Episode III: The Revenge of the Nitrate
The Saga Continues…

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Background

Healthy ecosystems consist of balanced relationships of organisms that can aid in each other's survival. However, a change in the conditions of the ecosystem could either severely damage or aid the relationships an organism uses to survive. Because the organisms in an ecosystem are interconnected, if organisms start to become extinct, all the predators of those organisms will have a food shortage. Therefore, any change could alter the entire ecosystem and re-arranging the relationships of the organisms in that environment. Changes to ecosystems can result from pollution, introduction of new organisms, or a number of other factors. Frequently, cars are associated with such pollution and cars are prevalent in many humans' lives. Our group questioned whether the byproducts from cars are beneficial or damaging to soil. The purpose of our experiment is to determine the effects of car exhaust on surrounding ecosystems, beginning with soil. We will test areas of soil that have increased concentrations of car exhaust toxins versus areas that have decreased concentrations. To test the affects, we will examine the protozoa population, a vital microorganism in soil ecology. In order to understand our data, we have researched a variety of topics, including the development and structure of ecosystems, general soil health, the makeup of soil, and pollution.

Ecosystems are a vital part of the environment. They can be greatly affected by the slightest change. The base of any food chain in any ecosystem begins with the microorganisms in the soil, including plants, organic matter, bacteria, fungi, nematodes, protozoa, arthropods, birds, and animals. The organic matter of plants is eaten by bacteria and fungi. Bacteria can be eaten by nematodes, arthropods and protozoa, while fungi are eaten by arthropods and nematodes only. Protozoa and nematodes are then eaten by nematode predators, while arthropods are eaten by arthropod predators. Nematode predators can be eaten by arthropod predators, which are in turn eaten by birds and animals. If even one minor element of the food web was to undergo any changes, the entire food web of the soil would change, preventing the organisms from getting the food they need to survive. From that point, the entire food web of a larger ecosystem would be affected in the chain reaction of events. The stability of any ecosystem relies on the consistency of organisms involved. Soil includes a vast range of organisms, thus making its intertwining web particularly complicated. (Elaine R. Inghram, 2004)

The organisms in the soil include bacteria, fungi, protozoa, nematodes, micro-arthropods, earthworms, insects, small vertebrates and plants. Most of these organisms are helpful to the soil and make it more suitable for growing plants. Soil organisms are also responsible for decomposing organic compounds (manure, plant residue, and pesticides) and preventing toxic compounds from entering water and becoming pollutants. Soil microorganisms also decompose waste and nutrients that have nitrogen and thus make the nitrogen available to plants. This cycle and functions of these organisms all depend on the food web of the soil. (Elaine R. Inghram, 2004) In order for soil to be healthy, all organisms that live in the soil must also be healthy. Microbes are a particularly important group to the health of soil.

Microbes are responsible for plant growth and soil health. Microbes that are essential to soil are actinomycetes, bacteria, fungi and protozoa. Specifically, microbes maintain decomposition, mineralization, nitrogen and carbon cycling, storage and release of nutrients and the taking of pollutants out of water before it reaches underground or the surface. The microbe we will test for, protozoa, feeds on bacteria and other protozoa.

Some examples of protozoa are amoebae, flagellates and ciliates. Protozoa are vital to all ecosystems because they are responsible for nitrogen fixation. They also make nutrients accessible to plants so that the nitrogen cycle can occur. Protozoa live near the roots of plants, so plants have easy access to nutrients (Basic-info-4-organic-fertilizers, 2005).

Specifically, protozoa further break down nutrients that have already be broken down by bacteria and fungi in order to make the nutrients accessible to plants. Protozoa consume bacteria and fungi as a source of energy, and while doing so, they break down the nutrients of the bacteria into another form (such as nitrate) that can be absorbed by plant roots. When protozoa populations are high, one can infer that bacteria (the food source of protozoa) populations are low because the many protozoa devour the bacteria, their food source. In addition, when protozoa populations are high, plants also have access to nutrients (because of the increased amounts of nutrients); plants then grow at a rapid rate using the nutrients they absorb. However, when protozoa population is low, there are fewer nutrients in the soil that can be absorbed by plant roots. Overall, the population of protozoa is the key factor of making nutrients available to plants (Soil Foodweb, Inc., 2005). Protozoa contribute heavily to the ecosystem by making plants healthy.

Protozoa are not the only aspect of soil that is important to the overall heath, a healthy composition of soil provides water, air, and nutrients for plant growth. Soil is made up of living and non-living matter. The healthiest soil contains of 45% rock particles (rock that has been broken down over time by wind, water and chemical process), 5% organic matter (decaying plant and animal matter), 25% water, and 25% air (Urban Programs Resource Network, 2005). Rock particles that make up the soil are cations, clay, minerals, sand and silt. Although these materials are important to soil health, the most important components of soil are the nutrients. The nutrients in the soil include nitrogen, calcium, phosphorus, magnesium, potassium, sulfur, copper, and iron (M. Ray Tucker, 1999), but the most important nutrients in soil are nitrogen, potassium and phosphorus.

Nitrogen helps the development of a plant and aids leaves in growing. Nitrogen also contributes to leaves' dark green color, an indicator of chloroplasts. Chloroplasts undergo photosynthesis and utilize energy to control and maintain the growth and structure of a plant (Peduto, Greg, 1998). Nitrogen also contributes to the manufacturing of amino acids, proteins and nucleic acids in organisms. Therefore, an enzyme, a type of protein that controls the chemical reactions in a cell, is also made of nitrogen. When enzymes instigate chemical reactions, the four main tasks of the cell take place: regulation of the environment, production of proteins, reproduction, and energy transformation. Without the four tasks, a cell cannot live. Therefore, when nitrogen is not present, enzymes are not created, the four main tasks do not occur, and the organism dies. Specifically relating to plants, the enzymes allow the chloroplasts to perform photosynthesis in order for the plant to survive and create the energy necessary to perform the four main tasks.

In addition to aiding in the performance of the four main tasks of the cell, nitrogen has other vital functions. Nitrogen is integrated into the ecosystem through the nitrogen cycle, which transfers the nitrogen throughout the environment. In the soil, nutrients that are from waste, such as nitrogen, are broken down and are absorbed by plant roots. Therefore nutrients are within the plants. When consumers of plants, like cows, eat plants, they get a portion of the nutrients. Then when predators of cows, like humans, eat the cows, the predators get a smaller portion of nutrients than cows. Each organism, as it receives nutrients, uses the nutrients for the four main tasks, leaving some to be consumed by the next consumer. (Pidwirny, Michael, 2005). If plants, the beginning of the food chain, do not receive enough nutrients to perform the four main tasks, the organisms that eat the plants receive fewer nutrients. Nitrogen is passed on from organism to organism in the food chain, thus making nitrogen a vital building block for ecosystems. Nitrogen could not stabilize ecosystems or spread through so many levels of the food chain without the nitrogen cycle.

The nitrogen cycle, specifically, moves the nitrogen from the air (where it is a gas), to the soil through a mixture of nitrogen and rain. The rain with nitrogen is absorbed by the ground. In the ground, nitrogen fixation occurs, where bacteria and protozoa create nutrients that allow plants to absorb the nitrates and turn them into organic material. Nitrates are passed on when the plants are eaten. Animals such as herbivores that eat the plants, then turn the nitrates into ammonia (NH_3). The waste from the animal then seeps back into the ground where nitrogen fixation occurs again, and the cycle repeats itself. (Tiscali, 2005) Nitrogen compounds, while traveling through the digestive system of the organisms, are broken down for growth of the organism. The nitrogen compounds that do not get broken down in the animal's body, returns to the soil in the form of waste. Nitrogen is spread throughout food chains and ecosystems by following this cycle, thus making it vital to the environment. An excess or paucity of nitrogen could unbalance an ecosystem and eventually cause harm to organisms. Such an unbalance of nitrogen in the nitrogen cycle could be caused by car exhaust.

Car exhaust is made up of toxic pollutants. Some of the chemicals in car exhaust include carbon monoxide, carbon dioxide, nitrogen dioxide, nitrogen monoxide, sulphur dioxide, benzene, formaldehyde, and polycyclic hydrocarbons. The most harmful of these chemicals are carbon monoxide, carbon dioxide, nitrogen dioxide, and sulphur dioxide. (Gislason, Dr. Stephen. 1995) These chemicals can pose a danger to humans as well as the environment. Car exhaust contributes to acid rain and global warming among other environmental problems.

In our experiment, we would like to test the affects of car exhaust on soil. We are using one chemical, nitrate, and testing how it changes the protozoa population in the soil. We chose nitrate because it is the form that nitrogen dioxide takes when mixing with rain to enter the soil, thus imitating the effect of nitrogen dioxide from car exhaust. Because we are only testing for one compound of car exhaust, we can only infer whether the whole mixture of car exhaust is beneficial or harmful to the soil based on our experiment testing the affects of nitrate. Therefore, we can only attest to the effects of nitrate in car exhaust. We are using nitrate combined with water to put into soil plots. We are not using nitrogen dioxide because it is a gas and we cannot insert a gas into the soil. However, a trait of nitrogen dioxide is that when it is released in the atmosphere and it is mixed with rain, it is enters the soil in a form of nitrate. Therefore, in our experiment, we are essentially testing the effects of car exhaust, pollutants combined with rain, once it enters the soil.

Through research, we have found the influence of excess car exhaust in the ecosystem is negative. When excess nitrogen dioxide (a form of nitrate) is present in the soil, it causes the soil to be acidic which kills the plants and then harms the entire ecosystem. (National Environmental Research Council, 2005) Because the environment is acidic, protozoa populations would be low. Therefore, by testing to find the population of protozoa, we can indirectly find the health of the soil, which determines the health of plants because plants need nutrients to create enzymes.

In our experiment, we will have two plots of land outside, and we will test both plots for the population of protozoa and nitrate. Next, we will add a solution of water and zinc nitrate to one plot, and an equal amount of water to the other plot to imitate an area with more car exhaust and an area with less car exhaust. We will then wait for one day to pass allowing the environment to react to the excess nitrate. Again, we will test for the population of protozoa and amount of nitrate in each plot. We will repeat our experiment two more times for accuracy. We think access nitrate will harm the environment of the soil, making it acidic; therefore, we surmise that the plot with the nitrate solution will have a lower population of protozoa. If the levels of nitrate were to increase drastically, similarly to the effect that excess car exhaust may have, the protozoa would be affected, leading to plants, which leads to animals, and then to humans. We hypothesize that car exhaust lowers levels of protozoa, ultimately harming the ecosystem.

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Experiment

Problem: How do increased levels of nitrate from car exhaust change the number of protozoa in soil?

Hypothesis: Increased levels of nitrate will decrease the number of protozoa in soil.

Independent variable: Presence of zinc nitrate in soil

Dependent variable: density of Protozoa in soil

Negative Control: Plot two (soil with only water added)

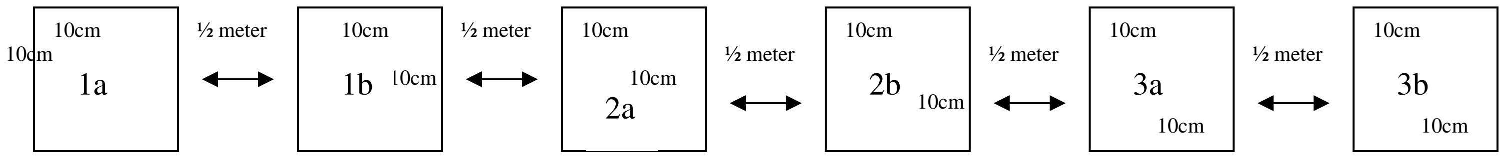
Positive Control: plots tested before adding chemicals

Controlled variables:

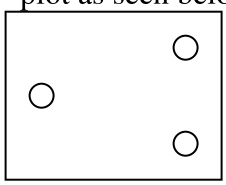
- proximity of soil samples
- size of plots
- proximity of plots
- number of soil samples from each plot
- amount of water in each plot
- amount of light
- elapsed time between soil sampling before and after nitrate addition
- size of soil samples
- Depth of soil samples
- Amount of soil from sample in each Petri dish
- Time elapsed between covering Petri dish and placing soil sample in modified Uhlig
- Amount of ul in each microscope slide
- Amount of H_2O added to Petri dish
- how long Petri dish sits
- timing of testing both nitrate and density of protozoa of a soil sample
- using Lamatte Nitrate test on all samples

Procedure

1. At 39.35808° N and 76.63602° W, label 6 sets of 2 plots of soil. Each plot should be a 10cm by 10cm square, each plot ½ meter apart. As seen below:



2. Collect 3 soil samples from these locations in each plot in a triangular formation. Two soil samples should be next to one side of edge of the plot, and the third soil sample should be across from the other soil samples on the opposite side of the plot as seen below:



   Make sure all plots have consistent soil sample formations.
3. Samples should be 15 cm deep in the soil cylinder and 2 cm diameter. Place each sample in a separate plastic bag
4. Measure all soil samples for Protozoa and Nitrate
   a. Place 15 cm sample from each separate soil sample from the plots into the bottom of a clean, empty Petri dish, each sample should have its own Petri dish; and allow to dry completely for 24 hours.
   b. Sift 9-10 g of each soil sample into a separate 2nd clean Petri dish using a 1 mm squared nylon screen or mesh
   c. Add 20 ml of distilled water to each dish in order to saturate the soil
   d. Cover the Petri dishes with lids and allow to sit for 7 hours
   e. Place the soil samples in separate modified Uhlig extractor containing 30 ml of distilled water for 24 hours
   f. Perform steps h-l while simultaneously doing g, the test for nitrate.
   g. Test for Nitrate:
      i. Use the soil from inside of the Uhlig extractor
      ii. Use a 1 mL pipet (0354) to transfer 1 mL of the general soil extract to one of the larger depressions on a spot plate (0159)
      iii. Add 10 drops of *Nitrate Test Reagent #1 (5146)
      iv. Use a .5 g spoon (0698) to add one level measure of *Nitrate Reagent #2 (5147)
      v. Stir thoroughly with a clean stirring rod (0519). Allow to stand five minutes for full color development.
      vi. Match sample color with the Nitrate Nitrogen Color Chart (1315). Record as parts per million nitrate nitrogen
   h. Filter the filtrate from the Uhlig extractor a 2nd time using 12.5 c, qualitative filter paper

i. Using a capillary tube, deposit 7 **ul** of methyl-green stain on a clean microscope slide (1 **ul=** 1 drop from the caililllary tube). Then using a disposable graduated Beral-type pipette, add 18 **ul** (the first demarcation on the pipette) of the 2^{nd} filtrate from step 6 to the stain on the microscope slide and cover with an 18 x 18 mm squared cover slip
j. Examine under a light microscope at 40x (for quantitative) observations of the various protozoa living in the soil
    i. Count protozoa from five fields of view and average the number of protozoa for the five fields
k. Use the following equations to determine the population density of protozoa in the soil sample (the 40x is the preferred method; use 100x formula only when absolutely necessary):
    i. [(# per field of view at 40x) x (total ml of 2^{nd} filtrate) x 747] / (grams of sifted soil) = # of protozoa per gram of soil;***
l. Record all data.

5. Mix .01g of Zinc Nitrate and 1 liter of water. Then pour the solution into each Plot A and pour 1 liter of water into each Plot B.
6. Wait 24 hours while the Nitrate is in the soil (without rain), if it does rain then follow step 7 immediately.
7. Repeat steps 2-4.

* Protozoa Extraction developed by Kate Brockmeyer

*Formula for finding moles of Zinc Nitrate:

Conversation with Nancy Lentz (May 5, 2005) “Finding Moles to Match Acidity”

Data Before Adding Nitrate

Level of Nitrate (ppm) (Group A)

Soil Sample	Level of Nitrate (ppm)
1 A. #1	7.5
1 A. #2	10
1 A. #3	10
2 A. #1	10
2 A. #2	20
2 A #3	10
3 A. #1	10
3 A. #2	20
3 A. #3	10

Level of Nitrate (pounds per acre nitrate nitrogen) (Group B)

Soil Sample	Level of Nitrate (ppm)
1 B. #1	7.5
1 B. #2	10
1 B. #3	5
2 B. #1	20
2 B. #2	20
2 B. #3	7.5
3 B. #1	20
3 B. #2	20
3 B. #3	20

Number of Protozoa (per gram of soil) (Group A)

Soil Sample	Number of Protozoa (per gram of soil)
1 A. #1	25246
1 A. #2	9809
1 A. #3	16769
2 A. #1	10299
2 A. #2	14940
2 A. #3	19487
3 A. #1	15181
3 A. #2	7622
3 A. #3	16868

Number of Protozoa (per gram of soil) (Group B)

Soil Sample	Number of Protozoa (per gram of soil)
1 B. #1	10564

1 B. #2	8492
1 B. #3	24900
2 B. #1	6567
2 B. #2	4718
2 B. #3	14483
3 B. #1	7409
3 B. #2	12852
3 B. #3	12322

Data After Adding Nitrate

Level of Nitrate (ppm) (Group A)

Soil Sample	**Level of Nitrate (ppm)**
1 A. #1	0
1 A. #2	0
1 A. #3	0
2 A. #1	10
2 A. #2	10
2 A #3	10
3 A. #1	10
3 A. #2	7.5
3 A. #3	0

Level of Nitrate (pounds per acre nitrate nitrogen) (Group B)

Soil Sample	**Level of Nitrate (ppm)**
1 B. #1	Unavailable
1 B. #2	2.5
1 B. #3	7.5
2 B. #1	0
2 B. #2	10
2 B. #3	0
3 B. #1	0
3 B. #2	10
3 B. #3	0

Number of Protozoa (per gram of soil) (Group A)

Soil Sample	**Number of Protozoa (per gram of soil)**
1 A. #1	741437
1 A. #2	25983
1 A. #3	202869
2 A. #1	120966
2 A. #2	45282

2 A. #3	48555
3 A. #1	62625
3 A. #2	126368
3 A. #3	20543

Number of Protozoa (per gram of soil) (Group B)

Soil Sample	**Number of Protozoa (per gram of soil)**
1 B. #1	Unavailable
1 B. #2	123255
1 B. #3	45794
2 B. #1	7077
2 B. #2	172203
2 B. #3	443432
3 B. #1	346190
3 B. #2	232935
3 B. #3	21054

Averages

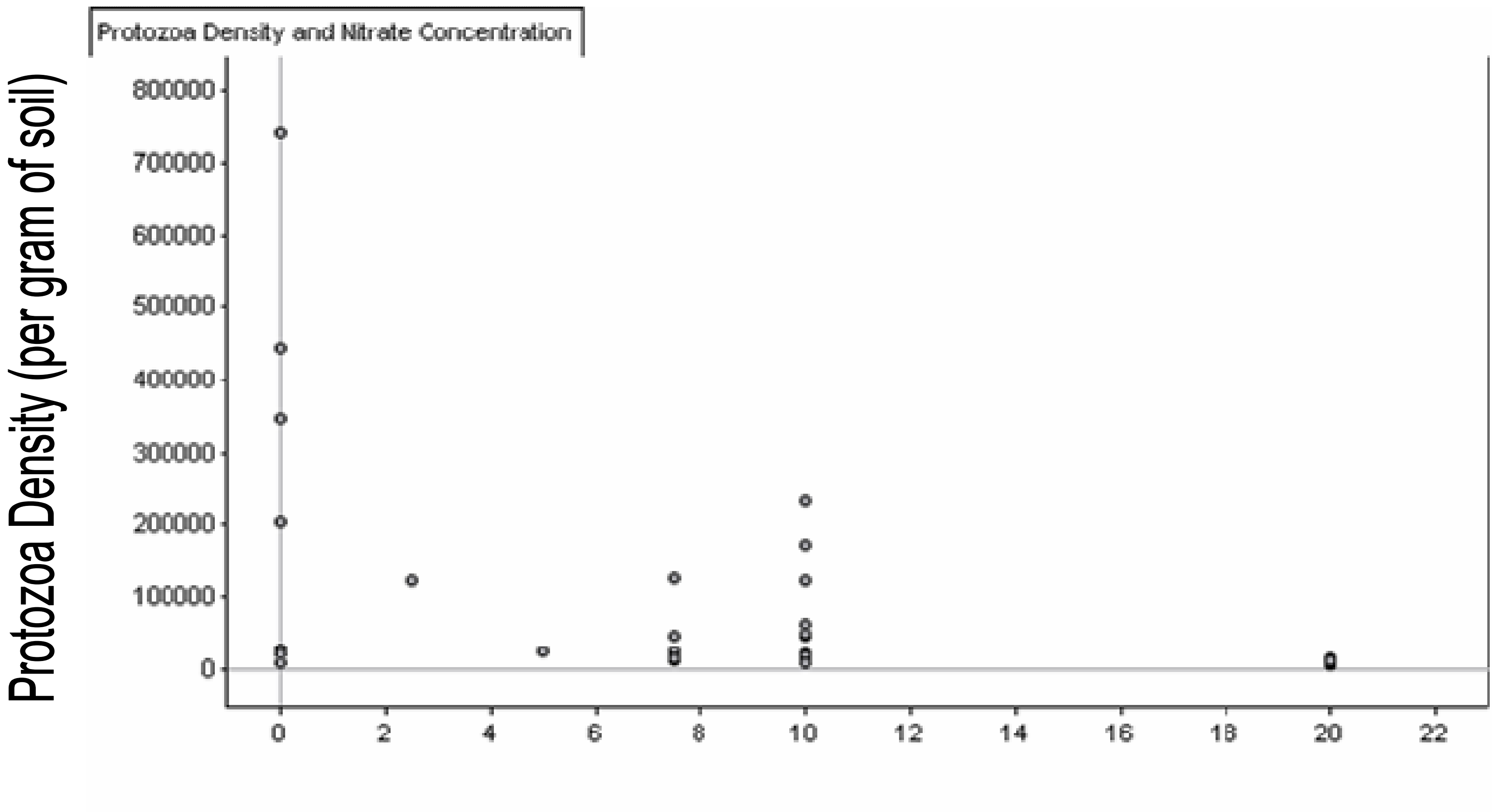
Average Level of Nitrate (ppm) and Average Level of Protozoa (per gram of soil)

	Before Adding Nitrate		**After Adding Nitrate**	
Soil Plot	**Average Level of Nitrate (ppm)**	**Number of Protozoa (per gram of soil)**	**Average Level of Nitrate (ppm)**	**Number of Protozoa (per gram of soil)**
1 A	9.16	17275	0	323430
1 B	7.5	14652	5	84525
2 A	13.3	14909	10	71601
2 B	15.83	8589	3.33	207571
3 A	13.3	13224	5.83	69845
3 B	20	10861	3.33	200060

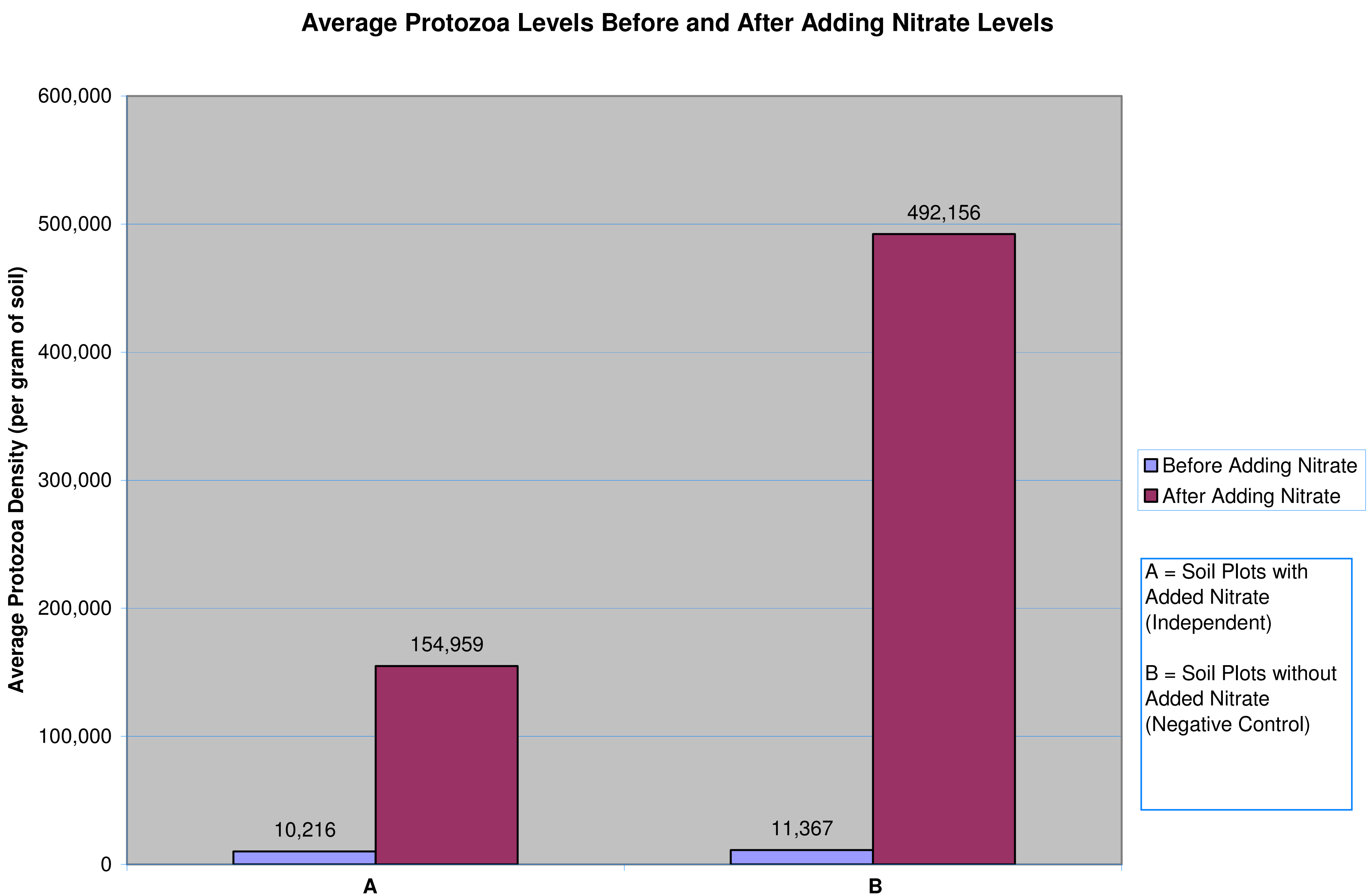
FINAL AVERAGE

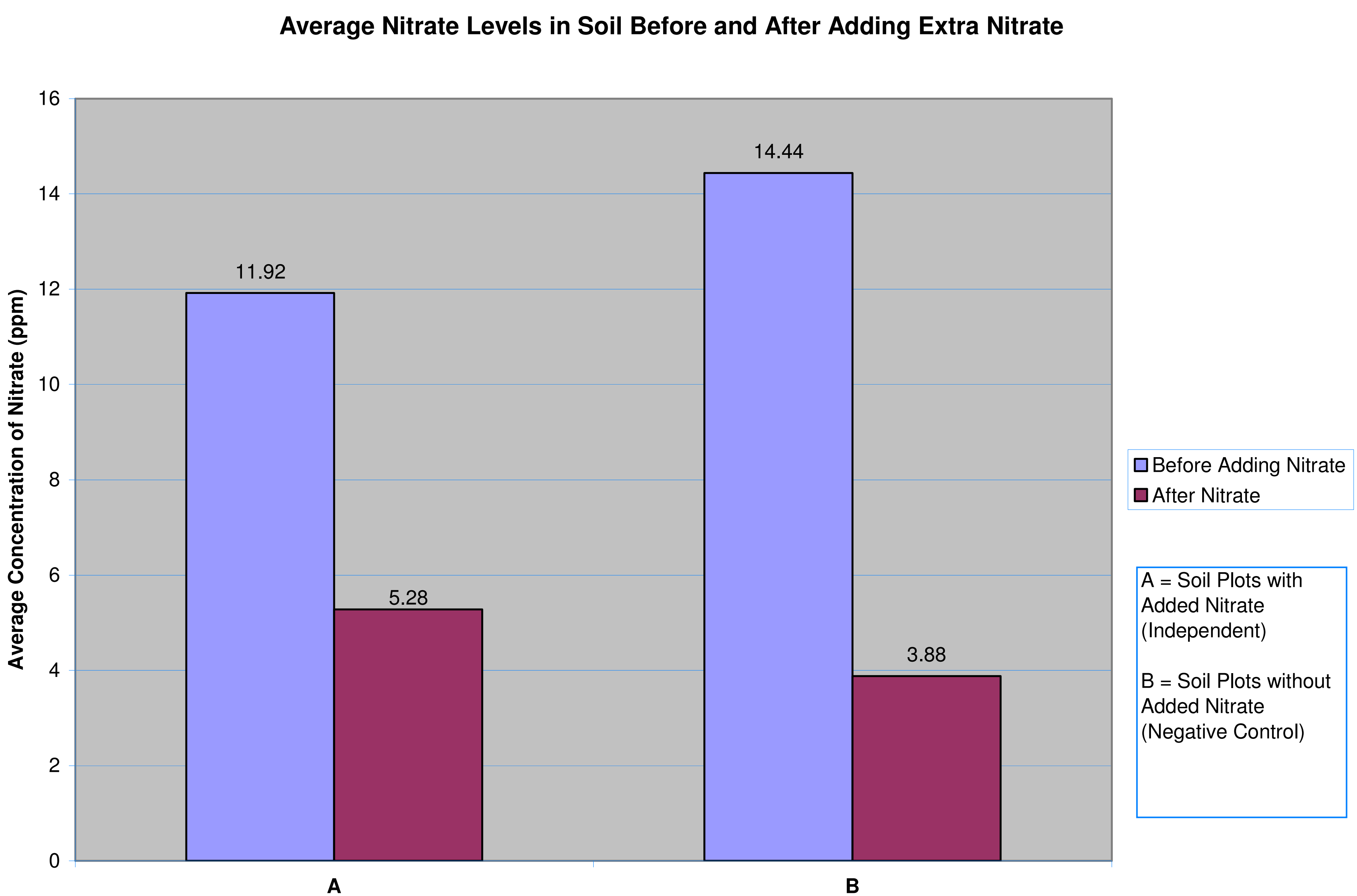
	Before Adding Nitrate		**After Adding Nitrate**	
Soil Plot	**Average Level of Nitrate (ppm)**	**Number of Protozoa (per gram of soil)**	**Average Level of Nitrate (ppm)**	**Number of Protozoa (per gram of soil)**
A	11.92	10,216	5.28	154,959
B	14.44	11,367	3.88	492,156

Figure 1



Protozoa Density and Nitrate Concentration
Protozoa Density (per gram of soil)
800000
700000
600000
500000
400000
300000
200000
100000
0
0
2
4
6
8
10
12
14
16
18
20
22


Average Protozoa Levels Before and After Adding Nitrate Levels
Average Protozoa Density (per gram of soil)
600,000
500,000
400,000
300,000
200,000
100,000
0
10,216
154,959
11,367
492,156
A
B
Before Adding Nitrate
After Adding Nitrate
A = Soil Plots with Added Nitrate (Independent)
B = Soil Plots without Added Nitrate (Negative Control)


Average Nitrate Levels in Soil Before and After Adding Extra Nitrate
Average Concentration of Nitrate (ppm)
16
14
12
10
8
6
4
2
0
11.92
5.28
14.44
3.88
A
B
Before Adding Nitrate
After Nitrate
A = Soil Plots with Added Nitrate (Independent)
B = Soil Plots without Added Nitrate (Negative Control)

Conclusion

Initially, we examined our data in the form of a scatter plot, as seen in Figure 1. The pattern in Figure 1 contradicts the normal relationship between protozoa and nitrate. Usually, as nitrate concentration in parts per million increases, so does the protozoa density per gram of soil. The more protozoa, the more bacteria they consume and the more nitrate is released. However, in Figure 1, as nitrate concentration increases, protozoa density decreases.

To further examine the unusual pattern and contradictory statistical analysis in Figure 1, we studied the proportions of nitrate concentration changes before and after we added additional nitrate in A Plots. A plots are experimental plots where we added a nitrate solution into the soil; B plots are negative control plots where we only added water; such information is displayed in Figure 2. In both A and B plots, averages of our nitrate data show that nitrate concentration dropped after additional nitrate/water was added to their respective plots; however, nitrate in A plots did not drop nearly as much as B plots. Figure 2 graph shows that before adding the nitrate, A plots' average of nitrate concentration was 11.92 ppm, and B plots' average of nitrate concentration was 14.44 ppm. After we added nitrate to A plots and we added water to B plots, an average of 5.28 ppm of nitrate were present in A plots and an average of only 3.88 ppm of nitrate were present in B plots. The nitrate concentration in A Plots decreased 55.7% after adding nitrate, and the nitrate concentration in B Plots decreased 76.1%. Therefore, our added nitrate created a higher concentration of nitrate in the soil, proving that our experimental manipulation had an impact.

Nitrate concentration dropped drastically in both plots after we added nitrate to A plots, and water to B plots, so we investigated the reason for the decrease in nitrate, but the unusual increase in protozoa. As seen in Figure 3, before adding nitrate, the average protozoa density was low in both A and B plots (10,216 per grams of soil in Plot A and 11,367 per grams of soil in Plot B). However, after adding nitrate, average protozoa density increased in both experimental and negative control plots (protozoa density increased to an average of 154,959 protozoa per gram of soil in A plots and in B plots, an average of 492,156 of protozoa per gram of soil). Our negative control plots, B plots, had a dramatically higher protozoa density average than the average of A plots (A plots increased by 1416.8% percent, and B plots increased by 4229.7% percent.) further proving our manipulation of increased nitrate had an impact on the density of protozoa. Before adding the nitrate, the average protozoa density in plots A and B were close in number, but after adding the nitrate (manipulating the soil) there was a much larger discrepancy of average protozoa density between A plots and B plots.

We then wondered why there was a dramatic decrease of nitrate concentration in the environment (present in both experimental and negative control plots), but a dramatic increase in protozoa in both plots. After consulting another source, we found that there was another component in the nitrogen cycle that had an impact on the protozoa density of our experiment: denitrifying bacteria. Denitrifying bacteria, as well as plants, consume nitrate as an energy source. Other bacteria cannot utilize nitrate as a food source, rather it is a poisonous waste. In the soil, we infer that denitrifying bacteria levels went up, which would be responsible for the decreased concentration of nitrate, since denitrifying bacteria would be consuming the nitrate. However, because we added an excess of nitrate to A Plots, the bacteria did not have sufficient time to consume all of the nitrate in the time we allotted them. Nitrate is a waste of both protozoa and bacteria, and therefore is negative component in the surrounding environment of the protozoa. Since protozoa were surrounded in waste, the nitrate poisoned the protozoa and hindered their ability to thrive in that environment, which explains why the protozoa levels were so low in our experimental plots. In our negative control plots, no excess nitrate was present that could have poisoned protozoa. Furthermore, our soil samples were contained to a small petri dish, which limited the parameter of the protozoa and their capability to migrate to a healthier environment.

In conclusion, our hypothesis was correct. We initially thought that nitrate would hurt the protozoa. Later, when our data contradicted itself, we began to think that nitrate helped protozoa, because it is a food source for denitrifying bacteria, which is a food source for protozoa. However, after even more research, we once again came to the conclusion that excess nitrate hinders protozoa's performance of the four main tasks that keep an organism alive,(transforming energy, reproduction, regulation of environment, and the manufacture of proteins). Nitrate is not a food source for protozoa, it is instead a harmful waste product. Over a long period of time, if the denitrifying bacteria had the chance to consume all the nitrate, then the protozoa density would eventually increase drastically and surpass the protozoa density in the negative control plots. However, car exhaust continually creates nitrogen dioxide, which mixes with rain to form acid rain that causes nitrate to enter the soil. Since cars continually produce exhaust and rain continues to fall, protozoa density is continually suppressed. So, the nitrogen dioxide in car exhaust is harmful to the density of protozoa.

The low density of protozoa can harm all other organisms in the ecosystem. Nitrate poisons protozoa, and if protozoa density is low, then not as many bacteria are eaten. When protozoa eat bacteria, nitrate is released. Then, plants absorb the released nitrate through their roots, and use it to make amino acids, which are building blocks for proteins, specifically enzymes. Without enzymes there are no chemical reactions, and without chemical reactions, the four tasks of each cell (transforming energy, reproduction, regulation of environment, and the manufacture of proteins) cannot be performed. One of the main tasks, transforming energy, is highly important for plants because through the process of photosynthesis, plants essentially convert sunlight, water, and ADT into a food source. If something hurts the density of protozoa, it also harms the health of plants.

Plants are the basis for any food web because they can create their own energy through the process of photosynthesis; also, all other animals depend on the plants' for energy. So, when the animal eats the plant, the animal can breakdown and utilize the plant's stored energy. But if the plant is unhealthy and grows very little because it has fewer nutrients, all the animals that eat plants have fewer nutrients. Then the animals that eat those animals have even fewer nutrients. In conclusion, when the protozoa density decreases, it indirectly hurts the health of plants, and higher food chain organisms.

To further research this issue and formulate more accurate results, we can record how much plants grow when nitrate is added to their soil compared to how much plants grow without the added nitrate. We could also research the density of denitrifying bacteria and how it affects protozoa density. Also, we only tested the result of one component of car exhaust. We can conclude that nitrogen dioxide, when it mixes with rain and enters the soil through the form of nitrate, is harmful to the protozoa population. Yet, we cannot conclude whether all of the other gases in car exhaust are harmful to the soil. So, in the future, more research could be conducted to determine if all components of car exhaust are harmful to the soil.