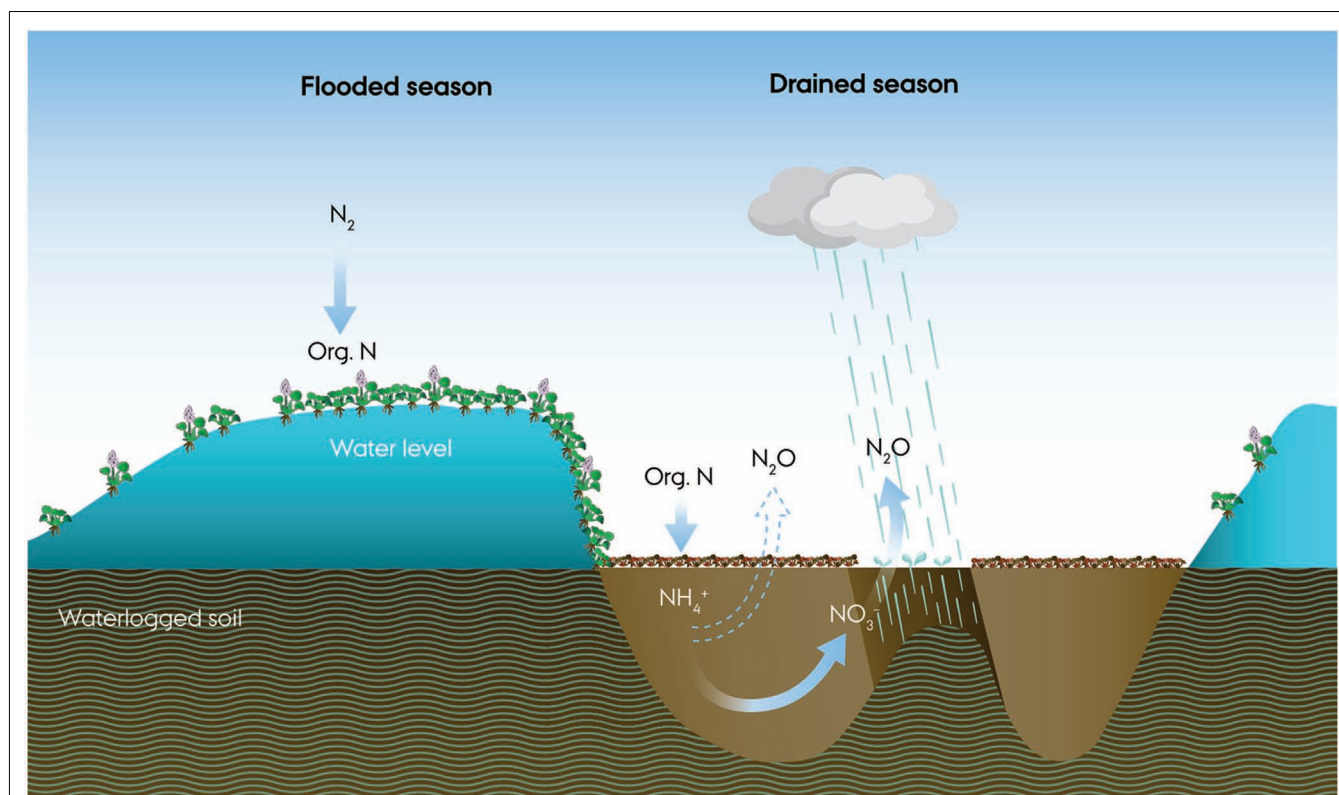
requiring an annual input of at least 158.5 mmol N m<sup>-2</sup> or 22.2 kg N ha<sup>-1</sup> to balance this loss assuming that the system is in steady-state. So where does this nitrogen come from?

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<sub>2</sub>-fixation, would have to occur in a nitrogen rich environment (Hedin et al., 2009). A spatial decoupling of the N<sub>2</sub>-fixation and the nitrogen rich soil due to epiphytic N<sub>2</sub>-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<sub>2</sub>O emission (Figure 11)?

Floating mats of *E. crassipes* have a seasonal biomass production of 10–20 t dry weight ha<sup>-1</sup> with an estimated nitrogen content of 30–50 kg N t dry weight<sup>-1</sup> 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<sup>-1</sup> year<sup>-1</sup> 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<sub>2</sub>-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<sub>2</sub>-fixation of 2.88 mmol N m<sup>-2</sup> day<sup>-1</sup> in a floating *E. crassipes* mat suggesting an input of 65–85 kg N ha<sup>-1</sup> year<sup>-1</sup>. Our calculated loss of nitrogen of 22.2 kg N ha<sup>-1</sup> from the Pantanal wetland soil via N<sub>2</sub>O would thus be in the range of 2–34% of the above estimated input of nitrogen to the soil via N<sub>2</sub>-fixation and aquatic macrophytes. This estimate is comparable to managed tropical soil where up to 28% of the applied N was lost as N<sub>2</sub>O (Veldkamp et al., 1998), but represents a much higher fraction than known from temperate soils. Fertilizer-induced N<sub>2</sub>O 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<sub>2</sub>O 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<sub>4</sub> 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<sub>2</sub>O 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<sub>2</sub> becomes available for intense nitrification in the soil while rain showers frequently deplete the O<sub>2</sub> and elicit denitrification with bursts of N<sub>2</sub>O until the drained season ends with re-flooding of the soil.

period (Bastviken et al., 2010) suggesting an annual loss of CH<sub>4</sub> to the atmosphere of 450–500 kg C ha<sup>-1</sup> year<sup>-1</sup>, this also makes the Pantanal a significant source of the greenhouse gas CH<sub>4</sub>.

## CONCLUSION

Studies of nitrogen dynamics and N<sub>2</sub>O emissions from tropical freshwater wetlands are noticeably scarce. Furthermore, the contribution of N<sub>2</sub>O 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<sup>2</sup> that is flooded annually (Hamilton et al., 2002), while globally tropical wetlands are estimated to cover 5,000,000 km<sup>2</sup> (Neue et al., 1997). Based on our measurements of the N<sub>2</sub>O flux from wetland soil we suggest that the Pantanal may be contributing 1.7% to the annual global N<sub>2</sub>O emission budget during the drained season; this is a significant hitherto ignored single source of N<sub>2</sub>O.

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<sub>2</sub>O 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<sub>2</sub>O suggesting that South America has a much higher emission of N<sub>2</sub>O than expected, supporting our

observation of the Pantanal as a significant, but hitherto unknown source of N<sub>2</sub>O.

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<sub>2</sub>O emission comparable to heavily fertilized agricultural soils. This first study of the dynamics of soil nitrogen pools and emission of N<sub>2</sub>O 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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## REFERENCES

- Abbasi, M. K., and Adams, W. A. (2000). Gaseous N emission during simultaneous nitrification-denitrification associated with mineral N fertilization to a grassland under field conditions. *Soil Biol. Biochem.* 32, 1251–1259.
- Abdo, M. S. A., and Da Silva, C. J. (2002). "Nutrient stock in the aquatic macrophytes *Eichhornia crassipes* and *Pistia stratiotes* in the Pantanal–Brazil," in *Proceedings of the German-Brazilian Workshop on Neotropical Ecosystems*, eds R. Lieberei, H. K. Bianchi, V. Boehm, and C. Reisdorff (Hamburg: GKSS-Gestacht), 1019.
- Allen, D. E., Dalal, R. C., Rennenberg, H., Meyer, R. L., Reeves, S., and Schmidt, S. (2007). Spatial and temporal variation of nitrous oxide and methane flux between subtropical mangrove sediments and the atmosphere. *Soil Biol. Biochem.* 39, 622–631.
- Andersen, K., Kjaer, T., and Revsbech, N. P. (2001). An oxygen insensitive microsensor for nitrous oxide. *Sens. Actuators B Chem.* 81, 42–48.
- Anthony, W. H., Hutchinson, G. L., and Livingston, G. P. (1995). Chamber measurements of soil–atmospheric gas exchange – linear vs. diffusion-based flux models. *Soil Sci. Soc. Am. J.* 59, 1308–1310.
- Assine, M. L., and Soares, P. C. (2004). Quaternary of the Pantanal, west-central Brazil. *Quat. Int.* 114, 23–34.
- Baldwin, D. S., and Mitchell, A. M. (2000). The effects of drying and re-flooding on the sediment and soil nutrient dynamics of lowland river-floodplain systems: a synthesis. *Regul. Rivers Res. Manage.* 16, 457–467.
- Bastviken, D., Santoro, A. L., Marotta, H., Pinho, L. Q., Calheiros, D. F., Crill, P., et al. (2010). Methane emissions from Pantanal, South America, during the low water season: toward more comprehensive sampling. *Environ. Sci. Technol.* 44, 5450–5455.
- Bateman, E. J., and Baggs, E. M. (2005). Contributions of nitrification and denitrification to N<sub>2</sub>O emissions from soils at different water-filled pore space. *Biol. Fertil. Soils* 41, 379–388.
- Blackmer, A. M., and Bremner, J. M. (1978). Inhibitory effects of nitrate on reduction of N<sub>2</sub>O to N<sub>2</sub> by soil microorganisms. *Soil Biol. Biochem.* 10, 187–191.
- Blicher-Mathiesen, G., and Hoffmann, C. C. (1999). Denitrification as a sink for dissolved nitrous oxide in a freshwater riparian fen. *J. Environ. Qual.* 28, 257–262.
- Bollmann, A., and Conrad, R. (1998). Influence of O<sub>2</sub> availability on NO and N<sub>2</sub>O release by nitrification and denitrification in soils. *Glob. Chang. Biol.* 4, 387–396.
- Bouwman, A. F. (1996). Direct emissions of nitrous oxide from agricultural soils. *Nutr. Cycl. Agroecosyst.* 46, 53–70.
- Braman, R. S., and Hendrix, S. A. (1989). Nanogram nitrite and nitrate determination in environmental and biological materials by Vanadium(III) reduction with chemiluminescence detection. *Anal. Chem.* 61, 2715–2718.
- Calheiros, D. F., Seidl, A. F., and Ferreira, C. J. A. (2000). Participatory research methods in environmental science: local and scientific knowledge of a limnological phenomenon in the Pantanal wetland of Brazil. *J. Appl. Ecol.* 37, 684–696.
- Carignan, R., and Neiff, J. J. (1992). Nutrient dynamics in the floodplain ponds of the Parana River (Argentina) dominated by the water hyacinth *Eichhornia crassipes*. *Biogeochemistry* 17, 85–121.
- Cervantes, F., Monroy, O., and Gomez, J. (1998). Accumulation of intermediates in a denitrifying process at different copper and high nitrate concentrations. *Biotechnol. Lett.* 20, 959–961.
- D'Amelio, M. T. S., Gatti, L. V., Miller, J. B., and Tans, P. (2009). Regional N<sub>2</sub>O fluxes in Amazonia derived from aircraft vertical profiles. *Atmos. Chem. Phys.* 9, 8785–8797.
- de Neiff, A. P., Neiff, J. J., and Casco, S. L. (2006). Leaf litter decomposition in three wetland types of the Parana river floodplain. *Wetlands* 26, 558–566.
- Dobbie, K. E., McTaggart, I. P., and Smith, K. A. (1999). Nitrous oxide emissions from intensive agricultural systems: variations between crops and seasons, key driving variables, and mean emission factors. *J. Geophys. Res.* 104, 26891–26899.
- Forbrich, I., Kutzbach, L., Hormann, A., and Wilmking, M. (2010). A comparison of linear and exponential regression for estimating diffusive CH<sub>4</sub> fluxes by closed-chambers in peatlands. *Soil Biol. Biochem.* 42, 507–515.
- Frunzke, K., and Zumft, W. G. (1986). Inhibition of nitrous oxide respiration by nitric oxide in the denitrifying bacterium *Pseudomonas perfectomarina*. *Biochim. Biophys. Acta* 852, 119–125.
- Garcia-Montiel, D. C., Stuedler, P. A., Piccolo, M., Neill, C., Melillo, J., and Cerri, C. C. (2003). Nitrogen oxide emissions following wetting of dry soils in forest and pastures in Rondônia, Brazil. *Biogeochemistry* 64, 319–336.
- Gruber, N., and Galloway, J. N. (2008). An Earth-system perspective of the global nitrogen cycle. *Nature* 451, 293–296.

- Hall, S. J., and Matson, P. A. (1999). Nitrogen oxide emissions after nitrogen additions in tropical forests. *Nature* 400, 152–155.
- Hamilton, S. K., Sippel, S. J., Calheiros, D. F., and Melack, J. M. (1997). An anoxic event and other biogeochemical effects of the Pantanal wetland on the Paraguay River. *Limnol. Oceanogr.* 42, 257–272.
- Hamilton, S. K., Sippel, S. J., and Melack, J. M. (2002). Comparison of inundation patterns among major South American floodplains. *J. Geophys. Res.* 107, 8038.
- Hedin, L. O., Brookshire, E. N. J., Menge, D. N. L., and Barron, A. R. (2009). The nitrogen paradox in tropical forest ecosystems. *Annu. Rev. Ecol. Evol. Syst.* 40, 613–635.
- Hernandez, M. E., and Mitsch, W. J. (2006). Influence of hydrologic pulses, flooding frequency, and vegetation on nitrous oxide emissions from created riparian marshes. *Wetlands* 26, 862–877.
- Houlton, B. Z., Sigman, D. M., and Hedin, L. O. (2006). Isotopic evidence for large gaseous nitrogen losses from tropical rainforests. *Proc. Natl. Acad. Sci. U.S.A.* 103, 8745–8750.
- IPCC. (2007). *Climate Change 2007: The Physical Science Basis*. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge, NY: Cambridge University Press.
- Iswaran, V., Sen, A., and Apte, R. (1973). Azotobacter chroococcum in phyllosphere of Water Hyacinth (*Eichhornia crassipes* Hort Solms). *Plant Soil* 39, 461–463.
- Junk, W. J., Da Cunha, C. N., Wantzen, K. M., Petermann, P., Strussmann, C., Marques, M. I., et al. (2006). Biodiversity and its conservation in the Pantanal of Mato Grosso, Brazil. *Aquat Sci* 68, 278–309.
- Junk, W. J., and Wantzen, K. M. (2004). “The Flood Pulse Concept: New Aspects, Approaches, and Applications – an Update,” in *Proceedings of the Second International Symposium on the Management of Large Rivers for Fisheries*. Food and Agriculture Organization and Mekong River Commission (Bangkok: FAO Regional Office for Asia and the Pacific).
- Keller, M., Veldkamp, E., Weitz, A. M., and Reiners, W. A. (1993). Effect on pasture age on soil trace-gas emissions from a deforested area of Costa Rica. *Nature* 365, 244–246.
- Kern, J., Darwich, A., Furch, K., and Junk, W. J. (1996). Seasonal denitrification in flooded and exposed sediments from the Amazon floodplain at Lago Camaleao. *Microb. Ecol.* 32, 47–57.
- Klimant, I., Meyer, V., and Kuhl, M. (1995). Fiberoptic oxygen microsensors, a new tool in aquatic biology. *Limnol. Oceanogr.* 40, 1159–1165.
- Kort, E. A., Patra, P. K., Ishijima, K., Daube, B. C., Jimenez, R., Elkins, J., et al. (2011). Tropospheric distribution and variability of N<sub>2</sub>O: evidence for strong tropical emissions. *Geophys. Res. Lett.* 38, L15806.
- Koschorreck, M. (2005). Nitrogen turnover in drying sediments of an Amazon floodplain lake. *Microb. Ecol.* 49, 567–577.
- Kroon, P. S., Hensen, A., van den Bulk, W. C. M., Jongejan, P. A. C., and Vermeulen, A. T. (2008). The importance of reducing the systematic error due to non-linearity in N<sub>2</sub>O flux measurements by static chambers. *Nutr. Cycl. Agroecosyst.* 82, 175–186.
- Marani, L., and Alvala, P. C. (2007). Methane emissions from lakes and floodplains in Pantanal, Brazil. *Atmos. Environ.* 41, 1627–1633.
- Markföged, R., Nielsen, L. P., Nyord, T., Ottosen, L. D. M., and Revsbech, N. P. (2011). Transient N<sub>2</sub>O accumulation and emission caused by O<sub>2</sub> depletion in soil after liquid manure injection. *Eur. J. Soil Sci.* 62, 497–655.
- Martinelli, L. A., Piccolo, M. C., Townsend, A. R., Vitousek, P. M., Cuevas, E., McDowell, W., et al. (1999). Nitrogen stable isotopic composition of leaves and soil: tropical versus temperate forests. *Biogeochemistry* 46, 45–65.
- Matson, P. A., McDowell, W. H., Townsend, A. R., and Vitousek, P. M. (1999). The globalization of N deposition: ecosystem consequences in tropical environments. *Biogeochemistry* 46, 67–83.
- Matson, P. A., and Vitousek, P. M. (1987). Cross-system comparisons of soil nitrogen transformations and nitrous oxide flux in tropical forest ecosystems. *Global Biogeochem. Cycles* 1, 163–170.
- Matson, P. A., and Vitousek, P. M. (1990). Ecosystem approach to a global nitrous oxide budget. *Bio-science* 40, 667–672.
- McClain, M. E., Boyer, E. W., Dent, C. L., Gergel, S. E., Grimm, N. B., Groffman, P. M., et al. (2003). Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. *Ecosystems* 6, 301–312.
- Mørkved, P. T., Dörsch, P., and Bakken, L. R. (2007). The N<sub>2</sub>O product ratio of nitrification and its dependence on long-term changes in soil pH. *Soil Biol. Biochem.* 39, 2048–2057.
- Morley, N., Baggs, E. M., Dörsch, P., and Bakken, L. R. (2008). Production of NO, N<sub>2</sub>O and N<sub>2</sub> by extracted soil bacteria, regulation by NO<sub>2</sub>- and O<sub>2</sub> concentrations. *FEMS Microbiol. Ecol.* 65, 102–112.
- Neue, H. U., Gaunt, J. L., Wang, Z. P., Beckerheidmann, P., and Quijano, C. (1997). Carbon in tropical wetlands. *Geoderma* 79, 163–185.
- Purchase, B. S. (1977). Nitrogen fixation associated with *Eichhornia crassipes*. *Plant Soil* 46, 283–286.
- Ravishankara, A. R., Daniel, J. S., and Portmann, R. W. (2009). Nitrous Oxide (N<sub>2</sub>O): the dominant ozone-depleting substance emitted in the 21st Century. *Science* 326, 123–125.
- Ruser, R., Flessa, H., Russow, R., Schmidt, G., Buegger, F., and Munch, J. C. (2006). Emission of N<sub>2</sub>O, N<sub>2</sub> and CO<sub>2</sub> from soil fertilized with nitrate: effect of compaction, soil moisture and rewetting. *Soil Biol. Biochem.* 38, 263–274.
- Schlesinger, W. H. (2010). On fertilizer-induced soil carbon sequestration in China's croplands. *Glob. Chang. Biol.* 16, 849–850.
- Simek, M., and Cooper, J. E. (2002). The influence of soil pH on denitrification: progress towards the understanding of this interaction over the last 50 years. *Eur. J. Soil Sci.* 53, 345–354.
- Smith, K. A. (1980). A model of the extent of anaerobic zones in aggregated soils, and its potential application to estimates of denitrification. *J. Soil Sci.* 31, 263–277.
- Smith, K. A. (1997). The potential for feedback effects induced by global warming on emissions of nitrous oxide by soils. *Glob. Chang. Biol.* 3, 327–338.
- Stevens, R. J., Laughlin, R. J., Burns, L. C., Arah, J. R. M., and Hood, R. C. (1997). Measuring the contributions of nitrification and denitrification to the flux of nitrous oxide from soil. *Soil Biol. Biochem.* 29, 139–151.
- Swarts, F. A. (2000). *The Pantanal: Understanding and Preserving the World's Largest Wetland*. St. Paul: Paragon House Publishers, 301.
- Veldkamp, E., Davidson, E., Erickson, H., Keller, M., and Weitz, A. (1999). Soil nitrogen cycling and nitrous oxide emissions along a pasture chronosequence in the humid tropics of Costa Rica. *Soil Biol. Biochem.* 31, 387–394.
- Veldkamp, E., Keller, M., and Nunez, M. (1998). Effects of pasture management on N<sub>2</sub>O and NO emissions from soils in the humid tropics of Costa Rica. *Global Biogeochem. Cycles* 12, 71–79.
- Velthof, G. I., Oudendag, D., Witzke, H. P., Asman, W. A. H., Klimont, Z., and Oenema, O. (2009). Integrated assessment of nitrogen losses from agriculture in EU-27 using MITERRA-EUROPE. *J. Environ. Qual.* 38, 402–417.
- Weiss, R. F., and Price, B. A. (1980). Nitrous oxide solubility in water and seawater. *Mar. Chem.* 8, 347–359.
- Wetselaar, R. (1960). Capillary movement of nitrate towards tropical soil surfaces. *Science* 186, 572–573.
- Xie, Y. H., Yu, D., and Ren, B. (2004). Effects of nitrogen and phosphorus availability on the decomposition of aquatic plants. *Aquat. Bot.* 80, 29–37.
- Zhou, Y., Pijuan, M., Zeng, R. J., and Yuan, Z. (2008). Free nitrous acid inhibition on nitrous oxide reduction by a denitrifying-enhanced biological phosphorus removal sludge. *Environ. Sci. Technol.* 42, 8260–8265.
- Zumft, W. G. (1997). Cell biology and molecular basis of denitrification. *Microbiol. Mol. Biol. Rev.* 61, 533–616.

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