The Effect of Soil Compaction on the Mold: Yeast Ratio in the Soil

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Soil Ecology Background Report

Fungi are essential heterotrophic organisms that play many critical roles in ecosystems. By releasing enzymes into their environment, they digest organic matter into forms that the fungi can use for their own metabolic needs as well as forms that are beneficial to other organisms, and soil fungi are actually grouped into three categories based on how they meet these metabolic needs: decomposers, pathogens, and mutualists (Ingham, n.d.). Decomposers convert dead organic material into fungal biomass, carbon dioxide, and other small molecules and are essential to ecosystems because they break down some pollutants and retain nutrients in the soil for other organisms living there to use (Ingham, n.d.). Pathogens colonize roots, and as they digest them, these fungi damage or kill the plant host, adding to the food supply of the decomposers for their carbon compounds (Hoorman, 2016). Finally, Mutualists, colonize plant roots in order to receive carbon from that plant in a fashion that is beneficial to both organisms (Hoorman, 2016).

Known as mycorrhizal fungi, these specialized microbes form a symbiotic relationship between the fungi and the plants in which the fungus spreads out its thin filaments known as hyphae, increasing the surface area that makes phosphorus, water, and other soil nutrients more absorbable, giving the plant easier access to them, and in return, the fungus is able to obtain nutrients in the form of sugars produced by photosynthesis from the roots of the plant (Pace, n.d.; Ingham, n.d.). Therefore, without this critical fungal–plant partnership, the plants in an ecosystem would struggle to survive, putting everything living there at risk (since as producers, the plants are the foundation of the rest of the food chain).

Given the importance of soil fungi to the health of the ecosystem, anything that might negatively impact them would consequently negatively impact the rest of the ecosystem, and

one-way fungi may be threatened by a common human behavior is soil compaction. This phenomenon can potentially harm soil fungi because when the particles in the soil are forced together under pressure, it can have negative consequences for the things living there. These repeated pressures can be activities such as walking or riding a bike, and as these regular actions compact a given area, it increases the bulk density of that soil (Sjoerd Willem Duiker, 2005). This makes the soil too firm and compacted for roots to grow which causes them to require more force to penetrate the compacted soil, inhibiting root growth (Dejong-Hughes, 2018). Fewer roots, though, means that the fungi will not be able to obtain as much energy and nutrients from their host plant, lessening the fungi population which reduces the nutrients available to plants, creating a negatively reinforcing cycle.

In addition, compaction affects soil fungi because the increase of bulk density makes it more difficult for them to reach the gas and nutrients they need to survive because there is less room in the soil for gas and nutrients to move. Furthermore, compaction affects the water within the soil by decreasing its saturated hydraulic conductivity, which is the movement of water through the soil. (Sjoerd Willem Duiker, 2005). Essentially, soil compaction shrinks the size and number of pores in soil (Wolkowski, Lowery, Schuler, & Bundy, 2008), and smaller and fewer pores mean a reduction in the amount of water in the soil which is an essential resource to soil fungi. Without water, the fungi cannot survive or be successful in decomposing organic matter (Kerr, 2108), and since decomposition produces nutrients that are essential to plant growth (such as nitrogen) (Hoyle, Murphy, & Sheppard, 2019), the reduction in soil fungi will cause plants to become less healthy and harder to grow; again, impacting all the other organisms in an ecosystem depending on the oxygen and food they need to survive which the plants produce (Science and Technology Concepts Middle School).

On the Roland Park campus, there are several places where there are areas visibly affected by compaction, one of which is in front of the benches on the front lawn. When people sit down on the bench, they put their feet on the ground in front of them. The constant weight of people's feet on the soil causes significant compaction since everyone who sits down on the bench puts their feet in the same spot. After learning about the major impact soil compaction and discovering it decreases plant growth, we chose to test the soil around the benches on the front lawn after observing the lack of grass around the area. We used soil from three distances away from one of the benches to find how far away from the bench's point of compaction, the soil fungi were no longer affected. We will compare the soil fungi from the bench locations to that of a faraway spot surrounded by a small post and a few small plants which we determined did have any compaction to the area. We expect to find that the soil farther away from the bench that we test will have a higher density of soil fungi, closer to that of the soil near the post.

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Little Things Experiment

I. Problem: How far from the area of compaction in front of the Roland Park bench does the soil fungi display a higher mold to yeast ratio?

II. Hypothesis: The display of a higher mold to yeast ratio will be observed 6 meters away from the area of compaction in front of the Roland Park bench.

III. Procedure:

A. Independent Variable: distance away from the area of compaction in front of the bench

B. Dependent Variable: density of soil fungi

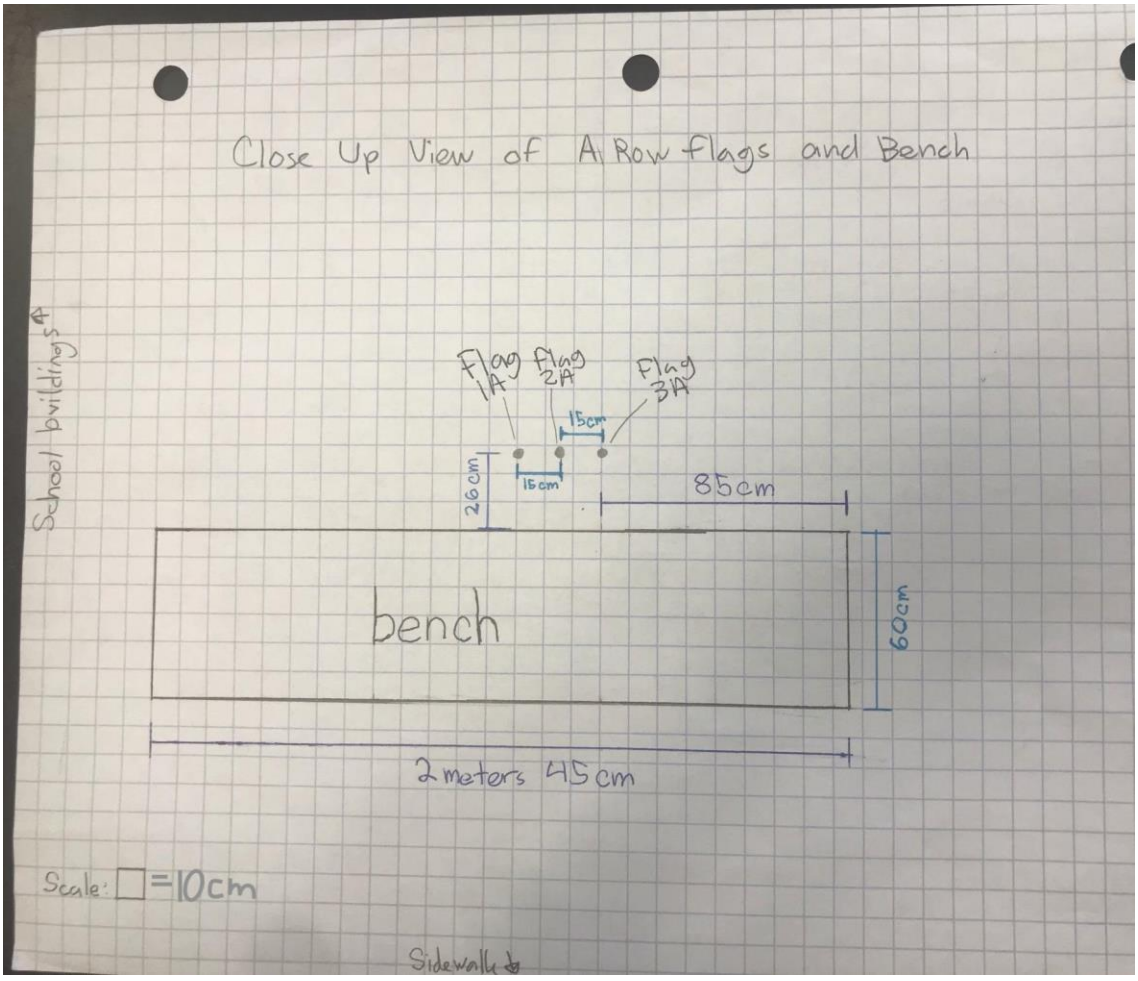
C. Negative Control: soil samples from a non compacted area

D. Controlled Variables: location of negative control, date and time when soil samples are taken, type of water for serial dilution, amount of water for serial dilution, size of soil samples, how deep soil extractor goes, type of petrifilm, size of transfer pipette, time between extracting soil and testing for fungi, location of

the benches, type of pipette, amount of soil in sterile solution, time allowed to grow on the mold and yeast plate, amount of solution on mold and yeast plate, how hard you shake the cap, degree to which sample is diluted, which dilutions are plated

E. Step-by-step:

1. Collect 12 yellow flags and label three of them 1A, 2A, and 3A respectively. Label another three 1B, 2B, and 3B respectively, label three 1C, 2C, and 3C respectively. Finally, label three NC1, NC2, and NC3 for negative control respectively.
2. Go to the coordinates N 39° 21.501’ W 076° 38.169.



(see picture above for steps 3-7)

3. Place flags 1A, 2A, and 3A (A set) 26 cm in front of the bench, 15 cm apart from each other. Flag 3A should be 85 cm away from the edge of the bench that is farthest from the school. Flag 1A should be closest to the school building.
4. Use a meter stick to measure and place flags 1B, 2B and 3B 3 meters south of the A set. Make sure the numbers on the B flags correspond to the numbers on the A flags. Each of the B flags should also be 15 cm apart.
5. Use the meter stick to take the meter stick to measure and place flags 1C, 2C 3 meters further south of the B set, each of the C flags 15 cm apart from each other. Remember to line up the numbers with the matching previous set.
6. Next, bring the 3 flags labeled NC 1, NC 2, and NC 3 for the negative control and go to the coordinates N 39° 21.460’ W 076° 38.117’
7. Place the flags into the ground, 15 cm apart.
8. At all locations in steps 1-7, complete steps 9-11 at the same time on the same day.
9. Temporarily remove the flags to take a sample of the soil beneath. Insert a soil extractor, 2cm wide in diameter, vertically into the ground up until the soil reaches the first mark (15 cm deep)(use a hammer if needed).
10. Pull the soil extractor vertically upwards until it is out of the soil. Then, dump the soil collected by the extractor into a plastic bag, seal the bags as soon as the soil is put inside, and immediately labeled each bag according to the flag label once it is sealed.

11. Do this for every flag (1B, 1C, 2A, 2B, 2C, 3A, 3B, 3C, NC1, NC2, and NC3), making sure to collect all of the samples at the same time on the same day.
12. Go inside after collecting all soil samples to use a lab table to test for soil fungi.
13. For steps 14-28 you MUST perform them at the same time on the same day.
14. Use a new clean 10 ml transfer pipette to add 10 ml of sterile water to a 15 ml culture tube.
15. Label the tube "1A 10^{0}".
16. Use the same pipette to add 9 ml of sterile water to a second 15 ml culture tube.
17. Label the tube "1A 10^{-1} ".
18. Use the same pipette to add 9ml to a third 15 ml culture tube, only label it "1A 10^{-2}".
19. Repeat steps 14-18 for every soil sample just make sure to label it according to the flag label and put each sample into different culture tubes.
20. Using a cubic centimeter scoop, place 1 cc of soil sample 1A into the "10^{0} 1A" culture tube.
21. Cap the tube and shake vigorously.
22. Using a new clean pipette remove 1ml of the soil/water mixture from the "10^{0} 1A" tube and place into the "10^{-1} 1A" tube.
23. Cap the tube and shake vigorously.

24. Using the same pipette in step 22, remove 1 ml of the soil/water mixture from the "10^{-1} 1A" tube and place into the "10^{-2} 1A" tube.
25. Repeat steps 20-24 for each set of soil samples, using the culture tubes that are labeled for that soil sample.
26. Using P200 micropipette with tips, Plate 100 µl samples of the 1st, 2nd, and 3rd tubes (dilutions "10^{0} 1A", "10^{-1} 1A", and "10^{-2} 1A") onto their own separate correspondingly labeled 3M Petrifilm™ Yeast and Mold Count Plate. Use a petrifilm spreader to flatten out the solution AFTER it has put onto the petrifilm.
27. Repeat step 26 for each soil sample set ("10^{0} 2A", "10^{-1} 2A", "10^{-2} 2A", and so on with each set)
28. Allow the soil fungi to grow for 72 hours.
29. After 72 hours, lay out each set of 3M Petrifilm™ Yeast and Mold Count Plates by their flag and dilution number.
30. Using a 5x magnifying glass, examine plate "1A 10^{-2}" for yeast specks and count the total amount. Write this number down with the correct label (flag and dilution).
31. Using the same 5x magnifying glass, examine plate "1A 10^{-2}" for mold. Count the total account and write this number down with the correct label (flag and dilution).
32. If there is both mold and yeast present, you do not need to examine the other dilutions in that flag group.

33. If there is either yeast or mold, not both, move to plate "1A 10^{-1}" and repeat steps 30-32.
34. After doing a flag set, Perform steps 30-33 for each flag set of plates.
35. Apply the following equation to the yeast and mold to find the final data for your chart:

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

Impact of Compaction on Soil Fungi Density

Location of Sample	Yeast (# of microbes in 1 cc of soil)	Mold (# of microbes in 1 cc of soil)	Total Fungi Count
1A- underneath the bench (0m)	10,000	2,000	12,000 # of microbes in 1 cc of soil
2A- underneath the bench (0m)	1,000	800	1,800 # of microbes in 1 cc of soil
3A- underneath the bench (0m)	600	0	600 # of microbes in 1 cc of soil
1B- 3m south of bench	20,000	2,000	22,000 # of microbes in 1 cc of soil

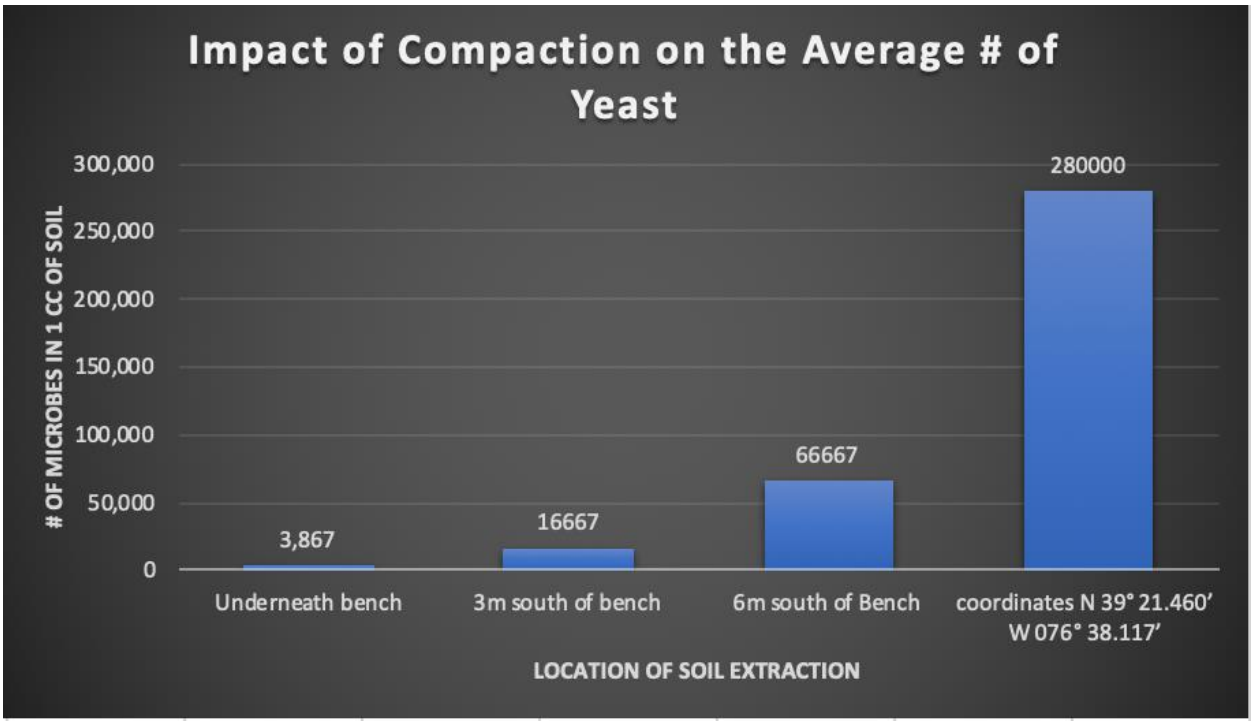
2B- 3m south of bench	20,000	10,000	30,000 # microbes in 1 cc of soil
3B- 3m south of bench	10,000	4,000	14,000 # microbes in 1 cc of soil
1C- 6m south of bench	140,000	60,000	200,000 # microbes in 1 cc of soil
2C- 6m south of bench	20,000	11,000	31,000 # microbes in 1 cc of soil
3C- 6m south of bench	40,000	6,000	46,000 # microbes in 1 cc of soil
NC1- coordinates N 39° 21.460' W 076° 38.117'	180,000	130,000	310,000 # microbes in 1 cc of soil
NC2- coordinates N 39° 21.460' W 076° 38.117'	70,000	50,000	120,000 # microbes in 1 cc of soil
NC3- coordinates N 39° 21.460' W 076° 38.117'	30,000	6,000	36,000 # microbes in 1 cc of soil

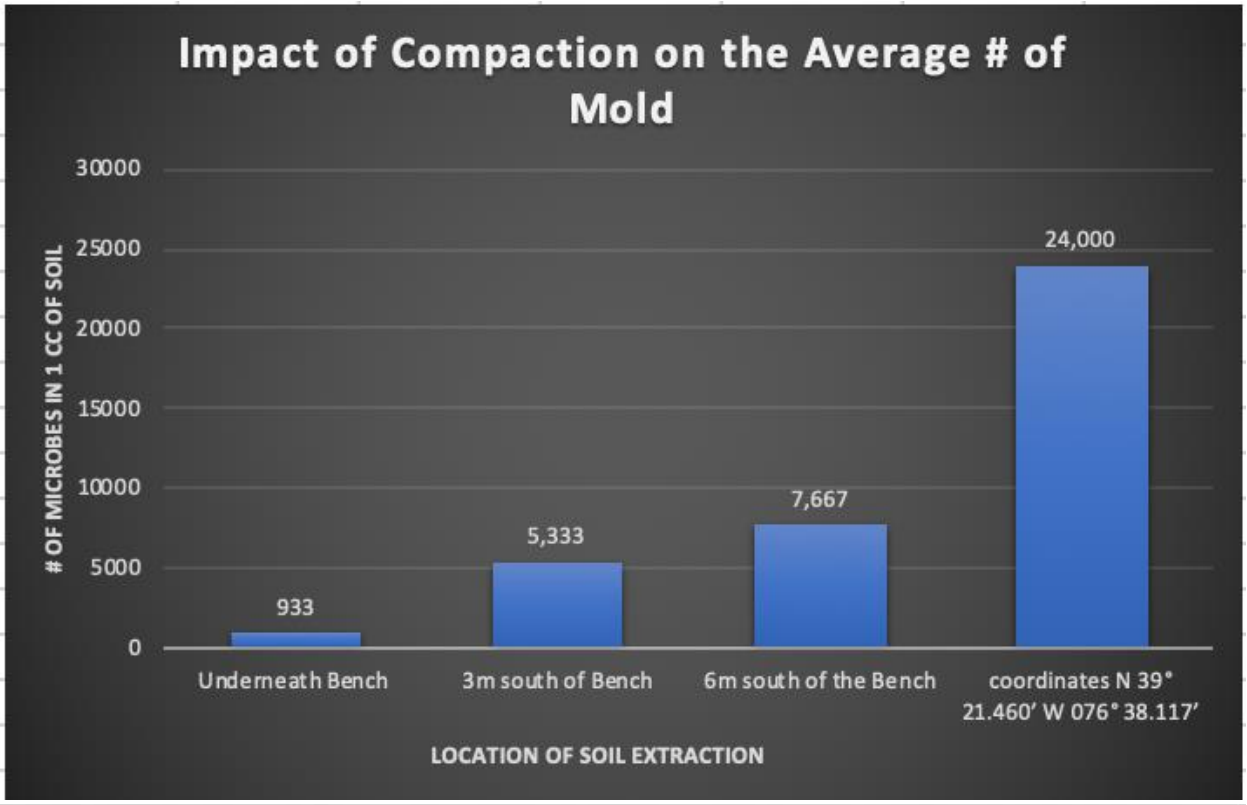
Impact of Compaction on Average Soil Fungi Density

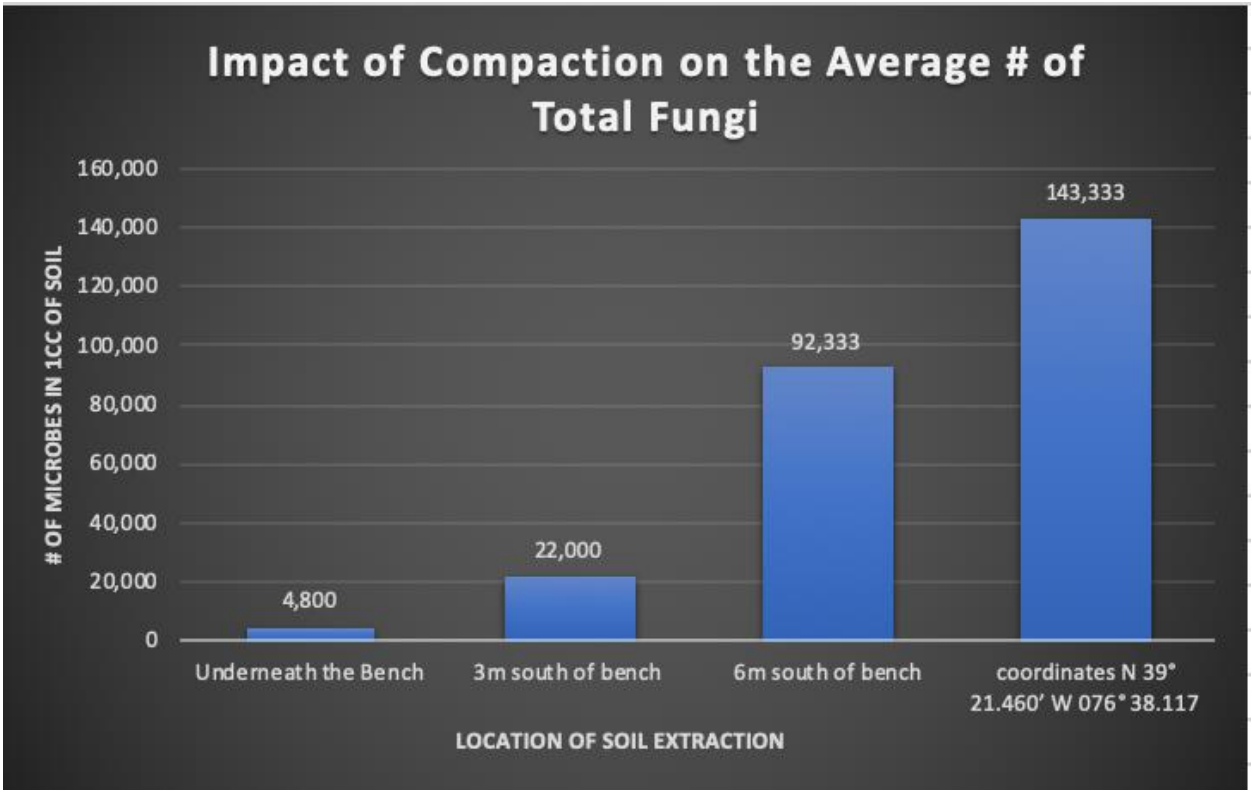
	Yeast	Mold	Total Fungi Count	Mold: Yeast Ratio

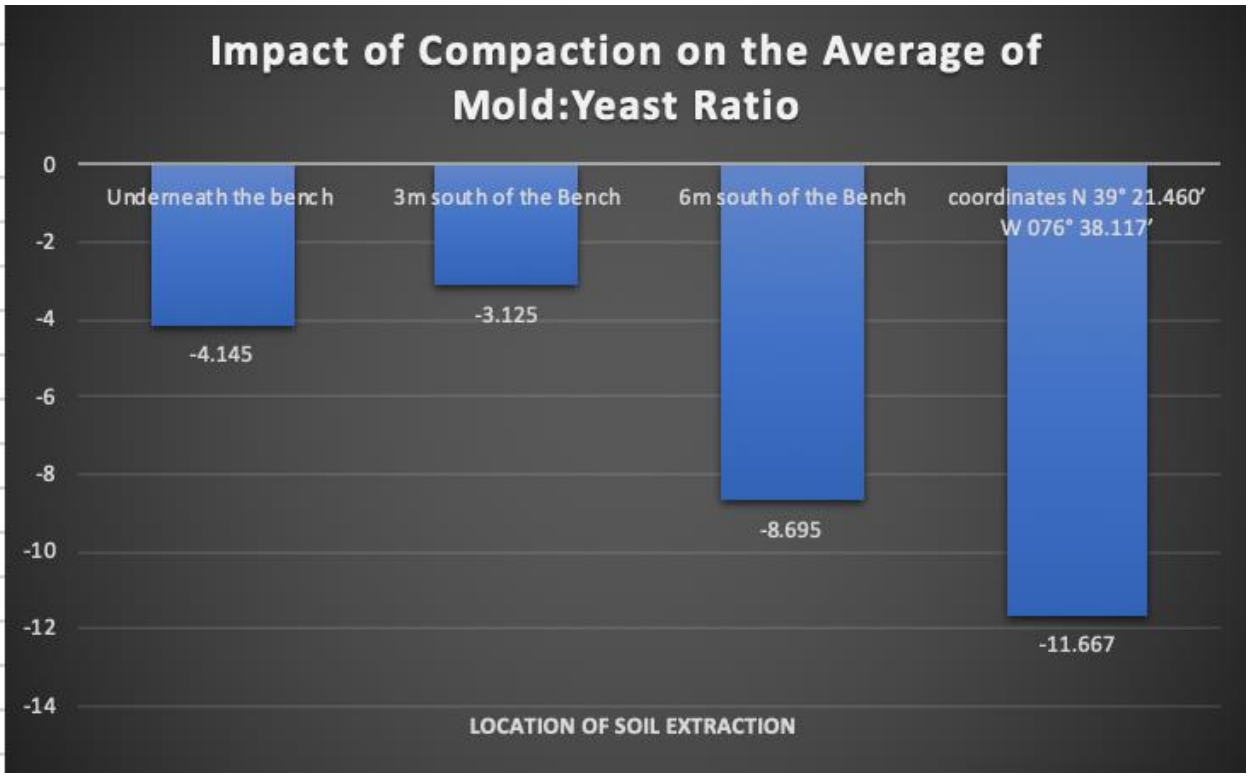
Underneath the bench	3,867 # of microbes in 1 cc of soil	933 # of microbes in 1 cc of soil	4,800 # of microbes in 1 cc of soil	1:- 4.145
3m south of bench	16,667 # of microbes in 1 cc of soil	5,333 # of microbes in 1 cc of soil	22,000 # of microbes in 1 cc of soil	1: -3.125
6m south of bench	66,667 # of microbes in 1 cc of soil	7,667 # of microbes in 1 cc of soil	92,333 # of microbes in 1 cc of soil	1: -8.695
coordinates N 39° 21.460' W 076° 38.117'	280,000 # of microbes in 1 cc of soil	24,000 # of microbes in 1 cc of soil	144,333 # of microbes in 1 cc of soil	1: -11.67

Graphs




Impact of Compaction on the Average # of Mold
OF MICROBES IN 1 CC OF SOIL
30000
25000
20000
15000
10000
5000
0
933
5,333
7,667
24,000
Underneath Bench
3m south of Bench
6m south of the Bench
coordinates N 39° 21.460' W 076° 38.117'
LOCATION OF SOIL EXTRACTION


Impact of Compaction on the Average # of Total Fungi
OF MICROBES IN 1CC OF SOIL
160,000
140,000
120,000
100,000
80,000
60,000
40,000
20,000
0
4,800
22,000
92,333
143,333
Underneath the Bench
3m south of bench
6m south of bench
coordinates N 39° 21.460' W 076° 38.117
LOCATION OF SOIL EXTRACTION



Conclusion

Our hypothesis was incorrect because the mold to yeast ratio was higher at the 3m south flag set (B flags), not the 6m south flag set (C flags). Our hypothesis stated that the 6 meter south flags would display the highest mold to yeast ratio. The average ratio of mold to yeast for the flags 3m south was -3.125. All of our ratios were negative, so whichever number is closest to zero is the highest. The Mold to Yeast ratio of the flags that are 6 meters south is -8.695. This number is farther from zero than the ratio from the flags 3 meters south. One possible reason why the mold: yeast ratio is the highest ratio is because of allelopathy. Allelopathy is when plants attack other plants in order to obtain nutrients from the soil. The "B" set of flags is 11m and 33 cm away from the large Catalpa tree on the front lawn. It is also 7m from one of the small cherry trees and 4m from the neighboring small Cherry tree. The roots of the 3 trees overlap in

the area where we tested the soil. The trees are engaging in allelopathy which causes the fungi to protect themselves from the chemicals being released. They do this by turning from a mold into a yeast. Further research could be done in order to test the impact of allelopathy on soil fungi.

However, our thought that compaction would decrease the number of fungi in the soil was correct. Looking at the graph, *"Impact of Compaction on the Average Total Fungi"*, the area directly underneath the bench has the smallest fungi count. Compaction decreases the number of soil fungi in the affected area. This explains why the "A" flag section of soil has the least amount of fungi. Looking at the total fungi count present at each of the testing locations, the number of fungi is highest in the area with the smallest amount of foot traffic (coordinates N 39° 21.460' W 076° 38.117). This helps to prove that compaction does, in fact, impact the total amount of fungi in the soil. Further research, without the presence of allelopathy, could occur in order to test the results of compaction alone.

Furthermore, compaction affects the fungi to a point where the fungi go into protection mode, also known as yeast. In each area with varying levels of compaction, (A-C flags), there was a significantly higher amount of yeast in the soil than there was mold. Since this is true, if allelopathy had not impacted our results, our hypothesis may have been correct because of this point. This point makes it necessary to complete an experiment in a more controlled environment in order to properly test the effect of compaction.

The results of allelopathy on the soil fungi was unintended and it altered our results. Each set of flags had contact with tree roots. The "A" flags came in contact with the roots of the large Catalpa tree. The "C" flags were in between the two cherry trees, causing the roots from those trees to interact with the soil. The "B" flags, however, were in close proximity two both the roots of the Catalpa and the two Cherry trees. While each set of flags interacted with at least one tree,

but the levels of interaction were not equal. This discrepancy means that the variable was not controlled, which means it may have strongly affected the results of the experiment. Our data was collected from the outside, making it extremely difficult to control. If we were to redo the experiment, we would plan for these inconsistencies or find a more controlled environment.

The experiment we performed solely focused on one type of compaction: foot traffic from humans. There are many other types of compaction, including compaction caused by machinery, animals, and weather. The different types may have different results on soil fungi. It would be interesting, for further research, to compare these results. That way we could find out which is the most damaging to the soil fungi.

There are a various amount of reasons why this is, that could have us go into further research for why. These reasons include allelopathy and foot traffic. If we were to continue our research we would have more controlled variables, such as the location of the experiment without plants nearby, less heavy foot traffic, the location not being exposed, and so on. Finally, we can conclude that the mold to yeast ratio is the highest 3m south of the Roland Park bench for a various amount of possible reasons.