Ammonium and Nitrate's Affect on Protozoa

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May 28, 2008

Background

Fertilizer is a compound that can come in either an organic or inorganic form and is used to improve the growth of plants by giving them extra nutrients through the soil. Organic fertilizer can be made from compost, manure and other natural nutrient filled resources, while non-organic fertilizers are made of chemicals and minerals. For example Ammonium, Phosphate, and Potassium are three macronutrients found in most fertilizers and are key components in promoting plant growth (How Stuff Works, 2008). Fertilizers basically provide nutritional “building blocks” for the plants, while the actual energy to ‘eat’ the food comes from photosynthesis. Fertilizer dissolves in the water within the soil and enters the plant through its roots. The nitrogen in the fertilizer gives plants ‘building blocks’ to create their proteins and DNA as well as biological compounds which are used to ‘build’ the plant (Kenneth Joergensen, 2006). These molecules are so important to the plant because its cells need them to perform the chemical reactions, the four tasks, which keep the cell and hence the plant alive.

While highly valuable to the plant world, the nitrogen in fertilizer may be directly or indirectly decimating the protozoa populations that live in the soil. “Protozoa are a varied group of single-celled animal-like organisms belonging to the kingdom Protista” (Advameg Inc, 2007). The excess nitrogen is harmful to protozoa or at least undesirable when inside or around their bodies. Basically, adding the fertilizer is comparable to shutting a human in a room full of carbon dioxide. Even if it is not directly harmful to the protozoa, if fertilizer upsets one link in the food chain it could negatively affect the protozoa population by removing a food source such as bacteria. Conversely, the fertilizer may instead have a beneficial effect on the protozoa as it increases plant growth and the dead plant material which protozoa consume to live. But in most cases, only a natural balance of protozoa helps plants get the nitrogen nutrients they need.

Whatever the result, it is estimated that humans have recently doubled the amount of fixed nitrogen introduced into the world yearly making it essential to find all of the effects of fertilizer on the environment (John Arthur Harrison, Ph.D., 2003). In the soil at RPCS, the protozoa levels behave strangely; where there are supposed to be high levels of protozoa, the levels are too low, and where there should be low levels of protozoa, the levels are too high. In many cases the levels of protozoa in the soil have been too low regardless of what they should have been (E.S.S.R.E. Microclimate Databases, 2007). Therefore we decided to study the protozoa levels and how ammonium affects them because the ammonium in fertilizer could be decreasing the amounts of protozoa in the soil, causing an upset in the balance of protozoa within the soil.

From our experiments we hope to discover whether or not the ammonium is the true culprit in our school's problem, and whether this is a problem that can be fixed. If ammonium does have a negative effect on our protozoa population, we can use less ammonium-rich fertilizer to promote healthy soil in our school's ecosystem, or attempt to find less toxic organic fertilizers for the plants.

To see if there is a negative effect, our group decided to stake off some land on campus to test the levels of nitrogen and ammonium in the soil. Then, we tested a different section of land, putting an ammonium nitrate solution into the soil. This will show us how an increase of ammonium affects the protozoa population. This test, hopefully, will show us what we need to do to return our protozoa population to its normal levels, which could create a healthier environment for the protozoa.

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Procedures

Question: are high levels of ammonium in the soil harmful to protozoa?

Hypothesis: if we add ammonium from fertilizer to soil containing protozoa, then there will be less protozoa than before the addition of the ammonium

Independent Variable: additional ammonium added to soil

Dependent Variables:

- number of protozoa per gram found in soil plot
- amount of nitrogen in soil plots
- amount of ammonium in soil plots

Negative control: addition of only water to 3 of the plots

Positive Control: the soil taken before the addition of any of the treatments

Controlled Variables:

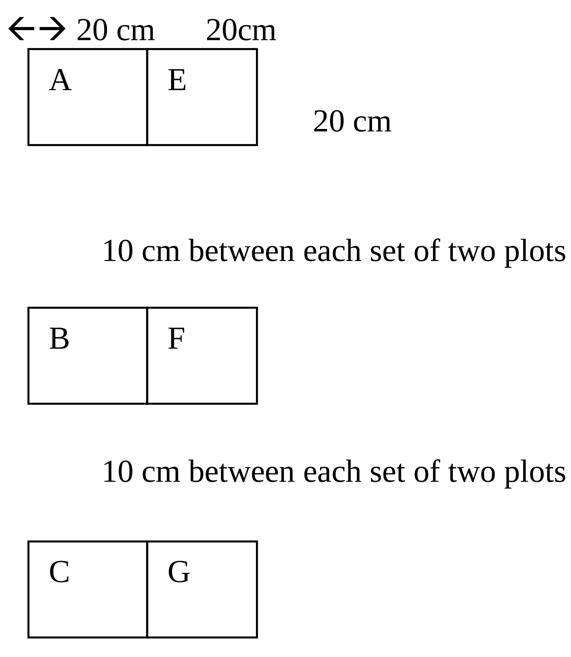
- plant life on and around the soil plots
- location of plots (must be close to each other)

- amount of liquid added
- concentration of ammonium
- size and dimensions of plot
- amount of soil collected and sifted through for each plot (g)
- timing of collection
- size of Petri dishes used
- amount of distilled water added to soil (20 ml)
- amount of soil sifted for protozoa count (9-10 g)
- size of Uhlig extractors used
- amount of liquid added for the protozoa extraction with the Uhlig extractors (30 ml)
- Amount of universal extracting solution used for extracting he general soil extract
- Size of plastic soil measure and amount of soil added to the extracting solution
- Size of filter paper, plastic funnel and time we shook the cap to mix the soil up (1 min)
- Size of 1 ml pipets and spot plates
- Amount of Nitrate Test Reagent #1 added to spot plate (10 drops)
- Amount of Nitrate Reagent #2 added (0.5 g spoon of it)
- Nitrate Nitrogen color chart used
- Amount of general soil extract used (4 drops)
- Amount of Ammonia Nitrogen Test Solution added (1 drop)

- Ammonia Nitrogen Color chart used
- Microscope view used for counting protozoa (60X)
- Type of microscope used (digital blue)
- Equation used for counting protozoa
- Amount of dye added to slide
- Amount of filtrate added to slid

Procedure:

1. Gather materials
2. label 12 flags ammonium and 12 flags water
3. go the coordinates N- 39.35799 and W- 076.63534
4. use a meter stick to map out 6 square 20 by 20 cm boxes with 10 cm in between each row of 2 boxes (see diagram below)



5. place the flags labeled “water" at the corners of three boxes in the plot area (see diagram above) water flags should be at the A,B and C spaces and take the labeled ammonium flags and put them at the corners of the other boxes (E,F,G)
6. label 18 plastic bags with the corresponding plot that its from and its trial number (for example Ammonium 1a)
7. Take 3 samples of soil out of each of the 6 plots using a 15 cm soil cylinder with a 2 cm diameter. ***These should all be taken at the same time of day and on the same day.***
8. place each soil sample into its own corresponding plastic bag
9. label 9 Petri dishes with “water” and 9 Petri dishes with “ammonium” and with their corresponding plot and trial number
10. Place each 15 cm cylinder of soil into the bottom of its own separate, labeled, clean, empty Petri dish and allow it to dry completely for 24 hours
11. Sift 9-10 g of each soil sample and put it into its own individual clean Petri dish using a 1 mm^2 nylon mesh and record how many grams were sifted for each soil sample. Make sure that they are also labeled so that you know which plot each sample came from.
12. Simultaneously as the chemical testing is occurring add 20 ml of distilled water to each plot sample to saturate the soil, while using the chemical testing kit STH-14 test for Ammonia Nitrogen and Nitrate Nitrogen. Be sure that the test is done on the remaining dry samples
13. cover the Petri dishes with their lid and allow to sit for 7 hours

14. place each soil sample in its own separate modified Uhlig extractor containing 30 ml of distilled water and let it sit for 24 hours

    Note: steps 15-21 need to be performed on the same day at the same time

15. remove the filtrate of sample Water 1a and filter a 2^{nd} time using a 12.5 cm qualitative filter paper filter and make a microscope slide of the filtrate immediately

16. using a capillary tube deposit 7 µl of methyl-green stain on a clean microscope slide (1 µl = one drop from the capillary tube) Then using a disposable graduated Beral-type pipette add 18 µl of the Water 1a sample to the stain on the microscope slide and cover with an 18 x 18 mm^2 cover slip

17. examine under a light microscope at 60X (for quantitative data) take a “picture” of the protozoa from Water 1a sample under the microscope using the digital blue microscope and count how many protozoa are in the one picture

18. use the following equation to determine the population density of protozoa in the soil sample Water 1a

19. [(# per field of view at 60X) × (total ml of water used) × 2165] ÷ (grams of sifted soil) = # of protozoa per gram of soil

20. Record number of protozoa for soil sample Water 1a

21. repeat steps 16-20 testing with all of the other soil samples

22. Take .625 grams of ammonium carbonate and add 1 liter of water mixed in with it

23. shake the solution to mix it up

24. repeat 23-24 so that there are a total of 3 bottles that are filled with 1 liter of water and 3 other 1 liter bottles with the ammonium solution

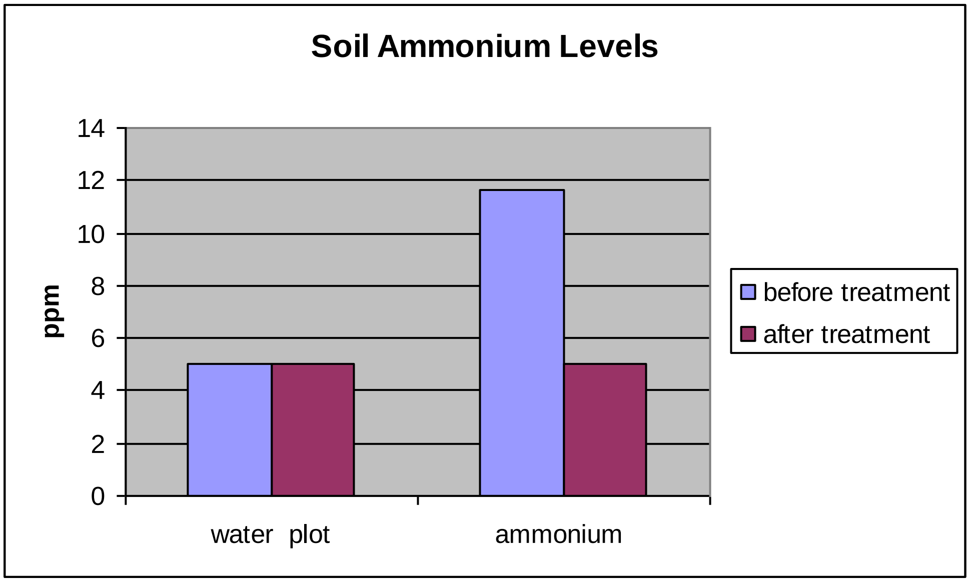
25. Take the 6 1 liter bottles filled 3 with water and 3 ammonium solution out to the plot area and add it to the corresponding plots. 1 liter of ammonium solution into each ammonium plot and 1 liter of water per plot labeled water
26. 2 days later get 3 15 cm by 2 cm in diameter soil samples from each plot repeating steps 6-21 using the new soil from the sample after the liquids have been added
27. record data and compare the number of protozoa for each

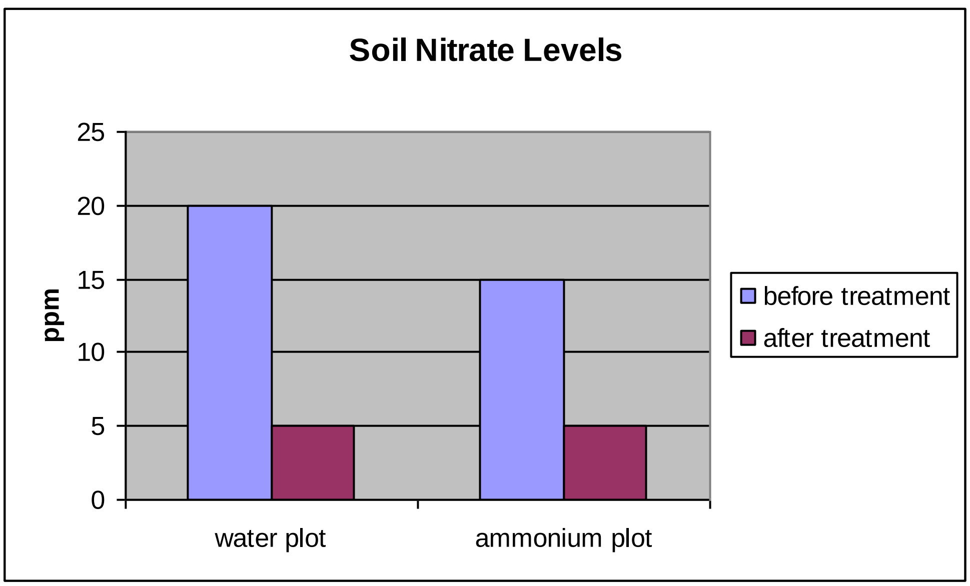
*Note due to lack of time we did not use the "b and c" soil samples in our data or analysis

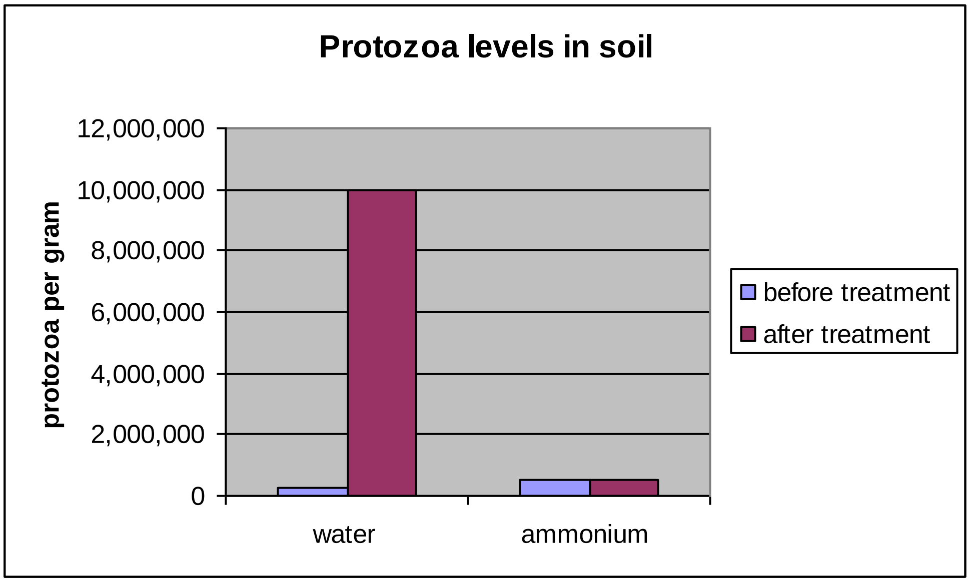
<u>Data Table for Ammonium, Nitrate and Protozoa Levels</u>

Condition of Plot Before	**Ammonium Levels Before (ppm)**	**Ammonium Levels After (ppm)**	**Nitrate Levels Before (ppm)**	**Nitrate Levels After (ppm)**	**Protozoa Levels Before (# of protozoa per gram**	**Protozoa Levels After (# of protozoa per gram)**
Water	1A: 5 2A: 5 3A: 5	1A: 5 2A: 5 3A: 5	1A: 30 2A: 20 3A: 10	1A: 5 2A: 5 3A: 5	1A:310,930 2A:116,397 3A:442,311	1A: 546,717 2A: 1,323,055 3A: 1,139,473
Average	5	5	20	5	289,879	10,003,081
Ammonium Carbons	1A: 25 2A: 5 3A: 5	1A: 5 2A: 5 3A: 5	1A: 5 2A: 30 3A: 10	1A: 5 2A: 5 3A: 5	1A: 941,304 2A: 349,193 3A: 273,473	1A: 79,763 2A: 356,868 3A: 1,176,630
Average	11.667	5	15	5	521,323	537,753

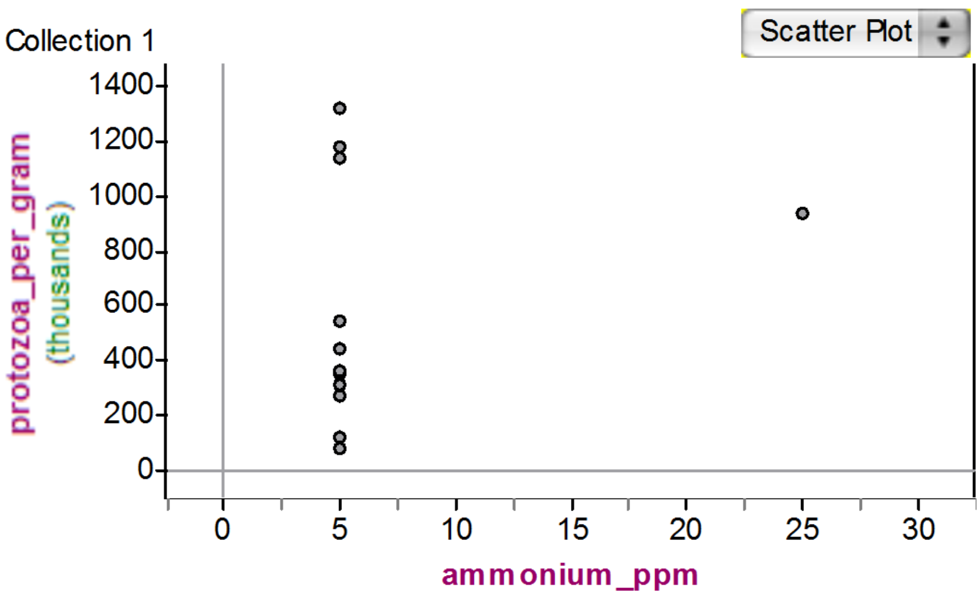
Ammonium, Nitrate and Protozoa Before and After Graphs



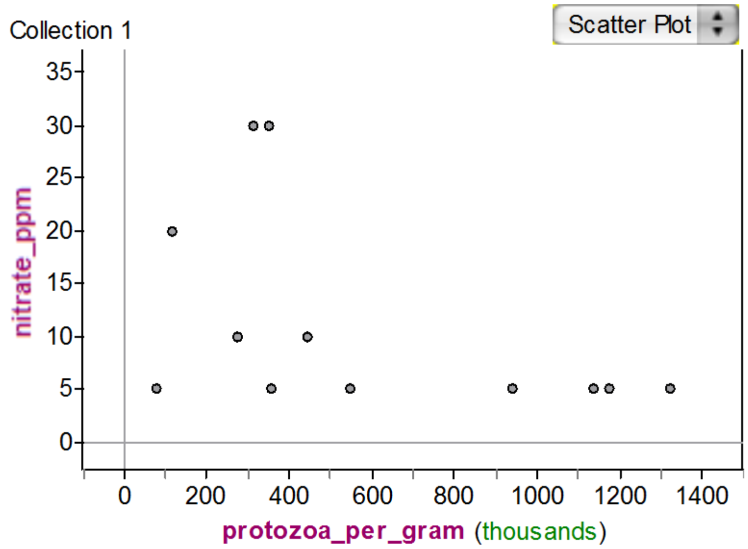




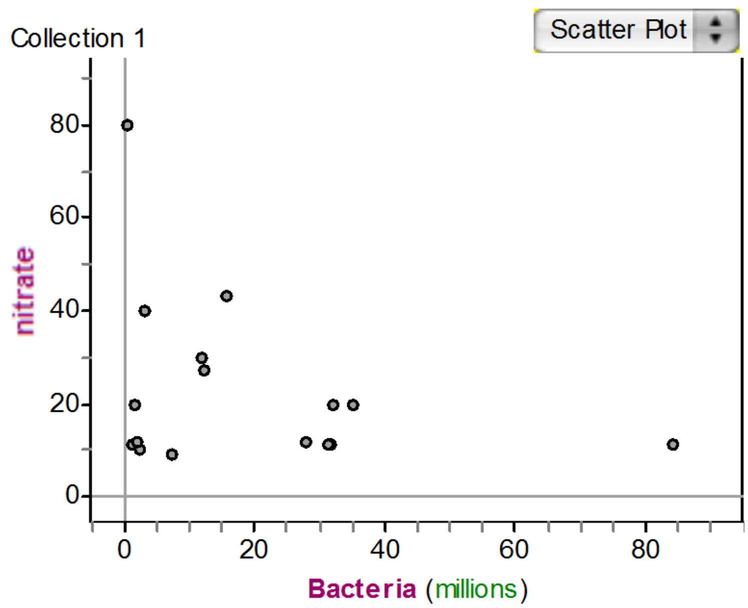
Relationship between protozoa and ammonium in the soil



Relationship between protozoa and nitrate in the soil



Relationship between nitrate and bacteria from another group's data



*Note ppm stands for parts per million

Conclusion

To conclude this experiment, our hypothesis is incorrect. Our hypothesis states: The ammonium in fertilizer could be decreasing the amounts of protozoa in the soil, resulting in an upset in the balance of protozoa to bacteria. We tested if the amount of ammonium and nitrate in the fertilizer is what caused the protozoa levels to be off balance. Our ammonium and nitrate tests show that the levels of protozoa after the ammonium was put onto the soil were lower than they were when we first took the samples. So, this proves that our hypothesis was incorrect because one of our scatter plots shows that it the ammonium that had no affect on the levels of protozoa in the soil. However, we proved that we were able to change the ammonium levels in the soil because they decreased after the addition of excess ammonium. In fact, they decreased like we predicted, we were just incorrect in the reasoning for the decrease. Then when analyzing we looked at our nitrate levels to see if they could be the reason for the drop of protozoa levels. However, the soil nitrate levels also decreased in both plots after the addition of any liquid, which shows that the nitrate does not affect the protozoa either. After this analysis the reason for the lack of protozoa after the addition of ammonium is still unknown.

We then studied the other group's data and saw that phosphate did not have an impact on either protozoa or bacteria. So we now know that it was not the nitrogen, ammonium, phosphate or bacteria that changed the protozoa count. However, we did discover that the key to finding out why the protozoa levels are low was in the relationship between protozoa, bacteria and nitrogen. We also found that another group's bacteria count was unusual because as the amount of bacteria went up the levels of nitrogen went down (see graph labeled "Relationship between nitrate and bacteria from another group's data"). Then we discovered that what these factors have in common is plants. We also know that on our campus we have foreign grass and we think that it could be the reason for the strange bacteria levels and therefore since they are connected, the protozoa levels. Hence, to further explain why our protozoa levels went down we need to test how different plants affect the protozoa count. It is possible that the foreign grass or the type of plants around the plots could have a strong impact on the protozoa. To further investigate why our protozoa and bacteria levels were so unusual we would have to perform tests using different types of plants and native and non-native samples from our campus. Overall in our experiment even though our hypothesis was incorrect with further testing of our problem we could possibly find the solution.

We have acted honorably,