

Natural Treatment of Septic Tank Effluent: *Distribution of Nitrogen-Transforming Bacteria in Infiltration Mound Systems*

Nicholas Pajerowski¹⁻³, Zack Jones^{2,3}, Kathryn Lowe^{2,3}, Junko Munakata-Marr^{2,3}

¹Villanova University; ²Colorado School of Mines; ³ReNUWIt ERC

Abstract:

Infiltration mound systems provide onsite wastewater treatment that serves to both recharge groundwater supply and remove nitrogen from wastewater as it percolates through the mound system to meet the groundwater table. The soil that comprises these mounds harbors a community of microorganisms that mediate the reactions of nitrogen transformation and removal. This study will seek to quantify the functional genes that code for these nitrogen transforming-reactions in the microbial community in an infiltration mound to gain insight into the process of nitrogen removal.

Nitrification, and more specifically, the step of ammonia oxidation of NH_3 to NO_2^- is carried out by autotrophic ammonia oxidizing bacteria such as *Nitrosomonas*. The functional gene *amoA* codes for the enzymes that allow ammonia oxidizing bacteria to carry out the reaction. Denitrification of NO_3^- to N_2 gas is mediated by a variety of heterotrophic bacteria, which express the *nirS* or *nirK* functional gene, both of which code for the enzymes for denitrification. The abundance and distribution of the genes *amoA*, *nirS*, and *nirK*, as well as total bacterial life, represented by 16s rDNA, were determined at various depths in soil cores taken from two types of infiltration mounds.

Soil cores were collected from the Florida Onsite Sewage Nitrogen Reduction Strategies (FOSNRS) site at University of Florida Gulf Coast Research and Education Center (GCREC) that was designed, constructed, and operated by Hazen and Sawyer. Cores were obtained from two similar systems for comparison. The Septic-Tank Effluent (STE) system consisted of a mound of Seffner fine sand atop native soil left in its natural state. The Passive Nitrogen Reduction System (PNRS) system involved a more heavily engineered design, with the removal of the natural soil horizon and addition of an “enhanced carbon source” zone that included wood chips mixed into soil, with the goal of promoting enhanced denitrification. Both systems receive septic tank effluent water under a dosing regimen of 6 doses per day, resulting in conditions that fluctuated between saturated and unsaturated in the soil. Cores from each system were frozen and shipped to Colorado School of Mines. STE cores were sampled at depths of 0, 3, 6, 9, 15, 30, and 60 cm from the infiltrative surface, located within the mound at the point of effluent release. PNRS cores were sampled at depths of 0, 3, 6, 15, 30, 46, 56, and 67 cm from the infiltrative surface. Soil sub-samples were freeze dried and weighed for dry mass. DNA was then extracted from all soil sub-samples using MoBio PowerSoil DNA Isolation kits. Quantitative polymerase-chain reaction (qPCR) was used to quantify the concentration of the gene sequences for *nirS*, *nirK*, *amoA*, and 16s rDNA, with results shown below.

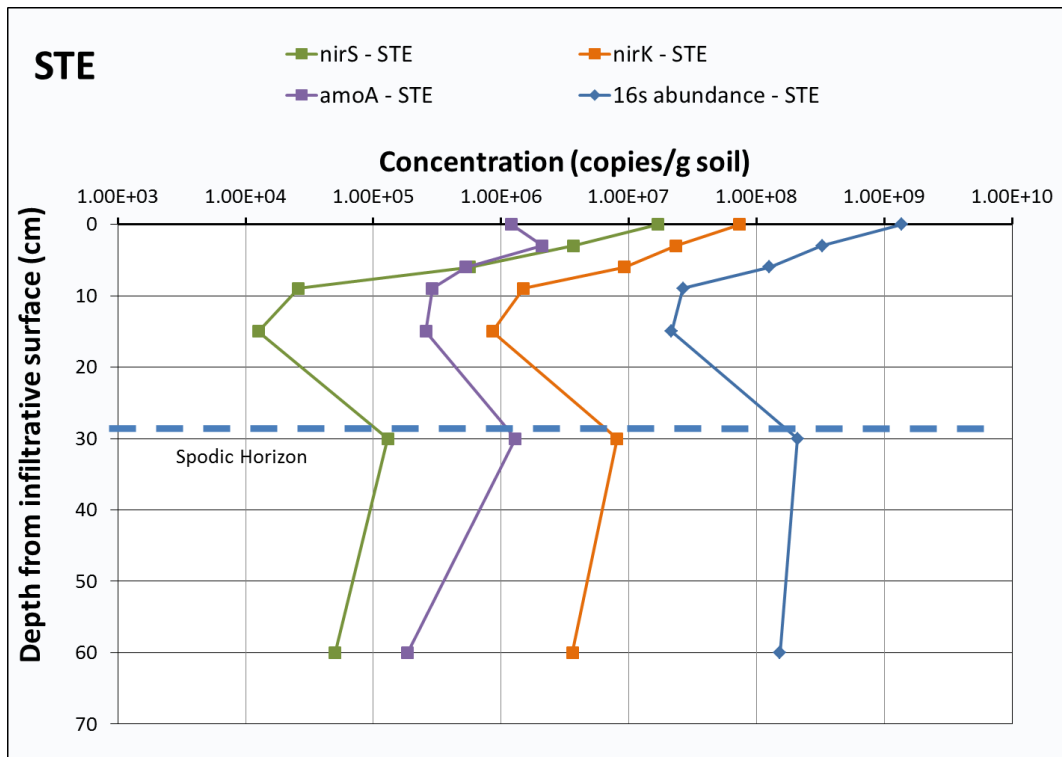


Figure 1: Concentration (copies/g of dry soil) of functional genes *nirS*, *nirK*, *amoA* and of 16s bacterial abundance at increasing depth (cm) from the infiltrative surface in STE system infiltration mound soil core.

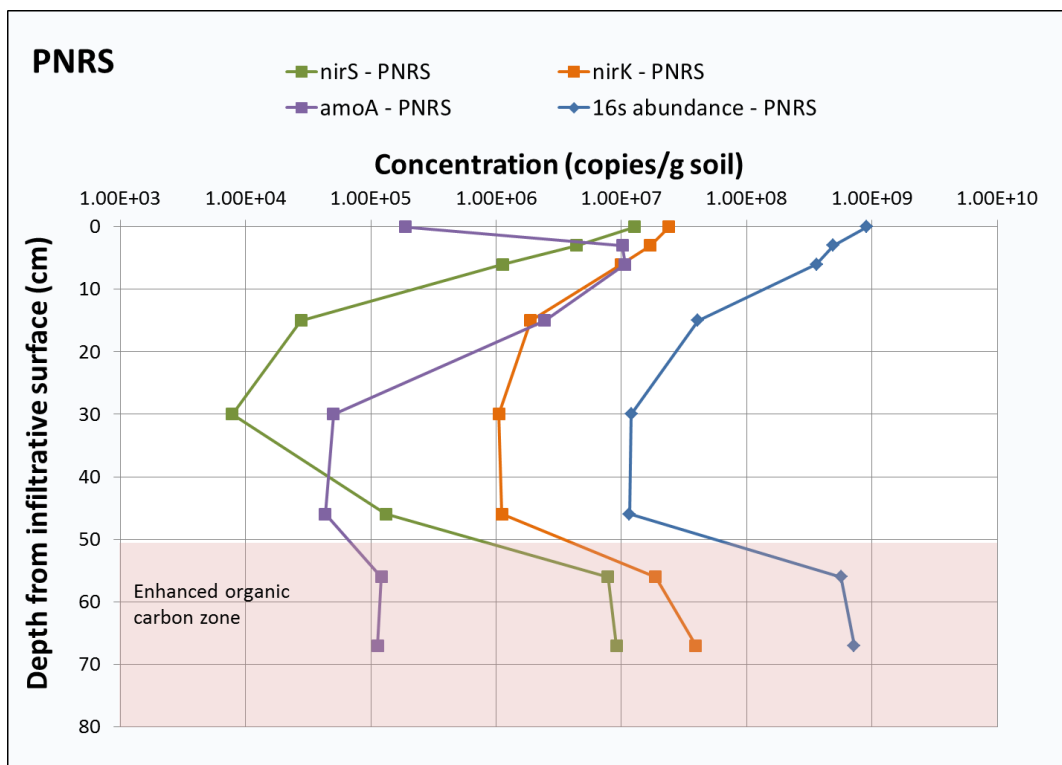


Figure 2: Concentration (copies/g of dry soil) of functional genes *nirS*, *nirK*, *amoA* and of 16s bacterial abundance at increasing depth (cm) from the infiltrative surface in PNRS system.

Results show that in both systems, the nirK gene (associated with soil systems) was the dominant denitrification gene, as opposed to nirS (associated with aqueous systems). In both systems, highest concentrations of all genes were generally found in the top 10 cm of the soil, indicating the existence of a “biozone,” near the infiltrative surface, where there is a high density of microbial life. This finding suggests that the microbial community thrives in the biozone and that much of the nitrogen transformation and removal likely occurs within the top 10 cm. At the infiltrative surface at 0 cm, the amoA was present at a relatively low concentration compared to the nirS and nirK genes. This indicates that at the infiltrative surface, the autotrophic ammonia oxidizing bacteria are likely out-competed by the heterotrophic bacteria that express the denitrification genes. In both systems, it was found that abundance of functional genes and total microbial life decrease below the biozone. In the STE system, abundance of functional genes and microbial life rebounded at the spodic horizon, a naturally occurring soil zone with greater amounts of organic matter. In the PNRS system, abundance of the denitrification genes nirS and nirK rebounded drastically at the “enhanced organic carbon zone,” indicating that the addition of a carbon source was successful in promoting enhanced denitrification, and thus increasing nitrogen removal from the water.

This analysis reveals the complexity of infiltration mound systems. The dynamics of the microbial community may be controlled by a number of variables including the system hydrology, dosing regimen, moisture content, carbon content, oxygenic conditions, and reaction kinetics. Further chemical analysis of nitrogen speciation in the cores will complement this study and provide insight into the nitrogen transformation and removal facilitated by the microbial community. While this investigation succeeds in determining abundance and distribution of the functional genes involved nitrogen cycling, further study of these mini-mound infiltration systems will contribute to a more thorough understanding and more effective design of on-site wastewater treatment systems in order to reduce the impact of nutrients on groundwater resources.