UNIVERSITY OF FLORIDA

IFAS EXTENSION

Biological Nitrogen Fixation [1]

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The Importance of Nitrogen

Molecular nitrogen (N_2) is the major component (approximately 80%) of the earth's atmosphere. The element nitrogen is an essential part of many of the chemical compounds, such as proteins and nucleic acids, which are the basis of all life forms. However, N_2 cannot be used directly by biological systems to build the chemicals required for growth and reproduction. Before its incorporation into a living system, N_2 must first be combined with the element hydrogen. This process of reduction of N_2, commonly referred to as "nitrogen fixation" (N-fixation) may be accomplished chemically or biologically.

Nitrogen (N) is the nutrient element most frequently found limiting to the growth of green plants. This results from the continual loss of nitrogen from the reserve of combined or fixed nitrogen, which is present in soil and available for use by plants. It is continually depleted by such processes as microbial denitrification, soil erosion, leaching, chemical volatilization, and perhaps most important, removal of nitrogen-containing crop residues from the land. The nitrogen reserve of agricultural soils must therefore be replenished periodically in order to maintain an adequate (non-growth limiting) level for crop production. This replacement of soil nitrogen is generally accomplished by the addition of chemically fixed nitrogen in the form of commercial inorganic fertilizers or by the activity of biological nitrogen fixation (BNF) systems. The significance of BNF as the major mechanism of recycling of nitrogen from the unavailable atmospheric form to available forms in the biosphere cannot be overemphasized.

The Mechanism of Nitrogen Fixation

Atmospheric nitrogen (N) is a molecule composed of two atoms of nitrogen linked by a very strong triple bond. Large amounts of energy are required to break this bond and the molecule is therefore quite chemically unreactive. The general chemical reaction for the fixation of nitrogen ($N + 3H_2 + Energy \rightarrow 2NH_3$) is identical for both the chemical and the biological processes. The triple bond of N must be broken and three atoms of hydrogen must be added to each of the nitrogen atoms. Living organisms use energy derived from the oxidation ("burning") of carbohydrates to reduce molecular nitrogen (N_2) to ammonia (NH_3). The chemical process of nitrogen fixation involves "burning" of fossil fuels to obtain the electrons, hydrogen atoms and energy needed to reduce molecular nitrogen.

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The Energy Cost for Nitrogen Fixation

The reduction of nitrogen, whether accomplished chemically or biologically, requires a large amount of energy. The chemical process used to produce fertilizers utilizes vast amounts of fossil fuels as an energy source. These materials are nonreplaceable and, ultimately, exhaustible. BNF obtains the required energy from the oxidation of carbohydrates which have been formed by the photosynthetic activity of green plants. The energy for photosynthesis comes from sunlight. The energy for BNF is therefore indirectly derived from a universally available and inexhaustible source. The direct source of energy (carbohydrate) for BNF is therefore potentially available wherever conditions permit the growth of photosynthetic organisms.

The ability of a biological system to fix nitrogen is dependent on the presence of a particular enzyme system known as nitrogenase, which catalyzes the conversion of N_2 into a reduced form (ammonia combined with certain organic compounds) which can then be used for growth by microorganisms and higher life forms. The nitrogenase system consists of two different protein molecules (enzymes) which must function together in the nitrogen fixing process. One of the enzymes (azoferredoxin) is an iron-containing protein. The second enzyme (molybdoferredoxin) contains both iron and molybdenum. The two components combine and function together as a single system. Components of the nitrogenase system from different organisms have been combined to form an "active hybrid" nitrogenase system, indicating an apparent general similarity in BNF systems derived from different organisms. Nitrogenase has so far been detected only in certain microorganisms.

The ammonia resulting from fixation is rapidly incorporated into certain amino acids, such as glutamine or alanine. The nitrogen may then be transferred to other amino acids and nitrogen-containing compounds by a variety of commonly occurring amino transfer reactions.

The Magnitude of BNF

It is estimated that BNF on a global scale may reach a value of 175 million metric tons of nitrogen fixed per year. The amount of nitrogen fixed in any given situation would depend upon the environmental conditions and the nature of biological system(s) present which are capable of nitrogen fixation. For particular situations, nitrogen fixation rates may vary from barely detectable to several hundred kilograms per hectare per year (Figure 1). The significance of the contribution of any BNF system to the nitrogen economy in any situation is a function of the supply and demand of the biological community for nitrogen.

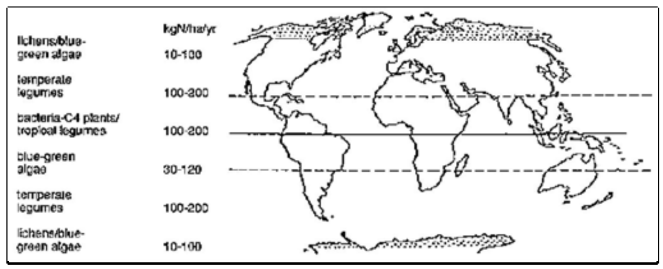

Figure 1.

The Diversity of BNF Systems

BNF is known to occur to a varying degree in many different environments, including soils; fresh and salt waters and sediments; on or within the roots, stems, and leaves of certain higher plants; and within the digestive tracts of some animals. The potential for nitrogen fixation exists for any environment capable of supporting growth of microorganisms. Biological systems which are capable of fixing nitrogen are historically classified as nonsymbiotic or symbiotic, depending on the required involvement of one or more than one organism, respectively, in the process.

Symbiotic Nitrogen Fixation

The most important contribution to BNF comes from the symbiotic association of certain micro-organisms with the roots of higher plants. A classic example is that of the bacteria (*Rhizobium*) which characteristically infect the roots of leguminous plants (e.g., bean, soybean, clover, peanut) with a high degree of host specificity. Small nodules are formed on the roots and these become filled with an altered form of the bacteria (bacteroides) which fix appreciable amounts of nitrogen. This symbiosis alone accounts for 20% of global biological nitrogen fixed annually. The legumes represent a major direct source of food for man and forage for livestock and therefore represent a critical contribution to world food production.

It is significant that agricultural scientists have learned to manipulate this symbiotic relationship in agronomic practice, employing selected combinations of bacteria and legumes in specific situations to obtain maximum crop production on land which is of low fertility and frequently unsuitable for growth of non-legume crops. Nitrogen fixation rates from 75 to 300 kilograms of N per hectare per year (70 to 275 pounds of N per acre per year) are common in various combinations. In Florida, the growth of white clover in combination with a tropical forage grass may result in the fixation of 180 kg N/ha/yr (160 lb N/a/yr) under favorable conditions.

Numerous genera of non-leguminous angiosperms, such as *Alnus*, *Casuarina*, *Coriaria*, *Myrica*, etc., form root nodules in response to infection by the actinomycete *Frankia*. These associations may achieve fixation rates as high as 100 kg N/ha/yr and may occur as climax vegetation or as pioneer species in adverse soil environments. Some gymnosperms, such as *Cycas*, *Macrozamia*, and *Podocarpus* , are capable of forming similar nitrogen fixing root nodule associations.

A variety of additional plant-symbiotic nitrogen fixing associations have been reported. Examples include the bacterium *Kiebsiella* in leaf nodules of *Psychotria* , and associations of cyanobacteria with fungi (lichens), liverworts (*Blasia*), angiosperms (*Gunnera*) and the water fern *Azolla*.

Increasing knowledge concerning the genetic information in various microorganisms which confers nitrogen-fixing ability now make the "creation" of new and perhaps more efficient nitrogen fixing organisms and symbiotic associations a possibility.

Non-symbiotic Nitrogen Fixation

There is great diversity in the metabolic types of free-living microorganisms which are capable of BNF. This includes about 20 genera of non-photosynthetic aerobic (*Azotobacter* , *Beijerinckia*) and anaerobic (*Clostridium*) bacteria and about 15 genera of photosynthetic cyanobacteria (blue-green algae) such as *Anabaena* and *Nostoc*.

Free-living, non-photosynthetic bacteria depend on soil organic matter as a food source whereas the photosynthetic microorganisms may derive their food from the products of photosynthesis.

The nitrogen fixing activity of free-living, non-photosynthetic, aerobic bacteria is strongly dependent on favorable moisture conditions, oxygen, and an organic food source. Anaerobic representatives (*Clostridium*) predominate in grassland and waterlogged soils and soil aggregates where moisture conditions and organic substrates are available but oxygen supply to the micro-environment of the bacteria is severely restricted.

The amounts of nitrogen fixed by free-living non-photosynthetic bacteria in the soil may achieve an approximate maximum of 15 kilograms per hectare per year. This relatively low estimated contribution is the result of limited availability of suitable organic substrates (energy sources) and low bacterial populations in the soil environment. Nitrogen fixation is characteristically higher in environments such as tropical soils, where such factors as substrate availability, temperature and moisture are more favorable to the maintenance and activity of a high bacterial population. Amendment of soil with a readily used organic substrate generally results in some increase in nitrogen fixation. Attempts to increase fixation in unamended soil by addition of high populations of bacteria (soil inoculation) are generally unsuccessful. The increased population of N-fixing bacteria resulting from inoculation is temporary and will rapidly die back to the original number found in an unamended soil, where no provision has been made to create environmental changes which will favor a higher microbial population.

Very little information is available concerning the possible contribution of the free-living, photosynthetic cyanobacteria to the nitrogen economy of soils but maximum gains of fifty kilograms per hectare per year have been reported. Nitrogen-fixing activity of these organisms is, of course strongly dependent on adequate sunlight in addition to favorable moisture conditions.

Living plant roots release a wide variety of simple organic compounds which may be used as food by free-living soil bacteria. This continuous supply of food supports a higher microbial population in the soil immediately surrounding the plant root (rhizosphere). Evidence indicates that native nitrogen-fixing bacteria are common in the rhizosphere of certain plants and that they may fix significant amounts of nitrogen in some cases. This effect may be related to the closeness of the root-microorganism association. Food material released from the roots would be available in greater concentration to those microorganisms more closely associated with the root surface (*Azospirillum*). A striking example is seen in certain combinations of bacteria with some tropical grasses which have a high photosynthetic efficiency and grow under environmental conditions favoring high photosynthetic activity. The roots of such plants may supply the nitrogen-fixing microorganisms with a relatively high and sustained supply of food (photosynthate) which is available in limited supply in the rhizosphere of most plants.

Under appropriate environmental conditions, aerobic cyanobacteria may contribute significantly to nitrogen gains in fresh water environments and anaerobic *Clostridium* may play a similar important role in fresh water sediments. Most of the nitrogen fixation in marine environments (about 20% of the total amount of annual biological fixation) is attributed to the cyanobacteria but many other kinds of nitrogen-fixing microorganisms have also been found in such environments. The significant contribution of photosynthetic (cyanobacteria) and non-photosynthetic (*Clostridium*) microorganisms to nitrogen fixation in the rhizosphere of rice is well recognized. In addition, nitrogenase activity has been detected in the soil surrounding the mycorrhizal roots of several conifers and in the rhizospheres of marine (*Thalassia*) and freshwater (*Glyceria*) angiosperms.

The leaf surface (phyllosphere) of certain plants in warm, humid tropical regions may provide an additional favorable environment for the growth and nitrogen fixing activity of such free-living bacteria as Azotobacter, *Beijerinckia* and *Kiebsiella.*

Free-living nitrogen-fixing anaerobic bacteria are present and fix nitrogen in the intestinal contents of a variety of animals (herbivores) and also man. Nitrogenase activity is generally quite low and its significance in terms of satisfying nutritional requirements of the host appears doubtful.

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