A Study on Earthworms and Their Effect on Protozoa and Bacteria

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Abstract

Earthworms, bacteria, and protozoa frequently interact with each other in a wide variety of soil environments, forming a complete food chain with earthworms at the top, protozoa in the middle, and bacteria at the bottom. During the E.S.S.R.E 2018 Biota Survey, earthworm density in Microclimate 3 was extremely high (115/m^2), which was unexpected for this typically dry location; while the bacteria and protozoa densities were almost identical (373,166/cc; 300,355/g respectively), unusual because the predator-prey relationship between the bacteria and protozoa is normally an inverse one. It was hypothesized that the increase of earthworm density was causing a decrease in protozoa density, which was unbalancing the bacteria density, and 3 locations were selected randomly in each of the 4 Quadrants of E.S.S.R.E Microclimate 3 and tested for earthworm, protozoa, and bacterial densities using direct inspection, a modified Foissner/Uhlig extraction, and serial dilutions respectively. We discovered that the density of the protozoa significantly varied every day but the changes in the density of the earthworm and bacteria populations were statistically insignificant. We believe that nematodes, which are also known predators of protozoa and are found in the majority of soil conditions, may be the cause of this fluctuation in protozoa density.

Introduction

Bacteria, protozoa, and earthworms co-exist in various soils throughout the world in a classic predator-prey relationship. Earthworms are at the top of this food chain, consuming both soil protozoa and bacteria (as well as nematodes and fungi), and a higher density of earthworms will normally lead to decreased densities of both bacteria and protozoa as earthworms must consume up to a third of their body weight each day to survive (Journey North, 2018). Protozoa come next in the environmental hierarchy, and while they are a frequent prey of the earthworms, they are the primary consumers of bacteria in the soil (also eating some fungi as well as other protozoa). Bacteria remain at the bottom of any soil food chain, where these unicellular prokaryotes break down both dead plant and animal material, releasing important nutrients like phosphorus, nitrogen, and carbon into the soil (Egbunine, *et al.* 2017). A high density of bacteria in the soil is good for a healthy ecosystem, as so many other organisms rely on the bacteria for their food. In addition, all three organisms -earthworms, protozoa, and bacteria- serve as food for many other terrestrial species and improve soil quality by breaking down nutrients that enrich the soil for plant life.

A healthy food chain is so vital to healthy soil because the food chain allows for nutrients in the soil, often sourced from plant debris, to be recycled through different organisms back into the plants in the environment. For example, protozoa are commonly found near the roots of plants as they prefer areas that are damp and rich in bacteria to feed on, and they benefit the plants because when protozoa eat the bacteria in the soil they mineralize nutrients and get rid of the excess nitrogen they receive from digesting the bacteria, releasing all of it back into the soil for the plants to reabsorb and recycle (Ingham, 2018). Hence, as a result, disruptions to the food chain could be dangerous for the health of the soil.

That is why we were intrigued when the 2018 ESSRE biota data survey revealed that the normally inverse relationship between bacteria (375,000 per cm3) and protozoa (300,000 per gram of soil) densities was not observed in Microclimate 3 (N 39.35797; W 076.63836). However, the survey also revealed an unusually high density of earthworms (115 per m2) given the extremely dry conditions found in Microclimate 3 (since earthworms prefer damp soils [Uday Bhawalker, 2008]). Given the known predator/prey relationships between these three groups of organisms, we wanted to see if the new bacteria and protozoa relationship might have been caused by the earthworm population eating more of the protozoa, therefore reducing the soil bacteria predator density.

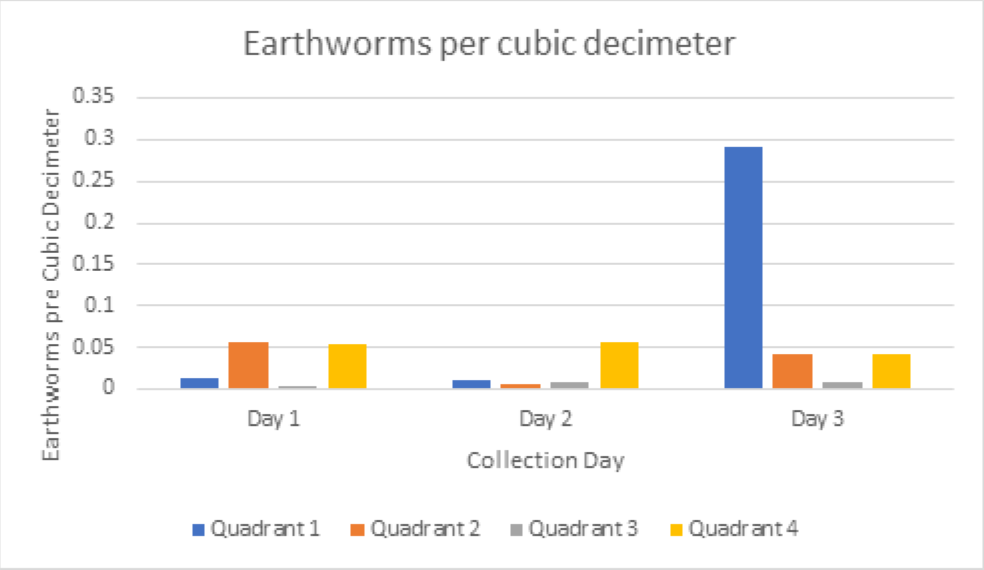
Methods

12 different locations in ESSRE Microclimate 3 (N 39.35797; W 076.63836) were chosen randomly, 3 in each of the 4 quadrants. Cylinders of soil 15 ½ cm deep and 11 ½ cm diameter were extracted from each location and sorted to count earthworm density (#/dm). Simultaneously, a soil core 15 cm deep and 2 ½ cm in diameter was collected at each location to test for protozoa and bacteria densities. Protozoa density (#/g) was determined using a modified Foissner/Uhlig Extraction Method (Brockmeyer, 2008) at the 40X power, observing 5 fields of view (the four corners and center of the slide) and averaging the counts. While that data was being gathered, serial dilutions were performed with sterile water on one cubic centimeter of

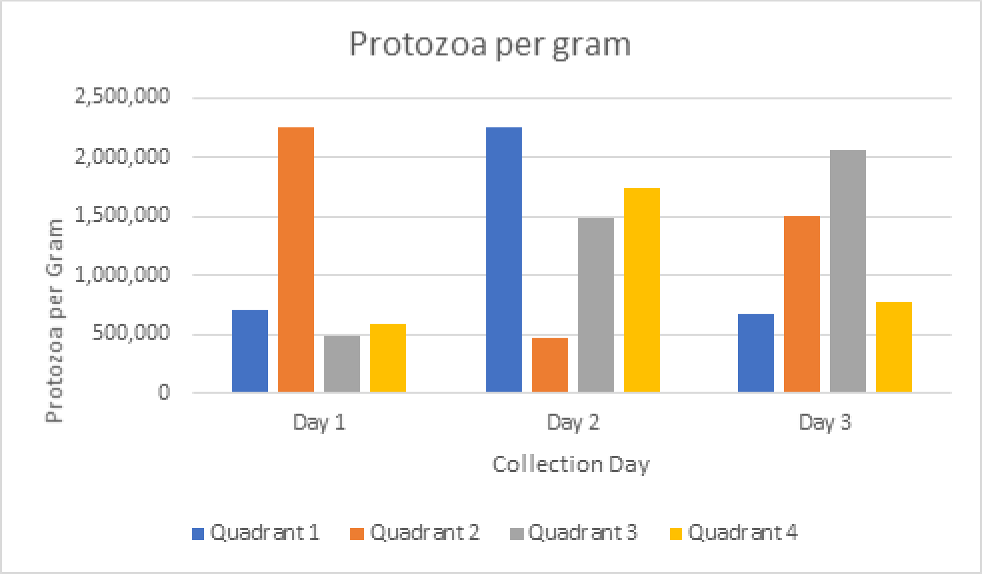
each sample to the 10-3 value. 100 Ml samples of each dilution were plated on 3M Petrifilm Aerobic Count plates and grown for 3 days to determine bacteria density (#/cm3).

Results

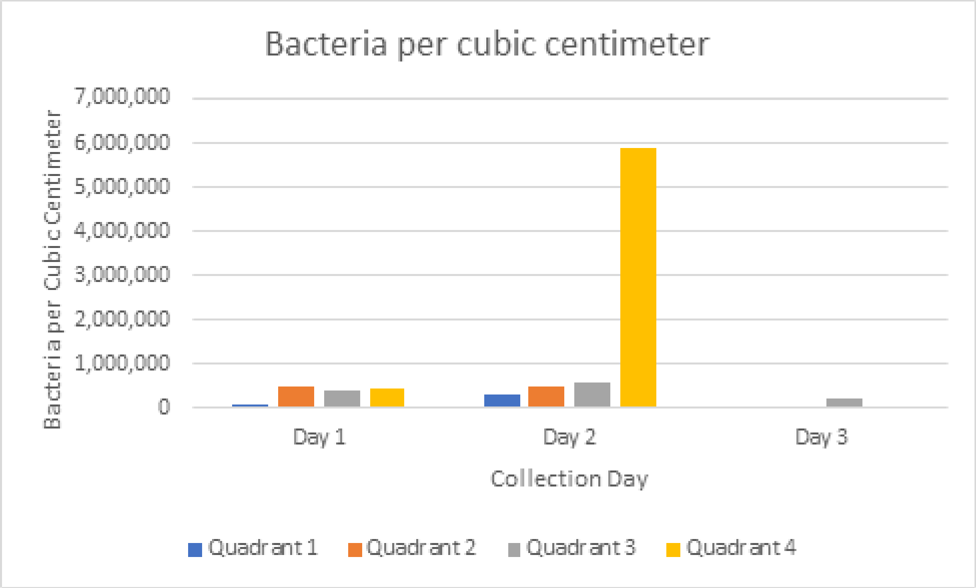
Graph 1: The Density of Earthworms in the Quadrants 1-4



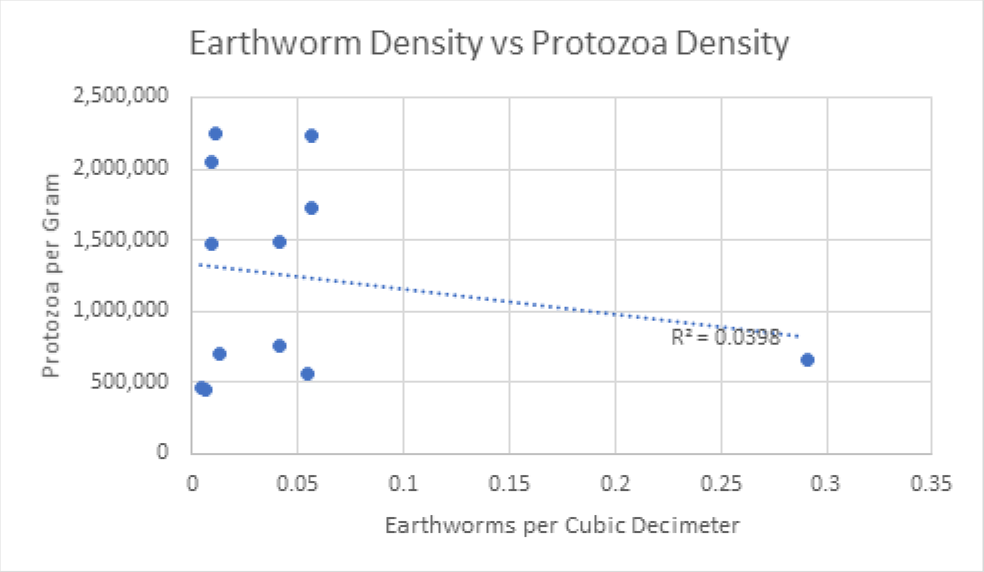
Graph 2: The Density of Protozoa in the Quadrants 1-4



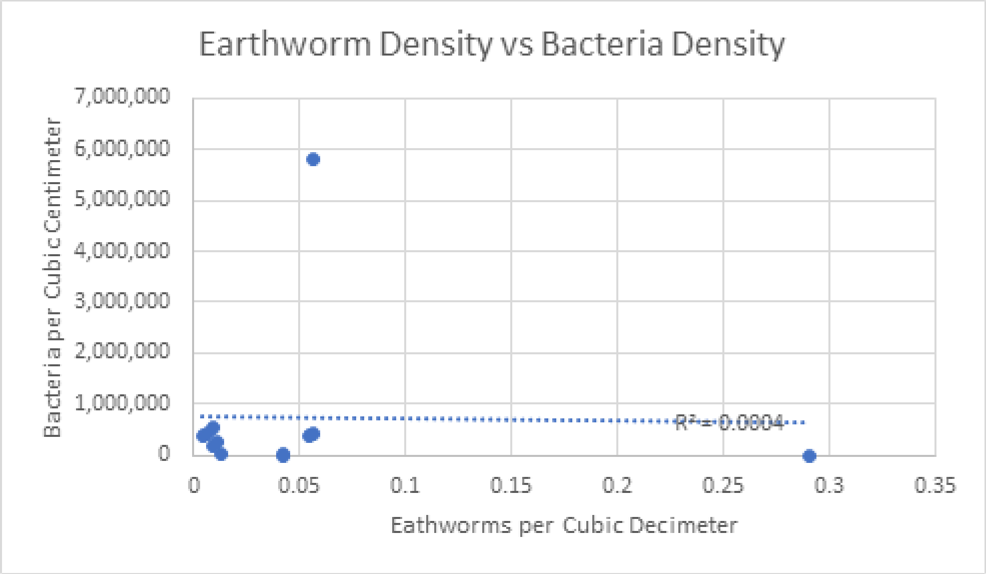
Graph 3: The Density of Bacteria in Quadrant 1-4



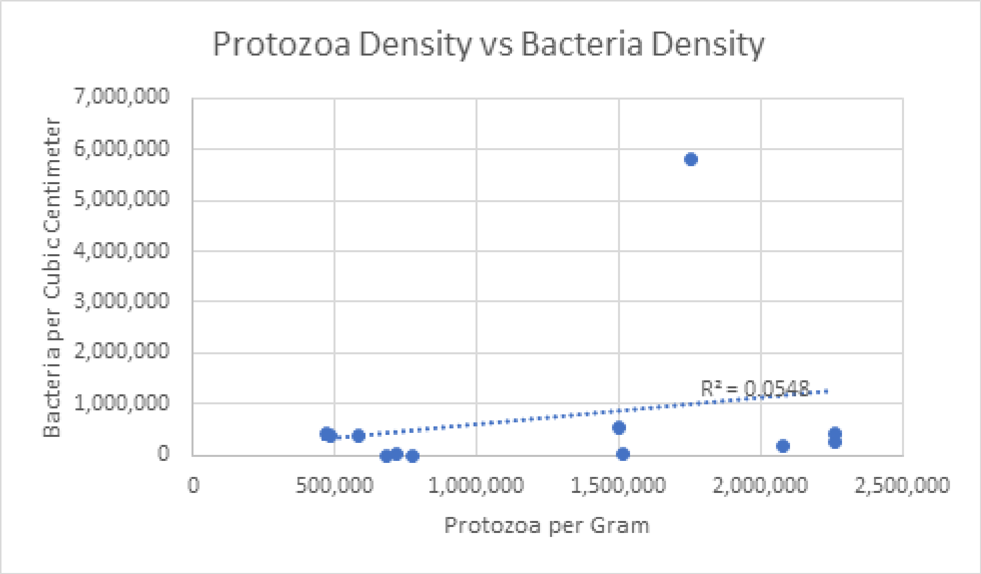
Graph 4: The Correlation Between the Earthworm Density and the Protozoa Density in the Soil



Graph 5: The Correlation Between the Earthworm Density and the Bacteria Density in the Soil



Graph 6: The Correlation Between the Protozoa Density and the Bacteria Density in the Soil



Discussion

Our initial hypothesis that an increase in earthworm density would cause a decrease in protozoa density and, therefore, a consequent increase in bacteria density was not supported. As seen in Figure 1, the earthworm densities throughout the collection days remained stable (p= 0.461-0.964). However, the protozoa density varied significantly in the different quadrants throughout the collection days (Figure 2; p= 0.011-0.357). Hence, it could not be the earthworms causing the disruption of the protozoa and bacteria relationship observed in the original ESSRE 2018 Biota Survey. Figure 4 further supports this absence of any significant correlation between earthworm densities and protozoa densities found during this study (r^2= 0.0398), and while the bacteria densities varied in some quadrants over the course of the three collection days, remaining stable in others (see Figure 3; p = 0.089-0.351), Figure 5 clearly indicates that earthworms were not significantly responsible for any of the observed changes in bacteria density either (r^2=0.004).

The data did reveal a small correlation between the protozoa and bacteria densities, (see Figure 6; r^2=0.054). Hence, there are grounds to claim that some of the normal predator-prey relationship between these two organisms was taking place. However, this correlation is not strong enough to be the cause of the significant variation seen in the protozoa densities seen in Figure 2 over the course of the 3 days of the study. Therefore, the population density of soil bacteria in Microclimate 3 is not having a strong impacting on the protozoa density there.

Since neither earthworms nor bacteria appear to be causing the fluctuation of protozoa during the experiment, some other factor must be the cause. A possible culprit could be nematodes. Large nematodes feed on protozoa fattened from eating bacteria and nematodes are found in the majority of soil types (Nardi, 2003). Therefore because heavy predation by protozoa can cause soil bacteria to reproduce more rapidly (resulting in the kind of small increase in bacteria density observed in Figure 6 [Rønn R, *et all*. 2018]), it is possible that nematodes might be causing the fluctuations in protozoa we observed in Microclimate 3. In the future we would test for both protozoa densities and nematode densities in the soil to see if the days that showed low protozoa also showed high nematodes levels and vice-versa.

Biblio

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