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Background

Biodiversity is the variety of the forms of life in a specific area, for example, undersea or in high elevations. The greatest amount of biodiversity is actually found below ground level, among microscopic life, rather than the more visible life forms above ground. Most biodiversity in the soil, however, does not effect the functioning of the soil: soil decomposes matter whether or not it is diverse in life forms. Studying biologically diverse life in soil, as well as the functions of soil, is called soil ecology. Microbiology of the soil greatly influences plant establishment, competitiveness, and plant growth, thus affecting the soil's miniature ecosystem (Daniell, 2005). If the microbiology of the soil is unbalanced, for example, by an excess of one type of bacteria, and the other bacteria are becoming extinct, then the change in the soil ecosystem will affect the greater ecosystem around it—and therefore humans (Daniell, 2005). The soil microbiology affects its ecosystem, as the microbes living in the soil determine all other life within that ecosystem.

Soil is composed of decaying matter and microorganisms that decompose the dead matter, recycle elements like hydrogen, and circulate water throughout the soil. These microorganisms supply the soil with necessary nutrients for plant growth, like nitrogen to build amino acids in the cells, which then allows the cell to reproduce and help the plant grow with the new cells reproduction causes. Plants then flourish in the soil according to the amounts of minerals, vitamins, nitrogen, and amino acids in the soil; the microbes, which supply these nutrients to the soil through their biological processes, allow more plant life to grow in the abundance of resources for growth and nutrition

(Comm Tech Lab, 2000). Organisms like rhizobium and azotobacter both take nitrogen from the atmosphere and convert it to ammonia for the plants to use as an amino acid in building its cells; again, this is important because the amino acids create proteins that can form new cells in mitosis, and the new cells create new growth in the plant, which can then feed the primary consumers and the rest of the food web. Mycorrhizal fungi supply plants with phosphorus, a mineral required to support further growth and development of a young plant by supplying phosphates for ATP production (ATP provides energy for the plant to grow larger and therefore pass more energy on to the next level of the food pyramid) and energy synthesis (Comm Tech Lab, 2000).

Bacteria also help provide plants with nutrients by taking elements like carbon and nitrogen from dead organic matter. Bacteria consume intricate forms of carbon and degrade them so that plants can use the carbon, hydrogen, nitrogen, and other molecules to build their own cells. Bacteria are eaten by protozoa, the larger life forms in the soil. Protozoa help circulate nutrients through the soil by eating bacteria and breaking down the nutrients inside the bacteria for the plants' use. Protozoa living in a specific plot of soil determine the type of bacteria living there through natural selection, as they are the main predators of bacteria (90% of bacteria consumed are by protozoa). The influence of protozoa and their internal nutrient cycles determine the health of the soil for plants and other life forms; therefore, a sudden change in bacteria population density would cause a change in the protozoa and the rest of the ecosystem. Bacteria, in short, control the health of an ecosystem by means of the soil.

The presence of aliphatic hydrocarbons, complex carbons from spray paint used on fields, can affect the carbon cycle, a very important process that supplies different

organisms in an ecosystem with the carbon for growth and development. The carbon cycle, according to Encyclopedia Britannica (2005), is how plants produce energy: they take carbon dioxide from the air (some of which has come from decaying matter in the soil) and water from the soil to form sugar, which then becomes energy in energy synthesis. Humans and other, larger animals all produce carbon dioxide as a waste product; plants absorb the CO2 byproduct from the air. If a large amount of complex carbons were in the soil, then bacteria that decompose the carbons would increase in number to try to break down all of the carbon and other materials, like phosphorus and hydrogen. The carbon could then be used in plants in their own energy production. This also impacts humans: if more bacteria produce more carbon dioxide so more plants can grow, then spray paint used on athletic fields is safe for other uses on the soil, and even beneficial to it, increasing the health of the ecosystem by supplying another food resource for the smaller organisms in the greater ecosystem (Encyclopedia Britannica, 2005).

Another element, hydrogen, supplies the bacteria in the soil with material to build amino acids, which then supervise the cell's four tasks (reproduction, respiration, energy synthesis, and homeostasis). The hydrogen cycles throughout the ecosystem through the processes of nitrogen fixation, nitrogen assimilation, ammonification, nitrification, and denitrification (Encyclopedia Britannica, 2005). Nitrogen fixation occurs when nitrogen from the atmosphere is converted into ammonia and inorganic nitrates through organisms like bacteria. These nitrates are used by plants to build their own cells, and primary consumers use the converted nitrates in the plant cells in their own cellular structures. Ammonification is the conversion of all dead matter from plants and animals into ammonia or ammonia compounds by decomposers in the soil; these ammonia molecules

are also then converted into nitrates and used by plants. Nitrification is the conversion of ammonia into nitrate compounds by bacteria in the soil; existing proteins and amino acids in plant cells convert the nitrates into forms usable in the plant's tissue. This process is denitrification, breaking down nitrates into nitrogen compounds that return to the atmosphere (Encyclopedia Britannica, 2005). All the nitrates used in plant cells can be converted to use inside a higher organism, for example, a cow. The cow digests plant cells and uses nitrates within its own cells, and when a human eats the cow or other secondary consumer, that consumer converts the nitrates to suit its cellular structure.

Phosphorus, a byproduct of energy synthesis, is the source of energy in cells. Three phosphates together equal ATP, the basic molecule that provides proteins in the cell with energy for homeostasis and other tasks. One of the phosphates from the ATP breaks off and attaches itself to the protein, allowing the protein to take its energy to use in the cellular processes. The remaining ADP (two phosphates) then returns to the ribosome for another phosphate and the process begins anew. Without phosphate from the soil, plants would not grow. If plants did not grow, then primary consumers would have no food source and would die out. If primary consumers died out, each successive hierarchy of consumers would die out and the ecosystem would be destroyed. Phosphate, carbon, and hydrogen each compose a key element in the growth of plants, which then affects the larger ecosystem, including humans (Encyclopedia Britannica, 2005).

Our hypothesis for this experiment is that the presence of aliphatic hydrocarbons from spray paint positively influences the populations of bacteria in the soil. Aliphatic hydrocarbons are loose carbon atoms, bonded to no more than two other carbons (Alkane, 2005). They are an organic compound. Organic compounds are classified into

two broad groups, the aromatic and aliphatic (Alkane, 2005). Aromatic compounds have a fragrance and a low hydrogen to carbon ratio; aliphatic act like alkanes, alkenes, and alkynes. Alkane, alkene, and alkyne are all type of hydrocarbons; an alkene is an unsaturated hydrocarbon, alkynes contain three or more carbon triple bonds, and an alkane is a single-bonded hydrocarbon (Alkane, 2005). These hydrocarbons can feed bacteria in the soil (bacteria's main food sources are forms of carbon (Daniell, 2005)): therefore, we can hypothesize that the presence of aliphatic hydrocarbons cause populations of bacteria to flourish.

References

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Procedure

Problem: How do aliphatic hydrocarbons in spray paint change the population density of bacteria in the Roland Park Country School fields?

Hypothesis: The aliphatic hydrocarbons in spray paint are increasing the population density of bacteria in the Roland Park Country School Fields.

Independent variable: Soil samples with spray paint applied to them.

Dependent variable: Population density of bacteria per gram of soil

Primary Negative Control: Soil samples from plots 1, 2, and 3 where no spray paint is applied

Experimental Negative control: Soil samples from plots 4, 5, and 6 before spray paint is applied to the plots

Control List:

A. Amount of Spray paint applied to each plot
B. Distance between each plot
C. Amount of water that is poured on each plot
D. Location of the soil samples
E. Time that the soil samples were taken
F. Type of spray paint applied to the plots
G. Temperature that bacteria is grown at
H. Length of time that bacteria is allowed to grow before it is examined
I. Time that the serial dilutions are performed
J. Size of soil plots
K. Number of soil samples taken
L. Size of soil samples
M. Amount of sterilized solution used in the serial dilutions
N. Amount of soil diluted
O. Amount of times that the soil is diluted
P. How vigorously the transformation tubes are shaken during the dilution processes
Q. Condition of the Petri film plates
R. Type of Petri film plate

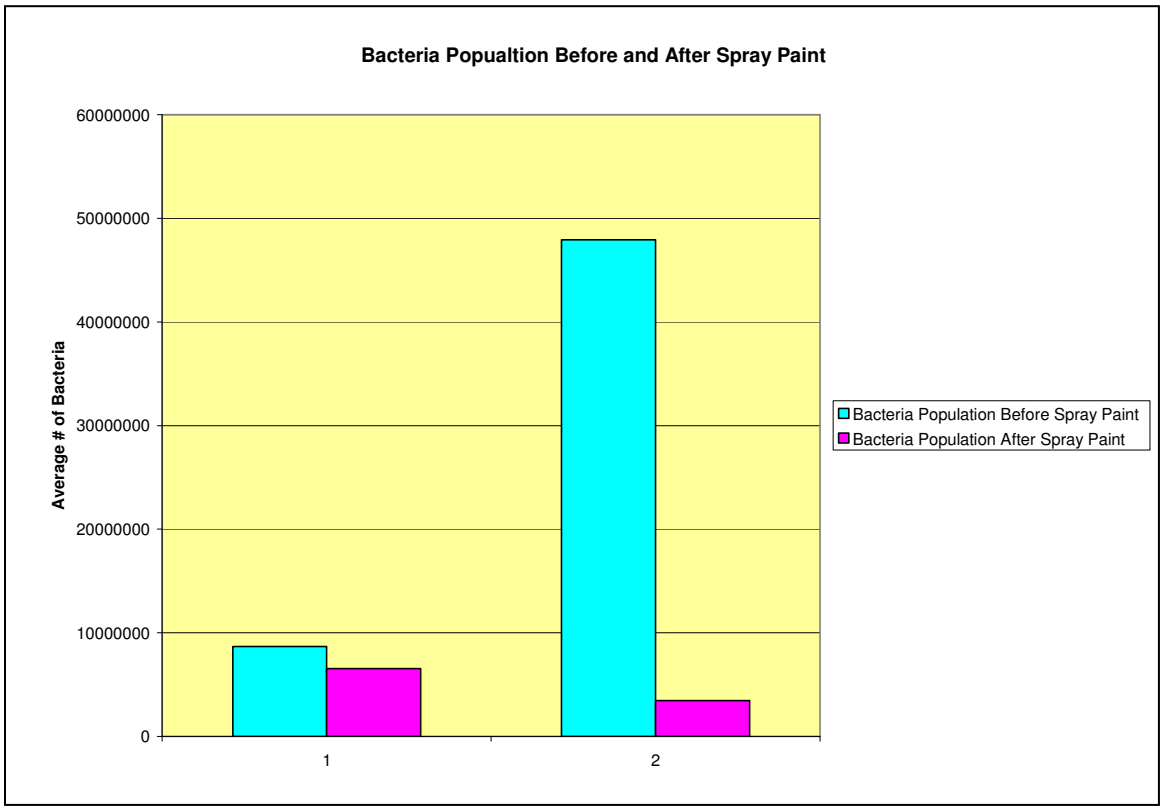
Procedure

1. At N 39.35774º W 076.63530º mark three 6cm by 6cm plots 5cm apart from each other
2. Name these plots 1, 2, and 3.

3. At N 39. 35752º W 076.63544º mark three 6cm by 6cm plots 5cm apart from each other
4. name these plots 4, 5, and 6
5. In plot 1 use a twisting action to embed a soil core with a diameter of 2cm in the soil
6. push the soil core into the soil until you have gathered 15cm of soil
7. Twist the soil cylinder 360 degrees to isolate sample
8. Pull straight up to remove soil core
9. Place the soil core sample in a clean plastic bag,
10. label the bag pre plot 1
11. As soon as your are done step 10 repeat steps 5-9 in plot 2 and label the bag pre plot 2
12. As soon as you are done step 11 repeat steps 5-9 in plot 3 and label the bag pre plot 3
13. as soon as you are done step 12 repeat steps 5-9 in plot 4 and label the bag pre plot 4
14. As soon as you are done step 13 repeat steps 5-9 in plot 5 and label the bag pre plot 5
15. As soon as you are done step 14 repeat steps 5-9 in plot 6 and label the bag pre plot 6
16. take all of the samples back to the lab
17. Spray "Pioneer Quick Stripe Athletic Field Marking Paint" on plot 4 for five seconds, do the same for plot 5 and 6
18. wait 24 hours for the spray paint to dry
19. After 24 hours pour 1 liter of water on plot 4, one liter on plot 5, and one liter on plot 6
20. allow the water to soak in for 28 hours
21. after 28 hours in plot 4 embed the soil core into the soil
22. push the soil core with a diameter of 2cm into the soil until you have gathered 15cm of soil
23. Twist the soil cylinder 360 degrees to isolate sample
24. Pull straight up to remove soil core
25. Place the soil core sample in a clean plastic bag
26. label the bag plot 4
27. As soon as your are done step 26 repeat steps 22-25 in plot 5 and label the bag plot 5
28. As soon as you are done step 27 repeat steps 22-25 in plot 6 and label the bag plot 6
29. As soon as your are done step 28 repeat steps 22-25 in plot 1 and label the bag plot 1
30. As soon as you are done step 29 repeat steps 22-25 in plot 2 and label the bag plot 2
31. As soon as you are done step 30 repeat steps 22-25 in plot 3 and label the bag plot 3
32. Take all of the samples back to the lab
33. label
34. perform a serial dilution for the sample labeled pre plot 1, the sample labeled pre plot 2, the sample labeled pre plot 3, the sample labeled pre plot 4, the sample labeled pre plot 5, and the sample labeled pre plot 6 at the same time, steps 35-44 show how the dilution should be performed
35. Use a micro centrifuge tubes to create a 1cc soil scoop
36. collect and 1cc sample of the soil that you are performing the serial dilution on
37. place the 1cc soil sample into a culture tube containing 10ml of sterile water, cap the tube and shake vigorously
38. Using a serological pipette, remove 1ml of the soil/water mixture and place into a fresh culture tube
39. Add 9ml of fresh sterile water to this second tube; cap and shake vigorously.

40. Repeat step 38 using the second, diluted tube then repeat step 39 with a third tube.
41. continue step 40 with each additional tube until you have diluted the original soil/water mixture 4 times(there should be a total of 5 culture tubes)
42. Plate 100ul samples from the 4^{th} and 5^{th} tubes onto their own separate, individual Petri film plates.
43. label each Petri film plate
44. Allow all of the Petri film plates to grow at room temperature for 2 days
45. Perform serial dilutions for the sample labeled plot 1, the sample labeled plot 2, the sample labeled plot 3, the sample labeled plot 4, the sample labeled plot 5, and the sample labeled plot 6 at the same time, steps 35-44 show how the serial dilutions should be performed
46. allow all of the Petri film plates to grow a temperature for 2 days
47. Examine each plate from the dilutions series to find the ones between 5 & 30 colonies: count the colonies on only those petria films & use the formula to calculate the density of bacteria in the original cc of each soil sample. The formula for population density is, Population density = # of colonies • 10 squared • 10 to the |dilution factor| an example of how to use the formula is below in step 48
48. If there are 30 bacteria colonies on a Petri film plate with a dilution factor of 10 to the -3 this is how you would use the formula. Multiple 30 by 10 squared. 30 times 10 squared is 3,000. Take this number and multiply it by 10 to the $3^{rd.}$ 3,000 times 10 to the 3^{rd} is 3,000,000. This is the bacteria population density.

Data & Analysis



1= Negative Control (Plots 1,2,3)

2= Independent Variable
(Plots 4, 5, 6)

Data and Analysis

The tables and graph shows that the bacteria population greatly decreased in plots four five and six when spray paint was applied to them. The bacteria population decreased by 3089333 colonies when the spray paint was applied. When the spray paint was applied to four five and six, the negative control increased, meaning the four five and six should have increased as well, but they did not.