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Soil Ecology Final Project

Ecology Project: Background

Protozoa are soil microorganisms that benefit the growth of plants and the health of the larger ecosystem. Protozoa are unicellular eukaryotes, which live in water-filled pores, and reproduce by binary fission. The four types of protozoa are amoebas, ciliates, flagellates, sporozoans. They move around by the flagella attached to each protozoa, and can only move in aquatic locations. Protozoa primarily consume bacteria and make nutrients available for plants. When protozoa eat bacteria, they take some nutrients for themselves, but then they release excess nutrients into the soil (National Science & Technology Center, 2014). Without protozoa, soil would lack major nutrients and bacteria would overpopulate the soil.

Protozoa are one of the smallest organisms with a large impact on the environment. Protozoa are usually located in water near the roots of plants, which allows plants to absorb additional nutrients to perform daily functions (National Science & Technology Center, 2014). Since protozoa consume bacteria, they are responsible for controlling the decomposition rate and maintaining the nitrogen cycle in the soil. Protozoa release ammonium to soil upon eating the bacteria, and then nitrifying bacteria transform this into nitrates for plants to eat. Nitrates provide nitrogen to the plants, which is a critical element in their growth and development. Nitrogenous bases, which make up RNA and DNA, as well as amino acids, are made out of nitrogen. Amino acids make up proteins or enzymes that stop and start chemical reactions. Chemical reactions allow us to perform the four tasks of life including, homeostasis, reproduction, transformation of energy, and synthesis of new material, make up the living cell. Therefore, nitrogen is a necessary component in all living cells. After protozoa have performed their necessary duties, they can also be used as a food source for invertebrates such as fish and other water inhabitants, that aid in decomposition process. More protozoa in soil, leads to more invertebrates and producers in the ecosystem, which supports more consumers, which provides more organic material for decomposers, and eventually leads to a healthier ecosystem.

One gas that comes from fossil fuels is carbon dioxide, which is a greenhouse gas. Other major byproducts of fuel combustion include nitrous oxide, sulfur dioxide, and methane gas (Environmental Protection Agency, 2014). Every gallon of gas burned emits pounds of these byproducts into the atmosphere. (The Effects of Air Pollution on Plants and Animals, 2010). All greenhouse gases that are released into the air contribute to global warming. Carbon dioxide is one chemical that is thinning the ozone layer. Because of this, people do not have as much protection from ultraviolet radiation from the sun, which can lead to skin cancer, eye damage, and weakening of the immune system (Environmental Protection Agency, 2014). Global warming is particularly dangerous for humans because it puts our food and water supply at risk, endangers our health, and threatens other basic human needs. We have already seen the impacts of global warming, which include record high temperatures surpassing the toxic ozone, melting glaciers and severe flooding and droughts. Car exhaust helps with the production of global warming.

The effects of chemicals produced in car exhaust are worsened when mixed with moisture. Nitrous oxide and sulfur dioxide from the exhaust interact with the water and oxygen in the atmosphere to produce sulfuric and nitric acid, which then fall to earth in the form of acid rain. (Ophardt, 2003 & Upper Midwest Aerospace Consortium, 2006) Substances like sulfur dioxide dissolve in water easily, which makes it easy to transport through precipitation. Sulfur dioxide can be carried through rain, sleet, snow and fog. (United States Environmental Protection Agency, 2014) When there is too much car exhaust close to soil, the water pores within the soil will become more acidic.

This can be seen through a decrease in the pH level of soil. (U.S Environmental Protection Agency, 2012) pH measures how acidic or how basic the substance is. A pH scale goes from 0-14, 7 being neutral, anything lower than 7 is acidic, and anything higher than 7 is basic. (Charles E. Ophardt, 2003) Soil functions the best between a pH ranges of 5.0 to 7.5. Plant nutrients are most available in the pH range of 5.5 - 6.5. (Leonard Perry, 2003) Most bacteria grow best around the neutral pH values (6.5-7.0), but some can live in very acidic environments (Professor John Blamire, 2000). As acidity of the soil increases and the pH level decreases, this has a profound impact of the functioning of microorganisms in the soil. In protozoa, their enzymes in the protozoa can become denatured or disabled, such that they do not properly start and stop chemical reactions. If in their cells the enzymes can't start and stop chemical reactions, then the protozoa cannot perform the four properties of life (reproduction, homeostasis, transformation of energy, synthesis of new material) which means the protozoa would die. Without protozoa, the entire soil ecosystem would suffer. This is why our group has decided to test the protozoa population in soil, depending on the exposure to car exhaust. We have hypothesized that, as the exposure to car exhaust increases, the population density of protozoa in the soil will decrease.

Outline of Experiment

Problem: How does proximity to car exhaust change the protozoa population density in the soil?

Hypothesis: The closer proximity to car exhaust, the lower the population protozoa density

Independent Variable: the proximity to the soil of the car exhaust

Dependent Variable: the population density of the protozoa in 1 gram soil

Negative Control: the courtyard soil, farthest distance from car exhaust

Controlled Variables: location, the amount of soil extracted, time of day, actual day soil is collected, size of the soil plot, size of petri dish, type of microscope, magnification of microscope, size of microscope slide, amount of methyl green dye on microscope slide, amount of soil placed in the Uhlig extractor, type of mesh, amount of distilled water in bottom of petri dish, type of filter paper, amount of time left to hydrate the soil, amount of time in Uhlig extractor, amount of water to put into the Uhlig extractor, type of pipette and size, amount of µl in pipette

Step- By-Step

1. Make soil plot 45x45 cm by RPCS island by carpool lane located at N: 39.35817 degrees and W: 076.63554 degrees, label this plot A
2. Make another soil plot 45x45 cm by the front lawn located at N: 39.35822 degrees and W: 076.63604 degrees, label this plot B
3. Make another soil plot 45x45 cm by the courtyard located at N: 39.35790 degrees and W: 076.63661 degrees, label this plot C
4. Make sure to collect all three samples on the same day at the same time
5. Use the soil core sampler to collect 15 cm of soil from Plot A which has a 2 cm diameter
6. Use a hammer to push the soil core sampler into the ground 15 cm
7. Then turn the soil core sampler clockwise and extract from the soil in order to get your soil sample
8. Then place your soil sample into a sterile Ziploc bag and label it "Plot A trial one"
9. Repeat steps 5-7 but extract soil from Plot B (not Plot A) and label soil samples "Plot B trial one" respectively
10. Repeat steps 5-7 but extract soil from Plot C (not Plot A) and label soil samples "Plot C trial one" respectively
11. Place each of the three 15 cm soil samples (from "Plot A trial one", "Plot B trial one", and "Plot C trial one") into the bottom of a clean, empty petri dish; and allow to dry completely for 24 hours (label each petri dish by plot and trial number)
12. Use mortar and pestle to grind each soil sample, into small particles
13. Sift 9.8 g of soil from "Plot A Trial 1" into 2nd clean petri dish using 1 mm^2 nylon screen or mesh and label "Plot A trial one"
14. Repeat step 12 for trials Plot B Trial one and Plot C Trial one
15. Add 20 mL of the distilled water to saturate the soil in each petri dish
16. Cover each petri dish with its lid and allow to sit for 7 hours
17. Place the soil sample in a modified Uhlig extractor containing 30 mL of distilled water for 24 hours. Add 5 mL of water if it does not reach the top of the Uhilg extractor legs, then mark the total number of mL in data table
18. Remove the filtrate and filter a 2nd time using 12.5 cm qualitative filter paper
19. Using a capillary tube, deposit 7 µl of methyl-green stain on a clean microscope slide (1µl= 1 drop from the capillary tube). Then using a disposable graduated Beral-type pipette, and 18 µl (the first demarcation on the pipette) of the 2nd filtrate from the step 18 to stain on the microscope slide and cover with 18x18 mm^2 cover slip
20. Examine under a light microscope at 40X to count the amount of protozoa living in the soil sample. Count 3 fields of view, then calculate an average field of view for the microscope slide
21. Using the following equation to determine the population density of protozoa in the soil sample:

[(average# per field of view at 40X) x (total mL of water used) x 747] ÷ (grams of sifted soil) = # of protozoa per gram of soil

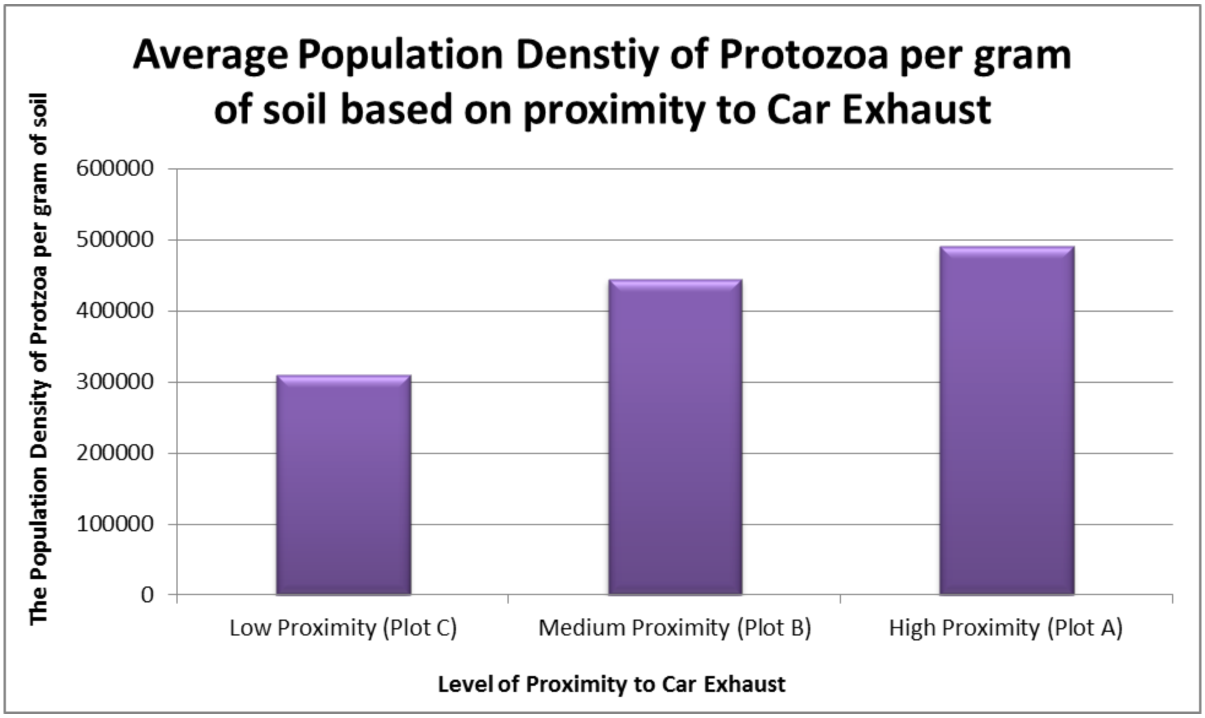
22. Record data number of protozoa per grams of soil in data table
23. Repeat steps 4-22 for trials 2 and 3 respectively, making sure to label all samples by individual plot name and trial number

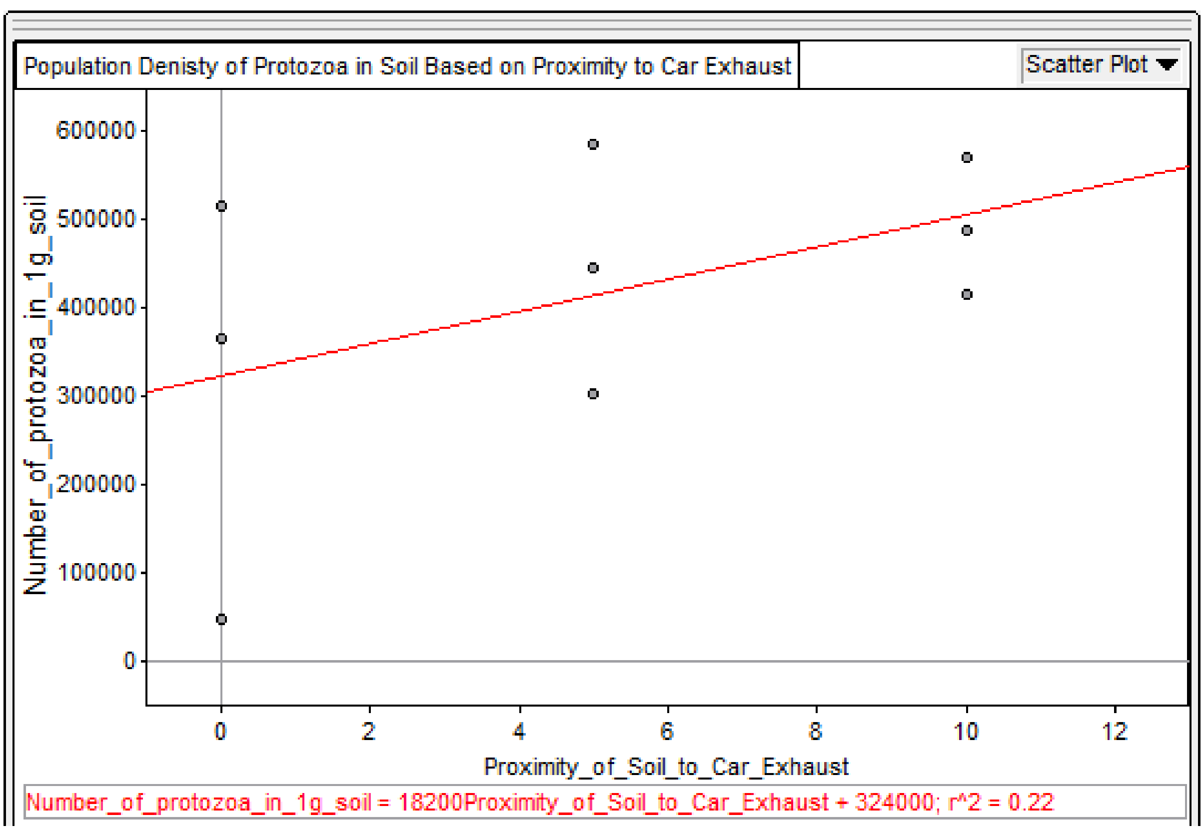
Population Density of Protozoa in the Soil based on Proximity to Car Exhaust

Soil Sample	# of protozoa in 1 gram of soil
Plot A Trial 1	487836
Plot B Trial 1	586928
Plot C Trial 1	46115
Plot A Trial 2	570159
Plot B Trial 2	444388
Plot C Trial 2	515658
Plot A Trial 3	415042
Plot B Trial 3	301848
Plot C Trial 3	364734

Average Population Density of Protozoa Based on Proximity to Car Exhaust

Plot	Average number of protozoa in 1 gram of soil
A	491012
B	444388
C	308835


Average Population Denstiy of Protozoa per gram of soil based on proximity to Car Exhaust
The Population Density of Protzoa per gram of soil
600000
500000
400000
300000
200000
100000
0
Low Proximity (Plot C)
Medium Proximity (Plot B)
High Proximity (Plot A)
Level of Proximity to Car Exhaust


Population Denisty of Protozoa in Soil Based on Proximity to Car Exhaust
Scatter Plot
Number_of_protozoa_in_1g_soil
600000
500000
400000
300000
200000
100000
0
0
2
4
6
8
10
12
Proximity_of_Soil_to_Car_Exhaust
Number_of_protozoa_in_1g_soil = 18200Proximity_of_Soil_to_Car_Exhaust + 324000; r^2 = 0.22

Conclusion

In conclusion, our hypothesis of, the higher the car exhaust exposure, the lower the protozoa population will be, was proven wrong over the course of this experiment. Our group collected soil samples in three different plots around Roland Park Country School based on exposure to car exhaust. Plot A was located on the island in between two carpool lanes as high exposure, Plot B was located in the courtyard as medium exposure, and Plot C was located in the courtyard as low to no exposure. After we collected these samples and filtered them thoroughly, our group counted the population of protozoa in each trial, for each plot. The plot that was exposed to the most car exhaust, Plot A, had the highest amount of protozoa which **estimated to be** 491,012 protozoa in one gram of soil. Plot B had the next highest number of protozoa, **which estimated to be** 444,388 protozoa in one gram on soil. Leaving Plot C to have the least protozoa, **estimated as** 308,835 in one gram of soil. Looking at our scatter plot we saw that our data in the trials were spread far apart. When graphing the line of best fit, r^2 value was 0.22, which means there was a very poor correlation. This shows that the pattern seen in the bar graph with the least amount of protozoa in the low proximity site, and the highest population of protozoa in the highest proximity site, was not very strongly supported. In the future our group would like to advise the upcoming researchers of some conflicts we think may have affected our data. To perform the experiment, we thought there would be more precise data if the surrounding environment was controlled to be the same for each plot. For future research, we would test the pH levels of the soil, which would show if the car exhaust was actually affecting the soil. The pH test would show the correlation between the car exhaust and the acidity levels in the soil. the In conclusion, the data determined the more exposure to car exhaust, the higher levels of protozoa population.

Bibliography

Bradley, J. (Ed.). *The Harmful Effects of Vehicle Exhaust*. Retrieved from Environment and Human Health website: http://www.ehhi.org/reports/exhaust/exhaust06.pdf

The environmental impact of different types of transport. Retrieved from South African History Online website: http://www.sahistory.org.za/article/history-transport-grade-4-5

Ingham, E. R. The Living Soil: Protozoa. Retrieved from Soil Biology website: http://urbanext.illinois.edu/soil/SoilBiology/protozoa.htm

Knorrel. The Effect of Vehicle Exhaust on Plants and Humans. Retrieved from Serendip Studio website: http://www.serendip.brynmawr.edu/exchange/node/8250

National Science & Technology Center. Soil Protozoa. Retrieved from Soil Biological Communities website: http://www.blm.gov/nstc/soil/protozoa/

Ophardt, C. E. (2003). pH Scale. Retrieved from Virtual Chembook Elmhurt College website: http://www.elmhurst.edu/~chm/vchembook/184ph.html

Perry, L. (2003). pH for the Garden. Retrieved from University of Vermont Extension website: http://pss.uvm.edu/ppp/pubs/oh34.htm

Safer Solutions. (2014). Car exhaust gases. Retrieved from Safer Solutions website: http://www.tec.org.au/safersolutions/a/15-a-z-of-chemicals/a-z-of-chemicals-c/46-car-exhaust-gases.html

Stout, J. D. (2014). Protozoa and the Soil. Retrieved from Victoria University of Wellington website: http://nzetc.victoria.ac.nz/tm/scholarly/tei-Bio04Tuat03-t1-body-d3.html

Union of Concerned Scientists. (14, January 31). Car Emissions and Global Warming. Retrieved from Union of Concerned Scientists website: http://www.ucsusa.org/clean_vehicles/why-clean-cars/global-warming/

Brock, D. Brockmeyer, K. Loya, K. and Torres, M. (2008) Soil Ecology Lab Manual. Batavia, IL: Flinn Scientific, Inc.