Soil Ecology Project

Brock 9H

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Background of Soil Compaction and Bacteria

Bacteria are single celled microbes with a simple structure that does not include a nucleus, or any other membrane bound organelles. They can be found in every ecosystem on the planet, including the soil, and in the soil, there are four main groups of bacteria: decomposers, mutualists, pathogens, and lithotrophs (Ingham 2009). The first of these, the decomposing bacteria, consume carbon compounds along with simple sugars, integrating nutrients such as nitrogen into their cell membranes which can then be a source of nitrogen for the organisms that consume them. Mutualistic bacteria include four types of bacteria that convert nitrogen from the atmosphere into nitrogen that can be used by plants and other organisms, and while pathogenic bacteria can cause disease in plants and other organisms, there are few of this kind of bacteria present in the soil. Finally, lithotrophs are bacteria present in the soil that get their energy from inorganic substances such as sulfur and nitrogen. (Lowenfels & Lewis, 2006)

One of the most important functions bacteria in the soil perform is the cycling of nutrients, (Microbiology Society 2019) and one of the most important of these is the element nitrogen (Dick R. 2009). However, most nitrogen exists as nitrogen gas in the atmosphere and is therefore inaccessible to most organisms (Kahl, 2004). However special nitrogen fixing bacteria such as Diazotrophs and Rhizobia can take this nitrogen gas from the atmosphere and convert it into ammonium. (Kahl, 2004) Other nitrifying bacteria then change this ammonium into nitrite and nitrate through the process of nitrification. Both ammonium and nitrate are forms of nitrogen that plants use to carry out their cellular functions. Then, with the nitrogen now present in the plants, it can move into the animals that consume them and on through the food chain, and when the plants and animals die, they release waste which is converted into ammonium by decomposing bacteria through the process of ammonification, releasing it back into the soil for the plants to reuse. Finally, denitrifying bacteria convert any excess ammonium back into atmospheric nitrogen through the process of denitrification. (Adnan 2010)

The reason Nitrogen is so important is that it is used as a building block towards creating proteins and nucleic acids. Proteins and nucleic acids are needed for stopping and starting the chemical reactions that cause the four properties of life (Pidwirny M. 2006). Hence, nitrogen is needed for plant growth and development as it makes up the chlorophyll as well as their amino acids. Low levels of nitrogen can cause stunted growth in plants.

Since nitrogen and its cycling through the soil to ecosystem is so important, anything that may interfere with it could harm the environment, and one such factor is soil compaction. This process is the compression of molecules in the soil into a smaller volume. Thus, soil compaction could affect the amounts of ammonium nitrogen along with the density of bacteria in the soil. People do this for the purpose of construction and these areas are often close to buildings, sidewalks, or roads. The reason construction workers compact soil is to make their foundation and surface more stable.

Because soil compaction, decreases the amount of pore space available for the exchange of air and water (Briggs and Stratton, 2019) it could be very detrimental to bacteria because they survive better in highly oxygenated areas, and soil with large pore spaces and with larger air spaces have more oxygen than soil that is compacted. Ammonium can only be converted into nitrate when aerobic conditions are present (Pidwirny M. 2006) (Dick, W. 2009), and this is an aerobic process that can only be performed when oxygen is also present in the soil (Elaine R. Ingham). Thus, compaction has the potential to greatly reduce the fertility of the soil and has very negative effects on both plants and organisms in the soil (Dick R. 2009).

We decided to choose the topic of seeing soil compaction's effects on the levels of ammonia nitrogen and the levels of soil bacteria in the Roland Park environment. In our project we should expect to find more bacteria in areas with less compacted soil. We believe that the soil nearest to sidewalks will show low levels of ammonia as well as low levels of soil bacteria. This can therefore show the effects that construction can have on the soil of our school environment.

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Experiment

Problem: Does the level of soil compaction alter levels of ammonia nitrogen and bacteria?

Hypothesis: The soil sample closest to the sidewalk of the RPCS campus will have the lowest levels of bacteria and the lowest levels of Ammonia Nitrogen.

Independent Variable: the distance away from the sidewalk that soil is collected from (more compacted to less compacted)

Dependent Variable:

1. Amount of Ammonia Nitrogen present in soil sample
2. Density of Bacteria present in soil sample

Negative Control: Soils samples from directly next to the sidewalk.

Controlled Variables: environment soil is collected from, type of nitrogen (ammonia) tested for, when the soil is collected, color chart used for level of ammonia nitrogen, time when experiment is conducted, Soil extraction process used, location of extracting area, type of microbes tested for (bacteria), amount of soil sample tested (1-cc), size of soil samples (14.5cm by 2cm column), use 15ml culture tubes, use serological pipettes for extractions, Petrifilm Aerobic Count Agar Plates, use micropipette for microliter extractions, allow bacteria and chemicals to sit for 72 hours before taking data, use magnifying glass to count bacteria colonies, amount of solution plated, degree which soil is diluted, which dilutions were plated, type of magnifying glass (5x).

Soil Sample Marking Instructions

1. Go to the location 39.35806 North and 076.63633 West on the Roland Park Country School campus. Go to the sidewalk next to the ward house

2. Stick a yellow flag, labeled “sidewalk 1” east next to the side walk

3. Measure 1 meter from the yellow flag south and place another yellow flag, labeled “sidewalk 2” in the soil directly next to the sidewalk

4. Measure 1 meter from the flag labeled “sidewalk 2” south, and place another yellow flag labeled “sidewalk 3”, directly next to the sidewalk

Measure 1.5 meters, east, from each of the 3 flags and place a yellow flag in each spot

Label one yellow flag “1.5 meter #1”, another yellow flag “1.5 meter #2”, and the other yellow flag “1.5 meter #3”, respective of the corresponding flags.

7. Measure 1.5 meters east, from each flag labeled 1.5 meter and place a yellow flag in each spot

8. Label one yellow flag “3 meters #1”, “3 meters #2”, “3 meters #3”

9. Use a soil extractor to extract soil from the places where there are flags. **Take all soil samples on the same day, at the same time.**

10. Place a 14.5 by 2cm column of soil in a plastic bag labeled with the location and sample number. (e.g.: Sidewalk #3)

Bacteria Procedure: **(Perform Bacteria Procedure and Ammonia Nitrogen Procedure simultaneously on all soil samples on same day at same time)**

1. Gather 36 15 ml culture tubes and divide them into sets of 4

2. Label one culture tube in each set of four 10^0, another test tube $10^{-1,}$ another $10^{-2,}$ and the last 10^{-3}

3. Repeat step 2 until all of the culture tubes are labeled with the dilutions

4. Next, label the groups of (4) culture tubes with a corresponding soil sample. Label one group “sidewalk, trial 1”, another group “1.5-meter trial 1”, and another group “3-meter trial 1”

5. Repeat step 4 two more times except changing the trials to “trial 2” and “trial 3” the last time
6. Place all of the culture tubes in a test tube rack
7. Get a clean, new transfer pipette and add 10 ml of sterile water to each of the culture tubes that have the “10^0” labeling
8. Use the same transfer pipette to transfer 9 ml of sterile water into each of the rest of the test tubes
9. Place 1cc of the “sidewalk trial 1” soil into the corresponding culture tube with the dilution 10^0 (the tube should be labeled “sidewalk trial 1, 10^0”)
10. Cap the culture tube and shake vigorously
11. Get a clean new transfer pipette, and remove 1 ml of the soil/water mixture from the sidewalk trial 1 10^0 tube and place it in the $10^{-1,}$ sidewalk $^{\text{trial}}$ 1 tube
12. Cap tube and shake vigorously
13. Still using the same pipette, remove 1ml of the mixture from the 10^{-1} sidewalk trial 1 tube and place it into the 10^{-2} tube sidewalk trial 1 tube
14. Cap tube and shake vigorously
15. Use the same pipette to remove 1ml of the mixture from the 10^{-2} sidewalk trial 1 tube and place it in the 10^{-3} sidewalk trial 1 tube
16. Cap tube and shake vigorously
17. Repeat step 9 with each 10^0 tube using its corresponding soil samples and a new pipette for each soil sample
18. Then repeat steps 11-16 with each set of (4) culture tubes. The soil sample must correspond to the trial and distance on the tube.

19. After all the tubes have been diluted, get 18 agar 3m Petrifilm TM aerobic count plates and label 9 of them 10^{-2} and the other 9 with 10^{-3}

20. Then put them in groups by pairing one 10^{-3} plate with one 10^{-2} plate.

21. Label each set of agar plates with a corresponding soil sample (sidewalk trial 1, sidewalk trial 2, sidewalk trial 3, 1.5-meter trial 1, 1.5-meter trial 2, 1.5-meter trial 3, 3-meter trial 1, 3-meter trial 2, 3-meter trial 3)

22. Get a micro pipette and place a brand-new micro pipette tip onto the micro pipette

23. Remove 100 microliters from the 10^{-2} tubes and the 10^{-3} tubes a new microliter tip each time

24. After the 100 microliters is removed, put it on the agar plate that has the same labeling as the tube that you removed it from

25. After you lift the film to place the 100 microliters on the agar plate, put the film back down and press down on the plate with a smooth, hard surface to flatten out the liquid

26. Allow the bacteria colonies to grow for 48-72 hours

27. For each soil sample, observe the # of bacteria colonies on the 10^{-3} dilution plates confirming that there are at least 5 colonies, if so, disregard the 10^{-2} plate of the corresponding sample. If there are less than 5 colonies than disregard the 10^{-3} plates and instead use the corresponding 10^{-2} plate for that sample.

28. Count and record the number of bacteria colonies on the chosen plate using a 5x magnifying glass (the red dots indicate the bacteria colonies).

29. Estimate the number of bacteria in the original 1 cc soil sample using the following formula.

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

Ammonia Nitrogen Test Procedure **(Perform Bacteria Procedure and Ammonia Nitrogen Procedure simultaneously)**

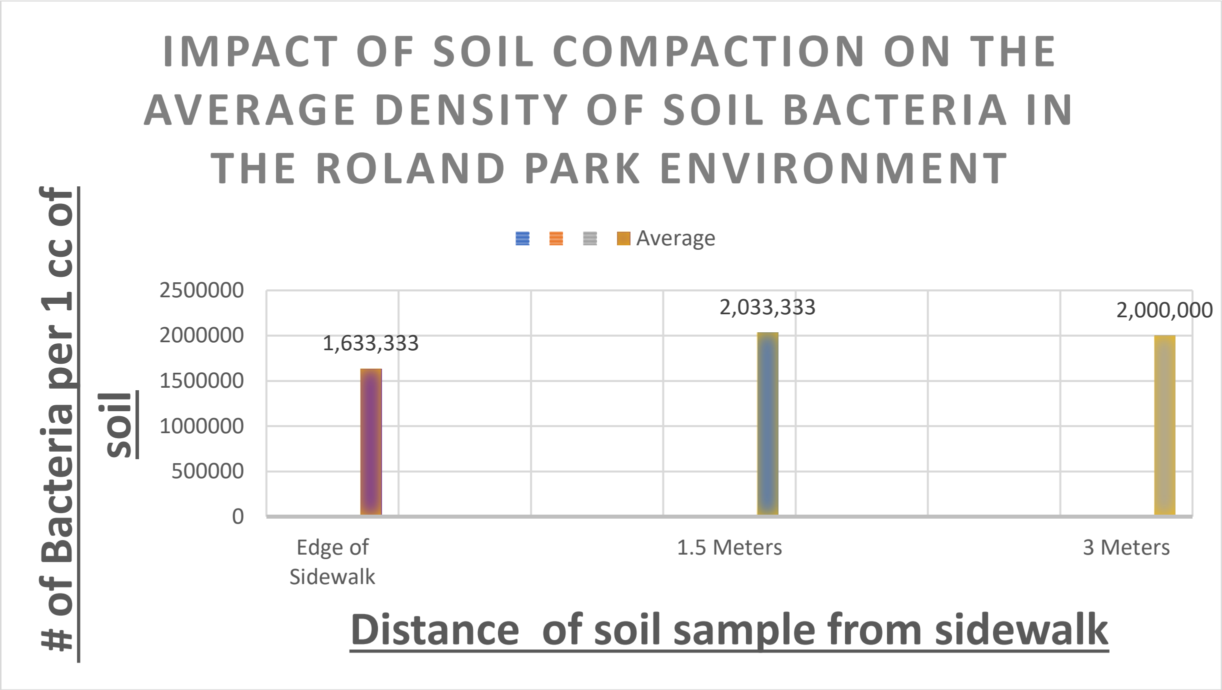
1. Use the LaMotte STH professional soil testing kit to perform the Ammonia Nitrogen test.

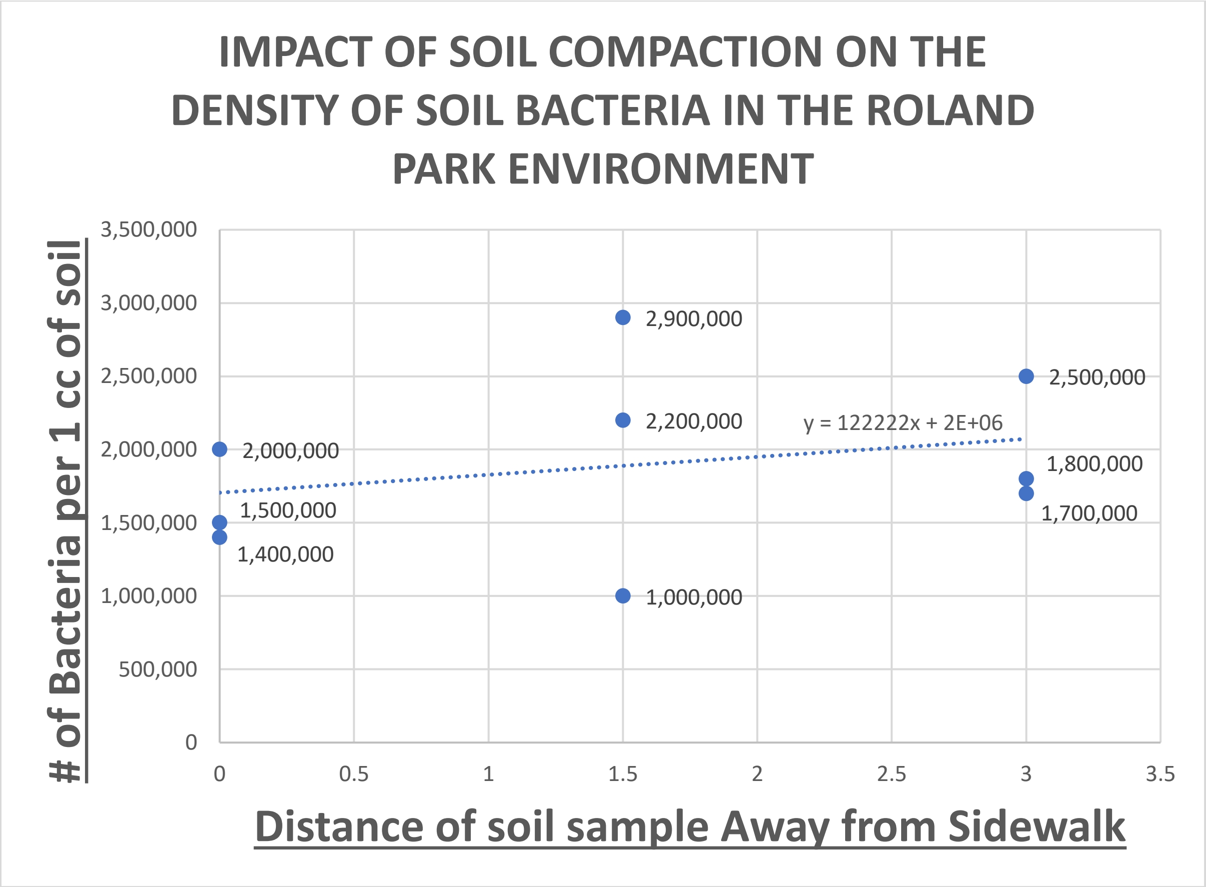
Impact of Soil Compaction on Levels of Ammonia Nitrogen and Density of Soil Bacteria in Roland Park Environment

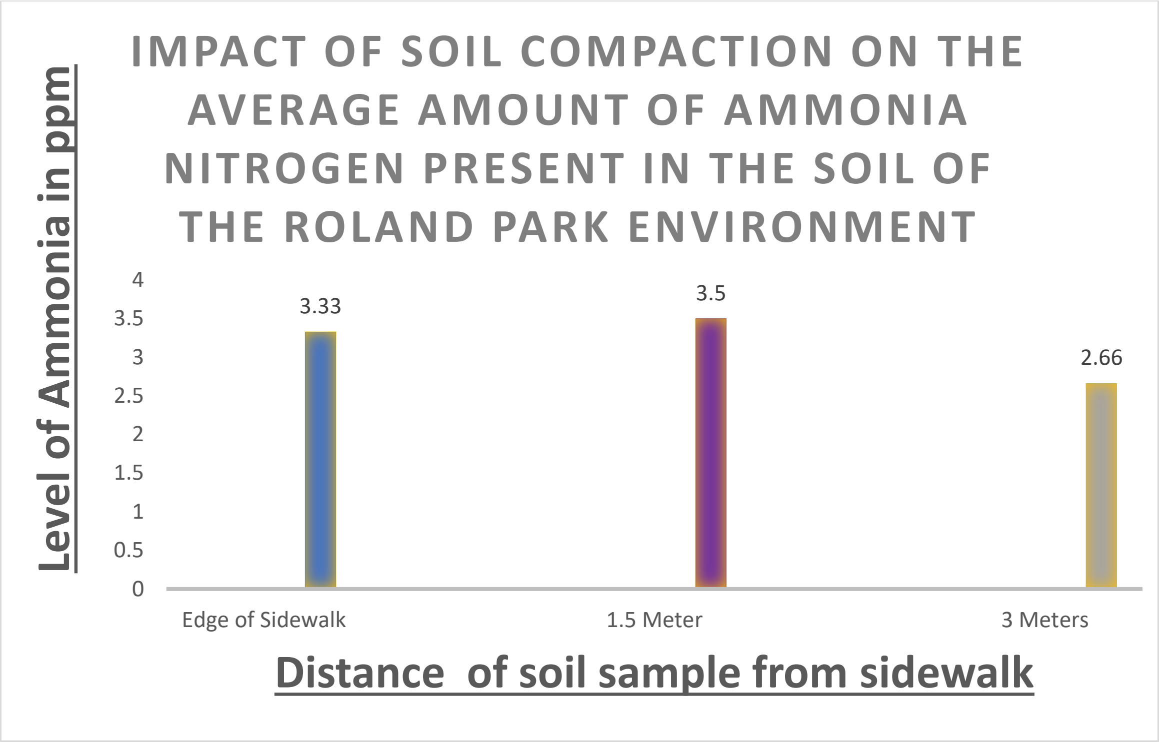
Data:

Edge of Side Walk	# of bacteria microbes in 1cc of soil	Ammonia Nitrogen Level in ppm	1.5 Meter from the Sidewalk	# of bacteria microbes in 1cc of soil	Ammonia Nitrogen Level in ppm	3 Meters from the Sidewalk	# of bacteria microbes in 1cc of soil	Ammonia Nitrogen Level in ppm
Sample #1	1,500,000	4	Sample #1	1,000,000	2.5	Sample #1	1,700,000	3
Sample #2	1,400,000	4	Sample #2	2,200,000	5	Sample #2	2,500,000	5
Sample #3	2,000,000	2	Sample #3	2,900,000	3	Sample #3	1,800,000	0
Average	1,633,333	3.33		2,033,333	3.5		2,000,000	2.66

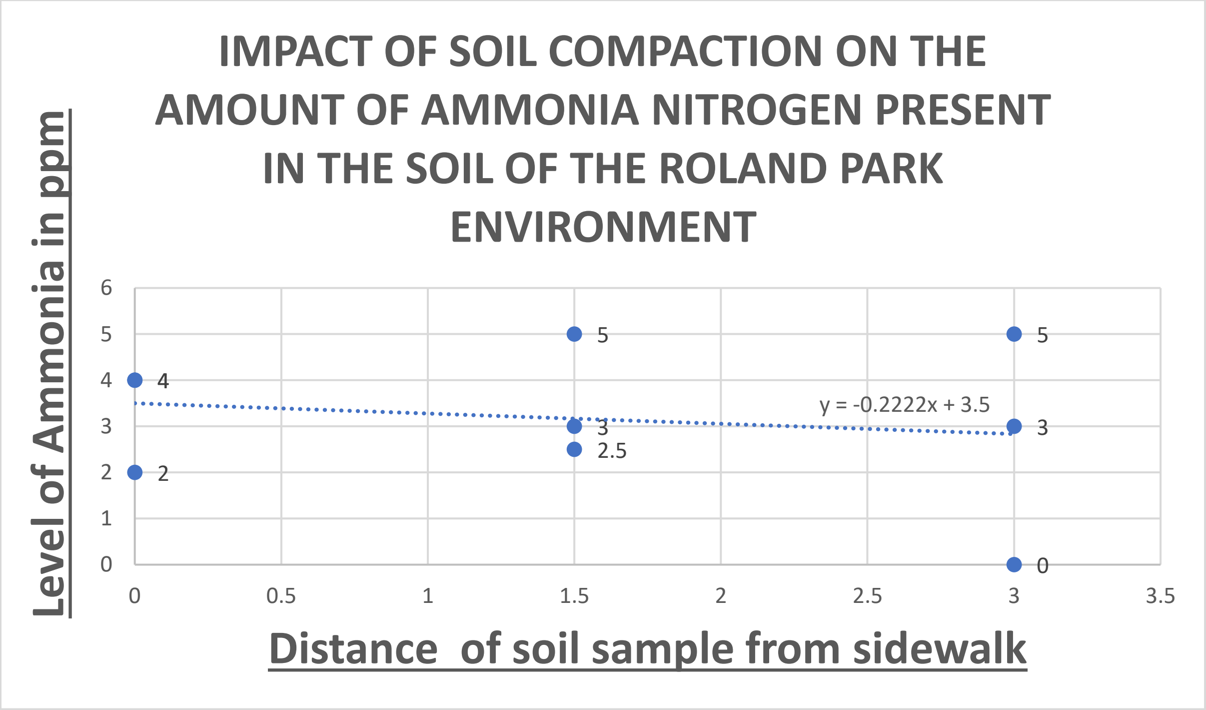
Graphs:


IMPACT OF SOIL COMPACTION ON THE AVERAGE DENSITY OF SOIL BACTERIA IN THE ROLAND PARK ENVIRONMENT
Average
# of Bacteria per 1 cc of soil
2500000
2000000
1500000
1000000
500000
0
1,633,333
2,033,333
2,000,000
Edge of Sidewalk
1.5 Meters
3 Meters
Distance of soil sample from sidewalk


IMPACT OF SOIL COMPACTION ON THE DENSITY OF SOIL BACTERIA IN THE ROLAND PARK ENVIRONMENT
# of Bacteria per 1 cc of soil
3,500,000
3,000,000
2,500,000
2,000,000
1,500,000
1,000,000
500,000
0
2,900,000
2,500,000
2,200,000
y = 122222x + 2E+06
2,000,000
1,800,000
1,500,000
1,700,000
1,400,000
1,000,000
0
0.5
1
1.5
2
2.5
3
3.5
Distance of soil sample Away from Sidewalk



IMPACT OF SOIL COMPACTION ON THE AVERAGE AMOUNT OF AMMONIA NITROGEN PRESENT IN THE SOIL OF THE ROLAND PARK ENVIRONMENT



IMPACT OF SOIL COMPACTION ON THE AMOUNT OF AMMONIA NITROGEN PRESENT IN THE SOIL OF THE ROLAND PARK ENVIRONMENT

Conclusion:

The hypothesis that the soil sample closest to the sidewalk of the RPCS campus will have the lowest levels of bacteria and the lowest levels of Ammonia Nitrogen was both supported and refuted. The section that said the soil sample closest to the sidewalk of the RPCS campus will have the lowest levels of bacteria was supported, while the section that stated that the soil closest to the sidewalk will have the least amount of ammonia nitrogen was not supported.

Based on the graphs we found that samples of soil closest to the sidewalk, which are more compacted, contain a lower density of bacteria microbes. The average number of bacteria in 1 cc soil samples that were directly next to the sidewalk was 1,633,333 bacteria per 1 cc of soil. The average number of bacteria microbes in 1 cc of soil that were 1.5 meters away from the sidewalk was 2,033,333 bacteria per 1 cc of soil, and the average number of bacteria in 1 cc of soil samples that were 3 meters away from the sidewalk was 2,000,000 bacteria per 1 cc of soil. On average, the soil samples that were taken from 1.5 meters away from the sidewalk (more compact soil) had more microbes than the soil that was taken from 3 meters away from the side walk (less compact). The soil that was most compact (next to the side walk) had less bacteria microbes than the soil that was least compact (3 meters from sidewalk).

Our data also shows that the average amount of ammonia nitrogen levels in the soil that was located directly next to the side walk was 3.33 ppm, the average amount of ammonia nitrogen levels in the soil that was 1.5 meters away from the side walk was 3.5 ppm, and the average level of ammonia nitrogen in the soil that was 3 meters away from the sidewalk was 2.66 ppm. This proves the part of our hypothesis stated about Ammonia Nitrogen not to be supported because on average, the soil sample that was most compact contained the most ammonia nitrogen while the soil sample that was the least compact contained the least ammonia nitrogen. both soil samples that were less compact.

Our data for the ammonia nitrogen proved the complete opposite of the section of the hypothesis that pertained to ammonia nitrogen with a negative R-squared value of y = -0.2222x + 3.5 when it was expected to be positive. Furthermore, our data proves that the section of our hypothesis stating that more compact soil will have lower levels of bacteria is supported. We inferred that bacteria counts would be higher in the soil further away from the sidewalk, in less compacted areas, and this was supported because as found in our research on soil compaction, the soil further away contained more oxygen.

From our background research we found that in less compacted areas, the soil particles are further spread out which allows more oxygen to be present area. Bacteria require aerobic conditions in order to convert atmospheric nitrogen into ammonia and nitrate. Since this soil was less compact there was more oxygen in the soil allowing more bacteria to live. We also suspected that the less compacted soil would have more ammonia nitrogen, because more bacteria could live there and therefore, produce more nitrogen. This assumption, however, was not supported by our data. The less compact soil should have had more ammonia nitrogen because there will be more bacteria able to live in the soil due to the more aerobic environment. This would mean that larger quantities of nitrogen would have been converted to ammonia by these bacteria. We suspected that the soil closest to the sidewalk would have low levels of ammonia since few bacteria would be present. One of the only types of bacteria that lives in anaerobic conditions are denitrifying bacteria which would convert the little ammonia nitrogen present in the soil back into atmospheric nitrogen. We believe that our data did not support this part of our hypothesis due to the plants growing on our plotting area.

We found after observing the plots that more plants were growing further away from the sidewalk, in the less compacted areas. This could be due to the reasoning that more space in the soil leads to more bacteria, more bacteria leads to more nitrogen and nutrients, and of if more nutrients are present then more plant life in the area will be a result. This could mean that the plants are absorbing the nitrogen from the less compacted soil causing the levels to not be as high as in the more compacted soil which has little to no plants or grass growing in it. To test this, we could use the chemical testing kits to extract plant material from the plants growing on each of our plots (next to the sidewalk, 1.5 meters away, and 3 meters away). We could then test the plant material for its level of ammonia nitrogen. The plants on the plots where we found low levels of ammonia nitrogen and high bacteria counts should have high levels of ammonia as we are inferring that the plants are absorbing all the ammonia nitrogen from the soil, while the plants on the plots where we discovered high levels of ammonia nitrogen and low bacteria counts should have low levels of ammonia since we are predicting that the plants will not have as much ammonia nitrogen to absorb in these areas.