How is the Global Increase in Carbon Dioxide Changing the Population Density of Soil Bacteria?

Soil Ecology Project

Madeline Kim

Katherine Harrison

Foula Protopapas

Annie Riley

Mr. Brock

Biology

18 May 2011

How is the global increase in carbon dioxide changing the population density of soil bacteria?

Soil Ecology Project

1. Background

Bacteria are living things that belong to a group all by themselves. They are prokaryotes which mean they are single celled organisms that do not have a nucleus. Bacteria are usually found in big groups because they can multiply quickly, and not every strain of bacterium is the same which is why they are separated into different groups based on the qualities that set them apart from all the rest. However, all bacteria have an outer enclosure called the cell membrane, and inside the membrane there is a fluid called cytoplasm that is about 70% water. The other 30% is filled with proteins, specifically enzymes, that the cell makes for itself in order to use energy. The DNA, which tells the cell how to make its proteins and other biological molecules, is found in the middle of the cell. Without this DNA, the bacteria would not be able to function.

In order to create the necessary chemicals and structures to survive, the bacteria in the soil take in nitrogen in the form of N_2 gas from the atmosphere. They then use it along with carbon from decomposing dead matter to produce their enzymes and biological molecules essential for their survival. Hence, without nitrogen from the atmosphere bacteria could not make amino acids and nucleotides, and since amino acids and nucleotides are the building blocks of the molecules that control life (DNA, RNA, and proteins), without fixed nitrogen, the bacteria could not survive: because where there are no proteins, there are no enzymes. Without enzymes, the chemical reactions between the five key biological molecules that cause a cell to perform the four key tasks of life (homeostasis, reproduction, synthesis, respiration) cannot take place.

Once bacteria use N_2 gas to create their own molecules, any leftover nitrogen is then converted into NH_4+ (ammonium) and NO_3- (nitrate) and released back into the soil (Ophardt,

2003). The extra ammonium and nitrate which the bacteria created are now absorbed by the plants from the soil through their roots. They in turn use these chemicals to create their own amino acids and enzymes which allow them to complete a process called photosynthesis (NCDA, 2011). This process creates sugars out of CO_2 (because carbon is an element used in every molecule essential for life), which are then used to make the other biological molecules the plants need. Then, the animals eat the plants, using the plants' biological molecules to produce the animals' own, and sooner or later, both the plants and animals die, and are decomposed by the bacteria in the soil which use these decomposed biological molecules to create their own enzymes and other molecules (Ophardt, 2003), and the cycle repeats: releasing CO_2 throughout the whole process for the plants to reabsorb in the photosynthesis stage.

However, there are other factors that now impact this complex cycling. As humans continue to burn fossil fuels, the CO_2 levels in the atmosphere continue to rise, and there are two possible consequences for soil bacteria due to this sudden increase in CO_2. The excess CO_2 could help the bacteria or it could suffocate them, depending on whether they are aerobic or anaerobic (Ophardt, 2003). Aerobic bacteria are found in soil and cannot live without oxygen and anaerobic bacteria are bacteria that can survive without oxygen. Both types of bacteria are an essential part of the nitrogen and carbon cycle, impacting both plants and animals in the environment, hence the increases in CO_2 emissions due to the burning of fossil fuels has the potential to have a profound impact on all life on this planet.

The potential positive effect is due to the greenhouse effect. Solar radiation passes through the clear atmosphere, and while most of the radiation is absorbed by the earth's surface and warms it, some solar radiation is reflected by the earth and the atmosphere. As this radiation passes through the atmosphere, some of its energy is absorbed and re-emitted in all directions by

greenhouse gas molecules. The more carbon that is in the atmosphere means the more radiation/ light is trapped in the atmosphere, warming the earth (Ingram, 2011). This steady increase in temperature can cause certain organisms to die because they are not suited to live in a hotter environment. But the anaerobic bacteria prefer the increase in CO_2 because they can live without oxygen and because the increase in temperature enables them to reproduce at a faster rate. Yet the more bacteria there are means the faster organisms are decomposed, and when bacteria decompose organisms faster, they can give nutrients to the plants and animals that are suited for the environment quicker, potentially increasing the bacteria and plants survival rate.

However, the increase in CO_2 could also have a negative effect on the environment by upsetting the aerobic bacteria that cannot live without oxygen. By potentially increasing the amount of CO_2 in the soil, these bacteria might begin to suffocate. Currently there is too much fossil fuel being converted into carbon dioxide and not enough plant life to convert it back into stored biological matter. Since some of the bacteria are aerobic, they need oxygen to survive. But any excess load of carbon dioxide in the soil might make it almost impossible for the bacteria to breathe because the CO_2 displaces the oxygen, making it difficult for respiration. But without the bacteria, the nitrogen in the atmosphere and the dead material would not have been converted into a substance the plants could use. Plants, animals, and bacteria are all made of cells which is why they need the five biological molecules in order to survive. Without bacteria, the organisms would not be able to build these molecules and amino acids to start and stop chemical reactions therefore, not performing the four key tasks causing the ecosystem to collapse.

We are wondering whether or not the increase in CO_2 emissions really does have an effect on the bacteria. By putting large loads of carbon dioxide directly into the soil, our group believes that the bacteria will not be able to breathe. So to see if it does this we are testing the soil's

bacteria population before and after the CO_2 is poured into the soil. What we researched lead us to believe that the increase in CO_2 would have a negative impact on the density of the bacteria in the soil.

2. Lab Outline

I. Problem: How is the global increase in carbon dioxide changing the population density of soil bacteria?

II. Hypothesis: The more carbon dioxide, the less densely populated the soil will be with bacteria.

III. Procedure:

A. Independent Variable: Amount of extra CO_2 added to the soil plot

B. Dependent Variable: Density of bacteria in the soil

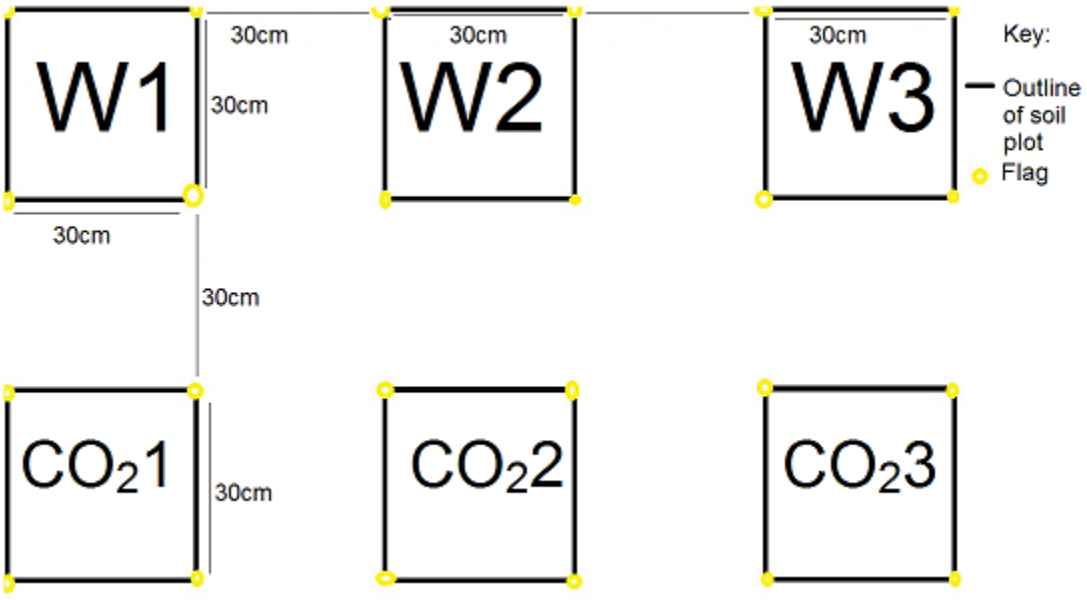
C. Negative Control: No CO_2 added to the soil plot; adding water only

D. Controlled Variables: size of soil plots, amount of baking soda added to soil plots, amount of water added to soil plots, time between soil samples taken and soil dilutions, depth of soil, number of flags for each square, amount of soil taken from ground, type of auger, size of bottles being used to measure amount of water going onto patch, method of applying water to soil plot, method of applying baking soda to soil plot, time between water and baking soda added to patch and soil samples taken, make sure soil samples are taken on the same day and at the same time to control for weather, amount of time shaking the culture tube, amount of soil added to dilute, amount of diluting, amount of sterile water added to culture tubes in the beginning, amount of soil and water mixture taken from one culture tube to the next dilution, size of culture tubes, amount of dilution plated on

3M Petrifilm Aerobic count plates, type of nutrient agar plates, which dilutions plated on 3M Petrifilm Aerobic count plates, length of time that the bacteria on 3M Petrifilm Aerobic count plates are allowed to grow

E. Step By Step Instructions:

1. Using yellow flags, go outside to 39 degrees and 21.414 N. 76 degrees and 38.184 W, and find an area of soil with common and evenly distributed vegetation.
2. In the area, measure six 30cm by 30cm plots of soil, each 30cm apart. Mark the corners of the six different soil plots, using the yellow flags, (two rows of three columns), to have a total of 24 flags. See diagram below.



3. Label the flags of the top row of soil plots, "W." Individually label the flags of the bottom row of soil ploys, "CO_2," (the "W" stands for water being added to the soil, the "CO_2" stands for the CO_2 and water being added to the soil) See diagram above.

4. Next to the labels from step 3, individually label the flags of the first column, "1;" the second column's flags, "2;" the third column's flags, "3." (the numbers stand for each trial) See diagram above.
5. On the same date and at the same time, for each patch, using the auger, take two separate soil samples from each soil plot, digging the auger, with a 2cm diameter, into the soil until the 15 cm line is level with the ground. Place each soil sample in its corresponding separate plastic baggy labeled, "before; substance/s added to soil; trial number; soil sample A or B." e.g.: "before W1A."
6. Follow steps a-p for each soil sample individually, labeling the culture tubes corresponding to the correct substances being added to the soil (W or CO2), trial (1, 2, or 3), soil sample(A or B), and dilution(10^{0}, 10^{-1}, 10^{-2}, or 10^{-3}). **Be sure to perform the soil dilutions for every one of the individual soil samples on the same date, and the same time.**
    a. Using a clean, new transfer pipette, add 10 ml to a 15 ml culture tube, and label the tube 10^{0} and according to its soil sample. E.g.: "beforeW1A10^{0}"
    b. Using the same pipette, add 9 ml to a second 15 ml culture tube, and label the tube, 10^{-1} and according to its soil sample, e.g.: "beforeW1A10^{-1}."
    c. Repeat step b two more times to two additional 15 ml culture tubes, only label them "10^{-2}," and "10^{-3}" respectively and

individually, along with the other corresponding information, e.g.: "beforeW1A10^{-2}," and "beforeW1A10^{-3}".

d. Place 1 cc of the soil sample that you are testing the number of bacteria for (e.g.: bag labeled "beforeW1A") into the correspondingly labeled "10^{0}" culture tube.
e. Put the cap on the tube and shake the tube thoroughly.
f. Use a new clean pipette to remove 1 ml of the soil/water mixture from the "10^{0}" tube (e.g.: culture tube labeled, "beforeW1A10^{0}") and place into the "10^{-1}" tube, labeled, (e.g.: culture tube labeled, "beforeW1A10^{-1}").
g. Put the cap on the tube and shake the tube thoroughly.
h. Use the same pipette in step f to remove 1 ml of the soil/water mixture form the "10^{-1}" tube (e.g.: culture tube labeled, "beforeW1A10^{-1}") and place into the "10^{-2}" tube (e.g.: culture tube labeled, "beforeW1A10^{-2}")
i. Put the cap on the tube and shake the tube thoroughly.
j. Use the same pipette in step f to remove 1 ml of the soil/water mixture form the "10^{-2}" tube (e.g.: culture tube labeled, "beforeW1A10^{-2}") and place into the "10^{-3}" tube, (e.g.: culture tube labeled, "beforeW1A10^{-3}")
k. Put the cap on the tube and vigorously shake the tube thoroughly
l. You should have a total of four culture tubes.

m. Plate separate 100 µl samples from each of the 3rd and 4th tubes (dilutions 10^{-2} and 10^{-3}) onto their own separate correspondingly, labeled 3M Petrifilm Aerobic count plate. e.g.: plate labeled, "beforeW1A10^{-2}," and "beforeW1A10^{-3}," individually.

n. Allow to grow for 72 hours.

o. Examine each of the plates, starting with lowest dilution plates. If the 10^{-3} dilution plate has at least five colonies, use the information (count the number of bacteria colonies) from this plate in the equation below to calculate the density of bacteria. If the 10^{-3} dilution plate does not have at least five visible colonies, refer to the 10^{-2} plate and count the number of colonies. Place that information (the number of bacteria colonies) into the equation below to make your 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 sheet x 10^2 x $10^{|\text{dilution \# at which these colonies were found}|}$

p. Record # of microbes in the before section of the data table.

7. For each soil plot, place .5 liter of water into a separate bottle for each individual patch, labeled, "1 CO_2," "1 NO," "2 CO_2," "2 NO," "3 CO_2," and "3 NO" respectively.
8. For each CO_2 patch of soil, evenly spread 50 grams of baking soda across the whole soil plot.
9. Pour the .5 liter of water from each bottle evenly onto its corresponding patch.

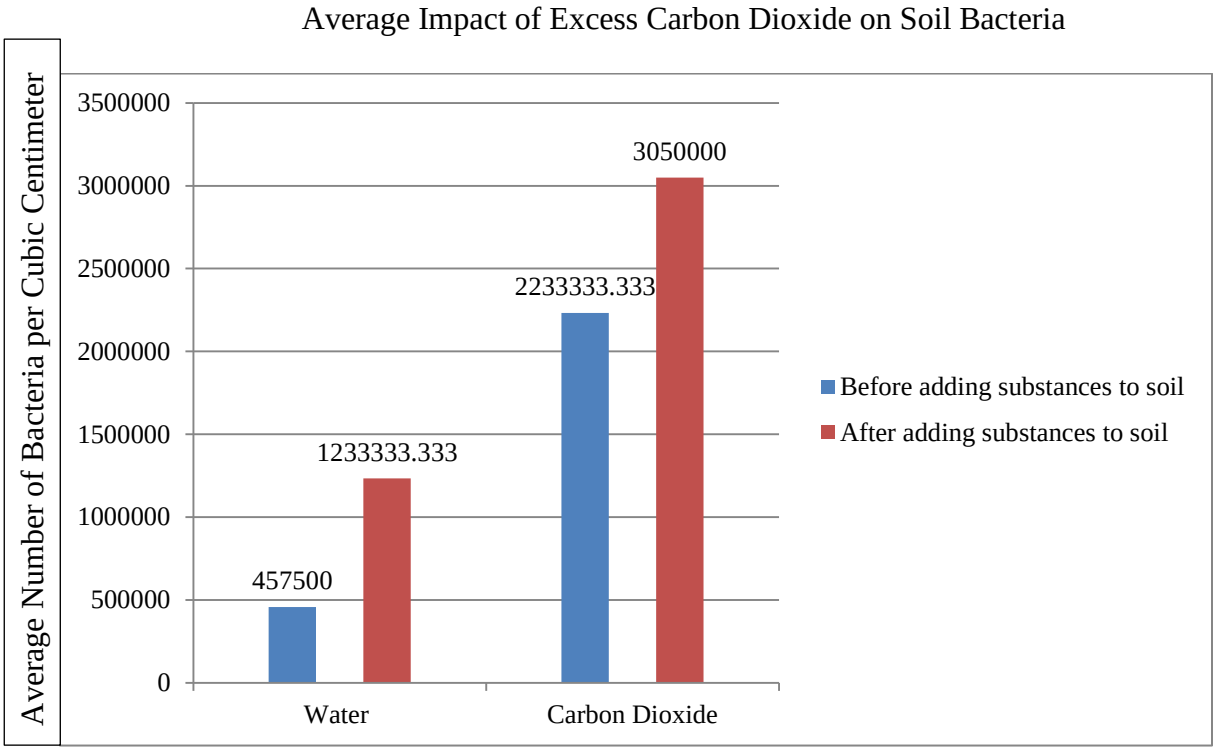
10. Wait 72 hours.
11. On the same date and at the same time, for each patch, using the auger, take two separate soil samples from each soil plot, digging the auger, with a 2cm diameter, into the soil until the 15 cm line is level with the ground. Place each soil sample in its corresponding separate plastic baggy labeled, "after; substance/s added to soil; trial number; soil sample A or B." e.g.: "afterW1A."
12. Repeat step 6, but use the soil samples from step 11, and record the number of microbes in the after section of the data table. **Be sure to perform the soil dilutions for every one of the individual soil samples on the same date, and the same time.**

3. Data

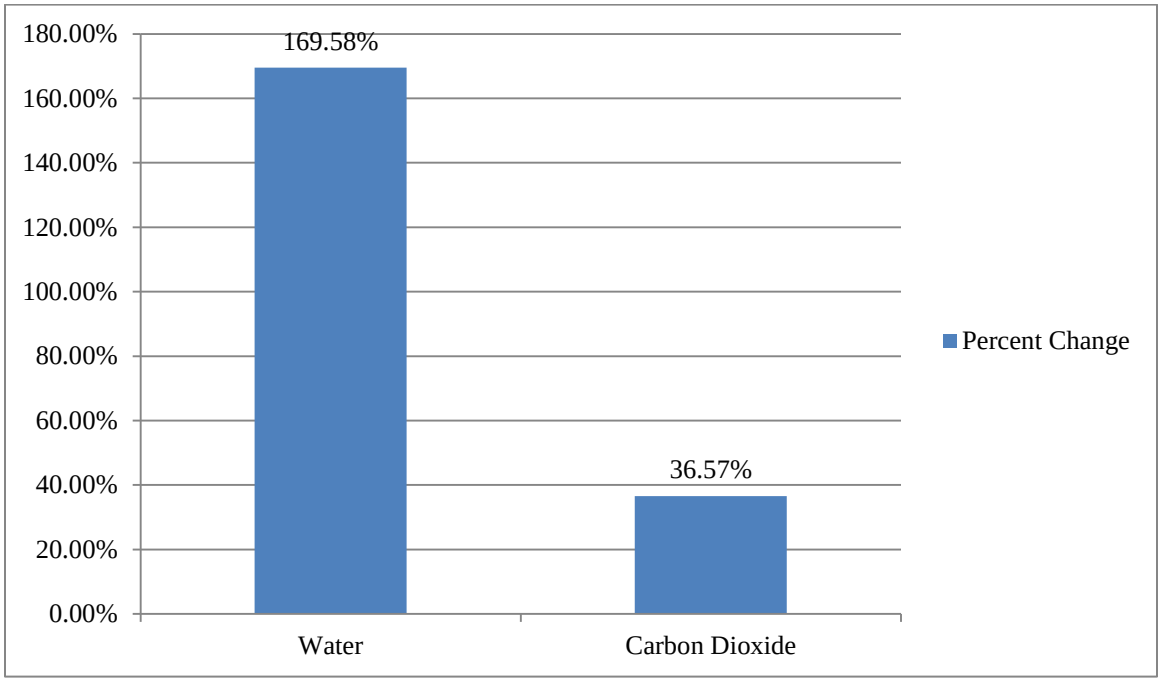
Impact of Excess Carbon Dioxide on Soil Bacteria

Substance/s added to soil plot:	Trial:	Soil Sample:	Number of bacteria per cubic centimeter:		Percent Change:
			Before adding substances to soil:	After adding substances to soil:	
Water	1	A	N/A	900,000	N/A
		B	640,000	1,800,000	181.25%
	2	A	N/A	1,700,000	N/A
		B	700,000	1,000,000	42.857%
	3	A	280,000	800,000	185.714%

		B	210,000	1,200,000	471.429%
	Average:		457,500	1,233,333.333	169.581%
Carbon dioxide	1	A	800,000	2,700,000	237.5%
		B	600,000	3,000,000	400%
	2	A	2,900,000	5,600,000	93.103%
		B	6,700,000	2,800,000	-58.209%
	3	A	800,000	2,400,000	200%
		B	1,600,000	1,800,000	12.5%
	Average:		2,233,333.333	3,050,000	36.567%



Average Percent Change in Bacteria Density After Adding Solutions



4. Conclusions

The hypothesis was right; the more carbon dioxide, the less densely populated the soil will be with bacteria. We tested for aerobic bacteria, which cannot live without oxygen. By increasing the amount of carbon dioxide in the soil, we suffocated a lot of the bacteria, causing the density of bacteria in the soil plots that received carbon dioxide to increase only 36.567%, while the bacteria density of the soil plots that received only water increased 169.581%. In the first graph which shows the average density of bacteria per cubic centimeter before and after adding the substances, it is difficult to determine whether the bacteria survival rate was greater with or without carbon dioxide because both plots had a dramatic increase in bacteria after adding the substances possibly due to a change in the weather. Therefore, a graph showing the percent changes in bacteria density was necessary. Based on the graph which shows the average percent change of the bacteria density after adding water to the soil, the bacteria density, on average, increased 169.581%; however, after adding the water and baking soda (which creates carbon

dioxide) to the soil, the density of bacteria only increased 36.567%. Both soil plot samples had an increase in the bacteria density. However, the increase for both was probably due to the weather. The bacteria density of the soil plots that only received water more than doubled from the original bacteria density.

Although we took every 3M Petrifilm Aerobic count plate from the corresponding bag, a few fungi plates were mixed among the bacteria plates so two samples could not be counted for. After waiting three days, we saw fungi growing instead of bacteria colonies. Our data is not as accurate as it could have been with the two bacteria counts, but we used the information we had to make the conclusions for our data.

These findings mean that as humans create and release more and more carbon dioxide into the atmosphere, the density of aerobic soil bacteria will decrease. For further research, one may investigate the effect of the increase in carbon dioxide on anaerobic soil bacteria, which are bacteria that do not need oxygen in order to survive and are harmed by oxygen. Also, how the reduction of aerobic soil bacteria will influence anaerobic soil bacteria, other microbes, plants, and other animals. By adding more carbon dioxide to the soil, the aerobic bacteria will suffocate due to a lack of oxygen. As we discovered that aerobic bacteria are poorly impacted by the increase in carbon dioxide, we can experiment if other microbes, like fungi and protozoa, will also affected by the increase in carbon dioxide. The discovery that aerobic bacteria are being harmed by the increase in carbon dioxide can lead us to experiment and discover other effects of global warming.

References:

All images taken from: http://www.google.com/imghp?hl=en&tab=wi

Ingram, E. (2011) Soil Biology. Natural Resources Construction Service. <http://soils.usda.gov/sqi/concepts/soil_biology/bacteria.html>

NCAGR.gov. (2011) Plant Nutrients. North Carolina Department of Agriculture and Consumer Services. <http://www.ncagr.gov/cyber/kidswrld/plant/nutrient.htm>

Ophardt, Charles. (2003). The Carbon Cycle. Elmhurst College Virtual Chem Book. http://www.elmhurst.edu/~chm/vchembook/306carbon.html