Analyzing the Health of Green Spaces in Brooklyn Through Fungal Species

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Abstract

The proliferation of trees in urban environments is essential for mitigating climate change effects, such as reducing air temperatures and managing stormwater. Despite their critical role, trees in New York City face health challenges, notably evidenced by a visual increase in fungal species. This project investigated the implications of fungal presence on tree health in Brooklyn's green spaces. We hypothesize that the prevalence of *Ganoderma* and other harmful fungal species will correlate with a deterioration in tree health. A total of 15 fungal and soil samples were collected from trees in Brooklyn's greenspaces. Among the 15 samples there were six different tree species, Thornless Honeylocust, Sweetgum, Pin Oak, Northern Red Oak, Littleleaf Linden and Japanese zelkova. DNA extraction and PCR amplification were performed on the fungal samples. Of the 15, seven were successful in DNA amplification, allowing for fungal species identification. These included *Trametes versicolor*, *Ganoderma sessile*, and species from the *schizophyllum* genus. Next, soil samples were analyzed for pH, nitrogen (N), phosphorus (P), and potassium (K) levels using LaMotte Soil test kits. Our results indicate that soil from the 15 trees were acidic, a condition favored by the fungi according to previous studies. The identified fungal genera *Schizophyllum, Ganoderma, and Trametes* are known to cause white root rot decay, indicating deteriorating tree health. Soil tests also revealed varied K, N, and/or P deficiencies in sample collection locations. Thus, our hypothesis was confirmed, the prevalence of *Ganoderma* and other harmful fungal species does correlate with a deterioration in tree health.

Introduction

- Brooklyn New York has approximately 610,000 trees whose canopies cover 11.4 percent of the borough's area (1).
- According to the US Environmental Protection Agency (EPA), trees and vegetation can, on average, lower surface and air temperatures by 2.9°F (2).
- The cooling effect of trees is of import since New York City's 10-year temperature averages, 2010 to 2019, for the months of June, July and August are, 80°F, 86°F and 84°F respectively (3).
- Additionally, September 2023 was the wettest in over a century, with the City accumulating 14 inches of rain which caused severe flash flooding (4).
- The EPA also suggests that leaf canopies and water uptake by roots of trees help reduce soil erosion and serve as a proven method of stormwater management (5).
- It is widely recognized that evidence of fungal growth on trees is a sign of deteriorating tree health.
- Our preliminary research of green spaces in Brooklyn via the New York Mycological Society's Fungi of NYC INaturalist project database, found a visual increase of mushrooms from the *Ganoderma* genus from 2020 to 2021, with the number of new sightings rising from 26 to 56, respectively (6, 7) (**Figure 1**).

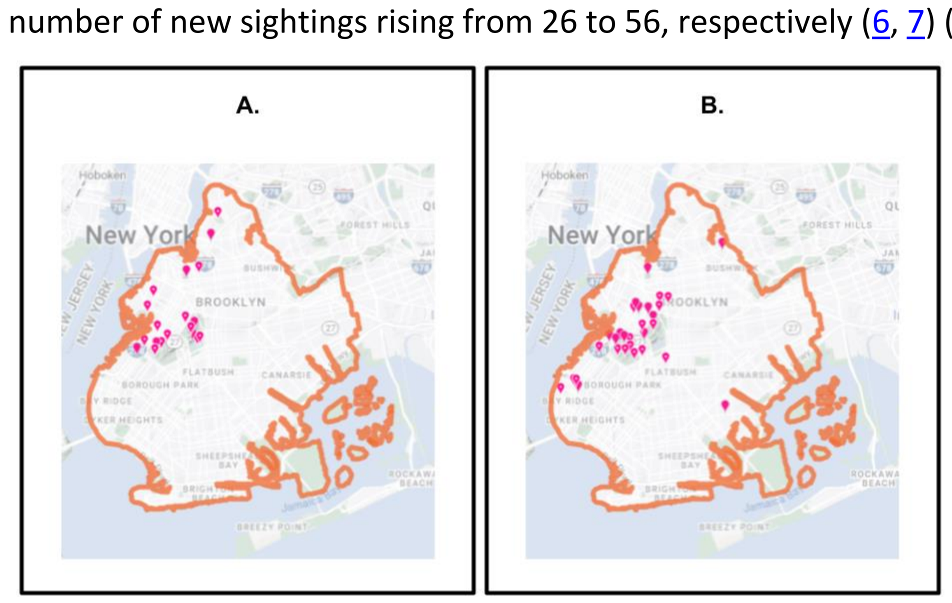

Figure 1. *Observations of Ganoderma in Brooklyn for 2021-2022 and 2022-2023.* Observations from the New York Mycological Society's "Fungi of NYC" INaturalist project database. (A) Shows the 26 sightings of *Ganoderma* in 2020. (B) Shows the 56 sightings of *Ganoderma* in 2021. Adapted from INaturalist project database (6, 7).

- Researchers note however, that little has been published about the health of green spaces and the larger ecological landscape of Brooklyn.
- We hypothesize that the prevalence of *Ganoderma* and other harmful fungal species will correlate with a deterioration in tree health.
- DNA Barcoding will allow us to unequivocally identify species of fungi on unhealthy trees and understand the distribution of different fungal species.
- We also hypothesized that a correlation between fungal species, soil nutrient levels (K, N, P), and environmental factors (pH, temperature, precipitation, humidity), indicate a broader ecological impact of climate change on New York City's green spaces.

Results

DNA Barcoding and Tree Identification Results

Sample Collection				Barcoding Analysis		Existing information on Fungal Species Enviomment			Existing information on Host Tree Species and Ideal Enviornment		
Sample Name	Relative Location	Image of Fungi	Image of Tree	Fungi: DNA Identification	ID%	Ideal Tree	Ideal pH	White Rot	Tree Identification	Hardwood	Ideal pH
1	70 Lafayette Ave								Northern Red Oak	Yes	4-7
2	36 Fort Greene Pl			Trametes versicolor	99.83%	Hardwoods such as beech and oak.	3-5	Yes	Not Listed		
3A	320 2nd St			Ganoderma sessile	99.84%	Hardwoods such as beech and oak.	5-9	Yes	Japanese zelkova	Yes	5.5 to 6.5
3B											
4	707 Fulton Street								Thornless Honeylocust	Yes	6-8
5	501 Carlton Avenue			Ganoderma sessile	99.51%	Hardwoods such as beech and oak.	5-9	Yes	Thornless Honeylocust	Yes	6-8
6A	242 Bay 17th st			Ganoderma sessile	98.23%	Hardwoods such as beech and oak.	5-9	Yes	Pin Oak	Yes	5.0-6.5
6B											
7	8860 18th Ave								Thornless Honeylocust	Yes	6-8
8	136 DeKalb Avenue			Schizophyllum radiatum	99.66%	Sickly hardwood trees	5-9	Yes	Littleleaf Linden	No	5.5 to 7.5
9	153 Dekalb Avenue								Pin Oak	Yes	5.0-6.5
10	199 Washington Park								Littleleaf Linden	No	5.5 to 7.5
11	152 Carlton Ave								Sweetgum	Yes	6.1-6.5
12	131 Carlton Ave								Not Listed		
13	93 Carlton Ave								Pin Oak	Yes	5.0-6.5
14	379 Myrtle Ave			Schizophyllum commune	99.83%	Sickly hardwood trees	5-9	Yes	Littleleaf Linden	No	5.5 to 7.5
15	129 Clermont Ave			Schizophyllum radiatum	99.66%	Sickly hardwood trees	5-9	Yes	Littleleaf Linden	No	5.5 to 7.5

Table 1. *Results from DNA Barcoding of Fungal Samples and Identification of Tree Species.*The fungi collected are identified, so are the species of the respective host trees. Existing information about the fungal species (ideal soil, ideal wood and pH tolerance) is noted in addition to existing information about tree species (ideal soil and pH tolerance). Tree species identification was retrieved from the NYC Parks department street tree map. Link: https://tree-map.nycgovparks.org/

- 15 fungal and soil samples were collected from Brooklyn's greenspaces: samples were collected in Park Slope (**Figure 2**).
- Among the 15 samples there were six different tree species, Thornless Honeylocust, Sweetgum, Pin Oak, Northern Red Oak, Littleleaf Linden and Japanese zelkova (**Table 1**).
- Of the 15 fungal samples, seven were successfully PCR amplified as confirmed via gel electrophoresis (**Figure 3**).
- Those seven samples were identified as specific fungal species via NCBI's BLAST search. Sample 2 was found to be *Trametes versicolor*. Samples 3, 5, and 6 were found to be *Ganoderma sessile*. Samples 8 and 15 were found to be *Schizophyllum radiatum* and sample 14 was found to be *Schizophyllum commune* (**Table 1**).
- Additionally, data shows that pH trends to be acidic (**Table 2**).
- Trees with fungal presence do not have sufficient nutrients to be healthy and thus established the correlation between not only fungal presence but also nutrient levels and a decline a tree health (**Figure 4, Table 2**).

Sampling Results

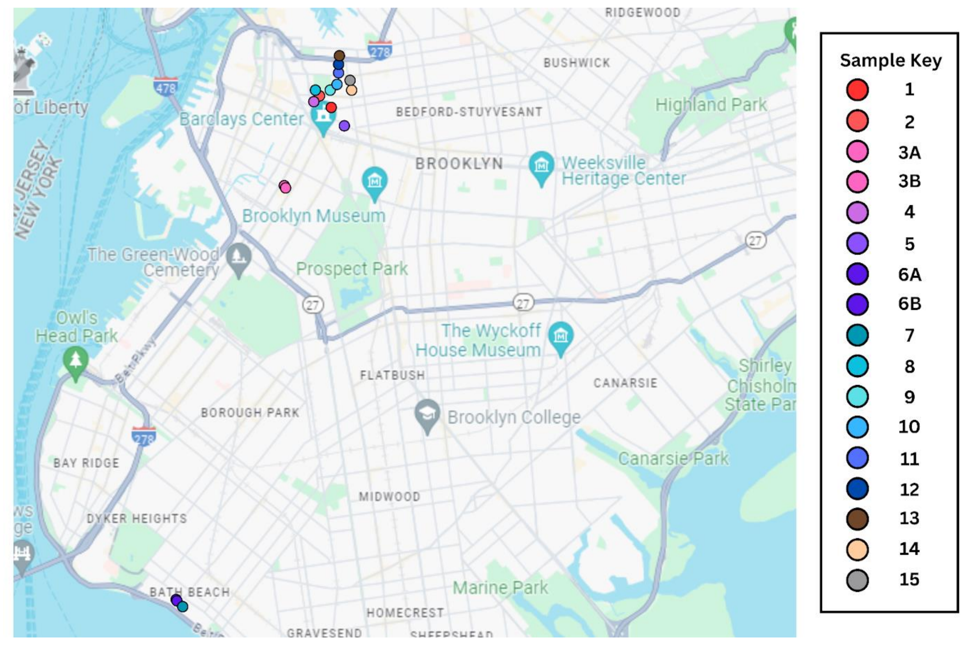

Figure 2. *Relative Location of Samples Collected in Various Neighborhoods of Brooklyn.* Map illustrates the relative location of samples. Samples were collected in Park Slope, one sample, Fort Greene, 12 samples, and Bensonhurst two samples. See inset key for samples and their color-coded pin.

Gel Electrophoresis Results

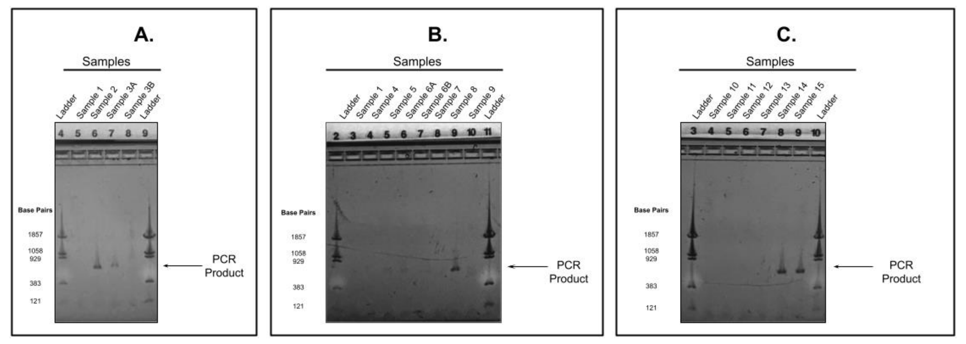

Figure 3. *Gel electrophoresis to Confirm the PCR Amplification of the Fungal Samples.* After DNA extraction and PCR amplification using IGS primers, DNA bands were confirmed by gel electrophoresis. Samples 1, 2, 3A and 3B (**A**), Samples 1, 4, 5, 6A, 6B, 7, 8, and 9 (**B**), and Samples 10, 11, 12, 13, 14, and 15 (**C**) were loaded onto 1.2% agarose gel in 1X TAE at 150 V for 30 min and visualized using a transilluminator.

Soil Test Results

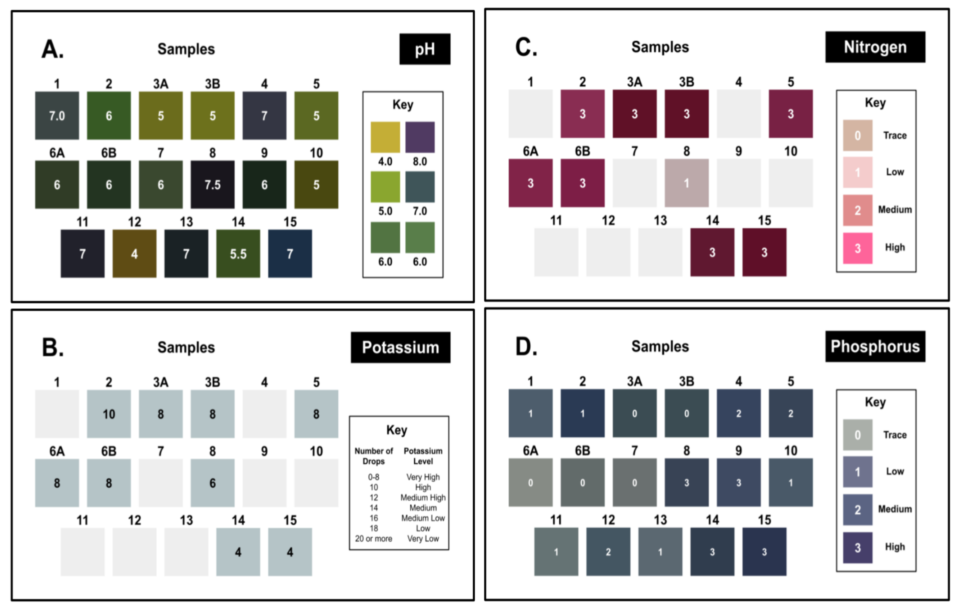

Figure 4. *Data from Soil Testing.* Experimentally determined soil data for levels of nutrients and pH by using the LaMotte Soil Test Kit. (**A**) pH raw data. (**B**) Potassium raw data. (**C**) Nitrogen raw data and analysis, (**D**) Phosphorus raw data. See inset key for panels A, B, C, and D for the color charts displaying known values for reaction results. For K and N only fungal samples which were identified via DNA Barcoding were tested.

Sample Name	Identification	Soil Test Results			
		pH	Potassium	Nitrogen	Phosphorus
2	Trametes versicolor	6	High	High	Low
3	Ganoderma sessile	5	Very High	High	Trace
5	Ganoderma sessile	5	Very High	High	Medium
6	Ganoderma sessile	6	Very High	High	Trace
8	Schizophyllum radiatum	7.5	Very High	Low	High
14	Schizophyllum commune	5.5	Very High	High	High
15	Schizophyllum radiatum	7	Very High	High	High

Table 2. *Identified Fungal and their Respective Soil Test Data.* The results highlight the K, N, and/or P deficiencies for samples 2, 3, 5,6 and 8. Additionally, data shows that pH trends to be acidic as the average across the seven identified staples is 6.

Materials and Methods

Sample Collection

- Sampling consists of two parts: a 2 cm cube of each fungal fruiting body and 2 cups of soil scooped from the base of the tree.
- For each sample, the day, time, and location were recorded, photographs of the fruiting body, tree trunk, and soil were taken, and then placed into a labeled Ziplock bag.

DNA Barcoding

- Fungal sample was taken and used for the DNA Identification process, using the Carolina Biological DNA Barcode Amplification Kit and its respective protocol (8).
- The protocol was modified to use sonication during the lysis stage of DNA extraction to disrupt the fungal cell wall.
- After DNA extraction, PCR amplification was performed with Carolina Biological Fungal Primers.
- Next, gel electrophoresis was performed to confirm PCR amplification.
- Next, DNA samples were sequenced by Azenta Genewiz using M13 forward primers.
- Using the National Center for Biotechnology Information (NCBI), a Standard Nucleotide BLAST search was performed using the sequencing results, thus identifying the species of each of the fungal samples.

Soil Testing

- Analysis of the soil was conducted using the LaMotte Soil Test kit and its protocols (9).
- This was used to measure the pH of each soil sample taken in addition to the levels of potassium (K), nitrogen (N), and phosphorus (P).

Conclusions

- Visual identification is only accurate to an extent.
- All host trees in this project naturally prefer slightly lower pH levels, and pH testing of the soil from these trees confirms this preference. We are not certain if preference for lower pH in both the trees and the fungi is the cause of the fungal presence or increases their susceptibility to fungal growth.
- Of the seven identified fungal samples, all three genera (*Schizophyllum, Ganoderma, and Trametes*) cause white rot root decay, indicating that the tree health has been or is currently deteriorating (10, 11, 12). This is further corroborated by the soil test results, which show that the trees associated with samples 2, 3, 5, 6, and 8 lack one or more essential nutrients for plants (13). Thus, our hypothesis was true: the prevalence of Ganoderma and other fungal species does correlate with a deterioration in tree health.
- Samples 14 and 15 are outliers in our data. There could be other environmental factors which may have influenced fungal presence.
- For the scope of this project, we can correlate fungal species, soil nutrient levels (K, N, P), and environmental factors (pH) to analyze the impact of fungal presence however, to fully analyze the ecological impact of climate change on Brooklyn's green spaces via fungal presence, we would need a larger sample size and data on temperature, precipitation, and humidity.

Acknowledgments

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