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Initial Evidence of the Relationships between the Human Postmortem Microbiome and Neighborhood Blight and Greening Efforts

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The microbiome is important in human health, yet its connection to the built environment remains understudied. Little is known about the potential influence of neighborhood environments on the bacterial and archaea communities that live in and on the human body, henceforth the microbiome. Thus, we examined relationships between the microbiome and features of the urban environment. To do this, we first quantified neighborhood levels of blight (e.g., abandoned buildings) and green remediation (e.g., tree plantings) using parcel data in Detroit, Michigan, and then compared neighborhood status to the composition and diversity of the human postmortem microbiome. The postmortem microbiome served as a surrogate for biological signatures and lifestyles of living neighborhood residents. We observed significant clustering of microbial composition by neighborhood blight, with significantly higher abundances of potential pathogens associated with unhealthy living conditions. We also observed significant clusters between high and low green remediation for the mouth and eye communities only, with high levels of commensals (or nonharmful bacteria) in green remediation neighborhoods. Microbial biodiversity was significantly and positively correlated with green remediation and negatively correlated with blight. Regression models yielded the largest positive effects of green remediation on microbial richness (rectum) and diversity (nose) for women; the largest negative effects of blight were observed for evenness (eyes) among women and richness and diversity (mouth and nose) among men. These results provide evidence of a relationship between the human microbiome and neighborhood conditions, establishing the foundation for novel research opportunities into the effects of green remediation and urban blight on health. **Key Words:** *blight, Detroit, greening, microbiome, postindustrial.*

微生物在人类健康中相当重要，但其与建成环境的关联却鲜少受到研究。有关邻里环境对于生活在人体之内与之上的细菌与古菌社群——随后称为微生物——的潜在影响鲜为人知。我们因而检视微生物与城市环境特征之间的关联性。于此，我们首先运用密西根底特律的包裹数据，将邻里层级的衰颓（例如遗弃的建筑）和绿色整治（例如植栽）进行量化，接着比较邻里状态与人类死后的微生物的组成与多样性。人类死后的微生物作为活着的邻里居民的生物特征与生活风格的代理人。我们观察到依照邻里衰颓的显著微生物组成集群，显著较多的潜在病原体并与不健康的生活条件相关。我们同时观察到仅存在于嘴与眼社群的高度与低度绿色整治之间的显著集群，绿色整治邻里中则存在高度的共生者（或不具害处的细菌）。微生物生态多样性与绿色整治显著且正向相关，并与衰颓呈现负相关。迴归模型生产出绿色整治对女性的微生物丰富度（直肠）和多样性（鼻）的最大正向影响；衰颓的最大负面效应，则在女性的平均（眼睛）和男性的丰富性与多样性（口与鼻）中观察到。这些结果为人类微生物与邻里条件之间的关联性提供了证据，为崭新的研究机会发展成健康的绿色整治和城市衰颓效应建立基础。 **关键词:** 衰颓, 底特律, 绿化, 微生物, 后工业。

Aunque el microbioma es importante para la salud humana, su conexión con el entorno construido continúa sin suficiente estudio. Poco se sabe de la potencial influencia de los entornos vecinos sobre las comunidades bacterianas y arqueas que habitan dentro del cuerpo humano y en su exterior, en lo sucesivo denominados microbioma. Por eso, examinamos las relaciones entre el microbioma y los rasgos del medio ambiente urbano. Para hacerlo, primero cuantificamos los niveles de deterioro del vecindario (por ejemplo, edificaciones abandonadas) y el remedio verde (por ej., arborización) usando datos por parcela en Detroit, Michigan, para luego comparar el estatus del vecindario con la composición y diversidad del microbioma humano post mórtem. El microbioma post mórtem sirvió como un sustituto de las marcas biológicas y estilos de vida de los residentes del vecindario vivos. Observamos un agrupamiento significativo de la composición microbiana por deterioro urbano en el vecindario, con abundancias significativamente más altas de patógenos potenciales asociados con condiciones de vida insalubres. También observamos significativos agrupamientos entre alta y baja remediación verde únicamente para las comunidades bucales y oculares, con altos niveles de comensales (o de bacterias inofensivas) en los vecindarios con remediación verde. La biodiversidad microbiana estaba positivamente correlacionada de modo significativo con la remediación verde y negativamente correlacionada con el deterioro urbano. Los modelos de regresión rindieron los efectos positivos más grandes de la remediación verde sobre la riqueza microbiana (recto) y diversidad (nariz) para las mujeres; los efectos negativos más grandes del deterioro fueron observados por regularidad (ojos) entre las mujeres y riqueza y diversidad (boca y nariz) entre los hombres. Los resultados proveen evidencia de una relación entre el microbioma humano y las condiciones del vecindario, estableciendo la fundamentación para novedosas oportunidades de investigación de los efectos de la remediación verde y el deterioro urbano sobre la salud. *Palabras clave:* deterioro urbano, Detroit, reverdecimiento, microbioma, posindustrial.

Human health can be influenced by our neighboring environment, both positively and negatively. Evidence abounds on the close relationships between residents' health and the neighborhood built environment (i.e., human-made features and structures) and the social and ecological milieu of neighborhoods (Cohen et al. 2003; Galea et al. 2005; Kruger, Reischl, and Gee 2007; South et al. 2015). On the negative side, neighborhood poverty and deteriorating built environments have been associated with a multitude of health risks (Kruger, Reischl, and Gee 2007), notably poor mental health (Galea et al. 2005). On the positive side, neighborhood green space might lower anxiety (e.g., Nutsford, Pearson, and Kingham 2013) or encourage physical activity (e.g., Coombes, Jones, and Hillsdon 2010). Both of these competing neighborhood influences are operating in many postindustrial cities. Postindustrial cities, such as those throughout the Rust Belt of the United States, often have high levels of neighborhood vacancy, abandoned homes, and lower tax revenue to maintain public spaces. At the same time, some Rust Belt cities, such as Detroit, Michigan, are also experiencing urban revival efforts to promote a sense of community and to make use of abandoned or unmaintained spaces, largely at the grassroots level and driven by volunteers.

Both blight features (e.g., vacant homes, broken windows) and signs of urban recovery (e.g.,

neighborhood greening efforts, hereafter green remediation) directly affect stress levels and heart rate, in opposing directions (South et al. 2015). Indirectly, deteriorating neighborhood conditions affect anxiety, depression, and mood disorders by lowering perceptions of neighborhood safety, social cohesion, and trust between neighbors (Cohen et al. 2003). In contrast, neighborhood greening efforts commonly include the maintenance of blighted properties through mowing (Sadler et al. 2017), signage, removal of waste, tree plantings, and installation of gardens (White 2011) on vacant properties, which serve as visual indications of care (Sampson et al. 2017) and improved feelings of safety (Garvin, Cannuscio, and Branas 2013). Greening efforts reduce visible signs of disorder, which have both a knock-on effect to further reduce dumping of litter (Joo and Kwon 2015) and other forms of neighborhood blight and also increase collective efficacy and empowerment of residents (Johansen Neal, and Gasteyer 2015), leading to cardiovascular benefits (South et al. 2015) and reductions in stress levels assessed via salivary cortisol (Roe et al. 2013) and through self-reporting (Branas et al. 2011). In fact, a recent review of the health benefits of greening efforts concluded that such efforts improve many health outcomes and related behaviors for residents (World Health Organization 2017). In addition to stress reduction and lowered blood pressure, to date,

studies quantifying health benefits of green spaces have found associations with improved self-reported outcomes (Amoly et al. 2014) and symptoms (Beyer et al. 2014), cognition (Berman, Jonides, and Kaplan 2008), and cardio-metabolic outcomes (Kardan et al. 2015). Green spaces can also serve as locations to engage in physical exercise, which has health benefits, including lower blood pressure and risk of a suite of chronic diseases (Centers for Disease Control and Prevention 2016). Moreover, physical activity when in direct contact with nature—called *green exercise*—has been shown to improve mental health and lower perceived stress and negative emotions (Pretty et al. 2005; Olafsdottir, Cloke, and Vogeley 2017). Finally, greening efforts such as community gardens have shown benefits including social cohesion and interactions, increased safety, and life satisfaction (for a review, see Guitart, Pickering, and Byrne 2012).

In these ways, neighborhood conditions can influence perceptions through visual exposure but might also shape the ways in which residents interact with one another and with the built infrastructure. Another understudied, yet plausible, mechanism through which neighborhood conditions influence health conditions is via the human microbiome (defined here as the bacterial and archaeal communities in and on the body; Huttenhower et al. 2012). In theory, microbes might live in and on the built and ecological features of neighborhoods and also in and on neighbors. The neighborhood conditions can and do shape the way in which we interact with those features and with one another and thus the exchange of microbes in the environment (Lax et al. 2014).

The microbiome might, in fact, serve as a subclinical indicator of both physical and mental health. For example, the composition or diversity of one's microbiome could play pivotal roles in metabolism, immune function, and mental health (Rook 2013). The microbiome of the gut, which is the most extensively studied microbial habitat in humans, has been linked to what has become known as the gut-brain axis, "a bidirectional intercommunication between the gut and the brain" (Bervoets et al. 2013; Farmer, Randall, and Aziz 2014, p. 2982). A lack of diversity in the gut microbiome has been associated with depression, anxiety, and Alzheimer's disease (Foster and McVey Neufeld 2013; Hsiao et al. 2013; Hill et al. 2014; Mayer et al. 2014; Naseribafrouei et al. 2014). Furthermore, the role of built environment sources of microbes and the

potential association with human health have been reviewed and suggests important interactions between environmental microbes and health that can be understood using high-throughput sequencing technologies (Prussin and Marr 2015). One classical example is that of Legionnaires' disease, where the pathogen, *Legionella* spp., grows in building water systems (Fields, Benson, and Besser 2002) and is dispersed via aerosol through air conditioners and other sources of misting water (e.g., mist sprayers), where the host inhales it and becomes infected. The built environment microbiome could hold important implications for pathogen and other microbe transfer in society and thus population health (Hoisington et al. 2015). Although research is moving in the direction of built environment associations with human microbiomes and health, geographic studies characterizing the neighborhood environment, particularly in urban, understudied, low-income settings are needed.

Geography becomes central to the study of the human microbiome because microbe sharing and transfer and conditions to support microbial life are intrinsically spatially defined. Studies of the human microbiome and surrounding environments are emerging but currently sparse. Still, some evidence suggests that this is both a reasonable and an exciting area of inquiry. Environmental exposures do play a key role in the development of microbial communities (Turnbaugh et al. 2007), and human microbiomes can be detected in the built environment itself (Lax et al. 2014). Humans both release and obtain microbes (Prussin and Marr 2015) through shared contact with features of their built environment (Lax and Gilbert 2015) and one another. The microbiomes of the built environment also play an important role in mental health (Galea et al. 2005). In one popular example linking the human microbiome to the built environment, samples were taken to characterize the microbiological profiles of the New York City subway system, a canal, and public parks ($n=1,457$). The authors were able to detect legacy indicators of the human-related microbes found in the samples based on the U.S. Census demographic profile of the area from which the samples were taken (Afshinnekoo et al. 2015), supporting the notion that residents are sharing microbes through neighborhood public spaces. Evidence is also emerging that land use (as a proxy for environmental biodiversity) in one's neighborhood is associated with the biodiversity of the skin microbiome of residents

(Hanski et al. 2012). This is akin to how environmental conditions are critical to the life of multicellular organisms that influence disease (e.g., land use influences mosquitos and thus malaria; Meade 1976). Such research promotes the theory that human contact with our surroundings—built, social, and natural—influences our microbiome and that the microbiome affects human health. In this way, the microbiome might exemplify the “fundamental permeability of the body” and could blur the boundaries between human and environment, similar to other geographic work on epigenetics (Guthman and Mansfield 2012, 496). Yet, to date, research on the connections between the human microbiome and neighborhood conditions is very limited.

Little is currently known about the composition and biodiversity of the microbiome of urban residents from neighborhoods with varying conditions. It is unclear whether those living in blighted neighborhoods might have unique microbial signatures, which could act as potential biological markers for the higher levels of mental illness in these settings (Kruger, Reischl, and Gee 2007), and whether green remediation has the potential to improve health via, for example, the changes in the biodiversity of the microbiome. Therefore, studies characterizing the composition and diversity of microbial communities in differing neighborhood environments could inform our ability to manipulate these features and might serve as an opportunity to address the disparate health outcomes observed in disadvantaged neighborhoods (e.g., Marmot 1997; Kennedy et al. 1998; Diez-Roux, Link, and Northridge 2000; Gordon-Larsen et al. 2006; Pearson et al. 2013).

The city of Detroit, Michigan, has many heavily blighted, high-vacancy neighborhoods, with up to 25 percent population loss since 2000. Once a major industrial center, the city has experienced rapid decline both economically and in population since the waning of the automobile industry (Martinez-Fernandez et al. 2012). Abandoned buildings have led to higher neighborhood crime in Detroit (Raleigh and Galster 2015), with implications for social cohesion among neighbors (Portes 1998). In response, recovery projects and activism have become increasingly prevalent and are largely centered on low-price land acquisition, improved maintenance, tree planting, urban farming, and street gardens in Detroit's vacant lots, collectively considered green remediation.

Here, we used a unique opportunity to partner with a medical examiner's office of a large urban

metropolis where postmortem microbiome characterization is part of ongoing death investigation, to quantify and model associations of the human microbiome with levels of neighborhood blight and green remediation. A previous study has shown the postmortem microbiota in adults is (1) structured by anatomic location, (2) stable within the first forty-eight hours after death and (3) potentially linked to antemortem health conditions (i.e., cardiovascular disease; Pechal et al. 2018). Given the absence of consensus on what constitutes a healthy microbiome and the exploratory nature of this novel area of inquiry, we did not develop specific hypotheses about the presence or specific pattern or direction of associations between microbial diversity or composition and neighborhood conditions. To conduct this study, we combined geospatial data on the extent of neighborhood blight and green remediation and microbial samples collected from five anatomic regions, less than forty-eight hours postmortem, of all cases received by the Wayne County Medical Examiner's Office in 2014 and 2015 in the City of Detroit ($n=48$) to examine the relationship between blight and green remediation and microbial composition and diversity.

Materials and Methods

Study Sample and Demographic Data

Microbial samples were collected from bodies ($n=48$) received during routine death investigations in 2014 and 2015 at the Wayne County Medical Examiner's Office in Detroit, Michigan. Inclusion criteria included being a resident of Detroit and having successful microbial DNA extraction of the five anatomic regions (see later for details). Demographic data were recorded at death, including manner of death, body mass index (BMI), age, sex, ethnicity, and day of death. Note that when used in forensic pathology *manner of death* categorizes the death as natural, accident, suicide, homicide, indeterminate, and pending, whereas *cause of death* indicates the medical cause of death. Here, we use *circumstances of death* to further define categories that we consider to relate to the microbiome: drug-related, violence and injury, and chronic disease deaths. Drug-related deaths included drug overdoses. Violence and injury included gun-related deaths and accidental injuries. Chronic disease deaths included cardiovascular

disease and diabetes. We created these categories based on potential relationships with the microbiome. For example, violent or injury-related deaths are likely to occur suddenly and unrelated to existing disease, which might affect the microbiome. Yet, these individuals might be exposed to risky social conditions, which could influence the microbiome. In contrast, those living with chronic diseases (e.g., obesity, diabetes, Crohn's disease, depressive disorders) might be taking prescription medication, have poorer nutrition, and have decreased physical activity, leading to effects on microbial diversity, as reviewed by Cho and Blaser (2012). Likewise, those habitually using drugs are likely to have altered microbiomes as a result of drug use and its related lifestyle, similar to evidence showing that the colonic microbiome is altered by alcoholism (Mutlu et al. 2012).

Microbiome Data Collection and Laboratory Analysis

Microbial samples were collected by swabbing five anatomic locations of each body. Briefly, each anatomic region was individually sampled with a DNA-free cotton-tipped applicator with a wood handle (Puritan, 25-8061WC FDNA, Guilford, Maine). The cotton tip portion was carefully removed from the handle and placed in a sterile collection tube (VWR, 1.7 mL, 87003-294, Radnor, Pennsylvania) containing 0.2 mL 100 percent molecular-grade ethanol. The five anatomic locations included the external auditory canal (ears), eyes, nose, mouth, and rectum. Samples were stored at -20°C until further processing for microbial (bacteria and archaea) genomic DNA. Sampling occurred most often for these cases at the death scene, except for the rectum, which was routinely swabbed at the medical examiner's office.

DNA extractions were performed on individual swabs as described in detail elsewhere (Pechal et al. 2018). Individual samples were extracted using a modified protocol for PureLink Genomic DNA Mini Kits (Thermo Fisher Scientific, Waltham, Massachusetts), quantified using Quant-iT dsDNA HS Assay kit and Qubit 2.0 (Thermo Fisher Scientific), and Illumina MiSeq 16S library construction ($2 \times 250\text{bp}$, paired-end reads) and sequencing was performed at the Michigan State University Genomics Core Facility using a modified version of

the protocol adapted for the Illumina MiSeq (Caporaso, Bittinger et al. 2010). The V4 region of the 16S rRNA gene was amplified with region-specific primers (Claesson et al. 2010; Caporaso et al. 2011; Caporaso et al. 2012). We classified sequences into operational taxonomic units (OTUs) based on the May 2013 Greengenes 97 percent reference data set (see <http://greengenes.secondgenome.com>; Second Genome Inc., n.d.). Data were filtered to remove chloroplast, mitochondria, and singleton OTUs, with any remaining low-abundance OTUs making up <0.0005 percent of reads in the total data set also removed (Bokulich et al. 2013). Not all samples returned the same number of sequences and thus samples were rarefied to 1,000 sequences for subsequent analyses to remove the effect of sample size bias on community composition; there were no microbial libraries containing fewer than 1,000 sequences. In preliminary analyses we determined little change in the postmortem microbiome within forty-eight hours of death. Although not perfect, the postmortem human microbiome is a suitable proxy of the living individual's microbiome (for further details, see [Supplementary Materials](#)) and allows insight into the microbiomes of large and diverse demographic groups. Microbial community composition and estimates of biodiversity (observed richness, Simpson Index $[1 - D]$ diversity, and Simpson's evenness) were calculated using QIIME (Caporaso, Kuczynski et al. 2010).

Quantifying Neighborhood Blight and Green Remediation

First, coordinate locations for each individual's home were obtained by manually entering the address of residence into Google Maps. For cases where the decedent's home address was unknown, the location of death was considered the sleep location ($n=6$, 13 percent). Sleep locations include areas where the homeless sleep and research indicates that the characteristics of sleep neighborhoods parallel those where homeless were previously housed (Culhane, Lee, and Wachter 1996; Alexander, Pollio, and North 2013). Last, in the event of a discrepancy or typography error in the address, Google Earth Pro was used for verification.

Next, spatial data on blight were compiled via the Motor City Mapping project, a thorough evaluation of the condition of Detroit's parcels (both lots and structures). First completed in 2014 and continually

updated via volunteer participation and a freely available smartphone application, the Motor City Mapping data set used in this study consists of volunteer-collected data supplemented by the organization with twenty-four additional data sets pertinent to city functions. This study used the Motor City Mapping Enhanced data set released 1 October 2014. The blight features for lot parcels ($n=112,040$) include inactivity, presence of dumping, and lack of maintenance. The blight features for structure parcels ($n=267,509$) include needed demolition, vacancy, presence of dumping, fire, and lack of security.

Public records from the City of Detroit were used to compile most of the green remediation data. These included lots sold and monitored through the Detroit Land Bank Authority Side Lot Sales (2014–2015, $n=731$), city parks listed as in “good” or “fair” condition (2015, $n=182$), and tree locations provided by The Greening of Detroit’s OpenTreeMap (2015, $n=15,191$). In addition, the locations of independent urban farming projects were collected and geocoded. These data were collected by an Internet search of the terms “urban farm[ing]” and “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). The timing of the blight and green remediation data overlap with the microbiome data (2014–2015).

Features of blight and green remediation were compiled and validated using Google Street View (Figure 1). Then, we assigned each parcel a high or low value for blight and green remediation, based on the following criteria. For blight, we summed features of disrepair or abandonment for lots and structures separately. Specifically, we summed inactivity, presence of dumping, and lack of maintenance for each lot parcel and found that only 25 percent ($n=27,636$) had no features of disrepair or abandonment. For structure parcels, we summed demolition, vacancy, presence of dumping, fire, and lack of security (could be entered by a vagrant) and found that 81 percent ($n=216,862$) had no features of disrepair or abandonment. We defined parcels in major disrepair or poor condition or maintenance as having one or more negative features, yielding a total of 135,051 parcels (36 percent). For green remediation, we assigned binary variables to each parcel where 1 indicated that



Figure 1. Google Street View images used for validation. (A) The case home location that was missing from the Motor City Mapping data set. (B) Examples of parcels in the sample that were defined as having high blight or high green remediation.

trees had been planted, the presence of a maintained park, community garden, or urban farm (only 3 percent of parcels; $n=12,649$). For both blight and green remediation, these binary variables were linked to each parcel in ArcGIS Version 10.3 (ESRI, Redlands, California). Last, for each buffer, the proportion of blighted parcels and the proportion of green remediation parcels were calculated as assigned to each individual. Neighborhoods with high blight and high green remediation were not mutually exclusive.

In our study, neighborhood was defined by a radius of 0.40 km around the home for green remediation and 0.72 km for blight (Figure 2), which echoes other research in postindustrial Michigan. Other researchers have quantified the number of buildings in disrepair or not salvageable and found that these features are associated with changes in mental health (Kruger, Reischl, and Gee 2007), premature births,

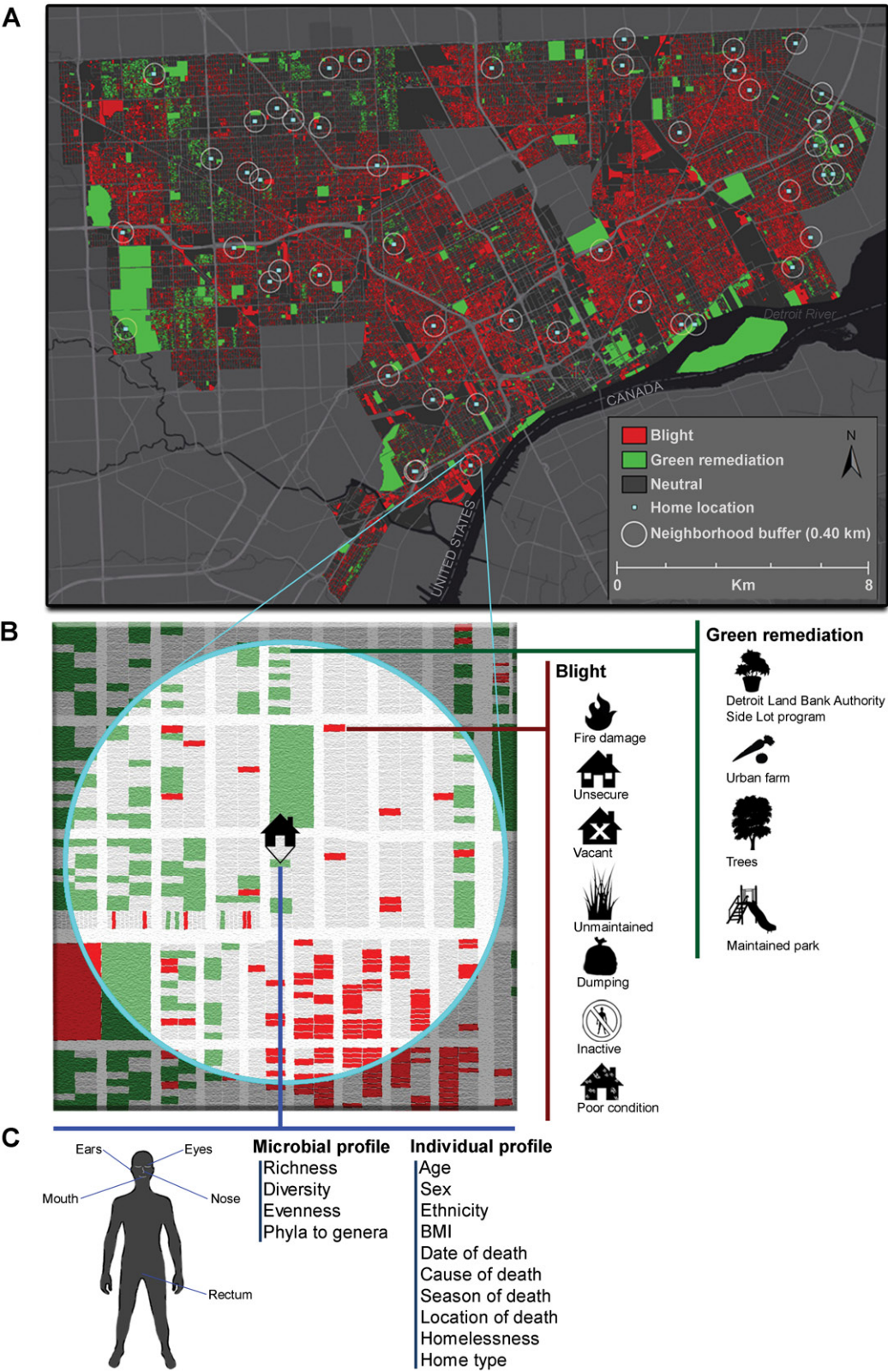


Figure 2. Study methodology. (A) Map of blight and green remediation for all parcels in Detroit, including the home locations and neighborhood buffers for all individuals in the study. (B) Example home location and neighborhood buffer for one individual, including each feature of blight and green remediation captured. (C) The individual-level characteristics and microbial profiles used in the study.

and low birth weight (Kruger, Munsell, and French-Turner 2011) at a distance of 0.25 miles (0.40 km) for residential structures.

Neighborhood Poverty and Monthly Average Temperature Data

We also compiled data on a number of potential confounders. For example, there might be an association between wealth and microbial diversity (although the direction is unclear based on previous evidence) and between wealth and neighborhood conditions. In some developing countries, diversity was higher among the poorest (Chong et al. 2015), whereas in other contexts diversity was lower among the poor (Conlon and Bird 2014). The built environment also tends to be more blighted in poor areas (Weaver and Bagchi-Sen 2013). With this in mind, we compiled poverty data for census block groups. Poverty data were obtained from the U.S. Census American Community Survey 2010 five-year estimates for Michigan (U.S. Census 2010). We used median income and percentage of neighborhood households living in poverty.

In addition, the microbiome could be affected by season, in that plant life and human behaviors (e.g., interactions with neighbors or walking through the neighborhood might be less frequent in the winter) vary across seasons. Evidence suggests that microbial diversity is dependent on the environment, including both biotic and abiotic factors (Thompson et al. 2017). Because our sample involved people who died at different times throughout the study period, we created a variable for average temperature during the month of death. Temperature data were collected from the archived monthly weather summary for Detroit, Michigan (42, 13 N, 83, 20 W, WBAN number: 94847), from the National Weather Service Office (2016).

Analytical Methods

First, we calculated descriptive statistics on the sample characteristics (e.g., mean age, BMI). Next, to assess differences in microbial community composition for each anatomic region, we used the Bray–Curtis dissimilarity, one of the most common approaches to quantify β diversity (Bray and Curtis 1957). It is robust even with counts of zero, as is often the case for microbiome data. Once

dissimilarities between samples have been computed, they can be positioned (ordinated) in a low-dimensional space (in this case two dimensions) to observe clustering patterns. One ordination technique in microbial community ecology to visualize sample clustering is nonmetric multidimensional scaling (NMDS; Rees et al. 2004). In this study, we used NMDS with Bray–Curtis dissimilarities and calculated p values using ADONIS in R *vegan* (Oksanen et al. 2007) to identify significant clustering of phyla by status of neighborhood conditions (i.e., high vs. low green remediation and high vs. low blight). Mean blight or green remediation values were used as thresholds for high and low binary indicators.

We then used Kendall's tau and corrected p values to test correlations between measures of microbial biodiversity (each anatomic region) and between abundances of selected genera (all anatomic regions) and case and neighborhood characteristics, using Bonferroni corrections for the p values based on multiple comparisons (for full results see Table S.1). Genera shown were selected because they exhibited five or more significant correlations across the case and neighborhood variables. Next, we examined mean relative abundance of each phylum- and genus-level OTU for each anatomic microbiome and for the overall microbiome and calculated p values using the Wilcoxon signed rank sum test, which allowed for repeated measure analysis by neighborhood blight and green remediation status (for full results see Table S.1). We also used indicator species analysis using the default parameters in R (*labdsv* library) to examine clustering and correlations between genera relative abundance and individual-level and neighborhood-level characteristics.

Finally, we fitted separate linear regression models using three metrics of biodiversity (taxon richness, Simpson's diversity [$1 - D$], or Simpson's evenness), as calculated in QIIME using default parameters, as the dependent variable and neighborhood blight or green remediation as the independent variable of interest, adjusted for age, ethnicity, BMI, circumstance of death, summer death, outdoor death, and poverty, stratified by sex and anatomic region (except ears). In total, we fitted forty-eight models. We stratified the cases by sex to examine possible differences in neighborhood effects on the microbiome, as has been found in other research (Haro et al. 2016). We stratified by anatomic region for two reasons. First, microbial biodiversity has distinct community

Table 1. Descriptive statistics for cases

Characteristics	Female (n = 14)	Male (n = 34)	Total (n = 48)
Age			
M	43	44	43
SD	15	17	16
Age 18–29			
n	4	9	13
%	29	26	27
Age 30–49			
n	4	12	16
%	29	35	33
Age 50–69			
n	5	11	16
%	36	32	33
Age 70+			
n	1	2	3
%	7	6	6
Ethnicity			
African American			
n	9	28	37
%	64	82	77
White			
n	5	6	11
%	36	18	23
BMI			
M	27	27	27
SD	8	8	8
Year of death			
2014			
n	11	18	29
%	79	53	60
2015			
n	3	16	19
%	21	47	40
Homeless			
n	1	5	6
%	7	15	13
Site of death			
Outdoors			
n	4	6	10
%	29	18	21
Indoors			
n	10	28	38
%	71	82	79
Circumstance of death			
Chronic disease			
n	4	9	13
%	29	26	27
Drugs			
n	6	10	16
%	42	29	33
Violent/injury			
n	4	15	19
%	29	45	40
Summer death			
n	5	10	15
%	36	29	31

(Continued)

Table 1. (Continued)

Characteristics	Female (n = 14)	Male (n = 34)	Total (n = 48)
Lived in apartment			
n	5	3	8
%	36	9	17
Median household income in thousands			
M	29.3	32.3	31.5
SD	12.0	12.9	12.7
% Block group in poverty			
M	30	31	31
SD	14	16	15

Note: BMI = body mass index.

composition and function based on anatomic region (e.g., gut, vagina, skin, mouth), and studies have shown significant microbial community differences among the sampled anatomic regions (Eckburg et al. 2005; Grice et al. 2008; Peterson et al. 2009; Cho and Blaser 2012; Knight et al. 2017). Second, we theorize that neighborhood conditions might be more likely to influence the microbiome of the mouth, nose, and eyes, because dust particles have been shown to carry microorganisms (Yamaguchi et al. 2012). Therefore, environmental microbes might enter these areas through respiration or wind blowing particles into the eyes. In contrast, the internal gut microbiome (rectum) has reduced contact with the physical environment. We omitted regression results related to the ears because environmental exposures were less likely to be relevant and for the clarity of the figure (results available on request). We also examined a possible interaction between blight and green remediation, but the interaction was not statistically significant and thus was not included in the final models. We used chronic disease deaths as the reference group. We included age as an independent variable because the gut microbiome has been shown to change drastically from infancy to older adulthood, which is thought to, in part, relate to changing diet and geography (Langille et al. 2014). Of the few studies in humans, age was shown to be positively associated with diversity (Yatsunenko et al. 2012). BMI was included because diet-induced obesity is associated with an imbalance in the normal microbial composition of the gut (Etteberria et al. 2015). Adults eating diets characterized by high saturated fat, low nutrients, and low plant intake also tend to have lower microbial diversity, particularly of the gut (Conlon and Bird 2014). Of course, obesity is also related to non-diet-related factors including physical activity (Ladabaum et al. 2014) and epigenetic

conditions (for a discussion, see Guthman and Mansfield 2012). To account for the possibility that the microbiome of cases discovered outdoors (rather than dying inside a building) might be affected by environmental microbes, we also included this variable. We reported standardized β coefficients and 95 percent confidence intervals (CIs), using the standardized standard errors, to make comparisons across the models. Significant results were reported at the $p < 0.10$ level.

Results

In our study sample, most cases were male (71 percent), African American (77 percent) and, on average, overweight (mean BMI = 27, SD = 8; Table 1). For females, the most common circumstance of death was drug related (42 percent), whereas for males it was violence or injury (45 percent). On average, individuals were from neighborhoods characterized by high percentage poverty (~30 percent of the population below poverty level).

For measures of microbial biodiversity, we found consistent negative correlations with neighborhood blight (Figure 3). In the mouth, we observed negative correlations for all features of blight and green remediation and both diversity and evenness. The ears showed inconsistent results but consistent negative correlations between blight features and evenness and almost entirely positive correlations with green remediation. The eyes showed almost all positive correlations with blight and strong positive correlations between diversity and evenness and green remediation. The nose showed opposite trends, whereby blight features were positively correlated with richness but negatively correlated with evenness. The nose exhibited positive correlations between richness and diversity and green

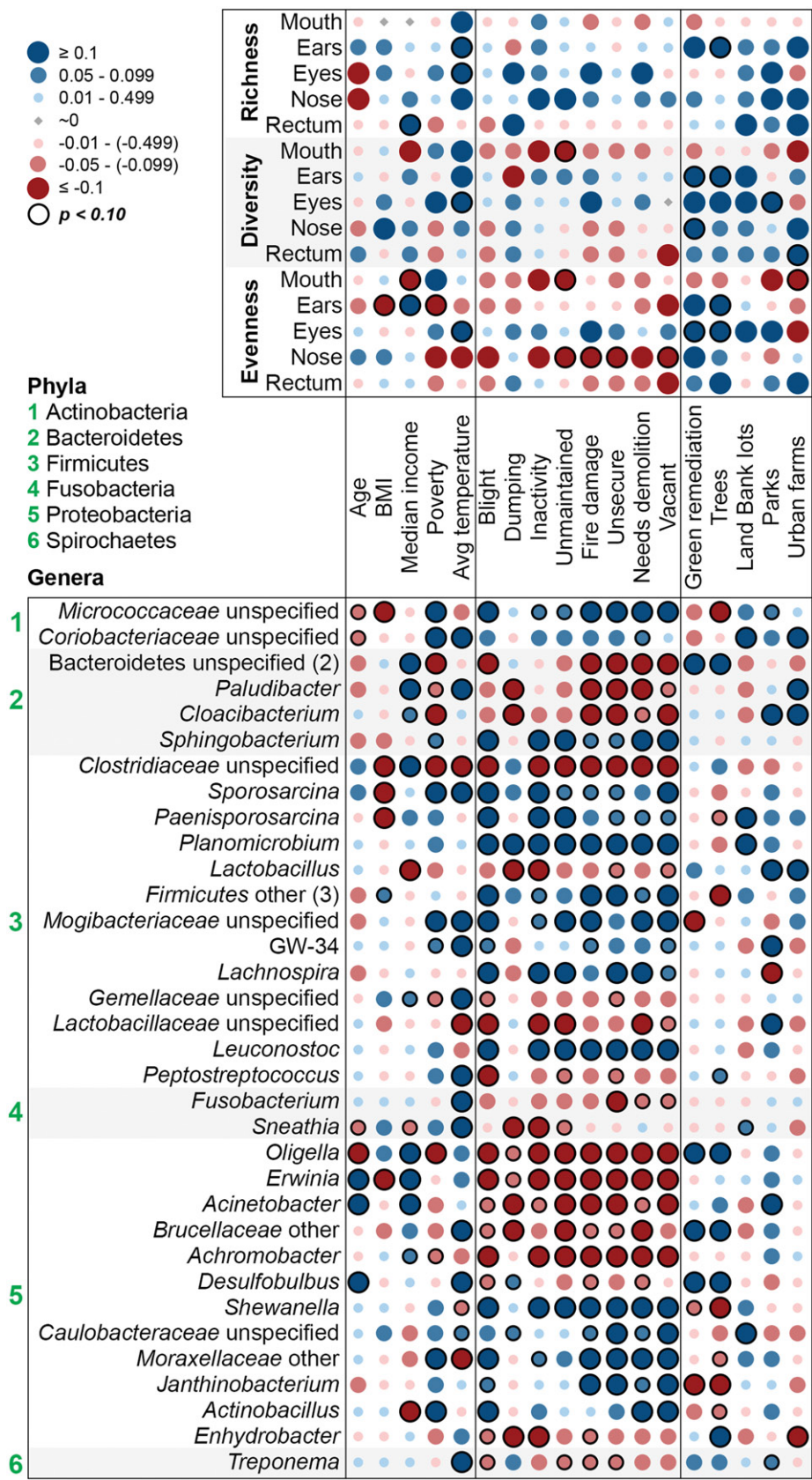


Figure 3. Correlations between microbial measures and individual and neighborhood characteristics. Significant rank correlations between individual and neighborhood characteristics and biodiversity measures by anatomical region and relative abundances for phyla and selected genera. Blue and red colors indicate negative and positive correlations, respectively. Circle size indicates correlation strength and a black perimeter indicates $p < 0.10$.

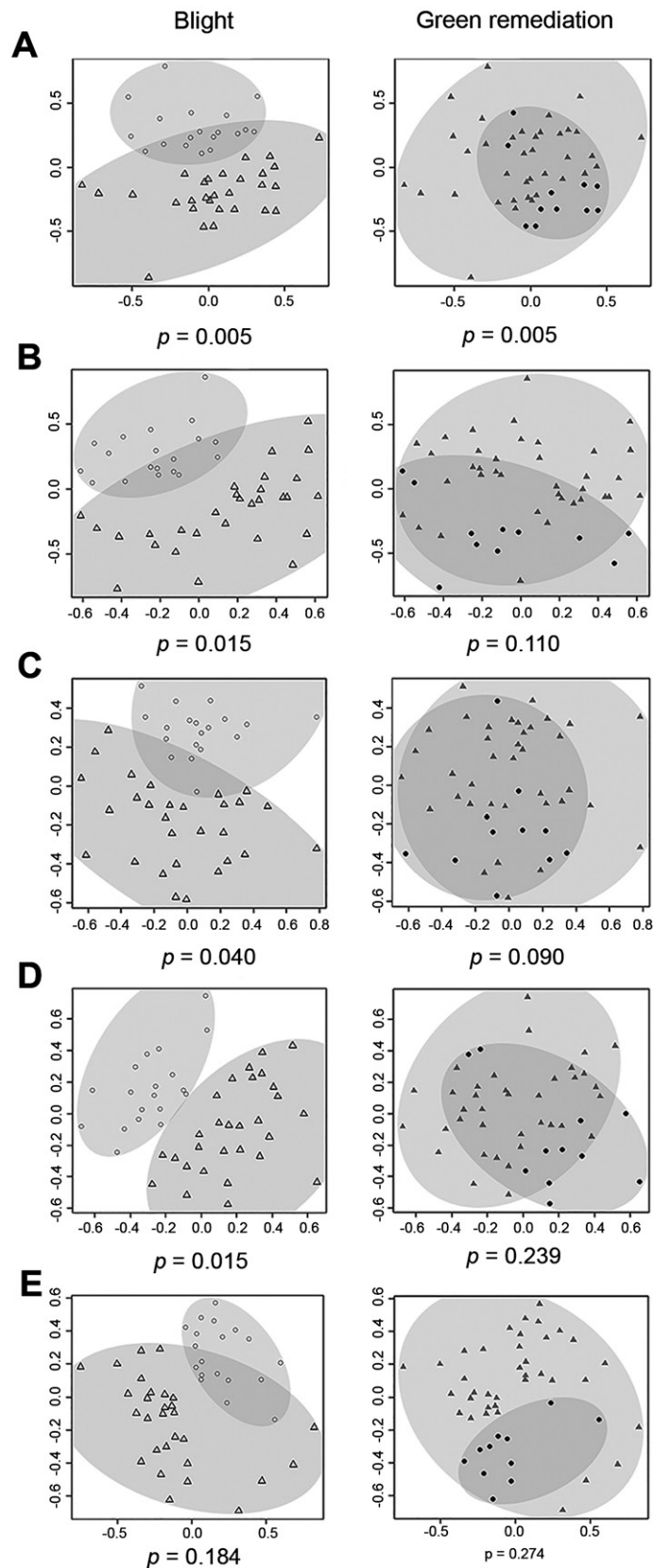


Figure 4. Clustering of microbial community composition. Nonmetric multidimensional scaling of microbial communities based on Bray-Curtis dissimilarities, in terms of neighborhood blight high/low and neighborhood green remediation high/low. Triangles indicate low values; circles indicate high values. Points in greater proximity correspond to similar community composition. (A) Mouth. (B) Ears. (C) Eyes. (D) Nose. (E) Rectum.

				Anatomic region								Anatomic region				
				High	Low	p-value			High	Low	p-value					
A	Phylum								Phylum							
	Other	21.26	0.03	0.047					Firmicutes	678.00	526.30	0.028				
	Genus								Genus							
	<i>Fusobacterium</i>	5.42	18.55	0.036					<i>Acidobacteria</i> unspecified	2.82	0.00	0.009				
	<i>Coriobacteriaceae</i> unspecified	0.47	0.07	0.009					<i>Bacteroides</i>	33.18	7.30	0.022				
	<i>Collinsella</i>	1.11	0.00	0.011					<i>Faecalibacterium</i>	2.27	0.57	0.007				
	<i>Rikenellaceae</i> unspecified	0.37	0.00	0.029					<i>Veillonellaceae</i> other	2.09	0.16	0.009				
	<i>Cloacibacterium</i>	0.68	0.03	0.045					<i>Lactobacillus</i> °	125.27	13.97	0.025				
	<i>Granulicatella</i>	0.63	3.07	0.047					<i>Blautia</i> °	2.82	0.92	0.019				
	<i>Anaerococcus</i>	78.95	22.31	0.002					<i>Comamonadaceae</i> unspecified	2.00	0.24	0.019				
	<i>Peptoniphilus</i>	30.89	13.03	0.049					<i>Gemella</i>	2.09	0.16	0.016				
	<i>Moryella</i>	0.26	3.41	0.030					<i>Gemellaceae</i> other	0.36	0.00	0.009				
	<i>Ruminococcaceae</i> unspecified	4.74	0.69	0.016					<i>Firmicutes</i> other (2)	0.36	0.03	0.010				
	<i>Psychrobacter</i>	2.26	0.00	0.029					<i>Streptococcaceae</i> unspecified	2.64	0.46	0.043				
	<i>Frigoribacterium</i>	0.16	0.00	0.029					<i>Lautropia</i>	0.27	0.00	0.009				
	<i>Firmicutes</i> other (3)	5.47	4.10	0.032					Other	1.36	0.73	0.039				
	<i>Peptococcus</i>	0.11	0.62	0.033					<i>Blautia</i> °	0.00	1.54	0.019				
	<i>Erysipelotrichaceae</i> unspecified	0.05	0.79	0.028					<i>Alloicoccus</i>	128.45	35.78	0.044				
	<i>Leptotrichia</i>	3.95	0.72	0.044					<i>Pedobacter</i>	1.45	0.03	0.001				
	<i>Tannerella</i>	0.47	0.00	0.029					<i>Lactobacillaceae</i> unspecified	6.00	6.46	0.044				
	B	<i>Firmicutes</i> other	0.89	1.90	0.026					<i>Agrobacterium</i>	0.36	0.00	0.001			
<i>Enterococcaceae</i> other		1.42	0.00	0.029					<i>Comamonadaceae</i> other	0.73	0.22	0.049				
<i>Bradyrhizobiaceae</i> unspecified		0.00	0.79	0.037					<i>Pseudomonas</i>	38.00	28.54	0.027				
<i>Comamonadaceae</i> unspecified		1.68	0.31	0.024					<i>Xanthomonadaceae</i> unspecified	1.09	2.16	0.040				
<i>Eikenella</i>		0.58	0.00	0.004					<i>Lactobacillus</i> °	91.73	17.35	0.048				
<i>Erwinia</i>		0.05	1.24	0.047					<i>Luteimonas</i>	0.91	0.11	0.044				
<i>Sporosarcina</i>		1.53	0.00	0.011					<i>Clostridium</i> ‡	31.09	1.76	0.034				
<i>Leuconostoc</i>		3.05	0.28	0.024					<i>Enterococcus</i> ‡	0.27	0.00	0.009				
Other		0.05	0.55	0.051					<i>Brucellaceae</i> other‡°	0.09	0.00	0.067				
C		<i>Achromobacter</i> †	0.00	0.21	0.095					<i>Vagococcus</i> ‡	1.45	0.00	0.067			
		<i>Proteus</i> †	0.00	14.66	0.059					<i>Bacteroidetes</i> unspecified (2)‡°	0.45	0.00	0.067			
		<i>Micrococcaceae</i> unspecified‡	0.68	0.07	0.051					<i>Oligella</i> ‡°	0.73	0.00	0.067			
		<i>Tepidimonas</i> ‡	0.84	0.00	0.011					<i>Oligella</i> ‡°	1.00	0.00	0.067			
		<i>Bacteroidetes</i> unspecified‡	0.16	0.00	0.029					<i>Bacteroidetes</i> unspecified (2)‡°	0.27	0.05	0.070			
		<i>Serratia</i> ‡°	1.89	0.00	0.078					<i>Oligella</i> ‡°	0.82	0.00	0.067			
		<i>Meiothermus</i> ‡	0.32	0.00	0.078					<i>Firmicutes</i> other (4)‡°	0.91	0.16	0.036			
		<i>Roseomonas</i> ‡	1.00	0.00	0.029					<i>Firmicutes</i> other (4)‡°	0.64	0.14	0.011			
		<i>Anoxybacillus</i> ‡	13.11	0.17	0.061					<i>Bacteroidetes</i> unspecified (2)‡°	0.18	0.00	0.067			
		<i>Shewanella</i> ‡	5.11	0.00	0.078					<i>Peptostreptococcus</i> ‡	2.00	0.43	0.037			
		<i>Sphingobacterium</i> ‡°	1.16	0.00	0.078					<i>Brucellaceae</i> other‡°	0.09	0.00	0.067			
		<i>Acinetobacter</i> †	23.16	54.38	0.021					<i>Bifidobacteriaceae</i> other†	0.09	0.00	0.067			
	<i>Lysinibacillus</i> †	0.00	1.62	0.059					<i>Gemellaceae</i> unspecified‡	no significant differences						
	<i>Sphingobacterium</i> ‡°	2.21	0.00	0.078					<i>Clostridiaceae</i> unspecified‡	no significant differences						
	<i>Paenisporsarcina</i> ‡	0.53	0.00	0.029					<i>Jeotgaliococcus</i> ‡	no significant differences						
	<i>Enterobacteriaceae</i> unspecified‡	69.42	37.45	0.059					† Indicator genera for low bright/green remediation							
	<i>Enterococcus</i> ‡	1.00	0.07	0.056					‡ Indicator genera for high bright/green remediation							
	<i>Planomicrobium</i> ‡	0.00	4.93	0.059					° Significant differences for multiple body sites							
	<i>Moraxella</i> ‡	0.00	17.14	0.095												
	<i>Serratia</i> ‡°	0.21	0.00	0.077												
	<i>Catenibacterium</i> †	no significant differences														
<i>Pseudomonas</i> ‡	no significant differences															
<i>Janthinobacterium</i> ‡	no significant differences															
<i>Lachnospira</i> ‡	no significant differences															

Figure 5. Significant ($p \leq 0.05$) mean differences in relative abundances of phyla and genera between high versus low (A) neighborhood blight or (B) green remediation for each anatomic region: Mouth shown first (in purple). Ears shown second (in gray). Eyes shown third (in dark pink). Nose shown fourth (in light pink). Rectum shown last (in green). Mean values and significant ($p \leq 0.10$) differences also shown for indicator genera for (C) blight and (D) green remediation.

remediation. The rectum showed inconsistent results for blight and positive correlations between richness and diversity and green remediation (full results are shown in [Supplementary Materials](#), Tables S.1A1–S.1F3).

In terms of patterns in correlations between neighborhood conditions and genera abundances, we observed higher versus lower across blight versus green remediation features (or vice versa) for the following genera: *Sporosarcina*, *Lactobacillus*, *Lactobacillaceae*

unspecified, *Oligella*, *Erwinia*, *Acinetobacter*, *Brucellaceae* other, *Shewanella*, *Caulobacteraceae* unspecified, *Moraxellaceae*, *Janthinobacterium*, *Actinobacillus*, and *Treponema* (Figure 3). Average temperature exhibited primarily positive correlations with diversity and genera, whereas most other case characteristics showed varied trends.

We observed distinct and significant clustering of microbial community structure for high versus low values for neighborhood blight for every anatomic

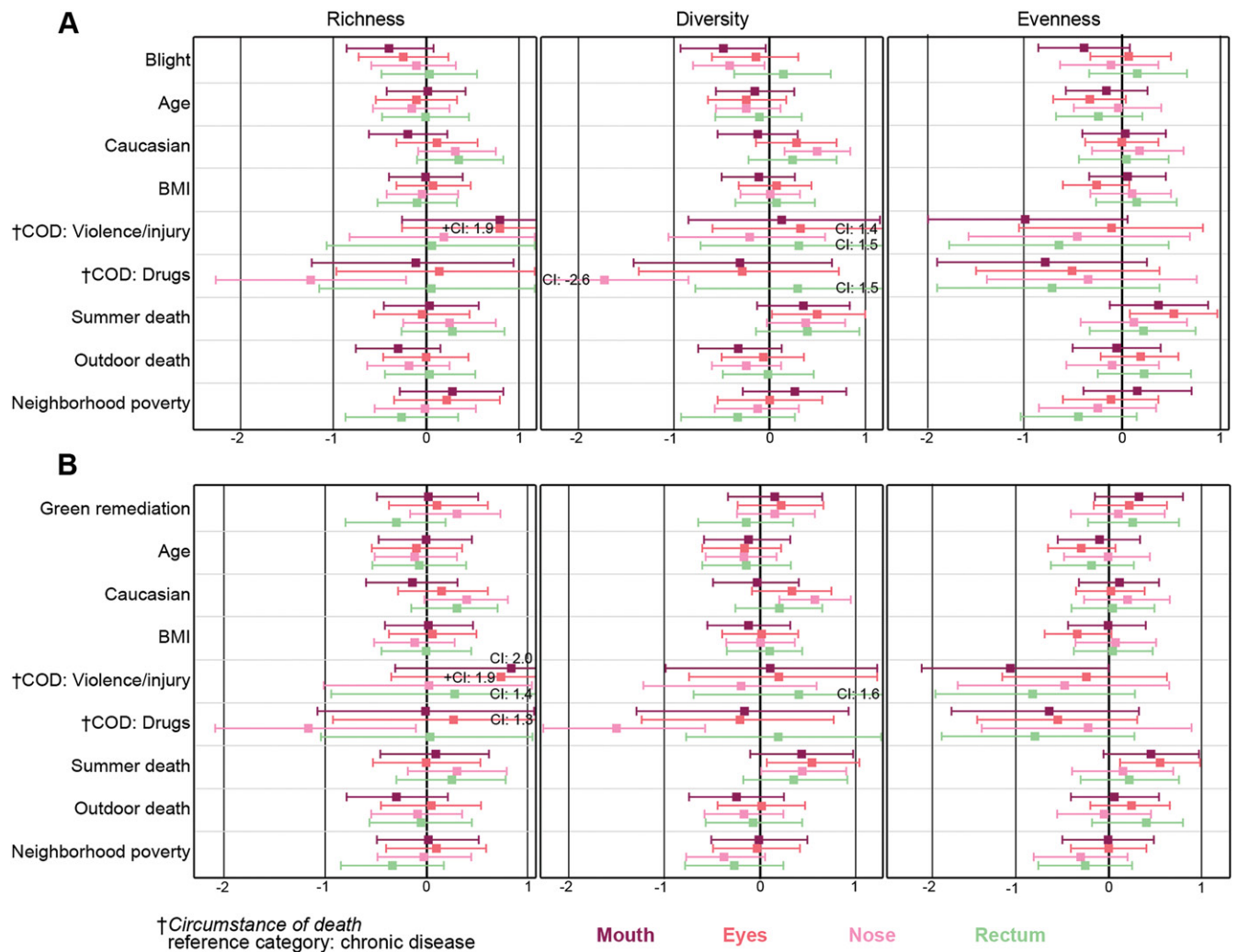


Figure 6. Relationships between measures of microbial biodiversity and neighborhood conditions, adjusted for individual- and area-level covariates, males. Standardized β coefficients and 95 percent confidence intervals for separate regression models shown. Each measure of biodiversity and (A) neighborhood blight or (B) green remediation.

region, except the rectum ($p=0.184$). We observed less distinct clustering for high versus low green remediation (Figure 4) but significant clustering for the mouth ($p=0.005$) and the eyes ($p<0.10$).

Almost all significant differences in genera abundances reflected higher levels in high-blight neighborhoods (Figure 5), except *Granulicatella* (normal oral and urogenital flora that can cause septicemia, endocarditis, or bacteremia) and *Moryella* (common oral flora) in the ears; *Peptococcus* (normal human gut flora) and *Erysipelotrichaceae* unspecified (found in the human gut) in the eyes; *Firmicutes* other (a group of most gram positive bacteria), *Bradyrhizobiaceae* unspecified (family containing potential pathogens), and *Erwinia* (potentially

pathogenic) in the nose; and other (unknown taxonomy) in the rectum. Almost all significant differences in genera abundances reflected higher levels in high green remediation neighborhoods (Figure 5), except *Blautia* (found in the gut) in the eyes and *Lactobacillaceae* unspecified (lactic acid-producing bacteria) and *Xanthomonadaceae* unspecified (nonpathogenic and pathogenic γ -proteobacteria), both in the rectum. Additionally, neighborhoods with high levels of green remediation had significantly higher relative abundances of both commensals (nonharmful) and potentially pathogenic bacteria: *Pedobacter* (oral cavity), *Alloiococcus* (a sometimes pathogenic common bacteria of the inner ear), *Lactobacillus* (commensal),

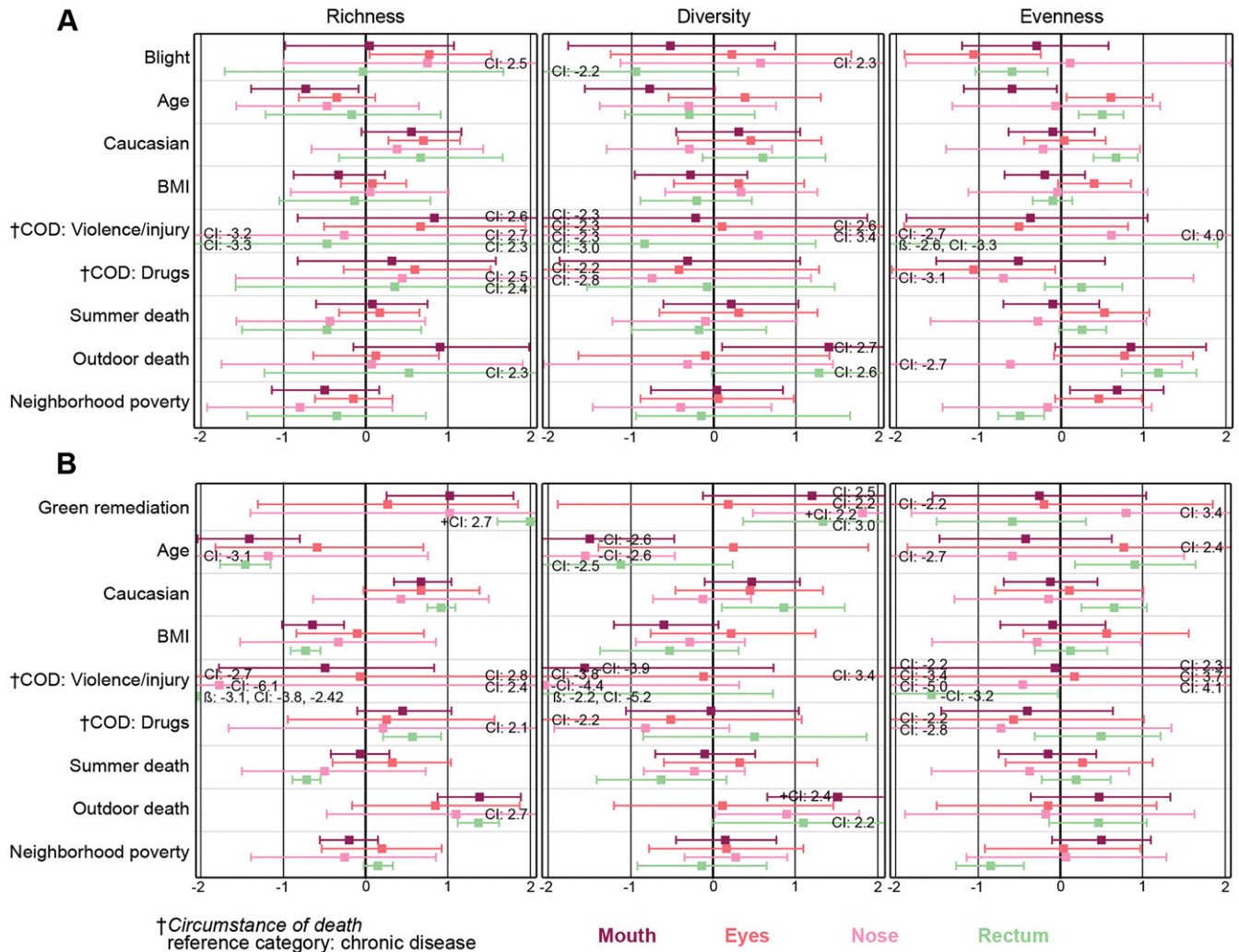


Figure 7. Relationships between measures of microbial biodiversity and neighborhood conditions, adjusted for individual- and area-level covariates, females. Standardized β coefficients and 95 percent confidence intervals for separate regression models shown. Each measure of biodiversity and (A) neighborhood blight or (B) green remediation.

and *Clostridium* (genus that contains several human pathogens).

In our regression modeling, we observed that, for males, both richness and diversity showed negative associations with blight for all anatomic regions (Figure 6A), with opposing findings for the rectum. Results were statistically significant for diversity of the mouth ($\beta = -0.48$, 95 percent CI, -0.94 , -0.02) and nose ($\beta = -0.42$, 95 percent CI, -0.80 , -0.05). Betas for each anatomic region exhibited a positive association between green remediation and biodiversity measures (Figure 6B), except the rectum, again, which showed the opposite trend for both richness ($\beta = -0.30$, 95 percent CI, -0.80 , -0.19) and diversity ($\beta = -0.15$, 95 percent CI, -0.66 , 0.35).

For females, statistically significant, negative associations were observed between blight and evenness for the eyes ($\beta = -1.08$, 95 percent CI, -1.90 , -0.25 ; Figure 7A) and the rectum ($\beta = -0.60$, 95 percent CI, -1.04 , -0.17). Also, for females, richness was positively and significantly associated with green remediation (Figure 7B) for the mouth ($\beta = 1.03$, 95 percent CI, 0.26 , 1.80) and the rectum ($\beta = 1.99$, 95 percent CI, 1.61 , 2.37), and diversity was positively and significantly associated with green remediation for the nose ($\beta = 1.84$, 95 percent CI, 1.61 , 2.37). Associations were all negative for evenness, except for the nose. The significance of these findings to the broader understanding of human microbiomes and the spatial environment is discussed next.

Discussion and Conclusion

In cities throughout the Rust Belt, structural deterioration, blight, and tax delinquency are widespread. The cost of complete blight removal is high and the question of what should take its place remains. Efforts to enhance the condition of empty lots, such as gardening, tree planting, ecological restoration, and farming, might not only mitigate the negative effects of blight but also lower crime (Bervoets et al. 2013) and increase social cohesion among neighbors (Farmer, Randall, and Aziz 2014), all of which appear to benefit mental health (Foster and McVey Neufeld 2013; Hill et al. 2014). There also appears to be a synergy whereby greening efforts lower levels of illegal dumping, which reduces future blight (Joo and Kwon 2015). Compared to demolition, green remediation efforts are far more economically feasible and often done by volunteers (Price, Smith, and Gilbert 2014). Efforts to improve neighborhood conditions might inadvertently also influence the microbial environment.

Our findings support the possible relationship between neighborhood conditions and both the composition and diversity of the human microbiome. Such findings include our observed distinct and significant clustering of microbial composition by neighborhood blight for each anatomic region (except the rectum) and significant clustering by green remediation for the mouth and eyes. We also found significantly higher abundances of potential pathogens and microbes associated with unhealthy living conditions. We acknowledge that the detection of microbial nucleotides does not imply pathogenicity for the microorganism. These taxa often result in human disease (e.g., periodontal disease; Colombo et al. 2009). *Sporosarcina* expresses urease and is associated with areas with significant concentrations of urine. In contrast, we observed high levels of gut commensals, those bacteria living in close association with the host that can benefit the host without harming it (i.e., nonpathogenic microorganisms), in green remediation neighborhoods. Consistently, microbiome biodiversity was positively correlated with green remediation and negatively correlated with blight.

Across all regression models, the largest effects were observed for green remediation on higher microbial biodiversity for women. Overall, this finding might provide a mechanistic reason why many emerging studies in the psychological sciences have

identified a positive effect of neighborhood green spaces on mental well-being across the globe (Portes 1998; Cohen et al. 2003; Martinez-Fernandez et al. 2012). More specifically related to women, our finding might highlight differences in neighborhood-related behaviors and activities by sex. In fact, other research has shown that women frequently participate in urban gardening and green volunteerism, including in Detroit (White 2011). Still, further research is warranted to understand the differences between men's and women's interactions with their neighborhood physical environments, built features, and social networks.

When looking at findings by anatomical region, we often observed differing trends for the rectum, in comparison to other anatomic regions. This was true for the cluster analyses and regression results for males. This is potentially important because these results underscore our hypothesized microbial transmission route via neighborhood conditions and the microbiome. Facial areas are nearly always exposed to the environment and breathing (via the nose and mouth) and thus are prone to transmission of microbes from the environment. In contrast, the microbiome of the rectum (or gut) might have less contact with environmental conditions and therefore could be more influenced by individual characteristics, including eating behaviors. It is possible that some microbes entering the body through breathing could be transported to the gut via swallowing, but the associations with the neighborhood might be less pronounced for the rectal microbiome, as found here.

Some findings warrant further exploration, as scientific consensus on features of "healthy" microbiomes across populations emerge. For example, we found a positive correlation between potentially pathogenic taxa (*Desulfovibrio*, *Kingella*, and *Kocuria*) and neighborhood land bank lots, parks, and farms, and we found higher levels of gut and vaginal commensals in neighborhoods of high blight. Additionally, neighborhoods with high levels of green remediation had significantly higher relative abundances of potentially pathogenic bacteria: *Pedobacter* (oral cavity), *Alloiococcus* (a sometimes pathogenic common bacteria of the inner ear), and *Clostridium* (genus that contains several human pathogens).

This study has several strengths and limitations that are important to consider. The postmortem microbial data are advantageous in that they do not rely on volunteers (as commonly conducted in

microbiome research). Most microbiome research involves targeted populations of volunteers concerning a specific health condition or disease status (e.g., obesity, diabetes, Crohn's disease, depressive disorders). Our study also allows for the sampling of sensitive anatomic regions (e.g., the surface of the eye), and our study is the only one to our knowledge that evaluates the relationship between the microbiota on different anatomic areas (e.g., ears, nose, eyes), which are routinely exposed to the surrounding environments (e.g., neighborhood green vs. blight). Some data related to the postmortem microbial communities are limited, however, because the life histories of cases are unknown. For example, it is unknown whether cases had a diet of highly processed food, used pro- or antibiotics, or used antibacterial products, all of which could affect microbial composition and diversity. This weakness might be somewhat diminished through the simultaneous sampling of five anatomic regions, which would unlikely be uniformly affected by such habits.

Next, our sample size might be considered small by statistical standards. Even though our sample size is less than fifty, this study would be considered large among many postmortem microbiome researchers, which typically have characterized the postmortem microbiome from fewer than fifty cases. One collaborative effort to characterize the microbial communities inhabiting the human body of "healthy individuals" has collected multiple samples from 300 adults, called The Human Microbiome Project. This project is the result of work from 300 scientists from more than eighty organizations and has been continuously funded by the National Institutes of Health for over a decade. Only one article to date has evaluated the postmortem variability in close to 200 cases (Pechal et al. 2018).

Important, this study is limited in that it is cross sectional and only includes measures at time of death. For this reason, our study cannot evaluate whether exposure to neighborhood conditions precedes microbial conditions or the required length of exposure required before effects can be seen. In addition, this study only evaluated the conditions of the residential neighborhood, omitting other potentially relevant places that might influence the microbiome, including those related to work, recreation, and social contacts. Although exclusive evaluation of residential neighborhoods is common within the neighborhoods and health research, it is commonly cited as a limitation (e.g., Chambers

et al. 2017). Finally, the spatial measures of green remediation were not as comprehensive as those for blight, which was conducted for every parcel throughout the city.

Because the human microbiome appears to play an important role in human systems functioning (e.g., immunity, metabolism) and possibly mood and mental health, our research findings, particularly if confirmed by other future studies, pose broader theoretical and empirical considerations for future research, highlighted here. First, this line of research calls into question the separate nature of human and environment and underscores the permeability of the body. Some recent theoretical groundwork has been laid by geographers on this topic (e.g., Guthman and Mansfield 2012; Mansfield and Guthman 2015), with potential expansion to explore the meaning and drivers of shared consciousness or mood in certain spaces and the false dichotomy of human versus nonhuman.

Future empirical research could use live samples and a longitudinal design (making use of an experimental design similar to recent greening intervention research South et al. 2018). Such prospective or experimental studies could compare microbial composition and diversity between residents in neighborhoods with either greening efforts or heavily blighted conditions to reduce bias related to behaviors and the consequential microbial colonization patterns. This would allow for the consideration of potential confounders such as life habits (e.g., diet, hygiene) and provide temporal support for the possible causal effects of neighborhood conditions on the microbiome. It will be important for future studies to elucidate behavioral differences among women and men regarding their participation and interactions with their built environments and green spaces in particular. Other future work might usefully use ethnographic and other qualitative methods to understand the lives of living cases, to better understand associations among the living microbiome, behaviors, and neighborhood conditions.

Our results help pave the way for such future studies, because they provide initial evidence of a relationship between the human microbiome and neighborhood conditions. This study established a foundation for novel research opportunities into the possible microbial mechanisms through which green remediation efforts and urban blight affect health.

Supplemental Materials

Supplemental materials for this article can be accessed on the publisher's [website](#).

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