

## Denitrification in Marsh-Pond-Marsh Constructed Wetlands Treating Swine Wastewater at Different Loading Rates

P. G. Hunt,\* M. E. Poach, T. A. Matheny, G. B. Reddy, and K. C. Stone

### ABSTRACT

Denitrification in constructed wetlands can be very important in the treatment of swine lagoon effluent when land application areas are limited. The objectives of this investigation were to determine (i) the denitrification enzyme activity (DEA) in the marsh sediments of marsh-pond-marsh (MPM) constructed wetlands, (ii) changes in DEA with additions of carbon and nitrate, and (iii) the response of DEA to different wastewater N loading rates. Swine wastewater was applied to six MPM wetlands located at North Carolina A&T State University, Greensboro, NC, at rates of 4 to 35 kg N ha<sup>-1</sup> d<sup>-1</sup>. Soil samples were obtained from the top 25 mm of the marsh sections on four dates for determination of DEA via the acetylene blockage method (blocked at N<sub>2</sub>O). Headspace N<sub>2</sub>O was measured via gas chromatography. In the control treatment, they ranged from 0.06 to 1.13 and 0.16 to 0.79 mg N<sub>2</sub>O-N kg<sup>-1</sup> soil hr<sup>-1</sup> in the first and second marshes, respectively. In both marshes, the DEA rate was significantly increased with the addition of nitrate but not by glucose, indicating that nitrate was a clear limiting factor for denitrification. The DEA in both the control and the amended treatments increased dramatically with increased wastewater N loading, and the increases were generally more pronounced in the first marsh. The DEA values produced in the absence of acetylene blockage did not increase with wastewater N loading rate. Denitrification enzyme activity levels in the marsh sections of the MPM were generally consistent with a highly denitrifying environment.

CONSTRUCTED WETLANDS have been investigated and used for treatment of animal waste in the USA and around the world for over 10 yr (Cathcart et al., 1994; McCaskey et al., 1994). The understanding of their treatment processes, designs, and operations have advanced significantly (Kadlec and Knight, 1995; Knight et al., 2000; Hunt et al., 2002; Stone et al., 2002). Constructed wetlands have been found to generally provide somewhat limited P removal capacities but significant N removal capacities (Knight et al., 2000; Hunt et al., 2002). The effectiveness of N removal has varied considerably with wetland design and loading rates (Poach et al., 2003, 2004b). Their research indicated that the continuous marsh wetlands were able to treat higher loads of N than the MPM wetlands. Furthermore, the continuous marshes were subject to less ammonia volatilization than the MPM wetlands (Poach et al., 2002, 2004a).

While soil accumulation and plant uptake of N were important at low N loading rates (Szogi et al., 2000), they

were responsible for smaller portions of the removal at higher N loading rates ( $\geq 10$  kg ha<sup>-1</sup> d<sup>-1</sup>) (Hunt et al., 2002). At the higher loading rates, denitrification became the apparent predominant pathway for N removal. Hunt et al. (2003) reported high levels of DEA in continuous marsh wetlands used to treat swine wastewater. These wetlands had sloped bottoms, and denitrification was higher at the upper slope area where oxygen was more available to facilitate nitrification of the ammonia in the wastewater. These apparent high losses via denitrification are in agreement with the recently reported very high N removal by constructed wetlands in Ontario, Canada (Kadlec and Bishay, 2005).

At the higher N loading rates, gaseous losses were also the major N removal pathway for N on MPM wetlands (Poach et al., 2003, 2004a). Whereas Poach et al. (2003) measured the volatilization of ammonia at various N loading rates, it was also important to assess denitrification at these varying loading rates. To make this assessment, we chose to measure denitrification enzyme activity via the acetylene blockage method. The objectives of this investigation were to determine (i) the DEA in the marsh sediments of MPM constructed wetlands; (ii) changes in DEA with additions of C and nitrate; and (iii) the response of DEA to varied wastewater N loading rates.

### MATERIALS AND METHODS

#### Site Description

Denitrification enzyme activity was assessed on constructed wetlands that received swine wastewater. The wetlands were constructed at the swine facility of the North Carolina A & T State University farm in Greensboro, NC, in 1995 and consisted of six sets of MPM wetland cells (Poach et al., 2004a, 2004b). Each wetland cell was 40 m by 11 m and divided into two marsh sections (10 m by 11 m) with a pond section (20 m by 11 m) between the marsh sections. A complete description of the wetland's construction was presented by Reddy et al. (2001). The marsh sections were planted with broadleaf cattail (*Typha latifolia* L.) and American bulrush [*Schoenoplectus americanus* (Pers.) Volkart ex Schinz & R. Keller] in March 1996. The cattails became the predominate species. The pond section contained duckweed (*Spirodela polyrhiza*) and algae.

#### Experimental Design

Two on-site sources of wastewater from the lagoon/pond system were used to provide each wetland cell with a different N loading rate while maintaining the same hydraulic load. The first source was the primary lagoon of the two-stage anaerobic lagoon system connected in series. The lagoon was a collection reservoir for all effluent from a swine sow-farrow facility that had 130 to 250 head. The second source was the storage pond

P.G. Hunt, M.E. Poach, T.A. Matheny, and K.C. Stone, USDA-ARS, Coastal Plain Soil, Water, and Plant Research Center, 2611 W. Lucas St., Florence, SC 29501; G.B. Reddy, North Carolina A&T State Univ., Greensboro, NC 27411. Received 14 Mar. 2005. \*Corresponding author (Hunt@Florence.usda.ars.gov).

Published in Soil Sci. Soc. Am. J. 70:487–493 (2006).

Wetland Soils

doi:10.2136/sssaj2005.0075

© Soil Science Society of America

677 S. Segoe Rd., Madison, WI 53711 USA

**Abbreviations:** DEA, denitrification enzyme activity.

that had been receiving the outflow from the constructed wetlands since their initial operation.

Wastewater from the primary lagoon was pumped by use of a submersible pump to an 8000-L storage tank and then moved from the storage tank to the wetland cells via gravity. Wastewater from the storage pond was transferred to the wetland cells via a shallow-well pump. From September 2000 to September 2001, wastewater from the storage tank (primary lagoon) and the storage pond was applied at various ratios to provide different N loading rates for each of the six wetland cells. The initial N concentrations of the storage tank and storage pond were used to calculate the ratios necessary to provide varying N loading rates. These ratios were adjusted on a weekly basis because of slight changes in the N concentration of the storage tank and storage pond. All wetland cells received the same hydraulic loading rate, but the rate varied from 7.1 to 12.5 m<sup>3</sup> d<sup>-1</sup> throughout the study period because of variation in the N concentration in the storage tank.

For all wetland cells, wastewater was delivered to the inlet of the first marsh and flowed, via gravity, through the pond and second marsh. The treated wastewater was collected at the outlet of second marsh and recycled to the storage pond. Flows into and out of the wetland cells were measured with tipping buckets connected to electronic pulse counters.

### Denitrification Enzyme Activity

Composite soil samples were collected at the 0- to 25-mm depth from both marshes of each wetland cell on four dates during 2000 through 2001. Soil samples were placed in plastic bags, stored on ice, transported to the laboratory, and stored overnight at 4°C. Denitrification enzyme activity was measured by the acetylene blockage method (Tiedje, 1994). Field moist soil (10–15 g) was placed in 60-mL serum bottles (five bottles per sample in triplicate). Duplicate soil samples were dried at 100°C for 72 h and weighed to determine moisture content. Each bottle received one of the following four amendments: (1) 5 mL of chloramphenicol (1 g L<sup>-1</sup>) to inhibit protein synthesis; (2) 5 mL of chloramphenicol with nitrate N (200 mg NO<sub>3</sub>-N L<sup>-1</sup>); (3) 5 mL of chloramphenicol with glucose (2 g glucose L<sup>-1</sup>); or (4) 5 mL of chloramphenicol with nitrate N (200 mg NO<sub>3</sub>-N L<sup>-1</sup>) and glucose (2 g glucose L<sup>-1</sup>). Bottles were capped with rubber septa, evacuated, and purged with nitrogen gas three times. Fifteen milliliters of acetylene were inserted into four bottles with a syringe. The fifth bottle, which also received Amendment 4, did not receive any acetylene. The bottles were incubated on a horizontal shaker at 90 rpm. Samples of the headspace gases were removed after 1, 5, and 24 h with a syringe (Becton Dickinson Plastipak syringe with slip tip needle) and placed in vials (borosilicate glass, crimp top with butyl septum). The anaerobic condition of the incubations and the blockage of nitrification by acetylene simulate the continually flooded conditions of these flat bottom wetland cells. However, the values could be somewhat conservative for the control treatment because in the wetland soils there is likely to be some diffusion of nitrate from the surface waters and plant root zones as well as consumption of nitrate by the plants (Malone et al., 1998; Silvan et al., 2005).

A Varian Model 3600 CX gas chromatography<sup>1</sup> (Palo Alto, CA) with a 15-mCi<sup>63</sup>Ni electron capture detector operating at 350°C was used for measuring N<sub>2</sub>O in the gas samples. A 1.8 m by 2 mm ID stainless steel column packed with Poropak Q

(80–100 mesh) was used to separate CO<sub>2</sub>, N<sub>2</sub>O, and C<sub>2</sub>H<sub>2</sub>. The column and injector temperatures were 70°C. Samples were injected into the column by a Varian 8200 autosampler.

### Water and Soil Analysis

Wastewater from the storage tank (primary lagoon), the storage pond, and the outlet of the six wetland cells was collected daily using autosamplers (Model 3700, Isco, Lincoln, NE). Daily samples were combined into a weekly composite and concentrated sulfuric acid was added to lower the pH below 2.5. The wastewater samples were packed on ice and transferred to the laboratory for analyses. Ammonia-N, nitrate-N, orthophosphate-P, total Kjeldahl nitrogen (TKN), and total phosphorus (TP) were determined on the acidified wastewater samples using EPA methods 350.1, 353.1, 365.1, 351.1, and 365.4, respectively (Kopp and McKee, 1983). All N and P analyses were conducted by automated analyzers (Technicon Instruments Corp., Tarrytown, NY and Bran+Lubbe Corp., Buffalo Grove, IL).

The redox potential (E<sub>H</sub>) of the wetland surface water was determined by an Orion Model 290A meter (Thermo Electron Corporation, Beverly, MA) with an Ag/AgCl electrode. The electrodes were tested with quinhydrone in pH 4.0 and 7.0 buffers. Redox potential values were adjusted to the H electrode potential by adding the potential of the Ag/AgCl reference electrode, +200 mV, to the mV reading. Soil redox potential (mV) was measured with platinum tip electrodes installed at the 5-cm soil depth with three replications. Data were collected with a CR23X data logger (Campbell Scientific Inc., Logan, UT). An Orion Model 210A pH meter (Thermo Electron Corporation, Beverly, MA) was used to determine the pH of the surface water. The pH electrode was calibrated with a pH 4.0 and 7.0 buffer.

### Statistical Analysis

Physical and chemical characteristics were statistically analyzed on data from the water analyses of the lagoons and storage pond, inflow and outflow samples for each wetland cell, and surface water samples in each wetland cell by SAS Proc Means, Proc ANOVA, and Least Significant Difference (SAS Institute, 1999). The four sampling dates were used as replications in time. The DEA data were analyzed by the SAS procedures for least significant difference (LSD) and regression (SAS Institute, 1999).

## RESULTS AND DISCUSSION

### Wastewater Treatment

During the 4 wk of sampling for DEA, the lagoon effluent was added at rates of 2 to 53 kg N ha<sup>-1</sup> d<sup>-1</sup> (Table 1). There was a very good sequential spread of about 7 kg N ha<sup>-1</sup> d<sup>-1</sup> among the 4-wk means for the lowest five loading rates, which ranged from 4 to 33 kg N

**Table 1. Nitrogen loading rates of constructed wetland cells receiving swine wastewater.**

Sampling date†	Wetland cell loading rates					
	1	2	3	4	5	6
	kg N ha <sup>-1</sup> d <sup>-1</sup>					
11–15–2000	6	21	21	31	34	53
05–02–2001	6	11	19	24	37	35
07–14–2001	2	12	19	30	24	24
08–29–2001	3	13	15	28	35	26
Mean	4	14	19	28	33	35

† Date samples were collected for denitrification enzyme activity analysis.

<sup>1</sup>Mention of trade name, proprietary product, or vendor is for information only and does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

**Table 2. Nutrient concentration of swine wastewater flowing into and out of the constructed wetlands.**

Loading rate	Location	pH <sup>†</sup>	Redox mV	TSS <sup>‡</sup> g L <sup>-1</sup>	COD <sup>§</sup>	mg L <sup>-1</sup>		
						Organic N	NH <sub>3</sub> -N	NO <sub>3</sub> -N
4	inlet	8.90	206	137	325	16	7	1.5
	outlet	6.76	50	92	180	9	6	0.3
14	inlet	7.30	209	195	491	21	49	1.3
	outlet	7.15	39	96	280	17	22	0.5
19	inlet	8.04	210	224	559	23	70	1.2
	outlet	7.23	55	165	288	12	31	0.6
28	inlet	7.18	207	276	671	27	105	1.0
	outlet	7.12	42	187	425	20	50	2.2
33	inlet	7.19	210	308	766	30	131	0.7
	outlet	7.06	61	173	450	21	60	4.8
35	inlet	7.23	206	321	796	32	139	0.6
	outlet	7.47	36	215	471	21	66	1.1
LSD <sub>0.10</sub>		1.76	18	59	73	9	17	1.7
LSD <sub>0.05</sub>		2.24	23	71	88	11	20	2.1

<sup>†</sup> Mean of four sampling dates during the study.

<sup>‡</sup> Total suspended solids.

<sup>§</sup> Chemical oxygen demand.

ha<sup>-1</sup> d<sup>-1</sup>. However, highest 4-wk mean was only 35 kg N ha<sup>-1</sup> d<sup>-1</sup>, and it exceeded 37 kg N ha<sup>-1</sup> d<sup>-1</sup> in only one of the DEA sampling weeks. The mean N concentration reduction for the four sampling weeks ranged from 45 to 65% (Table 2). A thorough discussion of the N treatment efficiency (mass removal) during the entire study can be found in Poach et al. (2004b). These N removal rates, generally <75%, are somewhat lower than the removal rate reported by Hunt et al. (2002) for continuous marsh constructed wetlands used to treat swine anaerobic lagoon effluent in North Carolina. In that study, they found N removal to be generally >75% when N was loaded at <25 kg ha<sup>-1</sup> d<sup>-1</sup>.

The loading of TSS ranged from 137 to 321 mg L<sup>-1</sup>, and the removal ranged from 49 to 74% (Table 2). The more soluble C component in the COD was removed less effectively. The COD concentrations ranged from 325 to 796 mg L<sup>-1</sup>, and the removal ranged from 52 to 63% (Table 2). The organic N concentrations in both the inlet and outlet were highly correlated to the COD (organic N = 0.0315 COD + 6.04; r<sup>2</sup> = 0.96), and its removal rate ranged from 56 to 81%. In other constructed wetlands investigated by Hunt et al. (2003), similar levels of C added to the wetland in the lagoon effluent along with that added from plant dry matter

degradations were found to be generally sufficient to support denitrification. Ammonia concentration removal was 86% at the lowest loading rate and varied only from 44 to 48% at all other loading rates. Nitrates were generally <2.0 mg L<sup>-1</sup> at both the inlet and outlet.

The physicochemical characteristics of the lagoon effluent were consistent with those expected for both lagoon effluent and a wetland environment (Table 2). The pH of the lagoon effluent was mildly alkaline, and it dropped slightly from the inlet to outlet. Values varied most (6.8–8.9) for the cell loaded at 4 kg N ha<sup>-1</sup> d<sup>-1</sup>. The pH range of the other cells was 7.1 to 8.0. The E<sub>H</sub> of inlet lagoon effluent was only mildly reduced (206–210 mV). However, the E<sub>H</sub> of the wetland outlet effluent was more indicative of a denitrifying environment (36–61 mV) (Szogi et al., 2004). The soil E<sub>H</sub> in the first marsh had a mean of -24 mV ± 92, with a range of -143 to 250 mV. The second marsh soil had a mean E<sub>H</sub> of 10 mV ± 103, with a range of -119 to 228 mV.

## Denitrification

Denitrification enzyme activity in first marsh for the control treatment ranged from 0.06 to 1.13 mg N<sub>2</sub>O-N kg<sup>-1</sup> soil h<sup>-1</sup> (Table 3). If a bulk density of 1.52 g cm<sup>-3</sup>

**Table 3. Denitrification enzyme activity of constructed wetlands with varying N loading rates and amendments.**

N Load <sup>†</sup> kg ha <sup>-1</sup> d <sup>-1</sup>	Marsh	Amendments				LSD <sub>0.10</sub>	LSD <sub>0.05</sub>
		Control	N <sup>‡</sup>	C <sup>‡</sup>	N + C		
		mg N <sub>2</sub> O-N kg <sup>-1</sup> Soil h <sup>-1</sup>					
4	1	0.061 <sup>§</sup>	0.597	0.134	0.600	0.190	0.230
4	2	0.204	1.028	0.116	1.195	0.233	0.282
14	1	0.190	1.388	0.311	1.774	0.196	0.237
14	2	0.164	1.806	0.114	2.112	0.735	0.887
19	1	0.248	1.082	0.254	1.826	0.232	0.279
19	2	0.221	1.580	0.358	1.774	0.718	0.866
28	1	0.518	1.848	0.814	4.795	1.000	1.206
28	2	0.695	1.795	0.608	2.196	0.676	0.817
33	1	0.701	2.188	0.966	2.787	1.051	1.267
33	2	0.424	1.021	0.624	2.233	0.479	0.578
35	1	1.127	4.864	1.803	6.562	0.845	1.020
35	2	0.786	2.196	0.932	4.544	0.952	1.318
LSD <sub>0.05</sub>		0.322	1.167	0.350	1.546	0.457	

<sup>†</sup> Mean N loading rate for four sampling dates.

<sup>‡</sup> N = Nitrate; C = Glucose.

<sup>§</sup> Mean denitrification enzyme activity for four sampling dates.

is assumed, these DEA values can be converted to a  $\text{kg N ha}^{-1} \text{d}^{-1}$  for the top 25 mm by multiplying by 9.12. This conversion suggests actual denitrification could be 0.55 to 10.33  $\text{kg ha}^{-1} \text{d}^{-1}$  in only the top 2.54 cm of the first marsh. The range of DEA values of the control was somewhat lower in the second marsh sections; values ranged from 0.16 to 0.79  $\text{mg N}_2\text{O-N kg}^{-1} \text{soil h}^{-1}$ . Thus, the DEA values suggest the top 25 mm alone could have had actual denitrification of 1.46 to 7.20  $\text{kg N ha}^{-1} \text{d}^{-1}$ . The DEA values of this study are in the range of those reported for continuous-marsh constructed wetlands used to treat swine lagoon effluent in North Carolina (Hunt et al., 2003). In that study, they found the mean DEA value of the top 50 mm for the cattail wetlands to be 0.21  $\text{mg N}_2\text{O-N kg}^{-1} \text{soil h}^{-1}$  when wetlands were loaded at 3 to 40  $\text{kg N ha}^{-1} \text{d}^{-1}$  (Hunt et al., 2002). Whereas denitrification was also likely occurring at lower soil depths, within the detrital material, and among the floating sludge; these values are consistent with very high levels of N removal via denitrification (Hunt et al., 2000).

The DEA values of the control treatment are also in the range of DEA values (0.07–0.37  $\text{mg N}_2\text{O-N kg}^{-1} \text{soil h}^{-1}$ ) reported for soil surface samples obtained from the stream edge of a riparian zone in North Carolina that was heavily impacted by a contiguous swine wastewater spray field (Hunt et al., 2004). However, the DEA values of the control treatment were much greater than those found for the similar control treatment by Ambus and Lowrance (1991) for riparian zones contiguous to row crops in Georgia ( $\leq 0.03 \text{ mg N}_2\text{O-N kg}^{-1} \text{soil h}^{-1}$ ).

Whereas denitrification requires anaerobic conditions, a C source, and nitrate as well as a population of denitrifying microorganism, the assessment of DEA with the addition of nitrate and C can provide an estimate of the potential for denitrification. In constructed wetlands used for livestock wastewater treatment, nitrates are normally low in concentration or so transitory that they are difficult to measure, but wetland N treatment responds greatly to even partial nitrification of the wastewater (Poach et al., 2003). These generally poor nitrification conditions in constructed wetland used to treat swine wastewater were also indicated by the higher DEA values found in the shallow upslope regions, which were exposed to intermittent drying (Hunt et al., 2003). In this current MPM study when nitrate was added, the DEA increased significantly relative to the control, which indicated the wetland soils were both nitrate limited and prevented from potential replenishment by the incubation conditions (Table 3). The range was from 0.60 to 4.87  $\text{mg N}_2\text{O-N kg}^{-1} \text{soil h}^{-1}$  for the first marsh section. The increase in  $\text{N}_2\text{O}$  from nitrate was not as great for the second marsh section; it ranged from 1.02 to 2.20  $\text{mg N}_2\text{O-N kg}^{-1} \text{soil h}^{-1}$ . However, the increased  $\text{N}_2\text{O}$  production was sufficient to implicate nitrate as a limiting factor to denitrification in the second marsh as well as the first. The increase in DEA with the addition of nitrate is very typical of natural and constructed wetlands, in general, and those receiving swine lagoon effluent, in particular (Hunt et al., 2005).

The addition of C to the soil samples of the first marsh section resulted in a small but consistent response in all of the wetlands (Table 3). The DEA values ranged from 0.13 to 1.80  $\text{mg N}_2\text{O-N kg}^{-1} \text{soil h}^{-1}$ . In contrast to the first marsh, no consistent response was produced by the addition of C to the soil samples of the second marsh. In fact, the second marshes frequently had smaller DEA values after the addition of C; DEA ranged from 0.12 to 0.93  $\text{mg N}_2\text{O-N kg}^{-1} \text{soil h}^{-1}$ . This generally inconsistent and small response to C additions was indicative of the relatively higher availability of C in the wetlands compared with nitrate. This carbon could have been provided and renewed by the applied lagoon effluent as well as by the annual production of large amounts of plant dry matter (16–25  $\text{Mg ha}^{-1} \text{yr}^{-1}$ , G.B. Reddy, NCA&TSU, Greensboro, NC, unpublished data, 1998-2002).

The highest DEA values were obtained with the addition of both nitrate and C to the marsh soil samples (Table 3). This was true for all the wetlands and marsh sections. Furthermore, the largest increases occurred in the wetlands loaded with  $\geq 28 \text{ kg N ha}^{-1} \text{d}^{-1}$ . These responses indicate that the wetlands had significant denitrifying population and good denitrifying conditions for potentially higher denitrification, particularly at the higher loading rates. The potential for higher removal of N and lower volatilization of ammonia has been documented in wetlands that receive even partially nitrified wastewater (Poach et al., 2003). The need for additional C at very high rates has also been found in wetland microcosms treated with N loading rates of nearly 50  $\text{kg ha}^{-1} \text{d}^{-1}$  (Hunt et al., 2000).

Some incomplete denitrification was indicated by the 0.32 to 1.06  $\text{mg N}_2\text{O-N kg}^{-1} \text{soil h}^{-1}$  produced when C and nitrate were added without the acetylene. The potential incomplete denitrification was generally greater in the second marsh, which had a median, mean, and standard deviation of 0.62, 0.68, and 0.27  $\text{mg N}_2\text{O-N kg}^{-1} \text{soil h}^{-1}$ , respectively. The first marsh had a median, mean, and standard deviation of 0.50, 0.50, and 0.16  $\text{mg N}_2\text{O-N kg}^{-1} \text{soil h}^{-1}$ , respectively. These values are in the range of  $\text{N}_2\text{O}$  production found in a DEA investigation of the continuous marsh wetlands in North Carolina (Hunt et al., 2003). However, these potential  $\text{N}_2\text{O}$  values are considerably higher than actual emission measurements of  $\text{N}_2\text{O}$  found by Fey et al. (1999) in constructed wetlands used to treat dairy wastewater under cold temperatures (23–339  $\mu\text{g N}_2\text{O m}^{-2} \text{h}^{-1}$ ). Previous studies have shown that production of  $\text{N}_2\text{O}$  can be affected by many factors; in a review of the topic, Conrad (1996) reported that the production and consumption of nitrous oxide were controlled by many different types of microbes and that the controlling processes of particular environments were varied and generally poorly defined.

### Response to Nitrogen Loading Rates

In addition to changes in DEA with the presence or absence of nitrate and C, there were significant relations associated with the rates of wastewater N applications

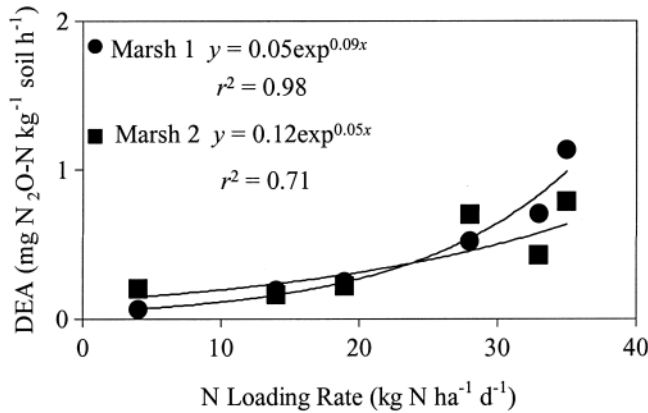


Fig. 1. Denitrification enzyme activity in non-amended marsh soils with varying wastewater N applications.

(Fig. 1). The DEA level in the control treatment of the first marsh was very highly and positively correlated to the rate of wastewater application [DEA =  $0.05\exp(0.09x)$ , where  $x = \text{kg N ha}^{-1} \text{d}^{-1}$ ;  $r^2 = 0.98$ ]. This exponential response to higher N loading is consistent with higher rates of denitrification after the capacity of soil accumulation and plant removal mechanisms have been surpassed (Hunt et al., 2002; Silvan et al., 2005). The DEA level in the control treatment of the second marsh was also positively correlated to the wastewater N application. However, the rates of DEA were lower, and the correlation was weaker [DEA =  $0.12\exp(0.05x)$ , where  $x = \text{kg N ha}^{-1} \text{d}^{-1}$ ;  $r^2 = 0.71$ ]. The lower DEA levels in the second marsh likely resulted from lower N loading because the first marsh and the pond sections removed substantial C and N. This DEA response is somewhat similar to that found in continuous marsh wetlands in North Carolina for DEA and cumulative N load. In those wetlands, the DEA of the control treatment increased from near zero to about  $0.5 \text{ mg N kg}^{-1} \text{ soil d}^{-1}$  as the cumulative N load increased to  $1.6 \text{ kg m}^{-2}$  (Hunt et al., 2003).

In the treatment receiving nitrate, there was a greater difference between the first and second marshes relative to their DEA responses to increased wastewater N application (Fig. 2). In the first marsh, there was a sig-

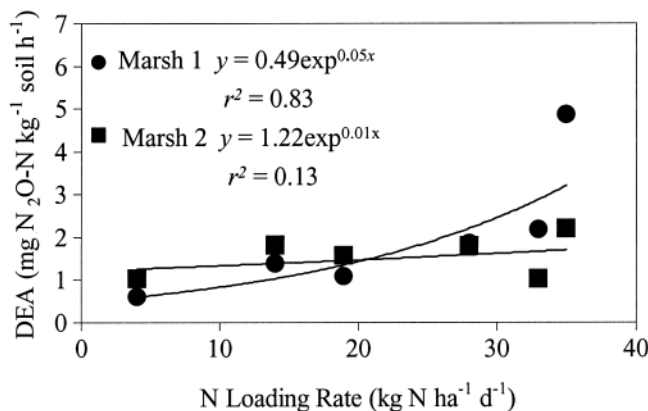


Fig. 2. Denitrification enzyme activity in nitrate-amended marsh soils with varying wastewater N applications.

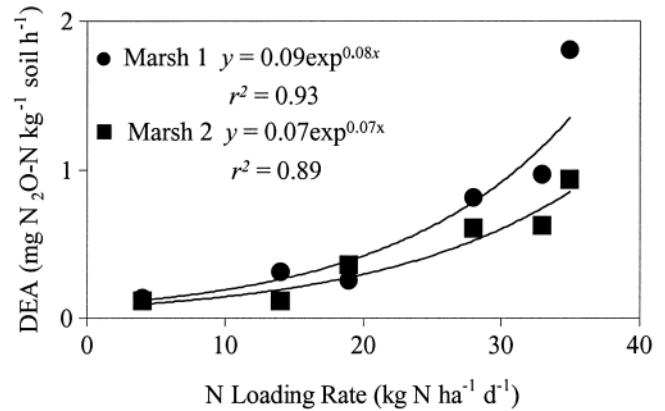


Fig. 3. Denitrification enzyme activity in glucose-amended marsh soils with varying wastewater N applications.

nificant and positive response to increased application of N [DEA =  $0.49\exp(0.05x)$ , where  $x = \text{kg N ha}^{-1} \text{d}^{-1}$ ;  $r^2 = 0.83$ ]. This response indicated that there was a significant increase in the denitrifying population with the addition of higher levels of wastewater N and that there was insufficient nitrate in the control soil samples for the expression of the full denitrifying potential. This increase in the denitrification population in the first marsh may have also resulted from either the higher application of soluble C associated with the wastewater or possibly higher plant C associated with plant growth and exudations. In the second marsh, the DEA response to nitrate addition was very different. Denitrification in the treatments with lower loading rates increased relative to the control with the addition of nitrate. However, there was little difference between the control and the nitrate addition in the treatments with higher loading rates. Thus, with the addition of nitrate, there was a somewhat uniform DEA rate at all levels of N application; DEA was generally above  $1.0 \text{ mg N}_2\text{O-N kg}^{-1} \text{ soil h}^{-1}$  and not well correlated to increases in wastewater N applications [DEA =  $1.22\exp(0.01x)$ , where  $x = \text{kg N ha}^{-1} \text{d}^{-1}$ ;  $r^2 = 0.13$ ].

When C was added, the DEA in both marshes increased as wastewater N application increased (Fig. 3). Furthermore, their increases were somewhat similar

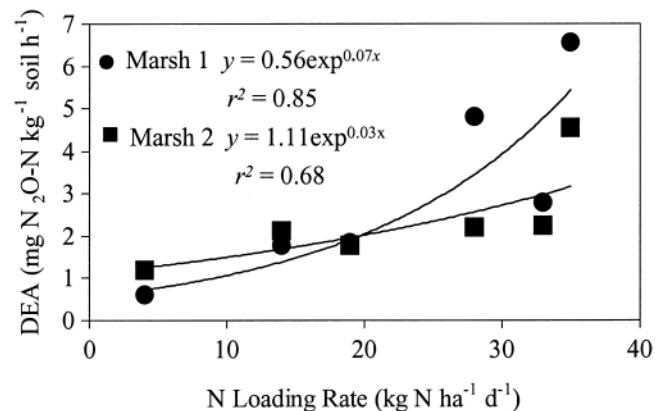


Fig. 4. Denitrification enzyme activity in nitrate and glucose-amended marsh soils with varying wastewater N applications.

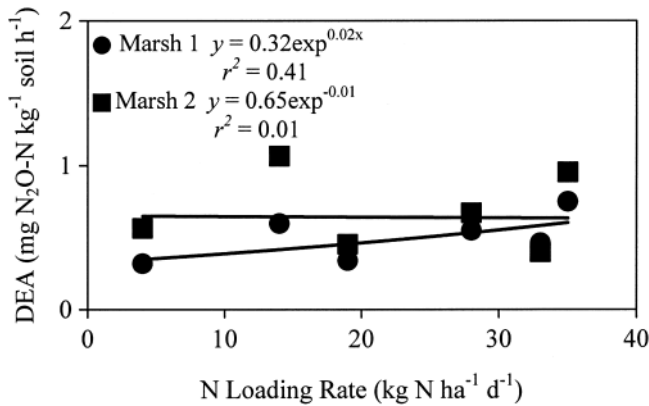


Fig. 5. Denitrification enzyme activity in nitrate and glucose-amended marsh soils without acetylene blockage at varying wastewater N applications.

to that of the control. The first marsh had a slightly higher correlation [DEA =  $0.09\exp(0.08x)$ , where  $x = \text{kg N ha}^{-1} \text{ d}^{-1}$ ;  $r^2 = 0.93$ ]. The second marsh had only slightly lower DEA levels and correlation [DEA =  $0.07\exp(0.07x)$ , where  $x = \text{kg N ha}^{-1} \text{ d}^{-1}$ ;  $r^2 = 0.89$ ]. Thus, C was not a significant limiting factor for denitrification if the nitrate was not increased.

When both nitrate and C were added, DEA levels were again positively correlated to increased wastewater N applications (Fig. 4). The correlation was strongest in the first marsh [DEA =  $0.56\exp(0.07x)$ , where  $x = \text{kg N ha}^{-1} \text{ d}^{-1}$ ;  $r^2 = 0.85$ ]. While the correlation in the second marsh was not as strong [DEA =  $1.11\exp(0.03x)$ , where  $x = \text{kg N ha}^{-1} \text{ d}^{-1}$ ;  $r^2 = 0.68$ ], it was substantially better correlated than the increase due to addition of nitrate alone. The nitrous oxide accumulation values for the treatment with nitrate and C but no acetylene were very poorly correlated to wastewater N loading rates (Fig. 5). The values were all  $<1 \text{ mg N}_2\text{O-N kg}^{-1} \text{ soil h}^{-1}$ . These data, thus, indicated that the percentage of incomplete denitrification decreased as the wastewater N load increased.

## CONCLUSIONS

1. Denitrification enzyme activity levels in the marsh section of the MPM wetlands were somewhat similar to those found in continuous marsh wetlands. In the control treatment, they ranged from 0.06 to 1.13 and 0.16 to 0.79  $\text{mg N}_2\text{O-N kg}^{-1} \text{ soil h}^{-1}$ , in the first and second marshes, respectively.
2. The DEA rate was significantly increased with the addition of nitrate in both marshes. This response was consistent with that expected for a generally reductive (low  $E_H$ ) environment where oxygen availability limited nitrification.
3. In contrast to nitrate, the DEA rate was not consistently increased by addition of C, which was supplied from both the applied wastewater and accumulated plant dry matter.
4. The DEA in both the control and the amended treatments increased dramatically with increased wastewater N loading. The increases were generally more pronounced in the first marsh.

5. The nitrous oxide produced in the absence of acetylene did not increase with wastewater N loading rate, indicating that the increased denitrification with increased load was primarily proceeding to dinitrogen gas.
6. Improved denitrification and nitrogen treatment could likely be obtained by nitrification of the wastewater because this would both reduce the potential for ammonia volatilization as well as provide the nitrate necessary for denitrification.
7. This nitrification could be obtained by a retrofit of the pond section, which seems to be the most promising avenue for using the marsh-pond-marsh wetlands for livestock wastewater treatment.

## REFERENCES

- Ambus, P., and R.R. Lowrance. 1991. Comparison of denitrification in two riparian soils. *Soil Sci. Soc. Am. J.* 55:994-997.
- Cathcart, T.P., D.A. Hammer, and S. Triyono. 1994. Performance of a constructed wetland-vegetated strip system used for swine waste treatment. p. 9-22. In P.J. DuBow and R.P. Reaves (ed.) *Constructed wetlands for animal waste management*. Purdue Research Foundation, W. Lafayette, IN.
- Conrad, R. 1996. Soil microorganisms as controllers of atmospheric trace gases ( $\text{H}_2$ , CO,  $\text{CH}_4$ , OCS,  $\text{N}_2\text{O}$ , and NO). *Microbiol. Rev.* 60: 609-640.
- Fey, A., G. Benckiser, and J.C.G. Ottow. 1999. Emissions of nitrous oxide from a constructed wetland using a groundfilter and macrophytes in waste-water purification of a dairy farm. *Biol. Fertil. Soils* 29:354-359.
- Hunt, P.G., A.A. Szogi, F.J. Humenik, G.B. Reddy, M.E. Poach, E.J. Sadler, and K.C. Stone. 2000. Treatment of swine wastewater in wetlands with natural and agronomic plants. p. 187-192. In Proc. 9th Int. Workshop of the European Cooperative Research Network (RAMIRAN), Gargnano, Italy, 6-9 September. European Coop. Res. Network.
- Hunt, P.G., A.A. Szogi, F.J. Humenik, J.M. Rice, T.A. Matheny, and K.C. Stone. 2002. Constructed wetlands for treatment of swine wastewater from an anaerobic lagoon. *Trans. ASAE* 45: 639-647.
- Hunt, P.G., T.A. Matheny, and A.A. Szogi. 2003. Denitrification in constructed wetlands used for treatment of swine wastewater. *J. Environ. Qual.* 32:727-735.
- Hunt, P.G., T.A. Matheny, and K.C. Stone. 2004. Denitrification in a coastal plain riparian zone contiguous to a heavily loaded swine wastewater spray field. *J. Environ. Qual.* 33:2367-2374.
- Hunt, P.G., M.E. Poach, and S.K. Liehr. 2005. Nitrogen cycling in wetland systems. p. 93-104. In E.J. Dunne and K.R. Reddy (ed.) *Nutrient management of agricultural watersheds: A wetlands solution*. Wageningen Academic Publishers.
- Kadlec, R.H., and R.L. Knight. 1995. *Treatment Wetlands*. Lewis Publishers, Boca Raton, FL.
- Kadlec, R.H., and F. Bishay. 2005. Wetland treatment at Musselwhite Mine, Ontario, Canada. p. 176-198. In J. Vymazal (ed.) *Nutrient Cycling and Retention in Natural and Constructed Wetland*. Backhuys Publishers, Leiden, the Netherlands.
- Knight, R.L., V.W.E. Payne, Jr., R.E. Borer, R.A. Clarke, Jr., and J.H. Pries. 2000. Constructed wetland for livestock wastewater management. *Ecological Eng.* 15:41-55.
- Kopp, J.F., and G.D. McKee. 1983. *Methods for chemical analysis of water and wastes*. Rep. EPA-600/4-79020. Environ. Monitoring and Support Lab., Office of Res. and Development, USEPA, Cincinnati, OH.
- Malone, J.P., R.J. Stevens, and R.J. Laughlin. 1998. Combining the  $^{15}\text{N}$  and acetylene inhibition techniques to examine the effect of acetylene on denitrification. *Soil Biol. Biochem.* 30:31-37.
- McCasky, T.A., S.N. Britt, T.C. Hannah, J.T. Eason, V.W.E. Payne, and D.A. Hammer. 1994. Treatment of swine lagoon effluent by constructed wetlands operated at three loading rates. p. 23-33. In P.J. DuBow and R.P. Reaves (ed.) *Constructed wetlands*

- for animal waste management. Purdue Research Foundation, W. Lafayette, IN.
- Poach, M.E., P.G. Hunt, E.J. Sadler, T.A. Matheny, M.H. Johnson, K.C. Stone, and F.J. Humenik. 2002. Ammonia volatilization from constructed wetlands that treat swine wastewater. *Trans. ASAE* 45:619–627.
- Poach, M.E., P.G. Hunt, M.B. Vanotti, K.C. Stone, T.A. Matheny, M.H. Johnson, and E.J. Sadler. 2003. Improved nitrogen treatment by constructed wetlands receiving partially nitrified liquid swine manure. *Ecological Eng.* 20:183–197.
- Poach, M.E., P.G. Hunt, G.B. Reddy, K.C. Stone, T.A. Matheny, M.H. Johnson, and E.J. Sadler. 2004a. Ammonia volatilization from marsh-pond-marsh constructed wetlands treating swine wastewater. *J. Environ. Qual.* 33:844–851.
- Poach, M.E., P.G. Hunt, G.B. Reddy, K.C. Stone, M.H. Johnson, and A. Grubbs. 2004b. Swine wastewater treatment by marsh-pond-marsh constructed wetlands under variable nitrogen loads. *Ecological Eng.* 23:165–175.
- Reddy, G.B., P.G. Hunt, R. Phillips, K.C. Stone, and A. Grubbs. 2001. Treatment of swine wastewater in marsh-pond-marsh constructed wetlands. *Water Sci. Technol.* 44:545–550.
- SAS Institute. 1999. SAS Version 8.2. SAS Inst., Cary, NC.
- Silvan, N., E-S. Tuittila, V. Kitunen, H. Vasander, and J. Laine. 2005. Nitrate uptake by *Eriophorum vaginatum* controls N<sub>2</sub>O production in a restored peatland. *Soil Biol. Biochem.* 37:519–526.
- Stone, K.C., P.G. Hunt, A.A. Szogi, F.J. Humenik, and J.M. Rice. 2002. Constructed wetland design and performance for swine lagoon wastewater treatment. *Trans. ASAE* 45:723–730.
- Szogi, A.A., P.G. Hunt, and F.J. Humenik. 2000. Treatment of swine wastewater using a saturated-soil-culture soybean and flooded rice system. *Trans. ASAE* 43:327–335.
- Szogi, A.A., P.G. Hunt, E.J. Sadler, and D.E. Evans. 2004. Characterization of oxidation-reduction processes in constructed wetlands for swine wastewater treatment. *Applied Eng. Agric.* 20: 189–200.
- Tiedje, J.M. 1994. Denitrifier enzyme activity (DEA). p. 256–257. *In* R.W. Weaver et al. (ed.) *Methods of Soil Analysis*. Part 2. 2nd ed. SSSA Book Ser. 5. SSSA, Madison, WI.