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Human decomposition: A mosaic model for community succession and implications for future forensic research

Purpose of the Project

Decomposition is a mosaic ecosystem with an intimate association of biotic factors such as the corpse, intrinsic and extrinsic bacteria (Galloway 1997; Micozzi 1997; Pless et al. 1997; Campobasso et al. 2001), insects (Catts and Haskell 1990; Catts and Goff 1992; Byrd and Castner 2000; Haskell and Williams 2008), and abiotic factors such as weather and climate. It is important to consider the action of these organisms in maintenance of the intrinsic ecosystem. As normal decomposition proceeds, the cadaver passes through approximately five stages of decomposition, although these stages can be greatly altered in duration by many factors (Galloway 1997; Micozzi 1997; Pless et al. 1997; Campobasso et al. 2001). While this overall result of decomposition—reduction of biomass—is the ordinary progression, our preliminary research has led to the observation that the route taken to get to the skeletal end stage can vary based on the conditions of the cadaver prior to the initiation of decomposition (fresh, refrigerated, or frozen; autopsied vs. non- autopsied) as well as conditions of the cadaver during decomposition. We propose that the differences seen during decomposition can be directly traced to the action of bacteria present in the earliest stages of decomposition. The gaseous by-products of bacterial metabolism then recruit (or repel) insects, which, if given the opportunity, can subsequently eliminate any remaining flesh. Understanding the bacterial basis of decomposition is crucial to understanding decomposition. Despite the integral role of bacteria and subsequent role of insects in the decomposition process, very little is understood regarding the bacterial basis of decomposition or its function in insect recruitment (or repulsion) to (or from) a cadaver (see Vass 2001). To confound this problem, studies have estimated that up to 99% of bacterial species found in nature cannot be cultured by conventional means (Amann et al. 1995, Vass 2001). Since all previous literature in this field is based upon culture-dependent techniques, this represents a gap in knowledge that needs to be addressed. We hypothesize that bacterial species guilds (i.e., complexes of species in an ecosystem that exploit the same resources) changes over time will modulate the tempo and mode of decomposition. By understanding the microbial communities involved in the stages of decomposition a more precise understanding of the overall process will be gained.

Project Design and Methods

The project tested microbial community structure and its change through time, or succession, in human cadavers. There are three main sources for bacterial biodiversity in the cadaver environment: the intrinsic cadaver microbiome, the soil the cadaver is lying on, and bacteria brought to the cadaver by flies. To test our hypothesis that bacterial species communities changes over time and ultimately modulate the tempo and mode of decomposition, four hypotheses were tested. We documented bacteria in and on the cadaver and compared that to documented bacteria brought to the cadaver by flies and acquired from the soil through time. We also identified gasses and insects associated with each stage of decomposition.

- Hypothesis A: Decomposition varies as result of successive changes in bacterial species guilds (i.e., complexes of species in an ecosystem that exploit the same resources) inhabiting the cadaver antemortem and colonizing the cadaver postmortem.
 - Prediction A: Decomposition (as determined by accumulated degree hour) will possess different species guilds of bacteria.
- Hypothesis B: Gasses emanating from the cadaver are a byproduct of bacterial metabolism.

- Prediction B: As bacterial species change through time, so will the gasses emanating from the cadaver.
- Hypothesis C: Fly species attracted to the cadaver are recruited (or repelled) by gasses emanating from the cadaver.
 - Prediction C: As gasses change through time, so will the species of fly recruited (or repelled) to the cadaver.
- Hypothesis D: Microbial soil composition underneath a cadaver changes through time.
 - Prediction D: As decomposition proceeds, bacterial species change through time.

Habitat

Cadavers were placed outdoors to decompose under natural conditions at the Southeast Texas Applied Forensic Science (STAFS) facility at the Center for Biological Field Studies (CBSF), Sam Houston State University, Huntsville, Texas. The STAFS facility is a state-of-the-art, morgue-like laboratory designed to accommodate research on cadavers and available for SHSU faculty use. The approximately 9-acres of fenced-off land is exclusive to non-authorized personnel but allows all manner of native scavengers access to cadavers to simulate normal Pineywoods Ecoregion conditions (characterized by a large distribution of pine trees and acidic soils (Lindgren et al. 2010)). Two bodies were used for each experiment with four experiments per year with each experiment corresponding to the four seasons (winter, spring, summer, and fall). We repeated this for a total of three years. Cadavers were unclothed, placed supine, with head oriented north. All measurable aspects of the ecosystem were documented: by noting gross condition of the cadaver, the cadaver temperature for sample sites, the maggot mass temperature, and weather conditions. Accumulated degree hour, which measures the total physiological time rather than calendar time, was documented.

Specimen Collection

Bacteria, gasses, and insects were sampled at regular intervals according to the scheme presented in Tables 1 and 2 (Appendix 1). Bacteria were sampled via sterile swabbing, soil was sampled via small scoops and then swabbed, gasses were samples using SPME fibers, and insects were collected via aerial net.

Specimen Identification

The 16S rRNA gene sequence has been used extensively to study the evolution of bacterial species. We will used this next generation deep sequencing approach to test our hypotheses related to the microbial community structure in the decomposing. Briefly, the microbiota from 1244 samples from the cadaver and associated insects were measured by deep sequencing of 16S rRNA genes. Total microbial genomic DNA was isolated from samples using the HMP- benchmarked MoBio PowerSoil Kit protocol, and sequencing of the bacterial encoded 16S rRNA genes contained in every sample were performed by amplifying the 16S rRNA genes with barcoded, degenerate primers that target the V1-V5 regions of the gene. Rarefaction and collector's curves were constructed using sequence data for each sample to ensure that we are sampling the majority of the bacterial diversity present. Using QIIME, all sequences were associated with their sample of origin based on their unique barcodes, and then sequences were quality trimmed and processed. One quality trimmed, a metadata file describing all samples and environmental data were input into the QIIME pipeline for taxonomic and OTU-based metagenomic analysis including rarefaction curves and various diversity indices (alpha diversity), UniFrac-based 3D Principal Coordinate Analysis (PCoA) plots (beta diversity), taxonomic composition-

based stacked histograms and heat maps (beta diversity), a phylogenetic tree, and various output tables.

Identification of the volatile organic compounds produced during the various stages of decomposition was documented using SPEME fibers from emanating gases during the various stages of decomposition. Gasses were collected near the head, chest, and abdomen of each cadaver to determine any differences in gases produced in different areas of the cadaver and compared with the identified bacteria and insects for each cadaver at each stage of decomposition. The analysis of the samples was conducted by gas chromatography-mass spectrometry for the VOCs and the cotton swabs were sent to Baylor College of Medicine for sequencing. The mass spectra of VOCs were identified and confirmed utilizing the NIST08 database and the NIST website. Data for identified compounds were cross-referenced with ChemSpider or NIST database for structures and boiling points. Statistical analysis was performed using R to examine the relationships between the VOCs and microbes identified. Using the relative populations of specific microbes present and time lapse as a basis, a comparative analysis was performed to identify a possible link between VOCs detected and microbes present during the decomposition process.

Adult flies were collected via sweep-netting, pinned or be mounted and labeled. All specimens were identified in house. The tarsi, labellum, and oocytes were dissected using sterile techniques and bacteria were identified using the above sequencing protocols.

TO BE COMPLETED: *Establish correlation between bacterial succession, gasses of metabolism, insect recruitment and abiotic factors.* This portion of the grant represents a major undertaking and it is still underway.

Results

Shifting Community Structure

Early analysis of the first sets of cadavers have shown that bacterial community structure of initial samples will vary (likely due to variation of microbiome between individuals) but as decomposition progresses, all samples became more similar. As seen in Fig. 1, there is a wide separation between samples on PC1 initially, but as decomposition progresses (left to right across the x-axis), the samples begin to cluster closer together. The cadavers also were characterized by similar changes in the relative abundance of phyla present through time for all body sites (Fig. 2). When looking at the mean relative abundance of all samples by site, we see a few genera predominate (Fig. 3). *Ignatzschineria* and *Wohlfahrtimonas* were abundant in most samples regardless of sample site.

As seen in Fig. 4, at bloat and purge and until tissues began to dehydrate or were removed, bacteria associated with flies, such as *Ignatzschineria* and *Wohlfahrtimonas*, were common. After dehydration and skeletonization, bacteria associated with soil, such as *Acinetobacter*, were common at most body sites sampled.

When looking at principle coordinate analysis for all samples by cadaver ID, we do not see any observable trends (Fig 5A). This would indicate that, while there is some variation among different cadavers, it was not the major driving factor in the successional change of the community structure. When looking at seasonal effect, we see a strong grouping of samples associated with each season (Fig 5B). This is likely due to differences in average temperatures the cadavers were exposed to. The most striking difference is seen in winter months. We hypothesize that this difference is due to lack of insect activity during colder winter months and see a lack of insect associated bacteria with these samples. When we look at samples split by stage of decomposition, we see a clear trend of samples grouping together by stage showing the progression as decomposition progresses (Fig 5C). Finally, when looking at samples by site, we see clear clustering by site (skin, fecal, oral), indicating that as decomposition

progresses, the community structure of skin sites remains more similar than that of fecal or oral.

We used Random Forest regression to model the PMI as a function of the changes in OTU abundances. 10-fold cross-validation (CV) was performed to tune and evaluate the models, i.e., the input samples are partitioned into 10 subsets and models are built on 9 subsets and validated using the rest subset. This cross-validation process is then repeated 10 times, with each of the 10 subsets used exactly once as the validation data. The accuracy of models is measured as root mean squared error (RMSE). It is calculated as the standard deviation of the differences between the predicted and observed values, representing the average prediction error in the same unit of original data (in this case, days). The most parsimonious model (in order to avoid overfitting to the input training data) within one standard deviation of the optimal accuracy were chosen as the final model (Fig 6)

Fly-associated bacteria

We have demonstrated that wet stages of decomposition have a high abundance of the fly associated bacteria *Ignatzschineria* and *Wohlfahrtimonas* and have begun to investigate the source of these bacteria relative to adult flies attracted to the cadavers. To assess diversity, sample processing, 16S rRNA gene amplification, and Illumina sequencing were performed following protocols benchmarked as part of the Human Microbiome Project and detailed above. 16s data were processed and analyzed using QIIME version 1.7.0. Samples were grouped according to fly identity (genus and species), fly body site, cadaver of origin, and accumulated degree hours. Special attention is paid to bacteria that have only been recorded in association with flies before. Sites show an abundance of *Ignatzschineria* and *Wohlfahrtimonas* on the labellum, tarsi, and oocytes. This is the first time these bacterial genera have been associated with human remains (Fig 6-9).

Volatile Organic Compounds Emitted During Decomposition

We have begun to analyze volatile chemical data collected in conjunction with bacterial samples. These data show varying compounds and different time points. As with our fly data, these data are still quite preliminary but will be the focus of further investigation (Fig 10).

Scholarly Products

Our data collection was highly successful and we have collect well over 10,000 bacterial, VOC, and insect samples with only a fraction having been sequenced and/or identified.

To date our scholarly output includes 5 papers publishes and 51 presentations (oral and poster format) (see Appendix C for a complete list). We are pursuing completion of the papers in preparation (6 more in total) and will be submitting those soon for publication.

We are working on developing an Internet database (“Virtual Museum”) detailing microbial succession. Because we are still analyzing data, we are not finished with the Virtual Museum.

Publications

Metcalf, J.L., Xu, Z.Z., Weiss, S., Lax, S., Van Treuren, W., Hyde, E.R., Song, S.J., Amir, A., Laresen, P., Sangwan, N., Haarmann, D.P., Humphrey, G.C., Ackerman, G., Thompson, L.R., Lauber, C., Bibat, A., Nicholas, C., Gebert, M.J., Petrosino, J.F., Reed, S.C., Gilbert, J.A., Lynne A.M., Bucheli, S.R., Carter, D.O, and Knight, R. 2015. A Universal Clock for Estimating the Postmortem Interval. Science. DOI 10.1126/science.aad2646

Hyde, E.R., Haarmann, D.P., Petrosino, J.F., Lynne, A.M., and Bucheli, S.R., 2014. Initial Insights into Bacterial Succession During Human Decomposition. *International Journal of Legal Medicine*. DOI 10.1007/s00414-014-1128-4

Zhang, X., Glennie, C.L., Bucheli, S.R., and Lynne, A.M. 2014. Terrestrial Laser Scanning and a Degenerated Cylinder Model to Determine Gross Morphological Change of Cadavers under Conditions of Natural Decomposition. *Forensic Science International*. 241:35-45.

Bucheli, S.R., Z. Pan, C.L. Glennie, A.M. Lynne, D.P. Haarmann, J.M. Hill. 2014. Terrestrial Laser Scanning to Model Sunlight Irradiance on Cadavers Under Conditions of Natural Decomposition. *International Journal of Legal Medicine*. 128(4):725-732.

Hyde, E.R., Haarmann, D., Lynne, A.M., Bucheli, S.R., and Petrosino, J.F. 2013. The Living Dead: Bacterial Community Structure of a Cadaver at the Onset and End of Bloat Stage of Decomposition. *PLoS ONE*. 8(10): e77733. Doi10.1371/journal.pone.0077733.

Implications for Criminal Justice Policy and Practice in the United States

Our long term goal is to be able to provide a model for decomposition that accounts for the mosaic nature of the process by studying intrinsic and extrinsic bacteria and its relationship to insect recruitment. By showing that bacterial species guilds change over time, characterization of this change will provide us and future researchers with a more precise understanding of modulation of tempo and mode of decomposition.

Understanding the identity of bacteria, insects, and gasses produced will provide insight into how a cadaver decomposes. These data will have later significance when incorporated into models that will be aiding in determination of the postmortem interval by providing a more precise method of analysis. These data will be used as markers for comparison when evaluating change in the microbiome as decomposition progresses. In the future, scientists will likely be able to distinguish the antemortem microbiome from the postmortem microbiome as well as determine exogenous bacterial species (i.e. brought by flies or already present in the soil) from endogenous bacteria. The novelty of this research will allow for the development of a predictive diversity program.

Meeting this goal will introduce a completely novel form of physical evidence to the justice system that can be used to complement well-established analyses such as anthropology, entomology, pathology, and taphonomy. Furthermore, our project has great potential to provide data that can be used as stand-alone evidence when well-established analyses are not available or not relevant. Our project is designed so that all aspects of reliability are addressed, such as those used in admissibility hearings in the United States. Thus, the findings from this research are poised for admission as evidence as rapidly as possible, should we find that they are accurate, precise and reproducible.

Finally, the data generated for this project will be useful for forensic microbiome applications beyond estimating PMI, which satisfies NIJ-2015-3985 solicitation's Innovative Areas of Research Opportunities on the microbiome. We have shown previously that gravesoil microbial communities hold great promise for locating clandestine graves, and that some soil decomposer microbes appear universal regardless of soil type (Metcalf et al. submitted, Supporting Data).

APPENDIX A

Tables

TABLE 1. Locations sampled on the cadaver before decomposition, during active decomposition, and after decomposition. Soil and gas samples were at the same time as cadaver samples. All insects were collected as well.

		Internal		External		Soil	Gas
		Deep Colon	Upper Esophagus	Superficial Face	Superficial Torso		
Initial		1	1	1	1	1	1
During Decomposition							
	Fresh	1	1	1	1	1	1
	Bloat	3	3	3	3	3	3
	Fermentation	3	3	3	3	3	3
	Dry	1	1	1	1	1	1
Post						1	

TABLE 2. Flies collected and sampled for bacteria survey.

Flies		Sites sampled		
		Internal	External	
		Ocytes	Tarsi	Labellum
<i>Lucilia</i>	<i>mexicana</i>	1	1	1
	<i>eximia</i>	1	1	1
	<i>coeruleiviridis</i>	1	1	1
<i>Phormia</i>	<i>regina</i>	1	1	1
<i>Cochliomyia</i>	<i>macellaria</i>	1	1	1
<i>Chrysomya</i>	<i>rufifacies</i>	1	1	1

APPENDIX B

Figures

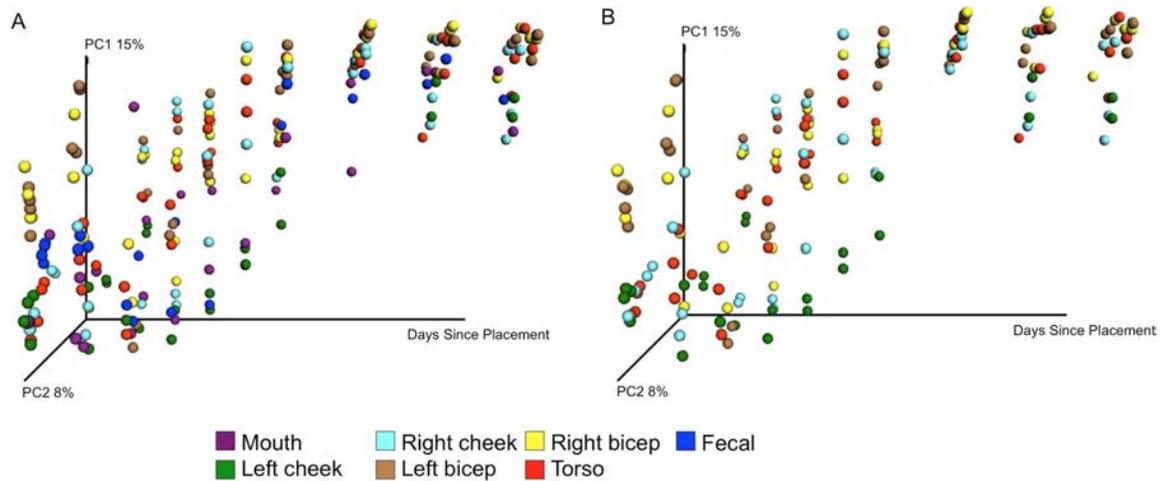


Fig. 1 Unweighted UniFrac-based PCoA plot for a all samples and b only skin samples (right and left cheek, right and left bicep, torso). The x-axis is defined by days since placement.

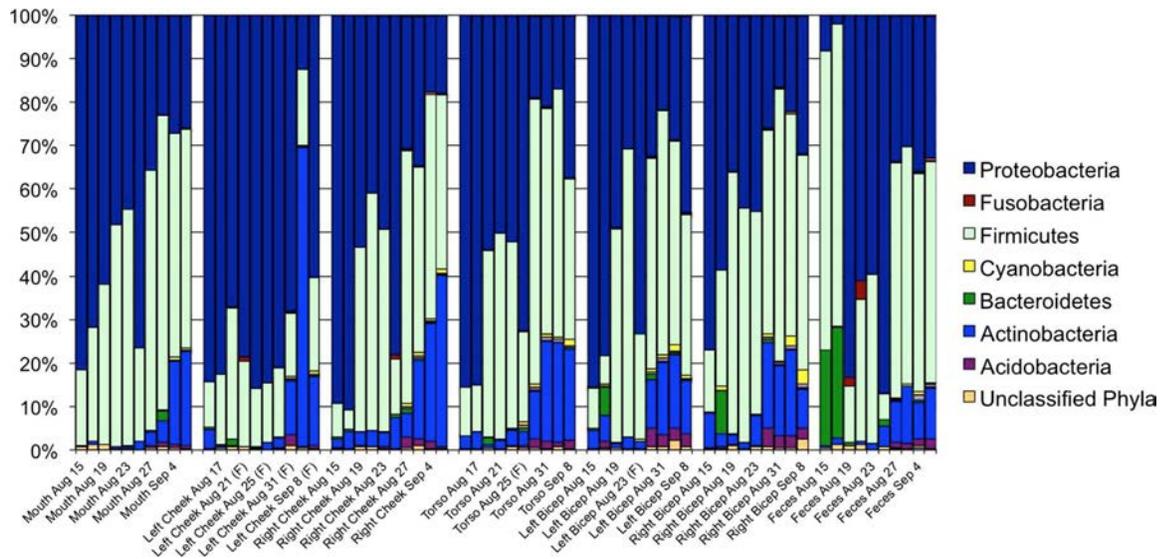


Fig. 2 Relative abundances of phyla present in each body site through time. The percent abundance of each phylum was determined for each body site for each body at each sampling date. The percent abundances for the two bodies were then averaged to obtain the mean relative abundance of phyla present at each body site at each sampling date. (F) denotes samples that were only obtained from the female cadaver (STAFS 2012-021) due to accelerated decomposition at the site in the male cadaver (STAFS 2012-023).

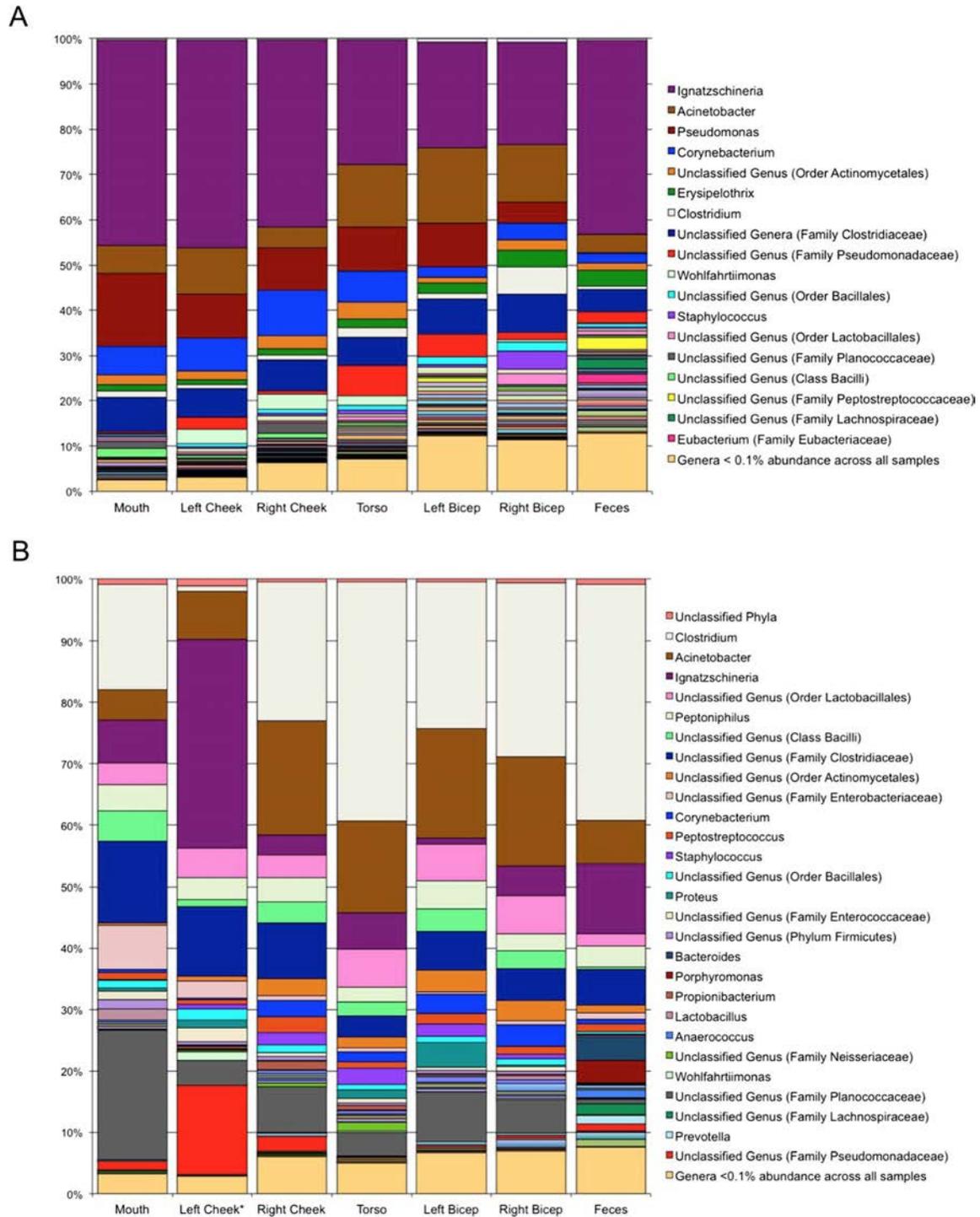


Fig. 3. Mean relative abundances of genera present, all sampling dates inclusive, of body sites for a STAFS 2012-021 and b STAFS 2012-023.

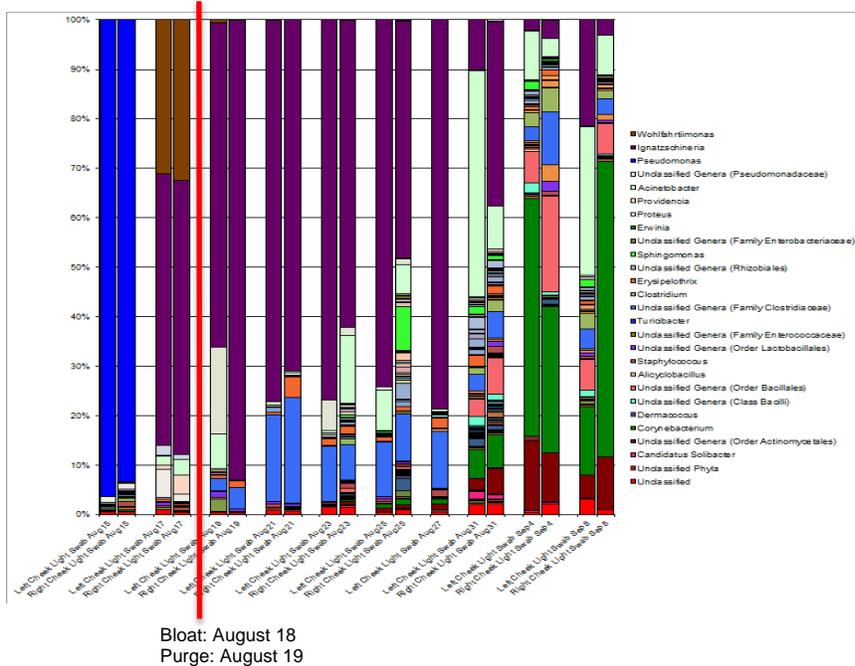


Fig. 4. Relative abundances of genera present on cadavers over time. The red line indicates where bloat and purge occur.

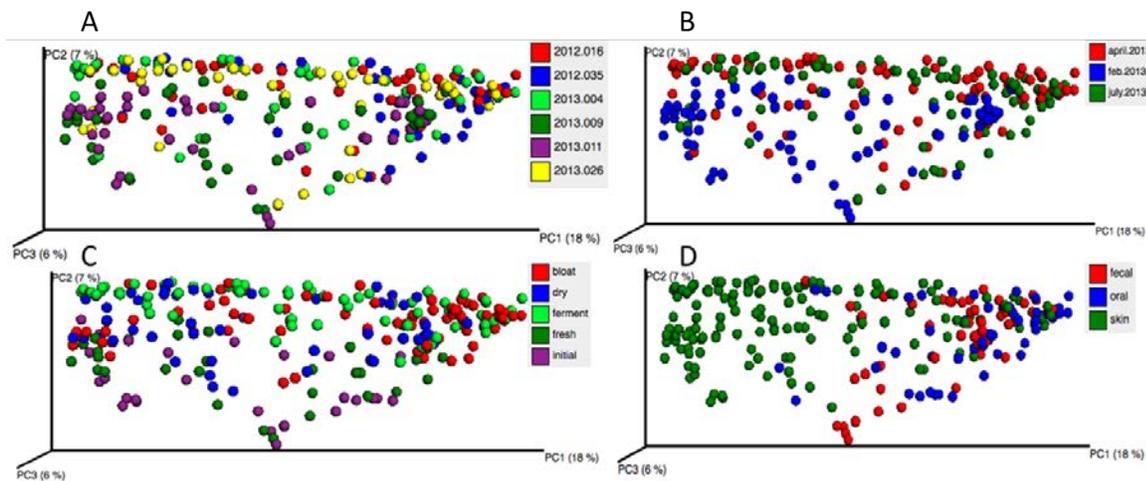


Fig. 5. Unweighted Unifrac-based principle coordinate analysis for all cadaver samples. A) Cadaver ID. B) Season of cadaver placement. C) Decomposition stage. D) Sample habitat type.

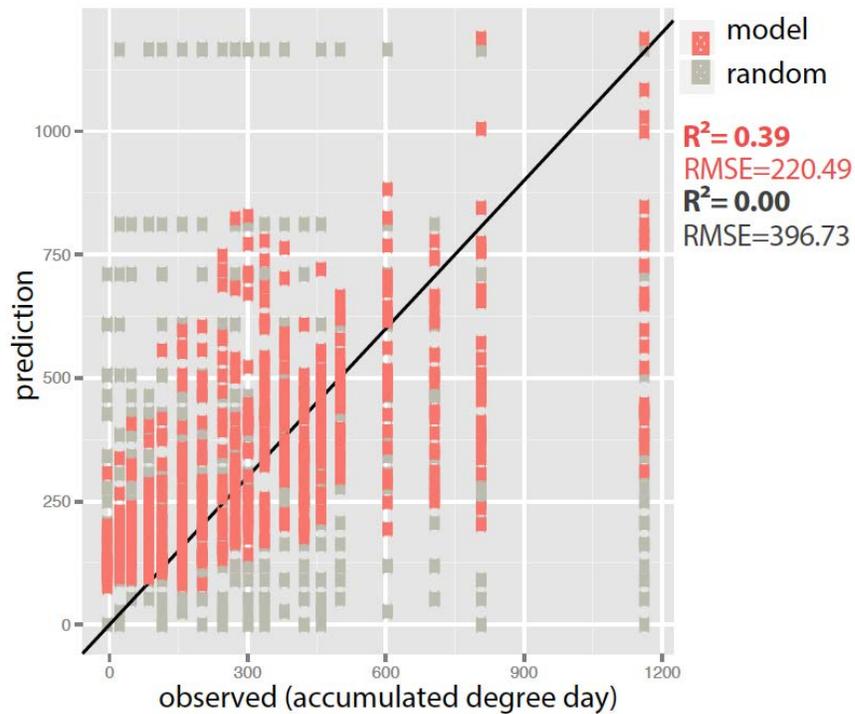


Fig 6 A 16S rRNA-based Random Forests (RF) model using mouse decomposition data to train the model and predict the PMI of human body. Each point in the plot indicates a sample collected at a certain PMI, with RF predicted PMI in red and randomly guessed PMI in gray.

Fig. 6. Relative abundances of genera of bacteria present on the tarsi, labellum, and oocytes of the first 40 flies collected from cadavers in 2014.

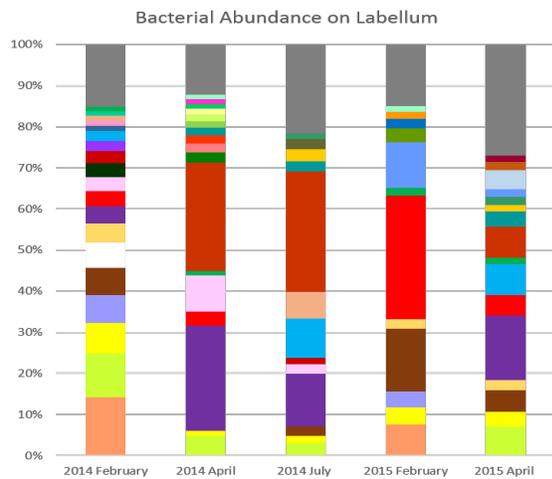
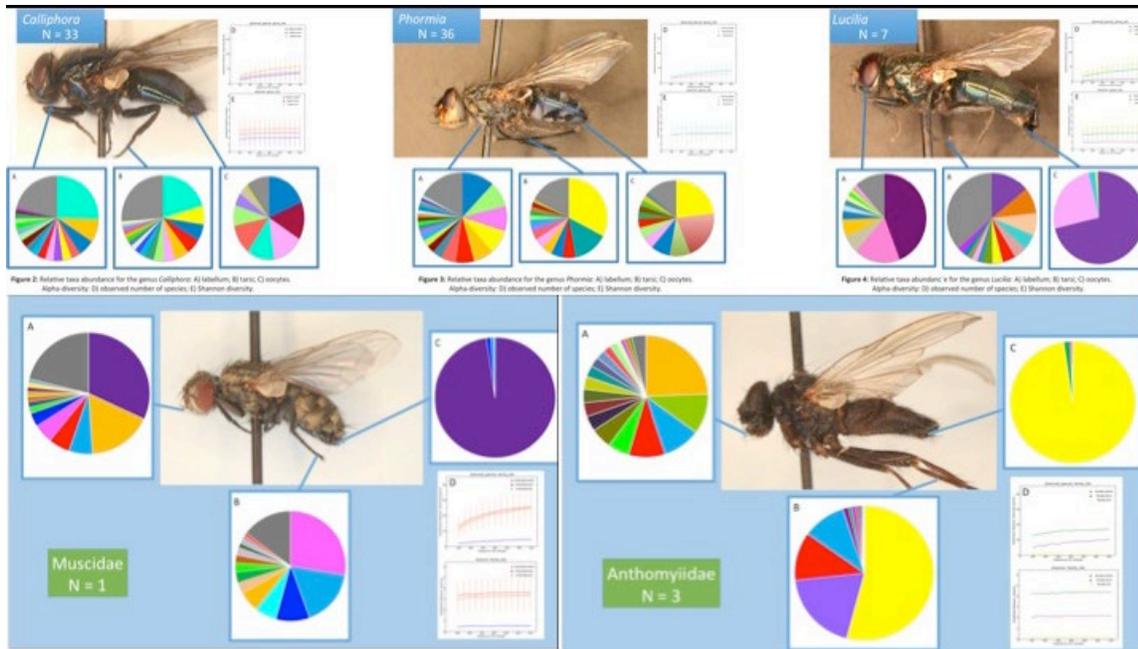


Fig. 7. Relative abundance of bacteria found on fly labellum collected for 5 different cadaver sets

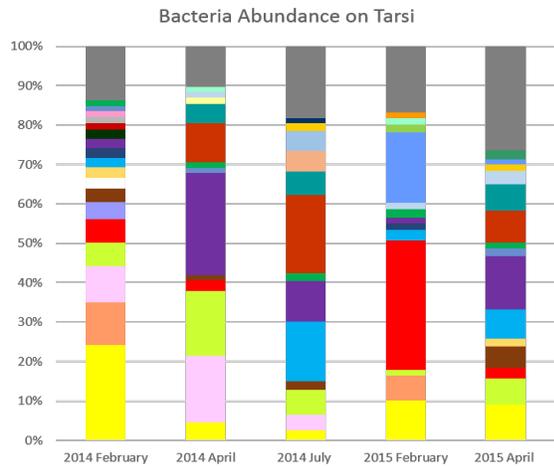


Fig. 8. Relative abundance of bacteria found on fly tarsi collected for 5 different cadaver sets

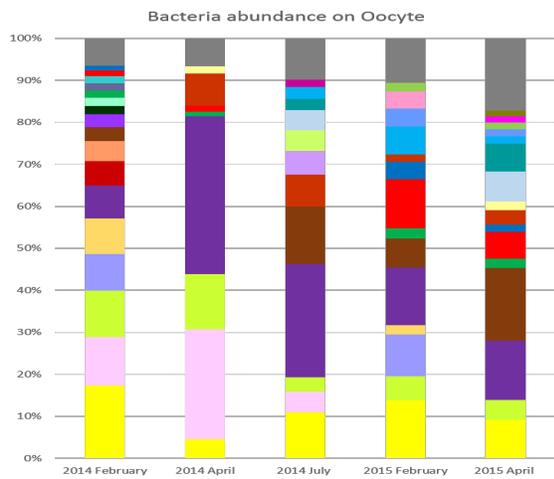


Fig. 9. Relative abundance of bacteria found on fly oocyte collected for 5 different cadaver sets

k_Bacteria;p_Bacteroidetes;c_[Saprospirae];o_[Saprospirales];f_Chitinophagaceae;g_Sediminibacterium
 k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Porphyrimonadaceae;g_Dysgonomonas
 k_Bacteria;p_Bacteroidetes;c_Flavobacteriia;o_Flavobacteriales;f_Flavobacteriaceae;g_Myroides
 k_Bacteria;p_Firmicutes;c_Bacilli;o_Bacillales;f_g_
 k_Bacteria;p_Firmicutes;c_Bacilli;o_Bacillales;f_Alicyclobacillaceae;g_Alicyclobacillus
 k_Bacteria;p_Firmicutes;c_Bacilli;o_Bacillales;f_Bacillaceae;g_
 k_Bacteria;p_Firmicutes;c_Bacilli;o_Bacillales;f_Planococcaceae;g_
 k_Bacteria;p_Firmicutes;c_Bacilli;o_Bacillales;f_Planococcaceae;g_Kurthia
 k_Bacteria;p_Firmicutes;c_Bacilli;o_Bacillales;f_Planococcaceae;g_Sporosarcina
 k_Bacteria;p_Firmicutes;c_Bacilli;o_Bacillales;f_Staphylococcaceae;g_Staphylococcus
 k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Enterococcaceae;g_Enterococcus
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 k_Bacteria;p_Proteobacteria;c_Betaproteobacteria;o_Neisseriales;f_Neisseriaceae;g_Vitreoscilla
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 k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Pseudomonadales;f_Moraxellaceae;g_Acinetobacter
 k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Pseudomonadales;f_Moraxellaceae;g_Psychrobacter
 k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Pseudomonadales;f_Pseudomonadaceae;g_
 k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Pseudomonadales;f_Pseudomonadaceae;g_Pseudomonas
 k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Xanthomonadales;f_Xanthomonadaceae;g_Ignatzschineria
 k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Xanthomonadales;f_Xanthomonadaceae;g_Wohlfahrtiimonas
 k_Bacteria;p_Tenericutes;c_Mollicutes;o_Entomoplasmatales;f_g_
 Taxon < 1%

Color coded key for figs 1 – 9.

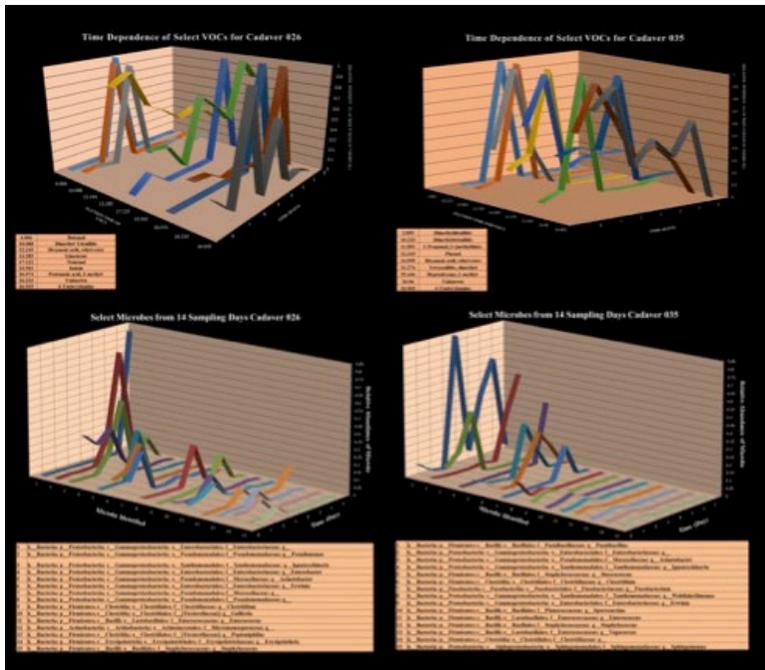


Fig. 10. Preliminary volatile chemical data.

APPENDIX C

Detailed list of Presentations

Presentations

Bucheli, S.R. The Living Dead: Cadavers as Ecosystems. University of Michigan Biological Station. Pellston, MI. July 2016.

Bucheli, S.R. The Living Dead: Cadavers as Ecosystems. University of California San Diego. San Diego, CA. May 2016.

Deel, H., Petrosino, J.F., Bucheli, S.R., and Lynne, A.M., The Core Microbiome associated with Human Decomposition. Tri-Beta South Central Regional Convention. Cedar Hill, TX April 2016

Woelfel-Monsivais, C.H., Greenwood, M.J., Haarmann, D.P., Petrosino, J.F., Bucheli, S.R., and Lynne, A.M. A seasonal comparison of shifting bacterial communities during human cadaver decomposition in southeast Texas. ASM Texas Branch Spring Meeting. New Braunfels TX, April 2016.

Vasquez, J.K, Petrosino, Lynne A.M., and Bucheli, S.R. A Study of Shifting Bacterial Communities during Human Cadaver Decomposition in Southeast Texas: A Male and Female Comparison. ASM Texas Branch Spring Meeting. New Braunfels TX, April 2016.

Paez, L.M., Smith, L.R., Petrosino, J.F., Bucheli, S.R., and Lynne, A.M. Study of shifting oral and fecal skin bacterial communities during human cadaver decomposition in southeast Texas. ASM Texas Branch Spring Meeting. New Braunfels TX, April 2016.

Olsen, K.M., Petrosino, J.F., Bucheli, S.R., and Lynne, A.M. Effects of Postmortem Storage Conditions on Shifting Skin Bacterial Communities during Human Cadaver Decomposition in Southeast Texas. ASM Texas Branch Spring Meeting. New Braunfels TX, April 2016.

Munoz, B., Petrosino, J.F., Bucheli, S.R., and Lynne, A.M. Succession of soil bacterial communities during human cadaver decomposition in southeast Texas. ASM Texas Branch Spring Meeting. New Braunfels TX, April 2016.

Smith, L.R., Petrosino, J.F., Bucheli, S.R. and Lynne, A.M. A Study of the Spatial and Temporal Features of the Human Face Microbiome during Decomposition in Southeast Texas. ASM Texas Branch Spring Meeting. New Braunfels TX, April 2016.

Lynne, A.M., The Living Dead: The Microbiome of Human Cadavers and Its Forensic Implications. University of Tulsa. Tulsa, OK, April 2016.

Deyne, T.A., Haines, D.C., Lynne, A.M., and Bucheli, S.R. Association Between Volatile Organic Compounds and Microbes Present During the Decomposition of a Cadaver. AAFS Annual Scientific Meeting. Las Vegas, NV. February 2016

Smith, L.R., Petrosino, J.F., Bucheli, S.R. and Lynne, A.M. A Preliminary Study of Shifting Bacterial Communities of the Face During Human Cadaver Decomposition in Southeast Texas. AAFS Annual Scientific Meeting. Las Vegas, NV. February 2016

King, K.L, Lynne, A.M., Bucheli, S.R., and Petrosino, J.F. Bacteria Triggering a Preference in Flesh Flies (Diptera: Sarcophagidae) Associated With Human Cadavers. AAFS Annual Scientific Meeting. Las Vegas, NV. February 2016.

Bucheli, S.R. The Living Dead: Cadavers as Ecosystems. Museum of Natural History. Bulawayo, Zimbabwe. December 2015.

Lynne, A.M., The Living Dead: The Microbiome of Human Cadavers and Its Forensic Implications. Southeastern Louisiana State University. Hammond, LA, Nov 2015.

Smith, L.R., Lynne, A.M., and Bucheli, S.R., The Living Dead. ASM General Meeting, New Orleans, LA. May 2015.

Smith, L.R., Petrosino, J.R., Buchlei, S.R., and Lynne, A.M. A Preliminary Study of Shifting Bacterial Communities of the Face During Human Cadaver Decomposition in Southeast Texas. ASM General Meeting, New Orleans, LA. May 2015.

King, K., Smith, L.R., Bucheli, S.R., and Lynne, A.M. Preference Behavior of Flesh Flies (Diptera; Sarcophagidae) Associated with Human Cadavers. ASM General Meeting, New Orleans, LA. May 2015.

Berry III, R., King, K., Haarmann, D., Petrosino, J.F., Bucheli, S.R., and Lynne, A.M. Microbiome of Flies (Diptera) Associated with Human Cadavers. ASM General Meeting, New Orleans, LA. May 2015.

Greenwood, M.J., Haarmann, D.P., Petrosino, J.F., Bucheli, S.R., and Lynne, A.M. A Preliminary Study of Season Effect on Bacterial Communities During Human Cadaver Decomposition in South East Texas. SHSU Undergraduate Research Symposium, Huntsville, TX 77341. April 2015.

Paez, L.M., Vasquez, J.K., Haarmann, D.P., Petrosino, J.F., Bucheli, S.R., and Lynne, A.M. A Preliminary Study of Shifting Skin Bacterial Communities During Human Cadaver Decomposition in Southeast Texas. SHSU Undergraduate Research Symposium, Huntsville, TX 77341. April 2015.

Lueck, Z.T., Plummer, D.A., Haarmann., D.P., Petrosino, J.F., Bucheli, S.R., and Lynne, A.M. A Preliminary Study of Shifting Oral and Fecal Bacterial Communities During Human Cadaver Decomposition in Southeast Texas. SHSU Undergraduate Research Symposium, Huntsville, TX 77341. April 2015.

Lueck, Z.T., Plummer, D.A., Haarmann., D.P., Petrosino, J.F., Bucheli, S.R., and Lynne, A.M. A Preliminary Study of Shifting Oral and Fecal Bacterial Communities During Human Cadaver Decomposition in Southeast Texas. ASM Texas Branch Spring Meeting. New Braunfels TX, Mar 2015.

Smith, L.R., Haarmann, D.P., Petrosino, J.F., Lynne, A.M, and Bucheli, S.R. A Preliminary Study of Shifting Bacterial Communities of the Face during Human Cadaver Decomposition in Southeast Texas. ASM Texas Branch Spring Meeting. New Braunfels TX, Mar 2015.

King, K., Berry, R., Petrosino, J.F., Bucheli, S.R., and Lynne, A.M.. Comparison of the Microbiomes of Non-Calliphoridae Flies and Accompanying Cadaver Sites Associated with Human Cadavers. ASM Texas Branch Spring Meeting. New Braunfels TX, Mar 2015.

Woelfel-Monsivais, C.H., Greenwood, M.J., Haarmann, D.P., Petrosino, J.F., Bucheli, S.R., and Lynne, A.M. A Preliminary Study of Season Effect on Bacterial Communities During Human Cadaver Decomposition in South East Texas. ASM Texas Branch Spring Meeting. New Braunfels TX, Mar 2015.

Vasquez, J.K., Smith, L.R., Petrosino, J.F., Lynne, A.M., and Bucheli, S.R. A Study of Shifting Bacterial Communities during Human Cadaver Decomposition in Southeast Texas: A Male and Female Comparison. ASM Texas Branch Spring Meeting. New Braunfels TX, Mar 2015. Honorable Mention Undergraduate Poster Award

Paez, L.M., Vasquez, J.K., Haarmann, D.P., Petrosino, J.F., Bucheli, S.R., and Lynne, A.M. A Preliminary Study of Shifting Skin Bacterial Communities During Human Cadaver Decomposition in Southeast Texas. ASM Texas Branch Spring Meeting. New Braunfels TX, Mar 2015.

Bucheli, S.R. The Living Dead: Cadavers as Ecosystems. Montgomery County Lawyers Association. Conroe, TX February 2015

Smith, L.R., Haarmann, D.P., Petrosino, J.F., Lynne, A.M, and Bucheli, S.R. A Preliminary Study of Shifting Bacterial Communities of the Face during Human Cadaver Decomposition in Southeast Texas. The 2014 Biological Sciences Graduate Research Symposium. Huntsville, TX, Dec 2014

Woelfel-Monsivais, C.H., Greenwood, M.J., Haarmann, D.P., Petrosino, J.F., Bucheli, S.R., and Lynne, A.M. A Preliminary Study of Season Effect on Bacterial Communities During Human Cadaver Decomposition in South East Texas. Texas Association of Biological Anthropologists. Huntsville, TX, Nov 2014.

Smith, L.R., Haarmann, D.P., Petrosino, J.F., Lynne, A.M, and Bucheli, S.R. A Preliminary Study of Shifting Bacterial Communities of the Face during Human Cadaver Decomposition in Southeast Texas. Texas Association of Biological Anthropologists. Huntsville, TX, Nov 2014. Co-First Place Poster Presentation

Paez, L.M., Vasquez, J.K., Haarmann, D.P., Petrosino, J.F., Bucheli, S.R., and Lynne, A.M. A Preliminary Study of Shifting Skin Bacterial Communities During Human Cadaver Decomposition in Southeast Texas. Texas Association of Biological Anthropologists. Huntsville, TX, Nov 2014. Co-First Place Poster Presentation

Lueck, Z.T., Plummer, D.A., Haarmann, D.P., Petrosino, J.F., Bucheli, S.R., and Lynne, A.M. A Preliminary Study of Shifting Oral and Fecal Bacterial Communities During Human Cadaver Decomposition in Southeast Texas Texas Association of Biological Anthropologists. Huntsville, TX, Nov 2014. Co-First Place Poster Presentation

Haarmann, D.P., Hyde, E.R., Petrosino, J.F., Lynne, A.M., and Bucheli, S.R. The Fly Associated Bacteria *Ingnatchineria* and *Wohlfahrtiimonas* on Cadavers Through Time. ASM Texas Branch Fall Meeting. Houston TX, Nov 2014. Second Place Graduate Student Oral Presentation

Smith, L.R., Haarmann, D.P., Petrosino, J.F., Lynne, A.M, and Bucheli, S.R. A Preliminary Study of Shifting Bacterial Communities of the Face during Human Cadaver Decomposition in Southeast Texas. ASM Texas Branch Fall Meeting. Houston TX, Nov 2014.

King, K., Berry, R., Petrosino, J.F., Bucheli, S.R., and Lynne, A.M. Microbiome of Blow Flies Associated with Human Cadavers. ASM Texas Branch Fall Meeting. Houston TX, Nov 2014.

Berry, R., King, K., Haarmann, D.P., Petrosino, J.F., Bucheli, S.R., and Lynne, A.M. Microbiome of Blow Flies Associated with Human Cadavers. ASM Texas Branch Fall Meeting. Houston TX, Nov 2014.

Woelfel-Monsivais, C.H., Greenwood, M.J., Haarmann, D.P., Petrosino, J.F., Bucheli, S.R., and Lynne, A.M. A Preliminary Study of Season Effect on Bacterial Communities During Human Cadaver Decomposition in South East Texas. ASM Texas Branch Fall Meeting. Houston TX, Nov 2014.

Paez, L.M., Vasquez, J.K., Haarmann, D.P., Petrosino, J.F., Bucheli, S.R., and Lynne, A.M. A Preliminary Study of Shifting Skin Bacterial Communities During Human Cadaver Decomposition in Southeast Texas. ASM Texas Branch Fall Meeting. Houston TX, Nov 2014.

Lueck, Z.T., Plummer, D.A., Haarmann., D.P., Petrosino, J.F., Bucheli, S.R., and Lynne, A.M. A Preliminary Study of Shifting Oral and Fecal Bacterial Communities During Human Cadaver Decomposition in Southeast Texas. ASM Texas Branch Fall Meeting. Houston TX, Nov 2014.

Haarmann, D.P., Hyde, E.R., Petrosino, J.F., Lynne, A.M., and Bucheli, S.R. The Fly Associated Bacteria *Ingnatchineria* and *Wohlfahrtiimonas* on Cadavers Through Time. ASM Texas Branch Fall Meeting. Houston TX, Nov 2014.

Lynne, A.M., The Living Dead: The Microbiome of Human Cadavers and Its Forensic Implications. ASM Texas Branch Fall Meeting. Houston TX, Nov 2014.

Smith, L.R., Haarman, D.E., Hyde, E.R., Petrosino, J.F., Bucheli, S.R. and Lynne, A.M., A Preliminary Study of Shifting Bacterial Communities During Human Cadaver Decomposition in Southeast Texas. ASM Texas Branch Spring Meeting. New Braunfels, TX April 2014. Honorable Mention Undergraduate Poster Presentation

Bucheli, S.R. The Living Dead: Cadavers as Ecosystems. AAFS Annual Meeting. Seattle, WA. February 2014

Haarmann, D.E., Hyde, E.R., Bucheli, S.R., Petrosino, J.F., and Lynne, A.M. A Preliminary Study of Shifting Bacterial Communities during Human Cadaver Decomposition in Southeast Texas. SHSU Graduate Research Symposium, Huntsville, TX. November 2013.

Haarmann, D.E., Hyde, E.R., Bucheli, S.R., Petrosino, J.F., and Lynne, A.M. A Preliminary Study of Shifting Bacterial Communities during Human Cadaver Decomposition in Southeast Texas. ASM Texas Branch Fall Meeting. New Orleans, LA. November 2013.

Baker, J., Haarmann, D., Alicki, E.R. Petrosino, J., Bucheli, S.R. and Lynne, A.M. Microbiome of Human Decomposition. ASM General Meeting, Denver, CO. May 2013.

Bucheli, S.R. The Living Dead: Cadavers as Ecosystems. University of Colorado-Boulder. Boulder, CO May 2013.

Baker, J., Haarmann, D., Alicki, E.R. Petrosino, J., Bucheli, S.R. and Lynne, A.M. Microbiome of Human Decomposition. ASM Texas Branch Fall Meeting. Waco TX 76798 . October 2012. Second Place Undergraduate Poster Presentation

Publications In Preparation

Madamba, D., Berry III, R., Walton, A., Lynne, A.M., and Buchlei, S.R. The Significance of the Fly-Borne Bacteria *Ignatzschineria* During Decomposition

Carrol, Z., Smith L.R., Petrosino, J.F., Bucheli, S.R., and Lynne A.M. A Study of the Spatial and Temporal Features of the Human Face Microbiome during Decomposition in Southeast Texas.

Brumlow C., Carlson, J., Metcalf, J. Knight, R., Bucheli, S.R., and Lynne A.M. Spatial and Temporal Change of the Soil Microbiome under Human Decomposing Cadavers.

Bucheli, S.R., Haines, D., Petrosino, J.F., and Lynne A.M. Human Decomposition: A Mosaic Model for Community Succession and Implications for Future Forensic Research.

Xu, Z.Z. Metcalf, J., Bucheli, S.R., Lynne, A.M. and Knight, R. Comparison of insect occurrence models to microbial occurrence models for estimating the postmortem interval.

Bucheli, S.R., Petrosino, J.F, and Lynne, A.M., The microbiome of decomposing burned cadavers.

Database

Virtual Museum of Human Decomposition.