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Measurement of Important Soil Microbial Process Such as Nitrification and Nitrogen Fixation

(*Sangeeta Danga and Narendra Danga)
College of Agriculture, Ummedganj, Kota, Rajasthan, India
*Corresponding Author's email: sdsd675314@gmail.com

Abstract

Nitrogen (N) is widely distributed in the lithosphere, hydrosphere, atmosphere and biosphere. It is a basic component of every plant cell as well as microorganisms, as a component of proteins, nucleic acids and chlorophyll. It enters soil with organic and mineral fertilizers, plant and animal residues and biological nitrogen fixation. There are various forms of nitrogen in soil, and this element is usually transformed by microorganisms. The transformation of nitrogen compounds (ammonification, nitrification and immobilization) is significantly influenced by climatic conditions and the physicochemical properties of soil. Microbial mineralization of nitrogen organic matter results in the enrichment of soil with this element, which is necessary to generate a yield. The amount of nitrogen entering soil through the mineralization of crop residues ranges from 15 to 45 kg N/ha in cereal residues and from 80 to 144 kg N/ha in winter rape residues. Biological nitrogen fixation can increase the nitrogen content in soil by 30–50 kg/ha/year. In recent decades, the mismanagement of mineral fertilizers has drastically changed the natural balance of the nitrogen cycle. Every year huge amounts of nitrogen compounds enter the aquatic ecosystems and cause their eutrophication. That is why it is important to have adequate knowledge of sustainable fertilization so as to practice integrated crop management.

Keywords: Ammonification, Biological nitrogen fixation, Eutrophication, nitrification

Introduction

Microbial activities important to effects on crop productivity and nutrient cycling can be altered by agriculture management practices. The productivity and stability of soil as a medium for plant growth depend greatly on the balance between living and non-living components. Nitrification is the aerobic conversion of ammonium (NH_4^+) in to nitrite (NO_2^-) and nitrate ($NO_3^{-)}$ nitrifying bacteria. There are several chemoautotrophic bacteria such as Nitrosomonas, Nitrospira, Nitrosococcus, and Nitrosovibrio involved in the first step of the process whereby ammonium is oxidized to nitrite (Schmidt 1982; Paul and Clark1996). In soil systems, Nitrobacter and Nitrospira -like bacteria are involved in the second step whereby nitrite is converted to nitrate (Schmidt 1982). The ammonia-oxidizing bacteria and the nitrite-oxidizing bacteria are usually found together, and as a result, nitrite rarely accumulates in soil. There are several reasons why it is important to know the nitrification rate in soils. The conversion of NH_4^+ to NO_3^- increases the mobility of nitrogen (N) in soil (because of the dominance of negatively charged surfaces on soil colloids), potentially increasing availability to plants, but also potentially increasing N loss via leaching. Furthermore, the formation of NO_3 from NH_4 via nitrification also increases the potential for gaseous loss of N from the soil via the process of de-nitrification. The nitrification process

has a net acidifying effect on the soil and the resulting drop in soil pH may alter the availabilities of other plant nutrients. In addition, standard methods to measure nitrification are required when various nitrification inhibitors or fertilizer formulations (e.g., polymer coated N fertilizer, super granules, etc.) are evaluated. Considerable information is available in the literature concerning the key variables (temperature, moisture, pH, NH_4 and oxygen) that affect the nitrification process in soil. It is a component of biological molecules, such as DNA, RNA, ATP, and phospholipids, and on a macro level, it affects root development, stalk and stem strength, crop maturity, and nitrogen fixation in legumes.

Measurment of Nitrification Process

Nitrification is the aerobic conversion of ammonium (NH_4^+) in to nitrite (NO_2^-) and nitrate ($NO_3^{-)}$ nitrifying bacteria. There are several chemoautotrophic bacteria such as Nitrosomonas, Nitrospira, Nitrosococcus and Nitrosovibrio involved in the first step of the process whereby ammonium is oxidized to nitrite (Schmidt 1982; Paul and Clark1996). In soil systems, Nitrobacter and Nitrospira -like bacteria are involved in the second step whereby nitrite is converted to nitrate (Schmidt 1982; Bartosch *et al.* 2002). The ammonia-oxidizing bacteria and the nitrite-oxidizing bacteria are usually found together, and as a result, nitrite rarely accumulates in soil (Paul and Clark 1996). There are several reasons why it is important to know the nitrification rate in soils.

Maximum nitrification rates occur at: 1. Neutral pH 2. High temperature

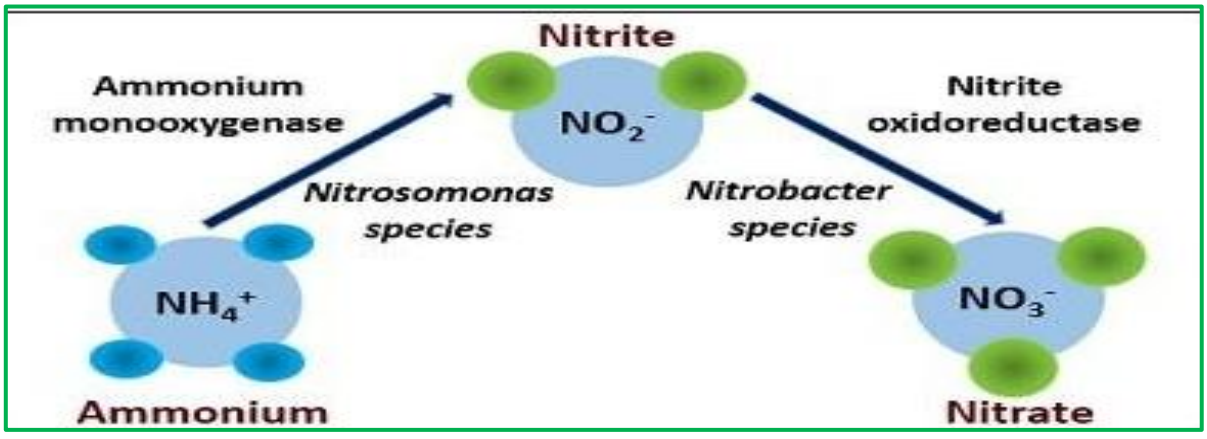
Nitrification bacteria: Nitrosomonas sp., Nitrosococcus sp., Nitrobacter sp., Nitrosospira sp.

Characters of nitrifying bacteria: Aerobic, Alkaline PH**,** Temperature 20-30 c**,** Motile (Flagella)**,** Gram negative, Different cell shape such as spindly and bacilliform

Nitrification reaction:

$2NH_4^+ + 3O_2$ ------ $2NO_2^- + 2H^+ + H_2O$

$2NO_2^- + O_2$ ------ $2NO_3^-$

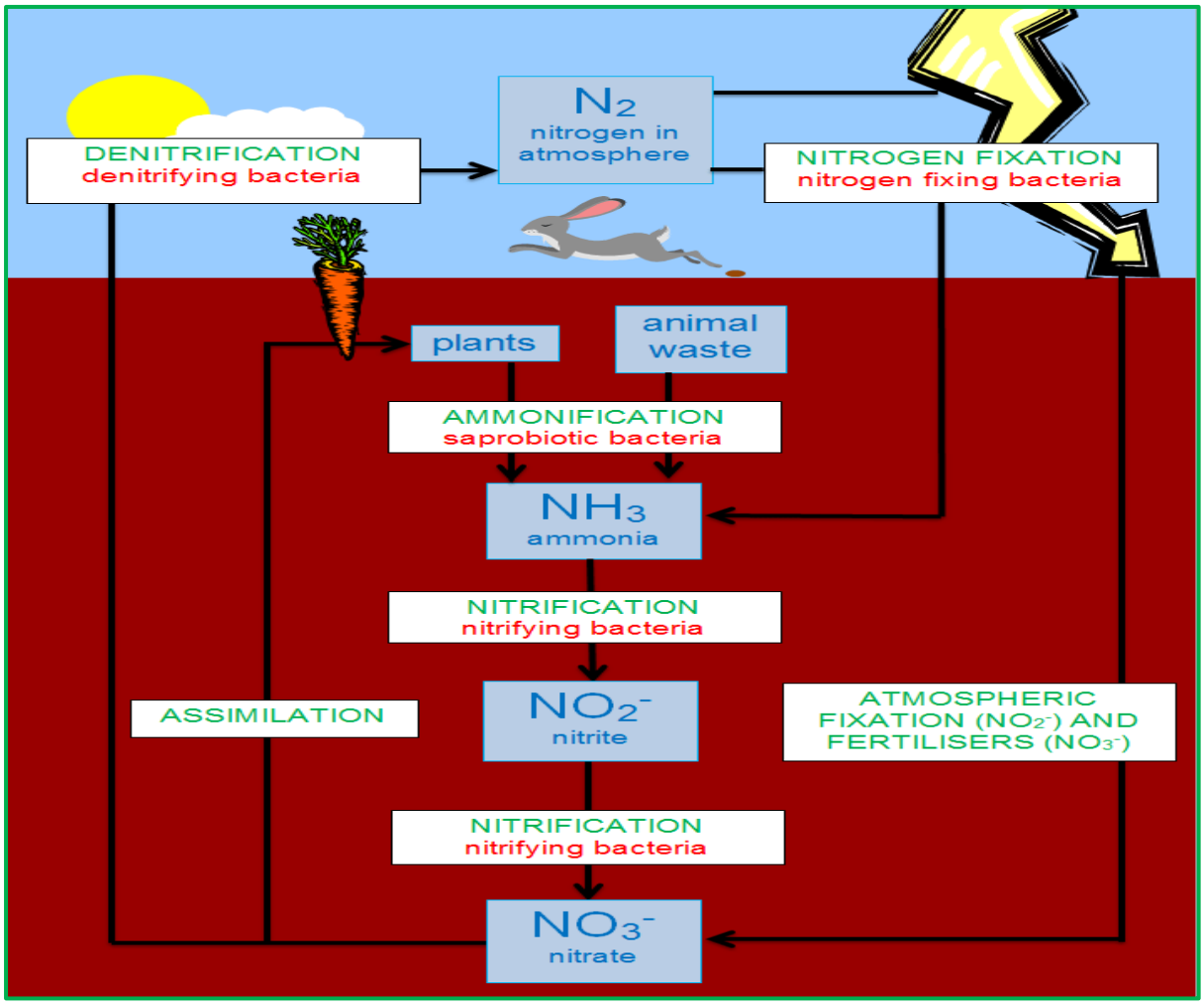


Materials for nitrification measurement: Soil, Ammonium sulfate broth (20ml), Nitrite broth (20ml), Nessler's reagent, Trommsdorf's reagent, Diphenylamine, Spot plate, Sulfuric acid (1 part conc. Sulfuric acid to 3 parts water)

Methods

1. Inoculate the ammonium sulfate and nitrite broth bottles with pinches of soil (1g). Label the bottles and shake vigorously for 5 minutes.
2. Shake the bottles for 7 days at room temperature.
3. Place a drop of sulfuric acid and 3 drops of Trommsdorf's reagent in a well on a spot plate. Add drop of culture from the ammonium broth and mix. Use a Pasteur pipette and not an inoculating loop. A blue – black color indicates the presence of nitrite.

4. Test the ammonium broth for ammonia with Nessler's reagent
5. Test the nitrite broth for residual nitrite
6. If no blue black color was present test for nitrate. Add 1 drop of diphenylamine, 2 drops of sulfuric acid and 1 drop of nitrite broth culture in a well on the spot plate and mix. A blue black color indicates the presence of nitrate.
7. Grams stain the organisms in the broth cultures. Record your results (Harmsen *et al.* 1955).



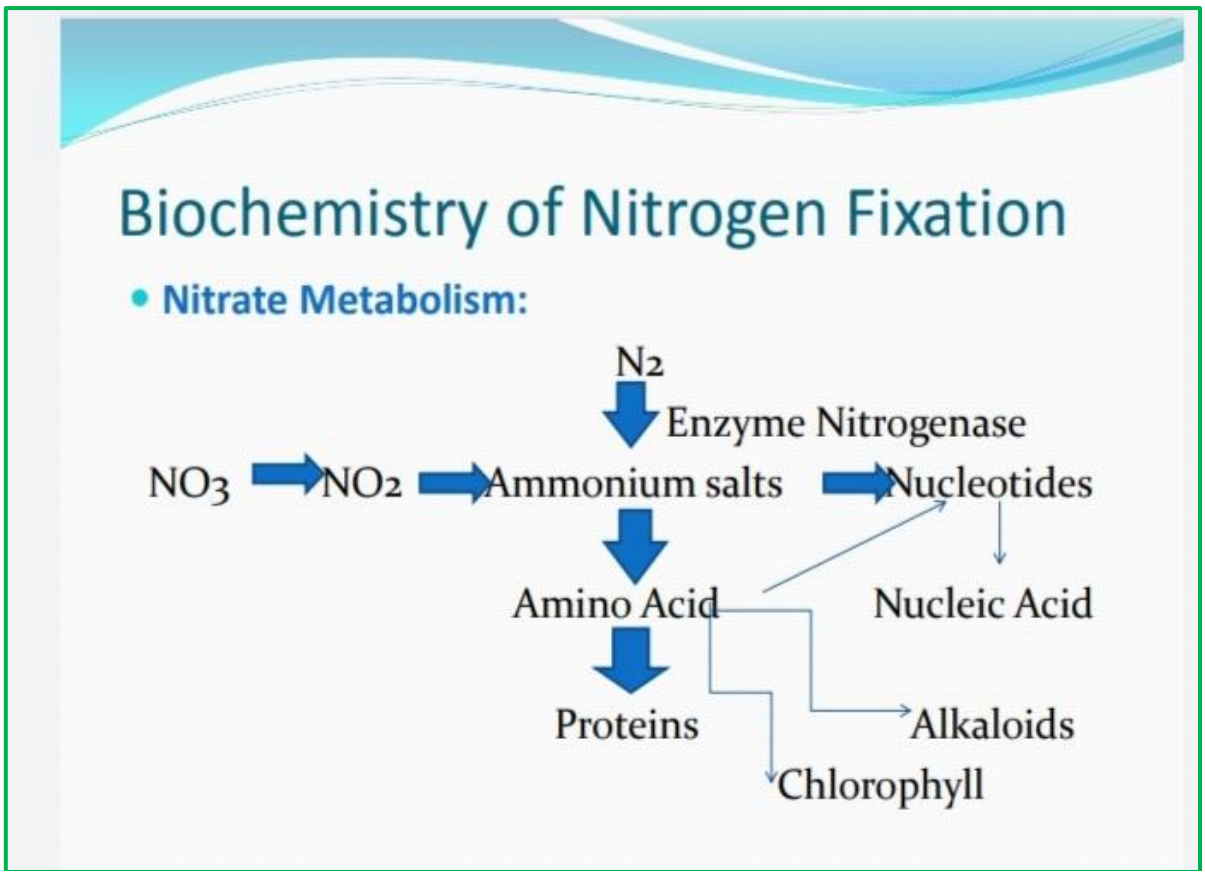
Denitrification and Nitrogen Fixation in Environment

Nitrogen Fixation

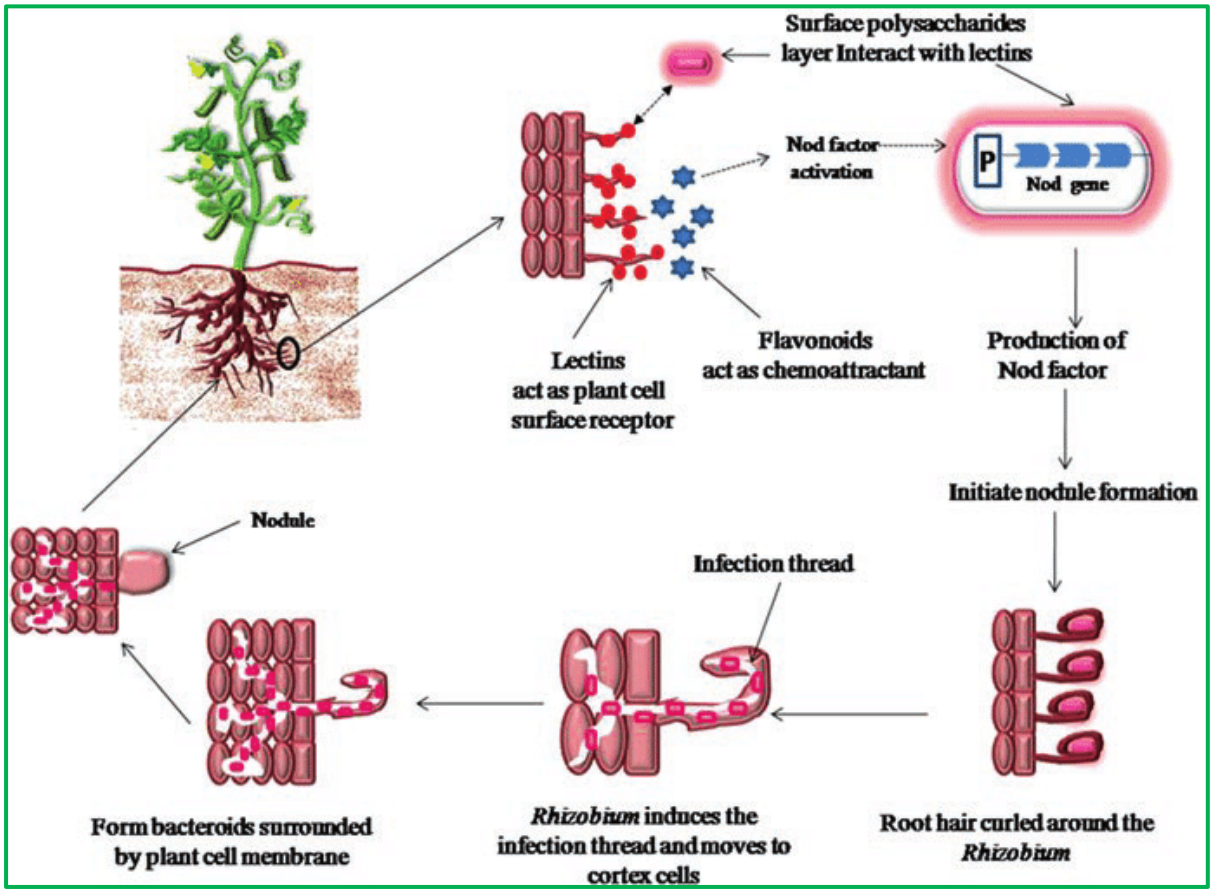
Nitrogen is highly inert gas thus why it cannot be used directly by the higher plants, and therefore has to be fixed. The phenomenon of conversion of free Nitrogen (molecular & elemental) into Nitrogenous compounds which become available to the plants for absorption is known as Nitrogen Fixation. It is of two types: Physical and Biological nitrogen fixation

Biological nitrogen fixation: Inorganic salts of Nitrogen taken from the soil are converted to various organic forms which are used for the formation and functions of cellular components. In soil it is found occur in highly oxidized forms, such as Nitrate and must be reduced by various processes, before they are incorporated into cellular component.

(A) A symbiotic Nitrogen Fixation
(B) Symbiotic Nitrogen Fixation (Legumes)
(C) Associative Nitrogen Fixation (Non legumes).



Nodule Formation



Method for Measuring Biological Nitrogen Fixation

(a) Dry matter yield method (DM): If nitrogen is the primary limiting factor for growth, dry matter yield of the legume plant should be positively correlated with the amount of N from fixation. This method is often used to screen large numbers of rhizobium strains and host plant lines. It has the advantage of being simple and cheap to carry out and non-destructive if only fresh weight is being recorded and the plants can be replanted for seed production.

(b) Total N difference method: This method is related to the previous one with the additional quantification of % N in the fixing plants and non-fixing reference crop plants. Although the need for Kjeldahl N analyses of the samples makes this method more time consuming, it provides more information on the amount of nitrogen fixed. Recently infrared reflectance methods calibrated against Kjeldahl N have been used to determine plant N, resulting in considerable time savings and similar accuracy. The main disadvantage of this method is that legume and reference crops must absorb similar amounts of nitrogen from the soil. When this is not the case erroneous estimates of N_2 fixation may result. Further information on this method can be obtained from Peoples et al. (1989).

(c) Acetylene reduction assay (ARA): In this method nodule roots are incubated in acetylene and ethylene produced after a specific time as measured by gas Chromatograph. Although simple to use, it provides only point measurements of nitrogenase activity as measured by gas Chromatograph. It is not advisable to calculate N_2 fixation from these measurements since the calibration factors are variable for different fixing systems and also variable with time. Further errors may arise due to acetylene- induced decline in nitrogenase activity during assay. Additional information on this method may by from Bergersen(1980).

Conclusion

Nitrogen is an essential nutrient for plant growth and development but is unavailable in its most prevalent form as atmospheric nitrogen. Plants instead depend upon combined, or fixed, forms of nitrogen, such as ammonia and nitrate. Much of this nitrogen is provided to cropping systems in the form of industrially produced nitrogen fertilizers. Use of these fertilizers has led to worldwide, ecological problems, such as the formation of coastal dead zones. Biological nitrogen fixation, on the other hand, offers a natural means of providing nitrogen for plants. It is a critical component of many aquatic, as well as terrestrial ecosystems across our biosphere.

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