The Effect of Fertilizer on pH and the Density of Bacteria within Soil

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1 June 2017

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Biology

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Background

Bacteria are single-celled prokaryotes that inhabit every ecosystem on the planet, and those found in the soil are often found inside the roots of plants or right next to them. They all form microaggregates by binding soil particles together with their secretions of glycoproteins, and these microaggregates act as the building blocks for improving soil structure (Hoorman 2017) by improving water infiltration. This allows for a higher water holding capacity for the soil, increasing the decomposition of organic soil matter (Johnson and White 2017), and in fact, bacteria can alter the soil's environment so much through this process that the soil environment will favor certain plant communities over others (Hoorman 2017).

Among those bacteria that live in the soil, there are four main types: decomposers, lithotrophs, mutualists, and pathogens; each helping the ecosystem within which they live. The decomposers, for example, help create nutrients for plants by processing dead organisms and releasing their carbon, nitrogen, and phosphorus back into the soil for the plants to absorb, and by making nitrogen in particular accessible to plant life, decomposers help the nitrogen cycle continue working properly (Sundareshwar 2017). Similarly, lithotrophs aid in nitrogen cycling in plants (Hoorman 2017) by converting gaseous nitrogen from the atmosphere into nitrates which plants can use as well. The mutualists interact with a variety of host plants, but in all cases, the relationship is symbiotic and both the plant and bacteria benefit (Wall and Moore 1999). Furthermore, while the pathogenic bacteria in soil cause diseases in plants and other organisms

living there, they help control the population sizes of the soil's many inhabitants to maintain proper ecological balance there (Hoorman 2017).

Of these four main types of bacteria, though, the ones involved in the nitrogen cycle are among the most significant in terms of the overall health of an ecosystem. The nitrogen cycle is so immensely important due to the fact that nitrogen is a critical element in the proteins and nucleic acids of all living things. Proteins control the chemical reactions that occur between the biological molecules of the cell and nucleic acids control the production of proteins. Therefore, without nitrogen, plants could not manufacture proteins, chemical reactions could not happen; and the four tasks of a plant's cells could not occur. Thus, without nitrogen, cells could not function, and the plant would die. But without plants, the rest of an ecosystem will not be able to survive as other organisms get their biological molecules either directly (Primary consumers) or indirectly (Secondary consumers) from plants. Hence, without the nitrogen cycle moving this element through the atmosphere, hydrosphere, biosphere, and lithosphere, life itself would not be possible.

However, almost all nitrogen on earth is unavailable as inert nitrogen gas in the atmosphere, and therefore it must be converted to forms that living things- particularly plants- can use. The organisms in the soil that do this are the bacteria. Nitrogen fixing bacteria include symbiotic bacteria in legume's root nodules, and these and other soil bacteria work to convert nitrogen gas from the air into ammonium in the soil, a form which plants can then absorb. Once this has occurred, other bacteria convert any remaining ammonium into nitrate in a process known as nitrification. Nitrification works when types of certain bacteria first create nitrite, then turn this nitrite into nitrate (the other form of nitrogen plants can consume). Any remaining

excess fixed nitrogen then goes through denitrification where microscopic bacteria and fungi convert nitrates back into nitrogen gas that is released into the atmosphere (Thomas N.D.).

Because plants cannot absorb nitrogen that has not been fixed, without all these different groups of bacteria performing their duties throughout the nitrogen cycle, plants would not be able to absorb nitrogen, and nothing else in the food chain would be able to make their biological molecules. Hence, without bacteria's involvement in the nitrogen cycle, the entire ecosystem would collapse.

However, during the nitrogen cycle, the compounds this process creates can alter the acidity of the soil. As nitrogen gas goes into the ground and nitrogen fixation occurs, the ammonium that results can make pH levels go up, while the later nitrification that generates nitrate makes the pH levels go down. Since normally the ammonium causing the pH to go up is cancelled by the nitrate causing the pH to go down, a healthy nitrogen cycle helps maintain the balance of the soil's pH. But if something is introduced into the soil that can upset that balance, then the soil's pH could be thrown off, and although bacteria are found almost everywhere, most bacteria grow best around these neutral pH values. Therefore, anything introduced into the soil that can alter its pH might harm bacterial growth (Blamire 2000), negatively impacting the health of the rest of an ecosystem (Kundi 2010).

The reason the soil pH can have such a dramatic impact on bacteria and everything else living there is because pH affects the shape, function, and activity of enzymes. While different enzymes have different optimum pH levels, when there is an extremely high or low pH in the soil, there can be a complete loss of enzyme function (Freiden 1969), and since enzymes control the chemical reactions of cells, a change in soil pH could prevent bacteria from performing the four tasks of a living thing, causing them to die.

Yet, as mentioned earlier, when the amount of nitrogen in soil is changed, soil pH will change and therefore any additional nitrogen added to the soil may change the density of bacteria living there as well. One such source of additional nitrogen which humans frequently employ is commercial fertilizer. Fertilizers work by providing chemical elements that are needed for plants to develop and grow more rapidly and one of their main ingredients is nitrogen (Savonen 2008). The addition of this extra nitrate and ammonium has the potential to disrupt the soil's pH balance and therefore, the extra amounts of nitrate and ammonium applied to the soil through the application of fertilizer may disrupt the nitrogen cycle, with all the consequences that would have on the overall environment.

Thus, for our soil ecology project, we wanted to find out how heavy amounts of nitrogen in fertilizer would affect the pH level and the density of bacteria when applied to a certain plot of soil. To determine this, we tested the nitrogen heavy fertilizer on one plot and just water in another to see how each affected the pH levels and the amount of bacteria living in the soil. We hypothesized that the nitrogen heavy fertilizer will increase the level of soil pH and decrease the bacteria living in the soil.

Soil Ecology Lab Report

I. Problem: Do nitrogen fertilizers increase or decrease the levels of soil pH and the density of bacteria living in the soil?

II. Hypothesis: Nitrogen fertilizers will increase the level of soil pH and decrease the density of bacteria living in the soil.

III. Procedure:

A. Independent Variable: The application of nitrogen fertilizer to the soil.

B. Dependent Variable: The pH levels and density of bacteria (#/cm^3) in the soil.

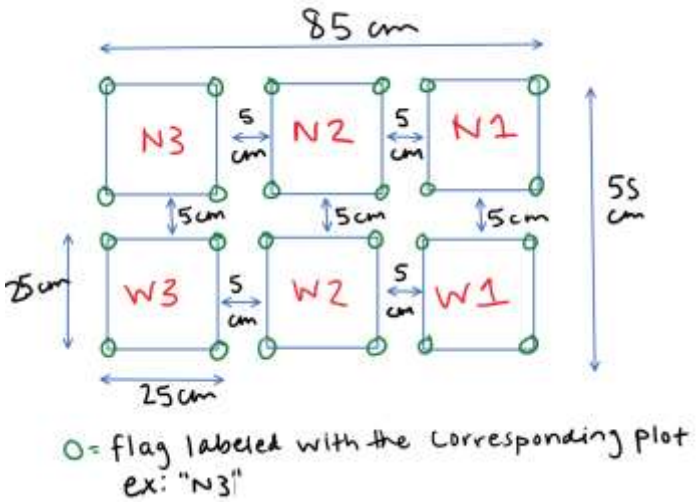
C. Negative Control: The soil with only water and not the nitrogen fertilizer added to it.

D. Positive Control: Testing for the pH level and density of bacteria in the soil before adding fertilizer or water to it.

E. Controlled variables: The type of fertilizer, type of soil, amount of water added, location of soil, type of water added to soil, taking soil samples from the same location at the same time, size of soil sample extracted, size of plot, distance in between each plot, tool used to extract soil, amount of fertilizer added to soil, time waited between collecting data after adding independent variable, amount of nitrogen in the fertilizer, control of plant life by placing plots in same place, use of same chemical test kit for pH test, use of serial dilution for bacteria, size of culture tube, amount of sterile water added to each culture tube, amount of soil used in dilutions, amount of soil mixture added from one tube to another, size of soil sample used when testing pH and bacteria density, equation used to estimate the density of bacteria in the original 1 cc soil sample, degree of dilution, which dilutions are plated, type of nutrient agar on petri plates, amount of soil/water mixture plated on petri plates, how long the bacteria are allowed to grow for, and the temperature grown at.

F. Step by step:

1. Go to “N 39.35814, W076.63618” located on the RPCS campus, and measure out six plots using the diagram below. Mark the corners of each plot with plastic, orange flags with its corresponding plot. (ex; “N 1”)



2. Complete steps 2-5 simultaneously at the same time on the same day. Collect three separate soil samples that are 15.2 cm deep and 2.25 cm in diameter each from each of the "N" plots, using a oakfield apparatus soil core extractor. Clean the extractor with a damp paper towel in between collecting each sample.
3. Place each of the soil samples into their own separate plastic bag that is labeled with its corresponding plot. For example, "N 1", "N 2", and "N 3". You will have 9 plastic bags from the "N" plots, three "N 1" bags, three "N 2" bags, and three "N 3" bags.
4. In each "W" plot, collect three separate 15.2 cm deep and 2.25 cm in diameter soil samples. Collect these using the same oakfield apparatus soil core extractor, making sure to clean it with a damp paper towel between each soil sample.
5. Place each of the soil samples into their own separate plastic bag that is labeled with its corresponding plot (ex: "W 1"). You will have 9 plastic bags from the "W" plots, three "W 1" bags, three "W 2" bags, and three "W 3" bags.
6. Complete steps 6-22 simultaneously at the same time on the same day. Mush all three soil samples with the same label for all "N" samples and "W" samples. To mush the soil samples, grind them with clean hands until the samples have the texture of wet sand.

7. For all six types of soil, test for pH levels at the same time, and add the collected data in data table. To test for pH levels in soil, use the LaMotte STH-14 Outfit soil test kit.
8. Test for the density of bacteria in all six soil samples, and add the data into the data table. To test for the density of bacteria, use the following instructions in steps 9-22.
9. Using a clean, new transfer pipette, add 10 ml of sterile water to a 15 ml culture tube making sure to label the tube "N 1 10^{0}".
10. Using the same pipette, add 9 ml of sterile water to a second 15 ml culture tube. Label the tube "N 2 10^{-1}" and with the plot the soil that will be added came from.
11. Repeat step 10 three more times to three additional 25 ml culture tubes, only label them "N 1 10^{-2}", "N 1 10^{-3}", and "N 1 10^{-4}" and with their plot the soil came from.
12. Place 1 cc of your soil sample into the "10^{0}" culture tube.
13. Cap the tube and shake vigorously.
14. Using a new, clean pipette, remove 1 ml of the soil/water mixture from the "10^{0}" tube and place into the "N 1 10^{-1}" tube.
15. Cap and shake vigorously.
16. Using the same pipette in step 13, remove 1 ml of the soil/water mixture from the "10^{-1}" tube and place into the "N 1 10^{-2}" tube.
17. Cap and shake vigorously.
18. Using the same pipette in step 13, remove 1 ml of the soil/water mixture from the "10^{-2}" tube and place into the "N 1 10^{-3}" tube.
19. Cap and shake vigorously.
20. You should now have a total of four culture tubes.

21. Place 100 µl samples from the 2nd, 3rd, and 4th tubes (dilutions N 1 10^{-1},N 1 10^{-2}, and N 1 10^{-3}) onto their own separate, labeled petri plates containing nutrient agar.
22. Repeat steps 8-21 with each of the remaining five soil samples, replacing the "N 1" with the soil sample's corresponding plot.
23. Allow to grow for 72 hours.
24. After 72 hours, examine each of the plates for individual bacteria colonies and choose the plate with the fewest colonies at the lowest dilution level (but at least 5) to make your estimates of the density of bacteria in the original 1 cc soil sample using the following formula:

Microbes in 1 cc of soil = # Colonies on sheet x 10^2 x 10 |dilution #at which these colonies were found|

25. After testing for pH and the density of bacteria in the soil, return to "N 39.35814, W076.63618" location with the plots labeled "N1", "N2", "N3", "W1", "W2", "W3".
26. Complete steps 26-27 simultaneously at the same time on the same day. Scatter 0.9764 grams of Sta-Green lawn fertilizer 29-2-5 into each of the three plots, "N 1", "N 2", and "N 3" plots. Using a beaker, and all at the same time, pour 0.5 liters of tap water into each of the three plots, "N 1", "N 2", and "N 3" plots. During that same time period, all at the same time, pour 0.5 liters of tap water into each of the three "W 1", "W 2", and "W 3" plots.
27. Without changing the soil, wait 72 hours.
28. Complete steps 28-30 simultaneously at the same time on the same day. After waiting 72 hours, repeat step 2-7 with the same "N" and "W" plots at the same time.
29. Repeat step 7 to test pH level in all 6 soil samples. Add data in data table.

30. Repeat step 9-22 to test the density of bacteria in each of the six soil samples. Add data in data table.
31. Repeat steps 23 and 24 in each of the six soil samples. Add data to data table.

IV. Data and Analysis:

A. Data Table

Impact of Nitrogen Heavy Fertilizer on pH levels and Bacteria Density in Soil

Trial #	pH of the plot before the fertilizer treatment	pH of the plot after fertilizer treatment	Bacteria density (number per cc in soil) in plot before adding fertilizer and water	Bacteria density (number per cc in soil) in plot after adding fertilizer and water	pH level in plot before water treatment	pH level in plot after water treatment	Bacteria density (number per cc in soil) in plot before adding water	Bacteria density (number per cc in soil) in plot after adding water
1	7.1	6.3	700000	270000	6.6	6.4	18100000	9400000
2	7.3	6.9	2700000	160000	6.3	6.4	700000	7300000
3	7.1	6.5	100000	2800000	5.7	5.8	2200000	8800000
Average	7.167	6.567	1166667	1076667	6.2	6.2	7000000	8500000

B. Graph

Figure 1:

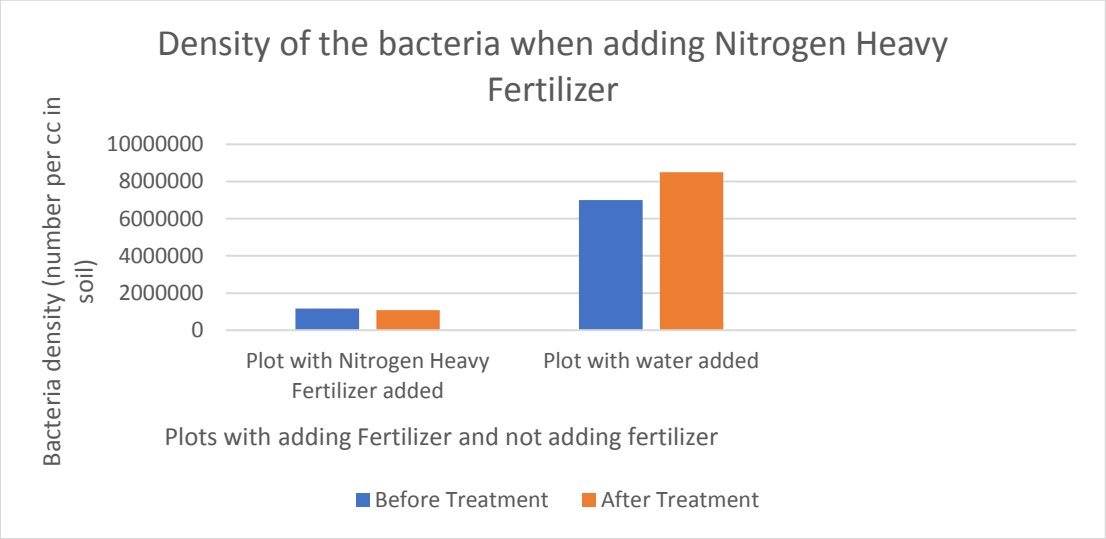


Figure 2:

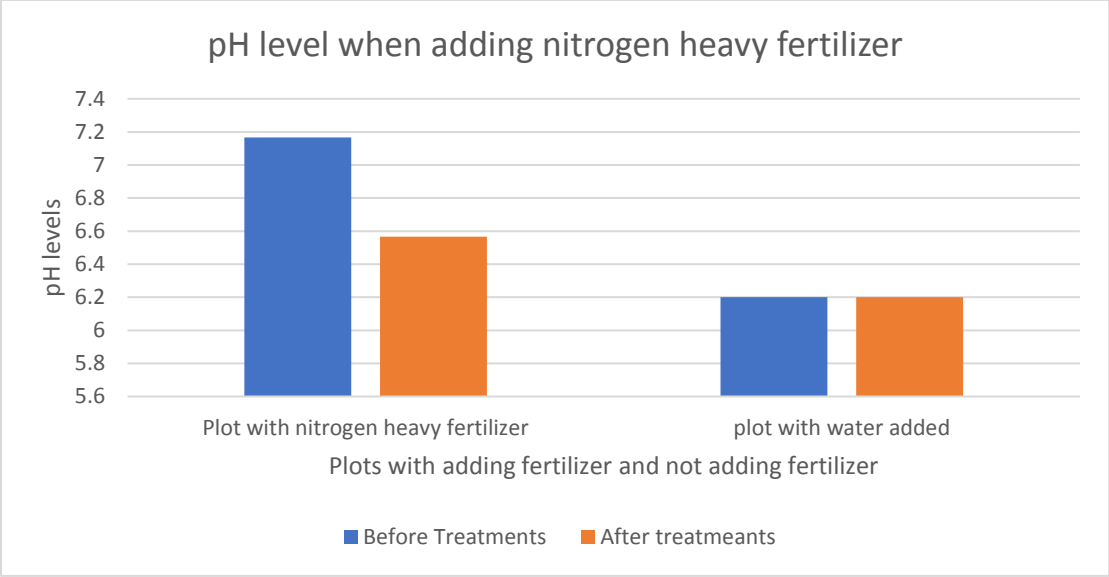
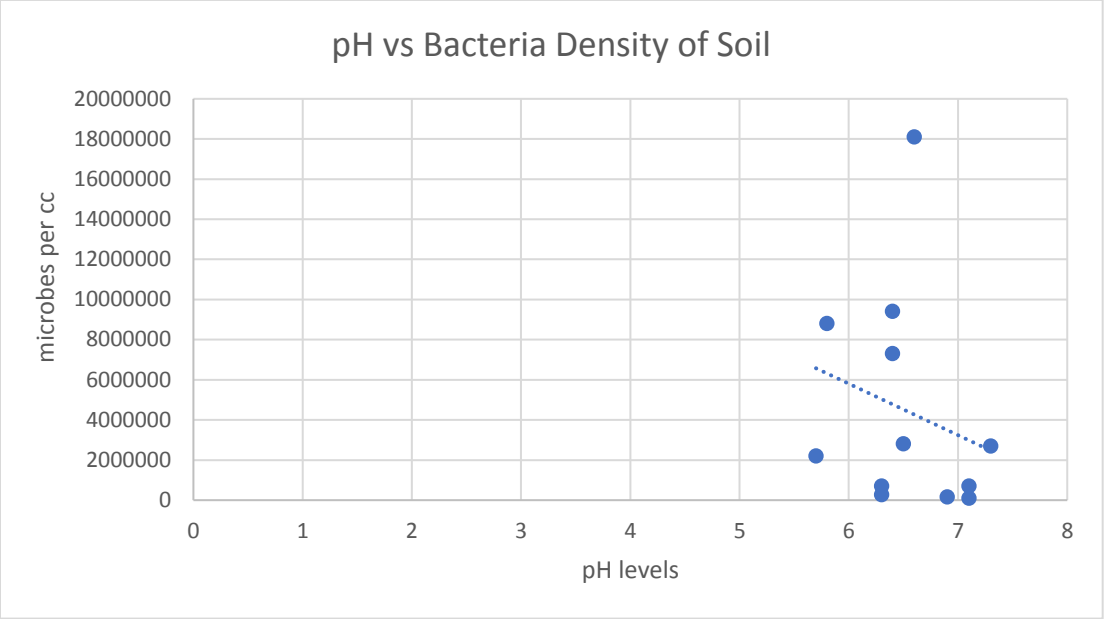


Figure 3:



V. Conclusion

Our hypothesis proved to be incorrect because we predicted that nitrogen fertilizers increase the level of soil pH and decrease the density of bacteria living in the soil. In this experiment, we tested to see if adding nitrogen heavy fertilizer to one plot of soil and water to another plot within five centimeters of each other would affect the pH of the soil and therefore change the density of bacteria. In the plot where nitrogen fertilizer was added, when comparing the data found from the positive control where there was no added fertilizer and the data found in the independent variable where the fertilizer was added, on average, the pH dropped 0.6, from 7.167 to 6.567, and the density of the bacteria dropped 90,000/cm^3, from 1,166,666.667/cm^3 to 1,076,666.667/cm^3. Within the negative control plots, where only water was added, when comparing the data found between the positive control where there was no added water to the negative control where half a liter was added to the plot, on average, the pH remained at 6.2 and the density of the bacteria grew 1,500,000/cm^3, from 7,000,000/cm^3 to 8,500,000/cm^3. This data

disproves our hypothesis because we predicted that the fertilizer would cause the pH to increase and therefore cause the density of the bacteria to decrease; however, the pH decreased and when this happened, the bacteria decreased. Based on the scatter plot seen above (figure 3), the normal relationship between pH and density of bacteria was observed in all of our plots and therefore, we can be confident that normal bacteria activity was occurring. Because of this, the density of the bacteria should have increased, when the pH decreased, but this did not occur; potentially due to the fertilizer applied to the soil. The fertilizer affected the pH level in the soil by causing it to decrease. This occurred because there was 27% more nitrate than ammonium in the fertilizer, nitrate causing pH to go down. During our experiment, although we hypothesized that the pH would increase when the nitrogen fertilizer was added, we realized that our hypothesis may turn out to be incorrect. We realized this may occur because we learned that ammonium causes pH levels to go up and nitrate causes pH levels to go down, and within the fertilizer used in our experiment, 28.2% of the nitrogen is nitrate and 0.8% of the nitrogen is ammonium. Because there is such a large difference between the two, and there is 27.2% more nitrate than ammonium, the pH would potentially be lower, or more acidic. Although the pH decreased, it was still within normal pH levels, meaning the chemicals in the fertilizer caused the density to decrease. Because there was 27% more nitrate nitrogen in the fertilizer, and the density of the bacteria decreased, by adding the fertilizer, the bacteria were living in their own waste instead of using the nitrogen as food. Because of this, they died, causing the density to decrease.

In the future, our group wants to research which of the two nitrogen products, ammonium or nitrate, caused the bacteria within our experiment to die because they were living within their own waste. Because the fertilizer caused the bacteria density to decrease and act against normal bacteria activity, and because there was a presence of both ammonium and nitrate, we need to

determine an experiment to test which of the products cause bacteria to die. The experiment would be similar to ours except, one plot would have a fertilizer with a significant amount more of nitrate than ammonium and the other plot would have a fertilizer added with a significant amount more of ammonium than nitrate. After collecting all the data, we would then compare how the fertilizer affected the bacteria density to see whether the bacteria density increased or decreased, determining which type of nitrogen causes the bacteria to die. In the future, we could also test to see if adding fertilizer to soil changes the amount of nitrate and ammonium in the soil. To do this, we would have a negative control that has a plot with water added to it and a plot where fertilizer was added. We would then perform an experiment similar to ours except we would test the amounts of nitrate and ammonium in the soil before and after adding the fertilizer in the plots, find the data, and compare how the fertilizer versus the negative control plot affected the nitrate and ammonium amounts within the soil, helping us determine which type of nitrogen was more affected.

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