All Dried Up

Soil Ecology Project
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Background

The world beneath our feet, i.e. the soil, is the grounding from which all life abounds. The soil consists of many components all of which play a significant role in maintaining and balancing a productive and fruitful ecosystem. The soil nutrients, air, water, microorganisms and physical structure of the soil all work collectively to work towards maximizing plant growth and ecological success ("What are Microbes," 2003). All organisms no matter the size, contributes to the soil's success.

Many different factors need to be in balance in order for soil to be healthy. One factor is that nutrients need to be run in systems maintaining nutrient cycles and the retention of nutrients within the soil ("The Soil Foodweb," 1996). The major nutrients that play a role in the health of the soil and in turn plant life are carbon (C), nitrogen (N), magnesium (Mg), calcium (Ca), iron (Fe), and phosphorus (P) ("Nutrient Cycles," 1996). Another factor necessary for sustaining a healthy soil is proper microbe interaction. Microbes, which are the life of the soil, include archaea, bacteria, fungi, and protists. Two of these microbes, bacteria and fungi, are responsible for retaining nutrient levels in the soil as soil decomposers. The process in which these soil decomposers redistribute nutrients to the plants and animals in the ecosystem is called mineralization. The system is responsible for getting nutrients from their retained form to and from plants. They do this by converting or retaining nutrients into the necessary form for plant uptake ("What are Microbes," 2003). If the soil decomposers do not retain nutrients, the ecosystem will have a productivity problem, jeopardizing all life forms and negatively affecting other surrounding areas ("The Soil Foodweb," 1996). The protozoa, nematodes, micro-

arthropods, and earthworms are all predators to bacteria and fungi within the soil. The predator's role along with the prey is to redistribute the nutrients through the soil to other living things, in order to enhance the ecosystems productivity. The activity of these interactions may be controlled by higher-level predators such as millipedes, centipedes, beetles, spiders, or small mammals in the soil food chain ("The Soil Foodweb," 1996). The interactions amongst all of these organisms are important to maintain a rich healthy soil and allow plant growth to flourish to serve as a basis for animal life.

Bacteria are vital microorganisms in the soil. They are prokaryotic organisms serving an important role at the bottom of the food chain. Their roles in the soil include rapidly enhancing and building good soil structure, releasing nutrients into the soil, disease control, and they are essential for nutrient cycling ("What are Microbes," 2003). Bacteria as well breakdown crop residues and chemical toxins that would otherwise decrease plant growth (Agulia, 2003). Bacteria are also crucial for plant growth by providing them with usable nutrients and performing nutrient retention. Bacterium plays a critical role in maintaining the earth as a suitable place for inhabitation by other forms of life ("Protozoa," 1998).

Protozoa is an important microbe throughout mineralization and other processes that occur within the soil. The word Protozoa literally means, "first animals." They are eukaryotic and are the most abundant "animals" in the world in terms of population and biomass. The primary importance of protozoa is as consumers of bacteria however they also prey for unicellular or filamentous algae, microfungi and nematodes. Protozoa are a significant food source for micro-invertebrates, such as earthworms and nematodes. They play a role as both herbivores and consumers in the decomposer link of the

ecological food chain. They are also responsible for transferring bacterial and algal assembly and sustaining consecutive trophic levels in the soil (Lipscomb, 2000). Normal protozoa levels involve a balance of three kinds of protozoa: ciliates, flagellates, and amoebae. Ciliates, hence the name, are characterized by having cilia which are usually arranged in rows all beating in a stroke in the same direction. Ciliates as well have two different types of nucleuses and undergo transverse fission ("Protozoa," 1998). Flagellates use flagella as their organelle of locomotion and like amoebae, undergo binary fission. Many flagellates can feed both autotrophically engaging in photosynthesis and heterotrophically. Amoebae are characterized by their pseudopodium, which can be used either for locomotion or to take up food. Based on this organelle, the amoebae are then divided into two other groups Rhizopoda and Actinopoda. Amoebae as well sometimes live in shells ("Protozoa," 1998). These three types of protozoa: flagellates, amoebae, and ciliated are essential to the mineralization process and making nutrients available to plants in their ecosystem ("The Soil Foodweb," 1996).

Protozoa have the ability to live virtually anywhere. They have been found all over the world in a variety of diverse environments and ecosystems. As for protozoan existence in the soil, they have been found in both peat-rich soils and dry sands of deserts (Lipscomb, 2000). Ideal soil conditions for protozoa however are moistness, high temperatures, plentiful nourishment, rich organic material, and high levels of bacteria. Protozoa are in their greatest quantity within 15cm from soil surface but they have been counted at depths of a meter or more (Lipscomb, 2000).

Protozoa are an important natural soil-health indicator. By studying the three types of soil protozoa: ciliates, flagellates, and amoebae and finding which types are high

or low, many important things can be concluded about the soil. The counts for these three types also assess whether the soil is aerobic or anaerobic (Agulia, 2003). Studying counts of bacteria versus counts of protozoa would also be beneficial to find the health of soil. Both high bacteria and high protozoa levels are desirable. A decrease in the number and diversity of protozoa in the soil is one of the first indicators that a particular ecosystem is in danger.

Maryland's past years of drought may have been the cause for disturbed levels of protozoa and bacteria previously measured in RPCS's backwoods. A drought can interrupt the normal cycling of nutrients that are caused by movements of water by altering protozoa and bacteria levels. Protozoa thrive in very moist environments so with decreased water density in the soil protozoa levels would decrease. The dampness in soil causes room for the microorganisms to move about so with a drought microorganisms would be hindered in their mobility. With the breaking of the drought perhaps protozoa levels on the RPCS campus would return back to normal.

Using the National Weather Service (2001), we found and averaged the Maryland climate records and PCPN amounts from places around Maryland to find an average rainfall amount per year. That amount was then used to find that 26 $\frac{2}{3}$ mL of water was an average amount of rain per day. This was then used so that appropriate amounts of water could be added to our indoor plots simulating average conditions of rainfall.

In order for soil to be healthy, a grand scale of factors needs to be in sync. The soil's water content, microbe levels, nutrient levels, chemical levels, and air density all depend and are resultant on each other's counts. The soil is a complex network that

contains both biotic and abiotic elements, which must harmoniously co-exist to create a perfect balance.

Lab Outline

I. **Problem**: Will the protozoa density in soil on the RPCS campus return to normal soil protozoa levels when soil samples are isolated and exposed to precipitation levels that simulate normal average precipitation levels in Maryland?

II. **Hypothesis**: The protozoa density will increase to normal levels when the precipitation level is increased.

III. **Experiment**:

A. Variables
   1. Independent Variables: The amount of precipitation the soil receives, isolation of soil from natural plot.
   2. Dependent Variable: The density of protozoa in the soil.

B. Controls
   1. Negative Control: the plot outside (on the edge of the lower playing field) that receives natural precipitation and is not isolated
   2. Controlled Variables: density of water, type of water used, when the samples are taken, exposure to fertilizers, wind, pollution, foot traffic, runoff, density of water per amount of soil, amount of soil in sample, size and type of petri dishes, size and type of nylon mesh, the size of the Uhlig extractor, the amount of water used to saturate soil samples, the amount of methyl green stain per sample, the balanced used, the type and size of the microscope slide air, amount of sunlight, time between watering, time soil spends drying, temperature, interval between saturating the soil and filtering, Uhlig extractor used, source of water used, unit of measure, type of core samplers, type and size of cylindrical canisters, location of plots, location of isolated plots, time between samples were taken, when isolated plot were watered, soil treatments, contact with natural ecosystem, contact with animals, humidity, temperature, runoff.

C. Procedure
   1. Use a GPS device to find the location of the plot 39.35858 North, 76.63760 West.

2. Stake out 3 plots of flat ground covered with grass on a field with the dimensions of .5 meters by .5 meters square in a line, next to each other.
3. Place one flag in each corner of each plot to mark the sample area (total of 4 flags).
4. Designate which plot is which by naming them Outdoor Plot #1, Outdoor Plot #2, and Outdoor Plot #3 by writing the number (1, 2, or 3) on the plot's flags.
5. In the southeast corner of each plot, take a 15 cm deep core sample with a diameter of 10 cm. (For this we used Pirouette cookie tins)
6. Rotate the tins 360 degrees while in ground.
7. Remove the tins from the ground and make sure the soil samples are secured in the tin by placing your hand on the bottom openings.
8. Label the tins according to which plot they came from, as Indoor Plot #1, Indoor Plot #2, and Indoor Plot #3 accordingly.
9. Cut the top edge of the tins at the grass line of the soil boundary with a tin cutter if the soil does not reach the top of the can so that the top of the grass reaches the top of the can.
10. Place all three indoor plots in the lab in a spot where they will receive the same amount of sunlight as the plots in the field.
11. 30 minutes after all six plots have been set, go to the outdoor plots and randomly take a 15 by 2 cm core sample from each plot from anywhere within the plot, making sure to rotate the core samplers 360 degrees before removing.
12. Place each sample in a plastic baggie and label them according to their plot names (Outdoor Plot #1, Outdoor Plot #2, and Outdoor Plot #3), and the words Sample #1.
13. Immediately go to the indoor plots and randomly take a 15 by 2 cm soil sample from each one, making sure to rotate the core samplers 360 degrees before removing.
14. Place each sample in a plastic baggie and label them according to their plot names (Indoor Plot #1, Indoor Plot #2, and Indoor Plot #3) and the words Sample #1
15. On the day the samples are taken, begin drying all six soil samples by placing each of them in a petri dish in a windowsill according to the Brockmeyer Protozoa Extraction procedure.
16. Use the Uhlig/Brockmeyer Protozoa Extraction procedure on all six soil samples.
17. Once the microscope slides are made, they can be viewed at 40X or 100X. Each slide should be looked at in five different fields of view and in each field of view the number of protozoa seen are to be counted.
18. Average the five different counts from each slide (i.e.: slide 1 Outdoor Plot sample 2: 45*36*23*30*34/5). This number will be put into the appropriate equation below as the # of protozoa per field of view.

19. To collect the number of protozoa per gram of soil, use the following equation:
    a. For 40X - [(# of protozoa per field of view) * (total ml of H20 used) * 747] / (grams of soil) = # of protozoa per gram of soil
    b. For 100X - [(# of protozoa per field of view) * (total ml of H20 used) * 5102] / (grams of soil) = # of protozoa per gram of soil
20. Every 3 days water each indoor plot with 80 ml of water each to simulate average precipitation levels for the course of the experiment.
21. Repeat steps 11-18 every 3 days* four more times, so that the experiment has been run a total of five times
    *Because of a weekend, our Sample 2 from all plots was taken 4 days after the initial sample (Sample 1) was taken
22. Use the data collected (# of protozoa per gram of soil) to compare and contrast the protozoa levels in the soil.

Data and Analysis

Population density of Protozoa from averages protozoa counts (5/9/03)

	Outdoor	**Isolated**
Plot 1	26,679	1,127,811
Plot 2	94,358	912,989
Plot 3	78,632	375,937
AVERAGE	***66,556***	***805,579***

Population density of Protozoa from averages protozoa counts (5/13/03)

	Outdoor	**Isolated**
Plot 1	7,863	217,106
Plot 2	11,434	53,705
Plot 3	15,726	*No Data*
AVERAGE	***11,674***	***135,406***

Population density of Protozoa from averages protozoa counts (5/16/03)

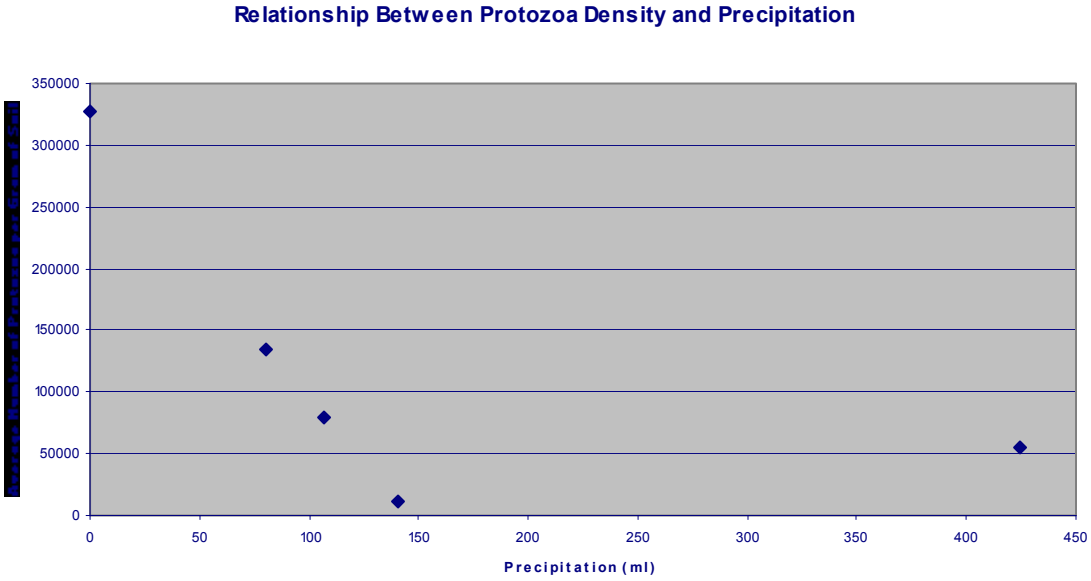
	Outdoor	**Isolated**
Plot 1	188,716	102,221
Plot 2	70,768	78,632
Plot 3	62,905	220,168
AVERAGE	***107,463***	***133,674***

Population density of Protozoa from averages protozoa counts (5/19/03)

	Outdoor	**Isolated**
Plot 1	47179	70768
Plot 2	66837	90426
Plot 3	51111	78632
AVERAGE	***55,042***	***79,942***

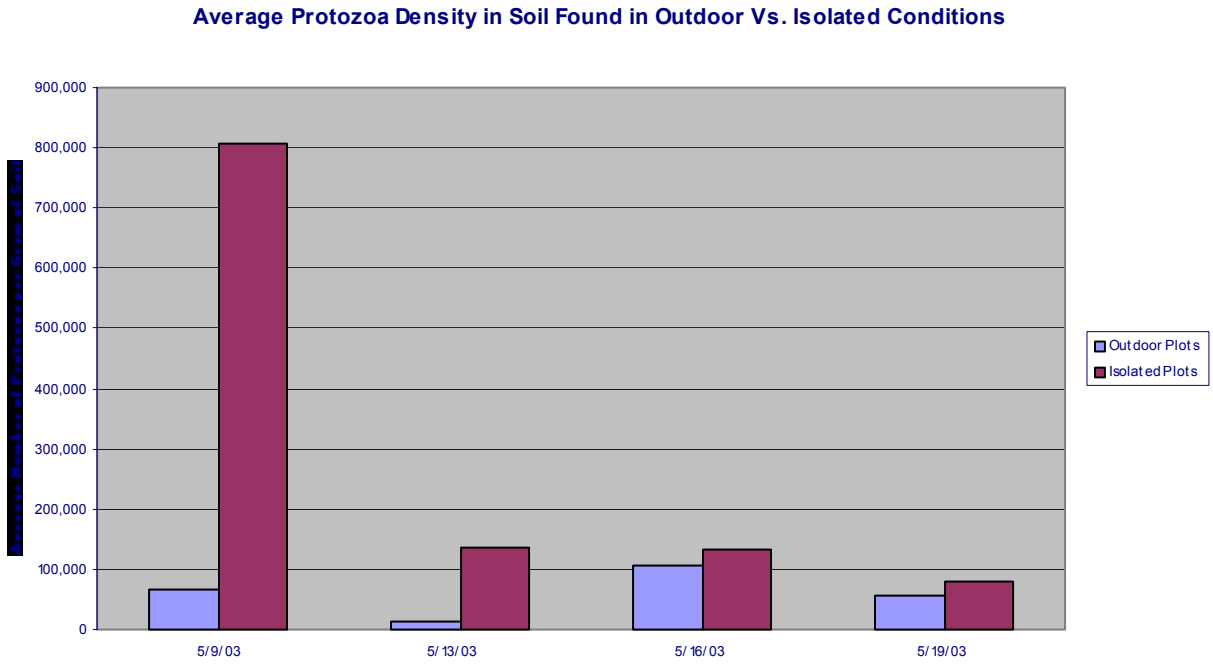
Amount of precipitation (ml) received by the plots

	Outdoor	**Isolated**
5/9/03	99	N/A[1]
5/10/03	229	N/A
5/11/03	10.3	N/A
5/12/03	4.12	80
5/13/03	Trace	N/A
5/14/03	0	N/A
5/15/03	Trace	80
5/16/03	404	N/A
5/17/03	12	N/A
5/18/03	8	N/A
5/19/03	0	106.7



[1] Instead of watering with the average precipitation everyday we added the total average precipitation for every 3 days every 3 days

We preformed a Sample T Test on the amount of protozoa per gram of soil, comparing outdoor and isolated conditions. The P value for this test was .0561, which, technically is not valid because for two sets of numbers to have a valid relationship, the P value would need to be .05 or less. Our P value is greater than .05, but because it is only slightly off, we have decided to continue with the analysis. Our P value tells us that there is a 94.4% chance that the isolated data has a defined relationship with the outdoor data.



Conclusion

Our Hypothesis was incorrect. The protozoa density in the soil did not increase to normal levels when the precipitation increased as seen in the "Relationship Between Protozoa Density and Precipitation" graph. Through our research, we learned that protozoa thrive in moist climates, but our data from the RPCS fields is a counterexample of this belief. The protozoa density fluctuated with the increases and decreases of water availability. When samples had access to 0 ml of water they achieved the highest average protozoa density, at 326,532 protozoa per gram of soil. After receiving 80 ml of precipitation, the average protozoa density dropped significantly to 134,366 protozoa per gram of soil. This negative trend continued; when the soil received 106.7 ml the protozoa density per gram of soil decreased to 79,942 protozoa per gram of soil and then decreased further to hit a low (for our experiment) of 11,674 protozoa per

gram of soil when the soil had access to 141 ml of water. When the soil had access to 424 ml of water however, the protozoa density defied the trend and went up, bringing the average protozoa density to 55,042 protozoa per gram of soil. We are 94.4% sure that the fact that the protozoa density for the isolated samples were significantly higher because of a valid relationship. Something outdoors, not the amount of precipitation, is affecting the protozoa density in the soil. The collected data shows that the initial average protozoa densities were 66,566 protozoa per gram of soil for the outdoor plots and 805,579 protozoa per gram of soil for the isolated plots. Since the samples were taken thirty minutes after being isolated from each other, and had received the same precipitation (0 ml), it seems unlikely that these figures are totally accurate. This is a source of error in our data that is due to lack of experience with protozoa counting. Both samples that received 80 ml of precipitation had relatively close protozoa densities. Sample 2 Isolated had an average of 135,406 protozoa per gram of soil and Sample 3 Isolated had an average of 133,674 protozoa per gram of soil. The consistency of these figures shows that the amount of precipitation has a slight effect on the protozoa density. We can conclude that the amount of precipitation did not directly affect the protozoa density in the soil. There are many possible indirect effects that the precipitation could have caused. Further and extended experiments would eventually answer this question. This would expand the amount of data and would allow the protozoa to change even more. This would also extend our knowledge of the correlation between protozoa and water levels. The protozoa were shocked by the drought and began to have abnormal behaviors. Now that the drought has ceased, our experiment can be done again with improvements and hopefully show us what is specifically causing the low protozoa levels on the Roland Park Country School's campus. The short generation span of protozoa assures us that the healthy protozoa will begin to thrive and reproduce and the mutated protozoa will die off in a typically short amount of time. Another improvement could be to study the properties in water of the tap water and of the natural precipitation. Although the increase in the amount of water didn't increase the amount of protozoa, another property of the water potentially could have. Nutrient tests could be done alongside chemical tests as well.

Further experiments are necessary to accurately solve and understand the correlation between water and protozoa.

References

Agulia, Johnny. *Healthy Soil Ecology and Beneficial Microorganisms Soil Food Web Baseline Assay* (2003) Retrieved May 9, 2003 from http://www.abcorganics.com/Functional%20Groups.htm

Lipscomb, Diana. (2000). *Protozoa*. Retrieved May 4, 2003, from http://biology.usgs.gov/s+t/noframe/m2083.htm

National Weather Service. (2001). *Annual 2001 Statistics For The Washington/Baltimore Area.* Retrieved May 3, 2003, from http://www.nws.noaa.gov/om/presto/presto2001/2001dectable.pdf

Nutrient Cycles (1996). Retrieved May 20, 2003, from http://www.swifty.com/apase/charlotte/soil7.html

Protozoa. *(1998) Retrieved May 6, 2003, from http://www-biol.paisley.ac.uk/courses/Tatner/biomedia/units/prot1.htm*

The Soil Foodweb: It's Importance In Ecosystem Health *(1996) Retrieved May 6, 2003, from http://www.rain.org/~sals/ingham.html*

What Are Microbes? (2003) Retrieved May 5, 2003, from http://www.superbio.com/science/science-microbes.shtml