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Soil Ecology Final Report

Background Report

Protozoa are single-celled eukaryotic organisms that are very important for the health of the soil, and they come in many different shapes and sizes that are traditionally classified according to their mode of motility. In soils, there are three main types of protozoa: ciliates, amoebae, and flagellates, and depending on the type of soil, the ratio of the three types can vary. For example, in fungal-dominated soils, such as forests, testate or "shelled" amoebae and ciliates are most common while in bacteria-dominated soils, flagellates and naked amoebae are more common. Finally, in coarse-textured soils, all three types of protozoa are common.

One important role protozoa play in soil health is their place in the soil food web. Protozoa regulate the bacteria population (Ingham N.D.), eating up to five million bacteria a day (Hoorman 2011) as well as consuming other kinds of protozoa, nematodes, and deadly pathogens (such as parasitic fungi). They, in turn, are also an important food source for other soil organisms, including nematodes, fungi, actinomycetes, and earthworms. In addition, protozoa help mineralize key elements for other organisms to consume, and they compete with nematodes in consuming bacteria, which leads to soils typically having a high population of either protozoa or nematodes, but not both (Ingham N.D.). Hence, protozoa play a crucial role in the soil food web and, therefore, in the general health of the soil.

Protozoa also play a crucial role in the nitrogen cycle, the process in ecosystems for circulating this vital element among living things. The majority of naturally occurring nitrogen occurs as nitrogen gas in the atmosphere and is unusable by most organisms. However, soil microbes can alter this form of the nitrogen into forms plants can use, which in turn, makes it available to the rest of life on earth (The Editors of Encyclopedia Britannica, 2018).

There are 5 major steps to this process. The first is when nitrogen-fixing bacteria take in the nitrogen gas from the atmosphere and produce ammonia with it. Next, other bacteria convert the ammonia into ammonium, that is then converted into nitrites and eventually into nitrates. Nitrates and ammonium can be absorbed by plants to make amino acids and nucleotides, which they then use to make proteins. That is what makes nitrogen important to all living things: without it, living things would be unable to create their biological molecules, and without these biological molecules, living things would be unable to complete the four basic survival tasks. Because plants are the first multicellular organisms to incorporate the nitrogen from the bacteria, they are the first to build these molecules. Primary consumers then eat the plants, using the plant's amino acids and nucleotides to make their own and so on up the food chain. The final step is that decomposers break down dead organisms, converting their amino acids and nucleotides back into ammonia and return it to the soil so the process can repeat itself (Partnership For Environmental Education and Rural Health 2000). Finally, any excess fixed nitrogen in the soil is then returned to the air as nitrogen gas by the denitrifying bacteria.

The part of this cycle that protozoa contribute to is that protozoa eat the bacteria that take in the nitrogen that produces ammonia. Protozoa have a lower concentration of nitrogen in their cells than the bacteria they eat; so, after they eat bacteria, protozoa release excess nitrogen (Ingham N.D.) as a part of the nitrogen cycle. The nitrogen then passes to the plants to absorb. Therefore, since protozoa are so critical to soil health, anything that might disturb or harm them could threaten the entire ecosystem.

One such possible threat is construction, which has a huge impact on the soil, including the potential to have harmful effects on otherwise healthy soil. During construction, the topmost layer of the soil is affected the most, and since this is where the highest population density of

protozoa reside, here is where construction can have its biggest impact. Topsoil, as the term implies, is about 8-12 inches in depth, and when construction occurs, this layer of topsoil can suffer in several ways (Soil Quality Institute, 2000). Machinery used in construction can lead to the topsoil simply blowing away (Department of Environmental Quality, 2001), totally devastating the ecosystem. But more often, construction leads to erosion, a "process in which earthen materials are worn away and transported by natural forces such as wind or water" to other locations, often rivers and streams (National Geographic, 2019). This erosion can harm or even kill the soil protozoa as the loss of topsoil carries away the nutrients they need to survive.

Another major problem construction causes is soil compaction. This is when soil particles get pressed together, creating issues with water infiltration and drainage (University of Minnesota, 2018). Soil compaction makes it harder for the soil to absorb water, and since protozoa need water to move, moist soil is crucial to their survival. This is because, as earlier stated, a moist environment is absolutely vital to the health of protozoa. Without moisture, they are unable to move around successfully, leaving them immobile and therefore unable to swim in order to get food to eat (Department of Environmental Quality, 2001). Harmful compaction and excess water buildup is sometimes enough to suffocate entire populations of protozoa. This surplus of water can also lead to oxygen deficiency, making survival conditions even tougher for organisms inhabiting compacted soil. Essentially, construction sites leave soil with unbalanced levels of water buildup, whether it be too much or too little, and this imbalance to a typical amount of water is extremely harmful to populations of protozoa (Department of Environmental Quality, 2001).

When picking a topic for our soil ecology project, we took into consideration the unique history of construction within our RPCS campus. Our school has added several new buildings

over the course of its many existing years, and our group wondered what the difference in levels of protozoa density around previous construction sites would be. Hopefully, we will be able to observe a difference in population density of protozoa between soil that has been allotted more recovery time since construction and soil that has been disturbed more recently. We will be testing the soil at three different places around our campus. Our first location is next to the oldest building on our campus. This soil has had many decades potentially to recover. The second oldest place we tested is the soil next to the lower school wing. The last place we will be testing is the athletic complex, the most recent building on our campus. The soil has only had about 10 years to recover. In addition, we also need to test soil that is not near any buildings. We will be testing the soil in the middle of the front lawn, which will be our negative control for the experiment. We think that the soil near the oldest buildings, therefore, the soil with the longest recovery time will have a higher population density of protozoa.

Lab Outline

I. Problem:

How does the amount of recovery time following construction alter the population density of protozoa in the surrounding soil?

II. Hypothesis:

Soil surrounding older construction sites with longer recovery time will have a higher population density of protozoa.

III. Procedure

A. Independent Variable:

Recovery time of soil sample following construction of building site

B. Dependent Variable:

Population density of soil protozoa

C. Negative control:

Soil sample taken from location never exposed to construction

D. Control Variables:

- Amount of sunlight
- Control for the distance from cars
- Amount of water is used to saturate the soil
- Amount of soil taken in each sample
- Environmental conditions (soil samples taken all on same day at same time)
- Type of plants surrounding soil sample
- Size of petri dish
- Size of nylon screen/mesh
- Amount of time the soil saturates for
- Type of water added to saturate the soil
- Tool used to extract soil
- Amount of filtrate on slide
- Size of cover slip
- Temperature of room where the petri dishes lay out
- How long the water saturates the soil for
- How long the soil is in the Uhlig extractor
- Magnification of microscope

- Amount of Methyl green stain
- Temperature of water

E. Step-By-Step:

1. Place 4 different flags at each of the 4 locations from which soil samples are being collected. Make sure they are each 15 cm away from the respective building that is being tested next to; The flags should be located at the Ward House (N39.35786, WO76.6351), The Lower School Wing (N39.35749, WO76.63536), The Athletic Complex (N39.35827, WO76.63646), and the front lawn (N39.39796, WO76.63598).
2. Collect three samples that are 15 cm deep and 2 cm diameter of soil at each of the 4 flags. Make sure the samples are taken within 5 cm of the flag in any direction. These samples should all be taken on the same day at the same time of day. To extract the soil, use a Soil Core Extractor that has a diameter of 2 cm.
3. Label 12 petri dishes that have a 9-centimeter diameter with the location from which the soil sample was taken and the respective number for the trial. For example, label the first petri dish Ward House #1. Place each of the soil samples that were taken in step two into the bottom of its corresponding labeled clean, empty petri (cm diameter) dish; and allow to air dry for more than 24 hours.

4. Sift 9-10 grams of each soil sample into its own new separate correspondingly labeled clean petri dish (9 cm diameter) for each using a 1 mm^2 nylon screen or mesh.
5. Add 20 mL of distilled room temperature water to each of the soil samples at the same time to saturate each soil sample.
6. Cover each of the petri dishes with their lid at the same time and allow them all to sit for 7 hours at room temperature.
7. Place each soil sample in a modified Uhlig Extractor containing 30 mL of distilled water for 24 hours in room temperature, all soil samples should go into a Uhlig extractor on the same day at the same time.
8. Remove the filtrate and filter each soil sample at the same time a second time using 12.5 cm qualitative filter paper. Refrigerate samples until ready to proceed to step 9.
9. Using a capillary tube, deposit 7 µl of methyl-green stain on a clean microscope slide (1 µl = 1 drop from the capillary tube).Then using a disposable graduated Beral-type pipette, add 18 µl (the first demarcation on the pipette) of the 2nd filtrate from step 8 to the stain on the microscope slide and cover with an 18 x 18 mm^2 cover slip. Do this for each of the samples using a new microscope slide and cover slip every time.
10. Repeat step 9 for all of the samples and make sure you do it in the same day at the same time.

11. Examine each microscope slide in 5 different spots under a light microscope at 40X and count number of protozoa you are observing. The first spot you observe on the microscope slide is the top right corner. After you count how many protozoa are in that spot move to the second spot which is the bottom right corner. Count the number of protozoa and move to the bottom left corner. Count the number of protozoa and move to the top left. Count the number of protozoa and move to the center of the microscope slide and count protozoa. Take the average of the 5 field views into the equation below in step 12.
12. Use the following equation to determine the population density of protozoa in each of the soil samples:

[(# per field of view at 40X) • (total ml of water used) • 747] ÷ (grams of sifted soil) = # of protozoa per gram of soil.

13. Record the data in the table

Citation:

Brockmeyer, K. (2008) Chapter 3. Soil Ecology Lab Manual. Batavia, IL: Flint Scientific, Inc.

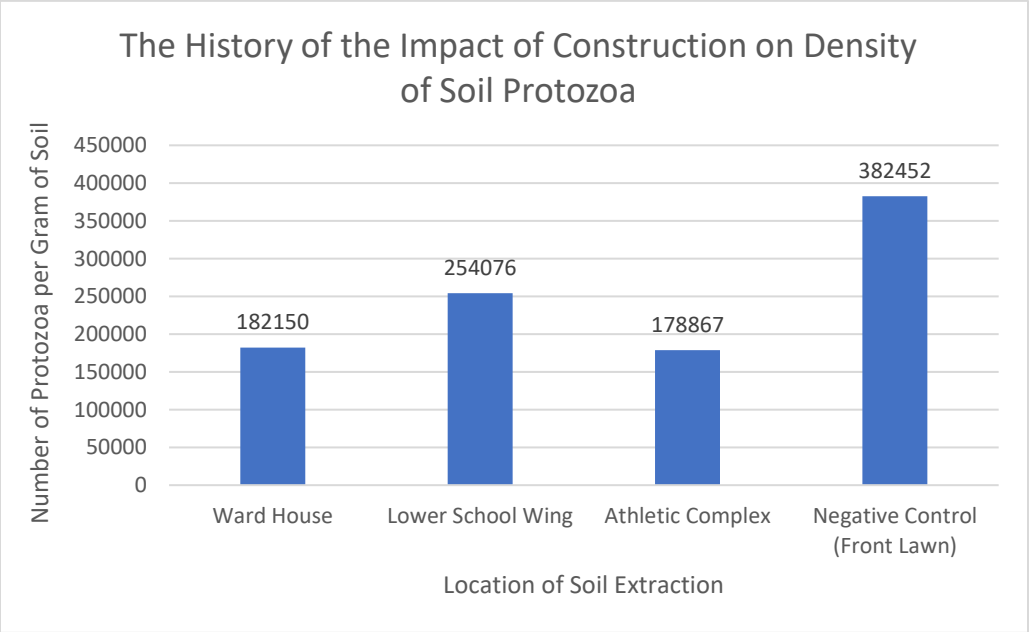
Analysis

Data Table

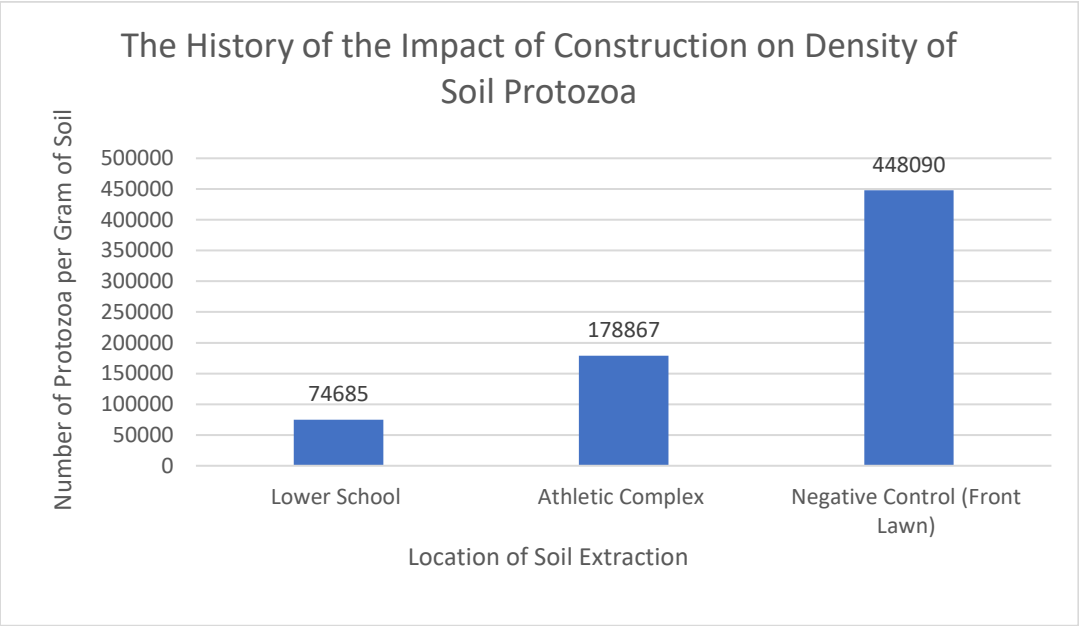
The History of the Impact of Construction on Density of Soil Protozoa

	Ward House	Lower School Wing	Athletic Center	Front Lawn
Trial 1	371141 per gram	174571 per gram	200066 per gram	348700 per gram
Trial 2	120982 per gram	174798 per gram	194220 per gram	251179 per gram
Trial 3	54327 per gram	412858 per gram	142315 per gram	547479 per gram
Average	182150 per gram	254076 per gram	178867 per gram	382452 per gram

Graphs:



Data Corrected Graph (without the outliers):



Conclusion

Our hypothesis stated that soil surrounding older construction sites with longer recovery time will have a higher population density of protozoa. After doing our experiment, we can conclude that our hypothesis was incorrect. The Ward House, the location with the longest recovery time, had 182,150 protozoa per gram of soil, and the location with the second longest recovery time: the lower school wing, had 254,076 protozoa per gram of soil. Since the Ward House had a longer recovery time than the lower school wing, it should have had a higher population of protozoa density. One thing that could have affected the results is that we took the sample for the Ward House in the Courtyard which is a highly trafficked area. Since there are so many people walking in the courtyard every day, it could have affected how many protozoa were living and multiplying in the soil. The Athletic Complex, which is the location with the least

amount of recovery time, had 178,867 protozoa per gram of soil, which is some evidence of our hypothesis being correct. This is because it had the least amount of protozoa per gram of soil as well as the least amount of recovery time. The front lawn, which was our negative control, had 382,452 protozoa per gram of soil. We expected it to have the most protozoa per gram of soil and it did because it has had the longest amount of time to recover. This is another piece of evidence that shows our hypothesis was partially correct. Even though our hypothesis was overall incorrect, there is some evidence that if we took the samples from a different spot, the results could have been different. When counting protozoa, there were certain trials that had many more or many less protozoa than the rest of that sample making there be some outlier data. We decided to make another graph that took out the outlier data and analyze it. When looking at this graph you can see that it is the complete opposite from the graph including the outliers. The only thing that stayed constant throughout our both graphs is that the negative control continuously had the highest population density of protozoa. Since the negative control has had the longest recovery time, it means that there must be another factor about construction that makes all the locations that are younger than the front lawn, have different numbers of population density. This being said, future research on this topic could include testing the soil for different factors about the construction that could be affecting the soil population. One factor that could affect the density of soil protozoa is the material the construction is being built out of. Different building materials, like brick, concrete, or stone, could have different effects on soil protozoa. In addition, other future research could include doing the same exact experiment again just picking a different spot for the Ward House location. By doing this, the results would most likely turn out how we expected them because the soil would be taken from a less disturbed area. Overall, our

hypothesis was incorrect but there is evidence that future experiments could result in our hypothesis being correct.

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