Chelbi Dickens, Betsy Hebert,
Meredith von Paris and Kayla Alevizatos
Mr. Brock
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Ammonia Invasion

Soil Ecology Project

Chelbi Dickens

Background

Nitrogen is a key element found in living things like plants and animals. It is also an important part of non-living things like air and soil. A vital ingredient in the life of the cell, every living thing needs some form of nitrogen to survive because they need nitrogen to make amino acids, proteins and DNA. Without these molecules, cells could not survive. Without DNA, one cannot have enzymes, and without those enzymes, the cells cannot perform the chemical reactions. The chemical reactions are crucial to the cell functions and performing the four tasks in order to keep the cell, and ultimately the organism, alive. Therefore, nitrogen is vital to all living organisms, especially plants and animals.

Most nitrogen (N_2) is in the atmosphere, though, is in a form that cannot be used by plants and animals, but, it can become useful when broken apart. This breaking apart can be caused by lightning strikes, fires, and bacteria. Nitrogen broken down by the bacteria in the soil is how most plants receive their nitrogen from the soil, and animals then obtain theirs by eating plants or animals that contain this nitrogen (Gardiner 2005).

Atoms of nitrogen move between living things (plants and animals), dead organisms (decomposed animals), and non-living things (air and water), allowing plants and animals to access them. The movement of these atoms is the nitrogen cycle (Gardiner 2005). The nitrogen cycle includes four processes in cycling of nitrogen through the biosphere (nitrogen fixation, decay, nitrification, and dentrification), and microorganisms play major roles in all four processes. The first three processes remove nitrogen from the atmosphere and pass it through ecosystems, while the last, dentrification, reduces nitrates to nitrogen gas by restoring it to the atmosphere (Kimball 2007). When organisms die, their bodies decompose and dispense nitrogen into the soil or water in the form of ammonia. Then, bacteria change that nitrogen into the nitrate form which is usable by plants. Other bacteria can change

nitrogen dissolved in water into a form so that it can return to the atmosphere, and the nitrogen cycle can occur again. This process and the balance of nitrogen among the air, soil, and organisms are critical to life. They are also heavily impacted by the actions of humans.

"Certain actions of humans are causing changes to the nitrogen cycle and the amount of nitrogen that is stored in the land, water, air, and organisms," says Lisa Gardiner, (2005). Changes in the nitrogen cycle have a blatant impact on natural environments and human health (Gardiner 2007) because the use of fertilizers rich in nitrogen can add too much nitrogen to the environment. Too much nitrogen can be a bad thing (Loftus 2003). Another way in which humans negatively influence the nitrogen cycle is by having livestock (Gardiner 2005). While most ammonia in the environment is from the natural breakdown of manure, dead plants, and animals (IDPH 2005), the waste of the livestock raised by humans adds an even greater amount of nitrogen to the soil. This increased amount in nitrate causes plants to grow more rapidly to the point at which they use up their resources and die. The number of plant-eating animals will increase if the number of plants goes up, but if all the plants die, those animals are left without food (Gardiner 2007). Therefore, the actions of humans are directly related to the changes or lack of changes in the levels of ammonia nitrogen in the soil.

Ammonia (NH_3) is a colorless liquid or gas with an overpowering and nauseating odor. It is found in water and soil and also in the air (IDPH 2005). It is formed as a result of the decomposition of most nitrogenous organic material (Sisler 2007) and, along with nitrate, it is a nutrient which is essential for plants, animals, and humans (CTIT 2001, IDPH 2005). It is the most familiar compound made of elements of hydrogen and nitrogen (Sisler 2007), and when combined with water, it becomes ammonium. However, ammonia does not last long in the environment because it is recycled naturally, and even when fertilizer containing ammonia is added to soil, the level of ammonia drops within a few days (ATSDR 1970). Ammonia is most commonly used as a nutrient for plants since, as we have seen, it

is one of the seventeen chemical elements needed to complete the plants' life cycle (Loftus 2003, PPH 2004). Because of this, it is in most fertilizers which are applied to soil to enhance the growth of plants.

Plants, though, do not access the chemicals found in fertilizer (such as ammonia) directly. Instead, plants benefit from fertilizer though the activities of soil protozoa. Protozoa means "first animals" and they are the hunters and grazers of the microbial world and the keystone of taking care of the balance of bacterial, algal and other microbial life that live in the soil. They are single-celled eukaryotic organisms that have high rates of reproduction, and there are over fifty thousand species of them (ASM 2006, PGAU 2007). Protozoa play a major role in maintaining the balance of bacteria's life and are the food source for larger creatures and the basis of many soil food chains. They can live in many different environments including deserts, bogs, deep in the ocean, and in the Arctic (ASM 2006).

One of protozoa's most significant roles in the environment includes mineralizing nutrients, which make them available for use by plants and other soil organisms. Protozoa regulate bacteria populations as they consume bacteria, and as they eat bacteria, they release excess nitrogen in the form of ammonia that will then be used by plants and other members of the soil food web (JEE n.d.). The way it works is that bacteria fix nitrogen. Protozoa eat bacteria and receive the nitrogen from those bacteria. It then converts the extra nitrogen to ammonium and releases it in the form of ammonia near the roots of plants. Bacteria, plants and other organisms then benefit and receive the ammonium (NRCS 2007). This process of converting ammonia is what we focused and based our project on.

For our experiment we investigated the question "What part of the fertilizer is harming the nitrogen cycle?" We knew that the protozoa levels did not change according to the levels of bacteria in the soil here on the RPCS campus (ESSRE Microclimate Databases, 2001-2007). Of the three ingredients in fertilizer (potassium, phosphate and ammonia), we decided to look at ammonia. We predicted that the levels of bacteria were related to the amount of ammonia in the soil. The ammonia could be killing the protozoa in the soil, providing the bacteria with more food (dead organisms) and causing a growth in

population of bacteria. This would explain there being more bacteria than protozoa. There could also be a greater amount of bacteria due to lack of water or moisture in the soil which would prevent the protozoa from accessing the bacteria. If we discover either of these events to have occur, we would have data that supports our hypothesis which is "If we increase the level of ammonia in the fertilizer that is applied to the soil, the number of bacteria will increase after 2 days. " If we were to find that there were more protozoa than bacteria, the data would not support our hypothesis.

We investigated this question by taking Before Soil Samples from the plot of land we chose to use for the experiment. With those soil samples we performed Serial Dilutions of Bacteria and we tested the presence of Ammonia and Nitrate in that soil. Then we created an Ammonia Carbonate Solution to purposefully add ammonia to half of the plot. The other half received only water so we could compare it to the results we received from the plot that did receive the ammonia. In doing so, we sought to confirm or deny the hypothesis we developed.

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Betsy Hebert

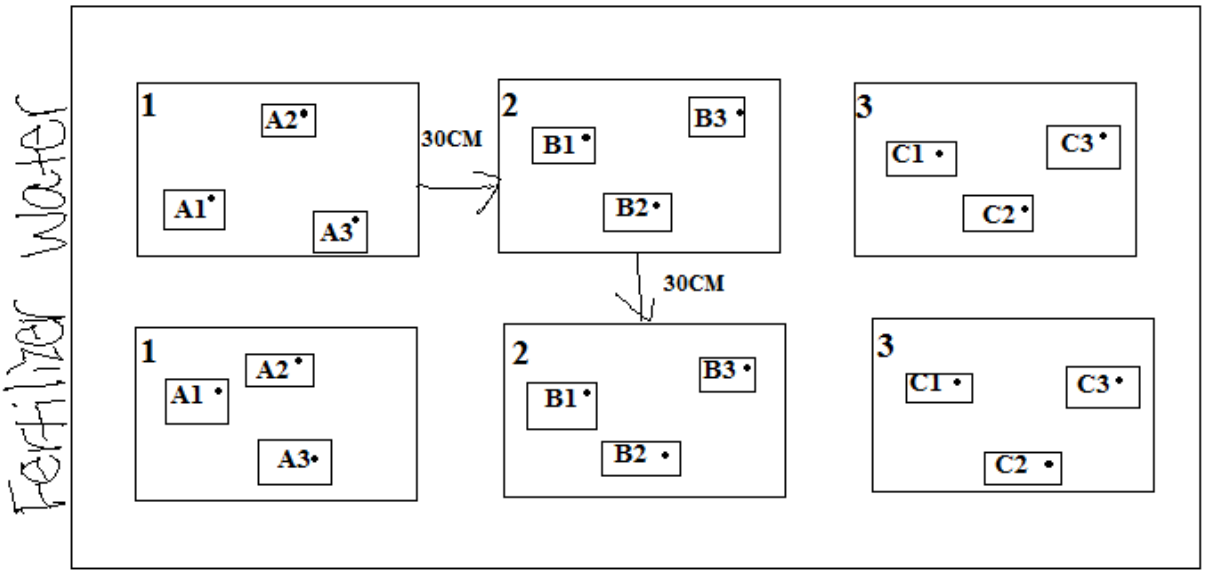
Procedures

Question: What component of fertilizer is harming the nitrogen cycle?
Hypothesis: If we increase the level of ammonia in the fertilizer that is applied to soil, the number of bacteria living there will increase after two days.
Independent Variable: land that receives ammonia treatment
Dependent Variable: Amount of bacteria/ cm^3 in soil
Negative control: Land that receives water treatment only
List of Controlled Variables:

- How much fertilizer
- How long to let bacteria grow
- Type of nutrients
- How big of a plot of land
- Length of treatment of soil
- Location of soil plots
- Type of fertilizer applied to soil plot
- Number of trials
- Intervals of time between observing/recording data
- Size of soil samples
- Time soil samples are taken
- Size of culture tubes
- Size of pipettes
- Condition of pipettes (clean, fresh)
- Number of culture tubes
- mL's of soil removed from each mixture of soil/water
- Amount of soil added to culture tube
- Amount of µl plotted
- Items used from as STH-14 LaMotte tests for ammonia and nitrate

Step-by-step Instructions:
Plotting Land

1. Go to N39^o 21. 479' W076^o 38. 121'
2. Measure two 20cm by 20cm plots for each trial (6 plots total) within the overall plot.
3. Make sure there is 30cm between each plot
4. Label one plot 'Fertilizer' and the other 'Water'.
5. In each plot sampled place flags labeled A1,A2,A3(Plot 1Fertilizer) A1,A2,A3 (Plot 1 Water) B1,B2,B3(Plot 2 Fertilizer) B1,B2,B3(Plot 2 Water) C1,C2,C3(Plot 3 Fertilizer) C1,C2,C3(Plot 3 Water)
6. In corner of each flag draw a black dot representing the before sample



7. Label 18 bags. One bag will be labeled “Fertilizer 1A” another bag will be “Water 1A” the next bag will be “Fertilizer 2A” and then the next “Water 2A” etc.

Making Ammonia Solution

1. Retrieve 3 liter bottles, a balance, a container of Ammonium Carbonate, a metal spatula, marker, sticker labels, weigh boat, goggles and gloves.
2. Using the sticker labels, label each liter bottle with group name and “Ammonium Carbonate”.
3. Fill each liter bottle to the 1000mL mark with tap water.
4. Place the weigh boat onto the balance and reset the weight so that it reads “0.0g”.
5. Using the metal spatula, scoop up some of the Ammonium Carbonate and gently tap it into the weigh boat until the balance reads “0.7g”.
6. Fold the weigh boat to pour the Ammonium Carbonate into the liter bottle.
7. Cap the bottle and shake it vigorously until the Ammonium Carbonate dissolves into the water.
8. Repeat steps 3-7 for each of the liter bottles.

Before Samples

1. Take all soil samples at same time
2. Take 3 separate cylinders of soil that are 15 ½ deep cm by 2 cm wide from each individual plot (18 total samples) and place each sample in its corresponding bag.
3. Return to plots after 2 days

Applying to soil

1. Using the ammonia carbonate made previously, pour the 1L bottle of the solution into “plot one fertilizer”.
2. Using the water prepared the previous day, pour the 1L of water into “plot one water”.

3. Repeat steps 1&2 for all three plots
4. Return to plots after two days to take after samples

After Samples

1. Take all soil samples at same time
2. Take 3 samples of 15 ½ cm by 2 cm of soil from each individual plot (18 total samples)
3. Repeat steps 7 from "Plotting Land" procedures and adding the word after onto each bag
4. Take soil samples from fertilizer and the other from water
5. Be sure to match samples with corresponding pair from the other plot

Serial Dilutions for Bacteria

1. Do this test at same time as STH-14 LaMotte tests for "before" and "after" samples
2. Do all sample A's at same time and repeat for B's and C's
3. Use a clean, new transfer pipette to add 10mL to a 15mL culture tube. Label the tube "10^0 Water 1A"
4. Use the same pipette to add 9mL to a second 15mL culture tube. Label the tube "10^{-1}Water 1A"
5. Repeat step 2 three more times to three additional 15mL culture tubes, only Label them "10^{-2}Water 1A", "10^{-3}Water 1A " and "10^{-4}Water 1A"
6. Repeat these steps for B's and C's
7. Place 1cc of the "Water 1A" samples into the "10^0Water 1A" culture tube
8. Cap the tube and shake vigorously
9. Using a new clean pipette remove 1mL of the soil/water mixture from the "10^0Water 1A" tube and place in the "10^{-1}Water 1A"
10. Cap the tube and shake vigorously
11. Using a new clean pipette remove 1mL of the soil/water mixture from the "10^{-1}Water 1A" tube and place in the "10^{-2}Water 1A"
12. Cap the tube and shake vigorously
13. Using a new clean pipette remove 1mL of the soil/water mixture from the "10^{-2}Water 1A" tube and place in the "10^{-3}Water 1A"
14. Cap the tube and shake vigorously
15. Using a new clean pipette remove 1mL of the soil/water mixture from the "10^{-3}Water 1A" tube and place in the "10^{-4}Water 1A"
16. You should now have a total of 5 culture tubes
17. Plate a 100µl samples from the 4^{th}&5^{th} tubes (dilutions 10^{-3} and 10^{-4}) onto their own separate, labeled "3M Petrifilm Aerobic Count Plate" containing nutrient agar (NOTE: on your first sample, plate ALL five dilutions to determine which two dilution values will give you the best data; Dilutions 10^{-3} and 10^{-4} are only the most probable ones).
18. Allow to grow for 48 to 72 hours
19. Examine each of the plates for individual bacteria colonize and choose the plate with the lowest dilution that has at least five, colonies to make estimates of the number of bacteria in the original 1cc soil sample using the following formula:

a. # Microbes in 1cc of soil = #Colonies on sheet x 10^2 x $10^{|\text{dilution number at which these colonies were found}|}$

20. Repeat steps 3-20 for all other samples to complete this process

Extraction Procedures

1. Perform tests at the same time as Bacteria test s
2. Fill an extraction tube (0704) to the 7mL line with *Universal Extraction Solution (5173).
3. Use the plastic soil measure (0819) to add 1 level measure of the soil sample. Cap and shake for one minute.
4. Use a piece of filter paper (0465) and a plastic funnel (0459) to filter the soil suspension into a second extraction tube (0704). (Fold the filter paper in half and then in half again to form a cone which is fitted into the funnel.) The filtrate in the second extraction tube is the general soil extract for use in the test procedure for ammonia nitrogen.

Ammonia Procedures

1. Use a transfer pipette (0364) to transfer four drops of the general soil extract to one of the larger depressions on a spot plate (0159).
2. Add one drop of *Ammonia Nitrogen Test Solution (5103). Stir with a clean stirring rod (0519). Allow to stand for one minute.
3. Compare the resulting color against the Ammonia Nitrogen Color Chart (1302). The test result is expressed in relative values of ammonia nitrogen from very low to very high. For approximate corresponding values in parts per million or pounds per acre, see chart below.

	Approximate Value Expressed in ppm (parts per million)				
Test Factor	Very Low	Low	Medium	High	Very High
Ammonia Nitrogen	5	10	40	100	150

Nitrate Procedures

1. Use a 1mL pipette (0354) to transfer 1mL of general soil extract to one of the large depression on a spot plate (0159).
2. Add 10 drops of *Nitrate Test Reagent #1 (5146).
3. Use a 0.5g spoon (0698) to add one level measure of *Nitrate Reagent #2 (5147).
4. Stir thoroughly with a clean stirring rod (0519). Allow to stand for five minutes for full color development.
5. Match sample color with nitrate nitrogen color chart (1315). Record as ppm (divide number on chart by “0.5”). (For example, if the color is similar to level “10”, the value in ppm is “5”.

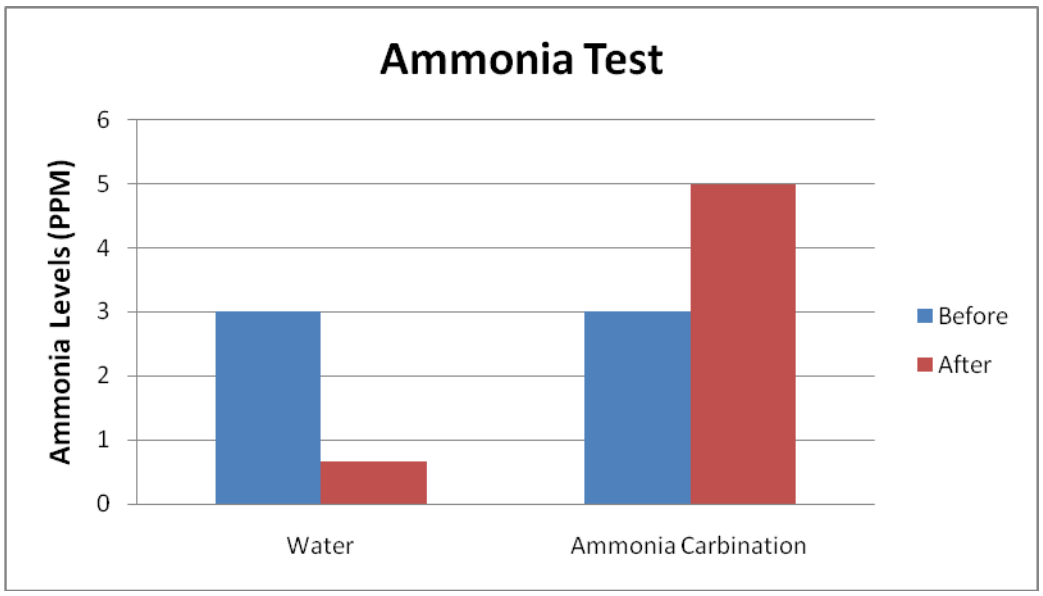
	Approximate Value Expressed in ppa (Pounds per Acre)					
Test Factor	Very Low	Low	Medium	High	Very High	Extremely High
Nitrate Nitrogen	10	20	40	60	100	150

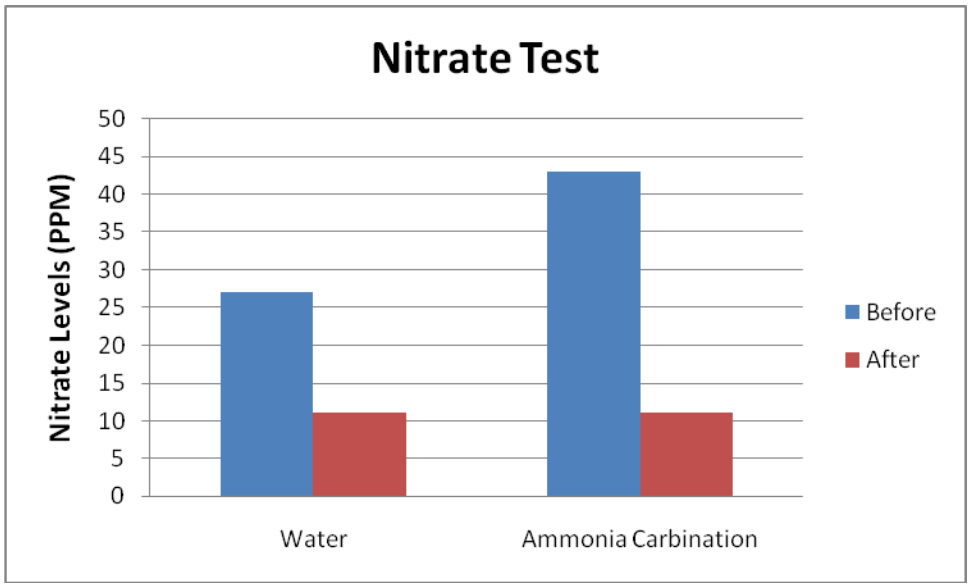
Meredith von Paris

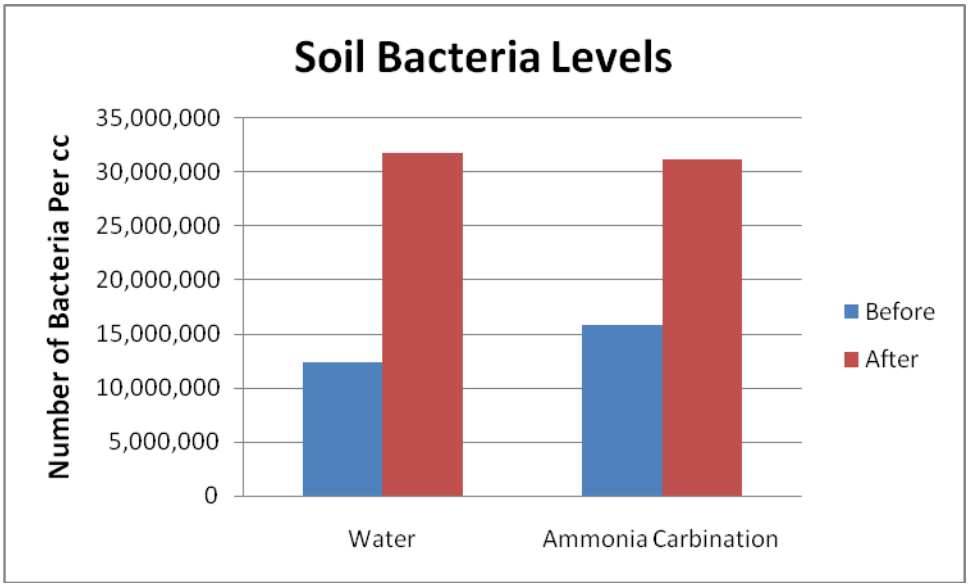
Data Charts and Graphs

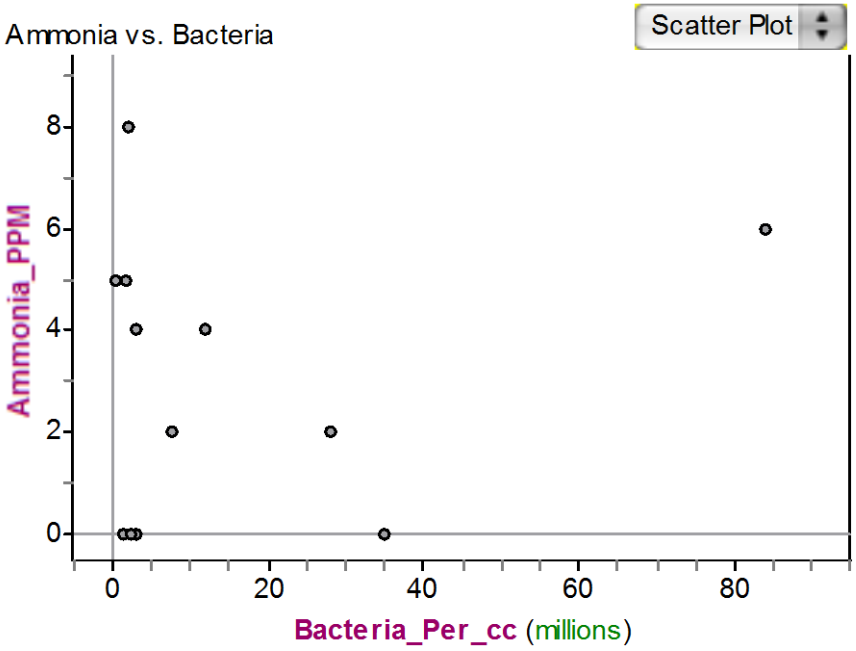
Bacteria Where?

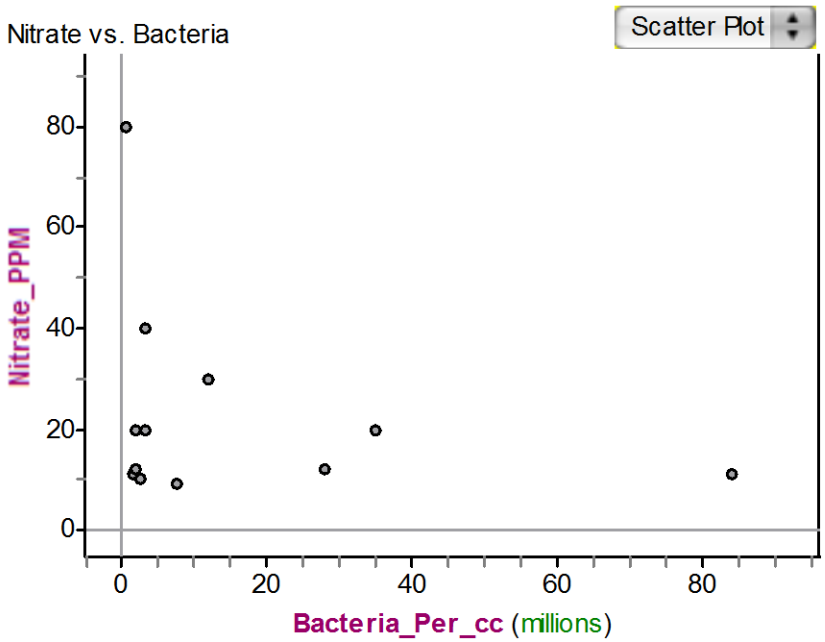
Condition of Plot	Ammonium Before (PPM)	Ammonium After (PPM)	Nitrate Before (PPM)	Nitrate After (PPM)	Bacteria Before	Bacteria After
Water	1A: 5 ppm,	1A: 2 ppm	1A: 20 ppm	1A: 12 ppm	1A: 1,800,000 cm^3	1A: 28,000,000 cm^3
	2A: 0 ppm	2A: 0ppm	2A: 40 ppm	2A: 11 ppm	2A: 3,200,000 cm^3	2A: 1,400,000 cm^3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


Ammonia Test
Ammonia Levels (PPM)
6
5
4
3
2
1
0
Water
Ammonia Carbination
Before
After


Nitrate Test
Nitrate Levels (PPM)
50
45
40
35
30
25
20
15
10
5
0
Water
Ammonia Carbination
Before
After


Soil Bacteria Levels
Number of Bacteria Per cc
35,000,000
30,000,000
25,000,000
20,000,000
15,000,000
10,000,000
5,000,000
0
Water
Ammonia Carbination
Before
After


Ammonia vs. Bacteria
Scatter Plot
Ammonia_PPM
8
6
4
2
0
0
20
40
60
80
Bacteria_Per_cc (millions)



Kayla Alevizatos

Conclusion

Our hypothesis was that the bacteria levels would increase after two days with the use of ammonium carbonate. This hypothesis was incorrect. Contrary to what we thought would be the results, the bacteria levels actually decreased with the use of ammonium carbonate, but went up with the use of water on the plots. Specifically, 2/3 of the plots' bacteria went down with ammonium carbonate use and 2/3 of the water plots' bacteria went up. On water plot 1A before anything was applied, there was a bacteria count of 1,800,000 per cubic centimeter. After water was applied, there was a bacteria count of 28,000,000 per cubic centimeter. That is a 26,200,000 increase in the bacteria. On ammonium carbonate plot 2A before anything was applied, there was a bacteria count of 35,000,000 per cubic centimeter. After applying the fertilizer and waiting two days, there was a bacteria count of 2,000,000 per cubic centimeter. That is a 33,000,000 decrease in the bacteria over a two day period. The increase and decreases indicate something about not only the bacteria, but also the protozoa. A raise in the bacteria can be associated with lower protozoa levels. This is because the protozoa eat the bacteria

and if there are fewer protozoons, fewer bacteria will be eaten. Likewise, a decrease in bacteria indicates that there are more protozoa. Using this knowledge, one can safely infer that the ammonium carbonate feeds the protozoa allowing them to flourish and reproduce. Water weakens them but gives the bacteria a better chance at multiplying. In addition, this information shows that water is good for the protozoa, and the ammonium carbonate is good for the bacteria. Regarding the protozoa, before water was added to the plot, there were almost no protozoa. After adding water, the protozoa levels shot up significantly to 10,000,000 protozoa per gram. Before the ammonium carbonate was added, there was also very little protozoa. This level did not change after the solution was added.

We have acted honorably.