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Honors Biology 9

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Fungi and Soil Compaction

Soil Fungi are microscopic organisms that do many important things for the soil. They help with water dynamics, disease suppression, and nutrient cycling, and they consume organic-material that is hard to digest, releasing it in forms other organisms can use. Fungi also help hold all the soil particles together and help keep the nutrients in the soil from washing away (Ingham 2014).

Traditionally, these fungi have been grouped into three categories- decomposers, mutualists, and parasites- each with its own separate role in the soil (Ingham, 2014). The first group, the decomposers, break apart rotting organisms and other organic objects such as food, dead bodies, dead plants (grass, flowers, trees, etc.) and paper, and when they do so, they add nutrients to the soil which plants then absorb to help them live and grow (Lavelle, 2001). Mutualists, meanwhile, are a type of fungi that connect with plants directly in a symbiotic relationship that helps each other survive. The fungi link the soil to the plants and they exchange nutrients the other needs (Lavelle, 2001). The fungi give plants chemicals such as phosphorus, nitrogen, and water which are needed for survival and in return the plants give fungi carbohydrates for energy (C.V. Starr Virtual Herbarium). The last group, parasitic fungi, are organisms that connect themselves with other plants to take nutrients from them without giving anything in return. They steal nutrients from hosts to survive and continue this until the host

dies. However, mutualistic fungi help defend plants against parasitic fungi using antibiotics that they emit (Wise Geek, 2014), and so the impact of parasitic fungi in the soil is limited.

Since Fungi live in the soil, they are constantly being affected by soil compaction. The compaction of the soil is caused whenever something puts pressure onto the soil, weighing it down and pushing the soil particles closer together, causing the soil to become stiffer. Compressed soil has a massive and blocky structure, where the particles seem to be bonded into one group on the top and a structure where soil particles form blocky shapes (Jordán, 2014). Hence soil compaction reduces the total pore space of soil and reduces the amount of large pore space. Air and water cannot move through the soil easily (Colorado Master Gardener Program, 2011), but air is needed for fungi because it has the oxygen fungi need to produce energy in order to survive and grow (Fogel, 2002), and they need water because without its stabilizing environment, fungi cannot perform the chemical reactions they need in order to live (Karch, 2010). Therefore, where soil has been compacted, the fungi have a difficult time surviving, and so soil compaction is the primary factor that limits plan growth. Soil conditions like soil compaction contribute to 80% of the plant disorders in landscape settings (Colorado Master Gardener Program, 2011).

One source of is compaction of soil when they walk on the soil and construct on the soil. Humans may cause the population density of fungi to go down on the top layer of the soil because of these actions. Compaction can be minimized by adding organic matter, managing traffic flow, using mulch, aerate lawn and around tree area, avoiding excessive cultivation, and avoiding cultivating overly wet or dry soil (Colorado Master Gardener Program, 2011).

Loosing fungi is a major problem to the environment and humans. Without fungi plants cannot sustain life because fungi do a major part in keeping them alive (Lavelle, 2001). Fungi

also improve the soil in many ways such as keeping nutrients in it (Ignham, 2014). Fungi also naturally decompose to help the soil (Lavelle, 2001). Without fungi, waste could build up and plants could die. This is a negative impact on the environment and humans. Humans cannot survive without plants and cannot survive in conditions with high waste.

As a group, we would like to see how our actions as humans impact fungi. We could be decreasing the population of fungi daily. We would like to see the different populations of fungi at different soil depths in compressed and uncompressed soil. These soil depths would be 15.24 cm and 30.48 cm below the surface. Using the uncompressed soil, we could see what the normal population amount for fungi is at these soil depths. Using the compressed soil we could see if humans affect this population number in a negative way by decreasing it with our compaction on their home. With this experiment we will be able to see if we impact fungi's lives and if we do, we can see the point under the surface where we no longer affect fungi with compression. We predict that humans do decrease the population of fungi on top layers of soil by compressing it.

Lab Report

Problem: Does humans induced compression of the soil change the population density of fungi on the top layers of the soil?

II. Hypothesis: The population density of fungi on the top layers of soil will decrease where humans construct upon the soil but will increase where human construction does not occur.

III. Procedure:

A. Independent Variable: Soil samples taken from soil immediately next to a building

B. Dependent Variable: Population density of Soil Fungi

C. Negative Control: Soil samples taken behind bushes near the RPCS turf field.

D. Controlled Variables:

1. Specific plant life at locations
2. Sterile water (10 ml)

3. Time soil is collected
4. Day soil is collected
5. Time serial delusion is taken place
6. The size of the soil samples
7. 10 milliliter (ml) serological pipettes
8. P200 micropipette with tips
9. 15 milliliter (ml) culture tubes with caps (3 per dilution)
10. Amount of soil used (1 cubic centimeter (cc))
11. Nutrient agar plates called 3M Petrifilm™ Yeast and Mold Count Plate
12. Amount of Nutrient agar plates called 3M Petrifilm™ Yeast and Mold Count Plate used (half)
13. Amount of soil sample placed onto Nutrient agar plates called 3M Petrifilm™ Yeast and Mold Count Plate (100 µl)
14. Amount of time soil fungi grows (48 hours)
15. Materials used

E. Step-by-step:

1. Perform steps 1-8 at the same time and the same day
2. Collect soil (by pushing the soil extractor into the ground until the surface of the soil is at the line closest to top of extractor) next to the RPCS building N 39.35786 degrees and W

076.63605 degrees using a soil test core to get 30.48 cm deep and 2.3 cm wide into the soil

3. Put the bottom 15.24 cm of soil into one bag and label it "bottom half", also label it independent variable and the trial number (ex: Bottom Half, IV, Trial 1)
4. Put the top 15.24 cm of soil into one bag and label it "top half", also label it independent variable and which trial it is (ex: Top Half, IV, Trial 1)
5. Collect soil (by pushing the soil extractor into the ground until the surface of the soil is at the line closest to top of extractor) on the lawn at RPCS N 39.35876 degrees and W 076.63548 degrees using a soil test core to get 30.48 cm deep and 2.3 cm wide into the soil
6. Put the bottom 15.24 cm of soil into one bag and label it "bottom half negative control", also label which trial it is (ex: Bottom Half, NC, Trial 1)
7. Put the top 15.24 cm of soil into one bag and label it "top half negative control", also label which trial it (ex: Top Half, NC, Trial 1)
8. Repeat steps 2-7 twice more for 2 more separate samples
9. Take the samples inside
10. Perform steps 10-23 at the same time and on the same day
11. For the Negative control soil bags (non compressed soil) and Independent Variable bags (compressed soil), perform steps 12-42 separately for each bag of soil.
12. Use a clean, new transfer pipette to add 10 ml of sterile water to a culture tube. Label the tube "10^0". Also add a label of the trial, where the soil is from (negative control or compressed soil), and soil depth (top/bottom), (ex: Trial 1 NC (negative control) 10^0).

13. Use the same pipette to add 9ml to a second 15 ml culture tube. Label the tube "10^{-1}", also label which trial it is, independent variable or negative control, and top or bottom (ex: Trial 1 NC (negative control) 10^{-1}).
14. Use the same pipette to add 9ml to a second 15 ml culture tube. Label the tube "10^{-2}", also label which trial it is, independent variable or negative control, and top or bottom (ex: Trial 1 NC (negative control) 10^{-2}).
15. Place 1cc of the soil sample that matches the label of the tubes (ex: Trial 1 IV (independent variable) 10^{0}) into the "10^{0}" culture tube
16. Cap the tube and shake vigorously
17. Using a new clean pipette, remove 1 ml of the soil/ water mixture from the "10^{0}" tube and place into the "10^{-1}" tube.
18. Cap and shake vigorously.
19. Using the same pipette in step 17, remove 1 ml of the soil/water mixture from the "10^{-1}" tube and place into the "10^{-2}" tube.
20. You should now have a total of 3 culture tubes
21. Shake the 10^{0} and the 10^{-1} tubes for 5 seconds
22. Plate 100 µl sample from the 10^{0}, 10^{-1}, and 10^{-2} tubes (dilutions 10^{0}, 10^{-1}, and 10^{-2}) onto their own separate nutrient agar plates called 3M Petrifilm™ Yeast and Mold Count Plate, which will be labeled 10^{0}, 10^{-1}, and 10^{-2} with their trial numbers, independent variable or negative control, and top or bottom, ex. Trial 1 IV (Independent Variable) 10^{-1}
23. Use a spreader to spread the solution onto the nutrient agar plate
24. Allow to grow for 48 hours
25. Line your plates up in order from 10^{0} to 10^{-2} for each of the trials

26. Use your magnifying glass to examine the first plate, 10^{-2}, for yeast dots
27. If you find yeast dots in plate 10^{-2}, record how many you found and the dilution number from which you found it
28. If no yeast dots in plate 10^{-2} then go to the next plate, 10^{-1}, and search for yeast dots.
29. If you find yeast dots in plate 10^{-1}, record how many you found and the dilution number from which you found it
30. If no yeast dots in plate 10^{-1}, then go to the next plate, 10^{0}, and search for yeast dots
31. If you find yeast dots in plate 10^{0}, record how many you found and the dilution number from which you found it
32. Now take the first set of plates that you examined
33. Line them up in order from 10^{0} to 10^{-2}
34. Use your magnifying glass to examine the first plate, 10^{-2}, for mold
35. If you find mold in plate 10^{-2}, record how many you found and the dilution number from which you found it
36. If no mold in plate 10^{-2}, then go to the next plate, 10^{-1}, and search for mold.
37. If you find mold in plate 10^{-1}, record how many you found and the dilution number from which you found it
38. If no mold in plate 10^{-1}, then go to the next plate, 10^{0}, and search for mold
39. If you find mold in plate 10^{0}, record how many you found and the dilution number from which you found it

40. When all data is recorded, use the following formula to calculate the total number of fungi: number Microbes in 1 cc of soil = number of Colonies on the sheet X 10^2 X $10^{|dilution\ number\ at\ which\ these\ colonies\ were\ found|}$
41. Record all of your data in your data table

Data/ Observations

Trial 1

	# of yeast in Top 15.24cm of Soil	# of mold in Top 15.24cm of soil	Final calculations in Top 15.24cm	# of yeast in Bottom 30.48cm of soil	# of mold in Bottom 30.48cm of soil	Final calculations in Bottom 30.48cm of soil
Independent Variable (Compressed Soil)	10,000	300	10,300	10,000	4,000	14,000
Negative Control (Uncompressed soil)	3,000	400	3,400	10,000	0	10,000

Trial 2

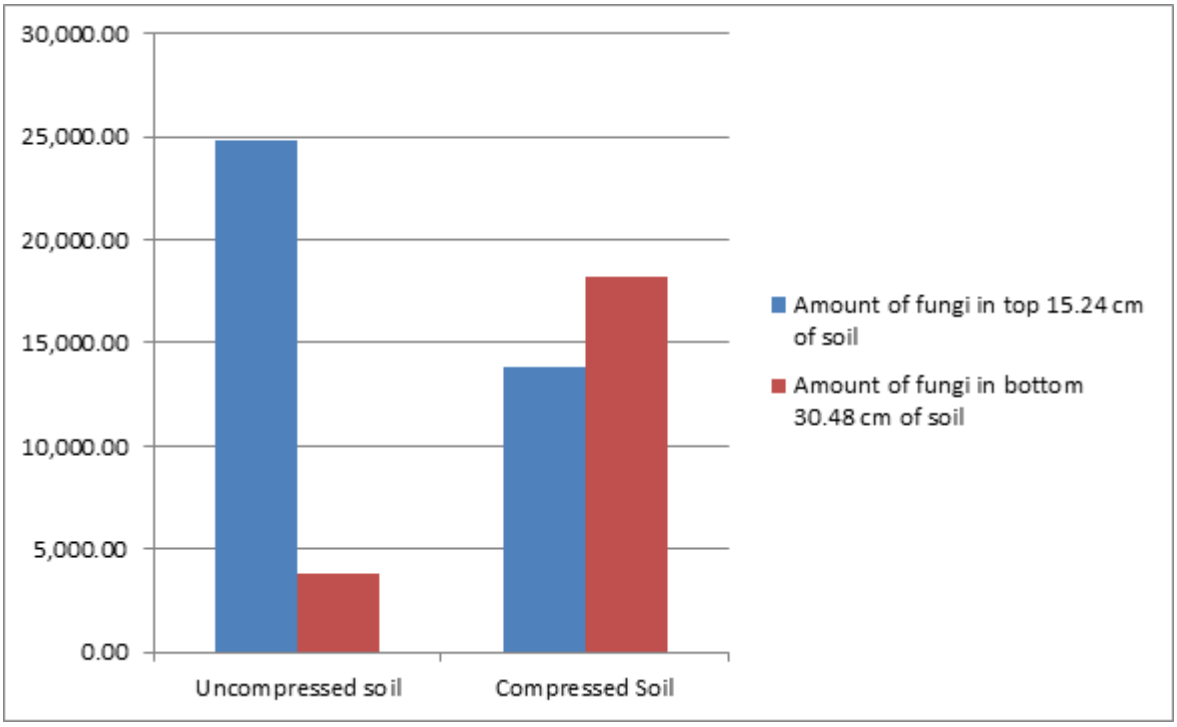
	# of yeast in Top 15.24cm of Soil	# of mold in Top 15.24cm of soil	Final calculations in Top 15.24cm	# of yeast in Bottom 30.48cm of soil	# of mold in Bottom 30.48cm of soil	Final calculations in Bottom 30.48cm of soil
Independent Variable (Compressed Soil)	30,000	0	30,000	30,000	0	30,000
Negative Control (Uncompressed soil)	10,000	60,000	70,000	300	0	300

Trial 3

	# of yeast in Top 15.24cm of Soil	# of mold in Top 15.24cm of soil	Final calculations in Top 15.24cm	# of yeast in Bottom 30.48cm of soil	# of mold in Bottom 30.48cm of soil	Final calculations in Bottom 30.48cm of soil
Independent Variable (Compressed Soil)	600	600	1,200	10,000	500	10,500
Negative Control (Uncompressed soil)	1,000	100	1,100	1,000	100	1,100

Averages of Fungi at Different Soil Depths

	Total amount of Fungi in Top 15.24cm of soil	Total amount of Fungi in Bottom 30.48cm of soil
Independent Variable (Compressed Soil)	13,833.333	18,166.667
Negative Control (Uncompressed soil)	24,833.333	3800



Conclusion:

In conclusion, our hypothesis was correct. Our hypothesis was correct because we predicted that humans change the population density of fungi at different soil depths by compressing it. By constructing on top of the soil, humans change the population density of soil fungi. This is proven in our data table and reiterated in our graph. In our averages table, one can see that in the final calculation in the bottom 30.48cm of the soil, there are 18,166.667 fungi in the compressed soil. Since there are 18,166.667 fungi at the bottom of the compressed soil, compared to 13,833.333 fungi in the top layer of the compressed soil, this proves that soil compression from humans negatively affects the population of fungi. The amount of fungi that live on the top layer of the soil decreases because of oxygen loss. In the averages chart, one can also see that the total amount of fungi in the top 15.24cm of uncompressed soil there are 24,833.33 fungi, compared to the bottom where there are only 3,800 fungi living. These numbers also prove our hypothesis to be correct because in the uncompressed soil, there was a large amount of fungi at the top (24,833.333 fungi). This means that fungi are able to live on the top layers of uncompressed soil. Normally, fungi do not have to live on the bottom of the uncompressed soil because they can get all their oxygen from the top layer of soil.

In the future, to continue our research on the effect of fungi growth under different layers of fungi compression, our group will test to see where the degree of compression begins to be affected. Our experiment showed that there are more fungi at the bottom of compressed soil, rather than on the top layer of soil because the compression from humans, construction sites, and other man made obstacles that prevent oxygen from getting to the fungi at the top layers of the soil. In order to see when the effect of compression begins to affect fungi, our group will need to take soil samples from the top layer of soil until we reach a measurement of 30.48cm deep. After

we have the measurements, our group will use the same dilution and calculation processes to find the amount of fungi present at each soil depth. Once our group discovers exactly when compression affects the number of fungi, we will analyze and compare the results to our current data. Using this method, our group will be able to clearly see how compression affects the number of fungi. Finally, once this question is answered we will find a way to prevent a decrease of fungi in the top layers of soil.

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