The Effect of Heat Sinks on Protozoa Population Density

Paige Carey, Ally Cirelli, Grace Laria, and Emma McCormack

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Mr. Brock

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Background of Protozoa and Its Importance

Microbes are the foundation of any ecosystem. They decompose organic matter, break down inorganic substances, and make all these various nutrients available for all the other organisms in the ecosystem either directly (such as plants) or indirectly (such as animals). One of these microbes, protozoa, are among the most diverse eukaryotes in the evolutionary tree, and in fact, they are so diverse that the only distinguishing attributes they share as a group are the fact that they are unicellular, have no cell wall, and employ motility to go acquire their food. It is these differences in motility that scientists actually use to classify the various kinds, and so for example, amoebas use pseudopodia, or extensions of their plasma membrane and cytoplasm, to move and engulf their prey; flagellates use a whip-like tail; the ciliates use fine hair-like structures which are extensions of the membrane to move; and sporoza move by means of gliding (Wiser, 2008).

Because they are heterotrophs, the way protozoa contribute to the decomposition and breakdown process in the soil is to eat bacteria and release carbon and nitrogen compounds during the process of mineralization. Specifically, what the protozoa do is to break down the organic material of the consumed organism, using these nutrients to complete each crucial task needed for their own existence while releasing excess materials necessary to the other organisms. (Wiser, 2008; Conant, 2008; Education, 2004).

Specifically, protozoa are responsible for moving through the ecosystem two particularly important nutrients that are critical for all life: carbon and nitrogen (Zuberer, 2008). Carbon is the foundation of all biological molecules, while nitrogen is a key component of the amino acids and nucleotides that are the monomers of the enzymes, proteins, and nucleic acids which direct the chemical reactions that enable a cell to perform its four fundamental tasks of life (respiration, regulation, reproduction, and synthesis). Hence, without nitrogen, the chemicals that support all life and determine an organism's phenotype would simply not exist, and life as we know it would not be possible.

The nitrogen that is necessary for all life, though, is made available through the nitrogen cycle, and that is where the soil protozoa play such a crucial role. First, despite the fact that pure nitrogen makes up 70% - 80% of the earth's atmosphere, it cannot be utilized directly by any complex living organism and must be converted into other forms that the plants and then the consumers can use for their own biological needs (Brock, 2012; Ho, Leonard, 2002). Certain groups of bacteria in the soil, many located within the roots of plants, take the nitrogen from the air and convert it into the organic form of ammonia (NH_3), later releasing it in its inorganic form ammonium (NH_4^+), which is one of the forms that plants can use to make their own nucleic acids and proteins. Still other bacteria then take any excess released ammonium and convert it into nitrite ($N0_2^-$), followed by a third type of bacteria that must then convert it into nitrate (NO_3^-), the other form of nitrogen plants can utilize.

At all points in this cycle, protozoa play a key role by eating the bacteria that are producing the ammonium, nitrite, or nitrate. By eating the bacteria, the protozoa release any extra ammonium, nitrate, or nitrate remaining inside the bacteria, thereby increasing the availability of these key nutrients for use by plants (Brock, 2012; Ophardt, C. 2003; Brencas-Moreno, 2009),

and what is more, by eating and killing bacteria, protozoa stimulate the growth of the bacterial population, which increases the decomposition rate, making even more nitrogen available for plants to use as well to carry out their biological processes.

In addition, protozoa also regulate algae and fungi populations, and because some of these species are pathogenic, by eating these pathogens, protozoa thereby prevent disease in the soil that can affect a whole host of organisms. Furthermore, protozoa themselves are an important link in the soil food chain, serving as food for organisms such as nematodes and earthworms (Brock, 2012; Hoorman, J., 2011). It is in this latter role that protozoa play an indirect but important role in the movement of water through the ecosystem. By needing to plow through the soil in order to capture their protozoan prey, earthworms create pathways for water to reach the roots of producers, which increases the rate at which the ecosystem can cycle water (Conrad, 2011). This, in turn, results in a process known as evapotranspiration, in which plants release moisture through transpiration and the evaporation of water from the soil, thereby cooling the environment (Saxena, 2011; Dictionary.com LLC, 2012). In fact, areas such as parks or other plant-filled regions have been shown to have so much lower temperatures over areas containing no plant life (Loehrlein, 2012) that "the net cooling effect of a young, healthy tree is equivalent to ten room-size air conditioners operating 20 hours a day" (Arbor Day Foundation., n.d.). Thus, if there are fewer protozoa and consequently fewer earthworm trails, the cooling effect of plants on the areas surrounding them is reduced as well.

Unfortunately, the human population has replaced once green, unpolluted regions with its own synthetic structures, exposing areas of soil to the heat often created by these materials. Increased urbanization, specifically the building of roads and other structures that utilize asphalt, cement, or turf, has led to the rise of temperature in the soil, and materials such as asphalt and

concrete conduct and trap heat from the sun, causing their surfaces to become very hot (Heimbuch, 2008; Larson, 2012). In fact, the typical thermal conductivity of asphalt is 0.75 W/(mK) and even the thermal conductivity of the rubbers used in turf fields range from 0.14-0.35 W/(mK). But the archetypal conductor of heat is cement at1.73 W/(mK), and that is a principal reason why cities and other urban areas are higher in temperature than more rural regions (*Engineeringtoolbox.com*, n.d; Lasance, 2001).

Heat sinks, then, such as turf, cement, or asphalt near areas of soil can cause the soil itself to become warmer. Yet what impact is this having on the density of microbes, including protozoa, in soils near these locations? Moreover, studies have shown that, when exposed to warmer temperatures, protozoa tend to reproduce more quickly and, as protozoa reproduction rates increase, the rate of decomposition and mineralization may correspondingly increase as well. (Education, 2004). Hence as a consequence, more nitrogen may be fixed in the soil, thereby increasing the number of producers and consumers in the environment, as crucial nitrogen moves up through the food chain (Brock, 2012), stabilizing the environment.

In our experiment, we decided to test the effects of heat sinks on the population density of protozoa. We believed that areas of soil near heat sinks constructed of turf, asphalt, and concrete would have a greater population density of protozoa than an area of soil farther away from such structures.

Soil Ecology Experiment & Procedure

I. Problem:

- How do soil heat sinks impact soil protozoa density?

II. Hypothesis:

- Areas with more pronounced heat will cause an increase in protozoa population density.

III. Procedure:

A. Independent Variable:

- Location of heat sink by which the soil sample is taken

B. Dependent Variable:

- Population density of protozoa in each sample of soil

C. Negative Control:

- Soil sample taken from the center of the front lawn (the site farthest from the potential heat sinks on campus)

D. Positive Control

- Temperature of the soil at each location being studied

E. Controlled Variables:

1. Amount of soil taken from each area (15 centimeters in depth, 2 centimeters in width)
2. Type of plant life in areas tested
3. Amount of soil sifted from each petri dish (9-10 grams)
4. Amount of methyl-green stain (7µL)
5. Rehydration time (7 hours)
6. Amount of distilled water used for saturation
7. Amount of distilled water used for Uhlig Extractor filtration
8. Type of plastic bag (using different ones each time)
9. Number of samples from each location (3)

10. Day and time of collection of soil
11. Size/shape/type of petri dish
12. Time at which they are put in and taken out of refrigerator (same time)
13. Type of pipettes used to prepare microscope slides
14. Environment in which soil samples are observed and tested on (lab room)
15. Time waited for drying (24 hours)
16. Size/ shape/ type of nylon mesh cover slip (1mm squared) used for sifting
17. Type of Uhlig extractor
18. Time allowed for filtration period in Uhlig Extractor (24 hours)
19. Size of qualitative filter paper (12.5 cm)
20. <u>All soil samples that have water or other chemicals added to them or must sit for a specific period of time must be tested upon at the same time (Filtration periods)</u>
21. Capillary tube used
22. GPS coordinate of each separate soil sample location
23. Amount of filtrate (18µL) used in microscope observation
24. Size of cover slip (18 X 18 mm squared)
25. Magnification of light microscope (40x)
26. Distance of soil sample from the heat sink (20 centimeters)
27. Thermometer used to measure temperature of soil (Electronic digital thermometer inserted twenty centimeters into the soil)

F. Procedure 1 (Measurement of Protozoa Population Density)

1. **For steps 1-5, all soil samples need to be taken from each location on the same day at the same time, and each sample should be placed in its own separate, new Ziploc bag**
2. At GPS location N 39° 21.482, W 076° 38.153 (the center of the front lawn) take three 15 centimeter (depth) by 2 centimeter (width) samples of soil using the soil auger and place each sample into its own separate Ziploc sandwich bag labeled “Negative Control 1,” “Negative Control 2,” “Negative Control 3” respectively
3. At GPS location N 39° 21.503, W 076° 38.170 (the area of soil/grass 20 centimeters from the concrete walkway) take three 15 centimeter (depth) by 2 centimeter (width) samples of soil using the soil auger and place each sample into its own separate Ziploc sandwich bag labeled “Concrete 1,” “Concrete 2,” “Concrete 3” respectively
4. At GPS location N 39° 21.512, W 076° 38.169 (the area of soil/grass 20 centimeters from the hill to the right of the turf field) take three 15 centimeter (depth) by 2 centimeter (width) of soil using the soil auger and place each sample into its own separate Ziploc sandwich bag labeled “Turf 1,” “Turf 2,” “Turf 3” respectively
5. At GPS location N 39° 21.441, W 076° 38.227 (the area of soil/grass 20 centimeters from the asphalt road in the back driveway) take three 15 centimeter (depth) by 2 centimeter (width) samples of soil using the soil auger and place each sample into its own separate Ziploc sandwich bag labeled “Asphalt 1,” Asphalt 2,” “Asphalt 3” respectively

6. Label 12 separate clean petri dishes corresponding to the respective labels of each Ziploc bag
7. **For step 8, all soil sample should be left to air dry at the same time**
8. Pour each soil sample into its own respectively labeled, separate petri dish and allow each soil sample to air-dry (each petri dish has its lid off) for twenty four hours
9. Grind each soil sample using a mortar and pestle and then place each soil sample into its own separate, small plastic cup, correspondingly labeled according to step 6, and cover each cup with a $1mm^2$ nylon mesh (make sure to wash and dry each mortar and pestle thoroughly between each soil sample)
10. Sift 9-10 grams of each soil sample into its own new, clean petri dish correspondingly labeled according to step 6
11. **For step 12, the distilled water should be added to all soil samples at the same time (if necessary, all soil samples can be placed in the refrigerator after step 12 to stop microbe activity, but all soil samples must be placed in the refrigerator at the same time. This is true for step 12, steps 14-15, step 17, and steps 21-22)**
12. Saturate each soil sample with 20 milliliters of distilled water and allow all the samples to sit for seven hours at room temperature
13. **For steps 14-15, the distilled water should be added to all soil samples at the same time, and all soil samples should be filtered in the Uhlig extractor at the same time**

14. Add 30 milliliters of distilled water each into separate 100x15 millimeter petri dishes, labeled according to step 6
15. Place the Uhlig extractors in the water-filled petri dishes and scoop each rehydrated soil sample from step 12 into its own separate extractor (each extractor should be correspondingly labeled according to step 6), allowing each sample to filter for twenty four hours
16. **For step 17, all soil samples should be filtered again at the same time**
17. Filter each sample a second time, using qualitative filter paper, into its own separate small plastic cup correspondingly labeled according to step 6
18. Prepare microscope slides for viewing the second filtrates; **slides should be prepared as shown in steps 18a-18f, making sure that all 2nd filtrates are examined at the same time with each sample on its own, separate slide**
    - 18a. Add 7ul (7 drops) of methyl green dye to a slide labeled "Asphalt 1" using a capillary tube
    - 18b. Add 18ul (18 drops) of the Asphalt 1 filtrate to the microscope slide labeled "Asphalt 1" each using a graduated Beral-type pipette and cover the slide with an 18x18mm^2 cover slip
    - 18c. Examine the slide labeled "Asphalt 1" under a light microscope at 60x
    - 18d. Count the number of protozoa per field of view by looking at five different fields of view (i.e. the microscope slide's center, top

corner, bottom corner, left corner, and right corner) and take the average of those numbers

18e. Use this average field per view in the equation "[(# per field of view at 60X) • (total ml of water used) • 2165] ÷ (grams of sifted soil)" to find the number of protozoa per gram of soil

18f. Record the number of protozoa per gram of soil and repeat process with each soil sample for the number of allotted days

G. Procedure 2 (Measurement of Heat Sink Temperature)

1. **For steps 2-9, each temperature measurement should be taken from each location on the same day and at the same time (preferably at the end of the day, around 3:45-4:00 P.M.)**
2. At GPS location N 39° 21.482, W 076° 38.153 (the center of the front lawn) use an electronic digital thermometer inserted 20 centimeters into the soil to take its temperature
3. Record soil temperature data
4. At GPS location N 39° 21.503, W 076° 38.170 (the area of soil/grass 20 centimeters from the concrete walkway) use an electronic digital thermometer inserted 20 centimeters into the soil to take its temperature
5. Record soil temperature data
6. At GPS location N 39° 21.512, W 076° 38.169 (the area of soil/grass 20 centimeters from the hill to the right of the turf field) use an electronic digital thermometer inserted 20 centimeters into the soil to take its temperature

7. Record soil temperature data
8. At GPS location N 39° 21.441, W 076° 38.227 (the area of soil/grass 20 centimeters from the asphalt road in the back driveway) use an electronic digital thermometer inserted 20 centimeters into the soil to take its temperature
9. Record soil temperature data

IV. Data Tables & Graphs

a. Figure 1: Measurement of Protozoa Population Density (Number of protozoa/gram of soil)

Heat sink near which soil sample is taken	Number of protozoa/gram of soil in Sample 1		Number of protozoa/gram of soil in Sample 2	Number of protozoa/gram of soil in Sample 3		Soil Sample Average
Turf	Turf1	Turf1a	297688	Turf3	Turf3a	175517
	82042	380584		29318	87953	
Front Lawn (Negative Control)	2016868		1474479	1117419		1536255
Asphalt	87953		37177	36083		53738
Concrete	682638		546717	182316		470557

*Note: The samples Turf1 and Turf3 were examined two separate times under the microscope.

b. Figure 2: Measurement of Heat Sink Temperature (degrees Celsius)

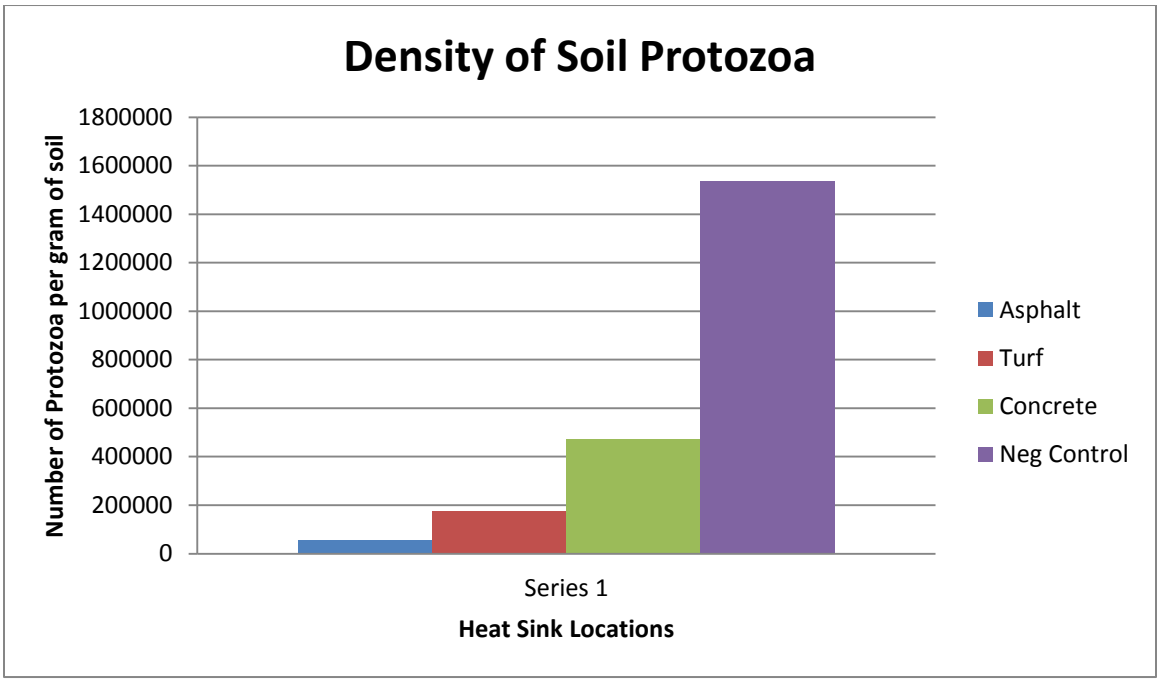
Heat Sink	Temperature taken, 5.15.2012	Temperature taken 5.16.2012	Temperature taken 5.17.2012	Temperature taken 5.18.2012
Front Lawn (Negative Control)	19.7°C	19.2°C	19.9°C	18.4°C
Turf	22.4°C	23.6°C	24.6°C	23.3°C
Asphalt	20°C	19.7°C	21.7°C	21.6°C
Concrete	19°C	19.4°C	19.9°C	17.7°C

c. Figure 3: Heat Sink Temperature Average (degrees Celsius)

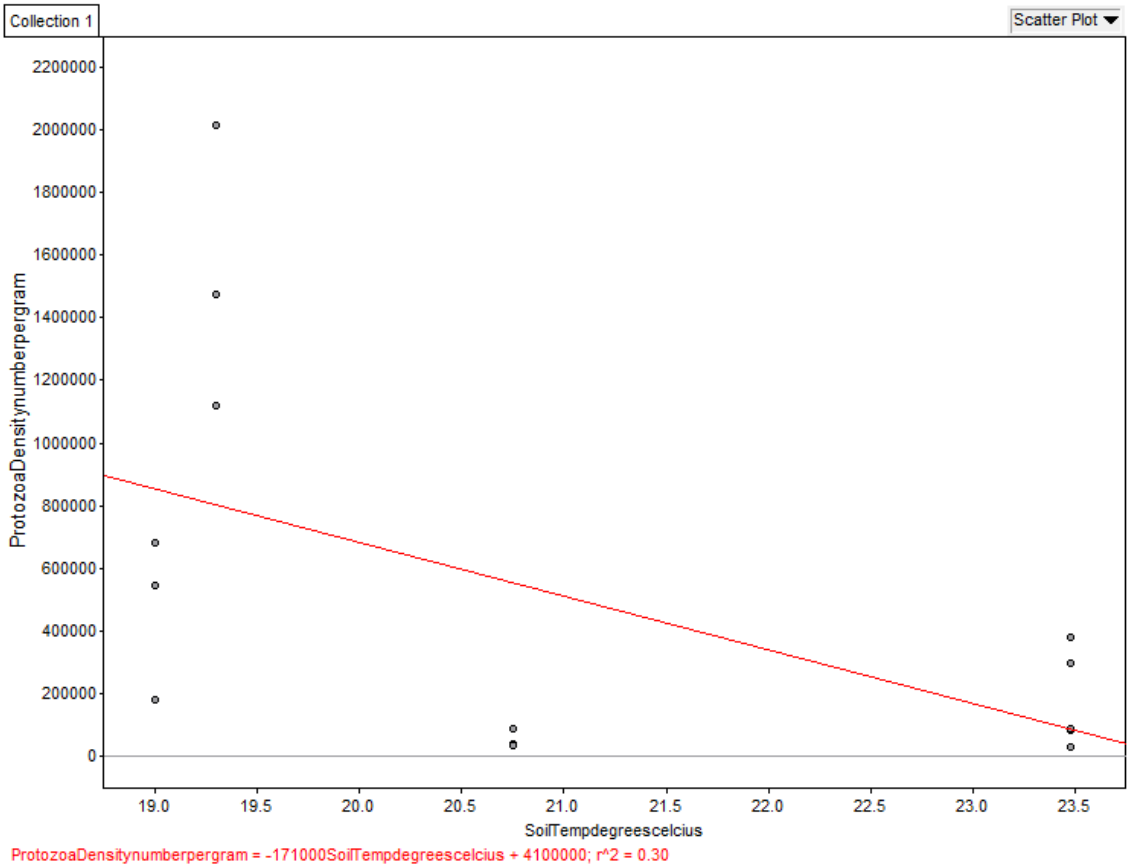
Heat Sink	Average

Front Lawn (Negative Control)	19.3°C
Turf	23.475°C
Asphalt	20.75°C
Concrete	19°C

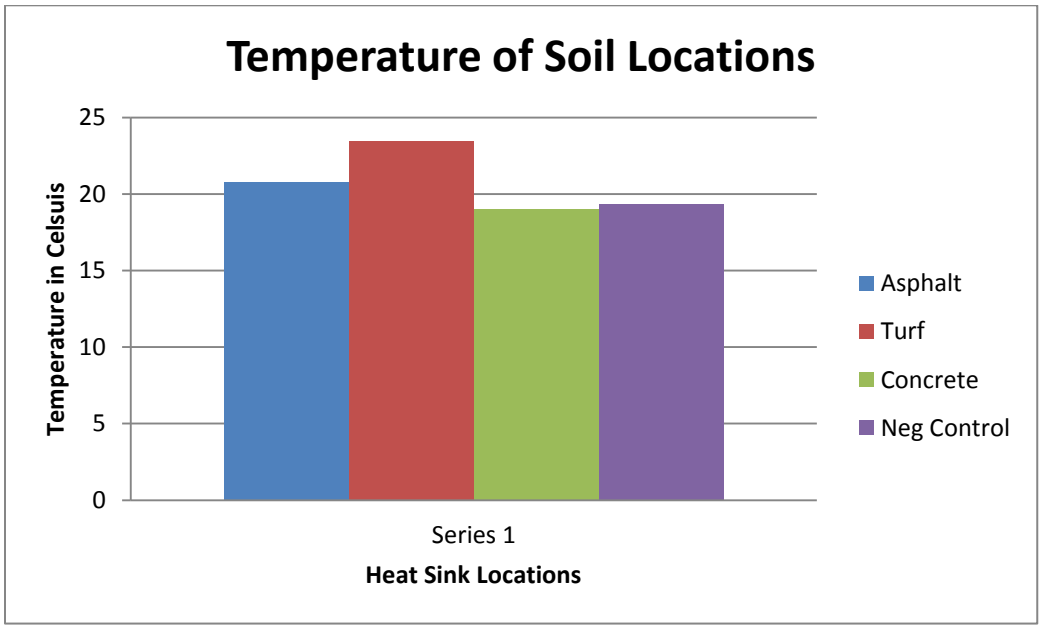
d. Figure 4: Density of Soil Protozoa (Number of protozoa/gram of soil)



e. Figure 5: Density of Soil Protozoa Regression Graph (Number of protozoa/gram of soil)



f. Average Soil Temperature Graph (degrees Celsius)



V. Conclusion

Soil near turf had the highest temperature at 23.475° Celsius, followed by soil near asphalt at 20.75° Celsius, soil near the front lawn (the negative control) at 19.3° Celsius, and finally the soil near concrete at 19° Celsius. While it was expected that the soil near concrete would have the highest temperature, our data shows that, in fact, it was the lowest in temperature. We believe that this is true because, at the time of day that the temperatures were taken (3:45-4:00 P.M.), the area of soil near concrete was shaded, while the front lawn (the negative control) was situated in direct sunlight.

The execution of this experiment proves the hypothesis to be incorrect. It was hypothesized that the soil near the hottest heat sink would have the highest protozoa population density. After completing this experiment, we found that the soil taken from the front lawn (the negative control) had the highest protozoa population density at 1,536,255 protozoa per gram of soil, therefore proving the hypothesis incorrect as the front lawn did *not* conduct the most heat. The soil samples taken from the area near concrete had the second highest protozoa with 470,557 protozoa per gram of soil. The area of soil with the lowest protozoa population density was near asphalt at 53,738 protozoa per gram of soil, followed by the samples taken from the soil near turf at 175,517 protozoa per gram of soil. Although the prediction that heat causes protozoa population density to increase was incorrect, it can still be said that heat impacts protozoa populations.

Instead of increasing the density of protozoa populations, heat sinks appear to cause a decrease in the amount of protozoa per gram of soil. Above, in Figure 5, the regression data shows that there still is a 30% likelihood that as heat goes up, the number of protozoa goes down. Ecologically speaking, 30% is a large possibility and therefore it can be assumed that building heat-conducting structures negatively affects soil protozoa by decreasing their population

density. For example, the soil near turf conducted the most heat and had the second lowest protozoa count at 175,517 protozoa/gram of soil. Despite the 30% likelihood of heat reducing protozoa populations, there are still two anomalies within the data that must be explained.

Although soil samples taken near asphalt did not have the highest temperature, they had the lowest protozoa population density at 53,738 protozoa/gram of soil. It would be expected that the soil with the lowest protozoa population density would have the highest temperature (i.e. turf at 23.475° Celsius). Instead, soil near asphalt had the most dramatic drop in protozoa, with 148,2517 protozoa/gram of soil less than the negative control while having a temperature 2.725° Celsius lower than the soil near turf. This may have occurred because, unlike turf, there is no drainage system near asphalt that removes water from its surface. Therefore, possibly dangerous chemicals located on the asphalt's surface could potentially seep into the soil as the water flows off the asphalt structure. This could result in a decrease of protozoa populations as these chemicals kill soil protozoa. So, while turf has the highest temperature, it does not pollute the soil with chemicals as asphalt does, therefore killing fewer protozoa. While chemicals on the surface of asphalt may explain its drop in protozoa population, compaction may be the reason behind the decrease in protozoa in the soil near concrete.

Even though the soil near concrete was 0.3 degrees Celsius less than the soil in the front lawn (negative control), it still had a smaller protozoa count at 470,557 protozoa/gram of soil. This seems to disprove the theory that, as heat rises, the number of protozoa decreases. However, this pattern change may have occurred because the building of concrete structures creates compaction in the soil. In past experiments conducted on this same campus, compaction has been shown to result in fewer protozoa in the soil near such structures (Gore, Futrell, Faust & Julio, 2011).

Clearly, the building of heat sinks damages the environment as it disrupts protozoa populations by decreasing them in density. Therefore, the amount of nitrogen in the ecosystem is decreased due to the lack of protozoa in the soil. As more protozoa die, they are unable to release enough excess nitrogen for plants and other organisms to use. Nitrogen is vital to all living things, as it is a key component in the structure of DNA. To explore the concept of heat sinks further, it would be helpful to research the effects of compaction and chemical runoff produced by materials like asphalt on protozoa populations in the soil. To do so, separate plots could be compacted to different degrees, while others could be exposed to chemicals associated with asphalt runoff. We would hypothesize that the more compacted and chemically treated areas of soil would have the least amount of protozoa.

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