



A157 Forensic Exploration of Temporal and Spatial Differences of Soils Using Next-Gen Sequencing

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After attending this presentation, attendees will understand how both time and distance can influence bacterial composition of soil and their impact on how evidentiary soil can be interpreted.

This presentation will impact the forensic science community by outlining how variability in the bacterial populations of both diverse and similar habitats over time and space can impact the identification of soil evidence. Further, advantages of using next generation sequencing coupled with Non-Metric Multidimensional Scaling (NMDS) will be discussed.

The traditional methods for forensic soil analysis — identifying physical and chemical characteristics of a soil sample such as color, chemical makeup, etc. — have been successfully utilized, but can suffer from ambiguous results and often require large samples. Recently, there have been advances in the field of soil identification aimed at investigating the bacterial populations endemic to soils. The most common forensic genetic analysis of soil microbial populations is Terminal Restriction Fragment Length Polymorphism (TRFLP), which establishes a “DNA fingerprint” that can potentially be used to compare known and questioned samples. However, TRFLP suffers from several limitations, including complicated profile interpretation, inability to identify specific bacterial composition, and difficult statistical analysis. Given this, a more powerful technique that allows for straightforward statistical examination and the investigation of the actual species present in a soil sample is needed.

In the past 20 years, DNA-sequencing technologies have advanced substantially, allowing examination of complex media such as soil. Large amounts of DNA can be sequenced simultaneously at a relatively low cost. Furthermore, vast quantities of data can be generated from the small samples realistic in forensics. Additionally, microbial species identification is a possible technique that can be useful for inclusion or exclusion of questioned soil samples when comparing to a known. Next generation sequencing has the potential to revolutionize how evidentiary soil is examined because of the depth of information that can be gained from small amounts of sample.

Nine lawns at various distances from the Michigan State University campus were sampled on the same day to study the effect space has on bacterial populations. The previously conducted temporal study, on the other hand, entailed the sampling of three habitats — a woodlot, a lawn, and a marsh — quarterly for a year. DNA extracts were amplified with barcoded universal 16S rRNA primers and sequenced on a high-throughput 454 pyrosequencing platform. Sequence files were filtered with the open source software mothur and aligned and grouped by sample. Sequences were then clustered into operational taxonomic units and pairwise comparisons were calculated using various diversity indices based on bacterial community composition, which were input for NMDS analysis.

All samples from the spatial study were differentiated on a 2D MDS plot. Some samples were within standard error of each other, which tended to be the sites within the closest proximity. This trend existed for all diversity indices calculated. Conversely, for the temporal study, all samples from the same habitat clustered together distinctly from the other habitats on two-dimensional MDS plots for all diversity indices investigated (Figure 1). For both studies, the same bacterial phyla and classes existed in most samples, but at different abundances, and were used for additional comparison along with the MDS plots.

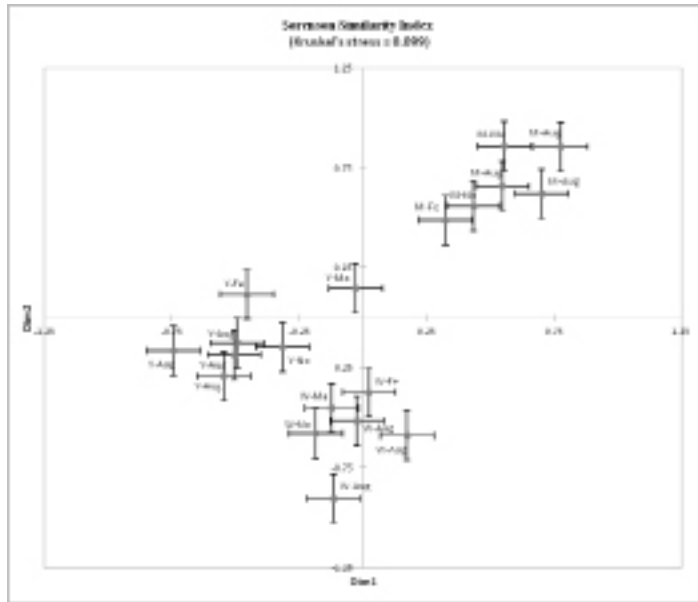
This research represents an initial investigation into how time and space influence species composition of soil bacteria using advanced sequencing technology. From the temporal study sequence data, bacterial composition of soil did not change statistically season to season, indicating that a known sample taken months after a crime occurred could still be associated with a questioned sample taken from a suspect-associated item. Additionally, similar habitats, like the yards investigated in this study, can be differentiated to varying degrees, allowing a questioned sample to be potentially traced back to a specific location. These findings have the potential to dramatically change the way soil is utilized forensically, including statistically linking a suspect to a crime scene.

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Figure 1



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