Nitrogen and Legumes: A Meta-analysis

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Abstract

The current progress in agricultural production does not really cater to the demand of the burgeoning human population. Consequently, this puts global food and nutritional security at a great risk. This challenge calls for concerted efforts of all stakeholders to produce required quantity and quality of assured foods for ensuring food security. In the past, the principal driving force was to increase the yield potential of food crops and to maximize productivity. Today, the drive for productivity is increasingly combined with a desire for sustainability. For farming systems to remain productive and to be sustainable in the long term, it will be necessary to replenish the reserves of nutrients which are removed or lost from the soil. The nitrogen (N) inputs derived from atmospheric N via biological N fixation (BNF). Therefore, current farming systems need sustainable intensification through the inclusion of legume crops. This facilitates the precise use of nitrogen (N) by reducing their losses into the environment and ensures self-sufficiency in protein. The relevance of legumes in this context is enhanced as these crops offer numerous amenities that remain in line with prevalent sustainability principles. Legume crops provide protein-rich food, oil and fibre while supplying the 195 Tg N year^{-1} (also includes actinorhizal species) to the agroecosystem through the process of biological nitrogen fixation (BNF). Besides serving as the fundamental global source of good-quality food and feed, legume crops contribute to 15% of the N in an intercropped cereal and mitigate the emission of greenhouse gases (GHGs) by reducing the application demand of synthetic nitrogenous fertilizers. Legume cultivation releases up to seven times less GHGs per unit area than non-legume crops. Legumes allow the sequestration of carbon (1.42 Mg C ha^{-1} year^{-1}) in soils and induce the conservation of fossil energy inputs in the system. The other benefits of legume crops include their significant positive impacts on biodiversity and soil health. Rotating legume crops with non-legume crops has the dual advantage of cultivating the legumes with slight or no extra N fertilizer. Care should be taken to ensure the availability of adequate N for the succeeding non-legume crops. The legume crops respond very well to conservation of agricultural practices. Overall, these characteristics are crucial to agriculture both in developing and developed countries apart from the conventional farming systems. Legumes in rotation promote exploration of nutrients by crops from different soil layers. They also help in reducing pressure on soil created by monocropping. Thus, crop rotation acts like a biological pump to recycle the nutrients. Hence, inclusion of legumes in the cropping system is inevitable to advance soil sustainability and food and nutritional security without compromising on the long-term soil fertility potential.

Keywords
Legumes' effects on succeeding crops · Legumes mitigate GHGs · Nitrate leaching · N fixation · Residual N in soil

Abbreviations

ADP	Adenosine diphosphate
ATP	Adenosine-5′-triphosphate
BNF	Biological nitrogen fixation
GHGs	Greenhouse gases
GWP	Global warming potential
N	Nitrogen
NUE	Nitrogen use efficiency
SOC	Soil organic carbon
SPA	Soil-plant-atmosphere

9.1 Introduction

Legumes belong to the Leguminosae or Fabaceae family and rank third in global production after cereal and oilseed. These hold immense agricultural significance worldwide contributing an area ~14% of total land under cultivation (Sulieman and Tran 2015). They largely contribute to global food and nutritional security, besides soil health. Moreover, they generate income for millions of smallholder farmers at the regional and global level, and their role in environmental safety measures is well documented (Peoples et al. 2009; Yadav et al. 2015; Guardia et al. 2016). Legumes fix the atmospheric N through symbiotic associations. They are also important sources for proteins, minerals and micronutrients suitable for human and animal consumption besides being sources of fibre and oils (Voisin et al. 2014; Stagnari et al. 2017). Half of the entire N used in agriculture production system is delivered by the legume crops (Graham and Vance 2003). So, the biologically fixed N remains adequate to cater to the requirements of the plant. This is apart from leaving some N (as residual N) in the soil for the succeeding non-legume crops (Mayer et al. 2003; Peoples et al. 2009; Dhakal et al. 2016). This underlines the great potential of legume crops for use in soil restoration and stabilization. The scope of legume crops to agricultural systems could be further enhanced manifold. This could be done by attending to soil constraints such as soil acidity, salinity and drought and through undertaking modern plant breeding programmes (Graham and Vance 2003).

The use of legume crops as green manure in non-legume-based cropping system was prevalent since agriculture began to be developed. However, a drastic decline in the practice was seen with the increasing availability of industrially produced fertilizers (especially the N fertilizer). Green manure adds N to the soil and improves quality by increasing the soil organic carbon (SOC), macro- and micronutrients and humus content (Graham and Vance 2003; Jensen et al. 2012; Hajduk et al. 2015). Legumes enrich the soil with N and thus facilitate a better environment to subsequent crops for better growth and productivity (Meena et al. 2015a). Legumes can fix substantial amounts of free atmospheric N, which allows them to be grown in N-stored soils without using synthetic N fertilizers. The BNF by legumes and actinorhizal species is estimated for about 195 Tg year^{-1} (Vitousek et al. 2013). According to Frame (2005), with an estimated capacity to fix 72–350 kg N ha^{-1} year^{-1}, the legumes facilitate the transformation of environmental N into various nitrogenous compounds including amino acids and proteins. These amino acids and proteins, which are being used by the growing plants, also contribute to improved soil fertility (Nulik et al. 2013). Peoples et al. (2009) reported that nearly 30–40 kg of N is fixed on a whole plant basis for each ton of dry matter produced by legume crops. According to Mayer et al. (2003), total N uptake by the following non-legume crops is strongly influenced by the preceding legume crop. It relies upon the residual N input and the N fixation capacity of the different legume crops. Up to 12% of the residual N is recovered from the succeeding crops at maturity. Berg (1997) highlighted that wheat (*Triticum aestivum*) yield of 3070 kg ha^{-1} year^{-1} over 5 years following alfalfa, 2580 kg ha^{-1} year^{-1} following milk vetch and 950 kg ha^{-1} year^{-1} following grass with N uptake was attributed to the residual effect from legumes averaged 34 kg N ha^{-1} year^{-1} from alfalfa and 25 kg ha^{-1} year^{-1} from milk vetch. Mineral N in root-zone soil is often 30–60 kg N ha^{-1} higher when crops are preceded by legumes as compared to cereal crops (Dalal et al. 1998; Meena et al. 2015d). This enhancement is accounted to both nitrate sparing by the legume species and mineralization of the N-rich residues (Evans et al. 1991). The increasing cost of industrial fertilizers and the availability of fertilizers at the proper time raise serious concerns among farmers. This is particularly felt by the farming community with resources of a poor and marginal nature (Luce et al. 2015). These in turn incorporate legumes in the cropping systems as alternate ways to minimize the use of synthetic fertilizers (Yadav et al. 2000; Ram and Meena 2014). In this context, incorporating legumes in the cropping and intercropping system can contribute substantially to improved soil fertility and better plant growth. This in turn could improve the productivity of succeeding non-legume crops (Banyong et al. 2000; Yusuf et al. 2009; Bonilla et al. 2017).

Furthermore, Jeuffroy et al. (2013) observed that the legume crops release approximately five to seven times lower greenhouse gases (GHGs) to the atmosphere per unit area compared to non-legume crops. This process highlights their growing significance in the face of the global climate change. Further, it is reported that the peas (*Pisum sativum* L.) released 69 kg N_2O ha^{-1}, which is far less when compared to rape (*Brassica napus*) (534 kg N_2O ha^{-1}) and wheat (*Triticum aestivum*) (368 kg N_2O ha^{-1}) (Stagnari et al. 2017). Similarly, Clune et al. (2017) further reinforced the relevance of legume crops in the climate change scenario (Meena et al. 2017a). This was done by stating their extremely low global warming potential (GWP) values (0.50–0.51 kg CO_2 eq. kg^{-1} produce). Schwenke et al. (2015) showed that the emissions of N_2O from mineral N-fertilized canola (385 N_2O ha^{-1}) largely exceeded those from the faba bean (*Vicia faba* L.) (166 N_2O ha^{-1}), chickpea (*Cicer arietinum* L.) (166 N_2O ha^{-1}) and field pea (135 N_2O ha^{-1}). The authors also reported that the N fixed by legumes represented a less-emissive form of N input to the soil when compared to the nitrogenous fertilizers of industrial origin.

Given the above description, the objective of this chapter is to offer an overview of the mechanism of N fixation by legume crops. This is followed by a brief description of the impact of legumes on soil fertility and productivity of succeeding crops. The role of legume crops in mitigating environmental N emission is also highlighted. Hence, the food and nutritional security can achieve great heights under intense agriculture and thereby improve long-term soil fertility potential.

9.2 Legumes and Nitrogen Cycle

9.2.1 Mechanics of the Nitrogen Cycle

The predominant form of N, the seventh most abundant element on the earth, is the N gas (that constitutes ~78% of the earth's atmosphere). This form of N is relatively inert and therefore warrants its conversion to available form for its subsequent use by different organisms and plants (Carroll and Salt 2004; Sergei 2012) (Table 9.1). As illustrated in Fig. 9.1, this occurs through a process called the "N cycle" that converts N into compounds to be used by living organisms (plants and animals). In the atmosphere, N is the most important limiting nutrient for plant growth and development. N is generally taken in two forms, i.e. ammonium (NH_4^+) and nitrate (NO_3^-) (Sergei 2012; James 2013). Also, N is a constituent of organic molecules like proteins, amino acid and nucleic acids. In the N cycle, the important processes include N fixation, ammonification, nitrification, assimilation of N and denitrification (James 2013; Varma et al. 2017).

Table 9.1 N inputs, outputs and cycling in the soil-plant-atmosphere (SPA) system

N inputs (gain)	N output (loss)	No gain or loss in net N (cycling)	References
BNF	N uptakes by plants	Mineralization	Gonzalez et al. (2005)
Physical N_2 fixation	Denitrification (N_2O, NO, N_2)		Groffman (2012)
1. Industrial (Haber-Bosch process)			
2. Electrical (lighting)			
3. Combustion (fossil fuel)			
Animal manures	Volatilization (NH_3)	Immobilization	Rochette et al. (2009)
Crop residues	Leaching (NO_3^-)	Nitrification	Weil and Brady (2017)
	NH_4^+ fixation		Weil and Brady (2017) and Dhakal et al. (2016)

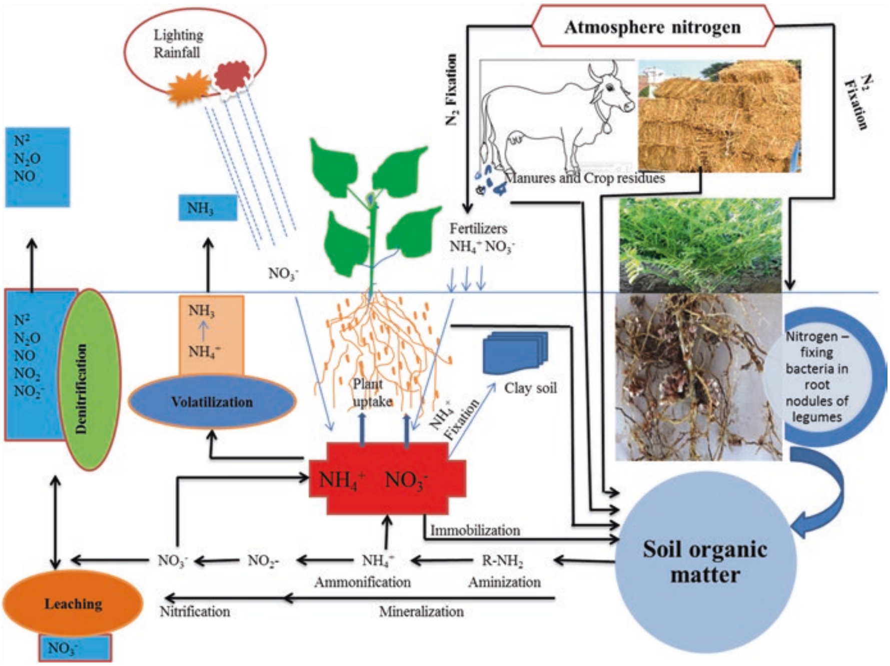

Fig. 9.1 The N cycle; most of the nitrogen conversions are facilitated by various microorganisms and cycle through the organic fraction in the soil (The complete N cycle is also described in brief as steps A–F)

Step A: N is found in manures and plant residues and is derived from the atmosphere through the process of physical electrical discharges/lighting, industrial processes and biological N fixation through living organisms, i.e. legumes. Consequently upon the completion of the N fixation, N is added to the soil (Weil and Brady 2017).

Step B: Organic N is mineralized to ammonium (NH_4^+) by certain organisms in the soil. Abundant ammonia is then transformed to nitrate (NO_3^-) by bacteria through the process called “nitrification” (Weil and Brady 2017).

Step C: Nitrate and ammonium ions are taken up by active roots of plants in soil solution (Weil and Brady 2017).

Step E: Soil solution ammonium and nitrate are converted back to N compounds through immobilization. The solution of NO_3^- can be lost by leaching to groundwater drainage system as a result of the vertical movement beneath the root zone in percolation water (Weil and Brady 2017).

Step D: Ammonium is absorbed and fixed by clay colloids (Weil and Brady 2017).

Step E: Ammonium can be volatilized into the gaseous NH_3 (Weil and Brady 2017).

Step F: Nitrate (NO_3^-) derived from nitrification, fertilization or rainfall can be converted by denitrifying bacteria to N_2, N_2O and NO gases which are emitted into the atmosphere (denitrification) (Weil and Brady 2017; Meena et al. 2014a, b).

9.2.1.1 Soil Nitrogen Forms

N is a nutrient that is usually deficient in most of the crop rotations involving non-leguminous crops (Ladha and Peoples 1995). A variety of sources of N including organic and inorganic types are supplied to non-legume crops (Shaha et al. 2003; Meena et al. 2015b). The quantity of N fixed by legumes is usually adequate to allow their growth and development. The N in soils exists in two forms: (i) organic and (ii) inorganic nitrogen (Weil and Brady 2017). Nearly all N is present in the organic form in contrast to the inorganic form which constitutes only around 2%. According to Weil and Brady (2017), clay form is represented by 8% and 40% of the total N in surface and sub-surface soils, respectively.

Organic Nitrogen

The organic form of soil N is represented by compounds such as amino acids, amino sugars, proteins and more resistant N compounds such as humus. This organic N in soil (mostly in hydrolysable form) is gradually mineralized and converted into mineral N through the process of aminization, ammonification and nitrification, thus ultimately rendering N available to plants (Gonzalez et al. 2005). The organic soil N is found in manure, compost, crop residues, green manure, bio-fertilizer and several waste materials (Ladd et al. 1983). Amino acids, proteins and polypeptides are the most common organic constituents of living organisms including plants (Gonzalez et al. 2005).

Inorganic Nitrogen

The inorganic forms of N are represented by ammonium (NH_4^+), ammonia (NH_3), nitrate (NO_3^-) and nitrite (NO_2^-) (James 2013), which can be utilized by plants as plant roots absorb N from the soil in the form NO_3^- and NH_4^+. Also, NH_4^+, NO_2^-, NO_3^-, N_2O, NO and elemental N are important sources of nutrition for N-fixing microorganisms (Havlin et al. 2014; Meena et al. 2014a, b). N in its inorganic forms remains “available” to plants and microorganisms or could move downward in the soil along with the movement of water. By contrast, the majority of the N in the soil remains unavailable to plants due to its organic form (Havlin et al. 2014). The N that is absorbed by plants or any other living organism is incorporated into soil organic matter after the death and subsequent decomposition of the organisms. Nitrate is the dominant form of N in aerobic soil, while N remains predominantly as ammonium in case of anaerobic soils (Sqrensen and Sessitsch 2007).

The N Cycle Involves the Following Processes

1. Nitrogen fixation
2. Ammonification
3. Nitrification
4. Denitrification
5. Volatilization
6. Leaching of nitrate

The conversion of N can be accomplished through both biological and physical processes.

Fixation of Nitrogen

N fixation yields ammonia (NH_3) and then N-containing organic compounds as a result of the transformation of earth's atmospheric N. This is a process that makes N accessible to the entire living organisms (Postgate 1998). In nature, the process of N fixation is mediated by certain N-fixing rhizobia bacteria (*Rhizobiaceae*, *α-Proteobacteria*) (Sqrensen and Sessitsch 2007; Buragohain et al. 2017). Alternatively, the N fixation can be accomplished by natural means like lightning and/or processes including the Haber-Bosch that is used to produce fertilizers such as urea and other chemical fertilizers (Havlin et al. 2014). Among all N fixation processes, BNF is the most common one in plants. Due to their property to fix atmospheric N and accumulate a great quantity of N in their organs, legumes serve as bio-fertilizers in crop production systems (Peter et al. 2002). Accordingly, a leguminous crop when applied as green manure in the soil confers the subsequent non-legume crops with a huge quantity of sources comprising N and C (Stagnari et al. 2017).

Ammonification/Mineralization of Nitrogen

The N mineralization generates inorganic N (NH_4^+) from organic N involving two major processes, viz. ammonization and ammonification.

(i) *Aminization*: An enzymatic reaction mediated by soil microorganisms (aerobic and anaerobic bacteria, fungi and actinomycetes) converts proteinous and protein compounds into amino acid and amines (James 2013; Stagnari et al. 2017).

$$\text{Proteins} \underset{\text{Soil microbes}}{\overset{\text{Enzyme}}{\rightarrow}} \text{polypeptide} \underset{H_2O}{\overset{\text{Enzyme}}{\rightarrow}} \underset{\text{Amino acid}}{R-\overset{\overset{\displaystyle NH_2}{|}}{\underset{\underset{\displaystyle H}{|}}{C}}-COOH} + \underset{\text{Amine}}{R-NH_2} + CO_2 + \text{Energy}$$

9.2.1.2 Ammonification

Ammonification driven by certain soil microorganism enables organic N compounds to be transformed into ammonia (NH_3) or ammonium (NH_4^+). The NH_4^+ ions are produced as a waste of animal, organic matter, crop residues and manure by bacteria (aerobic and anaerobic), fungi and actinomycetes (Sergei 2012; Meena et al. 2014a, b). The process of ammonification takes place in aerobic environments with the liberation of NH_3 or NH_4^+ ions, which are either released into the atmosphere or used by selective plants (e.g. rice) and microorganisms. Also, the ions may be oxidized to nitrites and finally to nitrates under favourable soil conditions (Havlin et al. 2014).

The process of ammonification as shown below is commonly mediated by *Clostridium* spp., *Micrococcus* sp., *Proteus* spp., etc. (Groffman 2012).

$$R-NH_2+H_2O \underset{\text{Hydrolysis}}{\overset{\text{Enzyme}}{\rightarrow}} NH_3 \overset{H_2O}{\rightarrow} NH_4^+ + OH^- + R-OH + \text{Energy}$$

Nitrification

Enzymatic oxidation of NH_4^+ to NO_2^- and ultimately to nitrate (NO_3^-) by certain soil microorganism is termed as nitrification. Two groups of bacteria participate in the process of nitrification: the one that causes oxidation of ammonia to produce nitrite (NO_2^-) and the other that further oxidizes nitrite to NO_3^- (Sergei 2012). These bacteria obtained energy from N compound (proteins, polypeptides and amino acids) and carbon from CO_2. Nitrate (NO_3^-), the end product of nitrification, is extremely important for plant growth (Bundy 1998).

As mentioned above, the oxidation process is completed in two steps, and each step is performed by different groups of bacteria as follows:

Step I: The process referred to as "nitrification" leads to the generation of nitrite and is mediated by ammonia-oxidizing bacteria (*Nitrosomonas*, *Micrococcus*, *Europaea*, *Nitrosococcus*, *Nitrosospira*, *Briensis*, *Nitrosovibrio* and *Nitrocystis*). The chemical reaction underlying the process is denoted as follows (James 2013; Meena et al. 2014a, b).

$$\underset{\text{Ammonia}}{2NH_3} + ½O_2 \rightarrow \underset{\text{Nitrite}}{NO_2} + 2H + H_2O\left(\text{Nitrosomonas bacteria}\right)$$

Step II: In the second step, nitrite is oxidized to nitrate by nitrite-oxidizing bacteria (*Nitrobacter winogradskyi*, *Nitrosococcus mobilis*, *Nitrocystis*, *Nitrospina gracilis*, etc.) and some fungi (e.g. *Penicillium*, *Aspergillus*) and actinomycetes (e.g. *Streptomyces*, *Nocardia*).

$$\underset{\text{Nitrite ions}}{NO_2^-} + ½O_2 \rightarrow \underset{\text{Nitrate ions}}{NO_3^-} \left(\text{Nitrobacter}\right)$$

Nitrification Is Affected by Several Factors, Which Include

(i) Supply of ammonium ions
(ii) Soil moisture
(iii) Soil temperature
(iv) Soil pH
(v) Soil aeration
(vi) C/N ratio

9.2.1.3 N Losses from Soil-Plant System

Denitrification

As a reverse process to nitrification, denitrification causes reduction of NO_3^- and NO_2^- by anaerobic bacterial, thus resulting in the release of nitric oxide (NO), nitrous oxide (N_2O) and N_2 that eventually are lost to the atmosphere (Seitzinger et al. 2006; Groffman 2012). Consequently, the plant-available N (an inorganic form of N) in the soil is lost to the atmosphere; this process is also called dissimilarity nitrate reduction, and NO_3^- is also reduced to NH_4^+, and this is assimilated to the protein through the formation of amino acids. This process is called assimilatory nitrate reduction.

The process of denitrification is as follows (Seitzinger et al. 2006; Datta et al. 2017):

$$\text{Denitrification reaction series}$$
$$\underset{\text{Nitrate}}{NO_3^-} \rightarrow \underset{\text{Nitrite}}{NO_2} \rightarrow \underset{\text{Nitric oxide}}{NO} \rightarrow \underset{\text{Nitrous oxide}}{N_2O} \dashrightarrow \underset{\text{N}}{N_2} -$$

When oxygen is depleted in the soil, some of the NO_3^- can change to N_2O, and N_2 gaseous forms are lost to the atmosphere. The sequence of intermediate products of denitrification is as follows. Some of the organisms (*Thiobacillus thioparus*, *Thiobacillus denitrificans*, *Pseudomonas*, *Micrococcus*, *Bacillus* and *Achromobacter*) are involved in this process (Groffman 2012).

A range of factors influence the process of denitrification such as:

(i) Supply of nitrate substrate
(ii) Soil texture
(iii) Aeration and water status
(iv) Soil pH
(v) Available soil organic carbon
(vi) Temperature

Ammonium Volatilization

Ammonia gas (NH_3) is produced in the soil-plant system from the mineralization of crop residues, organic matter, farmyard manure (FYM), compost and industrial chemical fertilizer (like anhydrous ammonia and urea) (Rochette et al. 2009). This process reflects a reversible reaction as follows:

Reversible Process

$$\underset{\text{Dissolved ions}}{NH_4^+} + OH \leftrightarrow H_2O + \underset{\text{Ammonia gas}}{NH_3} -$$

Volatilization of NH_3 depends on the concentration of ammonium and ammonia ions in the soil solution and the soil pH. At pH 9.5, the ammonium and ammonia are

Table 9.2 Global N fixation from different sources

Source of N fixation	N fixation rate kg ha^{-1}	Nitrogen fixed (10^6 tons $year^{-1}$)
Legume crops	140	35
Non-legume crops	8	9
Meadows and grassland	15	45
Forest and woodland	10	40
Other vegetated lands	2	10
Ice-covered land	0	0
Total land		139
Sea	1	36
Total biological fixation		175
Lightning		8
Fertilizer industry		77
Total non-biological fixation		85
Grand total		260

Source of data: The Nature and Properties of Soils (2002): Weil and Brady (2017)

of equal concentration (50% each) with ammonia increasing constantly with increasing soil pH (Havlin et al. 2014; Varma and Meena 2016). A large proportion of mineral N fertilizer applied can be lost through the process of volatilization, if not properly managed, and in this way, it is not incorporated into the soil. When soil pH reaches above 7.5, a percentage of the NH_4^+ can be converted into ammonia gas (NH_3) and thus released to the atmosphere (Havlin et al. 2014).

In addition to soil pH, higher soil moisture and temperature contribute to NH_3 loss. Sometimes, NH_3 volatilization can occur under neutral and acidic soils.

Nitrogen Fixation in Soil

As mentioned earlier, notwithstanding the existence of nearly 80% of N in the atmosphere, the maximum abundant forms cannot be accessed directly by plants (Ladha and Peoples 1995). This makes the N often a limiting factor in the agricultural production system, especially for non-legume crops that show a greater demand for a high amount of N. The free atmospheric N gas can become available to plant through N_2 fixation.

Biological N fixation, the most common N fixation process, facilitates fixation of an estimated 175 × 10^6 tons of N each year worldwide (Table 9.2).

9.2.2 Types and Process of Soil Nitrogen Fixation

Atmospheric N is fixed by two major means, viz. (i) physicochemical and (ii) biological processes, which enable nearly 10 and 90% of natural N fixation, respectively.

9.2.2.1 The Low Type of Soil Nitrogen Fixation

Physical Nitrogen Fixation

Natural Nitrogen Fixation

With the influence of lightning strikes (i.e. electric discharge in the clouds) and thunder, N and oxygen (O_2) of the air react to form nitric oxide (NO). The nitric oxides are again oxidized with oxygen to form N peroxide (NO_2) (Sergei 2012; Havlin et al. 2014).

$$N_2 + O_2 \text{ Lightning} \rightarrow \text{Thunder } 2NO\left(\text{Nitric Oxide}\right);$$
$$2NO + O_2 \rightarrow 2NO_2 \text{Oxidation}\left(\text{N peroxide}\right)$$

During the rains, NO_2 combines with rainwater to form nitrous acid (HNO_2) and nitric acid (HNO_3). The acids fall on the soil with rainfall and react with the alkaline radicals to form water-soluble nitrates (NO_3^-) and nitrites (NO_2^-) (Sergei 2012; Havlin et al. 2014).

$$2NO_2 + H_2O \rightarrow HNO_2 + HNO_3$$
$$HNO_3 + \text{Ca or K salts} \rightarrow \text{Ca or K nitrates}$$

The nitrates are soluble in water and are directly absorbed by the roots of the plants (Vitousek et al. 2013).

Industrial N Fixation

Ammonia is produced industrially by direct combination of atmospheric N with hydrogen (obtained from water) at high temperature (400–500 °C) and pressure (15–25 MPa). Further, it is converted into different types of fertilizers, such as urea, etc.

Biological Nitrogen Fixation

Mechanism and Process of BNF

N is an essential element for plant growth and development (Sergei 2012). Plants instead depend upon combined or fixed forms of N, such as ammonium and nitrate. A considerable proportion of this N is supplied to the cropping systems in the form of industrially produced N fertilizers. The prime sources of N include N available in the soil, the BNF and synthetic fertilizers. Soil organic N in natural or human-made ecosystems is constantly lost through plant exclusion and further losses through leaching, denitrification and NH_3 volatilization. BNF is known to be a key to sustain agriculture production and to increase soil fertility (Vitousek et al. 2013; Meena et al. 2017b).

Research on microorganisms and plants capable of fixing atmospheric N contribute fundamentally to bio-fertilizer production. Thus, it is important to ensure that BNF research and development will take into account the needs of smallholder farmers in the developing countries (Bhat et al. 2015). BNF refers to the conversion of atmospheric N_2 into NH_3 and then to N-containing organic compounds that can become available to form life through the N cycle (Herridge et al. 2008).

Globally, a huge amount of N is fixed biologically each year and ranges from 130 to 180 × 10^6 tons, with 50% fixed by *Rhizobium* (Havlin et al. 2014). In contrast,

Table 9.3 A short list of *Rhizobium* species and their corresponding hosts

Nodulating bacteria	Host crop/plant	References
Rhizobium phaseoli, *R. leguminosarum* biovar *phaseoli* and *R. tropics*	*Phaseolus vulgaris* (common bean)	Kahindi et al. (2009)
R. leguminosarum bv. *viciae*	*Lens* (lentils), *Vicia* (vetch), *Pisum sativum* L. (peas)	
R. leguminosarum bv. *trifolii*	*Trifolium* sp. (clovers)	
Mesorhizobium loti	*Lotus*	
R. leguminosarum bv. *phaseoli*	*Vicia faba* (broad bean)	
Bradyrhizobium japonicum, *B. elkanii*, *R. fredii*	*Glycine max* (soybean)	Kahindi et al. (2009) and Meena et al. (2017b)
Azorhizobium caulinodans	*Sesbania* sp., *Sesbania rostrata* (stem nodulating)	
R. meliloti, *Sinorhizobium meliloti*	*Medicago sativa* (alfalfa), *Trigonella* (fenugreek)	
R. loti	*Lotus* (trefoils), *Lupinus* (lupin), *Cicer* (chickpea), *Leucaena*	
"Cowpea rhizobia" group or *Rhizobium* sp.	*Vigna unguiculata* (cowpea)	Kahindi et al. (2009)
Bradyrhizobium sp.	*Arachis hypogaea* (peanut), *Cajanus* (pigeon pea) and *Crotalaria* (crotalaria)	

global fertilizer N use was about 109 × 10^6 tons in 2014 (FAOSTAT 2014), about twice as much as is industrially fixed in the manufacture of N fertilizers.

BNF was discovered by the German agronomist Hermann Hellriegel and Dutch microbiologist Martinus Beijerinck. In the BNF equation, 2 moles of NH_3 ions are made by a single mole of N_2 gas, at the expense of 16 moles of ATP and a supply of electrons and protons (hydrogen ions). The nitrogenase enzyme is the key to biological N fixation, which catalyses the reduction of N gas to ammonia.

$$N_2 + 8H^+ + 8e^- + 16\,ATP = 2NH_3 + H_2 + 16ADP + 16\,Pi$$

The NH_3, in turn, is combined with organic acids to amino acid and, finally, protein:

$$NH_3 + \text{organic acids} \rightarrow \text{amino acids} \rightarrow \text{protein}$$

The BNF occurs through a number of microorganisms in the system, with or without direct association with higher plants (Tables 9.3 and 9.4); while the legume-bacteria symbiotic system has received the most attention, recent findings suggest that the other system involves many more families of plants worldwide and may even rival the legume-associated system as supplier of biological N to the soil. Each major system will be discussed below briefly (Timothy 1999; Herridge et al. 2008). As mentioned already, large quantities of N can be fixed through the process of symbiosis of microorganisms and legumes (Meena et al. 2014a, b). In this process, the plants produce the energy through the process of photosynthesis, and the

Table 9.4 Range in quantity of N_2 fixed by selected legumes

Sr. no.	Legumes	Botanical/ scientific name	Associated organism	N fixing (kg ha^{-1} $year^{-1}$)	References
1.	Soybean	*Glycine max* L.	Bacteria (*Bradyrhizobium*)	100–150	Ahlawat and Gangaiah (2004), Mugwe et al. (2011) and Meena et al. (2017b)
2.	Chickpea	*Cicer arietinum* L.	Bacteria (*Rhizobium*)	40–50	Ahlawat and Gangaiah (2004) and Seymour et al. (2015)
3.	Lentil	*Lens esculents* Medik.	Bacteria (*Rhizobium*)	40–68	Shaha et al. (2003) and Mugwe et al. (2011)
4.	Groundnut	*Arachis hypogaea* L.	Bacteria (*Bradyrhizobium*)	150	Mugwe et al. (2011) and Seymour et al. (2015)
5.	Field pea	*Pisum sativum* L.	Bacteria (*Rhizobium*)	65–100	Peoples et al. (2009)
6.	Pigeon pea	*Cajanus cajan* L.	Bacteria (*Bradyrhizobium*)	100,200	Mugwe et al. (2011)
7.	Mung bean	*Vigna radiata* L.	Bacteria (*Rhizobium*)	60,112	Shaha et al. (2003) and Seymour et al. (2015)
8.	Urdbean	*Vigna sinensis* L.	Bacteria (*Rhizobium*)	30	Ahlawat and Gangaiah (2004)
9.	Cowpea	*Vigna unguiculata* L.	Bacteria (*Bradyrhizobium*)	90	Mugwe et al. (2011)
11.	Lupins	*Lupinus* sp. L.	Bacteria (*Rhizobium*)	60–100	Havlin et al. (2014)
13.	Beans	*Phaseolus vulgaris* L.	Bacteria (*Rhizobium*)	20–80	Mugwe et al. (2011) and Havlin et al. 2014
14.	Alfalfa	*Medicago sativa* L.	Bacteria (*Rhizobium*)		Carlsson and Huss-Danell (2003), Aranjuelo et al. (2009) and Mugwe et al. (2011)
15.	Cluster bean	*Cyamopsis tetragonoloba* L.	Bacteria (*Rhizobium*)	60–150	Mugwe et al. (2011) and Meena et al. (2017b)
17.	Fenugreek		Bacteria (*Rhizobium*)	45	Mugwe et al. (2011)
18.	Black gram	*Vigna mungo* L.	Bacteria (*Rhizobium*)	100	Mugwe et al. (2011)

(continued)

Table 9.4 (continued)

Sr. no.	Legumes	Botanical/ scientific name	Associated organism	N fixing (kg ha^{-1} year^{-1})	References
20.	Faba bean	*Vicia faba* L.	Bacteria (*Rhizobium*)	130	Peoples et al. (2009) and Seymour et al. (2015)
21.	Clover	*Trifolium pratense* L.	Bacteria (*Rhizobium*)	100–150	Aranjuelo et al. (2009)
23	Red clover	*Trifolium pratense* L.	Bacteria (*Rhizobium*)		Carlsson and Huss-Danell (2003)
24	White clover	*Trifolium repens* L.	Bacteria (*Rhizobium*)		Carlsson and Huss-Danell (2003)
Non-legumes (nodulated)					
25	Species of *Gunnera*		Cyanobacteria[a] (*Nostoc*)	10–20	Weil and Brady (2017)
26	Alders	*Alnus* sp.	Actinomycetes (*Frankia*)	50–150	Weil and Brady (2017)
Non-legumes (non-nodulated)					
27	Pangola grass	*Digitaria decumbens*	Bacteria (*Azospirillum*)	5–30	Weil and Brady (2017)
28	Bahia grass	*Paspalum notatum*	Bacteria (*Azotobacter*)	5–30	Weil and Brady (2017)
29	Azolla		Cyanobacteria[a] (*Anabaena*)	150–300	Weil and Brady (2017)
	Non-symbiotic		Bacteria (*Azotobacter*, *Clostridium*)	5–20	Weil and Brady (2017)
	Non-symbiotic		Cyanobacteria[a] (various)	10–50	Weil and Brady (2017)

[a]Sometimes referred to as blue-green algae

microorganisms utilize this energy to fix N. The process of BNF is carried out by a different group of bacteria that are either free living or in symbiotic associations with plants (*Rhizobium* and actinomycetes). Legume-bacteria symbiosis is the major form of N fixation that delivers N to crops.

The BNF can take many forms in nature, including blue-green algae (a bacterium), lichens and free-living soil bacteria. These kinds of N fixation contribute significant quantities of ammonia (NH_3) to ecosystems but not to most cropping systems, except paddy rice. Their contributions are less than 6 kg N ha^{-1} year^{-1} (Fig. 9.2). However, N fixation by legumes can be in the range of 25–190 kg N ha^{-1} year^{-1} in a natural ecosystem and several hundred kilogrammes in a cropping system (Frankow and Dahlin 2013). BNF of the atmospheric N can be estimated at around 175 million metric tons year^{-1} or nearly 70% of all N fixed on the soil in each year; the remaining is by some microorganisms, autotrophs or heterotroph "free" fixers (Peter et al. 2002).

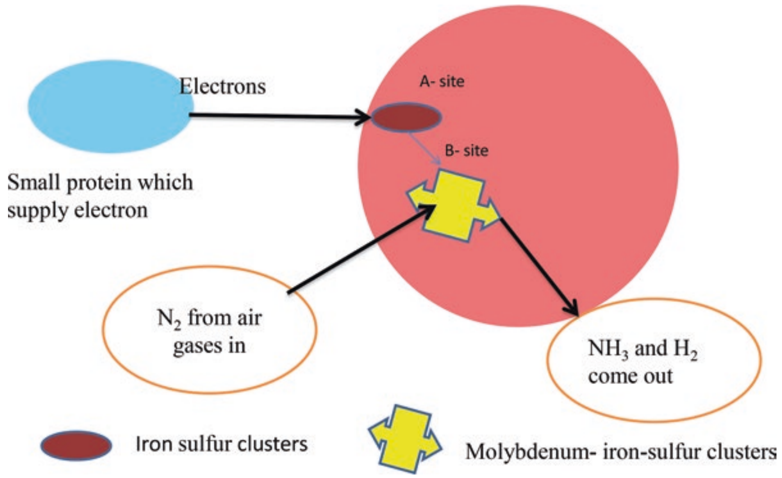

Fig. 9.2 Nitrogenase enzyme consists of two proteins. The bigger protein converts atmospheric N into ammonia using electrons which are provided by the smaller protein. The B sites on the bigger protein arrest N_2 from the air; however, the A site gets the electrons from the small protein, so finally N_2 can be reduced to NH_3

The conversion or fixation of N is from the unavailable gaseous form in the atmosphere to forms that plants and other living organisms can take (either ammonia or nitrate) and is mediated by (i) bacteria in symbiotic relationships with vascular plants, (ii) symbioses between cyanobacteria and fungi (lichens) or plants, (iii) free-living autotrophic or heterotrophic bacteria that are naturally associated with soil or litter and (iv) abiotic reactions that occur with lightning in the atmosphere (Timothy 1999; Meena et al. 2017b).

The Following Are Three Types of N-fixing Microorganisms of Symbiotic and Asymbiotic Nature

1. Symbiotic N fixation: fixes N_2 only by the formation of nodules in legume, e.g. *Rhizobium*, *Bradyrhizobium* and *Sinorhizobium*, and in some selective non-legumes, e.g. *Anabaena*, *Azolla* and *Frankia*
2. Associative N fixation: requires oxygen for growth and fixes N in the existence of oxygen (*Azospirillum*)
3. Free-living N fixation: fixes N both in aerobic and anaerobic (*Azotobacter*, *Thiobacillus*, *Bacillus* and *Clostridium* and *Klebsiella*)

Symbiotic Nitrogen Fixation

Many microorganisms fix N_2 symbiotically by partnering with a host plant. The plant provides food (sugars) from photosynthesis that is used by the N-fixing microorganism for the energy it needs for N_2 fixation. In exchange for these carbon sources, the microbe provides fixed N_2 to the host plant for its growth (Peter et al. 2002).

The symbiotic microorganisms are not only bacteria but also involve fungi, actinomycetes (e.g. *Frankia*) and cyanobacteria (e.g. *Anabaena*). These microorganisms create multiple kinds of relationships with different parts of plants and develop a special structure. Species from angiosperm family Leguminosae such as *Pisum sativum*, *Cajanus cajan*, *Glycine soja* and *Cicer arietinum* build a symbiotic relationship with *Bradyrhizobium*, *Azorhizobium*, *Allorhizobium*, *Photorhizobium*, *Sinorhizobium*, *Mesorhizobium* and *Rhizobium* (Herridge et al. 2008: Havlin et al. 2014; Meena et al. 2017b).

For the first time, rhizobia were isolated from root nodules by M. Beijerinck and were shown to have the capacity to reinfect their legume hosts and to fix N in symbiosis. N-fixing bacteria (rhizobia) are known for their ability to establish symbiotic interactions with leguminous plants through the development and colonization of root nodules, where the bacteria fix N to ammonia and make it available for the plant. The bacteria are mostly rhizospheric microorganisms, in spite of their ability to live in the soil for a long period (Gonzalez et al. 2005).

Rhizobium is a free-living, gram-negative, aerobic, non-sporulating and rod-shaped (0.5–0.9 mm and 1.2–3 mm) bacteria, which produce nodules in the leguminous plant. It is a fast-growing bacterium; however, *Bradyrhizobium* is a slow-growing strain which possesses subpolar flagella (Vieira et al. 2010).

Acetobacter diazotrophicus colonizes the stem apoplast in maize (*Zea mays*). The N_2-fixing microorganism forms a symbiotic association with the grasses without nodule formation, and such association is called associative N fixation. The *Azotobacter paspali* remains alive in the rhizospheric zone of *Paspalum notatum*, a tropical grass (Yusuf et al. 2009; Frankow and Dahlin 2013).

The *Beijerinckia* living in the rhizosphere of sugarcane (*Saccharum officinarum*) and *Klebsiella* in leaf nodules of *Psychotria*, *Casuarina equisetifolia*, *Alnus*, *Myrica* and *Parasponia* do not form nodules but fix N_2 through harbouring *Frankia* and *Rhizobium*. They show host specificity. The two partners in the N_2 fixation recognize each other with the help of the chemical substance lectins which are phytohaemagglutinins (carbohydrates having plant proteins). All the 42 bacterial isolates (grouped in the genera *Sinorhizobium* (27), *Rhizobium* (13) and *Agrobacterium* (2)), *Sinorhizobium* sp. strains STM 4036, STM 4034 and STM 4039 forming the most effective symbiosis are potential candidates for inoculants in revitalisation programmes (Mahdhi et al. 2008).

Nitrogen-Fixing Mechanism in Legumes

When legume root growth is initiated, N-fixing bacteria in soil enter root hair and multiply (Figs. 9.3 and 9.4). Legume root responds by developing tumour-like structure called nodules on the plant root surface (Kahindi et al. 2009). The particular bacteria called rhizobia inside the nodule absorb N_2 from soil air and convert it to ammonia. Rhizobia use the enzyme nitrogenase and energy from the transformation of adenosine-5′-triphosphate (ATP) to adenosine diphosphate (ADP) to break the strong triple bond in nitrogen (Peix et al. 2010). The symbiotic connection between nodule bacteria and the legume host plant is mutually beneficial. Growth-stimulating substances like biotin, thiamine, amino acids, etc. are secreted by the root of the symbiotic bacteria which enhances the growth of rhizobia and other microbes

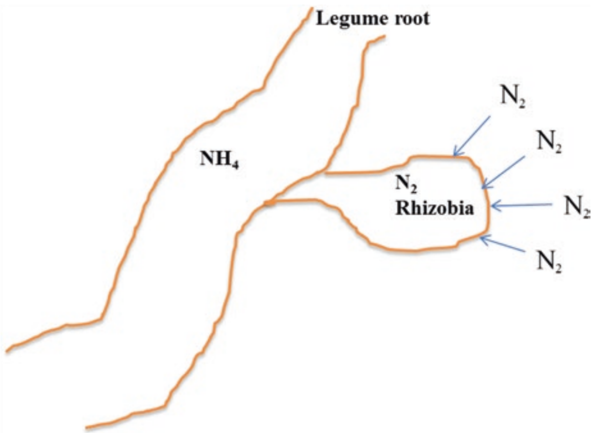

Fig. 9.3 Conversion of N_2 to NH_4 by rhizobia inside legume root nodules

Fig. 9.4 Example of nodules on lucerne (left) and clover (right) showing difference in nodulation

(Skorupska et al. 2010; Meena et al. 2014a, b). The reactions between polysaccharide (callose) present on the surface of the rhizobial cell and the lectin secreted by the plant root hairs help in the recognition of the correct host plant by the specific *Rhizobium* (Skorupska et al. 2010). Some other compounds (specific flavonoids) are also secreted by plant root hairs, e.g. alfalfa secretes luteolin which activates the "*nod*" genes in the bacteria, which results in nodule formation. Except for the two *Agrobacterium* isolates, all strains induced nodulation on *Argyrolobium* uniformly, but the number of nodules and N fixation efficiency varied among them (Mahdhi et al. 2008). The host plant delivers energy (carbohydrates, sugar and ATP) for rhizobia to fix N, and rhizobia, in turn, provide ammonium for production of protein by the host plant, and most of the fixed N is utilized by the host plant. However, some may be excreted from the nodule into the soil and used by nearby plants or resealed as nodules which decompose after the plants die (Sindhu et al. 2010).

Many rhizobium species exist in soil, each requiring a specific host legume plant. For example, *Rhizobium leguminosarum* biovar. *trifolii* will only nodulate clover (*Trifolium*), while *Rhizobium meliloti* will only nodulate alfalfa. This host specificity is referred to as cross inoculation group cell signalling between the legume host and the bacteria. The above-mentioned Nod factors have been identified as lipochition oligosaccharides. Dissimilarities in the structures of these oligosaccharides determine the host specificity for the bacterium. The presence of nodules on legume root does not necessarily indicate N_2 fixation by active rhizobia. Mature, effective chickpea nodules tend to be elongated and clustered on the primary roots and have pink to red centres. This red colour is due to leghaemoglobin and indicates that the rhizobia are actively fixing N. The main cross inoculation groups of bacteria are presented in Table 9.4.

Curling of Root Hairs

Certain soil bacteria release Nod factors; this results in curling of root hairs, which is accompanied by the formation of the infection thread by the hair tip (Kahindi et al. 2009). There is a continuation of the wall of the infection thread with the cell wall of the root hair. The branching of infection thread then occurs, and bacteria continue to produce nod factors which result into the stimulation of root cells to proliferate, resulting in nodule formation (Peix et al. 2010; Sindhu et al. 2010). Thousands of N-fixing bacteria (rhizobia) inhabit the root nodule and form the bacteroids (the bacterial cells become dormant and are called bacteroids). The membrane that is formed by the plant cell, which surrounds the bacteroid, is called symbiosome or peribacteroid (Kahindi et al. 2009).

Nodule Formation and Leghaemoglobin

The mucopolysaccharide released by N-fixing bacteria reacts with a component of root hair cell to form a compound which induces the production of polygalacturonase. When all these processes occur, rhizobia enter into the cell (Kahindi et al. 2009). The inner cortical tissue stimulates by bacteria divide and forms an organized mass of infected plant tissue which is protruded out and appears as the nodule. Rhizobia are released from the infection tube and occupy the central position in the nodule. The central nodule is tetraploid which is a peculiar characteristic (Peix et al. 2010; Dhakal et al. 2016). The available space in the host cell is totally filled. The free-living microorganisms develop mechanisms to protect the enzyme of nitrogenase from oxygen such as high rates of metabolism, physical barriers, etc.; the level of oxygen in nodules is controlled by leghaemoglobin. Nodules have an

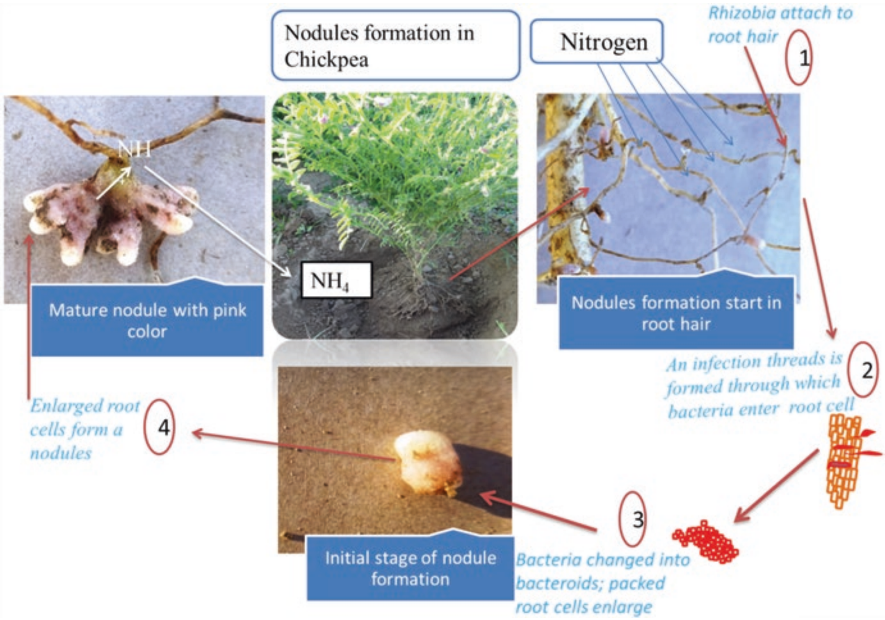

Fig. 9.5 Schematic view of N_2-fixing bacterial association with leguminous plant and development of root nodules by *Rhizobium* sp.

oxygen-binding heme protein, i.e. leghaemoglobin, and hence the colour of nodules appears pink (Fig. 9.5). When the nodule is fully mature after that it dies, bacteria are released into the soil. The bacteroids are the main sites of the N fixation (Kahindi et al. 2009; Peix et al. 2010). Bacteroids may be swollen, irregular, star shaped, branched, etc. Leghaemoglobin has about ten times higher affinity for oxygen than human haemoglobin. The prosthetic group protohaem synthesizes the bacteroids, while the synthesis of the protein part involves the plant cell (Peix et al. 2010). It supplies O_2 to the respiring symbiotic bacterial cells. It enhances the transport of oxygen at low partial pressure and also provides protection to nitrogenase against oxygen and stimulates ATP production needed for N_2 fixation (Peix et al. 2010; Skorupska et al. 2010).

Nitrogen Release to the Soil and Other Crops

Almost the entire N fixed is taken directly by the plant; the minute leaks into the soil for a neighbouring non-legume plant (Herridge et al. 2008). Nevertheless, N finally returns to the soil for a neighbouring plant when vegetation (roots, stem, leaves and fruit) of the legume dies and decomposes. The yield of non-legume crops is often increased when grown following legumes. For example, when maize is grown after soybean, the N requirement is far less than that required for maize after maize (Havlin et al. 2014; Ram and Meena 2014). Similarly, less N is required to improve wheat yield following legumes (Stagnari et al. 2017).

Reduced N rates with the non-legume crop follow legumes and then continuous non-legume rotations. This is mainly due to:

- Readily decomposition of legumes residue providing plant-available N (Stagnari et al. 2017).
- Greatly reduced N immobilization drives continuous legume rotation (Shaha et al. 2003; Peoples et al. 2009).
- Improved soil microbial activity results in increased N mineralization in legume rotation optimum (Stagnari et al. 2017).

Asymbiotic N_2 Fixation

Certain free-living microorganisms present in soil and water can fix atmospheric N_2 because these organisms are not directly associated with plants; the conversion is called non-symbiotic or free-living microorganism. The N fixation by tropical grasses including some cereals by a non-symbiotic process was first time recognized by a Brazilian scientist Johanna Döbereiner in the 1960s. She found out the considerably greater population of *Beijerinckia* and *Azotobacter* in the rhizospheric zone of batatais grass (*Paspalum notatum*) under acidic soil environment (Ruschel and Döbereiner 1965). Some of the bacteria and majority of the cyanobacteria involve this class of microorganisms. These microorganisms are generally called free-living diazotrophs. Among the cyanobacteria, unicellular, filamentous non-heterocystous and filamentous heterocystous fix N independently. Both aerobic and anaerobic bacteria are free-living diazotrophs (Table 9.5). Water, nutrients and oxygen are required in an appropriate amount, so that the microorganism can grow. Cyanobacteria grow commonly in the crop fields. The site of N fixation in the cyanobacteria is the heterocyst because of the nitrogenase enzyme required for N fixation which acts under an anaerobic situation (Kumari and Rajeshwari 2011). Asymbiotic N_2-fixation process is complete through two major groups of bacteria which are as follows:

Asymbiotic N_2 Fixation by Heterotrophs

The major fixation is brought about by species of two genera of heterotrophic aerobic bacteria, *Azotobacter* and *Beijerinckia*, which belong to temperate zones and

Table 9.5 Bacterial types fixing N symbiotically

Aerobic bacteria	Anaerobic bacteria	Facultative bacteria	Photosynthetic bacteria
Azomonas	*Clostridium*	*Bacillus*	*Chlorobium*
Azotobacter	*Desulfovibrio*	*Enterobacter*	*Chromatium*
Beijerinckia		*Klebsiella*	*Rhodomicrobium*
Derxia			*Rhodopseudomonas*
Methylomonas			*Rhodospirillum*
Mycobacterium			

Source: Kumari and Rajeshwari (2011) and Havlin et al. (2014)

tropical soils, respectively. Other aerobic bacteria of the genus *Clostridium* are also able to fix N_2. Because pockets of low O_2 supplies exist in the soil, despite good tilth, the anaerobic bacteria may work side by side in many well-drained soils. The amount of N_2 fixed by these heterotrophs varies greatly with soil properties such as pH, soil N level and sources of organic matter available. Because of their limited energy supply, under normal agriculture conditions, the rate of N fixation by these organisms is brought to be in the range of 5–20 kg N ha^{-1} $year^{-1}$ only a small fraction of the needed N by crops.

Asymbiotic N_2 Fixation by Autotrophs

Among the autotrophs able to fix N are certain photosynthetic bacteria and cyanobacteria. With the presence of light, these organisms can fix CO_2 and N_2 simultaneously. The contribution of the photosynthetic bacterias are uncertain, if bacteria are thought to be of some significance, particularly in wetland areas and in a rice field. In some case, the algae have been found to fix sufficient N_2 for reasonable rice yields, but the usual level may be no more than 20–30 kg N ha^{-1} $year^{-1}$. Cyanobacteria also fix N_2 in the upland soil, but the level is much lower than that which is found under wetland conditions.

9.2.3 How to Increase BNF and N_2-Fixing Ability

Biological N_2 fixed represents N advantage and determines mineral N fertilizer savings in cropping systems. Legumes can fix more than 40–250 kg N ha^{-1}. However, the amounts of N_2 fixed can differ considerably in time and space.

Four common approaches to enhance biological N fixation are:

- Inoculation with proven strains (covered above)
- Microbial screening for improved strains
- Host plant screening and breeding and adoption of cropping systems and cultural practices

9.2.4 Factors Affecting N_2 Fixation

The following factors will affect the rate of N_2 fixation by legume-bacteria symbiosis: the amount of applied manure or fertilizer N; because N_2 fixation requires higher energy, the amount of N fixed through BNF will be much less when the soil contains large inorganic N from other sources (Meena et al. 2013). The N_2 fixation increases with the decrease of N availability in soil. Excess NO_3^- availability reduces nitrogenase activity, which resulted in reduction of N_2 fixation by competition for photosynthate between NO_3^- reduction and N_2^- fixation reactions (Havlin et al. 2014).

Soil fertility: the deficiencies of some nutrients in soil such as Mo, Fe, P, Mg and S will result in a reduced N_2 fixation as these elements are part of the nitrogenase complex which permits N_2 fixation to take place. N fixation requires more Mo than the host plant; because of this, Mo is a main component of the nitrogenase (Hungria and Vargas 2000; Verma et al. 2015).

Soil pH: N fixation reduces when soil pH is lower than 6.7 (Hungria and Vargas 2000).

Soil temperature: soil temperature from 24 to 30 °C is optimum for N_2-fixing bacteria (rhizobia). Effective N_2 fixation will be inhibited below 10 °C (Havlin et al. 2014).

9.3 Leguminous Effect on Succeeding Crops

Legume crops that generate cash or economic income will fit better in the production systems practised by small farmers with limited resources (e.g. maize-mung bean-wheat and rice-mung bean-wheat). Legumes can be successfully accommodated into cereal-based cropping systems through the following means:

1. Crop rotation
2. Green manure
3. Intercropping

9.3.1 Crop Rotation

Crop rotation is the system of growing a sequence of different crops on the same ground so as to maintain or increase soil fertility and crop productivity. In crop rotation, legumes contribute to the diversification of cropping systems and act as free atmosphere N_2-fixing plants; it can reduce the synthetic N fertilizer demand. In the rotation of crops, leguminous crops like pulses, chickpea, beans, peas, groundnut, soybean, lentil, Bengal gram and cluster bean are sown in between the seasons of cereal crops like rice, wheat, maize, sorghum and pearl millet and cash crop like cotton, sugarcane, etc. (Shaha et al. 2003; Mayer et al. 2003; Luce et al. 2015).

Presently, several groups are intensively researched on sustainable reintroduction of grain legumes into non-legume crop rotations, based on their economic advantage on crop yield and quality characteristics on succeeding non-legume crops (Kirkegaard et al. 2008; Luce et al. 2015; Yadav et al. 2017). Legumes could be competitive crops regarding ecological and socioeconomic benefits with the potential to be introduced in modern cropping systems, which are characterized by decreasing crop diversity (FAO 2011). Legumes enrich the soil with N which provides a more favourable environment to succeeding cereals or non-legume crops for better growth and grain yield (Fig. 9.6). However, their ability to fix atmospheric N_2 did not make them independent of other sources of N, even when symbiosis was

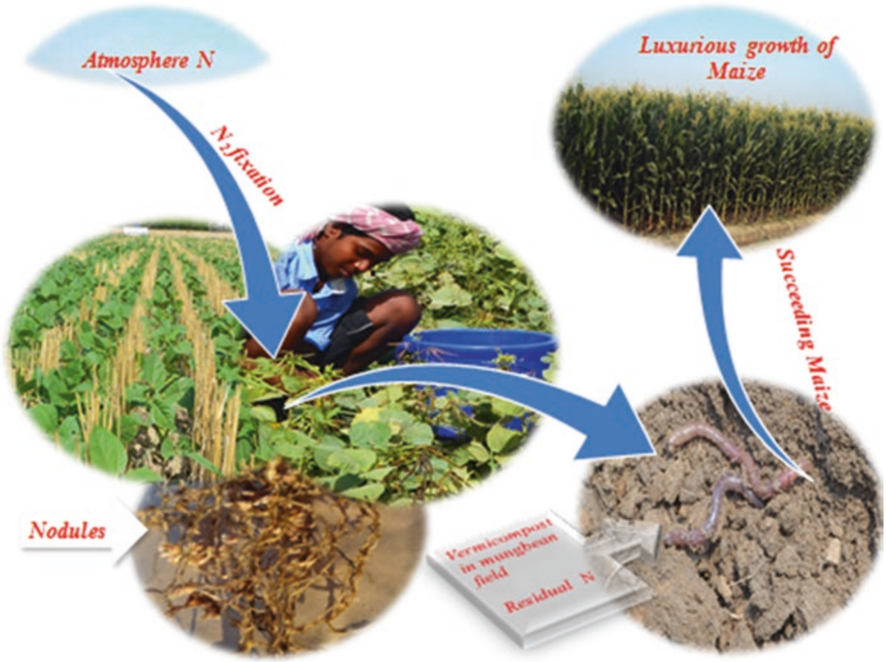

Fig. 9.6 Beneficial effects of legumes on succeeding crop in maize/wheat cropping systems

fully effective (Gibson 1976). Legumes cause significant, positive effect on growth and yield on subsequent non-legumes when compared with rotations with non-legumes (Chalk 1998; Adeleke and Haruna 2012; Dhakal et al. 2016). In addition to its beneficial factors, such as improving soil biodiversity, breaking pest and disease cycles and the phytotoxic and allelopathic effects of crop residues, N is a key factor in the positive response of cereals following legumes (Chalk 1998).

Several studies have reviewed the yield advantage of legumes for the following cereal and cash crops; the example is described as follows:

9.3.1.1 Maize-Based Cropping Systems

Banyong et al. (2000) while examining the amount of N fixed by preceding legume crops observed that the amount of N fixed varied from 20 to 104 kg N ha^{-1} and the net N benefit to the subsequent crops was up to 51 kg N ha^{-1}. Concerning the grain yield advantage in succeeding maize, an increase of up to 34% was obtained using legume crops when compared to the non-legume treatment (Yusuf et al. 2009).

Bonilla et al. (2017) concluded that incorporation of legumes and cover crops causes reduced requirements of N-based chemical fertilizers (by 13–30% for wheat and 49–61% at the rotation level) without experiencing any deterioration in wheat yield and quality. Similarly, the use of green manure to rice was reported to increase the yield of the subsequent wheat crop due to residual effect (Yadav et al. 2000;

Meena et al. 2015d). A residual benefit of legume incorporation is commonly assessed on increased grain and dry matter yields of succeeding crops (Mubarak et al. 2002).

9.3.1.2 Rice-Based Cropping Systems

The practice of using crop rotations with green/brown manure crops and farmyard manure (FYM) is critical to the sustenance of soil fertility and enhanced soil microbial productivity. The addition of leguminous crops into the cereal cropping system is vital for their long-term sustainability, primarily for the legume-led fixing of atmospheric N_2 (Chalk 1998). Further, legumes in rotation with crops increase the organic matter content of the soil and also improve the soil fertility status (Schulz et al. 1999). Biological N-fixing systems offer an economically attractive and ecologically sound means of reducing internal inputs of industrial N fertilizers and in saving internal resources (Ladha and Peoples 1995; Meena et al. 2017b).

Crop rotation with legumes improves soil physical properties and the microbial population (Yusuf et al. 2009) and might, therefore, reduce mineral fertilizer requirements of succeeding leguminous crops. In an experiment involving sequential cropping, a significantly higher N_2 fixation and residual N effect on the succeeding non-legume rice crop were noted for groundnut (*Arachis hypogaea*) than black gram, mung bean and pigeon pea (Ahmad et al. 2001). The growth and N yield of the rice crop were positively correlated with the quantity of N_2 fixed by the preceding legume crop, which led rice yield to become 0.6–1.1 t ha^{-1} higher in the legume-cereal rotation than in the cereal-cereal sequence (Ahmad et al. 2001).

9.3.1.3 Wheat-Based Cropping Systems

Higher yield (by 30%) of wheat was recorded after legumes (field peas, lupins, faba beans, chickpeas and lentils) compared to wheat monocropping (wheat-wheat yield of 4.0 t ha^{-1}) (Angus et al. 2015; Meena et al. 2015d). In temperate environments, cereal yield is on an average 17 and 21% higher in legume-based systems than the wheat-wheat-based system, under standard and moderate fertilization levels, respectively (Jensen et al. 2004).

9.3.1.4 Cotton-Based Cropping Systems

A 3-year cotton-corn-soybean rotation with 134 kg N ha^{-1} $year^{-1}$ had higher soil organic matter (SOM) and crop yield compared to cotton grown every year without a legume crop (Entry et al. 1996). However, the cotton crop cultivation after legume produced with higher oil content (22.87%), seed cotton yields (2428 kg ha^{-1}) and N intake increased up to 91.17 kg ha^{-1}. For improvement in crop productivity, the inclusion of the leguminous crop at least once in a 2-year cropping rotation was suggested, because leguminous crops enrich soil fertility (Kumbhar et al. 2008).

9.3.1.5 Sugarcane-Based Cropping Systems

Leguminous plants can accumulate 5 t ha^{-1} of dry mass in a short period during the summer season and subsequently accumulate more amounts of N and potassium. Most of this N comes from the association of legumes with N-fixing bacteria rhizobia. In this context, crop rotation with legume crops can replace partially or fully the N mineral fertilization of sugarcane, at least for the first ratoon (Ambrosano et al. 2005).

9.3.2 Green/Brown Manures

The addition of crop residue into soils, with the objective of sustaining or improving productivity and soil fertility for the succeeding non-legumes, is known as green manuring. The introduction of green manure's biomass in crop rotation improves soil quality and their beneficial N effects (Jannink et al. 1996). Incorporation of legumes residue using ^{15}N label highlights that 10–34% of the legume N can be recovered in the succeeding rye or wheat crop, 42% in rice, 24% recovery from velvet bean by corn crop, around 15% of N recovery from sunn hemp by corn plants in no-till system, 30% by maize (Ambrosano et al. 2009) and 5% of N recovery from sunn hemp by sugarcane (Ambrosano et al. 2005) and ranged from 19% to 21% when the recovery was observed from sunn hemp by two sugarcane harvests (Ambrosano et al. 2011). Legumes develop deep root systems which enable the acquisition of nutrients from deeper soil layers, and symbiotic N_2-fixing bacteria convert the environmental N into a form. This form is directly available for plant intake.

9.3.3 Intercropping Systems

Intercropping systems consist of synchronized growth of two or more crop species in the same area and at the same time (Brooker et al. 2015). Legumes can contribute up to 15% of the N in an intercropped cereal (Li et al. 2009), thus increasing biomass production (Pappa et al. 2012; Ram and Meena 2014) and reducing synthetic mineral N fertilizer use and mitigating N_2O fluxes. Osman et al. (2011) reported that intercropping with two rows of cowpea and one row of millet gave significantly higher economic benefit than a mixture with one row of each of the crops. Nair et al. (1979) revealed that legumes like cowpea, soybean, pigeon pea and groundnut when grown as intercrops with corn had a beneficial residual effect on the grain yield of the succeeding wheat crop. Similarly, intercropping of sorghum with groundnut, green gram and cowpea reduced by 61, 83 and 38 kg ha^{-1}, respectively, the mineral N fertilizer requirements of wheat for a target yield of 4.0 tons ha^{-1}.

9.4 Leguminous Residual N in Field

The benefits of legumes are usually associated with their N contribution to succeeding crops. Fixation of atmosphere N_2 by legumes in symbiosis with *Rhizobium* bacteria contributes to subsequent non-fixing crops upon decomposition of legume shoot (above portion of the ground) and root material (Bruulsema and Christie 1987; Meena et al. 2015d). Maize grown without mineral N fertilizer following crimson clover produced a higher yield of maize as maize grown following rye with 44 kg N acre^{-1} (Mitchell and Teel 2007). Bruulsema and Christie (1987) reported 56 kg N acre^{-1} contribution from alfalfa residues which resulted in a 2717 kg acre^{-1} maize yield and 56 kg N acre^{-1}contribution from red clover residues that resulted in a 2870 kg acre^{-1} maize yield. Hestermann et al. (1986) observed that crimson clover could replace 48 kg acre^{-1} of N fertilizer. Peanut residues were reported to release 17 kg N acre^{-1} to a succeeding maize crop (Mubarak et al. 2002).

Yano et al. (1994) reported that peanut residue contributed 11.2% N for succeeding wheat (*Triticum aestivum* L.) crop upon decomposition. This was comparable with the application of 30 kg N acre^{-1} as fertilizer. A small amount of N (5–15 kg) is recommended for legume at the initial stage of plant growth. This N gets the host plants off to a vigorous start, allowing rapid development of nodules and subsequent N fixation. However, studies have shown that a large amount of residual N in the soil, either from carry-over or added N, reduces N fixation (Havlin et al. 2014). In general, the host plant expends less energy by utilizing residual soil N than by fixing N through the rhizobia.

Mayer et al. (2003) reported that total N intake of the subsequent crop influenced by the legume used as preceding crop determines the residual N input and the N_2 fixation capacity of the legumes. The succeeding crops recovered 8.6–12.1% of the residual N at harvesting. Similar patterns were found for the microbial biomass, which recovered 8.2–10.6% of the residual N. Berg (1997) highlighted that wheat hay yields averaged 3.1 t ha^{-1} year^{-1} over 5 years following alfalfa, 2.6 t ha^{-1} year^{-1} following milk vetch and 0.95 t ha^{-1} year^{-1} following grass with N intake attributed to the residual effect from legumes averaged 34 kg N ha^{-1} year^{-1} from alfalfa and 25 kg ha^{-1} year^{-1} from milk vetch. Mineral N in root-zone soil following legumes is often 30–60 kg N ha^{-1} higher than after cereal crops in the same environment (Dalal et al. 1998).

9.5 Leguminous Residual Nitrogen in Field

Crop residue of legumes as a source of carbon and N for subsequent non-legume crops was found in low-input agriculture production systems. Several studies have reported that the increase of crop yield with legume residue inclusion in the field (Paré et al. 1992) and enhanced soil fertility by the providing of BNF (Ladd et al. 1983; Dhakal et al. 2016). Crop residues provide SOC and N to soil organism and physically protect soil from erosion. Legume crop residues are robust; they protect soil from erosion (wind and water) and help in improved soil physical properties and fertility. The ecosystem's nutrient retention, conserve soil moisture, help in carbon sequestration, reduce weed, help in hydraulic conductivity, help in water holding capacity of the soil and water infiltration and can contribute to climate change adaptation and mitigation. On the whole, they help ensure food, soil health and water security over a long term (Kabir and Koide 2002).

Legume crop residues decompose due to the presence of microorganism. This is done through the process of mineralization or immobilization and the release of plant nutrients into soil solution. They are easily available to subsequent non-legume crops. If properly incorporated, these crops do not require the application of N fertilizer. Legume residues contain huge amounts of N and have a relatively low C/N residue, leading to the more rapid release of N than lower N-containing cereal residues. Work by Sawatsky and Soper (1991) at the University of Manitoba reported that up to 44% of N fixed by legumes remained in the soil. This fixed N continuing in the soil would become available for succeeding non-legume crops. Enhanced N (N) availability to crops following legumes may also be due to reduced immobilization, as legume crops commonly produce lower amounts of residues along with higher N concentration than do cereal crops.

9.6 N Leaching

An appropriate cropping system and best management practices can help minimize the leaching risk besides improving N use efficiency (NUE). Legume intercropping in cereal-based system grown in wider crop rows can reduce the nitrate leaching risk (Weil and Brady 2017). Parallel multiple cropping (a system of growing two crops with dissimilar growth habits with minimum competition) of sugarcane and black gram or pigeon pea and maize resulted in low nitrate content in the soil profile when compared to sole cropping (Yadav et al. 2000). Soybean (*Glycine max*) seems to reduce the nitrate concentration in the soil profile more than maize.

9.7 Legumes and Soil Properties

Legume-based cropping systems improve several aspects of soil fertility, such as SOC, and major and micronutrient availability (Jensen et al. 2012). With respect to SOC, grain legumes can increase it in several ways, by supplying biomass, organic C and N (Lemke et al. 2007; Garrigues et al. 2012), as well as releasing the H_2 gas as by-product of BNF, which promotes bacterial legume nodules' development in the rhizosphere (La Favre and Focht 1983; Ram and Meena 2014). Although there is a general agreement on the influence of grain legumes on rhizosphere properties in terms of N supply, SOC and P availability, the magnitude of the impact varied across legume species, soil properties and climatic conditions. Among these, the soil type represents the major factor determining plant growth, rhizosphere nutrient dynamics and microbial community structure (Stagnari et al. 2017). The pattern of depletion and accumulation of some macro- and micronutrients differed also between cropping systems (i.e. monoculture, mixed culture, narrow crop rotations) as well as among soil management strategies (i.e. tillage, no tillage) (Shaha et al. 2003). Legume-based cropping system increased the soil organic matter. The average rate of sequestration or addition is ~1.42 Mg C ha^{-1} $year^{-1}$ in the soil profile of metre depth in soil with legume-based cropping system (Ahmad et al. 2001)

9.8 Legumes Mitigate Environmental N Emission

The nitrogen fertilization accounts for 60% of N_2O anthropogenic emissions through agricultural practices. Agriculture is also contributing to other greenhouse gas (GHG) emissions, such as CH_4 and CO_2. In this context, Rees et al. (2013) considered that potential strategies for reducing GHG emissions in a cropping system could be developed by making changes in the variables/interventions (Meena et al. 2015c). These interventions influence the biochemical processes that trigger GHG emissions from soils, as a result of agricultural operations (e.g. tillage, fertilization, irrigation and crop rotation). Total emissions of CO_2 and N_2O from legumes are less than those from N-fertilized crops. Legumes contribute to the mitigation of climate change.

9.8.1 Legumes and Mitigation Potential of GHGs

Nitrous oxide is an important anthropogenic GHG which contributes around 5–6% of the total atmospheric GHGs, but it is abundantly more active than CO_2 (Crutzen et al. 2007). It plays a major role in ozone depletion (Ravishankara et al. 2009). Agriculture is considered as the largest N_2O source (Robertson et al. 2004; Takle et al. 2008). Total global N_2O emissions from agricultural soils are estimated to be 2.1 million tons N year^{-1} (Jensen and Hauggaard 2003) and will continue to increase annually at a rate of 0.25% (Kaiser et al. 1998). In general, N_2O is produced from the soil by microbial conversions, that is, ammonification, nitrification and denitrification, especially when N availability exceeds plant requirements (Smith and Conen 2004).

Globally, it is estimated that 150–200 million tons of mineral N is required annually by the plants, out of which nearly 100 million tons of N is fixed through the industrial Haber-Bosch process (Unkovich et al. 2008) and 175 million tons of N fixed through biological N fixation of atmospheric N_2 yearly (Chafi and Bensoltane 2003). Undoubtedly, the N_2-fixing ability of the legumes minimizes synthetic N input in soil and does minimization of negative environmental impact (Lupwayi et al. 2010; Kumar et al. 2016).

9.8.2 The Role of Cropping Systems and Inclusion of Legumes in Mitigation of GHGs

Appropriate management of cropping systems allows greater carbon sequestration; intensification of the cropping system is one of the strategies used to mitigate climate change. It may include incorporation of pulses such as field pea, common bean, soybean, faba bean and lentil; rotation of forages such as alfalfa, meadow brome, timothy grass and cocksfoot; and use of cover crops such as annual clover, red clover, hairy vetch, ryegrass and yellow sweet clover. Cropping systems provide

Table 9.6 Total N_2O emissions from field-grown legumes, N-fertilized grass pastures and crops or unfertilized soils

S. no.	Crop/species	Total N_2O emission per growing season or year (kg N_2 ha^{-1}) Range	Mean	References
N-fertilized crops	Rice	0.2–5.0 (3)[a]	2.21	Gupta et al. (2016)
	Wheat	0.09–8.57 (18)	2.73	Jensen et al. (2012)
	Maize	0.16–12.67 (22)	2.72	Jensen et al. (2012)
	Canola	0.13–8.60 (8)	2.65	Jensen et al. (2012)
N-fertilized pasture[a]	Grass	0.3–18.16 (19)	4.49	Jensen et al. (2012)
Mean of fertilized systems				
Pure legume stands[b]	Alfalfa	0.67–4.57 (14)	1.99	Jensen et al. (2012)
	White clover	0.50–0.90 (3)	0.79	Jensen et al. (2012)
Mixed pasture sward[b]	Grass-clover	0.10–1.30 (8)	0.54	Jensen et al. (2012)
Legume crops[b]	Faba bean	(1)	0.41	Jensen et al. (2012)
	Chickpea	(5)	0.05	Jensen et al. (2012)
	Lupin	0.03–0.16 0 (1)	0.06	Jensen et al. (2012)
	Field pea	0.38–1.73 (6)	0.65	Jensen et al. (2012)
	Soybean	0.29–7.09 (33)	1.58	Jensen et al. (2012)
Mean of all legumes			1.29	
Soil	No N fertilizer or legume	0.03–4.80 (33)	1.20	Jensen et al. (2012)

[a]Parenthesis values are highlighting the total number of experimentation sites
[b]Data come from systems where either zero N fertilizer was used or legume crops were provided with just 5 kg mineral N fertilizer ha^{-1} as starter N at the time of sowing. This is barring two experiments with grass-clover pastures and three soybean studies where 35–44 kg N fertilizer ha^{-1} had been applied

opportunities in reducing N_2O while improving energy use efficiency and in increasing C sequestration (Table 9.6). The introduction of legume crops could have an impact on soil microorganisms, including symbiotic and asymbiotic N_2-fixing bacteria, mycorrhiza and soil fauna. Legume crops contribute to mitigation of GHGs by replacing the N requirement of the cropping system than without the inclusion of pulses (Lupwayi et al. 2010; Ram and Meena 2014; Gregorich et al. 2005) found that emissions of N_2O from soils increased linearly with the quantity of mineral N fertilizer applied and because systems containing legumes produce lower annual N_2O emissions. Legumes emit around five to seven times less GHGs per unit area when compared to non-legume crops. Measuring N_2O fluxes, it was shown that peas emitted 69 kg N_2O ha^{-1}, much less than wheat (368 kg N_2O ha^{-1}) and rape (534 kg N_2O ha^{-1}) (Jeuffroy et al. 2013).

Guardia et al. (2016) reported that the emission of N_2O was higher for barley (non-legume) compared to vetch and lentil (legumes); moreover, the N_2O fluxes derived from the chemical fertilizers added to the crops were 2.5 times higher in barley than vetch. Schwenke et al. (2015) demonstrated that the cumulative N_2O

emissions from N-fertilized canola (385 g N_2O ha^{-1}) greatly exceeded those from chickpea (166 g N_2O ha^{-1}), faba bean (166 g N_2O ha^{-1}) and field pea (135 g N_2O ha^{-1}). When faba bean (441 g N_2O ha^{-1}) was grown through monocropping, it led to three times higher cumulative N_2O emissions than that of unfertilized wheat (152 g N_2O ha^{-1}); conversely, when faba bean was mixed with wheat (intercropping system), cumulative N_2O emission fluxes were 31% lower than that of N-fertilized wheat (Senbayram et al. 2016). The mitigation of GHG emissions is also achieved by adopting sustainable agricultural systems, such as conservation tillage and conservation agriculture systems, which are suitable for the cultivation of both grain and green manure legumes. Emissions of N_2O tend to be lower under legumes when compared to N-fertilized crops and pastures, mainly when commercially relevant rates of N fertilizer are applied.

9.9 Conclusion

Restoring the essential plant nutrients that are removed or lost from the soil remains central to long-term productivity, profitability and sustainability of any farming system. The emerging role of legume crops becomes evident in enhancing crop productivity along with retaining soil fertility and environmental quality. Besides serving as high-quality food and feed worldwide and allowing BNF, legume crops offer a range of other benefits. These benefits include considerable positive impact on biodiversity and soil health. Introducing legumes to modern cropping systems will not only improve crop diversity but also contribute to reduced use of imported inputs (especially N fertilizer). This would be in conjunction with enhanced yields of succeeding non-legumes. This also demands great efforts to broaden the scope of legume crops and their numerous positive advantages. These advantages are aimed towards sustainable intensification of agriculture besides the livelihoods of millions of farmers across the world.

Future Prospective

Pulses are always praised for dual benefits of soil health and human health. The dual benefits are directly related to ecological and economical upliftment of society. In a bid to heighten the public awareness of the environmental and nutritional benefits of pulses, as part of sustainable food production for achieving food, nutrition and environmental security, the 68th United Nations General Assembly declared 2016 as the International Year of Pulses. To envisage the importance of pulses which are equally good for people and soil, their sustainability should be given priority. It is also well understood and proved by the researchers globally that pulses have the ability to fix hefty quantities of nitrogen in the soil, by boosting soil fertility and reducing dependence on external nutrients (millions of tons globally). It is likely, of the other crops, pulses use half the non-renewable energy inputs including nitrogen. This results in remarkably small carbon footprints. The key message “Soil and Pulses: Symbiosis for Life” is the prelude to the sustainability, which will remain unfulfilled without pulses. Additionally, the importance of pulses in crop rotation, cropping system, intensification and diversification has also been explained in the text.

The vagaries of unforeseen negative factors of productivity need to be tackled smartly and cleverly. There are a few lines of research milestones that can help in augmenting soil and environmental benefits via pulses. Some of them are:

1. Developing short-duration high-yield yielding pulse varieties: Having the pulses and their varieties matching crop maturity duration to existing cropping window could be beneficial to cereals and vice versa. This way crop intensification, diversification and mutual sharing of critical natural resources especially water, inherent soil nutrients, sunlight, etc. may be utilized efficiently.
2. Pulses in new niches: Popularizing pulses in unexploited or untapped area, viz. rice fallows, which have approximately 14.3 million hectare area in Indo-Gangetic Plains (IGP), especially in Eastern India, spread over four Asian countries—Pakistan, India, Nepal and Bangladesh. These rice fallows offer a huge potential and scope to expand pulses, thereby improving soil, environmental and nutritional health. Adding more area under pulses directly helps in adding more SOC and releasing external nutrient input dependence. Further, development of short-duration/extra-early/super-early pigeon pea varieties for different agro-ecologies might enhance cropping intensity through pulses.
3. Nodulation engineering: As nodulation degeneration starts in pulses after the flowering initiation stage, this leads to declining or stoppage of nitrogen fixation. If the pulse roots are so engineered and nodulation period is prolonged, then it may be a marvellous nitrogen economy for the pulses as well as the succeeding crops.
4. Smart microbial strains: Due to non-availability and production of effective and efficient strains of *Rhizobium*, the full potential on nitrogen fixation by the legumes is tingling. Additionally, developing efficient strains for other nutrients (P, K, S, Zn, Fe, Mo) might have positive interaction with N, and thereby it may save millions of tons of respective nutrient fertilizers too.
5. Smart pulses: The development of smart pulse plant types could tackle the future problems of climate change and speedy depletion of natural resource base. Further, developing multi-nutrient-efficient genotypes/cultivars of pulses through the use of high-end technologies, viz. transgenic, next-generation genomic tools, coupling with classical breeding might become a boon to avoid external nutrient use.

Summing up, this chapter points out that pulses are not only N factory but also could be futuristic nutrient factory.

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