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MICROBIAL PROCESSES CONTROLLING THE FATE OF NITROGEN IN VEGETATIVE BUFFER STRIPS

by

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MICROBIAL PROCESSES CONTROLLING THE FATE OF NITROGEN IN
VEGETATIVE BUFFER STRIPS

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INTRODUCTION

The nature of non-point source pollution

The movement of pollutants from terrestrial environments into water bodies is a critical threat to water quality in many areas, including Narragansett Bay. Terrestrial land uses can deliver substantial loads of sediments, nutrients and toxic compounds into water bodies, leading to eutrophication, sedimentation, and biological decline. Understanding the terrestrial sources of pollutants, and the biological, chemical and physical factors affecting their fate and transport in terrestrial environments is essential to the maintenance of water quality for drinking supplies, navigation, fisheries and recreational uses.

Extensive basic and applied research has addressed specific "point source" pollutants over the last 20 years, and many advancements have been made towards their control. Non-point source (NPS) pollutants have proven more difficult to study and control however. These pollutants have multiple, diffuse sources and are affected by a wide range of chemical, physical and biological factors as they travel across the landscape. Small, diffuse non-point sources can multiply and interact to cause significant degradation of ground and surface water bodies however, and these pollutants have become proportionately more significant as point source controls have increased. The diverse origins of NPS pollutants greatly complicate their control because a wide range of control strategies are required, and these controls must be applied in a total landscape or watershed context. Many of these controls are expensive and difficult to justify at a local scale, given the diffuse and often poorly defined nature of NPS pollutants. Furthermore, landscape and watershed scale approaches to control are seldom attempted in scientific studies and are difficult to implement in a legislative context. Control of NPS pollution thus provides significant challenges to scientists and policy makers.

NPS pollutants can be classified based on their source, their chemical nature or their mode of transport across the landscape. In a heterogeneous landscape, NPS pollutants from agriculture, industrial areas, highways and suburban development often become highly mixed, making it difficult to pinpoint original sources. Chemically, NPS pollutants can be conveniently classified as nutrients, metals or organics. These pollutants can either be transported primarily by water flowing across the surface of the land (surface runoff), or by subsurface flow (groundwater). Different chemical types of pollutants with different modes of transport are affected by different physical, chemical and biological processes as they move across the landscape and thus pose distinct challenges for control.

Control of NPS pollution requires a mixture of engineering approaches with maintenance and augmentation of pollutant mitigation processes inherent in the environment. Surface runoff is frequently controlled by engineered drainage systems and detention basis that reduce the erosive power of runoff and allow for settling and stabilization of pollutants contained therein. Runoff control systems are often dependent on and/or are designed to augment natural infiltration and biological degradation processes present in soil. Groundwater borne pollutants are more difficult to control since subsurface flow is difficult to isolate and treat. Biological and chemical pollutant degradation mechanisms in the subsurface are poorly understood and are not readily amenable to engineering solutions.
Vegetated buffer strips

Vegetated buffer strips (VBS) are an example of a NPS pollutant control mechanism that relies heavily on pollutant degradation mechanisms inherent in the environment. VBS are defined as "small strips of grass or other vegetation that are used to trap pollutants moving from land areas before they enter water bodies (SCS 1989)". VBS potentially can serve to intercept pollutants moving in both surface runoff and subsurface flow, and can facilitate a variety of biological and chemical pollutant attenuation mechanisms. The maintenance of riparian VBS was adopted as a "best management practice" (BMP) by the USDA SCS (Dillaha et al. 1988) and VBS are recommended for use as a complement to structural stormwater control devices in Rhode Island (Scott 1988).

Despite the emerging widespread use of VBS, there are several unresolved scientific issues relating to their effectiveness for controlling both surface runoff and groundwater pollutants (Hayes et al. 1988). Understanding of the physical processes that intercept pollutants, and of the chemical and biological processes that degrade pollutants in VBS are incomplete. Generating this understanding is essential for evaluation of the effectiveness of VBS in different situations and for developing management strategies to enhance their performance.

The concept of buffer strips originated from research that found that strips of riparian forest vegetation were important in maintaining stream water quality in areas of intensive agriculture (Karr and Schlosser 1978, Lowrance et al. 1984, Jacobs and Gilliam 1985). Riparian strips were found to effectively impede surface runoff moving out of agricultural fields, reducing sediment delivery to water bodies and increasing infiltration of surface flow. Soluble pollutants in both surface and subsurface flow moving through riparian zones were found to be subject to plant uptake, microbial degradation and chemical immobilization by soil particles. Major unresolved questions relating to the effectiveness of riparian filters center around the long term fate of pollutants trapped in sediments, soil and vegetation in the zones and to the effects of pollutants on biological resources in the buffer. The latter question has increased in importance as interest (and legislation) in wetland preservation has increased in recent years (riparian zones are often dominated by wetland ecosystems).

Research on the use of grass VBS to trap sediment and nutrients moving from agricultural operations began in the 1980's (Magette et al. 1987). Most studies have focused on removal of sediment in surface runoff by grass VBS (Young et al. 1980, Magette et al. 1987, Dillaha et al. 1988, Lee et al. 1989). The long term effectiveness of these strips, and their ability to remove soluble pollutants from surface and subsurface flow are not well established (Doyle et al. 1977, Thompson et al. 1978, Young et al. 1980). The performance of either grass or forested VBS in non-agricultural zones is largely untested.

Factors controlling the effectiveness of VBS

For VBS to be effective NPS pollutant control mechanisms they must physically intercept pollutants and then either chemically or biologically remove or degrade them. Physical interception of surface runoff is complicated by the strong tendency of water to move in discrete channels. Such channelization can completely eliminate pollutant attenuation by VBS as water and pollutants can rapidly flow through the channels into either receiving
water or wetlands. Once begun, channelization tends to increase in severity. Some type of engineered control system is necessary to insure that the erosive, channelizing force of runoff is dissipated before runoff enters the VBS. If uniform, "sheet flow" is achieved, sediments will be deposited and soluble pollutants will be subject to biological and chemical attenuation.

The ability of VBS to physically impede groundwater flow is low. Indeed, the major control mechanism for surface runoff in VBS is to increase infiltration of surface flow into soil, stimulating movement of soluble pollutants into groundwater. The ability of VBS to affect groundwater pollutants is dependent on the ability of plant roots, microorganisms and chemical binding processes to be active in the saturated zone through which groundwater flows. In upland areas, groundwater is usually well below the area of highest tree root and microorganism density.

Chemical mechanisms of pollutant attenuation that operate in VBS arise from the ability of soil mineral and organic components to absorb certain chemical species. Clay and organic matter surfaces contain negative charges that can absorb cations and many polar organic compounds. Most toxic metals are cations as is ammonium (\(\text{NH}_4^+\)), a major inorganic form of soil nitrogen (N). Cation absorption by soil is a dynamic process however, and any cations absorbed on a soil particle can be displaced and released to the soil solution. Furthermore, the cation absorbing capacity (cation exchange capacity, CEC) of soil is finite, and is controlled by the amount of clay and organic matter present. Clay and organic matter are in short supply in Rhode Island soils in general, and in subsurface soils in particular.

Biological pollutant attenuation mechanisms in VBS include plant uptake of nutrients and microbial processing of nutrients, metals and organics. Plant uptake of nutrients is dependent on the ability of plants to intercept and remove nutrients from either surface or subsurface flow. Plants differ greatly in their selectivity for particular nutrient forms and in the rate at which they take up nutrients. More importantly, nutrients trapped in plant tissues can later be released back into the soil solution as these tissues decompose. Storage of nutrients in structural tissues of trees (Ehrenfeld 1987), or in grass tissues that are later harvested (Brown and Thomas 1978) represent effective pollutant removal mechanisms. Clearly, some form of plant community management is necessary to maintain an effective plant uptake sink for nutrients in VBS.

Microorganisms have the ability to degrade organic compounds as food resources and to absorb (immobilize) nutrients and metals into their tissues to support growth. Microbial immobilization is reversible; nutrients that are absorbed can later be released, or mineralized, depending on the amount of nutrient available in soil. Nitrate (\(\text{NO}_3^-\)), the most mobile form of N, can be converted to \(\text{N}_2\) gas by certain microorganisms that respire \(\text{NO}_3^-\) in the absence of oxygen. Wet (oxygen poor), organic rich wetland soils are thought to be excellent sites for this process (denitrification), but its significance in wetland soils may be greatly overestimated (Bowden 1987, Neely and Baker 1989). Microbial processes in subsurface soils are poorly understood but are likely strongly inhibited by a lack of organic carbon to support growth. Research is needed to establish if microbial processes can significantly contribute to pollutant attenuation in VBS and if strategies can be devised to favorably manage these processes in the environment.
Landscape aspects of VBS

While most research on NPS pollutants has focused on field scale dynamics of specific pollutants and control mechanisms, a larger scale approach is needed when considering the cumulative impacts of land use changes on large water bodies such as Narragansett Bay. The use of VBS must be considered in relation to upstream and downstream sources of pollutants and biological resources. VBS and other NPS control mechanisms must be considered in the context of total watershed management since receiving water quality is the product of integrated, watershed scale factors.

A major landscape scale issue concerns the role of upland VBS as buffers for wetlands versus the role of wetlands as buffers for streams, lakes and coastal water bodies. Protection of wetlands is explicitly mandated by both federal and state legislation, yet the role of wetlands as landscape scale pollutant attenuation mechanisms has been extensively studied (Lowrance et al. 1984, Neely and Baker 1989) and is frequently cited. Wetlands are effective as buffers because pollutants in both surface and subsurface flow come into contact with surface soil and vegetation, maximizing the potential for biological and chemical attenuation of pollutants. Given that it is difficult to obtain this type of contact in upland areas (especially for subsurface flow), the development of VBS for wetland protection is problematic. Roman and Good (1985) presented a comprehensive approach for establishing upland buffers for wetlands in the New Jersey pinelands but they stressed that the mechanisms operating in upland buffers were not well characterized, and that much further research was required. The ability of upland areas to act as buffers is a critical question. We must determine if we will need to implement stronger controls on upland pollutant sources to protect wetlands, or if we will rely on wetlands to buffer receiving water bodies from upland land uses (with the potential for wetland degradation).

Landscape analysis of VBS must also consider aesthetic and wildlife habitat values of buffer areas. The maintenance of open space and wildlife (including rare and/or endangered species) can be advanced by the use of VBS for water quality maintenance, and can provide additional justification for their implementation as part of watershed management plans (Brown et al. 1987). Quantitative evaluation of aesthetic values is problematic however since it is based on subjective factors, and there are no generally accepted criteria or models currently available. The wildlife value of buffers is difficult to assess since wildlife habitat suitability is affected by many factors that vary widely in different types of VBS. Food, nesting and roosting resources, and buffer width and edge, will control wildlife abundances and densities in VBS. Research in existing VBS is required to assess their value as wildlife habitat.

Objectives of this research

This research addressed the microbial processes that control the fate of N in VBS. Our objective was to measure the potential of grass and forested VBS to immobilize and denitrify NO$_3^-$, and to determine the factors controlling these processes in the field. The research was part of a larger effort directed towards comprehensive evaluation of VBS as NPS pollutant control and wildlife maintenance techniques. Other components of this effort (some of which are presented in this report), addressed the ability of different grass species to remove NO$_3^-$ from surface runoff (funded by the Soil Conservation
Service), the attenuation of NO$_3^-$ and metals in groundwater moving through forested buffers (funded by the Narragansett Bay Project) and the wildlife habitat values of forested VBS (also funded by the Narragansett Bay Project).

MATERIALS AND METHODS

Field plots

This experiment was conducted at the University of Rhode Island's Peckham Farm, in Kingston, Rhode Island. Experimental VBS were established as part of an ongoing USDA SCS study on soil classified as a Typic Dystrochrept. This soil contained 0.92% organic carbon, 2.53% organic matter, and a pH of 5.89. The full scope of the buffer strip study consisted of 10 different species of grasses, each replicated three times, making a total of 30 plots (Table 1). Each plot measured 3 m x 5 m in size, with a 0.70 m alleyway between alternate plots. The switchgrass and big bluestem were both propagated in a greenhouse and when they had grown to a height of approximately four inches, were then transplanted into their respective plots. The remainder of the grasses were grown from seed in the field. Situated above the grass plots was a 25 m x 100 m oat field which was graded to a 2% slope to provide runoff onto the grass plots.

On April 20, 1988, the grass plots, along with the oat field, were treated with 33 kg N/ha in the form of urea. Additional treatments with urea were confined to the grass plots. On July 22, 1988, 96 kg N/ha was added as a top dressing, and on September 30, 1988 another 48 kg N/ha was added to boost root and rhizome production for the winter. The plots were sprayed on June 8, 1988 with 0.28 kg/ha of Buctril and .07 kg/ha of Banvel, both of which are for broadleaved weed control. Additional spot treatment for Agropyron repens, was accomplished using a 2% glyphosate solution. The plots were mowed four times to a height of three inches to suppress weeds, allow the grasses to fill the plots, and to prevent the grasses from going to seed.

Ceramic lysimeter plates were installed below each grass plot to collect water percolating below the root zone as part of the ongoing SCS study. The plates were installed in triplicate below each of the ten species at an average depth of 70 cm. Leachate samples were collected for selected events in late 1988 and have been collected for every percolation event since March 10, 1989 as part of the SCS study. Nitrate in leachate samples was quantified using an ion chromatograph.

For microbial process studies, only two of the grass treatments, common reeds canarygrass (Phalaris arundinacea) and tribute tall fescue (Festuca arundinacea) were used. The reeds canarygrass was chosen because it is well adapted to a moist environment (such as riparian areas) and has the ability to uptake large quantities of nutrients. The tall fescue was chosen because it is a common plant used in many other studies. We also located microbial process study plots in well and poorly drained riparian forest sites adjacent to the grass VBS study area. The poorly drained site was situated in a soil that was classified as an Aquic Dystrochrept, with a depth to mottles of 25 cm. This soil contained 8.5% organic carbon, 0.32% total N, and a pH of 3.52. In the well drained site, the soil was classified as a Typic Dystrochrept, with a 66 cm. depth to mottles. This soil contained 5.1% organic carbon, 0.25% total N, and a pH of 4.23. Sub-plots of the forest sites were treated with lime (20 lbs/ft$^2$ in the poorly drained, 10 lbs/ft$^2$ in the well drained) to test for pH limitation of microbial N processes.
TABLE 1

Grass species and plot numbers in vegetative buffer strip experiment. Plots established as part of USDA SCS funded research.

<table>
<thead>
<tr>
<th>Grass species</th>
<th>Plot numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Big bluestem</td>
<td>10, 15, 27</td>
</tr>
<tr>
<td>Bromegrass</td>
<td>4, 11, 24</td>
</tr>
<tr>
<td>Garrison creeping foxtail</td>
<td>2, 19, 30</td>
</tr>
<tr>
<td>Kentucky bluegrass</td>
<td>8, 16, 25</td>
</tr>
<tr>
<td>Orchardgrass</td>
<td>7, 20, 22</td>
</tr>
<tr>
<td>Perennial ryegrass</td>
<td>1, 13, 23</td>
</tr>
<tr>
<td>Reeds canarygrass</td>
<td>5, 17, 29</td>
</tr>
<tr>
<td>Sweet vernalgrass</td>
<td>9, 14, 26</td>
</tr>
<tr>
<td>Switchgrass</td>
<td>3, 12, 21</td>
</tr>
<tr>
<td>Tall fescue</td>
<td>6, 18, 28</td>
</tr>
</tbody>
</table>
Microbial process experiments

Denitrification was measured using soil core techniques described by Groffman and Tiedje (1989). Cores of 15 cm depth and 2 cm diameter were removed from soil, placed in plexiglass tubes and sealed with rubber serum stoppers. Cores were subjected to a variety of experiments.

Experiment 1. The first experiment began on July 6, 1988 and consisted of three parts carried out over a three day period.

Part 1:

The first part of the experiment measured in situ rates of denitrification and N₂O production in the different plots. Ten soil cores were sampled from each of the forest plots and each of the grass treatments. These cores were immediately capped and 5 ml of acetylene was added to every other core. Acetylene was added to inhibit the final step in the denitrification process, allowing us to quantify denitrification rates by measuring the accumulation of nitrous oxide (N₂O) in the sealed cores (Yoshinari and Knowles 1976). The remainder of the cores received 5 ml air. To ensure a proper diffusion of the acetylene throughout the core sample, the atmosphere in each core was mixed by pumping five times with a 60 cc syringe. These cores were then placed in the ground and left to incubate for a total of 6 hours. A 3 ml gas sample was taken from each of the cores after two and six hours of incubation. The samples were placed into 10.25 x 65 mm evacuated glass tubes and were returned to the laboratory. After taking the six hour sample, the caps were removed and the cores brought to the laboratory where they were stored at 4° C overnight.

Part 2:

In part 2 of the experiment, the cores were incubated under anaerobic conditions to test for oxygen limitation of denitrification. The following day, the cores were recapped using the rubber septum stoppers and were alternately evacuated, by means of a vacuum pump, and refilled using 99.999% pure nitrogen gas. All cores received 5 ml acetylene, were mixed and were incubated at 22° C for 6 hours, with gas samples taken at two and six hours. After the second sampling, the caps were once again removed from the cores, and the cores were stored at 4° C overnight.

Part 3:

In part 3, cores were amended with either NO₃⁻ or NO₂⁻ and glucose to test for NO₃⁻ and carbon limitation of denitrification. The following day, five cores from each of the plots were subjected to treatment with a 100 ppm NO₃⁻-N solution, while the other five cores from each of the plots were treated with a 100 ppm NO₂⁻-N and 1000 ppm glucose solution. Cores were sealed, made anaerobic and incubated and sampled as described above.

Experiment 2. The second experiment was conducted on the three days between July 25-27, 1988. The objective was to assess how much denitrification would occur following an addition of NO₃⁻, thus simulating a runoff event. Ten cores were taken from each of the reeds canary grass, tall fescue, well drained forest, and poorly drained forest sites. Five cores from each of these sites were then treated with 10 ml of a 100 ppm NO₃⁻-N solution, and the other five cores were treated with 10 ml of a 100 ppm NO₂⁻-N and 1000 ppm glucose solution. The cores were capped, amended with acetylene and incubated and sampled as described above. After the six hour sample was taken, the cores were uncapped and stored at 4° C overnight. The cores were resealed, and incubated on the following two days to follow the response of the cores to the amendments over a three day period.
**Experiment 3.** This experiment was designed to determine if low pH limited denitrification in the forest plots. On August 9, 1988, two new 100 ft² forest plots were created, one located in the poorly drained section and the other located in the well drained section. These plots received 20 lbs/ft² and 10 lbs/ft² of lime, respectively.

Denitrification rates were measured in the well drained forest on September 6, 1988, and in the poorly drained forest on September 23, 1988. The experimental procedure consisted of taking 20 cores from each of the four forest plots, and returning these cores to the lab. Each set of 20 cores were then broken into 4 sets of 5, with each set receiving one of the following treatments: 10 ml distilled water, 10 ml of a 100 ppm NO₃⁻-N solution, 10 ml of a 100 ppm NO₃⁻-N and 1000 ppm glucose solution, and the forth set receiving no amendment. All cores were then capped, amended with acetylene and incubated and sampled as described above.

Before any of the cores from experiments 1, 2 and 3 were discarded, their weight and length were recorded. The head space of each core was then measured by calculating the difference between the pressure within the core, as measured with a pressure transducer, at atmospheric pressure and with 5 ml air added. Gas samples were analyzed for N₂O on a Tracor model 540 gas chromatograph equipped with two electron capture detectors and four 2 m columns packed with Porapak T M.Q. The data were expressed on an areal basis using bulk density values determined on each core. Results were analyzed using the ANOVA procedure in the SAS statistical package. A duncans multiple range test and an LSD test of significance with a 0.05 confidence interval, were used to determine differences between treatments both within and between plots.

**Experiment 4.** In this experiment, denitrification was measured over an 8 day period in NO₃⁻ amended (4 µg/g soil) soils held in 946 ml mason jars in the laboratory. Levels of mineral N in soil were also measured to assay immobilization and re-mineralization of the added NO₃⁻. Each jar contained 100 g soil, and denitrification and soil mineral N levels were measured 1 day before addition of NO₃⁻ and 1, 3 and 8 days following the addition. Denitrification was measured by sealing the jar with a lid containing a black rubber septum, adding 10 ml of acetylene, and taking gas samples at 2 and 6 hours following sealing. Acetylene was removed from the jars by evacuating and refilling the jar with air three times. The jars were left unsealed between denitrification measurements. Mineral N was extracted from 5 g subsamples with 2 N KCl and analyzed on an Alpkem RFA 300 continuous flow analyzer.

**RESULTS**

The average concentrations of NO₃⁻ in leachate from the 10 grass species ranged from 0.9 to 31.6 mg/L. Loading rates of NO₃⁻ to groundwater ranged from 2.2 to 106.1 kg/ha. The 10 grasses can be broken into four groups based on their loading rates (Table 2). All grasses received 177 kg N/ha as fertilizer. N removal efficiencies (calculated as N not leached/total fertilizer N input) for the grasses thus ranged from 40 to 99% of the N applied.

In experiment 1, aerobic, in situ rates of denitrification and N₂O flux were insignificant in all plots (Table 3). Anaerobic, unamended rates were also very low, but were somewhat higher in the poorly drained forest plot than...
TABLE 2

Nitrate leaching losses (kg N/ha) and approximate N removal efficiencies for grasses in vegetative buffer strip experiment, Spring 1988 - Spring 1989. Data collected as part of USDA SCS funded research.

<table>
<thead>
<tr>
<th>Grasses</th>
<th>Nitrate leaching losses</th>
<th>N removal efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>orchardgrass, sweet vernalgrass</td>
<td>2.0 to 10.0 kg/ha</td>
<td>94-99%</td>
</tr>
<tr>
<td>tall fescue, creeping foxtail</td>
<td>10.0 to 25.0 kg/ha</td>
<td>86-94%</td>
</tr>
<tr>
<td>perennial ryegrass, big bluestem</td>
<td>25.0 to 50.0 kg/ha</td>
<td>72-86%</td>
</tr>
<tr>
<td>kentucky bluegrass, reed canarygrass</td>
<td>&gt; 50.0 kg/ha</td>
<td>&lt;70%</td>
</tr>
<tr>
<td>bromegrass, switchgrass</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE 3
Denitrification rate (g N ha\(^{-1}\) d\(^{-1}\)) in soil cores in response to amendments, 880706

<table>
<thead>
<tr>
<th></th>
<th>Well drained forest</th>
<th>Poorly drained forest</th>
<th>Tall fescue</th>
<th>Reeds canary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No amendment</td>
<td>-4.6(^c)</td>
<td>-8.7(^c)</td>
<td>-4.0(^b)</td>
<td>2.0(^b)</td>
</tr>
<tr>
<td>C(_2)H(_2)</td>
<td>-21.3(^c)</td>
<td>-16.3(^c)</td>
<td>-9.0(^b)</td>
<td>-2.0(^b)</td>
</tr>
<tr>
<td>Anaerobic with C(_2)H(_2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No amendment</td>
<td>1.1(^c)</td>
<td>13.1(^c)</td>
<td>1.0(^b)</td>
<td>1.0(^b)</td>
</tr>
<tr>
<td>NO(_3)(^-)</td>
<td>1306(^b)</td>
<td>1402(^b)</td>
<td>17208(^a)</td>
<td>15208(^a)</td>
</tr>
<tr>
<td>NO(_3)(^-) and glucose</td>
<td>2155(^a)</td>
<td>2951(^a)</td>
<td>21702(^a)</td>
<td>15819(^a)</td>
</tr>
</tbody>
</table>

\(^1\)Values followed by different superscripts within columns are significantly different at p < 0.05. The tall fescue and reeds canarygrass plots had significantly higher (p < 0.05) denitrification than the forest plots when all treatments were combined.
in the other plots (Table 3). Nitrate amended denitrification rates were higher \((p < 0.05)\) than either aerobic or anaerobic unamended rates in all plots (Table 3). Nitrate and carbon amended rates were higher \((p < 0.05)\) in all plots other than the reeds canary grass (Table 3).

In experiment 2, denitrification rates in \(\text{NO}_3^-\) and \(\text{NO}_3^-\) and carbon amended cores were in the order of: tall fescue \(\geq\) reeds canary grass \(\geq\) poorly drained forest \(\geq\) well drained forest (all differences \(p < 0.05\), Table 4). Nitrate and carbon amended rates were higher than \(\text{NO}_3^-\) only amended rates in all plots, but the differences were significant in the poorly drained forest and reeds canary grass plots only (Table 4). The \(\text{NO}_3^-\) amendment in experiment 2 simulated a 31.8 kg N/ha addition. Denitrification N removal efficiencies for the different plots, (calculated as denitrification rate/total N addition), ranged from 1 to 50% per day (Table 4). Results from experiment 3 indicated that the lime amendment significantly increased soil pH in the forest plots, but denitrification did not increase accordingly (Table 5).

When \(\text{NO}_3^-\) was added to soils held in mason jars in the laboratory (experiment 4), soil from the reeds canarygrass plot showed the strongest denitrification response followed by the tall fescue and well drained forest plots (Figure 1). The poorly drained forest soil showed no denitrification response to added \(\text{NO}_3^-\) in this experiment. All soils other than the poorly drained forest were able to immediately absorb the added \(\text{NO}_3^-\) (Figure 2). However, levels of mineral N increased in all soils over the eight day incubation.

**DISCUSSION**

The results from the lysimeters suggest that the different grasses have dramatically different abilities to take up N. It is important to note that all grasses received 177 kg N/ha as fertilizer and therefore all grasses demonstrated a significant ability to take up N. However, N removal efficiencies (calculated as N not leached/total N fertilizer input), were rather low in many cases. These results were obtained during spring and summer, when N removal efficiencies should be highest due to high plant uptake of N and low leaching during this period. Results from a related SCS study conducted at the URI agronomy farm with VBS established and fertilized during winter found much lower N removal efficiencies by grass. In this study, tall fescue-perennial ryegrass VBS were established and fertilized with 33 kg N/ha in September. An average of 21 kg N/ha (64%) was leached overwinter. These results suggest that winter N removal efficiencies by VBS will be much lower than summer removal efficiencies. It is also important to note that the ability of grasses to take up N fertilizer does not necessarily predict their ability to remove N from runoff. We had hoped to directly measure removal of N from runoff during the summer of 1988, but technical difficulties hindered our efforts. These experiments will be done during 1989.

The ultimate fate of N taken up by vegetation in VBS is uncertain. N in plant tissues can be released during senescence and decomposition and can be re-mineralized and released into the soil solution. Some type of plant harvest is necessary to minimize this problem (Brown and Thomas 1978). Harvest of grasses is more straightforward than for trees since it is physically easier to accomplish and it stimulates regrowth of the grasses. Tree removal, or woodlot management, is more complex to accomplish and can involve considerable disturbance to soil in the VBS. Tree regrowth is often not immediate following harvest and thus tree removal can temporarily reduce VBS performance.
**TABLE 4**

Denitrification (g N ha\(^{-1}\) d\(^{-1}\)) response and N removal efficiencies for soil cores treated with simulated runoff containing either NO\(_3^-\) or NO\(_3^-\) and glucose, 880725\(^1\). Total NO\(_3^-\)-N addition = 31,800 g N/ha.

<table>
<thead>
<tr>
<th>Plot</th>
<th>NO(_3^-) amended</th>
<th>NO(_3^-) and glucose amended</th>
<th>N removal efficiency (% N denitrified/day) NO(_3^-) amended</th>
<th>NO(_3^-) and glucose amended</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well drained(^c) forest</td>
<td>311</td>
<td>408</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>Poorly drained(^{bc}) forest</td>
<td>365</td>
<td>*</td>
<td>1.2</td>
<td>4.5</td>
</tr>
<tr>
<td>Tall fescue(^a)</td>
<td>7889</td>
<td>16186</td>
<td>25</td>
<td>51</td>
</tr>
<tr>
<td>Reeds canary(^b) grass</td>
<td>4537</td>
<td>*</td>
<td>14</td>
<td>29</td>
</tr>
</tbody>
</table>

\(^1\)Plots followed by different superscripts showed significantly different denitrification activity over both to amendments at p < 0.05. *Indicates significant difference between NO\(_3^-\) and NO\(_3^-\) and glucose treatments at p < 0.05.
TABLE 5

Denitrification (g N ha$^{-1}$ d$^{-1}$) in soil cores from limed and unlimed forest plots treated with simulated runoff, September 1988

<table>
<thead>
<tr>
<th>Plot</th>
<th>pH</th>
<th>$\text{NO}_3^-$ amended</th>
<th>$\text{NO}_3^-$ and glucose amended</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poorly drained forest</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limed</td>
<td>4.79</td>
<td>365</td>
<td>1102</td>
</tr>
<tr>
<td>Unlimed</td>
<td>3.52</td>
<td>336</td>
<td>1606</td>
</tr>
<tr>
<td>Well drained forest</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limed</td>
<td>4.94</td>
<td>31</td>
<td>72</td>
</tr>
<tr>
<td>Unlimed</td>
<td>4.23</td>
<td>101</td>
<td>685</td>
</tr>
</tbody>
</table>
Figure 1 - Denitrification response to added NO$_3^-$ (4 ug/g soil) over 8 days in soil held in mason jars.
Figure 2 - Mineral N in soils held in mason jars that received 4 ug/g soil NO₃⁻-N immediately before day 1 sampling.
Data from experiment 1 found that in situ rates of denitrification in the plots were very low and were limited by the presence of oxygen and an absence of NO$_3^-$ and/or glucose. Oxygen and NO$_3^-$ control of denitrification have been reported in many studies (Tiedje 1988). However, the strong stimulation caused by glucose that was observed in the forest plots and not in the grass plots was surprising since the forest plots have high levels of organic matter relative to the grass plots. It is possible that tillage, fertilization and liming over time have increased substrate availability (both physical and biochemical) to microbes in the grass plots. Since the carbon amended incubations in experiment 1 were carried out under anaerobic conditions, the stimulation that we observed was due only to increasing the supply of substrates to denitrifiers and not to a reduction in soil oxygen levels caused by general stimulation of heterotrophic microbes as has been observed in other studies (Rice et al. 1988, Groffman and Tiedje 1988). The data suggest that carbon availability to denitrifiers may limit denitrification activity in forest soils more than has previously been thought.

We expected the forest plots to have a much higher potential for denitrification than the grass plots, since forest soils (especially poorly drained forests) generally have higher moisture and organic matter levels than upland agricultural soils. In contrast to our expectations, soils from the grass plots exhibited consistently higher denitrification activity than soils from the forest plots. In experiment 1, the grass plots had higher activity than the forest plots in anaerobic incubations of soil amended with either NO$_3^-$ or NO$_3^-$ and glucose. In experiments 2 and 4, the grass plots had higher activity than the forest plots in response to NO$_3^-$ additions made to simulate runoff. These results suggest that carbon availability to microbes is higher in the grass plots than in the forest plots (discussed above), and that the population of denitrifiers is bigger and/or more active in the grass plots than in the forest plots.

Experiment 3 was done to test the hypothesis that low pH limited denitrification activity in the forest plots relative to the grass plots. Although we successfully raised the pH in the forest plots from 3.5 to 4.8 in the poorly drained plot and from 4.2 to 4.9 in the well drained plot (pH in the grass plots was 5.3), denitrification rates did not increase accordingly. Denitrification was actually lower in the limed plots than in the unlimed plots in most cases. This is likely due to the fact that microbes in soil are adapted to in situ physical and chemical conditions, and changing the pH thus reduced their activity (Koskinen and Keeney 1982). In the long term, raising soil pH should lead to the development of a different microbial community, with higher denitrification activity (Parkin et al. 1985). These results suggest that forested buffers in Rhode Island may not always be effective denitrification sinks for NO$_3^-$, and that managing these zones to increase denitrification is not simple and requires long-term study.

Denitrification N removal efficiencies calculated from experiment 2 (Table 4) were quite high in the grass plots, suggesting that up to 50% of a very large N addition (> 30 kg N/ha) could be denitrified per day. These results must be interpreted with great caution however, since they were obtained with soil cores and are not field measured fluxes. Adding amendments to confined soil cores does not allow for free drainage and thus stimulates the development of anaerobiosis in the cores and maximizes the accessibility of NO$_3^-$ to denitrifiers. In experiment 4 (soil held in mason jars), simulated runoff was less confined and the denitrification response was less intense and was relatively brief. Measurements of denitrification in soil cores taken from field VBS that have received runoff are necessary to validate these results.
The simulated runoff experiments were useful in several regards however. First, the role of carbon in increasing N removal efficiency in all plots is clear. The carbon effect is likely due both to increasing substrate availability to denitrifiers (discussed above) as well as to depletion of soil oxygen levels resulting from a general stimulation of heterotrophic activity. These results suggest that runoff containing high levels of available carbon (feedlot or manured field runoff for example) may be more amenable to treatment in VBS than runoff that is low in available carbon. Second, the results suggest that if free drainage can be prevented, significant denitrification can occur in VBS. Flow of runoff through the VBS could be controlled either by surface contour engineering or subsurface drainage manipulation, creating a hybrid NPS pollutant control mechanism that utilizes aspects of VBS and common detention basins.

Results from the mason jar experiment suggest that immobilization of N by soil microbes is not a reliable N removal mechanism in VBS. All soils (other than the poorly drained forest) were able to absorb a spike of 4 ppm NO₃⁻, but all soils showed net N mineralization over the next 8 days. These results suggest that N that is immobilized during runoff events, may later be released to the soil solution and may either leached to groundwater, or carried out of the VBS in the next runoff event. On the other hand, immobilization may be useful as a temporary sink for NO₃⁻, allowing plant access to the N that is re-mineralized. This is especially important in light of the fact that N moving rapidly in runoff may not be particularly accessible to plant roots.

In summary, we demonstrated that there are major differences in the nature and extent of microbial processes in VBS of different types of grasses and soils. Our results suggest that low pH may limit the ability of forest soils to act as buffers, and that availability of carbon sources may ultimately limit denitrification in VBS. The study also suggests that N that is immobilized in VBS may be subject to re-mineralization and release. Management of VBS should focus on maximizing N removal by denitrification, perhaps by manipulation of flow through the VBS, and on maximizing N removal by plant harvest.

LITERATURE CITED


