A Seminar Paper on

Consumption of Biologically Fixed Green Nitrogen and Agricultural Sustainability

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Consumption of Biologically Fixed Green Nitrogen and Agricultural Sustainability[1]

By

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ABSTRACT

Nitrogen (N) is a crucial component of soil fertility and productivity but its uncontrolled and excessive use can lead to environmental contamination and financial losses. Therefore, the objective of the study was to review the role of consumption of biologically fixed green nitrogen in sustainable agricultural systems for efficient use of nitrogen**.** Biologically fixed nitrogen, facilitated by nitrogen-fixing microorganisms, converts atmospheric nitrogen into forms accessible to plants. Biological nitrogen fixation (BNF) occurs through various relationships between bacteria and plants, including symbiotic, associative, and non-symbiotic interactions. BNF has significantly contributed to agriculture by offering a sustainable solution to nitrogen deficiency in soils. The efficiency of bacterial partners in nitrogen fixation varies across symbiotic interactions, ranging from 1-465 kg N ha^{-1} y^{-1}. Different legume species exhibit annual N fixation through BNF, with values ranging from 14 to 465 kg N ha^{-1} yr^{-1}. Legume crops transfer nitrogen to the soil or neighboring plants in varying quantities, ranging from 0-650 kg ha^{-1}. Biofertilizers have shown potential in saving nitrogen fertilizer, ranging from 20 to 100 kg per hectare, while increasing grain yield from 1.2 to 2.08 tons per hectare, depending on the crop and biofertilizer used. BNF plays major roles in reducing the use of synthetic nitrogen fertilizer in agriculture, increasing soil and plant nutrient content, and soil health reclamation, which contribute to the long-term viability of agricultural systems and environmental sustainability.

Keywords: Biological fixed green nitrogen; legumes and non-legume crops; soil microbes; agricultural sustainability

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CHAPTER 1

INTRODUCTION

Today's agricultural systems have difficulties in producing enough food for a growing global population while also enhancing soil and water quality, preserving farmer profitability (Stephens *et al.*, 2018), and mitigating climate change (Niles *et al.*, 2018). Optimal plant development for crop production depends on nutrient cycling, which is ensured by a healthy, functioning soil (Ney *et al.*, 2019). The amount of soil nutrients, particularly nitrogen, that are accessible frequently limits agricultural output (Vitousek and Howarth, 1991). Despite the fact that nitrogen is the most abundant of all atmospheric gases, it is not found in soil parent material (Hedin *et al.*, 2009). Thus, organic matter decomposition, synthetic fertilizer inputs, and biological nitrogen fixation (BNF) via nitrogenase enzyme activity all play a role in soil nitrogen input for plant nutrition and crop yield (Galloway *et al.*, 2008; Vitousek *et al.*, 2013). In this context, feeding the world's growing population will be impossible without a significant increase in agricultural production through the use of inorganic nitrogen fertilizers (Liu *et al.*, 2016). Nitrogen is a necessary nutrient for plant growth and development, and agricultural intensification often supports higher usage of nitrogen to increase yields. On the other side, over usage of inorganic nitrogen fertilizer has resulted in global disruption to the environment (Yang and Fang, 2015). Unfortunately, nitrogen is also directly linked to negative environmental impact via direct (N_2O) and indirect (NH_3) greenhouse gas emissions, ozone depletion (NO), and water pollution (NO_3^-) (Galloway *et al.*, 2003; Lal, 2015). This situation is contrary to the philosophy and principles of the sustainable agriculture system (Li *et al.*, 2009; Lal, 2015). Consequently, nitrogen must be key to the sustainable transformation of the world's food production (Ying *et al.*, 2017). Most, if not all, agricultural production systems are limited by nitrogen availability, hence the widespread - and increasing use of fertilizers (Rütting *et al.*, 2018). Fertilizers are a source of nitrogen that is costly, of only moderate efficiency and with a relatively large CO_2 footprint, due to the energy intensity of the synthesis of chemical fertilizers by the Haber-Bosch process (Da Silva *et al.*, 1978). Therefore, reducing dependence on nitrogenous fertilizers in agriculture is necessary to achieve sustainable agricultural goals.

Biological nitrogen fixation emerges as the second largest source of nitrogen needed by field crops and as a key process on earth in the development of sustainable agriculture (e Castro *et al.*, 2016). Compared to chemical fertilizers, BNF is cheaper and may result in a lower carbon footprint. In addition, the nitrogen provided by biological nitrogen fixation is more environmentally friendly than the nitrogen to be applied chemically, and it also prevents a great loss of expense. Therefore, it is a complementary element for sustainable agriculture (Bhattacharjee and Dey, 2014; Rashid *et al.,* 2015; Soumare *et al.,* 2020). Davies-Barnard and Friedlingstein (2020) conducted a meta-analysis on biological nitrogen fixation on a global scale and stated that biological nitrogen fixation in terrestrial ecosystems ranged from 52–130 Tg N y^{-1} and an average of 88 Tg N y^{-1} in natural ecosystems. Green nitrogen that has been biologically fixed is one of the main sources of nitrogen for plants and an essential step in the distribution of this nutrient in the ecosystem. Therefore, the objectives of the study are:

1. To highlight the biologically fixed green nitrogen system;
2. To ascertain the role the biologically fixed green nitrogen in agricultural sustainability.

CHAPTER 2

MATERIALS AND METHODS

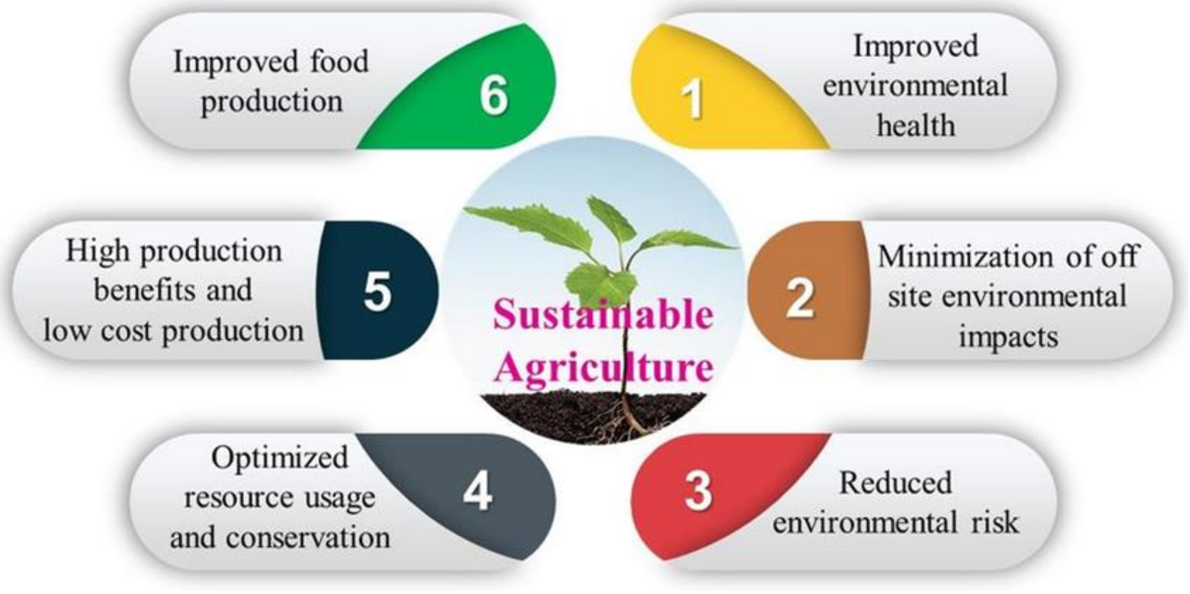
This is exclusively a review paper. Therefore, it was prepared using secondary sources, including different published reports, articles, conference papers in various journals, websites, and other books available on the internet. Moreover, I improved this paper with valuable suggestions from my respected major professor and course instructors. The collected information was compiled and arranged chronologically to provide better understanding and clarity.

CHAPTER 3

REVIEW OF FINDINGS

3.1 Sustainable Agriculture

Sustainable agriculture is the practice of farming in a way that makes better use of natural resources, lessens the impact of agriculture on the environment, and increases capacity for adaptation to climatic change and variability without sacrificing the ability of the current or future generations to meet their own needs (FAO, 2014) (Figure 1). It combines three major purposes; environmental health, economic profitability, and social equity (Sarker, 2017). In sustainable agricultural systems, the adoption of various technologies for soil, nutrients, and pests management has been advocated. These technologies include the use of dung, crop residue, and other bio-solids, as well as practices like biological nitrogen fixation (BNF), mixed cropping, and crop rotations (Lal, 2007; Narwal, 2010). These measures led to improved soil quality and nutrient pools, biological diversity, climate resilience and ecosystem restoration by increasing organic inputs to the soil along with a balanced application of inorganic fertilizers (Lal, 2015), and also increasing socio-economic status of the farmers (Mtengeti *et al.*, 2015). Threrefore, these practices have been suggested to be effective in achieving sustainable agriculture goals.



Source: (Aslam, 2015)

Figure 1. The concept of sustainable agriculture.

3.2 Role of nitrogen in Sustainable Agriculture

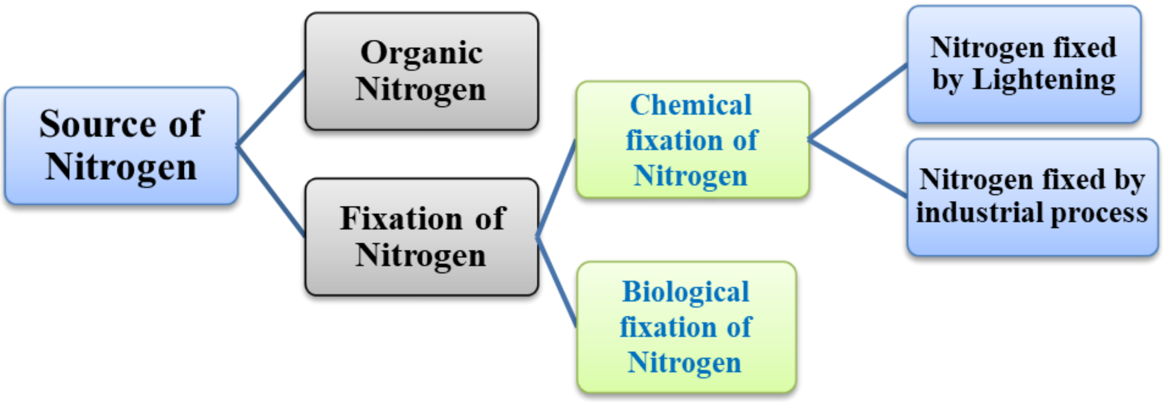
Nitrogen (N) is a crucial component of soil fertility and productivity (Fang *et al.,* 2018; Richard *et al.,* 2018). About 65% of the mineral nitrogen applied to the soil is lost in the soil and plant system by emission gas and erosion (runoff and leaching) (Rejesus and Hornbaker, 1999). These losses can lead to environmental contamination and financial losses (*Reddy et al.,* 2002). Incorrect or uncontrolled use of N causes serious environmental problems with the leakage of reactive N into surface and subsurface waters and the release of N (as N2O) into the atmosphere (Galloway *et al.,* 2003; Lal, 2015). This is contrary to the principles of sustainable agriculture and it is important to ensure that nitrogen is used in the most effective way possible and where it is needed (Li *et. al.* 2009; Lal, 2015).

3.3 Source of Nitrogen

Nitrogen is a crucial nutrient for crop production, and it is often deficient in soil. It is needed to add nitrogen inputs to the soil system to ensure good crop yields. Plants take nitrogen, which is vital for plants, as NO_3^- and NH_4^+ ions. (Bloom, 2015; Cui *et al*., 2017).The nitrogen source of the plants is organic nitrogen (Bloom, 2015; Stevens, 2019), and nitrogen fixation (Igarashi and Seefeldt, 2003; Soumare *et al.*, 2020; Mahmud *et al.*, 2020) (Figure 2).

Various organic nitrogen sources such as animal manure, compost, sewage sludge, and crop residues have been extensively studied for their effectiveness as soil fertilizers and their impact on crop yields (Manikandan and Thamizhiniyan, 2016).

Although lightning and UV radiation may combine nitrogen to form nitric oxide, soil microbes are more important in fixing nitrogen as ammonia, nitrites, and nitrates (Britannica, 2023). When lightning supplies the energy necessary for N_2 to combine with oxygen to produce nitrogen oxide, NO, and nitrogen dioxide, NO_2, a tiny quantity of nitrogen (5-8%) can be fixed. These forms of nitrogen then enter soils through rain or snow. Nitrogen can also be fixed through the industrial process that creates fertilizer. The Haber-Bosch method is now one of the largest and most-basic processes of the chemical industry throughout the world. This process of nitrogen fixation takes place under extreme heat and pressure, combining atmospheric nitrogen and hydrogen to create ammonia (NH_3), which may then be further processed to create ammonium nitrate (NH_4NO_3), a form of nitrogen that can be applied to soils and used by plants (Aczel, 2019). The majority of nitrogen fixing takes place naturally in the soil by microorganisms. They influence more than 90% of nitrogen fixation overall (Britannica, 2023).



Source: (Aczel, 2019; Mahmud *et al.*, 2020)

Figure 2. Source of Nitrogen for plants.

3.4 Biological Nitrogen Fixation (BNF)/Biologically Fixed Green Nitrogen

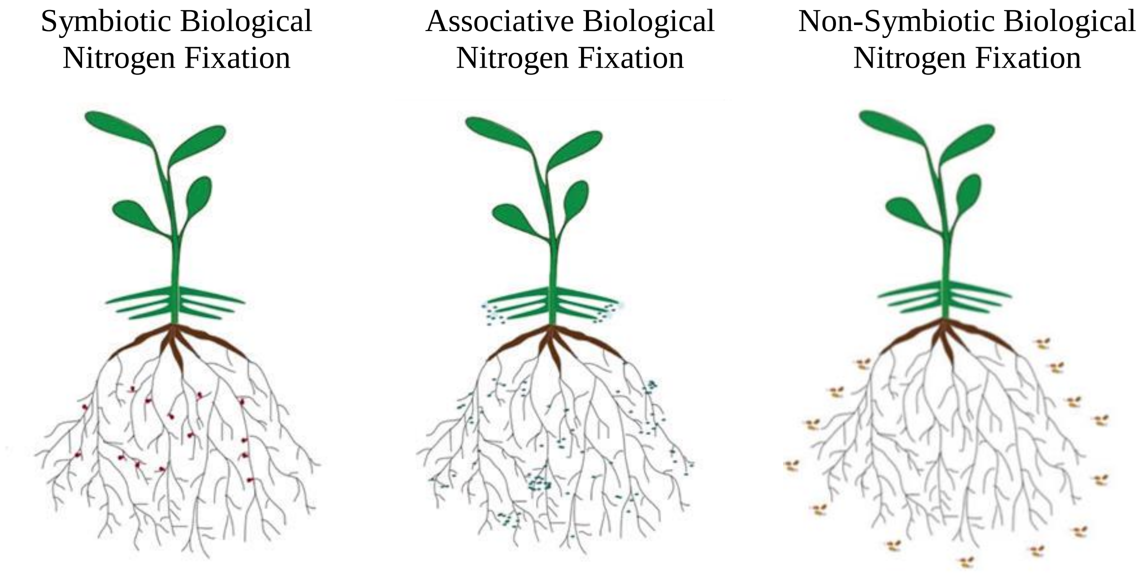
Nitrogen fixation is a dynamic and energy-demanding process (Rosenblueth *et al.*, 2018). Eq. 1 below illustrates the process by which inert N_2 is biologically converted into the reactive molecule NH3 (ammonia) in micro-aerobic conditions.

$$N_2 + 8H^+ + 8e^- + 16Mg\text{-}ATP \longrightarrow 2NH_3 + H_2 + 16Mg\text{-}ADP + 16\ P \text{ --------- } (1)$$

Source: (Lodwig and Poole, 2003)

Thus, conversion of this form of nitrogen into plant available form is a complex process, and large amount of energy is required to carry out this reaction. As a result, nitrogen-fixing bacteria need 16 moles of ATP to change one mole of gaseous nitrogen into a form that plants can use. The microbial oxidation of soil-based organic compounds provides the energy needed for this process. Free-living non-photosynthetic nitrogen fixer take these molecules released from other organisms, while associative microbes and symbiotic microorganism obtain these organic substances from the rhizosphere of host plants.

In general, BNF refers to a microbially driven process in which nitrogenase is present and atmospheric N_2 is reduced into ammonia (NH_3). A class of NF creatures known as diazotrophs exhibit this enzymatic conversion in a large variety of different ways. Some diazotrophs can fix N_2 in a free-living condition, while others work with plants, such as endophytic (within plant tissues) and symbiotic bacteria, which alter the physiology and structural characteristics of both the microbe and the plant roots in specialized structures called nodules (Unkovich *et al.*, 2008). Thus Biological Nitrogen fixation may be categorized into following types (Figure 3).


Symbiotic Biological Nitrogen Fixation

Associative Biological Nitrogen Fixation

Non-Symbiotic Biological Nitrogen Fixation

Source: (Pankievicz *et al.*, 2019)

Figure 3. Different types of Biological Nitrogen Fixation.

3.4.1 Symbiotic Nitrogen Fixation

In a symbiotic nitrogen fixation relationship, plants give bacteria a habitat and fixed carbon in return for fixed nitrogen. As the microbial partner fixes atmospheric nitrogen, such a relationship between the bacteria and the host is ecological, long-lasting, and advantageous to both partners (Oldroyd and Dixon, 2014). Symbiotic relationship is common in different species of plants and microorganism. These microbes use the photosynthates that these plants supply as a source of carbon and energy. In exchange, plants utilise the nitrogen fixed by these microorganisms for growth. For instance, the cyanobacterium *Anabaena azollae* establishes a symbiotic connection with the *Azolla* fronds' base cavities and produces a sizable quantity of nitrogen fixation in specialized cells known as heterocysts (Adams, 2000; *Rai et al.*, 2000). This association is being used for at least a thousand years as a biofertilizer for rice growth. Similarly, certain microbes develop symbiosis with trees and shrubs, for example, actinomycete *Frankia* live in association with alder (*Alnus* sp.) and helps. The most significant symbiosis relationship has been established between *Rhizobium* and *Bradyrhizobium* bacteria and legume plants, and they contribute more to BNF compared to other associations, even though the aforementioned interactions between microbes and plants are crucial for fixing atmospheric N. Different types of BNF with microbes and host plants are shown in Table 1.

Table 1. Different types of BNF with microbes and host plants.

Symbiotic Nitrogen Fixation		**Associative Symbiotic Nitrogen Fixation**		**Free-living Nitrogen Fixation**	
Microbes	**Host Plants**	**Microbes**	**Host Plants**	**Microbes**	**Host Plants**
Rhizobium *Sinorhizobium* *Mesorhizobium* *Bradyrhizobium* *Azorhizobium*	Legume plants (common bean, peas, lentils, peanut, soybean, vigna chickpea, vetch, medicago, sesbania)	*Azospirillum brasilense* *Beijerinckia*	Cereal roots Sugarcane roots	Heterotrophic (Aerobic) *Azorhizobium* *Azotobacter* *Azospirillum* *Beijerinckia* Heterotrophic (Anaerobic) *Clostridium* *Desulfobirio*	Host Plants Sesbania Tropical grass roots Cereal roots Sugarcane roots
Frankia	Non legume plants (*Alnus* sp, *Myrica gale)*			Phototrophic (Cyanobacteria) *Anabaena* *Nostoc*	Azolla Anthoceros
Anabaena	Azolla			Photosynthetic bacteria *Rhodospirillum* *Chlorobium*	
Nostoc	Anthoceros				
Fungi and algae	Lichens				

Source: (Khan *et al.*, 2019)

3.4.2 Associative Nitrogen Fixation

Nitrogen is fixed by bacteria that live in close contact with the roots of cereals and grasses. This is a loose mutualism known as associative symbiosis. The bacteria live in the rhizosphere (the transition zone between soil and root) and occasionally enter the roots (Oldroyd and Dixon, 2014). Different species of *Azospirillum* have the ability to thrive in the rhizosphere of the various members of *Poaceae* family and fix appreciable amount of atmospheric nitrogen into plant available N. Important agronomical crops which are found in close association with these bacteria include wheat, corn, oats, and barley. In associative nitrogen-fixing relationships, the ability of *Azospirillum* to fix nitrogen depends on the temperature of the rhizosphere, the availability of a suitable amount of carbon source, low atmospheric oxygen pressure in the rhizosphere, the competitiveness of the bacteria, and the efficiency of the bacterial nitrogenase enzyme (Bashan and de-Bashan, 2010).

3.4.3 Non- Symbiotic Biological Nitrogen Fixation

Non-symbiotic Biological Nitrogen Fixation refers to biological nitrogen fixation by microorganisms that live freely or outside of plant cells (Oldroyd and Dixon, 2014). Numerous heterotrophic bacterial species, including *Azotobacter, Bacillus, Clostridium,* and *Klebsiella,* freely coexist in soil and fix a significant amount of nitrogen without the assistance of other organisms or symbiotic relationships. As previously noted, these organisms obtain their energy from the oxidation of organic matter in soil; however, there are certain species which have chemo-lithotrophic capabilities and use inorganic compounds for energy production (Reed *et al.,* 2011). Because oxygen inhibits the activity of nitrogenase enzyme, therefore, these free-living nitrogen fixing organisms behave as anaerobes or microaerophiles during nitrogen fixation. Due to less availability of organic carbon for oxidation, the role of these microbes is supposed to be very minor in contributing BNF on global scale. However, proper maintaining of crop residues which serve as carbon source for these microbes and low available N in soil would facilitate the activity of free-living microbes.

3.5 Contribution of BNF to Agriculture

BNF has made significant contributions to agriculture by offering a natural and sustainable solution to the challenges of nitrogen deficiency in soils. In interactions between plants and bacteria, photosynthates from the plant are used to power the energy-intensive nitrogen fixation in return for some of the fixed nitrogen. Most of the time, "symbiotic nitrogen fixation" has referred only to symbioses leading to the development of root nodules. By definition, however, symbiosis is a long-term association between two different organisms that is beneficial for at least one of them (Martin and Schwab, 2013). This criteria is clearly met by associative nitrogen fixation, since the plant receives from growth promotion (both through greater nitrogen nutrition and various other advantages) while the bacterium gains carbon through plant photosynthesis. These kinds of interactions-energy supply, oxygen protection, and transport of fixed nitrogen to the plant-solve the three difficulties of biological nitrogen fixation with varying degrees of effectiveness (Table 2). The efficiency of each bacterial partner is indicated by low, moderate, or high. The nitrogen fixation rates depend on the efficiency of the interaction. a Root nodule symbiosis, 52-465 kg N ha^{-1} y^{-1} (Anglade *et al.,* 2015) ; (b) associative nitrogen fixation, 2-170 kg N ha^{-1} y^{-1} (Ladha *et al.,* 2016; de Morais *et al.,* 2012; Herridge *et al.,* 2008) ; and (c), (d) free-living nitrogen fixation, 1-80 kg N ha^{-1} y^{-1} (Reed *et al.,* 2008).

Table 2. Plant-bacteria interaction.

Types of nitrogen-fixing association	**Symbiotic Nitrogen Fixation**	**Associative Symbiotic Nitrogen Fixation**	**Free-living Nitrogen Fixation**	
			Photosynthetic	**Heterotrophic**
Energy source	+++	++	++	+
Oxygen production	+++	++	+	+
Transfer of fixed nitrogen	+++	++	+	+
Estimates of nitrogen fixationrates (kg N ha^{-1} y^{-1})	52-465 (a)	2-170 (b)	1-80 (c)	1-10 (d)

Note: + (low), ++ (moderate), or +++ (high).

Source: (Pankievicz *et al.,* 2019)

3.5.1 Contribution of BNF In legumes and non-legumes crops

Accurately estimating global N_2 fixation for the symbioses of the forage and fodder legumes is challenging because statistics on the areas and productivity of these legumes are almost impossible to obtain. As we transition to various agricultural production systems, such as extensive grazed savannas, rice (*Oryza sativa)*, sugar cane (*Saccharum spp*.), cereal and oilseed crop lands, and sugar cane (*Saccharum spp*.), the level of uncertainty rises (Table 3). Nonetheless, the estimates of annual N2 fixation inputs are 21 Tg (pulse and oilseed legumes) 12-25 Tg (pasture and fodder legumes), 5 Tg (rice), 0.5 Tg (sugar cane), <4 Tg (nonlegume crop lands) and <14 Tg (extensive savannas) (Herridge *et al.,* 2008).

Table 3. N fixation in different agricultural systems.

Agent	Agricultural system	Rate of N_2 fixation(kg N/ha/year)	Crop N fixed (Tg/year)
Legume-rhizobia	Crop (pulse and oilseed) legumes	115	21
Legume-rhizobia	Pasture and fodder legumes	110–227	12–25
Azolla-cyanobacteria, cyanobacteria	Rice	33	5
Endophytic, associative & free-living bacteria	Sugar cane	25	0.5
Endophytic, associative& free-living bacteria	Crop lands other than used for legumes and rice	<5	<4
Endophytic, associative& free-living bacteria	Extensive, tropical savannas primarily used for grazing	<10	<14

Source: FAOSTAT (2007); Smil (1999); Cleveland *et al.* (1999)

Alfalfa and clover fix more nitrogen than pulse crop species, according to the computation of N fixation using our novel connections (Table 4). Within grain crops, faba bean revealed a slightly higher amount of fixed N. The discrepancy between alfalfa and clover was due to differences in cutting regimes, because in this area red clover is generally grown as green manure, whereas alfalfa was harvested from three or four cuts per season. White clover used as a cover crop between winter and spring cash crops is able to fix similar amounts of N as in-field pea (Anglade *et al.*, 2015).

Table 4. Estimated of N fixed in 33 in field crop production in the Paris basin (France).

Species	Grain or herbage DM yield (t/ha)	BNF in shoot (kg N/ha/yr)	Total BNF (kg N/ha/yr)
Alfalfa	10.3 ± 2.2	274 ± 60	465 ± 102
Red clover	7.0 ± 2.8	148± 59	252 ± 100
White clover (CC)*	2.0 ± 1.2	60 ± 41	102 ± 16
Faba bean	3.1 ± 0.9	127 ± 35	165 ± 45
Field pea	2.4± 0.9	85 ± 29	111 ± 38
Lentil	1.1 ± 0.3	40 ± 11	52 ± 15

Note: *CC means cover crop; the other species are grown as a main crop.

Source: (Anglade *et al.*, 2015

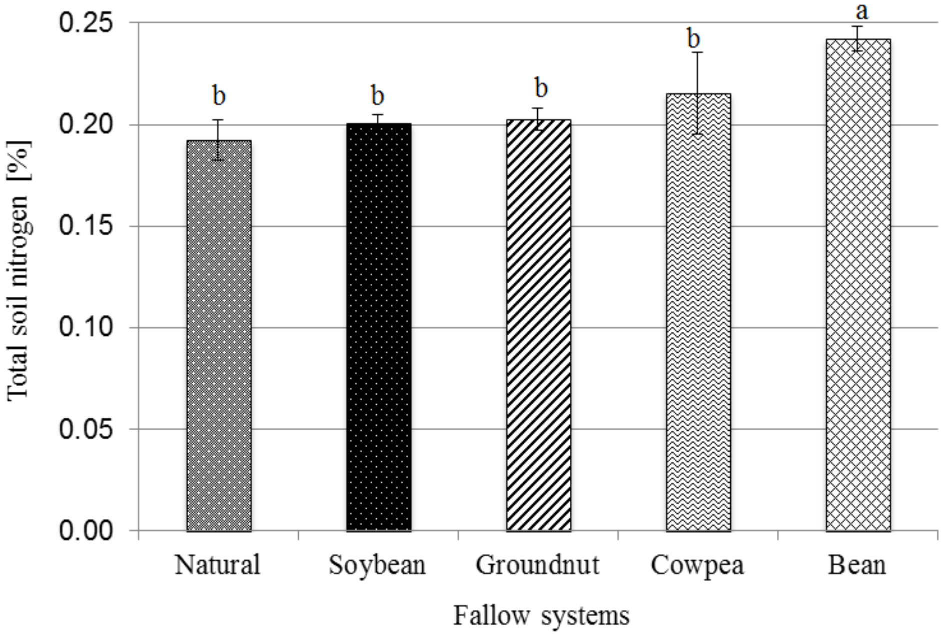
The annual N fixation of several legume species by BNF varies substantially, with chickpea (*Cicer arietinum*) and pinto peanuts (*Arachis pintoi*) fixing 103 kilogram N ha^{-1} and 14 kg N ha^{-1}, respectively (Table 5). Most of the legume crops could satisfy their N requirement from the BNF.

Table 5. Nitrogen fixation by different legumes.

Legume	**Annual N from BNF (kg N ha–1)**	**References**
Velvet bean (*Mucuna pruriens*)	59.6	Okito *et al.* (2004)
Pinto peanuts as green manure	14	Mendonça *et al.* (2017)
Pigeon pea as green manure	44	Mendonça *et al.* (2017)
Lablab beans (Dolichos lablab) as green manure	33	Mendonça *et al.* (2017)
Chickpea	64–103	Fatima *et al.* (2008)
Mung bean	19–54	Hayat *et al.* (2008)
Black gram	16–79	Hayat *et al.* (2008)

Source: (Rahman et al., 2022)

The most notable effect was the total nitrogen content that ranged from 0.19–0.24%, and differed ($P < 0.001$) significantly with the highest in common bean fallow system followed by cowpea, groundnuts, soybean and natural weed fallow (Figure 4). Grain legumes improved soil physicochemical properties by fixing the atmospheric nitrogen converting it from an inert form to forms that are available for plants uptake. This biological fixed nitrogen can replace nitrogen fertilization wholly or in part (Dwivedi *et al.*, 2015).



Source: (Nanganoa *et al.*, 2019)

Figure 4. Effect of five fallow systems (Natural, soybean, groundnut, cowpea, and bean) on total soil nitrogen content after nineteen weeks of crop cultivation. Data with different letters are significantly different (P < 0.05).

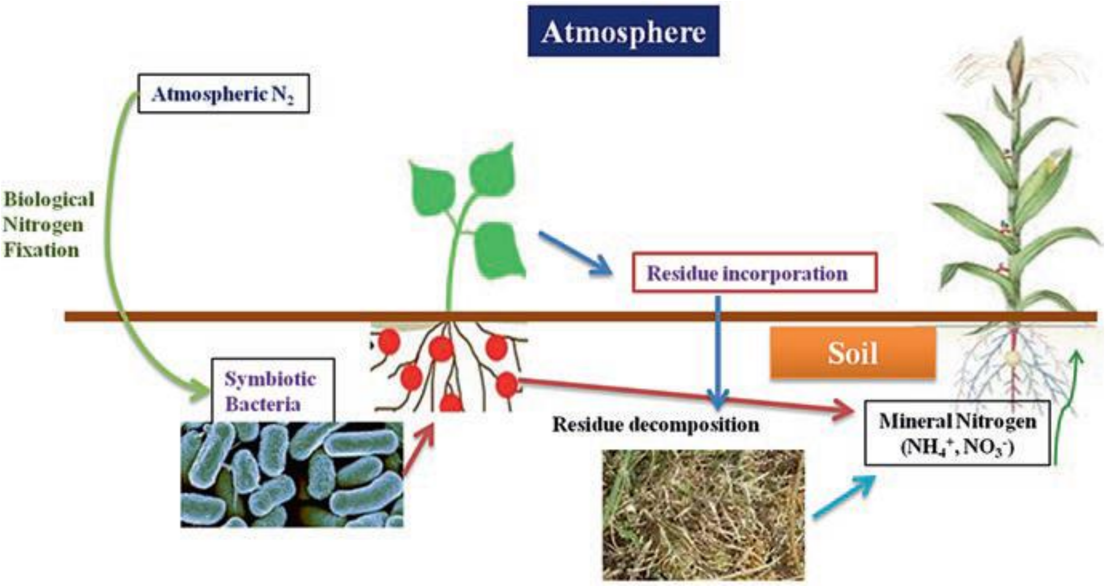
A comparison of global estimated of BNF in agriculture provided by grain legume-rhizobium symbioses, and/or freeliving/endophyte/symbiotic associations in rice and other major cereals (Table 6). The calculated estimates of BNF by grain legumes increased from 10 Tg N in the mid-1990s (Smil, 1999) to 35.5 Tg N by 2018 (Herridge *et al.*, 2022). This growth was largely associated with increased areas of grain legume production over time. Of the 35.5 Tg N deemed to be fixed in 2018, soybean accounted for 70% of the total. According to estimates of BNF for non-leguminous systems published by Smil (1999), Herridge *et al.* (2008), and Battye *et al.* (2017), the annual inputs of BNF from non-symbiotic sources in cereal cropping systems may have totaled 14.8 Tg of fixed N in 2010 (3.3, 5.6, and 5.9 Tg N yr^{-1} for wheat, rice, and maize, respectively. The extrapolated estimates of non-symbiotic BNF calculated by the authors for 2018 by applying the relationships developed by Ladha *et al.* (2016) to updated production data was 15.7 Tg of fixed N (3.1, 5.7 and 6.9 Tg N yr-1 each for wheat, rice and maize, respectively). The change in estimates of BNF by maize and rice were due to increases in total harvested areas (by 18.2% and 3.7% for maize and rice, respectively) from 2010 to 2018, and a decrease in wheat harvested area (1.3%).

Table 6. Estimated of annual inputs of BNF (Tg N) by symbiotic and non-symbiotic diazotrophs in cereal-based cropping systems.

Crop system	Diazotroph	BNF (Tg N)	References
Grain legumes	Rhizobium-legume symbiosis	10 (8–12)	Smil, (1999)
		21.5	Herridge *et al.*, (2008)
		32.5	Battye *et al.*, (2017)
		35.5	Herridge *et al.*, (2022)
Rice cultivation	free-living, endophytic and/or symbiotic (Azolla)	5 (4–6)	Smil, (1999)
		5	Herridge *et al.*, (2008)
		5.6	Ladha *et al.*, (2016)
		10	Battye *et al.*, (2017)
Cereals other than rice	free-living, endophytic	4 (2–6)	Smil, (1999)
		< 4	Herridge *et al.*, (2008)
		9.2	Ladha *et al.*, (2016)

Source: (Ladha *et al.*, 2022)

The BNF is the most effective method or technique for supplying the majority of N needed by legume crops or plants, as demonstrated by several researchs (Sulieman and Tran, 2014). Figure 5 (Meena *et al.*, 2018) shows the soil mineralization and BNF of a leguminous green manure crop.



Source: (Meena *et al.*, 2018)

Figure 5. N fixation and mineralization of leguminous green manure crop in soil.

The inherent Rhizobium in the soil is introduced externally as seed inoculums when the legume seed is sown, shortly after its germination. Later, the *Rhizobium* penetrates the legumes' root hairs and travels along an infection thread into the main root. Following invasion, these bacteria grow quickly in the roots of legumes, causing the root cells to expand and eventually form nodules. Legumes' root hairs "fix" atmospheric nitrogen by combining it with other elements and transforming it into an ammonical form that is usable for plants. In the process of converting atmospheric nitrogen (N) into ammonia, the Rhizobium bacteria utilise carbohydrates as a source of hydrogen (Fageria, 2007), and this symbiotic relationship between legumes and Rhizobium is responsible for 40% of all N fixation worldwide (Ladha *et al.,* 1992; Meena *et al.,* 2015a). According to a thorough review of the literature, grain legume crops may typically supply 50-80% of their own N needs through BNF. The symbiotic association of Rhizobium with seed legume crops, forage leguminous cover crops, non-Rhizobium N-fixing bacteria, cyanobacteria in rice, and endophytic N-fixing organisms in sugarcane contribute 10, 12, 4, 6, and 3 Tg N yr^{-1}, respectively, to the 33 Tg N added by legume crop cultivation (Smil, 1999). Therefore, it is evident that the amount of nitrogen fixed by various legume species varies and depends on the legume species, its variety, the number of functional root nodules, the type of soil, agronomic practices, water management techniques, and current climatic conditions, as well as their interactions with other factors (Fageria *et al.,* 2005). Sharma and Ghosh (2000) studied dhaincha in flood-prone lowland conditions as an intercrop with direct-seeded rice as well as incorporated pure dhaincha before transplanting rice and discovered that dhaincha accumulated 80-86 kg N ha^{-1} in pure stand and 58-79 kg N ha^{-1} when intercropped with direct-seeded rice in alternate rows at 50 days of growth.

The table 7 provided information on the amount of nitrogen transferred by different crop combinations. The data shows that different crop combinations transfer nitrogen in varying amounts, ranging from 0-650 kg ha^{-1}. The information presented in this table can be useful for designing legume-based cropping systems for sustainable intensification, as it provides insight into the nitrogen transfer potential of different crop combinations.

Table 7. Amount of nitrogen (N) fixed and proportion transferred to soil or neighboring plants in agricultural systems.

Sl. No.	**Crop(s)**	Amount of N transferred (% of fixed N)	Reference(s)
1.	Caragana (*Caragana arborescens* Lam.)-oat (*Avena sativa* L.)	38-45 kg ha^{-1} (60–70)	Issah *et al*. (2015)
2.	Alfalfa-tall fescue (*Schedonorus arundinaceus* (Schreb.) Dumort.)	0-650 kg ha^{-1} (0–12)	Louarn *et al*. (2015)
3.	White clover-perennial ryegrass	0-340 kg ha^{-1} (0–47)	Louarn *et al*. (2015)
4.	Mung bean-oat	12.8 mg plant^{-1} (9.7)	Zang *et al.* (2015)
5.	Soybean-maize	7.84 mg pot^{-1} (7.57)	Meng *et al.* (2015)
6.	Soybean-maize	10.77-13.72 mg pot^{-1} (1.26-2.17)	Yong *et al.* (2015)
7.	Faba bean-wheat	0.17 mg plant shoot^{-1} (14.9)	Wahbi *et al.* (2016)
8.	Red clover-bluegrass (*Poa pratensis* L.)	35.85 mg plant^{-1} (1.5)	Thilakarathna *et al.* (2016)
9.	Red clover-perennial ryegrass and forbs	25-58 kg ha^{-1} (9.5–15)	Dhamala *et al.* (2017)

Source: (Islam and Adjesiwor, 2018)

3.5.2 Contribution of BNF as a biofertilizer

A living fertilizer consisting of microbial inoculation agents or groups of microorganisms is able to fix atmospheric nitrogen in biofertilizer or a N fixing agent is used for biofertilization (Kyaw *et al.,* 2018). They are grouped together into free living bacteria (Azotobacter, Azospirillum), blue green algae, *Rhizobium*, *Frankia*, and *Azolla* symbionts. On the other hand N_2-fixing bacterium related to non-legume are *Achromobacter, Alcaligenes, Arthrobacter, Acetobacter, Azomonas, Beijerinckia, Batillus, Clostridium, Enterobacterium, Erwinia, Derxia, Disulfovibrio, Corynebacterium, Campylobacterium, Herbaspirillum, Klebsiella*, and *Rhodopseudobacterium.*

The table 8 showed that the recommended crops and their corresponding bio-fertilizers were used to save the N fertilizer. For lowland rice, fresh Azolla pinnata and blue-green algae (BGA) are recommended to save up to 30-50 kg of nitrogen fertilizer per hectare. For wheat, potato, and tobacco, fresh Azolla pinnata and dry BGA are also recommended to save the same amount of nitrogen as for lowland rice. Azotobacter chroococcum is recommended for pearl millet, sorghum, rajma (amaranthus), sugarcane, maize, potato, pigeonpea, onion, and cotton crops, and can save 20-40 kg of nitrogen fertilizer per hectare . Similarly, Azospirillum lipoferum is recommended for pearl millet, finger millet, paddy, sorghum, maize, tobacco, and onion crops, and can save 20-40 kg of nitrogen fertilizer per hectare. Acetobacter diazotrophicus is recommended for sugarcane crops and can save up to 100 kg of nitrogen fertilizer per hectare. Rhizobium spp is recommended for pigeonpea, chickpea, and green gram crops, and can save 30-50 kg of nitrogen fertilizer per hectare. Overall, the use of these biofertilizers can help reduce the use of synthetic nitrogen fertilizers in various crops (Bhattacharjee and Dey, 2014).

Table 8. Contribution of bio-fertilizer to N fertilizer saving.

Biofertilizer	**Recommended crop**	**Fertilizer saving**
Azolla pinnata (fresh)	Low land rice	30-50 kg N
Azolla pinnata (dry)	Wheat, potato, tobacco	30-50 kg N
Blue green algae	Low land rice	30-50 kg N
Azotobacter chroococcum	Pearlmillet,sorghum, rajma, sugarcane, maize, potato, pigeonpea, onion, cotton	20-40 kg N
Azospirillum lipoferum	Pearlmillet, fingermillet, paddy, sorghum, maize, tobacco, onion	20-40 kg N
Acetobacter diazotrophicus	Sugarcane	100 kg N
Rhizobium spp	Pigeonpea, chickpea, greengram	30-50 kg N

Sourse: (Bhattacharjee and Dey, 2014)

Biofertilizer and inorganic nitrogen influence was significant on lentil yield (Table 9). *Rhizobium* treated seed produced signifi-cantly highest grain, straw and biological yield (Begom *et al.*, 2021).

Table 9. Influence of *Rhizobium* and different amounts of urea on lentil yield.

Fertilizer	**Grain yield (t /ha)**	**Straw yield (t /ha)**	**Biological yield (t /ha)**
Rhizobium50mL/kg seed	2.08±0.11a	1.72±0.05a	3.88±0.16a
Urea20 kg /ha	1.33±0.11b	1.44±0.05bc	3.56±0.16ab
Urea40 kg /ha	2.04±0.11a	1.53±0.05b	2.91±0.16cd
Urea60 kg /ha	1.39±0.11b	1.42±0.05bc	3.34±0.16bc
Urea80 kg /ha	1.29±0.11b	1.33±0.05c	2.83±0.16d

Source: (Begom *et al.*, 2021)

The different bacterial fertilizers exerted significant effect on the seed yield and stover yield (Table 10). The use of *Bradyrhizobium* inoculants or *Azotobacter* inoculants produced significant increase over uninoculated control and 20 kg N ha^{-1} application (Table 9). The highest seed yield was noted when both *Bradyrhizobium* and *Azotobacter* inoculants were used together. The highest and the lowest stover yield was found in (*Bradyrhizobium*+*Azotobacter*) and in control, respectively (Nazmun *et al.*, 2009).

Table 10. Effect of bacterial fertilizers on seed yield and stover yield of mungbean.

fertilizers	Seed yield (kg ha^{-1})	Stover yield (kg ha^{-1})
Control	1.17^{c}	4.6^{cd}
Bradyrhizobium	1.45^{a}	5.2^{b}
Azotobacter	1.29^{b}	4.6^{d}
Bradyrhizobium+Azotobacter	1.37^{ab}	6.4^{a}
20 kg N ha^{-1}	1.30^{b}	5.1b^{c}
SD	0.026	0.124

Source: (Nazmun *et al.*, 2009)

3.6 Positive effects of diazotrophic bacteria (N-fixer) other than N-fixation

Diazotrophic bacteria, known for their nitrogen-fixing abilities, have been found to also promote plant growth and increase crop yield, as well as improve soil structure and microbial community (Gopalakrishnan *et al.,* 2015).Various strains of diazotrophic bacteria, such as *Rhizobia, Bradyrhizobia, Ensifer, Azotobacter, Azospirillum, Pseudomonas, Klebsiella,* and *Bacillus*, have been reported to enhance the growth and yield of crops like chickpea, bean, pea, wheat, and rice through the production of phytohormones and secondary metabolites (Gopalakrishnan *et al.*, 2017). *Rhizobia*, for example, have been found to produce indole acetic acid (IAA), siderophores, and organic acids, leading to stimulation of stem and root growth in chickpea (Gopalakrishnan *et al.*, 2018). Overall, diazotrophic bacteria play a crucial role in agriculture by not only fixing nitrogen but also promoting plant growth and crop yield, as well as acting as biocontrol agents against pathogenic fungi.

3.7 Synergistic benefits of BNF

According to Musyoka *et al.* (2020), arbuscular mycorrhizal fungi (AMF) have a positive effect on biological nitrogen fixation (BNF) through direct and/or indirect interaction with nitrogen-fixing microorganisms. AMF also play an important role in the uptake of water and nutrients from soil, which are necessary to generate energy required for BNF. Furthermore, through their hyphal networks, AMF can facilitate the colonization of legume roots by symbiotic N-fixing bacteria and transfer of nutrients and symbiotically fixed N between plants, as cited by Dellagi *et al.* (2020). On the other hand, bacteria can also be beneficial to AMF. Agnolucci *et al.* (2019) found that a commercial AMF inoculum contained many bacteria with important plant growth-promoting properties such as nitrogen fixation, inorganic phosphate solubilization, and AIA production. The synergistic effects between AM fungi and soil microbial communities can increase plant biomass and N acquisition from organic matter, as suggested by Musyoka *et al.* (2020)

CHAPTER 4

CONCLUSIONS

- Nitrogen is a crucial nutrient for crop production. It can be obtained mainly from two sources: organic nitrogen and fixed nitrogen. Biologically fixed nitrogen is one type of fixed nitrogen in which atmospheric nitrogen is converted into plant-available forms by nitrogen-fixing microorganisms. Biological nitrogen fixation (BNF) can occur through symbiotic, associative, and non-symbiotic relationships between bacteria and plants.
- The biological nitrogen fixation (BNF) process has made significant contributions to agriculture by providing a natural and sustainable solution to nitrogen deficiency in soils. The efficiency of bacterial partners in nitrogen fixation varies from 1-465 kg N ha^{-1} y^{-1} across different symbiotic interactions. Annual N fixation of different legume species through BNF vary from 14 to 465 kg N ha^{-1} yr^{-1}.The annual nitrogen fixation inputs are estimated to be 21 Tg for pulse and oilseed legumes, 12-25 Tg for pasture and fodder legumes, 5 Tg for rice, 0.5 Tg for sugar cane, 4 Tg for non-legume crop lands, and 14 Tg for extensive savannas. Different legume crops transferred nitrogen to the soil or neighboring plants in varying amounts, ranging from 0-650 kg ha^{-1}. Biofertilizers have shown the potential to save significant amounts of nitrogen fertilizer, ranging from 20 to 100 kg per hectare, and grain yield increased from 1.2 to 2.08 tons per hectare, depending on the crop and biofertilizer used. Biological nitrogen fixation significantly contributes to global nitrogen inputs in agricultural systems and can help reduce the reliance on synthetic nitrogen fertilizers, which play a vital role in promoting sustainable agriculture.

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