Impact of Compaction on Soil Fungal Density

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Background Report

One important group of microorganisms in the soil are the fungi. These eukaryotic creatures can be single-celled or multicellular depending on environmental conditions, and they live by decomposing and absorbing the organic materials in which and on which they grow. Sometimes plantlike in their physical appearance, they actually lack chlorophyll and are heterotrophs that get their nutrients directly from other living things or the dead remnants of living things. Common types of fungi include mushrooms, molds, mildews, smuts, rusts, and yeasts (Dictionary.com 2017), and most species belong to one of three large categories: decomposers, mutualists, or pathogens. The first group convert dead organic matter into fungal biomass, along with carbon dioxide and other organic compounds; while the second group develop mutually beneficial relationships with plants, and the last kind cause disease by penetrating plants and other creatures and directly destroying their living tissue.

All fungi, though, perform many important tasks that immediately help the soil in which they live including decomposing woody organic matter, increasing nutrient uptake, improving plant resilience, and improving soil structure. Woody plant material, in particular, is hard for most of the other microorganisms living in the soil to digest (Jenkins 2005), and so the fungi help the soil and the organisms living there by converting the nutrients in the woody material into mineral forms (such as carbon dioxide and ammonium) that are more accessible for the other organisms to consume (Fogel 2002). Because fungi decompose hard to digest material to help soil and plants, they can be found wherever there is hard, carbon-rich woody organic matter, and these places can include dead rotting trees in a forest, leaf litter, and even plant roots. (Jenkins 2005).

Because fungi can decompose and digest things other soil microbes cannot, they play a key role in all of the major biogeochemical cycles, including the nitrogen, carbon, oxygen, phosphorus, and water cycles. For example, in the carbon cycle, the fungi decompose organisms and waste which allows carbon dioxide to be released into the air, which plants can then use to complete photosynthesis and produce oxygen, glucose, and biological molecules. Plants, in turn, are consumed, moving their biological molecules up the food chain. Hence, without the fungal decomposers, there would be no raw material (carbon dioxide) to make the biological molecules all the living things in an ecosystem, and what is more, in the nitrogen cycle, the soil fungi return ammonium, a form of nitrogen, back into the ground, where nitrifying bacteria in the soil turn the ammonium into nitrate - a form plants use to help their growth and development. Again, consumers will eat the plants, moving the biological molecules containing the nitrogen up the food chain and throughout the ecosystem. Similar patterns occur with water, phosphorus, and all the other critical nutrients life needs to survive. Hence, put simply, if fungi did not exist in the soil, an ecosystem would collapse (Campbell, Williamson, Heyden 2004).

One type of fungi known as mycorrhizae play an especially significant role in this element cycling and are usually found naturally in all soils (Jenkins 2005). These specialized fungi help to colonize the roots of plants in a symbiotic relationship by producing finger-like projections known as hyphae that can retrieve nutrients easier than the roots can themselves, and the fungi give some of these nutrients to the plants in exchange for the carbon the plants give the fungi.

The size and mass of fungal hyphae can also help to decrease a plant's susceptibility to pests, diseases, and drought, and they can improve soil structure by binding soil particles together to create water-stable aggregates. These aggregates in turn create pore spaces in the soil

that enhance water retention and drainage and reduce the risk of erosion (Jenkins 2005), all of which promotes the root branching that increases the amount of nitrogen, phosphorus, and water a given the plant can access (Kahl 2004).

The reason this improved access to these three substances is so helpful to plants is because each plays a critical role in the life of a plant. For example, nitrogen is important because it forms proteins, nucleic acids, and other cellular components that are essential for all forms of life (Kahl 2004). Phosphorous is important because it is a component of the complex nucleic acid structure of plants, which regulates protein synthesis. Phosphorus is also essential to cell division and development of new tissue, as well as being associated with complex energy transformations in the plant, including cellular respiration where ATP donates its phosphate to transform into ADP, causing cellular work to happen (NSF, USDA, NIFA 2017). Finally, water uptake for transpiration is critical for photosynthesis and water retention provides a pool of dissolved nutrients that are readily available for plants to absorb and use to grow (University of Hawaii 2007 - 2017). Hence because fungi increase the amount of nutrients in the soil and provide additional access to these nutrients, they are vital to the health of the soil ecosystem.

Given how critical soil fungi are, anything that can help or harm them is going to have a profound impact on the well-being of the overall ecosystem, and one such process that can affect the growth of the fungi in the soil is compaction. During compaction, soil and weathered rock are mechanically pounded together to create suitable ground that is able to bear the weight of whatever structure humans are wanting to build there. Compaction is mainly used in construction in order to create a solid foundation for structures to stand on, and the soil is usually placed in layers that are typically 75 mm to 450 mm thick.

In order to complete compaction successfully, it is necessary to ensure proper moisture conditioning, the correct placement materials, and enough pounding on the soil with the correct equipment. The first of these, moisture conditioning, is critically important because according to The American Society of State Highway Transportation Officials (ASHTO) and the American Society of Testing Materials (ASTM) dry soil resists compaction and is hard to press down; while soil that is overly-moistened gets pushed down too easily and causes the mechanical energy to start oozing out to the sides (Gale 2011). Hence, the right amount of water in the soil is vital to the success of a building project. The optimum water content working range is more or less than 2 percent of what is left and the optimum air-voids content tolerance more or less than 1.5 percent (Atkinson 2000). Moreover, if compaction is performed incorrectly or not well enough, it will result in design failure or a decreased service life for any structure built on that soil.

However, while compaction done properly allows people to build roads, buildings, dams and more, it can also be harmful. For example, it can hurt production of crops as it makes root growth and movement very difficult, and it can also deprive the soil of access to enough oxygen which is necessary for the vast majority of organisms living in the soil to survive (Blanchfield 2011). Compaction causes the pores in the soil to become smaller which reduces the amount of atmospheric gas exchange in the soil even more and also decreases the space through which the water can travel lowering the amount of water available in the soil (AgriInfo.in 2015). Therefore, since fungi are dependent on oxygen and water to help them survive, compacted soils can cause a decrease in the population of fungi (Nardi 2003), which as already discussed would harm plant life because plants need the nutrients the fungi provide (McKenzie 2017).

Because fungi are very important microorganisms that help to keep the ecosystem alive, we chose our experiment to determine the impacts that compaction has on the amount of fungi in the soil. We hypothesized that the population of fungi in the soil will decrease the most when being compacted by a building. A building is much heavier in relation to a road or sidewalk. The buildings at RPCS are made of concrete which is a greater weight than the asphalt that the roads are made of, and the sidewalks are even lighter. Hence, we believe that the heaviest form of compaction will have the most impact on the fungal population in the soil.

Procedure

Question: How do different types of human sources of compaction increase or decrease the population of the fungi in the soil?

Hypothesis: The population of fungi in the soil will decrease the most in soil being compacted by a building.

Procedure:

A. Independent Variable: different types of compaction (building, road, sidewalk)

B. Dependent Variable: density of fungi in soil

C. Negative Control: soil sample taken from areas without human compaction

D. Controlled Variables:

- Type of plants growing on the surface
- Distance between soil sample and form of compaction
- Same road
- Same sidewalk
- Same building

- Size of soil sample
- Time and day when soil is taken out to be tested
- Time and day soil is taken out and diluted
- Size of culture tube
- Type of serological pipette
- Size of soil scoop
- Type of nutrient agar plates
- Amount of nutrient agar on each plate
- Number of nutrient agar plates
- Size of micropipette
- Size of transfer pipette
- Amount of sterile water used
- Type of transfer pipette
- Source of sterile water
- Amount of soil added to water
- Amount of water being transferred
- Time water is put in culture tube
- Size of culture tube
- Time from when soil is added to culture tube to when it is transferred
- Time samples sit on nutrient agar plate before analyzing
- Amount of solution plated on agar plate
- Temperature of room where fungi is growing
- How far mixture was diluted to

- Which dilutions were plated

E. Step by Step

1. Complete steps 1-8 on the same day at the same time.
2. Go to N 39.35805° W 076.63602° on RPCS campus (Negative Control).
3. Place flag in this spot, find coordinates of plot using Garmin GPS III Plus.
4. Put a 15.5 cm deep by 2 cm diameter soil core sampler into the soil and use a hammer to dig into the soil until the soil reaches the first marking. Twist the soil core sampler clockwise to retrieve the soil from the ground.
5. Place the soil from the soil core sampler into a plastic bag labeled with the location you took the soil from and what trial number it is "Negative Control (trial number)"
6. Repeat steps 3 - 4 with soil sample at N 39.35835° W 076.63622° (sidewalk).
7. Repeat steps 3 - 4 with soil sample at N 39.35857° W 076.63586 ° (road).
8. Repeat steps 3 - 4 with soil sample at N 39.35782° W 076.63613° (building).
9. Complete steps 10-18 on the same day at the same time.
10. Label all the culture tubes with the following process, but include the correct type of compaction and trial number
11. One day after collecting the soil samples, use a clean, new transfer pipette to add 10 ml of sterile water to a 15-ml culture tube label the tube "10^0 Negative Control Trial 1"
12. Use the same pipette to add 9 ml to a second 15 ml culture tube and label the tube "10^{-1} Negative Control Trial 1"

13. Repeat step 13 one more time to additional 15 ml culture tube but this time label it "10^{-2} Negative Control Trial 1"
14. Place 1 cc of soil from the "Negative Control Trial 1" bag into the "10^{0} Negative Control Trial 1" using a 1 cc soil scoop.
15. Cap the tube and shake vigorously.
16. Using a new clean pipette remove 1 ml of the soil/water mixture from each "10^{0} Negative Control Trial 1" tube and place it into the "10^{-1} Negative Control Trial 1" tube
17. Cap the tube and shake vigorously.
18. Using the same pipette from step 16, remove 1 ml of the soil/water mixture from "10^{-1} Negative Control Trial 1" and place into the "10^{-2} Negative Control Trial 1"
19. You should now have a total of 3 culture tubes filled with a soil/water mixture.
20. Immediately after completing the transferring of soil/water mixture between the culture tubes, place 100 µl samples from each of the "10^{0}, 10^{-1}, 10^{-2} Negative Control Trial 1" tubes onto their own separate, correspondingly labeled 3M Petrifilm Yeast and Mold Count Plate nutrient agar plate.
21. Repeat steps 11-20 labeling the tubes "10^{0} Sidewalk Trial 1" "10^{-1} Sidewalk Trial 1" "10^{-2} Sidewalk Trial 1" "10^{0} Road Trial 1" "10^{-1} Road Trial 1" "10^{-2} Road Trial 1" "10^{0} Building Trial 1" "10^{-1} Building Trial 1" "10^{-2} Building Trial 1"
22. Allow all agar plates to grow for 72 hours.
23. Repeat steps 1 - 22 two more times, gathering soil for trial 2 on the same day as completing steps 1-22, and gathering soil for trial 3 on the same day as completing the serial dilutions for trial 2.

24. After it has been at least 72 hours since trial 3 samples were plated, arrange all plates by trial number and type of compaction (there should be 36 total plates).
25. Begin examining the 10^{-2} plate for each type of compaction and each trial
26. Count the number of yeast spots on the plate and record the number and the dilution value that they were found at
27. If there is no yeast on the 10^{-2} plate, then flip that plate over and examine the 10^{-1} plate.
28. Count the number of yeast spots on the plate and record the number and the dilution value that they were found at
29. If there are no yeasts on the 10^{-1} plate, then flip that plate over and examine the 10^{0} plate.
30. Count the number of yeast spots on the plate and record the number and the dilution value that they were found at
31. Use the following equation to calculate the total number of fungi in 1 cc of soil: # of microbes in 1 cc of soil = # of colonies on sheet x 10^{2} x $10^{|\text{dilution \# at which these colonies were found}|}$
32. Repeat steps 23 - 27, but this time counting the number of mold spots.
33. Add the calculation for yeasts with the calculation for molds to get the total amount of fungi for that trial.

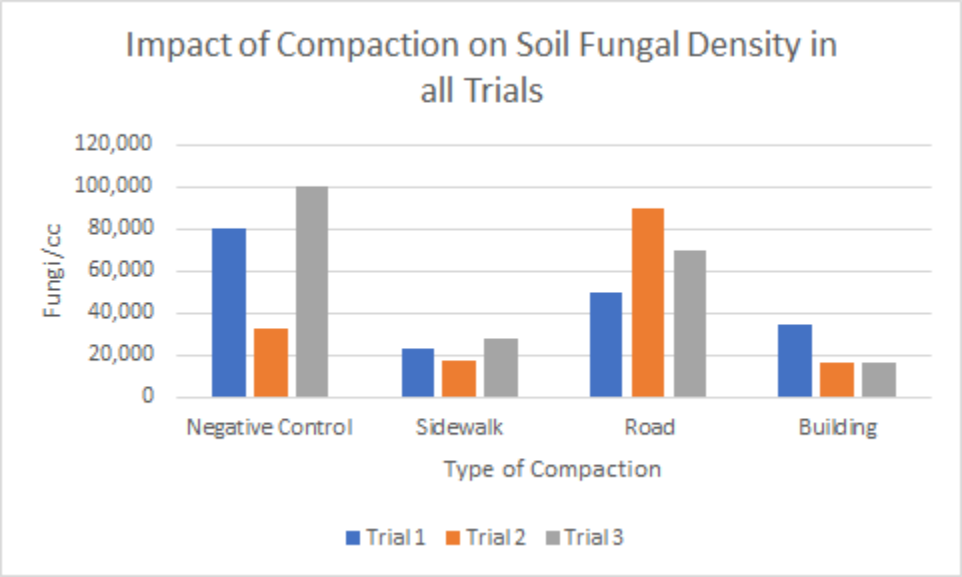
Data and Observations

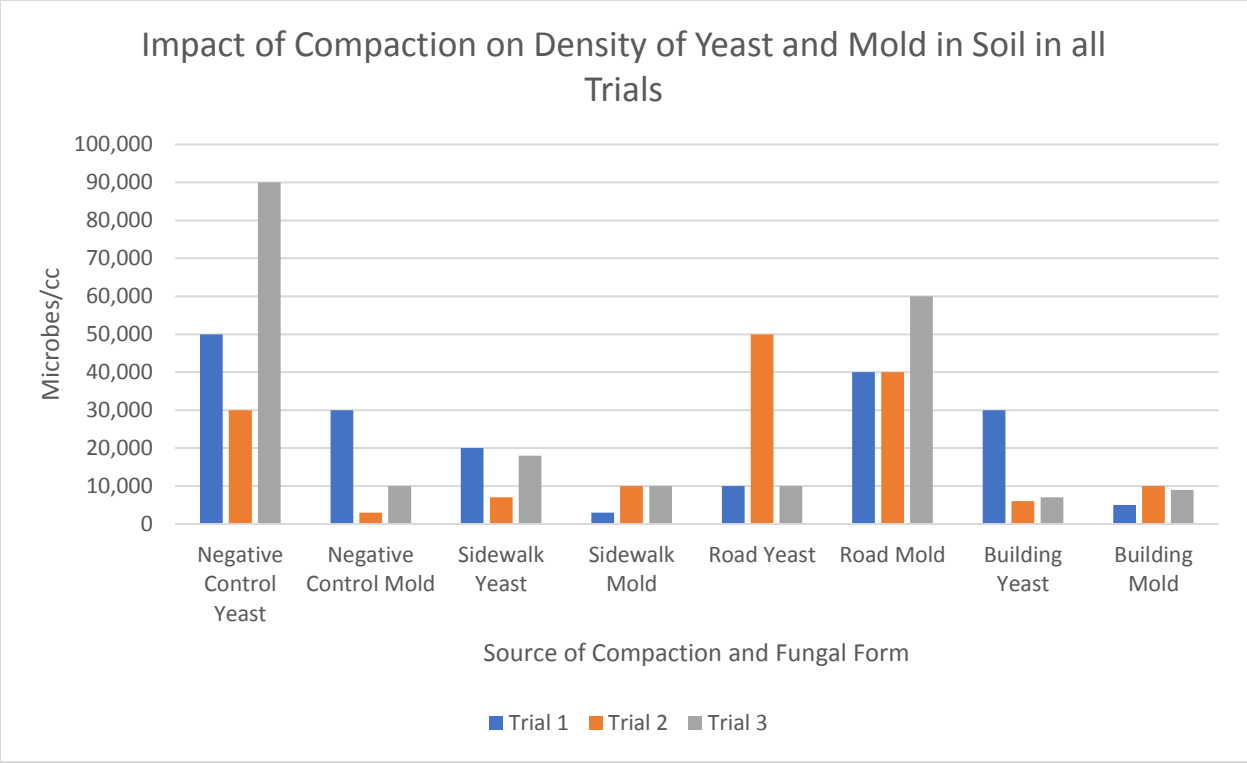
Impact of Compaction on Fungal Density in Soil

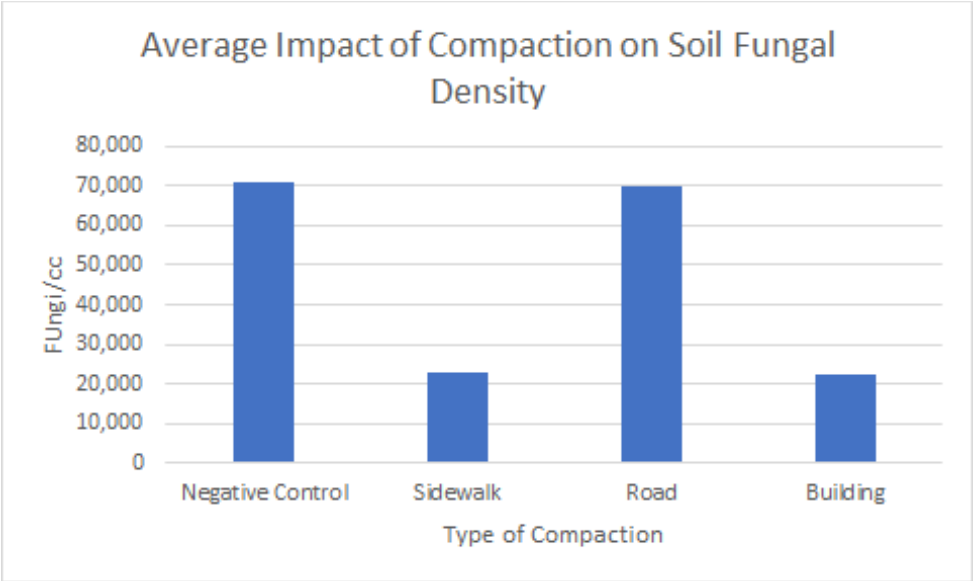
Source of Compaction												
	Negative Control			Sidewalk			Road			Building		
	Fungal Form			Fungal Form			Fungal Form			Fungal Form		
Trial #	Yeast Density (#/cc)	Mold Density (#/cc)	Total Fungi (#/cc)	Yeast Density (#/cc)	Mold Density (#/cc)	Total Fungi (#/cc)	Yeast Density (#/cc)	Mold Density (#/cc)	Total Fungi (#/cc)	Yeast Density (#/cc)	Mold Density (#/cc)	Total Fungi (#/cc)
Trial 1	50,000	30,000	80,000	20,000	3,000	23,000	10,000	40,000	50,000	30,000	5,000	35,000
Trial 2	30,000	3,000	33,000	7,000	10,000	17,000	50,000	40,000	90,000	6,000	10,000	16,000
Trial 3	90,000	10,000	100,000	18,000	10,000	28,000	10,000	60,000	70,000	7,000	9,000	16,000
Average	56,667	14,333	71,000	15,000	7,667	22,667	23,333	46,667	70,000	14,333	8,000	22,333

Average Soil Fungal Density (#/cc)

Type of Compaction	(#/cc)
Negative Control	71,000
Sidewalk	22,667
Road	70,000
Building	22,333




Impact of Compaction on Density of Yeast and Mold in Soil in all Trials
100,000
90,000
80,000
70,000
60,000
50,000
40,000
30,000
20,000
10,000
0
Microbes/cc
Negative Control Yeast
Negative Control Mold
Sidewalk Yeast
Sidewalk Mold
Road Yeast
Road Mold
Building Yeast
Building Mold
Source of Compaction and Fungal Form
Trial 1
Trial 2
Trial 3


Average Impact of Compaction on Soil Fungal Density
80,000
70,000
60,000
50,000
40,000
30,000
20,000
10,000
0
FUngi/cc
Negative Control
Sidewalk
Road
Building
Type of Compaction

Conclusion

Our hypothesis was incorrect. We know that our hypothesis was incorrect because we predicted that the population of fungi in the soil would decrease the most in soil being compacted by a building, and after testing different soil samples, we proved this to be false. To test this, we completed three trials, and in each trial, we collected a soil sample from next to a building, road, sidewalk, and an area with no compaction to use as our negative control. Once we collected these samples we completed serial dilutions to find the yeast density and the mold density, and then added those two numbers to end up with the total amount of fungi in one cubic centimeter of that certain soil sample. When examining the data for the average impact of compaction on soil fungal density, we found that the building had the lowest fungal density out of all four areas. The building had an average of 22,333 fungi per cubic centimeter. The sidewalk had just higher than that, at an average of 22,667 fungi per cubic centimeter. The road had an average of 70,000 fungi per cubic centimeter, and the negative control had an average of 71,000 fungi per cubic centimeter. Although the average for the building location was in fact lower than the average for the sidewalk location, it was by such a small amount that it was hard to prove fully, so we had to take a look at the averages for each individual trial. When taking a look at each individual trial, the graph seems to confirm our hypothesis correct because there is a dropoff in the amount of fungi by the building and the fungi that are by the sidewalk fluctuate a little bit. We cannot conclude that our hypothesis is correct because we do not know which physical form of fungi, yeast or mold, caused the drop off in density of fungi by the building, so we must look at the graph which includes the density of yeast and mold in all of the trials in one cubic centimeter of soil. When looking separately at the impact of compaction on the density of yeast and mold in the soil, we found that our hypothesis proved to be incorrect. After examining the amount of

yeast in the soil that was compacted by a sidewalk, the amount of yeasts decreased and then fluctuated, while the amount of molds fluctuated and stayed higher. For the building location, there was a significant drop in the amount of yeast during the three trials, while for mold, there was a slight fluctuation. Fungus lives in two forms: mold and yeast. When a fungus is in its yeast form, it pulls in all of the hyphae because it cannot thrive in the environment it is currently in. When it is in its mold form it can spread out its hyphae and is satisfied with the conditions of the current environment. A higher level of mold shows that the fungi can thrive in the environment they were in. A higher level of yeast shows that the fungi disliked the environment. In the soil compacted by the sidewalk there was a higher population of yeast than in the soil compacted by the building showing that the sidewalk had the most negative impact on the fungal population growth.

We discovered from our graphs that even though we hypothesized that the building would compact the soil the most, the sidewalk had the most negative impact on the fungal population growth. This could have occurred because with a building, the taller it is, the deeper the foundation of it is. The building that we used for our experiment was much taller than the sidewalk and therefore has a deeper foundation than the sidewalk. Since a sidewalk is thin and sits on top of the ground, the most compacted soil will be found right below it, where we were testing. Whereas the building has a deeper foundation, and we were only digging 15.5 centimeters into the ground. Since we were not digging deep enough to reach where the building foundation sits, the soil was not as compacted. We believe that if we had collected soil from underneath the building foundation, the soil would have been more compacted than the soil from the sidewalk, since it would have been directly under the building.

After performing our experiment we have come up with a future research direction to further increase our knowledge on compaction and how it affects microbes in the soil. We could perform an experiment to find how the depth of the collected soil next to a building and sidewalk affects the number of fungi in the soil. We could compare the fungal density levels by collecting soil from different depths in the soil from both the topsoil and subsoil from both the building and the sidewalk. We would hypothesize that the subsoil from near the building would have the lowest fungal density. We believe that the subsoil of the building would be the most compacted because it is the closest to the base of the building. Since the foundation of the building is situated deeper in the ground the soil in the subsoil section would have the least number of fungi. On the other hand, the foundation of the sidewalk does not reach all the way to the subsoil so there would be a greater fungal population. During our experiment, we learned that the soil from the topsoil near the sidewalk had a more negative impact on the fungal population in the soil then the building did. If we conducted this future experiment, we think that the subsoil near the building would most negatively impact the fungal population growth.

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