RPCS

Little Things Project

Does deforestation change the population density of fungi in the soil?

By: Lily Mitchell, Blaire Miran and Lilah Noyes

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Background

Fungi microbes have multiple important roles in the environment including recycling chemical elements from dead organic material back into the soil. The plants get energy from the organic material acquired from the nutrients (Soil Health, 2004). Mycorrhizae, one helpful type of fungi, need nutrients and colonize plant roots by physically binding to a host plants' roots. It also creates stable aggregates, which helps plants absorb more water and retain nutrients. The relationship between plant roots and mycorrhizae is symbiotic because the fungi can receive carbon, water, and nutrients from the host plant and the fungi helps to solubilize phosphorus which brings the different nutrients to the plant. Although the fungi receive these necessities from the plants, the most important thing it receives from the plants is sugar.

The plants get theses sugars when the fungi brings them phosphorus, and in return the plant gives the fungi sugars it had created through photosynthesis so the fungi can transform them into energy (Kendrick B 2002). Also, fungi gives the plants magnesium, iron, and other elements to help them with the process of photosynthesis (The compost gardener, 2013). When there are fewer fungi, there will be fewer producers, giving less energy to the consumers, which make an unhealthy ecosystem.

There are two major groups of mycorrhizae fungi that work alongside the fungi, ectomycorrhizae and endomycoorhizae. Ectomycorrhizae grows on the top layers of the roots and commonly is associated with tress; where endomycoorhizae grows within the root cells and is usually associated with grass, crops, and shrubs (Ingham, n.d.). If an area is deforested, the fungi can no longer be supported because of the lack of plants (their food source), and the fungi microbe population will decrease. Forested areas offer an environment that sustains the fungi

population by providing it with sugar and carbon from the plant roots allowing the fungi to survive and reproduce.

The relationship between plant roots and soil structure is important so that fungi live in an environment beneficial to their growth. Forestation helps to improve soil structure by protecting the soil from harsh rains or extreme temperatures because the weather does not have direct contact with the soil. Plant roots hold soil particles together and create pockets in the soil. Inside these pockets, the crops release the organic molecules and produce fungi networks (Penn State Extension, 2013). Fungi thrive and reproduce in the pockets between the soil particles, because it is the perfect environment for them. When these roots are taken away by deforestation, it not only takes away the fungi's main food source, but also the soil structure necessary for microorganism to live. Without the roots there to establish this structure and home for fungi, the fungi cannot survive, and thus their role in the soil is not fulfilled, affecting all of the surrounding plants.

The human impact of deforestation plays a major role in the health of soil and the microorganisms within, specifically fungi. Deforestation happens for many reasons, but occurs most often because of construction or farming. In order to make room for buildings in the industrialized world, trees are cut down, which adds CO_2 to the atmosphere in two ways. Since plants take in CO_2 and release oxygen in photosynthesis, a decrease in plants through deforestation leads to an increase in carbon dioxide and decrease in O_2 left in the atmosphere. In addition, the building that has replaced the cut down trees adds more CO_2 through the burning of fossil fuels. In order for there to be enough space for farming, trees have to be cut down. This disrupts the natural environment as crops that do not normally grow in that area are replacing the trees that originally grew there. This therefore changes the fungi populations in the soil of that

environment. Although farming adds plants to the land, the plants take away nutrients that are necessary to the fungi and soil. Therefore, deforestation is causing an even greater amount of CO_2 and less O_2 in the atmosphere, which is harmful to every aerobic organism that requires oxygen to perform the necessary four tasks of life (Collins, 2001).

Deforestation which is taking out trees and foliage from an area has many negative consequences on the surrounding environment. When people cut down trees, water then pushes the soil into streams and rivers. This clogs the rivers and streams and deprives them of needed nutrients. Most of the soil that is pushed into streams and rivers is top soil, which has the most nutrients. A decrease in nutrients decreases plant growth, which means growth and reproduction of fungi is also diminished (Collins, 2001).

Within the deforested sites, the removal of trees causes the soil to dry out, and become inhospitable to fungi. This occurs because of the decreasing amount of humus and organic material entering the soil. Humus, which is the top layer of soil, is important because it regulates soil functions and plant growth; the more humus in the soil, the healthier and more productive the soil will be (Lippman, 2003). In a deforested area, the soil is exposed to light for the majority of the day, which means the sun evaporates the water in the soil, and leaves the remaining soil excessively dry, changing the texture to a sandier quality which retains much less water than clay soil (Collins, 2001). Since fungi live in the water channels between soil particles, this means that sandier soils do not support larger populations of fungi in result of poor water retention. The reason the water retention is diminished in the sandy soil is because they have large particles and pores, this causes the retained water to leak out of the large pores after a short amount of time. The clay soil is more effective in retaining water because their particles and pores are very small so the water cannot move through the soil as quickly. (Tuller, 2003) After forest canopies are

removed and deforested, the fungus that surrounds the tree roots will not grow in the drier soil (Lynn, 1999). In conclusion, one would expect that in deforested areas, the fungi population density decreases because of the lack of plants to provide the fungi with the necessary nutrients, sugars, and soil structures to survive.

In this experiment, we will investigate the change in fungi population density between forested and deforested environments. We hypothesize that in a deforested environment there will be a lower population density of fungi in the soil compared to that of a forested environment. To complete this experiment we will extract soil from three different sites for each environment and dilute samples from this soil to count the number of fungi in each location.

Lab Report

I. Problem- Does deforestation change the population density of fungi in the soil?

II. Hypothesis: In a deforested environment there will be a lower population density of fungi in the soil compared to that of a forested environment.

III. Procedures

A. Independent: Soil from a deforested environment

B. Dependent: Population density of fungi in the soil

C. Negative Control: Soil from a forested environment

D. Controlled Variables

- Type of microbe being tested for
- Type of trees in forested area and deforested area
- Day and time of soil extraction
- Weather on day of extraction
- Location of each site for extraction

- Materials used for extraction
- Amount of sterile water in each tube
- Size of the plot of land being tested
- Time when dilution is performed for soil samples
- Amount of soil diluted
- Number of dilutions
- Amount of soil extracted from each site
- Dilution levels plated
- Spacing between plots
- Type of water used for dilution
- Amount of water used in dilution
- Type of petrifilm
- Size of petrifilm
- How much diluted soil/water sample on plate
- Amount of time for fungi to grow

Soil Extraction

1. Travel to the deforested environment at coordinates north 39.35771° and west 76.63514°.
2. Place three 60 cm by 60 cm sites 3 meters away from each other in a line along the deforested area, and mark with flags (see Diagram 1).
3. Before proceeding to steps 4-14, make sure there is time to extract all samples of one trial at the same time on the same day before moving onto another trial

4. For deforested site 1 trial 1, press the 2 cm diameter soil extractor 15 cm into the ground (use hammer to push into ground if difficult)
5. Rotate clockwise while pushing down
6. Place each soil sample in its own sterile plastic bag, labeled by location, site #, and trial # (ex: Deforested site 1 trial 1). See diagram 1 for location and site names.
7. Repeat step 4-6 to extract trial 1 from deforested sites 2 and 3
8. Then travel to the forested environment at coordinates north 39.35674° and west 76.63512°
9. For forested site 1 trial 1, press the 2 cm diameter soil extractor 15 cm into the ground (use hammer to push into ground if difficult)
10. At the 15 cm mark, twist clockwise and lift soil extractor out of ground
11. Place each soil sample in its own sterile plastic bag, labeled by location, site #, and trial # (ex: Forested site 1 trial 1). See diagram 1 for location and site names.
12. Repeat step 9-11 to extract trial 1 from forested sites 2 and 3
13. Repeat steps 2-11 to collect trials 2 and 3 from all sites in the deforested environment and the forested environment in the soil underneath the cyprus trees, be sure to move mulch off of soil before extraction
14. Place each soil sample in its own sterile plastic bag, labeled by location, site #, and trial # (example: forested site 1 trial 1). See diagram 1 for site names and locations.
15. Bring soil samples inside and follow steps 16-31 for dilution and counting of fungi
16. Before beginning soil dilution, note that all samples of one trial must be diluted at the same time on the same day before moving on to diluting another trial of samples.

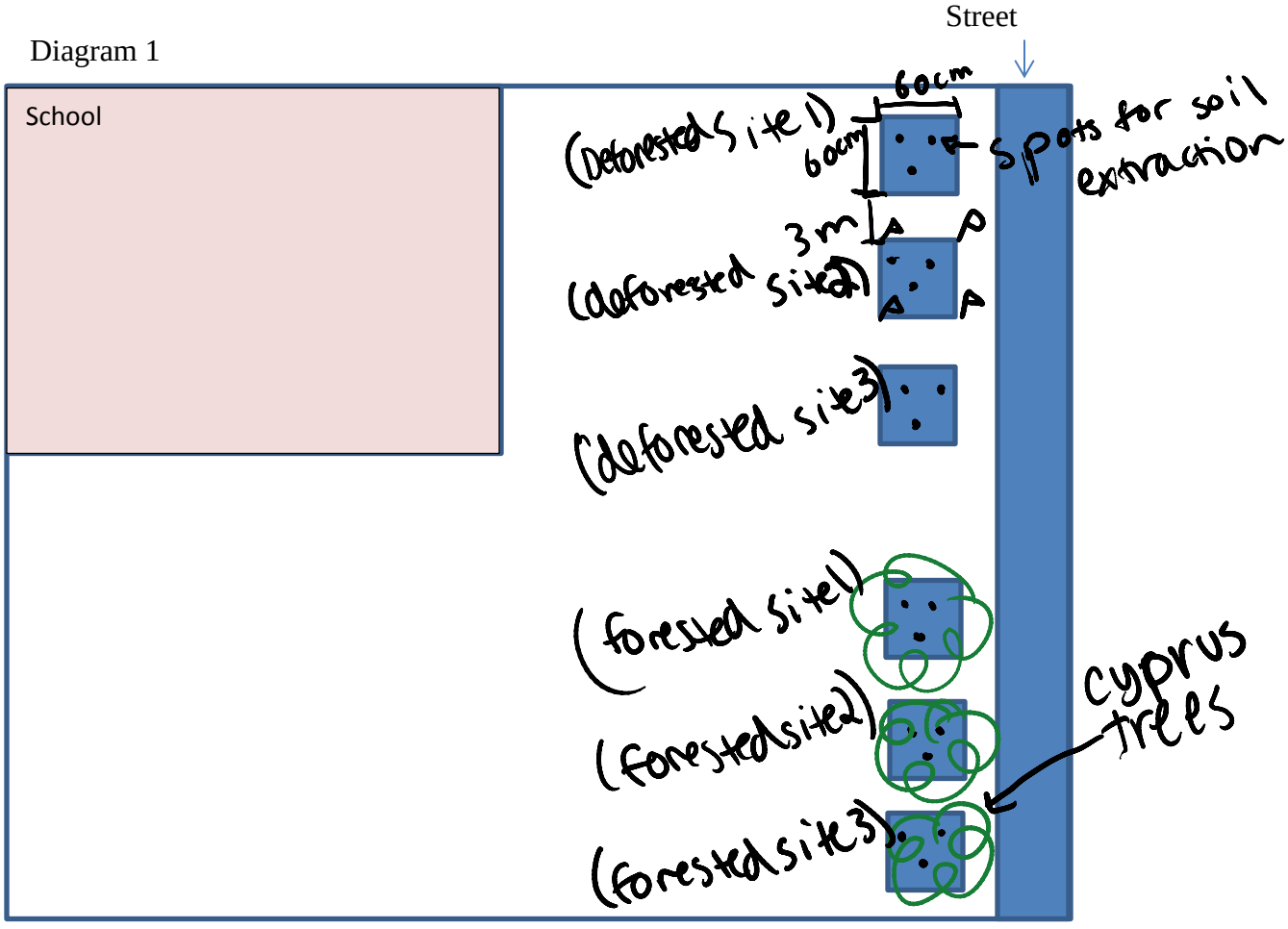
17. Use a clean, new transfer pipette to add 10 ml sterile water to a 15 ml culture tube. Label the tube "10^0 deforested site1 trial 1".
18. Use the same pipette to add 9ml of sterile water to a second 15 ml culture tube. Label the tube "10^{-1} deforested site1 trial 1".
19. Repeat step 18 one more time to one additional 15 ml culture tube, label it "10^{-2} deforested site1 trial 1".
20. Place 1cc of your deforested site 1 trial 1 soil sample into the "10^0" culture tube.
21. Cap the tube and shake vigorously.
22. Using a new clean pipette, remove 1ml of the soil/water mixture from the "10^0" tube and place into the "10^{-1}" tube.
23. Cap and shake vigorously.
24. Using the same pipette in step 22, remove 1ml of the soil/water mixture from the "10^{-1}" tube and place into the "10^{-2}" tube.
25. Cap and shake vigorously.
26. You should now have a total of three culture tubes.
27. Plate 100 µL samples from all three tubes (dilutions 10^0, 10^{-1}, and 10^{-2}) onto their own separate, 3M Petrifilm™ yeast and mold count plate, labeled "deforested site 1 trial 1" and their respective dilution number and press top film gently with spreader.
28. Allow fungi to grow for 96 hours, this period of waiting must be the same for every trial.
29. Examine the plates for sharp edged bluish or yellow-brown colonies (which are yeast) and blurry edged colonies (which are mold). Look at the "10^{-2}" plate first for at least one colony of yeast and at least one colony of mold. But if you cannot find at least

one colony of yeast or mold, look at the "10^{-1}" plate first, then if necessary look at the "10^{0}" plate. Make your estimates of the number of yeast, mold and total fungi in the original 1 cc soil sample using the following formula:

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

30. Record data of how many yeast, mold and total microbes from every dilution and trial in chart
31. Repeat steps 16-30 to perform dilutions and collect data for trials 2 and 3 at all sites for the deforested environment and forested environment

Diagram 1



Data tables

Amount of Yeast, Mold and Total Fungi at Each Site for Trial 1

Sample F=forested D=deforested	Yeast			Mold			Total Fungi
	Dilution	Colonies	Total	Dilution	Colonies	Total	
F_1	10^{-2}	5	50,000	10^{-2}	1	10,000	60,000
F_2	10^{-1}	10	10,000	10^{-2}	3	30,000	40,000
F_3	10^{-2}	4	40,000	10^{-2}	2	20,000	60,000
D_1	10^{-2}	32	320,000	10^{-1}	5	5,000	325,000
D_2	10^{-2}	11	110,000	10^{-2}	8	80,000	190,000
D_3	10^{-2}	15	150,000	10^{-2}	1	10,000	160,000

Amount of Yeast, Mold and Total Fungi at Each Site for Trial 2

Sample F=forested D=deforested	Yeast			Mold			Total Fungi
	Dilution	Colonies	Total	Dilution	Colonies	Total	
F_1	10^{-1}	3	3,000	10^{-1}	2	2,000	3,200
F_2	10^{-2}	2	20,000	10^{-2}	5	50,000	70,000
F_3	10^{-1}	9	9,000	10^{-1}	8	8,000	17,000
D_1	10^{-2}	26	260,000	10^{-2}	4	40,000	300,000
D_2	10^{-2}	12	120,000	10^{-2}	5	50,000	170,000
D_3	10^{-2}	4	40,000	10^{-2}	1	10,000	50,000

Amount of Yeast, Mold and Total Fungi at Each Site for Trial 3

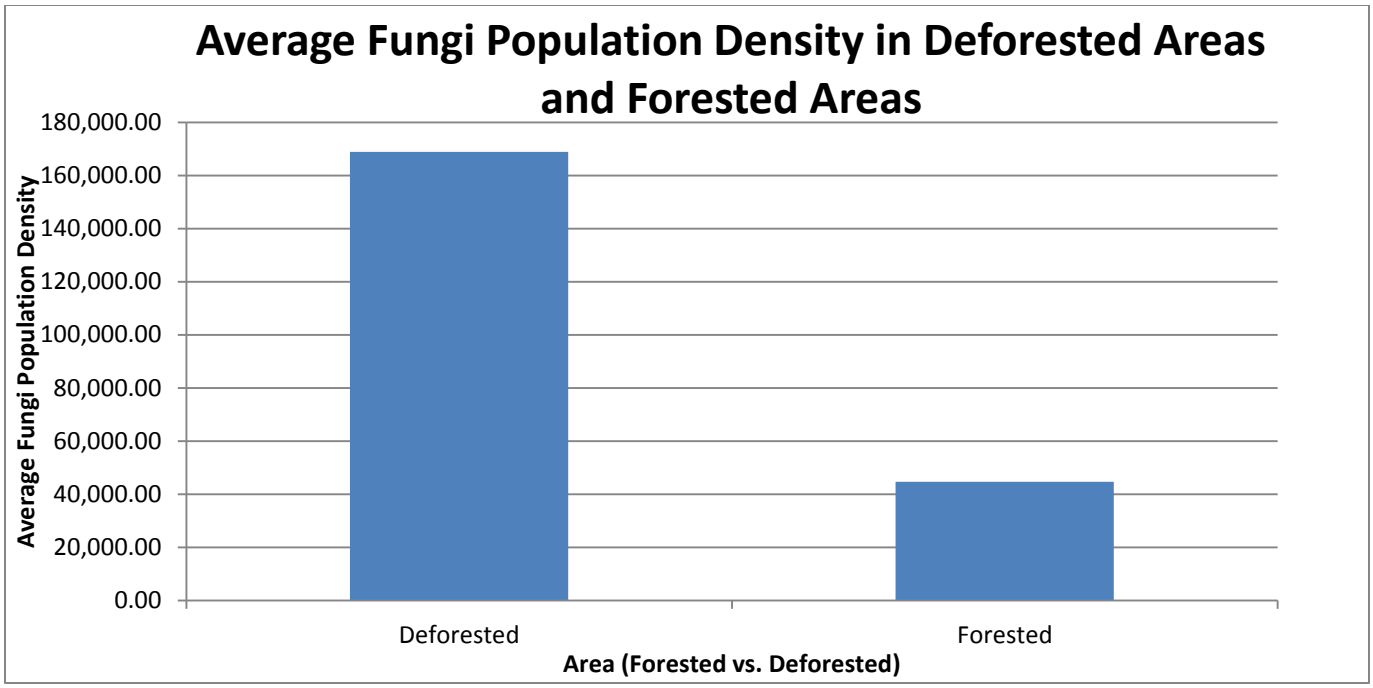
Sample F=forested D=deforested	Yeast			Mold			Total Fungi
	Dilution	Colonies	Total	Dilution	Colonies	Total	
F_1	10^{-2}	5	50,000	10^{-2}	3	30,000	80,000
F_2	10^{-1}	12	12,000	10^{-2}	2	20,000	32,000
F_3	10^{-2}	2	20,000	10^{-2}	2	20,000	40,000
D_1	10^{-2}	11	110,000	10^{-1}	8	8,000	118,000
D_2	10^{-2}	6	60,000	10^{-1}	7	7,000	67,000
D_3	10^{-2}	11	110,000	10^{-2}	3	30,000	140,000

Average Population Density of Yeast and Mold in Forested and Deforested Areas

	Total Yeast	Total Mold
Forested	23,778	21,111
Deforested	142,222	26,667

Average Fungi Population Density in Deforested Areas and Forested Areas

	Number of Fungi in 1cc of soil
Forested	44,689
Deforested	168,889



Conclusion

In conclusion, our hypothesis was not supported. We stated that in a deforested environment the population density of fungi would be lower compared to that of a forested environment, however this was the not the case. In our forested environment the average population density of fungi was 168,889 fungi's in 1cc of soil, and in our deforested environment the average population density of fungi was 44,689 fungi's in 1cc of soil. Although there was a much higher population density of fungi in the deforested environment, the ratio of yeast to mold in this environment was 5:1. This means that for every 5 yeasts there were 1 mold, and the yeast is the "sad" fungus that is stressed because their specific environment does not supply them with the correct needs to survive. The mold is the "happy" fungus that is not stressed and their environment is giving them all the appropriate needs to survive. However, in the forested environment the ratio of yeast to mold was 1:1, meaning for every 1 yeast there was 1 mold and in successful soil there must be an about even ratio of both. Thus, although the population

density of fungi in the deforested environment was greater, there was also a high amount of fungi that was not thriving for every fungus that was. In result of these ratios, we can conclude that in the forested soil the fungus is not as "stressed" as those in the deforested soil, so there is something in the deforested soil's environment that is preventing the fungus from thriving.

In the future to better support our hypothesis there are many things that could have been done. If we had tested the amount of water in each environment and how the water effects the fungi population, that could have changed our results because in our experiment the forested environment had large trees blocking our soil sights from obtaining rain water, while in the deforested environment there were no trees blocking the rain from being absorbed into the soil. In addition, if we had tested the amount of mulch in each environment this could have altered our data. Fungi cannot live in the mulch, so if there is mulch in our soil samples were are extracting part mulch when we should be extracting all soil and therefore fungi and no mulch. If we tested the amount of mulch in the soil then we could have chosen places where mulch was not present, so we are getting samples with all soil and therefor all fungi. Finally, we could have tested the amount of CO_2 in the soils. Both of our sites, deforested and forested, were right next to a road. The deforested environment had no trees around protecting it from the CO_2 released by the passing cars, while the forested environment did. This could have been a potential reason why our hypothesis was wrong, because CO_2 normally stimulates improved growth of soil mycorrhizae fungi. (Steward, 2013)

Citation

Collins, Jocelyn. (2001). Deforestation.

http://www.botany.uwc.ac.za/envfacts/facts/deforestation.htm

Ingham, E. (N.D.) Chapter 4: SOIL FUNGI. Oregon State University: Urbanext.

http://urbanext.illinois.edu/soil/SoilBiology/fungi.htm

Kendrick, B. (2002). How do fungi form partnerships with most plants? Mycologue Publications. http://www.mycolog.com/mycorrhiza.htm

Lippman (2003) The importance of Humus. Global Marketing, Ltd. http://www.globalmkg.com/humus.htm

Lynn, V. (1999) Effects of Deforestation. The guardians: Satellite Events Enterprises Inc. http://www.theguardians.com/Microbiology/gm_mbr10.htm

N.D. (2004) Soil Fungi. The University of Western Australia: Soil Health. http://www.soilhealth.see.uwa.edu.au/components/fungi

N.D. (2013) Soil Structure improvement. Penn State Extension: College of Agricultural Sciences. http://extension.psu.edu/agronomy-guide/cm/sec10/sec102c

N.D. (2013) The Plant Fungi Partnership - Benefits of Trade. The Compost Gardener. http://www.the-compost-gardener.com/plant-fungi.html

Steward, L. - Symbiotic Soil Fungi - Benefits of CO_2 - Carbon Dioxide. *Plants Need CO_2 - First Page*. http://www.plantsneedco2.org/default.aspx?menuitemid=337

Tuller, M. (2003) Retention of Water in Soil and the Soil Water Characteristic Curve. University of Connecticut http://www.engr.uconn.edu/environ/envphys/pdf/vadose_pdf/SWC_revised01.pdf

Vitousek, P. (N.D.) The Effects of Deforestation on Air, Soil, and Water. Scope environment. http://www.scopenvironment.org/downloadpubs/scope21/chapter07.html