Wetlands and Aquatic Processes

Denitrification in South Carolina (USA) Coastal Plain Aquatic Sediments

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ABSTRACT

Denitrification rates in aquatic sediments from three sites upland of high salinity estuaries were measured to compare nitrate (NO_3^-) removal by sediment bacteria. The study sites included two blackwater creeks that drain to coastal inlets (one in an undeveloped coastal forest and the second in a suburban residential development) and a drainage pond on a golf course. Two to 20 mmol NO_3^- kg^{-1} soil was added to microcosms and the acetylene block technique was used to estimate denitrification. Nitrate, nitrous oxide (N_2O) and ammonium (NH_4^+) concentrations were monitored over time. The rate and efficiency of denitrification was low at the 2 mmol NO_3^- kg^{-1} addition and increased proportionally with NO_3^- added. Across all treatments, the golf course sediment that received consistent N inputs in situ had the most rapid and greatest N_2O production, while the salt marsh in the undeveloped area had the least. The NH_4^+ concentrations remained constant and low (2 mmol N kg^{-1}) except at the undeveloped, forested Oyster Creek (8 mmol NH_4^+–N kg^{-1}). In most cases, the majority of NO_3^- removal did not occur until 24 to 48 h of incubation, regardless of N_2O production. During typical low- and no-flow periods, this time frame may be adequate for slow diffusion of nutrients and removal of NO_3^- prior to transport to coastal estuaries or ground water, particularly at the golf course site. However, this time frame may not be adequate during rapid water and nutrient transport due to severe storm events typical of this region.

THE influence of anthropogenic activities on microbial nitrate removal has implications for potential contamination or mediation of nitrate contamination of ground water, surface waters, and estuaries. The coast of South Carolina was relatively undeveloped until the 1970s and is currently undergoing rapid residential and tourism-related development. This has increased nutrient loadings from nitrogenous fertilizers, particularly in recreational areas such as golf courses, and increased runoff from impermeable surfaces such as roads and parking lots (Vernberg et al., 1992; Wahl, 1996). Channelization projects to drain land may further concentrate contaminants and reduce residence times in streams (Smock and Gilinsky, 1992; Wahl et al., 1997). Sediments underlying coastal creeks and periodically flooded estuaries may be receiving areas for highly soluble contaminants. Anthropogenic activities affect not only nitrate loading, however, but also microbial processes and may increase or decrease the capacity of native bacteria to remove nitrate.

Nutrients affect the amount of primary productivity in aquatic ecosystems and excess nutrient inputs correspond to areas of eutrophication (Radach, 1992; Valiela et al., 1992; Nixon, 1995). Nitrate is of particular concern because it is water soluble, can be carried through runoff into surface waters, and can migrate rapidly through sediments and reach ground water. Ground water in turn can discharge nitrate contamination to surface waters (McMahon and Böhlke, 1996) and coastal embayments (LaPointe and Clark, 1992; Millham and Howes, 1994; McClelland et al., 1997; McClelland and Valiela, 1998).

Microbial denitrification is a primary mechanism for removing excess nitrate (Seitzinger, 1988) and occurs primarily in sediments under anaerobic conditions (Atlas and Bartha, 1993). The objectives of this study were to compare rates of denitrification by bacteria in water-saturated sediments collected from three sites in South Carolina's coast that differ in the level and type of land development and anthropogenic NO_3^- addition. The study sites included two blackwater creeks, one in an undeveloped forested site (Oyster Creek) that drains to North Inlet estuary and the second in a suburban residential subdivision bordered by uncultivated farmland (Dog Creek) that drains into Murrells Inlet estuary (Fig. 1). The third site was a small drainage pond on a golf course in Pawleys Island, SC that received fertilizer and pesticide applications. All sites were in slow-moving or nonflowing aquatic systems (creek or small artificial pond) and all the sediments were completely water-saturated. It was hypothesized that bacteria associated with the golf course sediments that received regular anthropogenic inputs of nitrogenous fertilizers in situ would remove NO_3^- at a greater rate relative to the other two sites, because they would be adapted to the nitrate inputs and a denitrifying community would be more established. Dog Creek was expected to have less denitrification than the golf course, followed by the undeveloped low-gradient Oyster Creek, which has lower average nitrate concentrations in ground water and surface water than Dog Creek (Aelion et al., 1997; Wahl, 1996).

MATERIALS AND METHODS

Study Locations and Sediment Collection

Sediment samples were collected from three coastal South Carolina sites within 30 km of each other (Fig. 1). Two of the sites are slow-moving, low-gradient blackwater creeks (approximately 2 to 4 m wide) that drain to high salinity estuaries characteristic of the Coastal Plain of the southeastern United States. Blackwater streams tend to have high dissolved organic carbon concentrations and low concentrations of dissolved

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oxygen and nutrients. Although many of these streams have seasonal no-flow periods, they represent an input of nutrients to the high salinity, ocean-dominated estuaries along South Carolina's coast, particularly during storm events (Wahl et al., 1997).

The first site, Dog Creek, drains to Murrells Inlet, a bar-built estuary system. The 11-ha Dog Creek watershed has a highly developed infrastructure for tourism, with community septic tanks and roads nearby, and is adjacent to uncultivated farm land (Wahl et al., 1996, 1997; Cresci, 1997). The land cover consists of 82% turf grasses and impervious surfaces. Although most development is downstream from this residential community, land use has changed dramatically in the vicinity of the study site as recently as from 1993 to 1997. At the collection site behind the residential development, the blackwater creek is approximately 4 m wide. The creek channel was dug 2 to 3 m below the water table across the natural topographic divide for drainage for the development and an agricultural field. During dry periods there was minimal flow in the creek (channel slope of 0.34%), but it had water for the entire year. Average dissolved organic carbon concentrations in the water of 13 mg C L^{-1}, average NO_3^- of 130 μg N L^{-1}, and average annual stream flow volume of 162 × 10^4 m^3 yr^{-1} have been reported (Wahl, 1996).

Oyster Creek, which drains into North Inlet estuary, is a blackwater creek located in an undeveloped forested area near Georgetown, SC. The 6880-ha property includes 2883 ha of 100% forested uplands and a receiving estuary. The 37-ha catchment was originally used for indigo and then for rice cultivation. The rice cultivation, which was abandoned approximately 90 yr ago, required a hand-dug ditch network that exists today. The property was acquired in 1958 and for the last 30 yr has been owned by the Belle W. Baruch Foundation to be permanently maintained in its natural state (Vernberg et al., 1992).

The creek is in a low-gradient (0.13% slope vs. 0.34% for Dog Creek) watershed characterized by dense stands of pine and hardwoods, with cypress wetlands (Wahl et al., 1997). During dry times, typically in the hot summers, there was limited flow and approximately 0.5 m of water in the creek. An average dissolved organic carbon (DOC) concentration in the creek of 26 mg L^{-1}, average NO_3^- concentration of 45 μg N L^{-1}, and average annual streamflow volume of 94 × 10^3 m^3 yr^{-1} have been reported (Wahl, 1996). Sediment samples were collected at three locations in Oyster Creek from the forested uplands to the saline estuary. The first sediment collection location was in the creek bed approximately 400 m upland of the mouth of the estuary in a densely forested area, above any region receiving tidal influx. The second area was the mouth of Oyster Creek, before it discharged into North Inlet. This site received tidal influx and the salinity of the overlying water was approximately 16 to 23 dS m^{-1} at low tide and 47 to 50 dS m^{-1} at high tide. The third area was further downstream in a channel in the salt marsh in North Inlet, where the salinity was approximately 47 to 50 dS m^{-1}. Unlike the first area, the last two areas were vegetated, but not forested.

The third site was on a golf course in Pawleys Island, SC. Approximately 360 condominiums surround the golf course. Like Dog Creek, ditches were cut below the water table to provide drainage and the soils were predominantly sands. Sediments were collected from a constructed drainage pond on a golf course receiving periodic fertilizer input. The pond was 2 m deep and 15 m across and was located at the edge of one of the fairways. The collection site was adjacent to the area where fertilizer spraying tanks were washed out and near the storage facilities for pesticides and fertilizers used on the property (Aelion and Cresci, 1999). The greens (total area 9295 m^2) were fertilized once a month (21–4–11 N–P–K) at an application rate of 49 kg per 1000 m^2. The 21 ha of fairways, tees, and roughs were fertilized twice a year at an application rate of 618 kg per ha (10–21–21 N–P–K). Ammonium was spot applied as grasses became yellow (21% NH_4 solution) (Cresci, 1997).

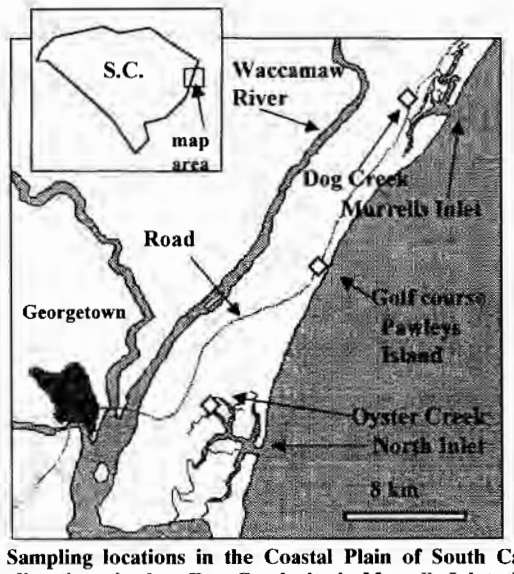

Fig. 1. Sampling locations in the Coastal Plain of South Carolina including the suburban Dog Creek site in Murrells Inlet, the golf course site in Pawleys Island, and the undeveloped preserve in forested Oyster Creek in North Inlet. Samples were collected at three different times at each site.

At all the sites, after surface detritus was scraped away, sediment was collected 15 cm below surface in the creek beds with a hand auger and transferred to autoclaved, sterile Mason jars. This depth was chosen to sample the anoxic region through which NO_3^- may be transported after application at the land surface, and to complement previous studies examining ground water quality (Aelion et al., 1997). The jars were filled completely with sediment to minimize any headspace when the jars were sealed. The samples were transported on ice and stored in the laboratory at 4°C. After returning to the laboratory, subsamples of sediment were dried (90°C, 72 h) to determine water content, ashed to determine organic content (loss on ignition), and sieved for particle size analysis (Table 1). Sediment was collected from each site on three occasions between February 1996 and January 1997. Three complete sets of denitrification experiments were carried out using sediments collected from each site from each sampling trip. Sediments generally were used within the first 2 wk of collection and were rarely stored for more than 1 to 2 mo.

Denitrification Experiments

Sediment denitrification was measured using the acetylene block technique (Lensi et al., 1985; Yoshinari et al., 1977). Sediments were incubated by replacing 5% of the microcosm headspace with acetylene (C_2H_2) to inhibit nitrous oxide reductase. The resulting N_2O accumulation was measured by gas chromatography (GC). Subsamples of sediment (average dry weight approximately 8.0 g) were placed in 125-mL serum vials and spiked with one of three levels of nitrate to give final concentrations of 2, 10, or 20 mmol NO_3^- kg^{-1} dry sediment. For the Oyster Creek site, the salinity of the nitrate solution was adjusted with Instant Ocean (Aquarium Systems, Mentor, OH), a synthetic sea salt, to equal the salinity mea-

Table 1. Sediment characteristics from the golf course, suburban Dog Creek, and undeveloped forested Oyster Creek sites. Porewater and surface water nitrate concentrations (μmol NO_3–N L^{-1}) were ranges from repeated sampling trips.

Site	Moisture	Sand†	Silt/Clay†‡	OM†§	Porewater	Surface water
	%				μ*M* NO_3–N	
Golf course	26	98	<1	2	10–27	13–1214
Dog Creek	31	87	4	6	14–32	2–18
Oyster Creek						
Creek bed	36–41	87–90	5–6	4–8	14–25	4–17
Creek mouth	38	85	6	6	ND¶	64
Salt Marsh	30	92	5	3	7	57

† Percent w/w of dried sediment.
‡ <63-μm diameter.
§ Organic matter, loss on ignition determined by ashing sample at 550°C.
¶ ND = not determined.

sured in situ. To ensure anaerobic conditions, the headspace was flushed with two to three volumes of N_2 using a cannula, and 750 μl of Oxyrase (Mansfield, OH) was added, a sterile enzyme additive that produced and maintained anaerobic conditions by reducing any O_2 in solution. The vials were sealed with Mininert Teflon valves (Dynatech Corp., Baton Rouge, LA) that allowed repeated headspace gas sampling without exposing the sediment to atmospheric O_2. Approximately 5% of the headspace was replaced with high-purity C_2H_2 and the vials were incubated in the dark at room temperature (20–22°C).

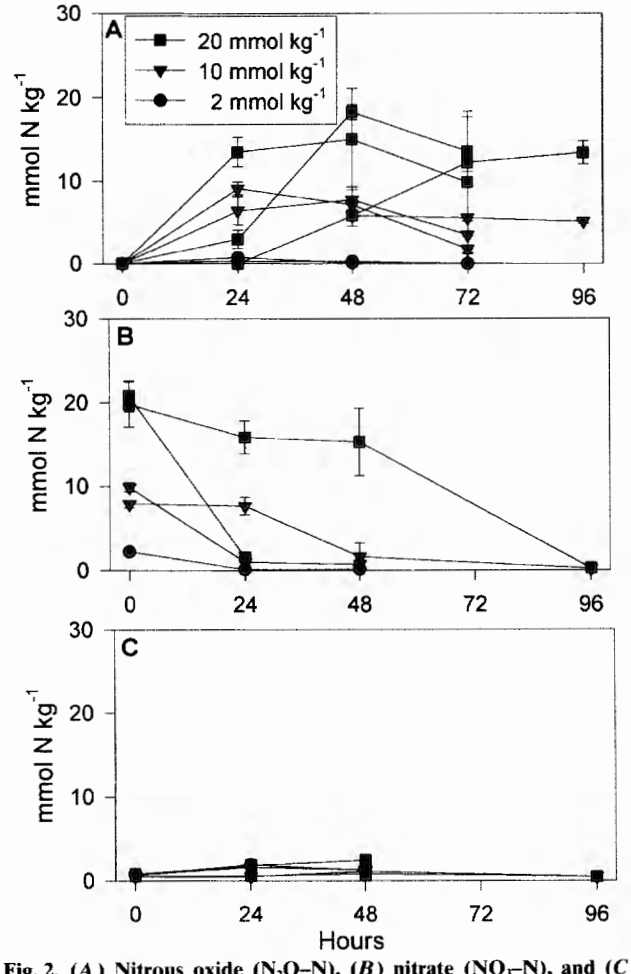

Fig. 2. (*A*) Nitrous oxide (N_2O–N), (*B*) nitrate (NO_3–N), and (*C*) ammonium (NH_4–N) concentrations (mmol kg^{-1} dry sediment) measured over time in golf course sediment microcosms incubated at three added nitrate concentrations (20, 10, and 2 mmol NO_3–N kg^{-1} dry sediment). Each line represents a separate experiment (N_2O–N, mean of at least four replicates ± s.d.) carried out using sediment collected on each of three sampling dates.

At the beginning of each experiment, 8 to 10 experimental replicates were measured for N_2O at each NO_3^- concentration. Four vials from each concentration were destructively sampled for nutrient concentrations during the time course of the experiment, leaving a minimum of four experimental replicates at the end of the incubation. Abiotic control vials (n = 3) were used for each experiment by metabolically inhibiting the bacteria with $HgCl_2$ (750 μL of a saturated solution; final concentration 6.6 g $HgCl_2$ kg^{-1} dry sediment), but were otherwise treated identically to the experimental vials. Headspace N_2O concentrations were measured in each vial at 1, 24, 48, 72, and 96 h of incubation using a Varian 3700 gas chromatograph equipped with a 1/8 in × 3 m ss Porapak Q column (Supelco, Bellefonte, PA), a Valco (Houston, TX) gas sampling valve, and a ^{63}Ni electron capture detector (Aelion et al., 1997). Gas chromatograph conditions were: column at 80°C (isothermal), detector at 350°C, and carrier gas at 35 mL min^{-1} (5% CH_4 in Ar). The N_2O–N concentrations were computed from peak areas using a standard curve derived from N_2O standards (Scott Specialty Gases, Plumsteadville, PA). Total N_2O concentration in each vial was calculated using Henry's Law to estimate the proportion dissolved in the liquid phase, assuming equilibrium between the slurry and headspace (Davidson and Firestone, 1988). All values were corrected for headspace concentrations in abiotic control vials.

Surface water was filtered (0.2 μm) and analyzed on-site to determine in situ NO_3^- concentrations using a Hach kit (Aelion and Cresci, 1999). After returning to the laboratory, 10 g of sediment were removed and the pore water and sediment were analyzed for NO_3^- by adding 30 mL of 2 *M* KCl, shaking, and allowing the samples to sit overnight. The samples were then centrifuged at 1000 rpm for 15 min (120 × *g*), decanted, and filtered through a 0.2-μm membrane filter. The total filtrate volume was measured to determine the volume of the original liquid sample by difference and analyzed for NO_3^- as described below for the experimental vials. For NO_3^- and NH_4^+ measurements during the denitrification experiments, duplicate vials were destructively sampled at 0, 24, 48, or 96 h. The bacteria were metabolically inhibited with $HgCl_2$ and sediments were extracted overnight with 30 mL of 2 *M* KCl as described above. Aliquots of the sediment extracts were filtered through a 0.2-μm filter and frozen until analysis using a Shena Lasca (Norcross, GA) 6880 Automated Chemistry Analyzer. Nitrate + nitrite concentrations were determined colorimetrically by the cadmium reduction method (USEPA Method 353.2) and NH_4^+ concentrations were determined using the salicylate method (USEPA Method 350.1) (USEPA, 1993).

Table 2. Denitrification rates (mmol N_2O–N kg^{-1} h^{-1}) estimated from N_2O production, and denitrification efficiencies (% NO_3–N converted to N_2O–N) at three added NO_3^- concentrations (mmol NO_3^- kg^{-1} dry wt.)(mean of three experiments, each with $n = 4 \pm$ s.d.)

Site	2.0 mmol NO_3 kg^{-1}	10 mmol NO_3 kg^{-1}	20 mmol NO_3 kg^{-1}
Golf course†			
Rate (mmol N_2O–N kg^{-1} h^{-1})	0.03 ± 0.01	0.30 ± 0.07	0.51 ± 0.16
Conversion (%)	28 ± 12	75 ± 12	79 ± 9
Dog Creek			
Rate (mmol N_2O–N kg^{-1} h^{-1})	0.01 ± 0.00	0.23 ± 0.05	0.42 ± 0.11
Conversion (%)	7 ± 2	53 ± 13	51 ± 13
Oyster Creek			
Creek bed			
Rate (mmol N_2O–N kg^{-1} h^{-1})	0.00 ± 0.00	0.21 ± 0.13	0.44 ± 0.16
Conversion (%)	7 ± 4	60 ± 28	67 ± 10
Creek mouth			
Rate (mmol N_2O–N kg^{-1} h^{-1})	0.02 ± 0.00	0.23 ± 0.06	0.51 ± 0.08
Conversion (%)	19 ± 2	56 ± 9	65 ± 10
Salt Marsh			
Rate (mmol N_2O–N kg^{-1} h^{-1})	0.00 ± 0.00	0.15 ± 0.10	0.26 ± 0.20
Conversion (%)	1 ± 1	43 ± 25	41 ± 31

† For all data combined, differs from Dog Creek, Oyster Creek, Oyster Salt Marsh ($p < 0.05$).

Data Analysis and Statistical Analyses

The dependent variables, lag time, potential denitrification rate, and denitrification efficiency were compared using a two-way ANOVA (site × concentration; $\alpha = 0.05$) using SigmaStat 1.0 (Jandel Scientific, 1994). Pairwise multiple comparisons (Student–Newman–Keuls; $\alpha = 0.05$) were used to identify statistical differences in the dependent variables. In general, the N_2O concentrations demonstrated an initial lag time in which no N_2O was measured in the headspace, followed by a rapid increase in N_2O that leveled off asymptotically (Fig. 2A). The denitrification rate for each vial was calculated as mmol N_2O–N produced kg^{-1} dry sediment h^{-1} during the period of most rapid production (i.e., the maximum slope of the plot of N_2O–N concentration vs. time). This generally occurred between 0 and 24 h, or between 24 and 48 h if a lag period occurred. Denitrification efficiency of each vial was calculated as the maximum percent of the original N added (as NO_3–N) that was measured as headspace N_2O–N. To assess differences in denitrification rate as a function of sampling location and nitrate concentrations, data from replicate experiments (three per site) were pooled for each site for each NO_3^- concentration added. Denitrification rates also were estimated from NO_3^- depletion over time as an independent measure and used for an order-of-magnitude comparison with rates determined from N_2O production.

RESULTS

All sediments were predominantly sand, with very little silt or clay (Table 1). The golf course sediment had the lowest organic content (2%) and the greatest percentage of sand. For Oyster and Dog Creeks, surface water NO_3^- concentrations were of the same order of magnitude as the pore water NO_3^- concentrations on all sampling dates and did not exceed 64 μM NO_3–N. Although pore water NO_3^- concentrations at the golf course did not differ from the other sites (range of 10–27 μM NO_3–N), surface water samples were variable and up to two orders of magnitude greater than those at Dog and Oyster Creeks (i.e., as high as 1.2 mM NO_3^- in September 1996).

The N_2O, NO_3^-, and NH_4^+ concentrations are presented in Fig. 2 through 6 from three series of experiments conducted for each study location at each NO_3^- concentration added. In all cases, initial headspace N_2O concentrations measured at 0 to 1 h were at or near zero. Following a lag phase of up to 48 h, N_2O concentrations rapidly increased, then leveled off. The length of the lag phase varied depending on site ($p < 0.001$). The mean lag time for the golf course sediment (Fig. 2A) was shorter than that of the other sediments. The longest lag times were measured in Oyster Creek Salt Marsh sediments at the highest NO_3^- level (Fig. 6A). Nitrous oxide production in abiotic controls was negligible (data not shown).

Denitrification rates were determined from the linear portion of the N_2O produced over time (Table 2). When a two-way ANOVA was performed (level × site), there were statistically significant main effects for each factor (level [$p < 0.001$] and site [$p < 0.001$]). For all sites, N_2O production was minimal at the lowest treatment and increased significantly with increasing NO_3^- addition. When N_2O production rates were compared between sites combining data from all experiments regardless of NO_3^- additions, the golf course had greater denitrification than Dog Creek, Oyster Creek, and Oyster Creek Salt Marsh. When compared at each NO_3^- concentration, there were no differences between site at the lowest NO_3^- addition. At the intermediate NO_3^- addition, few differences existed between site, except that the golf course had greater denitrification than Oyster Creek and Oyster Creek Salt Marsh. At the highest NO_3^- addition, the golf course and Oyster Creek mouth had greater denitrification rates than the other sites and the Oyster Creek Salt Marsh had lower N_2O production rates.

There was a statistically significant interaction between site and concentration for denitrification efficiencies. For all sites, denitrification efficiencies were minimal at the lowest treatment and increased at the higher NO_3^- concentrations ($p < 0.01$). When tested using pairwise multiple comparisons combining data from all experiments at all NO_3^- additions, the golf course sediment had significantly ($p < 0.05$) greater efficiencies than the other sediments (Table 2).

The production of headspace N_2O was accompanied

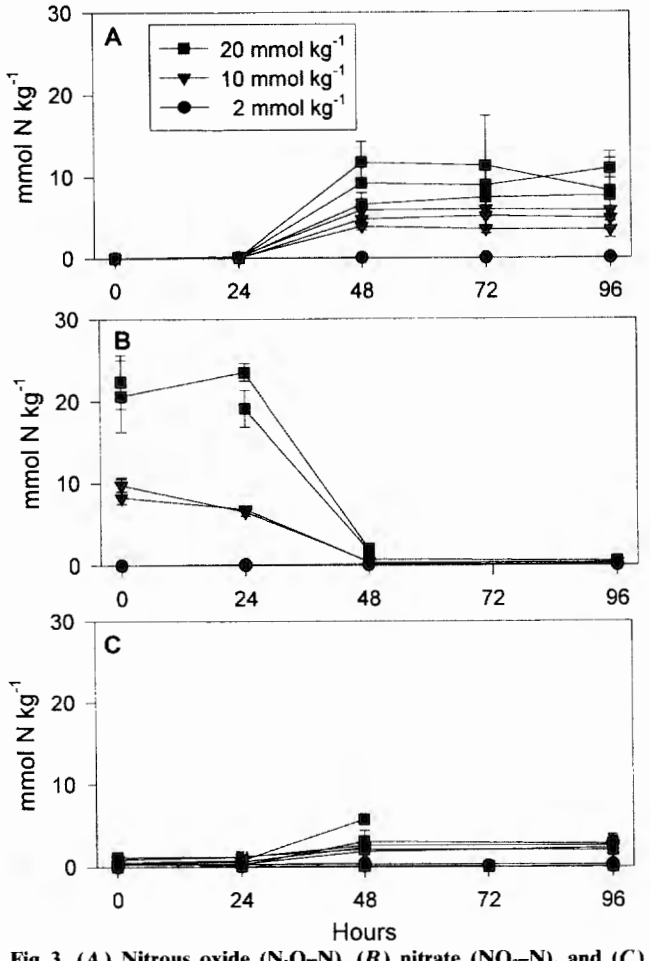

Fig. 3. (*A*) Nitrous oxide (N_2O–N), (*B*) nitrate (NO_3–N), and (*C*) ammonium (NH_4–N) concentrations (mmol kg^{-1} dry sediment) measured over time in residentially developed Dog Creek sediment microcosms incubated at three added nitrate concentrations (20, 10, and 2 mmol NO_3–N kg^{-1} dry sediment). Each line represents a separate experiment (N_2O–N, mean of at least four replicates ± s.d.) carried out using sediment collected on each of three sampling dates.

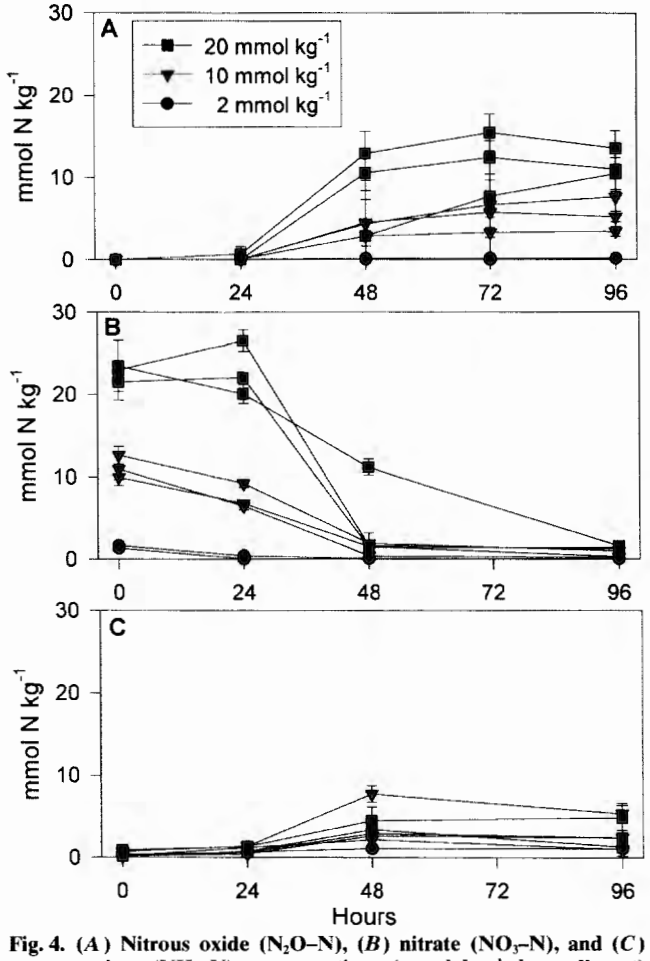

Fig. 4. (*A*) Nitrous oxide (N_2O–N), (*B*) nitrate (NO_3–N), and (*C*) ammonium (NH_4–N) concentrations (mmol kg^{-1} dry sediment) measured over time in forested, undeveloped Oyster Creek sediment microcosms incubated at three added nitrate concentrations (20, 10, and 2 mmol NO_3–N kg^{-1} dry sediment). Sediments were sampled in the upland fresh water creek bottom. Each line represents a separate experiment (N_2O–N, mean of at least four replicates ± s.d.) carried out using sediment collected on each of three sampling dates.

by a depletion of NO_3^- measured over time in duplicate vials (Fig. 2B–6B). Nitrate concentration decreased more rapidly at the 20 mmol kg^{-1} NO_3^- addition at Dog and Oyster Creeks (Fig. 3B–4B). At the three other sites including the golf course, the decrease in NO_3^- concentration was more gradual in general and more variable (Fig. 2B, 5B, 6B). The change in NO_3^- concentrations over time was used as a gross estimate and independent measure of denitrification rates, which were similar in magnitude to those based on N_2O production, but ranged more widely. Essentially no NO_3^- depletion was measured in the abiotic control vials (data not shown).

In samples from Dog Creek, Oyster Creek, and the golf course to which no NO_3^- was added (data not shown), small concentrations of NH_4^+ were measured over time (<1 μmol NH_4–N kg^{-1} dry sediment). The amount of NH_4^+ generated in NO_3–amended vials was less than ≤2 mmol N kg^{-1} and was relatively constant over time (Fig. 2C–6C), with the exception of Oyster Creek (Fig. 4C), which had greater NH_4^+ production than the other sites at the 10 mmol NO_3^- kg^{-1} treatment levels ($p < 0.05$). Ammonium was a greater percentage of inorganic nitrogen initially present in the vials at the lowest levels of NO_3^- addition, up to 63% at the Oyster Creek salt marsh, but decreased with increasing NO_3^- additions. At the highest NO_3^- addition, NH_4^+ was a small percentage (ranging from 8 to 17%) of the inorganic nitrogen present in sediment from all sites.

DISCUSSION

The addition of NO_3^- to sediment samples from all sites sampled in this study induced nitrate reduction, as evidenced by the removal of NO_3^- and production of N_2O in the presence of 5% acetylene. Denitrification rates and efficiencies compared favorably with estimates reported by Seitzinger (1988) in her extensive review of denitrification studies. All samples showed a lag time before N_2O accumulation could be detected and this lag phase varied by site. However, denitrification rates were calculated after this lag period as though community

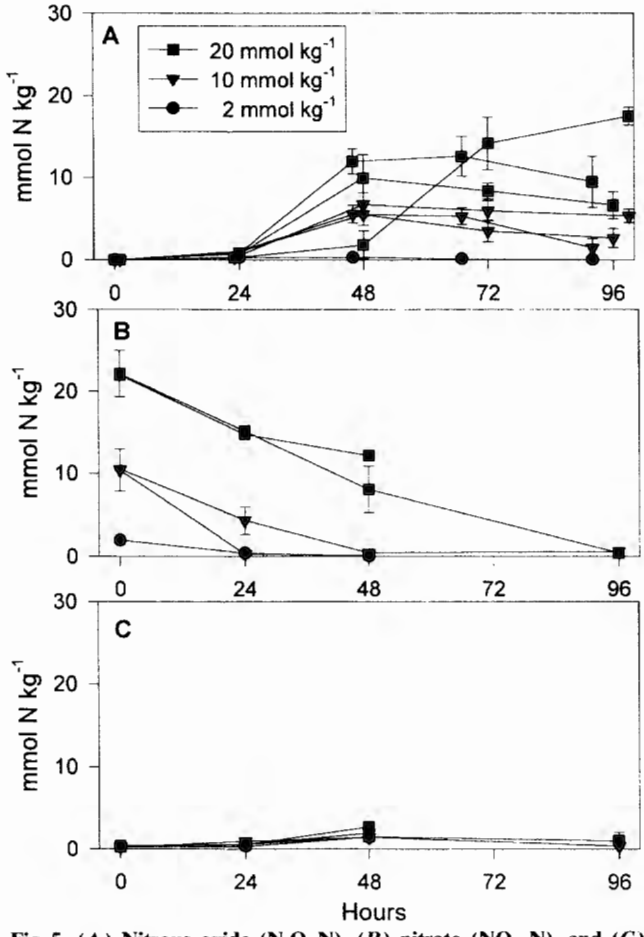

Fig. 5. (*A*) Nitrous oxide (N_2O–N), (*B*) nitrate (NO_3–N), and (*C*) ammonium (NH_4–N) concentrations (mmol kg^{-1} dry sediment) measured over time in undeveloped Oyster Creek mouth sediment microcosms incubated at three added nitrate concentrations (20, 10, and 2 mmol NO_3–N kg^{-1} dry sediment). Each line represents a separate experiment (N_2O–N, mean of at least four replicates ± s.d.) carried out using sediment collected on each of three sampling dates.

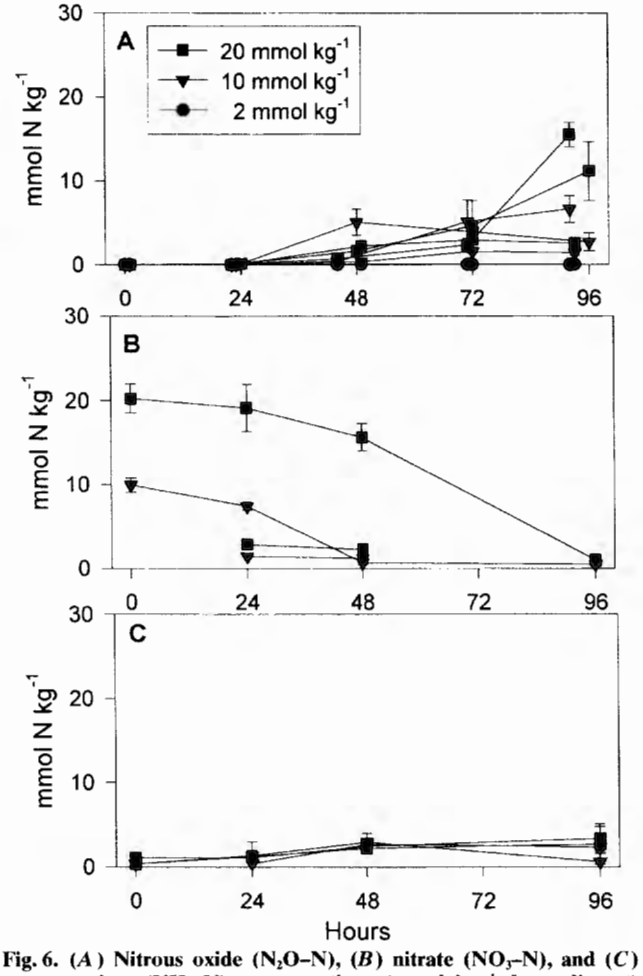

Fig. 6. (*A*) Nitrous oxide (N_2O–N), (*B*) nitrate (NO_3–N), and (*C*) ammonium (NH_4–N) concentrations (mmol kg^{-1} dry sediment) measured over time in undeveloped Oyster Creek salt marsh sediment microcosms incubated at three added nitrate concentrations (20, 10, and 2 mmol NO_3–N kg^{-1} dry sediment). Each line represents a separate experiment (N_2O–N, mean of at least four replicates ± s.d.) carried out using sediment collected on each of three sampling dates.

response was immediate. Thus, our estimated rates are potential rates, and although comparisons of rates and efficiencies between study locations are valid, rates calculated in this laboratory study may not represent those occurring in situ. In addition, we were not able to measure nitrification, which also is inhibited by acetylene (Knowles, 1990) and may be an important source of nitrate during times of the year when freshwater streamflow is extremely low in these tidal creeks (Wolaver et al., 1984).

In our study, at the lowest NO_3^- addition that resembled in situ concentrations, denitrification was low and did not differ by site. With increasing NO_3^- addition, the rate and extent of denitrification increased significantly as was found by Seitzinger et al. (1993), and differences between site became more evident. The golf course sediments had less than a 24-h lag time for N_2O production and greatest denitrification rates and efficiencies in comparisons combining data for all NO_3^- concentrations added. In contrast, the undeveloped salt marsh had the lowest and most variable N_2O production rates, perhaps due to sulfide, which can inhibit denitrification in sulfate-reducing bacteria that can reduce nitrate (Dalsgaard and Bak, 1994).

Ryals et al. (1998) suggested that NO_3^- was effectively removed from surface waters at three golf course sites in the Coastal Plain of North Carolina. In our study, although the golf course sediments produced N_2O more efficiently than the other sites in general, NO_3^- removal occurred on a similar time frame as the other sites (i.e., after 48 h). While some studies have reported almost complete NO_3^- removal in sediment (>90%) by denitrification (Davidsson et al., 1997; Blicher-Mathiesen and Hoffmann, 1999), lower removals in highly affected areas have been reported (35% of N input in rivers and 20–50% of input to estuaries) (Seitzinger, 1988). In our study, N_2O–N accounted for approximately 50 to 80% of NO_3^- removal at the higher NO_3^- concentrations, and removal occurred by 96 h. The blackwater streams and high salinity inlets found on South Carolina's coast, although undergoing rapid land use changes and increased nutrient loading, are not as highly affected as those reported by Seitzinger (1988).

An additional fate of the NO_3^- could have been dissimilatory nitrate reduction to ammonium. Ammonium also can be generated from ammoniafication in which NH_4^+ is released from organic material in sediment due to microbial mineralization. Production of NH_4^+ from dissimilatory nitrate reduction to ammonium and ammoniafication does not remove N from the system, rather this process maintains N in a bioavailable form that may undergo subsequent nitrification. In some estuarine sediments, dissimilatory nitrate reduction to ammonium may be insignificant for NO_3^- removal (Binnerup et al., 1992). The process responsible for the generation of NH_4^+ was not identified in this study, but NH_4^+ was a small part of the total dissolved inorganic nitrogen present at the greatest NO_3^- addition. Davidsson et al. (1997) found that total ammoniafication in peaty soil was twice as great as in sandy soil causing NH_4^+ to accumulate at depth in the peaty sediment (35 cm). Peaty soil was an N source, while sandy soil was an N sink. All the sites in this study, but particularly the sandy golf course sediments, were N sinks as a result of the enhanced NO_3^- reduction to N_2O, with only small increases in NH_4^+. Ammonium production appeared to play a greater role at the forested Oyster Creek upland creek bed than at the other sites, and ammonium is the major form of dissolved inorganic nitrogen measured in ground water at this site (Aelion et al., 1997). Ground water NH_4^+ concentrations were approximately 10 times greater than those of NO_3^- and average NH_4^+ concentrations were significantly greater in Oyster Creek ground water (56 μmol L^{-1}) than in Dog Creek ground water (23 μmol L^{-1}) (Aelion et al., 1997). The role of conversion to NH_4^+, whether associated with dissimilatory nitrate reduction to ammonium or ammoniafication, merits further study, particularly at Oyster Creek.

Diffusion is an important process for transferring NO_3^- between overlying water and shallow subsurface sediments. At the golf course site, large anthropogenic inputs of NO_3^- to surface water may increase the concentration gradient between surface water and sediments, and may episodically supply sediment microorganisms with a source of NO_3^- and enhance denitrification. At Oyster Creek and Dog Creek during low-flow conditions, NO_3^- concentration gradients may be less than those at the golf course. Alternatively, during storm events, nutrients can be transported quickly to receiving estuaries and advection, not diffusion, may dominate nutrient transport. Wahl (1996) measured rising and falling limbs of water discharge of approximately 10 to 20 h duration for storm events for Dog and Oyster Creek. He estimated that due to runoff during storm events, 11 times more NO_x loading occurred at the urbanized stream than the forested stream due to a combination of increased streamflow and higher NO_x–N concentrations in runoff at Dog Creek. In this case, bacterial NO_3^- removal may be reduced as water is rapidly carried to the estuary and salt marsh without adequate time for diffusion into upland creek sediments, which have potentially greater capacities to remove NO_3^- than estuarine sediments. Thus, both the magnitude of the loading and the time frame in which nutrient loading occurs are important considerations for denitrification and subsequent bacterial NO_3^- removal.

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Long-Term Wastewater Treatment Effectiveness of a Northern Wisconsin Peatland

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ABSTRACT

Secondary effluent from the Drummond, WI wastewater stabilization lagoon system was applied to an acidic, nutrient-poor, 8.3-ha peatland for the purpose of advanced wastewater treatment. Application occurred from June through October at an average rate of 10 cm yr^{-1} from 1979 to 1982, 24 cm yr^{-1} from 1983 to 1988, and 40 cm yr^{-1} from 1989 to 1996, increasing surface flow from the application area by 47, 78, and 154%, respectively. The pH of the peatland outflow increased from 4.2 to 6.7, chloride rose from 1.1 mg L^{-1} to 80 mg L^{-1}, and total phosphorus increased from 0.05 mg L^{-1} to 0.6 mg L^{-1}. Because raw sewage input was only one-half of the lagoon system's design capacity, the lagoons provided a high degree of sewage treatment, removing 95% of suspended solids (SS), biochemical oxygen demand (BOD), and nitrogen from the wastewater stream. Phosphorus removal in the lagoons, which was 98% in the first years of operation, declined to 85% by 1995. The peatland contributed little additional treatment, removing only 37% of the nitrogen and 17% of the phosphorus remaining in the lagoon effluent. The peatland's capacity to retain phosphorus was exhausted after a few years of application. Increased pH, nutrient availability, and water levels altered the peatland vegetation community. The *Sphagnum* ground cover was reduced or eliminated in many places, and dense stands of cattail (*Typha* sp.) developed in some areas. Small peatlands such as this one seem poor candidates for use as tertiary treatment systems.

Since 1979, secondary effluent from the sewage stabilization lagoon system at Drummond, WI, a town of about 225 people in the northwestern part of the state, has been applied to a small peatland for advanced treatment before disposal of this wastewater into a high-quality trout stream. Northern Wisconsin, adjacent portions of Minnesota and Michigan, and parts of Canada abound in such peatlands. The potential of these peatland areas to provide simple and cost-effective wastewater treatment for small communities is the subject of recurring interest. A considerable body of knowledge exists on the basic processes occurring in wetlands that could influence the capacity of wetlands to provide wastewater treatment (for example, Nichols, 1983; Godfrey et al., 1985). However, relatively little research has been done to quantify this capacity in specific types of wetlands over extended periods of time. This paper describes some of the effects on the Drummond peatland of almost 20 yr of secondary effluent application and evaluates the capacity of this peatland and similar peatlands to provide long-term tertiary treatment of municipal wastewater.

Due to heavy texture and slow permeability, many of the soils in and around Drummond have severe limitations for use in septic tank adsorption fields. Because failure of individual septic systems was a continual problem, the Wisconsin Department of Natural Resources (WDNR) ordered that a municipal system for secondary treatment of sewage be designed for the community. Construction of sewers and a waste stabilization lagoon system was completed in 1978.

The original permit for operation of the sewage treatment facility called for disposal of the stabilization lagoon effluent into the nearby Long Lake Branch of the White River. The Long Lake Branch is designated by the WDNR as a Class 1 trout stream and supports self-sustaining populations of brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*).

Concerns about potential adverse effects on this high quality stream caused by nutrients, organic materials

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Abbreviations: BOD, biochemical oxygen demand; SS, suspended solids; WDNR, Wisconsin Department of Natural Resources.