The Study of Water's Effects on Protozoa and Bacteria Populations in the Soil

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Abstract:

Bacteria are prokaryotic microorganisms that help plants grow; protozoa are eukaryotic microbes that eat bacteria, releasing excess nitrogen, as well as other nutrients, as they do so. In the 2017 ESSRE Biota survey, the bacteria densities were extremely low, even though the protozoa density levels were high. Furthermore, while the differences between protozoa densities in the different microclimates were statistically significant, the corresponding significance was not observed in the bacteria populations. We speculated that the very dry conditions in the microclimates might be the source of this anomaly, and we hypothesized that adding water to the soil would make the bacteria and protozoa populations return to their normal predator/prey relationship. In order to test this, we set up 3 1m x 1m plots in the ESSRE Microclimate 3, and we took 3 soil samples from each of the 3 plots before and after adding 0,4, and 8 liters of water respectively to the 3 plots. All samples were tested for protozoa and bacteria densities using serial dilutions and a modified Foissner-Uhlig extraction technique. We found that the addition of water did in fact restore the expected relationship between bacteria and protozoa populations. However, there was no statistically significant difference in densities for both microbes between the 4 liter and 8 liter plots ($p= 0.84$). Hence to research this topic further, we would test to find the specific amount of additional water needed to improve the relationship between bacteria and protozoa populations.

Introduction:

The Nitrogen cycle is a complex biogeochemical process in which nitrogen is altered from the primary form of nitrogen gas in the atmosphere to ammonium and nitrate for use by plants, animals, and the rest of the ecosystem (Kahn Academy, N.A). This process must occur because the plants and animals in an ecosystem cannot use atmospheric nitrogen directly, but can use the fixed nitrogen the nitrogen cycle produces (Environmental Literacy Council, N.A). The cycle contains five major stages: nitrogen fixation, nitrification, assimilation, ammonification, and denitrification (Environmental Literacy Council, N.A) Soil bacteria and protozoa are especially important for their specific roles in the different parts of the cycle because they share a predator/prey relationship. Without this relationship, the nitrogen cycle would not function.

Bacteria, the prey in the relationship, are prokaryotes that perform a number of tasks which keep an ecosystem functioning (such as decomposing). However, they play their most significant role in the nitrogen fixation step of the nitrogen cycle since they are the only organisms capable of converting the nitrogen gas into other forms.. Their role is to change the nitrogen in the atmosphere (N_2) to ammonia, (NH_3) that converts to ammonium (NH_4^+) which other bacteria in the soil process into nitrite (NO^-_2) and nitrate (NO^-_3) which plants absorb through their roots (Kahn Academy, N.A). The plants and animals in the environment need this nitrogen to produce certain acids and proteins, which re-enter the nitrogen cycle following decomposition (Layne, 2006).

The release of nitrogen, though, depends to a great extent on two unique soil protozoa types: shelled and unshelled amoeba. Shelled amoeba, or Testate amoeba, possess a shell-like covering, known as tests (Ingham, N.A), and this type of amoeba go into this shelled state when they are environmentally stressed. Shelled, unshelled, and the other types of protozoa play a vital part in the Nitrogen cycle because they eat bacteria as their primary source of food. Since bacteria contain more nitrogen than the protozoa need, they are able to release the transformed excess nitrogen for plants and animals to use (Ingham, N.A)**.** In addition, since the

protozoa eat the bacteria, they are able to regulate the bacteria populations in the environment that are responsible for the nitrogen fixation process. Hence, protozoa regulate the amount of available nitrogen by regulating how much gets fixed in the first place.

The data found in the 2017 E.S.S.R.E Biota Survey (E.S.S.R.E, 2017) revealed an unusual ratio between the protozoa and bacteria densities in the microclimates. Normally, since they exist in a predator/prey relationship, when the protozoa populations are high, the bacteria levels are low and vice versa. But the 2017 data showed that the bacteria densities were extremely low in comparison to previous years, and even more importantly, while there were statistically significant differences in protozoa densities between the various microclimates, the corresponding statistical difference for bacteria densities was not observed.

We believe that this anomaly is a result of high levels of Testate amoeba and dry conditions. The dry soil could potentially be causing the amoeba to “go into hibernation” and take their shelled form, thus disrupting the predator-prey ratio because the protozoa in this form are not feeding on the bacteria. Therefore, we hypothesized that the lack of water in the soil may be the root cause of the problem, and we believe that when a greater amount of water is added to the plots, the protozoa and bacteria population’s relationship will return to its expected values, displaying a more accurate predator/prey relationship.

Methods:

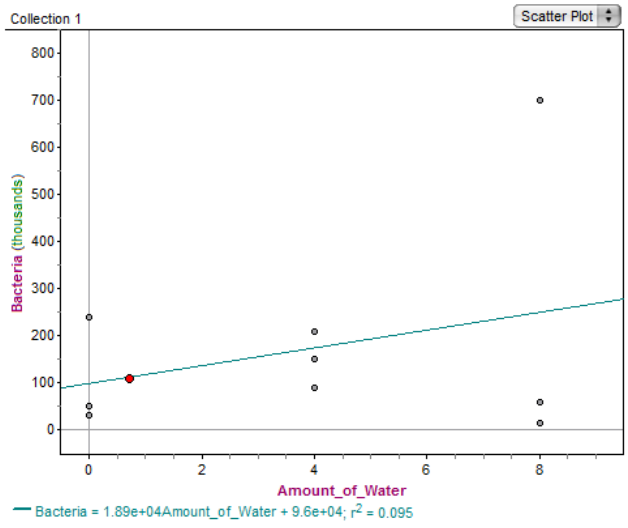
In E.S.S.R.E. Microclimate 3 (N 39.35797; W 076.63836), 3 1m x 1m plots were cleared of all ground clutter along the same contour line in quadrant 3. Next, 9 soil samples 2 cm in diameter and 15 cm deep were simultaneously collected on July 19th, 2017; 3 from each plot. Each plot was subsequently saturated with either 0 liters, 4 liters, or 8 liters of distilled water

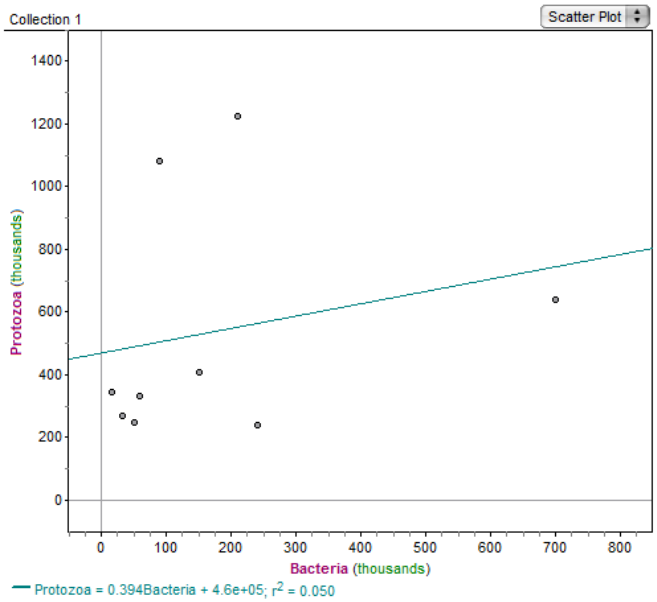
respectively. After 24 hours, 9 additional soil samples were simultaneously collected on July 20th, 2017.

Both sets of soil samples were allowed to dry completely. Then each sample was simultaneously tested for bacteria and protozoa densities. Serial dilutions were used to determine bacteria density, and all samples were diluted using sterile water to 10^{-4}. 100 µl of each dilution was plated on its own individual 3M™ Petrifilm Aerobic Count Test Plate and grown for 72 hours. Protozoa were extracted using a modified Foissner-Uhlig extraction technique (Brockmeyer, 2008).

Results:

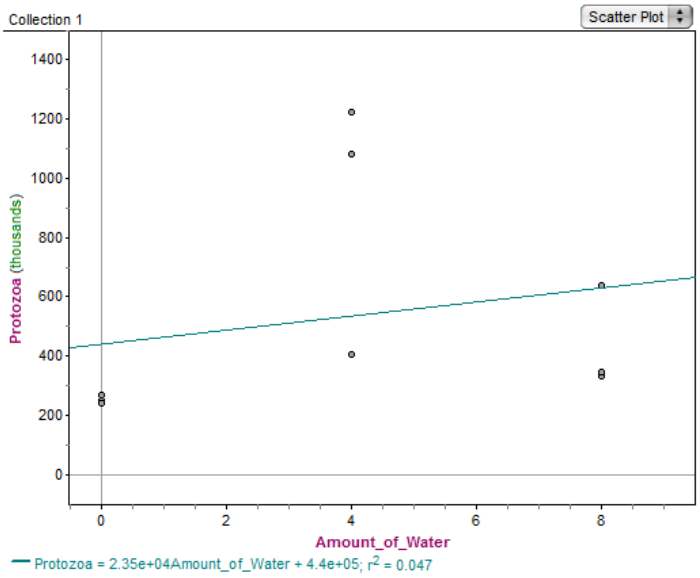
Graph 1: Shows the Correlation Between Any Pre-existing Moisture Levels in the Soil and the Density of the Bacteria in the Research Plots Prior to Addition of Water



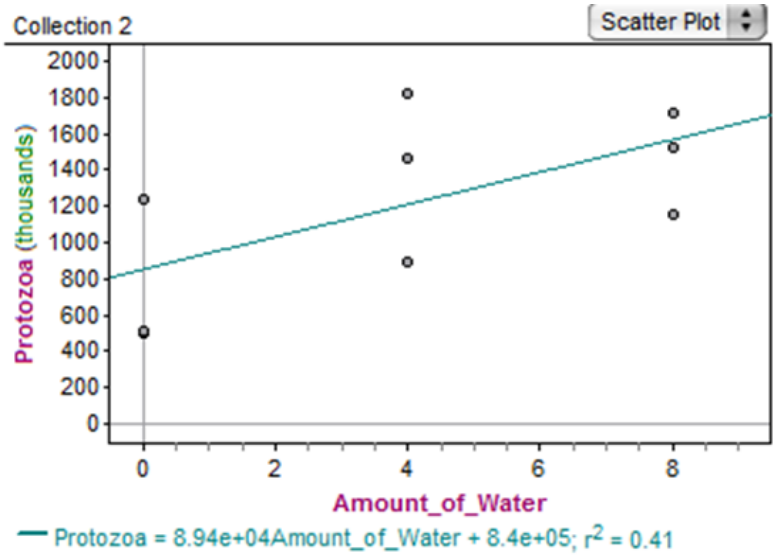


Graph 2: Shows the Correlation Between the Bacteria and Protozoa Populations in the Research Plots Prior to Addition of Water

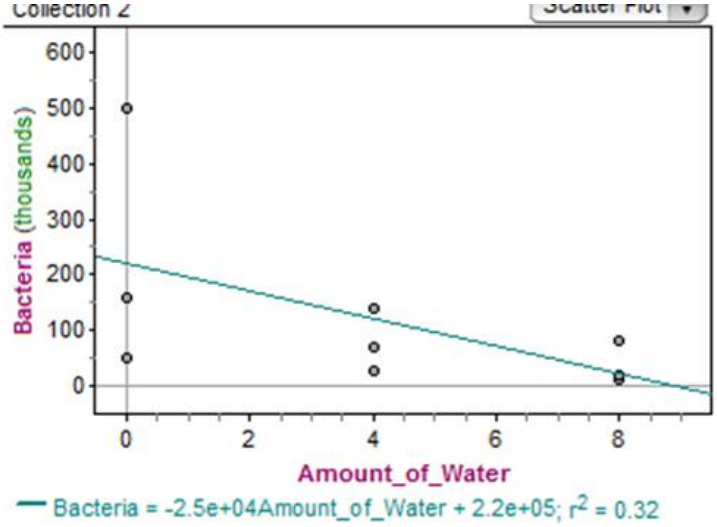
Graph 3: Shows the Correlation Between Any Pre-existing Moisture Levels in the Soil and the Density of the Protozoa in the Research Plots Prior to Addition of Water



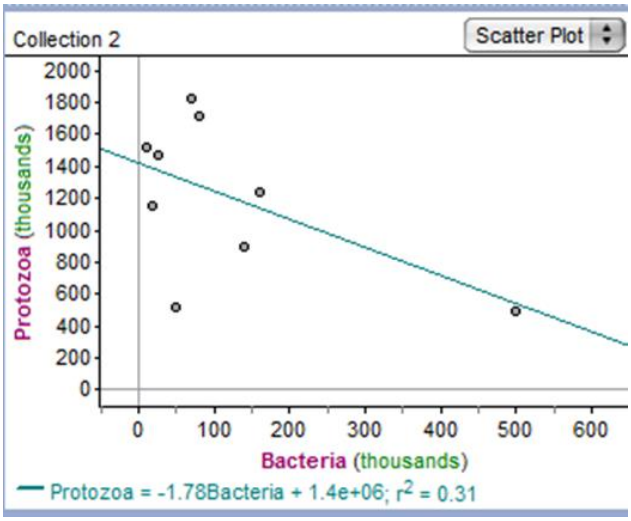
Graph 4: Shows the Correlation Between Moisture Levels in the Soil and the Density of the Protozoa in the Research Plots After Addition of Water



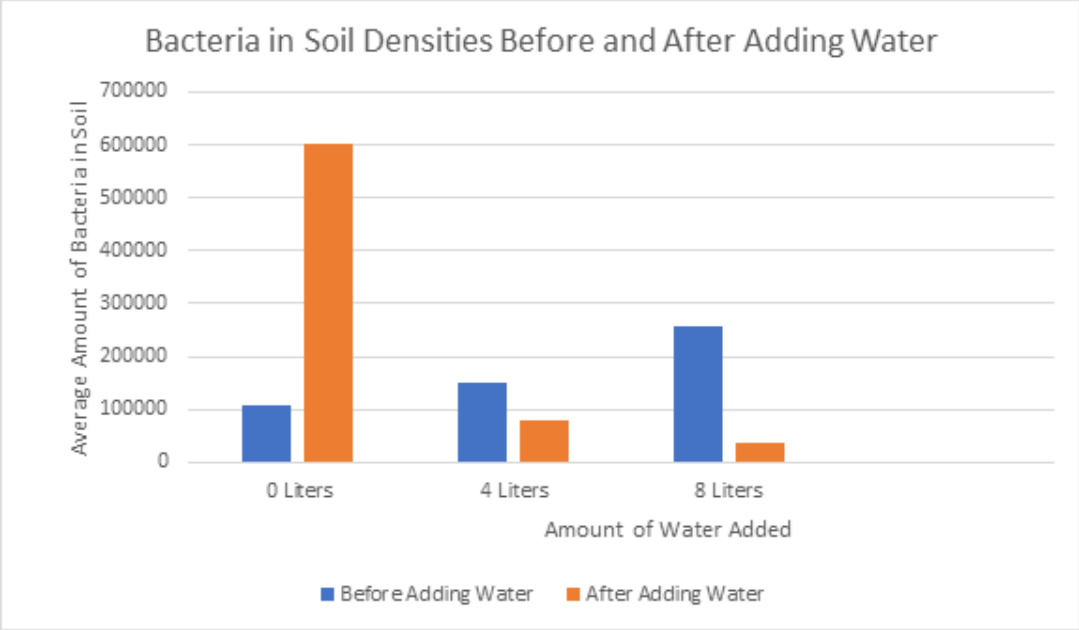
Graph 5: Shows the Correlation Between Moisture Levels in the Soil and the Density of the Bacteria in the Research Plots After the Addition of Water



Graph 6: Shows the Correlation Between the Bacteria and Protozoa Populations in the Research Plots After the Addition of Water



Graph 7



Graph 8

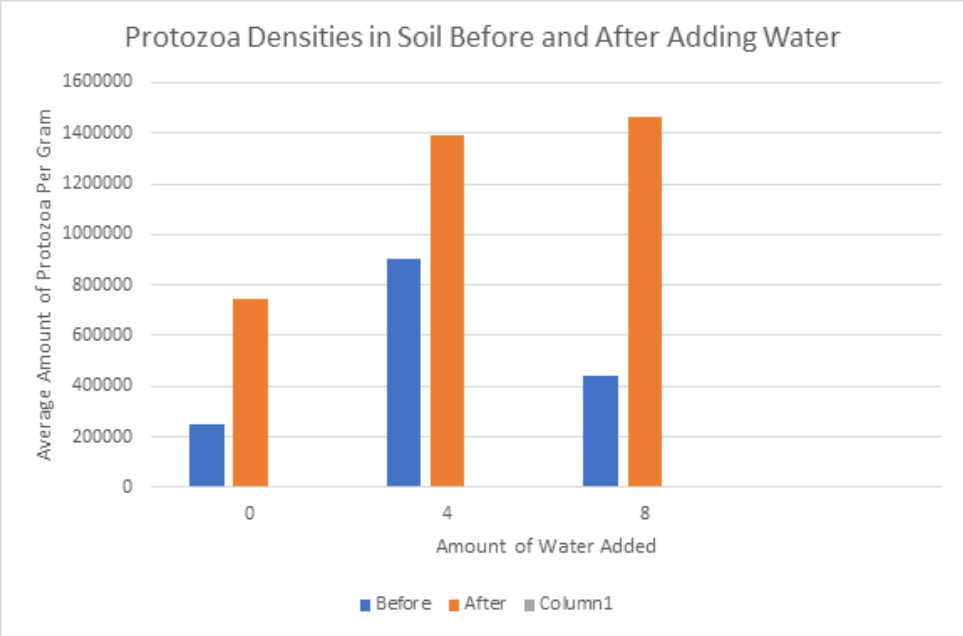


Table 1: p-values calculated during t-testing

Title/Description	Plots Compared	p-value of Soil
Bacteria Populations Before Adding Water	0 - 4	0.60991758915
Bacteria Populations Before Adding Water	0 - 8	0.5702592512
Bacteria Populations Before Adding Water	4 - 8	0.6731877312
Title/Description	Plots Compared	p-value of Soil
Protozoa Populations Before Water was Added	0-4	0.1221007877
Protozoa Populations Before Water was Added	0-8	0.1991618185
Protozoa Populations Before Water was Added	4-8	0.1981865985
Title/Description	Plots Compared	p-value of Soil
Protozoa Populations After Water was Added	0-4	0.1502639831
Protozoa Populations After Water was Added	0-8	0.0796947516 ,
Protozoa Populations After Water was Added	4-8	0.8403235092
Title/Description	Plots Compared	p]-value of Soil
Bacteria Populations Between Plots After Adding Water	0-4	0.3630762833
Bacteria Populations Between Plots After Adding Water	0-8	0.2757965992
Bacteria Populations Between Plots After Adding Water	4-8	0.3619041581

Discussion:

When analyzing the protozoa and bacteria populations found in the soil, our hypothesis that water added to the research plots would generate a more accurate predator/prey relationship between the protozoa and bacteria living there was supported. As Graphs 1 and 3 show, our belief that this relationship had been disturbed (as found during the original Biota survey) was supported because our samples taken before we manipulated the environment displayed no statistically significant correlation between whatever water was present beforehand and the population densities of bacteria and protozoa located in our designated research plots ($r^2 = 0.095$ and 0.047 respectively).

However, Graph 4 shows that water added to soil did effectively induce an increase in protozoa density ($r^2 = 0.41$), and therefore we can attribute the change in protozoa density to our manipulation of the sites. Thus it would be logical that we should have seen a similar increase in bacteria density due to the known direct correlation between water and bacterial growth. Yet Graphs 5 and 7 show a statistically significant correlation between the amount of added water and an actual decrease in the bacteria density in our plots ($r^2 = 0.32$).

This apparent anomaly, though, actually supports our hypothesis because Graph 6 suggests that the observed inverse relationship seen in Graph 5 is due to the return of the natural predator/prey relationship. Graph 6 supports the notion that as the additional water caused the protozoa populations in the saturated soil to increase, these additional protozoa consumed the bacteria population in our soil plots, and thus the expected increase in the bacteria populations in the saturated soil was not observed ($r^2 = 0.31$).

Further confirmation of our hypothesis can be found in Table 1. As our statistical analysis shows, before adding any water, the populations of bacteria and protozoa in our plots displayed no significant difference between plots (bacteria before water, p= 0.57 to 0.67; protozoa before water, p= 0.12 to 0.20 r). After adding any water, the populations of bacteria and protozoa in our plots did display significant difference between the plots (bacteria after

water, p= 0.28 to 0.36; protozoa after water, p=0.07 to 0.15). Hence we can be confident in our analysis of our data.

However, as Graph 8 shows, there was no statistically significant difference between the average amount of protozoa per gram of soil found in the plots with 4 L of water added and 8 L of water added (p=0.84). Therefore in the future, we would conduct a similar experiment using smaller increments of water between 0 L and 4 L to determine the point where soil can be considered to be in drought conditions.

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