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Background

Fungi are microscopic organisms that can consist of a single cell (also known as yeasts) or many cells (Chamberlain, 2011). The multicellular fungi are made out of tiny filaments called hyphae (Fogel, 1995), which can range in length from a few cells long to many yards. These hyphae grow either into large unconstructed masses, called mycelium or molds (Chamberlain, 2011), or they grow into structures called rhizomorphs, which are similar to roots, and both are important ecologically because in the soil, they bind the particles of soil together, which increases water's ability to permeate into the soil and the soil's ability to retain it once it has (Ingham, 2011). Increasing the soil's water permeation and ability to retain water not only benefits organisms like plants, but helps the fungi as well. Fungi need moisture to survive, and a lack of moisture can mean a lack of fungi (Canadian Centre for Occupational Health and Safety, 2006).

There are three main types of soil fungi: decomposers, mutualists, and pathogens (or parasites) (Ingham, 2011). The decomposers release enzymes to break down the complex organic materials that surround them into simpler forms (such as nitrogen, phosphorus, and iron) so that these nutrients can be absorbed by the fungi and other organisms (Amaranthus, 2011), and the group of fungi responsible for this process can even decompose carbon rings that are found in some pollutants (Ingham, 2011). The absorption of nitrogen especially is critical, because nitrogen makes is used by plants to make amino acids (Ophardt, 2003). Amino acids are the

monomers that make proteins, and proteins, specifically enzymes, cause chemicals reactions. Chemical reactions are the basis of a cell functioning properly, and cells work together to form an organism. This means that nitrogen is essential to the functioning of plants, because it helps cause the chemical reactions that cause the plants to survive (Campbell, N; Williamson, B; Heyden, R., 2004). Furthermore, decomposers increase the accumulation of humus-rich organic matter (Ingham, 2011) which improves the uptake of nutrients (such as iron) in plants and improves the growth of plants and their roots (Tew, 2008).

The second type of fungi, Mutualists (also known as mycorrhizae) (Ingham, 2011) are specialized fungi that release strong enzymes into the soil and join with a plant's root system (Amaranthus, 2011). The plant roots these fungi attach to provide carbohydrates and other nutrients for the fungal partner which it cannot produce for itself, and in return, the mycorrhizae enable the plants to absorb more water and critical nutrients from the soil, thus making the plant healthier (Fogel, 1995). In fact, plant species without mycorrhizal fungi have to consume more of their own photosynthesis products to help them absorb water and nutrients and, as a consequence, are often less healthy (Amaranthus, 2011). There are two types of mycorrhizae: endomycorrhizae, which grows inside of plants, and is commonly associated with grass, vegetables, and row crops, and ectomycorrhizae, which grows on the surface of plant roots, and is commonly associated with trees (Ingham, 2011).

The final type of fungi are pathogens or parasites. These type of fungi are detrimental to the plants around them and reduce the plant's ability to produce by colonizing on their roots and robbing the plant of nutrients without returning any nutrients of their own (Ingham, 2011). For example, the rye ergot, a parasitic fungus, can infect wheat and damage or even kill it (Campbell, N; Williamson, B; Heyden, R, 2004).

Overall then, fungi play many critical roles in ecosystems. They can promote the growth of other organisms by helping them receive nutrients, decompose dead organisms and pollutants (Ingham 2011) and, without them, plants could not get the nutrients that they needed and dead matter and waste would accumulate (Northwestern University). Moreover, without plants, the food chain falls apart because plants support the entire flow of energy through an ecosystem. They are the producers in the food chain, which the primary consumers depend upon for food. The secondary, tertiary, etc. consumers depend, in turn, on the primary consumers and the producers for food as well, and so without the producers, the food chain would collapse, and thus ruin ecosystems everywhere (Campbell, N; Williamson, B; Heyden, R, 2004).

The presence of litter can also destroy ecosystems. The most common forms of litter, also known as slow or non-decomposable trash or discarded solid waste, are food wrappers, cigarette butts, and aluminum cans. Litter is harmful to the environment in that it can contaminate water and destroy natural beauty (Hartmann and Brown, 2006). It can also clog waterways, and sometimes leak hazardous chemicals such as lead, mercury, and cadmium into the soil (Town of Braintree Massachusetts), which, as these chemicals decompose, can kill microorganisms (such as mycorrhizae (Crane S, Dighton J, Barkay T, 2010)) and animals (Nonpoint Source Pollution, 2006). Killing microorganisms, of course, interrupts energy flow because the microorganisms support the life of the producers, and without them, the producers wither and die. Dead producers mean that there is no food source for primary consumers, secondary consumers, tertiary consumers, and etc., and with the collapse of a food chain, an ecosystem will fall apart (Campbell, N; Williamson, B; Heyden, R, 2004). Hence, when litter impacts microbes in the soil, it impacts the entire environment. Furthermore, all of these problems that litter can cause are

not only harmful but long-lasting, because litter can take from 2 weeks to 800 years to decompose (Waste Management & Recycling Program, 2006).

In our experiment, we will be observing the affects that litter has on the population of fungi in the soil at RPCS, to see if litter does in fact cause the problems previously mentioned. This is an important issue because if litter does in fact have a negative impact of the fungi, then litter could potentially kill the fungi at RPCS, and prove that litter can harm the ecosystem.

Lab Report

I. **Problem**

a. Does the presence of slow decomposing or non-decomposable trash increase or decrease the density of fungi in the soil found around tree roots?

II. **Hypothesis:**

a. The presence of slow decomposing or non-decomposable trash will cause the population density of the fungi to decrease in the soil found around tree roots.

III. **Procedure**:

a. *Independent Variable* – presence of slow decomposing or non-decomposable trash on the soil

b. *Dependent Variable* – the density of the fungi found in the soil around the tree roots, the amount of moisture found in the soil around the tree roots

c. *Negative Control* – the absence of slow decomposing or non-decomposable trash on the soil

d. *List of Controlled Variables* – Amount of time the slow decomposing or non-decomposable trash is present on the area, tree the soil is being extracted and tested from/near, arrangement of the slow decomposing or non-decomposable trash on the plots, types and amount of slow decomposing or non-decomposable trash used, types, brands and sizes of soda cans, bottles, and chip bags used as slow decomposing or non-decomposable trash, amount of slow decomposing or non-decomposable trash, dimensions of each testing plot, distance between each testing plot in the same trial, distance between each testing plots for different trials, depth of the soil tested, amount of soil extracted, type of tools used, amount of soil being tested for fungi test, amount

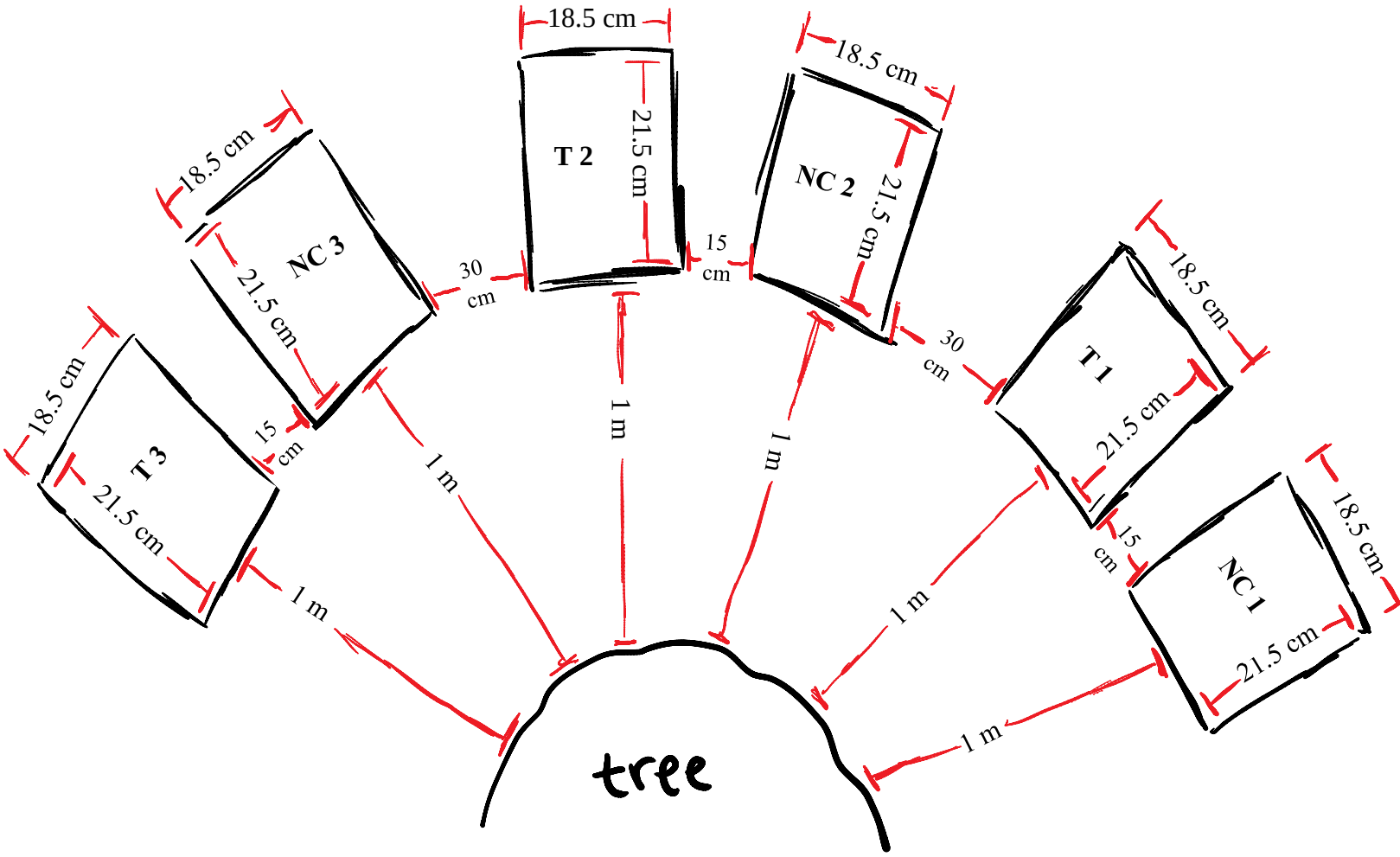
of soil being tested for moisture test, method of extracting moisture from soil, type of water (sterile), temperature of sterile water, amount of time for the soil solutions to sit on the media during the fungi test, amount of time for the soil to sit out during the moisture test, equation used to estimate the total amount of fungi in 1 cc of the soil sample, amount of sterile water added to each culture tube per test, degree of dilutions, type of media fungi grown on, size of crate/box put over slow or non-decomposable trash, amount of soil mixture plated on the fungi media, dilution levels tested for fungi, labels on corresponding culture tubes/plastic bags/fungi media used for the same tests, equation used to find the change in mass of soil after moisture tests, equation used to find the percentage change in mass after moisture tests

e. *Step-by-Step Instructions*:

- ***Setting Up Experimental Plots:***
    1. Find a large patch of flat surface near the tree at the coordinates N 39.35679^{o} W 076.63631^{o}
    2. ***NOTE: keep all plots 1 meter away from the base of the tree***
    3. Set up 1 plot for negative control with dimensions of 18.5 cm x 21.5 cm (see Figure A)
    4. Place yellow flags on the corners or this plot and label the flags "NC 1" (NC = negative control)
    5. Move 15 cm away from the plot labeled "NC 1" to set up 1 plot for slow decomposing or non-decomposable trash with dimensions of 18.5 cm x 21.5 cm (see Figure A)

6. Place yellow flags on the corners of this plot and label the flags "T 1" (T = slow decomposing or non-decomposable trash)
7. Move 30.5 cm away from the plot labeled "T 1" to set up the 2nd plot for negative control with dimensions of 18.5 cm x 21.5 cm (see Figure A)
8. Place yellow flags on the corners of this plot and label the flags "NC 2"
9. Move 15 cm away from the plot labeled "NC 2" to set up the 2nd plot for slow decomposing or non-decomposable trash with dimensions of 18.5 cm x 21.5 cm (see Figure A)
10. Place yellow flags on the corners of this plot and label the flags "T 2"
11. Move 30.5 cm away from the plot labeled "T 2" to set up the 3rd plot for negative control with dimensions of 18.5 cm x 21.5 cm (see Figure A)
12. Place yellow flags on the corners of this plot and label the flags "NC 3"
13. Move 15 cm away from the plot labeled "NC 3" to set up the 3rd plot for slow decomposing or non-decomposable trash with dimensions of 18.5 cm x 21.5 cm (see Figure A)
14. Place yellow flags on the corners of this plot and label the flags "T 3"
15. ***Your plot should look like this:***

FIGURE A



- ***Extraction of Soil Procedures*:**
  1. Get two soil samples that are each 15 cm deep x 2 cm wide from each of the 6 plots on the same day at the same time that the plots were set up.
  2. Place one soil sample from plot “NC 1” in plastic bag labeled “NC 1 Before”
  3. Place another soil sample from plot “NC 1” in plastic bag labeled “NC 1.5 Before”

4. Repeat steps 2-3 with the plots “NC 2” and “NC 3” placing each individual soil sample into its own bag with the corresponding label
5. Place one soil sample from plot “T 1” in plastic bag labeled “T 1 Before”
6. Place another soil sample from plot “T 1” in plastic bag labeled “T 1.5 Before”
7. Repeat steps 5-6 with the plots “T 2” and “T 3” placing each soil samples in the bag with the corresponding label
8. On the same day as the soil was extracted from the plots, go to “Moisture Test Procedures” to determine how much moisture is in the “before” testing samples

- ***Moisture Test Procedures:***

1. On the same day as the soil was extracted and put into labeled plastic bags, weigh one uncovered unlabeled petri plate (8.75 cm in diameter and 1.5 cm tall) and take note of its mass
2. Take each soil sample and put it in its own uncovered petri plates (8.75 cm in diameter and 1.5 cm tall) that has been labeled with the corresponding label from its bag
3. Weigh each soil samples on a scale and record its weight in grams
4. Follow this equation to determine the soil’s mass:

Mass of soil (grams) – mass of petri plate (grams) = mass of soil (grams)

5. Place all petri dishes next to a window
6. Let petri dishes sit next to the window for 4 to 5 days to let the moisture evaporate from the soil

7. After 4 to 5 days, weigh each soil sample to determine its new mass
8. Use the equation in step 4 to determine the soil's final mass
9. Record the resulting weight in grams
10. Determine the change in mass to determine the mass of moisture in the original sample
11. Find out the percentage change in mass of the soil after drying

- ***Examining Fungi Procedures:***
    1. ***NOTE: use the soil from after the moisture test is completed AND complete all procedures of this test within one time period in the same day for all samples***
    2. Use a clean, new transfer pipette to add 10 ml (milliliters) of sterile water to a 15 ml culture tube and label the tube "10^{0} NC 1"
    3. Use the same pipette to add 9 ml to a second 15 ml culture tube. Label the tube "10^{-1} NC 1"
    4. Repeat step 2 to an additional 15 ml culture tube, only labeling the third culture tube "10^{-2} NC 1"
    5. Place 1 cc of the "NC 1" soil sample into the "10^{0} NC 1" culture tube
    6. Cap the tube and shake vigorously for 30 seconds
    7. Using a new clean pipette, remove 1 ml of the soil/water mixture from the "10^{0} NC 1" tube and place into the "10^{-1} NC 1" tube
    8. Cap and shake vigorously for 30 seconds
    9. Using the same pipette in step 6, remove 1 ml of the soil/water mixture from the "10^{-1} NC 1" tube and place into the "10^{-2} NC 1" tube

10. Cap and shake vigorously for 30 seconds
11. There should now be a total of 3 culture tubes
12. Place separate100 µl samples from all three dilutions onto their own separate, correspondingly labeled 3M Petrifilm^{TM} Yeast and Mold Count Plates
13. Repeat steps 2-11 with the soil samples "NC 1.5", "NC 2", "NC 2.5", "NC 3", "NC 3.5" "T 1.5", "T 2", "T 2.5", "T 3", and "T 3.5" making sure that all the samples are tested in appropriately corresponding culture tubes and fungi media
14. Allow all samples to grow for 72 hours
15. Start with the lowest dilutions of the "NC 1" soil sample (10^{-2})
16. Examine the amount of yeast fungi in each sample and record the amount of yeast fungi and the dilution value. If there are no yeast fungi in the lowest dilution, move up one dilution higher, until yeast fungi are visible to count. Then record the number observed and the new dilution value.
17. Examine the amount of mold fungi in each sample and record the amount of mold fungi and the dilution value. If there are no mold fungi in the lowest dilution, move up one dilution higher, until mold fungi are visible to count. Then record the number observed and the new dilution value.
18. Follow this equation to find how much yeast was present in 1 cc of the "NC 1" soil sample:

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

19. Use the same equation in step 18 to find how much mold was present in 1 cc of the soil
20. Follow this equation to find the total number of fungi was in 1 cc of the soil sample:

Total # of Fungi in 1 cc of Soil Sample = # Yeast Microbes + # Mold Microbes

21. Record all results
22. Repeat steps 15 – 21 for soil samples “NC 1.5”, “NC 2”, “NC 2.5”, “NC 3”, “NC 3.5”, “T 1”, “T 1.5”, “T 2”, “T 2.5”, “T 3”, and “T 3.5”

- ***Setting Up Slow or Non-Decomposable Trash Procedures:***
    1. After the before soil sample has been taken, start setting up the slow or non-decomposable trash on the corresponding boundaries
    2. The slow decomposing or non-decomposable trash should consist of:
        a. One slightly crushed 12 ounce empty Coke soda can
        b. One 20 ounce empty Vitamin Water or Gatorade bottle
        c. One empty UTZ chip bag that, when full, holds 1 ounce
    3. Make sure to do the following things:
        a. Make sure there is direct contact with the ground and the soda can, bottle, and empty chip bag
        b. Do not leave large patches of dirt, or space in between the different pieces of slow decomposing or non-decomposable trash
    4. Place the different types of slow decomposing or non-decomposable trash on the ground in each of plots “T 1”, “T 2”, and “T 3”. Cover all plots

with a box/crate with the dimensions of: 21.5 cm (length) x 18.5cm (width) x 15.5 cm (height)

5. Let the slow decomposing or non-decomposable trash sit on the boundaries for 6 days
6. After 6 days, complete all steps in "Extraction of Soil Procedures"
7. After the soil samples are taken, on the same day complete all steps in "Moisture Test Procedures"
8. After the moisture testing is complete for the new soil samples, complete all steps in "Examining Fungi Procedures"

IV. **Data and Analysis:**

a. *Data Tables*:

Effects of Slow Decomposing or Non-Decomposable Trash on Fungi Density

***KEY*:** NC = Negative Control Samples

T = Slow Decomposing or Non-Decomposing Trash Samples

Location/ Label/ Trial of Slow or Non-Decomposable Soil	**Before Adding Slow Decomposing or Non-Decomposable Trash**			**After Adding Slow Decomposing or Non-Decomposable Trash**		
	Number of Yeast Per cc of Soil	**Number of Mold Per cc of Soil**	**Number of Fungi Per cc of Soil**	**Number of Yeast Per cc of Soil**	**Number of Mold Per cc of Soil**	**Number of Fungi Per cc of Soil**
T 1	200	300	500	10000	10000	20000
T 1.5	600	1000	1600	N/A	N/A	N/A
T 2	200	100	300	5000	10000	15000
T 2.5	300	400	700	N/A	N/A	N/A
T 3	700	1000	1700	7000	2000	9000
T 3.5	200	600	800	N/A	N/A	N/A
Average	367	567	933	7333	7333	14667

Effects of Slow Decomposing or Non-Decomposable Trash on Fungi Density

***KEY*:** NC = Negative Control Samples

T = Slow Decomposing or Non-Decomposing Trash Samples

Location/ Label/ Trial of Negative Control Soil	Before Adding Slow Decomposing or Non-Decomposable Trash			After Adding Slow Decomposing or Non-Decomposable Trash		
	Number of Yeast Per cc of Soil	Number of Mold Per cc of Soil	Number of Fungi Per cc of Soil	Number of Yeast Per cc of Soil	Number of Mold Per cc of Soil	Number of Fungi Per cc of Soil
NC 1	200	1000	1200	1000	10000	11000
NC 1.5	300	20000	20300	N/A	N/A	N/A
NC 2	3000	3000	6000	1000	10000	11000
NC 2.5	200	1000	1200	N/A	N/A	N/A
NC 3	2000	1000	3000	1000	1000	2000
NC 3.5	600	1000	1600	N/A	N/A	N/A
Average	1050	4500	5550	1000	7000	8000

Amount of Moisture in the "Before" Slow Decomposing or Non-Decomposable Trash Soil Testing Samples

***KEY*:** NC = Negative Control Samples

T = Slow Decomposing or Non-Decomposing Trash Samples

Location/ Label/ Trial of Slow Decomposing or Non-Decomposable Trash Manipulated Soil	Percentage Change in Mass		Mass of Water in Slow Decomposing or Non-Decomposable Trash Manipulated Soil Samples (grams)	
	Before Added Trash	After Added Trash	Before Added Trash	After Added Trash
T 1	- 17.008 %	- 24.449 %	14.1 g	11.1 g
T 1.5	- 16.317 %	N/A	10.1 g	N/A
T 2	- 23.139 %	- 19.094 %	11.5 g	11.8 g
T 2.5	- 20.273 %	N/A	11.9 g	N/A
T 3	- 22.088 %	- 23.619 %	11.0 g	12.4 g
T 3.5	- 20.942 %	N/A	12.0 g	N/A
Average	- 19.9612 %	- 22.3873 %	11.7667 g	11.7667 g

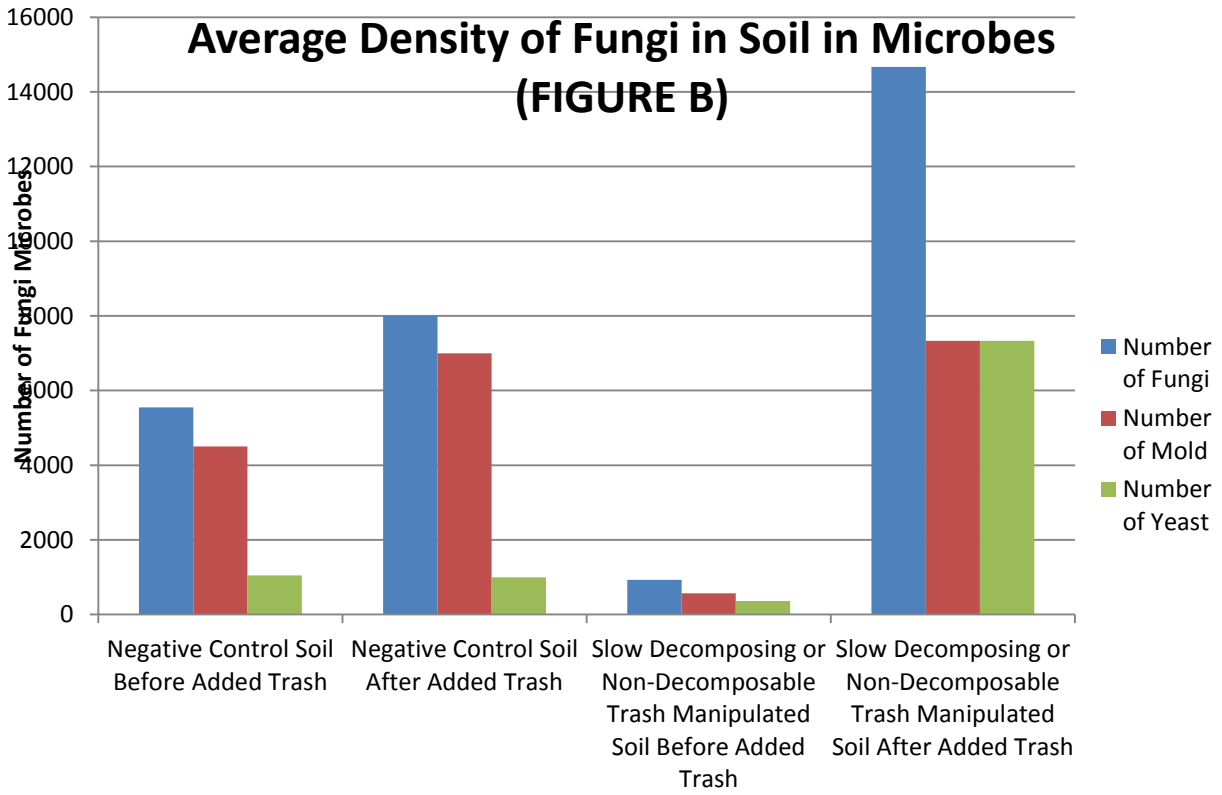
Amount of Moisture in the "Before" Negative Control Soil Testing Samples

***KEY*:** NC = Negative Control Samples

T = Slow Decomposing or Non-Decomposing Trash Sample

Location/ Label/ Trial of Negative Control Soil	Percentage Change in Mass		Mass of Water in Negative Control Soil Samples (grams)	
	Before Added Trash	After Added Trash	Before Added Trash	After Added Trash
NC 1	- 18.132 %	- 25.913 %	9.9 g	14.9 g
NC 1.5	- 20.370 %	N/A	12.1 g	N/A
NC 2	- 20.078 %	- 23.968 %	11.9 g	15.1 g
NC 2.5	- 20.612 %	N/A	12.8 g	N/A
NC 3	- 22.736 %	- 21.739 %	11.3 g	13.5 g
NC 3.5	- 21.386 %	N/A	10.8 g	N/A
Average	- 20.5523 %	- 23.8733 %	11.4667 g	14.5 g

b. *Analysis → Graphs:*

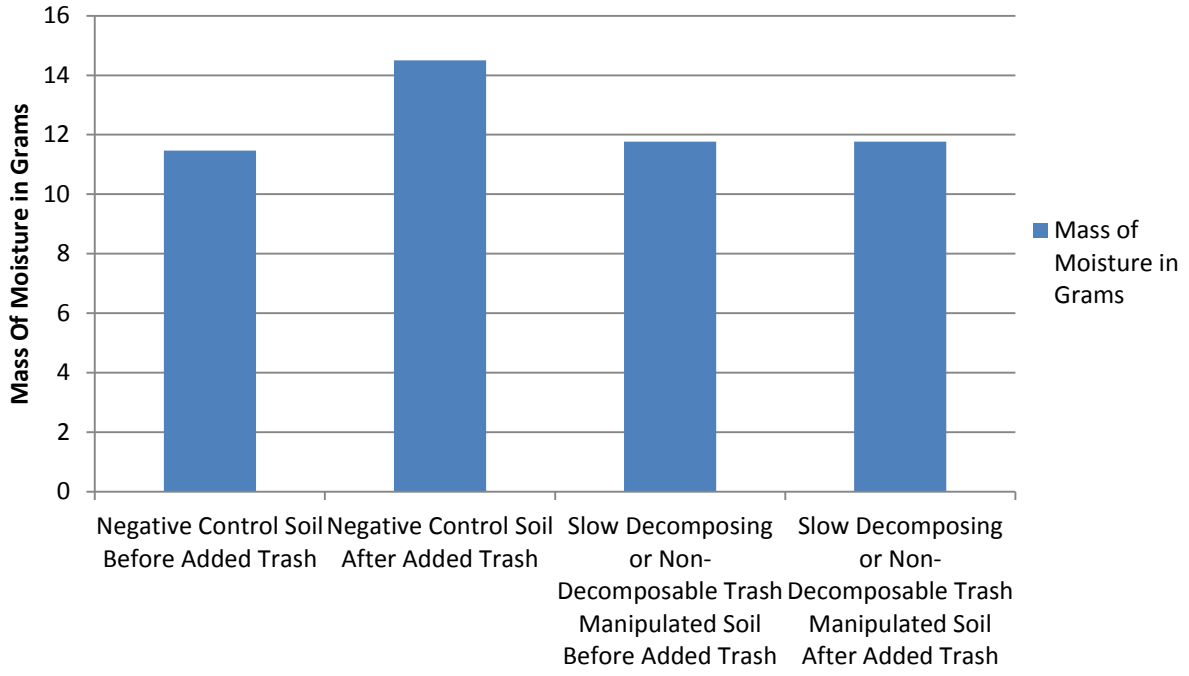


T-tests for Experimental Conditions (FIGURE C)

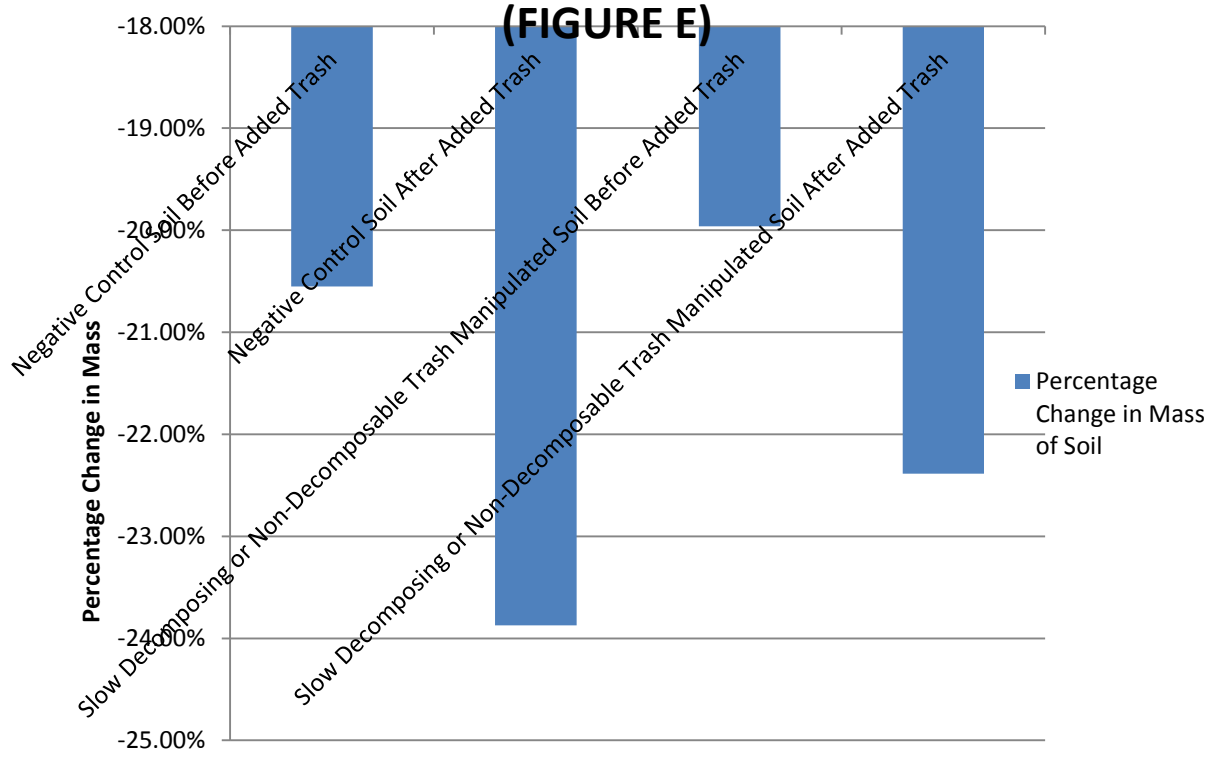
Comparison of Number of Microbes	P Value
Yeast Test Plots	0.04
Mold Test Plots	0.13
Fungi Test Plots	0.05
Yeast Control Plots	0.92
Mold Control Plots	0.58
Fungi Control Plots	0.59

This data in, Figure C, in spite of the missing data in the "After" trials, the data still proved to be true.

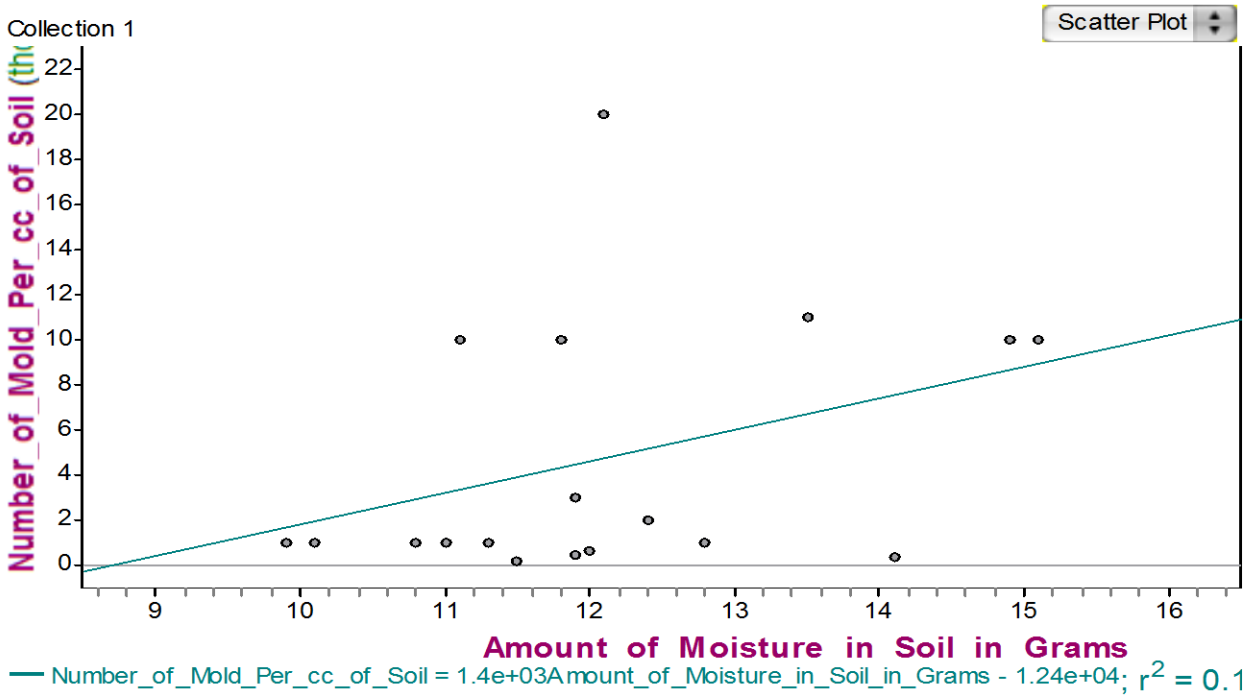
Average Amount of Soil Moisture (FIGURE D)



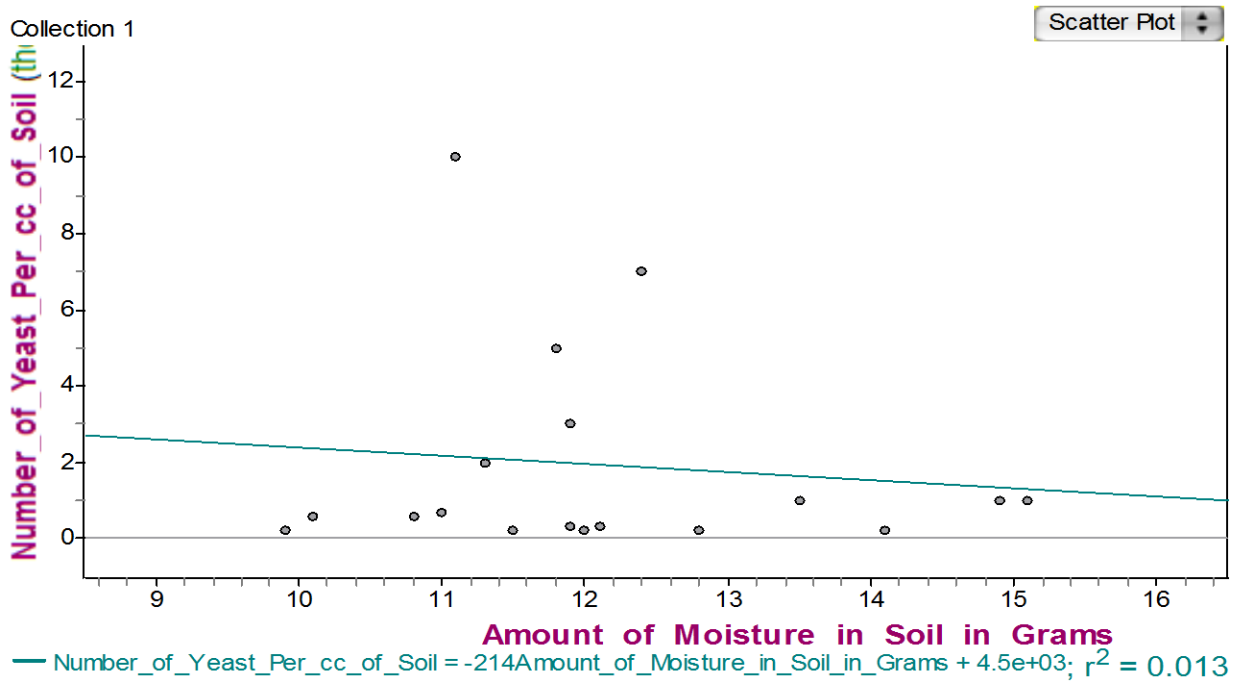
Average Percentage Change in Mass of Soil (FIGURE E)



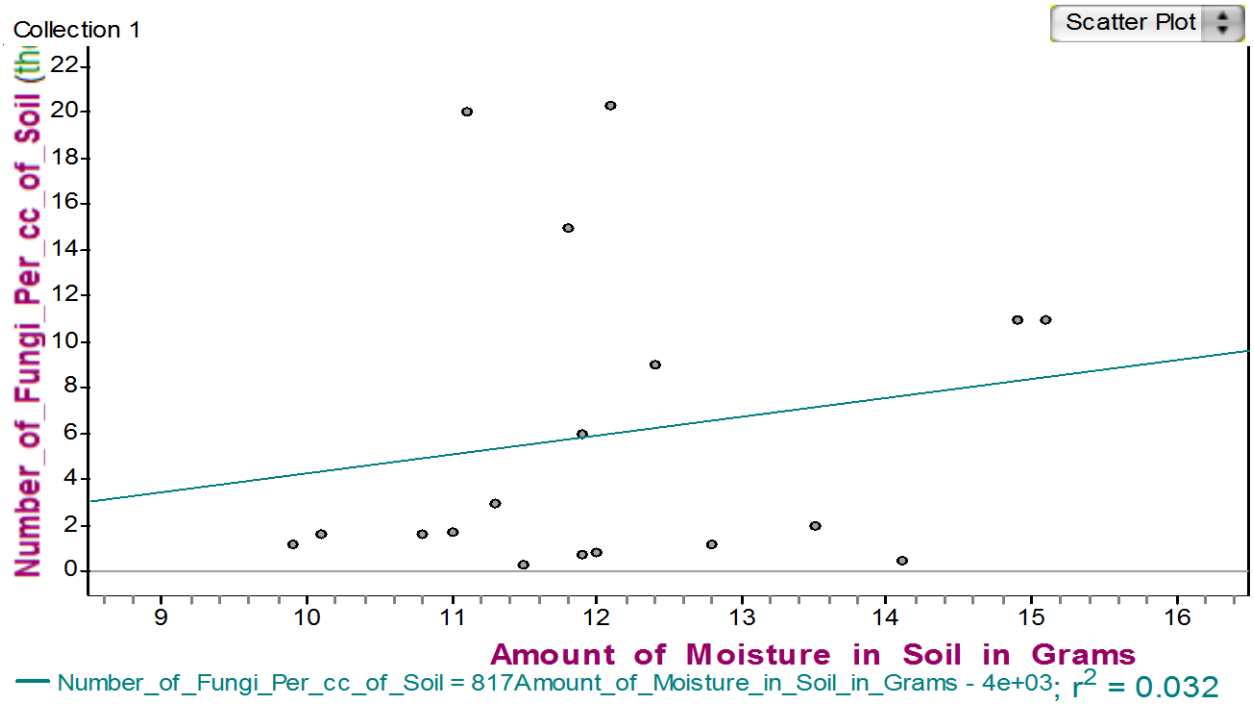
Number of Mold Fungi Per cc of Soil vs. Amount of Moisture in Soil in Grams (FIGURE F)



Number of Yeast Per cc of Soil vs. Amount of Moisture in Soil in Grams (FIGURE G)



Number of Fungi Per cc of Soil vs. Amount of Moisture in Soil in Grams (FIGURE H)



V. Conclusion:

a. The hypothesis "The presence of slow decomposing or non-decomposable trash will cause the population density of the fungi to decrease in the soil found around tree roots." is incorrect. The environment as a total functioned in a healthy manner during this experiment. As you can see in Figure G, the amounts of yeast fungi in the soil samples decreased as the amount of moisture in the soil samples increased, and in Figure F the amounts of mold fungi in the soil samples increased as the amount of moisture in the soil samples increased. These results are to be expected because as the fungi interacts positively with its environment and the amount of moisture in the soil increases, the amount of mold fungi increases as the amount of yeast fungi decreases. As shown in Figure B, the average amount of fungi for the manipulated plots were low during our "Before" trials, but the average amount of fungi for after we manipulated the plots skyrocketed dramatically; but when those results from Figure B are compared to Figure D, the average amount of moisture found in the soil samples for the manipulated plots hardly changed. This means that the slow decomposing or non-decomposable trash did not increase or decrease the population density of fungi due to the amount of moisture. In Figure C, you can see that there was a change in the number of microbes from the experimental conditions. If a P-value in a T-test is below 0.20, then there was a dramatic change due to the conditions, and if the P-value in a T-test is below a 0.05, then the results were expected. The P-values in the T-test for the test plots were all below 0.20, and two of them were equal to if not lower than 0.05 proving that the added slow decomposing or non-decomposable trash did guarantee the change in the density of

fungi. The P-values for the control plots where all significantly above 0.20 in the T-test proving that were was no significant change, and that the environment was doing its job. These results lead us to the questions: what properties of the slow decomposing or non-decomposable trash were the causes in the dramatic increase of the amount of fungi in the manipulated plots? Was it the type of slow decomposable and non-decomposable trash that we used that caused the dramatic increase in the amount of fungi in the manipulated plots? What properties of the slow decomposing or non-decomposable trash caused the moistures in the manipulated plots to stay the same?

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