Report as of FY2006 for 2006KY64B: "Evaluating Denitrifier Stratification in Fragipan Soils"

Publications

- Water Resources Research Institute Reports:
    - Wu, Tingting and Mark S. Coyne, 2007, Denitrifier Ecology in Fragipan Soils of Kentucky, in Proceedings of the Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, p 69-70.

Report Follows

Problem and Research Objectives

Very little is known about denitrification processes in subsurface environments. Fragipans are a significant subsurface feature in Kentucky that result in seasonal perched water tables. These environments may contribute to substantial loss of NO_3^- through leaching past the root environment. Our objectives were to evaluate whether surficial land treatments influenced the distribution of denitrifiers by depth in fragipan soils and whether population changes in denitrifiers could be driven by the selection of soil amendment and incubation condition. Our original proposal called for sampling three sites with fragipans. We reduced this number to two sites, but maintained our ability to compare sites with historical evidence of animal waste application.

Methodology

Intact soil cores (4.4 cm diameter, 90 cm long) were taken in fragipan soils (Zanesville silt loam) identified in a formerly cultivated soil with poultry litter amendment (one treatment with 22.4 Mg ha^{-1} litter added; another treatment with no litter added) and an unamended pasture site in Princeton, KY. Soil cores were divided into subsamples by distance relative to the estimated fragipan/soil interface (5 cm above, 5 cm and below, and in 15 cm increments from the interface to the soil surface). Subsamples were analyzed in terms of denitrifier population (by most probable number analysis), and denitrifier activity (by potential denitrification enzyme activity assay). In addition, DNA was isolated from the subsamples using UltraClean Soil DNA Isolation Kits (MoBio Laboratories, Solana Beach, CA.) as specified by the manufacturer. Commercial NESTLÉ® CARNATION® NonFat Dry Milk was added (40 mg g^{-1} soil) to the bead solution tube of the kit to improve extractions. Isolated DNA was quantified using a spectrophotometer. Fragments of *nirK*, *nirS* and *nosZ* genes were amplified using primer pairs *nirK*1aCuF (5'ATC ATG GT(C/G) CTG CCG CG 3')-*nirK*3CuR (5'GCC TCG ATC AG (A/G) TTG TGG TT 3') for *nirK; nirS* cd3aF(5'GT(C/G) AAC GT(C/G) AAG GA(A/G) AC(C/G) GG 3')- *nirS* 3cdR (5' GA(C/G) TTC GG (A/G) TG(C/G) GTC TTG A 3') for *nirS*; and *nosZ*-F (5'CG(C/T) TGT TC (A/C) TCG ACA GCC AG 3') - *nosZ*1622R (5'CGC (G/A) A (C/G) GGC AA (G/C) AAG GT (G/C) CG) for *nosZ* (Throbäck et al., 2004). A 33-bp GC-clamp (5' GGC GGC GCG CCG CCC GCC CCG CCC CCG TCG CCC3') was attached to the 5' end of 3CuR, 3cdR and 1622R primer respectively, for denaturing gradient gel electrophoresis (DGGE) analysis. Intact cores (with plastic liners) from the unammended pasture site with average fragipan depth at 70 cm were used for the treatment application assay with three treatments (1mM glucose, 1mM KNO_3, and water only) in duplicate. All cores were maintained with a 15 cm water table above the fragipan/soil interface. Subsamples were taken at 0, 4, and 8 weeks after the application of amendments and analyzed as previously described to assess whether the amendment influenced the number and distribution of denitrifying bacteria.

Results and Significance

Depth to fragipan at the formerly cultivated site varied from 53 to 62 inches. Depth to fragipan at the pasture site ranged from 43 to 81 cm and for the purposes of this study was further divided into shallow (43-54 cm), moderate (70-72 cm), and deep (75-81 cm).

Within every soil core, soil organic carbon (SOC) and total N of the surface soil were significantly different from subsurface soils ($P<0.05$) (Table 1). However, SOC and total N of soil at the same depth showed no difference between the two poultry litter amendment levels from the formerly cultivated site (Fig. 1), which indicated no permanent effect of the litter amendments on C and N levels at the rates employed.

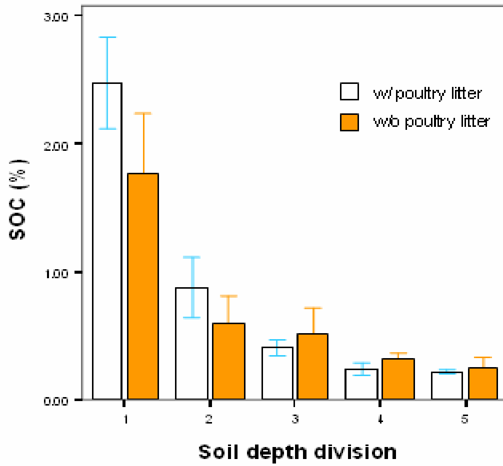
Table 1 Soil water content, SOC, and TN of the soil samples†.

Properties	Soil core divisions				
	Layer1	Layer2	Layer3	Layer4	Layer5
	Plot with poultry litter amendment				
Soil water content (%)	24.83±3.04	24.59±1.01	25.44±0.6	21.91±0.64	23.71±1.2
Organic carbon (SOC%)	2.47±0.36	0.88±0.23	0.41±0.07	0.24±0.05	0.22±0.02
Total Nitrogen (TN%)	0.16±0.021	0.06±0.012	0.04±0.004	0.03±0.005	0.03±0.004
	Plot without poultry litter amendment				
Soil water content (%)	25.52±4.43	24.37±1.27	25.16±1.56	24.49±2.51	22.57±2.31
Organic carbon (SOC%)	1.76±0.47	0.61±0.21	0.51±0.20	0.33±0.04	0.25±0.08
Total Nitrogen (TN%)	0.11±0.03	0.05±0.014	0.04±0.01	0.03±0.009	0.02±0.007
	Native pasture site				
Soil water content (%)	27.07±4.68	23.71±2.52	22.99±2.33	20.67±2.61	21.01±1.61
Organic carbon (SOC%)	2.73±1.20	0861±0.28	0.37±0.11	0.30±0.07	0.40±0.32
Total Nitrogen (TN%)	0.84±1.75	0.20±0.38	0.09±0.15	0.11±0.14	0.06±0.09

† Layer 1: surface soil; layer 2: 35 cm above the fragipan; layer 3: 20 cm above the fragipan; layer 4: 5 cm above the fragipan; layer 5: 5 cm below the fragipan.

Evidence of denitrifiers, as revealed by consumption of NO_3^- in facultatively anaerobic conditions, was found in all depths in all soil cores. As anticipated, the population of denitrifiers declined exponentially from the surface to the depth of the fragipan (Fig. 2).

If the relative populations of denitrifiers to heterotrophs were plotted, in some cases it appeared that denitrifiers made up a greater portion of the microbial population at the fragipan interface (Fig. 3). However, the evidence that the denitrifiers had stratified above the fragipan in either site was inconsistent, most probably because of the extended period in which neither site received supplemental organic C and N. This contrasts with previous findings that fragipan soils receiving continual manure applications do demonstrate stratification. Given the current microbial distribution we observed in these samples, we should be able to observe a significant effect of surface amendment on denitrifier population distribution if such an effect does in fact occur.



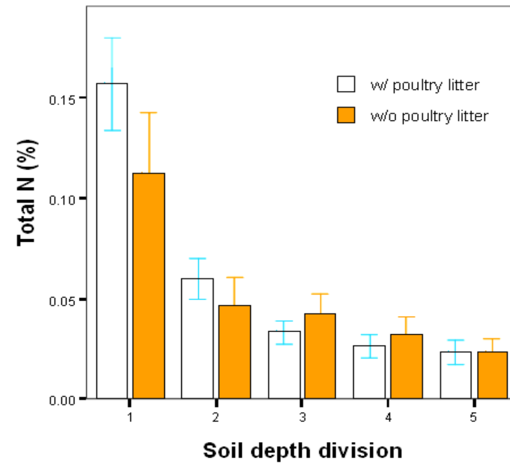


Figure 1. SOC and total N of soils of two treatments from Site 1. Soil depth division: 5: 5cm below fragipan/soil interface; 4: 5cm above fragipan/soil interface; 3: 20cm above fragipan/soil interface; 2: 35cm above fragipan/soil interface; 1: surface soil.

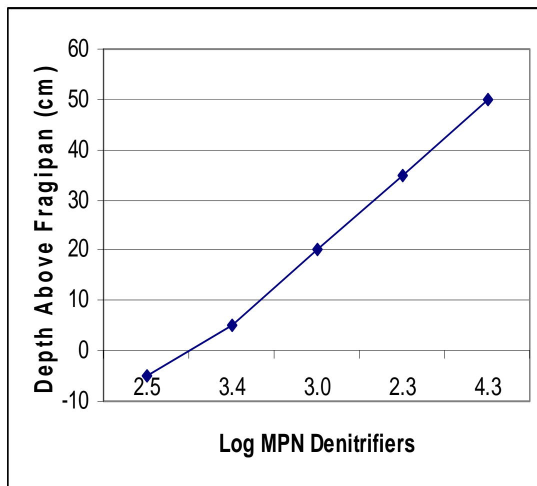


Figure 2. Distribution of denitrifiers by depth from an intact soil core removed from a fragipan soil in Princeton, KY. Depth to fragipan was approximately 50 cm. No amendments had previously been applied to this soil. This represents one-of-twelve unique cores for which MPN analyses were performed at each depth.

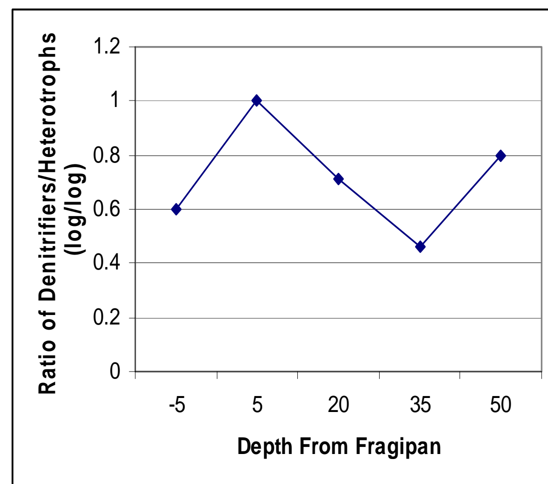


Figure 3. Ratio of denitrifiers to total facultatively anaerobic heterotrophs in soil with depth. Soil core is the same as reported in Fig. 2.

With amendment of 10 mM NO_3-N and 10 mM Glucose, DEA rates of 0.5-0.9 ng of N_2O-N per g dry soil per min were recorded within the first 4 hours of incubation for most of the surface soils, which was 2 to 3 fold higher than the rates of the subsurface soils, which were sometimes not detectable. No significant differences were found in the DEA rate between soils 5 cm above and 5 cm below the fragipan/soil interface. Even though in some samples we found

denitrifier population (determined by MPN) to be stratified above the fragipan, it was not necessarily true that the denitrification rate also stratified, as shown by DEA assay. Besides the anaerobic environment, sufficient substrates such as C and N also affect both denitrifier population and activity.

DNA extraction from soils above the fragipan was greatly improved by adding skim milk to the reaction mix of the commercial DNA extraction kits (Fig. 4). This has not been previously noted. Recovered DNA were significantly different between the subsurface and surface soils, ranging from 3 μg ml^{-1} to 128 μg ml^{-1}, and were stratified in some samples, showing the same population distribution pattern indicated by MPN.

Primers used in this study did not work for all samples. Adjustments and new primers may be necessary for subsequent study.

Soil cores for the amendment experiment have been incubated at room temperature for at least 6 weeks. Samples were taken from the area where the 15 cm-water table was maintained above the estimated fragipan/soil interface at time 0 and after 4 weeks incubation, and were stored in Eppendorf tubes at -80°C for future DNA analysis.

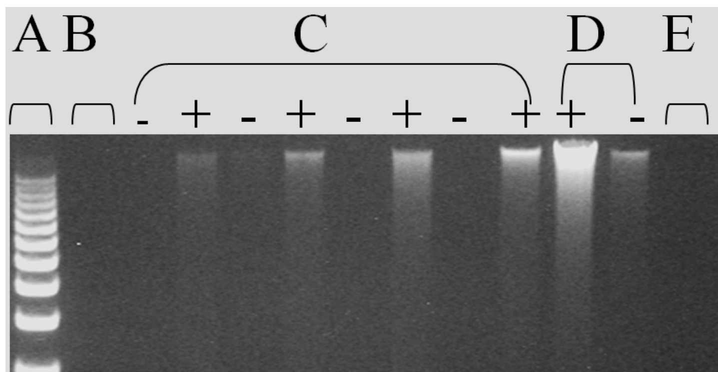


Figure 4. Agarose gel electrophoresis of DNA isolated using a commercial kit: without skim milk (-); with skim milk (+); A, Ladder; B, Skim milk control; C, Subsurface soil; D, surface 15 cm soil; E, H_2O control.

We successfully isolated denitrifier-specific DNA from above the fragipan of agricultural and pasture soil. This has not previously been accomplished. The amount of extracted DNA reflects the paucity of microbial populations at these interfaces in the absence of substantial additions of organic C. Different primers for the amplification of Cu-type (*nirK*) and heme type (*nirS*) nitrite reductases suggested that the Cu-type was more prevalent in the subsurface soil environment. Prior addition of organic material in the form of poultry litter had little effect on the population of denitrifiers above the fragipans, which suggests that frequent amendment is required to manipulate these bacteria. The DEA and MPN assays both suggested that in the absence of active denitrifier populations, the rates of NO_3^- reduction at these interfaces may be low even if perching occurs.