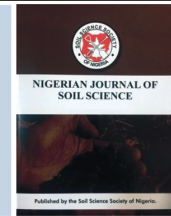




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## Biological nitrogen fixation contributes to soil productivity in tropical agroecologies

Bello, S.K.,<sup>1,2</sup> Shobayo, A.B.,<sup>1</sup> Ibrahim, M.M.,<sup>3,4</sup> Alasinrin, S.Y.,<sup>5</sup> Aliyu, I.A.<sup>1</sup> and Yusuf, A.A.<sup>1</sup>

*1*Department of Soil Science, Faculty of Agriculture, Ahmadu Bello University, Zaria, P.M.B. 1044, Kaduna State, Nigeria

*2*Department of Arid Land Agriculture, Faculty of Meteorology, Environment and Arid Land Agriculture, King Abdulaziz University, Jeddah-80200, Kingdom of Saudi Arabia

*3*Department of Soil Science, University of Agriculture Makurdi, Nigeria

*4*Key Research Laboratory of Soil Ecosystem Health and Regulation, College of Resources and Environment, Fujian Agriculture and Forestry University, Fuzhou, China

*5*Department of Agronomy, Faculty of Agriculture, University of Ilorin, Nigeria

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### ABSTRACT

Biological nitrogen fixation (BNF) is described as a sustainable and economically friendly source of N for crop productivity and soil biodiversity. However, its contributions to tropical soil have not been fully harnessed especially in the African tropics. There is therefore need to review the successes and contributions of this beneficial source of N in tropical agroecologies. Thus, this review investigated the contribution of BNF to soil N pool in legumes as well as N in rice and sugarcane cultivation. It was gathered that most BNF systems are restricted to scientific researches and has not been fully utilized by farmers on a large scale especially in tropical Africa. If BNF is to be optimally utilized for improved productivity of soils and crops, its use must be matched with the requirements of the prevailing environmental condition. In the near future, particularly in tropical regions of the world, tremendous opportunities exist for improving the impact of BNF on N economy in agricultural ecosystems through improved farming practices and molecular advancement in organisms, crop and environment.

Corresponding Author's E-mail Address:

[abshobayo@yahoo.com](mailto:abshobayo@yahoo.com) +234 818 260 9577

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### 1.0 Introduction

Nitrogen (N) is an essential macronutrient required for plant growth and is an integral part of protein which is essential for optimal plant growth. Nitrogen fertilizer ranks first among the external inputs to maximize output in plant production. Smil (2002) estimated that nitrogen fertilizers have contributed approximately 40 % to the increase in per-capita food production over the past 50 years. The effect of N on crops is profound and it is the element that soils most often lacks and limits potential yield of crops. Despite N abundance in the atmosphere, ammonium and nitrate N are limited in most soils

, its plant-absorbable forms, ammonium and nitrate, are limiting in soil systems (Herridge, Peoples, and Boddey 2008). Fertilizer N is a major input in global crop production and farmers have become increasingly dependent on off-farm supplies of N which requires cash and may not always be available on time (Bohlool et al. 1992). Besides, the economic and environmental costs of the heavy use of chemical N fertilizers in agriculture are a global concern. 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 (Nair and Garrity 2012).

Sustainable crop production relies on the continuous renewal of soil fertility through a balance between N demand and supply in cropping systems (Yousaf *et al.* 2016). Nitrogen is the most yield-restraining nutrient in crop production globally (Guo *et al.* 2016). It is required in the greatest quantity by plants and is the most mobile. In sustainable agriculture the main goal is to maintain production at levels necessary to meet the increasing aspirations of an expanding world population without degrading the environment (FAO 1989). Current agricultural production systems require the input of high N fertilizer to increase food, fiber, feed and fuel production for consumption by humans and livestock (Bohlool *et al.* 1992). An appropriate amount of N fertilizer encourages photosynthesis (Hu *et al.*, 2007; Hussain *et al.* 2016), enhances resistance to biotic stress (Guo *et al.* 2009), improves dry matter accumulation and nutrient uptake (Barlóg and Grzebisz, 2004), and increases grain and oil yields (Juan *et al.* 2009). Meanwhile, over-fertilization has become a major concern for sustainable intensive agriculture in some parts of the world (Meng *et al.* 2013). Input efficiency of N fertilizer is one of the lowest among plant nutrients and, in turn, contributes substantially to environmental pollution (Bohlool *et al.* 1992).

Tropical cropping system is characterized by intensive cultivation with little or no fallow (Lal, 2000). This has grave consequences on soil fertility level, destroys soil organic matter, increases soil acidity and accelerates desertification (Sparling *et al.*, 2003). Moreover, most of these tropical soils which have inherently low N are experiencing decline in soil N (Sanginga, 2003). This has resulted in a renewed interest in biological nitrogen fixation. BNF is a microbiological process in the biosphere 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 (Werner 2005). Biological nitrogen fixation offers an environmentally friendly and efficient source of N in farming systems on tropical soils. Nitrogen input through BNF can help maintain soil N reserves as well as substitute for N fertilizer to attain large crop yields (Peoples and Craswell 1992). The amount of N fixed by legumes varies due to the differences in legume genotypes, soil N, effectiveness and presence of rhizobial bacteria, amount of N accumulated as biomass during growth and the area of the cultivated legume (Giller and Mapfumo 2005). As reported by van Kessel *et al.* (2000), 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. Since most tropical soils are low in N, the contributions of BNF in these soils were reviewed to establish the successes and limitations regarding harnessing this cheap and environmentally friendly source of N in legumes, rice and sugarcane cultivation.

### 1.1 Tropical Agro-ecologies

One-third of cultivable soils of the world are in the tropics and these support more than three-quarters of the world population and yet more is known about the soil resources in the temperate regions (Hartemink 2004). The three main causes are that research lags behind; persistent myths continue to hinder development and over generalization hin-

der the advancement of soil science in tropical regions (Lal and Sanchez 1992). Agroecology varies across the world from the tropics to the temperate and the subarctic regions, hence, it is important to give some details about the characteristics and practices of the tropics having great effect on the agroecology of this region.

According to Buringh (1970), there is a very great range in annual precipitation, usually 3000-6000 mm in the humid tropics, but over 10000 mm in some wet equatorial regions and almost zero in some extremely arid regions. Besides these variations, there is also an extreme diversity in precipitation between seasons (alternating dry and wet seasons) or during the day. In addition, some soils in the tropics are quite old, lack bases and even without weatherable minerals. Others are very young, with soil formation at its early stage. In the humid tropics, where soil formation proceeds almost continuously, soils can be altered in a short period; yet in arid regions, lack of moisture slows down many soil processes, so that changes are hardly detected, even over hundreds of years. Under tropical conditions, which include abundant rainfall and high temperatures, soils are heavily weathered and characterized by enrichment in aluminum and iron oxides (Qafoku *et al.* 2004). The amount of free forms of iron oxide in tropical soils is significantly larger than in temperate soils (Zhang *et al.* 2009), resulting in a greater redox potential under similar soil moisture conditions (Ding 2008). In addition, many naturally humid tropical soils are highly acidic (Xu and Cai 2007), infertile Oxisols and Ultisols with variable-charge minerals and with microbial populations often dominated by fungi (Sanchez *et al.* 1989). In most tropical soils, sustainable agriculture faces large constraints due to low nutrient contents and accelerated mineralization in soil organic matter (Zech *et al.* 1997). Consequently, the cation exchange capacity of the soils which is often low due to their clay mineralogy decreases further. Therefore, efficiency of applied mineral fertilizers like N is very low when the loss of mobile nutrients from the top soils is enhanced by high rainfall (Cahn *et al.* 1993). In addition, many farmers cannot afford the cost of regular applications of mineral fertilizers. Therefore, nutrient deficiency is prevalent in many soils of the tropics.

The most practiced form of land use in the tropics is shifting cultivation using slash and burn techniques whose nutrient additions have positive effects on soil fertility only for a short period (Kleinman, Pimentel, and Bryant 1995). In addition, under tropical conditions, organic matter is usually mineralized rapidly (Tieszen, Cuevas, and Chacon 1994), therefore only small amounts of applied organic materials will be stabilised in the soil in the long term and successively released to the atmosphere as  $CO_2$  (Fearnside 2000). Xu and Cai (2007) reported that humid tropical soils were characterized by generally small denitrification capacities, based on  $NO_3^-$ -N reduction. This may be essential in the N economy of tropical soils by sustaining the N balance of the soil after biological N fixation. The reasons for the low denitrification capacity of the acidic tropical soils could be attributed to: larger oxidation capacity values in tropical soils which would result in a higher level of soil redox potential, thus suppressing denitrification (Zhang *et al.* 2009), small amounts of organic C and mineral N which may not be enough to sustain denitrifying populations (Wang and Cai 2008) or low soil pH, which is far from the favourable soil pH (6 to 8) for most denitrifiers growth and activity (Aulakh, Khera, and Doran 2000). Considering the fact that the production of N ferti-

lizer is the costliest both economically and energetically, that less than half of the N applied is recovered by the first crop under tropical conditions and that it strongly contributes to pollution. Then, N availability must be made a priority in any attempt to increase the production of nutritious food in a clean environment.

### 1.2 Soil Nitrogen Pool

The increased crop yields that are widely observed following N<sub>2</sub>-fixing associations may arise from breaking cycles of pests and diseases, through changes in soil microbial, chemical and physical characteristics or through encouraging the activity of soil macrofauna such as earthworms (Wani, Rupela, and Lee 1995). Like wise, rotational benefits of N<sub>2</sub>-fixing legumes and cereals mixture can be attributed to an improvement in the N economy of soils. The key to the long-term sustainability and productivity of soils is organic matter, in particular organic C and N (Peoples, Ladha, and Herridge 1995). In the western and southern parts of Australian wheat belt, annual pasture legumes were introduced into pastures grown in rotation with crops in the 1940's and 50's, and more recently, perennial legumes in particular Lucerne have also been used. These legume-based pasture systems were successful in increasing total (organic) soil N (Reeves 1991; Dalal et al. 1994; Peoples, Ladha, and Herridge 1995). Annual increment of soil N of between 25 and 100kg N ha<sup>-1</sup> appear to be common in subterranean clover-based pastures but average rates of soil N accretion much greater than 100kg N ha<sup>-1</sup> have been reported under Lucerne despite the removal of large amounts of shoot N by grazing animals or as hay (Gault et al. 1995). However, once the pasture phase is replaced by cereal cropping, N fertility quickly declines. Thus the system demands alternating pasture-cropping phases (Peoples, Ladha, and Herridge 1995).

### 1.3 Biological Nitrogen Fixation (BNF)

High cost of N fertilizers and their non-availability at proper time have renewed the interest of farmers on BNF. Biological N<sub>2</sub> fixation provides continuous supply of N for plant growth in situ, adds organic matter to the soil and is economically viable (Yakubu, Kwari, and Ngala 2010). BNF is a key process in which molecular N is reduced to form ammonia, which is the form of N that is used by living systems for the synthesis of many bioorganic compounds. *Rhizobium* inoculation is a cheaper and usually more effective agronomic practice for ensuring adequate N supply compared to the use of high N fertilizer amounts. This process of BNF accounts for 65% of the N currently utilized in agriculture, and will continue to be important in future crop productivity, especially in sustainable systems (Matiru and Dakora 2003).

Several researchers have demonstrated the ability of N fixing bacteria to provide N to crops with yields similar to 70 kg ha<sup>-1</sup> of N-urea (Soares et al. 2006; Lacerda et al. 2004) or 50 kg N-urea ha<sup>-1</sup> (Almeida et al. 2010). Nodulation, shoot biomass, and grain yield can be improved by legume seed inoculation with *Bradyrhizobium* (Egamberdiyeva, Qarshieva, and Davranov 2004; Fatima, Zia and Chaudhary 2007; Hayat et al. 2008). What ultimately determines the success of a BNF practice is the genetic potential of the organisms and how that interacts with components of the environment (Thies, Singleton and Bohlool 1991). Results obtained by Yakubu, Kwari, and Ngala (2010) showed that seeds inoculation with suitable *Rhizobium* strains enhanced the performances of crops significantly as reflected by the interactions between

crops and *Rhizobia*.

Soil organic matter is important for BNF because of its influence on soil physical, chemical and biological properties and processes. It helps to create a favourable medium for physical processes, chemical reactions, and biological activity (Coskan and Dogan 2011). The multi-faceted role of soil organic matter (SOM) must therefore be taken into consideration in any assessment of soil quality and land management. Low concentration of organic matter can have a deleterious effect on offsite environment because it is often associated with decreased soil fertility, water holding capacity and water infiltration, and increased erosion.

Further, SOM turnover controls the fluxes of nutrients. Microbial biomass measurements combined with soil respiration have frequently been used as an index of soil development or degradation (Insam and Domsch 1988; Coskan and Dogan 2011) and to assess the quality of organic matter input. Interactions between soil microorganisms and soil microfauna, particularly Protozoa and Nematode, largely control SOM turnover (Elliot et al. 1984; Coskan and Dogan 2011). Harvested crop residues and rotation have a major impact on the soil organic matter content. However, some features, such as the type and degree of decomposition of organic matter affects BNF in different ways. Many field trials which applied organic material by Gok et al. (2001) show that with the organic material application, organic matter content of soil increased in the short term but at the end of the trial, soil organic matter content decreased due to high rate of mineralization. To gain long-term soil organic matter, all kind of organic substrates should be regularly added to the soils under the effect of semi-arid climate condition (Coskan and Dogan 2011).

### 1.4 Contribution of Biological Nitrogen Fixation in Tropical Soils

The 'Law of the Minimum' states that plant productivity can be defined by a single limiting factor in the system. Amelioration of the limiting factor will increase productivity until another essential growth factor in the system becomes limiting. Therefore, no amount of biologically or chemically fixed nitrogen can increase production as long as some other factor is limiting growth. Major considerations affecting the microbe, the host, or their symbiotic interaction include soil acidity (Munns 1977), other acid-related factors including aluminium and manganese toxicity, calcium (Singer and Munns 1987), and phosphorus deficiency (Cassman et al. 1981; Leung and Bottomley, 1987).

Symbiotic activity within a plant community is also conditioned by the amount of N mineralized from organic sources (George et al. 1988). One of the major obstacles to successful establishment and effective performance of introduced N<sub>2</sub>-fixing systems is competition from native organisms. This has been extensively demonstrated for legume inoculants (Bohlool et al. 1992). The yield and nodulation of a promiscuous host cowpea (*Vigna unguiculata* (L) Walp) can be increased with inoculation less frequently due in part to the population size and competitive ability of indigenous *Bradyrhizobium* sp. in tropical soils (Danso and Owiredo, 1988).

High soil temperatures in tropical and subtropical areas are a major problem for BNF of legume crops (Michiels et al. 1994). Selection and development of crops species and cultivars that grow and produce well at lower levels of

available soil nutrients (high nutrient use efficiency) seems an important strategy for sustainability in tropical soil systems (Baligar and Fageria 1997). Instead of productivity alone, tropical agriculture in the next century must favour biodiversity, the preservation of soil organic matter and efficient cycling of nutrients. Biological N fixation may play an important role on restoring fertility and sustainability under those conditions (Franco and Balieiro 2012).

Many agronomic plant species associate with diazotrophic microorganisms which are able to fix atmospheric nitrogen. Among the legume species are the crops with highest potential for BNF already available to be used in the productive systems. Blue green algae, free-living in the soil or associated with the fern *Azolla* and endophytic associations between diazotrophic bacteria and gramineous and starchy species also have potentials to be used in agriculture (Dobereiner, Baldani, and Reis 1999). Table 1 below

presents some values on BNF in some crops. There are also experiments showing BNF contribution of 30 kg of N ha<sup>-1</sup> year<sup>-1</sup> in free-living cyanobacteria and up to 3 kg of N per ha per day in the symbiosis *Azolla* with cyanobacteria in a tropical soil in Brazil (Watanabe and Roger 1984). In Africa, grain legumes fix about 15-210 kg N ha<sup>-1</sup> seasonally, while tree legumes fix about 43-581 kg N ha<sup>-1</sup> year<sup>-1</sup> (Dakora and Keya 1997).

Biological N fixation is an essential, cost-effective alternative or complementary solution to industrially manufactured N fertilizers for many poor farmers in the tropical region of the world. Many grain legumes are major sources of protein for the subsistence of poorest farmer households. When legume production exceeds household requirements, it can be readily traded to generate income, making significant and direct contributions to livelihoods. In addition to the considerable economic interest and positive impact on human health, BNF technologies have the

Table 1: Amount of nitrogen fixed (kg/ha/year or cycle) by some leguminous crops and tree species

| PLANT SPECIE                        | BNF kg/ha/year or cycle | DAYS  | COUNTRY                |
|-------------------------------------|-------------------------|-------|------------------------|
| Soybean (glycine max)               | 114-188                 | 66    | Nigeria <sup>1</sup>   |
|                                     | 85-154                  | 110   | Brazil <sup>1</sup>    |
| Beans (Phaseolus vulgaris)          | 25-65                   | 60-90 | Brazil <sup>1</sup>    |
|                                     | 3-32                    | 61    | Brazil <sup>1</sup>    |
|                                     | 11-53                   | -     | Brazil <sup>2</sup>    |
|                                     | 18-36                   | 47    | Colombia <sup>2</sup>  |
|                                     | 92-125                  | -     | Guatemala <sup>2</sup> |
| Pigeon pea (Cajanus cajan)          | 68-88                   | -     | India <sup>1</sup>     |
|                                     | 168-208                 | -     | 3                      |
| Cowpea (Vigna uingiculata)          | 9-51                    | 110   | Brazil <sup>1</sup>    |
|                                     | 73-354                  | -     | 3                      |
|                                     | 66-120                  | 57    | Nigeria <sup>1</sup>   |
| Mung bean (V. mungo)                | 119-140                 | 66    | Thailand <sup>1</sup>  |
| Chickpea (Cicerarietinum)           | 60-84                   | 160   | Australia <sup>1</sup> |
| Leucaena (Leucena leucocephala)     | 500-600                 | -     | 3                      |
| Centrosema (Stylosanthes pubescens) | 80-280                  | 1     | Several <sup>1</sup>   |
| Stylo (Stylosantes spp.)            | 20-263                  | 1     | Several <sup>1</sup>   |
| S. capitata                         | 3-46                    | 1     | Brazil <sup>1</sup>    |
| Kudzu (Pueraria phaseoloides)       | -                       | -     | Colombia <sup>1</sup>  |
| Acacia (Acacia mearnsii)            | 200                     | -     | 3                      |

potential to generate global environmental benefits by reducing greenhouse gas emissions and water pollution from inorganic N fertilizers. Research on BNF, particularly molecular genetic understanding of *Rhizobium*-legume symbiosis, has recently made significant progress, opening new possibilities to design strategies aimed at enhancing N-fixing capacity and legume productivity in tropical soils.

### 1.5 Benefits of Biological Nitrogen Fixation in the Tropics

In Africa, most farmers cannot afford the inorganic nitrogen fertilizers due to their high cost and non-availability. This has renewed the interest of farmers on biological nitrogen fixation, which provides a continuous supply of N for plant growth and is economically viable (Yakubu *et al.* 2010). The success of a legume grain crop is dependent on

its capacity to form effective nitrogen-fixing symbioses with root-nodule bacteria. Many soils however, do not have adequate amount of native rhizobia in terms of number, quality or effectiveness to enhance biological nitrogen fixation (FAO, 1984). These situations call for provision of external source of rhizobia. However, the benefits, production and utilization of rhizobia inoculants for legumes improvement has been recognized for a very long time in developed countries, but still developing in sub-Saharan African countries. Therefore, there is hardly any country in Africa, where rhizobial inoculants are regularly used by farmers, except in experiments that are limited to research farms (Bala, 2011). This could be attributed to handling and storage capacity of these inoculants by the mostly rural farmers as well as inadequate extension services regarding the po-

tential benefits of microbial fertilizers. However, there have been reports of positive results in the use of rhizobial inoculants in various parts of Africa on symbiotic effectiveness of legumes. According to Dakora and Keya (1997), grain legumes fix about 15-210 kg N ha<sup>-1</sup> seasonally in Africa. Studies in humid regions of western Nigeria have shown that cowpea can fix 66-120 kg N ha<sup>-1</sup> in 57 days (Awosaike *et al.* 1990). Yusuf *et al.* (2006) reported that cowpea fixed 16-34 kg N ha<sup>-1</sup> and soybean between 41 and 50 kg N ha<sup>-1</sup> in the northern Guinea Savanna zone of Nigeria.

The amount of N fixed by *Rhizobium*-plant association varies considerably with the environmental factors such as soil temperature, moisture, and pH which affect the host-plant (macrosymbiont) and the *Rhizobium* (microsymbiont) (Rayar, 2000). Inoculation with specific strains of indigenous or exotic rhizobium or bradyrhizobium has also resulted to improved productivity of legumes in several regions of Africa. In cowpea, inoculation caused increase in nodule number by 21%, N content by 33% and amount of N fixed by 38% over the control (Yakubu, Kwari, and Ngala 2010). These results concur with the reports of Bello *et al.* (2018), Chude *et al.* (2006) and Uzoma *et al.* (2006) that cowpea responded positively to *Rhizobium* inoculation in tropical savanna soils of Nigeria. Ibrahim *et al.* (2017) reported high N fixation in groundnut after inoculation with the exotic rhizobia NC 92 alongside fertilizers and soil amendments. Similar reports were also given by Yusuf *et al.* (2012) for groundnut; Sanginga *et al.* (2000); Osunde *et al.* (2003); and N'cho *et al.* (2013) for soybean, indicating that tropical Africa has the potential of harnessing BNF to stimulate productivity of legumes as observed in other parts of the developed tropical regions like Australia, Brazil and parts of the United States.

The direct availability of biologically fixed N to the host plant, through the BNF process, allows the plant to grow in environments that are low in N and at the same time reduce losses from denitrification, volatilization and leaching, improving the sustainability of agricultural system that involves such symbiosis (Yakubu *et al.* 2010). Biological nitrogen fixation can be a major source of N in tropical agriculture when symbiotic N<sub>2</sub>-fixing systems are used; the amount of N input is reported to be as high as 360 kg N ha<sup>-1</sup> (Bohloul *et al.* 1992). Among symbiotic N<sub>2</sub>-fixing systems, nodulated legumes have been used in cropping systems for centuries. The N fixed by rhizobia in legumes can also benefit associated non-legumes via direct transfer of biologically-fixed N to cereals growing in intercropping (Englesham *et al.* 1981), or to subsequent crops rotated with symbiotic legumes (Dakora and Keya 1997). Azolla, a nitrogen-fixing symbiotic water fern, is of particular practical value in rice production systems. Azolla in symbiosis with cyanobacterium (*Anabaena azollae*), can fix 2-4 kg N ha<sup>-1</sup> day<sup>-1</sup> (Lumpkin and Plucknett 1982). Recently other benefits of Azolla have been recognized such as weed suppression, potassium scavenging from floodwater, source of animal and fish feed, phosphorus scavenging in sewage-treatment plants and suppression of ammonia volatilization (Watanabe and Liu, 1992).

Biological nitrogen fixation can reduce the need for N fertilizers, resulting in an economy estimated in US\$ 3 billion per crop season (Nicolás *et al.* 2006). Yamada and Lopes (1998) estimated that, from 1993 to 1996, 1.05 million tons of N approximately US\$ 680 million were used per year in Brazil while BNF in the soybean crop alone has contributed to an estimated saving of US\$ 1.5 billion every year in re-

placing N that would be required in nitrogen fertilizers. To further elaborate more on specific contribution of BNF to N economy in tropical soils.

#### *1.6 Proper inoculation and genetic improvements increases the efficiency of biological nitrogen fixation in the tropics*

Rhizobium inoculants are the main contributors to the biological nitrogen fixation Singh and Tarafdar (2002). Full legume N-benefits can only be achieved in the presence of efficient rhizobial strains, which can be native to the soil or introduced in-form of commercial inoculants. It is widely acknowledged that inoculation of legumes with effective rhizobia can improve yields and provide a substitute to inorganic fertilizers. In Brazil, inoculation with commercially produced *Bradyrhizobium* completely replaces application of chemical fertilizer in soybean. In 2006, with soybean yield of 57million mega gram, about US\$3.3 billion in fertilizer expenses were saved (Moreira, 2008). Similarly in Australian agriculture, that in recent years has seen the introduction of several new legumes, specific inoculant strains are sold commercially to promote N<sub>2</sub> fixation particularly when these crops are first cultivated in soils. This practice is necessary because the indigenous rhizobia are usually unable to form effective symbioses with exotic legumes (Howieson *et al.*, 2000). In soils where there is a low indigenous population of N-fixing bacteria and limited N within a soil such as conditions of the soils of the Cerrados of Brazil, the nodulation of the common bean is poor but can be improved by inoculation with a commercial inoculant PRF 81 (Hungria and Vargas 2000). Also in soils where there is no history of legume cultivation or a legume is being cultivated that is exotic (as clearly illustrated by the widespread cultivation of Mediterranean legumes in Australia) and the indigenous rhizobia could not effectively nodulate these legumes, exotic commercial rhizobia were and continue to be imported to use as inoculants (Baldock and Ballard 2004; Howieson *et al.*, 2000). In sub-Saharan Africa, inoculation of soybean with *Bradyrhizobium japonicum* increased yield from 500 to 1500 kg ha<sup>-1</sup> (Mpeperekki *et al.*, 2000). In 12 trials with chickpea in India, inoculated plots gave on an average 116 kg ha<sup>-1</sup> more grain as compared to uninoculated plots (Wani *et al.*, 1995). In another set of field demonstrations, inoculation resulted in grain yield increase in the range of 112-227 kg ha<sup>-1</sup> (Chandra and Ali, 1986). The results of 1500 demonstrations on farmers' fields with pigeon pea conducted in India showed 100% increase in yield (1035 vs. 516 kg ha<sup>-1</sup>) due to balanced use of DAP and rhizobium inoculation (Chinmulgund and Hegde, 1987). On research stations in 16 trials inoculation of chickpea with rhizobium increased grain yield by 342 kg ha<sup>-1</sup>. Significant improvement in chickpea grain yield was reported from 7 out of 16 locations (Subba Rao, 1976) and 6 out of 12 locations (Subba Rao and Tilak, 1977), predominantly in central and northern India with yields varying from -14 to 30% compared to the control plots yield. Increase in grain yield of the pigeon pea inoculated with effective *Rhizobium* ranged from 19 to 68% over uninoculated controls (Nambiar *et al.*, 1988).

The usefulness of inoculation in different crop sequence has been investigated by various research workers. Singh and Tarafdar (2002) reported that residual effect of rhizobium inoculation of summer mung bean clearly indicated that they have not only enhanced the yield of subsequent

rice crop but have also improved the physical and chemical properties of soil. Saxena and Tilak (1975) observed increased soybean yield as well as that of succeeding wheat crop due to rhizobium inoculation. This increase in yield was equivalent to the direct application of 30 kg N ha<sup>-1</sup> to wheat crop. Singh *et al.* (2004), Goss *et al.* (2002) and Stefanescu and Palanciuc (2000) reported increased yield and N balance in soil after inoculated soybean. They further observed that the residual effect of rhizobium gave 54.4% higher wheat grain at a considerable saving of fertilizer nitrogen. In addition to increase in yields of grain legumes due to rhizobium inoculations, Subba Rao and Tilak (1977) reported yield increase of wheat or rice following rhizobium inoculation which was always more in rhizobium inoculated series than in un-inoculated ones. Maximum residual effect was seen in soybean which increased the yield of the subsequent crop of wheat by 65.93% over corresponding un-inoculated controls. Singh and Tarafdar (2002) reported that when summer mung inoculated with specific strain of rhizobium, not only improved the yield of legume crops, but also leave behind sufficient quantity of 25.95 kg ha<sup>-1</sup> nitrogen for use of subsequent crop grown in rotation. Barea (2015) observed that in double or multiple cropping systems, inclusion of rhizobium inoculated legumes results in saving of fertilizer N.

However, the quality of commercially produced inoculants depends on both the number of rhizobia they contain and effectiveness in fixing N with target host (Amarger, 2001). Symbiotic effectiveness is one of the most important factors when selecting an inoculant strain (Date, 2000; Stephens and Rask, 2000). Other characteristics to be considered are genetic stability, ability to survive in inoculant, to persist in the soil, and to compete in nodule formation with soil rhizobia (Graham and Vance, 2000). The quality of an inoculant is evaluated by the number of viable rhizobia it contains (Hiltbold *et al.*, 1980; Brockwell and Bottomley, 1995; Stephens and Rask, 2000). High quality inoculants can provide approximately  $2 \times 10^{11}$  to  $4 \times 10^{11}$  rhizobia ha<sup>-1</sup>. This is about 1 % of the indigenous population of rhizobia present in the top 20 cm of soil (Catroux and Amarger, 1992).

## 2.0 Legumes and the symbiotic process

The legume-*Rhizobium* symbiosis allows many species to obtain their nitrogen nutrition from N<sub>2</sub>. This has been known since the Greeks discovered the value of legumes in enhancing soil fertility, some 2300 years ago. Since 1888, it has been known that this is due to the nodules on legume roots and the bacteria inside the nodules (Date 2000; Abd-Alla, Issa, and Ohyama 2014). This nitrogen fixation is very hard to quantify in economic (Lanyon 1995) or ecological terms (Hoglund and Brock 1987), but it is estimated to be responsible for between 45% (Danso 1995) and 85% (Vance, Heichek, and Phillips 1988) of the BNF in agricultural areas. Biological nitrogen fixation has the advantage of being environmental friendly and therefore would be ideal for sustainable agriculture. Biological nitrogen fixation, a key source of N for farmers using little or no fertilizer, constitutes one of the potential solutions and plays a key role in sustainable grain legumes production (Abd-Alla, Issa, and Ohyama 2014). Given the high cost of fertilizer in developing countries and the limited market infrastructure for farm inputs, current research and extension efforts have been directed to integrated nutrient management, in which legumes play a crucial role (Chianu *et al.* 2008; Abd-Alla, Issa, and Ohyama 2014).

Certain microorganisms have the ability to use the renewable source of energy to fix atmospheric nitrogen under mild conditions, such as normal temperature and normal pressure. Biologically-fixed N could be directly “absorbed” by plants and keep the environment almost “untouched”. Crop rotation with legumes has been recognized to increase soil fertility and agricultural productivity since ancient China and Rome (Cheng, 2008). Rhizobia live in the rhizosphere of leguminous plants such as soybeans and faba bean, forming colonies that produce root nodules (Foth, 1990). The symbiosis between soybean and *Bradyrhizobia* results from a complex process involving many genes of both partners that leads to the formation of N<sub>2</sub>-fixing nodules in roots (Provorov and Vorob'ev, 2000). Biological nitrogen fixation is an oxygen dependent reaction and therefore is prevalent in legumes growing in aerated, upland soils. Currently, approximately 2 tons of industrially-fixed nitrogen is needed as fertilizer for crop production to equal the effects of 1 ton of N biologically-fixed by legume crops (Chianu *et al.*, 2008). Therefore, biologically-fixed nitrogen influences the global N cycle substantially less than industrially fixed nitrogen. Research in this field has pivotal importance and would be significantly beneficial.

## 3.0 Cereals

Efforts at extending N<sub>2</sub>-fixing ability to important non-leguminous crops such as cereals have long been a major goal of researchers working on BNF (Matiru and Dakora 2004). N<sub>2</sub>-fixing bacteria have been isolated and characterized from a variety of wild and cultivated cereal crops (Stoltzfuz *et al.*, 1997). Some of those microbes so far identified in non-legumes include *Gluconacetobacter diazotrophicus* (formerly *Acetobacter diazotrophicus*) from sugarcane (Gillis *et al.*, 1989; Fuentes-Ramirez *et al.*, 1993; Caballero-Mellado and Martinez-Romero, 1994; James *et al.* 1994; Sevilla *et al.* 2001; Reis, Olivares and Dobereiner 2001; Riggs *et al.* 2001; Muthukumarasamy, Revathi, and Loganathan, 2002). Strains of *G. diazotrophicus* have also been isolated from roots and stems of coffee (Jimenez-Salgado *et al.*, 1997). *Azospirillum* is another much studied diazotroph, especially the species *lipoferum* and *brasilense*, which have been shown to infect a number of cereal plants including wheat, maize and sorghum (Pacovsky, Paul and Bethlenfalvay 1985; Dobereiner and Boddey 1981; Christansen and Vanderleyden 1993; Fallik and Okon 1996; Malik *et al.* 1997; Kapulnik *et al.* 1983; Weber *et al.*, 1999; Dobbelaere *et al.* 2001). Plant inoculation with *Azospirillum brasilense* promoted greater uptake of NO<sub>3</sub><sup>-</sup> in corn, sorghum and wheat (Murty and Ladha 1988; Zavalin, Kandaurova, and Vinogradova 1998; Saubidet, Fatta, and Barneix, 2000), and subsequently increase the N economy of the soil.

## 4.0 Rice and sugarcane cultivation

Associative and free-living microorganisms are believed to contribute to production sustainability of flooded rice production systems (Roger and Ladha, 1992). Approximately 50% of the N requirement of a flooded rice crop is met from the soil N pool (Bouldin, 1986) which is believed to be maintained through BNF by associative and free-living microorganisms (Koyama and App 1979). Contributions from non-symbiotic N<sub>2</sub> fixation in upland agriculture is generally not substantial (Bohloul *et al.*, 1992), although N<sub>2</sub> fixation to the order of 160 kg N ha<sup>-1</sup> has been reported for sugarcane. *G. diazotrophicus* have been reported to fix economical amounts of N<sub>2</sub> in sugarcane (Boddey and

Dobereiner 1988; Dobereiner *et al.*, 1993; Sevilla *et al.* 2001).

In Brazil, sugarcane crops accumulate N between 100 and 200 kg N per hectare per year, while N fertilization rates are relatively low, usually less than 60 kg N per hectare (Reis, Olivares, and Dobereiner, 2007). Also, the response of sugarcane crops to N fertilizers is usually very weak (Boddey 1995; Reis, Olivares, and Dobereiner, 2007). The use of low N fertilizer input was justified by the results of 135 field experiments in all of main cane growing areas of Brazil, with only 19% of the studies showing a significant increase in yield owing to the application of N fertilizer (Azeredo *et al.*, 1986). In some sites of Brazil, sugarcane has been grown continuously for more than 100 years without any N fertilizer being applied at all (Dong *et al.*, 1994). This circumstantial evidence suggests a high potential for BNF in sugarcane.

Using a  $^{15}\text{N}$  dilution technique involving the supply of a  $^{15}\text{N}$ -labeled fertilizer, Lima, Boddey, and Döbereiner (1987) evaluated the contribution of BNF in four sugarcane varieties planted in large-size pots and concrete tanks. The result showed that 40-60% of plant N was derived from BNF in the variety CB 47-89. Urquiaga, Cruz, and Boddey (1992) also calculated the contribution of BNF in several cultivars of sugarcane, and found it to be about 70% for the most promising genotypes. Again, using the  $^{15}\text{N}$  natural-abundance method, Boddey *et al.*, (1991) showed that 25-60% of the N assimilated in sugarcane at various sites in Brazil was derived from BNF. The analysis showed very large BNF inputs to several sugarcane varieties, especially the wild non-commercial species Krakatau (*S. spontaneum*) used in plant breeding in Brazil, as well as the commercial varieties SP 70-1143 and CB 45-3 in low-fertility soils. Yoneyama *et al.* (1997) examined the contribution of BNF using a  $^{15}\text{N}$  natural-abundance method in Brazil, the Philippines and Japan, comparing the abundance of  $^{15}\text{N}$  in sugarcane with that in neighbouring weeds as control plants. At many but not in all of the sites in Brazil, a contribution from BNF was indicated.

#### 4.1 Agroforestry Systems

In addition to the common occurrence within the legume family (Fabaceae), nitrogen-fixing rhizobium symbiosis has been found in a small genus of tropical trees found in the family Cannabaceae called Parasponia (Trinick 1973; Rik *et al.* 2012). Almost all measurement of BNF by  $\text{N}_2$ -fixing trees, either on farms or as part of natural communities, have been restricted to estimates of P fixation, from studies or surveys undertaken in Africa, Australia, Brazil, Indonesia, the Philippines, Thailand or USA (Peoples *et al.* 1995). Although amounts of  $\text{N}_2$  fixed cannot be calculated in most cases because tree biomass is hardly determined, the inputs of N could be substantial because of the large annual yields of dry matter and N of woody species (from 150-850 kg N  $\text{ha}^{-1}$ ) in natural communities (Sandhu *et al.*, 1990), in alley cropping systems (Sanginga *et al.*, 1995) or where trees and shrubs are cut for forage (Blair *et al.*, 1990).

## 5.0 Conclusion

Biological  $\text{N}_2$  fixation is of high importance in sustainable and environmentally friendly food production systems with long-term crop productivity in tropical agroecologies. However, if BNF is to be utilized, it needs to be optimized to meet the requirements of the prevailing environmental conditions. In the near future, particularly in tropical regions of the world, tremendous opportunities exist for improving the contribution of BNF to N economy across all agricultural sys-

tems through improved farming practices and molecular advancement in organisms, crop and environment. Although, there is no simple and easy approach to increase the contribution of BNF to N economy in agricultural systems under realistic farm field conditions, it is an attainable goal if pursued holistically. Numerous (micro) climatic variables, soil physical properties, agronomic management, host-*Rhizobia* association and socioeconomic aspects play an important role in controlling BNF in tropical soils.

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