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Soil Ecology: Herbicide Background Report

One of the types of microbes that plays a significant role in soil is fungi. Fungi benefit soil and plants in various ways, aiding in decomposition and soil aggregation (Parris, n.d.). Decomposition is where fungi break down dead organic matter, such as dead plants, animals, or insects; into its simple components: water, nutrients, and carbon dioxide. Water is an important compound that plants take up from the soil in order to engage in photosynthesis. Certain nutrients from the soil will also help plants to grow and develop (Simmons, 2009). Nitrogen is one of the essential elements plants need to perform photosynthesis, which creates their own food, along with water and oxygen for the environment (Higgins, n.d.). The other major process fungi participation soil participate in, called soil aggregation, fungi use their hyphae (long slender tubes that develop from their germinated spores) to clump together soil particles, which creates spaces between the clumps that can be used to store things such as air, water, nutrients, microbes, and organic matter. Soils that have many aggregates are healthier, as aggregated soil remains stable against water movement due to its water and nutrient-holding pores located between aggregates (Collins, 2004).

One of the most important types of fungi in soil is called mycorrhizal fungi. There are two major classes of mycorrhizal fungi: endomycorrhizae and ectomycorrhizae (Reid, n.d.) Endomycorrhizae are involved in helping vascular plants, such as flowers and certain vegetables like asparagus (Fiore, n.d.) Endomycorrhizae grow at the cortical cells of the roots of these plants. The endomycorrhizae aid plants in capturing micronutrients like phosphorus from the soil,

and playing a crucial role in the initial colonization and evolution of vascular plants. Ectomycorrhizae are typically found on the roots of woody plants, such as birch trees or pine trees. The ectomycorrhizae form an extensive network within the soil and leaf litter in order to move nutrients between different plants (Aurea, 2012).

Mycorrhizal fungi have a symbiotic relationship with the plants growing in the soil, in which the fungi and plants mutually benefit from providing something to the other organism. Mycorrhizal fungi assist plant roots in retaining moisture and soaking up nutrients. They also link to the cell walls of the plant roots and grow into them, which creates structures that allow nutrients to transfer between them. Without these fungi to support the plants, it will be much harder for plants to get nutrients and water; which will slow down their growth and ability to photosynthesize (Erdimer, n.d.). Mycorrhizal fungi also provide plants with the nutrient phosphorous. Phosphorous plays a key role in many critical plant processes; such as metabolism, respiration, and photosynthesis. It also enhances plant development with flowering, fruiting, and root growth. In return for this help, the plant supports the mycorrhizal fungi by providing them with the sugar it needs to keep growing, because fungi are heterotrophic and cannot photosynthesize (Uhde-Stone, 2004).

Aside from phosphorus, mycorrhizal fungi helps the plants get iron and organic nitrogen. Iron is important to plants because it is essential for the formation of chlorophyll (NCDA&CS, n.d.). Chlorophyll is important because it yields light energy to be turned into chemical energy necessary to survive (McIntosh, 2013). Nitrogen benefits plants by strengthening their roots, which allows them to take in more water and nutrients. Aside from this, nitrogen is crucial to DNA production. DNA is what transcribed into RNA, which is then translated into amino acids. Amino acids make proteins, which start and stop all chemical reactions; which involve the use

and release of energy to make and break bonds to form new substances. Chemical reactions are one of the four tasks of life; necessary to create a cell. Cells are what build up the plants and keep them alive, so without cells plants would die. So, if there was no nitrogen in the plant to compose cells, then the plant would die (House and Garden, n.d.).

Aside from contributing to plant growth, mycorrhizal fungi also benefit the general soil health. In 2010, a soil microbiologist named Kris Nichols observed that mycorrhizal fungi are able to bind soil together into clumps and increase the amounts of long lasting carbon in the soil. Mycorrhizal fungi do this by wrapping soil particles together and then creating a sticky substance called glomalin, which is composed by forms of carbon. This process creates clusters of soil that build air pockets in the soil, which stabilizes the soil structure. This keeps organic matter and nutrients within the air pockets of the soil instead of on top of the soil. This prevents the nutrients from being lost when the top of the soil is lost through erosion (Ziegler, 2010).

However, mycorrhizal fungi are being harmed by certain herbicides. Herbicides are one of the most common pesticides; specifically used to kill unwanted plants, such as weeds. Humans use herbicides to kill off these weeds because weeds use the same nutrients, water, space, and sunlight that other plants growing in people's gardens need. Therefore, the weeds and other plants will need to compete for these resources (Frick, 2012). One of the most commonly used herbicides is called Roundup™ (Handzo, n.d.). Roundup™ is used by many gardeners and homeowners to kill weeds. It typically comes in the form of a spray product, premixed and ready to use. Roundup™ was the most used herbicide in 2007, as it is successful in eliminating weeds down to their roots. Roundup™ is a non-selective herbicide, meaning that all plants it has contact with will be destroyed (Handzo, n.d.). This herbicide kills plants it is sprayed on by inhibiting one of them enzymes needed to grow, called EPSP synthase. Without this protein

plants are unable to produce proteins they need to grow, and die within a few days (N.N., 2000). If these plants are being killed off, the symbiotic relationship between them and the mycorrhizal fungi will be broken; and they won't be able to give sugar to the fungi anymore. Therefore, the fungi will die as well.

One of the most active ingredients in the herbicide Round Up is a chemical called glyphosate. Although glyphosate is what contributes most to the killing of gardeners' unwanted plants, it is actually very harmful to certain parts of the soil. Glyphosate is quite harmful to the soil because it kills beneficial soil microbes that have crucial roles in the soil, one of which is mycorrhizal fungi. As humans, we need to be aware of the harmful effects of Roundup™ weed killer. The more Roundup™ that is used, the less these fungi can contribute to their environment. Without fungi to support them, producers will no longer be able to grow and flourish, and consequently die off. Therefore, the primary consumers, secondary consumers, and so on would have no source of energy because the energy provided by the fungi would be lost and the ecosystem would collapse (Ziegler, 2010).

In conclusion, fungi are beneficial to the soil in multiple ways; in ways such as decomposition, soil aggregation. Aside from benefitting the soil, mycorrhizal fungi also benefit the plants by helping them gain nutrients such as iron, nitrogen, and phosphorus. However, this herbicide Roundup™ is harmful to the soil because when it is used to kill off plant in the soil, it breaks the symbiotic relationship between the mycorrhizal fungi and plants. Without that symbiotic relationship between them, the fungi will die off because they will no longer have the sugar they need to survive. From this research one can hypothesize that when exposed to the herbicide Roundup™, the mycorrhizal fungi population in soil will decrease.

I. Problem: Does the mycorrhizal fungi population in soil change after the use of the herbicide Roundup™?

II. Hypothesis: The mycorrhizal fungi density in soil will decrease after exposure to the herbicide Roundup™.

III. Procedure

A. Independent variable: soil with the herbicide Roundup™

B. Dependent variable: the yeast and mold population density

C. Negative control: soil with water

D. Controlled: the amount of the Roundup™ used, the amount of soil collected from each site, time the soil is exposed to Roundup™, location of soil, time we collect the soil sample, size of site, location of site, diameter of soil cylinder, amount of water used to spray, temperature of water used to spray, where the soil sample was stored after collection, size of culture tubes, amount of sterile water, source of sterile water, amount of soil put into test tube, amount of solution plated, size of plates, type of plates, how long we let the fungi grow on the plates

E. Step-by-step Instructions

1. Plot six 20x20 cm sites on a grassy/ soil area, each 10 cm apart at a coordinate 39.35722° north and 076.63510° west. See Diagram 1 to observe set – up and how to label the sites.

2. Be sure to take each trial's samples on the same day at the same time – so take all the same colored samples shown in the diagram above on the same day at the same time

   Ex: take all "red" trial 1 before samples from each site on the same day at the same time; take all "yellow" trial 2 after samples on the same day at the same time

3. Take 3 soil samples from each site using a 15 cm. deep soil cylinder with a 2 cm. diameter by sticking the soil cylinder into the soil and pounding down with a mallet, then twisting clockwise and pulling up out of the ground – do this until the soil cylinder is 15 cm. full of soil

4. Place each separate sample in a plastic bag labeled with "before", its site location (either IV site 1, IV site 2, IV site 3, NC site 1, NC site 2, NC site 3), and its trial #

5. Dilute your "NC site 1 T1 before" soil sample by following steps 7-22, and be sure to dilute all of the other trial 1 samples in one day at the same time

6. Use a clean, new transfer pipette to add 10 ml of sterile water to a 15 ml culture tube. Label this tube "10^{0}", and label it "NC site 1 T1 before"

7. Use the same pipette in step 7 to add 9 ml of sterile water to a second 15 ml culture tube. Label the tube "10^{-1}" and label it NC site 1 T1before"

8. Repeat step 8 one more time for one additional 15 ml culture tube, label it "10^{-2}" and "NC site 1 T1 before"

9. Place 1 cc of your NC site 1 trial 1 soil sample into the "10^{0} NC site 1 T1 Before" culture tube

10. Cap the tube and shake vigorously

11. Using a new pipette, remove 1 ml of the soil/water mixture from the "10^{0}" tube and place into the "10^{-1}" tube

12. Cap and shake vigorously

13. Using the same pipette in step 11, remove 1 ml of the soil/water mixture from the "10^{-1}" tube and place into the "10^{-2}" tube

14. Cap and shake vigorously

15. You should now have a total of three culture tubes

16. Place 100 µl samples from each culture tube onto their own separate, 3M Petrifilm™ yeast and mold count plates containing nutrient agar, labeled "NC site 1 T1 before" and its respective dilution number

17. Allow the yeast and mold to grow on the 3M Petrifilm™ yeast and mold count plates for 72 hours

18. First look at the most dilute plated sample, 10^{-2}, and examine this plate for individual mold and yeast colonies (mold colonies will be a blurry greenish-blue color and yeast colonies will have sharper edges and be a shade of dark teal or brownish yellow). If this plated sample does not contain both mold and yeast colonies, then examine the 10^{-1} plated dilution sample for individual mold and yeast colonies. If this plated sample does not contain both mold and yeast colonies, then examine the 10^{0} dilution sample for individual mold and yeast colonies. However, if you cannot find one single dilution plated sample that contains both mold and yeast colonies, examine two plates (from the

same soil sample, but they can be different dilutions), one with mold and one with yeast colonies.

To find the number of mold colonies in each sample, use the equation below:

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

To find the number of yeast colonies in each sample, use the equation below:

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

Then, add together the products you found for the # of mold colonies in 1 cc of soil and the # of yeast colonies in 1 cc of soil to find the total number of yeast and mold colonies in 1 cc of soil

19. Record how many colonies of yeast and mold you see on each plate
20. Repeat steps 6-19 for trials 2 and 3 of all before sites respectively
21. Next, go back to coordinate 39.35722° north and 076.63510° west, and spray 30 sprays of Roundup™ directly and evenly onto each of the sites 1IV, 2IV, and 3IV.
22. Next, to apply the NC to sites, Spray 30 sprays of tap water directly and evenly onto each of the sites 1NC, 2NC, and 3NC
23. Wait 48 hours after you've sprayed the Roundup™ and water before extracting the soil samples
24. Take 3 soil samples from each site using a 15 cm. deep soil cylinder with a 2 cm. diameter by sticking the soil cylinder into the soil and pounding down with a mallot, then twisting clockwise and pulling up out of the ground – do this until the soil cylinder is 15 cm. full of soil
25. Place each separate sample in a plastic bag labeled with what site it was from (either IV site 1, IV site 2, IV site 3, NC site 1, NC site 2, NC site 3), that it was an "after" sample, and its trial number
26. Repeat steps 6-19 to dilute all three trials of the "after" samples for all sites

IV. Data Table

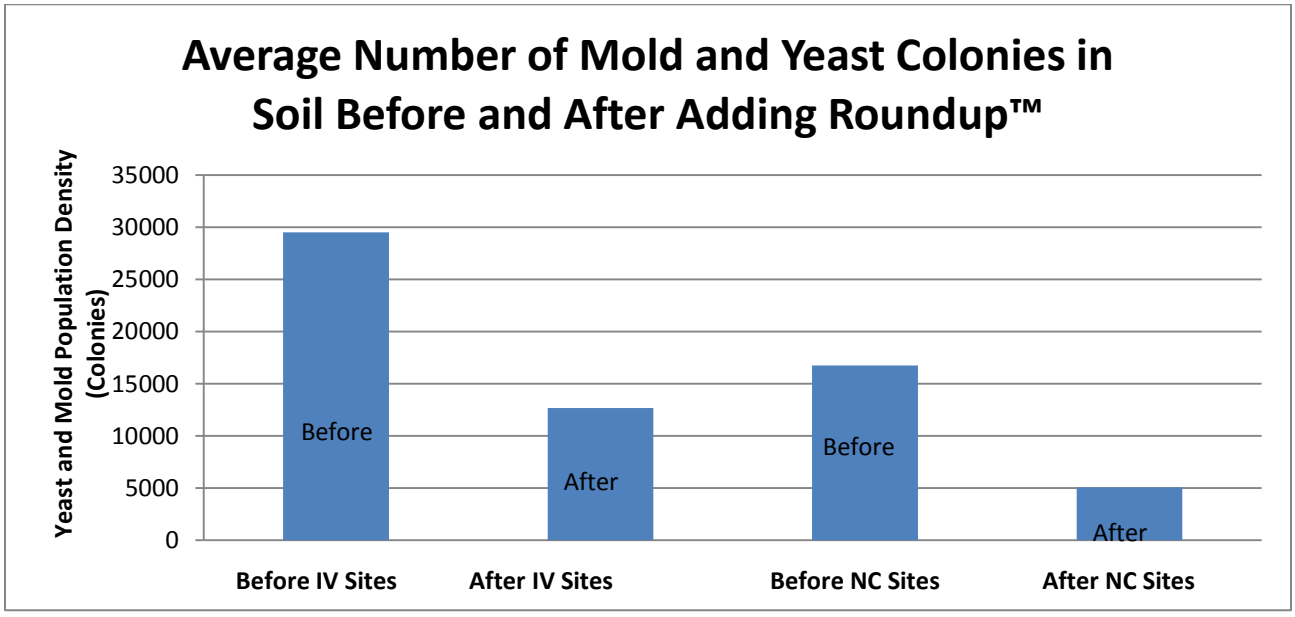
Yeast and Mold Count in Soil Before and After Adding Roundup™								
	Soil Sample	Dilution #	Yeast # on plate	# of yeast per 1 cc	Dilution #	Mold # on plate	#of mold per 1 cc	Total
Before	IV S1 T1	10^{-1}	1	1000	10^{-1}	1	1000	2000
	IV S2 T1	10^{-2}	2	20000	10^{-2}	1	10000	30000
	IV S3 T1	10^{-2}	1	10000	10^{-2}	16	160000	170000
	NC S1 T1	10^{-2}	2	20000	10^{-2}	4	40000	60000
	NC S2 T1	10^{-2}	1	10000	10^{-2}	4	40000	50000
	NC S3 T1	10^{-1}	5	5000	10^{-1}	None	None	25000
	NC S3 T1	10^{-2}	None	None	10^{-2}	2	20000	
	IV S1 T2	10^{-1}	21	21000	10^{-1}	7	7000	28000
	IV S2 T2	10^{-1}	2	2000	10^{-1}	6	6000	8000
	IV S3 T2	10^{0}	1	100	10^{0}	4	400	500
	NC S1 T2	10^{0}	3	300	10^{0}	4	400	700
	NC S2 T2	10^{-1}	2	2000	10^{-1}	6	6000	8000
	NC S3 T2	10^{-1}	1	1000	10^{-1}	8	8000	9000
	IV S1 T3	10^{-1}	2	2000	10^{-1}	5	5000	7000
	IV S2 T3	10^{-1}	3	3000	10^{-1}	10	10000	13000
	IV S3 T3	10^{-1}	2	2000	10^{-1}	5	5000	7000
	NC S1 T3	10^{-2}	2	20000	10^{-2}	3	30000	50000
	NC S2 T3	10^{0}	2	200	10^{0}	10	1000	1200
	NC S3 T3	10^{0}	3	300	10^{0}	6	600	900
After	IV S1 T1	10^{-1}	1	1000	10^{-1}	4	4000	5000
	IV S2 T1	10^{-1}	2	2000	10^{-1}	3	3000	7000
	IV S3 T1	10^{0}	1	100	10^{0}	1	100	200
	NC S1 T1	10^{-1}	5	5000	10^{-1}	2	2000	7000
	NC S2 T1	10^{-1}	1	1000	10^{-1}	3	3000	4000
	NC S3 T1	10^{0}	2	200	10^{0}	8	800	1000
	IV S1 T2	10^{-2}	3	30000	10^{-2}	1	10000	40000
	IV S2 T2	10^{-1}	2	1000	10^{-1}	8	8000	9000

	IV S3 T2	10^{-1}	3	3000	10^{-1}	4	4000	7000
	NC S1 T2	10^{-1}	1	1000	10^{-1}	2	2000	2000
	NC S2 T2	10^{0}	3	300	10^{0}	2	200	500
	NC S3 T2	10^{-1}	1	1000	10^{-1}	3	3000	4000
	IV S1 T3	10^{-1}	1	1000	10^{-1}	1	1000	2000
	IV S2 T3	10^{-2}	1	10000	10^{-2}	3	30000	40000
	IV S3 T3	10^{-1}	1	1000	10^{-1}	3	3000	4000
	NC S1 T3	10^{-1}	2	2000	10^{-1}	4	4000	6000
	NC S2 T3	10^{-1}	4	4000	10^{-1}	4	4000	8000
	NC S3 T3	10^{-1}	6	6000	10^{-1}	7	7000	13000

Averaged out Data Table:

Average Number of Mold and Yeast Colonies in Soil Before and After Adding Roundup™	
Before IV Sites	29500
After IV Sites	12688
Before NC Sites	16755
After NC Sites	5055

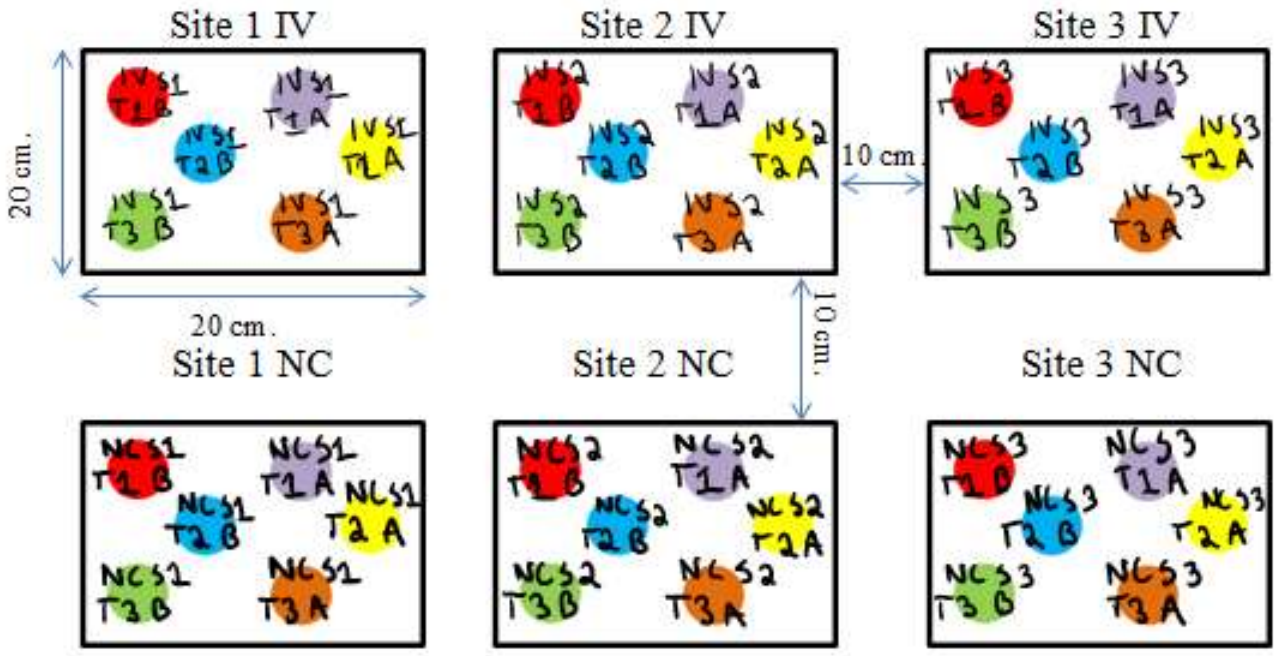
Graph:



V. Conclusion:

Our hypothesis; that the mycorrhizal fungi density in soil will decrease after exposure to the herbicide Roundup™, was proven correct. On average, there were 29,500 mold and yeast colonies in 1 cc of soil from our "before IV" site soil samples. On average, there were 12,688 mold and yeast colonies in 1 cc of soil from our "after IV" site soil samples. This data shows that on average, the number of mold and yeast colonies in 1 cc of soil from our IV site soil samples after adding Roundup™ decreased by 16,812 colonies. We also observed that on average, there were 13,494 individual mold colonies in each of our "IV before" sites. We then noticed that on average, there were 4,950 individual mold colonies in each of our "IV after" sites. This data shows that there were 8,544 mold colonies less in our "IV after" sites. Mold tends to appear more in non-stressed environments; so from this observation we can further conclude that Roundup™ was threatening the soil environment. To alter our experiment, we could perform it in the winter. This would alter our experiment because the initial amount of yeast and mold depends on the season it is in. Yeast will appear in a more stressed environment, for example when it is cold, while mold will appear more in a non-stressed environment, such as the spring. We conducted our experiment in the spring; a season that provides a non- stressful environment for the soil and its microbes. This may have caused an increase in the mold count, because it was not in a stressed environment. So, if we conducted this experiment in the winter, there could potentially be less of a change in fungi population since the population would have had less mold in it. However, since it would have had more yeast since the environment would be stressed, there may not be a significant difference in data. In future experiments we could test by varying the intensity of Roundup™, used. Based on this experiment, one might hypothesize that the larger amount of Roundup™ used on the soil, the lower the fungi population will be.

Diagram 1:



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