Jane Hollon Ann Margaret Millspaugh
Allie Weiss Caroline Atwater

Background Report

"*We know less about life in the earth under our feet than we do about the far side of the moon*" (Discovery School, 2002).

Knowledge about soil and the organisms living in it has been expanding, however it was not until recently that the study of these ecosystems became prevalent. The studies of “the life under our feet” is called soil ecology and since scientists realized the significant impact microorganisms have on everything, especially humans, it has become one of the most important fields of scientific study. The study of soil ecology is also essential because plants use the soil to sustain life and plants are the base of the food chain. Thus soil affects every other organism on the planet. Therefore it is imperative in order to understand our lives and science in general that we learn more about soil ecology.

Soil makes up a large part of the world in which we live. Its variation and multiple microorganisms make it consist of much more than just dirt and rock particles; it is a habitat to many living organisms. This habitat, soil, is a complex mixture of sand, silt, clay, and decaying animal and plant tissue. (Essenfeld, Carol, and Moore, 1994) Most soil organisms and plants tend to make their habitats in topsoil, which is mostly made up of mostly organic materials (Johnson, 1998) Below the topsoil is a mixture of mineral particles and humus (partly or completely decayed plants and animals), which contains most of the soil’s nutrients and is called subsoil. After the subsoil the organic material and microorganisms begins to decrease as the soil begins to get closer to the core of the earth. In fact, the organisms which live in this environment are so microscopic and numerous, scientists maybe never name them all. When the organic material and

microorganisms are almost completely absent, it is the point of Bedrock where the earth has become solid rock. (Essenfeld, Carol, and Moore, 1994)

Soil is critical in maintaining an environment. Soil is a living space for a manifold of living creatures such as bacteria, fungi, worms, insects, and larger burrowing animals. (Schraer and Stoltze, 1983) Bacteria and fungi break down organism remains into simple inorganic and organic compounds (such as nitrate, ammonia, and simple sugars) that enrich soil chemically. Larger creatures, such as worms and burrowing animals, supply air to the soil as well as enriching it with their excrements. But ultimately it is the microorganisms found in the soil that continue to keep it alive; they are the basis of the entire ecosystem. (Johnson, 1998)

In the soil, fungi, bacteria, and algae and other organisms interact with one another resulting in complex ecosystems. Each species struggles for food and space and each species has their own niche. Because there is such a variety of microorganisms, identifying the various species can be challenging, especially since the most critical microorganisms can often be found in the smallest forms. (USGS Canyonlands Field Station, 2003) Microorganisms perform the vital function of decomposing the organic matter and releasing nutrients from that matter for plants to use such as nitrogen, phosphorus, potassium, calcium, and magnesium. (Pidwirny, 2003) Not only do they help the plants perform their various functions, but they also increase the soil's ability to hold water and air. Out of the millions of microorganisms found throughout the soil, one example is the thread-like fungi, *Albegia*, which are partially attached to the soil and partially attached to the cells of the plant's root; they help deliver nutrients to plants. (Pidwirny, 2003)

Microorganisms also play a key role in directing the circulation of carbon dioxide. Fixation (the process of incorporating carbon dioxide into the molecules of living matter) is vital procedure in plants and animals. Carbon-containing substances consumed and used by plants and animals trigger biochemical reactions, critical to their daily function. Plants convert inorganic carbon dioxide and water into simple carbohydrates and the molecules that make up every living thing on the earth. An animal, which ate the plant containing these substances, then consumes the simple carbohydrates and uses the molecules to create and repair its tissues. If an animal eats that animal, the simple carbohydrate and molecules are passed to the carnivore that ate it. Once the simple carbohydrate is in the body and respiration occurs, the carbohydrate becomes oxidized; releasing energy and originating the chemicals back to their previous form of water and carbon dioxide.

This same process occurs if the plant or animal dies: in order to satisfy their own cell-building and energy needs, microorganisms break down the organic molecules of a plant or animal thus returning them to their previous form of water and carbon dioxide. The decomposition of organic matter is the primary process in which nutrients are released back into the soil. The decomposition begins with larger soil organisms such as earthworms, arthropods, and gastropods; they break down the soil particles into smaller pieces, which are then decomposed by fungi and bacteria, specifically yeast. The yeast decompose the soil by fixating themselves on the organic matter and growing. They then begin to eat the organic matter and while they do this, they release carbon dioxide, water, nitrogen, phosphorus, and sulfur; these are all materials that are needed by plants. By releasing more carbon dioxide in the air, they allow the carbon cycle to continually take

place. The carbon dioxide then goes back into the plants, and cycle of the production of molecules of all living things begins again.

In the plant world yeast also benefits the growth level of vegetation in the environment by aerating the soil as it expands and also giving off carbon dioxide. The effects yeast have on plants is positive feedback, yeast eats sugar, and then as a result, makes carbon dioxide. The plants need carbon dioxide to survive, and as they respire using that carbon dioxide, the produce sugar. Therefore, the yeast and the plants have a mutual relationship, they both benefit off the other. In their niche, yeasts survive in watery environment, which is why they are also prevalent in aquatic environments. Therefore the amount of water in the soil also affects the amount of yeast in soil. *(Saacharomyces* Genome Database, 2003)

In our experiment, we are testing whether plant diversity or plant density have an impact on the population of yeast in the soil. In the bigger picture, we are using this information to decide if the human altered soil with varying plant diversity and/or density has an impact on the population density of yeast. The amount of yeast in these human altered plots on the RPCS campus will determine which has created a better soil environment, more plants or more diverse plants. To test this we will be choosing three different gardens (with different diversity and different densities) and taking three samples from each, then finding the yeast content using the soil identification procedure. By testing these plots, as well as using a positive and negative control (the backwoods and a plot without any plants), we can determine where the most yeast lives. Elements such as the amount of water and sunlight also have an affect on the yeast population,

therefore we are controlling this by taking all the samples at the same time, that way they would all have had the same environmental affects.

Using our knowledge about yeasts and their production of carbon dioxide, we can make a rather accurate determination on whether plant density or diversity provides a better environment for yeasts to thrive in. We believe that our findings could in fact hold a beneficial conclusion in the field of soil ecology because human alterations in the environment have become such a large part of our surroundings. Gardens are everywhere and by knowing if it is better for the yeast population to plant more of the same plant or only a few of many different plants, we can plant gardens that will have the best growing conditions. Yeast is a crucial part in the plant growth process because of its ability to aerate the soil and produce CO2, and although plants can live without it, yeast is a growing factor that all successful plants will need.

Jane Hollon
Allie Weiss
Ann Margaret Millspaugh
Caroline Atwater

Work Cited

Discovery School. The Dirt On Soil; What's Really Going on Underground. (2002) Retrieved 10 May 2002, http://school.discovery.com/schooladventures/soil/index.html

Essenfeld, Bernice, Carol, Gontang, and Moore, Randy. (1994) *Biology.* California: Addison-Wesley Publishing Company, Inc.

Johnson, George B. (1998) *Biology Visualizing Life.* Texas: Harcourt Brace & Company.

Pidwirny, Michael. (2003) Fundamentals of Physical Geography. Retrived 23 May 2003, from http://www.geog.ouc.bc.ca/physgeog/contents/9q.html

Saacharomyces Genome Database. (2003) What are yeasts? Retrieved 15 May 2003, from http://www.yeastgenome.org/VL-what_are_yeast.html

Schraer, William and Stoltze, Herbert. (1983) *Biology the Study of Life.* New Jersey*:* Viacom Company.

USGS Canyonlands Field Station . (2003) An Introduction of Biology Soil Crust. Retrieved 18 May 2003, from http://www.soilcrust.org/crust101.htm

Jane Hollon
Allie Weiss
Ann Margaret Millspaugh
Caroline Atwater

Experiment Lab

I. Problem: Which method of creating a garden creates a better environment for yeasts to survive in the soil, a garden with more plant density or a garden with more plant diversity?

II. Hypothesis: Gardens with higher plant density will have a higher yeast density than gardens with higher plant diversity.

III. Experiment:

a. Variables~
   i. Independent Variable 1: plant density
   ii. Independent Variable 2: plant diversity
   iii. Dependant Variable: density of yeast in the soil

b. Controls~
   i. Positive Control: a plot in the back woods
   ii. Negative Control: plot with no plant life
   iii. Control variables:
      1. Time when samples are taken
      2. How often samples are taken
      3. Duration of experiment
      4. How samples are taken
      5. Size of samples
      6. Amount of soil in each culture tube
      7. Amount of sterile water in each tube
      8. Amount of soil/water to be moved to each different tube
      9. Level of dilution
      10. Temperature the tubes are in over night
      11. Formula used to calculate the yeasts/cubic centimeter
      12. How plant density and diversity is measured
      13. Make sure they all get watered equally
      14. Take samples on same day so the sunlight and temperature is the same.
      15. When you measure the yeast levels
      16. Which samples are measured at the same time

c. Procedure:
   i. Find gardens with different plant densities and diversity; label them plots 1, 2 and 3. Measure each garden (plot) with measuring tape and find the area in square meters. Compare their plant densities by dividing the number of plants by the area. Then identify the type of plants in each garden and record the number of each various species of plants.
   ii. Take 3 random soil samples from every garden with the soil sample taker. Drive the soil sample taker into the ground15 cm deep with a diameter of 2½ cm. *to take a soil sample,

drive the soil sample taker into the ground as far as the required distance. Twist the soil sample taker to secure the soil in it. Then put the soil sample in a plastic bag and label the bag with the day, plot, and soil sample number.

iii. After the soil samples have been taken, stick a flag where the soil sample was taken and mark it with the day number, plot number, and soil sample number.
iv. Take three random soil samples (see directions above, at the asterisk) from soil in the back woods with the soil sample taker, 15 cm deep with a diameter of 2½ cm, North 39.35705 and West 76.63677.
v. Take three random samples (see directions above, at the asterisk) from the negative control plot (no plant life) with the soil sample taker, 15 cm deep and 2½ cm in diameter on day 1. It is critical that the soil samples for all the plots are taken around the same time (within the same hour).
vi. Perform the serial dilution tests at level 10^{-3} and 10^{-2} to the all the soil sample "1"s (the soil samples which were taken first at each plot) collected on day 1 from each plot. Perform this test the same day the soil samples are taken. Grow yeast in the Yeast and Mold count Petrifilm plate for 6 days. At the end of 6 days, count the number of yeasts colonies on the lowest dilution plate where they grew. Use the following formula to determine the number of yeasts in each cubic centimeter of soil: , $(\#\text{ of colonies} * 10^2) * 10^{|\text{dilution number}|} = \#\text{ of yeast/cm}^3$.
vii. Record the number in the data chart
viii. On day 2 collect 3 samples from each plot (negative control, positive control, plot 1,2, and 3).
ix. On day 2, perform the serial dilution tests at level 10^{-3} and 10^{-2} to the soil sample "2"s and "3"s collected on day 1 from each plot. Grow yeast in the Yeast and Mold count Petrifilm plate for 6 days. At the end of 6 days, count the number of yeasts colonies on the lowest dilution plate where they grew. Use the following formula to determine the number of yeasts in each cubic centimeter of soil: , $(\#\text{ of colonies} * 10^2) * 10^{|\text{dilution number}|} = \#\text{ of yeast/cm}^3$.
x. Record this number in the data chart.
xi. On day 3, perform the serial dilution tests at level 10^{-3} and 10^{-2} to all the soil samples collected on day 2. Grow yeast in the Yeast and Mold count Petrifilm plate for 6 days. At the end of 6 days, count the number of yeasts colonies on the lowest dilution plate where they grew. Use the following formula to determine the number of yeasts in each cubic centimeter of soil: , $(\#\text{ of colonies} * 10^2) * 10^{|\text{dilution number}|} = \#\text{ of yeast/cm}^3$.

xii. Record this number in the data chart.
xiii. Four days after day 3 perform the serial dilution tests at levels 10^{-3} and 10^{-2} to the soil sample "1"s, collected on day 3. Grow yeast in the Yeast and Mold count Petrifilm plate for 6 days. At the end of 6 days, count the number of yeasts colonies on the lowest dilution plate where they grew. Use the following formula to determine the number of yeasts in each cubic centimeter of soil: , $(\#\text{ of colonies} * 10^{2}) * 10^{|\text{dilution number}|} = \#\text{ of yeast/cm}^3$.
xiv. Record this number in the data chart.

Jane Hollon
Allie Weiss

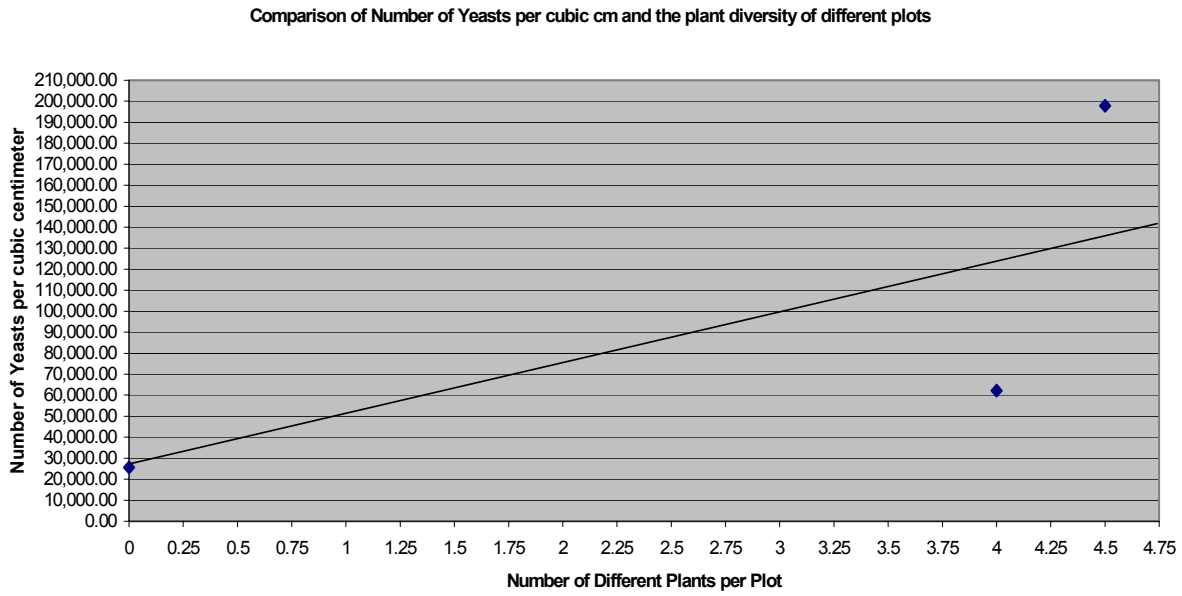
Ann Margaret Millspaugh
Caroline Atwater

IV. Data and Analysis

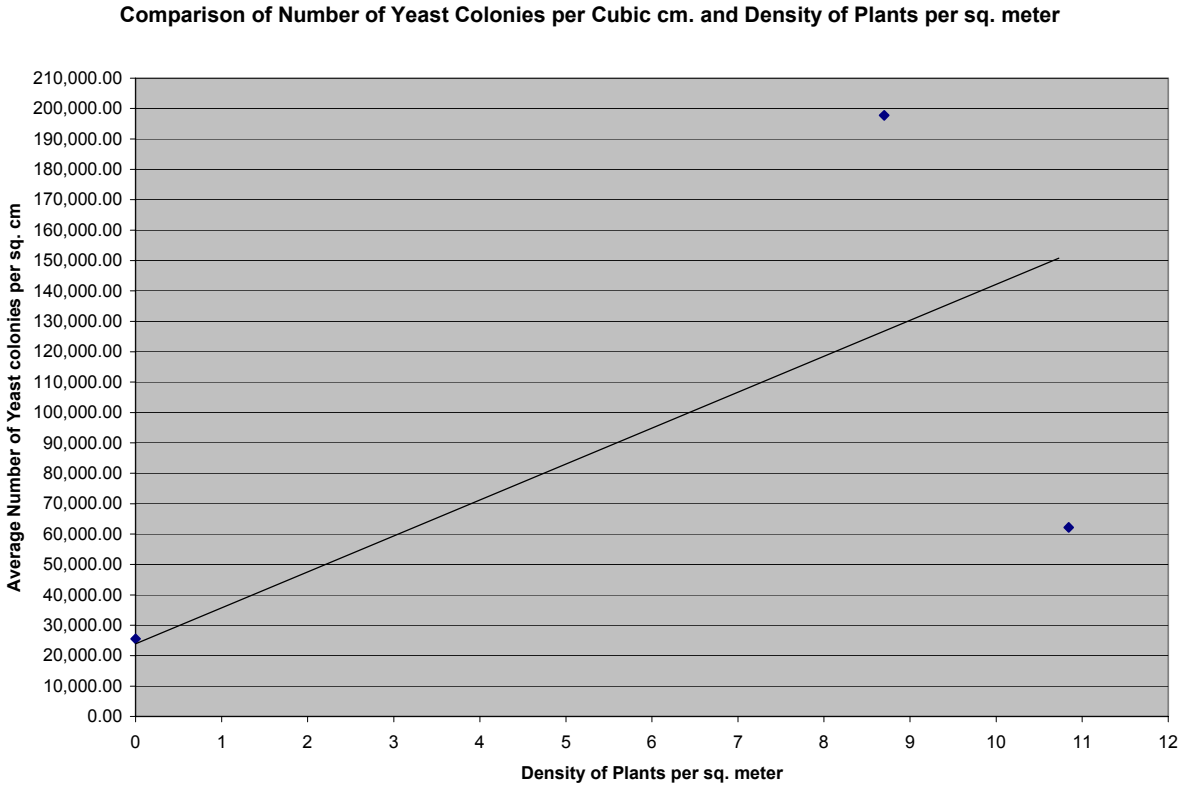
Yeast Sample Per Cubic cm	Density of Plants (number per m2)	Diversity					
			Test 1 Yeast Count	Test 2 Yeast Count	Test 3 Yeast Count	5/19 Test 4	5/21Test 5
Plot 1 Sample 1	9.899	6	300,000		200,000	190,000	
Plot 1 Sample 2				300,000	100,000		120,000
Plot 1 Sample 3				110,000	200,000		100,000
Plot 2 Sample 1	7.5	3	100,000		300,000	220,000	
Plot 2 Sample 2				200,000	90,000		100,000
Plot 2 Sample 3				200,000	700,000		30,000
Plot 3 Sample 1	10.844	4	40,000		10,000	50,000	
Plot 3 Sample 2				70,000	70,000		100,000
Plot 3 Sample 3				80,000	100,000		40,000
Positive Control Sample 1	3	1	100,000		80,000	130,000	
Positive Control Sample 2				40,000	200,000		100,000
Positive Control Sample 3				100,000	40,000		50,000
Negative Control Sample 1	0	0	20,000		20,000	90,000	
Negative Control Sample 2				20,000	30,000		0
Negative Control Sample 3				30,000	10,000		10,000

2 Sample T Tests			Diversity				
1 vs. 2	0.6302278783`	Plot 1/2	4.5	197,777.78			
1 vs. 3	0.001915326	Plot 3	4	62,222.22			
1 vs. NC	2.87E-04	Negative	0	25,555.56			
2 vs. 3	0.0509208558'		Density				
2 vs. NC	.0214379896'	Plot 1/2	8.6995	197,777.78			
3 vs. NC	.0137229873'	Plot 3	10.844	62,222.22			
NC vs. PC	.00391655'	Negative	0	25,555.56			

a. Data



Jane Hollon
Allie Weiss
Ann Margaret Millspaugh
Caroline Atwater



When analyzing the data collected from the Yeast Petrifilms we had to use the 2 Sample T Test to determine whether or not the data was simply by chance or that there was a real difference in the data we collected among each plot. In order to prove which plots were statistically different from each other and able to be used for comparison the p value had to be less then .05 (5%), meaning there had to be less then a 5% chance that the data collected was simply because of chance. In our specific 2 Sample T Tests plot 1 and plot 2 had a p value of about .63, therefore, their data was too similar and could not be compared to each other, but instead combined together because they were so similar. All of the other data checked out however with p values all of less then .05: plot 1 vs. plot 3 was .0019, plot 1 vs. negative control was 2.87e-4, plot 2 vs. plot 3 was .05, plot 2 vs. negative control was .02, and plot 3 vs. negative control was .01. When comparing the negative and positive controls, it was to serve the purpose that the experiment actually had validity and that the two opposite ends of the experiment were different, therefore there was something causing changes in the number of yeast. Negative vs. Positive control had a p value of .003, therefore, our experiment was valid. When graphing our data, we made sure to average plot 1

and plot 2 together and use them together to compare against plot 3 and the negative control. Because the graphs had very similar results, in order to determine whether there was one that was truly better then the other we had to determine the slope of the line of best fit. For diversity the slope was 24575. For density the slope was 11604. Therefore diversity had the greatest affect on yeast population in the soil.

V. Conclusion

Our hypothesis was wrong, the more plant diversity, not plant density, has higher yeast density per cubic centimeter. As the graphs proved, in the areas with the most diversity (plots 1 and 2) there was the greatest average yeast count of 197,777.78 yeast cultures per cubic centimeter, in plot 3 there was a lesser diversity and therefore a lower average yeast count of 62,222.22 yeast cultures per cubic centimeter, and in the negative control with no plant diversity or plants there was the lowest average yeast count of 25,555.56 yeast cultures per cubic centimeter. However, the plant diversity did not have a great difference between how many plants were in each plot, but there was still a correlation in the data. But, density did not affect the yeast population as we had expected with the middle density with the highest average yeast count, the highest density with the middle average yeast count, and the lowest density with the lowest average yeast count. Although both did show that the higher diversity and density did cause for greater yeast population and the data that made it seem wrong in density could have just been a fluke, we had to prove which had a greater yeast population by finding the slope for the line of best fit on each graph.

We didn't have any sources of error, however it would have been easier to perform all the serial dilutions for each day of sample taking in the same day. When performing this experiment in the future you should make sure you do the serial dilutions all in the same day to make the data tables and charts less complex. Also, if possible and if time permits it would be more controlled of an experiment to grow the plants in a controlled environment, therefore, we could control water intake and weather. Water and weather were in some ways a source of error, but because we took all the samples in the same day we reduced the source of error.