The Effects of Turf Beads (Styrene Butadiene) on the Density of Bacteria

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Background Report

Heterotrophic bacteria are the most abundant bacteria in the soil, and there are three main types of these bacteria that play significant roles in the ecosystem: azotobacter, azospirillum, and clostridium. Together, these groups of bacteria convert stored chemical energy in the soil into organic matter which can be used by other organisms for their specific needs. These bacteria accomplish this by decomposing dead organisms and cycling nitrogen. (Hoorman 2016).

To decompose and cycle nitrogen, these groups of bacteria go through several complex chemical processes. Once an organism dies, living organisms called decomposers, which use oxygen, feed on the dead organisms' organic nitrogen, phosphorus and some of their carbon. To do this, the decomposers release a digestive enzyme which breaks down the dead organism's polymers (which are the organism's biological molecules) to form monomers. The decomposers then eat these monomers and then use them in order to carry out metabolic processes. Since the decomposers essentially have an external digestive system, the digestive enzymes continue breaking down polymers into monomers, some of which the decomposers do not eat. Instead of being eaten, the monomers fall off onto the soil and interact with different soil particles to form humus. This humus then is converted by other bacteria in the soil to become nitrogen that plants are able to absorb. This process is called humification. Once the decomposers die, they are eaten by protozoa, which excrete the excess nitrogen into the soil, thus continuing the nitrogen cycle.

The other way nitrogen is cycled in the soil begins with atmospheric nitrogen gas (N_2) which is first accessed and collected by nitrogen-fixing bacteria in the soil that convert this atmospheric nitrogen gas into ammonium (NH_4+), a form of organic nitrogen which some producers can absorb. However, since ammonium can only be used by some producers, bacteria in the soil called nitrobacter convert the excess ammonium into nitrite and then into

nitrate (NO_3-) which is the other form of nitrogen that producers can absorb from the soil. This process is called nitrification and it is followed by denitrification in which the last group of bacteria in the soil are involved. Denitrifying bacteria are active when there is a lack of oxygen in the soil and these microbes convert the excess nitrate in the soil back into gas again or into nitrous oxide gas (N_2O).

Plants, after nitrification, absorb the ammonium and nitrates converted by the nitrifying bacteria and use them to form proteins and nucleic acids. These biological molecules are critical for controlling the essential chemical reactions of the cell (respiration, synthesis, homeostasis, and reproduction). These enzymes are eventually passed up the food chain as the plants and other producers are eaten by consumers. Once the consumers and producers who have obtained their nitrogen eventually die, the process of decomposition begins, cycling the nitrogen yet again. Bacteria, being involved in the nitrogen cycle and decomposition, are clearly necessary to many aspects of the ecosystem, because without the usable nitrogen to make DNA, RNA, and enzymes, all organisms would be harmed because the cells they are made of could not stay alive. The plants would not be able to survive because they would be unable to make proteins and therefore keep their cells alive by carrying out one of the four tasks. Furthermore, since animals consume plants in order to gain the nitrogen they need to make proteins and nucleic acids for their cells (and so on up the food chain), animals would also die for the same reasons, as well as the decomposers who depend on the dead bodies of plants and animals to obtain the nitrogen to control their essential chemical reactions. All of this would mean the ecosystem would fail completely.

Since heterotrophic bacteria are so vital to the nitrogen cycle and decomposition, anything that harms them could greatly harm the entire ecosystem, and one possible danger that humans have created are the excess scraps of rubber that make up artificial turf. Artificial turf is 20-40% made up of carbon black, which is extremely detrimental to the environment

and can kill bacteria (Zhang, Lin, Tang, Xu, 2010). Artificial turf beads also contain benzothiazole, which has been tested on various types of bacteria and deemed toxic to them. (Wever, Verachtert, 1998), as well as a toxin called Polycyclic aromatic hydrocarbons (PAH) that is found in the rubber tire remains out of which the turf beads are made (Cheng, Hun, Reinhard, 2014). PAH is a chemical found in oil which is used in the manufacturing of car tires, and when turf beads are exposed to sunlight and/or rain, these harmful chemicals can seep into the soil and come in contact with bacteria, which could potentially be detrimental (Cheng, Hun, Reinhard, 2014).

Our project tests how styrene butadiene (turf beads) being tracked onto soil increases or decreases the density of heterotrophic bacteria after the beads are exposed to rainfall or a form of heat/sunlight. Heterotrophic bacteria is involved in many specific and absolutely essential processes, and since we have a turf field at Roland Park that is surrounded by soil and inhabited by different types of bacteria and microorganisms, the impact of the dangerous chemicals contained in turf beads on heterotrophic bacteria in the soil directly affects our surrounding ecosystem and therefore community's wellbeing. By seeing how how styrene butadiene (turf beads) being tracked onto soil affects the density of heterotrophic bacteria after being exposed to rainfall or extreme heat, we can hopefully educate and make others aware of their negative or positive impact on the environment.

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Lab Report

I. Problem: How will excess styrene butadiene (turf beads) tracked onto soil by humans change the density of heterotrophic bacteria in the soil?

II. Hypothesis: The excess styrene butadiene (turf beads) tracked onto the soil by humans will decrease the density of heterotrophic bacteria in the soil.

III. Procedure:

A. Independent Variable: The addition of turf beads to the surface of the soil plot.

B. Dependent Variable: The density of the heterotrophic bacteria in the soil

C. Positive Control: The soil samples collected before the addition of the turf beads.

D. Negative Control: Soil plots without the addition of turf beads.

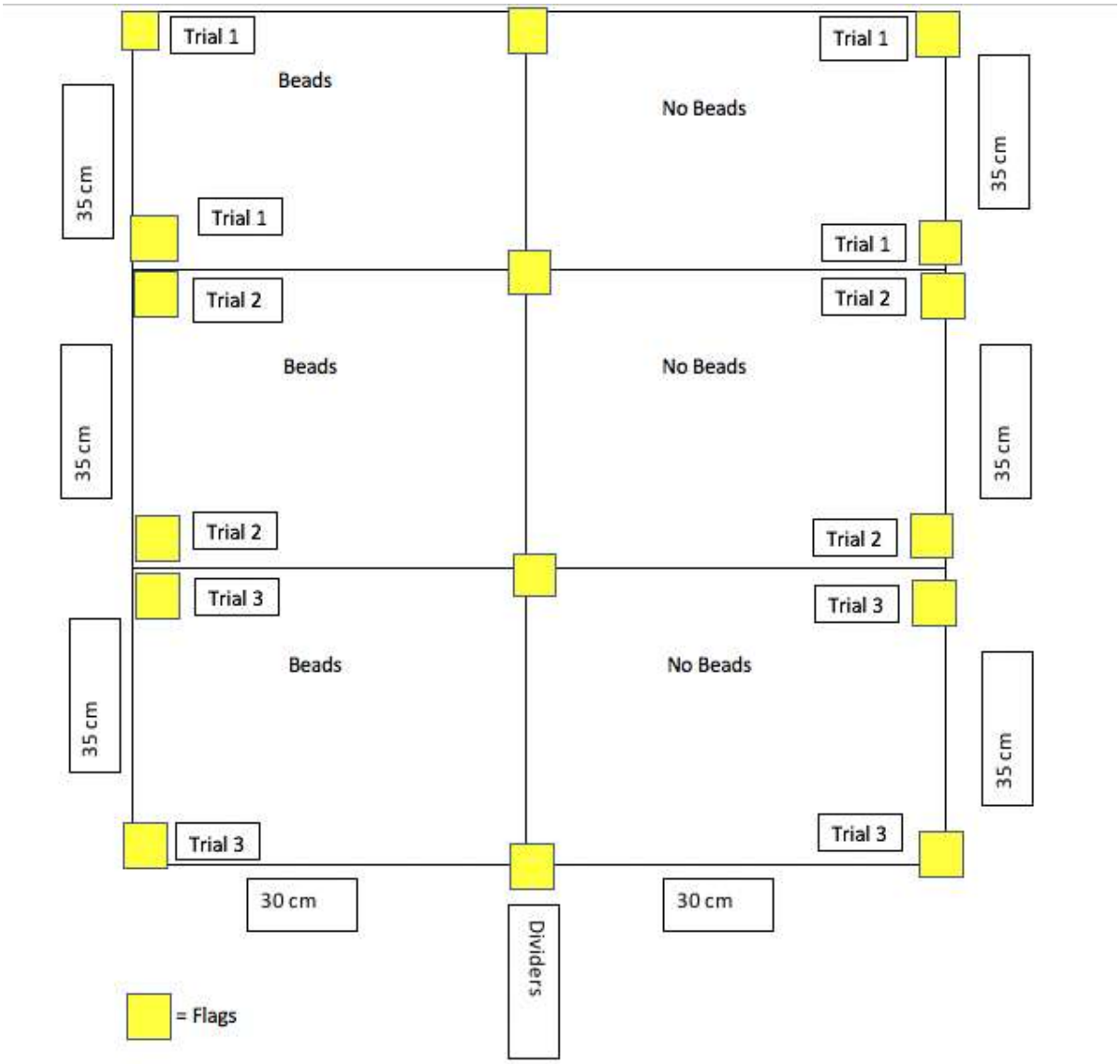
E. List of controlled variables:

- Location (39°21.427 North and 076°38.211 West)
- Amount of beads added onto each plot (20.8 grams)
- How long beads stay on soil (72 hours)
- Time of day when the turf beads are put onto the soil (9 am)
- (if it doesn’t rain) spread equal amounts of water on each plot
- Amount of soil tested (1 cc)

- Nutrient agar (called 3M Petrifilm™ Aerobic Count Plate)
- All soil dilutions must occur on the same day at as close to the same time as possible
- Number of soil samples from each plot (3 separate samples)
- Size of each soil sample (15 cm mark on the 2 cm in diameter core soil extractor)
- Time sequence each soil sample is taken (all on the same day at same time)
- Type of water used in possible rain simulation (tap water)
- Amount of water used in possible rain simulation (2 liters)
- What the water in the possible rain simulation is poured from (watering can capable of holding 2 liters of water)
- Size of culture tubes (15 ml)
- How long bacteria are given to grow on nutrient agar (48 hours)
- Size of plots (35 by 30 cm)
- Number of flags used to mark plots (16 flags)
- Size of serological pipette (10 ml)
- How much turf beads in each cup (20.8 grams)
- How much turf beads to collect in the main cup/in all (67.9 grams)
- Type of tool used to sift debris from turf (metal sifter)
- Amount of sterile water in the 10^{0} culture tube (10 ml)
- Amount of sterile water in the $10^{-1,-2 \text{ and } -3}$ culture tubes (9 ml)
- Amount of diluted soil transferred from each culture tube (1 ml)
- Amount of diluted soil placed onto the petrifilm (100 microliters)
- Material used to transfer the soil dilution from the $10^{-2 \text{ and } -3}$ culture tubes (micropipettes)
- Size of micropipette (p200)
- Type of pipette for diluting (15 ml serological pipette)
- Which dilutions plated (10^{-2} and 10^{-3})
- Degree diluted to (10^{-3})

Procedure for flags:

1. Obtain 16 yellow flags.
2. Go to the exact coordinates North 39°21.427 and West 076°38.211.
3. Insert the flags into the soil according to the pattern in the diagram below



Procedure for turf beads and soil samples:

1. Obtain 3 plastic cups. Label one cup “turf 1”, label the second “turf 2”, and label the third “turf 3”.
2. Take 67.9 grams of turf beads from a synthetic turf field.
3. Sift the turf beads to get rid of any debris using a metal sifter.

4. Once the turf beads are sifted, distribute them equally among the three cups labeled “turf 1”, “turf 2”, and “turf 3”. Put 20.8 grams in each cup.
5. Obtain 18 plastic bags.
6. Label 3 plastic bags “negative b-1”, another 3 plastic bags “negative b-2,” and another 3 plastic bags “negative b-3.” (“B” stands for “before”)
7. Label another 3 plastic bags “with beads b-1”, another 3 plastic bags “with beads b-2”, and the final 3 plastic bags “with beads b-3”. (“B” stands for “before”)
8. To take soil samples, use a compact one soil probe/soil core extractor.
9. All soil samples (steps #10-17) must be taken on the same day around the same time.
10. Insert the soil core extractor into the soil of the plot labeled “no beads trial 1” (see diagram “A”) and take a core of soil. (15 cm in depth and 2 cm in diameter). Place the core of soil into the plastic bag labeled “negative b-1”.
11. Repeat step #10 with the plot “no beads trial 2”, (see diagram “A”) and empty the contents into the plastic bag labeled “negative b-2”.
12. Repeat step #10 with the plot “no beads trial 3”, (see diagram “A”) and empty the contents into the plastic bag labeled “negative b-3”.
13. Repeat step #10 with the plot “beads trial 1”, (see diagram “A”) and empty the contents into the bag labeled “with beads b-1”
14. Repeat step #10 with the plot “beads trial 2”, (see diagram “A”) and empty the contents into the bag labeled “with beads b-2”
15. Repeat step #10 for the last time with the plot “beads trial 3”, (see diagram “A”) and empty the contents into the bag labeled “with beads b-3”.
16. Repeat steps #10-15 two more times, so that you have 3 soil samples for each plot.
17. Steps #18-21 must be completed all on the same day at the same time
18. Take the cups labeled “turf 1”, “turf 2”, and “turf 3” with the 20.08 grams of turf beads to the land plots located at 39°21.427 North and 076°38.211 West.
19. Distribute by sprinkling evenly all of the contents of the cup labeled “turf 1” onto the plot labeled “beads trial 1” (see diagram “A”)

20. Repeat step #19 with the cup labeled “turf 2”, with the plot labeled “beads trial 2” (see diagram “A”)
21. Repeat step #19 with the cup labeled “turf 3”, with the plot labeled “beads trial 3” (see diagram “A”)
22. Leave the newly distributed turf beads along the plots alone for 72 hours. Make sure during these 72 hours it will rain.
23. If it does not rain during the waiting period, 2 liters of “rain” (tap water) must be simulated by pouring (by watering can able to hold 2 liters of water) the 2 liters of tap water equally on all of the plots, all on the same time. It must be poured 36 hours from initial turf spreading (steps #18-22).
24. Wait 72 hours before taking soil samples again.
25. During the 72 hours of waiting, obtain 18 new plastic bags.
26. Label 3 of the plastic bags “negative a-1”, 3 more of the plastic bags “negative a-2”, and 3 more of the plastic bags “negative a-3” (“A” stands for “after”)
27. Label 3 of the plastic bags “with beads a-1”, 3 more of the plastic bags “with beads a-2”, and the last 3 plastic bags “with beads a-3”. (“A” stands for “after”)
28. After 72 hours, return to the plots and take soil samples from each plot.
29. All soil samples (steps #30-36) must be completed on the same day at the same time.
30. Do step #10 with the plot “no beads trial 1” (see diagram “A”) into the bag “negative a-1”
31. Do step #10 with the plot “no beads trial 2” (see diagram “A”) into the bag “negative a-2”.
32. Do step #10 with the plot “no beads trial 3” (see diagram “A”) into the bag “negative a-3”.
33. Do step #10 with the plot “beads trial 1” (see diagram “A”) into the bag “with beads a-1”
34. Do step #10 with the plot “beads trial 2” (see diagram “A”) into the bag “with beads a-2”
35. Do step #10 with the plot “beads trial 3” (see diagram “A”) into the bag “with beads a-3”.
36. Repeat steps #30-35 two more times, so that you have 3 soil samples from each plot.

Procedure for extracting bacteria:

1. All soil dilutions (steps #2-31) must be completed on the same day at the same time.

2. Before diluting bacteria, combine all of the soil within the bags labeled “negative b-1” into a bag.
3. Repeat step #2 with the bags labeled “negative b-2” into a different bag.
4. Repeat step #2 with the bags labeled “negative b-3” into a different bag.
5. Repeat step #2 with the bags labeled “with beads b-1” into a different bag.
6. Repeat step #2 with the bags labeled “with beads b-2” into a different bag.
7. Repeat step #2 with the bags labeled “with beads b-3” into a different bag.
8. Repeat step #2 with the bags labeled “negative a-1” into a different bag.
9. Repeat step #2 with the bags labeled “negative a-2” into a different bag.
10. Repeat step #2 with the bags labeled “negative a-3” into a different bag.
11. Repeat step #2 with the bags labeled “with beads a-1” into a different bag.
12. Repeat step #2 with the bags labeled “with beads a-2” into a different bag.
13. Repeat step #2 for the final time with the bags labeled “with beads a-3” into a different bag.
14. Use a clean,10 ml serological pipette to add 10 ml of sterile water into a 15 ml culture tube. Label the tube “10^0 negative b-1”.
15. Use the same pipette to add 9 ml of sterile water to a second 15 ml culture tube. Label the tube “10^{-1} negative b-1”.
16. Repeat step #14 two more times to two additional 15 ml culture tubes, only label them “10^{-2} negative b-1”, “10^{-3} negative b-1”
17. Place 1 cc of the soil sample from the bag labeled “negative b-1” into the “10^0 negative b-1” culture tube.
18. Cap the tube and shake vigorously.
19. Using a new clean 10 ml serological pipette, remove 1 ml of the soil/water mixture from the “10^0 negative b-1” tube and place into the “10^{-1} negative b-1” tube.
20. Cap and shake vigorously.
21. Using the same pipette in #19, remove 1 ml of the soil/water mixture from the “10^{-1} negative b-1” pipette tube and place into the “10^{-2} negative b-1” tube.
22. Cap and shake vigorously.

23. Using the same pipette in #19, remove 1 ml of the soil/water mixture from the "10^{-2} negative b-1" tube and place into the "10^{-3} negative b-1" tube.
24. Cap and shake vigorously.
25. There should now be a total of 4 culture tubes.
26. Place 100 microliters of the sample from the 3rd tube (dilution "10^{-2} negative b-1") onto its own correspondingly labeled 3M Petrifilm™ Aerobic Count Plate
27. Place 100 microliters of the sample from the 4th tube (dilution "10^{-3} negative b-1") onto its own correspondingly labeled 3M Petrifilm™ Aerobic Count Plate
28. Repeat steps #14-27 two more times using "negative b-2" the first time, then using "negative b-3" the second time, labeling the culture tubes corresponding to the names of the soil sample. Also label the plates containing nutrient agar corresponding to the name of the culture tubes.
29. Repeat steps #14-27 three more times using the bag labeled "with beads b-1" the first time, "with beads b-2" the second time, and "with beads b-3" the last time, labeling the culture tubes corresponding to the name of the soil sample. Also label the plates containing nutrient agar corresponding to the name of the culture tubes.
30. Repeat steps #14-27 three more times using the bag labeled "negative a-1" the first time, "negative a-2" the second time, and "negative a-3" the third time, labeling the culture tubes corresponding to the name of the soil sample. Also label the plates containing nutrient agar corresponding to the names of the culture tubes.
31. Repeat steps #14-27 three more times using the bag labeled "with beads a-1" the first time, "with beads a-2" the second time, and "with beads a-3" the third time, labeling the culture tubes corresponding to the name of the soil sample. Also label the plates containing nutrient agar corresponding to the names of the culture tubes.
32. Allow time for the bacteria to grow for 48 hours.
33. Examine each of the plates for individual bacteria colonies and choose the plate with the lowest dilutions and fewest colonies (but at least 5 colonies) to make estimates of the number of bacteria in the original 1 cc soil sample using the following formula:

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

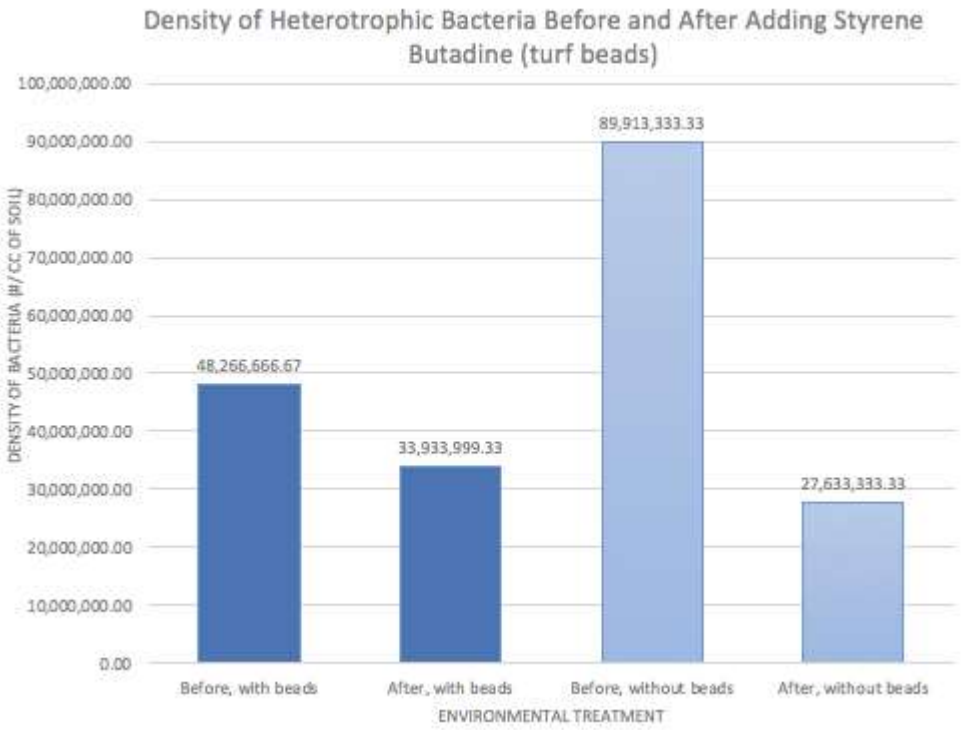
34. Record all data in the data tables.

Data Table

The Impact of Turf Beads on the Density of Soil Bacteria (#/cc of soil)

	Density of Bacteria with Beads Present		Density of Bacteria with No Beads	
	Before adding turf	After adding turf	Before adding turf	After adding turf
Trial #1	3,540,000	10,400,000	32,000,000	8,800,000
Trial #2	32,200,000	67,500,000	104,500,000	28,000,000
Trial #3	234,000,000	5,000,000	8,300,000	65,000,000
Averages	89,913,333.33	27,633,333.33	48,266,666.67	33,933,333.33

Graph



Conclusion

Our hypothesis was: The excess styrene butadiene (turf beads) tracked onto the soil by humans will decrease the density of heterotrophic bacteria in the soil. Our hypothesis was correct. The recorded data (how many colonies of bacteria on each nutrient agar plate) strongly supported our hypothesis. Our data showed that on average, the number of colonies of bacteria (density) of the soil collected from the corresponding plots (plots labeled “beads trial 1, 2, and 3”) was 89,913,333.33 bacteria microbes per 1 cc of soil before we distributed the turf beads on the plots. After we distributed the turf beads onto the corresponding plots (plots labeled “beads trial 1, 2, and 3”) and left the plots alone for 72 hours, the average number of bacteria microbes per 1 cc of soil was 27,633,333.33. This exhibits a 62,280,000 decrease in the number of colonies from before distribution and after distribution of the turf beads. Before the 72 hours of leaving the plots alone, the soil from the corresponding plots (the plots labeled “no beads trial 1, 2, and 3”) on average had 48,266,666.67 bacteria microbes per 1 cc of soil. After leaving the corresponding plots alone (plots labeled “no beads

trial 1, 2, and 3") for 72 hours, we returned, and collected soil samples from the plots. The average number of bacteria microbes per 1 cc of soil was 33,933,333.33, showing a 14,333,333.34 difference in the number of bacteria colonies from the before and after. Already, proven by the decrease in bacteria density over the 72 hour time period for the negative control plots in which turf was not added (roughly 14 million colonies), survival became very hard for the bacteria, prompting them to decrease in numbers and density. For the plots with the turf added, there was an even greater drop in bacteria density over the 72 hours after the turf beads were distributed on the plots (roughly 62 million colonies). This proves that survival was extremely hard for bacteria, much more than the bacteria in the soil of the plots that had no turf beads distributed on them before the 72 hour waiting period. Because of this drastic drop exhibited after the 72 hour time-lapse for the plots with beads added (roughly 62 million colonies) compared to the negative control where turf was not added (roughly 14 million colonies), concludes that the styrene butadiene added on the soil was the cause for the bacteria dropping in density, proving our hypothesis to be correct. This happened because the styrene butadiene was harming the bacteria and making it very difficult for them to live and thrive in the soil with the effects of the styrene butadiene.

These results from our experiment allowed us to further question how excess styrene butadiene (turf beads) increase or decrease the density of heterotrophic bacteria in the soil. The first question we established was how the location/orientation of the plots help to increase or decrease the density of heterotrophic bacteria in the soil. For our particular experiment, our specific location allowed us to control for amounts of fertilizer, growth of plants and species, and exposure to sunlight. This allowed us to manipulate only the turf beads without any additional influencers on the soil. If we were to carry out this experiment again, we could place our plots in an area that has limited sunlight, seeing how these conditions would further affect the density of the bacteria. We could also place our plots in an area on campus that would have fertilizer and an abundance of growing plant species in order to observe the effects of turf beads on the soil in any location.

The previous question of location also brought up another question: size of the plots in relation to how many turf beads were distributed. For our experiment, we decided that each of our plots would be 35x 30 cm. We distributed 20.08 grams of turf beads onto each plot. If we were to

manipulate the size of plot, it would result in the beads either being more pact together or more spaced out. How, if we were to control this, would it further affect the data outcomes of our experiment? If we were able to manipulate the spacing of the turf beads, we may be able to observe how this would affect our overall results of our experiment.

The next question we asked was how the amount of time given for the turf beads to increase or decrease the amount of heterotrophic bacteria in the soil play a role in how much bacteria forms over that given time. For our specific experiment, we allotted 72 hours for the turf to effect the soil in the plots. After the elapsed 72 hours, we collected the soil samples from the plots and diluted them. We waited an additional a 48 hours for bacteria colonies to form. In order to further understand the importance of timing in this experiment and how it affects the data outcomes, we could possibly lengthen or shorten the time allotted for the turf beads to increase/decrease the amount of heterotrophic bacteria in the soil before taking soil samples and diluting them. If we were to manipulate this aspect, we may be able to observe how time allotted affects the outcomes of our experiment.

Finally we wanted to further question how the season played a role in the increase or decrease of heterotrophic bacteria in the soil. Our experiment took place in the springtime, during late May. During the 72 hours that we waited after applying turf beads, the plots experienced various amounts of rain. In order to manipulate the temperature and effects of weather, we could conduct our experiment in a different season such as fall or winter, in which temperatures are generally cooler and different forms of weather may occur. During the spring and summertime, the temperatures increase. Turf beads attract sunlight due to their distinct dark color and rubber material. This means that when turf beads are distributed onto the soil, there is a greater chance of drying out the soil. In the fall or winter, the chances of sunlight and warm temperatures affecting the soil is less likely. Therefore, if our experiment was conducted in fall or winter, we may experience different data outcomes in our experiment due to the different atmospheric effects.

To conclude, our hypothesis that turf beads will decrease the amount of heterotrophic bacteria within the soil was correct. We know this because during the 72 hours of waiting after the turf beads were distributed, there was a greater decrease in heterotrophic bacteria density within the soil of the

plots that turf beads were distributed on, opposed to the soil of the plots that received no turf beads. This proves, as we stated, that after turf bead distribution, life became much harder for heterotrophic bacteria in that soil, causing them to decrease in density. Although this particular experiment proved our hypothesis to be correct, altering and manipulating certain aspects of our experiment may exhibit different data results. This may include altering location of the plots in which we conducted our experiment, the size of the plots and amount of turf beads spread on that plot, time given for turf beads to affect the density of bacteria, and the season in which we conducted our experiment. Our particular experiment allowed us to learn how turf beads affect the density of bacteria, however, there are still many outcomes we did not consider, or test for, concerning how turf beads (styrene butadiene) affects the density heterotrophic bacteria in the soil.