Background

Bacteria are prokaryotic cells that have no nucleus or membrane bound organelles. They can be categorized by three main shapes including rods, spheres, and spirals. Two major types of bacteria are gram positive and gram negative which differ in their cell wall structure. Bacteria reproduce by binary fission which allows them to multiply into billions in a very short period of time. Bacteria are much simpler than other organisms, and are affected much more by a decrease in temperature or a change in acidity (Aggie Horticulture, unknown). When an environment becomes unfavorable, it can cause the bacteria to become inactive or die. This would be particularly dangerous in environments such as the soil ecosystem, where bacteria perform a large variety of chemical transformations to control soil health including decomposition, maintaining soil structure, and nitrogen fixation. (University of Western Australia, 2004). In this experiment, our group is looking at how the chemical transformations are impacted when the soil environment is changed by humans (Aggie Horticulture, unknown).

Bacteria live mainly in the pores of soil and serve as important contributors to the nitrogen cycle. In the nitrogen cycle, nitrogen fixing bacteria turn ammonia (which comes from the process of decomposition) into ammonium, which is then converted by nitrifying bacteria into nitrites. Afterwards the nitrifying bacteria turn the nitrites into nitrates, which help the plant to grow. Plants need nitrogen to grow and synthesize new proteins. Specifically, DNA and RNA have nitrogenous bases and make up every living being. Amino acids in proteins also contain nitrogen. Enzymes are special proteins whose job is to start and stop chemical reactions, which are necessary to perform the 4 tasks of life. The 4 tasks of life are reproduction, production of new materials, homeostasis/regulation of the environment, and transformation of energy. Since the ability to perform the 4 tasks of life qualifies an organism as living, nitrogen is a crucial element in cells. Without nitrogen neither plants nor any other organism would be able to live.

Another major task that bacteria perform in the soil is decomposition (The University of Western Australia, 2004). Decomposition is the process that breaks down large organic matter into smaller pieces. First, larger invertebrates break down matter into smaller pieces which is then given to bacteria to fully decompose. Bacteria use special enzymes to transform polymers into monomers, which can be re-used to form the five biological molecules (water, carbohydrates, lipids, nucleic acid, and proteins) in new cells. In addition, bacteria extract nutrients from decomposed material for their own food and then leave excess nutrients for other organisms to use. Without decomposition, there would be a countless amount of unused biomass in the soil (NET Industries, 2014).

Soil is composed of many nutrients, elements, and materials including water, oxygen, charged ions, silicon, aluminum, iron, magnesium, calcium, sodium, and potassium. Some of these elements come together in different forms to create minerals. Soil chemical properties are greatly influenced by carbon, hydrogen, nitrogen, and sulfur found within organic matter. The organic matter develops the structure and collection of the soil and stores necessary nutrients that need to be provided for organisms to live and grow (Texas A&M University, unknown). The physical properties of the soil structure are determined by the size, shape, and the placement of the soil particles. These key factors in particle arrangements create pores in the soil. The size of the pores affects the velocity of air and water, traveling through the soil, as well as the amount of water and nutrients that the plant can absorb (Baker, unknown). Soil aggregates are about a thousandth of a millimeter to 2 millimeters across in size. Aggregates make the soil more stable and less likely to erode. Bacteria can be involved in soil aggregation in two ways. One way is by producing organic compounds called polysaccharides that “[resist] decomposition long enough to be involved in holding soil particles together in aggregates.” Bacteria are also involved through developing an electrostatic charge that brings the small aggregates of soil together (University of Western Australia, 2004). The pore space stores oxygen and water. The stored oxygen is used by bacteria in the process of cellular respiration to extract energy and the water is used by the plant in the process of photosynthesis so the plant can make its own food. A plant’s growth can be affected by the soil’s physical and biological properties. Overall, these microorganisms play a crucial role in maintaining the physical properties of soil structure, which can have a profound effect on plant growth.

When the microorganisms in the natural ecosystem cannot support enough producers, human fertilizers speed up plant growth. Fertilizers are mainly used by farmers in order to produce crops more quickly. When the same plot of soil is used repeatedly for a long period of time, the soil loses its nutrients and can no longer sustainably support plants. The USPEA states “Fertilizer application is required to replace crop land nutrients that have been consumed by previous plant growth. It is essential for economic yields.” (USEPA, 2010). Fertilizers recondition the soil and create an environment where plants can thrive. Humans can increase the rate of plant growth by giving these necessary nutrients directly to plants through fertilizer (University of Tennessee, date unknown). Specifically, fertilizers provide plants with a larger amount of essential chemical elements including nitrogen, phosphorus, and potassium. Nitrogen improves the growth and yield of crops since it is necessary in nucleic acid synthesis and protein synthesis. Potassium speeds up crop maturity and strengthens the immune system of the plant in order to fight crop disease. Phosphorous is used in the creation of ATP so cells have energy to perform tasks (EuroChem, 2013). Extra nitrogen can be added through the use of fertilizers.

Organic fertilizers are made from a variety of organic materials including animal manures, compost, bone meal and blood meal. This fertilizer must be broken down by microorganisms before it can be used by plants. While this is an extra step, it does not disrupt the natural cycle of nutrients in the soil, and maintains the role of microorganisms. Inorganic fertilizers, on the other hand, introduce important compounds to the soil which can be immediately used by the plant. Therefore, a significant portion of the bacteria population is not being used to transform nitrogen or extract nutrients. In addition, inorganic fertilizers are most likely to cause leaching; this is when the rain washes the irrigation water down to the plant roots. Chemical fertilizers can "burn" or “dry out” the plant seeds when it washes down to the roots (Boston Gardening, 2014). Then again, chemical fertilizers can benefit the soil as well.

When chemical fertilizers are introduced into the soil, all they introduce ammonium and extra of nitrogen which feeds the nitrifying bacteria. The population of nitrifying bacteria increases, which increases the amount of nitrate produced. As the amount of nitrate increases in the soil, the more plants can grow. In order to continue to feed this growing population of nitrifying bacteria, it is necessary that the fertilizer is continually added into the soil. Once fertilizer is no longer used, this reduces the food source for nitrifying bacteria, which causes some bacteria to die and reduces the amount of nitrate produced. When the nitrate levels fall, the plants begin to die. The system will crash once the fertilizer is no longer used because the fertilizer is no longer providing a food source for the large bacteria population. Therefore the plants have become dependent on chemical fertilizer to survive and will die without it (National Geographic, 2014). Organic fertilizers, on the other hand, add a lower concentration of nutrients which are released overtime. Organic fertilizers also add microorganisms, allowing the bacteria population to transform nutrients for plants to grow.

The inorganic fertilizer will work faster than the organic fertilizer because it will provide an immediate food source for the bacteria, but over a longer period of time the bacteria density influenced by the organic fertilizer will surpass the inorganic plots because it gradually releases its nutrients. In our experiment, we are testing whether inorganic or organic fertilizer will increase the population density of bacteria after 72 hours. We hypothesized that the organic fertilizers will ultimately increase the population density of bacteria compared to inorganic fertilizers.

Lab Report

I. **Problem**: How does organic fertilizer change the population density of bacteria in the soil compared to inorganic fertilizer?

II. **Hypothesis**: Organic fertilizer will increase the population density of bacteria more than inorganic fertilizer.

III. **Variables**:

   a. **Independent**: type of fertilizer added to the soil (inorganic or organic)

   b. **Dependent**: the of population density of bacteria in 1cc of soil

   c. **Negative control**: soil without fertilizer added

   d. **Controlled**: location of soil, elevation of soil, moisture of soil, the amount of fertilizers, the brand of fertilizers, the amount of soil, amount of water added to the culture tubes, depth of soil, temperature of room, distance between plots, type of water used in dilution, type of pipette, amount of sterile water in the culture tubes, amount of soil that is being diluted, amount of soil/ water mixture placed on the petri plates, size and type of petri plates

IV. **Step-by-Step**:

1. Go to N 39.35801^0, W 076.63576^0 on RPCS front lawn.
2. Measure out a square in the grass that is 60x60cm and divide this in to nine 20x20cm boxes. Reference diagram 1 on final page of report.
3. Put flags in each corner of the 9 boxes and label flags according to plot number (see diagram 1).
4. Label 9 sterile Zip Lock bags: “negative control A”, “negative control B”, “negative control C”, “inorganic A”, “inorganic B”, “inorganic C”, “organic A”, “organic B”, and “organic C”.
5. In the “negative control A” plot, put the soil extractor in the ground 15 cm deep and turn clockwise to pull up soil sample. Put the soil in plastic bag labeled “negative control A.”
6. Repeat step 5 for the other eight plots, putting each soil sample in its corresponding Zip Lock bag.
7. Perform the dilution procedure in steps 8-22 to calculate the population density of bacteria in 1cc of each soil sample. Make sure all dilutions for nine samples are done on the same day at the same time.
8. Use a clean, new transfer pipette to add 10 ml of sterile water to a 15ml culture tube. Label the tube “10^0 negative control A. ”
9. Use the same pipette to add 9ml of sterile to a second 15 ml culture tube. Label the tube “10^{-1} negative control A ”.
10. Repeat step 9 two more times to two additional 15 ml culture tubes, only label them “10^{-2} negative control A” and “10^{-3} negative control A”.

11. Place 1 cc of your “negative control A” before soil sample into the “10^{0}” culture tube.

12. Cap the tube and shake vigorously.

13. 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.

14. Cap and shake vigorously.

15. Using the same pipette in step 13 remove 1 ml of the soil/water mixture from the 10^{-1} tube and place into the 10^{-2} tube.

16. Cap and shake vigorously.

17. Using the same pipette in step 13, remove 1ml of the soil/water mixture from the “10^{-2}” tube and place into the “10^{-3}” tube.

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

19. Plate 100µL samples from the 3rd and 4th tubes (dilutions 10^{-2} & 10^{-3}) onto their own separate, 3M Petrifilm™ plates containing nutrient agar label “Negative control A 10^{-2}” and “Negative control A 10^{-3}”

20. Allow bacteria colonies to grow for 48 hours.

21. Examine the “10^{-3}” plate for red dots representing bacteria colonies. If this plate does not have 5 or more bacteria colonies, then examine the “10^{-2}” plate. To make your estimates of the number of bacteria in the original 1 cc soil sample using the following formula:

    # Microbes in 1cc of soil = # Colonies on sheet x 100 x $10^{|\text{dilution \# at which colonies were found.}|}$

22. Then record the population density of bacteria in your “Negative control A before” sample in the data table.

23. Repeat steps 7-22 for Negative control B before, Negative control C before, Inorganic A before, Inorganic B before, Inorganic C before and Organic A before, Organic B before, Organic C before.

24. Then put 1.9 grams of inorganic fertilizer on plot, “Inorganic A, Inorganic B” Respectively, “Inorganic C”. Spread evenly. Then Put 11.7 grams of Organic fertilizer on Plot “Organic A”, “Organic B”, “Organic C” respectively and spread evenly.

25. Repeat steps 1-22 for all after plots: “Organic A after,” “Organic B after,” “Organic C after,” “Inorganic A after,” “Inorganic B after,” “Inorganic C after,” and “Negative control A after,” “Negative control B after,” “Negative control C after”. Remember to label the bags “after” instead of “before.”

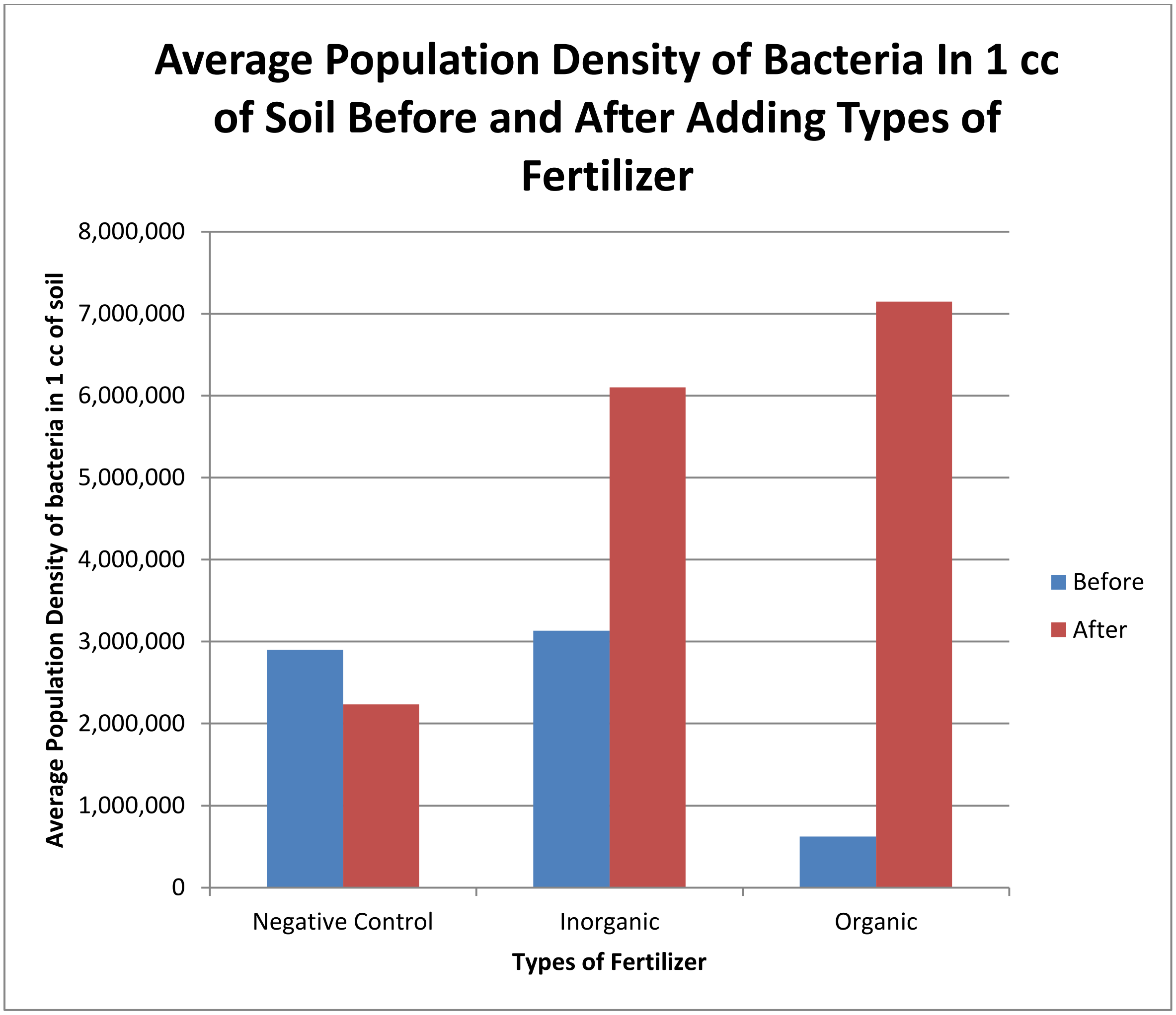
V. Data and Graph

Averge Number of Bacteria In 1 cc of Soil Before Adding Different Types of Fertilizer

Soil Sample	# of bacteria colonies in 1 cc of soil
Negative Control	2,900,000/1 cc
Inorganic	3,133,333/1 cc
Organic	623,333/1 cc

Average Number of Bacteria In 1 cc of Soil After Adding Different Types of Fertilizer

Soil Sample	# of bacteria colonies in 1 cc of soil
Negative Control	2,233,333/1 cc
Inorganic	6,100,000/1 cc
Organic	7,146,666/1 cc



VI. Conclusion

Our hypothesis, organic fertilizer will increase the population density of bacteria more than inorganic fertilizer, was proven correct. The average number of bacteria in 1 cc of soil before adding organic fertilizer was 623,333/1cc. Then after adding the organic fertilizer it was 6,100,000 /1cc. The average for the "before negative control" was 2,900,000 bacteria colonies/1cc soil. The average for the "after negative control" was 2,333,333 bacteria colonies/1cc soil. Therefore, the average number of bacteria colonies per cc of soil decreased by 566,667 in the negative control plots because of outside factors in the environment. The average for the “before inorganic” was 3,133,333 bacteria colonies/1cc soil. The average for the “after Inorganic” was 6,100,000 bacteria colonies/1cc soil. Therefore, the average number of bacteria colonies per cc of soil increased by 29,666,667 in the inorganic control plots. The average for the “before organic” was 623,333 bacteria colonies/1cc soil. The average for the “after organic” was 7,146,666 bacteria colonies/1cc soil. Therefore, the average number of bacteria colonies per cc of soil increased by 6,523,333 the organic control plots. Since the population density of the bacteria for the negative control decreased, whereas the population density of bacteria in inorganic and organic fertilizer increased, both fertilizers had an even greater impact on the population density of bacteria, which cannot be measured only by the amount that they increased in the “after” trials.

For our future research directions, our group chose to further study the use organic fertilizer because it had the greatest impact on the population density of bacteria. If one wanted to test a future experiment on the multiple types of organic fertilizers, one could test differences in their nitrogen, phosphorus, and potassium ratios. Ultimately, we would want to find out which one had the greatest population of bacteria growing after the application of the different types of organic fertilizer.

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Diagram 1)

60 cm

20 cm	20 cm	20 cm
Negative Control A	Negative Control B	Negative Conrol C
Inorganic Fertilizer A	Inorganic Fertilizer B	Inorganic Fertilizer C
Organic Fertilizer A	Organic Fertilizer B	Organic Fertilizer C

60 cm (each row 20 cm)