In Situ Measurements of Denitrification in Constructed Wetlands

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ABSTRACT

Quantitative estimates of denitrification are needed in designing artificial wetlands to optimize nitrate (NO_3^-) removal. Acetylene blockage and ^{15}N-tracer methods were employed to quantify denitrification in constructed wetlands receiving agricultural tile drainage, using plastic tubes to enclose in situ mesocosms. Estimates were also made through NO_3^- disappearance from mesocosm water columns. The ^{15}N and C_2H_2 methods yielded comparable rates. At 4 to 25°C, and with 9 to 20 mg NO_3^-–N L^{-1} initially in the mesocosm water columns, denitrification rates by the C_2H_2 technique ranged from 2.0 to 11.8 mg N m^{-2} h^{-1}. In the June-August ^{15}N experiment, when wetland NO_3^- was below detection, a time series of denitrification rates followed a bell-shaped curve after a pulse input of NO_3^- (~15 mg N L^{-1}, 70 atom% ^{15}N). The maximal denitrification rate (9.3 mg N m^{-2} h^{-1}) was observed 5.4 d after the pulse. After 33 d, 58% of the $^{15}NO_3^-$ had been evolved as N_2, only ~0.1% as N_2O; 6 to 10% was recovered in plant shoots and as organic N in the upper 5 cm of sediment. From 32 to 36% of the $^{15}NO_3^-$ spike was not recovered, and presumably seeped into the sediments. The NO_3^- disappearance rates in the water column ranged from 12 to 63 mg N m^{-2} h^{-1} at 11 to 27°C. Because water infiltration carries NO_3^- through the anaerobic sediment/water interface for denitrification, a subsurface-flow wetland may denitrify more NO_3^- than a surface-flow wetland.

WETLANDS have recently been recognized as having extremely high ecological and economic values (Costanza et al., 1997). Because most natural wetlands have been drained for agriculture and other development (Mitsch, 1994), constructed wetlands are receiving increasing attention (Kadlec and Knight, 1996). One use of constructed wetlands is to remove NO_3^- from agricultural runoff to reduce pollution of drinking water supplies and perhaps also alleviate eutrophication of coastal waters (Anonymous, 1996).

A significant proportion of agricultural land in the Midwest is artificially drained with subterranean tiles (clay or perforated plastic pipe) to allow farming to be practical and economically viable. Many studies have linked agricultural tile drainage to surface water N concentrations (Logan et al., 1994; Fausey et al., 1996; Drury et al., 1996; David et al., 1997b). More specifically, Gentry et al. (1998) showed a strong relationship between high inorganic soil N pools associated with corn (*Zea mays* L.) production and high tile and river NO_3^- concentrations, especially after a poor growing season. All of these studies found NO_3^- concentrations greater than the USEPA drinking water standard of 10 mg N L^{-1} in agricultural tiles that directly drain into surface waters.

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Lateral wetlands (Osborne and Kovacic, 1993) may be constructed on the economically marginal riparian zone to receive tile drainage water. The NO_3^- carried in the tile drainage may be removed in these artificial lateral wetlands through biochemical processes. Lateral wetlands are built by excavating surface soil to make a pond surrounded by artificial berms made of excavated soil. Little maintenance on the wetlands is required after their construction.

A substantial proportion of the NO_3^- entering a wetland is believed to undergo denitrification. This view is based largely on the finding that NO_3^- disappears from treatment wetlands, often with little direct evidence that the disappearance is due to denitrification (Hsieh and Coultas, 1989; Kadlec and Knight, 1996). There is a need for more field data to directly and quantitatively verify the importance of denitrification as a pathway for NO_3^- removal in constructed wetlands (Groffman, 1994). Some researchers have studied denitrification in the riparian soils. For example, Hanson et al. (1994) estimated that denitrification in riparian wetlands receiving groundwater NO_3^- inputs may remove up to 50% of the NO_3^- loads. Denitrification rate, in units of mg N m^{-2} h^{-1}, is directly useful for wetland design. By multiplying the rate with wetland area and retention time of water in the wetland, one can calculate the amount of NO_3^- that may be removed from the tile drainage water by the wetland. Given NO_3^- loading in a tile, one can calculate the size of the wetland that will be needed to achieve certain NO_3^- load reduction in the tile drainage water.

Denitrification may be described by the following scheme: $NO_3^- \rightarrow NO_2^- \rightarrow N_2O \rightarrow N_2$ (Weeg-Aerssens et al., 1987; Tortora et al., 1995). Acetylene (C_2H_2) inhibition and ^{15}N isotope tracing are two common techniques to quantify denitrification (Mosier and Heinemeyer, 1985). The first method uses C_2H_2 to prevent N_2O from reducing to N_2; thus, accumulated N_2O may be measured to quantify denitrification. Nitrogen-15 is used in the second method to measure N_2O and N_2 evolution. The objectives of this study were to: (i) estimate in situ denitrification rates using the ^{15}N tracer and C_2H_2 inhibition techniques and compare the denitrification rates with the NO_3^- removal rates in the water column, and (ii) to evaluate the significance of denitrification as a sink for NO_3^-, in constructed wetlands that received agricultural tile drainage.

MATERIALS AND METHODS

The constructed wetlands were located in Champaign County, Illinois [see David et al. (1997b) and Gentry et al. (1998) for detailed description of the site]. The sediment soil (Colo, a fine-silty, mixed, mesic Cumulic Haplaquolls) con-

Abbreviations: GC, gas chromatograph; IC, ion chromatograph; MS, mass spectrometer; TKN, total Kjeldahl nitrogen; PVC, polyvinyl chloride.

tained 36 g kg^{-1} organic-C and had a C/N ratio of 13; the pH of the wetland water was ~8 (David et al., 1997a). The dominant plant species in the wetlands were reed canarygrass (*Phalaris arundinacea* L.), barnyard grass [*Echinochloa crusgalli* (L.)], smartweed (*Polygonum persicaria* and *P. coccineum*), yellow nutsedge (*Cyperus esculentus* L.) and pigweed (*Amaranthus spinosus*); total biomass in 1995 and 1996 measured as dry weight at the end of the growing season was approximately 500 g m^{-2}. We studied denitrification in two constructed wetlands, 0.8 and 0.6 ha, respectively, both built in 1993. Each wetland was fed by one 20 cm diam. drainage tile. The maximum tile flow rate was approximately 30 L s^{-1} and there were times during heavy rainfall when surface runoff entered the wetlands. Average water retention time was about 7 d. The two wetlands under study were adjacent to each other and were similar in their hydrologic and biogeochemical characteristics.

Nitrogen-15 Technique

White polyvinyl chloride (PVC) pipes (20.3 cm i.d., cross-sectional area 324 cm^2) served as mesocosms in the field denitrification experiment using ^{15}N as a tracer. The mesocosms were buried upright to a depth of 15 cm into the plant-free sediment to enclose a 10.4-L water column that was 32.0 cm tall (David et al., 1997a). $Ca(^{15}NO_3)_2$ (isotope purity 99%) (Isotec, Inc., Miamisburg, OH) served as the tracer, and was mixed with unenriched $Ca(NO_3)_2$ to prepare an enriched solution 70 atom% in ^{15}N. This solution was then added to the wetland water in the mesocosm to achieve ~15 mg NO_3^-–N L^{-1} (labeled plus unlabeled NO_3^-), which was a typical NO_3^- concentration in the tile drainage water that entered these wetlands (Gentry et al., 1998). No background NO_3^- was detected in the wetland water throughout the ^{15}N experiment period. Duplicate ^{15}N mesocosms were used in the experiment.

A plastic cover, fitted with a sampling port, sealed the top of the PVC pipe. A plastic air-tight syringe was used to draw 30 mL of gas from the mesocosm air head-space. In the laboratory, the gas in the syringe was injected into the inlet of a mass spectrometer (MS) for isotope analysis (Mulvaney, 1993). Each time after a gas sample was taken, the mesocosm cover was removed and the air head-space was ventilated quickly before recapping the pipe. The sediment in the mesocosms was mildly shaken by kicking each mesocosm 60 times on the side immediately before each gas sample was taken. This agitation may accelerate gas release from the sediment into the air head-space. Preliminary ^{15}N experiments showed that the denitrification rates (N_2O and N_2 emitted/total NO_3^- spiked) were 0.50% on average for a starting concentration of 15 mg NO_3^-–N L^{-1} after 22 h with mesocosm agitation, as compared to an average 0.24% denitrification rate for a starting concentration of 20 mg NO_3^-–N L^{-1} at 26 h without agitation. Lindau et al. (1988b) previously reported the entrapment of N_2 evolved during soil denitrification in a laboratory experiment.

The volume of air head-space was calibrated by weighing water in the cover that enclosed the head-space. The N_2O and N_2 evolution was calculated using MS data based on algorithms developed by Mulvaney and Boast (1986). The denitrification rate was calculated by dividing the amount of N_2O or N_2 evolved by the duration of the experiment and the cross-sectional area of the mesocosm. During the ^{15}N experiments, water levels in the mesocosms dropped slowly because the overall water level in the wetland declined gradually due to outflow from the wetland, lack of rain, decreased input from tiles, seepage through the sediment, and evapotranspiration. Some plants emerged and grew in the mesocosms during the study period. No standing water remained in mesocosms after 500 h of the experiments, but the sediment was moist throughout the experiment.

Nonlabeled NO_3^- was used in some mesocosm experiments, separate from ^{15}N experiments, to calculate NO_3^- removal rates in the water column. Nonlabeled NO_3^- concentrations in the water column at different times were analyzed by ion chromatograph (IC) to calculate the NO_3^- removal rate, which was calculated by dividing the amount of NO_3^- disappeared from the water column by the experiment duration and the cross-sectional area of the water column. We also mixed Br^-, a chemically conservative anion in the wetland environment, with NO_3^- in the mesocosms to compare their behavior (Gold et al., 1998). Bromide concentration was also determined by IC.

Measurement of Plant and Microbial Uptake of Nitrogen-15 Labeled Nitrate

At the end of the mesocosm experiment, plants in the mesocosms were harvested. The plant materials from each mesocosm were composited and oven-dried at 105°C overnight for total Kjeldahl N (TKN) and ^{15}N MS analysis. From each mesocosm, three sediment cores (5.5 cm i.d.) were extracted and samples were taken from each core to represent the top 5 cm of the sediment for TKN and ^{15}N MS analysis. These solid samples were digested and the nitrogenous constituents were converted first to NH_4^+ (to determine TKN), and then to N_2 for MS analysis (Mulvaney, 1993; Mulvaney et al., 1996, 1997). The proportion of labeled N in a sample was calculated by (M-B)/(L-B), where M is the measured ^{15}N atom percentage in a sample, L is the ^{15}N enrichment of the NO_3^- spiked in the water column (70 atom% in our study), and B is the natural abundance of ^{15}N in plant and soil samples (0.370 atom% was used in our calculation). This proportion was multiplied by TKN to obtain the amount of N in a plant or soil sample that was derived from the labeled N spike in the mesocosm.

Acetylene Inhibition Technique

Acetylene inhibition technique was adapted from Sorensen (1978), and Christensen and Sorensen (1986). Sediment cores were taken by forcing a blue PVC tube (26.5 cm long and 5.3 cm i.d.) into the sediment to a depth of 15 cm (numbers of replicates are listed in Table 1). These tubes were inserted in locations with or without plants, in areas where the original sediment layer was kept intact during wetland construction, and in the areas where the original top sediment layer had been removed during wetland construction. The composited rates are reported here. Each tube was extracted from the sediment, and rubber stoppers were placed in both ends while submerged. Tube design allowed for a 200 mL water column above a 15 cm long sediment core after stoppers were inserted into both ends of the PVC tube. The tube was fabricated with a series of vertical holes spaced at 1 cm intervals adjacent to the sediment within the core and two holes adjacent to the top and bottom of the water column above the sediment. Septa placed in the holes served as ports to add C_2H_2 solution or remove water samples.

Calcium carbide added to water produced C_2H_2 used to saturate deoxygenated deionized water. A 1.0 mL aliquot of C_2H_2 solution was injected with a syringe and needle into the sediment at each of the 15 ports. The stopper on the water column was then removed; the water was decanted and replaced with ambient wetland water containing 9 to 20 mg of NO_3^-–N L^{-1}. A 7.0 mL aqueous sample was drawn from the water column and injected into a 10 mL Vacutainer for N_2O

Table 1. Mean *in situ* denitrification rates measured by C_2H_2 blockage method in constructed wetlands with other supporting measurements.

Location	Date	Background NO_3^- concentration†	Temperature‡	Denitrification rate††		Extractable organic C in sediment††	
		mg N L^{-1}	°C	mg N m^{-2} h^{-1}	*n*	g m^{-2}‡‡	*n*
Wetland 1	29 June 1995	not detected	24	2.1 (0.2)	12		
	29 May 1996	10.0	18	6.2 (1.4)	3	9.1 (1.4)	3
	14 June 1996	10.5	25	11.8 (1.0)	3	9.5 (1.6)	3
Wetland 2	29 June 1995	not detected	25	2.7 (0.5)	14		
	6 Feb. 1996	8.4	4	2.0 (0.4)	6	2.1 (0.2)	6
	29 May 1996	6.5	17	3.9 (0.8)	6	5.0 (0.6)	6
	14 June 1996	4.3	20	9.0 (1.0)	6	9.3 (2.9)	6

† Nitrate concentrations in the wetlands before the denitrification experiments started.
‡ At sediment-water interface.
†† Standard error in parentheses.
‡‡ Extractable organic C in the top 5 cm in the sediment core.
Note: Starting NO_3^- concentrations in the water columns in the experimental PVC tubes ranged from 9 to 20 mg N L^{-1}. Experiments on 29 June 1995 lasted for 16.5 h in the field; all the other experiments lasted for 3 h.

analysis by gas chromatograph (GC) for denitrification measurement. The stopper was replaced, displacing some water in the tube, and 20 mL of water sample was taken with a syringe for NO_3^- analysis by IC for determination of NO_3^- removal rate. Twenty milliliters of C_2H_2 solution was then injected into the water column. The final C_2H_2 concentration in the soil pore water and water column above the soil core was 10% of the C_2H_2 saturation concentration. The core was returned to the wetland and incubated in situ for 3 or 16.5 h. These times were short enough to minimize C_2H_2 decomposition, the development of organisms that can denitrify in the presence of C_2H_2, and the inhibition on nitrification (Rolston, 1986); the times were long enough to measure N_2O accumulation, however.

Immediately following incubation, a 7.0-mL aqueous sample was drawn from the water column with a syringe through the upper sampling port and injected into a 10-mL Vacutainer that had been flushed with N_2. The water sample was preserved with 0.06 mL of saturated $HgCl_2$ solution for N_2O analysis by GC for denitrification rate measurement. Samples were also taken from the water columns for NO_3^- analysis by IC to calculate NO_3^- removal rates. The sediment cores were cooled to between 0 and −20°C by dry ice in the field. In the laboratory, the upper 5 cm segment of the frozen cores (−20°C) was cut with an electric miter saw using a carbide tipped blade. Preliminary analysis of the entire core revealed that 95% of N_2O evolved from the upper 5 cm of the core; therefore, only the top 5 cm segment of each core was analyzed in these measurements reported here. The cut frozen core pieces were placed in a Nalgene bottle and 50 mL of 1 *M* KCl extracting solution was added, and the bottle was sealed with a rubber stopper. Bottles were shaken for 30 min until the sediment cores were thawed and the N_2O was in equilibrium between the aqueous and gaseous phases. A 4 mL gas sample was taken from each bottle with a syringe and placed in a 4-mL Vacutainer for N_2O analysis. A 7 mL aqueous sample from each bottle was also removed with a syringe and placed in a Vacutainer for N_2O analysis. Aqueous samples for N_2O analysis were preserved with 0.06 mL of saturated $HgCl_2$. Vial septa were covered with silicone sealant to prevent leakage.

Nitrous oxide was analyzed using a GC fitted with an electron capture detector (Lindau et al., 1988a). Nitrous oxide in the liquid samples was determined by extracting gas from the vial head-space several times until no N_2O was detected and summing the results of all extractions. Usually five extractions were sufficient. The denitrification rate was calculated as follows:

$$(N_{post} - N_{pre})/(T \times A)$$

where N_{post} and N_{pre} are the amounts of N_2O–N (in the water column and sediment core combined) after and before the in situ incubation, respectively; T is the duration of the experiment; and A is the cross-sectional area of the PVC tube.

Extractable organic C was determined to examine its relationship with denitrification rate. Field moist sediment soil was extracted with 0.5 *M* K_2SO_4 (Brooks et al., 1985). A Dohrmann DC-80 carbon analyzer (Xertex Corp., Santa Clara, CA) was used to determine dissolved organic C in these K_2SO_4 extracts, following passage through GF/C (1.2 μm) glass fiber filters. The NO_3^- removal rate in the water column was calculated as follows:

$$(N_{after} - N_{before})/(T \times A)$$

Where N_{after} and N_{before} are the amounts of NO_3^-–N in the water column after and before in situ incubation, respectively. For all 3 h C_2H_2 inhibition incubations, NO_3^- removal rates were not calculated because N_{after} values were not statistically different from N_{before} values, given the variability of these data with such a short incubation time.

RESULTS AND DISCUSSION

The disappearance of NO_3^- in wetland water could be due to (i) movement out of the water column hydrologically, (ii) biochemical reactions, and (iii) plant uptake and microbial use. Bromide is a conservative anion whose transport and fate are subject to hydrologic conditions but not chemical reactions in the wetland. If we assume that the hydrologic behavior of NO_3^- is the same as that of Br^-, then the decline in the concentration ratio $[NO_3^-]/[Br^-]$ with time indicated that NO_3^- was transformed by nonhydrologic reasons in the mesocosm in which Br^- and nonlabeled NO_3^- had been spiked at the same concentration of 14 mg L^{-1} (Fig. 1).

Nitrogen-15 Isotope Tracing Experiment

Nitrate concentrations in the water columns in the mesocosms decreased linearly with time (Fig. 2A). The ^{15}N isotopic analysis confirmed the formation of denitrification products (i.e., labeled N_2O and N_2) (Fig. 2B). Emission of N_2O was not observed after 172 h of the experiment and total N_2O emissions accounted for only ~0.1% of the total denitrification gas emissions (hence Fig. 2B shows N_2 emission only). The N_2O from agricultural sources has been a concern as an ozone depletion

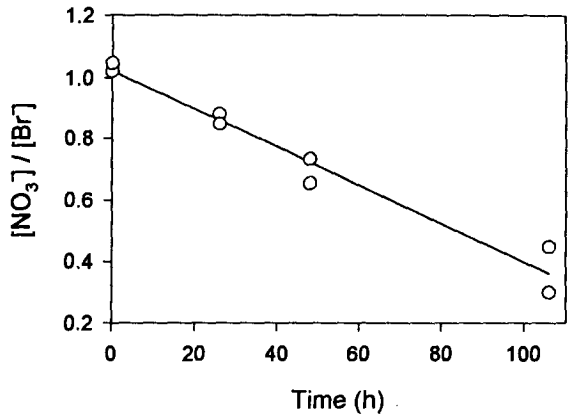

Fig. 1. Concentration ratio of $[NO_3^-]/[Br^-]$ in the wetland mesocosm experiment. Results from two replicate mesocosms shown.

and greenhouse gas (Bremner and Blackmer, 1978). Our data were consistent with Bremner and Blackmer's (1979) conclusion that denitrifying microorganisms produce little N_2O when reducing NO_3^-. Comparing the two curves in Fig. 2A and 2B, there was a lag of gas evolution behind the aqueous NO_3^- removal. By ~800 h (33 d), evolution of N_2 completely stopped and 58% of the spiked NO_3^- was recovered as N_2 (Fig. 2B). This percentage is smaller than the 77% value reported in a laboratory study by Lindau et al. (1988b) using a closed system.

The sigmoidal shape of the denitrifying curve (Fig. 2B) appears to indicate the nature of the microbial activity. Before the ^{15}N mesocosm experiment started, the background NO_3^- concentration in the wetland water had not been detectable for at least 5 d. Thus, the initial denitrifier populations were perhaps small, and denitrification proceeded slowly as a result. When denitrifier populations increased in response to the NO_3^- pulse in the mesocosm, the reaction accelerated. Eventually, after all NO_3^- available to denitrifiers was consumed, the denitrification curve (Fig. 2B) flattened. Our constructed wetland contained abundant organic matter in the anaerobic sediment under inundation, which formed an ideal biochemical environment for denitrifier metabolism (David et al., 1997a). Thus, in these constructed wetlands, denitrification can be a major mechanism for NO_3^- removal if the residence time is sufficient. During heavy rain events, water can flow through constructed wetlands in a few hours and the water residence time in the wetlands will be short; therefore, little denitrification will occur. Because we shook the mesocosms to help release gases trapped in the sediment, the noninterfered gas evolution rate from the sediment could have been slower, but the effects of factors such as surface waves and water currents are unknown. With or without mesocosm shaking, the final denitrification percentage in Fig. 2B would likely be the same; shaking would only help release the gases that the denitrification produced into the air head-space.

By multiplying the derivative of the curve in Fig. 2B (derivative was calculated using Mathematica, Wolfram Media, Champaign, IL) with the total amount of NO_3^- spiked, the curve in Fig. 2C was obtained which showed that, in response to an instantaneous NO_3^- input,

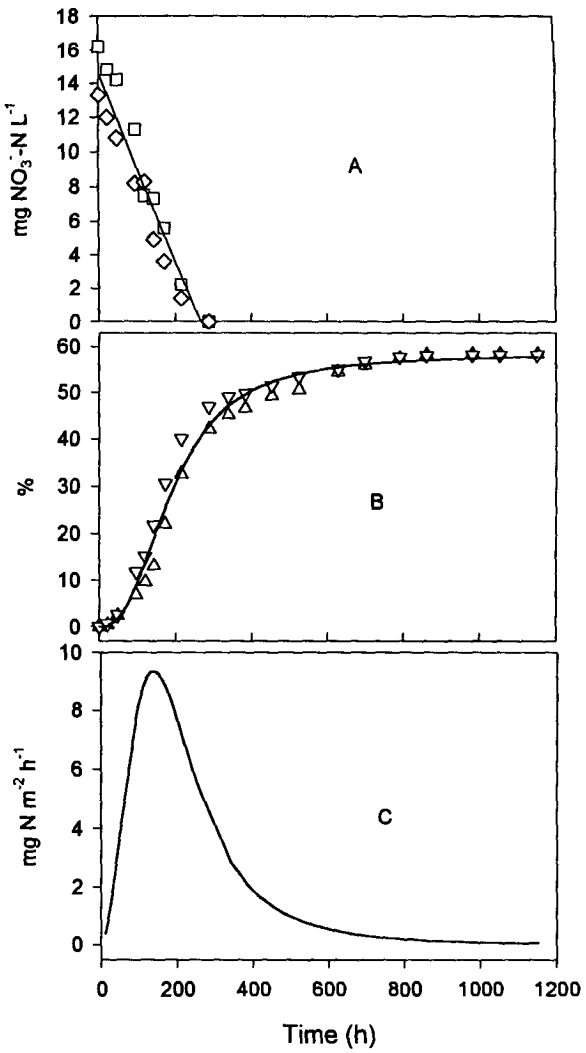

Fig. 2. Disappearance of NO_3^- from the water column measured by ion chromatography and denitrification measured by ^{15}N-tracing technique in constructed wetland in June-August. Results from two replicate mesocosms shown. (*A*) NO_3^- concentration in water column. (*B*) N_2 evolved/total N spiked. (*C*) Denitrification rate.

the denitrification rate changed with time. The rate peaked at 9.3 mg N m^{-2} h^{-1} at 130 h (5.4 d) of the experiment. As a comparison with denitrification rates in some natural wetlands, this peak rate was much faster than 0.15 mg N m^{-2} h^{-1} for fresh and saline ponds, but comparable with 3 mg N m^{-2} h^{-1} for salt marshes (Howard-Williams and Downes, 1993). Mosier et al. (1989) previously noted a lag between the time fertilizer was applied and the time of peak N emissions. In their study in a rice (*Oryza sativa* L.) field, the N_2 flux peaked 2 d after urea fertilization.

Acetylene Block Experiment

In our February 1996 experiment, N_2O as a denitrification product was only found in the sediment of the cores; whereas in May and June experiments, N_2O was found in both sediment and water column. This indicated that diffusion of N_2O from the sediment into the overlying water column was slower in winter than summer. In situ denitrification rates in the constructed wet-

lands measured by C_2H_2 inhibition ranged from 2.0 to 11.8 mg N m^{-2} h^{-1} (Table 1). For 1996 data in Table 1, the rates were significantly related to temperature (r^2 = 0.71, $P < 0.05$, $n = 5$) and extractable sediment organic C ($r^2 = 0.50$, $P < 0.001$, $n = 24$). Duxbury (1986), Keeney (1986), and Rolston (1986) cautioned about the potential problems associated with the C_2H_2 block technique when applied in flooded soils: it may be difficult to distribute C_2H_2 evenly in the soil matrix; C_2H_2 may block nitrification; and C_2H_2 may degrade in the soil. Because we injected C_2H_2 solution at depths of 1-cm intervals into the PVC tube and into the water column in the PVC tube and our experiment lasted for only 3 or 16.5 h, the potential problems were expected to have been minimized.

Despite the potential shortcomings of the C_2H_2 blockage method, it has been recognized that the C_2H_2 technique, when its potential problems are adequately addressed, can yield reasonable denitrification rates (Keeney, 1986) and the denitrification rates measured by the C_2H_2 inhibition technique are comparable with the rates determined by the ^{15}N technique (Mosier et al., 1986). This seems to be true in our study. The 2.0 to 11.8 mg N m^{-2} h^{-1} range of the denitrification rates determined by C_2H_2 block method as shown in Table 1 fall in the range of the rates determined by the ^{15}N tracing technique in Fig. 2C. Lowrance et al. (1995) reported an average annual denitrification rate of 0.78 mg N m^{-2} h^{-1} using C_2H_2 inhibition method for a restored riparian forest wetland. Christensen and Sorensen (1986) reported denitrification rates of 3.2 mg N m^{-2} h^{-1} under light and 4.8 mg N m^{-2} h^{-1} in dark in a littoral lake in Denmark in August by C_2H_2 inhibition. Their soil cores were amended with 8.4 to 12.6 mg NO_3^-–N L^{-1}. These rates are similar to our rates in constructed wetlands. Moreover, Lindau et al. (1990) reported denitrification rates ranging from 1.4 to 14 mg N m^{-2} h^{-1} in a KNO_3-treated flooded rice soil as determined in a 21-d field experiment using the ^{15}N technique.

Nitrate Removal Rate in the Constructed Wetlands

During the time when the field C_2H_2 inhibition measurements were conducted, NO_3^- removal rates were also determined; the average NO_3^- removal rates in the water column in the two wetlands determined at the same time and in the same blue PVC mesocosms as the C_2H_2 inhibition experiments in June 1995 were 21 mg N m^{-2} h^{-1} ($n = 14$, SE = 1.4) and 25 mg N m^{-2} h^{-1} ($n = 12$, SE = 2.0), respectively, at 24 to 25°C measured at the water/sediment interface. The wetland background NO_3^- concentrations were below detection at the time of these experiments and the incubations lasted for 16.5 h. The NO_3^- removal rate in the water column, determined with nonlabeled NO_3^- in white PVC mesocosms identical to the ^{15}N experiments (the PVC tubes were not capped), averaged 12 mg N m^{-2} h^{-1} (six replicates, SE = 1.48); the field experiments lasted for 120 h and the background NO_3^- concentrations were below detection with a day time temperature of 16 to 17°C at water/sediment interface. Previously David et al. (1997a) reported NO_3^- removal rates of 14 mg N m^{-2} h^{-1} at 11 to 12°C in April and 63 mg N m^{-2} h^{-1} at 27°C in June at the same field site.

Summarizing the above NO_3^- removal rates, they ranged from 12 to 63 mg N m^{-2} h^{-1} at a temperature range of 11 to 27°C. Compared to the denitrification rates measured by C_2H_2 inhibition technique (2.0–11.8 mg N m^{-2} h^{-1} in Table 1) and by ^{15}N isotope tracing ($\leq$9.3 mg N m^{-2} h^{-1} in Fig. 2C), the NO_3^- removal rates in the water column were significantly greater. This was probably because a portion of the NO_3^- that disappeared from the water column infiltrated into the sediment, and had not been denitrified.

Plant and Microbial Uptake of Labeled Nitrate

Recovery of the labeled N in the plant and soil samples at the end of the ^{15}N mesocosm experiment is summarized in Table 2. At the end of this experiment, NO_3^- concentrations in the water columns had been below detection for a month. Therefore, the labeled N detected in the sediment by TKN and MS analysis would be in organic forms produced through root or microbial uptake. The plants (mixture of barnyardgrass and yellow nutsedge) harvested in the two mesocosms weighed 0.792 and 3.29 g for mesocosm 1 and 2, respectively. Assuming a bulk density of 1 g cm^{-1} for the sediment column in the mesocosm, a 5 cm deep sediment core (20.3 cm i.d., 324 cm^2 cross-sectional area) weighed 1.62 kg. The last column in Table 2 was calculated using these data. Summing labeled N in plants and the top 5 cm of sediment in each mesocosm in Table 2, 6.73 mg + 0.217 mg yields 6.95 mg N in mesocosm 1, and 12.0 mg + 0.607 mg yields 12.6 mg N in mesocosm 2. Therefore, 5.5% of 127 mg of spiked NO_3^- was recovered in the top 5 cm of sediment and plant shoots in mesocosm 1; in the second mesocosm the recovery was 9.9%. Combining the recovery in the air head-space as N_2, in the plants, and in the top 5 cm of the sediment, the total recovery for spiked N in mesocosm 1 was 64%, as compared to 68% for mesocosm 2. The rest of the spiked N probably moved with seepage water to depths lower

Table 2. Recovery of labeled N in plants and the top 5 cm of sediment in mesocosms buried in a constructed wetland.

Mesocosm		TKN† (dry wt.)	^{15}N atom	Total N resulting from spiked NO_3^-
	Top 5 cm soil core	mg g^{-1}	%	mg N per mesocosm
1	I	3.87	0.3985	2.56
	II	4.15	0.5259	15.1
	III	3.41	0.4023	2.56
				x = 6.73
2	I	4.50	0.5247	16.2
	II	3.30	0.5138	11.0
	III	3.54	0.4762	8.75
				x = 12.0
1	Plants above soil surface	28.7	1.0350	0.217
2	*Ibid.*	36.5	0.7222	0.607

† Total Kjeldahl nitrogen.

Table 3. Denitrification capacities and agricultural tile NO_3^- loads in three constructed wetlands. Each tile drained cropland under maize-soybean rotation.

Wetland	Area	Seasonal denitrification capacity		Mean monthly NO_3^- loads (1995–1997 water years)†	
		Jan. (4°C)	June (25°C)	Jan.–Feb.	May–June
	ha	kg N		kg N	
1	0.60	8.8	52	46 (6–101)	180 (8–431)
2	0.30	4.4	26	12 (4–28)	44 (4–99)
3	0.78	11	65	54 (8–127)	210 (17–451)

† Range in parentheses. 1995–1996 data from David et al. (1997b); 1997 data from M.B. David (unpublished data).

than 5 cm in the wetland sediment. The transport and fate of NO_3^- in the seepage was not determined.

Wetland Denitrification Capacity

The highest denitrification rate found in this study was 11.8 mg N m^{-2} h^{-1} (Table 1). This average rate was obtained in June, the temperature at water/sediment interface was 25°C, and there was a background NO_3^- concentration of 10.5 mg N L^{-1} at the time of the experiments in the wetland. Because these conditions perhaps represented the most favorable scenario for denitrification in the wetland, this rate was likely to be the maximal denitrification rate for summer in the study wetlands. At 4°C in February when the wetland background NO_3^- concentration was 8.4 mg N L^{-1}, the denitrification rate was 2.0 mg N m^{-2} h^{-1} (Table 1). These two rates were assumed to be typical for summer and winter, respectively, to estimate seasonal denitrification capacities (Table 3). Also shown in Table 3 are the NO_3^- loading data to these wetlands, which were highly variable due to the great range in precipitation amounts during a given month. In winter and summer for the 1995–1997 water years, tile NO_3^- loads were greater than these wetlands' denitrification capacities on average. The ratios between denitrification capacity and mean load for the three wetlands ranged from 19 to 59% with an average of 33% based on the data in Table 3. We would estimate that for months with low inputs, nearly all of the NO_3^- could be denitrified. However, for months with high inputs, a small percentage would be removed. The ultimate wetland denitrifying efficiency depends on both wetland capacity and the water residence time in each wetland.

Infiltration may enhance denitrification because it carries NO_3^- in the water column gradually through the sediment/water interface where denitrifier populations are likely to be the most active because of the high organic matter content; mixing in the wetland can also bring NO_3^- to the sediment surface for denitrification. In contrast, the denitrification rate in a stagnant wetland underlain by an impermeable sediment layer is expected to be low. In addition, background NO_3^- concentration was important in determining denitrification rates because it affects the pre-existing denitrifying enzyme activity.

This study suggests that NO_3^- removal rate in the water column was much faster than the production rate of the denitrification gases in the constructed wetland. About 60% of the NO_3^- removed from the water column in the study wetland was recovered as N_2 given enough residence time. The other pathways for NO_3^-–N included 6 to 10% in the plants and soil organic matter; presumably about 30% was lost to seepage water. Further research is needed on the gas and NO_3^- diffusion processes in the water column and the sediment, the biochemical reaction rate of denitrification per se, the influence of pulsing vs. the continuous NO_3^- load on denitrification rate, long-term pathways for NO_3^- removal through plant and microbial uptake, and denitrification of the seepage NO_3^- in the subsurface. The current world-wide tendency to use constructed wetlands as a water treatment tool certainly demands a more thorough understanding of the kinetics and mechanisms of the denitrification processes.

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