SOIL ECOLOGY PROJECT: SOIL COMPACTION AND BACTERIA

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Background:

Whiting, Wilson, and Reeder say, "Soil compaction can change a block or aggregate structure (with good infiltration and drainage) into a massive structure (with poor infiltration and drainage)." (Whiting, Wilson, Reeder. 2011) Foot traffic, like that in a garden, is a prime example of gradual, yet equally damaging soil compaction. The College of Agricultural Sciences defines soil compaction as, “the reduction of soil volume due to external factors.” (College of Agricultural Sciences. 2014) As a result of soil compaction, the volume of pore space, specifically large pore space, lessens considerably in addition to the increase in restriction of air and water movement in and throughout the soil. This means that less oxygen can be stored or used in the soil. The restriction of oxygen prevents bacteria from performing cellular respiration, which is the process in which glucose and oxygen produce carbon dioxide, water, and ATP (energy). Unable to perform cellular respiration, bacteria are deprived of the energy necessary to complete their daily tasks and reproduce. If bacteria can't perform tasks and reproduce, the plants have no alternative source of energy. As a result, bacteria, as decomposers in the ecosystem, are prevented from fulfilling their environmental duty of aiding in the growth of producers. Producers go on to then give energy to the primary consumers. Primary consumers give secondary consumers energy. This transferring of energy continues through the tertiary consumers and quaternary consumers. When creatures at the top of the food chain die, decomposers now have food, the cycle begins again.

In the nitrogen cycle, nitrogen is recycled in different forms to be used by different organisms. First, animal waste and decaying organic matter gets absorbed into the soil, as ammonia. Then the nitrogen fixing bacteria eats the ammonia and produces ammonium. Afterwards nitrifying bacteria changes ammonium into nitrites, which are later converted into nitrates. Nitrates are taken up by the plant to live and grow. Nitrogen is crucial to a plants life because DNA and RNA are made from nitrogen bases. After DNA is transcribed into RNA, RNA is translated into proteins or enzymes, which contain amino acids, also made out of nitrogen. Enzymes are what start and stop chemical reactions to perform the 4 tasks of life, including homeostasis, synthesis of new material, reproduction, and transformation of energy. The 4 task of life is what makes a cell and effectively the organism living. This process would not be possible without bacteria; therefore plants could not live without bacteria. (Duckster 2014)

The nitrification process is what would be the most impacted by the compaction of soil. The nitrification process is when a specific group of bacteria in the soil pulls nitrogen from the air and bring it into the soil. The nitrogen is made into nitrate which is what feeds the plants. The more soil bacteria in a specific area, the less compacted the soil will be because for the nitrification process to happen the soil must have pores. The pores are where the nitrification process takes places, meaning that the pores have to be big so that there is space for this task to happen. Compaction shrinks and eventually eliminates pores in the soil, depending on the level of compacted. (Bryan Morse and Kevin Norton 2014)

Compaction of the soil changes pore space size, distribution, and soil strength. The compacted soil will have few pores and decreases the rate of infiltration of water and drainage. Roots can't penetrate compacted ground; water can't drain into the earth and instead runs off,

causing erosion. This will impedes the growth of the root, therefore decrease the ability to take up nutrients and water. (DeJong-Hughes, Moncrief, Voorhees, and Swan 2001) The individual soil particles assembled as aggregates and there are soil pores between them. There are three main types of soil structure, single-grain structure, granular structure and massive structure. The single-grain structures are the individual soil particles which do not bind together. The granular structure, where clay particles and humus substances plays an important role in the formation of it. The compaction will push air and water out of soil so that it becomes denser. There will be less pores, spaces, air, nutrient in compacted soil. (Kulmala, 2012)

Humans do many different things that cause soil compaction. For example, intensive agriculture may lead to a major compaction of soil through increased traffic on the surface of the soil, and the mass of the farm machinery being increased (Grieve, 2001). Also, heavier equipment involved with farming increases soil compaction, along with multiple field operations going on at one time. When a machine is carrying heavy equipment then the wheel increases the level of soil compaction. Tractors alone have increased their weight over the past 70 years from less than 3 tons to just about 20 tons (Schuler 2009). Human-induced soil compaction is most often caused by wheel traffic by off-road vehicles. In mechanized agriculture, vehicles with high axle load are one of the major causes of compaction as well (Soane and van Ouwerkerk, 1994.) Another cause of soil compaction is road construction, which increases soil compaction up to 200 times more than a place without road construction going on nearby (Riley 1984). Compaction of soil can also be caused by deforestation. The machinery that is used to remove trees from land is very large and they are used in all types of weather conditions which results to high level of soil compaction**.** Building houses, roads, sidewalks, and

other parts of infrastructure also lead to soil compaction (Cranfield University, 2014). Foot traffic, like that in a garden, is a prime example of gradual, yet equally damaging soil compaction. Foot traffic is even more apparent in moist soils (if compacting occurs while soil is still moist). This type of compaction typically occurs during the assembly of a home. (Whiting, Wilson, Reeder. 2011) Soil compaction is almost irreversible—it's very difficult to undo its negative influence on the soil, which is why it's better to put forth all efforts in its prevention. (Whiting, Wilson, Reeder. 2011)

Soil Compaction Lab

I. Problem: Does the level of soil compaction change the population density of bacteria in the soil? Hypothesis: The higher the level of soil compaction, the lower the population density of bacteria in the soil.

II. Independent Variable: the level of compaction of the soil—high compaction and semi-compaction

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

IV. Negative Control: non-compacted soil

V. Controlled Variables: type of environment, light exposure of soil, temperature of soil, size of plot, amount of soil being extracted, amount of solution, amount of soil (in each culture tube), amount of soil/water mixture removed from tube, amount of trials/culture tubes, amount of samples plated from culture tubes, amount of time allotted for growth of each diluted sample, day/time soil is extracted, type of petri plates

VI. Step-by-Step Instructions:

1. Mark 3 plots of soil (H, M, and N), each measuring 40 cm x 40 cm, located with coordinates: Plot H at N39.21484°, W076.38176°; Plot M at N39.35812°, W076.63611°; Plot N at N39.35816°, W076.63644°.
2. Extract one 15 cm sample of soil from plot H using soil core sampler, 2 cm in diameter, by pressing sampler down into soil (15 cm deep) and turning sampler clockwise until it resurfaces. Then proceed to pull sampler straight out of ground.
3. Place extracted soil sample from Plot H into a sterile Ziploc bag labeled with Plot H, Trial 1
4. On the same day at the same time, repeat steps 2-3 using Plot M and Plot N (labelled with plot letter and Trial 1)
5. In lab-room, dilute all samples from the same trial on the same day, beginning with sample from Plot H, Trial 1 using the process noted in steps 6-22:
6. Use a clean, new transfer pipette to add 10 ml of sterile water to a 15 ml culture tube. Label the tube "Plot H trial 1 10^{0}".
7. Use the same pipette to add 9 ml of sterile water to a second 15 ml culture tube. Label the tube "Plot H Trial 1 10^{-1}".
8. Repeat Step 7 two more times to two additional 15 culture tubes, only label them Plot H trial 1 "10^{-2}" and Plot H trial 1 "10^{-3}",.
9. Place 1 cc of your Plot H trial 1 into the "Plot H trial 1 10^{0}" culture tube.
10. Cap the tube and shake vigorously.

11. Using a new clean pipette, remove 1 ml of the soil/water mixture from the "Plot H trial 1 10^{0}"tube and place into the "Plot H trial 1 10^{-1}" tube.
12. Cap and shake vigorously.
13. Using the same pipette used in Step 11, remove 1 ml of the soil/water mixture from the "Plot H trial 1 10^{-1}" tube and place into the "Plot H trial 1 10^{-2}" tube and place into the "Plot H trial 1 10^{-3}" tube.
14. Cap and shake vigorously.
15. Using the same pipette used in step 11, remove 1 ml of the soil/water mixture from the "Plot H trial 1 10^{-2}" tube and place into the "Plot H trial 1 10^{-3}" tube.
16. Cap and shake vigorously.
17. You should now have a total of four culture tubes.
18. Plate 100μl samples from the 3rd and 4th tubes (dilutions 10^{-2} and 10^{-3}) onto their own separate, labeled petri plates containing nutrient agar (3M Petrifilm™ Aerobic Count Plate)
20. Allow bacteria to grow for 72 hours.
21. Examine the 10^{-3} plate for individual bacteria colonies, seen as red dots (needs at least 5 colonies—if less than 5, examine the 10^{-2} plate). Make estimate 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 $10^{|\text{dilution \# at which these colonies were found}|}$

22. Repeat steps 5-22 using soil samples from Plot M (Trial 1) and Plot N (Trial 1) respectively on the same day at the same time

23. Extract Trial 2 samples by repeating Steps 2-3, now labeling samples with respective plot letter and Trial 2
24. Dilute Trial 2 samples by repeating Steps 6-21 on the same day at the same time
25. Extract Trial 3 samples by repeating Steps 2-3, now labeling samples with respective plot letter and Trial 3
26. Dilute Trial 3 samples by repeating Steps 6-21 on the same day at the same time

Data Tables:

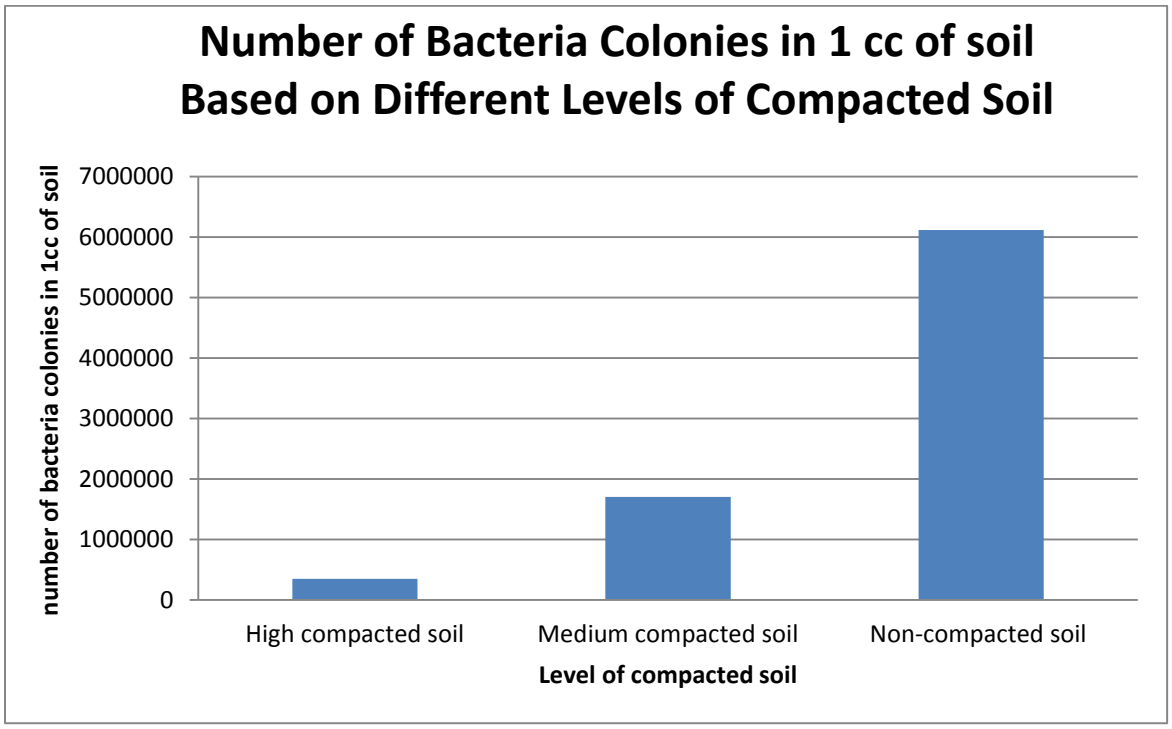
Population density of bacteria in 1cc soil based on the level of soil compaction

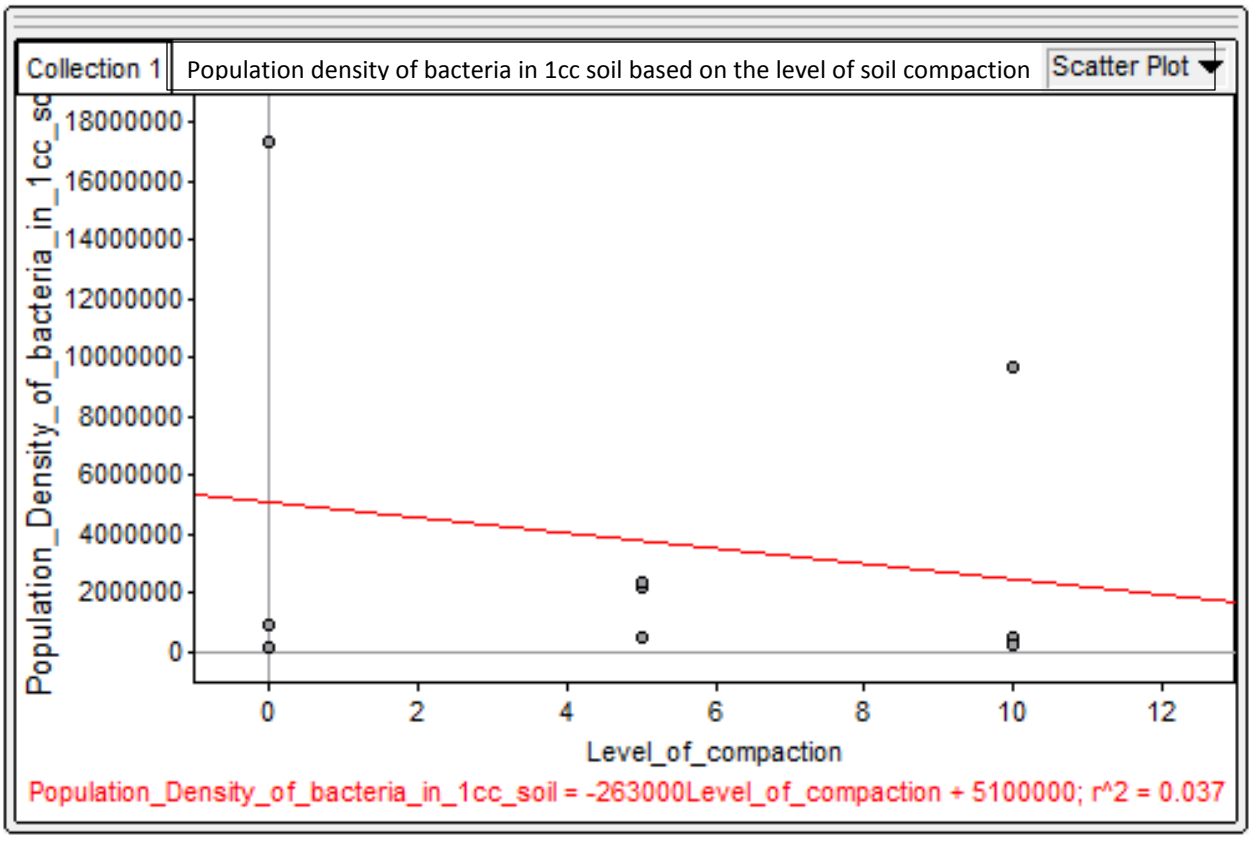
Soil sample	# Dilution	# bacteria on the plate	# colonies in 1 cc of soil
High compaction soil	Trial 1: 10^{-3}	Trial 1: 5	Trial 1: 500000
	Trial 2: 10^{-2}	Trial 2: 26	Trial 2: 260000
	Trial 3: 10^{-3}	Trial 3: 97	Trial 3: 9700000
Medium compaction soil	Trial 1: 10^{-3}	Trial 1: 22	Trial 1: 2200000
	Trial 2: 10^{-3}	Trial 2: 5	Trial 2: 500000
	Trial 3: 10^{-3}	Trial 3: 24	Trial 3: 2400000
None compaction soil	Trial 1: 10^{-3}	Trial 1: 9	Trial 1: 900000
	Trial 2: 10^{-2}	Trial 2: 14	Trial 2: 140000
	Trial 3: 10^{-3}	Trial 3: 173	Trial 3:17300000

Average of population density of bacteria in 1cc soil based on the level of soil compaction

Soil sample	# bacteria colonies in 1 cc of soil
High compacted soil	348666
Medium compacted soil	1700000
None compacted soil	6113333

Graphs:


Number of Bacteria Colonies in 1 cc of soil
Based on Different Levels of Compacted Soil
number of bacteria colonies in 1cc of soil
7000000
6000000
5000000
4000000
3000000
2000000
1000000
0
High compacted soil
Medium compacted soil
Non-compacted soil
Level of compacted soil



Conclusion

Our hypothesis was supported in this experiment because we saw that the higher the level of soil compaction, the lower the population density of the bacteria in the soil. In the high compaction soil there was an average of 348,667 bacterial colonies per cc of soil. In the medium compaction soil there was an average of 1,700,000 bacterial colonies per cc of soil. In the non-compacted soil, there was an average of 6,113,333 bacterial colonies per cc of soil. However, after looking at the scatter plot of our data, there are a couple large outliers in our graph that led to a very poor correlation. The r^2 value of the least squares line, or line of best fit, is 0.037. This poor correlation of our data means that while our hypothesis was supported, it is not very strongly supported.

A future experiment that we could do is to compact our own soil to see if the results would change if we altered the amount of compaction. This would be a controlled experiment because of our ability to compact the soil ourselves. We would then find one big plot, leaving one soil sample not compacted at all, then compacting one sample soil to a medium amount, and then the last sample of soil we would compact the most to make it the high compaction soil sample. We would compact these soil samples by using 15 pound weights shaped like a raffle ticket. The medium compacted soil would have one of the 15 pound weights on top of the soil for 7 full days. For the high compacted soil, we would place two of the 15 pound weights on top of this soil sample, and leave the weights on top of this soil for 7 full days to allow the compaction to occur. Doing this experiment would give us more accurate results and give us a chance to see the levels of soil compaction when we compact the soil ourselves.

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