Impact of Compaction on the Density of Protozoa

By: Hadley Saba, Casey Feinstein, Hannah Rhea, and Christyn Robinson

We have acted honorably.

Christyn Robinson
Hadley Saba
Casey Feinstein
Hannah Rhea

Background

Protozoa are single-celled, eukaryotic organisms that feed primarily on bacteria and are found in almost every type of soil environment where there is adequate moisture (Ingham, n.d.). These single-celled creatures are essential to many larger organisms in an ecosystem because when they digest their food, they release many different nutrients into the soil in forms which other living things such as plants (and eventually even animals) can use. Protozoa that live in soil vary in size and are classified into three major categories: flagellates, ciliates, and amoebae. Of the amoebae, there are two different types: testate amoebae and naked amoebae (Ingham, 2016), and while they range in size from smallest (the flagellates) to largest (the ciliates) (The Microbe World, 2014), all categories of protozoa are involved in aiding an essential process called the nitrogen cycle (Ingham, 2016).

Nitrogen is a required element for all living organisms to produce a number of complex organic molecules, including proteins and nucleic acids. But most of the nitrogen on this planet is in the form of nitrogen gas in the earth's atmosphere, which is not accessible to most living things. Certain soil bacteria, though, can capture this nitrogen gas from the air and convert it into ammonia. The ammonia becomes ammonium when it reacts with the water in the soil, and then other bacteria convert the ammonium into nitrate (Harrison, 2003). While this is happening, decomposers in the soil also produce ammonium as they break down dead organisms into simpler organic forms, and plants can use both the nitrate and the ammonium in the soil to grow (Ingham, 2016). Any excess nitrate in the soil is then converted by the denitrifying bacteria back into nitrogen gas again, which they release into the air, completing the cycle. (Campbell, Williamson, Heyden, 2004). Because protozoa regulate bacterial populations, they contribute to this regulation of nitrogen levels in the environment by eating the bacteria in the soil, which

stimulates the growth of the bacterial population, consequently increasing the rates of decomposition and nitrogen fixation.

The nitrogen cycle is so important because without nitrogen, plants wouldn't be able to create enzymes, since nitrogen is a main component in amino acids, the monomers of proteins. Without enzymes, though, the cells of plants could not perform chemical reactions, and without chemical reactions, there is no photosynthesis or any other cellular activity. Hence, no nitrogen, no cells, and without cells, plants do not survive; they will not be able to produce oxygen into the atmosphere (which is a key component for organisms to live) (Ingham, 2016); and they cannot provide for the rest of the consumers.

However, both plants and protozoa need large pores in the soil because such pores play key roles in moving water throughout the soil when it is saturated, as well as determining the ability of soil to hold and conduct nutrients and air (DeJong-Hughes, Moncrief, Voorhees, and Swan, 2001). Soil compaction is the compression of soil particles (Whiting, Wilson, and Reeder, 2014). Heavily compacted soils don't have many large pores and have a reduced rate of water infiltration and drainage from the compacted layer (DeJong-Hughes, Moncrief, Voorhees, and Swan, 2001). Since protozoa *need* water to be able to move through the soil, compaction reduces the number of protozoa in the soil and without the protozoa, the bacteria populations become unstable, disrupting the nitrogen cycle (Ingham, 2016)**.**Therefore, soil compaction decreases the plant's ability to absorb nutrients and water. In dry seasons, soil compaction can cause dried out, stressed plants from decreased root growth, and in wet seasons, soil compaction decreases soil aeration, which increases the loss of nitrate-nitrogen in the atmosphere, another important component of the nitrogen cycle. The result of protozoa dying will also be a limited food source for protozoan consumers, such as nematodes and micro arthropods. (NSTC 2001). Finally,

animals eat the plants, and plant residues return nitrogen into the soil to start the cycle again (Killpack and Buchholz, 1993). Hence, because compaction can disrupt both the protozoa living in the soil and the plants that depend on them, it can be concluded that there is a large chain reaction of bad events that can follow the disruption of protozoa.

From a substantial amount of research and many experiments, it is already known the harmful effects that soil compaction has had on plants and microorganisms as a whole. This project will isolate the impact soil compaction is having on protozoa, a key part of the ecosystem, in order to determine how the human population can treat the environment better for a better world in the future.

Outline of Soil Ecology Experiment (C2H2)

I. Problem: What impact do different types of compaction have on the density of protozoa that live in grass that abuts the areas of compaction?

II. Hypothesis: Areas exposed to the compaction caused by a building will contain the smallest density of protozoa**.**

III. Independent Variable: type of compaction (road, sidewalk, building)

IV. Dependent Variable: density of protozoa in soil

V. Negative Control: Soil that is not being compacted (front lawn)

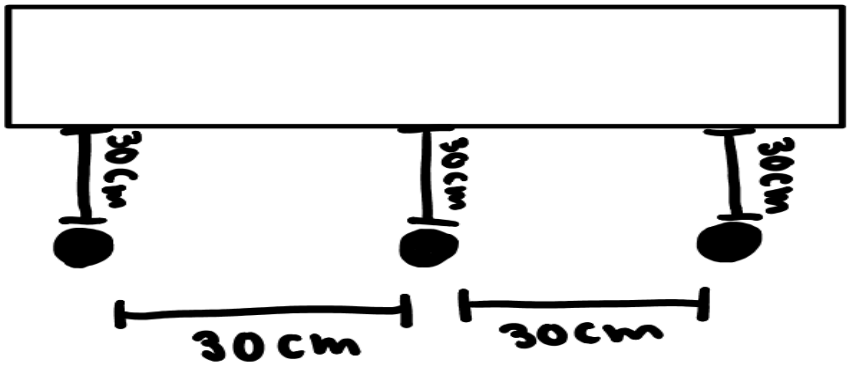
VI. Controlled Variables:

- Amount of soil extracted
- Type of grass soil is extracted from
- Type of vegetation covering soil
- Distance between flags
- Distance between tested soil and source of compaction

- Collected on the same day at the same time
- Amount of soil sifted in each petri dish
- Amount of distilled water added to saturate soil
- Amount of time soil is saturated
- Number of samples taken from each location
- Type of petri dish
- Size of petri dish (9 x 1.5cm)
- Type of Uhlig extractor
- How long soil is filtered in Uhlig extractor
- Size of qualitative filter paper
- Type of capillary tube
- Magnification of microscope
- Amount of dye put on microscope slide
- Amount of filtrate put onto the microscope slide
- Amount of sample put on the microscope slide
- Size of cover slip
- How long the soil is left out to dry

Step-by-step:

1. Go to the coordinates N39.3523° and WO76.63562°, which is the negative control.
2. Place 3 yellow flags that are 30 centimeters directly horizontal away from each other, and also 30 centimeters parallel away from the source of compaction at N39.3523° and WO76.63562°. See diagram below.



3. Go to the coordinates N 39.35737° and WO76.63581° , which is near the building, then repeat step 2 for.

4. Go to the coordinates N39.35786° and WO76.63582° , which is near the sidewalk, then repeat step 2.

5. Go to the coordinates N39.35703° and WO76.63645° , which is near the road, then repeat step 2.

6. Steps 7-8 must be completed the same day and same time of day

7. Use a soil core extractor to extract 15 centimeters deep by 2.2 cm wide of soil from each flag site making sure each sample from each location is extracted at the same time.

8. Place each soil sample in its own individually labeled plastic bags by putting the soil core extractor in this bag and scraping the dirt off of the extractor into the bag. For example, label the bag “road 1 day 1”.

9. Do steps 10-11 on the same day and at the same time.

10. Place 15 cm of the day one trial 1 road compaction soil sample into the bottom of a clean, empty 9x1.5 cm petri dish labeled “day one road 1”.

11. Repeat step 10 for the other two road trials and all three trials of building, negative control, and sidewalk, making sure to label the petri dishes with the corresponding type of compaction, trial number, and which day the soil was collected.

12. Allow soil in steps 10 and 11 to dry completely for 2 days.

13. Using a 1 mm^2 mesh, sift 9-10 g of the soil into a 2nd clean petri dish labeled “day one road 1” and record how many grams of soil was sifted.

14. Repeat step 13 for the other two road trials and all three trials of building, negative control, and sidewalk making sure to label the petri dishes with the corresponding type of compaction, trial number, and which day the soil was collected on the same day.

15. Do steps 16-18 on the same day at the same time.

16. Add 20 ml of distilled water to the day 1 road 1 petri dish to saturate the soil.

17. Cover the petri dish with its corresponding lid and allow to sit for 7 hours on the same day at the same time.

18. Repeat steps 16-17 for the other two road trials and all three trials of building, negative control, and sidewalk on the same day.

19. Do steps 20-21 on the same day at the same time.

20. Place the day 1 road 1 soil sample in a modified Uhlig extractor with nytex nylon containing 30 ml of distilled water for 24 hours.

21. Repeat step 20 for the other two road trials and all three trials of building, negative control, and sidewalk on the same day.

22. Do steps 23-24 on the same day at the same time.

23. Remove the filtrate and filter a 2nd time using 12.5 cm qualitative filter paper.

24. Repeat step 23 for the other two road trials and all three trials of building, negative control, and sidewalk.

25. Do steps 26-28 on the same day at the same time.

26. 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 pipet, add 18 µl (the first demarcation on the pipette) of the day 1 trial 1 road filtrate from step 11 to the stain on the microscope slide and cover with an 18 x 18 mm2 coverslip, labeling the microscope slide day 1 road 1.

27. Examine all microscope slides under a light microscope at 40X (for quantitative data) observations of the various protozoa living in the soil. View each corner and the center of the microscope slide, for each specific area count how many protozoa there are. Calculate the average amount of protozoa there is between each field of viewing.

28. Use the following equation to determine the population density of protozoa in the soil sample: [(# per field of view at 40X)(total ml of water used) 747]/(grams of sifted soil) = # of protozoa per gram of soil.

29. Repeat steps 26-28 for the other two road trials and all three trials of building, negative control, and sidewalk making sure to label the petri dishes with the corresponding type of compaction, trial number, and which day the soil was collected.

30. Compare number of protozoa per gram of soil from each compaction surface and record in data table.

31. Complete steps 6 through 30 two more times for a total of three trials for each type of compaction using different sets of soil from each location.

Data Tables

Day 1

Impact of compaction of Density of Soil Protozoa

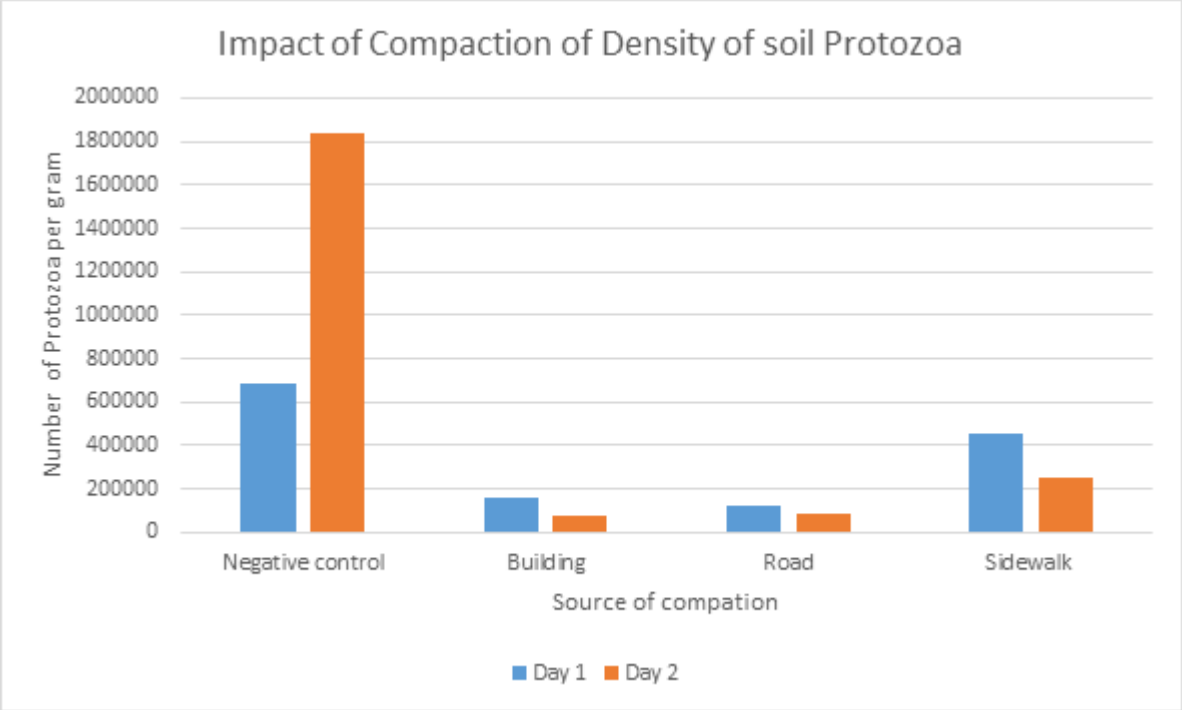
Source of Compaction				
Trial	Building	Road	Side Walk	Negative Control
1	251263.636 per gram of soil	183876.923 per gram of soil	991920.638 per gram of soil	1141842.857 per gram of soil
2	89961.290 per gram of soil	87691.304 per gram of soil	124229.348 per gram of soil	615270.968 per gram of soil
3	134263.62 per gram of soil	106366.304 per gram of soil	245787.097 per gram of soil	291018.75 per gram of soil
Average	158496.18 per gram of soil	125978.177 per gram of soil	453979.0277 per gram of soil	682710.8583 per gram of soil

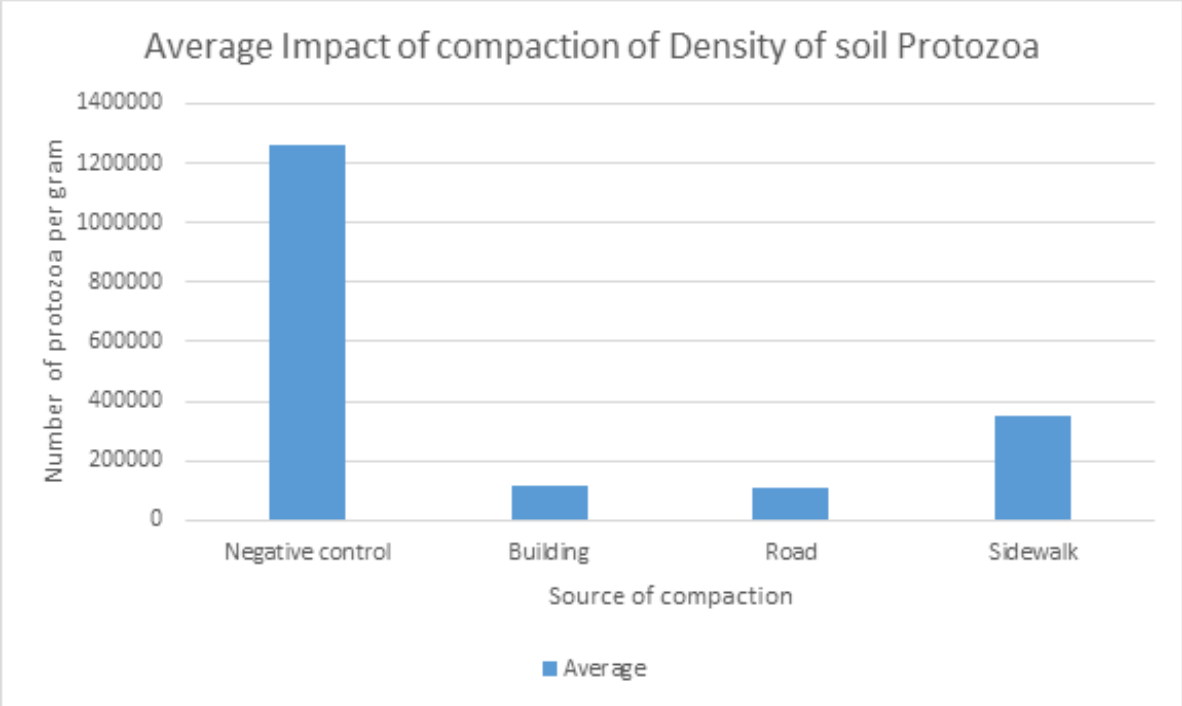
Day 2

Impact of compaction of Density of Soil Protozoa

Source of Compaction				
Trial	Building	Road	Side Walk	Negative Control
1	69309.278 per gram of soil	96622.826 per gram of soil	100968.132 per gram of soil	1877511.34 per gram of soil
2	73896.774 per gram of soil	131868.367 per gram of soil	229846.154 per gram of soil	1286767.742 per gram of soil
3	78472.7272 per gram of soil	31787.234 per gram of soil	93580.22 per gram of soil	2342206.452 per gram of soil
Average	73892.9266 per gram of soil	86759.476 per gram of soil	251736.264 per gram of soil	1835495.178 per gram of soil

Graphs:


Impact of Compaction of Density of soil Protozoa
Number of Protozoa per gram
2000000
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
Negative control
Building
Road
Sidewalk
Source of compation
Day 1
Day 2


Average Impact of compaction of Density of soil Protozoa
Number of protozoa per gram
1400000
1200000
1000000
800000
600000
400000
200000
0
Negative control
Building
Road
Sidewalk
Source of compaction
Average

Conclusion

We hypothesized that areas exposed to the compaction caused by a building will contain the smallest density of protozoa**,** and it was supported. According to our second graph, our negative control had the highest amount of protozoa overall for both days, with an average of 1259103.01815. This proves that compaction does in fact harm protozoa density. The amount of soil protozoa in the location of our negative control was 1142908.465 protozoa per gram of soil higher than the amount of protozoa per gram of soil located around the building, which was 158498.18. In addition, the amount of soil protozoa in the location of our negative control was 1142908.465 protozoa per gram of soil higher than the amount of protozoa per gram of soil located around the road which was 212737.653, and 906245.3721 protozoa per gram of soil higher than the amount of protozoa per gram of soil located around the sidewalk, which was 352857.64585.

From Day 1 to Day 2, the amount of protozoa in the soil for the negative control increased by almost 300%, which means life got generally better for the protozoa living in that soil. This is because it rained more on the second day, and as explained in the background, protozoa need water to live. However, from the first day to the second day, the protozoa density in the building, road, and sidewalk in fact dropped, which further proves that compaction is really bad for protozoa. From Day 1 to Day 2, for the building, the amount of protozoa per gram of soil dropped by about 53%, for the road,31%, and for the sidewalk about 45%. When comparing the Day 1 averages of building and road, the road appeared to have a lower density of protozoa than the building. The building had about 32518.003 protozoa per gram of soil more than the road, which is about 26% more. But, when looking at the overall averages of both days for the building and road, the amount of protozoa in the soils are about equal. The average

amount of protozoa per gram of soil for the building was 116194.5533, and the average amount of protozoa per gram of soil for the road was 106368.8265. This is only a 9825.7268 per gram of soil difference, or a 9% difference. When looking back at the Day 1 and Day 2 soil, though, the percent decrease in protozoa density appears higher in the building than in the road, which means the building had a greater percent decrease in protozoa from Day 1 to Day 2 than the road.

Between the building, road, and sidewalk, the sidewalk had the second highest percent decrease in protozoa density of the three from Day 1 to Day 2, about 45%. Since this percent is very close to the percent decrease from Day 1 to Day 2 for the building (53.379%), whether the sidewalk was actually having the worst impact of the protozoa density comes into question**.** We also observed shelled testate amoebae in only the sidewalk soil. This is their "protective state", which means something in the soil was putting them in so much danger that they had to shield themselves from it (Brock, 2016). This is probably because the sidewalk that the soil was extracted from was actually downhill from the main school driveway. From this, the effects of runoff most likely played a key role in this great percentage decrease in the protozoa density and their shelled state. Because it rained on Day 2, runoff came down from the driveway and into the soil that abutted the sidewalk. Runoff contains motor oil, which interferes with the movement of water by clogging pores in the soil. This prevents gases, like oxygen and nitrogen from exchanging, and without this movement of gases and water, protozoa are not able to live (Brock, 2016). So, because the sidewalk soil protozoa was being negatively impacted by the effects of runoff, the only sources of compaction that are actually being affected by *only* compaction are the building and road. Hence, since the building showed a greater percent decrease in protozoa density from Day 1 to Day 2 as opposed to the road, it can be concluded that, like our hypothesis,

the building has the worst impact on the protozoa in the soil that abuts it because of its compaction.

Since one of three of our "compaction locations" wasn't actually being affected by only compaction, for future research we would use a sidewalk location that is not affected by runoff, instead of our original sidewalk location which was. We would then repeat the experiment to see if we would get different results because of the new sidewalk location. This experiment was very interesting, and our group learned a lot about what compaction can do to the environment and atmosphere. Hopefully in the future, mankind will work together to end this important problem to make the world a better place for everyone and everything that inhabits it.

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