Biological Nitrogen Fixation in Nutrient Management

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Abstract

The use of costly chemical nitrogen fertilizers for increased food production is a global concern due to their economic and environmental effects. It is the dire need of the day to find out some alternative to the nitrogen fertilizers which is economical and environmentally safe. Biological fixation of atmospheric diatomic nitrogen into a form useable by the plant is a possible alternative to the chemical nitrogen fertilizer which is economically viable, ecologically desirable, and environmentally safe with reduced external inputs. In most of the symbiotic systems, *Rhizobium*-legume association contributes its major part in providing the N to most of the cropping system, whereas *Anabaena* and *Azolla* can be important in reduced conditions such as flooded rice. Despite the importance of nitrogen fixation, there are a number of sociocultural and scientific constraints that limit the adoption of BNF system in agriculture. The major limitation is the hindrance in the management of nutrients in the soil using the BNF as sustainable system. However, if these limitations are handled carefully on scientific basis, then BFN can be a potential source for the management of soil nutrients. Crop residues from nodulated crops also provide nutrients especially nitrogen to the subsequent crops. By adopting the BFN as cropping system, it can cut the heavy use of nitrogen fertilizer which is not only costly but also polluting the environment especially the groundwater. However, optimization of nitrogen fixation can balance the use of fertilizer and thus can help to manage the nutrients for the crops in a sustainable manner. In the present chapter, it is discussed how BNF can be crucial in managing the nutrients.

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8.1 Introduction

Over the last century, the world population has multiplied many times. In 1915, the world population was 1.8 billion which is estimated to be 7.3 billion in the first two decades of the twenty-first century (Melorose et al. 2015). This rising population demands increased food production worldwide. To fulfill this increased demand, farmers are trying to increase crop production, either by increasing the cultivated land to grow crops by using less fertilized uncultivated lands or by enhancing the crop production on already cultivated land through artificial fertilization techniques. Over cropping and extensive use of chemicals for crop fertilization and protection not only increase the input cost but also decrease the soil fertility by depleting the soil nutrients. However, in the recent past, chemical fertilizers play a key role in increasing the global food production and became the indispensable portion of the agricultural systems. The green revolution was also brought about using the practices heavily dependent upon chemical fertilizers. It is also a fact that most of the areas of the developing nations are deprived from the availability of synthetic fertilizers or they are too costly to be used by poor farmers. Even in developed countries, many social, economic, and environmental constraints compel the scientists to find out the biological or organic alternatives to chemical fertilizers especially to N fertilizer. One option is to use natural biological nitrogen fixation (BNF) systems, an alternative to nitrogen fertilizers. Under BNF phenomenon, atmospheric N is fixed to ammonia and other organic compounds that could be uptaken by the plants. It is estimated that globally 346 thousand tons of N is fixed each year through the process of BNF (Table 8.1).

Adoption of cropping system with biological nitrogen fixation not only can help to manage the soil N but will enhance the productivity, eliminate the risk of nitrate contamination of groundwater, and enhance the quality of dietary food. This chapter

Table 8.1 Estimated global N fixation by different biological and non-biological processes

Source	Amount of N fixation (000 tons)
Legume	39
Non-legume	10
Land	153
Sea	40
Others	104
Total biological	346

Source: Brady and Weil (2002)

focuses on the role of biological nitrogen fixation in crop productivity and nutrient management of soils. After introduction, the process of biological nitrogen fixation is described briefly (Sect. 8.2), while in Sect. 8.3, the role of BNF in nutrient management through BNF is elaborated. Section 8.4 discusses the different constraints in the adoption of BNF, and the last section explains the different strategies that can be used to promote BNF as a nutrient-providing system.

8.2 Biological Nitrogen Fixation

Nitrogen is one of the essential nutrients for plant growth. Chlorophyll pigment which is responsible for photosynthesis is largely composed of N. It is also a component of many other essential biomolecules such amino acids which are building blocks of proteins and also found in the synthesis of ATP and nucleic acids. It is predominately found in gaseous form (N_2) in the Earth's atmosphere. However, this form is not directly available to plant, and it must be transformed to nitrate and ammonium forms before plants use it. Thus, these forms of nitrogen are available to plant by the following ways: (1) addition of ammonia- and/or nitrate-containing fertilizers (from the Haber-Bosch process), (2) the release of inorganic form of N ($NH_4^+$and NO_3^-) during decomposition of soil organic matter, (3) fixation of atmospheric nitrogen into plant available form by natural processes like lightning, and (4) BNF (Vance 2001).

Nitrogenase is an enzyme that mediates BNF process that involves the fixation of atmospheric nitrogen (N_2) to ammonia (NH_3) (Beijerinck 1901). The prokaryotic organisms include in BNF are (a) aquatic prokaryotes such as cyanobacteria; (b) bacteria freely living in soil, such as *Azotobacter*; (c) associative bacteria such as Azospirillum; and (d) most importantly bacteria, those developing symbiotic relationship with legumes or other crops such as *Rhizobium* and *Bradyrhizobium*. These organisms are presented in Fig. 8.1.

8.2.1 Nitrogen Fixation Process

Nitrogen in gaseous form composed of two N atoms which are strongly attached to each other with a triple covalent bond. 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. Therefore, nitrogen fixing microorganisms utilize 16 moles of ATP to convert one mole of gaseous nitrogen in plant available form. The energy used in this process comes from the microbial oxidation of organic molecules present in soil. 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.

For industrial production of nitrogenous fertilizer, Haber-Bosch process is followed to reduce nitrogen which results in many consequences, including use of fossil fuels to produce energy and emission of CO_2 leading to pollution and global warming (Erisman et al. 2008).

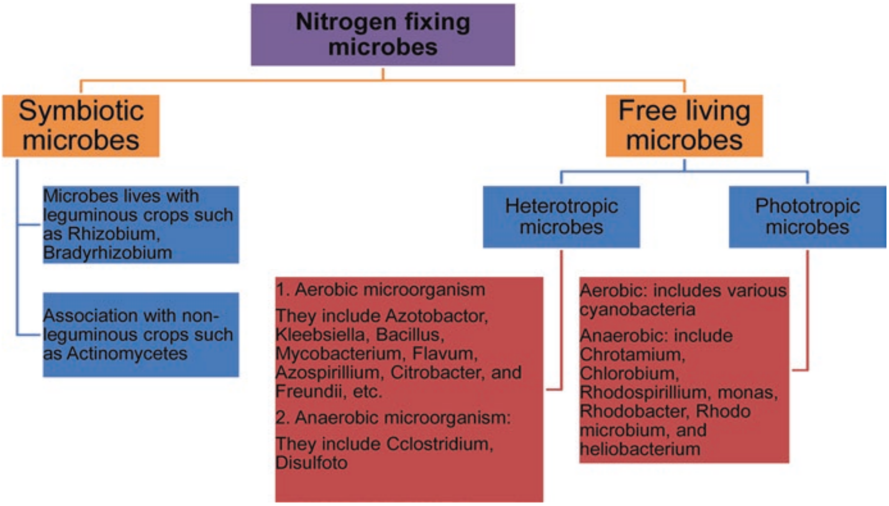

Fig. 8.1 Nitrogen fixing process of microbes in the soil

Overuse of synthetic fertilizers has some environmental constraints and upsets N cycle that resulted in surface and groundwater pollution. Excessive uses of N fertilizers cause nitrate leaching and contamination of groundwater. Surface runoff losses cause accumulations of nutrient into rivers and lack and initiate the process of eutrophication. That causes the proliferation of green algae, and when they die, microbes use water oxygen during the process of decomposition which results in depletion of O_2, thereby causing the death of aquatic animals and further aggravating the problem of water pollution.

8.2.2 Biological Nitrogen Fixation by Free-Living Microorganisms

Some species of heterotrophic bacteria such as *Azotobacter*, *Bacillus*, *Clostridium*, and *Klebsiella* freely live in the soil and fix a significant amount of nitrogen without any symbiotic or direct relationship with other organisms. 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. It has been documented that proper management in wheat rotation farming system free-living microbes has fixed up to 20 kg per hectare each year to fulfill the long-term N needs of the cropping system in Australia (30–50% of the total needs) (Vadakattu and Paterson 2006).

8.2.3 Associative Nitrogen Fixation

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 relationship, the ability of *Azospirillum* to fix N depends upon the temperature of rhizosphere which is important for the optimum growth of these microbes, availability of suitable amount of carbon source and low atmospheric oxygen pressure in rhizosphere, the competitiveness of the bacteria, and the efficiency of nitrogenase (Van Dommelen and Vanderleyden 2007).

8.2.4 Symbiotic Nitrogen Fixation

Symbiotic relationship is common in different species of plants and microorganism. These plants provide photosynthates that are utilized by these microorganisms as source of carbon and energy. In return nitrogen fixed by these microbes is used by plants for their growth. For example, a cyanobacterium *Anabaena azollae* colonizes in the cavities formed at the base of *Azolla* fronds and forms a symbiotic relationship which results in a significant amount of nitrogen fixation in specialized cells called heterocysts (Adams 2000; Rai et al. 2000).

In South Asia, rice paddies are usually covered with *Azolla* "blooms" for the purpose of N fixation, and it results in fixation of up to 600 kg N ha^{-1} per growing season (El-Refai et al. 2005), and 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. Though these aforementioned interactions between microbes and plants play an important role in fixing atmospheric N, however, among all of them, the most important symbiosis relationship is established between *Rhizobium* and *Bradyrhizobium* bacteria and legumes plants, and they contribute more in BNF compared with other associations. Some *Rhizobium* species living in association with legumes are shown in Table 8.2. Alfalfa, clover, beans, lupines, cowpeas, soybean, peanut, and vetches are the important legumes, and these are grown throughout the world of agriculture. Among these soybean contributes to 68% of the total legumes produced in the world, and 50% of the total cropping area of the word is devoted to the legume production (Vance 2001).

Table 8.2 Specific rhizobia species live in association with legumes

Crop	*Rhizobium* species	References
Alfalfa	*Rhizobium meliloti*	Wall and Favelukes (1991)
Chickpea (*Cicer arietinum* L)	*Mesorhizobium ciceri*	Martínez-Abarca et al. (2013)
Lentil (*Lens culinaris* M)	*Leguminosarum* biovar *viciae*	Rashid et al. (2012)
Soybean (*Glycine max* L.)	*Bradyrhizobium elkanii*	
	Bradyrhizobium japonicum	Mishra et al. (2009)
	Sinorhizobium fredii	Krishnan (2002)
Drybean (*Phaseolus vulgaris* L.)	*Rhizobium etli*	Martínez-Romero et al. (1998)
	Rhizobium leguminosarum	Broughton et al. (2003)
	Rhizobium tropici	Karaca and Uyanöz (2012)
Pea (*Pisum sativum* L.)	*Leguminosarum* biovar *viciae*	Novak et al. (2002)
Mung bean (*Vigna radiata* L.)	*Rhizobium leguminosarum*	Chudasama and Mahatma (2016)
Cowpea (*Vigna unguiculata* L.)	*Bradyrhizobium japonicum*	Nyoki and Ndakidemi (2013)

8.2.5 Nodulation in Legumes

Rhizobium colonization in the root system of host plants causes the development of nodules which provide habitat for these bacteria where they start to fix atmospheric nitrogen. During nodule formation, uptake of this nitrogen by plants increases the photosynthetic activity of plant that results in nitrogen-rich seeds. On the other hand, if leguminous plants failed to develop nodulations, they suffered from N deficiency and resulted in stunted growth and low seed production.

In legumes like alfalfa, clover, and soybeans, when the process of nodulation begins, flavonoids are released from the host plants that attract the *Rhizobium* toward those plants, and these microbes attached with the epidermal cell of root hairs. In the first step, bacteria attach to root hair using Ca^{2+} binding protein called rhicadhesin. Second, strong association occurs due to cellulose fibrils and/or lectins and fimbriae produced by the bacteria and host plant, respectively.

Rhizobium releases a certain type of chemicals, called NOD factors that stimulate the plant to produce curl root hair called as shepherd's crook. Afterward, penetration of *Rhizobium* into root hairs forms a tubular structure which is called an infection thread. Rhizobia stimulate the cortical cell divisions that cause nodule formation, and as it happens, plant-derived membrane surrounds the *Rhizobium* and bacteria are released inside plant cells forming the nodule. These bacteria inside the nodules loss their cell wall and exist in large masses with irregular-shaped branching cells called bacteroids. After it, establishment of relationship entirely depends on the host plant for food and energy, and in return these bacteroids fix atmospheric nitrogen for the host plant.

This association is entirely host specific, and a particular species of *Rhizobium* develops interaction with specific plant genera. For example, *Rhizobium meliloti* and *Rhizobium leguminosarum* biovar *trifolii* will only develop nodulation in alfalfa clover (*Trifolium*).

The abovementioned NOD factors are basically lipochition oligosaccharides, and variations in their structures are responsible for the host specificity. Besides these lipochition oligosaccharide structures, production of leghemoglobin also plays an important role for this relationship between the rhizobia and the host legume (Appleby 1984). It is produced in fully functioning nodules and has similar function just like the hemoglobin. This heme protein is formed by combination of apoprotein and heme (porphyrin ring bound to an iron atom) produced by the legume and bacterium, respectively. It transports the oxygen to the *Rhizobium* in the nodules. As it has been discussed that nitrogenous enzyme activity is sensitive to oxygen and is restricted in excess of oxygen, thus, leghemoglobin carries oxygen to *Rhizobium* for cellular respiration but is regulated to avoid the inactivation of nitrogenase.

8.3 The Role of N2-Fixing Symbiotic Association in Agricultural Nutrient Management

Role of BNF in contributing N in terrestrial ecosystem has been estimated to range from 139 to 170 × 10^6 tons of nitrogen per year. However, this amount of N fixed by BNF is very small compared with total N reserves, (105,000 × 10^6tons N), while this amount is still greater than the N added by the synthetic fertilizer, i.e., 65 × 10^6 tons of nitrogen per year (Paul 1988). Considerable amount of this fixed N comes from various symbiotic associations such as legumes/*Rhizobium* spp., actinorhizal associations of plants, and microorganisms. However, free-living fixers (diazotrophs) contribute little to the aforementioned amount of fixed N into ecosystem. BNF associations in different cropping systems are presented in Fig. 8.2.

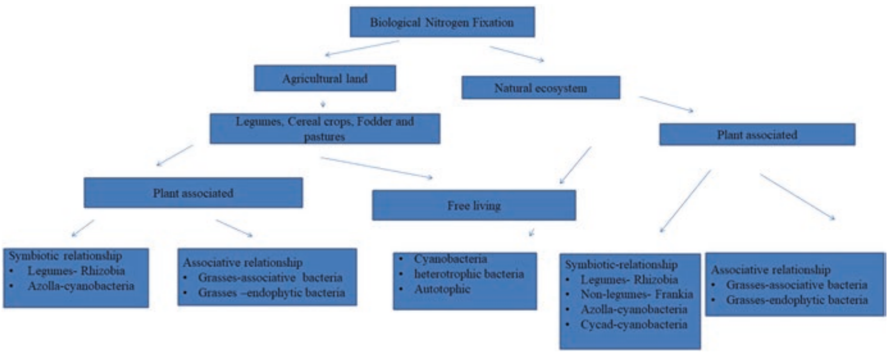

Fig. 8.2 Biological nitrogen fixation under different systems

The relative contribution of N fixation by symbiotic relationships and free-living organism contributes to 70% of the global N fixation (Paul 1988). However, in cropping system the share of symbiotic nitrogen fixation has been considered not less than 80% of the global fixed nitrogen (Burns and Hardy 1975; Lobell et al. 2009). In ancient time legumes and their subsequent incorporation in soil had played an important role in traditional farming systems. However, introduction of synthetic fertilizers led to the widescale abdication of legume pasture mostly used for green manuring in Europe and North America. Similarly, recently in China and Japan, use of milk vetch (*Astragalus sinicus*) and *Azolla* as fertilizers has declined because of high labor charges and easy availability of synthetic fertilizers.

In developed countries, adaptations of organic farming and reduction in excessive use of nitrogenous fertilizers have resulted in the increased use of BNF systems. In humid and subhumid upland soil due to higher rainfalls, excessive leaching causes the depletion of both total and plant available nitrogen. Moreover, due to improper land management, deforestation and over grazing may result in rapid losses of nitrogen. This trend is widespread due to intensive cultivation for increasing crop production to feed the increasing human beings. Yield of major crops is often limited by N supply, and considering the economic value due to higher prices, N fertilizer is being used in limited extent (Myers and Wood 1987). However, introduction of BNF system into farming systems assures that it will fulfill all or at least its own requirements of N, and additionally improvement in rhizosphere will facilitate the growth of companion crops and also on subsequent crops. However, capacity of BNF system for nitrogen fixation depends upon environmental, nutritional, and biological factors and disturbances in any of these factors limit nitrogen fixation, and it cannot be assumed that BNF contribute largely in global N cycle (Chalk 1991).

8.3.1 Role of Legumes

Legumes are important source of vegetable protein and are widely used for food, shade, fodder, timber, fuel, and green manuring. They are grown in rotations with other crops and are incorporated into soil as green manure to improve various physiochemical properties of soil. These are grown as cover crops and intercrops in tree crops such as coffee, cocoa, rubber, tea, and oil palm. Various tree legumes are grown in agroforestry systems and used for grazing purpose to increase the sustainability and productivity of farming systems.

8.3.1.1 Food Legumes

About 90% of human dietary protein comes from plants, while 17–34% of protein is contributed by legume seeds. The distribution of crop legumes is generally determined by their adaptation to particular climates and environments (Ludwig and Asseng 2006; Hatfield et al. 2011). The variations in consuming legumes as source of food depend upon the introduction of legumes into cropping systems and also their uses as fodder crops (Byth et al. 1986). Different legume species are widely grown in lowland and upland cropping systems. Some of them are grown for oil seed purpose such as groundnut and soybean, while some are important pulses such as chickpea, cowpea, gram, and bean.

The amounts of N fixation by legumes depend upon various factors like microbial inoculation, water supply, fertilizer application, crop rotation, and soil fertility. In a situation where legumes have access to adequate amount of N in soil, it would result in low level of N fixation. It is evident from previous reported studies the proportion of nitrogen fixation is reduced with increasing level of soil available N, and this is the most important factor in reducing the nitrogen fixation by microbes.

Legume crops are not only growing as single crop, but in many parts of the world, they are grown in intercropping systems with other crops, and this results in intensification of crops because it more effectively exploits the environment. The selections of crops for intercropping depend on the length of season, but generally crops with early and late maturity are selected in order to better utilize resources in mixed cropping pattern (Ofori and Stern 1987). The amount of N fixation in legume systems depends upon the morphology of legumes, number of legume plants and management practices, and the competition of component crops in intercropping. Generally, legumes with indeterminate growth and climbing habit have the most successful N fixation.

8.3.1.2 Green Manures

Leguminous tree and shrubs are widely used to reclaim problematic lands, as mulching material slowing down the process of soil erosion, as source of fuel wood, and as green manure. The potential for the integrated use of these species is well illustrated in alley-farming systems. This cropping system involves the growing of agricultural crops in rows between these leguminous trees and shrubs which are maintained as hedge rows and the leaves and twigs of these plants which are incorporated into soil as green manure. In the humid regions, these hedge rows are established along the contours on slope lands to reduce water erosion, and these hedgerows serve as natural terraces (Mutegi et al. 2008). In many countries of tropics, rubber and oil palm are widely cultivated, and during the initial period of the year, the row spaces between these tree species which represent 70–80% of the land are widely occupied by weeds which cause diseases and pests attack. In order to control this situation, perennial leguminous cover crops are grown which protect from weed infestations and soil erosion, and incorporation of these cover crops increases fertility and quality of soil. The use of legumes as cover crop is not only restricted with the long-term alley system, they are widely grown in rotation with short-term agronomic crops such as rice. For this purpose both food and perennial legume species are grown. Species of *Aeschynomene* and *Neptunia* and *Sesbania rostrata* could be successfully grown in rice system because they can grow well and produced nodules in flooding situation. Organic matter inputs into the soil are of great importance in terms of increasing soil fertility and retaining nutrient and moisture, thereby increasing crop productivity in degraded soils (Tschakert et al. 2004). Incorporation of leguminous crops into soil increases the organic matter of soil and improves the supply of nitrogen on decompositions of the organic residues. Integration of legumes in cropping system has the potential to improve the growth and yield of crops. Advantage of legumes over grown non-leguminous crop is that they show exceptional ability to uptake and utilize inaccessible soil phosphorus and potassium, thus maximizing the availability of these nutrients for subsequent crops. Use of legumes as green manure has great advantage when the time of decomposition of incorporated residues matches the nutrient requirement of subsequent crop, thereby increasing the supply of essential nutrients for crop growth.

8.3.1.3 Contribution of Legume Residues

The amount of nitrogen utilized by the legume crop (Nl) emanates either from the N_2 fixation (Nf) or is uptaken from the soil solution. The total amount of nitrogen in food legumes is divided into two parts, i.e., seed nitrogen (Nls) and vegetative nitrogen. The vegetative portion includes stems, leaves, and nodulated roots. All these are generally rendered as crop residues.

The net nitrogen fixation that is contributed to N balance of a soil subsequent to legume crop can be calculated as:

$$\text{Net nitrogen balance} = \text{Nf} - \text{NIs}$$

During the growth and development of legume crop, leaf fall and roots each can contain up to 40 kg of nitrogen per hectare. The food crops usually require more nitrogen than the N fixed by the *Rhizobium*. In many cases, the level of fixed nitrogen in the field might be too high, but these levels are always lower than the nitrogen removed with the harvested seed. Obviously, if the food legumes contribute substantial amount of N to the soil, then net nitrogen balance must be high after harvesting. The crops with high seed production need high amount of nitrogen and thus cause net loss to soil nitrogen, and this loss would be high if the crop residues are removed and used as animal fodder. However, the persistent use of legume crops increases the fertility of the soil and thus improves the nutrient status of the soil. If the cereal crops were grown after monocropping legume system, then increase in yield is about 30–35% when compared to cereal-cereal cropping system. When addition of nitrogen from the legume is measured as nitrogen fertilizer, then as much as 70 kg N per hectare was needed in cereal-cereal cropping system to gain the similar yield increase. Different factors can affect the increase in yield that might be cropping system, season of the crop, and soil type used for the cropping.

The surplus yield might result because of:

1. Legumes break the insect cycle that is detrimental in yield reduction. Further, many crops and their residues also have allelopathic effects.
2. Growing of legumes improves the soil structure, thus improving the nutrient availability due to the incorporation of residues.
3. Crop residues enhance the organic matter that ultimately increases the water holding capacity and buffering capacity of the soil.
4. Soil microbial activity enhanced many times when nitrogen-rich residues are incorporated in the soil following the legume crop.

5. When compared with the non-legume crops, legume crop leaves more nitrates in the soil after harvesting.

The net high nitrate level following legume crop might be due to the lower uptake by the legume crop or enhanced mineralization rate under a legume crop. When in the case of legume much of the N is removed with harvested seed and no or very little net N is gained by the soil, then how much the loss might be expected from the non-legume crop when seeds are also harvested.

However, to measure the equivalence of fixed N to N fertilizer, much care is required because fertilizer use efficiencies can vary, and amount of added N can be volatized. This might show the over efficiency of legume over N fertilizers. When crop is harvested and seed is removed, then ratio of nitrogen in different organs of plants varies. These organs also vary in their potential to release the N to the soil, and this would affect the yield of subsequent crop. This release of nitrogen from legume residues or its transformation to plant usable form is affected by physical and chemical properties of the soil, temperature of the area, and method of crop residue management, and in the case of rice crop, flooded condition may also be important in N transformation. Many other factors have direct influence in N release including the lignin and polyphenol content. However, the major factors are the water status of soil, C/N ratio of legume residues and its nitrogen concentration that determine the rate of mineralization, and thus availability of released nitrogen to following crop. Leaves of most legume crops contain high C/N ratio that enhances the mineralization process and so increases the availability of N to subsequent crop.

To enhance the nitrogen fixation at its maximum that will give the maximum yield of nitrogen, the legume crop must be grown in a favorable growing season. It may be wet season with enough irrigation water. However, in Pakistan, the winter wet season or area under irrigation is mostly reserved for cereal crops, and legume crops were grown in dry areas with no irrigation that leads to lower legume yield. Other factor of legume low yield is the application of lower rates of fertilizers by the farmers to the legume field (Craswell et al. 1987). As a result, yield of legume crop is critically low due to nutrient deficiencies.

When a green legume is grown and entire crop is incorporated to the soil, then the amount of nitrogen incorporated and concentration of nitrogen in the legume are higher than the crop where seed is harvested from the legume crop. In this scenario the rate of decomposition might also be high due to lower C/N ratio. Experiments on decomposition of residues from the alley crop reveal that half of the N from incorporated legume is released to the soil within 1–9 weeks. This release of nitrogen is affected by the environmental condition and amount of nitrogen already present in the soil. It is obvious that incorporation of green legumes can provide enough amount of nitrogen to the soil that significantly increases the yield of following crops.

Soil nutrient of flood areas during the transition period from dry to wet season can be managed by the flood-tolerant species of green manure legumes where they tolerate the short-term waterlogging. Due to the reluctance of farmers in the use of inorganic fertilizer because of uncertainty of the climatic conditions, alternative crops cannot be grown.

Legume crops are also grown as a source of green manure in conditions other than exhaustive agriculture. Local legume species are cropped to improve the fertility of soil in shifting cultivation system. Legume tree can also be used to restore eroded or degraded land. It is recorded that litter and leaf fall from the *Leucaena* in the dry tropics can increase more than ten tons of organic matter to the soil per hectare annually. This organic matter contributes up to 250 kg of N to the soil (Sandhu et al. 1990). However, decomposition of this organic matter under hard dry condition is tough and slower than the continuous agriculture system, and rates of nitrogen release depend upon the C/N ratio of organic matter from leaf and litter. Therefore, it can be predicted that out rate for N release for fruit leaf and litter with lower C/N ratio (17–18) would be higher for woody parts and twigs with higher C/N ratio (>33). Even so, it is expected that up to 80% of the N in the leaf and litters is released to the soil annually that shows a major portion to the N cycling in infertile, eroded, and degraded soil (Sandhu et al. 1990).

8.3.2 Nutrient Management and Non-legumes

8.3.2.1 Actinorhizal N Fixation

Other than nitrogen fixing legumes and those species that form nodules by *Rhizobium* sp., approximately more than 200 plant species comprising of 8 families and nearly 17 genera in arid and semiarid areas fix atmospheric nitrogen by forming nodules by actinomycetes. The actinorhizal plants are much fewer than N_2-fixing legume plants. However, they have potential to regenerate the poor soils and preserve the land surfaces from erosion. Among these, Casuarinas have too much potential for agriculture. However, very scanty information is available regarding *Frankia* inoculation.

8.3.2.2 Azolla

Azolla is an aquatic fern which is found everywhere in the world and has N_2-fixing heterocyst cell. *Azolla* can grow in N limiting conditions where other water plants cannot grow due to their N_2-fixing ability. In tropic zones, it forms dense layers on the surfaces of drainages, ditches, marshes, ponds, and rice paddies. *Azolla* is also being used to suppress weeds and as green manure in paddy fields. It is also being used as feed for dairy animals and cattle, fish, or farm ducks. Under favorable conditions, it can regrow with the same mass in 2–3 days. Its dry matter content comprises of 4–6% of nitrogen, and its one reap can uptake 30–100 kg of N per hectare. Therefore, it can contribute annually 450–840 kg N per hectare under optimum conditions. This too much nitrogen percentage shows a high increase in N of rice-based agricultural soils. Many factors can affect the potential on *Azolla* that include very high or very low temperature, high light intensity, and deficiency of essential nutrients especially phosphorus. Rate of decomposition of *Azolla* depends upon the method and time of addition and its quantity incorporated. However, under ideal conditions, its decomposition rate is rapid. Its incorporation and release of nitrogen is nearly equal to inorganic fertilizer efficiencies especially in flooded paddy field.

8.3.2.3 Nutrient Management Through Nitrogen Fixing Grasses and Cereals

There are many genera of associated nitrogen fixers (diazotrophs) that are found in the roots of many cereals and grasses. Acetylene-reduction assay has been used to demonstrate the N_2 fixation in cereals such as sorghum and maize. Now it has been confirmed from N15 techniques that many plants from the grass family such as wetland rice, sugarcane, and grasses under some conditions get nitrogen from associated N_2-fixing bacteria. Inoculation of these bacteria can help to manage nitrogen deficiency in the soil.

8.3.2.4 Nutrient Management Through Nitrogen Fixing Forage Grasses

Many studies provide the data regarding the N fixation in C_4 forage grasses especially Kallar grass. Kallar grass is widely distributed in Pakistan. It is observed that N_2 fixation from these grasses can contribute up to 40 Kg of N per hectare per annum in the production of pasture (Chalk 1991).

8.3.2.4.1 Sugarcane

Many experiments have shown associative nitrogen fixation with sugarcane crop. As sugarcane crop is a most exhaustive crop, therefore associative N_2 fixation can play a key role in managing the nutrient needs of the crop. Each crop harvest of sugarcane can remove up to 200 kg of nitrogen per hectare. With this so much N removal if compensated from associative nitrogen fixation, then a good yield can be obtained even in continuous cropping (Thompson 2004). Further, the potential of sugarcane for associative N_2 fixation can be improved through breeding techniques.

8.3.2.4.2 Rice

Acetylene-reduction assays on different field experiments have confirmed measurable quantities of associative N_2 fixation in rice plants. But extensive importance of associative nitrogen fixation with paddy crop is tough to measure because many of the investigations did not confirm the N_2 fixation activity. However, some experiments show varietal differences in the potential of rice to establish potential associations regarding N_2 fixation. In tropical and subtropical agriculture, it is tough to evaluate accurately the role of N_2-fixing non-legumes because very few studies are conducted to measure the ability of N_2 fixation using reliable methodology. However, it can be concluded that the potential of non-symbiotic plants for biological nitrogen fixation is too low as compared to symbiotic actinorhizal plants and *Azolla*.

8.4 Constraints Associated with Utilization of BNF

Although much work has been done on N_2 fixation, still there are many unknowns in the subject which should be explored for improving fixation mechanism in the future, and some of these unknowns are restricting the application of N-fixation technologies. Much of the explored work is still not being practiced particularly in developing countries. Implementation of BNF technologies in field crops is difficult due to practical, socioeconomic, and human-resource barriers. These complexes can be resolved through scientific research, education and awareness, training, and growth of private enterprise. To attain full benefit of BNF system, constraints should be properly addressed and resolved so that farmers can easily adopt the technology. These constraints can be named as biological, environmental, methodological, and sociocultural constraints.

8.4.1 Environmental Constraints

The modern biotechnology improves the life of humans by introducing genetic engineering techniques, i.e., one can change and modify the genetic composition of organisms. Techniques are available, but due to lack of knowledge how to match the genetic buildup of the living systems to the environment. For instance, how N-fixing organisms will respond in different soil environment. For successful implementation of N-fixation techniques at farming levels, complete understanding of N-fixing systems is prerequisite. Numerous environmental factors affecting the activity of legume-rhizobia symbiosis (Provorov and Tikhonovich 2003) and actinorhizal (*Frankia*) symbiosis (Torrey 1978) have been reported. Soil constraints to symbiotic performance have been reported by a number of researchers. The host-microbe symbiotic interaction is mainly affected by soil pH, aluminum and manganese toxicity at low soil pH (Panhwar et al. 2015) and calcium deficiency, phosphorus (Kabir et al. 2013), salinity (Soussi et al. 1998), and flooding (Choudhury and Kennedy 2004). Nitrogen release from organic sources is also a limiting factor for symbiotic interaction within a plant community. Competition between native microbes and N-fixing microbe is one of the major barriers to successful implementation and efficiency of N_2-fixing systems especially for legume inoculants. Soil temperature or P and K enrichment is among such environmental factors which can influence the competition patterns. Moreover, population size of indigenous rhizobia and crop response to applied rhizobia affect the establishment and activity of applied inoculants. For example, in tropical environment, inoculation-induced increase in yield and nodulation of cowpea is dependent on population and competitive nature of native *Bradyrhizobium* sp. (Danso and Owiredu 1988). However, very less information is available to support the misconception-based idea that leguminous crops grown in tropical regions do not respond to rhizobial inoculation. Whereas response of soybean to *Bradyrhizobium* rhizobia inoculation was significant, when applied rhizobia become naturalized, no response was observed in the following crops in rotation (Salvagiotti et al. 2008). For this purpose, ecological models have been proposed based on population size of indigenous rhizobia and soil nitrogen status to predict the likelihood and response of legume crops to applied inoculants of rhizobia.

8.4.2 Biological Constraints

The major biological constraints to BNF practices' implementation include microbial genetic potential and their interaction with environmental factors. In symbiotic interactions, biological constraints affect the association of both partners, e.g., quantity of fixed N is directly or indirectly affected by disease and predation, and consequently amount of N becomes available to other parts of the cropping system. Quantity of fixed N and host plant growth potential are directly related to each other especially in leguminous crops. For example, when crop growth is restricted by disease or by other constraints, N fixation will be reduced accordingly.

8.4.3 Methodological Constraints

Difficulty in identification of a specific nitrogen fixing bacteria for different BNF systems to make this strategy successful is one of the main constraints for failure of this technology. However, due to development of serological methods, it is relatively easier to identify and monitor the specific rhizobia for different legumes which involve symbiosis. With the genetic analysis with DNA probe (Holben et al. 1988), this system will be simplified in the near future. Inoculation technology is fully developed for legume inoculant, but for the inoculants of actinorhizal and other BNF systems, there are still many issues. Nevertheless, *Frankia* can be grown successfully in pure culture, but large-scale production is always a problem. Even for legume, large-scale production of inocula still has many issues such as selection and availability of appropriate carrier material, shelf life of packed materials, and preservation of microbial germplasm in developing countries due to load-shedding problems. Another important methodological constraint is the accurate measurement of BNF due to the lack of reliable techniques under field conditions.

8.4.4 Production-Level Constraints

There are various field level production constraints related to the introduction of BNF systems into the farming system; thus there is no guarantee that development of BNF system will successfully prevail in a system. Cereals are dominantly grown in cropping system all over the world, and introduction of legumes into this system is a task of challenging complexity regardless of the nitrogen fixing abilities of legumes. In humid tropic regions, higher precipitation and humidity in rainy season and uncontrollable proliferation of insects, pest, and diseases are the major factors that constrained the production of leguminous crops at large scale (Ratnadass et al.

2012). Similarly due to hydrophilic characteristic of grain legumes, seed quality deteriorates very rapidly in such conditions (Shanmugasundaram 1989). Therefore, precautionary measures are needed for grain legumes compared to the cereal crops. Besides leguminous plants are indeterminate types, and they have low grain to plant biomass ratio in wet season, thus resulting in low seed yield in humid climate. Therefore, in order to get good yield they must be grown in the in humid uplands of all tropical continents severe acidity and P in ultisols and oxisols soils results in lower production of legumes unless nutrients are added and soil reclamation strategies are adopted (Sanchez and Uehara 1980). Moreover, low BNF in these soils is due to excessive available N. In humid tropics leguminous green manures have played a critical important role in nitrogen fixation in rice production system, but the contribution of legumes in BNF declined with the introduction of synthetic fertilizer in cropping systems. Although *Azolla* is one of the potential candidates responsible for BNF in rice production system, however, due to many farm-level constraints, such difficulty in maintaining availability of inocula of *Azolla* throughout the year and its susceptibility to insects and diseases limit the adaptation of this technology by farmers. Legume crops flourish well and play an important role in BNF in the semiarid tropics compared to the humid tropics, and they are mostly grown with cereals. For example, in India, pigeon peas are intercropped with sorghum, while in West Africa cowpeas are grown commonly with sorghum or maize.

8.4.5 Sociocultural Constraints

It is important to note that BNF technologies not only have scientific constraints but are also influenced by cultural, educational, economic, and political values. Therefore, efforts should be made for training, education, and technical assistance in order to make BNF system successful. Socioeconomical restrictions should be evaluated and provide information publicly to remove or reduce these constraints. Many farmers in developing countries have no knowledge about the nodulations in legumes and their potential benefits in nutrient managements and soil fertility. Farmers have been growing leguminous crops since ancient time just considering them as valuable component of cropping system rather than their nodule characteristics and importance in BNF. Normal extension mechanisms are unable to transfer difficult BNF technology effectively at the farm level because of insufficient illustrative and explanatory materials and other aids. Furthermore, in developing countries, only few of the senior decision-makers who are responsible for determining the agricultural policies have knowledge of opportunities for legume-based BNF technology in the agriculture sector of their countries. Among those only a smaller number of peoples recognize that adaptation of such technology will be beneficial for their prevailing farming systems. Hence, special educational material should be developed for such group of peoples to bring their attentions to adapt this technology. The lack of technical persons who can disseminate and transfer BNF technology to farmers at field level is also a big constraint. A subsidy on nitrogenous fertilizers is also a factor for avoidance of BNF by farmers. Similarly, most of the subsidy programs for crop productions are limited to cereal crop productions around the world which create a wide gap of interests for legume productions. For example, in America most of the farming systems are cereal based. However, in some parts of the world like in Australia, there is no discrimination for subsidies among crops exploitation of cereal-legume systems is a dominant feature of agriculture.

8.5 Strategies to Enhance N_2 Fixation

The increment in quantity of N_2 fixation should be achieved by:

(a) Improving leguminous crop yield as affected by cultural, fertility, supervisory, and environmental obstacles.
(b) Minimizing quantity of nitrate in rhizosphere through tillage management, time of sowing, and grazing management.
(c) Selection and inoculation of rhizobial strains to attain optimum population and breeding approach for selective nodulation.
(d) Breeding techniques to minimize the inhibitory effects of nitrate on nodule formation or by improving nodulation through introduction of required inoculants in rhizosphere and properly manage the soil under different environments, two important practices of which are discussed below.

8.5.1 Tillage

Land cultivation enhances the decomposition of soil organic matter and commonly increases nitrate N in the soil profile and minimizes the process of denitrification, immobilization, and nitrate leaching. Under no till, cereal crops need additional N to overcome deficiency of soil nitrate N, whereas N_2 fixation by legume crops enhanced at lower nitrate in soil. Additionally, soil structure is also improved under no till system favoring soil moisture and temperature for plant growth. Soybean grown in subtropical environment showed higher nodule formation and N fixation under no till as compared to disturbed soil. Although N balance is found positive for both no till and tillage systems, but sudden increase is noted under no till systems.

8.5.2 Removal of Plant or Animal Products

Grazing management and cropping pattern influence the availability of nitrate N to legumes. Growing leguminous crops in rotation to cereal crop can fix higher amounts of N as compared to fallow land cultivation (Van Kessel and Hartley 2000). For example, the N harvested by soybean seeds was significantly enhanced from -44 kg N ha^{-1} after fallow to +39 kg N ha^{-1} in previously cropped land (Bergersen et al. 1985). Thus along with other factors, crop rotation is also important for N_2 fixation. On the other hand, intercropping maize and rice bean has resulted in obtaining higher P levels as attained during single cropping. This is due to competition between legume and maize crop for indigenous soil N. The intercropping resulted in higher total N harvested by intercropping with legumes as compared to combined weighted N yield of single crops of maize and rice (Chu et al. 2004; Yilmaz et al. 2008). Similar strategies might be followed for forage systems. To maintain lower levels of soil nitrate N and to improve N_2 fixation, legume crops can be introduced in competition with vigorous grasses or through grazing to remove leguminous N or sequential cut and carry practices. On the basis of compatibility to rhizobial strains, there are three main groups of legumes (Peoples et al. 1989). First group includes leguminous crops which can perform effective symbiosis with variety of strains. These species are enriched in tropical soils, and members of this group are nodulated by cowpea-like rhizobial. Some host-strain association specificity in this group is also observed. Second group members can nodulate with several rhizobial species, but some strains result in effective N_2 fixation. Third group legumes are highly specific to strains especially when grown to new areas, and their association is usually successful. Factors/reasons restricting effective host-strain association include (1) lack of similar legume crop in previous cropping pattern, (2) same crop in rotation resulting in poor nodulation, (3) legume-non-legume rotation, (4) during land amelioration, and (5) unsuitable environment for *Rhizobium* survival (e.g., variation in soil pH, long-term flooding, and drying before planting). The effective and successful host-strain association in field is dependent on procedure followed, technicality of operator and presence of toxic agrochemicals, and variation by soil factors (Brockwell et al. 1988). These strains are practically used in Australia and the USA. They establish legume-based pasture and cropping systems. Two countries in Latin America use inoculants to any extent; in Brazil, the main producer of seed legumes, common beans, did not use inoculants but rather use N fertilizer (Freire 1982).

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