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Tillage Practices and Rhizobium Inoculation Effect on Soil Nitrogen and Soybean Nodulation in Savanna Alfisol of Nigeria

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ABSTRACT

Tillage and rhizobium inoculation may affect nodulation and subsequent N release to the soil, thereby influencing soil productivity. The response of an improved soybean variety (TGX-1448-2E) on nodulation and soil nitrogen to rhizobium inoculation under different tillage practices in a Savanna Alfisol of Nigeria was evaluated at the Institute for Agricultural Research, Ahmadu Bello University, Samaru. Treatments consisted of two tillage systems; conventional tillage (CT) and reduced tillage (RT) and rhizobium inoculation; [inoculated (IN) and uninoculated (UNIN)]. These were tested in a split-plot experimental design with three replications. Data obtained were evaluated for total nitrogen (soil property), nodule number, nodule weight, grain nitrogen, protein (plant parameters) and statistically analysed using general linear model procedure of SAS 9.3 software. Results of soil N and protein content were higher in RT compared to CT with 3.98 % and 24.89 % differences respectively. Soil N and grain N obtained under inoculated plots were significantly higher than uninoculated plots with differences of 44.04 % for soil N and 24.44 % for grain N. Similar observation was obtained for protein content with 46 % difference. The results showed that RT recorded lower nodule number (34 nodule plant-1) and lower nodule weight (239.31 mg plant-1) compared to CT with 36 nodule number plant-1 and 272.86 mg plant-1 of nodule dry weight. The nodule number and nodule dry weights were consistently higher in inoculated plots with 89 % (nodule number) and 68.74 % (nodule dry weight) significantly difference. These findings suggest the need for rhizobium inoculation to improve soil N and soybean nodulation under reduced tillage practice for sustainable crop production.

Key words: Tillage, Rhizobium Inoculation, Soil N, Nodulation, Alfisol

INTRODUCTION

Soybean (*Glycine max* L. Merr) production has gained prominence in the Northern Guinea savanna zone of Nigeria, stimulated by demand for oil and raw material for poultry feed (Chiezey and Odunze 2009). The total area under soybean production in Nigeria is estimated at 659,000 ha with a total output of 1031,000 MT, giving an average yield of 1500 kg/ha with estimated increased by 17 % over 2014 estimated land area (NAERLS, 2015). Soybean is perhaps one of the oldest food crops of the world and ranks first among oilseed crops. It contains 18-22 % cholesterol free oil, having 85 % unsaturated fatty acids and 40-44 % proteins (Government of Pakistan, 2002). Compared to other protein rich foods such as meat, fish and eggs, soybean is far the cheapest. It can be sown as a sole crop, intercropped, rotation with cereals or mixed with cereals in irrigated and rain fed production systems in all agro-ecological zones of Nigeria. It improves soil fertility by fixing atmospheric nitrogen through root nodules. Soybean seeds inoculated with commercial inoculant produced more nodules in no-tilled soil and had a greater nodule mass per plant than soybeans grown in cultivated soils (Kemal *et al*., 2011). Inoculated soybean seeds produced well-nodulated plants with average grain yield value of approximately 2233 kg/ha as compared with uninoculated soybean seeds with 11.65 % difference (Tran *et al*., 2006).

Among various factors that can contribute to soybean success, phosphorus and inoculation had quite prominent effects on nodulation, promote soil nitrogen, growth and yield parameters (Kumaga and Ofori, 2004; Singaga *et al*., 2002). Symbiotic nitrogen fixation is definitely beneficial to agriculture and is a major source of fixed nitrogen in agricultural soils. Inoculation of soybean seeds with proper bacterial strains increased grain production by 33-83 % over uninoculated soybean seeds (Omeke *et al*., 2017). Reduced soil disturbance, for example, improves soil physical parameters; such as structure, and chemical and biological parameters that affect nitrogen fixation. Soil moisture and temperature, are known to be influenced by tillage, all of which affect biological nitrogen fixation (Salvagiotti *et al*., 2008). According to Kemal *et al.,* (2011), No-Tillage with Direct Seeding (NTDS) plots gave root weights (6.9 g/plant), number of nodules (96 number/plant) weight of nodules (0.318 g/plant) and root nitrogen content (% 0.71), which were statistically higher than with the other tillage methods. In reduced tillage with rotary tiller (RTR) plots, values of root dry weight (51.3 g/plant), mean nodule weight (3.91 mg/nodule), root N content (2.38 %) were higher than in NTDS plots.

However, legumes such as soybean that transport N fixed from roots to shoots in the form of ureides (allantoin and allantoic acid) are particularly susceptible to water stress (Sinclair *et al*., 2007) and conservation practices involving reduced tillage and surface crop residue application, are expected to positively influence nitrogen fixation (Kemal *et al.,* 2011). Similar studies also show that zero and reduced soil tillage methods increased soil microbial activity and population (Alvarez *et al.*, 1995). Average yield increase over ten years of research in soybean inoculation returned a profit of over 300 percent (Beuerlein, 2004). Increases in fresh weight, dry weight and seed weight were also observed as compared to the control in several instances (Jalaluddin, 2005). The high N fixing property of soybean can be seen in plant coloration and weight of nodules. Colour differences were obvious in a previous study, as inoculated plots appeared much greener compared to uninoculated (Thelen and Schulz*,* 2009). In view of the above discussion, a field study was conducted with a view to evaluate the effect of tillage practices (reduced and conventional tillage) and *rhizobium* inoculation (inoculated and uninoculated soybean) on soil nitrogen and soybean nodulation as it influenced fertility status of a savanna Alfisol of Nigeria

MATERIALS AND METHODS

Experimental site

A field study was conducted at Research Farm of the 'Institute for Agricultural Research, Ahmadu Bello University (IAR/ABU)', Samaru, Zaria during year 2011 and 2012 cropping seasons. The research field was located within latitude 11°11'19.3"N and Longitude 7°37'02"E (Fig. 1). Samaru has an altitude of 686 m above sea level and is located in Northern Guinea savanna ecological zone of Nigeria. Samaru received a total rainfall amount of 1207 mm in year 2011 and 1333 mm in year 2012. Also, in both years, the third decadal of June witnessed reduced rainfall events, suggesting dry spell occurrence at this period (Fig 2). The rainfall data for 2011 and 2012 (Fig. 2) were obtained from the Meteorological Unit of the Institute for Agricultural Research, Ahmadu Bello University, Samaru, Zaria. The weather station is located about 100 m away from the experimental field. Rainfall and temperature data obtained for both seasons fell within the long-term range, with temperature of 21.05°C (minimum) and 33.47°C (maximum) and annual rainfall of 1011 ± 161 mm. The main soil sub-group is Typic Haplustalf or Chromic Cambisols according to the FAO system of soil classification (FAO, 2001).

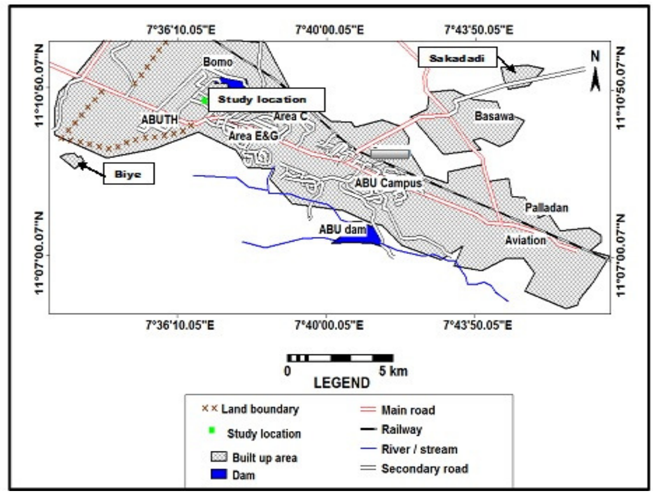

Figure 1: Map of Samaru, Zaria showing the study location

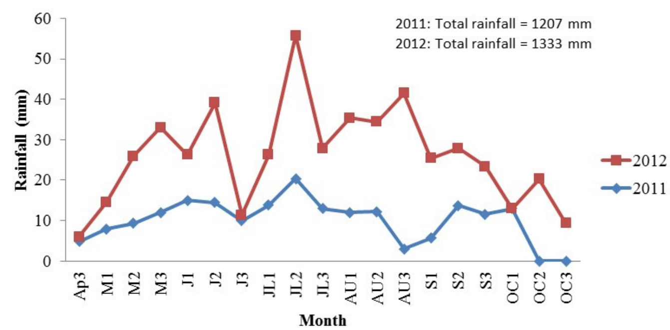

Figure 2: Decadal rainfall patterns in Samaru during 2011 cropping season

Field experiment

The experiment was laid in a randomised complete block design with split plot arrangement and three replicates. The treatments were tillage practice as main plot (reduced and conventional tillage) and rhizobium inoculation as sub plot (inoculated and uninoculated). The conventional tillage (CT) was manual ridging at 0.75 m apart using hoe and was remoulded at 8 weeks after sowing. For reduced tillage (RT) treatment seeds were sown directly without ridging at 0.75 m interval between the ridges and was not remoulded. Soybean (TGX 1448-2E) was used as a test crop. Phosphorus and potassium 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, without nitrogen fertilizer application. Weeds were controlled by the application of glyphosate at two weeks before land preparation at 4 l/ha.

Subsequently, manual weeding was employed two times before harvesting. Each plot size measured 8 m by 5 m and soybean was sown on 1st July, 2011 by drilling after on-set of rains have been established. The crops were harvested manually when soybean had reached physiological maturity on 12th October, 2011 (102 days after sowing).

Inoculation and planting

The soybean seeds were surface sterilised and inoculated with a Legume Fix bradyrhizobia strain using the method of IITA (2014) as shown in Fig. 3. Soybean seeds were drilled in open grooves on the ridges and covered lightly with soil. The uninoculated soybean treatment rows were planted first in order to avoid cross contamination between rows. The seedlings were thinned to one plant per hill at 10 cm within row spacing two weeks after planting. Subsequent field operations such as weeding were cautiously done manually to avoid transfer of rhizobia from inoculated rows to uninoculated rows.

Figure 3: Soybean seeds before inoculation (left) and inoculated with Bradyrhizobia (right)

Soil sampling and analysis

Soil samples were collected at 0-20 cm depth from the experimental field before planting for the determination of its physicochemical properties. Sixteen soil samples were randomly taken from the experimental field, thoroughly mixed, bulked and a representative sample drawn for initial soil analysis (soil pH, organic carbon, total nitrogen, available phosphorus, exchangeable bases, exchangeable acidity and particle size distribution) following standard procedures. At maturity, four disturbed surface soil samples (0-15 cm depth) were taken at alternate points from four inner ridges per plot using a soil auger and used for determination of total nitrogen by micro-kjeldahl digestion method (Bremner and Mulvaney, 1982).

Soybean nodulation assessment

The soybean nodulation was investigated at 50 % podding using a quadrant measuring 0.5 m by 0.5 m. It was placed on the border ridge of each plot, aboveground shoots were then cut and roots carefully excavated to 0.2 m depth, and nodules were sorted out by hand. After washing to soil-free, nodules were counted, fresh weights were determined, and dry weights were also determined after oven-drying at 60 °C for 48 hours.

Plant samples and analysis

All plants within the net ridges of each plot at crop physiological maturity (when 95 % of plants were brown) were cut at ground level, bagged, air-dried and manually threshed. The following measurements were obtained: weight of dry haulms, grain yield and 100 grains weight, as well as shoot weight and root weight per plot. The grains were ground in a hammer mill, passed through a 0.5 mm sieve and stored at -5 ^{0}C in bags for analysis. The nitrogen content of the grains was determined by micro-kjeldahl digestion method (Bremner and Mulvaney, 1982) and multiplied by a factor (5.7) to obtain the seed protein percent (Halvorson *et al.,* 2004)

Statistical analysis

Data obtained from the study were subjected to analysis of variance (ANOVA) using mixed linear model procedure of SAS, (Institute Inc., 2009). The effects of various factors and their interactions were compared by computing least square means and standard errors of difference (SED) at 5 % level of probability.

RESULTS AND DISCUSSION

Initial soil properties

Pre-experiment soil physical and chemical analysis are shown in Table 1. The bulked soil was sandy loam in texture and mildly acidic, low in organic carbon (5.37 g kg^{-1}), total N (0.45 g kg^{-1}) and available P (9.44 mg kg^{-1}) as well as exchangeable cations (3.07 cmol/kg) and acidity (1.8 cmol kg^{-1}). The soil is earlier considered to be low in inherent fertility, organic matter content, cation exchange capacity and dominated by low activity clays in the same agro-ecological zone (Odunze, 2003). Northern Guinea savanna of Nigeria is characterised by intensive cultivation; coupled with low input use and low capital base, resulting in reduced soil fertility and productivity. The situation is further worsened when soil resources are continuously mined by cultivation with little or no replacement of exported nutrients. This situation is supported by the rapid declined of organic matter, followed by extensive leaching of basic cations and development of soil reaction (Yusuf *et al.*, 2009), and coupled with high temperature and short duration of heavy rainfall of the NGS.

Soil total nitrogen (TN), nitrogen concentration in grain (NCG) and Protein Content

Results of total soil nitrogen (TN), nitrogen concentration in grain (NCG) and protein content of soybean as influenced by tillage practices and rhizobium inoculation are presented in Table 2. The result shown that tillage practices did not significantly ($P<0.05$) influence the grain

N concentration and protein content except TN which was significantly higher under reduced tillage treatment with difference of 67.69 %.

Table 1: Physico-chemical analysis (properties) of the site soil before commencement of the experiment

Properties	Unit	Test Values
pH (H_2O)		5.8
pH ($CaCl_2$)		4.5
Organic Carbon	g/kg	5.37
Total nitrogen	g/kg	0.45
Phosphorus	mg/kg	11
Exchangeable cations	cmol/kg	3.07
Exchangeable acidity	cmol/kg	1.8
Sand	%	67.12
Silt	%	19
Clay	%	13.88
Textural Class		Sandy loam

Table 2: Soil nitrogen, grain nitrogen and protein content as influenced by tillage and rhizobium inoculation

Treatment	Total Soil Nitrogen (g kg^{-1})	Grain N (%)	Protein Content (%)
Tillage system			
CT	0.65	3.69	23.05
RT	1.09	3.77	23.57
SE ±	**0.16****	0.07	0.41
Rhizobium Inoculation			
Inoculated	4.22	3.98	24.89
Uninoculated	2.7	2.49	20.74
SE ±	1.33***	0.07***	0.41***
Interaction			
TP*RI	**	NS	NS

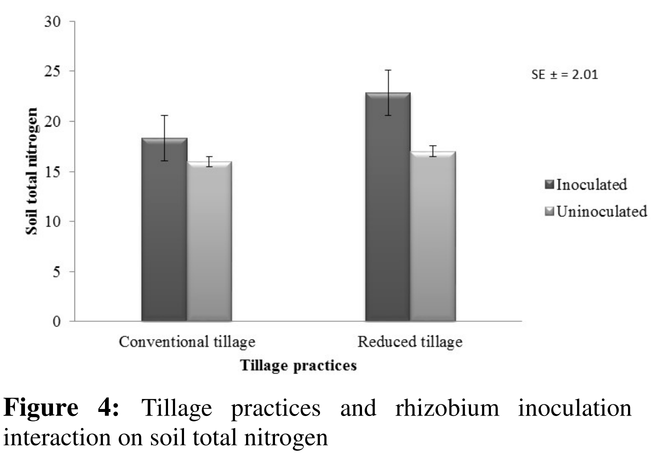

Figure 4: Tillage practices and rhizobium inoculation interaction on soil total nitrogen

The significant effect of tillage on TN could be due to the differences in nature of the soil cover (Buschiazzo *et al.*, 1998) which relatively reduced topsoil TN losses through volatilization as compared to CT. Similar observation has earlier been reported in south-western Nigeria (Agbede and Ojeniyi, 2009). The present results regarding the effects of the RT and CT on TN, NCG and protein content of soybean were consistent with the findings obtained in five-year study in the semi-arid region of Tanzania (Shemdoe *et al.*, 2009). Rhizobium inoculated soybean treatment resulted in significantly ($P<0.0001$) greater values of TN, NCG, and protein content of soybean as compared to soybean plots without rhizobium inoculation treatment with differences of 56.30 % for TN, 14.04 % for NCG and 14.50 % for protein. The tillage practices and rhizobium inoculation interaction was significant on TN (Fig. 4) which was significantly high under reduced tillage and inoculated soybean treatment combination as compared to other treatments combination. The higher total grain N and protein content recorded in inoculated plots was due to effectiveness of the rhizobium inoculant used. The significant increase in nodulation resulted in greater N fixation (Seneviratne *et al*., 2000; Sarr *et al.,* 2005; Majid *et al.,* 2009) which might have contributed to the higher N in grain and percent protein. Rhizobium inoculation increased soybean seed N by 9 % over uninoculated control (Majid *et al.,* 2009) and 23 and 10 % increase in protein content and 19 % increase in oil content of soybean following the inoculation with rhizobia. This implies that the higher soil N and grain N observed for inoculated plots 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 *et al*., 2016).

Table 3: Effect of tillage and rhizobium inoculation on soybean nodulation

Treatment	Nodule Number per plant	Nodule Dry Weight (mg/plant)
Tillage		
CT	36.14	178.25
RT	33.83	239.31
SE ±	2.52	13.57
Rhizobium Inoculation		
Inoculated	48.86	272.86
Uninoculated	31.11	144.69
SE ±	2.52**	13.57**
Tllage*Rhizobium Inoculation		
Significance	**	**

Soybean nodulation

Effects of tillage practices and Rhizobium inoculation on soybean nodulation are presented in Table 3. The effect of tillage practices on nodule number was not significant but effect of rhizobium inoculation was highly significant. The percentage difference between inoculated and uninoculated plots was 25 % indicating higher nodule number in inoculated soybean. Results of nodule dry weight obtained under tillage practices was significantly ($P < 0.05$) higher for reduced tillage (RT) than conventional tillage (CT) with 34 % contrast. Results of tillage practices and rhizobium inoculation interaction on nodule number (Fig. 5) and nodule dry weight (Fig. 6) was significantly difference ($P < 0.05$). The values were higher under RT and inoculated soybean interaction followed by CT and inoculated soybean nitration and least under CT with uninoculated soybean treatment combination.

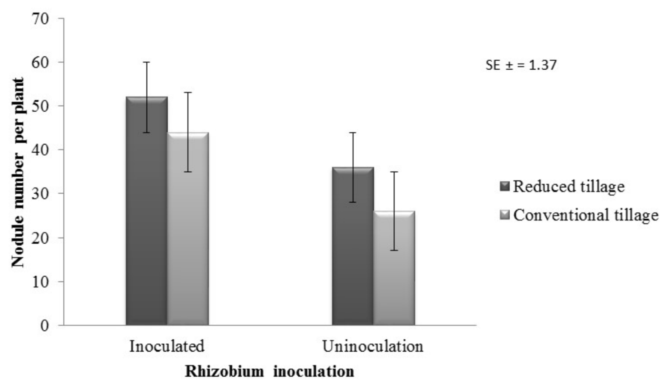

Figure 5: Tillage practice and rhizobium inoculation interaction on nodule number

Significantly higher amount of dry nodule weight obtained under reduced tillage condition could have been due to minimal disturbance of soil leading to increase in rhizobial activity. In addition, Zhang *et al.,* (2012) stated that rhizobial population was high under reduced tillage condition. Ferreira *et al.,* (2000) further affirmed that rhizobia isolates from reduced tillage condition plots fixed more atmospheric nitrogen and Omondi *et al*., (2014) in their studied also asserted that reduced tillage leads to stimulation of nitrogen fixation due to higher dry nodule weight. Generally, these arguments reflected high nodule number and dry nodule weight under reduced tillage and inoculated soybean interaction condition justifies the high amount of nitrogen that would be fixed compared to other tillage practices and rhizobium inoculation interactions. Results also show that inoculated soybean had higher nodule number and dry nodule weight compared to uninoculated soybean plots with difference of 57.06 % for nodule number and 89 % for nodule dry weight. The positive results from this study might be attributed to the efficiency of the rhizobium inoculants strain used which stimulated nodulation process. A study conducted by Okogun *et al.,* (2004) showed that effectiveness of rhizobial inoculation were responsible for the differences in parameters observed. This is supported by low nitrogen status of the soil as reported in Table 1 and the field was not previously used for soybean production 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 (Hungria *et al*., 2006; Salvagiotti *et al.,* 2008).

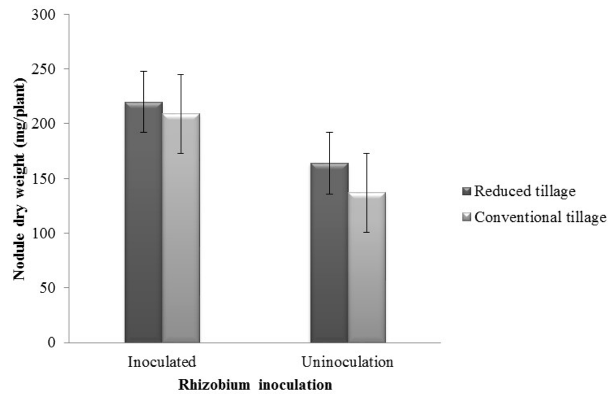

Figure 6: Tillage practice and rhizobium inoculation interaction on nodule dry weight

CONCLUSION

In conclusion, tillage practices and rhizobium inoculation had positive impacts in increasing soil total nitrogen, grain N, protein content and soybean nodulation in the savanna Alfisol of Nigeria. These practices in one way enhanced the availability of soil N and nodulation which was superior under reduced tillage and inoculated soybean interaction plots. This would be beneficial to soil productivity improvement in the savanna zones of Nigeria; which are characterised by declined in fertility due to intensive cultivation coupled with low input use, low capital base and total removal of crop residues after harvest.

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