

Legumes—Nitrogen “Fixers”

10.11.03

Name _____

Partner _____

Hour _____

One aspect of soil microbiology that has been studied extensively is the nitrogen cycle. All organisms need nitrogen for the synthesis of proteins, nucleic acids, and a few other nitrogen-containing compounds. The recycling of nitrogen by different organisms is called the nitrogen cycle. Microbes play a fundamental, irreplaceable role in the nitrogen cycle by participating in many different metabolic reactions involving nitrogen-containing compounds.

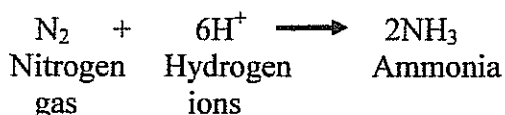
One way nitrogen is recycled.... When plants, animals, and microorganisms (all of which are made of proteins) die, microbes decompose (rot) them by proteolysis (destruction of the proteins from which they are made) and return the nitrogen to the soil for re-use.

Name two biological molecules that need nitrogen for their synthesis: _____, _____

Tell what the nitrogen cycle does: _____

Describe proteolysis: _____

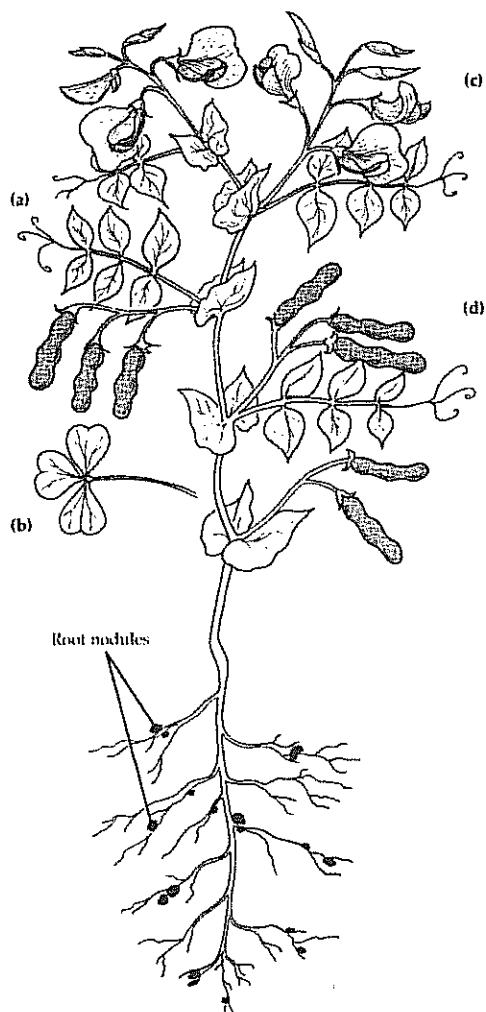
A second way nitrogen can be made available to living things..... Atmospheric nitrogen (N_2), which is of little value to plants (as part of the air), can be returned to the soil by the conversion of nitrogen gas into ammonia, a process called nitrogen fixation. Here is how it works....



Ammonia is then converted to the ammonium ion (NH_4^+) because of water in the soil.

Some free-living prokaryotic organisms, such as *Azotobacter* and cyanobacteria, can fix nitrogen. However, many of the nitrogen-fixing bacteria live in close association with the roots of plants in the rhizosphere, where root hairs contact the soil.

Symbiotic bacteria serve a more important role in nitrogen fixation. One such symbiotic relationship (two organisms living together) is a mutualistic (they HELP each other) relationship between *Rhizobium* and the roots of legumes (such as soybeans, beans, peas, alfalfa, and clover). There are thousands of legumes. Farmers grow soybeans and alfalfa to replace nitrogen in their fields. Many wild legumes are able to grow in the poor soils found in tropical rain forests, arid deserts, and poor South Carolina soils. *Rhizobium* species are specific for the host legume that they infect. When a root hair and rhizobia make contact in the soil, a root nodule forms on the plant. The nodule provides



Some characteristic features of legumes. The leaves are opposite and may be (a) pinnately or (b) palmately compound. The flowers (c) have five asymmetrical petals, and the fruits (d) are pealike.

the anaerobic environment necessary to host the bacteria and for nitrogen fixation.

Give the symbol for atmospheric nitrogen: _____ Tell what the process of nitrogen fixation, as conducted by bacteria, does: _____

Name two free-living prokaryotes (bacteria) that can carry-on nitrogen fixation in the soil or water: _____ and _____ Where is the rhizosphere and why is it important? _____

Describe the process of symbiosis: _____

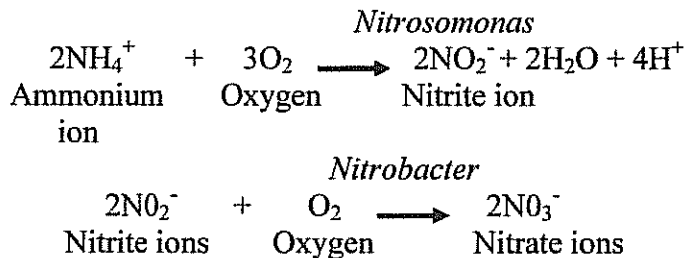
Describe mutualism (a type of symbiosis): _____

Give the name for the types of plants that can host bacteria for nitrogen fixation: _____

Describe the part of the plant that will host the bacteria: _____

Any break in the nitrogen cycle would be critical to the survival of all life. Some of the ammonium (NH_4^+) ions are used directly by plants and bacteria for the synthesis (making) of amino acids which are used to make new proteins. However, there are other types of soil bacteria that can take over and convert the ammonium to other nitrogen compounds.

Two genera of soil bacteria are capable of oxidizing the ammonium ion in the two successive stages, shown as follows:



Nitrates are an important source of nitrogen for plants.

Name the material living things make from the nitrogen-rich compounds listed above: _____

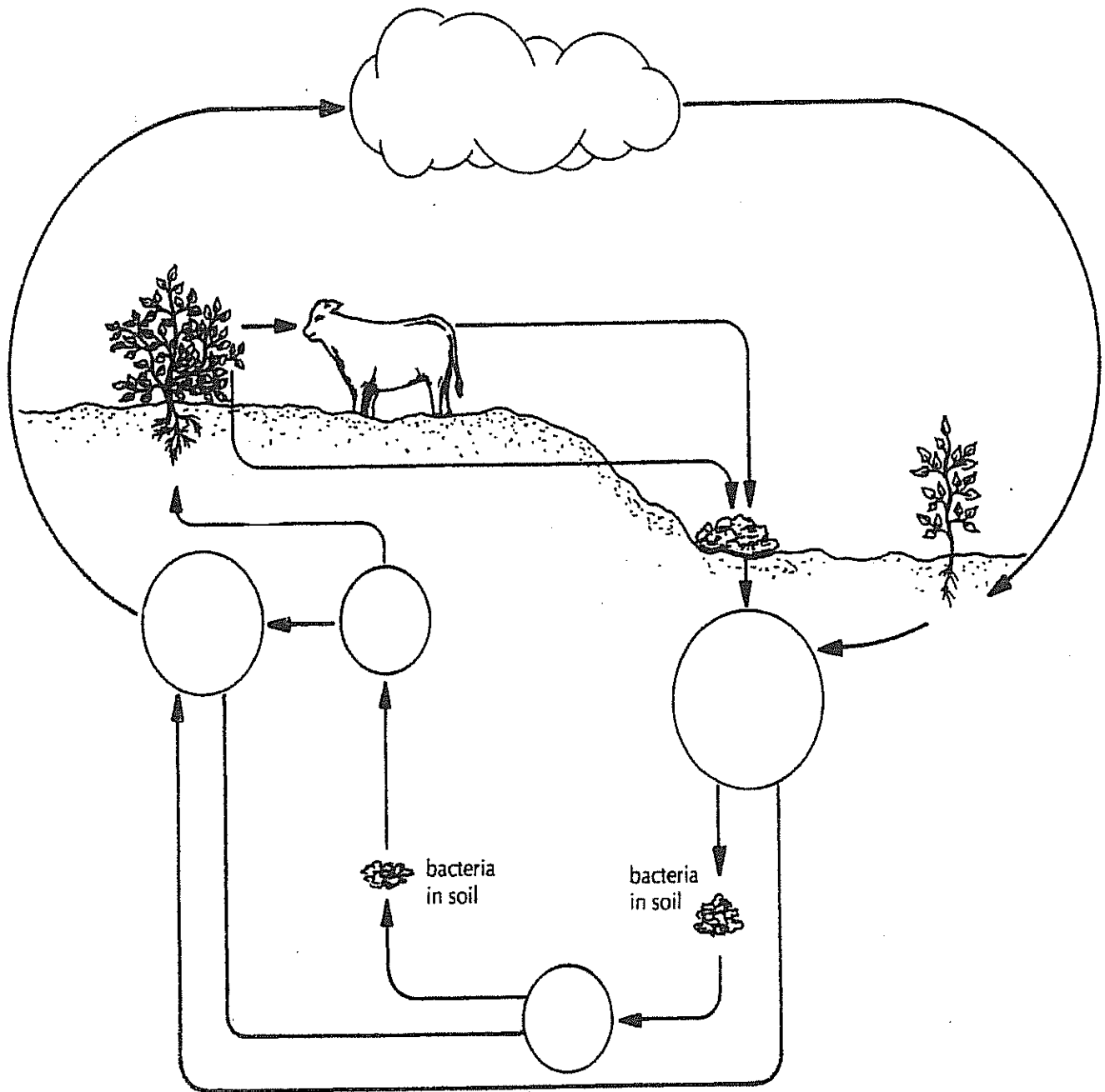
Name two soil bacteria that that process ammonia to other nitrogen-rich compounds (mainly for use by plants): _____ and _____

Write the equation for nitrogen fixation:

Write the equation for nitrite production by *Nitrosomonas*:

Write the equation for nitrate production by *Nitrobacter*:

Below is a diagram summarizing the nitrogen cycle. Use the diagram available in class (or on the internet) to label all parts of the cycle.



Procedure:

As a part of this activity, EACH partner will grow a legume.

OUTDOORS—Collect ~2L of “forest soil” from a designated area of campus for your lab group. The soil naturally contains nitrogen-fixing bacteria and will supply our inoculum.

Prepare the growth chambers (pots) as demonstrated by your instructor. Label the outside of your container with your name, the date, and the legume (seed) you will grow.

On a piece of newsprint (use your growth chamber (pot) to measure) thoroughly mix one-half measure of forest-soil with one-half measure of prepared growth medium. Fill your growth chamber to within ~1cm of the top.

Push 4-12 seeds just under the surface of the growing mixture. LIGHTLY top-water the growth medium—check carefully to make sure your seeds were not “exposed” during the watering.

Seed sources (circle the seed-type you planted for your lab group):

Clover (*Trifolium sp*)

Soybeans (*Glycine max*)

Peas (*Pisum sativum*)

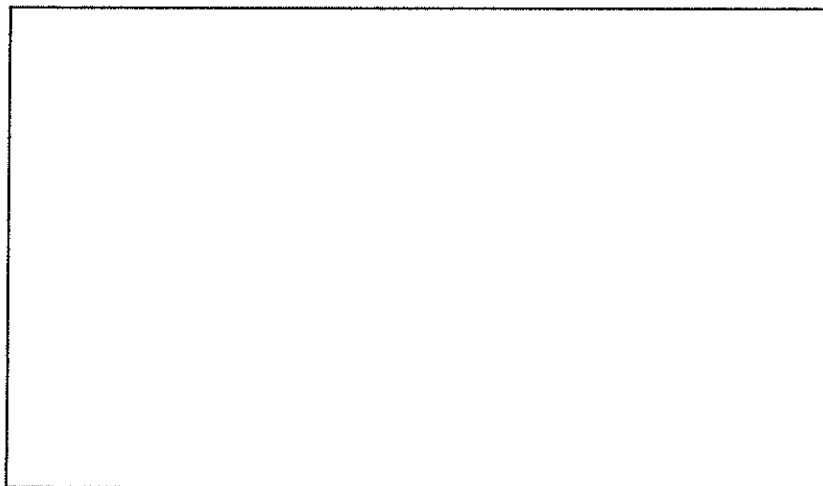
Lima Beans (*Phaseolus lunatus*)

Inoculum (from the forest soil): *Rhizobium sp*

Now we wait....Plant growth and maturity will take several weeks. Weekly care will involve filling the water reservoirs, thinning and trellising the plants, and, possibly, adding a nitrogen-deficient fertilizer.

At the end of the growing period carefully remove one of the mature plants WITH THE ROOT SYSTEM IN ONE PIECE. Wash the roots in a 3-L bottle to remove excess growth medium. DO NOT pour the wash water down the drain—follow instructions concerning disposal.

Examine the roots with a magnifier....Draw a sample of the roots and any nodules you observe in the space below. IF you don't observe any nodules on your plant, use the “sample” plant available in class for your drawing. Label the nodules.



Observing the bacteria in the nodules:

Place a drop of congo red stain on a microscope slide. Remove several nodules and transfer them to the stain. Using a razor blade, finely dice the nodules allowing the contents to mix with the stain.

Use a toothpick to move a sample of the milky-red fluid to a second microscope slide. Spread the material so it forms a THIN layer and allow it to air-dry for a few minutes.

Add a drop of acid-alcohol and a cover slip. View at high power under the compound light microscope. Do a quarter-field drawing of what you observe in the space below. Have your instructor verify your drawing when you're done.

Verified _____

