Ecological Engineering 35 (2009) 1514–1522

Contents lists available at ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng

Denitrification of nitrified and non-nitrified swine lagoon wastewater in the suspended sludge layer of treatment wetlands☆

P.G. Hunt*, K.C. Stone, T.A. Matheny, M.E. Poach, M.B. Vanotti, T.F. Ducey

Coastal Plains Soil, Water, and Plant Research Center, USDA-ARS, 2611 W. Lucas Street, Florence, SC 29501, United States

ARTICLE INFO

Article history:
Received 30 September 2008
Received in revised form 13 June 2009
Accepted 3 July 2009

Keywords:
Denitrification
Nitrification
DEA
Suspended sludge
Nitrous oxide
Swine wastewater
Treatment wetlands

ABSTRACT

One method for managing livestock-wastewater N is the use of treatment wetlands. The objectives of this study were to (1) assess the magnitude of denitrification enzyme activity (DEA) in the suspended sludge layers of bulrush and cattail treatment wetlands, and (2) evaluate the impact of nitrogen pretreatment on DEA in the suspended sludge layer. The study used four wetland cells (3.6 m × 33.5 m) with two cells connected in series. Each wetland series received either untreated or partially nitrified swine wastewater from a single-cell anaerobic lagoon. The DEA of the suspended sludge layers of the constructed wetlands was measured by the acetylene inhibition method. The control DEA treatment for the sludge layer had a mean rate of 18 $\mu g\ N_2O\text{-}N\ g^{-1}$ sludge h^{-1}. Moreover, the potential DEA (nitrate-N and glucose-C added) mean was very large, 121 $\mu g\ N_2O\text{-}N\ g^{-1}$ sludge h^{-1}. These DEA rates are consistent with the previously reported high levels of nitrogen removal by denitrification from these wetlands, especially when the wastewater was partially nitrified. Stepwise regression using distance within the wetland, wastewater nitrate, and wastewater ammonia explained much of the variation in DEA rates. In both bulrush and cattail wetlands, there were zones of very high potential DEA.

Published by Elsevier B.V.

1. Introduction

Animal production plays a vital role in USA agriculture, both in terms of economic prosperity and food stability. However, the concentration of animal feeding operations makes the treatment of the resultant wastes a more complex matter than the historical land spreading of manures on croplands (Sánchez and González, 2005; Peu et al., 2007; Vanotti et al., 2007; Gilley et al., 2008; Stone et al., 2008). Prior to land spreading, swine wastewater producers often treat the wastewater via anaerobic lagoons (Bicudo et al., 1999; Westerman and Bicudo, 2002). This practice proves effective as long as sufficient land is available for balanced application of nutrients and reasonably non-offensive odors (Stone et al., 1995; Vanotti et al., 2007; Lopez-Ridaura et al., 2009). If land application rates exceed crop uptake rates, excess nutrients can produce elevated greenhouse emissions along with contaminated surface and ground waters (Stone et al., 1998; Bender and Wood, 2007; Gilley et al., 2007; Dukes and Evans, 2006). Thus, there has been interest in practices that can keep the nutrient load in balance with the available cropland.

For the past several decades, wetlands have been utilized for the treatment of agricultural, municipal, and residential wastewaters (Hammer, 1989; Kadlec and Knight, 1996; Knight et al., 2000; Meers et al., 2008; Mustafa et al., 2009). For the treatment of these wastewaters, wetlands were considered to be natural, operationally passive, relatively cost effective, and simple in design and operation. In regard to animal wastewater, the use of constructed wetlands has been particularly effective in reducing nutrient mass load, especially N; the associated result has been the reduction of cropland necessary to assimilate the remaining nutrients. These treatment wetlands have been reported to remove N at 70–95% efficiency when N loading rates were in the range of 3–36 $kg\ ha^{-1}\ d^{-1}$ (Hunt et al., 2002). Adsorption, ammonia volatilization, microbial and plant assimilation, nitrification–denitrification, and sedimentation are a few of the ways by which constructed wetlands removed N from wastewater (Vymazal, 2007).

In regard to ammonia volatilization, it has been previously demonstrated that ammonia volatilization rate can be responsible for an appreciable rate of N removal, 7–16% (Poach et al., 2002). However, ammonia volatilization was not responsible for the removal of the majority of N from swine wastewater treated in constructed wetlands (Poach et al., 2002, 2004). These results coincide with other research results that indicate denitrification was likely the process responsible for the majority of the N removal in

☆ The mention of firm names or trade products does not imply that they were endorsed or recommended by the U.S. Department of Agriculture over other firm names or similar products not mentioned.

* Corresponding author. Tel.: +1 843 669 5203x100; fax: +1 843 669 6970.
E-mail address: Patrick.Hunt@ars.usda.gov (P.G. Hunt).

0925-8574/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.ecoleng.2009.07.001

the soils of constructed wetlands (Hunt et al., 2002; Dong and Sun, 2007). Additional research by Poach et al. (2003) revealed that partially nitrified animal wastewater was more amenable to treatment in constructed wetlands than unaltered wastewater. They found that inflow nitrate was very effectively removed in the first portion of the wetland system. They concluded that very high rates of denitrification were likely occurring. Although no assessments of denitrification were reported, DEA measurements within the wetlands were made for both the partially nitrified and unaltered swine wastewater treatments. The suspended sludge layer was found to be particularly important for denitrification. This paper reports the results of the DEA assessments of the suspended sludge layer in the treatment wetlands reported by Poach et al. (2003). The specific objectives were to (1) assess the magnitude of DEA in the sludge layers in both bulrush and cattail treatment wetlands; (2) evaluate the impact of nitrogen pretreatment on DEA in the suspended sludge layer.

2. Materials and methods

2.1. Study location

The study was conducted from July 2000 through August 2001 on treatment wetlands at a swine farm in Duplin County, North Carolina. The farm included a 2600-pig nursery with an average pig weight of 13 kg. Waste generated in the swine facility was flushed to a single-stage anaerobic lagoon with a volume of 4100 m^3 and a residence time of 120 d.

2.2. Constructed wetland design

A schematic of the entire pre-wetland nitrification and treatment wetland system is presented in Fig. 1. Additionally, the system is described in more detail in previous publications (Hunt et al., 2003; Poach et al., 2003). The constructed wetland systems consisted of two parallel wetland systems, each containing two wetland cells (3.6 m × 33.5 m), connected in series. The cells were constructed in 1992 by removal of the topsoil, grading to a 0.2% slope, sealing the cell bottoms with 0.30 m of compacted clay, and covering with 0.25 m of loamy sand topsoil. The first cell in each series received inflow of wastewater from the lagoon and fresh ground water; the second cell in each series received wastewater from the outflow of the first cell. The effluent of the second cell was pumped back to the lagoon.

Wetland System 1 was planted with *Schoenoplectus tabernaemontani* (K.C. Gmel.) Palla (softstem bulrush), *Schoenoplectus americanus* (Pers.) Volkart ex Schinz & R. Keller (American bulrush), *Scirpus cyperinus* (L.) Kunth (woolgrass bulrush), and *Juncus effusus* L. (soft-rush). Wetland System 1 will be hereafter referred to as bulrush wetlands. Wetland System 2 was planted with *Typha latifolia* L. (broadleaf cattail), *Typha angustifolia* L. (narrowleaf cattail), and *Sparganium americanum* Nutt. (American bur-reed). Wetland System 2 will be hereafter referred to as cattail wetlands. At the time of the experiment, *Schoenoplectus* sp. and *Typha* sp. dominated their respective systems. After 7 years of operation the surface of the wetland cells contained three distinct layers: the soil, detritus, and suspended sludge. A schematic of a cell including the three layers is depicted in Fig. 2.

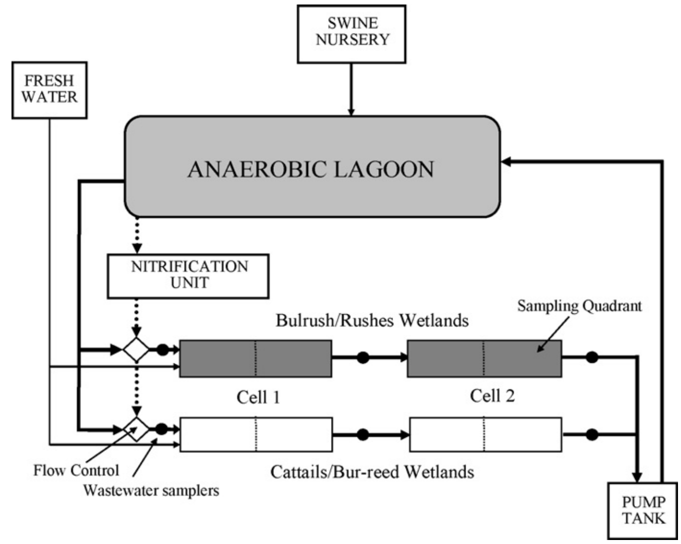

Fig. 1. Overhead view of the constructed wetlands.

The nitrified wastewater was produced in a nitrification chamber (1.3 m^3) that contained a high level of immobilized nitrifying bacteria that provided rapid nitrification (Vanotti and Hunt, 2000; Vanotti et al., 2007). During the study period, the wetlands received a cycle of standard lagoon wastewater effluent and a cycle of partially nitrified wastewater (Poach et al., 2003). Wastewater inflow into each cell of a series was measured using tipping buckets equipped with reed switches and electronic counter. Outflow from the second cell in each series was measured by use of V-notch weirs with ultrasonic depth detectors (Control Electronics, Morgantown, PA) and pressure transducers (Druck, Inc., PDCR 950, New Fairfield, CT).

2.3. Water analyses

Water samples were collected from the inlet of the first cell and the outlet of each wetland system (two cells per wetland system) by ISCO automated water samplers (ISCO Corp., Lincoln, NE). Samples were collected daily, composited weekly, and refrig-

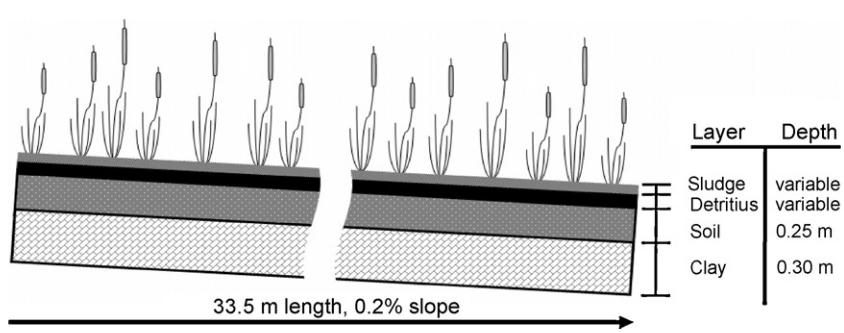

Fig. 2. Schematic of a cell from the constructed wetland experimental site. The cells were 3.6 m × 33.5 m, with a 0.2% slope (the cell is not drawn to scale). The depth of the wastewater that flowed through the detritus and sludge layers ranged from 25 to 175 mm.

erated for later analyses. Wastewater analyses were performed according to Standard Methods for the Examination of Water and Wastewater (Clesceri et al., 1998). Total suspended solids (TSS) and chemical oxygen demand (COD) were measured by Standard Methods 2540D and 5220D, respectively. Kjeldahl N (TKN), ammonia-N (NH_4-N), nitrate-N (NO_3-N), and total phosphate (TP) were measured with Standard Methods 4500-N_{org} D, 4500-NH_3-G, 4500NO_3 F, and 4500-P H, respectively.

2.4. Denitrification enzyme activity

To provide a comparison to previous investigations of DEA in the soil layer, soil samples were collected from the 0- to 25-mm soil depth of four quadrants for each wetland system on July 16, 2000, and August 15, 2001. The respective distances of each quadrant (half of one cell) from the inlet of the first wetland were (1) 0–17 m, (2) 17–34 m, (3) 34–51 m, and (4) 51–68 m. The detritial layer just above the soil surface was also sampled. The suspended sludge layer existed from the water surface to the detritus/soil layer. Samples of this sludge layer were collected from its top inch in four quadrants of each wetland system on September 9, 2000, during a cycle of nitrification for the bulrush wetlands. Similar samples were collected on August 15, 2001, during a cycle of nitrification for the cattail wetlands. Samples for each quadrant were a composite of 6–8 samples taken throughout the quadrant. The Eh and pH measurements of the sludge layer were made at the time of DEA sampling by use of a YSI multi-parameter pH/ORP meter (YSI Incorporated, Yellow Springs, OH). After collection, samples were placed in plastic bags, stored in ice, transported to the laboratory, and stored at 4 °C until analyses.

Denitrification enzyme activity (DEA) was measured by the acetylene inhibition method (Tiedje, 1994). All analyses were performed in triplicate. Field moist sludge, detritus, and soil (10–15 g) from each sample location were placed into five 60-ml serum bottles that contained 5 ml of chloramphenicol (1 g L^{-1}) to block protein synthesis. The sample received one of the following treatments:

(I) Acetylene (15 ml, produced from calcium carbide) to block denitrification at the nitrous oxide phase for measuring actual DEA—the control treatment.
(II) The control treatment plus a 5-ml amendment (200 mg L^{-1} NO_3-N) to examine nitrate limitation.
(III) The control treatment plus a 5-ml amendment (600 mg L^{-1} glucose-C) to examine C limitation.
(IV) The control treatment plus a 5-ml amendment (200 mg L^{-1} NO_3-N and 600 mg L^{-1} glucose-C) to measure potential DEA.
(V) The control treatment plus a 5-ml amendment (200 mg L^{-1} NO_3-N and 600 mg L^{-1} glucose-C) without acetylene to block denitrification at the nitrous oxide phase to measure potential incomplete denitrification.

Division of the DEA rate in treatment (V) by the DEA rate in treatment (IV) gives the percentage of potential incomplete denitrification in the system.

The serum bottles were capped with rubber septa, evacuated, and purged with purified N gas three times. Acetylene was added to the appropriate serum bottles after purging with N gas. The serum bottles were incubated on a horizontal shaker at 1.5 cycles s^{-1} and 24 °C. After 1 and 5 h of incubation, 5 ml of the headspace gases were removed from the serum bottles with a syringe (Plastipak, Franklin Lakes, NJ) and injected into vials (borosilicate glass, crimp top with butyl septum). The N_2O-N in the headspace gas was measured with a Model 3600 CX gas chromatograph (Varian, Palo Alto, CA) equipped with a 15-mCi ^{63}Ni electron capture detector operating at 350 °C. Chromatographic separation of the headspace gases was obtained by use of a 1.8-m-long × 2-mm-i.d. stainless steel column packed with Poropak Q (80–100 mesh; Alltech Associates, Deerfield, IL). The column and injector temperatures were 70 °C; and the carrier gas was purified N. Samples were injected into the column by a Model 8200 auto-sampler (Varian).

The DEA treatments for the suspended sludge layer were analyzed via analysis of variance (ANOVA) using the General Linear Model of SAS (SAS Institute, 2002). For this analysis of variance for the DEA treatments, there was pooling of the two plant communities, two nitrification treatments, and four quadrants. To better assess the influence of nitrate-N and ammonia-N on DEA, the DEA treatments of the suspended sludge layer were also analyzed by stepwise regressions. The analyses were done for both the cattail and bulrush wetlands under both nitrified and non-nitrified treatments for each of the DEA treatments (I–V). The regressed variables were (1) distance in the wetland system; (2) wastewater nitrate-N within the quadrants; and (3) wastewater ammonia-N within the quadrants. This resulted in 10 stepwise regressions for both the cattail and bulrush wetlands. All data analyses were conducted with Version 9.1 of Statistical Analysis System (SAS Institute, 2002).

3. Results and discussion

3.1. Wastewater characteristics

The lagoon wastewater used for wetland treatment during the study period was typical for a moderately loaded swine lagoon. The pH was 7.8 ± 0.3, and the TSS and the COD were 1494 ± 1062 and 1027 ± 407 mg L^{-1}, respectively. The total N content of 333 ± 89 mg L^{-1} was predominately ammonia (271 ± 72 mg L^{-1}). Conversely, the nitrate content was <1 mg L^{-1}. Ortho-phosphorus was 44 ± 1 mg L^{-1}; about 40% of the 111 ± 83 mg L^{-1} total phosphorus.

The treatment efficiencies for the wetlands during this study period were discussed in detail by Poach et al. (2003). They reported that the partially nitrified treatment was more effective in removing N than the non-nitrified treatment. This was particularly true in the first cell of the treatment wetland system, where total N removal was increased from 32 to 64% with the nitrified wastewater. There was less additional removal after the wastewater had passed through second cell where total N removal was increased from 68 to 78% with nitrified wastewater.

Additionally, the bulrush wetlands were more effective than the cattail wetlands. To provide summary insight of the treatment efficiencies associated with the DEA values reported herein, the overall mass removal treatment means are presented in Table 1. The wetlands were quite effective in removing the TSS. The TSS load ranged from 33 to 188 kg ha^{-1} d^{-1} at the inlet and 4–12 kg ha^{-1} d^{-1} at the outlet. These solids likely contributed to the formation of the active sludge layer. The COD load ranged from 103 to 120 kg ha^{-1} d^{-1} at the inlet to 74–120 kg ha^{-1} d^{-1} at the outlet. Despite the fact that the COD was removed somewhat less effectively than the TSS, it likely provided significant C to the wetland for denitrification. During the time of sampling for DEA, the wetlands were efficient in the removal of all N fractions. The total N ranged from 34 to 51 kg ha^{-1} d^{-1} at the inlet to 4–14 kg ha^{-1} d^{-1} at the outlet. Nitrate-N was essentially removed in the first cell. Conversely, as mentioned earlier, the wetlands were not effective in the removal of P (Hunt et al., 2002).

3.2. Soil and detritus layer DEA

In the assessment of DEA in the sludge layer, it was important to establish that the underlying soil layer had DEA similar to pre-

Table 1
Nutrient loading at the inlet and outlet of the bulrush and cattail constructed wetlands.

Plant type	Parameter	Nitrified[a]		Non-nitrified	
		Inlet (kg ha^{-1} d^{-1})	Outlet (kg ha^{-1} d^{-1})	Inlet (kg ha^{-1} d^{-1})	Outlet (kg ha^{-1} d^{-1})
Bulrush	TSS	33 ± 8	4 ± 3	188 ± 35	11 ± 12
	COD	103 ± 25	–	120 ± 20	46 ± 40
	Total N	34 ± 2	4 ± 3	44 ± 8	7 ± 5
	NO_3-N	9 ± 10	<1	<1	<1
	TKN	25 ± 10	3 ± 3	44 ± 8	2 ± 1
	Total P	9 ± 4	8 ± 4	16 ± 7	10 ± 8
Cattails	TSS	–	12 ± 10	115 ± 95	12 ± 7
	COD	103 ± 11	74 ± 57	107 ± 14	–
	Total N	51 ± 6	11 ± 9	37 ± 3	14 ± 4
	NO_3-N	17 ± 2	<1	<1	<1
	TKN	33 ± 4	10 ± 8	37 ± 3	14 ± 3
	Total P	7 ± 1	9 ± 5	10 ± 6	12 ± 3

[a] The nitrified sampling cycle for bulrush and cattail wetlands occurred on September 9, 2000 and August 15, 2001, respectively.

Table 2
Soil and detritus layer DEA values.

Treatment	Soil[a]			Detritus[b]		
	Mean (μg N_2O-N g^{-1} soil h^{-1})	S.D. (μg N_2O-N g^{-1} soil h^{-1})	Median (μg N_2O-N g^{-1} soil h^{-1})	Mean (μg N_2O-N g^{-1} detritus h^{-1})	S.D. (μg N_2O-N g^{-1} detritus h^{-1})	Median (μg N_2O-N g^{-1} detritus h^{-1})
I	0.6	0.6	0.5	1.1	1.6	0.4
II	1.2	0.8	0.9	3.1	2.4	2.7
III	0.5	0.5	0.5	1.3	2.2	0.3
IV	1.7	1.2	1.1	4.9	3.9	3.9
V	0.8	0.6	0.6	2.0	1.3	1.9
Mean I–IV	1.0	1.0	0.7	2.6	3.1	1.8

[a] Based on 16 soil samples.
[b] Based on 8 detritus samples.

viously reported values. Based on 80 measurements, the soil layer had a DEA mean of 1.0 ± 1.0 μg N_2O-N g^{-1} soil h^{-1} for treatments I–IV (Table 2). The median value was 0.7 μg N_2O-N g^{-1} soil h^{-1}. The control treatment had a mean of 0.6 ± 0.6 μg N_2O-N g^{-1} soil h^{-1}. Its median value was 0.5 μg N_2O-N g^{-1} soil h^{-1}. These DEA values for the soil layer are generally similar to previously reported DEA values of the soil layer in treatment wetlands (Hunt et al., 2003, 2006). They are also in the range of the DEA reported for tidal wetlands of the Potomac River and the *Hole-in-the-Donut* within Everglades National Park, 0.15–3.23 μg N_2O-N g^{-1} soil h^{-1} (Smith and Ogram, 2008; Hopfensperger et al., 2009). In those studies, the DEA of the control treatment ranged from 0.06 to 1.13 μg N_2O g^{-1} soil h^{-1}. These rates of DEA in wetland soils are substantial. For instance, the DEA of the soil layer was about 10-fold higher than the 0.059 μg N_2O-N g^{-1} soil h^{-1} DEA of riparian buffer soils of the watershed in which the treatment wetlands existed (Hunt et al., 2007).

The addition of glucose-C did not increase the DEA (0.5 ± 0.5 μg N_2O-N g^{-1} soil h^{-1}). The addition of a C source almost never increases the DEA of constructed wetlands used to treat swine lagoon wastewater. However, the C level can affect the amount of incomplete denitrification (Hunt et al., 2007). The addition of nitrate caused a modest increase in DEA to 1.2 ± 0.8 μg N_2O-N g^{-1} soil h^{-1}. This increase was consistent with the long recognized view that low nitrate typically limits denitrification in wetland systems (Reed and Brown, 1995). The addition of both nitrate-N and glucose-C (treatment IV) resulted in an increase of DEA to 1.70 ± 1.3 μg N_2O-N g^{-1} soil h^{-1}. These values were also in the range of those previously reported for both continuous marsh and marsh–pond–marsh treatment wetlands (Hunt et al., 2003, 2006).

If the acetylene blockage was not added, there was still considerable nitrous oxide production of 0.86 ± 0.62 μg N_2O-N g^{-1} soil h^{-1}. An assessment of the potential incomplete denitrification can be obtained by dividing the DEA of treatment V by DEA of treatment IV. Thus, these rates of nitrous oxide in treatment V indicated that a substantial portion (51%) of the DEA was proceeding in an incomplete manner. Incomplete soil denitrification would be consistent with a soil C/N ratio below 25 (Klemedtsson et al., 2005; Hunt et al., 2007; Ernfors et al., 2008). The mean N for the bulrush was 789 ± 297 mg kg^{-1} while the cattails had a mean of 575 ± 88 mg kg^{-1}. The mean C for the bulrush was 5295 ± 814 mg kg^{-1} while the cattails had a mean of 3955 ± 379 mg kg^{-1}. Thus, the C/N ratios were 6.6 and 6.9 for the bulrush and cattail soils, respectively. These ratios in the soil layers would be consistent with a substantive amount of incomplete denitrification.

In addition to the soil layer, there was a layer of more defined plant residue/detritial material (Table 2). Based on 60 measurements, this layer had a mean of 2.6 ± 3.1 μg N_2O-N g^{-1} detritus h^{-1} for treatments I–IV. The median DEA was 1.8 μg N_2O-N g^{-1} detritus h^{-1}. It had a DEA rate of 1.1 ± 1.6 μg N_2O-N g^{-1} detritus h^{-1} for the control treatment. When nitrate was added to our treatment wetland detritial samples, an increase in DEA was observed, 3.1 ± 2.4 μg N_2O-N g^{-1} detritus h^{-1}. As with the soil layer, the addition of C did not increase the DEA rates which were 1.3 ± 2.2 μg N_2O-N g^{-1} detritus h^{-1}. The addition of both glucose-C and nitrate-N caused the greatest increase. In this treatment, the mean DEA value was 4.9 ± 3.9 μg N_2O-N g^{-1} detritus h^{-1} for the glucose-C and nitrate-N added treatment IV. The median DEA for treatment IV was 3.9 μg N_2O-N g^{-1} detritus h^{-1}. These values were lower than the 6–18 μg N_2O-N g^{-1} detritus h^{-1} reported for the detritial layer of the Everglades wetlands by White and Reddy (2003). However, their DEA values were obtained on soil samples that had been in a 25-d aerobic-nitrification condition. When no acetylene blockage was added, the nitrous oxide accu-

Table 3
Suspended sludge layer DEA for the amendment treatments.

Treatment	Plant type[a]				All wetlands[b]		
	Bulrush		Cattail		Mean		Median ($\mu g\, N_2O$-$N\, g^{-1}$ soil h^{-1})
	DEA ($\mu g\, N_2O$-$N\, g^{-1}$ soil h^{-1})	S.D. ($\mu g\, N_2O$-$N\, g^{-1}$ soil h^{-1})	DEA ($\mu g\, N_2O$-$N\, g^{-1}$ soil h^{-1})	S.D. ($\mu g\, N_2O$-$N\, g^{-1}$ soil h^{-1})	DEA ($\mu g\, N_2O$-$N\, g^{-1}$ soil h^{-1})	S.D. ($\mu g\, N_2O$-$N\, g^{-1}$ soil h^{-1})	
I	30	24	6	7	18	20	13
II	84	26	52	22	68	29	69
III	43	48	9	12	26	38	8
IV	167	66	75	29	121	69	110
V	38	40	31	35	35	37	23
Mean (I–IV)	73	66	35	35	58	58	43

[a] Based on 8 suspended sludge samples.
[b] Based on 16 suspended sludge samples.

mulation was $2.0 \pm 1.3\, \mu g\, N_2O$-$N\, g^{-1}$ detritus h^{-1}. Although lower than the soil, this 40% potential incomplete denitrification was substantial.

3.3. Sludge layer

The overwhelmingly highest rate of DEA was found in the sludge layer (Table 3). The sludge could have developed from decayed plant material, suspended solids of the swine wastewater, bacterial cells of the heterotrophic bacterial community, or most likely, a combination of all three components. In any case for these wetland systems, it had a mean DEA of $58 \pm 58\, \mu g\, N_2O$-$N\, g^{-1}$ sludge h^{-1} for treatments I–IV. This is more that 50 times greater than the previously discussed soil mean of $1.0 \pm 0.9\, \mu g\, N_2O$-$N\, g^{-1}$ soil h^{-1}. The median DEA for the sludge was $43\, \mu g\, N_2O$-$N\, g^{-1}$ soil h^{-1}. The DEA treatments were significantly different at the $P \leq 0.05$ level via the ANOVA. The DEA for the control treatment was $18 \pm 20\, \mu g\, N_2O$-$N\, g^{-1}$ sludge h^{-1}. This was much greater than the soil DEA values reported in our previous investigations of soil DEA in swine wastewater treatment wetlands (Hunt et al., 2003, 2006). When expressed on an area basis by using the measured bulk density of 0.3275 and a 25-mm depth, the sludge mass was $8.3\, kg\, m^{-2}$. Using this mass, the DEA of this layer would be $3.7\, kg\, N_2O$-$N\, m^{-2}\, d^{-1}$. Within the standard deviation, the DEA would have ranged from 2.1 to $5.7\, g\, N_2O$-$N\, m^{-2}\, d^{-1}$. This very high level of DEA in the sludge layer was consistent with the treatment efficiency of the treatment wetlands: these findings would be expected given the reported very high rates of nitrate removal. Poach et al. (2003) reported that the nitrates in the nitrified wastewater entering either the cattail or bulrush wetlands were typically removed within the first 4 m. They noted that this would be equivalent to a removal rate for the first 4 m of 10 to $19\, g\, N\, m^{-2}\, d^{-1}$.

DEA rates of this magnitude were obtained in the nitrate-added DEA treatment II; the mean was $68 \pm 29.0\, \mu g\, N_2O$-$N\, g^{-1}$ sludge h^{-1}. If this DEA is expressed on a square-meter area basis and a 25-mm sludge depth basis (i.e., $0.025\, m^3$), the DEA for the nitrate-added treatment would be $14 \pm 18\, g\, N_2O$-$N\, m^{-2}$ wetland surface d^{-1}. While there are large variations inherent to scaling up, it is evident that the mean value is very close to the mean denitrification value of $14.4\, g\, N\, m^{-2}\, d^{-1}$ for the first 4 m determined by Poach et al. (2003). Accordingly, these data provide evidence that they were likely correct in their assertion that a large portion of the N was removed via denitrification, particularly when the wastewater was partially nitrified. The addition of C resulted in very little increase in DEA, $26 \pm 38\, \mu g\, N_2O$-$N\, g^{-1}$ sludge h^{-1}. However, the addition of both C and N revealed an extremely high level of potential DEA, $121 \pm 69\, \mu g\, N_2O$-$N\, g^{-1}$ sludge h^{-1}. Furthermore, the majority of this denitrification was complete (75%). The mean for the no-acetylene treatment was $35 \pm 19.4\, \mu g\, N_2O$-$N\, g^{-1}$ sludge h^{-1}. Whereas the vast majority of denitrification appeared to be associated with the sludge layer, it is reasonable to assume that most of the nitrogen was lost as di-nitrogen gas.

These DEA values suggest that the wetlands were easily capable of denitrification in the range of the $2.5\, g\, m^{-2}\, d^{-1}$ reported for wood-based denitrification drainage water reactors (Van Driel et al., 2006). Moreover, they were also well in the range of the 7–$10\, g\, N\, m^{-3}\, d^{-1}$ for a porous wood-based filter called "nitrex" when used for a septic system (Robertson et al., 2005). However, even the highest rate of the sludge layer was an order of magnitude lower than that of a denitrification-sludge that was immobilized in polyvinyl alcohol and used in a drainage water bioreactor (Hunt et al., 2008). Their sludge was developed from an inoculum from a contiguous overland flow treatment system at the same treatment site. Thus, in addition to very good N treatment, the sludge layer of swine wastewater treatment wetlands could potentially provide broadly useful treatment inoculum.

3.4. Impact of nitrification pretreatment on nitrate-N and ammonia-N within the treatment wetlands

There was relatively little correlation with either nitrate-N or ammonia-N vs. distance for the cattail wetlands (Table 4). Similarly, there was little correlation with nitrate-N vs. distance for the bulrush wetlands. However, for bulrush wetlands, ammonia in the wastewater flowing through the wetlands was very different—there was substantial removal of ammonia with increased distance through the wetlands. With pre-wetland nitrification, there was a very good correlation between ammonia-N and distance (mg NH_4-N = −0.91 m + 58; $R^2 = 0.97$). Likewise, when the wastewater was non-nitrified before passing through the wetlands, there was also good removal of ammonia-N (mg NH_4-N = −0.65 m + 67; $R^2 = 0.96$). This would be consistent with the generally high oxidative/reductive condition of the bulrush wetlands suspended sludge layer relative to the cattail wetlands, -15 ± 68 mV vs. -52 ± 37 mV, respectively. A similar difference for the wetland soil Eh has been reported by Szogi et al. (2004).

3.5. Impact of nitrogen pretreatment on the sludge layer DEA

The sludge layer was further analyzed for the cattail and bulrush wetlands under both nitrified and non-nitrified conditions by stepwise regression. The parameters used in the stepwise regression were "distance from the first wetland inlet" along with wastewater ammonia-N and nitrate-N content. The wastewater nitrogen components and DEA values for cattail and bulrush wetlands are presented in Tables 4–6. With the stepwise analyses, there was a first step linear regression of the best linear fitted variable. Subsequently, the regression was expanded by stepwise regressions to

Table 4
Nitrate-N and ammonia-N of the wastewater effluent through the cattail treatment wetland.

Wastewater treatment	Distance (m)	Cattail[a]				Bulrush[a]			
		NO_3		NH_3		NO_3		NH_3	
		Mean ($mg\,L^{-1}$)	S.D.	Mean ($mg\,L^{-1}$)	S.D.	Mean ($mg\,L^{-1}$)	S.D.	Mean ($mg\,L^{-1}$)	S.D.
Nitrified	8.4	4.3	5.5	56.6	28.5	8.2	1.9	53.3	13.9
	25.1	8.5	11.2	58.3	25.6	0.0	0.0	33.5	11.6
	41.9	0.7	0.6	59.0	8.9	0.6	0.0	15.7	3.1
	58.6	0.2	0.0	54.8	7.2	0.0	0.0	8.2	2.6
Non-nitrified	8.4	0.2	0.3	88.3	21.3	0.6	0.2	59.9	15.4
	25.1	0.1	0.0	78.0	16.0	1.6	0.8	52.2	18.4
	41.9	0.3	0.5	86.4	2.9	1.2	0.7	43.1	21.0
	58.6	0.2	0.0	71.0	0.8	1.4	1.1	26.7	3.8

[a] Two or three replicates.

determine if additional parameters provided significant improvement to the regression. Generally, one or more of the three variables were significant for the linear regression with a P value of ≤ 0.05. Additionally, the Cp values for the final step of the stepwise regressions were typically near the desired value that corresponded to the number of variables used in the final regression step. Thus, these Mallow's Cp values were consistent with an acceptably low collinearity in the stepwise regression model. Generally, if the R^2 was ≥ 0.70 for the first step, this linear regression formula was presented.

3.6. Cattails

For the nitrified cattail wetlands with the control DEA treatment (I), none of the variables provided good prediction ($R^2 < 0.28$) (Table 7). Moreover, the DEA values were somewhat low in the first cell. Yet, there was rapid consumption of the nitrate within the first 4 m as describe by Poach et al. (2003). This rapid removal of nitrate-N was consistent with the rapid consumption of nitrate in the typically reduced oxidative/reductive environment of cattails (Szogi et al., 2004; Gebremariam and Beutel, 2008). The low DEA values suggest that another microbial process might have been involved (Hunt et al., 2003; Raghoebarsing et al., 2006; Sumino et al., 2006; Dong and Sun, 2007). For the control DEA treatment when the wastewater was not nitrified, distance through the wetlands was effective in predicting the DEA value (DEA = 0.44 m − 6.3; $R^2 = 0.72$). The DEA increased as the effluent moved through the wetlands. This response was likely related to within wetland nitrification prior to denitrification.

In the nitrate-added treatment (II) when the wastewater was nitrified, distance provided good predictions of DEA (DEA = 1.19 m + 17.1; $R^2 = 0.70$). With the inclusion of ammonia-N, the stepwise regression improved the R^2 to 0.82. When the wastewater was not nitrified, the nitrate-added DEA treatment was again well predicted by distance (DEA = 0.66 m + 26.4; $R^2 = 0.72$). With the inclusion of ammonia-N in the stepwise regression, the accounting for DEA variation became very good ($R^2 = 0.94$).

In treatment III, the DEA rates in sludge layer of neither the nitrified nor non-nitrified wastewater were well predicted by the stepwise regression; the R^2 values were below 0.36. However, when the data were log transformed, there was good correlation of DEA with distance for both the nitrified and non-nitrified wastewater treatments. The non-nitrified wastewater treatment had an R^2 value of 0.71 (DEA = 0.06 m − 0.40). The nitrified wastewater treatment also had an R^2 value of 0.71 (DEA = 0.03 m + 3.24). For both the nitrified and non-nitrified wastewater treatments, it was the higher value of DEA in the last quadrant that likely made the relation change to logarithmic.

With the nitrate- and carbon-added treatment (IV) when the wastewater was nitrified, regressions were similar to the nitrate-added treatment. Distance was again a good predictor (DEA = 1.39 m + 22.2; R^2 of 0.69). Moreover, the inclusion of ammonia-N and nitrate-N in the stepwise regression improved the R^2 to 0.93. When the wastewater was not nitrified, distance provided an R^2 of 0.44. The inclusion of ammonia-N and nitrate-N to the analysis improved the R^2 to 0.90.

The DEA treatment (V) without acetylene provided insight into the amount of incomplete denitrification. As with treatment IV for the nitrified wastewater, the amount of nitrous oxide production increased substantially with distance. There was good linear correlation of the nitrous oxide production in the absence of acetylene in the nitrified wastewater treatment with distance (N_2O-N = 1.35 m + 12.7; $R^2 = 0.72$). In the first quadrant, the incomplete denitrification was approximately 50%, and it increased to over 90% in the last quadrant. In contrast, the non-nitrified wastewater pretreatment had only an R^2 of 0.32 for distance vs. DEA in treatment V. This poor correlation was likely related to the very low nitrous oxide production—none of the quadrants exceeded 8 $\mu g\ N_2O\text{-}N\ g^{-1}$ sludge h^{-1}.

This is a very interesting result because the means for the potential DEA (treatment IV) of the nitrified and non-nitrified treatments were somewhat similar, 68.7 ± 32 and 81.5 ± 24.5 $\mu g\ N_2O\text{-}N\ g^{-1}$ sludge h^{-1}, respectively. Yet, the means for treatment V of the nitrified and non-nitrified were extremely different, 58.0 ± 31.3 and 4.4 ± 2.6 $\mu g\ N_2O\text{-}N\ g^{-1}$ sludge h^{-1}, respectively. The reason for this difference in potential incomplete denitrification is not clear. The COD values of the influent wastewater were about the same, but the differences may have been caused by something other than the carbon or nitrogen. In any case, it is evident that the potential for incomplete denitrification in the cattail wetlands was affected by wastewater and wetland conditions.

3.7. Bulrush

When the DEA values of the bulrush wetland were analyzed via stepwise regression using the same variables (distance from the first wetland inlet along with wastewater effluent ammonia-N and nitrate-N content), they were somewhat effective in explaining treatment variation (Table 8). As with the cattail wetlands, the Cp values were generally similar to the number of variables in the final step.

In the case of the nitrified bulrush wetlands with the control DEA treatment (I), nitrate-N was a good predictor; it provided an R^2 of 0.92. This regression was dominated by the high nitrate and DEA in the first quadrant. When the wastewater was not nitrified, most of the variation in DEA was explained by ammonia-N.

Table 5
DEA by distance of the sludge layer for cattail plant type wetlands.

WT[a]	Distance[b] (m)	DEA[c]									
		I		Ii		III		IV		V	
		Mean ($\mu g N_2O$-$N g^{-1}$ soil h^{-1})	S.D.[d] ($\mu g N_2O$-$N g^{-1}$ soil h^{-1})	Mean ($\mu g N_2O$-$N g^{-1}$ soil h^{-1})	S.D. ($\mu g N_2O$-$N g^{-1}$ soil h^{-1})	Mean ($\mu g N_2O$-$N g^{-1}$ soil h^{-1})	S.D. ($\mu g N_2O$-$N g^{-1}$ soil h^{-1})	Mean ($\mu g N_2O$-$N g^{-1}$ soil h^{-1})	S.D.	Mean ($\mu g N_2O$-$N g^{-1}$ soil h^{-1})	S.D. ($\mu g N_2O$-$N g^{-1}$ soil h^{-1})
Nitrified[d]	8.4	1.0	0.2	23.2	10.6	1.0	0.4	28.3	12.5	14.9	5.0
	25.1	1.5	0.4	44.6	13.4	2.1	0.6	52.3	1.1	54.4	2.6
	41.9	5.5	4.2	83.7	8.8	3.1	0.6	106.5	3.6	81.2	29.1
	58.6	11.6	14.6	76.7	16.6	24.0	26.2	87.9	14.7	81.5	7.6
	Mean	4.9	7.9	57.1	27.8	7.6	14.9	68.7	32.8	58.0	31.3
Non-nitrified[d]	8.4	1.0	0.0	30.0	2.6	1.3	0.2	54.5	14.3	1.3	0.1
	25.1	1.3	1.1	37.7	2.8	2.2	1.3	71.9	12.6	4.6	3.5
	41.9	7.5	4.5	65.2	6.1	7.4	3.8	114.1	4.2	7.0	0.1
	58.6	23.3	7.3	57.9	4.0	31.7	36.5	85.6	4.9	4.5	0.6
	Mean	8.3	10.2	47.7	15.7	10.6	19.2	81.5	24.5	4.4	2.6

[a] Wastewater treatment.
[b] Distance from the inlet of the first cell to the middle of the quadrant.
[c] Treatment I = control, II = nitrate added, III = glucose added, IV = nitrate and glucose added and V = treatment IV without acetylene.
[d] The number of measurements was 3 for the nitrified and 2 for the non-nitrified.

Table 6
DEA by distance of the sludge layer for the bulrush plant type wetlands.

WT[a]	Distance[b] (m)	DEA[c]									
		I		II		III		IV		V	
		Mean ($\mu g N_2O$-$N g^{-1}$ soil h^{-1})	S.D.[d] ($\mu g N_2O$-$N g^{-1}$ soil h^{-1})	Mean ($\mu g N_2O$-$N g^{-1}$ soil h^{-1})	S.D. ($\mu g N_2O$-$N g^{-1}$ soil h^{-1})	Mean ($\mu g N_2O$-$N g^{-1}$ soil h^{-1})	S.D. ($\mu g N_2O$-$N g^{-1}$ soil h^{-1})	Mean ($\mu g N_2O$-$N g^{-1}$ soil h^{-1})	S.D. ($\mu g N_2O$-$N g^{-1}$ soil h^{-1})	Mean ($\mu g N_2O$-$N g^{-1}$ soil h^{-1})	S.D. ($\mu g N_2O$-$N g^{-1}$ soil h^{-1})
Nitrified	8.4	71.9	1.3	123.9	8.7	80.6	25.1	278.4	47.9	31.2	37.3
	25.1	20.5	10.2	71.9	0.7	21.4	8.0	173.4	17.2	6.4	1.5
	41.9	22.1	2.6	100.1	27.5	7.6	5.3	187.1	3.4	4.4	0.1
	58.6	13.8	14.2	41.3	1.7	1.7	0.5	134.1	17.9	10.9	0.1
	Mean	32.1	25.7	84.3	34.8	27.8	35.0	193.3	60.1	13.2	18.1
Non-nitrified	8.4	4.4	3.0	99.6	65.4	7.7	6.3	232.2	16.7	131.2	68.0
	25.1	13.7	13.4	67.4	39.0	7.7	5.7	67.5	19.2	31.7	14.6
	41.9	35.2	17.0	100.2	62.6	107.9	58.9	144.3	100.6	49.0	32.7
	58.6	58.1	21.4	69.7	35.4	109.0	7.6	122.4	40.3	42.2	18.5
	Mean	27.8	25.3	84.2	47.6	58.1	58.5	141.6	78.1	63.5	53.3

[a] Wastewater treatment.
[b] Distance from the inlet of the first cell.
[c] Treatment I = control, II = nitrate added, III = glucose added, IV = nitrate and glucose added and V = treatment IV without acetylene.
[d] The number of measurements was 3 for the nitrified and 2 for the non-nitrified.

Table 7
Stepwise regression of DEA for the sludge layer of the cattail wetlands.

Treatment	Nitrification	Step	Variable	Partial R^2	Model R^2	*Cp*	Prob. *F*
I	Nitrified	1	Distance	0.28	0.28	0.41	0.07
I	Non-nitrified	1	Distance	0.73	0.73	6.46	0.01
II	Nitrified	1	Distance	0.70	0.70	7.56	0.00
	Nitrified	2	NH_3	0.11	0.82	3.66	0.04
II	Non-nitrified	1	Distance	0.72	0.72	24.17	0.01
	Non-nitrified	2	NH_3	0.22	0.94	3.91	0.01
III[a]	Nitrified	1	Distance	0.71	0.71	1.68	0.01
III[a]	Non-nitrified	1	Distance	0.71	0.71	0.32	0.01
IV	Nitrified	1	Distance	0.69	0.69	30.50	0.00
	Nitrified	2	NH_3	0.19	0.87	9.70	0.01
	Nitrified	3	NO_3	0.06	0.93	4.00	0.02
IV	Non-nitrified	1	Distance	0.44	0.44	19.48	0.07
	Non-nitrified	2	NH_3	0.34	0.77	7.45	0.04
	Non-nitrified	3	NO_3	0.13	0.90	4.00	0.08
V	Nitrified	1	Distance	0.72	0.72	5.05	0.00
	Nitrified	2	NH_3	0.11	0.82	2.10	0.04
V	Non-nitrified	1	Distance	0.32	0.32	5.67	0.14

[a] Log transformed data.

Table 8
Stepwise regression of DEA for the sludge layer of the bulrush wetlands.

Treatment	Nitrification	Step	Variable	Partial R^2	Model R^2	*Cp*	Prob. *F*
I	Nitrified	1	NO_3	0.92	0.92	0.59	0.00
I	Non-nitrified	1	NH_3	0.73	0.73	0.28	0.00
II	Nitrified	1	Distance	0.57	0.57	13.51	0.03
III	Nitrified	1	NO_3	0.86	0.86	2.75	0.00
	Nitrified	2	NH_3	0.06	0.91	2.06	0.13
III	Non-nitrified	1	Distance	0.65	0.65	6.61	0.00
	Non-nitrified	2	NO_3	0.08	0.73	5.14	0.13
	Non-nitrified	3	NH_3	0.08	0.81	4.00	0.11
IV	Nitrified	1	NH_3	0.79	0.79	3.25	0.00
IV	Non-nitrified	1	NO_3	0.62	0.62	0.25	0.00
V	Nitrified	1	NO_3	0.36	0.36	0.19	0.11
V	Non-nitrified	1	NO_3	0.57	0.57	0.55	0.00

It decreased with distance through the wetland (DEA = $-1.67NH_4$-N + 103; $R^2 = 0.73$).

The stepwise regression of treatments II–IV provided relatively little additional insight for either the nitrified or non-nitrified wastewater treatments. When the carbon-added treatment (III) was used for the nitrified wetlands, nitrate-N was a good predictor (DEA = $8.69NO_3$-N + 8.67; $R^2 = 0.86$). The inclusion of ammonia-N improved the R^2 to 0.91. For the non-nitrified wastewater receiving the carbon-added treatment, distance provided modest prediction with R^2 values of 0.65. The inclusion of nitrate-N and ammonia-N provided an R^2 of 0.81. When the nitrate- and carbon-added treatment (IV) was used for the nitrified wetlands, nitrate-N provided a moderately good prediction (DEA = $14.4NO_3$ + 161.7; $R^2 = 0.79$).

The results of the stepwise regression for nitrified wastewater with treatment V were converse to those of the cattail wetlands. The variables used in the stepwise regression provided poor prediction of nitrous oxide. This was likely related to the low production of nitrous oxide in this treatment. The mean for treatment V was 13.2 ± 18.1 $\mu g\ N_2O$-N g^{-1} sludge h^{-1}. When compared to treatment IV to obtain an estimate of the percentage of incomplete denitrification, first quadrant the incomplete denitrification was 11%. In the remaining quadrants, it was <8% of treatment IV. The non-nitrified wastewater was somewhat better predicted by nitrate-N with an R^2 of 0.57. This may have been related to the fact that production of nitrous oxide in this treatment was somewhat high relative to the DEA of treatment IV. The mean was 63.5 ± 53.3 $\mu g\ N_2O$-N g^{-1} sludge h^{-1}; this level of nitrous oxide production was 45% of the DEA in treatment IV. Our data were not sufficient to show the cause of the difference in incomplete denitrification between the nitrified and non-nitrified wastewater treatments. However, they do clearly show that changes of the inflow wastewater or wetland conditions can affect the extent of potential nitrous oxide production for either the cattail or bulrush wetlands.

4. Conclusion

These wetlands had a substantial suspended sludge layer that was likely formed from a combination of decayed plant materials, cells of the heterotrophic bacterial community, and suspended solids of the swine wastewater. This suspended sludge layer had very high DEA rates. It was probably the critical component in effective nitrogen treatment by these wetlands. The control DEA treatment for the sludge layer had a mean rate of 18 $\mu g\ N_2O$-N g^{-1} sludge h^{-1}. Moreover, the potential DEA (nitrate-N and glucose-C added) mean was very large, 121 $\mu g\ N_2O$-N g^{-1} soil h^{-1}. These DEA rates are consistent with the previously reported high levels of nitrogen removal by denitrification from these wetlands, especially when the wastewater was partially nitrified. When the DEA rate was expressed on an area or volume basis, the rates were comparable to those expected for media-based treatment wetlands. The soil DEA rates were typical for those published for the soil layer; control treatment had a mean of 0.6 $\mu g\,N_2O\text{-}N\,g^{-1}$ soil h^{-1}. The potential DEA (nitrate-N and glucose-C added) mean was about double the control treatment, 1.7 $\mu g\,N_2O\text{-}N\,g^{-1}$ soil h^{-1}. In the sludge layer, the potential DEA rate of 121 $\mu g\,N_2O\text{-}N\,g^{-1}$ sludge h^{-1} was 70 times greater than in the soil. When the DEA of the suspended sludge layer within the wetland systems was assessed via stepwise regression; distance within the wetland, wastewater nitrate, and wastewater ammonia accounted for much of the variation among the DEA treatments. This was true with the nitrified and non-nitrified wastewater for both the cattail and bulrush wetland systems. The cattail DEA rates were generally lower than those of the bulrush. With either nitrified or non-nitrified wastewater, cattail wetland system DEA rates tended to increase with distance from the inlet. The reverse was often the case with the bulrush wetlands. Yet, in both wetland systems, there were zones of very high potential DEA.

References

Bender, M.R., Wood, C.W., 2007. Above and below ground measurements of greenhouse gases from swine effluent amended soil. Commun. Soil Sci. Plant Anal. 38 (17–18), 2479–2503.

Bicudo, J.R., Safley Jr., L.M., Westerman, P.W., 1999. Nutrient content and sludge volumes in single-cell recycle anaerobic swine lagoons in North Carolina. Trans. ASAE 42 (4), 1087–1093.

Clesceri, L.S., Greenberg, A.E., Eaton, A.D. (Eds.), 1998. Standard Methods for the Examination of Water and Wastewater, 20th ed. APHA, AWWA, WEF, Washington, DC, 1220 pp.

Dong, Z., Sun, T., 2007. A potential new process for improving nitrogen removal in constructed wetlands—promoting coexistence of partial-nitrification and ANAMMOX. Ecol. Eng. 31, 69–78.

Dukes, M.D., Evans, R.O., 2006. Impact of agriculture on water quality in the North Carolina Middle Coastal Plain. J. Irrig. Drain. Eng. 132 (3), 250–262.

Ernfors, M., Arnold, K.V., Stendahl, J., Olsson, M., Klemedtsson, L., 2008. Nitrous oxide emissions from drained organic forest soils—an up-scaling based on C:N ratios. Biogeochemistry 89 (1), 29–41.

Gebremariam, S.Y., Beutel, M.W., 2008. Nitrate removal and DO levels in batch wetland mesocosms: Cattail (*Typha* spp.) versus bulrush (*Scirpus* spp.). Ecol. Eng. 34 (1), 1–6.

Gilley, J.E., Eghball, B., Marx, D.B., 2007. Nutrient concentrations of runoff during the year following manure application. Trans. ASABE 50 (6), 1987–1999.

Gilley, J.E., Sabatka, W.F., Eghball, B., Marx, D.B., 2008. Nutrient transport as affected by rate of overland flow. Trans. ASABE 51 (4), 1287–1293.

Hammer, D.A. (Ed.), 1989. Constructed Wetlands for Wastewater Treatment: Municipal, Industrial and Agricultural. Lewis Publishers, Chelsea, MI, 831 pp.

Hopfensperger, K.N., Kaushal, S.S., Findlay, S.E.G., Cornwell, J.C., 2009. Influence of plant communities on denitrification in a tidal freshwater marsh of the Potomac River, United States. J. Environ. Qual. 38 (2), 618–626.

Hunt, P.G., Matheny, M.A., Ro, K.S., 2007. Nitrous oxide accumulation in soils from riparian buffers of a coastal plain watershed—carbon/nitrogen ratio control. J. Environ. Qual. 36, 1368–1376.

Hunt, P.G., Matheny, T.A., Ro, K.S., Stone, K.C., Vanotti, M.B., 2008. Denitrification of agricultural drainage line water via immobilized denitrification sludge. J. Environ. Sci. Health A 43 (9), 1077–1084.

Hunt, P.G., Matheny, T.A., Szögi, A.A., 2003. Denitrification in constructed wetlands used for treatment of swine wastewater. J. Environ. Qual. 32 (2), 727–735.

Hunt, P.G., Poach, M.E., Matheny, T.A., Reddy, G.B., Stone, K.C., 2006. Denitrification in marsh–pond–marsh constructed wetlands treating swine wastewater at different loading rates. Soil Sci. Soc. Am. J. 70 (2), 487–493.

Hunt, P.G., Szögi, A.A., Humenik, F.J., Rice, J.M., Matheny, T.A., Stone, K.C., 2002. Constructed wetlands for treatment of swine wastewater from an anaerobic lagoon. Trans. ASAE 45 (3), 639–647.

Kadlec, R.H., Knight, R.L., 1996. Treatment Wetlands. Lewis Publishers, Boca Ration, FL.

Klemedtsson, L., Von Arnold, K., Weslien, P., Gundersen, P., 2005. Soil CN ratio as a scalar parameter to predict nitrous oxide emissions. Global Change Biol. 11 (7), 1142–1147.

Knight, R.L., Payne Jr., V.W.E., Borer, R.E., Clarke Jr., R.A., Pries, J.H., 2000. Constructed wetlands for livestock wastewater management. Ecol. Eng. 15 (1–2), 41–55.

Lopez-Ridaura, S., Werf, H.V.D., Paillat, J.M., Le Bris, B., 2009. Environmental evaluation of transfer and treatment of excess pig slurry by life cycle assessment. J. Environ. Manage. 90 (2), 1296–1304.

Meers, E., Tack, F.M.G., Tolpe, I., Michels, E., 2008. Application of a full-scale constructed wetland for tertiary treatment of piggery manure: monitoring results. Water Air Soil Pollut. 193 (1–4), 15–24.

Mustafa, A., Scholz, M., Harrington, R., Carroll, P., 2009. Long-term performance of a representative integrated constructed wetland treating farmyard runoff. Ecol. Eng. 35 (5), 779–790.

Peu, P., Birgand, F., Martinez, J., 2007. Long term fate of slurry derived nitrogen in soil: a case study with a macro-lysimeter experiment having received high loads of pig slurry (Solepur). Biores. Technol. 98 (17), 3228–3234.

Poach, M.E., Hunt, P.G., Reddy, G.B., Stone, K.C., Matheny, T.A., Johnson, M.H., Sadler, E.J., 2004. Ammonia volatilization from marsh–pond–marsh constructed wetlands treating swine wastewater. J. Environ. Qual. 33 (3), 844–851.

Poach, M.E., Hunt, P.G., Sadler, E.J., Matheny, T.A., Johnson, M.H., Stone, K.C., Humenik, F.J., Rice, J.M., 2002. Ammonia volatilization from constructed wetlands that treat swine wastewater. Trans. ASAE 45 (3), 619–627.

Poach, M.E., Hunt, P.G., Vanotti, M.B., Stone, K.C., Matheny, T.A., Johnson, M.H., Sadler, E.J., 2003. Improved nitrogen treatment by constructed wetlands receiving partially nitrified liquid swine manure. Ecol. Eng. 20 (2), 183–197.

Raghoebarsing, A.A., Pol, A., Van De Pas-Schoonen, K.T., Smolders, A.J.P., Ettwig, K.F., Rijpstra, W.I.C., Schouten, S., Sinninghe Damste, J.S., Op Den Camp, H.J.M., Jetten, M.S.M., Strous, M., 2006. A microbial consortium couples anaerobic methane oxidation to denitrification. Nature 440 (7086), 918–921.

Reed, S.C., Brown, D., 1995. Subsurface flow wetlands—a performance evaluation. Water Environ. Res. 67 (2), 244–248.

Robertson, W.D., Ford, G.I., Lombardo, P.S., 2005. Wood-based filter for nitrate removal in septic systems. Trans. ASAE 48 (1), 121–128.

Sánchez, M., González, J.L., 2005. The fertilizer value of pig slurry. I. Values depending on the type of operation. Biores. Technol. 96 (10), 1117–1123.

SAS Institute, Inc., 2002. Statistical Analysis System. SAS Institute, Inc., Cary, NC.

Smith, J.M., Ogram, A., 2008. Genetic and functional variation in denitrifier populations along a short-term restoration chronosequence. Appl. Environ. Microbiol. 74 (18), 5615–5620.

Stone, K.C., Hunt, P.G., Coffey, S.W., Matheny, M.A., 1995. Water quality status of a USDA water quality demonstration project in the eastern Coastal Plain. J. Soil Water Conserv. 50 (5), 567–571.

Stone, K.C., Hunt, P.G., Humenik, F.J., Johnson, M.H., 1998. Impact of swine waste application on ground and stream water quality in an Eastern Coastal Plain watershed. Trans. ASAE 41 (6), 1665–1670.

Stone, K.C., Hunt, P.G., Millen, J.A., Johnson, M.H., Matheny, T.A., Vanotti, M.B., Burns, J.C., 2008. Forage subsurface drip irrigation using treated swine wastewater. Trans. ASABE 51 (2), 433–440.

Sumino, T., Isaka, K., Ikuta, H., Saiki, Y., Yokota, T., 2006. Nitrogen removal from wastewater using simultaneous nitrate reduction and anaerobic ammonium oxidation in single reactor. J. Biosci. Bioeng. 102 (4), 346–351.

Szogi, A.A., Hunt, P.G., Sadler, E.J., Evans, D.E., 2004. Characterization of oxidation–reduction processes in constructed wetlands for swine wastewater treatment. Appl. Eng. Agric. 20 (2), 189–200.

Tiedje, J.M., 1994. Denitrifier enzyme activity (DEA). In: Mickelson, S.H., Bigham, J.M. (Eds.), Methods of Soil Analysis—Part 2, 2nd ed. SSSA Book Series 5. SSSA, Madison, WI, pp. 256–257.

Van Driel, P.W., Robertson, W.D., Merkley, L.C., 2006. Denitrification of agricultural drainage using wood-based reactors. Trans. ASABE 49 (2), 565–573.

Vanotti, M.B., Hunt, P.G., 2000. Nitrification treatment of swine wastewater with acclimated nitrifying sludge immobilized in polymer pellets. Trans. ASAE 43 (2), 405–413.

Vanotti, M.B., Szogi, A.A., Hunt, P.G., Millner, P.D., Humenik, F.J., 2007. Development of environmentally superior treatment system to replace anaerobic swine lagoons in the USA. Biores. Technol. 98 (17), 3184–3194.

Vymazal, J., 2007. Removal of nutrients in various types of constructed wetlands. Sci. Total Environ. 380, 48–65.

Westerman, P.W., Bicudo, J.R., 2002. Application of mixed and aerated pond for nitrification and denitrification of flushed swine manure. Appl. Eng. Agric. 18 (3), 351–358.

White, J.R., Reddy, K.R., 2003. Nitrification and denitrification rates of everglades wetland soils along a phosphorus-impacted gradient. J. Environ. Qual. 32 (6), 2436–2443.