The Correlation Between Mold, Sulfur, and Ammonia Nitrogen

By Sydney Sauls, Annie Ho, and Sophia Koontz

Abstract:

Decomposition of plant matter is conducted by mold and other microorganisms breaking down the organic material. Through this chemical process, ammonia and sulfur, among other chemicals, are produced in soils. From the data taken from the E.S.S.R.E 2019 Biota Survey, it was observed that significantly different levels of mold were found in different microclimates, as were significantly different levels of ammonia. It was hypothesized that these varying mold and ammonia levels were caused by decomposition in the contrasting densities of dead plant matter. Three E.S.S.R.E microclimates were chosen varying on their statistically different mold densities, and soil samples, 15 cm deep by 2 cm in diameter, were taken from plots set at varying distances from a decaying log at each of these locations. Serial dilutions were performed to test for the mold densities using the 3M Petrifilm^{TM} Yeast and Mold Count Plates, and ammonium and sulfate levels in the soil were tested using a LaMotte STH-14 test kit. We discovered that neither the distance from the log nor the mold level had a significant effect on the ammonia and sulfate levels in the soil. We predict that the bacteria, however, would have shown a significant effect on the ammonia and sulfate levels in the soil. Additionally, we believe that the distance observed from the log was not significant enough to display notable results.

Introduction:

Decomposition is the breakdown of organic matter by a variety of microorganisms, such as fungi and bacteria, into chemical nutrients that are released back into the soil, water, and atmosphere (NHPBS, 2019). These nutrients include carbon dioxide and inorganic states of the elements in the soil such as nitrogen. The process starts when earthworms and other invertebrates break down the decomposing matter in a process called fragmentation, increasing the surface area of the decayed material which allows bacteria and fungi to grow and reproduce more easily. Fungi in particular play a vital role in the process of decomposition and the cycling of carbon and other elements in soil. These microbes lack chlorophyll and cannot make their own energy; hence, they must consume dead and decomposing material in order to survive (The City of Euless, 2019). They do this by releasing enzymes that break down the dead matter and then directly absorb the released nutrients from the decomposed plants and animals for their own metabolic needs (NHPBS, 2019).

Table 1		
Microclimate	Coordinates	Average
Microclimate 2, Quadrant 2	N 39.35740; W 076.63893	average = 22491.67 #/cc soil;
Microclimate 3, Quadrant 1	N 39.35797; W 076.63836	average = 62833.33 #/cc soil
Microclimate 5, Quadrant 3	N 39.21511; W 076.38397	average= 41283.33 #/cc soil,

The rate of fungal decomposition is dependent on a number of different environmental factors, including temperature, moisture, and the chemical content of the organic matter (Utah State University, n.d.). These factors, in turn, directly impact the morphology of all fungi, causing them to take one of their two forms: unicellular yeasts or multicellular molds (Brock, 2006). In the soil, fungi take the form of yeast when they need to protect themselves but take the form of mold in the conditions more conducive to higher levels of decomposition. Therefore, when there is a higher ratio of mold to yeast, more decomposition is taking place.
Two of the key nutrients fungi release during the decomposition process are nitrogen and sulfur. Both sulfur and nitrogen play major roles in making the amino acids needed to make proteins, and these proteins allow plants to grow and survive. Yet when a plant (or any other organism) dies, the nitrogen in their bodies is in the form of organic nitrogen that is not accessible by other plants. Fungi, though, break down the plant matter into inorganic nitrogen in the form of ammonium. This process, known as ammonification, allows for ammonium to become available for plants and other microorganisms for growth (Biology Dictionary, 2019). Meanwhile, the organic sulfur that is decomposed is mineralized into sulfate (SO_4^{-2}), the form that is taken up by the roots of growing plants ("Soils - Part," 2019).
These two nutrients, therefore, are key indicators of decomposition taking place in the soil, which is why during the ESSRE 2019 Soil Biota Survey, when significantly different levels of mold were found in the different microclimates that were tested, we wanted to look further into what variable could be causing the mold count to be higher in some locations rather than all of

the locations to be similar. After looking at each of the sites with higher mold counts (see Table 1), we noticed that they all had fallen logs and decaying plant matter. Knowing the relationship between fungi and decomposition, we hypothesized that the levels of mold in the soil will decrease as the distance from decomposing plant matter increases (ESSRE 2019).

Methods:

Three of the E.S.S.R.E Microclimates were chosen based on their statistically higher mold densities observed in the E.S.S.R.E 2019 Soil Biota Survey (ESSRE, 2019): Microclimate 2, Quadrant 2 (N 39.35740; W 076.63893), average = 22491.67 #/cc soil; Microclimate 3, Quadrant 1 (N 39.35797; W 076.63836), average = 62833.33 #/cc soil; Microclimate 5, Quadrant 3 (N 39.21511; W 076.38397), average= 41283.33 #/cc soil. 3 plots were created 0.5 m away from each other parallel to and 0 meter from the decomposing log located at each site. 3 more plots were created perpendicular to the log 3 meters from each of the "0 meter" plots, and 3 plots were created perpendicular to the log 6 meters from the "0 meter" plots.

Soil samples, 15 cm deep by 2 cm in diameter, were taken from one row of the 0 meter, 3 meters, and 6 meters plots on July 18th. 3 more samples were taken on July 19th from the second row of 0, 3, and 6 meters plots, and 3 more on July 22nd from the final row of 0, 3, and 6 meters plots. All soil samples were tested for ammonia and sulfur levels (ppm) using a LaMotte STH Series professional soil testing outfit. Simultaneously, serial dilutions of 1 cubic centimeter of each sample were done with sterile water mixed to the 10^{-3} dilution. Then, 100 µl samples of each dilution were plated on its own individual 3M Petrifilm^{TM} Yeast and Mold Count Plate and grown for 72 hours before counting.

Results:

Figure 1: This line graph shows the average mold count at each location within the soil in relation to the distance from a fallen log.

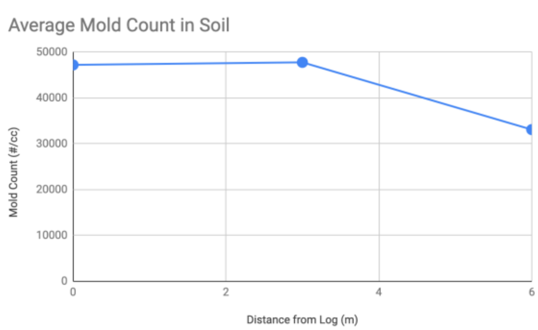

Figure 2: This scatter plot shows the relationship between the mold count and sulfur levels in the soil from all 3 locations and trials.

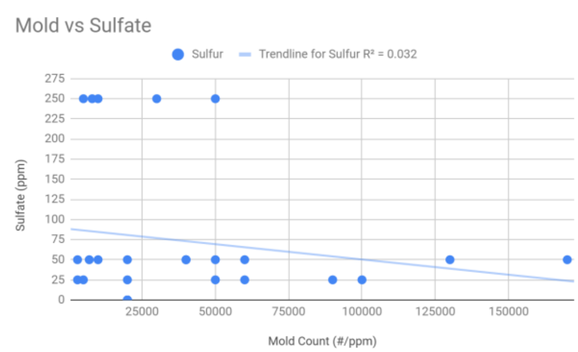

Figure 3: This scatter plot shows the relationship between the mold count and ammonium levels in the soil from all 3 locations and trials.

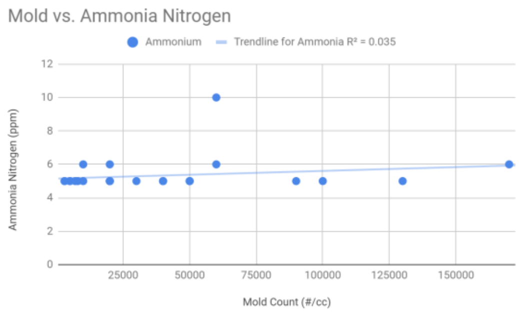

Figure 4: This line graph shows the average ammonium levels at each location within the soil in relation to the distance from a fallen log.

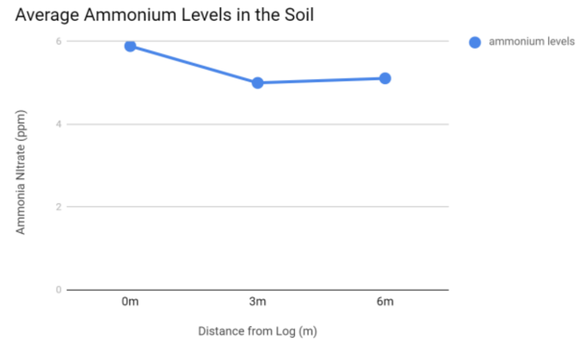

Table 2: P-values for Ammonia Nitrogen, Sulfate, Mold, and Yeast

Distance from log (m)	Ammonia nitrogen	Sulfate	Mold	Yeast
0 - 3	**p = 0.14**	**p = 0.69**	**p = 0.69**	**p = 0.66**
3 - 6	**p = 0.35**	**p = 0.95**	**p = 0.68**	**p = 0.37**
0 - 6	**p = 0.19**	**p = 0.64**	**p = 0.39**	**p = 0.36**

*****2-Sample T-tests were performed to find p-values comparing the distances from the fallen logs to both the chemicals and microbes that were tested for.

Discussion:

Our initial hypothesis, that as the distance from decomposed plant matter increased, the mold densities in the soil, along with ammonium levels, and sulfate levels would decrease, was not supported. While Figure 1 would seem to support our hypothesis, there was no statistically significant difference between the distance from the log and the mold density found there (see Table 2; $p = 0.39$-0.69). Figures 2 and 3 also show that there was effectively no correlation found between the mold, ammonium, and sulfate levels in our study ($r^2 = 0.032$ and 0.035 respectively).

However, the data did reveal statistically significant differences in the amount of ammonium at each location. As Figure 4 shows, ammonium levels dropped in soil the farther away from the log samples were taken ($p = 0.14$-0.19), and since our data strongly suggests that this correlation is not due to the density of mold in the soil (see Figure 2), there is most probably another variable responsible for the higher ammonium levels closer to the log.

One possibility for this factor may be bacteria. Like molds, bacteria also break down proteins in decomposing matter and release ammonium ions, and they are heavily involved in the nitrogen cycle. Therefore, in the future, we would test each sample for bacteria counts as well as the other forms of nitrogen found in the soil (nitrite and nitrate).

References:

Biology Dictionary. (2019). Ammonification. Retrieved July 19, 2019, from https://biologydictionary.net/ammonification/

Bot, A., & Benites, J. (2005). The importance of soil organic matter. Retrieved July 19, 2019, from http://www.fao.org/3/a0100e/a0100e00.htm#Contents

Brock, D. (2006). *Infectious fungi (deadly diseases & epidemics)* (E. I. Alcamo, Ed.). Chelsea House.

The City of Euless. (2019). Microbial decomposers. Retrieved July 18, 2019, from http://www.eulesstx.gov/composting/bc_microbial.htm

Department of Land, Air, and Water Resources. (n.d.). Decomposition (University of California, Davis, Ed.). Retrieved July 18, 2019, from http://lawr.ucdavis.edu/classes/ssc219/biogeo/decomp.htm

ESSRE. (2019). E.S.S.R.E microclimate database (Environmental Science Summer Research Experience for Young Women, Ed.). Retrieved July 19, 2019, from http://essre.rpcs.org/ESSREMicroclimateSurvey.htm

Kamprath,E. J., & Smyth, T. J. (2004). *Liming*. https://doi.org/10.1016/B0-12-348530-4/00225-3

McClaugherty, C. (2001). *Soils and decomposition*. Retrieved July 19, 2019, from https://www.geneseo.edu/~hartvig/popcommlab/papers/soils%20and%20decomposition%20-%20Nature%20ELS%202001.pdf

McGinnis, M. R., & Tyring, S. K. (1996). Introduction to mycology. In B. S (Ed.), *Medical microbiology*. (4th ed.). Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK8125/

Microbiology Society. (2019). Fungi. Retrieved July 19, 2019, from https://microbiologyonline.org/about-microbiology/introducing-microbes/fungi

NHPBS (Ed.). (2019). Decomposers. Retrieved July 18, 2019, from https://nhpbs.org/natureworks/nwep11b.htm

Soils – Part 7: Soil and plant considerations for calcium, magnesium, sulfur, zinc, and other micronutrients. (2019). Retrieved July 19, 2019, from https://passel.unl.edu/pages/informationmodule.php?idinformationmodule=1130447044&topicorder=3&maxto=7

Utah State University (Ed.). (n.d.). Decomposition. Retrieved July 18, 2019, from http://herbarium.usu.edu/fun-with-fungi/decomposition

Acknowledgments

We would like to thank Larry & Kathy Jennings as well as the VWR Charitable Foundation for funding our research. We would also like to thank the Teaching Assistants this year Peyton Flannigan, Elizabeth Peace, Ava Drum, and Taja Washington. Lastly, we would like to thank Mr. Brock.