The Effect of Fertilizer on the Biodiversity of Bacteria in the Soil

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Background

Bacteria are microscopic single celled prokaryotes, and they are usually classified according to either their shape (cocci, bacilli, spirochetes, or vibrios); their cell wall structure (gram positive vs. gram negative); or their metabolic processes (aerobic, anaerobic, autotrophic, or heterotrophic) (Andreae, 2010). They are found in every environment on earth, including the soil, and those living there are even further classified based on their role in the environment: decomposers, mutualists, and lithotrophs (with a few types of pathogenic bacteria that do not make much of an impact on the soil ecosystem).

The decomposers are an essential component to natural cycles involving the soil, such as the nitrogen and carbon cycles, and groups of these bacteria break down organic material in soil to convert its energy into forms of organic matter useful for other organisms in the ecosystem, enabling the things living there to thrive. Decomposers also break down pesticides and pollutants in soil, helping to keep it clean and an ideal environment for plants to grow (Ingham, n.d.), which also contributes to the overall health of the environment.

Mutualistic or symbiotic bacteria form partnerships with plants by attaching to nodules on the root of plants where the bacteria and the plants carry out nutrient transfers between them. The nodules collectively increase the surface area of the root which allows the host plant to obtain more nutrients from the soil. The extra nutrients enable the plant to photosynthesize more, which in turn increases the amount of photosynthetic byproducts the plant can make available to the bacteria, thus making it a mutually beneficial, symbiotic relationship.

The last of the major groups of soil bacteria, lithotrophs, sometimes known as chemoautotrophs, are organisms that obtain their energy from inorganic compounds such as nitrogen, sulfur, iron, etc. via electron transfer (Ingham, n.d.). This means that the electrons

move from an inorganic donor molecule to an acceptor molecule through a pathway that conserves the energy released when the electrons are transferring by “trapping it in a form that the cell can use for its chemical or physical work” (The Editors of Britannica, n.d.). The main form of energy that is captured from the transfer of this energy is ATP (The Editors of Britannica, n.d.), and that is why most lithotrophs are “aerobic respirers that produce energy in the same manner as all aerobic respiring organisms” (Todar, 2008). Today, they are among the most diverse group of prokaryotes on earth, only united in their ability to oxidize an inorganic compound as an energy source (Todar, 2008).

All of these types of bacteria can be greatly impacted by any changes to the chemistry of the soil because all are involved in the cycling of soil nutrients, and one of the major ways humans interfere with the chemistry of the soil is the application of fertilizer. Fertilizer is made up of chemical ingredients that aid the natural fertility of the soil and allow more plants to grow more efficiently. They do this because they have nutrients that contain three key elements: nitrogen, phosphorus, and potassium. The nitrogen, usually in the form of ammonium or nitrate, is a source of nitrogen plants use to make their proteins and nucleic acids, and the phosphorus is key to photosynthesis, respiration, cell division, and cell growth. Potassium, meanwhile, is important to photosynthesis because it regulates the most crucial factors used during it: the uptake of CO_2 and water and the regulation of a plant’s temperature.

Although fertilizer helps the plants get the nutrients they need to grow and thrive, it does not help the bacteria and other living things in the soil around the plants. When the fertilizer is applied to the soil, it changes the bacteria’s community structure and their ability to function correctly (Deobhani, S., & Cuttack, 2018, July 11). Furthermore, all the different types of fertilizers applied to the soil have this negative effect on the bacteria living there. Inorganic

fertilizer can make the bacteria population decline dramatically, and though organic fertilizer is slightly better, causing less of a decrease the bacteria population, it still causes a decrease (Nakhro, N., & Dkhar, M. S. 2010).

It can also affect the biodiversity of bacteria living in the soil. A diverse bacteria population in the soil increases the quality of the soil because each bacteria has a different role and job (Nakhro, N., & Dkhar, M. S. 2010). So, when fertilizer abuses the soil by killing the bacteria, it hurts the soil even more in the long run because the decrease in bacterial diversity will decrease the supply of nutrients to the plants (Schiffman, R. 2017, May 3). Overall, fertilizer may help plants grow more efficiently, but by harming soil bacteria, it removes the natural help plants need to grow, requiring even more fertilizer the next time, becoming a vicious cycle.

For this experiment, the objective is to find out how fertilizer impacts the biodiversity of bacteria in soil. We have hypothesized that fertilizer will kill bacteria unequally and decrease the diversity of the bacteria by killing one type more than another. We will test this hypothesis by sampling soil from two plots, one negative control plot and one plot to put fertilizer on. We will then use the serial dilutions process to test for the diversity of bacteria within the samples. We will then place fertilizer on one plot (F plot) and leave the other alone (NC plot) and wait 2 days to take more samples to be tested in the same way. This will allow us to see how fertilizer affects the diversity of bacteria within the soil.

Experiment Outline

Problem: How does fertilizer alter the diversity of bacteria in the soil?
Hypothesis: The fertilizer will decrease the diversity of the bacteria in the soil.
Procedure:

Independent Variable: the presence or absence of fertilizer applied to the soil
Dependent Variable: the density of the different color and size of colonies bacteria
Positive Control: soil samples collected before the fertilizer was added

Negative Control: soil being tested with only water applied to soil

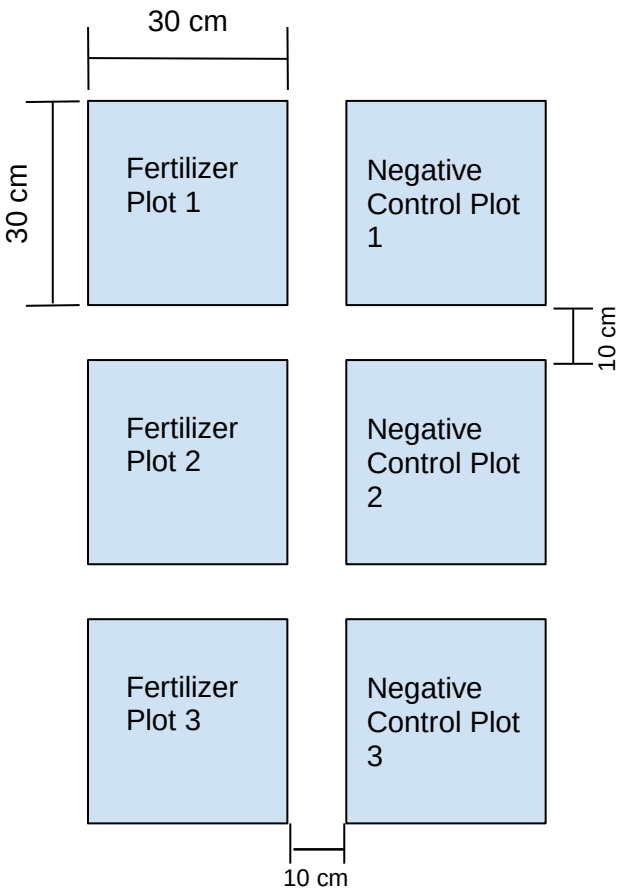
Controlled Variables:

- Location of soil plots (N 39°21.484 W 076°38.177)
- Type of fertilizer: Sta-Green 29-2-5
- Size of plot: 30cm X 30cm
- Distance between plots: 10cm
- Concentration of fertilizer applied to soil: 43 grams per 30cm X 30cm plot
- Day and time soil samples are taken
- Amount of water applied: 1 liter per 30cm X 30cm plot (water in fertilizer solution)
- Time fertilizer is on soil before after samples are taken: 48 hours
- Size of serological pipette: 10 mL
- Size of culture tube: 15 mL
- Source/type of water used for dilution: sterile water
- Nutrient agar plate used: 3M Petrifilm™ Aerobic Count Plate
- Amount of water in first culture tube (10^0): 10 mL
- Degree the bacteria is diluted: 10^{-3}
- Amount of water in all other culture tubes (10^{-1}, 10^{-2}, 10^{-3}): 9 mL
- Amount of soil added to first culture tube (10^0): 1 cc
- Amount of time bacteria grew on agar plate before collecting data: 96 hours/ 4 days
- Amount of dilution on agar plate: 100 µl
- The dilution of bacteria plated: dilutions 10^{-2} and 10^{-3}
- Type of bags used to carry soil samples: 16.5 by 14.9 cm Great Value ziploc bags
- Temperature of fertilizer solution: about 70°F (room temperature)
- Magnifying glass used to observe agar plates

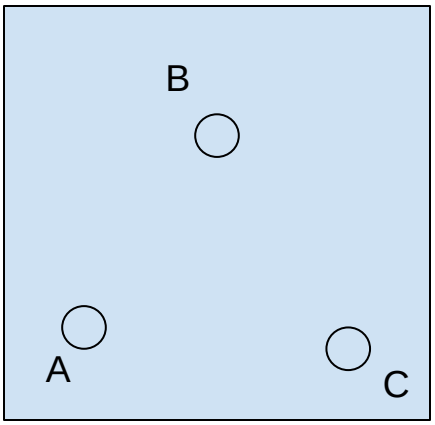
Steps:

1. Go to coordinates N 39°21.484 W 076°38.177
2. Label 4 flags “F1” (fertilizer plot 1), label 4 flags “NC1” (negative control plot 1), label 4 flags “F2” (fertilizer plot 2), label 4 flags “NC2” (negative control plot 2), label 4 flags “F3” (fertilizer plot 3), label 4 flags “NC3” (negative control plot 3)

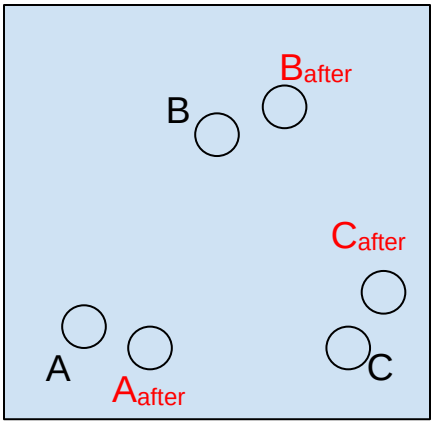
3. Mark off six 30 by 30 cm plots of grass by using flags at the corners of the boxes below and follow the model below:



4. Label 18 ziploc bags (16.5 by 14.9 cm) according to which soil sample will go into that bag (e.g. "$F1_a$" means fertilizer Plot 1, sample a)



5. Using the 2.5 cm diameter soil core extractor, take 3 separate samples of 2.5 cm in diameter X 15 cm deep from each plot and place in its correspondingly labeled bag, making sure to do this all on the same day at the same time.
6. Mix 43 grams of Sta-Green 29-2-5 fertilizer and 1 liter of water in a 1 liter bottle.
7. Add 1 liter of water to another bottle of the same size.
8. Label each bottle according to the plot it will be put on (e.g. “F1” for fertilizer, plot #1 or “NC1” for negative control plot #1)
9. Repeat steps 7-8 two more times for total of 3 times.
10. Pour each bottle of fertilizer or water onto its corresponding plot (bottle “F1” would be poured onto fertilizer plot 1) on the same day at the same time.
11. 48 hours after applying fertilizer to the F plots, return to the plots.
12. Label 18 ziploc bags (16.5 by 14.9 cm) according to which soil sample will go into that bag by using the following method (be sure to include the “A” so that it is clear these bags are from the AFTER fertilizer period of the experiment)(e.g. “$AF1_a$” means after fertilizer plot 1, sample a)
13. Using the 2.5 cm diameter soil core extractor, take 3 separate samples of 2.5 cm in diameter X 15 cm deep from each plot and place in its correspondingly labeled bag on the same day at the same time.

a. 

b. *Note that the new samples taken 48 hours after the application of the fertilizer have been taken within centimeters of the original sample locations to ensure accuracy of testing bacteria.

14. While completing the steps for number 13, label 12 3M Petrifilm™ Aerobic Count Plates according to the samples that will be on them (e.g. "$F1^{10\text{-}2}$" would have a 100-microliter sample of solution from the fertilizer, plot #1, dilution 10-2 culture tube)
15. Bring the bags of soil that were gathered before the fertilizer was put down and bring them back to the lab. Use the following steps to test the biodiversity of the bacteria for all before samples on the same day at the same time.
16. Label 6 Great Value ziploc bags (16.5 by 14.9 cm) according to the samples that will be put in them (e.g. "F1" for all of the soil samples from plot #1)
    a. Mix the three samples from each plot (e.g "$F1_a$", "$F1_b$", and "$F1_c$") together thoroughly in the corresponding bag (e.g. "F1") to make one sample for each plot, a total of 6 samples in 6 bags.
    b. Gather 24 15-mL culture tubes, 7 clean transfer pipettes, a 1-cc scoop, 1 micro pipette (with 12 disposable tips), sterile water, and a test tube holder.
    c. Label each culture tube according to the soil that will diluted in them (e.g. each dilution will need 4 culture tubes, one culture tube "$F110^{0}$", one "$F110^{-1}$", one "$F110^{-2}$", and the last one "$F110^{-3}$" than repeat for samples F2, F3, NC1, NC2, and NC3.
    d. Label one of the transfer pipettes "W" and use it to add 10 mL of sterile water to all of the tubes labeled with 10^{0}. This transfer pipette will be used only for sterile water.

e. Use the same pipette to add 9 mL of water to all of the 10^{-1} tubes.
f. Use the same pipette to add 9 mL of water to all of the 10^{-2} tubes.
g. Use the same pipette to add 9 mL of water to all of the 10^{-3} tubes.
h. Place 1 cc of the F1 soil sample, using the 1-cc scoop, into the "$F110^{0}$" culture tube. Repeat for all of the other samples washing out the 1 cc scoop every time you switch samples (F2, F3, NC1, NC2, and NC3).
i. Cap the tubes and shake vigorously.
j. Label another transfer pipette "F1" and use it to remove 1 mL of the soil/water mixture from the "$F110^{0}$" tube and place into the "$F110^{-1}$" tube. Then repeat for all of the other soil samples (F2, F3, NC1, NC2, and NC3) using different transfer pipettes corresponding to the soil samples they will be transferring (e.g. "F1"). There are now 7 transfer pipettes in total.
k. Cap the tubes and shake vigorously.
l. Using the "F1" pipette, remove 1 mL of the soil/water mixture from the "$F110^{-1}$" tube and place into the "$F110^{-2}$" tube. Repeat for all other soil samples using their corresponding transfer pipettes (F2, F3, NC1, NC2, and NC3).
m. Cap the tubes and shake vigorously.
n. Using the "F1" pipette once again, remove 1 mL of the soil/water mixture from the "$F110^{-2}$" tube and place into the "$F110^{-3}$". Repeat for all other soil samples using their corresponding transfer pipettes (F2, F3, NC1, NC2, and NC3).
o. Cap the tubes and shake vigorously.
p. Place a new, clean tip onto the P200 micropipette and use it to plate a 100 µl sample from the "$F110^{-2}$" culture tube onto a correspondingly labeled 3M Petrifilm™ Aerobic Count Plate.
q. Using the petrifilm spreader flatten out the solution.
r. Replace the tip of the micropipette with a new, clean tip and use it to plate a 100 µl sample from the "$F110^{-3}$" culture tube onto another correspondingly labeled 3M Petrifilm™ Aerobic Count Plate.
s. Then repeat steps 13q-13s for all of the other soil samples (F2, F3, NC1, NC2, and NC3).
t. Using the petrifilm spreader flatten out the solution.

u. Allow to grow for 96 hours (four days).

v. Examine the "F110^{-2}" and "F110^{-3}" 3M Petrifilm™ Aerobic Count Plates for large red, small red, large pink, small pink, and tiny colonies of bacteria and make your estimates of the number of bacteria in the original 1 cc or soil sample using the following formula: # of microbes in 1 cc of soil = # colonies on sheet x 10^2 x $10^{|\text{dilution \# at which these colonies were found}|}$ and record in the data table.

w. Repeat step v for all of the other soil samples (F2, F3, NC1, NC2, and NC3).

17. Repeat step 16, making sure to test the biodiversity of the bacteria in the "after" soil samples.

Data & Observations

Amount and Diversity of Bacteria in Soil Before Application of Fertilizer (per 1 cc of soil)

Sample Tested	Large Red Colonies	Small Red Colonies	Large Pink Colonies	Small Pink Colonies	Tiny Colonies
NC1	700,000 bacteria per cc of soil	600,000 bacteria per cc of soil	500,000 bacteria per cc of soil	1,200,000 bacteria per cc of soil	4,700,000 bacteria per cc of soil
NC2	200,000 bacteria per cc of soil	100,000 bacteria per cc of soil	200,000 bacteria per cc of soil	300,000 bacteria per cc of soil	600,000 bacteria per cc of soil
NC3	900,000 bacteria per cc of soil	800,000 bacteria per cc of soil	1,200,000 bacteria per cc of soil	2,100,000 bacteria per cc of soil	5,900,000 bacteria per cc of soil
NC Averages	**600,000 bacteria per cc of soil**	**500,000 bacteria per cc of soil**	**633,333 bacteria per cc of soil**	**1,200,000 bacteria per cc of soil**	**3,733,333 bacteria per cc of soil**
F1	400,000 bacteria per cc of soil	200,000 bacteria per cc of soil	200,000 bacteria per cc of soil	400,000 bacteria per cc of soil	500,000 bacteria per cc of soil
F2	200,000 bacteria per cc of soil	300,000 bacteria per cc of soil	200,000 bacteria per cc of soil	400,000 bacteria per cc of soil	700,00 bacteria per cc of soil

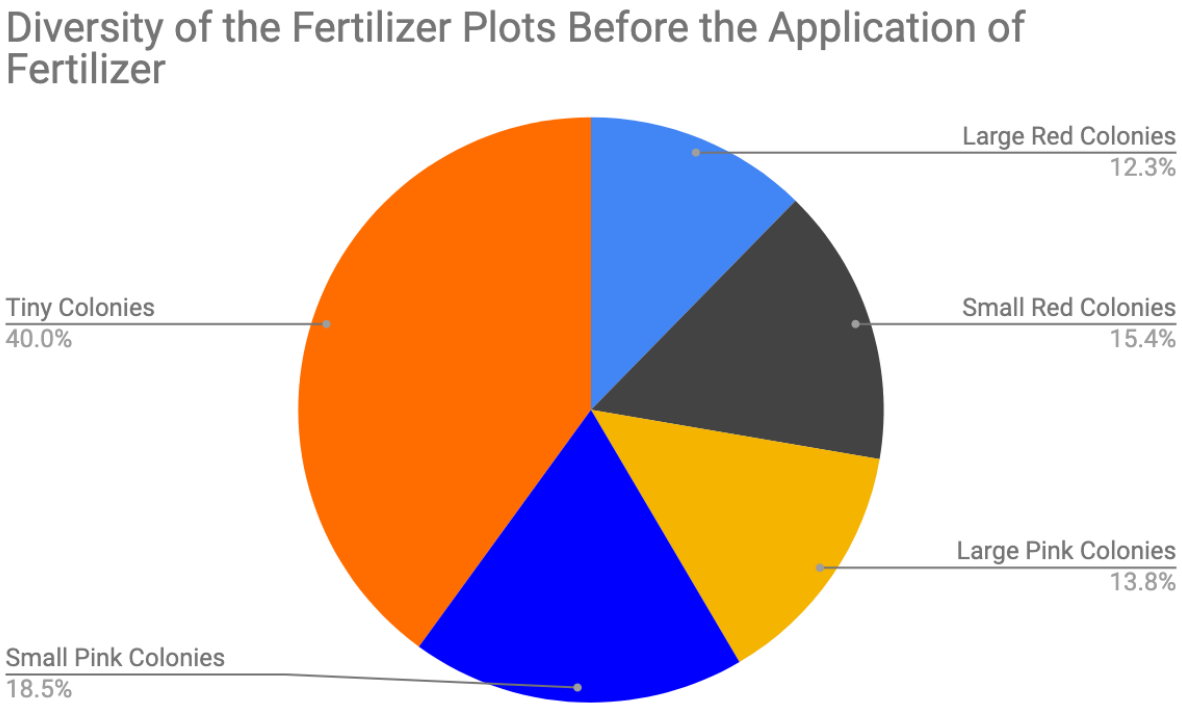
F3	200,000 bacteria per cc of soil	500,000 bacteria per cc of soil	500,000 bacteria per cc of soil	400,000 bacteria per cc of soil	1,400,000 bacteria per cc of soil
F Averages	**266,667 bacteria per cc of soil**	**333,333 bacteria per cc of soil**	**300,000 bacteria per cc of soil**	**400,000 bacteria per cc of soil**	**866,667 bacteria per cc of soil**

Amount and Diversity of Bacteria in Soil After Application of Fertilizer (per 1 cc of soil)

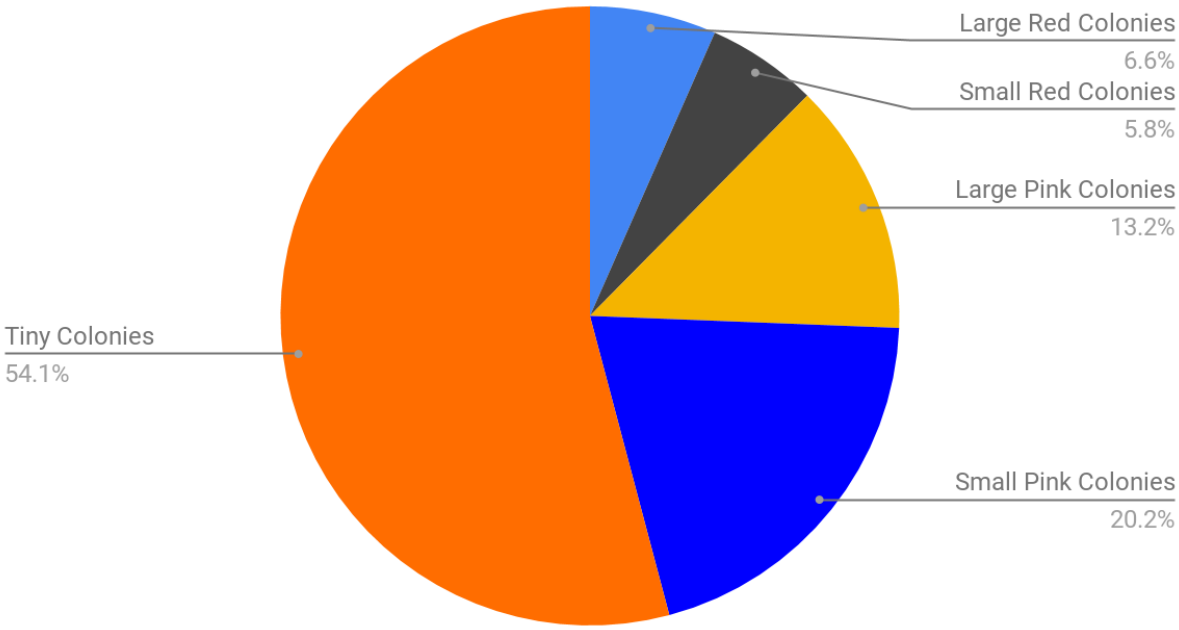
Sample Tested	Large Red Colonies	Small Red Colonies	Large Pink Colonies	Small Pink Colonies	Tiny Colonies
ANC1	200,000 bacteria per cc of soil	400,000 bacteria per cc of soil	200,000 bacteria per cc of soil	500,000 bacteria per cc of soil	3,000,000 bacteria per cc of soil
ANC2	300,000 bacteria per cc of soil	500,000 bacteria per cc of soil	400,000 bacteria per cc of soil	400,000 bacteria per cc of soil	1,900,000 bacteria per cc of soil
ANC3	400,000 bacteria per cc of soil	300,000 bacteria per cc of soil	500,000 bacteria per cc of soil	600,000 bacteria per cc of soil	900,000 bacteria per cc of soil
NC Averages	**300,000 bacteria per cc of soil**	**400,000 bacteria per cc of soil**	**366,667 bacteria per cc of soil**	**500,000 bacteria per cc of soil**	**19,333,333 bacteria per cc of soil**
AF1	500,000 bacteria per cc of soil	600,000 bacteria per cc of soil	1,900,000 bacteria per cc of soil	3,300,000 bacteria per cc of soil	5,300,000 bacteria per cc of soil
AF2	800,000 bacteria per cc of soil	400,000 bacteria per cc of soil	500,000 bacteria per cc of soil	700,000 bacteria per cc of soil	3,200,000 bacteria per cc of soil
AF3	300,000 bacteria per cc of soil	400,000 bacteria per cc of soil	800,000 bacteria per cc of soil	900,000 bacteria per cc of soil	4,600,000 bacteria per cc of soil

F Averages	**533,333 bacteria per cc of soil**	**466,667 bacteria per cc of soil**	**1,066,667 bacteria per cc of soil**	**1,633,333 bacteria per cc of soil**	**4,366,667 bacteria per cc of soil**

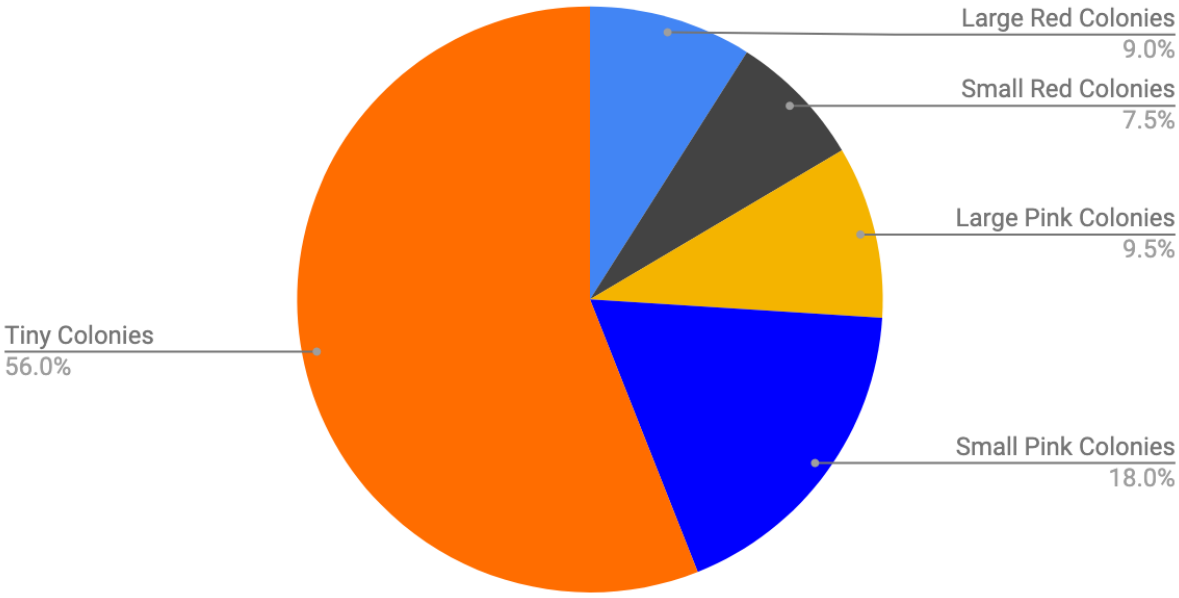
Key:
F- Fertilizer
NC - Negative Control
- plot # (e.g. NC1 means Negative Control Plot #1)

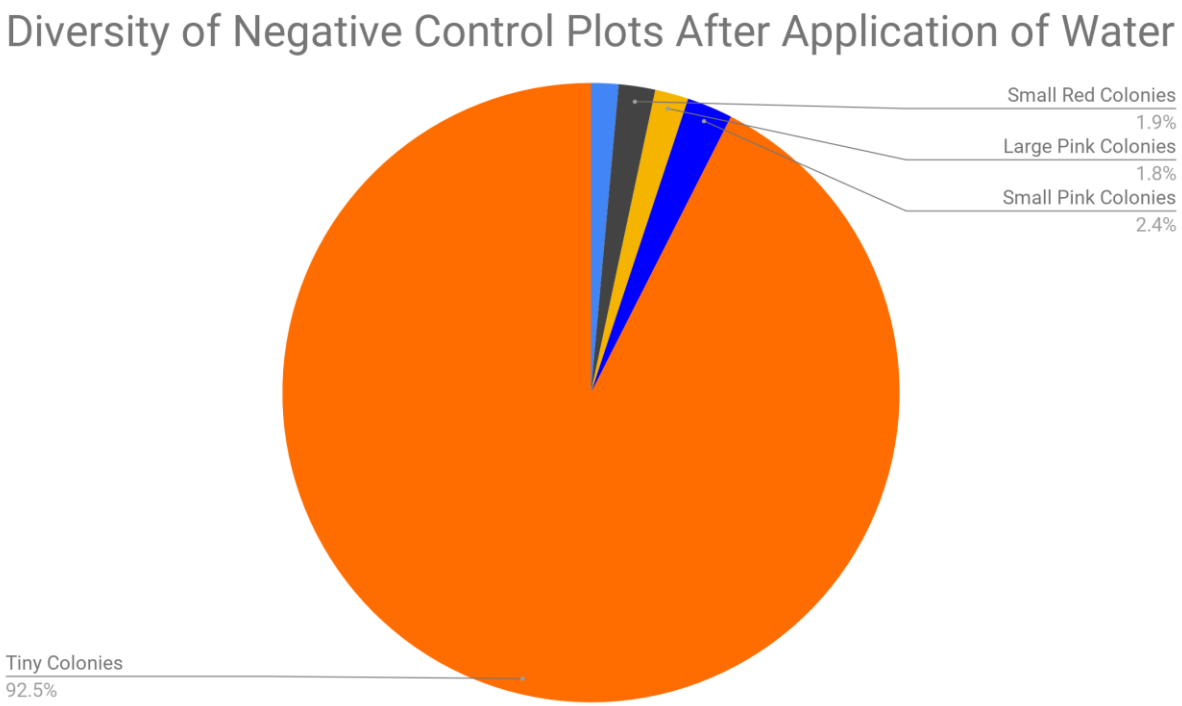


Diversity of the Fertilizer Plots After the Application of Fertilizer



Diversity of Negative Control Plots Before the Application of Water





Conclusion

Our hypothesis, which stated that fertilizer will decrease the diversity of bacteria in the soil, was proven incorrect because instead of decreasing the diversity of the soil, the fertilizer stabilized the diversity of the population. Our data indicates that, at the time that the positive control samples were taken, the bacteria in the fertilizer plots as opposed to the negative control plots were relatively similar. Based on the pie charts above, one can conclude that the diversity of bacteria in the fertilizer plots before fertilizer was applied was similar to that of the the negative control plots before water was applied. The positive control samples were taken on May 6th and two days later on May 8th, fertilizer was applied to the fertilizer plots, and water was applied to the negative control plots. On May 10th samples were taken again from the fertilizer and negative control plots. Over the course of the 4 days between taking the positive control

samples and the samples taken after the application of fertilizer or water, approximately 0.56 inches of rain fell (NOAA, 2019). We also poured 1 liter of water onto each negative control plot and 1 liter of water mixed with 43 grams of fertilizer onto the fertilizer plots. This resulted in heavy saturation of water on both the negative control fertilizer plots from the rain and the extra water poured for the experiment onto each plot.

The saturation of the soil due to so much excess water coupled with the poor drainage on our campus resulted in the closing of pores in the soil so that oxygen could not get through to the bacteria in the soil, thus creating an anaerobic environment. Anaerobic bacteria thrive in environments with little to no oxygen while aerobic bacteria need oxygen to survive (Lowenfels & Lewis, 2006). Because of this anaerobic environment, the bacteria that require less oxygen were able to thrive and take over the population in the case of the negative control plots. However, in the case of the fertilizer plots the diversity of bacteria in the soil stayed relatively similar to the percent populations taken from the positive control samples. The fertilizer was able to stabilize the diversity of the population of bacteria within the soil by providing the bacteria with an alternate source of energy that did not require the intake of oxygen, preventing the anaerobic bacteria to take over and decrease the diversity of the soil.

The three main elements in fertilizer that help plant growth are phosphorous, nitrogen, and potassium. Phosphorous aids in the production of ATP which allows the bacteria to make energy without oxygen. If bacteria that has make contact with fertilizer is able to create its own energy without oxygen, it is more likely to survive in an anaerobic environment, such as the one created by the oversaturate soil. The population of bacteria in the negative control plots did not remain diverse because there was no phosphorus provided by any fertilizer to help the aerobic bacteria survive without oxygen, so the anaerobic bacteria took over the population, thus

decreasing the diversity of the soil. The diversity of the fertilizer plots changed slightly by comparison to the diversity of the positive control samples. The pink colonies were able to maintain their percent population while the tiny colonies grew and the red colonies shrunk because each type of bacteria has a differential in their ability to absorb nutrients which gives the tiny colonies and the pink colonies an advantage in the competition for the limited nutrients in the soil.

For further research in order to understand the results of our experiment further, a new experiment would have to be designed in which the problem might be "how does oversaturating the soil with water affect the diversity of bacteria within the soil?" In this experiment, the steps would be similar to those of our above procedure. Positive control samples would be taken, but instead of applying fertilizer, we would apply a great deal of water to half the plots and no water at all to the negative control plots. Further serial dilution tests would be done to determine the diversity of the soil and how the water affected the diversity of the population of bacteria in the soil.

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