Soil Ecology Project

IMPACT OF COMPOST ON BACTERIA POPULATION DENSITY

By: Sydney Benfield, Landry Carrera, and Emily Roebuck

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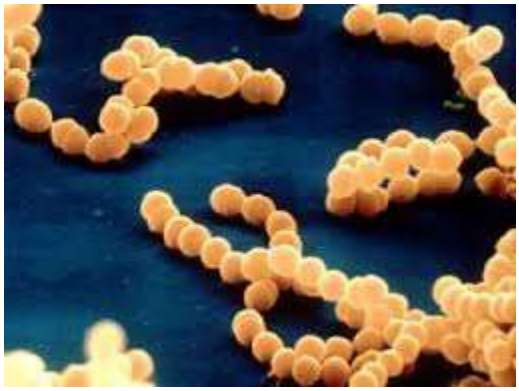


http://seven-eight-science.wikispaces.com/aerobic+bacteria

Background Information

In any ecosystem, there are three main types of microbes present: fungi, protozoa, and bacteria. But of these, bacteria are the most critical because of their many roles: decomposing dead organic matter, creating fertilizer for the soil by enabling the nitrogen cycle to happen, and providing food for more complex organisms in the soil food chain (Lawerence, 2013). They also suppress disease, degrade inorganic substances (Reid and Wong, 2005), and create detritus and other products beneficial to plant growth (University of Michigan, 2010). However, the main job of bacteria in the soil is transforming the inorganic components found there from one chemical form to another.

Perhaps the most significant of these transformations is the nitrogen cycle. Nitrogen-fixing bacteria take the nitrogen gas (N_2) from the atmosphere and convert it into nitrate in a process called nitrification. This nitrate is crucial for the development of plants and the organisms that eat them because "it is the basic constituent of proteins" (Brown, J.R., 1993). Proteins, though, are vitally important to an ecosystem because enzymes, they start and stop the chemical reactions that cells use to function. Without them, a cell would not be able to survive, and since plants are made up of cells, they would die without their enzymes (as would everything that depends on plants for food). Hence, without the nitrate that plants need to make their proteins, the entire ecosystem would collapse.

Nitrogen is so important that there are even other ways that it can be recycled. In addition to the atmosphere, another critical source of nitrogen comes from the decomposition of dead plants and animals into ammonia (NH_3) as part of the composting process. Aerobic bacteria take the ammonia out of the compost to create nitrite (NO_2^-), which other bacteria then convert into

nitrate (NO_3^-). The nitrate then enters the food chain through the plants (as indicated earlier), and the cycle continues. Any excess nitrite and nitrate left in the soil is then converted back into the atmosphere, enabling the nitrogen fixation process to begin again.

In addition to playing a role in the nitrogen cycle, compost also improves the quality of all of the parts of the soil by acting as a "conditioner" for it. It helps the soil to absorb other nutrients and retain them for future use. It helps the soil maintain air and moisture to improve the growth of the plants, and it also attracts earth worms and other detritivores that benefit soil structure by making the soil more porous. Compost therefore makes the soil easier to drain, more crumbly, and able to retain moisture more readily. For example, sandy soils have a hard time retaining water and are usually very dry, but adding compost to them greatly increases the amount of water that the sandy soil can keep in for any plants living there (Friend, 2013). Likewise, clay soils are very heavy and tightly compacted, and therefore, when compost is added, the soil becomes lighter, thereby creating more spaces for air and moisture to flow in and out of the soil.

Because of the significant benefits of compost to the soil, our school, Roland Park Country School, made the decision to start sorting our trash in order to send compostable trash to a facility that will turn our trash into compost. The facility that Roland Park Country School sends their trash to is called The Peninsula Compost Group and the first step of making compost at this facility is weighing the trucks filled with compost. Next, the compost is taken out of the trucks and inspected by specialists who mix yard and food waste to place into a shredder to be cut into the proper size and shape. Once the waste comes out of the shredder, workers pick out any plastic to begin the actual composting process and this final mix of waste to an area where the waste is covered.

Phase 1 of this process, the stack of compost is covered for four weeks, during which microorganisms decompose the mixed waste, which heats the stack up, killing any pathogenic bacteria living there. After this, in Phase 2, workers keep the compost covered for two more weeks to strengthen the biological elements of the decomposing process, and then finally, in Phase three, the compost is left uncovered for two weeks to dry and cool the compost. After the eight weeks have passed, the compost is placed on a magnet to remove any metals (The Peninsula Compost Group, 2010), and then Roland Park Country School receives a certain amount of the finished product to use in the various gardens around the school.

Given that compost is such a central part of our school life, we were interested to see if it was beneficial or not to the soil. We believe that the addition of compost to soil will have a positive impact on the ecosystem. We also believe that more bacteria can be found in soil with compost in it, which therefore benefits the ecosystem. To test our theory, we decided to place different amounts of compost on varied plots within the same location. Then we extracted soil samples from these plots and diluted them in order to make a proper estimate of the number of bacteria per cm^3 living there.

Lab Outline

I. Problem: how does the amount of compost present change the density of the bacteria population in soil?

II. Hypothesis: The bacteria population will be the densest in the site with the greatest amount of compost.

III. Procedures

A. Independent variable: amount of compost applied to the soil

B. Dependent variable: density of bacteria in the soil

C. Negative control: plot with no compost applied to it

D. Positive control: initial density of bacteria in the soil before adding given amounts of compost to the sites

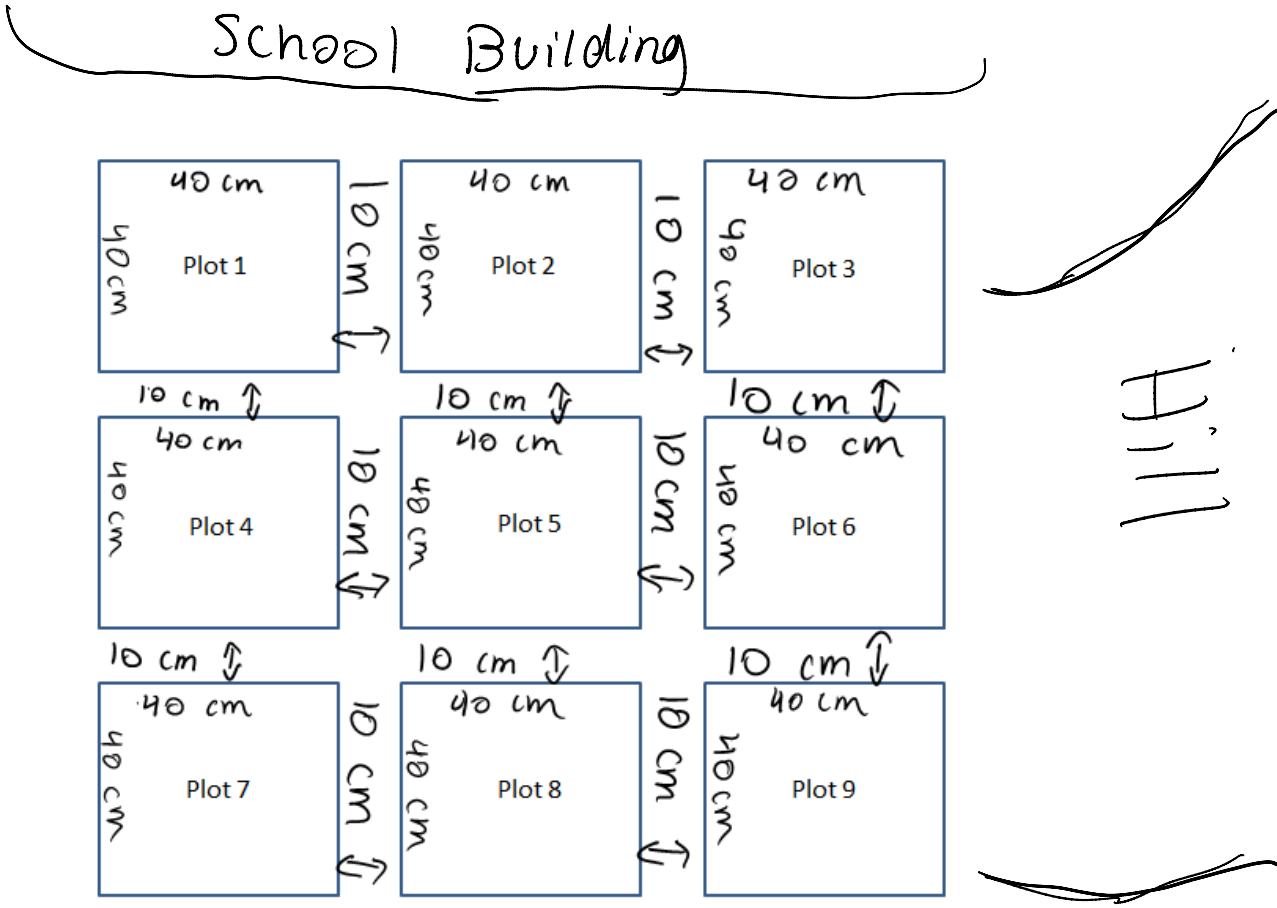
E. Controlled variables:

- size of site
- all samples are taken on the same time and the same day
- amount of soil samples extracted from soil
- location of site
- given time for compost to sit in soil
- type of GPS device
- size of pipette used for serial dilutions
- size of culture tubes
- type of nutrient agar
- size of petri dishes
- time allowed for nutrient agar plates to grow
- amount of soil samples put in the first tube
- amount of water in the 10^0 tube during each dilution is 10ml
- amount of water in 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} tube during each dilution is 9ml
- number of dilutions per sample
- source of compost
- which samples are plated

- amount of dilutions plated
- size of soil samples

F. Step-by-step instructions:

1. Go out to the GPS coordinates 39^0 21.468 N, 76^0 38.162 W. at Roland Park Country School in Baltimore, Maryland. Make your site according to the following diagram:



2. Use flags to mark each of the corners surrounding each individual plot. Label them as the following: (g = grams)

- Plot 1: “Trial 1, 0g of compost”
- Plot 2: “Trial 1, 40g of compost”
- Plot 3: “Trial 1, 80g of compost”
- Plot 4: “Trial 2, 0g of compost”
- Plot 5: “Trial 2, 40g of compost”
- Plot 6: “Trial 2, 80g of compost”
- Plot 7: “Trial 3, 0g of compost”
- Plot 8: “Trial 3, 40g of compost”
- Plot 9: “Trial 3, 80g of compost”

3. Get 18 Ziploc bags and label them according to step 2. Each plot should have two labeled bags – one for the positive control sample and one for the applied compost sample. For the bags that will be used to hold the positive control samples, make sure you label somewhere on the bag that it is a positive control sample bag in order to avoid confusion.
4. Get 6 more Ziploc bags. Three bags should be labeled “40g of compost”. Three bags should be labeled “80g of compost”.
5. Fill each of the bags from step 4 with the corresponding amount of compost (i.e. fill the bags that say 40g of compost with 40g of compost, etc.). Store these bags for later use.
6. On the same day at the same time, use a soil core extractor with a 2.5 cm diameter to extract soil samples from each plot. When extracting the soil, put the soil core extractor 15 cm deep into the plot. Then, twist the handle clockwise and then pull the soil core extractor up and out of the soil. Place

these samples in their corresponding bags. These samples are the positive control samples.

7. On the same exact day at the same exact time, extract the bacteria from each soil sample using steps 8-21. Start with the "Trial 1, 0g of compost positive control" soil sample.
8. Use a clean, new transfer pipette to add 10 ml of sterile water to a 15 ml culture tube. Label the tube "10^0 Trial 1, 0g of compost PC" (PC meaning positive control).
9. Use the same pipette to add 9ml to a second 15ml culture tube. Label the tube "10^{-1} Trial 1, 0g of compost PC".
10. Repeat step 9 three more times to three additional 15 ml culture tubes, only label them "10^{-2} Trial 1, 0g of compost PC", "10^{-3} Trial 1, 0g of compost PC", and "10^{-4} Trial 1, 0g of compost PC" respectively.
11. Place 1 cc (cubic centimeter) of your "Trial 1, 0g of compost positive control" soil sample into the "10^0 Trial 1, 0g of compost PC" culture tube.
12. Cap the tube and shake vigorously.
13. Using a new clean pipette, remove 1ml of the soil/water mixture from the "10^0 Trial 1, 0g of compost PC" tube and place into the "10^{-1} Trial 1, 0g of compost PC" tube.
14. Cap and shake vigorously.
15. Using the same pipette in step 13, remove 1 ml of the soil/water mixture from the "10^{-1} Trial 1, 0g of compost PC" tube and place into the "10^{-2} Trial 1, 0g of compost PC" tube.

16. Cap and shake vigorously
17. Using the same pipette in step 13, remove 1 ml of the soil/water mixture from the "10^{-2} Trial 1, 0g of compost PC" tube and place into the "10^{-3} Trial 1, 0g of compost PC" tube.
18. Cap and shake vigorously
19. You should now have a total of 4 culture tubes.
20. Place 100 µl samples dilutions 10^{-2} and 10^{-3} onto their own separate correspondingly labeled 3M Petrifilm^{TM} Aerobic Count plates
21. Repeat steps 8-20 for each of the remaining 8 plots, changing the labels according to which plot samples you are diluting and plating.
22. Allow all plates to grow for 48 to 72 hours.
23. Line up your all of your plates so that the 10^{-3} column is on the right and the 10^{-2} column is on the left. Have each corresponding trial next to each other.
24. First look at the 10^{-3} plate. If that plate contains at least 5 red dots, then continue to count the bacteria on that plate. The bacteria are the red dots. However, if the 10^{-3} plate contains less than 5 dots, then count the red dots on the 10^{-2} plate. Do this for each of your plot sample plates.
25. After examining plates for dilutions 10^{-2} and 10^{-3} for each plot, use the following formula to make your estimates of the number of bacteria in the original 1 cc soil sample:

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

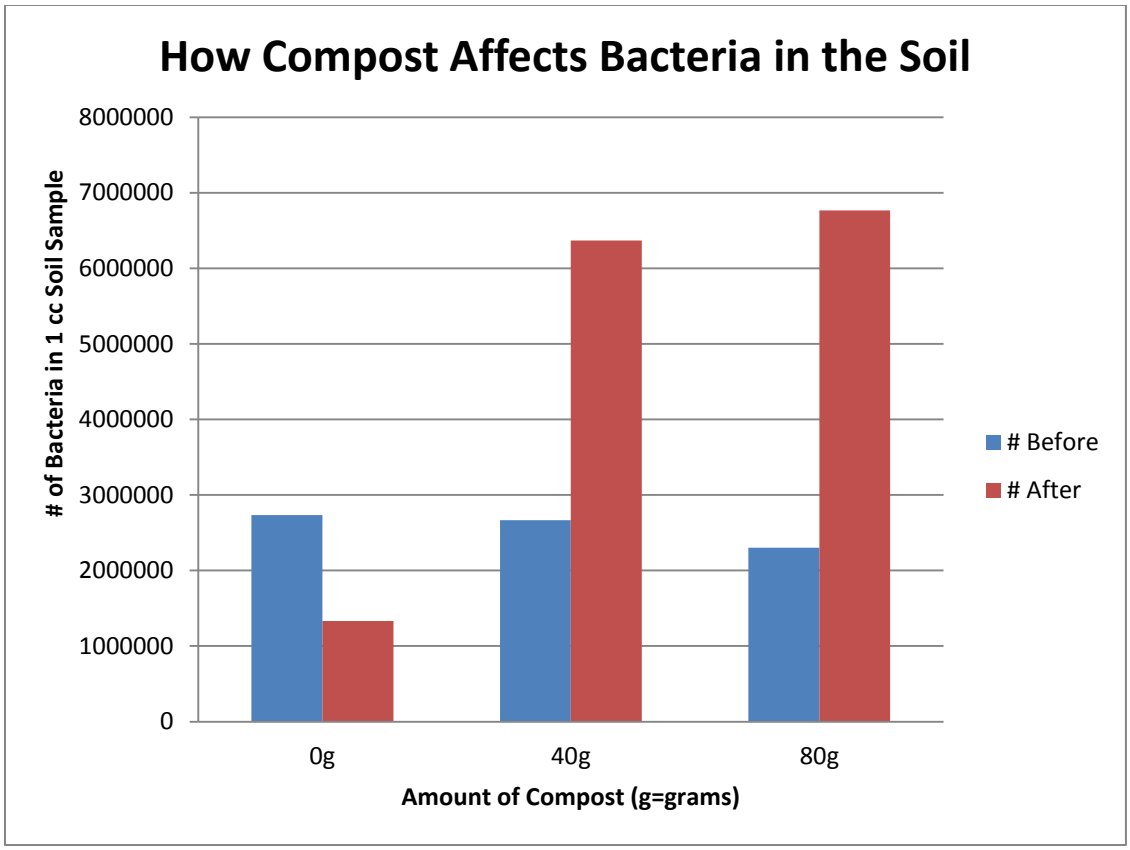
26. Record this information about the bacteria density of plot 1 in a data table.

27. On the same day at the same time, brings the bags (from steps 4 and 5) that you filled with compost to your site. Evenly disperse the compost onto its corresponding plot according to bag and flag label (i.e. empty the bag that says “Trial 1, 40g of compost” into the plot with the flags labeled “Trial 1, 40g of compost”). Make sure that the compost of evenly spread throughout the plot. Rake the compost into the soil using a hand fork. Do this for each plot.
28. Allow the compost two days to sit.
29. On the same day at the same time, use a soil core extractor with a 2.5 cm diameter to extract soil samples from each plot. When extracting the soil, put the soil core extractor 15 cm deep into the plot. Then, twist the handle clockwise and then pull the soil core extractor up and out of the soil. Place these samples in their corresponding labeled bags.
30. On the same day at the same time, use steps 8-26 to extract and count the bacteria levels from the “after” soil samples of plots 1-9. Record the population density of the bacteria from each soil sample in a data table.

Data and Analysis

Impact of Compost on Soil

Trial # and amount of compost added	Total # of bacteria/ cm^3 (Before adding compost)	Total # of bacteria/cm^3 (After adding compost)
Trial 1- 0 grams of compost	3,500,000	900,000
Trial 2- 0 grams of compost	3,000,000	1,800,000
Trial 3- 0 grams of compost	1,700,000	1,300,000
Average of 0 grams of compost	2,733,333	1,333,333
Trial 1- 40 grams of compost	2,000,000	2,400,000
Trial 2- 40 grams of compost	2,400,000	3,500,000
Trial 3- 40 grams of compost	3,600,000	13,200,000
Average of 40 grams of compost	2,666,666	6,366,666
Trial 1- 80 grams of compost	1,900,000	5,000,000
Trial 2- 80 grams of compost	2,600,000	6,000,000
Trial 3- 80 grams of compost	2,400,000	9,300,000
Average of 80 grams of compost	2,300,000	6,766,666



Conclusion

In conclusion, our hypothesis was correct. We predicted that the bacteria population will be the densest in the site with the greatest amount of compost, and this was proven true by our data. The positive control sample for the 0g (g=grams) of compost plot's average number of total bacteria/cm^3 was 2,733,333. However, after adding compost to the other plots, but giving these particular three plots no compost, the number of total bacteria/cm^3 decreased by 51.22% from 2,733,333 to 1,333,333 bacteria/cm^3. This suggests that something destructive happened in the environment, causing the bacteria levels to decrease. Since compost was not a factor in these particular plots, this leads us to think that something else in the environment caused the bacteria to die. However, despite the clear destructive environmental forces, we still saw a great increase

in the total number of bacteria/cm^3 in the 40g of compost plots and the 80g of compost plots. Before adding compost, the 40g of compost plot's average number of total bacteria/cm^3 was 2,666,666. But, after we added 40g of compost to each of these three plots, the average number of total bacteria/cm^3 increased from 2,666,666 to 6,366,666 bacteria/cm^3. It is evident in multiple ways that the added 40g of compost benefitted the density of the bacteria population. To start, the average number of bacteria per cm^3 in these particular plots increased by 138.75%. Furthermore, we know from our previous data that something destructive happened in the environment during this time period. So, the fact that the bacteria population still increased greatly despite the negative environmental forces at this given time shows that the compost had an even greater positive impact on the population than we had realized before. Similarly, the average number of bacteria/cm^3 in the 80g of compost plots greatly increased as well. In the positive control samples from these plots, there were 2,300,000 bacteria/cm^3 on average. However, after adding 80g of compost to each of these three sites, the population increased to a total of 6,766,666 bacteria/cm^3 on average. This is a 194.2% increase in bacteria/cm^3. Also, as explained previously, we know that the compost had an even greater impact because the population still managed to increase by 4,466,666 bacteria/cm^3, regardless of the environmental forces fighting against it at that time. Thus, with 6,766,666 being the greatest number of bacteria/cm^3, we see that our hypothesis was indeed correct since this average came from the 80g of compost plots.

From our data, we were also able to draw more interesting observations. As shown in the graph titled "How Compost Affects the Bacteria in Soil", one can see that after adding compost, the gap between the numbers of bacteria/cm^3 in 0g of compost to 40g of compost is very drastic with a difference of 5,033,333 bacteria/cm^3. However, it is also evident that after adding

compost, there is a meager gap between the 40g of compost numbers of bacteria/cm^3 to that of the 80g of compost with a difference of only 400,000 bacteria/cm^3. Thus, these differences show that the numbers of bacteria/cm^3 begin to level off after adding 40g of compost. So, this brings up the question: is there a point at which the amount of compost added no longer has a drastic effect on the density of the population of the bacteria? To further investigate this, one could add a wider variety of different amounts of compost to numerous plots and then see how these amounts affect the bacteria population. In our experiment, we tested 0g of compost, 40g of compost, and 80g of compost. However in this new experiment, one would test the effect of 0g of compost, 10g of compost, 20g of compost, 30g of compost, 40g of compost, and so on until reaching 110g of compost. Then, one would count and calculate the average number of bacteria/cm^3 in these varied plots. When observing this data, one would especially look to see if the numbers of bacteria/cm^3 begin to level off starting at a certain amount of added compost. This would lead one to discover if and where the amount of added compost no longer greatly affects the population density of bacteria. Thus, our hypothesis was not only proven to be correct, but it also leads us to considering and investigating possible future experiments regarding the effect of compost on bacteria population density.

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