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Effects of flooding cycles in the Pantanal on the turnover of soil nitrogen pools and emission of N_2O

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Abstract

The global nitrous oxide (N_2O) budget remains unbalanced. Currently, ~25 % of the global N_2O emission is ascribed to uncultivated tropical soils, but the exact locations and controlling mechanisms are not clear. In this study, we present the first detailed study of the dynamics of soil nitrogen pools and flux of N_2O from the world's largest wetland Pantanal, South America. At three long-term measurement sites we measured porewater pH, NO_3^-, NH_4^+ , N_2O and O_2 as well as N_2O dynamics in soil slurry, and in situ fluxes of N_2O and CO_2. The pool of inorganic nitrogen changed (7.1–92 µg NH_4^+-N g dw^{-1}, and 0.1–201 µg NO_3^--N g dw^{-1}) with the seasonal flooding and drying cycles, indicating dynamic shifts between ammonification, nitrification and denitrification. In the field, O_2 penetrated to a depth of 60 cm in dry soil, but O_2 was rapidly depleted in response to precipitation. Soil pH fluctuated from pH 7–7.5 in flooded soil to pH 3.5–4.5 in the same drained soil. Microsensor measurements showed rapid N_2O accumulation reaching >500–1000 Pa in soil slurries due to incomplete denitrification. In situ fluxes of N_2O were comparable to heavily fertilized forest or agricultural soils. The dominating parameter affecting N_2O emission rate was precipitation inducing peak emissions of >3 mmol $N_2O\,m^{-2}\,d^{-1}$, while the mean daily flux was 0.43 mmol $N_2O\,m^{-2}\,d^{-1}$. Single measurement based screening of in situ activity at 10 Pantanal sites during dry conditions averaged 0.39 mmol $N_2O\,m^{-2}\,d^{-1}$. The in situ N_2O fluxes were only weakly correlated ($r^2 = 0.177$) with NO_3^- and pH value, showing a tendency ($p = 0.063$) for NO_3^- concentration to be positively correlated with the in situ N_2O flux and a weaker tendency ($p = 0.138$) for the pH value to be negatively correlated with the in situ N_2O flux. Over 170 days of the drained period we estimated non-wetted drained soil to contribute 70.0 mmol $N_2O\,m^{-2}$, while rain induced peak events contributed 9.2 mmol $N_2O\,m^{-2}$, resulting in a total N_2O emission of 79.2 mmol $N_2O\,m^{-2}$. The total nitrogen loss via emission of NO, N_2O and N_2 was estimated to be 206 mmol $N\,m^{-2}$ over 170 days, representing 0.7–1.6 % of the total nitrogen in the top 6.5 cm soil layer.

BGD
8, 5991–6030, 2011

1 Introduction

The atmospheric concentration of nitrous oxide (N_2O) is increasing at an accelerating rate with anthropogenic sources estimated to account for ~38 % of the current N_2O emission (IPCC, 2007). This is concerning because N_2O is a powerful greenhouse gas (IPCC, 2007) and the most important ozone depleter of the 21st century (Ravishankara et al., 2009). Modelling of the future global climate is dependent on our understanding of the mechanisms that control the atmospheric concentration of greenhouse gases and our ability to obtain an accurate budget of anthropogenic and natural sources and sinks. Although our knowledge of sources and sinks of N_2O in different environments is increasing, the global N_2O budget remains unbalanced (Smith, 1997).

Currently, ~25 % of the global N_2O emission is ascribed to uncultivated tropical soils, but the exact locations and controlling mechanisms are not clear (D'Amelio et al., 2009). Several studies indicate that tropical forest ecosystems simultaneously accumulate, recycle and loose nitrogen in far larger quantities than temperate ecosystems (Martinelli et al., 1999; Matson et al., 1999; Hedin et al., 2009). In spite of being rich in nitrogen, this recycling may include high N_2 fixation activity, a nitrogen paradox (Hedin et al., 2009) enabling tropical forest ecosystems to sustain large gaseous nitrogen losses (Houlton et al., 2006). Half of the world's wetland areas are found in the tropics (Neue et al., 1997) and tropical wetlands may thus have a major and yet unresolved role in the global budget of atmospheric N_2O.

In both natural and impacted environments, the same microbial processes, i.e., nitrification and denitrification are responsible for N_2O release to the atmosphere. Release of N_2O from nitrification in soil may be stimulated by low O_2 availability, under high soil moisture content (Bollmann and Conrad, 1998), and low pH (Mørkved et al., 2007). Denitrifying bacteria use nitrogen oxides as electron acceptors in their anaerobic respiratory pathway:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \quad (1)$$

BGD
8, 5991–6030, 2011

A useful conceptual model of such nitrogen turnover is the "Hole-in-Pipe" model (Firestone and Davidson, 1989) that describes the denitrification process as a leaky pipe with NO_3^- entering one end and N_2 exiting at the other end. The gaseous intermediates NO and N_2O can exit via holes along the pipe, where the size of these holes is controlled by environmental parameters such as O_2, NO_3^- and pH. Release of N_2O often occurs in environments with rapidly shifting O_2 availability, e.g. soils undergoing changing water content (Davidson, 1992). Both nitrification and denitrification can occur simultaneously in different soil microsites making it difficult to associate the N_2O emission with a specific process (Stevens et al., 1997). However, denitrification is often considered to be the main N_2O producing process in soils, although production by nitrification (Bateman and Baggs, 2005) and nitrifier denitrification (Wrage, 2004) may also be important.

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

In this study we present the first detailed study of the dynamics of the flux of N_2O, O_2, soil nitrogen pools and pH from Pantanal wetland soils. Three different sites were analysed over three seasons, and an additional 10 sites were screened for N_2O flux and soil characteristics including NO_3^-, PO_4^{3-} and pH.

BGD
8, 5991–6030, 2011

2 Materials and methods

2.1 Study sites

The Pantanal, a pristine tropical wetland in central South America, is shaped by the deposition of sediments into a tectonic depression in the Upper Paraguay River Basin, which formed during the last Andean compressive event (~2.5 Ma) (Assine and Soares, 2004). The Pantanal supports a lush vegetation of floating and emersed herbs and is dominated by an annual flooding and precipitation cycle, alternately inundating and draining ~140 000 km^2 of soil, thus representing the world's largest wetland (Fig. 1). 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 when the water floods the area annually, 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 floodplain soil as the water level decreases (Junk et al., 2006). The flood pulse thus leads to a regular set-back of community development maintaining the system in an immature, but highly productive stage (Junk and Wantzen, 2004). The annual flooding cycle is driven by a distinct dry/wet season in the ~500 000 km^2 watershed, but due to a north-south slope of only 2–3 cm km^{-1}, there is a lag period of up to several months between precipitation in the watershed and the flooding of the Pantanal (Junk et al., 2006). The Pantanal thus receives the highest precipitation in the non-flooded season (Fig. 2a).

During three field campaigns in 2008, 2009 and 2010, we investigated the nitrogen cycling and N_2O emission 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 (Fig. 1). A main site (site A: 19°01.16′ S; 57°32.99′ W) was chosen for monitoring in 2008 and 2009, while two additional sites where chosen for monitoring in 2008 (site B: 19°0.61′ S; 57°33.51′ W) and 2009 (site C: 18°59.28′ S; 57°25.17′ W).

BGD
8, 5991–6030, 2011

In addition, ten sites representing different soil and habitat types were screened in 2010 (Fig. 1). Measurements and sampling in 2008 and 2009 were conducted during the season of retreating water, while the screening in 2010 was conducted during the season of rising water level (Fig. 2a). A weather station was installed at site A in 2009 (Fig. 2a) and monitored the local precipitation and soil water content for 170 days of the drained period 2009–2010 (Fig. 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 site 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 flooded initially but then drained during the period. Three levels were selected along each transect: Level 1 at the lowest end, Level 2 in the centre and Level 3 at the highest end, thus representing 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 waterlogged during all measurements, while early flooding of site C in 2010 left it inaccessible.

2.2 Precipitation, soil moisture and soil temperature

The autonomous weather station installed at site A collected seasonal changes in precipitation, soil temperature, volumetric soil water content and height of the water level. Precipitation was measured using a "Tipping Bucket" rain gauge. Soil temperature was measured with Campbell Scientific 107 Temperature probes (Campbell Scientific Ltd., Loughborough, UK) installed in 5 depths; 2.5 cm, 5 cm, 10 cm, 30 cm and 60 cm below the soil surface, respectively. The volumetric soil water content was logged using soil moisture sensors (Theta Probes ML2x, Delta-T Devices Ltd, Cambridge, UK) installed in 4 depths; 5 cm, 10 cm, 30 cm 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. The height of the water level was monitored using a pressure transducer (Druck PDCR 1830 Series, Campbell Scientific Ltd., Loughborough, UK) installed at a known depth in a 2.5 m long perforated plastic tube. The Theta-probes were logged every 6 h, while all

BGD
8, 5991–6030, 2011

other sensors were logged at 10 min intervals (CR10X Datalogger, Campbell Scientific Ltd., Loughborough, UK).

2.3 Soil parameters and nutrient pools

2.3.1 KCl extracted soil NH_4^+

Soil cores (ø = 3 cm, $n = 3$) were collected at each level, cut in 2 cm thick slices and subsamples (sample size ~0.5 g) were stored at −20°C. Soil subsamples for NH_4^+ extraction were dried at 105 °C for 2 h. Subsequently, 1.5 ml 0.1M KCl and 1 % ZnCl was added and the samples were incubated on ice for 30 min to extract NH_4^+. After centrifugation, the supernatant was transferred to 1.5 ml tubes and stored at −20 °C until further analysis. The concentration of the extracted NH_4^+ in the sample was determined by fluorometric method according to Holmes (1999).

2.3.2 Soil NO_3^-, pH and PO_4^{3-}

Soil porewater was collected in situ at each level by inserting 0.2 µm Rhizon filters (Rhizosphere Research Products, Netherlands) at 1.5 cm, 3.5 cm and 6.5 cm below the soil surface through the side of whole soil cores (ø = 5.5 cm, length = 15 cm, $n = 9$). Samples of ~0.5 ml of porewater were extracted at each depth by suction with a 60 ml syringe. Extracted porewater samples were immediately transferred to 1.5 ml tubes. Additional water samples were taken from rivers and water bodies and filtered (0.2 µm filter, Sartorius AG, Germany). The samples were kept on ice for transport to the field lab. Porewater pH was measured with an Argus ISFET pH-meter equipped with a standard probe (RL Instruments Inc., USA) by placing 150 µl of porewater on the tip of the probe. The remaining sample was 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). Additionally, in 2009 soil and water pH

BGD
8, 5991–6030, 2011

was measured in situ along a transect at site A and C by inserting the probe directly into water or moist soil.

Data from each soil NO_3^- and NH_4^+ profile were averaged over the upper 6.5 cm soil column. Detailed depth profiles are available as Supplement.

During the third field campaign (2010), soil NO_3^- was measured in a field lab with a NO_3^- biosensor (Unisense A/S, Denmark), calibrated at 5 different concentrations of NO_3^-. Soil samples were collected and refrigerated up to 48 h until $NO_3^-$was extracted with 1 % (wt/wt) NaCl solution in the field lab. The reason for adding NaCl was primarily a higher stability of the NO_3^- biosensor reading in a saline solution. Soil pH values were determined by mixing fresh soil samples with demineralized water (ratio 1:2.5) and measuring with an Argus ISFET pH-meter equipped with a standard probe after 1 min of stirring.

Soil samples collected during the third field campaign (2010) were screened for PO_4^{3-} by the Olsen method (do Carmo Horta and Torrent, 2007).

2.3.3 Total carbon and nitrogen

Soil samples from the top 6.5 cm soil layer were collected in 2008 at site A and B at each level, dried in an oven (105°C for 2 h) and then weighed into ~100 mg subsamples. The subsamples were analysed by combustion and gas analysis on a C/N analyser (TrueSpec CN, Leco, USA).

2.3.4 Distribution of O_2 in soil

In 2009, the depth distribution of O_2 concentration was measured at site A and C at Level 2 (centre of transect) with custom built fibre-optic O_2 optodes (ø = 2 mm) (Rickelt et al., 2011). The optic fibres were installed in the soil at 13 fixed depths (2.5 cm, 5 cm, 10 cm, 15 cm and 20–100 cm with 10 cm interval). The optodes were connected to a 4-channel fibre optic O_2 detector system (OXY-4, Presens GmbH, Germany) at each visit to the sites (site A: $n = 22$, site C, $n = 9$).

BGD
8, 5991–6030, 2011

2.4 Microsensor measurements in soil slurry

Soil cores were collected from Level 3 at site A and B. As a proxy for a wetting event, slurries were made by mixing the upper 6 cm of a soil core with water (site A: 97 g soil wet weight plus 70 ml water, site B: 52 g soil wet weight plus 60 ml water). After mixing the slurry, O_2 and N_2O microsensors were inserted ~5 mm into the slurry measuring the concentration of O_2 and N_2O in the wetted soil continuously for 36 h. Oxygen microoptodes (detection limit ~1 µmol l^{-1}) were constructed according to Holst (2000) and connected to a fibre-optic O_2 meter (Microx TX3, Presens GmbH, Germany). Nitrous oxide microsensors (detection limit ~1 µmol l^{-1}) were constructed according to Andersen (2001) and connected to a picoammeter (PA2000, Unisense A/S, Denmark). The O_2 sensors were calibrated from measurements in an O_2 free solution (0.2 M ascorbate, pH 12), and in water equilibrated with atmospheric air. The N_2O sensors were calibrated from measurements in water saturated with 0 % and 1 % N_2O, respectively. Sensor signals were collected on a PC via an A/D converter using the SensorTrace Pro software (Unisense A/S, Denmark).

2.5 In situ flux measurements

At each site, the in situ flux of N_2O and CO_2 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 (ø = 24 cm, height = 20 cm) were inserted ~15 cm into the soil. In situ flux measurements were performed every 2–14 days by placing a lid on the tube and measuring the N_2O and CO_2 concentration for 30 min in each chamber with a photo-acoustic gas monitor (INNOVA 1312, Lumasense Inc., Denmark). Flux chambers were lined with reflective material on the outside and were shaded during measurements to prevent a rise in temperature. The flux in each chamber at $t = 0$ was estimated by fitting the partial pressure increase to a three-parameter exponential function ($y = y^0 + a(1 - e^{-bx})$) in Sigmaplot (Systat Software Inc., USA).

BGD
8, 5991–6030, 2011

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

2.6 Statistical tests

Correlation between in situ N_2O drained soil flux with corresponding NO_3^- and soil pH ($n = 43$) was tested by performing a multiple linear regression analysis (Sigmaplot, Systat Software Inc., USA). Furthermore, correlation between soil N_2O and CO_2 fluxes was tested with linear regression analysis (Sigmaplot, Systat Software Inc., USA).

3 Results

3.1 Precipitation, soil moisture and soil temperature

Precipitation resulted in an increase in soil moisture at site A. This was most pronounced near the soil surface and progressively less in the deeper soil layers (Fig. 2b). A decrease in soil moisture occurred after each rain event, most rapidly at the surface due to evaporation. The mean temperature of the soil during 170 days of drained period was 30.0 °C at the surface decreasing to 29.4 °C at 60 cm depth. Soil temperature plots are available in the Supplement (Fig. S1).

3.2 Soil parameters and nutrient pools

3.2.1 Soil NH_4^+, NO_3^-, pH and PO_4^{3-}

At both site A and B, Level 1 showed a higher soil NH_4^+ content (2.8 and 2.6 µmol NH_4^+ cm^{-3} soil) in 2008 than at Levels 2 and 3 (0.3–1.1 µmol NH_4^+ cm^{-3} soil), but the NH_4^+ content decreased over time at both sites to 1.1 and 0.8 µmol NH_4^+ cm^{-3} soil,

BGD
8, 5991–6030, 2011

respectively as the soil drained (Fig. 3a–b). The NO_3^- content at site A (Fig. 3e) increased over time at Level 1, from below detection limit to 0.1 µmol NO_3^- cm^{-3} soil, but decreased at Level 2 and 3 from 1.5 and 2.1 µmol NO_3^- cm^{-3} soil to 0.3 and 0.1 µmol NO_3^- cm^{-3} soil, respectively. The NO_3^- content at site B (Fig. 3f) increased over time at all levels from 0.01, 0.2 and 0.6 µmol NO_3^- cm^{-3} soil to 0.9, 0.5 and 1.1 µmol NO_3^- cm^{-3} soil, respectively.

In 2009, the soil NH_4^+ content at site A (Fig. 3c) was also higher at Level 1 (1.7 µmol NH_4^+ cm^{-3} soil), than at Level 2 and 3 (0.6–0.7 µmol NH_4^+ cm^{-3} soil). The NO_3^- content at site A (Fig. 3g) increased over time at Level 1 from below detection limit to 0.6 µmol NO_3^- cm^{-3} soil. At Level 2 and 3, the NO_3^- content fluctuated over time between 0.3 and 8.7 µmol NO_3^- cm^{-3} soil with very high NO_3^- content found at Level 3. At site C, the soil NH_4^+ content (Fig. 3d) varied little between levels ranging from 1.1 to 1.5 µmol NH_4^+ cm^{-3} soil, while the NO_3^- content (Fig. 3h) at all levels remained low over time, probably reflecting that this site was completely waterlogged throughout the field campaign.

In 2008, soil porewater pH at site A ranged from pH 5.37 to 6.87 at Level 1, from pH 4.51 to 6.15 at Level 2 and from pH 4.43 to 5.15 at Level 3 (Fig. 4a). At site B soil porewater pH ranged from pH 4.79 to 5.97 at Level 1, from pH 5.14 to 6.01 at Level 2 and from pH 4.85 to 5.16 at Level 3 (Fig. 4c). Porewater samples from waterlogged soil contained visible iron oxide precipitation after freezing indicating a high content of ferrous iron in the flooded and waterlogged soil.

In 2009, soil porewater pH at site A ranged from pH 5.05 to 8.31 at Level 1, from pH 4.51 to 5.22 at Level 2 and from 4.57 to 4.74 at Level 3 (Fig. 4b), while at site C soil porewater pH ranged from pH 5.00 to 5.97 at Level 1, from pH 4.38 to 5.81 at Level 2 and from pH 4.25 to 6.31 at Level 3 (Fig. 4d).

Detailed profiles of soil NH_4^+, soil NO_3^-, soil pH and NH_4^+ and NO_3^- in rivers and water bodies are available in the Supplement (Fig. S2–S6). The porewater NO_3^- content in

BGD
8, 5991–6030, 2011

soil samples from the screening of 10 sites ranged from 0.02 to 5.38 µmol NO_3^- cm^{-3} (Table 3) and porewater pH from pH 4.13 to 7.48. The 10 sites thus represented a wide range of soil environments in terms of NO_3^- availability and pH (Fig. S7). Soil PO_4^{3-} for the ten sites varied between 0.82 and 4.37 µmol P gdw^{-1} (Table 3).

3.2.2 Soil total nitrogen and carbon

At site A and B, the total pool of nitrogen in the soil was 100-1000 times larger than the pool of inorganic nitrogen (Table 1 and Fig. 3). At site A, total nitrogen and carbon ranged from 242 to 451 µmol N cm^{-3} soil and from 4054 to 6775 µmol C cm^{-3} soil, respectively, while the C:N ratio (mol:mol cm^{-3} soil) ranged from 15 to 21. At site B, total nitrogen and carbon ranged from 196 to 353 µmol N cm^{-3} soil and from 2740 to 3415 µmol C cm^{-3} soil, while the C:N ratio ranged from 10 to 21. Detailed profiles of total nitrogen and carbon are available in the Supplement (Fig. S8).

3.2.3 Depth distribution of O_2 in soil

At site A, O_2 penetrated to a depth of ~60 cm, but fluctuated throughout the field campaign in response to precipitation (Fig. 5a). At site C, O_2 was not detected in the soil except the first measurement (Fig. 5b) in consistency with the soil being waterlogged during the field campaign.

3.3 Microsensor measurements in slurries

After O_2 depletion, both slurries remained anoxic for the rest of the experiment (Fig. 6). From the onset of anoxia, N_2O could be measured in each slurry increasing from 0 Pa to 800 Pa and 1400 Pa in the site A and site B slurry, respectively. The concentration of N_2O in the slurries peaked after ~20 h (site A) and ~13 h (site B) and then decreased until N_2O was no more detectable after ~30 h (site A) and ~19 h (site B) reflecting a delayed consumption of the N_2O accumulated in the soil slurry. In the soil slurry from

BGD
8, 5991–6030, 2011

site B O_2 was consumed within <1 h while there was some O_2 intrusion for ~12 h in the soil slurry from site A.

3.4 In situ flux measurements of N_2O and CO_2

At site A in 2008, the in situ flux of N_2O ranged from 0.1 to 3.1 mmol N_2O m^{-2} d^{-1} and 0.1 to 1.0 mmol N_2O m^{-2} d^{-1} (Fig. 7a) at Level 1 and Level 2, respectively, and in 2009 from 0.03 to 1.1 mmol N_2O m^{-2} d^{-1} and 0.6 to 3.7 mmol N_2O m^{-2} d^{-1} (Fig. 7b). At site B, the in situ flux of N_2O (Fig. 7a) ranged from 0.4 to 1.5 mmol N_2O m^{-2} d^{-1} at Level 1 and from 0.4 to 2.4 mmol N_2O m^{-2} d^{-1} at Level 2. At site C, the in situ flux of N_2O (Fig. 7b) ranged from 0.02 to 0.1 mmol N_2O m^{-2} d^{-1} at Level 1 and from 0.03 to 0.1 mmol N_2O m^{-2} d^{-1} at Level 2. The total emission of N_2O from each level was integrated for the period and the mean daily flux based on this integration is shown in Table 2. However, the in situ flux of N_2O at site A, B and C fluctuated over time with some peak event measurements being several times higher than measurements before and after. These peak events were closely associated with sudden and heavy precipitation causing increasing soil water content in the upper 10 cm soil layer at site A (Fig. 8). The in situ flux of N_2O at 10 different sites determined during the screening field campaign ranged from 0.2 to 1.1 mmol N_2O m^{-2} d^{-1} (Table 3).

The in situ flux of CO_2 at site A in 2008 ranged from 0.1 to 0.2 mol CO_2 m^{-2} d^{-1} and 0.1 to 0.4 mol CO_2 m^{-2} d^{-1} at Level 1 and Level 2, respectively, and in 2009 from 0.1 to 0.4 mol CO_2 m^{-2} d^{-1} and from 0.2 to 0.5 mol CO_2 m^{-2} d^{-1} (Fig. S9). At site B the in situ flux of CO_2 ranged from 0.5 to 0.7 mol CO_2 m^{-2} d^{-1} at Level 1 and from 0.2 to 0.4 mol CO_2 m^{-2} d^{-1} at Level 2. At site C, the in situ flux of CO_2 ranged from 0.1 to 0.2 mol CO_2 m^{-2} d^{-1} at Level 1 and from 0.1 to 0.3 mol CO_2 m^{-2} d^{-1} at Level 2. The total emission of CO_2 from each level integrated for each field campaign and the mean daily flux is shown in Table 2.

BGD
8, 5991–6030, 2011

3.5 N_2O flux correlation with NO_3^- and pH

Multiple linear regression analysis of in situ N_2O flux from drained soil, NO_3^- and pH showed a weak correlation ($r^2 = 0.177$). There was a tendency ($P = 0.063$) for NO_3^- to be positively correlated with the in situ N_2O flux, while there was a weaker tendency ($P = 0.138$) for pH to be negatively correlated with the in situ N_2O flux. Data on other parameters such as soil temperature, soil moisture content, organic matter would be necessary to obtain a better correlation.

4 Discussion

Our study represents the first detailed study of the nitrogen cycle in Pantanal and revealed a large and hitherto unknown source of N_2O emission in the largest wetland of the world. In this discussion, we first relate our findings of soil porewater chemistry to other studies of tropical systems. Thereafter, we discuss our findings of wetted soil N_2O production and accumulation in relation to studies of parameters influencing the reduction of N_2O in soil. We relate our observed in situ N_2O fluxes to the soil porewater chemistry and weather data and compare these results with those found for other tropical systems. Finally we speculate on how such a large loss of nitrogen could be supported in a natural system like the Pantanal.

4.1 Dynamics of the soil nitrogen pools and pH

The fluctuating pool of inorganic nitrogen indicated dynamic shifts between ammonification, nitrification and denitrification in the soil. Inorganic nitrogen in the top 6.5 cm soil from all sites in the Pantanal varied with 7.1–92 µg NH_4^+-N g dw^{-1}, and 0.1–201 µg NO_3^--N g dw^{-1}. In a study of a range of tropical ecosystems by Vitousek and Matson (1988) similar NH_4^+ (0.7–102 µg NH_4^+-N g dw^{-1}) and NO_3^- (0.5–38 µg NO_3^--N g dw^{-1})

BGD
8, 5991–6030, 2011

contents were found, while Koschorreck (2005) found wetland soil in the Amazon to be rich in NH_4^+ (40–100 µg NH_4^+-N g dw^{-1}), but not NO_3^- (<0.6 µg NO_3^--N g dw^{-1}).

At the end of the flooded period at the long-term monitoring sites, we found that the wetland soil was rich in inorganic nitrogen in the form of NH_4^+, but contained no NO_3^-. After 1-2 weeks of draining the soil contained less NH_4^+, but NO_3^- was found (0.1–0.5 µmol NO_3^- cm^{-3} soil) presumably due to nitrification. Nitrification is dependent on O_2 and our microsensor data showed that drained soil was aerated to a depth of 20–50 cm interrupted only by short anoxic spells caused by precipitation and an increase in soil moisture. Soil that had drained for longer time was further enriched in NO_3^-.

Soil rich in NO_3^- was found both at the beginning and the end of the drained season indicating that NO_3^--rich soil and a dynamic inorganic nitrogen pool was prevalent throughout the drained season. The concentration of soil porewater NO_3^- in our study shifted dramatically from being undetectable in still flooded soil to >1000 µmol NO_3^- $liter^{-1}$ in drained soil with some samples reaching 10 000–30 000 µmol NO_3^- $liter^{-1}$ as the soil gradually dried up. In contrast, Kern (1996) found high concentrations of NH_4^+ (146–520 µmol NH_4^+ $liter^{-1}$), but little NO_3^- (0–5 µmol NO_3^- $liter^{-1}$) in the porewater of Amazonian soil and sediment.

Total nitrogen in the Pantanal soil ranged from 196–451 µmol N cm^{-3} soil, which is comparable to findings of Hedin (2003) in Hawaiian tropical soil (132-220 µmol N cm^{-3} soil). In contrast, Cenciani (2009) found <100 µmol N cm^{-3} soil in Amazonian pasture, fallow and forest soil in both dry and wet season, while Fernandes (1999) found even less (~25 µmol N cm^{-3} soil) in a non-flooding savannah soil in Pantanal.

As the wetland soil drained at the long-term monitoring sites, only sparse plant growth (typically *Panicum maximum*) was observed, and by the end of each field campaign most of the soil surface was still covered with decaying macrophytes (largely *E. crassipes*) left from the last flooding. In the absence of nitrogen uptake by macrophytes, the large pool of nitrogen in the decaying plant residues was largely available for nitrification and denitrification during the drained period. The water level during the

BGD
8, 5991–6030, 2011

2009 flood was, however, lower than average and areas that had not been flooded were covered by thick plant growth (e.g. *Costus spiralis, P. maximum*) by the end of the drained period. Another consequence of the periodic flooding and draining was that pH fluctuated from pH 7–7.5 in the water phase and flooded soil to pH 3.5–4.5 in the drained wetland soil. The pH fluctuations were probably dominated by oxidation and reduction of iron and nitrogen in the soil. The drained wetland soil was visibly mottled with precipitated ferri-hydroxides typically known from iron rich gleisoil's (Bridges, 1997), where ferric iron is reduced and re-oxidized with every flooding cycle. Freezing of extracted porewater from the flooded wetland soil also produced an orange-red precipitate indicating a high content of dissolved ferrous iron. Therefore, oxidation of dissolved ferrous iron was likely important in the acidification of the draining wetland soil, and nitrification in the drained soil also lowers pH.

4.2 N_2O production in soil slurry

High concentrations of N_2O accumulated in the soil slurries during the first hours due to constant denitrification and an inhibited or retarded reduction of N_2O to N_2. After 13-20 h, N_2O reduction was enhanced leading to rapid consumption of the remaining N_2O in the slurries. This was caused by depletion of NO_3^- and NO_2^- in the slurries. As these electron acceptors were no longer available, there was a rapid transition from accumulation of N_2O to consumption of N_2O. Ruser (2006) found that in an agricultural soil fertilized with high NO_3^- concentrations and a pH value of 6.1, the primary end product of denitrification upon wetting was N_2O. The soil at our long-term sites, although not fertilized, similarly contained high concentrations of NO_3^-. Furthermore, Koskinen (1982) found that N_2O was the primary end product of denitrification in soil at pH 4.6–5.4, similar to the range of pH measured in the drained Pantanal wetland soil, while N_2 was the predominant product in soil at pH >6.9.

It is well known that sudden onset of anoxia (Bollmann and Conrad, 1998), high concentrations of NO_3^- (Blackmer and Bremner, 1978), NO (Frunzke and Zumft, 1986), and low soil pH (Simek and Cooper, 2002) can increase the emission of N_2O. The

BGD
8, 5991–6030, 2011

four step reduction of NO_3^- to N_2 is governed by four specific enzymes. The gene of each enzyme has multiple transcriptional promoters that are activated by different environmental parameters, and each enzyme has different substrate requirements and inhibitors (Zumft, 1997). Thus, differences in production and consumption rates of each intermediate can lead to accumulation of these intermediates, which can be promote or inhibit steps further downstream (Frunzke and Zumft, 1986; Cervantes et al., 1998; Zhou et al., 2008).

4.3 In situ flux of N_2O and CO_2 at site A, B and C

The measured fluxes of N_2O from Pantanal wetland soil were generally high and comparable to fluxes reported from heavily fertilized forest or agricultural soils receiving regular inputs of nitrogen (Hall and Matson, 1999; Ruser et al., 2006). At the long-term monitoring sites, the daily N_2O emission averaged 0.69, 0.78 and 0.07 mmol $N_2O\,m^{-2}\,d^{-1}$ at site A, B and C, respectively. Single measurements of in situ activity at 10 sites during dry conditions averaged 0.39 mmol $N_2O\,m^{-2}\,d^{-1}$. In a cross-system comparison of tropical forest soils of different ages and soil types, the N_2O flux was found to be ~0.001 mmol $N_2O\,m^{-2}\,d^{-1}$ in Hawaii, 0.01–0.03 mmol $N_2O\,m^{-2}\,d^{-1}$ in Costa Rica and 0.006–0.01 mmol $N_2O\,m^{-2}\,d^{-1}$ in Amazonia (Matson and Vitousek, 1987). Thus, the average daily emission of N_2O from Pantanal wetland soil was 10–100 times higher than found in other pristine tropical systems.

Koehler (2009) found that fertilization of lowland and montane tropical forest soil in Panama induced a peak of N_2O emission of up to 0.36 and 0.25 mmol $N_2O\,m^{-2}\,d^{-1}$, respectively, increasing the transitory N_2O emission five-fold compared to non-fertilized control plots. Hall and Matson (1999) found that adding nitrogen fertilizer to phosphorus limited tropical forest soils increased the N_2O flux from <0.01 mmol $N_2O\,m^{-2}\,d^{-1}$ to 0.15 mmol $N_2O\,m^{-2}\,d^{-1}$ in first time fertilized soil and 0.13 mmol $N_2O\,m^{-2}\,d^{-1}$ in long term fertilized soil. No such effect was seen in tropical forest soil that was nitrogen limited (Hall and Matson, 1999).

BGD
8, 5991–6030, 2011

The measured fluxes of CO_2 from the wetland soils were much more constant than the fluxes of N_2O. Garcia-Montiel (2002) found a linear relationship between N_2O and CO_2 emissions from Amazonian forest soil and suggested that nitrification and denitrification were tightly coupled to labile soil carbon pools as estimated by CO_2 emissions. No relationship between N_2O and CO_2 emissions ($r^2 = 0.07$) was found in the Pantanal soil, but the N_2O fluxes were 10–100 times higher in the Pantanal soil than in the Amazonian forest soil, while the CO_2 fluxes were of comparable magnitude.

It is difficult to attribute the measured N_2O flux in the drained Pantanal soil to either nitrification or denitrification. At site A, O_2 penetrated 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 decrease in NH_4^+, increase in NO_3^- and low pH suggest that nitrifiers could contribute to the production of N_2O throughout the drained soil. Anaerobic microsites in the generally oxic layers may, however, cause locally intense denitrification (Smith, 1980). A relative increase of the anoxic volume in the soil, e.g. by higher soil temperature or increased soil moisture, would further favour denitrification.

The in situ flux of N_2O from the Pantanal wetland soil at sites A, B and C showed a high degree of temporal variability (Fig. 7), which was apparently closely coupled to precipitation events and variations in water content in the soil matrix (Fig. 8). Increased soil moisture did not result in a complete depletion of O_2 in the soil (Fig. 5a), but rather increased the anoxic soil volume where heterotrophic turnover of organic matter proceeded via denitrification. Such a mixed situation of water filled and gas filled pore space has previously been associated with high N_2O fluxes, even facilitating an efficient transport of N_2O to the atmosphere (Markfoged et al., 2011) Accumulation of N_2O in the Pantanal soil occurred when reduction of N_2O was delayed or inhibited due to high concentrations of NO_3^-, sudden onset of anoxia and low pH in the soil. Our multiple linear regression analysis further supported this tendency for NO_3^- to be positively correlated and the pH value to be negatively correlated with the N_2O flux.

BGD
8, 5991–6030, 2011

As precipitation was apparently the predominant parameter affecting N_2O emission, each flux measurement was attributed to either a drained soil flux or a peak event flux triggered by sudden wetting and anoxia. The soil slurry experiment (Fig. 6) and the in situ flux measurements (Fig. 7a–b) suggest that a typical peak event lasted ~1 day. Therefore, cumulative emissions were conservatively calculated assuming linear changes between subsequent measurements of drained soil fluxes, while rain wetted soil fluxes (peak events) were assumed to last 1 day. Accordingly, the mean daily flux of 0.56 mmol $N_2O\,m^{-2}\,d^{-1}$ at site A, B and C could be divided into contributions from drained soil or rain wetted soil (Fig. 9), with a mean of 0.43 mmol $N_2O\,m^{-2}\,d^{-1}$ coming from drained soil and 0.13 mmol $N_2O\,m^{-2}\,d^{-1}$ coming from rain wetted soil during the measurement period. Although rain wetted soil emitted in excess of 3 mmol $N_2O\,m^{-2}\,d^{-1}$, such peak events were relatively rare and short-lived and eventually contributed only ~20 % of the mean daily flux of N_2O measured during the long term studies at site A, B and C.

4.4 Screening of multiple sites and extrapolation to seasonally flooded soils

To the best of our knowledge, all screening measurements were performed on drained soil that had not been wetted for at least several days. Therefore, these measurements were considered as drained soil measurements and not as part of peak events. The mean daily flux of 0.39 mmol $N_2O\,m^{-2}\,d^{-1}$ from the screened wetland soils was strikingly similar to the mean daily flux contribution from drained soils at site A, B and C. Furthermore, seven of the ten screened sites had a soil pH < 6 and six sites contained more NO_3^- than was found to cause >70 % inhibition of N_2O reduction in soil with a pH < 6 (Blackmer and Bremner, 1978). This leads us to assume that the long term pattern of drained soil and rain wetted soil fluxes can be extrapolated to the screened sites and other seasonally flooded soils in the Pantanal.

Precipitation and soil moisture data from the weather station at site A showed that, during 170 days of the drained season 2009–2010, there were at least 6 events of

BGD
8, 5991–6030, 2011

heavy precipitation and increased soil moisture. As the drained soil at site A, B and C had fluctuating concentrations of NO_3^- and low pH at both the beginning and end of the drained seasons we assume that this was the case throughout the 170 days of weather monitoring. We can thus expect that precipitation triggered at least 6 peak events of N_2O emission from wetting of the drained soil during these 170 days (Fig. 8).

In total, 116 flux measurements (each representing a mean of 5 chambers) were performed during three field campaigns, of which we attributed 22 measurements to peak events, while 94 measurements were considered non-wetted drained soil fluxes. An average peak event lasted ~1 day with a mean flux of 1.54 mmol $N_2O\,m^{-2}\,d^{-1}$ (mean of 22 measured peak events). With 6 precipitation triggered peak events we thus assume a contribution of 9.2 mmol $N_2O\,m^{-2}$ from peak events to the total emission during 170 days. The flux from non-wetted drained soil (mean of 94 flux measurements) was 0.43 mmol $N_2O\,m^{-2}\,d^{-1}$. Therefore, we assume that the remaining 164 days contributed 70.0 mmol $N_2O\,m^{-2}$. The N_2O emission from the wetland soil during 170 days of the drained season was thus 79.3 mmol $N_2O\,m^{-2}$, with wetting events contributing ~12 % of the total N_2O emission. In contrast, wetting events in forest soil in Rondônia, Brazil contributed <2 % of the annual emissions (Garcia-Montiel et al., 2003).

Based on these calculations, the N_2O source strength of the seasonally flooded soils (140 000 km^2) in the Pantanal would be 0.30 Tg N yr^{-1} (0.27 Tg N yr^{-1} from dry, drained soil and 0.04 Tg N yr^{-1} from rain wetted soil) or 1.7 % of the global emission budget. This can be considered a conservative source strength estimate as it is based on just 170 days of the drained period excluding the remainder of the drained season as well as the flooded season. Climate change may alter the existing precipitation patterns which would likely influence the emission of N_2O from wetlands such as the Pantanal due to the tight coupling between rain showers and N_2O peak events.

BGD
8, 5991–6030, 2011

4.5 Nitrogen balance

Does the wetland soil contain enough nitrogen to support such a large loss through N_2O emission? If we assume that the N_2O emitting processes occurred in the upper 6.5 cm soil layer, a flux of 79.3 mmol N_2O m^{-2} represents a loss of 0.5–1.2 % of the total organic nitrogen present in that soil layer. However, we have not taken into account the nitrogen loss via NO and N_2. Several studies have found NO to be an important component in emission of nitrogenous gases from tropical soil, but it is not clear whether nitrification (Davidson et al., 1993) or denitrification (Davidson et al., 1991) is the most important source of NO. In a phosphorus limited tropical forest soil fertilized with nitrogen the emission ratio NO/(NO+N_2O) (mol N/mol N) was 0.55–0.92 (Hall and Matson, 1999), while in a review of nitrogen oxide emission from Indonesian, Hawaiian and Panamanian tropical forest soils the emission ratio NO/(NO+N_2O) (mol N/mol N) was 0.11–0.93 (Koehler et al., 2009). Furthermore, Garcia-Montiel (2003) found that NO constituted 42 % of the annually emitted nitrogen oxides from a Brazilian tropical forest soil.

A denitrification enzymatic assay described by Smith (1978) was performed on the Pantanal wetland soil from site A, B and C to estimate the relative product ratio of N_2O and N_2 (data not shown). The mean N_2O/(N_2O+N_2) ratios were 0.49 (site A, 2008), 0.56 (site A, 2009), 0.64 (site B) and 0.88 (site C). For the purpose of calculating the total loss of nitrogen from the system, a conservative estimate of the product ratios from the Pantanal wetland soil is 0.10 for NO/(NO+N_2O) and 0.80 for N_2O/(N_2O+N_2). The loss of nitrogen from the soil over 170 days of the drained period via emission of NO, N_2O and N_2 would thus be 15.9 mmol N m^{-2} (NO), 158.5 mmol N m^{-2} (N_2O) and 31.7 mmol N m^{-2} (N_2). The total loss would thus be 206.1 mmol N m^{-2}, representing a loss of 0.7–1.6 % of the total organic nitrogen present in the top 6.5 cm soil layer. While there is apparently enough N in the soil to sustain high N_2O emission, this means that the system needs an annual input of 206.1 mmol N m^{-2} or 28.9 kg N ha^{-1} to balance the loss. What is then the source for such nitrogen input?

BGD
8, 5991–6030, 2011

Floating mats of *E. crassipes* can have a net biomass production per season of 10 t dry weight ha^{-1} with an estimated nitrogen content of 50 kg N t dry weight^{-1} (de Neiff et al., 2006). Such a decaying mat would supply the soil with 500 kg N ha^{-1} y^{-1}, which eventually must be mineralized. The source of such nitrogen could be N_2 fixing bacteria associated with the aquatic macrophytes that dominate the flooded period. *E. crassipes* growing in nutrient poor water have previously been associated with N_2 fixing *Azotobacter* (Iswaran et al., 1973; Purchase, 1977). Carignan (1992) measured a total N_2 fixation of 2.88 mmol N m^{-2} d^{-1} in a floating *E. crassipes* mat and made a tentative seasonal nitrogen budget for the 7 month period of low nitrogen availability suggesting an input of 65–85 kg N ha^{-1} y^{-1} from this source, somewhat lower than the above-estimated 500 kg N ha^{-1} y^{-1}.

According to these estimates the loss of nitrogen via NO, N_2O and N_2 would be in the range of 6–34 % of the total input of nitrogen to the soil, which could thus easily support the nitrogen loss from the wetland soil estimated above.

Not all flooded areas are covered with decaying macrophytes by the end of each flooding cycle and we have not included other nitrogen exporting mechanisms such as leaching. Conversely, neither have we included nitrogen input from N_2 fixing phytoplankton communities in the Pantanal (Loverde-Oliveira and Huszar, 2007), nor nitrogen input from other N-fixers such as legumes that are abundant over vast areas of the Pantanal. While our calculations are not an attempt to present a closed nitrogen budget for the Pantanal, they do demonstrate that the amount of nitrogen needed to support the extremely large loss of nitrogen from the soil can be supplied by N_2 fixation associated with macrophyte biomass growth.

5 Conclusion

The six major tropical freshwater wetlands in South America are estimated to cover an area of 500 000 km^2 that is flooded annually (Hamilton et al., 2002), while globally tropical wetlands are estimated to cover 5 000 000 km^2 (Neue et al., 1997). It is currently

BGD
8, 5991–6030, 2011

unknown to what extent the Pantanal wetland system with its dynamic cycling of nitrogen can be compared to other wetlands. The global N_2O budget is not balanced, which has been attributed to either a major unknown source or uncertainties in the quantification of one or more known sources (Smith, 1997). Until now, the contribution of N_2O from tropical freshwater wetlands has largely been considered negligible. However, our study underscores the direct and indirect importance of flooding and precipitation patterns in tropical watersheds and wetlands, where nitrification in combination with incomplete denitrification due to sudden natural wetting events can cause very high N_2O emission from the soil. This first detailed study of the nitrogen cycling and emission of N_2O from the world's largest wetland thus emphasizes the current lack of knowledge about nitrogen cycling in natural and pristine systems, and about how such systems may alter in response to a changing global climate.

Supplementary material related to this article is available online at: http://www.biogeosciences-discuss.net/8/5991/2011/ bgd-8-5991-2011-supplement.pdf.

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BGD
8, 5991–6030, 2011

Table 1. Soil Total Carbon and Nitrogen in the top 6.5 cm of soil. Mean ± S.E. ($n = 9$).

Site	Level	Nitrogen (μmol N cm^{-3} soil)	Carbon (μmol C cm^{-3} soil)	C:N (mol:mol)
A	1	451 ± 47	6775 ± 684	15.6 ± 1.7
	2	418 ± 33	6115 ± 328	15.0 ± 0.7
	3	241 ± 49	4054 ± 714	21.0 ± 4.3
B	1	259 ± 30	2740 ± 254	10.9 ± 0.9
	2	353 ± 53	2905 ± 159	9.6 ± 1.3
	3	196 ± 31	3415 ± 373	20.5 ± 4.0

BGD
8, 5991–6030, 2011

Table 2. Integrated flux for field campaign and mean daily flux of N_2O and CO_2 at each level. Mean ± S.E. ($n = 5$).

Site	Level	days	Integrated N_2O flux (mmol N_2O m^{-2})	Mean N_2O flux (mmol N_2O m^{-2} d^{-1})	Integrated CO_2 flux (mol CO_2 m^{-2})	Mean CO_2 flux (mol CO_2 m^{-2} d^{-1})
A	1[a]	42	22.0 ± 5.6	0.52 ± 0.13	8.1 ± 1.5	0.19 ± 0.04
	2[a]	42	14.0 ± 3.4	0.33 ± 0.08	8.3 ± 0.7	0.20 ± 0.02
	1[b]	23	12.9 ± 1.7	0.55 ± 0.07	4.6 ± 0.4	0.20 ± 0.02
	2[b]	23	32.0 ± 5.0	1.37 ± 0.21	5.8 ± 0.5	0.25 ± 0.02
B	1	29	17.8 ± 1.8	0.64 ± 0.06	17.6 ± 0.6	0.61 ± 0.02
	2	29	26.7 ± 4.0	0.92 ± 0.14	10.4 ± 0.5	0.36 ± 0.02
C	1	23	1.0 ± 0.1	0.04 ± 0.00	1.9 ± 0.2	0.09 ± 0.01
	2	23	2.1 ± 0.4	0.09 ± 0.02	3.9 ± 0.2	0.17 ± 0.01

N.B. site C was completely waterlogged during the entire field campaign.
[a] 2008, [b] 2009

BGD
8, 5991–6030, 2011

Table 3. Screening of in situ N_2O and CO_2 flux, pH and porewater NO_3^- and PO_4^{3-} in 2010 at 10 sites. Mean ± S.E. ($n = 5$).

Location	Position (WGS 84)	Level	in situ N_2O flux (mmol $N_2O\,m^{-2}\,d^{-1}$)	In situ CO_2 flux (mol $CO_2\,m^{-2}\,d^{-1}$)	pH (µmol $NO_3^-\,cm^{-3}$ soil)	NO_3^- (µmol gdw^{-1})	PO_4^{3-}
Site A	19°01.16′ S 57°32.99′ W	1	0.41 ± 0.05	1.32 ± 0.13	5.32	0.19	2.66
		2	0.31 ± 0.02	0.98 ± 0.05	4.96	0.91	1.51
		3	0.61 ± 0.29	0.61 ± 0.11	4.43	2.69	2.57
Site B	19°00.61′ S 57°33.51′ W	1	0.21 ± 0.03	0.49 ± 0.04	4.37	0.16	4.29
		2	0.48 ± 0.12	0.60 ± 0.07	4.13	3.45	3.24
Site D	18°43.56′ S 57°32.12′ W	–	0.27 ± 0.02	0.66 ± 0.11	4.50 ± 0.12	0.07 ± 0.04	1.87
Site E	18°44.08′ S 57°32.38′ W	1	0.77 ± 0.18	0.53 ± 0.03	5.08 ± 0.11	0.55 ± 0.08	2.73
		2	1.08 ± 0.17	1.14 ± 0.12	4.75 ± 0.06	5.38 ± 2.94	
Site F	19°04.26′ S 57°20.08′ W	1	0.22 ± 0.01	0.42 ± 0.10	6.90 ± 0.06	0.03 ± 0.00	2.10
		2	0.41 ± 0.02	0.34 ± 0.01	6.25 ± 0.03	0.05 ± 0.03	
Site G	19°06.03′ S 57°16.85′ W	–	0.18 ± 0.04	0.25 ± 0.11	5.33 ± 0.06	0.79 ± 0.12	1.25
Site H	19°15.15′ S 57°04.83′ W	–	0.20 ± 0.02	0.70 ± 0.14	5.98 ± 0.10	0.02 ± 0.01	0.82
Site I	19°15.03′ S 57°04.04′ W	–	0.45 ± 0.09	1.18 ± 0.42	5.18 ± 0.08	0.07 ± 0.02	3.62
Site J	19°18.53′ S 57°03.29′ W	–	0.09 ± 0.01	0.31 ± 0.06	7.48 ± 0.08	0.29 ± 0.11	1.29
Site K	19°34.50′ S 57°01.22′ W	1	0.30 ± 0.01	0.87 ± 0.02	–	0.06	1.01
		2	0.30 ± 0.01	1.01 ± 0.04	–	0.31	

BGD
8, 5991–6030, 2011

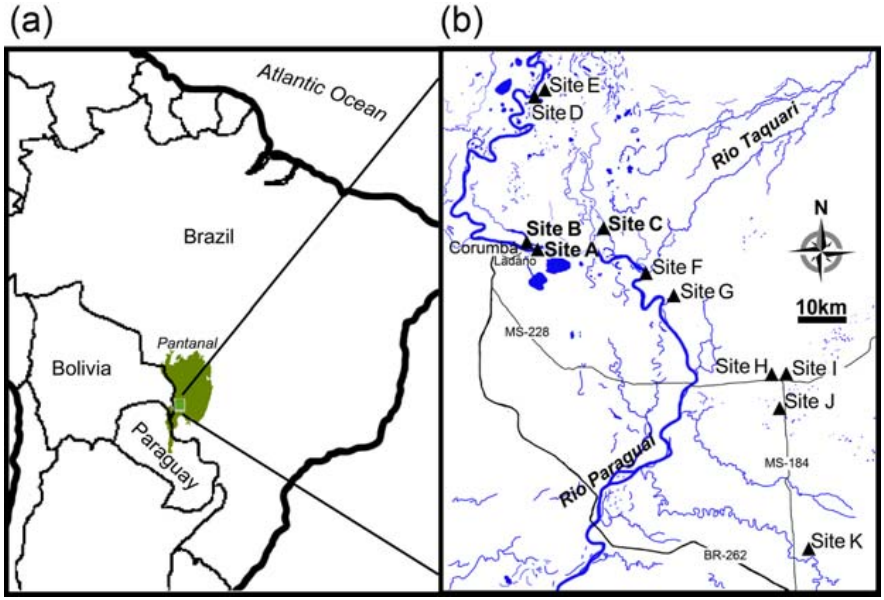

Fig. 1. Location of Pantanal and map insert of the study sites. **(a)** Pantanal is shown as green area in the centre of South America. **(b)** The course of Rio Paraguai flowing from north to south in the Pantanal and locations of the study sites.

BGD
8, 5991–6030, 2011

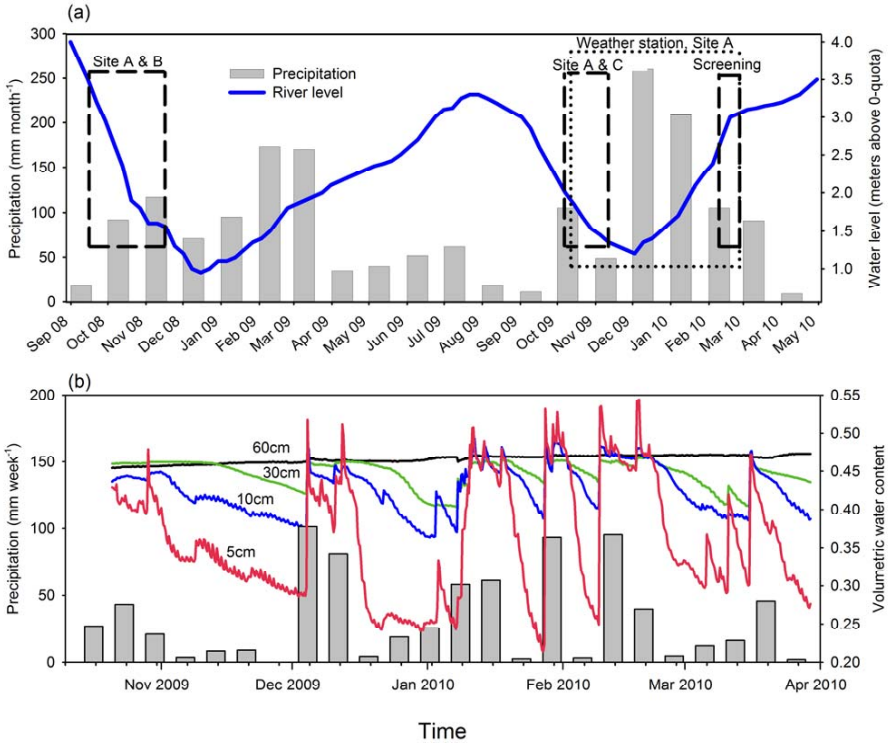

Fig. 2. Precipitation and Rio Paraguai water level in 2008–2010. **(a)** Monthly precipitation at Corumbá Airport is shown as grey bars (source: www.inmet.gov.br). Daily water level of Rio Paraguai at Ladário Naval Station is shown as blue line (source: www.cprm.gov.br). The period of each field campaign in 2008, 2009 and 2010 is indicated with black dashed boxes, while the dotted box indicates the period of local weather station monitoring at site A. **(b)** Weather station data of precipitation and soil water content in 2009–2010 at site A. Weekly precipitation is indicated as grey bars. Soil water content at different depths is shown as lines; 5 cm (red), 10 cm (blue), 30 cm (green) and 60 cm (black).

BGD
8, 5991–6030, 2011

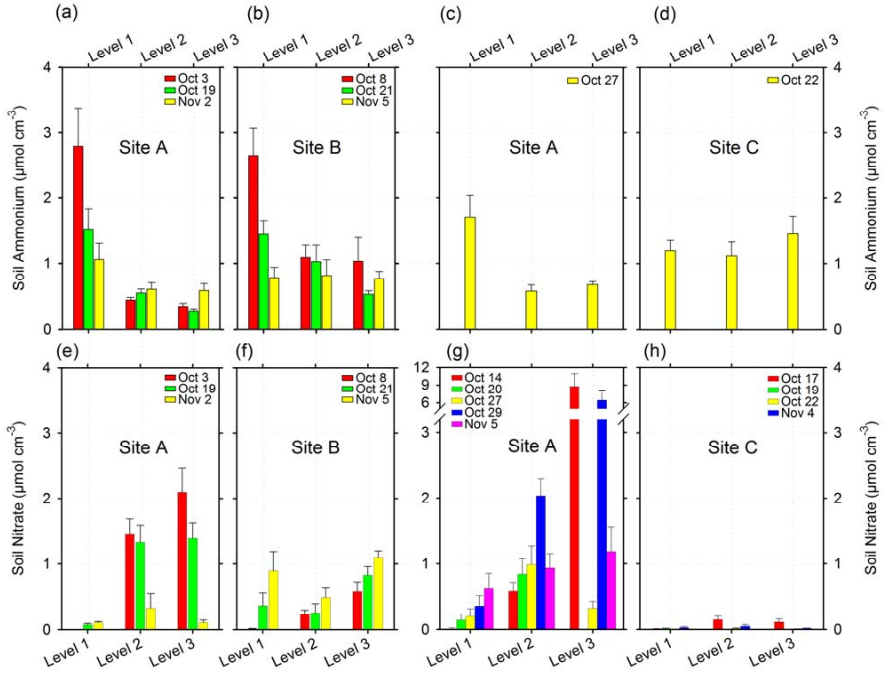

Fig. 3. Changes over time in soil NH_4^+ and porewater NO_3^- per soil volume (µmol N cm^{-3} soil) measured along transects at site A, B and C. **(a, b, c, d)** Soil NH_4^+. **(e, f, g, h)** Porewater NO_3^-. Mean ± S.E. ($n = 9$). Please note discontinuous scale on **(g)**.

BGD
8, 5991–6030, 2011

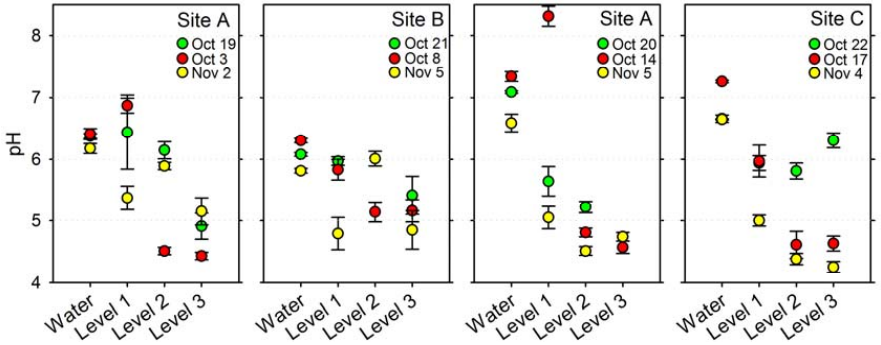

Fig. 4. Porewater pH changes over time at site A, B and C measured in water, water logged soil (Level 1) to drained soil (Level 3) in 2008 and 2009. Mean ± S.E. ($n = 9$).

BGD
8, 5991–6030, 2011

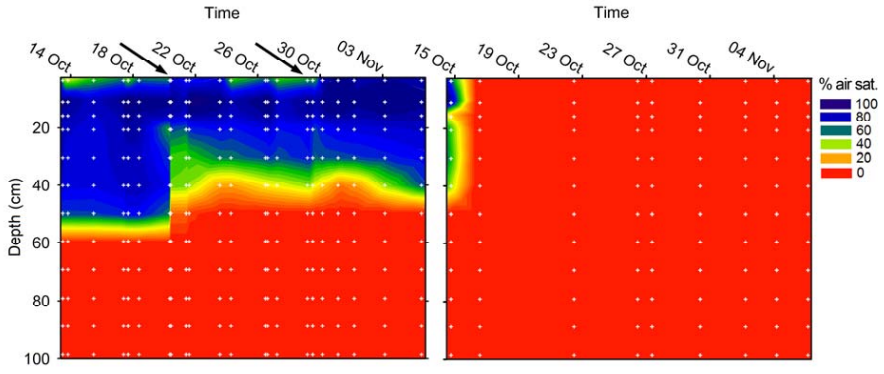

Fig. 5. Soil O_2 concentration at site A **(a)** and site C **(b)** during field work in 2009. The O_2 concentration maps were compiled from individual oxygen profiles ($n = 22$ at site A, $n = 9$ at site C) composed of measurements at 12 depth (white dots). The black arrows on the time scale in subfig **(a)** indicate heavy precipitation events as measured by the weather station.

BGD
8, 5991–6030, 2011

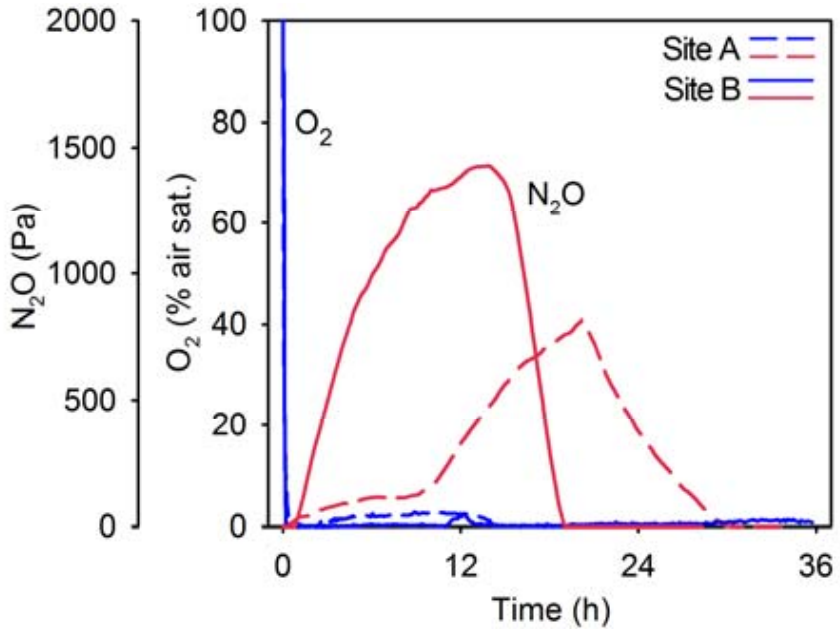

Fig. 6. Production of N_2O in soil slurries from Level 3 at site A (short dash line) and site B (solid line). Oxygen in the slurry is shown in blue and N_2O in red.

BGD
8, 5991–6030, 2011

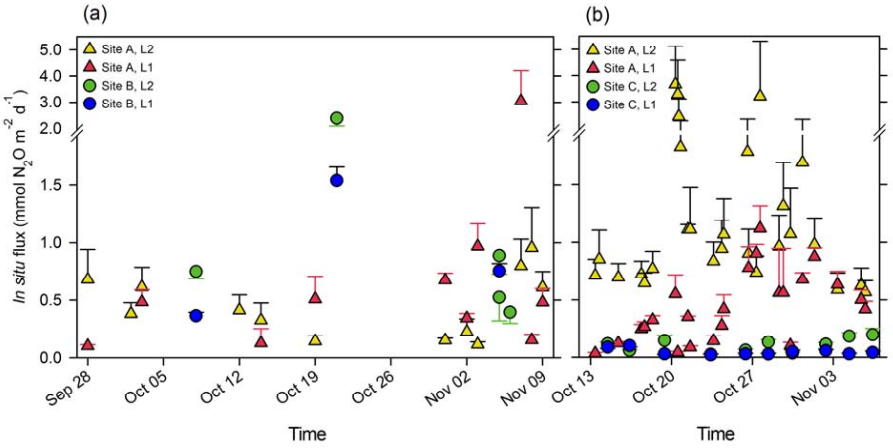

Fig. 7. in situ flux of N_2O from site A and B (2008) **(a)**, and site A and C (2009) **(b)**. Flux rates at site A are from Level 1 (▲) and Level 2 (▲), and at site B and C from Level 1 (●) and Level 2 (●). Symbols indicate mean ± S.E. ($n = 5$). Note scale break on y-axis.

BGD
8, 5991–6030, 2011

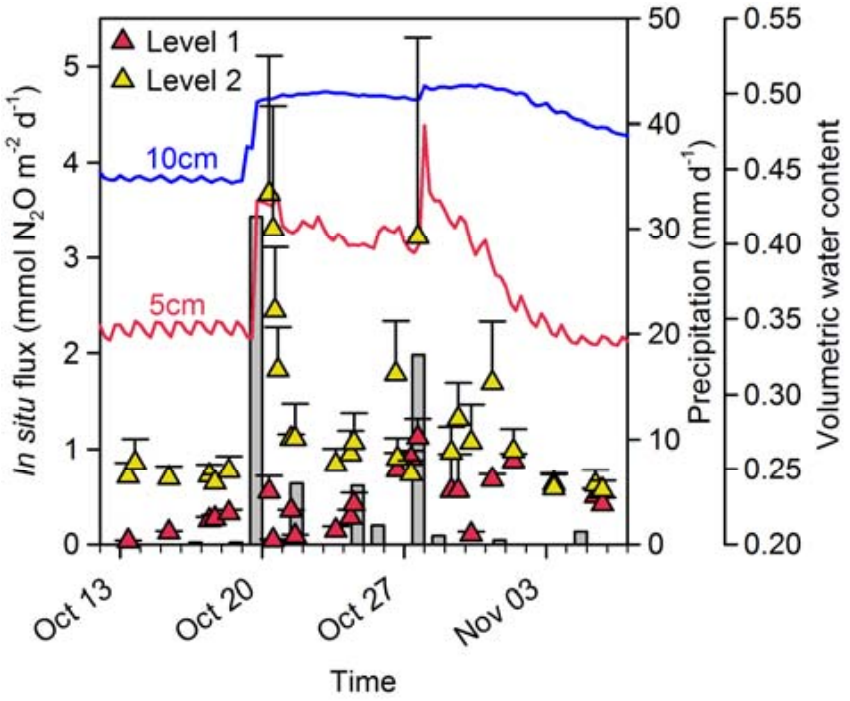

Fig. 8. in situ N_2O flux, precipitation and soil water content at site A in 2009. Heavy precipitation (grey bars: daily precipitation) caused a sudden increase in soil water content (5 cm: red line, 10 cm: blue line) in the top 10 cm soil layer and triggered an increase in the in situ flux of N_2O at Level 1 (▲) and Level 2 (▲).

BGD
8, 5991–6030, 2011

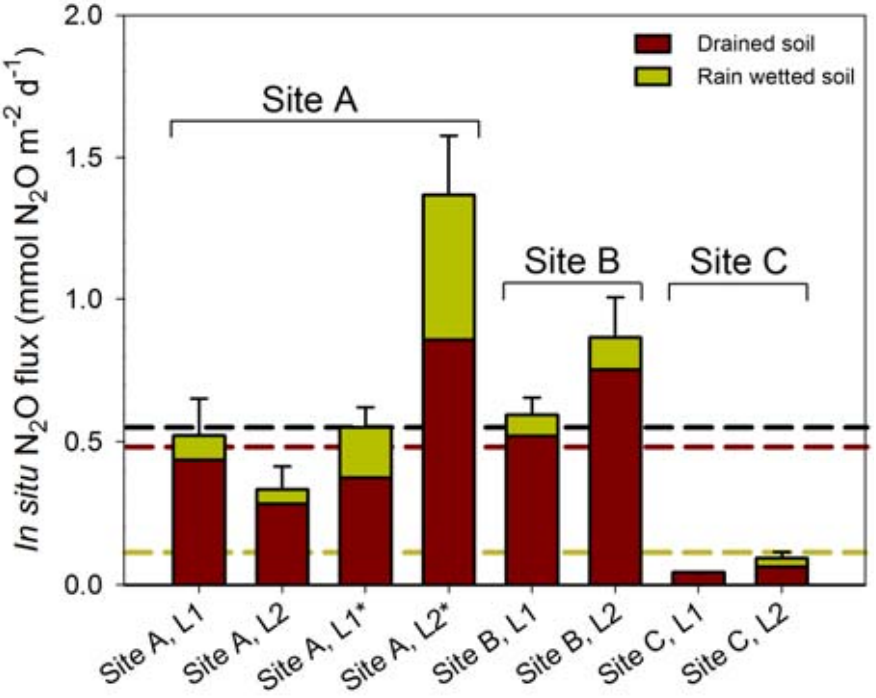

Fig. 9. The annual contribution of N_2O from drained soil and rain wetted soil to the mean in situ flux of N_2O at site A, B and C. The lines indicate total mean flux (black), mean flux from drained soil (red), and mean flux from rain wetted soil (yellow). Mean ± S.E. ($n = 3 – 27$).

BGD
8, 5991–6030, 2011