Background Information

Soil is the place from which all living things originate and from which all organisms directly or indirectly take their sustenance. It contains billions upon billions of living organisms, all acting in different ways that happen to make the land suitable for growing the crops that feed humanity. But not being satisfied with nature, humans have found ways to increase the production of crops from the land and the speed of production. However, while the increased productivity and the increased speed of productivity have helped feed humanity's masses, the methods employed to perform this task are essentially harming the soil by killing an entire group of bacteria and in the process making the soil chemically dependant on humans. (*Fertilizers*, July 2003; *Soil pH and Fertilizers*, November 2002) This altering of the eco-systems is occurring in many places throughout the world, even in our own neighborhoods.

Roland Park Country School covers its campus with a variety of plants. For example, Bermuda grass covers the athletic fields, while the playgrounds are covered by woodchips. Most of the soil on our school campus that is under vegetation of any kind is subjected to chemicals in the form of fertilizers. There is a range of chemicals used with different chemical ratios on the grounds. Each different chemical and ratio of chemicals has a different affect on the soil ecosystem. (*Fertilizers*, July 2003; *Soil pH and Fertilizers*, November 2002) Soil is in its healthiest state when it is untouched by chemicals. (*Soil pH and Fertilizers*, November 2002) Therefore, ecosystems altered by a fertilizer with a lower chemical ratio are healthier than ones where the chemical ratios are higher. (*Soil pH and Fertilizers*, November 2002; *Soil Chemistry*, November 12, 2002)

Fertilizers contain several chemicals that alter the ecosystems, with nitrogen, phosphorus and potassium being the most prevalent. (*Soil pH and Fertilizers*, November 6, 2002; *Soil Chemistry*, November 12, 2002) By adding excess nitrogen in the form of ammonium to the natural nitrogen cycle in soil, the cycle is thrown out of balance. A natural nitrogen cycle would operate as follows: Decomposing fungi would break down organic matter in the soil, ammonia being the byproduct. The ammonia would then be transformed by nitrifying bacteria into a nitrate that can be used by plants. (*Life and Biogeochemical Cycles*, May 3, 2004; *Biogeochemical Cycles*, March, 2004; *Nitrogen Cycle*, Unknown Date) The plants absorb the usable nitrogen into their tissue, which then helps the cells manufacture proteins increasing its productivity. (*Life and Biogeochemical Cycles*, May 3, 2004; *Nitrogen Cycle*, Unknown Date)

By adding fertilizer to this cycle, humans are destroying the nitrogen cycle of this eco-system because the fertilizer, which contains excess ammonium, kills the decomposing fungi because they are made to live in excess amounts of their own waste. At the same time the population of the nitrifying bacteria increases because of the increasing amounts of food supply in the form of fungi. Then when the fertilizer is not applied to soil that has been treated with fertilizers, the soil cannot function on its own, because there are no decomposing fungi to convert organic matter into ammonium for the cycle to begin and with no ammonium. As a result the nitrifying bacteria cannot do their job of converting ammonium into nitrates. (*Life and Biogeochemical Cycles*, May 3, 2004; *Biogeochemical Cycles*, March, 2004) This in turn leaves the plants in the eco-system without a source of usable nitrogen, and without it they will die because plants use nitrogen to make proteins which are the building blocks for every living activity.(*Life*

and Biogeochemical Cycles, May 3, 2004; *Biogeochemical Cycles*, March, 2004; *Soil Chemistry*, November 12, 2002) Consequently, plant life as well as microbes becomes dependant on chemicals for their continued existence. Thus while adding nitrates to the soil seems to increase its productivity, the ecology of the soil is destroyed by the excess of this chemical.

The ecology of the soil is dependent on nitrogen cycle to a great extent. (*The Nitrogen and Carbon Cycles*, December 1997) This is because nitrogen is a key ingredient of enzymes. Nitrogen is an important constituent of many structural, genetic, and metabolic compounds, which helps plants undergo photosynthesis. (*Soil Chemistry*, November 12, 2002; *The Nitrogen and Carbon Cycles*, December 1997) Photosynthesis in turn aids in the growth and reproduction of plants. (*Soil Chemistry*, November 12, 2002) Nitrogen is also a component of energy-transfer compounds, such as ATP (adenosine triphosphate), which allows cells to use the energy released in metabolism. Nitrogen is a significant component of nucleic acids such as DNA, the genetic material that permits cells (and eventually whole plants) to grow and reproduce. When the nitrogen cycle is not efficient in producing useable nitrogen for plants, the cells in the plants cannot produce proteins, because nitrogen is also a major component of amino acids, which are the building blocks of proteins. Some proteins act as structural units in plant cells while others act as enzymes, initiating all of the biochemical reactions on which life is based. . (*Soil Chemistry*, November 12, 2002; *The Nitrogen and Carbon Cycles*, December 1997) Consequently, the four major functions of a cell, cellular respiration, reproduction, regulation of environment, and making of protein, will not occur and the plant will die.

The nitrogen cycles and most of the activities in the soil are performed by the soil microbes. They affect the efficiency of the soil ecology. They are essential to the health and longevity of an ecosystem by interacting with many multi-cellular organisms, such as animals or humans. Microbes aid multi-cellular organisms in their acquisition of food, resources, digestion, nutrition, and even reproduction. They even play an important role in the other global geochemical cycles, *i.e*. carbon, iron and sulfur cycles other than nitrogen. These cycles are the processes in which elements and chemical compounds are moved from one organism to another and rotate in the biosphere making it available for every kind of living life. (*Benefits of Soil Microbes,* August 2001; *Soil Microbes,* August, 2001) Life and the earth's climate depend on the biogeochemical cycles.

There are several different types of microbes that take part in these cycles. However, for our experiment, we will be measuring the amount of bacteria and fungi that take part in the nitrogen cycle to determine the affect of the fertilizers on the campus of Roland Park Country School.

Bacteria have the greatest influence on the soil environment and on all organisms. Bacteria and fungi decompose or break down and gain energy from organic matter in the soil. The organic matter, which they decompose, becomes food for other organisms as well. Bacteria and fungi also provide carbon dioxide and nitrogen for their environment. (*Soil Chemistry*, November 12, 2002; *Soil pH and Fertilizers*, November 6, 2002.) In addition, bacteria have a very important role in enzymatic transformations. (*Soil Chemistry*, November 12, 2002) Two different species of bacteria transform nitrogen from ammonia to nitrite and nitrate, which is absorbed by the soil thus basically

controlling the nitrogen cycle. (*Soil Chemistry*, November 12, 2002; *Soil pH and Fertilizers*, November 6, 2002.)

When the nitrogen cycle is efficiently working, there should be a correlation between the fungi, nitrifying bacteria, and the ammonium. If the bacteria and fungi have a relatively high and similar population density combined with a medium to low number of parts per million (ppm) of ammonium, the soil is healthy and the ecosystem is self-sustaining. However, in an eco-system that is chemically dependant, the levels of decomposers and bacteria differ greatly in proportion, with the decomposers having a very low count and the nitrifying bacteria having a very high count. Also, the ammonium level is at a medium to high number of ppm. The high ppm level indicates that fertilizer has been added to the soil. In addition, the unbalanced levels of decomposers and nitrifying bacteria illustrate that the decomposers has been dying off because of the excess fertilizer. On the other hand, the high count of the nitrifying bacteria indicates that the food source has increased, which has caused an increase in the population.

Experiments can be done to test whether or not an ecosystem is healthy. Soil samples are taken from the desired places and tests are done to observe the number of decomposing fungi and nitrifying bacteria. A serial dilution is done on the two main kinds of fungi in the soil, mold and yeast. The fungi are grown for 5 days and then the levels are compared. Another test is done on the soil to test for the ppm of ammonium. At the end of the experiment, graphs are done and the levels of the fungi, bacteria, and ammonium are all compared to see whether the ecosystem is healthy or not.

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Lab Report

Problem: Which PKN ratio in fertilizer disturbs the ecosystem of the soil more?
Hypothesis: The PKN ratio 24-3-11 will disturb the soil ecosystem more than the PKF ratio 24-3-7

Procedure:

Variables:

Independent Variable: Location where soil sample is taken based on difference in type of fertilizer applied to it
Dependent Variable 1: Population density of yeast
Dependent Variable 2: Population density of mold
Dependent Variable 3: Population density of bacteria
Dependent Variable 4: Ammonium level in soil

Controls:

Negative control 1: Bare soil
Negative control 2: Grass without fertilizer

Control Variable List:

- Location of bare soil samples
- Location of grass without fertilizer samples
- Location of playing field samples
- Location of campus grass samples
- Size of soil sample
- Soil sample diluted to the 10^{-1} and 10^{-2} for the yeast/mold plates
- Soil sample diluted to the 10^{-3} and 10^{-4} for the bacteria plates
- Different serological pipette tip for each soil sample
- Different micro-pipette tip for each extraction
- Clean soil scoop each time it is used
- Amount of light where petri-film plates are stored
- Date and time which soil samples are collected
- Use of Petri-film plates
- Amount of dilution applied to Petri-film
- Petri-film plates stored at room temperature

Step by Step:

1. Collect 3, 15 cm soil samples from each location with a soil core sampler with a diameter of 2.5 cm.
    a. Bare soil – (N = 39.35696^{o}, W = 76.63625$^{o)}$
    b. Grass without fertilizer – (N = 39.35709^{o}, W = 76.63641$^{o)}$
    c. Playing field grass with PKN ratio of 24-3-7 – (N = 39.35855^{o}, W = 76.63622^{o})
    d. Campus grass with PKN ratio of 24-3-11- (N = 39.358109^{o}, W = 76.63612^{o})
2. Place each soil core sample in a separate clean, plastic storage bag and label each bag with the name of the location it is taken from and the soil sample number. Before you start step number 3 note that steps 3-4 and 5-20 must be completed simultaneously for each number of soil samples
3. Test the 1st playing field sample for ammonium with the LaMotte STH-14 ammonia nitrogen test kit
4. Using the ammonia nitrogen test chart, record results in parts per million

5. Place 1 cc of from the 1st playing field sample into a 15 mL culture tube containing 10 ml of sterile water
6. Cap the tube and shake vigorously.
7. Using a serological pipette remove 1 ml of the soil/ water mixture and place into a fresh culture tube containing 9 ml of sterile water
8. Cap the tube and shake vigorously.
9. Repeat steps 7-8 two more times until you have five test tubes and your original soil sample is diluted to 10^{-4}
10. Lift lid of bacteria Petri-film plate and use a micropipette to place a 100 µl sample from the 10^{-3} tube onto the plate
11. Drop the lid and use plastic circle to spread sample
12. Label plate with dilution number, location of sample, and sample number
13. Store sample in a dark area which is room temperature and let sit for 5 days
14. Repeat steps 10-13 for the 10^{-4} dilution
15. Repeat steps 10-13 with yeast/mold Petri-film plates using the 10^{-1} dilution
16. Repeat steps 10-13 with a yeast/mold Petri-film plate using the 10^{-2} dilution
17. Examine the 2 bacteria plates and chose plate with the least amount of bacteria colonies
18. Record number of bacteria colonies and the dilution factor for the plate which was used
19. Repeat steps 17-18 for the yeast/mold plates
20. Use this formula: number of colonies x 10^{2} x $10^{|\text{dilution factor used}|}$
21. Record number of yeast, mold and bacteria colonies for the 1cc of soil taken from the playing field sample
22. Repeat steps 3-21 for the 1st campus, grass and bare soil samples which must be performed simultaneously with the first playing field sample
23. Repeat steps 3-21 for the 2nd and 3rd samples for each location. The second samples must be performed simultaneously. The 3rd soil samples must also be performed simultaneously
24. Repeat steps 1-23, 2 more times

Data and Analysis:

Data for 1cc of soil

		Bacteria (number of colonies per cubic centimeter of soil)	Yeast (number of colonies per cubic centimeter of soil)	Mold (number of colonies per cubic centimeter of soil)	Ammonia in ppm
Trial 1	Playing field 1	2000000	9000	11000	5
	Playing field 2	55000000	5000000	10000	70
	Playing field 3	800000	17000	5000	40
Trial 2	Playing field 1	7000000	3500000	70000	70
	Playing field 2	12000000	3000000	70000	70
	Playing field 3	48000000	380000	70000	70
Trial 1	Bare soil 1	6000000	4000	30000	7.5

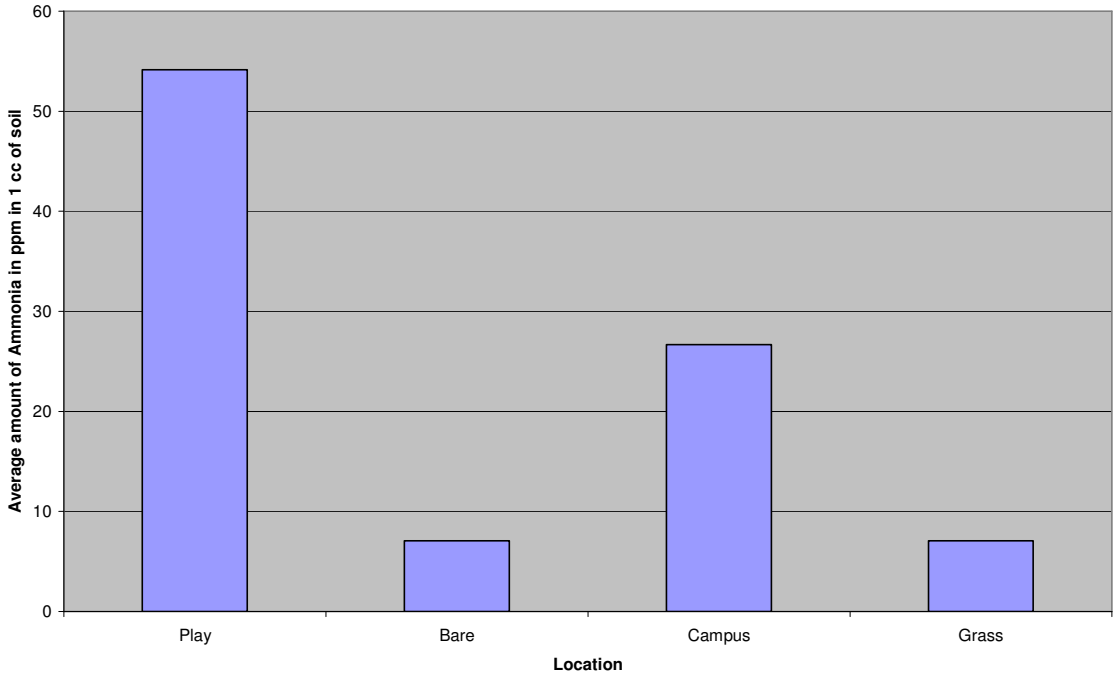
	Bare soil 2	2300000	45000	13000	5
	Bare soil 3	1500000	13000	12000	7.5
Trial 2	Bare soil 1	16000000	80000	60000	7.5
	Bare soil 2	9000000	150000	10000	5
	Bare soil 3	8000000	40000	70000	10
Trial 1	Campus grass 1	6000000	70000	70000	5
	Campus grass 2	7000000	90000	10000	40
	Campus grass 3	7000000	40000	60000	25
Trial 2	Campus grass 1	15000000	110000	50000	25
	Campus grass 2	6000000	90000	40000	40
	Campus grass 3	20000000	90000	30000	25
Trial 1	Unfertilized grass 1	6000000	40000	20000	7.5
	Unfertilized grass 2	8000000	30000	70000	5
	Unfertilized grass 3	8000000	30000	70000	10
Trial 2	Unfertilized grass 1	10000000	2100000	50000	5
	Unfertilized grass 2	400000000	2600000	50000	10
	Unfertilized grass 3	18000000	210000	100000	5

Average Data for 1cc of soil

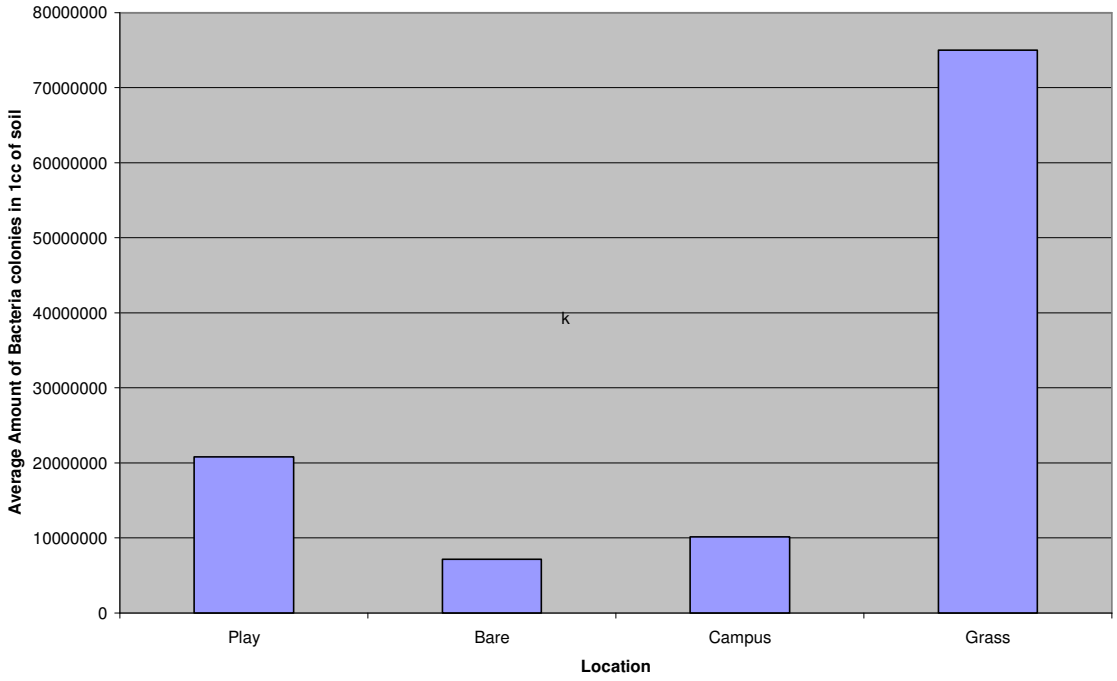
	Bacteria (number of colonies per cubic centimeter of soil)	Yeast (number of colonies per cubic centimeter of soil)	Mold (number of colonies per cubic centimeter of soil)	Ammonia in ppm
Play	20800000	1984333	39333.33	54.167
Bare	7133333	55333.33	32500	7.083
Campus	10166667	81666.67	43333.33	26.67
Grass	75000000	835000	60000	7.083

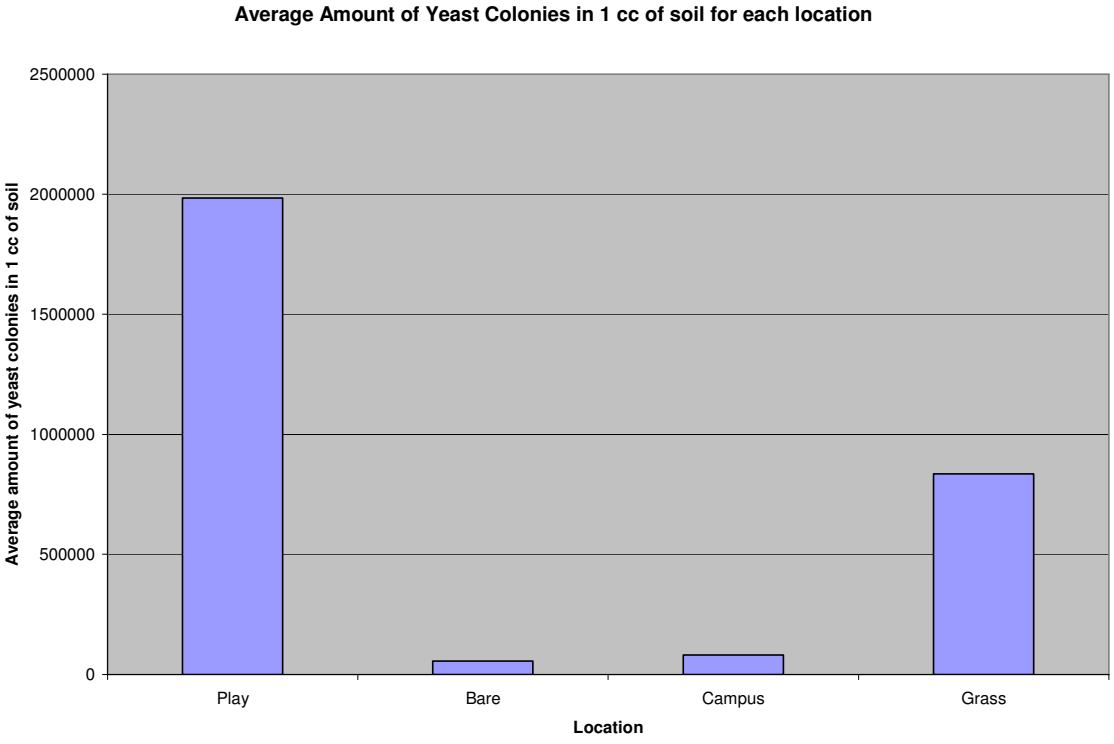
Graphs

Average Amount of Ammonia in 1 cc of soil for each location

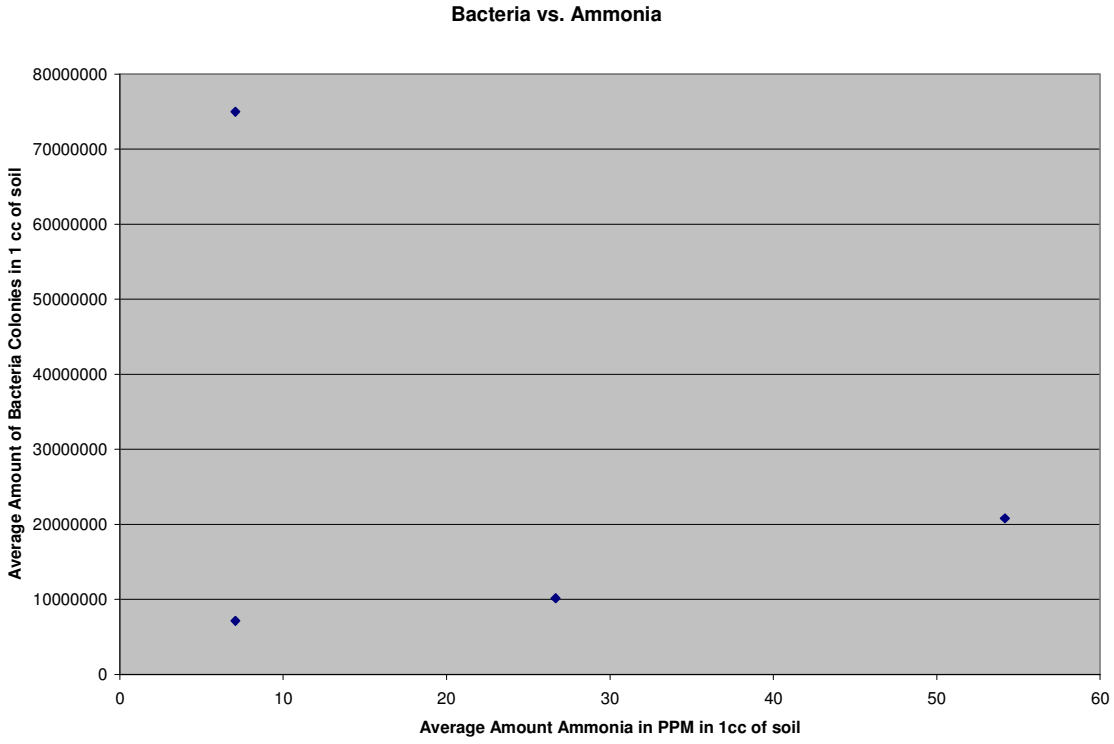


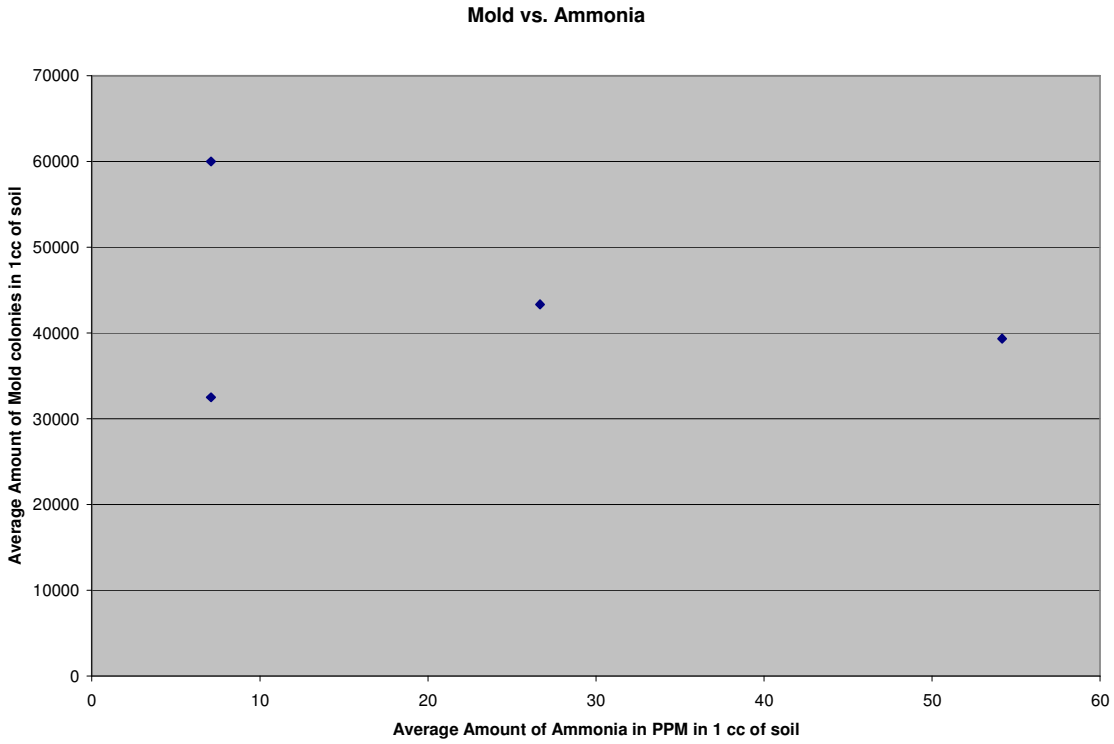
Average Amount of Bacteria colonies in one cubic centimeter of soil for each location

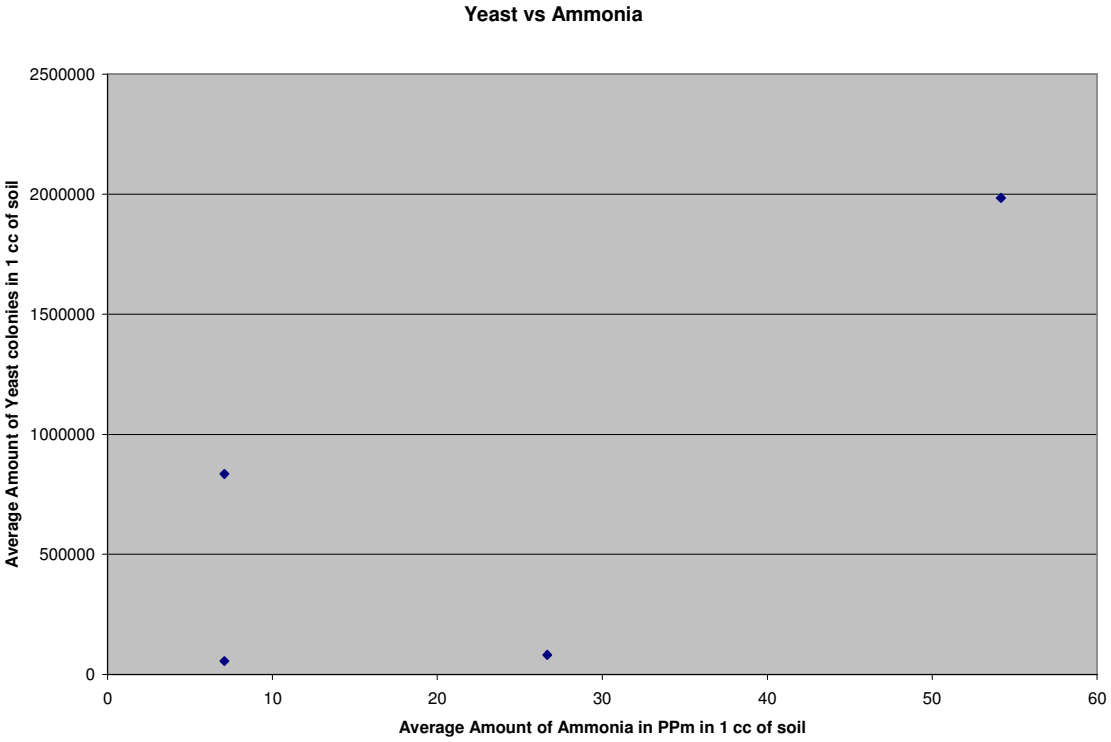



Average Amount of Yeast Colonies in 1 cc of soil for each location
Average amount of yeast colonies in 1 cc of soil
2500000
2000000
1500000
1000000
500000
0
Play
Bare
Campus
Grass
Location


Average Amount of Mold colonies in 1cc of soil for each location
Average Amount of Mold colonies in 1cc of soil
70000
60000
50000
40000
30000
20000
10000
0
Play
Bare
Campus
Grass
Location


Bacteria vs. Ammonia
Average Amount of Bacteria Colonies in 1 cc of soil
80000000
70000000
60000000
50000000
40000000
30000000
20000000
10000000
0
0
10
20
30
40
50
60
Average Amount Ammonia in PPM in 1cc of soil


Mold vs. Ammonia
Average Amount of Mold colonies in 1cc of soil
70000
60000
50000
40000
30000
20000
10000
0
0
10
20
30
40
50
60
Average Amount of Ammonia in PPM in 1 cc of soil



Preliminary Analysis

The bare soil, when looking at its average amount of bacteria, yeast and mold, has a fairly low amount. This is predicted pattern of unfertilized soil with no plants. The level of ammonia is also low in comparison to the campus and playing field grass which is also in the predicted pattern.

The unfertilized grass, when looking at the average amount of bacteria, mold and yeast, has a fairly high amount in comparison to the bare soil. The unfertilized grass also has a fairly low amount of ammonia. This is the predicted pattern for unfertilized soil with grass.

The playing field grass has a high amount of ammonia in comparison to the bare soil and unfertilized grass. The playing field grass has a low amount of mold and a high amount of bacteria. This data would fit in the predicted pattern for fertilized grass. The playing field grass has a high amount of yeast in comparison to the bare soil and unfertilized grass. This data does not fit with the predicted pattern for fertilized grass.

The campus grass has a high amount of ammonia in comparison to the bare soil and the unfertilized grass. The campus grass also has a low amount of yeast. The campus grass has less bacteria than the unfertilized grass and more bacteria than the bare soil. This data fits with the predicted pattern for grass with fertilizer. The campus grass has less mold than the unfertilized grass and more mold than the bare soil.

When looking at the Ammonia vs. Bacteria graph, one observes that when the amount of ammonia increases the number of bacteria in the soil increases. This was the predicted trend.

When looking at the mold vs. ammonia graph, one observes that when the amount of ammonia in the soil is increased, the amount of mold decreases. This data follows the predicted trend.

When looking at the yeast vs. ammonia graph, one observes that the as the amount of ammonia in the soil increases, the amount of yeast increases. This was not the predicted trend for the data..

Conclusion:

Our hypothesis, which stated that the PKN ratio of 24-3-11 would disturb the soil ecosystem more than the PKN ration 24-3-7, was incorrect. The PKN ratio 24-3-11 corresponds with the Campus grass while the PKN ratio 24-3-7 corresponds with the Playing Field grass. When fertilizer is added to soil, ammonia levels increase. The increase in Ammonia levels usually decreases the amount of decomposers (yeast and mold) and increases the number of bacteria. Therefore soil with fertilizer should have higher ammonia levels and bacteria than soil without fertilizer. Soil with fertilizer should also have lower amounts of mold and yeast than soil without fertilizer. The campus grass has 19.587 more ppm of ammonia than the bare soil and the unfertilized grass. The campus grass also has 24.497 less ppm of ammonia than the playing field grass. The campus grass should therefore have fewer bacteria than the playing field grass and more mold and yeast. The campus grass follows the appropriate pattern for its level of Bacteria, yeast and mold. The campus grass has an average of 3033334 more colonies of bacteria than the bare soil and 648333333 less colonies than the unfertilized grass. This is expected because the unfertilized grass is a very healthy eco-system and would therefore have more activity and more bacteria, mold and yeast. The campus grass also has 10633333 less bacteria colonies than the playing field grass which is expected because it has a lower ammonia level and therefore fewer bacteria. The campus grass has 10833.33 more mold colonies than the bare soil and 4000 more mold colonies than the playing field grass. This is expected because the campus grass has less ammonia than the playing field grass and therefore more mold colonies. The campus grass also has more activity than the bare soil and therefore more mold colonies. The campus grass has 16666.67 less mold colonies than the unfertilized grass which would be predicted because the unfertilized has much more activity than the campus grass. The campus grass has 753333.33 less yeast colonies than the unfertilized grass which would be expected because the unfertilized grass has less ammonia than the campus grass and therefore more yeast colonies. The

campus grass also has 26333.34 more yeastcolonies than the bare soil which would be expected because the campus grass has a higher activity level than the bare soil and therefore more yeast colonies. The playing field grass follows the predicted trend for its amount of bacteria and mold. The playing field grass has 13666667 more bacteria colonies than the bare soil and 197833333 more bacteria colonies than the campus grass. This expected because the playing field grass has more ammonia than these two locations and therefore more bacteria colonies. The playing field grass also has 54200000 less bacteria colonies than the unfertilized grass which is expected because the unfertilized grass has more activity and therefore more bacteria, yeast, and mold colonies. The playing field grass has 20666.67 fewer mold colonies than the unfertilized grass which is expected because the unfertilized grass has a higher level of activity and therefore more mold colonies. The playing field grass has 4000 less mold colonies than the campus grass. This is expected because the playing field grass has more ammonia than the campus grass and therefore fewer mold colonies. The playing field grass has 6833.33 more mold colonies than the bare soil which is expected because the playing field grass has a higher level of activity than the bare soil. However, the level of yeast for the playing field grass does not follow the expected trend for soil with fertilizer. The playing field grass has at least 1149333 more yeast colonies than any other location. This does not follow the expected trend because the playing field has the highest ammonia level and should therefore have fewer molds than any other location. Thus our hypothesis was incorrect and the 24-3-7 PKN ratio, which corresponds with the Playing field grass, disturbs its soil ecosystem more. In the future, we would like to research why the yeast level in the playing field soil is so high. We also might pursue the question of why the bacteria in the campus and playing field grass is so low in comparison to the unfertilized grass when the playing field and campus grass have been exposed to a high amount of ammonia.