Soil Ecology Project

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Background

Fungi are microscopic organisms that play a critical role in the soil food web and the larger ecosystem. Hyphae, a long thread or strand, push their way between soil particles, roots, and rocks. Some fungi, such as yeast and mold, are single celled organisms, whereas fungal fruiting structures, like mushrooms multicellular organisms. (Elaine R. Ingham, 2014) Yeast is a unicellular organism that reproduces asexually while molds reproduce sexually and asexually. Both yeast and mold are considered parasites since they are opportunistic organisms, and they have to be in their host to grow, live, and reproduce. (DifferenceBetween.net, 2010) The soil food web is the community of organisms living all or part of their lives in the soil. Food webs describe the transfer of energy between species in an ecosystem. Therefore, the soil food web is dependent on fungi to decompose dead organic matter, maintain soil structure, and give plants key nutrients. (Elaine R. Ingham, 2014)

First, invertebrates break down large pieces of dead, organic matter, and then the fungi colonize these fragments. They extract food for their own production of energy, and leave excess nutrients in the soil for other organisms to take in. The nutrients are brought up through the roots of the plants which benefit is the plants' growth. This in turn, increases the food source for the consumers, and more energy will be passed up the food chain. (Fogel, 2001) Hyphae are long strands attached to the fungi which extract nutrients from the dead material and they use organic waste as food to produce energy. Fungal hyphae physically bind soil particles together, creating stable aggregates which are a material formed from a loosely compacted mass of fragments or particles that help increase water infiltration and soil water holding capacity. (Elaine R. Ingham, 2014) It is important to hold oxygen and water within the pores so the processes of cellular

respiration and photosynthesis can take place. When the hyphae bundle together, they create mycelium. (Countryside Information, 2014) This is the decomposition process. A decomposer breaks down dead plants and animals. A decomposer is important for maintaining any ecosystem, because without decomposers, plants wouldn't receive the essential nutrients and waste would begin to build up. (Lovett, 2012)

Fungi cannot synthesize their own food. They mostly grow on trees or on roots of trees**.** Fungi that have a symbolic or mutualistic, relationship with plants are known as mycorrhizae. Mycorrhizae colonize plant roots, and in exchange for carbon from the plant, the fungi solubilize phosphorus and bring soil nutrients to the plant. There are two common types of mycorrhizae, ectomycorrhizae and endomycorrhizae. Ectomycorrhizae are characteristic of certain temperate trees such as oaks, willows, and conifers. They surround the root tips with hyphae penetrating between the cells of the root cortex. Endomycorrhizae are commonly found on herbaceous plants and many tropical and some temperate trees. (San Francisco State University, 2014) Many fungi help control diseases, including the nematode-trapping fungi which parasitize disease-causing nematodes. In fact, the fungi which feed on insects and invertebrates may be useful as biocontrol agents. (Elaine R. Ingham, 2014)

Like most organisms, a fungus is dependent on oxygen in order to live and reproduce. When the air is polluted, the fungi population is at risk of not being able to perform their crucial tasks in the soil. Air pollution occurs when harmful amounts of gases, dust, fumes, or odors are emitted into the atmosphere. Some air pollution occurs from the wind blowing dust, debris, and smoke from house and forest fire, however, the main cause is car exhaust. (Air-Quality.org.uk, 2014) The major chemicals found in car exhaust are sulfur dioxide, nitrogen oxides, and carbon

monoxide. (Air-Quality.org.uk, 2014) These chemicals can get into the soil by a chemical process called chemodenitrification and change the acidity of the soil. (Bremner 1997). When mixed together with the moisture of the air, these chemical gases are turned into sulfuric and nitric acids which precipitate into the ground as acid rain. (Barbarick, 1997). Acid rain makes the soil more acidic, and therefore decreases the pH level of the soil. pH is a measurement of the acidity in the soil, or more specifically the total hydrogen ion concentration in soil water. It is measured on a scale of 0-14, 0-6 being acidic, 7 being neutral, and 8-14 being basic. (Singapore garden society 2009) When pH levels fall, plants have difficulty growing from the lack of nutrients caused by the acid. (Singapore garden society 2009) On a larger scale, acid rain will cause a slower growth, injury, or death of forests, putting the entire ecosystem in jeopardy. The enzymes of organisms, including fungi and plants, are denatured or disabled by a low plot, which prevents them from performing basic functions. (U.S Environmental Protection Agency 2014). Without enzymes as crucial proteins that allow chemical reactions to occur, all living things could not perform the 4 properties of life including, homeostasis, transformation of energy, synthesizing new material, and reproduction, therefore the organism would eventually die.

Knowing that chemicals such as sulfur dioxide, nitrogen oxides, and carbon monoxide are heavily present close to high traffic areas, it is likely that these plots of soil closest to the cars would be most affected. Through our research, we have determined that these primary pollutants harm the organism in the soil by reducing their enzymatic reactions. We proposed a hypothesis stating that the closer proximity to the car exhaust, the lower population density of fungi in the soil. Therefore, the further the soil, from the areas of car exhaust, the more fungi will be able to live healthily and perform their important functions in the soil.

Lab Outline

Problem:

How does car exhaust impact the fungi population density in the soil?

Hypothesis:

The closer proximity to the car exhaust, the lower population density of fungi in the soil.

Variables and Controls:

Independent Variable: Proximity of soil to the car exhaust: Island (closest), front lawn (semi-close), courtyard (farthest)

Dependent Variable: Population density of fungi in 1cc of soil

Negative Control: Courtyard plot (Farthest distance from the car exhaust)

Controlled Variables:

- Amount of car exhaust
- Amount of soil sample
- Location of soil samples
- Time of day
- Amount of soil extracted
- Size of plotting area
- Size of culture tubes
- Amount of sterile water added to transfer pipette
- Amount of soil added to culture tubes
- Amount of soil/water mixture removed from culture tubes
- Amount of soil/water mixture put on the petri plates
- Amount of time that yeast and mold can grow on plate

Procedure:

1. Plot a 20x20 cm spot in the area of grass by the carpool line at coordinate points: N: 39º 21.486’ W: 076º 38.131’. This will be called plot 1.
2. Extract a soil sample for trial one by inserting the soil core sampler 15 cm into the soil then turning it clockwise. Then, lift the 15 cm deep and 2 cm across in diameter soil core sampler out of the soil and place the soil sample into a sterile Ziploc bag labeled “plot 1 trial 1”.
3. Plot a 20x20 cm spot on the front lawn at coordinate points:

N: 39º 21.486’ W: 076º 38.166’. This will be called plot 2.

4. Extract a soil sample for trial one by inserting the soil core sampler 15 cm into the soil and turn clockwise. Then, lift the soil core sampler out of the soil and place soil sample into a sterile Ziploc bag labeled “plot 2 trial 1”.
5. Plot a 20x20 spot in the courtyard at coordinate points:
   N: 39º 21.468 W: 076º 197. This will be called plot 3.
6. Extract a soil sample for trail one by inserting the soil core sampler 15 cm into the soil and turn clockwise. Then, lift the soil core sampler out of the soil and place soil sample into a sterile Ziploc bag labeled “plot 3 trial 1”.
7. Then begin the dilution process for all of trial one. All trial 1 samples must be diluted on the same day at the same time.
8. Use a clean, new transfer pipette to add 10 mL of sterile water to a 15 mL culture tube. Label the tube “10^0 plot 1.”
9. Use the same pipette to add 9 mL of sterile water to a second 15 mL culture tube. Label the tube “10^{-1} plot 1.”
10. Repeat step 9 one more time to make a 15 ml culture tube labeled “10^{-2} plot 1”.
11. Place one cc of your “plot 1 trial 1” soil sample into the “$10^{0"}$ culture tube.
12. Cap the tube and shake vigorously.
13. Using a new clean pipette, remove one mL soil/water mixture from the “10^{0}” tube and place into the “10^{-1}” tube.
14. Cap and shake vigorously.
15. Using the same pipette in step 13, remove one mL of the soil/water mixture from the “$10^{-1"}$ tube and place into the “10^{-2}” tube.
16. Cap and shake vigorously.
17. You should now have a total of three culture tubes.
18. Plate 100 ml samples from all three tubes onto their own separate petri plates containing nutrient agar labeled “10^0 plot 1 trial 1”, “10^{-1} plot 2 trial 1”, and “10^{-2} plot 3 trial 1”.
19. Allow yeast and mold to grow for 72 hours.
20. Examine each of the plates to count the individual yeast and mold colonies. Yeast colonies are sharp, little blue or yellow, brown circles. Mold colonies are big, fuzzy circles that are green. First look at the 10^{-2} plate for at least 1 yeast and mold colony. If one or both colonies are missing, look to the 10^{-1} plate for at least 1 yeast and mold colony. If one or both are missing in the 10^{-1} plate, look to the 10^0 plate. To make your estimates of the number of fungi in the original 1 cc soil samples, use the following formula:

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

21. Repeat step 2 to extract plot 1 trial 2.
22. Repeat step 4 to extract plot 2 trial 2.
23. Repeat step 6 to extract plot 3 trial 2.

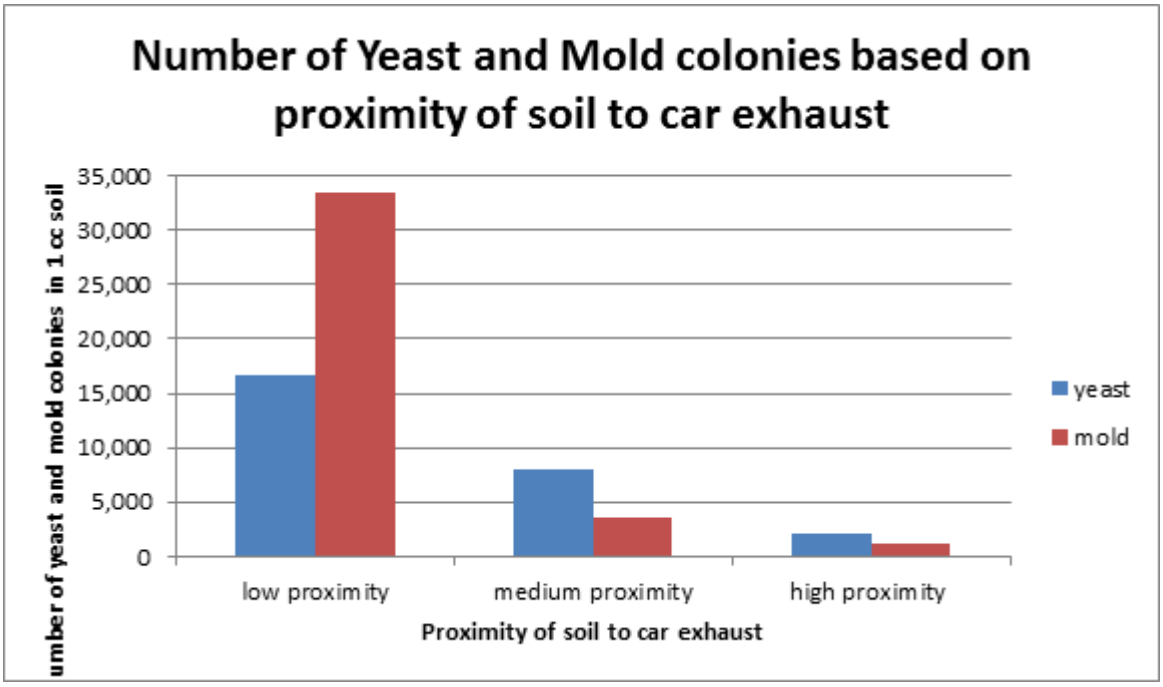
24. Then begin the dilution process for all three trial two samples, repeating steps 8-20. All trial 2 samples must be diluted on the same day at the same time.
25. Repeat step 2 to extract plot 1 trial 3.
26. Repeat step 4 to extract plot 2 trial 3.
27. Repeat step 6 to extract plot 3 trial 3.
28. Then begin the dilution process for all three trail three samples, repeating steps 8-20. All trial 3 samples must be diluted on the same day at the same time.

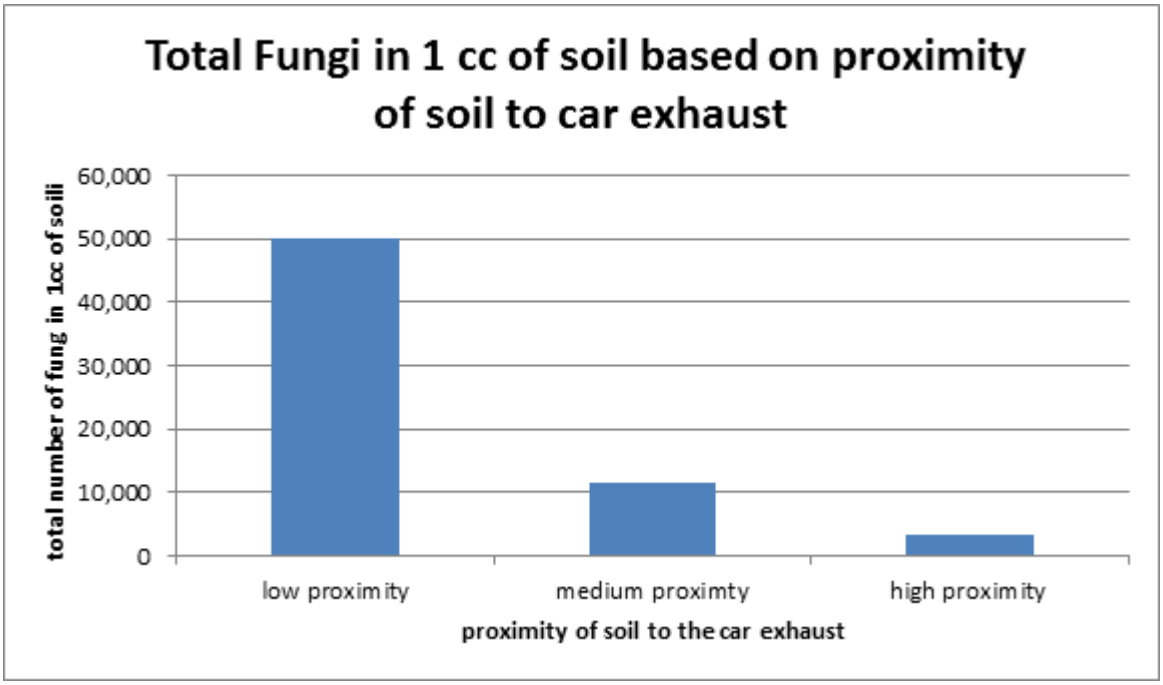
Data & Observations

Yeast and Mold Colonies Data Table

Soil Sample	Yeast Dilution number	Number of Yeast on plate	Mold Dilution Number	Number of mold on plate	Number of yeast colonies in 1cc soil	Number of mold colonies in 1cc soil	Number of fungi colonies in 1cc soil
Plot 1 Trial 1	10^{0}	2 colonies	10^{0}	4 colonies	200	400	600
Plot 2 Trial 1	10^{-1}	3 colonies	10^{-1}	8 colonies	3,000	8,000	11,000
Plot 3 Trial 1	10^{-2}	2 colonies	10^{-2}	7 colonies	20,000	70,000	90,000
Plot 1 Trial 2	10^{-1}	4 colonies	10^{-1}	2 colonies	4,000	2,000	6,000
Plot 2 Trial 2	10^{-2}	2 colonies	10^{-1}	1 colony	20,000	1,000	21,000
Plot 3 Trial 2	10^{-2}	2 colonies	10^{-2}	2 colonies	20,000	20,000	40,000
Plot 1 Trial 3	10^{0}	19 colonies	10^{0}	13 colonies	1,900	1,300	3,200
Plot 2 Trial 3	10^{-1}	1 colony	10^{-1}	2 colonies	1,000	2,000	3,000
Plot 3 Trial 3	10^{-2}	1 colony	10^{-2}	1 colony	10,000	10,000	20,000

	Average # of total fungi in 1 cc of soil	Average # of total yeast in 1 cc of soil	Average # of total mold in 1 cc of soil
Plot 1	3,267	2,033	1,233
Plot 2	11,667	8,000	3,667
Plot 3	50,000	16,667	33,333


Number of Yeast and Mold colonies based on proximity of soil to car exhaust
umber of yeast and mold colonies in 1 cc soil
35,000
30,000
25,000
20,000
15,000
10,000
5,000
0
low proximity
medium proximity
high proximity
Proximity of soil to car exhaust
yeast
mold


Total Fungi in 1 cc of soil based on proximity of soil to car exhaust
total number of fung in 1cc of soili
60,000
50,000
40,000
30,000
20,000
10,000
0
low proximity
medium proximty
high proximity
proximity of soil to the car exhaust

Conclusion

In conclusion our hypothesis was proven correct as we stated the lower proximity the greater population of fungi the car exhaust. The average number of fungi with low proximity (plot 3) was 50,000 colonies in 1 cc soil, in medium (plot 2) proximity there were 11,667 fungi colonies in 1 cc of soil, and in high proximity (plot 1) of the soil to the car exhaust there were 3,267 fungi colonies in 1 cc of soil. In the higher proximity in the yeast to mold colonies there was a ratio of 2 to 1, in the medium proximity the ratio of the number of yeast to mold colonies was 2 to 1, in the high proximity the yeast to mold colonies had a ratio of 1 to 2. The lower the proximity of the soil to the car exhaust the more mold colonies were present proportional to the yeast colonies. This shows that the lower proximity soil is the healthiest, as the mold grows in healthy environments with nutrients, whereas the yeast are fungi are in a survival mode and only performs the most necessary tasks. For future research, we would test the pH of the soil to test the acidity of the soil from the car exhaust. For the current experiment we assumed, from the car exhaust that the proximity of the soil to the car exhaust would make the soil acidic but had no proof of this. To better see the relationship between the number of fungi in the soil and the acidity of the soil due to car exhaust, we would test the pH level.

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