Denitrification below the Crop Rooting Zone as Influenced by Surface Tillage

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ABSTRACT

In recent years, there has been increased concern over pollution of groundwater by NO₃⁻, yet little is known about microbial N transformations below the crop rooting zone. This study investigated the importance of microbial denitrification below the crop rooting zone of conventional and no-till corn plots. The soil was a well-drained Matapeake silt loam located in the Atlantic Coastal Plain. Plots were sampled in 30- and 60-cm depth increments down to the water table, which occurred at ca. 420 cm. Total viable bacteria and numbers of denitrifying bacteria decreased exponentially with increasing soil depth down to 150 cm. From 180 cm to the top of the water table total bacterial numbers were very low (< 100 organisms/gram). No CO₂ production activity was observed below the 180-cm depth in unamended soil incubated aerobically at 25 °C for 24 h. Anaerobic incubations with added NO₃⁻ and glucose showed no denitrifying activity below the 180-cm depth. These results indicate that C levels below the rooting zone in this well-drained, low organic matter soil are too low to support anaerobic conditions necessary for denitrification or to sustain a microbial population. Therefore, denitrification is not a mechanism of significant NO₃⁻ loss below the crop rooting zone at this location. Surface tillage practices had little influence on microbial activity below the root-zone.

NITRATE ENRICHMENT of groundwater resulting from agricultural practices is a topic of increasing concern, yet microbial N transformations below the crop rooting zone are poorly understood. Biological denitrification of NO₃⁻ leached below the rooting zone may be a process that ameliorates groundwater contamination. Indirect evidence that subsoil denitrification may be an important process in the lower coastal plain soils of North Carolina has been provided by Gilliam et al. (1974). This early evidence was corroborated by later work in a poorly drained soil in the lower coastal plain, which documented decreased NO₃⁻/Cl⁻ ratios with increasing soil depth; indicating that denitrification was an important process in reducing NO₃⁻ loading to the groundwater (Gambrell et al., 1975). Similar declines in NO₃⁻/Cl⁻ ratios with depth were observed by Gast et al. (1974) for a Webster soil (fine, loamy, mixed, mesic Typic Hapludult) in Minnesota. However, subsequent N balance work suggested that denitrification was of minor importance in removing fertilizer N (Gast et al., 1978).

Although indirect evidence suggests that denitrification below the rooting zone may be an important process in some systems, there have been few direct measurements of denitrifier numbers or denitrification activity below the crop rooting zone. In a study of the vadose zone above the Chalk aquifer in England, it was found that NO₃⁻ reducing bacteria were present at high levels down to 10 m at an unfertilized grassland site (Whitelaw and Rees, 1980). Balance sheet calculations of N and S in a European aquifer have indicated that the aquifer is a NO₃⁻ sink and that Thiobacillus denitrificans used reduced S compounds in the denitrification of NO₃⁻ (Kolle et al., 1986). Denitrifying bacteria have also been isolated from limestone material obtained from two drilling sites in England and from this information, along with data on the relative concentrations of NO₃⁻ and thermonuclear tritium, it was concluded that denitrification in the groundwater was a likely possibility (Foster et al., 1985).

Due to the diversity of subsoil environments and the paucity of data, it is difficult to generalize or predict the importance of subsurface denitrification at a given site. More studies are needed to provide a better data base to increase our understanding of microbial processes in the vadose zone. This study was initiated to (i) obtain information on the physico-chemical characteristics and microbial activities of the subsoil environment, (ii) determine the importance of denitrification in the vadose zone and the upper region of the water table in reducing NO₃⁻ loading to groundwater, and (iii) determine if surface tillage practices influence denitrification and microbial activity below the root zone.

MATERIALS AND METHODS

Study Site and Sample Collection

The study site was located in the Eastern Shore region of Maryland at the Wye Research and Education Center near Queenstown, MD. The soil was a well-drained Matapeake silt loam (fine-silty, mixed, mesic Typic Hapludult) underlain by sand. Field plots (4.6 m by 12.2 m) had been cropped to continuous corn for 4 yr using either conventional moldboard plow tillage or no-tillage practices. Four replicate plots were established in each tillage treatment. Fertilizer N had been applied to all plots in 1980 through 1984 at a rate of 270 kg N/ha. In 1985 plots were fertilized at a rate of 180 kg N/ha. Soil samples down to the water table (ca. 420 cm) were collected in April 1986 at the end of the groundwater recharge period, and before the soil had been tilled, fertilized, or planted.

A crucial aspect of this study was to obtain subsoil samples uncontaminated by surface soil material. The 0- to 30-cm sample was taken with a 2.4-cm diam. hand probe, then a larger 7.6-cm diam. core was removed and a plastic tube (35 cm long by 7.6 cm diam.) was inserted in the resulting hole. This plastic liner protruded ca. 5 cm above the soil surface and minimized contamination due to surface soil material falling into the open hole when deeper samples were collected. Samples from the 30- to 180-cm depths were collected using a 5-cm diam., 180 cm long coring tube. Stacked inside this tube were six 30-cm lengths of polycarbonate tubing, which served...
as liners to contain the soil material. This sampling tube was driven into the ground to the 180-cm depth with a 68 kg drop-hammer and then removed using an electric winch. The polycarbonate tubes containing the intact cores were removed from the sample tube and the ends of each soil core were shaved with a sterile knife.

The intact soil cores were then subcored in the field to eliminate contamination by surface soil, which may have been smeared along the sides of the samples. Subcoring was done using a 2-cm diam. chrome-plated steel tube that was flame sterilized in the field. The subcores were then immediately removed from the chrome-steel tube and placed in sterile plastic bags that were stored on ice until returned to the laboratory.

The sandy texture of the material below 180 cm prohibited the use of the long soil core tube. Samples from 180 cm to the water table were collected at 60-cm depth intervals using a 60-cm long, 5-cm diam., split core sampler that was driven into the ground with the drop hammer described above. The soil material contained in the cutting bit of this sampler was then removed and discarded using a sterile knife, and the soil core was subcored using the procedure described above. Four deep cores were collected from each of three replicate tillage plots. Each deep core consisted of 10 samples: 30-cm increments down to 180 cm and 60-cm increments down to teh top of the water table (ca. 420 cm). Samples were returned to the lab and stored at 4°C overnight before processing.

### Biological Analyses

The biological parameters analyzed were (i) CO₂ production, (ii) denitrification enzyme activity, (iii) numbers of denitrifying bacteria, and (iv) total viable bacteria. Carbon dioxide was determined by incubating 50 g of fresh soil (water contents shown in Fig. 2) in flasks over a 24-h period of 25 °C and periodically measuring CO₂ concentrations using a gas chromatograph equipped with a thermal conductivity detector. These incubations, which were conducted under an aerobic headspace with no amendments, indicate the general microbial activity as well as the available organic C at depth.

Denitrification enzyme activity was determined over a 3-h incubation by monitoring N₂O produced from 50 g of soil in flasks over a 24-h period of 25 °C and amended with nitrate, glucose, chloramphenicol, and acetylene (Smith and Tiedje, 1979). Nitrous oxide was analyzed using a gas chromatograph equipped with an electron capture detector. These incubations yield information on the maximum potential activity of existing denitrifying enzymes in the samples (Smith and Tiedje, 1979; Tiedje, 1982).

Denitrifying bacteria were enumerated using the most probable number dilution tube method (Tiedje, 1982; Alexander, 1982). Numbers of total viable bacteria were determined by plate counts on nutrient agar (Wollum, 1982).

### Physico-Chemical Analyses

Soil samples were analyzed for NO₃⁻, total C, water content, pH, and particle size. Soil NO₃-N (NO₃⁻ + NO₂⁻) was determined by an automated cadmium reduction method (Technicon Autoanalyzer II, Industrial Method no. 100-70W, 1973; Technicon Industries, Tarrytown, NY) on KCl soil extracts (5 g soil:10 mL 1 M KCl). Total organic C was determined on pulverized soil samples (0.1–0.5 g) using a model 700 total organic carbon analyzer (O.I. Corp., College Station, TX). This procedure utilizes a persulfate oxidation of the organic matter at 100 °C (0.5 h) followed by the determination of CO₂ in the sealed head space by gas infrared absorption. Water content was determined gravimetrically after air-drying moist samples. Soil pH was measured in a 1:1 (water/soil) extract by glass ele-

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**Physico-Chemical Environment**

The texture profile of the site (Fig. 1) shows that the top 120 cm of soil contained an average of 20, 60, and 20% sand, silt, and clay, respectively. From 120 to 180 cm, a marked texture change occurred and sand content increased to 90% with a corresponding drop in silt content. The clay content was more uniform with depth, although somewhat higher clay contents occurred in the B horizon (ca. 60 cm). Bulk densities of the surface layer (0–30 cm) were ca. 1.50 Mg/m³, 1.68 Mg/m³ for the 30-60 180-cm layer and 1.48 Mg/m³ for the 180-360-cm depth layer.

Soil water contents were not affected by tillage treatments since sampling was done during the end of the groundwater recharge period (Fig. 2). Soil water content decreased from 15 to 18% at the surface to 12% at 300 cm, in concert with the decreasing silt and increasing sand content, and then increased regularly as the top of the water table was approached at ca. 420 cm below the soil surface.

Soil pH decreased from 6.5 at the surface to 5.4 at 180 cm, where the texture change occurred, and remained relatively constant down to the water table (Fig. 3). At the top of the water table, pH increased slightly to 5.8. Similar pH profiles were observed under both tillage treatments.

Average O₂, CO₂, and N₂O concentrations in the gas-filled pore space of the no-till plots are shown in Fig. 4. Gas composition at the zero depth are concentrations in ambient air at the soil surface. With increasing soil depth, N₂O concentrations increased only slightly over ambient
Fig. 2. Gravimetric soil water content as a function of soil depth under conventional and no-till plots. Values are means of three replicates of four samples at each depth.

Fig. 3. Soil pH as a function of soil depth under conventional and no-till plots. Values are means of three replicates of four samples at each depth.

Fig. 4. Concentrations of N$_2$O, CO$_2$, and O$_2$ under no-till plots. Values are means of two replicates of two samples at each depth.

Fig. 5. Nitrate concentration in the soil solution under conventional and no-till plots. Values are means of three replicates of four samples at each depth. Significant differences ($P < 0.05$) in nitrate concentration between the tillage treatments at a given depth are indicated by (*).

levels. Carbon dioxide increased markedly with depth to levels nearly 100-fold greater than ambient concentrations at 240 cm. The precise reason for the elevated CO$_2$ concentrations in the subsoil pores is unclear since CO$_2$ production was observed to be greater in the surface soil (Table 1). It is possible that dissolved CO$_2$ was transported from the surface soil to the deeper soils in recharge water and that slow gas exchange between the atmosphere and soil pores of the deeper soils resulted in the observed high soil pore CO$_2$ concentrations. In any case, the CO$_2$ data do indicate that the gas sampling probe was not sampling air drawn down the sides of the probe from the soil surface. Only slight decreases in O$_2$ levels were observed with increasing depth. Oxygen concentrations dissolved in the groundwater were ca. 7 mg/L, which is approximately 74% of saturation at a temperature and O$_2$ partial pressure of 17°C, and 21 kPa, respectively.

Nitrate profiles under conventional and no-till plots suggested that tillage differences influenced NO$_3^-$ leaching (Fig. 5). Under no-tillage, NO$_3^-$ decreased from 30 mg NO$_3^-$-N/L soil solution at the 15-cm to 20 mg NO$_3^-$-N/L at 45 cm, and below this depth NO$_3^-$ concentrations remained relatively constant at ca. 20 mg NO$_3^-$-N/L soil solution. Under conventional till, NO$_3^-$ concentrations were similar at the first two depth increments; however, a broad peak of NO$_3^-$ was observed in the 80- to 200-cm region. Near the water table, NO$_3^-$ was observed in the 80- to 200-cm region. Near the water table, NO$_3^-$ concentrations in the two tillage systems were similar.

Total organic C profiles were similar under both tillage systems (Fig. 6). Organic C decreased exponentially from 6.0 to 7.2 mg C/g soil at the surface to 0.3 mg C/g at 180 cm. Below 180 cm, organic C levels were nearly constant at 0.2 mg C/g.

**Biological Properties**

Average rates of CO$_2$ production appeared to be higher under no-till as compared to conventional till (Table 1); however, no significant difference was observed between tillage systems at any depth. Carbon dioxide pro-
Table 1. Average daily CO₂ production rates as related to soil depth and surface tillage practice.

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>CO₂ Production†</th>
<th>Depth (cm)</th>
<th>CO₂ Production†</th>
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<tr>
<td></td>
<td>Mean ± SD</td>
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<tr>
<td>0-30</td>
<td>8.9 ± 1.3</td>
<td>30-60</td>
<td>1.8 ± 1.7</td>
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<tr>
<td>30-60</td>
<td>1.0 ± 1.7</td>
<td>60-90</td>
<td>2.0 ± 2.3</td>
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<tr>
<td>90-120</td>
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<td>120-150</td>
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<tr>
<td>120-150</td>
<td>&lt;0.4  —</td>
<td>150-180</td>
<td>&lt;0.4  —</td>
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<tr>
<td>150-180</td>
<td>&lt;0.4  —</td>
<td>190-240</td>
<td>&lt;0.4  —</td>
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<td>190-240</td>
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<td>310-360</td>
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<td>310-360</td>
<td>&lt;0.4  —</td>
<td>370-420</td>
<td>&lt;0.4  —</td>
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</table>

† 24-h aerobic incubations with no amendments. Conventional and no-till CO₂ production rates were not significantly different (P < 0.05) at any soil depth.

Means of three replicates of four samples each.
§ Detection limit of the assay is 0.4 mg C/kg soil per day.

Denitrification enzyme activity showed trends similar to CO₂ production (Table 2). Activity was significantly higher under no-till as compared to conventional till in the 0- to 30-cm zone. The high activity observed in the surface soils of both tillage treatments decreased substantially with depth down to 150 cm. At the 120- to 150-cm depth increment, denitrification enzyme activity was undetectable under conventional till; however, under no-till some activity was observed. Under both tillage systems, below the 150 cm depth, active denitrifying enzymes were not detected.

Numbers of denitrifying bacteria decreased nearly exponentially down to 180 cm, and there was no significant difference in denitifier counts between the two tillage systems (Fig. 7). From 180 to 330 cm, denitrifying organisms were undetectable under both tillage treatments (<20 organisms/g soil). A slight increase in numbers of denitrifying bacteria were detected at the top of the water table. Total viable bacteria (Fig. 8) also followed a near-exponential decline down to 180 cm. Below this depth, numbers of bacteria were low but detectable.

It is possible that, due to the relatively high C content of the growth media used, numbers of viable bacteria may underestimate total numbers of microorganisms present in the subsoil. However, if low-carbon adapted microorganisms were present, their activity was very low as indicated by the absence of measurable CO₂ production in unamended samples. Balkwill and Ghiorse (1985), using direct microscopy, observed high numbers of microorganisms in subsoil samples from several sites; however, very few of the microorganisms exhibited any respiratory activity (INT reduction), indicating that most of the observed organisms were either dead or inactive.

Results of this study are similar to previous studies investigating denitrification as a function of depth within the crop rooting zone. Khan and Moore (1968) observed...
a trend of decreasing denitrification potential with increasing soil depth down to 100 cm in anaerobic incubations of six Alberta mineral soils. Incubations of subsoil under water-saturated conditions revealed that denitrification capacity as well as CO$_2$ production decreased exponentially with increasing soil depth down to 150 cm in three Alberta soils (Cho et al., 1979). McGarity and Myers (1968), however, observed high denitrifying activity in the fine-textured, slowly permeable, low organic B horizon of some solodized solonetz soils and postulated that this high activity was supported by soluble organics leached from the surface soils (Myers and McGarity, 1971). It should be noted, however, that the physical and chemical conditions in the subsoil of a solonetz soil are markedly different from the above studies and from the soil of our study. Brar et al. (1978) concluded that the added C in wastewater had little influence on the mass of NO$_3^-$ leaching from the surface soil to the groundwater at four different wastewater irrigation sites.

Although our data represent only one point in time, our study was conducted in the spring when, due to the release and subsequent transport of soluble C from the surface soils, microbial activity should have been at a maximum. Also, past studies indicate that temporal variability of denitrification enzyme activity is relatively low, and that highest enzyme activities occur in the spring (Rice and Smith, 1980; Smith and Parsons, 1985). Our results show that denitrification enzyme activity was undetected below 160 cm, and denitrifying bacteria were in very low numbers below the rooting zone. In the intermediate vadose zone (90-270 cm), even if maximum potential rate of 0.02 $\mu$g/kg per day is being expressed, <0.3 kg N/ha per year would be denitrified in the intermediate vadose zone. It is highly unlikely, given the aeration state and low organic C levels of this site, that the maximum potential denitrification rate would be expressed. The actual rate is likely to be much lower. Thus, we conclude that, at this site, denitrification is not a mechanism of significant NO$_3^-$ loss below the crop rooting zone. Further, these data indicate that surface tillage practices did not significantly influence subsurface biological activity, which implies that gross C leakage from the two tillage systems is not substantially different. The dominant factors influencing NO$_3^-$ concentrations below the root zone apparently results from differences in NO$_3^-$ transformations from the surface soil rather than any direct NO$_3^-$ transformations within the intermediate vadose zone.

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REFERENCES


