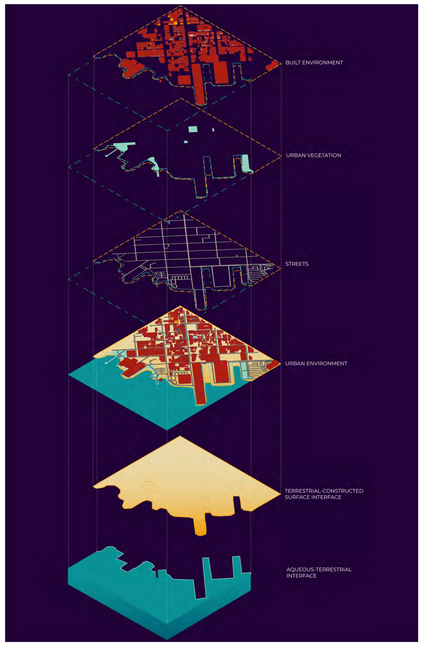
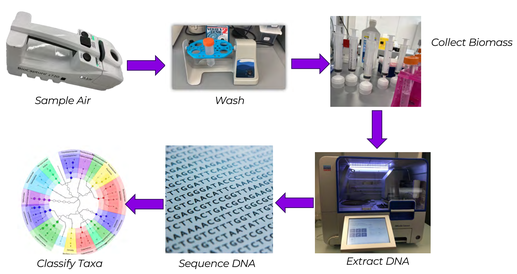
ASSESSMENT OF HYPERLOCAL URBAN AEROBIOME MICROCLIMATES

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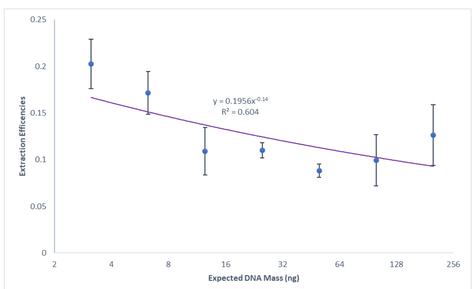


Figure 1. Our developed DNA extraction protocol (top) and calculated DNA extraction efficiencies at a $\log_2$ scale using the ZymoBiomics Microbial Community Standard (bottom). DNA extraction efficiencies increase for lower biomasses when using our adapted PowerWater Pro Extraction Protocol.

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INTRODUCTION

Defined as the population of microorganisms inhabiting a specific environment, **environmental microbiomes** surround us. A growing body of literature shows that **low microbiome diversity exposure has been linked to negative health outcomes, including atopic skin conditions and allergies[1,2], asthma[1,3,4], obesity[4-6], cancer[7], depression[8,9], and the transmission of potential pathogens[10-12]**. Similarly, microbial taxa with enhanced aerosolization capabilities are likely to exert greater influence on climate variables and weather patterns, with prior studies noting such species as potential plant and human pathogens[13,14].

Nearly 70% of the world's urban population is expected to inhabit cities by 2050, resulting in increased density of buildings and people and depletion of climate-mitigating natural resources[15]. A consequence of this rapid urbanization is the impact it will have on urban microclimates and public health effects (i.e., illness and death)[16]. As our environments shape human health, **access to diverse environmental microbiomes is a question of environmental justice**.

After developing a novel DNA extraction protocol to optimize DNA yields for low-biomass aerobiome samples (Figure 1), **our team conducted a pilot study in Fort Greene Park this summer to observe the dynamics of airborne microbial communities and identify correlations between this metagenomic data and urban spatial features.**

METHODOLOGY

Geospatial Site Analysis for Site Selection

Given the challenges of low-biomass aerobiome sampling and a desire to use a site that serves as a *crossroads* of wildlife and people (i.e., higher potential microbial diversity), our team used comparative geospatial analysis of civic and earth observation data to select sampling sites. Spatial characteristics considered are based on social justice and environmental health metrics. These include **urban surface temperature, pollution, vegetation density, proximity to parks, distance to the ocean, population density, and zoning districts**. Based on this analysis, we selected two sites in **Fort Greene Park (FGP Site A. The Visitors Center, Site B. The Tennis Courts)** for sampling (Figure 2). Additionally, as this was a pilot study, FGP provided more controlled conditions for our team to evaluate both in terms of the potential for higher microbial diversity and environmental conditions of the urban microclimate.

Aerobiome Sample and Environmental Metric Collection

Over one month **(July 2023),** our team collected air and environmental samples from the two sites in FGP. Samples and environmental metrics were collected for each day and site at **10 AM, 12 PM, and 2 PM**. In analysis, **different days were treated as sample replicates for the same time point**.

Aerobiome DNA Extraction[17] (Figure 1 top)

Air samples were collected onto electret filters using a **BioFlyte BioCaptureTM z720 Air Sampler Unit (rate 200L/min)** (Figure 3 right). The filters have a quasi-permanent charge to collect dust particles containing microbes through electrostatic interactions. After air sampling, **using a detergent mix (PBS and Triton X-100 (0.1%)), the filters are washed three times** before the collected **supernatant is filtered through a 0.22 µm PES membrane**. This membrane is retained and used as our sample input for the **Qiagen Dneasy PowerWater Pro Kit, and DNA concentration is measured using Qubit Fluorometric quantification** against our standard curve for extraction efficiency developed from a $\log_2$ serial dilution of the ZymoBiomic Microbial Community Standard (Figure 1 bottom). **DNA standard spike-ins** are used to counteract observed variability within field samples due to lower biomass and to control for minimum quantities needed for sequencing. Next, the **extractions are sent away for metagenomic sequencing via 16s rRNA and shotgun analysis**. From this data, we will perform data quality control using FastQC and downstream analyses using MetaPhlan for **taxonomic visualization.**

Urban Aerobiome Microclimate Metrics

For each location in FGP, environmental metrics **(air temperature, relative humidity, wind speed, wind direction, and particulate matter (PM2.5) with Purple Air Sensors and Reed Vane Anemometers)** were collected concurrently with aerobiome samples (Figure 3 left). Currently, a co-analysis of environmental metrics and the frequency and abundance of microbial taxa is being performed.

PRELIMINARY FINDINGS & FUTURE WORK

After DNA extraction, initial observations revealed that **higher DNA mass concentrations were contained in the Tennis Courts** samples (Figure 4). However, a **two-way factorial ANOVA failed to find significant differences** between any time point or location variables. The highest observed significance (p=0.06) was between the two locations at 10 AM. This suggests that recorded environmental metrics may contribute to the high DNA quantity variability for the same time across the sampling period. These data suggest that microbial species identification in the aerobiome must be purely qualitative without co-analyzing environmental metrics and DNA quantity measurements. Reliable control data on DNA extraction efficiencies is necessary for future aerobiome studies. DNA spike-in standards for downstream bioinformatic processing may assist in controlling interpretations of microbial relative abundances.

The properties of urban aerobiomes are not isolated to the microbial scale; they participate in interactions between people, space, and cycles of change. Therefore, the impact of this project is revealing the connections between microbial agents and societal processes, behaviors, and other ecometrics[11]. We aim to apply this framework to future aerobiome microclimate impact studies relating to vulnerable populations, the spread of epidemics, and the confluence of microbial dispersal characteristics and resulting weather patterns.

POLICY RECOMMENDATIONS

- **Equitable funding to expand the scope of the NYC Mayor's Office of Climate & Environmental report to include sampling and analysis of environmental microbiomes.**
  - Existing studies already include socioeconomic, public health, and climate variability metrics but do not include long-term microbial sampling.
    - Using existing COVID-19 air sampling, this can be accomplished.
- **Raise public awareness through community involvement, education, and partnership in existing Remediation-Restoration-Revitalization (R2R2R) programs such as PlaNYC.**
  - Use existing R2R2R assessment and public programs to emphasize the microbial impact and connections between improving ecology, improving human access, and increasing human health and wellbeing (Figure 5).
  - Increase support to existing and new academic and community partnerships for citizen science for all.
    - Addresses local issues in several timescales (past, present, and future), advancing community resilience goals.
    - The outcomes of these partnerships further the environmental goals of districts to decrease the harms they are exposed to and provide researchers and students with skills beyond academic training.

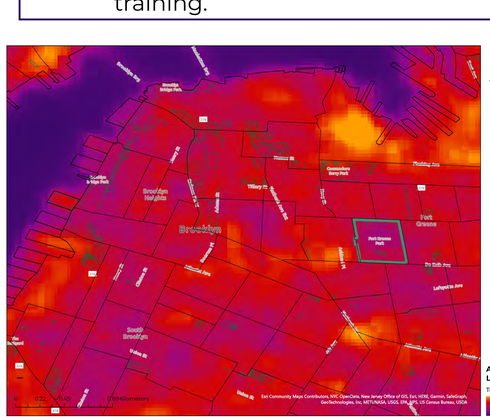


Figure 2. Site map illustrating average land surface temperatures measured from observations by Landsat 8 for 2020.





Figure 3. Our in-field setup at the Fort Greene Park Visitors Center for environmental metrics (left) and aerobiome sampling (right).

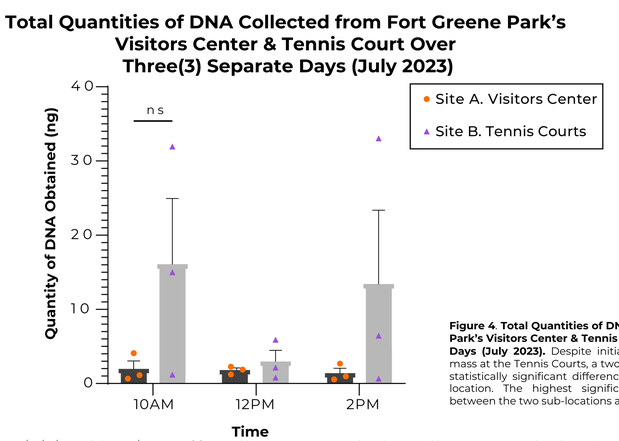


Figure 4. Total Quantities of DNA Collected from Fort Greene Park's Visitors Center & Tennis Court Over Three (3) Separate Days (July 2023). Despite initial observations of higher DNA mass at the Tennis Courts, a two-way factorial ANOVA found no statistically significant differences between any time point or location. The highest significance observed (p=0.06) was between the two sub-locations at 10 AM.



Figure 5. Example of how researchers can engage the community through fieldwork.

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