Environmental Science Laboratory, Fall 2006

Laboratory #4: Bacterial Activity in Forest Soil*

The numbers and kinds of microbes present in a soil will vary considerably from one site to another as a function of each soil's chemical composition, aeration, moisture, temperature, and so forth. An understanding of the ways these and other factors affect the microbial communities of soils is important to both environmental studies and to agriculture.

Today, we will start our investigation of microbes involved in the nitrogen cycle of the forest floor. Nitrogen is one of the four most important elements needed for the construction of organic molecules. In addition, nitrogen compounds serve as electron donors or acceptors in the metabolic activities of certain specialized kinds of bacteria. As a consequence of these many processes, nitrogen compounds are constantly being converted from one form to another, differing mainly in their state of reduction or oxidation. This flux or traffic of nitrogen constitutes the <u>nitrogen cycle</u>, - one of the most important of the biogeochemical cycles, and one in which microbes play essential roles.

The two steps of the nitrogen cycle which we will focus on this week and next week are ammonification and nitrification. <u>Ammonification</u> is the release of ammonia during the degradation of organic molecules by heterotrophic microbes. This ammonia is only minimally usable as a nitrogen source by plants, which rely much more upon the nitrate ion for their nitrogen. <u>Nitrification</u> is the conversion of ammonia to nitrate.

Before starting today's lab ask yourself the following questions:

> *Why is important to learn about soil microbial communities?*
> *What type of ecosystem service does a healthy microbial community provide?*
> *What hypotheses will you be testing by comparing microbes between forest and non-forest soils?*
> *What are your expectations?*

<u>Field procedure:</u>

- You will work in groups of three students (6 groups total).
- Three groups will collect soil samples from one of the plots used for the vegetation analysis. The other three groups will collect soil samples from a non forested area near the pavilion.
- Each group will collect three soil samples (total samples 6* 3 = 18)
- Bring the soil samples to the lab and perform the following qualitative tests in order to identify these microbially-mediated processes.

*Adapted from Exercise 8 in Dr. Hill's "Microbiology Laboratory Manual, 1995-1996"

Part I: Ammonification

We will use a qualitative test for ammonification, by placing a sample of soil in a medium that provides only peptone as a nutrient source. Since nitrogen is in excess in such a food source, microbes degrading the peptone will discard extra nitrogen in the form of ammonia.

Procedure

1. Obtain a tube of sterile 4% peptone broth (5 ml).

2. Inoculate the broth with about one-tenth of a gram of soil, mix thoroughly, let it equilibrate for about 5 minutes, and test for initial ammonia content as follows:

    a. Add one drop of Nessler's solution to a depression on a porcelain spot-plate.

    b. Clean a glass stirring rod with water, and then sterilize one end of it by dip-ping it into 95% ethanol, and then touching it briefly to a flame in order to burn the solvent away.

    c. Dip the sterile end of the rod into the peptone broth culture, and mix the adhering drop into the Nessler's reagent in the well of the spot plate.

    d. The development of a yellowish to orangish precipitate is indicative of the presence of ammonia. (Look for a precipitate, not just a color change.)

    e. Wash the spot plate and glass rod with soap and hot water, and set them aside to dry.

3. Incubate the culture at 25°C, and repeat the ammonia assay **on every other day starting on Friday until you get a** very strong, distinct precipitate forms**. You must record your observations every time.**

Part II: Nitrification

The conversion of ammonia to nitrate is a two-step process, each part of which is carried out by its own specific genus of bacterium. *Nitrosomonas* first oxidizes ammonia to nitrite, and then members of the genus *Nitrobacter* carry out the conversion of nitrite to nitrate.

For detection of the activity of *Nitrosomonas,* we'll use Nitrosomonas Medium, which contains the ammonium ion as its sole nitrogen source. Following inoculation of this media, we'll monitor the culture periodically for nitrites using Trommsdorf's reagent.

To detect conversion of nitrite to nitrate, we'll use Nitrobacter Medium, whose nitrogen source is sodium nitrite. Unfortunately, we can't just do a quick test for nitrates, because all the nitrate-detecting reagents available to us will also react with nitrites, and of course the medium is well supplied with those from the very beginning. Therefore, we have to wait first until the medium has been depleted of nitrites, presumably through the action of *Nitrobacter*, before using diphenylamine reagent for detection of nitrates.

Procedure

NOTE: The tests using Trommsdorf's reagent and diphenylamine reagent involve strong acids. Be sure to BE CAREFUL! Wash any spills immediately with water, and clean the spot-plates in such a manner that the acids are confined to the sink and are completely washed down the drain.

1. For every sample of soil to be tested, obtain one tube of Nitrosomonas Medium and one tube of Nitrobacter Medium.

2. Inoculate each with about 0.1 gram of soil, and mix well.

3. Before beginning the incubation, aseptically test both media with Trommsdorf's reagent in order to establish a base-line point of reference.

   a. With a sterile glass rod, add a drop of culture to a spot-plate well, which already contains:

      i. Trommsdorf's reagent 3 drops
      ii. 1/4 concentrated sulfuric acid solution 1 drop

4. Incubate the cultures at 25°C, and test each broth using the Trommsdorf's reagent **every other day starting on Friday. The appearance of** an intense blue-black color in the Nitrosomas medium indicates the production of **nitrites** (NO_2^-). The **disappearance** of blue block color in the Nitrobacter medium shows that NO_2^- has been converted to another form.

5. After nitrites can no longer be detected in the Nitrobacter Medium, test it for the presence of **nitrates** (NO_3^-)by transferring one drop of medium to a spot-plate well, to which has already been added

diphenylamine reagent	1 drop
concentrated sulfuric acid	2 drops

Keep performing this assay using diphenylamine reagent every other day until an intense blue-black color indicates the presence of **nitrates.**

As always, be sure to **record your observations every time**.