The impact of Inorganic and Organic fertilizers on the bacteria population in the soil

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Background

Bacteria are prokaryotic microorganisms that play an important role in the soil food web. Specifically, they help with water dynamics, the cycling of nutrients, disease suppression, and maintaining soil structure. They are categorized into different shapes such as spherical, rod, or a spiral twist. Bacteria are very small, generally 4/100,000 of an inch wide (Ingham, n.d.). Some live-in colonies, but they can also be free-living. They reproduce very quickly using binary fission: a single colony of bacteria can reach the billions in a few minutes. One generation of bacteria generally only lives 20 to 30 minutes, though this depends on the species. Bacteria are very nutritionally diverse, meaning that they can eat almost anything though mostly they are looking for sources of nutrients and energy. (Lineberger, 2009). Protozoa are the main consumers of bacteria and in doing so release nutrients into the soil (Melendrez, 2003). They also maintain the bacteria population size.

Most bacteria in the soil are decomposers that consume simple carbon compounds, such as root exudates and fresh plant litter. They convert energy from soil organic matter into forms that can be used by every organism in the soil food web, which benefits the producers and the larger ecosystem. Through decomposition, bacteria break down dead plants or animals and release nitrogen back into the environment. Earthworms called detritivores feed on decaying material and start to break down organic matter in the soil. After they have done this, the bacteria act as saprophytes and completely break down the materials. They break down the polymers of organic material into monomers in order to recycle the biological molecules. Bacteria are adept at producing a variety of enzymes that help to digest or break down material (Lineberger, 2009). Decomposers retain nutrients in their cells which prevents the loss of such nutrients, like nitrogen, from the soil. Not only do decomposers break down materials to release energy, but also materials that can be harmful to the soil (such as pesticides and pollutants). Certain strains of bacteria also produce compounds that inhibit the growth of pathogens and reduces the invasion of the plant by a pathogen (Kennedy, n.d.). For example, bacteria produce phenazines which prevent diseases like Take-all, a harmful fungal disease that impacts wheat, soybean, and other crops (Hamilton, 2014-2015). Bacteria help the ecosystem through decomposition, not only by providing nutrients, but also by protecting plants from harm.

In addition to providing nutrients bacteria also help the ecosystem by improving soil structure. Bacteria produce sticky substances, or polysaccharides, that bind soil particles into aggregates (Ingham, n.d.). Soil aggregates are groups of soil particles that are strongly bonded together which improves soil structure (Nimmo, 2004). Aggregates improve water infiltration and water storage capacity. Water is one of the key reactants of photosynthesis, and the soil aggregates allow plants quick and easy access to it. Aggregates create space in the soil, which allows for pockets of oxygen. Oxygen is a necessary reactant in the process of making ATP, which is the molecule we use for energy. Seeds need both oxygen and water to germinate and be able to grow. Furthermore, the roots of plants must use stored oxygen to take up nutrients (Daum, 2017). Another important function of bacteria is altering the soil environment to accommodate different plants. On fresh sediments, the bacterial community establishes first before the plants can become established. As the plant community grows, different types of organic matter enter the soil. This changes the bacteria's food source and it alters the soil environment to accommodate the new additions (Ingham, n.d.) Without bacteria, new plant populations often do not exist or survive (Hoorman, 2011) Bacteria may be small, but ultimately help to enrich, protect, and aid the soil and all the organisms in it in major ways.

Bacteria also aids the soil and ecosystem because of the key role it plays in the nitrogen cycle. The nitrogen cycle starts when a type of bacteria called nitrogen-fixing bacteria consumes nitrogen gas from the atmosphere and produces ammonia (Peer Curricula, 2003). Ammonia is gas compound of nitrogen and hydrogen. (Encyclopedia Britannica, 2017). After ammonia is produced, other bacteria use the ammonia to make nitrites and nitrates which are compounds of nitrogen and oxygen. Plants use the nitrates to make nucleic acids and amino acids, which are then converted to plant proteins. Plant proteins or enzymes are essential because they accomplish different functions and start chemical reactions. Once plant proteins are made, other organisms eat the plants and use the amino acids from the plants to form their own amino acids. (Khan Academy, 2017) Then bacteria changes excess nitrogen into ammonia and returns it to the soil (Peer Curricula, 2003). Finally, some bacteria release a very small amount of nitrogen back into the atmosphere through a process known as denitrification (Khan Academy, 2017). Bacteria are very helpful but commonly used materials such as fertilizers can be harmful to the bacteria population and detrimental to the ecosystem as a whole.

The three main nutrients in fertilizers are nitrogen, phosphorus, and potassium. Different fertilizers have different ratios of these nutrients, noted as N-P-K. Phosphorus is used so plants can form new roots and make seeds. Nitrogen, in addition to forming nucleic acids and amino acids, is used so plants can have leaf growth and a solid green color. (University of Illinois Board of Trustees, 2017). The most important function of phosphate is energy storage or creation of ATP, adenosine triphosphate (Net Industries and its Licensors, 2017). Lastly, potassium is used so plants can make strong stems and keep growing fast (University of Illinois Board of Trustees, 2017). Inorganic fertilizer and organic fertilizer are two different fertilizers. Organic fertilizer is derived from animal or vegetable matter (Farlex, Inc, 2017). Inorganic fertilizer is a mineral fertilizer which will help a plant grow faster (Dave's Garden, n.d). If too much inorganic fertilizer is applied, it can burn plant roots and create chemical imbalances. Inorganic fertilizer also causes leaching which occurs when fertilizers are washed by rain or irrigation water below the level of plant roots (Hearst, n.d). Organic fertilizer progressively works over time to create a healthy environment, while inorganic fertilizer provides quick nutrition (Hearst 1994). For humans, organic fertilizer will provide clean food that will not harm humans since there are no chemicals in the products. If people consumed non-organic products they are potentially at a higher risk for cancer, strokes, and other diseases. Organic fertilizers will also help prevent environmental pollution since they are easily biodegradable. They also are cheaper than chemical fertilizers, since most organic fertilizers can be made locally or on a farm (Organic Facts, 2017). Organic fertilizers can also build up concentrations of nutrients such as Potassium, Nitrogen, and Phosphorus. It is unlikely for a build up to be toxic as long as the material is able to decompose (Hearst, n.d). Organic fertilizers decrease the danger of "over-fertilizing" since the nutrients are released very slowly. This also causes the fertilizer to last longer, thereby reducing the number of applications required (DoItYourself.com, n.d). Although both fertilizers have many differences, they also have some comparisons. Both fertilizers can contain hydrogen and carbon, although if they were to contain both, it would be organic (Bhalinge, 2016). Since each fertilizer has different ingredients and each fertilizer is used in different ways, there are not that many comparisons.

Even though bacteria are often resilient organisms, since they are smaller, less mobile, and less complex than most organisms, it is harder for them to escape an environment, if the conditions change and become harmful. Even the smallest change in conditions, such as a decrease in the temperature or a change in acidity of the soil could become unfavorable and lead to a decrease in the bacteria populations (Lineberger, 2009). Considering how important bacteria is to the soil, this is problematic. Bacteria provide key nutrients, improve soil structure, and are essential to the protection and growth of plants. If the bacteria colonies are being harmed, then the plants will not be able to grow, which will lead to fatal effects in the ecosystem, such less food for consumers. Sadly, commonly used products such as inorganic fertilizer can provide a change in condition and harm the bacteria population (Ingham, n.d.). While organic products mimic natural conditions. Because of this, we hypothesize that inorganic fertilizer will decrease the bacteria population in the soil and organic fertilizer will increase the bacteria population in the soil.

Lab Report

Problem: How do organic fertilizer and inorganic fertilizer impact the bacteria population in the soil respectively?

Hypothesis: Inorganic fertilizer will decrease the bacteria population in the soil, whereas and organic fertilizer will increase the bacteria population in the soil.

Independent Variable: Type of fertilizer (organic or inorganic) applied to each plot of soil

Dependent Variable: Population density of bacteria in 1 cc of soil

Negative Control: Soil plots without any fertilizer added

Controlled Variables:

- The amount of fertilizer applied to the plots of land

- The number of plots of land
- The size of the plots of land
- The location of the plots of land
- The amount of soil extracted from each plot of land
- The brand of fertilizers applied to the soil
- How many flags are bordering each plot of land
- The length of time the fertilizers sit on the soil
- Amount of test tubes holding diluted soil
- The amount of sterile water used to dilute the soil samples
- The amount of test tubes
- How many serological pipettes are used
- The amount of liquid transferred to each different dilution tube
- How much liquid is distributed onto the 3M petrifilm^{TM} aerobic plates
- What is holding the test tubes
- Location of dilution process
- The amount of nutrient agar plates used
- How long the bacteria grows on plates
- Where the nutrient agar plates are left to grow

Procedure:

1. Go to N:39.35811° and W: 076.63627° and place 16 flags that are 20 centimeters apart in three columns and three rows, forming 9 plots. See diagram below

Diagram

Plot 1 (inorganic)	Plot 2 (organic)	Plot 3 (negative control)
Plot 4 (inorganic)	Plot 5 (organic)	Plot 6 (negative control)
Plot 7 (inorganic)	Plot 8 (organic)	Plot 9 (negative control)

2. Label 9 plastic bags respectively with plot names: Plot 1 before inorganic fertilizer, Plot 2 before organic fertilizer, Plot 3 before negative control, Plot 4 before inorganic fertilizer, Plot 5 before organic fertilizer, Plot 6 before negative control, Plot 7 before inorganic fertilizer, Plot 8 before organic fertilizer, Plot 9 before negative control.
3. Take a soil sample from plot 1 using a soil extractor and a hammer. Put soil extractor in the soil with the handle facing up, hold it while hammering the top until it is 15 cm in the soil. Then twist the handle clockwise and pull up from the soil
4. Put the end of the soil extractor in the bag and use your finger outside of the bag to push the soil out of the extractor and into the bag
5. Place soil samples in a plastic bag labeled “plot 1 before inorganic fertilizer”
6. Repeat step 3-5 eight more times for the eight other soil plots, putting each soil sample in its respectively labeled bag
7. Test all of the “before fertilizer” soil samples using the bacteria dilution process seen in steps 8-28
8. Place five 15ml culture tubes in a test tube rack.

9. Use a clean, new transfer pipette to add 10 ml of sterile water to a 15-ml culture tube and label the tube “10^0 Plot 1”

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

11. Repeat step 10 to two additional 15 ml culture tubes, only label them “10^{-2} plot 1” and “10^{-3} plot 1” respectively.

12. Place 1 cc of plot 1 soil sample into the “10^0” culture tube.

13. Cap the tube and shake vigorously.

14. 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.

15. Cap and shake vigorously.

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

17. Cap and shake vigorously.

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

19. Cap and shake vigorously.

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

21. Take two 3M Petrifilm™ aerobic count plates

22. Label one plate “Plot 3 10^{-2}” and another “Plot 3 10^{-3}”

23. Plate 100 mL samples from the 3rd and 4th tubes (dilution “10^{-2}” and “10^{-3}”) onto their own separate, labeled 3M petrifilm™ aerobic count plate

24. Repeat steps 8-23 8 more times for 8 different soil samples, labeling culture tubes and plates with the given plot number and dilution number

25. Let bacteria colonies grow for 48 hours

26. Examine each of the plates for individual bacterial colonies. Start with the most diluted plate (10^{-3}) and count the number of red dots on the plate, or bacteria colonies. If there are less than 5 red dots, observe the 10^{-2} plate. Remember to record the number of dots found. Make 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 * "10^2" * $10^{|\text{dilution at which these colonies were found}|}$

27. Place 1.872 grams of Sta-Green Lawn inorganic fertilizer, with a nitrogen-phosphorus-potassium ratio of 29-2-5 on plots 1, 4, 7 of land respectively.

28. Place 11.712 grams of Plant-tone organic fertilizer with a nitrogen-phosphorus-potassium ratio of 5-3-3 on plots 2, 5, 8 of land respectively.

29. Leave plots 3, 6, 9 of land without fertilizer (these are the negative control plots).

30. Let fertilizer sit on soil for 72 hours

31. After 72 hours, label nine plastic bags respectively, Plot 1 after inorganic fertilizer, Plot 2 after organic fertilizer, Plot 3 after negative control, Plot 4 after inorganic fertilizer, Plot 5 after organic fertilizer, Plot 6 after negative control, Plot 7 after inorganic fertilizer, Plot 8 after organic fertilizer, Plot 9 after negative control.

32. Take the soil extractor and extract a total of 9 soil samples by repeating the same process as in steps 3-6, placing the soil samples in the newly labeled plastic bags.

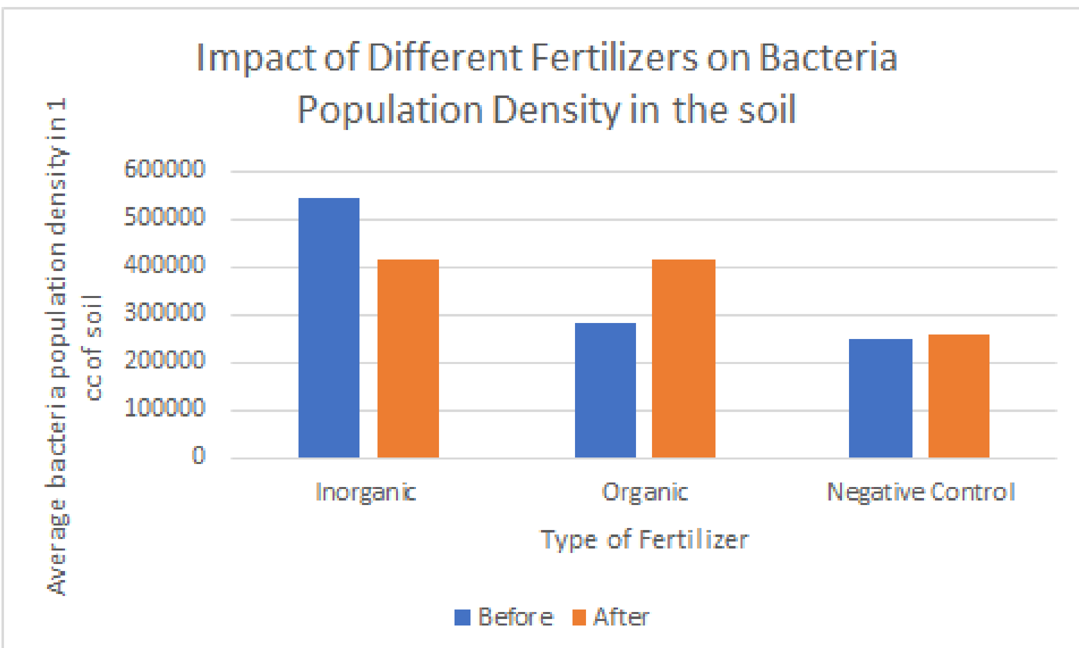
33. Bring the nine soil samples into lab room and repeat steps 8-26 to complete dilutions for "after" samples

Bacteria Population Density in the Soil Before and After Adding Different Fertilizers

Plot number	Type of fertilizer	Population density before	Population density after
Plot 1	Inorganic	860,000 colonies per 1cc soil	600,000 colonies per 1cc soil
Plot 2	Organic	280,000 colonies per 1cc soil	50,000 colonies per 1cc soil
Plot 3	Negative Control	10,000 colonies per 1cc soil	160,000 colonies per 1cc soil
Plot 4	Inorganic	500,000 colonies per 1cc soil	500,000 colonies per 1cc soil
Plot 5	Organic	480,000 colonies per 1cc soil	600,000 colonies per 1cc soil
Plot 6	Negative Control	700,000 colonies per 1cc soil	100,000 colonies per 1cc soil
Plot 7	Inorganic	270,000 colonies per 1cc soil	140,000 colonies per 1cc soil
Pot 8	Organic	90,000 colonies per 1cc soil	600,000 colonies per 1cc soil
Plot 9	Negative Control	40,000 colonies per 1cc soil	510,000 colonies per 1cc soil

Average amount of colonies in 1 cc of soil before and after adding inorganic or organic fertilizer

	Before Averages	After Average
Inorganic	543333 colonies per 1cc soil	413333 colonies per 1cc soil
Organic	283333 colonies per 1cc soil	416666 colonies per 1cc soil
Negative Control	250000 colonies per 1cc soil	256666 colonies per 1cc soil



Conclusion

Our hypothesis was that inorganic fertilizer would decrease the bacteria population in the soil and organic fertilizer would increase the bacteria population in the soil. In the end, our hypothesis was supported by our data. In the data, it was clearly shown hat in the inorganic fertilizer plots the bacteria population had decreased after the fertilizer was added to the soil. Before the fertilizer was added the average amount of bacteria colonies in 1 cc of soil for all three plots was 543333, after it was added the average amount of bacteria colonies in 1 cc of soil for all three plots was 413333. The data also shows an increase in bacteria population in the plots where organic fertilizer was added. Before the fertilizer was added the average amount of bacteria colonies in 1 cc of soil for all three plots was 283333 in 1 cc of soil, after it was added the average amount of bacteria colonies in 1 cc of soil for all three plots was 416666. The negative control plots were very close with a before average of 250000 bacteria colonies in 1 cc of soil and an after average of 256666 bacteria colonies in 1 cc of soil. This is important because it shows that no outside factor besides the fertilizer significantly affected the soil as the only difference between the negative control plots and the fertilizer plots was the addition of fertilizer. We used the negative control plots to confirm that the sole contributor to the change in the bacteria population in the soil was the fertilizer. The reason for this change can be found in the composition of both fertilizers and how these ingredients impact the soil.

Organic fertilizers are derived from animal or vegetable matter, meaning that they are more natural. This helps the bacteria population. Since most fertilizers contain Nitrogen, Phosphorus, and Potassium, in the ratio (N-P-K), and these ingredients are what help plants and the soil, the bacteria population has a higher chance of increasing than decreasing. However, when the inorganic fertilizer is added to the soil this means that the bacteria population is reduced and as a result the bacteria population suffers. With a smaller population, the bacteria cannot complete their jobs and the soil will suffer. Furthermore, organic fertilizer with increase the population of bacteria because it can decontaminate the land and water. Inorganic fertilizers will burn plant roots and cause leaching which will cause a decrease in the bacteria population. For organic fertilizer, bacteria work to process the organic fertilizer because it must be processed before being available to plants. On the other hand, inorganic fertilizer does not need to be broken down and gives the nutrients straight to the plants. This means that the bacteria are not exposed to the nutrients in this form.

In the organic fertilizer setting the bacteria population is increasing, while in the inorganic fertilizer setting the bacteria population is decreasing. If indeed, inorganic fertilizer decreases the bacteria population we need to watch how much inorganic fertilizer we use because, as we stated before bacteria is important in the soil and ecosystem. Bacteria is important because it decomposes providing and cycling essential nutrients, maintains plant health, and improves the structure of the soil. If the bacteria population is decreasing then that will have negative environmental consequences.

Another way bacteria affects the soil and entire ecosystem is its impact on the nitrogen cycle. Bacteria plays a large role in the nitrogen cycle because in the very beginning of the cycle, bacteria consumes nitrogen from the air and converts it to ammonia. Without ammonia, nitrites and nitrates would not be produced which would cause essential enzymes to not be made and available to the plants. Fertilizers different ingredients cause inorganic fertilizers to decrease the bacteria population and in turn be harmful to the nitrogen cycle and the ecosystem as a whole, while organic fertilizers are beneficial to the soil and increase the bacteria population.

If we were to do a future project to expand on the subject of fertilizers and their effect on bacteria in the soil we would expand on inorganic fertilizers. We would test different brands to see if they would impact the soil differently. Each type of fertilizer has different ratios of the nutrients and we would experiment to see if this changed the fertilizers effect on the bacteria and the soil. We would try to find an inorganic fertilizer that is not harmful to the soil. This is useful because if we can find a safe brand of inorganic fertilizer then people could use this fertilizer without having to worry about damaging the soil. This is helpful as inorganic fertilizer is less expensive and more popular. Inorganic fertilizer decreases the bacteria population which is fatal because bacteria is important to the ecosystem and an integral part of the nitrogen cycle; while organic fertilizer is helpful to the soil because it increases the bacteria population.

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