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Biological nitrogen fixation: Reducing the N footprints of the environment

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Abstract
Biological Nitrogen Fixation (BNF) is a vital ecological process where certain microorganisms, such as bacteria and archaea, convert atmospheric nitrogen gas into ammonia, which can be used by plants as a nutrient. The French chemist Antoine Lavoisier gave the name “azote” which translates to “without life” to the element nitrogen. Contrarily, it has been demonstrated that this component is present in all living things. The amount of nitrogen that is available to the plant has a big impact on how productive the crop is. Nitrogen is an important macronutrient, which plays an important role in the growth and development of plants. It is a major component of chlorophyll, the most important pigment needed for photosynthesis, as well as amino acids, the key building blocks of proteins. Due to its energy intensive manufacture, impact on air and water quality, soil and water acidification, and climate change, nitrogen-like fertilizer production and overuse come at a significant financial and environmental cost. There are significant potential gains to reduce our dependence on nitrogen fertilization in agriculture. BNF is an environmentally friendly solution to address nitrogen deficiencies and increase crop production. BNF is the only natural process that converts atmospheric nitrogen into biologically useful forms. Nitrogen – fixing bacteria support soil fertility by living in the soil or creating symbiotic interactions with plants, which in turn promotes the growth of numerous crops. This organic process is essential for maintaining global nitrogen cycles, agricultural production, and ecosystems health. It also lessens the demand for synthetic fertilizers.

Keywords: Nitrogen, environment, pollution, azote, microbe

1. Introduction

Nitrogen is a critical limiting element for plant growth and a vital component in plant development. About 2% of all plant dry matter that enters the food chain is made up of it. Although it makes up roughly 80% of the atmosphere, dinitrogen gas (N_2) is inaccessible to plants. Through their roots, plants take up nitrates and ammonium, which are two forms of the soil's available nitrogen. Since crop development depends on nitrogen, which has a limited bioavailability, there is a huge N-based fertilizer industry globally. Chemical fertilizers have recently had a significant impact on food production and are now an essential component of modern agricultural methods. The Green Revolution of the past half-century was fuelled by technologies heavily dependent on synthetic fertilizers.

In 1985, the use of 38.8 million Mt of N fertilizer on cereals globally resulted in increased world cereal production of 938 million Mt, more than half of the total cereal production in that year (Rusell *et al*., 1989) [15]. Although the scientific community as a whole agrees that N fertilizers are practically necessary in modern agriculture, there are serious concerns about their use. Some of problems include low N use efficiency (NUE), nitrous oxide (N_2O) emissions, and surface and subsurface water contamination.

As a result, N management in global food production has multiple facets. There are calls for more sustainable methods of fulfilling the N requirement of crops, such as climate-smart agriculture and sustainable intensification, despite the fact that N fertilizers are being used to crops to boost crop production. Biological Nitrogen fixation (BNF), discovered by Beijerinck in 1901, is carried out by a specialized group of prokaryotes. These organisms utilize the enzyme nitrogenase to catalyse the conversion of atmospheric nitrogen (N_2) to ammonia

(NH_3). Plants can readily assimilate NH_3 to produce the aforementioned nitrogenous biomolecules.

These prokaryotes include aquatic organisms, such as cyanobacteria, free living soil bacteria, such as Azotobacter, *Azospirillum*, and most importantly, bacteria, such as *Rhizobium* and *Bradyrhizobium* that form symbiosis with legumes and other plants. BNF is regarded as one of the most environmentally friendly methods for satisfying crop N requirements (Singh *et al*. 2023) [18]. For instance, it has been calculated that NUE increases exponentially with increasing soil levels of biologically fixed N_2, whereas NUE declines linearly with increasing soil levels of applied synthetic N fertilizers. In this article we will show that "Biological nitrogen fixation is a crucial ecological process that increases soil fertility, decreases the need for synthetic fertilizers, and supports sustainable agriculture. This organic nitrogen transformation by microbes highlights the crucial part that it plays in preserving ecosystem health and food production (Santoro 2010) [17].

2. Why biological N fixation is better than other process

Although atmospheric nitrogen, which is nitrogen's most common form, is unavailable, it is a crucial ingredient for plant growth and development. As opposed to this, plants rely on combined, or fixed, forms of nitrogen like ammonia and nitrate. A large portion of this nitrogen is supplied to cropping systems as nitrogen fertilizers made in factories. The use of these fertilizers has resulted in ecological issues on a global scale, such as the development of coastal dead zones. On the other hand, biological nitrogen fixation provides plants with nitrogen in a natural way (Fig 1). It is an essential part of numerous terrestrial and marine ecosystems throughout our biosphere (Wagner 2011) [23].

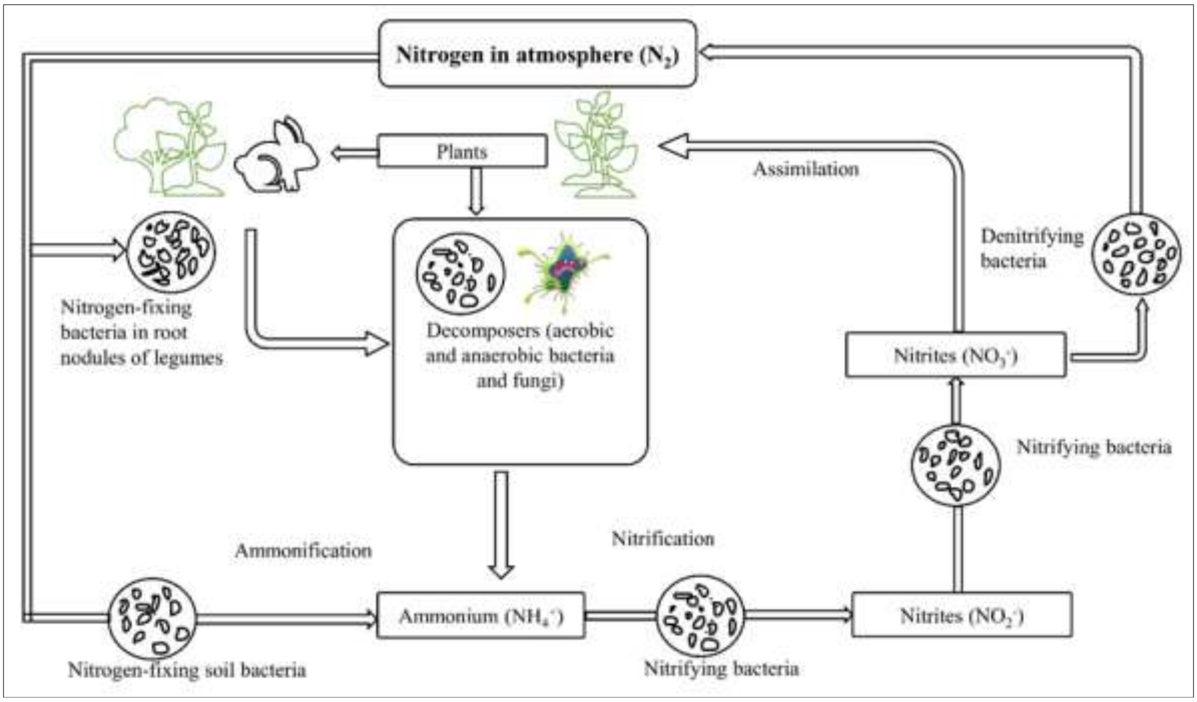

Fig 1: Biological nitrogen fixation

3. Types of Biological Nitrogen Fixation

Non symbiotic Biological Nitrogen Fixation

Biological nitrogen fixation by microorganisms that reside outside of a plant cell is referred to as non-symbiotic nitrogen fixation. The process of turning atmospheric nitrogen into nitrogenous compounds is known as biological nitrogen fixation. In soil, there are numerous free-living nitrogen-fixing microorganisms playing role in plant system (Table 1, Fig 2). They also include several type aerobic and anaerobic microbes, as well as blue-green alga (Soumare *et al*. 2020) [19].

The non-symbiotic nitrogen fixers fall into the following categories

1. Free-living aerobic Nitrogen-fixing bacteria:-
   Photosynthetic: Chlorobium, Chromatium
   Non-Photosynthetic: Azotobacter, Azomonas, Dexia, Beijerinckia.
2. Free-living anaerobic Nitrogen-fixing bacteria:-
   Photosynthetic: Rhodospirillum.
   Non-Photosynthetic: Clostridium.
3. Free-living chemosynthetic bacteria:
   Heterotrophic: Desulfovibrio.
4. Cyanobacteria or Blue-green algae:
   Heterocyst bearing: Nostoc, Anabaena, Rivularia, Calothrix.
   Non-Heterocyst bearing: Oscillatoria, Gloeocapsa, Lyngbya, Plectonema.
5. Free-living Fungi: Yeasts and Pullularia.
   As long as no hydrogen gas is produced, these organisms fix nitrogen more actively when there is low aeration.

Associative Symbiotic Nitrogen Fixation

Bacteria that are close to the roots of cereals and grasses (Poaceae) fix nitrogen. This adaptable mutualism is known as associative symbiosis. The bacteria occasionally enter the roots from the rhizosphere, or the region between the soil and the roots. The roots absorb some fixed nitrogen, which, through the carbohydrates the roots create, feeds the bacteria (Franche *et al*. 2009) [3].

Some examples are

1. *Azospirillum brasilense* in association with cereal roots.
2. *Beijerinckia* in association with the roots of Sugarcane.
3. *Azotobacter paspali* in association with roots of tropical grass- *Paspalum notatum.*

Symbiotic Biological Nitrogen Fixation

Many microorganisms fix nitrogen symbiotically by partnering with a host plant. The plant provides sugars from photosynthesis that are utilised by the nitrogen fixing microorganisms for the energy it needs for nitrogen fixation. The bacterium gives the host plant fixed nitrogen for growth in return for these carbon sources (Mus *et al*. 2016) [13].

The symbiotic nitrogen fixers fall into the following categories

1. Nitrogen Fixation in leguminous plants

The genus Rhizobium is primarily one of several legume plants' symbiotic nitrogen fixers. They establish themselves in root nodules, which are specialized structures on the roots. Only when the bacteria are present inside the nodules, they fix nitrogen. Because the host plant provides the nodule bacteria with organic carbon (carbohydrates), therelationship is classified as symbiotic. The microorganisms then supply fixed nitrogen to the host plant (Santi *et al*. 2013) [16].

2. Nitrogen Fixation via nodule formation in the non-leguminous plants

There are other plants outside of the Leguminosae family that are known to generate root nodules. The most significant of them are mainly trees and shrubs. The following are some prominent instances of non-leguminous plants that fix nitrogen and form root nodules:

i) Genus Frankia produces root nodules in association with *Alnus, Myrica gale, Casuarina equisetifolia, etc.*
ii) Rhizobium also has root nodules in the genus Parasponia.
iii) Leaf nodules are formed by bacteria Klebsiella in the genus Psychotria and by bacteria Burkholderia in genus *Pavetta zimermanniana.*
iv) Nitrogen Fixation through Non-nodulation: In some plants, symbiotic nitrogen fixation occurs, but nodules are not formed. Such associations are Pseudo symbiotic (Pseudo Symbiosis). Some of the examples are: Anthoceros, associated with Nostoc; Azolla living in association with Anabaena.

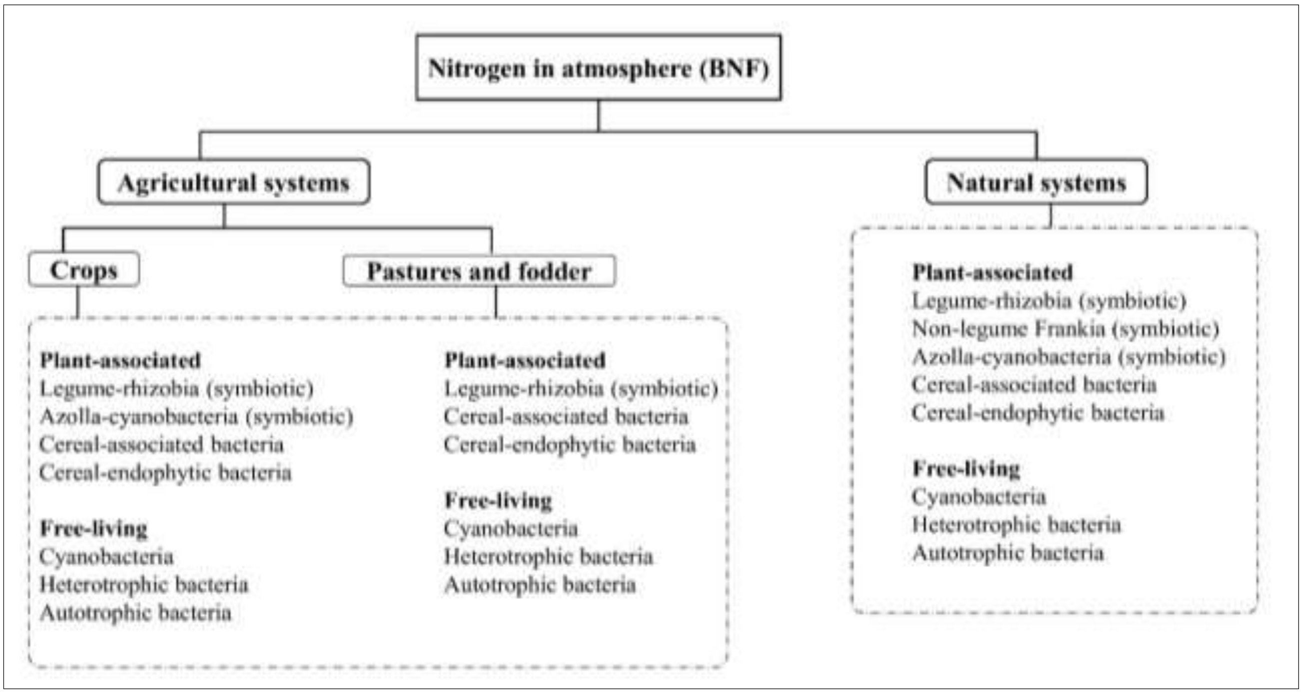

Fig 2: Types of biological nitrogen fixation

Table 1: Estimates of dinitrogen fixed in agriculture by various N_2-fixing systems

Sl. No	N_2-fixing system	N_2 fixed (kg N ha^{-1})	References
A. Free-living/associative			
1.	Rice-blue green algae	10-80 crop^{-1}	Roger and Ladha (1992) [14]
2.	Rice-bacterial association	10-30 crop^{-1}	Roger and Ladha (1992) [14]
3.	Sugarcane bacterial association	20-160 crop^{-1}	Urquiaga *et al.* (1989) [20]
B. Symbiotic			
1.	Rice-Azolla	20-100 crop^{-1}	Roger and Ladha (1992) [14]
C. Legume-Rhizobium			
1.	*Leucaena leucocephala*	100-300 yr^{-1}	Danso *et al*. (1992) [2]
2.	*Glycine max*	0-237 crop^{-1}	Keyser and Li (1992) [8]
3.	*Trifolium repens*	13-280 crop^{-1}	Ledgard and Steel (1992) [10]
4.	*Sesbania rostrata*	320-360 crop^{-1}	Ladha *et al*. (1990) [9]
5.	Non-legume-*Frankia Casuarina sp.*	100-300 yr^{-1}	Gauthier *et al*. (1985) [4]

4. Advantages of BNF

1) **Reducing synthetic N fertilizers:** The dependency on chemical N fertilizers currently felt by humanity will decrease when BNF is used more often. Anhydrous ammonia, a commonly used synthetic N fertilizer, must be produced using a sizable amount of energy from non-renewable sources of energy, such natural gas. Additionally, very considerable volumes of fossil fuels, including diesel fuel, are needed for the distribution and use of these fertilizers (Mahmud *et al*. 2020) [12].
2) **Pollution free:** Utilizing BNF more frequently can improve environmental quality by lowering issues with pollution in the air and water. In a number of sites in the United States, high groundwater nitrate (NO_3^-) levels have been related to the overuse of synthetic N fertilizers. Nitrate concentrations that are too high might be harmful to people's health. Manufactured N fertilizers are produced and applied by burning non-renewable fossil fuels including natural gas, petrol, diesel, and gasoline, all of which have been linked to increased air pollution (Gu *et al*. 2017) [5].
3) **Less production cost:** Increasing the use of BNF can assist reduce production costs and boost producers' profit margins. Nitrogen fertilizer requirements for crops in crop rotations can be greatly reduced by the use of nitrogen-fixing crops. For instance, following alfalfa with corn in a mix will result in profitable corn yields with lower fertilizer application and buying costs (Jensen and Hauggaard-Nielsen 2003) [6].
4) **Improving soil fertility and tilth**: By enhancing soil fertility and tilth, greater usage of BNF can contribute to increasing sustainable food production. Some farmers have discovered that using so-called green manure crops instead of synthetic fertilizer they buy is a more environmentally friendly option. Crops raised expressly for soil incorporation as opposed to harvesting are known as "green manure crops." Growing crops with green manure that fix atmospheric nitrogen may eventually raise the amount of organic matter and N in the soil. The tilth of a soil, which refers to desired physical characteristics of soil such good drainage, capacity for water retention, aeration, and structure, is typically improved by the addition of organic matter to soils (Kannaiyan and Kumar 2006) [7].

5. Disadvantages of BNF

1) **Energy:** Energy. Energy is required to fix nitrogen. If organisms can obtain what they need without having to fix nitrogen, they don't. When they do, the energy expenditures prevent them from growing as quickly as species that do not fix nitrogen. When provided enough nitrogen, maize produces higher yields than soybeans, demonstrating this (Brill 1977) [1].
2) **Shade tolerance:** Many legumes that are (trees, but not tropical ones) are less sensitive to shade due to the high cost of energy of N-fixation, which makes them less competitive in combinations with non-N-fixing plants (Vitousek *et al*. 2002) [22].
3) **Oxygen levels:** Low oxygen concentrations are needed for biological N fixing. To block oxygen, N-fixers must create structures like nodules that are part on legume roots. This is also expensive and not necessary for non-N-fixers (Vitousek *et al*. 2002) [22].
4) **Phosphorus and other nutrient levels:** Nutrient requirements are greater for nitrogen fixers than for non-N-fixers. Molybdenum, P, K, and Fe. Once more, this restricts N- fixation in settings where these nutrients are scarce. Although alfalfa producers may not need to use N fertilizer, they won't be able to harvest much hay without sufficient soil P levels. "Therefore, P (or another non-N nutrient) limitation may be mistaken for N limitation to primary utilization on a whole-system level" (Vitousek *et al*. 2013) [21].
5) **Grazing:** Herbivores (such as cattle, beetles, and others) prefer to consume N-fixers over non-N-fixers because the latter have a higher N content, which gives the latter a benefit. This is known to graziers as the issue of maintaining a steady legume-to-grass ratio over a long period of time (Vitousek *et al*. 2013) [21].

6. Future Prospect of BNF

Remaining gaps and future research needs despite the fact that a lot of research has been done, we have not made much progress in transferring all these technologies to farmers and therefore improving their cropping systems. Most of this research is done on research stations. There is little work done on farmer fields and therefore no benchmark practices from which to measure the impact of research innovations. There is a need to work in a holistic way on the entire cropping system to maximize the benefits of BNF to cropping systems in the developing countries (Mafongoya *et al*. 2009) [11]. We need further research on the following:-

i) Below-ground contribution of nitrogen by various legumes;
ii) Research on soil factors such as P and water availability, which affect growth of legumes and BNF.
iii) Economic impact of cropping systems involving all legumes.
iv) access and improvement of management practices such as residue management and selection of germplasm which affect N fixation;
v) Production of quality inoculants for various legumes;
vi) The role of P on improving thenutrient-use efficiency from legumes in crops especially with legumes that take P from insoluble forms;
vii) The long -term benefit of the legume–cereal rotations and quantification of the benefit in terms of yield stability.
viii) The non-rotational effects of legume–cereal rotations
ix) Better assessments of nitrogen balances in legume-based systems.
x) Increased inoculation efficiency of legumes with rhizobia under farmer field conditions;
xi) Quantification of the role of legumes in improving soil chemical, physical, and biological properties (Mafongoya *et al*. 2009) [11].

7. Conclusion

Nitrogen fertilizer is now a crucial component of crop production all over the world. Farmers are increasingly reliant on non-farm inputs, which can be expensive and occasionally unavailable in a timely manner. It is becoming increasingly clear that using a lot of N fertilizer has negative consequences on the ecosystem. Additionally, the fossil fuels that are needed to produce N fertilizer are becoming more expensive and in short supply. As populations rise, there is a parallel rise in food demand. Therefore, it is crucial to look into every option for enhancing biological nitrogen fixation and farmers' utilization of it. There is a need for sustainable farming, which preserves soil fertility by utilizing readily and affordably accessible renewable resources on the farm.

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