G46 Characterization of Cadaver Decomposition Islands Using Necrophilous Insect Diversity and Soil Metagenomic Analyses

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After attending this presentation, attendees will gain insight on vertebrate decay, insect scavenging, the effects of cadaver decomposition islands on topsoil microbial community structure, and the application of metagenomics in forensic science.

This presentation will impact the forensic science community by demonstrating the importance of understanding biological and ecological aspects of the carrion community.

Introduction: Decomposition of large vertebrates above ground is predominately driven by microorganisms and necrophilous insects. Scavenging activities of sarcosaprophagous insects dramatically alter Cadaver Decomposition Islands (CDIs) both mechanically and chemically, resulting in nutrient-loading of decomposition products in topsoils and exposure of internal resources for later-arriving species. In forensic taphonomy, site ecology influences the rate of decay while decomposing remains affect a site's microhabitat. Thus, analysis of soil microbial community structure, in conjunction with necrophilous insect diversity and succession, associated with CDIs is critical to fully understand the carrion habitat. The widespread availability and low-cost of next generation sequencing makes cadaver soil metagenomic analyses feasible and potentially a valuable tool in forensic science.

Materials and methods: Four 12-month long seasonal studies (winter, summer) were conducted in a hardbottom flatwoods forest in Hammond, Louisiana, from February 2011 to July 2013. Each field study included three adult swine carcasses (~45-122kg, with average weight ~68kg) placed directly on the leaf litter/soil surface. Sampling events and protocols varied for insect and soil core collections throughout the five stages of decay. Manual sampling and insect pitfall traps (four traps/carcass) were collected daily, every other day, biweekly, bimonthly, and monthly. Two soil cores (~12cc each) were collected beneath each carcass every 3 days, biweekly, bimonthly, and monthly. Control soils were collected per sampling event ~15m away from the carcasses.

Soil samples were processed for soil characterization and chemical composition (pH, KCl extractable organic carbon, total nitrogen, ammonia/ammonium, orthophosphate, and nitrite). Microbial characterization included enumeration of lipolytic and proteolytic colony-forming units (three serial dilutions in triplicate/sampling event) and 16S rDNA metagenomic analysis using semiconductor sequencing (Ion Torrent PGM™ with barcoded universal primers flanking the V4 region). Statistical analyses of insect and microbial data included classification of insects and amplicons, community structure (α/β diversity), and succession analysis and ordination using principle component analyses.

Results: A total of 35 and 34 soil sampling events (280 and 272 total soil cores, respectively) were performed during the winter and summer 2011 series, respectively. *Calliphoridae* and *Sarcophagidae* immatures were the predominant insect scavengers of swine carrion during the fresh to active decay stages for both winter and summer studies, including the following blow fly species: winter 2011-2012 studies: C*alliphora vicina* (Robineau-Desvoidy), *Phormia regina* (Meigen), and *Lucilia coeruleiviridis* Macquart (*Calliphoridae*); and summer 2011-2012 studies: C*ochyliomyia macellaria* (F.)*, Chrysomya rufifacies* (Macquart), and *L. Coeruleiviridis* (*Calliphoridae*). Later stages of decay were primarily associated with the following fly species: *Hydrotaea leucostoma* (Muscidae) and *Fannia scalaris* (F.) during active to putrid remains; and *Hermetia illucens* (*Stratiomyidae*) from advanced decay to putrid/dry remains.

Shifts in soil microbial biomass reflect, in part, the nutrient-loading of soils with cadaveric fluids due to necrophagous insect activity. For instance, increased lipolytic activity was observed from early advanced decay to late putrid/dry remains stages for both winter and summer studies (e.g., winter 2011: ~days 35-200; summer 2012: ~days 12-180 of decay). Trends in proteolytic microbial biomass were similar for control and cadaver soils during fresh, bloat, active, and late putrid/dry remains stages. Thus, increased proteolytic activity was observed during ~days 40-200 and ~days 57-130 of decay for winter 2011 and summer 2012 studies, respectively.

Family level shifts in microbial diversity correlated with the five stages of decay. For example, the winter 2011 soils demonstrated the following trends: (1) control, fresh, and bloat stages were dominated by

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Acidobacteria; (2) microbes present during active and advanced decay were predominately *Pseudomonadaceae* and *Flavobacteriaceae*; and, (3) putrid/dry remains were dominated by *Xanthomonadaceae* and *Chitinophagaceae*. Clustering of stages of decay based on microbial and insect data were observed using principle component analyses.

These results suggest that stages of decay could potentially be characterized using microbial topsoil community composition. From an evidentiary point of view, the more information one has, the stronger the case. Thus, investigating the biotic and abiotic aspects of CDIs is imperative to our understanding decomposition.

Decomposition, Forensic Entomology, Soil Metagenomics

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