UNDERSTANDING THE RELATIONSHIP BETWEEN THE HUMAN MICROBIOME AND URBAN DECAY/RECOVERY AMONG AUTOPSIED ADULTS IN DETROIT, MICHIGAN

By

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ABSTRACT

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Increased social cohesion within neighborhoods has been associated with better mental and physical health. This phenomenon is often associated with features of the built environment. The recent and rapidly expanding body of research surrounding the human microbiome has presented new approaches to understanding human health and behavior. In this study, microbiome data representing multiple body sites were collected from residents of Detroit, Michigan (n = 30), postmortem. A Detroit Index of Urban Decay (DIUR) and Detroit Index of Environmental Recovery (DIER) was then created and assigned to each city parcel to evaluate individual neighborhood conditions. Results showed the microbiome of the nares to be most associated with the condition of the environment and the microbiome of the ears to be unaffected. Significant relationships support that microbial diversity increases with environmental recovery and decreases in conditions of decay. By investigating the relationship between the human microbiome and the condition of the surrounding ecological environment, this research discusses the potential for these relationships to serve as a vector for promoting both human and ecological health at an accessible, subclinical level.

For my sister.

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Chapter 1

Introduction

Human health is closely linked to the surrounding environment, both built, social and ecological. Urban settings that are characterized by extreme poverty and have fallen into disrepair can result in a multitude of risks (Kruger, Reischl, & Gee, 2007). For example, poor neighborhoods in the urban built environment may contain ‘food deserts’, areas that lack accessible markets for healthy and affordable food choices (Cummins, 2014). Level of exposure and access to environmental features of the built environment, such as parks, have also been shown to affect mortality rates (Mitchell & Popham, 2008). High crime rates, which are a common component of the urban social environment, can cause stress and anxiety amongst urban residents (Truong & Ma, 2006). This is especially true for the city of Detroit, Michigan, which has garnered a worldwide reputation for high crime rates and heavily blighted neighborhoods. Once a flourishing industrial center, the city of Detroit has experienced rapid decline both economically and in population, an example of the shrinking cities phenomenon (Martinez-Fernandez, Audirac, Fol, & Cunningham-Sabot, 2012). Abandoned neighborhoods and high crime rates have contributed to poor cohesion of the social environment (Portes, 1998). Studying the human microbiome of urban residents in comparison to their neighborhood condition is an opportunity to address individual and community health at an accessible, subclinical level. It has been established that residents of urban areas have comparatively less diverse gut microbiomes, an indicator of poor health (Tyakht et al., 2013). The diversity and composition of microbial communities on other sites of the body are only beginning to be understood. The effect of the conditions of the neighborhood environment on human microbial profiles, and our ability to manipulate these features, may serve as an opportunity to address the disproportionate health outcomes observed in disadvantaged neighborhoods.

The conditions of the physical and social environment are extremely poor in the City of Detroit. A 2014 physical survey of all 380,233 city parcels revealed 78,506 (30%) of structures to be “blighted”, a term for multiple conditions of decay, the majority of which were constructed during the peak of the use of lead in construction material, rendering their decay especially hazardous (Price, Smith, & Gilbert, 2014). Additionally, at least 6,135 (5%) of vacant lots showed evidence of significant dumping of trash, chemicals and other debris (Price et al., 2014). Crime is also a major issue in the city. In 2013, Detroit led the nation in violent crime with a homicide rate 10 times the national average at 45 homicides per 100,000 people (Federal Bureau of Investigation [FBI], 2013). Although system reforms have been effective in reducing the violent (murder, rape, assault and robbery) crime rate, it is still the highest in the nation at the time of this study (Detroit Police Department [DPD], 2014).

Socioeconomic disparity is also prominent in the City of Detroit. Detroit became the most populous city to file for Chapter 9 bankruptcy in 2013, the largest municipal bankruptcy filing in U.S. history, with an estimated at $18–20 billion debt (Davey & Walsh, 2013). Spurred by the severity of its situation, the City of Detroit has experienced a cultural revival in recent years, including a growing number of grassroots and aid programs developing within the city. This revitalization includes the restoration and repurposing of Detroit’s thousands of vacant lots. Activism ranges from “mower gangs” that volunteer to mow overgrown parks to independent contractors transplanting hundreds of mature trees in targeted neighborhoods (Nardone, 2016; Hantz Woodlands, 2016). The urban farming movement has also gained considerable momentum within the city, which not only helps restore ecological diversity and naturally filter contaminated soil, but provides increased access to healthy food choices as well (Detroit Dirt LLC, 2012). The built and social environments may evolve as features such as community gardens and urban farmer's markets becoming increasingly popular.

As a method of studying this dynamic urban setting, this study investigated the relationship between the condition of urban neighborhoods on individual microbial profiles at multiple body sites, a potential indicator of health. This research uses components of the built environment as measures of neighborhood decay and ecological restoration, which also serve as proxies for the social environment. Data from a city-wide assessment of the condition of all visible structures and empty lots in Detroit, Michigan, USA (2014) were used to create the Detroit Index of Urban Decay [DIUD]. Available records of the locations of ecological restoration (2015) were compiled to create the Detroit Index of Ecological Recovery [DIER]. Postmortem microbiome data of Detroit residents was obtained through the Human Postmortem Microbiome Project (NIJ-HPMM) (2014-2015). I hypothesized that increased levels of urban decay would be associated with lower microbiome diversity, an indicator of poor health. Conversely, I anticipated that ecological restoration would be associated with higher microbiome diversity. This knowledge may help shape the restoration efforts taking place in shrinking cities, potentially reducing the disparity in health outcomes characteristic of poor urban communities.

Chapter 2

The Human Microbiome

The following chapter will discuss the many aspects of the human microbiome, defined as "the community of microorganisms, including prokaryotes, viruses, and microbial eukaryotes, that populate the human body" (Consortium, 2010). This section will also include what existing research has revealed about the human microbiome and its relationship to health, body site-specific characteristics, and the environment. Current research has mainly focused on the gut microbiome and how its composition may affect individual health; the current study aims to provide a more holistic understanding of the human microbiome and its interaction with the environment.

2.1 Introduction to the Human Microbiome

Humans are born microbially sterile but immediately begin the process of establishing personal microbial communities. Most recent findings estimate that the human body hosts one microbe in proportion to each of its own cells (Sender, Fuchs, & Milo, 2016). In 2005, technological advances in high-throughput sequencing greatly expanded the possibilities for studying the microbiome. Previously, microbiology relied upon being able to grow a culture in a laboratory. As this is no longer necessary, the number of known microorganisms continues to expand. It has been established that there are both abundant and prevalent—but no universally present—microorganisms in the human microbiome (Morgan & Huttenhower, 2012). Twin and parent studies of the gut microbiome have shown that the genetic inheritability of the microbiome is very low; instead, environmental exposures are key to the development of community structures (Turnbaugh et al., 2007). Humans and their co-habitants (including the family dog) share microbial communities, increasingly so as proximity between individuals decreases (Song et al., 2013). Features of the urban setting, such as decay and low ecological diversity, may potentially limit microbial exposure.

Additionally, the potential to manipulate the human microbiome of an individual is dependent upon its ability to change over time. In one of the most intensive studies of the temporal nature of the microbiome to date, Berg-Lyons et al. (2011) sampled four body sites of two individuals over 396 timepoints (span of 15 months for one subject and 6 months for second; average interval between sampling = 1.12 days). The authors established that while stable differences between body sites existed, no detectable 'core' temporal microbiome exists in high abundance for the gut (sampled fecally), mouth, or skin (right and left palms)—meaning diversity and abundance of species present change over time and individual. Differences occurred even within a few days. Furthermore, this temporal variability varies by site. For example, the salivary oral microbiome has been found to exhibit stable diversity over time, but fluctuate in relative abundance potentially by season (Cameron et al., 2015). This is a similar finding to Berg-Lyons et al. (2011), in that while different body sites possess independent microbial diversity profiles, the proportion of these profiles varies over time. Though microbial community structure varies over time, the variation of microbial profiles between individuals in the sample (n = 40) was greater than the variation of the temporal changes in a single individual.

2.2 Measures of the Microbiome

The analytical methods used to study microbial communities varies; however, these measures are rooted in taxonomic diversity. Microbial samples are most often sequenced using the 16S ribosomal gene, a method that is relatively inexpensive and simple compared to using other gene markers (Morgan & Huttenhower, 2012). The population is then described as the number of times each sequence was detected. Because of the likelihood of a sequencing error, in addition to the nature of genetic evolution, it is impractical to rely on a complete sequence match—instead, cutoffs of 95%, 97%, or 99% similarity are often used. The resulting groups are referred to as Operational Taxonomic Units [OTUs] (Morgan & Huttenhower, 2012). The number of groups detected in a sample is reported as observed species (Kuczynski et al., 2011). The frequency at which known bacterial communities are detected (called abundance) is often described at the biological taxon levels phylum and/or genus in addition to the OTU level.

Measures of within-community (sample site) diversity are characterized as alpha diversity (as opposed to between-community, beta diversity). Alpha diversity is defined as "the number (richness) and distribution (evenness) of taxa expected within a single population" (Morgan & Huttenhower, 2012, p. 4). Richness (count), evenness, and abundance are all considered features of microbial diversity. These values are determined quantitatively, based on abundance, versus qualitatively (presence/absence of species). Examples of these mathematical indexes include Heip's evenness and the Simpson index. Evenness (or relative abundance) describes how similar in number each species is. This measure of evenness was designed to correct for an issue present in alternative measures where values do not remain constant when used on communities with very little diversity (Heip, 1974). The Simpson index measures both the number of species present as well as the species abundance (Simpson, 1949).

The current study utilizes post-mortem human microbiome samples, which can also be referred to as the necrobiome. The necrobiome, "the community of species (e.g., prokaryotic and eukaryotic) associated with decomposing remains of heterotrophic biomass, including animal carrion and human corpses", was first defined by Benbow, Lewis, Tomberlin, and Pechal (2013). The necrobiome has been found to change in largely predictable ways in rodent and swine models, though these changes are also influenced by factors such as seasonality (Carter, Metcalf, Bibat, & Knight, 2015). This study samples the necrobiome within three days of death, when it is still representative of the human microbiome (Pechal, 2016, personal communication). This is advantageous as it allows body sites to be sampled that are difficult or impossible to collect on living persons. For example, the surface of the eye and the trabecular space (inside the skull). Sampling the microbiome at a single point in time, in this case time of death, is sometimes referred to as a snapshot sample (Smillie et al., 2011).

2.3 The Microbiome and Health

The measurement of the microbiome is important as community disruptions are thought to be precursors of disease. Diversity and abundance have been linked to various mental and physical health conditions, mostly in the gut microbiome and related sites (e.g., oral). Mental health conditions, such as schizophrenia, have been associated with lessened diversity of the oropharyngeal (portion of oral cavity near the throat) microbiome occurring amongst subjects diagnosed with schizophrenia in comparison to a healthy control group (Castro-Nallar et al., 2015). Physical health has been shown to benefit from increased diversity as well. It has been suggested that the increased gut microbiome diversity that results from consuming a more traditional (high in fiber) diet can explain the lack of infectious digestive disorders amongst African populations, in comparison to European populations that consume highly processed food (De Filippo et al., 2010). Demographic factors do not appear to influence microbial diversity, though some research has found gut diversity to increase for the duration of one’s lifespan when comparing Malawian, Amerindian (Venezuelan), and US populations (Yatsunenko et al., 2012). Similar to De Filippo et al. (2010), gut diversity was lowest amongst the US adults in comparison to the Malawian and Amerindian samples. In respect to the causal mechanisms of disease, it is theorized that low microbiome diversity facilitates an overgrowth of one or multiple microbes, resulting in diseases such as *C. difficile* colitis in the gut (S. Johnson et al., 1990; Morgan & Huttenhower, 2012).

In addition to studies of diversity, The Human Microbiome Project has been at the forefront of discovering the purpose of typical human microbial community members, how they are affecting humans, and their impact on health and disease. Ultimately, various taxa and their abundance may serve as biomarkers for future disease (Morgan & Huttenhower, 2012). The characteristic profile of taxa within the microbial community of healthy individuals has been shown to exhibits common trends. For example, Firmicutes dominate the oral microbiome of individuals with low amounts of periodontitis and dental cavities (Belstrøm et al., 2014). High abundance of Firmicutes and Actinobacteria are also associated with a healthy gut microbiome, while low levels of Bacteroides have been associated with metabolic diseases such as obesity and diabetes (Bervoets et al., 2013; Tyakht et al., 2013; Zhang et al., 2013).

2.4 Site-specific Characteristics of the Microbiome

The following section will review existing literature for the body sites sampled in the current study. This will include site characteristics and impacts on health, if known. The trabecular space, the inner surface of the skull, is not included in this review as it is a site novel to this research.

2.4.1 The Gut Microbiome

The gut microbiome (typically collected rectally or fecally) has been a major focus of microbiome research. The gut microbiome has been linked to a number of physical conditions, such as obesity, but of particular interest is what has become known as the gut-brain axis—"a bidirectional intercommunication between the gut and the brain" (Bervoets et al., 2013; Farmer, Randall, & Aziz, 2014). The gut microbiome has been linked to conditions such as depression, anxiety, and Alzheimer's disease (Foster (Foster & McVey Neufeld, 2013; Hill et al., 2014;

Hsiao et al., 2013; Mayer, Knight, Mazmanian, Cryan, & Tillisch, 2014; Naseribafrouei et al., 2014).

Diet plays a large role in the gut microbiome, and is a variable highly influenced by cultural and regional differences. For example, De Filippo et al. (2010) were able to identify a more diverse and potentially adapted gut microbiome amongst children from Burkina Faso, who consume a diet higher in fiber content in comparison to a European group. The authors hypothesized that a diet high in processed foods strips the gut microbiome of the important components for preventing inflammation of the lining of the digestive system.

2.4.2 The Oral Microbiome

The human oral cavity is a complex environment; previous research has established nine sites within this region that have distinct microbial profiles (tongue dorsum, lateral sides of tongue, buccal epithelium, hard palate, soft palate, supragingival plaque of tooth surfaces, subgingival plaque, maxillary anterior vestibule and tonsils) (Aas, Paster, Stokes, Olsen, & Dewhirst, 2005). Research of the oral microbiome using mouse models has found the buccal microbiome to be the most resistant to change (Rautava et al., 2015). The composition of the salivary oral microbiome was found to not be associated with diet, age, gender, body mass index [BMI], or alcohol consumption within a sample of 292 Danish individuals in good oral health (Belstrøm et al., 2014). Conversely, the analysis also suggested that smoking and socioeconomic status may have an effect on oral profiles, though the authors suggest that further research is necessary to confirm this result.

Changes in physical health have been associated with changes in the oral microbiome. In a study designed to be similar to the biological processes of humans, inflammatory bowel disorder [IBD] was replicated with mouse models and linked to changes in the oral microbiome, specifically the tongue, buccal mucosa, and saliva (Rautava et al., 2015). These findings support earlier research that compared children with IBD to a healthy control group (n = 114) and found lower oral diversity amongst children with the inflammatory disease (Docktor et al., 2012).

Mental illnesses such as schizophrenia have also been associated with differences in the diversity and evenness of the oral microbiome. A study by Castro-Nallar et al. (2015) found that individuals without schizophrenia had significantly more diverse oropharyngeal microbiomes, and a higher relative abundance of Bacteroidetes and Actinobacteria. The oral microbiome of schizophrenic individuals was characterized by low diversity and relative evenness in abundance, with higher proportions of Firmicutes than the control group.

Geography, specifically latitude, also appears to play a minor role in the species present in the salivary oral microbiome. Comparison of salivary microbial samples from twelve global locations (n = 120) presented some variance in the genera present (e.g. one of the most abundant genera in samples from one location was not present in many other countries) (Nasidze, Li, Quinque, Tang, & Stoneking, 2009). However, variance in overall diversity was attributed to individual differences and not geography. A follow-up study by Li et al. (2014) compared the salivary microbiomes of native Alaskan, German, and African populations at the OTU level and again found support for variations in microbial communities by latitude. The OTU network for Alaskans and Germans were similar, while the African group showed greater variation. This data was also used to investigate the existence of a core microbiome for the salivary microbiome; however, consistent with the findings of Berg-Lyons et al. (2011), no OTUs are shared by more than 90% of the individuals and only 14 OTUs were present in 50% of the sample (0.23% of the total OTUs).

2.4.3 The Ocular (Eye) Microbiome

Sampling procedures (swabbing) have limited the opportunity to study the microbiome of the surface of the eye; instead, swabbing the conjunctiva (mucous membrane on the eyelids) is done to represent to ocular surface (Dong et al. 2011). Deep sequencing of the conjunctiva of four individuals by Dong et al. (2011) yielded a bacterial community of five phyla and fifty-nine distinct genera; twelve genera were present in all four individuals. In regards to health, initial research has determined a healthy cornea to be largely composed of Proteobacteria, Actinobacteria, and Firmicutes and that the occurrence of diseases such as ulcerative bacterial keratitis will disrupt the community (Tuzhikov et al., 2013). Blindness caused by infection, predominantly *Chlamydia trachomatis*, has been linked to decreased diversity and overabundance of single microorganisms. However, it is unclear whether changes in the microbiome cause this disease or vice versa (Zhou et al., 2014). Additionally, the use of contact lenses appears to have a significant effect on the ocular microbiome, increasing diversity and abundance approximately three-fold and introducing bacteria typically found on the skin such as *Lactobacillus, Methylobacterium*, *Acinetobacter*, and *Psuedomonas* (Long, 2015; Shin et al., 2016). It is not yet known if these are related to ocular infections.

2.4.4 The Microbiome of the Skin

The microbiome of the skin is not only diverse, but presents high variability between individuals. This latter feature has been tested for potential forensic application by successfully matching the bacteria found on objects to the microbial community of the skin found on the person who touched the object (Fierer et al., 2010). A comparison of twenty sites on the skin found that like most sites, the skin is dominated by four phyla—Actinobacteria, Firmicutes, Proteobacteria and Bacteroidetes—and that the communities of physiologically similar sites were similar to one another (Grice et al., 2009). The authors suggested further study of the many microbial niches of the skin; doing so may provide insight into site-specific dermatological disorders such as psoriasis and eczema, as well as provide insight into the effects of health practices such as antibiotic use and hygienic behaviors. As with other sites, particular microbes, such as *Staphylococcus epidermidis,* are considered part of the healthy microbiome and act as a defense against disease, while others, such as *Staphylococcus aureus*, often cause infection (Kong, 2011).

2.4.5 The Microbiome of the Nares (Nose)

The nose can be considered a niche of the skin, characterized by hair, moisture, as well as being a component of the respiratory system (Boutin et al., 2015). The microbial community of the nose has been associated with skin and soft tissues infections [SSTIs], such as abscesses. Specifically, colonization of *Staphylococcus aureus* has been found to increase the likelihood of SSTIs while higher relative abundance of the phylum Proteobacteria is associated with the absence of SSTIs (R. C. Johnson et al., 2015). The role of the microbiome and health is not always straightforward; for example, the presence of *S. aureus* does not always result in infection; competition from *S. epidermidis* has been suggested as a mediator, however, this is potentially dependent upon a specific protein only found in a subset of strains (D. N. Frank et al., 2010; Iwase et al., 2010; Yan et al., 2013). Like the skin as a whole, the nose is dominated by Actinobacteria, Firmicutes, Proteobacteria, and to a much lesser extent, Bacteroidetes (Yan et al., 2013). Besides studies of *Staphylococcus*, research on the microbiome of the nares is limited.

2.5 The Microbiome and the Environment

The human microbiome is known to influence the microbial profile of the space that they occupy, but the understanding of this relationship is currently limited. For example, samples were taken of the microbiological profile of the New York City subway system, a canal, and public parks (n = 1,457). Almost half of the DNA collected did not match known organisms (48%), however, the authors were able to detect the relationship between the ancestry indicators in the genome of the human-related microbes found in the samples and the demographic profile of the area as indicated by U.S. Census data (Afshinnekoo et al., 2015).

Features of the environment have been found to affect the microbiome, but this has yet to be well-established. In two cross-sectional studies in Germany, Ege et al. (2011) compared the microbial diversity of children's rooms in rural (farm) and urban settings and found that exposure to a greater number of microbes (a characteristic of rural areas) and instance of asthma and/or allergies were inversely related. Similarly, residents of rural areas in Russia were found to have microbial profiles more characteristic of a healthy gut in comparison to their urban counterparts (Tyakht et al., 2013). However, this may be the effect of diet, culture, and socioeconomic status. The ingestion of airborne urban particular matter [PM] (360 micrograms per day mixed into food) was found to induce inflammatory response in the gut of mouse models after continued exposure (30 days) followed by reduced diversity of the gut microflora (Kish et al., 2011).

2.6 Contributions of the Current Study

Technological advances have only recently made studying the many components of the human microbiome economically feasible (Morgan & Huttenhower, 2012). Existing research primarily studies the gut microbiome, and little research exists that studies multiple body sides concurrently. Multi-site research is limited to the study of only three or four body sites or multiple sites within the same organ, such as the skin (Berg-Lyons et al., 2011; Grice et al., 2009). The current study adds to the literature by studying six unique locations of the human microbiome, including one that is new to microbial research. Additionally, this research uses post-mortem microbiome data, also called the necrobiome. While analysis has been done of the necrobiome of human gravesoils, the necrobiome has not yet been studied directly with human samples (Finley, Pechal, Benbow, Robertson, & Javan, 2016). Additionally, the study of the effect of the surrounding environment on the human environment is an approach unique to this study. Existing research has examined the microbiome of the environment in comparison to the ethnic profile of the human population, contrasted the microbiome diversity of residents of urban and rural areas, and studied microbial variation on a global scale (Afshinnekoo et al., 2015; De Filippo et al., 2010; Li et al., 2014; Nasidze et al., 2009). These contributions allow for the current study to provide insight into both within-individual site variation and within-environment variation—and how these characteristics relate to the external environment in ways that may reflect social inequality and influence health outcomes.

Chapter 3

Health and the Urban Environment

The following chapter will discuss the various components of the built environment (structural and green spaces) and social environments that affect mental and physical health in an urban setting and will help identify gaps in the literature. As of 2014, 54% of the world's population live in an urban setting (World Health Organization [WHO], 2014). However, many large urban areas known as 'shrinking cities' have seen a stark decrease in population that has resulted in a pattern of structural decay (Martinez-Fernandez et al., 2012). Income inequality and social issues, such as discrimination against minority groups, are often found in urban settings and have been associated with negative health outcomes (Meyer, 1995; Wilkinson, 1999). In an urban context, features of the built and social environments may also serve as proxies for one another. This chapter closes with discussion of how features of the built environment serve as proxies for the social environment. Focus is placed on the components of an urban setting relevant to the current study and thus is not an exhaustive representation of the features of urban environments that affect mental and physical health.

3.1 The Built Environment and Health

The built environment refers to the human-made components of where we live, including buildings, streets, infrastructure, and open spaces (Centers for Disease Control [CDC], 2011). The design and quality of these features has the potential to both directly and indirectly affect mental health ranging from psychological stress to clinical mental illness (Evans, 2003). In urban settings, the built environment can lead to psychological stress via crowded housing structures and living spaces, poor lighting, noise, little privacy, and close proximity to sources of pollution

(Evans, 2003). For example, a longitudinal study of five types of neighborhoods in Oslo, Norway, revealed one city with marked change in social characteristics over a period of ten years. Surveys of residents and key informants indicated that this site was initially poor in both city structure and the mental health of residents; significant improvements to city development and the social environment coincided with a significant improvement in mental health (Dalgard & Tambs, 1997). However, as exemplified in this study and the discussion of the social environment to follow, improvements in the built and social environment often coincide. A London study of 1,887 individuals, aged 16 or older, examined the effect of individually rated features of the built environment on depression. When controlling for socioeconomic status and internal features of the home, it was found that living in dwellings with predominantly deck access and newer construction (typical features of rented apartment complexes) was associated with survey items indicative of depression (Weich et al., 2002). The authors note that the associations between the built environment and depression were not explained by individual risk factors, such as unemployment, and that measures of the social environment were intentionally excluded from the study design.

The built environment has also been linked to physical health, most often by its role in either promoting or prohibiting physical activity. For example, urban settings with an infrastructure that supports bike- and walkability increase the opportunity for physical activity, contributing to fewer overweight or obese individuals (Sallis et al., 2009). An additional component of the aforementioned study was that both lower- and higher-income groups benefited from neighborhoods with high walkability. In addition, walking or biking as a means of daily transportation has been suggested to be more beneficial than vigorous forms of exercise (such as aerobics) because the behavior is more likely to be repeated (L. D. Frank & Engelke, 2001). Besides walkability, the built environment can affect health outcomes via one's proximity and access to 'physical activity facilities'. A survey of a nationally representative sample of adolescents (n = 20,745) found an increasing number of physical activity facilities within a census-block group to be related with a decreased number of overweight individuals (Gordon-Larsen, Nelson, Page, & Popkin, 2006). The urban built environment may also contain 'food deserts', which are areas that lack accessible markets for healthy and affordable food choices (Cummins, 2014). Lastly, the physical condition of the urban built environment may also affect health both directly and indirectly, such as via accidental injury or financial losses, respectively (Kruger et al., 2007). In addition to features such as buildings and transportation, the impact of urban infrastructure on health is also tied to the implementation of green spaces.

3.2 Green Spaces of the Built Environment and Health

Green space, features of the built environment such as parks, sports fields and gardens, has an effect on both mental and physical health (Mitchel & Popham, 2008). Humankind's connection with nature is based in an evolutionary perspective, when the features of the natural environment were necessary for survival (Frumkin, 2001). Green space has been extensively researched for its role in mental well-being, and research has found many mediating factors in this relationship, including but not limited to a person's age, gender, education level, childhood experience with nature, and frequency of visits to green space (Astell-Burt et al., 2014; Maas, Verheij, Groenewegen, de Vries, & Spreeuwenberg, 2006; Thompson, Aspinall, & Montarzino, 2008; van den Berg et al., 2016). While a Japanese study found "forest bathing" to decrease salivary cortisol levels (an indicator of stress) in comparison to the effects of urban stimuli (Lee et al., 2011), a Scottish study found living in a higher percentage of urban green space to have a similar physiological and mental effect on middle-aged persons in deprived urban neighborhoods as well (Roe et al., 2013). Visibility and quality are also realted to the effects of green space on mental health. Nutsford, Pearson, Kingham, and Reitsma (2016) found that visibility of urban green space did not have an effect on the psychological distress of adults in a New Zealand city. However, visibility of blue space (natural water bodies) was associated with lower psychological distress levels. The authors suggest that the type of green space, such as a lusher natural setting, may have a differing effect on mental health. Quality of green space in urban areas was assessed in Van Dillen, de Vries, Groenewegen, and Spreeuwenberg (2012) with a 10-item scale that included some non-natural items such as presence of litter and maintenance. Still, the authors found that while the quality of streetscape greenery was related to all self-reported health measures, the quality of green areas was not connected to mental health. Distance, accessibility, and interaction are theorized to play a role in these findings.

In addition to promoting mental well-being, green space has been linked to physical well-being. A primary example is a study by Mitchel and Popham (2008) that divided the population of England below retirement age (n = 40,813,236) into groups by income deprivation and amount of green space in the neighborhood. Using individual mortality records (n = 366,348), the authors found that higher neighborhood exposure to green space was associated with lower all-cause ad cardiovascular mortality. In turn, causes of mortality that would not likely be affected by green space, such as self-harm and lung cancer, did not have significant results. In addition to promotion of the circulatory system and health overall, time spent in green spaces has been credited with promoting the health effects of Vitamin D production via sun exposure. In this way, the physical environment helps prevent the effects of Vitamin D deficiency, including osteoporosis, rickets in children, type I diabetes, and increased risk of some cancers (Holick, 2004). However, factors that would prevent green spaces from being used in this capacity, such as restricted access and poor quality, may lessen the effect on physical health (Van Dillen et al., 2011). In turn, the use of public space in an urban setting, such as green space, impacts the social environment.

3.3 The Social Environment and Health

The relationship between social context and mental health has been extensively studied within the social sciences, a famous early example being Durkheim's *Suicide*, published in 1897 (McKenzie, Whitley, & Weich, 2002). The social environment refers to "social relationships, and cultural milieus within which defined groups of people function and interact" (Barnett, 2001, p. 465). However, this section will focus primarily on the effect of social relationships on health. Like the built environment, the condition of the social environment can affect the direction of its relationship to health. For example, crime rates are considered a component of the social environment, and high crime rates can cause stress and anxiety amongst urban residents (Truong & Ma, 2006). Minority status within a community, for example via race, sex, social class or sexual orientation, has also been associated with chronic stress and poor mental health outcomes (DeJonckheere, 2016). In contrast, the social environment can promote mental health outcomes as well. For example, a longitudinal study of Australian secondary school students (n = 2,678) found that good connectedness in early years, both socially and to school, continued to affect mood, substance abuse, and behavioral outcomes (such as completing school) in later years (Bond et al., 2007). In addition to mental health, social factors have the potential to influence physical health outcomes and behaviors.

The cultural component of the social environment, e.g. group norms, can affect physical health outcomes by encouraging or discouraging certain behaviors (Frank & Engelke, 2001). For example, community norms that support physical activity such as walking or biking may increase participation in a community, resulting in better health outcomes (Frank & Engelke, 2001). In contrast, negative social factors such as high crime rates may discourage these behaviors and prevent the associated health outcomes. Urban environments are prone to high crime rates due to there being many targets in a concentrated space and the absence of a ‘capable guardian’, i.e. any actor or feature whose presence deters crime (Hollis-Peel & Welsh, 2014). Within a community, the level of physical activity may also vary by social conditions. For example, physical activity amongst Australian individuals aged sixty or older increased among those whose friends and family who participated, those who had high self-efficacy, and those who had access to features of the built environment such as safe walkways and physical activity facilities (Booth, Owen, Bauman, Clavisi, & Leslie, 2000). Ultimately, the built and social environments interact to affect community health.

3.4 The Built Environment as a Proxy for Social

Features of the built environment are often considered to be indicators of the social environment (Barnett, 2001). Features of the social environment, such as supportive relationships and health outcomes such as the reduction of stress and fatigue have been argued to be affected by the built environment (Booth et al., 2000; Evans, 2003). A useful example is the concept of urban decay, which is typically characterized by decline in the built environment (e.g. unoccupied buildings, fire damage, etc.). These decayed, built features influence social contact, crime and cohesion in neighborhoods (Weich et al., 2002). Ultimately, neighborhood decay leads to decreased contact with one’s community, which then leads to negatively impacts mental health (Kruger, 2007). In this respect, neighborhood decay can serve as a physical manifestation of the social environment of a community. In contrast to urban decay discouraging social contact, green spaces may promote social connectedness.

Green spaces within the built environment are linked to the social environment. Features of green space, such as parks, sports fields, and walkways facilitate social contact (van den Berg et al., 2016). A measurement of the amount of green space and social contacts of residents of the Netherlands (n = 10,089) found less green space to be associated with feelings of loneliness and a perceived lack of social support (Maas et al., 2009). This relationship remained when controlling for socioeconomic and demographic factors. Additionally, residents of apartment buildings in inner-city neighborhoods report fewer incivilities, less fear, and less aggressive or violent behavior when neighborhood green space is increased (Kuo & Sullivan, 2001). This relationship—which also applies to both property and violent crimes—remains even after controlling for building height, the number of apartments per building, and occupancy and vacancy rates. In effect, existing research supports that green space may represent the social environment via the promotion of positive components, such as social contact, and the prevention of negative components, an example being crime rates. In sum, the built and social environments in an urban setting play a multi-faceted role in the manifestation of community health outcomes.

Chapter 4

Methods

This study investigates the relationship between the condition of the surrounding environment and the human microbiome. This relationship may have important implications for both mental and physical health. Previous research suggests a link between physical and mental health benefits from gut microbial diversity, as well as a link between the urban and rural environment and intestinal microbial diversity (Tyahkt et al., 2013). This study adds to this body of knowledge by performing analysis of the species diversity and known phyla of six anatomical sites in comparison to the level of urban decay and environmental recovery of residents of a major US city.

This study will address the following research questions:

1. Is there a difference in mean observed species diversity for each body site between young and old persons?
2. Is there a difference in mean observed species diversity for each body site by season of death?
3. Is there a difference in mean observed species diversity for each body site by cause of death?
4. Is there a relationship between the level of neighborhood decay and measures of diversity for each body site?
5. Is there a relationship between level of neighborhood environmental recovery and measures of diversity for each body site?
6. Is there a relationship between the level of neighborhood urban decay and log counts of the four most abundant phyla for each body site?
7. Is there a relationship between the level of neighborhood environmental recovery and log counts of the four most abundant phyla for each body site?

4.1 Study Site

The City of Detroit comprises 138.75 mi^2 in the southeast portion of Michigan, with the Detroit River serving as its eastern border. Founded by the French in 1701, the city has an extensive history as a hub for trade, manufacture, and immigration. The population of Detroit peaked in 1950, when 1.85 million residents occupied the city mostly for its role in the booming auto industry (Poremba, 2001). Following the decline of this industry, Detroit's population significantly declined to only 680,250 residents as of 2014 (U.S. Census Bureau, 2015). As the economic condition of the City continued to worsen from year to year, an increasing number of the City's homes were abandoned or subject to foreclosure. By 2010, the large but sparsely populated neighborhoods created additional economic stress on Detroit's resources, leading the Mayor to propose that Detroit's remaining population be concentrated and that "one-quarter of the City bulldozed" to better allocate resources (Snyder, 2010). By 2013, Detroit had filed for bankruptcy and the Obama Administration allocated $300 million in aid "to address key areas of importance, including blight removal, public works, and public safety" (Price et al., 2014, p.2). This initiative allowed for the creation of the Motor City Mapping project, a technology-based evaluation of all 380,233 of Detroit's parcels. Designed to be continuously updated, it took ten weeks for over 150 residents and volunteers to collect the first round of data in 2013. Of the 377,602 surveyed parcels (parcels that were not visible were excluded), 78,506 structures (30%) and 6,135 lots (5%) were found to meet the project's definition of "blight", which is any property that meets one or more of the following conditions: a public nuisance, fire hazard, lacks working utilities, tax-reverted, owned by the land bank, not to code, vacant for 5 consecutive years, or a health hazard (Price et al., 2014). In response to the processes of abandonment and urban decay, urban recovery projects and activism have become increasingly prevalent in recent years, allowing for the restoration of targeted neighborhoods and properties. Much of this activism is centered on the acquisition and subsequent maintenance of Detroit's 6,135 vacant lots, identified as overgrown and subject to dumping. To combat this problem, since July of 2014 the Detroit Land Bank Authority (DLBA) has allowed Detroit residents to purchase vacant adjacent properties for $100 under the agreement that the property will be maintained (DLBA, 2015). Those who purchase the property are then allowed to cultivate it however they may choose, which is conducive to the increasingly popular trend of "urban farming". Urban farming, which is considered anything from cultivating a small planter box to a fully operating produce market, serves as a critical mechanism in the City of Detroit for not only environmental restoration but improvement of the social environment as well. The ease of access to vacant land and low cost of personal farming have prompted many neighborhoods to develop their own "community garden" or "street gardens". The gardens have the potential to increase social capital in what are notoriously dangerous areas and may also provide better access to healthy foods. In addition to government regulated and grassroots efforts, private companies have acquired, restored and maintained many of Detroit's blighted properties. For example, Hantz Woodlands LLC purchased 1,755 parcels as of December 2015 and plans to purchase a total of two hundred acres within the City, to plant rows of mature trees, transplanted from outside of the City (Hantz Woodlands, 2016).

Despite concentrated recovery efforts, the population of Detroit declined by 4.7% between the years 2010 and 2014, compared to a 0.3% population increase for the entire state of Michigan within that same period (US Census Bureau, 2015). At the time of the 2010 census, the residents of Detroit were 82.7% black or African American, 7.8% white, 6.8% Hispanic or Latino, and 2.7% other. During the four-year period of 2009 to 2013, 39.3% of Detroit's residents lived below the federal poverty level, compared to 16.8% statewide. This site serves as an ideal location to study urban decay and recovery because it encompasses a wide range of both elements within a single study site. In addition, revitalization efforts within the City have made Detroit an accessible nexus for change and further research.

4.2 Data

4.2.1 Microbial Collections

Microbial samples were collected from 30 cadavers (21 male, 9 female) between May 11, 2014 and April 4, 2015. These cadavers were selected from those sampled by NIJ-HPMM for having been residents of Detroit. All cadavers were sampled within a three-day postmortem range, except for two cadavers that had postmortem interval estimates greater than 72 h. DNA-Free (sterile) cotton-tipped swabs were used by the Wayne County Medical Examiner’s Office to aseptically collect individual microbial samples from six anatomic sites: the external auditory canal (ears), nares (nose), buccal cavity (oral), eyes, rectum (gut), and the trabecular space. The following data were recorded for each cadaver: sex, race, age, date of birth, date of death, location of death, location of residence, manner of death, cause of death, date of autopsy, time of autopsy, height (m), and weight (kg) and body mass index (BMI: kg/m2).

4.2.2 Urban Decay

The Motor City Mapping project, the first thorough evaluation of the condition of Detroit’s properties, was completed in 2014 and continues to be updated via volunteer participation and a freely available smartphone application. Motor City Mapping supplemented the volunteer-collected data with 24 additional data sets pertinent to City functions, of which record of foreclosed and tax delinquent properties were used for the current study. As part of the project’s mission, the “enhanced” Motor City Mapping data set was made publicly available through the Data Driven Detroit (D3) organization (Price et al., 2014). This study uses the Motor City Mapping Enhanced dataset released October 1, 2014.

4.2.3 Environmental Recovery

Four sources of publicly available environmental data were collected with an internet search, which includes: a record of the lots sold and monitored through the DLBA's Side Lot Sales (2014-2015), the location of parcels in the Adopt-a-Garden program (2015), a shapefile of City parks (2015); and a shapefile identifying the location of trees (2015). The locations of urban farming projects within the City of Detroit were compiled specifically for this research.

4.3 Data Processing

4.3.1 Microbial Samples

4.3.3.1 DNA Extraction and 16s rRNA Gene Amplicon Sequencing

DNA was extracted using a modified protocol of a commercially available kit (Invitrogen PureLink™ Genomic DNA Mini Kit): an addition of 15 mg/ml of lysozyme was added during the lysis step for each DNA extraction reaction to improve efficiency. DNA quantification was performed using the Quant-iT dsDNA HS Assay kit and a Qubit 2.0 (Grand Island, NY, USA). All DNA preparations were stored at −20°C. The Michigan State University Genomics Core Facility performed the Illumina MiSeq 16S library construction and sequencing (2 x 250 bp paired-end reads) using a modified version of a protocol adapted for MiSeq (Caporaso et al. 2011a). The V4 region of the 16S rRNA gene was amplified with region-specific primers (515F/806R) (Claesson et al., 2010; Caporaso et al., 2011b; Caporaso et al., 2012).

4.3.3.2 Sequencing Analysis

Downstream process of the sequences was performed using the default settings in QIIME version 1.8.0 (Caporaso et al., 2010). The raw fastq files were assembled, quality-filtered, demultiplexed, and analyzed. After quality control, as previously described in Pechal and Benbow (2015), the remaining sequences were binned into operational taxonomic units (OTUs) at a 97% sequence cutoff based on the May 2013 Greengenes 97% reference dataset (http://greengenes.secondgenome.com) (DeSantis et al., 2006; McDonald et al., 2012; Werner et al., 2012). Finally, singleton OTUs were removed. Not all samples returned the same number of sequences, thus samples with fewer than 10,000 sequences were not used in subsequent analyses. Sequence files for all samples used in this study have been deposited in the Sequence Read Archive at the EMBL European Nucleotide Archive: TBA.

4.3.2 Detroit Index of Urban Decay (DIUD)

For this study, the data were compiled for every tax parcel for which all decay variables were available within Detroit (n=373,160 of 377,602 surveyed properties). These variables consisted of: structure condition, evidence of lot activity, evidence of lot maintenance, whether or not the structure was secured (no openly accessible doors/windows, etc.), occupied, tax delinquent/foreclosed or showed signs of fire damage, or dumping. These data were downloaded as shapefiles and exported as csv files, for analyses in Stata v13 software.

To develop a Detroit Index of Urban Decay (DIUD), I separated parcels into those that contain a structure (n = 261,120) and those that do not (i.e. lots; n = 112,040) because some indicators of decay were only applicable to one group (e.g., fire damage of structures). I ran principal components analysis (PCA) on variables representing urban decay for each of these parcel types. PCA is used to reduce the number of dimensions in data with a large number of variables so that trends in the data are more visible (Smith, 2002). Specifically, for empty lots, I analyzed a number of aspects of urban decay: visible dumping on site, indications of improvement (called “activity” by the MCM project), indications of lot maintenance, and a record of tax delinquency for the parcel (including foreclosure). I explored these aspects of urban decay for parcels with structures: structure condition, occupancy, whether the structure was secure, fire damage, dumping and record of tax delinquency.

The minimum Eigen value was set to 1 to organize the data into a single dimension with the largest variance. The primary component for lot decay had high loadings for lot activity (0.67) and lot maintenance (0.68). Thus, this component could be considered an indicator of decay for lot parcels. The primary component for structures had high loadings for condition (0.52), occupancy (0.48), secure (0.50), fire (0.40), and dumping (0.30). This component can be considered an indicator of decay for parcels with structures. These component values were assigned to every parcel in Detroit as DIUD values. Values for lots ranged from -1.42 to 0.65, where higher values indicate higher decay. Values for structures ranged from -0.55 to 0.85, where higher values indicate higher decay **(Figure 1)**.

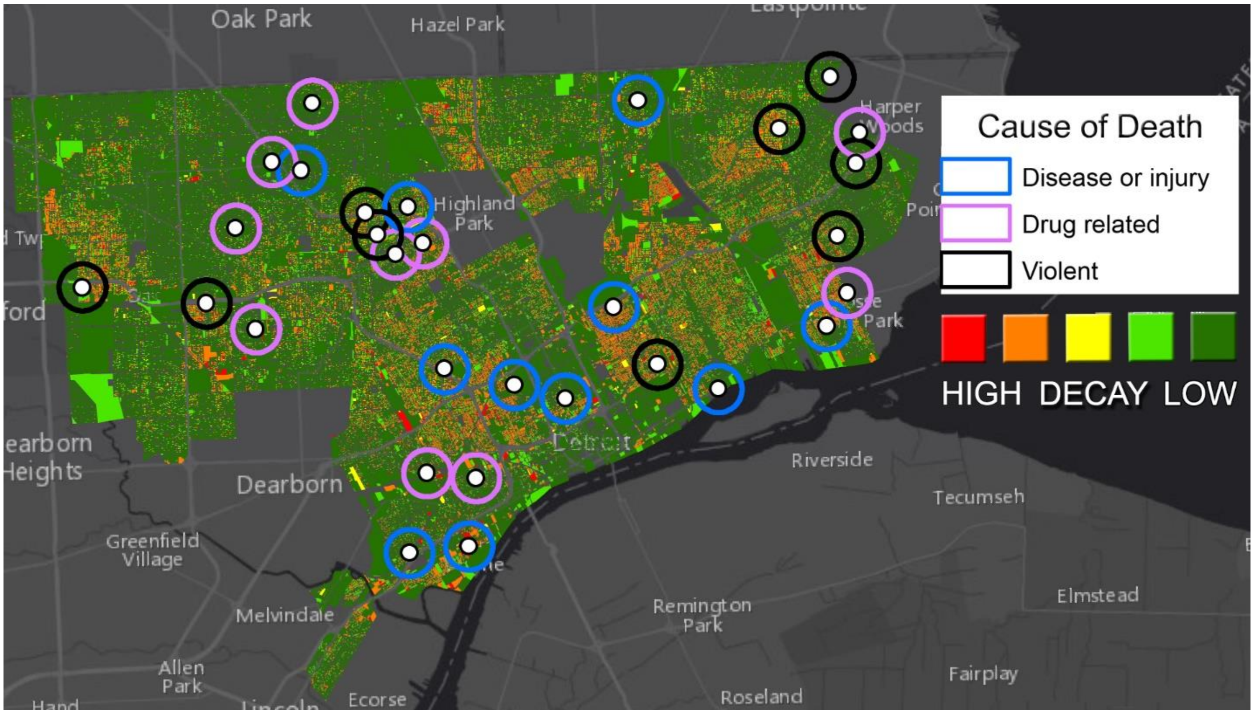

Figure 1. Map of DIUD Values for Study Area and Neighborhood Buffers of Sample

4.3.3 Detroit Index of Environmental Recovery (DIER)

A range of publicly available environmental data was used to create the Detroit Index of Environmental Recovery (DIER). A record of the lots sold and monitored through the DLBA's Side Lot Sales (2014-2015) was obtained and geocoded. Similarly, the location of parcels which are part of the Adopt-a-Garden program, in which residents may assume responsibility for a vacant lot for one year at no charge, were publicly available from the City of Detroit (2015). The City of Detroit also provided public access to a shapefile of city parks (2015); only those that were rated in “good’ or fair” condition were used to represent recovery (n = 182). Additionally, spatial data identifying the location of trees planted within the city, compiled by The Greening of Detroit’s OpenTreeMap (2015), were also obtained. Lastly, the addresses of independent urban farming projects were collected and geocoded. This data was collected by an Internet search of the terms “urban farm[ing]” for Detroit, which provided a list of organizations (2015). News articles and related organizations (i.e. The Greening of Detroit) were then searched for additional mention of urban farming projects (n = 22 parcels; some organizations owned multiple locations).

To develop the DIER, the environmental spatial data was compiled in ArcGIS and joined with the parcel data (n = 373,160). This allowed for the exploration of multiple parcel-level indications of urban recovery: trees per parcel, maintained side lots, adopted gardens, and urban farms. I used the primary component for all parcels, setting the minimum Eigen value at 1. The primary component had high loadings for parks (0.68), Adopt-a-Gardens (0.32), and number of trees per parcel (0.64). Thus, this component could be considered an indicator for environmental recovery. This component was used to assign DIER values to every parcel in Detroit. Values ranged from -0.05 to 0.2, where higher values indicate higher environmental recovery **(Figure 2).**

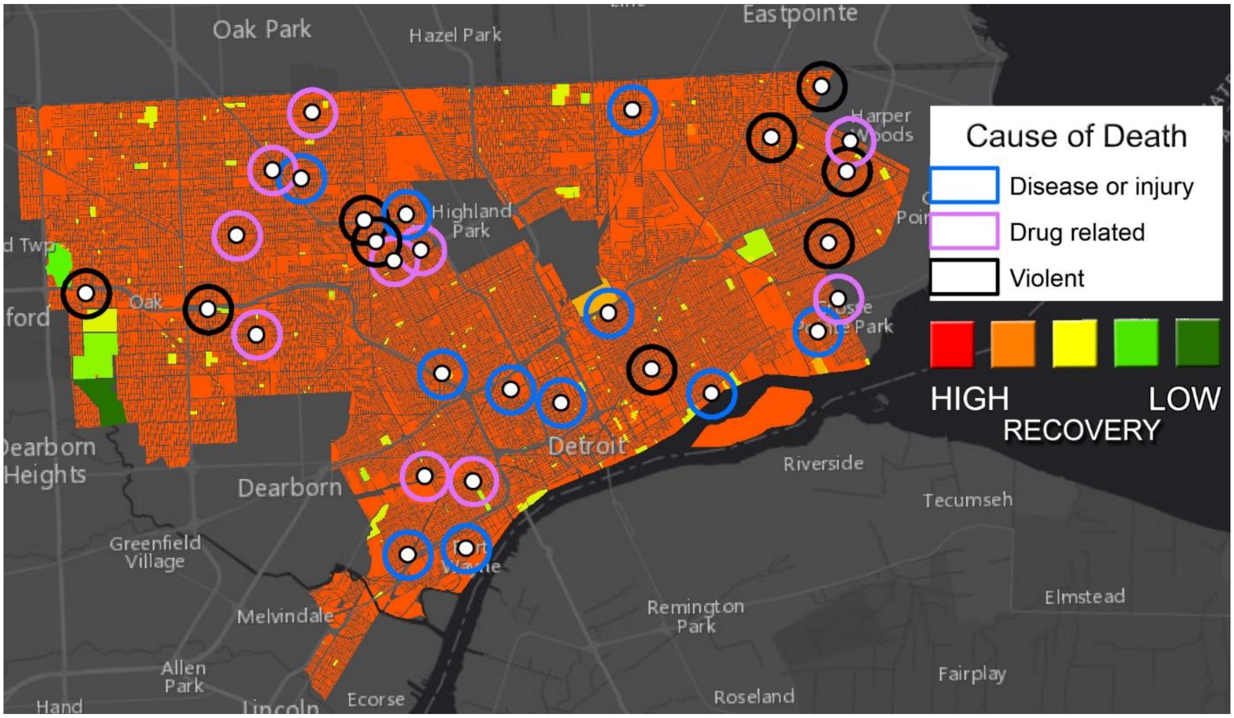

Figure 2. Map of DIER Values for Study Area and Neighborhood Buffers of Sample

4.3.4 Assigning DUID and DIER Values to Participants

Neighborhood Euclidean distance buffers of 0.5 miles (~15 minute walk) were created around each participant’s home. Each decedent was assigned a DIUD – Lots, DIUD – Structures and DIER value by summing the values of the parcels contained within the buffer of their home location and dividing this sum by the number of parcels [for example, (sum of DIUD Structures)/(# parcels defined as a structure)]. Individuals who were homeless or had an unknown residence were assigned values based on the location of their death (n = 4). These buffers were used to assign the following variables to decedents: i) the rate of structures with fire damage, ii) rate of parcels with dumping, iii) rate of tax delinquent parcels, iv) average condition of structures, v) rate of unmaintained lots, vi) rate of lots without signs of activity, vii) average DIUD value for lots, viii) average DIUD value for structures, iv) DIUD, and x) DIER.

4.4 Analytical Methods

In order to test for significant differences in mean observed species diversity by age, I used a series of Mann-Whitney-Wilcoxon tests (also called Mann-Whitney U test) on younger (age 19 – 44, n = 15) and older (age 45 – 71, n = 15) persons for each body site separately. A non-parametric measure, the assumptions of this test are that both groups are independent of one another and that the variables are ordinal level or above (Hettmansperger & McKean, 1998).

In order to test for significant differences in mean observed species diversity by cause of death, I used a series of Kruskall-Wallace H tests on cause of death (disease or injury, n = 11; drug related, n = 10; violent, n = 9) for each body site separately. Kruskall-Wallace is a non-parametric, rank-based test of two or more groups on an ordinal or higher dependent variable (Laerd Statistics, n.d.).

In order to test for correlations between microbial diversity and DIUD/DIER values, I used a series of Spearman’s rank-order correlation tests for each body site separately. This was done for all three measures of diversity (observed species, Heip’s evenness, and Simpson index). In order to test for correlations between log counts of the four most abundant phyla (Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria) and DIUD/DIER values, I also used a series of Spearman’s ran-order correlation tests for each body site separately. The data do not satisfy the assumptions of a Pearson’s correlation, which requires normally distributed data, homoscedasticity, and few outliers. The assumptions of Spearman’s rank (or rho), a non-parametric test, require only non-categorical data and a monotonic relationship between variables. In addition, Spearman’s rank is less sensitive to outliers (Laerd Statistics, n.d.).

Chapter 5

Results

5.1 Sample Characteristics

Table 1. Baseline Characteristics of Study Population by Cause of Death

		Disease or injury **n = 11**	**Drug related** **n = 10**	**Violent** **n = 9**	**Total** **n = 30**
Age, mean (sd)		49 (12)	48 (15)	36 (14)	45 (14)
Male, N (%)		7 (64)	6 (60)	8 (89)	21 (70)
Ethnicity, N (%)	Black	8 (73)	6 (60)	8 (89)	22 (73)
	White	3 (27)	4 (40)	1 (11)	8 (27)
BMI, mean (range)		29 (32)	27 (18)	25 (12)	27 (32)
Place of death, N (%)	Indoors	8 (73)	9 (90)	2 (22)	19 (63)
	Outdoors	3 (27)	1 (10)	7 (88)	11 (37)
Days between death and autopsy, mean (sd)		1 (0.40)	1 (0.32)	1 (0.44)	1 (0.40)
Homeless, N (%)		2 (18)	1 (10)	1 (11)	4 (13)
Season of death, N (%)	Spring	7 (64)	5 (50)	6 (67)	18 (60)
	Summer	4 (36)	5 (50)	3 (33)	12 (40)
Neighborhood Characteristics: Decay		**Disease or injury**	**Drug related**	**Violent**	**Total**
Rate of no lot activity, mean (sd)▲		633 (180)	521 (187)	750 (135)	630 (189)
Rate of no lot maintenance, mean (sd)▲		564 (180)	439 (175)	652 (154)	549 (187)
Average neighborhood structure condition, mean (range)♦		296 (449)	205 (432)	294 (412)	265 (455)
Rate of unoccupied structures, mean (sd)▲		446 (186)	312 (159)	494 (179)	416 (186)
Rate of unsecure structures, mean (sd)▲		114 (71)	76 (64)	134 (70)	107 (70)
Rate of structures with fire damage, mean (sd)▲		33 (25)	23 (19)	34 (17)	30 (21)
Rate of parcels with dumping, mean (sd)▲		34 (24)	28 (36)	35 (15)	32 (26)
DIUD lot value, mean (sd)		-0.163 (0.557)	-0.523 (0.554)	0.115 (0.448)	-0.20 (0.57)
DIUD structure value, mean (sd)		0.120 (0.420)	-0.143 (0.387)	0.197 (0.392)	0.06 (0.41)
Neighborhood Characteristics: Recovery		**Disease or injury**	**Drug related**	**Violent**	**Total**
Count of trees, mean (sd)		36.09 (38.56)	47.60 (48.96)	30.67 (73.23)	38 (53)
Count of Adopt-a-Gardens, mean (sd)		0.64 (0.67)	0.70 (0.95)	0.78 (0.83)	0.70 (0.80)
Count of park parcels, mean (sd)		4.64 (4.18)	4.00 (5.98)	5.78 (8.98)	5 (6)
DIER value, mean (sd)		0.006 (0.036)	-0.011 (0.031)	0.004 (0.081)	0.00 (0.05)

▲ Calculated as [# of variable]/[total # lot or structure] per 1,000 parcels

♦ Average values for all parcels within buffer where condition is rated 0 (good) through 4 (suggest demolition)

Sample characteristics are shown in Table 1. Participants ranged in age from 19 to 71 (mean 45; sd 14). A majority of the sample was black (73%) and/or male (70%). Most died indoors (63%), most individuals were autopsied the following day (87%). Only 13% of the sample was homeless or residence unknown (n = 4). A slight majority of sample members died in the spring (60%).

Lots without signs of activity were the most common indicators of decay (mean rate per 1,000 parcels: 630, sd 189). Structures with fire damage were the least common (mean rate per 1,000 parcels: 30, sd 21). Rates for parcels with dumping of debris were similar (mean rate per 1,000 parcels: 32, sd 26). The neighborhood of the average sample member included 38 registered trees (sd 53), and five parcels designated as a park (sd 6).

Descriptive statistics of the three measures of diversity for each body site are given in **Table 2.** Observed species is a measure of community richness, Heip's evenness index is a measure of relative abundance, and the Simpson index measures diversity using both richness and evenness.

Table 2. Diversity Descriptive Statistics for Each Body Site

	Observed species▲				**Heip's Evenness Index♦**				**Simpson Index■**			
Body site	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
Oral	189	1378	589	275	0.0040	0.0411	0.0182	0.0081	0.1908	0.9722	0.8220	0.1511
Ears	54	1270	534	316	0.0021	0.0027	0.0165	0.0214	0.0366	0.9840	0.6606	0.2574
Ocular	109	1486	545	341	0.0062	0.0240	0.0457	0.0334	0.4738	0.9847	0.8808	0.1253
Nares	208	1577	635	307	0.0033	0.0842	0.0197	0.0154	0.1614	0.9731	0.8167	0.1789
Gut	342	1831	1006	320	0.0078	0.0837	0.0335	0.0143	0.7673	0.9862	0.9429	0.0450
T. Space	49	1566	710	485	0.0080	0.1297	0.0508	0.0338	0.0653	0.9897	0.8817	0.1976

▲ count of observed species at the OTU level (richness)

♦ measure of relative abundance that does not depend on species richness

■measure of diversity that takes into account both richness and evenness

5.2 Tests of Microbial Diversity

A Mann-Whitney-Wilcoxon test of mean diversity (observed species) and age groups young (19 – 44) and older (45+) detected no significant differences between groups for any of the body sites **(Figure 3)**.

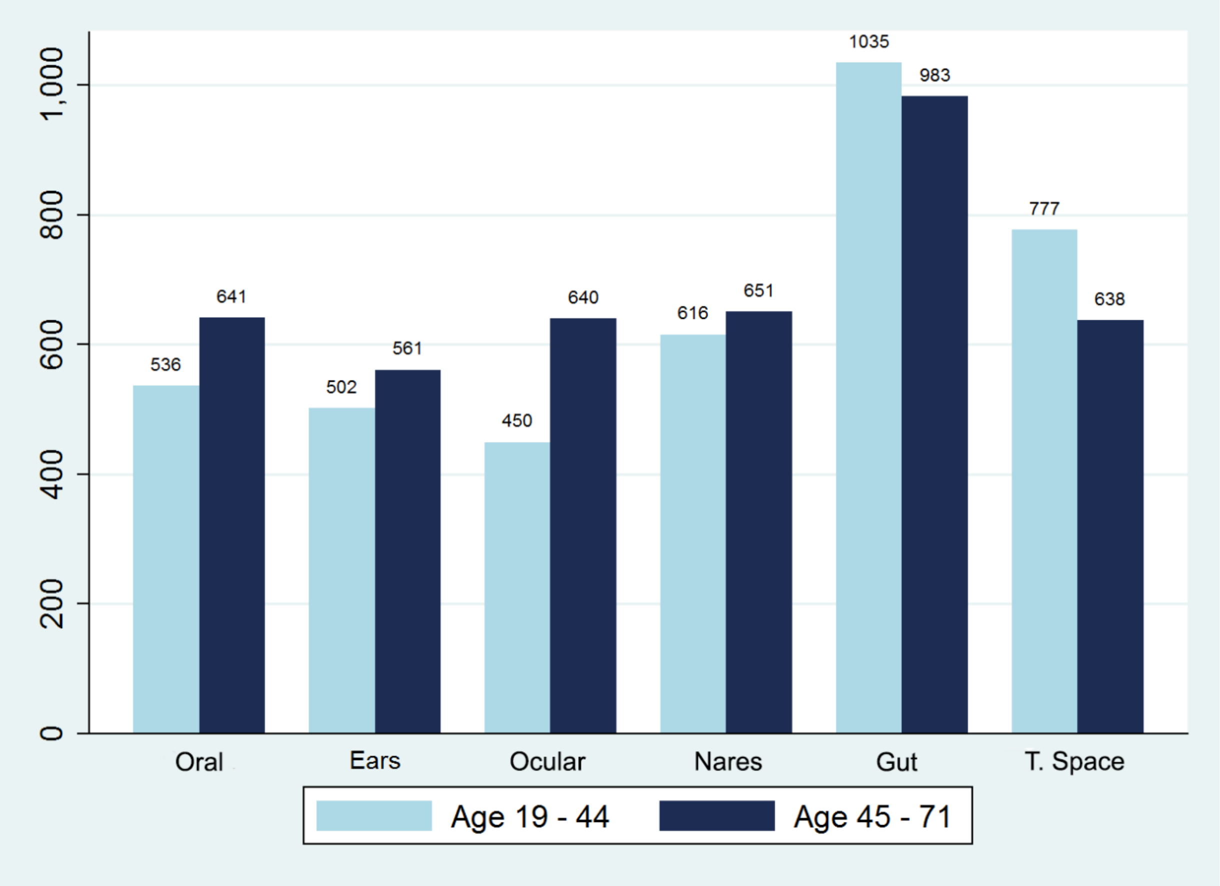

Figure 3. Mean Diversity in Observed Species by Age Group for Each Body Site

Figure 4 shows mean diversity (observed species) by body site and season of death (spring and summer). Significant differences were detected for the oral ($p = 0.02$) and gut ($p =$

0.02) microbiomes. Although only the oral and gut microbiomes were statistically significant, mean summer diversity was higher for all sites except for the gut microbiome.

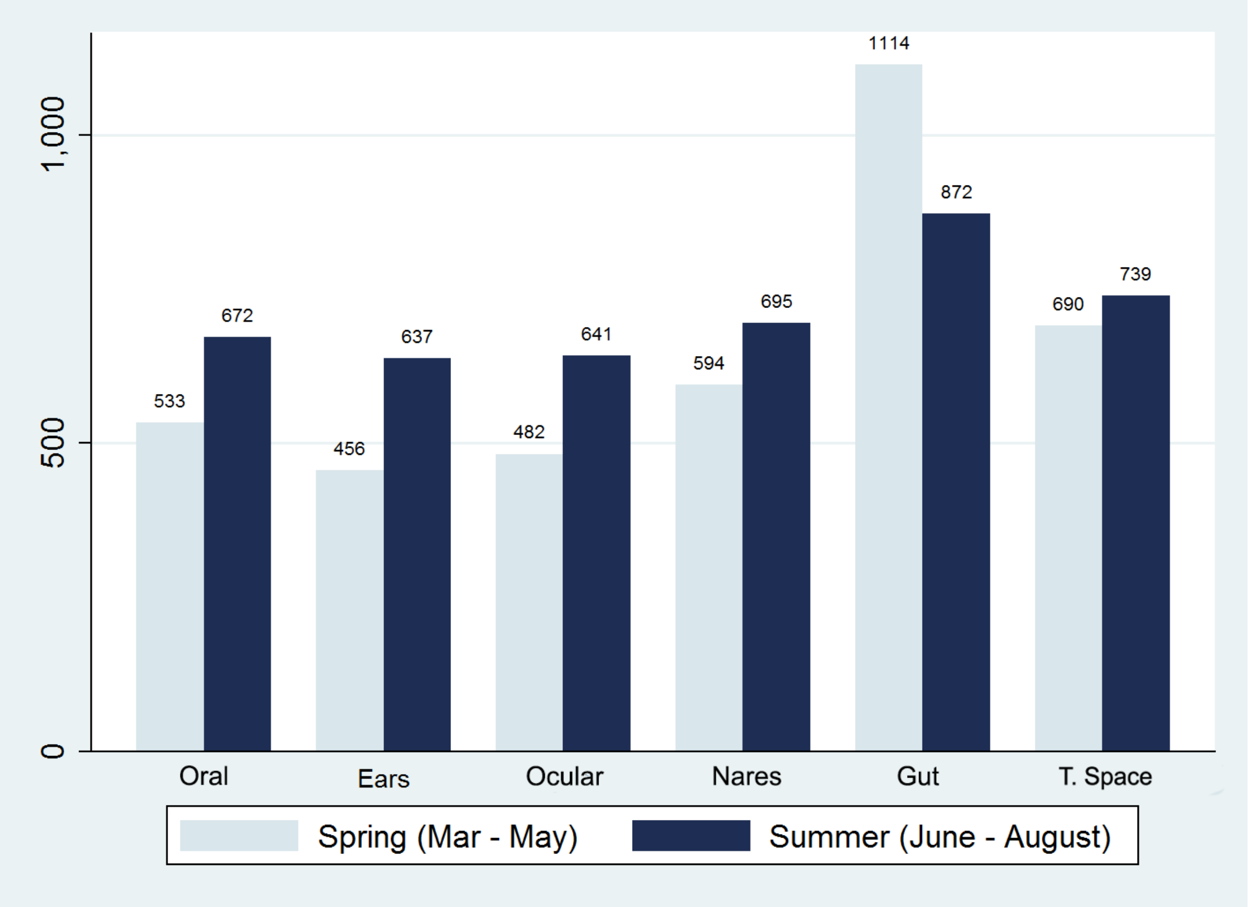

Figure 4. Mean Diversity in Observed Species by Season of Death/Sample Collection

Because this research used postmortem microbiome data, a unique opportunity is presented to examine microbial diversity in comparison to cause of death as shown in **Figure 5.**

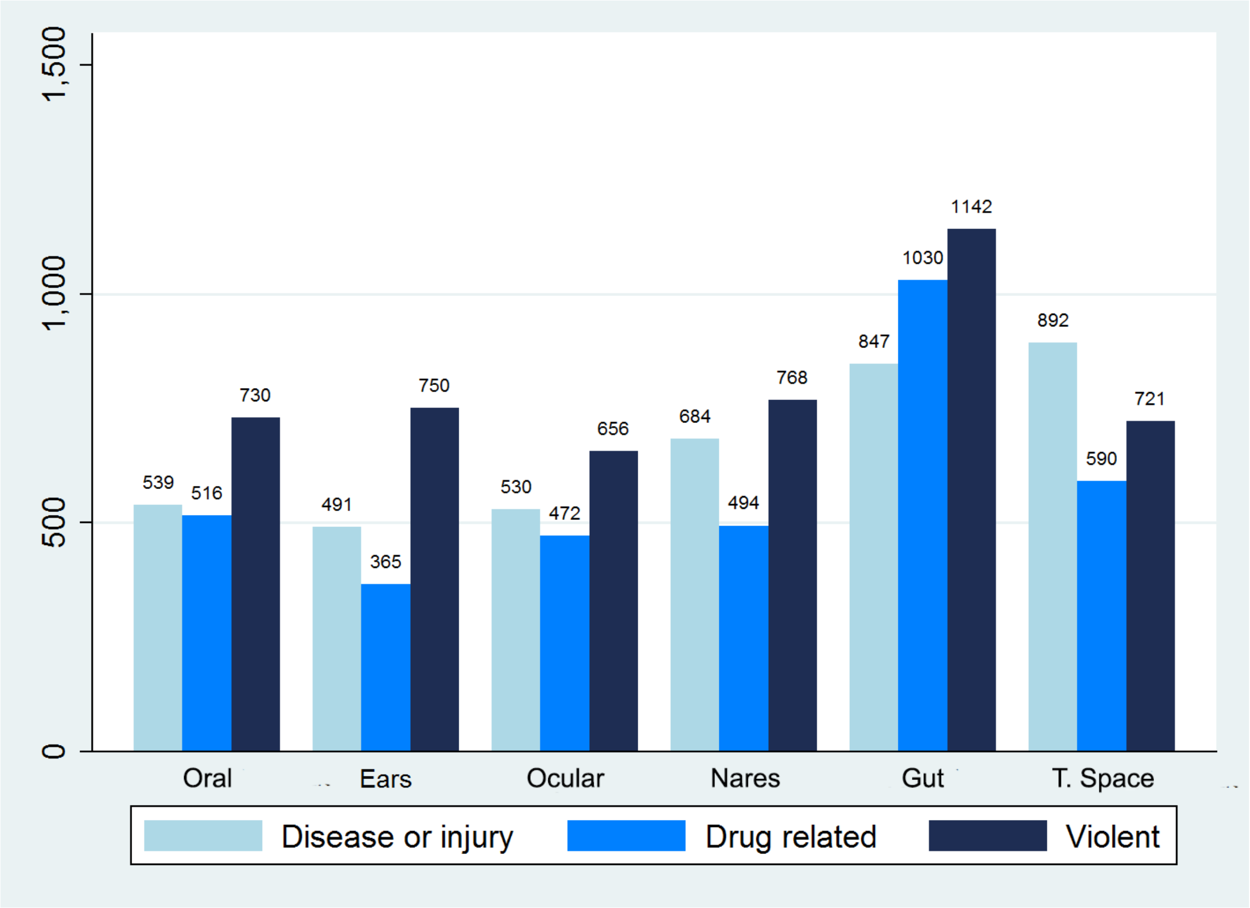

Figure 5. Mean Diversity in Observed Species by Cause of Death for Each Body Site

There is a significant difference in mean diversity (observed species) by cause of death at the $p < 0.10$ level for the ear microbiome ($p = 0.07$) and gut microbiome ($p = 0.08$). With the exception of the trabecular space, mean diversity (observed species) was highest for violent deaths. The gut microbiome shows the highest mean diversity for all causes of death except disease or injury.

The relationships between three individual measures of microbial diversity (observed species, Heip’s evenness index, and the Simpson index) and the Detroit index variables (DIER, DIUD – Lots, and DIUD – Structures) were tested for each body site **(Table 3).**

Table 3. Correlation of Index Variables and Measures of Diversity for Each Body Site

Index	**Diversity Measure**		**Oral**	**Ears**	**Ocular**	**Nares**	**Gut**	**T. Space**
DIER	Observed species	*rho =*	0.193	0.048	**0.348**	**0.373**	0.006	**0.386**
		p =	0.31	0.81	**0.07***	**0.05****	0.98	**0.05****
	Heip's evenness	*rho =*	0.074	-0.143	0.183	0.173	0.023	0.109
		p =	0.70	0.47	0.35	0.39	0.91	0.60
	Simpson index	*rho*	0.107	-0.077	0.250	0.264	0.034	0.294
		p =	0.58	0.70	0.20	0.18	0.87	0.15
DIUD - Lots	Observed species	*rho =*	-0.066	0.030	-0.176	-0.215	-0.286	-0.049
		p =	0.73	0.88	0.37	0.28	0.15	0.82
	Heip's evenness	*rho =*	-0.118	0.026	0.069	**-0.340**	-0.263	0.005
		p =	0.53	0.89	0.73	**0.08***	0.18	0.98
	Simpson index	*rho*	-0.214	-0.035	-0.037	**-0.364**	-0.172	-0.135
		p =	0.26	0.86	0.85	**0.06***	0.39	0.52
DIUD - Structures	Observed species	*rho =*	-0.053	0.189	-0.077	-0.226	-0.250	-0.277
		p =	0.78	0.34	0.70	0.26	0.21	0.18
	Heip's evenness	*rho =*	-0.117	0.065	0.229	**-0.355**	**-0.446**	0.026
		p =	0.54	0.75	0.24	**0.07***	**0.02****	0.90
	Simpson index	*rho*	-0.167	0.064	0.140	**-0.385**	**-0.380**	-0.184
		p =	0.38	0.75	0.48	**0.05****	**0.05****	0.38

*significant at p <0.10 level

**significant at the p < 0.05 level

The most significant correlation is between gut diversity (Heip's evenness) and DIUD – Structures (*rho* = -0.446, *p* = 0.02). There is also a significant relationship between gut diversity (Simpson) and DIUD – Structures (*rho* = -0.380, *p* = 0.05). In addition to gut diversity, nares diversity (Simpson) and DIUD – Structures are significantly correlated (*rho* = -0.3846, *p* = .05). Diversity (observed species) and DIER are significantly correlated with the microbiome of the nares (*rho* = .373, *p* = 0.05) and trabecular space (*rho* = .386, *p* = 0.05). Some relationships are significant at the *p* < .10 level. For the microbiome of the nares, these include DIUD – Lots and both the Simpson index (*rho* = -0.364, *p* = 0.06) and Heip's evenness index (*rho* = -.0.340, *p* = 0.08), as well as DIUD – Structures and Heip's evenness index (*rho* = -0.355, *p* = 0.07). The ocular microbiome and DIER are also correlated at this level (*rho* = 0.348, *p* = 0.07). The oral and ear microbiome sites have no significant relationships with any of the measures of diversity.

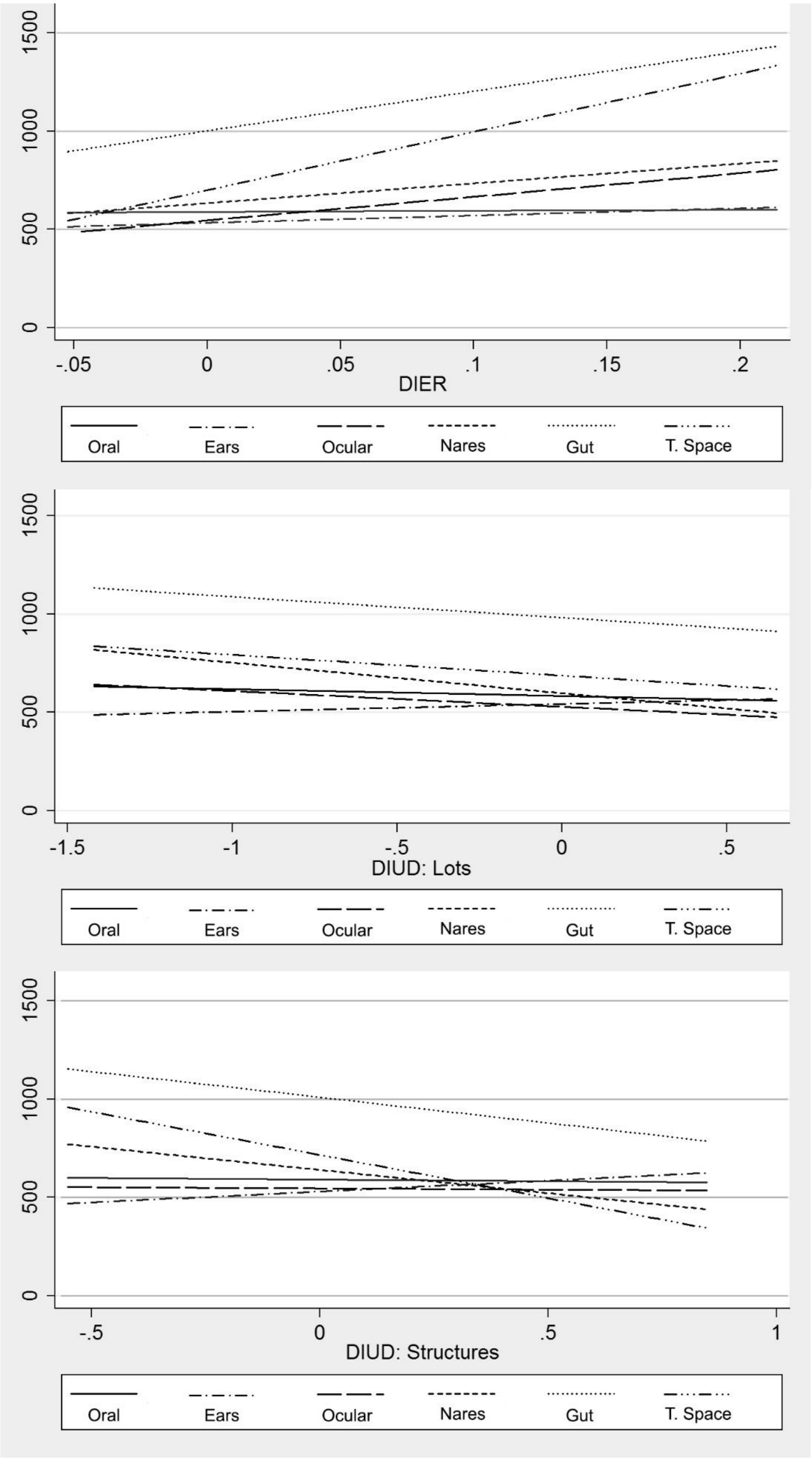

Figure 6. Fitted Values of Diversity (Observed Species) and Detroit Indexes

Figure 6 shows the directions of the relationships between diversity (observed species) and the Detroit indexes. Mean diversity is highest for the gut microbiome and the remaining sites are relatively similar in range. The oral microbiome remains relatively stable for all three indexes; the ocular microbiome shows a similar pattern for DIUD – Structures. The greatest variation between sites in observed in DIER; the amount of variation between body sites in the DIUD indexes are relatively similar to one another. Focusing on the gut microbiome as an example, diversity increases as DIER value increases and decreases as the DIUD values increase. The diversity of the trabecular space mirrors this pattern.

5.3 Major Phyla and Urban Decay and Recovery

Table 4 shows the relationships between the log counts (relative abundance) of the four most prevalent phyla (Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria) and the Detroit index values for each body site.

Table 4. Correlation of Index Variables and Log Counts of Four Major Phyla for Each Body Site

Index	**Phyla**		**Oral**	**Ears**	**Ocular**	**Nares**	**Gut**	**T. Space**
DIER	Actinobacteria	*rho =*	-0.068	0.002	0.166	**0.461**	-0.244	0.165
		p =	0.72	0.99	0.40	**0.02****	0.22	0.43
	Bacteroidetes	*rho =*	0.301	0.102	**0.357**	**0.534**	-0.023	0.268
		p =	0.11	0.61	**0.06***	**0.004*****	0.91	0.20
	Firmicutes	*rho*	-0.151	0.145	0.149	-0.117	0.174	-0.162
		p =	0.43	0.46	0.45	0.56	0.39	0.44
	Proteobacteria	*rho =*	-0.099	0.006	-0.266	-0.285	0.134	-0.035
		p =	0.60	0.98	0.17	0.15	0.51	0.87
DIUD - Lots	Actinobacteria	*rho =*	0.048	0.117	0.116	0.040	0.324	0.082
		p =	0.80	0.55	0.56	0.85	0.10	0.70
	Bacteroidetes	*rho =*	-0.032	0.230	-0.067	-0.204	-0.321	-0.176
		p =	0.87	0.24	0.73	0.31	0.10	0.40
	Firmicutes	*rho =*	-0.091	-0.227	-0.021	-0.131	0.130	-0.077
		p =	0.63	0.25	0.91	0.52	0.52	0.71
	Proteobacteria	*rho =*	**0.409**	0.089	-0.082	0.188	-0.080	0.205
		p =	**0.02****	0.65	0.68	0.35	0.69	0.33
DIUD - Structures	Actinobacteria	*rho =*	-0.054	0.043	**0.401**	0.121	0.168	-0.093
		p =	0.78	0.83	**0.03****	0.56	0.40	0.66
	Bacteroidetes	*rho =*	-0.012	0.275	0.086	-0.087	-0.245	-0.128
		p =	0.95	0.16	0.66	0.67	0.22	0.54
	Firmicutes	*rho =*	-0.152	-0.165	-0.305	-0.239	-0.033	-0.056
		p =	0.42	0.40	0.11	0.23	0.87	0.79
	Proteobacteria	*rho =*	**0.438**	0.091	0.008	0.186	-0.266	0.202
		p =	**0.02****	0.64	0.97	0.35	0.18	0.33

* significant at $p < 0.10$ level
**significant at $p < 0.05$ level
***significant at $p < 0.01$ level

The most significant relationship is between Bacteroidete abundance of the nares and DIER (*rho* = 0.534, *p* = .004). The abundance of Actinobacteria and DIER is also significantly correlated (*rho* = 0.461, *p* = .02). There is also a significant relationship between the Bacteroidete abundance in the ocular microbiome and DIER at the $p < .10$ level (*rho* = 0.357, *p* = .06). Actinobacteria of the ocular microbiome and DIUD – Structures (*rho* = 0.401, *p* = .03) are also significantly related. Lastly, there are significant relationships between the abundance of

Proteobacteria in the oral microbiome and DIUD for both lots (*rho* = 0.409, *p* = 0.02) and structures (*rho* = 0.438, p = 0.02). The four dominant phyla of the ear, gut, and trabecular space microbiomes do not have any significant relationships to the Detroit indexes. Only the ear microbiome has no significant relationships to either measures of diversity or abundance of major phyla.

Individual variation in the relative abundance of microbial communities at the phylum level is shown in **Figure 7**; the four most abundant phyla have been removed to highlight differences.

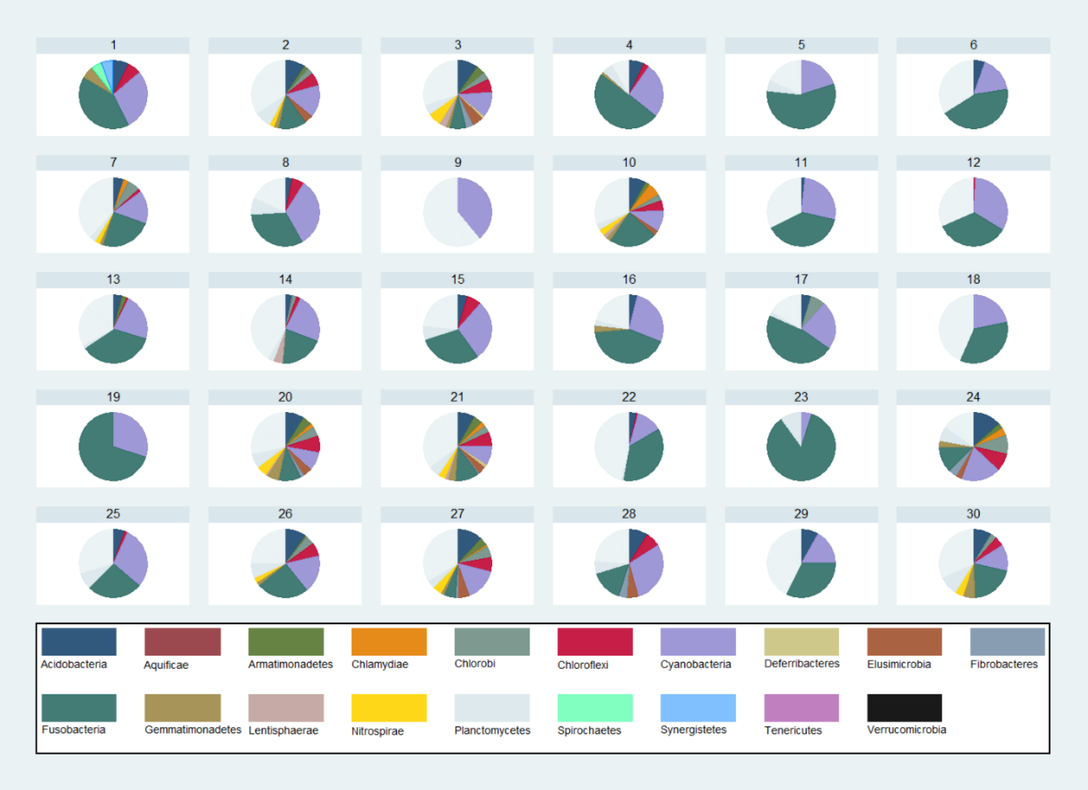

Figure 7. Phyla Composition of Each Participant Excluding the Four Most Prevalent Phyla

Variation in diversity and abundance can be observed across the sample. For example, persons 20 and 21 are similar to one another, but far more diverse than 11 and 12, who are similar to one another. However, some of these visual differences may be the result of individuals who are lacking data for one or more body sites.

Chapter 6

Discussion

The intention of this study was to explore potential associations of age, season of death, cause of death and neighborhood conditions with multiple measures of diversity and abundance for six independent sites of the human microbiome. The current study did not find any significant differences in mean diversity (observed species) between younger and older persons for any of the body sites tested. Previous research (Yatsunenko et al., 2012) suggests an increase in gut diversity with age, but measures age as a continuous variable from birth. The current study represents age with two groups of adults, so these results may not be comparable to the aforementioned research findings. Seasonal differences in mean diversity (observed species) were observed in the oral and gut microbiomes. Of all six body sites, only the gut microbiome had higher mean diversity in the spring. Existing research has also observed some seasonal differences in micro/necrobiome composition (Cameron et al., 2015; Carter et al., 2015; Berg-Lyons et al., 2011). Significant differences in mean diversity (observed species) by cause of death were observed for the ear and gut microbiomes at the $p < 0.10$ level. Mean diversity was highest for violent deaths at all body sites except for the trabecular space. Potential associations between microbial diversity and cause of death have not been explored in prior research.

Of the six body sites tested, the microbiome of the nares (nose) yielded the most significant relationships to the condition of the neighborhood. In contrast, only the microbiome of the ears did not yield any. This study supports the findings of previous research in multiple ways. First, the microbiome of the gut was consistently the most diverse region, using observed species, Heip’s evenness, and the Simpson index as measures. The exploration of the various roles of microbes in physical and mental health has established the gut microbiome to be a diverse community (Bervoets et al., 2013; Tyakht et al., 2013; Zhang et al., 2013) which was echoed in this study. The diversity of the buccal (inner cheek) microbiome, a site within the oral microbiome, was found to remain stable, regardless of neighborhood conditions. This is supported by previous research which found this site to be resistant to change in composition (Rautava et al., 2015). Despite this, an increase in the abundance of Proteobacteria in the oral microbiome was found to be significantly related to an increase in both structural and lot decay. This may be the result of a specific genus or species of Proteobacteria that is more abundant in conditions of decay, such as the intestinal bacteria *E. coli* (Kersters et al., 2006). Higher values of structural decay were significantly correlated with lower gut diversity using two of three diversity measures. This follows previous research that residents of urban areas have less diverse gut microbiomes (De Filippo et al., 2010), however, the same relationship did not occur for lot decay. Rather than being an effect of the condition of the built environment, the lower diversity may be related to other conditions of poor neighborhoods which also have poorer building conditions, such as lack of healthy food outlets. This explanation is supported by previous research that suggests an urban diet of processed foods decreases gut diversity (De Filippo et al., 2010).

Increased diversity (observed species) and abundance of Bacteroidetes of the nares and ocular microbiome were both significantly related to higher values of environmental recovery. Both of these sites are exposed to the outside. However, diversity in the ears was not significantly correlated with DIUD or DIER. Given the seasons of sampling, one possible explanation is that these results are in some way the effect of seasonal allergies. Should the symptoms of seasonal allergies result in increased contact of these sites with the diverse microbial niche on the skin of the hands, a change in diversity may result (Grice et al., 2009).

This is similar to observed changes in the composition of the ocular microbiome in wearers of contacts lenses (Shin et al., 2016). In addition, the diversity of the nares decreased in two of three measures (Heip's and Simpson) with the increase of both lot and structural decay. Of the six body sites studied, the microbiome of the nares appears to be the most influenced by neighborhood conditions.

This study has many strengths. It is the first to use human necrobiome data; this feature allowed for six unique body sites, including the trabecular space to be studied concurrently. Microbial niches such as the ears and ocular microbiome are not well represented within the human microbiome literature, and to the best of my knowledge, this is the first study to directly compare the microbial communities of the ears, ocular, and trabecular space to other sites on the body. This is also the first study to investigate how the condition of the neighborhood built environment may affect the human microbiome. The current study examined an American city that is both representative of the shrinking cities phenomenon and experiencing a revival, allowing for an evaluation of both components within a single context. Although all adults, the sample of the current study is not restricted to a certain group within the population, such as apartment dwellers or a certain age group such as grade school children or retired persons (Bond et al., 2007; Mitchel & Popham, 2008).

This research has multiple limitations. The variables used to quantify environmental recovery were not as comprehensive as those used to quantify urban decay. Parcel-level evaluation of the presence of features such as trees and gardens would be necessary to correct for this issue. This may have may have made it more difficult to detect significant associations between DIER and the human microbiome. Also, additional factors that may affect the composition of the human gut microbiome, such as diet, probiotic use, medical history, income, education, and occupation were not known for the participants in this study. One-third (n = 10) of the individuals died of drug related causes, which may or may not have an effect on the human microbiome. Pre- and post-mortem microbiomes have not yet been compared for drug-related or other causes of death. However, drug use is known to have an effect on postmortem scavengers, e.g. the growth rate of maggots, and thus may affect the micro/necrobiome (Catts, 1992). Lastly, sample size and the sampling of only a single point in time (‘snapshot’) may have influenced the relationships that were or were not detected in the current study. For example, differences in mean diversity (observed species) were associated with season of sampling for the oral and gut microbiomes in the current study. Future research would benefit from including an analysis of multiple time points to account for potential seasonal changes in microbial composition.

The findings of this study provide opportunities for policy implication and further study. While many relationships were found to be insignificant, the relationships that were significant were correlated in the predicted directions. Higher values of lot and structural decay were associated with lower microbial diversity; conversely, higher values of environmental recovery were associated with higher microbial diversity. This has several potential implications for cities of extreme socioeconomic disparity such as the City of Detroit. For example, structural decay is common and yet expensive to alter. Efforts to enhance the condition of the natural features of the built environment, such as gardening on empty lots, may then mitigate the effects of this urban decay. Compared to demolition, environment-based remedial efforts are far more economically feasible and often completed by volunteer groups, such as the Detroit Mower Gang (Nardone, 2016). As a part of this process, efforts to restore the built environment may also possibly impact the social environment with related mental health benefits.

This research has aimed to establish relationships between the condition of the surrounding environment and the human microbiome. However, to better understand these relationships and their effect on health, future research would benefit from replicating this process in a live population, making use of controls. For example, the study sample could include individuals from the same neighborhood who either are or are not involved in environmental recovery efforts. This would allow for a more in-depth measurement of exposure as well as the ability to control for possible intermediate variables such as diet, medical history, socioeconomic status, and length of residence in the community. Future research would also benefit from studying the microbial communities of urban residents at the genus and species level in comparison to neighborhood conditions. Doing so would elucidate which microbes are being affected (e.g. specific community members within the higher abundance of Proteobacteria in the oral microbiome that has been associated with conditions of decay). With this knowledge, the source and health implications of these microbes could be assessed. Should an aspect of urban decay be associated with pathogenic microbes, directly addressing the improvement of this feature has potential to positively impact community health.

This research has aimed to contribute to our knowledge of the human microbiome and its relationship to the built environment of a major city, one characterized by both extreme levels of decay and fervent revival efforts. Though exploratory, results are promising and warrant further investigation. From a perspective of social justice, this knowledge may help guide restoration efforts in other shrinking cities such as the City of Detroit. As the features of the built and social environments improve, the mental and physical health of disadvantaged urban residents may benefit as well.

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