The Impact of Pesticides on Bacterial Diversity in the Soil

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Biology H

Thursday, May 28th, 2015

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April 28, 2015

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Biology 9H

Soil Ecology Background

"Bacteria are any of a very large group of single-celled microorganisms that display a wide range of metabolic types, geometric shapes and environmental habitats" (Hogan,*et al,* 2012), and they participate in a variety of tasks in every ecosystem on earth. Of these tasks, decomposition and contributing to the nitrogen cycle are two of the most important. These responsibilities are crucial to maintaining the health of the natural world, and without soil bacteria to make them happen, life itself would not be possible.

The first step of nitrogen fixation is the conversion of atmospheric nitrogen into ammonia by soil bacteria. The ammonia then reacts with water in the soil and connects with a hydrogen ion from the water to form ammonium. The bacteria engaged in this nitrification will then convert ammonium to nitrate, which is a form of nitrogen usable by plants. This nitrate is important to plants because it contributes to the synthesis of amino acids. These amino acids are the building blocks for proteins, which are necessary to complete the four tasks of all cells: synthesis, homeostasis, transforming energy, and reproduction. Furthermore as enzymes, proteins start and stop the chemical reactions that make life possible. Hence, without nitrogen fixation, the cells of the plants could not function, and the plants would die. Furthermore, once a plant is consumed, the nitrogen goes through the whole food chain to all living things, and this provides amino acids and life for all living things. Any excess nitrate will be then converted by

denitrifying bacteria back into nitrogen gas and release it into the atmosphere, and the cycle then continuously repeats itself. (Campbell, N.; Williamson, B.; & Heyden, R, 2004)

During decomposition, bacteria break down dead animal and plant tissue, as well as other organic materials, and the process starts when, "colonies of microorganisms use enzymes to oxidize the organic matter to obtain energy and carbon," which they then use to grow. (University of Western Australia, 2004) Decomposition releases nitrogen to contribute to the nitrogen cycle. In the nitrogen cycle, soil bacteria play an important role. The ammonium, which provides nitrogen for the amino acids will return to the soil where it can be reused. In the process of decomposing ammonium is produced that is the second stage of the nitrogen cycle.

Due to the important role bacteria play in plant life cycles, anything that disrupts the presence of the bacteria can be potentially harmful for plant communities and consequently all the organisms that depend on them. One thing that can be harmful to bacteria are pesticides. Pesticides are used to kill or deter insects, bacteria, fungus and other things that are harmful to grass or crops. Carbaryl (l-naphthyl N-methylcarbamate) is the active ingredient in a pesticide which is highly toxic and attacks the nervous system of these insects and microorganisms. In the soil, bacteria decompose the carbaryl and help break it down, but there is a range of effects possible this process can have on microorganisms. For example, "some pesticides stimulate the growth of microorganisms, but other pesticides have depressive effects or no effects on microorganisms" (Taiwan Agricultural Chemicals and Toxic Substances Research Institute, 2010). Here, an abnormal amount of bacteria could possibly be growing where the pesticide is applied, which can lead to the disturbance of the other microorganisms in the area, the surrounding soil, and the plant itself. (NCBI, 2010)

We decided to test the effect of pesticides on bacteria in soil by observing if the biodiversity of the bacteria decreases or increases with the introduction of pesticides. We were interested in this topic because we learned about the harmful effects of pesticides and were curious about its impact on bacteria in the soil. Bacteria have a crucial role, whether it be contributing to the nitrogen cycle or decomposing organic matter. The introduction of pesticides in the soil can put the health of the affected plant community at risk either by stimulating or inhabiting bacterial microorganism's growth. We hypothesized that the population of soil bacteria will decrease after pesticides are added into the soil.

Soil Ecology Experiment

I. Problem

   a. Do pesticides increase or decrease the population diversity of soil bacteria?

II. Hypothesis

   a. Pesticides decrease the population diversity of soil bacteria.

III. Procedure:

   a. Independent variable

      1. Application of Pesticides vs. no application of pesticides

   b. Dependent variable

      1. Number of types of bacteria

   c. Negative control

      1. Application of only water

   d. Positive Control

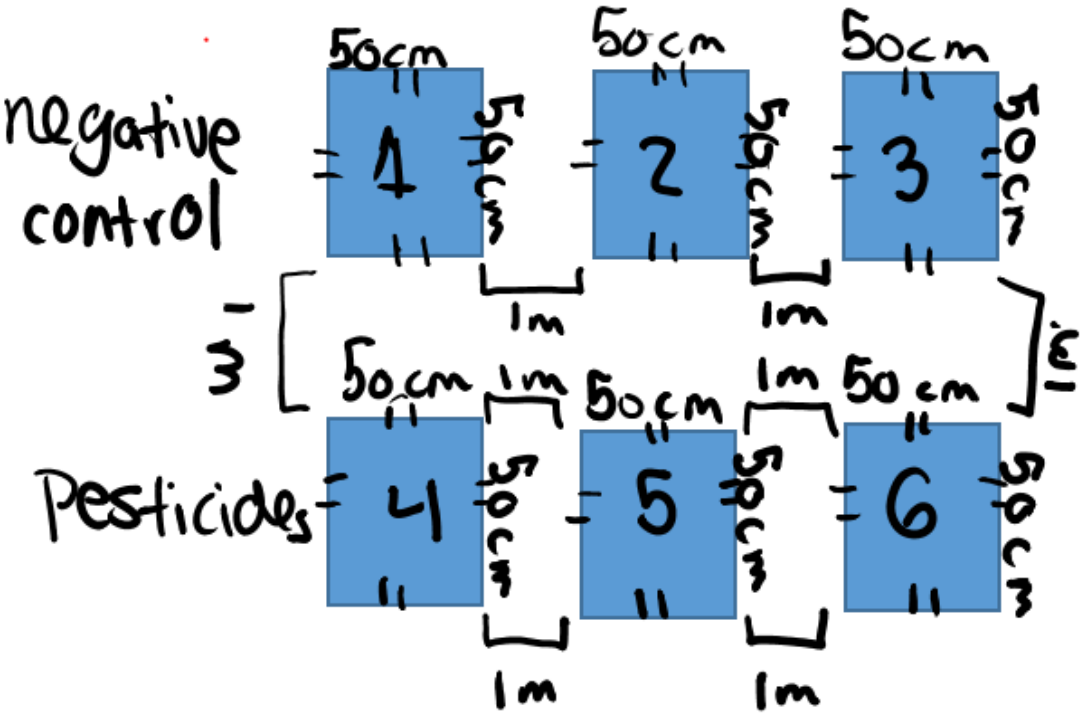
      1. Soil samples taken before independent variable is applied

e. Controlled variables

1. type of pesticides, location of soil plot, size of soil plots, amount of squirts of pesticides, kind of petrifilm, size of petrifilm, amount of squirts of water, type of sprayer, Amount of days water and pesticides sit before being sampled, amount of sterile water, size of culture tube, degree of dilution, size of soil sample both taken and diluted, soil diluted at the same time on the same day, amount being plated, any samples of soil collected on the same day at the same time, how long bacteria grows

f. Step by step instructions:

1. Plot six areas of soil on the lawn next to Deepdene Road (N 39.35661˚, W 076.63537˚) using flags. Place four yellow flags on



the four corners of each plot and place each flag 50 cm apart from each other. The flags should form a square and each plot should be a meter apart in two rows and three columns (see diagram below).

2. Collect all soil samples in step three on the same day at the same time
3. Using the soil core extractor that is 48cm long with a diameter of 2cm, collect three different sets of soil samples 17 centimeters deep from each of the three plots without water and each of the three plots without pesticides. Place each sample into its own plastic bag and label the bags “positive control” and according to the plot number and trial number (ex. Positive Control, Plot 1, Trial 1.) Collect all samples for the positive control on the same day at the same time to control for weather.
4. For the serial dilutions steps 5-18, do these steps on the same day at the same time
5. Use a clean, new transfer pipette to add 10 ml of sterile water to a 15 ml culture

   tube. Label the tube “10^{0} Plot 1 trial 1 positive control”
6. Use the same pipette to add 9 ml of sterile water to a second 15 ml culture tube.

   Label the tube “10^{-1} Plot 1 trial 1 positive control”
7. Repeat step 6 two more times to two additional 15 ml culture tubes, only label

them “10^{-2} Plot 1 trial 1 positive control”, “10^{-3} Plot 1 trial 1 positive control”

8. Place 1 cubic centimeter (cc) of your positive, plot 1, trial 1 soil sample into the “10^{0} Plot 1 trial 1 positive control” culture tube.
9. Cap the tube and shake vigorously.
10. Using a new clean pipette, remove 1 ml of the soil/water mixture from the “10^{0} Plot 1 trial 1 positive control” tube and place into the “10^{-1} Plot 1 trial 1 positive control” tube.
11. Cap the tube and shake vigorously.
12. Using the same pipette in step 10, remove 1 ml of the soil/water mixture from the “10^{-1} Plot 1 trial 1 positive control” tube and place into the “10^{-2} Plot 1 trial 1 positive control” tube
13. Cap the tube and shake vigorously.
14. Using the same pipette in step 10, remove 1 ml of the soil/water mixture from the “10^{-2} Plot 1 trial 1 positive control” tube and place into the “10^{-3} Plot 1 trial 1 positive control” tube.
15. Cap the tube and shake vigorously.
16. You should now have a total of four culture tubes.
17. Plate 100 µl samples from the 3rd and 4th tube (dilutions 10^{-2} and 10^{-3} Plot 1 trial 1 positive control) onto their own separate, correspondingly labeled 3M Petrifilm™ Aerobic Count Plate containing nutrient agar

18. Repeat steps 5-17 using the previous labeling system but for the negative or pesticides for every other trial and plot, changing labels to match soil samples
19. Allow to grow for 48 to 72 hours.
20. Once fully grown, examine the nutrient agar plates using a magnify glass to identify the different types of bacteria according to their shape, size, and color.
21. Record the amount for each type of colony and the dilution level it was observed at, then use the equation below to determine the total quantities of each type of bacteria: # Microbes in 1 cc of soil = # Colonies on sheet x 10^{2} x 10 | dilution # at which these colonies were found|
22. Record data in the data table
23. Add the pesticides and water in steps 23 and 24 to the correct plots on the same day at the same time
24. Add pesticides onto plots 1-3 using a squirt bottle and label them pesticides. Hold the squirt bottle 20.32 to 30.48 cm from the plots. Spray 20 times on each plot until surface is wet. Wait at least 24 hours for the additions to settle into the soil.
25. Add water on plots 4-6 using a different squirt bottle and label them negative control. Hold the squirt bottle 20.32 to 30.48 cm from the plots. Spray 20 times on each plot until surface is wet. Wait at least 24 hours for the additions to settle into the soil.

26. Collect all soil samples in step 26 and 27 on the same day at the same time
27. Using the soil core extractor that is 48cm long with a diameter of 2cm, collect three different sets of soil samples 17 centimeters deep from each of the three pesticide plots and label the bags “pesticides with the plot number and the trial number”
28. Using the soil core extractor that is 48cm long with a diameter of 2cm, collect three different sets of soil samples 17 centimeters deep from each of the three negative control plots and label the bags “negative control with the plot number and the trial number”
29. Go back to steps 4-21 and repeat the serial dilution process only this time using the previous labeling system but changing “before” to “after” for every other trial and plot for the negative control and pesticides.

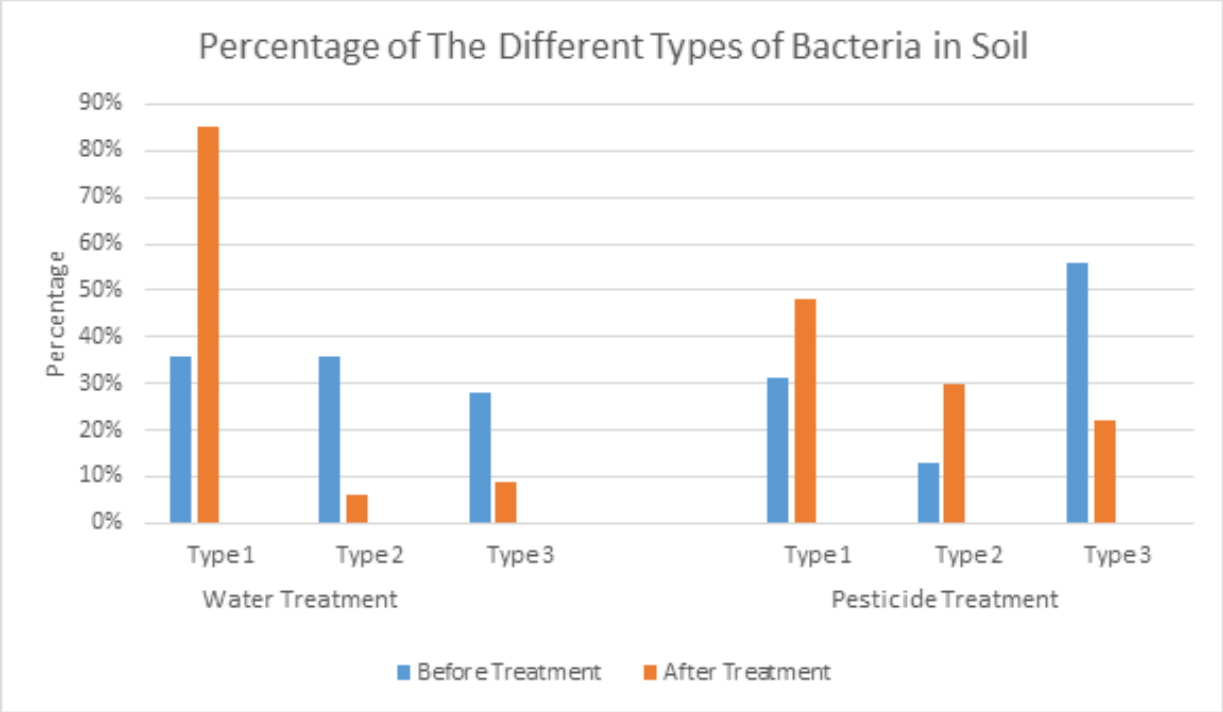
IV. Data and Analysis

a. Data table

Impact of Pesticides on the Diversity of Bacteria in Soil

	Water Treatment Plots						Pesticide Treatment Plots					
	# of bacteria per cubic centimeter before adding the treatment			# of bacteria per cubic centimeter after adding the treatment			# of bacteria per cubic centimeter before adding the treatment			# of bacteria per cubic centimeter after adding the treatment		
	Type 1	Type 2	Type 3	Type 1	Type 2	Type 3	Type 1	Type 2	Type 3	Type 1	Type 2	Type 3
Trial 1	110,000	10,000	110,000	10,000	10,000	20,000	110,000	120,000	0	50,000	20,000	10,000
Trial 2	47,000	7,000	47,000	190,000	0	0	40,000	20,000	10,000	40,000	20,000	40,000
Trial 3	600,000	733,700	433,333	90,000	10,000	10,000	833,333	266,666	1,766,666	20,000	30,000	0
Total	757,000	750,700	590,333	290,000	20,000	30,000	983,333	406,666	1,776,666	110,000	70,000	50,000
Total % by type	36%	36%	28%	85%	6%	9%	31%	13%	56%	48%	30%	22%

b. Analysis



V. Conclusion

In conclusion, our hypothesis was proven incorrect. We hypothesized that the diversity of bacteria in soil would decrease after pesticide treatment. After completing three trials of water treatment, the diversity of soil bacteria decreased. Specifically, type 1 took up most of the bacteria population being 85%. Our data shows that something in the environment made it difficult for the type 2 being 6% and type 3 being 9% of bacteria to live after the water treatment. In the bacterial environment, some type of substance in the soil made it untimely for type 2, and type 3 to remain diverse. When comparing the before and after treatment of pesticides we observed that the pesticides stimulated the growth of the bacteria in the soil. The after pesticide treatment data showed that the diversity of type 1 and type 2 increased by 17%. The data of type 3 showed that the diversity decreased by 34%. When comparing the data of the before and after of type 3 in the water treatment and type 3 in the pesticide treatment, we found that

proportionally speaking type 3 of the pesticide treatment was at a greater diversity level. For the type 3 water treatment, the after results of 9% were about ⅓ of the percentage of the before results which were 28%, for the diversity of the bacteria in soil. For the type 3 pesticides treatment, the after results of 22% were about ½ of the percentage of the before results which were 56% for the diversity of the bacteria in soil.

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