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Changing the Natural Landscape: How Roland Park Country School's Rain Drainage System Affects the Density of Protozoa in the Soil

We have acted honorably.

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Background:

According to dictionary.com, (2010) leaching is "the removal of soluble material from a substance, such as soil or rock, through the percolation of water." Typically, it results in a loss of substances present in the upper layers of the soil, and organic matter and soluble metals in particular are removed from the top soil horizons in this manner (Mifflin 2002). When this process occurs naturally through precipitation, the impact is minimal. Natural leaching is a part of a habitat's normal biological activity. But, when it is the result of human activity, leaching can dramatically affect the health of an ecosystem. Man-made leaching can change critical ecological cycles. It can cause excess nutrients from the soil to run down hill, and the negative consequences for the environment can be dramatic. For example, in areas that have large amounts of leaching, many of the plants lose their access to nutrients. Leaching removes quartz, hydroxides of iron, manganese, and aluminum, creating a type of soil known as laterite and the laterite then results in the accumulation of bauxite. High levels of bauxite lead to a loss of humus in the soil, and the result is the creation of tough and impermeable layers called duricrusts. These rigid layers make it impossible for the soil to absorb water, and consequently, to reach the roots of plants (Kellogg, 2010). In addition, this toughness of the soil makes it extremely difficult for the roots to grow in the first place, which is why they lose their access to the nutrients.

Leaching can also affect soil by altering its pH and reducing the amount of calcium in the soil, and this can make the soil become more acidic. Sometimes, leaching removes extra sodium salts, making the soil very alkaline instead. But either way, the changes in the soil pH

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caused by the leaching can lead to soil and surface waters becoming toxic because pH affects a critical component of all biological systems: the enzymes living things use to function. Enzymes are very sensitive to changes in pH, and any alteration in pH can change a shape, resulting in the loss of the enzyme's function. This prohibits them from carrying out the four fundamental tasks of life by stopping all of their chemical reactions (Bill, 2010). Another way leaching can hurt both the terrestrial and aquatic ecosystems is by generating large losses of nitrogen. The nitrate compounds can leach from the soil quickly and efficiently, and the consequent toxicity of the soil and water can leak into aquatic habitats, harming nature both on land and in streams, rivers, lakes, and oceans.

The deficit of critical nutrients which leaching causes takes a toll on many of the creatures in the soil, especially protozoa. These eukaryotic organisms are found throughout the upper soil horizon and are part of a natural organic cycle where energy is constantly being transformed. The three main types of protozoa in the soil (Ciliates, Amoeba, and Flagellates) constantly use, emit, and alter the chemical elements in the soil and one of the main tasks they help to accomplish is the turnover of carbon. They also interact with the various microbe populations and other living organisms that inhabit the soil (Protozoa and the Soil, 2008).

Soil protozoa, for example, help fuel the rate of carbon turnover, and the faster the rate of carbon turnover, the better. Carbon compounds are essential to life on this planet because they are the building blocks of all biological molecules. Hence, the cycling of those molecules through the food chain, decomposition process, and photosynthesis is what makes life on this

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planet possible. Since the faster the rate of carbon turnover the better off all life is and since the stimulation of carbon turnover increases with more protozoa, denser protozoa communities in the soil mean healthier soil because increased carbon turnover leads to more biological molecules being available for all organisms.

Although all types of protozoa can be affected through leaching, this reduction appears to affect the ciliate the most. This is especially severe because ciliates regulate the size and the composition of bacterial communities through nitrification (The Journal of Eukaryotic Microbiology, 2004). Nitrification is the transformation of ammonium to nitrate (Understanding Nitrogen in Soil, 2002). Protozoa aid this process of mineralization of nitrogen from soil organic matter by stimulating the turnover of bacterial biomass (Protozoan Predation and the Turnover of Organic Carbon and Nitrogen in the Presence of Plants, 1990). Mineralization is when bacteria take in organic material and release ammonium nitrogen, which increases with microbial activity (Understanding Nitrogen in Soil, 2002). In other words, the more protozoa in the soil, the more stimulation of nitrogen, and the more fertile the soil is. This is because without nitrate, plants cannot make DNA, RNA, or proteins. Without these essential molecules, the cells of plants cannot transcribe DNA into RNA and cannot translate the RNA into the most critical protein of all, enzymes. No enzymes and there are no chemical reactions that are necessary for life.

Soil health, then, depends on the density of protozoa in it. When a natural cycle is thrown off balance because of human impact, in this case the way that building run off non-naturally leaches, the protozoa in the soil may be negatively affected. Draining away natural minerals and

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nutrients in the soil through the process of leaching has an effect on protozoa in soil. Through leaching, the population of protozoa tends to fall (Protozoa And The Soil, 2008). The impact in population reduction is greater when the soil is leached.

This is why leaching harming protozoa can be a major problem for the soil; less nitrification which leads to less fertile soil which leads to bigger problems. This is bad because fertile soil is one of the most essential elements for life on earth (Fertile Soil, 2009). Without fertile soil, the population of plant life can dramatically lower. If one looks at the big picture, plants photosynthesize, creating oxygen. Fewer plants lead to less oxygen in the air and less food for organisms to consume. With less oxygen and less food the quality of life for organisms will lesser radically. This major problem can all be traced back to protozoan density in the soil.

Due to the importance of having protozoa-rich soil, we decided to test our school's grounds to determine the health of the soil on campus. Roland Park Country School and the surrounding land naturally has a difference in elevation. The slope goes from the front lawn down to the backwoods. The lie of the land was compromised, however, when the school buildings were placed near the top of the hill. Instead of the water soaking into the soil and feeding the organisms within it, the run-off is forced to channel down the hill, taking essential nutrients with it. To keep rainwater from eroding the hillside, all the water that lands on the buildings is channeled into large containers. These containers that lie within the ground gradually leak out water into the soil, and because of the ground's decline, the water soaks through the soil all the way down to the lowest part of campus, as true with the process of leaching. Leaching

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depletes the soil of critical nutrients, and we would like to see if the leaching lowers the density of the protozoa population, and therefore health, in the soil. We will test different elevations on the hill to see if the protozoa population is lower where the concentration of the roof run- off is higher and where leaching has rid the soil of natural nutrients, compared to farther down the hill, where the concentration of the roof run- off is lower and where leaching has not rid the soil of natural nutrients as much. After this experiment, we will know if the man-made structures on the top of the hill and the leaching that occurs because of them decrease the health of soil, specifically in the quantity of protozoa.

Experiment:

Question: Does the leaching of the water runoff collected from the school's impervious surfaces decrease the density of the protozoa population in the soil of the RPCS campus?

Hypothesis: The closer the soil protozoa population is to the building, the smaller it is.

Procedure:

A. Independent Variable: The different distances between the soil samples taken and the source of the water run-off on campus.
B. Dependent Variable: The density of soil protozoa in the soil samples
C. Negative Control: Soil samples from the top of the hill, outside the leaching field
D. List of Controlled Variables:

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a. size of soil sample taken
b. The way in which the soil is extracted
c. The way that the soil is stored after extraction
d. Amount of water used to rehydrate
e. The elevation of each soil sample within each testing site
f. Amount of time that soil dries before testing
g. Way that soil is sifted
h. Amount of time that rehydrated soil sits
i. Process of filtering used
j. Amount of dye on microscope slide
k. Amount of water used in Uhlig process
l. Amount of filtered Protozoa solution viewed under microscope
m. Amount of views that the formula is applied to for each slide
n. size of a cover slip on each slide
o. Dye used to test
p. Formula used to compute density

E. Step-by-Step Instructions

a. Go to the following sites on the RPCS campus with these coordinates: N.39.35832, WO.76.63610; N. 39.35718, WO.76.63650; N. 39.35706, WO.76.63665; and N.39.35713, WO.76.63751.

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b. In each site, defined by its coordinates, establish three different plots along the same line of elevation. Facing the school and going left to right, label the plots A, B, and C, respectively. A and C should be 1 m on either side of B. Each site (2-4) lies 60 m away from the next site, in a continuous line running west of the building, with similar plant life, and minimal direct sunlight.

c. At the same place, time, and day, use soil cylinders 12 cm deep and 2 cm wide to extract exactly 10 cm of soil from each plot at each site. Place each soil sample in its own fresh plastic bag, each labeled with the site and plot number. Refer to the site on the front lawn (N.39.35832, WO.76.63610) as 1, the site near the cafeteria (N. 39.35718, WO.76.63650) as 2, the site next to the road (N. 39.35706, WO.76.63665) as 3, and the site in the backwoods (N.39.35713, WO.76.63751) as 4. In each site, as previously stated in step b, facing the school and going left to right, label the separate samples “A”, “B”, and “C”, accordingly.

d. Immediately, place 10 cm of each soil sample into the bottom of a separate, clean, empty petri dish, labeled with the corresponding site and plot number as the sample.

e. Allow all of the soil samples to dry completely for 24 hours.

f. Sift 10 g of each soil sample into a 2nd clean petri dish, also labeled with each sample’s site and plot number, using a 1 mm^2 nylon screen. Put each

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sample in a different dish and use a different screen for each sample. Put the top on each dish.

g. When ready, but at the same place, time, and day, add 20 ml of distilled water to each sample to saturate the soil.

h. Cover each petri dish with its lid and allow to sit for 7 hours.

i. At the end of the 7 hours, put each soil sample in a modified Uhlig extractor, also labeled with each sample's site and plot number, containing 30 ml of distilled water for 24 hours. If 24 hours passes and the next steps cannot be completed, let the filtrates rest in the refrigerator until the steps j-n can be completed. When you remove the samples, remove them at them at the same time, place, and day.

j. At the end of the 24 hours or when the all of filtrates can be removed from the refrigerator at the same place, time, and day, remove the filtrate of each petri dish and filter each sample a 2nd time using 12.5 cm qualitative filter paper. Place each sample into a cup, also labeled with each sample's site and plot number.

k. Complete steps 1-o for each soil sample at the same time, place, and day,.

l. Using a capillary tube, deposit 7 µl of methyl-green stain on a clean microscope slide (1 µl = 1 drop from the capillary tube).

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m. Then using a disposable graduated Beral-type pipette, add 18 µl (the first demarcation on the pipette) of one of the samples after its 2nd filtrate from step j to the stain on the microscope slide and cover with an 18 x 18 mm^2 cover slip.

n. Examine the digital microscope at 40X to observe how many protozoa are living in the soil.

o. Examine the sample five different times, one time in the left top corner of the cover slip, one in the right top corner, one in the left bottom corner, one in the right bottom corner, and one in the center of the cover slip. Count the number of protozoa in each viewing of the slide in each different place, and find the average of those counts to find the average number of protozoa per field of view for that sample. Use the number in the following equation to determine the population density of protozoa in the soil sample: (# per field of view at 40X) x (total ml of water used) x (747) ÷ (grams of sifted soil) = # of protozoa per gram of soil.

Data Table

Protozoa Density in The Soil

Sample #	Distance Away from Building	# of protozoa per gram of soil
Site 1- Front Lawn- Plot A	NC (not in leaching field)	814230
Site 1- Front Lawn- Plot B	NC (not in leaching field)	705915
Site 1- Front Lawn- Plot C	NC (not in leaching field)	395910
Site 2-Near Dining Hall-Plot A	60 m	112050
Site 2-Near Dining Hall-Plot B	60 m	339885

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Site 2-Near Dining Hall-Plot C	60 m	470610
Site 3-Next to road, on backwoods side-Plot A	120 m	89640
Site 3- Next to road, on backwoods side –Plot B	120 m	97110
Site 3-next to road, on backwoods side-Plot C	120 m	1822680
Site 4-In Backwoods-Plot A	180 m	2095335
Site 4-In Backwoods Plot B	180 m	784350
Site 4-In Backwoods Plot C	180 m	Data point lost

Average Number of Protozoa per Gram of Soil in each Site

Sample Number	S1-front lawn	S2-near cafeteria	Site 3- next to road, on backwoods side	Site 4-in backwoods
Distance away from building	NC (not in leaching field)	60 m	120 m	180 m
Number of Protozoa per gram of Soil	638685	307515	669810	1439843

P-Values

Negative Control vs. 60 meters away from the building	.1148
60 meters away vs. 120 meters away from the building	.596
120 meters away vs. 180 meters away from the building	.88

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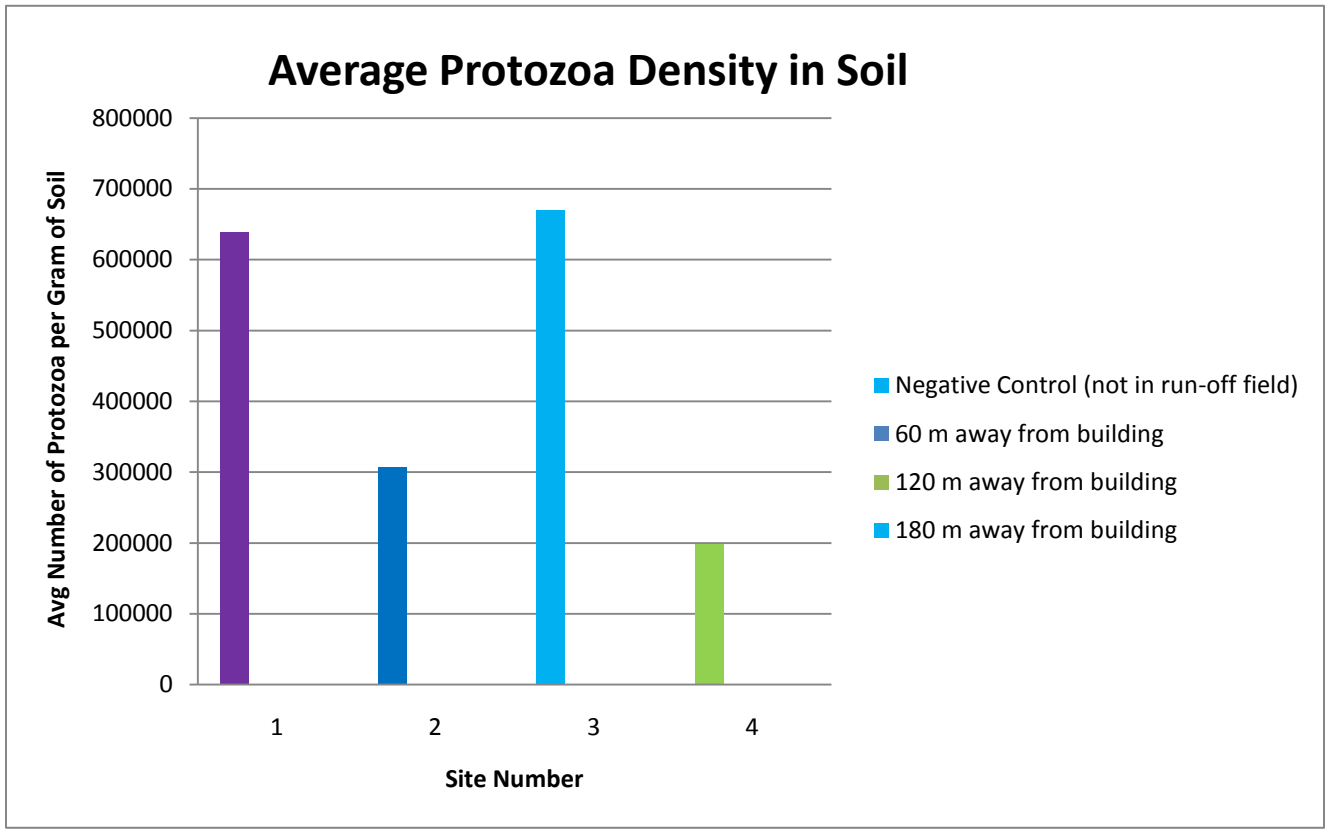
Graphs:



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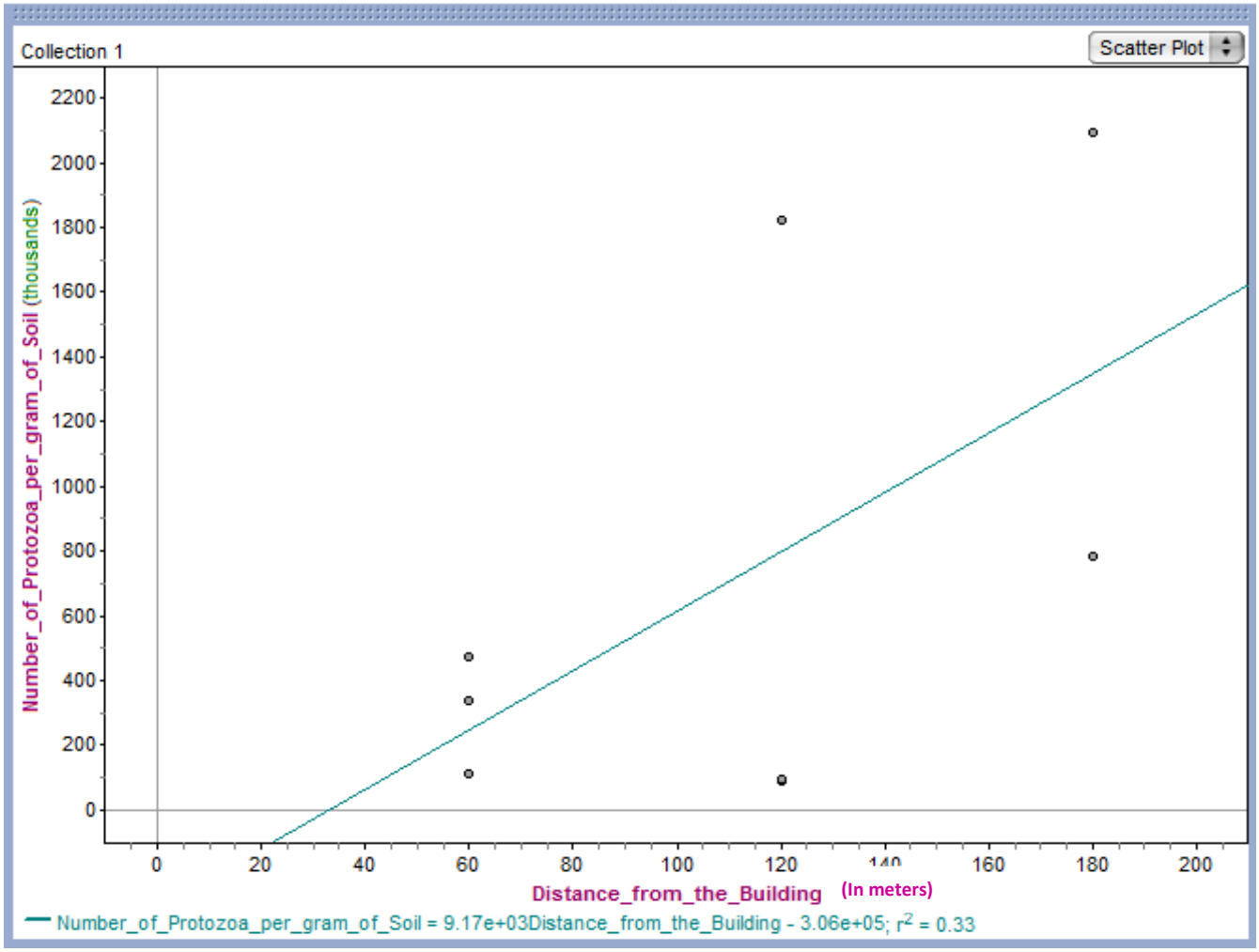
Distance from the RPCS Buildings vs Average Protozoa Density in the Soil



Conclusion:

Our hypothesis was correct. The closer the protozoa population to the building, the smaller it is. Evidence supporting this fact is found in our scatter plot. However, the bar graph does not

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support this hypothesis, with the average number of protozoa not steadily increasing as the plots moved further away the building. 60 meters away from the building, the average protozoa count was 307515, and 120 meters away from the building, the average protozoa count was 669810, but 180 meters away from the building, the average protozoa count was 198777, showing that more protozoa was present 120 meters away from the building than 180 meters. On the other hand, when we completed a T-test, we found that the P-value comparing the 60 meters away from the building test vs the 120 meters away from the building test was 0.596 and the P-value comparing the 120 meters away from the building test vs the 180 meters away from the building test was 0.88, both proving that the way that the data was presented in the bar graph was not as accurate as the scatter plot. The scatter plot showed that the average number of protozoa did steadily increase as the plots moved further away from the building at a rate of 9170 protozoa per gram per meter.

However, the r^2 value indicates that only 33 % of the upward rise shown in the scatter plot can be attributed to the distance from the building. This small r^2 value could indicate that the significantly larger density of protozoa that was observed in the bar graph at 120 m away from the building could be significant, even though the large P-value declared it insignificant. For further research to discover the reason for this contradictory data, one could test the soil for other factors that may have contributed to the spike in protozoa at 120 meters. Scientists could test how much water runs off the road near the backwoods, or how the road-run off affects the protozoa, as protozoa thrive in water-rich environments. Possibly, the large density of protozoa

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120 meters away from the school was due to the great amount of water that ran-off from the road.

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