The Impact of Water Levels on Fungal Density in Soil

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Abstract

Fungi are typically active in an area with moist soil, but in the E.S.S.R.E. Microclimate 2, the driest microclimate, fungi thrived. This observation led was to our experiment. In further research, we found that xerophilic fungi grew in dry areas. Therefore, we hypothesized that as the amount of water increased the fungal density would decrease. We created three rows on hill with plots 30cm x 30cm with a steep slope where we collected 27 soil samples. Various amounts of water were added to different plots depending on location on the hill. There was 1 liter and 2 liters of water added to plots in columns 2 and 3 while plots in column 1 received no additional water. After the rain, we collected our second set of samples and repeated the process. Our results showed that before any water was added to the soil that the fungi were functioning as they typically would in a typical environment. The results after water was added show that as the water amount increased the fungal density decreased. However, there was one piece of evidence that led us to believe that there could be another potential source providing water. In further research, the experiment would be repeated, but we would investigate several additional plots over a larger area to determine the potential presence of an additional water source affecting the data.

Introduction

Fungi are microscopic cells in soil particles and roots. They perform important duties related to water dynamics and nutrients in the soil (Ingham, 2012). Common fungi are decomposers and rely on dead organic matter for nutrients. They feed by absorbing nutrients from these organic materials in which they live. Some types of fungi, secrete acids and enzymes that break the surrounding organic materials into simple molecules they can easily absorb (Quinn and Fogel, 1999). Deceased organisms, such as trees, make perfect conditions for fungi to grow and develop. These fungi come in the form of visible mushroom-like blooms that occur on or around decaying organisms.

Fungi can exist in two forms: mold and yeast. Molds are fungi that grow in the form of multicellular filaments. Fungi generally grow best and exist in mold form in warm, moist environments (Deacon, n.d.). Yeasts are fungi that grow as single cells and typically grow in moist environments where there is a plentiful supply of simple, soluble nutrients such as sugars and amino acids. When the fungi are in an inadequate environment, such as the moisture from their nutrient source has been eliminated or is not sufficient enough, they revert back to their yeast form. The yeast form is the smaller, more condensed appearance of fungi.

There is a specific type of fungi called xerophilic fungi, which means "dry-loving." xerophilic fungi thrive in dry environments and are able to grow in dry environments at or below a water activity of 0.85 (Petterson, 2011). Water activity describes the energy status or escaping tendency of the water in a sample. Most yeast activity is inhibited at a water activity level of less than 0.8 (Association for Environmentally Conscious Building, 2015). However, xerophilic fungi are able to reproduce rapidly in these dry environments because they can metabolically increase the amount of dissolved solids in their cells when exposed to a highly concentrated solution of dissolved solids. Normally, water would begin to leave the cell with a lower concentration and cross over into the cell with a higher concentration via osmosis. However, since xerophiles can increase the concentration of dissolved solids in their cells at will, they have a built-in water preservation mechanism which allows them to survive and thrive in dry environments all while continuing to grow and form spores (Quinn and Fogel, 1999).

During the 2016 Biota Survey, unusually high numbers of fungi were discovered in the presence of almost no water in only one of the four microclimates surveyed, E.S.S.R.E. microclimate two (N 39° 21.484, W 76°38.370). Microclimate two also had the highest number of fungi in yeast form, 55,000 yeast in total, while the second highest number of yeast was in microclimate three, 10,000 yeast in total. This microclimate is located on a steep hillside in the middle of the woods, making the slope a key contributing factor to the amount of water absorbed into the soil due to runoff, therefore allowing little time for water absorption. The most fungi were found in microclimate 2 despite its dry conditions. We hypothesized that the reason why there were so many fungi in E.S.S.R.E. microclimate two was that it was inhabited with xerophilic fungi.

Methods

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Due North of Quadrant 2 of E.S.S.R.E. Microclimate 2 (N 39° 21.484, W 76°38.370), a 7m x 1.4m grid was created on the slope of the hill side located there. On the top of the slope, 3

30cm x 30cm plots were created running along the elevation line with 15cm between plots. 3m downhill from the first plots, 3 more 30cm x 30cm plots were created from the second plots running along the elevation line with 15cm between plots. Then 3m downhill, 3 more plots were created from the second plots running along the elevation line with 15cm between plots. (see Figure 1)

3 15cm by 2.5cm soil samples were collected from each plot at the same time on July 20, 2016, for a total of 27 samples. All samples were simultaneously tested for the level of moisture content and fungal density. Each sample was serial diluted to 10^{-2} and 100 µl aliquots of each dilution were grown on individual 3M Petrifilm™ Yeast and Mold Count Plates for 48 hours. After 48 hours the yeast and mold were examined and the density of each per cm^2 of soil calculated. To determine moisture content, each sample of soil was placed in an aluminum weigh boat, massed, and then baked at 107°C for 24 hours. After 24 hours, the soil was cooled, massed, and the percent change in mass calculated.

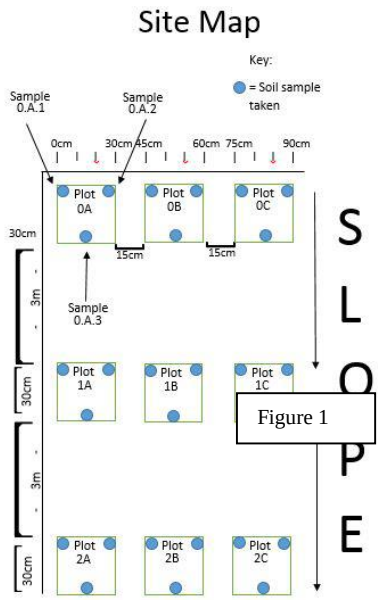
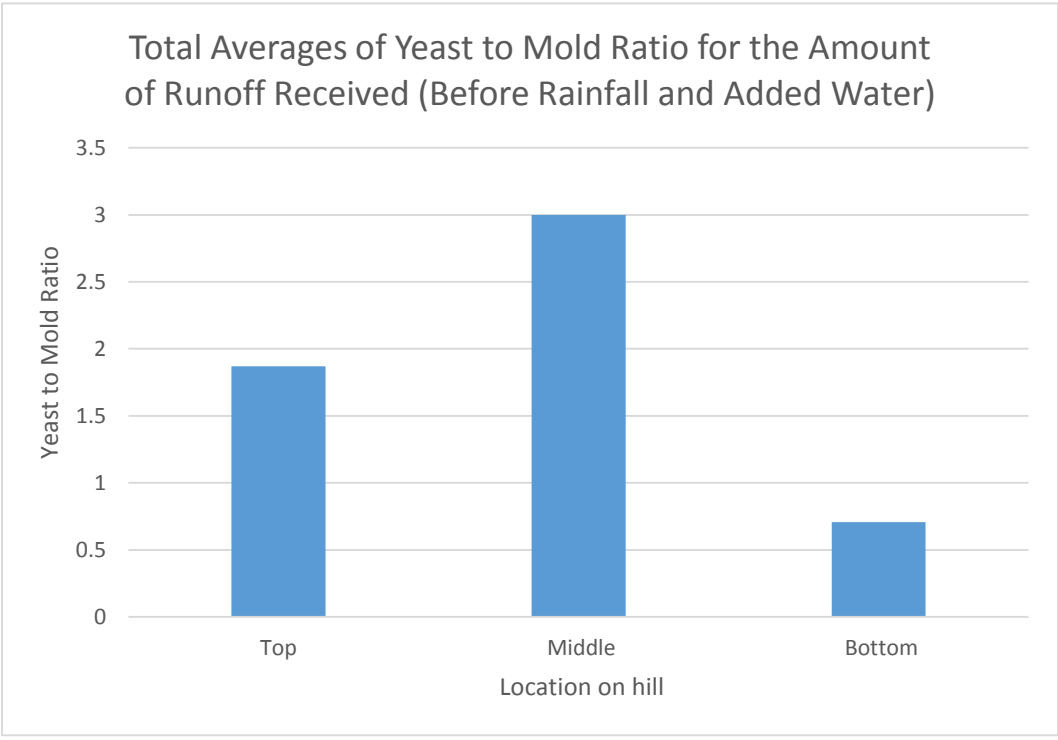


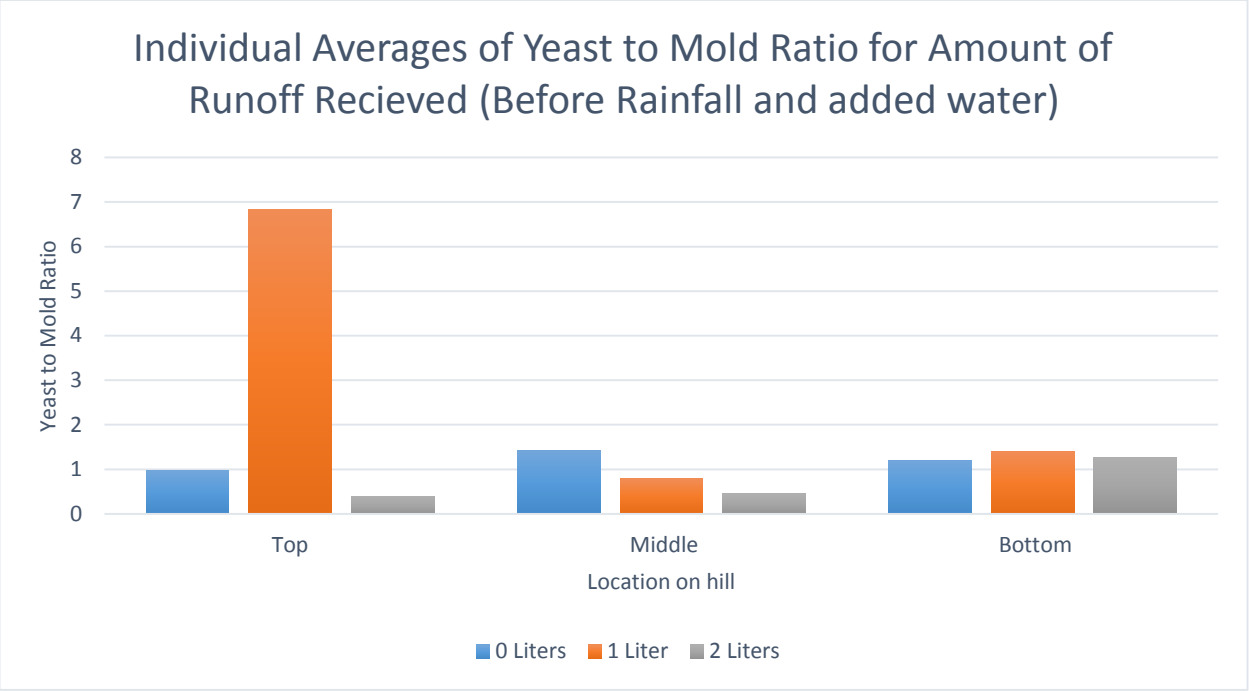
Figure 1

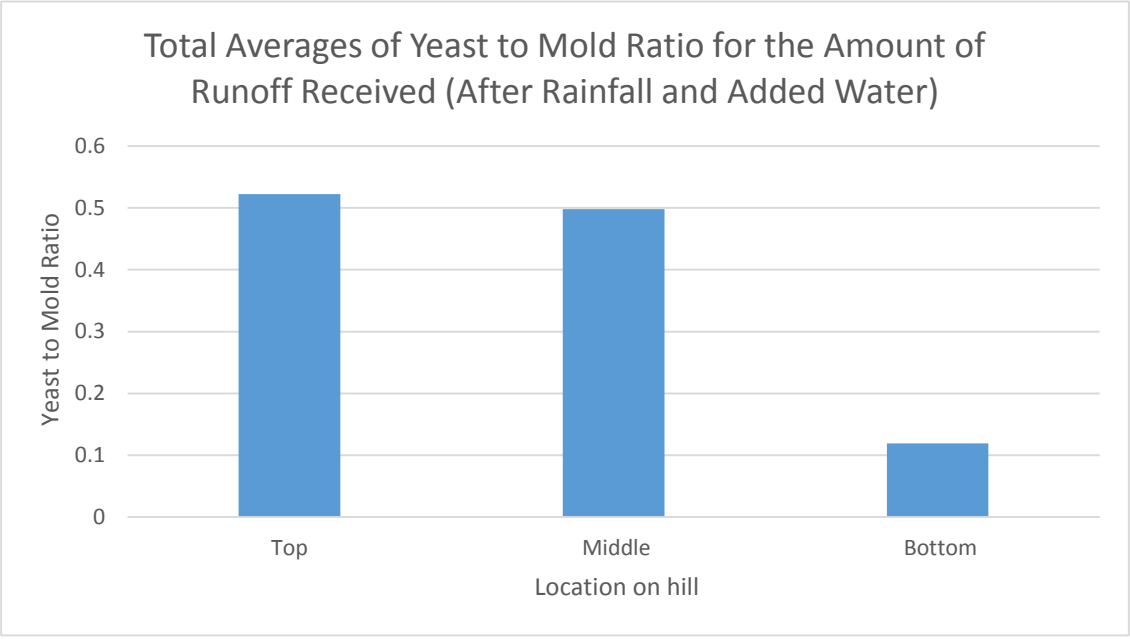
Following the initial soil extractions, stream water was poured on the same day at the same time onto the plots in two of the three columns. 1 liter was poured onto each of the plots in the middle column, and 2 liters were poured onto each of the plots in the last column. The next day, the process of adding the water was repeated. Following the next substantial rainfall, a second set of samples were collected using the same earlier extraction process used to extract the first set of samples. This second set of 27 samples were again simultaneously tested for the level of moisture content and fungal density. The ratio of yeast:mold was then calculated for all 54 samples.



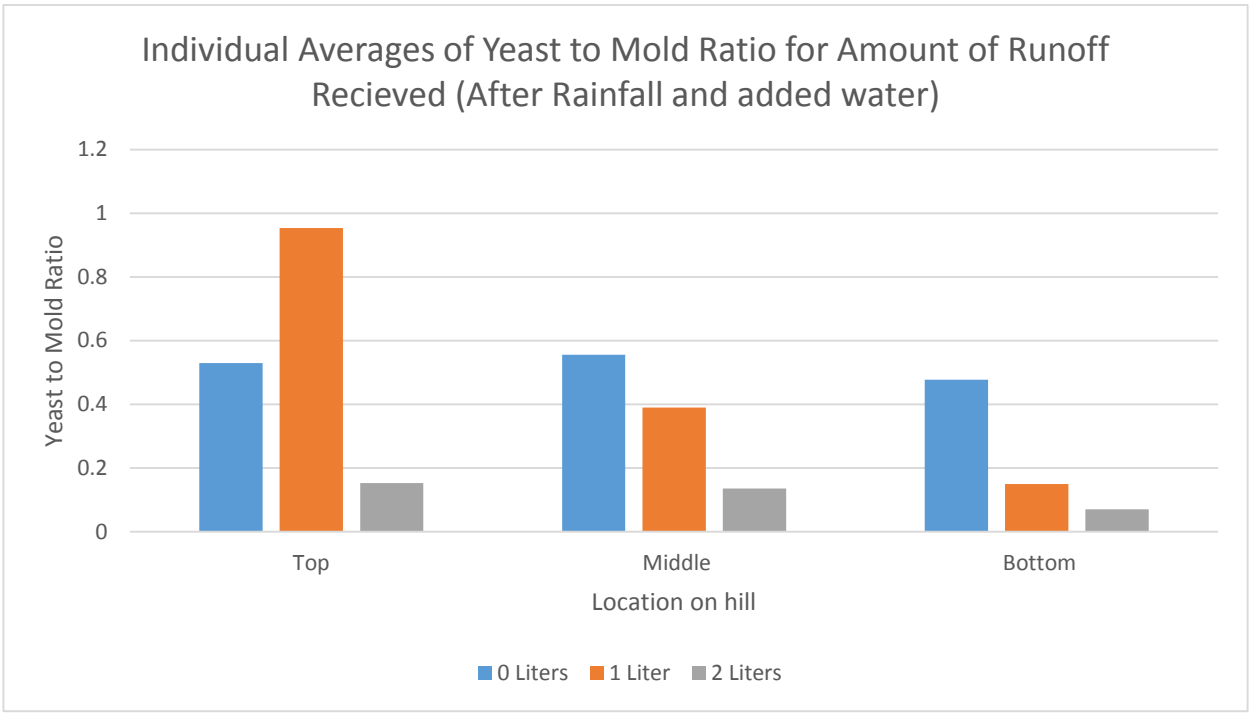
Graph 1

Graph 2

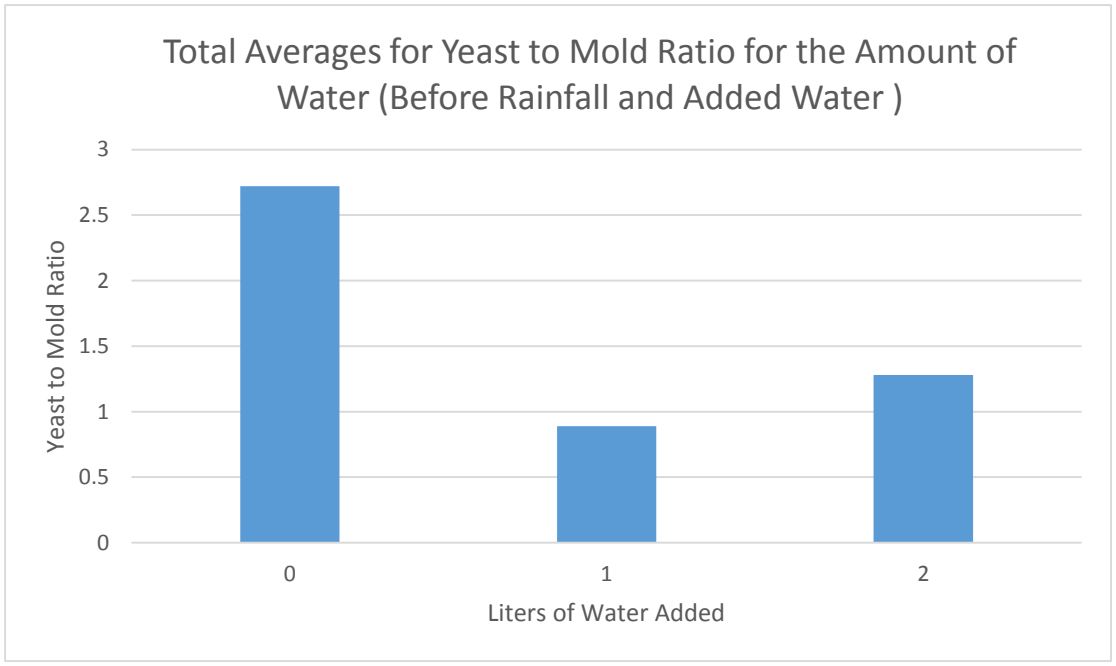




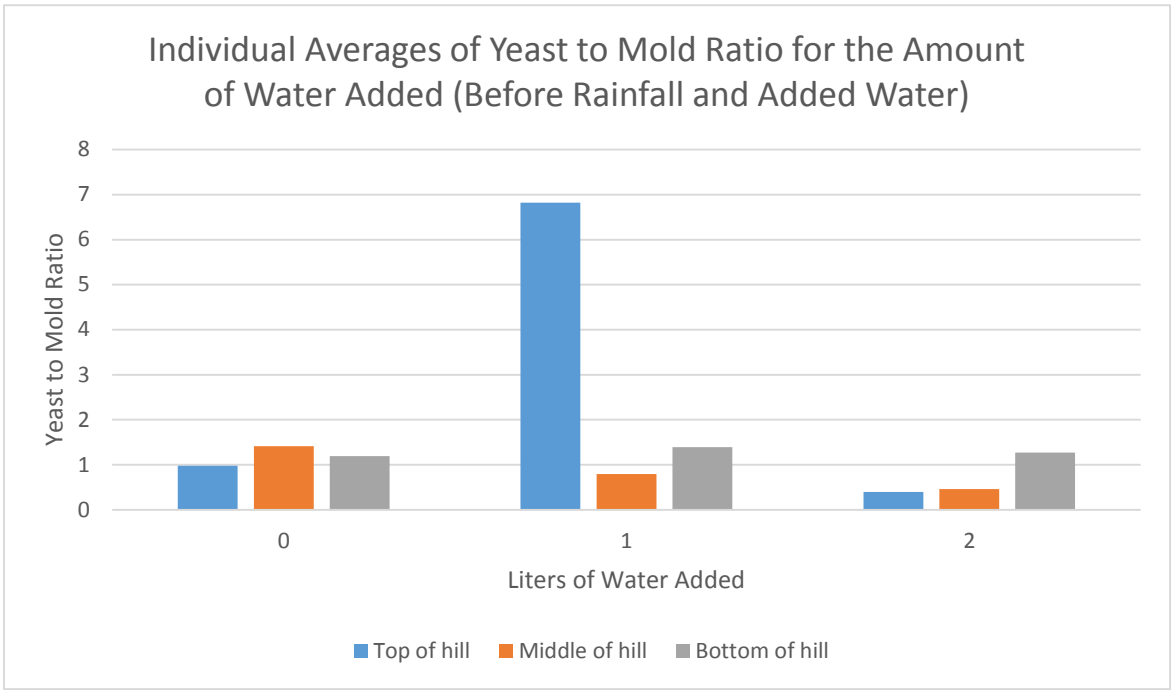
Graph 3



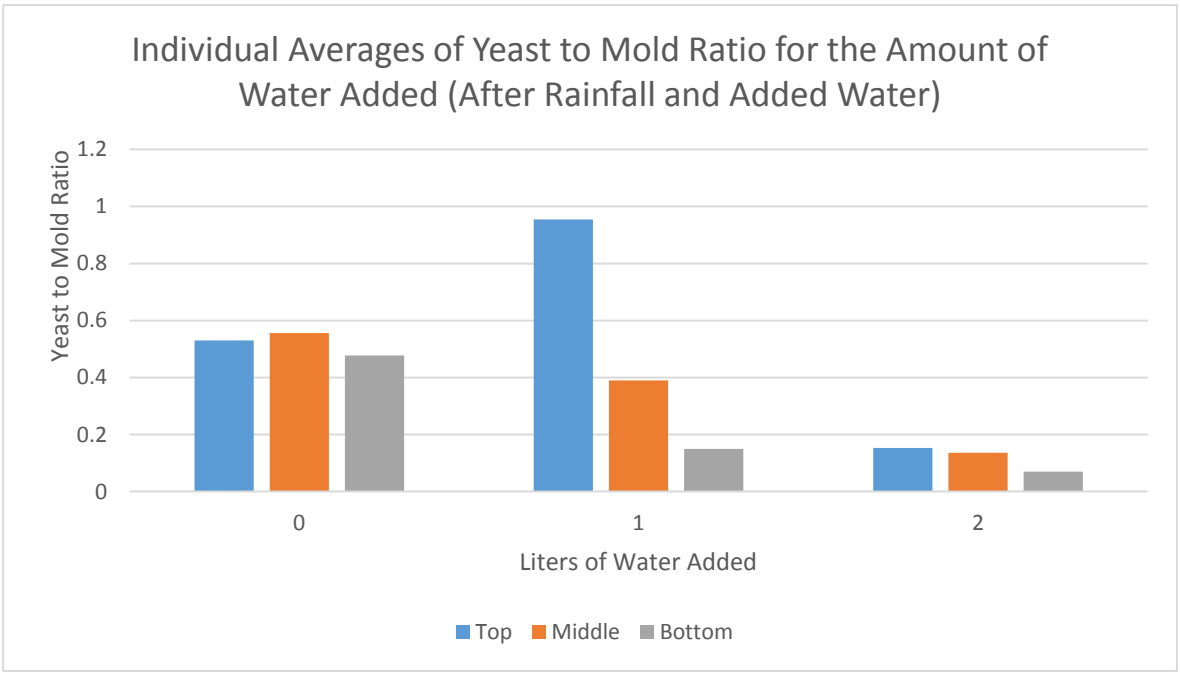
Graph 4



Graph 5

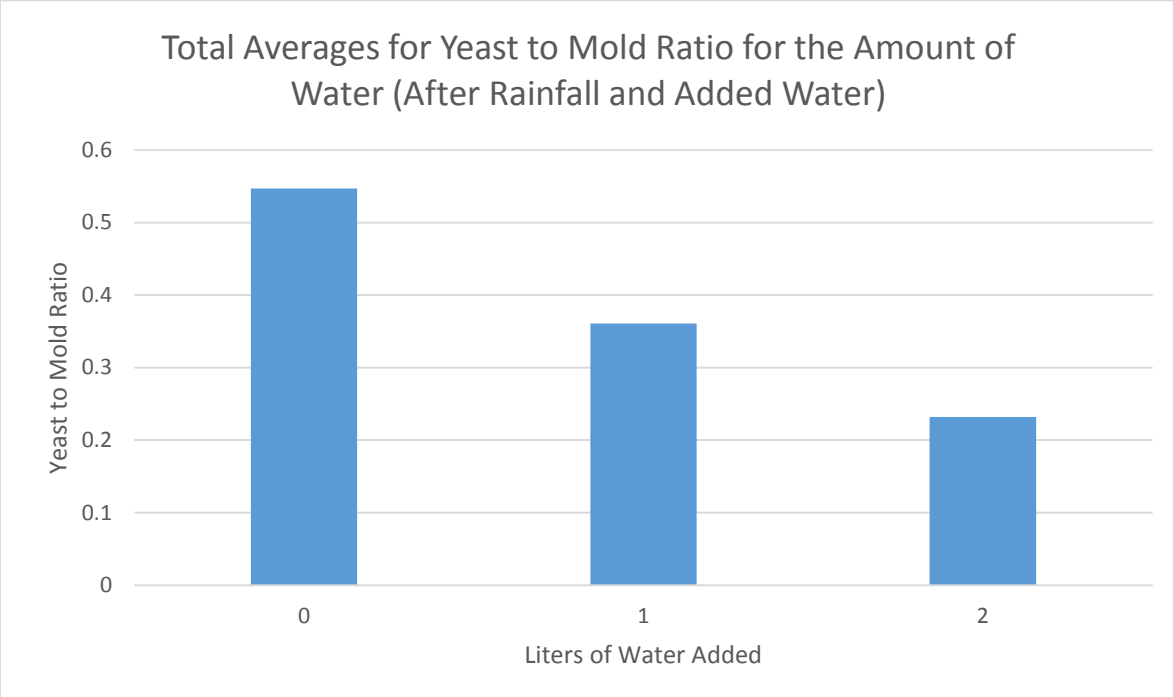


Graph 6



Graph 7

Graph 8



Discussion

As Graphs 1, 2, 5, and 6 show, there was no particular pattern to the yeast:mold ratio found in various research plots before the introduction of water to them. Hence, we can conclude that the fungi in these locations were behaving as they regularly would in their environment. In Graphs 3,4,7, and 8, there is one general trend showing in the plots: as the amount of water increases, the yeast:mold ratio decreases which supports our hypothesis. However, Graph 3 indicates the potential for either an outlier in the data for the plots from the middle of the hill or a challenge to our hypothesis because there is not a consistent decrease from the top of the hill to the bottom. Yet, there is a statistically significant difference between the yeast:mold ratio found at the plots in the middle of the hill and those found in the bottom of the hill ($p = 0.11$) which raises two questions: was the source of the fungal decrease due only to the water that we added instead of any possible contribution by the rainfall, or is there potentially another source of water that we are unaware of on the hillside of Microclimate 2?

Addressing the first question, Graphs 7 and 8 support and affirm the idea that it was our manipulation of water levels in the research plots that was the primary mechanism that made the yeast:mold ratio decrease. Specifically, the plots where 2L of water was added made a nearly statistical significant difference. In Graph 4, there was almost a significant change with the yeast:mold ratio in the 2L plots 1B, 1C, 2B and 2C on the middle and bottom of the hill with p values of 0.24 and 0.22. This near statically significant difference located on the bottom of the hill further supports the idea of an alternative source of water rather than simple precipitation. The hillside of Microclimate 2 is in a limestone area known for having seasonal springs, so the possibility that there is a spring is probable. Because the results did not completely support our hypothesis, further experimentation should be completed to resolve issues such as the true source of the change in fungal population, as well as manipulating other variables. To eliminate the

possibility of having a majority of the plots on a spring, multiple plot sites should be set up in different locations around the vicinity. In addition, the number of plots should be increased, such as creating five columns and five rows of 30 x 30 cm plots instead of three columns and three rows of 30 cm x 30 cm plots to increase the sample size. Because there could be a spring under the bottom row, the results were not completely consistent throughout the rows. By adding more sites in slightly varying locations and more plots to each site, we would potentially be able to prove whether there was a spring or another water source that would give a more accurate reason for the change in data.

References

Deacon, J. (n.d.). The Microbial World: yeasts and yeast-like fungi. Retrieved July 28, 2016, from The Microbial World website: http://archive.bio.ed.ac.uk/jdeacon/microbes/yeast.htm

Department of, S. N. R. (Ed.). (n.d.). *Soil Survey Standard Test Method*. Retrieved July 27, 2016, from Department of Sustainable Natural Resources website: http://www.environment.nsw.gov.au/resources/soils/testmethods/mc.pdf

Fact Sheet: Moisture Requirements for Mold Growth. (n.d.). Retrieved July 27, 2016, from http://Fact-Sheet-Moisture-Requirements-for-Mold-Growth-via-E-Rirsch.pdf

Ingham, E. R. (n.d.). Soil Fungi. Retrieved from Natural Resources Conservation Science website: http://www.nrcs.usda.gov/wps/portal/nrcs/detailfull/soils/health/biology/?cid=nrcs142p2_053864

Pettersson, O. V., & Leong, S. L. (2011, August). Fungal Xerophiles (Osmophiles). Retrieved July 28, 2016, from eLS website: http://www.els.net/WileyCDA/ElsArticle/refId-a0000376.html

Quinn, E., & Fogel, R. (1999, March). What exactly are fungi? Retrieved July 27, 2016, from Where Fungi Love and Grow website: http://herbarium.usu.edu/fungi/funfacts/live_grow.htm

Brock, David. Welcome to ESSRE! (2016, July). Retrieved July 27, 2016, from Environmental Science Summer Research Experience for Young Women website: http://essre.rpcs.org

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