The Impact of Deforestation on Soil Bacteria

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We have completed this assignment honorably:

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I. Background:

Deforestation is the removal of all plant material from an ecosystem, and in today's world, anywhere from 119,000 to 150,000 square kilometers of forest are burned, cut down, or degraded every year (World Wildlife Fund, 2016). The consequences for the larger environment are severe, and deforestation can lead to increased greenhouse gas emissions, the disruption of water cycles, increased soil erosion, and the collapse of people's livelihoods.

The first of these problems, an increase in greenhouse gas emissions, occurs because the trees and other plants that have been removed normally absorb excess carbon dioxide out of the atmosphere. But when they are cut down, they can no longer perform this function, and in fact the carbon dioxide which the plants had already absorbed is actually released back into the air because of their destruction. Since tropical forests alone hold about fifteen percent of the world's excess carbon dioxide, the loss of these forests is particularly problematic. Furthermore, because greenhouse gases also contribute to rising temperatures, the loss of these tropical forests (as well as deforestation elsewhere in the world) can cause changes in weather patterns and increase the likelihood of extreme weather events (World Wildlife Fund, 2016)-which simply worsens the potential impact that deforestation can have on another key part of the ecosystem, the water cycle.

The reason deforestation harms this particular cycle is because trees play a major role in keeping a balance between the amount of water in the soil and the amount in the atmosphere. Plants absorb water through their roots and produce evapotranspiration through their leaves, which returns this water to the atmosphere where it will condense to form clouds and eventually return to the earth in the form of rain, sleet, or snow. But when the trees are cut down, the available precipitation decreases, harming the soil by drying it out.

Changes in the water cycle, though, can lead to extreme weather events as well as drought. Hence, when it rains heavily, soil erosion can occur. This is when fertile soil is washed away by rainfall, and according to the World Wildlife Fund (2016) "as fertile soil washes away, agricultural producers move on, clearing more forest and continuing the cycle of soil loss." Because of this, about one-third of arable land has been lost through deforestation and soil erosion since 1960, leading to the loss of many people's livelihoods.

But humans and their livelihoods are not the only thing ecologically impacted by the effects of deforestation. Organisms below the earth's surface are impacted by it as well, including microbes such as bacteria. These tiny 1-10 micrometer prokaryotic cells have two major roles in the terrestrial ecosystem: they are a part of the decomposition cycle, and they are a part of the nitrogen cycle (Farabee, 2007). In the first of these, heterotrophic bacteria in the soil eat dead organic matter and break it down into simpler components such as carbon dioxide and ammonium, releasing these nutrients into the air and soil where plants can absorb them for their own processes (such as photosynthesis) (Simmons, 2009). These bacteria are able to decompose just about everything from naturally occurring compounds to human made compounds, and there can be as many as 33 billion of them in every 28.35 grams of fertile soil. Hence the heterotrophic bacteria help control the amount of carbon dioxide liberated into the atmosphere (Nardi, 2003) as well as the amount of ammonium released into the soil.

This ammonium from decomposition, though, contributes to the other important cycle in the soil that bacteria perform, the nitrogen cycle. Most of the nitrogen on earth is in the Earth's atmosphere as nitrogen gas where it is not accessible to living things (Flynn & Idowu, 2015). But nitrogen is a very important element for organisms because they need it for growth and reproduction. Nucleic acids and proteins are made with nitrogen; therefore, without nitrogen atoms, organisms such as plants could not make these biological molecules, and without DNA, RNA, and proteins, the chemical reactions of a plant's cells could not happen. No cellular chemical reactions and plants (and everything that depends on them) would not survive (Pidwirny, 2011).

However, plants cannot absorb nitrogen gas directly; instead, they require nitrogen in the form of ammonium or nitrate, chemicals which plants can take in through their roots to acquire this nitrogen that they need (Flynn & Idowu, 2015). But plants cannot make ammonium or nitrate; only the soil bacteria can through the process of mineralization when the bacteria modify nitrogen gas from the atmosphere into ammonium ion in the soil. Other bacteria then convert ammonium into nitrite, which another type of bacteria turn into nitrate (Pidwirny, 2011) - the other form of nitrogen which plants can then absorb for all their cellular processes. Excess nitrogen in the soil is then converted back into nitrogen gas through the process of denitrification, and the cycle repeats (Mcgraw, Williams, Heinzel, Whorl, 1997).

Yet because the nitrogen cycle is where the producers get their nitrogen from, it is where all the rest of the ecosystem does as well. The primary consumers eat the producers and break down their polymers into monomers, and so on up the food chain in the ecosystem. Hence, without the nitrogen provided by the bacteria, there would be no cells of any kind, meaning there would not be an ecosystem.

While bacteria enable plants to survive, plants also give bacteria what they need in return. Plants provide the bacteria with carbohydrates for energy and a safe environment in the nodules of their roots where the bacteria can live. When these plants are removed through deforestation, the soil bacteria no longer have the safe environment provided to them (Pidwirny, 2011). In addition, bacteria are very vulnerable to deforestation because they are sensitive to the moisture fluctuations and temperature increases caused by holes in the soil from the removal of trees (Rainforest Conservation Fund, 2016).

In conclusion, deforestation has many negative impacts on the Earth. It can lead to increased greenhouse gas emissions, the disruption of water cycles, soil erosion, and disrupted livelihoods. It also has a major impact on the bacteria in the soil, which means that there is a decreased amount of nutrients and energy for the rest of the plants and animals on Earth. Overall, bacteria are a vital part of any ecosystem, and since deforestation hurts bacteria, it therefore hurts ecosystems.

Because this is so important, we decided to test a simplified version of deforestation. In our experiment, we will be testing the problem, “How does the removal of grass on top of soil increase or decrease the density of bacteria in the soil the grass has been removed?” We will have three same sized plots of grass, and measure the density of bacteria before removing any plant life. In one section, there will be no plant life removed. In other section, we will remove 50% of plant life, and in our last section we will be removing all plant life. We will let those plots sit for 7 days before testing the density of bacteria for any change. We will repeat that at least one more time, and have 3 different trials running at the same time. We hypothesize to see a decrease in the density of bacteria, which will warn others of another reason on why deforestation harms the Earthly life.

II. Experiment:

I. Problem: How does the removal of plant life on top of soil increase or decrease the density of bacteria in the soil where the plant life has been removed?

II. Hypothesis: As the percentage of plant life removed increases, the population density of bacteria in the underlying soil will decrease.

III. Procedures:

A. Independent Variable: The percentage of plant life removed from a soil plot

B. Dependent Variable: Population density of soil Bacteria

C. Negative Control: a soil plot with 0% of plant life removed

D. Positive Control: samples of soil taken before plant material was removed

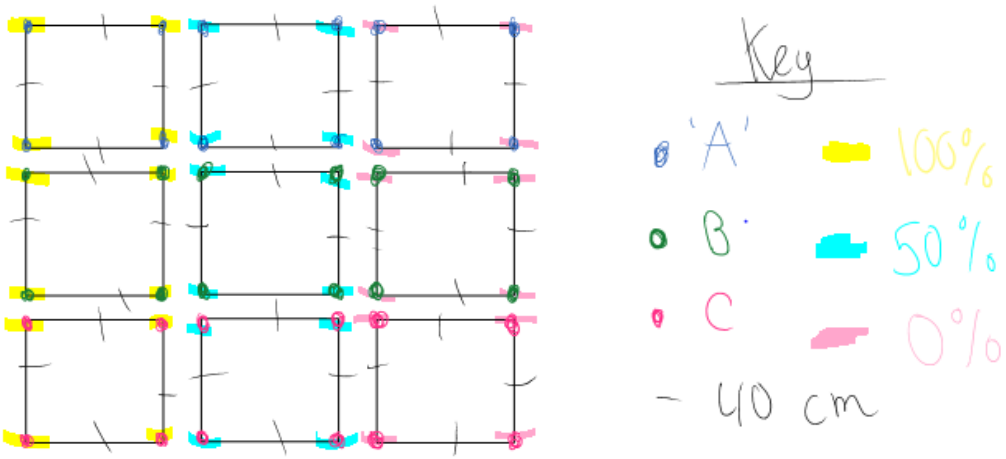
E. Controlled Variables:

1. Type of plant life
2. Marking area method
3. Size of plot (40cm by 40cm)
4. Distance between plots (20 cm)
5. Type of soil
6. Day and time soil sample is collected
7. Location of plots
8. Amount of soil collected
9. Method of counting bacteria population
10. Amount of plant life on soil before conducting experiment
11. Method of soil removal
12. Amount of time tubes are shaken
13. Amount of water in tubes
14. Type of water in tubes
15. Amount of soil in tubes
16. Which dilutions plated
17. How much it is diluted
18. Amount of water soil solution added into each tube
19. Type of plates used
20. Amount added to each plate

21. Time grown before examining bacteria colonies
22. Method of calculating amount of bacteria
23. Plates which are examined for bacteria
24. How long plates are grown

F. Step-by-Step:

1. Collect 36 flags and divide them into 12 groups of 4
2. With one group of 4, label them ‘ A 100%’ with your initials
3. With another group of 4, label them ‘A 50%’ with your initials
4. With another group of 4, label them ‘A 0%’ with your initials
5. With another 3 groups of 4 ,repeat steps 2-4 using the letters ‘B’ instead of ‘A’
6. With the last 3 groups of 4, repeat steps 2-4 using the letter ‘C’ instead of ‘A’
7. Find a location that has access to the same amount of sunlight, the same amount and type of plant life, and on flat ground (coordinates: N 39° 21.413 and W 076° 38.181)
8. Assemble a square with a length and width of 40cm using the ‘A 100%’ labeled flags (see diagram)
9. 20 cm to the east, assemble a square with a length and width of 40cm using the ‘A 50%’ labeled flags (see diagram)
10. 20 cm to the east, assemble a square with a length and width of 40cm using the ‘A 0%’ labeled flags (see diagram)
11. 20 cm south of the ‘A 100%’ square, assemble a square with a length and width of 40cm using the ‘B 100%’ labeled flags (see diagram)
12. 20 cm south of the ‘A 50%’ square, assemble a square with a length and width of 40cm using the ‘B 50%’ labeled flags (see diagram)
13. 20 cm south of the ‘A 0%’ square, assemble a square with a length and width of 40cm using the ‘B 0%’ labeled flags (see diagram)
14. 20 cm south of the ‘B 100%’ square, assemble a square with a length and width of 40cm using the ‘C 100%’ labeled flags (see diagram)



15. 20 cm south of the ‘B 50%’ square, assemble a square with a length and width of 40cm using the ‘C 50%’ labeled flags (see diagram)
16. 20 cm south of the ‘B 0%’ square, assemble a square with a length and width of 40cm using the ‘C 0%’ labeled flags (see diagram)
17. Collect 9 plastic bags, and label each bag corresponding to a different square
18. For each square, place a soil cylinder with a 2.5cm diameter 15 cm into the ground and pull up the soil, placing it into the corresponding labeled plastic bag. Repeat this process two times at different locations in the same square, placing it in the same corresponding plastic bag for a total of three samples in each bag (note: all soil samples must be taken at the same time on the same day)
19. Test the soil in the bag labeled A 100% for the number of bacteria (note: step 19 must be completed at the same time on the same day)
    a. Use a clean, new transfer pipette to add 10 ml of sterile water to a 15 ml culture tube. Label the tube and cap“10^{0} A 100%”
    b. Use the same pipette to add 9 ml of sterile water to a second 15 ml culture tube. Label the tube and cap “10^{-1}A 100%”
    c. Repeat step two three more times to three additional 15 ml culture tubes, only label the tubes and caps “10^{-2}A 100%”, “10^{-3}A 100%”, and “10^{-4}A 100%” respectively

d. Place 1 cc of your soil sample into the "10^0A 100%" culture tube
e. Cap the tube and shake vigorously
f. Using a new clean pipette, remove 1 ml of the soil/water mixture from the "10^0A 100%" tube and place it into the "10^{-1}A 100%" tube
g. Cap and shake vigorously
h. Using the same pipette in step 5, remove 1 ml of the soil/water mixture from the "10^{-1}A 100%" tube and place it into the "10^{-2}A 100%" tube
i. Cap and shake vigorously
j. Using the same pipette in step 5, remove 1 ml of the soil/water mixture from the "10^{-2}A 100%" tube and place it into the "10^{-3}A 100%" tube
k. Cap and shake vigorously
l. You should now have a total of 4 culture tubes
m. Plate 100 µI samples from the 10^{-2}A 100% and 10^{-3}A 100% tubes onto their own separate, correspondingly labeled 3M Petrifilm™ Aerobic Count Plate
n. Repeat steps a-m for A 50% labeling the tubes and 3M Petrifilm™ Aerobic Count Plate A 50% instead of A 100%
o. Repeat steps a-m for A 0% labeling the tubes and 3M Petrifilm™ Aerobic Count Plate A 0% instead of A 100%
p. Repeat steps a-m for B 100% labeling the tubes and 3M Petrifilm™ Aerobic Count Plate B 100% instead of A 100%
q. Repeat steps a-m for B 50% labeling the tubes and 3M Petrifilm™ Aerobic Count Plate B 50% instead of A 100%
r. Repeat steps a-m for B 0% labeling the tubes and 3M Petrifilm™ Aerobic Count Plate B 0% instead of A 100%
s. Repeat steps a-m for C 100% labeling the tubes and 3M Petrifilm™ Aerobic Count Plate C 100% instead of A 100%

t. Repeat steps a-m for C 50% labeling the tubes and 3M Petrifilm™ Aerobic Count Plate C 50% instead of A 100%

u. Repeat steps a-m for C 0% labeling the tubes and 3M Petrifilm™ Aerobic Count Plate C 0% instead of A 100%

20. Allow to grow for 48 hours

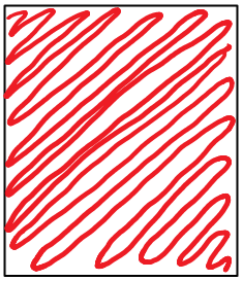
a. Examine each of the plates for individual bacterial colonies by looking at the dilution plate with the lowest value. Count the red dots on the plate using a magnifying glass

b. If there are more than five, find the total number of colonies and use that number in the calculation below.

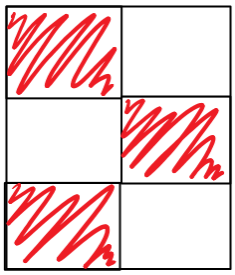
c. If it has less than five colonies move on to the dilution plate with the next highest value, find the total number of colonies, and use this plate for the calculation below.

Microbes in 1 cc of soil = # Colonies on sheet x 10^2 x $10^{|\text{dilution at which these colonies were found}|}$

21. In the three squares that read 100% (A, B, and C), remove all of the grass



in the plots using a hand trowel (see diagram below and remove the plant life of area that is shaded)



22. In the three squares labeled 50% (A, B, and C), remove half of the grass in a zigzag pattern: (see diagram below and remove the plant life of area that is shaded)
23. For the three squares labeled 0% (A, B, and C), do not remove any grass
24. Wait 12 days and repeat step 17-20 using the new soil samples
25. Compare population density of amount of grass before and after and draw conclusions

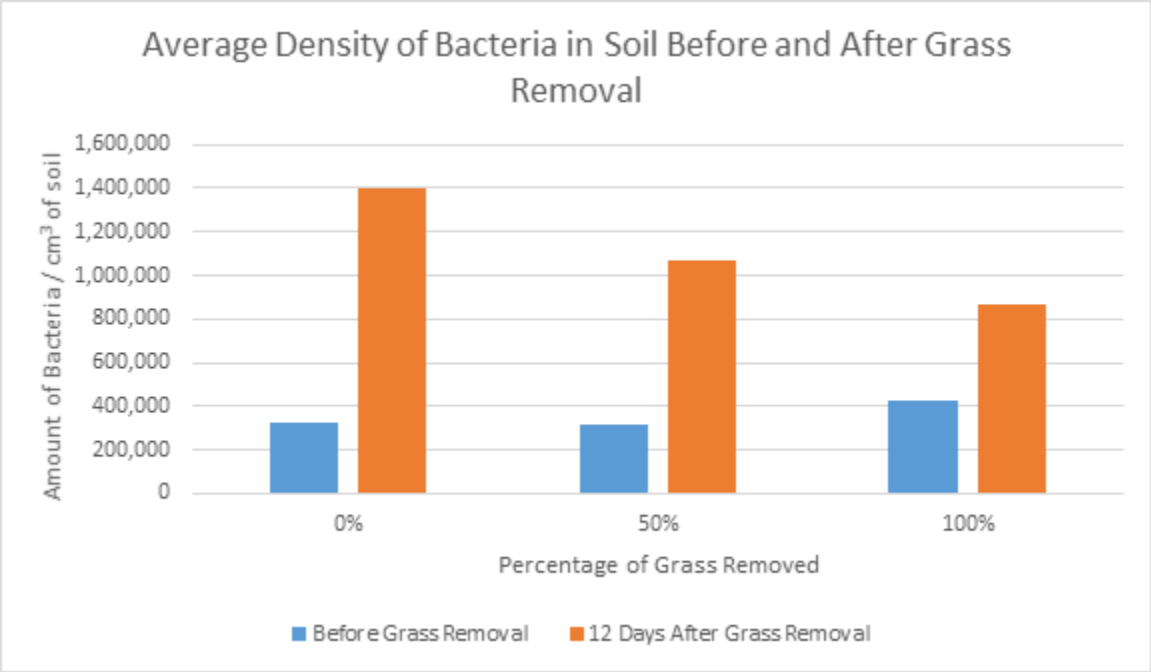
III. Analysis:

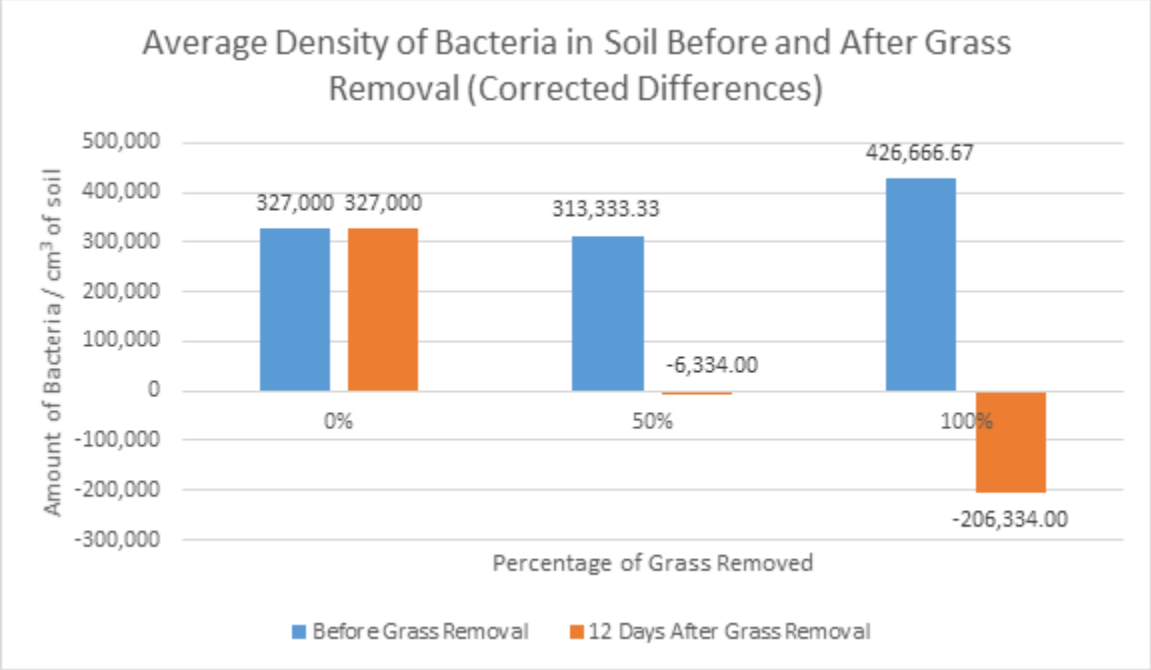
A. Data Table:

Bacteria Density Before and After Grass Removal

Trials	Grass Removal Condition					
	0% (#/cm^3)		50% (#/cm^3)		100% (#/cm^3)	
	Before	After	Before	After	Before	After
A	140,000	1,200,000	150,000	700,000	900,000	700,000
B	410,000	1,600,000	120,000	500,000	70,000	1,800,000
C	800,000	1,400,000	670,000	2,000,000	310,000	1,000,000
Averages	327,000	1,400,000	313,333	1,066,666	426,666	866,666

B. Graph:


Average Density of Bacteria in Soil Before and After Grass Removal
Amount of Bacteria / cm3 of soil
1,600,000
1,400,000
1,200,000
1,000,000
800,000
600,000
400,000
200,000
0
0%
50%
100%
Percentage of Grass Removed
Before Grass Removal
12 Days After Grass Removal


Average Density of Bacteria in Soil Before and After Grass Removal (Corrected Differences)
Amount of Bacteria / cm3 of soil
500,000
400,000
300,000
200,000
100,000
0
-100,000
-200,000
-300,000
327,000
327,000
313,333.33
-6,334.00
426,666.67
-206,334.00
0%
50%
100%
Percentage of Grass Removed
Before Grass Removal
12 Days After Grass Removal

IV. Conclusion:

The hypothesis that the population density of bacteria in the underlying soil will decrease as the percentage of plant life removed increases is supported by the data collected. This experiment proves the point that plants are necessary for bacteria to live. As stated in the background, plants provide bacteria with carbohydrates for energy, and a safe environment in the nodules of their roots where the bacteria can live. Also, when plants are removed, bacteria become more sensitive due to the moisture fluctuations and temperature increases caused by the opening of holes in the soil from where the plant's roots were. Bacteria is unequipped to live on their own, therefore when plant life is removed, many of them die.

This is true because according to our corrected differences graph, 12 days after we removed the plant life, there was a drastic decrease in the density of bacteria when we removed 100% of the plant life, and a smaller but still a drastic decrease in the density of bacteria when we removed 50% of the plant life. This is proven by the data shown on our corrected differences chart. From having all plant life to 50% removal, there was a 333,334 decrease in bacterial density. From having all plant life to 100% removal, there was a 533,334 decrease in bacterial density. There was also a decrease in bacterial density from 50% removal to 100% removal of 200,000 which shows that the amount of plant life removed impacts bacterial density.

This evidence is valid because deforestation does take a major toll on bacteria. When deforestation happens, species are not only losing their habits, but also because oxygen levels would have drastically dropped with an increase in carbon dioxide according to this data. This data has an exponential decrease versus a linear one, and this might be the case because there is a loss of energy when deforestation happens. This is the case because when plants are being removed from an ecosystem, they're the ones that make the energy for other organisms to eat. So when they are gone, there's no energy being consumed or going down to the decomposers. The ecosystem is not there as a whole. In addition, this might be because of a certain plant life being removed, but one should find out by doing further research.

There are many tests based on this one that can be conducted in the future. One experiment could be testing if the type of plant life removed on top of the soil has an effect on the population density of the bacteria. For example, different plots with different plant life could be tested for the bacteria population, and then after a few weeks the bacteria population could be tested again

to see which types of plants bacteria relies on the most for survival. Another test that could be conducted would be testing the population density of the bacteria in the soil at different points in time after removing the plant life overtop of it, such as testing the population density 1 day after, 5 days after, 10 days after, and so on to discover how long it takes for the plant life removal to have an effect on the bacteria. One more test that could be conducted would be to do the same experiment except test for another microbe population instead, such as fungi. Fungi also plays many major roles in an ecosystem so this test could determine if deforestation has an effect on other microbes as well as bacteria.

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