

Biological Nitrogen Fixation in Agricultural Systems

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## **ABSTRACT**

The purpose of this research paper is to assess the effectiveness of Biological Nitrogen Fixation (BNF) as an alternative source of nitrogen (N) in agricultural systems. N is found ubiquitously in the atmosphere, yet often it is the most limiting nutrient for plant growth. Biological Nitrogen Fixation (BNF) is the process by which microorganisms convert atmospheric nitrogen into its plant usable form. The production and transport of chemical fertilizers consumes large amounts of fossil fuels and releases potent greenhouse gases. BNF is a sustainable alternative to conventional N fertilizers that derives its energy from renewable energy sources and enhances nutrient cycling within the soil environment.

This paper compares the potential BNF rates of pulses, N<sub>2</sub> fixing trees and green manures and limitations. Through comparative analysis, results indicated that methods of utilization are abundant and diverse, thereby facilitating BNF incorporation in a wide range of agricultural systems. Furthermore, social and economic aspects revealed that there is no one size fits all approach. The case studies showed that overall nutrient budgets must be considered to ensure net positive effects and the future of soil nutrient management strategies necessitates integrative approaches.

## **Introduction**

Nitrogen (N) is an essential macronutrient required for plant growth and is an integral part of proteins; its presence is essential to supporting optimal plant growth. Although it is abundant in the atmosphere, its plant-absorbable forms, ammonium and nitrate, are limiting in soil systems. With the exponentially growing global population provoking a high demand on food production, soil fertility management is at the forefront of environmental concerns. Since the innovation of industrial fixation of nitrogen--the Haber-Bosch process--the use of chemical fertilizers has proliferated. Between 1960 and 2009 global fertilizer consumption increased more than tenfold: from 10.8 Mg (metric tons) per year to 113 million Mg per year (Crews and Peoples, 2004; FAO, 2014). Our society's existence relies heavily on these N fertilizers, yet their sustainability is questionable due to their high consumption of fossil fuels and high nitric oxide emissions during production. Furthermore, their overuse can lead to excessive runoff and leaching, which can result in contamination of surrounding surface and groundwater sources thus impacting the broader ecosystem (Connor, 2013).

In semi-arid, sub-humid, and humid sub-Saharan Africa, loss of soil fertility has been recognized as the central biophysical constraint to agricultural production (Smaling and Dixon, 2013). The UNDP (2012) outlines how the sole act of increasing yields can induce a multiplier effect on human development by enhancing food security. Without additional inputs, the nitrogen balance in most agricultural systems is at disequilibrium: outputs are greater than natural inputs. Losses in nitrogen are not only attributed to crop removal, but also to erosion, leaching, and denitrification (Giller *et al.* 1995). The search for methods to enhance soil fertility, with the goal of long-term sustainability, has

focused on the effective management of internal resources. Biological Nitrogen Fixation (BNF) is a sustainable alternative that has the potential to contribute significant quantities of N into agricultural systems while alleviating global fossil fuel reliance.

### **Biological Nitrogen Fixation**

BNF is the process through which organisms convert atmospheric nitrogen ( $N_2$ ) into ammonia ( $NH_3$ ). The ability to transform N, deeply rooted in evolutionary history, is observed in free-living, associative, symbiotic, and endophytic bacteria (Wagner, 2012). The scope of this paper is limited to the ability of symbiotic  $N_2$  fixing bacteria and their plant host to contribute N to agricultural cropping systems.

#### Symbiotic Relationships

##### *Legume-Rhizobia*

For centuries, legumes have been integrated into agricultural systems for their beneficial effects on soil fertility. This is largely due to their ability to utilize atmospheric N through BNF. In legumes this symbiotic process involves the formation of nodules stimulated by the presence of a specific bacteria, rhizobia. As the rhizobia infect the legume, specialized cells are developed that contain nitrogenase and leghaemoglobin, which work together to fix nitrogen. The amount of N fixed by legumes varies because of legume genotype, soil N, effectiveness and presence of rhizobial bacteria, amount of N accumulated as biomass during growth and the area of the cultivated legume (Mapfuno, 2011).

Table 1:  $N_2$  fixation potential of various categories of legumes

Legume Type	%N derived from fixation	Amount fixed (kg N ha <sup>-1</sup> )
Pulse	60-100	105-206
Green manure	50-90	110-280
Trees	56-89	162-1063

Adapted from (Giller, 2001)

Table 1 provides further support to Peoples (2009) statement that the Legume-*Rhizobia* relationship alone has the potential to input several hundreds of kilograms of N/ha into agricultural systems annually. Inputs from legume trees and green manures have yet to be fully exploited to maximize their N contributing potential; they will both be discussed in their respective categories. Currently, the most important and utilized N<sub>2</sub>-fixing relationship in agriculture is the symbiosis between legumes and *Rhizobia*.

#### *Actinomycetes-Frankia*

Similar to the Legume-*Rhizobia* association, actinorhizal plants form root nodules through a symbiotic relationship with the N<sub>2</sub> fixing bacteria, *Frankia*. Actinorhizal-*Frankia* symbioses can be found in a vast range of climates including the arctic, tropics, rainforests and semi-desert regions and have the potential to fix up to 300kg N ha<sup>-1</sup>yr<sup>-1</sup>. Additionally, these species tend to be aggressive colonizers who can reside in harsh conditions. However, the integration of these trees into agroecosystems is an area of research that remains largely unstudied (Batiano, 2012).

#### *Azolla-Anabaena*

*Azolla*, a free-floating freshwater fern, and *Anabaena-azolla*, a blue-green algae, form a symbiotic relationship where the algae fixes atmospheric N<sub>2</sub> in exchange for protection within *Azolla*'s leaf cavities. The genus *Azolla* includes six species, which are found in temperate, tropical and sub-tropical regions (FAO, 2008).

## **N releases into the soil**

The two ways that N can be released into the soil for subsequent crop uptake are biomass decomposition and rhizodeposition. Decomposition—the rate at which N is released into the soil—is controlled by the quality of the organic material, the environment, and the decomposers present. Leaf litter and other organic inputs that are high in N content, low lignin and low in polyphenol content not only release larger proportions of N, but do so at a faster rate. Whereas organic inputs that are low in N content, high lignin and high in polyphenol content release nutrients much more slowly and continuously resulting in lower potential losses to leaching. The discrepancy between slow and quick release litters can be utilized to maximize synchronicity between crop N demand and N release.

Furthermore, when litter with high C:N ratios are added to the soil, they can induce an overall N deficit and N immobilization is more likely to occur. On the other hand, C:N ratios less than 15:1 generally result in N mineralization, thereby releasing plant usable N (Gregory and Nortcliff, 2013).

Rhizodeposition, the release of N compounds directly from the plant symbionts roots, can occur in two ways: by the exudation of soluble N compounds and through the decomposition of nodules and root cells (Fustec et al. 2011).

## **Biophysical and Management Constraints**

Successful BNF within an agricultural system relies not only on the survival and growth of both the micro-symbiont and the host-plant, but also their interactions. Abiotic conditions in the soil environment that limit the effectiveness of BNF are: extreme temperatures, light availability, moisture content (water logging or drought), soil acidity and nutrient concentrations (Mulgoney, 1990). Although the degree of sensitivity varies with respective species and strains, knowledge regarding an organism's unique tolerances has enhanced selection for in field applications. Significant progress in the field of biotechnology has improved the understanding of BNF genes. Seed breeding and bacterial culturing have augmented N<sub>2</sub> fixing capacity and plant productivity (CGIAR, 2002). The use of inoculants has enabled the introduction of N<sub>2</sub> fixing plants into farming systems that lack the corresponding soil bacteria (Giller and Cadisch, 1995). With respect to nodulation, native bacteria can colonize plant roots and act as a barrier to BNF activity. Therefore inoculants must be aggressive colonizers who can outcompete ineffective native strains (Bohloul et al. 1992). In on farm trials in semi-arid Tunisia, Romdhane et al. (2008) tested the effectiveness of chickpea associated rhizobia inoculants characterized by their high N<sub>2</sub>-fixing capacity and salt stress tolerance. The nodule occupancy of the selected inoculants was determined using repetitive extragenic palindromic sequences amplified by polymerase chain reaction (REP-PCR). Comparatively, nodule occupancy for the native strains was determined using 16S rDNA typing (Romdhane et al. 2008). This experiment showed that the presence of inoculant strains resulted in increased nodulation and increased overall chickpea yields; Romdhane et al. (2008) states that these outcomes were equal to or better than N fertilizer application. Furthermore this study

exemplifies the role of molecular genetic knowledge in overcoming environmental limitations.

The usage of inoculants varies greatly with geographic location. In general, highly evolved markets and high quality inoculants predominate in North America, South America, Europe and Australia. Comparatively, in Africa and Asia less evolved and more fragmented markets result in inconsistent quality of inoculants (Herrigde et al. 2008; Peoples et al. 2009).

Rates of  $N_2$  fixation are highest when soil N concentrations are low. The host plant tends to use up available N in the soil, prior to intensifying  $N_2$  fixation (van Kessel et al. 2000). Therefore in N-depleted soils, relative rates of  $N_2$  fixation have been shown to be greater. Comparatively, high nitrate levels, a consequence of excessive tillage and fertilization, have an inhibitory effect on nodulation, nodule establishment and nitrogenase activity (Peoples et al. 2009). BNF is an energy intensive process that requires large amounts of phosphorous (P), hence P fertilization can often enhance  $N_2$  fixation (van Bloem et al. 2006).

### **Methods of Utilization**

The following section examines the practices utilized to integrate the above sources of N into agricultural systems where the benefits of BNF can be extended to crops.

## Crop Rotations

Crop rotation is an ancient agricultural practice that involves the long-term, permanent cultivation of an ordered series of crops on the same land (Carter, 2005). Crop sequences are a flexible alternative that involve similar management, yet are short to medium-term durations (FAO, 2003).

Crops are planted in accordance to their nutrient needs: deep-rooted plants are followed by their shallow-rooted counterparts in order to allow nutrients to regenerate at various depths (Mulvaney et al. 2011). For example, deep-rooted crop legumes such as the common bean, cowpea and pigeon pea can access nutrients below the cereal rhizosphere (1-3m) (Batiano, 2011). The incorporation of legumes into crop rotations has proven to increase yields of subsequent crops (Peoples et al. 2009). Beyond the advantage of soil fertility, crop legumes also provide a source of feed, supplying the farmer with an immediate source of income. For this reason, crop rotations and intercropping tend to be frequently adopted by farmers.

## Simultaneous Cropping

Intercropping is a traditional agricultural practice where two or more crops are planted simultaneously in a specific spatial organization – typically rows—where their ecological interactions are directly linked. Mixed cropping and relay cropping are two practices that also involve simultaneous cultivation of two or more crops. Mixed cropping is the same as intercropping, however it does not involve a specific geometric pattern such as rows

(Pearson et al. 1995). Relay cropping involves planting the second crop directly onto the first crop prior to harvesting, thus allowing the cultivation of two crops during the same year (Blanco-Canqui and Rattan, 2008). Due to spatial organization, close consideration of individual species' biotic and abiotic requirements must be managed to reduce competition for resources. Compared to sole cropping, the total amount of N fixed in intercropped or mixed cropping system tends to be lower because of the increased competition for light and nutrients as well as decreased legume populations densities (van Kessel et al. 1999). The process of rhizodeposition enhances the N-uptake by the companion crop (Frustec et al. 2011).

#### Improved Fallow

Natural fallows are defined as "land resting from cultivation." Improved fallows are an extension of this principle; the main difference between natural and improved fallows is that instead of leaving the soil to restore itself through natural vegetation with improved fallows, species of trees, shrubs and herbaceous cover crops are planted and managed to minimize the fallow period (Sanchez, 1999).

#### Green Manuring

Green manures are defined as locally produced, non-decomposed plant matter that is applied to the soil surface (as in conservation agriculture) or tilled into the soil (FAO, 2011). Hatfield and Sauer (2011) state that green manures are cultivated for the specific purpose of providing nutrients to the agricultural system through biomass decomposition.

Crop rotations, intercropping, improved fallows and green manuring are used globally to capitalize on the N<sub>2</sub> fixing symbiotic relationship. These methods can be implemented in isolation or in hybrid combinations. Selection of an appropriate method is only one management step; pairing the system with a N<sub>2</sub>-fixing symbiotic relationship adds dimension to the decision-making process. The following section examines the potential and limitations of the following categories of N<sub>2</sub>-fixing plants: pulses, trees and herbaceous legumes.

### **Pulses**

A pulse, in some contexts referred to as a ‘grain legume,’ is defined by the FAO (1994) as an annual leguminous crop that yields between one and 12 grains or seeds. Unlike other vegetable and oil leguminous crops, pulses are harvested exclusively for dry grain (FAO, 1994). In addition pulses provide a high quality source of dietary protein.

### **Potential**

The percent of N derived from the atmosphere (%Ndfa) reflects the legume’s relative reliance on BNF for growth. It is important to note that the BNF rate was derived from measures of plant shoot biomass, thereby ignoring the belowground N contributions.

Peoples (2006) states that N associated with the roots may increase the total N inputs by 50-100%.

### **Limitations**

Undoubtedly, pulses can fix significant amounts of N; however, for their cultivation to have a positive impact on the overall N-budget, the amount of N removed at harvest must be less than the amount of N fixed. The N harvest index (NHI) is the portion of N that is

partitioned into a seed (therefore removed), whereas the rest of the N (vegetative parts) usually remains in the system as a crop residue (Peoples and Craswell, 1992). Table 2 shows the % of N derived from atmosphere Ndfa values (experimental and field conditions), the BNF rate and the NHI of the most-widely cultivated pulses.

Table 2: Different legume %Ndfa, BNF rates and NHI

Legume	Experimental %Ndfa mean	Farmers' field %Ndfa mean	BNF rate (kg N ha <sup>-1</sup> yr <sup>-1</sup> )	NHI	Reference
Chickpea ( <i>Cicer arietinum</i> )	58	54	3-124	0.9	(Peoples et. al 2008; van Kessel and Hartely, 2000; Werner, 2005)
Common bean ( <i>Phaseolus vulgaris</i> )	39	31	0-125	0.16	(Peoples et. al 2008; Peoples and Craswell, 1992)
Cowpea ( <i>Vigna unguiculata</i> )	54	65	3-201	0.3-0.89	(Peoples et. al 2008; Peoples and Craswell, 1992)
Fababean ( <i>Vicia faba</i> )	75	68	13-252		(Peoples et. al 2008)
Groundnut ( <i>Arachis hypoge</i> )	62	52	17-200	0.42-0.47	(Peoples et. al 2008; Peoples and Craswell, 1992)
Lentil ( <i>Lens cu/inaris</i> )	65	74	4-152	0.42	(Peoples et. al 2008; van Kessel and Hartely, 2000)
Pea ( <i>Pisum sativum</i> )	65	70	11-183		(Peoples et. al 2008)
Pigeon pea ( <i>Cajanus cajan</i> )	57	78	7-88	0.21-0.5	(Peoples et. al 2008; Peoples and Craswell, 1992)
Soybean ( <i>Glycine max</i> )	68	58	88-193	0.8	(Peoples et. al 2008; Peoples and Craswell, 1992)

In general the %Ndfa values are higher for experimental trials compared to field conditions showing that the full N<sub>2</sub> fixing potential may not be fully exploited. These values can be limited by variances in soil N, soil rhizobia and rhizobial inoculation (Peoples et al. 2009). This is further emphasized by the range of BNF rates. This data is inclusive of a multitude of management practices in different farming systems and climatic regions around the world, furthering the idea that “BNF cannot be considered in isolation, but must be examined within the context of the farming system in which it is utilized” (Giller and Cadisch, 1995).

#### Current Usage

In the past decade pulse production has increased; this has been mostly centralized in countries-such as North America and Australia- where crops are cultivated commercially. In North America, the area cultivated by both lentils and dry peas has increased due to incentives for diversified cropping systems. Although global and local pulse demands are rising, few increases in production have been observed in small-scale subsistence-based agricultural settings, where the majority of pulse production occurs (Siddique et al. 2011). Akibode and Maredia (2011) connect this to agricultural policies that focus on cereal production for food security needs; this results in pulses receiving less resources and investment than cereals. The following case studies explore how pulses can be integrated into cereal systems.

At the Farakô-Ba agronomic research station-located in the savannah zone of Burkina Faso- over two seasons, Bado et al. (2005) analyzed the BNF capacities and the soil N-

fertility effects of cowpea (*Vigna unguiculata*) and groundnut (*Arachis hypoge*) sown in rotation with sorghum (*Sorghum bicolor*). Additionally, this study assessed the effects of various combinations of fertilizers on BNF rates and sorghum yields. This experiment showed that soil mineral N was positively affected by crop rotations. Although legume shoots were exported, the remaining residues and belowground plant components helped to improve soil mineral N and soil organic matter. In comparison to continuously cropped sorghum, groundnut-sorghum rotations soil mineral N increased by 36% and in cowpea-sorghum rotations soil mineral N increased by 52%. Consequently, this resulted in higher N uptake by succeeding sorghum. The soil in the experimental blocks was classified as utisols, a weakly acid, sandy soil with relatively low quantities of clay and organic C. In this experiment the N<sup>15</sup> dilution method was used to calculate Ndfa %. The Ndfa % values were highest for both cowpea and groundnut with NPK fertilizer applications, dolomite and manure applications. The inherent low soil fertility impedes BNF; these soil amendments can act as both a buffering agent and nutrient supplier to improve plant growth and rhizobial activities (Bado et al. 2005).

Intercropping pulses with cereals is practiced globally, yet it is most widespread the tropics of Asia, Africa and South America (Peoples et al. 2009). This practice is recommended for farmers with limited land and resources. For intercropping to enhance overall production and increase resource-use-efficiency a comprehensive understanding of aboveground and belowground interspecies interactions is required. In Northwest China, Fan et al. (2006) compared the effectiveness of N<sub>2</sub> fixation in three systems: wheat-fababean intercropping systems, fababean-maize intercropping systems and

fababean monocropping systems. Results showed increases in Ndfa% in both intercropping systems compared to the monocropping system; this is caused by the N-sparing effect where the non-legume component decreases the N concentration in the legume's rhizosphere, thus forcing intensification of BNF to meet N requirements (van Kessel et al. 2000). Fababean growth was suppressed in the wheat-fababean system and facilitated in the maize-fababean system (Fan et al. 2006). The shading effects and rapid root growth of wheat inhibited fababean biomass production, thereby resulting in a lower Ndfa% value compared to fababean intercropped with maize. The outcome of the wheat-fababean system supports the prevalent view that legumes are weak competitors-compared to cereals-and should be treated as secondary crops (Fan et al. 2006).

### **Nitrogen Fixing Trees**

Agroforestry “is a land use in which trees are grown on the same land as crops, either in a spatial arrangement or in a time sequence, and in which there are both ecological and economic interactions between the tree and the non-tree component” (Batiano et al. 2011). Although agroforestry is practiced globally, motivations vary greatly between industrialized and developing nations. In many industrialized nations, the growing public awareness of the negative environmental implications associated with high-input agriculture has fueled the demand for environmental accountability. In this context, agroforestry is seen as a form of environmental protection that maintains biodiversity, limits deterioration of water quality, and offers the potential to reduce erosion and pollution (Nair, 2008). Contrarily, in developing nations, where access to fertilizers is limited and soil degradation is prevalent, the emphasis is on the role of trees in improving

soil quality and crop productivity. Trees can also act as a source of fuel, food and fodder, while also aiding in soil conservation. The following section focuses on the benefit of the integration of trees into a cropping system in terms of soil N fertility.

## **Potential**

The purpose of agroforestry systems is to promote a closed loop of nutrient cycling, thereby relying on the natural variable abilities of trees to enhance soil fertility. In regards to the overall N budget, trees contribute to soil N through BNF, plant litter fall and nutrient cycling of deep soil stratum. Furthermore, the presence of trees also limits losses through leaching.

Nitrogen Fixing Trees (NFT) include both leguminous and actinorhizal species which form N<sub>2</sub> fixing root nodules through symbiosis with bacteria *Rhizobia* and *Frankia*, respectively. Atangana et al. (2014) categorized NFT in two groups: species with a high potential fixation rate, between 100-300 kg N ha<sup>-1</sup> y<sup>-1</sup>, and species with a low potential fixation rate, less than 20 kg N ha<sup>-1</sup> y<sup>-1</sup>. Nair (1993) further classifies species with a high potential fixation rate into intolerant or demanding species that require substantial amounts of P, K and Ca, and tolerant or non-demanding species that flourish in poor soils with low nutrient levels.

Table 3: N<sub>2</sub> fixing trees properties

Species	Potential BNF rate (N ha <sup>-1</sup> yr <sup>-1</sup> )	Classification of Fixing Potential	Environmental Tolerances	References
<i>Acacia mangium</i>	50-100	High	Tolerant to acidic soils with low nutrient levels	Chaturvedi et al. 2009; Atangana et al. 2014
<i>Casuarina equisetifolia</i>	362	High	Require large amounts of nutrients, drought and high temperature tolerance	Atangana et al. 2014; Nygren et al. 2012
<i>Faidherbia albida</i>	20	Low		Nair, 1993
<i>Glirucudua sepium</i>	50-100	Low		Chaturvedi et al.
<i>Leucaena leucocephala</i>	100-500	High	Require large amounts of nutrients	Chaturvedi et al. 2009; Atangana et al. 2014
<i>Sesbania rostrata</i>	100-500	High		Chaturvedi et al. 2009;

### Tree Biomass Decomposition

Tree biomass decomposition can enhance soil fertility through the recycling of nutrients mainly from leaf litter, pruning, and root components. Current crops will only uptake about 20% of the N released from the above sources, and the remaining N is temporarily held in the soil most commonly in association with soil organic matter (SOM) (Chaturvedi et al. 2009). This results in a slow release of N into the system, thereby acting as a long-term soil amendment. In comparison, the degree of this residual affect is much lower in inorganic fertilizers. Decomposition, and thus the rate at which N is released into the soil, is controlled by the quality of the organic material, the environment and the decomposers present. Leaf litter and other organic inputs that are high in N content, but low lignin and polyphenol content not only release larger proportions of N, but do so at a rapid rate. Organic inputs low in N content, but high lignin and polyphenol

content release nutrients much more slowly and continuously, result in lower potential losses to leaching. The discrepancy between slow and quick release litters can be utilized to maximize synchronicity between crop N demand and N release. Furthermore, when litter with high C:N ratios are added to the soil, they can induce an overall N deficit, and N immobilization is more likely to occur. On the other hand, C:N ratios less than 15:1 generally result in N mineralization, thereby releasing plant usable N (Gregory and Nortcliff, 2013). Soil N fertility from tree biomass decomposition is not limited to NFT; it has been found that several species of *Senna* can accumulate as much as or more N in their leaves than certain NFT, yet this is simply a mechanism of nutrient cycling not contributing inputs (Sanchez et Palm, 1995).

The root biomass of trees consists of 20-30% of total plant biomass, yet the rate of primary production is much greater due to the turnover of fine roots. These root residues inevitably return to the soil, adding to the soil organic matter content and aiding in further nutrient cycling (Chaturvedi et al. 2009).

#### Tree Uptake of N from deep soil stratum

The roots of trees extend deeper into the soil than most agricultural crops, allowing them to access untouched nutrient sources. Tree roots accessing untouched nutrient sources is exemplified in the work of Zaharah et al. (2008), where maize monocrops are compared to maize hedgerows of leguminous trees (*Paraserianthes falcataria* and *Gliricidia sepium*): with under-fertilized maize monocropping, N was found to accumulate in the subsoil, whereas accumulated N (mainly nitrate) in the subsoil underneath planted trees

decreased. With this evidence, Zaharah et al. (2008) inferred that trees were likely retrieving subsoil N, which was inaccessible by maize crops. Furthermore, research from Magongoya et al. (2003) states that continuous N-fertilization of shallow root crops (such as maize) may lead to N leaching and N accumulation in the subsoil layer, resulting in a loss of nutrients for crops as well as a multitude of environmental concerns. If paired in accordance to the depth of their rooting systems, trees offer the potential to access and redistribute this otherwise lost source of usable N back into the system. Contrarily, integrating trees and crops with similar root depths results in competition for water and nutrients consequently causing decreased crop yields (Magongoya et al. 2003).

The potential of trees to uptake N from deep soil horizons is maximized when the trees have deep root systems, high demand for nutrients, and grown in environments where water and nutrient stress is prevalent at near surface levels, but abundant reserves exist in subsoil (Nair et al. 1999). Although not directly contributing to soil fertility, the presence of deep rooted trees can contribute to improved soil physical properties and higher microbiological activities which in turn can amelioration N cycling potential.

### **Limitations**

The incorporation of trees into an agricultural system can result in the competition for resources both above and belowground. The shading effect of trees can have negative implications on the crops ability to capture light; in many cases this was noted to be of greater importance compared to belowground interactions (Atangana et al. 2014). As previously mentioned, belowground water and nutrient acquisition is largely affected by

the depth of the rooting system. In all areas, the competition for water is extreme, yet its intensity is amplified in semi-arid regions where the effects were more apparent than shading (Atangana et al. 2014). Some agroforestry species have been observed to release chemicals that are harmful to the growth of other plant species, thus further emphasizing the need for a holistic understanding of crop-tree interactions. Lack of short-term benefits, seed availability, unpredictable rainfall, additional labour inputs and loss of arable land can all act as barriers to agroforestry adoption (Mhango et al. 2012).

### **Current Usage**

The nature of agroforestry systems varies depending on climatic variables. For example, in comparison to temperate regions, the tropics have longer growing seasons allowing for more production cycles that favor rapid growth, and a large diversity of tree species resulting in a more complex agroforestry systems (Nair, 2008).

The ample benefits of trees to agricultural systems are not limited to soil fertility; additional unique benefits include shade, wind protection, erosion prevention and fuelwood production. Systems are tailored to the needs of a specific site, and therefore many tree species utilized do not significantly affect N balances. The widespread increase in yields can potentially be attributed to various components such as higher soil fertility, improved microclimate and better physical soil properties (Bryan, 2000). This paper focuses strictly on practices that are related to soil N fertility: improved fallows, alley cropping and parkland systems.

	Improved fallow	Alley cropping	Shaded Perennial-crop	Parkland system
Climate	Semi-arid to humid subtropics	Arid to wet tropics	Wet tropics	Semi-arid tropics
Primary tree species	<i>Acacia</i> , <i>Sesbania</i> , <i>Gliricidia</i> , <i>Tephrosia</i>	<i>Acacia</i> , <i>Faidherbia</i> , <i>Gliricidia</i> , <i>Caleandra</i>	<i>Inga</i> , <i>Erythrina</i> , <i>Eucalyptus</i>	<i>Faidherbia</i> <i>Parkia biglobosa</i> , <i>Vitellaria paradoxa</i> , <i>Melia volkensi</i> , <i>Prosopis cineraria</i>
Primary crops	Maize, beans	Maize, sorghum	Cocoa, coffee	
Management intensity	Low	High	Moderate	
Geographic distribution	Sub-Sahara Africa, Latin America	India, Sahel, Sub-Sahara Africa	Latin America, Southeast Asia, East and West Africa	Semi-arid West Africa, Sahel, India
Production orientation	Subsistence	Subsistence	Markets, subsistence	

Table 4: Agroforestry Systems  
Modified from Rosenstock et al. 2014

### Improved Fallows

As previously mentioned, improved fallows involve planting a beneficial crop during the fallow period to restore soil fertility in order to enhance subsequent crop production. In tree fallows, the wood can be harvested while N-rich components (leaves, pods and green stems) tend to be incorporated into the soil prior to the rainy season (Sanchez, 1999).

Agricultural production in some parts of Zambia is practiced by small-scale, resource-poor farmers in isolated communities where access to external inputs is limited. Soil

infertility is the major problem responsible for the low yields of their principal crop, maize. Due to land pressure, traditional fallow systems have been shortened from once every several decades to less than five years, thereby resulting in inadequate time for soil fertility restoration. The integration of *Sesbania sesban*—an indigenous, deep-rooted N<sub>2</sub>-fixing tree, as an improved fallow crop—has been widely accepted by farmers in Zambia mainly due to its potential to augment soil fertility in shorter fallow periods. In Zambia, Nair et al. (1999) observed that one year *S. sesban* fallows increased the yield of subsequent maize crops by 50-80% while two-year fallows showed increases of 150-270%. Residual benefits of these tree fallows were observed for four years after fallowing: yields were three times greater than monocropped maize. Additionally, economic analysis showed that a two-year fallow period of *S. sesban* was more profitable compared to continuous unfertilized maize crop (Smithson et al. 2002).

One of the major advantages of *S. sesban* in a short fallow cycle is its rapid early growth; *S. sesban* has been observed to reach a height of 4-5m in six months (ICRAF, 1996). This tree thrives under repeated cuttings and coppicing, in general 3-4 cuts per annum, and can yield up to 12t/ha of dry matter. Due to its woody biomass, *S. sesban* can be used as fuelwood and green manure. Furthermore, in many areas positive net N balances were observed for multiple cropping seasons (ICRAF, 1996).

#### Intercropping (Alley cropping)

Among the methods reviewed in this paper, alley cropping is the most extensively studied form of agroforestry used with annual crops. It is the simultaneous growing of crops between hedgerows of woody trees, including but not limited to NFT. Alley cropping compared to rotation with trees appears to have a greater impact on long-term nutrient acquisition from deep soil strata. The continued presence of perennial species can act as a safety net as well as prevent nitrate losses through leaching (Uphoff, 2006). In contrast to improved fallows where during the cropping season deep-rooted trees with such potential do not exist. Although variance between systems exists, the management intensity tends to be greater for alley cropping compared to improved fallow.

#### Trees in Cropland (Parkland) Systems

In tree cropland systems, trees are scattered in agricultural lands. Trees in this system tend to be derived from natural regeneration where farmers make a conscious effort to preserve pre-existing trees on their land; it is rare that trees are planted (Ramachandran et al. 1999). A strong example of a tree used in Parkland systems is *Faidherbia albida*, which is grown extensively in the Sahel. In these systems, crop productivity increases are more pronounced on soils of low fertility and water availability (FAO, 1999).

*F. albida* is a leguminous N<sub>2</sub>-fixing tree indigenous to Africa, that acts as a direct N-source through a variety of mechanisms: BNF, release of N in decomposing roots and nodules, accessibility to deep-soil N and N in groundwater through its root system. Furthermore, its unique “reverse leaf phenology” is compatible with cropping systems; it is dormant during the wet season and will drop its leaves to fertilize crops when seeds are

being planted. In Malawi, farmers reported that their maize yields increased up to 280% in the areas under the *F. albidia* tree canopy (Langford, 2009). In proximity to *F. albidia*, increases in maize yields, from 1.3 tonnes to 4.1 tonnes per hectare, were also reported in Zambia. The Departments of Agriculture in Malawi and Zambia seek to increase maize production with the use of this tree and it is currently recommended that farmers establish 100 trees per hectare of maize planted. The Director General of the World Agroforestry Centre, Dennis Garrity, states that "knowledge of this tree is farmer-driven...we are now combining the scientific knowledge base with the farmer knowledge base. There is sufficient research on both sides to warrant dramatically scaling-up the planting of this tree on farms across Africa through extension programs. The risks to farmers are low; it requires very little labor, and delivers many benefits" (Langford, 2009).

Undoubtedly, *F. albida* enhances yields within the vicinity of its cultivation, however to what degree is this related directly to its N contributions. Due to the limited presence of nodules, the BNF capability of this tree legume is relatively low (Mokgolodi et al. 2011; Atangana et al. 2014). Although this tree may not add significant amounts of N to the system, it still plays a role in nutrient cycling.

### **Green Manures**

A wide range of N<sub>2</sub> fixing plants that produce large amounts of biomass can be used as green manures. Many species that were previously discussed for their N<sub>2</sub> fixing potential can also be used as green manures. These include chickpeas, pigeon peas, cowpeas, *S. rostrata* and *S. sesban* (Batiano et al. 2011). The main difference is that these pulses and

trees are not harvested for alternative uses, rather they are uprooted and placed on the soil surface or incorporated into the soil. Many other herbaceous legumes that are not classified as pulses or trees have been used as green manures.

## Potential

Table 5: Tropical Leguminous Green Manures

Legume	Description	Potential Fixation (kg N per ha <sup>-1</sup> per yr)	Soil Fertility	References
<i>Cajanus cajan</i>	Semi-perennial, tall, shrubby	91	Tolerates drought and cold	(Werner, 2005; FAO 2010)
<i>Crotalaria grahamiana</i>		142		(Werner, 2005)
<i>Crotalaria juncea</i>	Annual, tall	129	Grows well on degraded soils	(Becker, 1995; FAO 2010)
<i>Mucuna pruriens</i>	Annual, herbaceous, climbing	130	Medium to high, does not develop well on degraded soils	(FAO 2010; Werner, 2005)
<i>Tephrosia vogelii</i>	Perennial, bushy, woody stems	100	Grows well on clay, acidic, sandy and low fertility soils	(FAO 2010; Werner, 2005)

Table 6: Temperate Green Manure Legumes

Legume	Description	Potential Fixation (kg N per ha <sup>-1</sup> per yr)	Reference
Alfalfa ( <i>Medicago sativa</i> )	Cool season perennial	300	(GS, 2013)
Crimson Clover ( <i>Trifolium incarnatum</i> )	Winter annual	84	(Hatfield et al. 2011)
Hairy Vetch ( <i>Vicia villosa</i> )	Winter cool season annual	113	(Hatfield et al. 2011)
Winter Pea ( <i>Pisum sativum</i> )	Winter annual	99	(Hatfield et al. 2011)

## Current Usages

Throughout the tropics, green manures have recently achieved ‘spontaneous diffusion’, mainly due to their ability to suppress weeds (Ajai et al. 2006). Velvet bean is a herbaceous N<sub>2</sub> fixing legume known for its vigorous growth and weed suppression abilities. Weed suppression can reduce labour requirements, thus enhancing green manure adoption. In eastern Uganda, Kaizzi et al. (2006) conducted research across four different agroecological zones with varying altitudes, degrees of soil fertility and land use intensities. The objective was to determine the most appropriate N restoring strategy for resource poor farmers. Overall, the use of velvet bean (*Mucuna pruriens*)-as a green manure and relay crop- and inorganic N fertilizer both proved to be equally effective sources of N for maize production. Differences in yields were observed between low productivity and high productivity areas: average increases for low productivity fields were 1.0 t ha<sup>-1</sup> and 2.2 t ha<sup>-1</sup> for high productivity fields. Through cost-benefit analysis, Kaizzi et al. (2006) concluded that *M. pruriens* relay cropping and green manuring is profitable regardless of the natural land quality. However, it is only economically advantageous for farmers cultivating land on productive soils to use fertilizer. This exemplifies the role of low cost and low input BNF strategies in improving soil fertility and providing food security in less productive area. In a few cases, in the eastern Uganda setting, decreased maize yields were observed under the simultaneous cropping system; this can partially be attributed to resource competition, yet the main factor was related to inadequate management of velvet bean that caused intertwining with maize stalks (Kaizzi et al. 2006).

As a result of increasing land pressure and farmer-farmer dissemination of knowledge mucuna-maize crop rotations replaced existing bush fallow systems throughout northern Honduras; this practice doubled maize yields, reduced the risk of drought stress and decreased land preparation and weeding requirements (Correia, 2014).

In temperate regions winter legumes grow well in the fall and in the spring. Research conducted at the Rodale Institute showed that red clover and hairy vetch incorporated into a wheat/corn/soybean rotation as winter green manure cover crops without fertilizer inputs produced yields equivalent to conventional crop systems (Bagley et al. 2006).

#### Limitations

Although green manures can enhance soil N fertility, their adoption is relatively low and their use has been widely replaced by chemical fertilizers. This is largely attributed to their additional management, labour and land requirements (Giller and Cadisch, 1995). Unlike pulses and trees, green manures do not provide any income-generating byproducts such as grain or fuelwood.

#### **Azolla**

Azolla, a free-floating freshwater fern; and Anabaena-azolla, a blue-green algae, form a symbiotic relationship where the algae fixes atmospheric N<sub>2</sub> in exchange for protection within Azolla's leaf cavities. The genus Azolla includes seven species, which are found in temperate, tropical and sub-tropical regions (Choudhury and Kennedy, 2004).

## Potential

Due to its ability to flourish in shallow freshwater environments, high N-content and rapid growth rate the *Azolla-Anabaena* complex is most widely used as a green manure or a dual crop in flooded rice fields (Vaishampayan et al. 2001). Until rice reaches maturity, there is no competition for space or light. However, after maturity the larger canopy begins to inhibit light acquisition by *Azolla*, thus causing death and decomposition of the fern. The decomposition release of N coincides with the rice's highest nutrient demand (FAO, 1979). In a single year, a sole continuous crop of *Azolla*, repeatedly harvested fixed up to 1000 kg N ha<sup>-1</sup> (Roger, 1996; FAO, 2008). Furthermore, when dual-cropped with rice this fern has the potential to exceed rice N requirements (Roger, 1996; Choudhury and Kennedy, 2004).

## Limitations

Environmental variables affect growth rates, yet adequate moisture is paramount to *Azolla*, which cannot withstand desiccation. For this reason their incorporation into flooded rice fields results in successful yields. However, in areas where water availability is low, irrigation networks are required which can prevent farmer adoption (Roger and Watanabe, 1986). In China and Vietnam, practices are labour-intensive and involve multiple incorporations. Roger (1996) states that the potential for implementation of *Azolla* as an alternative N source is greatest where the cost of labour is low.

## Current Usages

Azolla-as a green manure- has mostly been incorporated into rice systems in tropical and sub-tropical parts of Asian countries, including China, Vietnam and India. However, recent interest lies in expanding its application into temperate zones: Bocci and Malgioglio (2010) found a strain of Azolla-Anabaena that is resistant to winter temperatures and can produce sufficient inoculum for rice during the spring.

Pulses, N<sub>2</sub>-fixing legumes, green manure and Azolla all have the ability to fix significant amounts of N. Agricultural systems are not confined to one source of BNF; hybridization and mixtures can create positive effects on soil N fertility. In the words of Barry (1999), “the economy is where nature and society meet.” In many countries around the world this interface is agriculture. Marketable, income-generating products play a central role in BNF adoption and in some cases its economic advantage over conventional fertilizers. Although farmers understand the inherent link between soil fertility and crop productivity, this is not always reflected in decision-making. The following section addresses the social implications associated with BNF in agricultural systems.

## **Social Considerations**

Extensive research has proven that BNF has the potential to contribute significant amounts of usable N to agricultural systems while contributing to long-term soil fertility. Biophysical limitations can reduce this potential, yet research has focused on understanding agroecological niches best suited to unique symbiotic plants, thereby capitalizing on their diversity. Although an abundance of knowledge exists within the

realm of BNF science and technology, adoption in the some of the most vulnerable areas has lagged (Jerneck and Olsson, 2013). Agriculture is a “lifeworld”; modifications to practices must consider the social and economic dimension.

In developed countries, technological information tends to reach farmers rapidly, whereas Siddique et al. (2011) identified slow knowledge dissemination as a core issue in preventing the transmission of innovation to rural communities. Although the ineffectiveness of top-down approaches is widely recognized, such processes continue to dominate and create serious disconnects between researchers, extension programs and rural farmers (Siddique et al. 2011). The focus needs to be on participatory approaches that emphasize the incorporation of local perspectives and needs. The importance of the transmission of knowledge between generations and through existing social networks cannot be overlooked. BNF has been utilized in agricultural systems for centuries; traditional practices and innovative technologies can work synergistically to enhance soil N fertility.

Although knowledge dissemination and awareness is the first step towards unlocking the full potential of BNF, a farmer’s decision to adopt new technologies is influenced by perceived profitability of the proposed system, their household level of resource endowment and their prevailing social context.

Altering current practices is associated with uncertainty; vulnerable populations tend to opt for risk-aversion strategies with immediate profitability. When present needs are not

met, long-term soil fertility is not a focus; for instance, food secure farmers with an entrepreneurial orientation are more likely to adopt agroforestry practices compared to farmers whose first order priorities such as food security and health are not satisfied (Jerneck and Olsson, 2013). Many of the practices that utilize BNF described in this paper require additional labour. In areas where labour shortages are prevalent--perhaps due to migration or HIV-AIDS--farmers are not self-sufficient and are forced to diversify into other livelihoods (Jerneck and Olsson, 2013).

Beliefs and customs instilled within the prevailing social environment affect the perceived usefulness and perceived ease of BNF strategies (Ajayi et al. 2007). In Mozambique planting trees is an act of claiming ownership, therefore women, tenants and migrants are prohibited from planting trees, thus impeding the adoption of agroforestry. Furthermore, gender inequalities in access to financial resources, education and agricultural extension visits are apparent throughout Africa and can affect adoption decisions (Kiptot and Franzel, 2012).

Social protection must be at the forefront of the incorporation of BNF into agricultural systems. Will these practices enhance or hinder the farmer's lives? In essence, soil fertility is a social issue. In the words of Giller (1995), "BNF cannot be considered in isolation, but must be examined within the context of the farming system in which it is utilized." Ajayi et al. (2007) propose the use of Geographic Information System (GIS) to create suitability maps using two dimensions: the biophysical--characterized by elements such as rainfall, soil type and topography; and the socio-economic--characterized by

population demographics, existing infrastructure, market access, and property rights. The two maps are superimposed to produce 'hotspots' where optimal adoption would occur (Ajayi et al. 2007).

### **Comparative Analysis to other N-sources and Economic Limitations**

As the global population rises so too does the world's dependency on fertilizers; between 2002 and 2009 the global use of synthetic N fertilizer increased from 86 million Mg to almost 113 million Mg (FAO, 2014). The widespread use of fertilizers has drastically increased yields by effectively meeting crop N demands and reducing the need for fertility regenerating periods in rotations (Crews and People, 2004). The sustainability of these fertilizers is questionable due to their high consumption of fossil fuels and high nitric oxide emissions during production. Furthermore, their overuse can lead to excessive runoff and leaching, which can result in contamination of surrounding surface and groundwater sources as impacting the broader ecosystem (Connor, 2013).

Between 1981 and 2002, field investigations at the Rodale Institute were conducted to assess the change in soil N percentages in the three systems described above. Results showed that values in the conventional system were unchanged at 0.31 %. However, significant increases were observed in the organic animal and organic legume systems: 0.35% and 0.33%, respectively (Pimentel, 2005). These residual benefits may be attributed to the overall increased SOM from the organic sources.

Pimentel (2005) assessed the energy inputs in three systems: organic animal, organic legume and conventional corn production systems. The inputs included farm machinery, fertilizers, seeds and herbicides. In comparison to the conventional system, results showed that the energy inputs for the organic animal and organic legume systems were 28% and 32% lower, respectively. The greater energy requirements of conventional cropping systems was directly linked to fossil fuel consumption at the fertilizer production, transport and application phases. Manure applications also required fossil fuel for transport and application. BNF does not require extensive transportation of resources; it can provide agricultural systems with an on-site source of N, derived from solar energy.

The selection of N-sources is context specific and depends on market access, return on investment and subsidization. Market access can be hindered by poor infrastructure, most notably in landlocked rural areas. In many cases a minimum soil fertility threshold is required for the use of fertilizers to be economically viable. In a previously discussed example, Kaizzi et al. (2006) observed that green manuring and relay cropping were profitable regardless of the natural soil quality. However, fertilizer use was only cost-effective on productive lands.

In the 1980s, structural adjustment programs (SAPs) in Africa focused on the deregulation of the agricultural sector consequently inducing cutbacks on input subsidies (Mkandawire and Agunda, 2009). These effects were strongly felt in Zambia where prices were so high that it was unprofitable to use fertilizers. As a result, farmers adopted

agroforestry practices (Ajayi et al. 2005). In comparison, “the Government of India fixes minimum support prices for the main crops, controls the farm price of urea, and issues indicative selling prices of other fertilizers. The aim is that the farmers should receive a price for their crops and pay a price for fertilizers that make the use of fertilizers acceptable and remunerative” (FAO, 2006).

Within the framework of sustainable production, the ultimate goal of any agricultural system is maximizing yields while maintaining the natural resource capital. Resilient systems that rely less on fossil fuels and more on internal resources are essential given the current Climate Change crisis. Fertilizers, manures and BNF sources have the potential to act synergistically to enhance N-budgets. Capitalizing on individual strengths has the potential to produce balanced agricultural systems.

## **Conclusions**

N is found ubiquitously in the air, yet often the most limiting nutrient for plant growth. BNF can transform portions of the abundant atmospheric N stores into plant usable forms. This process derives its energy from renewable sources and as an in situ fertilizer, thus substantially decreasing the need for fossil fuel. The incorporation of BNF aligns with current shifts towards more sustainable management practices that conserve internal resources.

There is clear evidence that BNF has the potential to fix significant quantities of N. Yet when juxtaposed to inorganic fertilizers is this enough to satisfy global needs? Badgley et

al. (2006) estimate that N fixed through the sole addition of leguminous cover crops can contribute 140 million Mg of N per annum, surpassing the most recent global fertilizer consumption rate of 113 million Mg of N per annum (FAO, 2014). Even though this estimate does not include contributions from pulses, trees or azolla, it may be overshooting the capacity of BNF. This paper emphasizes how BNF rates are highly variable given the biophysical conditions. In the face of climate variability--where the frequency of extreme weather events is predict to increase--would BNF provide a stable source of N?

Furthermore, this comparison is drawn from current fertilizer consumption; what about the N depleted areas that do not utilize N fertilizers? The real question remains: what is the global N deficit in agricultural systems and what role does BNF play in alleviating it? A transition towards more sustainable nutrient management systems must incorporate BNF, especially where fertilizer use is lacking due to economic or physical barriers to access. An integrative approach that does not undermine current agricultural practices is the way forward.

#### Summary Points

- Research has proven that BNF can contribute significant amounts of N into agricultural systems
- Overall nutrient budgets must be considered to ensure net positive effects
- The large diversity of symbiotic relationships can be incorporated into a variety of different environments, this can be enhanced by advances in biotechnology
- Methods of utilization are abundant and diverse, thereby facilitating BNF incorporation in a wide range of agricultural systems
- “BNF cannot be considered in isolation, but must be examined within the context of the farming system in which it is utilized” (Giller and Cadisch 1995); there is no one size fits all approach
- Future soil nutrient management strategies necessitate integrative approaches

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