ROLAND PARK COUNTRY SCHOOL

Bacteria Density Lab

Landscaped vs. Natural Soil

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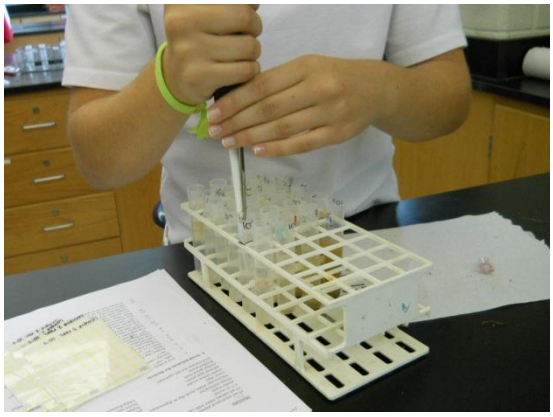

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Background:

Microbes are a critical part of any environment. They usually include protozoa, fungi, and bacteria, and each kind play important roles in the life of soil. In general, the more microbes there are in an ecosystem, the more stable its environment, and this is especially true in the soil where all the different microbes help decompose organic material and recycle the carbon, oxygen, and nitrogen that make up all living organisms (Todar, 2009).

One specific group of microbes, the bacteria, are the prokaryotic organisms that possess the enzymes that enable them to convert nitrogen compounds through the process of decomposition and nitrogen fixation into forms of nitrogen that can be used by plant cells. This process, called the nitrogen cycle, involves the bacteria first either capturing nitrogen gas from the atmosphere and turning into ammonium or releasing ammonium through the decomposition of once living organisms which have nitrogen in their tissues. This ammonium can be directly absorbed by plants for the biosynthesis of biological molecules, or it can be utilized in the process of nitrification. In nitrification, the other groups of bacteria turn the ammonium ultimately into nitrate which plants can also assimilate and convert into their amino acids and nucleotides. Then any excess nitrate is converted back into nitrogen gas and put back into the atmosphere. (Ho, 2002).

The reason the nitrogen cycle is so is necessary for a fully functioning ecosystem is because these amino acids and nucleotides are needed by plants as the monomers for their

proteins nucleic acids respectively. Nucleic acids and proteins are two of the five biological molecules, and without them, there would be no DNA or enzymes to start and stop the various chemical reactions that perform the four tasks keep cells alive. Here, without the nitrogen, plant cells cannot function and therefore the plant itself could not survive. Plants, though, are in turn, consumed by primary consumers that use the plant's amino acids and nucleotides for their cells followed by secondary consumers who eat the primary consumers to acquire the amino acids and nucleotides for *their* bodies. When all these plants and animals eventually die, their bodies are decomposed by the bacteria and fungi in the soil, releasing the ammonium once again, and the cycle continues.

Clearly, therefore, the survival of soil bacteria is critical to a properly functioning ecosystem. However, when humans landscape they can negatively affect the survival of the bacteria through the implementation of fertilizer and over watering. Fertilizers increase the rate of nitrogen input beyond what would normally occur during the nitrogen life cycle, and while the plants soak up this excess nitrate and grow at a rapid pace, the ultimate result is that the bacteria die. The fertilizer causes them to reproduce at a more rapid pace to process the extra nitrogen, and their population crashes. If the fertilizer is not then replaced the bacteria run out of this critical nutrient. Furthermore when the soil is over watered, the pH levels in the soil go up, making the soil less acidic than it is naturally. If the pH is not between 6 and 9, the enzymes inside of the bacteria are torn apart. Without these enzymes, the bacteria cannot convert the ammonium into nitrate and they will then die.

In our experiment, we will focus on population density of bacteria in soil. We will take soil samples from three different types of plants that have been landscaped, and the same type of plant but that has been left untouched. By finding the bacteria population difference in the soil,

we will determine how human tampering with nature affects the amount of necessary bacteria in the soil. This is an important to study because if the population of bacteria in the soil that has been landscaped is unhealthily low, it threatens the life cycle of the ecosystem around it, since it is not able to perform the nitrogen cycle and decomposition. An ecosystem with a low amount of nitrogen will lead to species extinction, diminishing our human resources as well.

Experiment:

I. Problem:

How does the deliberate landscaping in front of RPCS impact the population density of bacteria in the soil?

II. Hypothesis:

In the area where the soil is less actively landscaped on the school's grounds, there will be a higher density of soil bacteria population than where the soil is more actively landscaped.

III. Procedure:

a. Independent Variable: The landscaped soil from the front of the school alongside of the carpool lane
b. Dependent Variable: The population density of bacteria in the soil
c. Negative control: The grass
d. Controlled variables:

-Same type of landscaped and natural tree and grass pairs

-All soil samples collected on the same day at the same time

-Deepness into the ground

-Amount of soil tested on

-Amount of soil sampled

-Size of culture tubes

-Units used to measure amount of soil, sterile water, and number of bacterium

-Amount of time waited to allow for bacteria to grow

-Size of petri plates

-Amount of soil put into culture tubes

-Amount of sterile water put into each culture tube

-Amount of soil/sterile water mixture put onto petri plates

-Type of nutrient agar

-Number of dilutions performed

-Which dilutions plated

e. Procedure:

1. For steps 2 – 7, make sure to collect all soil samples at the same time on the same day.
2. Use a soil core that is 2 centimeters in diameter with a depth of 15 ½ centimeters to collect 3 soil samples from the base of a planted rose-bud tree on the RPCS campus at the location N 39.35723, W 76.63541; each sample should be taken 15 centimeters away from the coordinates of the base of the tree. Put the three collected soil samples into three separate plastic bags and label the bags "landscaped rosebud 1, 2, and 3" respectively.
3. Use the same soil core to collect 3 soil samples from a naturally growing rose-bud tree on the RPCS campus at the location N 39.35695, W 76.63598; each sample should be taken 15 centimeters away from the coordinates of the tree. Put the three collected soil samples into three separate plastic bags of their own and label the bags "natural rosebud 1, 2, and 3" respectively.

4. Use the same soil core to collect 3 soil samples from the base of a planted red maple tree on the RPCS campus at the location N 39.35811, W 76.63607; each sample should be taken 15 centimeters away from the coordinates of the base of the tree. Put the three collected soil samples into three separate plastic bags and label the bags "landscaped red maple 1, 2, and 3" respectively.
5. Use the same soil core to collect 3 soil samples from the base of a naturally growing red maple tree on the RPCS campus at the location N 39.35674, W 76.63550; each sample should be taken 15 centimeters away from the coordinates of the base of the tree. Put the three collected soil samples into three separate plastic bags and label the bags "natural red maple 1, 2, and 3" respectively.
6. Use the same soil core to collect 3 soil samples from the base of planted grass at the location N 39.35796, W 76.63587; each sample should be taken 15 centimeters away from the coordinates of the base of the tree. Put the three collected soil samples into three separate plastic bags and label the bags "landscaped grass 1, 2, and 3" respectively.
7. Use the same soil core to collect 3 soil samples from the base of naturally growing grass on the RPCS campus at the location N 39.35673, W 76.63567; each sample should be taken 15 centimeters away from the coordinates of the base of the tree. Put the three collected soil samples into three separate plastic bags and label the bags "natural grass 1, 2, and 3" respectively.
8. Bring these samples into your lab station.
9. Make sure to do all of the dilution processes for all the samples at the same time.
10. Use a clean, new transfer pipette to add 10 ml of sterile water to a 15 ml culture tube. Label the tube "Natural 10^0.".

11. Use the same pipette to add 9 ml to a second 15 ml culture tube. Label the tube "Natural 10^{-1}."
12. Repeat step 11 two more times to two additional 15 ml culture tubes, only label them "Natural 10^{-2}" and "Natural 10^{-3}" respectively.
13. Place one cc of your "Natural 1" soil sample into the "10^{0}" culture tube.
14. Cap the tube and shake vigorously.
15. Using a new clean pipette, remove one mL of the soil/water mixture from the "10^{0}" and place it into the "10^{-1}" tube.
16. Cap and shake vigorously.
17. Using the same pipette as step 15, remove one mL of the soil/water mixture from the "10^{-1}" tube and place it into the "10^{-2}" tube.
18. Cap and shake vigorously.
19. Using the same pipette in step 15, remove one mL of the soil/water mixture from the "10^{-2}" tube and place it into the "10^{-3}" tube.
20. Cap and shake vigorously.
21. You should now have a total of four culture tubes
22. Plate 100µl samples from the 3^{th} and 4^{th} tubes (dilutions 10^{-2} and 10^{-3}) onto their own corresponding separate 3M Petrifilm™ Aerobic Count Plate petri plates containing the labels "natural 10^{-2}" and "natural 10^{-3}" respectively.
23. Repeat steps 10-22 for all remaining/other soil samples.
24. Allow to grow for 48 to 72 hours.
25. Examine each of the plates for individual bacteria colonies and choose the plate with the fewest colonies (but at least 5) at the lowest dilution value to make your estimates of the

number of bacteria in the original 1 cc soil sample using the following formula:

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

26. Compare the amount of bacteria in the soil samples and record observations in data table.

Data and Analysis:

a. Table:

	Amount of Bacteria Per Cubic Centimeter of Soil			
		Rose-bud	**Red Maple**	**Grass**
Trial 1	**Landscaped**	6,200,000	7,300,000	10,300,000
	Natural	8,100,000	4,900,000	4,600,000
Trial 2	**Landscaped**	900,000	1,600,000	2,900,000
	Natural	N/A	3,400,000	4,300,000
Trial 3	**Landscaped**	9,400,000	3,300,000	5,500,000
	Natural	4,700,000	1,600,000	2,580,000
Average	**Landscaped**	5,500,000	4,066,666.667	6,233,333.333
	Natural	6,400,000	3,300,000	3,826,666.557

b. Graphs:

Figure 1:

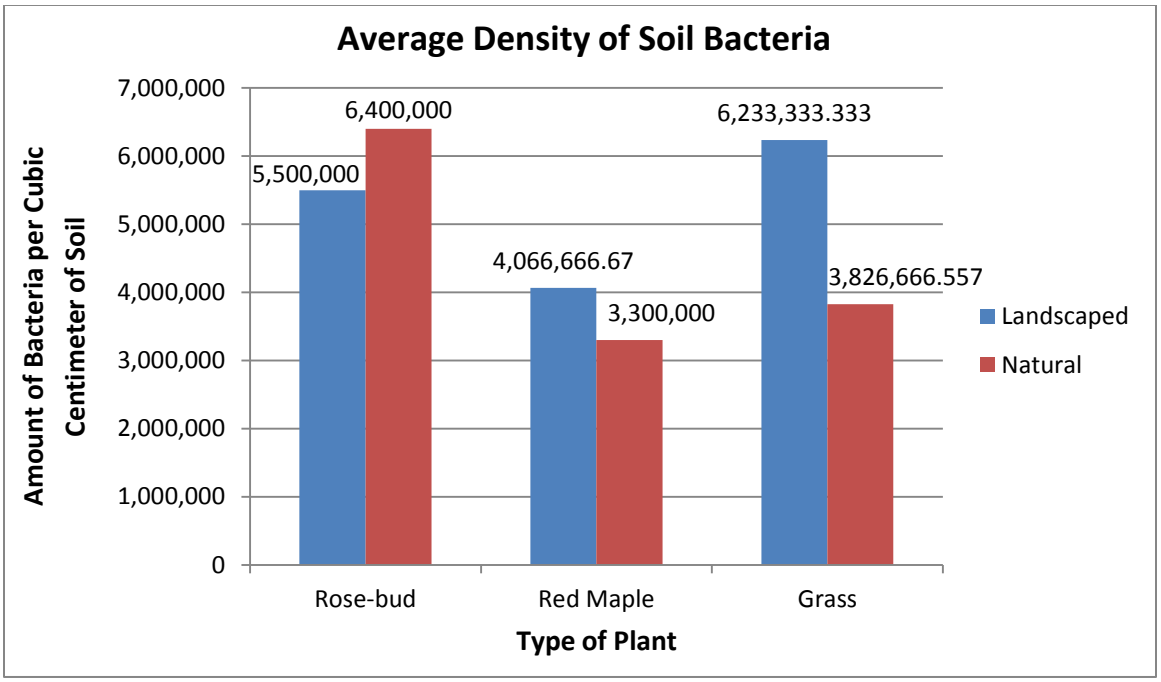
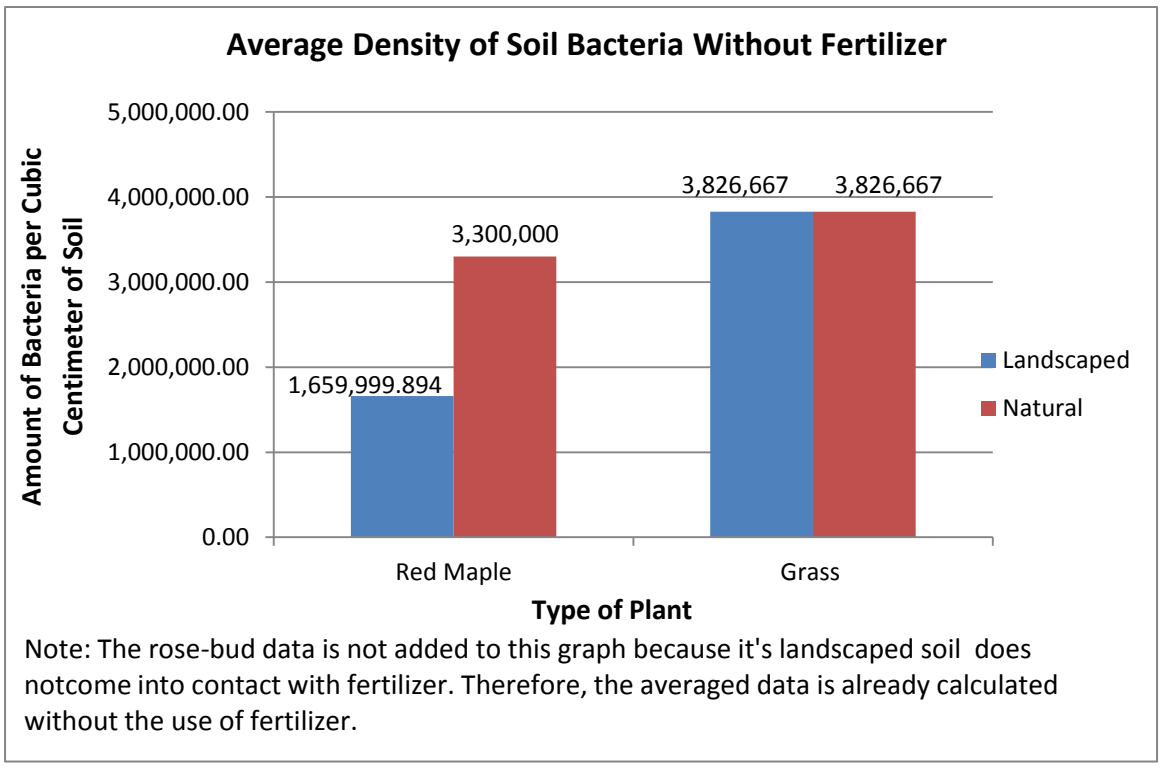


Figure 2:



Note: The rose-bud data is not added to this graph because it's landscaped soil does notcome into contact with fertilizer. Therefore, the averaged data is already calculated without the use of fertilizer.

Conclusion:

Our hypothesis was correct when we tested the rosebud tree. There were 5,500,000 bacteria per CC in the landscaped soil and 6,400, 000 bacteria per CC in the natural soil. This means that there were 900,000 more bacteria per CC in the natural soil. When we tested the landscaped soil there was no fertilizer because it was isolated from any fertilized grass by concrete (see figure 1). This means that the soil around the rosebud would have no fertilizer mixed in with it because it was not near any area grass where the application of fertilizer takes place. When we tested the maple tree, it seems that there was more bacteria in the more landscaped tree (4,066,666.667 bacteria per CC) than in the less landscaped tree (3,300,000 bacteria per CC) but this is because the maple tree's roots reached fertilized grass (see figure 2). The fertilizer in this grass would boost the population of bacteria. Our negative control, grass, showed us what the effect of fertilizer is on the amount of bacteria in an area, which is a difference of 2,406,666.776 bacteria per CC. This is because there were 6,233,333.333 bacteria per CC in the more landscaped grass soil and 3,826,666.557 bacteria per CC in the less landscaped grass soil. We are able to subtract this difference to see if without the effect of fertilizer there were more bacteria in the natural soil. When the effect of the fertilizer is subtracted from the amount of bacteria in the more landscaped soil from the maple tree bacteria count, it proves our hypothesis correct again because there would be 3,300,000 bacteria per CC in the natural soil and 1,659,999.891 per CC in the landscaped soil. This data proves that natural soil has a healthier amount of bacteria because landscaped soil, when unaffected by fertilizer, does not have as many bacteria as more natural soil.

Although fertilizer momentarily boosts the amount of bacteria, the population is not sustained unless the fertilizer is continually replenished. This happens because soon after the

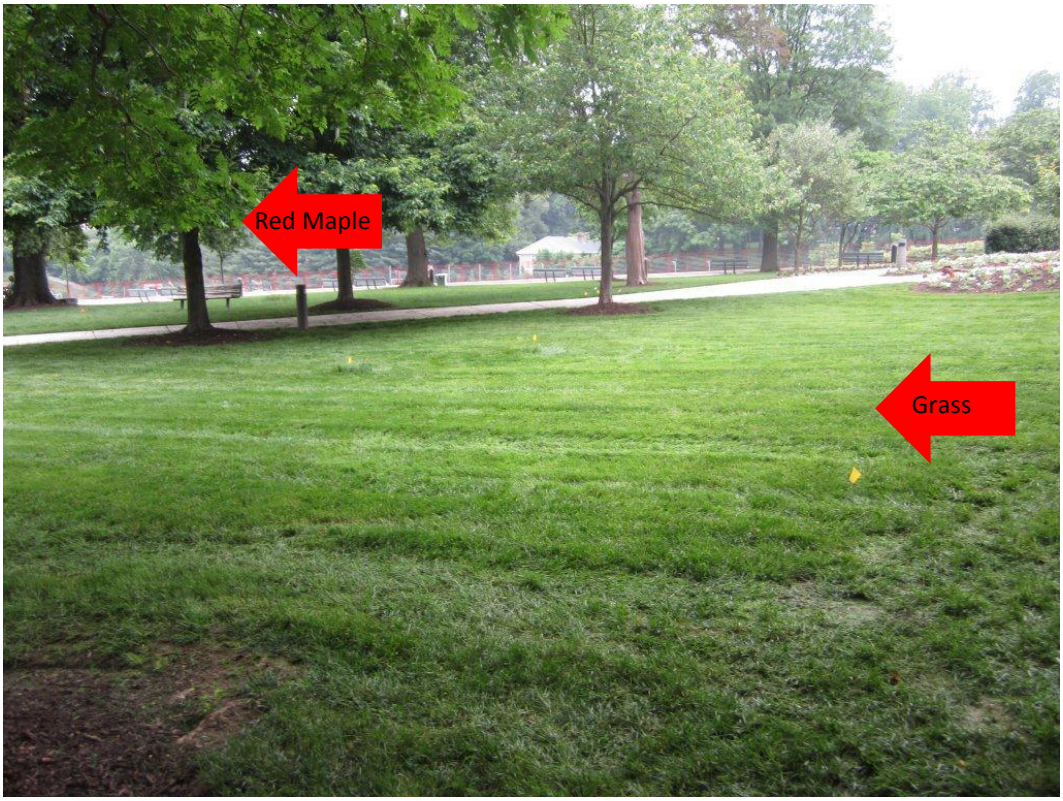
bacteria populations rapidly grow, they begin to plummet due to the nitrogen found in fertilizer. If this cycle continues, the bacteria population will eventually crash to a point where it altogether disappears and the soil becomes infertile. This is why it is healthier to have natural soil that has a steady population of bacteria.

It makes sense that the rosebud has a generally higher population of bacteria because it is the smaller tree and its roots are more compact. Therefore the bacteria would not have as much space to spread out and also would be more compact. In contrast, the red maple is much larger and its roots spread much farther; this allows the bacteria to spread out as well. Since there is a higher concentration of bacteria in the rosebud soil, because it's more compact, there was a higher density of bacteria when we took a small sample of the soil in comparison to the red maple soil, where the bacteria would have a lower concentration.

To continue taking steps in researching this issue, logical next experiments could involve using a plot of land with a designated spot for fertilizer where the maple tree's roots could reach it. There would also be a plot of land with another maple tree without any access fertilizer. This way we could confirm that fertilizer causes the increase of bacteria for the maple tree. Another experiment would be to find different maple trees of different ages to figure out if age affects the amount of bacteria that its soil contains.

Figure 1:

Figure 2:



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