H51 The Influence of Depth and Mixtures on the Bacterial Profiling of Soil Using Next Generation Sequencing

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After attending this presentation, attendees will understand how different soil depths and mixtures alter the bacterial profiles produced from soil samples and that these factors must be considered when collecting known soil samples in order to produce strong associations between evidentiary soils and the site of a burial.

This presentation will impact the forensic science community by examining how various depths of soil and mixed samples, as may be encountered in burials, affect the bacterial composition of soil, thus expanding the knowledge necessary to successfully individualize forensic soil samples.

Soil is a commonly recovered form of trace evidence found on items such as shovels, tires, or clothing that has the potential to be traced back to its place of origin. Successful identification of soil can be valuable for forensic applications, as it can link a suspect or victim to the scene of a crime. In the past, soil has been analyzed primarily using physical class characteristics. More recently, researchers have sought to individualize soil evidence by analyzing the huge number of bacteria that inhabit it.[1,2] Many factors affecting the bacterial profiling of soil have been studied, including temporal and spatial changes; however, potential vertical changes have yet to be rigorously examined. The bacterial composition of soil may change as depth increases, as was found over horizontal space.[3,4] This could be particularly important in burials, where soil from various depths would be mixed. Understanding how depth and mixtures affect the bacterial profiles of soil is imperative in understanding how to collect and analyze known soil samples.

In the research presented, soil samples were collected from the surface and 5, 10, 20, 40, and 60 inches deep at a central coring location and four nearby corings. Five habitats were examined: two separate agricultural fields, a coniferous forest, an untreated yard, and a deciduous woodlot. Using these depth samples, mixtures were created to simulate a burial by homogenizing equal masses of soil from all six depths for each of the 25 corings. Different combinations of depths were used as training sets for supervised classification to examine the best way to collect depth samples in the case of a mixture or burial.

Bacterial DNA was extracted from soil samples using a DNeasy PowerSoil® kit, and variable regions 3 and 4 of the bacterial 16S recombinant DNA (rRNA) gene were amplified using universal barcoded primers. Bacterial sequences were produced on an Illumina® MiSeq® and visualized via abundance charts and non-metric multidimensional scaling. A bagged trees algorithm was used to objectively associate depth and mixture samples to a habitat and to provide likelihood scores that each of the classifications was correct.

In four of the habitats, there were increases in the bacterial classes Betaproteobacteria and Nitrospira and decreases in Sphingobacteria and Spartobacteria with depth. In contrast, the coniferous forest displayed increases in Deltaproteobacteria and Nitrospira, and decreases in Sphingobacteria and Alphaproteobacteria. The mixture samples for all habitats exhibited a bacterial profile resembling the shallowest soils. Graphically, as depth increased, the soil samples drifted away from the surface samples in multidimensional space, with the 40" and 60" samples being furthest away from the shallow samples while the mixtures grouped closest to the surface, 5", and 10" samples.

The central coring mixtures were then compared to different depths from the four surrounding corings using supervised classification. When compared to surface, 5", or surrounding mixtures, 100% classification accuracy was achieved. This accuracy decreased as depth increased, with the 60" samples producing 20% classification accuracy when used as the training set. This mimics the bacterial composition differences seen between the mixtures and the deep samples, as well as the deep samples being far away from the others in multidimensional space. The results indicate that the deep soil samples contribute very little to the mixture's bacterial profile and, thus, may not need to be collected, even when soils are from a burial.

These results elucidate the effects of vertical spatial changes, along with mixtures, on the bacterial composition of soil via next generation sequencing. Our understanding of these changes aids in the determination of how to best collect and analyze known soil samples when a burial has occurred.

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