Soil Ecology

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Background

Fertilizers are a chemical or natural substance that is used to help the growth of plants by providing key nutrients. They can also fight off plant diseases that are caused by depletion of nutrients. Many people, including gardeners and landscapers, use fertilizers to help their lawns and gardens flourish (LaLiberte, 2019). As plants grow and develop, they absorb nutrients from the soil. Farmers use fertilizer to replenish the nutrients that were lost from the previous crop and to maximize crop output. Output is the total amount of crops produced by the farm, so a higher output means that more food can be sold, thereby increasing the farmer's profits. In order to be competitive in agriculture today, farmers need to use fertilizer in order to maximize their crop yield (The Fertilizer Institute, n.d.).

The macronutrients in fertilizer are nitrogen, phosphorus, and potassium. These three nutrients play vital roles in plant growth. Nitrogen is important for the development of a plant because nitrogen is needed for chlorophyll production, which is used in photosynthesis. For example, when the plant is deficient in nitrogen, they produce a yellow color, instead of a bright green color. In addition, nitrogen is necessary in nucleic acid and amino acid production. Nucleic acid production includes DNA and RNA. These nucleic acids are used to create proteins. Nitrogen is also used to make the amino acids, which are the building blocks of proteins. Enzymes are a type of protein that start chemical reactions. The ability to perform chemical reactions is what makes cells living. Without nitrogen, there is no life. Phosphorus is an important component of ATP. This will help the plant's energy production, so cells can perform chemical tasks. In addition, phosphorus supports the growth of the roots and helps the plant to bloom and fruit. Potassium helps the roots and the stems grow and develop. (Beaulieu, 2019). Together, these nutrients are found in a certain ratio called the NPK ratio. NPK stands for the amount of nitrogen, phosphorus, and potassium in fertilizer (Beaulieu, 2019).

There are two major types of fertilizer known as organic and inorganic. Organic fertilizers are made up of natural products, such as dehydrated manure, feather, crab, and bone meal, and dried blood. Inorganic fertilizers are synthetic chemical products. These two types of soil differ in how their nutrients are delivered because of the release time of nutrients. Because the nitrogen in inorganic fertilizers is quick-release, the nutrients in inorganic fertilizers are directly accessible for use by the plant compared to organic fertilizers. When organic fertilizers are applied to soil, microorganisms in the soil must break down the organic material in the fertilizers before nutrients can be used by the plants (Silva, 2018). This is because the nitrogen in organic fertilizer is slow-release (The Lawn Institute, n.d.). In addition, the NPK ratio of the fertilizer is an indicator of whether the fertilizer is organic or inorganic. In an organic fertilizer, the amount of the three nutrients are low and close together. For example, in the organic fertilizer, Espoma Plant-Tone, there is a NPK ratio of 5-3-3. This means that the fertilizer contains 5% nitrogen, 3% phosphorus, and 3% potassium. Comparatively, in an inorganic fertilizer, there are higher ratios of provided nutrients. For example, in the inorganic fertilizer Sta-Green, there is an NPK ratio of 29-2-5. This means that the fertilizer contains 29% nitrogen, 2% phosphorus, and 5% potassium (Beaulieu, 2019). Because inorganic fertilizers have a greater percentage of nitrogen, it is more likely that these fertilizers will burn plant roots, compared to organic fertilizer. In addition, leaching can be caused when an inorganic fertilizer is applied in excess, draining past the roots, and contaminating groundwater. This means that the nutrients are no longer accessible to the plants (Lehmann & Schroth, 2003).

While using inorganic fertilizer has many benefits, the chemicals in the fertilizer can also be harmful. Given the concentration of nutrients in inorganic fertilizer, it is more likely that not all of the nutrients are processed by the soil. This can pollute the air and water through excess

nitrogen and phosphorus. The pollution of nutrients in fertilizer, such as phosphorus and nitrogen, can cause serious environmental issues, including algal blooms and dead zones. When these nutrients infiltrate a body of water, the combination of excess nutrients and warm, calm water produces an algal bloom. An algal bloom is caused by the rapid reproduction of algae, a photosynthesizing protist. When this algae dies, dissolved oxygen in the water is depleted (St. Johns River Water Management District, n.d.). The dead algae and plants are decomposed by bacteria. This causes the bacteria to use the dissolved oxygen in the water. This decrease in oxygen is dangerous for the ecosystem because it can kill aquatic organisms that need oxygen (Regional Science Consortium, n.d.). Oxygen is necessary for organisms who use cellular respiration to produce energy. The lack of oxygen creates a dead zone in which plants and animals die or are forced to leave the area. This area changes from an area that supports life to an area that no longer can support life (National Ocean Service, n.d.). When environmental issues arise, especially in aquatic environments, bacteria play a role. Fertilizer impacts aquatic and soil environments and a wide variety of organisms that live there.

Bacteria are a group of microorganisms that supports soil productivity and health. Bacteria are prokaryotic cells that reproduce asexually through binary fission. Bacteria make up the largest number and biomass of the soil microorganisms. Many soil bacteria live in water films near the roots called the rhizosphere. The small size of bacteria helps them to grow and adapt quicker than more complex microorganisms in the changing environment. Bacteria help to maintain the soil structure by producing a layer of polysaccharides that bind the soil particles together, which results in the formation of microaggregates. Microaggregates create pore space in the soil that stores oxygen and provides better water filtration. The storage of oxygen is helpful because oxygen is necessary for bacteria and other organisms to perform cellular

respiration. Bacteria recycle nitrogen, carbon, phosphorus, and other nutrients stored in the soil, so the plants have more nutrients available. Without the help of bacteria, plant populations would die because bacteria help maintain a healthy soil environment. (Hoorman, 2016, June 6) If the plant population decreases, then the animals that consume the plants may also decrease which would create a domino effect within that food chain, impacting the larger ecosystem. (PBS, n.d.) One of bacteria's most important roles in the soil is their participation in the nitrogen cycle.

The nitrogen cycle is the process of nitrogen being transformed from into different chemical forms. Due to its toxicity, nitrogen gas is unable to be used by most living organisms, so it must be converted into a usable form. The first phase of the nitrogen cycle is nitrogen fixation, when nitrogen gases (N_2) diffuse into the soil. From there, nitrogen fixing bacteria converts nitrogen gas into ammonium (NH_4+) and ammonia (NH_3+) which can be used by plants. The next stage of the nitrogen cycle is ammonification. Throughout assimilation, plants collect ammonia or ammonium ions through their roots. Consumers are then able to eat these plants to gain nitrogen. When a plant or animal dies, or when an animal excretes waste, this organic matter is broken down by bacteria through decomposition. Certain types of bacteria such as decomposing bacteria release ammonia and ammonium (CK-12 Foundation, n.d.). The next phase of the nitrogen cycle is nitrification. During nitrification, nitrifying bacteria convert ammonia into nitrite (NO_{2-}) and nitrate (NO_{3-}). Nitrate (NO_{3-}) is the most common form of nitrogen taken up by plants also through assimilation. Finally, in denitrification, denitrifying bacteria converts nitrate in the soil back into nitrogen gas. (Science Learning Hub – Pokapū Akoranga Pūtaiao, 2013).

In our experiment, we are testing how the application of organic and inorganic fertilizer impacts the population density of bacteria in the soil. Since inorganic fertilizer has higher rates of

ammoniacal nitrogen than organic fertilizer, it causes a higher concentration of ammonia in the soil. Nitrifying bacteria thrive in this environment, using ammonia as a food source to produce energy. On the other hand, the nitrogen fixing bacteria continue to produce ammonia in the soil, expelling more of this waste product into the environment. This ammonia creates a toxic living environment for most bacteria in the soil, causing the population of the nitrogen fixing bacteria to decrease, while the population of nitrifying bacteria increases. (Geisseler & Scow, 2014). This effectively creates an imbalance of bacteria populations in the soil which damages the functionality of the nitrogen cycle. This damage not only affects the growth of the plant, but the primary consumers of the plants as well. Since the population density of bacteria in the soil decreases, protozoa, the microorganisms that eat bacteria are affected as well. The consumers of protozoa are affecting, damaging the soil food web (D. Brock, personal communication, April 26, 2019). Through this research, we hypothesize that the application of inorganic fertilizer will cause a greater decrease in the population density of bacteria in the soil compared to the organic fertilizer.

Experimental Design

I. Problem: How does the application of organic and inorganic fertilizer impact the population density of bacteria in the soil?

II. Hypothesis: The application of inorganic fertilizer will cause a greater decrease in the population density of bacteria in the soil compared to the organic fertilizer.

III. Procedure:

A. Independent Variable: type of fertilizer: organic or inorganic

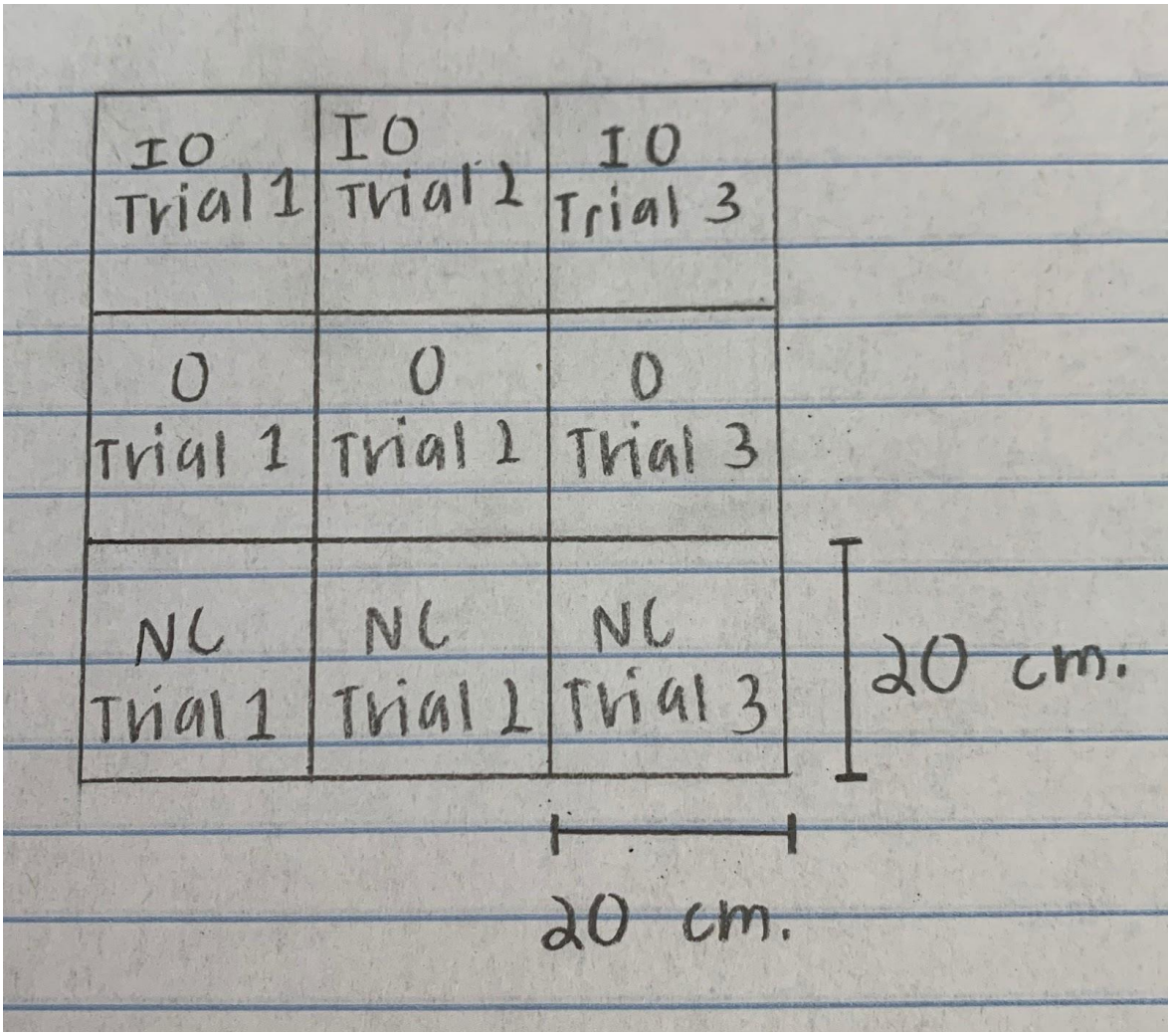
B. Dependent Variable: the population density of bacteria in 1 cc of soil

C. Negative Control: the population density of bacteria in 1 cc of soil without fertilizer

D. Controlled Variables: type of organic fertilizer, type of inorganic fertilizer, type of water (sterile), size of serological pipette, size of micro-pipette, size of culture tubes, size of scoop, type of agar plates, amount of time given for the bacteria to grow, amount of sterile water used, amount of sterile water transferred into the culture tubes by the serological pipette, amount of solution transferred between culture tubes by the serological pipette, amount of solution that is transferred onto the nutrient agar plates by the micro-pipette, size of grid, size of plots, location of extraction of soil, amount of soil added to the 10^0 culture tube, amount of time that passes after fertilizer is placed on soil and before the soil is extracted, time of dilution, amount of inorganic fertilizer spread, amount of organic fertilizer spread

E. Step-By-Step

1. Go to N 39.21490°, W 76.38157° and create a grid that is 60 centimeters by 60 centimeters with nine 20 by 20 centimeter squares within this grid. Each intersection of the grid lines should have a flag.



2. The top three squares (going across) will be used to test inorganic fertilizer. Label the four top flags “IO”.

3. The middle three squares (going across), will be used to test organic fertilizer. Label the four flags “O”.

4. The bottom three squares (going across), will be used to test no fertilizer. Label the four flags with “NC” for negative control.

5. On the same day at the same time, collect soil using the soil extractor from the three inorganic fertilizer plots. Insert the soil extractor 15 cm into the soil, rotate it clockwise, and pull

it straight up. Place the soil into separate plastic bags labeled “Before IO” with the respective trial number (see diagram).

6. On the same day at the same time, repeat step 5 using the organic fertilizer plots and label the separate plastic bags “Before O” with the respective trial number (see diagram).

7. On the same day at the same time, repeat step 5 using the negative control fertilizer plots and label the separate plastic bags “Before NC” with the respective trial number (see diagram).

8. Using the extracted soil samples, follow the serial dilution procedure in steps 9-27 . All soil samples must be diluted on the same day at the same time.

9. Use a clean, new serological pipette to add 10 ml of sterile water to a 15 ml culture tube. Label the tube “IO#1 10^{0}”.

10. Use the same pipette to add 9 ml of sterile water to a second 15 ml culture tube. Label the tube “IO#1 10^{-1}”.

11. Repeat step 10 three more times to three additional 15 ml culture tubes, only label them “IO#1 10^{-2}”, “IO#1 10^{-3}", and “IO#1 10^{-4}" respectively.

12. Place 1 cc of your “Before IO” soil sample into the “IO#1 10^{0}”culture tube.

13. Cap the tube and shake vigorously.

14. Using a new clean serological pipette, remove 1 ml of the “Before IO” soil/water mixture from the “IO#1 10^{0}” tube and place into the “IO#1 10^{-1}” tube.

15. Cap and shake vigorously.

16. Using the same pipette in step 14, remove 1 ml of the “Before IO” soil/water mixture from the “IO#1 10^{-1}” tube and place into the “IO#1 10^{-2}” tube.

17. Cap and shake vigorously.

18. Using the same pipette in step 14, remove 1 ml of the “Before IO” soil/water mixture from the “IO#1 10^{-2}” tube and place into the “IO#1 10^{-3}” tube.

19. Cap and shake vigorously.

20. Using the same pipette in step 14, remove 1 ml of the “Before IO” soil/water mixture from the “IO#1 10^{-3}” tube and place into the “IO#1 10^{-4}” tube.

21. You should now have a total of five culture tubes.

22. Plate 100 µl samples from the 4th and 5th tubes (dilutions IO#1 10^{-3} & IO#1 10^{-4}) onto their own separate, labeled 3M Petrifilm™ Aerobic Count Plate. Label the plates according to their plot name and dilution number.

23. Repeat steps 8-22 two more times to create a total of three trials for the other two IO soil samples. Label with respective trial number.

24. Repeat steps 8-22 using the no fertilizer samples rather than IO soil samples. Label with respective plot name and trial number.

25. Repeat steps 8-22 using the organic soil samples rather than IO soil samples. Label with respective plot name and trial number.

26. Allow the bacteria to grow on the plates for 96 hours.

27. Examine each of the plates for individual bacteria colonies. Start with the most dilute plate, 10^{-4}. If there are at least 5 bacteria colonies (red dots), count this plate. If there are less than 5 colonies, then go to the next plate, 10^{-3}, and count the number of bacteria colonies. For each plot/soil sample, write down the dilution number and number of colonies on the chosen plate.

28. 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 $10^{|\text{dilution \# at which these colonies were found}|}$

29. Record the amount of bacteria in each soil sample.

30. Spread 11.72 grams of Plant tone fertilizer in each “O” plot.

31. Spread 1.88 grams of Sta-Green fertilizer in each “IO” plot.

32. Allow 48 hours to pass before collecting soil.

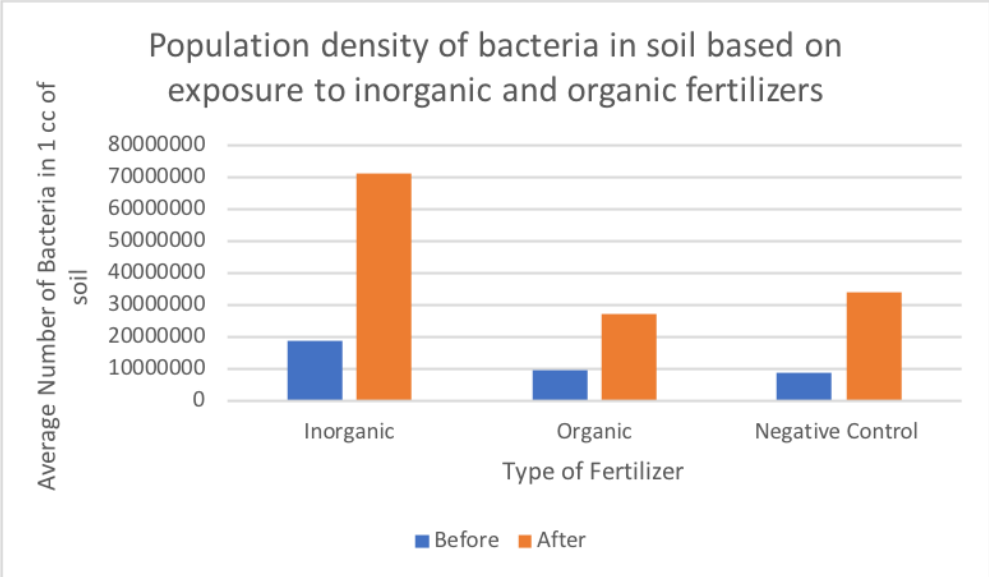
33. Label nine plastic bags. Three should be labeled “After IO”. Three should be labeled “After O”. Three should be labeled “After NC”. Each three bags should be labeled trial 1, 2 and 3 respectively.

34. Repeat steps 5-7 to collect soil from each plot, but change the labeling to “After IO”, “After NC”, and “After O”.

35. Repeat steps 8-29 using the “After NC” samples, the “After IO” samples, and “After O” soil samples.

Data and Analysis

Population density of bacteria in soil based on exposure to inorganic and organic fertilizers						
	Inorganic		Organic		Negative Control	
	Before	After	Before	After	Before	After
Plot 1	25000000 bacteria in 1 cc soil	50000000 bacteria in 1 cc soil	6000000 bacteria in 1 cc soil	44000000 bacteria in 1 cc soil	6000000 bacteria in 1 cc soil	25000000 bacteria in 1 cc soil
Plot 2	25000000 bacteria in 1 cc soil	153000000 bacteria in 1 cc soil	18000000 bacteria in 1 cc soil	13000000 bacteria in 1 cc soil	3000000 bacteria in 1 cc soil	72000000 bacteria in 1 cc soil
Plot 3	6000000 bacteria in 1 cc soil	10000000 bacteria in 1 cc soil	5000000 bacteria in 1 cc soil	24000000 bacteria in 1 cc soil	16900000 bacteria in 1 cc soil	5000000 bacteria in 1 cc soil
Average	18666666 bacteria in 1 cc soil	71000000 bacteria in 1 cc soil	9666666 bacteria in 1 cc soil	27000000 bacteria in 1 c soil	8633333 bacteria in 1 cc soi	34000000 bacteria in 1 cc soil



Conclusion

In our experiment, we found that the application of organic fertilizer caused a greater decrease in the population density of bacteria in the soil compared to the inorganic fertilizer. This does not support our hypothesis. Before applying the inorganic fertilizer to the soil, the average population density of bacteria in the soil was 18,666,666 microbes in 1 cc of soil. After applying the inorganic fertilizer to the soil, the average population density of bacteria in the soil increased by 280% to 71,000,000 microbes in 1 cc soil. Before applying the organic fertilizer to the soil, the average population density of bacteria in the soil was 9,666,666 microbes in 1 cc of soil. After applying the organic fertilizer to the soil, the average population density of bacteria in the soil increased by 179% to 27,000,000 microbes in 1 cc soil. When we originally tested the negative control, the average population density of bacteria in the soil was 8,633,333 microbes in 1 cc of soil. After we tested the negative control again, the average population density of bacteria in the soil increased by 294% to 34,000,000 microbes in 1 cc soil. The negative control shows the natural, environmental changes that occurred in the bacteria in the soil. Because the bacteria in the negative control plots increased by 294%, these natural environmental changes need to be

applied to our population densities. These environmental changes need to be taken into account. In the inorganic fertilizer plots, the population density of bacteria in the soil decreased by 14% based on the changes seen in the negative control. In the organic fertilizer plots, the population density of bacteria in the soil decreased by 115% based on the changes seen in the negative control. This means that the application of organic fertilizer caused a greater decrease in the population density of bacteria in the soil compared to the inorganic fertilizer.

In the future, we could research how environmental factors contribute to the population density of bacteria in the soil. For instance, we could research how rain would play a role in comparison to how sunlight would play a role. In addition, based on our results, we would like to do further research on a variety of inorganic and organic fertilizers, as well as their impact on the population density of bacteria in the soil. In the future, we could research several inorganic and organic fertilizers based on their chemical ratios and ingredients. For instance, Sta-Green inorganic fertilizer has an NPK ratio of 29-2-5, meaning that it has a higher concentration of ammoniacal nitrogen in comparison to phosphorus and potassium. However, we could instead direct our experiment using Pro Grow inorganic fertilizer with an NPK ratio of 19-4-10, or Jack's inorganic fertilizer with an NPK ratio of 20-20-20. In this, we could see how different ratios of nitrogen, phosphorus, and potassium in fertilizers affect the population density of bacteria in the soil, in comparison to different types of organic fertilizer in a further light. Also, in the future, we would like to research more on the topic of population of bacteria in the soil. We could research the normal activity of bacteria by extracting soil every day for one week from 3 GPS locations to see the natural changes of the population. We can dilute and plate each sample and observe the changes that have occurred in each soil sample. By completing these tests, we could see how bacteria populations change in different areas of soil.

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