

Reply to comments of Anonymous Referee #2

RESPONSE: We thank referee #2 for evaluating and reviewing our manuscript. We are especially grateful for the many constructive comments and suggestions and we have to the best of our abilities responded to them. We address the referee's comments in the following point by point response.

GENERAL COMMENTS

In general, the study reports very interesting data, especially very high N₂O emission rates, from a poorly studied, but ecologically and biogeochemically very significant part of the world, i.e. the tropical wetlands. Some parameters that are relevant for N₂O emissions, such as inorganic nitrogen content of the soil, pH and O₂ content, have been determined. However, this study suffers from several weaknesses. First of all, "long-term" measurement (if you want to call three to seven weeks long-term) have been conducted only at two sites each in 2008 and 2009, one of which (site A) was the only one that was sampled in both years, and the second site (site B in 2008 and site C in 2009) were only sampled within that one year. Moreover, site C was completely water-logged during the whole field campaign 2009, therefore the conditions there were not comparable to the other two sites and the other year. The "screening" in 2010 was only a short-term campaign.

RESPONSE: We thank the referee for commenting on the relevance of our findings from the Pantanal. Furthermore, after collegial discussions we fully agree that the use of 'long-term' is indeed misleading to the general audience. To make it clear that this is a first study of the dynamics of soil nitrogen pools and the emission of N₂O from the Pantanal we have exchanged 'long term sites' with 'sites of repeated sampling'. We also agree that due to water-logging in 2009, site C was not directly comparable to the other drained sites. However, in the beginning of the field campaign in 2008 a quick screening of site C showed high soil nitrate. This has now been mentioned in the MS. What we have termed a 'screening' in 2010 was an attempt at obtaining data on primarily soil nitrate, soil pH and soil N₂O flux from as many sites as possible with single measurements and not repeated sampling. If the term 'screening' gives other associations we are of course very willing to exchange it with a more adequate term.

The other obstacle is that not all parameters were determined at all sampling locations: soil pore water pH, and ammonium, nitrate and phosphate concentrations were determined at all sites at all three levels; total soil C and N content were determined at site A and B for all three levels, but only in 2008; the O₂ concentration profile was measured in 2009 at site A and C, but only at level 2; N₂O and CO₂ fluxes were quantified at sites A, B and C, but only at levels 1 and 2 etc. No flux data for inundated areas are presented, although they represent a major area of the Pantanal during the flooding period. Especially in view of the fact that the authors try to come up with an N₂O flux estimate for the whole Pantanal, this is a major drawback.

RESPONSE: We agree with the referee that it is a drawback that not all parameters were determined at all sampling locations. However, for the major part of the field campaign in 2008 and 2009 sampling was accomplished by only one person. Furthermore, the logistical challenges of working in the field in the Pantanal forced us to focus on a few key parameters that are important to adequately describe the dynamics of the soil nitrogen pools. We believe that this is also at the very core of the concerns of the referee; this study does not attempt to describe the complete cycling

of nitrogen in the Pantanal and does not cover the entire year. This is, however, not our goal. We wish to report the finding of an unexpected emission of N₂O from an undisturbed tropical wetland. Within the time frame of our project, we have tried to sample several sites as effectively as possible covering both temporal and spatial variability of the N₂O flux. Attempting to explain this unexpected N₂O emission, we have focused on determining a few key parameters (ammonium, nitrate and pH), and invested less energy into determining phosphate and total soil nitrogen and carbon. Acknowledging the lack of studies of nitrogen cycling in tropical freshwater wetlands in combination with the unexpected finding of significant N₂O emission we therefore strongly believe that the present study has merit.

The same is true for the fact that the authors did not measure N₂O (and CO₂) fluxes at level 3, which might also represent a significant part of the total area of the Pantanal. With respect to methodology the significance of the soil slurry incubation experiments was not clear, as the conditions applied do not correspond to natural conditions, namely the complete destruction of the soil structure and hence the total alteration of nutrient availability and diffusivities of dissolved substances and gases. Therefore, the explanatory power of these experiments can be questioned. The mere fact that N₂O is first formed under anaerobic conditions and then further reduced to N₂ with depleting nitrate pools is not new. Also the estimation of NO and N₂ losses is founded on a very weak basis.

RESPONSE: We agree with the referee that not measuring N₂O flux at Level 3 may have biased our areal estimates of N₂O emission (see our response to specific comment below). We also agree that the wetted mixed soil experiment does not reflect natural conditions (see specific comment below). However, such experiments have merit if they can improve our understanding of the mechanisms behind observations in the field. In this case, N₂O peak events associated with precipitation in the field were observed and continuously measuring the O₂ and N₂O concentration in the wetted mixed soil indicates that such peak events can be explained by the soil becoming suddenly anoxic and N₂O being accumulated by denitrification. It is true that N₂O formation under anoxic conditions is not new (and we do not claim this), however, the response time and amount of accumulated N₂O in our wetted soil was considerable under the given conditions and thus offer an explanation to size and duration of the observed in situ peak events.

In general the paper is quite well written, although especially the Materials and Methods section is sometimes imprecise.

RESPONSE: We can only agree, but hope that our revision, based on the very thorough reviews from the referees, have heightened the quality of the Materials and Methods section. Again, we are very grateful for the many suggestions and comments.

The number of figures should be perhaps reduced.

RESPONSE: We have reduced the number of figures in the revised MS according to the referee's comment. In the main text MS, the original figure 3, containing eight panels, has been split into two figures, each containing four panels. Figure 9 from the original MS has been removed in the revised MS. In the supplementary material nine figures have been reduced to four.

Although the general information, i.e. repeatedly very high N₂O emissions from a natural, unfertilized wetland, which are as high as the highest N₂O emission rates from fertilized

agricultural land, is very interesting and highly valuable, I have doubts whether this paper reaches the high standards a scientific journal like Biogeosciences should try to maintain. This is, inter alia, due to the poor temporal coverage of measurements, the lack of sufficient replication (only two sites in 2008 and 2009 with one transect each, and ten “screening” sites in 2010), that was aggravated by the fact that the measurement in the different years were performed in different seasons (with falling and rising water level, respectively), and the fact that there was no clear concept visible in determining the different parameters, i.e. several parameters were not determined in all years or at all three sites or at all three locations of the transects. If the authors could come up with more data for more sites and/or more locations at each site, I could recommend publication of this paper.

RESPONSE: We thank the referee yet again for commenting on the value of our findings. We believe that the doubts and concerns of the referee regarding poor temporal and spatial coverage is due to a combination of our incorrect use of term ‘long-term’ and the fact that we speculate on the N₂O source strength of the Pantanal. We agree that our extrapolation is based on a small data set and it is not our intention to mislead readers into thinking that the suggested N₂O source strength should stand untested. In response to the referee’s comments we have tried to clarify that our study is a report on an extraordinary finding of significant N₂O emission from undisturbed tropical wetland soil that we believe has the interest of the readership of Biogeosciences. We have attempted to validate our findings by covering both the spatial and temporal variability of the N₂O emission at several sites. In addition, we have determined key parameters to explain the cause of the observed N₂O emission. We believe that determining e.g. soil nitrate and N₂O flux at both the beginning of the drained season (2008 and 2009) and end of the drained season (2010) allows us to suggest a seasonal extrapolation of our N₂O flux measurements. We have further attempted to explain the observed dynamics of the soil nitrogen pools and the N₂O emission by suggesting a source of the nitrogen. We have placed the N₂O source strength calculations in a last, separate section of the discussion to make it absolutely clear that these calculations are speculative. However, we still believe that such speculations have merit, especially in the light of emerging new information indicating a much larger N₂O source strength of South America, recently published by Kort et al. (2011, DOI: 10.1029/2011gl047612) – cited in the revised manuscript.

SPECIFIC COMMENTS

p. 5994, l. 1-3: The hole-in-the-pipe model also refers to nitrification as one of the two sources of nitrous oxide.

RESPONSE: Correct, thank you! Changed as requested.

p. 5995, l. 23: It would be good to learn about the period of each field campaign here to be able to put the calculation of cumulative fluxes and other generalizations into the right perspective.

RESPONSE: This information has been added as requested.

p. 5995, 26-28: Unfortunately only one replicate was sampled in the years 2008 and 2009. Given the vast extension of the Pantanal, this appears to be too little replication for a representative description of the study region.

RESPONSE: We understand your concerns regarding the number of studied sites and the length of the field campaigns. However work in this area is logistically very difficult and our goal with this first study is to report the unexpected high N₂O emissions found in this remote and logistically challenging area (see Response to General comment).

p. 5996, l. 1-2: What is the difference between “field campaign” (2008 and 2009) and “screening” (2010). Was it only the difference in length of the measurement periods, or were different parameters measured?

RESPONSE: During the field campaigns in 2008 and 2009 we monitored various parameters on both a temporal and spatial scale. The aim of the screening in 2010 was to sample as many sites as possible to get some idea of the spatial variation between sites.

p. 5996, l. 2-4: This is the major drawback of this study: The data from the sampling in 2008 and 2009 are not comparable to the 2010 data, as in 2008/2009 sampling was done while the water was retreating, i.e. after a longer period of inundation, when soil nitrate pools are known to be exhausted due to continued denitrification activity, whereas in 2010 screening was done with rising water, i.e. when pulse emissions of NO and N₂O are to be expected after N accumulation during the drained phase.

RESPONSE: We disagree and actually consider this a strength of the study, as we cover both the beginning and the end of the low water period. During the 2008 and 2009 field campaigns the soil nitrate pools were building up in soil that was going through draining after a longer period of inundation. At the same time there was an increasing flux of N₂O from the draining soil. After having completed the 2008 and 2009 field campaigns we did not know whether the soil nitrate pools would become exhausted before the next flooding (due to e.g. plant growth during the low water period or denitrification activity following precipitation events) or would accumulate during the low water period. We therefore had no idea, whether the period of relatively high N₂O fluxes was limited to a few months after draining or could last throughout the low water period until re-flooding. The 2010 field campaign showed that by the end of the low water period the soil still had a relatively high content of nitrate and still emitted N₂O in comparable amounts to the 2008 and 2009 measurements, suggesting that the combined nitrification and denitrification (and emission of N₂O) could have continued all through the low water period. We never measured the in situ production of N₂O in recently flooded soil due to the technical challenges involved in this and therefore have not included any potential pulse emission from nitrate-containing flooded soil in our calculations. However, we agree that any nitrate in recently flooded soil would most likely give rise to a pulse emission of NO and N₂O and this would be interesting to investigate.

p. 5996, l. 18: It is not clear which part of the transects the location of the weather station is representative for, especially with respect to soil temperature and soil moisture. Is it more the upper, the middle or the lower part?

RESPONSE: This information has been added to the MS as requested. The weather station was placed at Level 2, the middle of the transect.

p. 5997, l. 5: At which sites and down to which depth were the soil cores sampled?

RESPONSE: This information has been added to the MS as requested. The soil cores were sampled at site A, site B and site C. At each site cores were sampled at Level 1, Level 2 and Level 3 to a depth of ~10 cm. Each core was cut into 2 cm slices and analysed down to a depth 6.5 cm.

p. 5997, l. 13-15: At which sites was soil pore water sampled? Furthermore, it is not fully clear to me how the Rhizon filters were inserted: With the soil core left in its place in the soil just with digging away the surrounding soil? Taking out the soil core and inserting the filters? Placing the soil core back to its original location and then sampling the pore water? Or leaving the soil core out of the soil during pore water sampling?

RESPONSE: This information has been to the MS added as requested. We have revised the description of the methods and procedures and hopefully it is more clear now. Porewater was sampled at site A, site B and site C. At each site cores were sampled at Level 1, Level 2 and Level 3. The actual sampling was done by extracting a whole soil core and inserting Rhizon filters into the side of the core. The porewater was then immediately extracted from the core with the core out of the soil.

p. 5998, l. 3-4: How did you calculate the average? Just the arithmetic mean? Or did you use a weighted average, i.e. attributing a certain part of the soil core to each of the three values?

RESPONSE: This information has been added as requested. The average was indeed calculated as the arithmetic mean.

p. 5999, l. 2f.: When were the soil cores sampled? Which diameter and depth? Moreover, the significance of these experiments is not clear to me. According to my understanding, converting soil samples to slurry does not reflect the natural conditions of inundation, where the soil stratification stays more or less intact, at least at the low water flow velocities in the Pantanal. This has implications for nutrient availability as well as diffusion coefficients for nutrients and oxygen. The authors should comment on this.

RESPONSE: This information has been added as requested. The soil cores were sampled at Level 3 at site A and site B. The diameter of the cores were 5.5 cm and the depth was 6 cm. Please notice that we have changed the coice of words from 'soil slurry' to 'wetted mixed soil', since we were under the impression that readers imagined a much thinner 'solution'. As described in the MS we mixed the upper 6 cm of a soil core and added approx. equal amounts of water and soil (weight weight⁻¹).

We fully agree that this type of laboratory wetted soil does not reflect the natural conditions of inundation. The purpose of the experiments were to investigate the response of the soil to a complete wetting (e.g. due to heavy precipitation) and measure the concentration of the produced N₂O in such a 'simulated peak event'. As such, the purpose was to understand the process behind the N₂O production in the soil when wetted, i.e. how fast does the soil begin to produce N₂O, when does it peak and when is all the produced N₂O gone.

p. 5999, l. 17-18: Why were N₂O and CO₂ fluxes not measured at Level 3? Depending on the areal representativeness of each level chosen in this field study (highest,

intermediate, lowest soil moisture) for the whole Pantanal you could have missed an important part of the N₂O source, or the areal estimate could have been also much lower due to lower N₂O emissions at Level 3.

RESPONSE: For logistical reasons, we were limited to 10 flux chambers per site. At the first measurements made in 2008 we divided the 10 chambers between all three levels with three chambers at each level. However, the fluxes measured at Level 2 and 3 were very similar at both site A and B while the measured fluxes at Level 1 at both site A and B were significantly lower. We agree that measuring only at Level 1 and Level 2 and not Level 3 may have biased our areal estimate, but as we expected Level 1 to undergo larger changes in soil moisture during the field campaign (it had been recently flooded and was still completely water-logged when we arrived) we decided to rearrange the chambers to better cover the heterogeneity of Level 1 and 2.

p. 6002, l. 3-4: You do not mention any phosphate data for sites A, B and C for the years 2008 and 2009 in the text.

RESPONSE: Phosphate was only sampled during the 2010 screening of several sites.

p. 6002, l. 6f.: It is unfortunate that there are no data for total C and N neither for site C nor for the ten screening sites in 2010.

RESPONSE: We fully agree. Such data would have been desirable but were not possible to sample due to logistic problems in the field..

p. 6003, l. 18-25: The relevance of the CO₂ flux data for this paper is not clear to me, as there is neither a correlation analysis with soil parameters nor a comparison with N₂O fluxes.

RESPONSE: Thank you for bringing this to our attention. We have included a correlation analysis of the CO₂ flux with the N₂O flux and compared them with other studies. In addition we have used the CO₂ flux data as an element in our speculation on the carbon balance of the system.

p. 6004, l. 2: “. . .from drained soil. . .”: Does this mean that data from site C were excluded, as this site was permanently water-logged during the field campaign in 2009?

RESPONSE: Thank you for pointing this out! No, although it was water-logged the data from site C were included as the soil was not flooded, but water-logged due to precipitation events. This has been clarified in the revised text.

p. 6004, l. 5-7: At least for site A you should have some data, as the weather station was located there, recording soil temperature and soil moisture data.

RESPONSE: We agree. However, there are too few data points from Level 2 at site A to make a valid correlation.

p. 6004, l. 9f.: “. . .detailed study of the nitrogen cycle. . .”: For a detailed study of the nitrogen cycle I would expect at least some numbers on (at the input side) nitrogen fixation, DIN, DON and particulate organic nitrogen import with flood water, and (on the

output side) NO and N₂ losses, as well as nitrate and DON losses with the retreating water, furthermore data on plant and microbial N uptake and release. As only data on soil ammonium, nitrate and total nitrogen as well as N₂O losses are available I would refrain from talking of a “detailed study of the nitrogen cycle”.

RESPONSE: Point taken! We have changed the phrase to “...the first study of the soil nitrogen transformations...”.

p. 6005, l. 3: What are the long-term monitoring sites? There is only one site that was monitored within two years (both 2008 and 2009, site A), the other two sites (B and C) were only sampled in one year (2008 and 2009, respectively). I would not speak of “long-term monitoring sites”.

RESPONSE: Term changed to “repeatedly sampled sites”.

p. 6006, l. 14f.: Again, how comparable are these slurry incubation studies with natural conditions?

RESPONSE: See answer above (p. 5999, l. 2f.).

p. 6006, l. 15: How do you know that denitrification was constant?

RESPONSE: We deleted ‘constant’.

p. 6006, l. 17: Did you measure nitrate and nitrite concentrations in the slurries?

RESPONSE: We did not measure nitrate and nitrite in the slurries. The phrase has been revised accordingly.

p. 6007, l. 23f.: Here a critical discussion of the phosphorus status of the studied soils is missing. A P deficiency in Pantanal soil together with high natural N fixation rates could explain the observed very high N₂O emission rates.

RESPONSE: Thank you for suggesting this. We have revised the discussion and included the P data.

p. 6009, l. 5-7: How did you calculate the magnitude of N₂O pulses from rain-wetted soils? Did you use one default value, which lasted for one day?

RESPONSE: Based on 22 observed peak events we calculated a mean value for a precipitation-triggered peak event.

p. 6009, l. 23-25: For this extrapolation you would need a good estimate of the spatial and temporal distribution of drained and wetted soils.

RESPONSE: We agree and have revised the phrase.

p. 6010, l. 1-5: This is an assumption on weak grounds, totally neglecting any other

dynamics like e.g. plant N uptake, thereby fostering plant–microbe competition for nitrogen.

RESPONSE: We have revised the phrase. We agree that the assumption neglects the plant-microbe competition for nitrogen. However, the 2010 flux measurements from drained soils at the end of the low water period showed the same (mean) N₂O flux as at the beginning of the low water period. This, to us, suggests that although plants were growing during the period of low water, they did not outcompete the bacteria to an extent where nitrifiers and denitrifiers could not continue producing N₂O.

p. 6010, l. 18-20: Do you have any estimate of the contribution of inundated soil to total N₂O emissions? Given the vast flooded area of the Pantanal this could be large. Or have you assumed that there is no N₂O emission from flooded soil, but only total reduction to N₂? Then I would say this should have been proven.

RESPONSE: We measured only on drained or draining soil and have no measurements from flooded soil. We agree that the contribution of flooded soils could be large, but since we have no data from flooded soil at all we did not wish to speculate on this contribution. The massive decomposition events that follow the flooding of the larger wetland plains (Hamilton et al., 1997) suggest that a pulse emission could take place immediately after flooding (days, weeks), but whether the N₂O produced in such an event would escape reduction to N₂ in the flooded soil and cause an increased emission of N₂O remains to be investigated. In the revised manuscript, we have revised any yearly (yr⁻¹) insertions and substituted them with ‘170 days⁻¹) to make it clear that we estimate only the emission from drained soil during the low water period.

p. 6011, l. 16-28: Again, what is the contribution of the period of inundation? Do you think there is no nitrogen loss during that time? Any estimate of a nitrogen balance has to include this period.

RESPONSE: See above.

p. 6012, l. 10-11: These plants will for sure not take up all of their nitrogen directly from N-fixing microorganisms, but only a smaller, albeit significant part. Thus, the large difference in estimated annual N fixation and total N content of the plant material is not surprising.

RESPONSE: We agree.

TECHNICAL CORRECTIONS

p. 6006, l. 7: Replace “gleisoil’s” with “gley soils”

RESPONSE: Replaced as requested.

p. 6007, l. 5: omit “be” between “can” and “promote”

RESPONSE: Omitted as requested.

REFERENCES

Hamilton, S. K., Sippel, S. J., Calheiros, D. F., and Melack, J. M.: An anoxic event and other biogeochemical effects of the Pantanal wetland on the Paraguay River, *Limnology and Oceanography*, 42, 257-272, 1997.

Kort, E. A., Patra, P. K., Ishijima, K., Daube, B. C., Jiménez, R., Elkins, J., Hurst, D., Moore, F. L., Sweeney, C., and Wofsy, S. C.: Tropospheric distribution and variability of N₂O: Evidence for strong tropical emissions, *Geophysical Research Letters*, 38, 15806, 10.1029/2011gl047612, 2011.