An Analysis of the Potential Relationship Between Humus and Protozoa Levels, in Urban Deciduous Forest Soils

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Abstract

As participants of E.S.S.R.E., we examined the correlation between the quantity of humus (organic matter) and protozoa levels between 2 neighboring microclimates. The 32 collected samples from each site were tested for levels of humus and of protozoa per one gram of soil. Yet after data analysis, we found that there was no actual correlation between the humus and protozoa levels. Despite this, t-tests show (see fig. 7) that there is a difference in the populations of protozoa (with only a 0.0001% chance that the populations are in fact the same). However there is no reliable chance that the humus populations are different; not even an 80 percent chance exists to prove that they are distinct from one another in terms of humus presence. Based on our research, we disproved our initial hypothesis; hence we are led to speculate that the diverse protozoa populations are caused by an alternative ecological factor. However what this other factor is could only be determined with additional research. Upon looking back at a preliminary biota survey, we have begun to redirect our attentions to levels of bacteria, ferric iron, and potassium as factors that determine the size of a protozoa community.

Introduction

Our research interest in studying the relationship between protozoa and humus levels was piqued by an initial biota survey of the 4 microclimate sites of the Backwoods behind Roland Park Country School in Baltimore, Maryland (essentially, an urban deciduous forest). The two populations (of sites 2 and 3) we ultimately chose to look into varied in the protozoa count results collected from each.

First, we will review the basic features of sites 2 and 3. Site 2, located at N 39.357 40 W 76.638 93, was bisected by a streambed, contained a large variety of life (e.g., in terms of plants) and rotting materials and had a soil texture of primarily loamy sand. Site 3, located at N 39.357 97 W 76.638 36, also had a considerable water supply, a streambed, and an uneven topography; yet, in addition, was characterized by a great amount of sedimentary rock material and invasive plant species (e.g., hillside rhododendron).

The sample counts of protozoa in sites 2 and 3 varied to the extent that there is an 80 percent likelihood that the Null Hypothesis can be refuted and hence that the 2 populations are indeed different. In site 2, 8 samples were collected while 12 were collected in site 3. Statistics in figure 1 below.

Statistics *figure 1.*

	Site 2	Site 3
Mean	28	263
s (standard deviation)	11	192
s^2 (variance)	113	36,980
Standard error	9; 19-37	114; 148-377
Range	34	751
n (sample number)	8	12

We performed t-tests on the 2 protozoa populations, and this led us to speculate which ecological factor(s) could have been responsible. After discussing the disparity in protozoa populations with Dr. Peter Groffman, we became interested in exploring the role of humus in determining protozoa population (Groffman, 2001).

Methods

To begin our research, we collected 8 samples from each quadrant in sites 2 and 3. On the first day we collected 4 samples each; then another 4 samples each on day 2. To collect the samples, we used a soil core sampler up to the first mark (to a soil depth of 13.5 cm). We took these samples back to the lab to set up a protozoa count.

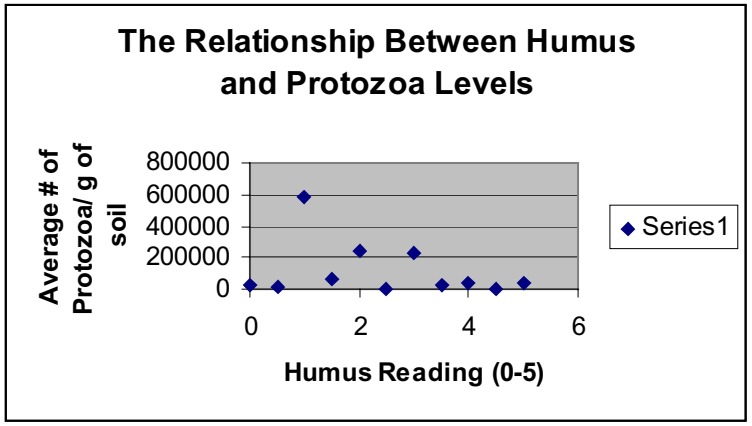
We first massed 10g of soil, which then went into labeled (by site, quadrant, and sample number) petri dishes. Each petri dish soil sample was then saturated with 14 mL of distilled water. To best saturate the soil, we tilted the dishes at a 45 degree angle to create a soil-water solution. Covered petri dishes were then stored at room temperature (about 30 degrees Celsius) for two days or, approximately 48 hours. After the 48-hour waiting period, we collected 1 drop of each sample's soil-water solution and placed it on a depression slide. We then added 1 drop of diluted methyl green dye solution (50 percent distilled water, 50

percent pure methyl green dye) to each depression slide, and covered with a cover slide. After preparing each of the 64 protozoa slides, we examined each under a light microscope. We first examined the slide at 10*x* and then 40*x* and, finally, 100*x*. Under the 100*x* magnification, we counted (and recorded) the number of protozoa in 5 individual field views. Protozoa counts were converted for each sample to number per effluent extract by summing the 5 field views (per sample) and then dividing this by 2.025. To convert the number of protozoa per effluent extract to per gram of soil, we multiplied the former data by 1400.

We then, using the core samples also used for the protozoa count, tested for humus presence. For each sample, we measured 2 level measures (i.e., 2 grams) of the soil, which was then placed into a soil testing tube. We then added demineralized water until the mixture reached the 14 mL mark, and shook gently for 5 seconds. We proceeded to add 1 gram of Humus Screening Reagent to each tube, and then shook the tubes vigorously for 60 seconds. Finally, we completed the original humus solution by adding 15 drops of Soil Flocculating Reagent and shook the tubes gently for 5 seconds. We allowed this solution to settle for a couple of minutes. The settled solution was then filtered into another soil testing tube through use of a funnel and quartered filter paper. We then matched the resulting color of the new humus liquid solution with a corresponding color on the Humus Color Chart (provided by a chemical soil testing kit). Although the Humus Color Chart only provided a "key" of colors in terms of integers 1-5 (5 being a most intense orange; 1, the lightest), we used not only the whole numbers but also data like 1.5, 2.5, and so on. We added 0 to our color scale since occasionally no color change was observed. This data was then recorded.

Results

Firstly, we looked for a general correlation between humus and protozoa levels, spanning both sites. A regression analysis indicated little connection between the 2 variables, as an r-value of 0.14 was found. We also calculated a t-bivariate of 1.1133, less than the table value for a 20 percent chance of 1.296.



Since we found only a weak (if any actual) correlation between humus and protozoa levels, we decided to isolate each variable and compare their presence in site 2 versus site 3. The following results were collected for humus presence: *see figure 2. Figure 3* indicates statistics found from *figure 2* results. A performed t-test (*see figure 4*) indicates that even for

a 0.2 t-value, the sample t-value (0.779) was less than the table value (0.1.296). Thus, there is not even an 80 percent chance that we can refute the Null Hypothesis; and the 2 populations are most likely from the same population in terms of humus levels.

A comparison of protozoa levels in each site was also performed. See *figures 5 and 6* for collected protozoa counts, and their statistics. While site 2 had an average of 12 885 protozoa, site 3 had 29 703. *Figure 7* indicates the resulting t-values from a t-test comparing the sites. The found t-sample value of 8.989 was greater than the t-alpha value of 4.169 (for a 0.0001 degree of error) signified a 99.999 percent likelihood that the Null Hypothesis could be refuted; hence the 2 sites are almost certainly statistically from 2 different populations in terms of protozoa levels.

Discussion

Although we hypothesized that a larger protozoa population would correlate with greater humus levels, our data and regression analysis pointed otherwise. The protozoa samples of sites 2 and 3 are statistically significant and thus from different ecological populations in terms of protozoa count (a t-alpha value . However, not even an 80 percent chance existed in our t-test analysis that the humus samples were collected from different ecological populations in terms of humus count. Since we performed a regression analysis and found an r-value of 0.14, only a weak to non-existent correlation exists between protozoa and humus levels in the 64 samples collected.

Therefore, protozoa and humus levels are not interconnected and our initial question of, What caused this difference in protozoa populations in sites 2 and 3? We can only conclusively say from our data, that amounts of humus (namely, organic matter) within a soil habitat is not the cause. Our experiment drastically oversimplified the situation since a single ecological factor cannot be the sole determinant for protozoa populations. We later hope to definitively acknowledge multiple factors, such as bacteria population and soil texture, in determining protozoa populations. Upon looking back at our initial biota survey, we noticed a new potential direction for our research. Higher levels of bacteria seemed to correspond to higher levels of protozoa, which seems entirely logical and probable–since protozoa feed off of bacteria. From the E.S.S.R.E survey, an average of 7.513x10^6 was found in site 2 and an average of 5.46x10^8 was found in site 3. Average Ferric Iron levels within sites 2 and 3 respectively, were as follows: 7.5, 10.625. Average potassium levels within sites 2 and 3 respectively, were as follows: 0. 87.5. An interesting future direction would be an exploration of a count of different types of protozoa in relationship to a count of different types of bacteria. We also noticed higher levels of ferric iron and potassium in correspondence with higher protozoa levels (between sites 2 and 3).

References

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