

Soil Ecology

Project: Erosion

and Bacteria

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"We have acted honorably on this assignment."

Amber Bustard

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Background

The earth is shaped in many different ways. One of them is the process of erosion, where the forces of water, wind, ice, and people can cause rocks and other surfaces to break into fragments and be carried away by rain and wind into lakes and streams, causing these ecosystems to be disrupted. Along the way, erosion also has a negative impact on the soil it is dislodging, including loss of arable land, desertification, and flooding. These three impacts are interrelated because as erosion carries away the topsoil, it decreases the amount of plant life that can survive in the remaining soil, which leads to desertification (World Wildlife Fund, 2016). Through desertification, plant roots are destroyed, causing a loss of plant life in the area, and without plants, fresh plant residue, or plant roots, the remaining dirt ceases to be well-aerated, leading to flooding because the soil can no longer absorb excess water like it formally could (World Wildlife Fund 2016).

Today, in addition to natural forces, human activity also contributes to the problem of erosion, causing 10 times more than the natural forces do (Landscape Planet, 2006). When humans cut down forests, build on hills, and plow fields, they loosen the soil in these locations, making it more susceptible to erosion, damaging the soil, and anything that lives there (Landscape Planet, 2006).

One of the organisms harmed by erosion are bacteria. Soil contains many different types of bacteria, the most common microbe living in it, and there are at least 1 million of them in every teaspoon's worth (Ingham, E. 2000). But all of the varieties of bacteria contribute something critical to plant life and the rest of the soil ecosystem.

These essential tasks include: converting energy into organic forms, transforming organic matter into plant nutrients, and creating a material that helps improve infiltration and the soil's water-capacity (Ingham, E. 2000). All of these bacteria live near fresh young plant residue where they receive the carbon they need from the plants in order to perform their own essential tasks and, in turn, provide nutrients from the soil to the plants. (World Wildlife Fund 2016).

Several kinds of bacteria that are particularly helpful for the soil are those involved in the nitrogen cycle. First, Nitrogen Fixation Bacteria convert nitrogen gas from the atmosphere into nitrogen compounds that the plants can use by changing the atmospheric nitrogen into ammonium (which can also result from bacterial decomposition) (Harrison, J.A., 2003). Then comes nitrification, where other groups of bacteria convert the ammonium into nitrates (the other form that plants can absorb), and then finally, through a process known as denitrification, any excess nitrate in the soil gets put back in the air by special bacteria that convert it back into nitrogen gas (Harrison, J.A., 2003.).

Once plants assimilate either the ammonium or the nitrate through their roots, they use these forms of nitrogen to create their amino acids and nucleotides (Harrison, J.A., 2003). Amino acids are the monomers of proteins and the nucleotides are the monomers of the nucleic acids, DNA, and RNA. DNA and RNA are responsible for creating the proteins known as enzymes, and enzymes are responsible for starting and stopping the chemical reactions within a cell. Therefore, when erosion causes bacteria to be carried away, there is less nitrogen available for the plants to make their enzymes and nucleic acids, and without these molecules, the cells of a plant do not function properly

since there is no control of the chemical reactions within the cell. Hence, no nitrogen, no cell, meaning no living plants, and if there are no plants, the consumers are not able to consume their primary source of energy, killing them off, etc. In short, no organically available nitrogen results in there being no ecosystem at all.

We are conducting an experiment to determine how the density of the bacteria is affected by erosion on the Roland Park Hill. We hypothesized that the density of the bacteria would increase as you descended down the hill. We determined this hypothesis because we thought that the topsoil containing the bacteria would be pushed from the top down to the bottom of the hill due to the forces of erosion. This means that the soil would be concentrated at the bottom of the hill; therefore we believe there will be more bacteria found at the bottom of the hill, than the middle or top. The negative control in our experiment is a flat area of ground where there is no runoff, but which has the same type of plants on it as the hillside being tested. We will compare the density of the bacteria in this area to the density of the bacteria on the eroded hill to determine how the effect of erosion on the population of bacteria.

Lab Outline

Question: Does the density of the bacteria in the top 15 cm of the soil increase or decrease as you move down the hill from a source of erosion?

Hypothesis: The density of the bacteria in the top 15 cm of the soil will increase as you descend down the hill from the source of the erosion.

Procedure

Independent Variable: Distance down the hill from the source of the erosion.

Dependent Variable: Density of bacteria found in the soil samples

Negative Control: A flat area of ground where there is no runoff, but which has the same type of plants on it as the hillside being tested.

Controlled Variables:

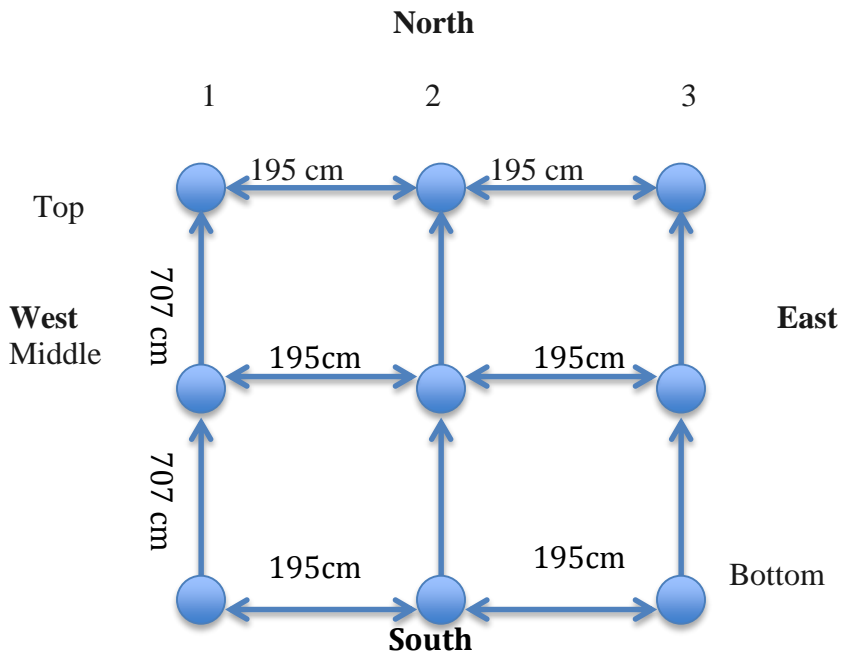
- Amount of soil collected and tested
- Increments of distance between where the soil is extracted
- Degree soil is diluted in the culture tubes
- Size of serological pipette
- Amount of sterile water added to culture tube
- Size of culture tubes to extract bacteria from soil
- Location of experiment
- Amount of time bacteria is left to grow
- Type of nutrient agar on the plates
- Amount of nutrient agar in the plates
- Equation used to calculate
- Labels on tubes
- Force of shaking the tubes
- Same day and time soil is extracted and combined
- Same day and time soils are diluted and plated
- Amount of dilution placed on plate (100 microliters)
- The dilutions being plated (10^{-1} and 10^{-2})
- Size of soil extractor
- Amount of soil diluted per flag

Step by step instructions:

1. Go to the site that is at N. $39^{\circ} 21.434$, W. $076^{\circ} 38.224$, to the top of hill in the back of RPCS.
2. Insert the yellow flag in the ground, and label this “source of erosion 1” (see diagram below).
3. Move East 195 cm and place down another yellow flag, labeled “source of erosion 2” (see diagram below).
4. Move East 195 cm from this point and insert another yellow flag, and label this “source of erosion 3” (see diagram below).
5. Go to the flag that is labeled “source of erosion 1”, and use a tape measure-to-measure South 707cm (see diagram below).
6. Insert a yellow flag in this location and label it “middle of hill 1”.
7. Move East 195 cm from this point and label this “middle of hill 2” (see diagram

- below).
8. Move East 195 cm from this point and label this “middle of hill 3” (see diagram below).
 9. Go to the flag that is labeled “middle of hill 1”, and use a tape measure-to-measure South 707cm (see diagram below).
 10. Insert a yellow flag in this location and label it “bottom of hill 1”.
 11. Move East 195 cm and insert another yellow flag, labeled “bottom of hill 2” (see diagram below).
 12. Move East 195 and insert another yellow flag, labeled “bottom of hill 3” (see diagram below).
 13. Go to an area with flat ground where no runoff has occurred, and have similar land, N. 39° 21.405, W. 076° 38.138, and insert and label the yellow flag, “Negative Control 1.”
 14. Move East 195 cm from the recent location, insert and label a yellow flag “Negative Control 2”
 15. Move East 195 cm from the recent location, insert and label a yellow flag “Negative Control 3”

Diagram 1



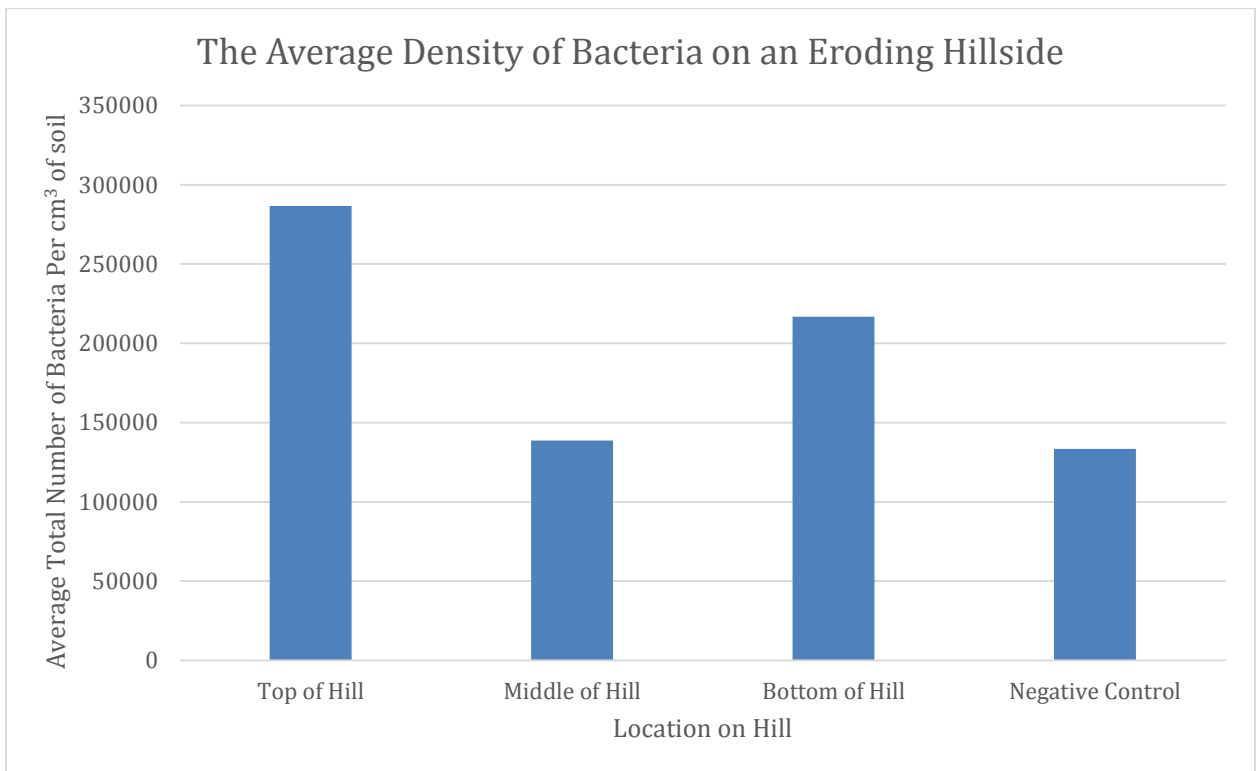
16. Gather 36 bags, and label each bag, according to its trial and location.
17. Using the soil core and mallet, extract 3 soil samples that are 2 centimeters in diameter, and 15 centimeters deep into the soil, from each flag location. All samples should be taken on the same day, at the same time.

18. Combine the three samples per flag site into one bag per the three trials, on the same day and at the same time.
19. On the same day, at the same time, do steps 18 through 34.
20. Use a clean, new transfer pipette to add 10ml of sterile water to a 15ml culture tube. Label this tube “10⁰ TH”.
21. Use the same pipette to add 9ml of sterile water to a second 15 ml culture tube. Label the tube “10⁻¹ TH”.
22. Use the same pipette in to add 9ml of sterile water to a third 15ml culture tube. Label the tube “10⁻² TH”.
23. Place 1 cc of soil from the “Trial 1 Top of Hill” bag into the “10⁰ TH” culture tube.
24. Cap the tube and shake vigorously.
25. Using a new clean pipette, remove 1 ml of the soil/sterile water mixture from the “10⁰ TH” tube and place into the “10⁻¹ TH” tube.
26. Cap the tube and shake vigorously.
27. Using the same pipette in step 17, remove 1 ml of the soil/ sterile water mixture from the “10⁻¹ TH” tube and place into the “10⁻² TH” tube.
28. Cap the tube and shake vigorously.
29. You should now have a total of three culture tubes.
30. Plate 100 microliters samples from both the “10⁻¹ TH” and “10⁻² TH” tubes onto their own separate, “3M Petrifilm™ Aerobic Count petri plates” labeled the same as their corresponding culture tubes
31. Repeat steps 20-30 two more times using the “Trial 2 Top of Hill” and “Trial 3 Top of Hill” soil samples.
32. Repeat steps 20-30, using the “Middle of Hill” soil samples, and label the culture tubes “MH”, versus “TH.”
33. Repeat steps 20-30, using the “Bottom of Hill” soil samples, and label the culture tubes “BH”, versus “TH.”
34. Repeat steps 20-30, using the “Negative Control” soil samples, and label the culture tubes “NC”, versus “TH.”
35. Allow it to grow for 48 to 72 hours.
36. Examine the plate with the lowest dilution per the four locations, and three trials, and if it has at least 5 bacteria colonies, use this plate to make your estimates of the number of bacteria in the original 1 cc soil sample from the “Trial 1 Top of Hill”, using the following formula “#Microbes in 1cc of soil + #colonies on sheet x 10² x 10⁻²”.
37. If the lowest dilution does not have at least 5 bacteria colonies, use the 10⁻¹ dilution plate and use the following formula to calculate the number of bacteria “#Microbes in 1cc of soil + #colonies on sheet x 10² x 10⁻¹”.
38. Record data into data table.

Data and Observations

Location on Eroded Hill	Trial 1	Trial 2	Trial 3	Average
Total density of bacteria in 1 CC of soil (cm ³)				
Top of the Hill	250,000	550,000	60,000	286,667
Middle of the hill	27,000	19,000	370,000	138,667
Bottom of the hill	490,000	80,000	80,000	216,667
Negative Control	210,000	70,000	120,000	133,333

The Effect of Erosion on the Density of Soil Bacteria



Conclusion

In our experiment, we hypothesized that “the density of the bacteria in the top 15 cm of the soil will increase as you descend down the hill from the source of the erosion.” This hypothesis was proven invalid. We predicted that the erosion would cause the amount of bacteria to decrease when descending down the hill, since the erosion would cause the top soil to be carried down to the bottom of the hill, where we predicted the density of bacteria would be the greatest. Our assumption was disproven by the graph of our data, as it shows the top of the hill (source of the erosion), contains the largest density of bacteria compared to the three other sites. The top of the hill bacteria density average was 286,667 bacteria colonies per 1 cc of soil. The middle of the hill bacteria density average was 138,667 bacteria colonies per 1 cc of soil. The bottom of the hill bacteria density average was 216,667 bacteria colonies per 1 cc of soil. The negative control bacteria density average was 133,333 bacteria colonies per 1 cc of soil. The data suggests, that the bacteria in the middle of the hill was carried away from its original site, for it is less dense compared to the other locations on the hill. To further explain why the top of the hill had the densest bacteria population, there is a slanted road at the top of the hill. Whenever rain falls the bacteria would be pushed across the slanted road to the top of the hill, causing there to be the most rainfall condensed in this area. Bacteria need water to survive, so therefore it makes sense this area had the greatest bacteria density. For the negative control the land is flat, meaning the only rain this area gets is if the rain hits directly, causing there to be less rain fall in this area. Since there is less water, less

soil bacteria life can be supported by the amount of water in the soil of the negative control. As the data shows the top of the hill has the most dense bacteria population, the bottom of the hill has the 2nd most dense bacteria population. The bottom of the hill would have the 2nd most dense bacteria population because the rain is causing the top soil of the hill to be carried away by the erosion to the bottom of the hill, as well as being carried onto the road that is at the bottom of the hill. The difference between the top of the hill and the bottom of the hill, is that at the top of the hill, extra soil is being carried from the road above to the top of the hill, while for the bottom of the hill, some of the bacteria will be carried onto the road versus just staying in the bottom of the hill.

Future researchers could compare the effect of the different types of erosion on the density of the bacteria on the same sites. In our experiment the same type of erosion occurred, which was wind, and water moving the topsoil down the hill. To test this, these researchers could try different areas where different types of erosion can occur. For instance, one test could be where glaciers are presence to test the effect of ice erosion on the density of bacteria. Another example could be the beach, where water erosion, washes up the sand on the beach. Besides the different types of erosion on the density of bacteria, researchers could test how the different temperatures of the seasons, played a role in the density of bacteria found in the soil. We would follow the same steps that we performed in our original experiment, but we could see how dense the bacteria is when the weather is 30°C during the summer months, or when the weather is -0°C during the winter months. Weather is known to be an environmental factor in the density of bacteria in the soil, as each kind of soil bacteria has a temperature that it survives best in (Acosta-Martinez, V. Van Pelt, S. Moore-Kucera, J. Baddock, M.C. Zobeck, T.M. 2015). One

more experiment, future researchers could conduct is the same steps of the current experiment, but avoid choosing a hill that has other factors contributing to the density of the bacteria in the areas of the hill. In our experiment the roads was a factor in the density of the bacteria per the top of hill and bottom of hill areas, so with just a hill and erosion minus any other factors, our results may have proved our experiment valid versus invalid. From conducting these other experiments, research scientists could find ways to prevent erosion, and other environmental factors from taking one of the most important microbial creatures, bacteria out of the soil.

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