Soil Ecology

May 31, 2018

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Background

Soil bacteria are tiny single celled prokaryotic organisms that are the most abundant microbes in the soil, and scientists frequently classify them into four functional groups: Decomposers, Mutualists, Lithotrophs and Pathogens. The first of these, the decomposers, consume carbon compounds such as dead plants and animals and convert them into energy and organic matter that is useful to other organisms in the soil food web. Mutualists meanwhile form partnerships with plants and help them gain access to nutrients to survive, and lithotrophs obtain their energy from nitrogen, sulfur, iron, or hydrogen and play an important role in nitrogen cycling and the degradation of pollutants. Lastly, the pathogenic soil bacteria cause unhealthy formations in plants called galls. (Pelly, 2018).

However, bacteria from all four of these groups perform very important tasks that are related to water dynamics, nutrient cycling, and disease suppression, and the most important of these tasks is the cycling of nutrients, including nitrogen, phosphorus, potassium, and calcium. (Ingham, 2018.) Nitrogen is a key component of chlorophyll in plants, and it improves the quality of leaf crops and stimulates the utilization of other nutrients, such as phosphorus and potassium. Of these, phosphorus is a nutrient necessary for the cellular activities associated with the growth of plants, while potassium helps control how that growth and development occur. (International Plant Nutrition Institute, 1998). Calcium, which raises the pH levels in soil, is important for stimulating root and leaf development. (LaMotte, 1994).

But of all of the nutrients bacteria help cycle, nitrogen is the most significant because it is the one critical element that only certain soil bacteria are capable of making accessible to the rest of life. They do so by transforming the gas form of nitrogen in the atmosphere into a form called ammonium that is usable by plants. This process is called biological fixation, and without it, the nitrogen would remain unavailable to the plants and everything that depends on them. Some ammonium is also produced through decomposition. But even this ammonium was originally created through the nitrogen fixation process, and once present in the soil, all of the ammonium is available to the plants and microbial producers. Any extra ammonium in the soil then goes through the nitrification process where the nitrifying bacteria convert this ammonium into nitrite, and then into nitrate, which is also a form that is usable by plants. Finally, denitrification completes the nitrogen cycle when the bacteria responsible for this process convert nitrate to obtain energy and release nitrogen gas back into the atmosphere, where the cycle repeats. (University of Waikato 2013).

The reason this nitrogen fixation and cycling is so important is because nitrogen is essential for all living things. It is a major part of amino acids, which are the building blocks for proteins, and a major part of nucleotides, the building blocks for nucleic acids. These biological molecules make up organelles, which interact to make and break chemical bonds using energy to create new substances. The substances are directly involved in the execution of the four tasks of the cell: regulation, reproduction, respiration, and synthesis. Hence, without these molecules, life itself is not possible, and therefore without the access to the usable nitrogen which the bacteria provide the other organisms to make these molecules, the entire ecosystem will collapse.

Because of the significance of this ecological role soil bacteria play, anything that can impact their health will impact the entire ecosystem's health as well. Factors that can have this impact are temperature, pH, dissolved gases, osmotic pressure, and water availability, and any changes in these factors can cause changes in the bacteria's density in the soil. Sometimes these changes can kill the bacteria in the soil environment, but most soil bacteria are usually extremely tough and are able to withstand these changes in the soil (Ingham, 2018).

Bacteria, though, like all living things, are extremely sensitive to the hydrogen ion concentration of their environment, and most soil bacteria grow best around neutral pH values (6.5 - 7.0). Some, called acidophiles, do prosper in very acidic conditions and can tolerate a pH of as low as 1.0. Some can even produce acid as they grow, expelling it and lowering the pH of the surrounding environment. (Blamire, 2000). But most soil bacteria need a more neutral pH to thrive.

One thing that can heavily influence pH levels in the soil is acid rain. Acid rain is any form of precipitation that is unusually acidic, and it occurs when gases emitted into the air, such as nitrogen oxides (NOx) and sulfur dioxide (SO2), react with the tiny droplets of water, oxygen, and oxidants high in the clouds, forming solutions of nitric and sulphuric acids. Sunlight helps increase the rate of most of these reactions, and while some of these chemicals come from natural resources, most acid rain is the result of air pollution, specifically the burning of fossil fuels such as coal (Young People Trust for the Environment, 2018).

The reason bacteria need to live in such a narrow range of pH is because their cellular enzymes have ideal pH conditions which give those enzymes their most active shape, and the optimal level of acidity for the vast majority of enzymes falls in the 6-8 pH range. If the pH level of an organism's environment is in its ideal zone, the enzymes in it are likely to be at their most productive shape. When the pH level is out of this range, the enzymes will be the wrong shape to connect with the substrates and will be unable to do their job controlling the chemical reactions of a cell. (Academic Brooklyn, 2018). Therefore, if the environment of soil bacteria is too acidic, its enzymes will stop working, causing the chemical reactions within them to stop, causing the bacteria to die. And, as discussed earlier, if the bacteria die, the ecosystem dies.

In addition to harming bacteria, acid rain can cause damage to an ecosystem in other ways as well. When acid rain falls on the ground, its hydrogen ions can react with the nutrients and helpful minerals in the soil such as potassium, phosphorus, magnesium, and calcium, causing them to wash away, This and this harms the trees and other plants that use these mineral nutrients to grow. Further, the change in acidity caused by this type of precipitation can also cause the release of toxic minerals that are actually harmful to plants such as aluminum (Georgia State University 2018).

Because of this potential relationship between acid rain and bacteria we decided to test if acid rain actually does decrease the amount of bacteria in the soil. There were six plots in an area away from the overwhelming presence of car exhaust, and we added acid to half of them and water to the other half to see what would happen to the bacteria levels. We are hypothesizing that acid rain will decrease the density of bacteria in the soil.

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Soil Ecology Lab

I. Problem: Does acid rain change the density of bacteria in the soil?

II. Hypothesis: Acid rain decreases the density of bacteria in the soil.

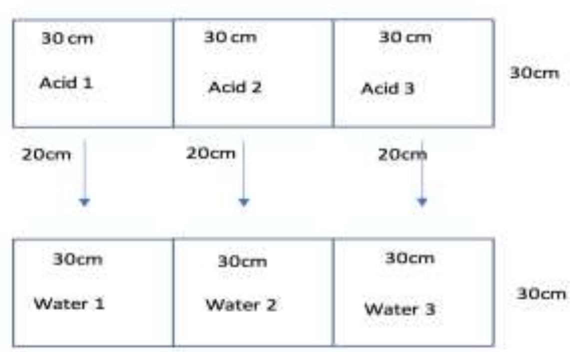
III. Problem

A. Independent Variable: sulfuric acid with a pH of 4 applied to soil plots
B. Dependent Variable: Density of soil bacteria (#/cc) , The pH of the soil samples
C. Negative Control: Soil plots with distilled water applied to it.
D. Positive Control: Soil samples tested for the bacteria density and pH before anything is added to the soil.

List of controlled variables: the location of the plots, size of the plots, size of soil sample taken, the coordination between the soil and the bag, the type of pipette, how much water is added to the tube, the size of the tube, the labeling of the tubes according to the substances inside, how much soil goes into each tube, how the tube is closed, how the tube is shaken, how much soil/ water mixture is removed, degree to which soil is diluted, from which dilutions the samples are taken from, how much is plated, what kind of nutrient agar is used, how long we let the bacteria colonies grow for, what equation we use for finding bacteria in the soil, type of acid used, pH level of the acid used, how much sulfuric acid is poured onto the acid plot, where on the acid plot the solution is poured, what type of water we pour on the water plot,taking all soil samples at the same time on the same day, where the water is poured on the water plot, how much water is poured, how long we let the solution sit on the plot for, use same kit to test for ph.

E. Step-by-Step:

1. Find a flat plot of land at N 39◦21.411' W076◦38.183' that is not near idling cars.
2. Obtain 24 flags and label 12 with the group name and an "a" indicating the acid plots.
3. Use the other 12 flags with the group name and a "w" indicating the water plots.
4. Place the flags in squares that are 30 cm by 30 cm sharing a side, and the line of water plots should be 20cm away from the line of acid plots (see diagram below).
5. Make sure the soil plots are in a line, and the acid plots are in a line with the flags marking each corner. The plots should be in a configuration that looks like this.



6. On the same day at the same time, complete steps 7-11
7. Place a metal soil core extractor with a diameter of 2 cm into the water 1 plot. Use a mallet to hit the extractor into the soil until it gets to the first marking which is 14 cm.
8. Twist the soil extractor 360 degrees clockwise and pull it out of the soil.
9. Remove the soil for the soil extractor and immediately place it in a plastic bag.
10. Label the bag according to the plot.
11. Repeat steps 7-10 taking three samples of soil from each plot and labeling each bag with the appropriate name.
12. Combine the bags of soil before performing the serial dilution on the same day at the same time so that all of the samples from a single plot are in the same bag.

13. Perform the Serial Dilution process for each bag of soil as seen below (steps A-0) all on the same day at the same time, and on the same day at the same time as the pH testing (Step 14) :

A. Use a clean new 10ml serological pipette to add 10 ml of sterile water to a 15 ml tube. Label the tube “10^{0}” and W1.

B. Use the same pipette to add 9 ml of sterile water to a second 15 ml culture tube. Label the tube “10^{-1}.” and W1.

C. Repeat Step B two more times to two additional 15 ml culture tubes, only label them “10^{-2}”, “10^{-3}” respectively.

D. Place 1 cc of W1 soil sample into the “10^{0}” culture tube.

E. Cap the tube and shake vigorously.

F. Using a different 10 ml serological pipette, remove 1 ml of the soil/ water mixture from the “10^{0}” tube and place into the “10^{-1}” W1 tube.

G. Cap and shake vigorously.

H. Using the same pipette in step F, remove 1 ml of the soil/water mixture from the “10^{-1}” tube and place into the “10^{-2}” W1 tube.

I. Cap and shake vigorously.

J. Using the same pipette in step F, remove 1 ml of the soil/water mixture from the “10^{-2}” tube and place into the “10^{-3}” W1 tube.

K. Cap and shake vigorously.

L. You should now have a total of four culture tubes.

M. Place 100 µl samples from the 3rd and 4th tubes (dilutions 10^{-2} and 10^{-3}) onto their own separate correspondingly labeled, 3M Petrifilm™ (Yeast and Mold Count Plate).

N. Allow to grow for 72 hours.

O. Repeat steps A-O for all 6 soil samples

14. On the same day at the same time test for the pH with the LaMotte STH using the Model STH-14 Outfit.

15. Make 3 liters of Sulfuric acid with a pH of 4

16. On the same day at the same time, pour 1 liter of the acid rain solution into the middle of each Acid Rain plot and pour 1 liter of distilled water into the middle of each Water Plot

18. Leave the solution on the plots for 72 hours.

19. After 72 hours, repeat steps 6-11, but labeling the bags with information indicating that the acid and water has already been poured on the plots.

20. After 72 hours of completing the first serial dilution, analyze the dilutions by counting the number of colonies of bacteria, and using the equation.

21. Record number of bacteria per cc of soil in data table

22. Repeat steps 12-14 doing the dilutions and pH test for the second set of soil samples

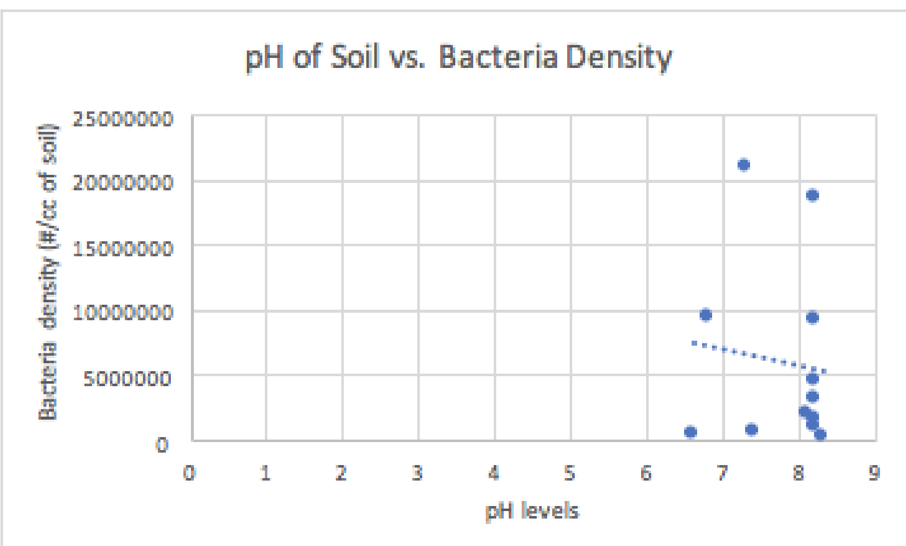
23. After 72 hours of completing the second serial dilutions, analyze the dilutions by counting the number of colonies of bacteria, and using the equation.

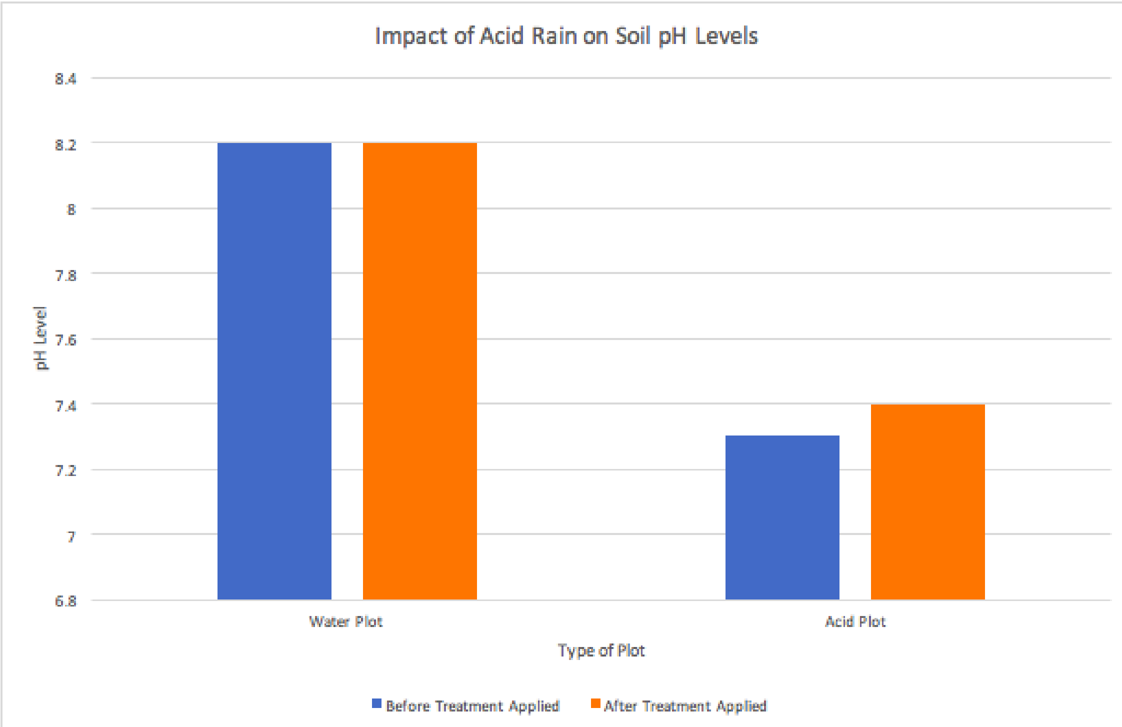
24. Record the number of bacteria per cc of soil in the data table

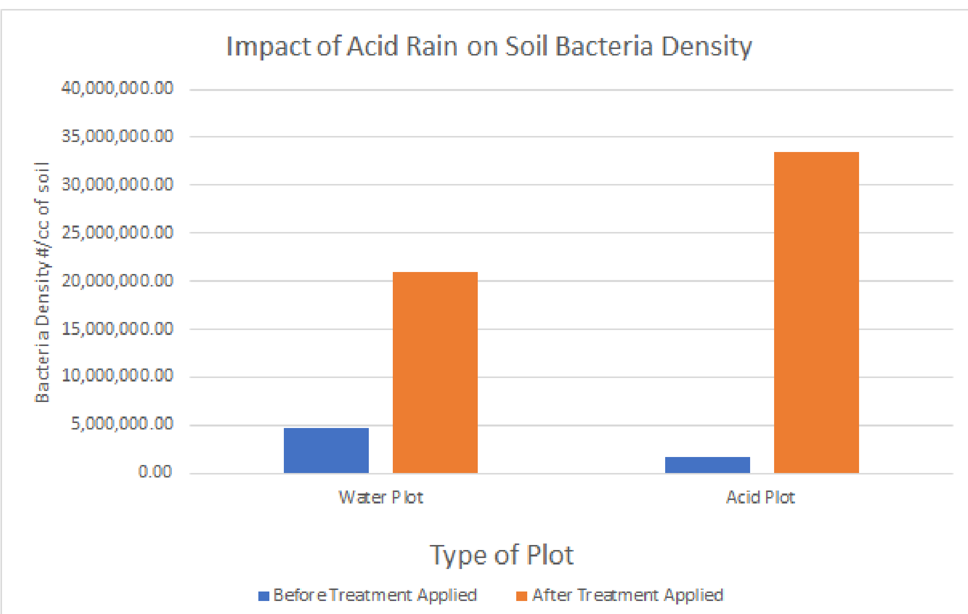
Data Tables and Graphs

Impact of Acid Rain on the Density of Soil Bacteria (# in 1cc of soil)

	Water Treatment Plots				Acid Treatment Plots			
Trials	Bacteria Density Before Treatment Applied	pH level Before Treatment Applied	Bacteria Density After Treatment Applied	pH level After Treatment Applied	Bacteria Density Before Treatment Applied	pH level Before Treatment Applied	Bacteria Density After Treatment Applied	pH level After Treatment Applied
1	1,500,000	8.2	980,000	8.2	700,000	7.4	9,400,000	6.8
2	3,200,000	8.2	18,500,000	8.2	320,000	6.6	21,000,000	7.3
3	170,000	8.3	4,500,000	8.2	1,900,000	8.1	9,200,000	8.2
Averages	4,756,666.6	8.2	20,980,000	8.2	1,653,333.3	7.3	33,466,666.7	7.4




Impact of Acid Rain on Soil pH Levels
8.4
8.2
8
7.8
7.6
7.4
7.2
7
6.8
pH Level
Water Plot
Acid Plot
Type of Plot
Before Treatment Applied
After Treatment Applied


Impact of Acid Rain on Soil Bacteria Density
40,000,000.00
35,000,000.00
30,000,000.00
25,000,000.00
20,000,000.00
15,000,000.00
10,000,000.00
5,000,000.00
0.00
Bacteria Density #/cc of soil
Water Plot
Acid Plot
Type of Plot
Before Treatment Applied
After Treatment Applied

Conclusion

In conclusion, our hypothesis was not correct. We said, "Acid rain decreases the density of bacteria in the soil", however, we found that pouring sulfuric acid on our plots increased the density of bacteria in the soil. Pouring sulfuric acid on our soil plots also decreased the pH of the soil compared to our negative control. Our average number of bacteria per one cc of soil before adding our negative control, water, to the soil plots was 4756666.6. Our average number of bacteria per one cc of soil for after adding the negative control to the soil plots was 20980000. Our average number of bacteria per one cc of soil for before adding sulfuric acid to the acid plots was 1653333.3 Our average number of bacteria per one cc of soil for after adding sulfuric acid to the acid plots was 33466666.7. This shows that after pouring sulfuric acid on our acid plots, the number of bacteria in the soil increased. Additionally, the average pH of the soil before the water was poured on the negative control plot was 8.2. After pouring water on the negative control plots it was still 8.2. On the acid plot, the average pH of the soil before the acid was poured on, was 7.3. After pouring acid on the acid plot, the average pH of the soil was 7.4. The water plot had a pH level of 8.2, and we expected those plots to see a low level of bacteria. But, when we added the acid to the environment as a whole, the acid actually helped the soil, which the bacteria liked, causing the pH level to go down in the alkaline soil. After adding the acid, it created a more ideal environment, giving more bacteria the ability to survive. After looking at the plots outside, some of the acid, rolled into the water plot, affecting how the solution flowed. As a result, both plots had a more normal pH level than before the treatment was applied allowing the bacteria to survive. A way to test this further, would be to test the pH level of the road runoff. We would then, pour it on the soil plots to observe what it does, and how it affects the bacteria in the soil. If the road runoff has a high pH level, it would explain why there were so many dead plants around all of the soil plots. It would also explain the increase of bacteria in the soil after adding acid to the soil plots.