

Uptake and Assimilation of Nitrogen By Plants

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In North Dakota, as throughout the rest of the world, the utilization of available soil nitrogen is the key to productivity of our crop and range lands. Consequently, the production of the different forms of biologically active nitrogen, their utilization and cyclization throughout various types of agricultural systems has been the subject of investigation by crop production specialists, soil scientists, plant and crop physiologists, microbiologists and ecologists just to name a few.

If we limit our discussion here to agricultural systems we can make the assumption that most of the agronomically important plant species obtain the nitrogen necessary for their metabolism from that which is dissolved in the soil solution. The only exceptions to this are the leguminous plants, which will be discussed later. This dissolved soil nitrogen is the net balance of all inputs and exports of the system but may not all be in the same form, state of oxidation/reduction or have the same energy content. Therefore, as nitrogen moves through the system, the transfer of considerable amounts of energy may be involved.

Today, the level of free or exchangeable soil nitrogen in North Dakota's agricultural land is partially the result of many years of application of one or more of the available forms of supplemental nitrogen (nitrate, ammonia, urea). However, it is also the result of naturally occurring biological processes in the soil such as nitrogen fixation, ammonification, nitrification and denitrification. The magnitude and output of these processes is dependent on the physiological activity of the various populations of soil microbes present. It should be kept in mind during this discussion that there are numerous other factors which may determine the form in which the nitrogen exists, whether or not it is retained by the system and whether it is readily available to plants. These include various environmental factors such as soil moisture, temperature, soil solution pH, carbohydrate content, carbon dioxide and oxygen concentrations of the soil atmosphere, etc.

Figure 1 is but one of several ways of diagrammatically representing the source, forms and transformations nitrogen can undergo within the soil and plants of a typical agricultural system. Since parental soil materials contain no endogenous nitrogen all the nitrogen found in a soil, organic or inorganic, has been added by the presence of

man and animals or has originated from atmospheric nitrogen by a process known as nitrogen fixation. The factors regulating transformations of nitrogen in the soil are treated in detail by Dr. B. R. Funke in his article in this issue. Consequently for our purposes it is sufficient to summarize them.

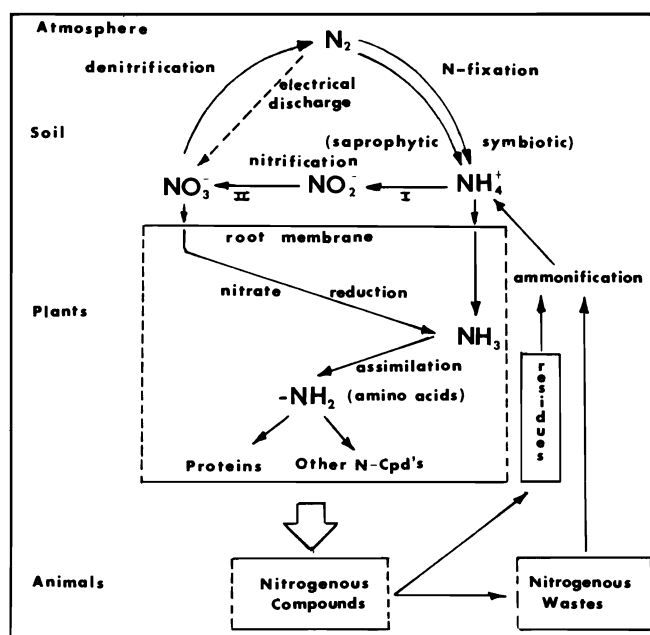


Figure 1. Nitrogen Cycle

Nitrogen fixation is the reduction of atmospheric denitrogen (N_2) into organic combinations of ammonia ($-NH_2$). This is accomplished by a number of free living bacteria such as *Clostridium* and *Azotobacter*, the blue-green algae and also by symbiotic associations between certain bacteria and their host plants. Except for the photosynthetic bacteria which can meet their own carbohydrate requirements through photosynthesis, the others are saprophytic in the sense that they obtain their energy needs from dead organic matter in the soil. Symbiotic bacteria such as the various *Rhizobia* have their carbohydrate requirements supplied by the host plant. In this symbiosis both organisms benefit mutually from the relationship since the microorganisms supply much needed reduced nitrogen, ammonia, to the host plant while the plant supplies carbohydrates to the *Rhizobia*. In such

symbiotic relations there are other advantages to the host plant in that some problems of absorption of fixed nitrogen and nitrates by the roots are overcome since the denitrigen when reduced to ammonia (NH_3) is already in the translocation stream of the plant. Also, this ammonia is the reduced form whereas nitrate taken up by a plant is an oxidized form and places an additional energy demand on the plant in order to reduce it to ammonia. The incorporation of this reduced nitrogen into organic compounds will be dealt with later. The fact that the process of nitrogen fixation by *Rhizobia* is decreased significantly by the presence of soil nitrates is easily demonstrated by the fewer numbers and smaller, less active nodules on the roots of legumes grown in soil where nitrate nitrogen is plentiful.

Reduced nitrogen in the form of ammonia can also originate from a process known as **ammonification**. This process, in which organic residues are broken down to simpler compounds with most of the nitrogen being released in the form of ammonia, is accomplished by ammonifying bacteria. The ammonia, which is dissolved in the soil solution as the ammonium ion (NH_4^+), may volatilize and be lost to the atmosphere, may be bound by exchanging with a cation in the soil matrix, may be absorbed and assimilated into the organic constituents of the plant or may be oxidized to nitrate by other soil microbes.

In the soil the process oxidation of ammonia to nitrite and nitrate is called **nitrification** and is exhibited by several nitrifying bacteria and some fungi. The overall oxidation requires at least two steps, each associated with specific microorganisms. In the first step microbes such as *Nitrosomonas* sequentially add electrons to the nitrogen, first forming hydroxylamine (NH_4OH) and then nitrite (NO_2^-). This nitrite acts as a substrate for another group of bacteria typified by *Nitrobacter*, which obtain an atom of oxygen from water and oxidize the nitrite to nitrate (NO_3^-). The nitrate so formed is that form which is most readily used by a great number of higher plants.

Nitrate not utilized by the plants can be lost from a given system by conversion to denitrigen gas or nitrous oxide (N_2O) through the process of **denitrification**. Several denitrifying bacteria can produce these gases which then can return to the atmosphere. In this type of reduction, organisms use the nitrogen rather than oxygen as the electron acceptor and thus this process proceeds best under anaerobic conditions. Denitrification can also be accomplished by its uptake and reduction by higher plants.

Regardless of the means by which nitrate nitrogen is generated in a given system, under most conditions it is absorbed readily by plant root systems. This absorption process is influenced to a large measure by the physiologic condition and age of the particular plant species. Nutritional studies, where both nitrate and ammonia are available, show that, depending upon the plant species, soil conditions, etc., either form may be taken up preferentially. Although there is a voluminous amount of literature depicting the use of ammonia vs. nitrate, by far the most predominant form of nitrogen used by plants is nitrate. Before the nitrate taken up by the roots can be converted into organic nitrogen it must be reduced to ammonia. Although there are some reports of root and etiolated tissues reducing nitrate, in the majority of cases it is transported through the vascular system to green leafy tissue before being reduced. This is supported by the fact that most

plants grown on nitrate nitrogen have significant levels of nitrate nitrogen in the xylem sap and leafy tissue. In the same leaf tissue are significant quantities of the enzymes essential for the reduction of this nitrate.

The reduction of nitrate to ammonia by higher plants is accomplished in two steps. In the first step nitrate is reduced to nitrite by the enzyme nitrate reductase (NR). This requires the provision of electrons or reducing power (NADH_2) which is usually supplied by the oxidative respiration of 3-carbon sugars in the plant cell. In the second step the nitrite is reduced to ammonia by the enzyme nitrite reductase which gets its reducing power (NADPH_2) directly from photosynthesis (Figure 2). This form of nitrate reduction is referred to as assimilative nitrate reduction because the net result is the formation of ammonia, which enters into organic combinations, including amino acids which form proteins, nucleic acids, alkaloids, growth regulators, etc. Since the respiration of triose sugars and photosynthesis generate reducing power and provide carbohydrate skeletons for amino acid synthesis, this form of nitrate reduction is aerobic. Under anaerobic conditions it has been shown that this system can function as an electron acceptor instead of oxygen, to support limited respiration. This is referred to as respiratory nitrate reduction during which ammonia, certain amino acids (glutamate and aspartate) and their amides (glutamine and asparagine) accumulate because there is a shortage of carbohydrate skeletons for the synthesis of other amino acids.

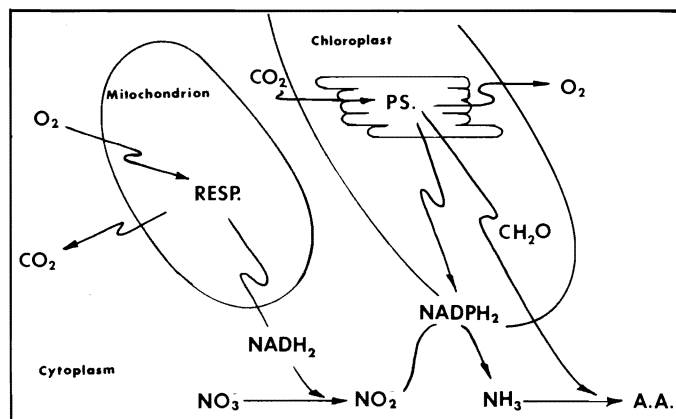


Figure 2. Nitrate Reduction, Photosynthesis and Respiration

Once ammonia is available within the plant tissues it is incorporated into amino acids and then into proteins and other organic combinations in a very orderly sequence of biochemical reactions. The key type reactions of assimilation include the reversible formations (amination of keto acids) of the two amino acids glutamate and aspartate and their amides, glutamine and asparagine. The other type reaction is the reversible transfer of ammonia (transamination) from one of these compounds to other appropriate keto acids to form the rest of the amino acids common to plants. Figure 3 illustrates the relationships between uptake, transport and assimilation of nitrogen by plants. It shows the uptake of nitrate nitrogen by roots and also the fixation of atmospheric nitrogen by root nodules. Although in some cases nitrates can be reduced by the roots, it is usually translocated in the anionic form through

xylem tissue to the leaves and shoot apices. Here the nitrate is reduced and incorporated first into amino acids and then into other structural and functional combinations as described.

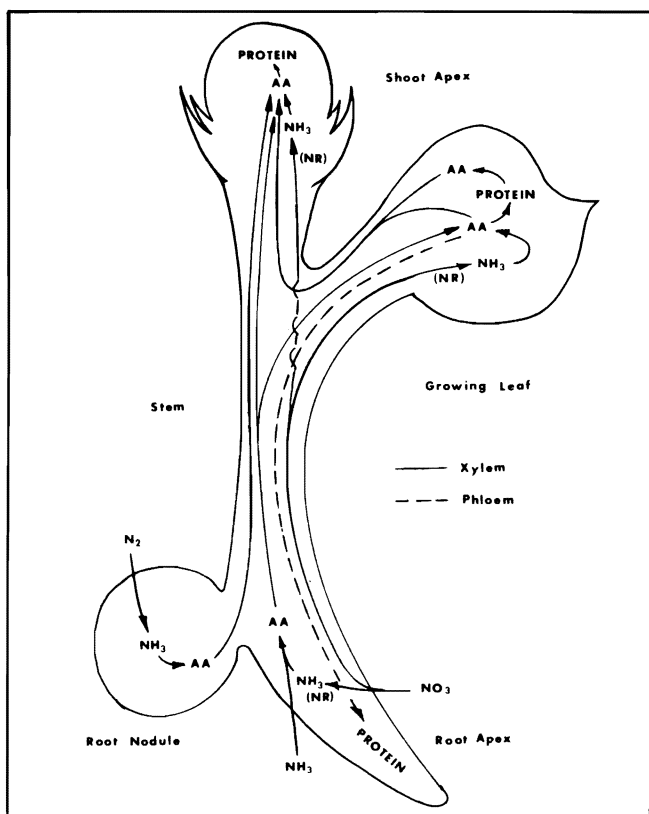


Figure 3. Mobilization of Plant Nitrogen

There are a number of environmentally induced physiologic stresses in which this system of metabolic transformations plays a vital part in determining the responses of plants to a given situation. Figure 4 summarizes the metabolic transformation involved. A couple examples of how this works might be helpful here.

First, consider normal vegetative growth and the accumulation of seed protein. Here there is the normal assimilation of reduced nitrogen (ammonia) into amino acids and the coupling of these into structural and functional proteins. During subsequent germination of the seed, these proteins are broken down (hydrolyzed) to amino acids, which in turn are deaminated and the sugar skeletons respired as a source of energy to support the commencement of growth of the seedling. The liberated nitrogen is not free ammonia but in the form of glutamate and aspartate or their amides. It is these forms which are readily transferred through xylem tissue to the growing regions of the shoot or through the phloem to the growing region of the roots where nitrogen is needed to support growth and the formation of new cells (see Figure 3).

A similar situation may be seen under conditions where nitrogen becomes limited during the growth of a plant. Nitrogen is considered a mobile nutrient, meaning that it can be transported from one plant part to another, in this case, from older leaf tissue to the younger leaves and growing regions. The mechanism by which this is accomplished again seems to be through the formation of glutamate and aspartate and their amides.

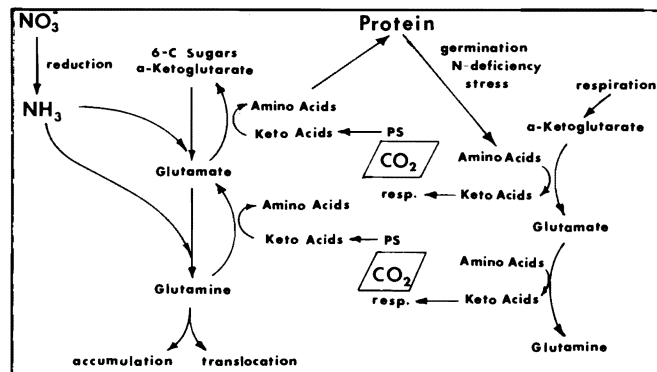


Figure 4. Stress and N-Transformations in Plants

Another situation where these metabolic transformations become critical to a plant is when the amount of ammonia in the tissues becomes high relative to the amount of sugar acids available to serve as carbohydrate acceptors. This situation can arise when excessive nitrogen has been applied, or when a moderate amount of ammonia is being produced but photosynthetic production of carbohydrates is significantly reduced because of very heavy cloud cover. It can also arise under temporary anaerobic conditions caused by flooding, in which case respiratory nitrate reduction is occurring and proteins are hydrolyzed to amino acids which are deaminated so as the carbohydrate skeletons can be respired. Both these processes add more ammonia to the system, which must be tied up in order to avoid accumulation to toxic levels.

If these stresses are alleviated, by sunlight increasing photosynthetic output, or by elimination of the anaerobic conditions, the reversibility of these transformations insures the plant of the capability of resuming growth and development, at least relative to its nitrogen requirements.

In summary, it can be said that nitrogen is a critical commodity, essential to the growth and development of all plants. In order to synthesize the protein and other nitrogenous compounds essential to growth, the nitrogen must be in a reduced form (ammonia). Plants can absorb nitrogen from the soil as nitrate as well as ammonia but in this case it must be reduced by the plant in order to be assimilated. This nitrate reduction requires energy or reducing power and carbohydrate skeletons to form the various amino acids. Therefore, the process is strongly dependent upon photosynthesis. Various metabolic transformations make nitrogen a mobile element in the plant.

The ultimate source of nitrogen to any living organism is atmospheric denitrogen. Since N_2 cannot be utilized by higher plants it must be converted to forms which can be absorbed and assimilated. These forms are primarily nitrate and ammonia. The necessary conversions are accomplished by specific soil microbes as discussed.

The balance of the different forms of nitrogen in the soil then is the result of the physiological status and activity of all the living organisms found in a defined agricultural system plus any supplemental applications and minus any nitrogen removed by cropping. Nitrogen is a natural resource, in a sense a renewable natural resource, and the productivity of our land is dependent on our understanding the processes by which it undergoes various transformations and our willingness to safeguard the balance of these processes, inputs and exports.