RPCS

The Little Things that Run the World

The Effect of Soil Compaction on Population Densities of Bacteria

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Background Information

Bacteria play many important roles in the soil ecosystem (Bot and Benites 2005). They are responsible for decomposing organic material in the soil, and transforming it into nutrients that other organisms can use to grow. Bacteria's ability to decompose organic material allows them to decompose toxins, and from them create completely harmless particles that are released back into the soil (Hoorman 2011). Bacteria can also release minerals from rocks, and make them available to other organisms that demand them for their very survival. Additionally, bacteria aid in keeping a stable soil structure that improves water infiltration, aeration, as well as enable plants to grow (Bot and Benites 2005).

The structure of soil is a key piece of its health. Only 50% of the soil is made of solid matter. The remainder of the soil is open pore space, which is where water, organisms, organic matter and nutrients are found. The small pores in the soil provide storage and protection and the large pores between the aggregates (pieces or chunks of soil), are paths for liquids, gases, roots, and organisms to move through (Victorian Recourses 2011). Bacteria produce a sticky substance, known as exudates, that helps to bind the soil together, thereby improving its structure, making it a better environment for organisms to grow (Lewandowski 2002). The exudates enable the soil to maintain a consistency that supports soil life. There are various types of structures, and they include very fine, thin, medium, thick and very course soils (Victorian Recourses 2011).

Presence of bacteria is very important to create a livable soil stricture for its inhabitants (Lewandowski 2002).

Finally, bacteria play a key role in the nitrogen cycle, which is very important for the growth of plants, and the health for the ecosystem as a whole. Bacteria capture organic nitrogen from the soil, and transform it into inorganic forms, known as nitrates, that plants use to develop (Killpack and Buchholz 1993). About 99 % of the nitrogen in soil is in organic matter form and only a small portion of that nitrogen is available to plants. When bacteria break down soil organic matter, ammonium is released. Ammonium is then transformed into nitrates (forms of nitrogen) that plants can use. To do this, bacteria uses the nitrogenize enzyme that has iron protein and molybdenum-iron protein to transform the ammonium into nitrate (Deacan, 2010). The plants need bacteria to make these nitrates because of nitrogen's essential role in sustaining life. Nitrogen is a large elemental component of DNA, RNA, and amino acids, which are used to make enzymes. Enzymes start and stop chemical reactions which are needed to perform the four task of life, which are reproduction, regulation of environment, synthesis of new material and transformation of energy. When the four tasks of life cannot be performed, there is no living cell, and the plant dies. This means aerobic organisms, including humans, cannot perform their daily functions. This is because crucial biological processes, such as cellular respiration, require oxygen, which are provided from the photosynthesis of plants. The ability of bacteria to decompose and transform nitrogen into nitrates is crucial to the health of the ecosystem.

An interference in the soil nitrification process, such as soil compaction, can greatly decrease the health and fertility of the soil ecosystem. Bacteria's ability to transform raw nitrogen into nitrates is most effective when the soil is moist (Jacob and Cordaro 2000). This is because without water, chemical reactions, such as transforming nitrogen into nitrates, do not have a stable environment in which to take place. Soil compaction occurs when the soil is pressed, resulting in removal of pores, elimination of water, and decrease in the ability to hold air capacity. Soil compaction can be caused by many things; almost all cases are results of human action. Humans and animals alike walk on soil, which results in soil compaction over time. Also, equipment such as cars and farming tools, including plows and tractors greatly compact the soil (Killpack and Buchholz 1993). Placements of sidewalks and roads as well as construction are more causes of soil compaction, as these all press down on the soil with a tremendous amount of weight (DeJong-Hughes and Moncrief 2001).

Soil compaction is dangerous to the environment because it damages to the soil structure. This is important because it determines the ability of the soil to hold and conduct water, nutrients, and air, which are necessary for the plant to grow. Soil that is heavily compacted has few pores and can have a reduced rate of water infiltration. Pores are most effective in moving materials through the soil when it is wet, and therefore it is more difficult for bacteria when soil structure has been disrupted and dried out (DeJong-Hughes and Moncrief 2001). This means that human activities, which have increased soil compaction, are ultimately destroying the ecosystems through their impact on soil structure.

The two major results of soil compaction related to soil structure are the decrease in water infiltration and decrease in aeration, which is the process where air is circulated through the soil. In order for bacteria to more effectively transform organic nitrogen into nitrates, oxygen and water are necessary reactants (Jacob and Cordaro 2000). These two components are needed for the process of cellular respiration, where oxygen and glucose are transformed into carbon dioxide, water and ATP. This is the process of creating energy. A lack of oxygen and water in the soil can kill bacteria because they will not be able to produce energy to perform chemical reactions. If these chemical reactions do not occur, the cell would not be able to perform the four tasks of life which are transformation on energy, synthesizing new material, regulation of environment, and reproduction. Therefore, soil compaction can significantly hinder bacteria's ability to nitrify, and reproduce, thereby damaging the health of the soil and ecosystem as a whole.

As the soil compaction increases, we hypothesize that the bacteria population density will decrease, due to a major change in soil structure which eliminates water and oxygen from the soil. When the soil is compacted, the bacteria do not have enough room to perform their daily functions and die. A significant decrease in bacteria population will lead to a decrease in the producer population, therefore decreasing the amount of animals that survive in consumer populations, and distorting the food chain as a whole. Limiting the producer population results in limited energy transfers from producer to organism and then from one organism to another, and can have a large negative effect on the entire ecosystem.

This experiment is designed to test the population densities of bacteria at different levels of soil compaction. In doing this, we are also seeking to determine the health of the soil at different locations in the RPCS backwoods. A greater population density of bacteria implies a healthier soil ecosystem. Compaction destroys soil structure and diminishes pores such that bacteria cannot perform their vital role in the soil. We hypothesize that an increase in soil compaction will decrease the population density of bacteria, and consequently, decrease the overall health of the soil.

Lab Outline

I. Question: Does increased soil compaction change the population density of bacteria?

II. Hypothesis: As the level of soil compaction increases, the population density of bacteria will decrease.

III. Procedure:

A. Independent Variable: Soil samples taken from areas with heavy soil compaction, and semi-soil compaction.

B. Dependent variable: Population density of bacteria.

C. Negative Control: Soil sample taken from a location where there is no soil compaction.

D. Controlled Variables:

1. Location of soil sample
2. Amount of dilution allowed to grow (100 uL)
3. Weather during extraction (per trial)
4. Size of plot (25 cm by 25 cm)
5. Amount of water in dilution (50 mL total)
6. Amount of soil and water solution plated per trial (100 uL)
7. Size of culture tubes (15 mL)
8. Amount of soil tested per trial (1 cc)
9. Depth of soil collected in core sampler
10. Time of day and date that soil is taken and tested (same for all trials)
11. Amount of time soil sits before testing (same for each trial)
12. Plates that are used for bacteria colony growth (3M, Petri film Aerobic count plate)

13. Diameter of soil core extractor (2 cm)
14. Time left for bacteria to grow (96 hours)
15. Number of dilutions performed
16. Dilutions plated (10^{-3} and 10^{-4})

E. Step-By-Step Instructions

1. Mark 3 plots of soil, each one measuring 25 cm by 25 cm. Plot C (Compacted) is located at GPS location N 39.35764°, W 76.63855°; Plot S (Semi- Compacted) is located at GPS location N 39.5722°, W 76. 63841°; Plot N (Non-Compacted) is located at GPS location N 39.35761°, W 76.63792°.
2. Steps 3-7 must be completed on the same day, at the same time!
3. Travel to plot C and extract first trial of soil, using a soil extractor with a 2 cm diameter, by pushing it 15 cm into the soil.
4. Rotate the soil extractor 360° clockwise
5. Pull the soil extractor from the ground
6. Place the soil from the soil extractor in a sterile, plastic Ziploc bag, and immediately label it with "Compact 1" in accordance to the plot that it was extracted from, and its trial number.
7. Repeat steps 3-6 to extract trial 1 soil samples for additional 2 plots (semi-compacted and non-compacted), labeling the plastic bags by their respective locations (“Semi-compacted, 1” and “Non-compacted, 1”).

8. Once all soil from trial 1 has been collected and labeled, return to the biology lab, to begin the serial dilution process (steps 9-24). Trials must be done on the same day, at the same time!
9. Use a clean, new transfer pipette to add 10 ml of sterile water to a 15 ml culture tube. Label the tube"C,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 “C,1 10^{-1}".
11. Repeat step #9 three more times to three additional 15 ml culture tubes, only label them "C,1 10^{-2}" "C,1 10^{-3}" and “C,1 10^{-4}" respectively.
12. Place 1 cc of your compacted trial 1 soil sample into the "C,1 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 "C,1 10^{0}" tube and place into the "C,1 10^{-1}" tube.
15. Cap the tube and shake vigorously.
16. Using the same pipette as in step #14, remove 1 ml of the soil/water mixture from the "C,1 10^{-1}" tube and place into the " C,1 10^{-2}" tube.
17. Cap the tube and shake vigorously.
18. Using the same pipette as in step #14, remove 1 ml of the soil/water mixture from the " C,1 10^{-2}" tube and place into the " C,1 10^{-3}" tube.
19. Cap the tube and shake vigorously.
20. Using the same pipette as in step #14, remove 1 ml of the soil/water mixture from the " C,1 10^{-3}" tube and place into the " C,1 10^{-4}" tube. (you should now have a total of 5 culture tubes)

21. Plate 100 μL samples from the 4th and 5th tubes (dilutions C,1 10^{-3} and C,1 10^{-4}) onto their own separate, labeled 3M, Petrifilm Aerobic count plates.
22. Allow bacteria to grow for 96 hours.
23. Examine each of the $10^{-3}/10^{-4}$ plates, looking at the 10^{-4} plate first, for individual bacteria colonies. If the 10^{-4} plate has more than 5 bacteria colonies, that plate may be used to calculate data; if not, examine the 10^{-3} plate for individual colonies. To make your estimates for the number of bacteria in the original 1cc soil sample using the following formula:

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

24. Record the dilution plate at which the colonies were found, the number of bacteria on plate and the number of bacteria in 1 cc of soil in a data table.
25. Repeat steps 9-24 for two additional times with the trial 1 Semi-compacted and Non-compacted soil samples.
26. Repeat steps 2-25 to extract, dilute, and collect data for trials 2 and 3 of compacted, semi-compacted and non-compacted sites.

Data and Observations

A. Data Table

Population Density of Bacteria in Different Levels of Soil Compaction

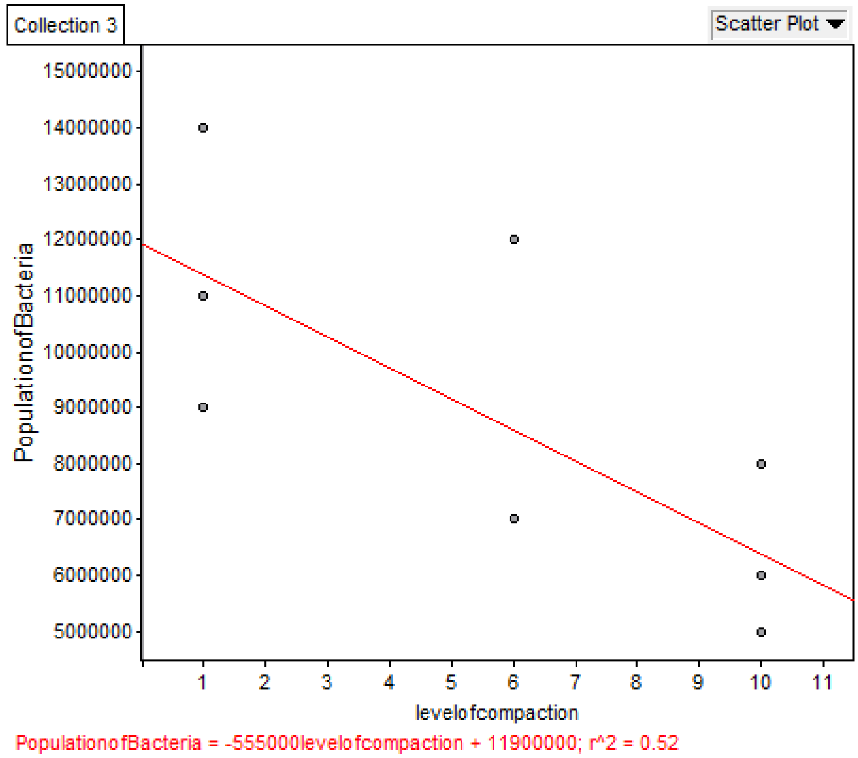
Trial Number	Soil sample	Dilution Number	Number of Bacteria on Plate	Number of Bacteria in 1 cc of soil
1	Compacted	10^{-4}	5	5,000,000
	Semi- Compacted	10^{-4}	7	7,000,000
	Non-Compacted	10^{-4}	11	11,000,000
2	Compacted	10^{-4}	6	6,000,000
	Semi- Compacted	10^{-4}	7	7,000,000
	Non-Compacted	10^{-4}	9	9,000,000
3	Compacted	10^{-4}	8	8,000,000
	Semi- Compacted	10^{-4}	12	12,000,000
	Non-Compacted	10^{-4}	14	14,000,000

Average Population Density of Bacteria in Different Level of Soil Compaction

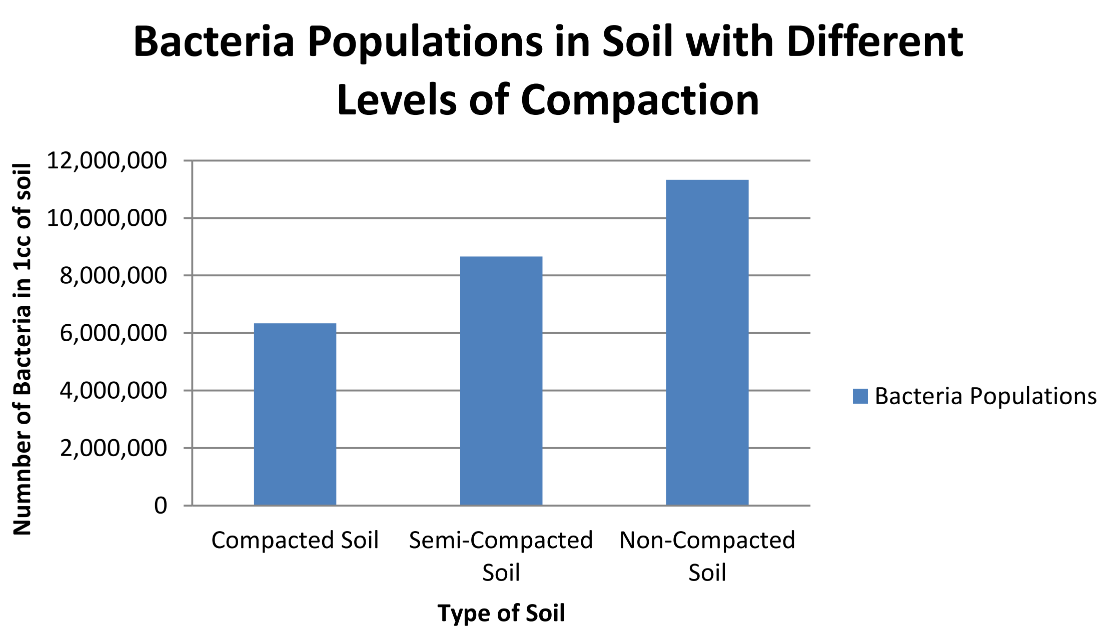
Soil Sample	Dilution Number	Bacteria on Plate	Bacteria in 1 cc of soil
Compacted	10^{-4}	6.333	6,333,333
Semi-Compacted	10^{-4}	8.666	8,666,667
Non-Compacted	10^{-4}	11.333	11,333,333

B. Graph

Scatter Plot 1:
Populations of Bacteria vs. Levels of Soil Compaction



Bar Graph 1



Conclusion

In conclusion, the hypothesis that soil compaction will cause the population density of bacteria to decrease was supported!

When an area of soil was affected by the human population of RPCS through compaction, the level of bacteria in the soil greatly decreased. On average, 11,333,333 bacteria inhabited soil with no compaction at all, and an area with semi-compacted soil, there were 8,666,667 bacteria. In contrast, only 6,333,333 bacteria inhabited an area with very much compacted soil. This proves that soil compaction decreases the populations of bacteria. This result was also supported by the bar graph, as it displayed the bacteria populations rising as the compaction level decreased from compacted to semi-compacted and to non-compacted.

When looking at the scatter plot, it also supported this trend. The line of best fit that was created possessed a negative slope. The equation, $y=-555000x+11900000$ (x being the level of soil compaction and y the population density of bacteria), shows this trend, and having an r^2 value of 0.52 tells that there is somewhat of a negative correlation between soil compaction levels and bacteria populations. The x-axis variable is the level of compaction, and on the y-axis is the population of bacteria in 1cc of soil. The downward slope shows that the more compacted the soil is, the lower the population of bacteria in the soil.

A T-test was also performed to determine the correlation between the levels of soil compaction. The only p-value that was an acceptable value was the one comparing compacted soil and non-compacted soil. The p value that was discovered for the test comparing these bacteria levels was 0.05, showing that there is a strong difference in the bacteria levels, also supporting the trend seen in the scatter plot, bar graph and averages. The other two tests (comparing compacted with semi-compacted soil and non-compacted soil with semi-compacted soil) resulted in a p-value of 0.3, showing that there was not a great difference in the population levels of bacteria.

According to the calculated averages, scatter plot and T-test results, we can draw the conclusion that soil compaction causes a decreased population of bacteria. This is likely because of the increased level of oxygen and moisture that is present in less compacted soil, which bacteria require to live. Therefore, they would flourish in an area with less compacted soil.

There are further experiments that could be performed based on our own experiment. From this experiment, an experiment could be designed where one could test the population density of bacteria by increasing soil aeration, or adding pores to the soil, which is the opposite of compaction. One might hypothesize that the bacteria populations would increase as the aeration increases. Because increasing aeration and compaction are opposite actions, one would see opposite results in the population densities of bacteria. This experiment would explore a compacted area, and see if it could regain the bacteria populations that it lost during compaction, when provided with additional aeration. Additionally, an experiment could be done to see the effect that soil compaction has on fungi populations to explore the idea that soil compaction would have a similar effect on fungi as it has on bacteria; decreasing its population as the levels of soil compaction increase. Because fungi greatly contribute to the overall health of the soil, its decreased population due to compaction can be fatal to soil health.

Work Cited

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