



## **How Do Soil pH Levels Affect Soil Sulfur Levels?**

Maria Folgueras, Shelby Smith and Julia Tich



## **Abstract**

Bacteria, sulfur, pH, and protozoa share a very complex relationship with one another. In the annual 2010 Biota Survey (E.S.S.R.E., 2010), an anomaly in the amount of sulfur in this relationship was observed. Our group sought to find out if deliberate manipulation of the pH in the soil would cause changes in the other key factors protozoa, bacteria, and sulfur. We expected that as we made the pH more acidic the population of protozoa would decrease, the population bacteria would increase, and the levels of sulfur would increase. We made five different plots, and took samples before we added pH solutions ranging 3-7, and after we added the solutions. We tested each sample for protozoa and bacteria densities as well as sulfur and pH levels. We found our hypothesis was correct on a small scale, but that larger environmental forces on the scale of the whole site where the experiment was performed had an adverse impact on our results.

## Introduction

Bacteria perform many major tasks linked to water dynamics, nutrient cycling, and decomposition (Ingham, 2010a), and when decomposing organic matter, they release a number of plant-available and plant-usable nutrients, one of which includes sulfur in the form of sulfur dioxide and sulfate (Regents of the University of Minnesota, 2002). Sulfur that remains in the soil after plant uptake often reacts with other factors to form sulfuric acid. Specifically, sulfur dioxide ( $\text{SO}_2$ ) is oxidized catalytically to form sulfur trioxide ( $\text{SO}_3$ ), which, when combined with water, forms sulfuric acid ( $\text{H}_2\text{SO}_4$ ) (Chemical and Engineering News, 2001). This form of sulfur can impact other vital elements in the soil, but its most significant impact is the lowering of pH levels. Acidic pH levels profoundly alter soil health, and in particular, acidic soils harm microbial life.

One such group of microbes which pH affects most significantly are protozoa. Protozoa survive and thrive in moist soils with neutral pHs, and so when soil becomes acidic, the protozoa living there can be severely harmed. However, the consequent decrease in protozoa density in the soil can become self-reinforcing. Since soil bacteria are the primary prey of soil protozoa (Ingham, 2010b), fewer protozoa mean higher levels of bacteria, and since a high density of bacteria increases the amount of decomposition, the consequent potential positive feedback loop can destabilize an environment. More decomposition can create higher levels of sulfur which can then lower pH levels (The Environmental Literary Council, 2006). The resulting drop in soil pH levels, though, can cause the protozoa population to decrease further, which can lead to a still further increase in the bacteria population. This increase, in turn, can cause an increase in the rate of decomposition, causing the sulfur levels there to increase, lowering the pH, and so forth. Hence, once pH levels drop below a certain level in the soil, this cycle of microbes and nutrients can steadily continue to reinforce itself to the detriment of the environment.

Because this complex relationship among bacteria, sulfur, pH and protozoa in the soil plays such a prominent role in maintaining the health of an ecosystem, any change in any component of this cycle has the potential to profoundly affect all the other components. The change can, in fact, destabilize an ecosystem. Hence, when the annual 2010 Biota Survey (E.S.S.R.E., 2010) revealed in Site 4 (E.S.S.R.E., 2001) the expected predator/prey relationship between protozoa and bacteria (low levels of bacteria [average density: 412, 500/cc soil] with correspondingly high levels of protozoa [average density: 837064.27/g soil]) and the expected nearly neutral soil pH level (6.63), the excessive amounts of sulfur (1916.67 ppm) found in Site 4 was a significant anomaly with enormous implications for the future health of this microclimate. We sought to determine if the deliberate manipulation of one of the other key factors (protozoa, bacteria or pH) might show how this anomaly was impacting the larger health of the soil ecology in this location.

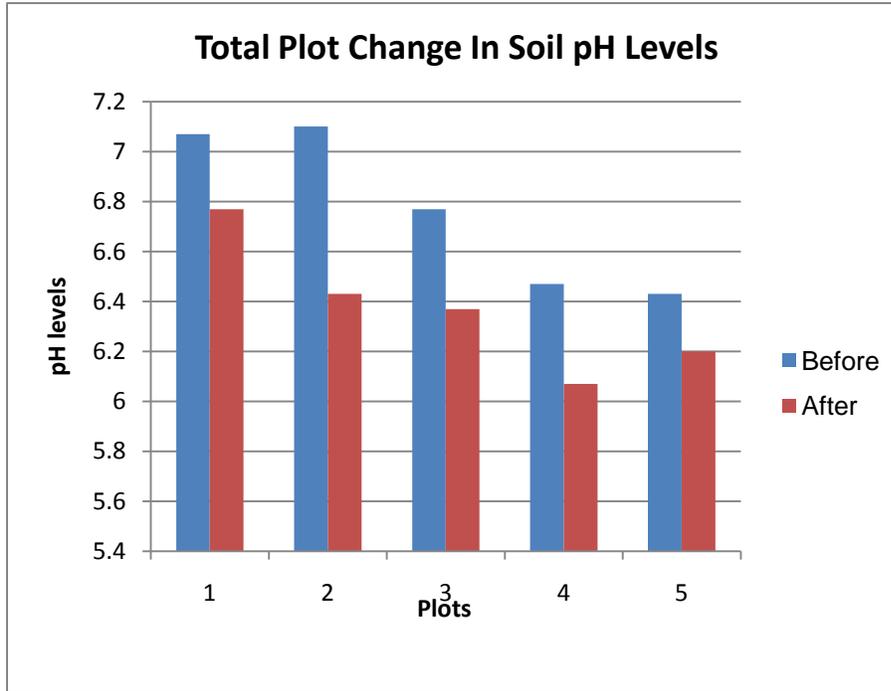
## Methods

5 adjacent 30.5 cm x 30.5 cm plots were created at N 39.35733, W 076.63840 in E.S.S.R.E. Site 4 (E.S.S.R.E., 2001) and were labeled according to their respective treatments. 3 separate soil cores 15 cm deep and 2.25 cm in diameter were then extracted from each plot. 5 separate 1-liter solutions of 1.0 M hydrochloric acid were diluted using distilled water to pHs of 3, 4, 5, 6, and 7 respectively made. These pH solutions were then applied to their corresponding data plots. The pH 7 solution was applied to Plot 1; the pH 6 solution was applied to Plot 2; the pH 5 solution was applied to Plot 3; the pH 4 solution was applied to Plot 4; and the pH 3 solution was applied to Plot 5. After 24 hours, 3 additional soil cores 15 cm deep and 2.25 cm in diameter were extracted from each data plot.

A modified Foissner/Uhlig extraction process (Brockmeyer, 2008) was first performed on all soil samples through the end of the sifting stage. The remaining dried soil was then used to execute a serial dilution to test for bacteria density, and chemical tests were performed on the dried soil to determine each sample's pH and sulfate levels (ppm). Each soil sample was diluted to  $10^{-3}$  using sterile water, and 100  $\mu$ l aliquots from the  $10^{-2}$  dilution and the  $10^{-3}$  dilution were each plated on their own individual 3M<sup>TM</sup> Petrifilm<sup>TM</sup> Aerobic Count Plates. All plates were then allowed to grow for 48 hours and examined for individual colonies to calculate bacteria density (#/cc soil). Simultaneously, all soil samples were tested for pH and sulfate using the LaMotte Chemical Test Kit Series Model STH-14. The rehydration step of the modified Foissner/Uhlig extraction process was then initiated on all soil samples, and the rest of the modified Foissner/Uhlig extraction process was completed. The resulting microscope slides were observed using a QXS Digital microscope at 60X. 5 fields of view for each sample were observed and the average protozoa count per sample was used to calculate that sample's protozoa density (#/g soil).

## Results

Graph 1: Change in soil pH levels between the five research plots.



### Key:

Plot 1 = pH 7

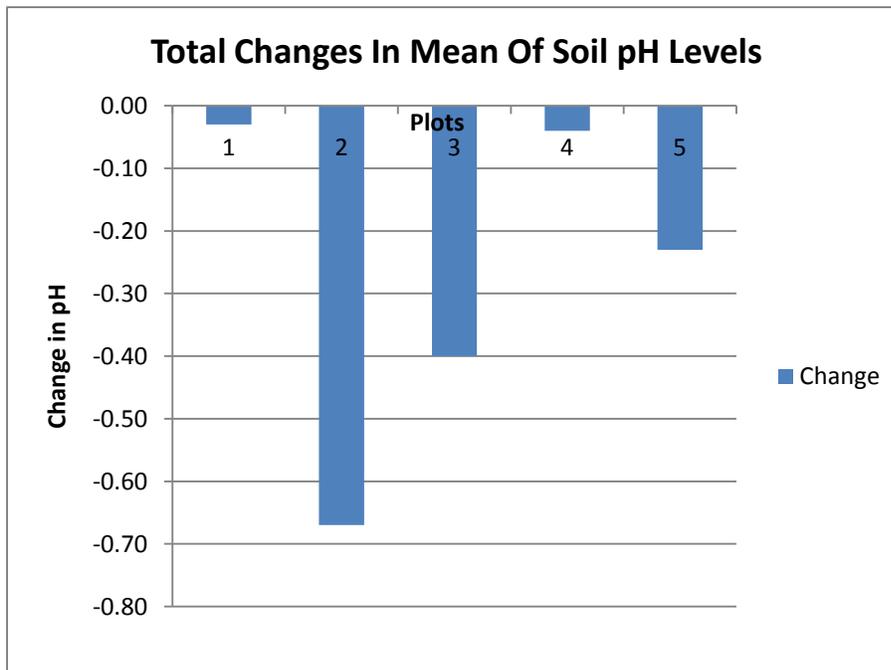
Plot 2 = pH 6

Plot 3 = pH 5

Plot 4 = pH 4

Plot 5 = pH 3

Graph 2: Significant difference in the average soil pH levels.



### Key:

Plot 1 = pH 7

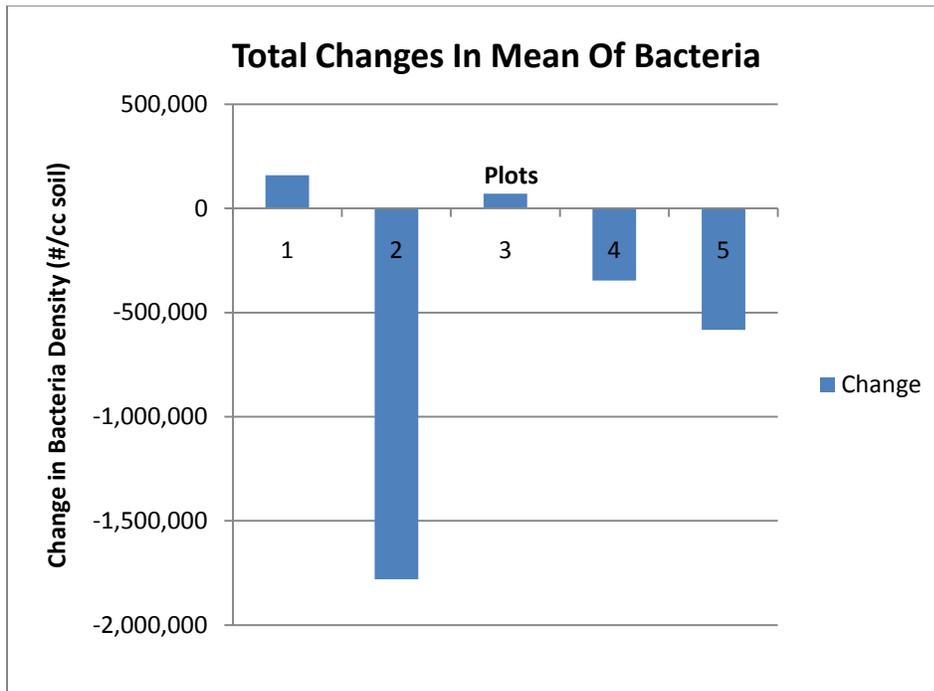
Plot 2 = pH 6

Plot 3 = pH 5

Plot 4 = pH 4

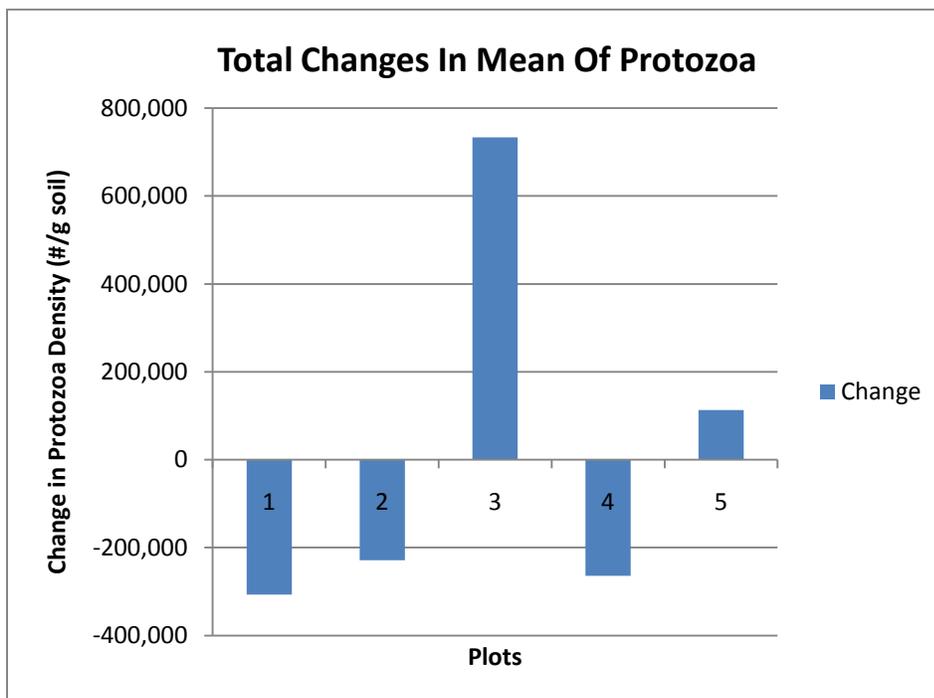
Plot 5 = pH 3

Graph 3: Significant difference in the average amount of bacteria.



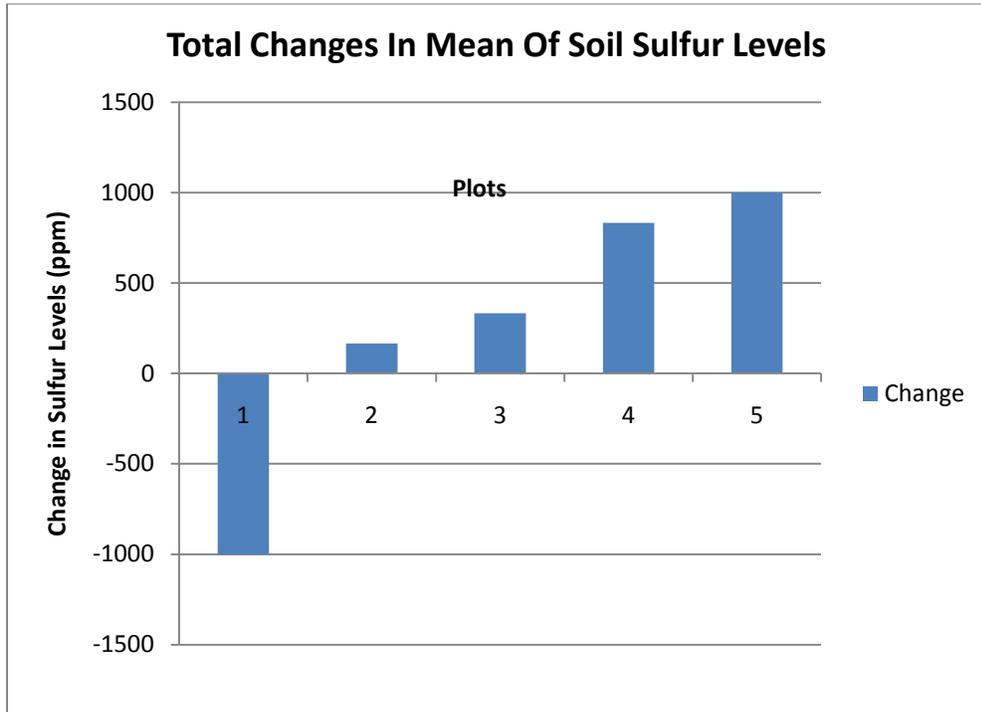
**Key:**  
Plot 1 = pH 7  
Plot 2 = pH 6  
Plot 3 = pH 5  
Plot 4 = pH 4  
Plot 5 = pH 3

Graph 4: Significant difference in the average amount of protozoa.



**Key:**  
Plot 1 = pH 7  
Plot 2 = pH 6  
Plot 3 = pH 5  
Plot 4 = pH 4  
Plot 5 = pH 3

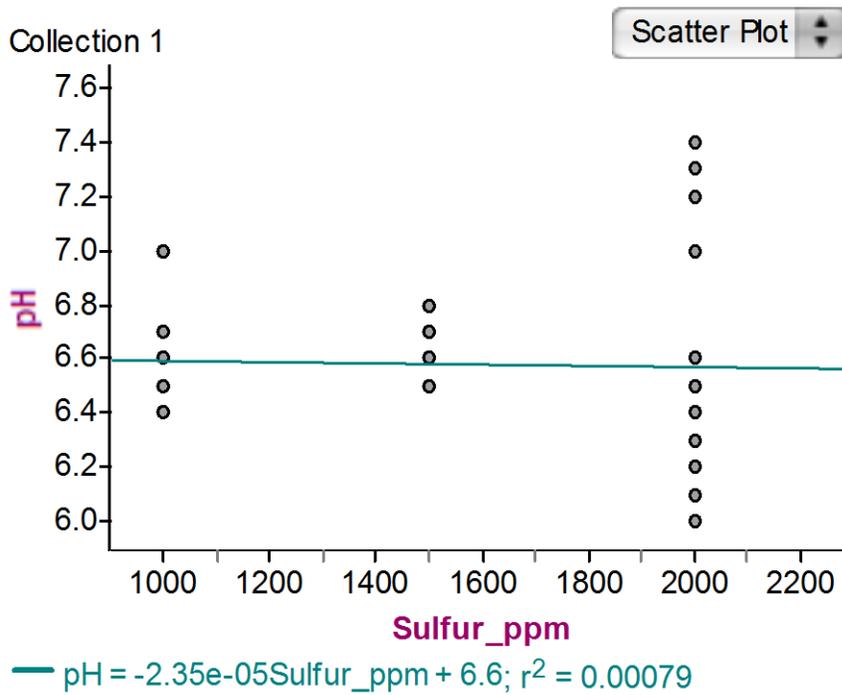
Graph 5: Significant difference in the average soil sulfur levels.



**Key:**  
Plot 1 = pH 7  
Plot 2 = pH 6  
Plot 3 = pH 5  
Plot 4 = pH 4  
Plot 5 = pH 3

Graph 6: Correlation between soil sulfur levels (ppm) and soil pH levels.

Sulfur vs. pH





protozoa and bacteria densities was also not seen in our overall data ( $r^2 = 0.0021$ ) (see graph 7). Therefore, our hypothesis is not fully supported.

It is probable that other environmental factors could have been affecting the amount of bacteria and protozoa in the soil as well as soil sulfur levels. Because graph 1 clearly shows that we were successful in manipulating the environment, the question we now face is what could be causing the observed anomalous relationships shown in graphs 6 and 7. Previous research in E.S.S.R.E. Site 4 shows that this site has been steadily undergoing a process of desertification (Geraghty, Mitchell, Nelson and Sabah, 2009). This drying, in turn, is probably affecting pH levels in the soil. As a result, the actively changing moisture levels in Site 4 and the possible consequent changes in soil pH levels could account not only for the abnormal differences in the pH levels between plots as displayed in graph 2 but also for the variance within graph 6. Furthermore, the moisture-affected pH levels could impact the amount of protozoa in the soil and, therefore, the bacteria density in the soil as well, thereby explaining the discrepancy in graph 7. Overall, we believe that although we were successful in manipulating the soil and, in doing so, were able to prove, to some extent, that the pH component of this cycle can have a profound impact on all other components in this cycle, we also think that the drying process occurring in Site 4 is another highly probable explanation for the anomalies observed in the original survey on which we based our research. Therefore, further research into how much of an impact the drying process in Site 4 is having on the bacteria, sulfur, pH and protozoa cycle there would be the most logical next step.

## **Acknowledgements**

We thank Dr. Holliday Cross Heine and Human Capital Development, Inc. for supporting the 2010 E.S.S.R.E. and for supporting young women pursuing science as a topic of interest and/or as a career. In addition, we thank Mr. Brock, Catherine, Molly and Ashley for continuously helping, supporting and encouraging us throughout this program. Lastly, we also thank Roland Park Country School for allowing us access to their campus in order to partake in this summer research experience.

## **References**

Brockmeyer, K. (2008). "Extracting Soil Protozoa." In Brock, et al. (Ed). *Soil Ecology Lab Manual: "It's the Little Things that Run the World."* Batavia: Flinn Scientific, Inc.

Chemical and Engineering News. (2001). *Chemical of the Week: Sulfuric Acid*. Retrieved from <http://scifun.chem.wisc.edu/chemweek/Sulf&top/Sulf&Top.html>

- Environmental Literary Council. (2006). *Sulfur Cycle*. Retrieved from <http://www.enviroliteracy.org/article.php/1348.html>
- Environmental Science Summer Research Experience for Young Women (E.S.S.R.E.). (2001). *General Description of the E.S.S.R.E. Survey Sites*. Retrieved from <https://faculty.rpcs.org/essre/ESSRE%20Locations.htm>
- Environmental Science Summer Research Experience for Young Women (E.S.S.R.E.). (2010). *E.S.S.R.E. Microclimate Databases*. Retrieved from <https://faculty.rpcs.org/essre/2010/TA%20Folder/Entire%20Site%204%20Summary%202010.htm>
- Geraghty, K., Mitchell, C., Nelson, E., & Sabah, S. (2009). *How Does Oxygen Affect Protozoa Density?* Available online <https://faculty.rpcs.org/essre/2009/TA%20materials/Final%20Copy%20of%20Research%20paper%20OXYGEN.pdf>
- Ingham, E. (2010a). *Bacteria*. Retrieved from [http://soils.usda.gov/sqi/concepts/soil\\_biology/bacteria.html](http://soils.usda.gov/sqi/concepts/soil_biology/bacteria.html)
- Ingham, E. (2010b). *Soil Protozoa*. Retrieved from [http://soils.usda.gov/sqi/concepts/soil\\_biology/protozoa.html](http://soils.usda.gov/sqi/concepts/soil_biology/protozoa.html)
- Regents of the University of Minnesota. (2002). *Organic Matter Management*. Retrieved from [http://www.extension.umn.edu/distribution/cropsystems/components/7402\\_02.html](http://www.extension.umn.edu/distribution/cropsystems/components/7402_02.html)