Nysa Gandhi, Zuri Hurley, Katie Gjerde, Ava Kulinski

Biology 9H

Mr. Brock

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The Impact of Acid Rain on Bacterial Population Density and pH of Soil

Background:

Soil bacteria are tiny, single-celled prokaryotes that grow and live in the thin water films which surround soil particulates (Hoorman, 2016). A teaspoon of soil usually contains between 100 million and 1 billion of them, and they frequently live near roots in an area called the rhizosphere. Their small size enables them to grow and adapt rapidly to changing environmental conditions (Ingham, n.d.). Hence, they play many essential functions in stabilizing the biotic ecosystems where they reside.

One such function is the creation of the actual structure of the soil. Bacteria produce a layer of polysaccharides or starches that coat the surface of soil particles, helping to cement inorganic sand, silt, and clay particles together to create the actual dirt. This is important because this soil structure determines the amount of nutrients that are available to plants. Furthermore, by improving soil structure, bacteria also play a role in water dynamics, creating the pores and openings that allow soil to absorb and deliver water to plants and the other organisms living there.

In addition to contributing to soil structure, bacteria also partake in nutrient cycling as they decompose dead organisms, animal waste, and simple carbon compounds (such as plant litter) (Ingham, n.d) to obtain nutrients for themselves and then recycle the waste. This process of decomposition releases elements such as carbon, nitrogen, and phosphorus back into the ecosystem for plants and animals for their own biological needs (Glausiusz, 2007). Thus, without bacteria contributing to a range of essential soil processes, terrestrial ecosystems would collapse.

The most important of these processes that the soil bacteria perform is the recycling of the element carbon. Carbon is the basis of all life on Earth, and its cycle through living things starts with plants. In the presence of sunlight, plants combine carbon dioxide from the air with water to create glyceraldehyde phosphate through the process of photosynthesis. Plants use this molecule to create their nucleic acids, lipids, proteins, water, and carbohydrates, the molecules that make up all of the living organisms on the planet (Rader, 2017). Plants use these biological molecules to grow and fuel all of their activities, and when they are eaten, the carbon in their own biological molecules is passed on up the food chain to the rest of the organisms in an ecosystem. Then, when the plant or animal dies, the soil bacteria break down these molecules by releasing enzymes to break apart the chemical bonds that hold the biological molecules together. Doing so releases nutrients including glucose that the soil bacteria then uses for their own growth, reproduction, and other activities, and along the way, they release carbon dioxide back into the air as waste which the plants inhale and the cycle continues (Kowalski, 2014).

A second process soil bacteria perform is the movement of nitrogen through the ecosystem. Known as the nitrogen cycle, it consists of a series of processes that convert nitrogen gas in the atmosphere into inorganic substances and these in organic substances back to nitrogen in the air. This process is known as fixation and is carried out by four types of soil bacteria: nitrogen-fixing bacteria, ammonifying bacteria, nitrifying bacteria, and denitrifying bacteria. ("Biogeochemical Cycles," n.d.). The nitrogen-fixing bacteria start the process by first converting nitrogen gas in the atmosphere into ammonia. This ammonia then reacts with water in the soil to form ammonium. Next, the nitrifying bacteria, a form of nitrogen plants that can absorb through their roots, can assimilate ammonium into nitrates. Finally, the denitrifying bacteria coverts some nitrates back into nitrogen gas to be released into the atmosphere.

The reason the fixing of nitrogen is so important is because livings things use ammonium and nitrates to build proteins and nucleic acids. These are the critical biological molecules that control the chemical reactions within a cell. These chemical reactions determine whether the cell lives or dies. Hence, without nitrogen and the bacteria that make it accessible, the living things in an ecosystem could not survive.

Because soil bacteria play so many roles in plant health, anything that harms them could potentially harm the entire ecosystem. One such factor is acid rain. Acid rain is liquid precipitation containing high levels of sulfuric and/or nitric acid, and while occasionally produced by natural factors such as volcanoes and rotting vegetation, it is most commonly produced by the human activity of burning fossil fuels, which releases sulfur dioxide and nitrogen oxide into the atmosphere ("What Is Acid," 2019). When these gases are emitted into the atmosphere, their chemical properties react with water in the atmosphere to form the sulfuric and nitric acid that gives this precipitation its additional acidity (Nunez, 2019). Normal rain is already slightly acidic, with a pH value of between 5.0 and 5.5 because as rain falls from the atmosphere, it reacts with atmospheric carbon dioxide to make H_2CO_3, which is a weak acid. Therefore, most rain water usually has a pH between five and seven, making it slightly acidic (Balun, 2017). However, when rain reacts with sulfur dioxide and/or nitrogen oxides, it becomes what is known as “acid rain” because it typically has a pH value of 4.0 - meaning it is 10 times more acidic than normal rain.

That level of acidity is enough to do significant damage to plants and microbial life ("Acid Rain," n.d.). Indeed, a laboratory experiment performed by scientists from China to discover the impact of simulated acid rain on microbial biomass found that, when compared with the control treatment, the concentration of bacteria density in the soil was significantly decreased by adding the acid rain, and therefore lowering the pH level (Hua-qin Xu et al., 2015).

The reason the extra acidity can be so harmful is because when the pH of a cell’s environment changes too much, the enzymes in that cell stop working. These enzymes control the stopping and starting of chemical reactions, which result in the four tasks that keep cells alive. Without the four tasks, the bacterial colonies will die. This loss of microbial life further depletes the amount of nutrients available to plant life because, as noted earlier, the micro-organisms play an important role in cycling carbon, phosphorous, and nitrogen. Hence, even if the soil is slightly acidic, it can be disastrous. ("Acid Rain," 2019).

To test these facts and the hypothesis we have formulated (that acid rain decreases the population density of bacteria in the soil around campus), we have decided to experiment on the effects of acid rain on the population density of soil bacteria and the pH levels of the soil. We are pouring sulfuric acid and water onto soil plots to observe the change in population density before and after the testing. We are also observing the pH levels of the soil before and after the tests to make sure there is a correlation between the acidity of the soil and the population density of the bacteria inhabiting the soil.

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Lab Outline:

I. Problem: How does acid rain alter the population density of bacteria in the soil on the Roland Park Country School campus?

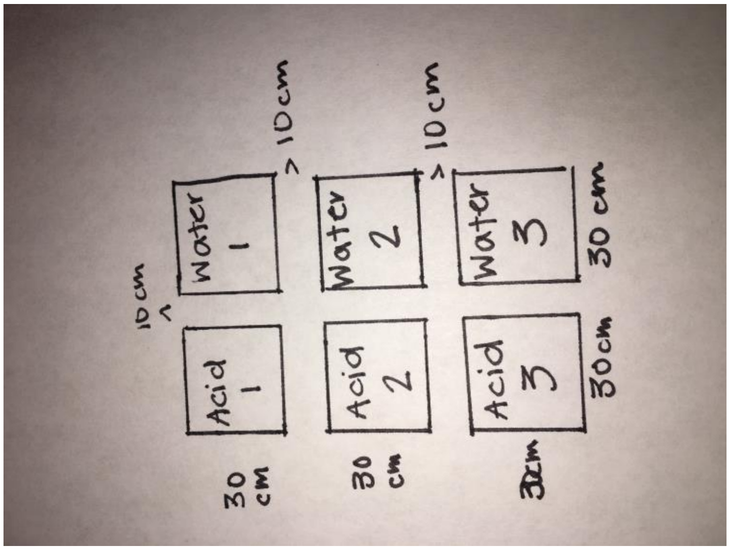
II. Hypothesis: Acid rain decreases the population density of bacteria in the soil on the Roland Park Country School campus.

III. Procedure:

   a. Independent Variable: Addition of acid rain to the soil plots
   b. Dependent Variable: Density of bacteria in the soil and the pH of the soil
   c. Negative Control: Addition of distilled water to the soil plots
   d. Positive Control: Soil samples taken before acid and water was added
   e. Controlled Variables: How big the plots are, temperature of acid, temperature of water, how far apart the plots are from each other, where the plots are, amount of time between before and after sampling, amount of acid rain added to soil plots, amount of water added to soil plots, amount of soil measured, type of vegetation on plots, amount of vegetation on plots, sterile water, type of pipette, type and size of test tubes, micropipette tip, amount of solution on the plates, type of plates, degree of dilution, amount of soil placed in first tube, size of soil samples taken, bacteria growth time, dilution levels that were plated,

f. Step by Step:

1. Use 3 molar concentrated sulfuric acid and distilled water to make 3 liters of sulfuric acid solution with a pH of 4.0
2. Pour 500 mL of the acid rain solution each into 6 different 500 mL plastic containers and label them acid 1A, acid 1B, acid 2A, etc…
3. Pour 500 mL of distilled water each into 6 different 500 mL plastic containers and label them water 1A, water 1B, water 2A, etc…
4. To make the soil plots, gather 24 marking flags and label them for 3 acid plots and 3 water plots, 4 flags for each plot. For example, label all 4 flags in the first water plot “W1.”
5. Go to the following coordinates: N 39º, 21.409 W 76º, 38.150
6. Measure out a 30 cm by 30 cm square and put a correctly labelled flag on each corner of the square by sticking the flag into the ground (see diagram)
7. Repeat step 10 five more times, making the plots each 10 cm away from the previously made plot (see diagram). There should be three “water” plots in a row and three “acid” plots in a row.



8. To take the “before” soil samples from the 6 soil plots, label 18 sandwich sized plastic Ziploc bags, for 3 samples from each of the 6 plots, for ex: “A1 sample 1, A1 sample 2, A1 sample 3, A2 sample 1, etc…”
9. Use a soil core extractor and a hammer to extract 15 cm deep and 5 cm wide of soil from the first plot and place it in the correctly labelled bag. Repeat this two more times for the first plot, and 3 times for each of the other 5 plots on the same day, at the same time.
10. Do steps 11-12 at the same time as steps 13-30 on all samples on the same day
11. Use the LaMotte STH-14 test kit to perform the pH test on all of the soil samples
12. Record data about the pH levels of the “before” soil samples
13. To find the bacterial population density before the tests, use a clean, new transfer pipette to add 10 mL of sterile water to a 15 mL culture tube. Label the tube “$A1^0$”

14. Use the same pipette to add 10 mL of water to a second 15 mL culture tube. Label the tube “$A1^{-1}$”
15. Repeat step 14 three more times to three additional 15 mL culture tubes, only label them “$A1^{-2}$,” “$A1^{-3}$,” and “$A1^{-4}$” respectively
16. Mix all of the samples from A1 together into one bag, and repeat for all of the other plots
17. Place 1 cc of the A1 soil sample into the $A1^0$ culture tube
18. Cap the tube and shake vigorously
19. Using a new clean pipette, remove 1 mL of the soil/water mixture from the $A1^0$ tube and place it in the $A1^{-1}$ tube
20. Cap and shake the 10^{-1} tube vigorously
21. Using the same pipette, remove 1 mL of the soil mixture from the A^{-1} tube and place it in the $A1^{-2}$ tube
22. Cap and shake the $A1^{-2}$ tube vigorously
23. Using the same pipette in step 19, remove 1 mL of the soil/water mixture from the $A1^{-2}$ tube and place it in the $A1^{-3}$ tube
24. Cap and shake the $A1^{-3}$ tube vigorously
25. Using the same pipette in step 19, remove 1 mL of the soil/water mixture from the $A1^{-3}$ tube and place it in the $A1^{-4}$ tube
26. There should now be a total of 5 culture tubes
27. Label a bacteria growth plate for each dilution of A^{-3} and A^{-4}

28. Plate 100 μl samples from the 4th and 5th tubes (dilutions A^{-3} and A^{-4}) onto their own separate, correspondingly labelled 3M Petri film aerobic count plates; we plated both 10^{-3} and 10^{-4}
29. Repeat steps 13-28 5 more times with the corresponding labelling for each soil sample ($A2^0$, $A3^0$, $W1^0$, $W2^0$, $W3^0$, etc…) using different tubes for each sample
30. Allow the samples grow for 72 hours
31. Use a magnifying glass to examine the lowest dilution for individual bacteria colonies, and find the plate with at least 5 colonies to make the estimates of the number of bacteria in the original 1 cc soil sample using the following formula: # of microbes in 1 cc of soil = # of colonies on sheet x 10^2 x $10^{|\text{dilution \# at which these colonies were found}|}$
32. Record data about bacterial population density before the acid and water tests
33. To conduct the acid and water tests, take the 12 bottles of water and sulfuric acid solution to the soil plots
34. Pour 1 liter of the acid solution onto each of the three soil plots labelled “acid”
35. Pour 1 liter of the distilled water onto each of the three soil plots labelled “water”
36. Wait 48 hours
37. Use a soil core extractor and a hammer to extract 15 cm deep and 5 cm wide of soil from the first plot and place it in the correctly labelled bag.

Repeat this twice more for the first plot, and 3 times for each of the other 5 plots on the same day, at the same time.

38. Repeat steps 10-30 for the "after" soil samples

Analysis:

Before acid and water were added and after acid and water were added pH levels

	Acid 1 plot	Acid 2 plot	Acid 3 plot	Water 1 plot	Water 2 plot	Water 3 plot
Before pH	6.6	6.6	6.8	6.8	6.8	6.8
After pH	5.8	6.0	6.0	6.6	6.8	6.8

AVERAGES OF PH

	acid	water
before	6.66	6.8
after	5.93	6.73
	0. 73 difference	0.07 difference

Before acid and water were added and after acid and water were added bacterial population density

Before

Plot Name	Population density of 1 cc scoop
W1 plot	5,000,000 cc
W2 plot	1,700,000 cc
W3 plot	2,100,000 cc
A1 plot	3,500,000 cc
A2 plot	600,000 cc
A3 plot	700,000 cc

After

Plot Name	Population density of 1 cc scoop
W1 plot	900,000 cc
W2 plot	7,000,000 cc
W3 plot	1,500,000 cc
A1 plot	100,000 cc
A2 plot	100,000 cc
A3 plot	100,000 cc

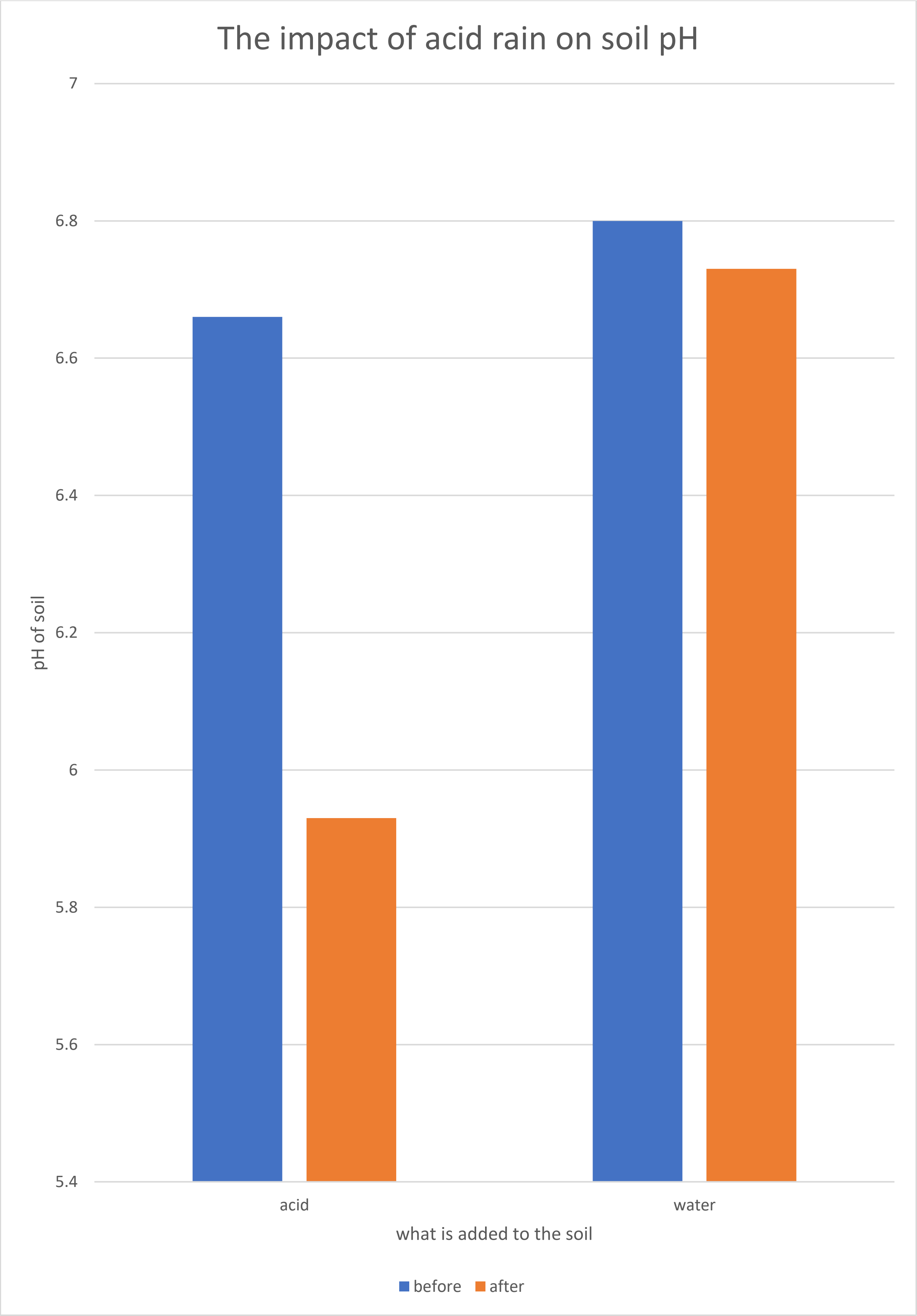
<u>Averages of population density</u>

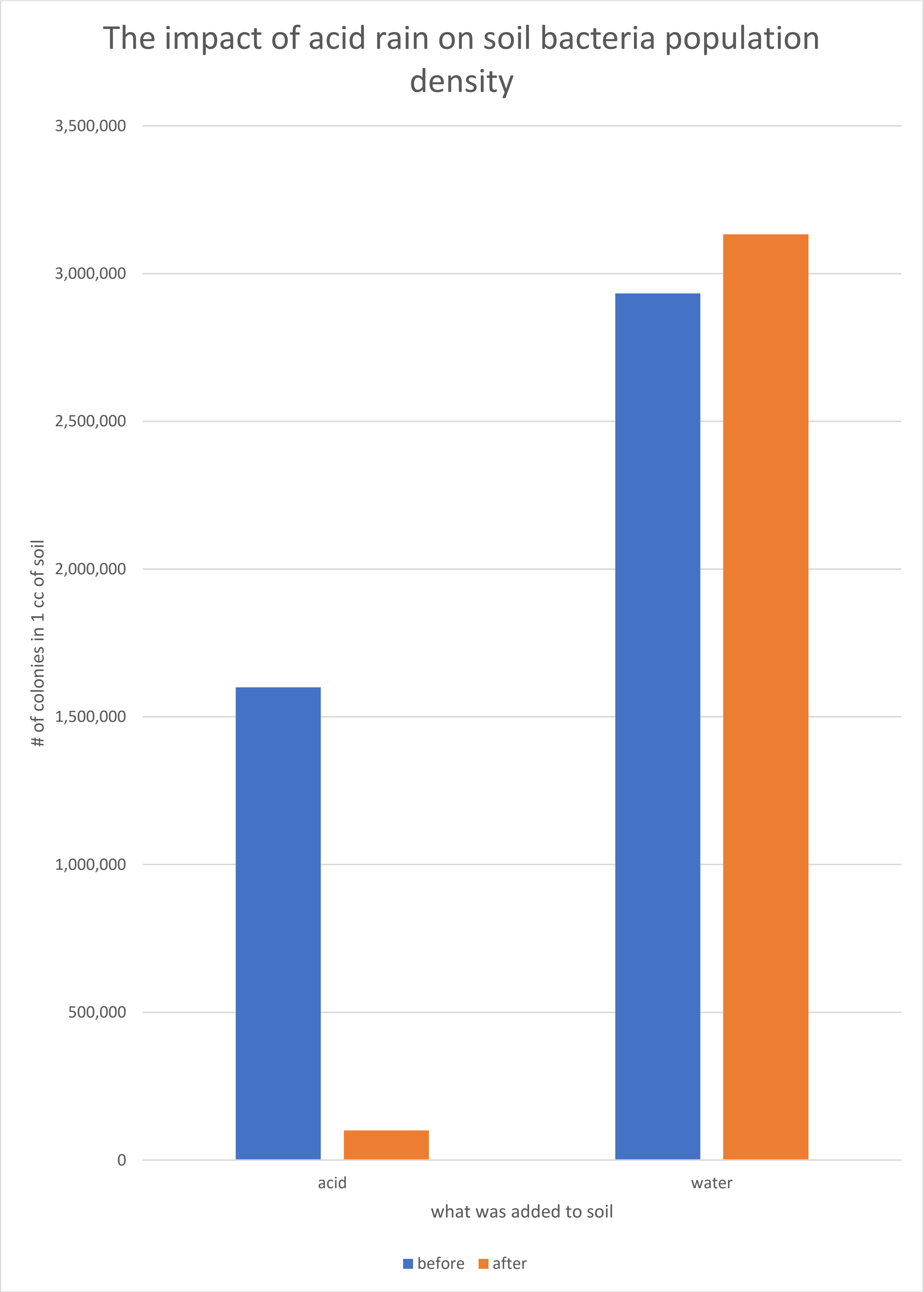
Before

Plot Name	Population density of 1 cc scoop
Water	2,933,333.33
Acid	1,600,000

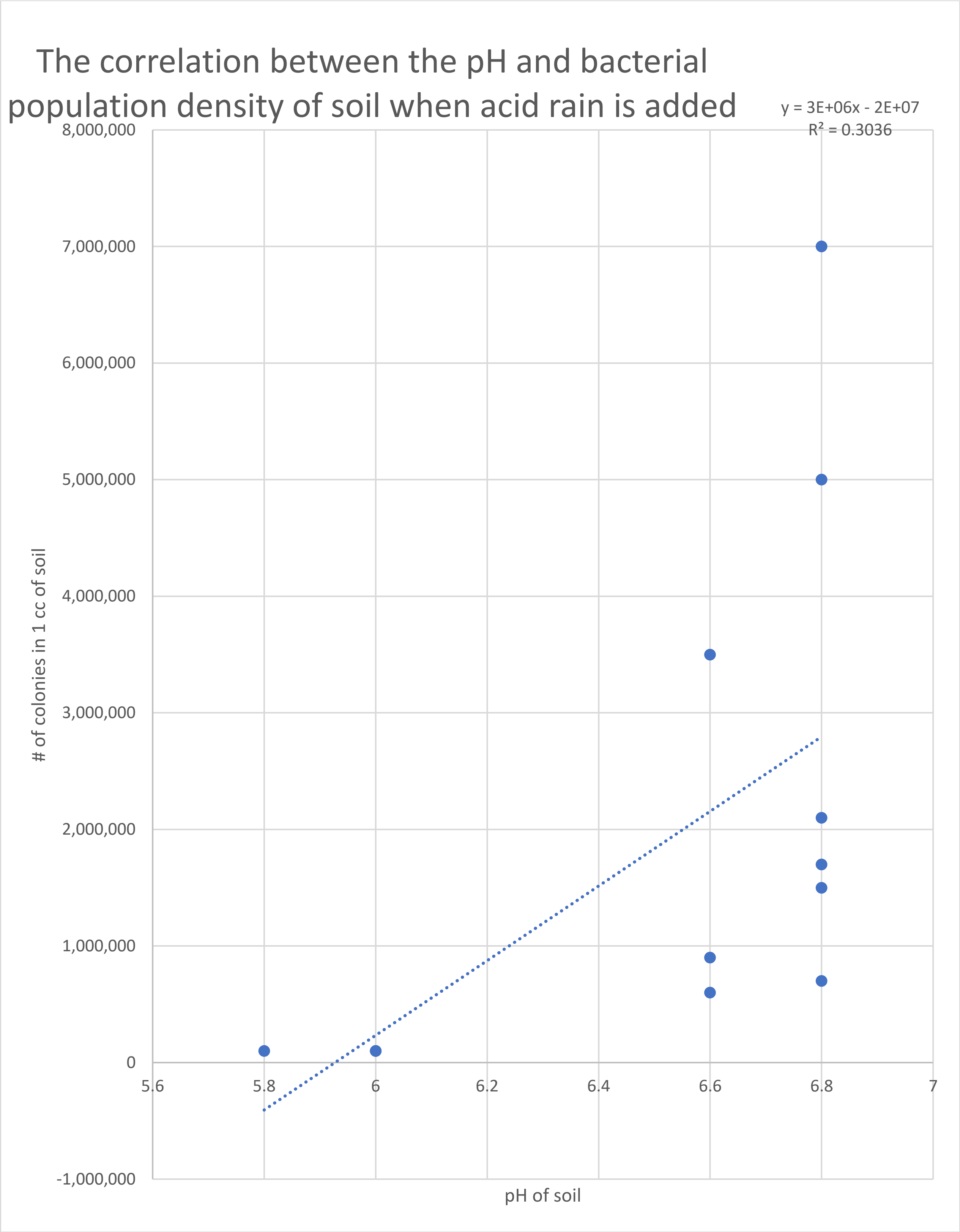
After

Plot Name	Population density of 1 cc scoop
Water	3,133,333.33
Acid	100,000


The impact of acid rain on soil pH
7
6.8
6.6
6.4
6.2
6
5.8
5.6
5.4
pH of soil
acid
water
what is added to the soil
before
after



The impact of acid rain on soil bacteria population density


The correlation between the pH and bacterial population density of soil when acid rain is added
y = 3E+06x - 2E+07
R² = 0.3036
8,000,000
7,000,000
6,000,000
5,000,000
4,000,000
3,000,000
2,000,000
1,000,000
0
-1,000,000
# of colonies in 1 cc of soil
5.6
5.8
6
6.2
6.4
6.6
6.8
7
pH of soil

Conclusion:

Our hypothesis that acid rain decreases the population density of bacteria in the soil on the Roland Park Country School campus was proven correct through the results of our experiment. To conduct this experiment, we first tested the pH levels of soil samples from the back lawn of Roland Park Country School before and after adding sulfuric acid solution (our simulation of acid rain) in order to observe how acid rain impacts the acidity of soil. Before applying the variable of acid rain with a pH of 4 and the negative control of distilled water with a pH of 7, we tested the pH and bacterial population density of the soil.

As the scatter plot shows, the expected correlation between pH levels and the bacterial population density is observed in all of the plots, meaning it is a stable ecosystem, so the other graphs can be trusted when explaining how the hypothesis is correct. Because the R^2 value of the scatter plot is so high, we can assume that the data set that we collected is representative of what is happening normally in the ecosystem.

To test the pH of the soil, we used the LaMotte STH-14 test kit to perform the pH test on all of the soil samples. The three soil samples in the first three plots labeled “acid” averaged a pH of 6.66, and the three soil samples in the other three plots labeled “water” averaged a pH of 6.8. After adding acid to the first plot there was a significant difference in the soil’s pH level than before the variable was applied. With the acid, the three soil samples averaged a pH of 5.93 indicating that the acid rain made the soil more acidic. After adding the negative control of distilled water to the second plot, there was no significant difference in the soil’s pH level than before the negative control was applied. With the distilled water, the three soil samples averaged a pH of 6.73 indicating that the distilled water did not make the soil more or less acidic. Our data shows a change in soil pH levels from adding acid rain, but no change in soil pH levels without adding acid rain. Our graph, “The impact of acid rain on soil pH,” displays the average of the change between the before and after pH of the soil on the acid plots and the water plots. From these results, it is evident that acid rain affects soil on the Roland Park Country School campus by lowering its pH levels. If soil has lower pH levels and therefore is more acidic, based on our background research, it is expected that the population density of bacterial inhabitants would decrease.

To test our theory and prove our hypothesis correct, our group observed the population density of bacteria in the soil before and after adding acid rain and distilled water. To perform serial dilution tests, we diluted the bacteria by a power of either -3 or -4, and chose the plate with at least 5 colonies, if there was one. We let the bacteria grow for 48 hours, and then went back to observe and choose our dilution plates. We did these tests for the “before” and “after” soil samples. The average number of bacteria in 1 cc of soil from our “before water” plots were 2,933,333.33. We found that the average number of bacteria per 1 cc of soil in our “after water” plots were 3,133,333.33. This data showed that the population density increased when water was added to the plots, although not by much. The average number of bacteria per 1 cc of soil from our “before acid” plots were 1,600,000. The average number of bacteria per 1 cc of soil after the tests from the acid plots was 100,000. After the acid rain was added, the average number of bacteria per 1 cc of soil was 16 times less than before the acid was poured on the soil. This shows that the number of bacteria in the soil before and after adding the water to our plots did not significantly decrease, whereas after adding the acid, the number of bacteria in 1 cc of soil decreased by a tenfold. Our graph, “The impact of acid rain on soil bacteria population density,” displays the average of the change between the before and after population density of the soil on the acid plots and the water plots. From these results, it is evident that acid rain affects soil on the Roland Park Country School campus by lowering its bacterial populations as a result of lowered pH levels.

Some future research directions could include researching what type of bacteria are killed when acid rain is poured onto soil, what other types of precipitation are harmful to soil, or how the decrease in bacteria affects the ecosystem in the long term. We might also perform this experiment under different conditions. Because there are two types of acid rain (sulphuric and nitric), we could try the same experiment using nitric acid to see f the results would be any different. Also, the plots that we tested on had a mustard type of leaves and clay-like soil, so we could test on another type of vegetation and soil to see if the results differ in any way, or if they additionally prove our point. These future research directions could lead to other experiments, new tests, and new data that can be used to further prove our results, lead into further discussions on the topic of acid rain and bacterial population density.