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Soil Ecology

It has been found that the number of protozoa level in Roland Park Country School's soil is quite low (D. Brock, Personal communication, May 29, 2003). What factors could cause this problem? We found it important that someone figure it out; so for "The Little Things that Run the World" project, we felt the need to research on whether the amount of fertilizer used on our campus is the source of this problem. It is known that our school uses a lot of fertilizer on our grass in order to make it look good, which could cause the protozoa level to decrease as a result. We would like to find out if the pampering of the grass causes the killing of the protozoa.

Protozoa are one of the many types of microbes, tiny organisms that cannot be seen by the naked eye. They come in many different varieties and live as individuals or group together in colonies. Protozoa are single-celled animals that are the length of .005 to .16 millimeters from the kingdom protests (American Society for Microbiology 1999).

Protozoa have a large population in soil, consisting of about 250 different species. They are particularly ample in well-drained surface soils, and often found near their prey, bacteria. Soil-dwelling protozoa live in almost every type of soil and environment. They can be found in great quantity near the soil surface in the upper fifteen centimeters of the soil (American Society for Microbiology 1999). There are ten billion protozoa estimated to live in the top twelve centimeters of a square meter of soil. In those centimeters are four different types of protozoa including, flagella (whip) protozoa, cilia (small hair) protozoa, amoeboid (changing form) protozoa, testa (shell) protozoa, and nardi (no shell) protozoa. The amoebae and testacea protozoa are commonly found in forest soil, and the amoebae that have pseudopods "false feet" live in wet soils. Protozoa are at risk and dry out when the soil doesn't receive enough moisture. This causes the protozoa to change form and become inactive (also known as a cyst). The protozoa can become activated again when the soil gets moisture.

If levels of protozoa in soil are altered, then there will not be as many protozoa to decompose organisms, and there could be a problem with the regulation of unicellular algae, bacteria, and micro fungi. The protozoa are supposed to eat the bacteria to help stop the spread of disease, and if there are too many bacteria in the soil, it can pose a problem for the plants. If protozoa levels are altered and are too high or low it causes the ecosystem to be unable to maintain a healthy environment. This shows that protozoa are essential to the environment and ecosystem.

Protozoa are very important in soil. One function is decomposing organisms. They also serve as a food source for micro invertebrates. Protozoa regulate the amount of unicellular algae, bacteria, and micro fungi by consuming them as a food source. Another purpose of protozoa is to transfer bacterial and algal production to successive trophic levels, or a continuous feeding habitat. With a low amount of protozoa not all of these tasks will be preformed as needed for the soil to maintain a healthy environment. Without healthy soil, plants do not grow to their full potential, which leads to a low amount of food for herbivores. Therefore, they have little food and the amount of herbivores decreases, causing the amount of food for carnivores to go down. Though, it is not as dramatic at RPCS, the quantity and health of the plants will decline leaving us with a vast wasteland.

Protozoa exist in the detritus food chain where they decompose organic waste and eat bacteria, dead plants, and dead animals. Protozoa consume bacteria, which consume organic matter. The protozoa are eaten by mites, which are then eaten by beetles and ants. Protozoa are “herbivores and “consumers in the decomposer link of the food chain” (George Washington University, 2001).

The following nutrient cycles are continuous movements of important nutrients relevant to the existence of protozoa. The cycles do not stop and the nutrient is always moving through all parts of the cycle. The carbon cycle is a gas cycle. The part of the carbon cycle which protozoa are involved is as a decomposer, which while decomposing the organic or dead matter converts organic molecules into carbon dioxide. They attack, digest, and decompose organic materials converting it to simpler and energy poor compounds. After the decomposers convert the molecules, it is received by the atmosphere through respiration, then plants store it by incorporating it into plant material then animals eat it and it is in waste, then the protozoa and other microorganisms decompose and the process starts over again. Carbon enters the soil from “plant sources” which is then immobilized by microbes such as protozoa then released. Their role in the carbon cycle is to “breakdown residue, immobilize nutrients” in the soil, and “create new organic compounds” (University of Minnesota, 2003) by decomposing organisms. Carbon may affect the protozoa level (University of California, 1990).

The water cycle is very important to protozoa because they become active and are able to swim around and eat the bacteria when the soil is moist and contains water. This allows the protozoa to have more food allowing them to become more numerous and as a result, they help the soil become healthier.

Protozoa release nitrogen to the plant. The nitrogen cycle is the path that nitrogen follows in the ecosystem in order to allow organisms to get the nitrogen to make proteins and nucleic acids. Nitrogen is an element made up of amino acids, which form proteins and nucleotides, which form nucleic acids. Most organisms in this world cannot use nitrogen gas directly so the nitrogen cycle is needed to make it possible for the organisms to get the correct amount nitrogen, which is needed for them to live. The organisms that are not able to use nitrogen gas directly use nitrogen compounds, which are usually in two inorganic forms for plants, ammonia and nitrate. With the nitrate and ammonia, the plants are able to make proteins and nucleic acids, an ability that animals do not have. Animals have to receive nitrogen in an organic form, by swallowing or eating plants or other animals. In order for the plants to get the nitrogen in the first place, the organism's remains and wastes are decomposed by decomposers, which break down the complex organic compounds. During this process, the nitrogen that is in the complex organic compounds is released as ammonia. Some of the ammonia is taken up by plants, but the rest of it is "converted by nitrifying bacteria to nitrite and finally back to nitrate."(Scharaer, Stoltze, 1993). The plants then receive nitrogen in the nitrate form. Not all of the nitrate is used and taken up by plants and animals. The rest of the nitrate and nitrite is converted by denitrifying bacteria into nitrogen gas, which is released into the atmosphere. Plants and animals cannot use nitrogen gas, but if they are in need of nitrogen, some kinds of bacteria are able to change the nitrogen gas into ammonia using the nitrogen fixation process. This is how the nitrogen is used and reused in a cycle in order to make it available for all organisms and the atmosphere in order to allow things to live.

The nitrogen cycle is put into effect when the protozoa along with bacteria, fungi, earthworms, and other soil organisms, eat organic residues and turn them into soil organic matter. These soil organisms, including protozoa, eat the organic residue for two reasons: "Growth which requires both carbon and nitrogen to build proteins and genetic material; and generating energy, which requires carbon (usually carbohydrates) but not nitrogen. When fed nitrogen-rich residues such as dung, the soil life burns some nitrogenous compounds [DNA and proteins] for energy, releasing the nitrogen in inorganic form, primarily ammonium" (Mark Schonbeck, 2003). This process is known as mineralization and it permits nitrogen to be available to plant roots. If the plant root is given nitrogen-poor materials (i.e. straw), "the soil life must take ammonium and/or nitrate from the soil to satisfy its nitrogen requirements" (Mark Schonbeck, 2003). This problem is called immobilization, also known as nitrogen tie-up. Plants need nitrogen to grow better; therefore, the plants need more bacteria. The bacteria go to a certain spot, the plant. This gives the protozoa more food and it is easier for them to find their food because they will know that the bacteria are at the plant. Having more to eat allows the protozoa to grow faster, which will cause them to break apart quicker and more often. The breaking apart is the reproduction of the protozoa, therefore, like the water cycle, there will be more protozoa in the soil to make it healthier. Plants use nitrogen to make proteins, which are composed of amino acids; the nitrogen works with other nutrients, such as sulfur, carbon, and potassium to make amino acids that plants need to grow. Without nitrogen, plants are unable to produce amino acids to make proteins. The plants will die because without protein the cell cannot make enzymes. The plant needs enzymes to start and stop chemical reactions between the biochemicals, carbohydrates, lipids, water, proteins, and nucleic acids. Without the biochemicals the cell cannot perform certain tasks including, homeostasis, synthesis of chemicals, reproduction, and the manufacturing of chemicals, which will ultimately make the plant so weak that it will die.

One of the ways people try to prevent this from happening is to use fertilizer. Fertilizer provides plants with a combination of the nutrients plants need to grow, and its three main components are potassium, phosphorus, and nitrogen (Potash Corporation 2002). Potassium is a mineral that helps crops fight stress and diseases, phosphorus aids the plants health and helps to germinate root growth, and nitrogen provides the nutrients and proteins as discussed above (Potash Corporation 2002).

In addition to phosphorus, potassium, and nitrogen, fertilizer also includes different minerals and salts, and these salts have led to the depletion of microorganisms in the soil, such as protozoa (University of Guam 2003). Calcium and potassium are example of salts that significantly influences the availability of other nutrients in the soil. The calcium can influence the nitrogen and phosphorus's availability in the soil due to high salt concentrations. When salt concentrations are high in fertilizer, the salt in fertilizer is then secreted by protozoa and other microorganisms. The environment inside the protozoa becomes hypotonic. The protozoa try to even out the levels of salt by making the levels isotonic. Fertilizer does not allow protozoa and other microorganisms in the soil to make salt concentrations isotonic, causing the protozoa to eventually burst and lead to the depletion of protozoa. Another factor to the depletion of protozoa occurs when potassium and phosphorus levels are too high in the soil. This causes inadequate organic matter, nutrients like potassium, nitrogen and others to quickly move out of the root zone and become unavailable to the plants (George Washington University 2003).

When there is an inadequate amount of organic matter in the soil, the protozoa levels begins to decrease, because there is less food for them to eat. Microorganisms are what bring the nutrients back in the root zone so that the nutrients from fertilizer can become available to plants.

The typical use of fertilizer is to put in extra amounts of nutrients for plant growth, such as grass growth. To protect the levels of microorganisms, one should put the recommended amounts of fertilizer on the soil. People continuously spread fertilizer on their yards, farms, and land, thinking that the fertilizer will have a positive effect on their plants and soil when actually they are damaging the most important part in the soil, microorganisms. The fertilizers are reducing the levels of microbial population in the soil; this includes protozoa levels (George Washington University 2003). Without protozoa the nutrient cycles do not work correctly in the ecosystem.

As a result, our group isolated four plots on the RPCS soil, each plot being ½ meter by ½ meter large. In each plot, we added different amounts of fertilizer in order to test if it affected the amount of protozoa. We counted the protozoa in each sample. We also took samples of our soil and tested for nitrate nitrogen in order to find out if the fertilizer increased the level of nitrogen.

Experiment Outline

Problem: Does a higher concentration of fertilizer and therefore nitrate decrease the number of protozoa in RPCS' soil?

Hypothesis: Yes, the higher the amount of the fertilizer put on the soil on the RPCS campus the larger the decrease in amount of protozoa.

Experiment:

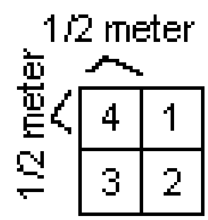
A. Variables

1. Independent Variable 1: Concentration of Fertilizer
2. Independent Variable 2: Amount of nitrogen in soil
3. Dependant Variable: Level of protozoa in soil

B. Controls

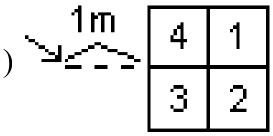
1. Negative Control 1: Plot not fertilized (only watered)
2. Negative Control 2: Samples Taken 1 meter from Plots
3. Controlled Variables:
   - amount of water in each solution
   - type of fertilizer
   - size of each plot
   - plot locations
   - depth of samples taken
   - type of water in solution
   - amount of time soil has to dry, sit, and extract
   - amount of time fertilizer solution to be absorbed into the soil
   - type of nitrate test
   - amount of soil in original sample taken from plot
   - size of screen used for first sifting
   - amount of time the saturated soil sits
   - nylex filter size and pore density
   - amount of methyl green stain put on slide
   - size of cover slip and slide
   - amount of soil sample put on slide
   - humidity
   - temperature
   - sunlight

C. Procedure



1. Go outside on the front lawn of RPCS.
2. Measure out 4 adjacent ½ meter by ½ meter square plots (see figure →)
   a. at N: 39.35809^{o}, W: 076.63599.
3. Put flags in the grass and write a number (1-4) to indicate which plot it is around the measured plots (see figure →).
4. Put 60 ml of tap water into a beaker, add 6.2 g of Turf Builder (24-2-11) fertilizer, then pour evenly (by holding the beaker at a 45^{o} angle and making a fluid motion over the plot) over plot 1.
5. Repeat step 4 using 3.1g of the same fertilizer and pour over plot 2 as above.
6. Repeat step 4 using 1.5g of the same fertilizer and pour over plot 3 as above.
7. Put 60 ml of tap water into a beaker; pour evenly (as in step 4) over plot 4.
8. Take each solution and evenly pour over each plot, accordingly.
9. Wait 48 hours and take 3 core samples 15 meters deep and 2 ½ cm wide from each of the four plots. To use the soil test core sampler dig in until the 15 cm line is at the surface then turn a full 360^{o} and pull out of ground.

10. Take the 12 soil samples and put in 12 labeled plastic Ziploc bags (with the corresponding plot and sample number).
11. Also after 48 hours take 4 core samples 15 meters deep and 2 ½ cm wide from 4 different places one meter away from each plot (see figure →)



12. Take each of the four samples and put in a labeled plastic Ziploc bag with 1-4 according to the plots closest to where the sample was taken (ex: from the figure, 3-4).
13. Follow Brokmeyer protocol to extract protozoa from each sample taken after 48 hours in the plots (not those taken a meter away) and count the protozoa. Use the equation on the protocol when counting at 40X. When counting at 100X use the following equation:

[(# per field view at 100X) · (total ml of water used) · (5102) ÷ (grams of sifted soil) = # of protozoa per gram of soil

14. Keep in mind the remaining soil (after step 2 of Brockmeyer procedure) needs to be used for chemical testing later so keep that dry for now.
15. Allow samples to dry to perform nitrate nitrogen testing.
16. Follow the La Motte test procedure for nitrate nitrogen for plots after fertilizing/48 hours and all four chemical tests done outside the plots..
17. Record the nitrate level as pounds per acre (convert to parts per million).

Concentration of Fertilizer	Plot. Sample	Amount of Protozoa After Fertilizing (per gram of soil)[1]	Nitrate Levels Before Fertilizing (ppm)[2]	Nitrate Levels After Fertilizing (ppm)
0.103	1.1	261450*	7.5	10
0.103	1.2	49800*	N/A[3]	15
0.103	1.3	153550	N/A	12.5
0.052	2.1	N/A	10	7.5
0.052	2.2	70550*	N/A	35
0.052	2.3	29050	N/A	30
0.025	3.1	717950*	2.5	7.5
0.025	3.2	99600*	N/A	17.5
0.025	3.3	99600	N/A	35
0	4.1	618350*	15	6
0	4.2	278050*	N/A	30
0	4.3	49800	N/A	30

Averages

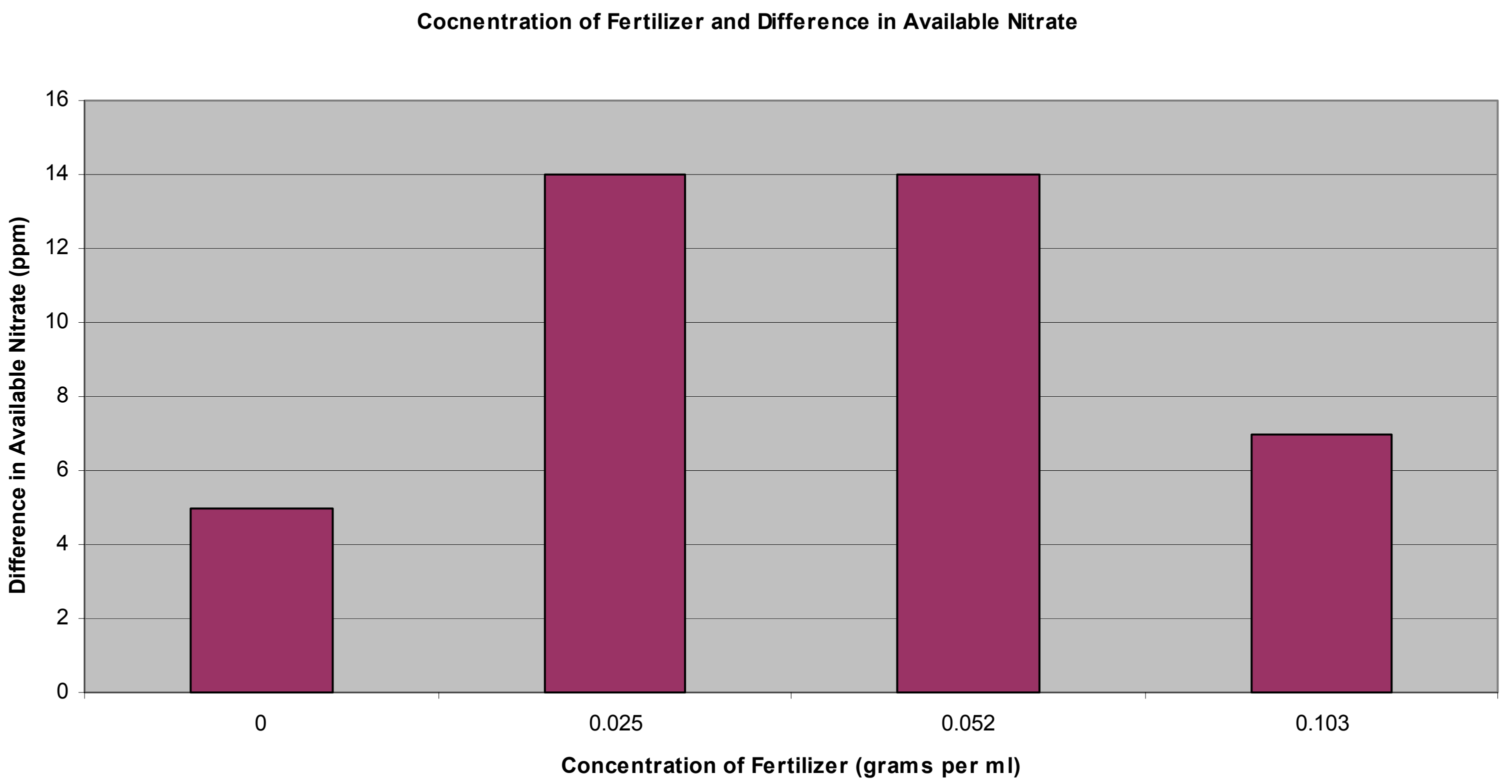
Concentration of Fertilizer (grams per ml)	Plot	Amount of Protozoa After	Nitrate Levels Before	Nitrate Levels After
0.103	1	154933.3333	7.5	12.5
0.052	2	49800	10	24.16666667
0.025	3	305716.6666	2.5	16.66666667
0	4	315400	15	22

[1] Any stared items mean that these samples in step 5 on the Brockmeyer procedure were done after 20 ½ hrs.
[2] In this column the samples with nitrate levels were taken a meter away from the plot.
[3] These samples were either contaminated or spilled.

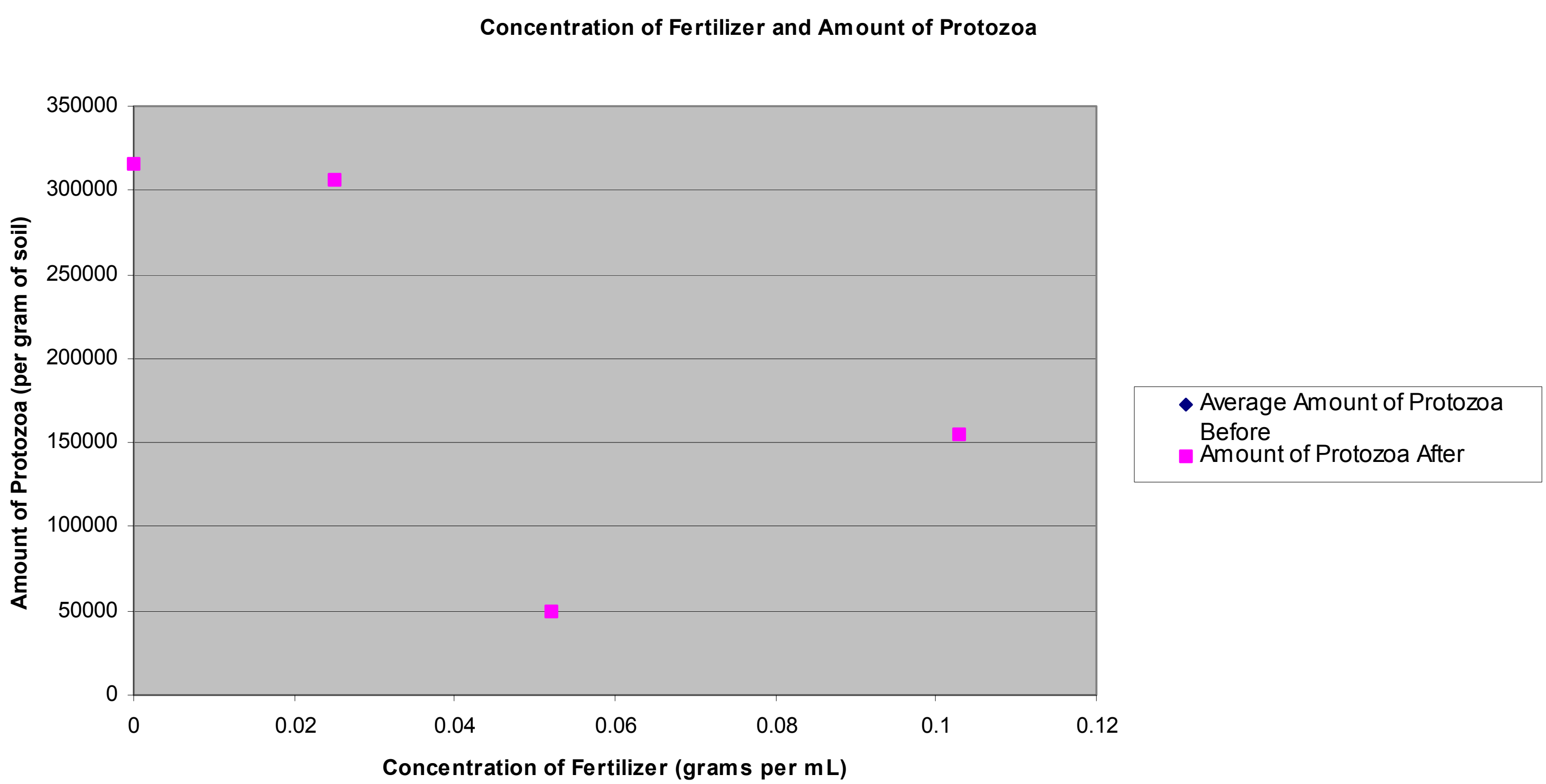
Concentration of Fertilizer	Amount of Protozoa After
0	315400
0.025	305717
0.052	49800
0.103	154933

Amount of Protozoa After Nitrate Increase			
Difference in Available Nitrate	5	14	7
Amount of Protozoa After	315400	177758	154933.3333

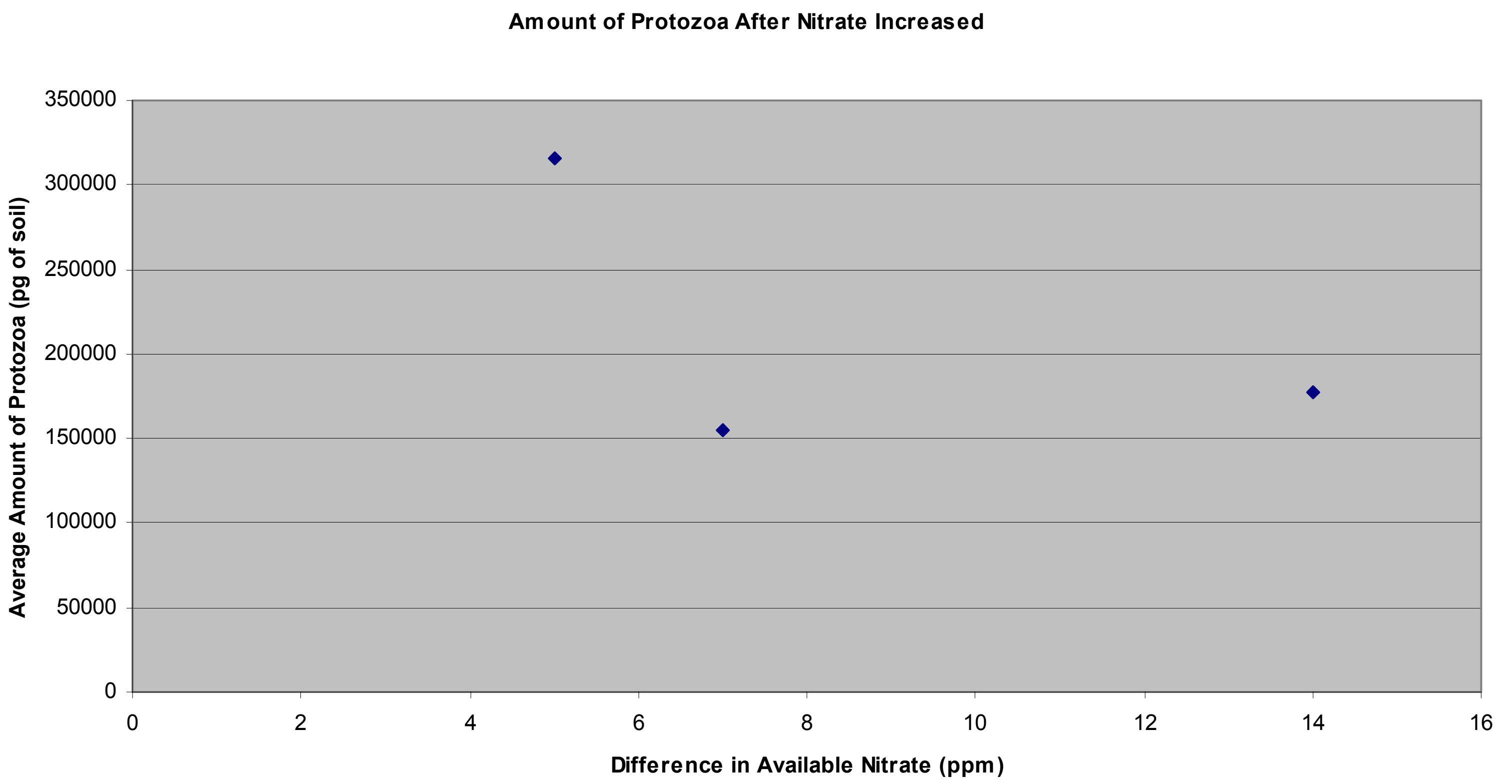
Concentration of Fertilizer and Difference in Available Nitrate				
Concentration of Fertilizer	0	0.025	0.052	0.103
Difference in Available Nitrate	5	14	14	7



Cocnentration of Fertilizer and Difference in Available Nitrate
Difference in Available Nitrate (ppm)
16
14
12
10
8
6
4
2
0
0
0.025
0.052
0.103
Concentration of Fertilizer (grams per ml)



Concentration of Fertilizer and Amount of Protozoa
Amount of Protozoa (per gram of soil)
350000
300000
250000
200000
150000
100000
50000
0
0
0.02
0.04
0.06
0.08
0.1
0.12
Concentration of Fertilizer (grams per mL)
Average Amount of Protozoa Before
Amount of Protozoa After



Conclusion

In conclusion to our experiment we have discovered that our hypothesis was incorrect. Our hypothesis was, the higher amount of fertilizer put on the RPCS campus the faster the level of the protozoa will decrease. We know that the fertilizer did not cause the depletion because in the plot we did not fertilize, the level had an average of 315,400 protozoa per gram of soil. In the amount of protozoa in the plot we fertilized, the recommended amount had an average of 49, 800 protozoa per gram of soil. We did find that the nitrate nitrogen levels increased. In the difference of nitrogen levels from plot 1, fertilized to the sample taken a meter away from plot 1, unfertilized was 5 parts per million, from plots 2 and 3, fertilized to samples taken a meter away from plots 2 and 3, unfertilized was 14 parts per million, and from plot 4, unfertilized to the sample taken one meter from plot 4, the difference of the nitrogen level was 7 parts per million. As the nitrogen level increased the protozoa level decreased. Other causes of our abnormal protozoa level may be, construction, temperature of the soil, or a large amount of predators to protozoa (i.e. mites).

During the process of our experiment, we encountered a couple errors, which altered our conclusion. These errors included: contamination of sample 1 unfertilized, spilled a sample (sample 2, plot 1, fertilized) and some liquid from other samples during the loss of electricity dilemma, and had a design flaw; we did not include a positive control in our experiment. We also had to take a sample for the chemical testing on unfertilized soil a meter away from the plots because we had already fertilized when we realized we needed to test for nitrate nitrogen in the soil. These errors could have made an impact/difference in our results because we were missing a sample and lacked testable material, which gave us less data to use to make our conclusions. For the all samples 2 and 3 we preformed step 5 of the Brockmeyer procedure after 20 ½ hours.

Resources

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