The Effects of Herbicide on Mycorrhizal Fungi

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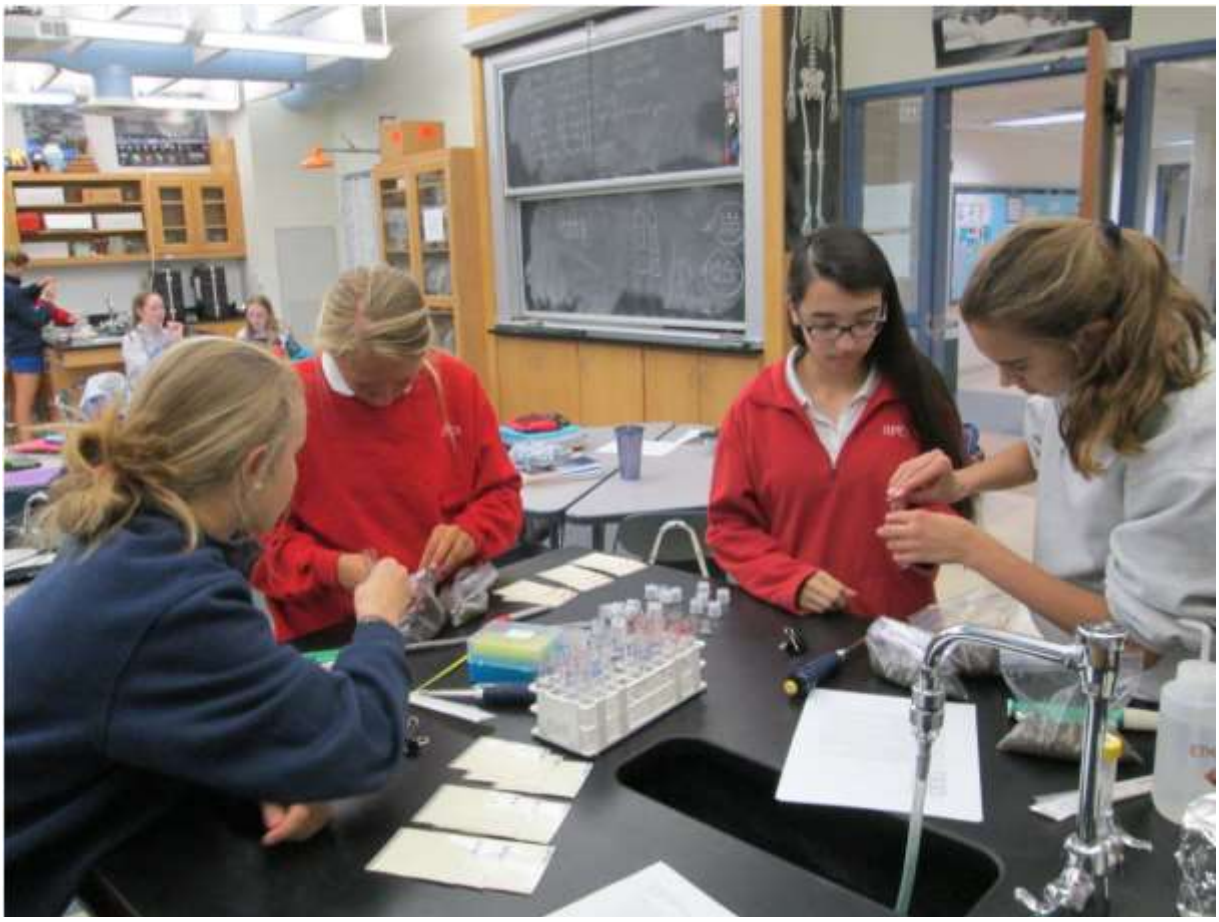

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Background Report

Fungi are a large and diverse group of eukaryotic organisms that play a significant role in many ecosystems including the soil. There, they improve fertility by releasing nutrients from organic matter (Grains Research & Development Corporation, *et al*, 2017 Soil Quality Pty Ltd, n.d.) and quicken the process of decomposition by breaking down dead animals and plants (The British Mycological Society, 2017). They also increase the rate of microbial biomineralization (Benzerara, Miot and Coradin, 2016), which is the process of the "formation and deposition of minerals directly or indirectly influenced by microorganisms" (C. Heim, n.d.). Furthermore, they enhance plant health and nutrition by using microscopic filaments called hyphae to branch out into the soil, beyond the plant's root tips, thereby giving plants access to a larger volume of soil and more nutrients (Mercola, 2015).

All fungi are heterotrophs, which means that they need carbon from other sources for energy and reproduction, and through the process of evolution, soil fungi have adopted three different ways to obtain this carbon. One way that certain fungi such as mushrooms and the other saprotrophs obtain carbon and energy is by "decaying organic matter and feeding off the results" (Finlay, 2008). Parasitic fungi, on the other hand, live off of various organisms and can cause disease in plants and various animals(The Free Dictionary Fungi By Farlex, 2003-2017). These necrotrophic fungi such as rusts "kill their host tissue as they thrive on dead or dying cells" (K. Laluk and T. Mengiste, 2010). Finally, the biotrophic fungi acquire carbon by taking "their

nutrients from living cells and maintaining host viability" (K. Laluk and T. Mengiste, 2010). These mutualistic fungi, such as lichen and mycorrhizal fungi, are among the most critical of the soil fungi because of their interdependence with their photosynthetic hosts.

In the case of mycorrhizae, the symbiotic relationship is with the roots of certain kinds of plants that increases the fitness for both the fungus and the plant. The fungal partner helps create a sustainable nutrient supply for the plant by increasing the surface area of the roots for absorbing nutrients and by releasing powerful enzymes into the soil that dissolve tightly bound soil nutrients such as organic nitrogen, phosphorus, and iron, which the plant can then absorb through its roots. In return, the plant exudes carbohydrates from its roots which the fungi to use for energy and their own metabolic activities(New York Botanical Garden, 2003 and Finlay, 2008). Mycorrhizal fungi also protect plants from parasites and other diseases "because the plant, being better nourished, is healthier and has better resistance to the invader." (A. O'Calalghan, n.d.).

Mycorrhizal fungi can take one of two forms: endomycorrhizae and ectomycorrhizae. The first of this use their hyphae to penetrate the cell wall of a plant's root cells and invaginate the cell membrane, while the ectomycorrhizal fungi, do not penetrate individual cells within the root but entwine themselves around the roots in a woven net of hyphae. Ectomycorrhizal hosts are usually trees such as oaks, pines, firs, hemlocks, beeches, and birches, and the endomycorrhizal hosts are the non-woody plants such as the various species of grasses. However, regardless of whether a specific mycorrhizal fungus is endo or ecto, both types help enhance root access to water and a sustainable nutrient supply to plants (Frey-Klett, Burlinson, Deveau, Barret, Tarkka, Sarniguet, 2011). Furthermore because of the better access to water,

plants with this fungal relationship can photosynthesize at a faster rate and overall, be healthier. Hence, Mycorrhizal fungi are critical to the survival of nearly all kinds of plants.

One particular way these fungi are so important is the way they increase a plant's access to the macronutrient nitrogen. This key nutrient is needed in order for a plant to survive because it makes up nitrates, which are the major source of nitrogen for the formation of DNA, RNA, and the amino acids that make up proteins in a plant. These structures are important because every cell needs DNA, RNA and amino acids to function. DNA copies its information into RNA which then makes specific proteins out of amino acids called enzymes. These enzymes then control the chemical reactions of the cell, enabling it to perform its essential four tasks: reproduction, homeostasis, synthesis and transformation of energy. Hence, without the aid of fungi, plants could not survive, nor could any of the rest of the food chain of consumers in an ecosystem that depends on the producers for their own biological molecules (Campbell, Williamson, Heyden 2004).

However , this relationship between fungi and plants may be negatively affected by a number of ways people commonly interact with soil, one of the most common of which is the use of herbicides. These toxic chemicals are frequently used to kill weeds or any other plant "objectionable to humans primarily because they reduce the quality and quantity of agricultural production, and produce allergens or contact dermatitis that affect public health" (Ware and Whitacre, 2004). But in killing their host plants, herbicides could also be harmful to the fungi that live with them, since damaging this symbiotic relationship with plants might "reduce the growth and function of the mycorrhizal fungi" (National Wildlife Refuge System, 2009).

The degree of damage, though, may depend on what type of herbicide is used: selective or nonselective. An herbicide is "classed as selective when it is used to kill weeds without

harming the crop and an herbicide is classed as nonselective when the purpose is to kill all vegetation" (Ware and Whitacre, 2004). When the herbicide is not selective it is applied to the crop and usually targets an enzyme to bind and inhibit its function, by stopping its chemical reaction. When a herbicide is selective it targets the weed, not the crop itself meaning "it must be metabolized, or broken down, by the crop plant, and not by the weed" (Martin, 2004).

A common herbicide such as Ortho Weed B Gon Max can contain multiple active ingredients, including 2,4-D dimethylamine salt, quinclorac, and dicamba dimethylamine salt. "The salt forms are derivatives of the parent acid" (Jervais, G.; Luukinen, B.; Buhl, K.; Stone, D. 2008) and kill "plants by causing the cells in the tissues that carry water and nutrients to divide and grow without stopping" (National Pesticide Information Center, 2009). The result is that the plants run out of energy and die.

The other ingredient in this herbicide, Quinclorac, is used after seedlings have emerged to control crabgrass and broadleaf weeds (University of Pennsylvania, n.d.), and there are three current hypotheses about how quinclorac functions. The first one "is supported by evidence of inhibition of incorporation of C^{14}-glucose into cellulose and hemicellulose, thus, affecting the cell wall synthesis"(Fipke, M.V., & Vidal, R.A.. ,2016). The second hypothesis says that in broadleaved weed species quinclorac acts as an auxin, which is a plant hormone that causes certain cells to grow larger than others,and the third and final current hypothesis says that in some species of plants the formation of reactive oxygen species may be harmful to the plants.(Fipke, M.V., & Vidal, R.A..,2016) While dicamba is a selective herbicide which acts on pre- and post-emergent seedlings,it acts similarly to the 2, 4D diethylamine salt because it is a hormone that causes plants to " grow in abnormal and uncontrollable ways",(National Pesticide Information Center, 2009).

For the experiment, selective herbicides will be used as they do not destroy all of the living things but get rid of the weeds. In our experiment we are testing for how herbicides affect the population of soil fungi. We have hypothesized that herbicides will decrease the population density of mycorrhizal fungi on our campus. To test this hypothesis, we will look at soil fungi levels before and after the application of herbicide. We hypothesized that herbicides would decrease the population density of mycorrhizal fungi on our campus because herbicides would kill weeds that the fungi would have a symbiotic relationship with. Since we controlled for the type of plants on our plots, the herbicides should have the same effect on the plants in all the plots.

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Experiment

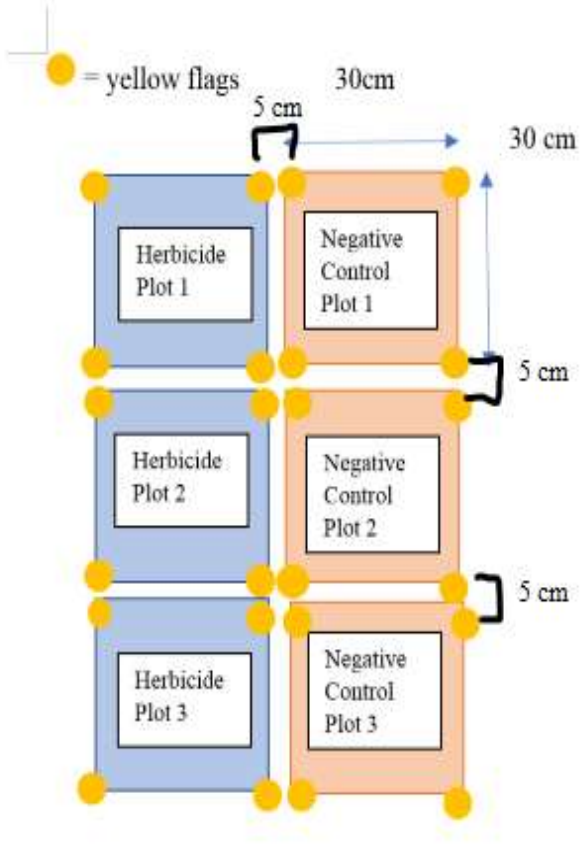
I. Problem: Do herbicides decrease the population density of mycorrhizal fungi on our campus?
II. Hypothesis: Herbicides will decrease the population density of mycorrhizal fungi on our campus.
III. Procedure:

A. Independent Variable: addition of Herbicides to the soil plot
B. Dependent Variable: population density of mycorrhizal fungi in soil samples (#/cc)
C. Negative Control: addition of only Water on one plot of soil
D. Positive control: Soil samples taken prior to adding herbicides or water
E. Controlled Variables:

1. Same area of soil
2. Amount of soil collected
3. Amount of water used during each test
4. Amount of time herbicides and water are left on soil plots
5. Amount of herbicide used during each test
6. Same serial dilution method
7. Plant life around plots of soil
8. Same material of bags for soil samples
9. Size of pipette
10. Amount of sterile water used
11. Size of culture tubes
12. Amount of nutrient agar and type of nutrient agar
13. Amount of liquid put on nutrient agar plates
14. Which dilutions plated on nutrient agar
15. Type of herbicide
16. Size of soil extractor
17. Strength tube is shaken
18. Plot sizes
19. Amount of hours that fungi grows on nutrient agar plate
20. Temperature fungi grows at on nutrient agar plate
21. Serial dilutions done on the same day at the same time
22. Soil collected the same way on the same day at the same time
23. Degree to which soil is diluted
24. Amount of soil diluted

F. Step-by-Step:

1. Use 4 yellow flags per square plot to mark off 6, 30x30cm squares at N 39.35698 W 076.63635 of the campus and label the plots “Negative control plot 1”, “Negative control plot 2”, “Negative control plot 3”, “Herbicide plot 1”, “Herbicide plot 2, “ Herbicide plot 3”. See diagram below.



2. Take all of the following samples at the same time on the same day: Use a 2x47cm 2 cm diameter soil core extractor and take 3 separate, 15 cm deep soil samples from each of the 6 soil plots and place each soil sample in its own separate correspondingly labeled plastic bag.

3. On the same day and at the same time take the positive control samples back to the lab station and mix the 3 samples from one plot together by combining them into one plastic bag and knead them together with your hands until they are the consistency of wet sand, and mix them together so you have a representative sample from that one plot then do that for each plot and soil samples, mix the three samples from one plot together so that in the end you have 6 total soil samples, 1 from each plot.

4. For the steps 4-14 you need to do all of this at the same time on the same day. Use a clean, new transfer pipette to add 10 ml of sterile water to a 15 ml culture tube. Label the tube “Negative control plot 1 before 10^0”.

5. Use the same pipette to add 9 ml of sterile water to a second 15 ml culture tube. Label the tube "Negative control plot 1 before 10^{-1}".

6. Repeat step 5 one more time but add 8 ml of sterile water and label the third tube "Negative control plot 1 before 10^{-2}". [1]

7. Place 1 cc of soil from the negative control, plot 1 before soil sample into the "Negative control plot 1 before 10^{0}**"**culture tube.

8. Cap the tube and shake vigorously.

9. Using a new clean pipette, remove 1 ml of the soil/water mixture from the "Negative control plot 1 10^{0}**"** culture tube and place it into the Negative control plot 1**10^{-1}**culture tube

10. Cap and shake vigorously.

11. Using the same pipette in step 9, remove 1 ml of the soil/water mixture from the "Negative control plot 1 before 10^{-1}**"** tube and place it in the Negative control plot 1 10-2 tube.

12. Cap and shake vigorously.

13. Plate 100 ul samples from the 1st, 2nd, and 3rd tubes (dilutions 100, 10-1,10-2) onto their own separate 3M Petrifilm™ yeast and mold count plate nutrient agar which are correspondingly labeled "Negative control plot 1 10^{0}**",** "Negative control plot 1 before 10^{-1}**",** and **"**Negative control plot 1 10^{-2}" containing 3M petrifilm yeast and mold count plate nutrient agar

14. After this repeat steps 4-13 five more times but label the tubes for each dilution series respectively: "Negative control plot 2 before" and "Negative control plot 3 before" and "Herbicide plot 1 before", "Herbicide plot 2 before", "Herbicide plot 3 before"

15. Allow to grow for 72 hours.

16. Start at lowest dilution and look for yeast, small blue/green dots, and if there are at least 1 colony record what dilution the yeast was found at and how many. If there are not any on the lowest dilution go to the next lowest dilution and look for yeast. Do this same process when looking for mold which are small areas covered by fuzzy fungi. Record all of this into your data table and then use the yeast and mold counts to plug into the formula below to find out the total yeast and mold densities in the 1 cc of soil:

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

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[1] Due to a non-standard serial dilution technique, we had to employ the following equation to the 10^{-2} dilutions when calculating fungal density: (# colonies x 90 x $10^{1.9}$)

17. Add together the calculated numbers of yeast and mold to get total fungi population density

18. Record the numbers found in the data table below.

19. You must do this step all at the same time on the same day. After taking the before soil samples from the 6 plots of soil measure out 28.02 milliliters of water and add 9.34 milliliters of water to each of the three of the plots which are the negative control plots. Mix together 0.54 milliliters of herbicide and 27.48 milliliters of water and then add 9.34 milliliters of this mixture to each of the other three plots which are labeled the herbicide plots.

20. Wait 48 hours, for the herbicides and water to sink into the soil

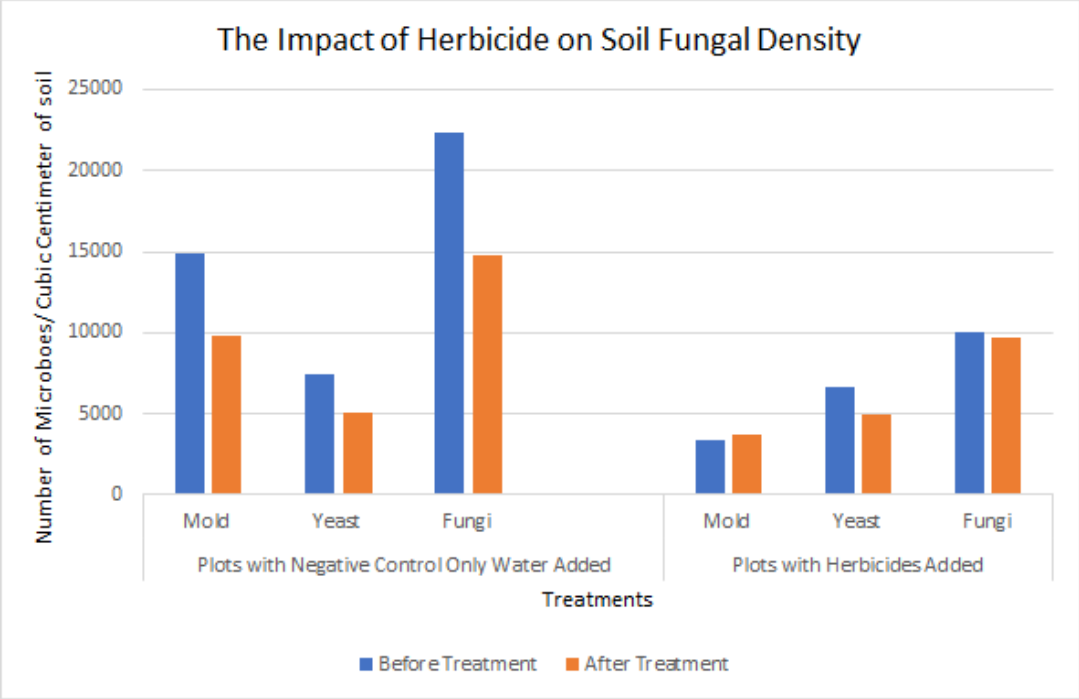
21. Do this step all at the same time on the same day. Use a 2x47cm soil core extractor and take 3, 15 cm soil samples from each soil plot and place the soil in plastic bags.

22. Repeat steps 3-17 but label the tubes for the different serial dilutions respectively: “Negative control plot 1 after”, “Negative control plot 2 after”, “Negative control plot 3 after”, “Herbicide plot 1 after”, “Herbicide plot 2 after”, “Herbicide plot 3 after” including the different dilution values, 100, 10-1,10-2, in each label.

Data and Analysis

The Impact of Herbicide on Fungal Density in Soil

Trials	Plots with Only Water Added						Plots with Herbicides Added					
	Mold levels (#/cc)		Yeast levels (#/cc)		Fungi levels (#/cc)		Mold levels(#/cc)		Yeast levels (#/cc)		Fungi levels (#/cc)	
	Before adding negative control	After adding negative control	Before adding negative control	After adding negative control	Before adding negative control	After adding negative control	Before adding herbicide	After adding herbicide	Before Adding herbicide	After Adding herbicide	Before Adding herbicide	After Adding herbicide
1	21,447	7,149	1,000	3,000	22,447	10,149	1,000	2,000	600	700	1,600	2,700
2	14,298	700	14,298	7,149	28,590	7,849	7,149	4,000	14,298	7,149	21,447	11,149
3	9,000	21447	7,149	5,000	16,149	26,447	2,000	5,000	5,000	7,149	7,000	12,149
Averages	14,915	9,765	7,482	5,050	22,395	14,815	3,383	3,667	6,633	4,999	10,016	9,666



Conclusion

Our hypothesis stated that herbicides will decrease the population density of mycorrhizal fungi on our campus, which was proven incorrect. In the negative control the average fungi population per cubic centimeter went from 22,395 to 14,815. In the negative control soil samples the average mold level decreased from 14,915 mold per cubic centimeter to 9,765 mold per cubic centimeter after water was added. The average yeast levels also decreased, from 7,482 yeast per cubic centimeter to 5,050 yeats per cubic centimeter after water was added. The fact that the decrease in mold was larger than the decrease in yeast shows that the something in the environment caused the fungi to be in their protective state. Based on the negative control this tells us that in the herbicide plots the herbicide helped because the average fungi population went from 10,015 fungi per cubic centimeter before treatment to 9,666 fungi per cubic centimeter after treatment. The average mold per cubic centimeter went from 3,383 to 3,667. The average yeast population per cubic centimeter went from 6,632 to 4,999. Because of what was exhibited in the negative control the average numbers of mold, yeast, and fungi per cubic centimeter should have plummeted because of the combination of the environmental factor present in the negative control, and our hypothesized negative effect of herbicide. Although, since the average mold per cubic centimeter increased from 3,383 to 3,667 this shows that the environment got better for the mold after adding herbicide. The environment got better for the molds because of the relationship that they have with decaying matter. When the herbicide kills the weeds, the molds decay the dead plant material and are able to reproduce quicker, increasing the population, because of the energy source that is created. This is also shown in the decrease of average yeast from 6,632 to 4,999 per cubic centimeter. It is shown that the herbicides counteracted the environmental factor because of the ratio of the decrease in the negative control plots and the

herbicides plots of yeast, mold and fungi per cubic centimeter. The average yeast per cubic centimeter decreased by 32.5% in the negative control plot while the yeasts per cubic centimeter decreased by 24.6% in the herbicide plots. This shows that the yeast in the herbicide plots were able to survive despite the environmental factor that was decreasing the mold per cubic centimeter in the negative control plots. The average mold per cubic centimeter decreased by 34.5% in the negative control plots, and the the mold per cubic centimeter increased by 8.39%. The fact that the mold per cubic centimeter decreased in the negative control plots and increased in the herbicide plots shows that the environment created by adding herbicide was beneficial to the mold. The average fungi per cubic centimeter decreased by 33.85% in the negative control plots, the average fungi per cubic centimeter decreased by 3.5% in the herbicide plots. The general fungi data supports this conclusion even further because of the the large difference between the percentages of decrease in the negative control and herbicide plots. For further research to explain what happened to cause this conclusion, this experiment could be moved to a different environment or we could stay in the same environment and change the type of herbicide. If we moved the location of the experiment, we would differ the plants in the location so we could see how the selective herbicide would affect different plants and their relationship with fungi. If we stayed in the same location but chose a non-selective herbicide we could see how it would affect the same plant life and the fungi population.