Effect of Sunlight on Bacteria in Soil

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Background

According to the National Resources Conservation Service, soil is “The unconsolidated mineral or organic material on the immediate surface of the Earth that serves as a natural medium for the growth of land plants (2008).” Soil is composed of decayed organic material, minerals and living organisms, and is crucial for the growth of plants (TQNCY, 2004). Many different factors have an effect on the health of the soil, including the amount of water it receives, the amount of fertilizer used to help in the growth of plants, and the amount of microbes living in the soil. Much like many other living things, the soil needs a certain amount of sunlight in order to provide a healthy, stable place for plants to grow. But does temperature have an effect on any other parts of the soil, such as the bacteria that live within it?

Bacteria are small, one-celled organisms that perform tasks within the soil such as services related to water dynamics and nutrient cycling. (National Resources Conservation Service, 2008) In addition to these tasks, soil bacteria decompose organic nitrogen forms in the soil to the ammonium form. These nitrogen fixing bacteria convert nitrogen from the atmosphere to ammonia, which is helpful because these bacteria possess enzymes that help to create this chemical reaction. These types of bacteria are members of the Genus Rhizobium group, and live on roots of leguminous plants (plants that bear their seeds in pods, such as peas, beans and clover.) The plants benefit from having bacteria on their roots because they use the ammonia to synthesize their amino acids. Amino acids can serve as a source of nitrogen in the soil, which increases the protein content in plants (National Vegetable Society, 1998).These bacteria also make great amounts of ammonia which is released in the soil in order to be used by other plants. These types of bacteria are crucial to the soil because they enrich the soil as they the nutrients they need in order to be helpful to other plants (Vision learning, 2009). Although bacteria have a great impact on the content and quality of the soil, it is not the only factor that has an effect on it.

The effects of sunlight are both good and bad for the soil; they can cause the soil to become dry, but many organisms depend on sunlight in order to survive. The heat of the sun can dry up the soil, evaporating the water on the top of the soil. Most soil needs about 6 to 8 hours of sunlight each day. Too much sunlight or intense sunlight for a certain period of time can be bad for the soil. Although sunlight has an effect on the contents of the soil, wind and water have bigger effects on the wearing of the soil. The sunlight also can be bad for the minerals in the soil, causing them to decompose, but unlike minerals, organisms such as bacteria depend on sunlight (International Maize and Wheat Improvement Center, 2004). While sunlight plays a very important role in the soil, it also penetrates more than a few millimeters into the soil.

As the sun light's intensity increases, the amount of moisture in the soil will decrease. This affects the entire water cycle; if there is too much sunlight in an area, there will not be enough moisture in the soil (it will all evaporate), resulting in fewer bacteria. While the sun can use its solar power to evaporate the water in the soil, the plants that live in the soil will also absorb moisture in the soil to photosynthesize. (Pearson Education, Inc 2004) The sunlight's intensity decreases both moisture and bacteria; however, many people attempt to protect the bacteria by trying to control the temperature of the soil This way the sunlight will not decrease the moisture in the soil;

most of the moisture (that would otherwise evaporate) will go into the plants to be used for photosynthesis while the amount of bacteria will increase because of the higher levels of moisture in the soil that will help them breed more easily.

The information that we collected through the process of learning about bacteria and its purpose in the soil led us to choose to perform an experiment in which we as humans purposefully manipulate the temperature of the soil. We will do so by placing an amount of mulch on top of the soil, in attempts to protect the bacteria in the soil by manipulating the temperature. If our hypothesis is correct, after placing mulch on top of a plot of soil, the temperature of the soil will decrease. We will then observe the temperatures both before and after, as well as count the amount of bacteria in the soil for samples that we take both before and after placing mulch on top of the soil. We chose to look at the effect bacteria have in the soil, and whether or not the temperature plays a crucial role in the life of bacteria in the soil here at RPCS.

Background –Works Cited

1. 1. Harrison, John Aurther. "The Nitrogen Cycle." Visionlearning. 17 May 2009 <http://http://www.visionlearning.com/library/module_viewer.php?mid=98>.

2. 2. Lafitte, et al. "Sunligh Effects." International Maize and Wheat Improvment Center. 16 May 2009 <http:// http://wheatdoctor.cimmyt.org/index.php?option=com_content&task=view&id=44&Itemid=42>.

3. Soil Science Glossary (Soil Science Society of America). "What is Soil?" Natural Resources Conservatoin Serive. 16 May 2009 <http:// Soil Science Glossary (Soil Science Society of America)>.

4. Enchanted Learning. "Bacteria." Bacteria. 29 May 2009 <http://http://74.125.47.132/search?q=cache:ehSUo6K709UJ:www.enchantedlearning.com/subjects/bacterium/+how+do+bacteria+make+ammonia&cd=3&hl=en&ct=clnk&gl=us>.

5. Mrs. Olesh. "What is Soil Masde Of?" Soil- It ain't Just Dirt. 29 May 2009 <http://http://www.tqnyc.org/2004/NYC040803//madeof.html>.

6. National Vegetable Society. "Soil Analysis." National Vegtable Society. 29 May 2009 <http://http://www.nvsuk.org.uk/medwyn-williams-vegetable-grow-show-336.html>.

7. Pearson Education, Inc.(2004). Biology: Exploring Life. Prentice Hall.

Lab Outline

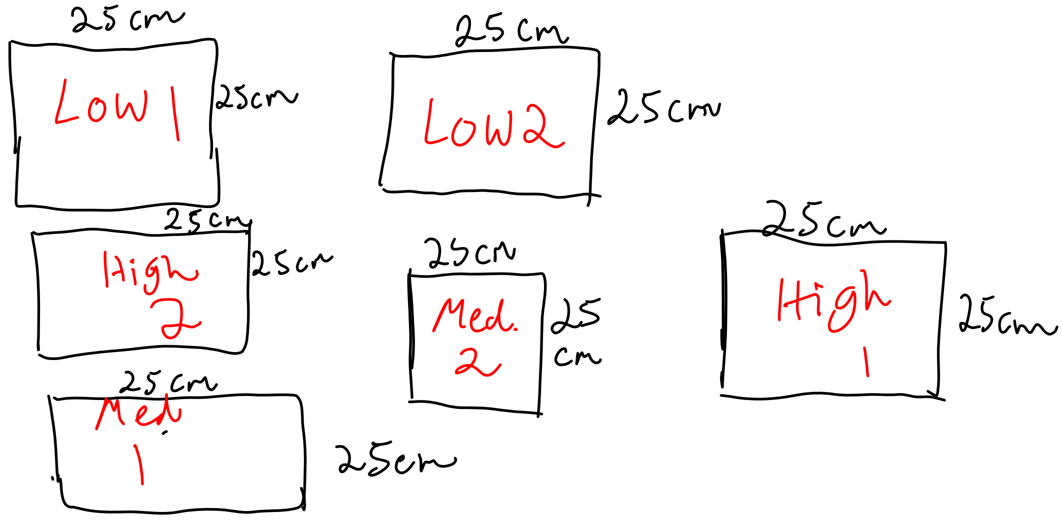
1. Question:
    a. Does the temperature of the soil alter the amount of bacteria in the soil?
2. Hypothesis:
    a. Cooler soil will have a higher density of bacteria.
3. Procedures:
    a. Independent variable
        i. Depth of mulch
    b. Dependent variable
        i. Number of bacteria colonies; temperature of soil
    c. Negative control
        i. Areas with 0 cm of mulch
    d. Controlled variables
        i. Size of plot, location of plot, depth in soil, take/ test all samples at the same time, type of mulch used, size of samples, amount of sterile water used in serial dilutions, amount of soil placed in test tubes during serial dilutions, number of flags used on plot, number of samples taken (before and after), amount of sample added to each nutrient plate, amount of days bacteria had to grow, type of nutrient plate used.

Experimental Steps

Getting Samples

1. Use meter stick to measure out 6 25 X 25 cm plots at N 39°29.493; W 76°38.117. Shape each plot like a square; place flags that label the test and the sample (low, medium or high) at each corner of the square

- Diagram of the Plot:



2. Take the temperatures of the soil from each plot/ record the data
3. Use soil core tester to get a soil sample that is 15 cm in depth, 3cm in width from the Low 1 plot; place in corresponding bag labeled Low 1
4. Repeat step 3 for remaining plots, putting each soil sample into its corresponding bag **BE SURE TO PERFORM STEPS 3 AND 4 ALL ON THE SAME DAY AT THE SAME TIME**
5. Pile mulch on each plot, covering each plot completely as follows: leave the plot called "low" with no mulch, the plot called "medium" with mulch (4cm deep), and the plot called "high" with mulch (8cm deep).
6. Wait three days before taking the temperatures of the plots for the "after" soil test
7. Go back out to plots to take the temperatures for the "after" test.; label new bags for "after" samples of soil
8. repeat steps 3 and 4 for the "after" test

   ***BE SURE TO PERFORM THIS STEP ON THE SAME DAY AT THE SAME TIME**

DO THE FOLLOWING SERIAL DILUTIONS ON THE SAME DAY, AT THE SAME TIME

Serial Dilutions

1. Use clean, new transfer pipette to add 10mL of sterile water to a 15mL culture tube. Label the tube “10^{0}”. You will use it for the soil from the bag labeled “before test, low sample”
2. Repeat labeling step for the next tubes
3. Use the same pipette to add 9mL to a second 15mL culture tube. Label the tube “10^{-1}”.
4. Repeat step 2 three more times to three additional 15mL culture tubes, only label them “10^{-2}”, “10^{-3}” respectively.
5. Place 1cc of your soil sample from the bag labeled “before test, low sample” into the “10^{-0}”culture tube.
6. Cap the tube and shake vigorously.
7. Using a new clean pipette, remove 1mL of the soil/water mixture from the “10^{0}” tube and place into the “10^{-1}” tube.
8. Cap and shake vigorously.
9. Using the same pipette in step 5, remove 1mL of the soil/water mixture from the “10^{-1}” tube and place into the “10^{-2}” tube.
10. Cap and shake vigorously.
11. Using the same pipette in step 5, remove 1mL of the soil/water mixture from the “10^{-2}” tube and place into the “10^{-3}” tube.
12. Cap and shake vigorously.
13. You should now have a total of 4 culture tubes.
14. Plate 100μl samples from the 3rd and 4th tubes (dilutions 10^{-2} and 10^{-3}) onto their own separate, labeled *Aerobic Count Petrifilm Plate* containing nutrient agar.
15. Repeat steps 1-14 for the rest of the “before” samples; **make sure you do the serial dilutions test on all “before” samples on the same day and at the same time.**

16. Allow the plates to grow for 48 to 72 hours
17. Examine each of the plates for individual bacteria colonies and choose the plate with the fewest colonies (but at least 5 and at the lowest dilution) to make your estimates of the number of bacteria in the original 1cc sample using the following formula:

#Microbes in 1cc of soil = #Colonies on sheet x 10^2x $10^{|\text{dilution \# at which the colonies were found}|}$

18. Repeat steps 1-17 for the after samples **(again, on the same day and at the same time)**

Data/Observations

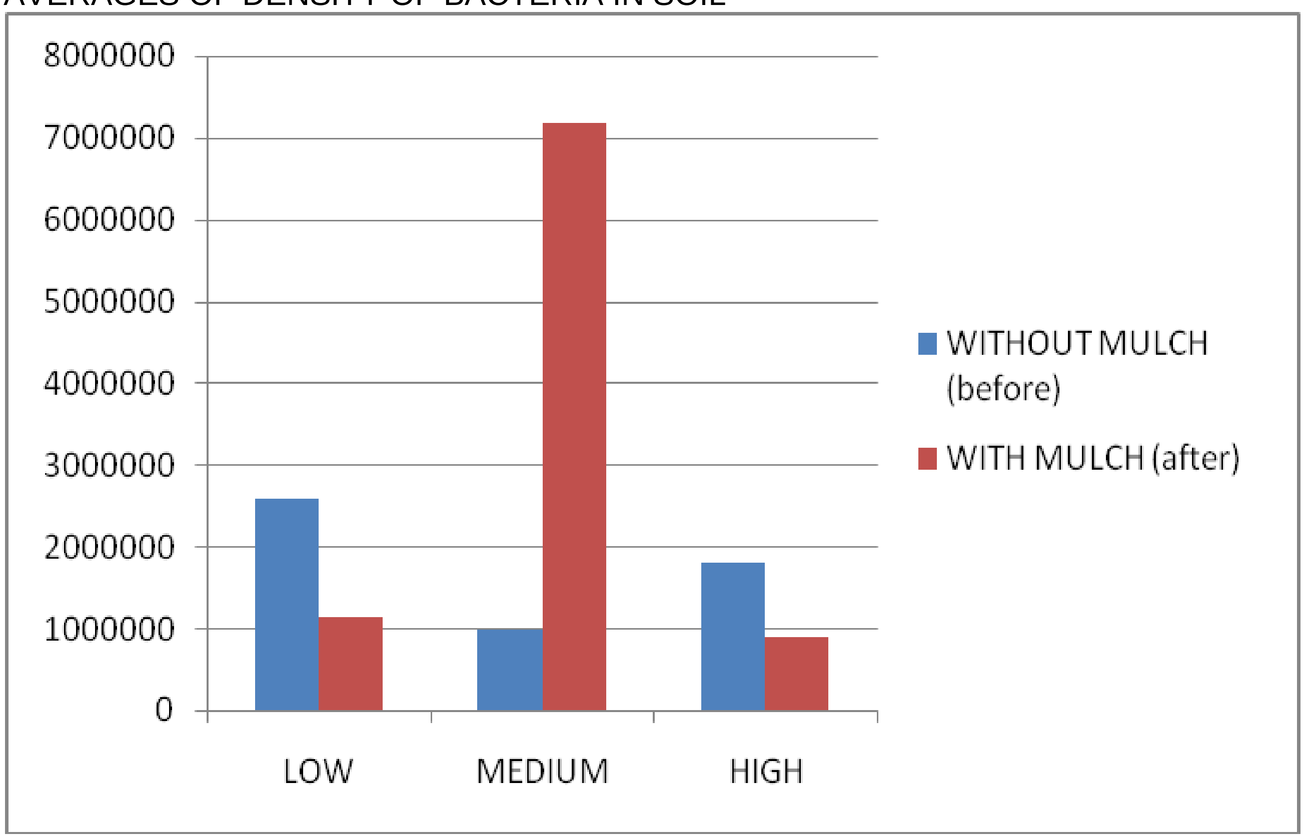
Density of bacteria→ before and after

		plot 1 sample 1	plot 1 sample 2	plot 2 sample 1	plot 2 sample 2
without mulch (before)	low	1,500,000	500,000	4,300,000	4,000,000
	medium	850,000	1,000,000	1,210,000	900,000
	high	4,400,000	1,900,000	480,000	460,000
with mulch (after)	low	250,000	600,000	3,600,000	110,000
	medium	340,000	500,000	730,000	1,300,000
	high	1,500,000	500,000	830,000	700,000

Averages of Density of Bacteria

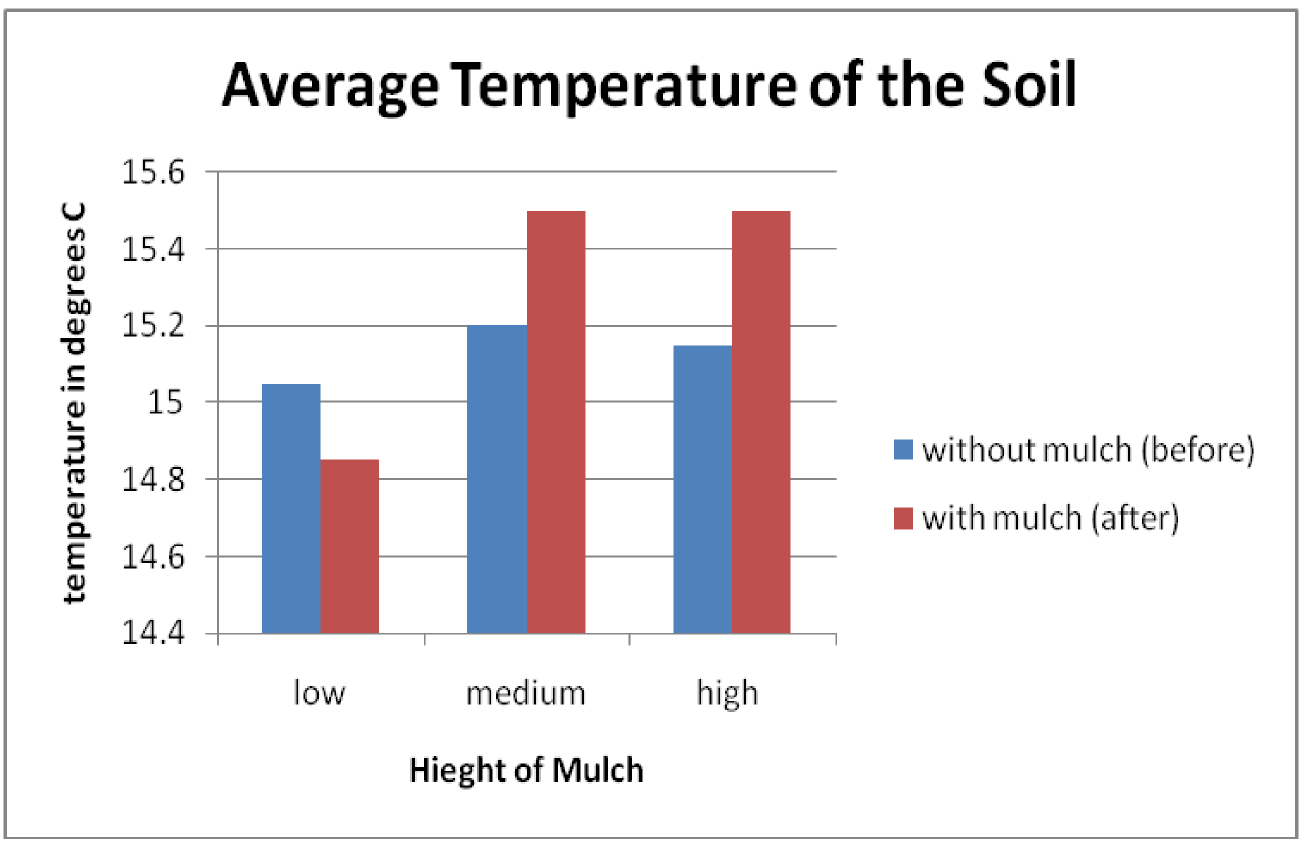
	Without mulch	With mulch
low	**2575000**	**1140000**
medium	*990000*	*7175000*
high	1810000	882500

AVERAGES OF DENSITY OF BACTERIA IN SOIL



(LOW= 0 cm of mulch; MEDIUM= 4 cm of mulch; HIGH= 8 cm of mulch)

AVERAGE TEMPERATURES (before and after)		
	WITHOUT MULCH (before)	WITH MULCH (after)
LOW	15.05 ° C	14.85° C
MEDIUM	15.2 ° C	15.5° C
HIGH	15. 15° C	15.5° C



Statistical Analysis

	t_s
Low	1.458
Medium	1.213
High	.974

Conclusion

For our experiment, we were testing to see if the amount of human interference [pertaining to the temperature of the soil] affected the amount of bacteria in the soil. To do this, we looked at the change in the density of bacteria from before putting mulch on, to after having the mulch be on the soil, then observing the change in temperature. We believed that putting the mulch on would hold in the temperature, then this would cause less bacteria to grow. This was not the case; proving our hypothesis to be false. To test our hypothesis we first made our plots and took before temperatures and 2 samples, from each plot, 15 cm into the soil. We put mulch on the plots; we had two low plots with 0 centimeters of mulch, two medium plots with 4 centimeters of mulch and two high plots with 8 centimeters of mulch. After leaving the mulch on for two days we then went back and removed the mulch to take temperatures and 2 samples from each plot. Then we did the serial dilution on the before samples and then the after samples. We next put the diluted solutions on *Aerobic Count Petrifilm Plate* containing nutrient agar so we could count the number of bacteria after culturing it. We were then able to count the number of bacteria and plug it in to an equation to get the total amount of bacteria in our 25 cm by 25 cm plot. After getting the data for the number of bacteria's in the plot before and after the mulch we next did The t Test which showed us if statistically there was any change in bacteria from before to after (the mulch). That showed us that even though in our data tables and graphs it looks like there was a noteworthy change in the density of bacteria there proved to be no significant change. In The t Test if the numbers were higher than 2.353 then that means there was a change in the density of the bacteria but because the numbers were: 1.458, 1.213 and .974, obviously all those numbers are less than 2.353; thus there was not a remarkable change in the density of the bacteria in the soil. We also looked at the temperature before and after the mulch was put on. We thought that the temperature would change significantly but the most it changed was by only .2. Therefore proving that out hypothesis was incorrect and that the mulch does not affect the temperature or the density of bacteria significantly.

For further research to build onto this experiment, one might follow the same procedures except instead of finding the density of bacteria, finding the density of protozoa in the soil. This would be a logical experiment because protozoa eat bacteria, which is another aspect of the experiment that we did not look into. Performing this experiment would show if the mulch affected the protozoa instead of the bacteria because bacteria is the prey of protozoa. This experiment could also show if bacteria are able survive normally in an environment where their predators are not. This research of bacteria and further research of protozoa could be very helpful for gardeners and everyday people who use mulch; in order for these groups of people to be fully educated on the effect temperature has on the soil. If we were to perform this experiment again, we would have chosen to make nine plots total, in order to have a complete set of data, which would cause us to not have to solve the mathematical equation which we did have to solve.

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