JOURNAL OF
EMERGING INVESTIGATORS

Article

Investigating ecosystem resiliency in different flood zones of south Brooklyn, New York

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SUMMARY

With climate change and rising sea levels, south Brooklyn is exposed to massive flooding and intense precipitation. Previous research discovered that flooding shifts plant species distribution, decreases soil pH, and increases salt concentration, nitrogen, phosphorus, and potassium levels. We predicted a decreasing trend from Zone 1 to 6: high-pH, high-salt, and high-nutrients in more flood-prone areas to low-pH, low-salt, and low-nutrient in less flood-prone regions. We performed DNA barcoding to identify plant species inhabiting flood zones with expectations of decreasing salt tolerance and moisture uptake by plants' soil from Zones 1-6. Furthermore, we predicted an increase in invasive species, ultimately resulting in a decrease in biodiversity. After barcoding, we researched existing information regarding invasiveness, ideal soil, pH tolerance, and salt tolerance. We performed soil analyses to identify pH, nitrogen (N), phosphorus (P), and potassium (K) levels. For N and P levels, we discovered a general decreasing trend from Zone 1 to 6 with low and moderate statistical significance respectively. Previous studies found that soil moisture can increase N and P uptake, helping plants adopt efficient resource-use strategies and reduce water stress from flooding. Although characteristics of plants were distributed throughout all zones, demonstrating overall diversity, the soil analyses hinted at the possibility of a rising trend of plants adapting to the increase in flooding. Future expansive research is needed to comprehensively map these trends. Ultimately, investigating trends between flood zones and the prevalence of different species will assist in guiding solutions to weathering climate change and protecting biodiversity in Brooklyn.

INTRODUCTION

Since the late 1800s, the global average temperature has, so far, increased by about 1.8°F (1.0°C), with 2020 being one of the warmest years on record (1, 2). This global warming generates an upsurge in rain; the warmer temperatures cause substantial amounts of moisture from the land and ocean to evaporate, thus regularly generating more intense storms (3). In the Northeast, the most extreme storms generate approximately 27 percent more moisture than they did a century ago (4). This generates rainfall at least 40 percent more likely and 10 percent more intense, increasing the potential of flooding due to climate change (4). Implications of heavy precipitation include soil erosion and shift of pH levels and nutrients (nitrogen, phosphorus, potassium) that can alter the levels of nutrients available in the soil by depositing sediments and leaching water-soluble nutrients (3, 5). Ultimately, this change in allocations inhibits normal plant function and makes plants more susceptible and vulnerable to infection and stresses (6). Furthermore, floodwater can also affect species distribution by carrying invasive species into new areas (7). An increase in invasive species can be detrimental to ecosystems, as it can modify energy flow, nutrient cycling, and hydrological cycling. The presence of invasive species creates more disturbances in the ecosystem and further reduces biodiversity by outcompeting native species and altering the soil composition to be less suitable for native species (8). Ultimately, these factors cause flood-prone areas to be more susceptible to changes in local biodiversity, warranting an investigation.

Being one of the largest metropolitan areas in the United States, New York City is bordered by more than 30 islands that lie within its five boroughs (9). The state's close proximity to bodies of water leads to potentially large impacts brought by climate change. For example, at the beginning of September 2021, Category 4 Hurricane Ida inflicted havoc on New York City, especially in Brooklyn. Regions in Brooklyn that belong to the lower evacuation zones were exposed to massive flooding; the coastal storm broke rainfall records, with more than three inches of rain in one hour (10). But since 1900, the average annual precipitation has only been increasing across the state by approximately 0.8 inches per decade (11, 12). This rising precipitation is projected to increase throughout the year, resulting in frequent storms and flooding that can impact communities and ecosystems. For instance, previous studies have observed plant species that are less tolerable to stress brought by climate change in New York City expand their range and move to other areas; instead, invasive plants proliferate as they can better tolerate the conditions, thus potentially disrupting coastal ecosystems and damaging plants (13). With the rise in global warming, The Federal Emergency Management Agency (FEMA) has been mapping areas of New York's flood risks in Flood Insurance Rate Maps (FIRMs) since the 1980s, using the topography and historical trends of storms (14). These flood hazards in New York City are organized into 6 distinct zones: Zone 1, neighborhoods that will most likely have to evacuate, to Zone 6, less likely to evacuate, and hence less susceptible to flooding **(Figure 1)**.

The increasing tidal and flooding levels in New York City make it a worthy study to examine ecosystem resiliency by investigating plant diversity in the flood evacuation zones located near coastal Brooklyn to collect a baseline study observing the effects of climate change. Factors caused

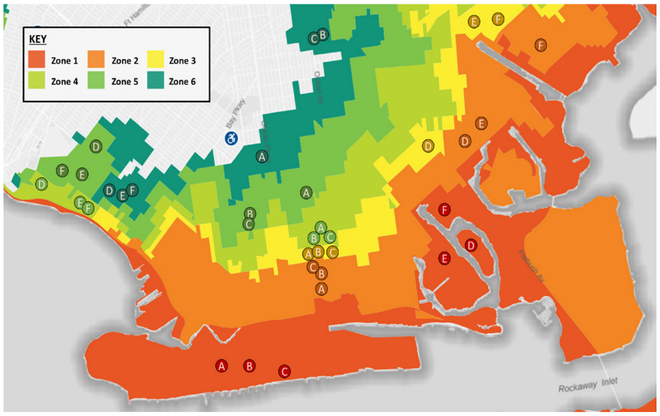


Figure 1: Approximate location of samples we collected based on the NYC hurricane evacuation zone map. Zones are color-coded and labeled 1, 2, 3, 4, 5, and 6. The samples are denoted as A, B, C, D, E, or F and represented as a point on the approximate collection location on the map. The NYC Federal Emergency Management Agency geographically mapped these zones. *Adapted from the publicly released map by NYC on NYC Hurricane Evacuation Zone Finder (30).*

by global warming, including flooding, rise in sea levels, and extreme storm events, are all suggested to decrease biodiversity due to shifts in species distribution (15-18).

In our study, we aimed to identify if this is also the case among the plants in the different evacuation zones of South Brooklyn, as the shifting population could be dangerous to the environment by affecting species distribution (13). By analyzing plant species from regions subject to flooding, we hope to identify trends in species invasiveness, ideal soil conditions, plant pH tolerance, plant salt tolerance, and soil nutrient levels (nitrogen, phosphorus, potassium).

pH is an important factor attributed to plant growth as it affects the availability of nutrients to plants (19). It has been found that nutrients are more readily available in acidic soils, specifically between the pH range of 6 to 7 (20). On average, seawater is at a basic pH of 8.1, and rainwater is at an acidic pH of 5.0-5.5 (21, 22). We hypothesized that evacuation zones closer to bodies of water, and therefore more susceptible to coastal flooding, would have a higher soil pH, while evacuation zones inland, and therefore more affected by flash floods, would have a lower soil pH. While floods can bring hazards, they can also carry newly deposited sediments and nutrients such as nitrogen (N), phosphorus (P), and potassium (K) into the soil (23).

Nitrogen is an essential micronutrient in plants, playing essential roles as the building blocks of plant proteins that stimulate growth and development, as well as an essential element of nucleic acid (24). Furthermore, it has been found that nitrogen is responsible for the utilization of nutrients, such as phosphorus (24). Phosphorus is a crucial element involved in photosynthesis, where a phosphorus deficiency would result in the lack of energy to fuel biochemical processes, thus affecting seed formation, plant growth, hardiness, and water-use efficiency (25). Another crucial nutrient for plant growth is potassium. Potassium is involved in several biochemical processes, such as increasing CO_2 uptake in photosynthesis and activating enzymes in ATP, protein, and starch synthesis (26). When flooding occurs, levels of plant nutrients may decrease, due to the leaching of nutrients and deposition of sediments (23).

In addition to analyzing pH and nutrient levels across the zones, we also want to examine the salt concentrations. Oceans have high salinity, with the surface water of the North Atlantic Ocean averaging at over 37 parts per thousand—the highest salinity of the oceans in the world (27). Hence, the runoff from nearby saltwater sources could have a greater influence on the salt concentrations of soil in coastal areas more susceptible to flooding.

Therefore, we predicted a decreasing trend from Zone 1 (high flood risk) to Zone 6 (low flood risk) in plant tolerance to pH, salt, and nutrients in the soil of plants; hence, flood-prone areas having higher tolerance to high changes in these indicators. Investigating if there is a correlation between the evacuation zones and the prevalence of different species will assist in guiding solutions to weathering climate change. Ultimately, these analyses can help develop policy measures attempting to integrate and protect biodiversity.

RESULTS

DNA Barcoding

We barcoded DNA found in plant tissue samples to identify plant species inhabiting various flood zones in south Brooklyn, New York (NY). This enabled us to do background research on certain characteristics of the species, such as invasiveness, ideal soil, pH tolerance, and salt tolerance **(Table 1)**. Based on our hypothesis, we expected a greater presence of invasive species, loam soil, pH tolerance, and salt tolerance in more flood-prone zones.

After identifying 36 plant tissue samples inhabiting various flood zones in south Brooklyn, NY with nucleotide BLAST, we discovered that our sample set represented 25 different plant species, with the majority of the plants being non-native to the U.S. and invasive species **(Table 1)**. Notably, some plant species were repeatedly identified throughout all the evacuation zones, such as Northern Bog Violet, Chickweed, Common Ivy, and Mugwort **(Figure 2A)**.

The ideal soil type for plants varied, but throughout the zones, the most reported optimal growth occurred in loam soil, which characteristically holds plenty of moisture and drains well **(Figure 2B)**. Northern Bog Violet were found 4 times in loam soil, making up 11% of sample species, while Chickweed was found 3 times in sandy clay loam soil, accounting for approximately 8% of species. Both Common Ivy and Mugwort were identified twice in sandy loam and loam soils respectively.

All plants were able to tolerate slightly acidic conditions, and over half of the samples were able to tolerate neutral and basic conditions **(Table 1)**. The average pH range of the plant samples was from 5.3 to 7.4. Most of the plant species were also able to tolerate elevated salt conditions, although the level of tolerance varied **(Figure 2C)**.

Soil Analysis

We collected samples and the soil of their environment from parks, residences, and sidewalks. We performed soil analysis to identify the levels of pH and nutrients, such as nitrogen (N), phosphorus (P), and potassium (K), found in soil samples. From our hypothesis, we were expecting pH and N, P, and K levels to decrease from Zone 1 to Zone 6. We found a relatively flat trend, with evacuation zones experiencing an average pH range of 6.8-7.6 **(Figure 3)**. The correlation

https://doi.org/10.59720/23-042

Sample Collection		Barcoding Analysis		Existing Information on Species' Environment			
Zone	Sample	% ID	Scientific Name (Common Name)	Invasive	Ideal Soil	pH Tolerance	Salt Tolerance
1	A	100.00%	Lamium amplexicaule (Common henbit)	Yes	Moist	6.0 - 7.0	—
	B	99.65%	Trifolium pratense (Red clover)	Yes	Wide Range	5.5 - 7.6	Low
	C	100.00%	Plantago lanceolata (Ribwort Plantain)	Yes	Compacted	6.5 - 7.3	Moderate
	D	86.18%	Quercus ningangensis (Ring-cupped Oak)	Yes	Loam, Sand, Clay	3.6 - 7.0	Yes
	E	—	—	—	—	—	—
	F	—	—	—	—	—	—
2	A	99.65%	Stellaria media (Chickweed)	Yes	Moist	4.8 - 7.3	High
	B	99.82%	Galium aparine (Cleavers)	Yes	Moist	5.5 – 8.0	Moderate
	C	99.31%	Gymnanthemum amygdalinum (Bitter Leaf)	Yes	Loam	5.0 - 7.5	—
	D	97.35%	Dysphania ambrosioides (Mexican Tea)	Yes	Sand, Loam, Clay	5.2 - 8.3	Yes
	E	91.18%	Perilla Frutescens (Beefsteak Plant)	Yes	Moist, Humus-rich	5.0 - 6.5	Low
	F	87.88%	Sonchus Oleraceus (Common sowthistle)	Yes	Sand, Loam, Clay	5.0 - 8.0	Yes
3	A	99.48%	Lamium purpureum (Red Deadnettle)	Yes	Moist	6.0 - 7.0	—
	B	100.00%	Hedera helix (Common ivy)	Yes	Loam	5.5 - 6.5	Moderate
	C	99.83%	Lunaria annua (Annual honesty)	Yes	Moist	5.0 - 8.5	—
	D	92.39%	Viola nephrophylla (Northern Bog Violet)	No	Wet/Moist	5.5 - 7.0	Low
	E	98.38%	Artemisia montana (Mugwort/Wormwood)	Yes	Dry, Loam	4.8 - 8.2	Moderate
	F	90.00%	Chenopodium album (Lambsquarters)	Yes	Moist, Loam	4.5 - 8.3	High
4	A	99.65%	Stellaria media (Chickweed)	Yes	Moist	4.8 - 7.3	High
	B	—	—	—	—	—	—
	C	—	—	—	—	—	—
	D	100.00%	Taraxacum sp (Common Dandelion)	Yes	Any	4.2 - 8.3	High
	E	96.46%	Artemisia princeps (Mugwort/Wormwood)	Yes	Dry, Loam	4.8 - 8.2	Moderate
	F	97.76%	Viola nephrophylla (Northern Bog Violet)	No	Wet/ Moist	5.5 - 7.0	Low
5	A	97.72%	Pachysandra Terminalis (Japanese Spurge)	Yes	Loam	5.5 - 6.5	Yes
	B	99.65%	Hedera helix (Common Ivy)	Yes	Loam	5.5 - 6.5	Moderate
	C	100.00%	Stellaria media (Chickweed)	Yes	Moist	4.8 - 7.3	High
	D	92.99%	Viola nephrophylla (Northern Bog Violet)	No	Wet/ Moist	5.5 - 7.0	Low
	E	82.63%	Malva neglecta (Common mallow)	Yes	Any	6.0 - 7.0	Yes
	F	90.64%	Dolomiaea/Saussurea costus (Indian costus)	Potentially	Moist	—	—
6	A	98.60%	Euonymus fortunei (Fortune's spindle)	Yes	Loam	6.0 - 8.0	High
	B	99.47%	Stachys byzantina (Lamb's ear)	Potentially	Dry, Sandy	6.0 - 6.5	Moderate
	C	99.82%	Chrysanthemum lucidum Nakai (Chrysanthemum)	Potentially	Moist	6.0 - 7.5	Low
	D	94.09%	Viola nephrophylla (Northern Bog Violet)	No	Wet/ Moist	5.5 - 7.0	Low
	E	98.25%	Ajuga forrestii (Bugleweed)	Yes	Moist, Humus-rich	4.5 - 6.5	Yes
	F	93.00%	Atractylodes macrocephala (Bai Zhu)	Potentially	—	—	—

Table 1: DNA barcoding results of plant samples. The data table reports sample collection information (zone, sample), barcoding analysis data (identification percentage, scientific name, common name), and existing information found regarding the species (invasiveness, ideal soil, pH tolerance, and salt tolerance). A "—" denotes no information was found.

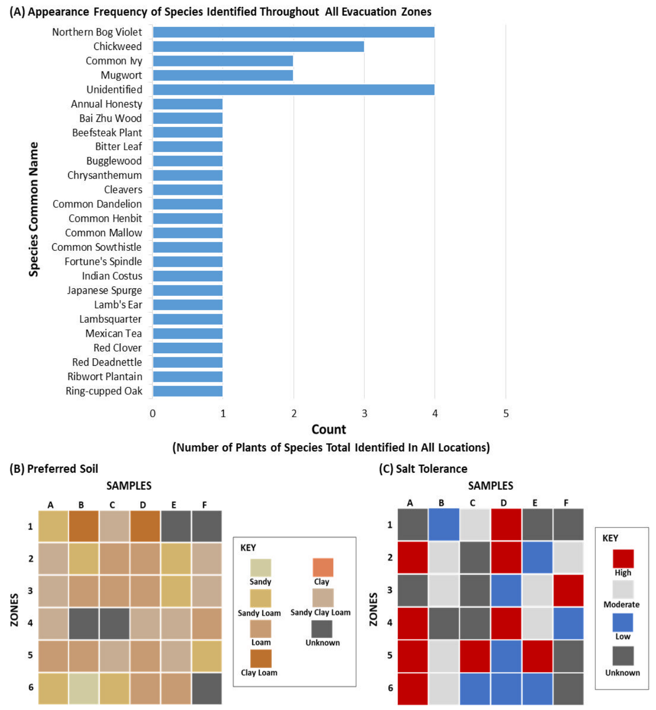
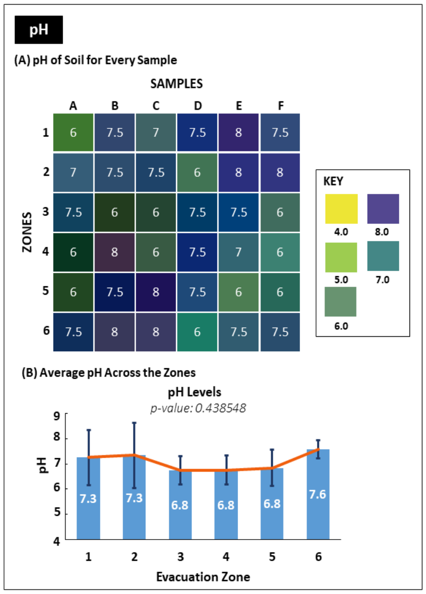


Figure 2: Existing information of barcoded species. Data including barcoding results of species and their ideal soil and salt tolerance were visualized into figures of a horizontal bar chart and heat maps respectively (31). (A) Appearance frequency of species identified throughout all evacuation zones. The count in x-axis refers to the number of plants of certain species that we found in all locations. (B) Soil preference of the identified species. (C) Salt tolerance of the identified species.



	A	B	C	D	E	F
1	6	7.5	7	7.5	8	7.5
2	7	7.5	7.5	6	8	8
3	7.5	6	6	7.5	7.5	6
4	6	8	6	7.5	7	6
5	6	7.5	8	7.5	6	6
6	7.5	8	8	6	7.5	7.5

Figure 3: pH levels of soil samples in various evacuation zones. Based on the statistical differences, there is no correlation between pH levels and evacuation zones. (A) The raw data of the pH of each sample is shown on the heat map. (B) Averages of the pH levels were calculated and graphed into a line-column combination graph with standard error bars. There is no significant difference in pH between the zones, with a p-value > 0.05 obtained from the Kruskal-Wallis test.

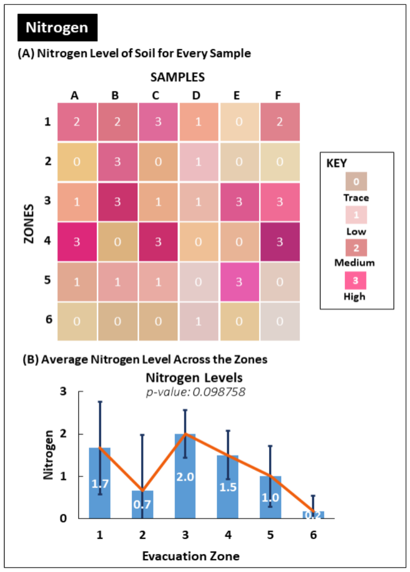
https://doi.org/10.59720/23-042

between the pH of the soil and the different evacuation zones was nonsignificant (p = 0.439, Kruskal-Wallis test).

There was a decreasing trend in nitrogen levels from Zone 1 to Zone 2, followed by a spike in Zone 3, where the N levels decreased until Zone 6 **(Figure 4)**. The general decrease in nitrogen levels as zones become less susceptible to flooding was nonsignificant but with an observable trend (p = 0.099). We discovered a generally decreasing trend in phosphorus levels as zones become less susceptible to flooding **(Figure 5)**. This correlation was found to be statistically significant (p = 0.045). A decrease in the number of potassium indicator drops signifies an increase in potassium levels from Zone 1 to Zone 4 and from Zone 5 to Zone 6 **(Figure 6)**. The decreasing trend was found to be nonsignificant (p = 0.344).

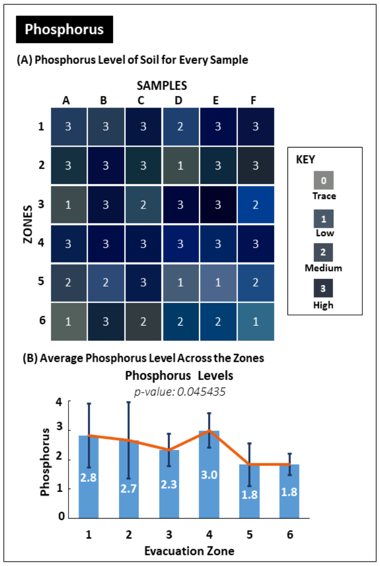
DISCUSSION

In support of our hypothesis of high-pH in more flood-prone areas to low-pH in less flood-prone regions, our results displayed a slightly basic average pH (7.3 - 7.6) in Zones 1, 2, and 6, and a slightly acidic average pH (6.8) in Zones 3,4, and 5. The slightly basic pH levels in Zones 1 and 2 can be explained by the effects of coastal flooding, and the slightly basic pH levels in Zone 6 can be explained by the fewer acidification effects of flash floods on inland regions (21). Consequently, this mix of flash floods and coastal flooding in Zones 3, 4, and 5 could have resulted in a slightly acidic but close to neutral pH of 6.8. But, without finding a general trend across the evacuation zones, the differences in pH were statistically non-significant (p = 0.439).



ZONES	A	B	C	D	E	F
1	2	2	3	1	0	2
2	0	3	0	1	0	0
3	1	3	1	1	3	3
4	3	0	3	0	0	3
5	1	1	1	0	3	0
6	0	0	0	1	0	0

Figure 4: Nitrogen levels of soil samples in various evacuation zones. The correlation in the differences in nitrogen levels amongst the evacuation zones is statistically non-significant. (A) The nitrogen level was indicated by the shade of pink in the resulting color of the solution after adding nitrogen indicator powder. The color is shown on the heat map. To quantify our data, we corresponded trace, low, medium, and high nitrogen levels with numerical values of 0, 1, 2, and 3 respectively. (B) Averages of the nitrogen levels in each zone were calculated and graphed into a line-column combination graph with standard error bars. There is no significant difference in nitrogen levels between the zones, with a p-value between 0.10 and 0.05 obtained from the Kruskal-Wallis test.

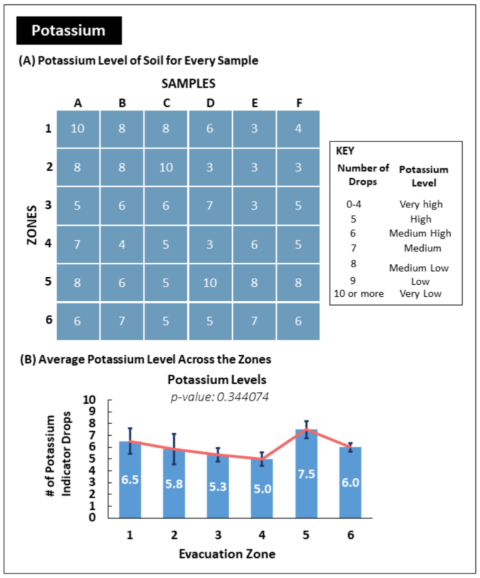


ZONES	A	B	C	D	E	F
1	3	3	3	2	3	3
2	3	3	3	1	3	3
3	1	3	2	3	3	2
4	3	3	3	3	3	3
5	2	2	3	1	1	2
6	1	3	2	2	2	1

Figure 5: Phosphorous levels of soil samples in various evacuation zones. The differences in phosphorus levels amongst the evacuation zones resulted in statistically significant analyses. (A) These qualitative assessments were determined by the shade of gray or blue returned after adding the phosphorus indicator. The color is shown on the heat map. We corresponded trace, low, medium, and high nitrogen levels with numerical values of 0, 1, 2, and 3 respectively to quantify our results. (B) Averages of the phosphorus levels in each zone were calculated and graphed into a line-column combination graph with standard error bars. There is a statistically significant difference in phosphorus levels between the zones, with a p-value < 0.05 obtained from the Kruskal-Wallis test.

We also predicted a decreasing trend in nitrogen levels from Zone 1 to Zone 6, where there would be high levels of nitrogen in more flood-prone areas and lower levels of nitrogen in less flood-prone areas. Our findings partially supported this hypothesis because although we found a statistically non-significant general decreasing trend of nitrogen levels from Zone 1-2 and Zone 3-6, N levels increased from Zone 2 to 3 (p = 0.099). This contradiction could be due to other aspects, including human intervention and sample locations. However, when evaluating the differences among N levels from Zones 3-6, the p-value calculated from the Kruskal-Wallis test was 0.052. The decreasing difference is much closer to being significant when excluding the sudden spike from Zone 2 to 3. Hence, raising suggestions of the possibility of rising N levels in more-flood prone areas. Increases in soil moisture brought by flooding can increase anaerobic N cycling, and this combination of soil moisture and nitrogen can help plants adopt efficient resource-use strategies (24). Nonetheless, the correlation we have discovered is statistically nonsignificant and will require further investigation to confirm our speculations. Furthermore, factoring in nutrient-rich depositions from flooding, we predicted a decreasing trend in phosphorus levels from Zone 1 to Zone 6. Our results support this hypothesis, as the graph illustrated a generally decreasing trend in phosphorus levels. Soil moisture has been previously found to enhance phosphorus uptake (25). Connecting this finding to the decreasing trend we observed, flood-susceptible areas could possibly have greater phosphorus usage to reduce water stress from the increase in flooding.

https://doi.org/10.59720/23-042



Potassium

(A) Potassium Level of Soil for Every Sample

ZONES / SAMPLES	A	B	C	D	E	F
1	10	8	8	6	3	4
2	8	8	10	3	3	3
3	5	6	6	7	3	5
4	7	4	5	3	6	5
5	8	6	5	10	8	8
6	6	7	5	5	7	6

KEY

Number of Drops	Potassium Level
0-4	Very high
5	High
6	Medium High
7	Medium
8	Medium Low
9	Low
10 or more	Very Low

(B) Average Potassium Level Across the Zones

Figure 6: Potassium levels of soil samples in various evacuation zones. Based on the differences, there is no correlation between potassium levels and evacuation zones. Levels of potassium were determined by the number of indicator drops required to titrate the solution, which was indicated by the visible color change from purple to blue. (A) The number of indicator drops to achieve the solution color for every sample is shown on the heat map. Levels of potassium decrease with the increase of potassium indicator needed. (B) Averages of the number of potassium drops in each zone were calculated and graphed into a line-column combination graph with standard error bars. The difference in potassium levels between the zones is non-significant, with a p-value >0.05 obtained from the Kruskal-Wallis test.

Previous research found that nutrient levels increase from flooding. Our results suggest otherwise, as it demonstrates a decreasing trend in the number of potassium indicator drops, and therefore, an increasing trend in potassium levels from Zone 1-6. This observation contradicts our hypothesis and could be due to losses of potassium from fixation in highly weathered soil, where an abundance of minerals can transform or release K (28). Interestingly, K fixation is also highly prominent in illite clays as the sulfide layers release exchangeable K to the solution and deplete K from the soil by the formation of jarosite and plant uptake (28). 75% of the plants we identified in Zone 1 had a soil preference for clay loam or sandy clay loam. This percentage decreases in zones 2-5, where two of the identified plants had soil preferences toward sandy clay loam or sandy loam. Contrary, in Zone 6, none of the identified plants preferred clay soil. The general decrease of K levels from less flood-prone to more flood-prone regions could be influenced by the clay texture containing minerals that encourage K fixation for plant uptake; hence the lower levels we observed in high flood areas. However, we found the correlation between differences in potassium levels and evacuation zones to be nonsignificant with 0.334. We were also unable to identify all the plants in Zones 1, 4, 5, and 6 and their characteristics. Nonetheless, the results paint an interesting picture of K levels, soil type, and moisture that could be further investigated with more samples.

Examining the barcoding results of the plants we collected, we found many of the same plant species inhabiting various evacuation zones, indicating that plant species were not restricted to certain flood zones. Furthermore, invasive and native plants were present throughout the zones. However, out of the identified plants, all species in Zones 1-2 were invasive, while Zones 4-6 had native species. These findings hinted at the impending consequence of flooding in carrying invasive species to the areas, outcompeting native species as they can better tolerate the conditions and shifting species distribution as we identified greater invasive species in flood-prone areas.

Furthermore, all identified plants had a similar range of pH tolerance, with our sample set averaging a pH tolerance of 5.3-7.4. We did not find a considerable disparity in the average soil pH in each evacuation zone: 7.3 (Zone 1,2), 6.8 (Zones 3, 4, 5) and 7.8 (Zones 6). Correspondingly, most of these plants were known to tolerate all these pH conditions since we found no pattern in soil pH preferences based on evacuation zones. The diversity of plants in the zones was roughly around the same regardless of levels of nutrients. Similar to the ideal soil of the identified plants, where a characteristic did not concentrate in a specific zone or evident trendline, the salt tolerance of the species varied throughout the zones except Zone 6. We discovered that in Zone 6, 60% of the identified samples had low salt tolerance. Salinization is a more pronounced issue in coastal areas where there is high salinity due to the proximity to saltwater sources (22). But in inland areas, like Zone 6, this is a lesser issue due to farther proximity; therefore, plants inhabiting those regions do not require a high salt tolerance to flourish, hence possibly accounting for the prevalent low salt tolerance (29).

Our soil analysis tests overall displayed a fairly weak or statistically nonsignificant correlation; plausible explanations include some of the samples we collected were near the border between two evacuation zones or because the majority of the samples were collected from parks, residences, or sidewalks, which may have been impacted by human activity like the addition of fertilizers that could influence nutrient levels. We also tried to not collect identical samples in each zone to fully capture the spectrum of tolerance levels of the plants in the zones. While we were able to focus on the broader comparisons of plant diversity across the zones, this limited us in being able to fully capture the diversity of samples in each zone. Furthermore, nutrient levels and pH are known to fluctuate by seasonality, and we collected half of the samples during the Atlantic hurricane season, so it may not capture a complete picture of the evacuation zones. In future investigations, we can collect a greater number of samples during different seasons instead of limiting them to six samples per zone and collect them from different areas within a zone to diversify our sample population.

Despite these limitations, our study provides a baseline for investigating the influences of climate change, specifically heavy precipitation and flooding, on ecosystem resilience. While occasional flooding is beneficial for plants, as it deposits nutrient-rich sediments, an increasing trend of flooding due to human-induced climate change can increase invasive species that will be more flood-prone—a pattern supported by our study where almost all the samples we collected were invasive. And although there were weak correlations between the increase in phosphorus and nitrogen levels in more flood-susceptible areas, previous studies found that soil moisture can increase nitrogen and phosphorus uptakes, helping plants adopt efficient resource-use strategies and reduce water stress from flooding (24, 25). These analyses hinted at the possibility that plants, especially those susceptible to flooding, are becoming tolerant to their changing ecosystem affected by climate change with the presence of drainage soils, such as loamy and sandy soils, and salt-tolerant species dispersed throughout the flood zones. Conclusively, our study will help serve as a baseline for monitoring the increasing effects of climate change on ecosystem resilience. Centering awareness and continuing to monitor the prevalence of species will help integrate and push forth calls for measures that will protect the biodiversity in our community against the impending detrimental consequences of global warming to the ecosystem.

MATERIALS AND METHODS

Sample Collection

In June 2021, the NYC City Office of Emergency Management released a revised version of the Hurricane Evacuation Zones, noting the vulnerability of coastal neighborhoods in Southern Brooklyn during the Atlantic hurricane season (32). We collected six plant and soil samples from each of the six evacuation zones in south Brooklyn, referring to the most updated (June 2021) version of the Hurricane Evacuation Zones map during sample collection. In the sample name, the first number indicates the flood zone location of the sample, and letters A-F denote the six different samples collected in the locations within a flood zone (e.g., Sample 3D - fourth sample collected in Evacuation Zone 3). We collected samples A, B, and C of each flood zone in February to April 2022. To increase our sample size, we collected samples D, E, and F of each flood zone in September to October 2022, during hurricane season. We collected samples from neighborhoods in south Brooklyn, including Midwood, Sheepshead Bay, Brighton Beach, Bath Beach, Marine Park, and Canarsie **(Figure 1)**.

To isolate the area of focus, we placed a 9-inch hoop over the plant and recorded observations, such as access to sunlight, soil, and plant crowding. We collected one to two leaves from each plant and two to three tablespoons of the top layer of soil to minimize the waste of samples in our experiments. Everything was labeled with the sample's evacuation zone, location, and assigned letter.

DNA Barcoding

DNA extraction was performed according to the instructions provided by the Carolina DNA Barcode Amplification Kit (Carolina Biological Supply Company). We extracted DNA from each sample twice to ensure we obtained a viable DNA sample for sequencing. We dissolved an Illustra Ready-To-Go PCR™ Bead with 22.5 µL of a pre-made master mix containing forward and reverse plant *rbcL* primers. Next, we added 2.5 µL of extracted DNA, and placed it in a thermal cycler programmed for 35 cycles of 94°C for 30 seconds, 54°C for 45 seconds, and 72°C for 45, followed by 4°C indefinitely after the 35 cycles. We performed gel electrophoresis to check for successful amplification using the invitrogen E-Gel®iBase™Power System at 100-240 V. We ran the Pre-Run setting for 2 minutes. Next, we loaded 5 µL of the PCR products into the wells and ran the E-Gel 0.8-2% setting for 26 minutes. Then, we used a UV transilluminator to visualize the results and photographed it. A yellow band indicated the presence of DNA, making the PCR product a viable sample for sequencing **(Figure 7)**.

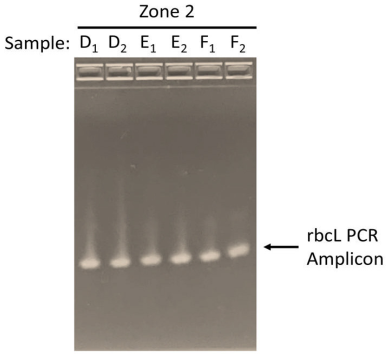


Figure 7: DNA from samples to aid in identifying plant species in zones. The above agarose gel confirms amplification of rbcL gene in the PCR products of Zone 2 samples D, E, and F using specific forward and reverse primers. DNA bands were confirmed using 1.2% agarose gel in 1X TAE run for 26 min at 120V.

After confirming that DNA was present in the samples, we sent samples to GENEWIZ for Sanger sequencing. We input the resulting FASTA sequences into National Center for Biotechnology Information (NCBI) Nucleotide Basic Local Alignment Search Tool (BLAST). After identifying the species of the plants and cross-checking with the images we took, we looked up existing information known about the plants including certain characteristics, such as invasiveness, ideal soil, pH tolerance, and salt tolerance.

Soil Analyses

Soil tests were used in this project to gauge the levels of pH, nitrogen, phosphorus, and potassium in the soil the plants resided in using a LaMotte Soil Test Kit (LaMotte Company, Item #: 5679) following standard procedures. For the nitrogen, phosphorous, and potassium tests, we scaled down the measurements by a factor of ½, ⅓, and ½ respectively, due to the limited amount of soil remaining. In all soil tests, the color charts provided by the soil testing kit were used to gauge the levels of the soil sample.

Received: March 1, 2023
Accepted: July 10, 2023
Published: March 23, 2024

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