



## EFFECTS OF TILLAGE AND BRADYRHIZOBIUM INOCULATION IN SOYBEAN/MAIZE INTERCROP ON MICROBIAL BIOMASS AND WATER-SOLUBLE CARBON IN SAMARU, NIGERIA

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### Abstract

A field study was conducted during 2011 cropping season to investigate the effect of tillage practices and bradyrhizobium inoculation of soybean on microbial biomass carbon ( $C_{mic}$ ), microbial biomass nitrogen ( $N_{mic}$ ) and water-soluble carbon (WSC) 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 soybean sole, inoculated soybean/maize intercrop, un-inoculated soybean sole and un-inoculated 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  $C_{mic}$  and  $N_{mic}$  were significantly higher under RT than CT by 31.97% and 17.28% respectively. The WSC was consistently higher in maize-soybean intercropping system with soybean inoculated with bradyrhizobium than in the un-inoculated plots. Values of WSC was 22% higher in inoculated sole soybean and 38% higher in inoculated maize-soybean intercrop than the un-inoculated sole and intercropping system respectively. Similarly,  $C_{mic}$  was 39.20% higher in the inoculated sole soybean than the un-inoculated sole and 24.43% higher in inoculated soybean/maize intercrop than in the un-inoculated soybean/maize intercrop. Also results of  $N_{mic}$  obtained under inoculated sole soybean and inoculated soybean/maize intercrop, respectively, were significantly higher than in un-inoculated sole soybean and soybean/maize intercrop by 45.13 and 56.78% respectively. The results demonstrated that inclusion of bradyrhizobium inoculants in soybean-maize intercropping systems under tillage practices will improve microbial biomass and water-soluble carbon, thereby enhancing the productivity of Alfisols of Northern Guinea Savanna of Nigeria.

**Keywords:** Tillage, Rhizobium inoculation, Soybean/Maize intercrop, Microbial Biomass and Water-soluble carbon

### Introduction

Recent interest in soil ecology has been focused on studying soil and fertilizer management in agricultural systems to improve soil productivity and minimize possible deleterious effects on the environment. Since crop residues are primary sources of organic matter, crop management and fertilizer regime and can have significant influence on soil productivity (Moor *et al.*, 2000). Compared with systems involving crop rotations, soils under monocultural systems in general contain significantly lower concentrations and qualities of soil organic matter, less soil structural stability, and reduced amounts of microbial biomass and activities.

The positive effect of inclusion of rhizobium inoculation in soybean-maize-based crop rotation on biological soil properties are related to higher C inputs, increase in soil nitrogen and diversity of plant residues returned to soils (Omeke *et al.*, 2020). The variability of microbial biomass carbon ( $C_{mic}$ ) and microbial biomass nitrogen ( $N_{mic}$ ) in soil among soybean-maize cropping systems could be attributed to differences in biomass production which influences mineralization and microbial activity. The rate of organic carbon input from plant biomass is generally considered as a dominant factor controlling amount of microbial biomass present in the soil (Campbell *et al.*, 2000). This implies that the

higher microbial biomass observed for inoculated soybean-maize intercrop and rotation as compared with plots of uninoculated and rotation might be attributed to the greater residues accruing from in-season and after harvest activities, which also serve as substrate for microbial pool (Omeke *et al.*, 2016). Sustainable soil productivity entails that the soil ecosystem be in good biological, physical and chemical conditions irrespective of its management and exploitation. Alteration of soil conditions by tillage practices can significantly affect soil productivity through influence on distribution and amount of soil organic matter, microbial activity and nutrients content of the soil (Omeke, 2016). Studies have reported that higher levels of  $C_{mic}$  and  $N_{mic}$  are found near the soil surface under no-tillage compared with conventional tillage and similar or lower levels at lower depths (Motta *et al.*, 2001; Salinas-Garcia *et al.*, 2002; Omeke *et al.*, 2016). However, the decrease in microbial biomass in soil under CT over RT could be attributed to rapid structural deterioration caused by slaking and dispersion due to soil disturbance which might expose the soil to higher temperature, reduction of moisture content and poor soil aggregation, thereby, lowering C and N contents of the soil. The lack of a major disturbance in RT likely provided a steady source of organic C to support the microbial community, compared to CT where a temporary flush of microbial activity with tillage resulted in a large loss of C as  $CO_2$  (Elcio *et al.*, 2003; Omeke, 2016). Soil microbial biomass C/N ratio is often used to describe structure and state of microbial community of soil (Moore *et al.*, 2000), subsequently determine the rate of microbial activity in soil. The objectives of this study were to estimate the proportions of the  $C_{mic}$  and  $N_{mic}$  in field experiments, where different tillage practices and rhizobium inoculation in soybean-maize intercrop and sole cropping systems resulted in field plots with significant differences in water soluble carbon contents of the soil.

### Materials and Methods

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

### Field experiment

The experiment was a randomized complete block design with a split-plot arrangement 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

soybean/maize intercrop, uninoculated sole soybean, and uninoculated soybean /maize intercrop) as sub-plots. The conventional tillage (CT) was manual ridging at 0.75m apart using hoe at 14days after spraying the field with glyphosate (4ltrs/ha) and re-moulded at 8weeks after sowing. For reduced tillage (RT), seeds were sown directed in rows maintaining 0.75m as row spacing without ridging and re-moulding.

### 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. 2. Test crops were soybean (variety; TGX 1448 2E) which matures within 85 – 95days, and maize (variety; SAMMAZ 15), which is a medium maturing variety. Planting was done on 1st July 2011. Two maize seeds were sown at 0.75m x 0.25m apart, while inoculated and uninoculated soybean seeds were drilled in open grooves at 0.75m x 0.05m 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 266,666plants/ha respectively. Phosphorus and K fertilizers were applied to all plots at the rate of 60kg $P_2O_5$ /ha-1 and 60kgK $_2O$ /ha-1 respectively at planting. Whereas N fertilizer at the rate of 20 kg N ha<sup>-1</sup> was applied to soybean and application of 0 kg N ha<sup>-1</sup>, 40 kg N ha<sup>-1</sup>, 80kgNha<sup>-1</sup> and 120kgNha<sup>-1</sup> were applied to maize crop at 2weeks after sowing. At 4 and 8weeks after sowing, manual weeding was done cautiously to avoid the transfer of rhizobia from inoculated rows to uninoculated rows.

### Soil and plant sampling and analysis

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

### Laboratory soil and plant analysis

Initial and end of year (2011) soil samples obtained at depths 0- 15cm from the field were air-dried, sieved through 2mm 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). Enumeration of soil bacteria and fungi

were done from soil samples stored in the refrigerator by weighing 10g into 90ml of sterilized distilled water in 205ml Erlenmeyer flask. Each soil suspension was then diluted serially (10-fold) and used in the estimation of bacterial and fungal population by the standard Pour-plate method described by Seeley and VanDemark (1981) in duplicates. The bulk density was measured using core method (Grossman and Reinsch, 2002). The moist core soil sample was oven-dried at 105°C for 24h until a constant dried weight was obtained

$$\text{Bulk density (BD Mgm}^{-3}\text{)} = \frac{\text{Oven dry soil sample}}{\text{Volume of core cylinder}} \dots (1)$$

Soil porosity was calculated using a mathematical relationship between bulk density and particle density (Foth, 1984). Porosity (P) was computed as follows thus;

$$P (\%) = \frac{1 - \text{BD}}{\text{PD}} \times 100 \dots (2)$$

Where;

P= Porosity, BD= Bulk density (Mgm<sup>3</sup>) and PD= Particle density (2.65g/cm<sup>3</sup>).

The soil microbial biomass C and N were estimated by the fumigation-extraction method (Brookes *et al.*, 1985; Sparling and West, 1988), using field-fresh, moist 2mm sieved soil sample. The extractable C and N in both fumigated and unfumigated samples were determined. Microbial biomass C was estimated by multiplying the difference in extractable C of fumigated and unfumigated samples using a conversion factor of 2.64 (Vance *et al.*, 1987). Microbial biomass N was calculated by multiplying the difference in extractable N of fumigated and unfumigated sample using a conversion factor of 1.46 (Brookes *et al.*, 1985). Enumeration of soil bacteria and fungi were done from freshly collected samples by weighing 10g into 90ml of sterilized distilled water in 205ml Erlenmeyer flask.

#### Statistical analysis

Statistical analyses, including analysis of variance, contrast comparisons, and separation of means by least significant difference were performed by using the general linear models (GLM) procedure (SAS, 1996). Effects of the various treatments and their interactions were compared by computing least square means and standard error of difference (SED) at 5% level of probability.

## Results and Discussion

### Results

#### Soil Microbial Biomass Nitrogen and Carbon

The results of C<sub>mic</sub> and N<sub>mic</sub> were significantly higher in plots under RT compared with those obtained from CT (Table 1). The mean values of microbial biomass values were 347.19mgkg<sup>-1</sup> for C<sub>mic</sub> and 53.12mgkg<sup>-1</sup> for N<sub>mic</sub>. Values of C<sub>mic</sub> and N<sub>mic</sub> were higher under RT than CT with differences of 31.97% and 17.28% respectively. Similar trend was found in water-soluble carbon (WSC) with difference of 22% higher under RT than CT. However, no significant effect on C<sub>mic</sub> / N<sub>mic</sub> ratio was

found between the two tillage practices.

Analysis of variance showed that C<sub>mic</sub>, N<sub>mic</sub> and WSC values were significantly ( $P \leq 0.05$ ) affected by Rhizobium inoculated soybean-maize intercropping systems (Tables 1). The study revealed that the highest C<sub>mic</sub>, N<sub>mic</sub> and WSC values were found in plots treated with Rhizobium inoculated soybean sole (INSS), while the lowest C<sub>mic</sub> and N<sub>mic</sub> values were obtained in plots under Rhizobium uninoculated soybean sole (UNSS), followed by Rhizobium uninoculated soybean-maize intercrop (UNSM). Values of WSC was 22% higher in inoculated sole soybean and 38% higher in inoculated maize-soybean intercrop than the uninoculated sole and intercropping system respectively. Similarly, C<sub>mic</sub> was 39.20% higher in the inoculated sole soybean than the uninoculated sole and 24.43% higher in inoculated soybean/maize intercrop than in the uninoculated soybean/maize intercrop. Also results of N<sub>mic</sub> obtained under inoculated sole soybean and inoculated soybean/maize intercrop were significantly higher than in uninoculated sole soybean and soybean/maize intercrop by 45.13 and 56.78% respectively.

#### Tillage and rhizobium inoculation on C<sub>mic</sub> and N<sub>mic</sub> Interaction

Results for tillage practices and bradyrhizobium inoculation in soybean-maize intercropping systems interaction was significant on C<sub>mic</sub> (Fig. 3) and N<sub>mic</sub> (Fig. 3) which were significantly high under RT and INSM follow by INSS treatment combinations as compared to other treatments combination with or without inoculation. Least values of soil C<sub>mic</sub> and N<sub>mic</sub> were obtained under UNSS in combination with CT followed by UNSM and CT combination. Whereas, highest values were recorded for INSM and RT treatment combinations. Values of C<sub>mic</sub> obtained for INSM and RT treatment combinations were 37.46%, and 29.54% difference compared with those obtained under CT in combination with UNSS and UNSM respectively. Similar observation was found under tillage in combination with rhizobium inoculation soybean/maize intercrop on N<sub>mic</sub> which indicated that plots without bradyrhizobium inoculation and CT combination with difference of 46.55% for RT/INSM and 33.6% for RT/INSS.

#### Interaction of tillage and rhizobium inoculation on water soluble carbon (WSC)

The results also show that tillage practices and bradyrhizobium inoculation in soybean/maize intercropping systems interaction was significant on WSC (Table 2), which was lower under CT and UNSS treatment combinations compared to other treatment combinations. Inoculated soybean/maize intercropping system in combination with RT had a highly significant value of WSC at probability levels of 5% with mean of 143.34gkg<sup>-1</sup> for RT with rhizobium inoculation and 128.67gkg<sup>-1</sup> for CT with rhizobium inoculation. The data also indicated percent differences between RT and INSM treatment combinations of 15% for CT and INSS, 33% for RT and UNSS and 31% for UNSM. Similar

trends were observed compared to the value for RT and INSM treatment combinations with that of with or without inoculated soybean/maize intercrop under CT combination, but with higher percent differences.

### Discussion

The study revealed lower significant values of SMBC and SMBN for plots under CT compared with RT; this might be attributed to less residues and high decomposition due to tillage operations and less ground cover of the soil. The result agrees with previous reports that higher levels of SMBC are found near the soil surface under no-tillage, compared with conventional tillage and similar or lower levels at lower depths (Motta *et al.*, 2001; Salinas-Garcia *et al.*, 2002). Tillage operations exposed top soil to higher temperature, reduction of moisture content and poor soil aggregation, thereby, lowering C and N contents of the soil (Omeke *et al.*, 2016). The absence of a major disturbance in RT likely provided a steady source of organic C to support the microbial community, compared to CT where a temporary flush of microbial activity with more soil disturbance during land preparation facilitates great loss of C as CO<sub>2</sub> and N as NO<sub>2</sub> (Adeboye, 2009). Further, less soil disturbance would favour the formation and stabilization of macro-aggregates to improve and protect the habitat for microbes. This implies that RT would improve microbial biomass carbon and nitrogen and WSC status of the soil to enhance its productivity. Soil microbial biomass C/N ratio is often used to describe structure and state of microbial community of soil (Moore *et al.*, 2000), subsequently determine the rate of microbial activity in soil. Differences in WSC values between the Rhizobium inoculated soybean sole and other treatments were 38% for UNSS, 22% for INSM and 32% for UNSM. The reasons for the variations are due to differences in C<sub>mic</sub> and N<sub>mic</sub> values as a result of variations in soil moisture and temperature, and available substrate (Chang and Juma 1996). Higher values obtained under plots treated with Rhizobium inoculation could be attributed to high supply of C source from crop residues which serve as an energy source for microorganisms (Govaerts *et al.*, 2007). This relatively improves the soil microbial biomass nitrogen and subsequently boosts the WSC of the soil. Generally, the amounts of SMBN obtained in this experiment were similar to that of Adeboye (2009) who reported 18.3mgkg<sup>-1</sup> for soybean-maize rotation, 18.9mgkg<sup>-1</sup> for cowpea-maize rotation, 18.7mgkg<sup>-1</sup> for centro-maize rotation and 20.4mgkg<sup>-1</sup> for fallow-maize rotation in the Guinea savanna Alfisol of Nigeria. Whereas, Vinzke *et al.* (2004) observed that soil microbial N contents decreased with increasing soil depth for both crops. For maize crop, the decrease was from 54 to 21mgkg<sup>-1</sup> and 71 to 28mgkg<sup>-1</sup> for soybeans, confirming lower value in soils under maize/soybean intercrop. The rate of organic carbon input from plant biomass is generally considered the dominant factor controlling the amount of microbial biomass present in soil (Campbell *et al.*, 2000). The results of WSC reported in this study are in line with that of Yusuf (2007) who reported greater WSC in the legume-maize systems than in the fallow-maize or

continuous maize systems, but in contrast with the results obtained by Adeboye (2009) in the same agro-ecological zone. The differences could be attributed to management practices, time of sampling, amount of rainfall, and soil types. These factors influence the organic matter content of the soil, which was the main constituent of WSC. Water soluble carbon originates from liter dissolution, root exudates, microbial and plant metabolic residues of stable soil organic matter, which facilitates energy source for microbial activity (Yusuf, 2007; Omeke 2016). Therefore, inclusion of rhizobium inoculated soybean in soils under maize based cropping systems would produce higher amounts of shoot, root and nodule biomass, which habitually reveal greater amount of WSC, and subsequently enhance microbial biomass contents of the soil. Differences observed in this study might be attributed to the effectiveness of rhizobia inoculants integrated in soybean/maize based intercropping systems which significantly enhance soybean nodulation and soil N (Seneviratne *et al.*, 2000; Sar *et al.*, 2005; Majid *et al.*, 2009; Omeke *et al.*, 2020). This might be responsible for the higher soil microbial biomass and WSC found in plots treated with inoculated soybean in combination with tillage practices. This is supported by higher residues accruing from nodules and root biomass and as in-season and after harvest residues, which serve as substrate for soil microbial pool (Omeke, 2017). Values of C<sub>mic</sub> observed in this study were comparable to 164-284gkg<sup>-1</sup> established in Northern Guinea Savanna agro-ecosystems (Adeboye, 2009). The results are also in line with that of Yusuf (2007) who reported greater WSC for legume-maize systems than under fallow-maize or continuous maize systems in the same agro-ecological zone of Nigeria. Differences in values reported could be attributed to differences in management practices, time of sampling, amount of rainfall, and soil types. These factors influence the organic carbon and nitrogen content of the soil, which strongly contributes to WSC and microbial activity. Water soluble carbon originates from liter dissolution, root exudates, microbial and plant metabolic and hydrolysis of stable soil organic matter and can be utilized by microorganisms as energy source (Yusuf, 2007). Therefore, integration of Rhizobium inoculation in soybean/maize intercropping systems would enhance higher production of shoot, nodules and root biomass which explained greater amount of microbial biomass and WSC obtained in all the plots treated with inoculated soybean in combination with RT practice. This relationship suggests that activity of microbial biomass in soil is strongly influenced by levels of TN in soil due to crop competition with microbes for N.

### Conclusion

Soil C<sub>mic</sub> and N<sub>mic</sub> contents of the soil were generally greater in inoculated soybean/maize intercrop than other soybean/maize plots treated with or without Rhizobium inoculation. Similar results were obtained for WSC. Also, higher values of C<sub>mic</sub> and N<sub>mic</sub> concentrations were found in RT plots than CT plots. These findings may indicate a close relationship between the amount and composition of root and shoot biomass that facilitates

the activity of microbial communities in the rhizosphere. Generally, the study revealed that integration of Rhizobium inoculation in soybean/maize intercropping systems in combination with tillage practices especially RT would enhance microbial activity and productivity of the soil.

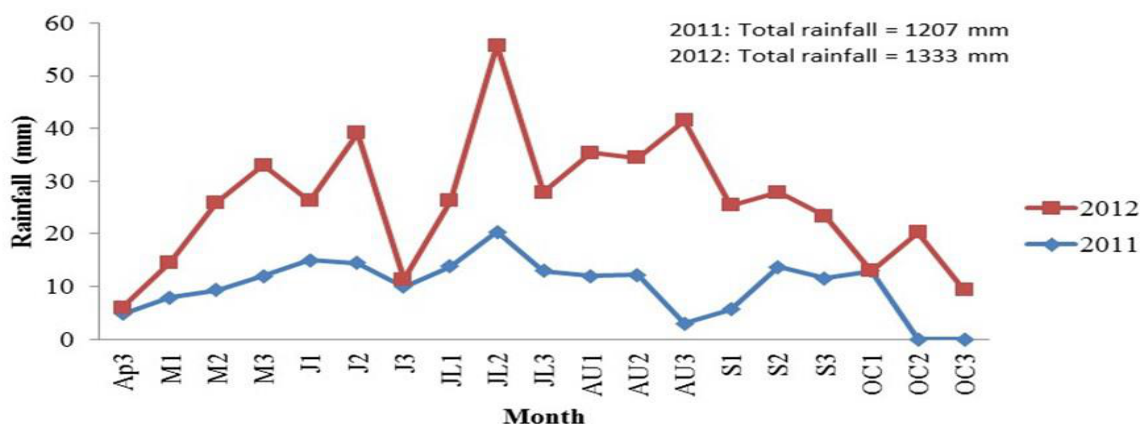
#### Acknowledgements

This study was partly supported by the Institute of Agricultural Research, Ahmadu Bello University, Zaria, Kaduna State, Nigeria, during my PhD research programme.

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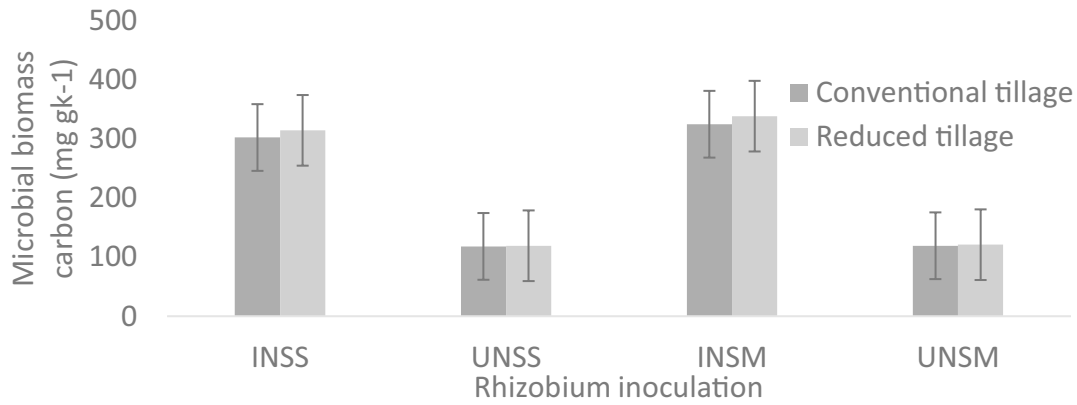
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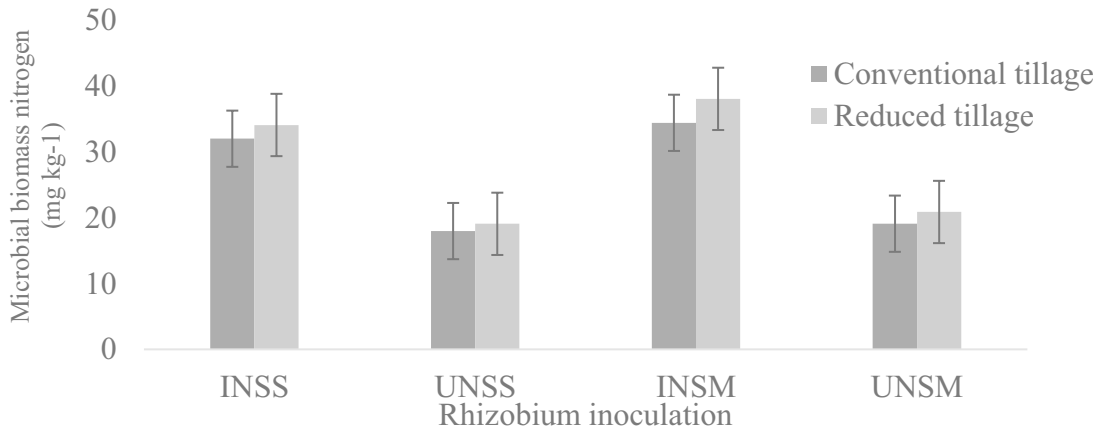
**Figure 1: Decadal rainfall pattern in Samaru during 2011 cropping season**  
Source IART/ABU Zaria (2011)



**Figure 2: Soybean seeds before inoculation (left) and inoculated with Bradyrhizobia (right)**



**Figure 3: Interaction between tillage and rhizobium inoculation on microbial biomass carbon**  
Error bar = Standard error



**Figure 4: Interaction between tillage and rhizobium inoculation on microbial biomass nitrogen**  
Error bar = Standard error

**Table 1: Soil microbial biomass and water-soluble carbon**

	Nmic (mg/kg)	Cmic (mg/kg)	Cmic/Nmic Ratio	WSC (g/kg)
<b>Treatment</b>				
<b>Tillage system (TS)</b>				
CT	53.12	236.21	4	147.01
RT	64.22	347.19	5	187.86
SE ±	2.13	56.67	0.04	1.01
<b>Rhizobium inoculation (RI)</b>				
Uninoculated Intercrop	25.55	229.88	9	127.56
Inoculated intercrop	59.12	304.21	5	188.07
Inoculated sole	51.43	289.44	6	147.31
Uninoculated sole	28.22	175.98	8	116.80
SE ±	3.42	67.91	0.04	6.01
<b>Interaction</b>				
TS x RI	**	**	NS	**

CT = Conventional tillage, RT = Reduced tillage, \*\* = Significant at  $P < 0.01$ , Nmic = Microbial biomass nitrogen, Cmic = Microbial biomass carbon, Cmic / Nmic = Microbial biomass nitrogen: Microbial biomass carbon ratio, WSC = Water soluble carbon

**Table 2: Interaction of tillage practices and bradyrhizobium inoculation on WSC (g kg<sup>-1</sup>)**

Tillage practice	Rhizobium inoculation				Mean
	Inoculated		Uninoculated		
	Soybean/maize Intercrop	Soybean Sole	Soybean/maize Intercrop	Soybean Sole	
Conventional tillage	145.71	138.99	117.32	112.67	128.67
Reduced tillage	178.80	152.80	122.61	119.21	143.34
Mean	162.26	145.90	119.97	115.94	

SE ± (1.39) for TP x RI = 2.66