Background Report

Microorganisms are so small that they cannot be seen with the naked eye. Yet these tiny organisms greatly impact our lives. Also known as microbes, microorganisms include algae, bacteria, molds, viruses, yeasts, and protozoa. Microbes can be found in a variety of areas, some of which are impossible for other organisms to inhabit. One place they can be found is in soil. There, they impact plant diseases, control soil fertility, and cause agricultural crops to spoil or flourish (*World Book*, 2000). These are some examples of the relevance of microorganisms to our everyday lives. In our experiment we will examine the effects that aeration, a form of human interference with the environment, has on one type of microorganism known as protozoa.

Protozoa are one of the many microbes that inhabit the soil. They are animal-like, single celled organisms, varying in length from 2-70 micrometers, and they frequently form colonies. There are many different types of protozoa, such as zoomastigina, crytomonad, dinoflagellates, flagellates, foraminifera, and sarodina. The most well known species of protozoa are paramecium, euglena, and amoeba. Most species of protozoa are motile and move by means of their flagella or cilia. They are able to survive in virtually any environment except very acidic ones. Most species live in aquatic habitats or in moist areas like soil, and they can inhabit the interstices of sediment and beach sand.

Protozoa are very beneficial to the environment because of the role that they play in soil chemistry. Their principal importance is as consumers of bacteria and of waste products from other organisms. The three main functions of soil protozoa are: they release excess nitrogen through the bacteria that they eat, they mineralize nutrients, and

they regulate bacteria populations (Ingham, 2002). By consuming bacteria in soil, protozoa aid in the breakdown of soil. This helps soil maintain normal bacteria levels and regulate other conditions in the soil. Because protozoa play a role in controlling soil chemistry, they affect the growth of crops, and since humans eat these crops and the animals that feed off them, protozoa have a greater impact on us than many people know. The word 'protozoa' sometimes carries a negative connotation. Humans do not usually appreciate the role these microorganisms play in the breakdown and balance of soil and its inhabitants.

Protozoa maintain balance not only in soil but also in the food chain. They serve as food for earthworms, and earthworms serve as food for birds and other larger organisms. Thus, protozoa are not only engaged in the decomposition process, they are also the basis of the food chain.

Given the importance of protozoa in the environment, we would like to find out the best way to maintain healthy levels of them in the soil. In order to do so, it is important to understand how protozoa survive. Protozoa absorb oxygen through their cell membranes while at the same time releasing carbon dioxide into the soil. Because oxygen is vital to the survival of protozoa and other microbes, allowing more oxygen to flow into the soil could increase the population of protozoa in a plot of soil. "Soil organisms live best in soils that contain almost equal amounts of air and water" (World Book 2000, "Soil", Chicago).

Aeration is one way to increase the levels of oxygen and water in soil. Aeration, or "mechanical methods of selective tillage that modify the physical or other characteristics of a turf" (Filebox, n.d.), is a process that is used to maintain healthy soil

conditions. Many agriculturalists consider the process of aeration a necessity for farming because it allows for better gas exchange around the root areas of certain crops. When aeration is not utilized, soil can become too compact, making water infiltration almost impossible and therefore causing excessive runoff (Chesnut, et al, 1997). Another effect of reduced soil aeration, or compacted soil, is that there is more carbon dioxide and less macropores in the soil (Filebox, n.d.). These conditions are harmful to the microorganisms that inhabit and regulate soil. They need oxygen to live, and aeration helps to increase the level of oxygen in soil. The holes made by the aeration process allow for oxygen, water, and nutrient penetration. They also "[improve] rooting, [enhance] rainfall and irrigation infiltration, and [help] prevent runoff of fertilizer and pesticides in heavily compacted areas" (The Garden Link, 2002). The benefits of aeration include the release of toxic gases from the soil, increase of water movement, and internal drainage. A disadvantage of aeration is that it causes a temporary disruption of the soil surface, resulting in damage to plants and/or other life forms.

Earthworms are responsible for carrying out one natural method of aeration. Their tunnels create air pockets, thus allowing for more oxygen and water to be distributed throughout the soil. This process has been imitated through various methods of aerating soil, some of which include slicing, spiking, drill and fill, hydro injection, forking, and hollow tine. Different methods are used depending on the state of the soil and the reasons for aeration. Slicing is used for shallow depths and causes little surface disruption and desiccation, or soil drying. Spiking is the least effective method of aeration and uses small cores to upturn the soil. It is often used to aid in seeding (Filebox, n.d.). The 'drill and fill' method is used primarily for hard soils. The holes removed are

filled in with synthetic soil, but this method is very pricey. The hydro injection process infuses water into the soil at an immensely high rate. This is effective because it causes very little surface disruption and the least soil desiccation of all of the processes, but it must have a close water source. Forking uses a shattering motion to punch holes in the soil, and it is ideal for small, dryer plots of soil. The hollow tine method involves removing cores from the soil, thus encouraging the exchange of oxygen and water. For this method to work well, the soil cannot be too dry or too wet. Farmers and other biologists use different methods of aeration depending on their needs.

For our experiment, we chose to implement the forking and hollow tine methods, each on its own plot of soil, as well as monitor a plot that we did not manipulate at all. These two different types of aeration will affect the soil in different ways, therefore producing diverse results on the level of protozoa in the soil.

The hollow tine method of aeration actually takes chunks of soil out of the ground, resulting in much soil disruption and desiccation. A possible outcome of this method of aeration is a drop in protozoa levels. The desiccation of the soil would leave little water in the ground; therefore the protozoa could not swim to get prey as easily as they could in a moister environment. A decrease in water, however, could stimulate levels of bacteria, therefore causing an increase in protozoa, since protozoa live off bacteria.

Forking, the second type of aeration that we will use, employs a pitchfork-like blade (a screwdriver, in our case) to cut the surface of the soil at an angle. This method does not result in as much soil desiccation or surface disruption as the hollow tine method; however, it does compact the soil more than actually opening it up (Fagerness,

2001). Though the compacted area around the holes would not cause as much water evaporation as the hollow tine holes would, it would make it hard for the protozoa to move through the tight soil. Also, since the holes made by the screwdriver are smaller than those in the hollow tine method, not as much oxygen would be let into the forking plot as would be let into the hollow tine plot. Since the results of the forking method are more short-term than those of the hollow tine method, we are led to believe that the levels of protozoa in the forking plot would decrease, but not as drastically as they would in the hollow tine plot.

For our experiment, we will take three initial soil samples from each plot after aerating the soil. Three days after aerating the plots, we will take samples from each plot of soil. Two days after that, we will take samples again, and two days after that we will take samples a fourth time. Eventually, we will examine our soil samples under a microscope to determine whether more protozoa are in the aerated soil than in the non-aerated soil. We will also examine the differences among the population densities of protozoa caused by the no aeration method, hollow tine method, and forking method. We already know that protozoa play important roles in humans' lives. We want to find out whether humans can use certain methods of aeration to change soil conditions in order to alter the number of protozoa that thrive in soil.

Works Cited

Chesnut, Timothy, et al. (1997, February) *Soil Aeration Systems- Do they really improve tree root zone conditions under fill and paving?* http://www.clemson.edu/hort/sctop/dsec/dsec-05.htm

Fagerness, Matthew J. *Aerating Your Lawn.* Kansas State University. January 2001.

Filebox. (n.d.) *Aeration.* Filebox.vt.edu/cals/cropsci/vstma/docs/aeration.doc

Garden Link, The. (2002) *Tips and Techniques.* http://www.thegardenlink.com/html/tips.htm

Ingham, Elaine R. (2000) *The Soil Biology Primer: Soil Protozoa.* http://soils.usda.gov.sqi/SoilBiology/protozoa.htm

The world book encyclopedia. Microbiology. (2000). Chicago: World Book, Inc.

The world book encyclopedia. Soil. (2000). Chicago: World Book, Inc.

Experimental Outline

I. Problem: Will different methods of aerating soil: hollow tine, forking, or no aeration, cause an increase or decrease in the population density of protozoa in a plot of soil?

II. Hypothesis: The population density of the protozoa in the hollow tine plot will decrease the most, the no aeration plot will have the highest population density of protozoa and the forking plot will have a higher population density of protozoa than the hollow tine but lower than the no aeration.

III. Experiment

A) Variables

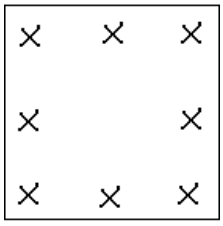
1. Experimental (Independent)
    a. IV_1: method of aeration
    b. IV_2: time elapsed from initial treatment
2. Dependent
    a. DV: population density of protozoa

B) Controls

1. Negative: no aeration
2. Positive: number of protozoa before treatment
3. Controlled Variables
    - Intervals between taking samples
    - Place where data is taken from
    - Depth at which soil samples are taken
    - Size of plot
    - Method of protozoa extraction
    - Size of soil sample
    - Method of collection of soil
    - Temperature at site of plots
    - Humidity at site of plots
    - Precipitation at site of plots
    - Slope of land at site of plots
    - Sunlight at site of plots
    - Drainage at site of plots
    - Plant density at site of plots
    - Human and animal interaction at site of plots

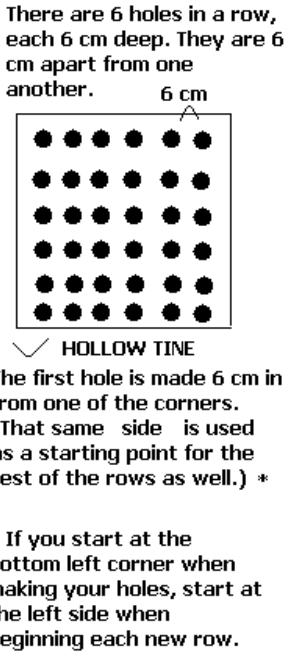
C) Procedure

1. Take three plastic 0.5 x 0.5 meter plots and place them in a row at GPS location N=39.35804, W=076.65598. Place a flag in each corner of each plot. Also place a flag in between the two corner flags on each side of each plot. See picture below.



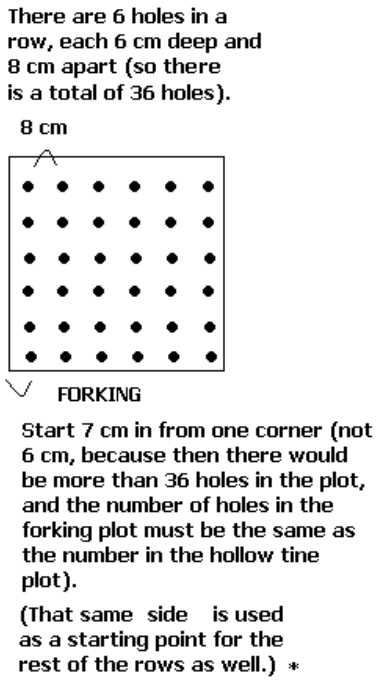
× stands for 1 flag

2. Label two of the flags in each plot with the plot's respective name ("Hollow tine," "Forking," or "No Aeration").
3. Use the three different methods of aeration to aerate each plot of soil, respectively. (For example, perform hollow tine method in the plot of soil labeled "Hollow tine.")
4. For hollow tine method: The soil core sampler is 2 cm wide, so begin making holes 6 cm in from one corner edge (start at the same side of the plot when beginning each new row).
5. Make 6 holes in a row, 6 cm deep and 6 cm apart from each other. Make 6 rows, each with 6 holes in it, following this design. There should be a total of 36 holes in this plot. See picture below.



6. Use the soil from three of the holes you just made in the hollow tine plot. These three soil samples will be the three random initial samples for the hollow tine plot.
7. Place each sample in its own Ziploc bag and label the bags "HT Day 0 Sample 1," "HT Day 0 Sample 2," and "HT Day 0 Sample 3," respectively.
8. For forking method: Start 7 cm in from one corner (not 6 cm, because then there would be more than 36 holes in this plot, and the number of holes in the Hollow tine plot must be the same as the number of holes in the Forking plot). See picture below step 9.
9. Make a mark at the point that is 6 cm from the tip of a Phillips head screwdriver with a circumference of 12.5 cm. Use the screwdriver to make 6 holes in a row, 6 cm deep and 8 cm apart from each other (the holes are 8 cm apart, not 6 as in the Hollow tine plot, so that there will be 36 holes in the Forking plot). Make the holes at a 45-degree angle

to the soil surface. All holes should face the same direction. See picture below.



10. Make 6 rows, each with 6 holes in it, following this design (start at the same side of the plot for each row). See picture above.
11. Use a 2 cm wide soil core sampler to take three random samples at depths of 6 cm from the forking plot of soil. Place each sample in its own Ziploc bag and label the bags "Forking Day 0 Sample 1," "Forking Day 0 Sample 2," and "Forking Day 0 Sample 3," respectively.
12. For the "no aeration" method, just leave the plot alone. Use a 2 cm wide soil core sampler to take three random samples at depths of 6 cm from the "no aeration" plot of soil. Place each sample in its own Ziploc bag and label the bags "NA Day 0 Sample 1," "NA Day 0 Sample 2," and "NA Day 0 Sample 3," respectively.
13. Wait three days.[1]
14. Use a 2 cm wide soil core sampler to take three random samples at depths of 6 cm from each of the three plots of soil. When taking samples, avoid the holes made by previous aeration and/or sampling.
15. Place each sample in its own Ziploc bag and label the bags with their respective labels, e.g. HT Day 3 Sample 1, Forking Day 3 Sample 1, NA Day 3 Sample 1.

[1] This is a source of error. We should have controlled for the intervals between taking samples. There is a three-day interval between the initial samples and the Day 3 samples. In step 16, there is a two-day interval between the Day 3 samples and the Day 5 samples. In step 18, there is a two-day interval between the Day 5 samples and the Day 7 samples. We should have made the intervals all equal. There was a weekend separating our initial samples and our Day 3 samples, so the interval was three days instead of two.

16. Wait two days.[1]
17. Repeat steps 14 and 15, but in step 15, substitute "Day 5" for "Day 3".
18. Wait two days.[1] (see footnote on above page)
19. Repeat steps 14 and 15, but in step 15, substitute "Day 7" for "Day 5".
20. Perform Brockmeyer Protozoa Extraction method on each sample from each plot and each day. Count protozoa. If you used 40X magnification, use the following formula:
    [(# per field of view at 40X) * (total ml of water used) * 747) / (grams of sifted soil) = # of protozoa per gram of soil
    If you used 100X magnification, use the following formula: [(# per field of view at 100X) * (total ml of water used) * 5102) / (grams of sifted soil) = # of protozoa per gram of soil
21. Record number of protozoa/g of soil for each sample.

IV. Data and Analysis

A) Data

Average Number of Protozoa Per Gram of Soil

No Aeration

	Sample 1	*Sample 2*	*Sample 3*
Day 0	841287	57732892	280715
Day 3	11041802	12056832	2824897
Day 5	2370626	331367	N/A*
Day 7	4685510	6830097	3690809

Forking

	Sample 1	*Sample 2*	*Sample 3*
Day 0	1809867	222591	2846379
Day 3	1365687	11904667	541121
Day 5	N/A*	211778	213451
Day 7	N/A*	3549918	257677

Hollow Tine

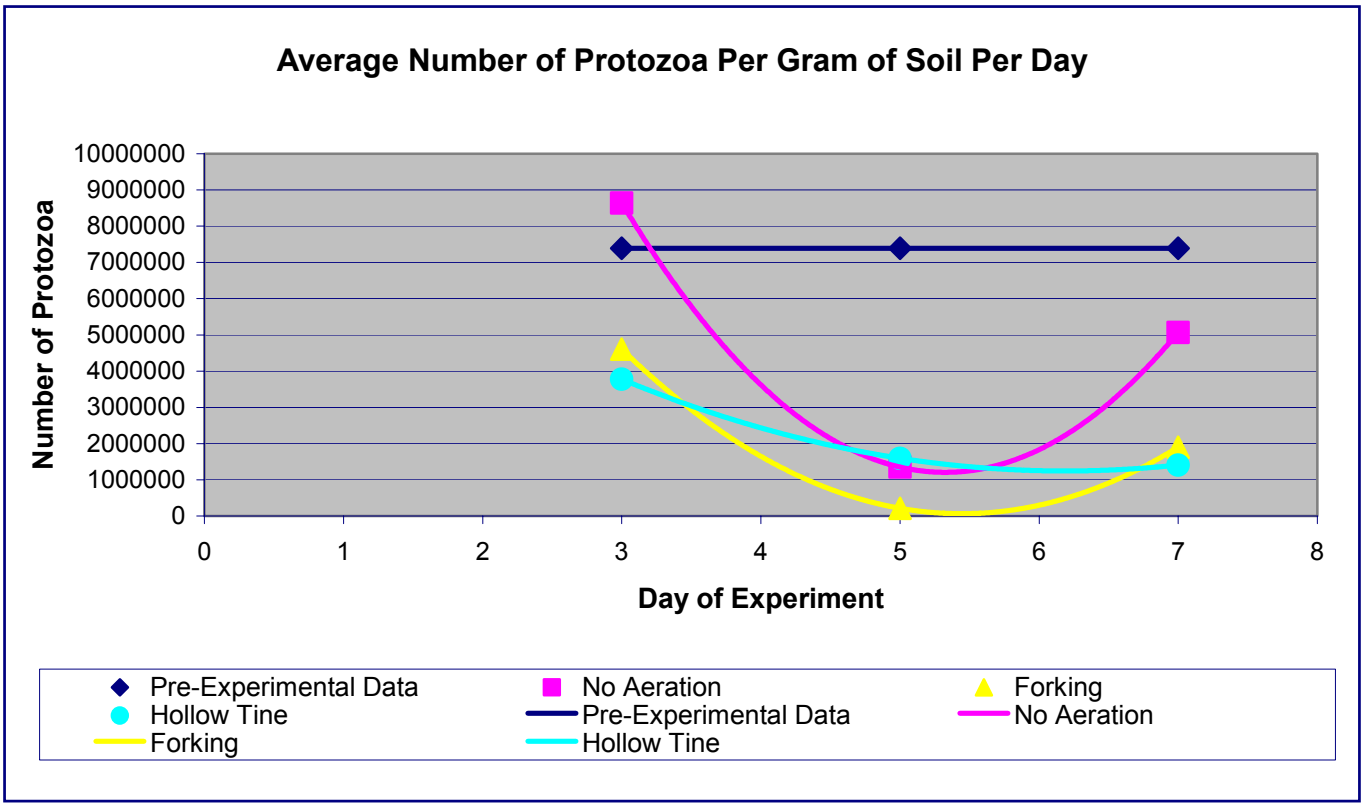
	Sample 1	*Sample 2*	*Sample 3*
Day 0	394485	1036858	1365687
Day 3	219386	6462533	4635688
Day 5	2657292	644463	1483745
Day 7	554226	2107348	1563516

*Lost Data

Average Number of Protozoa Per Gram of Soil Per Day

	Pre-Experimental	*No Aeration*	*Forking*	*Hollow Tine*
0	7392307	19618298	1626279	932343.33
3	7392307	8641177	4603825	3772535.7
5	7392307	1350996.5	212614.5	1595166.7
7	7392307	5068805.333	1903797.5	1408363.3

B) Analysis



V. Conclusion

Through the various trials of our experiment, our group confirmed our hypothesis was incorrect. We stated that the No Aeration plot would have the most protozoa, the Hollow Tine plot would have the least amount and the Forking plot would be somewhere in the middle. The No Aeration plot did have the highest amount of protozoa, however, the Forking and Hollow Tine plots were so close in data that we cannot say definitely whether or not one was higher than the other. The average number of protozoa of all of our pre-experimental data was 7,392,307 protozoa per gram of soil. Over the course of the first three days of the experiment, the No Aeration of the soil proved beneficial to the protozoa levels because there was an average of 8,641,177 protozoa per gram of soil on Day 3 of our experiment, 1,248,870 protozoa per gram of soil above the pre-experimental

data. From Day 3 to Day 5 the amount of protozoa in the No Aeration plot decreased again by an average of 7,290,181 protozoa per gram of soil, and then increased by an average of 3,717,809 protozoa per gram of soil from Day 5 to Day 7 to end at an average of 5,068,805 protozoa per gram of soil. On the other hand, the first three days of the experiment proved beneficial neither to the Forking plot, nor to the Hollow Tine plot. On Day 3, the Forking plot had an average 4,603,825 protozoa per gram of soil, which was 2,788,482 protozoa per gram of soil below the pre-experimental data. From Day 3 to Day 5, the average number of protozoa per gram of soil decreased 4,391,211, but then increased again an average of 1,691,183 protozoa per gram of soil from Day 5 to Day 7, thus ending at an average of 1,903,797 protozoa per gram of soil on Day 7. The Hollow Tine plot followed a similar pattern, starting at 932,343 protozoa per gram of soil on Day 3, and decreasing an average of 2,177,369 protozoa per gram of soil per day from Day 3 to Day 5. The number of protozoa continued to decrease from Day 5 to Day 7 by an average of 186,803 protozoa per gram of soil, ending on Day 7 at 1,408,363 protozoa per gram of soil.

These patterns of change over time show that, in general, aerating the soil is harmful to the amount of protozoa. On Day 3 and Day 7, the levels of protozoa per gram of soil were significantly larger than either that of Forking or Hollow Tine. The question is whether or not there is a considerable enough difference in the levels of protozoa in the Forking and Hollow Tine plots to conclude that one of them was more harmful than the other in regard to protozoa levels. Looking at the data, and the similar patterns in both plots, our group concluded that there was not substantial enough difference in the amounts of protozoa to confidently say that the difference was significant, and not just the result of some counting problem or other source of error. On Day 5, the levels of protozoa in all three plots were generally the same, but considerably lower than the amounts on Day 3 or Day 7. This shows that some factor influenced a change in all three plots. Looking back at conditions on that particular day, we noticed that it rained an extensive amount, enough to make a big difference in our data and the levels of protozoa. This goes to show that different weather conditions are also a factor in determining whether or not aeration is a good idea. For instance, on Day 3 and Day 7, when the weather was clear, no aeration was obviously the best method in obtaining high levels of protozoa. However, on Day 5 when it was raining, aerating the soil actually proved helpful in maintaining protozoa levels. Between the two aeration methods, Forking is better when the weather is clear and sunny because there is less surface desiccation and therefore less water is evaporated out of the soil. On rainy days, Hollow Tine aeration is best because bigger holes in the soil lead to more water infiltration. Water is important to protozoa levels because they “swim” through the soil and therefore more water would make it easier to move and catch prey.

Though careful in our experimentation, our group nonetheless encountered many sources of error over the course of our research. During the Uhlig extraction phase, we misplaced three of our Uhlig extractors and therefore, because they sat out too long, the data was lost. Those Uhlig extractors contained data for No Aeration: Day 5, Sample 3, Forking: Day 5, Sample 1, and Forking: Day 7, Sample 1. Although it led to interesting results, the rain during the last portion of our experiment did not make our data easier to interpret. The rain made the dirt wet so that it filled in some of our aeration holes, partially defeating the purpose of aerating in the first place. Another uncontrollable

circumstance was the fact that the refrigerator that we kept our protozoa petri dishes in went off during the power outage. Even though we sealed the petri dishes quickly, there was a short amount of time when the petri dishes were not being kept cold and the protozoa could have reproduced. One last thing we could have done to better our experiment would have been to have repetitions of plots. Had we had different plots set up all over campus with the aeration methods, we would have been certain that our data was not just due to its location.

After our research, our group still had many unanswered questions about the levels of protozoa and what exactly causes them. Though we did have time, repetition of plots would have given us a much deeper insight into what aeration actually does to the levels of protozoa. The same goes for the duration of the experiment; if we had had more time our data may have been different. To also further our research, we could have tested for nitrogen levels or bacteria levels. Since protozoa eat bacteria, and release excess nitrogen in the form of ammonium from the bacteria they consume, both would prove that certain levels of protozoa were not due to extra stimuli or causes. The rain, though difficult at first, led to interesting problems related to protozoa levels. As a second experiment, it would be fascinating to see what different levels of moisture do to the levels of protozoa, and if there is a point where there is too much water in the soil for the protozoa to live. By performing this experiment, we learned a lot about how human interaction affects things that we would not even think about. Though great for agricultural needs, aeration has a deeper consequence on protozoa that are essential for life that farmers should take into consideration.