Bacteria Density Lab

Plant Variety affecting the Nitrogen Cycle

Marisa Class, Caroline Hernandez, and Jordan Tapp

Mr. Brock

Ninth Grade Honors Biology

30 May 2013

I. Backround

Soil microbes are a critical part to the terrestrial ecosystem. They decompose organic residues and recycle soil nutrients, and all the different types, (bacteria, fungi, protozoa, actinomycetes, algae) all play important roles in the soil. However, "the smallest and most hardy microbe in the soil," (The Ohio State University) bacteria play the key role in the way in which soil, plants, and animals interact. Bacteria play the key role in the way in which soil, plants, and animals interact. This process is known as the nitrogen cycle.

In the nitrogen cycle, bacteria in the soil use enzymes to convert nitrogen gas from the atmosphere into the forms that plants can use as nutrients in a process called fixation (the National Academy of Engineering, 2012). Most producers can only use nitrogen in the form of compounds such as ammonium and nitrate, and the nitrogen fixing bacteria that convert the nitrogen gas to ammonia live throughout the soil and in nodules on the roots of certain species of plants. In the soil, the ammonia picks up another hydrogen ion from water, forming ammonium, and then other bacteria in the soil can convert this ammonium first to nitrites and then to nitrates . Producers then build, proteins, and nucleic acids using the nitrate as the primary source of the element nitrogen. These two biological molecules are important because if there is no DNA or RNA, then enzymes are not produced, and if there are no enzymes, no chemical reactions will take place to keep a cell alive and functioning. Since plants are made of cells, if the cells are not functioning then the plants will die. But consumers that eat the plants depend on their producers to obtain their own nitrogen in the form of digested biological molecules. Therefore if the producers are not present the consumers will die and then the entire ecosystem will fall apart. Finally the bacteria in the soil are also responsible for converting any excess nitrates not used by the bacteria for their own metabolism back to nitrogen gas in a process called denitrification.

This gas is then released into the atmosphere, where it almost 80 percent of the air we breathe completing the cycle.

So critical is this relationship between bacteria and plants that few seedlings can survive in newly wrought soil that begins on the surface of sand or rock until it has been prepared by bacteria, algae, lichens, and mosses. While such soils have most of the mineral nutrients that a plant needs to get started, they are deficient in one essential nutrient: nitrogen. Rocks and soils that contain living things contain nitrogen and organic matter. However some of the plants that pioneer these soils have the ability to do so because a certain rod shaped bacteria call rhizobia and other filamentous bacteria called actinomycetes have developed special partnerships with their early roots. The roots of these plants accommodate these bacteria in special knots or nodules; with millions of bacteria sheltered in each root. The nodules provide the perfect environment for the bacteria to convert nitrogen gas from the air into the ammonia which the bacteria and plants can use (Nardi, 2003).

Not every type of soil bacteria and not just any root can create these modules because each type of plant requires a special type of rhizobium or actinomycete, and only when plants secrete certain substances through their root hairs do the bacteria and the plants strike up an intimate relationship. Once formed, the compatible soil bacteria latch on to the hairs and spread into the root, where they stimulate root cells to divide and to form a knot or nodule of cells (Nardi (2003). Both plant and bacteria are nourished by the ammonia produced in the root nodules, and in return for the help fixing the nitrogen the plants supply the energy that the bacteria need to survive. According to Nardi (2003), this teamwork allows plants to settle in soils that other plants without nodules could never make into proper environments in which to live. Yet plants with root nodules leave enough nitrogen compounds in the soil to support a new wave

of plant settlers that have neither root nodules nor the ability to supply their own nitrogen (Nardi, 2003).

One of things that influence these especial relationships between bacteria and plants are the types of plants that are living in the environment. The diversity of plants is important to humans and to animals because both rely on plants for energy. The plants are able to provide that energy to the animals and humans because they are able to convert the sun's energy through photosynthesis. Many species of plants are used for fuel, food, herbs, oils and spice by humans but for animals, plants provide forage for domestic animals. Also plants provide shade and shelter at various places throughout the world. Having different plants is also very important because it is needed for provisions of ecosystems. Some of these provisions would be protection of watersheds, improvement of soils and moderation of climate. Therefore, plant diversity is so important because the different plants overall provide for the many needs for people and other animals. (Heywood and Davis 1992).

Furthermore, in our experiment, we are testing to see which gardens on the RPCS campus have the healthiest nitrogen cycle. The amount of bacteria varies in each garden by the different types of plants that were planted. The gardens that we looked at were the Butterfly Garden, the Food Garden, and the garden behind the school, the Natural Meadow, and our Negative Control, which was plain soil from construction. Our hypothesis was that the Natural Meadow is the garden where the nitrogen cycle is functioning best. We chose the natural meadow because it has the most native plants which will get the nitrogen cycle started. We predict that based on the amount of bacteria in each garden, the garden that has the most bacteria has the healthiest nitrogen cycle.

II. Lab Outline

A. Problem: Which of the gardens on the RPCS campus has the healthiest nitrogen cycle?

B. Hypothesis: The Natural Meadow is the garden where the nitrogen cycle is the healthiest.

C. Procedures:

a. Independent Variable: Gardens growing different types of plants

b. Dependent Variable: # of bacteria in 1 cc of soil

c. Negative Control: soil samples from a location without plants

d. Controlled Variables:

1. How deep the soil core is inserted in soil- 15 cm deep and 2 cm wide
2. Take soil samples on same day and at same time
3. Location of plant in garden
4. How much soil placed in tube
5. Amount of water added to tubes
6. Size of serological pipettes
7. Size of culture tube
8. How much soil is diluted
9. Type of water
10. # of levels planned to dilute
11. Type of agar
12. How long the bacteria grows
13. How much is placed on each agar plate

E. Step-by-Step Instructions:

1. Take 15 clear sandwich bags; label 3 “Butterfly”, another 3 “natural meadow”, another 3 “food garden”, another 3 “behind school”, and the final 3 “negative control”. Also label each set of bags “1” “2” and “3” for each of the different trials
2. For steps 3-22 be sure to make sure all samples are collected on the same day and at the same time

3. Go out to the vegetable garden which is located at the following coordinates: North 39.35733° and West 076.63574°.
4. In the middle of the garden, using the soil core extract 15cm in depth and 2 cm in width of soil
5. Put the soil in one of the three sandwich bags labeled “food garden.”
6. Repeat steps 4-5 twice more but using the other two sandwich bags labeled “food garden”
7. Travel to the back of the cafeteria on the hill where there is a garden which is located at the following coordinates: N 39.35715° and W 076.63627°.
8. In the middle of the garden, using the soil core extract 15 cm in depth and 2 cm in width of soil
9. Put the soil in one of the three sandwich bags labeled “behind school”.
10. Repeat steps 8-9 twice more but using the other two sandwich bags labeled “behind school”.
11. Travel to the Natural Meadow which is located at the following coordinates: N 39.35709° and W 076.63522°.
12. In the middle of the garden, using the soil core extract 15 cm in depth and 2 cm in width of soil
13. Put the soil in one of the three sandwich bags labeled “natural meadow”
14. Repeat steps 12-13 twice more but using the other two sandwich bags labeled “natural meadow”
15. Travel to the construction site located at the following coordinates: N 39.35751° and W 076.63521°
16. In the middle of the construction site, using the soil core extract 15 cm in depth and 2 cm in width of soil
17. Put the soil in one of the three sandwich bags labeled “negative control”
18. Repeat steps 16-17 twice more but using the other two sandwich bags labeled “negative control”
19. Travel to the butterfly garden which is located at the following coordinates: N 39.35769° and W 076.63533°.
20. In the middle of the garden, using the soil core extract 15 cm in depth and 2 cm in width of soil
21. Put the soil in one of the three sandwich bags labeled “butterfly garden”

22. Repeat steps 20-21 twice more but using the other two sandwich bags labeled “butterfly garden”
23. Go back to the lab on the same day the samples from the various gardens were extracted
24. You need to do the following steps on the same day and at the same time: steps 25-41
25. Use a clean, new 10ml serological pipette to add 10 ml of sterile water to a 15 ml culture tube. Label the tube “1 food garden 10^0”
26. Use the same serological pipette to add 9ml to a second (different) 15 ml culture tube. Label the tube “1 food garden10^{-1}”.
27. Repeat steps 2 three more times to a different 15 ml culture tube each time. Label 1 culture tube “1 food garden 10^{-2}”, another “1 food garden 10^{-3}” and.
28. Place 1 cc of your trial one “food garden” sample into the “ 1 food garden 10^0” culture tube
29. Cap the tube and shake vigorously
30. Using a new clean pipette, remove 1 ml of the soil/water mixture from the “1 food garden 10^0” tube and place it in the “1 fg 10^{-1}” tube.
31. Cap and shake vigorously
32. Using the same pipette in step 30, remove 1 ml of the soil/water mixture from the “1 fg 10^{-1}” tube and place it into the “1 fg 10^{-2}” tube.
33. Cap and shake vigorously.
34. Using the same pipette in step 30, remove 1 ml of the soil/water mixture from the “1 fg 10^{-2}” tube and place it into the “1 fg 10^{-3}” tube.
35. You should now have a total of four culture tubes.
36. Plate 100 µl samples from the 2nd and 3rd tubes (dilutions “1 fg 10-2” & “1 fg 10-3”) onto their own separate, labeled 3M Petrifilm™ Aerobic Count plate (you should label the petri dishes with the location and dilution #; ex: 1 fg10-2)
37. Repeat steps 24-36 twice more but using a different sample each time of the “food garden” for step 27 (in the end you should have no more samples of the “food garden” samples; also label the tubes for which trial you are doing → ex. 2 fg 10^0 or 3 fg 10^0)

38. Repeat steps 24-37 three times but using a different sample each time of the "**behind school**" (be sure to label the culture tubes "behind school") for step 27 (in the end you should have no more samples of the "behind school" samples)
39. Repeat steps 24-37 three times but using a different sample each time of the "**natural meadow**" (be sure to label the culture tubes "natural meadow") for step 27 (in the end you should have no more samples of the "natural meadow" samples)
40. Repeat steps 24-37 three times but using a different sample each time of the "**negative control**" (be sure to label the culture tubes "negative control" for step 27 (in the end you should have no more samples of the "negative control" samples)
41. Repeat steps 24-37 three times but using a different sample each time of the "**butterfly garden**" (be sure to label the culture tubes "butterfly garden") for step 27 (in the end you should have no more samples of the "butterfly garden" samples)
42. Allow all plates to grow for 48 to 72 hours
43. Examine each of the plates for individual bacteria colonies and first go to the lowest dilution. If the lowest dilution has at least 5 visible colonies, use that dilution to count the amount of colonies, but if not go to the next lowest dilution and use that one. Mark each colony with a pen as you are counting to keep track of which colonies you have already counted. Make your estimates of the number of bacteria in the original 1 cc of soil sample using the following formula:

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

III. Data/Observations

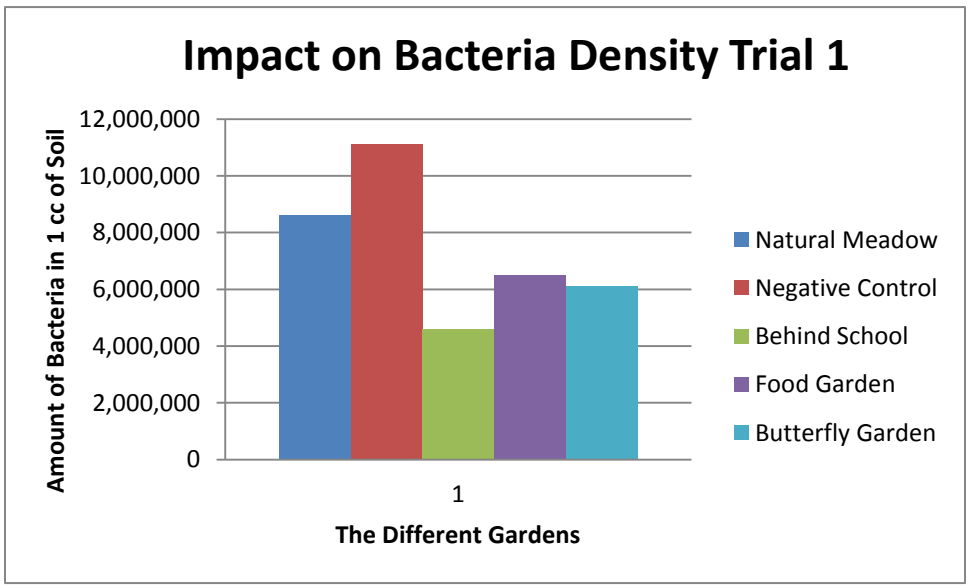
a. Data Table:

Impact of Plant Diversity on the Nitrogen Cycle

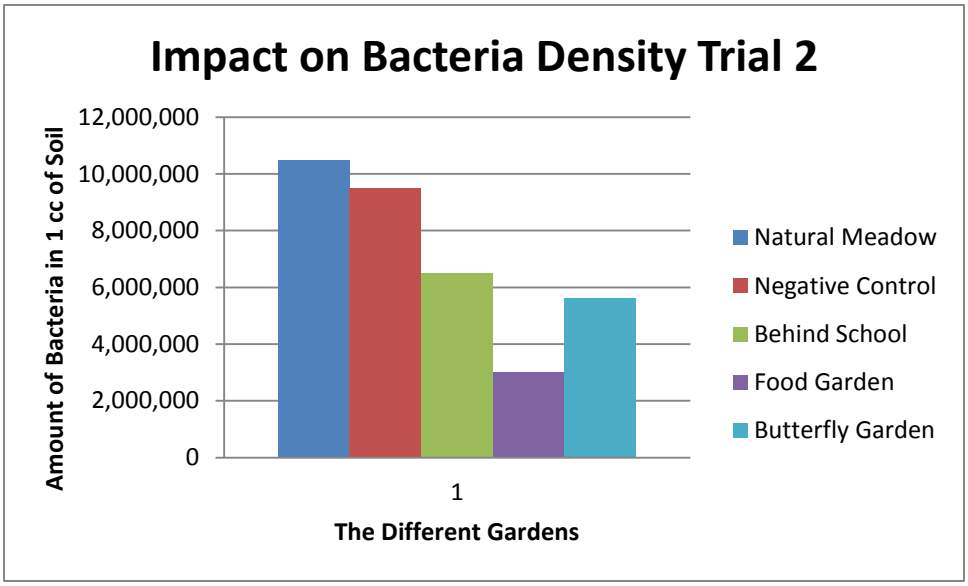
Trial #	Locations				
	Amount of Bacteria in 1 cc of soil "Natural Meadow"	Amount of Bacteria in 1 cc of soil "Negative Control"	Amount of Bacteria in 1 cc of soil "Behind School"	Amount of Bacteria in 1 cc of soil "Food Garden"	Amount of Bacteria in 1 cc of soil "Butterfly Garden"
1	8,600,000	11,100,000	4,600,000	6,500,000	6,100,000
2	10,500,000	9,500,000	6,500,000	3,000,000	5,600,000
3	12,300,000	9,100,000	9,800,000	4,600,000	5,400,000
Averages	10,466,666.67	9,900,000	6,966,666.667	4,700,000	5,700,000

b. Graphs

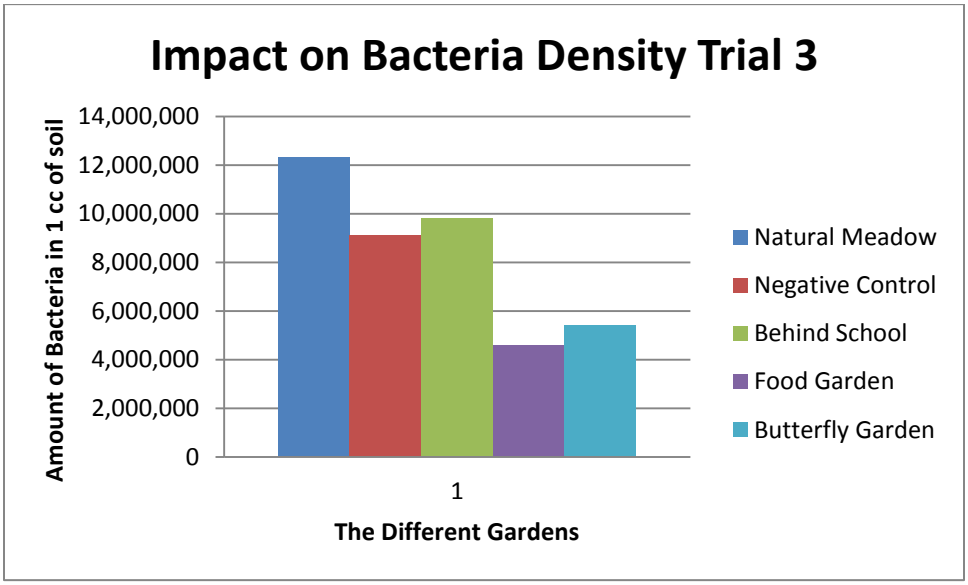
Trial 1:



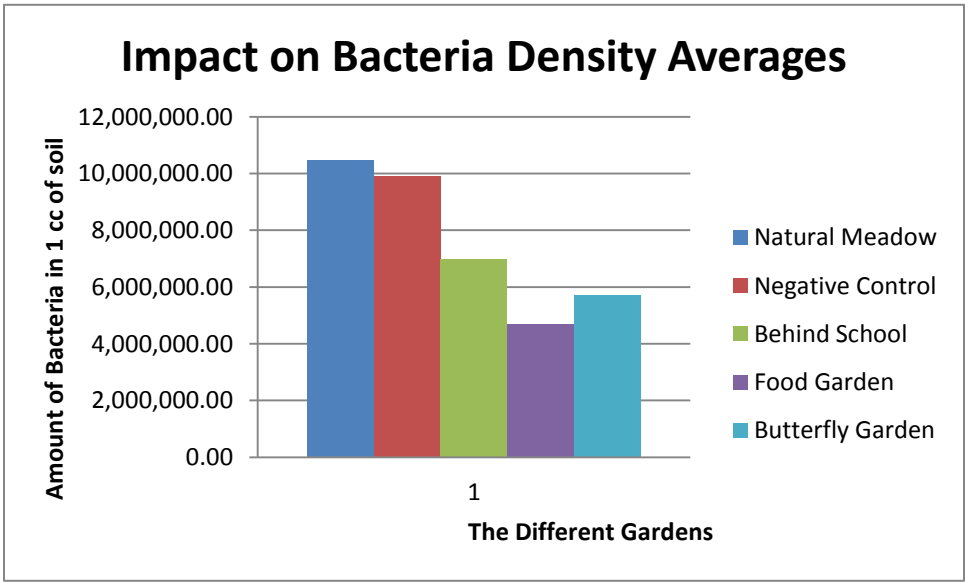
Trial 2:



Trial 3:



Averages:



IV. Conclusion

Therefore, our hypothesis is correct, the impact of Plant variety on the Nitrogen Cycle shows the Natural Meadow to obtain the most bacteria with an average of 10,466,66.67 bacteria in 1cc of soil. The native plants in the Natural Meadow cause the nitrogen cycle to get started faster because of the large amount of bacteria. The difference rate between the Negative Control, Butterfly Garden, Food Garden, Behind School, and Natural meadow varied. The Negative Control had the next greatest amount of bacteria with an average of 9,900,000 bacteria in 1cc of soil. In Comparison between the Natural Meadow and the Negative Control, the Natural Meadow had 566,666.67 more bacteria in 1cc of soil. Containing the third highest amount of bacteria is the Behind School soil. This soil had an average of 6,966,666.667 bacteria in 1cc of soil. The difference between the amount of bacteria in the Negative Control and Behind School decreased from the difference between the Natural Meadow and Negative control at 2,933,333.333 bacteria in 1cc of soil. The second to last highest amount of bacteria is the

Butterfly Garden with an average of 5,700,000 bacteria in 1cc of soil. Comparing the difference between the Behind School and Butterfly Garden; the amount of bacteria lowered from the last comparison of the Negative Control and Behind School. The difference between behind school and the butterfly garden is 1,266666.667 bacteria in 1cc of soil. Having the least amount of bacteria, the Food Garden has an average of 4,700,000 1cc of soil. There is only a 1,000,000 bacteria in 1cc of soil difference between the Butterfly garden and the Food Garden. Overall, there was no pattern from the different averages of the five gardens.

The other gardens in our study that had fewer bacteria than the negative control and the Natural Meadow is caused because fertilizer is used which interrupts the nitrogen cycle. Fertilizer is only helpful to plants, but not to the nitrogen cycle because it harms the microbes in the soil which are needed to continue the nitrogen cycle. After many applications of fertilizer there would be fewer bacteria present in the soil causing the nitrogen cycle to be unhealthy. Therefore, because the behind school garden, food garden, and butterfly garden are used with fertilizer to help the particular plants in each garden grow, it decreases the amount of bacteria present. This is why their nitrogen cycles are unhealthy compared to the Natural Meadow and Negative Control that does not use fertilizers.

For further research, it would be logical to chemically look at the chemicals in fertilizer to see what is contained that kills microbes in the soil. To do this we would take soil samples and test fertilizer levels to see what is so harmful about chemicals. By doing this we can see which type of fertilizer does not harm the bacteria the most. We would do this because if more bacteria are present when we stop using the fertilizer in that particular soil, the nitrogen cycle will be the healthiest.

Work Cited

- Campbell, N.A., Williamson B., Heyden, R.J. (2004) Biology Exploring Life. Boston: Pearson Education Inc.
- Heywood,V.H. and Davis, S.D. (1992). Centres of Plant Diversity. Botany: Smithsonian Institute. http://botany.si.edu/projects/cpd/introduction.htm#importance
- Nardi, J. (2003) The World Beneath Our Feet: A Guide To Life In The Soil. New York: Oxford University Press, Inc.
- The National Academy of Engineering. (2012) Manage the Nitrogen Cycle. National Academy of Sciences. http://www.engineeringchallenges.org/cms/8996/9132.aspx
- Smith, K. (2010) Understanding Soil Microbes and Nutrient Recycling. The Ohio State University. http://ohioline.osu.edu/sag-fact/pdf/0016.pdf
- Speer, B. R. Waggoner, B. (1995) Bacteria: Life History and Ecology. The Regents of the University of California. http://www.ucmp.berkeley.edu/bacteria/bacterialh.html