Role of Microorganisms in Nitrogen Cycling in North Dakota Soils

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We all live at the bottom of a vast ocean of nitrogen gas which represents about 79 per cent of the atmosphere we breathe. Over every single acre of soil in North Dakota stands a column of nitrogen weighing about 32 thousand tons. However, this nitrogen is in the form of a very stable molecule (N_2) which is unusable by plants and animals without fixation; which is the conversion of atmospheric nitrogen into chemical forms usable by plants and animals.

Nitrogen has always been one of the limiting resources for growth in agriculture. Production in agriculture was revolutionized by the development in the early 1900's of the Haber-Bosch process which provided a relatively cheap source of industrially fixed nitrogen. In fact, so much nitrogen is now fixed industrially that nitrogen is building up on land becoming a pollution problem in runoff to waters. Industrial fixation promises to become more and more expensive. This is, in part, because of the energy required to break the stable nitrogen bond. A more important reason is that natural gas is used as the main chemical feedstock; it provides the hydrogen which is added to nitrogen (N_2) to convert it to ammonia (NH_3). When we see photographs of natural gas being flared away in oil fields in Mexico and the Middle East we should think of this.

It is unlikely that biological nitrogen fixation will ever totally replace industrial fixation for intensive agriculture. However, research in biological fixation is being intensified because added efficiency in biological nitrogen fixation would help conserve irreplaceable types of fertilizer supplies. At the present the annual amount of biological nitrogen fixation is roughly equal to that fixed by industry, although much of it is unrelated to agricultural uses.

One would think that nitrogen, an element so important for growth and present in such immense amounts, would be available for direct use by many organisms. However, the energy requirements for breaking the nitrogen bond are so high that very few organisms are capable of it. No higher organism such as a plant, animal, protozoan, fungus or alga is able to fix nitrogen by itself. Only a few species of bacteria and the blue-green algae (which are a form of photosynthetic bacteria and not the same as higher algae) have this ability.

Farmers are most familiar with the *Rhizobium* bacteria which cause formation of nodules on the roots of legumes such as soybeans and alfalfa. The bacteria are fairly specific for certain plants – for example, the species which infects soybeans will not infect alfalfa. The bacterium attaches to a root hair of the plant and in response the plant forms a hollow thread leading into the root. Bacteria grow through this infection thread and eventually initiate formation of a nodule on the root. As much as 30 per cent of the weight of a nodule may be bacteria. The plant supplies the bacteria with nutrients and the bacteria supply the plant with nitrogen from the air in a form the plant can use. This is an example of symbiotic nitrogen fixation. There are over 12,000 leguminous species of plants, only a few of which are used in agriculture.

Rhizobium bacteria can be purchased commercially for inoculation of legumes at seeding. This inoculation results in better and more effective nodulation, particularly, if the soil has not been regularly cropped with the same leguminous plant.

Considerable research effort throughout the world is being expended on improving the efficiency of this type of nitrogen fixation. Commercial inoculants have always been selected for effectiveness in nodulation, but recent developments in microbial genetics have indicated that further improvements are possible.

These improvements will be based largely on use of genetic engineering, or as it is more exotically termed, recombinant DNA research. For example, it has recently become possible to transfer genetic information between one bacterial species and another; this is more than just selective breeding. The common intestinal bacterium *Escherichia coli* is not capable of fixing nitrogen from the air. However, another similar bacterium, *Klebsiella pneumoniae*, can fix nitrogen. The genes to fix nitrogen can be transferred by laboratory manipulation to *E. coli* from *K. pneumoniae*. These two bacteria are rather closely related, which simplifies procedures, but it is also possible to transfer some genes from higher animals to bacteria. There have been many newspaper and television reports on the fact that industry is even now preparing to manufacture hormones [which have previously only been available from slaughtered animals] for the treatment of diabetics using the bacterium *E. coli*. The bacterium produces the hormone using a gene transferred to it from a rat. As time goes on we will all see other examples of practical applications of this biological breakthrough. Agriculture can expect to share in the benefits.

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At the moment, most such research has centered around the improvement of the plant-bacteria nitrogen fixing symbiosis.

This research is taking a number of directions, for example:

1. The transfer of nitrogen fixation genes directly from bacteria into the cells of plants. The hope is to cause the plant to fix nitrogen directly. This is an extremely difficult problem because the nitrogen fixing enzyme (nitrogenase) must be protected from oxygen – which is structurally difficult in the plant. However at least one bacterium even now transfers a bacterial gene into a plant cell. The organism is *Agrobacterium tumefaciens*, the cause of crown gall disease in plants. The injected gene causes the formation of the typical tumorous growth (not unlike a nodule) and causes the plant to produce specialized nutrient requirements for the bacterium. Researchers hope to adapt this organism, which happens to be related to the rhizobia, to nitrogen fixation as well. However, many feel that this is an unlikely development.
2. Leguminous plants prefer to take up nitrogen from the soil rather than fix it from the atmosphere. Fixation is expensive to the plant in energy and is avoided if possible by the plant. Perhaps genetic manipulations can prevent the plant-bacteria system from shutting down nitrogen fixation when soil nitrogen levels are high.
3. When soil nitrogen is plentiful many plants are not properly infected and nodulated by *Rhizobium* bacteria. Genetic changes might make the nodulating bacteria less sensitive to this.
4. At the University of Wisconsin mutants of *Rhizobium* bacteria have been isolated which are able to fix twice the normal amounts of nitrogen. Whether these organisms will be competitive in practical field applications has not been established.
5. Legumes typically waste much of their hydrogen to the atmosphere rather than using it to change atmospheric nitrogen into fixed nitrogen. Some rhizobia have been isolated and tested which have an enzyme which minimizes these losses. Commercial inoculants are expected to incorporate this discovery in a few years.
6. Rhizobia used to inoculate legumes may be damaged by pesticides used to protect the seeds. Strains of bacteria resistant to specific pesticides are being developed. Some day the pesticides to which rhizobia in an inoculant are resistant may be listed on the package label.

The Bacteriology Department at North Dakota State University is currently doing work on the effects of pesticides on nitrogen fixation, development of pesticide-resistant strains, as well as research on the basic genetics of rhizobia bacteria.

Nitrogen fixation is carried out most efficiently in symbiosis with plants because the plant, through photosynthesis, provides the bacteria with the necessary conditions, energy and nutrients for fixation. Some free-living bacteria can fix nitrogen but lack the carbohydrate supplies necessary for energy and the hydrogen for fixation. The efficiency of free-living bacteria in fixing nitrogen is therefore only a small fraction of the *Rhizobium*-legume capability. However, these bacteria are rather common in the root region (rhizosphere) of grasses and many other plants. Individually their contribution of nitrogen is small but their cumulative effect is significant. This is more true of grasslands and forest areas than for intensive agriculture. As an example, climax grasslands in North Dakota probably receive only a few pounds per acre of nitrogen from this source each year. However, this is an essential element of the ecology of grasslands. Research by the Bacteriology Department has been aimed at determining the effects of variations in top soil dressings on reclaimed strip-mined land in North Dakota on populations of these nitrogen-fixing bacteria of the grass rhizosphere.

One free-living bacteria, *Azospirillum brasilensis*, was discovered a few years ago on the roots of tropical grasses in Brazil. It fixes nitrogen very efficiently for an organism of this type. There is some indication that the bacterium is so closely associated with the root system that it may sometimes grow inside the root cells. Several tests have been made in this country to see if it will fix useful amounts of nitrogen on grasses and corn. To date no useful increase in yields has resulted.

Non-rhizobial type seed inoculants are frequently marketed in this area in a way which suggests that they are capable of increasing crop yields by nitrogen fixation. A type of bacteria called *Azotobacter* is the most common example but many others, of defined or undefined composition, have made an appearance. *Azotobacter* is extremely capable of fixing nitrogen if supplied with ample carbohydrates – something not normally available in soils. There has been widespread testing of such inoculants in Russia and Europe over many years. The conclusion is that they do not fix significant amounts of nitrogen for cereal-type crops. There is some evidence that they produce plant growth hormones which may improve the growth of certain leafy plants such as cabbage or tomatoes.

In a day when forests are being considered as reserves of energy, as well as building material, the contribution of microorganisms to their nitrogen ecology is worth mentioning – although North Dakota is not noted for expanses of forest. When a forest is becoming established, for example following a fire, one of the early trees to appear is the alder. Alder trees are not legumes (although there are a number of trees and shrubs which are). The alder forms nodules of a size comparable to golf balls when infected by an actinomycete. These nodules fix nitrogen and enrich the surrounding soil. Even the falling leaves of the alder tree contain enough nitrogen to contribute to the fertility of the soil. Actinomycetes are bacteria which form long filaments and are quite common in soil. The typical musty odor of soil is due to a gas produced by some species of actinomycetes.

Lichens growing on forest trees and rocks often contain nitrogen-fixing blue green algae. These organisms with their accumulated nitrogen eventually enter the forest soil and make a significant contribution to the growth of forests. If the biology of some of these symbioses were as well understood and studied as the legume-rhizobia symbiosis this knowledge might lead to better forest cultivation practices.

Once nitrogen is fixed and incorporated into organic matter in plants, animals and bacteria, it must eventually be recycled. This recycling makes it available again for uptake by plants and animals. The first step involves microorganisms which degrade the organic matter as a source of energy. In the process the nitrogen is released, usually in the form of ammonia. The process is termed ammonification. Actually in soil this takes the form of the ammonium ion (NH_4+) which has a positive charge. This charge tends to bind the nitrogen to clay minerals of the soil, an advantage in that the nitrogen is not readily lost by leaching or runoff. It has the disadvantage that it cannot easily migrate to reach plant roots for uptake.

Most plants are adapted to nitrates as a nitrogen source and excessive amounts of ammonium may even be harmful. The conversion in the soil of ammonium to nitrate (nitrification) is done by a rather specialized and limited group of bacteria. They require oxygen for their activity and nitrification is inhibited in waterlogged soils. Activity is very slow in the cold but still proceeds at near freezing temperatures. The ammonium ion represents a source of energy for these bacteria, which convert it to nitrite (NO_2-). Nitrite is toxic to plants and animals and normally does not accumulate, but is immediately used in turn as a source of energy for another group of bacteria which convert it to nitrate (NO_3-). Fertilizers such as anhydrous ammonia or ammonium nitrate are also nitrified in soil. Ammonia is also formed by bacterial action on urea-based fertilizers.

Because nitrates are so readily lost by leaching and runoff there are times when nitrification inhibition is an advantage. Commercially available chemicals inhibit the first step of nitrification, the conversion of ammonium to nitrate. The result is a nitrate concentration in the field which is more stable over the growing season. This is preferable to a pulse of nitrate at each application of fertilizer, much of which might be lost. Climax grasslands appear to have arrived at this solution naturally. There is evidence that exudates by grasses tend to inhibit nitrification and that there is a greater tendency for plants in a mature grasslands to use nitrogen directly as ammonium rather than as nitrate. All of this helps conserve the limited inputs of nitrogen into grasslands.

Other than leaching or runoff another form of nitrogen loss from the nitrate form of nitrogen is by denitrification. This occurs largely by the action of bacteria in waterlogged soils. In the absence of oxygen the bacteria use nitrate as a substitute. The nitrate is converted eventually to nitrogen gas (N_2) and reenters the atmosphere. An intermediate in this process is nitrous oxide (N_2O) which is of special interest today. (Some nitrous oxide is formed as a minor byproduct of aerobic nitrification, also.) Nitrous oxide which is not converted further to nitrogen gas is released as a gas to the atmosphere. There is some concern that this gas will damage the ozone layer. Most of us recall the recent concern over possible destruction of the ozone layer (which intercepts the ultraviolet radiation from the sun) by fluorocarbons from aerosol-can propellants. Nitrous oxide is reputed to have a similar effect and it is feared that excessive use of nitrogen fertilizers may accelerate this trend. In any event denitrification is an important factor in loss of applied nitrogen fertilizer. Some estimates are that as much as half of applied fertilizer is lost either by denitrification or by water leaching and runoff.

Bacteria are also factors in immobilization of nitrogen. Microorganisms contain approximately one part of nitrogen for every 10 or 12 parts of carbon. The ratio of carbon to nitrogen in soil stabilizes at about this ratio. If excess carbon (plants usually have a considerably higher ratio of carbon over nitrogen) is added to soil the microorganisms will use it as an energy supply. To fill their nitrogen requirements of one part of nitrogen for every 10-12 parts of carbon, the organisms utilize soil nitrogen which would otherwise be available for plant use. Eventually, over a few microbial generations the excess of carbon is cycled into the atmosphere as carbon dioxide and the carbon-nitrogen ratio stabilizes at the normal ratio. In the meantime the nitrogen immobilized in microbial cells is not available for plant growth.

It can be seen that microorganisms are intimately involved with the nitrogen economy of soils everywhere. In fact they are essential to life and agriculture as we know it. Also, as knowledge of how to control and manipulate microbial processes increases agriculture should benefit from these scientific advances.