Effect of Acid Rain on the Ability of Soil Microbes to Decompose Organic Matter

Target Grade Level: High School

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UNIVERSITY OF MONTANA GK-12 PROGRAM

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2. NAME OF INQUIRY: EFFECT OF ACID RAIN ON THE ABILITY OF SOIL MICROBES TO DECOMPOSE ORGANIC NITROGEN

3. GOALS AND OBJECTIVES:

a. Inquiry Questions: How does changing environmental parameters influence essential processes such as nutrient cycling.

b. Ecological Theme(s): Nutrient cycling, environmental change

c. General Goal: Provide a hands-on activity that demonstrates the ecological themes stated above and how microbes are essential to the process of nutrient cycling.

d. Specific Objectives:

Academic – To provide an understanding of the importance of stable environmental conditions in microbial nutrient cycling. To teach the key concept of the importance of nutrient cycling in ecosystem function/maintenance.

Experimental – To provide basic skills and knowledge for working with microbes in the classroom.

Social – To work collaboratively in a group.

Communication – To generate written responses to questions that reflect an understanding of the exercise.

e. Grade Level: High School

f. Duration/Time Required:

→ Prep time: need 2 days incubation for microbial plates, then about 3-4 hours for autoclaving broth and preparing the cultures.

→ Implementing Exercise During Class: 1 to 1.5 hours

→ Assessment: Questions related to the exercise must be answered by the students to determine if they have learned the basic concepts.

4. ECOLOGICAL AND SCIENCE CONTEXT:

a. Background (for Teachers):

We developed the following inquiry template as a companion piece for the activity, The Influence of Acidification on Ammonification, by Brian R. Shmaefsky. We performed this lab with tenth grade classes at Big Sky High School in Missoula, Montana.

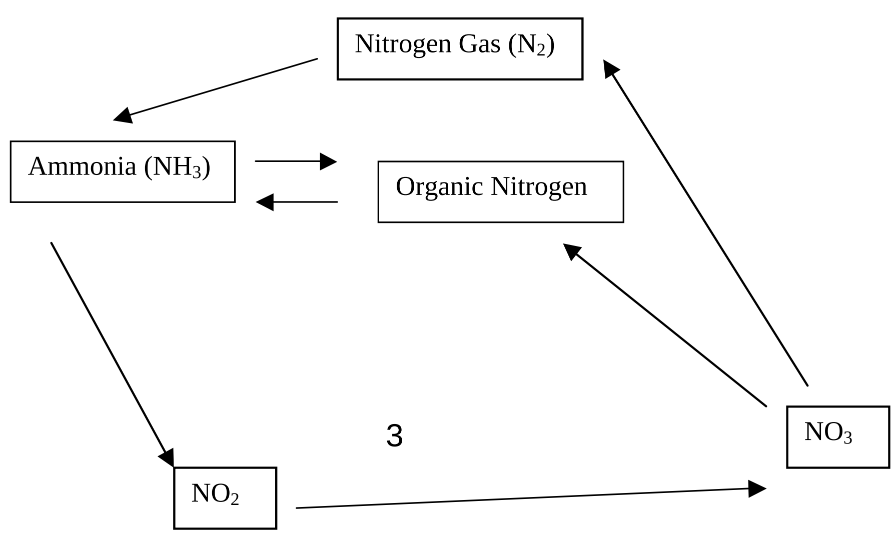
The interactions of human beings with their environment often results in unintended and undesirable consequences which drastically alter natural ecosystems. One well known example of this is the production of acid rain due to certain air pollutants. The primary cause of acid rain is the release of sulfur dioxide (SO_2) and a variety nitrogen oxides (NO_x) into the atmosphere. Sulfur dioxide is a by-product of burning coal and other fossil fuels. Nitrogen oxides are released by the combustion of fossil fuels. Power plants are responsible for most of the SO_2 and about one quarter of the NO_x produced in the U.S. Automobiles are the primary producers of NO_x, accounting for just over half of nitrogen oxide emissions. When these pollutants combine with water in the atmosphere, they form sulfuric (H_2SO_4) and nitric (HNO_3) acid. These acids lower the pH of rainwater, making it significantly more acidic than pure rainwater. Normal water on earth is just below a neutral pH of 7. It is not exactly neutral because carbon dioxide (CO_2) in the atmosphere reacts with water, much like the pollutants responsible for acid rain, to produce carbonic (H_2CO_3) acid. Acids created by these pollutants can lower the pH of atmospheric water significantly from normal levels.

When acid rain occurs, it lowers the pH of both soil and groundwater, making it acidic, and altering normal environmental conditions. The pH is one of many very important environmental parameters, including temperature and oxygen content, which should be kept relatively stable for proper ecosystem functioning. All fish and amphibians are directly affected by drops in pH, and death may result. Plants are also affected, trees do not grow as quickly, are less healthy, and may die. One effect which may be less obvious is that on the essential communities of bacteria present in the soil. Fluctuations in pH may slow or eliminate many natural life processes carried out by bacteria.

Lowering of the pH of the environment, which is an increase in the proportion of hydrogen ions present, can effect the following aspects of bacterial life. The activity of proteins, which are composed of amino acids, is often dependent on a certain pH range in order to work quickly and efficiently. Furthermore the electrochemical gradient, or difference in proportion and charge of ions between the inside and outside of the cell, will be altered. This can affect the ability of the cell to produce energy, as well as some forms of solute transport.

Properly functioning microbial communities are essential for the survival of all higher organisms in all ecosystems. This is due to the key roles they fill in the breaking down and building up of various elements in nutrient cycles. One such cycle is the carbon cycle. Bacteria play key roles in building up organic carbon from CO_2, and also in the breaking down of organic substances into CO_2, thereby contributing to both sides of the nutrient processing cycle. Another important cycle which relies even more heavily on bacterial activity is the nitrogen cycle. The nitrogen cycle is particularly important, since organic nitrogen (nitrogen bound to a carbon structure) is necessary for all protein and DNA synthesis.

The Nitrogen Cycle



Few organisms can use naturally occurring nitrogen gas (N_2). The nitrogen cycle begins when nitrogen fixing bacteria take N_2 gas from the atmosphere and change it into Ammonia (NH_3), which is usable by most other organisms. A great deal of ammonia can then be bound to carbon to create the organic nitrogen essential to life. Plants take ammonia from the soil for this purpose. The process of generating organic carbon from NH_3 is called assimilation, and can be done by all eukaryotic organisms.

Ammonification is the step in microbial nutrient cycling which is the counterpart to assimilation. This is the step we will be analyzing in this exercise. When an organism dies in the wild, its organic nitrogen is decomposed by bacteria to release Ammonia back into the soil, which can then be re-utilized by plants to create new organic nitrogen. Recycling and reusing of all available resources is essential for ecosystem functioning, which is why ammonification is such an important process for ecosystem health. (Other steps in the cycle include nitrification, which is the conversion of NH_3 to NO_2 and NO_3. NO_3 can also be converted back into Nitrogen gas by denitrification. These steps in the process are carried out by different bacteria, but an understanding of these steps is not necessary for this exercise.)

The presence of ammonia can be tested for with Nessler's reagent, which produces a color reaction with ammonia. Test tubes containing organic nitrogen and inoculated with common soil organisms can be tested for ammonification using this reagent. By inoculating broths at different pHs with the same organism and observing the reaction of the broth with Nessler's, we can determine the effects acid rain might have on soil organisms in an ecosystem. Judging by the magnitude of the Nessler's reaction at different pHs, it is possible to determine if soil bacteria are able to effectively carry out the process of ammonification, which in turn effects the amount of usable nitrogen present in the soil for use by plants and higher organisms.

b. Background (to present to Students):

Students should understand pH, as well as the source and general effects of acid rain on an ecosystem's plants and animals. Using this lab as part of a general introduction to microbiology can be effective. The necessity of bacteria to the continuation of all life on the planet due to their role in nutrient cycling should be stressed. The students should learn about nutrient cycling and decomposition in general, in particular the carbon and nitrogen cycles. The general slides for the presentation we used are attached to this document. (at a later date)

5. MOTIVATION AND INCENTIVE FOR LEARNING: Learning the importance of microbes and their proper functioning to our existence and the existence of all other higher organisms on the planet. Acid rain and its effects are locally relevant in several parts of the country.

6. VOCABULARY:
nitrogen cycle: process by which naturally occurring nitrogen (N_2) is converted into forms that are easily used by other organisms, and eventually converted back into N_2 gas.
nitrogen fixation: process (carried out by nitrogen fixing bacteria only) which converts N_2 gas into the form NH_3, which is easily used by eukaryotic organisms.
ammonification: decomposition process by which organic nitrogen is broken down, releasing NH_3 which can then be reused by other organisms.
assimilation: process of taking NH_3 and binding the nitrogen to a carbon structure to make organic nitrogen.
parameters: a set of variable factors which can be measured, such as temperature, pressure, pH, etc.

7. SAFETY INFORMATION:
NESSLER'S REAGENT IS A NEUROTOXIN AND CONTAINS MURCURY, GLOVES SHOULD BE USED WHEN HANDLING. THE ETHANOL USED IS HIGHLY FLAMMABLE, PROPER PRECAUTIONS SHOULD BE TAKEN. SOME OF THE BACTERIA, SUCH AS PSEUDOMONAS AERUGINOSA, ARE OPPORTUNISTIC PATHOGENS. THEREFORE BACTERIA SHOULD NOT BE TOUCHED WITH BARE SKIN, THESE SPECIES ARE UBIQUITOUS AND NOT USUALLY PROBLEMATIC, BUT IT IS BEST TO BE CAUTIOUS.

8. MATERIALS LIST (including any handouts or transparency masters):
MATERIALS
NESSLER'S REAGENT (CONTAINS MURCURY)
LATEX OR NITRILE GLOVES
95% ETHANOL
GLASS RODS
BUNSEN BURNER OR OTHER SOURCE OF FLAME
MATCHES
SMALL TEST TUBES AND TEST TUBE RACKS
BACTO-PEPTONE BROTH
SULFURIC ACID TO LOWER PH OF BROTH
PH INDICATOR (FOR PREP)
CULTURES
PSEUDOMONAS AERUGINOSA
BACILLUS CEREUS
ANY OUTDOOR SOIL
HANDOUTS
THE ORIGINAL LAB IS AVAILABLE AT: HTTP://WWW.ECOED.NET/. WE MADE COPIES OF THIS VERY WELL DONE ACTIVITY AND GAVE THEM TO THE STUDENTS. A COPY OF THE PDF OF THIS ACTIVITY IS AVAILABLE AT: HTTP://WWW.BIOED.ORG/ECOS/.

TRANSPARENCY
POWER POINT PRESENTATION.

9. METHODS/PROCEDURE FOR STUDENTS:

a. *Pre-investigation work*: none for students. For teachers, proper inoculation procedure for the broths is as follows. First plated bacterial cultures should be established: one to two days in an incubator should be sufficient to establish good colony growth. After all of the tubes have been labeled and filled with the correct pH broth, use a wire loop to perform the inoculations. Flame the loop over a Bunsen burner, then let it cool before touching the loop to the bacterial growth on the plate. Then take the top off of the test tube being used, pass the open end of the test tube through the flame, and dip the inoculating loop into the broth. Then remove the loop, cap the tube, and reflame the loop. Rinse and repeat until all cultures are done. The plate cultures should be covered when not being used. For the soil culture, merely dump a small portion (a small pinch) of dirt into the tube, make sure roughly the same quantity of dirt is used in all of the tubes.

b. Investigation work:

1) *What evidence (data, samples) do students collect:* First, drops of Nessler's reagent will be placed on glass slides. Students will use glass rods to transfer the bacteria to a glass slide for the Nessler's reaction. The rods will be dipped in ethanol and then passed through the flame of a Bunsen burner to sterilize them. They will then be dipped in one of the broths, and a drop of the culture will be placed one the drop of Nessler's reagent. The color reaction that does or does not occur will then be recorded. No reaction is indicated by a lack of color change. A mild reaction will result in shades of yellow. A larger reaction will result in a brown color, and brown color with precipitate indicates the greatest reaction. This procedure will be repeated for all of the pHs being tested for both pure bacterial cultures, the soil, and the control.

2) *How do students present the evidence (data*): In tables. There are tables both for predictions, and for actual results. Students can then analyze why their predictions were or were not correct. They then answer a list of questions based on the data.

3) *What conclusions are drawn from the evidence students collect?* The questions following the exercise make the students use what they have seen to draw conclusions. The questions are interpretive, and ask questions about such topics as the rate of decomposition of various organic substances at different pHs.

4) *Include examples of data sheets.* See activity sheet when it is ready.

10. ASSESSMENT:

Activity sheet with questions should be used to determine if the activity was well understood.

11. EXTENSION IDEAS:

SIMILAR SETUP COULD BE USED TO DETERMINE MICROBIAL UTILIZATION OF HEAVY METALS OR ARSENIC. WHERE WE PERFOMRED THIS ACTIVITY IN WESTERN MONTANA, THIS WOULD HAVE BEEN A MORE LOCALLY RELEVANT CAUSE OF ENVIRONMENTAL DISTURBANCE.

12. SCALABILITY
THIS IS AN ACTIVITY THAT SHOULD BE USED AT THE HIGH SCHOOL LEVEL AND POSSIBLY IN COLLEGE. DUE TO THE TOXIC NATURE OF THE NESSLER'S REAGENT AND ADVANCED ACADEMIC MATERIAL, THIS SHOULD NOT BE DONE WITH YOUNGER KIDS.

13. REFERENCES:
NAMOWITZ AND SPAULDING. EARTH SCIENCE. 1989. D.C. HEALTH AND COMPANY. (PAGES 91, 121, 465)
THE INFLUENCE OF ACIDIFICATION ON AMMONIFICATION, BRIAN R. SHAEFSKY. AVAILABLE AT: HTTP://WWW.BIOED.ORG/ECOS/.

14. LIST OF EXPERTS AND CONSULTANTS
The reagents used for this activity are commonly used for other microbiology lab activities. We were able to get pure cultures of both bacteria species from the University of Montana. We recommend trying to contact a local university and asking for a professor of microbiology for advice and help, if needed. In Missoula, if you are interested in trying to use heavy metals instead of pH as a treatment, contact David Nicholas (email if he says its okay).

15. EVALUATION/REFLECTION BY FELLOWS AND TEACHERS OF HOW IT WENT:
The activity went relatively well. We tried using two different set ups to get the class through the exericise. In one, the students were divided into groups of two, each having their own ethanol, burner, and set of inoculated tubes. There was an explanation directly before starting the experiment about nitrogen cycling. In addition to a discussion of the nitrogen cycle the opening discussion covered broad topics relating not only to general nutrient cycling, but also to the importance of bacteria to many varied ecosystems (for example, mutualistic relationships with cows for the digestion of cellulose, with humans for aiding in digestion, the production of vitamins, and out-competing pathogenic bacteria, coral reefs, etc.), as well as the ways in which humans have harnessed bacteria to meet their needs (food production, antibiotics). This discussion is a helpful way to introduce the experiment if class time allows. After giving basic instructions the students then worked on their own in groups. The teachers applied the Nessler's reagent due to its toxicity, and answered questions as needed. We discovered that this method did not work very well. Despite giving very specific instructions as to how to proceed, many of the groups did the experiment incorrectly, and much time was wasted.

A second method we tried was setting up only a few stations in the back of the room with the ethanol, burners, etc. In this way, two or three groups came back at a time, and we made sure each one of them did the experiment correctly, and we were able to offer explanations if there was some aspect of the experiment which they didn't understand. This worked much better, and even went quicker, despite the fact that some groups had to wait for a considerable amount of time before getting access to the materials.