How Does Oxygen Affect Protozoa Density?

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ABSTRACT:

Protozoa are microorganisms that live in soil. Traditionally they are found in areas with high amounts of water, iron and low levels of chloride. In the ESSRE microclimate Site 4 (N 39.35733; W 076.63840), the levels of protozoa were significantly lower than expected given that these ideal conditions were present. We hypothesized that there was too much oxygen and that this was causing the extremely low densities of protozoa in Site 4. We tested this hypothesis by examining soil samples for the density of protozoa in Site 4 and then aerated 5 plots with increasing volumes of air to expose the protozoa to oxygen. After three days, we then tested the soil for protozoa density again. Even though our hypothesis was proven to be incorrect, we discovered that a strong positive correlation between oxygen and protozoa does exist.
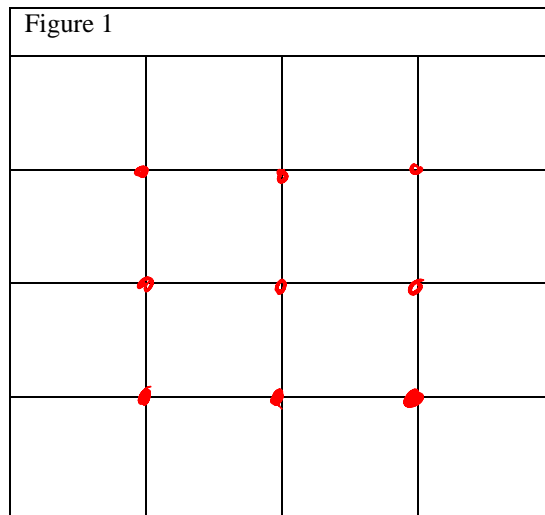
Introduction :

Soil protozoa are single-celled organisms that feed primarily on bacteria, other protozoa, organic matter, and sometimes fungi (Wikipedia, 2009). When consuming bacteria, they release excess nitrogen, in the form of nitrite into the surrounding soil (Ingham, E.R.), and since nitrite is oxidized to nitrate, almost immediately in most soils, there will be a high concentration of nitrate an environment with a lot of protozoa. In addition, high levels of iron and low levels of chloride are traditionally associated with large sums of protozoa (Weinberg, E., 1999), as well as significant quantities of water, since protozoa live in water (Microbiology Online, 2009). However, for protozoa to truly thrive, the chemical properties of the water have to be precise. The pH of the environment needs to be between 6.5 and 8.5 (Spellman, F.R., 1998) because anything higher or lower than that would be almost too acidic or basic for ANY organism to live.

During the annual E.S.S.R.E. biota surveys (2009), an interesting discrepancy arose. Since most protozoa are found where there is a high concentration of water, the extremely low density of protozoa found in one of the microclimates (Site 4; N 39.35733; W 076.63840) that has wetland properties was puzzling. Also, Site 4 had the perfect living condition for protozoa to be there. The environment had a low chloride level (79.17 ppm), a high iron concentrate (17.71 ppm), and a large amount of nitrate (42.08 ppm). Yet, the average density of protozoa in the soil at Site 4 was only 137,592.
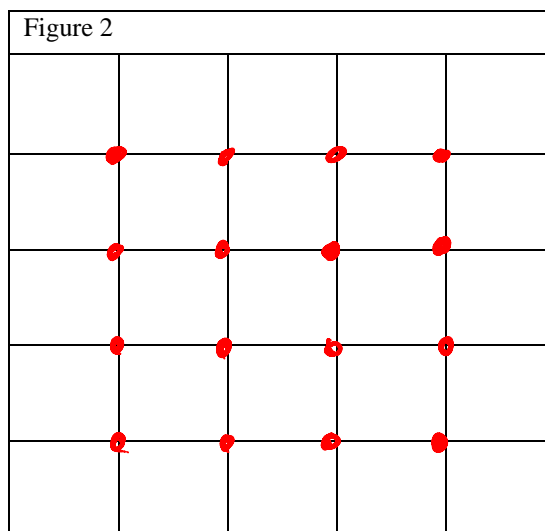
In an environment with too much oxygen, there will be fewer protozoa because a high concentration of oxygen is toxic to microbes (Lotus, n.d.). Yet, if the soil has too little oxygen, the amount of protozoa in it will decrease because they would not have enough oxygen for metabolic activity. Protozoa need to be in an environment with not too much, but not too little oxygen. One key environmental factor, though, that was not included in the original biota survey was the amount of oxygen available in the soil of Site 4. Hence, we hypothesize that the reason for the lower than expected density of protozoa in Site 4 may be due to the fact that there is too much oxygen in the soil there.
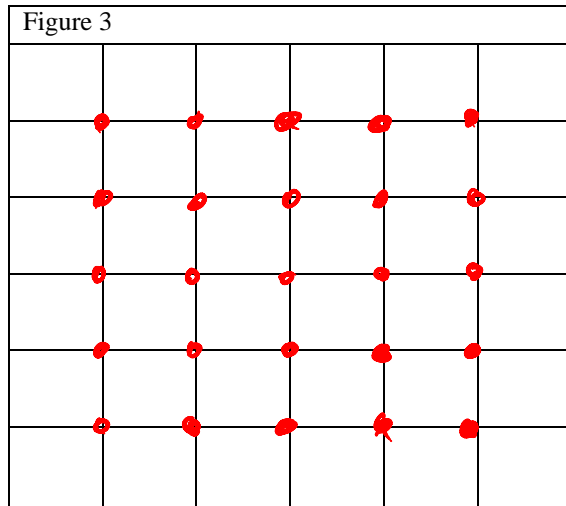
Methods:

Five 1m x 1 m plots were created in E.S.S.R.E. Microclimate 4 (N 39.35733; W 076.63840). In Plot 1 (the negative control), 3 soil extractions (2.5 cm in diameter and 15 cm deep) were taken to serve as the positive control ("before") samples. The holes were then collapsed to minimize aeration from occurring. In Plot 2, 9 holes (2.5 cm in diameter and 15 cm deep) were placed in a grid pattern at 25 cm intervals from the edge of the plot and each other (see figure 1). The soil from 3 of these extractions was reserved as positive control ("before") samples for protozoa extraction.
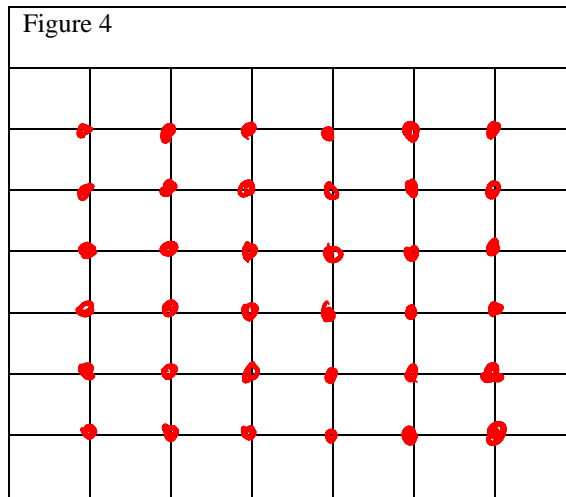
Figure 1

In Plot 3, sixteen holes (2.5 cm in diameter and 15 cm deep) were placed in a grid pattern at 20 cm intervals from the edge of the plot and each other (see figure 2). The soil from three of these extractions was reserved as positive control ("before") samples for protozoa extraction.

Figure 2

In Plot 4, twenty-five holes (2.5 cm in diameter and 15 cm deep) were placed in a grid pattern at 16.7 cm intervals from the edge of the plot and each other (see figure 3). The soil from three of these extractions was reserved as positive control ("Before") samples for protozoa extraction.

Figure 3

In Plot 5, thirty-six holes (2.5 cm in diameter and 15 cm deep) were placed in a grid pattern at 14.3 cm intervals from the edge and each other (see figure 4). The soil from three of these extractions was reserved as positive control ("before") samples for protozoa extraction.
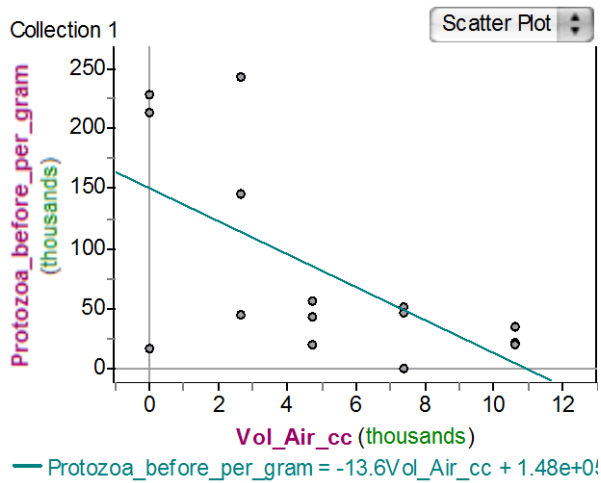

Figure 4

After the plots were left to aerate for two days, 3 additional ("after") samples from each plot (2.5 cm in diameter and 15 cm deep) were taken. Protozoa were extracted from all soil samples and the density in number per grams of soil was determined using a modified Fossiner/Uhlig process (Brockmeyer et al, 2007).
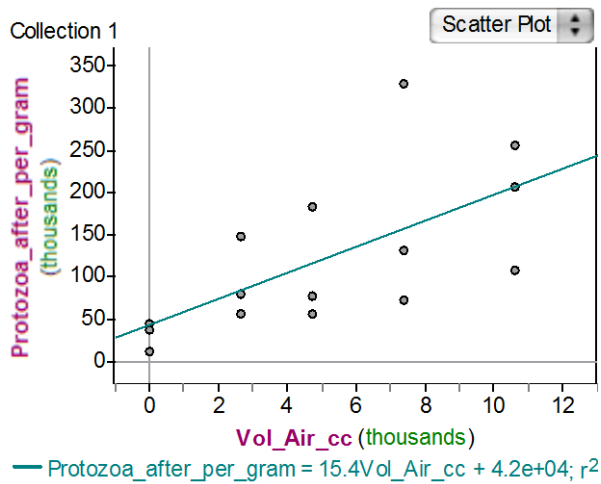
RESULTS:

Graph 1: The correlation between the research plots assigned aeration volumes and protozoa density before the experiment.
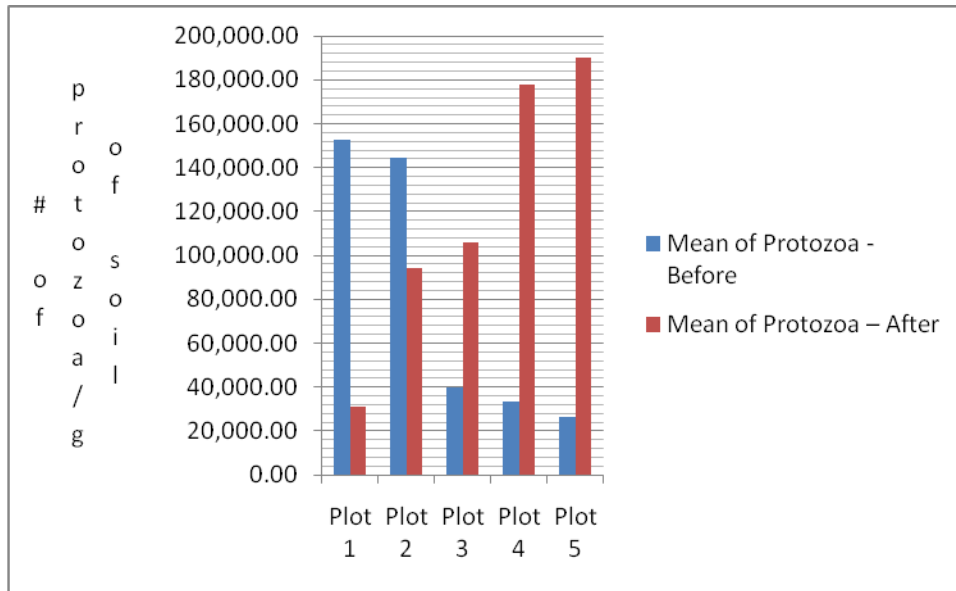
Volume vs. Protozoa: Before

Graph 2: The correlation between aeration volumes and protozoa during the experiment

Volume vs. Protozoa: After

Graph 3: The change in protozoa density between the five research plots
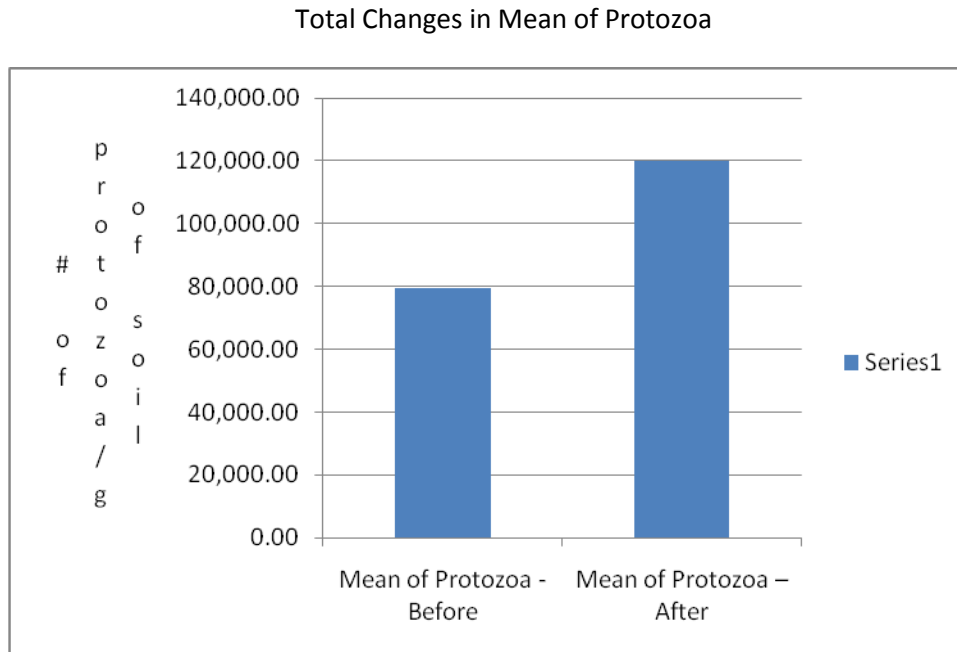
Total Plot Change in Protozoa

Plot 1= 0 cm^3

Plot 2= 2651 cm^3

Plot 3= 4712 cm^3

Plot 4= 7363 cm^3

Plot 5= 10,603 cm^3

Graph 4: The overall significant difference in the average overall amount of protozoa within the aerated plots

Total Changes in Mean of Protozoa

DISCUSSION:

The results of our experiment contradict our initial hypothesis. We hypothesized that too much oxygen was causing the extremely low levels of protozoa in Site 4, but, as graph 2 clearly shows, there was a strong positive correlation between the degree amount of aeration and the density of protozoa in the soil: As the volume of air in the soil increased, the amount of protozoa per gram also increased. Furthermore, graph 4 shows that while the collective average density of protozoa in all the aerated plots before aeration was approximately 79,370 protozoa/g, the collective average density afterwards was approximately 119,885 protozoa/g. Therefore, our hypothesis was technically incorrect.

But while our experiment invalidated our actual hypothesis, our results do demonstrate an extremely strong correlation between oxygen levels in the soil and the amount of protozoa living there. In fact, graph 3 shows that the more the soil was exposed to oxygen, the more protozoa there were. Indeed, as Graph 3 shows, Plot 1, our negative control, actually showed a decrease in protozoa density of 121,536 per gram, followed by less of a decrease in plot 2 (which was minimally aerated) and then increasingly dramatic increases in the number of protozoa in plots 3-5. Indeed, the ratio of protozoans present after aeration when compared to before aeration goes from 3:1 in Plot 4, to 5:1, and Plot 5 was to 7:1. This suggests that in fact there is an exponential relationship between the amount of oxygen present in the soil and the number of protozoa in the soil, and when the p-values of t-testing of the data are examined, the difference between the number of protozoa before and after the experiment in plots 3-5 becomes increasingly statistically significant.

However, it is possible that other environmental factors could have been affecting the amount of protozoa in the soil. In order to determine whether or not it was indeed oxygen that was causing the

differences in protozoa densities, we tested the soil before and after for dissolved oxygen using a modification of the Azide method of Winkler titration. Our results showed that the overall dissolved oxygen levels increased as predicted: the average amount of dissolved oxygen went from 1.34 ppm to 2.69 ppm. These results indicate that we did in fact alter the oxygen levels successfully in each of the aerated plots. Hence it was differences in oxygen levels in the soil of out five research plots that was causing the differences in protozoa densities.

Therefore, based on what we found, it would be logical to go back and look for the source of low oxygen levels in the soil. One such factor to examine first would be to check if the soil is compacted, given the amount of runoff and erosion in the research site. Compacted soil prevents oxygen and water from traveling through the ground, trapping carbon dioxide as well, and with the poor quality of the plant life in the research site, we think that soil compaction is the most probable root of the problem.

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