The Effect of Decomposition on Soil pH, Protozoa, and Bacteria Levels

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ABSTRACT

Decomposition is the natural process by which organic material and tissue are broken down and recycled into usable nutrients that are either consumed by other organisms or released into the soil. Both bacteria and protozoa work to break down decomposing material, both by consuming nutrients and releasing them. This work is the base of the food chain which provides nutrients for surrounding organisms to feed on (Project Learning Tree, n.d.). Bacteria are unicellular organisms that perform many important tasks for living organisms including decomposing single carbon compounds into necessary nutrients in the soil. Bacteria are a key food source for protozoa, unicellular organisms that also feed on other protozoa, organic materials, and occasionally fungi. As they consume things such as bacteria, they release nitrogen and phosphorus into the soil, key elements in plant growth. Protozoa do best in moist environments with fertile soil (Laybourn-Parry, Diaz, 2019). The role that bacteria and protozoa play in decomposition greatly affect the health and fertility of the soil in addition to other factors such as nutrient availability and soil pH. A soil with high acidity can inhibit organic matter from breaking down, creating an accumulation of organic matter and stunting plant growth (Bickelhaupt, n.d.). A soil with a pH over 6.0 typically has a higher rate of decomposition due to a higher rate of bacteria growth in a more neutral and basic pH (Thumma, 2018). Protozoa and bacteria aid in the process of decomposition by catalyzing its rate, making it more efficient. Decomposition is vital for ecosystems—Without decomposition, nutrients would not be able to be returned to the soil, making it nutrient-poor and infertile, which in turn would make it difficult for living organisms to survive in the ecosystem. To investigate decomposition, we designed an experiment in which we observed the population of protozoa and bacteria as well as soil pH to come to conclusions about their correlations and behavior concerning decomposition. We observed that protozoa and bacteria populations are inversely proportional and that a low pH (lower than 6) disrupts bacteria populations, however, we found that overall the data we observed refuted the hypothesis.

INTRODUCTION

During our initial Microclimate investigation, we noticed many fallen trees, specifically in Microclimates 1 and 2 due to both heavy rainstorms and high winds over the years (E.S.S.R.E. 2021). Our group was curious as to how this disturbance to the area and the resulting decomposition would affect the soil, and in turn, how fallen trees would affect the bacteria and protozoa populations in the area. We were also interested in how decomposition could possibly affect something as fixed and slow to change as soil pH. This was especially interesting to us because bacteria populations and pH are connected because the more neutral the pH, the more bacteria there will be in that area. To study how much the decomposition in the backwoods affects the concentrations of bacteria and protozoa and pH levels, we set up research plots at varying distances from the decomposing trees. Bacteria and protozoa catalyze the rate of decomposition, which means that larger numbers of both are indicative of a high rate of decomposition.

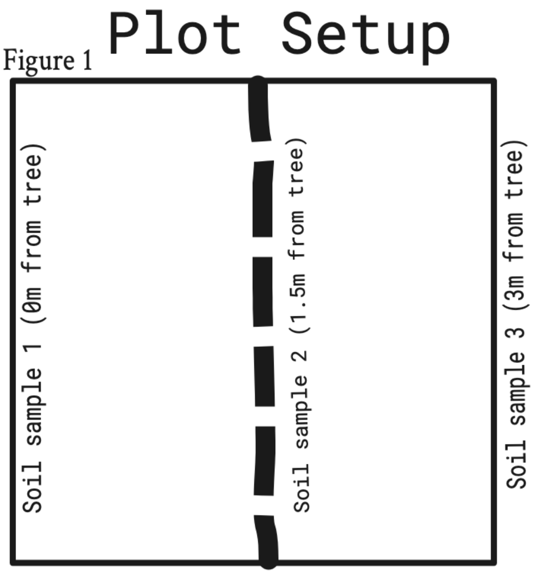
Decomposition is when organic materials are broken down and transformed into carbon dioxide and humus and release nutrients back into the environment (UC Davis n.d). Within these processes, bacteria, fungi (mold and yeast), protozoa, along other saprophytic organisms accelerate the decomposition rate by feeding on decaying materials. In soil, some protozoa are nitrogen fixers, making nitrogen available to several other organisms in the ecosystem (Ingham, n.d). Many protozoa also both control the populations of bacteria and accelerate the rate at which bacteria decompose organisms (Ingham, n.d.). In feeding on the bacteria, the protozoa keep the bacteria population in a younger, more active state. This causes the rates at which bacteria decompose organic materials to accelerate, and the rate of decomposition as a whole to increase. (Laybourn-Parry, Diaz, 2019). During decomposition, bacteria render nutrients reusable as well as fix nitrogen and sulfur into a usable form for plants (Hoorman, 2016). Bacteria often prefer a neutral soil pH, but some species prefer more acidic soil. (Blamire, 2000).

Typically the decomposition of organic matter—a process linked to bacterial populations— acidifies the soil, marginally lowering its pH (Zhang, 2017). The soil pH has an effect on the availability of chemicals and nutrients in the soil which affects the number of nutrients available for living plants. Along with and in collaboration with other factors such as the aforementioned bacteria and protozoa populations all play a large role in regulating the forest ecosystem. Our other hypothesis was that the soil samples closest to the area of decomposition would have higher levels of bacteria and protozoa, along with a more acidic pH, according to healthy decomposition cycles.

METHODS

The two research plots were located in Microclimates 2 and 4 (E.S.S.R.E. 2021). These two locations were chosen based on first-hand sightings of fallen trees decomposing, taking into account their similar age and size, and relative time since the tree fell. Two 3x3 meter plots were set up with one side running parallel to the decomposing tree. A core extractor was used to take three soil samples 15 cm deep and 2 cm in diameter per location per day. Each day one soil sample was taken randomly along the 0, 1.5, and 3-meter lines from the decomposing tree within each 3x3 meter plot as shown in Figure 1. 'This process was performed on three consecutive days, sampling randomly along the 0, 1.5, and 3-meter lines within each 3x3 meter plot.

Figure 1



Soil pH was measured by following the pH test instructions from the LaMotte Combination Soil Outfit Kit model STH-14.

Bacteria populations within the soil were measured using the serial dilution method. After the diluted solution was transferred to 3M™ Petrifilm™ Aerobic Count Plates, the colonies grew for 72 hours. The dilutions plated were either -3 or -4. Once 72 hours had passed, analysis of the plates was conducted by counting the number of colonies on the plate of a soil sample with the least amount of colonies grown but with a number of colonies greater than five and using that number to calculate the number of bacteria in the original 1 cc soil sample. The number of bacteria colonies in the original 1cc soil samples was calculated and recorded.

To measure the presence of protozoa within the soil, the protozoa were examined under a microscope. The rehydrated soil samples had to go through 2 filtration processes before being transferred to a microscope slide. To set up the microscope slides, the filtrate taken from the Uhlig extractor was filtered again and placed on a slide with a methyl-green stain before a glass coverslip was placed on top. Once all slides were completed, they were examined at 40X under the microscope and counted the number of protozoa in five separate fields of view. The number of protozoa counted per field was averaged together, and the equation (average # of protozoa x total water (g) x 747)/sifted soil (g) was used to determine the population density of protozoa per gram in the soil sample.

RESULTS

pH

Table 2.1 *T-test of pH comparing Plots 1 and 2*

Average pH of Plot 1	Average pH of Plot 2	T-Test value	P-value
4.9	6.6	13.92	0.0000000083

Table 2.2 *Statistical Data of pH vs. Distance from Decomposing Tree*

Distance from Tree (meters)	Mean pH	n	Range	Standard Deviation
0	5.8	6	2.2	1.083
1.5	5.7	6	1.6	0.816
3	5.7	6	2.2	0.926

Table 2.3 *T-tests of pH vs. Distance from Decomposing Tree*

Distance from Tree (meters)	T-Test Value	P-Value
0-1.5	0.18	0.86
0-3	0.057	0.955
1.5-3	0.132	0.897

After collecting the raw pH data from plots one (Microclimate 2) and two (Microclimate 4), we conducted a 2-sample T-test on a TI-84 calculator to determine if there was any significant difference in pH levels between these two plots. The P-value of this T-test was 0.0000000083 (Table 2.1), which is below 0.2 (the critical value for our research), meaning they are significantly different. There was not a significant difference, however, between any distances from the tree (Table 2.2, Table 2.3).

Protozoa

Table 3.1 *Statistical Data of Protozoa vs. Distance from Decomposing Tree*

Distance from Tree (meters)	Mean Protozoa in 1 cc of Soil	n	Range	Standard Deviation
0	1,764,198.23	6	2,459,697	956,481.97
1.5	1,342,904.65	6	1,584,944.05	651,439.75
3	1,565,758.02	6	2,384,954.55	913,307.45

Table 3.2 *T-tests of Protozoa density vs. Distance from Decomposing Tree*

Distance from Decomposing Tree (meters)	T-Test value	P-value
0-1.5	0.892	0.396

0-3	0.367	0.721
1.5-3	0.486	0.638

Table 3.3 *T-Test of Protozoa in Plot 1 vs. Distance from Decomposing Tree*

Distance from Tree (meters)	T-Test value	P-value
0-1.5	0.299	0.779
0-3	0.764	0.488
1.5-3	1.15	0.314

Table 3.4 *T-Test of Protozoa in Plot 2 vs. Distance from Decomposing Tree*

Distance from Decomposing Tree (meters)	T-Test Value	P-Value
0-1.5	1.035	0.359
0-3	1.976	0.119
1.5-3	0.728	0.507

Most of our protozoa data for our combined plots found no P-values less than 0.2, so there is no significant difference in the protozoa density in our soil based on distance from the decomposing trees. However, in the T-Test of Plot 2, we found a statistically significant difference between the distances of 0 meters and 3 meters.

Bacteria

Table 4.1 *Statistics of Bacteria vs. Distance from Decomposing Tree*

Distance from Tree (meters)	Mean Bacteria in 1 cc of Soil	n	Range	Standard Deviation
0	8,833,333.33	6	24,000,000	9,006,812.24
1.5	4,266,666.21	6	2,300,000	933,095.21
3	7,100,000	6	9,100,000	3,118,974.19

Table 4.2 *Statistics of Bacteria in Plot 1 vs. Distance from Decomposing Tree*

Distance from Tree (meters)	Mean Bacteria in 1 cc of Soil	n	Range	Standard Deviation
0	3,766,66	3	5,000,000	2,804,163.571
1.5	4,300,000	3	2,100,000	1,212,435.565
3	7,300,000	3	9,100,000	4,782,258.88

Table 4.3 *Statistics of Bacteria in Plot 2 vs. Distance from Decomposing Tree*

Distance from Tree (meters)	Mean Bacteria in 1 cc of Soil	n	Range	Standard Deviation
0	13,900,000	6	21,000,000	10,859,558.002
1.5	4,233,333.33	6	1,500,000	838,649.708
3	6,900,000	6	2,300,000	1,153,256.259

Table 4.4 *T-tests of Bacteria vs. Distance from Decomposing Tree*

Distance from Tree (meters)	T-Test value	P-value
0-1.5	1.235	0.27
0-3	0.445	0.671
1.5-3	2.132	0.078

Table 4.5 *T-tests of Bacteria in Plot 1 vs. Distance from Decomposing Tree*

Distance from Tree (meters)	T-test value	P-value
0-1.5	0.302	0.783
0-3	0.232	0.831
1.5-3	1.053	0.392

Table 4.6 *T-tests of Bacteria in Plot 2 vs. Distance from Decomposing Tree*

Distance from Tree (meters)	T-test value	P-value
0-1.5	1.537	0.263
0-3	1.11	0.38

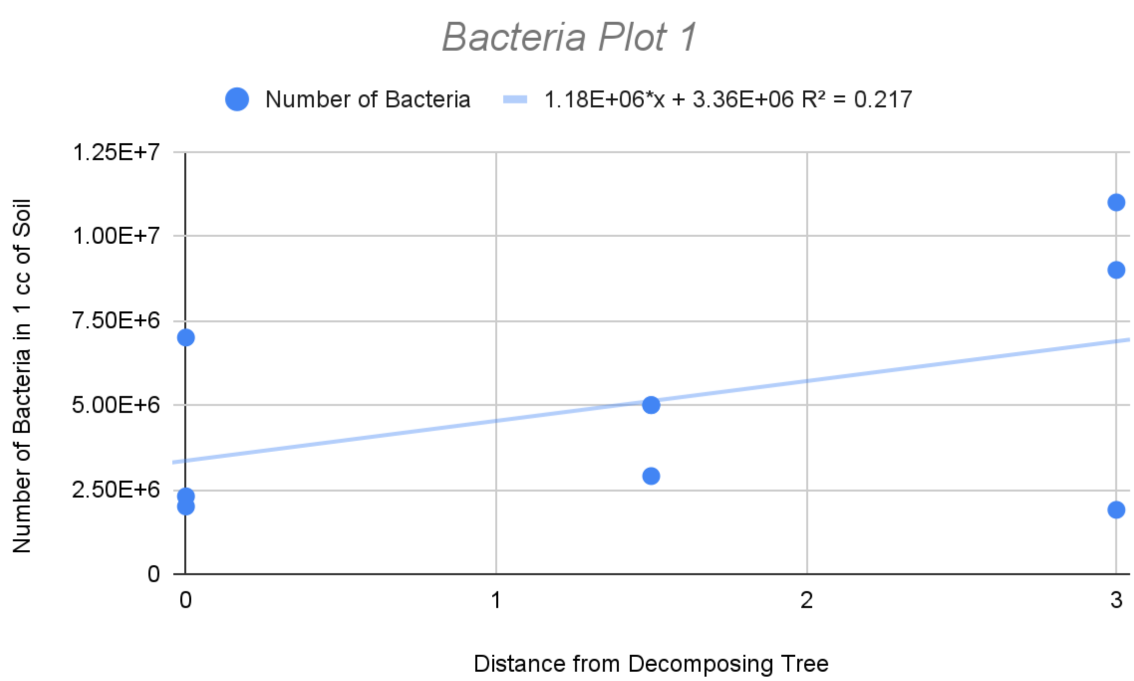
1.5-3	3.239	0.036

Table 4.7 *T-tests of Bacteria comparing Plots 1 and 2*

Average of Plot 1	Average of Plot 2	T-test value	P-value
5,122,222.22	8,344,444.44	1.253	0.235

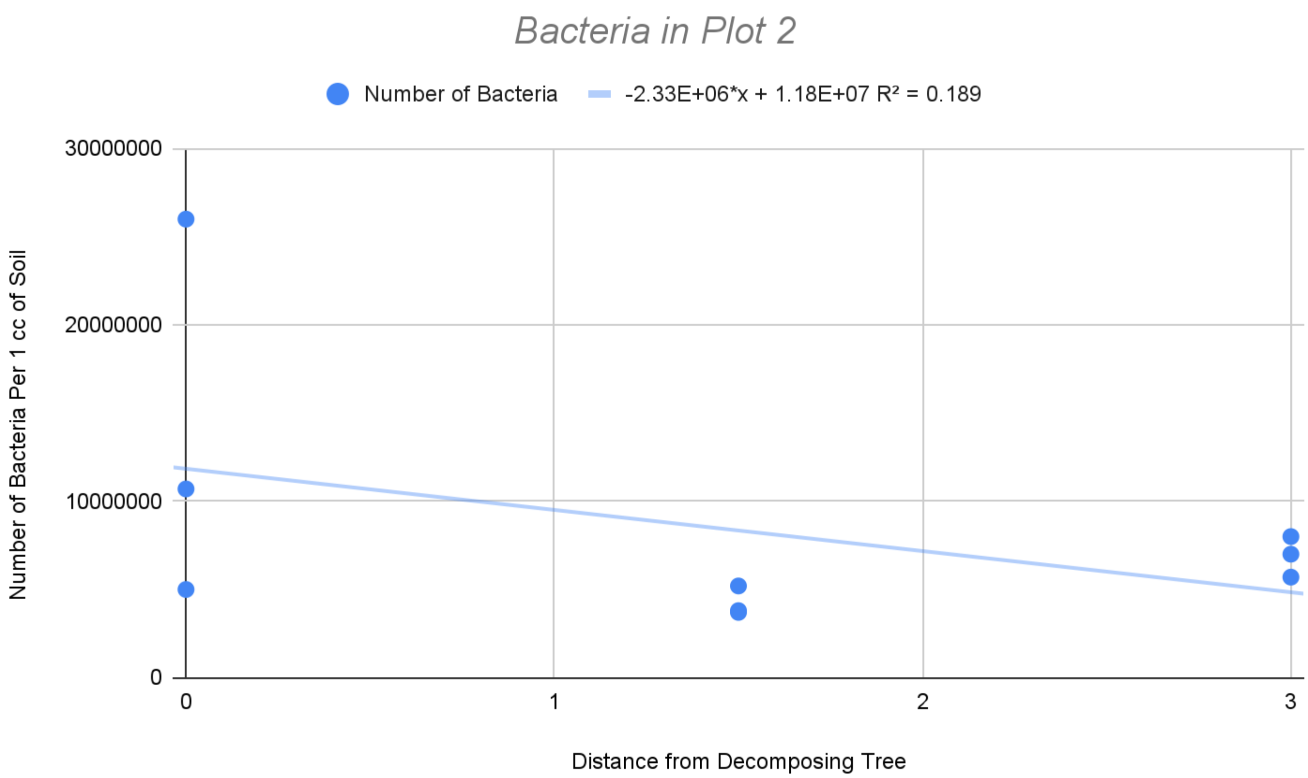
Throughout all our bacteria experiments with 2-sample T-tests, two of the only three comparisons that were found to be significant were the difference in bacteria levels in plot 2 between the data of 1.5 and 3 meters and the difference in bacteria levels between 1.5 and 3 meters of the combined data. Because no other P-value between any other distances in either of the plots was found to be significant, we concluded that the distance of soil from decomposing trees may have no effect on bacteria levels, however further testing may be required to come to a concrete conclusion, as the difference in pH between the plots most likely affected the data. It should be noted that plot 2 has a significantly higher pH than plot 1, and bacteria do best in pH levels of 6.5-7. Plot 2's average pH was 6.6, while plot 1's average pH was 4.9, which possibly disrupted the bacteria populations (Table 2.1).

Figure 4.8



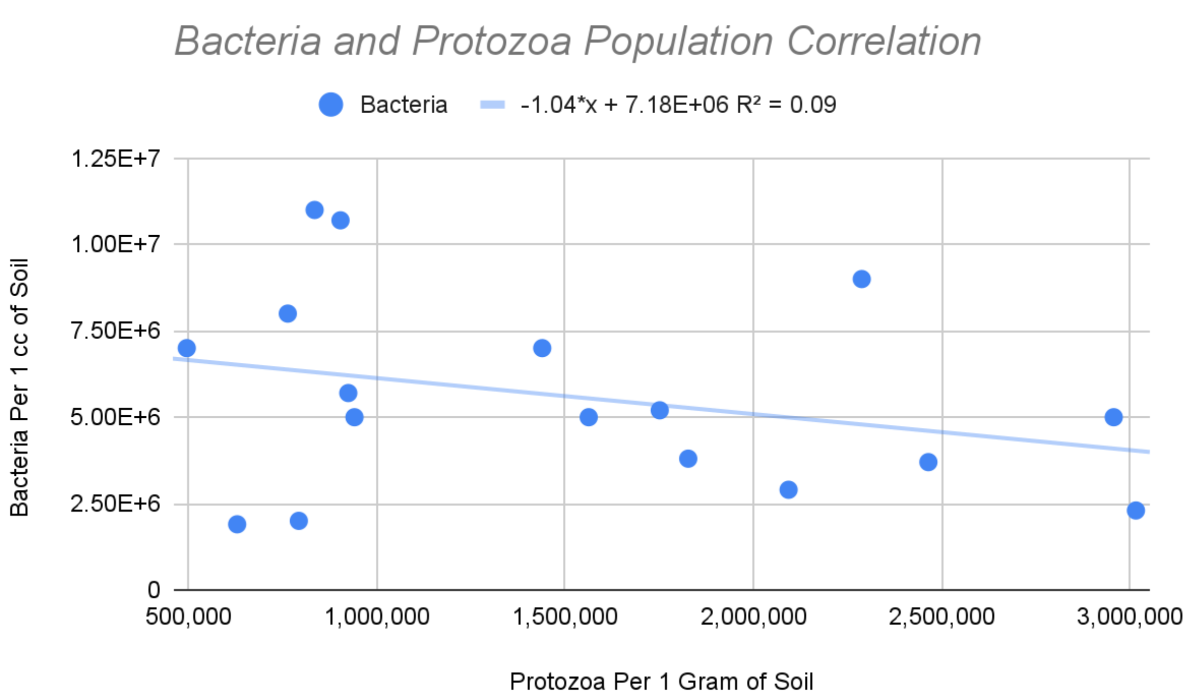
The graph above shows that the population of bacteria increased the farther from the decomposing log the soil samples were taken from.

Figure 4.9



In this graph of the bacteria present in plot 2, the A samples are 0 meters from the decomposing tree, the B samples are 1.5 meters away, and the C samples are 3 meters away from the tree. The roman numerals display which day the sample was taken from. As can be seen from the line of best fit, the amount of bacteria decreases the farther away from the decomposing log they are.

Figure 4.10



The graph above shows the correlation between protozoa and bacteria populations; it shows that protozoa levels are inversely proportional to bacteria levels, meaning that as one increases the other decreases. We removed outlier data from this data set that created an inaccurate best line of fit that showed a positive slope. The line of best fit shows that bacteria populations decreased as the protozoa population rose.

DISCUSSION

Our hypothesis that soil located closer to a decomposing tree would have higher populations of protozoa and bacteria and a lower pH, was refuted by our findings. As observed in Figure 4.5, the population of bacteria is higher when the soil sample was taken closer to the decomposing tree in our experimental plot with a higher pH. However, figure 4.9 showed that in the plot with the acidic pH the population of bacteria rose with distance from the decomposing tree. One trend that we found in our data was that protozoa populations, as shown by figure 4.8, fell with the rising bacteria populations.

With plots 1 and 2 combined, no significant differences in pH level, protozoa density, or number of bacteria colonies were found between different distances from the decomposing trees. We refute our original hypothesis with these findings.

As seen in Table 2.1, our plots have drastically different average pH levels. Our significant findings in our experiment were the difference in bacteria colonies in plot 2 between 1.5 and 3 meters along with the difference in protozoa density in plot 2 between 0 and 3 meters. We see these statistical differences only in plot 2 because the pH is higher, which is a more favorable environment to bacteria and protozoa populations. Soils with higher pH have higher rates of bacteria and therefore have higher rates of decomposition (Thumma, 2018). Our data supports this claim, with plot 2 having an average pH of 1.7 higher than plot 1, and the average bacteria levels of plot 2 were typically much higher than that of plot 1's (Table 4.2 and Table 4.3).

The statistics comparing distance from the log for pH are not entirely surprising. pH is a static property of soil, and the distances from the decomposing trees at which the pH was compared may not have been large enough to observe any statistical difference. As for protozoa, they regulate the populations of bacteria by feeding on them (Ingham, n.d), so this could be a possible explanation for why the populations of bacteria fell when the population of protozoa grew (Laybourn-Parry, Diaz, 2019).

One thing we would control for future research is the placement or presence of nearby bodies of water, such as a stream's location nearby. This could aid in the formation of plots with similar pH that are suitable for bacteria to thrive so that an unusually low or high pH for the area does not disrupt data. We would also adjust the size of our plot, as we think a larger plot and more distances at which samples are retrieved would yield more varied results so that any differences in bacteria or protozoa populations between distances would be more observable.

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Site coordinates --

Site 1
Top left corner - N 39˚21.478’ W 76˚38.364’
Top right corner- N 39˚21.475’ W 76˚38.363’
Bottom right corner - N 39˚21.473’ W 76˚38.362’
Bottom left corner - N 39˚21.470’ W 76˚38.363

Site 2
Top left corner - N 39˚21.468’ W 76˚38.344’
Top right corner - N 39˚21.465’ W 76˚38.345
Bottom right corner - N 39˚21.466’ W 76˚38.336’
Bottom left corner - N 39˚21.467’ W 76˚38.345’