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Urban heat and cool island effects on aerosol microbiome assemblages

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Abstract

Aeroecology, a field emerging since its conceptualization in 2008 and rooted in World War II radar technology, explores the ecological dynamics of the aerosphere. This interdisciplinary field holds promise for understanding pollution, disease transmission, and agricultural pest management. While the aerosphere comprises biotic and abiotic components influencing aerosol microbiome composition, gaps remain in characterizing bioaerosols, particularly amidst climate change challenges. This study investigates the impact of urbanization, specifically heat and cool island effects, on airborne microorganisms. We hypothesized that aerosol microbial richness, diversity, and evenness would vary with the time and location of heat and cool island effects. In La Verne, we observed changes in microbial richness related to heat and cool island effects with respect to time of day ($p = 0.0001$) and the interaction between time of day and location ($p = 0.0331$). However, microbial diversity and evenness remained unaffected by time of day, location, or the interaction between these factors ($p > 0.05$ respectively). Notably, the south campus of the University of La Verne exhibited the highest aerosol microbial richness ($p \ll 0.001$), whereas Las Flores Park displayed significant changes in aerosol microbial richness between the (morning) urban cool island effect and (afternoon) urban heat island effect ($p = 0.02$). Our findings emphasize the need for further research on aerosol microbiome dynamics in urban environments to better understand their impacts on human health and the environment, contributing to sustainable urban planning.

Keywords: atmospheric ecology; bioaerosols; biodiversity; environmental biology; microbial ecology

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Introduction

Aeroecology is an emerging scientific field that aims to understand the ecological function and biological importance of the aerosphere (Schmaljohann, 2020; Gagnon et al. 2022; Kearsley et al. 2022). Although the term "aeroecology" was coined in 2008 (Kunz et al. 2008), its roots can be traced back to the mid-20th century when the radio detection and ranging equipment used in World War II for tracking enemy aircraft was recognized as a tool for ecological research (Shamoun-Barnanes et al. 2019). The potential of this technology was realized after the war, revolutionizing fields such as Ecology, Meteorology, and Marine Biology (Brooks, 1945; Lack and Varley, 1945; Maynard 1945). The use of radio detection and ranging equipment provided novel insights into aerial movements, flight behavior, and the biomass of birds and insects, leading to a better understanding of species richness in the air and biodiversity estimates (Fox and Beasley, 2010).

In recent years, aeroecology has gained increased attention, especially with the SARS-COV-2 pandemic, due to its potential to further knowledge about important topics such as pollution, disease, crop damage, and pest control (Schmaljohann, 2020; Gagnon et al. 2022; Kearsley et al. 2022). Biotic components such as competition, predation, and biodiversity, as well as abiotic components including resources, habitat, and weather, are present in the atmospheric environment (Wei et al. 2021). Recent studies have shown predictable patterns of bacterial composition in the atmosphere across space and time (Fröhlich-Nowoisky et al. 2016; Smets et al. 2016).

While the ecological importance of microorganisms is well studied, more research is needed to better characterize airborne microbes, known as bioaerosols (Pearce et al. 2009). These bioaerosols are influenced by physicochemical properties of the atmosphere, which can be altered by climate change and impact their geographic distribution (American Society for Microbiology, 2020). Aguilera et al. (2018) has shown that airborne microorganisms play a role in maintaining the global climate system, biogeochemical cycles, and health through their metabolic activity and influence on atmospheric chemistry.

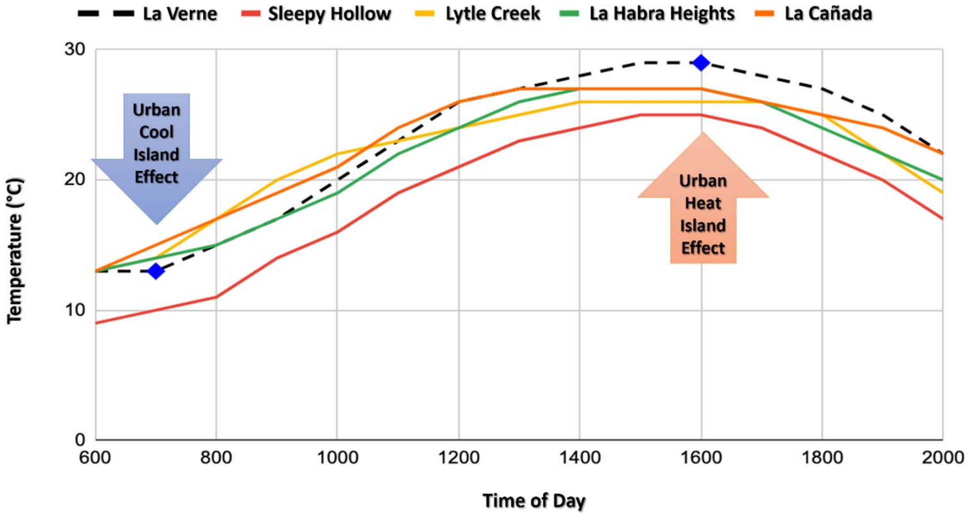

Figure 1. Hourly changes in temperature reveal the time of day for urban cool and heat island effects at La Verne relative to neighboring cities.

Our study aims to examine the impact of urbanization, specifically the heat and cool island effects, on the diversity of airborne microorganisms. The heat island effect, caused by factors such as reduced vegetation, heat-retaining infrastructure, and human activities, leads to higher temperatures in urban areas compared to rural areas (James, 2018). In contrast, the cool effect utilizes water bodies and green spaces to mitigate the heat (James, 2018). The heat and cool island effects can influence microbial diversity, affecting important ecological processes such as decomposition, nutrient cycling, soil aggregation, and pathogen control (Peng et al. 2016). Our study aimed to investigate the impact of urbanization on the diversity of microbes present in the atmosphere. We hypothesized that aerosol microbial richness, diversity, and evenness would change with the time of day and location of urban heat and cool island effects.

Methods

Time of urban heat and cool island effect

To identify the most suitable hours for conducting our microbial sampling in La Verne, we used online weather data (weather.com) to collect hourly temperatures (°C) between 06:00 hrs and 20:00 hrs on 14-Apr-2023 for La Verne, California, as well as four neighboring cities: Sleepy Hollow, Lytle Creek, La Habra Heights, and La Cañada. These cities are situated amidst rolling hills and the foothills of the San Gabriel Mountains. They comprise a diverse array of residential neighborhoods characterized by varying architectural styles, interspersed with bustling commercial districts. Urban planning in these areas emphasizes both functionality and aesthetic appeal. Their physical environment features scenic vistas, valleys, and hillsides, underpinned by a temperate climate suitable for year-round

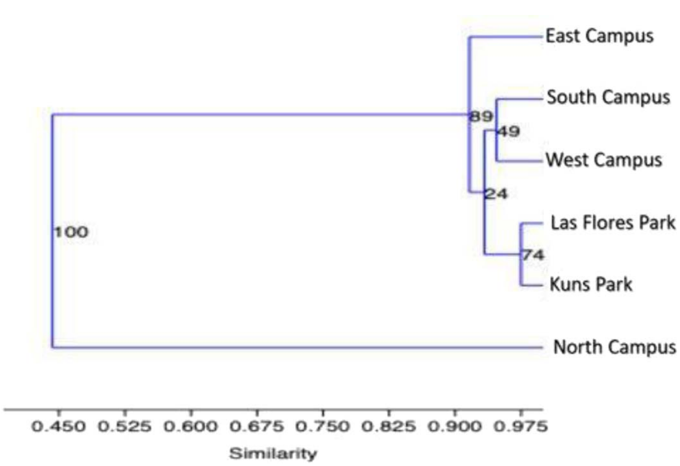

Figure 2. Hierarchical clustering of potential sampling locations in La Verne based on Bray Curtis similarity indices. Bootstrap values above 50% denote support for branch node interpretation.

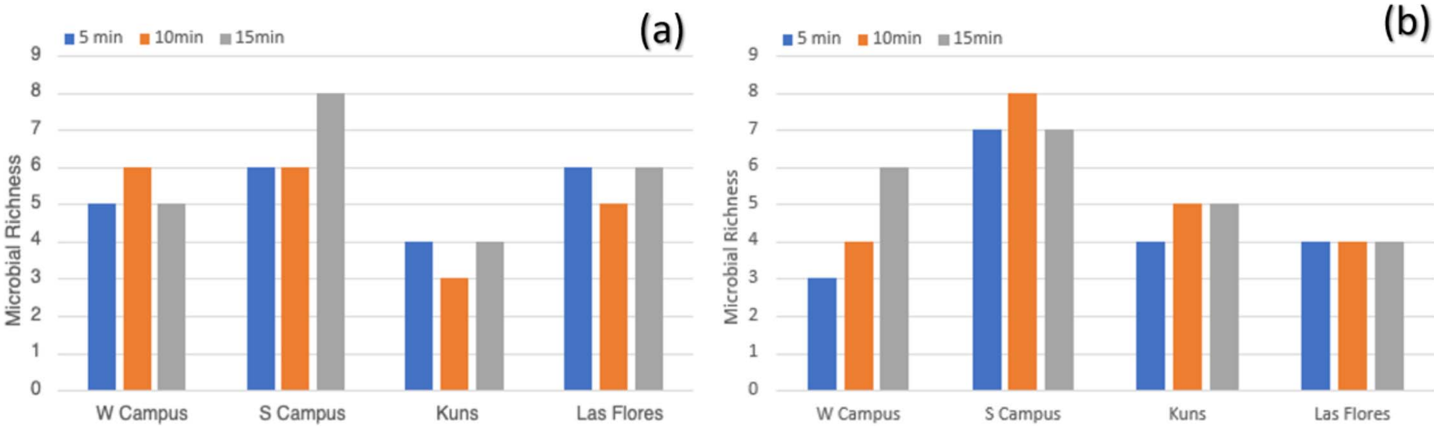

Figure 3. Microbial richness across sampling locations during the time of day for (a) urban cool island effect and (b) urban heat island effect at the city of La Verne.

outdoor activities. Their natural environment includes expansive green spaces, parks, and preserved habitats, promoting community engagement with local biodiversity. Hourly temperature trends were plotted using Excel to identify periods of warmer temperatures (Urban Heat Island Effect) and cooler temperatures (Urban Cool Island Effect) in La Verne relative to neighboring cities.

Biophysical similarity of sites

To identify the most biophysically similar locations for conducting our microbial sampling in La Verne, we evaluated potential sampling sites by measuring variables that characterized the built enviornment (distance to the nearest building [m] and density of buildings [No./m^2]), the physical enviornment (aspect of site [°] as well as the absolute difference between air temperature and ground temperature [°C]) and the natural environment (light intensity [%] and the number of tree canopy layers [No.]). We initially assessed six sampling-sites: North University of La Verne (ULV) campus, south ULV campus, east ULV campus, west ULV campus, Las Flores Park, and Kuns Park. The University of La Verne campus features a blend of historic and contemporary architectural styles set within a suburban landscape and a tapestry of academic buildings, libraries, and student amenities, enveloped by gardens and expansive green spaces that foster both scholarly pursuits and cultural activities. Las Flores Park offers a tranquil natural retreat with expansive lawns, mature trees, and winding pathways ideal for recreation and community gatherings. Kuns Park provides an intimate neighborhood setting characterized by landscaped grounds, benches, and peaceful walking trails, providing local residents a serene environment for relaxation and outdoor leisure.

The biophysical characteristics of the six sampling sites were assessed using a cluster analysis conducted in the software application PAST4 (Hammer et al. 2001). The cluster analysis generated a dendrogram, which organized the sampling sites into a hierarchical branching pattern based on a Bray-Curtis similarity index and supported by a Bootstrap value. We used the dendrogram to choose the sampling locations that exhibited the highest degree of biophysical similarity, while excluding sites that showed the least biophysical similarity.

Microbial assemblages

On 15-Apr-2023, three petri dishes with nutrient gradient agar were placed at ground level and exposed to the atmosphere at intervals of 5, 10, and 15 minutes, during the two pre-selected times of day (identified as having Urban Heat and Cool Island Effects) and at each of the four pre-selected sampling locations (identified as having similar biophysical characteristics). The total of 24 plates were then incubated at 26°C for one week. Following the

Table 1. Mean differences in microbial morphospecies richness with respect to sampling location and time of day.

Source of variation	d.f.	MS	F	*p*
Time of Day (TOD)	1	0.3750	0.5625	0.4641
Location	3	9.1528	13.7292	0.0001
TOD ∗ Location	3	2.4861	3.7292	0.0331
Error	16	0.6667		

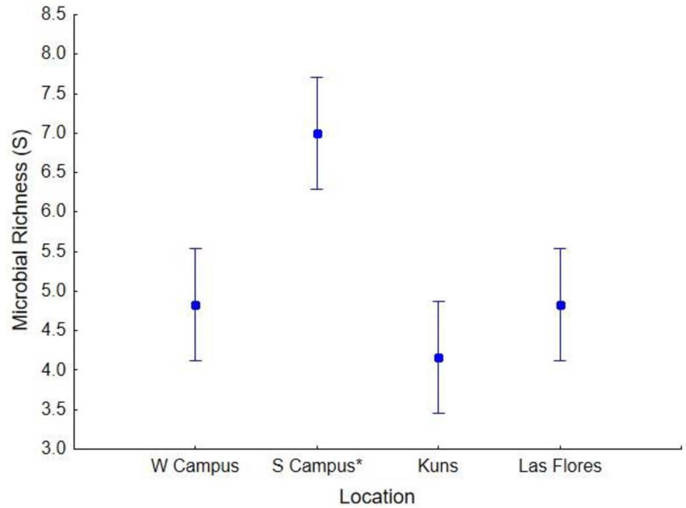

Figure 4. Differences in microbial morphospecies richness with respect to sampling location in La Verne. Bars denote 95% C.I.

incubation period, we digitally photographed the surface of each agar plate and identified microbial species richness (S) in terms of morphospecies (i.e. unique morphological characteristics). We used the measuring tool in the software application Adobe Acrobat to measure the proportional abundance of each morphospecies in a given agar plate relative to total petri dish area.

The microbial morphospecies richness and the respective proportional abundance were used to calculate the Shannon Diversity Index (H) for each petri dish as well as rarefy H-values across all petri dishes using the software application PAST4 (Hammer et al. 2001). Rarefaction is a mathematical sub-sampling technique that renders index values comparable by quantifying them based on an equal number of specimens across all samples (e.g. locations). For each plate, the values of morphospecies richness (S) and rarefied diversity index (H) were used to calculate the evenness (J) of morphospecies representation using equation (1):

$$J = \frac{H_{rarefied}}{ln(S)} \tag{1}$$

Differences in the observed distribution of microbial richness across the intervals of exposure to the atmosphere and sampling locations during both urban cool and urban heat island effects were evaluated relative to expected homogenous richness values using two-factor Chi Square tests. Interval of exposures resulting in microbial richness

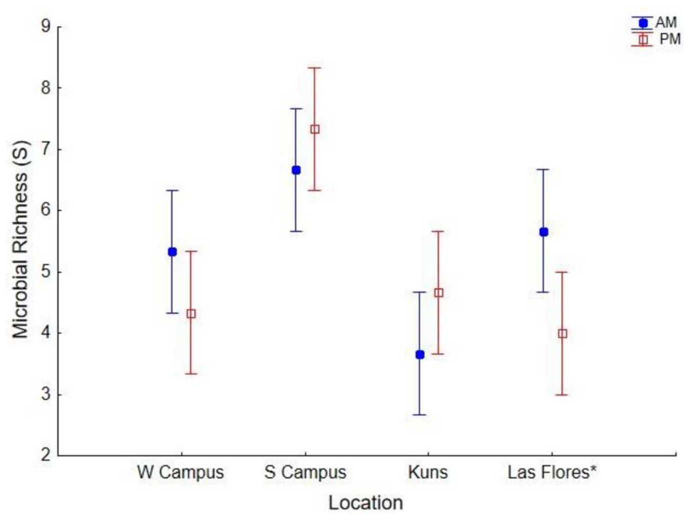

Figure 5. Differences in microbial morphospecies richness with respect to time of day in La Verne for urban cool island effects (AM) and urban heat island effects (PM) as well as sampling location. Bars denote 95% C.I. Asterisks (*) denote Fisher's LSD $p < 0.05$ and bars denote 95% C.I.

Table 2. Mean differences in microbial morphospecies diversity with respect to sampling location and time of day.

Source of variation	d.f.	MS	F	*p*
Time of Day (TOD)	3	0.0425	1.1244	0.3706
Location	1	0.0003	0.0084	0.9281
TOD * Location	3	0.0075	0.1977	0.8963
Error	15	0.037818		

values statistically indistinguishable from an expected homogeneous distribution (i.e., $p > 0.05$) were treated as sampling replicates for the given site (Sokal and Rohlf, 1995). The distribution of morphospecies richness, rarefied diversity, and evenness were evaluated using a Shapiro-Wilks test for normality. The mean differences in morphospecies richness, rarefied diversity indices, and evenness values were each evaluated with respect to time of day and sampling location using two-way ANOVA. Post-hoc analyses were conducted using a Fisher's Least Significant Difference (LSD) test. All statistical analyses were conducted with the software application Statistica (Tibco, 2017).

Results

Based when the city of La Verne was respectively cooler and warmer relative to neighboring cities, changes in hourly temperature over time indicated an urban cool island effect at 07:00 hrs and an urban heat island effect at 18:00 hrs (Figure 1). Based on the dendrogram branching patterns and corresponding bootstrap values, we found that four sites (south ULV campus, west ULV campus, Las Flores Park, and Kuns Park) shared the most similarity in terms of the six biophysical characteristics measured and were included in the study (Figure 2). However, the dendrogram also showed that two sites (North Campus and East Campus) had the least similarity across potential sites and were subsequently excluded from the study (Figure 2).

We identified a total of nine microbial morphospecies across all sampling sites (n = 24 plates). The observed distribution of microbial richness across the 5, 10, and 15 min interval exposure to the atmosphere across sampling locations did not differ significantly from an expected homogenous distribution during either the urban cool island effect ($\chi^2 = 0.611$, $p = 0.99$) or urban heat island effect ($\chi^2 = 1.057$, $p = 0.98$) (Figure 3). We detected normal underlying distributions for microbial morphospecies richness ($W = 0.928$, $p = 0.09$), diversity ($W = 0.974$, $p = 0.77$), and evenness ($W = 0.968$, $p = 0.63$).

While microbial morphospecies richness did not change significantly with respect to time of day, we detected significant differences in relation to location as well as significant differences in terms of the interaction between location and time of day (Table 1). West ULV Campus, Kuns Park, and Las Flores Park exhibited statistically similar levels of microbial morphospecies richness. However, the South ULV Campus location had significantly higher microbial richness compared to these sites (Fisher's LSD, $p \ll 0.001$, Figure 4). At Las Flores Park, microbial morphospecies richness was significantly highest at 07:00 hrs sampling time and lowest at 18:00 hrs sampling time (Fisher's LSD, $p = 0.02$, Figure 5). During the 07:00hrs sampling time, microbial morphospecies richness at Las Flores Park was statistically similar to the levels observed at South ULV Campus (Fisher's LSD, $p = 0.15$, Figure 5). However, at the 18:00 hrs sampling time, richness levels at Las Flores Park were statistically similar to those at West ULV Campus and Kuns Park (Fisher's LSD, $p = 0.62$, Figure 5). We did not detect a significant difference in microbial morphospecies diversity (Table 2) or evenness (Table 3) with respect to time of day, location, or the interaction between time of day and location.

Discussion

The city of La Verne exhibits distinct urban cool island effects during the early morning (07:00 hrs) and urban heat island effects during the late afternoon (18:00 hrs). We also identified sampling locations in La Verne that

Table 3. Mean differences in microbial morphospecies richness with respect to sampling location and time of day.

Source of variation	d.f.	MS	F	*p*
Time of Day (TOD)	3	0.0029	0.1932	0.8994
Location	1	0.0010	0.0669	0.7994
TOD * Location	3	0.0139	0.9431	0.4445
Error	15	0.0148		

shared similar biophysical urban characteristics, broadly describing the built, physical, and natural environment. At these biophysically similar locations, microbial richness across three 5-minute intervals of exposure denoted a statistically similar sampling population, allowing us to use these intervals as sampling replicates for a given site when evaluating the effects of time of day and location (Sokal and Rohlf, 1995). We found that aerosol microbial richness changed in relation to the times of day with urban cool and heat island effects as well as location. However, neither aerosol microbial diversity nor evenness were affected by either the time of day or location. The findings indicate that certain bioaerosols may exhibit spatiotemporal preferences influenced by the presence of heat and cool island effects.

During our study, we made an interesting observation that the south Campus location exhibited the highest richness compared to the other four sites. Additionally, we noticed a significant difference in foot traffic between morning and afternoon at several locations, such as Las Flores Park. In the morning, there was considerably less activity from both people and pets compared to the afternoon. It is worth noting that humans have been identified as one of the major sources of bioaerosols in the built environment (Prussin and Marr, 2015). These findings suggest a potential relationship between human activity and the abundance of bioaerosols in our sampled locations, which merits further study.

While urban cool and heat island effects on biodiversity have been reported for both aquatic and terrestrial ecosystems (Schaffer et al. 1997), our preliminary study documents such effects can also impact the biodiversity of the aerosphere both temporally and spatially. Urban cool and heat island effects have significant impacts on human health, primarily through increased air pollution levels, which are linked to various health issues such as respiratory problems, heat cramps, heat exhaustion, and heat strokes (Santamouris, 2020). As such, our findings have important implications for urban design and urban planning (Santamouris, 2014; Rosenzweig et al. 2006).

To address the challenges of urban cool and heat island effects, existing strategies draw heavily from terrestrial and aquatic biological principles (Santamouris, 2014). These approaches involve actions such as planting heat-resistant trees, incorporating green roofs and vegetation-rich areas, and utilizing tall trees to provide shade to building surfaces while facilitating evapotranspiration (Rosenzweig et al. 2006). However, our findings further underscore that the dynamics and consequences of aerosol microbiome assemblages within the context of urban environments warrants further investigation. Exploring the potential impacts of aerosol microbiomes on human health and the environment is crucial for developing effective strategies to manage and mitigate any associated health risks (Kim et al. 2018). Further research could explore cultivation-independent characterization of the microbial assemblages present in urban aerosols, investigating their sources, dispersal patterns, and potential interactions with other components of the urban ecosystem. For example, many bacteria require specific nutrients, growth conditions, or longer incubation times, so not all bacteria can grow on agar plates. As a result, our measurements of microbial alpha-diversity may underestimate the beta- and gamma- diversity of bioaerosol assemblages represented in urban environments (Sher and Molles, 2022). A metagenomics approach would also provide valuable insights for urban planning, public health interventions, and the development of sustainable urban environments (Shiraiwa et al. 2017).

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Author Contributions

Conceptualization, TP, EH, AO, JR, AA, CZ, AS, VDCG; methodology, TP, EH, AO, JR, AA, CZ, AS, VDCG; software, VDCG; validation, VDCG; formal analysis, TP, EH, AO, JR, AA, CZ, AS, VDCG; investigation, TP, EH, AO, JR, AA, CZ, AS, VDCG; resources, VDCG; data curation, TP, EH, AO, JR, AA, CZ, VDCG; writing – original draft preparation, TP, EH, AO, JR, AA, CZ, VDCG; writing – review and editing, TP, EH, AO, JR, AA, CZ, VDCG; visualization, TP, EH, AO, JR, AA, CZ, AS, VDCG; supervision, AS and VDCG; project administration AS and VDCG; funding acquisition, VDCG. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest Statement

Authors declare no conflict of interest.