The Effects of Glyphosate Herbicide on the Density of Fungi in the Soil

Mehek Singh, Payton Gore, Kathryn Kleiser

Background:

Fungi are a common type of microbe that survive by decomposing and absorbing the organic material in which and on which they grow in, and they play a large role in the health of an ecosystem. Mushrooms, molds, mildew, and yeasts are all common examples of the different types of fungi frequently found in the soil, and they are responsible for recycling dead organic material and returning it to the soil in a new form in order that it may be reused by plants and other organisms that live there (Alexopoulos 2017). In fact, without fungi, many types of dead organisms and plant remains would not decompose at all, trapping their nutrients from being available to the rest of the ecosystem.

Plants in particular depend on fungi to help them absorb nutrients from the soil through a mutual symbiosis known as mycorrhizae that help the two different types of organisms survive together. In this partnership, the fungi collect nutrients from the mineral parts of the soil, by releasing enzymes that break down the minerals so they can be dissolved by the plant including phosphorus, and give them to the plant so that it can survive. The structure of the hyphae and mycelium allow the fungi to reach more surface area and break down more minerals.The plant in return gives some sugar, which the plant produced during photosynthesis, to the fungi, so that the fungi has the energy it needs to survive. (Bryce 2002). In addition, the branching extensions of fungi, called hyphae, increase the surface area of the plant root system, enabling the plant to absorb more nutrient minerals than the plant could on its own. (Boundless 2016).

There are two types of mycorrhizae, endomycorrhizae and ectomycorrhizae. Endomycorrhizae involves microscopic fungi which penetrate the plant's root cells and make tiny organs inside it, living inside the plant itself. In ectomycorrhizae, on the other hand, fungi produce a web of hyphae around the plant's root and never penetrate the actual root cells. (Bryce

2002). Plants with endomycorrhizae are usually located in forests with a high abundance of species, whereas plants with ectomycorrhizae are located in forests with a low abundance of species. The roots of ectomycorrhizal trees support a number of fungi symbionts, amounting to more than 5,000 species around the world, while endomycorrhizal trees, have low fungal species richness, with only 30 species of fungi around the world. (D.W. Malloch, K.A. Pirozynski, P.H. Raven 1980). Yet the majority of land plants form endomycorrhizae. (D.W. Malloch, K.A. Pirozynski, P.H. Raven 1980). This is because in endomycorrhizal plants, the fungi physically penetrate the plants roots, and help plants to obtain nutrients such as phosphorus more directly and efficiently from the soil.

However, regardless of which type of Mycorrhizae a particular plant is associated with, this fungal partnership is crucial to the survival of plants. Even more importantly because plants provide and support life on earth, mycorrhizae are critical to the survival of every complex organism in an ecosystem. As producers in the food chains, plants directly and indirectly help organisms to obtain food, and as a major source of oxygen, plants are necessary for most organisms to be able to transform energy, therefore life without plants and soil, is impossible (Clark 2014). Producers, the plants, convert energy from the environment into carbon bonds, found in glucose. Then the primary consumers eat the plants, and obtain energy from the nutrients. Then, the secondary consumers eat the primary consumers, gaining energy. Hence, all the organisms in an ecosystem ultimately depend on plants for food and survival, and because the mycorrhizal fungi help plants to grow, these fungi are equally critical for all terrestrial life. (Boundless 2016).

Though the value of fungi in the soil ecosystem is well known, harmful chemicals are often used on plants to control their growth that may be detrimental to the health of these fungi and, hence, the soil ecosystem. Herbicides in particular are commonly used around the world, particularly in the United States, for agricultural and gardening to kill off undesired plants (typically weeds and other dicots) that can compete with crops or other desired plants, and one of the most effective, and therefore most frequently used, chemicals among the many types of herbicides is Glyphosate. (Ganzel 2009). Glyphosate is nonselective and, therefore, can kill any plant onto which it is sprayed. It works by stopping plant growth, causing the plant to die, and the mechanism behind its activity is that it prevents aromatic amino acids necessary for protein synthesis from being produced in the shikimic acid pathway (Anderson, A. M.; Gervais, J. A.; Luukinen, B.; Buhl, K.; Stone, D. 2010). And without the ability to build the proteins that serve as enzymes to control chemical reactions in the plant, photosynthesis cannot be performed; cells cannot perform the four tasks of life, reproduction, regulation, respiration, synthesis; and the plant dies.

But while Glyphosate is very effective in killing plants, it also may cause damage to the soil surrounding the undesired plant. There have been many studies done about the effects of Glyphosate on soil, with mixed results. Some studies have found that when a plant living with the support of Mycorrhizal Fungi is sprayed, the beneficial Fungi are killed as well (Zaller, J. G., Heigl, F., Ruess, L., & Grabmaier, A. 2014). On the other hand, Glyphosate in the soil has been shown to benefit other types of fungi and bacteria that can break down the chemical using enzymes in order to produce phosphorus, carbon, and nitrogen, which the organisms use to feed themselves. Hence Glyphosate has been shown to actually help some types of Fungus to survive because these organisms can live off of it. Indeed, a highly consistent result of research has shown that fungal populations, aside from mycorrhizae, usually increase when Glyphosate has been used (Soil Association, 2017).

Because fungi affect the health of the ecosystem in a positive way, we wanted to examine the effects of the herbicide, glyphosate, on the fungi in our lawns to see what impact the herbicide might be having. By comparing the soil before adding the herbicide and after, we will observe how it affected the density of fungi in the soil. We predict that applying the glyphosate herbicide will increase the density of fungi in the soil.

References

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Lab Report:

I. Problem: Does Glyphosate herbicide increase or decrease the density of fungi in the soil?

II. Hypothesis: Applying glyphosate herbicide will increase the density of fungi in the soil.

III. Procedure:

A. Independent Variable: The addition of glyphosate herbicide to the soil

B. Dependent variable: The density of fungi in the soil (#/cm^3)

C. Positive Control: Soil collected before adding any glyphosate

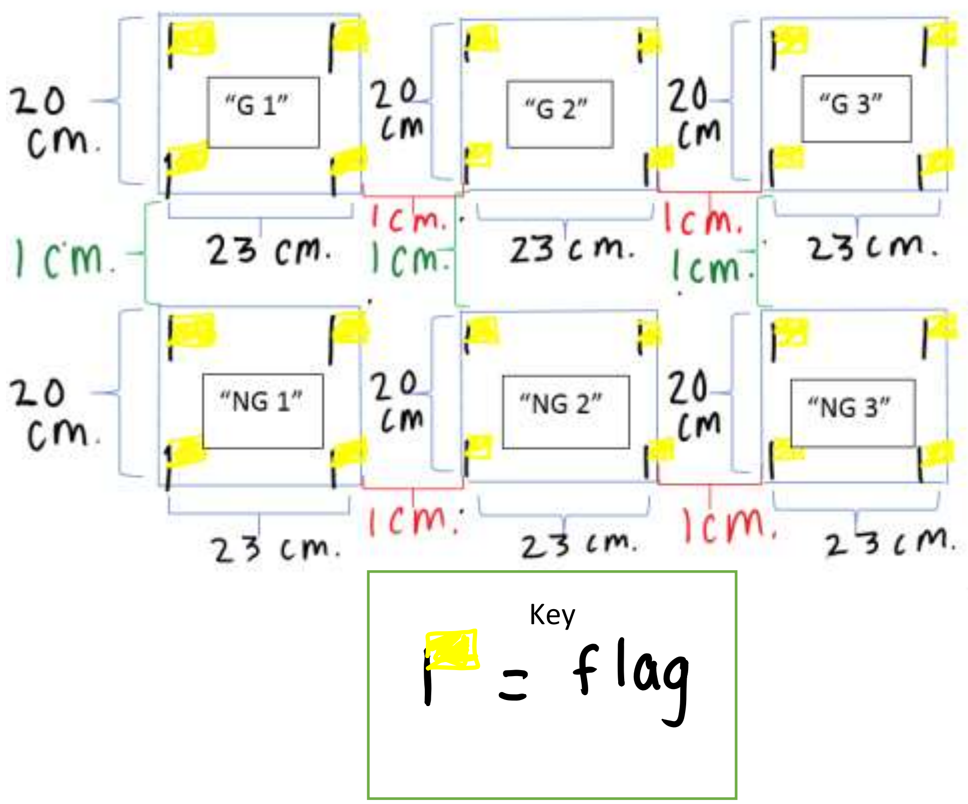
D. Negative control: A soil plot with only water and no glyphosate herbicide added to it

E. Control variables: amount of soil collected, amount of water given to soil, type of soil, type of plants growing on soil, size of plant, amount of glyphosate, source of glyphosate, time/day/location soil collection and dilution, serological pipettes, size of culture tubes, amount of sterile water used, amount/type of agar plates, amount of soil added to dilute, amount diluted, amount plated, amount of time given to grow, temperature it grows at, which dilutions were plated

F. Step by Step:

1. Label four flags “glyphosate (1)” and four flags “no glyphosate (1)”

2. Repeat step 1 two additional times, but the second time replace the "(1)" with "(2)" and the third time, write "(3)" as the number on the "glyphosate" and the "no glyphosate" flags.
3. At the coordinates N 39° 21.411, W0 76° 38.174, make a 20cm by 23 cm rectangular plot using the labeled flags, pushing the flags 5 cm deep into the soil. Make six of these plots to look like the diagram shown below:



4. Label 18 plastic bags, each one a different name. The names should be as follow: "Glyphosate 1-1", "Glyphosate 1-2", "Glyphosate 1-3", "Glyphosate 2-1", "Glyphosate 2-2", "Glyphosate 2-3", "Glyphosate 3-1", "Glyphosate 3-2", "Glyphosate 3-3", "No Glyphosate 1-1", "No Glyphosate 1-2", "No Glyphosate

1-3”, “No Glyphosate 2-1”, “No Glyphosate 2-2”, “No Glyphosate 2-3”, “No Glyphosate 3-1”, “No Glyphosate 3-2”, “No Glyphosate 3-3”

5. At the same time, on the same day, using a soil core extractor, take three 15 ½ cm deep by 2 cm in diameter soil samples from the “Glyphosate (1)” plot and place each individual soil sample into its corresponding plastic bag: “Glyphosate 1-1”, “Glyphosate 1-2”, “Glyphosate 1-3” plastic bag. Take 3 soil samples from each of the 6 plots and place each sample into its correspondingly labeled bag. There will be 18 samples total.
6. Back at a black lab bench, place all of the plastic bags with the soil samples on the bench.
7. Collect six new bags and label them as followed: “Glyphosate (1)”, “Glyphosate (2)”, “Glyphosate (3)”, “No Glyphosate (1)”, “No Glyphosate (2)”, and “No Glyphosate (3).
8. Steps 9-14 should occur at the same time, on the same day, and be sure to wash hands thoroughly between each step.
9. Take the soil samples from “Glyphosate 1-1”, “Glyphosate 1-2”, and “Glyphosate 1-3” and put the samples in the “Glyphosate (1)” bag and mix the soil samples thoroughly with one’s hands until the soil has a texture of wet sand.
10. Take the soil samples from “Glyphosate 2-1”, “Glyphosate 2-2”, and “Glyphosate 2-3” and put the samples in the “Glyphosate (2)” bag and mix the soil samples thoroughly with one’s hands until the soil has a texture of wet sand.

11. Take the soil samples from "Glyphosate 3-1", "Glyphosate 3-2", and "Glyphosate 3-3" and put the samples in the "Glyphosate (3)" bag and mix the soil samples thoroughly with one's hands until the soil has a texture of wet sand
12. Take the soil samples from "No Glyphosate 1-1", "No Glyphosate 1-2", and "No Glyphosate 1-3" and put the samples in the "No Glyphosate (1)" bag and mix the soil samples thoroughly with one's hands until the soil has a texture of wet sand.
13. Take the soil samples from "No Glyphosate 2-1", "No Glyphosate 2-2", and "No Glyphosate 2-3" and put the samples in the "No Glyphosate (2)" bag and mix the soil samples thoroughly with one's hands until the soil has a texture of wet sand.
14. Take the soil samples from "No Glyphosate 3-1", "No Glyphosate 3-2", and "No Glyphosate 3-3" and put the samples in the "No Glyphosate (3)" bag and mix the soil samples thoroughly with one's hands until the soil has a texture of wet sand.
15. Steps 16-30 should occur at the same time, on the same day.
16. Pour 50 ml of sterile water into a 250ml beaker.
17. Take a new pipette and label it "sterile water"
18. Using the "sterile water" pipette, add 10 mL of sterile water dilution to a 15 ml culture tube. Label the tube "10^0 glyphosate 1" and set the tube into holder
19. Use the "sterile water" pipette to add 9 ml of the solution to a second tube, labeling this tube "10^{-1} glyphosate 1" and set the tube into holder
20. Use the "sterile water" pipette to add 9 ml of the serial water dilution to a 15ml culture tube and label the tube "10^{-2} glyphosate 1" and set the tube into the holder
21. Repeat steps 18-20 five additional times changing the names of the tubes each time to as followed:

   a. First repeated time: “10^{0} glyphosate 2”, “10^{-1} glyphosate 2”, “10^{-2} glyphosate 2”
   b. Second repeated time: “10^{0} glyphosate 3”, “10^{-1} glyphosate 3”, “10^{-2} glyphosate 3”
   c. Third repeated time: “10^{0} no glyphosate 1”, “10^{-1} no glyphosate 1”, “10^{-2} no glyphosate 1”
   d. Fourth repeated time: “10^{0} no glyphosate 2”, “10^{-1} no glyphosate 2”, “10^{-2} no glyphosate 2”
   e. Fifth repeated time: “10^{0} no glyphosate 3”, “10^{-1} no glyphosate 3”, “10^{-2} no glyphosate 3”

22. Place 1 cc of the “Glyphosate 1” soil sample into the “10^{0} glyphosate 1” culture tube. Cap the tube and shake vigorously for 30 seconds.
23. Use a new pipette and label it “transfer” and then use it to remove 1 ml of the soil/water mixture from the “10^{0} glyphosate 1” tube and place it into the “10^{-1} glyphosate 1” tube.
24. Cap the “10^{-1} glyphosate 1” tube and shake vigorously for 30 seconds
25. Using the pipette labeled “transfer”, remove 1 ml of the soil water mixture “10^{-1} glyphosate 1” tube and place into the “10^{-2} glyphosate 1” tube.
26. There should now be a total of three culture tubes.
27. Label three separate 3M Petrifilm™ Yeast and Mold Count Plates respectively as follow: “10^{0} glyphosate 1”, “10^{-1} glyphosate 1”, “10^{-2} glyphosate 1”
28. Plate 100 ul samples using a micropipette from each the tubes onto their own separate, correspondingly labeled, 3M Petrifilm™ Yeast and Mold Count Plates.

29. Repeat steps 22-28 five more times with the soil samples from “Glyphosate (2)”, “Glyphosate (3)”, “No Glyphosate (1), “No Glyphosate (2)”, and “No Glyphosate (3)”, with the tubes that match the name of the soil sample and a new “transfer” pipette for each separate dilution.
30. Let the dishes with the soil/water solution sit for 72 hours in order for the solutions to grow
31. Using a magnifying glass, examine the plates for yeast and mold. Always start with the lowest diluted plate and observe for yeast and mold. If both yeast and mold are found, record the amount of yeast and mold in the data table, and move onto the next set of plates. If only yeast is found, record the amount of yeast in the data table and move to next highest diluted plate. If only mold is found, record the amount of mold in the data table and move to next highest diluted plate. Be sure to have more than one person examine the plates.
32. After recording the amount of yeast and mold in the data table, use the formula # of microbes in 1 cc of soil= # of colonies on sheet x 10^2 x $10^{|\text{delusion \# at which three colonies were found}|}$ to find the density of fungi in the soil
33. Record on the data table the number of microbes of fungi the soil had with the units cm^3
34. Steps 35-43 should occur at the same time, on the same day, at the same location
35. Go to N 39° 21.411, W0 76° 38.174 with “Roundup Herbicide” and a spray bottle filled with water. Use gloves and goggles when working with herbicide.
36. On the “Glyphosate (1)” plot, spray three mists of the “Roundup Herbicide” 30 cm above the plot, and careful to avoid getting the spray in any other plots.

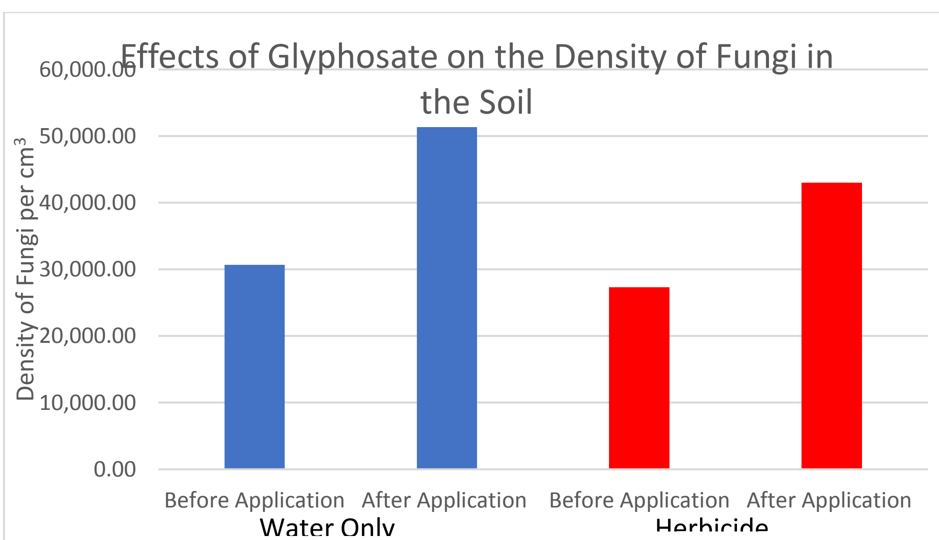
37. Spray three mists of water 30 cm above the “Glyphosate (1)” plot immediately after spraying the herbicide.
38. On the “Glyphosate (2)” plot, spray three mists of the “Roundup Herbicide” 30 cm above the plot, and careful to avoid getting the spray in any other plots.
39. Using the water spray bottle, spray three mists of water 30 cm above the plot labeled “Glyphosate (2)” immediately after spraying the herbicide
40. On the “Glyphosate (3)” plot, spray three mists of the “Roundup Herbicide” 30 cm above the plot, and be careful to avoid getting the spray in any other plots.
41. Spray three mists of water 30 cm above the “Glyphosate (3)” plot immediately after spraying the herbicide
42. Spray three mists of water 30 cm above the “No Glyphosate (1)” plot
43. Repeat step 42 two additional times, spraying three mists of water onto each of the “No Glyphosate (2)”, and “No Glyphosate (3)” plots
44. Wait 48 hours before returning to N 39° 21.411, W0 76° 38.174, where the plots are.
45. Repeat steps 4-33 with the plots with and without Glyphosate.

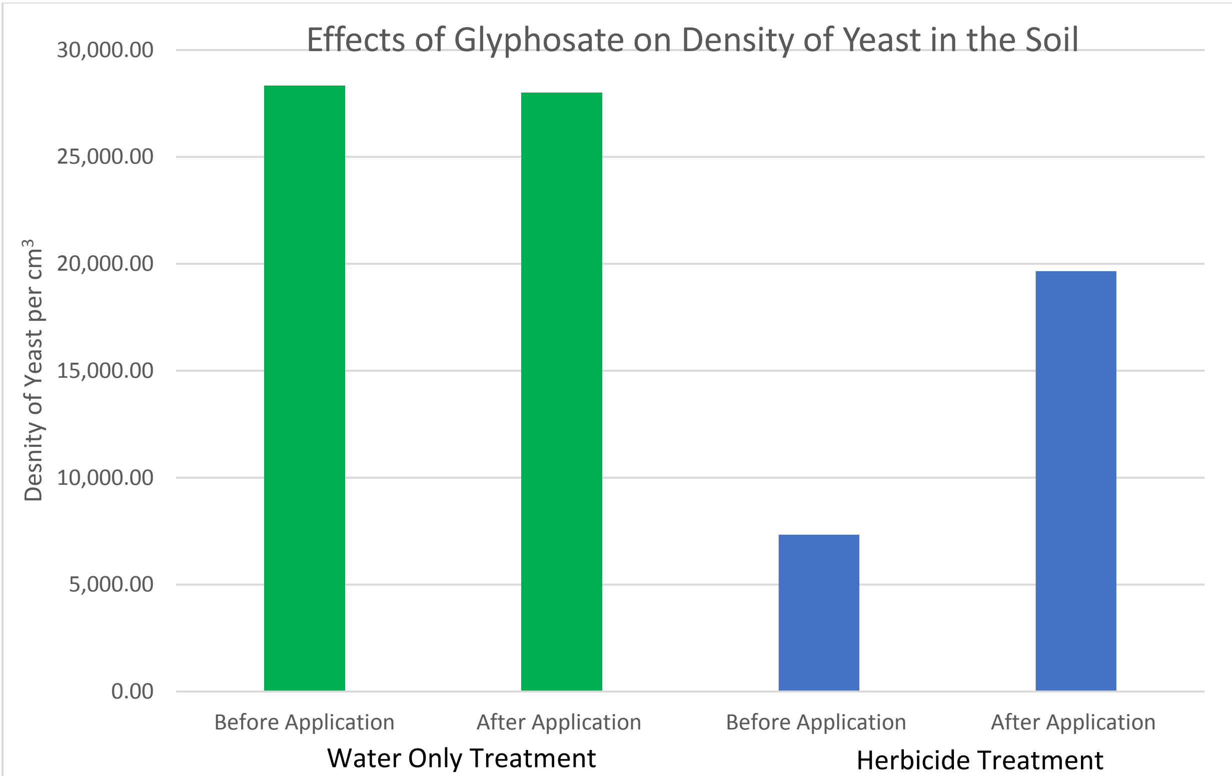
IV. Data and Analysis

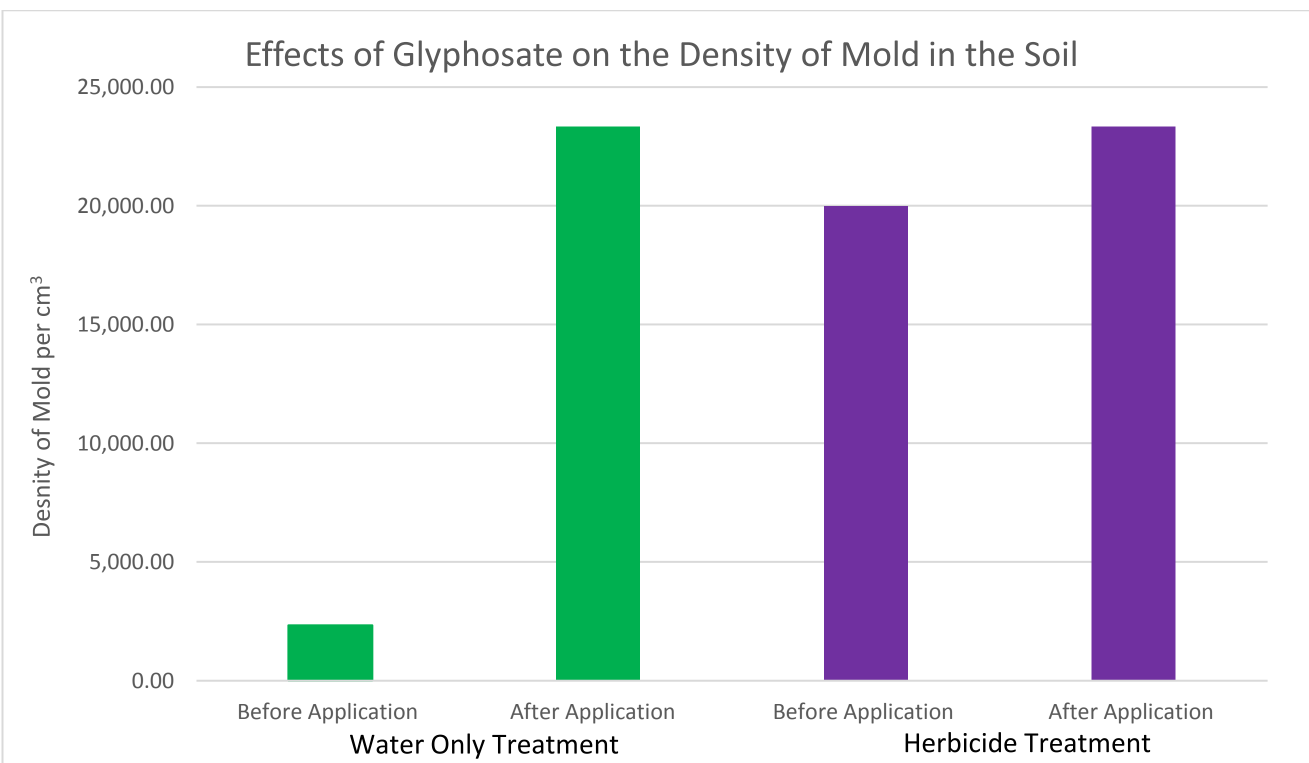
A. Data Table

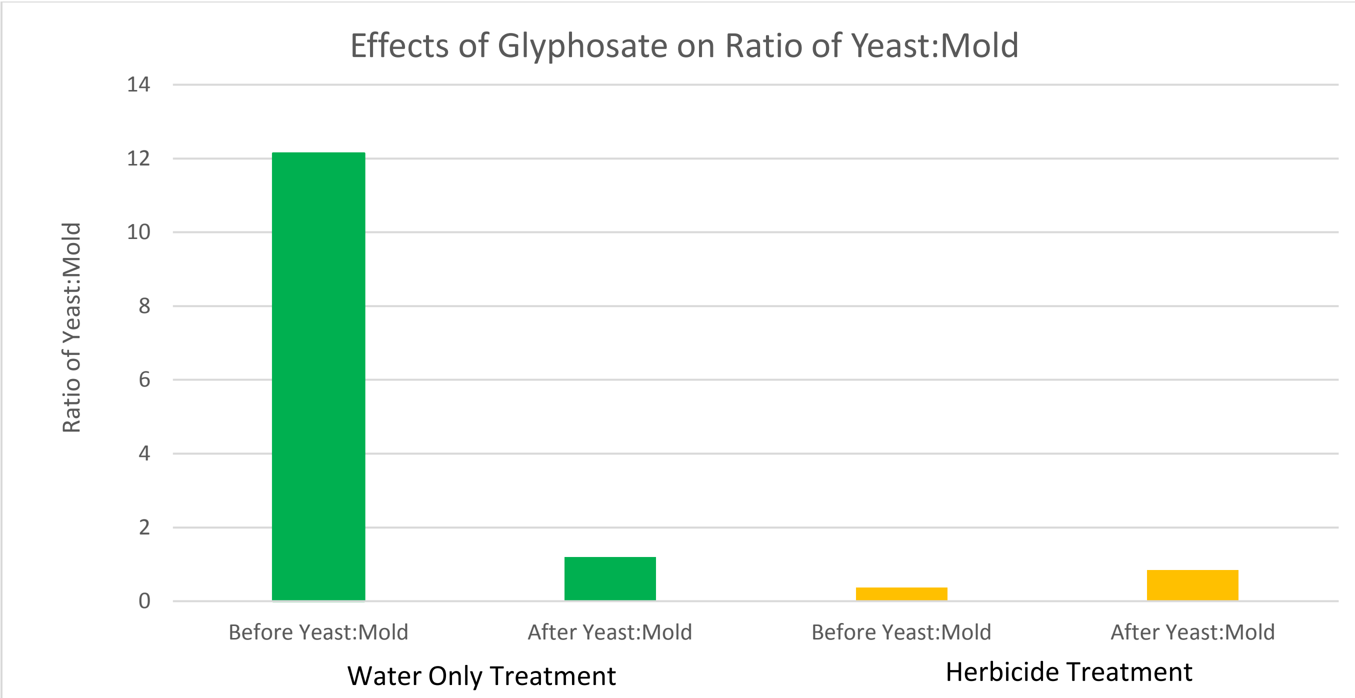
The Effects Of Glyphosate Herbicide on Fungi Levels in Soil												
	Negative Control: Application of H_20 on Plots						Application of Glyphosate on Plots					
	Yeast Density in microbes per cm^3		Mold Density in microbes per cm^3		Fungi Density in microbes per cm^3		Yeast Density in microbes per cm^3		Mold Density in microbes per cm^3		Fungi Density in microbes per cm^3	
Trials	Before Glyphosate	After Glyphosate	Before Glyphosate	After Glyphosate	Before Glyphosate	After Glyphosate	Before Glyphosate	After Glyphosate	Before Glyphosate	After Glyphosate	Before Glyphosate	After Glyphosate
1	50,000	50,000	1,000	20,000	51,000	70,000	8,000	5,000	20,000	20,000	28,000	25,000
2	5,000	4,000	1,000	20,000	6,000	24,000	10,000	4,000	20,000	10,000	30,000	14,000
3	30,000	30,000	5,000	30,000	35,000	60,000	4,000	50,000	20,000	40,000	24,000	90,000
Aver ages	28333.3	28,000	2333.3	23,333.3	30666.6	51,333.3	7333.3	19,666.6	20000	23,333	27, 333.3	43,000

B. Graph or Summary of Analysis




Effects of Glyphosate on Density of Yeast in the Soil
Desnity of Yeast per cm3
30,000.00
25,000.00
20,000.00
15,000.00
10,000.00
5,000.00
0.00
Before Application
After Application
Before Application
After Application
Water Only Treatment
Herbicide Treatment


Effects of Glyphosate on the Density of Mold in the Soil
Desnity of Mold per cm3
25,000.00
20,000.00
15,000.00
10,000.00
5,000.00
0.00
Before Application
After Application
Before Application
After Application
Water Only Treatment
Herbicide Treatment



V. Conclusion

Our hypothesis, which predicted that applying glyphosate herbicide will increase the density of fungi in the soil, was incorrect. Our data shows that glyphosate reduced certain components of fungi including yeast and mold. Prior to adding glyphosate to certain plots, our group took soil samples from all six of our plots. We did a serial water dilution to find levels of yeast and mold in each plot, and then calculated our total amount of fungi in the soil. The initial soil samples collected for the data served as our positive control variable, and showed our group whether fungi levels were changing as a result of glyphosate, or because of the environment. Before adding any glyphosate or water to our plots, the average density of yeasts for all three trials was 28,333.3 microbes per cm^3, and the average density of yeast after adding water was 28,000 microbes per cm^3. The average density of mold before adding water was 2,333.3 microbes per

cm^3, and the average density of mold after adding water was 23,333.3 microbes per cm^3. The density of fungi in the positive control plots, before adding water, was 30,666.6 microbes per cm^3, and the density of fungi after applying water was 51,33.3 microbes per cm^3. After waiting the appropriate time, we went to our plots and sprayed glyphosate on the three glyphosate plots, and immediately watered all the plots. After examining the soil, we gathered our data. In the negative control plots, which were plots with no glyphosate and just water applied to them, the average density of yeast for all three trials was 7,333.3 microbes per cm^3, and the average density of yeast after applying glyphosate increased to 19,666.6 microbes per cm^3. The average density for mold before applying glyphosate was 20,000 microbes per cm^3, and the average density for mold after applying glyphosate increased to 23,333 microbes per cm^3. The average density for fungi before adding glyphosate was 27,333.3 microbes per cm^3, and the average density for fungi after applying glyphosate was 43,000 microbes per cm^3.

When examining the data, the glyphosate seemed to have several effects on the fungi, especially when observing the density of yeast and mold. After observing the data from the density of fungi in the water only treatment, we concluded that the amount of fungi increased after adding water, and in the herbicide treatment, the amount of fungi also increased by a relatively similar ratio. From this data, we could infer that the environment played a role in this increase of fungi, but when taking a closer look at the types of fungi (yeasts, and microbes), the similar ratio is not shown. The density of yeast in microbes per cm^3 after adding water decreased a small amount, whereas the density of fungi after adding glyphosate in comparison to before adding glyphosate increased significantly. The density of mold in microbes per cm^3 after adding water increased significantly when compared to the density of fungi before the application of water. By this ratio, if glyphosate truly helped the fungi, the density of mold should have increased by somewhat the same ratio. Though the density of fungi in microbes per cm^3 increases after the application of glyphosate, it does not increase by a significant amount like it did when treated with water. So, glyphosate prevented the growth of mold. Mold are aerobic organisms, and are unable to live in detrimental conditions, especially when there is no oxygen, whereas yeast are able to grow and survive in such conditions. In an ideal, healthy environment, mold would be able to grow and prosper. So, perhaps the lower levels of mold indicate that the addition of glyphosphate did not provide a healthy environment for the organisms. When examining the ratio between yeast and mold, our data may be considered inconclusive. After the application of water only, the ratio of yeast to mold decreases a considerable amount, showing that there was a greater amount of mold, proving the soil was healthier. The ratio of yeast to mold increased after the application of glyphosate, proving that the amount of yeast increased, and mold decreased. Though this increase is small, it leads us to future research and experiments, that would explain what in the environment allowed glyphosate to effect mold and yeast in different ways.

To better understand our data, an experiment could be performed to test for the density of fungi, while influencing another part of the environment. In order to see what in the environment influenced the density of mold and yeast, we could test for the diversity of microbes and collect soil from several different locations. With more locations, we would observe areas with lots of plants, vs. places where there are fewer plants. We would be sure to control the time of our experiment, just altering the location. We could examine larger quantities of mold and yeast, and compare to our current data. By testing in different locations, it would allow us to see if the glyphosate had the effect on the density of fungi, or a different environmental factor. Another experiment that could be performed is testing for fungi in the soil in various weather conditions.

When the positive control samples for our experiment were collected, it was raining heavily, which could have impacted the density of mold and yeast, when compared to a sunny day of a windy day. This would also allow us to see is the glyphosate effected the fungi, or if it was the environment.