The Effects of Nitrogen-based Fertilizer on the Density of Bacteria in the Different Layers of Soil

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The Effects of Fertilizer on Soil and its Micro-organisms

Soil consists of many living organisms, including microorganisms such as bacteria, fungi, and protozoa. In fact, the soils of the entire planet contain 8 to 15 billion tons of bacteria, fungi, protozoa, nematodes, earthworms, and arthropods (Ohio State University, 2010). But the most prevalent of all the microbes are the bacteria, and in as little as a teaspoon of productive soil, there are usually between 100 million to 1 billion of them.

Traditionally, bacteria have been classified into four functional groups: the decomposers, the mutualists, the lithotrophs, and the pathogens. Most soil bacteria fall under the category of decomposers and consume simple carbon compounds and convert energy from the organic matter in the soil into forms that are useful to other soil organisms. The mutualists, meanwhile, work with the plants to aid in the process of cycling key nutrients (e.g. the nitrogen-fixing bacteria), and the lithotrophs, or chemoautotrophs, acquire their energy from compounds of nitrogen, sulfur, iron, or hydrogen instead of from carbon compounds, aiding the nitrogen cycle and the degradation of pesticides and other pollutants. Finally, the pathogens cause many of the different types of plant illnesses as well as attacking a variety of soil invertebrates (Ingham, n.d.).

Of the different categories of bacteria, conceivably the most significant are those involved in the cycling of nitrogen. Plants need nitrogen from the soil to grow because they are not able to use the gaseous nitrogen found in the atmosphere (Waggoner, n.d.). However, certain bacteria in the soil can access this gas, and these nitrogen-fixing bacteria frequently form symbiotic relationships with the roots of plants. The bacteria convert the nitrogen gas (N_2) from the air into a form, ammonium (NH_4^+), that the plant can use and, in return, the plant supplies the bacteria with carbon compounds in the form of simple sugars from photosynthesis. Another group of bacteria, the nitrifying bacteria, then convert the ammonium (NH_4^+) into nitrite (NO_2^-)

which is then transformed into nitrate (NO_3^-), the other form of nitrogen plants can absorb. This latter form, though, is primarily advantageous to the grasses, hence in forests, where there are few of these species and plants consume ammonium. There are fewer nitrifying bacteria in the soils. Finally, the last group of bacteria involved in the nitrogen cycle are the denitrifying bacteria. The denitrifiers are active when oxygen is absent in the soil (e.g. in saturated soils or soil aggregates), and it is these bacteria that convert any excess nitrate in the soil back into nitrogen (N_2) or nitrous oxide (N_2O) gas to release back into the atmosphere. (Encyclopaedia Britannica, 2018).

Usable forms of nitrogen are so important to the plants (as well as the animals that consume them and the detritivores who consume all of them) because nitrogen is the essential ingredient in nucleotides and amino acids. Hence, without usable nitrogen, plant cells could not make their proteins, including the most critical type of protein, enzymes, which control the chemical reactions that keep cells alive. Therefore, without nitrogen, plant cells cannot perform the chemical reactions necessary to complete the four tasks of life (respiration, homeostasis, reproduction, synthesizing new material), and as a consequence the cells of the plant (and therefore the plant itself) would die. Animals, too, would then begin to die as well because there would be no plants for the different consumers to get the nitrogen for their own cells. Even the decomposers would eventually die because there would no longer be a source of nitrogen for their cells either. Hence, without a source of nitrogen, there would be no ecosystem because all the cells that make up all the organisms living there would cease to function.

Since nitrogen is so critical to life, people have sought ever since the rise of agriculture to control how much of it is available to plants, and fertilizer is any natural or artificial substance containing chemical elements, specifically, nitrogen that is vital to the survival of plants.

Fertilizer improves the growth and development of plants, making crops grow faster and bigger, and the application of it can improve the natural fertility of the soil or replace the chemical elements taken from the soil by earlier crops. Furthermore, while the ingredients vary in different brands of fertilizer, three key chemicals, nitrogen, phosphorus, and potassium, are in every fertilizer. Indeed, they are actually identified by three numbers on the bag of fertilizer that specify the percent weight of these three main plant nutrients, and different percentages are used for different situations (e.g. lawn vs. garden) where a person is trying to promote plant growth. These ingredients fuel growth, promote root development, and defend against stress, and while other chemicals that are sometimes found in fertilizer, including calcium, magnesium, and sulfur, can promote the growth of young roots and shoots, along with the production of chlorophyll (LaLiberte, n.d.), the "big three" remain nitrogen, phosphorus, and potassium.

However, fertilizer is not always good for plants or the soil. The use of fertilizer impacts the soil's pH and can affect the health of soil-microbes. Fertilizer alters the level of acidity, and this can harm the microbial life that are beneficial to the health of the soils and plants for all the reasons discussed so far, and what is more, the use of chemical fertilizers can actually jeopardize the health of the bacteria that are necessary to the nitrogen-fixation process (Encyclopaedia Britannica, 2017). Since ammonium is the waste product of the first group of bacteria involved in the cycle, it is therefore toxic to these bacteria. Yet when fertilizer is added to the soil, it increases the ammonium present which forces the nitrogen-fixing bacteria live in their own waste, thereby decreasing their population. This means that there would now be less bacteria to convert the nitrogen from the atmosphere naturally, and therefore, it is going to require yet more fertilizer to be added to the soil if someone wants to grow plants using the same soil. Hence, the slightest change in conditions, such as a change in the acidity of the soil could make the

environment harmful to the bacteria, leading to a decrease in the bacteria population. (Lineberger, 2009).

Therefore, for our experiment, we chose to put nitrogen-based fertilizer on the grass out in the front lawn, and took soil samples before and after it had rained so the fertilizer had been absorbed into the soil, to see whether the density of bacteria in the soil increased or decreased. Considering how important bacteria is to the soil, we chose to do this particular experiment because our group wanted to see if fertilizer is actually damaging the soil and the microorganisms in it, especially because fertilizer is used regularly on the school's lawns.

Density of Bacteria in the Soil: Nitrogen-based Fertilizer Experiment

I. Problem: Does nitrogen-based fertilizer change the density of bacteria in the surface layer of the soil more than it does in the deeper layers?

II. Hypothesis: Nitrogen based fertilizer will decrease the density of bacteria in soil closer to the surface.

III. Procedure:

    A. Independent Variable: Application of fertilizer to soil plots

    B. Dependent Variable: The density of bacteria in the soil samples

    C. Negative Control: Soil plots with no fertilizer added

    D. Positive Control: Soil samples collected before fertilizer application

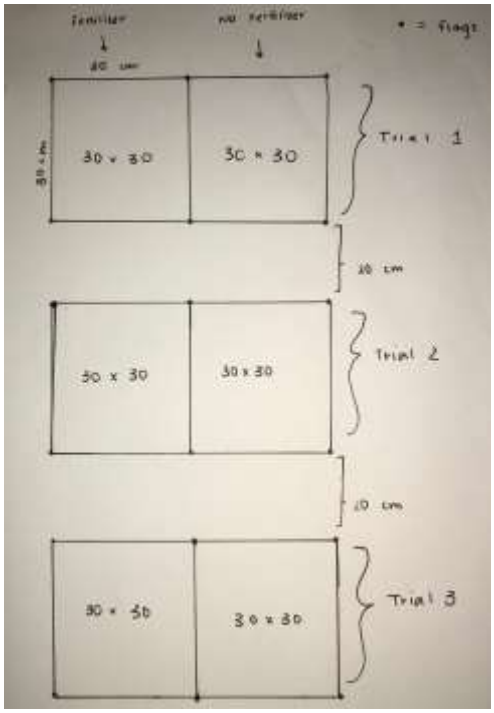
    E. Controlled Variables:

        1. Size of plots (30 cm by 30 cm)
        2. Space in between plots (20cm)
        3. Amount of fertilizer (1.4 grams)

4. Type of fertilizer (Vigoro Ultra Turf Lawn Fertilizer)
5. Distance between plots (right next to each other)
6. Amount of water added to both plots (rain)
7. Size of soil column (15 cm deep)
8. Distance between each trial plots (20 cm)
9. Location of plots (N° 39.35303, W° 076.63604)
10. Placement of plots of each trial in relation to each other
11. When the soil samples are taken
12. When serial dilutions are done
13. Size of pipette (10 mL)
14. Type of pipette (serological)
15. Size of culture tube (15 ml)
16. Amount of soil used in bacteria dilution (1 cc)
17. Type of water used in bacteria dilution (sterile water)
18. Type of test kit used for nitrate test (LaMotte Combination Soil Model STH-14)
19. Amount of sample placed on agar plate (100 microliters)
20. Degree to which diluted: 10^{-2} and 10^{-3}
21. Type of agar plate (3M Petrifilm™ Aerobic Count Plate)
22. How long the bacteria grow (72 hours)
23. Which dilutions plated

F. Step-by-Step Instructions:

1. Obtain 18 yellow flags.
2. With the yellow flags, plot the diagram shown at the right in the lawn at the coordinates, N°



39.35303, W° 076.63604.

3. Do steps 4-10 at the same time on the same day.
4. Using the soil core extractor collect a soil sample that is 2 cm in diameter and 15 cm deep from the No Fertilizer, positive control trial 1 plot.
5. When the soil core extractor has been removed from the ground, divide the soil into three 5-centimeter sections.
6. Put the soil from the first 5 centimeters section at the bottom of the soil core extractor into a bag labelled “No Fertilizer, positive control trial 1, bottom layer (10-15 cm deep)”
7. Put the soil from the middle section of the soil core extractor 1 into a bag labelled “No Fertilizer, positive control trial 1, middle layer (5-10 cm deep)”
8. Put the soil from the top of the soil core extractor into a bag labelled “No Fertilizer, positive control trial 1, top layer (0-5 cm deep)”
9. Repeat steps 3-8 in the plot next to the one that you just took soil from, that is marked for fertilizer. Label the bags the same except change “no fertilizer” to “fertilizer
10. Repeat steps 3-9 for each trial (2 more times [3 trials total]), changing the labelling number in positive control dependent on the trial number. For example, trial would be “No fertilizer, positive control 2, top layer (0-5 cm)
11. Now that the soil is collected and separated into its own bags, it is time to test for the bacteria in the soil.

12. It is crucial to test all bacteria in the soil on the same day at the same time as well as the chemical extraction process in the nitrate testing, steps 12-28.
13. Use a clean, new 10 mL serological pipette to add 10 mL of sterile water to a 15 mL culture tube. Label tube "10^0, no fertilizer, positive control 1, bottom layer".
14. Use the same pipette to add 9 mL of sterile water to a second 15 mL culture tube. Label the tube "10^{-1} no fertilizer, positive control 1, bottom layer"
15. Repeat step 14 two more times to two additional 15 mL culture tubes, label them "10^{-2} no fertilizer, positive control 1, bottom layer", and "10^{-3}no fertilizer, positive control 1, bottom layer" respectively.
16. Place 1 cc of the soil sample from the No fertilizer, positive control 1, bottom bag into the 10^0 mL tube.
17. Cap the tube and shake vigorously.
18. Using a new 10 mL serological pipette, remove 1 mL of the soil/water mixture from the 10^0 tube and place it into the 10^{-1} tube.
19. Cap and shake vigorously.
20. Using the same pipette in step 18, remove 1 mL of the soil/water mixture from the 10^{-1} tube and place it into the 10^{-2} tube.
21. Cap and shake vigorously.
22. Using the same pipette in step 18, remove 1 mL of the soil/water mixture from the 10^{-2} tube and place into the 10^{-3} tube.

23. Cap and shake vigorously
24. You should now have a total of four culture tubes.
25. Plate 100 ul (microliters) samples from the 3rd and 4th tubes (10^{-2} and 10^{-3}) onto their own separate and correspondingly labeled nutrient agar 3M Petrifilm™ Aerobic Count Plates.
26. Using the LaMotte Combination Soil Test Kit Model STH-14, perform a nitrate test on the middle 5 centimeters level of the soil samples taken.
27. Record the amount of nitrate in the soil in the corresponding sections of the data table.
28. Repeat steps 12-25 for each top and bottom layer soil sample and step 26 for each middle section.
29. Allow nutrient agar plates to grow for 72 hours.
30. Examine each of the plates for individual bacterial colonies and choose the plate with the lowest dilution (that has at least 5 colonies) to make estimates of the number 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 number at which these colonies were found|

31. Record bacteria level for each soil sample in corresponding section of the data table.
32. Measure 1.4 grams of nitrogen fertilizer using a petri dish
33. Place the 1.4 grams of nitrogen fertilizer into a plastic bag.
34. Repeat steps 32-33 2 times so there are 3 bags total.

35. Sprinkle the 1.4 grams of fertilizer on each plot so that it is scattered throughout the plot labelled for “Fertilizer” at the same time on the same day.
36. Allow at least 24 hours to pass and wait to take samples until after it rains.
37. Retake soil samples from each plot, repeat steps 3-10.
38. Repeat steps 12-31 to retake the bacteria and nitrate levels in the soil.
39. Record new bacteria and nitrate levels for each soil sample in data table.

IV. Data and Analysis:

A. Data Table

The Impact of Fertilizer on Different Bacteria Density and Nitrate Levels in Layers of Soil

	Plots with No Fertilizer Applied							
	Top Layer of Soil (0-5 cm deep)				Bottom Layer of Soil (10-15 cm deep)			
	Before Application of Water		After Application of Water		Before Application of Water		After Application of Water	
Trial	Bacteria (#/CC)	Nitrate (ppm)	Bacteria (#/CC)	Nitrate (ppm)	Bacteria (#/CC)	Nitrate (ppm)	Bacteria (#/CC)	Nitrate (ppm)
1	1,400,000	10 ppm	6,100,000	20 ppm	1,200,000	10 ppm	6,100,000	20 ppm
2	700,000	30 ppm	18,400,000	50 ppm	3,300,000	30 ppm	8,100,000	50 ppm
3	2,100,000	30 ppm	3,700,000	50 ppm	9,900,000	30 ppm	2,100,000	50 ppm
Averages:	1,400,000	23 ppm	9,400,000	40 ppm	4,800,000	23 ppm	16,300,000	40 ppm

	Plots with Fertilizer Applied							
	Top Layer of Soil (0-5 cm deep)				Bottom Layer of Soil (10-15 cm deep)			
	Before Application of Fertilizer		After Application of Fertilizer		Before Application of Fertilizer		After Application of Fertilizer	
Trial	Bacteria (#/CC)	Nitrate (ppm)	Bacteria (#/CC)	Nitrate (ppm)	Bacteria (#/CC)	Nitrate (ppm)	Bacteria (#/CC)	Nitrate (ppm)
1	1,400,000	30 ppm	1,100,000	20 ppm	9,700,000	30 ppm	10,400,000	20 ppm
2	48,800,000	5 ppm	6,800,000	30 ppm	12,600,000	5 ppm	1,360,000	30 ppm
3	800,000	20 ppm	3,200,000	10 ppm	1,100,000	20 ppm	6,400,000	10 ppm
Averages:	17,000,000	18 ppm	3,700,000	20 ppm	7,800,000	18 ppm	6,053,333	20 ppm

B. Graph

Figure 1.

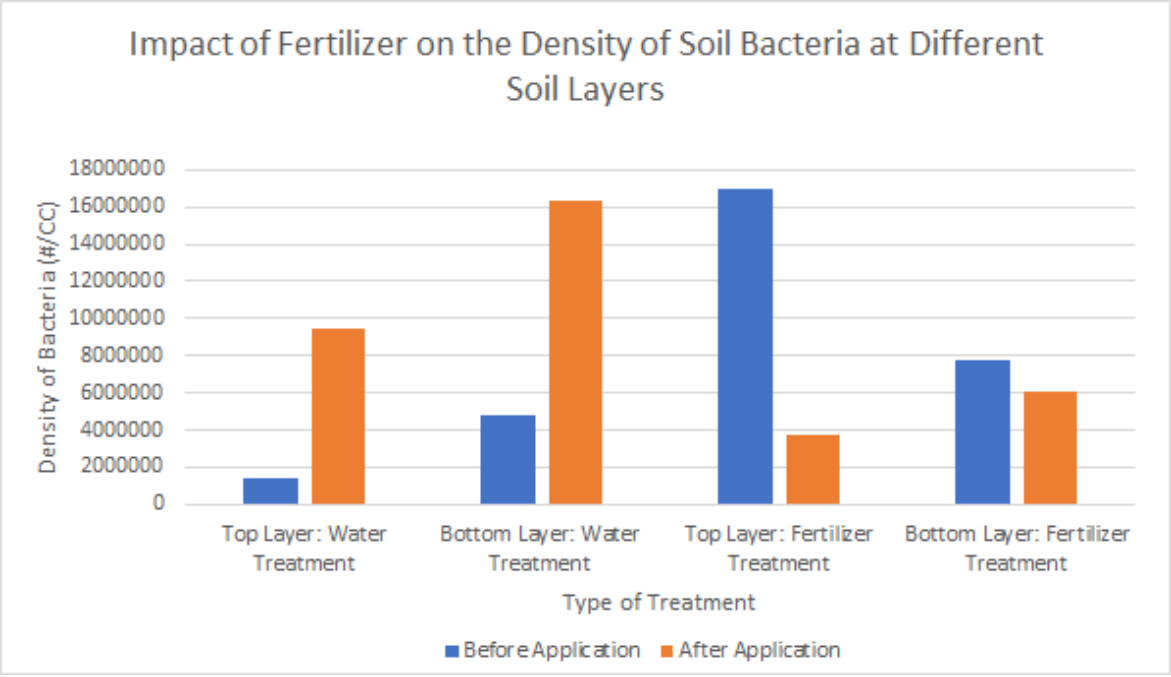


Figure 2.

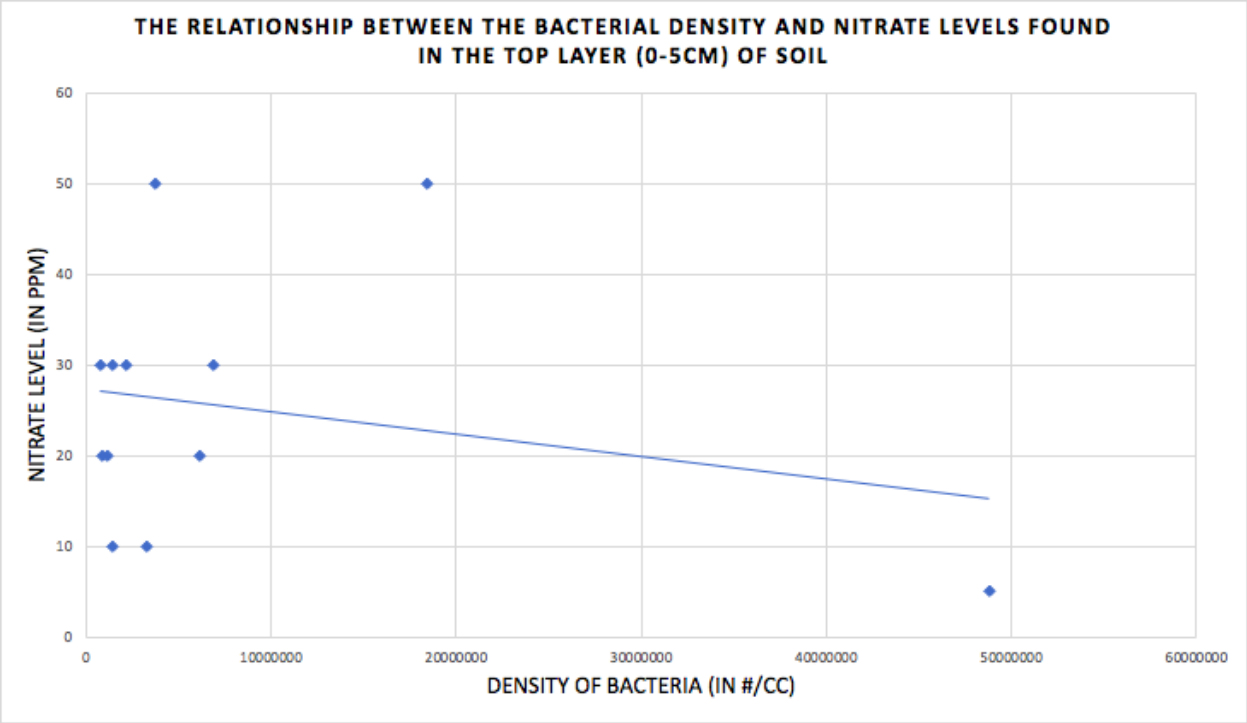


Figure 3.

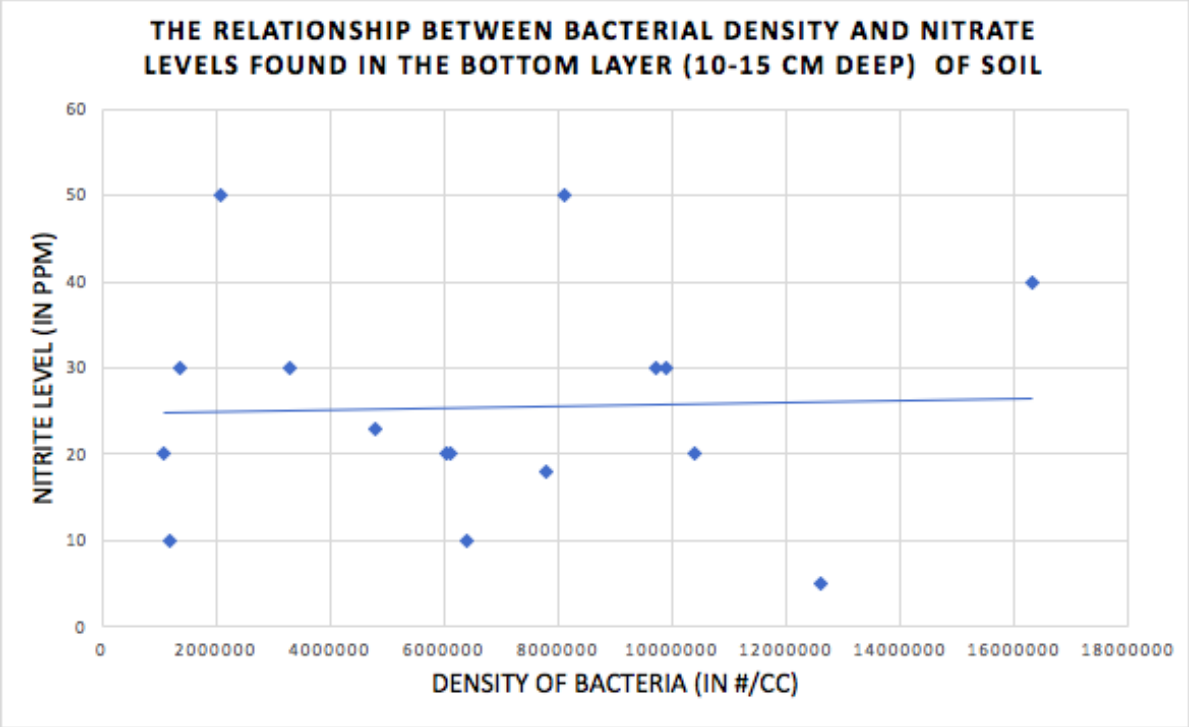
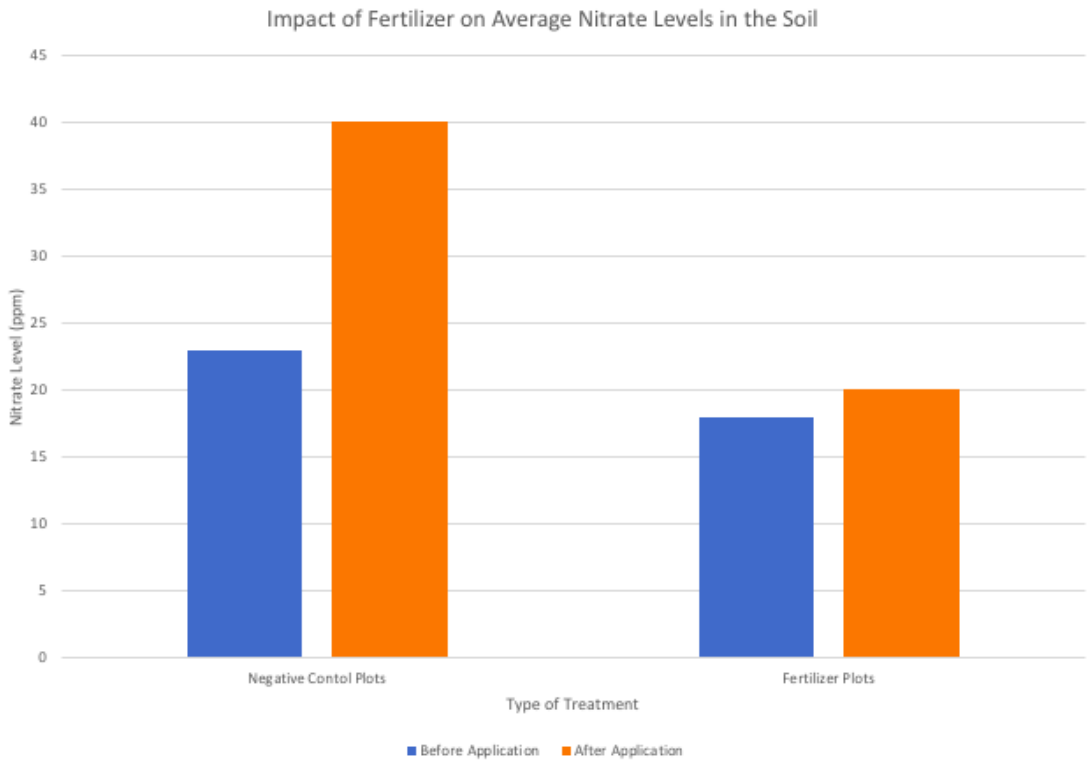


Figure 4.



V. Conclusion:

Our hypothesis was proven correct by our experiment because the density of bacteria decreased after the application of fertilizer and the density of bacteria decreased more in the top level of the soil, which is closer to the surface. In the plots where nitrogen fertilizer was added, there was significant decrease in the average density of bacteria in the top layer of soil after the application of fertilizer. On average, in the top layer of soil (0-5 cm), the density of bacteria decreased from 17,000,000 per 1 cc to 3,700,000 per 1 cc after fertilizer was applied which is a difference of 13,300,000 bacteria colonies per 1 cc. There was also a decrease in the bottom layer of soil (10-15 cm) after fertilizer was applied; the average density of bacteria decreased from 7,800,000 per 1 cc to 6,053,333 per 1 cc which is a difference of 1,746,667 bacteria colonies per 1 cc. This shows that the density of bacteria decreased more in the top layer of the soil than it decreased in the bottom layer. In the negative control plots in which only water was

added, there was an increase in the average density of bacteria in both layers of soil. Before the application of water to the top level of soil (0-5 cm deep), the average density started as 1,400,000 per 1 cc and increased to 9,400,000 per 1 cc after the application of water which is a difference 8,000,000 per 1 cc. In the bottom layer of the soil (10-15 cm deep) that was treated with water, the average density of bacteria increased from 4,800,000 per 1 cc to 16,300,000 per 1 cc which is a difference of 11,500,000 bacteria colonies per 1 cc. This is important because it shows that there was no other factor except for the fertilizer that significantly affected the soil, since the only difference between the negative control plots and fertilizer plots was the application of fertilizer. Therefore, this data proves our hypothesis is correct because not only did the density of bacteria decrease after the application of fertilizer, it decreased significantly more in the soil closer to the surface.

In this experiment, we also discovered that the chemical fertilizer is harming the nitrogen cycle in the soil closer to the surface by decreasing the density of the bacteria population in the soil, but it is not disrupting the nitrogen cycle deeper down in the soil. In Figure 1, it shows that the density of bacteria in both the top and bottom layers of soil in the negative control plots increased after the application of water, especially in the bottom layer. This corresponds to Figure 4, in which, in the negative control plots, on average the nitrate levels went from 23 parts per million to 40 parts per million after the application of water, which shows the nitrate levels went up by 17 parts per million, showing the normal relationship between nitrate levels and the density of bacteria. The key factor in the increase of the nitrate levels was the increase of the density of bacteria in the bottom layer of soil, 10 to 15 cm deep. Figure 1 also shows that in the fertilizer plots, in both the top and bottom layers, the fertilizer caused the density of bacteria to decrease, specifically in the soil closer to the surface. This corresponds to Figure 4 as the average

nitrate levels went from 18 parts per million to 20 parts per million which only increased 2 parts per million. This increase is not as large of a difference as found between the nitrate levels of the negative control plots. There is not a significant change in the nitrate levels since there was such a large decrease in the density of bacteria in the top layer of soil. The normal relationship between the density of bacteria and nitrate levels should be as the density of bacteria increases, the nitrate levels should increase as well, since more bacteria allows for more nitrogen to be converted to nitrate, and therefore, the nitrate levels would increase. This relationship was observed in the bottom layer of soil (10 to 15 cm deep), shown in Figure 3, in which the trendline shows as the density of bacteria rose, the nitrate levels rose as well. This trendline was a result of the data that is shown in Figure 1, since in the negative control plots for the bottom layer of soil (10 to 15 cm deep), the average density of bacteria significantly increased and after the application of fertilizer, there was not a significant decrease in the density of bacteria. This shows that there was not a disruption in the nitrogen cycle deeper down in the soil, since the relationship between the density of bacteria and nitrate levels was normal. However, this normal relationship was not observed in the top layer of soil (0 to 5 cm deep) shown in Figure 2, in which the trendline shows that as the density of bacteria went up, the nitrate levels dropped. The results of this trendline in figure 2, are due to the significant decrease in the density of bacteria in the top layer of soil, as shown in Figure 1, after fertilizer was applied to the soil. Therefore, our data and graphs show that nitrogen-based fertilizer is disrupting the nitrogen-cycle in soil closer to the surface but not deeper down in the soil. If this pattern were to continue in a longer period of time, it would show that the fertilizer is indeed not helping. However, since we completed this experiment in a short period of time, there is not a definite answer as to if the fertilizer is helping.

From this experiment, we learned that nitrogen-based fertilizer negatively impacts the nitrogen cycle in the soil closer to the surface by decreasing the density of bacteria which are vital to the nitrogen cycle but not deeper in the soil. If further research were to be conducted, it would be logical to continue to look at the effects of fertilizer on the nitrogen cycle in different layers of the soil. The next step would include testing to find the exact level of soil where you see the switch over from the fertilizer disrupting the nitrogen cycle to where it stops disrupting the cycle since we did not test the middle layer of soil (5 to 10 cm deep), for the density of bacteria. This experiment would be similar to the one we already conducted but testing the middle layer of the soil or the soil levels in smaller increments. Another logical concept to research is if a longer amount of time would lead to the same pattern we found; since we gave the fertilizer 48 hours to sink in after a rainfall, the data could have changed if we had let it sink in longer or if different patterns and amounts of rainfall had occurred. If the pattern we found continued after a longer amount of time, we could conclude that the fertilizer is actually not helping the soil.

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