

## Runoff and Its Impact on the Ecosystem

We are looking at the effects that soil nutrients in runoff, specifically nitrogen, have on bacteria in the soil. Before we started our experiment, we researched information on soil nutrients, microbes, runoff, and fertilizer. We found a lot of information on the Internet and after researching, designed an experiment to discover more about soil ecology.

When rain, snow, and any other type of precipitation falls, a portion of that seeps into the ground while the other parts flow downhill as runoff towards larger bodies of water (Storm Runoff Prediction, 1996). The rainfall characteristics that impact runoff are the duration of the fall, amount and intensity of precipitation, and how the runoff is spread out over an area (Earth's Water: Runoff, 2003). How long and how powerful the rainfall is can also greatly increase how much water is running off surfaces at one time. For example, if it rained hard for three days, more water would run off of a certain surface at one time than if it drizzled for an hour. Where this runoff is directed also changes how much runoff there is in one area. Climatic conditions such as temperature, wind, and season also affect runoff (Earth's Water: Runoff, 2003) because these are all factors that affect rainfall, which in turn, change the runoff characteristics.

The different surfaces that runoff flows over greatly influences how runoff can be accessible to plants (Managing Urban Runoff, 2003). For example, steep slopes reduce the time that the runoff is in one area and reduces the volume of runoff. Rough surfaces increase surface storage and allows for more dispersion into the soil (Managing Urban Runoff, 2003). In areas such as forests, wetlands, and grasslands, rainwater is trapped and it is allowed to slowly filter into the ground (Managing Urban Runoff, 2003). In urban

areas with roads and bridges, however, the non-porous surfaces do not allow runoff to filter into the ground and instead, the runoff remains above the surface, accumulates, and flows in very large amounts (Managing Urban Runoff, 2003). These large amounts of water can affect our environment in various ways and as a result, ways to control runoff are developed.

Sewer systems are developed to try and redirect runoff, which can actually impair watersheds. Once water enters the system, the runoff gathers speed. When it leaves the sewer system and enters a stream, its large quantities erode stream banks, damage vegetation near the stream, and widen the stream channels (Managing Urban Runoff, 2003). These highly altered streams have many components in their soil that are also altered when runoff flows through a stream, microorganisms being some of these components.

A countless number of different microorganisms are in soil that is carried in runoff. These microorganisms help the soil immensely in many activities such as growing plants and decomposition. Some microorganisms contribute to the soil by making vitamins and plant hormones. Not all microorganisms take part in these activities but their jobs are just as important. Algae, fungi, protozoa, bacteria, and viruses are among the most common microorganisms (Cultivated Soil Composition, 2004), bacteria and fungi being the most common. Bacteria's primary function is to break down materials. *Bradyrhizobium*, a certain kind of bacteria, fixes nitrogen and helps plants grow (Cultivated Soil Composition, 2004). It is able to turn nitrogen into a form that plants are able to use, like nitrate-nitrogen. The plants, in turn for receiving the nitrate-nitrogen, give the bacteria sugar for energy (Cultivated Soil Composition, 2004). This energy helps

the bacteria continue converting the nitrogen. Bacteria in the soil also assist in the nitrogen and carbon cycles to enhance the environment plants can grow in. Besides helping plants, bacteria help in the destruction of harmful fertilizer and pesticides that kill off plants. Bacteria and the other microorganisms in the soil are very beneficial, but in order for the large amount of microorganisms to thrive there must be a sufficient amount of nutrients within the soil. Along with the amount of nutrients in the soil, the type and health of these nutrients can alter the impact that bacteria have on soil.

Some of the major nutrients in soil are nitrogen, phosphorus, potassium, calcium, magnesium, and sulphur (Lindsell, 2000). These nutrients are all very beneficial for plant growth and aid in the protection of the plants and strength of the plants internally.

Nitrogen is a nutrient in the soil that is very beneficial to the soil and plants that grow in it. Because it is so helpful in plant growth, it is used in fertilizer. This affects the runoff that flows from the areas where nitrogen-rich soil is used. In very large quantities, nitrogen can be harmful to the soil. Nitrogen is recycled and re-used through the biogeochemical cycle called the nitrogen cycle. Nitrogen, in its gaseous form, makes up 79% of the Earth's atmosphere (The Nitrogen Cycle, 2003) and can only be used by plants in the forms of nitrate ions, ammonia, and urea (The Nitrogen Cycle, 2003). So, the nitrogen must be converted into forms plants can use. As the nitrogen slowly seeps into the ground, bacteria on the nodes of vegetation convert the nitrogen into different forms, most commonly ammonia and ammonium (Nardi, 2003). Bacteria convert the nitrogen into ammonia by consuming the energy their plant hosts produce in turn for giving up the ammonium they converted for the plant's use. This process is known as nitrogen fixation. Along with this process, another conversion must happen because very

few plants can use ammonia, and only in small quantities. (Brock, 2004) So in order for the plants to get the form of nitrogen they need, bacteria convert the ammonia into nitrite and again into nitrate, which all plants can use. (Nardi, 2003) When fertilizers, filled with nitrogen, are applied to soil, most of the ammonium-nitrogen is eventually converted to nitrate-nitrogen. An acid residue then forms over the surface of the soil and an “acid roof” is created, actually slowing plant growth (Soil Fertility Management, 2003). From our research, we wondered if the components of fertilizer (especially nitrogen) that are carried in runoff alter the natural balance of the soil ecosystem. Because bacteria are such important microorganisms to plant growth, we wanted to see if nitrogen from fertilizer in our campus’ runoff alters bacteria density. To look at this relationship, we took soil samples in the stream and soil samples moving further away from the stream and tested for nitrate-nitrogen and bacteria density in each sample. Below is the experiment that we designed to further study nitrogen in runoff and its impact on bacteria density in the soil.

### **Experiment Description**

- I. Problem: How does fertilizer runoff change the soil ecosystem as it flows down the hill?
- II. Hypothesis: As you move outward from the stream, nitrate-nitrogen levels decrease and bacteria population density increases and as you move further down the stream, nitrate-nitrogen levels increase and bacteria population density decreases.
- III. Procedure:
  - A. Variables
    - Independent Variable 1- distance outward from the stream
    - Independent Variable 2- distance downward through the stream
    - Dependent Variable 1- amount of nitrate-nitrogen in the soil
    - Dependent Variable 2- population density of bacteria in soil
  - B. Controls
    - Baseline Negative Control- test at highest point of stream in the stream

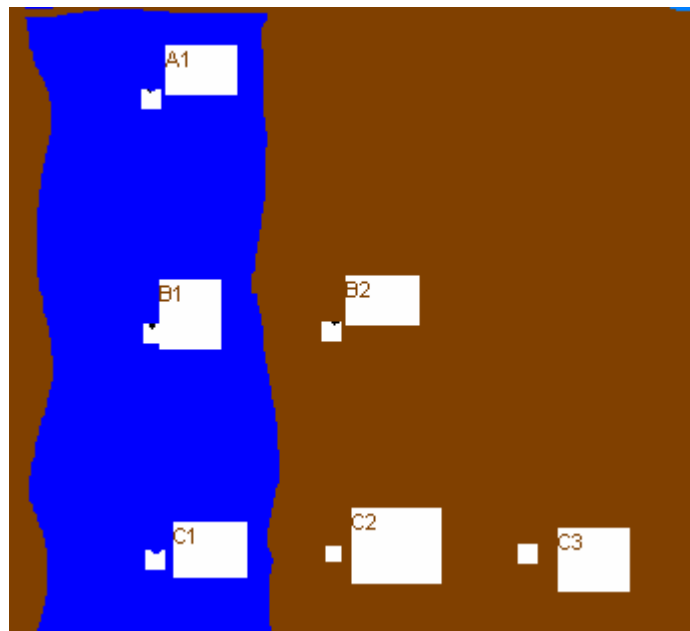
-Experimental Negative Control- all tests taken in the stream

-Controlled Variables List:

- How far apart samples are taken
- Time samples are taken
- Type of core sampler
- Amount of soil sample
- Depth of soil sample
- Testing processes of testing nitrate-nitrogen and bacteria population density
- Process through which soil samples are taken
- How we diluted the soil samples

### C. Step-by-step

1. Mark 3 plots at the following locations:  
Plot A (top of stream): (N39.35816, W076.63355)  
Plot B (middle of the stream): (N39.35303, W076.63851)  
Plot C (bottom of the stream): (N39.35817, W076.63900)
2. For plot B, mark another plot 1 meter away from plot B that is perpendicular to the streambed. For plot C, mark another plot 1 meter away from plot C that is perpendicular to the streambed and then another that is 1 meter away from that so you have 6 plots.
3. Mark all plots with a stake flag. The plots are labeled as follows:



4. \*All soil samples for one trial MUST be taken on the same day\*  
Collect 3 soil samples from each of the six plots with a soil core sampler with a diameter of 2.5 cm by inserting the soil core sampler 15 centimeters into the ground.

- Put each separate soil sample in a plastic bag and label the bag. In labeling, “a”, “b”, and “c” stand for the three different samples from each plot. The plots and bags are labeled as follows:



- Complete the serial dilutions and nitrate-nitrogen tests for each set of samples at the same time and within one hour. (Do all tests for “a” samples at the same time, do all tests for “b” samples at the same time, and do all tests for “c” samples at the same time.)
- For the serial dilution, place 1 cc of each soil sample into a culture tube with 10 ml of sterile water.
- Cap the tube and shake vigorously.
- Use a serological pipette to remove 1 ml of soil/water mixture and place into a fresh culture tube containing 9 ml of sterile water. Repeat this step until each soil sample has been diluted to, at least, the fourth power. There should now be 5 culture tubes per soil sample.
- Place 100 ul from the tubes diluted to  $10^{-4}$  and  $10^{-5}$  onto their own Petri-film® strips and allow it to incubate at room temperature for at least 24 hours.
- Use the LaMotte STH-4 Combination Soil Outfit Kit to do a nitrate-nitrogen test.
- Record the amount of nitrate-nitrogen in pounds per acre and then convert it to parts per million using this equation:  
$$\text{Lb/acre} \times 0.5 = \text{ppm}$$
- 24 hours after completing the serial dilutions look at the bacteria in the Petri-film® and count the number of bacteria colonies. Convert this number into  $\text{cfu/cm}^3$  by using this equation:  
$$\# \text{ of colonies} \times 10^2 \times 10^{\text{dilution factor}} = \text{cfu/cm}^3$$
- Record this data as  $\text{cfu/cm}^3$ .
- Repeat steps 1-14 one additional time.

IV. Data Tables and Analysis:

**Key**

A- Samples at uppermost plot of stream

B- Samples at middle plots of stream

C- Samples at lowest plots of stream

1- Samples taken from plots in the stream

2- Samples taken from plots 1 meter away from plots in the stream

3- Samples taken from plots 2 meters away from plots in the stream

a- One set of samples taken from each plot

b- Another set of samples taken from each plot

c- Last set of samples taken from each plot

Trial	Plots	Nitrate Nitrogen (parts per million)	Bacteria Population Density in cfu/cm <sup>3</sup>
1	A1a	7.5	9x10 <sup>6</sup>
	A1b	7.5	5x10 <sup>6</sup>
	A1c	20	3.1x10 <sup>7</sup>
2	A1a	10	2x10 <sup>6</sup>
	A1b	10	3x10 <sup>6</sup>
	A1c	15	2x10 <sup>6</sup>
1	B1a	10	1.1x10 <sup>7</sup>
	B1b	10	5x10 <sup>6</sup>
	B1c	10	4x10 <sup>6</sup>
2	B1a	10	1.2x10 <sup>7</sup>
	B1b	8.5	7x10 <sup>6</sup>
	B1c	10	1x10 <sup>7</sup>
1	B2a	8.5	1x10 <sup>7</sup>
	B2b	10	4x10 <sup>6</sup>
	B2c	8.5	1.07x10 <sup>4</sup>
2	B2a	9	9x10 <sup>6</sup>
	B2b	10	6x10 <sup>6</sup>
	B2c	10	5x10 <sup>6</sup>
1	C1a	7.5	1x10 <sup>7</sup>
	C1b	8.5	2x10 <sup>6</sup>
	C1c	10	7x10 <sup>6</sup>
2	C1a	10	2x10 <sup>6</sup>
	C1b	9	7x10 <sup>6</sup>
	C1c	10	1x10 <sup>6</sup>
1	C2a	7.5	1x10 <sup>7</sup>
	C2b	8.5	4x10 <sup>6</sup>
	C2c	10	1x10 <sup>6</sup>
2	C2a	7.5	6x10 <sup>6</sup>
	C2b	8.5	6x10 <sup>6</sup>
	C2c	10	5x10 <sup>6</sup>
1	C3a	7.5	4x10 <sup>6</sup>
	C3b	7.5	3x10 <sup>6</sup>

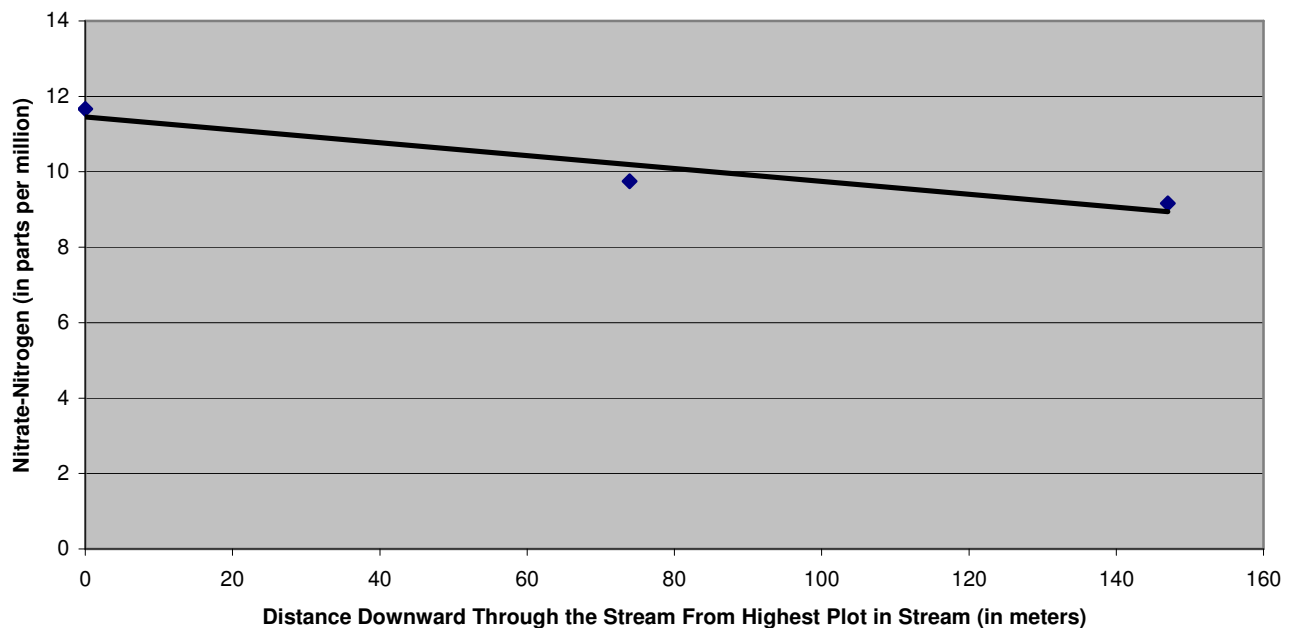
	C3c	8.5	$4.3 \times 10^7$
2	C3a	8.5	$4 \times 10^6$
	C3b	10	$2 \times 10^6$
	C3c	8.5	$4 \times 10^6$

Averages:

Plots	Nitrate-Nitrogen (parts per million)	Bacteria Population Density in cfu/cm <sup>3</sup>
A1- uppermost plot of the stream, in the stream	11.67	$8.66 \times 10^6$
B1- middle plot of stream, in the stream	9.75	$6.66 \times 10^6$
B2- middle plot of stream, 1 meter out	9.83	$5.668450 \times 10^6$
C1- lowest plot of stream, in the stream	9.166	$4.83 \times 10^6$
C2- lowest plot of stream, 1 meter out	8.67	$5.33 \times 10^6$
C3- lowest plot of stream, 2 meters out	8.416	$1 \times 10^7$

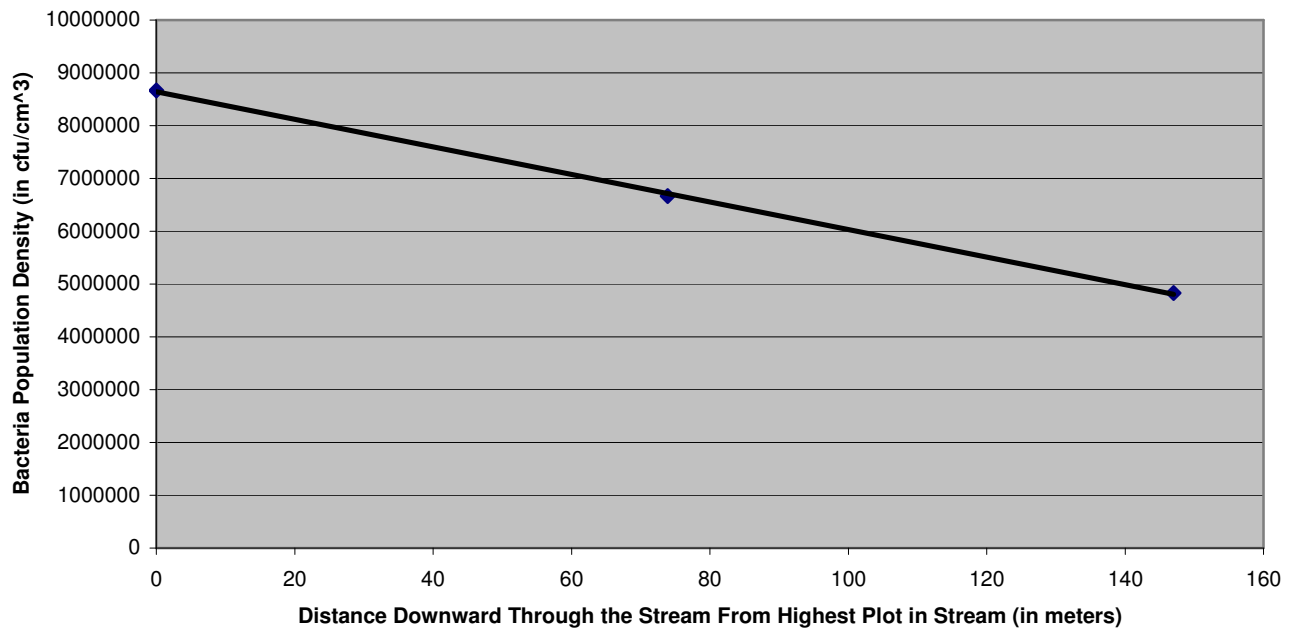
Analysis:

Nitrate-Nitrogen In the Soil As You Move Downward Through The Stream

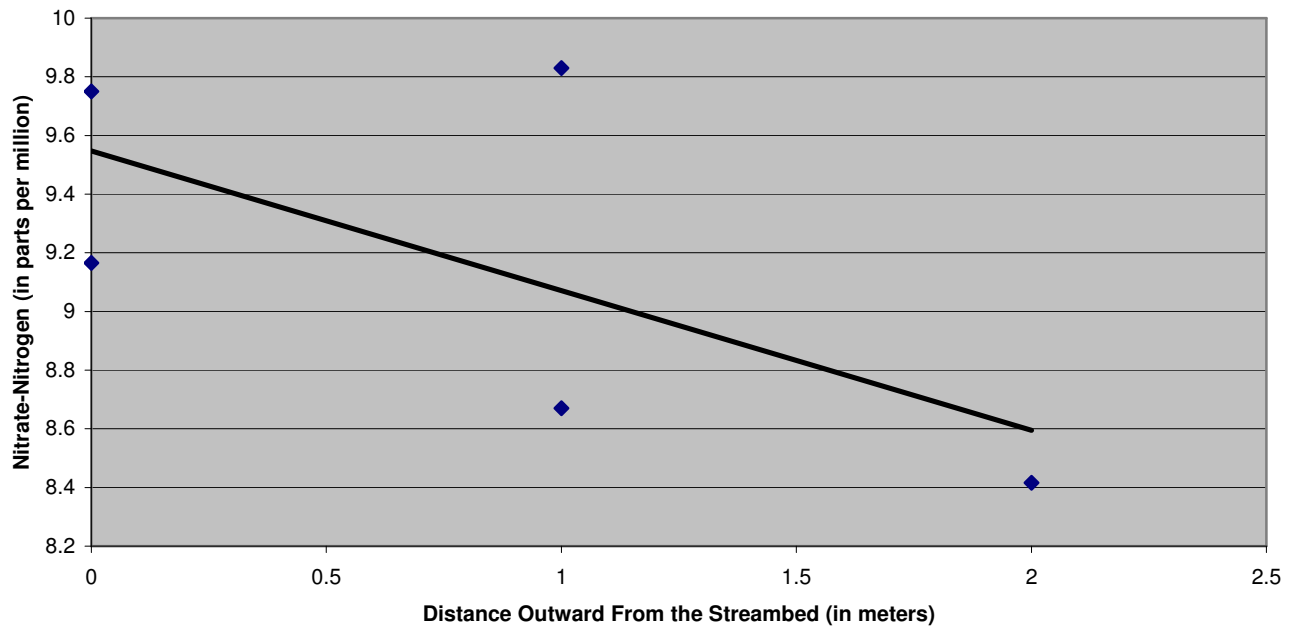




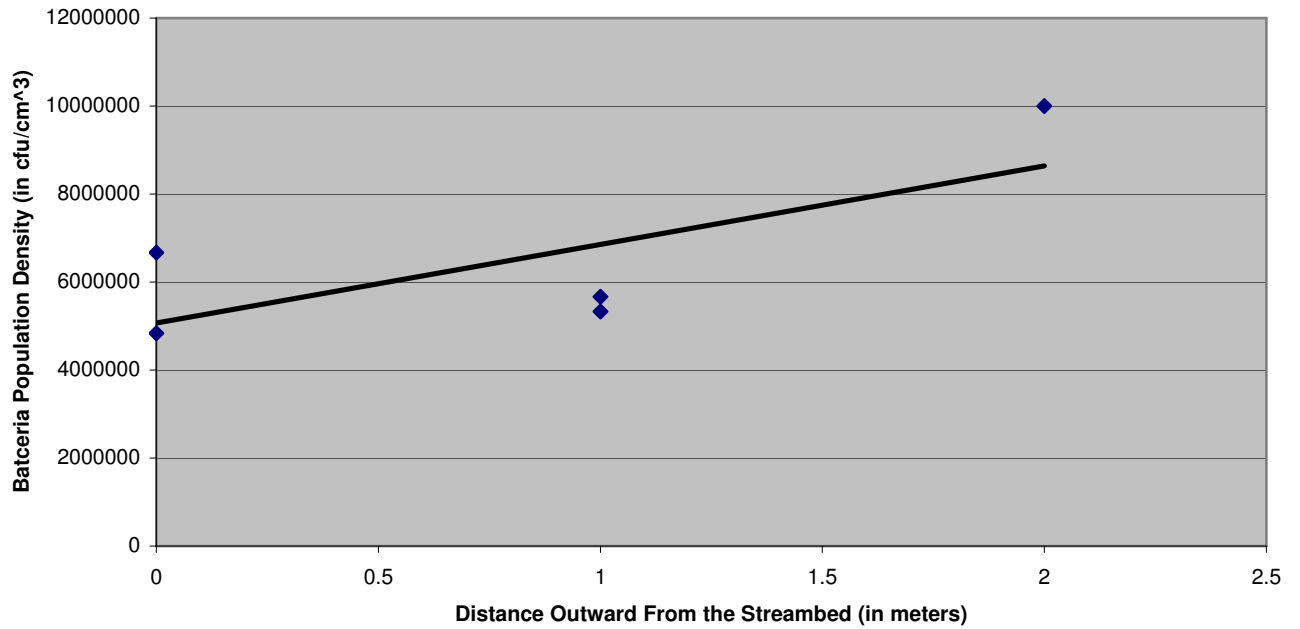
**Bacteria Population Density In The Soil As You Move Downward Through the Stream**



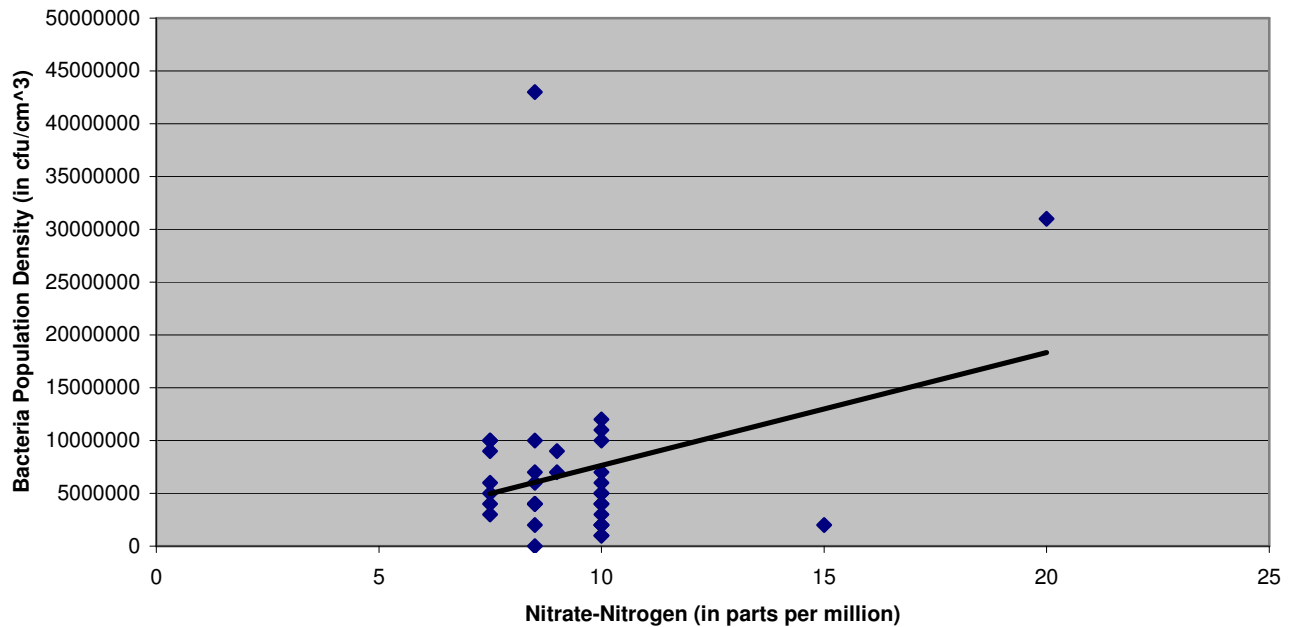
**Nitrate-Nitrogen In The Soil As You Move Outward From the Streambed**



Bacteria Population Density As You Move Outward From the Streambed



Nitrate-Nitrogen In Relation to Bacteria Population Density In the Soil



In this graph, we saw that as nitrate-nitrogen increased, bacteria density did as well. It was not a very large increase. We looked at the residual to see how much of the “rise” was controlled by the nitrogen. The  $r^2$  value was greater than .09, showing that about 10% of the “rise” in bacteria was controlled by the nitrogen.

## V. Conclusion

In this experiment, our hypothesis was: As you move outward from the stream, nitrate-nitrogen levels decrease and bacteria population density increases and as you move further down the stream, nitrate-nitrogen levels increase and bacteria population density decreases. The data we collected from our tests showed that as we moved further down the stream, bacteria density decreased and nitrate-nitrogen levels also decreased. Our tests also showed that as we moved further outward from the streambed, bacteria density increased and nitrate-nitrogen levels decreased. We originally thought that nitrogen would increase as we moved down the stream because of a build-up of fertilizer runoff. Because there was not an increase in nitrate-nitrogen in the stream, we concluded that it was not *fertilizer* runoff that was changing bacteria density but runoff in the stream, not necessarily from fertilizer.

The point where bacteria population density was lowest was when it was about 4.8 million cfu/cm<sup>3</sup>. When we moved two meters away from the stream, the bacteria population density increased to 10 million cfu/cm<sup>3</sup>. The drastic increase in bacteria density indicates that something in runoff is harmful to bacteria, but not necessarily fertilizer. The decrease in bacteria density as we moved down the stream is also a possible indication that there is a substance in runoff that is harmful to bacteria.

There are some flaws in our experiment design that may have altered our data. Our data did show us that nitrate-nitrogen decreased as we moved down the hill, from about 11 parts per million to about 9 parts per million. This decrease is not an immense reduction; one flaw may be that our plots in the stream were not far enough apart. Maybe if they were further apart than about 74 meters, we would see a definite increase or

decrease in nitrate-nitrogen levels. Other design flaws were results of not having much time to do our tests. Because of limited time, we had to take out 3 plots that could have given us more data to look at. Another result of this time limit was that we only got to complete two trials. A third trial could have changed or further emphasized our conclusions that we made from our collected data. In conclusion, our hypothesis was partially correct because we incorrectly hypothesized that nitrate-nitrogen levels would increase as we moved down the stream, but the rest of our hypothesis was correct. Through our tests, we found that nitrate-nitrogen from fertilizer runoff does not change this specific aspect of the soil ecosystem (bacteria population density). In a future experiment, we would like to test soil samples from these same plots for bacteria population density along with a nutrient, different from nitrogen, to see what actually harms the bacteria in the stream's soil.

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