Soil Ecology Project

Background Report

Bacteria are prokaryotic, unicellular organisms that play numerous critical roles in the soil ecosystem, including producing nitrogen plants can use, performing bioremediation, and helping with decomposition. While there are many different types of bacteria that engage in these activities, the four groups that do most of the work are nitrogen fixing bacteria, nitrifying bacteria, denitrifying bacteria, and actinomycetes. Bacteria from all four groups perform important services related to water dynamics, nutrient cycling, and disease suppression that aid plant growth indirectly, but some, such as the nitrogen fixers, frequently actually form direct symbiotic associations with the roots of legumes (such as clovers and trees). In these relationships, the plant supplies simple carbon compounds (Kennedy and Ingham, 2013) such as sugars (Ohio State University, 2017) to the bacteria while in return the bacteria convert nitrogen gas from the air into a form the host plant can use for its own needs.

However, not all nitrogen fixing bacteria are symbiotic, and those that are not can still directly aid plants by supplying the plants with the nitrogen they need. Nitrifying bacteria, for example, change the ammonium from decomposition to nitrite NO_{2^-} and then to nitrate NO_{3^-}, which is the preferred form of nitrogen for grasses and most row crops.

Furthermore, nitrogen is not the only nutrient plants need, and the actinomycetes bacteria are a large group that decompose a wide array of substrates back into the carbon dioxide plants need for photosynthesis, including hard to degrade compounds such as chitin and cellulose (Kennedy and Ingham, 2013). These bacteria live as pseudo-like projections known as hyphae and are responsible for the characteristically “earthy” smell of freshly turned healthy soil.

But the nitrogen cycling is the most critical role bacteria play in the soil ecosystem because they are essential to every single stage of this biogeochemical cycle - from the initial

nitrogen fixation that makes this element available to living things to the final denitrification that converts nitrogen back to its inorganic form (Brenton W. Thomas, N.A). The cycle begins when rhizobia bacteria in the soil capture the very abundant nitrogen gas (N_2) found in the atmosphere and convert this gas into a biologically usable form known as ammonium, using the enzyme, nitrogenase; Ammonium (NH_4^+) is a form of nitrogen that plants and other organisms in the soil can use (Jacob and Cordaro, 2000). Hence, when the atmospheric nitrogen gas is converted into ammonium through nitrogen fixation, the plants are able to obtain the nitrogen they need to synthesize proteins and nucleic acids, and in return, and the bacteria benefit as well because they eventually obtain carbon from the plants and a secure environment to inhabit within their roots (Boundless, 2016).

The next major process of the nitrogen cycle is nitrification. When animals eat plants, they acquire usable nitrogen compounds. However, the nitrogen doesn't remain permanently in their bodies, and the nitrogenous wastes from these organisms (along with their decomposing bodies after they die) are converted into ammonium by decomposers, which the nitrifying bacteria can then convert into nitrites (NO_2^-) and nitrates (NO_3^-) (Jacob and Cordaro, 2000). Nitrification occurs most rapidly in warm, moist, and well-aerated soils (Regents of the University of Minnesota, 2017), and the process is carried out by two different types of bacteria. Nitrosomonas carry out the first step, producing nitrite NO_2^-, and the resulting nitrite is then converted to nitrate NO_3^- by nitrobacters. The rates of nitrification are highly dependent on a number of environmental factors, including the substrate and oxygen concentration, temperature, pH, and the presence of toxic or inhibiting substances (Renay Jacob and Emily Cordaro, 2000). This process is an important part of the nitrogen cycle because for most plants, nitrate is the

preferred chemical form of nitrogen over ammonium to absorb for the production of their biological molecules.

The last major step of the nitrogen cycle is denitrification, which according to Merriam Webster Dictionary (2017), is "the loss or removal of nitrogen or nitrogen compounds; *specifically* : reduction of nitrates or nitrites commonly by bacteria (as in soil) that usually results in the escape of nitrogen into the air." Denitrification is essentially the reverse process of nitrification, when nitrates (NO_{3^-}) are reduced to nitrites (NO_{2^-}) and then back to nitrogen gas (N_2) (Agriinfo 2007), and the bacteria responsible for this process are basically converting any excess nitrate in the soil that the plants did not use back into nitrogen gas that is released into the atmosphere, thus bringing the nitrogen cycle full circle.

The reason all this nitrogen processing is so critical is because nitrogen is a vital component of the bodies of all living organisms. It is an essential element in proteins and the nucleic acids, DNA and RNA. (Kahn Academy, 2017), and therefore, it is necessary for virtually every process which the plant engages in. Without nitrogen, a plant cannot make the biological molecules its cells need to complete the four tasks of life (synthesis, homeostasis, transformation of energy, and reproduction), and without nitrogen, a plant cannot make the chlorophyll molecules it needs for creating its food through photosynthesis (Meredith Corporation, 2017). Hence, without nitrogen, a plant dies. Furthermore, since the process of photosynthesis creates the biological molecules and chemical energy that move up the trophic levels of an ecosystem, without nitrogen, the entire ecosystem would fail. Therefore, without the access to nitrogen which soil bacteria make available to all living things, life itself would not be possible.

Because of this significance, nitrogen is a major part of the artificial "food for plants" that is inorganic fertilizer. These manufactured forms of fixed nitrogen help to provide plants with all

the nutrients they need for survival, and they can come in a variety of physical forms (solid, liquid, or gas). They are either applied to soil, directly to the foliage of a plant, or added to aqueous solutions that are injected into the ground (International Fertilizer Association, 2015), and they frequently contain two other basic elements plants need: phosphorus and potassium. Together, these three elements are the most important ones a plant requires for some of the key reasons already discussed, with the phosphorous also helping plants to form new roots, make seeds, and fight disease, and the potassium helping plants make strong stems and regulate rates of photosynthesis (Fertilizer Canada, 2017).

Although inorganic fertilizers provide the critical nutrients in a convenient and inexpensive form that everyone can use to promote plant growth, they may also potentially harm the environment as well. Inorganic fertilizers have been proven to lead to oxygen loss in the waterways due to runoff in the street, and the nitrogen in them can also find its way into the waterways and causes an excess in algae. Along with doing damage in waterways, nitrogen from inorganic fertilizers can do damage when it sinks into soils because it often creates conditions that favor the growth of weeds rather than native plants. Soil impacted by commercial fertilizers also results in low organic carbon content, low microbial counts, and low microbial carbon biomass.

Furthermore, when the inorganic fertilizers are added to soil, the forms of nitrogen they increase in the soil are primarily ammonium and nitrate (NO_{3}^{-}), which are the waste products of the rhizobia and Nitrobacters respectively. Therefore, when more of these forms of nitrogen are added to the environment, these types of bacteria are essentially living in their own waste, which can harm and eventually kill them and in turn throw offbalance the natural ratio of rhizobia, nitrobacters, ammonium, and nitrate found in normal, healthy soil. In addition, ammonium is the

food for nitrosomonas and nitrate NO_3^- is the food for denitrifying bacteria, meaning that these two types of bacteria are receiving a surplus of their food, causing their population densities to increase when fertilizer is added, which throws off the balance of bacteria species and levels of nitrogen in the soil even further. Basically, the plant is the only part of the cycle that is benefiting from receiving the excess nitrogen from the fertilizer, and because its use unbalances the natural nitrogen cycle, the land will always need to keep being fertilized in order to support any type of planting living there. Put plainly, if the land is not fertilized, the plant life there will die because there will not be the correct balance of natural nitrogen fixing bacteria to feed it, with all the negative impacts on any consumers in the environment as well.

Yet while artificial fertilizers can be harmful, compost is another type of fertilizer that is naturally made of any decomposed organic material, from plants to kitchen scraps. Compost can be used in soil to improve its quality and to help the growing plants receive all their necessary nutrients for survival (University of Illinois Board of Trustees, 2017: The Composting Process.). Compost helps soil by speeding up decomposition, thus creating the ideal environment for the breakdown of organic materials. Microscopic bacteria and fungi account for most of the chemical decomposition in compost, while larger macro-organisms, like worms and snails, account for most of the physical decomposition of compost (University of Illinois Board of Trustees, 2017 and The Science of Composting n.d.). Because there are already natural bacteria in compost, when compost is added to soil, the amount of bacteria already living in the soil are not harmed and the bacteria to nitrogen ratio remains balanced. Compost, unlike inorganic fertilizers, actually promotes healthy microbe growth within the soil, feeding the soil food web and increasing the health of the natural soil (Science Encyclopedia, 2008), and over time, this

creates a more nutrient rich soil that is beneficial for the plants that grow within it (Kahn Academy, 2017).

Given the potential impacts of compost versus artificial fertilizer, we wanted to know which fertilizer, inorganic or organic, increases the nitrate nitrogen levels and population density of bacteria in soil more. In the experiment we will take soil samples and test for the population density of bacteria and the levels of nitrate nitrogen. We will then add organic fertilizer and inorganic fertilizer to different plots of the soil and retake the soil samples, also testing the bacteria population density and nitrate nitrogen levels again. Next we will compare the data. From our research we are expecting the nitrate levels and bacteria population density to increase more in the plots with compost than with the inorganic fertilizer. We are performing this experiment in order to illustrate the impact of human's use of fertilizer on the nitrate nitrogen levels and bacteria population in the soil. We plan to further explore how fertilizer affects the nitrogen cycle, thus affecting the entire ecosystem.

Experiment Outline

I. Problem: Which fertilizer, inorganic or organic, increases the nitrate nitrogen levels and population density of bacteria in soil more?

II. Hypothesis: Organic fertilizer increases the nitrate nitrogen levels and population density of bacteria in soil more than inorganic fertilizer.

III. Procedure:

Independent Variable: the type of fertilizer added to the soil, either inorganic (Vigoro Ultra Turf Phosphorus Free Turf Fertilizer 29-0-4) or organic (compost)

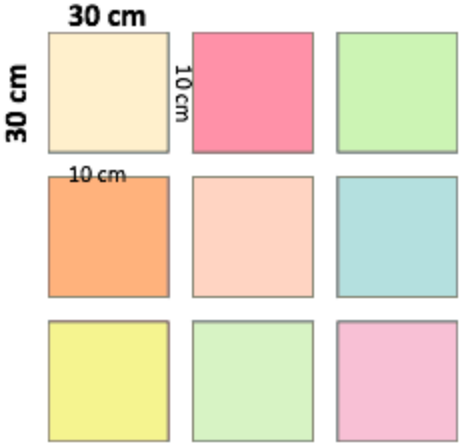
Dependent Variable: the nitrate nitrogen levels of the soil (ppm) and the population density of bacteria in the soil (#/cm^3)

Negative Control: a plot of land without any fertilizer, only water added to it

Controlled Variables: size of plots, size of space between each plot, the location of the plots, lighting of plots, topography of plots, climate of plots, the order in which the plots are placed, the type of compost used, the amount of compost used, the type of fertilizer used, the amount of fertilizer used, the type of water used to water the plots, the amount of water used to water the plots, the nutrient agar plates used, type of water used in bacteria testing, the number of soil samples taken from each plot, the amount of soil extracted from each plot, size of micropipettes, size of test tubes, method of mixing soil samples together, type of soil, method of testing for bacteria population density, method of testing for nitrogen levels, size of pipettes, amount of soil placed in 15mL plastic culture test tube during serial dilution, method and amount of time 15mL culture test tubes are shaken during serial dilution, amount of time bacteria samples grow, the temperature of the environment in which the bacteria samples grow, number of diluted cultures tubes made, amount of material diluted between culture tubes, amount of time fertilizer, compost, and water sit on soil plots, method of applying fertilizer, compost, and water, amount of sterilized water added to culture tubes, longitude and latitude of testing, amount of dilution mixture placed on nutrient agar plate, which dilution mixtures are placed on nutrient agar plates

Step-by-Step Instructions:

1. Choose a flat and mainly sunny location at N.39.35803, W.076.63625 on the Roland Park Country School campus that is entirely covered by grass. Create 9 plots that are 30 centimeters by 30 centimeters. They should be arranged in 3 rows, with 3 in each row, and are 10 cm apart from each other on all sides. See diagram below:



2. Label 36 yellow flags with sharpie. 4 flags should read “1 N.C” for the negative control of trial 1. 4 flags should read “1 O” for the organic fertilizer plot of trial 1. 4 flags should read “1 I.O” for the inorganic fertilizer plot of trial 1. 4 flags should read “2 N.C” for the negative control of trial 2. 4 flags should read “2 O” for the organic fertilizer plot of trial 2. 4 flags should read “2 I.O” for the inorganic fertilizer plot of trial 2. 4 flags should read “3 N.C” for the negative control of trial 3. 4 flags should read “3 O” for the organic fertilizer plot of trial 3. 4 flags should read “3 I.O” for the inorganic fertilizer plot of trial 3.

3. Place the sets of flags around each corresponding plot, one flag on *each* of the corners of *each* plot to differentiate the plots from each other. The column oriented on the east side of the plots should be marked with the N.C. flags, with trial 1 N.C at the top, trial 2 in the middle, and

trial 3 at the bottom. The middle column should be marked with the O flags, with trial 1 O at the top, trial 2 in the middle, and trial 3 at the bottom. The column oriented on the west side of the plots should be marked with the I.O flags, with trial 1 I.O at the top, trial 2 in the middle, and trial 3 at the bottom. See diagram below:

4. All of the following samples must be collected on the same day at the same time. Collect 3 samples of soil from EACH of the 9 plots. Do this by using a mallet to hammer a soil core extractor with a 2 cm diameter into the soil. Take a core that is 15.5 cm deep with a diameter of 2 cm. This is the 'before' soil sample. Each of the three samples from each of the nine plots should be placed in its own individual plastic bag labelled identically to the flag from the plot from which the soil was taken. Each bag should also be labelled with a 'B' to make it clear it is from the before trial. In total there should be 27 labelled bags containing soil samples.

5. The following process must take place on the same day at the same time. Once all the soil samples are collected, return to an indoor laboratory. Combine the 3 samples taken from each plot by pouring 2 of the samples into the remaining sample's plastic bag and kneading them with your fingers until the soil is fine instead of chunky and has the texture of wet sand. Do this until the 27 plastic bags are reduced to only 9 plastic bags, one from each plot.

6. The entirety of steps 7a-7p and step 8 must occur on the same day at the same time. Step 9 does not need to occur on the same day at the same time as steps 7a-7p and step 8.

7. Now use the soil samples to test for bacteria density in **each** plot by doing the following:

7a. Use a clean, new transfer pipette to add 10 ml of sterile water to a 15 ml culture tube. Label the tube "1 N.C. 10^{0}"

7b. Use the same pipette to add 9 ml of sterile water to a second 15 ml culture tube. Label the tube "1 N.C. 10^{-1}."

7c. Repeat step 7b two more times to two additional 15 ml culture tubes, only label them "1 N.C. 10^{-2}" and "1 N.C. 10^{-3}" respectively.

7d. Place 1 cc of the trial 1 negative control soil sample into the "1 N.C. 10^{0}" culture tube.

7e. Cap the tube and shake vigorously.

7f. Using a new clean pipette, remove 1 ml of the soil/water mixture from the "1 N.C. 10^{0}" tube and place into the "1 N.C. 10^{-1}" tube.

7g. Cap the tube and shake vigorously.

7h. Using the same pipette from step 7f, remove 1 ml of the soil/water mixture from the "1 N.C. 10^{-1}" tube and place into the "1 N.C. 10^{-2}" tube.
7i. Cap the tube and shake vigorously.
7j. Using the same pipette from step 7f again, remove 1 ml of the soil/water mixture from the "1 N.C. 10^{-2}" tube and place into the "1 N.C. 10^{-3}" tube.
7k. Cap the tube and shake vigorously.
7l. You should now have a total of four culture tubes.
7m. Using a P200 micropipette and sterile micropipette tips, plate a 100 ul sample from the 3rd tube (dilution 1 N.C. 10^{-2}) on one half of a 3M Petrifilm™ aerobic count plate by dripping the dilution onto the plate and then flattening it with a petrifilm spreader. The nutrient agar petrifilm should be labeled "1 N.C. 10^{-2}" to indicate that it is the 10^{-2} dilution from the negative control plot of trial 1.
7n. Repeat step 7m, this time plating a 100 ul sample from the 4th tube (dilution 1 N.C. 10^{-3}). This petrifilm should be labeled "1 N.C. 10^{-3} to indicate that it is the 10^{-3} dilution from the negative control plot of trial 1.
7o. Repeat steps 7a-7n using the soil samples from the other plots. Each set of culture tubes and petrifilms should be labeled according to which plot the soil being diluted came from.

-For the negative control plot of trial 2, all culture tubes should be labeled with "2 N.C. 10^{x} (x being the dilution of the tube) and the 2 petrifilms should be labeled "2 N.C. 10^{-2}" and "2 N.C. 10^{-3}".

-For the negative control plot of trial 3, all culture tubes should be labeled with "3 N.C. 10^{x} (x being the dilution of the tube) and the 2 petrifilms should be labeled "3 N.C. 10^{-2}" and "3 N.C. 10^{-3}".

-For the organic fertilizer plot of trial 1, all culture tubes should be labeled with "1 O. 10^{x} (x being the dilution of the tube) and the 2 petrifilms should be labeled "1 O. 10^{-2}" and "1 O. 10^{-3}".

-For the organic fertilizer plot of trial 2, all culture tubes should be labeled with "2 O. 10^{x} (x being the dilution of the tube) and the 2 petrifilms should be labeled "2 O. 10^{-2}" and "2 O. 10^{-3}".

-For the organic fertilizer plot of trial 3, all culture tubes should be labeled with "3 O. 10^{x} (x being the dilution of the tube) and the 2 petrifilms should be labeled "3 O. 10^{-2}" and "3 O. 10^{-3}".

-For the inorganic fertilizer plot of trial 1, all culture tubes should be labeled with "1 I.O. 10^{x} (x being the dilution of the tube) and the 2 petrifilms should be labeled "1 I.O. 10^{-2}" and "1 I.O. 10^{-3}".

-For the inorganic fertilizer plot of trial 2, all culture tubes should be labeled with "2 I.O. 10^{x} (x being the dilution of the tube) and the 2 petrifilms should be labeled "2 I.O. 10^{-2}" and "2 I.O. 10^{-3}".

-For the inorganic fertilizer plot of trial 3, all culture tubes should be labeled with "3 I.O. 10^{x} (x being the dilution of the tube) and the 2 petrifilms should be labeled "3 I.O. 10^{-2}" and "3 I.O. 10^{-3}".

7p. Place in a safe storage place and allow to grow for 48 hours.

8. Use the LaMotte basic Model STH-14 kit to perform the universal extracting procedure for each of the soil samples collected.

9. Use the LaMotte basic Model STH-14 kit to perform the nitrate nitrogen test for each of the soil extracts created in step 8, recording the values in parts per million (ppm) in the data table.

10. Finish testing for the population density of bacteria by doing the following:
10a. After the bacteria has grown for 48 hours, examine the 10^{-3} plate first of each of the plates for individual bacterial colonies, verifying that they have at least 5 colonies PER PLATE. If they do, ignore the 10^{-2} plate. If they do not, ignore the 10^{-3} plate and look at the 10^{-2} plate instead. Make estimates of the number of bacteria in the each of the original 1 cc soil samples 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|
10b. After calculating the number of microbes in 1 cc of soil from one of the plots, record that number in the corresponding box in the data table. In total, you should be calculating 9 numbers, one for each plot.

11. Use the LaMotte basic Model STH-14 kit to perform the nitrate nitrogen test for each of the soil extracts created in step 8, recording the values in parts per million (ppm) in the data table.

12. Gather 4,500 mL of room temperature tap water, 4.2 grams of Vigoro Ultra Turf Phosphorus Free Turf Fertilizer (29-0-4), and 4.2 grams of natural compost. Divide and weigh out the 4.2 grams of each fertilizer into individual three 1.4 gram quantities. Each of the 1.4 grams should be stored individually in its own separate Plastic Sandwich Bag. The 3 bags containing Vigoro Ultra Turf Phosphorus Free Turf Fertilizer should be labeled 'I.O', and the 3 bags containing natural compost should be labeled 'O'.

13. This step must occur on the same day at the same time. Return to your plots outside. Place the 1.4 grams of inorganic fertilizer directly in the center of each of the I.O. plots. Place the 1.4 grams of compost directly in the center of each of the O. plots. Pour 500 mL of room temperature tap water directly over the substances PER plot, and in the negative control plots, directly in the center of the plot.

14. Wait 48 hours for the inorganic and organic fertilizers to sink into the soil, and then repeat steps 4-10. This, instead, is the 'after' soil sample. All of the plastic bags should be labeled identically except with an 'A' instead of a 'B' to indicate they are from the after trial.

15. Compare and analyze data.

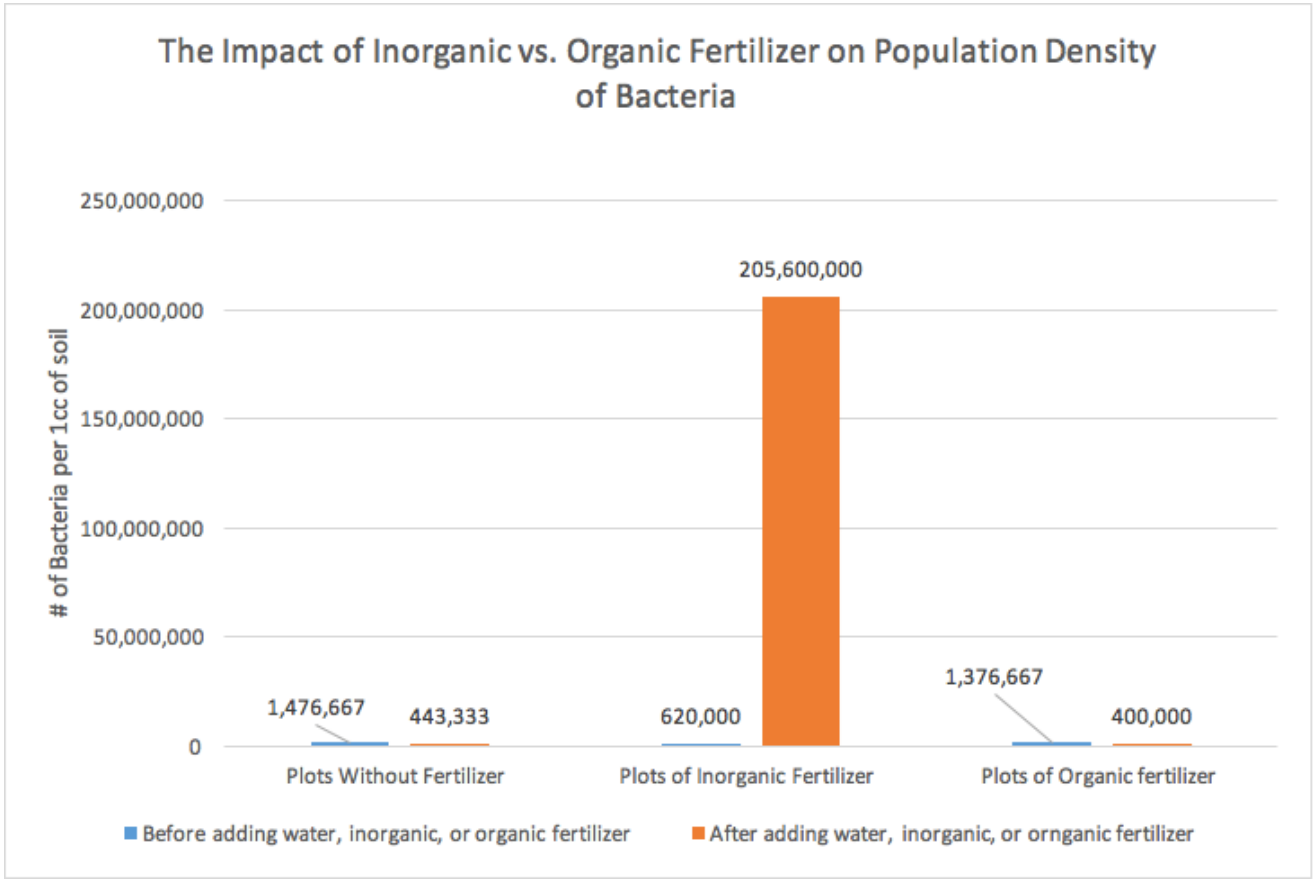
IV. Data and Analysis

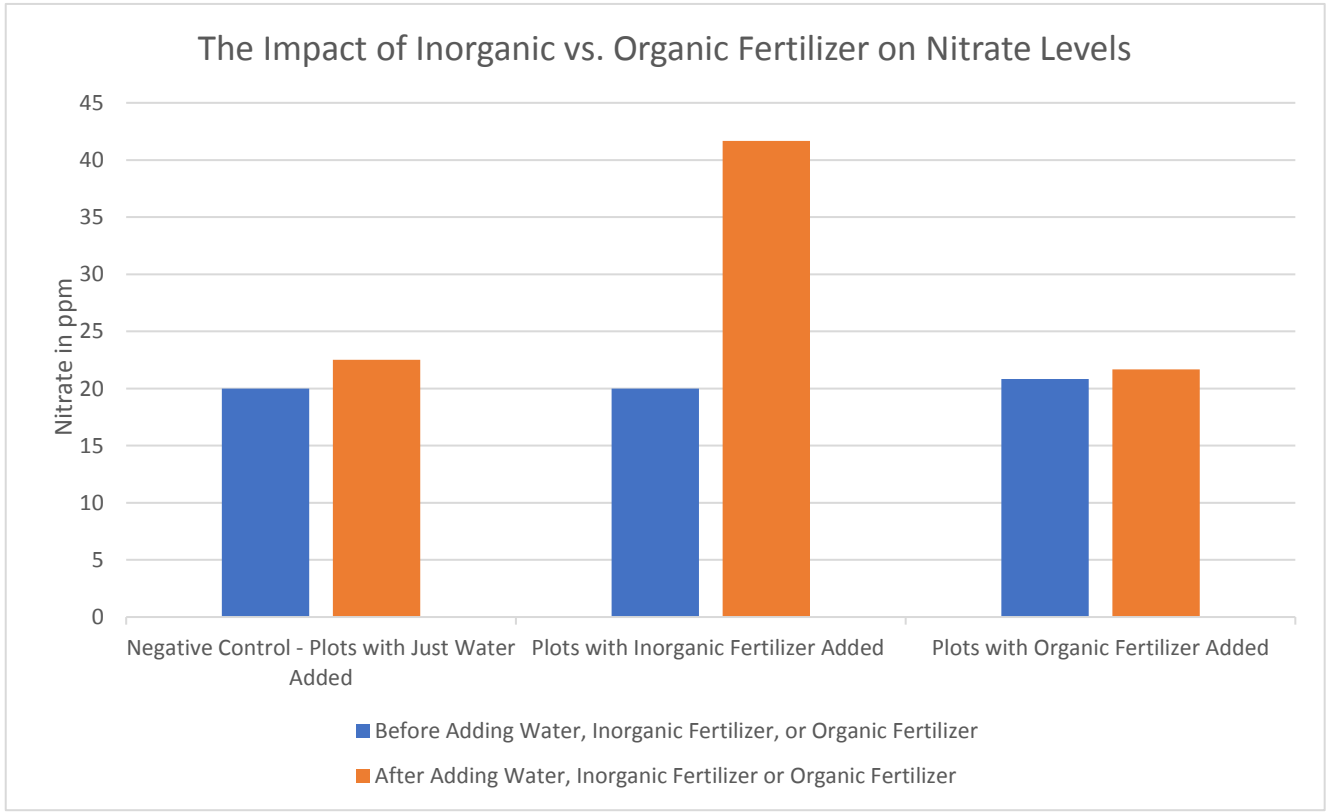
A. Data Table:

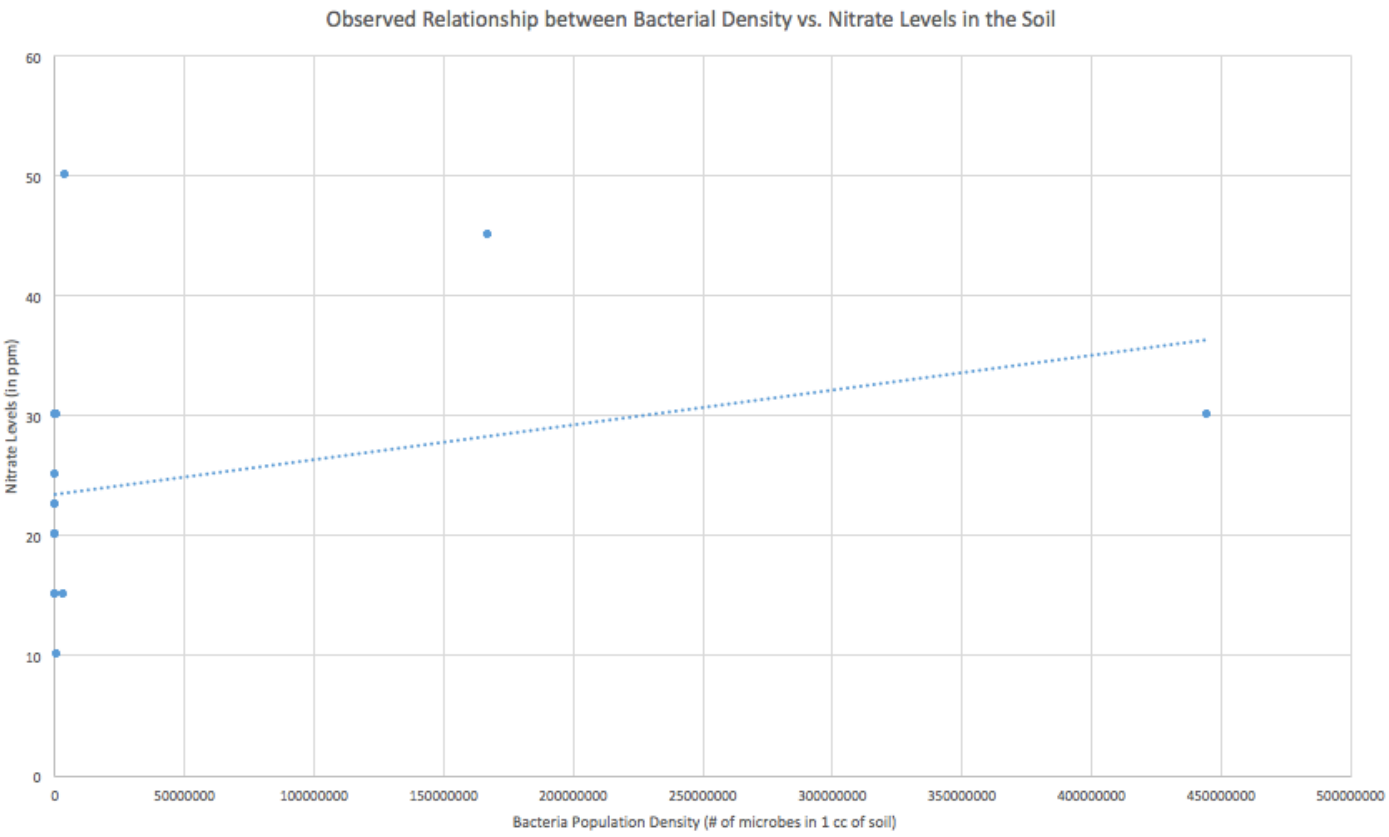
The Impact of Organic vs. Inorganic Fertilizer on the Population Density of Bacteria and Soil Nitrate Levels

Trial #	Plots with No Fertilizer Applied				Plots with Organic Fertilizer Applied				Plots with Inorganic Fertilizer Applied			
	Bacteria Density (# of bacteria in 1 cc. of soil)		Nitrate Levels (parts per million)		Bacteria Density (# of bacteria in 1 cc. of soil)		Nitrate Levels (parts per million)		Bacteria Density (# of bacteria in 1 cc. of soil)		Nitrate Levels (parts per million)	
	Before Adding Water	After Adding Water	Before Adding Water	After Adding Water	Before Adding Organic Fertilizer	After Adding Organic Fertilizer	Before Adding Organic Fertilizer	After Adding Organic Fertilizer	Before Adding Inorganic Fertilizer	After Adding Inorganic Fertilizer	Before Adding Inorganic Fertilizer	After Adding Inorganic Fertilizer
1	3,500,000	430,000	15	15	390,000	370,000	20	20	800,000	4,300,000	30	50
2	500,000	280,000	15	22.5	340,000	280,000	22.5	20	160,000	445,000,000	20	30
3	430,000	620,000	30	30	3,400,000	550,000	20	25	900,000	167,500,000	10	45
Averages	1,476,667	443,333	20	22.5	1,376,667	400,000	20.83	21.667	620,000	205,600,000	20	41.67

B. Graphs:


The Impact of Inorganic vs. Organic Fertilizer on Population Density of Bacteria
# of Bacteria per 1cc of soil
250,000,000
200,000,000
150,000,000
100,000,000
50,000,000
0
1,476,667
443,333
620,000
205,600,000
1,376,667
400,000
Plots Without Fertilizer
Plots of Inorganic Fertilizer
Plots of Organic fertilizer
Before adding water, inorganic, or organic fertilizer
After adding water, inorganic, or ornganic fertilizer


The Impact of Inorganic vs. Organic Fertilizer on Nitrate Levels
Nitrate in ppm
45
40
35
30
25
20
15
10
5
0
Negative Control - Plots with Just Water Added
Plots with Inorganic Fertilizer Added
Plots with Organic Fertilizer Added
Before Adding Water, Inorganic Fertilizer, or Organic Fertilizer
After Adding Water, Inorganic Fertilizer or Organic Fertilizer



V. Conclusion

In this experiment, we first proved that the expected relationship between soil bacteria population density and nitrate levels was observed. Our graph showing this relationship indicates that a normal nitrogen cycle process was taking place in the soil, prior to when we began our experiment. We know this because as the population density of bacteria increased, the nitrate levels did as well, which is shown in the graph by an increasing trend line with steady upward growth. We know, therefore, that any changes in the other graphs of bacterial population density and nitrate levels are valid, and must have been reactions to a force in the environment or one of the three substances we added to the plots: water, inorganic fertilizer, or organic fertilizer.

By performing our experiment three times, we proved our hypothesis to be false. We predicted that organic fertilizer (compost) would increase the nitrate nitrogen levels and

population density of bacteria in soil more than the inorganic fertilizer. We believed this because from our previous knowledge of fertilizers, we knew that fertilizers increased nutrients in the soil, but also that inorganic fertilizer was worse than organic fertilizer for the environment because it was not natural. Therefore, we assumed that compost would increase the nitrate nitrogen levels and population density of bacteria more than inorganic fertilizer, and in the process, would be more environmentally friendly. From our data, our hypothesis was proved false, meaning inorganic fertilizer increased the nitrate nitrogen levels and population density of bacteria in soil more than the organic fertilizer. If our hypothesis was correct, the data collected for organic fertilizer would have shown that the bacteria population density increased more than the population density of the bacteria in the inorganic soil plots. The nitrate levels would also have risen by a greater percent in the organic fertilizer plots as compared to the inorganic fertilizer plots, however this was not the case. To help prove why our experiment was valid we had to show the normal change in bacteria population and nitrate levels over a 48 hour period before showing how fertilizers impacted this change. In the graph of the impact of inorganic versus organic fertilizer on nitrate levels, the negative control shows the data for the plots with only water applied. The nitrate levels in the negative control went up by 12.5% or 2.5 parts per million showing that there was a force present in the soil that produces nitrate causing the levels to rise. In the bacteria chart however, the negative control data shows that the bacteria population density decreased by 69.98% due to some force that was killing off the bacteria.

The outside force that was causing the bacteria population density to decrease, and the nitrate levels to increase was protozoa. Protozoa are unicellular microorganisms that can cause disease in animals and humans. Protozoa primarily feed on free-swimming bacteria in the soil. As they consume the bacteria, they release ammonium, a type of nitrogen (Ingham, 2013). All of

this excess ammonium is then converted to nitrate during nitrification, a process of the nitrogen cycle, which helps to explain why the nitrate levels increased as the bacteria levels decreased in our experiment.

As mentioned above, the population density of bacteria decreased in the plots without fertilizer because the protozoa in the soil were eating some of the bacteria. This in turn, increased the nitrate nitrogen levels because the protozoa released ammonium nitrogen in the process of eating bacteria, which was then converted to nitrate nitrogen. The same pattern occurred in the plots with compost. The population density of bacteria decreased by about 71% because the protozoa in the soil were eating some of the bacteria, which increased the nitrate levels by 5% because the protozoa released ammonium nitrogen that was then converted to nitrate. The graphs of the plots with inorganic fertilizer, however, show a sharp increase in both the population density of bacteria and the nitrate levels. The population density of bacteria increased by 33,061% because the bacteria in the soil were given a greater supply of their food (the ammonium nitrogen) when they were fertilized, which allowed them to survive and reproduce. The nitrate levels increased by 105% because ammonium was added to the soil with the fertilizer, and with a higher bacterial population density, more of the ammonium was converted to nitrate.

From our experiment, we have learned that although, when applied to soil, inorganic fertilizer causes an increase in both bacterial density and nitrate levels, it is harmful, not beneficial, for the environment. Excess nitrogen levels that are produced by the fertilizer interacting with the soil have been proven to provide nutrients for plant growth, but they also harm the environment. Ultimately, the inorganic fertilizers causes forms of ammonia and nitrate to increase in the soil, which are either the waste products or food for certain types of bacteria in

the soil. This either leaves the bacteria to live in their own waste, eventually killing them, or gives bacteria a surplus of food, leading to the population density dramatically increasing, all of which unbalances the nitrogen cycle. Through our experiment, we discovered these negative effects that fertilizer has on the nitrogen cycle, which we learned consequently negatively impacts the entire ecosystem.

If future research were to be conducted in response to this experiment, one would continue to look at the relationship between bacteria and nitrate, but would also need to examine the relationship between protozoa, bacteria, and nitrate, because protozoa largely affected the results of our original experiment.

Works Cited

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