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Biology 9H

Mr. Brock

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Final Report

Background:

Bacteria are prokaryotic organisms that are some of the smallest and most abundant microbes in soil, and they play a huge role in decomposing of organic materials, suppressing diseases, and breaking down organic compounds such as the pesticides and pollutants which humans often dump there (Ingham, 2015). For example, the nitrogen fixing bacteria regulate the amount of nitrogen in the soil, and they fix nitrogen to make it available for other organisms by extracting nitrogen gas from the air and converting it into forms that plants can use (Reid, Wong, 2005). Meanwhile other bacteria help the ecosystem by immobilizing nutrients in their cells to prevent loss through runoff and erosion (Ingham, 2015), and certain bacteria also affect the water dynamics of soil by producing substances that bind soil particles into small aggregates that improve water infiltration and soil's water holding ability (Ingham, 2015).

Of their many tasks in the soil ecosystem, though, none is perhaps as significant as the role bacteria play in the nitrogen fixation process. One of the most important elements on earth is nitrogen and as a gas, it makes up 78% of the earth's atmosphere. But most living things in an ecosystem, including the producers, (the plants) cannot use any of this form of nitrogen, and in order for the plants and everything else in the food chain to be able to use it, the nitrogen in the atmosphere needs to be chemically mixed with oxygen or hydrogen to "fix it" to make it more accessible. This is the role different groups of soil bacteria play in the ecosystem. For example, bacteria called Rhizobia can convert the unusable nitrogen gas into nitrogen compounds that can be used to help plants, and it is one of the reasons, the plant and the rhizobia are co-dependent on each other because the rhizobia keeps the

plant working at its best while the plant offers protection and nutrients to the rhizobia (Science Buddies Staff, 2013).

But all plants are dependent on some type of fixed nitrogen, and the way the Rhizobia and other groups of bacteria complete this task is through six different processes that include nitrogen fixation, nitrification, assimilation, ammonification, and denitrification (Ecosystem: The Nitrogen Cycle, 2015). In the first step of the process, bacteria produce the enzymes (nitrogenases) responsible for converting the nitrogen gas into ammonium (Postgate, John R. 2014; Ecosystem: The Nitrogen Cycle.2015). But these proteins are denatured by oxygen; so they require an anaerobic environment to operate properly, which is why nitrogen fixation must take place deep within the soil (Freedman, B. 2014.B). Following this initial fixation, nitrification occurs in which the ammonium is converted into nitrates by bacteria by changing the number of the electrons of the nitrogen in the ammonia to produce nitrous and nitric acids or salts through oxidation (Ecosystem: The Nitrogen Cycle. 2015; Access Science, 2015). The plants then absorb the nitrates from the soil into their roots through the process of assimilation. This nitrogen is crucial to the plants survival, as it makes up the base of all amino acids, which are needed in proteins. The nitrogen is also in nucleic acids, which make DNA, RNA and enzymes. (Campbell, N., & Williamson, B. (2004); Ecosystem: The Nitrogen Cycle. 2015). These things aid in helping the plant do things such as reproduction, and in the case of chlorophyll, help with the process of photosynthesis.

Once assimilation has been completed, the now biological forms of nitrogen move through the food chain, providing primary consumers, secondary consumers, and tertiary consumers with nutrients that help them form amino acids, DNA, RNA, and enzymes, enabling all the organisms in the ecosystem to copy their genetic instructions into RNA to create the enzymes needed start and stop chemical reactions between the biological molecules that enable a cell to perfrom its four tasks: reproduction, transforming energy, homeostasis, and synthesis. IN other wods, assimilation allows all the organisms in an ecosystem to live and when all these organisms (including the producers), die and their remains and

their waste products are decomposed by microorganisms in the process of ammonification (Nitrogen Cycle. 2015) , accessible nitrogen can reenter the nitrogen cycle, and any excess nitrate undergoes denitrification, in which special bacteria convert some of the nitrates back into the nitrogen gases, which is released into the atmosphere.

Like the nitrogen cycle, the phosphorus cycle is essential to the process of plant growth. The phosphorus cycle begins with rocks or other deposits such as bone and bird dropping that erode, and when the rocks are eroded and weathered upon, they release phosphate. This phosphate is essential to life, and shows up in ATP. Phosphate is also the base of DNA and RNA. When the phosphate comes out of the rocks it enters into the soil and then goes into plants and waste products. When these decay, they release the phosphate back into the environment. This phosphorus goes into the water, where it aids in the growing of water plants and algae. Before eventually sinking down to the deep sediments where it goes into rocks and is uplifted through continental drift, which starts the cycle over again (Ophardt, 2003). Since phosphorus is a key ingredient in the natural growth of plants, it makes sense to see that is one of the key ingredients in commercial fertilizers.

Fertilizers are used in soil to improve the growth and productiveness of plants (Fertilizer, 2015). They are designed to enhance the natural fertility of the soil and replace the chemical elements taken from the soil by previous crops (Fertilizer, 2015). All fertilizers supply three key elements that are useful in plant nutrition: nitrogen, phosphorus, and potassium. As seen above, nitrogen and phosphorus are critical for the growth and development of plants. But potassium is also vital because it plays a fundamental role in the water management of the plants (Longacre, Alan, Papendick, R. I., and Parr J. F. 2014). Futhermore, Potassium is involved in the oxidative reactions that are the carriers for the iron need by enzymes to convert sugar to starch and amino acids to proteins (Longacre, Alan, Papendick, R. I., and Parr J. F. 2014). Many people use fertilizer to keep their plants green and healthy, but most cases, this is also harmful to the plant, especially in excess amounts.

The use of fertilizers can reduce the use of herbicides and pesticides as well as aide plants in surviving difficult situations by increasing their capability to hold more water and increasing root depth. But when commercial cropping is used, the availability of nitrogen in the soil diminishes (Longacre, Alan, Papendick, R. I., and Parr J. F. 2014; Fertilizer, 2012), and due to this human activity, the normal nitrogen cycle is altered by additional nitrogen the fertilizer adds to the soil. When fertilizers are added to the ground, ammonium is added with them, and ammonium is the waste product of the bacteria that produce fixed nitrogen naturally. Therefore when humans put fertilizer in, they are essentially drowning the bacteria in their own waste. When this happens, the bacteria cannot do what they need to, and the plants don't prosper as the humans expect them to, so humans put in more fertilizer, therefore putting in more waste product. This disrupts the natural nitrogen cycle even further. If this continues, the effects on the nitrogen cycle could be harmful for the plants, and even cause long term damage.

In testing, we hope to conclude that the fertilizer impacts the bacteria population in the soil, either positively or negatively. It is important to test this because the fertilizers may impact other good bacteria that are crucial to the plant's growth. In this experiment we will search for relationships between nitrogen, bacteria, and fertilizer and how it impacts the bacterial life in the soil. The RPCS community relies on its fertilizer, and it is important to know how it impacts the soil life.

Lab Report:

I. Problem: Does the population of soil bacteria increase when the chemicals in fertilizer are added to soil?

II. Hypothesis: The density of soil bacteria increases when fertilizer is added to soil.

III. Procedure:

A. Independent Variable: applying fertilizer to the soil

B. Dependent Variable: amount of bacteria per cubic centimeter of soil

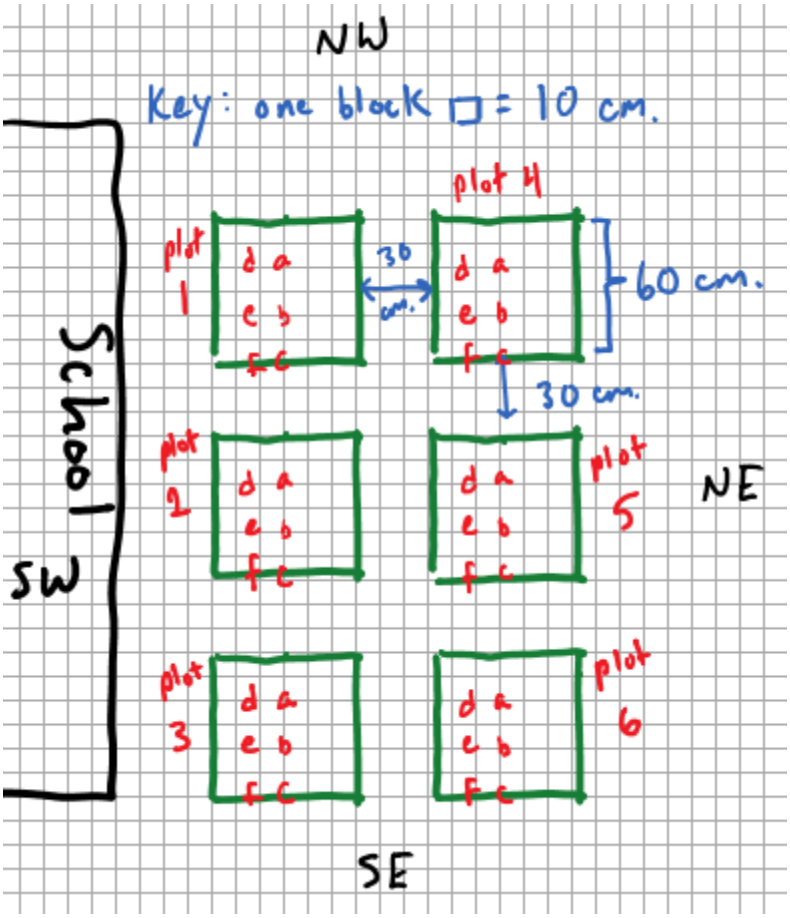
C. Negative Control: add only water instead of fertilizer to the soil

D. Positive Control: soil samples before applying treatment

D. Controlled Variables: area where soil is taken from, amount of soil collected, soil is collected same time of day and same day, brand of fertilizer, size of soil extractor, amount of time fertilizer and water is left on the grass, amount of time bacteria is growing, amount of fertilizer and water added to soil, size of culture tubes, season of testing, size of plots, number of plots, sterilization of water, cleanliness of dilution tubes, distance between plots, how shaken the dilution is, pipette size, amount of sterile water used for dilutions, amount of soil-water solution put on the plate, type of nutrient agar plate, dilute to the same dilution level, clean pipette used for each test, which dilution levels plated, amount of soil added to dilutions, serial dilutions done same time same day

E. Step-by-Step Instructions

1. On the RPCS campus front lawn (GPS location N 39.358° W 76.6361°) , measure out six 60 cm. by 60 cm squares, that are each 30 cm. apart (horizontally and vertically on the ground). Set the plots so that there are 2 columns that each have three plots. Mark the four corners of each plot with flags. Refer to the following diagram for guidance.



2. Label the plots in the south west column plots 1, 2, and 3. Label the plots in the north east column 4, 5, and 6.

3. Begin collecting the positive control soil samples at 10:00 a.m. Be sure to collect all the samples by doing steps 4-6 on the same day. Using the 28 cm tall soil cylinders, extract three samples of soil, 14 cm deep and 2.3 cm wide, from each plot.

4. Collect samples from locations 1a, 2a, 3a, 4a, 5a, and 6a in the middle of each plot and 20 cm. from the north west side of the plot. Place each of the soil samples into a fresh plastic bags, corresponding to its label 1a, 2a, 3a, 4a, 5a, or 6a. Be sure to set up plots and collect samples on the same day at the same time.

5. Collect samples 1b, 2b, 3b, etc. from locations in the middle of each plot and 40 cm. from the north west side of the plot. Place each of the corresponding samples into fresh plastic bags and labeled 1b, 2b,or 3b.

6. Collect samples 1c, 2c, 3c, etc. from locations in the middle (30 cm over) of each plot on the southeast edge of the plot. Place each of the corresponding samples into fresh plastic bags and labeled 1b, 2b,or 3b.

7. Mark each spot where the soil sample is collected with a flag.

8. In plots 4, 5, and 6, evenly apply to each plot 16.8 grams of Sta-Green lawn fertilizer (three times the recommended amount).

9. Fill six 1 liter of bottles with tap water. Using the 1 liter bottles, pour 1 liter of tap water evenly on each plot.

10. Let soil in plots 4, 5, and 6 absorb the fertilizer for at least 2 days. Plots 1, 2, and 3 are being used for the negative control of just water.

11. While the soil is absorbing the fertilizer, use serial dilutions in steps 12-25 to test for population of bacteria in all positive control samples. Be sure to perform the serial dilutions for all samples of all plots at the same time on the same day.

12. For each plot, combine the three samples (a,b,c) in a plastic bag. Shake the bag to evenly mix the three samples together.

13. Use a clean new transfer pipette to add 10 ml of sterile water to a 15 ml culture tube. Label the tube "10^{o}" and "plot 1".

14. Use the same pipette to add 9 ml of sterile water to a second 15 ml culture tube. Label the tube "10^{-1}" and "plot 1"

15. Repeat step 14 two more times to two additional 15 ml culture tubes, only label them "10^{-2}", and "10^{-3}" respectively along with labeling them all with "plot 1".

16. Place 1 cc of your soil sample from plot 1 into the "10^{0}" culture tube.

17. Cap the tube and shake vigorously.

18. Using a new clean pipette, remove 1 ml of the soil/water mixture from the "10^{0}" tube and place 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}" and place 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}" and place into the "10^{-3}" tube.

23. You should now have a total of four culture tubes.

24. Shake the 3rd and 4th tubes. Plate 100 µl samples from the 3rd and 4th tubes (dilutions 10^{-2} & 10^{-3}) onto their own separate3M Petrifilm™ Aerobic Count nutrient agar Plate , labeled "plot 1" and their corresponding dilution number. Use spreader to spread the bacteria sample out flat on the nutrient agar plate. Be sure to label the sample and the dilution on the nutrient agar plates at the top.

25. Repeat steps 13-24 for the five other plots, changing the labels to correspond to the correct soil sample.

26. Allow bacteria to grow for 48 to 72 hours.

27. Examine each of the plates for individual bacterial colonies and choose the plate with the fewest colonies (but at least 5) and at the lowest dilution value to make your 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 $10^{|\text{dilution \# at which these colonies were found}|}$. Record data.

28. After 2 days of fertilization, using the 28 cm tall soil cylinders, extract three samples of soil, 14 cm deep and 2.3 cm wide, each from plots 4, 5, and 6 at the same time. Be sure to do this at 10:00 am, and be sure to collect all of these samples on the same day. At the same time and day, collect three samples each from plots 1, 2, and 3 with just water added. Do this to control for steps 29-31.

29. Collect samples 1d, 2d, 3d, 4d, 5d and 6d from locations 15 cm. from the south west side of the plot and 20 cm. from the north west side of the plot. Place the samples in fresh plastic bags. Be sure to label them 1d, 2d, 3d, etc. Be sure to collect samples on the same day at the same time.

30. Collect samples 1e, 2e, 3e, 4e, 5e, and 6e from locations 15 cm from the south west side of the plot and 40 cm. from the north west side of the plot. Place each of the the samples in fresh plastic bags corresponding to its label them 1e, 2e, or 3e.

31. Collect samples 1f, 2f, 3f, 4f, 5f, and 6f from locations 15 cm from the south west side of the plot and on the southeast edge of the plot. Place each of the samples in fresh plastic bags labeled 1f, 2f, or 3f.

32. For each plot, combine the three samples (a,b,c) in a plastic bag. Shake the bag to evenly mix the three samples together.

33. Repeat steps 12-27 for the samples for each plot mixture to test for the population of bacteria for soil with water and soil with water and fertilizer using the serial dilutions process. Clean dilution tubes thoroughly between each sample. Be sure to dilute all samples on the same day at the same time.

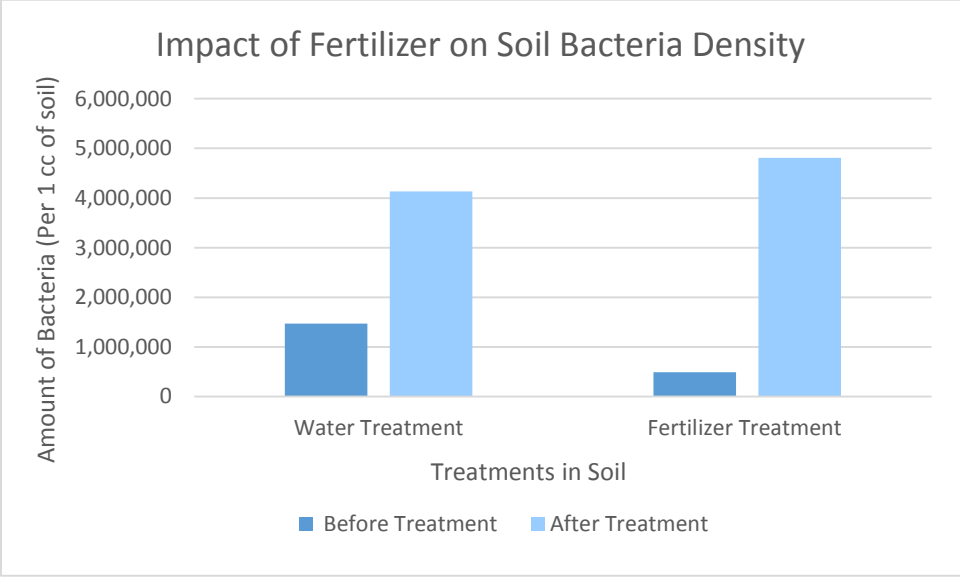
IV. Data and Analysis

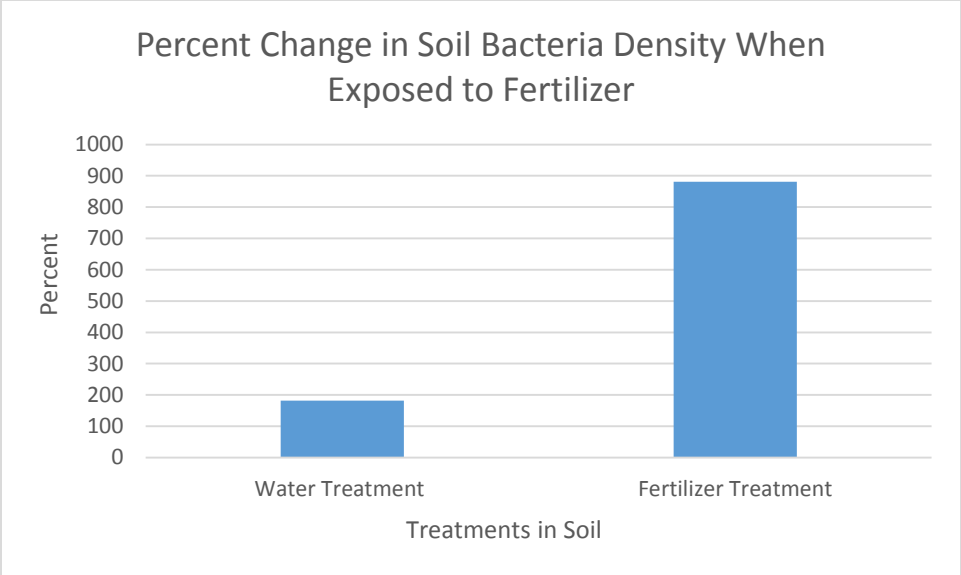
A. Data Table

Bacteria Population Change Following Fertilizer Treatment (Bacteria per 1 cc of soil)

	Water Treatment Plots		Fertilizer Treatment Plots	
Trial	Before Treatment	After Treatment	Before Treatment	After Treatment
1	1,700,000	1,700,000	700,000	3,200,000
2	1,100,000	6,700,000	270,000	10,100,000
3	1,600,000	4,000,000	500,000	1,120,000
Averages	**1,466,666**	**4,133,333**	**490,000**	**4,806,666**

B. Graphs





V. Conclusion

Our hypothesis was supported because the density of soil bacteria increased when we added Sta- Green fertilizer to the soil. For our negative controls plots 1-3, where just water was added, the average soil bacteria density increased by 2,666,667 microbes per 1 cc of soil. When we added three times the recommended amount of the Sta-Green fertilizer to plots 4-6, the average soil bacteria density increased by 4,316,666 microbes per 1 cc of soil. The average percent change for our negative control was 181.818%. Due to the steep change in bacterial density for our negative control, we can conclude that adding water in addition to natural events in the environment affected the density of bacteria. The average percent change for our plots that went through our Sta-Green fertilizer treatment was 880.952%. However, as a result of the environmental factors and water that caused a change in bacterial density, the Sta-Green fertilizer treatment only caused an average of 699.134% increase in bacterial density. The Sta-Green fertilizer increased the average bacterial density for our independent variable plots by approximately 7 times the original density because the fertilizer contains nitrogen supplements for the plants. In the Sta-Green Lawn fertilizer that was used in our experiment, the NPK (nitrogen- phosphate- potassium) ratio was 29-2-5. The nitrogen was made of 0.8% ammoniacal nitrogen and 28.2% urea nitrogen (7.2% derived from the slowly available nitrogen from the polymer-

coated urea). Urea nitrogen, once applied on the soil's surface, causes a chemical reaction that converts the urea to ammonium bicarbonate. The ammonium is then changed into the gas form of nitrogen (Rose, L. 2015). In order to take in the ammonium compounds, the rhizobia bacteria must fix the gas nitrogen to make it usable for the plants' root absorption. The bacteria is essentially eating the ammonium compounds to convert the ammonium into available nitrate that makes up plants' amino acids. One of the main roles of soil bacteria is to fix nitrogen to make it available for other organisms. Soil bacteria is needed to regulate the amount of nitrogen available for the plants. As the nitrogen content in the soil has increased because of the fertilizer, the bacteria density must also increase in order to regulate the amount of usable nitrogen for the plants (Science Buddies Staff, 2013). To test this theory that the application of urea nitrogen from the fertilizer causes an increase in bacterial density, we would pose an experiment where potassium, phosphate, and urea nitrogen are applied to multiple plots. Then, using serial dilutions, we could test which nutrient supplement caused the greatest increase in bacterial density. This future experiment could determine which element in the fertilizer causes the greatest increase and growth in bacterial density.

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