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BACKGROUND, SHORT-TERM AND POTENTIAL LONG-TERM DENITRIFICATION CAPACITY OF SOILS IN URBANIZED COASTAL WATERSHEDS ON KIAWAH ISLAND, SOUTH CAROLINA, USA

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ABSTRACT

Urbanization is escalating in many coastal areas of the US and is associated with deteriorating water quality. Often the associated changes in land use result in an overabundance of nutrients and other types of pollution entering ground and surface waters. It is important that we understand biogeochemical transformation processes on urbanizing watersheds if we are to develop management practices that can improve nutrient attenuation on the landscape. In this study denitrification capacity was estimated for two watersheds with residential and resort land uses on Kiawah Island, South Carolina. Potential for soils and sediment to reduce nitrogen (N) in runoff was estimated, and ways to improve denitrification capacity were tested. Background denitrification capacities (ambient) were substantially less than the potential short-term capacity (nutrient enriched) that ranged from 0.03 to 1.82 nM nitrous oxide (N_2O) gdw^{-1} h^{-1} in soil and 0 to 2.48 nM N_2O gdw^{-1} h^{-1} in pond sediments. Denitrification rates were considerably higher near the soil surface than at the water table, rates were stimulated by adding NO_3^- to the surface but not by adding it to the subsoil, and added glucose did not affect rates. Potential long-term rates were explored using soil amendments and 340 hour incubations. Denitrification capacity was stimulated with added nitrate (NO_3^-) and with added NO_3^- plus carbon (C) in the form of glucose and wood fiber, but denitrification rates dropped to zero after addition of only glucose and wood fiber to soil. This suggested that heterotrophic microbes grew on the carbon substrate and reduced N availability through immobilization, and this low N availability was confirmed for C amended soil. These results suggest that 1) immobilization may be an important process in the removal of anthropogenic NO_3^- from stormwater runoff, 2) the N processing efficiency of a landscape can be sustained with high soil organic matter (SOM) content, as on forested watersheds, and 3) N processing efficiency can be improved on SOM depleted soils by incorporating wood fiber into the soil to provide substrate for microbial activity.

KEYWORDS. Denitrification, constructed wetlands, eutrophication, nitrous oxide

INTRODUCTION

Denitrification is an important part of the global N cycle that removes mineral N from surface and groundwater and biogeochemically transforms it into N gas (Payne, 1981; Ma and Aelion, 2005). However, many landscapes have diminished denitrification capacities due to drainage, loss of vegetated buffers, and stormwater management practices that bypass environmental processing of runoff. At the same time, nutrient loads in some watersheds have increased from turf and landscape fertilization and other residential and resort practices. Because primary productivity in

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coastal waters is commonly N limited (Casey and Klaine, 2001), and this non-point source (NPS) pollution tends to be N rich, eutrophication is a major concern for coastal land and water managers (Poe et al., 2003). To sustain and improve coastal water quality it is necessary to manage the N processing capacity of coastal landscapes.

In an effort to mitigate deteriorating water quality, created wetlands are being tested for their capacity to process NPS pollution (Cardoch et al., 2000; Havens et al., 2002; Strosnider, 2005). Because wetlands tend to have high N removal efficiencies (Bachand and Horne, 2000; Mitsch and Gosselink, 2000) the construction of wetlands to intercept and process nutrient rich stormwater may be a way to improve water quality along the coast. However, at present information is not available to adequately evaluate how effective these wetlands might be at reducing mineral N loads in runoff.

This study compared denitrification rates in two watersheds feeding impaired coastal waters. In a test of ways to help mitigate poor water quality the background (ambient), short-term (nutrient enriched) and potential long-term (soil amended and incubated) denitrification capacities were tested.

STUDY SITE

Kiawah Island is a barrier island located approximately 34 miles south of Charleston, South Carolina, at 32° 37’ 10” N, 80° 3’ 40” W. Kiawah was relatively pristine prior to the onset of residential and resort development in 1976 (Combes, 1975). Soils within are classified by the USDA Soil Survey Report (1971) as Crevasse-Dawhoo complex, and predominant vegetation was maritime live oak forest prior to development (Hosier, 1975).

The two watersheds studied drain into detention ponds K67 and K70 (Figure 1). The watershed feeding pond K67 is 5.7 ha and is the site of a planned constructed wetland to mitigate N loading of the pond (Strosnider, 2005). The watershed feeding pond K70 is16 ha (Bunker, 2004). Both watersheds contain residential areas and pond K67 also is bordered by a golf course. Both ponds are terminal end members in their pond network: the last pond in the interconnected series of ponds to receive water from tidal surges (Shea, 2001). Stormwater is piped directly from impervious surfaces into both ponds by way of an underground drainage system.

There were three sites in each pond where sediment samples were collected: K67 site 1, 2, and 3 and K70 site 1, 2, and 3 (Figure 1). Also, soil was collected from the terrestrial part of each watershed at K67 sites 4, 5, and 6 and K70 sites 4 and 5.

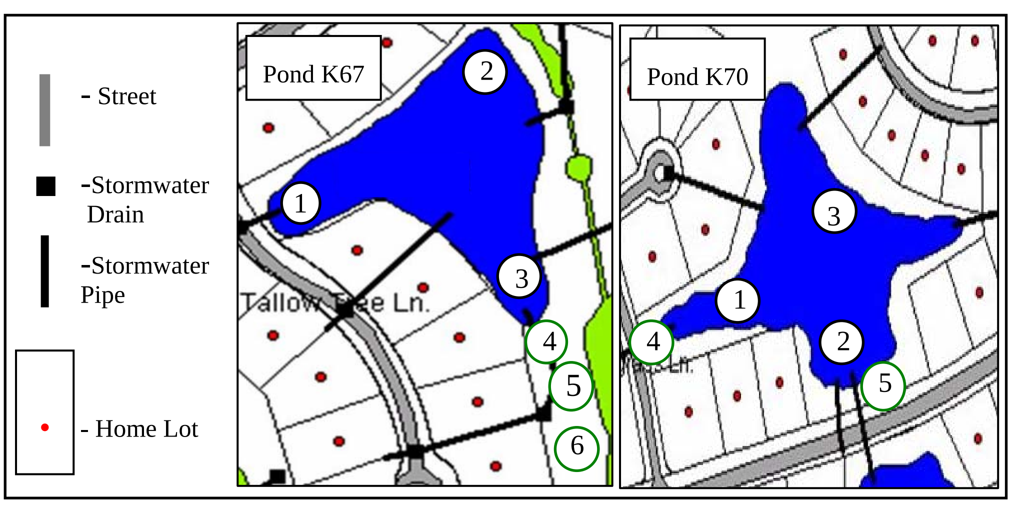

Figure 1. Experimental pond K67 sediment sample sites (1, 2, and 3) and terrestrial soil sample sites (4, 5, and 6) and reference pond K70 sediment sample sites (1, 2, and 3) and terrestrial soil sample sites (4 and 5) on Kiawah Island, South Carolina.

METHODS

Studies of background, short-term, and long-term denitrification potential were conducted at several soil depths to compare difference between surface and subsurface soils. Factors that might limit denitrification rates were explored, and a long-term experiment tested how soil amendments could influence the N processing ability of the planned constructed wetland.

Soils and sediments were sampled monthly during 2004 and quarterly during 2005. Duplicate terrestrial soil samples were collected with a hand bucket auger, while pond samples were collected using a polyvinyl tube (8.9 cm i.d. by 30 cm length) as a push tube. Cores were taken to a depth of 15 cm below the surface, and then were separated into 5 cm depth intervals. All samples were transported on ice and stored at 4°C in polyethylene bags until processing. Temperatures (T) of samples were measured using a digital thermometer and redox potentials (E_h) were measured with a redox potential probe and silver chloride (AgCl) reference electrode in a 1:1 soil to deionized (DI) water slurry (Patrick et al., 1996).

Sediment pore water samples were collected every other month during 2004 and were analyzed for ammonium (NH_4^+), nitrate and nitrite (NO_3^- + NO_2^-), orthophosphate (PO_4^+), and dissolved organic carbon (DOC) using a Quik Chem +8000 flow injection analyzer (FIA) (Lachat Instruments, Milwaukee, Wisc.). Pore water was collected using diffusion chambers that were incubated *in situ* for at least 30 days according to Sundareshwar and Morris (1999) at 0-2, 5, 10, 15, and 20 cm pond sediment depths. These diffusion chambers were Kimble 30 mL borosilicate glass vials (Vineland, N.J.) covered with 0.45 µm nitex nylon mesh.

Soil and sediment pH were measured according to Peech (1965). Percent SOM was estimated as total volatile solids by combustion in a muffle furnace at 545°C for 15 minutes. Soil N availability was estimated on soil within 24 hours of sampling using KCl extractable NO_3^- and NH_4^+ according to Mulvaney (1996). The supernatant was frozen until nutrient analysis for NH_4^+ and NO_3^- + NO_2^- by FIA.

Background denitrification capacities were estimated for terrestrial soils between August 2004 and April 2005 and for pond sediments during October 2004. Anaerobic assays were conducted in serum bottles containing 25 g fresh soil or sediment and the headspace was purged with ultra high purity helium (UHP He). After 48 hours of incubation at 22°C ± 2 °C, N in the soils and sediments was considered removed (Yoshinari et al., 1977; Murray and Knowles, 2003). Subsequently, 25 mL of 10 mM NO_3^- was added unless otherwise noted. Finally, the liquid and gas within the serum bottle were purged with UHP He and the bottles were stored in the dark until sampling. Denitrification rate was measured as N_2O produced with (n=3) and without (n=1) a nitrous oxide reductase inhibitor (acetylene, C_2H_2) in the headspace during the incubation. N_2O concentrations were quantified using a new detection method (Drescher, 2005; Drescher and Brown, 2005) modified from Poli et al. (1999) employing solid-phase microextraction (SPME)-Gas Chromatograph (GC)-Mass Spectrometer (MS) using a ThermoFinnigan Trace GC coupled with a Finnigan Polaris Q MS and LEAP CombiPal autosampler for SPME. Background N removal capacities by denitrification were calculated assuming a bulk density value of 1.2 g cm^{-3}, considered standard for sandy soil (Brady, 1974).

To compare whether potential short-term denitrification rates were greater at the soil surface or at the groundwater table, and to determine whether short term rates were limited by NO_3^- or C substrate availability, experiments were conducted from the 0 to 15 cm soil terrestrial surface and at the surface of the groundwater table. Soil from K67 sites 4, 5, and 6 were collected in March 2005, and 25g of soil sample was amended with 25 mL of either 10 mM NO_3^- (+N) or 278 mM glucose without pre-incubating the soil to exhaust the mineral N. For each sample there were assays with (n=3) and without (n=1) C_2H_2 as a nitrous oxide reductase blocker.

Potential long-term denitrification rates were explored by amending 500 g of soil (n=3) with 500 mL of 10 mM NO_3^- (+N), 500 mL of 14 mM glucose plus 7 g sawdust mixed into the soil (+C), both +N and +C, or just with 500 mL DI water, and allowing the microcosms to incubate for about 340 hours before conducting the assay. This experiment was conducted in the greenhouse at the Center for Forested Wetlands Research in Charleston, South Carolina. Soil used in this experiment was collected in March 2005, from a 0 to 10 cm soil depth at K67 site 4 and was incubated in high density polyethylene tanks. Microcosms were replenished with treatment solution as needed to maintain anaerobic conditions during the two week period. Microcosm temperatures were maintained at 29°C ± 3°C. Subsequently, a 72 hr assay was carried out for each sample during which denitrification was measured as described above. At the end of the experiment, N availability for the previously incubated soil was estimated using KCl extractions as described above, to measure the change in soil N availability during the incubation.

One-way analysis of variance (ANOVA) was performed using Student-Newman-Keuls when variances were homogeneous, and the non-parametric test Kruskal-Wallis was used when variances were heterogeneous. Because one datum was suspected of being an outlier, a test for identifying significant outliers was conducted according to Neter et al. (1985) using SigmaStat (SPSS, 1998). Statistical significance levels were α=0.05 unless otherwise reported.

RESULTS AND DISCUSSION

Baseline characterization showed that the two watersheds were similar in soil and sediment T ($\bar{x}$ =21°C) and pH ($\bar{x}$ =8.2) but the SOM tended to be greater and the soil E_h was lower in K70 ($\bar{x}$ =2% and $\bar{x}$ =-112 mV respectively) than in K67 ($\bar{x}$ =1.3% and $\bar{x}$ =-50 mV respectively). In both watersheds, SOM decreased with depth for both the terrestrial (soil) and pond (sediment) sites, and sediments were consistently more reduced than were soils (data not shown).

In general, soil N availability was low in soils of both watersheds (<5µg N g^{-1}) (n=132), but spikes (> 20 µg N g^{-1}) were recorded in K67 and K70 soils seven times during the study. These values were low compared to estimates of 26 to 56 µg N g^{-1} reported for other sites in the lower coastal plain of South Carolina (Burke et al., 1999; Burke and Eisenbies, 2000).

In contrast, N availability was substantially greater in sediment pore water (Table 1). During 2004, NH_4^+ concentrations in sediment pore water ranged from below the limit of detection (LOD, <0.05 µM) to 731 µM, NO_3^- + NO_2^- concentrations from below LOD, (<0.002 µM) to 122 µM, PO_4^+ ranged from 2 to 271 µM, and DOC ranged from 348 to 2635 µM. The N:P ratio (based on dissolved inorganic nutrients) in sediment pore water was low (relative to the Redfield Ratio of 16) for both pond K67 (4) and pond K70 (3). Ironically, although N concentrations were high in pore water, the overabundance of P in the pore water made N the nutrient likely to be the more limiting to primary production.

Table 1. Mean (and standard error) pore water nutrient concentrations of pond sediments 0 to 20 cm deep for pond K67 and K70.

Sample Site	Mean NH_4^+ (µM)	Mean NO_3^- + NO_2^- (µM)	Mean PO_4^+ (µM)	Mean DOC (µM)
K67	175 (27)	5 (2)	63 (6)	1235 (85)
K70	279 (24)	6 (1)	73 (8)	1150 (53)

Background (ambient) denitrification rates were correlated with both soil water content (r=0.632, p<0.001), and SOM (r=0.540, p<0.001), and were weakly negatively correlated with pH (r=-0.285, p=0.067). Soil extractable N was positively correlated with denitrification rates in August (r=0.887, p=0.008) and September (r=0.594, p=0.020) 2004. These results were similar to those of Tuerk and Aelion (2005) who found that sediment C and N were associated with denitrification rates on their South Carolina study site.

Background denitrification rates declined with depth in soils and sediments in both watersheds. Also, almost no N_2O was produced in the background assays (detected in only one of 42 samples) and instead, complete transformation of NO_3^- to N_2 gas appeared to occur. Usually, denitrification rates were higher for the K70 watershed than for the K67 watershed (eg. Figure 2), but occasionally denitrification rates at K67 were higher, possibly due to fertilizer from turf management entering the system. Background rates in K67 soils ranged from 0.03 to 1.82 nM

N_2O gdw^{-1} h^{-1}, while rates in K70 soils ranged from 0.10 to 0.83 nM N_2O gdw^{-1} h^{-1}. Average background denitrification rates in soil were 0.28 and 0.37 nM N_2O gdw^{-1} h^{-1} on K67 and K70, respectively. There were no differences detected for background denitrification rates for pond sediments (p=0.66) with rates at K67 ranging from 0.04 to 2.48 nM N_2O gdw^{-1} h^{-1}, and rates at K70 ranging from 0 to 1.57 nM N_2O gdw^{-1} h^{-1}. Average rates were 0.70 nM N_2O gdw^{-1} h^{-1} for K67 and 0.50 nM N_2O gdw^{-1} h^{-1} for K70. Using background denitrification rates it was estimated that a 15 cm deep slice from watershed K67 could remove 123 kg NO_3^--N ha^{-1} y^{-1} from soil and 163 kg NO_3^--N ha^{-1} y^{-1} from sediments based on mean denitrification rates from August, September, and October of 2004, and March of 2005.

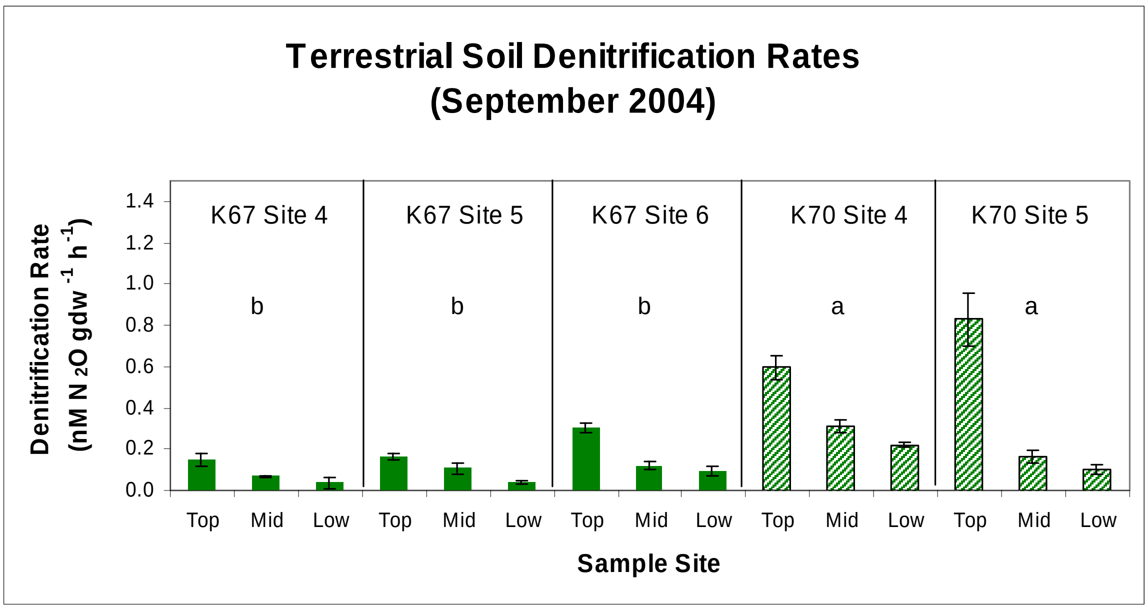

Figure 2. Denitrification rates (mean and standard deviation) for terrestrial K67 and K70 sites on Kiawah Island, South Carolina, in September 2004. Denitrification rates for site K70 were higher (a) than for site K67 (b) (p=0.03, Student-Newman-Keuls method one-way ANOVA). Sites refer to top (0 to 5 cm), mid (5 to 10 cm), and low (10 to 15 cm) depths (n=3).

Potential short-term denitrification capacities were rates that could be expected if nutrient rich water came in contact with the soil rather than bypassing the soil in the drainage pipes. Potential short-term rates were limited at the soil surface by NO_3^- but not by C substrate but neither amendments limited denitrification rate at the groundwater table (Figure 3). These results show that potential short-term (nutrient enriched) denitrification capacity is greater than background level estimates. Hence, the landscape has a greater capacity for removing NO_3^- from runoff than is currently occurring, and by piping stormwater directly into the ponds the environment is not helping to attenuate the nutrients.

The relationship between SOM and denitrification originally identified for the soil was not retained after the long-term incubations (p=0.315). Instead, after the long-term incubations only soil N availability was correlated with denitrification rate (r=0.733, p=0.007). Soil N availability after incubation in the +C treatment ($\bar{x}$ =0.3 µg N g^{-1}) was $\simeq$ DI water treatment ($\bar{x}$ =0.7 µg N g^{-1}) < +N ($\bar{x}$ =513 µg N g^{-1}) $\simeq$ +N+C ($\bar{x}$ =262 µg N g^{-1}) with n=6 for each treatment. This relationship, and the fact that denitrification was substantially greater in the long term +N treatments than in the short term +N treatments suggests that incubating soil with NO_3^- additions stimulated the growth of denitrifying soil bacteria in both the +N and +N+C treatments (Figure 4). Similarly, long-term incubation of soil with C appeared to stimulate the growth of heterotrophic microbes that immobilized N in their biomass and as a result reduced soil N availability. Immobilization can help explain the lack of correlation between SOM and denitrification rate in soil after incubation (p=0.315).

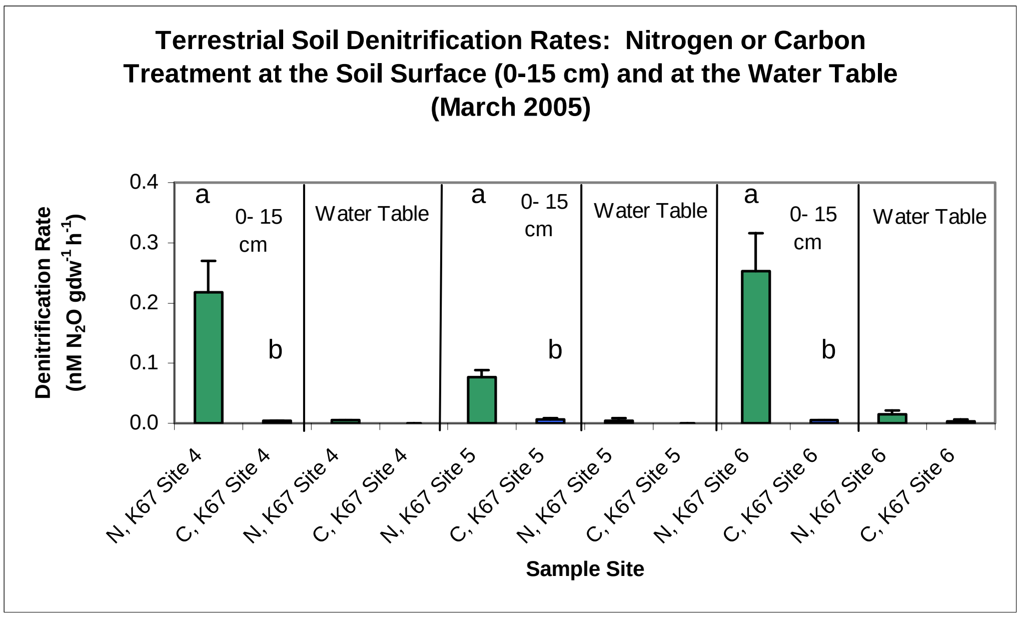

Figure 3. Denitrification rates for K67 soil on Kiawah Island, South Carolina, in March 2005. Denitrification rates were measured at the soil surface (0 to 15 cm) and at the groundwater table with the following two treatments: 1) NO_3^- (N) or 2) glucose (C). Different letters denote statistically different values ($p<0.05$, Student-Newman-Keuls method one-way ANOVA). Note that the y-axis maximum value is less than in the scale in Figure 2.

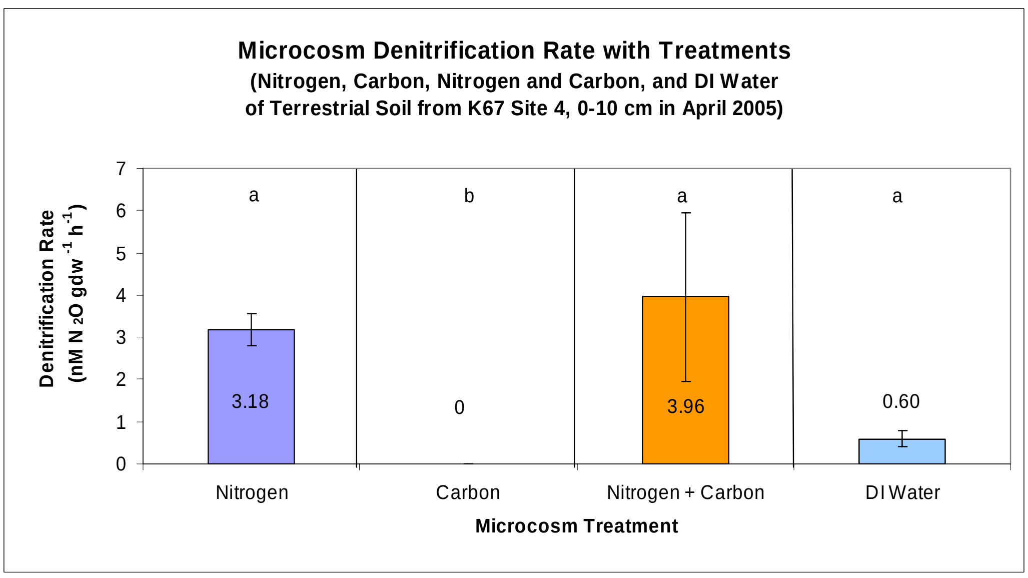

Figure 4. Denitrification rates of microcosms with added N, C, N + C, or DI water treatments. All soils were collected from terrestrial K67 site 4 at a 0 to 10 cm depth below the soil surface on March 25, 2005. Different letters denote statistically different values ($p<0.05$, Student-Newman-Keuls method Kruskal-Wallis one-way ANOVA).

These results support the findings of Ma and Aelion (2005) that 1) denitrification rate was enhanced with the addition of NO_3^- to soil, 2) addition of glucose alone did not enhance denitrification rate in short-term experiments, and 3) addition of NO_3^- and glucose did not increase denitrification rate more than the addition of only NO_3^-. However in this study we showed that addition of wood fiber, a less labile C source than glucose, can reduce denitrification rate, probably due to competition between heterotrophic and denitrifying bacteria for mineral N and net immobilization. The substantially longer incubation period in this study (340 vs. 56 hours) and the less labile C source gave the microbes more of an opportunity to colonize the organic matter and immobilize N than in Ma and Aelion's study.

It was expected that N availability in soils would decline as the C/N ratios increased (Stevenson, 1986) after adding wood fiber, so it was not surprising that denitrification rates were low in the +C microcosm treatment. Also it was expected that over time, as immobilization occurred, the C/N ratio of the SOM would decline, N availability would increase and subsequently denitrification rates would increase. There is evidence that this occurred on a more rapid time scale in the +N+C microcosm treatment because this treatment had among the highest values for extractable NH_4^+, denitrification rate, and SOM.

These results suggest that immobilization of N should be recognized with the previously identified processes of denitrification and dissimilatory nitrate reduction (Ma and Aelion 2005) as important to the removal of mineral N from runoff with a high nutrient load. Also, these results suggest that the N processing efficiency of a landscape can be sustained when SOM remains high as is the case on forested watersheds. Finally, it is clear that N processing efficiency can be increased on SOM depleted soils by incorporating wood fiber into the soil to increase substrate for microbial activity.

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