H99 Storage Conditions and Time Alter the Association of Known and Questioned Soil Evidence Derived Via Next Generation Bacterial DNA Profiles

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After attending this presentation, attendees will understand how storage conditions and time change bacterial profiles generated from soil evidence, which, if not considered, has the potential to negatively influence the association of a suspect, victim, or evidentiary item with a crime scene.

This presentation will impact the forensic science community by revealing how the transient nature of the bacterial makeup in soil and its traceability to a location of origin is affected by aging and storage conditions, which must be understood if bacterial profiling is to be used to individualize soil evidence. Further, the objective association of soil evidence with its habitat of origin, using supervised classification, is examined, as the ability to produce strong associations will increase the value of soil as trace evidence.

Soil is a common form of trace evidence, recoverable from clothing, tires, shovels, etc., although its forensic analysis is usually based on class characteristics. To overcome this, past researchers have tested microbiological methods to assess if the microbial makeup, primarily bacterial, of soil might be used for forensic identification; however, almost all of these studies involved assaying the microbial DNA shortly after soil collection or freezing the soil until processing. In contrast, soil evidence will not be submitted to a laboratory until sometime after a crime is committed, and during that interlude, the bacterial composition of the soil may change, perhaps substantially. Likewise, known soil samples will not be collected at the same time the crime occurred, and may be stored for extended periods prior to analysis. If soil microbial profiling is to be a viable forensic technique, understanding how soil profiles from different evidence types change temporally is requisite, as is identifying how known soil samples should be stored prior to analysis, such that the evidentiary soil best associates with them.

In the research presented, T-shirts, shovels, sneakers, and jeans were exposed to soil from one of three habitats (yard, agricultural field, and dirt road) and stored at ambient temperature. Three and ten months later, known soils were collected from the same three habitats at a center point and 5', 10', 15', and 20' in each cardinal direction. The knowns were stored in plastic bags at room temperature, 4°C, -20°C, and -80°C for one day, one week, one month, and two months prior to processing.

DNA was isolated from soil samples using a MO BIO PowerSoil® kit. A 16S rRNA gene fragment containing variable regions 3 and 4 was amplified using universal barcoded primers. Bacterial sequences were generated on an Illumina® MiSeq®. Bacterial DNA profiles from the evidence were associated with the known soils via non-metric multidimensional scaling. A random forests algorithm was used to objectively assign the aged soil evidence to a habitat, and scores were generated to measure the likelihood the given classification was correct.

The bacterial composition of the soil on evidence displayed specific and consistent bacterial changes over time in all habitats, most notably increases in the taxonomic classes Actinobacteria and Bacilli, and decreases in Acidobacteria and Sphingobacteria. In contrast, known soil samples maintained the overall bacterial makeup from the time of collection regardless of storage temperature. On the other hand, when the known soils stored at room temperature were exposed to air, bacterial composition changes mirrored those of the aged soil evidence.

As time passed, the aged soil evidence became less similar to their habitat of origin, with the evidence soils drifting away from the known soils in multidimensional space; however, the groupings between evidence and known soils tightened when knowns were stored at room temperature and exposed to air. This was further reflected in supervised classification, in which the highest median classification accuracy (98.7%) was obtained when known soils were stored under the same conditions as the evidence, which was approximately ten percentage points higher than known soils stored at -80°C. Therefore, unlike most biological evidence, cold storage may not be optimal for known soils.

Overall, next-generation bacterial DNA profiling proves to be a viable technique for forensic soil analysis and is more individualizing than traditional methods. Further, our understanding of bacterial composition changes in known and questioned soils, including how to best handle soils when submitted to a laboratory, will allow for highly informative comparisons.

Soil Evidence, Bacterial Profiling, Supervised Classification