Background Essay

Microbes, a shorter way of saying microorganisms, are tiny creatures that cannot be seen with the naked eye. Microbes have been around for billions of years because they are able to adapt to the ever-changing environment (Microbe Zoo, 2002). Contrary to popular belief, not all microbes live in the soil, nor only live in the soil. Microbes live in a variety of places because of their trait of adaptation. Microbes have an immense impact on human life in different ways. Some microbes harm humans by causing disease, such as viruses and prions. But, also some microbes help humans. Many microbes are used to make medicine, break down oil from oil spills, and some even help in the production of the oxygen that we breathe (American Society for Microbiology, 1999). Therefore, microbes are extremely relevant to our everyday lives.

The soil represents a favorable habitat for microbes and is inhabited by a wide variety of microbes, including bacteria, fungi, algae, viruses, and protozoa (UCC, 2003). Microbes are found in large numbers in soil, with bacteria being the most prevalent (AEHS, 2001). However, the availability of nutrients is often limiting for microbial growth in soil and most soil microbes may not be actually active or properly functioning in soil at a given time (UCC, 2003). Soil microbes are extremely important, as almost every chemical transformation that takes place in the soil involves active contributions from soil microbes. In particular, they play an active role in soil fertility as a result of their involvement in the cycle of nutrients like carbon and nitrogen, which are required by plant growth (UCC, 2003).

Soil microbes are responsible for the breakdown of organic matter, the conversion of inorganic components from one form to another, and the production of humus (AEHS,

2002), which is a "brown or black organic substance consisting of partially or wholly decayed vegetable or animal matter that provides nutrients for plants and increases the ability of soil to retain water" (dictionary.com, 2003). Furthermore, soil microbes are a major part of soil fertility because of their active role in the cycle of nutrients. This includes carbon and nitrogen to nutrients, which are required for the growth of plants (UCC, 2003). Yet, as in most cases, there is an up to every down; the same applies with soil microbes.

There are beneficial and detrimental soil microbes. Beneficial microbes have numerous tasks and responsibilities. Some of their tasks include the decomposition of organic matter, increasing the availability of mineral nutrients, and increasing the amount of nutrients (UCC, 2003). An example of the decomposition of organic matter is plant litter entering the soil and therefore in the recycling of nutrients, like nitrogen in the soil. Increasing the availability of mineral nutrients includes phosphorus to plants and increasing the amount of nutrients includes potassium, which is present in the soil. In order to decompose the organic matter, microbes secret enzymes that essentially dissolve the plant material left as trash (MSU, 2001). These microorganisms, which improve the fertility status of the soil and contribute to plant growth have been termed "biofertilizers" and are receiving more and more attention for use as microbial inoculants, in agriculture (UCC, 2003).

In contrast to the beneficial tasks of soil microbes are soil microbes that are pathogenic, capable of causing disease to plants and may cause considerable damage to crops, such as row crops. These microbes infect the plant through its roots (UCC, 2003). However, certain native microbes pre-existing in the soil are antagonistic to these

pathogens and can prevent the infection of crop plants (MSU, 2001). All in all, soil microbes play a sufficient number of roles that the beneficial microbes dominate the detrimental microbes. A significant number of soil microbes are bacteria and they overpower both detrimental and beneficial microbes in several significant ways.

Bacteria are tiny, one-celled, prokaryotic organisms, but what they lack in size, they make up in numbers. For example, 4.72 grams of productive soil generally contains between one hundred million and one billion bacteria (American Society for Microbiology, 1999). Bacteria perform so many tasks that all of the tasks cannot be grouped into one category. In fact, bacteria fall into four functional groups. Most bacteria are decomposers, which consume simple carbon compounds (Soil Quality Institute, 2000).

By consuming simple carbon compounds, they convert energy in soil and organic matter into forms useful to the rest of the organisms in the soil food web (Soil Quality Institute, 2000). Many decomposers break down pesticides and pollutants in the soil, too, and in addition decomposers are especially important in immobilizing, or retaining, nutrients in their cells, thus preventing the loss of nutrients, such as nitrogen, from the rooting zone (Soil Quality Institute, 2000). Preserving nitrogen is vital because nitrogen is an element that plants and all living matter must have to make proteins and DNA (Johnson, 1998).

Separate from the decomposers is another group of bacteria, called the mutualists. The mutualists form affiliations with plants, the most well known of these being nitrogen-fixing bacteria (Soil Quality Institute, 2000). Almost opposite from the mutualists is another group of bacteria, the pathogens, many of which cause awkward formations in

plants (Soil Quality Institute, 2000). The last group of bacteria, called lithotrophs or chemoautotrophs, obtains its energy from compounds of nitrogen, sulfur, irony, or hydrogen as an alternative of carbon compounds. Some of these species are important to nitrogen cycling and degradation of pollutants (Soil Quality Institute, 2000). All four groups of bacteria play roles of impact in the nitrogen cycle, a major cycle that is performed throughout the soil.

All life requires nitrogen compounds in order to live because nitrogen is an element that everything living must have to make proteins and DNA (Johnson, 1998). Nothing can live without DNA or proteins. DNA copies itself into RNA, which makes proteins. Proteins cause chemical reactions that cause the chemicals of the cell (lipids, carbohydrates, water, proteins, nucleic acids) to react between each other. These chemical reactions are how the cell performs its four tasks: reproduction, manufacture of chemicals, respiration, and synthesis.

Air, which is seventy-nine percent nitrogen gas, is the major reservoir and most abundant source of nitrogen (Nitrogen Cycle, 2001). However, most organisms, including plants, cannot obtain nitrogen from the air. And as stated before, everything needs nitrogen to live. Plants must secure their nitrogen in “fixed” form, such as nitrate ions, ammonia, or urea (Nitrogen Cycle, 2001). Plants covert nitrogen into a form that they can use through a process called Nitrogen Fixation (Nitrogen Cycle, 2001). This is where bacteria come into play. Four processes participate in the cycling of nitrogen through the biosphere. These include nitrogen fixation, decay, nitrification, and dendrification, all of which microbes play major roles in (Nitrogen Cycle. 2001). These four processes keep everything alive and break apart the nitrogen molecules so they are usable.

The nitrogen molecule is motionless; to break it apart so that its atoms can combine with other atoms requires the input of substantial amounts of energy (Nitrogen Cycle, 2001). Three processes are responsible for nitrogen fixation in the biosphere. These processes are atmospheric fixation by lighting, biological fixation by certain microbes, along or in a symbiotic relationship with plants, and industrial fixation (Nitrogen Cycle, 2001). The proteins made by plants enter and pass through food webs just as anything else does. At each trophic level, their metabolism produces organic nitrogen compounds that return to the environment, mainly in excretions, the act or process of discharging waste matter (Nitrogen Cycle, 2001). The final recipients of these materials are microbes of decay; they break down the molecules in excretions and dead organisms into ammonia (Nitrogen Cycle, 2001). Plants, usually through their roots, can directly take up ammonia, but most of the ammonia produced by decay is converted into nitrates by the accomplishment of two steps (Nitrogen Cycle, 2001). Through nitrifying bacteria's activities, nitrogen is made available to the roots of plants. The three processes stated above remove nitrogen from the atmosphere and pass it through ecosystems. Dentrification reduces nitrates to nitrogen gas (Nitrogen Cycle, 2001). Again, bacteria are the agents. Bacteria use nitrates as an alternative to oxygen for the final electron acceptor in their respiration (Nitrogen Cycle, 2001). An acceptor receives two electrons to form a chemical bond with another atom (dictionary.com). Consequently bacteria close the nitrogen cycle.

The big picture of the nitrogen cycle is to show that bacteria play significant roles in this process that all life needs and by altering one or two of the factors (in the nitrogen cycle or number of bacteria, amount of food available to bacteria in order for them to

perform their responsibilities) there can be a significant or even drastic change. Humans cause this change and we hope to find the change as a result of performing our experiment.

Fertilizer has the number one effect on plants, soil, and microbes. The nutrients and chemicals that fertilizer consists of alter the soil composition and thereby affecting everything that relies on, or works for soil. The three key nutrients of fertilizer are Nitrogen, Phosphorous, and Potassium (Fertilizer University, 2002). Other nutrients in fertilizer are Calcium, Magnesium, and Sulfur (Fertilizer University, 2002). By using fertilizer the nitrogen cycle is modified because of the input of nitrogen quantity (from the fertilizer). Hence, having an affect on the bacteria as well by giving them more "food" or energy to be able to perform their job in the nitrogen cycle. If the "food" is cut short then the bacteria will theoretically not have enough energy to perform their job. By using different concentrations of fertilizer we want to out what impact it has on bacteria and the relating nitrogen cycle.

For our experiment we are going to test different concentrations of fertilizer to see what impact it has on the density of bacteria in a certain plot. The different fertilizer concentrations are: .9 grams for 1000 mL of water, 1.88 grams for 1000 mL of water, and 3.8 grams for 1000 mL of water; we also used plain water as a solution as well. How we arrived at the particular numbers for the amount of Miracle-Gro was by using the miracle-gro box as a guide. The box said to mix one tablespoon of Miracle-Gro for every gallon of water. We found out that one tablespoon was equal to 7.1 grams and then we put this into proportion with a liter. One gallon is equal to 3.78 liters thus our proportion looked as follows: 7.1 grams/3.78 liters=X grams/1 liter. By solving the proportion we

found out that .88 grams was proportionate to one liter. We rounded .88 grams to .9 grams, which was ½ of the recommended amount of Miracle-Gro. The recommended amount was approximately 1.88 grams and that doubled was approximately 3.8 grams. Each amount of Miracle-Gro is mixed with the same amount, 1000 mL, of water.

The purpose of our experiment is to determine whether adding additional fertilizer to a plot of soil will increase or decrease the density of bacteria in that plot and also have an effect on the soil composition, especially the nitrogen cycle. We will see if different concentrations of fertilizer have a different effect on the total number of bacteria along with the nitrate level in the soil. Then, by performing the Serial dilutions test with easy gel plates and the Lamotte Nitrate Nitrogen Test we anticipate to make some conclusions about the different concentrations of fertilizer in comparison to bacteria and the nitrate level. This may help us in finding the link between bacteria and the nitrogen cycle as well. Also, by performing our experiment we hope to see what particular impact humans carelessly have on the microbe environment they rely on so much. People have little or no knowledge about microbes and how much they are needed in and for the human life, and by adding fertilizer to their grass they could really be hurting something very significant. We hope to draw many conclusions from our experiment.

Lab Report

I. Problem: Does the concentration of fertilizer on a set plot of soil increase or decrease the density of bacteria in that certain plot?

II. Hypothesis: If we increase the concentration of fertilizer in a given plot of soil, then the density of bacteria will increase in that given plot of soil.

III. Experiment:

A) Variables

1. Independent Variables

IV1: Concentration of fertilizer
IV2: Concentration of nitrate

2. Dependent Variable

DV1: Density of bacteria per cubic centimeter

B) Controls

1. Negative Control

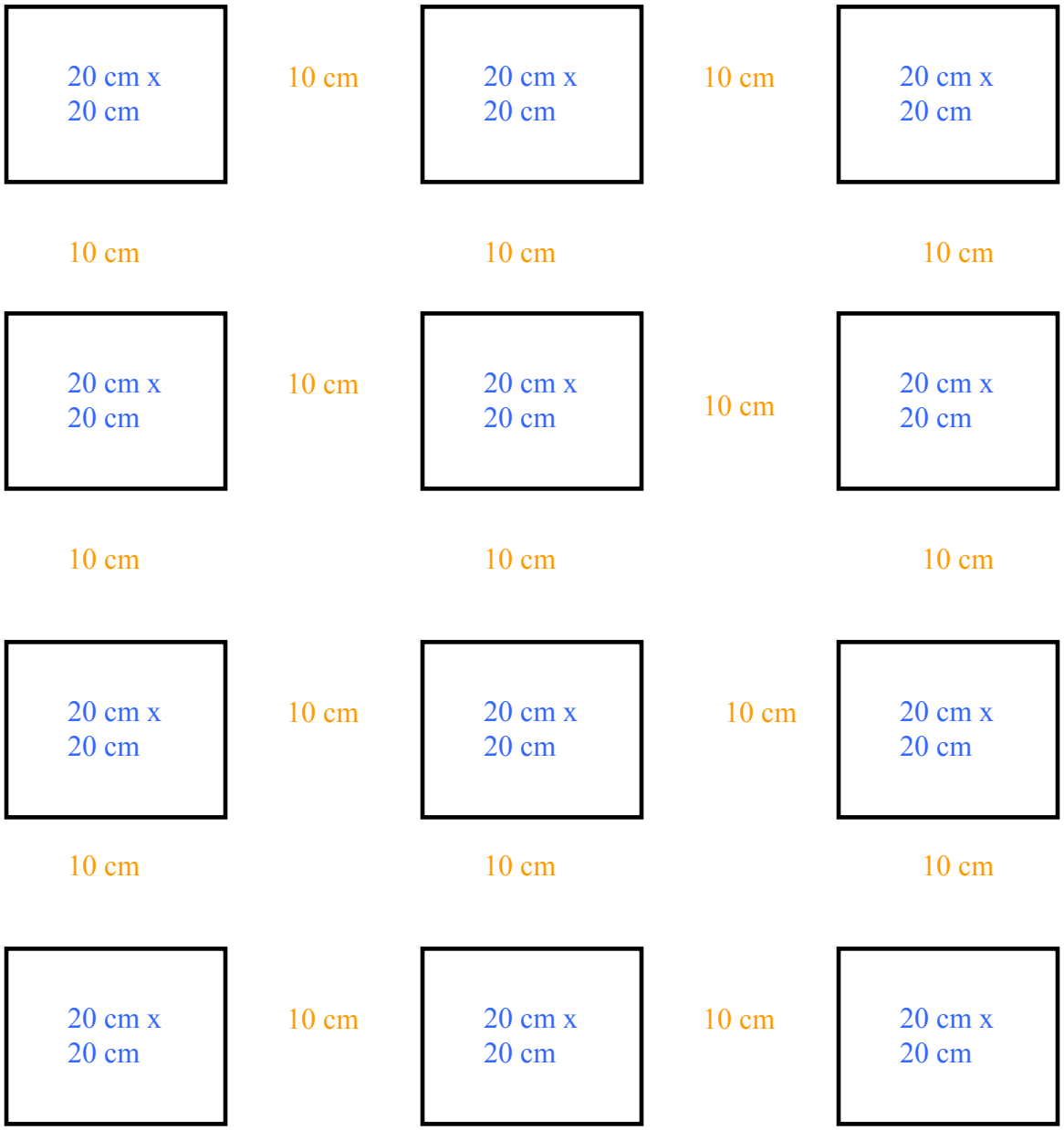
NC1: Soil plots without any additional fertilizer
NC2: Bacteria and nitrate levels in plots before additional fertilizer added

3. Controlled Variables

- Plot size
- Time of data collection
- Location of plots
- Method of data collection
- Depth of data collection
- Amount soil collected
- Places the sample is taken in the plot
- Type of fertilizer
- Water amounts
- Volume of liquid
- Temperature of plot environment
- Light
- Touch
- Movement
- Moisture
- Sound
- Food source
- Precipitation
- Runoff
- Topography of land
- Type of soil
- Spacing between plots
- Plot location
- Size of bottle used

C) Procedure

1. Using a meter stick, mark off with marker flags 12, 20 cm x 20 cm plots with 10 cm in between each plot at North 39.35693, West 076.63500. Use the diagram below as a reference and directions on how to set up the plots.



2. Get a package of Miracle-Gro and four 1000 mL bottles
3. In one of the 1000 mL bottle put 1000 mL of water into it, labeling the bottle “H2O”.
4. Using a balance, mas.9 grams of Miracle-Gro and put in another one of the 1000 mL bottles. In that same bottle put in 1000 mL of water. Shake the bottle until all of the fertilizer is dissolved. Label this 1000 mL bottle “½”.
5. Using a balance, mass 1.88 grams of Miracle-Gro and put it in another one of the 1000 mL bottles. In that same bottle put 1000 mL of water. Shake the bottle until all of the fertilizer is dissolved. Label this 1000 mL bottle “1”.
6. Using a balance, mass 3.8 grams of Miracle-Gro and put it into the last of the 1000 mL bottles. In that bottle put 1000 mL of water in it. Shake until all of the fertilizer is dissolved. Label this 1000 mL bottle “2”.
7. Make 3 bottles of each treatment the same way as in steps 3, 4, 5, and 6.
8. At the 12 plots located at North 39.35693, West 076.63500, randomize the four treatments over the 12 plots. Do this by randomly (you can map it out on a sheet of paper before hand by randomly picking certain plots for each treatment so you know where to pour) pouring one full bottle of each treatment into different plots, consequently having 3 plots with the “H2O” treatment, 3 plots with the “ ½” treatment, 3 plots with the “1” treatment, and 3 plots with the “2” treatment. Make sure to mark the marker flags at the corners of the plots with the particular treatment it is receiving so you can distinguish between all of the plots. (Label the marker flags for the first plot that receives H2O treatment, H2OA and so on). Pouring instructions in the next step.
9. Pour 1 full 1000 mL bottle (with a particular treatment for a particular plot) in the center of each plot. Repeat steps 8 and 9 for all 12 plots, pouring the contents of the 1000 mL bottle the same way, in the center each time.
10. Let the treatments sit on the plots for 30 hours.
11. After 30 hours go out to the plots with a soil test core.
12. In each of the 12 plots take 3 samples of soil, 15 cm deep by 2 cm wide soil sample (each), using the soil test core. The three sample locations are: the north corner, center, and northeast corner of the plot.
13. Place each sample in a separate plastic bag. Using a, b, and c to distinguish between the individual plots of soil for each treatment and 1 (north), 2 (center), and 3 (northeast) for the location of the soil sample in the plot. (Example: H2OA1=H2O treatment, 1st plot with H2O treatment, north corner)
14. At the end of the soil collections you should have 3 soil collections for each of the 12 plots; one collection from the north corner of the plot, one from the center of the plot, and one from the northeast corner of the plot; all of which are 15 cm deep by 2 cm wide. There will be a total of 36 soil collections.

15. Take H2OA1 and simultaneously perform the LaMotte Nitrate Nitrogen Test, and the Serial dilutions test using easy gel plates. Follow the LaMotte Nitrate Nitrogen Test directions.
16. As for the Serial dilutions test you will have to plate all dilutions the first time on easy gel plates, and for the times after the first plate only 2 of the dilutions, which you think is the best for counting the bacteria. (As for us, we plated 10^-3 and 10^-4 for most except sometimes 10^-5 and 10^-6 after the 1st test because those were the dilutions that were easiest to count.) When plating the dilutions plate it right side up on the easy gel plates (using the easy gel solution) then about 45 minutes later flip the plates over so the bacteria can grow.
17. About 2 days later count the bacteria colonies that have grown in the plates, pick the lowest dilution that you have plated and is easy enough to count.
18. Repeat steps 16-18 for each of the remaining soil collections from each location in the plot from all 12 plots. Both tests must be done simultaneously.
19. For the Lamotte Nitrate Nitrogen Test record the result pounds per acre then convert it to parts per million using the formula: pounds/acre x .5 Record this in a data table.
20. Also record the number of bacteria per cubic centimeter of soil (in the easy gel plate) in each plate (this is what you counted). Use that number the following formula: # colonies on plate x 10^2=# of bacteria in dilution tube; # of bacteria in dilution tube x 10^(# of dilution colonies)=# of bacteria in original sample tube

IV. Data and Analysis

A. Data

NITRATE (PPM) AND BACTERIA (DENSITY PER CC OF SOIL) COUNTS				
Fertilizer (g/mL)	**Location**	**Sample Location**	**Nitrate (ppm)**	**Bacteria (# per cc of soil)**
Plots with 0 grams per mL of miracle-gro	Plot A	North Corner	15	78000000
		Center	15	5000000
		NE Corner	15	14000000
	Plot B	North Corner	15	279000000
		Center	15	10000000
		NE Corner	15	13000000
	Plot C	North Corner	30	4000000
		Center	62.5	31000000
		NE Corner	25	24000000
Plots with .0009 grams per mL of miracle-gro	Plot A	North Corner	25	38000000
		Center	62.5	27000000

miracle-gro		NE Corner	62.5	20000000
	Plot B	North Corner	75	25000000
		Center	75	8000000
		NE Corner	62.5	2700000000
	Plot C	North Corner	50	28000000
		Center	50	125000000
		NE Corner	62.5	1300000000
Plots with .00188 grams per mL of miracle-gro	Plot A	North Corner	75	7200000
		Center	15	5600000
		NE Corner	62.5	23000000
	Plot B	North Corner	62.5	5800000
		Center	75	18000000
		NE Corner	75	29000000
	Plot C	North Corner	62.5	6000000
		Center	25	8100000
		NE Corner	40	11600000
Plots with .0038 grams per mL of miracle-gro	Plot A	North Corner	75	9000000000
		Center	62.5	650000000
		NE Corner	75	12100000
	Plot B	North Corner	75	321000000*
		Center	75	350700000*
		NE Corner	75	1700000000
	Plot C	North Corner	75	392300000*
		Center	50	463700000
		NE Corner	75 (+)	260000000

*To count the bacteria densities in these dishes we mapped 1square cm on the dish and counted the number of bacteria in that square and then multiplied that answer by the total number of square cm in the petri dish. Therefore we cannot verify and be certain about these bacteria densities per cc of soil.

B. Analysis

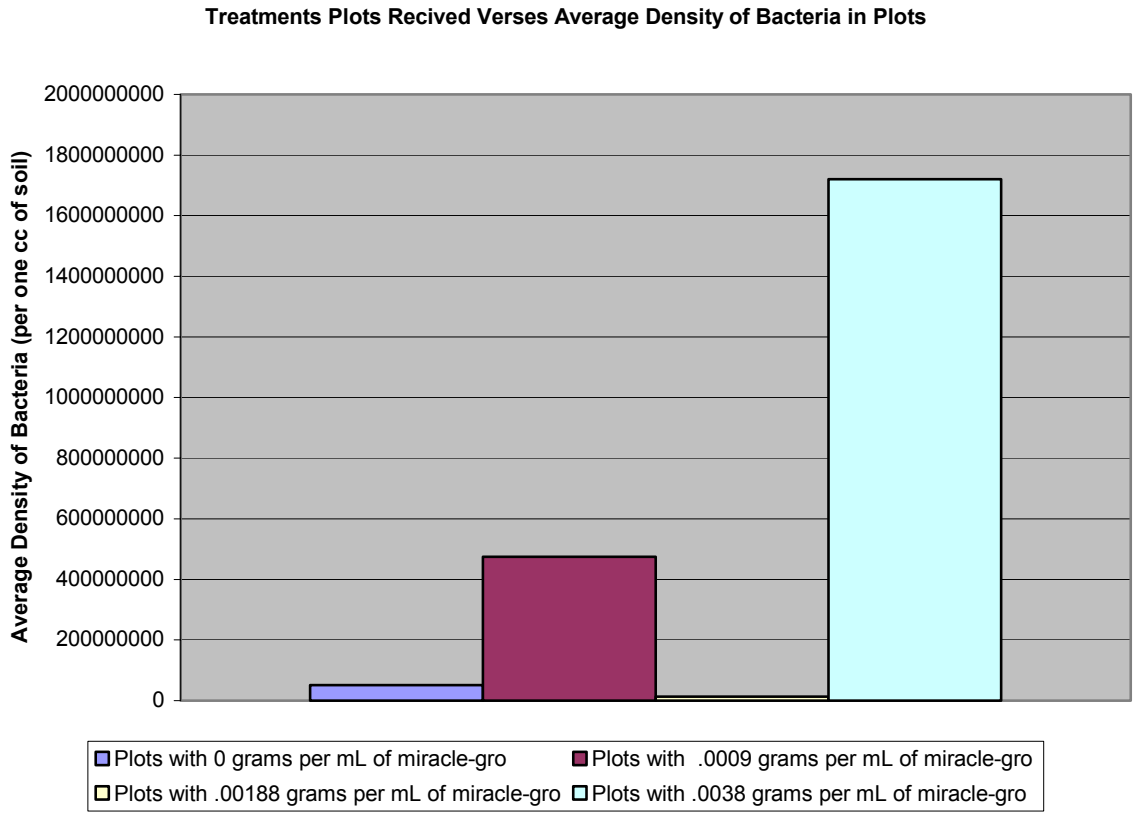
AVERAGES

COndition	Nitrate (average ppm)	Bacteria (avg. # per cc of soil)
Plots with 0 grams per mL of miracle-gro	23.056	50888888.890
Plots with . 0009 grams per mL of miracle-gro	58.333	474555555.600
Plots with .00188 grams per mL of miracle-gro	54.722	12700000.000

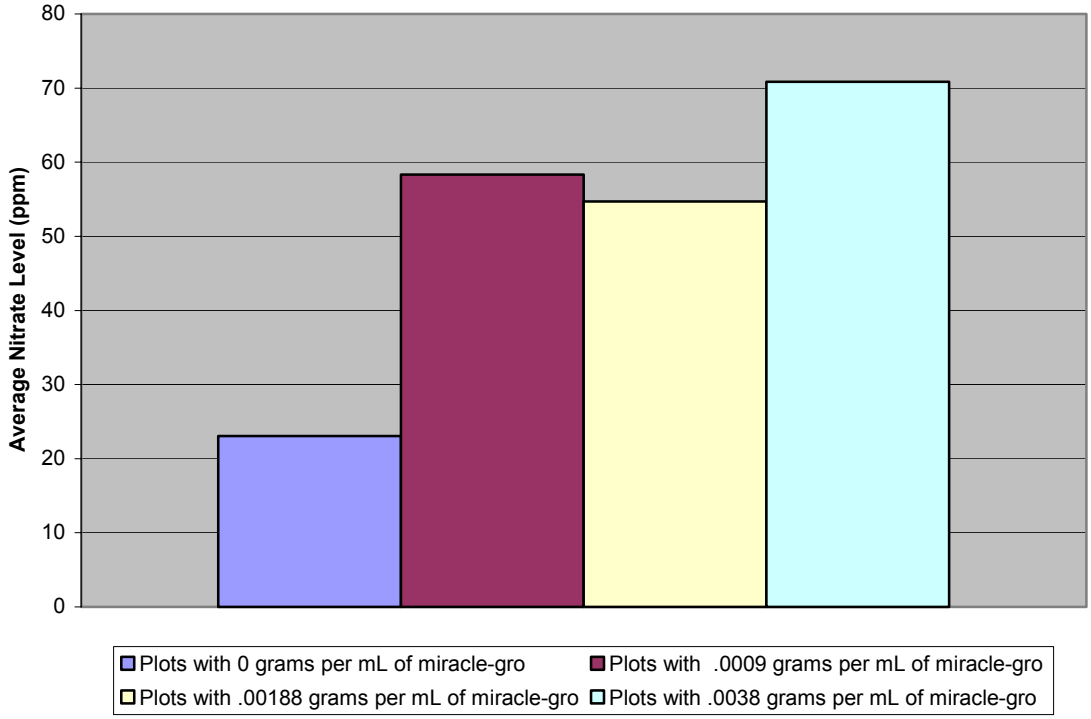
Plots with .0038 grams per mL of miracle-gro	70.833	1721088889.000

NITRATE LEVELS (PPM) COMPARED TO AVERAGE BACTERIA DENSITY (PER CC OF SOIL)

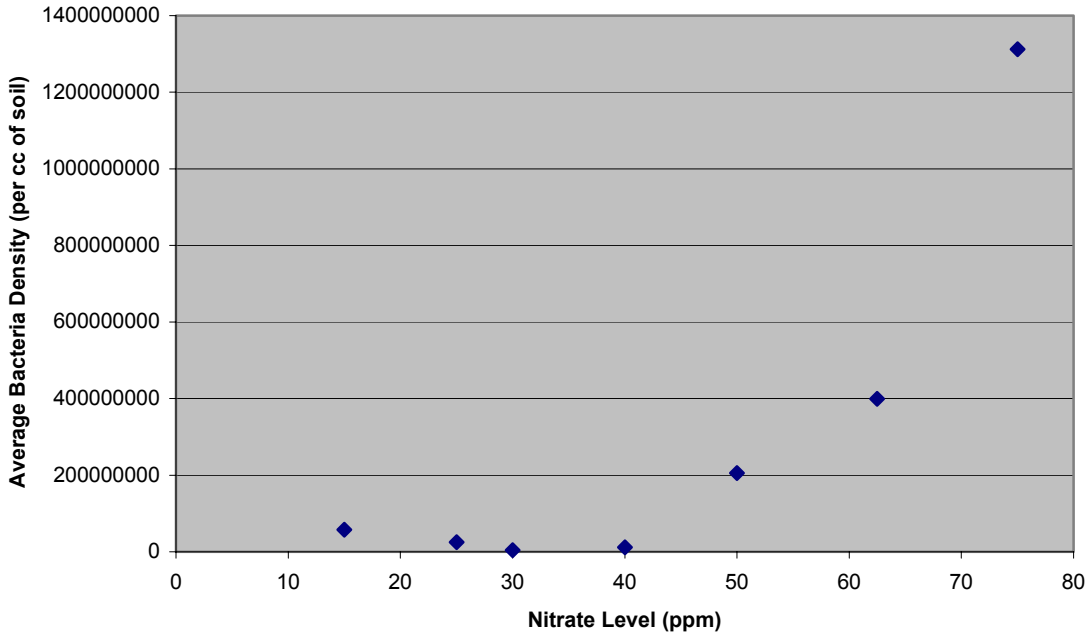
Nitrate Level (ppm)	Bacteria (avg. # per cc of soil)
15	57800000
25	24775000
30	4000000
40	11600000
50	205566666.7
62.5	399200000
75	1312209091



Treatments on Plots Verses Average Nitrate Levels in Plots



Nitrate Levels (ppm) Compared to Average Bacteria Density (per cc of soil) of That Nitrate Level



V. Conclusion

Our hypothesis was wrong because the average density of bacteria did not increase in a linear fashion as the concentration of the fertilizer increased. The average bacteria density per cc of soil increased from the condition of 0 grams per mL of miracle-gro to the condtion with .0009 grams per mL of miracle-gro. The average increase of bacteria density per cc of soil, between these two conditions was 423666666.7. The average bacteria density per cc of soil also increased from the condition of .00188 grams per mL of miracle-gro to .0038 grams per mL of miracle-gro. This average increase of bacteria density per cc of soil between these two conditions was 17083888899. However, there was no increase in average bacteria density per cc of soil between the .0009 grams per mL of miracle-gro condition the .00188 grams per mL of miracle-gro condition. In fact, there was a decrease; the decrease of average bacteria density per cc of soil between these two conditions was 461855555.6.

Consequently, as we increased the concentration of fertilizer there was an overall increase of the average nitrate levels as well. From the plots with 0 grams per mL of miracle-gro to the plots with .0009 grams per mL of miracle-gro, the average nitrate level increased 35.277 parts per million. From the plots with .0188 grams per mL of miracle-gro to the plots with .0038 grams per mL of miracle-gro, the increase of the average nitrate level was 16.111 parts per million. Conversely, there was an average nitrate level decrease between the plots with .0009 grams per mL of miracle-gro and the plots with .00188 grams per mL of miracle-gro. The average nitrate level between these two conditions decreased 3.611 parts per million. However this decrease of 3.611 parts per million is a very insignificant decrease and nevertheless there is still an overall increase in the average nitrate levels as the concentration of fertilizer is increased.

The nitrate levels compared to the average bacteria density per cc of soil had a positive correlation. The average bacteria density per cc of soil for nitrate levels 15 parts per million, 25 parts per million, 30 parts per million, and 40 parts per million were fairly constant and linear. At a nitrate level of 15 parts per million the average bacteria density per cc of soil was 57800000. At a nitrate level of 25 parts per million the average bacteria density per cc of soil was 247750000. At a nitrate level of 30 parts per million, the average bacteria density per cc of soil was 4000000. At a nitrate level of 40, the average bacteria density per cc of soil was 11600000. Although there were some increases and decreases within these four nitrate levels, nonetheless the average bacteria density per cc of soil stayed moderately and relatively constant. Between the nitrate levels of 40 parts per million and 50 parts per million, there was an average bacteria density per cc of soil increase of 193966666.7. From the nitrate level of 50 parts per million to the nitrate level of 75 parts per million then was a consistent increase. From 50 parts per million to 62.5 parts per million the average bacteria density per cc of soil increased 193633333.3. From 62.5 parts per million to 75 parts per million the average bacteria density per cc of soil increased 913009091.

There are several valid possible explanations for why the average bacteria density per cc of soil did not show an increase as the concentration of fertilizer increased, even though the average nitrate levels increased as the concentration of fertilizer increased and the average bacteria density per cc of soil increased as the nitrate levels. The data averages showed that the average bacteria density per cc of soil in the plots with .00188 grams per mL of soil had the most dramatic decrease. One reason for this is that

all three of the plots with this concentration of fertilizer were located on the outskirts of the site. This enabled the microbes in these plots to interact with the organisms outside of our site, which could have stimulated the decrease of average bacteria density within these plots. Another justification for this decrease is that our site was on an incline, and the probable upper left to lower right runoff pattern of the fertilizer put the plots with .00188 grams per mL of soil in a position of being the least likely treatment to receive supplementary fertilizer. The plots with .0038 grams per mL of soil were in a position of being the most likely treatment to receive supplementary fertilizer, which could be a reason for the dramatic increase of bacteria density per cc of soil in those plots.

As a direction to those who might perform or expand on our experiment in the future, the following directions are advice for continued research. First, perhaps more significant intervals between the different fertilizer concentrations would help to distinguish the pattern of bacteria and nitrate growth. Second, while using random distribution of treatments, make sure that any sort of probable runoff pattern would ensure supplementary fertilizer to at least one plot for each treatment. Another possibility would be not using random distribution at all. Lastly, the site where the plots are located should be as free as possible of obstacles that may hinder the process of taking soil samples.

Works Cited

American Society for Microbiology (1999). http://www.microbe.org

Discovery Zoo, (2002). "Bacteria".
http://school.discovery.com/schooladventures/soil/fg_bacteria.html

Fertilizer University (2002). http://fertilizer.com/University

Johnson, George B. (1998). *Biology: Visualizing Life*. New York: Holt, Rinehart and Winston.

Microbe Zoo (Michigan State University, 2000). "What is a Microbe?"
http://commtechlab.msu.edu/sites/dlc-me/zoo/ziwim.html

Montana State University (1998). "What do Fungi and Bacteria do in the Soil?"
http://www.montana.edu/wwwpb/ag/baudr150.html

Natural Resources Conservation Service (NRCS, 2001).
http://soils.usda.gov/sqi/sqw.html

Nitrogen Cycle (2001).
http://users.ren.com/jkimball.ma.ultranet/BiologyPages/N/NirtogenCycle.html

Soil Quality Institute (2000). "Chapter 3: Bacteria."
http://soils.usda.gov/sqi/SoilBiology/bacteria.htm

Soil Sediment and Water (AEHS, 2002). "Soil Microorganisms."
http://www.aehsmag.com/issues/2002/january/microorgansims.htm

University College Cork (UCC, 2003). "The Microbial Community of the Soil Ecosystem." http://www.ucc.ie/impact/agri2f.html