Denitrification in Constructed Wetlands Used for Treatment of Swine Wastewater

Patrick G. Hunt,* Terry A. Matheny, and Ariel A. Szögi

ABSTRACT

Constructed wetland treatment of swine wastewater probably involves substantial denitrification. Our objective was to assess denitrification and denitrification enzyme activity (DEA) in such wetlands in relation to plant communities, N loading, carbon or nitrogen limitations, and water depth. Two wetland cells each 3.6 m wide and 33.5 m long were connected in series. One set of cells was planted with rushes and bulrushes, including soft rush (*Juncus effusus* L.), softstem bulrush [*Schoenoplectus tabernaemontani* (K.C. Gmel.) Palla], American bulrush [*Schoenoplectus americanus* (Pers.) Volkart ex Schinz & R. Keller], and woolgrass bulrush [*Scirpus cyperinus* (L.) Kunth]. Another set was planted with bur-reeds and cattails, including American bur-reed (*Sparganium americanum* Nutt.), broadleaf cattail (*Typha latifolia* L.), and narrowleaf cattail (*Typha angustifolia* L.). The sets will be referred to herein as bulrush and cattail wetlands, respectively. Denitrification and DEA were measured via the acetylene inhibition method in intact soil cores and disturbed soil samples that were taken during four years (1994–1997). Although DEA in the disturbed samples was greater than denitrification in the core samples, the measurements were highly correlated ($r^2 \geq 0.82$). The DEA was greater in the bulrush wetlands than the cattail wetlands, 0.516 and 0.210 mg N kg^{-1} soil h^{-1}, respectively; and it increased with the cumulative applied N. The DEA mean was equivalent to 9.55 kg N ha^{-1} d^{-1} in the bulrush wetlands. We hypothesized and confirmed that DEA was generally limited by nitrate rather than carbon. Moreover, we determined that one of the most influential factors in DEA was wetland water depth. In bulrush wetlands, the slope and r^2 values of the control treatment were −0.013 mg N kg^{-1} soil h^{-1} mm^{-1} depth and $r^2 = 0.89$, respectively. Results of this investigation indicate that DEA can be very significant in constructed wetlands used to treat swine wastewater.

Swine waste management systems have generally been based on the N assimilative capacity of cropland. One of the common systems involves waste storage and treatment in an anaerobic lagoon followed by wastewater application to crop land. This method has the advantages of natural treatment, nitrogen utilization, and relatively low cost. However, it requires substantial crop land close to the facilities because of the high cost of either pumping or transporting wastewater. When available crop land is insufficient to assimilate the N generated by concentrated swine production, alternatives to the traditional lagoon-land application are necessary. One alternative treatment system is constructed wetlands.

Wetlands have been used around the world for municipal wastewater treatment for more than 20 years (Kadlec and Knight, 1996). They have more recently been used for treatment of animal wastewater (Knight et al., 2000; Hunt and Poach, 2001; Reddy et al., 2001; Hunt et al., 2002a,b). The wetlands used for animal wastewater treatment are constructed on upland sites specifically for treatment. Their primary purpose is to remove N before land application and, thereby, lower the cropland necessary for wastewater application and N assimilation. Generally, the wetlands are surface flow to avoid clogging problems associated with the high solids content of animal wastewater. Wetland vegetative cover varies, but it often contains a mixture of cattails, reeds, and sedges. Such vegetation thrives in the flooded wetland conditions that also promote the anaerobic condition required for denitrification. However, removal of N via denitrification also requires oxygen for the precursor process of nitrification. Part of the required oxygen can be supplied by transport through the plant stem and root, but the extent of this oxygen supply can vary greatly (Kadlec and Knight, 1996).

Whereas high rates of denitrification could be limited by insufficient oxygen for nitrification, some have suggested that ammonia volatilization could be a major mechanism for nitrogen removal in wetland treatment of swine wastewater (Knight et al., 2000). However, Poach et al. (2002) reported that although ammonia volatilization occurred in swine wastewater treatment wetlands, it was a relatively small portion of the total N loss. They postulated that denitrification was the primary loss mechanism. Significant denitrification in the wetlands is consistent with general wetland function (Hunt and Lee, 1975; Patrick and Reddy, 1976; Hammer, 1989; Kadlec and Knight, 1996), but the magnitude of denitrification is affected by wetland operational parameters and conditions within the wetland. Thus, the objectives of this investigation were to assess the denitrification and DEA of the surface-flow, constructed wetlands used for swine wastewater treatment in relation to plant communities, N loading, carbon or nitrogen limitations, and water depth.

MATERIALS AND METHODS

Design

Denitrification and denitrification enzyme activity (DEA) were assessed on constructed wetlands that received swine effluent. The wetlands were constructed in Duplin County, North Carolina, in 1992. They consisted of four cells (3.6 × 33.5 m) (Fig. 1). The cells were constructed by removing the topsoil, sealing the cell bottoms with 0.30 m of compacted clay, and covering with 0.25 m of loamy sand topsoil. The wetland cells had a bottom slope of 0.2%. Two cells connected in series were planted with rushes and bulrushes. Another two cells in series were planted with bur-reeds and cattails.

The wetlands received swine effluent from an anaerobic

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Abbreviations: DEA, denitrification enzyme activity.

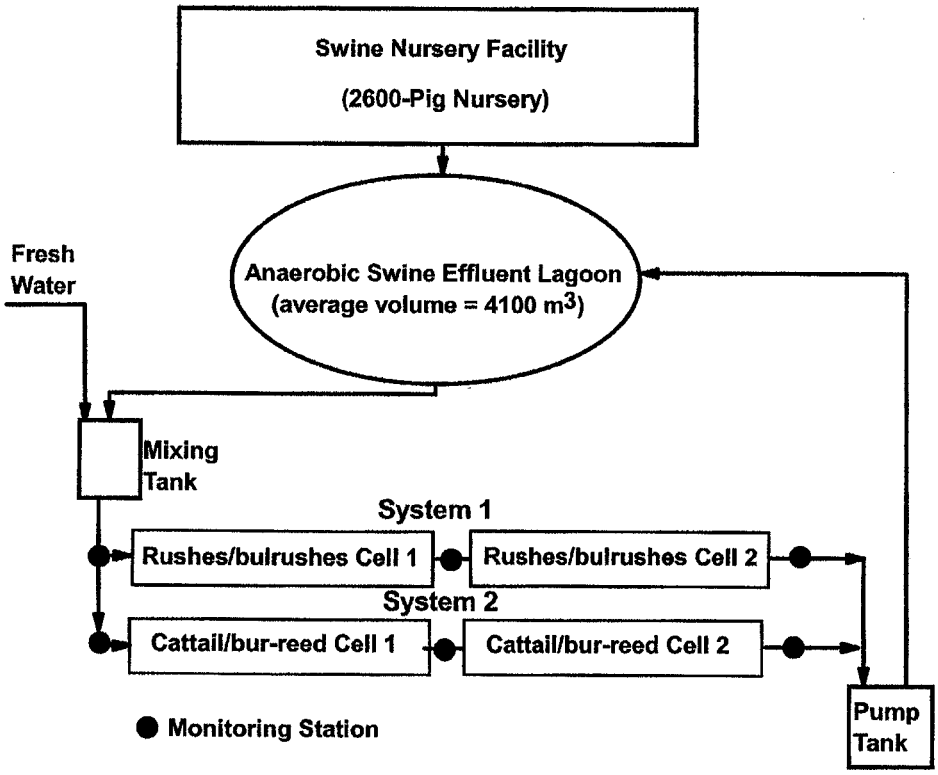

Fig. 1. Schematic of constructed wetland experimental site.

waste lagoon that collected wastewater from a swine nursery facility that had 2600 pigs with an average weight of 13 kg. The facility used a flushing system to recycle wastewater from a single stage anaerobic lagoon with a volume of 4100 m^3. Residence time of the wastewater in the lagoon was 120 d. The first cell in each series was flooded with swine effluent that had been diluted with fresh water to obtain nitrogen concentrations ranging from 25 to 127 mg L^{-1}. The dilution prevented potential ammonia toxicity to the wetland plants. The diluted wastewater was applied to the first wetlands during June through December in 1993, January through December in 1994, 1995, 1996, and March through December in 1997. The second cell in each series received wastewater from the outflow of the first cell. The effluent from the second cell was pumped back to the lagoon. The daily hydraulic loading rates ranged from 8 to 11 mm d^{-1} with a mean residency time of 12.5 d per cell (Szögi et al., 2000). The wetland operation, treatment effectiveness, and component function are reported in Hunt et al. (2002b).

Denitrification Rate and Enzyme Activity

Soil samples were collected at the 0- to 25.4-mm depth from four quadrants of each cell of the constructed wetlands on 12 sampling dates over four years (1994–1997) for analysis of DEA. Whereas the wetland treatment was more active in the spring and summer, samples were taken on four dates in both seasons, but only two sample dates were taken in either the fall or winter. After collection, soil samples were placed in plastic bags, stored on ice, transported to the laboratory, and stored overnight at 4°C. The DEA was measured by the acetylene inhibition method (Tiedje, 1994). Field-moist soil (10–15 g) from each sample location was placed in five 60-mL serum bottles that received 5 mL of chloramphenicol to inhibit microbial protein synthesis. In addition, the soil samples received one of the following treatments: (A) nonamended control, (B) 200 mg L^{-1} NO_3–N, (C) 2 g L^{-1} glucose C, (D) 200 mg L^{-1} NO_3–N and 2 g L^{-1} glucose C, or (E) the previous treatment without acetylene added. Bottles were capped with rubber septa, evacuated, and purged with nitrogen gas three times. Acetylene was then used to block the conversion of nitrous oxide to dinitrogen gas. Acetylene (15×10^{-3} L) was injected into bottles containing the four amendments. The bottles were incubated on a horizontal shaker at 1.5 cycles s^{-1}. After 1, 5, and 24 h of incubation, five 10^{-3} L samples of the headspace gases were removed with a syringe (Plastipak syringe with eccentric tip; Becton, Dickinson and Company, Franklin Lakes, NJ) and placed in vials (borosilicate glass, crimp top with butyl septum). Rates of N_2O accumulation were expressed on a dry soil weight basis. The rates for each incubation time were similar; therefore, the time with the highest value was used for each sample.

Intact soil cores (25.4-mm diameter by 50.8-mm length) were obtained from four quadrants of each cell on the same days as disturbed samples. The bulk density of the soil was 1.52 g m^{-3}. The core samplers were modified 60-mL syringes. The headspace of the syringe was adjusted to 30 mL. Once collected, the soil cores were sealed, packed in ice, transported to the laboratory, and stored overnight at 4°C. At the laboratory, each core headspace was purged with air to maintain an aerobic atmosphere, but 3×10^{-3} L of the headspace air was replaced with acetylene. The cores were incubated at 25°C, and samples of the headspace were removed and analyzed as stated above for the disturbed samples.

Soil, Effluent, Gas, and Data Analysis

Field-moist soil samples were collected, dried at 100°C for 72 h, and weighed to determine moisture content. Field-moist soil samples were extracted for water-soluble nitrate and solu-

ble carbon by (i) weighing 5 g of soil into a 25-mL flask, (ii) adding 20 mL of deionized water, (iii) shaking on a wrist-action shaker for 30 min, (iv) filtering through #2 filter paper (Whatman, Maidstone, UK), (v) acidifying with sulfuric acid, and (vi) storing at 4°C.

The redox potentials (Eh) of the wetland surface effluent were determined by a Model 290A meter with an Ag–AgCl electrode (Thermo Orion, Beverly, MA). The electrodes were calibrated with quinhydrone in pH 4.0 and 7.0 buffers. Redox potential values were corrected to the H electrode potential by adding the potential of the Ag–AgCl reference electrode, +200mV, to the mV readings. Dissolved oxygen of the surface water was determined with a Model 55 dissolved oxygen meter (YSI, Yellow Springs, OH). The dissolved oxygen electrode was calibrated with both 0 and 100% saturated dissolved oxygen solutions. A Model 210A pH meter (Thermo Orion) was used to determine the pH of the surface water. The pH electrode was calibrated with a pH 4.0 and 7.0 buffer. Wastewater analyses were conducted as reported in Hunt et al. (2002b).

Soluble nitrates and carbon were determined on a TRA-ACS 800 autoanalyzer (Bran+Luebbe, Norderstedt, Germany) and a DC190 carbon analyzer (Tekmar-Dohrmann, Mason, OH), respectively. Total nitrogen and carbon were determined on a Model CN2000 carbon and nitrogen analyzer (LECO Corporation, St. Joseph, MI). The N_2O in the gas samples was measured with a Model 3600 CX gas chromatograph (Varian, Palo Alto, CA) equipped with a 15-m $Ci^{63}Ni$ electron capture detector operating at 350°C. Chromatographic separation of N_2O was obtained by use of a 1.8-m-length by 2-mm-i.d. stainless steel column packed with Poropak Q (80-100 mesh; Alltech Associates, Deerfield, IL); the column and injector temperature was 70°C. Samples were injected into the column by a Model 8200 autosampler (Varian).

Data were analyzed by analysis of variance, regression, and least significant difference (LSD) with Version 6.12 of Statistical Analysis Systems (SAS Institute, 1997).

RESULTS AND DISCUSSION

Wastewater and Soil Characteristics

Before dilution and application to the wetland, lagoon wastewater effluent added to the wetlands was typical of a moderately loaded anaerobic lagoon (Table 1). The BOD and ammonia contents were 287 and 347 mg L^{-1}, respectively; nitrate N was <1 mg L^{-1}. The wastewater pH was buffered by both its ammonia and carbonate content. Consequently, the pH of the treated effluent varied only slightly as it moved through the wetlands. The pH dropped from 7.5 to 7.2 in the first 11 m of the wetlands, and it was essentially neutral (6.9–7.2) in the remainder of all wetland cells (Table 2). The dissolved oxygen content of the surface effluent was highest (1.3–1.8 mg L^{-1}) in the first one-third of all wetland cells where the water depth was the most shallow. The dissolved oxygen content decreased to a range of (0.3–0.7 mg L^{-1}) in the middle and bottom one-third of all wetland cells. The redox potential (Eh) of the surface effluent followed a similar trend to oxygen (Table 2). The Eh of the surface effluent in the first one-third of the cells was typical of an environment that contained microsites where nitrification–denitrification could occur; values ranged from 111 to 175 mV. The lower two-thirds of the wetland had surface effluent Eh values more characteristic of an environment that contained microsites where denitrification and iron reduction could occur; values ranged from −2 to 88 mV.

Although the Eh of the wetland surface effluent was similar for the bulrush and cattail wetlands, the soil Eh was higher in the bulrush wetlands (130 and 308 mV in the first and second wetlands, respectively) compared with the cattails (105 and 196 mV in the first and second wetlands, respectively) (Hunt et al., 2002b). Higher Eh is consistent with the higher O_2 transport capacity of bulrush stems and roots relative to cattail (Reddy et al., 1989). Transport of O_2 from leaves and stems to roots along with the associated rhizosphere microbes is important to wetland processes (Good and Patrick, 1987). Oxygen transport can be particularly important in the denitrification process of wetlands because it is required for nitrate formation and nitrate can be the limiting factor for denitrification. Since nitrification occurs in oxidized environments and denitrification in anaerobic environments, the juxtaposition of the oxidized zone around the root with the reduced environment of the wetland soil is very important for the function of constructed wetland treatment of N (Armstrong, 1964; Hunt and Lee, 1975; Kadlec and Knight, 1996; Hunt et al., 2002b). These oxidative–reductive conditions and the potential rapid formation and consumption of nitrate are consistent with the general absence of nitrate N in soil pore water samples (Szögi and Hunt, 2001).

During our study period, the wetlands both accumulated a surface, plant litter layer and doubled the C and N contents of the underlying mineral soil. Our denitrification measurements focused on the mineral soil layer. The mean soil total N ranged from 367 to 537 mg kg^{-1} (Table 3). The mean soil C content values ranged from 5.0 to 7.6 × 10^3 mg kg^{-1}, and mean soluble carbon content ranged from 32 to 42 mg kg^{-1} of soil (Table 3).

Table 1. Characteristics of swine wastewater after anaerobic lagoon treatment.

Parameter	Mean	Standard deviation
pH	7.83	0.14
	mg L^{-1}	
Total organic carbon	235	124
Biochemical oxygen demand	287	92
Total Kjeldahl nitrogen	365	41
Ammonia nitrogen	347	52
Nitrate nitrogen	0.04	0.03

Table 2. Dissolved oxygen, pH, and redox potential of surface water in constructed wetlands.

Cell	Plant community	Distance from inlet	pH	Dissolved oxygen	Redox potential
		m		mg L^{-1}	mV
1	rushes and bulrushes	0	7.46 (0.17)†	1.49 (0.61)	147 (45)
		11	7.15 (0.16)	0.26 (0.17)	39 (122)
		22	7.06 (0.26)	0.26 (0.09)	57 (98)
2	rushes and bulrushes	34	7.14 (0.28)	1.40 (1.05)	111 (105)
		45	7.19 (0.26)	0.52 (0.26)	66 (96)
		56	7.05 (0.12)	0.44 (0.09)	58 (101)
3	bur-reed and cattails	0	7.47 (0.29)	1.31 (0.87)	147 (41)
		11	7.16 (0.38)	0.52 (0.35)	43 (89)
		22	6.91 (0.36)	0.35 (0.17)	−2 (68)
4	bur-reed and cattails	34	7.14 (0.13)	1.75 (0.52)	175 (65)
		45	7.12 (0.18)	0.52 (0.52)	81 (132)
		56	7.08 (0.19)	0.70 (0.70)	88 (136)

† Values in parentheses are standard deviations of the mean.

Table 3. Carbon and nitrogen content of constructed wetland soil.

Cell	Plants	Nitrate N	Total N	Total C	Soluble C
		mg kg^{-1}			
1	rushes and bulrushes	1.0	537	7624	42
2	rushes and bulrushes	1.8	455	7274	32
3	bur-reed and cattails	0.9	367	5018	36
4	bur-reed and cattails	0.8	388	5965	39
$LSD_{0.05}$		1.1	50	637	6

These soil C and N values remained in the range of mineral soils; they are typical of those found in a sandy coastal plain mineral soil managed by traditional clean-tillage methods rather than organic soils (Hunt et al., 1996; Kadlec and Knight, 1996). The soil N and C contents increased more in the bulrush wetlands; this difference is consistent with the soil pore water data of Szögi and Hunt (2001).

Cell, Season, and Core versus Disturbed Sample Effect on Denitrification

The first and second wetland cells were not significantly different for denitrification as measured in either the intact cores or disturbed samples for either the bulrush or cattail wetlands ($P \leq 0.10$), so the measurements of the first and second cells were pooled. Similarity of the first and second wetland cells probably related to the similarity of their water levels, redox conditions, litter layers, and plant community.

Denitrification in core samples was not significantly different for seasons of the year ($P \leq 0.10$). Additionally, in the core samples, there was no significant plant community by season interaction ($P \leq 0.26$). The effect of season was only somewhat more pronounced in the disturbed samples; denitrification was highest (LSD 0.05) in the spring, 0.55 mg N kg^{-1} soil h^{-1}. The measurements in the other seasons were not significantly different from each other, and they were <0.32 mg N kg^{-1} soil h^{-1}. The higher rate in the spring could have been related to the release of C and N from the litter of plants, which had succumbed to previous winter kill. As with the cores, there was no significant interaction between plant community and season ($P \leq 0.33$).

The most dramatic difference in the core samples was between the plant communities. The mean denitrification of the bulrush wetlands was significantly higher ($P \leq 0.01$) than the mean of the cattail wetlands, 3.54 and 1.68 mg N m^{-2} soil h^{-1}, respectively. Similar results were obtained in the control treatment of the disturbed samples, which measured DEA without amendments. Bulrush wetlands had twofold higher DEA means ($P \leq 0.001$) than the cattail wetlands, 0.516 and 0.210 mg N kg^{-1} soil h^{-1}, respectively. The core and disturbed samples were highly correlated (bulrush DEA = 5.95 core rate + 9.5, $r^2 = 0.82$; and cattail DEA = 8.65 core rate − 5.5, $r^2 = 0.77$) (Fig. 2). Thus, one obtains similar insight into relative denitrification from either the core sample or the control sample of the DEA measurements. Higher values obtained in the disturbed samples relative to the cores were expected (Ambus and Lowrance, 1991).

The higher values for the disturbed sample relative to the core samples reflect better contact and more complete denitrification inhibition by acetylene as well as better efflux of N_2O into the bottle headspace. Therefore, the control treatment of the disturbed samples probably represents a better measure of the actual denitrification rate. The DEA treatments also give insight into the limiting factors. Consequently, we will only use the DEA in the remainder of the manuscript. Using the bulk density of 1.52 g cm^3, the disturbed samples can be converted to a kg N ha^{-1} d^{-1} basis by multiplying by 18.5. Thus, the mean value for the bulrush wetlands was equivalent to 9.55 kg N ha^{-1} d^{-1} (0.516 mg N kg^{-1} soil h^{-1} × 18.5). Even if these estimates of denitrification are high, they only represent the mineral soil. The total denitrification in the wetlands comes from the mineral

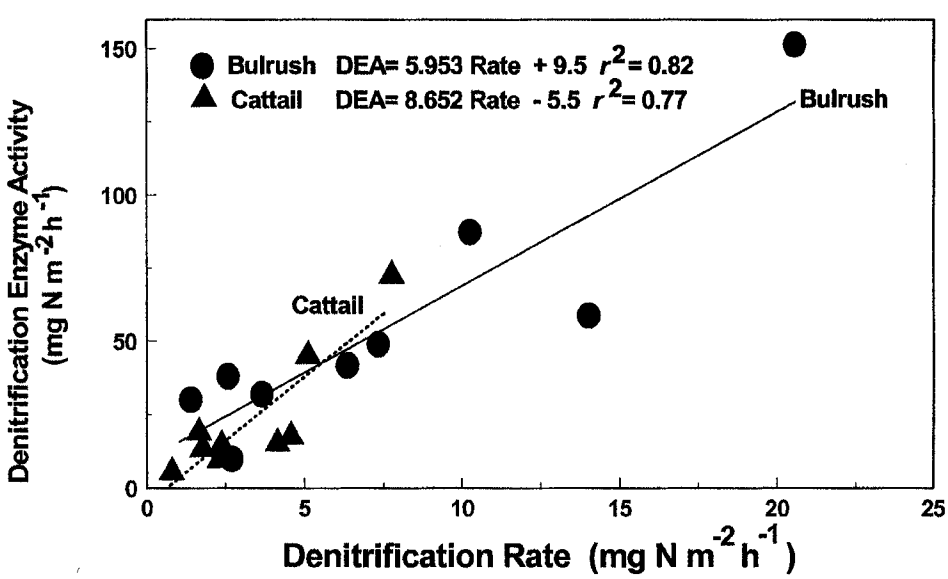

Fig. 2. Comparison of denitrification as measured in intact cores and disturbed soil samples from the constructed wetlands.

soil, litter layer, and floating sludge matrix. Furthermore, the denitrification measurements from the surface litter and floating sludge matrix are much higher (Hunt et al., 2002a). The high levels of DEA in the disturbed samples are also consistent with the projected high level of denitrification involved in the treatment effectiveness of these wetlands (Hunt et al., 2002b).

Soil Carbon Effect on Denitrification

As previously stated, the soil C content doubled during the experiment. Soil carbon would be expected to increase over time with the addition of organic carbon from the wastewater. Moreover, C was added to the wetlands from about 17 Mg ha^{-1} yr^{-1} of aboveground plant dry matter along with C from plant roots and root photosynthate exudate (Kadlec and Knight, 1996; Hunt et al., 2002b). Insight into the effect of this increased soil C on denitrification can be gained by evaluating the correlation of DEA to the mean soil C content of each of the 12 sampling dates. The DEA was only moderately correlated with total soil C. The r^2 values were 0.38 and 0.55, respectively, for Treatment A (the control) of the cattail and bulrush wetlands (Fig. 3A). Neither the correlations nor slopes were significantly changed in bulrush wetlands by addition of either nitrate or carbon (Treatments B and C, respectively), which indicated that the total soil C was supplying a sufficient available C for the denitrification even with additional large quantities of nitrate in Treatment B (Fig. 3B,C). Similarly, addition of more C did not change the correlation or DEA rates in samples from the cattail wetlands. However, addition of nitrate dramatically increased both the correlation with total soil C and DEA in the cattail-wetland samples (DEA = 0.418 C − 1.7, r^2 = 0.71) (Fig. 3B). These results reveal that the total soil C was supplying sufficient C for the denitrifying population to consume the existing nitrates and that the available C was sufficient to drive the denitrification when large amounts of nitrate were added to the cattail wetland. The difference between the cattail and bulrush wetlands may relate to differences in their litter mineralization. The apparent sufficiency of C notwithstanding, sustained high N loading of the wetlands will require large amounts of C, and at some N loading rate external C will be necessary to supplement C supplying capacity of the wetland system (Hunt et al., 1999).

When soil C was low in either the bulrush or cattail wetland, the values of Treatments D and E were nearly equal (Fig. 3D,E). This result indicated that even when the blocking agent (acetylene) was absent, most of the N_2O was not being converted to dinitrogen, which indicated that incomplete denitrification was prevalent. However, as total soil C increased, the relative amount of incomplete denitrification decreased. This response is consistent with the expected more complete denitrification with higher carbon relative to nitrate.

Soil Nitrogen Effect on Denitrification

Insight can also be gained from analyses of the mean total soil N and denitrification values for each sampling date. The concentration of total soil N increased during the experiment from about 200 to >500 or 800 mg kg^{-1}, respectively, in the cattail and bulrush wetlands. Since

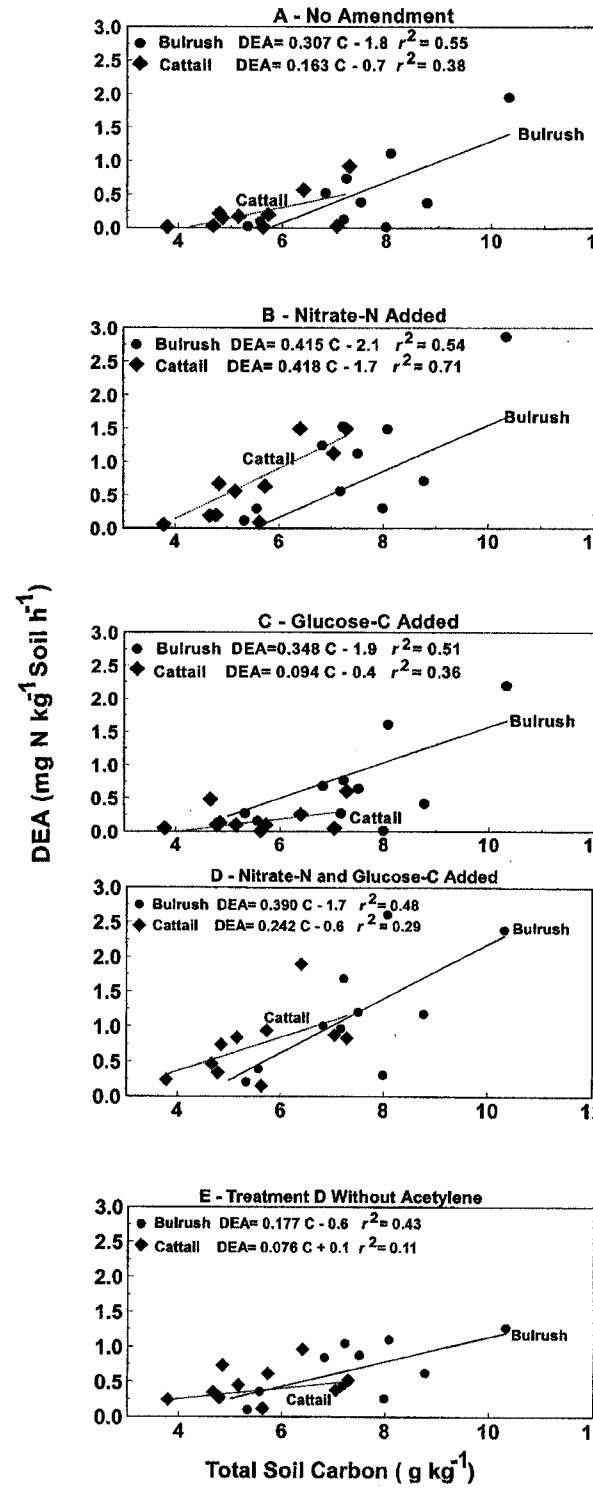

Fig. 3. Denitrification enzyme activity rate in constructed wetlands as affected by total soil carbon. (*A*) Nonamended control, (*B*) 200 mg L^{-1} NO_3–N, (*C*) 2 g L^{-1} glucose C, (*D*) 200 mg L^{-1} NO_3–N and 2 g L^{-1} glucose C, or (*E*) the previous treatment without acetylene added.

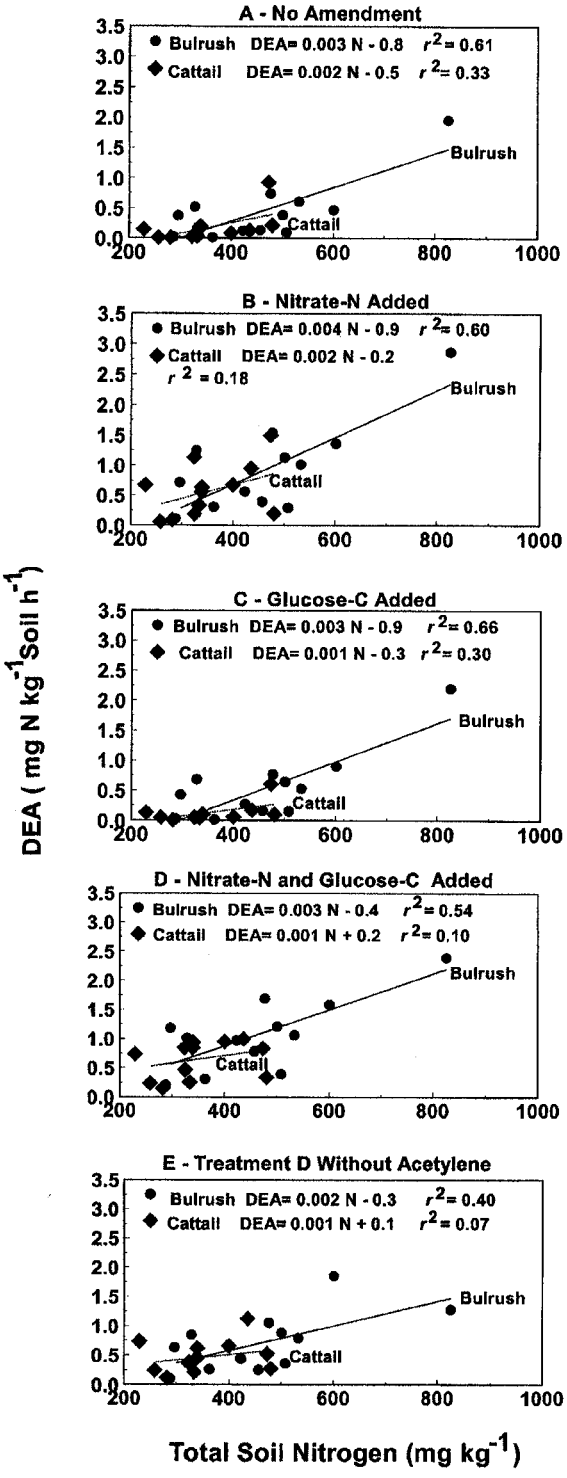

Fig. 4. Denitrification enzyme activity in constructed wetlands as affected by total soil nitrogen. (*A*) Nonamended control, (*B*) 200 mg L^{-1} NO_3–N, (*C*) 2 g L^{-1} glucose C, (*D*) 200 mg L^{-1} NO_3–N and 2 g L^{-1} glucose C, or (*E*) the previous treatment without acetylene added.

total soil C and N are closely related, the correlation of total soil N and DEA was somewhat similar to that of C. The DEA increased with increased soil N; the r^2 values for bulrush ranged from 0.66 to 0.40 (Fig. 4). The DEA was increased by addition of nitrate, but it was unchanged by the addition of C even at the higher levels of soil N (Fig. 4B,C). Production of N_2O in Treatment E indicated that substantial (50%) incomplete denitrification occurred across the range of soil N concentrations, despite the slightly diminished N_2O production as the soil N increased (Fig. 4E). Similar trends were found for the cattail wetlands in all treatments, but the correlation was weaker ($r^2 < 0.30$), particularly when nitrate was added (Fig. 4).

Cumulated Total Application of Nitrogen

In contrast to total soil C and N, which had significant recalcitrant N, the applied N was predominately in the readily available ammonia form. Additionally, the cumulated total application of N is a function of both the rate and duration of application, which makes it a good measure for the influence of both factors. Accordingly, the cumulated total application of N was better correlated to DEA than just time. The cumulated total applied N increased from the initial value of approximately 0.5 to 1.6 kg m^{-2} during the study period. The DEA was also strongly correlated ($r^2 > 0.73$) to cumulative total N applied to the bulrush wetlands (Fig. 5A). Furthermore, addition of nitrate (Treatment B) increased the DEA relative to the control indicating that the system was nitrate limited. This increase was prevalent even as the cumulated application of N approached 1.6 kg m^{-2}. On the other hand, addition of the C source, glucose, in Treatment C (Fig. 5C) did not increase DEA relative to the control, which indicated that the wetlands were not C limited. When both nitrate and glucose were added (Treatment D), the slope for DEA increased by 36% as it increased by fivefold from about 0.5 to 2.5 mg N kg^{-1} soil h^{-1}. The cattail wetlands responded in a similar fashion, but with lower rates and correlation values (r^2 = 0.48–0.63). These findings are consistent with a wetland system where DEA is limited by the conversion of ammonia to nitrate (Reed, 1993). This increased DEA was probably related both to the maturation of the wetland system and to the associated increase in surface area of the litter layer along with the availability of more N within the litter surface and pore water of the wetland. These findings are in agreement with pore water data (Szögi and Hunt, unpublished data, 2002) that revealed that (i) movement of N was from the water to the soil after the initially low amounts of cumulative N applications and (ii) movement of N was from the soil to the water after greater cumulative N applications. Initially, incomplete denitrification, as measured by Treatment E, was nearly 100% for both the bulrush and cattail wetlands (Fig. 5E). As the total cumulated N application increased, the percentage of incomplete denitrification decreased, but it remained a significant percentage of the denitrification.

Water Depth

One of the most influential factors on denitrification rates in the wetlands was water depth. The influence of

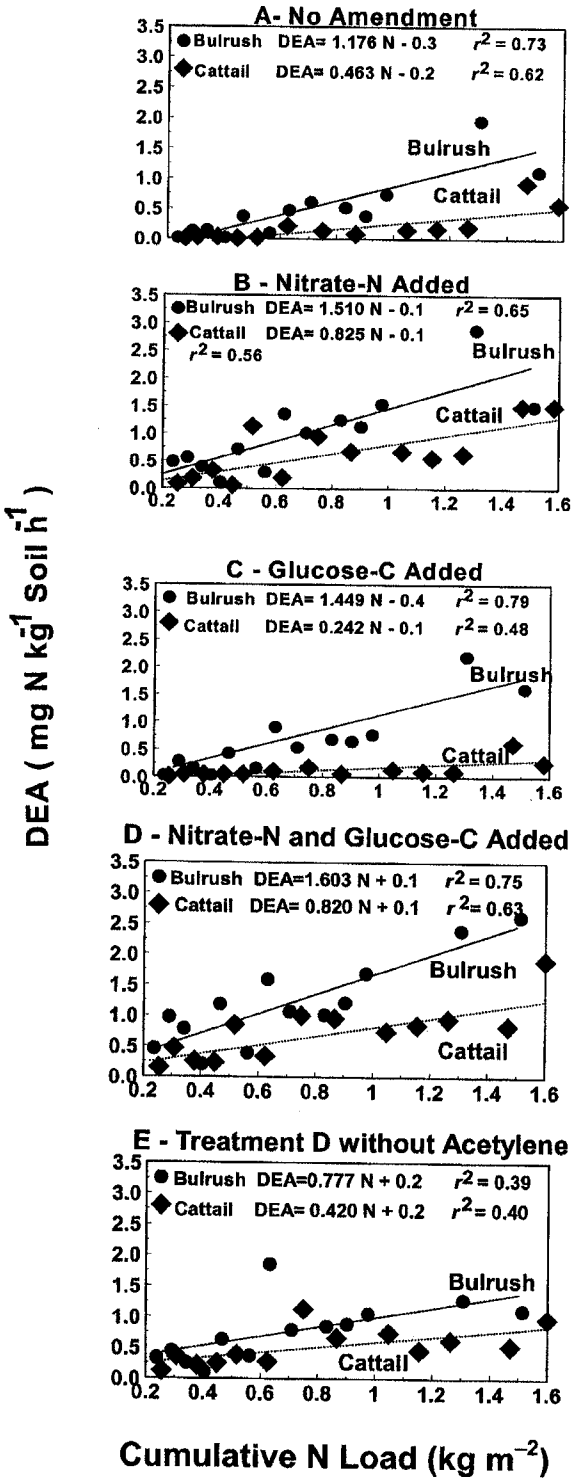

Fig. 5. Denitrification enzyme activity in constructed wetlands as affected by cumulative total nitrogen. (*A*) Nonamended control, (*B*) 200 mg L^{-1} NO_3–N, (*C*) 2 g L^{-1} glucose C, (*D*) 200 mg L^{-1} NO_3–N and 2 g L^{-1} glucose C, or (*E*) the previous treatment without acetylene added.

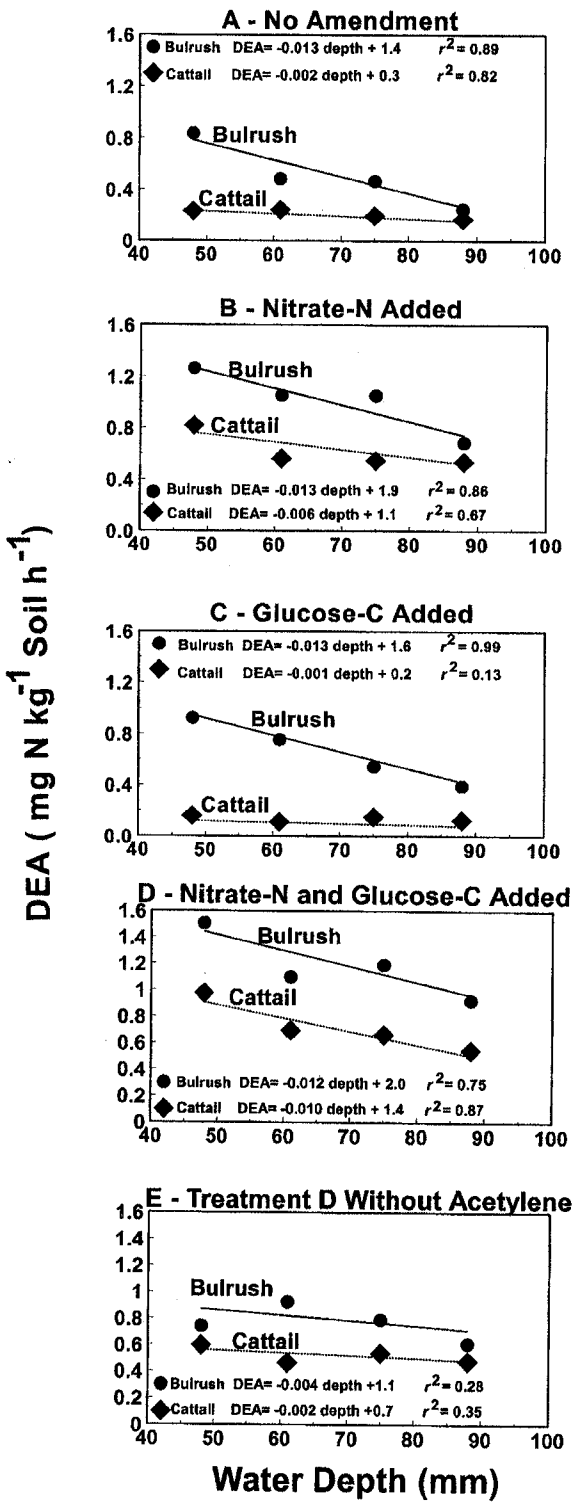

Fig. 6. Denitrification enzyme activity in constructed wetlands as affected by water depth. (*A*) Nonamended control, (*B*) 200 mg L^{-1} NO_3–N, (*C*) 2 g L^{-1} glucose C, (*D*) 200 mg L^{-1} NO_3–N and 2 g L^{-1} glucose C, or (*E*) the previous treatment without acetylene added.

water depth was much more dramatic for bulrush (Fig. 6). In bulrush wetlands, the slope and r^2 values of the control treatment were −0.013 mg N kg^{-1} h^{-1} mm^{-1} depth and 0.89, respectively. Furthermore, the effect of depth on DEA in the bulrush wetlands was extraordinarily consistent among treatments. The slopes varied by only 0.001 mg N kg^{-1} h^{-1} mm^{-1} depth, and the r^2

values ranged from 0.75 to 0.99. Addition of nitrate increased DEA by 0.48 mg N kg^{-1} h^{-1}, which further documents nitrate as a limiting factor for denitrification. Inasmuch as nitrate serves as a terminal electron acceptor of anaerobic microbial respiration, its consumption can also be limited by carbon, which is an energy source for microbial respiration. The addition of a carbon source (glucose) produced a small increase in DEA of 0.16 mg N kg^{-1} h^{-1} relative to the addition of nitrate, indicating that the system was only slightly limited by carbon. When nitrate and glucose were added simultaneously, the DEA increase was 0.61 mg N kg^{-1} h^{-1}. These results indicate a response to carbon when the nitrate load was very high.

Cattail wetlands were very different in relation to water depth and denitrification (Fig. 6). In the control treatment, there was very little effect of water depth on denitrification. The slope was −0.002 mg N kg^{-1} h^{-1} mm^{-1}, and the r^2 was 0.82. The DEA in the cattail wetlands at the deeper depth was about the same as in the bulrushes, but at the more shallow depth it was nearly fourfold less. With the addition of nitrate, the cattail wetlands more closely matched the bulrush wetlands in their DEA changes with depth. The slope increased to −0.006 mg N kg^{-1} h^{-1} mm^{-1}, and the difference relative to bulrush wetlands diminished by 50%. In contrast, the DEA changed very little after the addition of a glucose carbon source. The addition of both carbon and nitrate caused cattail wetlands to even more closely resemble the bulrush. The slope was −0.010 mg N kg^{-1} h^{-1} mm^{-1}, and the r^2 was 0.87. This indicated that the cattails were somewhat C limited at the high nitrate levels, particularly at the shallow depths.

These data document that DEA was consistently more active at the shallower depths of the bulrush wetlands. They also document that the cattail wetlands were not affected by effluent depth unless nitrate was added to the shallow depths. Whereas these wetlands have been operated in a more shallow than normal manner, even the deeper depths are relatively shallow. It is common for constructed wetlands to be operated at depths of 150 to 300 mm (Kadlec and Knight, 1996). If the regression lines from these data were projected to such depths, the DEA rates would approach zero. Deeper depths are useful because they increase residence time and promote more uniform water flow. However, in our wetlands, increased water depth clearly lowered denitrification in the bulrush, and it decreased the potential DEA rates in both the bulrush and cattail wetland systems.

CONCLUSIONS

- Denitrification as measured by the acetylene inhibition method was active in both the intact and disturbed soil samples of the wetlands. There was good correlation ($r^2 = 0.87$) between the rates of denitrification measured by both sampling methods, but the disturbed samples gave the higher estimates.
- The denitrification mean of the intact samples from the bulrush wetlands was significantly higher ($P \geq 0.01$) than the mean of the cattail wetlands, 3.54 and 1.68 mg N m^{-2} h^{-1}, respectively. Similarly, in the control treatment of the disturbed samples, bulrush wetlands had higher DEA means ($P \leq 0.001$) than did cattail wetlands; 0.516 and 0.210 mg N kg^{-1} soil h^{-1}, respectively. When converted to an area basis, the mean value for the disturbed samples of the bulrush wetlands was equivalent to 9.55 kg N ha^{-1} d^{-1}. Such a rate of N removal by denitrification would have been very significant in the wetland treatment of swine wastewater during the study period.
- The DEA rates increased over time as the rate of applied N increased and the wetlands matured. The DEA in the control treatment was well correlated to the cumulative total N applied to both the bulrush and cattails ($r^2 = 0.73$ and 0.62, respectively).
- Nitrate was generally the limiting factor, especially in the bulrush wetlands. On the other hand, carbon provided by the wetland plants and the wastewater was generally sufficient unless high additions of nitrate were made.
- Water depth was a very significant factor in the control of DEA in the bulrush wetlands. In bulrush wetlands, the slope and r^2 values of the control treatment were −0.013 mg N kg^{-1} h^{-1} mm^{-1} depth and $r^2 = 0.89$, respectively. Furthermore, the effect of depth on DEA in the bulrush wetlands was extraordinarily consistent among all treatments. The slopes varied by only 0.001 mg N kg^{-1} h^{-1} mm^{-1} depth, and the r^2 values ranged from 0.75 to 0.99. Decreased DEA with depth was probably caused by decreased O_2 and Eh of the effluent as well as the increased diffusion path associated with the greater water depth.
- Cattail wetlands were very different than bulrush wetlands in relation to water depth and denitrification. In the control treatment, there was very little effect of water depth on denitrification. The slope was −0.002 mg N kg^{-1} h^{-1} mm^{-1}, and the r^2 was 0.82. The lack of response to water depth change was probably because the cattails were not able to establish oxidative conditions sufficient for nitrification even with the relatively more oxidized condition associated with the shallower depth. This conclusion is supported by the fact that addition of nitrate and C to cattail wetlands produced DEA responses to depth much more similar to bulrush wetlands.
- Denitrification can be very significant in removal of N from constructed wetlands used for treatment of swine wastewater.

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