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Nitrogen Fixation and Transfer in Agricultural Production Systems

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Abstract

There is a consensus within the scientific community that nitrogenous fertilizers are almost indispensable in today's agriculture. However, the geometric increase in nitrogenous fertilizer applications and the associated environmental concerns call for focus on more sustainable alternatives. Biological dinitrogen (N_2) fixation (BNF) is one of the most sustainable approaches to meeting crop nitrogen (N) demands. The BNF is, especially, important in low value crops (e.g., forages) and in developing economies. However, just like synthetic N fertilizers, BNF has issues of its own. Among the issues of great importance is the low and highly variable proportion of fixed N_2 transferred to non- N_2 -fixing plants. The proportion of transfer ranges from as low as 0% to as high as 70%, depending on a myriad of factors. Most of the factors (e.g., N fertilizer application, species, and cultivar selection) are management related and can, therefore, be controlled for improved N_2 fixation and transfer. In this chapter, we discuss current trends in BNF in selected legume crops, the global economics of BNF, and recent reports on N_2 transfer in agricultural production systems. Additionally, factors affecting N_2 transfer and management considerations for improving N_2 fixation and transfer are discussed.

Keywords: biological nitrogen fixation, nitrogen transfer, fertilizers, legumes, grass-legume mixtures

1. Introduction

Plants require N in relatively large quantities to grow and reproduce. In fact, N is the third most important factor in the growth and development of crop plants [1]. This made N one of the most important nutrients in agricultural production systems. The important role N plays in global food production is evident in the ever-increasing amounts of N fertilizers applied annually. It has been estimated that approximately 100 Tg of synthetic N fertilizers

were applied in 2009 [2]. The geometric increase in N fertilizer use worldwide is in part, attributable to the need to produce enough food to feed the over 7 billion people currently living on earth. Although there is a consensus within the scientific community that N fertilizers are almost indispensable in today's agriculture, there are great concerns with the use of N fertilizers. Some of these include pollution of surface and underground waters, greenhouse gas (e.g., nitrous oxide: N_2O) emissions, and low N use efficiency (NUE). There is, therefore, a multi-pronged approach to N management in global food production. While N fertilizers are being increasingly applied to crops to increase crop productivity, there are calls for more sustainable approaches to meeting N demand of crops such as climate-smart agriculture and sustainable intensification.

The BNF, the process whereby micro-organisms use nitrogenase enzyme to convert atmospheric inert N_2 to plant usable forms [3, 4], was the main source of N prior to the industrial revolution [5]. It is generally agreed that BNF is one of the most sustainable approaches to meeting crop N demands. For example, it has been estimated that NUE increases exponentially with increasing levels of biologically fixed N_2 in soils while NUE decreases linearly with increasing levels of applied synthetic N fertilizers [2]. There are concerns about the best approach for quantifying inputs of fixed N_2 . Conservative estimates based on harvested areas and yields from 2005 Food and Agricultural Organization (FAO) database on world crop production (FAOSTAT) showed that 2.95 and 18.5 Tg N was fixed annually by pulses and oilseed crops, respectively [6]. Soybean (*Glycine max* (L.)) fixed 16.4 Tg N, representing 77% of total N_2 fixation by legume crops in 2005 [6]. Although BNF contributes ~25 Tg N which is dwarfed by the ~100 Tg contributed by synthetic N fertilizers [2], the importance of BNF to the global N budget is substantial.

Just like synthetic fertilizers, BNF has issues of its own. Among the issues of great importance is the transfer of fixed N_2 to non- N_2 -fixing plants. The proportion of biologically fixed N_2 transferred to neighboring plants can range from as low as 0% to as high as 73%, depending on a myriad of factors [1]. The biology, chemistry, and processes involved in BNF have been extensively described in the literature [7–12]. Therefore, in this chapter, we discuss briefly the organisms involved in BNF and then proceed to current trends in global N_2 fixation and value of BNF transfer in agricultural production systems with special emphasis on N_2 fixation from *Rhizobia*-legume symbiosis. Finally, we summarize current findings on N transfer in agricultural systems, discuss the factors responsible for low and variable transfer of biologically fixed N_2 and provide some suggestions for improved transfer of fixed N_2 .

2. Biological dinitrogen fixation: importance and economics

Several micro-organisms can convert inert atmospheric N_2 to plant usable forms. These organisms may exist in association and symbiosis with host plants or independent of a host plant (Table 1). Organisms relying solely on atmospheric N_2 as their N source for growth are referred to as diazotrophs [7]. Biological N_2 fixation is a significant source of N in agricultural and natural ecosystems. The N input from BNF is particularly important in low value crops (e.g., forages)

Micro-organism	Properties and importance
Rhizobia	Symbiosis with roots of legumes (nodules); important source of N for legumes; proper Rhizobia strains required for effective nodulation and N ₂ fixation
Frankia (Actinomycetes)	Symbiosis with non-legume angiosperms (e.g., <i>Alnus</i> , <i>Myrica</i> , <i>Alder</i> , <i>Casuarina</i>); important source of N in agroforestry
Anabaena	Autotrophic; mostly aquatic but can be terrestrial; symbiosis with non-legumes (e.g., <i>Azolla</i> sp.); important in paddy rice (<i>Oryza sativa</i> L.) production; can be utilized as green manure
Bradyrhizobium	Aerobic, heterotrophic, free-living N ₂ -fixer
Azospirillum	Microaerophilic; heterotrophic; free-living N ₂ -fixer or in association with grass roots; can be important source of N for non-legumes
Acetobacter	Heterotrophic; endophytic, can be important source of N for sugarcane (<i>Saccharum officinarum</i> L.) and some tropical grasses
Azotobacter	Aerobic; heterotrophic; free-living N ₂ -fixer
Cyanobacteria	Autotrophic; free-living N ₂ -fixer (e.g., <i>Escherichia coli</i>) or symbiotic; symbiosis with lichens (fungi), cycads, etc.

[†]Modified from [3, 7, 18].

Table 1. Properties of selected micro-organisms involved in biological N₂ fixation in agriculture and natural ecosystems[†].

and developing economies, where farmers either have limited access to synthetic N fertilizers or are unable to afford N fertilizers. In fact, forage accumulation and profitability from grass-legume mixtures have been reported to be equal or greater than N-fertilized grass monocultures [13–15]. Aside direct N input from BNF, N from BNF reduces the amount of synthetic N fertilizers applied in agriculture and natural ecosystems. This, in turn, reduces cost of production, greenhouse gas (GHG) emissions, and pollution of surface and underground waters. Low NUE and N recovery are major issues associated with use of N fertilizers [16, 17]. In a comprehensive analysis, Lassaletta et al. [2] showed that the efficiency of N use of biologically fixed N₂ is greater than synthetic N. Among the micro-organisms involved in BNF, N₂ fixation from *Rhizobia*-legume symbiosis is a significant source of N in agriculture. Needless to say, BNF from associative and free-living bacteria and diazotrophs are important in natural ecosystems and water-logged production areas (e.g., paddy fields) [6].

2.1. Amount and value of N₂ fixed by legumes

The amount of N₂ fixed from *Rhizobia*-legume symbiosis varies greatly depending on many factors. These include, but not limited to, plant species and cultivar, residual soil N, *Rhizobia* strains, and environmental conditions. Generally, perennial forages fix greater amounts of N₂ compared to annual forages since they live longer in the field [18]. For example, estimated total BNF from alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), and white clover (*Trifolium repens* L.) are 465, 252, and 102 kg N ha⁻¹ year⁻¹ while from faba bean (*Vicia faba* L.), field pea (*Pisum sativum* L.), and lentil (*Lens culinaris* Medik.) are 165, 111, and 52 kg N ha⁻¹ year⁻¹, respectively [19]. Estimates of N₂ fixation from selected crops has shown that in 2014, up to 29

Tg N was fixed by eight crops (**Figure 1**). Soybean (*Glycine max* (L.) Merr.) alone contributed 23.4 Tg, representing 81% of total N₂ fixed by these crops (**Figure 1**). While these might not be precise estimates, there is a clear indication that the contribution N₂ fixation to the global N budget is enormous. Though N₂ fixation from peas, lentils, common bean (*Phaseolus vulgaris* L.), faba bean, cowpea (*Vigna unguiculata* (L.) Walp.), chickpeas (*Cicer arietinum* L.), and groundnut

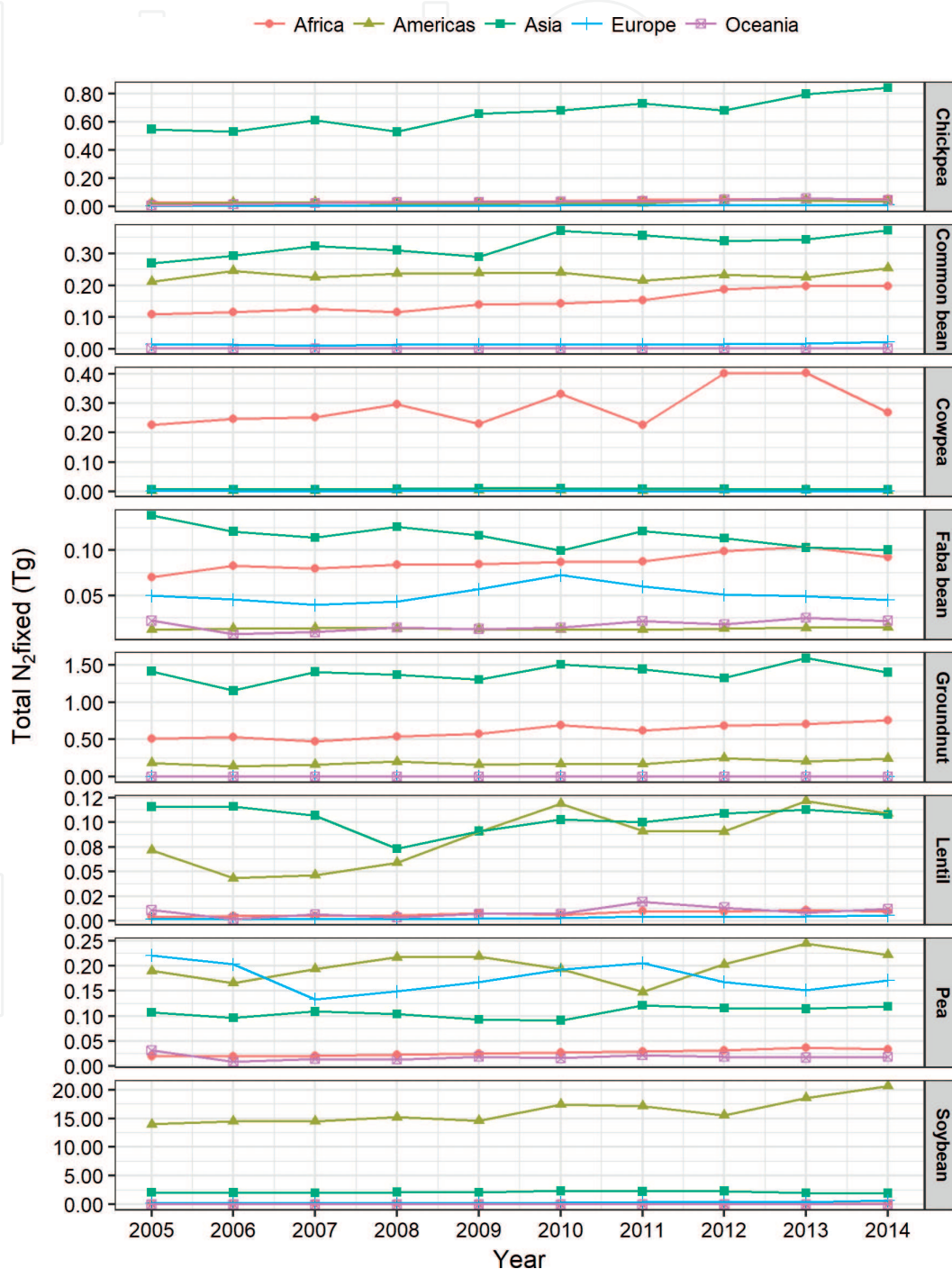


Figure 1. Estimates of global trends in biological N₂ fixation for selected legume crops. The N₂ was estimated based on harvested areas and yield data from Food and Agricultural Organization (FAO) database on world crop production (FAOSTAT) [21]. This follows the procedure described by [6].

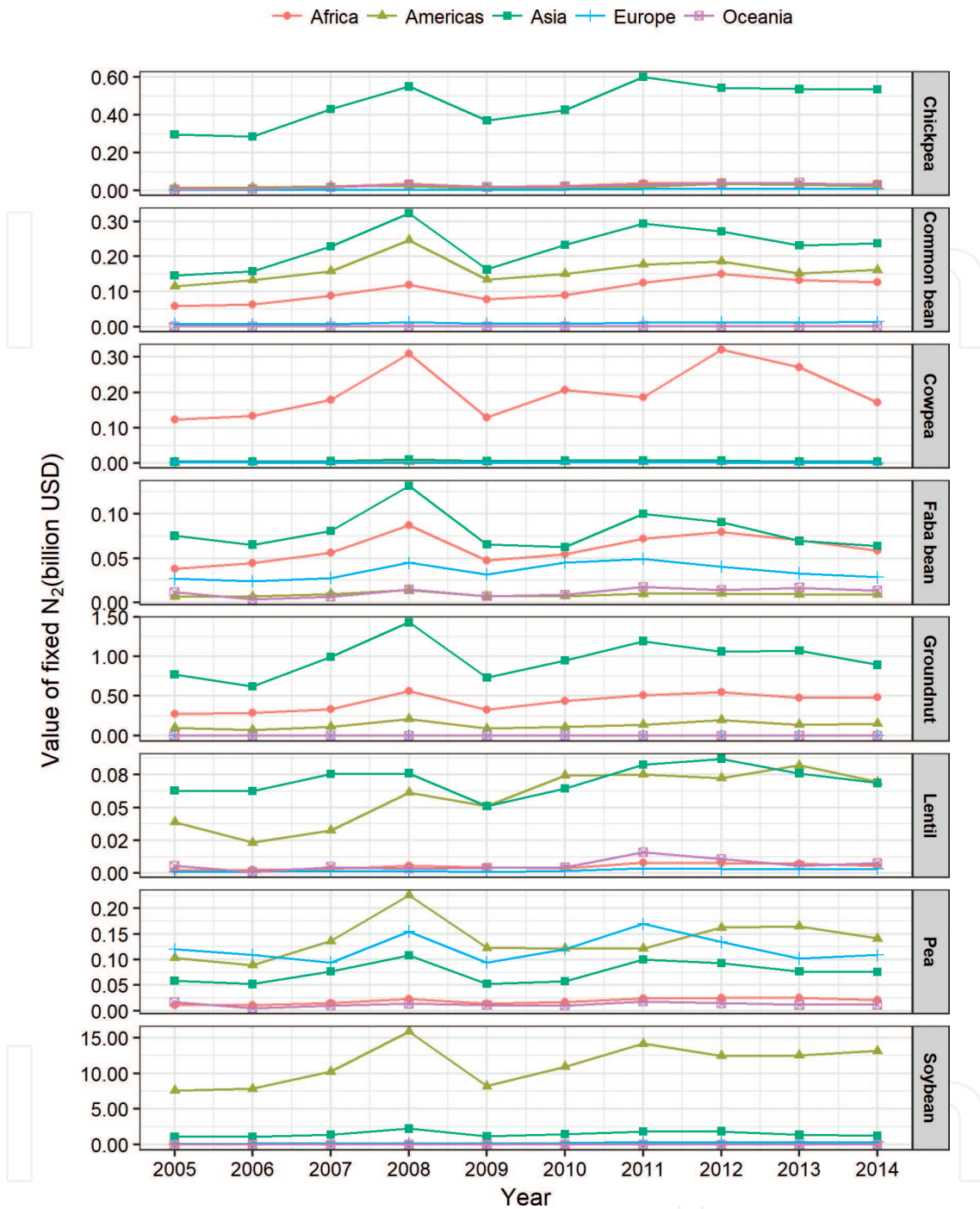


Figure 2. Trends in global economics of biological N_2 fixation. Value of fixed N_2 was calculated based on estimated N_2 fixation (Figure 1) and price of urea fertilizer from 2005 to 2014 reported by the World Bank [20].

(*Arachis hypogaea* L.) is dwarfed by soybean (because of the larger area planted to soybean) based on these estimates, the contribution of N_2 fixation from these crops (e.g., cowpea) to farmers in developing countries is substantial. Unlike forages, grains from grain legumes are harvested and removed from the field. Thus, grain legumes usually remove more soil N than forages [18]. The uncertainties associated with estimating N_2 fixation from forages, extensively grazed savannas, sugarcane (*Saccharum officinarum* L.), and rice (*Oryza sativa* L.) production

systems have been acknowledged [6]. Nonetheless, the estimated annual N_2 fixation from these systems are 5 Tg from rice, <4 Tg from non-legume crops, 12–25 Tg from pasture and fodder legumes, 0.5 Tg from sugarcane, and <14 Tg from extensive savannas. It is worth mentioning that biologically fixed N_2 must be transferred to neighboring and subsequent non- N_2 -fixing crops in the cropping systems for optimum benefits. Nitrogen transfer in cropping systems is often low. Thus, all the estimated N_2 fixed (**Figure 1**) may not be transferred to neighboring and subsequent non- N_2 -fixing crops.

The economic value of N_2 fixation is extraordinarily large. Of course, the value of biologically fixed N_2 is directly related to the amount N_2 fixed. Using estimates of N_2 fixation from **Figure 1** and cost of urea N fertilizer from the World Bank [20], it is estimated that in 2014, the value of N fixed by these eight crops is about 18.5 billion US dollars (**Figure 2**). Of this amount, about 14.9 billion (81%) is contributed by soybeans.

3. Management considerations for improving biological dinitrogen fixation

There are several management practices that influence BNF in agricultural production systems. These include but not limited to N-fertilization [22], species [23], genotype and cultivar [24], and seeding ratios (intercropping systems). Adopting best management practices can, therefore, improve N_2 fixation. In mixed swards, perennial ryegrass (*Lolium perenne* L.) competition for available soil N was reported to be important in determining N_2 fixation in birdsfoot trefoil (*Lotus corniculatus* L.), alfalfa, and white clover [25]. Species may differ in their reliance on soil N and fixed N_2 . In a red clover-grass-forbs mixture, grass relied mostly on fixed N_2 , while forbs relied on soil N [23]. Selecting compatible cultivars (**Figure 3**) and species may improve N_2 fixation and N_2 transfer in agricultural production systems [26]. For example, the proportion of N_2 derived from BNF was 75–94% in white clover monoculture compared to 85–97% in white clover-ryegrass mixtures [27]. The relatively greater N_2 -fixation in grass-legume mixtures compared to legume monocultures might be attributable to greater competition for soil N from non- N_2 -fixing plants [28]. In an extensive review, Rouquette and Smith [29] asserted that BNF in forage legumes may vary depending on the legume cultivar, species, soil nutrient composition, prevailing environmental conditions, and climate. The myriad of factors influencing BNF might explain the varied amounts of N_2 fixed by legumes even at same locations reported by many researchers [30–35]. For example, at the same location, the proportion of plant total N derived from BNF was reported to range from 12 to 96% on grazed plots [36]. Application of N fertilizers has been found to suppress BNF in legumes [22]. For example, the application of N fertilizer decreased atmospheric derived N_2 of clover from 77 to 43% [37].

The strain of *Rhizobia* also determines the level of N_2 fixation [38]. Most of these *Rhizobia* strains are highly specialized and due to this specialization and the intricacy of interaction between N_2 -fixing plant species and bacteria involved in N_2 fixation, any disturbance or manipulation may be detrimental to the amount of N_2 fixed [39]. Thus, inoculation with the right strains of *Rhizobia* would improve N_2 fixation. There are three major constraints to BNF in grass-legume

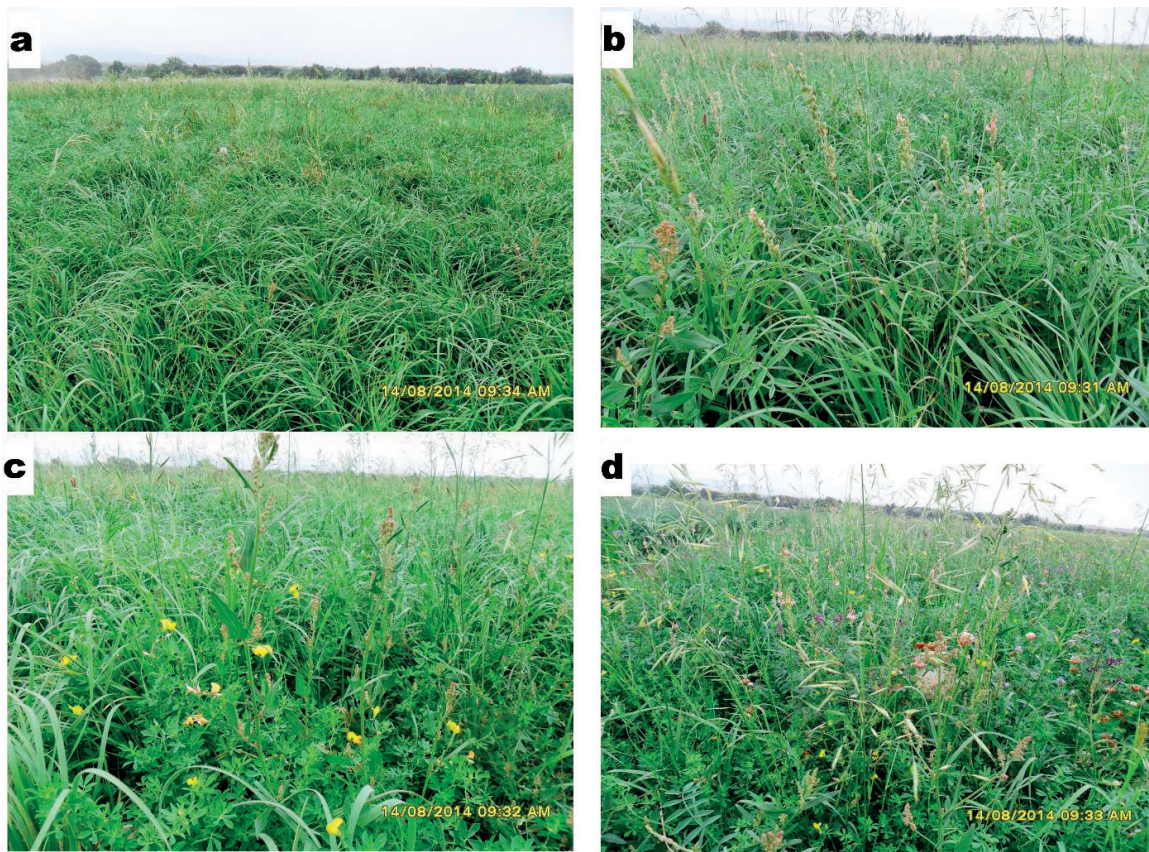


Figure 3. Established stands of meadow bromegrass (*Bromus biebersteinii* Roem. & Schult.) monoculture (a) and meadow bromegrass: 50% sainfoin (*Onobrychis viciifolia* Scop.) (b), 50% meadow bromegrass:50% birdsfoot trefoil (c), 50% meadow bromegrass:16.7% sainfoin:16.7% birdsfoot trefoil:16.7% alfalfa (d) seeding ratios in 2014 at the University of Wyoming Sheridan Research and Extension Center, WY, USA.

mixtures and these include: low forage yield, low proportion of legumes in mixtures, and low reliance of the legume on N_2 fixation [40]. To maintain optimal N_2 fixation, sufficient legume populations must be maintained in grass-legume stands. This might be difficult to achieve because of the selective grazing of legumes by livestock (in grazing systems), poor soil conditions, and pest and disease problems [28]. However, using optimal seed mass ratios and good grazing and haying practices may help maintain optimal legume proportions [15].

4. Transfer of biologically fixed nitrogen in agricultural production systems

Biologically fixed N_2 satisfies the immediate N needs of the host plants. However, the fixed N_2 can be transferred to other crops in the cropping system, especially non- N_2 -fixing plants. The transfer is accomplished through three main routes, viz.: decomposition of nodules and secondary roots that are not thickened, exudates of soluble N compounds, and transfer mediated by mycorrhizal fungi [1, 41–43]. The transfer of N through nodule and root decomposition and exudation of N compounds is termed as rhizodeposition [44]. The proportion of biologically

fixed N₂ transferred to neighboring or succeeding crop plants is highly variable [45]. This can range from as low as 0% to as high as 73%, depending on a myriad of factors [1]. In an extensive review, rhizodeposition was reported to vary from 4 to 71% [44]. Review of literature from 2015 to 2017 on transfer of N in selected crops has shown that N transfer ranged from 0 to 70% (**Table 2**). Among the three main N transfer routes, rhizodeposition through decomposition of the nodules and roots represents the main pathway of N transfer.

Nitrogen transfer from signal grass (*Brachiaria decumbens* Stapf.) to stylo (*Stylosanthes guianensis* (Aublet) Sw.) was reported to be mainly through decomposition of roots compared to root exudates and transfer mediated by mycorrhizae [46]. This might be particularly true for forage species since aboveground biomass is the economic part of the plant. Additionally, non-tree legumes have relatively greater proportion of fine roots that have faster turnover rate. It must be noted that despite the greater contribution of decomposition of the nodules and roots to N transfer, this transfer route is relatively slower compared to exudates of soluble N compounds and transfer mediated by mycorrhizae [1]. Nitrogen transfer from the tropical legume, gliricidia (*Gliricidia sepium* (Jacq.) Kunth ex Walp.) to yellow-blue stem (*Dichanthium aristatum* (Poir.) C.E. Hubb.) was reported to be mainly via root exudates [47]. In a short-term rhizodeposition study, 3.5 and 5.3% N was rhizodeposited through root exudates in white clover monocrop and white clover-perennial ryegrass mixture, respectively, over a 3-day period [42]. This significant N transfer within a short period is an indication of the importance of exudation of N compounds in meeting N needs of crops, especially during early growing stages [42]. It is well documented that mycorrhizae can facilitate the transfer of biologically fixed N₂

Crop(s)	Amount of N transferred (% of fixed N)	Reference(s)
Caragana (<i>Caragana arborescens</i> Lam.)-oat (<i>Avena sativa</i> L.)	38–45 kg ha ⁻¹ (60–70) [§]	[62]
Alfalfa-tall fescue (<i>Schedonorus arundinaceus</i> (Schreb.) Dumort.)	0–650 kg ha ⁻¹ (0–12) [†]	[66]
White clover-perennial ryegrass	0–340 kg ha ⁻¹ (0–47) [†]	[66]
Mung bean-oat	12.8 mg plant ⁻¹ (9.7)	[68]
Soybean-maize	7.84 mg pot ⁻¹ (7.57)	[53]
Soybean-maize	10.77–13.72 mg pot ⁻¹ (1.26–2.17)	[55]
Faba bean-wheat	0.17 mg plant shoot ⁻¹ (14.9)	[52]
Red clover-bluegrass (<i>Poa pratensis</i> L.)	35.85 mg plant ⁻¹ (1.5)	[24]
Pigeon pea (<i>Cajanus cajan</i> (L.) Millsp.)-coffee (<i>Coffea arabica</i> L.)	21.8 g kg ⁻¹ (na)	[63]
Crotalaria-coffee	13.5 g kg ⁻¹ (na)	[63]
Velvet bean (<i>Mucuna pruriens</i> (L.) DC.)-coffee	19.7 g kg ⁻¹ (na)	[63]
Red clover-perennial ryegrass and forbs	25–58 kg ha ⁻¹ (9.5–15)	[23]

na, could not be estimated from data.

[§]4 m distance from caragana shelterbelt.

[†]Cumulative over 3-year period.

Table 2. Amount of nitrogen (N) fixed and proportion transferred to soil or neighboring plants in agricultural systems.

from N₂-fixers to non-N₂-fixing plants [48–51]. In a rice and mung bean (*Vigna radiata* L.) intercropping study, arbuscular mycorrhizal fungi (AMF) inoculation increased N transfer from 5.4 to 15.7% [49]. Proportion of fixed N₂ transferred from faba bean to wheat (*Triticum aestivum* L.) was 50% when inoculated with AMF compared 15% in uninoculated stands [52]. Similar results were also reported in garden pea-barley (*Hordeum vulgare* L.) and soybean-maize (*Zea mays* L.) intercropping studies [48, 53]. The AMF-mediated transfer of N can be both unidirectional and bidirectional [48, 54] and often along with a concentration gradient [47]. Thus, transfer of N from N₂-fixing plants to non-N₂-fixers is often expected to be greater than from non-N₂-fixing plants to N₂-fixers [55].

5. Factors affecting nitrogen transfer

It has long been acknowledged that since plant N composition is partitioned into various plant organs or parts, not all the N₂ fixed by plants will be transferred to neighboring plants or succeeding plants in cropping systems [56]. However, there are a number of biotic and abiotic factors influencing N transfer in agricultural production systems [1]. Environmental factors such as water, temperature, and light have direct and indirect effects on N transfer in cropping systems. Soil moisture has a great influence on decomposition and it is required for the uptake of N. Thus, moisture stress affects both the mineralization of fixed N₂ and uptake of mineralized N by plants. However, moisture stress promotes nodule senescence, implying that more nodule biomass will be available for mineralization during moisture stress conditions [57]. Nitrogen is highly soluble. Thus, excess water can result in N leaching out of the rooting zone of plants making it unavailable for uptake. Flooding (e.g., low land rice production systems) results in anaerobic conditions, and thus could result in gaseous N losses in the form of N₂O [18]. Optimum light conditions (quality, quantity, and duration) and temperature have a direct effect on photosynthesis and hence, promote both N₂ fixation and transfer. For example, nodule activity and N exudation from roots of soybean and sesbania (*Sesbania cannabina* (Retz.) Poir.) were the greatest at 30 and 35°C day and night temperatures, respectively [58]. Prolonged dark treatment affected nodule functioning in barrel medic (*Medicago truncatula* Gaertn.) and induced nodule senescence [59]. This condition is common in intercropping systems (e.g., grass-legume mixtures) [1], especially in species with varied canopy heights.

A common practice in agricultural production systems is intercropping N₂-fixing legumes with non-N₂-fixing crops (**Figure 3**) [15]. This is particularly important in low value crops (e.g., forages) and in developing countries. In intercropping systems, the proximity of the N₂-fixing crop to the non-N₂-fixing determines the amount of N transferred. The concentration of N in the rhizosphere is the greatest closer to the root surface [60]. Therefore, N transfer predominantly occurs in upper soil layers [23]. Since N uptake is along with concentration gradients [47], close proximity between N₂-fixing legumes and non-N₂-fixing crops reduces the distance of travel for dissolved N compounds [1]. Close proximity is achieved either through direct root contact or mycorrhizal hyphae connections [61]. However, Issah et al. [62] reported that maximum oat productivity was obtained when grown 4 m from caragana shelterbelt compared to 2 m from the shelterbelt.

Aside proximity, species (**Table 2**) of N₂-fixing legumes as well as the non-N₂-fixing crops (when grown in mixtures) influence the amount of N₂ fixed and transferred to neighboring crops. The amount of N transferred to Arabian coffee (*Coffea arabica* L.) ranged from 13.5 to 21.8 g kg⁻¹ depending on the N₂-fixing legume (**Table 2**) [63]. There was no observable N transfer from berseem clover (*Trifolium alexandrinum* L.) to annual ryegrass (*L. perenne* L. subsp. *multiflorum* [Lam.] Husnot) when grown in mixtures [64]. This was attributed to the greater efficiency of annual ryegrass in the uptake of available soil N which resulted in berseem clover becoming reliant on fixed N₂ [64]. In an alfalfa-Bermudagrass (*Cynodon dactylon* (L.) Pers.) intercrop, alfalfa fixed 80 to 222 kg N ha⁻¹ year⁻¹ and transferred about 18 kg N ha⁻¹ year⁻¹ to Bermudagrass [65]. Alfalfa fixed twice as much N as white clover but transferred only 59 kg N ha⁻¹ compared to 147 kg N ha⁻¹ transferred by white clover over a 3-year period [66]. Although decomposed alfalfa roots released greater N than that of birdsfoot trefoil, the opposite was true for decomposed nodules [41]. There was no transfer of N from any of seven legumes [snail medick (*M. scutellata* L.), common vetch (*V. sativa* L.), squarrosom clover (*T. squarrosom* L.), hairy vetch (*V. villosa* Roth), sulla (*Hedysarum coronarium* L.), and fenugreek (*Trigonella foenum-graecum* L.)] to annual ryegrass under Mediterranean conditions [67]. N transfer is also influenced by crop cultivars. For example, red clover cultivars differed in amount of N transferred to Kentucky bluegrass [24]. Compatibility of species grown in mixed swards affects the amount of N₂ fixed and the proportion transferred. A recent study has shown that grass N demand in grass-legume mixtures might be more important than legume N supply in determining N transfer efficiency [26].

Other factors such as age or stage of growth [68], season or year [69–71], proportion of N-fixing species [71], compatibility [45], and stand persistence [35] affect N transfer in cropping systems. For example, N in naked oats (*Avena nuda* L.) derived from N₂ fixed by mung bean was 7.6% at pod setting and increased to 9.7% at maturity [68]. The proportion of N transferred from red clover to Kentucky bluegrass was reported to have increased over time [24]. This is particularly true for perennial forages because of relatively low N₂ fixation in establishment year compared to well-established stands [1]. It is generally expected that as the proportion of legumes in mixed swards increases, N₂ fixation and transfer increases [1]. However, in a continental-scale field study with two perennial N₂-fixing legumes (red clover and white clover) and four perennial grasses (perennial ryegrass, Timothy (*Phleum pratense* L.), Kentucky bluegrass, and orchardgrass (*Dactylis glomerata* L.)), it was reported that N gained in mixed swards increased with increasing legume proportion up to 30% [71]. This supports the assertion by [26] that grass N demand in grass-legume mixtures might be more important than legume N supply in determining N transfer efficiency. In an annual garden pea-barley intercropping system, greatest N transfer was obtained in 1:1 garden pea: barley compared to 2:1 system [72].

6. Conclusions

It is generally agreed that BNF is one of the most sustainable sources of N in agricultural production systems. The BNF is especially important in low value crops (e.g., forages) and in developing economies. Estimated N₂ fixation from selected crops showed that the

contribution of N_2 fixation to the global N budget is enormous. Though N_2 fixation from peas, lentils, common bean, faba bean, cowpea, chickpeas, and groundnut is dwarfed by soybean (because of the larger area planted to soybean) based on these estimates, the contribution of N_2 fixation from these crops (e.g., cowpea) to farmers in developing countries is substantial. Unlike forages, grains from grain legumes are harvested and removed from the field. Thus, grain legumes usually remove more soil N than forages. There are, however, several issues related to BNF that are of concern to the scientific community. Among the issues of great importance is the low and highly variable proportion of fixed N_2 transferred to non- N_2 -fixing plants. Proportion of fixed N_2 transferred to non- N_2 -fixing plants ranges from as low as 0% to as high as 70%, depending on a myriad of factors. This was not different than the range of values reported from previous reviews. However, most of the factors (e.g., N fertilizer application, species, and cultivar selection) are management related and can, therefore, be controlled for improved N_2 fixation and transfer. Most *Rhizobia* strains are highly specialized and due to this specialization, inoculation with the right strains of *Rhizobia* would improve N_2 fixation. One of the constraints to BNF in grass-legume mixtures is low proportion of legumes in the mixtures. It is, therefore, important to maintain sufficient legume populations in the grass-legume systems for optimal N_2 fixation. This might, however, be difficult to achieve because of the selective grazing of legumes by livestock (in grazing systems). Nonetheless, using optimal seed mass ratios and good grazing and haying practices may help maintain optimal legume proportions.

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