Soil Ecology Project:
Effects of Acid Rain on the Number of Protozoa in the Soil

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Biology/Brock

May 5, 2015

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Background

Soil protozoa are small single-celled organisms that eat mostly bacteria. They are classified into three groups based on their structural peculiarities, and although they are much larger than their prey, one pinch of soil can still have thousands of protozoans and they can only be seen through a microscope. Of the three groups, ciliates are the largest and use tiny hairlike projections to move through soil and water; while amoebae, which are smaller, use their temporary feet to move and flagellates, the smallest, use a tiny, whip like tail to swim. (Soil Biological Communities, 2001)

Why are protozoa in the soil important? First, they are a critical part of the soil food web. They both eat smaller organisms and are food themselves for larger organisms, and as part of this process, they help regulate bacterial populations by stimulating the growth of the bacterial population when they consume them, and so a second reason protozoa in the soil are important is because they play an important role in the cycling of elements in the soil, making nutrients such as nitrogen, phosphorus, and potassium available to different plants by eating different fungi and bacteria. For example, since soil protozoa maintain a lower concentration of nitrogen in their cells than the bacteria they eat, they release the leftover nitrogen in the form of ammonium (which usually occurs near the root system of the plant). Most of the ammonium released is taken up from the soil by plants and some of the ammonium is incorporated into plant and animal protein. This process is significant because nitrogen is extremely important in all cells.

Without it, plants cannot make their amino acids, and without amino acids, they cannot make their proteins, especially their enzymes. Without enzymes, cells cannot start and stop the chemical reactions needed to perform homeostasis, reproduction, synthesis, and transforming energy, and if these four tasks do not happen in cells, an organism dies (Science Learning, 2013).

Furthermore without nitrogen, plants cannot make the nucleic acids to provide the genetic information to make the proteins in the first place, and since plants are the foundation for the food web, all other organisms depend on the plants for their source of nitrogen for their proteins and nucleic acids to live and develop. Hence, if soil protozoa are harmed in any way, plants and other organisms would not be able to receive the nutrients they need to survive including nitrogen (Science Learning, 2013).

One way protozoa might be harmed is by the presence of acid rain. Acid rain is any form of precipitation that possesses elevated levels of hydrogen ions or has a low pH. Although it is called acid rain, this precipitation can occur as snow, fog, or other forms of moisture as long as it has a pH of 5 or lower. When it seeps into the ground, it can disrupt a variety of ecologic functions, and one of the most significant things a low soil pH can alter is the quantity and form of chemicals present and available to the organisms living there. For example, acid rain can dissolve nutrients such as magnesium and calcium, and when the pH is too low, it causes heavy metals such as arsenic and lead to become soluble. Once soluble, these heavy metals can attach to the biological molecules in the organisms living there and cause toxicity (Holifield, 2015).

One of the most detrimental chemicals that acid releases when it falls on the land and is absorbed by the soil is aluminum. The presence of active aluminum in the soil makes it more difficult for the trees and other plants to take up water and can damage the roots of plants and trees, interfering with the amount of nutrients that the plants can absorb, leading eventually to the death of the plant and any organism depending upon them. Hence, the presence of active aluminum in the soil brought on by acid rain can upset the food chain and thereby bring about the collapse of the entire ecosystem (EPA, 2012).

But what causes acid rain? It starts when compounds such as sulfur dioxide and nitrogen oxides are released into air from vehicles and power plants. These substances soar into the atmosphere where they combine and react with water, oxygen, and other chemicals to form acidic pollutants known eventually as acid rain. Specifically, when nitrogen oxides are released into the air and combine with water and oxygen, they produce nitric acid, and when sulfur dioxide is released into the air, it produces sulfuric acid. Both of these compounds then precipitate out of the air and into the soil where they affect soil greatly and can kill different organisms living in the soil, including protozoa (EPA, 2012).

The way the nitric and sulfuric acid harm the protozoa is by altering the pH of the moist areas of the soil where protozoa live and thrive. When soil protozoa are exposed to liquids with a low pH, they become less active, begin to stop functioning and eventually die. The enzymes in the protozoa begin to fall apart when they are exposed to the low pH of acid rain. If the enzymes fall apart, the protozoa are unable to start and stop chemical reactions which means that the protozoa will ultimately die. (EPA, 2012).

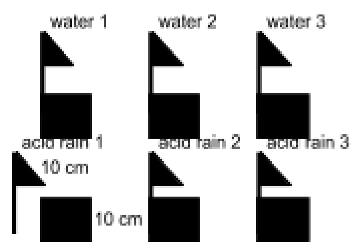
After studying acid rain and soil protozoa, our group decided to do a study on how acid rain affects the protozoa in the soil. First, we created six separate plots of soil and took soil samples from each of them to figure out the number of protozoa in those plots. Then we exposed half of the plots to acid rain and took new soil samples from the 6 plots to figure out the number of protozoa in them. The number of protozoa in the first samples from the six plots will be compared to the number of protozoa in the second samples from each plot. Then, we will calculate the difference in the number of protozoa to see how the acid rain had an effect on the number of protozoa in the soil. We hypothesize that the acid rain will decrease the amount of protozoa in the soil.

<u>Experiment Outline</u>

I. Problem: Does acid rain decrease the amount of protozoa in the soil?
II. Hypothesis: Acid rain decreases the amount of protozoa in the soil.
III. Procedure:
   A. Independent Variable- Presence of acid rain applied to the soil
   B. Dependent Variable - Number of protozoa per gram of soil
   C. Negative Control- 1 liter of tap water applied to soil
   D. Controlled Variables- amount of acid rain poured on the soil, chemicals used to create acid rain, concentration of acid rain, amount of soil placed in petri dish, exact location where soil was taken from, all samples tested same day and same time, all samples taken same day and same time, size of microscope cover slip, amount of time soil is in uhlig extractor, size of soil plots, number of soil plots, amount of flags around each soil plot, how far the soil extractor goes into the ground, degree that the soil extractor gets twisted, direction that the soil extractor gets twisted, amount of distilled water poured on the soil, amount of methyl-green stain put on slide, magnification, amount of space in between soil plots, set up of soil plots, size of soil extractor, size of capillary tube, amount of protozoa solution put on slide, number of fields of view counted
   E. Step by Step Instructions-

<u>Getting the Soil</u>

1. Steps 1-10 must be done on the same day at the same time
2. Make six square 20cm x 20cm plots in the backwoods at the location with the coordinates of N39.35688 degrees and W76.63628 degrees, in table form with 2 columns and three rows, 10 cm apart (See diagram)



3. Place one flag at each corner of each plot.
4. Label all of the flags in one column “water” and all of the flags in the other column “acid rain.” (see diagram)

5. Label one flag in the plot in the south “water 1”. Label at least one flag in the plot to the north of that plot, “acid rain one.” Repeat this process with the appropriate label with all the other plots. (see diagram)
6. Put the soil core extractor into the Acid Rain 1 plot.
7. Use a mallet to push it down into the ground until it gets into the 1st marking, 15 cm deep and 2¼ in diameter.
8. Twist the soil extractor 360 degrees clockwise and then pull it out of the ground.
9. Remove the soil from the soil extractor and put in a plastic bag. Label the bag “Acid Rain 1.”
10. Repeat steps 5-8 to take a sample from each soil plot and label each bag with the corresponding soil sample.

<u>Examining the Protozoa</u>

11. Place a 15 cm sample of soil from each of the plots into the bottom of its own separate, clean, empty petri dish that is labeled the same as the corresponding label on the plastic bag (e.g. soil from plastic bag labeled “water 1”is placed into soil is placed into a petri dish labeled “water 1”); and allow them to dry completely with the lid of the petri dish off.
12. Sift 9-10 grams of the soil collected from each plot into its own separate 2nd clean petri dish using a 1 mm^2 nylon screen or mesh. Label the petri dish with its corresponding soil sample. Use a different nylon screen or mesh for every sample of soil.
13. Add 20ml of distilled water to each petri dish in order to saturate the soil and be sure to do all soil samples on the same day at the same time.
14. Cover all the petri dishes with their lids and allow all of them to sit for seven hours.
15. Place each soil sample collected from each plot into its own modified Uhlig extractor containing 30 ml of distilled water for 24 hours. Label each Uhlig extractor with its corresponding soil sample and be sure to do all soil samples on the same day at the same time.
16. After 24 hours, remove the filtrates from each Uhlig Extractor and filter each sample separately a 2nd time using 12.5cm, qualitative filter paper into a dixie cup. Use a different qualitative filter paper for each soil sample and be sure to do all soil samples on the same day at the same time.
17. Steps 18-19 must be performed on the same day at the same time for each soil sample.Any untested soil samples on a given day can be placed in the fridge.
18. Using a capillary tube, deposit 7 µl (1ul=1 drop from the capillary tube) of methyl-green stain on clean microscope slides labeled with the corresponding soil sample (e.g. “water 1”). Then using a disposable graduated Beral-type pipette, add 18 µl (the first demarcation on the pipette) of the 2nd filtrate from that same soil sample to the stains on the microscope slides and cover with 18 x 18 mm^2 coverslips (e.g. “water 1”).
19. Examine under a light microscope at 40X observations of the various protozoa living in the soil.

20. Count the number of protozoa on five different sections of the microscope slide; count each corner and in the middle. Average the five fields of view.
21. Use the following equation to determine the population density of protozoa in the soil samples: [(# per field of view at 40X) x (total ml of water used) x 747]/ (grams of sifted soil) = # of protozoa per gram of soil. Average all of the densities together after all the calculation have been performed.
22. Record findings in the data table.

Testing with Acid Rain

23. Make three liters of acid rain by following steps 24-26
24. Put 1 liter of tap water into a container
25. Add drops of concentrated sulfuric acid until the solution reaches a pH of 5
26. Repeat steps 24-25 two more times to create a total of 3 liters of acid rain.
27. Pour 3 liters of acid rain on each of the three plots with flags labeled "acid rain", one liter per plot.
28. Pour 3 liters of tap water on each of the three plots with flags labeled "water", one liter per plot.
29. After 77 hours, extract and test soil from all six plots at the same time on the same day by repeating steps 6-22.
30. Record findings in data table

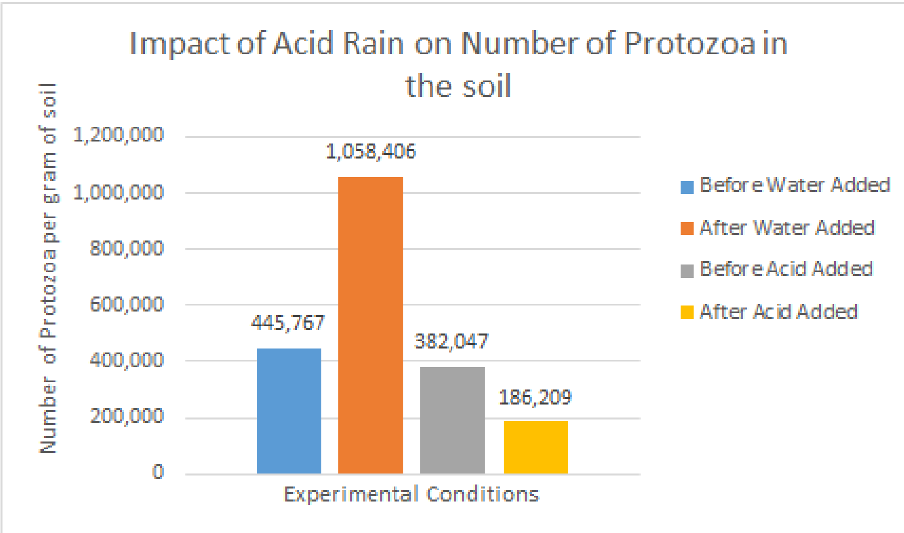
IV. Data and Analysis

A. Data Table

Impact of Acid Rain on Protozoa in the soil

Trials	Negative control soil plots before water added (Number of Protozoa per gram of soil)	Negative control soil plots after water added (Number of Protozoa per gram of soil)	Soil plots before acid rain added (Number of Protozoa per gram of soil)	Soil plots after acid rain added (Number of Protozoa per gram of soil)
1	995,735	2,488,647	794,179	366,192
2	153,291	199,465	153,291	102,010
3	188,274	487,106	198,670	90,426
Averages	445,767	1,058,406	382,047	186,209

B. Graph



V. Conclusion

Our hypothesis was correct. Our hypothesis was that acid rain decreases the amount of protozoa in the soil which we found was correct according to the data we found after completing our experiment. We know that acid rain decreases the number of protozoa in the soil because the average number of protozoa in the soil before we poured acid rain was 382,047 but the average number of protozoa after acid rain was 186,209. Therefore, there was less protozoa in the soil after the acid rain was poured on the soil plots since the number of protozoans after the acid rain was poured decreased by 195,838. This data shows that acid rain did decrease the amount of protozoa in the soil since there was less protozoans in the soil after the acid rain was poured on the plots. Since the number of protozoa per gram of soil is much larger than the number of protozoa per gram of soil after the acid rain, it proves that acid rain does decrease the number of protozoa in the soil. In trials 1-3 before acid rain was applied, the number of protozoa in the soil were 794,179; 153,291; and 198, 670. After pouring acid rain on the soil the number of protozoa per gram of soil were 366,192; 102,010; and 90,426. Because the data decreased by 195,838 protozoa per gram of soil, it is certain that acid rain decreases the number of protozoa in the soil. Our hypothesis tested to be correct because acid rain has chemicals in it that are harmful to protozoa. These chemicals can dissolve nutrients in the soil and alter the soil pH. The way the nitric and sulfuric acid harm the protozoa is by altering the pH of the moist areas of the soil where protozoa live and thrive. When soil protozoa are exposed to liquids with a low pH, they become less active, begin to stop functioning and eventually die. The enzymes in the protozoa begin to fall apart when they are exposed to the low pH of acid rain. If the enzymes fall apart, the protozoa are unable to start and stop chemical reactions which means that the protozoa will ultimately die. This process is how acid rain harms and kills the protozoa in the soil. For further research, we could experiment to see if nitric acid also decreases the number of protozoa in the soil. To research this, we could follow the experiment listed above, but we could replace sulfuric acid with nitric acid. Another research question we could study is if the concentration of acid rain poured on the soil alters the number of protozoa in the soil. To conduct this experiment, we could conduct a series of trials in which we alter the concentration of acid rain poured on the soil.We could pour acids with concentrations of 4, 5, 8, and 9 on the soil plots. Each concentration of acid rain will be poured on a different plot. In our experiment, while we were counting the number of protozoa in the soil, we noticed that the soil sample from the water 1 plot, before water was added, had many shelled amoebas. This means that the environment in which the shelled amoebas were living was very toxic. If we performed this experiment again, we would want to do a better job of controlling the location in which we made our plots. In conclusion, after we collected the data from our experiment, we discovered that acid rain does decrease the amount of protozoa in the soil.

Works Cited

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