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NOTES AND SHORT COMMUNICATIONS

Microbial Signatures of Cadaver Gravesoil During Decomposition

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Abstract Genomic studies have estimated there are approximately 10^3–10^6 bacterial species per gram of soil. The microbial species found in soil associated with decomposing human remains (gravesoil) have been investigated and recognized as potential molecular determinants for estimates of time since death. The nascent era of high-throughput amplicon sequencing of the conserved 16S ribosomal RNA (rRNA) gene region of gravesoil microbes is allowing research to expand beyond more subjective empirical methods used in forensic microbiology. The goal of the present study was to evaluate microbial communities and identify taxonomic signatures associated with the gravesoil human cadavers. Using 16S rRNA gene amplicon-based sequencing, soil microbial communities were surveyed from 18 cadavers placed on the surface or buried that were allowed to decompose over a range of decomposition time periods (3–303 days). Surface soil microbial communities showed a decreasing trend in taxon richness, diversity, and evenness over decomposition, while buried cadaver-soil microbial communities demonstrated increasing taxon richness, consistent diversity, and decreasing evenness. The results show that ubiquitous Proteobacteria was confirmed as the most abundant phylum in all gravesoil samples. Surface cadaver-soil communities demonstrated a decrease in Acidobacteria and an increase in Firmicutes relative abundance over decomposition, while buried soil communities were consistent in their community composition throughout decomposition. Better understanding of microbial community structure and its shifts over time may be important for advancing general knowledge of decomposition soil ecology and its potential use during forensic investigations.

Keywords Microbial diversity · Cadaver-soil · Decomposition · High-throughput genomic sequencing · Necrobiome

Introduction

Microbial decomposers are important recyclers of vertebrate remains including humans [1–4]. There is an estimated eight million bacterial species per gram of soil [5] and an inherent relationship between environmental and decomposition associated microbial communities. Early in decomposition, the microorganism abundance at the carcass-soil interface rapidly increases after carcass rupture events [1], and the decomposition associated microbial communities can have longer-term influences on soil dynamics [2, 3, 6–8]. The rate of decay is further accelerated in terrestrial environments via arthropods [9–11]. Insects, such as flies, are thought to deposit microbes onto the cadaver decomposition island [12] and may influence the existing epinecrotic communities [11]. There are

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Table 1 Forensic Anthropology Research Facility (FARF) case number, demographic data (sex and ethnicity), soil sampling time, stage of decomposition, location of bodies in soil, and collection date

FARF case number	Sex	Ethnicity	Soil sampling time (days)	Stage of decomposition	Location of body in the soil	Collection date
Control						
D31-2014	F	W	8	Active decay	Burial	07.14.2014
D18-2014	F	W	96	Advanced decay	Burial	07.14.2014
D63-2013	M	B	214	Advanced decay	Burial	07.14.2014
D48-2013	M	W	303	Advanced decay	Burial	07.14.2014
D56-2013	F	W	3	Fresh	Surface	10.25.2013
D57-2013	M	W	6	Fresh	Surface	10.25.2013
D34-2014	M	W	8	Active decay	Surface	07.14.2014
D53-2013	M	W	15	Active decay	Surface	10.25.2013
D28-2014	F	H	18	Active decay	Surface	07.14.2014
D30-2014	F	W	22	Active decay	Surface	07.14.2014
D52-2013	M	W	28	Active decay	Surface	10.25.2013
D51-2013	M	W	33	Active decay	Surface	10.25.2013
D50-2013	M	W	34	Active decay	Surface	10.25.2013
D47-2013	F	W	74	Advanced decay	Surface	10.25.2013
D37-2013	M	W	99	Advanced decay	Surface	10.25.2013
D35-2013	M	W	106	Advanced decay	Surface	10.25.2013
D16-2013	M	W	240	Advanced decay	Surface	10.25.2013

additional biotic and abiotic factors [13–16], such as temperature, humidity, and scavenging, which add to the complexity of decomposition and microbial community composition in natural environments. Despite previous studies, there is a paucity of data documenting microorganism community dynamics in soil associated with human decomposition [6, 13, 17, 18] (Table 1).

The goal of many decomposition studies is to generate reliable means to estimate postmortem intervals (PMIs) [2, 6, 7, 10, 13, 14, 19–25]. Studies using gravesoil from human surrogates, such as swine [11, 13, 14, 26] and mice [6, 7, 18], have demonstrated the correlation between microbial diversity and PMI estimates. Lauber et al. [7] demonstrated that soil microbial communities increase mice decomposition rates by two- to threefold. A Carter et al. [13] study of microbial succession in soil associated with swine decomposition reported seasonality differences between summer and winter. Cobaugh et al. [19] investigated gravesoil of four human cadavers in an outdoor anthropological research facility, and demonstrated aerobic taxa were predominant during active decay while anaerobic decomposers dominated during advanced decay. Metcalf et al. [18] used 16S ribosomal RNA (rRNA) and 18S rRNA gene amplicons and internal transcribed spacer (ITS) region sequencing to describe the microbial communities of four human cadavers in an outdoor anthropological research facility. This study proved that the microbes in cadaver tissue and gravesoil become analogous during decomposition and suggested that soil communities mediate cadaver decomposition. However, there is a scarcity of empirical data from human cadavers decomposing in natural ecosystems. In the present study, our aim was to characterize the microbial communities of 18 cadavers at various decomposition time periods in a terrestrial habitat.

Materials and Methods

Gravesoil was collected at the Forensic Anthropology Research Facility (FARF) at Texas State University (97°56′28″ W, 29°52′59″N). FARF is a willed donation facility in Edwards Plateau with a soil type: Rumple-Comfort association, Comfort-Rock outcrop complex, and Mollisolls soil order [27, 28]. Sample details and decomposition stages, categorized according to classifications used by FARF, are found in Table S1. This study was approved by Alabama State University Institutional Review Board, 2013CMST004A.

Cadavers were placed on the surface or buried at specific time points to provide a range of decomposition and burial times (3–303 days). Bodies were kept at 2–4 °C during transportation to FARF. The study included 14 unclothed bodies placed on the soil surface (hereafter referred to as “surface cadavers”), and four unclothed bodies buried at a depth of 45 cm (hereafter referred to as “buried cadavers”) to simulate clandestine gravesites [27, 29]. Approximately 5 g of cadaver-soil were collected from each surface cadaver at a uniform depth of 5 cm under the cranium and feet [30] using sterile soil scoops. Soil was collected from between the legs for three

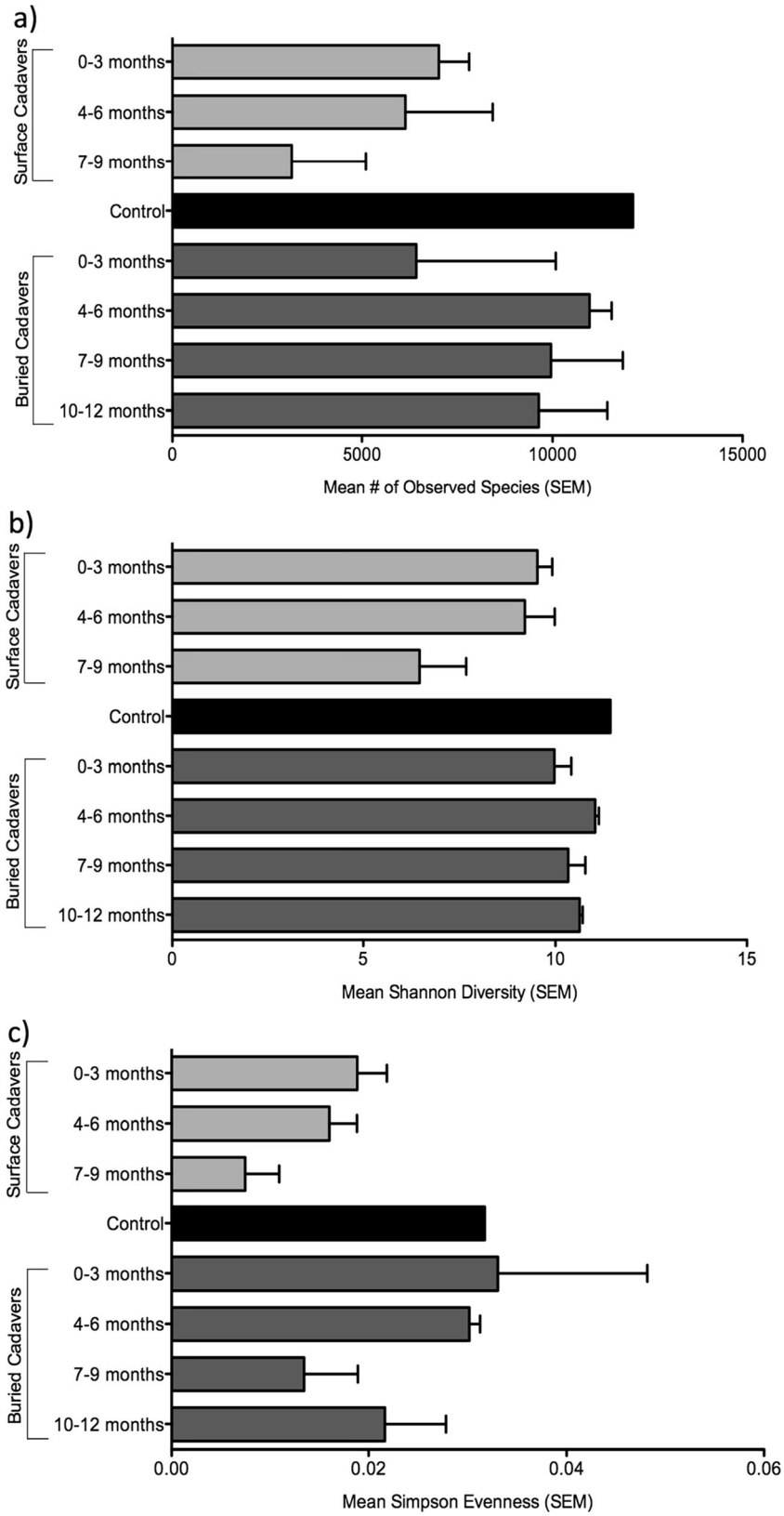

Fig. 1 The mean number of **a** observed number of species based on OTU frequencies, **b** Shannon-Wiener Diversity, and **c** Simpson Evenness decreased over time (0–3 to 4–6 to 7–9 months) for cadavers placed on the surface of the soil, but there was a variable microbial community response for the soil collected from the cadavers that had been buried

surface cadavers. For each buried cadaver, soil was obtained once from above the cadaver using soil corers 30.5 × 5 cm (AMS). Soil was stored in polyethylene bags at −80 °C until analysis [31]. A single surface control soil sample, void of

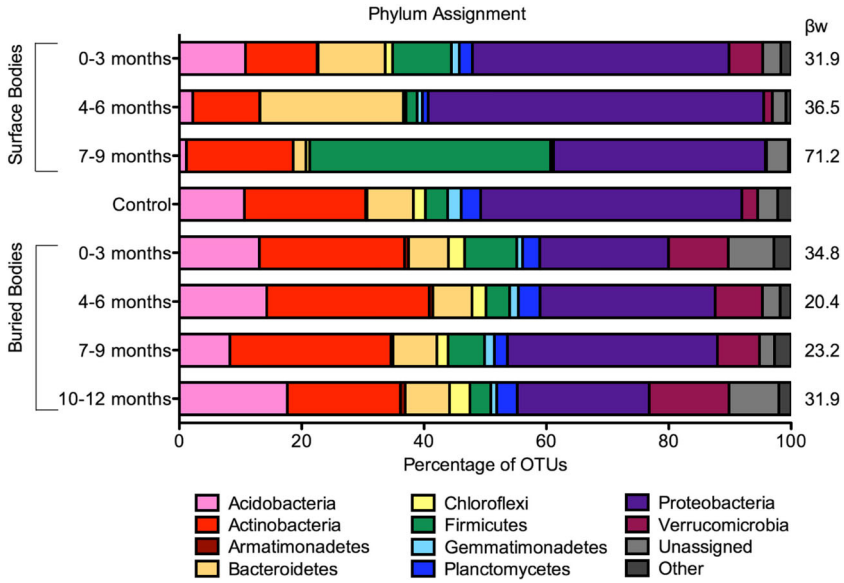

Fig. 2 Relative abundance of 16S rRNA gene sequences (OTUs) from soils collected beneath cadavers (surface) or from soils above cadavers (buried). Community composition is based on the top 12 phyla with a classification of *Other*; the *Other* phyla are those phyla that were present in some but not all samples, unlike the 11 phyla with classifications that were present in every sample. Results from the Whitaker's beta diversity (βw) calculations are on the column on the right side *y*-axis

contact with cadavers, was collected at least 5 m away from all cadavers at the facility.

DNA was extracted in triplicate from 0.25 g of cadaver-soil using Nucleospin® Soil Kit (Macherey-Nagel), as described

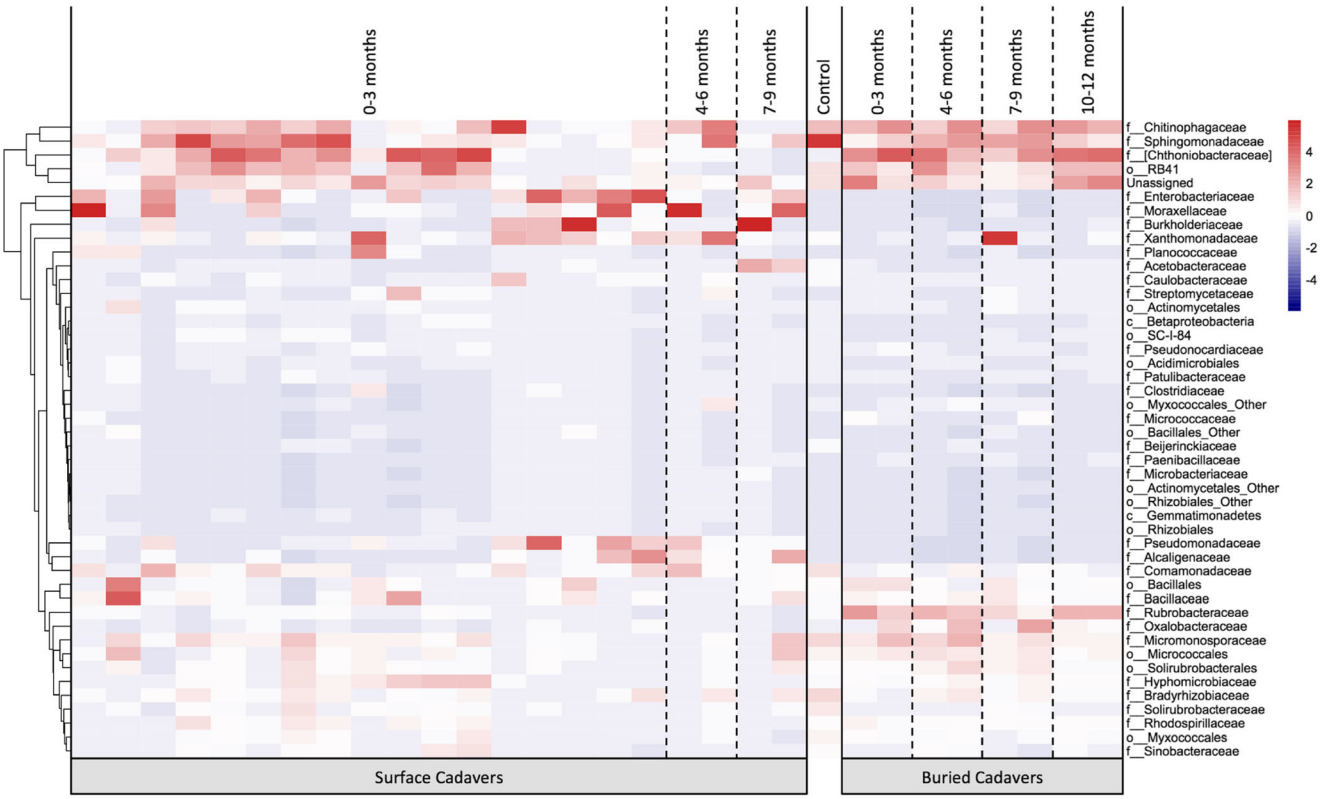

Fig. 3 Heatmap of the V4 region of the microbial 16S rRNA gene sequences (OTUs) at the familial taxonomic resolution. The *solid lines* separating the columns break the surface placed, buried bodies, and the control sample while the *dashed lines* indicate the temporal sampling frequency in months. The *top row* indicates the standard deviation from the mean number of sequences that correspond to the *different colors* of the heatmap

previously [30]. DNA quantification was performed using Quant-iT dsDNA HS Assay kit and Qubit 2.0 (Life Technologies) and by measuring absorbance at 260 nm using NanoDrop2000™ (Thermo Scientific). DNA was stored at −20 °C until further analysis. Illumina MiSeq 16S library construction and sequencing were performed by the Michigan State University Genomics Core Facility using a modified version of the previously described protocol [32]. The V4 region of 16S rRNA genes was amplified with region-specific 515F/806R primers [33–35]. Raw fastq files barcoded 16S rRNA paired-end reads were assembled, quality-filtered, demultiplexed, and analyzed using default settings in QIIME [36, 37]. Chimeric reads and singleton operational taxonomic units (OTUs) were also removed [38]. After quality control, the remaining high-quality sequences were binned into OTUs at a 97 % sequence similarity cutoff using UCLUST [39]. Sequence files were deposited in the European Bioinformatics Institute Sequence Read Archive (PRJEB9166).

DNA isolation for 40 samples was performed with 31 successfully sequenced after quality filtering, which were used in subsequent analysis. Temporal changes in microbial community composition for surface and buried cadavers were visualized in 3-month intervals using relative abundances at phylum and family taxonomic level resolutions and evaluated using weighted phylogenetic diversity (UniFrac) metrics. The time intervals were chosen based on natural taphonomic processes in decomposition time periods (Figure S1). A heatmap was constructed using the pheatmap package (R version 3.0.2, pheatmap version 1.0.2; R Core Team). Community differences between surface and buried remains were analyzed by non-parametric one-way analysis of variance Kruskal-Wallis tests using Prism 5 (GraphPad Software). The alpha value (significance level) was 0.05 for all tests.

Results and Discussion

A total of 1,729,482 reads were detected for all samples. Surface cadaver soil microbial community mean OTU richness (i.e., number of individual OTUs), diversity, and evenness decreased over time (Figs. 1, S1, and S2). Whitaker's beta diversity for surface cadavers exponentially increased, while buried cadavers demonstrated a U-shaped trend over decomposition time (Fig. 2). Proteobacteria, Actinobacteria, and Acidobacteria were the most predominant phyla in all samples (Fig. 2). Surface cadavers soils contained communities that had lower mean relative abundance of Acidobacteria and Verrucomicrobia. However, buried cadaver soil microbial communities did not show distinguishable shifts in the predominate phyla. Our study confirms Roesch et al. (2007) findings that Proteobacteria was the most abundant phylum in all soil types [40, 41], as it was most abundant in control and gravesoils. Ordinal level results were consistent with phyletic level assessments of soil microbial communities (Figure S3). Rhodospirillales had an overall increase in relative abundance for surface cadavers throughout decomposition time periods with a similar trend detected for Burkholderiales; Lactobacillales had the largest increase in relative abundance. There were two predominate orders that decreased in relative abundance: Rhizobiales and Chthoniobacterales. Further, changes in microbial communities for surface cadavers were detected at the family level taxonomic resolution, while buried cadavers maintained a consistent composition across decomposition time (Fig. 3).

Our study provided additional insight into soil ecology of human decomposition by identifying key microbial taxa and community changes that were detected in gravesoil of 18 cadavers naturally decomposing in a terrestrial habitat, making it the largest survey of cadaver gravesoil to date. Our results support the suggestion by Metcalf et al. [18] and others that these microbial communities can be useful in forensic research [6, 10, 42–45]. Additional studies are needed with true replication of cadavers of similar decomposition time periods to confirm our results and validate the findings of other recent papers focusing on the human postmortem microbiome.

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Compliance with Ethical Standards

Competing Interests The authors declare that they have no competing interests.

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