Aeration's Affect on Protozoa

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Background Report

Protozoa, microscopic single-celled organisms, play a very important role in the soil. They feed on bacteria in the soil, and because the bacteria contain too much nitrogen for the protozoa to use for their own needs, the excess nitrogen is released into the soil, allowing plants and other organisms that live there to use it **(NRCS, n.d).**

The cycle that follows nitrogen in and out of the soil is known as the nitrogen cycle. The movement of nitrogen in this cycle is important because it allows the nitrogen to be recycled, so the plants and animals can always get it, ultimately allowing the plants and animals to thrive. In the nitrogen cycle, nitrogen in the soil goes through denitrification and becomes part of the nitrogen in the atmosphere (nitrogen makes up about 79% of the atmosphere). The atmospheric nitrogen then is put back into the soil either through atmospheric fixation, biological fixation, or industrial fixation. Instead of going through denitrification, some of the nitrogen in the soil is used by plants to build proteins and nucleic acid (which are made up of nitrogen); the plants with the nitrogen are then eaten animals and the nitrogen is once again put back into the soil through the waste of the animals **(University of Minnesota Extension, 2002).**

Plants consume and use two different forms of nitrogen: ammonium ($NH_4^{+)}$ and nitrate (NO_3^-). Ammonium, which is positively charged, is attracted to clay and other things found in soil, which have negative charges. The charge binds it to the clay particles in the soil, and from there plants can consume it and use it in order to grow. Nitrate, on the other hand, has a negative charge, making it repel clay and other particles in the soil. This allows the nitrate to flow with water as it drains through the soil. As the roots of a plant take up this water, the nitrate in the water is consumed by the plant as well **(University of Nebraska Cooperative Extension, 1996-2001)**.

Protein and Nucleic acid, two important biological molecules, are composed of nitrogen. Proteins in particular are important because as enzymes, they are in charge of starting and stopping chemical reactions between the biological molecules in a cell. The chemical reactions caused by protein enzymes make or break chemical bonds using energy to make a new substance, allowing the four tasks (homeostasis, reproduction, transform energy, and synthesis) to occur; ultimately making up a cell. So, without protein to start the chemical reactions, the cells plants and the organisms that eat them could not run. Since cells are the "building blocks" of organisms, organisms would not be able to exist without the nitrogen that makes up proteins and nucleic acids. If the nitrogen supply used by plants and animals were to be cut off or relocated to a place unreachable to other organisms, there would be a ripple effect on the entire food chain. For example, if the protozoa in a certain soil area relocated deep into the soil (where there are no bacteria), then the protozoa would not have bacteria to eat, so nitrogen would not be released. Then, the plants that use that nitrogen source in order to survive would die. As a result, the deer and other animals that eat the plants will not have anything to eat and they will die. Then, the animals that ate the deer will no longer have deer to eat, so they will starve, and so on.

The bacteria eaten by protozoa need both oxygen (O_2) and water (H_2O) in order to survive **(Jim Deacon, n.d).** Aeration effects the concentration of both these compounds in the soil. Although it was not designed to help the health of plants, it also has an effect on both protozoa and bacteria. Many people aerate their lawn in order to get air into the soil and lessen the compaction of the soil. To aerate, one makes many small holes (about 15 cm deep) into the soil, in order to get air into the soil. This oxygen is helpful ~~in order~~ to lessen the compaction of the soil and it is also a necessity of bacteria **(Master Garden Products, 2001).** Although it does

help to provide oxygen for bacteria, too much air causes the water in the soil to evaporate, making it harder for protozoa to get the water they need in order to survive. So, while aeration is useful in providing oxygen to the bacteria, it hurts the water supply for the protozoa. If the protozoa are not present because of lack of H_20, then the protozoa will not be there to eat the bacteria to release nitrogen, and the ripple effect will occur. Lack of water also greatly affects protozoa. Since protozoa prefer to live in water rather than dry soil, if the water in the soil relocates to a different area, the protozoa will most likely travel with it **(MCWDN, n.d).** Thus, if aeration causes the water to relocate to deeper soil, protozoa will also travel to the deep soil, away from the majority of bacteria, causing a potential serious problem.

In our experiment, we are testing if aeration changes the location (depth) of the majority of protozoa. Since aeration decreases the water found in the soil towards the surface, our group hypothesized that, after aeration, the protozoa would move deeper into the soil, where there is more moisture. If the Protozoa do in fact move downward into the soil, the nitrogen released might not be able to be used by the plants. The Protozoa could also relocate deeper into the soil, making them unable to even find enough bacteria to eat. If the protozoa are unable to reach bacteria, then the nitrogen would not be able to be produced, and it is possible that the uneaten bacteria could build up at the surface of the soil, which could become harmful.

Work Cited:

1. Deacon, Jim. (n.d) "The Microbe World: The Nitrogen Cycle and Nitrogen Fixation" http://www.biology.ed.ac.uk/research/groups/jdeacon/microbes/nitrogen.htm
2. Master Garden Products. (2001) "Aerating Your Lawn" http://www.mastergardenproducts.com/gardenerscorner/aerating_your_lawn.htm
3. MCWDN. (n.d) "Protozoa" http://www.mcwdn.org/Animals/PROTOZOA.html
4. NRCS. (n.d) "Soil Biology" http://soils.usda.gov/SQI/concepts/soil_biology/protozoa.html

5. University of Minnesota Extension. (2002) "Understanding Nitrogen in Soils" http://www.extension.umn.edu/distribution/cropsystems/DC3770.html

6. University of Nebraska Cooperative Extension. (1996-2001) "Nitrogen Sources" http://lancaster.unl.edu/ag/factsheets/288.htm

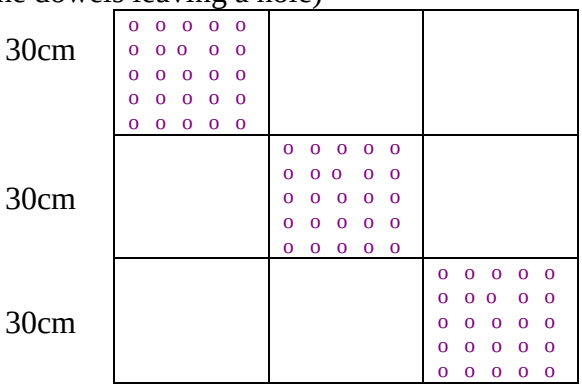
Outline of Experiment

Question: How does aeration alter the population density of protozoa in different depths of the soil?
Hypothesis: After aeration, the population of protozoa will be greater in deeper soil compared to shallow soil.
Independent Variable: Presence of aeration
Dependant Variable: number of protozoa per gram of soil at different depths
Negative Control: plot without aeration/ plot with sticks in it
Controlled Variables: size of dowel, area of plot, depth of dowel in the soil, location, how close the dowels are to each other, amount of soil taken for sample, the day and time we take our samples, what we use to take the samples from the soil, what we use to get the soil out, what we use to grind the hardened soil, what we put the soil in, how we label the petri dishes and cups, how we aerate the plots that get aerated, the Uhlig extractor method, the counting method of the slides, the magnification of microscope, how much water used to rehydrate, the 5mm of water we add in just case in there wasn't enough water to rehydrate the soil already
Step-by-Step

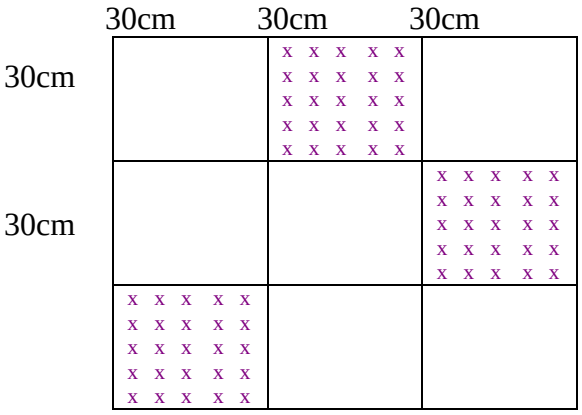
1. Get ten dowels
2. With a red sharpie, make a marking on each dowel every 15 cm
3. With a saw, cut the dowels on the red marks, until you reach 75 dowel pieces, each 15cm long
4. On each piece, mark the 7.5 cm mark in blue sharpie
5. In the front lawn, at location N 39◦ 21.484, W 076◦ 38.178 place 9 plots (using 16 flags, each 30 cm x 30 cm) so it looks like this…

	30cm	30cm	30cm
30cm	1	2	3
30cm	4	5	6
30cm	7	8	9

6. Using a soil test core sampler, take two samples from each plot (take 15 cm deep, 2.5 cm in diameter) and put the top half in a labeled plastic bag and the bottom half in a different plastic bag, all on the same day at the same time for accuracy and avoid change in weather, which could affect the process.
7. Aerate plots 1, 5, and 9 (aerate by placing the dowels 7.5 cm into the ground- 6 cm away from other dowels – and take out the dowels leaving a hole)



8. in plots 2, 6, and 7: put 25 dowels in the ground (7.5 cm deep and 6 cm away from each other) and leave them in the ground for the whole time



9. At least two days after aerating, take two samples from each plot again on the same day at the same time for accuracy(take 15 cm deep)
10. For the samples taken in step 6:
    - Place each sample of soil into the bottom of its own clean, empty petri dish; and allow them to dry completely for 24hrs. Make sure this happens at the same time (to make sure they all dry for the same amount of time)
    - Using a 1 mm^2 nylon screen or mesh, sift anywhere from 9g to 10g of each soil sample into its own second clean petri dish.
    - Add 20 ml of distilled water to saturate the soil into each sifted sample
    - Cover the petri dishes with lids and allow to sit for 7 hours.
    - Place each soil sample in its own modified Uhlig extractor containing 30 ml of distilled water for 24 hours.
    - Remove the filtrate from each sample and filter a 2nd time using 12.5 cm qualitative filter paper.

- Using a capillary tube, deposit 7 µl of methyl-green stain on a clean microscope slide (1 µl = 1 drop from the capillary tube). Then using a disposable graduated Beral-type pipette, add 18 µl (the first demarcation on the pipette) of the 2nd filtrate from one of the soil samples; repeat step 6 (6^{th} bullet point) to the stain on the microscope slide and cover with an 18 x 18 mm^2 cover slip.
- Examine each slide under a light microscope at 60X and count the number of protozoa in four fields of view.
- For each sample use the average of the four fields of view in the following equation to determine the population density of protozoa in the soil sample: [(# per field of view at 40X) • (total ml of water used) • 2165] (grams of sifted soil) = # of protozoa per gram of soil.

11. Repeat step 10 with all samples collected in step 9
12. fill in the data collected in steps 10-11 into a data table

Data Tables

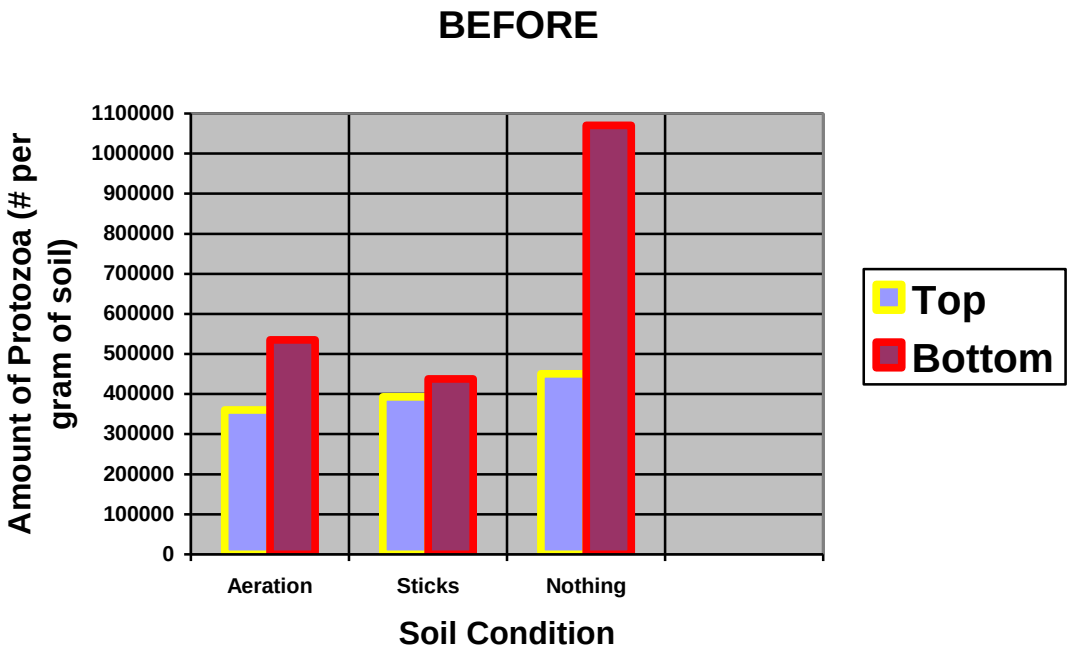
BEFORE:

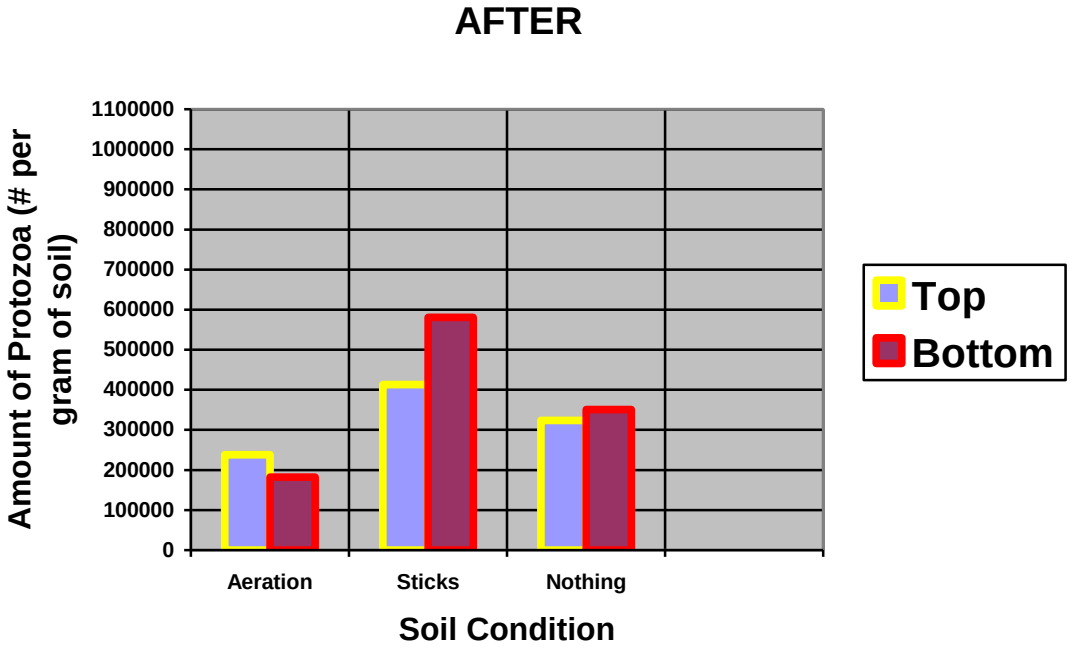
Soil Condition	Soil Depth	Amount of Protozoa (# per gram of soil) Trial 1	Trial 2	Trial 3	Average
Aeration	Top 7.5 cm	227,325	403,059	451,042	360,475
	Bottom 7.5 cm	553,146	732,601	320,632	535,459
Sticks	Top 7.5 cm	114,721	44,639	1,018,986	392,782
	Bottom 7.5 cm	330,103	349,899	634,277	438,093
Nothing	Top 7.5 cm	558,342	46,559	746,355	450,419
	Bottom 7.5 cm	403,231	1,223,695	1,584,660	1,070,529

AFTER:

Soil Condition	Soil Depth	Amount of Protozoa (# per gram of soil) Trial 1	Trial 2	Trial 3	Average
Aeration	Top 7.5 cm	319,830	143,597	250,892	238,106
	Bottom 7.5 cm	124,488	47,065	373,853	181,802
Sticks	Top 7.5 cm	850,145	65,606	322,563	412,771
	Bottom 7.5 cm	445,222	349,557	947,188	580,656
Nothing	Top 7.5 cm	401,837	142,147	426,439	323,474
	Bottom 7.5 cm	372,800	121,505	557,652	350,652

Graphs


BEFORE
Amount of Protozoa (# per gram of soil)
1100000
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
Aeration
Sticks
Nothing
Soil Condition
Top
Bottom


AFTER
Amount of Protozoa (# per gram of soil)
1100000
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
Aeration
Sticks
Nothing
Soil Condition
Top
Bottom

Conclusion

Our group hypothesized that aeration would cause protozoa to move deeper into the soil. In our research, we learned that protozoa prefer to live in water, which prompted this hypothesis. We predicted that aeration would cause the soil toward the surface to dry up, so we assumed the protozoa would relocate deeper into the soil, where there is more moisture. After setting up nine plots (3 aerated, 3 with sticks, and 3 with nothing done to them) and testing for protozoa, it turns out that our hypothesis was incorrect. Our results show that aeration actually causes protozoa to move toward the surface of the soil, instead of moving deeper into it. Before doing anything to the plots (aeration, etc), we tested to see the number of protozoa in the top 7.5 cm of the soil and the amount in the bottom 7.5. We found that all three of the different "sets" of plots had more protozoa in the bottom 7.5 cm than in the top 7.5 cm. Then, looking at our data after aerating and putting in sticks; the plots with sticks and the plots without anything both remained the same as the before samples (there were more protozoa in the bottom 7.5 cm). The plots with aeration, however, had different results in the after samples than in the before samples. After aerating, there were more protozoa in the top 7.5 cm of soil than in the bottom 7.5 cm. Obviously, this argued against our hypothesis. Our group wondered why this might have happened. We then realized that even though there is more moisture deep in the soil, the protozoa go to the top of the soil in order to eat the bacteria. The bacteria are located towards the surface because oxygen is more plentiful there. Because the protozoa are at the top of the soil, that means that the bacteria are being eaten and nitrogen is being released for plants and other organisms to use. So, instead of aeration causing major problems, it actually helps bacteria, protozoa, plants, and ultimately the entire nitrogen cycle.