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Bio 9H

25 May 2011

Background

Mycorrhizae are fungi that have a symbiotic association with plant roots (Ingham 2011). The fungi form outside of the tree roots and use structures called hyphae to penetrate the pores in the soil. The hyphae then help plants get water and nutrients more effectively, and they do so by first attaching to the roots to increase the size of the root system. These structures then release compounds that help decompose organic matter and give the newly formed nutrients to the plant in return for carbon and sugar from the plant's cells and the photosynthesis process. Furthermore, the hyphae from different mycorrhizae can be linked together to transport nutrients to different plants throughout the ecosystem, extended the aid they give to plants even more.

One of the key nutrients that fungi help provide is nitrogen. Nitrogen, one of the most essential elements in soil, promotes growth and is needed for the chloroplasts in plants for photosynthesis. As a key element in amino acids, nitrogen is crucial for the construction of the enzymes which create chemical reactions. If there is no nitrogen then there can be no chemical reactions, and the cells of a plant die along with all the other organisms that depend on it. Consequently, the more abundant nitrogen is in an ecosystem, the better the soil, food supply, and health of the organisms living there.

To get nitrogen, special plants called legumes contain bacteria that take the nitrogen from the air and convert it to a form that plants can absorb. Fungi are needed in this process as well because the fungi for most plants act as an ancillary to aid them in accessing the nitrogen these bacteria produce. Fungi also help plants receive nitrogen through the decomposition of these legumes and other organic matter, and in return for the nitrogen the mycorrhizae distribute, the hyphae transfer phosphorus back to the bacteria legume for its own respiration purposes (Wallace 2004).

Soil fungi are also critical to an ecosystem because they also supply compounds other than nitrogen to support life. By binding the soil particles together, fungi actually increase the efficiency of water and nutrients going into plants (Ingham 2011). Therefore, they reduce the need to water or fertilize plants, and this means that there will be less pollution on the ground or surface water and less wasted nutrients. In addition to aiding the surrounding plants, mycorrhizae also protect plants through droughts, cold temperatures, and other bad conditions (Wallace, 2004).

Many people, though, do not understand the roles fungi play in the ecosystem. As a result, when they cut down forests or allow fields to fallow, the soil stops being as fertile because without sugar from plants the number of mycorrhizal fungi in soil will decline. Here, in areas where the plants and trees that fungi depend on have been extracted, tilling the soil and pesticides also pose a toxic threat to mycorrhizal fungi. Pesticides upset the balance of nitrogen in the soil and basically cause a nitrogen surge so that the fungi soak it all up. Yet as the pesticide amount fades, it causes the population of now too numerous fungi to crash because of the lack the nitrogen of soil to fill their own biological needs. As a consequence, because of the reduction in fungi caused by deforestation and other land misuses of the ecosystem disrupt the symbiotic relationship, causing the plants to suffer (Ingham 2011).

Misuses of the land such as deforestation, therefore, pose a major threat to the function of the ecosystem and the fungi there. In the process of deforestation, the top layers of soil are removed which contain the majority of the minerals and sugars that fungi need to live, and that along with when the trees that have a symbiotic relationship with fungi are removed, the fungi have to fight to survive. To make it an even more difficult struggle, as the trees are removed, erosion starts to happen which causes even more rich soil to be moved and taken away from the fungi that depend on it, and now without the trees and other plants, the fungi are exposed to parasites and protozoa which battle them for the limited amount of food they have left. A fungi's prosperity depends on the amount of organic matter present for it to eat, and without deforestation, the organic matter and soil quality would increase causing the fungi to prosper (Chidumayo; Kwibisa, 2002)

Unfortunately deforestation takes a toll on the mycorrhizal community in any ecosystem. So to test the effects of it from a recent fire in the Roland Park ecosystem in Baltimore, Maryland, we performed an experiment to see what happens to the fungi after their main source of food is taken away. The ecosystem being tested is a rectangular plot of land in front of Roland Park Country School. Where half of the number of evergreen trees were destroyed due to an unexpected fire. Two tests were conducted to try and prove if deforestation had an effect on the amount of mycorrhizal fungi. Based on prior knowledge (Groffman, 2011), we performed a humus test to determine the amount organic matter in the soil at the test site, and we counted the number of fungi per gram of soil in the area. Both of these tests were necessary because if the humus levels are high in an area, then the fungi would be able to decompose the matter and help the plants around it grow and feed off of the decaying matter. If the humus levels are low, then the fungi will not have enough food to stay alive and help other plants do the same. The serial delusion test is crucial because if the amount of fungi in the forested area is more than the amount in the deforested area it proves that fungi need trees to survive and that trees need fungi as well. We predict that the density of fungi in the area with trees will be larger than the area without trees.

Experiment

I. Problem: What is the impact of deforestation on the density of soil fungi?

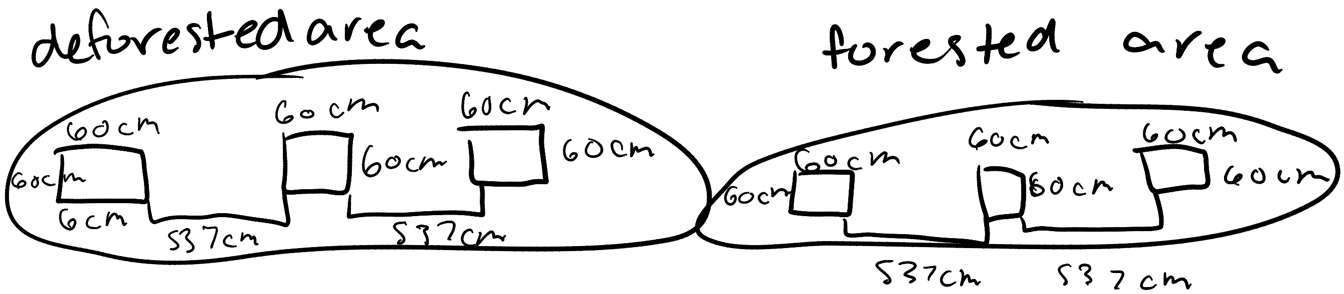
II. Hypothesis: Deforestation causes soil fungi to decrease in density.

III. Procedure:

a. Independent Variable: Soil samples from an area that has been deforested
b. Dependent Variable: Temperature of soil (◦C), amount of humus (LaMotte Ordinal scale), density of fungi (#/cm^3)
c. Negative Control: Soil samples from an area with trees that are of the same species as originally found in the deforested area
d. Controlled Variables: Same environment, used the same chemical test kit for every humus test, same degree diluted each time, same type of trees that were there before deforestation, same size sample of soil taken, serial dilution and humus test performed at the same time on the same day, same amount of soil diluted, concentration of solution, age of tree, taking the soil samples at the same time on the same day, same degree of dilution each time, same amount of soil/water mixture moved from the previous test tube each time, amount of soil diluted each time, same size test tubes, same amount of time shaking each test, same amount put on fungi/yeast plates, same dilution sample put on fungi/yeast plates, same time spent on fungi/yeast plates, same type of fungi plates

e. Step-By-Step Instructions:

1. Using flags to mark the corners, plot flags in a square in the deforested area on soil areas that are 60cm on each side and each 537cm apart.
    a. The center of trial 1 is 39° 21.503 N, 076° 38.112 W (See diagram)
    b. For trial 2 coordinates are: 39° 21.571 N, 076° 38.120 W (see diagram)
    c. For trial 3 coordinates are: 39° 21.515 N, 076° 38.120 W (see diagram)
2. Using flags to mark the corners, plot 3 squares in the forested area on soil areas that are 60cm on each side and each 537cm apart.
    a. The center of trial 1 is 39° 21.503 N, 076° 38.120 W (see diagram)
    b. For trial 2 coordinates are: 39° 21.501 N, 076° 38.120 W (see diagram)
    c. For trial 3 coordinates are: 39° 21. 498 N, 076° 38.120 W (see diagram)
3. Label 12 separate Ziploc bags respectively with the following: trial 1, 1 deforested; trial 1, 2 deforested; trial 2, 1 deforested; trial 2, 2 deforested; trial 3, 1 deforested; trial 3, 2 deforested; trial 1, 1 forested; trial 1, 2 forested; trial 2, 1 forested; trial 2, 2 forested; trial 3, 1 forested; trial 3, 2 forested



4. Steps 5-10 should be performed at the same time on the same day.
5. Put a thermometer in the marked soil in the deforested area with the flag marked “trial 1” right before you take the soil out and leave it there for 1 minute. Record temperature into table
6. Take out a cylinder of soil that 15cm deep X2cm wide from the same spot that you measured the temperature into a Ziploc bag labeled Trial 1, 1 deforested.
7. Repeat steps 5-6 with the following bags: trial 1, 2 deforested; trial 2, 1 deforested; trial 2, 2 deforested; trial 3, 1 deforested; trial 3, 2 deforested
8. Put a thermometer in the marked soil in the forested area with the flag marked “trial 1, 1 forested” right before you take the soil out and leave it there for 1 minute. Record temperature into table
9. Take out a cylinder of soil that is 15cm deep X2cm wide from the same spot that you measured the temperature place it into a Ziploc bag labeled “Trial 1, 1 forested.”
10. Repeat steps 8-9 with the following bags: Trial 1, 2 forested; trial 2, 1 forested; trial 2, 2 forested; trial 3, 1 forested; trial 3, 2 forested
11. Steps 12-20 must be done at the same time in the same period for all of the bags.

12. Using a sterile transfer pipette, add 10 mL of sterile water to a 15 mL culture tube labeled “10^0 Trial 1, 1 Forested”.
13. With the same pipette, add 9 mL of water to another 15mL culture tube labeled “10^{-1} Trial 1, 1 Forested”.
14. Next repeat step 10 but with a culture tube labeled “10^{-2} Trial 1, 1 Forested”.
15. Next add 1 cc of “Trial 1, 1 Forested” soil sample to the “10^0” culture tube. Continue to cap the tube and shake in a vigorous manner.
16. With a new pipette, take 1mL of soil/water mixture from the 10^0 tube and put into the 10^{-1} tube (each group should have separate pipettes). Continue to cap the tube and shake vigorously.
17. Using the pipette from step 14, remove 1mL of soil/water mixture from the 10^{-1}tube and place into the 10^{-2} culture tube (each group should have separate pipettes). Then cap and shake the tube hard.
18. Now plate separate 100ul samples from these (10^{-1} and 10^{-2}) tubes their own separate correspondingly labeled 3M Petrifilm yeast/mold count plates.
19. Repeat steps 11-17 with new 15 mL culture tubes. Each tube should have a fresh pipette: Trial 1,2 Forested 10^0, 10^{-1}, 10^{-2} ; Trial 2,1 Forested 10^0, 10^{-1}, 10^{-2} ; Trial 2,2 Forested 10^0, 10^{-1}, 10^{-2} ; Trial 3,1 Forested 10^0, 10^{-1}, 10^{-2} ; Trial 3,2 Forested 10^0, 10^{-1}, 10^{-2} ; Trial 1,1 Deforested 10^0, 10^{-1}, 10^{-2} ; Trial 1,2 Deforested 10^0, 10^{-1}, 10^{-2} ; Trial 1,1 Deforested 10^0, 10^{-1}, 10^{-2} ; Trial 2,1 Deforested 10^0, 10^{-1}, 10^{-2} ;

Trial 2,2 Deforested 10^0, 10^{-1}, 10^{-2} ; Trial 3,1 Deforested 10^0, 10^{-1}, 10^{-2} ; Trial 3,2 Deforested 10^0, 10^{-1}, 10^{-2}

20. Use the LaMotte Combination Soil Outfit Model STH-14 to perform the humus count test. Do every test at the same time.
21. Within a 144 hour period the fungi will grow.
22. Count the number of yeast colonies and mold on the lowest dilution plate (10^{-2}) for "Trial 1, 1 Forested". If the 10^{-2} plate is not countable, count the yeast colonies and mold on the 10^{-1} plate. Record the
23. Use the following formula to approximate the number of fungi: # of microbes in 1cc of soil= #colonies on the sheet x $10^{2(\text{or } 1)}$ x $10^{|\text{number of dilution at which the colonies were found}|}$
24. Repeat step 21 for every trial.

IV. Data and Analysis

[Impact of Deforestation on Soil]

Day	Deforested / Forested	Trial Numbers	Number of Yeast (per 1cc)	Number of Mold (per 1cc)	Total number of Fungi (per 1cc)	Humus Level (per 1cc)	Temperature (°C)
Day 1	Deforested	1	20,000	30,000	50,000	2	17.1
		2	10,000	30,000	40,000	1	17.8
		3	30,000	30,000	60,000	4	17.6
Day 2		1	60,000	90,000	150,000	1	17.7
		2	50,000	50,000	100,000	2	18.3
		3	140,000	10,000	150,000	2	17.5
Day 1	Forested	1	80,000	50,000	130,000	5	16.2

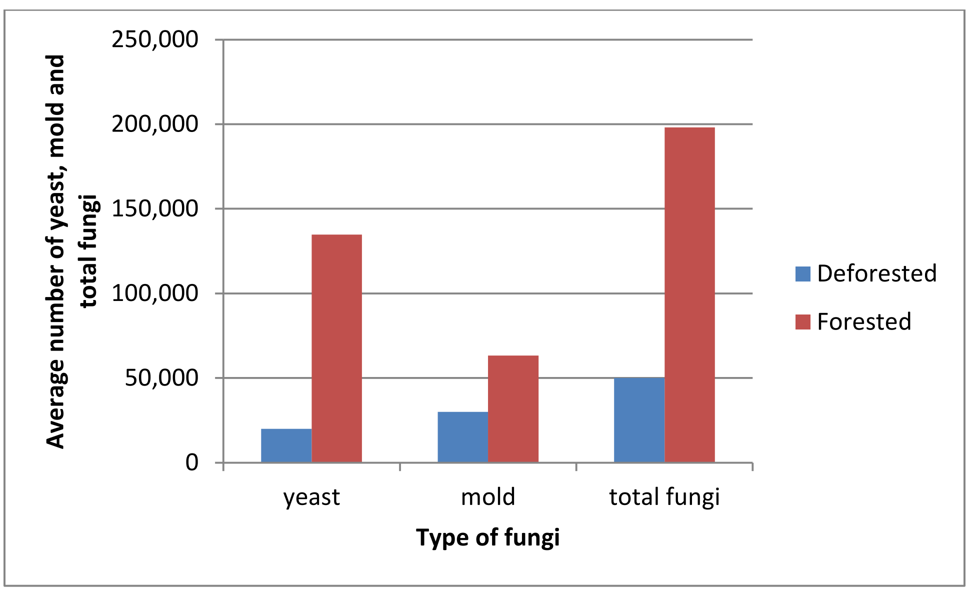
		2	320,000	70,000	390,000	5	15.3
		3	4,000	70,000	74,000	4	15.1
Day 2		1	40,000	150,000	190,000	4	15.2
		2	120,000	60,000	180,000	4	15.8
		3	120,000	20,000	140,000	5	15.4

[Average impact of deforestation on Soil]

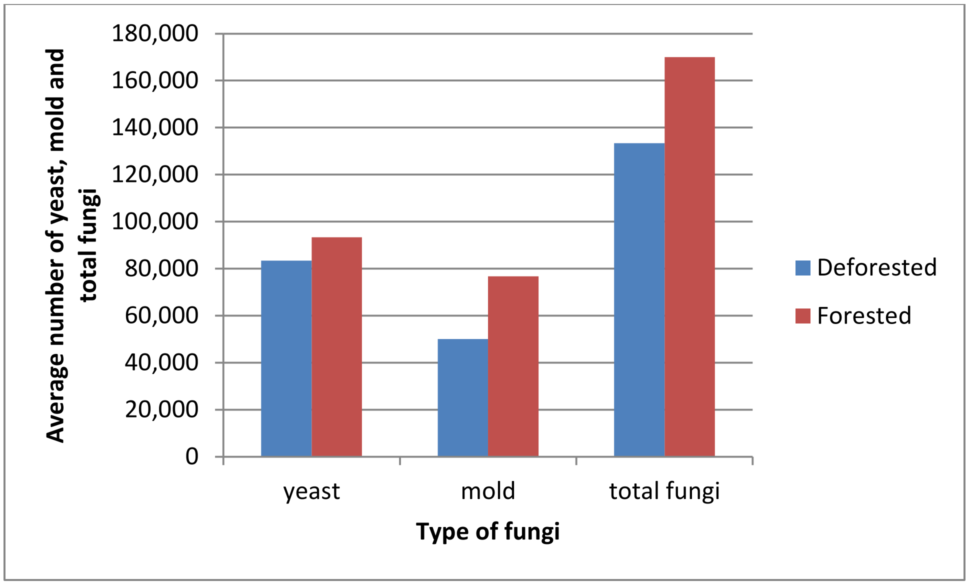
Deforested/ Forested	Day	Average number of yeast (per 1cc)	Average number of mold (per 1cc)	Average number of total fungi (per 1cc)	Average humus level (per 1cc)	Average temperature (◦C)
Deforested	Day 1	20,000	30,000	50,000	2.33	17.5
	Day 2	83,333	50,000	133,333	1.67	17.83
Forested	Day 1	134,667	63,333	198,000	4.67	15.53
	Day 2	93,333	76,667	170,000	4.33	15.47

Graphs

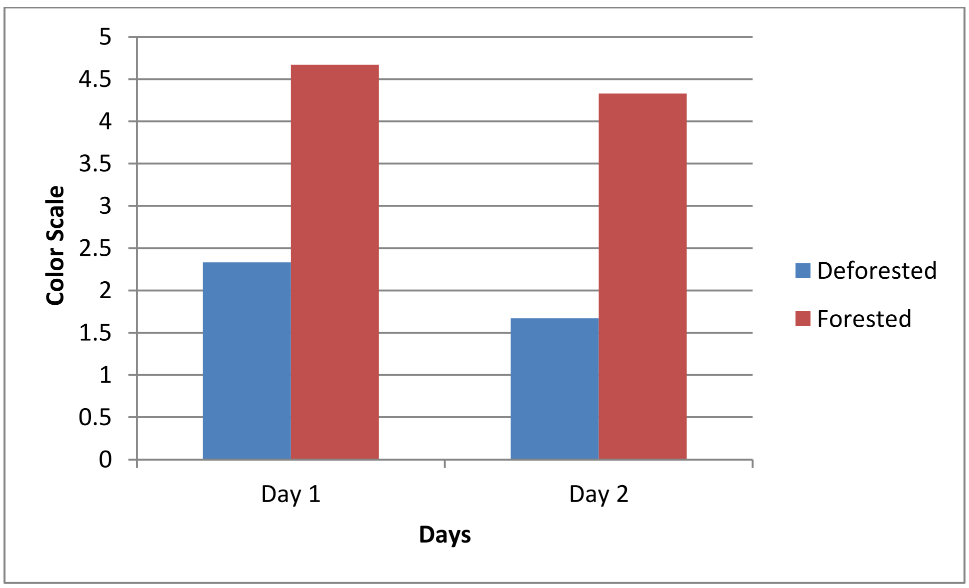
[The impact of deforestation on the average number of yeast, mold, and total fungi on Day 1]



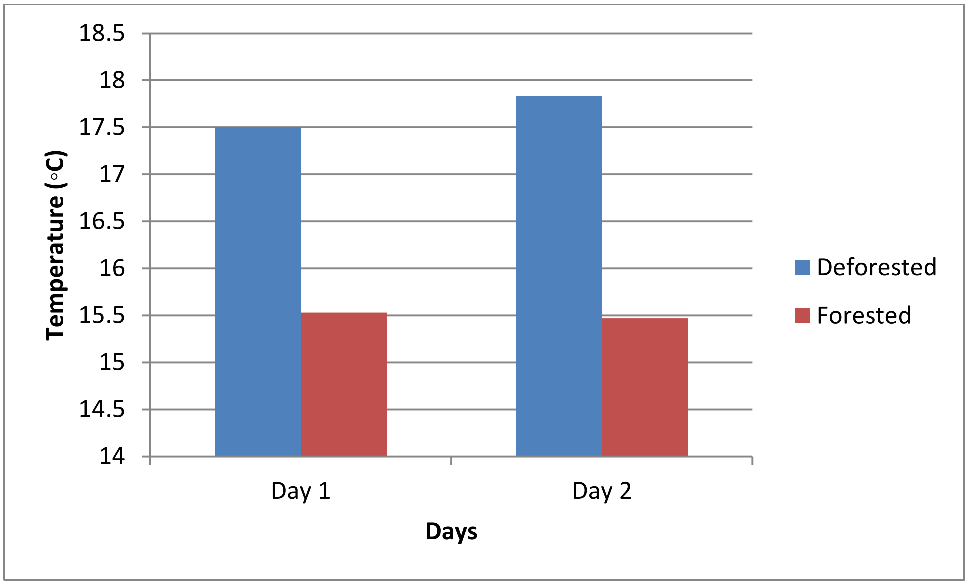
[The impact of deforestation on the average number of yeast, mold, and total fungi on Day 2]



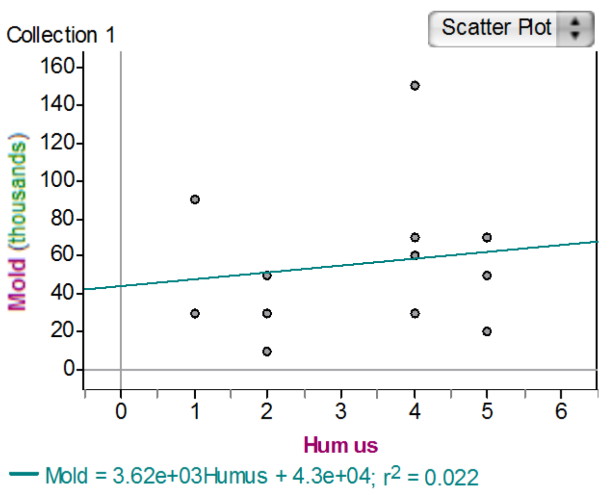
[The impact of deforestation on the average humus level of soil]



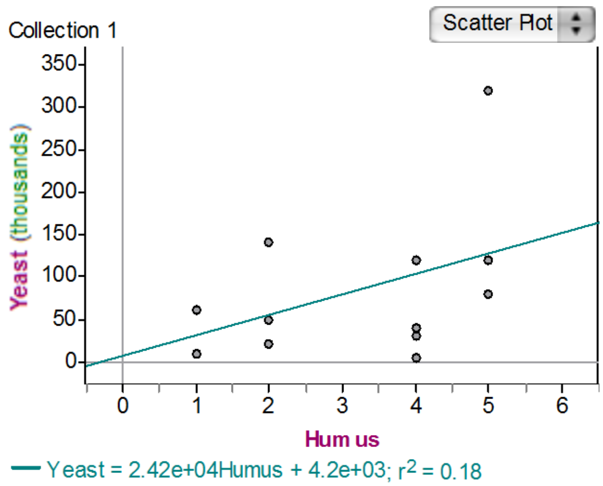
[The impact of deforestation on the average temperature of soil]



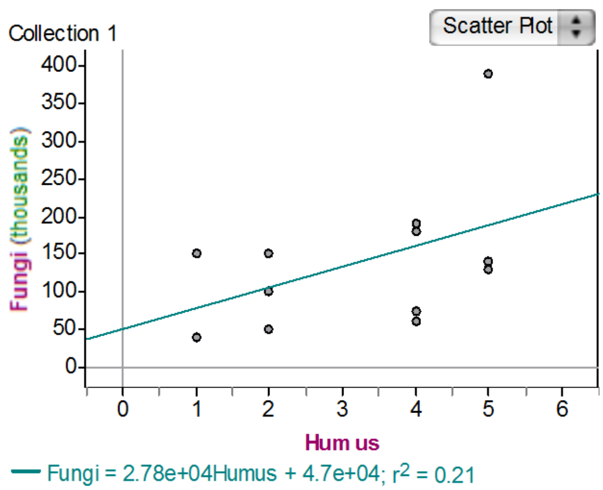
[The relationship between the number of mold and the humus level]



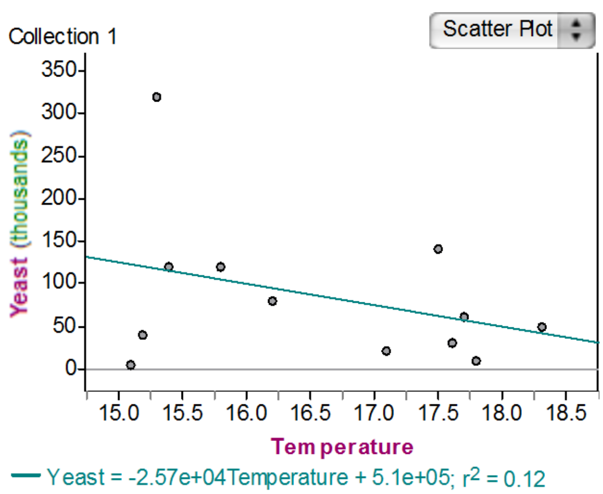
[The relationship between the number of yeast and the humus level]



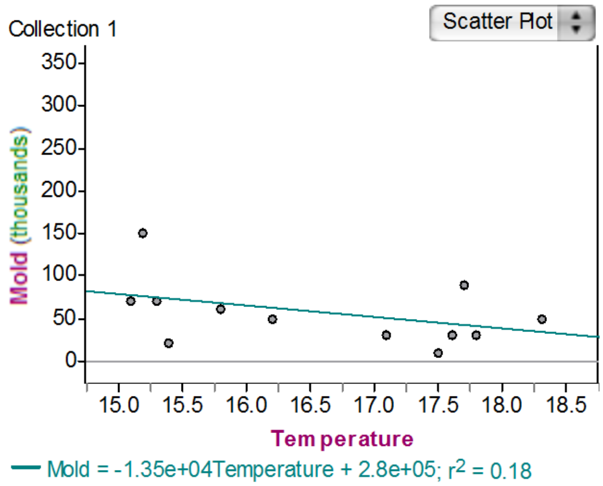
[The relationship between the number of total fungi and the humus level]



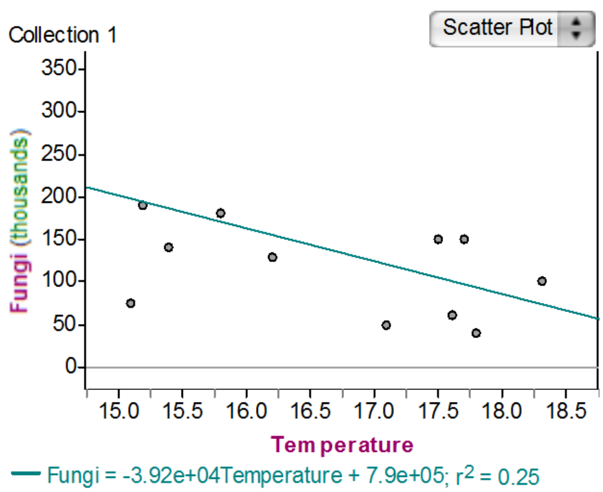
[The relationship between the number of yeast and the temperature]



[The relationship between the number of mold and the temperature]



[The relationship between the number of total fungi and the temperature]



V. Conclusion

Our hypothesis states that deforestation causes soil fungi to decrease in density. After performing the necessary tests, we found out that our hypothesis is correct. The density of fungi decreases because trees and mycorrhizal fungi have a symbiotic relationship; without one another, neither could live. The hyphae created formed by fungi boost the root system of trees, helping them get water and nutrients from the soil. Also, fungi help the plants receive nitrogen, which is a critical element that helps photosynthesis in the plants' chloroplasts, through the decomposition of legumes. In return, the trees give carbon and sugar to the fungi. When trees are removed from this picture, nothing is providing food for the fungi, and it also affects the carbon and nitrogen cycle in the ecosystem and determines the prosperity of the entire ecosystem. This information is relevant and present in the data.

The first experiment was measuring the temperature of the soil in deforested and forested areas. Fungi flourish in lower temperature with moisture. The [Average Temperature Graph] illustrates the difference in temperature between deforested and forested areas; deforested soil has higher temperatures than forested soil. The average temperature in deforested areas on day 1 was 17.5 ◦C while the average temperature in forested areas on day 1 was only 15.5 ◦C. On day 2, the average of deforested areas was 17.8◦C while for the forested area, it was 15.5◦C. When areas are deforested, the temperature rises, causing the soil to become too warm and dry for mycorrhizal fungi to survive.

The second test we performed was the humus test which determines how much humus, or organic matter, is in the soil. If there is more organic matter in the soil, it means there are more fungi, because fungi produce organic matter. In the test, the water extracted from the soil is labeled by the color. The scale is from 1 to 5, 1 being the lightest color with the least amount of humus and 5 being the darkest color with the most amount of humus. On day 1, the average humus level for deforested areas was 2.33. The average humus level for forested areas was 4.67. On day 2, the average humus level for deforested areas was 1.67, and the average humus level for forested areas was 4.33. There was a significant difference between the humus level in deforested soil and the humus level in forested soil. There are fewer fungi in deforested areas because there is less organic matter, or less humus.

The last experiment was serial dilution, which counts the number of yeast, mold, and total fungi in the soil sample. On day 1, the average number of total fungi was 50,000 yeast and mold in deforested areas. The average number of total fungi in forested areas was 198,000 yeast and mold. On day 2, the average number of total fungi in deforested areas was 133,333 yeast and mold, while the forested areas had 170,000 yeast and mold. In total, deforested areas have fewer fungi in the soil than forested areas.

Overall, the deforested areas had worse conditions of soil than the deforested areas. The temperature of deforested areas was much higher than the forested areas. The humus levels in deforested areas were very low compared to the forested areas. Consequently, the amount of total fungi in the deforested soil is low, while the fungi flourish in the forested soil. Deforestation affects the temperature and humus level of the soil, causing the density of fungi to decrease. So in effect, deforestation decreases the density of soil and makes the soil less prosperous for other plants and organisms that support the ecosystem.

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