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Soil Ecology Project

Background

There are many reasons why soil bacteria are beneficial. First, they help create compost by breaking down dead plants and animals into nutrients such as iron, zinc, copper, boron, and manganese (Nelson, 2012). Second, bacteria also convert compounds of nitrogen, sulfur, phosphorous, and carbon into forms that other organisms can use (Hoorman, 2011). Third, bacteria are a source of food for more complex soil organisms, such as protozoa. Fourth, they can form mutualistic relationships with plants as a part of the nitrogen cycle (Hoorman & Islam, 2010).

Plants rely on the nutrients from the organic matter which bacteria provide to their roots for their cells. The bacteria recycle nutrients which other organisms cannot access, and plants then use these elements for repair and growth (Lovejoy, 2014). For example, the nutrient potassium is heavily involved in photosynthesis while nitrogen and phosphorous are necessary for the formation of amino acids (the building blocks of protein) and nucleotides (the building blocks of DNA and RNA). Without these critical biological molecules, cells could not perform the chemical reactions that allow them to survive. Therefore, if it weren't for the nutrients provided by the organic matter that bacteria provide to the roots of plants, the cells of the rest of the plant

wouldn't be able to live. (El Dorado Chemical Company, 2013). Hence the nutrients from soil bacteria allow the plant to be healthy and perform their tasks efficiently.

Two of these nutrients that are specifically the result of the composting process are the elements nitrogen and carbon. Bacteria break apart dead organisms during the decay process. Ammonia, though, is a part of the nitrogen cycle, and so bacteria in that cycle has to convert ammonia in the soil into a chemical called nitrate. It is this nitrate that can be absorbed from the ground into the roots of plants to be able to form amino acids and nucleotides, which are needed to undergo chemical reactions. Once plants and any organisms that eat the plants die, the bacteria responsible for composting take the amino acids and nucleotides and break them down into the original ammonia they started as (Brock, 2014). The interaction between decomposition and the cycling of nitrogen keeps this critical element in the ecosystem for living things to use in their cells.

The second essential element involved in composting is carbon. All living things make cells out of biological molecules that are made up of the element carbon (the main compound in organic matter), and when they are broken apart through the process of decomposition, carbon dioxide is left. Then the plants recreate the biological molecules from the carbon dioxide and sunlight through the process of photosynthesis. Primary consumers eat plants in order to get their own biological molecules to live, and secondary consumers eat the primary consumers. Then when organisms and the bacteria perish, new plants absorb the released carbon dioxide to engage in photosynthesis, completing the carbon cycle. This ties back to bacteria because bacteria create the compost during this vital process of decomposition, which allows this carbon cycle to happen. (The National Center for Atmospheric Research, 2014).

Humans can impact the carbon cycle and other vital processes such as decomposition by altering the amount of organic material that is in the soil. By impacting organic matter, humans therefore impact the bacteria in soil in many ways. One way is by planting mulch, which is done to make the soil underneath stay wet and suppress weeds. Organic mulches decompose and help increase soil's fertility (Iannotti, 2014). Another way humans alter organic matter in soil is by planting crops which use the organic matter to grow and therefore cause more bacteria to be around them (Gabet & Fierer, 2002).

In our experiment, we will be testing whether or not adding organic matter to soil will cause the population density of bacteria in that area to increase. This problem is important because the way we manipulate the environment around us affects the organic matter in the area. Therefore, this affects the population of soil biota, such as bacteria in the soil. Our group thinks that more organic matter in the soil will cause a higher population density of bacteria. We are taking soil from the corn garden and a dirt area near a road without plants. We predict that the soil in the corn garden with added organic matter will have a lot of bacteria, but the dirt area without added organic matter will have a smaller amount of bacteria.

Lab Outline

I. Problem: Does adding additional organic matter to soil cause the population density of bacteria in that area to increase?

II. Hypothesis: Adding organic matter to soil causes the population density of bacteria in that area to increase.

III. Procedure:

A. Independent variable: whether or not additional organic matter has been added to soil

B. Dependent variable: population density of bacteria in soil

C. Negative control: patches of bare soil where extra organic matter has not been added

D. Controlled variables:

- Amount of soil extracted from marked areas
- Amount of soil tested in each trial
- Amount of sterile water
- Type of water
- Size of culture tubes
- Distance between each soil extraction location
- Size of micropipette
- Amount of nutrient agar added to petri dish
- Type of nutrient agar
- Size of scoop for soil that is put into the 10^0 culture tubes
- Amount of time dilutions are allowed to grow in petri dishes before examination
- Size of containers used to hold soil
- Amount of time culture tubes are shaken
- Which dilutions are plated
- How much is plated on each nutrient agar plate
- How far soil/water mixture is diluted each trial

E. Step by step:

1. Go to location N39.35707 W076.63686 and mark the first soil extraction location using a flag. Label flag “Soil 1”

2. 100 cm to the right when facing south of the first flag, mark a second flag at N39.35710 W076.63696. Label flag “Soil 2”
3. 100 cm to the right when facing south of the second flag, mark a third flag at N39.35712 W076.63690 so that all three flags are aligned in a straight line. Label flag “Soil 3”
4. Go to location N 39.35754 W 076.63690 and mark the first soil extraction location using a flag. Label flag “ Organic Matter 1”
5. 100 cm to the right when facing north of the first plot, mark a second soil extraction location at N 39.35741 W 076.63609 using a flag. Label flag “Organic Matter 2”
6. 100 cm to the right when facing north of the second flag, mark a third soil extraction location at N 39.35746 W 076.63626 using a flag so that all three flags are aligned in a straight line. Label flag “Organic Matter 2”
7. Steps 8-10 should all be done on the same day at the same time
8. Extract a separate cylinder of soil 15 cm deep and with a diameter of 2 cm using a soil core extractor from each marked area, putting each extraction into separate Ziploc bags
9. Label each Ziploc bag according to the area they were taken from (“Soil 1 Trial 1”, “Soil 2 Trial 1”, “Soil 3 Trial 1”, “Organic Matter 1 Trial 1”, “Organic Matter 2 Trial 1”, “Organic Matter 3 Trial 1”)
10. Bring Ziploc bags with soil extractions to lab station
11. Steps 12-26 should all be done on the same day at the same time

12. Use a clean, new transfer pipette to add 10 ml of sterile water to a 15 ml culture tube. Label the tube "Soil 1 Trial 1 10^{0}"
13. Use the same pipette to add 9 ml of sterile water to a second 15 ml culture tube. Label the tube "Soil 1 Trial 1 10^{-1}"
14. Repeat step 13 two more times to two additional 15 ml culture tubes, only label them "Soil 1 Trial 1 10^{-2}," "Soil 1 Trial 1 10^{-3}" respectively.
15. Place 1 cc of your "Soil Trial 1 1" soil sample into the "Soil 1 Trial 1 10^{0}" culture tube
16. Cap the tube and shake vigorously for 2 seconds
17. Using a new clean pipette, remove 1 ml of the soil/water mixture from the "Soil 1 Trial 1 10^{0}" tube and place into the "Soil1 Trial 1 10^{-1}"
18. Cap the tube and shake vigorously for 2 seconds
19. Using the same pipette in step 17, remove 1 ml of the soil/water mixture from the "Soil 1 Trial 1 10^{-1}" tube and place into the "Soil 1 Trial 1 10^{-2}" tube
20. Cap the tube and shake vigorously for 2 seconds
21. Using the same pipette in step 17, remove 1 ml of the soil/mixture from the "Soil 1 Trial 1 10^{-2}" tube and place into the "Soil 1 Trial 1 10^{-3}" tube
22. Cap the tube and shake vigorously for 2 seconds
23. You should now have a total of four culture tubes
24. Plate 100 µl samples from the 3rd and 4th tubes (dilutions "Soil 1 Trial 1 10^{-2}" & "Soil 1 Trial 1 10^{-3}") onto their own separate, labeled 3M Petrifilm™ Aerobic Count Plates. The Aerobic Count Plates should be labeled according to the dilutions that are plated on them ("Soil 1 Trial 1 10^{-2}" & "Soil 1 Trial 1 10^{-3}")

25. Repeat steps 12-24 five more times, each time using the soil from a different Ziploc bag until the soil in all six Ziploc bags has been experimented on. Make sure to change the labels on the culture tubes and according to which Ziploc bag the 1 cc of soil is taken from. Also make sure to label the Aerobic Count plates based on which culture tube the dilution is taken from
26. Allow all the samples to grow for 48 to 72 hours
27. Examine each of the plates for individual bacteria colonies and choose the plate at the lowest dilution that has at least 5 colonies. Count the total number of colonies on that plate and use the following formula to estimate the number of bacteria in the original 1 cc soil sample:

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

28. Record the estimated number of bacteria in the original 1 cc soil sample in data table
29. Repeat steps 7-28, using new Ziploc bags to gather the soil samples and new culture tubes to test the soil. Make sure that you change the trial number on the labels on the Ziploc bags and the culture tubes to “2”. Also make sure that all the soil extractions are done on the same day at the same time and all the serial dilutions for bacteria testing are done on the same day and at the same time. (The extractions and the testing do not have to be on the same day)
30. Repeat steps 7-28, using new Ziploc bags to gather the soil samples and new culture tubes to test the soil. Make sure that you change the trial number on the labels on the Ziploc bags and the culture tubes to “3”. Also make sure that all the soil extractions are done on the same day and all the serial dilutions for bacteria

testing are done on the same day and at the same time. (The extractions and the testing do not have to be on the same day)

Data/Observations

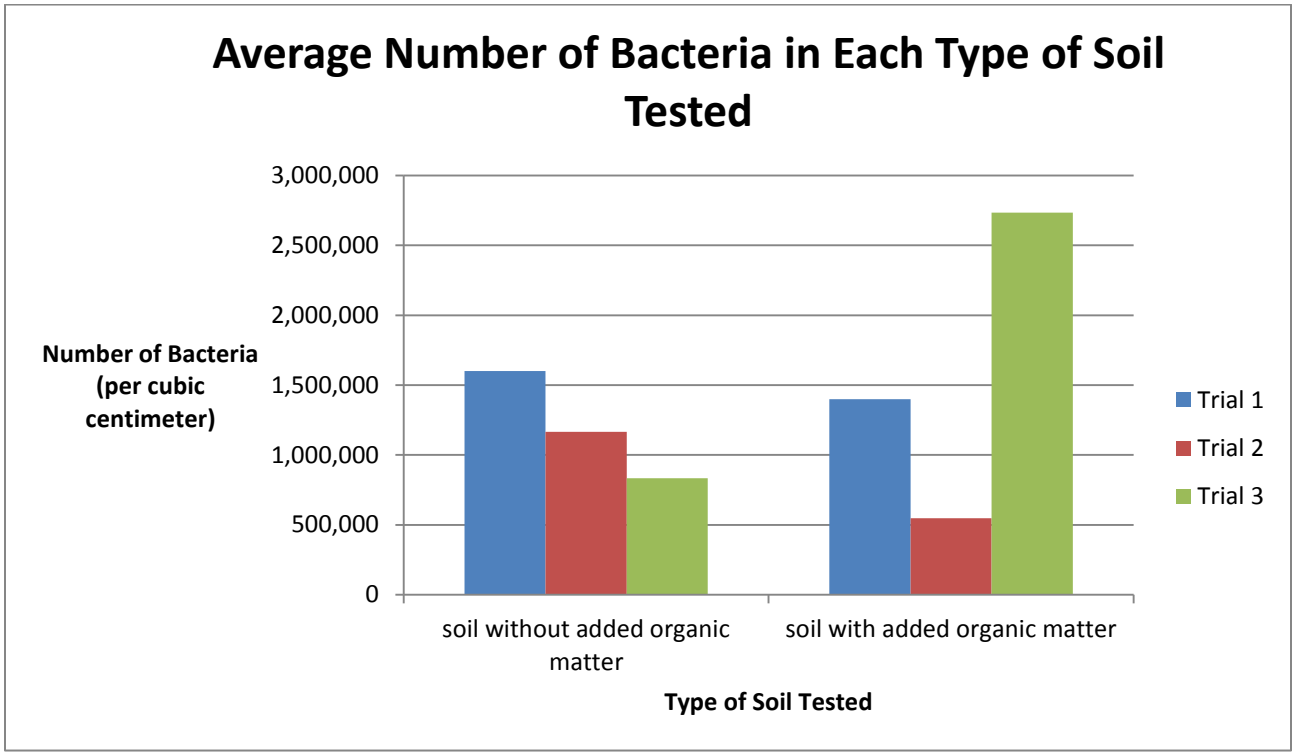
Population Density of Bacteria in Soil

	The Number of Bacteria in Soil per cubic centimeter					
	Soil without added organic matter			Soil with added organic matter		
Labels on flag marks	"Soil 1"	"Soil 2"	"Soil 3"	"OM 1" (Organic Matter 1)	"OM 2" (Organic Matter 2)	"OM 3" (Organic Matter 3)
Trial 1	1,300,000	2,400,000	1,100,000	1,800,000	1,800,000	600,000
Trial 2	1,300,000	800,000	1,400,000	370,000	800,000	470,000
Trial 3	400,000	1,500,000	600,000	3,600,000	2,200,000	2,400,000

Average Number of Bacteria in each Location Tested

	Average Number of Bacteria per cubic centimeter	
Type of soil tested	Soil without added organic matter	Soil with added organic matter
Trial 1	1,600,000	1,400,000
Trial 2	1,166,666.667	546,666.667
Trial 3	833,333.333	2,733,333.333

Graph:



Conclusion

Our hypothesis was disproven based on the data that was collected. We predicted that there would be a higher population density of bacteria in soil with added organic matter than there would be in soil without added organic matter. However, our data proved this prediction to be wrong. In trial 1, the average number of bacteria in soil without added organic matter was 1,600,000. In soil with added organic matter the average population density of bacteria was 1,400,000. In trial 2, soil without added organic matter again had a greater average population density than soil with added organic matter (1,166,666.667 vs. 546,666.667). In trial 3, however, the data offered evidence that would suggest our hypothesis to be true. The average population

density of bacteria in soil without added organic matter was 833,333.333 while the average population density of bacteria in soil with added organic matter was 2,733,333.333.

We originally thought our hypothesis would be correct after researching bacteria. We found that they eat nutrients such as simple sugars, carbons, and carbohydrates. The main part of organic matter is carbon, which is brought into the soil by the carbon cycle. Bacteria use this nutrient to reproduce and settle in the soil. This led us to believe that in soil with added organic matter (and therefore more carbon), the population density of bacteria will be higher than in soil without this added matter.

Soil does not originally have organic matter in it, however humans add it to soil to increase the nutrients for plants to grow. This human influence impacts the organic matter and therefore the bacteria in soil in many ways. One way is planting mulch, which is done to make the soil underneath stay wet and suppress weeds (Iannotti, 2014). Organic mulches decompose and help increase soil's fertility. Humans plant crops which use organic matter to grow and therefore cause more bacteria to be around the crops since there is supposedly an increase in bacteria population when organic matter is added to soil (Gabet & Fierer, 2002).

These facts support our hypothesis well, yet our hypothesis was disproven. There are many factors to consider when asking why this could have happened. The area we tested where organic matter was added to the soil was not in a secluded area. Therefore it was easily accessible to people walking over it, compressing the soil. Compression of the soil squeezes all of the spaces where water and oxygen would be, taking away two major chemicals that the bacteria would need to make energy in order to survive. This site was also on a hill, causing runoff to be a possible influence that lessens the amount of organic matter in the soil by flushing it away.

Runoff also consists of more protozoa because protozoa, which eat bacteria, prefer to live in moist areas, which means more bacteria would be eaten in wetter soil. Finally, the area where we drew our soil without added organic matter was next to a road, creating a possible influence on the bacteria population. This is because the oil which is deposited from cars mixes with water in the soil. This mixture of oil and water is a source of hydrocarbons, and therefore is a source of energy for molecules such as bacteria, causing more bacteria to live in the soil near the road (Brock, D. 2014).

Our hypothesis is based on carbon having a major effect on the bacteria population. For further research, we could test for carbon in the soil by using a humus test in addition to counting the bacteria. This would allow us to see if carbon really does have an effect on bacteria population density in soil. We could also control for the variables such as car oil, runoff, and the location of soil extraction in reference to humans.

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