URBAN FLOWERING PLANT MICROBIOMES IN RESPONSE TO TEMPORAL VARIATIONS

By

Daniella Lu

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APPROVED

Dr. Quinn McFrederick

Department of Entomology

Dr. Jason Rothman

Department of Microbiology and Plant Pathology

Dr. Begoña Echeverria, Howard H Hays, Jr. Chair

University Honors

Abstract

Urbanization leads to the cultivation of non-native horticultural and weedy plant species, which provide abundant floral resources and may dominate pollinator diets. Flowers are major conduits of microbial transmission, shaping the potential transfer of microbes between pollinators. Studying how the abundance of microbes within floral microbiomes changes over a season may help us understand some of the impacts of global climate change on pollinators and plants. To investigate these trends, I monitored urban gardens on and around the University of California, Riverside campus, recording ambient temperatures, humidity, wind speed, and I conducted pollinator surveys at a specified time once per week. At the same time, I aseptically collected whole flower head samples for culturing on Pollen Extract (PE) medium, a modified de Man, Rogosa, and Sharpe with Pollen Extract (MRS-PE) medium, and a modified Tryptic Soy Agar with Pollen Extract (TSA-PE). I tested how bacterial Colony Forming Units (CFU) correlated with environmental factors. As temperatures lowered, CFU counts increased, while rising temperatures correlated with decreased CFU counts. Urban environments are known as heat islands due to large areas of impervious, heat retaining surfaces. Higher temperatures led to fewer culturable floral microbes, suggesting that climate change may negatively affect floral microbiomes, thereby posing an increased epidemiological risk to plants and pollinators.

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I. Introduction

Urbanization is increasing worldwide as more of the population congregates into cities, replacing natural ecosystems with large industrial and residential settlements (Lowry, 1990). Humans have largely changed their environments based upon habitat fragmentation, the consequences of development, and preference (Potgieter et al., 2017). This has led urban environments to contain high concentrations of impervious surfaces and varying levels of sunlight or precipitation (Godefroid & Koedam, 2007). These urban environments historically experience higher temperatures for prolonged periods in comparison to undeveloped areas, leading to the well documented phenomenon of "Urban Heat Islands" (UHI) (Oke, 1967). Along with urbanization changing local climates, humans have built greater numbers of residential areas, causing the proliferation and spread of non-native plant horticulture (Cadotte et al., 2017). With humans often preferring to raise exotic garden flowers and horticultural cultivars, urban environments may provide relatively low amounts of pollen or nectar (Matteson et al., 2013). In combination with development intensity, this can cause the indirect decrease of the diversity of insect species visiting these urban gardens while also increasing the number of insect visits as floral resources increase (Gonzalez-Cesped et al., 2021). This disrupts local food chains and modes of pollination, causing the local environment to become less resilient to disease or invasive species.

Flowers are visited by many insects; as such, flowers act as major hubs for microbial transmission between many different species (Keller et al., 2021). The microbes found within the nectar of the flower are only a small subset of those that may be found on the insect body (Adler et al., 2021). This suggests that the microbes found within the nectar likely are those that can either survive in a sugar-rich environment or multiply very quickly, as they are the only ones that

are able to survive within the nectar long enough to be cultured and transmitted. Along with this, other interactions between these microbes can also occur. A priority effect can transpire, as the microbes that arrive first and have the higher abundance within the nectar will suppress the others (Vannette et al., 2021). The flower itself may have other mechanisms that change the viability of different microbes, causing huge variability especially amongst plants of different species. These microbes that are viable within the flower nectar or on the flower surface can then be taken in small amounts by visiting insects to other flowers, causing transmission and proliferation.

As there is little knowledge on how the abundance of these microbial communities change due to temporal variation, an outstanding but important question remains unanswered: how is the flower microbiome affected by urban environments? How do highly selective environments such as urban areas cause changes in the viability and transmissibility of microbes? Urban gardens experience higher ambient temperatures due to the UHI phenomenon and this may have cascading effects on the number of insect visitations and the number of microbes present due to this. To understand these epidemiological relationships, an investigation over time is necessary to fully grasp how humans have unintentionally shaped the ecosystems in their own backyards. In this research project I will provide preliminary information on how the network of plant, pollinator, and microbe interactions have been affected in a high human population environment.

II. Materials and Methods

A. Flower and Pollinator Surveys

Four common urban ornamental plants with longer flowering periods within the Lamiaceae family were chosen as the focus of this study. Three of the four were non-native species; *Salvia leucantha*, *Lavandula dentata*, and *Lavandula stoechas*. The fourth plant, *Salvia clevelandii*, is the only species which is native to Southern California. *Lavandula dentata*, *Lavandula stoechas*, and *Salvia clevelandii* plants were chosen to be collected from the Entomology Teaching Garden, Riverside CA, (33.9699, -117.3255) while *Salvia leucantha* was collected from Webber Hall, Riverside CA, (33.9735, -117.3256) on the University of California, Riverside campus. These plants were monitored once per week at approximately the same time (10 A.M. Pacific Daylight Time, UTC-7) on the same day (Monday). The ambient temperature, humidity, and wind speed was then recorded from the real-time observations broadcasted from the Riverside Municipal Airport (16.6 kilometers from the University of California, Riverside campus) which can be accessed from the National Weather Service by the National Oceanic and Atmospheric Administration website. For ten minutes a small section of the plant that hosts a minimum of four flower heads was watched and each insect visitation was then recorded. A visitation was defined as: if the insect landed on the flower for a minimum of five seconds, if pollen was witnessed to be collected by the insect, or if the insect had interacted with the floral structure at all for a prolonged period of time in a purposeful way. This was repeated on all four plant species.

B. Sample Collection

Flowers which had received visitations were selected for lab analysis. While wearing sterile gloves, forceps and scissors were dipped in 2 mL of ethanol then flame sterilized. Tools were allowed to cool, then whole flowering heads were cut and placed directly into 15 mL Falcon Tubes which had been sterilized using UV light (254 nm/>40 μW per square centimeter) for 10 minutes (McFrederick et al., 2017).

These 15 mL tubes with the whole flowering heads were then taken into the lab and mixed with physiologic buffered saline (PBS) until the total volume measured at 13 mL. This was then shaken at max speed in the orbital incubator shaker for a minimum of two minutes. 0.1 mL of each plant sample was then plated on three different types of selective media. This was left to incubate at 30 ℃ for two days and then the number of Colony Forming Units (CFU) was counted to determine the abundance.

C. Media

Three different selective media were used; Pollen Extract (PE) Agar, a modified de Man, Rogosa, and Sharpe with Pollen Extract (MRS-PE) Agar, and a modified Tryptic Soy Agar with Pollen Extract (TSA-PE). PE Agar was chosen and designed in collaboration with my faculty mentors to select for pollen specialist bacteria and to represent the narrowest diversity of microbial species. MRS-PE Agar was chosen to select for *Lactobacillus* bacterial species which may be beneficial for insect species (Gallus et al., 2022), and TSA-PE was chosen to show the broadest range of microbial diversity while still favoring pollen specialists (Figure 1).

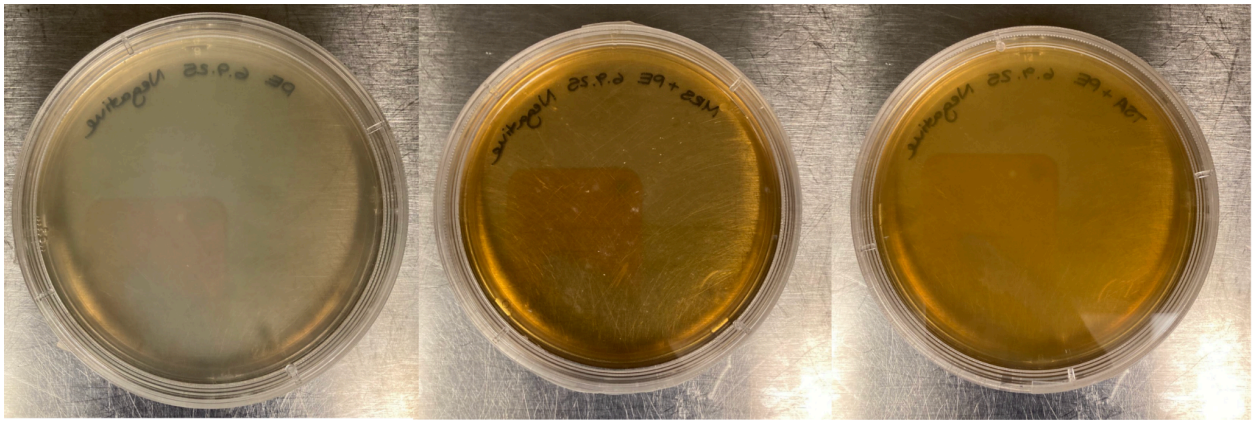

Figure 1. Pollen Extract (PE) Media, a modified de Man, Rogosa, and Sharpe with Pollen Extract (MRS-PE) Agar, and a modified Tryptic Soy Agar with Pollen Extract (TSA-PE). All were poured into the same 100 mm petri dishes and allowed to cool, lids open, under UV light in sterile Biological Safety Cabinet. PE Media targets pollen specialists, MRS-PE media targets Lactobacilli, and TSA-PE is a broad-spectrum media.

To create the pollen extract, 2500 mg of frozen sterile pollen was crushed into a fine powder using a clean mortar and pestle. This was then added into a 50 mL UV sterilized falcon tube with 50 mL of autoclave sterilized de-ionized (DI) water. Ten 5 mm stainless steel beads were added before vortexing solution at max speed for a minimum of three minutes. The solution was then added into a 80 ℃ hot water bath for a minimum of three minutes, followed by another three minutes of vortexing at max speed. The whole 50 mL falcon tube was centrifuged at 20,000x g for five minutes at 4 ℃. The supernatant was then transferred into a new clean UV sterilized 50 mL falcon tube to be centrifuged at 20,000x g for thirty minutes at 4 ℃. The supernatant pulled from this is the final pollen extract.

PE Agar with a 10% pollen extract concentration was created by adding 50 mL of pollen extract to 500 mL of DI water and 10 g of agar. This was then autoclaved, poured into 100 mm petri dishes, and allowed to dry in the sterile Biological Safety Cabinet under UV light. This

process was repeated with MRS-PE Agar and TSA-PE. MRS-PE and TSA-PE were created using the recipes given with the premade mixes of MRS and TSA, but modifying with pollen extract but subtracting the amount of water required in the recipe by pollen extract added to achieve a 10% ratio.

D. Plate Analysis

CFU was determined after two days of incubation at 30 ℃. Each plate was placed under a light microscope and each colony counted. As the CFU was relatively low due to the selective media, no serial dilutions were necessary. This was then plotted on a graph against each variable tested; temperature, humidity, wind speed, and insect visitation to determine which variables had an impact on the abundance of microbes.

E. Statistical Analysis

All statistical analyses were conducted in R Studio version 2025.05.1+513. A multicollinearity test was first used to determine if any of the variables were collinear and to visualize each variable's impact in relation to each other (Figure 2) (Makowski et al., 2020). A Principal Component Analysis (PCA) was then conducted on the variables; temperature, humidity, and wind speed as they were highly collinear variables and therefore violated the assumptions of the downstream statistical tests (R Core Team, 2024). The results of the PCA led us to using the first principal component axis (PC1) (temperature and humidity) and the second principal component axis (PC2) (wind speed) within our analysis as they encompassed ~95% of the variance within the data (Figure 3).

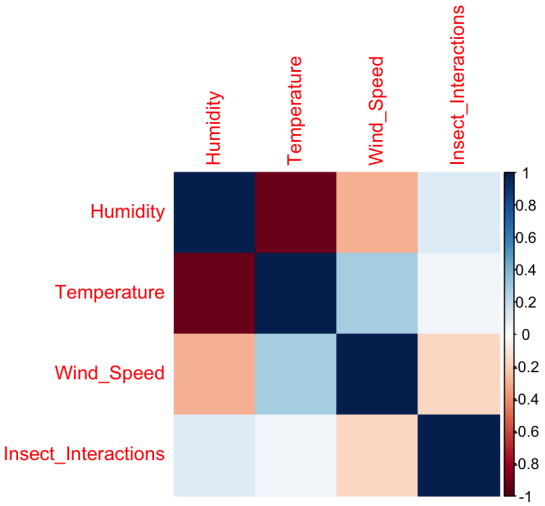

Figure 2. Results of multicollinearity analysis plotted using *corrplot* package (Wei & Simko, 2024). The more blue a box is the more positively collinear it is while the more red the more negatively collinear. Humidity and temperature are highly negatively collinear. Wind speed is slightly negatively collinear with humidity and slightly positively collinear with temperature. Insect interactions are not significantly collinear with any variable other than itself.

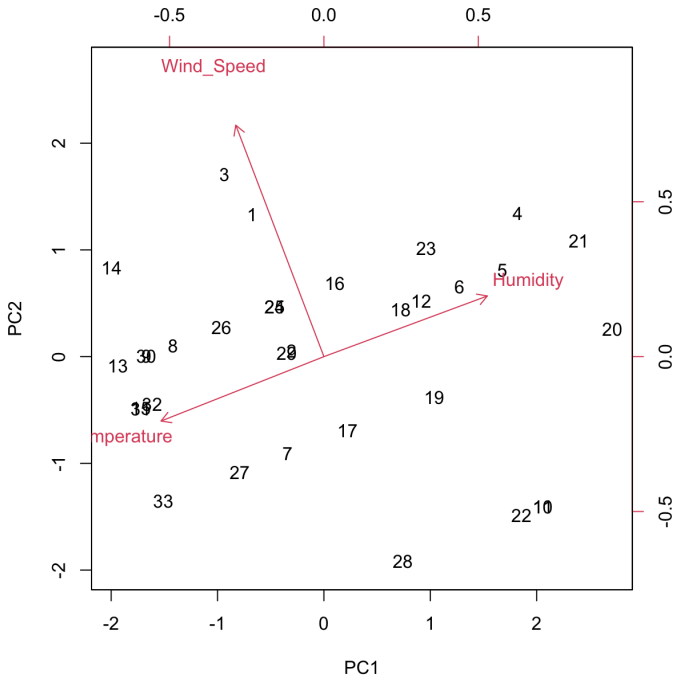

Figure 3. Results of Principal Component Analysis (PCA) plotted. temperature and humidity occupy one axis which is denoted as PC1 while wind speed represents another axis which is denoted as PC2. In PC1 temperature and humidity are on opposite sides of one axis as when PC1 increases humidity increases while temperature decreases. As PC1 decreases, temperature increases and humidity decreases.

To examine variations with CFU counts, we used a generalized linear mixed-effects model (GLMM) fitted with a Poisson distribution and log-link function using the glmer function from the *lme4* package (Bates et al., 2015). We used the calculated PCA axes along with insect interaction as our fixed effects. Plant species, sample volume, and observation-level random intercepts were used as our random effects which accounted for overdispersion.

III. Results

A. Predicted Effects of Abiotic and Biotic Factors

Overall, the results from our GLMM showed slightly differing results depending on the media used, however there is still a clear trend throughout. PC1 represented a temperature-humidity axis with higher values resulting in higher humidity and lower temperatures (cool-humid environment). This was seen to cause CFU to increase as the value of PC1 increased, indicating that the abundance of microbes increased in cool, humid ambient conditions and lowered in hot, dry environments. The significance of this effect on all three media differed as PC1 explained a significant amount of variation in PE agar and TSA-PE microbes ($P < 0.05$; Table 1) but did not have a significant effect on the abundance of microbes grown on MRS-PE agar ($P > 0.05$; Table 1).

Media	Fixed Effect	Estimate	Std. Error	Z Value	Pr(>|Z|)
PE	PC1	0.33763	0.16981	1.988	0.046783 *
PE	PC2	-0.52769	0.26061	-2.025	0.042887 *
PE	Insect Interactions	0.06257	0.05596	1.118	0.263541
MRS-PE	PC1	0.27826	0.17105	1.627	0.104
MRS-PE	PC2	-0.27963	0.26741	-1.046	0.296
MRS-PE	Insect Interactions	-0.03950	0.06149	-0.642	0.521
TSA-PE	PC1	0.302845	0.140768	2.151	0.0314 *
TSA-PE	PC2	-0.119074	0.217098	-0.548	0.5834
TSA-PE	Insect Interactions	-0.007299	0.043399	-0.168	0.8664

Table 1. The different factors; PC1, PC2, and insect interactions of each media type were analyzed using a generalized linear mixed-effects model (GLMM) fitted with a Poisson distribution and log-link function to determine their significance to predicting change in CFU count. * indicate significant P values.

PC2 primarily represented wind speeds with higher values showcasing higher wind speeds. Our results showed that higher wind speeds had a negative effect on microbial abundance, as CFU increased as wind speeds lowered. The significance of this effect was primarily seen in the microbes grown on PE media ($P < 0.05$; Table 1) as both MRS-PE agar and TSA-PE had weak negative associations with this environmental factor ($P > 0.05$; Table 1).

In our model, both PC1 and PC2 were deemed as abiotic factors, the only biotic factor deemed was insect interactions. The number of insect interactions a flower experienced was seen to have no significant impact on the CFU for all three media types ($P > 0.05$; Table 1). In all three media, there was also no consistent weak positive or negative associations between insect interactions and the abundance of microbes.

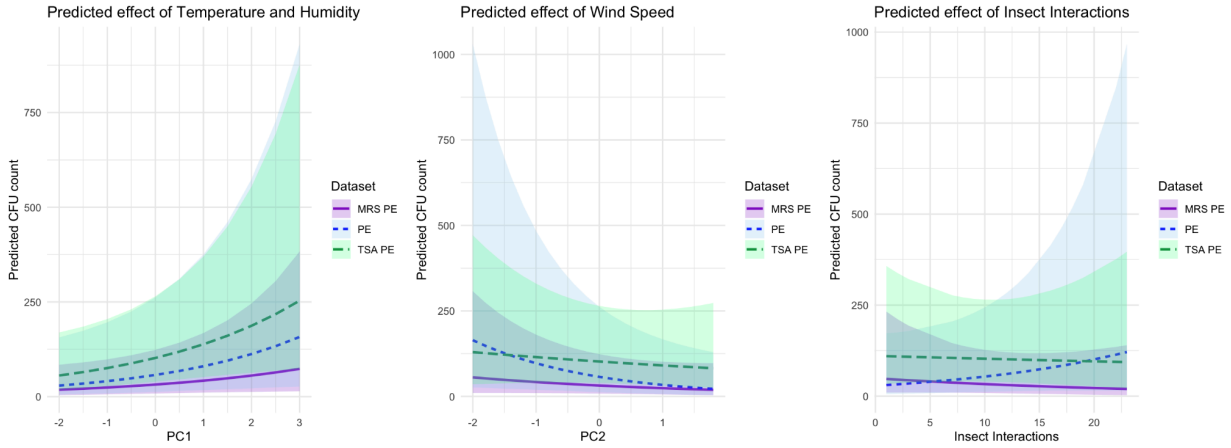

Figure 4. Visualization of predicted effects of abiotic and biotic factors. Results from a generalized linear mixed-effects model (GLMM) fitted with a Poisson distribution and log-link function were plotted. From left to right; PC1 (temperature and humidity) plotted against predicted CFU count, PC2 (wind speed) plotted against predicted CFU count, and finally insect interactions plotted against Predicted CFU count. As PC1 increases, CFU count increases, meanwhile as PC2 increases, CFU count decreases. Insect interactions did not have a clear trend.

B. Temperature and Humidity

As temperature and humidity were the greatest factors that drove the variability in the abundance of microbes within the flowering structures, we chose to visualize them in plots (Figure 5). There is a clear trend within all three media types that as temperature rose and

humidity lowered, CFU lowered with the effect being the strongest on the microbes grown on PE media ($P < 0.05$; Table 1).

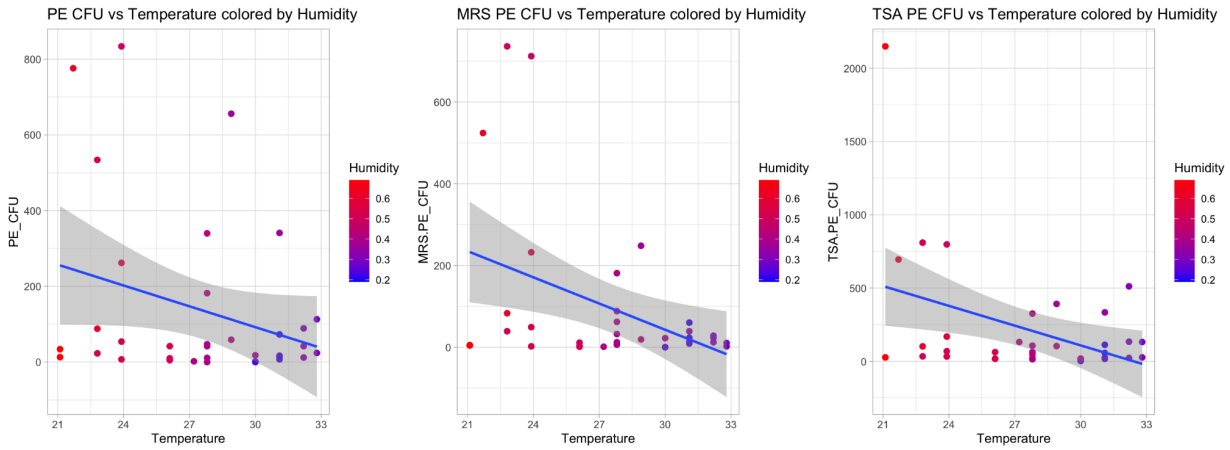

Figure 5. CFU count by media type vs. temperature colored by humidity. From left to right; PE CFU, MRS-PE CFU, TSA-PE CFU. Overall there is a clear trend, as temperatures increase and humidity decreases, CFU counts decrease while the opposite is true when temperatures decrease and humidity increases.

C. Temperature and Plant Species

Due to utilizing four different plant species, we chose to visualize the variability in CFU amongst species even within the same family (Figure 6). CFU counts consistently dropped as temperatures increased, however the strength of this relationship was highly variable amongst the different plant species. The negative trend was present fo*r Lavandula stoechas, Salvia Leucantha*, and *Salvia clevelandii*, however *Lavandula dentata* was not negatively or positively impacted by temperature increase.

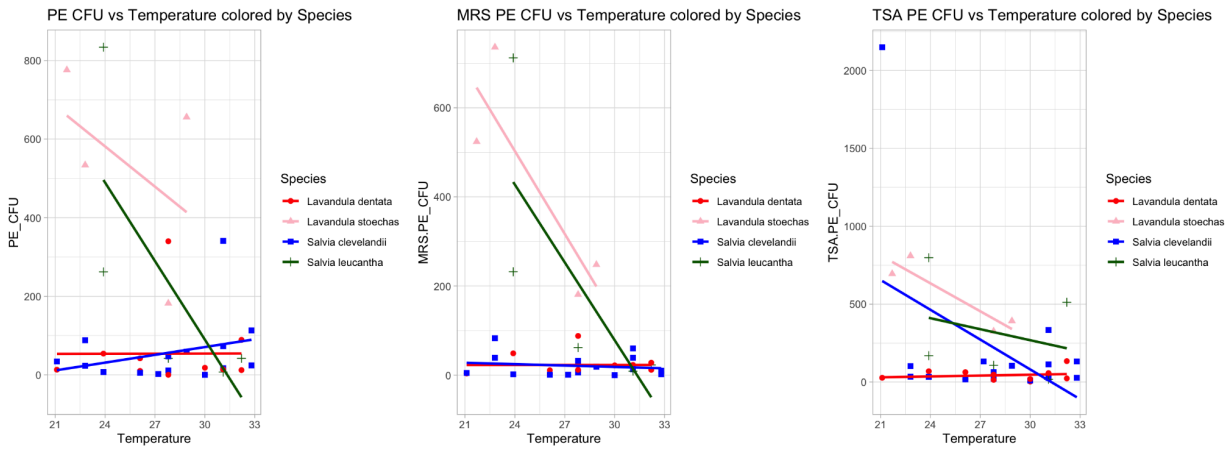

Figure 6. CFU counts by media type vs. temperature colored by plant species. From left to right; PE CFU, MRS-PE CFU, TSA-PE CFU. Temperature still negatively affects CFU counts overall, however the gravity of the effect is dependent on the plant species and the physiology of the plant's flowering structure.

IV. Discussion

This study sought to provide preliminary information on how the network of plant, pollinator, and microbe interactions have been affected in a high human population environment and the trends that could be found. Overall, I found that flower-associated microbial abundance is primarily driven by abiotic factors such as temperature, humidity, and wind, with temperature and humidity being the strongest driving forces. The additional effects of wind speed were likely dependent on the microbial functional group. Responses varied depending on the media used, as each different media type was designed to target different microbes, indicating functional differences in how different microbial communities respond to environmental conditions.

Within our model PC1 represented temperature and humidity, this was seen to have the greatest impact on microbial growth likely due to the role that they play in microbial desiccation.

A more hot and dry environment causes microbes to become more prone to drying out (Alpert, 2006). This likely causes either their death or the stress of the microbes on the flower's surfaces, limiting growth.

Wind speed was seen to impact only the microbial community which could colonize the PE agar, however there were weak negative trends on MRS-PE agar and TSA-PE. This is likely due to the wind speed contributing to the physical removal of microbes on the exposed pollen reservoirs. This can then disrupt deposition and accumulation of microbes from these structures. Along with this mechanical removal, high winds may further aggravate the desiccation stress of the microbes which are already in environments that are hot and dry by increasing evaporation. I hypothesize that this is why we see the effect of wind speed the greatest in the PE agar cultured microbes as we are selecting for pollen specialist microbial communities while the microbes which colonize TSA-PE and MRS-PE agar may have other mechanisms of adaptation.

Microbes grown on PE agar were seen to have the strongest environmental sensitivity overall. All abiotic factors significantly impacted CFU counts on this media. TSA-PE was chosen as a broad spectrum medium which could grow both specialist and generalist. It represented the overall carrying capacity of the floral environment, as such, our results showed that moisture and temperature were the strongest factors in determining that number. Wind speed did not affect these communities likely due to harboring more resilient species or species which recolonize quickly after disturbance. Finally, the microbes which grew on MRS-PE agar showed no significant change due to environmental effects. Although they experienced similar trends, all of them were weak and we could not conclude if the environment was truly the cause of the variability. This media is primarily targeting lactobacilli which are more specialized species that tend to be associated with nectars, sugars, and the internal more protected floral resources. This

leads me to hypothesize that that is the reason we did not see as great an effect as the other two types of media, and that the microbes grown on this media are potentially more affected by the plant sugar composition and species traits than the environment. Overall, I conclude that although there is a common trend, different microbial functional groups may respond differently to the same environment.

The role of the biotic factors measured in this study, insect visitations, were observed to be negligible to CFU abundance. This may have been due to several factors such as that the effects that insect visitations cause are indirect or context dependent such as that there needs to be a greater number of visitations which were not measured in this study to see a significant impact on abundance. The impact of biotic factors such as insect visitations could possibly be overshadowed by the abiotic stresses that the environment poses, as such they are not noticed.

Overall, I conclude that the environment and climate determines which microbes can persist on floral structures. Factors such as wind speed and other mechanical disturbances matter selectively. Wind speed primarily affects the communities which exist on exposed and vulnerable floral resources. Finally, functional specialization within microbial communities has an impact on abundance also as not all microbes respond the same or equally to environmental factors.

This study experienced several limitations which could be addressed. For example, the insect interaction counts could be refined by including insect species or capturing the pollinators which had interacted with the flower sample to compare microbes present on the flower and those on the insect. As this study was culture based, I was limited by the media that I used. Not all microbes are able to be cultured on media, giving us an ultimately incomplete picture of the full interactions that may be occurring. To expand on current findings and gain further insight on how global climate change driven by human activity has affected the network of plant, pollinator,

and microbe interactions, further study is required. A key study would be to determine the specific species present using genomic analysis and determine how they are affected by environmental factors both abiotic and biotic over a longer time scale.

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