DENITRIFICATION POTENTIALS IN SOILS UNDERLYING A RIPARIAN FOREST AND AN AGRICULTURAL FIELD IN THE COASTAL PLAIN OF VIRGINIA

by

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(ABSTRACT)

While research has shown that riparian forests are effective in reducing shallow groundwater nitrogen levels, the relative importance of the mechanisms responsible for this reduction have not been adequately addressed. This project focused on the microbial mediated process denitrification, which has been hypothesized to be a major factor responsible for decreased groundwater nitrate levels observed in forested regions.

The study site was located on Virginia's Eastern Shore and incorporated a transect extending from a field under agricultural use through a mesic forest to a distance of 91.4 meters. Groundwater flowed from a well drained agricultural field of Bojac sandy loam (coarse-loamy, mixed, thermic Typic Hapludults) and Munden sandy loam (coarse-loamy, mixed, thermic Aquic Hapludults) to a poorly drained forest soil, Nimmo sandy loam (coarse-loamy, mixed, Typic Ochraquults). Previous work along this transect reported mean nitrate (NO_3-N) levels of 1,161 $\pm$ 393 $u$mol$\bullet$liter^{-1} for shallow groundwater underlying the agricultural field, whereas shallow groundwater 91.4 meters into the forest had a mean concentration of 2.2 $\pm$ 2.6 $u$mol$\bullet$liter^{-1}. Groundwater nitrate (NO_3-N) levels below ~3 meters of the water table 91.4 meters into the forest 559.5 $\pm$ 101.9 increased to approximately 250 $u$mol$\bullet$liter^{-1}. In addition to nitrate levels, other water quality parameters and soil characteristics suggested that vertical variations of soil environments existed and therefore, must be incorporated into experimental design.

Denitrification activiy was measured at various depth increments in the agricultural

field and forest using an acetylene blockage technique. In addition, denitrification activity was measured after subjecting the soils to carbon and nitrate amendments. Denitrification activity from the forest was limited by nitrate at the water table and were carbon limited as vertical depth increased. Denitrification activity measured with nitrate amendments at the water table in the forest were two orders of magnitude higher than those in the field (7.37 $n$mol$\bullet g^{-1}\bullet hr^{-1}$ vs 0.074 $n$mol$\bullet g^{-1}\bullet hr^{-1}$). Dentrification activity measured with nitrate+glucose amendments were higher at the water table in the forest, 6.88 $n$mol$\bullet g^{-1}\bullet hr^{-1}$, as compared to the field, 0.15 $n$mol$\bullet g^{-1}\bullet hr^{-1}$. Denitrifier microbial densities were measured at various vertical depths in the forest and agricultural field. Results demonstrated that denitrifiers densities at the water table in the forest were greater than those at the water table in the field. The number of denitrifying organisms per cubic centimeter of soil at the water table in the field averaged 2850 $\pm$ 1553(SD) as compared to 14,350 $\pm$ 13,369(SD) at the water table in the forest. At 0.91 meters below the water table in the field and in the forest the number of denitrifying organisms per cubic centimeter of soil were 1343 $\pm$ 1086(SD) and 3922 $\pm$ 3919(SD), respectively. The differences in denitrification measurements were due to location of the water table. The water table in the forest was located in the A horizon as compared to the water table in the field which was located in the C horizon. Results demonstrated that denitrification was an active mechanism that affected nitrate reduction in shallow groundwater in this system. Thus, riparian vegetation can be quite beneficial in reducing shallow groundwater nitrogen levels through microbially mediated processes such as denitrification. As a result nonpoint source nitrogen loadings from groundwater discharge can be reduced.

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1. INTRODUCTION & LITERATURE REVIEW

1.1 Study Objectives

Nutrients can have a degrading effect on a surface body of water. Nutrients coming from nonpoint sources such as surface runoff and groundwater discharge are significant in the Chesapeake Bay watershed. Riparian vegetation can be beneficial in reducing shallow groundwater nutrient concentrations. The reduction of nutrients in shallow groundwater may occur via plant uptake, microbial respiration (i.e. denitrification), and/or physical dilution. The purpose of this study was to quantify the importance of denitrification in the removal of nitrate in groundwater underlying a coastal mesic forest through the following objectives.

1. To measure groundwater quality and soil characteristics in an agricultural field and adjacent forest.
2. To measure and relate denitrifier microbial densities in saturated soils at the water table and below the water table in an agricultural field and adjacent forest to objective 1.
3. To measure and relate denitrification potentials in saturated soils at the water table and below the water table in an agricultural field and adjacent forest to objective 1.

1.2 Nutrient Management in the Chesapeake Bay

The degrading effect that nutrients can have on a surface water body has been documented (Wetzel, 1983). An over abundance of nutrients is one of the contributing mechanism's degrading the living resources and aesthetic beauty of the Chesapeake Bay and its tidal tributaries (Figure 1.1). Excess nutrients such as nitrogen, in the form of inorganic nitrogen, and phosphorus, in the form of phosphates, can lead to eutrophication of the Chesapeake Bay and its tidal tributaries (Chesapeake Executive Council, 1988). Eutrophication is a process whereby the primary productivity in a body of water is

increased in response to additional inputs of nutrients to the system. High concentrations of nutrients in eutrophic waters can cause algal blooms of blue-green algae or other detrimental species. Nuisance algal blooms are damaging to the water quality in the Chesapeake Bay. These algae blooms prevent light from reaching important aquatic vascular plants that provide nursery habitat and are home to many fisheries, such as the blue crab, in the Chesapeake Bay system. The biomass from the algal bloom settles to the bottom and/or into the hypolimnion. Microbial communities consume oxygen during decomposition of the algal bloom biomass. The hypolimnion is thus depleted of oxygen which results in harmful side effects, such as fish kills, to the organisms in the ecosystem.

In the 1987 Chesapeake Bay Agreement, Virginia, Maryland, Pennsylvania, the District of Columbia, the Chesapeake Bay Commission, and the U.S. Environmental Protection Agency formally agreed to reduce and control point and nonpoint sources of pollution to attain water quality conditions necessary to support the living resources of the Chesapeake Bay (Chesapeake Executive Council, 1988). To achieve this goal, the previously mentioned parties agreed to a 40% reduction in nitrogen and phosphorus entering the mainstem of the Chesapeake Bay by the year 2000. In 1992 amendments were added to the 1987 Chesapeake Bay Agreement to include tributary-specific nutrient reduction strategies to meet the original 40% reduction goal (Chesapeake Bay Agreement, 1992). Extensive research and time have been devoted to identifying and quantifying nitrogen and phosphorus inputs to the Chesapeake Bay system. Sources of nitrogen and phosphorus have been classified into three basic groups; first, point sources such as sewage discharges, second, non-point sources such as surface runoff, and third, the realization of a large atmospheric deposition component.

1.3 Groundwater Inputs of Nutrients

Groundwater inputs of nitrogen and phosphorus to the Chesapeake Bay have not been included in any of the previous estimates. Preliminary estimates on the amount of

groundwater discharge from the shallow water table aquifer have been made by Simmons (1989). Several studies have been conducted documenting nutrient inputs from groundwater. Submarine groundwater discharge, from shallow aquifers, may significantly contribute nutrient inputs to the Chesapeake Bay (MacIntyre *et al.*, 1989; Simmons 1988 and 1989; Reay and Simmons, 1992; Libelo *et al.*, 1991). Simmons *et al* (1992) found a definite relationship between land use, groundwater quality, and sediment flux to the adjacent surface body of water. Figures 1.3 and 1.4 are adapted from Simmons *et al* (1992). Figure 1.3 shows groundwater quality under three types of sites, non-buffered agricultural, non-buffered urban, and a reference (marsh or forested system). Agricultural sites had groundwater with high concentrations of nitrate averaging 375.6 $u$mol$\bullet$liter^{-1} over the period of the study. Urban sites had groundwater with high ammonium concentrations averaging 629 $u$mol$\bullet$liter^{-1} over the period of the study. High ammonium concentrations are not typically found in the environment because ammonium is rapidly nitrified. Stewart and Reneau (1988) found ammonium concentrations to be minimal around on-site wastewater disposal systems in the Atlantic coastal plain. Simmons (unpublished) has also found that as distance from septic systems increases the ammonium concentrations decrease and nitrate concentrations increase. The high ammonium concentrations measured by Simmons *et al* (1992) are due most likely to the proximity of sampling near septic systems. Sediment flux nutrient concentrations, reflective of the corresponding groundwater quality, are shown in Figure 1.4. The water moving across the sediment\water interface at agricultural sites and urban sites had elevated concentrations of nitrate (avg=334.4 umol$\bullet$m$^{-2}\bullet$hr^{-1}) and ammonium (avg=718.3 umol$\bullet$m$^{-2}\bullet$hr^{-1}), respectively. Nutrient concentrations measured in the groundwater and from sediment fluxes from the reference sites were negligible in comparison to the agricultural and urban sites. The effect of these two major types of land use, agricultural and urban, can contribute significantly to the nitrogen entering the Chesapeake Bay via groundwater transport.

1.4 Groundwater Flow in Coastal Environments

Groundwater flows from a region of higher hydraulic head to a region of lower hydraulic head (Freeze & Cherry, 1979). Because hydraulic head is the sum of elevation head and pressure head, groundwater flow is often in the direction from high elevation to low elevation except when pressure head affects are substantial (Freeze & Cherry, 1979). In the Atlantic Coastal Plain, groundwater flows from uplands toward the adjacent surface body of water as depicted in Figure 1.2. Groundwater discharge in intertidal zones and offshore environments is governed by two basic principles. First, groundwater is flowing from a region of higher hydraulic head, in the uplands, to an area of lower hydraulic head, the sediment-water interface. Second, as the less dense fresh water intersects the denser salt water a zone of dispersion is created. The zone of dispersion is a mixture of fresh and salt water. This mixture, less dense than salt water, overrides the salt water, forcing the mixture vertically toward the sediment/water interface, (Figure 1.2). In nearshore environments the water table is generally closer to the surface as proximity to the land-water interface increases. As a result, groundwater discharge to nearshore marine environments may intersect biologically active communities such as wetlands and riparian forests.

1.5 Importance of Vegetative Filter Strips

Vegetative filter strips used in Best Management Practices (BMPs) are essential to reducing the degradation of water quality in the Chesapeake Bay (Dillaha, 1990). Previous research has focused mainly on the benefits of vegetative filters in reducing nutrients in surface water flows (Dillaha *et al.*, 1987; Magette *et al.*,1987). It is only within the past few years that the benefits of vegetative filter strips, with respect to nutrient reduction on subsurface groundwater flow, has been examined.

Previous research on vegetative filter strips in the Chesapeake Bay has been conducted by Peterjohn and Correll (1984), Hershner (1987) and Reay *et al* (1991). Results of these studies showed that vegetative filter strips can significantly reduce

nutrients in shallow subsurface groundwater. Vegetative filters employed as BMPs for nutrient reduction in subsurface groundwater flow could be quite beneficial in reducing nonpoint source nutrient loadings. While recent work has documented the reduction of nitrogen in shallow groundwater under vegetative filters, little work has been done on the mechanisms of nutrient reduction. Mechanisms of nutrient reduction may include plant uptake, microbial respiration (denitrification), and/or physical dilution.

Fail (1986) reported that nitrogen uptake by plant vegetation can be on the order of 50 $kg \bullet ha^{-1} \bullet yr^{-1}$ for a coastal plain riparian forest. Henderickson (1981) reported that denitrification can account for approximately 30 $kg \bullet ha^{-1} \bullet yr^{-1}$ of nitrogen removed. These studies, however, examined only the upper few centimeters of soil. Smith and Duff (1988) measured denitrification potentials from saturated groundwater soils in a sand and gravel aquifer contaminated with treated sewage. Smith and Duff (1988) concluded that denitrification occurred in this groundwater system and was carbon limited. Ambus and Lowrance (1991) measured denitrification potentials in two coastal plain riparian forests and found that denitrification in a shallower aquifer was more important to nitrate removal than in a deeper aquifer. Lowrance (1992) found that denitrification potentials in groundwater were highest when the saturated zone was within 60cm of the surface.

1.6 Denitrification

Denitrifying bacteria are facultative anaerobes that will use NO_3^- as an electron acceptor when oxygen concentration is low. The following pathway lists the intermediate compounds, along with their respective oxidation states, for denitrification.

$$\underset{(+5)}{NO_3^-} \rightarrow \underset{(+3)}{NO_2^-} \rightarrow \underset{(+2)}{NO} \rightarrow \underset{(+1)}{N_2O} \rightarrow \underset{(0)}{N_2}$$

Conditions do not have to be anaerobic for denitrification occur (Bartholomew and Clark, 1965), but may only have to be low, on the order of one to three $milligram \bullet liter^{-1}$, in oxygen.

Denitrification requires an oxidizable substrate to supply the energy for growth. This oxidizable substrate is organic matter. Many forms of organic matter, such as glucose, sucrose, dextrose, etc., are used to provide rapid growth of denitrifiers in cultures.

Other factors that may influence denitrification are pH, moisture content, temperature, and nitrate concentration. Denitrification rates are highest in the pH range of 7-8 (Bremner, 1958). Chemodenitrification, which is abiological, occurs in extremely acid soils pH < 6 (Tiedje, 1982). The product of chemodenitrification is most commonly NO (nitric oxide), and therefore can be distinguished from biological denitrification by this end product (Tiedje, 1982). Biological denitrification is more likely to occur in waterlogged soils (Bartholomew and Clark, 1965). Soil moisture increases denitrification by decreasing oxygen diffusion into interstitial pore space. Denitrifying bacteria prefer a temperature gradient between 2-25°C. Denitrification rates increase with increased temperature in this range (Bremner, 1958). Nitrate concentration can also affect denitrification. The nitrate concentration determines the reaction rate of denitrification.

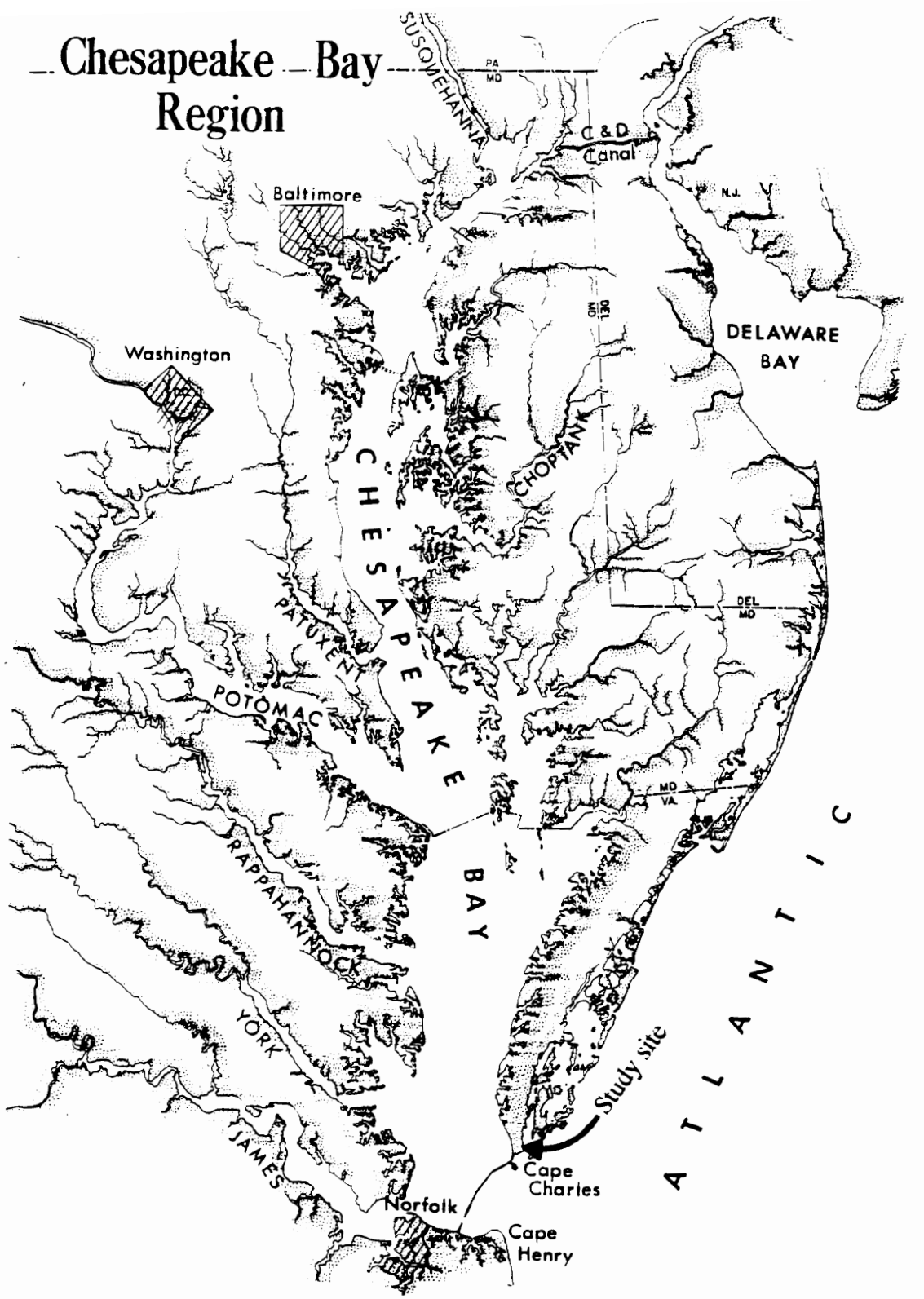

Figure 1.1 The Chesapeake Bay and it's tidal tributaries. Study site located on Virginia's Eastern Shore.

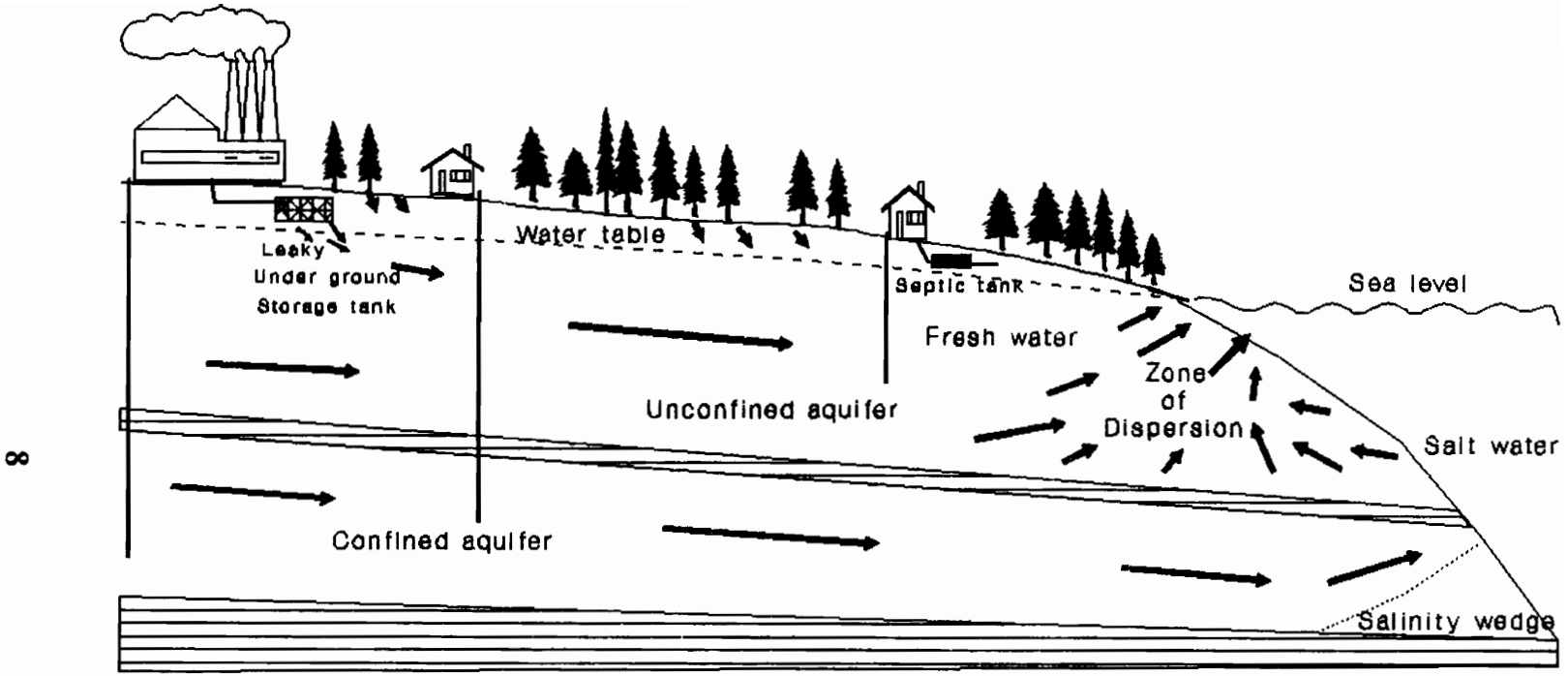

Figure 1.2 Coastal groundwater flow patterns.

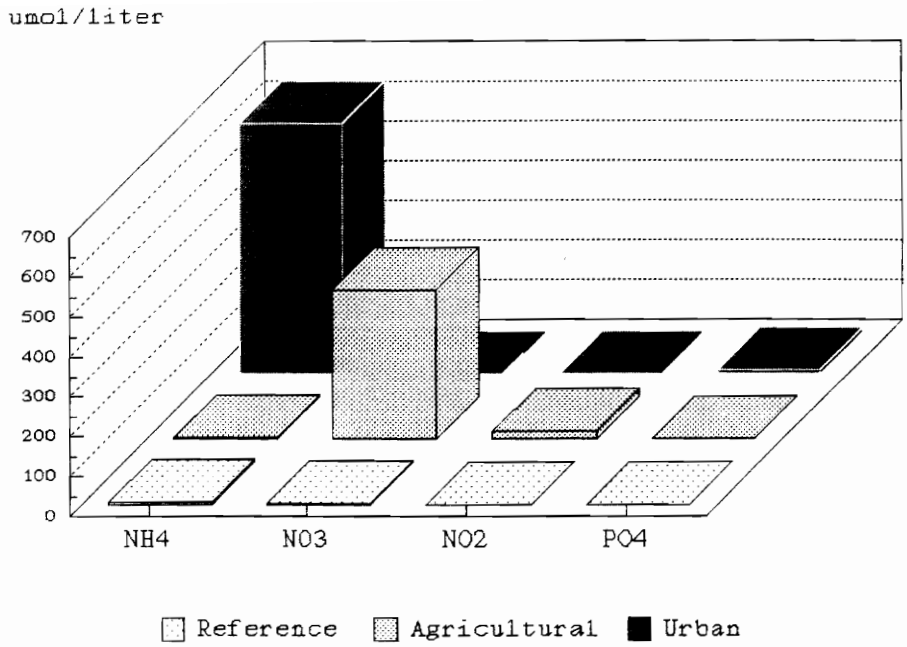

Figure 1.3 Groundwater quality in the southern Chesapeake Bay from three types of land use. Figure adapted from Simmons *et al* (1992).

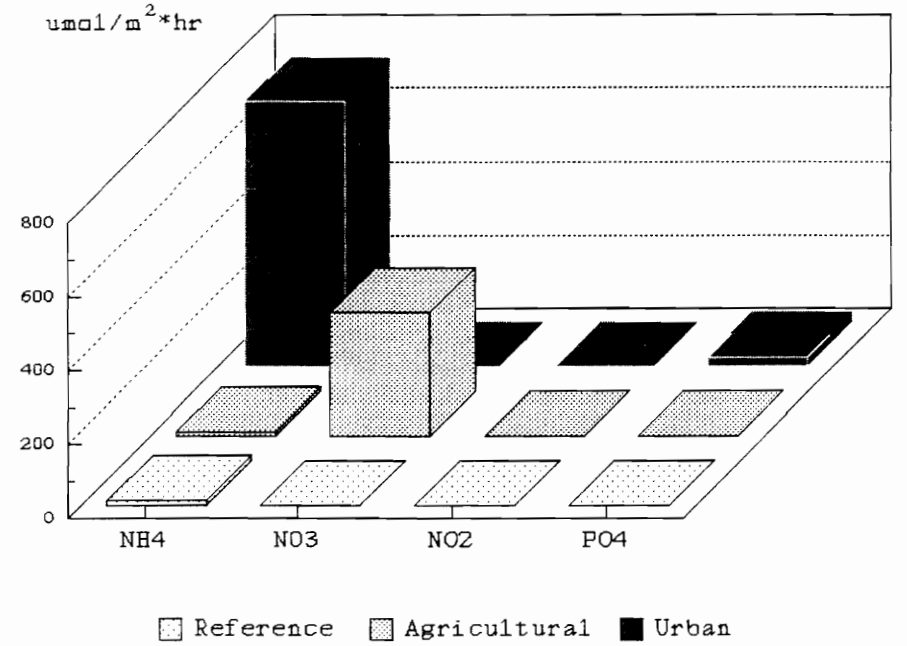

Figure 1.4 Sediment flux of nutrients offshore from three types of land use. Figure adapted from Simmons *et al* (1992).

2. STUDY SITE DESCRIPTION

The study site was located on Virginia's Eastern Shore, Figure 1.1, in Northampton County approximately 0.4 kilometers north of Townsend. An agricultural field was adjacent to a mesic forest of approximately 300 meters in width, which bordered a tidal marsh of approximately 300 meters in width also.

2.1 Floristic Survey

2.1.1 Methodology. The floristic survey identified the forest as mature coastal mesic forest (Reay *et al.*, 1991). Tree and shrub species importance values were calculated using a modified point centered quarter method (Mueller-Dombois and Ellenberg, 1974) along transects 10 meters south of, and parallel to, well transects one and two.

2.1.2 Results. Table 2.1 lists the dominant tree (canopy) and shrub species(subcanopy) found in the forest. The canopy of transect one was dominated by *Liquidambar styraciflua* (Sweet gum), which was also ranked third in importance in the subcanopy. The subcanopy was dominated by *Ilex opaca* (Holly) along transect one. The canopy at transect two was dominated by *Pinus taeda* (Loblolly Pine). The subcanopy was also dominated by *Ilex opaca* (Holly) along transect two. *Nyssa sylvatica* (Black gum) was prevalent in transect one and two occurring second in importance in both the canopy and subcanopy. Figure 2.1 and 2.2 show the dominant species of trees and shrubs along transect one and two, respectively. Herbaceous layer raw cover averaged less than 40% in both transects one and two. These plants were classified as facultative wetland species that have adapted to saturated or near saturated soil conditions along with low dissolved oxygen (Reed, 1988). Mesic forests dominated by a facultative wetland canopy are a common vegetation community found in Mid-Atlantic Coastal Plain.

Table 2.1 Dominant tree, shrub, and herbaceous species, with common name, from the forest buffer.

Species	Common name
Acer rubrum L.	Red maple
Aralia spinosa L.	Devil's walking stick
Ilex opaca Aiton	Holly
Ilex verticillata (L.) Gray	Winterberry
Lyquidamber styraciflua L.	Sweet gum
Myrica cerifera L.	Wax myrtle
Nyssa sylvatica Marshall	Black gum
Pinus taeda L.	Loblolly pine
Prunus serotina Ehrhart	Wild black cherry
Campsis radicans (L.) Seemann	Trumpet vine
Leersia oryzoides (L.) Swartz	Rice cutgrass
Lonicera japonica Thunberg	Japanese honeysuckle
Parthenocissus quinquefolia (L.)	Virginia creeper
Rhus radicans L.	Poison ivy
Smilax rotundifolia L.	Green briar
Thelypteris palustris (Michaux) Holub	Marsh fern
Woodwardia areolata (L.) Moore	Netted chain fern

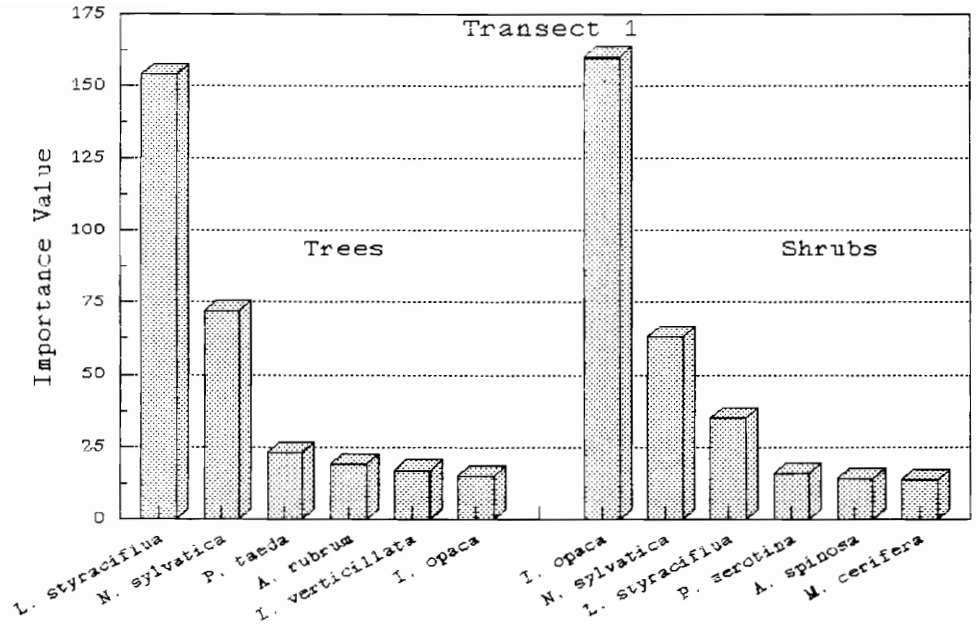

Figure 2.1 Dominant canopy and shrub species in the forest buffer along transect 1.

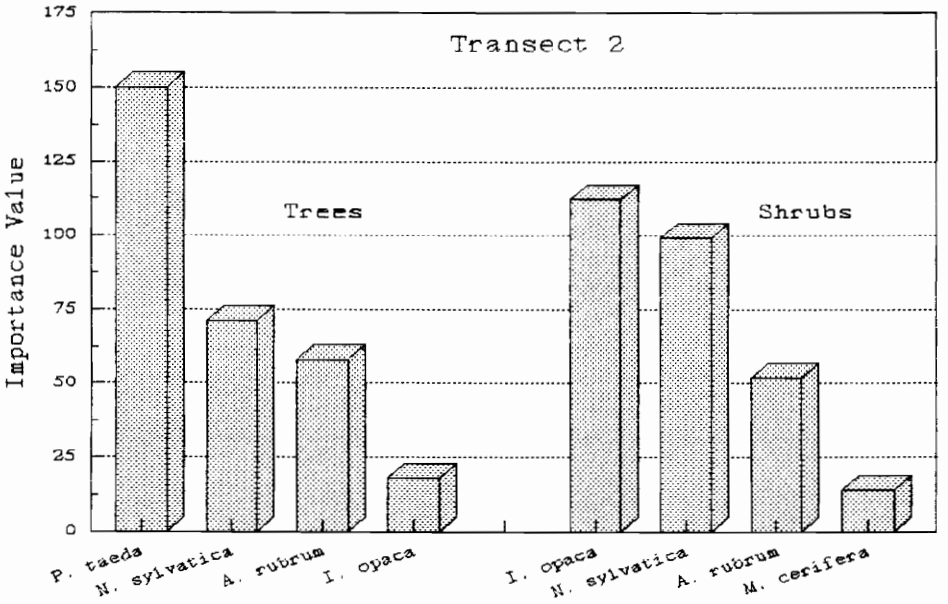

Figure 2.2 Dominant canopy and shrub species in the forest buffer along transect 2.

3. GEOHYDROLOGY & GROUNDWATER QUALITY

3.1 Methodology

3.1.1 Well and Transect Design. Three wells were located in the agricultural field along with two well transects extending from the field/forest interface to horizontal distances of 15.2, 30.5, 45.7, 61.0, and 91.4 meters into the forest, Figure 3.1 . This study focused on transect 1 where deeper wells had been installed. Illustrated in Figure 3.2 is a cross-sectional diagram of transect 1 which includes position and vertical depth of wells along the transect.

Well transect 1 had thirteen wells altogether. One well was placed in the field. A cluster of three wells at the field/forest interface at vertical depths (below land surface) of 3.05, 7.62, and 15.25 meters. One well at 15.2, 30.5, and 61.0 meter distances along the transect. A cluster of three wells each at 45.7 and 91.4 meters along the transect, at vertical depths of 3.05, 3.66, and 7.62 meters. Wells were installed by two different methods. Shallow wells were installed with a hand auger, and deeper wells were installed with a hydraulic jet drill. In both cases, wells were constructed of 5.08cm schedule 40 PVC casing with 0.3 meters of 0.025 centimeter slotted screen. All wells were backfilled with pea gravel and sealed with bentonite pellets.

3.1.2 Geology. Soil samples were collected using the hand auguring system. Soil organic matter was determined by combusting dried samples at 500°C for five hours followed by reweighing (Dean, 1974). Organic matter is expressed as a percentage weight loss from combustion of dried samples. Grain size distribution was determined by wet sieve and pipet analysis (Folk 1980). The Wentworth grain size scale was used to separate soil into gravel, sand, silt, and clay size fractions (Folk 1980).

3.1.3 Water Chemistry. Groundwater nutrient and dissolved oxygen samples were collected prior to the denitrification study and also when denitrification potentials were measured. Wells were purged for several minutes to allow for fresh groundwater to be sampled. Nutrient samples were filtered with pre-washed Gelman 0.45 um membrane

filters. Dissolved inorganic nitrogen and dissolved oxygen concentrations were measured in the laboratory on the Eastern Shore of Virginia. Ammonium was measured within six hours of collection, by using a modified phenate method (Strickland and Parsons, 1972). Nitrite was measured by diazotizing with sulfaniamide and coupling with N-(1-naphyl)-ethylenediamine (APHA, 1989). Nitrate was measured by Cu-Cd reduction method (APHA, 1989). Dissolved oxygen was measured by using the iodometric method with an azide modification (APHA, 1989.)

Quality control guidelines were followed in accordance to those outlined in the Standard Operating procedure for Coastal Groundwater Research Program (Miles, 1992). Reagents used for water chemistry analysis were prepared and stored in accordance with recommendations in APHA (1989). Duplicate samples were collected on 10% of samples. Standards for water chemistry analysis were analyzed during each sample collection. Triplicate samples of each standard were analyzed.

3.1.4 Statistical Analysis. An ANOVA (analysis of variance) was used to test statistically significant differences between sample sets. An alpha level of 0.05 ($\alpha = 0.05$) was chosen for statistical significance. Thus samples sets compared were considered significantly different if the probability computed from the ANOVA was less than 0.05 ($p < 0.05$). The null hypothesis tested was: Horizontal distance into the forest did not affect nitrate or dissolved oxygen concentrations.

In all cases, data values were transformed by taking the logarithm of the values. Data values were transformed to increase the homogeneity of variances between samples compared. This logarithmic transformation allowed for the use of parametric statistics on all samples compared. Bartlett's test for homogeneity of variances was used to compare sample set variances. Bartlett's test was used to assure that all sample sets had probabilities, of sample variances being equal, greater than 0.05 ($p > 0.05$).

3.2 Results

3.2.1 Geohydrology. At the surface in both the agricultural field and forest there was a higher percentage of silt, clay, and organic matter which decreased with increasing vertical depth. The soils in both the agricultural field and forest were sand dominated. The soils, described by Cobb and Smith (1989), in the agricultural field were classified as a Bojac sandy loam (coarse-loamy, mixed, thermic Typic Hapludults) and Munden sandy loam (coarse-loamy, mixed, thermic Aquic Hapludults), which are both well drained soils. Table 3.1 lists the values for grain size and percent organic matter measured in the field and forest. Figure 3.3 shows the results of grain size analysis in the agricultural field. The field was dominated by sand (>95%) at a vertical depth of approximately 2.0 meters where the water table was located. The forest soil was a Nimmo sandy loam (coarse-loamy, mixed, thermic Typic Ochraquults), which is a poorly drained soil (Cobb and Smith, 1989). The root zone in the Nimmo soil extended to a vertical depth of at least 1.52 meters. Figures 3.4 and 3.5 show results of grain size analysis from 53.3 and 91.4 meters into the forest. The forest was also dominated by sand (>60%) at a vertical depth of approximately 0.6 meters where the water table was located. The forest was characterized by a much higher percent organic matter in the vertical soil profile than the agricultural field. Figure 3.6 shows results of percent organic matter analysis from the agricultural field. Figures 3.7 and 3.8 show results of percent organic matter analyses in the forest from 53.3 and 91.4 meters. Percent organic matter in the field at the water table (approximately 1.9-2.5 meters vertical depth) was approximately 0.5%, as compared to the forest at the water table (approximately 0.1-1.1 meters vertical depth) in which percent organic matter was approximately 1.5% (Figures 3.7 & 3.8).

Depth to the water table in the forest was approximately 0.1-1.1 meters, whereas in the agricultural filed depth to the water table was approximately 1.9-2.5 meters. Reay *et al* (1991) also found that the water table in the forest rose rapidly in response to rainfall. The rapid response of the water table in the forest to rainfall is reflective of the

shallow water table. Calculated horizontal groundwater velocities using hydraulic conductivities and hydraulic gradients from Reay *et al* (1991) for the agricultural field were 0.01 meters•day^{-1} and 0.022 meters•day^{-1} for the forest. The general direction of groundwater flow was in a North-Easterly direction from the agricultural field to the forest (Reay *et al*, 1991). The average horizontal hydraulic gradients ($\Delta h_x/\Delta l_x$) in the forest between January, 1990 to October, 1990 are listed in Table 3.4. Average vertical hydraulic gradients ($\Delta h_z/\Delta l_z$) from March, 1992 to November, 1992 in the forest at 0.0, 45.7, and 91.4 meters along the transect are also listed in Table 3.1.

Groundwater flow was dominated by horizontal rather than vertical flow as distance into the forest increased. Horizontal flow dominance can be seen by comparing hydraulic gradients in the horizontal, $\Delta h_x/\Delta l_x$, and vertical, $\Delta h_z/\Delta l_z$, directions in the forest (Table 3.1). Positive values in the $\Delta h_z/\Delta l_z$ column indicate groundwater moving downward, whereas negative values indicate groundwater moving upward. In the field, at the field/forest interface (0.0m), and at 45.7 meters, groundwater flow was to the east and downward. At 91.4 meters along the transect groundwater flow was also toward the east but was moving toward the surface.

3.2.2 Groundwater Quality. Table 3.2 lists all groundwater nitrate concentrations measured. Table 3.4 lists all groundwater dissolved oxygen concentrations measured. Figures 3.9 & 3.10 show nitrate concentrations and dissolved oxygen concentrations in the shallow water table aquifer in the agricultural field and forest. Each bar in Figure 3.9 are averages of nitrate concentrations from a sixteen month sampling period between December, 1989 to March, 1991. Each bar in Figures 3.10 are averages of dissolved oxygen concentrations from a sixteen month sampling period between February, 1990 to June, 1991. Previous research by Reay *et al* (1990) and sampling conducted during this study found decreasing nitrate concentrations and decreased dissolved oxygen concentrations with increasing distance into the forest in the shallow water table. Nitrate concentrations in the shallow water table averaged 1,161.0 (SD=393, N=16) *u*mol•liter^{-1} in the field as compared to 2.2 (SD=2.6, N=16) *u*mol•liter^{-1} in the forest (91.4m).

Dissolved oxygen concentrations in the shallow water table in the field averaged 8.4 (SD=1.3, N=16) mg•liter^{-1} and 2.5 (SD=1.6, N=16) mg•liter^{-1} in the forest (91.4m).

In the forest, nitrate and dissolved oxygen concentrations were greater deeper in the aquifer than in the upper shallow region of the water table. Samples collected between March, 1992 - June, 1992 for nitrate concentrations and dissolved oxygen concentrations at various horizontal and vertical depths in the forest are shown in Figures 3.11 and 3.12, respectively. At the 45.7 meter distance along the transect, average nitrate concentrations were 6.0 (SD=7.5, N=5), 233.0 (SD=67.3, N=5), and 176.6 (SD=120, N=5) μmol•liter^{-1} at the respective vertical depths of 3.05, 3.66, and 7.62 meters. At the 91.4 meter distance along the transect, average nitrate concentrations were 2.2 (SD=2.6, N=5), 559.5 (SD=101.9, N=5), and 404.0 (SD=215.4, N=5) μmol•liter^{-1} at the respective vertical depths of 3.05, 3.66, and 7.62 meters. At the 45.7 meters distance along the transect, average dissolved oxygen concentrations were 1.60 (SD=1.25, N=15), 4.19 (SD=0, N=1), and 3.63 (N=1) mg•liter^{-1} at the respective vertical depths of 3.05, 3.66, and 7.62 meters. At the 90.1 meter distance along the transect, average dissolved oxygen concentrations were 2.50 (SD=1.6, N=16), 5.13 (N=1), and 2.14 (N=1) mg•liter^{-1} at the respective vertical depths of 3.05, 3.66, and 7.62 meters.

ANOVA (analysis of variance) was used to test whether there was a significant difference in nitrate and dissolved oxygen concentrations between samples, with respect to distance into the forest. There was a significant decrease in nitrate concentrations ($p < 0.001$) and dissolved oxygen concentrations ($p < 0.001$) in the shallow water table as distance into the forest increased.

3.3 Discussion

The vertical soil profiles of the agricultural field and forest were similar. Both the agricultural field and forest were characteristic of having an A horizon of sand, slit, clay, and organic matter at the surface (<1.0meters) overlaying a >95% sand dominated system. Soil characteristics at the water table in the agricultural field differed from soil

characteristics in the forest due to the relatively shallow water table in the forest. More important, with respect to denitrification, the percentage of organic carbon in the forest at the water table was three to four times the percentage of organic carbon in the agricultural field. As vertical depth increased below the water table in the forest, percent organic carbon decreased and percentages of sand increased to values similar to those found in the agricultural field at the water table. Below 1.1 meters in the forest, soil characteristics were very similar to those found at the water table in the field.

Fluctuations of the water table in the forest resulted in the flooding of soils closer to the surface. The water table fluctuated between 0.1 and 1.1 meters in the forest and between 1.9 and 2.5 meters in the agricultural field over a one year period. This process helped to facilitate denitrification in two ways. First, the diffusion of oxygen vertically was decreased. Second, the percentage of organic carbon in the soil increased as proximity to the surface increased. The direction of groundwater flow with respect to horizontal vs vertical dominance was important for understanding whether the groundwater coming from the agricultural field intersected the biologically active communities of the forest or simply flowing beneath them. If the groundwater flowed beneath the biologically active communities of the forest, then physical dilution would most likely be the dominant form of nutrient reduction. Measurements at the field-forest interface showed vertical flow was dominant, in the downward direction. But at 45.7 and 91.4 meters into the forest, horizontal flow was dominant. At 91.4 meters groundwater was moving toward the surface. From these measurements; the horizontal flow component was an order of magnitude greater than vertical flow component in the forest as compared to in the agricultural field. More important groundwater moving from the agricultural field, intersected the biologically active communities of the forest.

Decrease nitrate concentrations in the shallow water table demonstrated that one or more of the previously mentioned mechanisms (physical dilution, plant uptake, and/or denitrification) was responsible for this reduction. The effects of physical dilution were minimized due to the counter effects of evapotranspiration. Stanhill (1970) found that

60% of precipitation was released back into the atmosphere through evapotranspiration and the other 40% became surface and subsurface runoff in a temperate forest in the Eastern United States. Corell and Ford(1982) found precipitation to contain high amounts of nitrogen. In an eight year study conducted from 1973-1980 Corell and Ford (1982) found nitrate (NO_3-N) concentrations averaging 29.5 $\pm$ 7.2 $u$mol$\bullet$liter^{-1} and total nitrogen averaging 65.5 $\pm$ 13.0 $u$mol$\bullet$liter^{-1} in the upper Chesepeake Bay region. Reay *et al.* (1992) also found precipitation high in nitrogen concentrations, nitrate (NO_3-N) and ammonium (NH_4-N) averaging 32.7 $\pm$ 29.4 $u$mol$\bullet$liter^{-1} and 29.7 $\pm$ 11.6 $u$mol$\bullet$liter^{-1} respectively. Therefore the amount of nitrogen contained in precipitation would have increased nitrogen concentrations in the groundwater, not diluted them.

The biologically active zone of the forest, with respect to below the land surface, encompassed the root zone and the microbial communities associated with the root zone. In regions where the water table was relatively shallow, conditions could be very conducive to denitrification. As a result of the water table being shallow, oxygen diffusion into the vertical soil profile was prevented. Organic carbon was available from decomposing material and exudation from roots in the forest. Measurement of soil characteristics indicated that organic matter was more available at the water table in the forest as compared to the field, which was due directly to the proximity of the water table to the surface. Also, dissolved oxygen concentrations in the forest at the water table were in the range for denitrification. Facultative anaerobic denitrifiers used nitrate as an electron acceptor, through respiration, when oxygen concentrations became low. As nitrate became available in this biologically active zone it was reduced. Therefore the decrease in nitrate concentrations in the shallow water table indicated that plant uptake and denitrification were primarily responsible for the reduction of nitrate in this system.

Table 3.1 Grain size and percent organic matter in the agricultural field and forest.

Location	Depth (meters)	Grain Size Mass Ratio				Percent Organic Matter
		Gravel	Sand	Silt	Clay	
Agricultural Field	0.3	0.00	0.56	0.36	0.08	0.9
	0.6	0.00	0.46	0.41	0.13	1.6
	0.9	0.00	0.61	0.26	0.13	1.4
	1.2	0.00	0.96	0.03	0.01	0.2
	2.1	0.00	0.97	0.02	0.01	0.5
Forest 91.0 meters	0.15	0.00	0.36	0.54	0.10	13.0
	0.3	0.00	0.29	0.57	0.14	1.3
	0.6	0.00	0.62	0.25	0.13	1.9
	1.3	0.00	0.93	0.05	0.02	0.5
Forest 53.3 meters	0.1	0.00	0.67	0.25	0.07	11.7
	0.2	0.00	0.53	0.38	0.09	4.4
	0.3	0.00	0.51	0.38	0.11	0.9
	0.4	0.00	0.48	0.38	0.14	1.2
	0.5	0.00	0.48	0.36	0.15	1.3
	0.6	0.00	0.63	0.21	0.16	1.3
	0.7	0.00	0.76	0.08	0.16	1.3
	0.8	0.00	0.84	0.04	0.13	1.1
	0.9	0.00	0.91	0.01	0.07	1.0
	1.0	0.00	0.96	0.02	0.02	0.7
	1.1	0.00	0.99	0.01	0.00	0.3
	1.2	0.11	0.99	0.01	0.00	0.3
	1.3	0.00	0.99	0.00	0.00	0.3
	1.4	0.00	0.99	0.00	0.00	0.3
	1.5	0.00	0.99	0.00	0.00	0.5
	1.6	0.00	0.97	0.02	0.00	0.9
	1.7	0.00	0.80	0.03	0.16	1.1
	1.8	0.00	0.87	0.03	0.09	1.3
	1.9	0.00	0.83	0.06	0.11	1.3
	2.0	0.00	0.79	0.08	0.12	1.7
	2.1	0.00	0.70	0.18	0.11	0.8
	2.2	0.00	0.75	0.19	0.06	0.2
	2.3	0.00	0.98	0.01	0.01	0.3
	2.4	0.00	0.99	0.00	0.00	0.3
	2.5	0.00	0.99	0.00	0.01	0.2
	2.6	0.00	0.99	0.00	0.00	0.2

Table 3.2 Groundwater nitrate (NO_3-N) concentrations in the agricultural field and forest.

Date	Nitrate Concentrations (NO_3-N umol • liter^{-1}) along transect 1 from wells at 3.05 meter vertical depth						
	FW1	0.0	15.2	30.5	45.8	61.0	90.1
11/12/89	----	----	417.1	77.6	59.1	----	----
12/1/89	603.9	555.1	224.8	9.3	0	115.9	7.0
12/19/89	765.3	726.0	172.8	9.0	0.1	118.0	5.3
1/19/90	1126.0	692.8	29.3	22.0	7.3	104.1	0.0
2/23/90	1244.8	763.2	12.9	0.9	8.4	89.3	0.2
3/10/90	1214.2	745.7	8.5	1.0	0.1	107.4	0.0
4/14/90	1555.3	786.7	3.1	0.4	0.8	54.3	0.0
5/16/90	1317.9	507.5	10.6	2.9	3.0	33.6	3.1
6/13/90	1541.8	487.6	137.7	1.7	6.8	57.0	7.4
7/9/90	1285.0	466.1	380.8	34.8	4.0	162.1	4.9
8/15/90	1727.5	606.6	415.8	48.1	7.8	131.3	3.0
9/15/90	1462.2	345.0	189.8	8.2	0.7	18.1	0.7
10/20/90	1418.8	429.6	218.3	87.9	18.7	118.1	1.4
11/17/90	1235.6	-----	226.3	70.6	27.2	21.4	0.8
12/17/90	300.3	234.7	28.7	22.2	7.3	9.1	0.2
1/19/91	----	-----	-----	-----	-----	----	-----
2/15/91	639.0	518.1	17.1	10.0	0.9	55.7	0.4
3/12/91	1147.0	815.5	15.7	10.8	2.4	66.6	0.7
2/92	-----	736.6	48.2	13.4	1.6	12.2	0.5
3/92	----	704.8	45.0	17.7	----	15.9	3.6
3/13/92	-----	619.8	12.7	8.8	0.0	33.4	0.5
3/29/92	----	689.5	19.7	1.6	0.0	36.4	0.5
6/30/92	-----	649.8	454.6	0.8	0.1	29.9	0.2

Date	Well Depth (meters)	Nitrate Concentrations (NO_3 umoliter^{-1}) at well clusters along transect 1		
		0.0	45.7	90.1
3/13/92	7.62	638.6		
3/29/92	7.62	673.7		
6/30/92	7.62	663.8		
3/13/92	15.24	0.5		
3/13/92	15.24	0.7		
3/13/92	15.24	0.7		
2/92	3.66		125.8	532.28
3/92	3.66		207.2	397.9
3/13/92	3.66		270.1	589.3
3/29/92	3.66		281.9	665.3
6/30/92	3.66		280.1	603.7
3/13/92	7.62		294.1	595.8
3/29/92	7.62		180.9	171.0
6/30/92	7.62		54.7	445.2

Table 3.3 Groundwater dissolved oxygen concentrations in the agricultural field and forest.

Date	Dissolved oxygen concentrations (mg • liter^{-1}) along transect 1 from wells at 3.05 meter vertical depth						
	FW1	0.0	15.2	30.5	45.8	61.0	90.1
2/23/90	7.58	5.41	1.01	1.48	0.48	0.83	0.64
3/10/90	9.58	3.83	2.93	2.4	0.00	1.18	2.12
4/14/90	9.34	5.34	1.45	2.43	0.16	1.39	0.91
5/16/90	8.8	1.85	1.09	0.94	0.00	2.22	0.47
6/13/90	9.4	2.27	2.37	1.71	1.30	1.96	2.79
7/9/90	8.58	2.12	----	2.93	1.78	2.21	1.28
8/15/90	8.44	2.89	4.53	4.91	2.47	3.94	2.69
9/15/90	8.08	1.43	1.5	1.74	0.16	1.27	0.52
10/20/90	8.83	5.24	4.4	2.40	3.80	3.39	4.43
11/17/90	8.62	7.75	3.36	4.14	4.38	5.49	4.52
12/17/90	8.60	6.89	7.7	3.95	1.96	5.45	2.58
1/19/91	4.59	4.04	2.63	7.18	1.22	3.07	2.26
2/15/91	9.23	4.77	2.35	6.26	1.02	6.24	2.83
3/12/91	----	----	----	----	----	----	----
4/19/91	----	3.7	3.97	3.74	1.54	4.81	5.33
5/24/91	----	9.26	3.46	3.54	1.40	2.62	2.48
6/17/91	9.04	7.5	2.56	----	1.68	0.52	5.0

Date	Well Depth (meters)	Dissolved oxygen concentrations (mg • liter^{-1}) at well clusters along transect 1		
		0.0	45.7	90.1
6/30/92	7.62	5.17		
6/30/92	15.2	2.01		
5/24/91	3.66		2.1	2.0
6/17/91	3.66		2.52	1.65
6/30/92	3.66		4.19	5.13
6/30/92	7.62		3.63	2.14

Table 3.4 Horizontal and vertical hydraulic gradients in the forest.

Location	$\Delta h_x/\Delta l_x$	Location	$\Delta h_z/\Delta l_z$
Field	0.006*	0.0m	0.016
Forest	0.016*	45.7m	0.002
		91.4m	-0.001

* Reported by Reay *et al*(1991)

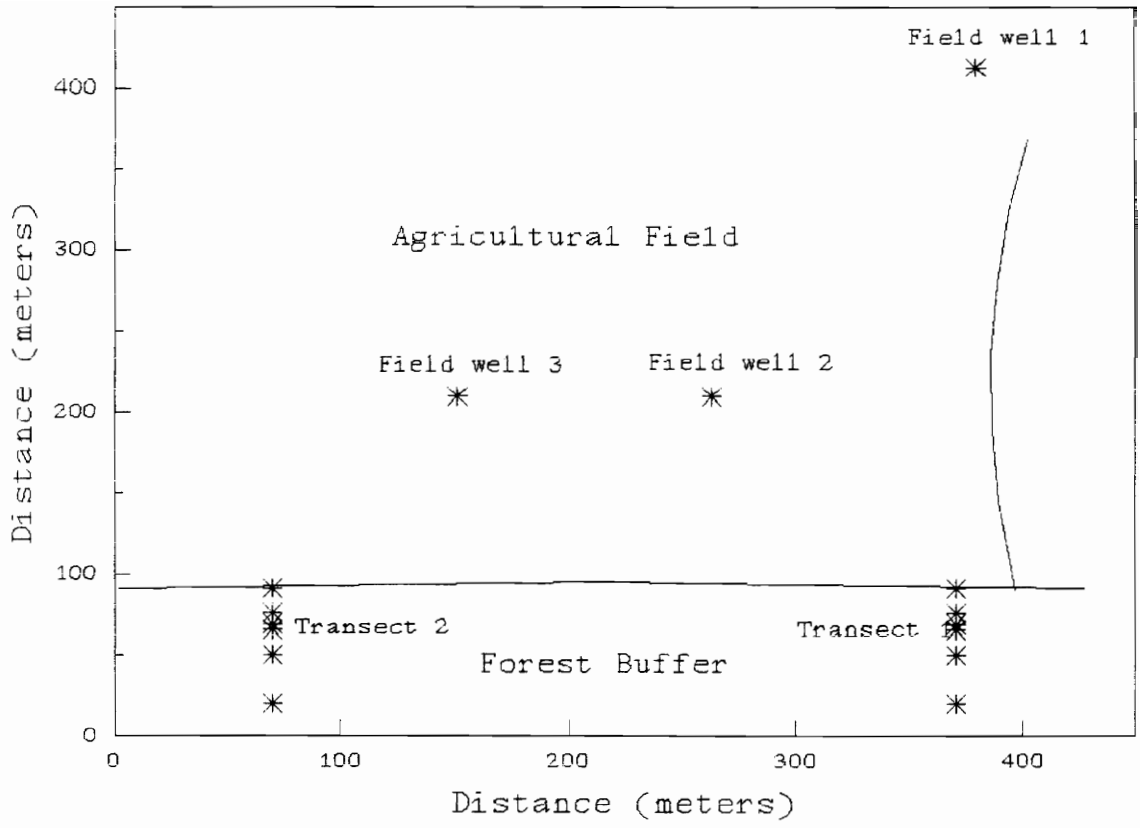

Figure 3.1 Aerial view of agricultural field, forest buffer, and well transects 1 & 2. (wells are denoted by asterisks)

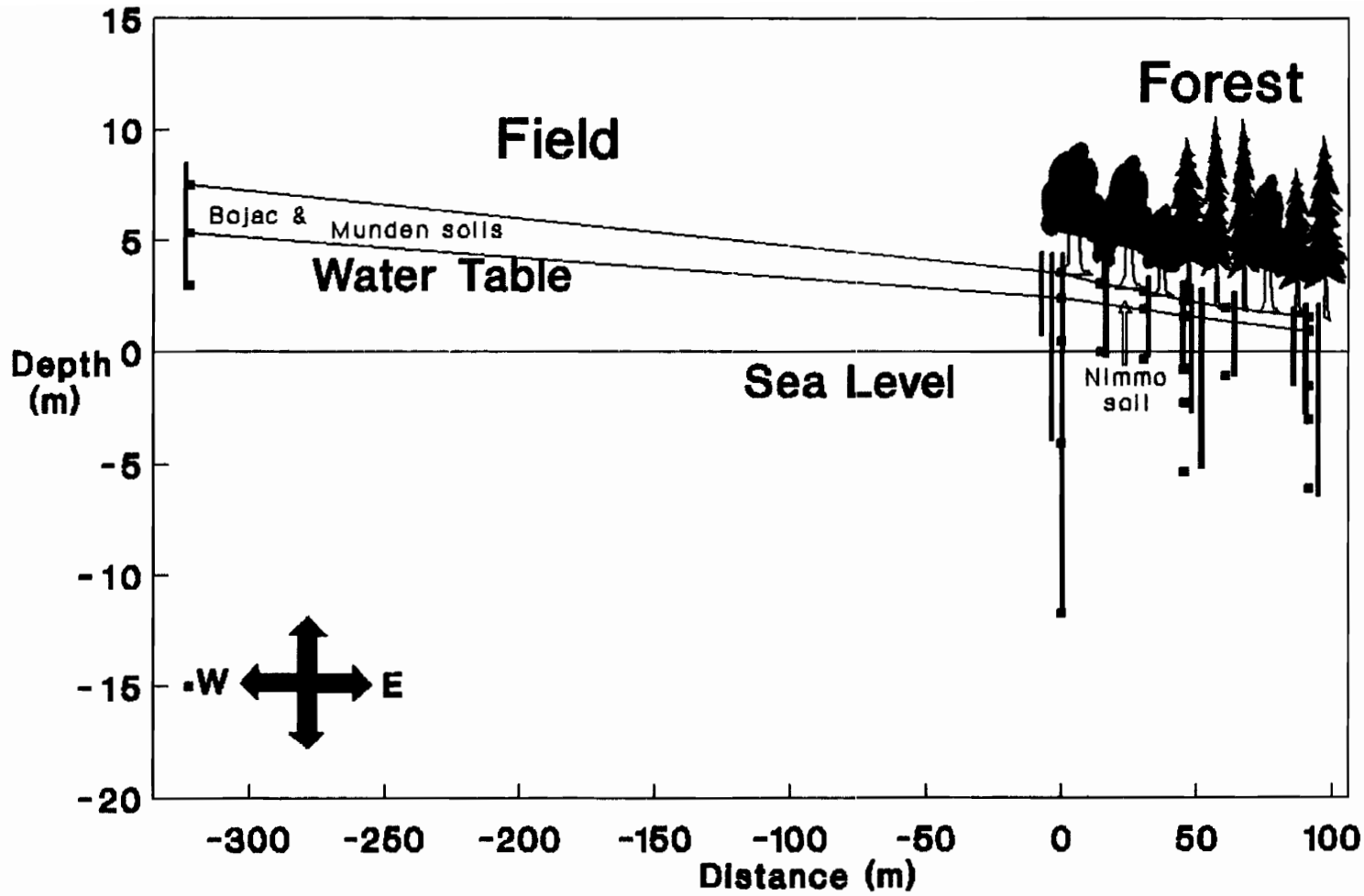

Figure 3.2 Cross-section of transect 1 at study site.

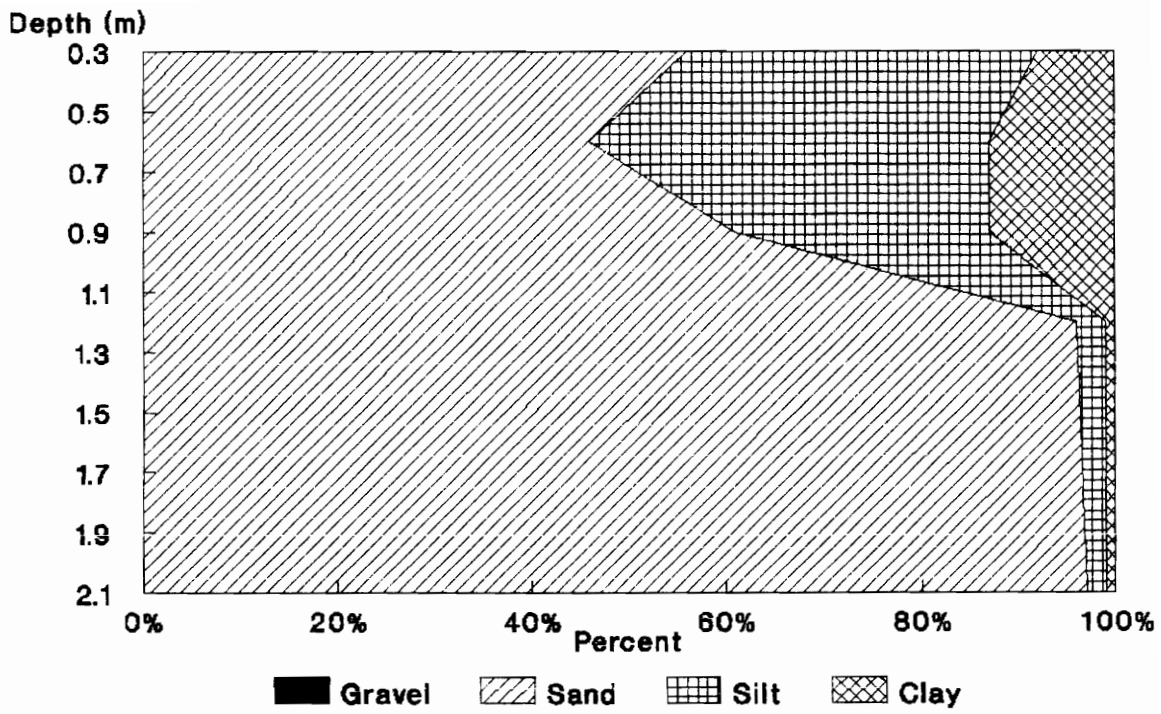

Figure 3.3 Field grain size vs vertical depth

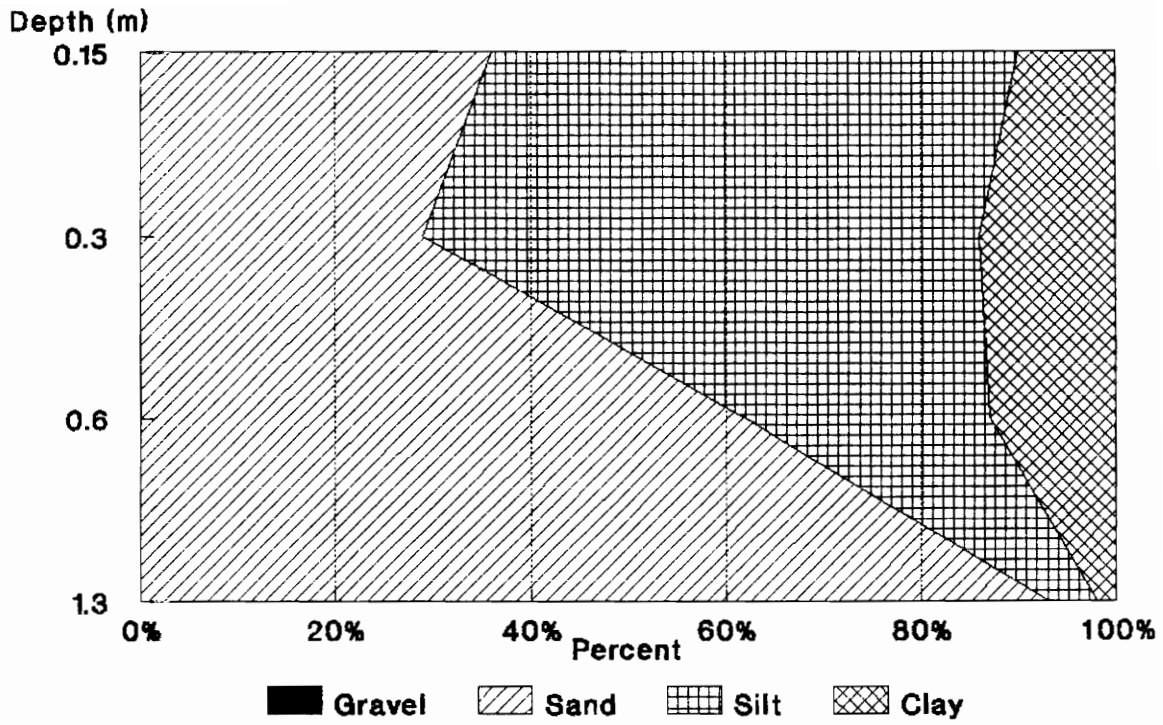

Figure 3.4 Forest 91.4m (300ft) grain size vs vertical depth

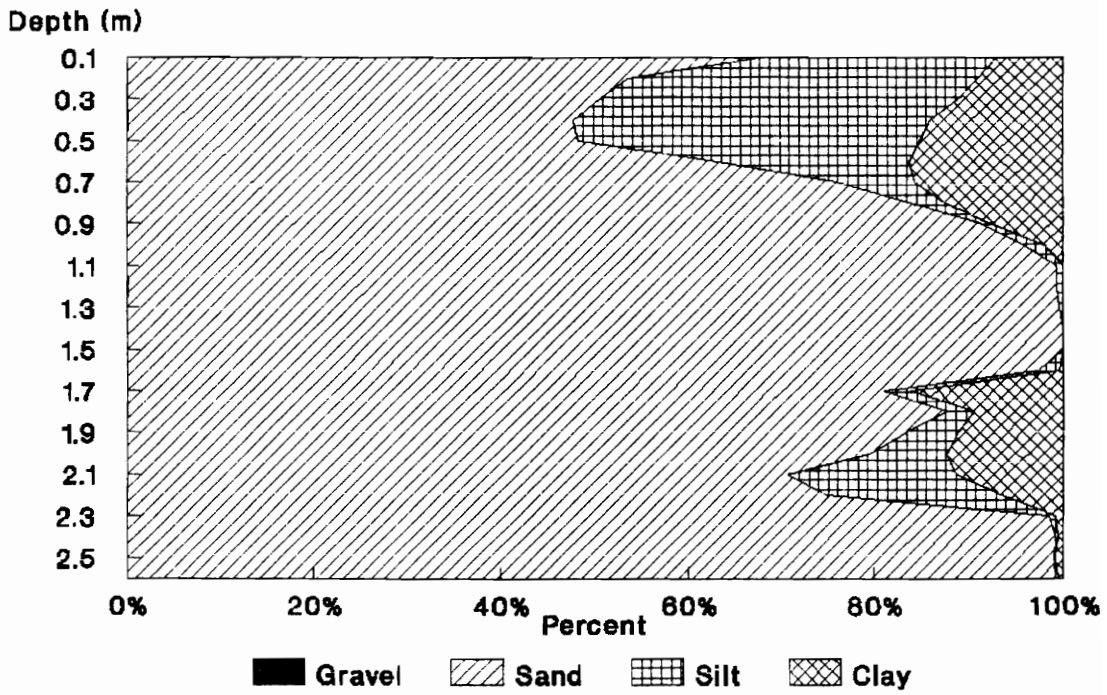

Figure 3.5 Forest 53.3m (175ft) grain size vs vertical depth

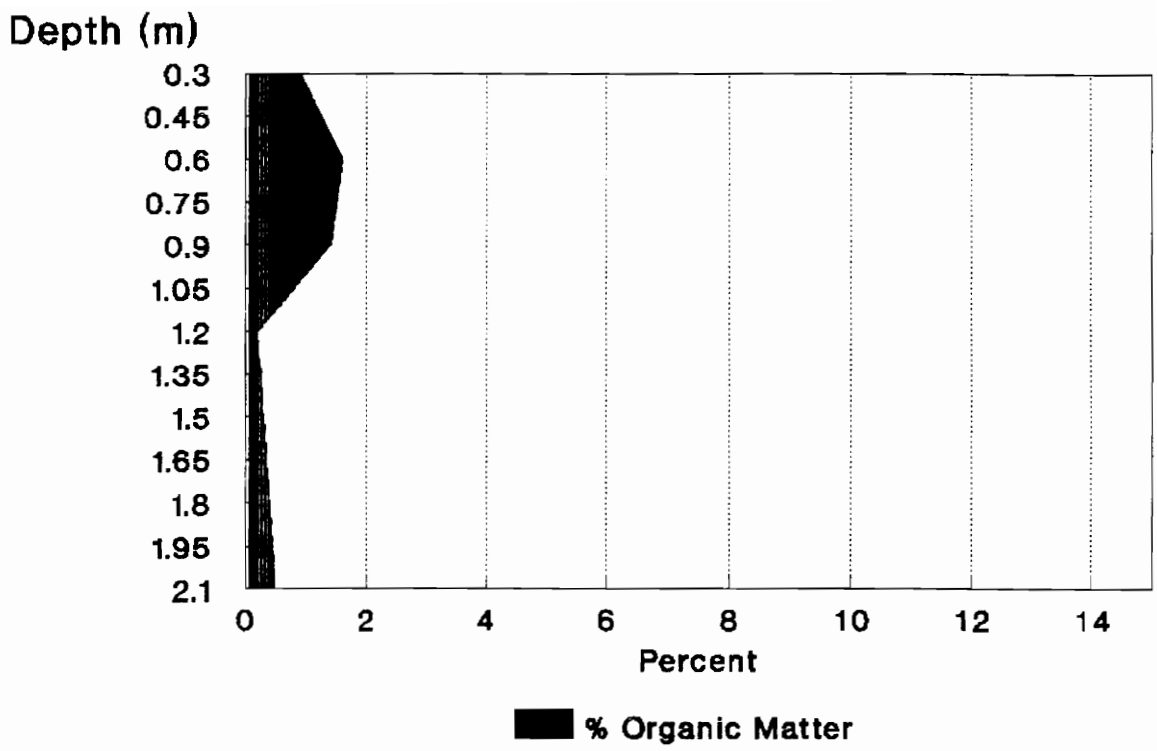

Figure 3.6 Field percent organic matter vs vertical depth

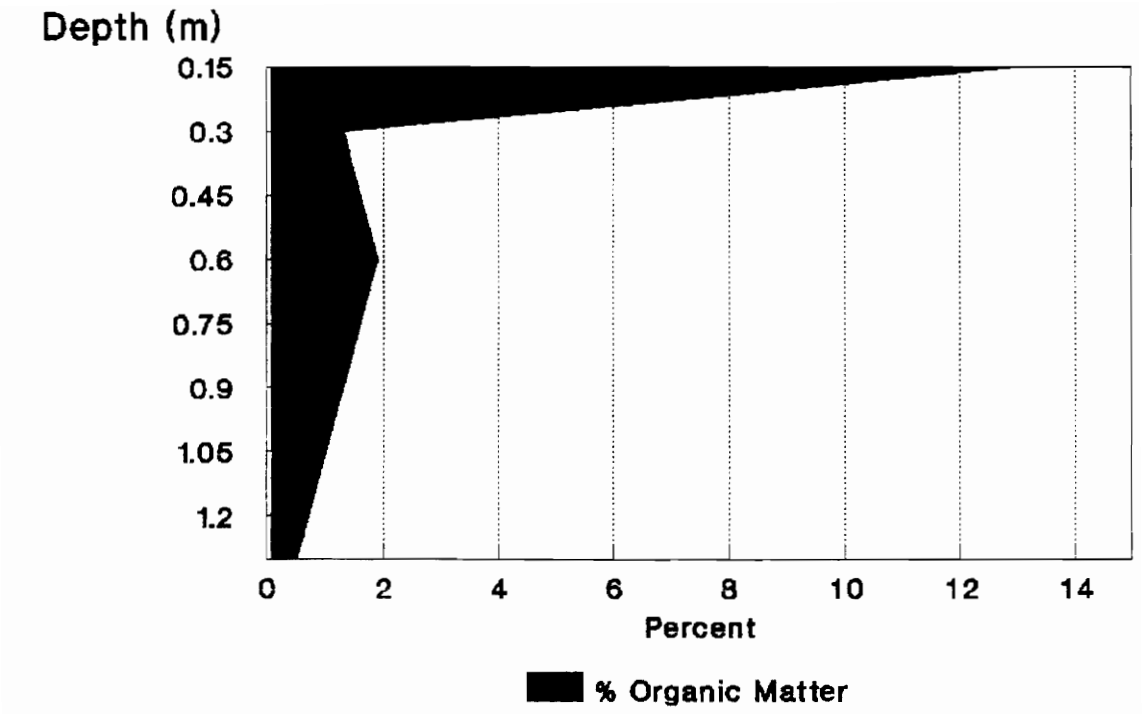

Figure 3.7 Forest 91.4m percent organic matter vs vertical depth

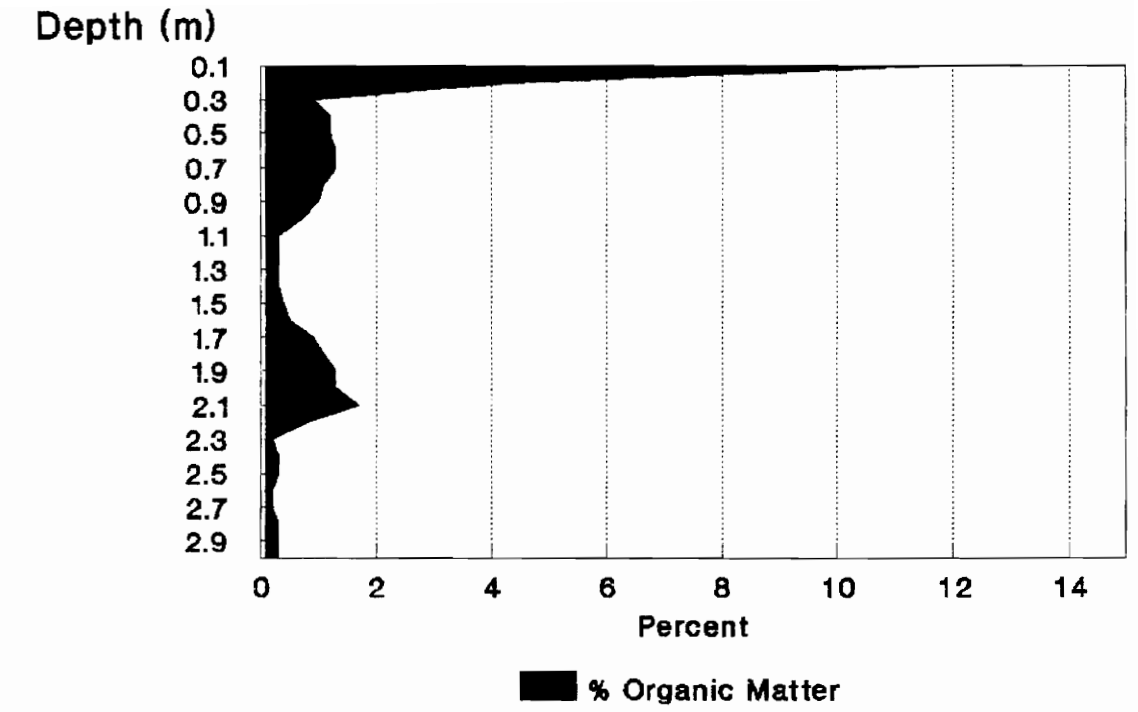

Figure 3.8 Forest 53.3m percent organic matter vs vertical depth

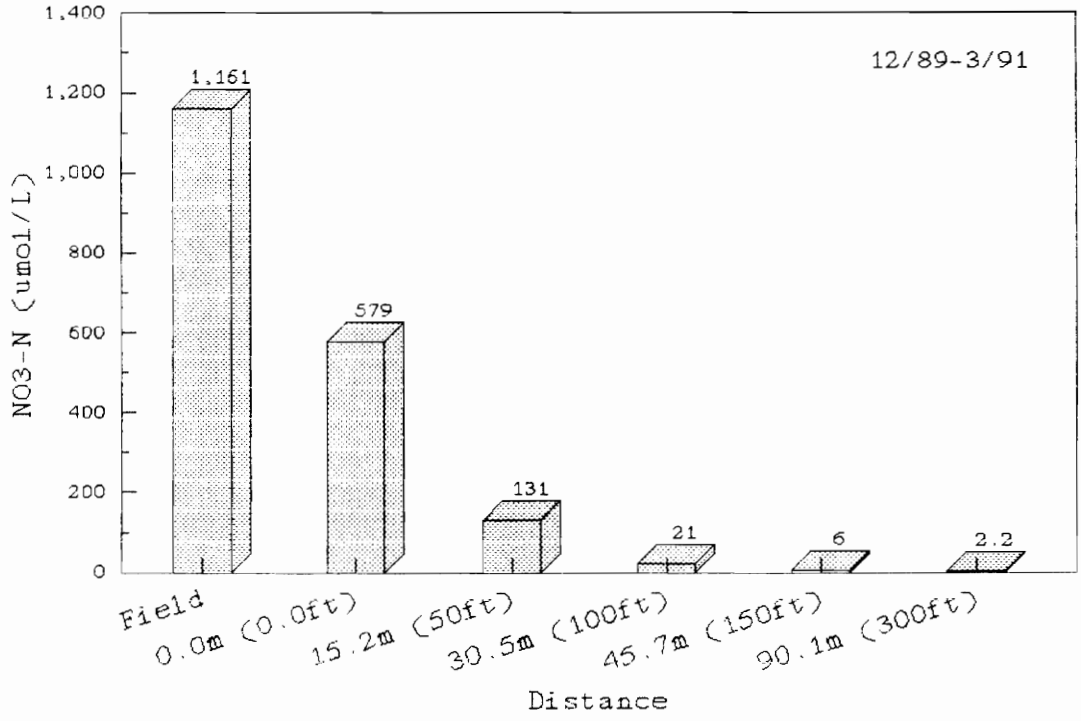

Figure 3.9 Shallow water table nitrate concentrations.

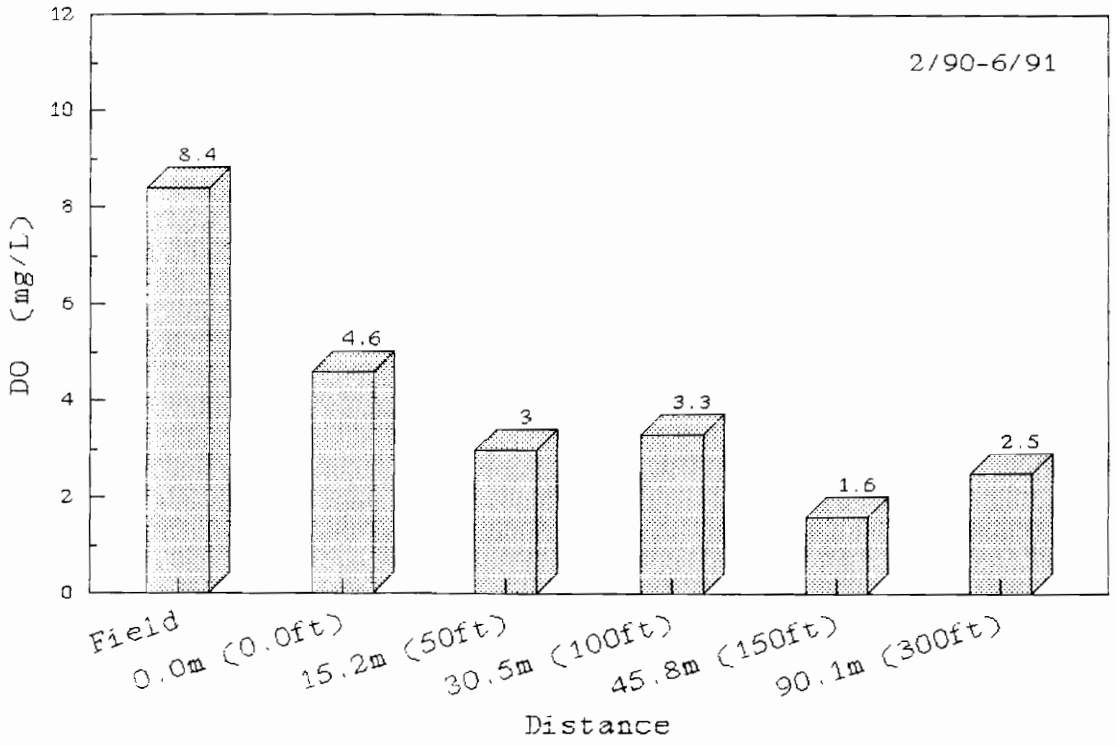

Figure 3.10 Shallow water table dissolved oxygen concentrations.

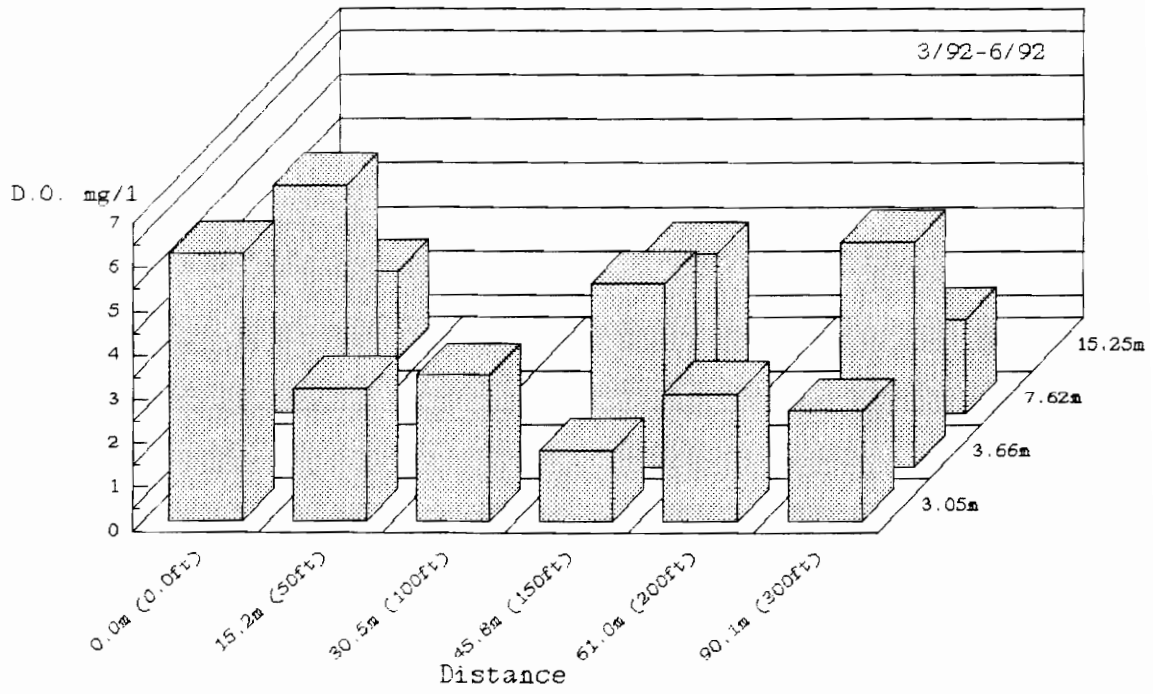

Figure 3.11 Dissolved oxygen concentrations at various horizontal and vertical depths in the forest.

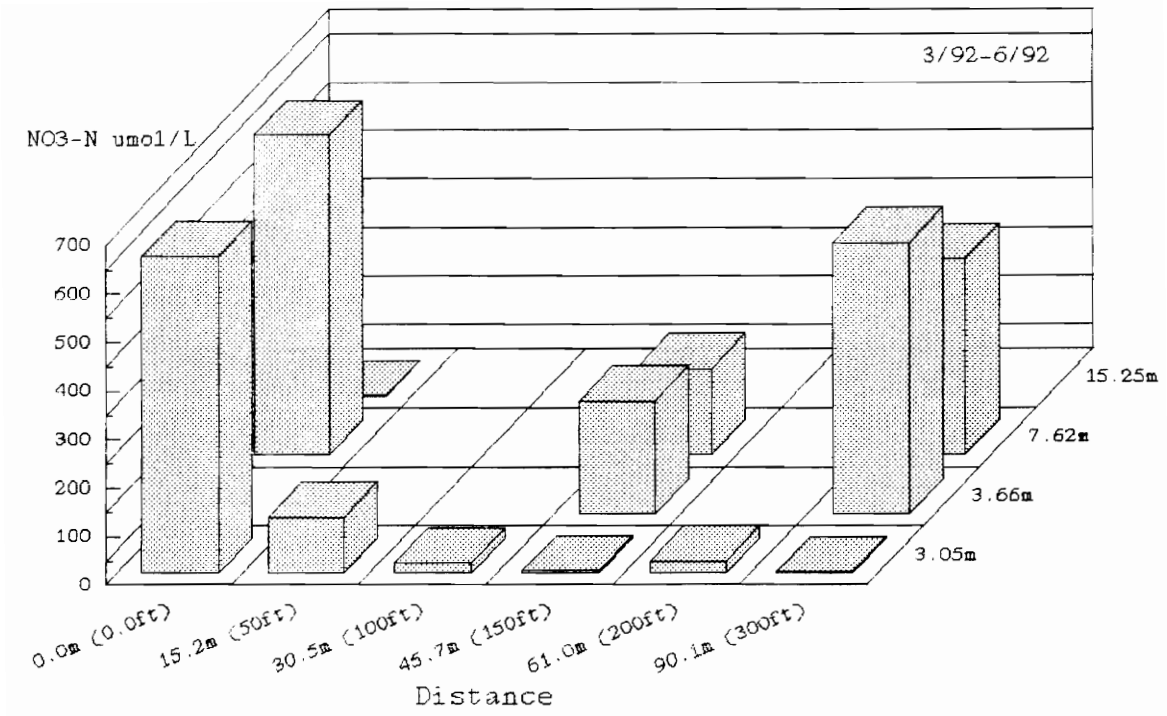

Figure 3.12 Nitrate concentrations at various horizontal and vertical depths in the forest.

4. DENITRIFIER DENSITIES & DENITRIFICATION POTENTIALS

4.1 Experimental Approach

The first objective was to determine the effectiveness of the forest in reducing nutrient contaminated groundwater flowing from the agricultural field by monitoring groundwater quality for almost a two year period. Some of this work was reported by Reay *et al.*(1992). The second objective was to determine the mechanisms responsible for nutrient reduction. Possible mechanisms responsible for nutrient reduction were physical dilution, plant uptake, and/or microbial respiration (i.e. denitrification). The extent of denitrification occurring in groundwater was unknown as compared to the effects of plant uptake or physical dilution. Therefore the third objective was to determine the importance of denitrification occurring in groundwater in the forest. Table 4.1 shows the sampling of denitrification activity and denitrifier densities in the field and forest with respect to location (field or forest), and vertical depth. The temperature range of groundwater in the field and forest over an annual temperature cycle was 11-16°C (Reay *et al*, 1992), but no attempt was made to account for seasonality affects on denitrification because samples were processed in the lab at 25°C. Samples were collected in the field and at various horizontal distances into the forest. Samples for denitrification activity and denitrifier densities were collected at the surface, at the water table, and 0.91 meters below the water table when sampling was conducted. The exception is the month of December 1992 when samples deeper than 0.91m below the water table were also collected.

4.2 Methodology

4.2.1 Denitrifier densities. Enumeration of denitrifying microbial densities were measured by the Most Probable Number (MPN) method similar to Tiedje (1982). Serum bottles of 25ml volume were filled with 15ml of nutrient+nitrate broth. The serum bottles were capped with a septum and sealed with aluminum rims. Serum bottles were then autoclaved at 120 °C for 15 minutes. Acetylene was then added to the serum bottles to give a final concentration of 10.1 KPa. Soil samples were collected in the field and forest at various vertical depths. Ten cubic centimeters of soil were mixed with 90ml of

deionized-distilled water and shaken vigorously for two minutes. Serial dilutions between 10^{-2} to 10^{-6} were then prepared from the previous dilution, with five replicates per dilution. Thus for each soil sample collected twenty-five bottles were incubated. Disposable tuberculin syringes were used to inject 0.15ml of the dilutions into the serum bottles. The serum bottles were incubated at 30°C for 2 weeks. After incubation the serum bottles were shaken to allow equilibration of N_2O between gas and aqueous phases, then 0.5 ml was withdrawn from the head space and analyzed on a Varion 3700 gas chromatograph. Positive bottles were those with $>20\%$ of the nitrogen in the form of NO_3 converted to N_2O. MPN values were calculated using an MPN table (Tiedje, 1982).

Standards were analyzed on the gas chromatograph after 70 serum bottle samples or once a day if less than 70 samples were analyzed. Standards of 10^{-6}, 10^{-8}, 10^{-10}, and 10^{-12} moles$\bullet$0.5 milliliters^{-1} were analyzed on the gas chromatograph with three replicates per standard. The gas chromatograph detection limit fluctuated around the 10^{-12} standard, as a result standards were analyzed after every 70 samples.

4.2.2 Denitrification Activity. Replicate (N=3) soil samples were taken at various depths in the vertical soil profile extending into the water table. Soil samples were collected with a hand auguring system and placed in 500ml sampling jars. A period of no more than a half hour elapsed between soil sample transportation from the field to the laboratory. Once in the lab, samples equilibrated for approximately one hour to the incubation temperature of 25°C. Soil slurry microcosms were made by mixing approximately 200g of soil and 50ml of one of three treatments. Trial experiments using different amendments were conducted to determine the best concentrations to use for these soils. The treatments consisted of deionized distilled water (no amendment), a 500 *u*mol$\bullet$liter^{-1} solution of NO_3-N, and a solution of 500 *u*mol$\bullet$liter^{-1} NO_3-N and 5000 *u*mol$\bullet$liter^{-1} of glucose. These treatments were chosen to determine whether nitrate and/or carbon was a limiting factor. Controls were treated with 50ml of 30% formaldehyde. Thus ten microcosms were used per sampling depth. The microcosms were then sealed in sampling jars that had two septa placed in the lid for sampling headspace. A small

vacuum was placed on the microcosm to reduce the amount of time needed to flush with nitrogen. The microcosm was then flushed with nitrogen for approximately five minutes. Acetylene was added, after removal of the same amount of headspace, to the microcosms to give a final concentration of 0.1 Atm. Acetylene was found to effectively inhibit the reduction of N_2O to N_2 when used in concentrations of 0.1 Atm or higher (Smith *et al*, 1978). The microcosms were shaken manually and incubated at 25°C for a 36 hour period. Gas samples were taken at 6, 12, 24 and 36 hours from the headspace of the microcosm. Microcosms were shaken manually after each sampling period. A gas sample volume of 4ml was placed in a 5ml vacutainer tube that contained 1ml of 2.5N NaOH. Excess CO_2, which can interfere with the GC analysis, was removed with the NaOH . Vacutainer tubes were then taken back to VPI & SU laboratory to be analyzed for N_2O (nitrous oxide). At the VPI & SU laboratory, 0.5ml was withdrawn from the vacutainer tubes and analyzed on a Varion 3700 gas chromatograph with an electron capture detector. Standards were analyzed in the same manner as previously mentioned for denitrifier density measurements. Calculated N_2O concentrations take into account the amount of N_2O dissolved in water by using the Bunsen absorption coefficient (Tiedje, 1982). Microcosms were then dried at 105°C to a constant weight. Denitrification activity was integrated over a 36 hour period and expressed as N2O production • gram dry weight^{-1} • hour^{-1}. Denitrification activity was integrated over a 36 hour period because this was the maximum rate produced for the treatments in this experiment.

4.2.3 Statistical Analysis. An ANOVA (analysis of variance) was used to determine statistically significant differences between sample sets. An alpha level of 0.05 ($\alpha = 0.05$) was chosen for statistical significance. The hypotheses tested were whether location, vertical depth, and/or treatment affected denitrification potentials. Thus samples sets compared were considered significantly different if the probability computed from the ANOVA was less than 0.05 ($p < 0.05$). In all cases data values were transformed by taking the logarithm of the values. Data values were transformed to increase the homogeneity of variances between samples being compared. This logarithmic

transformation allowed for the use of parametric statistics on all samples being compared. Bartlett's test for homogeneity of variances was used to compare sample set variances. Bartlett's test was used to assure that all sample sets had probabilities, of sample variances being equal, greater than 0.05 ($p > 0.05$).

4.3 Results

4.3.1 Denitrifier densities. All values measured for denitrifier densities are listed in Table 4.2. Denitrifier microbial densities were measured at the land surface, at the water table, and 0.91m below the water table in the agricultural field and the forest during August 8, 1992 and September 5, 1992. Samples where collected at vertical depths of 0.0 (surface), 2.4 (water table), and 3.3 meters (0.91 meters below water table) in the agricultural field. Samples in the forest (45.7m) where collected at vertical depths of 0.0 (surface), 0.8(water table), and 1.7 meters (0.91 meters below water table). Figure 4.1 shows the average for MPN (Most Probable Number of Organisms) values measured in September and October. Sampling emphasized the saturated soils in the agricultural field and forest. Samples collected at the land surface in the field (N=1) and forest (N=1) measured 35000 and 17000 per cm^3 of soil, respectively. Samples collected at the surface of the water table in the field (N=5) and forest (N=6) averaged 2850 $\pm$ 1553(SD) and 14,350 $\pm$ 13,369(SD) per cm^3 of soil, respectively. Samples collected 0.91 meters below the water table in the field (N=3) and in the forest (N=4) averaged 1343 $\pm$ 1036(SD) and 3922 $\pm$ 3919(SD) per cm^3 of soil, respectively. Samples collected in September and October, 1992 at the water table in the forest and field were significantly different (AVOVA, $p = 0.046$). Samples collected 0.91 meters below the water table in the field and forest during the same period were not significantly different (ANOVA, $p = 0.327$).

Soil samples were collected in December 1992 from greater depths in the water table aquifer from the forest. Three cores were taken from the surface to a vertical depth of 4.6 meters at 45.7 meters along transect 1 in the forest buffer. Previously the deepest soil samples collected in the forest were from a vertical depth of 1.82 meters, which was

0.91 meters below the water table. Denitrifier densities were measured at vertical depths of 0.15, 0.91, 1.82, 3.05, and 4.60 meters. The depth of the water table during this field exercise was 0.15 meters below the land surface. Denitrifier density (MPN's) results are shown in Figure 4.2. Denitrifier densities at the water table averaged 377,000 $\pm$ 152,000(SD) (N=3), during this sampling exercise. A regression between denitrifier density and vertical depth can be seen in Figure 4.2. A strong correlation existed between denitrifier density and vertical depth (r^2=0.8984).

In sum denitrifier densities measured in saturated soils were highest at the water table. The highest denitrifier densities measured were from December, 1992 where the water table was 0.15 meters from the surface. Denitrifier densities at 0.8 meters vertical depth in the forest were significantly different from denitrifier densities at the water table in the agricultural field. Denitrifier densities measured at 1.7 meters in the forest were not significantly different from denitrifier densities measured in the field at the water table.

4.3.2 Denitrification activity. All denitrification activities measured are listed in Table 4.3. Denitrification activity was measured in both the agricultural field and forest during December (1991), February (1992), and March (1992) at the land surface, at the water table, and 0.91 meters below the water table. Denitrification activity was also measured in the forest during the months of May (1992), and December (1992). Table 4.1 lists the location, with respect to distance into the forest, date, and vertical depth at which samples were collected.

Comparison of denitrification activity in the field and forest is shown for December (1991) in Figures 4.3, 4.4, and 4.5, for February (1992) in Figures 4.6, 4.7, and 4.8, and for March (1992) in Figures 4.9, 4.10, and 4.11. Two-way ANOVA (analysis of variance) probability values for comparison of denitrification activity between the agricultural field and forest are listed in Tables 4.2, 4.3, and 4.4 for December (1991), February (1992) and March (1992), respectively. During all three months denitrification activity at the water table in the forest were significantly higher than

denitrification activity at the water table in the field. Denitrification activity was also significantly higher at 0.91 meters below the water table in the forest as compared to the field during the month of March.

Comparison of denitrification activity within the field is shown in Figures 4.16, 4.17, and 4.18 for the months of December (1991), February (1992), and March (1992), respectively. ANOVA probabilities for denitrification activity with respect to vertical depth and treatment are listed in Table 4.5 and 4.6. Denitrification potentials in the agricultural field decreased significantly as vertical depth increased. Soils in the field at the water table, or 0.91 meters below the water table, did not respond significantly to treatments of nitrate and nitrate+glucose. Soils in the field at the surface did respond significantly to treatments of nitrate and nitrate+glucose except for the month of March.

Comparison of denitrification activity within the forest are shown in Figures 4.12, 4.13, 4.14, 4.15, and 4.19 for the months of December (1991), February (1992), March (1992), May (1992) and December (1992), respectively. Denitrification activity treatments at the water table in the forest were significantly different from one another at all samplings (Table 4.6). At the water table, treatments with nitrate were significantly different from treatments with no amendment, but were not significantly different from treatments with nitrate+glucose for December (1991) ($p=0.001$ and $p=0.475$, respectively), February (1992) ($p=0.027$ and $p=0.109$, respectively), March (1992) ($p=0.004$ and $p=0.883$, respectively), and May (1992) ($p=0.023$ and $p=0.082$, respectively). At 0.91 meters below the water table in the forest, denitrification activity treatments were significantly different from one another during the month of March (1992). March (1992), treatments with nitrate were significantly different from treatments with no amendment and were also significantly different from treatments with nitrate+glucose ($p=0.002$, $p=0.05$ respectively). Denitrification activity measured in the month of December (1992) was also significantly affected by increasing vertical depth for microcosms with no amendments ($p=0.002$) and potentials with nitrate amendment ($p=0.014$).

4.4 Discussion

The biologically active zone, with respect to denitrification, in the forest extended to a vertical depth of at least 0.8 meters, but less than 1.7 meters. Denitrifier densities at the water table in the forest were significantly different from those at the water table in the agricultural field. Though denitrifiers were present in the water table in the agricultural field and at greater vertical depths in the forest, these communities were limited extensively by carbon. Moreover dissolved oxygen concentrations were much higher in the water table in the field and at deeper vertical depths in the forest. Denitrification occurring below the biologically active zone would be limited in comparison to denitrification occurring in the biologically active zone.

All denitrification activity measurements decreased with increasing vertical depth, whether in the field or in the forest. Denitrification activity in the forest at the water table was always greater than denitrification activity measured at the corresponding water table location in the agricultural field. This due to the location of the water table in the forest in the A horizon and the water table in the field in the C horizon. All denitrification activity measured at the water table in the forest was nitrate limited, with no significant carbon limitation present. At 0.91 meters below the water table some carbon limitation was present during the month of March (1992) at 91.4 meters along the transect. During March (1992), the 0.91 meter sampling occurred at 1.30 meter vertical depth, the shallowest of all the 0.91 meter samplings. The other samplings did not demonstrate significant carbon limitations, most likely due to the lower microbial densities at greater vertical depths which resulted in a less of a response to the treatments. Denitrification activity measured in December (1992) to depths of 4.60 meters did not reveal any higher microbial activity at deeper vertical depths.

These denitrification results coincided with the soil characteristics and groundwater quality, in which the majority of denitrification was confined to the root zone. The root zone in the forest soil, Nimmo sandy loam, extended to a vertical depth of 1.52 meters (Cobb and Smith, 1989). Denitrification did not occur at the water table in the field or

from deeper soils in the forest due to higher oxygen concentrations. The denitrification potentials and denitrifier densities measured, would suggest the biologically active zone in the forest was approximately 1.3 meters deep. Thus the biologically active zone with respect to denitrification was confined to the root zone.

Table 4.1 Location and sampling date for denitrification activity and denitrifier densities in the field and forest.

Location		Dec 1991	Feb 1992	Mar 1992	May 1992	Sept 1992	Oct 1992	Dec 1992
Field	Denitrification activity	●	●	●				
	Denitrifier density					●	●	
Forest	Transect location	45.7m	61.0m	91.4m	15.2m	61.0m	61.0m	61.0m
	Denitrification activity	●	●	●				●
	Denitrifier density					●	●	●

Table 4.2 Enumeration of denitrifiers in the vertical soil profile in the agricultural field and the forest (45.7m).

	Field		Forest (45.7m)	
Date	**Depth (meters)**	**MPN**	**Depth (meters)**	**MPN**
8/92-9/92	0.0	35,000 (N=1)	0.0	17,000 (N=1)
	2.4	2850 ± 1553 (N=5)	0.8	14,350 ± 13,369 (N=6)
	3.3	1343 ± 1036 (N=3)	1.7	3922 ± 3919 (N=4)
9/92	----	----------	0.15	377,000 ± 152,000 (N=3)
	----	----------	0.91	2200 ± 0 (N=2)
	----	----------	1.83	360 ± 184 (N=2)
	----	----------	3.05	1550 ± 1202 (N=2)
	----	----------	4.57	230 ± 0 (N=2)

Table 4.3 Denitrification activity ($nmol \bullet g^{-1} \bullet hr^{-1}$) sampling date, location, and vertical depth.

	Field (Avg±Std, N=3)				Forest (Avg±Std, N=3)		
Date	Depth (meters)	no amendment	w/nitrate	w/nitrate+gluc	Depth (meters)	no amendment	w/nitrate	w/nitrate+gluc
12/91							Forest 45.7m	
	0.00	291.8 ± 325.7	128.3 ± 98.6	411.7 ± 218.3	0.00	0.349 ± 0.233	53.3 ± 20.4	55.0 ± 20.4
	2.59	0.124 ± 0.071	0.0744 ± 0.023	0.150 ± 0.123	0.76	0.0581 ± 0.008	7.37 ± 8.26	6.88 ± 8.67
	3.35	1.13 ± 1.75	1.15 ± 1.06	1.033 ± 1.54	1.60	1.51 ± 2.01	2.34 ± 2.91	10.9 ± 7.89
2/92							Forest 61.0m	
	0.00	19.3 ± 10.6	46.9 ± 72.5	74.5 ± 54.6	0.00	20.8 ± 32.0	14.5 ± 21.5	39.1 ± 20.5
	2.10	0.078 ± 0.011	0.105 ± 0.026	0.078 ± 0.014	0.61	0.083 ± 0.073	4.49 ± 5.96	24.2 ± 17.0
	3.05	0.072 ± 0.008	0.154 ± 0.095	0.087 ± 0.047	1.52	0.14 ± 0.064	0.104 ± 0.009	0.211 ± 0.159
3/92							Forest 91.4m	
	0.00	0.097 ± 0.011	19.1 ± 1.50	14.6 ± 3.78	0.00	0.0097 ± 0.0028	16.2 ± 2.44	11.4 ± 3.80
	2.29	0.0038 ± 0.0031	0.0023 ± 0.0019	0.0028 ± 0.0006	0.41	0.0018 ± 0.0003	0.101 ± 0.089	0.182 ± 0.240
	3.20	0.0004 ± 0.0001	0.0004 ± 0.0004	0.0049 ± 0.0004	1.30	0.0012 ± 0.0003	0.024 ± 0.034	0.531 ± 0.693
5/92							Forest 15.2m	
		----------	----------	----------	0.00	0.143 ± 0.137	11.2 ± 2.40	6.44 ± 5.63
		----------	----------	----------	0.93	1.45 ± 1.44	0.940 ± 0.550	0.115 ± 0.198
		----------	----------	----------	1.70	0.0081 ± 0.0065	0.0058 ± 0.0046	0.160 ± 0.078
12/92							Forest 61.0m	
		----------	----------	----------	0.15	0.0085 ± 0.0093	4.123 ± 2.80	2.773 ± 0.758
		----------	----------	----------	0.91	0.232 ± 0.311	0.931 ± 0.201	2.426 ± 1.241
		----------	----------	----------	1.82	0.503 ± 0.465	0.549 ± 0.193	0.505 ± 0.238
		----------	----------	----------	3.05	0.501 ± 0.0685	0.353 ± 0.166	1.179 ± 0.741
		----------	----------	----------	4.60	0.431 ± 0.177	0.928 ± 0.394	1.883 ± 1.154

Date	Field Controls (30% formaldehyde) (Avg±Std, N=3)	Forest Controls (30% Formaldehyde) (Avg±Std, N=3)
12/91	0.006 ± 0.003	0.006 ± 0.002
2/92	0.0004 ± 0.00005	0.0003 ± 0.0001
3/92	0.0003 ± 0.0002	0.0004 ± 0.0004
5/92	----------	0.0003 ± 0.0002

Table 4.4 ANOVA probability values between the field and forest for December, 1991.

H_o : Location (field and forest) did not affect denitrification activity.

H_o : Denitrification activity was not affected by treatments of no amendment, nitrate, or nitrate+ glucose.

H_o : Denitrification activity was not affected by an interaction of location (field or forest) and treatment.

Field vs Forest	Probability
Surface	
Location	<0.001
Treatment	<0.001
Location•Treatment	<0.001
Water Table	
Location	0.002
Treatment	<0.001
Location•Treatment	<0.001
0.91 meters Below Water Table	
Location	0.013
Treatment	0.366
Location•Treatment	0.597

Table 4.5 ANOVA probability values between the field and forest for February, 1992.

H_o : Location (field or forest) did not affect denitrification activity.

H_o : Denitrification activity was not affected by treatments of no amendment, nitrate, or nitrate+glucose.

H_o : Denitrification activity was not affected by an interaction of location (field or forest) and treatment.

Field vs Forest	Probability
Surface	
Location	0.258
Treatment	0.164
Location•Treatment	0.893
Water Table	
Location	<0.001
Treatment	<0.001
Location•Treatment	<0.001
0.91 meters Below Water Table	
Location	0.142
Treatment	0.747
Location•Treatment	0.190

Table 4.6 ANOVA probability values between the field and forest for March, 1991.

H_o : Location (field or forest) did not affect denitrification activity.

H_o : Denitrification activity was not affected by treatments of no amendment, nitrate, or nitrate+ glucose.

H_o : Denitrification activity were not affected by an interaction of location (field or forest) and treatment.

Field vs Forest	Probability
Surface	
Location	<0.001
Treatment	<0.001
Location●Treatment	<0.001
Water Table	
Location	<0.001
Treatment	0.007
Location●Treatment	0.003
0.91 meters Below Water Table	
Location	<0.001
Treatment	<0.001
Location●Treatment	0.001

Table 4.7 ANOVA probability values within the field and forest for December (1991), February (1992), March (1992), and May (1992).

H_o : Denitrification activity being compared within the field or forest was not affected by depth.

Date	Field	Forest
December,1991	<0.001	<0.001
February, 1992	<0.001	<0.001
March, 1992	<0.001	<0.001
May, 1992		0.092

Table 4.8 ANOVA probability values within the field and forest for December(1991), February(1992), March(1992), and May (1992).

H_o : Denitrification activity being compared within the field or forest was not affected by treatment.

	Field Treatment	Forest Treatment
December 1991		
Surface	0.273	<0.001
Water Table	0.551	<0.001
0.91m Bel WT	0.640	0.202
February 1992		
Surface	0.505	0.328
Water Table	0.203	0.003
0.91m Bel WT	0.299	0.509
March 1992		
Surface	<0.001	<0.001
Water Table	0.620	0.008
0.91m Bel WT	0.555	0.004
May 1992		
Surface		0.199
Water Table		0.022
0.91m Bel WT		0.235

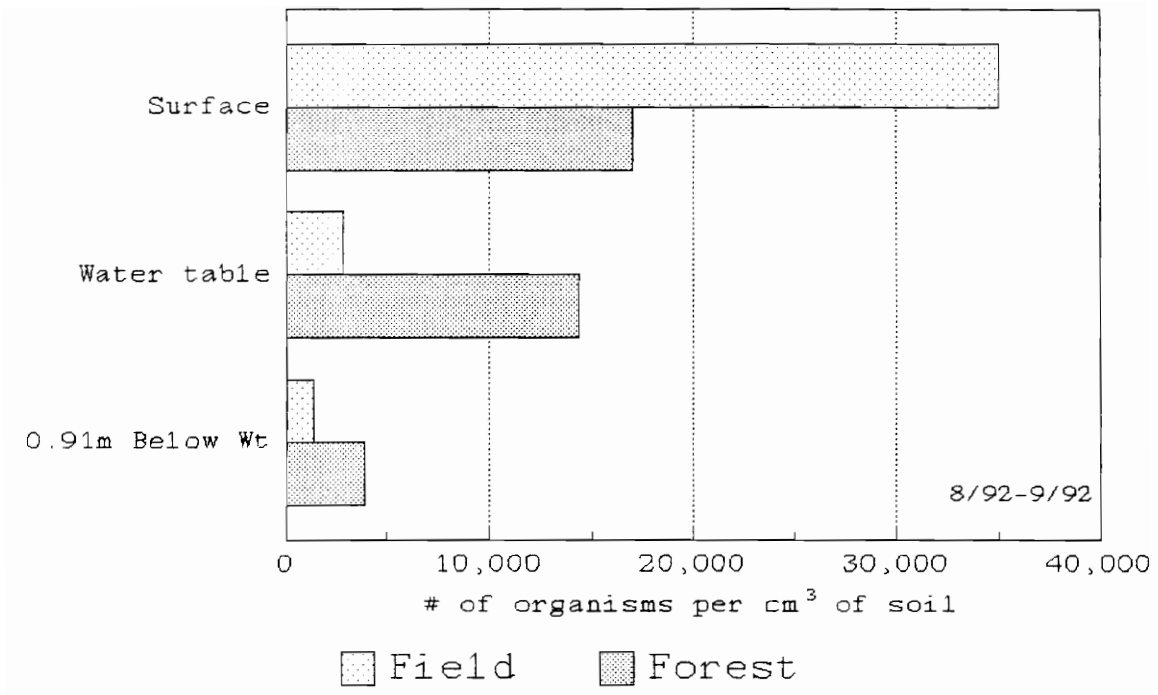

Figure 4.1 Denitrifier density vs vertical depth in the field and forest(45.7m).

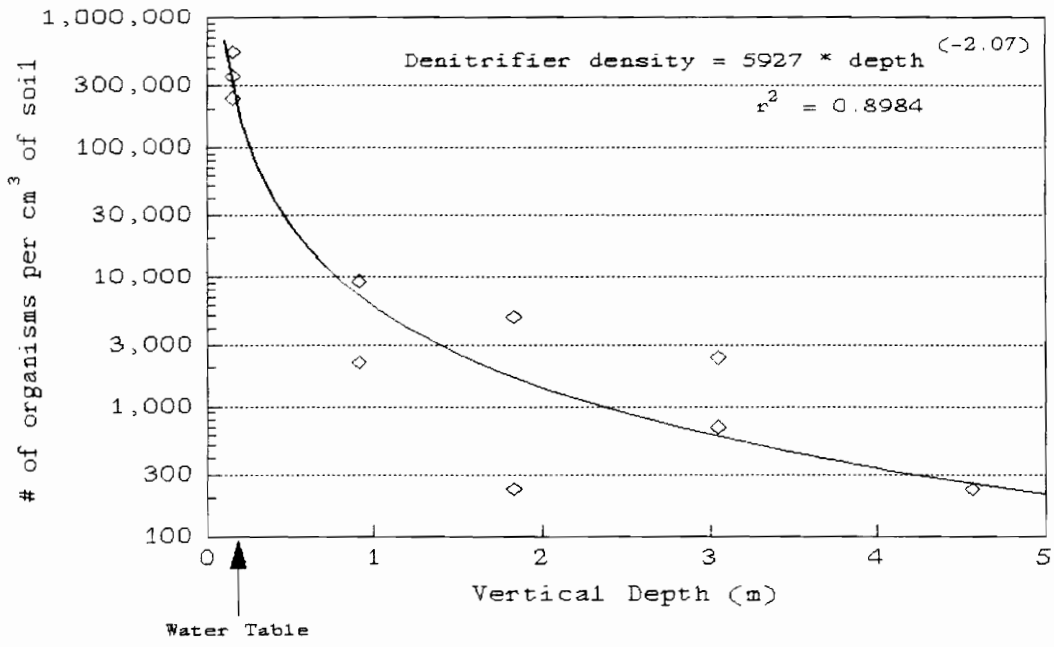

Figure 4.2 Denitrifier density vs vertical depth in the forest at 45.7m along the transect.

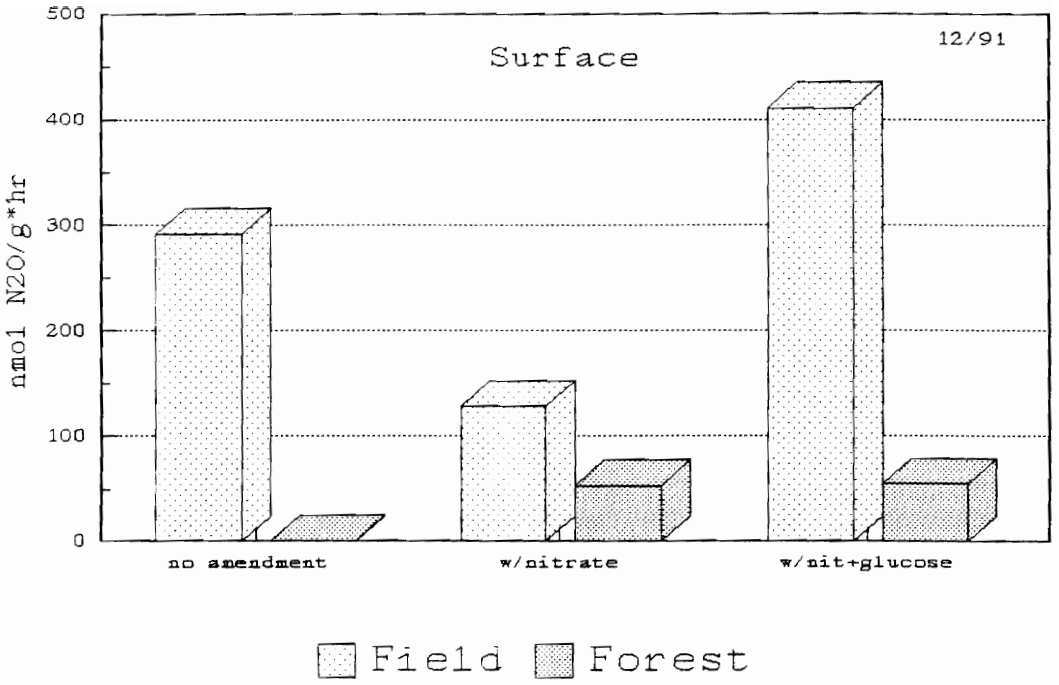

Figure 4.3 Denitrification activity from the surface of the field and forest(45.7m) during December, 1991.

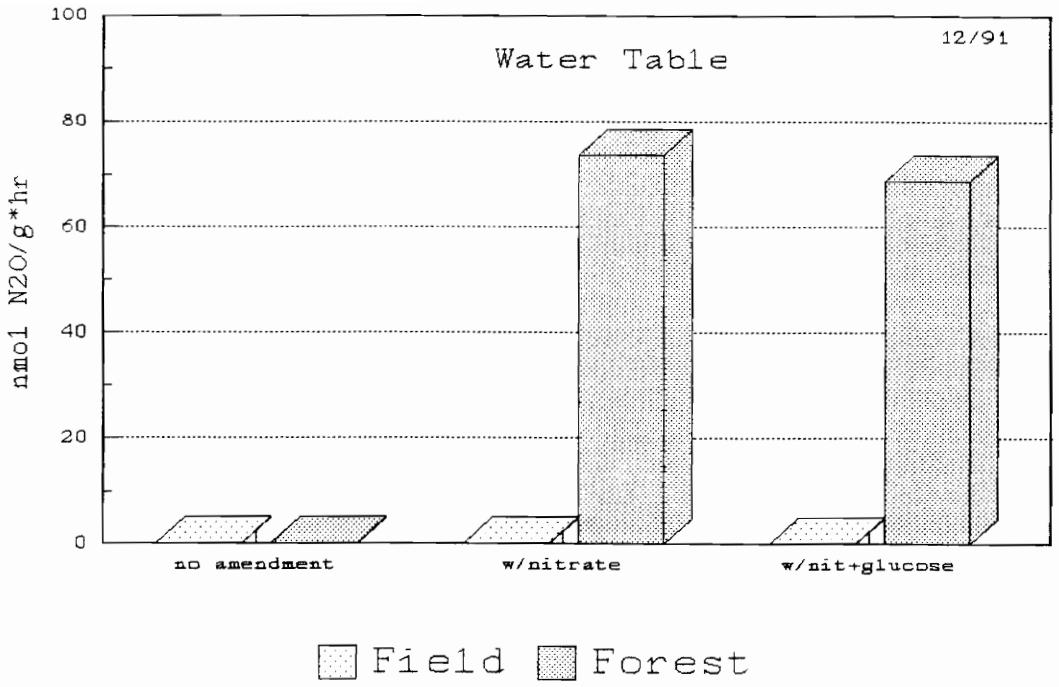

Figure 4.4 Denitrification activity from the water table in the field and forest(45.7m) during December, 1991.

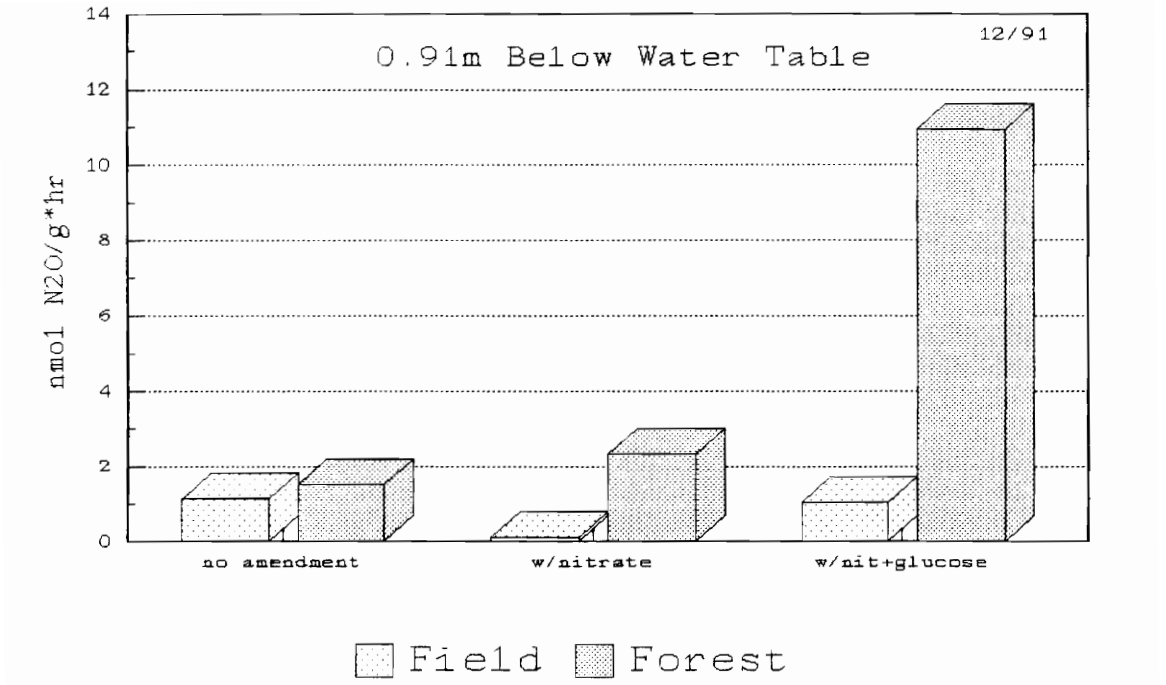

Figure 4.5 Denitrification activity from 0.91m below the water table in the field and forest(45.7m) during December, 1991.

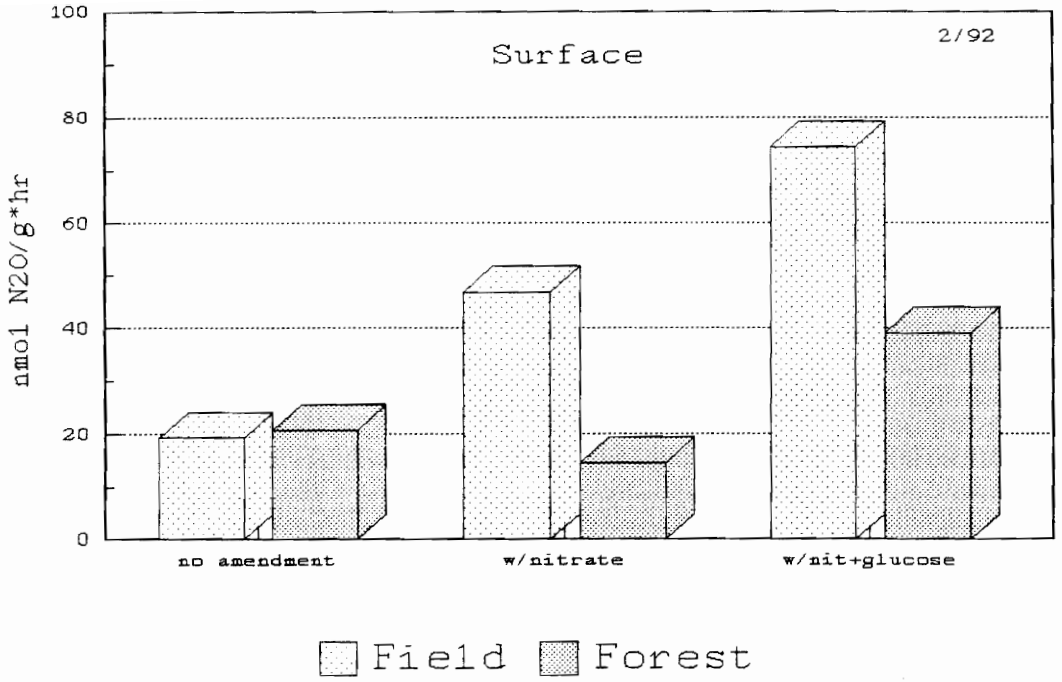

Figure 4.6 Denitrification activity from the surface of the field and forest(61.0m) during February, 1992.

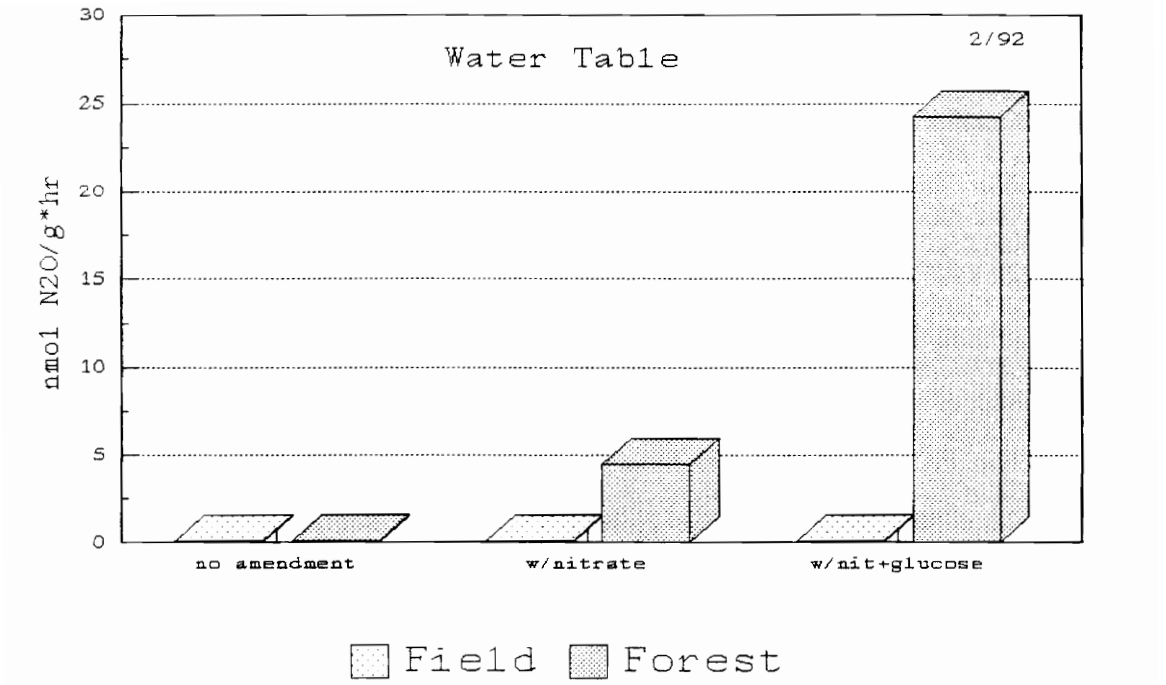

Figure 4.7 Denitrification activity from the water table in the field and forest(61.0m) during February, 1992.

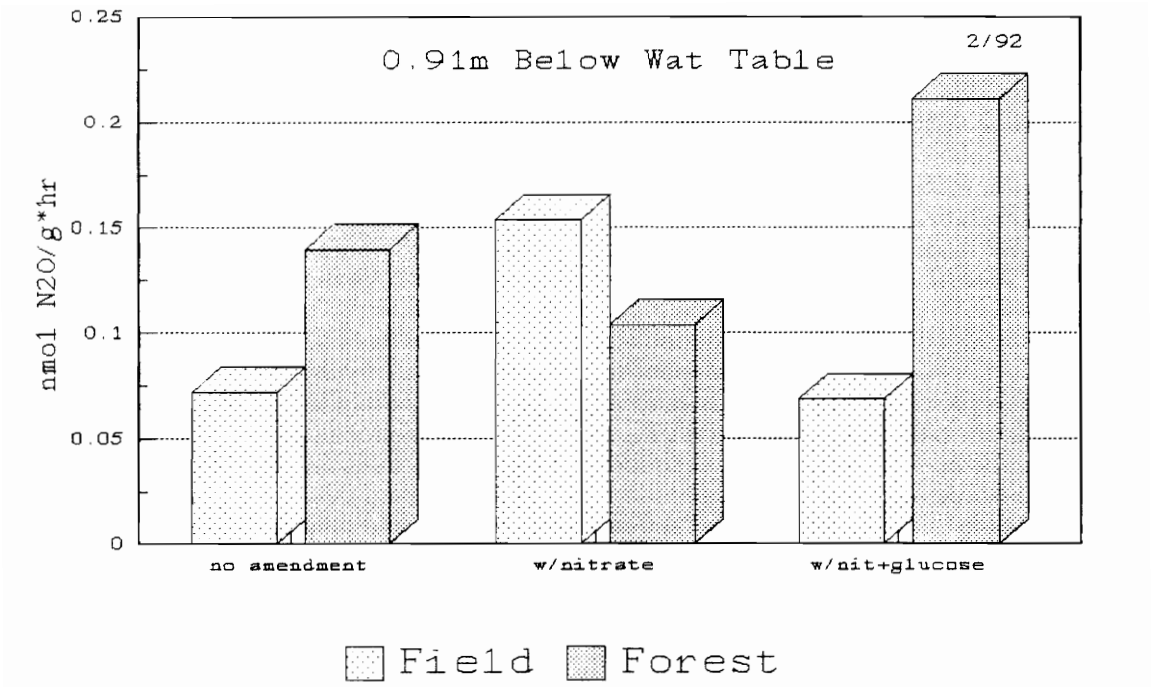

Figure 4.8 Denitrification activity from 0.91m below the water table in the field and forest(61.0m) during February, 1992.

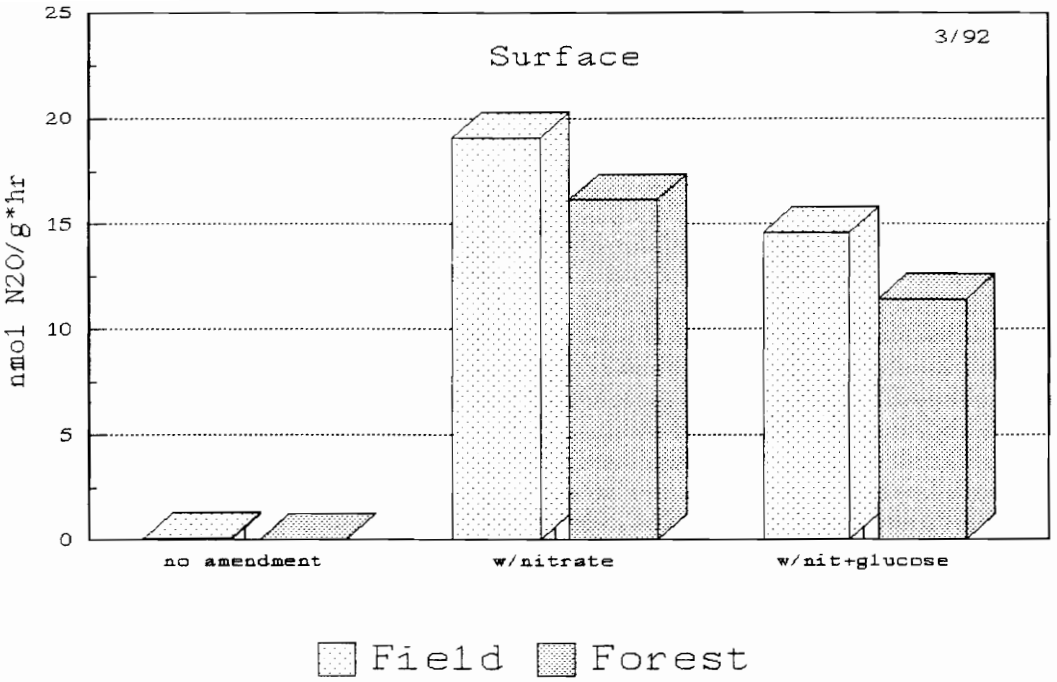

Figure 4.9 Denitrification activity at the surface from the field and forest(91.4m) during March, 1992.

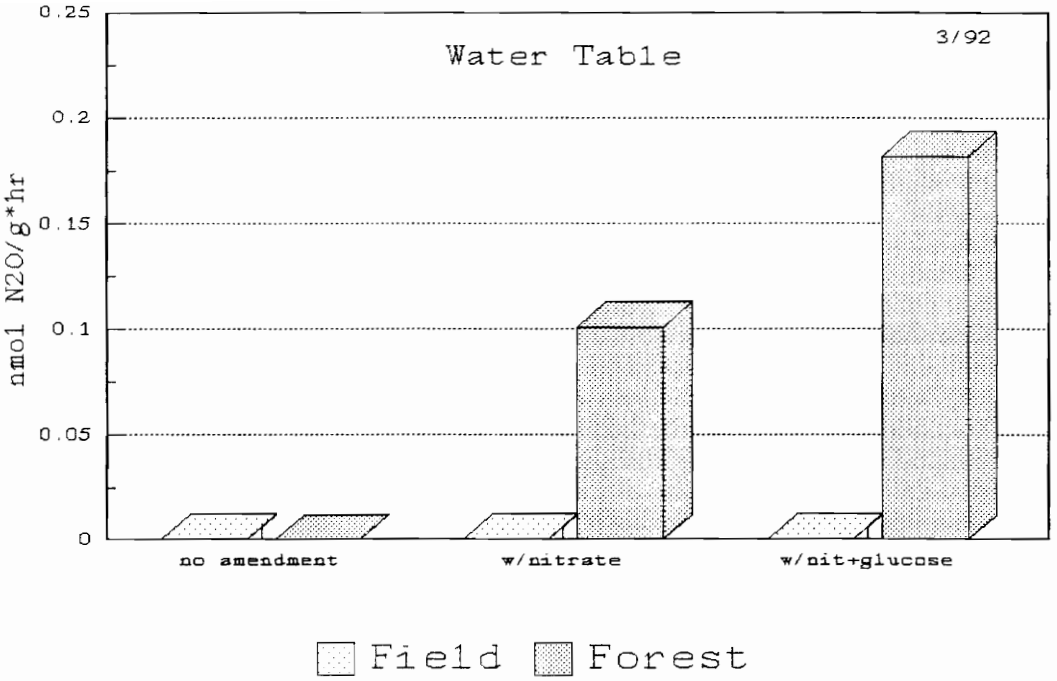

Figure 4.10 Denitrification activity from the water table in the field and forest(91.4m) during March, 1992.

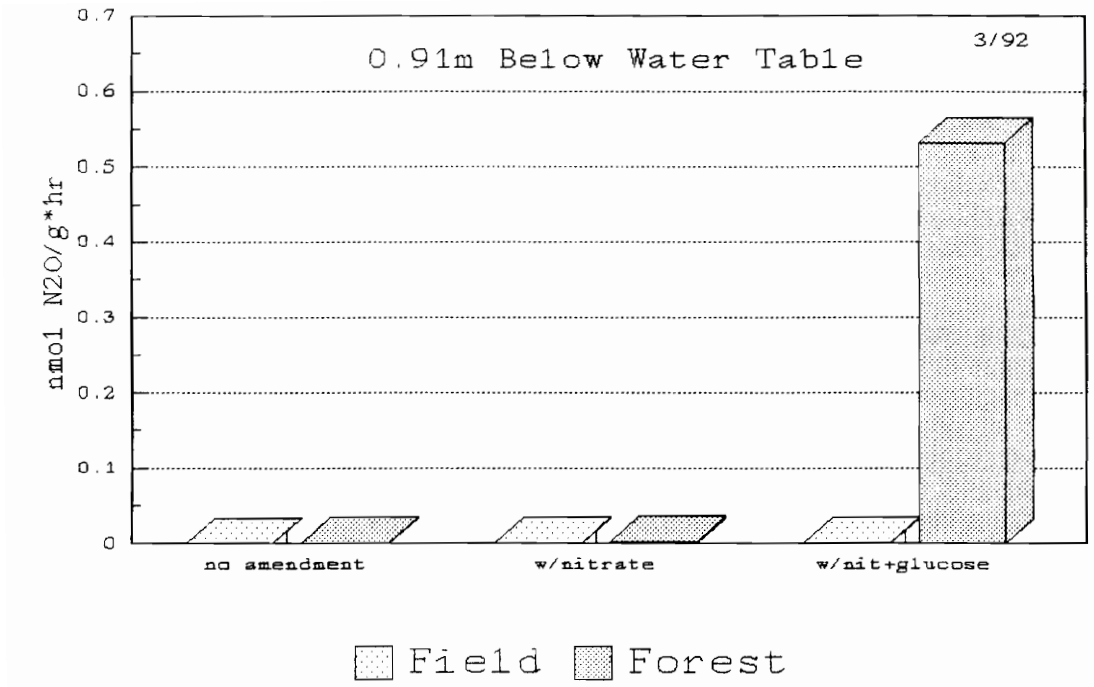

Figure 4.11 Denitrification activity from 0.91m below the water table in the field and forest(91.4m) during March, 1992.

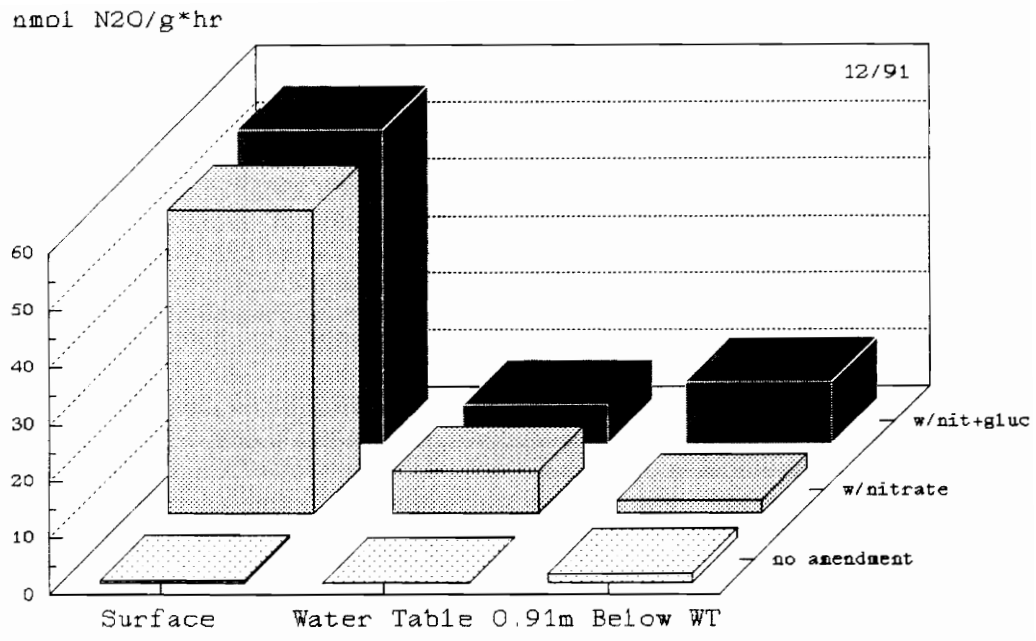

Figure 4.12 Denitrification activity vs vertical depth in the forest(45.7m) during December, 1992.

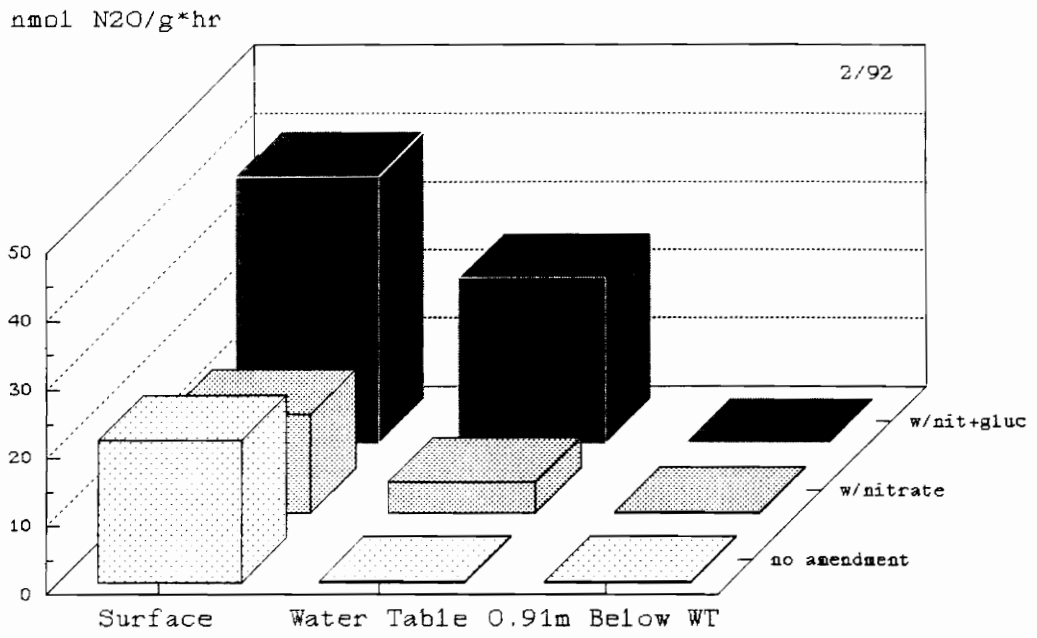

Figure 4.13 Denitrification activity vs vertical depth from the forest(61.0m) during February, 1992.

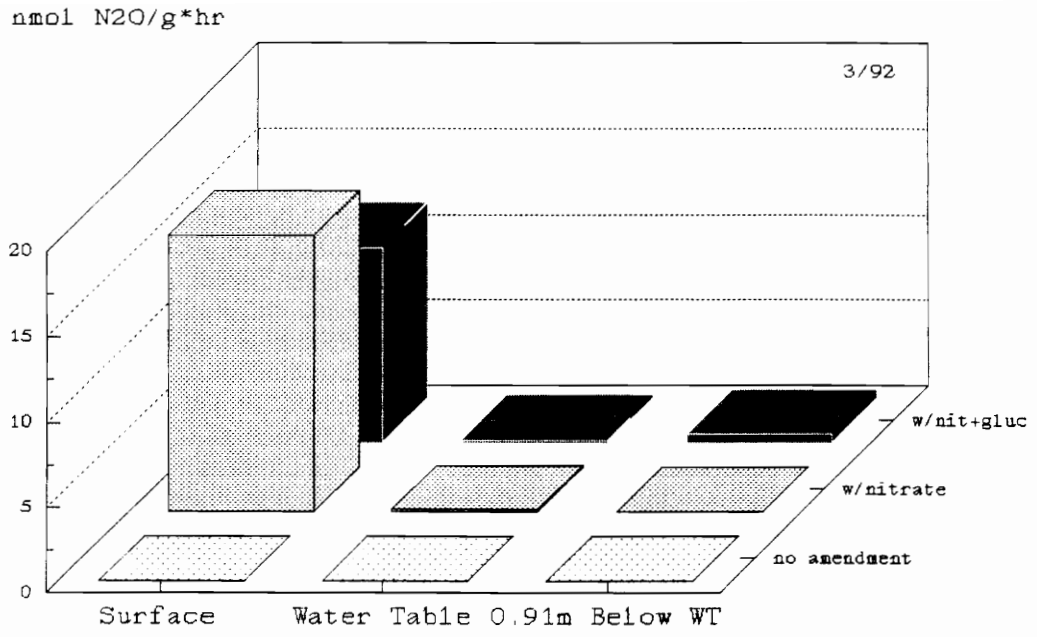

Figure 4.14 Denitrification activity vs vertical depth from the forest(91.4m) during March, 1992.

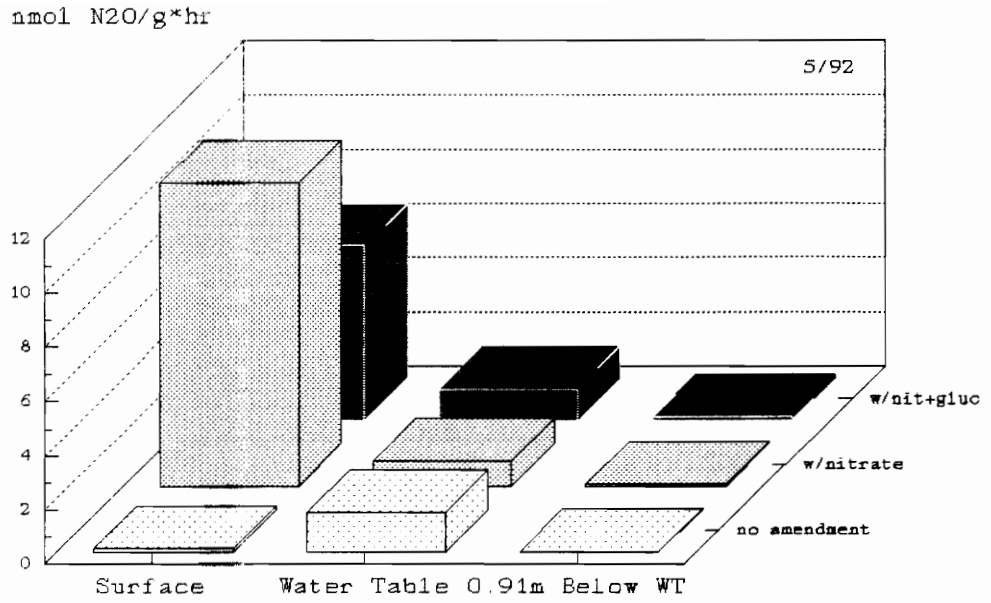

Figure 4.15 Denitrification activity vs vertical depth from the forest (15.2m) during May, 1992.

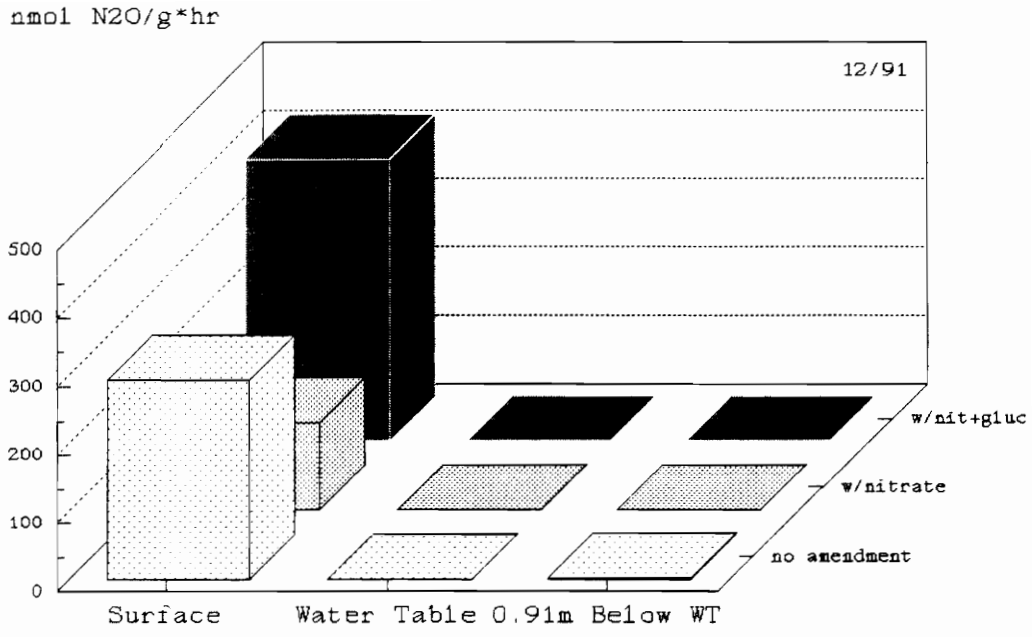

Figure 4.16 Denitrification activity vs vertical depth from the field during December, 1992.

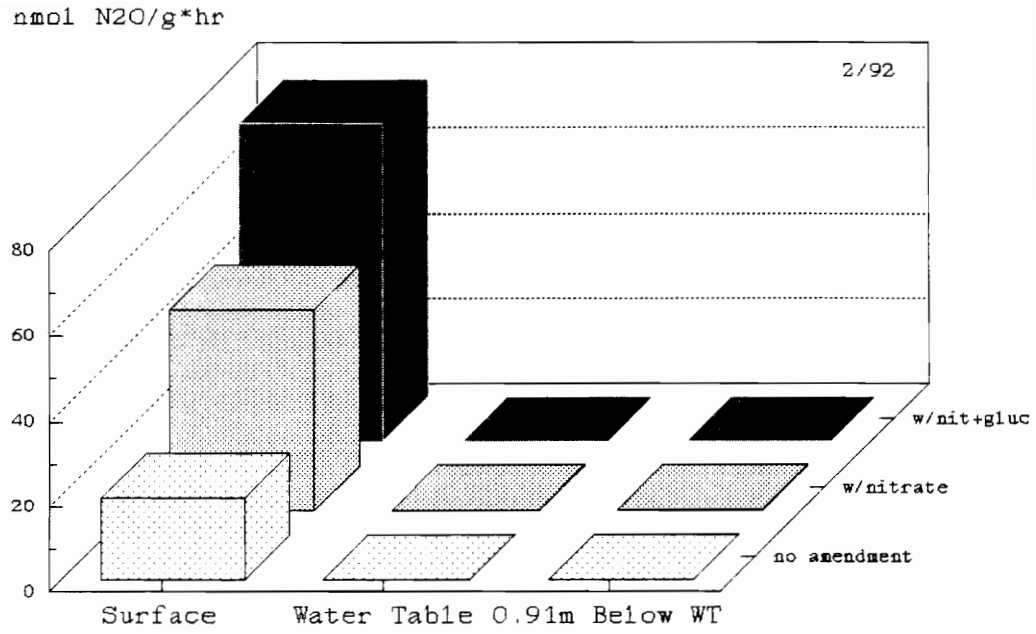

Figure 4.17 Denitrification activity vs vertical depth from the field during February, 1992.

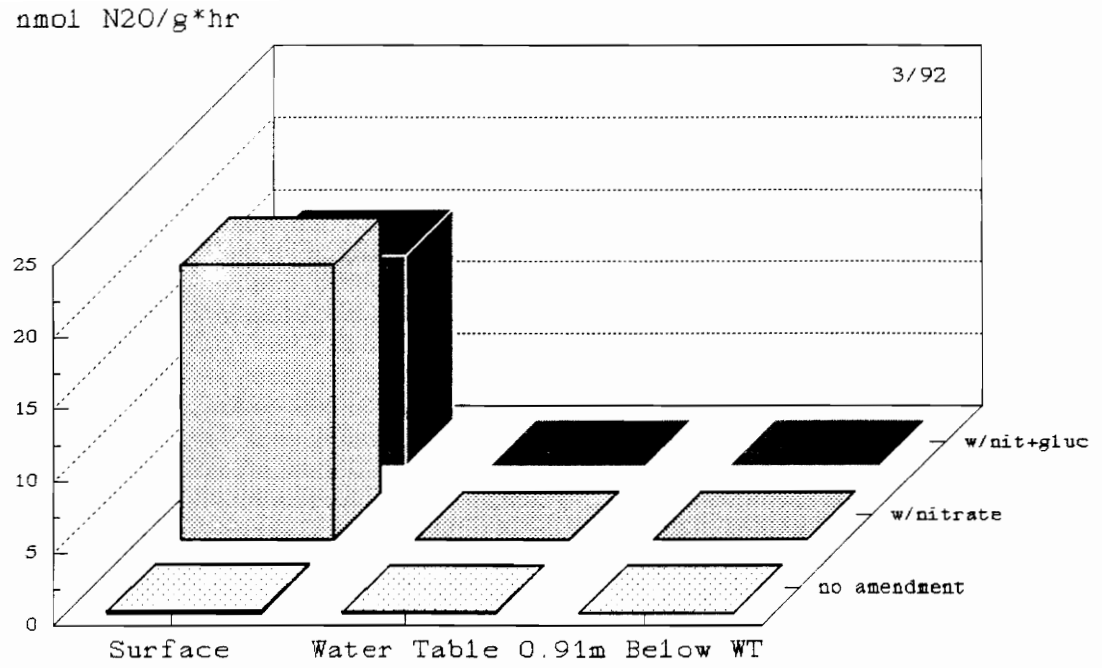

Figure 4.18 Denitrification activity vs vertical depth from the field during March, 1992.

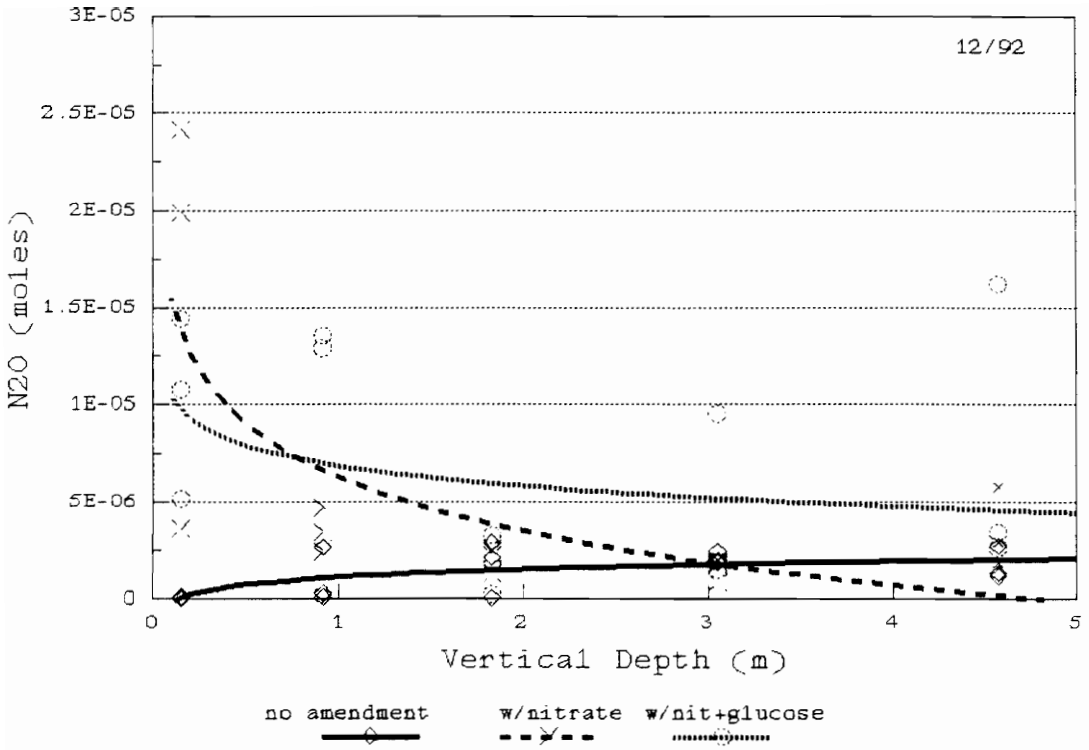

Figure 4.19 Denitrification vs vertical depth (meters) at 45.7 meters into the forest. Denitrification activity in moles of N2O produced over a 36 hour period.

5. DISCUSSION

The effectiveness of a mature coastal mesic forest to reduce nitrogen coming from agricultural subsurface drainage was limited to the upper regions of the vertical surface profile. Ground water flowed from a well drained agricultural field of Bojac sandy loam (coarse-loamy, mixed, thermic Typic Hapludults) and Munden sandy loam (coarse-loamy, mixed, thermic Aquic Hapludults) to a poorly drained forest soil, Nimmo sandy loam (coarse-loamy, mixed, Typic Ochraquults). The water table in the forest was relatively shallow, 0.1-1.1 meters, in contrast to the field were the water table was much deeper, 1.9-2.5 meters. As a result, the soil characteristics of the water table in the forest differed from soil characteristics at the water table in the agricultural field. The forest had approximately three times the amount of organic matter as the field at the water table.

Nitrate concentrations and dissolved oxygen concentrations decreased significantly along the transect into the forest in the shallow water table. Nitrate concentrations and dissolved oxygen concentrations in the forest were higher at deeper vertical depths as compared to in the shallow water table. The groundwater coming from the agricultural field intersected the biological active community of the forest as determined from the measurement of vertical and horizontal hydraulic gradients. Physical dilution was presumed to be a minor factor due to the counter effects of evapotranspiration. As a result plant uptake and denitrification were presumed to be the dominant factors responsible for the reduction of nitrate in the shallow water table.

All the conditions necessary for dentrification to occur were present in the shallow water table, except for nitrate. Dissolved oxygen concentrations were low, approximately 2-3 $mg \bullet liter^{-1}$ and organic carbon was available from decomposing organic matter and exudation from roots. Deeper in the unconfined aquifer, conditions were not as optimal. At greater vertical depths in the forest dissolved oxygen concentrations were higher and organic carbon was less available. Denitrifier densities measured in the forest at the water table, a vertical depth of 0.8 meters, were significantly higher than those measured in the

agricultural field at the water table. At a vertical depth of 1.7 meters, in both the agricultural field and forest, where soil conditions were very similar, denitrifier densities were not significantly different. Denitrification potentials measured in the forest at the water table and 0.91 meters below the water table were significantly greater than those measured at the corresponding depths in the agricultural field. Denitrification potentials in the forest at the water table, vertical depth of 0.41-0.93 meters, were limited by nitrate. At 0.91 meters below the water table, vertical depth of 1.3 meters, some carbon limitation was found. The vertical depth to which the biologically active zone extends, with respect to denitrification, was between 0.93 and 1.3 meters.

The denitrification activity measured in surface soils of this study corresponded with denitrification activity measured in surface soils of other studies (Table 5.1). Two studies most closely resembling this study were Ambus and Lowrance (1991) and Lowrance (1992). Both of these studies were conducted in riparian forests in the Gulf-Atlantic Coastal Plain. The geologic characteristics from those two studies and this study were very similar in that all were sandy loam soils. The plant communities were also very similar in all the studies (i.e. *Pinus taeda*, *Nyssa sylvatica* Marshall). Ambus and Lowrance(1991) and Lowrance(1992) only measured denitrification potentials to the water table, they did not extend below the water table. Lowrance(1992) did find, however, that denitrification potentials were the highest when the water table was within 0.6 meters of the surface.

This study, along with previous studies, enforces the importance of vegetative filters and the root zones that create suitable environments for nitrogen reduction in shallow groundwater.

Table 5.1 Comparison of denitrification activity from surface soils of other studies.

Denitrification activity (nmol $N_2O \bullet g^{-1} \bullet hr$)

Soil type	Denitrification potential	Reference
VA Agricultural (sandy loam)	104*	Smedley, 1993
VA Coastal forest (sandy loam)	5.3*	Smedley, 1993
N. Carolina Hardwood (sandy loam)	3.0	Tiedje *et al* 1982
Intertidal sediment	0.05-2.0	Tiedje *et al* 1982
Michigan muck (agricultural)	40	Smith & Tiedje 1979

* Averages over period of study.

6. SUMMARY

Nonpoint sources of nitrogen entering the Chesapeake Bay and it's tidal tributaries are substantial. Controlling nonpoint sources is quite difficult due to the to the broad and random nature of nonpoint source locations. Vegetative forest buffers have been used in the past as BMP's for nutrient reduction in surface drainage and for erosion prevention. Previous research has shown that two major land use practices, agricultural and urban, can contaminate groundwater with significant amounts of nitrogen. This nitrogen in the groundwater can enter the adjacent surface body of water through unbuffered zones with little interference. Along riparian zones vegetative forest buffers have the potential to intersect contaminated groundwater as it moves toward the land/water interface.

The importance of microbially mediated processes such as denitrification was examined in this study. Denitrifier densities and denitrification activity was measured at various vertical depths in an agricultural field and the adjacent coastal mesic forest. The biologically active zone under the forest comprising the root zone and the microbial activity of the root zone was the zone were nutrient reduction occurred. From the measurements made in this study the biologically active zone, with respect to denitrification, under the forest was between 0.9 and 1.3 meters. Below this depth denitrifier densities were not significant and conditions were not conducive to denitrification. The root zone in the forest extended to a vertical depth of at least 1.52 meters.

Vegetative forest buffers can be beneficial in reducing nitrogen in shallow groundwater. When contaminated groundwater is moving vertically through vegetative buffers, as in nearshore environments, the reduction of nitrogen is greatest. Vertical transport is a characteristic feature of submarine groundwater discharge in nearshore environments. With the amount of nitrate contaminated groundwater in the Chesapeake Bay watershed and the projected population increases to coastal regions, the use of vegetative buffers will be crucial to controlling nonpoint source nitrogen loadings from

groundwater. The integration of man and nature along our coastlines in a manner that is beneficial to both is not impossible as long as the common good out weighs individual desires.

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