Soil Ecology Project

Population Density of Fungi

In

Forested and Reforested Areas

By Lexi Wolfe, Casey Wong, and Julia Potter

Soil Ecology Project- May 2012- Biology 9 Honors – Mr. Brock

Background

Microbes in the soil are extremely important. They assist plants in the uptake of water, phosphorus, nitrogen, and many other key elements and nutrients plants use to grow, and without microbes, the lack of processed nutrients would eventually cause all the living things in an ecosystem to die (Jenkins, 2005). This is because plants provide the source of energy, as well as oxygen for all of the consumers in an ecosystem, and without the nutrients that the microbes provide the plants would not survive to provide these things. For this reason, populations of microbes are the basis to all healthy ecosystems, and among them, one of the most helpful are the soil fungi.

One group of fungi, in particular, is especially critical to the environment. Known as mutualists, these fungi have an interdependent symbiotic relationship with plants in which the fungi provide benefits to the plants and vice versa. Among these many mutual benefits, fungi decompose organic material into forms of carbon that can then be passed on to the plant for use in photosynthesis, and in return, the plant provides the fungi with a source of carbohydrates for energy. Because of their mass of mycelium, fungi can also provide plants with protection from pathogens and pests, and in return, the plant roots help the mycelium to be able to spread out and create pathways in the soil to increase their exposure to potential nutrients (Ingham, 2011).

The most significant of the mutualist fungi are the mycorrhizae. These organisms grow inside or outside of plant roots and are known for aiding plants in the uptake of three particularly critical compounds: phosphorus, nitrogen, and water. (Jenkins, 2005). The way it works is that when mycorrhizal fungi colonize the roots of plants, the hyphae of the fungus increases the surface area of the roots of the plants. This increase in surface area then allows plants to take in more water, and it provides the plant with greater access to the nitrogen compounds available from decomposition. Nitrogen is essential to plants because nitrogen is needed for the cell to make amino acids. Amino acids are then used to manufacture enzymes which are responsible for starting and stopping chemical reactions. Without enzymes, the cells of a plant would not be able to perform chemical reactions, and without being able to perform chemical reactions, the plant cannot live. In fact, 90 % of

healthy plants are associated with these types of fungi, and for this reason, it is very important for both mycorrhizal fungi as well as plants to be present in order to have a healthy ecosystem. (Ingham, 2011)

In order for soil fungi to thrive, though, there must be enough "food" or organic material for the fungi to be able to get their nutrients from, and most of this organic matter comes from things such as dead trees or old plant roots. Deforestation, therefore, can be very problematic for fungi. If trees from a forest are burned down or removed from a forest, fungi do not have the same access to the normal organic matter to feed on that the mycorrhizal relationship provides and therefore are unable to thrive (Jenkins, 2005). In addition, the plants are not be able to provide the fungi with the carbohydrates which the fungi use for energy. Hence when the trees in an area are eliminated, it removes the vital plant sources on which mycorrhizal fungi depend on in order to live.

Unfortunately, deforestation is a major problem in the world today. According to *Save America's Forests* (2011), over the course of the past 200 years, 95% of the original forests in the United States have been removed by logging, and what logging hasn't damaged, forest fires regularly destroy, particularly in the western United States. According to data from the National Interagency Fire Center displayed through the National Climatic Data Center (2011), there were 73, 484 forest fires in 2011 alone in which 8,706,852 acres of trees were destroyed-meaning that an area greater than the size of the state of Maryland was destroyed in just 2011 alone.

Many people are not aware of the negative impact that humans have on an ecosystem when the forests there are logged or burned down. Eliminating trees eliminates the major food source which soil fungi need to survive (Jenkins, 2005), and if the soil fungi cannot survive, then the whole ecosystem is put at risk. As seen earlier, soil fungi provide nutrients to plants that are essential to their survival. But plants essentially provide a food source for the rest of the ecosystem because primary consumers eat plants and then secondary and third level consumers either eat plants or other consumers who got their energy from plants. These consumers also

get the oxygen which they need to live from plants. Therefore without soil fungi, an ecosystem simply cannot function.

If deforestation happens, then, it is important for people to attempt to restore successfully what they can of a habitat so that the ecosystem can go on functioning properly. In 2011, at Roland Park Country School in Baltimore, Maryland, a fire wiped out a significant area of trees that served as a road buffer at the front of our school. Tests were done that spring to see the effects of it on the population of soil fungi living there, and as expected, the tests showed that the population density of these soil microbes dramatically decreased (Ahn, Frankel, and Olsson, 2011). However, the school took action and replanted the same area where the fire occurred over the course of the summer, and the purpose of this study is to see whether or not these newly planted plants helped to increase the population density of soil fungi where the fire occurred. We predict that the soil fungi population density will have increased from what it was after the fire occurred since the food source for the soil fungi has now been replenished.

Experiment

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

II. Hypothesis: Reforestation will cause the population density of soil fungi to increase.

III. Procedure

A. Independent Variable: The independent variable is the reforestation of a previously deforested area.

B. Dependent Variable: The dependent variable is the population density of the soil fungi.

C. Negative Control: The negative control is the population density of soil fungi from a previously deforested area (Ahn, Frankel, and Olsson, 2011).

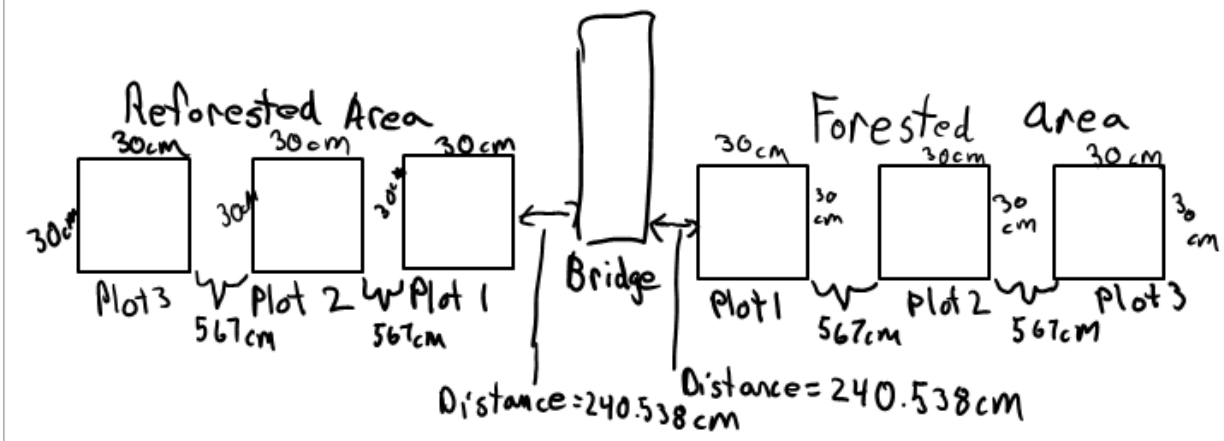
D. Controlled Variables:

- Same size sample of soil taken
- Same time and day soil samples are taken
- Temperature
- Type of pipette used
- Concentration of nutrient agar

- Same temperature of water
- Same source of water (Sterile)
- Time allowed to grow in nutrient agar plate
- Size of culture tubes
- Amount of soil added to water
- Amount of each dilution added to nutrient agar plates
- Diluted to the same level each time
- Amount of water used to dilute the soil

E. Step by Step Procedure

1. Go outside of Roland Park Country School in Baltimore Maryland and walk to the bridge that connects it to the Gilman school but stay on the RPCS side. Stand in front of the bridge facing the stairs
2. Turn 90 degrees to the right and walk 28.6 meters from the bridge.
3. Plot your first plot using four flags labeled "Plot 1-F". Place the four flags in a square formation with 30 cm on each side of the square (see diagram). The coordinates of the center of "Plot 1-F" are 39^0 21.471 N, 076^0 38.117 W.
4. Continue walking in the same direction as step 2 in order to mark Plot 2. Walk 567 cm from Plot 1-F.
5. Begin to plot Plot 2. Use four new flags labeled "Plot 2-F" and place them in a square formation with 30cm as each of the side lengths for the square. The coordinates for the center of Plot 2-F are 39^0 21.466 N, 076^0 38.116 W. (see diagram).
6. Continue walking in the same direction which as steps 2 and 4. Walk 567 cm away from Plot 2-F.
7. Create Plot 3 using four new flags labeled "Plot 3-F". Place the four flags in a square formation with 30 cm as the side lengths for the square. The coordinates for the center of Plot 3-F are 39^0 21.459 N, 026^0 38.116 W. (see diagram)
8. After plotting Plot 3-F in this area, return to the bridge and face the stairs again. This time, turn 90 degrees to your left and walk in the direction that you are facing until you are 28.6 meters from the bridge.
9. Plot your first plot in this area using four flags that are labeled "Plot 1-R". Place the four flags in a square formation so that there is 30 cm on each side of the square. The coordinates for the center of Plot 1-R in this area are 39^0 21.503 N, 076^0 38.112 W. (see diagram)
10. Continue walking in the same direction as you walked in step 8. Walk 567 cm away from Plot 1-R.
11. Using four new flags labeled "Plot 2-R" plot a square area so that the side length of each side of the square is 30 cm. The coordinates for the center of Plot 2-R are 39^0 21. 571 N, $076^0$38 W (see diagram).
12. Continue walking in the same direction you walked in steps 8 and 10 and walk 567 away from Plot 2-R.
13. Using four new flags labeled "Plot 3-R", plot the flags to make a square area so that all four of the side lengths of the square are 30 cm. The coordinates of the center of Plot 3-R are 39^0 21.515 N, 076^0 38.120 W (see diagram).



14. Label 18 separate Ziploc bags each with a different label. Each separate bag should get its own individual label. The 18 labels are as follows: "Reforested 1, Plot 1", "Reforested 2, Plot 1", "Reforested 3, Plot1", "Reforested 1, Plot 2", "Reforested 2, Plot 2", "Reforested 3, Plot 2" "Reforested 1, Plot 3", "Reforested 2, Plot 3", "Reforested 3, Plot 3", "Forested 1, Plot 1", "Forested 2, Plot 1", "Forested 3, Plot 1", "Forested 1, Plot 2", "Forested 2, Plot 2", "Forested 3, Plot 2" "Forested 1, Plot 3", "Forested 2, Plot 3", "Forested 3, Plot 3"
15. Steps 16-17 must be done at the same time and day.
16. Take out a cylinder of soil that is 15cm deep by 2cm wide from the plot 1 from the forested area (see diagram) and place in its correspondingly labeled plastic bag.
17. Repeat step 16 for all 17 other soil samples, taking 3 soil samples from each of the six plots that you created. On the Ziploc bag labels, these three samples are represented by the number (1, 2, or 3) that follows the "Forested" or "Reforested" label. Make sure to place the correct soil sample in its corresponding Ziploc bag.
18. Steps 19-34 must be done in the same time and day per trial. Therefore, six bags will be tested at the same day in time. On the first test day, the first samples taken from each plot will be tested. On the second test day, the second samples taken from each plot will be tested. On the third day, the third samples taken from each plot will be tested.
19. Label 18 different 15 ml culture tubes with the soil sample that you are testing as well as the dilution number. The respective labels are as follows:
    "F1 P1 10^{0}", "F1 Pl 10^{-1}", "F1 P1 10^{-2}", "F1 P2 10^{0}", "F1 P2 10^{-1}", F1 P2 10^{-2}", "F1 P3 10^{0}", "F1 P3 10^{-1}", "F1 P3 10^{-2}", "R1 P1 10^{0}", "R1 Pl 10^{-1}", "R1 P1 10^{-2}", "R1 P2 10^{0}", "R1 P2 10^{-1}", R1 P2 10^{-2}", "R1 P3 10^{0}", "R1 P3 10^{-1}", "R1 P3 10^{-2}"
    The F represents the soil from the forested area and the R the soil from the reforested area. P represents the word "Plot". On the bag which corresponds to its respective tubes, the words "forested" and "reforested" as well as "plot" are written out.
    These tubes are labeled for trial 1.
20. Use a clean, new transfer pipette to add 10 ml of sterile water to the culture tube1 labeled "F1 P1 10^{0}."
21. Use the same pipette to add 9 ml to a second labeled "F1 P1 10^{-1}."
22. Repeat step 21 one more time only add water to the tube labeled "F1 P1 10^{-2}".

23. Place 1 cc of your soil sample from the "forested 1, plot 1" bag into the "F1 P1 10^{0}"culture tube.
24. Cap the tube and shake vigorously.
25. Using a new clean pipette, remove 1 ml of the soil/water mixture from the "F1 P1 10^{0}" tube and place into the "F1 P1 10^{-1}" tube.
26. Cap and shake vigorously.
27. Using the same pipette in step 25, remove 1 ml of the soil/water mixture from the "F1 P1 10^{-1}" tube and place into the "F1 P1 10^{-2}" tube.
28. Cap and shake vigorously.
29. You should now have a total of three culture tubes.
30. Plate 100 µl samples from the all three culture tubes onto their own separate, labeled 3M Petrifilm™ Yeast and Mold Count Plates. Label the petri plates to their corresponding culture tube using the same labels as are on the tubes.
31. Repeat steps 20-30 for the remaining 5 soil samples for trial 1 (Forested 1, plot 2; Forested 1, Plot 3; Reforested 1, Plot 1; Reforested 1, Plot 2; Reforested 1, Plot 3), changing the soil sample you use in step 23 each time. When repeating the steps, use the culture tubes that are labeled to correspond to their respective soil sample (see step 19). Also use new pipettes when repeating the steps.
32. Repeat steps 18-31 for all the remaining 12 soil samples (trials 2 and 3). Preform trial 2 on test day two. Preform trial 3 on test day 3. Label 18 different culture tubes per trial using the same labels in step 19, only changing the number soil sample (trial). For example, the first culture tube would be labeled "F2 P1 10^{0}" in trial 2. Change the soil sample you use in step 23 each time.
33. Allow all samples to grow for 5 days.
34. Examine each of the plates for individual yeast and mold colonies and choose the plate with the lowest dilution value to make your estimates of the number of yeast and mold in the original 1 cc soil sample using the following formula:

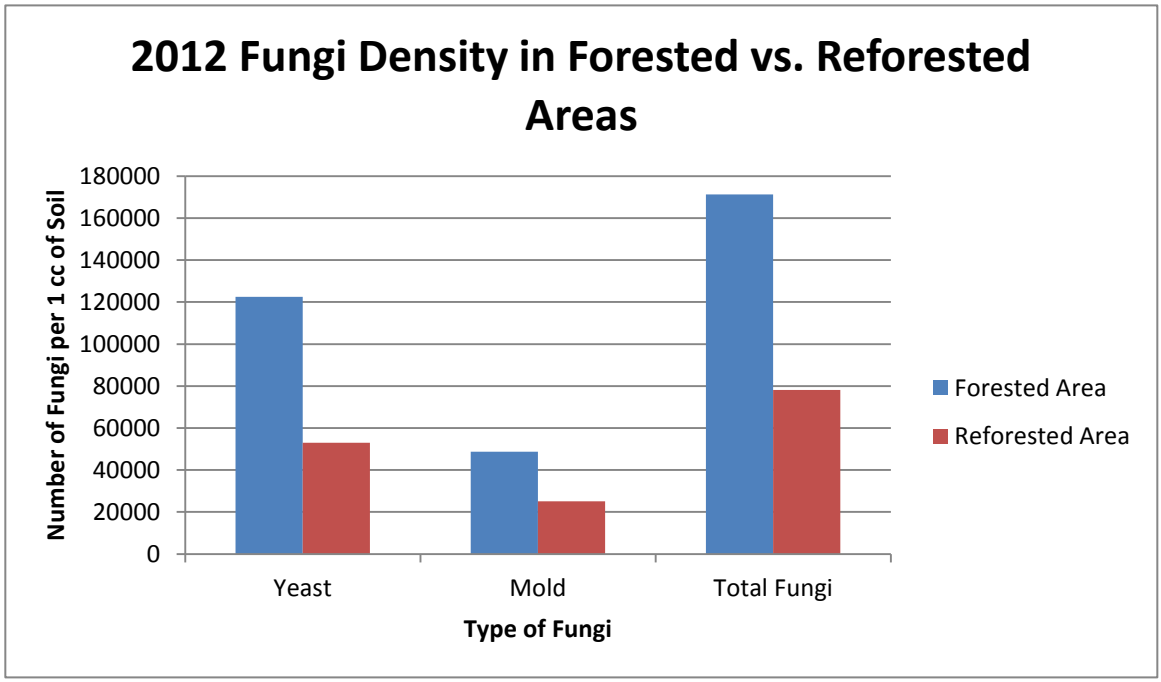
Microbes in 1 cc of soil = # Colonies on sheet x 10^{2} x $10^{|\text{dilution \# at which these colonies were found}|}$

Data and Analysis

2012 Population Density of Fungi Found in RPCS's Roland Avenue Landscape Buffer

Location	Trials	Number of Yeast (per 1cc of soil)	Number of Mold (per 1cc of soil)	Total number of Fungi (per 1cc of soil)
Forested Area Plot 1	1	51,000	6,000	57,000
	2	10,000	3,000	13,000
	3	320,000	90,000	410,000
Forested Area Plot 2	1	320,000	70,000	390,000
	2	120,000	50,000	170,000
	3	41,000	10,000	51,000
Forested Area Plot 3	1	100,000	40,000	140,000
	2	80,000	120,000	200,000
	3	60,000	50,000	110,000
Forested Area Averages		122444	48778	171222
Reforested Area Plot 1	1	40,000	10,000	50,000
	2	14,000	4,000	18,000
	3	50,000	20,000	70,000
Reforested Area Plot 2	1	8,000	10,000	18,000
	2	44, 000	40,000	84,000
	3	170,000	70,000	240,000
Reforested Area Plot 3	1	70,000	20,000	90,000
	2	21,000	2,000	23,000
	3	60,000	50,000	110,000
Reforested Area Averages		53000	25111	78111

Figure 1:



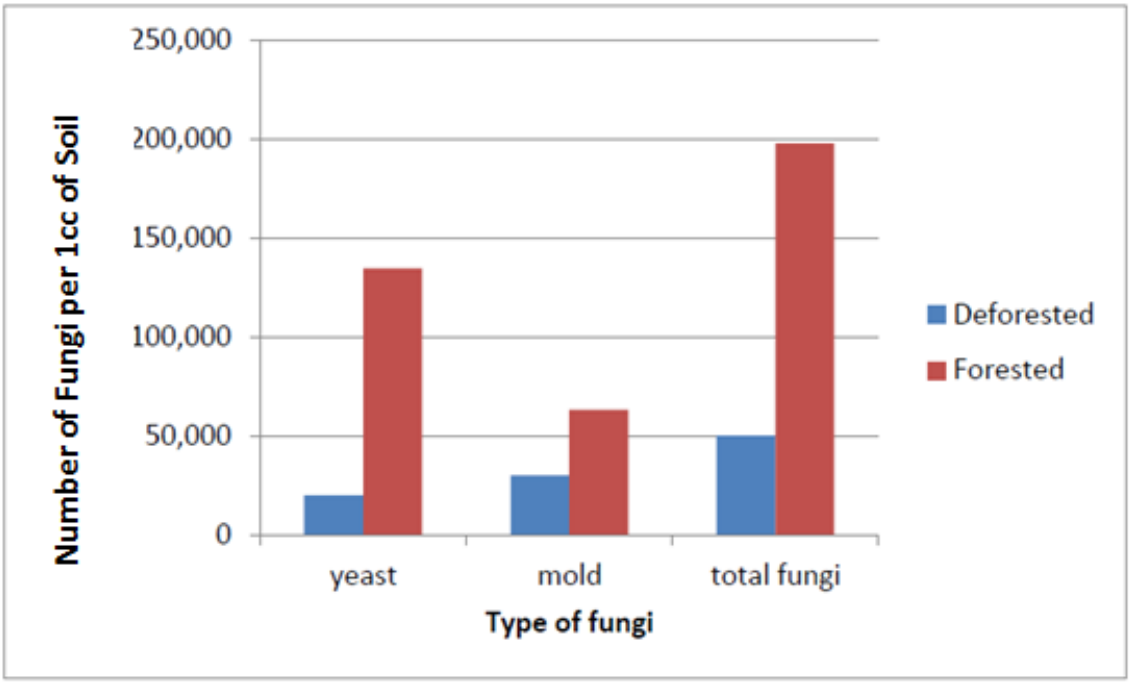
2011 Average Impact of Deforestation on Soil[1]

Deforested/ Forested	Day	Average number of yeast (per 1cc)	Average number of mold (per 1cc)	Average number of total fungi (per 1cc)
Deforested	Day 1	20,000	30,000	50,000
	Day 2	83,333	50,000	133,333
Forested	Day 1	134,667	63,333	198,000
	Day 2	93,333	76,667	170,000

[1] Ahn, J., Frankel, E., and Olsson, M. (2011). How Does Deforestation Affect Soil Fungal Populations?. Baltimore: RPCS Publishers. http://www.rpcs.org/LittleThings/Reports%20Archive/reports.htm

Figure 2:

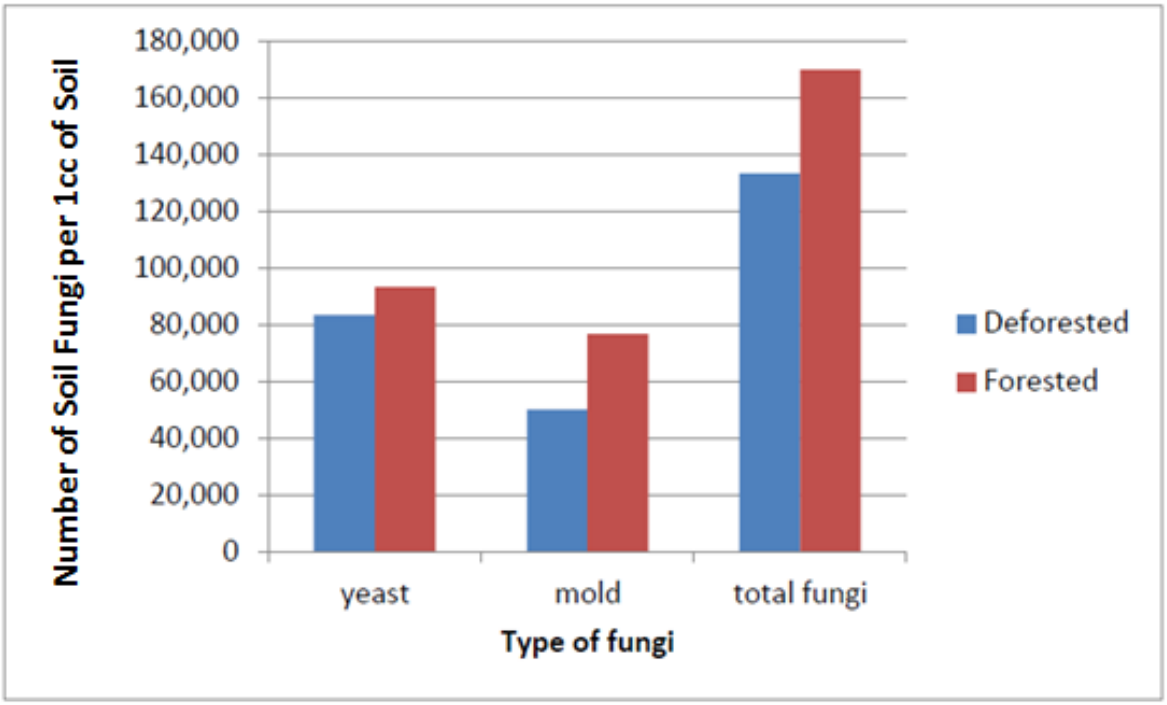
The 2011 Impact of Deforestation on the Average Number of Yeast, Mold, and Total Fungi on Day 1[2]



[2] Ahn, J., Frankel, E., and Olsson, M. (2011). How Does Deforestation Affect Soil Fungal Populations?. Baltimore: RPCS Publishers. http://www.rpcs.org/LittleThings/Reports%20Archive/reports.htm

Figure 3:

The 2011 Impact of Deforestation on the Average Number of Yeast, Mold, and Total Fungi on Day 2[3]



[3] Ahn, J., Frankel, E., and Olsson, M. (2011). How Does Deforestation Affect Soil Fungal Populations?. Baltimore: RPCS Publishers. http://www.rpcs.org/LittleThings/Reports%20Archive/reports.htm

Figure 4:

Comparison of 2011 vs. 2012 Fungi Density in a Forested Area

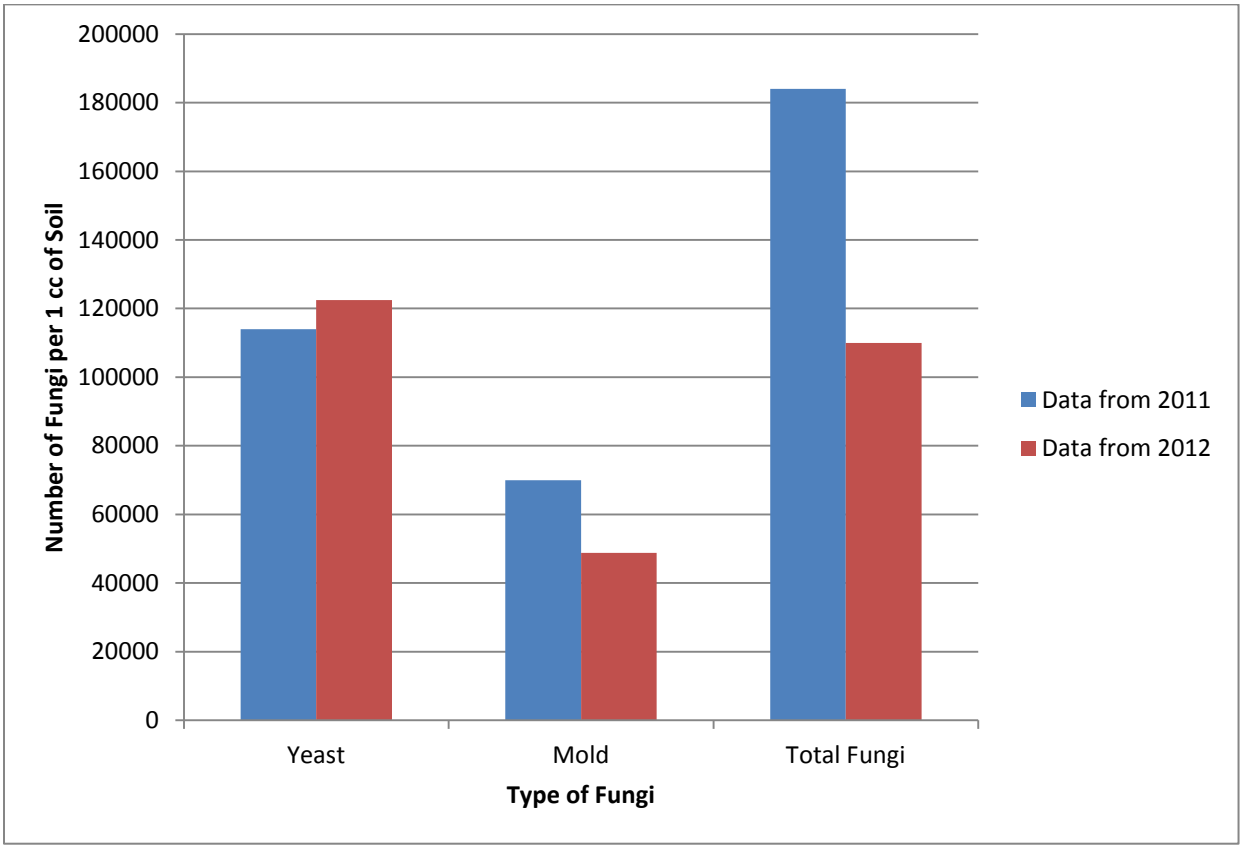
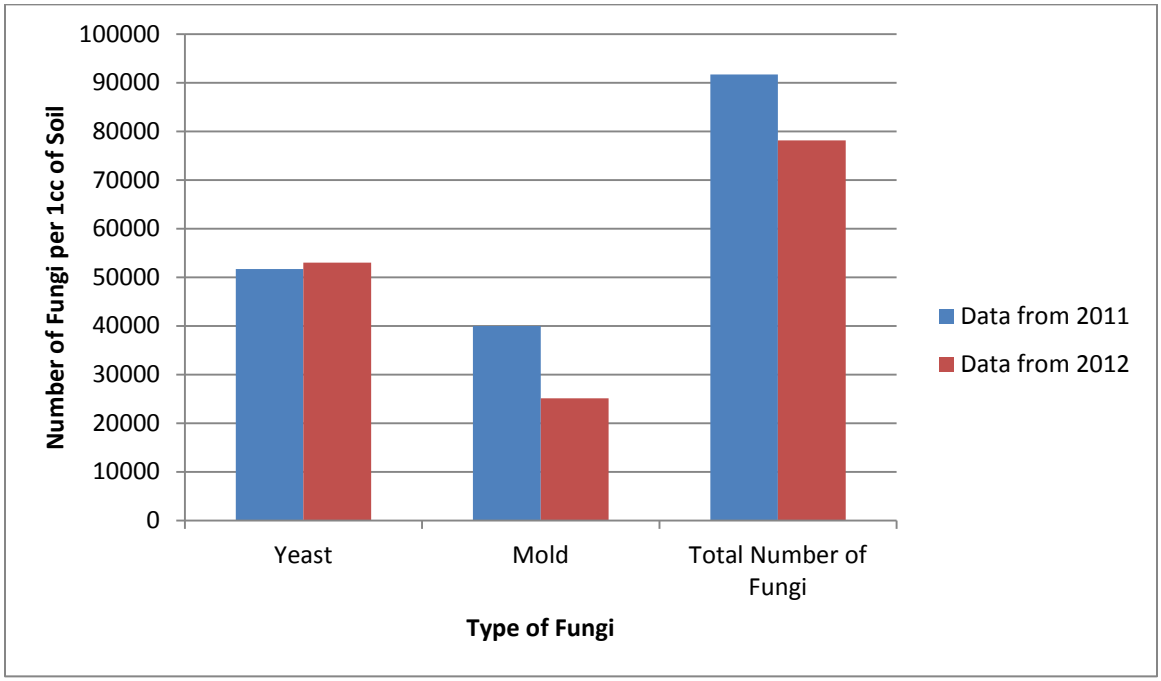


Figure 5:

Comparison of 2011 vs. 2012 Fungi Density in a Reforested Area



Conclusion

Our hypothesis, "reforestation will cause the population density of soil fungi to increase", was proven wrong by this experiment. In fact, the fungi population density decreased in a reforested area from that of the fungi population density in the spring of 2011 when the area was not yet reforested. This proves that the reforestation did not cause the population density to increase. The data for the total number of fungi in figure 5 shows that there was an average of 91,667 total fungi in 1cc of soil in 2011 before the reforestation while there was only an average of 78,111 total fungi in 1cc of soil in 2012 after the reforestation. However, by looking at our data in figures 4 and 5, we can see that the fungi population density is somewhat rebounding in the reforested area. We can see this because in figures 4 and 5, the total number of fungi in the area that has remained forested also decreased from the data taken last spring, and in fact, the population decreased more than that of the reforested area. The decrease in the total amount of fungi in the forested area which was 74,000

was significantly larger than the decrease of total number of fungi in the reforested area which was 13,556 fungi. Because the drop was greater in the forested area, it proves that the fungi are somewhat rebounding in the reforested area.

Through this experiment, we were also able to draw other interesting conclusions. Our data showed that clearly in both the forested and reforested environments this year, there were more yeasts than molds. In the forested area that we tested, we found an average of 122,444 yeasts per 1cc of soil, while there were only 48,778 molds in 1cc of soil. Similarly in our reforested area, we found that there were an average of 53,000 yeasts per 1cc of soil and only 25,111 molds per 1cc of soil. Because there were many more yeasts than molds, this data shows that there is obviously a stressor in the environment that is causing the more fungi to be in their yeast form than their mold form. When looking at the data in figures 2 and 3 that was taken in the spring of 2011, we can see that the yeast and mold levels were fluctuating between the two days in which the data was taken in both the forested and deforested areas. Because the levels of yeast and mold were not stable between these two days, we can see that a stressor was affecting the fungi last spring, as well. Furthermore, we can see that there is an ongoing stressor in the environment because it has affected the fungi populations in both the spring of 2011 and the spring of 2012. However, by looking at our data in figures 4 and 5, we are able to see that the fungi in the reforested environment seem to be somewhat handling the stressor better than the fungi population in the forested environment. In both the forested and reforested environments, the number of yeasts in 1cc of soil increased from last year. In the forested area, the number of yeasts in 1cc of soil increased by 8,444. In the reforested area, the number of yeasts in 1cc of soil increased by 1,333. The fact that the increase in yeasts from last year to this year was larger in the forested environment than the reforested environment shows us that the fungi in the reforested environment are handling the stressor better than the fungi in the forested environment.

Using the data that we gathered this year, multiple experiments can be done in the future to better our understanding of the fungal population in both the forested and reforested areas. One thing that can be done is to duplicate the experiment which we performed this year in the spring of 2013 to test to see if the fungi population density would further or completely be restored in the reforested area. The data from the new experiment could then be compiled with the data which we gathered this year as well as the data that was taken in the spring of 2011. All of this data could be compared in order to draw new and interesting conclusions. Tests can also be performed to identify the stressor in the environment that is causing more fungi to be in their yeast form than their mold form. Considering that the location of the forested and reforested environments is right next to Roland Avenue, a high amount of traffic could be causing the fungi to be stressed. A camera could be set up to count the number of cars that drive along Roland Avenue daily to see if the traffic on this road is more than average. A severe drought which has been affecting the state of Maryland for the past year could also be a possible stressor since our tests were performed in this state. In order to test this, an experiment could be done in which the water amounts given to the areas of soil are varied to see if the fungi which received less water are more commonly found in their yeast form than the mold form. It would also be interesting to research whether the diverse array of plant life located in the reforested area is causing the fungi to be able to handle the stressor better than the fungi in the forested area with a less diverse array of plant life. In order to test this, we could add new species of plants to the forested area which has a less diverse array of plant life. The soil in this area could then be tested to see if the yeast levels decrease. Although our hypothesis, "reforestation will cause the population density of soil fungi to increase", was wrong, our experiment helped us to draw very interesting conclusions and could be helpful to use in possible future experiments.

References

Ahn, J., Frankel, E., and Olsson, M. (2011). How Does Deforestation Affect Soil Fungal Populations?. Baltimore: RPCS Publishers. http://www.rpcs.org/LittleThings/Reports%20Archive/reports.htm

Brundrett, M. (2008). Importance of Mycorrhizas. Mycorrhizal Associations: The Web Resource. http://mycorrhizas.info/index.html

Gale. (2011) Microbes (microorganisms). Gale Science. http://ic.galegroup.com/ic/scic/ReferenceDetailsPage/ReferenceDetailsWindow?displayGroupName=Reference&disableHighlighting=false&prodId=SCIC&action=e&windowstate=normal&catId=&documentId=GALE%7CCV2644150859&mode=view&userGroupName=balt23720&jsid=ab8e5314e495ed475a7e6263e677c028

Ingham, E. (2011) Soil Biology. Natural Resources Conservation Service: http://soils.usda.gov/sqi/concepts/soil_biology/fungi.html

Jenkins, A. (2005) Soil Fungi. New South Wales: Department of Primary Industries. http://www.dpi.nsw.gov.au/__data/assets/pdf_file/0020/41645/Soil_fungi.pdf

(NCDACS)North Caroline Department of Agriculture and Consumers Services (2012) Plant Nutrition. North Carolina. http://www.ncagr.gov/cyber/kidswrld/plant/nutrient.htm

NOAA National Climatic Data Center. . (2011). State of Climate: Wildfires Annual 2011. National Climatic Data Center. http://www.ncdc.noaa.gov/sotc/fire/2011/13
(SAF) Save America's Forests (2011). America's Beautiful Forests. Washington D.C.: Save the World's Forests. http://www.saveamericasforests.org/crisis.htm

Sommer, C. V. (2012). Microbiology. The New Book of Knowledge. Grolier Online. http://nbk.grolier.com/ncpage?tn=/encyc/article.html&id=a2019130-h&type=0ta