An Investigation of Soil Protozoa Diversity as a Result of Compaction

By: Cameron Bell, Caroline Pate, and Emily Song

A. Background

Soil is a variable mixture of broken and weathered minerals, decaying organic matter, varying amounts of gasses and liquids, and the microorganisms that live there (Coleman, 2008). This microscopic life consists of billions of different types of microbes such as algae, bacteria, fungi, and protozoa, and these latter, single-celled eukaryotes are especially critical to the soil. They usually live in the thin film of water that lines the pores in the soil but can live in dry environments, too (Hall, 2008), and as many as 10 billion of them can be found within the top 6 inches of the soil (Nardi, 2003). Traditionally, they are classified based primarily on their mode of motility, and the four major Classes that can be found in the soil are: mastigiophora, ciliophora, shelled amoeba, and unshelled amoeba (Jahn, 1949). The mastigiophora are usually smaller than other protozoa, with a tear drop shape and no visible organelles, and they use a whip-like tail called a flagella to move. The much larger Ciliophora, on the other hand use small, hair-like structures called cilia to move, while both shelled and unshelled amoeba move using small extensions of the cytoplasm or cell membrane, called pseudopodium. (Jahn, 1949 and Campbell, Williamson, Heyden 2004).

The main role of protozoa in the soil ecosystem is to control bacterial populations. Bacteria are responsible for most of the nitrogen cycle, releasing nutrients such as nitrate and ammonia to the plants which allows plants to produce the amino acids they need to create the enzymes they use to start and stop their chemical reactions. But when protozoa eat bacteria, they cause the bacteria to reproduce more, which enables the additional bacteria to release additional nitrate for the plants. Furthermore, as protozoa eat the bacteria, excess nitrate from the bacteria cytoplasm spills into the soil, making it available to the plants as well (Nardi, 2003; Campbell,

Williamson, Heyden 2004). Therefore, through their interaction with bacteria, protozoa help cycle organic matter for other organisms in the soil and larger ecosystems (Nardi, 2003).

The producers are then able to create their own bodies from the nutrients the protozoa help provide, and in turn, these plants provide other organisms that consume them with amino acids they need, allowing them to produce enzymes for the chemical reactions they will use to survive. Hence, without the nitrate that protozoa help deliver to plants, nothing would be able to live, including people.

People, though, often unintentionally harm these very microbes they need in order to survive by altering the amount of water they receive. The soil in which the Protozoa are living in can undergo changes that effect the life of these critical microbes. For example, soil often dries out causing the water films within the soil to evaporate. When this occurs, protozoa lose their shape and enter an immobile, inactive state called a cyst, where they will remain until moisture returns to the soil (Nardi, 2003). When this occurs Protozoa are unable to carry out their normal duties in the nitrogen cycle which in turn causes the plants to suffer, resulting in less food for the consumers in the food chain as well.

One way this loss of moisture can occur is when soil becomes compacted. Soil compaction has a large impact on the microorganisms within the soil. Before it is compacted its pores are widespread, and there is more water and oxygen flowing through the soil. This helps the protozoa because water and oxygen are both necessary for their survival, and without adequate supplies of these key chemicals, protozoa cannot make the energy they need to perform their cellular functions. But when soil is compacted it undergoes changes that alter its original form, compressing the mineral components, and reducing the amount of space in the soil.

Compaction can occur through organisms stepping on the soil, and in highly populated areas, soil is likely to be more heavily compacted as it is trampled by the many organisms living there. But in the case of people, compaction is usually a result of constructing roads or buildings.

Either way, once compacted, the materials within the soil are, in a way, trapped (DeJong-Hughes, J., Moncrief, J.F, Voorhees, W.B., and Swan, J.B., 2001). When the water is squeezed out of the soil, the protozoa have no moisture in which to prosper. Yet because each Class of Protozoa prefers to live in different levels of moisture, different Classes will live in different levels of compaction, and in some cases it has been shown that in places of high soil compaction there are less flagellates and unshelled amoebas and more ciliates (Hoorman, 2011). (DeJong-Hughes, J., Moncrief, J.F, Voorhees, W.B., and Swan, J.B., 2001)

Because soil compaction affects the diversity of protozoa, the question arises of whether or not humans, through landscaping, roads, sidewalks, architecture, and more are having an impact on the diversity of protozoa. Therefore, we decided to examine how the compaction on the Roland Park Country School campus is impacting the diversity of protozoa here. By examining soil from, the center of the front lawn, next to the sidewalk, and next to the front lawn, we sought to determine whether our hypothesis that there will be more protozoa diversity in less compact soil is correct.

B. Experiment

I. Problem: How does compaction impact the diversity of soil protozoa

II. Hypothesis: There will be more protozoa diversity in less compact soil.

III. Procedure

A. Independent Variable- Altering the degree of soil compaction by taking samples from next to a road and a sidewalk

B. Dependent Variable- Percentage of the different Classes of protozoa in each soil sample

C. Negative Control- Soil taken from the front lawn

D. Controlled Variables- Amount of soil/size of soil sample, plants surrounding soil, samples taken from same time and same day, types of plants in test plots, amount of water, amount of methyl-green stain, amount of filtrate, microscope magnification setting, size of nylon screen/mesh, amount of dye on microscope, amount of filtrate on microscope, size of the cover slip on microscope slides, number of fields of view on microscope, size of Nytex © nylon, amount of soil sifted, how long soil is filtered, how long soil has to dry

E. Step-by-step Procedure

1. Steps 1-10 must be done at the same time on the same day to control for weather and changes in the environment
2. Go to the plot next to the back service road (near the back woods) at Roland Park Country School (location 1). Coordinates: N 39.35749, W 076.63721
3. Using a soil extractor 2 centimeters in diameter, extract 15 centimeters of soil deep by putting the soil extractor directly into the ground.
4. Place the soil into a plastic bag. Label “Location 1 A: Compact Location”
5. Repeat steps 3-4 twice more, using the respective labels “Location 1 B: Compact Location” and “Location 1 C: Compact location” for each additional plastic bag.

6. Go to the plot next to the sidewalk on the front lawn at Roland Park Country School (location 2). Coordinates: N 39.35806, W 076.63583
7. Repeat step 3-5 but label one plastic bag “Location 2 A: Middle Location”, another bag “Location 2 B: Middle Location” and another bag “Location 2 C: Middle Location”. Make sure each soil sample goes into its corresponding bag.
8. Go to the plot in the middle of the front lawn at Roland Park Country School (location 3). Coordinates: N 39.35817, W 076.63609
9. Repeat steps 3-5 but label one plastic bag “Location 3 A: Loose Location” another bag “Location 3 B: Loose Location” and another “Location 3 C: Loose Location”. Make sure each soil sample goes into its corresponding bag.
10. Take plastic bags inside to lab station
11. Label all 9 petri dishes the same way that the plastic bags were labeled in steps 4-5, 7, and 9
12. On the same day at the same time, place each of the 15 cm samples of soil from all of the labeled bags into the bottom of separate, clean, empty petri with corresponding labels and allow to dry completely
13. Steps 14-17 should be completed on the same day for all samples at the same time
14. Sift 9-10 grams of the soil from petri dish labeled “Location 1: Compact Location” into a 2^{nd} new clean petri dish using a 1 mm^2 nylon screen or mesh. Label the dish “Location 1: Compact Location.”
15. Repeat step 14 with the other petri dishes with the dried soil, labeling the new petri dishes so that each petri dish corresponds to the appropriately labeled bag.
16. Steps 17-18 should be completed on the same day, at the same time.

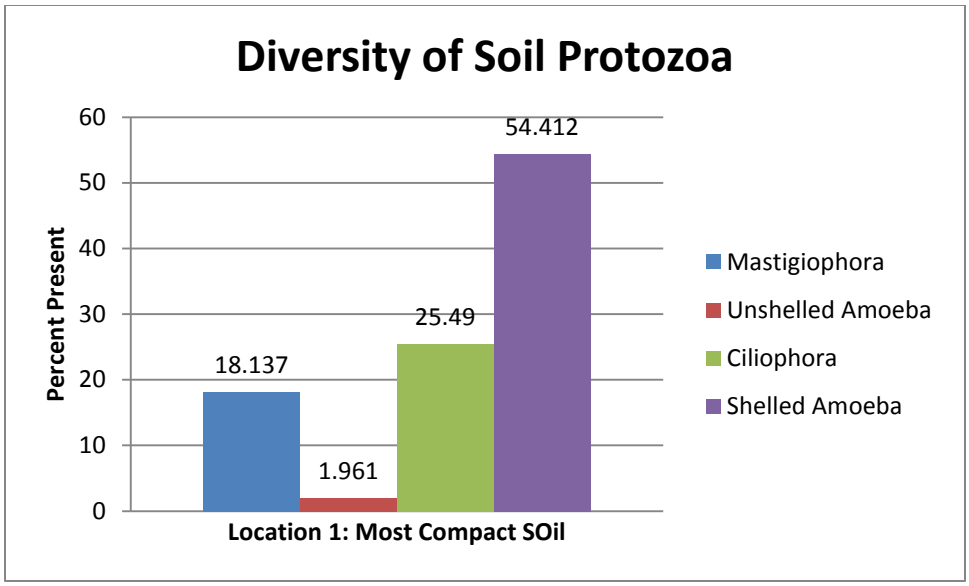
17. Add 20 ml of distilled water to each sample of sifted soil in each of the petri dishes in order to saturate the soil
18. Cover each petri dish with its lid and allow to sit for 7 hours
19. Steps 20-23 should be completed on the same day for all of the samples at the same time.
20. Place each of the rehydrated soil samples from step 18 in their own labeled, modified Uhlig extractor containing an additional 30 ml of distilled water for 24 hours. The Uhlig extractors should be labeled according to their corresponding petri dishes from step 16.
21. Steps 22-24 should be completed at the same time on the same day.
22. Remove the filtrates from each soil sample and filter each sample a 2^{nd} time using 12.5 cm qualitative filter paper
23. Using a capillary tube, deposit 5 µl of methyl-green stain on a clean microscope slide (1 µl- 1 drop from the capillary tube) labeled "Location 1: Compact Location." Then using a disposable graduated Beral-type pipette, add 18 µl (the first demarcation on the pipette) of the second filtrate from "Location 1: Compact Location" (from step 14 above) to stain on the microscope slide and cover with an 18 x 18 mm^2 cover slip.
24. Repeat step 23 with each remaining second filtrate making sure it is appropriately labeled so that the appropriate individual microscope slide corresponds to its correct second filtrate.
25. Examine each slide under a light microscope at 100X for 9 different fields of view, looking for the various Classes of protozoa living in the soil. The 9 different fields of view are shown in the diagram below.

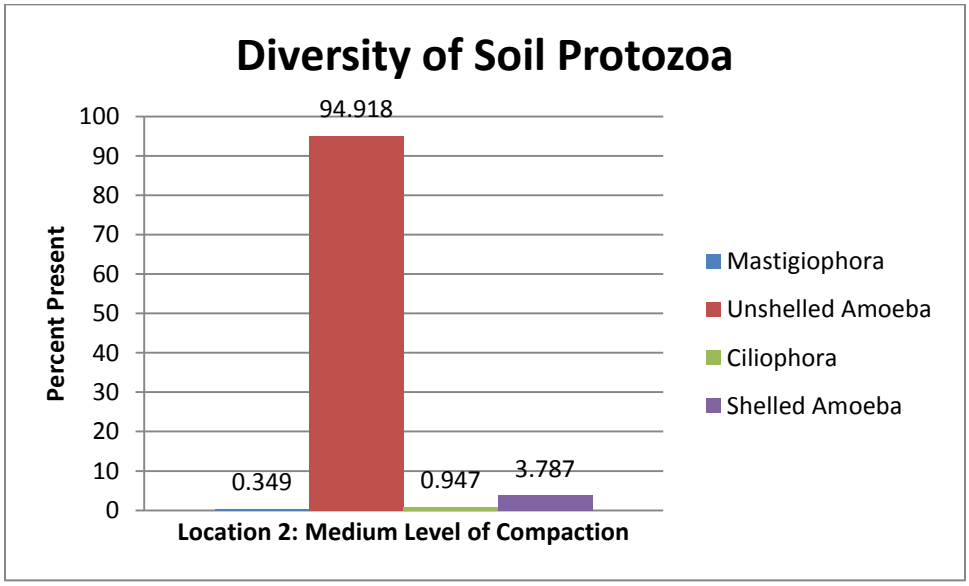
26. Record the number of the following type of protozoa that are observed in each field of view: Mastigophora, Sarcodina, Ciliophora, and shelled and unshelled Amoebas.
27. For location 1, add the total number of all of the protozoa together. Then divide the number of Mastigophora by the total number of protozoa from Location 1 and multiply that by 100 to determine the percent of that Class of protozoa at that location. Repeat this calculation for each Class of protozoa.
28. Repeat step 27 for each location.

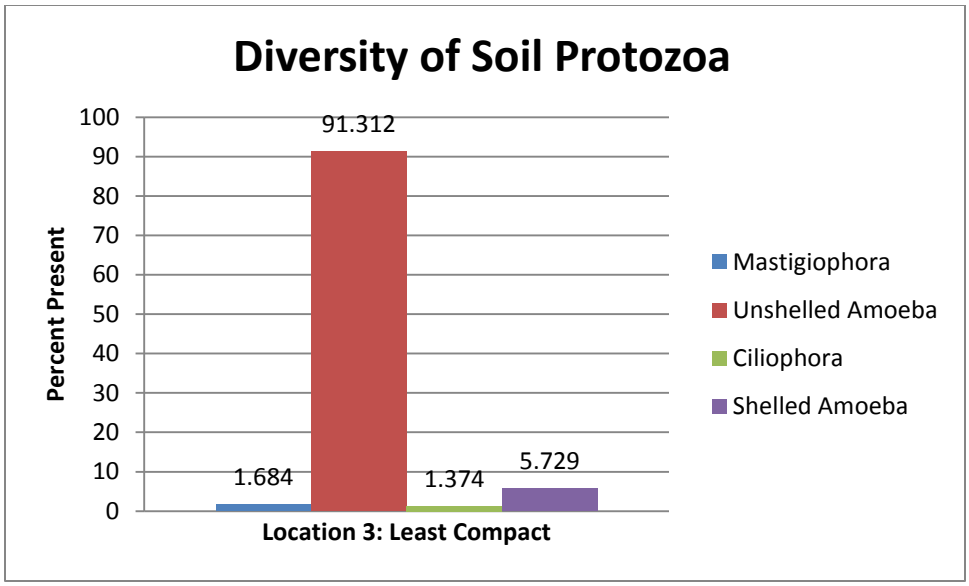
C. Data/ Analysis

Percent of Protozoa Found for Each Class in Locations

	Percent of Protozoa that were Mastigiophora	Percent of Protozoa that were Unshelled Amoeba	Percent of Protozoa that were Ciliophora	Percent of Protozoa that were Shelled Amoeba
Location 1	18.137%	1.961%	25.490%	54.412%
Location 2	0.349%	94.918%	0.947%	3.787%
Location 3	1.684%	91.312%	1.374%	5.629%


Diversity of Soil Protozoa
60
50
40
30
20
10
0
Percent Present
18.137
1.961
25.49
54.412
Location 1: Most Compact SOil
Mastigiophora
Unshelled Amoeba
Ciliophora
Shelled Amoeba


Diversity of Soil Protozoa
100
90
80
70
60
50
40
30
20
10
0
Percent Present
0.349
94.918
0.947
3.787
Location 2: Medium Level of Compaction
Mastigiophora
Unshelled Amoeba
Ciliophora
Shelled Amoeba



D. Conclusion

Our hypothesis that "There will be more protozoa diversity in less compact soil" was incorrect. Our result showed that compaction of soil has nothing to do with diversity. In places of maximum compaction, the protozoa were the most diverse, whereas in places of medium and low compaction, there was hardly any diversity. The diversity results of the medium and low compaction areas were nearly the same. We know this because the averages of our results showed that in "Location 1: Compact Location" 54.412% of the protozoa were shelled amoeba, 25.49% were ciliophora, 18.137% were mastigiophora, and 1.961% were unshelled amoeba. Shelled amoebas are typically protozoa that are trying to protect themselves from their surrounding environment. Their hard shells allow them to do this (Brock 2012). In "Location 2: Middle Location" the highest percentage of protozoa were unshelled amoeba at 94.918%. Next were shelled amoeba at 3.787%, ciliophora at 0.947%, and mastigiophora at 0.349%. At "Location 3: Loose location", we found very similar results to "Location 2: Middle Location." There were 91.312% of unshelled amoeba, 5.729% of shelled amoeba, 1.684% of mastigiophora,

and 1.374% of ciliophora. Both locations 2 and 3 showed very little diversity, with unshelled amoeba as the prominent Class of protozoa. Unshelled amoebas are typically free formed, very large, and unprotected from their environment. They don't have to worry about being protected as shelled amoebas do. They are more of a "happy-go-lucky" kind of amoeba as described by David Brock (2012). A logical explanation for these results is due to factors including pesticides and fertilizer. "Location 1: Compact location" (next to the back service road) is an area where there is no fertilizer and no pesticides. In locations two and three where compaction was minimal, there is no fertilizer or pesticides. Therefore, instead of compaction affecting diversity of protozoa, humans are a significant force, by adding chemicals to the soil.

In 2009 Elizabeth Knott, Jassmin Young, and Megan Lavin found that fertilizer can cause protozoa populations to drop and change in the soil, explaining why there were such big population changes from locations in the front of the school to the location by the back road. Fertilizers have this effect on the soil because of the bacteria that goes into the soil when it is added to surrounding plants. Unshelled amoebas are predators of these bacteria, so it is possible that this is the reason as to why there were so many more unshelled amoebas in the front lawn area. This would also explain the higher amount of diversity in the location behind the school. Since there are no fertilizers and pesticides put into the plants and soil in the back, there are a higher percentage of the other types of protozoa because there are no bacteria for the unshelled amoebas to eat. Though this is only one possible explanation for our data, it is supported by the fact that the bacteria in pesticides attract unshelled amoebas (Digrak and Özçelik, 1998). For further research we would go back out to these original locations and add varying amounts of pesticides and fertilizer to further test these results.

E. References

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