Exploring Phoenix's Urban Phyllosphere

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Introduction

- Motor vehicle transportation contributes to air pollution in the fine particle (PM 2.5) range, which is associated with adverse health effects and mortality
- A pollution removal strategy by the City of Phoenix is planting trees near roadsides
- Microorganisms inhabiting the surfaces of plant tissues (i.e. the phyllosphere) contribute to biogeochemical cycling, but little is known of their ability mitigate air pollution

Objectives

1) Characterize the composition and biogeochemical function of bacterial communities inhabiting the urban phyllosphere of city trees in Phoenix
2) Evaluate phyllosphere community variation due to differences in local air quality and host tree species.

Pollution Removal by Trees

City trees are strategically planted near roads because vehicles are pollution sources; air pollution is removed by trees through passive interception.

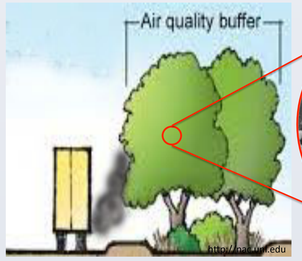


Figure 1. Roadside trees passively intercept motor vehicle pollution

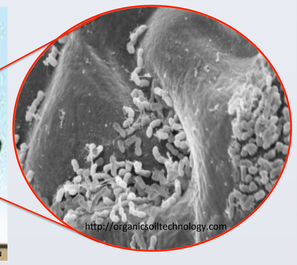


Figure 2. Electron microscope image of microbial phyllosphere colonizers.

According to the City of Phoenix's Tree and Shade Master Plan, the city's vision is to achieve 25% canopy coverage by 2030, doubling the current canopy coverage of 12%, comprised of 92,000 city trees. This will increase the surface area available to phyllosphere bacteria, which colonize leaves in densities of up to 10^7 cells per cm^2.

Figure 3. Satellite image of Washington St. and Gateway, where city trees line the streets. Each city tree is represented by a green dot.

Figure 4. Satellite images of Phoenix. Many city trees are planted along the transportation grid. Each city tree is represented by a green dot.

Phoenix City Trees

Host tree phylogeny selects for phyllosphere bacteria colonizers. A comparison of several tree species will illuminate differences in density, community composition, and function due to host species. Each of the species chosen for sampling are among the ten most common city trees Collectively they comprise almost 50% of all city trees.

Table 1. Ten most common city trees in Phoenix

Common Name	Proper name	% of public trees	Total trees
Mesquite	Prosopis velutina	8.8	9254
Blue Palo Verde	Parkinsonia florida	6.8	7196
Aleppo Pine	Pinus halepensis	5.8	6103
Palo Brea	Parkinsonia praecox	5.3	5712
Evergreen (Chinese) Elm	Ulmus parvifolia	4.3	4569
Indian Rosewood	Dalbergia sissoo	4.1	4342
California Fan Palm	Washingtonia filifera	3.8	3996
Shoestring Acacia	Acacia stenophylla	3.1	3238
Mexican Fan Palm	Washingtonia robust	3.1	3236
Arizona Ash	Fraxinus velutina	3	3176

Blue Palo Verde

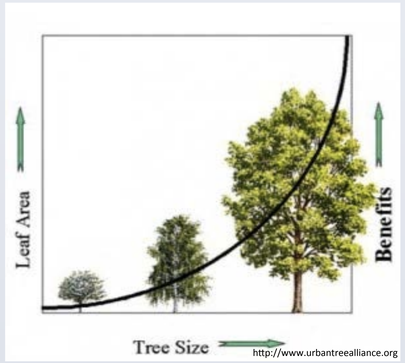
Arizona Ash

Mesquite

Indian Rosewood

Shoestring Acacia

Aleppo Pine



Mexican Fan Palm

Figure 5. Larger trees with more leaf area confer greater benefits, and also provide more phyllosphere area for microorganisms. Trees in Phoenix have varied canopy sizes.

Pollution or Food?

Phyllosphere bacteria are carbon-limited, and carbonaceous aerosols are the dominant species in fine particles (PM 2.5) found on roadsides. If bacterial communities can utilize atmospheric carbon from pollution sources, a higher density and different community composition of bacteria may be supported near roadsides. I sample trees of varied distances to the closest roadside to test the affect of pollution on bacterial communities.

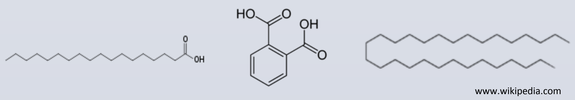


Figure 6. PM 2.5 molecules associated with motor vehicle sources: (left to right) stearic acid, phthalic acid, and nonacosane.

Methods: Extracting phyllosphere DNA

Leaves from city trees are extracted and washed to remove surface microorganisms. Each wash is filtered and its DNA isolated and the total leaf surface area is measured.

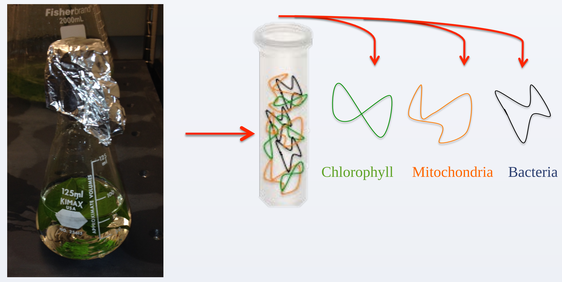
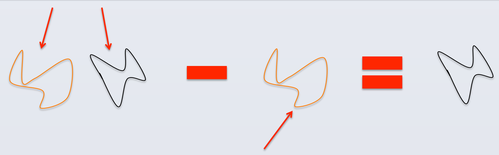


Figure 7. DNA isolated from leaf washes include chlorophyll, mitochondria, and bacteria.

Quantification of Bacterial Density

I use real-time PCR of the DNA from leaf washing samples to quantify the number of bacteria in each sample. Real-time PCR records a fluorescent signal for each copy of a DNA molecule present in a sample. The leaf washings have DNA from bacteria, chlorophyll, and mitochondria are present, so specific primers must be used.

Forward Primer 534 (5′-CCAGCAGCCGCGGTAAT-3′)
Reverse Primer 783 (5′-ACCMGGGTATCTAATCCKG-3′)
Oligonucleotide sequences that anneal with bacterial rRNA and also plant mitochondrial DNA.



Forward Primer 1345 (5′-AGTTTTTGGCCTTATCTTG- 3′)
Reverse Primer 1430 (5′-AAACCCCACTACGTACCACACCAC-3′)
Oligonucleotide sequences that anneal with plant mitochondrial DNA.

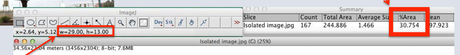
Figure 8. Subtracting the quantity of mitochondria from the bacteria and mitochondria mix elucidates the actual number of bacteria in the sample.

Scaling up

Each sampled tree is photographed from two angles and the color digital image processing method (CD) is used to estimate total leaf area.

Leaf Area = SA x CFA

Silhouette Area (SA):
Crown Area/Photo Frame Area



Crown Framed Area (CFA):
Height x Width of the canopy

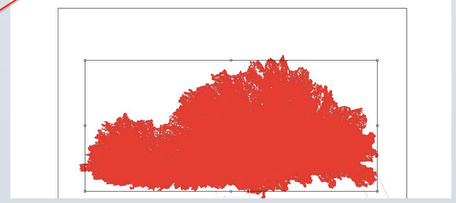


Figure 9. Photoshop and ImageJ are used to estimate the leaf area of photographed trees.

Future Research

The aforementioned techniques will provide estimates of how many bacteria inhabit a single tree. Ultimately this study also aims to understand the function of phyllosphere bacteria. Metagenomic analysis of functional genes and taxonomic community composition of phyllosphere bacterial colonizers are the next steps of this research.

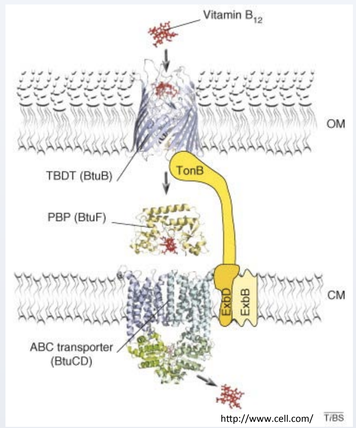


Figure 10. TonB receptor uptake of Vitamin B_{12}. TonB receptors are responsible for the uptake of many different molecules from outside the cell and are commonly found in phyllosphere bacteria.

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