The Effects of UV Radiation on Protozoa Population Densities

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Abstract

As part of the Environmental Science Summer Research Experience biota survey at Roland Park Country School, we found significantly lower protozoa levels in site 4 (N 39.35733; W 076.63840) than site 3 (N 39.35797; W 076.63836). This led our team to take a closer look at other environmental differences which may explain this considering site 4 is a wetland where protozoa are expected to thrive in comparison to the hillside of site 3. We observed an increased amount of sun exposure to the soil of site 4. Being that UV radiation is a main component of sunlight, we began studying the affects of UV radiation on protozoa levels at different depths of soil. To test this we controlled UV exposure at 5 degrees on individual plots at three areas in site 4. Our team counted the protozoa levels for the negative control before the experiment and again after the sun exposure. We expected the protozoa levels to be lower on the top 10 cm of the soil as more UV rays penetrated the soil and the protozoa levels to be higher in the 10 to 20 cm region as the protozoa migrated vertically avoiding the rays. After the experiment we found there was a significantly lower amount of protozoa in the 10 – 20 cm region and the top layer stayed relatively the same. Our data shows no relationship between this decrease and the UV radiation thus UV radiation did not have a direct influence on protozoa. Further research would lead us to study why there was no significant change in the top layer and such a great change in the 10-20 cm region. Studying other finding in the original biota survey and the affects of UV on organisms protozoa depend on may help us understand this trend

Introduction

Diverse populations of organisms live beneath the soil, each of which has a key individual role in the soil ecosystem. One of the most important organisms in the cycles of soil life is protozoa. Protozoa are unicellular eukaryotic organisms that have simplified systems which are key in keeping higher organisms alive. Protozoa consume bacteria and decompose organic matter. Protozoa's consumption of bacteria is important because it reduces the chance of pathogens infecting the soil. Their control of the bacteria populations has a prodigious influence on humus formation and the organic matter cycle. Protozoa consume 90% of the total consumed bacteria in the soil (Nardi, 2003). Furthermore, within the food web, protozoa are not only important for their consumption of bacteria, but larger protozoa sometimes feed on algae, fungi, and even plant debris. Just as protozoa are the main predators of bacteria, they provide a good source of nutrients and proteins for nematodes. This pattern makes up the food chain, a factor existent in all ecosystems.

Protozoa are also key to the environment as they play a significant role in biogeochemical cycles, particularly the nitrogen cycle. The consumption of bacteria is beneficial for protozoa, but bacteria have a much higher concentration of nitrogen containing compounds in their cells than protozoa. As the bacteria are eaten by the protozoa, the higher concentration of nitrogen in the bacteria is too much for the amount protozoa need (Ingham, undated). As a result, protozoa excrete excess nitrogen containing compounds into the soil in the form of ammonium. Usually, this excretion of nitrogen containing compounds occurs near the root system of plants and is therefore utilized by the plants for their own metabolic functions.

Because of the roles protozoa play, their absence would be detrimental to many aspects of ecosystems. First, the absence of protozoa would instigate a missing link in the food chain. There would be no regulation of bacteria and bacteria populations would grow to large numbers. Because of the growing population of bacteria, vegetation would be prone to infectious pathogens. Second, the absence of protozoa would create an interruption in the nitrogen cycle. This would produce a lack of ammonium in the soil for plants to use effectively causing a decrease in plant diversity and density. Third, the absence of protozoa would cause an interruption in the cycling of organic matter and humus formation.

In the 2004 biota survey we took of the back woods of Roland Park Country School at sites 3 (N 39.35797; W 076.63836) and 4 (N 39.35733; W 076.63840), we found statistically significantly lower protozoa levels in site 4 than site 3 (E.S.S.R.E. Microclimate Databases, 2004). This showed a discrepancy because site 4 is a wetland whereas site 3 is a hillside, and given that the ideal environment for protozoa is the thin layer of water that lines the numerous pores of the soil found in wetlands, one would expect more protozoa in site 4, particularly naked amoebae (amoebae that lack a shell and in turn have pseudopods) which thrive in wetlands. Furthermore, in 2003, the ratio of protozoa in site 3 to site 4 was 1:1.28 protozoa while in 2004, the ratio 1:0.46 protozoa, respectively (Ingham, undated). Also, site 4 exhibits many of the characteristics of low protozoa levels such as a distinctive smell from decaying matter, large amounts of bacteria in site 4, and there was a monoculture (Microclimates Data, 2004). Therefore, we began to wonder what significant environmental differences exist between site 3 and site 4 that might account for protozoa levels.

One significant difference is increased exposure to sun light. Ultraviolet (UV) Radiation, made up of invisible rays from the sun, is a major component of sunlight, and "In general, UV radiation of microorganisms causes chemical bonds to form in cellular DNA. The exposure thus interrupts normal DNA replication and organisms are killed or rendered inactive."(Northeast Midwest Institute, 2001) Thus, we thought perhaps the radiation from excess sun light could be the cause of low protozoa levels. Normally, there is a layer in the atmosphere of ozone molecules that absorbs these rays to prevent further damage (Nova, undated), but over the years there has been speculation that holes were being created in the ozone layer. It has been discovered that in various locations the ozone layer has thinned out severely due to several natural and manmade factors such as chemicals like chlorofluorocarbons (CFC). Several of these chemicals have been found to enter the atmosphere from industrial pollution. As chemicals like CFC enter the ozone layer, they break down. When CFCs break down they release atomic chlorine. One atomic chlorine molecule has the power to destroy thousands of ozone molecules (Environmental Protection Agency, 2004). These "holes" (literally areas where there are severely low levels of ozone molecules) in the ozone allow for more UVB and UVA rays to enter the troposphere and effectively cause higher degrees of UV Radiation. Holes such as these have been found above North America, Europe, and Antarctica, increasing in size every year. Being located in North America, site 4 (the wetland meadow)is also being exposed to growing amounts of UV Radiation.

As we have seen, the protozoa population density was unusually low. Because previous research has shown that excess UV Radiation was detrimental to mold levels in the soil at this site (Bartlett *et al*., 2002), we wondered if the fact that there has been an

increase in sun exposure due to a reduction in covering plants (as well as the North American hole in the ozone layer) might be the cause of the low numbers. But because protozoa are capable of vertical migration to reduce the negative effects of UV radiation(Sanders, undated), we weren't sure if excess UV radiation was reducing population densities by killing off the protozoa or forcing them to move deep into the soil.

We hypothesized that UV radiation causes protozoa to vertically migrate in the soil causing population densities of protozoa to increase at greater depths of the soil. Therefore we decided to test for protozoa levels at different depths of soil (0-10 cm, and 10-20cm) at different intensities of sunlight.

Methods

To test for the effect of UV radiation on protozoa density, we cleared three different 1m x 1m areas in Site 4 of all plant life and detritus over the soil. We marked off five adjacent 15cm x 15cm plots and took separate soil samples from each plot to act as a positive control. Samples were cores 2 cm diameter X 20 cm deep. We placed the first 0 – 10 cm of each core, the upper column of the soil measured from the surface in one clean plastic bag and the last 10 – 20 cm of each core in a second clean unused bag. We repeated the process for the other two areas.

To test if the degree of UV rays had any impact on the protozoa levels we controlled the ultraviolet radiation in five different ways. Based on Bartlett, *et al*(2002) we used black plastic trash bags (completely blocking UV rays), white unpainted canvas (blocking most UV rays), mesh (blocking only a few UV rays), clear plastic (blocking very little UV rays) and Saran® wrap being our negative control allowing all of the UV radiation in the soil.

We covered each of the 5 adjacent 15cm x 15cm plots as follows: first plot with Saran wrap® as degree 1, second plot with clear plastic as degree 2, third plot with mesh, fourth plot with white canvas and the fifth plot with plastic trash bags, all cut into 15cm x 15cm pieces. We secured the different ground covers by flags labeled with the corresponding degree and area and let the plots sit for 20.5 hours of sunlight giving them still as much time possible for UV exposure. Following exposure we collected a second set of 2cm diameter x 20 cm deep soil samples from each plot and site, dividing it again into the 0 – 10 cm and 10 – 20 cm levels.

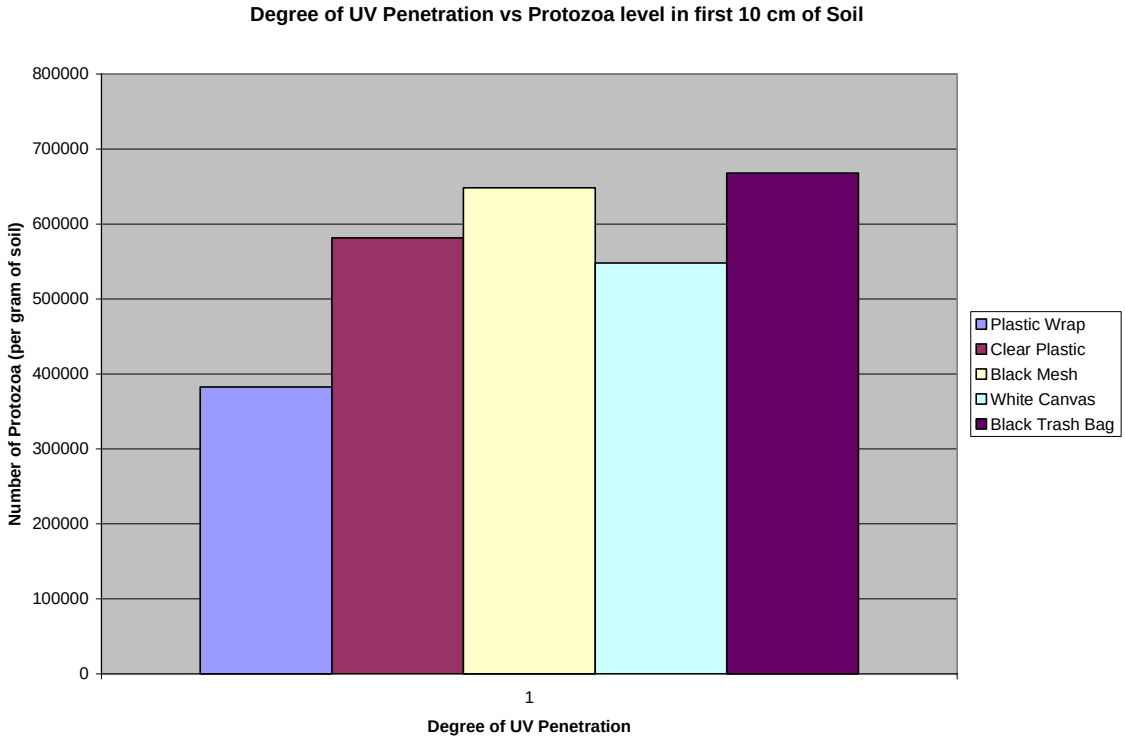
We extracted protozoa from all samples to determine approximate population densities using a modified Foissner/Uhlig protocol (Brockmeyer 2003). We first emptied each sample soil from its sealable bag into a clean empty Petri dish marked according to sample location, treatment and depth and allowed it to dry completely. We then sifted 9 g of each sample into a clean Petri dish using a $1mm^2$ nylon mesh and added 20 ml of distilled water to saturate the soil and covering it with a lid and allowing it to sit for 7 hours. We then placed each rehydrated soil sample in separate Uhlig extractors containing 30 ml of distilled water for 24 hours. We removed the filtrates and filtered them a second time using12.5 qualitative filter paper.

We made individual microscope slides from the second filtration by depositing 7 µl of methyl – green stain and adding 18 µl of the 2^{nd} filtrate to the stain on the microscope slide and covering it with an 18 x 18 mm^2 cover slip. We examined 5 different randomly chosen fields of view on the slide under a light microscope at 40X. Averaging the count from the 5 fields of view, we calculated the population density of the protozoa in each soil sample as follows:

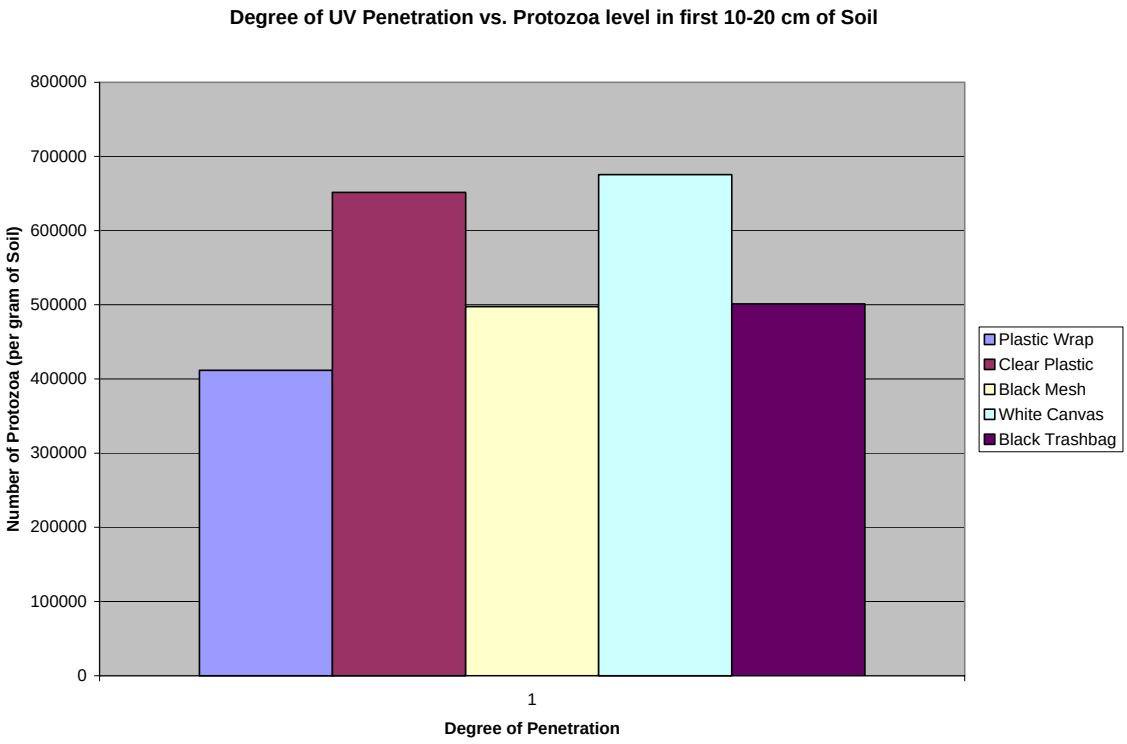
[(# per field of view at 40X) • (total ml of water used) • 747] (grams of sifted soil) = # of protozoa per gram of soil.

Analysis

Our data for the positive controls proves our hypothesis correct. The population density of protozoa in the 0 – 10 cm was 537,660 and in the 10 – 20cm were 815,269. We can be 89% sure that the difference in the levels was significant because of the p – value of .11. Thus the protozoa do migrate vertically when exposed to UV radiation.



The graph above shows the relation between the protozoa levels and exposure to the UV radiation in the first 10 cm of the soil. The protozoa densities follow a general trend of increasing as the exposure to UV rays decreases. However the trend is not consistent and when compared to the negative controls, the lowest p – value is 0.28. Therefore while the graph implies that there is a relationship between UV rays and protozoa levels in the soil, the relationship is not statistically significant.



The graph above shows the relation between the protozoa levels and exposure to the UV radiation in the second 10 – 20 cm of the soil. The protozoa densities do not follow any trend and are completely incoherent and therefore UV radiation can not be the reason for the decrease in the protozoa levels. The protozoa levels in the positive control were 815,269 and the controlled samples were 547,468. Thus the p value - 0.13 implies there was something else that affected the protozoa populations dramatically beside the UV radiation.

Discussion

Our experiment proved our hypothesis was wrong. We had two points we needed to prove in order to support our hypothesis. The first was that as the UV Radiation increased the overall protozoa populations would decrease. The second was that as the degree of UV penetration increased, the protozoa population in the 0-10 cm region would decrease and that the population in the 10-20 cm region would increase as the protozoa migrated vertically. Our results showed that in the 0-10 cm region the expected pattern for the first point was observed, but the student t-testing of the data provided p values ranging from .28 to .51. Hence the pattern is not statistically significant. Deeper into the soil from 10-20 cm, not only was there no significant difference but there was no pattern at all. In addition, in the 10-20 cm region it appears that something else dramatically affected the population beside UV Radiation because there was a 34.05% *decrease* from 815,269 before to 537,660 protozoa per gram of soil after ($p= 0.13$). The trend in the graph has no correlation to the expected decrease in protozoa levels. We can conclude that UV radiation did not directly affect protozoa levels.

None of the experimental data was what we expected. The first ten cm (0-10 cm) of soil had a population density of protozoa of 565,688 per gram of soil and the second set of ten cm of soil (10-20) had a population density of 547,468 per gram of soil. We expected the protozoa to vertically migrate so that the bottom set of 10 cm of soil would have significantly more protozoa than in the first ten cm of soil, but as seen above the numbers actually decreased. Our positive control plot had a total of 1,352,929 protozoa per gram of soil, while the experimental data had a total of 1,113,156 protozoa per gram of soil. There were 239,773 more protozoa per gram of soil before the experiment than

after. Along with this data and the graph of the conditions, the expected direct correlation between decreasing UV exposure and protozoa populations was not observed. Our results show that the direct result of the UV exposure is not causing these protozoa levels and something else is.

Considering other environmental factors of site 4, there was a strong smell similar to the smell of decaying matter (evidence for an increase in bacteria populations). Furthermore, from our 2004 biota survey, we found an unusually large amount of bacteria in site 4 (Microclimates Data, 2004). Also, there are more bacteria in the first fifteen cm of soil than anywhere else in the soil (Nardi, 2003). We therefore think a possible explanation for our results is that the protozoa migrated towards the top fifteen cm of soil because of this apparent increase in bacteria populations. As the protozoa reached the top layers in the soil, they experienced higher intensities of UV radiation in the sunlight killing them. Essentially, their death rate was faster than their reproduction rate. This explains the greater level of protozoa in the upper level than the lower levels of soil after the experiment, but also explains why there were 239,773 protozoa per gram of soil less in the experimental data than in the positive control data. The only decrease in the first 10 cm of soil of protozoa levels from the positive control to the experimental data was in degrees 1 and 2, which is expected because these two degrees allowed the most sunlight to pass.

For further research, we need to perform the experiment again including sampling bacteria populations as well as protozoa levels. We may also look back to the original biota survey for other differences or factors impacting protozoa in site 3 and 4 which may have resulted in our data, such as statistically higher pH levels.

References

Bartlett, E.; Edison, P.; Sorace, C. (2002) *The Effects of Ultraviolet Radiation of the Mold Population Density in Soil.* Retrieved July 9, 2004, from http://faculty.rpcs.org/brockda/ESSRE%20Research.htm

Environmental Protection Agency. (n.d.). *Ozone Science: The Facts Behind the Phaseout.* Retrieved July 9, 2004, from http://www.epa.gov/ozone/science/sc_fact.html

E.S.S.R.E Microclimate Databases, (2004). Retrieved July 9, 2004, from http://faculty/brockda/ESSRE%20Microclimate%20Survey.htm

Ingham, Elaine R. (n.d.) Soil Protozoa. In *The Soil Biology Primer* (chap.5). Retrieved June 9, 2004, from http://soils.usda.gov/sqi/soil_quality/soil_biology/protozoa.html

Nardi, James B. (2003) *The World Beneath Our Feet.* New York, NY: Oxford University Press

Northeast Midwest Insitute. (2001, April). *2.0 UV Treatment Technology Description.* Retrieved June 8, 2004, from http://www.nemw.org/Balsurv2_UV.htm

Nova.(n.d.). *Earths Suncreen-The Ozone Layer*. Retrieved June 12, 2004, from http://www.science.org.au/nova/004/004key.htm

Sanders, Bob. (n.d.). *Protozoa and UV Radiation.* Retrieved June 8, 2004, from Lehigh University, Website: http://www.lehigh.edu/~inuvlake/UVecology/Protist/protozoa.html.

Statistics Canada, (n.d.) *Case study: Ozone layer depletion and the Montréal Protocol.* Retrieved June 9, 2004, from www.statcan.ca/english/edu/power/ch5/case_study/ozone.htm