The Little Things That Run the World Biology Report
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Background: By Julia Sunderland *Source:* http://soils.usda.gov/sqi/concepts/soil_biology/protozoa.html

Protozoa play a main part in the life of soil which impacts the outside world around it. Protozoa are single-celled organisms which are slight larger then bacteria, and protozoa can be found in the soil. There are different types of Protozoa that are found in the ground. Some examples are Flagellates, they are small protozoa that stretch out and pull there way through the soil in a "whipping" way. There are also Ciliates, which are the largest protozoa but there are less Ciliates then there are Flagellates found in the soil. Protozoa play a role in regulating the environment in which it lives. What enables the protozoa to stay alive and play such an important role is moisture. Moisture is an essence in the world of protozoa, with out moisture the protozoa would be "stuck" in the same place. The protozoa then would not have the ability to "swim" throughout the soil causing the amount of protozoa to decrease because they would not have the ability to eat what protozoa eat in order to continue living.

Protozoa need bacteria to survive. Bacteria are the main source of food for protozoa. The reason that protozoa eat bacteria is because there are many bacteria's that are found in the soil. The more bacteria there are the more protozoa there will be, because that is the main source of food for protozoa. Bacteria eat dead organisms that have decomposed in the ground. Such as leaves, dead grass clippings, bark that had fallen from trees and many other dead organisms. Not only to protozoa need moisture to be able to swim and move around bacteria need moisture as well. The bacteria are able to swim around in the soil because of the moisture, as well as protozoa. This creates the environment that protozoa need to stay alive. The more moisture that is held in the ground, the better the environment is for the organisms that live in the soil. Not only does moisture help the protozoa move around and swim and stay "hydrated" but it also helps the nutrients in the ground. So therefore Moisture has a main impact on the amount of protozoa that are found in the soil.

Grass and mulch also play a key role in the amount of protozoa found and how the moisture stays in the ground. Grass can not hold moisture for a long period of time. The only time a grass comes in contact with water that is then able to seep into the ground is during a rain storm; the rain hits the ground then is absorbed by the ground creating a path for the protozoa to swim through. Another time when grass gets water is when the grass is being watered. However there is nothing to trap the moisture in so after it had gotten wet the ground dries and the protozoa die off. Mulch however can hold in moisture for an extended amount of time. Because there is something on top of the ground the moisture can be absorbed and then let back into the ground. Mulch will get wet when the grass does however the mulch is able to hold in the moisture for a lot longer then the grass can. Since the mulch can hold in moisture for an extended amount of time the protozoa can live longer and are able to move around much more efficiently.

Our experiment was which the soil under grass, or the soil under mulch would have the closest amount of protozoa in comparison to the backwoods. Our hypothesis was that if the grass has the more protozoa then the mulch then the grass is closest to the backwoods. We picked to test for the amount of protozoa because we thought that human contact (cutting grass, watering plants, laying mulch) would make a change in the amount of protozoa found in the soil. How we went about solving this problem was that we took samples from 3 different coordinates for each type of soil that we were planning on testing. We took three samples form three different places of mulch in the front lawn. We took three different samples of soil from three different places under grass in the front lawn. Then we went to the backwoods and took three different samples from three different parts of the back woods. Then we came back to the lab and tested the soil to find out the average of protozoa from each different place and which one, grass or mulch was the closest amount to the soil in the backwoods, which would reflect which one was closer to the natural environment of the protozoa.

Lab Outline: By Amena Chaudri

Hypothesis= When we test the soil under human generated mulch and grass for protozoa, then the soil under the grass will have a density closer to the one in the backwoods.

Problem: Are the number of protozoa in soil under grass closer to the natural soil in the backwoods?

Independent Variable: Location of samples based on grass or mulch

Dependant: Amount of Protozoa in soil

Negative control: soil from the backwoods

Controlled Variables:

- Amount of water put into each Petri dish
- Amount of dye used
- Grinding soil before sifting Uhlig Extractor
- Using 30 ml of distilled water for 24 hours
- Using the same filtering paper
- Using 12.5 cm qualitative filter paper
- Using 7 ml of methyl-green stain
- Adding 18 ml
- Covering the slide with an 18 x 18 mm^2 cover slip
- Looking through a 40X microscope
- Looking at 5 different views to count the protozoa
- Using the equation [(#per field of view at 40X)•(total ml of 2^{nd} filtrate) • 747] ÷ (grams of sifted soil)= #of protozoa per gram of soil; to find the average number of protozoa in each area (backwoods, mulch, and grass)
- Using distilled water
- Using a modified U
- Weather- if it rains we have to make sure we get all our samples at the same time
- How much soil to extricate from the ground (15 cm)
- Distance below Surface

- How far into the backwoods (plots have to be in areas with a few trees)—both
- No plots can be near a water source
- Clear surface of mulch/grass before extricating soil

Procedure:

1. Gather materials (walkie talkie, marking flags) and seek plot to test experiment on
2. Go to the first grass plot at (N. 39.35780-W. 76.63584) and place a flag in the plot
3. Go to the second grass plot at (N. 39.35793-W. 76.63584) and place a flag in the plot
4. Go to the third grass plot at (N. 39. 35816-W. 76.63617) and place a flag in the plot
5. Go to the first mulch plot at (N. 39.35769-W.76.63587) and place a flag in the plot
6. Go to the second mulch plot at (N. 39.35805-W. 76.63587) and place a flag in the plot
7. Go to the third mulch plot at (N. 39.35812-W.76.63623) and place a flag in the plot
8. Go to the first backwoods plot at (N. 39.35772-W. 76.63747) and place a flag in the plot
9. Go to the second backwoods plot at (N. 39.35739-W.76.63855) and place a flag in the plot
10. Go to the third backwoods plot at (N.39.35734-W.76.63786) and place a flag in the plot
11. The next day gather necessary materials to extricate soil (soil core sampler, GPS navigator, 9 plastic bags each labeled (Mulch1, Mulch2, Mulch3, Grass1, Grass 2, Grass3, Backwoods1, Backwoods2, Backwoods3), and walkie talkie)
12. That same day go plot 1 for grass (N. 39.35780-W. 76.63584) and extract 15 cm of soil with a diameter of 3.2 cm and put soil into plastic bag labeled grass 1
13. Go to plot 2 for grass (N. 39.35793-W. 76.63584) and extract 15 cm of soil with a diameter of 3.2 cm and put soil into plastic bag labeled grass 2

14. Go to plot 3 for grass (N. 39. 35816-W. 76.63617) and extract 15 cm of soil with a diameter of 3.2 cm and put soil into plastic bag labeled grass 3
15. Go to plot 1 for mulch (N. 39.35769-W.76.63587) and extract 15 cm of soil with a diameter of 3.2 cm and put soil into plastic bag labeled mulch 1
16. Go to plot 2 for mulch (N. 39.35805-W. 76.63587) and extract 15 cm of soil with a diameter of 3.2 cm and put soil into plastic bag labeled mulch 2
17. Go to plot 3 for mulch (N. 39.35812-W.76.63623) and extract 15 cm of soil with a diameter of 3.2 cm and put soil into plastic bag labeled mulch 3
18. Go to plot 1 for backwoods (N. 39.35772-W. 76.63747) and extract 15 cm of soil with a diameter of 3.2 cm and put soil into plastic bag labeled backwoods 1
19. Go to plot 2 for backwoods (N. 39.35739-W.76.63855) and extract 15 cm of soil with a diameter of 3.2 cm and put soil into plastic bag labeled backwoods 2
20. Go to plot 3 for backwoods (N.39.35734-W.76.63786) and extract 15 cm of soil with a diameter of 3.2 cm and put the soil into a plastic bag labeled backwoods 3
21. Once all of the soil samples have been collected, follow the protozoa extraction steps and do those steps for each soil sample

Protozoa Extraction Steps

1. Place 15 cm sample of soil sample into the bottom of a clean, empty Petri dish; and allow it to dry completely.
2. Sift 9-10g of the soil into a 2^{nd} clean Petri dish using a 1 mm^2 nylon screen or mesh.
3. Add 20 mL of distilled water to saturate the soil.
4. Cover the Petri dish with its lid and allow it to sit for 7 hours.
5. Place the soil sample in a modified Uhlig extractor containing 30 mL of distilled water for 24 hours.
6. Remove the filtrate and filter a 2^{nd} time using 12.5 cm qualitative filter paper.
7. 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 2^{nd}

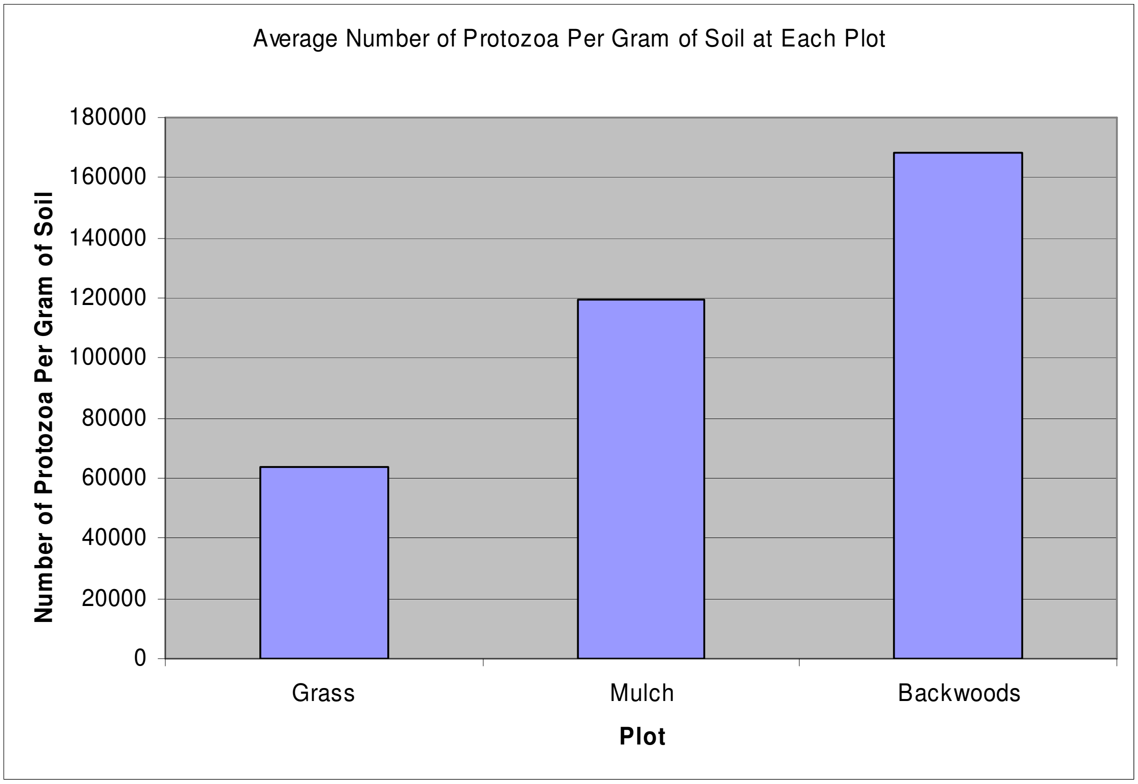
filtrate from step 6 to the stain on the microscope slide and cover with an 18 x 18 mm^2 cover slip.

8. Examine under a microscope at 40X (for quantitative) observations of the various protozoa living in the soil and count every blue spot in each of the five views.
9. Add up the total number of protozoa in each view and divide that number by 5 to get the average.
10. Use this equation to determine the population density of protozoa in the soil sample[(#per field of view at 40X)•(total ml of 2nd filtrate) • 747] ÷ (grams of sifted soil)= #of protozoa per gram of soil;
11. Record the data/ number of protozoa found in the soil samples.

Data/Observations: By Hanna Frank

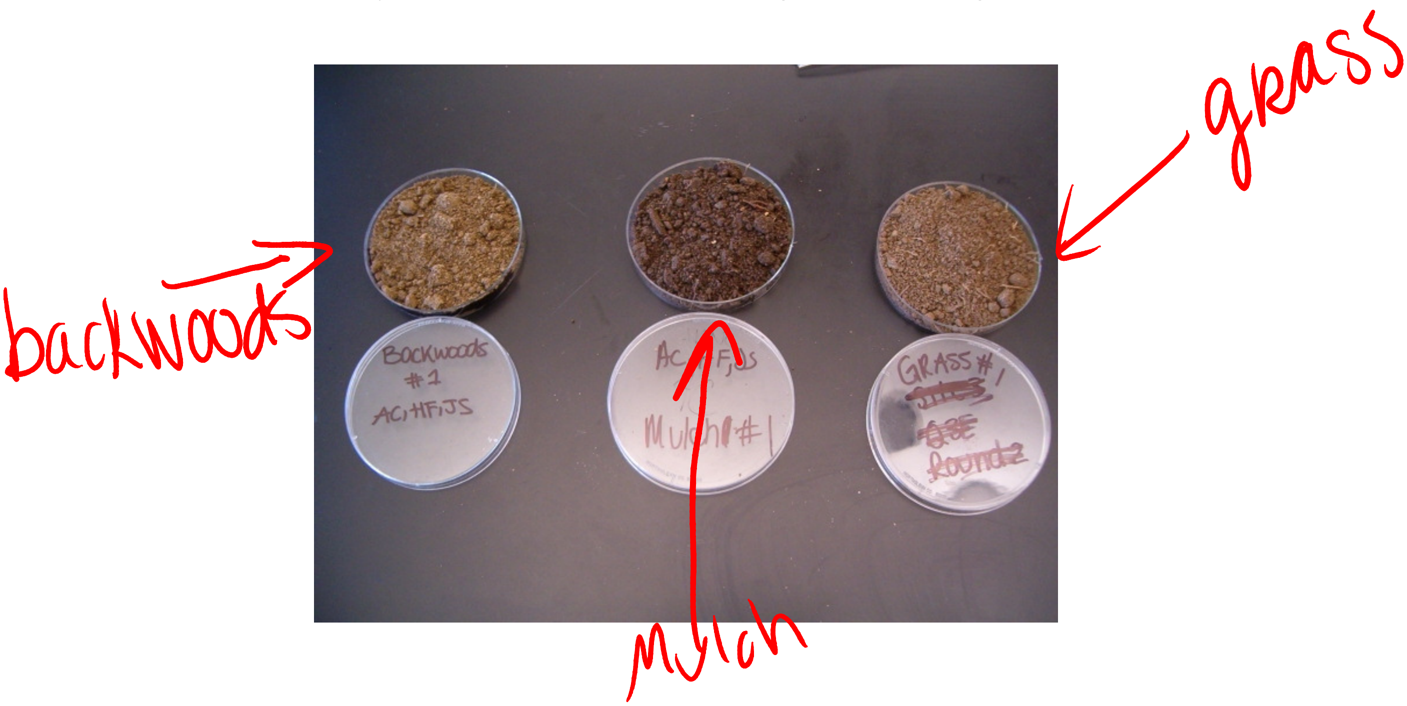
Number of Protozoa Per Gram of Soil at Each Plot

	Trial #1	**Trial #2**	**Trial # 3**	**Averages**
Grass	86591 Protozoa Per Gram of soil	33465 Protozoa Per Gram of soil	71955 Protozoa Per Gram of soil	64003 Protozoa Per Gram of soil
Mulch	106372 Protozoa Per Gram of soil	4980 Protozoa Per Gram of soil	247406 Protozoa Per Gram of soil	119586 Protozoa Per Gram of soil
Backwoods	84992 Protozoa Per Gram of soil	233899 Protozoa Per Gram of soil	186348 Protozoa Per Gram of soil	168413 Protozoa Per Gram of soil



Analysis-

When we first collected the soil, we observed the soil that was damper. Surprisingly, the mulch soil was the darkest soil, and then the backwoods soil was the next darkest. Finally, the soil from underneath the grass was the lightest in color.



The soil in the backwoods had the most protozoa, containing 168413 Protozoa Per Gram of soil on average. The soil under the human generated mulch had the second most number of protozoa, containing 119586 Protozoa Per Gram of soil on average. The soil under the human grown grass had the least number of protozoa, containing 64003 Protozoa Per Gram of soil on average.

Conclusion

In Conclusion, our hypothesis was incorrect. When we extracted and tested soil to see what was most like its natural environment, the backwoods, we found that mulch was most like the natural environment. This was because it had the largest number of protozoa per gram in comparison to grass.

In our hypothesis, we thought that soil under the grass would be closest to its natural environment. We had several reasons that supported this hypothesis. First of all, in the backwoods, there are many leaves that have fallen to the ground. Microorganisms decompose those leaves, which bring many microorganisms to that area. Since the protozoa eat microorganisms, there would be many more protozoa in the backwoods. We related this to the grass, because of grass clippings. With all of the grass clippings on the grass, there would be more decomposers and microorganisms in the soil under the grass. It made logical sense at that time, until we ran the experiment.

After we got the results back, we found that obviously the negative control, the soil from the backwoods, had the largest number of protozoa per gram of soil. The backwoods had an average of 168413 protozoa per gram of soil. We also found to our surprise, that the mulch had the second greatest number of protozoa per gram of soil, with 119586 protozoa per gram of soil on average. We came to the conclusion that the protozoa prefer to live in places that are damp and wet, because it is easier for them to navigate in. The habitat of the protozoa did not have much to do with the grass clippings. The mulch however, holds in dampness, creating the ideal environment for the protozoa.

With our results, we conclude that the soil under human generated mulch is much closer to the natural environment of protozoa, the backwoods. The mulch having the greatest number of protozoa was in comparison to the soil under human generated grass, proving to us that our hypothesis was incorrect, because in our hypothesis we said that the soil from under grass would have the number of protozoa, closest to it's natural environment.