# Soil Ecology Project:

## Impact of Compaction on the Density of Fungi

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# Background

"Little things make big things happen" (Wooden, 2013), and nowhere is this concept more applicable than to soil microbes. These microscopic organisms play a key role in maintaining the fertility, structure, drainage, and aeration of the soil, and they create energy for plants by converting nutrients in the soil such as water, nitrogen, zinc, and phosphorus into forms that plants can use (Soil Organism, 2013). Hence, microbes in the soil help keep plants healthy, and as a consequence, they also contribute to the balance and maintenance of the food chain. Without the microbes in the soil in an ecosystem, most plants would die and every other layer of foundation for all other organisms and life on Earth (Ingham, 2013).

Of all the different types of soil microbes, fungi are especially important because they directly benefit plants and many of the other organisms that live in the soil. Fungi engage in a number of activities that enrich the soil, including retaining nutrients, cleaning up pollution, decomposing carbon compounds, and improving the amount of organic matter in the soil. They are able to do all these things because fungi possess structures called hyphae. These cylindrical, finger-like extensions of a fungus serve as an environmental buffer for the rest of the fungal cell body, and all fungi p[use their hyphae to obtain the nutrients they need for survival. This process is able to happen because hyphae release enzymes into the soil that dissolve soil nutrients, enabling the fungi to absorb them (Brock, 2006). Even more importantly, hyphae are responsible through this process for fostering the symbiotic relationship between fungi and plants (Hall, 2004).

This special interdependent relationship is known as the mycorrhizae, and typically, there is a different species of this type of fungi associated with each type of plant. Plants equipped

with this helpful microbe can look forward to the benefits of heartiness, less disease, and easier transplants, and without them, plants could never colonize new land. One way this relationship works is that the fungi increase the surface area of the plant's roots (Moravec 2010) which increases the amount of water plants can uptake. In addition, fungi also absorb minerals such as phosphorus and iron from the soil and supply them to the plant in return for the protective environment of the root ball and a steady supply of sugar from their host plants. This sugar is what physically keeps the fungi alive and allows them to grow and reproduce and to continue providing for the plants (Wait, 2012). Hence, the healthy interaction between fungi and plants contributes to a healthy ecosystem because plants that perform their life functions, such as photosynthesis, at a high level increase the health and success of all the other species and organisms in an ecosystem by producing a greater amount of stored chemical energy for everything living there to use.

The second major type of fungi that lives in the soil is the decomposers, also known as saprophytes. These fungi convert dead organic material into substances that are useful to plants, such as carbon dioxide, and they also help keep nutrients in the soil. Decomposers are valuable to the environment because they recycle many nutrients like iron, phosphorus, potassium, and nitrogen and are more efficient at storing these nutrients than bacteria, partly due to the durable composition of their cell walls. Therefore, these types of fungi are more efficient at making these nutrients readily available to a plant and since these nutrients protect plants from disease, help them maintain a healthy dark green color, and allow them to grow without becoming dry or wilted (Wait, 2012), decomposing fungi play a critical role in plant health as well (The University of Western Australia Soil Club, 2004).

Finally, the last major category of fungi includes the pathogens which can cause death and disease in other plants and microbes. While directly harmful to other species, even these fungi play a positive role in the larger ecology by keeping the densities of other microorganisms in the soil in balance and killing off old and weak plants to make room for new growth (Natural Resources Management and Environment Department, 2005).

Sadly, many people do not realize the important roles all these types of soil fungi play in ecosystems and, as a consequence, are often harmful to them. One way humans do this is by compacting the soil in which the fungi live. Manual labor such as tilling, harvesting, grazing, construction, and physically moving the soil increases the pressure applied to the soil (University of Georgia, 2000), squeezing and consolidating it into a smaller volume than its original volume. In addition, transportation such as wheel traffic by machinery and force applied by humans from walking or running also increases the pressure being applied to the soil (USDA Natural Resources Conservation Service, 1996).

All this compaction, though, negatively affects the soil's density, pore space, water infiltration, water storage, plant growth, and plant nutrient cycling. It can cause increased runoff and erosion (USDA, Natural Resources Conservation Service, 2001), and together these consequences can harm the role of fungi in the larger ecosystem (International Society for Microbial Ecology, 2007). The reason why is because soil fungi cannot live without taking in the nutrients that make up soil organic matter (Michigan State University, 2004), and yet, when soil is compacted, its density increases (USDA Natural Resources Conservation Service, 1996) squeezing out the soil's organic matter (USDA Natural Resources Conservation Service, 2008). As a result, fungi do not have access to the natural organic matter they need in order to survive. This then becomes a vicious cycle: there is a decrease in the amount of organic matter the soil

contains because of the compacted soil pushing out the organic matter, causing the decreased amount of fungi in the soil because of their loss of an energy source. This causes even less organic matter to be available since there are now fewer fungi engaging in the decomposition that produces this organic matter in the first place.

In addition to the need for organic matter, fungi also need water. Unfortunately, soil compaction decreases the amount of macro pores which act as passageways for water throughout the soil, and because it decreases the amount of these passageways, compaction causes the amount of water able to flow through the soil to decrease (Beata, 2012). Yet without sufficient water, many fungi will die, and since the main producers, the plants, cannot live without the presence of fungi, subsequently, neither can the primary and secondary consumers that rely on them. Hence, with the loss of water from compaction, once again, the entire ecosystem is at risk and therefore, a decrease in fungi population has dangerous consequences for all the ecological relationships.

This problem has increasingly become potentially hazardous in today's world, even at Roland Park Country School. In 2009, Roland Park Country School redesigned and rebuilt their entire athletic complex building, and while rebuilding, many heavy trucks and construction vehicles drove through the campus. Due to the fact that since the 1940s the average weight of tractors and other machinery increased by 50 tons (University of Minnesota, 2013) and because of the increase in number of trucks and machinery at the school, soil compaction has potentially become a serious problem at our school. Therefore, because of the fact that soil compaction can severely harm ecosystems and because compaction is a potential problem out our school, we designed our experiment to compare the difference between two types of compaction in order to see which one has the largest effect on fungi density. The experiment looks at the differences

between compaction caused by benches and compaction caused by sidewalks. We believe that the density of fungi under the benches will most likely be higher than the density of fungi microorganisms near the sidewalk. This is because a sidewalk will probably cause more compaction then benches because it is walked on more often and more pressure is placed upon it.

## Experiment

I. Problem:

Is the result of soil compaction from sidewalks or benches more harmful to the density of fungi?

II. Hypothesis:

Soil compaction caused by sidewalks will be more harmful to the density of fungi than benches.

III. Procedure

a. Independent Variable:

The difference in level of compaction at each individual testing site

b. Dependent Variable:

The population density of fungi in the soil

c. Negative Control:

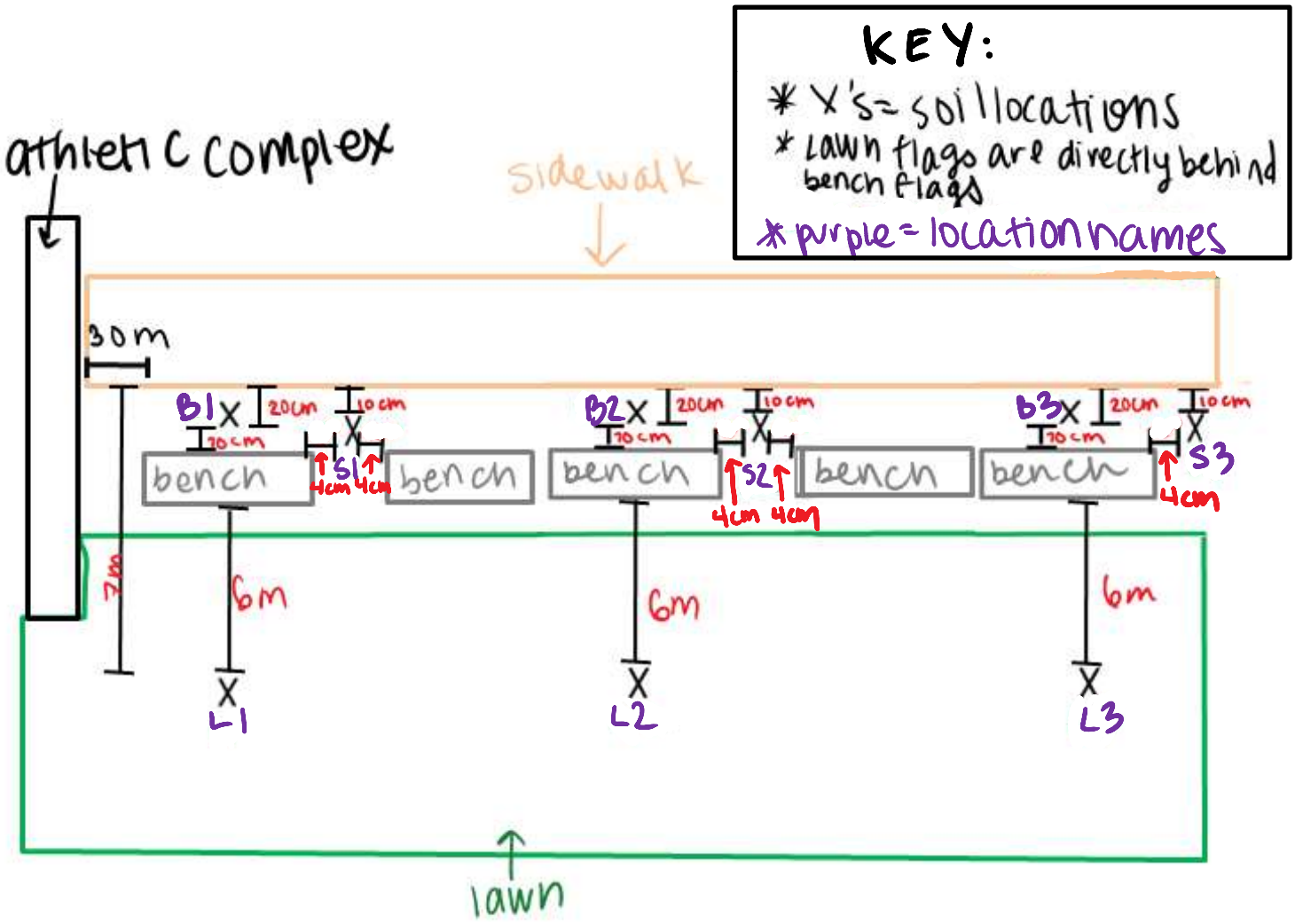
Soil from an area with minimal compaction (The Front Lawn)

d. Controlled Variables: Take all soil samples on a day in early May when the temperature is 12 degrees Celsius at 8:30 in the morning **(all samples on the same day and at the same time)**, devices used to extract soil, exact locations of each individual sites, amount of soil extracted from each site, size of transformation tubes, the way in which all transformation tubes, nutrient agar plates, and bags of soil are

labeled, materials used to mark each testing site, compass used to denote directions relative to the flag, type of nutrient agar plates used, time the fungi colonies have to grow before being counted, measuring units used, process of serially diluting the fungi, type of water used in serial dilution process, amount of water used to dilute soil, amount of soil that is mixed with water during serial dilution, type of petri dishes used, type of pipette used, amount of pipet caps/tips used during serial dilution, amount of water/soil mixture transferred to the succeeding tube during serial dilution, amount of soil/water solution put onto petri dishes during serial dilution, formula used during serial dilution, temperature of sterilizing solution and sterile water, amount of dilution solution placed on nutrient agar plates, temperature that the plates are grown in.

e. Step-By-Step Instructions

    1. Go outside of Roland Park Country School in Baltimore, Maryland at 8:30 AM in early May by exiting the Athletic Complex Door. Then, go to the first bench on the right side of the sidewalk, closest to the building, and west of the athletic complex door
    2. Locate all testing sites with a GPS; there will be 3 testing sites on the lawn, 3 testing sites near benches, and 3 near the sidewalk. The second bench used is the third closet bench to the athletic complex door on the right of the sidewalk and west of the athletic complex door. The third bench used is the bench that is the fifth closest bench to the athletic complex door on the right of the sidewalk and to the west of the athletic complex door. This is shown in the diagram below:




The exact locations of all sites are as follows

**L1:** N 39° 21.494, W 76°38.179

**L2:** N 39° 21.499, W 76°38.167

**L3:** N 39° 21.501, W 76°38.159

**S1:** N 39° 21.500, W 76°38.178

**S2:** N 39° 21.502, W 76°38.165

**S3:** N 39° 21.499, W 76°38.152

**B1:** N 39° 21.499, W 76°38.183

**B2:** N 39° 21.500, W 76°38.168

**B3:** N 39° 21.501, W 76°38.159

3. Mark the designated locations, located in step 2, with white flags
4. Label the flags with their first letter of their areas and number of the testing site; the first areas of lawn, under benches, and next to the sidewalk will be labeled L1, B1, and S1 respectively. There will be 9 sites total, and all the remaining flags should be labeled L1, L2, L3, S1, S2, S3, B1, B2, and B3, respectively
5. For each flag, label a Ziploc bag with the same letter and number as the flag.
6. Steps 7-11 must be completed at the same time of day, on the same day, and with the same weather and daylight conditions
7. With a compass above the L1 flag, find the north side of the flag
8. Then, remove soil from the ground directly to the North Side of the L1 flag using a cylindrical soil extractor that is 2.5 cm in diameter. Using the first stippling of the soil extractor, remove 15 cm deep of soil from the North side of the flag.
9. Place this soil into the bag marked L1
10. Repeat steps 7-9 for all 9 flags, placing each soil sample in its correspondingly labeled plastic bag; there will be a total of 9 soil samples
11. Bring all bags of soil samples inside
12. Steps 13-21 must be completed at the same time of day, on the same day, in the same room, with the same weather and daylight conditions

13. Begin the process of serial dilution by placing 1cc of soil from L1 into a 1cc scoop
14. Then, place the soil from the scoop along with 10 ml of sterile water into a 15 ml transformation tube
15. Label this $10^0$ L1
16. Cap the tube and shake vigorously, and then remove 1ml of the soil/water combination with a sterile pipette to add to a second transformation tube containing 9ml of sterile water
17. Label this tube $10^{-1}$ L1
18. Then, place 1ml of the solution with a sterile pipette from the second tube into a 3rd transformation tube containing 9ml of sterile water
19. Label this tube $10^{-2}$ L1
20. Place 100 microliters from each transformation tube onto its own correspondingly labeled 3M Petrifilm™ yeast/mold plate.
21. Repeat steps 12-20 for each individual soil sample except when labeling transformation tubs, switch the "L1" part of the label to the corresponding area that the soil was from. There will be a total of 27 labeled transformation tubes at the end of the experiment
22. Allow the fungi plates to grow for 72 hours
23. Then, after this time period, examine the $10^{-2}$ plate and look for distinct green dots. Record the number of these dots in a data table as "yeast"
24. Then, if no yeasts are present on the $10^{-2}$ plate, look instead at the $10^{-1}$ plate and make a note of this dilution change. If there are no fungi on the

$10^{-1}$ plate, then move to the $10^{0}$ plate, making a note of this dilution change. Record the number of yeast found in a data table.

25. Repeat steps 23-24, looking for molds instead of yeast. Molds will appear green and fuzzy, and look distinctly different than the yeast.
26. In order to make estimates of the number of fungi colonies in the original 1cc sample, this is the formula that should be used: Number of microbes in 1cc of soil = number of colonies on sheet x $10^{2}$ x $10^{|\text{dilution number at which these colonies were found}|}$
27. Once the estimates are made, record all numerical data in a data table

## Data and Observations

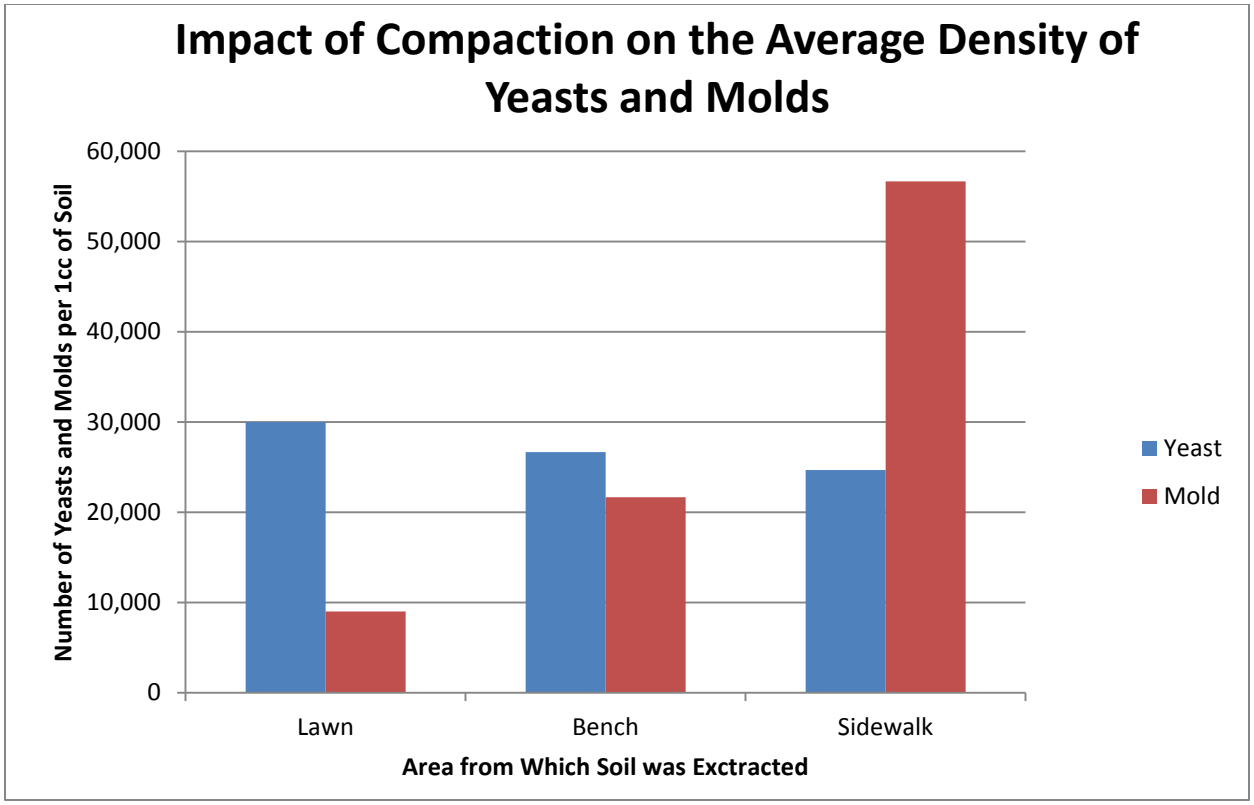
### Data

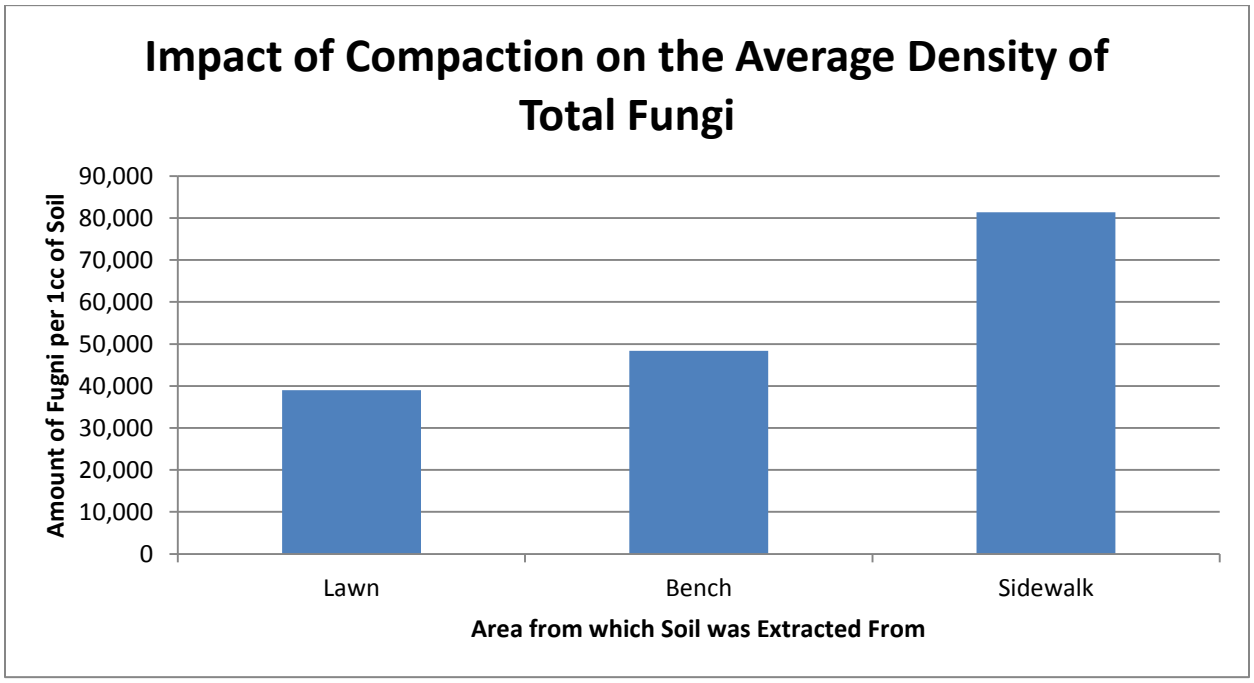
The Impact of Compaction on the Density of Soil Fungi

| Location of Soil Samples | Amount of Yeast (#/cc) | Amount of Mold (#/cc) | Total Number of Fungi (#/cc) |
|---|---|---|---|
| **Lawn Flag 1** | 10,000 | 7,000 | 17,000 |
| **Lawn Flag 2** | 40,000 | 10,000 | 50,000 |
| **Lawn Flag 3** | 40,000 | 10,000 | 50,000 |
| **Average Lawn Flags** | 30,000 | 9,000 | 39,000 |
| **Bench Flag 1** | 30,000 | 50,000 | 80,000 |

| | | | |
|---|---|---|---|
| **Bench Flag 2** | 40,000 | 10,000 | 50,000 |
| **Bench Flag 3** | 10,000 | 5,000 | 15,000 |
| **Average Bench Flags** | 26,667 | 21,667 | 48,333 |
| **Sidewalk Flag 1** | 30,000 | 120,000 | 150,000 |
| **Sidewalk Flag 2** | 4,000 | 40,000 | 44,000 |
| **Sidewalk Flag 3** | 40,000 | 10,000 | 50,000 |
| **Average Sidewalk Flags** | 24,667 | 56,667 | 81,333 |

Graphs


Impact of Compaction on the Average Density of Yeasts and Molds
Number of Yeasts and Molds per 1cc of Soil
60,000
50,000
40,000
30,000
20,000
10,000
0
Lawn
Bench
Sidewalk
Area from Which Soil was Exctracted
Yeast
Mold


Impact of Compaction on the Average Density of Total Fungi
Amount of Fugni per 1cc of Soil
90,000
80,000
70,000
60,000
50,000
40,000
30,000
20,000
10,000
0
Lawn
Bench
Sidewalk
Area from which Soil was Extracted From

## Conclusion

After concluding our experiment, our hypothesis that "Soil compaction caused by sidewalks will be more harmful to the density of fungi than benches" was proven to be incorrect. This is because as proven by our graphs, the highest density of soil fungi was located in our sidewalk samples. There was an average of 81,333 fungi in our 1cc samples of soil taken from next to the sidewalk, versus the comparative average of 39,000 fungi located in our 1cc samples of soil taken from the lawn. These numbers blatantly contradict our original hypothesis, therefore our hypothesis was rejected. These figures make sense for multiple reasons. Firstly, our negative control soil was extracted from the front lawn, where fertilizer is used on the grass. This synthetic fertilizer is extremely harmful to fungi populations for a number of reasons. Due to the fact that the type of fungi in the grasses is exomycorrhiza, the ammonia in the fertilizer affects the fungi in a negative way. As a result, they turn into their yeast form to protect themselves from this stressor of fertilizer. This explains why the highest average density of fungi in their yeast form, 30,000, was located in the lawn samples of our soil, and it shows that the presence of fertilizer can be harmful to the fungi. As proven in our background, fungi prefer areas with an excess of water. The fungi that we extracted were exomycorrhizae, which means that they live outside the roots and grasses on the lawn. This shows that the ammonia was being directly exposed to these fungi, and it was harmful enough to cause them to turn into their yeast form but not die. If there had been an excess of water in the lawn soil, the ammonia would have been flushed out and the harmful effects would have been minimized. Since the ammonia was still harmful to the fungi, it is known that there was not an excess of water. This absence of water could also be due to the minimal rainfall that we have received this year, which is 3.5 inches

below normal value. These facts also explains why the lowest average density of fungi in their mold form, 9,000, were found in our lawn areas.

Additionally, our data makes sense based on our knowledge of the function of the sidewalk. Since the sidewalk is an impervious surface, it does not absorb the water accumulated by rainfall. Instead, the water runs off the sides of the sidewalks, and is deposited into the soil directly adjacent to the sidewalk. This location is exactly where we collected our sidewalk soil samples. Due to this runoff from the sidewalk, there was a large amount of water deposited in the surrounding soil and the overall amount of water in the soil increased. The excess runoff water that the sidewalk fungi received flushed out any remaining ammonia that was originally there. This leads to "happier" fungi in the sidewalk environment, and causes them to expand into their mold forms because they are comfortable with their surroundings. The rate of fungi reproduction increases when there is an adequate or plentiful amount of water present in the soil, and this in turn leads to a higher overall density of fungi. This explains why the highest average total fungi, 81,333 were present in the areas directly adjacent to the sidewalk. It also explains why the highest average density of fungi in their mold form, 56,667 was present in our sidewalk test sites, and why the lowest average density of fungi in their yeast form, 24,667 were found in areas near the sidewalks.

Our bench data supports our previously mentioned theory for a few reasons. The areas that we extracted soil from near the benches are covered with mulch as opposed to grass, and therefore they are not fertilized in the same way that the front lawn is. The fungi however are still being exposed to some fertilizer, because when it is applied to the grass it spreads into other areas such as where we extracted our bench samples from. This means that the fungi are being exposed to a relatively small amount of fertilizer and ammonia, but they are also not receiving

the benefits of the runoff from the sidewalk. The average densities of yeast, mold, and total fungi should theoretically be in between all averages for sidewalk and lawn fungi, and they were. The average amount of total fungi in the original 1cc sample of bench soil was 48,333 which was in between the average amounts of total soil for both the lawn and sidewalk areas. This further proves our new theory because it shows that the runoff from the sidewalk is beneficial to the fungi and the fertilizer is very harmful, especially to the areas that had the most exposure to it. Also the benches were not receiving environmental benefits but they were also only minimally affected by the spread of environmental stressors, which is why it makes sense that the all data for benches was in between the data for the sidewalk and the lawn. The bench areas were physically closer to the sidewalk areas than the lawn areas, so they received a larger supply of water than the lawn areas. This water worked to flush out the ammonia that spread to the bench areas. This is shown by the fact that there is an average of 9,000 more fungi in the bench areas than the lawn areas. This means that some of the ammonia in the bench areas are flushed out by the water runoff from the sidewalk. Additionally, the smallest difference in the amount of average amount of yeast and molds, 4,000 fungi, was found at the bench sites, further showing that the bench environment was neither extremely beneficial nor extremely harmful to the fungi populations.

In the future, many other experiments can be created to increase our knowledge of both the positive and negative aspects of sources of compaction on the densities of soil fungi populations, and the effects of harmful substances such as fertilizer on the densities of soil fungi populations. Now that our research has shown that compaction and fertilizer are both harmful to fungi, the next logical step would be to compare an area of soil affected by compaction to an area of soil that fertilizer is frequently used on. In controlled variables it would be important to make

the distinction that the sidewalk areas cannot be exposed to fertilizer, and the fertilized areas should be in an area free of compaction. Also, since we are now aware of the effects of synthetic fertilizer on the soil fungi, an experiment could be performed in which synthetic and organic fertilizer are compared. Our hypothesis would be that areas fertilized with synthetic fertilizers would have a lower total density of fungi than areas fertilized with organic fertilizer. New conclusions could be drawn from this experiment, and new questions could potentially arise that could be formed into more experiments. Another way to further investigate compaction would be to conduct a similar experiment to the one we performed, but permeable and variable sources of compaction would be used as opposed to the sidewalk, which was constant and impermeable. For example, soil could be extracted from a frequently traveled grassy area, such as a sports field, or from a field where crops are grown that a heavy tractor frequently drives on. These new locations would change our data drastically, because they would not resist the water and would instead absorb it. The field with the tractor example is an instance of variable compaction, because the tractor would not be sitting upon the ground constantly, as the sidewalk did. It would be interesting to see if this was more helpful or destructive to the environment of the fungi than the sidewalk was. In another experiment, the densities of fungi could be tested and compared on a fallow field, and a field that was affected by the variable compaction of a tractor. Despite the fact that our hypothesis “Soil compaction caused by sidewalks will be more harmful to the density of fungi than benches” was proven to be false, our experiment allowed us to learn new and valuable information about sidewalks as a source of compaction and the effects of lawn fertilizer on the density of soil fungi populations.

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