



G79 Microbial Diversity in Swine (*Sus Scrofa Domestica*) Gravesoils Between Seasons

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After attending this presentation, attendees will understand how gravesoil microbial communities may change significantly over a short time period during the summer but not during the winter.

This presentation will impact the forensic science community by showing that postmortem microorganisms during summer investigations should be used differently than postmortem microorganisms during winter investigations.

It has recently been shown that soil microbial communities have potential to serve as physical evidence for estimating postmortem interval. Soil microbial communities respond to the presence of a corpse and the structure of their communities' changes in a predictable manner; however, we are only beginning to understand these changes. It is known that temperature is a significant modulator of corpse decomposition. A corpse typically decomposes more rapidly at warm temperature because insects and microorganisms are more active and chemical reaction rates are more rapid. Yet few decomposition studies have been conducted at colder temperatures when microorganisms and scavengers that drive decomposition and chemical reaction rates are less rapid, if not inhibited. This is a significant gap in knowledge because not all corpses are deposited during the warmer months. To address this gap this study conducted a replicated field experiment to characterize the microbial diversity in gravesoils during summer and winter decomposition.

The experimental unit was a 1.5 x 1.5m plot of pasture located approximately 48km north of Lincoln, Nebraska. The site was enclosed with steel fence to prevent the entry of mammalian scavengers. The climate at the experimental site is a Cold climate type with a mean annual temperature of 9.8°C. Maximum temperatures range from 0°C (January) to 31°C (July). Average annual precipitation is 695mm with approximately 75% of the precipitation between April and September. The soil at the site is a silty clay loam (Mollic Hapludalf) with a texture of 15.1% sand, 53.6% silt, and 31.3% clay. Swine (*Sus scrofa domestica*) carcasses were placed on February 25, 2008 (winter) and on June 11, 2008 (summer). Control (no carcass) units were also used. Swine carcasses (~20kg) were acquired within 20 minutes of death, which was caused by blunt force trauma to the skull with a bolt gun. Carcasses were placed on the soil surface on their right side facing west within 60min of death. Soil samples (0-5cm depth) were collected from underneath the carcass at the time of placement, 15 days, 30 days, and 60 days postmortem. The experiment was replicated three times resulting in a total of six carcasses (three summer and three winter).

It was hypothesized that soils associated with pig carcasses decomposing in the summer season exposed to 1,412 Accumulated Degree Days (ADD) would undergo more substantial microbial community change than soils associated with pig carcasses decomposing in the winter exposed to only 325 ADD. Culture-independent, next-generation sequencing approaches were utilized to characterize microbial community change in soil associated with carcass decomposition. Partial 16S and 18S ribosomal RNA genes were sequenced using Illumina® HiSeq and Miseq sequencing platforms, respectively.

Corpse-associated soil bacterial communities were discovered to have changed significantly compared to control soils during the summer, but not during the winter. The seasonal effect on gravesoil bacterial communities was not simply due to temperature as winter corpse-associated soils with an ADD 326 were highly similar to control soils, while summer samples at an ADD of 322 were marginally significantly different from control soils. Both summer and winter non-carcass control soils were dominated by bacteria of the Phylum *Verrucomicrobia* in the Class *Spartobacteria* and genus *Chthoniobacter*. The late decomposition summer soils increased in abundance of taxa in the Order *Sphingobacteriales* and decreased in the abundance of *Chthoniobacter*.

Microbial eukaryotic sequence data revealed that gravesoil microbial communities associated with summer carcasses became more differentiated from control soils than winter soils that were associated with carcasses. Summer and winter control soils were dominated by similar taxa such as *Cercozoa* in the Orders *Cryomonadida* and *Cercomonas*, fungi in the Classes *Sordariomycetes* and *Agaricomycetes*, as well as the amoeba of the genus *Acanthamoeba*. Late-stage decomposition gravesoils in the summer season showed a dramatic decrease in alpha diversity and became dominated by the cellular slime mold *Fonticula alba* and the



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endogenous soil bacterivore *Acanthamoeba*. Late stage winter gravesoils had comparable diversity to control soils.

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