Lecture 7
Nitrogen cycle

Although molecular nitrogen (N2) is abundant (i.e. 78-80 % by volume) in the earth´s atmosphere, but it is chemically inert and therefore, cannot be utilized by a source of combined or fixed nitrogen (eg. ammonia, nitrate) or organic nitrogen compounds for their nutrition and growth.

Plants require fixed nitrogen (ammonia, nitrate) provided by microorganisms, but about 95 to 98% soil nitrogen is in organic form (unavailable) which restrict the development of living organisms including plants and microorganisms. Therefore cycling/ transformation of nitrogen and nitrogenous compounds mediated by soil microorganisms of paramount importance in supplynic required forms of nitrogen to the plants and various nutritional classes of organisms in the biosphere.

In nature, nitrogen exists in three different forms viz. gaseous / gas(78 to 80% in atmosphere), organic (proteins and amino acids, chitins, nucleic acids and amino sugar) and inorganic (ammonia and nitrates).

Biological N2 fixation:-

A) Symbiotic:

eg. *Rhizobium* (Eubacteria) legumes, *Frankia* (Actinomycetes) and *Anabaena* (Cyanobacteria) non- legumes.

B) Non- symbiotic :

1- Free living: eg. *Azobacter*, *Derxia*, *Bejerinkia*, *Rhodospirillum* and BGA.

2- Associative: eg. *Azospirillum*, *Acetobacter*, *Herbaspirillim*.

Nutritional categories of N2 fixing bacteria

A) Heterotrophs

B) Photoautotrophs

Nitrogen cycle is the sequence of biochemical changes form free atmospheric N2 to complex organic compounds in plant and animal tissues and further to simple inorganic compounds (ammonia, nitrate) and eventual release of molecular nitrogen (N2) back to the atmosphere is called **nitrogen cycle.** In this Cycle a part of atmospheric nitrogen (N2) is converted into ammonia and then to amino acids (by soil microorganisms and plant –microbe associated) which are used for the biosynthesis of complex nitrogen –containing organic compound such as proteins, nucleic acids, amino sugars etc.

The proteins are then degraded to simpler organic compounds viz. **peptones** and **peptides** into **amino acid** which are further degraded to inorganic nitrogen compounds like **ammonia, nitrites, and nitrates.** The nitrate form of nitrogen is mostly used by plants or may be lost through leaching or reduced to gaseous nitrogen and subsequently goes into the atmosphere, thus completing the nitrogen cycle. Thus the process of **mineralization ((**conversion of organic form of nutrients to its mineral

/inorganic form**)) and immobilization ((**process of conversion of mineral/ inorganic form of nutrient elements into organic form**))** are continuously and simultaneously going on in the soil.

Several biological steps involved in the nitrogen cycle are:

1- Proteolysis:-

Proteolysis plants use the ammonia produced by symbiotic and non- symbiotic nitrogen fixation to make their amino acid &eventually plant proteins. Animals eat the plants and convert plant proteins into animal proteins. Upon death, plant and animals undergo microbial decay in the soil and the nitrogen contained in their proteins is released. Thus, the process of enzymatic breakdown of proteins by the microorganisms with the help of proteolysis enzymes is known as **proteolysis.**

The breakdown of proteins is completed in **two stages.** In **first stage** proteins are converted into peptides or polypeptides by enzyme **(proteinases)** and in **second stage** polypeptides/peptides are further broken down into amino acids by the enzyme **(peptidases).**

$$\text{Protein} \xrightarrow{\text{proteinase}} \text{Peptide} \xrightarrow{\text{peptidase}} \text{Amino acids}$$

The amino acids produced may be utilized by other microorganisms for the synthesis of cellular components, animated to yield ammonia. The most active microorganisms responsible for elaborating the proteolytic enzymes **(proteinases and peptidases**) are: ***Pseudomonas, Bacillus, proteus, Clostridium histolyticum, Micrococcus, Alternaria, Penicillium etc.***

2- Ammonification (Ammo acid degradation)

Amino acid released during proteolysis undergo deamination in which nitrogen containing amino (-NH2) group is removed. Thus, process of deamination which leads to the production of ammonia is termed as (**ammonification).** The process of ammonification is mediated by several soil microorganisms. Ammonification usually occurs under **aerobic** conditions (**known as oxidative deamination**) with the liberation of ammonia (NH3) or ammonium ions(NH4) which are either released to the atmosphere or utilized by plants (paddy) and microorganisms or still under favorable soil conditions oxidized to form nitrites. The processes of ammonification are commonly brought about by *Clostridium* sp. , *Micrococcus* sp. , *Proteus* sp. , etc. and it is represented as followes.

$$CH_3CHNH_2COOH + \tfrac{1}{2}O_2 \xrightarrow{\text{deaminase}} CH_3COCOOH + NH_3$$

Alanine $\xrightarrow{\text{deaminase}}$ Pyruvic acid + ammonia

3- Nitrification:-

Ammonical nitrogen/ ammonia released during ammonification are oxidized to nitrates and the process is called **(nitrification) soil conditions** such as well aerated soils rich

in calcium carbonate, temperature below 30°C, neutral PH and less organic matter are favorable for nitrification in soil. Nitrification is a two stage process and each stage is performed by a different group of bacteria as follows.

Stage I:

Oxidation of ammonia of nitrite is brought about by ammonia oxidizing bacteria viz. *Nitrosomonase europea*, *Nitrosococcus nitrostis*, *Nitrosospira briensis*, *Nitrosovibrio* and *Nitrocystis* and the process is known as nitrosification.

The reaction is presented as follows:

$2NH_3 + ½ O_2$……$NO_2 + 2H + H_2O$

Stage II:

In the second step nitrite is oxidized to nitrate by nitrite-oxidizing bacteria such as *Nitrobacter winogradsky*, *Nitrospira gracilis*, *Nirosococcus mobiiis* etc. and several fungi (eg. *Pencillium*, *Aspergillus*) and actinomycetes (eg. *Streptomyces*, *Nocardia):*

NO_2 (-) + ½ O_2……..> NO_3

Nitrate thus, formed may be utilized by the microorganisms, assimilated by plants, reduced to nitrite and ammonia or nitrogen gas or lost through leaching depending on soil conditions. **The nitrifying bacteria (**ammonia oxidizer and nitrite oxidizer) are aerobic gram – negative and chemoautotrophic and are the common inhabitants of soil, sewage and aquatic environment.

4- Nitrate Reduction:

Several heterotrophic bacteria (*E. coli*, *Azospirillum*) are capable of converting nitrates to nitrites to ammonia. Thus, the process of nitrification is reversed completely which is known as **nitrate reduction**. Nitrate reduction normally occurs under anaerobic soil conditions (water logged soils) and the overall process is as follows:

Reductase

$HNO_3 + 4 H_2$…….> $NH_4 + 3 H_2O$

Reductase

Nitrate……..> ammonia

Nitrate reduction leading to production of ammonia is called (**dissimilatory nitrate reduction**) assume of the microorganisms assimilate ammonium for synthesis of proteins and amino acid.

5- Denitrification

This is the reverse process of nitrification. During denitrification nitrates are reduced to nitrites and then to nitrogen gas and ammonia. Thus, reduction of nitrates to gaseous nitrogen by microorganisms in a series of biochemical reaction is called (denitrification). The process is wasteful as available nitrogen in soil is lost to atmosphere.

The overall process of denitrification as follows:

Nitrate…….> Nitrite……> Nitrouse oxide…….>Nitrogen gas

This process also called dissimilatory nitrate reduction as nitrate nitrogen is completely lost into atmospheric air. In the soils high organic matter and anaerobic soil conditions

(water logged or ill- drained) rate of denitrification is more. Thus, rice/paddy fields are more prone to denitrification.

The most important denitrification bacteria are *Thiobacillus denitrificans*, *Micrococcus denitrificans* and species of *Pseudomonase*, *Bacillus*, *Achromobacter*, *Serratia paracoccus* etc. Denitrification leads to the loss of nitrogen (nitrate nitrogen) from the soil which results into the depletion of an essential nutrient for plant growth and therefore, it is an undesirable process /reaction from the soil fertility and agricultural productivity. Although, denirtrification is an undesirable reaction from agricultural productivity, but it is of major ecological importance since, without denitrification thesupply of nitrogen including N2 of the atmosphere, would have not got depleted and NO3 (which are toxic) would have accumulated in the soil and water