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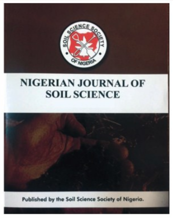

Biological Nitrogen Fixation and Productivity of Soybean in Maize-Soybean Intercrop under Tillage and Bradyrhizobium Inoculation on Alfisols of Northern Guinea Savanna, Nigeria

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ABSTRACT

A field study was conducted in the 2011 cropping season to investigate the effect of tillage and bradyrhizobium inoculation of soybean on biological nitrogen fixation (BNF) and yield components in maize-soybean intercropping systems. Treatments comprised of two tillage practices (conventional tillage (CT) and reduced tillage (RT)) as the main plot and bradyrhizobium inoculation at four levels (inoculated sole soybean, inoculated soybean/maize intercrop, uninoculated sole soybean, and uninoculated soybean/maize intercrop) as sub-plot. The treatments were laid in a split-plot under a randomized complete block design with three replications. Results showed that BNF and nitrogen derived from atmospheric (Ndfa) were significantly higher under RT than CT by 4.18 and 0.10 %, respectively. The BNF was consistently higher in the maize-soybean intercropping system with soybean inoculated with bradyrhizobium than in the uninoculated. BNF was 28.0 % higher in inoculated sole soybean and 80.2 % higher in inoculated maize-soybean intercrop than the uninoculated sole and intercropping system. Similarly, grain yield was 31.0 % higher in the inoculated sole soybean than the uninoculated sole and 33.7 % higher in inoculated maize/soybean intercrop than in the uninoculated intercrop. Biomass yields under inoculatedsole soybean and maize-soybean intercrop, respectively, were significantly higher than in uninoculated sole soybean and maize-soybean intercrop by 30.99and 33.66% for inoculated and uninoculated soybean sole and 34.44 and 30.40 % for inoculated and uninoculated intercrop. The results demonstrated that integrating bradyr*hizobium* inoculants and tillage will improve N fixation and productivity in maize-soybean-based intercropping systems in Alfisols of Northern savannah.

1.0. Introduction

Sustainability of soil productivity is imperative to attaining food security in Nigeria. Soil degradation is aggravated by land misuse, the rapid depreciation of natural resources and climate change (Hobbs and Gupta, 2003). Tillage is a primary activity in crop production and the tillage method adopted is dependent on crop species, weather, location and time of tillage (Martinez *et al*., 2008). Consequently, tillage is among the major practices that influence the physical, biological and chemical properties of the soil environment and subsequently affect nitrogen fixation (Kihara *et al*., 2012). Conventional tillage is a global practice for ages (Fowler and Rockstrom, 2001) due to its numerous advantages: improving soil tilt, soil drainage and aeration, root development and acceleration of organic matter decomposition by soil micro-organisms (Moussa-Machraou *et al*., 2010). It also enhances the early and fast development of arable crops like cereals (maize) in savanna ecology. Reduced tillage entails less pedoturbation and consequently, minimizes soil and nutrient losses through leaching and reduces erosion (Shipitalo *et al*., 2000; Schillinger, 2001). It also increases soil water storage through carbon sequestration and reduces production cost (Malhi *et al*., 2001; Singh *et al.,* 2008; Omeke, 2017). Northern Guinea savanna agro-ecological zone is characterized by unreliable on-set and cession of rainfall with a mono-modal pattern (Oluwasemine and Alabi, 2004). A tillage experiment conducted by Omeke (2017) on Alfisol in the savanna zone of Nigeria showed that reduced tillage significantly increased dry nodule weight higher than conventional tillage. The result corroborated other studies indicating that reduced tillage enhanced minimal disturbance of soil, increased rhizobial population and activity and stimulated nitrogen fixation due to higher dry nodule weight (Zhang *et al.,* 2012; Ferreira *et al.,* 2000 and Omondi *et al*., (2014).

Continuous depletion of soil nitrogen status among other nutrients are indices of low soil fertility and a major contributory factor to low crop yields in Nigerian agro-ecological zones (Hilhorst *et al*., 2000). Researchers have devised ways of alleviating this problem through the application of organic and inorganic fertilizers reduced tillage and the use of leguminous crops. However, the use of inorganic fertilizers by smallholder farmers in sub-Saharan Africa is limited by high costs, unavailability and sometimes lack of knowledge on usage (Omodi *et al*., 2014). Consequently, they stated that only 9 kg ha^{-1} inorganic fertilizers by smallholder farmers as compared to the global mean of 101 kg ha^{-1}. Materials for organic fertilizers are also difficult to acquire as farmers prefer supplying stovers to livestock and energy source rather than leaving them in the field to decay and consequently release nutrients (Baijukya, 2004). These challenges have led to the exploitation of other economical ways of supplying nutrients to crops and one of these ways is biological nitrogen fixation. Biological nitrogen fixation (BNF) in legumes has for a long time been a component of many farming systems throughout the world. Studies have shown that some soybean genotypes biologically fix 44 to 103 kg N ha^{-1} annually (Sanginga *et al*., 2003).

The use of bradyr*hizobium* inoculums in the establishment of legumes has been widely recognized, especially in soils where indigenous rhizobium was established to be poor. The benefits of the use of inoculants show that a quite good deal of money can be saved by marginal farmers by using quality tested inoculants on the farm (Zarin *et al.,* 2007). Rhizobial inoculation to seeds is well studied and exploitation of this beneficial nitrogen-fixing root nodule symbiosis represents a hallmark of successfully applied agricultural microbiology (Bruno, *et al*., 2003). Biological nitrogen fixation plays an essential role in crop establishment and yield since no N fertilizer is applied and it fulfills most of the plants' need for nitrogen. However, this BNF process is primarily controlled by four principal factors: effectiveness of rhizobia-host plant symbiosis, the ability of the host plant to accumulate N, amount of available soil N and environmental constraints (Van Kessel and Hartley, 2000). There is a need to enhance soil nitrogen through efficient biological nitrogen fixation which is a major limiting nutrient for plant growth, biomass and grain yields; as well as quality. The objective of this study was to determine the efficiency of rhizobium inoculation and tillage practices on biological N-fixation and productivity of soybean component crops in a maize-soyabean intercropping system on Savanna Alfisol of Nigeria.

2.0. Materials and Methods

Field study was conducted in 2011 cropping season on Alfisols at the Institute for Agricultural Research, Ahmadu Bello University, Samaru, in the Northern Guinea savanna ecological zone of Nigeria. Nigeria. The experimental site was located within latitudes 11°11’19.3”N and Longitudes 7°37’02”E (Fig. 1) on a physiographic surface rising 686 m above sea level. The climate is characterized by a total amount of rainfall of 1,207 and 1,333 mm, respectively in 2011and 2012. In both years, the third decadal rainfall witnessed a reduction in June, suggesting a dry spell occurrence at this period (Fig 2). Annual temperature ranges from 21.1 to 33.5^{o}C.

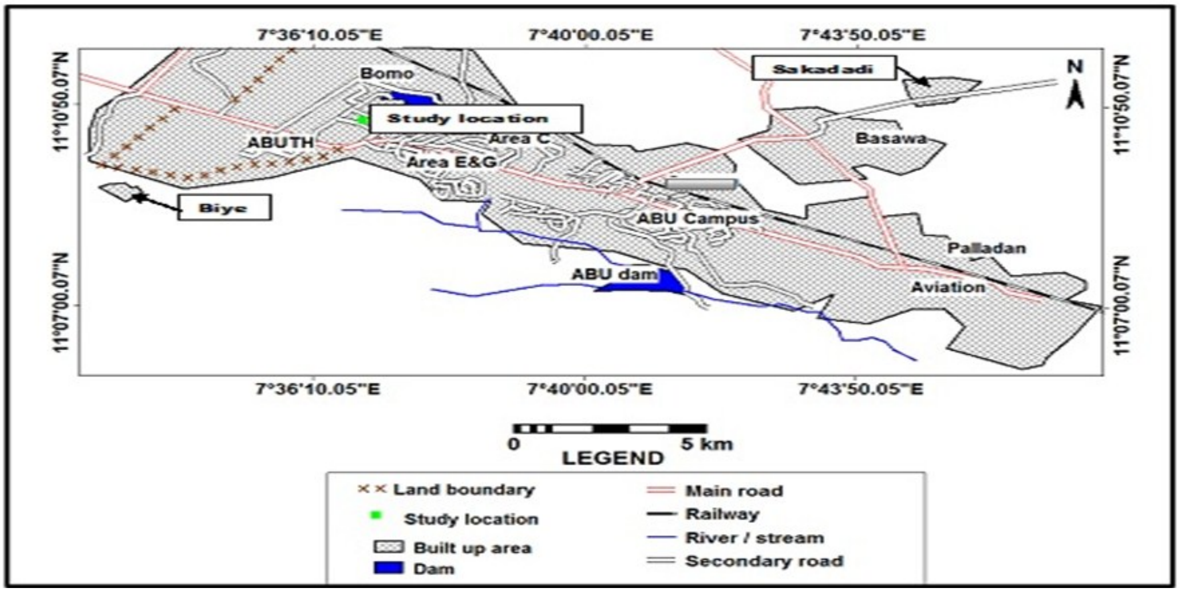

Fig.1: Map of Samaru, Zaria showing the study location.

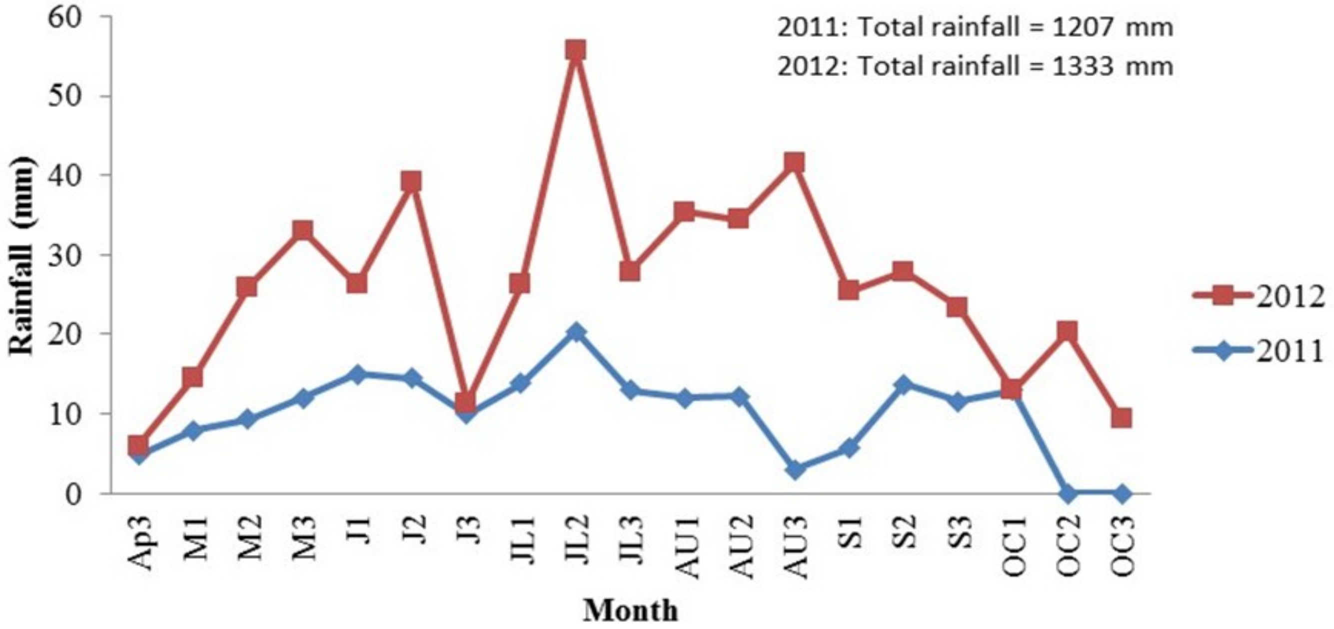

Figure 2: Decadal rainfall patterns in Samaru during 2011 cropping season
Source IART/ABU Zaria (2011)

2.1. Field experiment

The experiment was a randomized complete block design with a split-plot arrangement and replicated three times. Treatments comprised two tillage practices (conventional tillage (CT) and reduced tillage (RT) as main plots and bradyrhizobium inoculation at four levels (inoculated sole soybean, inoculated maize/soybean intercrop, uninoculated sole soybean, and uninoculated maize/soybean intercrop) as sub-plots. The conventional tillage (CT) was manual ridging at 0.75 m apart using hoe at 14 days after spraying the field with glyphosate (4 lits/ha) and remoulded at 8 weeks after sowing. For reduced tillage (RT), seeds were sown directed in rows maintaining 0.75 m as row spacing without ridging and remoulding.

2.2. Bradyrhizobium inoculation and planting

The soybean seeds were surface sterilized and inoculated with a Legume Fix *bradyrhizobia* strain using the method of IITA (2014) as shown in Fig. 3. Test crops were soybean (variety; TGX 1448 2E) which matures within 85 – 95 days, and maize (variety; SAMMAZ 15), which is a medium maturing variety. Planting was done on 1st July 2011. Two maize seeds were sown at 0.75 m x 0.25 m apart while inoculated and uninoculated soybean seeds were drilled in open grooves at 0.75 m x 0.05 m and covered lightly with soil. At two weeks after planting, maize and soybean seedlings were thinned to one plant/hill to maintain plant densities of 553,333 and 266,666 plants/ha, respectively. Phosphorus and K fertilizers were applied to all plots at the rate of 60 kg P_2O_5 ha^{-1} and 60 kg K_2O ha^{-1} respectively at planting. Whereas N fertilizer at the rate of 20 kg N ha^{-1} was applied to soybean and application of 0 kg N ha^{-1}, 40 kg N ha^{-1}, 80 kg N ha^{-1} and 120 kg N ha^{-1} were applied to maize crop at 2 weeks after sowing. At 4 and 8 weeks after sowing, manual weeding was done cautiously to avoid the transfer of rhizobia from inoculated rows to uninoculated rows.

Figure 3: Soybean Seeds Before Inoculation (Left) and Inoculated With Bradyrhizobia (Right)

2.3. Soil and plant sampling and analysis

Pre-cropping composite soil samples from 16 spots collected at 0-15 cm depth were collected using a soil auger. At maturity, composite soil samples were collected at 0-15 cm depth per treatment for the determination of total nitrogen. Harvesting of soybean grains was done at 3 months after sowing. Determination of the biomass and grain yields was done by oven drying at 70°C for 48 h. The dried samples were ground to powder and analyzed for N contents.

2.4. Laboratory soil and plant analysis

Initial and end of year (2011) soil samples obtained at depths 0- 15 from the field, were air-dried, sieved through 2 mm diameter sieve and subjected to laboratory analysis. Initial soil sample was analyzed in the laboratory of the Department of Soil Science Ahmadu Bello University for soil particle size distribution by hydrometer method (Gee and Bauder (1986). Soil pH electrometrically in 0.01M calcium chloride solution at soil-solution ratio of 1:2.5 and read on a pH meter (Hendershot *et al.*, 1993) Soil organic carbon by Walkley-Black wet oxidation method (Nelson and Sommers, 1982). Available phosphorus by Bray 1 method (Olson and Sommers 1982) and total nitrogen by the Kjeldahl digestion method (Bremner and Mulvaney 1982). The value of grain N concentration was multiplied by a factor (5.7) to obtain the seed protein content in soybean (Halvorson *et al.,* 2004) and recorded in percentage.

2.5. Determination of biological nitrogen fixation

The modified nitrogen-difference method (technique) as described by Peoples *et al*. (1989) was used in estimating biologically fixed nitrogen (N_2). In the N-difference technique, the difference between total plant nitrogen of an N_2 fixing legume and a control crop (non-N_2-fixing) is considered to be nitrogen that has been obtained through biological fixation. A modification to this basic principle is considered to improve the accuracy of measurements when the legume and control are not well matched (Evans and Taylor, 1987). In this study, a control crop was maize without fertilizer application. The quantity (Q) of biologically fixed N in a modified N-difference technique is calculated as follows:

Q = [N yield (legume) – N yield (control)] + [N soil (legume) – N soil (control)]

Where:

Q (kg ha^{-1}) = Quantity of the biologically fixed nitrogen

N yield [legume] (kg ha^{-1}) = Nitrogen yield of a legume (soybean)

N yield [control] (kg ha^{-1}) = Nitrogen yield of a non-fixing plant (maize at 0 N kg ha^{-1})

N soil [legume] (kg ha^{-1}) = Post-harvest soil nitrogen in a legume plots (soybean plots)

N soil [control] (kg ha^{-1}) = Post-harvest soil nitrogen in an unfertilized maize

The amount of nitrogen was determined per plant and converted to amount per hectare based on total shoot dry matter yield of each crop produced per hectare.

2.6. Statistical analysis

Data collected were subjected to analysis of variance (ANOVA) using a Mixed Linear Model procedure of SAS, (Institute Inc., 2009). Significant means were compared using the least significant differences (LSD) and standard errors (SED) at a 5 % level of probability.

3.0 Results and Discussion

3.1. Initial soil characterization

The initial characteristics of soils at the experimental site are presented in Table 1. The data showed that sand fraction was higher (671.20 g kg^{-1}), followed by silt (230.00 g kg^{-1}), while the lowest was clay (98.80 g kg^{-1}). The soil reaction was slightly acidic, while available P was medium. The soil was characterized by low organic carbon, total nitrogen, and carbon-nitrogen ratio, indicating that the soil was low infertility.

Table 1: Initial Soil Properties of the Experimental Site

Soil properties	**Unit**	**Test value**
Sand	g kg^{-1}	671.20
Silt	g kg^{-1}	230.00
Clay	g kg^{-1}	98.80
Textural class	Sandy loam	
pH (H_2O)	5.40	
pH ($CaCl_2$)	4.50	
Total nitrogen	g kg-1	0.46
Organic carbon	g kg-1	9.48
Carbon nitrogen ratio	12	
Available P	mg kg-1	9.14

3.2. Biological nitrogen fixation

The amounts of biological nitrogen fixation and percentage N derived from atmosphere (Ndfa) as influenced by tillage and bradyrhizobium inoculation in the soybean-maize intercrop systems are presented in Table 2. A significant difference between mean values was observed on BNF per plant at a 5 % level of probability. Generally, higher value BNF per plant was obtained under conventional tillage than the reduced tillage with an 11.45 % difference.

The results presented in Table 2 also show that the effect of bradyrhizobium inoculation in soybean-maize intercropping systems on biological nitrogen fixation (BNF) and percent N derived from the atmosphere (% Ndfa) were significantly different ($P \leq 0.05$). The values of biological nitrogen fixation (BNF) and percent N derived from atmosphere (% Ndfa) obtained under inoculated soybean sole and inoculated soybean-maize intercrop treatments were higher than those in other two soybean-maize cropping systems without bradyrhizobium inoculation. The highest amount of BNF per plant was obtained under inoculated soybean sole plots, followed by those in inoculated soybean-maize intercrop and least under uninoculated soybean-maize intercrop. The values of BNF observed for plots with soybean inoculated with bradyrhizobium either sole or intercrop was 28.00 % or 80.18 % higher than that insole or intercrop with uninoculated soybean, respectively. Similar trends were obtained for Ndfa uninoculated soybean-maize intercrop which gave significantly lower value as compared to other soybean-maize intercropping systems with or without inoculation. The differences were 11.80 % for the uninoculated sole, 27.30 % for inoculated intercrop and 71.31 % higher than the amount of Ndfa obtained from plots treated with uninoculated intercrop system. Combined effects of tillage practices and bradyrhizobium inoculation in soybean –maize intercropping systems on N fixation in g plant^{-1} (Table 3), kg ha^{-1} (Fig. 5) and Ndfa (Fig. 6) were significantly different ($P < 0.05$). The values were consistently higher under RT and inoculated soybean sole interaction followed by CT and inoculated soybean-maize intercrop and least under CT with uninoculated soybean-maize intercrop treatment combination. Biological N fixation obtained for g plant^{-1} was 17.48%, and 17.51 % under reduced tillage and maize-soybean intercrop and soybean sole inoculated combination as compared with those combined effect under conventional tillage. Similar observation was found under tillage in combination with maize-soybean without bradyrhizobium inoculation with a percent difference of 62.90 % and 27.66 % respectively.

Table 2: Effect of tillage practice and bradyrhizobium inoculation on N-fixation

Treatment	N biological fixed (g plant^{-1})	N biological fixed (kg ha^{-1})	Nitrogen derived from atmosphere (%)
Tillage practices			
Conventional tillage	1.16a	56.09	71.08
Reduced tillage	1.04b	58.74	74.50
SE±	0.07	1.22	0.25
Rhizobium inoculation			
Inoculated intercrop	1.15b	44.98	66.12
Uninoculated intercrop	0.81d	35.14	51.94
Inoculated Sole	1.42a	68.99	88.98
Uninoculated Sole	1.07c	38.29	58.07
SE±	0.04	1.09	1.01
Interaction			
TP x RI	**	**	**

* mean significant at $P < 0.05$ and ** mean significant at $P < 0.01$, SE = Standard Error

Table 3: Interaction of tillage practices and bradyrhizobium inoculation on BNF (g/plant)

Tillage practices	Bradyrhizobium inoculation				Mean
	Inoculated		Uninoculated		
	Intercrop	Sole	Intercrop	Sole	
Conventional tillage	1.43	1.77	0.62	0.94	1.19
Reduced tillage	1.68	2.08	1.01	1.20	1.49
Mean	1.56	1.93	0.82	1.07	

SE ± (0.05) for TP x RI = 0.23

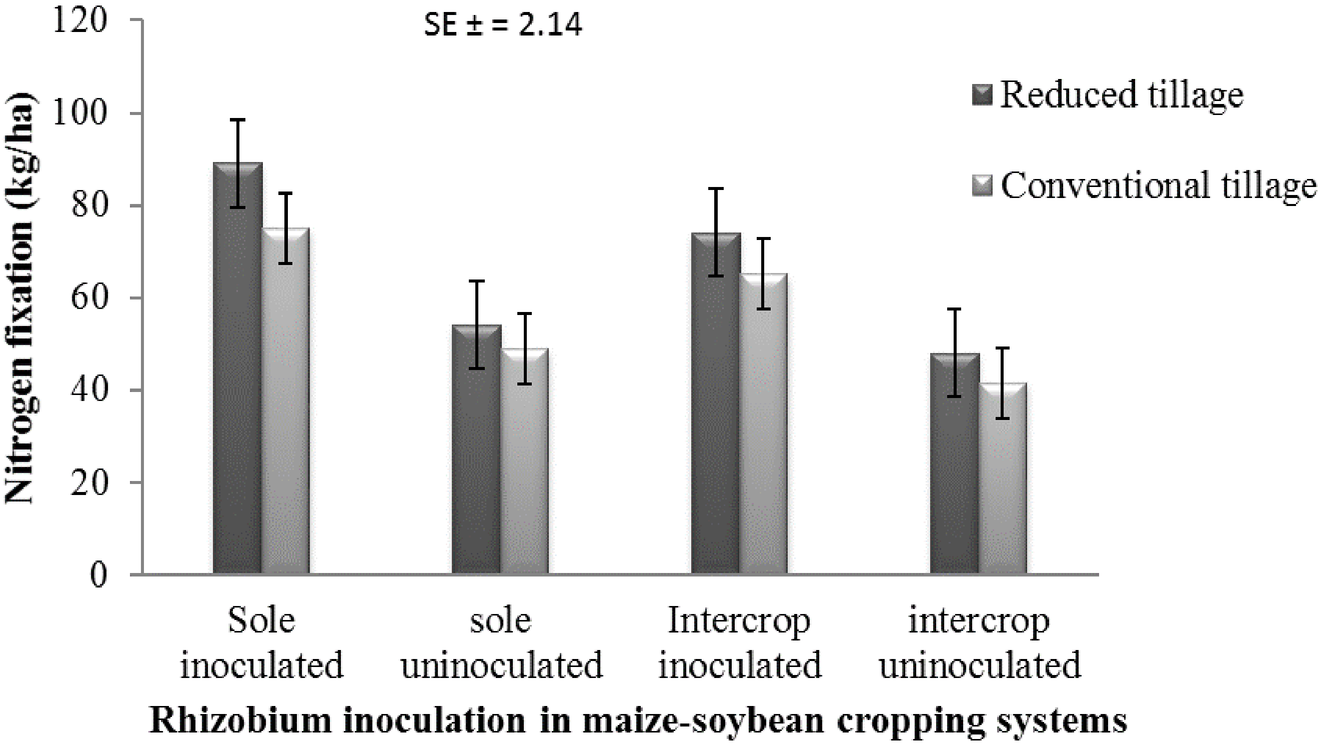

Fig 4: Tillage Practices and Bradyrhizobium Inoculation Interaction on N Fixation

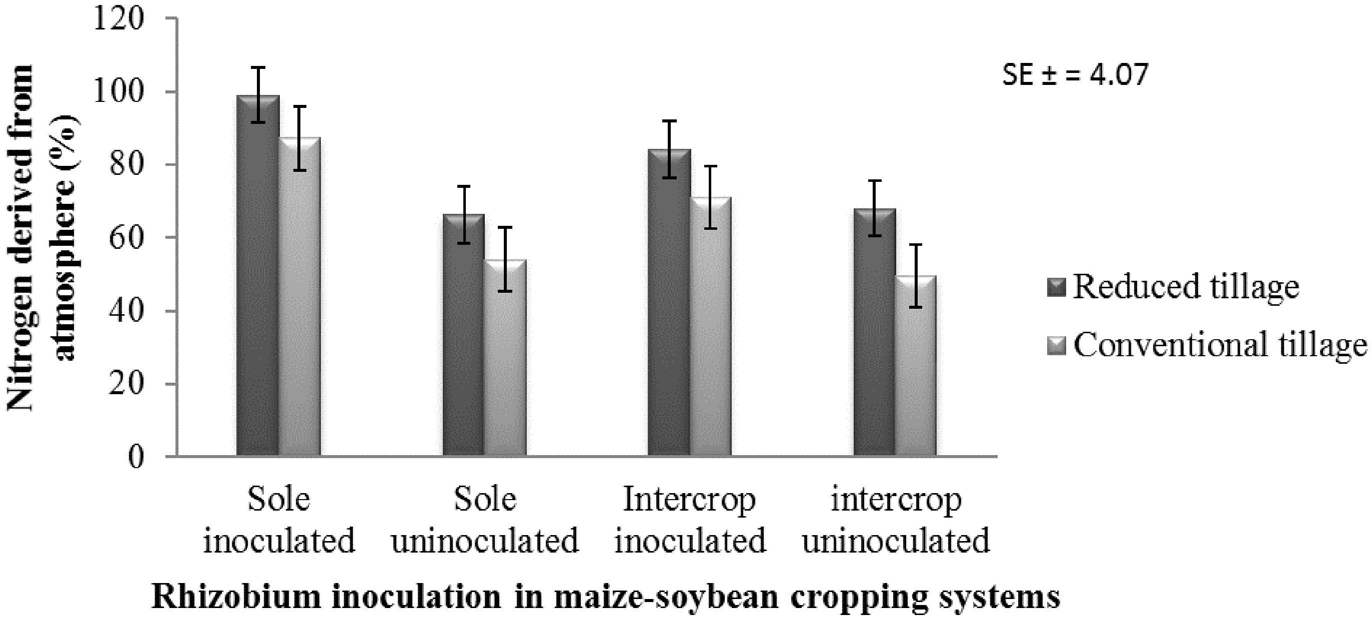

Fig 5: Tillage Practices and Bradyrhizobium Inoculation Interaction on Ndfa

3.3 Soybean Productivity

The results obtained for grain yield, biomass yield, nitrogen centration in grain (grain N) and percent protein of soybean under tillage practices are presented in Table 4. Tillage practices had no significant ($P < 0.05$) effects on soybean grain yield, grain N and percent protein. Whereas a significant difference was obtained between the two tillage practices on biomass yield which was significantly higher under conventional tillage with difference of 8.53 % as compared with reduced tillage. Similar observation was found in bradyrhizobium inoculation in maize-soybean-based intercropping systems effect on grain yield, biomass yield, grain N and protein content of soybean (Table 4). Inoculated soybeans sole cropping gave much higher grain yield (2116.01 kg ha^{-1}) and biomass yield (4231 kg ha^{-1}), followed by inoculated soybean-maize intercrop (1844.40 kg ha^{-1}) and least under uninoculatedsoybean-maize intercropping system (974.41 kg ha^{-1}). The percent differences observed between inoculated soybean sole or inoculated soybean-maize intercrop and uninoculated soybean sole or uninoculated soybean-maize intercrop were 78.87% or 89.52 % for grain yield. A similar trend was found for biomass yield which was significantly higher in inoculated soybean sole plots (4431 kg ha^{-1}) than other bradyrhizobium inoculation and uninoculated soybean-maize intercropping systems. The biomass yield was higher under bradyrhizobium inoculated soybean sole plots with a percent difference of 12 % for maize-soybean inoculated intercrop, 29 % for maize-soybean uninoculated intercrop and 24 % for uninoculated soybean sole cropping. The result also shows that N concentration in grain (grain N) and protein content were significantly higher under bradyrhizobium inoculated soybean sole plots but at par with bradyrhizobium inoculated soybean-maize intercrop for protein content as compared to other treatments plots.

3.4. Tillage Practices and Bradyrhizobium Inoculation Interaction on Grain and Biomass Yields

The tillage practices and bradyrhizobium inoculation under maize-soybean intercropping systems interaction was significant on grain and biomass yields (Table 5) which were significantly high under conventional tillage and inoculated soybean treatment combinations as compared to other treatments combination without inoculation. The higher grain and biomass yields were obtained under bradyrhizobium inoculated soybean sole in combination with conventional tillage followed by bradyrhizobium inoculated soybean-maize intercrop and conventional tillage combination and least values were recorded for uninoculated soybean-maize intercrop and reduced tillage interaction. The results also show that tillage practices and bradyrhizobium inoculation interaction was significant on grain N (Fig. 8) which was significantly lower under reduced tillage and uninoculated soybean-maize intercrop treatment combination as compared to other treatment combinations.

Table 4: Assessment of Soybean Productivity and Protein Content

Treatment	Grain yield (kg/ha)	Biomass yield (kg/ha)	Grain N (%)	Protein (%)
Tillage practices				
Conventional tillage	1986	4137a	4.53	25.85
Reduced tillage	1875	3815b	4.42	25.20
SE ±	30.57	77.46	1.19	1.42
Rhizobium inoculation				
Inoculation intercrop	1929b	3973b	4.15b	27.39a
Uninoculated intercrop	1828d	3441d	4.06c	23.13b
Inoculated soybean sole	2306a	4431a	4.89a	27.92a
Uninoculated soybean sole	1852c	3577c	4-08c	23.66b
SE ±	44.67	67.88	0.03	1.12
Interaction				
TS*RI	**	**	**	NS

Table 5: Tillage Practices and Bradyrhizobium Inoculation Interaction on Grain and Biomass Yields

Treatment	Grain yield (kg/ha)	Biomass yield (kg/ha)
Inoculation intercrop and Reduced tillage	2015d	3339d
Inoculation intercrop and Conventional tillage	2146cd	3413c
Uninoculation intercrop and Reduced tillage	1481f	2392g
Uninoculation intercrop and Conventional tillage	1488f	2766h
Inoculated soybean sole and Reduced tillage	2488b	4112b
Inoculated soybean sole and Conventional tillage	2629a	4973a
Uninoculated soybean sole and conventional tillage	1666e	3131c
Uninoculated soybean sole and reduced tillage	1559e	3077f
SE ±	64.67	77.88

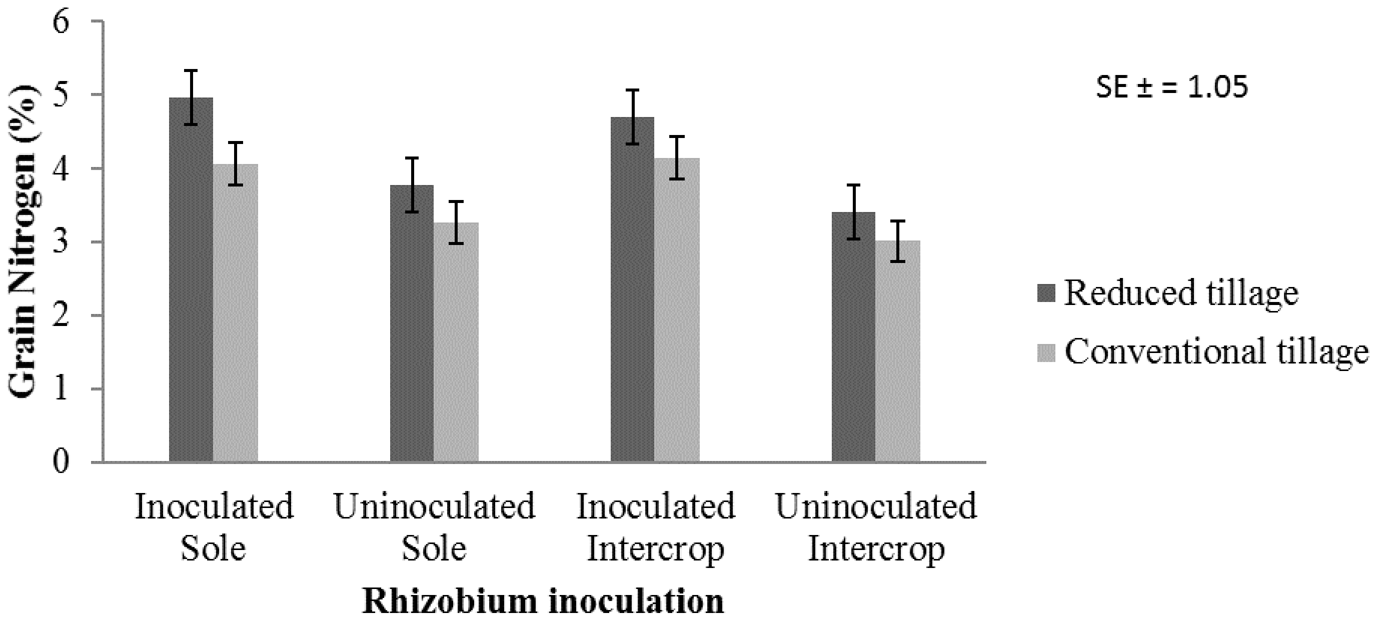

Fig. 6: Tillage Practices and Rhizobium Inoculation Interaction on Grain Nitrogen

4.0 Discussion

The low fertility status of the soil as reflected by slight soil acidic, low total N and low organic carbon could be attributed to nutrients depletion through continuous cultivation, overall removal of crop residues by farmer after harvest for livestock feeding, and occasional bush burning and climate change as earlier reported by Omeke (2017) and (Hobbs and Gupta, 2003). Northern Guinea savanna of Nigeria is characterized by intensive cultivation; coupled with low input use and low capital base, resulting in reduced soil fertility and productivity (Omeke, 2017b).

The bradyrhizobium inoculant strain used was effective as reflected in consistent higher significant BNF/plant, total biological N fixation and NDfa in the inoculated treatments when compared to the uninoculated. By corollary, the above parameters were also higher under reduced tillage than in conventional tillage as earlier reported by Zhang *et al.,* (2012) and Omeke (2017a), who found higher significantly amount of dry nodule weight under reduced tillage than in conventional tillage in the same agro-ecological zone. The result confirmed that reduced tillage enhanced minimal disturbance of soil and provided favourable soil environment for increased rhizobial activity and N fixation, corroborating the study by Ferreira *et al.,* (2000) who affirmed that rhizobia isolate from reduced tillage plots fixed more atmospheric nitrogen than conventional tillage.

The significantly higher biological nitrogen fixation parameters, grain N and protein content obtained under bradyrhizobium inoculated soybean sole cropping as well as in combination with tillage treatment plots as compared others treatments were due to effectiveness of the bradyrhizobium inoculants used as supported by low soil N and good rainfall situation (See Table 1). Effectiveness of rhizobia inoculants significantly increases soybean nodulation and N fixation (Seneviratne *et al*., 2000; Sarr *et al.,* 2005; Majid *et al.,* 2009) which might be responsible to the higher grain N and protein content. Short-term tillage operation also might have enhanced soil mineralization which positively influenced soybean biological fixation and yield. This implies that the higher grain and haulm yields found in inoculated plots with tillage practices might be attributed to the greater residues accruing from N fixation, in-season and after harvest residues, which also serve as substrate for soil microbial pool (Omeke, 2017a). Similarly, this was supported by the presence of inoculated soybean in the cropping systems, which enhanced a stable soil environment (Omeke, 2016). This stable environment can contribute to increasing soil N in the legume-cereal cropping systems through biological nitrogen fixation. This is supported by evidence of the field not previously used for legumes especially soybean production and low soil N. In soils with several years of soybean history, no increases in the proportion of N derived from fixation and nodulation were observed when comparing the inoculated treatment with the non-inoculated treatment, and the authors concluded that N supply from the natural *rhizobium* population was adequate (Salvagiotti *et al.,* 2008).

5.0. Conclusion

Integrating reduced tillage practice and bradyrhizobium inoculation could sustainably enhance soil fertility through N fixation and boost productivity of maize/soyabean intercrop on Alfisols in Northern Guinea savanna agro-ecological zone of Nigeria. The result is recommended as a low input, sustainable and environmentally friendly technology to be disseminated to farmers for sustainable soil health management to guarantee food security.

6.0. Acknowledgment

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