THE INFLUENCE OF ACIDIFICATION ON AMMONIFICATION

ESA lab

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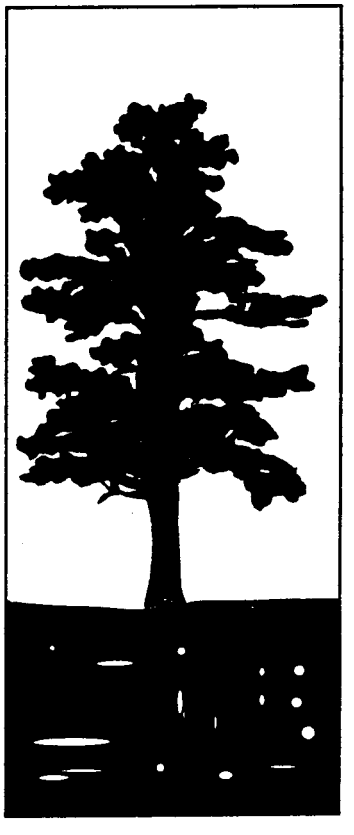
INTRODUCTION



Organic nitrogen deposited in the soil (in leaves, wood, etc.) is a major supply of nitrogen for terrestrial ecosystems. But much of this organic nitrogen is bound in proteins and other complex compounds that are not readily available sources of nitrogen for terrestrial ecosystems. To be used these compounds must first be converted into chemically simpler forms by soil microorganisms. **Ammonification** is a series of microbial metabolic activities that release organic nitrogen in the form of ammonia (in alkaline, agricultural soils) and as ammonium ions (in the more acidic soils of most terrestrial ecosystems). The ammonia and ammonium forms of nitrogen are suitable for many soil microbes and plants. Soil microbes convert much of the ammonia and ammonium ions into nitrite and nitrate ions through the process of **nitrification**. These ions are also readily used by a variety of the soil microbes and plants. The organisms using these inorganic nitrogen forms ultimately incorporate the nitrogen into organic substances, thus completing one aspect of the **Nitrogen Cycle** (Figure 1).

Nitrogen fixation, the microbial conversion of gaseous nitrogen into ammonia and ionic nitrogen, also supplies soil organisms with some usable nitrogen. However, in most terrestrial ecosystems, ammonification is critical to supply sufficient quantities of usable nitrogen.

Current research indicates that nitrogen availability in the soil is significantly influenced by **soil acidity**. Much of the effect may be due to the sensitivity of soil microorganisms to pH changes. This is supported by a

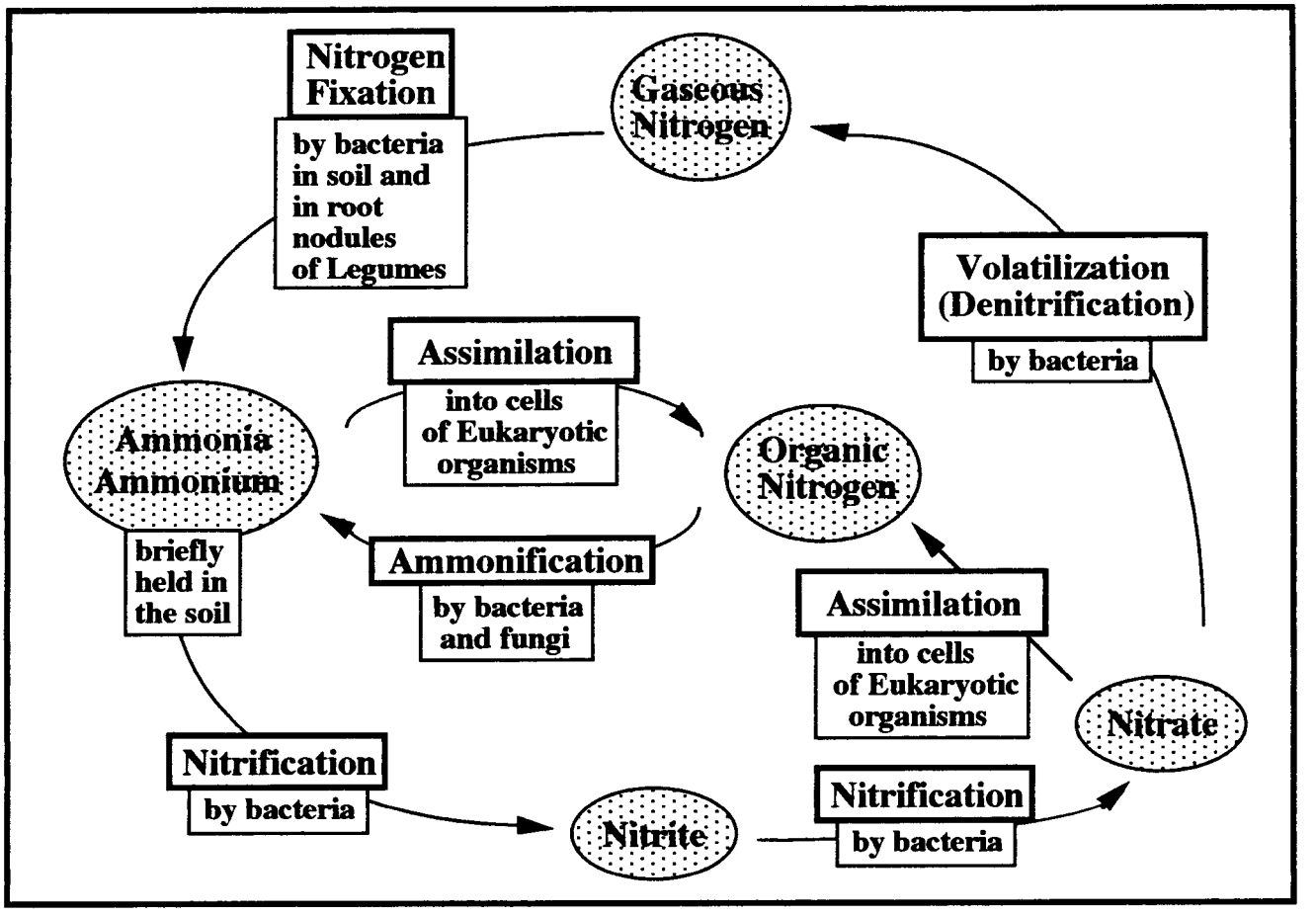


FIGURE 1. The Nitrogen Cycle

soil acidity and acid rain

growing body of evidence that suggests a link between decreasing pH (increasing acidity) and a decrease in the survivability of other soil inhabitants including some plants, worms and amphibians. One major source of soil acidification is the sulfuric acid present in acid rain generated by the combustion of fossil fuels. The use of high sulfur petroleum and coal in automobiles and industrial applications has already contributed to this destructive acidification of soils in many regions of the United States, Europe and Asia.

acidity and microbial activities

In the following in vitro laboratory experiment you will examine the influence of moderate (pH 5.5) to extreme (pH 3.5) sulfuric acid-induced acidification on the ability of soil microorganisms to conduct ammonification. You will analyze two common soil inhabiting bacteria, *Pseudomonas aeruginosa* and *Bacillus cereus*, for: 1. their ability to perform ammonification given only organic nitrogen and 2. their ammonification ability in an acidic environment. You will also look at the effect of acidification on the amount of ammonification in a local soil sample.

LABORATORY OBJECTIVES

experimental

1. Determine the ability of two soil bacteria (*Pseudomonas aeruginosa* and *Bacillus cereus*) to convert organic nitrogen to ammonia.
2. Determine the effect of acidification on the ammonification ability of the above species of bacteria.
3. Determine the effect of acidification on the ability of native soil microbes to convert organic nitrogen to ammonia.

procedural

4. Learn to manipulate bacterial cultures using a sterile technique.
5. Learn to distinguish different amounts of ammonification using a Nessler's reagent testing procedure.

MATERIALS

equipment

autoclave or pressure cooker

pH meter (optional)

supplies

3 microbiological culture tubes
caps for tubes
Nessler's reagent
alcohol burner
4 microscope slides
(or 12 small culture tubes and rack)
kimwipes

pH paper
peptone broth
5" sterile pipettes
waxed pencil or marker
70% ethanol
toothpicks
glass rod
beaker of 70% Ethanol (labeled "waste ethanol")

organisms

slant, plate or broth cultures of:
Pseudomonas aeruginosa
Bacillus cereus

soil sample

PROCEDURE

Your instructor may have you acidify nutrient broth with H_2SO_4 and establish your own cultures, or they (see the list on the following page) may already have been prepared for you to use. He/she will tell you to follow procedural method 1 or 2 depending on the supplies available for your lab. Both procedures test for the presence of ammonia with Nessler's reagent, a clear solution that undergoes colorimetric reactions in the presence of different concentrations of the substance.

cultures

You will prepare the following 4 sets of cultures or they will be available in the lab.

Set 1 - *Pseudomonas aeruginosa* cultures of pH 7, pH 5.5 and pH 3.5
Set 2 - *Bacillus cereus* cultures of pH 7, pH 5.5 and pH 3.5
Set 3 - native soil in cultures of pH 7, pH 5.5 and pH 3.5
Set 4 - uninoculated cultures of pH 7, pH 5.5 and pH 3.5

control

Set 4 is the control. Why?

predictions

Predict the expected outcome of the reactions for sets 1, 2 and 3. Use ++ for a strong reaction, + for a mild reaction, and - for no reaction.

culture	pH 7	pH 5.5	pH 3.5
Set 1 - Pseudomonas aeruginosa			
Set 2 - Bacillus cereus			
Set 3 - microbes in native soil			

METHOD 1: microscope slides and glass rods

procedure

1. Divide a microscope slide into thirds with a waxed pencil (see Figure 2a).

2. Bring one of the sets of three cultures to your lab table:

3. Using a sterile pipette, place a drop of Nessler's reagent in each section of one of the microscope slides (Figure 2b.)

4. Sterilize a glass rod by dipping it in 70% ethanol and carefully passing the rod through a flame until the alcohol is burned off. **Use caution to prevent the flaming alcohol from running onto your hand, clothing, or table top.** (A metal inoculating loop cannot be used because it will give false positive results in the following steps). Allow the rod to cool.

5. Dip the sterilized glass rod into the pH 7 culture and place a drop in the appropriately marked section on the microscope slide (Figure 2c). Thoroughly mix the drops of Nessler's reagent and culture with a clean toothpick.

6. Dip the rod into a waste ethanol and wipe it dry with a kimwipe.

7. Repeat steps 4, 5 and 6 with the pH 5.5 and 3.5 cultures.

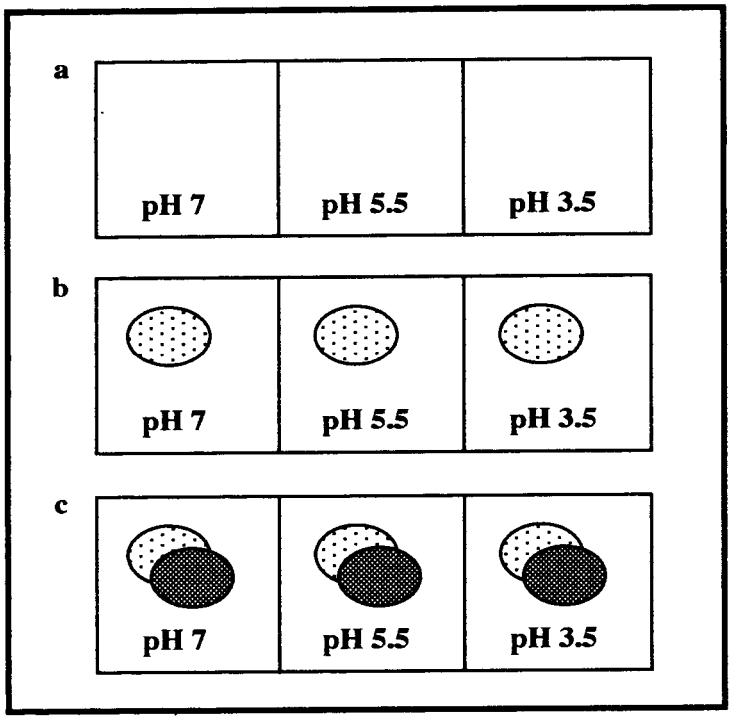
8. Examine the slide for color changes:
   clear = no reaction
   yellow = some reaction
   brown = more reaction
   brown + precipitate = greatest reaction

observations

9. Before continuing, record your results in Table 1 (at the end of Method 2).

10. Repeat steps 2 through 8 for the remaining three sets of cultures.

FIGURE 2: data collection method 1



Method 1: a. Microscope slide marked into three sections; b. one drop of Nessler's reagent in each section; c. one drop of culture plus Nessler's in each section.

METHOD 2: culture tubes and pipettes

1. Label 4 series of 3 small culture tubes as follows: (Figure 2):

   Pseudomonas aeruginosa - pH 7, pH 5.5 and pH 3.5
   Bacillus cereus - pH 7, pH 5.5 and pH 3.5
   soil - pH 7, pH 5.5 and pH 3.5
   control - pH 7, pH 5.5 and pH 3.5

procedure

2. Using a sterile, disposable pipette, place 4 drops of Nessler's reagent into each tube. (The exact number of drops isn't important as long as each tube has the same number).

3. Bring one culture series (each pH) to your lab table. Again, using a sterile disposable pipette, place 4 drops from each of these cultures into the appropriately marked culture tubes.

question

If you begin with the culture of the lowest pH, you can use one pipette for all three cultures in one set. Why? (Hint: see your predictions.)

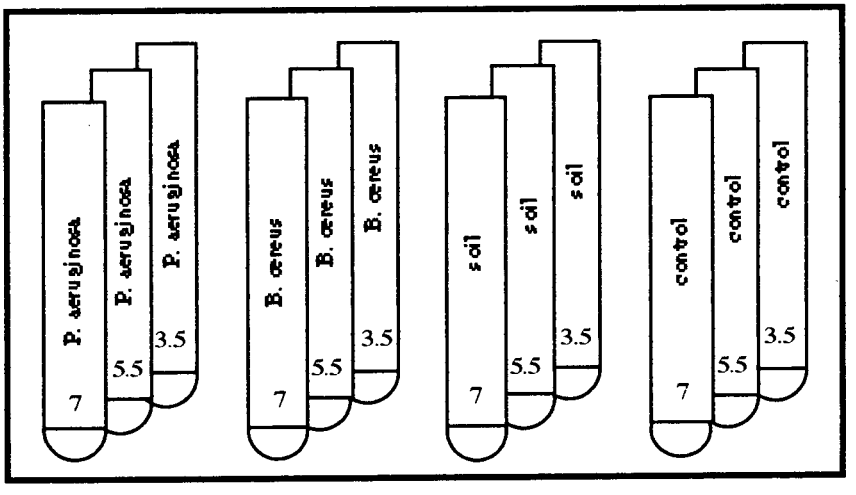
observations

4. Examine the tubes for color changes:
   clear = no reaction
   yellow = some reaction
   brown = more reaction
   brown + precipitate = greatest reaction

5. Before continuing, record your results in Table 1 (on the following page).

6. Repeat steps 3 through 5 for the remaining three culture series.

FIGURE 3: data collection method 2



Method 2. Culture tubes marked for data collection method two. One set of three tubes for each culture series.

TABLE 1: Experimental results acidification and ammonification experiment

culture	color of liquid on slides or in tubes		
	pH 7	pH 5.5	pH 3.5
Pseudomonas aeruginosa			
Bacillus cereus			
soil culture			
uninoculated control			

QUESTIONS

1. Did both pure bacteria cultures show ammonification? If not, which did?

2. What effect did pH have on ammonification of the pure bacterial sample(s)?

3. How did ammonification in the soil sample compare to the pure cultures of *Pseudomonas* or *Bacillus*?

4. What effect did pH have on ammonification in the soil sample?

5. Based on your results what would be the effect of acid rain on plant growth?

6. Based on your results predict some long term effects of acid rain on the following:

   a. leaf litter decomposition in a temperate forest

   b. regrowth of a forest harvest for paper in eastern Canada

   c. a stream passing through a dense forest

   d. populations of grazing or foraging animals

   e. the growth of crops in industrialized countries

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NOTES TO INSTRUCTORS

GENERAL COMMENTS

In this lab activity students will learn to complete a simple experiment involving the manipulation of one variable that influences the outcome of the physiological events of a population of organisms. They will also gain an understanding of the effect of acidic pH on the efficiency of organic degradation by soil microbes.

Bacterial cultures can be prepared in advance of the laboratory meeting or the students can be involved in setting up the cultures as well as in determining the results.

RECIPES

bacto-peptone broth

1. Mix a 4% solution of Bacto-peptone broth (40 g powder: 1000 ml distilled water).
2. Cap and autoclave the broth for 20 minutes at 240^0 F; allow it to cool.
3. Divide the broth into 3 three equal portions using 3 sterile flasks.
4. Using concentrated sulfuric acid, adjust one third of the broth to pH 7.0, one third to pH 5.5 and one third to pH 3.5. The pH cannot be adjusted until after the broth has been sterilized or it will be hydrolyzed.
5. Decant the broth into sterile test tubes; cap the tubes.
6. The sterile broth may be refrigerated for several days before use.

Nessler's reagent

SOLUTION A: Add 50 g of potassium iodide to 200 ml of distilled water. Drop by drop, add a saturated solution of mercuric chloride until only a slight precipitate remains.

SOLUTION B: Add 400 ml distilled water to 200 g of sodium hydroxide. **Use caution: this is an exothermic reaction!**

MIXING SOLUTIONS: Add solution A to solution B and dilute the mixture to 1000 ml with distilled water. The solution should be permitted to settle for at least 5 days. The completed solution should be clear with some sediment but with no floating particles. Store the solution in an amber glass bottle or in a covered, clear glass bottle.

pre-made Nessler's

Commercially available Nessler's can be purchased and used if preferred.

BACTERIAL TRANSFERS

materials

1. You will need the following materials:

   1 or 2 inoculating loop(s)
   bunsen burner or alcohol lamp
   4 test tubes of sterile pH 7.0, 4% bacto-peptone broth
   4 test tubes of sterile pH 5.5, 4% bacto-peptone broth
   4 test tubes of sterile pH 3.5, 4% bacto-peptone broth
   1 24 hour agar slant or plate culture of *Pseudomonas aeruginosa*
   1 24 hour agar slant or plate culture of *Bacillus cereus*
   1 fresh soil sample

procedure

2. Sterilize the inoculating loop by passing it through a flame until it is red hot.

3. Let the loop cool then scoop up a loopfull of *Pseudomonas aeruginosa.*

4. Swirl the inoculating loop into the pH 7.0, 4% bacto-peptone broth. Cap the tube.

5. Repeat steps 2-4 for the pH 5.5 and pH 3.5, 4% bacto-peptone broth.

6. Repeat steps 2-5 for *Bacillus cereus.*

7. Add an equal amount of soil to a culture of each pH. The amount will depend upon the type of soil. (Try 0.5g [about 1/8 tsp.]).

8. The remaining three test tubes are not inoculated , they are controls.

incubation

9. Incubate all test tubes for at least 2 days in a warm room, or in an incubator set at 37° C. The incubation time and temperature depends on how soon the students will examine the results and should be determined by pre-testing the experiment.

alternative

An alternative method of inoculation is to use sterile, disposable pipettes to transfer bacteria to the tubes.

VARIATIONS

This expment can ve varied in a number of ways. A few suggestions are given below.

different soils

Different soils give different results. Try:

1. garden soil
2. greenhouse soil
3, potting soil
4. forest soil
5. lawn soil, etc.

related studies

You could also test:

1. heavy metal poisoning
2. insecticide or herbicide pollution
3. salt accumulation

EDITOR'S NOTE

The nitrogen cycle diagram has been simplified for this lab exercise and does not reflect all of the interactions within this cycle. Research in this area is proceeding rapidly and the more recent literature will give the most detail.

EXPERIMENTS TO TEACH ECOLOGY FEEDBACK FORM

ACIDIFICATION AND AMMONIFICATION

Please complete this form after you have used this experiment and mail it to the address given on the reverse side of this page.

1. Was the introduction clear and informative? What changes would you suggest?

2. Was the list of materials complete? Would you suggest any additions or modifications?

3. Which procedure did you use? Did it work well? Are there changes you would suggest?

4. Were the illustrations and data charts adequate? What others would you include?

5. Were the instructor's notes complete? If you noted any omissions, what were they?

6. What level of students used the experiment? Was it suitable for this level? If not, what changes would you suggest?

7. Did the experiment work? Please explain any problems you had when using the experiment?

8. On a scale of 1-10, with 10 as outstanding and 1 as terrible, how would you rate this experiment? Would you recommend this experiment to others?

9. Was it helpful for this laboratory exercise to have been written for the student rather than as an instructor's guide?

Please mail the completed form to: Dr. Jane M. Beiswenger
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