Background

When given the assignment to pick a topic of specific research, our group decided to examine whether adding fertilizer, compost, or water increased the bacteria count in the soil the most. Fertilizer and compost can both be added to soil to increase levels of certain chemicals such as carbon and nitrate. Bacteria are always present in plant soil but the number of bacteria varies based on the level of chemicals such as nitrate and carbon. Since fertilizer, compost, and water change nitrate concentration, we examined how they would change the bacteria level over a series of soil testings, comparing nitrate concentration to the number of bacteria. Our group set aside several plots of land and examined bacteria count in the plots and then added fertilizer to one plot, compost to another, and left the third plot with adding only water. Using serial dilution method we examined the before and after bacteria count, to see which method increased the bacteria count the most. The fertilizer and compost use ingredients such as nitrate, phosphorus, and potassium, which are essential to plant growth so we also tested the nitrate levels before and after to examine the comparison between nitrate and bacteria growth. In short, our experiment examined the relationship between bacteria count and nitrate concentration and how adding fertilizer, compost, and water affected this relationship and growth.

We tested our soil from the RPCS backwoods at N 39.35698°, W 76.63667°. The soil provides a home for organisms such as bacteria, protozoa, fungi, algae, actinomycetes, nematodes, worms, insects, and other animals. Fertilizer and compost help plants and bacteria grow in the soil because they increase carbon and nitrate levels, thus making the carbon and nitrogen cycles activate at a greater amount. However, soil without fertilizer or compost also proves to be a significant part of the ecosystem. This soil without fertilizer or compost is able to grow based on cycles such as the carbon cycle, nitrogen cycle, and decomposition of dead organisms. (Soil Ecology Background Information, 2003)

The nitrogen cycle helps convert chemicals into usable nitrogen for plants and return ammonium to the soil. The process in which they do this begins in the atmosphere where there is "free N_2". The N_2 goes in to the ground where there are Nitrogen- fixing bacteria that converts the $N_{2\ \mathrm{to}}$ Nh_3. Then it goes deeper into the soil and the NH_3 becomes NH_4+ (ammonium) in soil. Nitrifying bacteria convert NH_4+ to NO_3- to usable nitrogen for plants. At the same time decomposers return the ammonium to the soil. Plants use (NO_3-) and NH_4+. Then the consumer eats plants. And at the same time Denitrifying bacteria convert NO_3- back into $N_{2,}$ which goes back to air. (Campbell, Neil A. Williamson, and Heyden, 2004). It is important that these waste products get recycled into the air because if they did not, the organisms would literally be swimming in their own feces. Waste products must be emitted into the air so that they do not build up inside the soil. (Mr. Brock, May 27, 2004). The nitrogen cycle makes nitrogen usable for plants while simultaneously allowing the carbon cycle to occur.

In the carbon cycle, the recycled carbon in plants, through photosynthesis, is used to produce oxygen for all other organisms. Carbon and nitrogen help keep the plant alive, each depending on each other. They use each other to recycle so they can both stay alive, thus keeping the soil alive. The carbon cycle and nitrogen cycle both allow the excretion of waste products and simultaneously the decomposers are excreting the waste products. (Campbell, Williamson, and Heyden, 2004).

The decomposition of dead organisms is important because the nutrients that come from the dead matter can also help the soil survive. The soil is also kept alive by the recycling of plant nutrients, controlling disease, absorbing water and nutrients, and the mixing of soil. These processes help soil without fertilizer or compost remain alive and efficient. (Campbell, Williamson, and Heyden, 2004).

In addition to the above cycles, bacteria growth can be stimulated by compost piles. Compost piles are the natural method which uses the combined natural ingredients containing nitrogen, phosphorus, and potassium to affect the natural cycles of plants living, dying, and breaking. The compost pile should be a balance between nitrogen and carbon, wet and dry, and acidic and basic components, and access to oxygen at all times. Nitrogen, which comes from grass clippings and dead green plants, is what allows the microorganisms to break down carbon and other organic matter to make it compost in the nitrogen cycle and the decomposers break nutrients into usable form. (Facts of the Case Composting 2004) Compost piles are a natural source of nutrients from other resources to improve fertility of the soil, thus increasing bacteria count. One aspect of our experiment is a test to see how the compost pile affects the nitrate concentration and thus the bacteria count.

Fertilizer, a factory made version of compost, also plays a role in improving the fertility of the soil based on its quantitative effects upon nitrate concentration and bacteria count. Although nutrients necessary for plant growth are available naturally from the earth's soil, the atmosphere, and animal manure, manmade fertilizer is often more beneficial for plants because sometimes the necessary nutrients are not available in their highest quantity in the natural environment. Factors that deplete the environment of its resources are overuse of the environment, and other animals, who often exonerate the needed nutrients. Fertilizer contains the correct ingredients, using a chemical balance to best stimulate plant growth. (What is fertilizer, 2004) These chemicals include nitrogen, phosphorus, and potassium, which are imperative for all forms of plant life since the cycles they induce are what keeps plant alive. Our group added the fertilizer Miracle-Gro Plant Food with the concentration of 15-30-15 to another plot of soil.

The nutrients that fertilizer, such as Miracle-Gro Plant Food, contains are necessary for plant life because they create proteins that make the cells which make up the entire plant. The proteins, one of the chemicals of the cell, act as catylists, allowing the cell performs its four tasks, regulation of the environment, transforming energy, manufacturing chemicals, and reproducing. Without those ingredients there would not be any plants because the cells in the plants would not be able to perform their tasks. Therefore the nitrogen cycle and carbon cycle are so important since they sustain these ingredients, enabling the cell to live. (What is fertilizer?, 2004) Fertilizer allows for a more precise balance which is important because the balance between the ingredients helps the proteins and nucleic acids make up the biomolecules. This benefit from the invention of fertilizer has greatly proven its aid to plant growth and thus bacteria count increase statistically. From the invention of fertilizer in 1955 to the present, the average US farm went from yielding enough crops to feed twenty seven people to yielding enough crops to feed one hundred twenty people because of the aid fertilizer provides. (What is fertilizer?, 2004).

The biomolecules make up cells which make up plant life. However, fertilizer can also throw off the biomolecules because fertilizer makes too much of the waste product ammonium which throws off the decomposition, thus throwing off the balance of the biomolecules (David Brock "conversations," 2004). Fertilizer also disrupts the nitrogen cycle because the excess ammonia starts killing off the decomposers, which disrupts the balance between decomposers and its waste product, ammonia (Mr. Brock "conversations", 2004).

In order to test our research of compost, fertilizers, and soil ecology, we created a specific protocol to measure the quantitative effects upon bacteria levels and nitrate concentration of adding compost, fertilizer, or just water to the soil. We predicted that fertilizer will increase the bacteria and nitrate concentration the most because fertilizer is produced in a factory so the specific chemicals and balances between these chemicals can be exacted to fit the needs of the environment. In compost, one does not know exactly what nutrients and how much of them you are adding. Fertilizer also is less dependent on external features such as the necessity for decomposers or the necessity for a balance between wet and dry. In conclusion, fertilizer, compost, and water in soil help induce the carbon and nitrogen cycle so they increase nitrate levels. We measured the nitrate levels in the RPCS backwoods soil and tested the nitrate concentrations compared to the bacteria numbers and we hypothesized that the greatest bacteria and nitrate count would come from adding fertilizer.

Works Cited

1. Campbell, Neil A. Williamson, Brad and Heyden, Robin J. (2004). *Biology: Exploring Life*, 797.
2. Facts of the Case Composting (2004). http://www.ajkids.com/main/followup.asp?aj_ques=snapshot%3Dkids%26kbid%3D1130752%26item1%3D1130391-1130418&aj_logid=26397FC15C436C46B84AF5D46050E215&aj_rank=1&aj_s

core=1&aj_list1=1130391-1130418&back=http%3A%2F%2Fwww.ajkids.com%2Fkids42.asp%3Fask%3Dcomposting%26qSource%3D0%26origin%3D0%26metasearch%3D1&en=ka&qid=1D4CA1E4FF33DD4AA1636ED974C3797C&frames=1&adcat=jeev&ac=24&ask=composting

3. Florida’s Online Composting Center, http://www.compostinfo.com/tutorial/QuickTutorial.htm
4. Interview with Mr. Brock (May 19, 24, 2004).
5. Soil Ecology Background Information (2003) <http://www.agronomy.psu.edu/Courses/SOILS101/Labs/ecology.html>
6. What is fertilizer? (2004) http://www.ajkids.com/main/followup.asp?aj_ques=snapshot%3Dkids%26kbid%3D1653772&aj_logid=CB34580BC111F249B3729D99A5045F02&aj_rank=1&aj_score=40.01&back=http%3A%2F%2Fwww.ajkids.com%2FKids42.asp%3Fask%3DWhat%2Bis%2Bfertilizer%253F%26site_name%3Dkids%26metasearch%3D1%26frames%3D1%26qSource%3D0%26spellcheck%3Don%26origin%3D&en=ka&qid=AFD65002E9FA284BAABA9DD7202C695C&frames=1&adcat=jeev&ac=24&ask=What+is+fertilizer%3F

Lab Outline

I. Problem: Which creates a higher density of bacteria in the soil: adding compost to soil or actual commercial fertilizer?

II. Hypothesis: Adding commercial fertilizer will produce a greater density of bacteria number.

III. Procedure

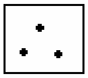
A. Variables
   1. Dependent variable: density of bacteria in soil
   2. Dependent variable$_{2:}$ nitrate concentration
   3. Independent variable: adding compost or fertilizer to the soil

B. Controls
   1. Negative control:
      i. Samples of site with only H_20 added
      ii. The samples taken before adding water, compost, or fertilizer.
   2. Control variable list
      i. Size of the plots, amount of soil in each sample taken, the way the fertilizer is applied to the soil, the way the compost is applied to soil, the amount and concentration of fertilizer, amount of compost and concentration, the conditions on top of the soil in the plots (whether mulch is on top or not, whether the roots of a plant are reaching into the soil being tested, etc.), using the same type of fertilizer the whole

time, using the same compost pile the whole time, taking the samples for a given test all on the same day and time, using sterile water, how you dilute the soil/water solution for counting the bacteria, the amount of time allowed for the bacteria to grow on the agar, the temperature in the incubator ; time elapsed after fertilizing and sampling; same test kit; type of agar used

C. Step by Step

1. Measure out 10 cm x 10 cm plots of land three times. (Leave 12 cm between each 10cm x 10 cm plot so the plots are not on top of each other, on a flat area, that doesn't have a lot of leaves, if there are leaves, remove them.)
2. Place a flag at the four corners of each of the plots.
3. Make 2 solutions (1w/fertilizer 1w/compost); each has a concentration of 1 gram /L
4. Before putting the fertilizer and compost on the plots, take three samples that are the size of the test sampler (2cm across in diameter, 15 cm long, which is the first line from the bottom of the test sampler, in length) from the plot that is going to have fertilizer and the plot that is going to have compost on it. Punch the sampler down into the ground by pushing firmly on the top of the sampler. Do all of the sampling on the same day at the same time for this step for your "before" samples.



Your plot should look like this after you take your first samples

5. Put fertilizer on one plot; mark the plot with "fertilizer" on the flags that are on its four corners. Just pour on the fertilizer on the plot, do not spray; you will burn the grass, a liter per 10cm.
6. Put compost on the second plot, mark it with "compost", and just pour on the compost solution a liter per 10cm.
7. Pour on water on the third plot, 1 liter per 10 cm. Mark it with a flag that says "nothing" on it.
8. Wait for 48 hours before taking "after" samples, be sure to keep the same amount of time in between trials so that you a get accurate density of bacteria and nitrate concentration. Take 3 "after" samples from compost, fertilization and the "nothing" plots.
9. Be sure to do the bacteria test and nitrate tests on the **SAME DAY** for the first trials' before and after samples. Do the same for the second trial.
10. Use the LaMotte STH-14 to test for nitrate levels and record nitrate concentration.
11. Then test for density of bacteria, to do this see steps below
    a. Place 1 cc of your soil sample into a culture tube containing 10 ml of sterile water; cap the tube and shake vigorously.
    b. Using a serological pipette, remove 1 ml of the soil/water mixture and place into a fresh culture tube.
    c. Add 9 ml of fresh sterile water to this second tube; cap and shake vigorously.

d. Repeat steps “b” using the second, diluted tube and then repeat step “c” with this third tube.
e. Continue step “d” with each additional tube until you have diluted the original soil/water mixture a minimum of four times (a 10^{-4} dilution). You should now have a total of five culture tubes.
f. Place 100 µl samples from the 4th and 5th tubes (dilutions 10^{-3} and 10-$^{4)}$) onto their own separate individual Petri plates filled with nutrient agar and allow to incubate at room temperature overnight.

12. Count number of colonies of bacteria on the 10 $^{3-}$and 10 $^{4-}$ look for the ones that had at least five colonies on the lowest dilution factor.
13. Then plug the number of colonies into this formula:

Of colonies x 10^{2} x $10^{|\text{the dilution \# on which the colonies were counted}|}$

14. Record that data that you got from the formula.

Data & Analysis

Bacteria (in bacteria/cc) vs. Nitrate (in ppm) in Soil

Before Adding the Fertilizer, Compost, or Water

Plot 1			**Plot 2**		
Water			**Water**		
	Bacteria (in bacteria/cc)	**Nitrate (in ppm)**		**Bacteria (in bacteria/cc)**	**Nitrate (in ppm)**
Trial 1	13,000,000	5	**Trial 1**	3,000,000	10
Trial 2	19,000,000	20	**Trial 2**	4,000,000	10
Trial 3	31,000,000	75	**Trial 3**	12,000,000	10
Averages	21,000,000	33	**Averages**	6,333,333	10

Compost			**Compost**		
	Bacteria (in bacteria/cc)	**Nitrate (in ppm)**		**Bacteria (in bacteria/cc)**	**Nitrate (in ppm)**
Trial 1	14,000,000	25	**Trial 1**	6,000,000	20
Trial 2	11,000,000	13	**Trial 2**	68,000,000	15
Trial 3	24,000,000	15	**Trial 3**	5,000,000	15
Averages	16,333,333	18	**Averages**	26,333,333	17

Fertilizer

	Bacteria (in bacteria/cc)	Nitrate (in ppm)
Trial 1	31,000,000	8
Trial 2	7,000,000	60
Trial 3	7,000,000	50
Averages	15,000,000	39

Fertilizer

	Bacteria (in bacteria/cc)	Nitrate (in ppm)
Trial 1	4,000,000	10
Trial 2	7,000,000	20
Trial 3	4,000,000	13
Averages	5,000,000	14

Final Averages of Plots 1 and 2

	Bacteria (in bacteria/cc)	Nitrate (in ppm)
Water	13,666,667	11
Compost	14,000,000	9
Fertilizer	10,000,000	14

Bacteria (in bacteria/cc) vs. Nitrate (in ppm) in Soil

After adding Fertilizer, Compost, or Water

Plot 1

Water

	Bacteria (in bacteria/cc)	Nitrate (in ppm)
Trial 1	0	10
Trial 2	3,000,000	10
Trial 3	4,000,000	10
Averages	2,333,333	10

Compost

Bacteria	Bacteria (in bacteria/cc)	Nitrate (in ppm)
Trial 1	28,000,000	10
Trial 2	13,000,000	20
Trial 3	16,000,000	10
Averages	19,000,000	13

Fertilizer

Plot 2

Water

	Bacteria (in bacteria/cc)	Nitrate (in ppm)
Trial 1	8,000,000	13
Trial 2	4,000,000	13
Trial 3	12,000,000	13
Averages	8,000,000	13

Compost

	Bacteria (in bacteria/cc)	Nitrate (in ppm)
Trial 1	53,000,000	20
Trial 2	9,000,000	15
Trial 3	20,000,000	25
Averages	27,333,333	20

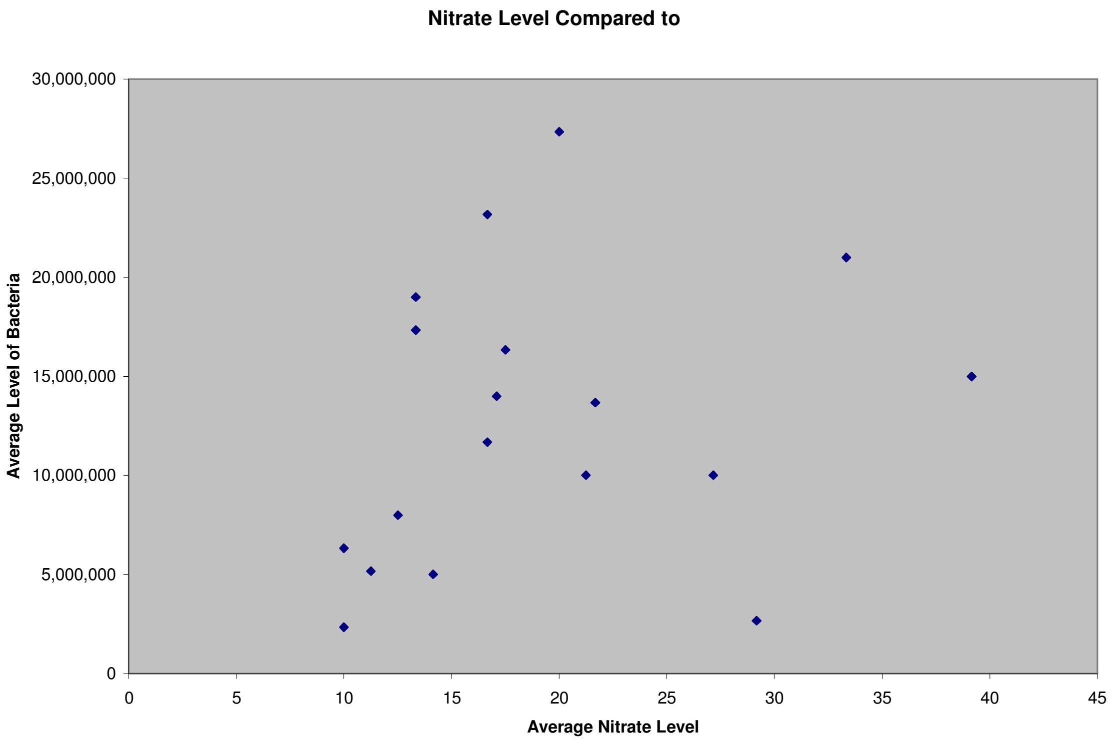
Fertilizer

	Bacteria (in bacteria/cc)	Nitrate (in ppm)
Trial 1	0	30
Trial 2	7,000,000	25
Trial 3	1,000,000	25
Averages	2,666,666	29

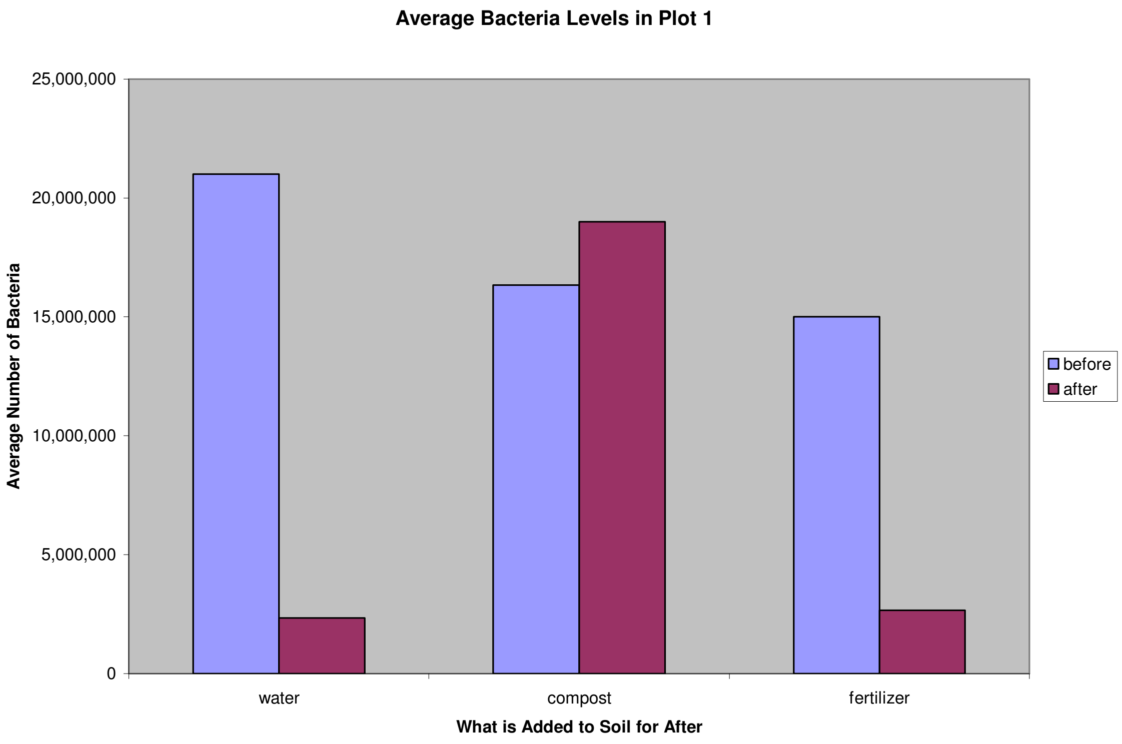
	Bacteria (in bacteria/cc)	Nitrate (in ppm)
Trial 1	29,000,000	10
Trial 2	9,000,000	10
Trial 3	14,000,000	20
Averages	17,333,333	13

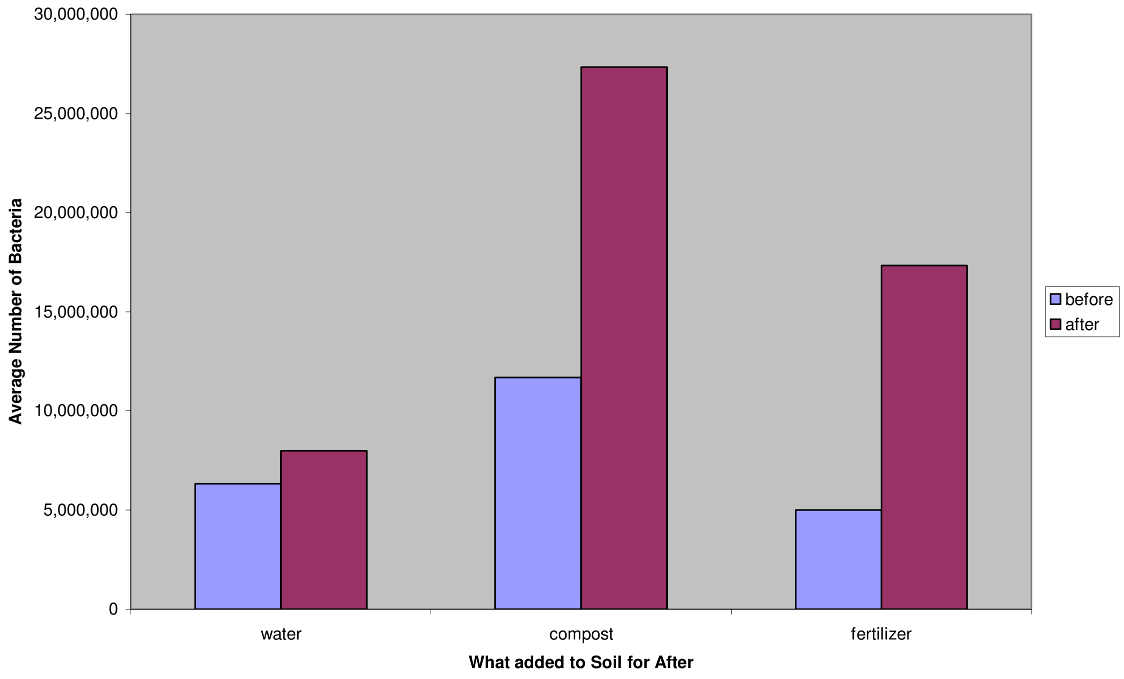
Final Averages of Plots 1 and 2

	Bacteria (in bacteria/cc)	Nitrate (in ppm)
Water	5,166,666	6
Compost	23,166,666	8
Fertilizer	10,000,000	11

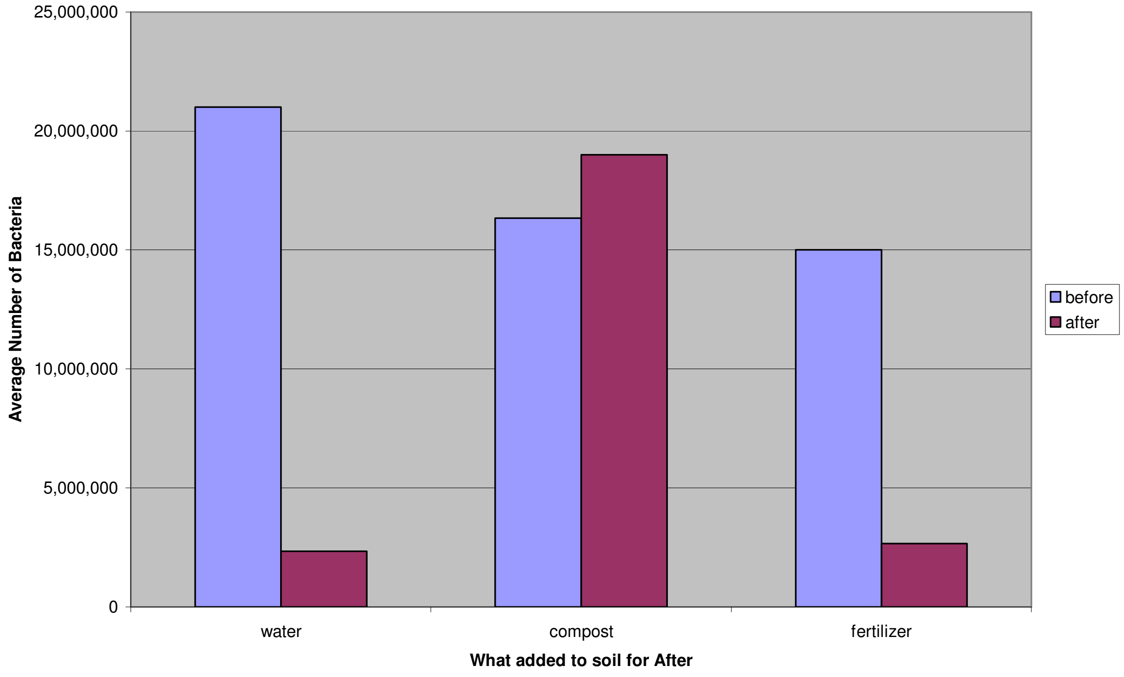


Because the slope of the above graph basically is always increasing, that shows that as the average nitrate concentration in ppm increases, the average number of bacteria (in bacteria/cc) also increases. The graph shows that the relationship is a normal trend and therefore the following graphs can be observed. Please note that in Plot 1 for all graphs, the results are errors made in our plot 1 procedure errors (see Conclusion).

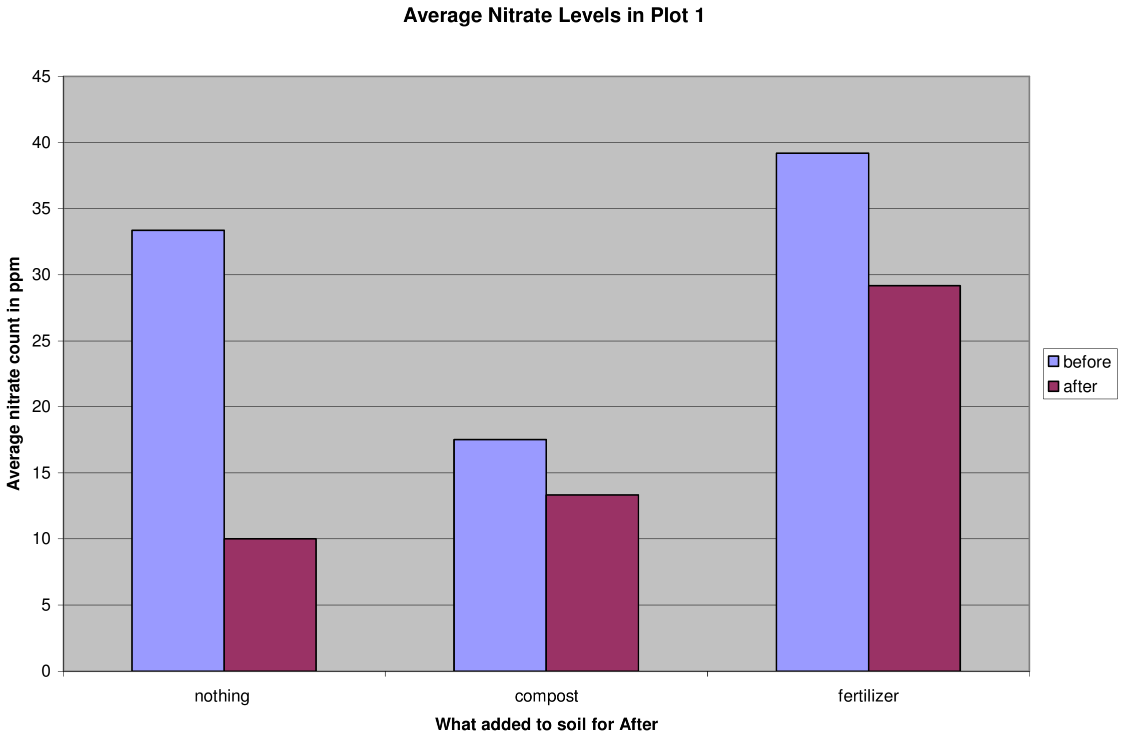

Average Bacteria Levels in Plot 1
25,000,000
20,000,000
15,000,000
10,000,000
5,000,000
0
Average Number of Bacteria
water
compost
fertilizer
What is Added to Soil for After
before
after



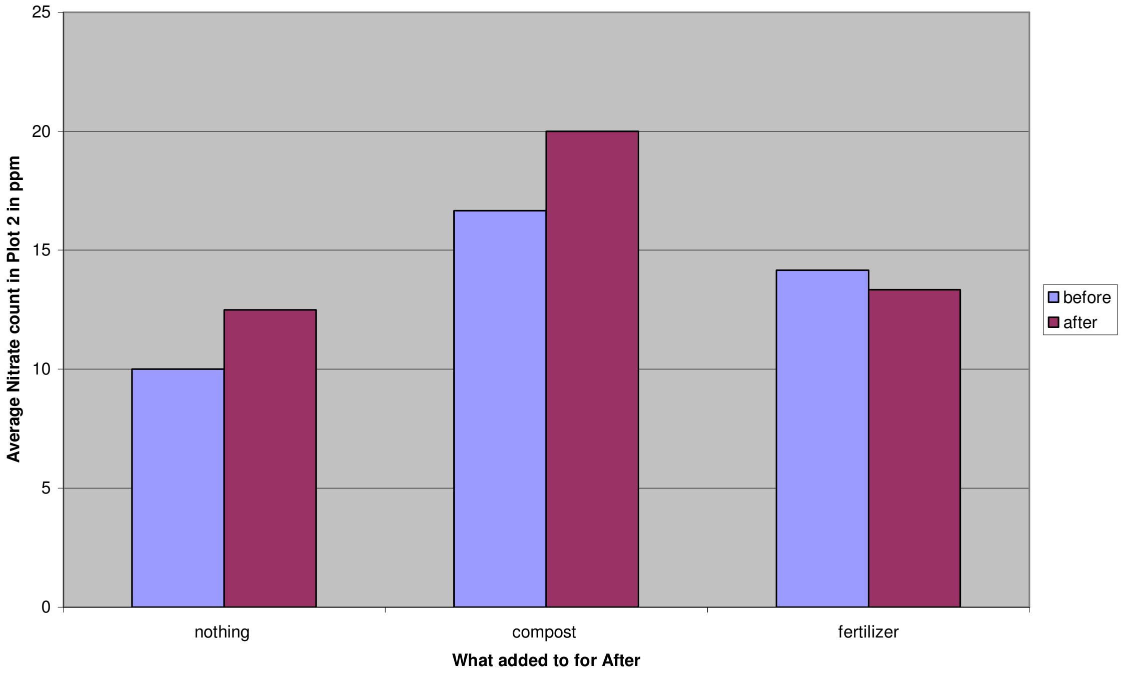
Average Bacteria Levels for Plot 2



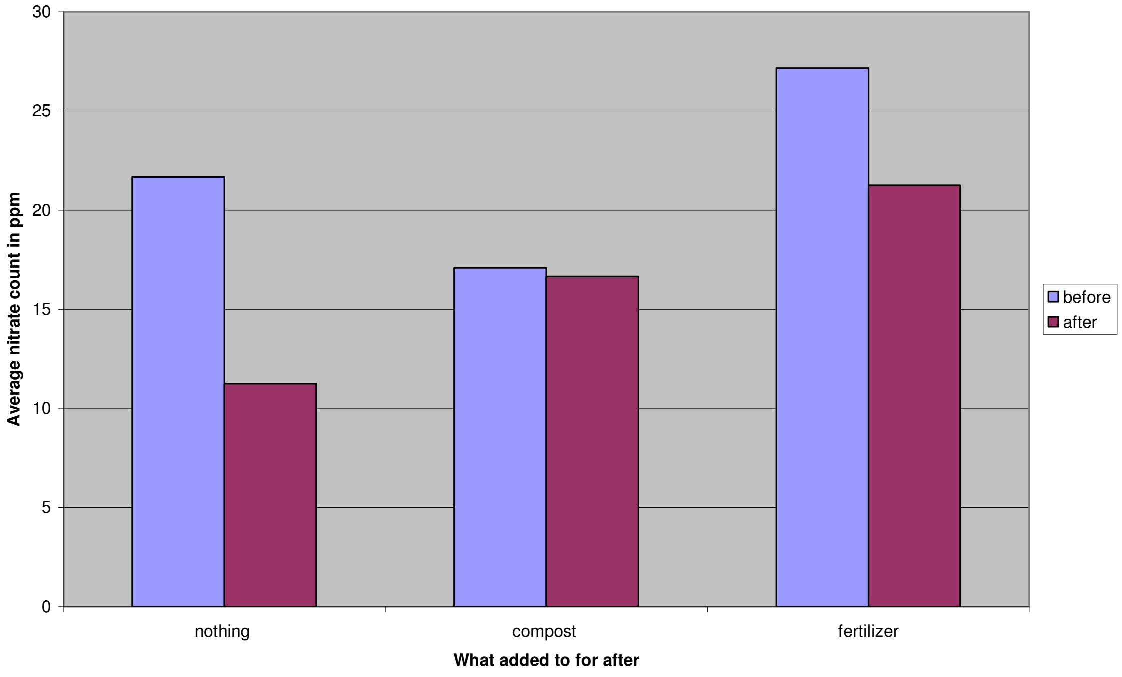
Average Bacteria Count for Plots 1 and 2 Averaged


Average Nitrate Levels in Plot 1
Average nitrate count in ppm
45
40
35
30
25
20
15
10
5
0
nothing
compost
fertilizer
What added to soil for After
before
after

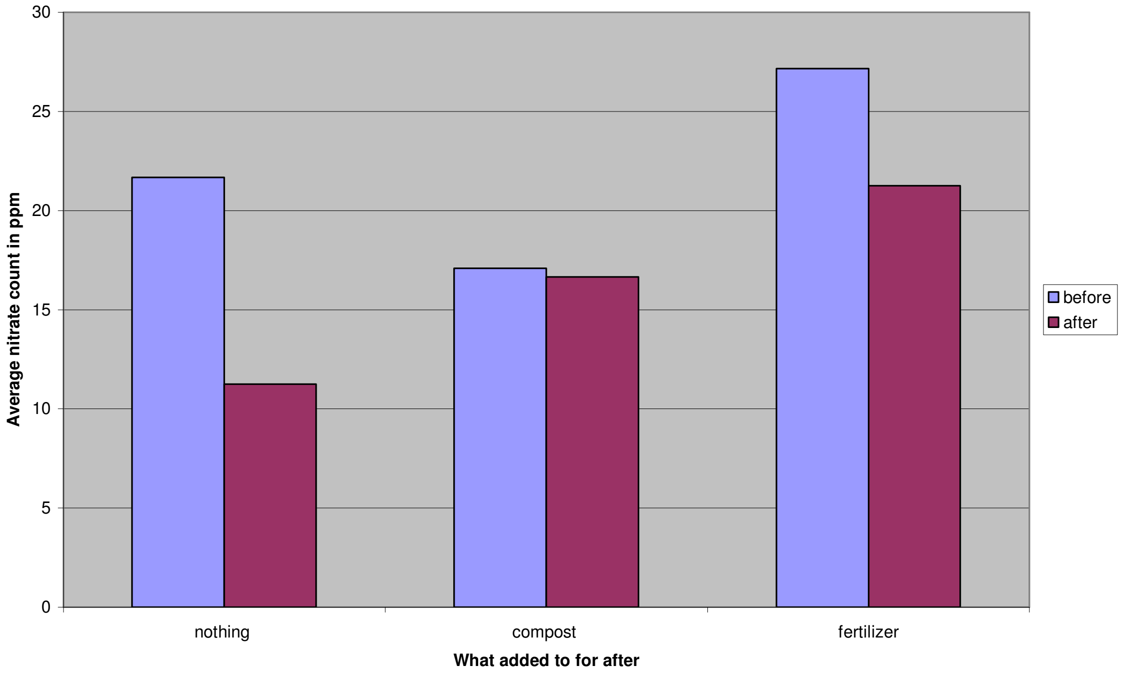
Average Nitrate Count in Plot 2



Average Nitrate Count for Plots 1 and 2 Averaged



Average Nitrate Count for Plots 1 and 2 Averaged



IV. Conclusion: Our hypothesis that adding commercial fertilizer would produce a greater density of bacteria number was incorrect. However, because of a large number of errors in our experiment, our results were not necessarily correct for our experiment. Our sources of error greatly influence if our hypothesis was correct or not. The average of our plot 1 and plot 2 would say thought our hypothesis was wrong, but the fact that for plot one we used the same soil sampler to dig up the samples after we already put fertilizer, compost and water on the soil influenced the bacteria count and possibly really damaged our results. If you look at plot 2, we fixed the problem and used different soil samplers and our hypothesis proved to be correct. Fertilizer had the greatest increase in bacteria count in the soil. Other sources of error in our experiment were we forgot to test the third dilutions, which created a potential to harm our bacteria count. Luckily, we ended up using the fourth dilution samples so that error did not harm us. We took our samples for plot 1 and plot 2 at separate times so weather, animals touching the soil, or other external affects could have affected the bacteria count. We did measure the change, so it should not be a huge source of error, but it definitely presents a question. For a future research direction, I would therefore perform our experiment in different weather conditions to see if factors such as rain, snow, or temperature increased the bacteria in the soil and how the affects varied depending on weather we added water, compost, or fertilizer. I would also attempt the experiment using different types of fertilizer and different ingredients and ingredient combinations in the soil. I would compare the bacteria count to other levels such as carbon concentration, rather than only nitrate. It is difficult to tell whether our experiment has any valid conclusions because of our drastic sources of error. In short, judging from solely plot 2, in which we used the correct procedure, our hypothesis was correct and fertilizer did increase the bacteria count the most by 12,333,333 bacteria/cc. However, judging from both plots and their average, our hypothesis was incorrect and compost had the greatest increase in bacteria count. From Plot 1 and 2 averaged together the fertilizer only increased by 10,000,000 and compost increased by 10,000,000 so they were the same. Overall the experiment proved a very interesting initial step in our attempt to essentially see weather fertilizer or compost or using only water was the most affective, by measuring bacteria count and comparing it to nitrate level. Based on this experiment, we would have to conclude that our hypothesis was incorrect. However, with future trials, altering other variables and performing the experiment with more trials and without the sources of error, we could easily come to a very different conclusion.