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## I. Background Information:

### Phosphate and Soil Bacteria vs. Phosphate and Soil Protozoa

In order to grow and thrive, plants need a variety of nutrients that are provided by fertilizer. For example nitrogen, phosphate, and potassium are all key ingredients in the life of a plant because they increase the health and growth of a plant. The first of these, nitrogen, is what makes lawns green and keeps them growing because it is involved in the protein and nucleic acid synthesis within a plant. Proteins and nucleic acids are vital because they are the necessary components to start and stop the chemical reactions that are used by cells to perform the four key tasks of reproduction, manufacturing of chemicals, regulation of the environment, and transforming energy (BBC, 2008 and Lisa Gardiner, 2007).

Potassium, another key nutrient, helps lawns fight "stress" and is responsible for the regulation of osmosis or water movement. It also helps form proteins and starches, as well as aiding in photosynthesis, fruit formation, and disease resistance **(**Canadian Organic Growers. Inc., 1992).

Finally phosphorus is vital for plants because it is one of the key elements in the transfer of energy (Gummerson, Boeke, Hubbard, and Ercolano, 2005) and in nucleic acid and ATP synthesis, which helps nourish a plant's roots (Oregon State University, 2007). ATP, for example, is required by organisms in order for them to function because phosphorus is needed in order for ATP to transfer its energy to cause chemical reactions (Luminultra, 2004-2008). Hence, without ATP, a plant cell can not engage in any of the cell's four tasks because ATP ultimately provides the energy needed to do so.

The nutrients found in fertilizer that are provided to the living organisms in the soil would be ineffective if it were not for the "biogeochemical cycles". These cycles are what provide nitrogen, phosphorus, and potassium compounds naturally. Phosphorus for example, can be delivered to the soil in the form of a phosphate rock, while in the nitrogen cycle, the nitrogen must be transferred into a usable form through the soil. Nitrogen "enters the food chain by means of nitrogen-fixing bacteria and algae in the soil" which means that nitrogen is available in a form that plants can take in because of the bacteria and algae living in the soil (Think Quest, n.d.). Nitrogen gas has to be converted into chemicals such as nitrate, ammonium salts, and urea through a process called nitrification (BBC, 2008), and it is this process that allows the plants to absorb nutrients from the soil (which is important because the nitrogen in the atmosphere is not in a form that the plants and animals can use).

Of all the nitrogen compounds, ammonia happens to be the key ingredient in nitrification. Once ammonia has been exposed to the soil it must be turned into nitrites. The nitrites are then turned into nitrates, which are eaten by the living organisms such as protozoa, bacteria, and plants. Indeed, the increased nitrate levels cause plants to grow rapidly, and the number of plant-eating animals will increase when the plant supply increases (Lisa Gardiner, 2007).

The third biogeochemical cycle related to the fertilizer is the potassium cycle. The potassium cycle affects the growth and health of plants just as nitrogen and phosphorus do. Potassium is made available to the plants through minerals but the availability of these minerals is scarce. Plants receive an exchangeable form of potassium through clay particles and minerals. These minerals, particularly feldspars and micas, release potassium as they are broken down into a

usable and exchangeable form. Although Potassium can be delivered through minerals already in the soil it can also be added to the soil from the rain (Balance, n.d.).

Humans try to supplement these biogeochemical cycles by applying fertilizer to the soil. Fertilizer is made from natural and synthetic materials, such as manure, nitrogen, phosphorus, and potassium compounds. The goal of fertilizer is to supply the nutrients above to living organisms in a form that has already been fixed for immediate use (How Stuff Works, Inc, 2008). Fertilizer is generally used to increase the soil's capacity to support plant growth, but can also be used to kill the weeds that inhabit the soil as well. The purpose of fertilizer is to provide necessary plant nutrients so that the plant can make its own food through photosynthesis. Choosing and using fertilizer properly will enhance plant growth and protect water quality depending on the levels of nitrogen, phosphorus, and potassium in it. In fact, on various fertilizer bags there is a three-number code. The first number is the percentage of nitrogen; the second number is the percentage of phosphorus found in the bag; and finally the third is the percentage of potassium (an example of this is "10-12-8" which is 10 parts available nitrogen, 12 parts available phosphorous, 8 parts available potassium). Hence fertilizers can provide a surplus of nutrients that help plant growth and health when the amount of nutrients provided naturally by biogeochemical cycles is too little (The Soil Science Society of America, 2005).

One compound found in fertilizer, however, that has been shown to be potentially harmful is phosphorus. Phosphorus has been shown to harm parts of the ecosystem such as the water quality and the organisms that live there. Bacteria thrive in moist areas of the soil but protozoa need that moisture to survive, and since protozoa play a role in its soil by either feeding off of bacteria, other protozoa and pieces of other plants (American Society for Microbiology, 2006), the run off

phosphorus into the soil could cause the conditions to be harmful to the entire soil ecosystem since it might unbalance this relationship between the bacteria and protozoa. Protozoa in particular increase the probability of the ecosystem running smoothly by regulating the population of bacteria in that ecosystem and releasing nitrogen as part of the nitrogen cycle to the plants. The fact that phosphorus could be harmful to the protozoa and bacteria levels can be tested by intentionally adding phosphorus to the soil.

The soil on Roland Park Country School's grounds is fertilized twice a year and it is our job to discover the affects these fertilizers are having on the microorganisms that reside in the soil. One can determine that the soil of Roland Park Country School should have levels of Bacteria in certain areas that increase on days when numbers of Protozoa are extremely low, but one should also find that on days when numbers of Protozoa have increased the amount of bacteria in that same area should decrease. Unfortunately what should be happening in our ecosystem is not currently happening as one would infer. The Bacteria levels are getting higher but the protozoa levels are staying at the same level when they should be decreasing but they are not. Also, when the Nitrogen levels are low the Bacteria levels change drastically by increasing and the protozoa levels by decreasing. So while, the nitrogen in the fertilizer may help protozoa and bacteria to exist in an area, the phosphorus could be causing an inconsistency in that data (The Environmental Science Summer Research Program for Young Women Microclimate Databases, 2007).

We believe that the phosphorus in the fertilizer is what is causing the number of Protozoa and Bacteria to vary incorrectly. Phosphorus is affecting the number of bacteria and protozoa because if we add more phosphorous to the soil, then the amount of protozoa will decrease in number

while the amount of bacteria will increase in number. To test this idea, we deliberating added phosphorus to the soil in order to see what changes in the bacteria and protozoa levels it would cause.

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## II. Lab Outline

I. Problem:

Does the amount of phosphorus found in fertilizer change the amount of protozoa and bacteria that live in the soil?

II. Hypothesis:

If we add more phosphorous to the soil, then the amount of protozoa will decrease in number while the amount of bacteria will increase in number.

III. Procedures:

A. Independent: ammonium phosphate added to the soil
B. Dependent: amount of protozoa/g and bacteria/$cm^3$ found in the soil
C. Negative Control: ammonium carbonate added to the soil
D. Controlled Variables:

- Number of Plots
- Number of samples from each plot
- Size of the sample taken from the ground
- Location of soil
- Number of plot flags
- Location of plot
- Number of Centimeters between each plot
- Date of before samples taken
- Date of after samples taken
- Type of light given to the soil
- Amount of light for soil
- Amount of soil tested
- Number of ziplock bags
- Number of petri dishes
- Type of petrifilms
- Amount of sterile water
- Amount of distilled water
- Type of pipette
- GPS
- Number of grams when sifting the soil
- Number of 15mL culture tubes with caps
- Size of culture tubes with caps

- Type Nylon mesh
- Number of Nylon mesh for each sample
- Number of Nytex Mesh for each Uhlig Extractor
- Type of balance
- Amount of ammonium phosphate
- Amount of ammonium carbonate
- Number of Nalgene bottles
- Amount of water added to the ammonium phosphate
- Amount of water added to the ammonium carbonate
- Type of micro-pipette
- 1-cc scope
- 10mL serological pipette
- Number of phosphorous tablets
- Number of phosphorous drops
- Location of soil
- Number of trials
- Size of Dixie cup
- The Lamotte Chemical Test Kit
- Temperature in Refrigerator
- Time
- Number of Modified Uhlig Extractors
- Number of Glass Funnels and Ring Stands
- Magnification 60X
- Amount of Methyl Green Staining Solution
- Amount of 2nd filtrate

E. Step-by-Step Procedure:

***For each of the following experiments I will do the test as if I were experimenting on Plot 1A but each of these test must be done with all of the soil A samples from each of the six plots.**

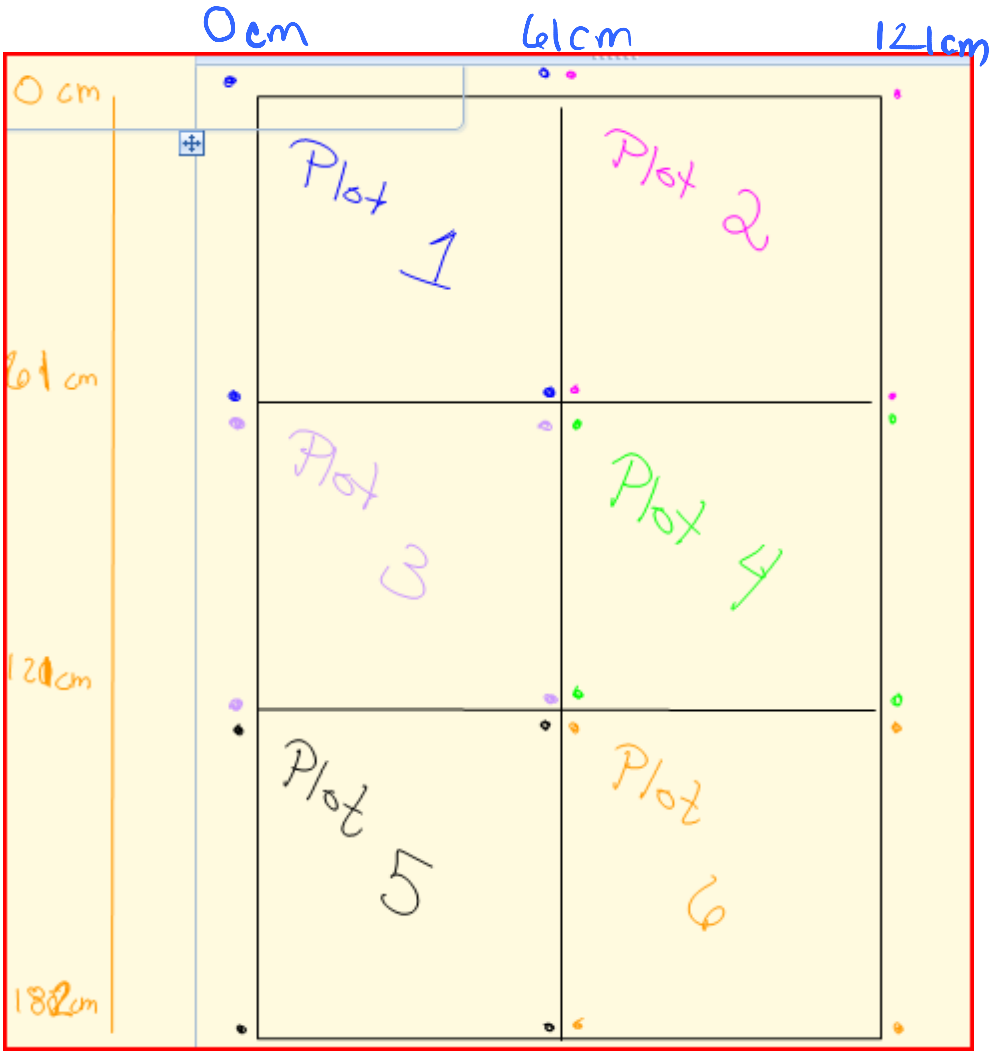
**Sample Collecting:**

1) Gather materials
2) Grab 4 flags
    a. Label each of the four flags with the following information
        i. Your initials
        ii. The Date that it is
        iii. The plot number
3) Grab 3 plastic ziplock sandwich bags
    a. Label each of the bags with the following information
        i. Your initials
        ii. The Date that it is
        iii. The plot number
        iv. The plot name
4) Repeat steps two and three 5 more times

*At the end of step 4 you should have a total of 24 flags and 18 ziplock bags ready to take outside

5) Go outside and create a 121 cm by 182 cm box
    a. Each plot should be 61 cm by 61 cm big

*HERE IS A DIARGRAM OF WHAT THE PLOT SHOULD LOOK LIKE




6) Take 3 samples of soil from each plot; each sample of soil must be 15 cm by 2cm- ***Make sure to collect all samples on the same date and time!!**
7) Put each of the samples into their own separate ziplock bag and label the bags with the corresponding plot number and letter as well the date, initials, and whether the soil was taken before or after the chemicals were added to the plots—this will allow you to transport the soil to the lab

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**Serial Dilutions: These tests have to be done at the same time as the protozoa extraction, starting at step 8 of that procedure, and phosphorous test**

1) Take a 15 ml culture tube and add 10 ml of sterile water to it
    a. Do so by using a transfer pipette
    b. Label the 15 ml culture tube "$10^{0}$" Plot 1A Before
2) Take a second 15 ml culture tube and add 9 ml of sterile water to it
    a. Do so by using the same pipette used in step 1
    b. Label this 15 ml culture tube "$10^{-1}$" Plot 1A Before
3) Repeat step 2 a total of two more times to two new additional 15 ml culture tubes
    a. Label them
        i. "$10^{-2}$" Plot 1A Before
        ii. "$10^{-3}$" Plot 1A Before
4) Take a 1cc soil scope to place 1cc of sample Plot 1A before into the $10^{0}$ Plot 1A before tube
5) Put a lid on the transformation tube and shake vigorously
6) Remove 1 ml of the soil/water mixture
    a. This should be removed from the "$10^{0}$" Plot 1A before tube and poured into the "$10^{-1}$" Plot 1A before tube
    b. Do this by using a new pipette
7) Put a lid on the tube and shake vigorously
8) Remove 1 ml of the soil/water mixture
    a. This should be removed from the "$10^{-1}$" Plot 1A before and pour it into the "$10^{-2}$" Plot 1A before tube
9) Put a lid on the tube and shake vigorously
10) Remove 1 ml of the soil/water mixture
    a. This should be removed from "$10^{-2}$" Plot 1A before tube and pour it into the "$10^{-3}$" Plot 1A before tube
11) Put a lid on the tube and shake vigorously
12) Now you should have 4 culture tubes
13) Take test tube $10^{-2}$ Plot 1A before and shake it vigorously
    a. Take a P200 micro-pipette and place a tip on it
    b. Open the cap to the test tube and extract 100µl from the test tube
    c. Open the labeled petrifilm
        i. Label it with your initial, the date, $10^{-2}$, the plot number and letter for example CP//5-7-08 $10^{-2}$ Plot 1A Before
    d. Eject the 100µl from the tip of the P200 micro-pipette onto the petrifilm
    e. Close the petrifilm and take a petrifilm spreader and spread the petrifilm spreader over the petrifilm
    f. Take the P200 micro-pipette and eject the tip into a beaker filled with sterile water
14) Take test tube $10^{-3}$ Plot 1A before and shake it vigorously
    a. Take a P200 micro-pipette and place a tip on it
    b. Open the cap to the test tube and extract 100µl from the test tube
    c. Open the labeled petrifilm
        i. Label it with your initial, the date, $10^{-3}$, the plot number and letter

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   d. Eject the 100µl from the tip of the P200 micro-pipette onto the petrifilm
   e. Close the petrifilm and take a petrifilm spreader and spread the petri film spreader over the petrifilm
   f. Take the P200 micro-pipette and eject the tip into a beaker filled with sterile water
15) Do steps 1-14 for each of the six plot labeled sample A's before
16) Allow all of them to grow for 48 hours
17) Do steps 18-19 sample A before of each plot
18) Take Plot 1A's before petrifilm $10^{-3}$
   a. Examine the plate
   b. If this plate has at least 5 colonies you will record how bacteria you found on the petrifilm and plug the number of the equation in step 19
   c. If this plate does not have at 5 colonies look at Plot 1A's petrifilm $10^{-2}$ before
   d. Record how many bacteria you found on this plate. Plug the number of bacteria you found into the equation in step 19
19) The formula is: # of Microbes in 1 cc of soil= # of colonies on sheet $(10^{2})(10^{|\text{dilution \# at which these colonies were found}|})$

**Protozoa Extraction:**

1) Go to the lab room and Gather Materials
2) Take a Petri dish and label it with the following
   a. Should include the following:
      i. Your initials
      ii. The Date the sample was taken
      iii. The Plot number and name
   - Example: C.P.// 5-07-08// Plot 1A
3) Do step two 17 more times.
   a. By the end of labeling you should have a total of 18 petri dishes each one's top and bottom should be labeled.
4) Take the petri dishes and match the soil bags' labels with the petri dishes' labels.
   a. Open the ziplock bag and pour the 15 cm of soil into the open petri dish
   b. Do this until each bag is emptied into its own petri dish
   c. Once the soil is in the petri dish allow the soil to dry completely
5) Take the 18 petri dishes filled with soil and place them on a tray.
   a. On the tray they will air dry for a minimum of 24 hours
   b. Once placed in the tray the petri dishes should not have the top on them
6) Take a Dixie cup and pour Plot 1A sample of dry soil into it and place a 1 $mm^2$ nylon mesh over top of the cup. Each time you do this step with the remaining samples make sure that you get a new Dixie cup and a new nylon mesh.
7) Sift 9-10 grams of Plot1A's soil into a new petri dish.
   a. Make sure you label each petri dish with the date, your initials, the plot number, and sample type

   *Example: C.P.//5-07-08//Plot 1A

   b. Make sure you record and observe how many grams are going into the new petri dish
8) Take Plot1A's sample of soil and fill the petri dish with 20 ml of distilled water
9) Cover the petri dish and set the sample aside and wait for 7 hours before you start the next step in the experiment
10) Make a modified Uhlig extractor by taking a plastic cup with the bottom cut out and place a white mesh over top the other plastic cup
11) Take a graduated cylinder and measure 30 mL of distilled water
12) Pour the 30 mL of distilled water in a new petri dish labeled Plot 1A before
    a. Make sure the petri dish is labeled with your initials, the date, and Plot 1A, label the rest of the petri dishes with the corresponding plot name and sample
13) Place the Uhlig into the petri dish filled with 30 mL of distilled water
14) Pour the soil from Plot 1A before found in the petri dish in step 9 into the Uhlig extractor; do the same for they remaining five sample A plots
15) Wait for 24 hours until you can start the next part of the experiment which is filtering the soil a second time
16) Get 6 Dixie cups and label each of them with the Plot Number and Sample Type
17) Set up 6 ring stands
18) Place a glass funnel inside of each of the ring stands
19) Fold a 12.5 cm qualitative filter paper in half and then in half again; repeat 5 more times
20) Put the filter papers into the funnels and they should be in a shape of a cone
21) Take the 6 labeled Dixie cups from step 16 and put one of them under each of the funnels
22) Now pour the before soil of Plot1A into the funnel that has the Dixie cup labeled Plot1A; repeat this steps 5 times with corresponding petri dish and Dixie cup label for each of the Plot A before petri dishes
23) Let the soil filter through to the properly labeled Dixie cup until there is no more liquid coming out of the funnel
    a. It will take anywhere between 10-30 minutes
24) Once all the liquid is in the Dixie cups label with the correct before plot sample and number you need to create a microscope slide
25) Grab a slide and dip a capalary tube into the methyl green staining solution
    a. Scrape off any excess dye and close the dye after use
26) Tap 7 drops of the methyl green staining solution onto the microscope slide
27) Use the Beral-type pipette and fill it with the protozoa extraction in the Dixie cup labeled plot 1A before to the first indentation; repeat this step for the remaining 5 protozoa extractions
28) Place a cover slip on top
29) Put the slide for Plot 1A before underneath the microscope that is connected to the computer screen; do the same for the other 5 protozoa extractions
30) Focus on the slide until the number of protozoa are visible; the little blue dots on the screen are the protozoa

31) When the microscope is in focus take a snap shot
32) Click on the export button and save under a plot number and letter of the protozoa extraction such as Plot 1A before; this step should be done for all 6 protozoa before extractions
33) Examine the slide for protozoa
34) Use the following formula to calculate the number of protozoa
    a. [(# per field of view at 60X)(total ml of water used)(2165)] / (grams of sifted soil)= # of protozoa per gram of soil
35) Repeat steps 22-34 for all six plots; specifically Plot 2A, Plot 3A, Plot 4A, Plot 5A, and Plot 6A.

**Extraction Procedure and Phosphorous Test: These tests have to be done at the same time as the protozoa extraction, and serial dilutions**

1) Use the Lamotte Combination Soil model STH-14 Code 5010-01 to perform the extraction procedure and the phosphorous test on Plot 1A, 2A, 3A, 4A, 5A, and 6A bfore soil.

**Second Part of the Experiment:**

**Preparing the solution for the soil:**

1) In our case we will need to put 5.8 grams of ammonium phosphate in a nalgene bottle with 1 Liter of water in it.
2) Cap the nalgene bottle and shake it until the ammonium phosphate has disappeared
3) Do steps 1 and 2 two more times. Each time use a new nalgene bottle.
4) Put 5.8 grams of ammonium carbonate in a new nalgene bottle with 1 Liter of water in it.
5) Cap the nalgene bottle and shake it until the ammonium carbonate has disappeared
6) Do steps 4 and 5 two more times. Each time use a new nalgene bottle.
7) After this you should have a total of 6 nalgene bottles.
    a. 3 labeled ammonium phosphate
    b. 3 labeled ammonium carbonate
8) Take the 6 bottles and go outside to your 6 plots
    a. Place each nalgene bottle in one of the 6 plots
        i. No plot should have more than one nalgene bottle in it
        ii. Place one of the nalgene bottles labeled ammonium carbonate in plot 1, another in plot 3, and the last in plot 5
        iii. Place one of the nalgene bottles labeled ammonium phosphate in plot 2, another in plot 4, and the last in plot 6
9) Pick up each nalgene bottle and pour it around the entire plot.
    a. Make sure you do not pour any of the ammonium carbonate in the plots listed for ammonium phosphate and vice versa.
10) Wait 2 days.

**Sample Collecting:**

1) On the third day:
2) Grab 3 plastic ziplock sandwich bags

a. Label each of the bags with the following information
   i. Your initials
   ii. The Date that it is
   iii. The plot number
   iv. The plot name

3) Repeat step two 5 more times
   a. *At the end of step 3 you should have a total of 18 ziplock bags ready to take outside
4) Take 3 samples of soil from each plot-- **Make sure to collect all samples on the same date and time!!**
5) put each of the samples into their own separate ziplock bag—this will allow you to transport the soil to the lab

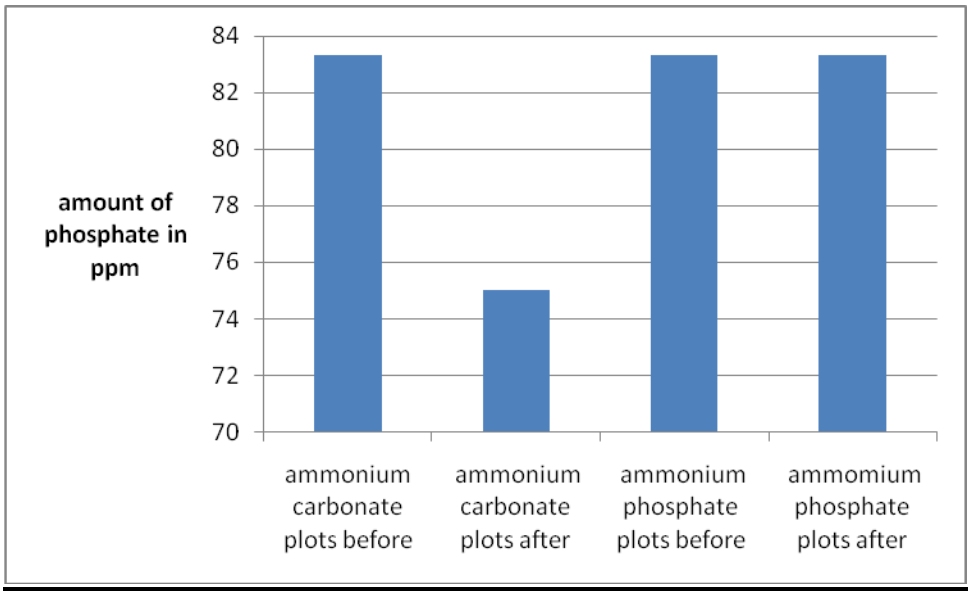
*For Plot 1A, 2A, 3A, 4A, 5A, and 6A After samples repeat steps 1-19 of the serial dilutions on the pages above, repeat steps 1-35 for the protozoa extraction process above, and repeat all steps of the phosphorus test for the after soil as well.

## III. Data

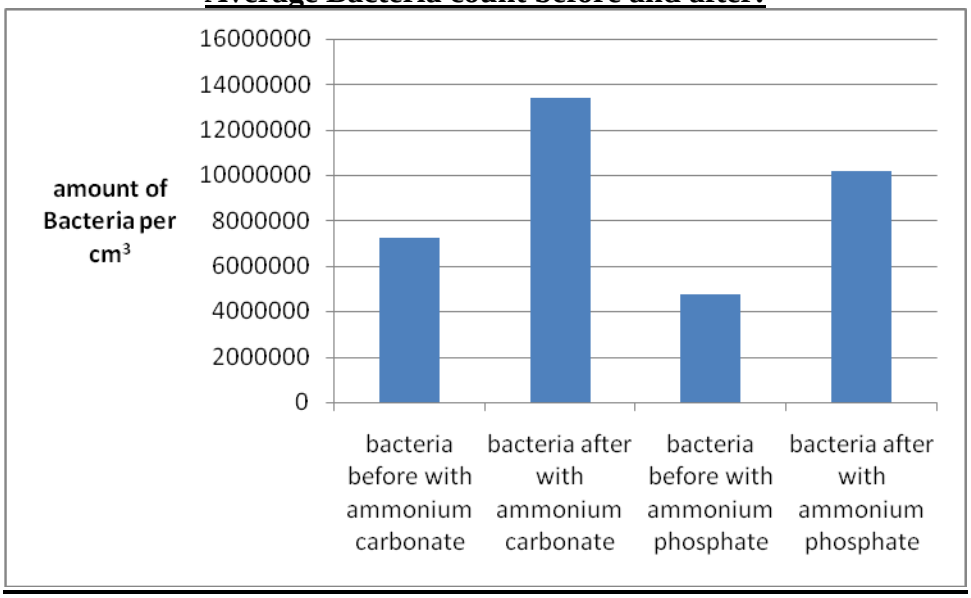
**Impact of Phosphate on soil bacteria and Phosphate:**

| Condition of Plot | Trial Number | Before Phosphate (ppm) | After phosphate (ppm) | Before bacteria ($cm^3$) | After bacteria ($cm^3$) | Before Protozoa (grams) | After Protozoa (grams) |
|---|---|---|---|---|---|---|---|
| Ammonium Carbonate | 1A | 75 | 100 | 1,200,000 | 9,700,000 | 126,675 | 139,677 |
| | 3A | 75 | 75 | 19,700,000 | 4,500,000 | 180,416 | 102,552 |
| | 5A | 100 | 50 | 800,000 | 26,000,000 | 144,333 | 111,597 |
| | Average | 83.3 | 75 | 7,233,333 | 13,400,000 | 150,474 | 117,942 |
| Ammonium Phosphate | 2A | 100 | 50 | 3,600,000 | 3,600,000 | 187,780 | 202,225 |
| | 4A | 75 | 100 | 10,100,000 | 5,600,000 | 205,105 | 211,793 |
| | 6A | 75 | 100 | 500,000 | 21,400,000 | 453,951 | 153,080 |
| | Average | 83.3 | 83.3 | 4,733,333 | 10,200,000 | 282,278 | 189,032 |

**Average Phosphate Levels before and After:**




**Average Bacteria count before and after:**




**Average Protozoa count before and after**

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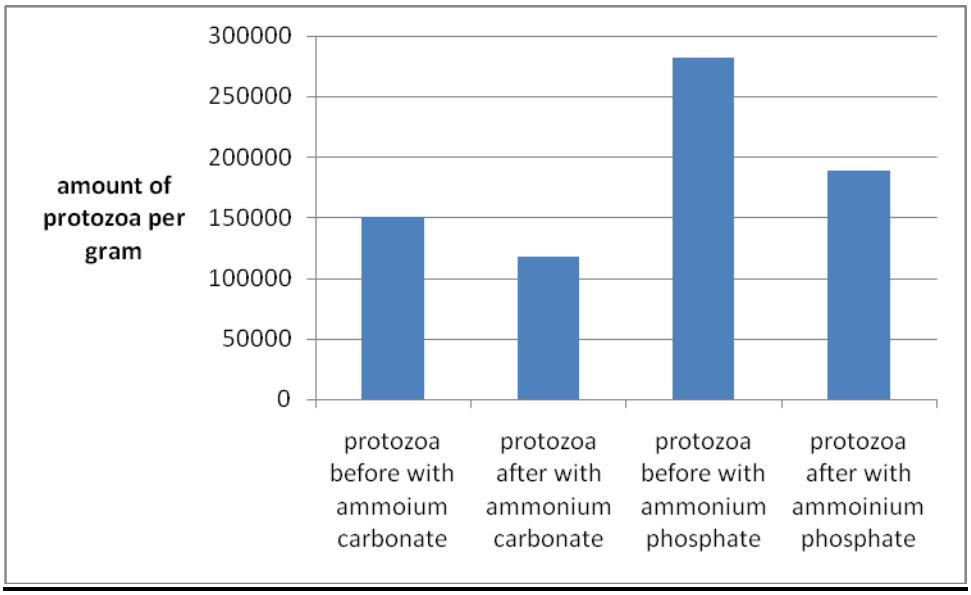
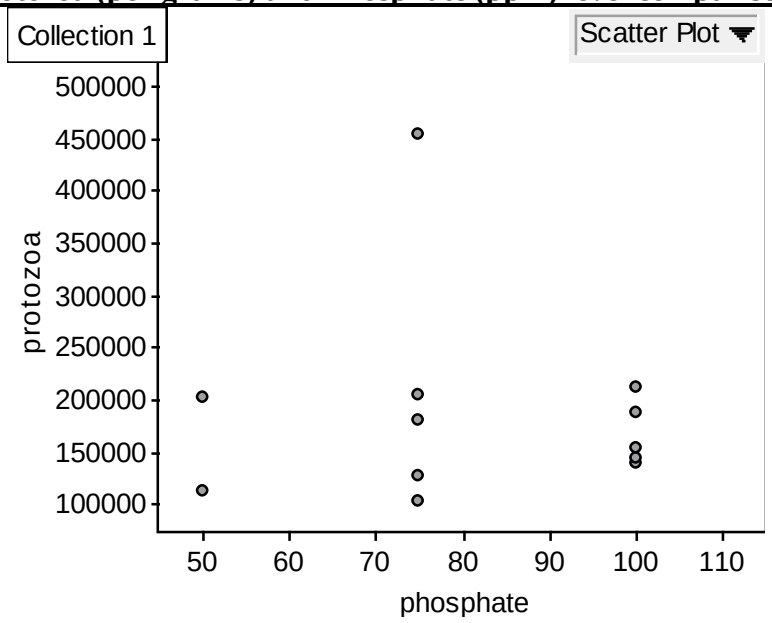
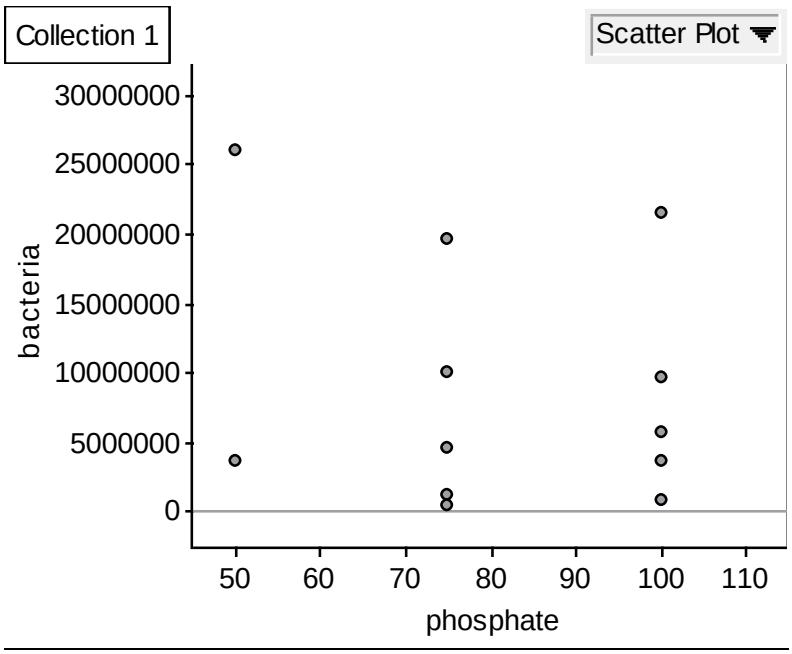


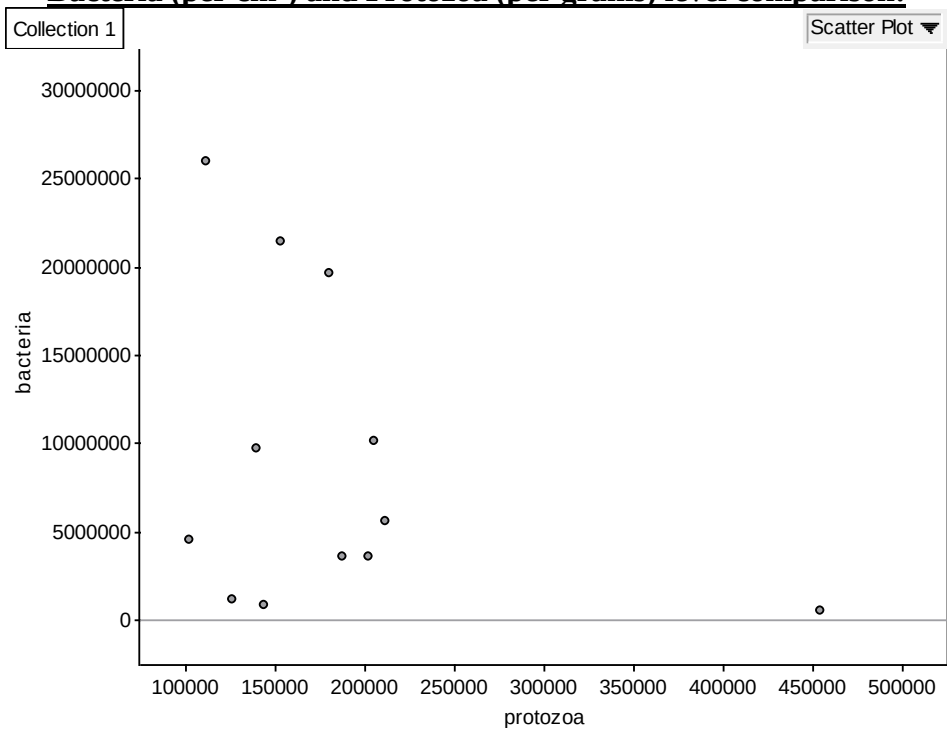

**Protozoa (per grams) and Phosphate (ppm) level comparison:**




**Bacteria (per $cm^3$) and Phosphate (ppm) level comparison:**




**Bacteria (per $cm^3$) and Protozoa (per grams) level comparison:**




## IV. Conclusion

Unfortunately our hypothesis was incorrect. We thought that if we added more phosphorus to the soil in the form of ammonium phosphate and ammonium carbonate, then the amount of protozoa would decrease in number while the amount of bacteria would increase in number. When understanding our question and proving our hypothesis either right or wrong, we had to understand protozoa, bacteria, plants, the biogeochemical cycles, fertilizer, and most importantly the affects of phosphorus. In order to test our plots for the affects of phosphorus on the soil we created two different solutions. One solution consisted of ammonium carbonate and water while the second solution consisted of ammonium phosphate and water. Three of our six plots were fertilized with the ammonium carbonate solution and the remaining three were treated with the ammonium phosphate solution. When the ammonium carbonate was poured onto the ground the carbonate rose in the form of carbon dioxide. Once the carbon dioxide left the soil then all six plots contained ammonium. The three plots that were fertilized with ammonium phosphate contained both phosphate and ammonium. Once the plots contained ammonium or ammonium phosphate depending on the given plot we counted the number of protozoa and bacteria after they were fertilized. Then we compared that data to the number of bacteria and protozoa before the plots were fertilized. We learned that by pouring the phosphate on the soil we created normal soil relations within our ecosystem. The graph titled “Average Phosphate Levels Before and After” on the previous page shows that when we added the ammonium phosphate to the soil, the amount of phosphate in the soil stayed the same. This graph also showed that when we added ammonium carbonate to the soil, the amount of phosphate decreased. When looking at the “Average Bacteria Count Before and After” and the “Average Protozoa Count Before and After” we found that we were able to create the normal soil relations, when the bacteria levels increased

the protozoa levels decreased. Essentially this graph showed that the phosphate was “harming” the bacteria and protozoa. The average number of bacteria in the soil before ammonium carbonate was added showed that fewer bacteria were present in the soil. After ammonium carbonate was added the number of bacteria increased. Before ammonium phosphate was added to plots 2A, 4A, and 6A the levels of bacteria was fewer than the number of bacteria before ammonium carbonate was added to Plots 1A, 3A, and 5A. After ammonium phosphate was added to plots 2A, 4A, and 6A the numbers of bacteria decreased. The average protozoa count before ammonium carbonate was added was greater than the protozoa count after ammonium carbonate was added. The protozoa count was greatest in the soil before the ammonium phosphate was added. After that solution was added the protozoa numbers decreased which proved phosphate “harmed” the microorganisms. Once we looked at all of our data and compared the before and after results, manipulating the phosphate in the soil actually proved that the phosphate had no affect on the amount of protozoa and bacteria. This means that something else was harming the protozoa and bacteria levels on campus. Although our hypothesis was incorrect we generated normal soil relations because in the past what should have been happening in the soil was not taking place. As our data proved it is not the phosphate that is causing this inconsistency in the numbers of bacteria and protozoa.

The nitrogen cycle as a whole could be a source of the protozoa problem, because of this, another group chose to test the affects of ammonium and nitrate on the protozoa. This could have been another possible reason for abnormal soil relations. They believed that the ammonium in fertilizer could be decreasing the amounts of protozoa in the soil, which resulted in an upset in the balance of protozoa to bacteria. Basically they inferred that the ammonium impacted the

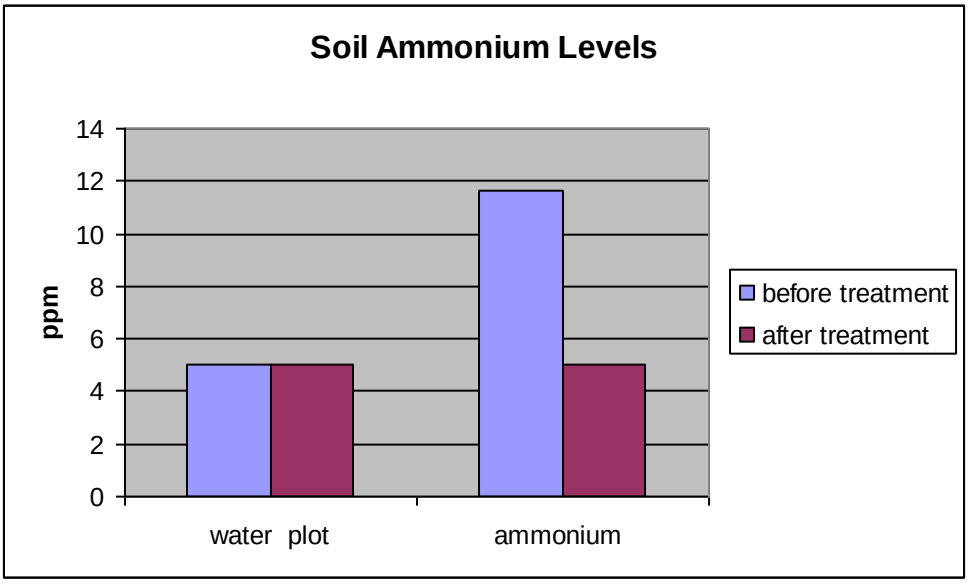
protozoa in a negative way and according to their data their hypothesis was incorrect. In order to test their hypothesis they created both an ammonium and a water plot. In both of these plots they added ammonium and nitrate. According to the graph, "Soil Ammonium Levels", once they added their solution to the soil the soil ammonium levels decreased in the ammonium plot and stayed the same in the water plot. The graph titled "Soil Nitrate Levels" shows that the soil nitrate levels decreased in both the water plot and the ammonium plot once their solutions were added to the soil. When looking at the graph titled "Protozoa Levels in the Soil" we noticed that the protozoa levels in the soil increased drastically after the fertilizer solution was added to the water plot while the number of protozoa in the ammonium plot did not change at all. Once they determined how the protozoa, nitrate, and ammonium levels increased they looked at the relationship between both protozoa and nitrate and protozoa and ammonium. They proved that the ammonium levels in soil affected the amount of protozoa neither negatively nor positively. In fact, the ammonium did absolutely nothing to the number of protozoa in the soil. The same is true for the nitrate levels in the soil, the amount of protozoa were not affected by the levels of nitrate in the soil. Although their hypothesis was incorrect, normal nitrate relationships were created by adding these solutions to the soil. The second group to observe ammonium and nitrate looked at the affects it was having on the bacteria. With that, they chose to test whether or not ammonium was the component of fertilizer that was harming the nitrogen cycle as a whole. If the ammonium was harming the nitrogen cycle then it would indirectly harm the levels of protozoa. According to the graph below, "Relationship between the Nitrate and Bacteria", there was no link between the numbers of bacteria and the levels of nitrate in the soil. They showed that the normal relationship in the soil is not being observed. Normally as bacteria levels go up the nitrate

levels go up as well. With the information of all three groups we concluded that nitrate, ammonium, phosphate, and bacteria are not upsetting the number of protozoa on our campus; therefore these parts of the nitrogen cycle are not responsible for the "protozoa problem".

In the future, studying nitrate, ammonium, phosphate, and bacteria would not aid us in finding an answer to the protozoa problem. With this information we believe that plants are causing an imbalance in the protozoa and bacteria levels. The grass on Roland Park Country School's campus is an invasive species called Bermuda grass, because of this we believe that the grass is causing an imbalance in the numbers of bacteria and protozoa. For future research we would test our experiment on other types of monocots and dicots to see if the results would be different.

## V. Data for Reference (Graphs of Two Other Groups)

**Ammonium, Nitrate and Protozoa Levels Before and After**



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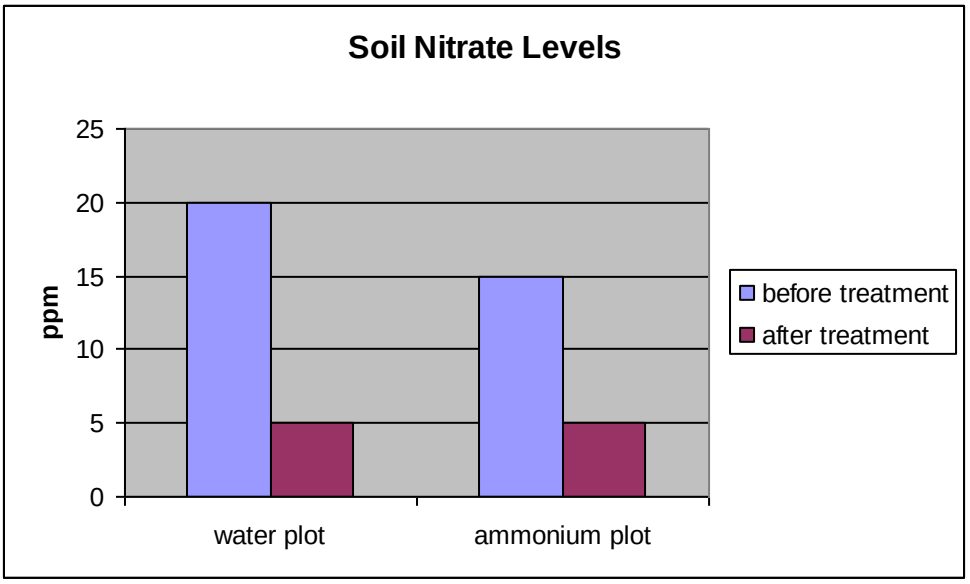


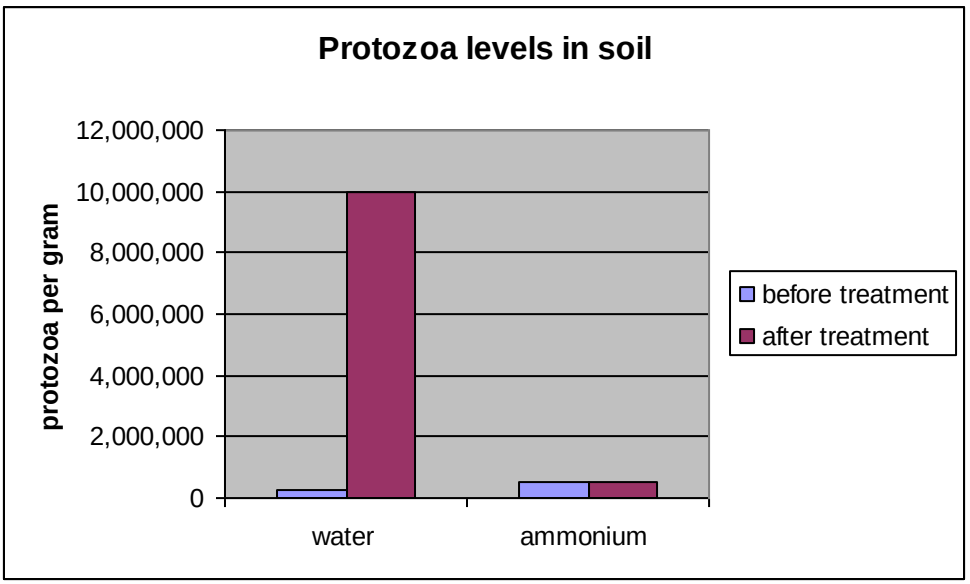


**Relationship between protozoa and ammonium in the soil**

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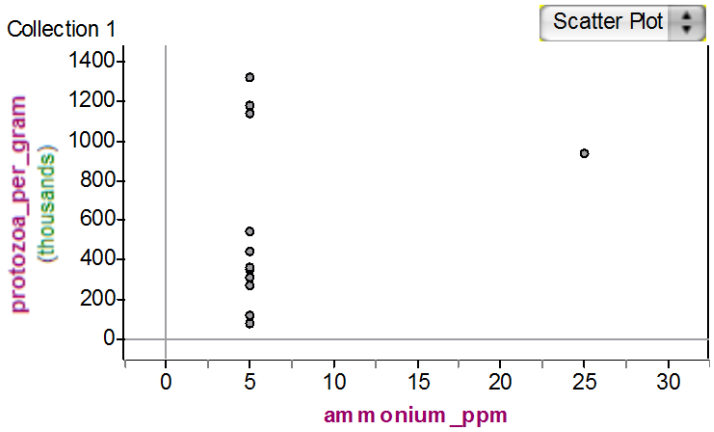
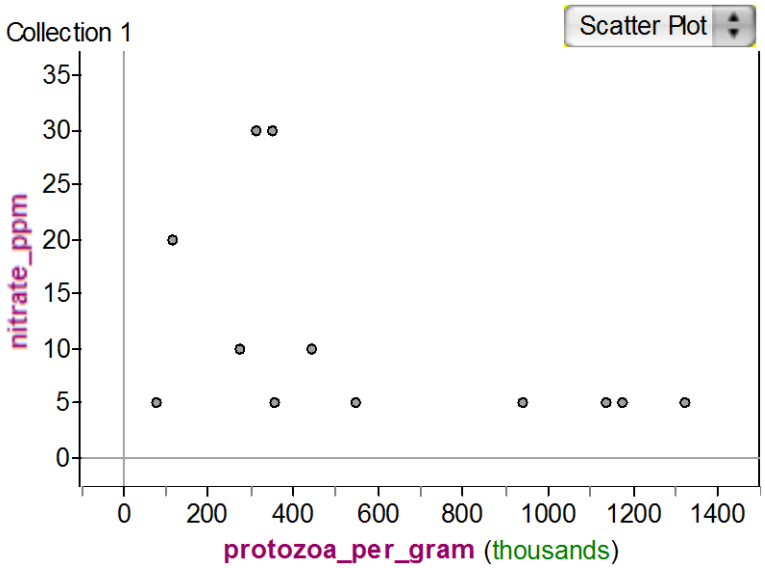



## Relationship between protozoa and nitrate in the soil




## Ammonium, Nitrate, and Bacteria Levels Before and After

| Condition of Plot | Ammonium Before (PPM) | Ammonium After (PPM) | Nitrate Before (PPM) | Nitrate After (PPM) | Bacteria Before | Bacteria After |
|---|---|---|---|---|---|---|
| Water | 1A: 5 ppm | 1A: 2 ppm | 1A: 20 ppm | 1A: 12 ppm | 1A: 1,800,000 $cm^3$ | 1A: 28,000,000 $cm^3$ |
| | 2A: 0 ppm | 2A: 0ppm | 2A: 40 ppm | 2A: 11 ppm | 2A: 3,200,000 $cm^3$ | 2A: 1,400,000 $cm^3$ |
| | 3A: 4 ppm | 3A: 0 ppm | 3A: 20 ppm | 3A: 10 ppm | 3A: 32,000,000 | 3A: 2,400,000 $cm^3$ |

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| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | $cm^3$ | |
| Average | 3 ppm | .67 ppm | 27 ppm | 11 ppm | 12,333,333 $cm^3$ | 31,800,000 $cm^3$ |
| Ammonia Carbonation | 1A: 5 ppm | 1A: 6 ppm | 1A: 80ppm | 1A: 11 ppm | 1A: 500,000 $cm^3$ | 1A: 84,000,000 $cm^3$ |
| | 2A: 0 ppm | 2A: 8 ppm | 2A: 20 ppm | 2A: 12 ppm | 2A: 35,000,000 $cm^3$ | 2A: 2,000,000 $cm^3$ |
| | 3A: 4 ppm | 3A: 2 ppm | 3A: 30 ppm | 3A: 9 ppm | 3A: 12,000,000 $cm^3$ | 3A: 7,500,000 |
| Average | 3 ppm | 5 ppm | 43 ppm | 11 ppm | 15,833,333 $cm^3$ | 31,166,666 $Cm^3$ |

## Relationship between Nitrate and Bacteria