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We have acted honorably

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Soil Ecology Project 2016

The Effects of Organic Fertilizer on the Population Density of soil bacteria

https://www.thedrinksbusiness.com/2015/03/soil-based-bacteria-key-to-wine-style/

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Final Report

Background

Bacteria, single celled microbes with no nucleus or membrane bound organelles, can be found on every surface on the Earth. But in the soil, they are most commonly found near the roots of the plants, and their interactions with these roots can affect plants both directly and indirectly (Microbiology Society, 2016). One way bacteria can affect plants directly is by producing organic material and supplying the plant with nutrients. In fact, while bacteria play many significant roles in the soil, none is as important as the role they play in this supply process through nitrogen fixation because in order for the plants in the ecosystem to be able to access the nitrogen they need, the bacteria in the soil have to chemically modify it first. Most of the nitrogen on earth is nitrogen gas found in the atmosphere, and bacteria in the soil convert this nitrogen gas into ammonium and nitrate ions for the plants to be able to absorb through their roots (Pidwirny, 2010).

Nitrogen is incredibly vital for the plants to thrive, and without it, plants will die because nitrogen is a critical element in the monomer of both nucleic acids and proteins which are responsible for day to day functioning of a plant's cells. Furthermore, since this nitrogen is passed on through the food chain to supply all the other organisms with the nucleic acids and proteins they need for their cells to function, all of the living things in the ecosystem would not be able to function without the nitrogen; hence, no fixed nitrogen, no ecosystem. (Science Buddies Staff, 2013)

Another way bacteria help plants (and everything else in the ecosystem) is through the phosphorus cycle. Phosphorous is a crucial element for the cells in plants because phosphate is a key component in ATP, which plays a major role in cellular respiration (as well as the nucleic

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acids mentioned earlier). While phosphorus enters an ecosystem as rocks are broken down, releasing phosphate ions and other minerals into the soil, the main source of phosphorus in an ecosystem comes when plants and animals die. The bacteria that decompose them release the organic phosphate back into the soil, and living plants then take up this phosphate; the animals eat the plants; and the cycle continues (Science Learning Sparking Fresh Thinking, 2013).

In addition to their many direct impacts on plants, bacteria also affect plants indirectly by helping to protect plants from disease by producing a sticky substance that will act as a coating for the roots that fights against pathogens, which enable the roots to function properly. Furthermore, bacteria cause soil particles to be more tightly packed together, improving water infiltration, allowing the soil to contain more water and providing plants with the water they need for growth (Ingham, 2016).

Because bacteria are so critical to soil health, anything that could upset their quantity in the soil could potentially upset the whole ecosystem. One such potential influence is the presence or absence of fertilizer. Fertilizer is typically used to increase the fertility of the soil and the amount of crops that can grow in it. Although most fertilizers contain the same nutrients all plants need to grow, there are three main types of fertilizers that are commercially produced depending on how those nutrients are created: chemical, organic, and biofertilizers. The first of these are made of manufactured nutrients, artificially fixed forms of nitrogen, phosphate, potash,iron, and chlorine which a plant can access to improve its growth and development (Savonen, 2008). Organic fertilizers are made of manure and composts, which contain essential nutrients created by the natural process of decay, and biofertilizers are made out of living fungi and bacteria that are beneficial to the soil.

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All excess fertilizer, though, has the potential to cause a significant decline in the population of the bacteria in the soil, which can lead to a disrupted ecosystem, because fewer bacteria would mean less nitrogen produced to help the plants living there. The bacteria that convert the nitrogen gas in the atmosphere into ammonium are releasing that ammonium into the soil because for those bacteria, it is essentially their "waste material". But when fertilizers are added, the excess ammonium they add to the soil increases the amount of "waste material" surrounding these bacteria, and when this happens, the bacteria responsible for the first stage of nitrogen fixation basically "drown" in their waste products and die. But without these bacteria to convert the nitrogen in the atmosphere into ammonium, the plants living in the soil would be unable to function properly; hence, more fertilizer must be added again, and the process becomes a vicious cycle, destabilizing everything in the ecosystem. As a result of this, any excess fertilizers, especially chemical ones can be said to be harmful not only to the bacteria living in the soil, but to all living things (Aktar W, Sengupta D, Chowdhury A, 2009).

Since both the bacteria and the fertilizers can be so beneficial to plant growth and the environment, we decided to explore if the addition of organic fertilizers to the soil increases or decreases the population density of the bacteria in the soil to help people determine which type of fertilizer they should use on their plants that will not only improve the growth but also not harm the soil microbe communities. Organic fertilizers are supposed to affect the microbial community positively because with the presence of more organic matter, it should increase the amount of microbial communities. (Cantoria C, 2012). Even though fertilizer increases the amount of plants, people overlook how it might affect the microorganisms living in the soil. This is important because the microorganisms are also essential to the growth of the plants.

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Lab Report

Problem: Does the presence of organic fertilizer increase or decrease the population density of bacteria in the soil of the front lawn on the RPCS campus?

Hypothesis: The presence of organic fertilizer will increase the population density of the bacteria in the soil of the front lawn on the RPCS campus.

Independent Variable: Application of organic fertilizer to the RPCS lawn

Dependent Variable: the density of bacteria in the soil samples collected

Negative Control: adding only water with no fertilizer to the soil sites on the front lawn

Positive Control: the samples of soil before the fertilizer/water mixture or negative control is added

Controlled Variables: size of soil patches, location of soil patches, number of days given for fertilizer to enable after it has been applied , type of fertilizer, amount of fertilizer, amount of fertilizer liquid added, amount of water added, method of testing for bacteria, size of pipettes, size of culture tubes, size of plates, type of nutrient agar used, same dilution level plated, type of water, degree to which diluted, size of soil samples extracted, concentration of fertilizer, size of soil extractor, time and day soil is extracted, amount of dilutions plated, amount of water in culture tubes, amount of soil added to first dilute

Step by step Instructions:

1. On the RPCS campus, go outside to a longitude of N39.35824° and a latitude of W076.63650°, making sure to determine your location with your GPS.

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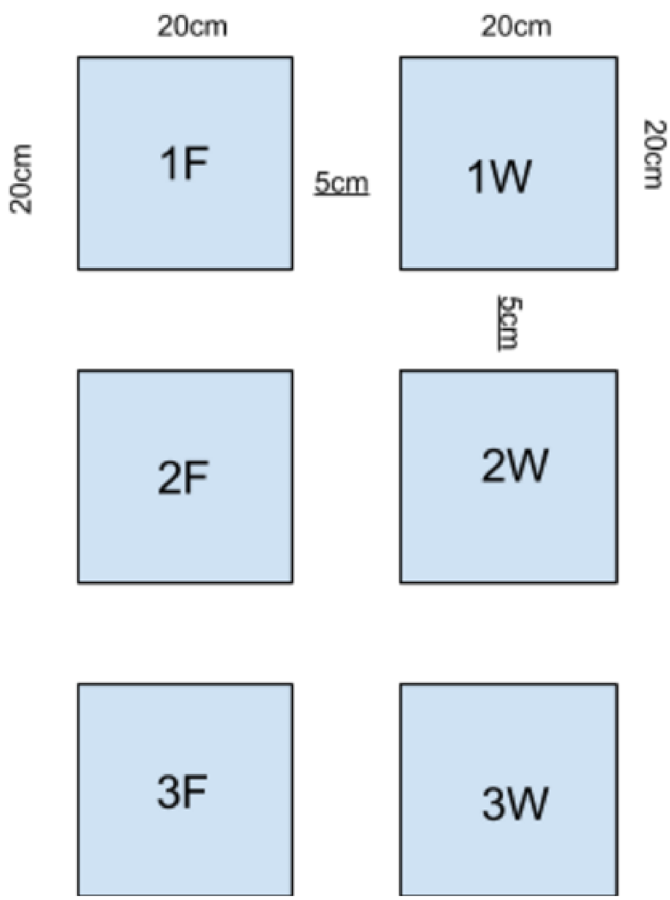
2. Using yellow flags, mark six 20cm by 20cm squares on the ground 5 cm horizontally and vertically apart from each other. (See the following diagram below.) Mark the squares in a 3x2 column,and label the plots to the west 1F, 2F, and 3F respectively, and the plots to the east 1w, 2w, and 3w respectively.



3. On the same day at the same time, collect the positive control from the each of the six plots using steps 4-5 for each plot.

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4. Collect 3 separate samples of soil from each of the squares using a soil extractor of 2cm in diameter, going 14cm deep into the soil.

5. Place each sample of soil collected into a clean plastic bag, making sure to label the bag based on which plot it is from and which soil sample it is. For example, your soil sample from soil plot 1F will be labeled 1Fa for the soil from the first hole in the ground you made, 1Fb for the soil in the second hole in the ground you made, and 1Fc for the soil from the third hole in the ground you made.

6. Once the soils are collected and placed properly in the ziploc bags, carry the soil samples back into the science room.

7. On the same day at the same time, carry out steps 7-21. Use a clean, new transfer pipette to add 10 ml of sterile water to a 15 ml culture tube. Label the tube and the pipette "10^0". Also label the tube with the soil sample you are using. For example, the tube testing the soil from plot 1F would be labelled 1F.

8. Use the same pipette to add 9 ml of sterile water to a second 15 ml culture tube. Label the tube "10^{-1}". Also label the tube with the soil sample you are using. For example, the tube testing the soil from plot 1F would be labelled 1F.

9. Using the same pipette in step 8, add 9 ml of sterile water to a third 15 ml culture tube. Label the tube "10^{-2}". Also label the tube with the soil sample you are using. For example, the tube testing the soil from plot 1F would be labelled 1F.

10. Using the same pipette in step 8, add 9 ml of sterile water to a fourth 15 ml culture tube. Label the tube "10^{-3}". Also label the tube with the soil sample you are using. For example, the tube testing the soil from plot 1F would be labelled 1F.

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11. Place 1 cc of the soil corresponding with the labeled “10^0” tube. For example, you will test the soil from plot 1F in the “10^0” tube labeled 1F.

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 labelled with the same soil plot. For example, you will remove 1 ml of the soil/water mixture from the “10^0” 1F tube to the “10^{-1}” 1F 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 labelled with the same soil plot. For example, you will remove 1 ml of soil/water mixture from the “10^{-1}” 1F tube to the “10^{-2}” 1F tube.

16. Cap and shake vigorously

17. Using the same pipette in step 13, remove 1 ml of the soil/water mixture from the “10^{-2}” tube and place into the “10^{-3}” tube, labelled with the same soil plot. For example, you will remove 1 ml of soil/water mixture from the “10^{-2}” 1F tube to the “10^{-3}” 1F tube.

18. Cap and shake vigorously

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

20. Shake the 3rd and 4th tubes. Plate 100µlsamples from the 3rd and 4th tubes (dilutions 10^{-2} and 10^{-3}) onto their own separate 3M Petrifilm™ Aerobic Count Plate, labeled with their plot, which soil sample, and their dilution number. For example, you will label your positive control 10^{-2} for soil plot 1F 1FB 10^{-2}. Spread the bacteria sample out on the 3M Petrifilm nutrient agar plate using the flat side of the spreader.

21. Repeat steps 4-20 for all the soil samples.

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22. Allow to grow for 48 to 72 hours

23. Examine each of your “10^{-3}” Petrifilm™ Aerobic Count Plates for individual bacterial colonies, but if there are less than five bacterial colonies on one of your plates examine your “10^{-2}” plates for that specific soil sample. For example, if your positive control 1F “10^{-3}” Petrifilm™ Aerobic Count Plate has less than 5 bacterial colonies, examine your 1F “10^{-2}”. Record the number of bacteria colonies observed and use that number in the following formula:

Microbes in 1 cc of soil= # Colonies on sheet $x10^2 x\ 10^{|\text{dilution \# at which these colonies were found}|}$

24. Record your data in the data table

25. Mix 7.8g of Espoma plant-tone organic gardening fertilizer with 500ml of water in a bottle

26. Repeat Step 25 two more times for a total of 3 fertilizer/water mixtures

27. At the same time and day, add 500ml each of the fertilizer/water mix to the soil plots labeled 1F, 2F and 3F and add 500ml each of water to the soil plots labeled 1W, 2W, and 3W.

28. Allow the fertilizer to sit for at least 48 hours.

29. After at least 48 hours repeat steps 3-5 for each square, labelling the bag based on which trial it is, and if it is the soil from the square of the negative control or the square where the fertilizer was added. For example, you will now label your plastic bag from soil plot 1F, 1FB

30. Repeat steps 7-24.

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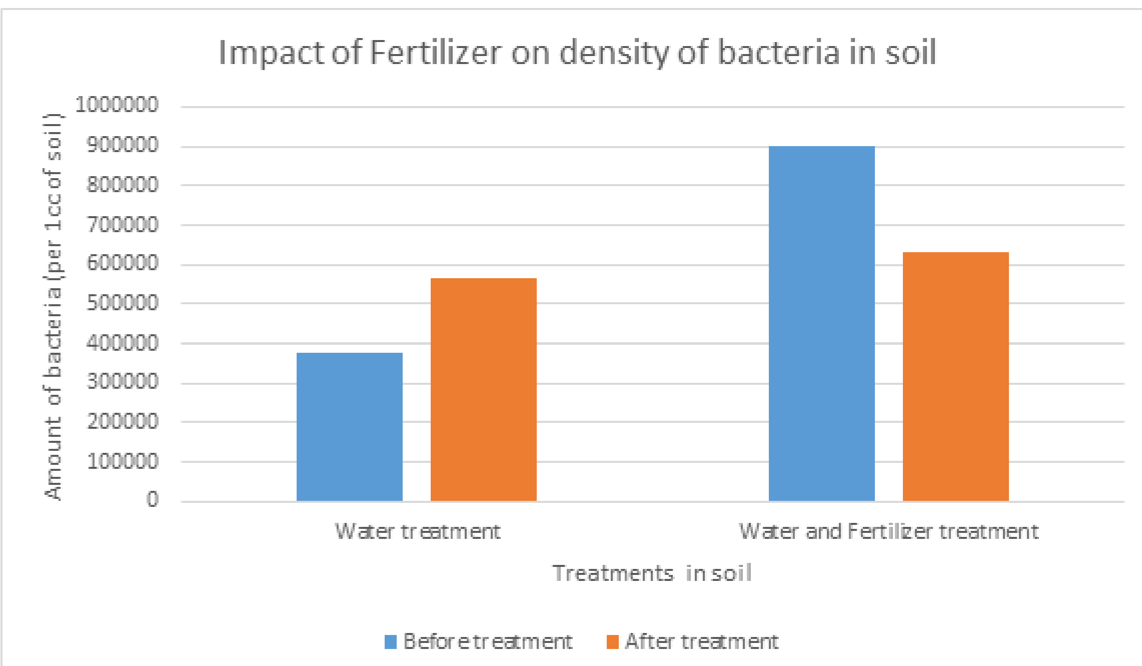
Data Table

Effect of fertilizer on bacteria (bacteria per 1cc of soil)

	Water treatment		**Water and Fertilizer treatment**	
Trials	**Before treatment**	**After treatment**	**Before treatment**	**After treatment**
1	350000	1000000	600000	550000
2	180000	100000	1000000	790000
3	600000	600000	1100000	550000
Average	376667	566667	900000	630000

Graphs



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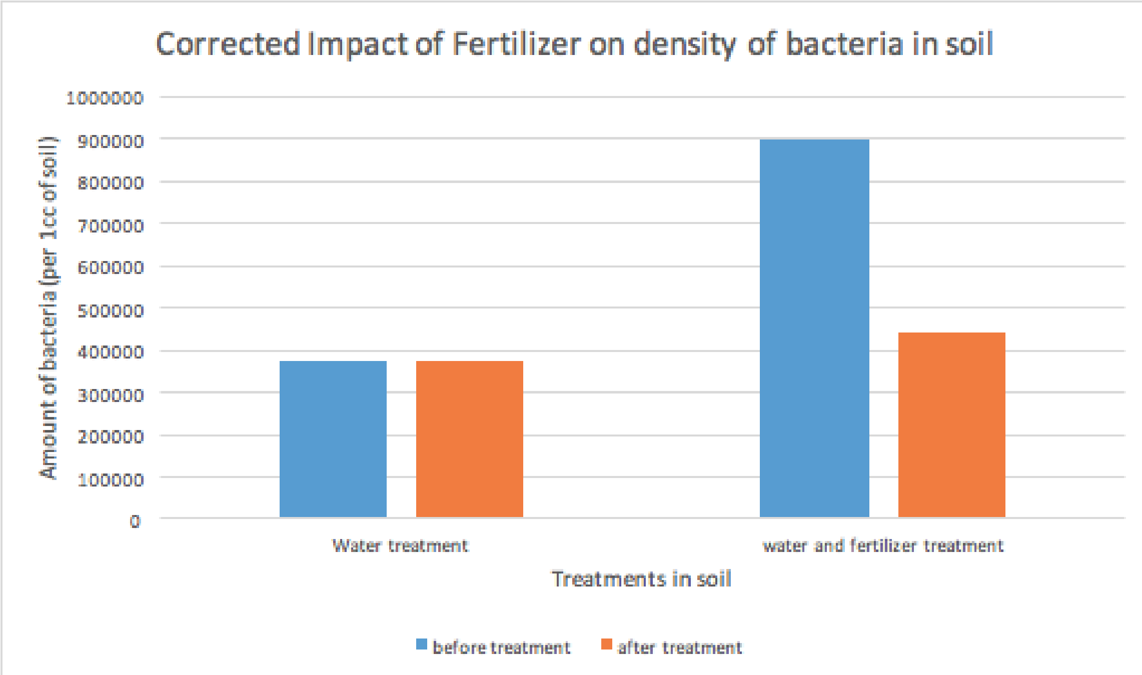

Conclusion

For our soil ecology project, we wanted to see how fertilizer affected the bacterial communities. Fertilizer is shown to help plant growth, but it is not as emphasized on what it does to the micro-organisms in the soil. In order to form a proper and logical hypothesis, we researched the different types of fertilizer, their different effects on bacteria, and how bacteria helps plants. Due to this research, we hypothesized that the presence of organic fertilizer will increase the population density of the bacteria in the soil of the front lawn at RPCS campus. We hypothesized this because we thought that since organic fertilizer only contains only compost and manure and no artificial nutrients, the population of bacteria would increase. However, our results proved our hypothesis to be false.

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In fact, while the water treatment increased the amount of bacteria in the soil, the combination of the water and the fertilizer decreased the amount of bacteria in the soil. The average of bacteria in the negative control soil plots for the positive control was 376667cc. Six days after we added 500 ml of water to the soil plots, the average actually increased by 50.5% to 566667cc. of bacteria. For the soil plots where we added the fertilizer/water mixture, the positive control for the three soil plots was 900000cc. of bacteria. Six days after we added the fertilizer/water mix to the soil plots, the results were much more surprising. Instead of the average increasing, like we hypothesized, the average actually decreased 30% to 630000cc. of bacteria.

In conclusion, our hypothesis was proved incorrectly because the water/fertilizer mix not only did not increase the amount of bacteria but also decreased it. We believe our hypothesis was incorrect because when organic fertilizer was added, the extra ammonium in the soil drowned and killed the bacteria, since ammonium is like the bacteria's waste product. However, a possible fault in our experiment is the weather. It rained every day the week of our experiment so the rain could have caused the fertilizer to dissolve much faster.

Since our hypothesis was wrong, in the future we could test many ways to increase the amount bacteria in the soil. We could test and see if biofertilizers would increase the amount of bacteria. This could prove to be more successful because biofertilizers are made of living fungi and bacteria beneficial to the soil while organic fertilizers are made of composts and manure. We would use the same procedure, but just replace the organic fertilizer with biofertilizer. Another way we could test to increase the amount of bacteria is to test different brands of organic fertilizer and see which brand increases the amount of bacteria the most.

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