The Influence of Compaction on the Density of Protozoa

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~ We have acted honorably ~

Background Information

Protozoa are single celled organisms that live in the soil, and although small, they play a significant role in the environment. Classified into three groups based on shape (ciliates, amoebae and flagellates), protozoa feed primarily on bacteria but can also eat other protozoa, organic matter, and fungi. They need plenty of water and oxygen to survive (Ingham 2010), and therefore they must live in soil that contains more water and oxygen than is needed by most other kinds of microbes. The soil in which they live in also needs to contain bacteria for food, and, each type of protozoa needs to inhabit certain types of soil based on the other organisms living there. For example, testate amoebae (which make a shell-like cover for protection) and ciliates tend to live in coarser textured soils where fungi dominate, while flagellates and naked amoebae (those without a "shell") are usually found in soils with high clay content where bacteria dominate (Ingham 2010).

Regardless of what soil they inhabit, all protozoa play the same important roles in the ecosystem. Perhaps the most significant of these is the process of mineralization, which makes the nutrients available for other plants and organisms to use. Mineralization is the process of turning organic matter into inorganic compounds (University of Michigan 2011), and protozoa do this by releasing excess nitrogen (in the form of ammonium) after eating bacteria (Ingham 2010). This ammonium is then consumed by other bacteria making the nitrogen chemically available for plants (who in turn make them available when eaten by consumers) (Ritter 2006). Nitrogen is extremely important to the survival of all living things because amino acids need nitrogen. Amino acids are able to make proteins, which make enzymes. Enzymes are responsible for the start and stop of chemical reactions, which make and break chemical bonds, leading to the completion of the four tasks of every cell. These tasks keep the cell running and alive, which therefore keep the organism alive. None of this would be possible without nitrogen, so protozoa's job of releasing nitrogen is very large and crucial.

In addition to providing ammonium for bacteria, protozoa regulate the populations of bacteria that control the rest of this nitrogen cycle. The protozoa do this by "grazing" on the bacteria. Grazing is the process in which the protozoa stimulate the growth of the bacteria. Grazing can be described as "pruning a tree". A small amount of grazing enhances the growth of the bacteria population, and too much grazing reduces this and unbalances the "mix of species in the bacterial community" (Ingham 2010). Protozoa can also be a food source for other organisms in the ecosystem; hence the loss or decrease of a protozoa population would be unfortunate and very damaging to the ecosystem, since protozoa impact the release of nitrogen, and therefore impact the survival of all organisms.

Because protozoa need more oxygen and water to live than other organisms, it is likely they would be greatly impacted by loss water and oxygen caused by compaction. Compaction is when soil is compressed together (Merriam Webster), and it is caused by forces that can include both human and natural causes (University of Michigan 2011). These forces have effects on the soil, which cause many problems, including limited air space and water flow. As the soil particles are being squished together from compaction, the pore space between those particles decrease (University of Michigan 2011). Soils that are greatly compacted do not have many large pores, and therefore they are not able to easily filter water. Furthermore such soil also cannot drain water from the layer of compaction as fast as non-compacted soil can because the soil needs the large pores in order to have good transportation of water through the soil when it is drenched.

Soil compaction can also cause major damage to the soil structure. This is important is because the structure gives the soil its ability to hold and transport nutrients, air, and water to plant roots (University of Michigan 2011). Compacted soil requires more force from roots to penetrate the soil, making it harder for plants to grow and stay healthy. Moreover roots in compacted soil also use up what little water and oxygen there is faster, depriving the protozoa of these critical chemicals. When the water and oxygen decrease in the soil, protozoa cannot survive. As stated before, protozoa are extremely important to the survival of all organisms because protozoa release the nitrogen that is ultimately needed to make enzymes which are crucial to the lives of all organisms. Compaction can ultimately end up affecting whole ecosystems.

In an ecosystem, all of the organisms are affected by the surrounding environment. Compaction can harm the protozoa population living in that particular area of compacted soil, since there is less water and oxygen, which are needed by protozoa. Both natural and human forces can cause compaction; some examples of human compaction include walking on top of the soil, construction, driving cars or tractors on the soil, and more. Sidewalks compact the soil, and therefore, harm protozoa. To see the effects of this compaction, we performed an experiment to see how the number of protozoa changes as tests are performed at different lengths away from the sidewalk. We expected that as tests are performed farther away from the sidewalk, there would be more protozoa, since the protozoa would not be as affected by the compaction.

Experiment

I. Problem: How does the compaction caused by a sidewalk alter the population density of protozoa?

II. Hypothesis: The lowest density of protozoa will be found closest to the sidewalk.

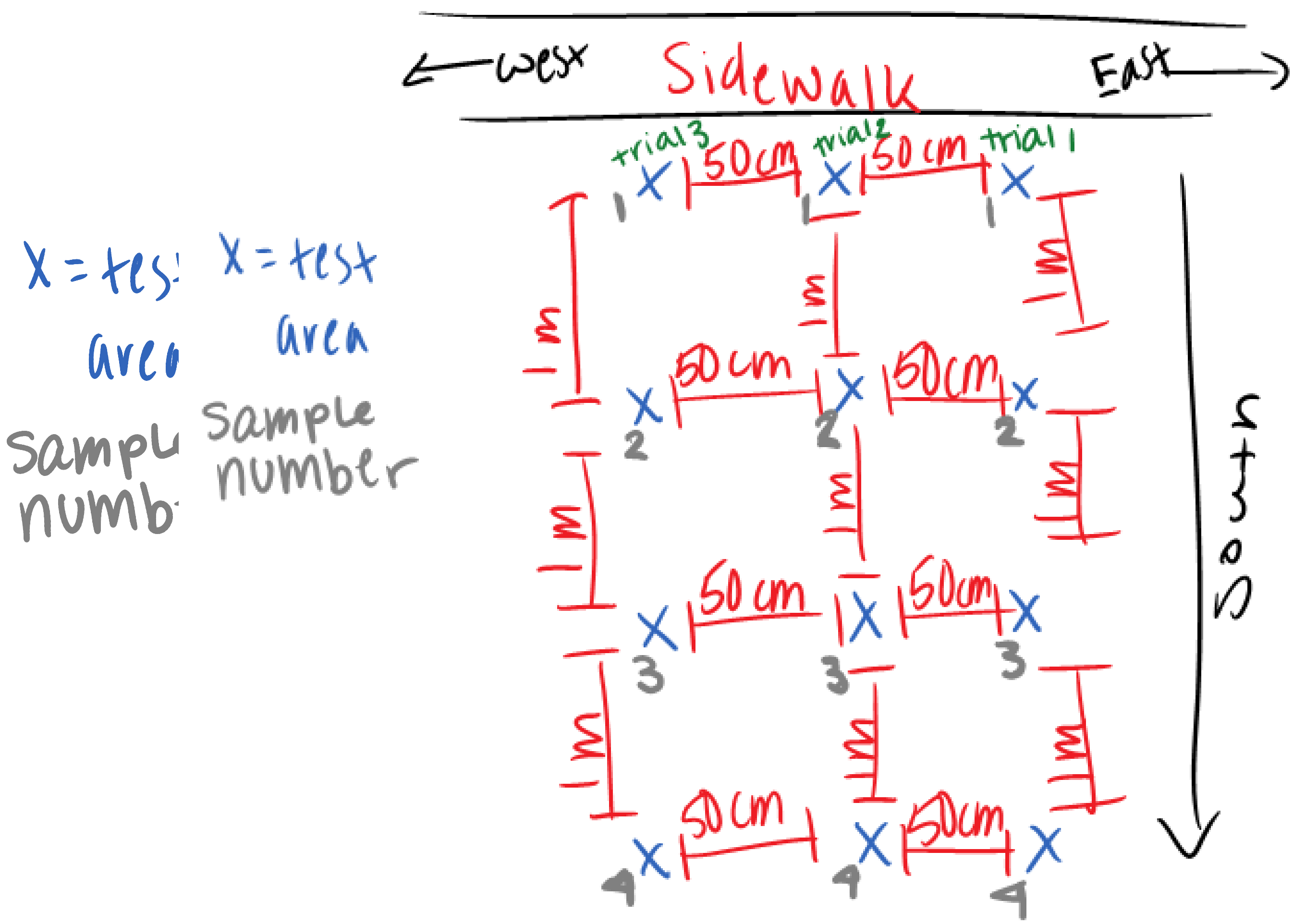
III. Experiment:

   a. Independent Variable: the distance soil samples are taken when measured from sidewalk in meters
   b. Dependent Variable: density of protozoa in the soil, amount of moisture in the soil
   c. Negative Control: soil samples taken 0 meters from sidewalk
   d. Controlled variables: Type of plant life, amount of plant life, temperature, location, season, length of distance between each of the trial areas, amount of soil taken for testing, samples taken on same time and same day, size of pipette, size of nylon screen, amount of water added to soil for rehydrating, time allowed for drying, time allowed for water to saturate, time allowed for soil to sit in Uhlig extractor, filter the second filtrate at the same time, strength of microscope, microscope, microscope slide, type of dye, amount of dye added to the

microscope slide, size of cover slip, amount of liquid soil added on cover slide, soil extractor, distance of petri dishes from window sill

e. Procedure :

1. Find the area N. 39.35837, W. 076.63602. Use the diagram below to know where to place the flags for soil sampling.



2. Label 12 separate bags respectively: sidewalk trial 1, sidewalk trial 2, sidewalk trial 3; test area 1 trial 1, test area 2 trial 1, test area 3 trial 1;

test area 1 trial 2, test area 2 trial 2, test area 3 trial 2; test area 1 trial 3, test area 2 trial 3, test area 3 trial 3.

3. Collect 2 ½ cm wide x 15 cm deep of soil from each location shown in the diagram with a soil extractor and put it into its corresponding labeled bag.
4. Label 12 separate petri dishes with the labels that correspond to each of the labeled bags.
5. Put each soil sample into its correspondingly labeled petri dishes, and weigh the mass of each soil sample; record data.
6. Allow all the soil to dry out for at 24 hours in the sunlight.
7. After 24 hours, label 12 new separate petri dishes that correspond to the each of the labeled bags.
8. During the protozoa extracting process(steps 9-13), be sure for any of these steps, that for each specific step, it is completed for all soil samples all at the same time.
9. Begin the protozoa extraction process by sifting 9-10 g of the soil from each of the drying petri dishes using 1mm^2 mesh into its corresponding new petri dish.
10. Weigh the remainder of each of the samples from the sifting process and leave it in its original petri dish to continue drying for at least 3 days in the sunlight.

11. Add 20 ml of distilled water to each petri dish in step 9 in order to saturate the soil, leave for 7 hours.
12. Place each soil sample from step 11 in its own separate modified Uhlig extractor that holds 30 ml of distilled water for 24 hours.
13. Remove the filtrate from each of the Uhlig extractors in step 12, and filter separately a 2nd time using 12.5 cm qualitative filter paper.
14. Use the following steps for creating a microscope slide to prepare a microscope slide for each of the soil sample filtrates in step 13, be sure to use a separate disposable cover slide and pipette for each soil sample:
    a. Using the capillary tube, deposit 7 ul of methyl- green stain on clean microscope slide. Then use a disposable graduated Beral-type pipette, add 18 ul of the 2nd filtrate from step 13 to the stain on the microscope slide and cover with 18 x 18 mm^2 cover slip.
    b. Examine under a light microscope at 60X observations of the number of protozoa living in the soil.
    c. Use [(# per field of view at 60X) * (total ml of water used) * 2165] *(grams of sifted soil) to find the number of protozoa per gram of soil.
    d. Repeat steps a-h with soil from different testing areas.
15. After the soil in step 10 has dried completely, reweigh the remaining soil to calculate the amount of moisture in the original soil sample.

Data and Analysis

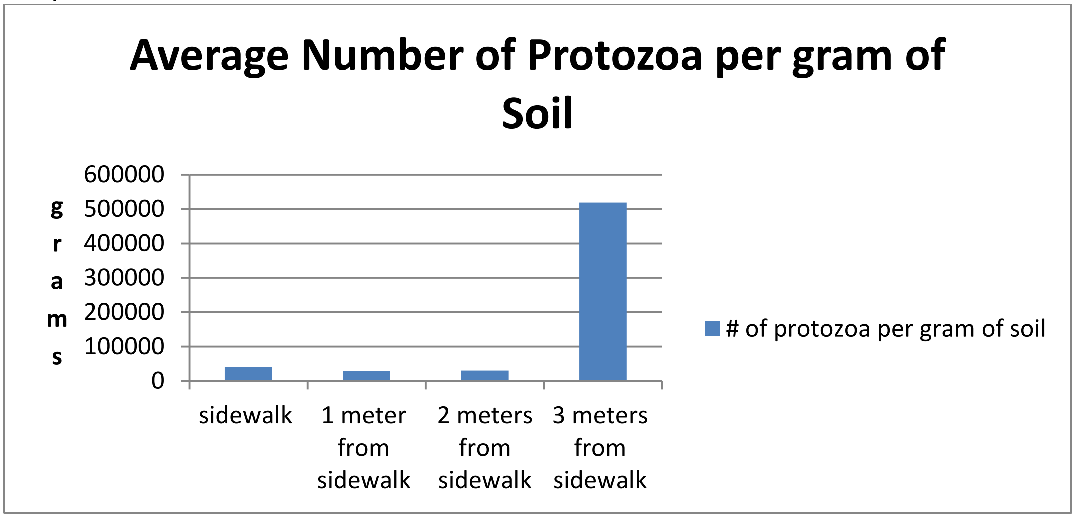
Impact of Soil Compaction on Water Content and Protozoa Density

Trial 1	% Change in Mass of the soil samples	Mass of H_2O in the soil samples	Number of protozoa per gram of soil
Sidewalk	79.845%	5.2	25068.421050 grams
1 meter from sidewalk	85.380%	7.5	26510.204080 grams
2 meters from sidewalk	86.355%	7.6	36463.157890 grams
3 meters from sidewalk	82.942%	8	1146310.526000 grams
Trial 2			
Sidewalk	81.743%	8.8	44639.175260 grams
1 meter from sidewalk	81.690%	7.8	22552.083330 grams
2 meters from sidewalk	86.180%	8.9	15464.285710 grams
3 meters from sidewalk	81.667%	5.5	363809.278400 grams
Trial 3			
Sidewalk	79.026%	5.6	52415.789470 grams
1 meter from sidewalk	83.634%	10.9	36083.333330 grams
2 meters from sidewalk	82.769%	11.7	37943.298970 grams
3 meters from sidewalk	81.288%	6.1	46392.857140 grams

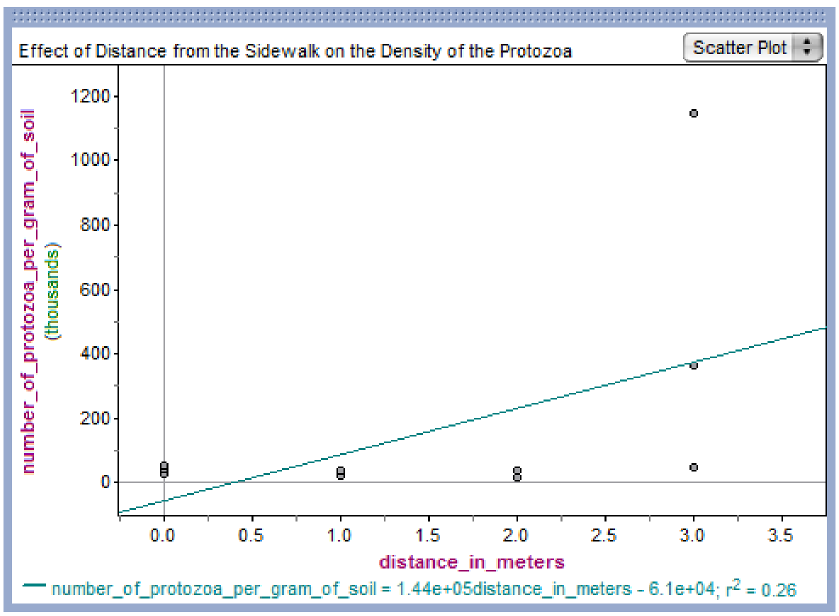
Averages in Impact of Soil Compaction on Water Content and Protozoa Density

	Percent Change in Mass of the soil samples Average	Mass of H_2O in the soil samples	Number of protozoa per gram of soil
Sidewalk	80.204%	6.53	40707.79526
1 meter from sidewalk	83.568%	8.73	28381.87358
2 meters from sidewalk	85.101%	9.4	29956.91419
3 meters from sidewalk	81.966%	9.4	518837.5538

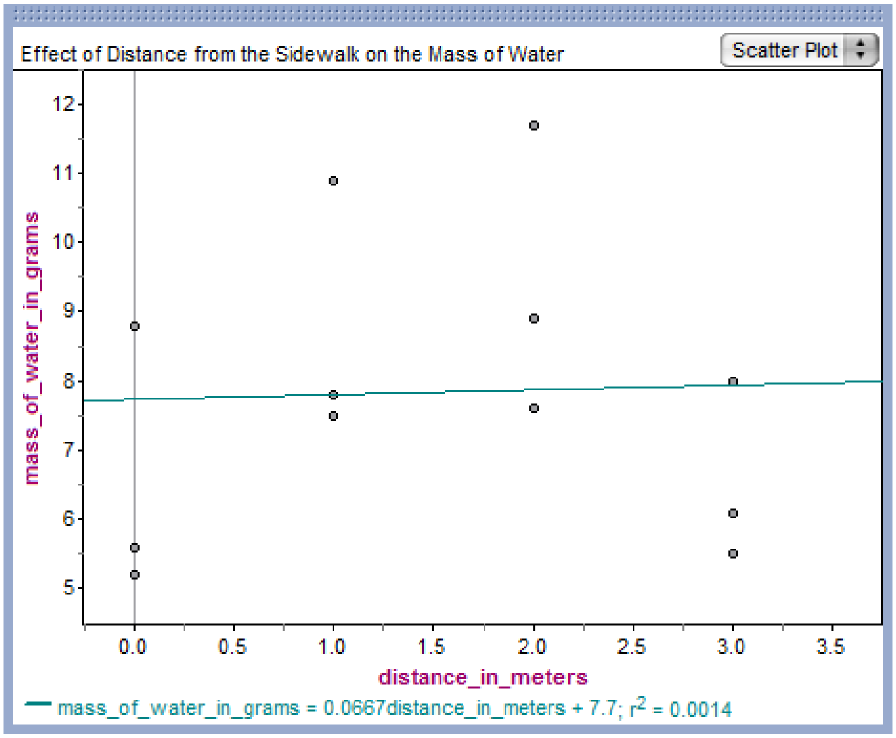
Graph 1



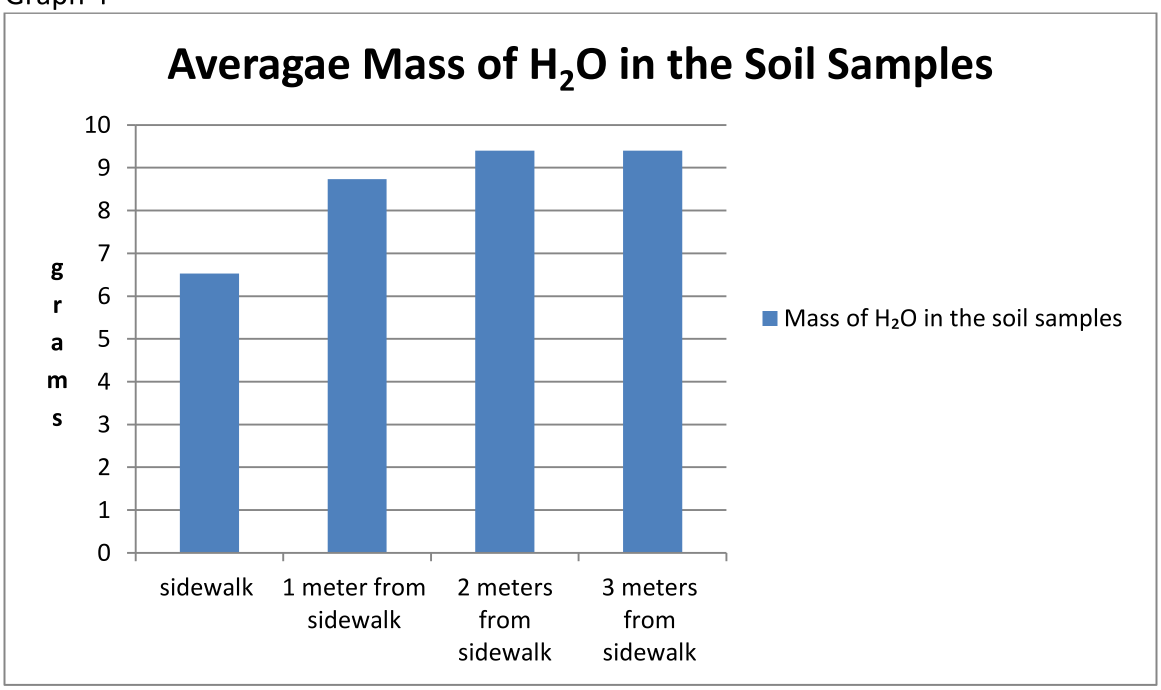
Graph 2



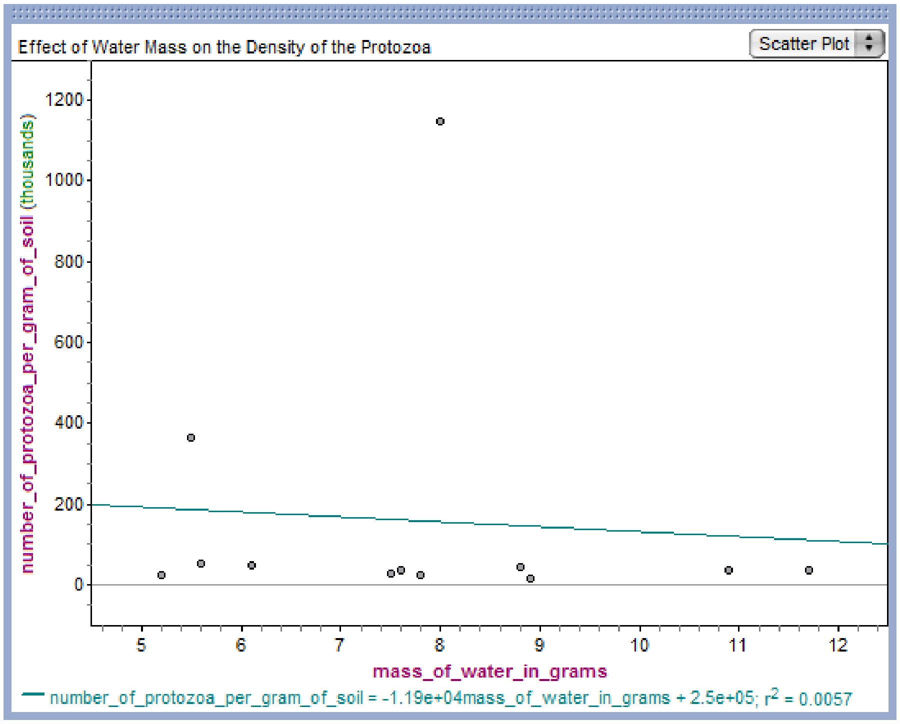
Graph 3



Graph 4



Graph 5



Conclusion

The hypothesis that the lowest density of protozoa would be found closest to the sidewalk was correct. As seen in Graph One and Graph Two, more protozoa were found three meters away from the sidewalk than were found less than three meters away from the sidewalk. There is an average of 518837.5538 protozoa per gram of soil three meters away from the sidewalk, as opposed to an average of 40707.79526 protozoa per gram of soil immediately next to the sidewalk. Predicted through research, there is less compaction of the soil as the distance from the sidewalk, since the sidewalk pressures the soil and increases the compaction of the soil closest to the sidewalk. The data shows that the degree of observed compaction of the soil influences the density of protozoa. Another comparison that can be made is the density of the protozoa one meter and two meters away from the sidewalk to determine if the compaction of the soil influences the protozoa more when the protozoa is closer to the source of the compaction, which is the sidewalk. The compaction of the soil influences the protozoa – and decreases the density of the protozoa found in the soil – more when the protozoa is closer to the sidewalk, since the sidewalk is the cause of the increased compaction of the soil. Although compaction of the soil greatly affected the density of the protozoa, the compaction of the soil caused by the sidewalk did not greatly alter the amount of water in the soil. As seen in Graph Three and Graph Four, the amount of water found in the soil did not differ as we tested soil farther away from the sidewalk. The average amount of water found closest to the sidewalk was 6.53 grams, compared to the average amount of water found three meters away from the sidewalk, which was an average of 9.4 grams. These numbers were very similar, which shows that, even though the amount of water found in between these testing areas was increased, compaction did not greatly influence the amount of water. This result could have occurred because of the large amount of rain prior to the periods of observation of the soil and measurement of water, or because of the constant watering of the grass, so the sidewalk could not have had any influence on the amount of water found in the soil. The amount of water found in the soil was not affected by the compaction of the soil, nor did it affect the density of protozoa found in the soil. As shown in Graph Five, the amount of water in the soil did not largely impact the density of protozoa found in the soil. As the amount of water in the soil increased, the density of protozoa in the soil stayed fairly constant. The protozoa density may not have been affected by the constant water in the soil because there may have been a change in oxygen in the soil. Although the compaction did not influence the amount of water found in the soil, there may have been in a decrease in oxygen in the soil closer to the sidewalk, which was the source of the compaction. Overall, the experiment was successful, and the hypothesis was correct.

If this experiment continued, future research would be needed. Unfortunately, during this experiment no tests were able to be conducted to determine the oxygen in the soil or the actual compaction of the soil, which are two tests which would have been beneficial. If tests were conducted to determine the oxygen in the soil, then the analysis of the data would have been more accurate, since it could have been determined whether compaction of the soil truly influenced the oxygen and water levels, which are two things needed for the survival of protozoa in the soil. Another useful change to this experiment would be to test greater distances between trials, as well as between testing areas. This would have provided more accurate data that could have been more accurately analyzed. For instance, a determination of whether the density of protozoa is independent of compaction of the soil could have been made if the maximum distance of the test area from the sidewalk were much greater than three meters.

References

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