The Effects of Herbicide on the Protozoa Population Density

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Background

Protozoa are single-cell eukaryotes that are part of the traditional taxonomic kingdom known as the Protista , and with over 50,000 species of them, they are found in almost every possible environment on earth, including the soil (Yaeger, R. G. 1996). There, they work to mineralize nutrients, making chemicals such as nitrogen available for plants and other organisms that live in the soil to use for their own metabolic purposes, and they serve as one of the two key organisms in the soil that regulate bacterial density. By eating the bacteria, protozoa help stimulate the growth in bacteria populations (Ingham n.d), which in turn increases the rates of nitrogen fixation. Hence, protozoa are a key factor in the Nitrogen cycle. Protozoa are also important because they help to limit disease in the soil by killing off pathogens (Ingham n.d), and they help to regulate the amount of algae in the soil as well (Hoorman, 2011).

However, of all their functions in the soil, the role protozoa play in the nitrogen cycle is the most critical for the organisms that live above the ground. The transformations that nitrogen undergoes as it moves between the atmosphere, the land, and living things are known as the nitrogen cycle, and the process starts when certain groups of soil bacteria, known as Nitrosomonas, capture nitrogen gas (N_2) (which makes up about 78% of Earth's atmosphere by volume) (Plants and Minerals. 2014) and convert it into ammonium, a form readily accessible by the other organisms living in the soil. In addition, certain other bacteria and fungi produce ammonium through the process of decomposition. Hence, there is always a slight excess of ammonium in the soil that is then converted to nitrate through a process called nitrification. The bacteria responsible for this task are called nitrobacter (Plants and Minerals. 2014), and they use the nitrification process for their own energy needs. But nitrification requires the presence of oxygen, which is what sets it off from denitrification. Denitrification is a process that converts

any excess nitrate found in the soil back to nitrogen gas (N_2), releasing it back into the atmosphere and it is the only nitrogen transformation that removes nitrogen from the biological components of ecosystems, thereby balancing the amount of fixed nitrogen in the environment. But denitrification must occur in anaerobic conditions because the bacteria that perform it use nitrate instead of oxygen when obtaining energy. Therefore, the protozoa have one of the most important roles in the function of the nitrogen cycle.

The critical role soil protozoa play in all of this as the protozoa eat the bacteria, who are doing the nitrogen fixation in the soil, they release nitrogen nitrate as a waste product to be used by plants and other organisms (The nitrogen cycle. n.d.). Protozoa vary in structure from bacteria, since their concentration of nitrogen is much lower than the bacteria they feed on (Portland State University, n.d.). Therefore, when the protozoa eat the bacteria, the protozoa have an excess amount of nitrogen. Due to this, the protozoa release this excess nitrogen into the soil, benefitting the plants. Protozoa are needed to release the nitrate to restart the nitrogen cycle and for other plants to utilize the nitrate for growth.

Nitrogen is so crucially important for all life because it is apart of many cells and processes, being a key factor in amino acids, proteins and DNA. If the plants cannot get access to the nitrates to make these things, it cannot supply the nitrates for the other organisms on top of them on the food chain. If no organisms can get nitrates to make the amino acids, proteins, and DNA, then they cannot control their chemical reactions and will die. Plants use nitrate to make amino acids. Amino acids are important for plant growth and plants that lack nitrate show stunted growth. It is also necessary in order to make chlorophyll in plants, which is a crucial chemical involved in photosynthesis to make their food (The nitrogen cycle. (n.d.).

The health of the protozoa must be maintained so that they can continue to play this key role in the nitrogen cycle, and therefore, anything that might cause harm to soil protozoa could harm the entire ecosystem. One such factor might be the common practice the people that use herbicides on their lawns and gardens.

Herbicides are chemicals that are used when there are other plants, commonly called weeds, that "tend to overgrow or choke out more desirable plants" (Cambridge University press, n.d.), and they come in two varieties: selective and nonselective. The first of these only kill specific weeds; while the latter kill all the plant material with which they come in contact (Penn State College of Agricultural Sciences, n.d.). The pros of using herbicides on crops are: they have the ability to kill weeds before they spread and can kill weeds at critical times. Also, they are able to ensure higher water use by crops that are in dry environments and reduce crop failure due to droughts. However, the cons of using herbicides are: an excess dosage of them can stunt plant growth (or even kill the plant) if not applied properly or at the proper time in the plant's life. Furthermore, herbicides can drift during application onto different vegetables or crops that people may eat, potentially posing a health risk, and once used, an herbicide can affect and limit what future crops are able to be planted in that soil.

Some common active ingredients in commercial herbicides are, dimethylamine salt and quinclorac which both target broadleaf weeds (2,4-D Technical Fact Sheet 2008). Dimethylamine salt works by imitating a chemical called Indole Acetic Acid, which is naturally produced by plants. This causes the plant to grow at such a rapid rate that it dies from overgrowth. (How do selective herbicides work? 2017) The mechanism for how Quinclorac works, on the other hand, is not yet understood. There are three hypothesis for how quinclorac works. One is that the chemical affects cell wall synthesis. A second is that quinclorac mimics an

auxin and causes uncontrollable plant growth, and the third is that is causes the growth of reactive oxygen species that are harmful to the broadleaf plants. (FIPKE, M., & VIDAL, R. 2016, June)

This experiment is testing the effects of herbicides on soil protozoa in the environment. We chose this experiment because the use of herbicide is common and our group was interested to see if the herbicides only harmed the plants or if they were damaging to the entire ecosystem. We chose to test for protozoa because of their role in the nitrogen cycle. Without protozoa, the balance in this crucial process would be broken. To test this, six plots will be set up, and one sample will be taken from each. We will examine the number of protozoa in the soil samples taken before and after the application of an herbicide, (Ortho Weed B Gon Max will be used in this experiment). We predict that the herbicide will kill off protozoa in the soil, lessening the population density.

Lab Report

I. Problem: How do herbicides increase or decrease the protozoa density in the soil?

II. Hypothesis: Herbicides will decrease protozoa density in the soil.

III. Procedure:

   A. Independent Variable: adding herbicide to the soil.

   B. Dependent Variable: # of protozoa per gram of soil

   C. Negative Control: Only adding water to the soil

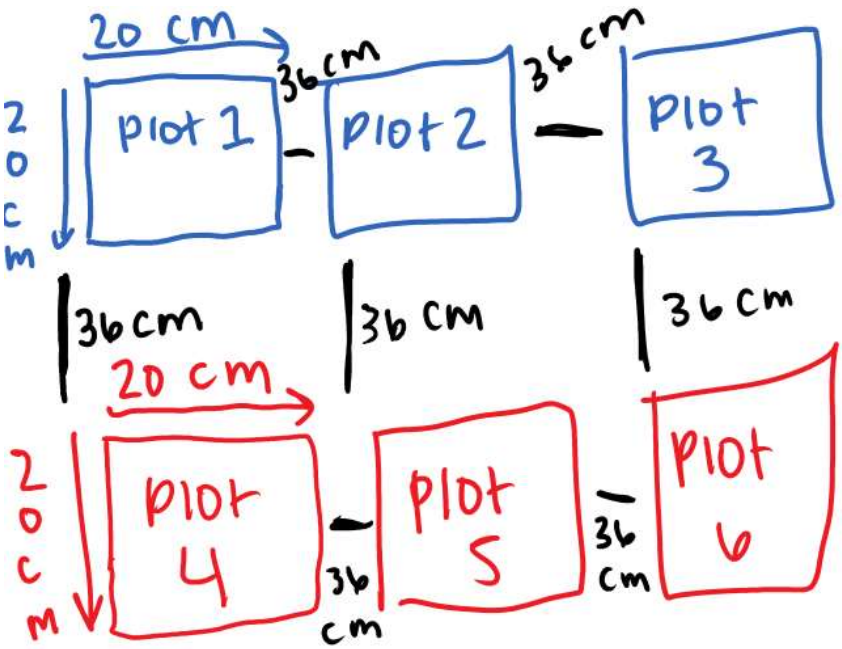
   D. Positive Control: The samples taken before anything is done to the soil

   E. Controlled Variables: method of application of herbicides, brand of herbicide, amount of herbicides added to soil, amount of water added to soil, where the soil was located, length of time waited after applying herbicides before collecting soil

samples, time waited after applying water before collecting soil samples, plot size, amount of soil put into the first petri dish, amount of soil put into the second petri dish, moisture of soil before herbicides are added, size of nylon screen, soil extraction method, size of soil extractor, type of soil, take soil samples the same day at the same time at the same location in order to control for the the environment,, surrounding plant life, size of soil samples taken, size of mesh used to sift, amount of soil sifted, amount of distilled water used to rehydrate, amount of water in Uhlig, type of microscope, power of microscope, rehydrate and filter same time and same time, same amount of dye placed on slide, amount filtered fluid put on slide, size of coverslip

F. Step-by-Step Instructions:

Step 1) Make six plots of land



that are

20 X 20cm each, that are also 36 cm apart at coordinates: (N 39° , 21.402) (W 076 ° , 38.1240) using the diagram below

Step 2) Place flags around this area to ensure that people will not step on and compromise the experiment

Step 3) At the same time on the same day, using the soil core extractor, push the extractor, with a diameter of 2cm, into the soil and wait until the soil fills up to the 1st mark on extractor. The first mark is at 15.25 cm from the bottom of the soil core extractor. Then, take 1 sample of soil from each of the 6 plots and place each soil sample into its own separate plastic ziploc bags labeled "positive control samples..." and the number corresponding to the plot number the soil was taken from (plots 1-6)

Step 4) Add 125 mL of water to each of the first three plots

Step 5) Add 123 mL of water mixed with 2 mL of herbicide (Ortho Weed B Gon Max) to each of plots 4-6

Step 6) Take the positive control samples back to the lab station and at the same time on the same day, place the six 15 cm samples of soil sample with nothing added to them into the bottom of six different clean petri dishes each labeled with a number corresponding to the number of the bag from which the soil sample came from and allow them to dry for at least 24 hours.[1]

Step 7) Sift 9-10 grams of each of the the soil samples from step 6 into six different clean petri dishes using a different 1 mm^2 nylon screen for each sample. Be sure to record the exact amount of soil sifted, for each sample.

[1] Steps 6-14 are taken from Brockmeyer, Kate. (2008) "Chapter 3 - Extracting Soil Protozoa." *Soil Ecology Lab Manual*. Batavia: Flynn Scientific,. 13-16.

Step 8) At the same time on the same day, add 20 mL of distilled water to each sample to saturate the soil

Step 9) Cover the petri dishes with their lids directly after adding the water and allow it to sit for 7 hours

Step 10) At the same time on the same day, place the six soil samples in six different modified Uhlig extractor containing 30 mL of distilled water for 24 hours at room temperature, label each extractor with a number corresponding to the number of the soil plot from which they came from.

Step 11) At the same time on the same day, remove the filtrate from each extractor and filter each sample into its own clean white plastic cup, a second time using 12.5 qualitative filter paper (different paper for each sample)

Step 12) One sample at a time, using a capillary tube deposit 7 µl of methyl-green stain on a clean microscope slide (1 µl is equal to 1 drop from the capillary tube). Then using a disposable Beral-type pipette add 18 µl (the first demarcation on the pipette) off the second filtrate for sample 1 from step 11 to the stain on the microscope slide and cover with a 18 x 18 mm^2 coverslip. Do this for each soil sample at the same time at the same day.

Step 13) Examine under a compound light microscope at 40X power until the microscope slide is focused and count the protozoa. To do this, look at the five different fields of view in order from view 1-5 (respectively: top left, top right, bottom left, bottom right, middle of the coverslip). Then find the average of the 5 views. Do this for each soil sample at the same time on the same day.

Step 14) Use the following equation to determine the population density of protozoa in the soil sample [(number per field of view at 40x) times (total mL of water used) times 747] divided by (grams of sifted soil) = number of protozoa per gram of soil. Record this number in the data table

Step 15) 48 hours after adding the herbicides, repeat step 3, labeling the samples from plots 1-3 as, “soil with water” and the number corresponding to the plot it was taken from. Do the same process for plots 4-6, but this time labeling the samples as “soil with herbicides” and the plot number the soil was taken from.

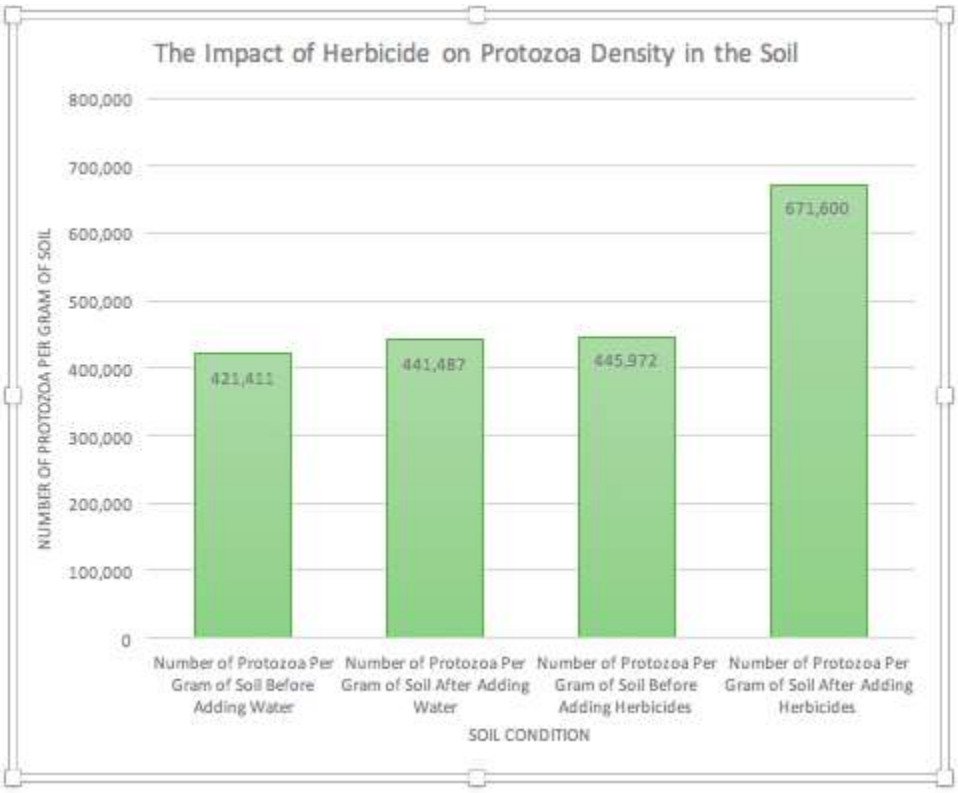
Step 16) Repeat steps 6-14 with the new soil samples recording all data in the data table

Data and Analysis

A. Data Table

The Impact of Herbicide on Protozoa Density in the Soil				
Trial #	Number of Protozoa Per Gram of Soil Before Adding Water	Number of Protozoa Per Gram of Soil After Adding Water	Number of Protozoa Per Gram of Soil Before Adding Herbicides	Number of Protozoa Per Gram of Soil After Adding Herbicides
Trial 1	536,906	366,030	585,278	627,480
Trial 2	361,948	836,640	468,78**0**	798,654
Trial 3	365,380	121,793	283,860	588,668
Average	421,411	441,487	445,972	671,600

B. Graph



V. Conclusion

The question posed in this experiment we conducted was "How do herbicides increase or decrease the protozoa density in the soil?" Our hypothesis was that herbicides would decrease protozoa density in the soil. Our hypothesis was proven to be incorrect. This is shown by the increase of protozoa, after our experiment. During our experiment, before we added water, there was an average of 421,411 protozoa per gram of soil. After we added water to the soil, there was an average of 441,487 protozoa per gram of soil. Before adding herbicides, there was an average of 445,972 protozoa per gram of soil and after adding the herbicides, there was an average of 671,600 protozoa per gram of soil. In the three plots where only water was added, the environment was stable with only a 4.76% change in protozoa density. In plots 4-6 where the herbicides were added however, the protozoa density increased by 50.59%. We believe that this

large change in density was due to a rise of bacteria. Though our group did not test for bacteria levels, we conferred with another group in our class (Flanigan P, *et al*, 2017) and their findings showed that the bacteria levels had significantly decreased, a drop of 40.74% when herbicides were added to the soil. We learned that bacteria decompose plant material. As the applied herbicides killed the plants in the area, the bacteria would have had a surplus of food causing them to multiply. The increase in population could have attracted protozoa to the area to eat the bacteria. This would then cause a decrease of bacteria and an increase of protozoa. We can make the assumption that our bacteria levels would have reflected theirs due to the fact that our experiments were conducted in similar environments, with the same plant life, sun exposure levels, temperature, precipitation levels, and the distance between the plot sites was within 10 meters.

An anomaly was noticed in the fourth slide view of the fifth positive control slide. The slide view showed that testate amoebae were present. These amoebae are a type of a protozoa that have a type of shell to protect themselves (Ingham n.d). This would indicate that in that part of the soil, the environment was harsher causing the protozoa to have to shield themselves. After pesticides were added the shelled amoebae were no longer present in plot five but appeared in plot six. A possible reason for this could be that as the food increased, more predators would be competing for the food source, in this case the bacteria. As the microbes engage in chemical warfare, it would be beneficial to the protozoa to have a shell to protect themselves. The disappearance of testate amoebae in the fifth slide after the addition of herbicides could be attributed to a decrease in other predators that the protozoa were competing with. The appearance of testate amoebae in the sixth plot after the addition of herbicides could suggest that there was an increase in the amount of soil predators that protozoa had to compete with.

For further research, we would test the effect of herbicides on bacteria at the same time as testing the effect on protozoa. This would allow us to see the effect the two organisms have on one another. It could prove or disprove our theory that the rise of protozoa was due to the initial increase of bacteria, due to the increase of dead plant material for the bacteria to decompose, which the protozoa then fed on. When analyzing our results, we would look for declined bacteria levels from the initial sampling from after adding the herbicide. In addition, we could fix our design flaw which is, the spacing between plots. If we were to redo this experiment, with a smaller distance between the plots, we might end up with different protozoa averages.

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