

# Nitrous oxide fluxes and denitrification in created wetlands receiving hydrological pulses

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## Introduction

Removal of nitrogen from water is one of the services that wetlands provide. Denitrification is the major process by which nitrogen is removed from water in wetlands; N<sub>2</sub> and N<sub>2</sub>O are the final products from this process. Formation and release of N<sub>2</sub>O into the atmosphere is a concern because of its contribution to the greenhouse effect and the ozone depleting effect (Johansson et al., 2003). On the other hand, N<sub>2</sub> is a desirable end product of denitrification because it is not harmful to the atmosphere, and the nitrogen cycle is completed. Riparian wetlands are exposed to seasonal flood pulses that cause changes in the physical and chemical environment of the soil, influencing microbial processes involved in the nitrogen cycle. When a flood pulse occurs, the total wetland area increases, causing changes in oxidizing and reducing conditions along the edges and terrestrial-wetland transitional zones. How flood pulses affect nitrogen gas fluxes dynamics in created wetlands is not well understood.

The aim of this study was to investigate the influence of hydrology and vegetation on gaseous nitrogen fluxes in two created experimental wetlands in the Midwestern USA.

## Material and methods

### Site Description

The study was carried out in two kidney-shaped 1-ha experimental wetlands, which were constructed in 1993 at the Olentangy River Wetlands Research Park at the Wilma H. Schiermeier Wetland Complex, in Columbus Ohio, USA. Seasonal hydrologic pulses were simulated by pumping river water at a high rate (1892-3785 L min<sup>-1</sup>) during the first week of each month, while in the remaining three weeks the wetlands received a low flow (378-757 L min<sup>-1</sup>). The pulse schedule operated from January through June, 2003 and 2004, and from July to December the wetlands received a steady rate of flow. An extraordinary simulated flood pulse was performed in August 2003. Natural flooding from the Olentangy River occurred on August 30, 2003 and June 13, 2004.

### Gas Flux Measurements

N<sub>2</sub>O fluxes were measured using a closed chamber technique (Smith et al., 1983). Nine plots in each wetland were sampled. The plots were distributed in transects along

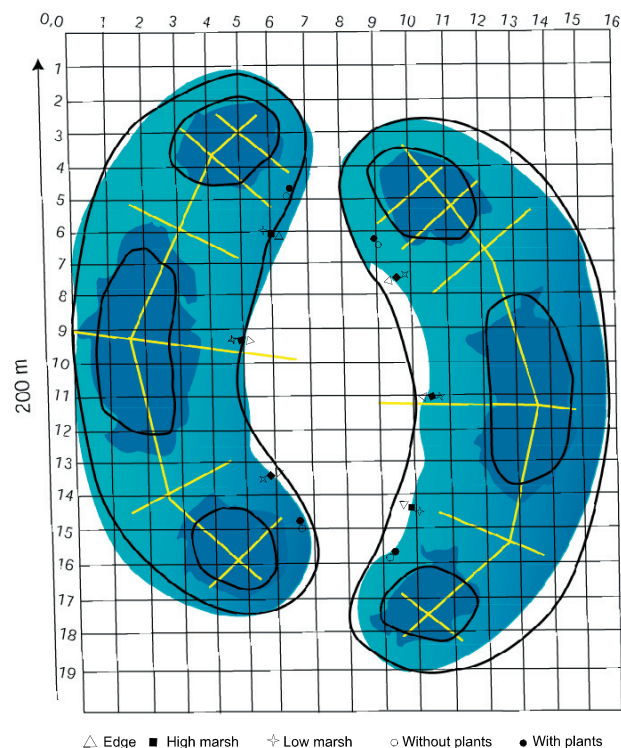


Figure 1. Map of sampling plots (grid 10 x 10 m). Dark areas indicate the open water zones. Gray areas indicate emergent vegetation zones. Areas outside these sections are Transitional/Upland Zones.

the two experimental wetlands (near the inflow, middle and outflow). Each transect contained plots at different elevations, including the edge and high marsh zones, which experienced alternating wet and dry conditions, and the low marsh zone which was continuously flooded (Figure 1). In each plot, a Plexiglas chamber (24.5 cm x 24.5 cm x 70 cm) was placed 10 cm into the soil. Gas fluxes were measured from June 2003 to December 2004. Samples were taken weekly, three times a month during pulsing months, and once per month when no pulses were performed. Chambers were sealed for 2 hours. Gas samples and internal chamber temperatures were collected or measured before the chambers were closed, and every 30 minutes after they had been closed.

To investigate the effect of vegetation on nitrous oxide fluxes, a different type of chamber was used (Altor and Mitsch, 2004). Circular HDPE bases (0.27 m<sup>2</sup>) with PVC frames were placed permanently in the edge zones. When

vegetation was included, frames were approximately 1.5m tall and where emergent vegetation was continuously removed, frames were approximately 0.5 m tall. During gas flux measurements, a 4-mil plastic bag with pressure vent was placed on the frame and attached to the base with two 3-cm wide elastic bands. Chambers were closed for 1 hr and gas samples were withdrawn as described above every 20 minutes during the hour. Internal air temperature, water table level and soil temperatures were also registered. The effect of vegetation on nitrous oxide fluxes was evaluated from May to September 2004.

Denitrification ( $N_2 + N_2O$  production) was measured in the field using the acetylene blockage technique (Ryden and Dawson, 1982). A PVC pipe (4 cm diameter x 75 cm high) with a water seal was placed 10 cm into the soil next to the Plexiglas chambers described above. Acetylene was injected 10 cm into the soil using a perforated 4mm PVC pipe to obtain a final concentration of 10% v/v. Thirty minutes after acetylene injection, the PVC pipes were sealed and gas samples were taken every 10 minutes over a 30 minute period.

### Analytical Methods

Nitrous oxide was analyzed using a gas chromatograph (Shimadzu GC-14-A) fitted with a 2 ml sampling loop, two Porapak-Q 1.8 m columns and an electron capture Ni-63 detector. Nitrous oxide fluxes were calculated using the closed chamber flux equation (Holland et al., 1999).

### Statistical Analysis

All statistical analysis were performed with SPSS software. The data were not normally distributed, therefore they were analyzed using nonparametric techniques. The Mann-Whitney-U test was used to examine differences in seasonal and spatial nitrous oxide fluxes. The Kruskal Wallis test was used to evaluate the effect of vegetation on  $N_2O$  fluxes. A 5% significance level was used to assess differences among treatments.

## Results and Discussion

### Seasonal Fluxes

During summer 2004, nitrous oxide fluxes in high marsh plots ( $40.3 \pm 15.0 \mu\text{g N m}^{-2} \text{h}^{-1}$ ) were significantly greater than fluxes obtained in these plots during the rest of the year ( $p < 0.05$ ). In low marsh and edge plots,  $N_2O$  emissions fluctuated between 3-10  $\mu\text{g N m}^{-2} \text{h}^{-1}$  and did not vary significantly among the different seasons (Figure 2).

### Spatial Patterns

Low marsh plots that were regularly flooded showed significantly lower average nitrous oxide fluxes than high marsh plots, which were affected by the flood pulses ( $3.82 \pm 0.78$  vs.  $14.46 \pm 8.65 \mu\text{g N m}^{-2} \text{h}^{-1}$  respectively,  $p = 0.027$ ). However, fluxes from marsh plots were not significantly

Table 1. Nitrous oxide emissions from plots at different elevations in created marshes receiving hydrological pulses. Values are means, and bars represent standard error.

Plot	$N_2O$ Fluxes ( $\mu\text{g N m}^{-2} \text{h}^{-1}$ )
Low marsh	$3.82 \pm 0.68$
High marsh	$14.46 \pm 8.63$
Edge	$5.96 \pm 1.14$

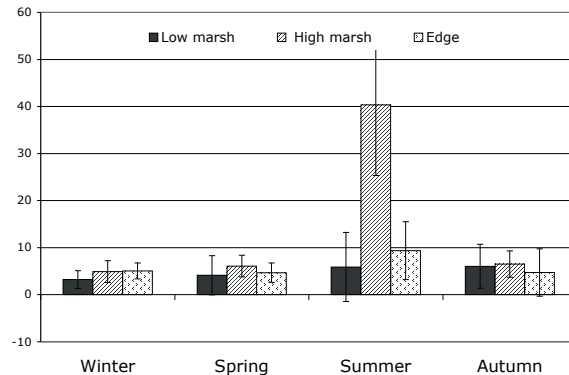


Figure 2. Seasonal nitrous oxide emissions from plots at different elevations in created marshes receiving hydrological pulses. Values are means and bars represent standard error.

lower than the fluxes from edge plots that were rarely flooded ( $5.96 \pm 1.14 \mu\text{g N m}^{-2} \text{h}^{-1}$ ,  $p = 0.10$ ) (Table 1).

### Short Term Effect of Flood Pulses on Gas Emissions

Low marsh plots that had continuous standing water had low rates of nitrous oxide fluxes regardless of the flood pulse condition (Figure 3). Nitrous oxide fluxes from high marsh plots were not significantly different before and during flood pulses ( $2.4 \pm 6.5$  and  $6.9 \pm 2.2 \mu\text{g N m}^{-2} \text{h}^{-1}$ ,  $p = 0.148$ ) when the water level averaged  $-22 \pm 3.0$  and  $+16 \pm 1.5$  cm respectively, but they increased significantly to  $25.9 \pm 13.8 \mu\text{g N m}^{-2} \text{h}^{-1}$  after the flood pulses, when the water table dropped to  $-9.2 \pm 3.3$  cm ( $p = 0.021$ ). In edge plots where surface flooding was infrequent but groundwater levels fluctuated, nitrous oxide emissions were significantly lower ( $p = 0.018$ ) before the pulse ( $4.1 \pm 1.8 \mu\text{g N m}^{-2} \text{h}^{-1}$ ) but increased significantly during ( $p = 0.013$ ) and after ( $p = 0.07$ ) the pulse ( $11.3 \pm 3.1$  and  $7.25 \pm 3.3 \mu\text{g N m}^{-2} \text{h}^{-1}$ ).

In our high marsh plots, the spike in nitrous oxide fluxes observed after flood pulses might be explained by a combined effect of a shallow water table and availability of inorganic nitrogen and organic carbon. When floods occurred, anoxic conditions favored denitrification ( $N_2 + N_2O$ ) in these wetland zones (Hernandez and Mitsch, in revision) and this could explain the slight increase in

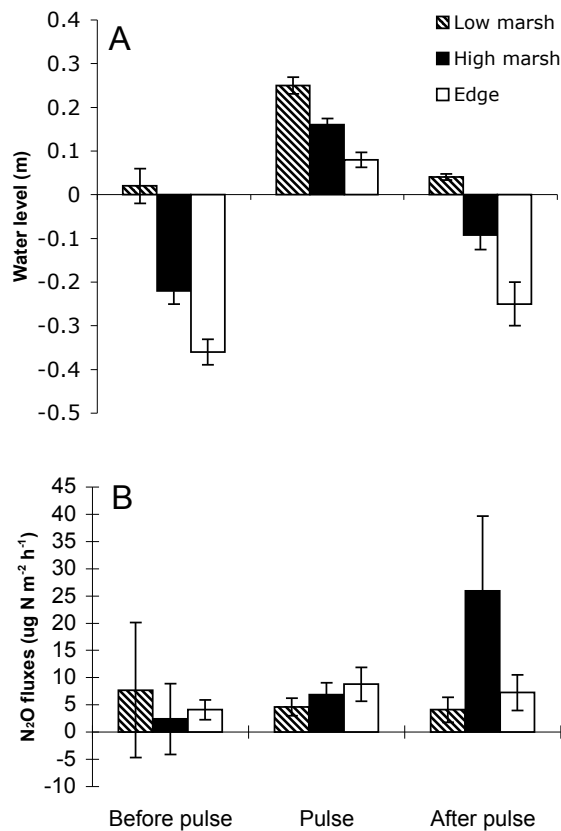


Figure 3. Short term effect of flood pulses on A: water level, and B: nitrous oxide fluxes in plots. Values are means and bars represent standard error

fluxes during the pulse. One week after the flood pulses, the water table was very low, but still maintaining anoxic conditions for denitrification and N<sub>2</sub>O production. Because no standing water was present, there was no restriction of N<sub>2</sub>O diffusion to the atmosphere, causing an increase in the fluxes. Likewise, the fact that the plots were exposed to air might have caused suboptimal conditions for denitrification. Under suboptimal conditions for denitrification, N<sub>2</sub>O is the predominant end product (Yoshinari, 1990). In forested riparian zones of the Netherlands, Hefting et al. (2003) found that N<sub>2</sub>O fluxes were higher in zones with a shallow water table compared to zones with deeper water levels. In our edge zones, nitrous oxide fluxes were affected by hydrology in a different way than in the high marsh plots. This might be due to the complex mechanisms controlling N<sub>2</sub>O emission; the gas is produced as both an intermediate product of denitrification and as a byproduct of nitrification (Yoshinari, 1990; Stevens et al., 1998; Muller et al., 1997). Since edge plots were exposed to air most of the time, aerobic conditions could have favored the establishment of a population of aerobic nitrifying bacteria; therefore, nitrous oxide produced when plots were exposed to air could be the byproduct of the nitrification of ammonium released during mineralization. Before flood pulses, fluxes from these plots were very low and the water table was not close to the surface. When floods occurred, denitrification

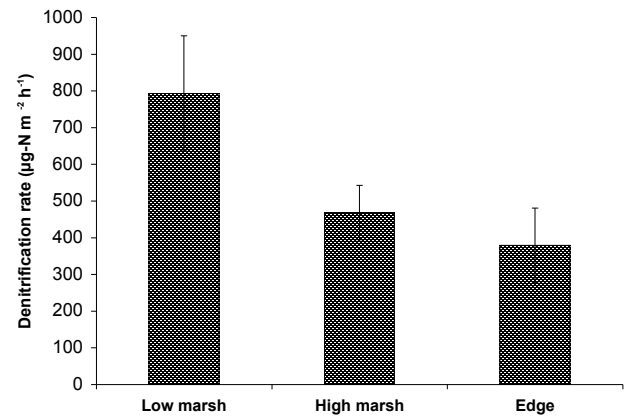


Figure 4. Average denitrification rates from May - December 2004, in plots at different elevations. Values are means and bars represent standard error.

Table 2. The effect of emergent vegetation on nitrous oxide fluxes from created marshes, under different hydrologic conditions. Values are mean flux rates ( $\mu\text{g N m}^{-2} \text{h}^{-1}$ ),  $\pm$  standard error. Asterick indicates a significant difference at  $\alpha=0.05$ .

Condition	Flooded	Exposed to air
<i>With plants</i>	39.64 $\pm$ 13.70	17.59 $\pm$ 15.80 *
<i>Without plants</i>	-3.58 $\pm$ 13.17	11.68 $\pm$ 1.46

and N<sub>2</sub>O production were stimulated and diffusion of N<sub>2</sub>O to the atmosphere was not restricted since flooding was very shallow. After flood pulses the water table dropped quickly, decreasing anoxic conditions for denitrification; therefore fewer fluxes were observed.

### Effect of Vegetation on Gas Fluxes

When wetland soils were exposed to air, the average nitrous oxide flux in plots with vegetation was 17.6  $\pm$  15.8  $\mu\text{g N m}^{-2} \text{h}^{-1}$ , and in plots without vegetation flux rates were 11.6  $\pm$  1.5  $\mu\text{g N m}^{-2} \text{h}^{-1}$  (Table 2). When plots were flooded, nitrous oxide fluxes were significantly higher where vegetation was present (39.6  $\pm$  13.70  $\mu\text{g N m}^{-2} \text{h}^{-1}$ ) compared to areas without vegetation (-3.57  $\pm$  13.17  $\mu\text{g N m}^{-2} \text{h}^{-1}$ ,  $p = 0.03$ ). In contrast, when no surface water was present, the presence of vegetation did not affect nitrous oxide fluxes ( $p = 0.312$ ).

Gas emissions through both wetland and upland plants have been previously described (Mosier et al., 1990; Chang et al., 1998). In wetlands plants, the aerenchyma system is involved in gas exchange with the atmosphere (Mitsch and Gosselink, 2000), and in upland plants gases are released to the atmosphere via plant transpiration (Chang et al., 1998). We found that N<sub>2</sub>O fluxes were higher in plots with plants than in plots without plants when they were inundated, but that plants did not have a measurable effect on nitrous oxide

fluxes when soils were exposed to air. This may be due to the fact that under flooded conditions the arechymatous system is more actively transporting oxygen from shoots to roots, and thus transporting gases in the soil more quickly to the atmosphere than under exposed conditions. Similar patterns have been described for N<sub>2</sub>O fluxes from rice paddies (Yan et al., 2000). When the soil was flooded, emissions were predominantly made through the rice plants, while in the absence of flood water; N<sub>2</sub>O was emitted mainly from the soil surface. Ulrike et al. (2004) found that N<sub>2</sub>O emissions from degraded fen soils planted with *Phragmites* were two times higher than emissions from unplanted soils, but that *Phalaris arundinacea* did not affect N<sub>2</sub>O emissions.

### Denitrification

Average denitrification rates were significantly higher in low marsh plots ( $793 \pm 156 \mu\text{g N m}^{-2} \text{h}^{-1}$ ) compared to high marsh and edge plots ( $468 \pm 74.15$  and  $379 \pm 101 \mu\text{g N m}^{-2} \text{h}^{-1}$ ), but no significant differences were observed in denitrification rates between high marsh and edge plots (Figure 4). This was due to the continuously flooded conditions that occurred in low marsh zones, which created prolonged anoxia that facilitated the total reduction of nitrates to dinitrogen.

### Conclusions

N<sub>2</sub>O emissions from created marshes were influenced by soil temperature, hydrologic conditions and the presence of emergent vegetation. Plots that were regularly inundated showed the lowest nitrous oxide fluxes when vegetation was not present. Flood pulses resulted in an increase in nitrous oxide fluxes from plots in the high marsh and edge zones of the wetlands. Vegetation facilitated nitrous oxide emissions from wetland soils only when soils were flooded. Total denitrification was higher in marsh plots which were regularly inundated.

### References

Altor, A. and W. J. Mitsch. 2004. Influence of hydrologic pulsing and macrophyte cover on exchange of carbon dioxide and methane in created riparian marshes. Abstracts, INTECOL International Wetlands conference, Utrecht, The Netherlands. 25-30 July.

Chang, C., H.H Janzen, C.M. Cho, and E. M. Nakonechny. 1998. Nitrous oxide emission through plants. *Soil Sci. Soc. Am J.* 62:35-38.

Hefting, M, R. Bobbink and H. Culawe. 2003. Nitrous oxide emissions and denitrification in chronically nitrae-loaded riparian buffer zones. *Journal of Environmental Quality* 32:1194-1203.

Hernandez, M.E. and W.J. Mitsch. in revision. Influence of hydrologic pulses and vegetation on seasonal and spatial nitrous oxide emissions from created riparian marshes in Midwestern USA. *Wetlands*.

Holland E, G.D. Robertson, J. Greenberg, M.P Groffman, R.D.Boone, and J. Gosz. 1999. CO<sub>2</sub>, N<sub>2</sub>O and CH<sub>4</sub> exchange. In: *Soil standard methods for long-term ecological research*. Robertson, G.P.,D.C. Coleman, C.S. Bledsoe, and P. Sollins (Eds). Oxford University Press. New York. pp. 187-199.

Johansson, E., K.A. Klemedtsson, L. Klemedtsson, and B.H. Sevansson. 2003. Nitrous oxide exchanges with the atmosphere of a constructed wetland treating wastewater. Parameters and implications for emission factors. *Tellus* 55B: 737-750.

Mitsch, W.J. and J.G Gosselink,. 2000. *Wetlands*. Third edition. John Wiley & Sons, New York.

Mosier, A.R., S.K. Mohanty, A. Bhadrachalam, and S. P. Chakravoti. 1990. Evolution of dinitrogen and nitrous oxide from the soil to the atmosphere through rice plants. *Biology and Fertility of Soils* 9: 61-67.

Muller, C., R.R. Sherlock, and P.H. Williams.1997. Mechanistic model for nitrous oxide emission and denitrification. *Biology and Fertility of Soils* 24: 231-238.

Ryden, J.C and Dawson, K.P 1982. Evaluation of acetylene inhibition technique for field measurement of denitrification in grasslands soils. *J. Scie.Food Agric.* 33-1197-1206

Smith, C.J., R.D. Delanue, and W.H. Patrick, Jr. 1983. Nitrous oxide emissions from Gulf Coast Wetlands. *Geochimica et Cosmochimica Acta.* 47: 1805-1814.

Stevens, J.R, R.J. Laughlin, and J.P. Malone, 1998. Soil pH affects the process reducing nitrate to nitrous oxide and dinitrogen. *Soil Biology and Biochemistry* 8/9: 1119-1126.

Ulrike, R., A. Jurrgen, R. Russow, and W. Merbach. 2004. Nitrate removal from drained and reflooded fen soils affected by soil N transformation process and plant uptake. *Soil Biology and Biochemistry* 36: 77-90.

Yan, X., L. Du, S. Shio, and G. Xing. 2000. Nitrous oxide emission from wetland rice soil as affected by the application of controlled availability fertilizer and mid-season aeration. *Biology and Fertility of Soils* 32: 60-66.

Yoshinari, T.1990. Emissions of N<sub>2</sub>O from various environments – the use of stable isotope composition of N<sub>2</sub>O as tracer for the studies of N<sub>2</sub>O biogeochemical cycling. In “Denitrification in Soil and Sediment”. Sorensen, J. ( Ed.), Plenum Press, New York, pp. 129-144.