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Biology 9

Mr. Brock

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Background

Microbes are crucial to the world we live in, especially soil microbes. These microbes consist of bacteria, protozoa, fungi, and algae, and among these, one of the most vital types of soil microbes living in the ground are fungi. Without fungi, litter and organic compounds such as dead plants and animals would build up in the soil and have a tremendous negative impact on the environment of other organisms. For example, fungi provide habitats for various invertebrates and perform tasks in the soil having to do with water, disease suppression and nutrient cycling (Guest Speaker report, 2004).

Fungi have very few needs, and they can survive in the soil on very little. Though they are not animals with stomachs, they still need food and energy, and because they do not have chlorophyll like plants do, it is impossible for them to make their own food. Therefore, they must eat living or non-living matter surrounding them (T 2003) by absorbing their food from the substrate in which they grow. Essentially, soil fungi decompose dead material and absorb their chemical matter by breaking down and converting large amounts of organic material into inorganic compounds**.** They then absorb these compounds through their cell membranes to make more biological molecules. (Elaine R. Ingham, 2009). In addition, fungi recycle any excess nutrients the do not consume themselves by making them available to plants, animals, and other organisms that are part of the soil food web. Chemical elements such as hydrogen, oxygen, nitrogen, carbon and phosphorus would be locked up in plants and animals permanently without fungi because as soil fungi obtain their own energy and carbon needs though degrading and composting organisms in the soil, they leave behind waste such as lignin, cellulose, and other complex molecules that the plants can use. (Dighton J, White JF & Oudemans P, 2005). These are very important because without these elements there would be no cells, and therefore, there wouldn't be biological molecules for plants and animals to survive, grow, and make more biological chemicals to give to the fungi and other organisms.

Fungi play a significant role in the soil, in two forms: ruderals and mycorrhizal. Ruderals are important because they associate with rainfall and the flushing of organic molecules from plants, microbes, and the surface litter and so do most of the mycorrhizal fungi. As a result, the fungi react instantly and grow while the soil is moist. Mycorrhizae are very different from ruderals, for they live a very stable life. The mycorrhizal fungi invade the roots of plants to get their organic energy, while supplying nutrients and water for the plants. Because of their way of obtaining energy, mycorrhizal fungi grow much slower than ruderals. However, because of the large population and dispersal of fungi, obtaining their energy is not hard for them to do (Dighton J, White JF & Oudemans P, 2005).

While obtaining energy and carbon in a regular environment for fungi is pretty simple regardless of whether they are ruderals or mycorrhizae, a range of complications can occur. Enemies such as other microbes, nutrient availability, and temperature have an effect on their productivity in the soil (Professor Lyn Abbott and Jen Slater, 2005). Without the proper environment it is harder for fungi to do their job in the soil. Healthy, biologically-active soil should have space between the soil particles and should be 25% air. It should be light, and not compacted, allowing it more oxygen to reach microbes such as fungi. Also soil temperature affects fungi, and soil between 70-80 degrees is best for fungi. Moisture affects fungi as well. If the soil is too wet, the water will fill the air spaces, and suffocate soil organisms. Too dry of soil is just as problematic (Harris 2003).

One of the setbacks that fungi microbes often face is soil compaction. But when soil compaction occurs, soil no longer has the right amount of oxygen, temperature, and water.

Soil compaction is caused by manmade and natural causes such as the impact from rain, cars, farm machines, and construction sites (University of Minnesota, 2001). Somewhat compacted soil improves germination, but compacted soil is generally bad for the ground. Compacted soil does not allow roots to grow as easily, and it can cause plants to die because the water cannot get to the roots (University of Minnesota, 2001). Compacted soil does not have a lot of large pores to breathe which does not allow for proper living conditions for most organisms that live there, including fungi (University of Minnesota, 2001). In fact compaction can often kill most fungi (The Blue Mountain Native Forest Alliance). The soil microbes and organisms need air to breathe which compaction disallows.

Fungi dying, though, cause unfortunate things to happen to the earth. One side effect would be that because the plants and trees would not retrieve enough nutrients, they would eventually die. This could cause the spread of disease and affect our entire ecosystem (The Blue Mountain Native Forest Alliance). In addition, if soil microbes and organisms were dying, plants would be unable to retrieve the biological chemicals that they need to survive and make more chemicals for all the other organisms, in the ecosystem to survive.

The great deal of causes and effects made this experiment exceptionally interesting, as a result we chose to test it. It was chosen to see how compaction affects soil fungi population. Tractors are drove over the soil during the construction of the new athletic center and compacted it. To test how the tractors are damaging the soil we set up three rows each has four plots starting closest to the road, and moving up the hill. We will test for the amount of fungi using the serial dilution experiment. Our data will then show us how the compaction is affecting the population. We also tested for the amount of humus, using the same plots. The Humus test is used to display how much decomposition occurs in each area and can help indicate how healthy the fungi are.

Bibliography

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3. Harris, Susanna. (2009) The Secrets to Great Soil- How Fungus Turned my Thumb Green. http://www.sustainable-gardening.com/Organics/GreatSoil.php
4. Ingham, Elaine R. (2009) Soil Fungi | NRCS SQ. http://soils.usda.gov/sqi/concepts/soil_biology/fungi.html
5. The Blue Mountain Native Forest Alliance. (n.d).Problems Related to Soil Damage or Loss. http://www.subtleenergies.com/ormus/bmnfa/soil.htm . Accessed May of 2009.
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Procedure

I. Question: Are the fungi microbes denser in compacted soil, or soil that has hardly been compacted.

II. Hypothesis: There will be more fungi microbes in the soil that is less compacted.

III. Procedure:

   a. Independent Variable- how compact the soil is based on its distance from the road

   b. Dependant- density of fungi per cubic centimeter of soil

   c. Negative control- the soil sample taken farthest from the road (i.e. least compacted soil)

   d. Controlled variable- amount of soil collected from each plot, amount of sterile water, amount of soil placed into each culture tube, amount of soil/water mixture removed from each tube, amount of soil/water mixture placed into each Petrifilm™ Yeast and Mold Plate, amount of time allowed for each culture to grow, collecting all samples on same time/day, tested on same day, LaMotte Combination Soil Model STH-14 Code 5010-01

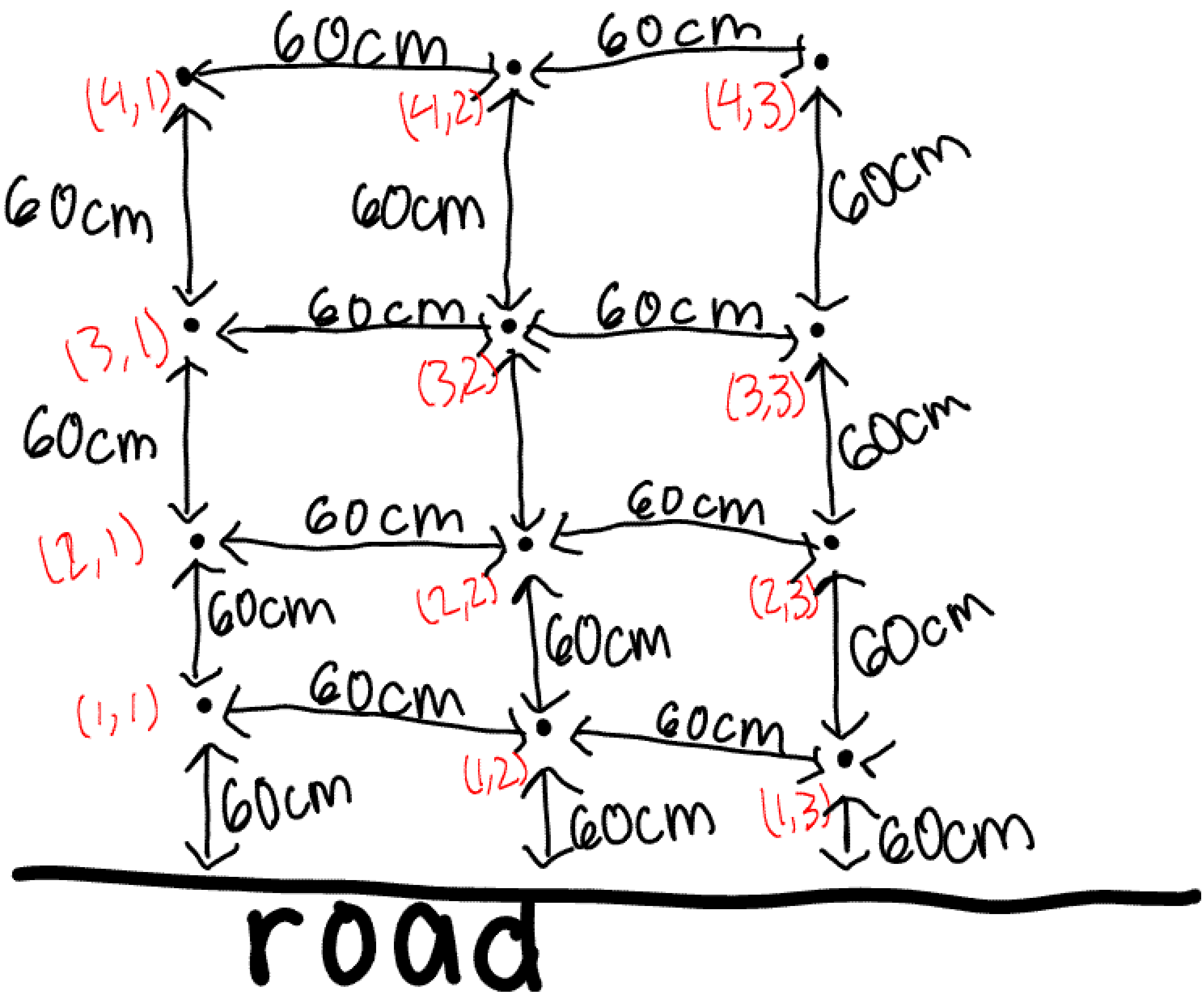
   a. Step-by-step instructions:

      i. Materials List:

         - Soil core tester
         - Yellow plotting flags

- Clean zip lock plastic bags
- GPS
- Permanent marker
- Stick

1. At North 39.35722° and West 076.63694°, 60 centimeters from the road, going perpendicular from it, put four flags, each of them 60 centimeters apart from each other. Parallel to that line of flag's, put another row of four flags 60 centimeters away from each other, and 60 centimeters away from the first perpendicular row. Do this one more time so that you have three perpendicular columns 60 centimeters away from each other with four flags in them and four parallel rows 60 centimeters apart with three flags in them.



See diagram above.

2. Once all of the flags are in the ground, label them according to the diagram above

3. Next to the plot flag, use a soil core tester and twist it 360 degrees clockwise down 15 centimeters with a diameter of 2.54cm (until the first dotted line is at the surface) into the soil.
4. Then pull the soil core tester straight out of the ground
5. Using a stick, place the soil you collected from the core tester, and place it in a clean zip lock bag to take back to the science room. As you place the sampled soil into the bag, be sure to label it with the plot location you collected it from.
6. Repeat steps three through five, eleven more times, until you have a soil sample from each flag making sure you take all samples the same time and same day.
7. Once you have collected the soil, follow the instructions to test for the population of fungi in each sample for each row. **Make sure you perform the Serial Dilutions and the Humus Test for all samples in each trial at the same time.** See the instructions below.

Serial Dilutions for Fungi

Materials List

- 10 ml serological pipettes **(2)**
- 15 ml tubes with caps **(3)**
- 1 – cc scoop **(1)**
- Glass Rod **(1)**
- Petrifilm™ Yeast and Mold Plate **(3)**
- P200 micropipette **(1)**
- Tips for the P200 micropipette **(3)**

1. Use a clean, new transfer pipette to add 10 ml of sterile water to a 15 ml culture tube. Label the tube "10^0" for test (1,1).
2. Use the same pipette to add 9 ml of sterile water to a second 15 ml culture tube. Label the tube "10^{-1}" for test (1,1).
3. Repeat step two, one more time to one additional 15 ml culture tube, only label the "10^{-2}"respectively for test (1,1).
4. Place 1 cc of your (1,1) soil sample into the "10^0" culture tube.
5. Cap the tube and shake vigorously.
6. Using a new clean pipette, remove 1 ml of the soil/water mixture from the "10^0" tube and place it in the "10^{-1}" tube.
7. Cap and shake vigorously.

8. Using the same pipette in step 5, remove 1 ml of the soil/water mixture from the "10^{-1}" tube and place it in the "10^{-2}" tube.
9. Cap and shake vigorously.
10. You should now have a total of three culture tubes.
11. Place 100 µl samples from each tube (dilutions 10^{0}, 10^{-1} and 10^{-2}) onto their own separate, labeled Petrifilm™ Yeast and Mold Plates containing nutrient agar. Allow yeast and mold to grow for 48 hours.
12. Repeat steps 1-11 for every other sample of soil in trial one.
13. Make sure to test and collect all of the samples on the same day to control for specific variables.
14. Examine each of the plates for individual fungi colonies, specifically mold and yeast, then choose the plate with the most dilution to make your estimates of the number of fungi in the original 1 cc soil sample using the fallowing formula:

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

Record the amount of yeast and mold observed in the 10^{1} Petrifilm. If either yeast or mold isn't present observe the 10^{2} Petrifilm plate. Record the amount of yeast and mold found then perform the formula above.

15. Use the LaMotte Combination Soil Model STH-14 code 5010-01 test kit to perform the Humus Test. The purpose of this test is to measure the amount of decomposed material. **Make sure you perform the humus test on every sample within each trial at the same time. Record the corresponding Humus Level(specific color) for each dilution tube.**
16. Repeat steps 1 – 14, for the remaining two trials.

Data/Observations

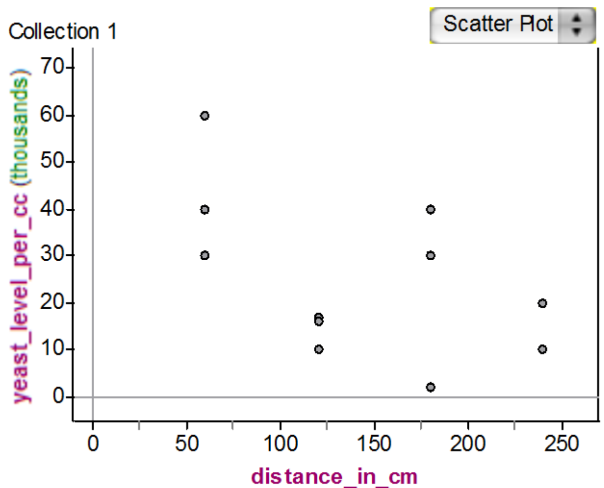
Serial Dilution Test

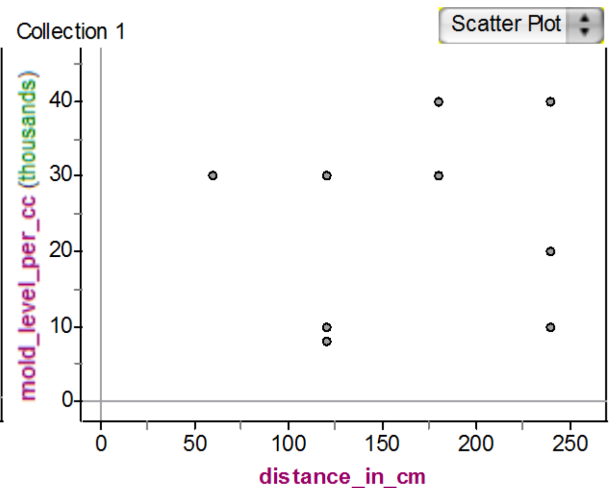
	Total Yeast	Total Mold	Total Fungi
Test 1	149000	100000	249000
Test 2	106000	140000	246000
Test 3	100000	78000	178000

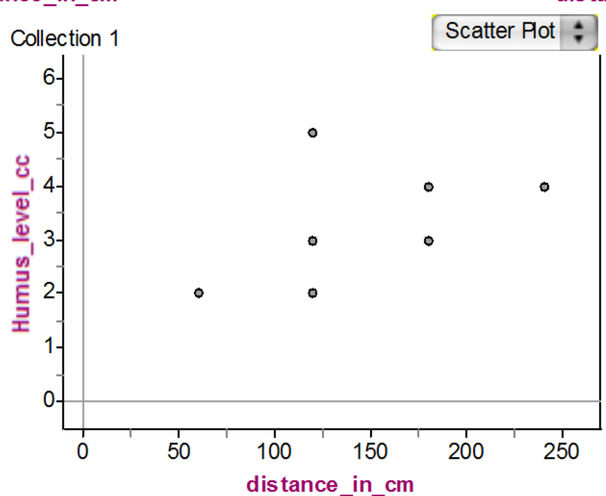
Humus Test

Plot				
	(1,1)	(2,1)	(3,1)	(4,1)
Level of Humus				
	2	2	3	4
Plot				

	(1,2)	(2,2)	(3,2)	(4,2)
Level of Humus				
	2	5	4	4
Plot				
	(1,3)	(2,3)	(3,3)	(4,3)
Level of Humus				
	2	3	4	4







Conclusion

Our hypothesis stating that there are more fungi microbes in the soil that is less compacted was proven correct. We know this because looking at the Petrifilm plates, the soil closest to the road contained the least amount of mold and more yeast. The further away from the road the soil contained even less yeast and even more mold. According to our data there are more fungi microbes in the soil 240 cm away from the road opposed to the soil 60 cm away from the road. More mold, means more decomposition. Fungi perform the action of decomposing, showing more Fungi in the soil. Furthermore, the soil furthest from the road is healthier and is least compacted. After our research, further work can be done. There needs to be more observations of other areas on campus that may be damaged by cars.