The Soil Ecology Project

"How Acid Rain Disrupts The Nitrogen Cycle"

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Biology

Brock

Background Information

Bacteria are single-celled prokaryotes that are the most abundant life on earth, and they play critical roles in everything from decomposing dead organisms to breaking down food in the human body. Traditionally, they are classified into three main types by their shapes: coccus (sphere-shaped), bacillus (rod-shaped), and spirochete (spiral-shaped) (Better Health Channel, 2014), and they can reproduce rapidly. So in a teaspoon of soil, there are typically between one hundred million and one billion bacteria (Ingham, E. R., n.d.).

There are many types of soil bacteria, and they are traditionally grouped according to the four main functions they do: decomposers, mutualists, pathogens, and chemoautotrophs. The first, the decomposers, break down carbon that can be found in the roots of plants or in dead animals and plants, and they prevent the soil from losing valuable nutrients such as nitrogen. The second group, the mutualists, work directly with plants to ensure they get the proper amount of nutrients, and among them, the nitrogen-fixing bacteria are perhaps the best example. The third group of bacteria, the pathogens, harm plants and can even kill them, and the fourth group, the chemoautotrophs, use compounds such as sulfur, nitrogen, and phosphorus instead of carbon to get energy, often helping to prevent the negative effects of soil pollutants.

But it is perhaps the mutualists involved in the nitrogen cycle that play the most critical role in the soil ecosystem, and these bacteria are subdivided even further into four major groups: the nitrogen-fixing bacteria, the nitrifying bacteria, the denitrifying bacteria, and the actinomycetes (Ingham, n.d.). Together, these 4 groups do four things: nitrogen fixation,

nitrification, ammonification, and denitrification. During the first of these processes, a special type of bacteria called diazotrophs use an enzyme called dinitrogenase to convert nitrogen gas from the atmosphere into ammonia (Thomas, n.d.). This ammonia then reacts with water in the soil to form ammonium, a form plants can absorb through their roots. In addition, when dead plants or animals are decomposed by the actinomycetes bacteria, they turn the nitrogen waste back into ammonia through the process of ammonification so it can be used once again. Following ammonification, nitrification takes the place, with the nitrifiers, nitrosomonas, nitrococcus, and nitrobacter converting ammonium first into nitrite ions and then into nitrates, the other form plants can directly intake as a nutrient (McGraw, n.d) (Solomon,1993). Finally, any excess nitrate is returned to the atmosphere during denitrification in which bacteria convert the nitrates back into nitrogen gas.

The cycling of nitrogen is important to plants because nitrogen is a key component in amino acids and nucleotides which are the monomers for proteins and nucleic acids. Without proteins doing the work in the plant and the nucleic acids telling the cells what to do, the plants would wither away and die. They need these biological molecules to perform the chemical reactions that allow the plants' cells to carry out the four tasks: reproduction, homeostasis, transform of energy, synthesizing new materials. Hence, without nitrogen, an ecosystem would have no plant life, and then there would be no oxygen or food for humans and other animals to use.

Due to the significance of the nitrogen cycle anything that might interrupt it could be catastrophic, and one such thing could be acid rain. Acid rain is any sort of precipitation that has a pH below 7, and while it can be caused by natural sources such as volcanoes, the major way acidic rain is formed is through the burning of fossil fuels. When cars are driven and planes are

flown, nitrogen dioxide and sulfur dioxide are released into the atmosphere, and when either mixes with falling precipitation, it forms acids, which is why despite the misleading title, acid rain does not have to be just rain; it can also be hail, dust, snow, or fog and comes in both wet and dry forms. Furthermore, whether it is wet or dry deposition, the acidic components can still be spread easily, as winds can blow the sulfuric and nitric oxides to many different places. Therefore, it is not only an issue for where the fossil fumes are produced, but for places anywhere downwind as well.

Living things can suffer because of acid rain, and one way is that as the acid rain flows through the soil, it can release aluminum from the clay particles, and this aluminum can lower the pH value level of the soil even further than the precipitation itself. All of which has the potential to severely impact the nitrogen cycle. When the pH is lowered and becomes more acidic, it messes with the optimal pH of the enzymes in the bacteria. Most enzymes work best in a neutral pH, but when acid rain sinks into the soil, the enzymes in the bacteria stop working. But thats mean the chemical reactions in the bacteria will not happen, preventing the bacteria in the soil from completing the four tasks of cells. If the bacteria in the soil die, then the nitrogen cycle will not be able to happen. The nitrogen in the atmosphere will continue to not be processed into a chemical that can be directly absorbed by plants, and since, as we have seen, plant life would not exist without nitrogen, acid rain has the potential to cause the entire ecosystem to collapse (Reece, 2011).

In order to find out more about the acid rain's effect on the nitrogen cycle, we will complete a test to see what affects it may have on the bacteria in the soil here on the RPCS Campus (Effect of Acid Rain, 2017). Therefore for our soil ecology project, our group has chosen to research how acid rain may disrupt the bacteria that may be involved in the nitrogen

cycle. We will test for how the ammonia in the soil is disrupted when the acid rain interacts with the environment, and we will test for the density of bacteria in the soil samples before and after the acid rain is added (Thomas, n.d). We are expecting to find how the acid rain will affect the density of the bacteria and the levels of ammonia and our research has predicted that the density of the bacteria will decrease as well as the ammonia levels lowering.

Lab Report

I. Problem:

A. How does acid rain alter the nitrogen cycle?

II. Hypothesis:

A. The acid rain will reduce the density of bacteria and lower the levels of ammonia in the soil.

III. Procedure:

A. *Independent Variable:* acid rain added to the plots

B. *Dependent Variable:* the density of bacteria in the soil and the levels of ammonia in the soil

C. *Controlled Variables:* amount of plant life, type of plant life, size of plot, distance between each plot, the pH of acid rain, the type of water added to plot, type of water added to test tube during serial dilutions, collect samples on same day at same time to control for impact of weather, the amount water added to plot, the size of soil extractor, the type of acid rain, time of year, pH of water, amount of time before collecting new soil after adding acid rain and water(48 hours), amount of acid rain added to each soil plot, temperature of the water, temperature of the acid rain, amount of sterile water added to test tubes, amount of soil added to test tubes, amount of time you let the bacteria grow for during serial dilution test, always dilute to same level each time, amount plated in agar, same chemical test that used to test for ammonia (LaMotte STH Series of Profession), the type of nutrient agar used.

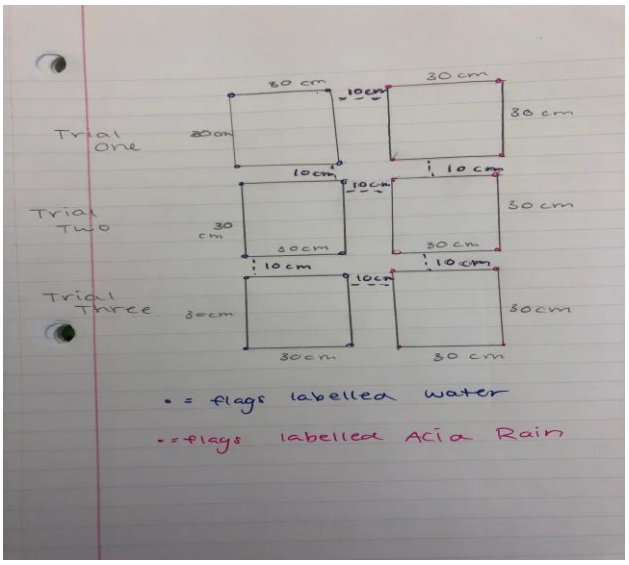
D. *Negative Control:* neutral distilled water added to plots,

E. *Positive Control*: soil samples collected before any acid or water was added

F. Step by Step:

1. Gather twenty four bright yellow mini flags and label them Water Trial and Acid Trial
2. Go to the coordinates (N 39.35664°, W 76.63561°) which is on the Roland Park Campus.
3. See Diagram A for the exact setup of the soil plots.

Diagram A:



4. From each of the soil plots marked by the flags, take three soil samples from each plot using a soil extractor that has a diameter of 2cm and when inserted into the ground the first line on the extractor should be equal to the place where the soil begins (depth of 15cm). Each of the soil samples should be placed into separate bags labelled as followed Before Water Trial 1a, 1b, 1c, Before Acid 1a,1b,1c, etc. for all 6 plots All these soil samples should all be collected at the same time on the same day.
5. After collecting the before samples, add one liter of neutral distilled water water to the three plots that have Water Trial flags surrounding them. The water should be evenly divided between the three plots. This should be taken on the same day and at the same time as the next step.
6. Add one liter of nitric acid which has a pH level of four to the plots labelled Acid Trial. The acid should be evenly divided between the three plots. The nitric acid should be added to the soil at the same time on the same day as the water being added to the soil from the previous step.
7. After both of the liquids have been added wait 48 hrs to take three more soil samples from each plot by using a soil extractor that has a diameter of 2cm and when inserted into the ground the first line on the extractor should be equal to the place where the soil begins (depth of 15cm). Place these soil samples in bags labelled After Acid Plot 1a,1b,1c, After Water Plot 1a,1b.1c.,etc. All these samples should be taken at the same time on the same day.
8. These samples can be stored, but when testing is ready to begin all the samples the three soil samples from within each single plot should be combined. The soil samples from different trials should not be combined.
9. Use the LaMotte STH Series of Profession soil testing to test for Ammonia for the Before Water Plot 1 and the Before Acid Rain Plot 1. Each trial should have its own test. The extraction process for the ammonia experiment should be done the same exact day and time as the serial dilution test. Record the data in the data table.

10. To start the serial dilution test, this should be done on the same day at the same time as the extraction process for the ammonia test and should be done on all before soil samples on the same day at the same time. Use a clean, new transfer pipette to add 10 ml of sterile water to a 15 ml culture tube. Label the tube "10^0" and *Before Water Plot 1*.
11. Use the same pipette to add 9 ml to a second 15 ml culture tube. Label the tube "10^{-1}" *Before Water Plot 1*.
12. Repeat steps 2 three more times to three additional 15 ml culture tubes, only label "10^{-2} "*Before Water Plot 1*,"10^{-3} "*Before Water Plot 1*, and "10^{-4}*Before Water Plot 1*respectively.
13. Place 1cc of your *Before Water Plot 1* soil sample into the "10^0"*Before Water Plot 1*.
14. Cap the tube and shake vigorously.
15. Using a new clean pipette, remove 1 ml of the soil/water mixture from the "10^0"*Before Water Plot 1* tube and place into the "10^{-1}"*Before Water Trial #* tube.
16. Cap the tube and shake vigorously.
17. Using the same pipette in step 15, remove 1 ml of the soil/water mixture from the "10^{-1}*Before Water Plot 1*" tube and place into the "10^{-2} *Before Water Plot 1*"tube.
18. Cap the tube and shake vigorously.
19. Using the same pipette in step 15, remove 1 ml of the soil/water mixture from the "10^{-2} *Before Water Plot 1*"tube and place into the "10^{-3}*Before Water Plot 1*"tube.
20. Cap the tube and shake vigorously.
21. Using the same pipette in step 15 , remove 1 ml of the soil/water mixture from the "10^{-3}*Before Water Plot 1*"tube and place into the "10^{-4}*Before Water Plot 1*"tube.
22. You should now have a total of five culture tubes.
23. Plate 100µl samples of the 4th and 5th tubes (dilutions "10^{-3}*Before Water Plot 1*& "10^{-4}*Before Water Plot 1*") onto their own correspondingly labeled separate, 3M $Petrifilm^{tm}$ Aerobic Count plates.
24. Allow to grow for 48 hours.
25. Examine each of the plates for individual bacteria colonies and choose the plate with the fewest colonies (but at least 5) to make your estimate of the number of the bacteria in the original 1cc soil sample using the formula:
    a) # Microbes in 1 cc soil = # Colonies on ⌊sheet x
    10^2 x $10^{/dilution\ \#\ at\ which\ these\ colonies\ were\ found\ /}$
26. Repeat steps 8 through 26 with *Before Water Trial 2 and 3, Before Acid 1,2,3, at the same time and same day as the Before Water Trial 1. The extraction process*
27. Repeat Steps 8) through 26) with *After Water 1,2,3, and After Acid Rain 1,2,3*

I. Data Table:

The Ammonia level in Each Plot Before and After Liquid Are Added (ppm)

Before and After Liquids Added to Soil Plot

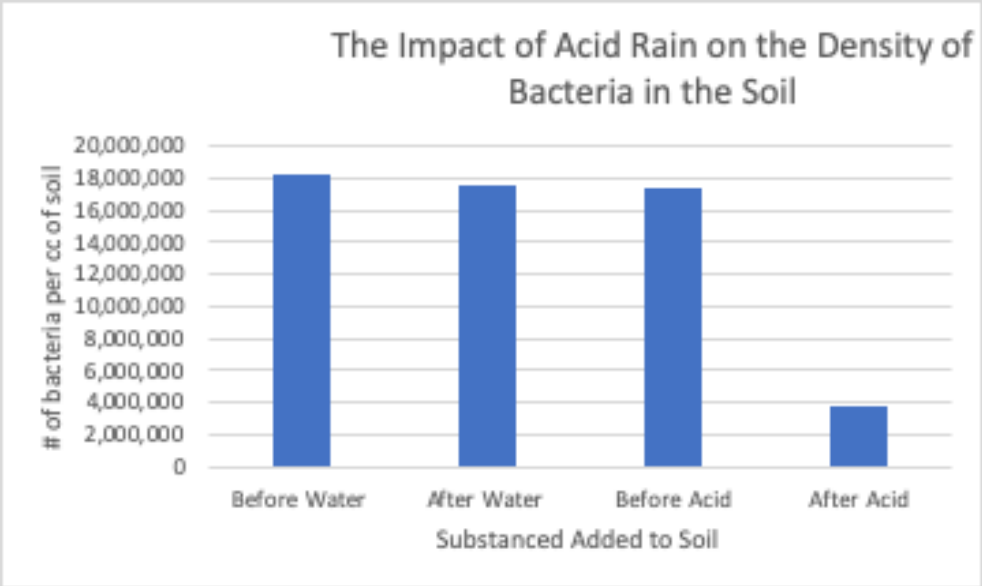
Trials	Before Water	After Water	Before Acid Rain	After Acid Rain
Trial 1	2 ppm	2 ppm	2 ppm	1 ppm
Trial 2	3 ppm	3 ppm	3 ppm	2 ppm
Trail 3	2 ppm	2 ppm	1 ppm	1 pppm
Average	2.333 ppm	2.3333 ppm	2 ppm	1.3333 ppm

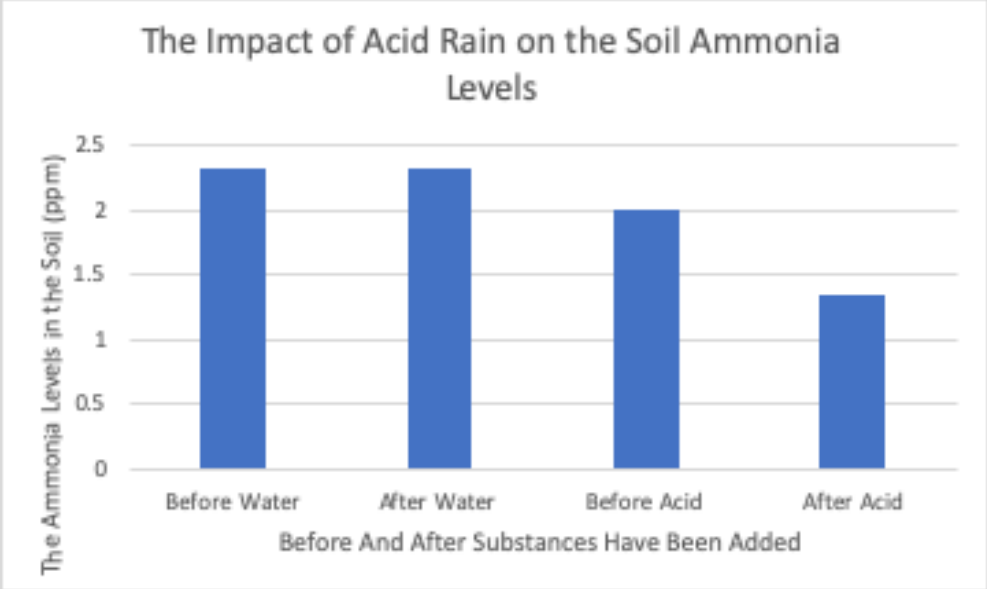
Amount of Bacteria In Each Plot Before and After Liquids Are Added (N)

Before and After Liquids Added

Trials	Before Water	After Water	Before Acid Rain	After Acid Rain
Trial 1	44,000,000 N	1,400,000 N	47,000,000 N	900,000 N
Trial 2	8,000,000 N	1,400,000 N	3,400,000 N	600,000 N
Trial 3	2,900,000 N	51,000,000 N	1,800,000 N	10,000,000 N
Average	18,300,000 N	17,933,333.33N	17,400,000 N	3,833,333.33 N

N= # number of microbes per cubic centimeter of soil





Conclusion

Our hypothesis stated that the acid rain will reduce the density of bacteria and lower the levels of ammonia in the soil. The ammonia levels decreased by 1 ppm when the acid rain was added to the soil and the significance of this is shown by the negative control not changing at all. This change in ppm shows that the ammonia levels were decreased by the acid rain. The bacteria were killed by the pH being lowered so they could not transform the nitrogen in the atmosphere into ammonia. The density of the bacteria was also lowered when the soil came in contact with acid rain. The density of bacteria changes from 17,400,000 bacteria per cubic cc of soil to 3,833,333.33 bacteria per cc of soil. This difference of 13,466,666.67 N is very significant because it is a big contrast between the negative control difference which is 366,666.67 N. This shows that the acid rain did negatively affect the density of bacteria.

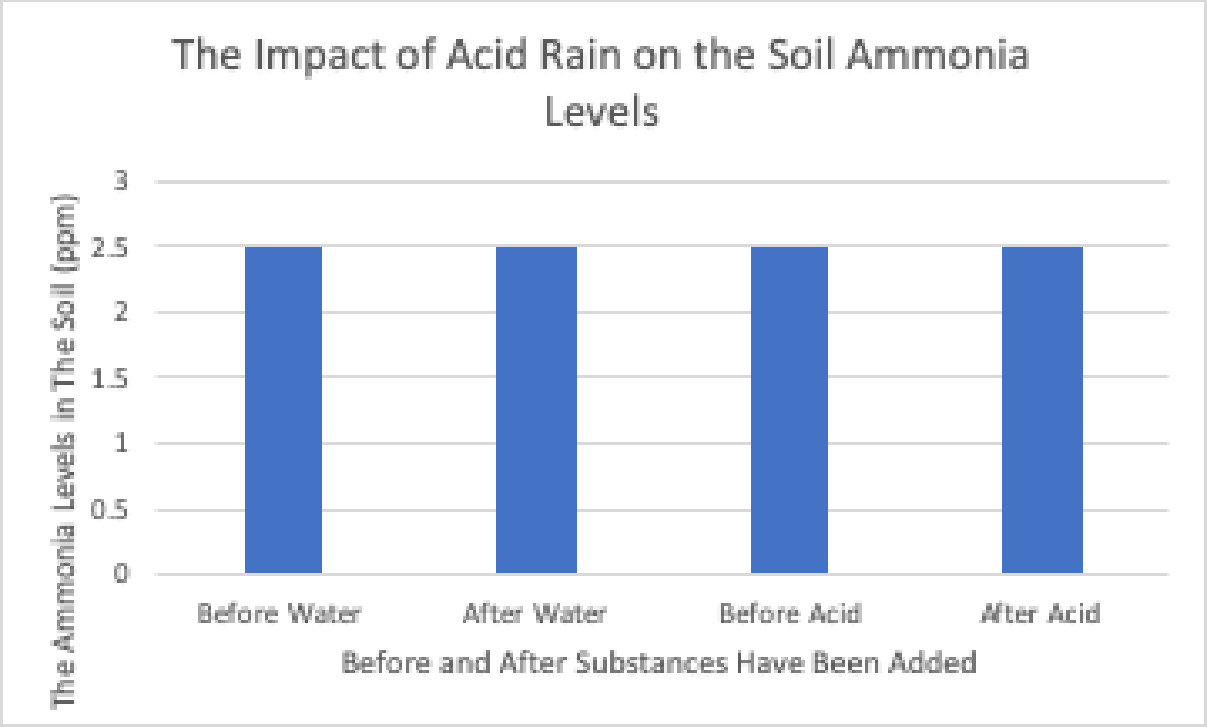
However our data may prove that our hypothesis is correct, an error occurred when we were recording the data causing us to have incorrect data. The error was the we chose numbers in between one and five, but we cannot do that because they do not exist on the ammonia level

scale. The only number that exist between the zero and five on the ammonia scale is two and a half so if our data table and graph was correct then they would look like the following:

The Ammonia level in Each Plot Before and After Liquid Are Added (ppm)

Before and After Liquids Added to Soil Plot

Trials	Before Water	After Water	Before Acid Rain	After Acid Rain
Trial 1	2.5 ppm	2.5 ppm	2.5 ppm	2.5 ppm
Trial 2	2.5 ppm	2.5 ppm	2.5 ppm	2.5 ppm
Trail 3	2.5 ppm	2.5 ppm	2.5 ppm	2.5 pppm
Average	2.5 ppm	2.5 ppm	2.5 ppm	2.5 ppm



Due to this error our hypothesis is incorrect since even though there was a slight color change the levels of ammonia did not change. It is clear that our hypothesis was incorrect and we should test for nitrate which is involved in the nitrification process because since the density of bacteria did decrease we know it did impact one of the stages of the nitrogen cycle. A possible

explanation for the density of bacteria decreasing and the ammonia levels still staying the same is that protozoa eats bacteria so when they eat bacteria they release nitrogen. This is a possible reason for our data, but this not a fact (Protozoa, n.d).

A way a future scientist could continue to research this topic is to test for the density of bacteria and the density of protozoa as well as testing for the levels of ammonia while doing the same experiment that we previously did. This future testing would be necessary to see if our explanation for why the bacteria levels decreased, but the ammonia levels did not. For another way to do future research, a scientist should repeat our experiment, but instead of using nitric acid like we did in our experiment use sulfuric acid. This will important to further research because it should be tested if it is the acidity that affects the nitrogen cycle or if it is the nitrogen oxide. This test would be necessary for further testing because we should know if both types of acid rain have an equally harmful impact on the environment or if one is more detrimental to the environment. Another way to do further research is to change the amount of plant life and the type of plant life in the experiment. In our experiment we had weeds from the RPCS background, but someone could redo this experiment and use grass. This would be necessary for further research because some plants do not have the bacteria involved in the nitrogen cycle in their roots. In conclusion this Soil Ecology Project we have learned valuable information about how acid rain disrupts the nitrogen cycle.

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