Outdoor Atmospheric Microbial Diversity is Associated with Three-Dimensional Urban Landscape Structure and Differs from Indoor-Transit Systems

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Abstract:

Microbes are abundant inhabitants of the near-surface atmosphere. Furthermore, urban areas are among the fastest growing ecosystems and host large and dense human populations. The distribution of microbial communities may benefit or hinder human wellbeing and ecosystem function. Surveys of airborne microbial diversity are uncommon. Studies which investigate these communities take place at isolated points in the city, thus missing the continuous exposure to microbes that covary with three-dimensional urban structure. Individuals in cities are generally mobile and would be exposed to diverse urban structures outdoors and within indoor-transit systems in a day. Microbiomes in these systems are especially relevant to public health. We used mobile monitoring of microbial diversity and geographic information systems, across Philadelphia, Pennsylvania, USA in outdoor and indoor-transit (subways and train cars) environments. This study is the first of its kind to complete an analysis of the role of three-dimensional urban structure in structuring atmospheric microbiomes. We found more

diverse communities outdoors that significantly differ from indoor-transit air in microbial community structure, function, likely source environment, and potentially pathogenic fraction of the community. Variation in the structure of the urban landscape was associated with diversity and function of the near-surface atmospheric microbiome in outdoor samples.

Main Text:

Microbes are abundant inhabitants of the near-surface atmosphere [1] with whose presence and distributions have implications on public health and ecosystem function. The atmosphere serves as both a source and a sink for microorganisms that may return to the surface through dry or wet deposition [2]. Urban areas also host these microbiomes; despite this, surveys near surface (~ 2 meter) diversity remain uncommon despite being the fastest growing ecosystems [3]. Furthermore, urban areas are composed of heterogeneous landscapes [4] with diverse land covers combined in various configurations and proportions. Structure of Urban Landscape (STURLA) studies have demonstrated that cities are composed of a limited number of three-dimensional composite land classes in which one class is responsible for large proportions of a city landscape [5, 6]. STURLA classes represent common combinations of urban composite urban structure. For example, the most frequently encountered class, *tgpl*, is heterogeneously composed of treecover (*t*), grass (*grass*), pavement (*p*), and low-rise buildings (*l*).

Urban areas host diverse microbiomes with unique selective pressures (e.g. pollution and chronic disturbance) that vary along spatial gradients [7, 8,] 9, 10]; however, relationships between urban landscape heterogeneity and atmospheric microbes remain largely unexplored. Aside from outdoor air, public transit systems also host unique microbiomes. These systems are

taxonomically diverse [11] and represent unique ecological niches due to their high usage rate by humans and rapid turnover in occupancy.

Microbiomes of both outdoor and indoor-transit air are assembled from diverse environments including soils [8, 12], water [13, 14], vegetation [15], and animals [16–18]. These communities host diverse bacterial phyla largely composed of *Proteobacteria*, *Actinobacteria*, *Firmicutes,* and *Bacteroidetes [10, 19]* that vary seasonally [20, 21] and by the environment surrounding the air [8, 22]. These studies are limited in that they take place at one point in space and do not reflect the average person's exposure or travel in the urban environments.

Recently, the health effects of microbial exposure in addition to pathogen identification are now being explored [23–25]. Microbially diverse outdoor and built environments offer protection against asthma and immune diseases [26–28]. Furthermore, low-diversity microbiomes in animals are associated with chronic-diseases including: asthma [24, 29–32], irritable bowel syndrome [33, 34], and obesity [35, 36]. Given these associations, discerning patterns of diversity in urban airs holds implications for human health.

Understanding how structural elements relate to atmospheric microbial diversity and function is necessary for understanding urban ecology [37], and can guide urban development in ways that utilize ecological functions to enhance environmental performance and human wellbeing. In this study we combine mobile monitoring of the atmospheric microbiome from 07/01/19 to 07/25/19 using marker gene sequencing (16s rRNA) and genome prediction to characterize the diversity and functional potential in outdoor and in indoor-transit air. This is complemented with analysis of how subtle variation in an urban landscape influences microbial communities.

We found diverse communities of 337 ASVs covering 22 phyla that varied significantly in community structure (Figure 1A, p=0.001) and functional potential (p=0.002, Figure 1B) between outdoor and indoor-transit air as well as between outdoor route driven. A diverse set of metabolic genes were identified including carbon fixation, sulfate reduction/assimilation/oxidation, nitrate reduction, and aromatic compound degradation. Despite differences in composition and functional potential, both indoor-transit and outside air hosted similarly dispersed communities (p=0.9631, Figure S1) where the majority of ASVs in outdoor and indoor-transit were found in all samples. No phylum was unique to a single sample, air type, or route (Figure 1C) thus suggesting that the atmospheric microbiome in general is highly dispersed with continuous mixing of microbial communities.

Figure 1: A. Principal Coordinates Analysis (PcoA) of phylogenetic beta-diversity (between sites) using unweighted UnifFrac distances with air type colored by site and shaped by indoor (circle) and outdoor (triangle) air. Shape/Color X Shape/Color indicates the factors used in hypothesis testing with the test above and p-value to the right. B. Principal Coordinates Analysis (PcoA) of functional potential beta-diversity (between sites) using Bray-Curtis dissimilarity with air type colored by site (green: indoor transit, dark brown: outdoor route 1, light brown: outdoor route 2) and shaped by indoor (circle) and outdoor (triangle) air. Shape/Color X Shape/Color indicates the factors used in hypothesis testing with the test above and p-value to the right. C. Relative abundances of phyla identified in samples separated by air type (outdoor route 1 vs outdoor route 2 vs indoor transit) with collection date above each column.

Indoor air hosted almost double the number of rare taxa (49 ASVs), an ASV found in just one sample, than outdoor air (26 ASVs). Outdoor air hosted significantly more phylogenetic (p=0.006, Figure 2A) and functionally diverse (p=0.002, Figure 2B), rich (p=0.045, Figure 2C), and evenly distributed (p=0.007, Figure 2D) communities as compared to indoor-transit air. This is most likely attributed to the unrestricted dispersal by wind at both the local (within-city)[38] and intercontinental scale[39]. For atmospheric communities it is expected that dispersal is the dominating process affecting community assembly as opposed to diversification, selection, and drift[40, 41]. Our finding that communities are highly dispersed in both outdoor and indoor-transit air suggests that atmospheric microorganisms are not endemic and have cosmopolitan distributions in their environment, likely a function of the large populations of microorganisms combined with their small size[42]. Furthermore, in semi-closed environments such as indoor-transit systems, physical dispersal limitation (e.g. a pillar or turnstile) should increase residence time for microbes[43, 44] and influence microbial diversity.

Figure 2. Alpha Diversity Metrics separated by air type (circle: indoor transit, triangle: outdoor) and route driven for outdoor samples (color) with date of collection on the x axis. Shape/Color X Shape/Color indicates the factors used in hypothesis testing with the test above and p-value to the right. A. Faith's phylogenetic diversity (PD). B. Functional diversity calculated using the Shannon index on PICRUSt functional pathways. C. Observed number of ASVs. D. Pielou's evenness metric.

The 40 most abundant ASVs accounted for 86.01% ± 11.06% of all taxa across all samples were identified as likely originating from animals, soil, or were generalists, in complement similar studies of urban air [7, 16]. Taxonomic composition by source environment significantly differed from outdoor and indoor-transit air (Figure 3A, p=0.003) but not by route driven (p=0.311) Proportions of source environments were not uniform between outdoor and

indoor-transit air as the most abundant ASVs in outdoor air were attributed to more evenly

distributed source environments.

Figure 3. A. Relative abundances of sources identified in samples separated by air type on the bottom
(outdoor route 1 vs outdoor route 2 vs indoor transit) with collection date above each column. Hypothesis test
results and factors are under the legend. B. Relative abundances of potentially pathogenic taxa identified in samples
separated by air type on the bottom (outdoor route 1 vs outdoor route 2 vs indoor transit) with collection date above
each column. Taxa in abundance smaller than .01% were grouped in the category "other" for visualization only.
Hypothesis test results and factors are under the legend.

Outdoor air hosted more soil and generalist taxa than the indoor-transit air (Figure 3A).

This is attributed to sampling across diverse landscapes that included water features, greenspace,

unpaved land as well as a dense urban core consisting of the built environment where humans

and animal companions primarily walk and drive outside. The closed environment (aside from

exits) of the indoor-transit system combined with high human occupancy seems to enrich the

taxa brought into the system by its riders as it does in other structures of the built environment [51]. It is expected to find bacteria disperse into and throughout the indoor-transit environment by three primary mechanisms: (1) contact with indoor surfaces; (2) particle emission from breath, clothes, skin and hair; and (3) resuspension of dust containing other microbial-loaded particles [45]. Given that humans carry 10^{12} microorganisms on the epidermis and 10^{14} microorganisms in the alimentary tract [46] and shed ~ 10^6 particles (>0.5 µm in diameter) an hour[47, 48] we likely serve as the dominant source of microorganisms in indoor-transit air. Complementing our findings of higher proportions of animal associated taxa in indoor-transit air, it has been seen that human occupancy increases the load of animal associated bacteria in the built environment. This includes subways [11, 49, 50], university classrooms [17, 51], public restrooms [52], dust [53], and kitchens [54]. Outdoor air microbes also seem to enter into the transit system, albeit to a lesser extent, given there were no unique phyla identified outside that were not encountered in transit air (Figure 1A). Furthermore, architectural design [44, 55] has been seen to influence microbial community structure and acts as a physical barrier differentiating outdoor versus indoor-transit air.

A total of 30 ASVs (Figure 3B) were identified as being potentially pathogenic where, on average, these taxa composed 33.89% ±0.19 % of outdoor air and 48.41% ± 0.21% of indoor-transit microbial communities. The majority of these taxa are commensal human bacteria that are also opportunistic pathogens. *Escherichia-Shigella spp., Corynebacterium spp.,* and *Pseudomonas spp.,* were absent in indoor-transit air. Structure and taxonomic composition of potentially pathogenic taxa was significantly different between indoor-transit and outdoor air (p=0.007) but not between route driven outdoors (p=0.661). High human occupancy in small spaces such as train cars combined with our finding of almost half of the microbiome being potentially pathogenic may alter the health state of the lung and respiratory tract microbiome. A

decrease in microbial diversity, richness and evenness, as we found in indoor-transit air compared to outdoor air, are frequent features of chronic inflammation of the lungs [30, 56]. This combined with the high percentages of potentially pathogenic taxa in indoor-transit air may have negative health effects on those with unstable lung microbial communities such as individuals who have a recurring cough, mechanically disrupting microbes, or have decreased diversity due to antibiotic use [57, 58].

In contrast, the lower proportions of potentially pathogenic taxa and relatively higher diversity of outdoor air may offer a buffer to any negative health effects of breathing indoor-transit air given that exposure to highly diverse atmospheric communities has been linked to decreased rates of asthma [24, 59]. The influence of outdoor air may be less than that of the indoor-transit system as 90% of urban individuals spend their time indoors [60]. One possible benefit of spending time outdoors aside from aesthetic and recreation [61] may be improving respiratory health.

The urban landscape consisted of 42 classes encountered while driving; however rare classes were common. The majority of the urban landscape from routes driven was homogenous in class composition where 92.16 % ± % 1.43 % was explained by 9 STURLA classes (Figure 4A). Despite this, routes significantly differed (p=0.0364) in STURLA classes encountered each day. STURLA class diversity was significantly correlated with microbial alpha diversity (Rho: 0.74, p= 0.045) and observed number of ASVs (Rho: 0.83, p= 0.011) but not with community evenness (Rho: 0.21, p= 0.620).

Figure 4: A. Barplot of the mean frequency of the 9 most common STURLA classes which comprise 92.16 % ± % 1.43 % of classes encountered in all outdoor samples. B. Scatterplot showing the results of a Mantel test on Bray-Curtis dissimilarity of STURLA classes for each route driven and microbial community dissimilarity (Unweighted UniFrac) with correlation coefficient and p-value in the top left.

While it is known that urban and rural/agricultural air host distinct microbial communities [7, 8], the role of within city variation where there are less obvious contrasts in landscape structure is largely unstudied. We found that variation of the STURLA classes was associated with beta diversity of microbial communities (Figure 4B, Mantel Test, r: 0.341, p=0.04) At the individual STURLA class level, functional diversity was significantly correlated (all $p < 0.05$) with 5 of the most frequent classes (Figure 5) as was the number of observed ASVs with class *tgbpl*. Furthermore, diversity of STURLA classes by day was strongly, albeit insignificantly, correlated with 14 metabolic pathways (Supplemental Figure 4), 25 biosynthesis pathways (Supplemental Figure 6), and 10 degradation pathways (Supplemental figure 5). Class richness was also correlated with 3 metabolic pathways, 9 biosynthesis pathways, and 4 degradation pathways. Of these, more were seen with STURLA diversity as opposed to

STURLA richness, suggesting the composition of heterogeneous 3D landscapes has greater influence on microbiome composition than the number of times the same landscape is encountered. These findings suggest that different structures of the urban landscape host functionally distinct microbiome.

Figure 5: Heatmap of Spearman's Rho correlation coefficients where lighter colors indicate magnitude of the correlation. X indicates a statistically significant p-value after Bonferroni correction.

In a rapidly urbanizing global community, humans spend the majority of their time in a urban landscapes of varying landscape structure, density and configuration. While both outdoors and in transit systems, we constantly emit and interact with microbes sourced from diverse environments that may influence our well being. In this study we found that microbial communities vary between outdoor and indoor-transit air with the former being more diverse and associated with the structure of the urban landscape. Likewise indoor-transit systems hosted largely homogenous communities with higher proportions of potentially pathogenic taxa likely emitted from animals. Limitations include addressing the bacterial and archaeal subset of the atmospheric microbiome but not fungal communities as well as inferred functional pathways instead of directly measuring gene expression. Future studies may consider exploring the variations of relationships to microbial diversity within a STURLA class.

Methods: 1022 words

Site Description and Sample Collection:

Philadelphia, PA, USA is a city flanked by two bodies of located in the Mid-Atlantic region of the United States of America with a 2018 population estimate of 1,584,138[62]. Samples were collected on sterilized (500°C for 3 hours) quartz fiber filters using a PDR1500 Air Quality Dust Monitor (Thermo-Fisher Scientific, United States of America) where the inlet tube and mesh filter holder was cleaned with 70% ethanol before sampling. Only particles smaller than 2.5 micrometers in diameter ($PM_{2.5}$) were collected using a blue cyclone and using a flow rate of 1.52 liters per minute. Particulate matter was impacted on quartz fiber filters where after collection the filter was removed using sterilized tweezers and stored in aluminum foil at -20 °C until DNA extraction. DNA was then stored at -80 °C until downstream analyses.

Outdoor Sampling:

Outdoor air samples (n=8) were collected using the same instrument (PDR1500) strapped to the roof of a van (~1.5 meters in height) with the inlet facing out to the side of the roof. Samples were collected from two routes (R1: Route 1, R2: Route 2) with R1 being more diverse in urban structure. Average sampling period for each of the routes was 600.88 ± 48.83 minutes for each of the outdoor air sample days, collecting an average of 913.33 L $\pm$ 73.42 L of air . Variation exists within routes to address the roles of both large (between route) and small (within-route) variation in structuring the atmospheric microbiome (Supplemental Figure 1).

Indoor-transit Sampling:

Three indoor-transit lines (Supplemental Figure 2, the Market-Frankford Line (above/below ground), Norristown Line (above ground), and BSL (below ground) of air were collected to comprise one sample. Collection (n=4) took place inside train cars for an average

288.25 ± 21.08 minutes impacting 438.14 ± 32.04 L of air . On average ~30 people were counted in each train car during sampling.

Urban Structure and Route Generation:

Urban structure was characterized in 120 m^2 blocks using the STructure of Urban LAndscapes (STURLA) system, a multi-dimensional spatial classification system that incorporates land cover with urban building/structure height as previously described[6]. Relative frequencies of STURLA classes were calculated and those with an abundance below 0.1% were grouped into the category "other". The 9 most prevalent classes encountered during sample collection can be found in Figure 4A.

Routes were generated to include at least 50 points in each of the composite classes represented in the city STURLA classification. Points were randomly selected based on a stratified random sample to ensure adequate representation of the most common composite classes. In addition, point of interest were added to the random sample including industrial sites, EPA TRI sites, and EPA air pollution monitoring station sites. Using ESRI ArcGIS 10.7.1 Network Analyst, an optimal route was generated, passing through the sample points. The route was then divided into two routes—R1 and R2 for feasibility of driving within one day. The routes were loaded to a professional-grade GPS unit (Thimble Juno 3b, USA), and the van was driven along each route. Each route was replicated 4 times for a total of 8 samples; however, variation of each route exists day to day to test the role of subtle variation in the urban landscape

DNA Extraction, Sequencing, Processing, and Genome Prediction:

DNA extraction and quantification was conducted as previously described [7]. Sequencing was performed at MR DNA (www.mrdnalab.com, Shallowater, TX, USA) using the

Illumina MiSeq platform with 20,000 reads. DNA sequences were analyzed and statistical analysis was conducted using (QIIME2 [65] and R. Briefly, raw reads were demultiplexed, denoised using the DADA2 algorithm [66], and truncated at 180 basepairs. Sequences were rarefied to 1,55,547 sequences aligned to the SILVA 132 99% reference taxonomy database[67] using a Naive Bayes classifier to Amplicon Sequence Variants (ASVs). Sequences were aligned using mafft[68] followed by construction of an unrooted phylogenetic tree using FastTree[69]. Alpha diversity metrics including observed number of ASVs, Pielou's evenness, and Faith's Phylogenetic Diversity [70] were calculated. Beta diversity was calculated using unweighted UniFrac for phylogenetic analysis [71]. PICRUSt2[72] was used to infer functional genes using the maximum parsimony hidden-state prediction methodology[74]. Functional gene predictions were rarefied to the minimum number of features found between all samples. Beta diversity for functional gene predictions was conducted using Bray-Curtis dissimilarity and Principal Coordinates Analysis (PcoA).

Data Analysis:

Identification of potentially pathogenic taxa was conducted comparing the average 40 most relatively abundant ASVs with a reference database of bacterial strains that are known human pathogens (UK Health and Safety Executive Advisory Committee on Dangerous Pathogens. 2004 The approved list of biological agents. UK Health and Safety Executive). Taxa representing less than 0.01 % of the pathogenic subset of taxa were grouped into the category "Other". Likely source environment for the top 40 ASVs was determined through a literature review (Supplemental Table 2). A taxon found in more than one environment was categorized as a "generalist".

Alpha diversity metrics including observed number of ASVs, Pielou's evenness, and Faith's Phylogenetic Diversity [70] were calculated. Beta diversity was calculated using unweighted UniFrac for phylogenetic analysis [71] and Bray-Curtis dissimilarity for functional gene predictions. Principal Coordinates Analysis (PcoA) was used to visualize beta diversity.

Kruskal-Wallis tests were used to test for significant differences indoor-transit, outdoor air, and variation between R1 and R2 in diversity. PERMANOVA tests identified if community structure (phylogenetic, by source enviriment, predicted functional genes, and potentially pathogenic taxa) varied between air types and route driven. Dispersal was measured by taking the sum of the presence of a taxon in outdoor and indoor-transit samples and breaking them into equal thirds.

Shannon diversity (H) was used to calculate the diversity of STURLA classifications encountered[75]. Richness of land cover was measured by taking the sum of the number of unique STURLA classes passed through on each route. Dissimilarity in routes was calculated using the Bray-Curtis dissimilarity metric[76]. Spearman correlations with Bonferroni correction were used to test for relationships between STURLA diversity and microbial structure/function. A mantel test was employed to test for correlation between microbial beta diversity and STURLA dissimilarity.

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Conflict of Interest

The authors declare no conflict of interest.

Supplemental:

Supplemental Table 1: Sample information with ID, air type, date, total sampling time in minutes, and volume of air collected.

Sample ID	Date	Air Type	Total Time (Minutes)	Volume (Liters)
P1	1-Jul-19	Outdoor-R1	635	965.2
P2	3-Jul-19	Outdoor-R2	655	995.6
P3	5-Jul-19	Outdoor-R2	536	814.72
P4	9-Jul-19	Outdoor-R1	645	980.4
P5	10-Jul-19	Outdoor-R1	632	960.64
P6	12-Jul-19	Outdoor-R2	604	918.08
P7	15-Jul-19	Outdoor-R1	562	854.24
P8	16-Jul-19	Outdoor-R2	538	817.76
S1	10-Jul-19	Subway	298	452.96
S2	11-Jul-19	Subway	279	424.08
S3	17-Jul-19	Subway	264	401.28
S4	25-Jul-19	Subway	312	474.24

Supplemental Figure 1: Dispersal distributions of OTUs between all samples separated by air type. Colors denote degree of dispersal as measured by the sum of ASVs found in the top, middle, and bottom 33% of the community. A Chi-Square test was used to see if dispersal patterns differ by air type (p=0.002).

Supplemental Figure 1: Maps of routes driven (gold: Route 1, purple: Route 2) with the route

number and date above .

Supplemental Figure 2: Map of indoor transit air sampling lines with the three lines ridden to collect a composite sample per day colored. White Border indicates the Philadelphia, PA, USA city limit.

Supplemental Figure 3: Dispersal distributions of OTUs between all samples seperated by air type. Colors denote degree of dispersal as measured by the sum of ASVs found in the top, middle, and bottom 33% of the community. A Chi-Square test was used to see if dispersal patterns differ by air type (p=0.002).

Supplemental Figure 4. Spearman correlations between metabolism associated functional genes identified using PICRUST2 and diversity and richness of STURLA classes. Strong correlations are set at a threshold of |0.70| with Bonferroni corrections (no significant relationships found).

Supplemental Figure 5. Spearman correlations between biosynthesis functional genes identified using PICRUST2 and diversity and richness of STURLA classes. Strong correlations are set at a threshold of |0.70| with Bonferroni corrections (no significant relationships found).

Supplemental Figure 6. Spearman correlations between degradation functional genes identified using PICRUST2 and diversity and richness of land cover (STURLA classes). Strong correlations are set at a threshold of |0.70| with bonferroni corrections (no significant relationships found).

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