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Assessment of microbial biomass and nitrogen fixation as influence by soybean genotypes and phosphorus fertilizer in the Northern Guinea savanna Alfisol of Samaru, Nigeria.

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ABSTRACT

Nigerian Northern Guinea savanna Alfisols are subjected to soil degradation problems, nutrient depletion resulting in low yields. Therefore, management practices that will enhance the soil fertility status and productivity such as the use of legumes with effective and competitive Rhizobium strains in addition to a supply of the appropriate amount of phosphorus fertilizer is encouraged. A Field experiment was conducted at the IAR Teaching and Research Farm of Ahmadu Bello University, Zaria, Kaduna State to determine the effect of phosphorus fertilizer application on soil microbial biomass and nitrogen fixation in four soybean genotypes. Treatment consists of four levels of phosphorus rates (0, 30, 60 and 90 Kg P_2O_5 ha^{-1}) and four soybean genotypes (TGx 1448-2E, TGx 1951-3F, TGx 1987-10F and TGx 1987-62F) in a 4×4 factorial combination arranged in a randomized complete block design and replicated thrice. Results obtained showed that 30 kg P_2O_5 ha^{-1} gave the highest microbial biomass carbon (246.98 mg kg^{-1}), nitrogen (26.91mg kg^{-1}) and phosphorus (8.40mg kg^{-1}) as well as nitrogen fixation (54.76kg ha^{-1}) and grain yield (2403 kg ha^{-1}). Soybean genotype TGx 1951-3F had the highest microbial biomass carbon (168.23 mg kg^{-1}), phosphorus (6.70 mg kg^{-1}) and grain yield (1680.78 kg ha^{-1}) while TGx 1987-62F had the highest microbial biomass nitrogen (18.01mg kg^{-1}). All four soybean genotypes were statistically similar in the amount of nitrogen fixed. This study, therefore, revealed that the addition of Phosphorus fertilizer beyond 30 kg P_2O_5/ha could be detrimental to soil microbial biomass, N_2 fixation and yield.

1.0 Introduction

Soil microbial biomass (bacteria, fungi and protozoa) is a measure of the mass of the living component of soil organic matter (Soil quality, 2022). It plays a crucial role in organic matter decomposition as well as in nutrient transformation and consequently influences ecosystem productivity (Brummer *et al.,* 2009). It also performs some important functions for plant production in an ecosystem; such as labile source and sink of C, N, P and S, agent of nutrient transformation and pesticide degradation and acts as a cementing agent, promoting soil aggregation and structure (Salinas-Garcia *et al.,* 2002). According to Insam (2001), the microbial biomass is an important indicator of soil productivity and its evaluation is invaluable in soil ecological studies. The knowledge acquired is also fundamental to sustaining the environment and productivity.

Studies have shown that soil microbial biomass is often influenced by soil depth, seasonal fluctuation, pH, heavy metal deposition and land management practices (Calbrix *et al*., 2007; Vásquez-Murrieta *et al*., 2007). High concentrations of heavy metals are known to affect the morphology, metabolism and growth of microorganisms in soils (Singh and Kalamdhad, 2011), as they disrupt the integrity of their cell membranes and cause protein denaturation (Lumen, 2020). Furthermore, microbial biomass has been reported to correlate positively with yield in organic farming compared to

conventional farming systems (Mäder *et al*., 2011).
Research on cereal-legume cropping systems has shown improvements in soil fertility and crop yields. However, little or no attention has been paid to some biological properties that may improve and maintain soil productivity. Due to this fact, the study was conducted to assess microbial biomass carbon, nitrogen and phosphorus under different phosphorus fertilizer rates and soybean varieties.

2.0 Materials and Methods

2.1 Experimental Site Description and Location

A field experiment was conducted at the Research Farm of the Institute for Agricultural Research (IAR), Ahmadu Bello University, Samaru, Zaria. The study location has an altitude of 686 m above sea level, latitude11^{o}11`N and longitude 07^{o}38'E in the Northern Guinea savanna of Nigeria. The soil type is Alfisol. Samaru is characterised by a unimodal rainfall pattern with a mean annual rainfall of 1060 mm concentrated mostly in May/June to September/October of the cropping season (Oluwasemire and Alabi, 2004).

Treatments and Experimental Design

The experiment was arranged in a Randomized Complete Block Design (RCBD). Treatment consisted of four soybean varieties (TGx 1448-2E, TGx 1951-3F, TGx 1987-10F, TGx 1987-62F) and four rates of phosphorus (0, 30, 60, 90 kg/ha P_2O_5) applied as single superphosphate (SSP) giving a total of 16 treatment combinations. This was replicated three times, giving an overall total of 48 treatments.

2.2 Agronomic Practices

The field was ploughed, harrowed and ridged at an inter-row spacing distance of 75 cm and intra-row spacing of 5 cm. Soybean was inoculated using nodumax. Before inoculation, the seeds were sterilized as outlined by Vincent (1970). The peat based inoculant was applied directly on the seed with some quantity of liquid gum Arabic as adhesive to coat the seeds with a little quantity of water which was gradually stirred to enhance uniform coating. The seeds were planted by hand drilling on the ridges. Fertilizer application was done a week after sowing (30 kg/ha of muriate of potash (60% K_2O) and 20 kg/ha N as Urea (46% N). These were applied to all plots. Hand weeding was done when needed.

2.3 Soil Sampling and Analysis

Disturbed soil samples were taken with an auger before the field was ploughed for the determination of some selected physical and chemical properties using standard laboratory procedures. Samples were taken at the depth of 0–15 cm. The samples were air-dried, grinded and sieved through a 2 mm mesh. At 6 weeks after sowing; a second sample was collected with an auger at a depth of 0-5 cm for determination of microbial biomass.

The soil microbial biomass C and N were determined using the chloroform fumigation method (Brookes *et al.,* 1985; Vance *et al.,* 1987; Anderson and Ingram, 1993; Okalebo *et al.,* 2002). The procedure entails the subjection of moist soil to chloroform fumigation which results in cell wall lyses thereby allowing the cellular content to become extractable in 0.5 M K_2SO_4. Two subsamples of soils were weighed into 50 ml beakers and uncovered plastic vials as unfumigated and fumigated samples respectively. The first sample in the beaker was extracted with 0.5 M K_2SO_4 for one hour which was filtered using Whatman No. 1 filter paper. The second sample was fumigated with 30 ml chloroform in a desiccator for 5 days at 25^{o}C and later extracted with 0.5M K_2SO_4. The organic C and total N content of the extracts were determined as described earlier. A k_C of 0.45 and a k_N of 0.54 was used for calculating the biomass C and N (Jörgensen and Müller, 1996), where K_C and k_N are extractable part of the total amount C and N fixed in the microbial biomass respectively.

Soil microbial biomass P was also determined using the fumigation-extraction method but extraction was carried out with Bray 1 (0.03M NH_4F- 0.025M HCl) extractant at a soil-to-solution ratio of 1:4 w/v and analyzing inorganic P instead of total P (Wu *et al*., 2000; Eche, 2011).

2.4 Estimation of Percentage Nitrogen Fixed

The technique that was used to estimate N fixation is the Total Nitrogen Difference (TND) method. This is done by comparing total nitrogen measured from the legume crop with that of a non-legume (maize) (Murray *et al*., 2008). The amount of N fixed was calculated by subtracting total nitrogen of the reference crop (maize) from that of the legume (soybean), and the difference value is assumed as N derived by biological nitrogen fixation (BNF).

Therefore, N_2 fixed = Total N in legume -Total N in reference crop

Where Total N in plants = $\frac{\text{(Dry matter weight (kg/ha) x \% N in plants)}}{100}$

% Ndfa = $\frac{\text{[Total N in legume -Total N in reference crop]}}{\text{Total N in legume}} \times 100$

Where % Ndfa is the percentage of nitrogen derived from the atmosphere.

2.5 Data Analysis

Data collected from various measurements were subjected to analysis of variance (ANOVA) using the SAS package (SAS, 2009). Treatment means were separated using Duncan's Multiple Range Test (Duncan, 1955) at 5% level of significance ($P< 0.05$).

3.0 Results and Discussion

3.1 Characterization of the Soil of the Experimental Site Used.

Some of the physical and chemical properties of the soil used is presented in Table 1 which shows that the soil is sandy loam in texture. The silt: clay ratio was 2.33 indicating a relatively young soil or low degree of weathering of the parent material (Eche, 2011). According to Van Wambeke (1962), who states that silt: clay ratio of less than 0.15 indicates an advanced weathering stage or senile soil. The soil was classified as Typic Haplustalf according to USDA Soil Taxonomy (Soil Survey Staff, 1999) as cited by Ogunwole *et al.* (2001). The soil reaction was slightly acidic. The Organic Carbon (5.50 g kg^{-1}), nitrogen (0.46 g kg^{-1}) and available P (2.27 mg kg^{-1}) and CEC were all low (Table 1). This is not surprising as the Guinea savanna soils are known to be low in organic matter, CEC, N and P (Odunze, 2003). This has been attributed largely to the rapid mineralization rate under high temperatures and rainfall in this agroecological zone. Exchangeable Ca^{2+} (2.5 cmol kg^{-1}), Mg^{2+} (0.7 cmol kg^{-1}) and K^{+} (0.18 cmol kg^{-1}) were medium according to the ratings of Esu (1991), while Na^{+} (0.11 cmol kg^{-1}) was observed to be low in the soil. The exchangeable acidity (1.5 cmol kg^{-1}) and CEC (5.0 cmol kg^{-1}) and effective cation exchange capacity ECEC (3.5 cmol kg^{-1}) were all low. This low ECEC will make the soil susceptible to soil acidification because the ability to hold on the basic cations is low.

Effects of Phosphorus rate and Variety on Soil Microbial Biomass Carbon, Nitrogen and Phosphorus.

The result of soil microbial biomass carbon (SMBC) is shown in Table 2. Soil microbial biomass carbon gives a sensitive indicator of the deterioration or improvement of soil quality as a result of different management practices (Powlson, 1994; Eche, 2011). The values obtained ranged

Table 1: Initial Soil Properties of the Experimental Site

Soil properties	Unit	Test value
Bulk density	Mg m-3	1.41
Moisture content	g kg-1	229.55
Total porosity	%	52.83
Sand	g kg^{-1}	671.20
Silt	g kg^{-1}	230.00
Clay	g kg^{-1}	98.80
Textural class		Sandy loam
pH in H_2O		6.10
Available P	mg kg^{-1}	2.27
Total nitrogen	g kg^{-1}	0.46
Organic carbon	g kg^{-1}	5.50
C:N ratio		12
Exchangeable Cations	cmol kg^{-1}	
Ca	"	2.50
Mg	"	0.70
K	"	0.18
Na	"	0.11
Exchangeable Al	"	1.50
CEC	"	5.0
ECEC	"	3.5

Note: CEC= Cation exchange capacity; ECEC= Effective cation exchange capacity

between 66.65 mg/kg to 246.98 mg/kg for the different rates of phosphorus with the control plots having the lowest values while 30 kg P_2O_5/ha (246.98 mg/kg) gave the highest value, followed by 60 kg P_2O_5/ha (230.47 mg/kg). There was a general decrease in microbial biomass C, N and P with an increase in phosphorus rate above 30 kg P_2O_5/ha, which could be due to the use of single superphosphate (SSP) fertilizer, which is actually rock phosphate activated with concentrated H_2SO_4. The calcium from the rock phosphate will increase calcium concentration in the soil which could result in nutrient imbalance in the soil, thereby hindering mineralization. This also indicates that an increase in phosphorus will invariably increase calcium. Calcium can raise pH to alkaline which can lead to a reduction in phosphorus availability due to the formation of calcium phosphate. As a result, microbial activity starts declining. Reduction in microbial activity will lead to a decrease in microbial biomass. Graham *et. al*. (1981) reported that too high level of phosphorus reduces root exudation and plant carbon allocation to Arburscular mychorrizal fungi network, which are two important sources of carbon for soil microorganisms (Bowen and Rovira, 1999).

A linear correlation between SMBC and soil organic C have been reported by several authors (Witter *et al*., 1993; Moore *et al*., 2000). Increased productivity also means high root biomass, growth and density (Yusuf, 2006). Perfect *et al*. (1990) also reported higher SMBC in crops with intensive root growth and density. The low values obtained from the control plots could be due to low soil organic matter. Variety TGx 1951-3F gave the statistically highest value while the other varieties were statistically ($P < 0.05$) the same. Similar trend was observed in soil microbial biomass nitrogen (SMBN) (Table 2) with 30 kg P_2O_5/ha having the highest biomass (26.91 mg/kg), followed by 60kg P_2O_5/ha (19.88 mg/kg). The values ranged from 10.62 mg/kg to 26.91 mg/kg. The result showed that adding P beyond 30 kg P_2O_5/ha is of no benefit to microbial biomass nitrogen. TGx 1987-62F was recorded for the highest SMBN but was statistically the same with other varieties. There was no significant interaction between the varieties and phosphorus rates.

Table 2 also shows the effect of phosphorus rates and variety on soil microbial biomass phosphorus (SMBP). The result showed that 30 kg P_2O_5/ha gave the highest biomass phosphorus (8.4 mg/kg) followed by 60 kg P_2O_5/ha (6.7 mg/kg), while 0 kg P_2O_5/ha had the lowest biomass. There was no significant interaction between the variety and phosphorus levels. Saini *et al*. (2004) reported a positive correlation between microbial biomass P, sorghum (*Sorghum vulgare*) and chickpea (*Cicer arietinum*) P uptake in field experiments in India. Significant positive relationships were also found between biomass P and maize yield in field experiments in sub-Saharan Kenya (Ayaga *et al*. 2006). In their experimental fields, available P in the soils was as low as 2.1–7.5 mg P kg^{-1} similar to what was obtained at the onset of this research (2.27 mg P kg^{-1}). Also, it has been considered that biomass P plays an important role in P supply to plants in soils with low P availability (Saini *et al*. 2004). Chen *et al*. (2000) also conducted pot experiments with soils of various levels of P fertility and found that the P concentration in ryegrass (Lolium spp.) was more significantly correlated ($r = 0.91$) with biomass P than with any other tested chemical indices of soil P (total P, organic P and Bray (I) P in Red soils (Oxisols, Ultisols and some Alfisols). Therefore, these results all suggest that biomass P is an important source of available P and could be an indicator of P availability for plants growing in soils with strong P adsorption. Variety TGx 1951-3F gave the highest SMBP while other varieties were statistically similar.

3.2 Effects of Phosphorus rate and Variety on Amount of N_2 Fixed and Percentage of N_2 Derived from the Atmosphere (% Ndfa)

The amount of nitrogen fixed and percentage of nitrogen derived from the atmosphere decrease with increasing phosphorus rate (Table 3). The highest amount of nitrogen fixed and percentage of nitrogen derived from the atmosphere were 54.76 kg / ha and 56.1 % respectively, when phosphorus was applied at the rate of 30 kg P_2O_5 / ha. There was significant ($P<0.01$) difference between the amount and percentage of N_2 fixed between the phosphorus rates. This indicates that P deficiency does not only limit plant growth, it can also limit biological N_2 fixation which has been noted to have a higher

Table 2: Effects of Phosphorus Rate and Variety on Soil Microbial Biomass Carbon, Nitrogen and Phosphorus.

Treatment	MBC (mg/kg)	MBN (mg/kg)	MBP(mg/kg)
Phosphorus rate (P; kg P_2O_5/ha)			
0	66.65d	10.62c	3.2c
30	246.98a	26.91a	8.4a
60	230.47b	19.88b	6.7b
90	85.84c	11.46c	3.8c
LOS	***	***	***
SE±	3.162	0.777	0.192
Variety (V)			
TGx 1951-3F	168.23a	16.97	6.7a
TGx 1448-2E	158.79b	16.48	5.0b
TGx 1987-10F	150.18b	17.1	4.8b
TGx 1987-62F	152.74b	18.01	5.5b
LOS	*	NS	*
SE±	3.162	0.777	0.192
Interaction			
V x P	NS	NS	NS

Means within same column and treatment group followed by same letter(s) are not significantly different at 5% level of probability using DMRT. * Significant at P < 0.05. *** Significant at P < 0.001. MBC= Microbial Biomass Carbon. MBN= Microbial Biomass Nitrogen. MBN=Microbial Biomass Phosphorus.

P requirement for optimal functioning than either plant growth or nitrate assimilation (Abdul- Aziz, 2013). N_2 fixation has been reported to increase with increasing P rate and crop duration (Ogoke *et al*., 2006). Rahman *et al*. (2014) reported that soil N supply through biological nitrogen fixation (BNF) by associated microbial populations is the principal source of N for legume production. Likewise, the nitrogen uptake could affect the crude protein and crude fiber of fodders as stated by Khogali *et al*. (2011).

The varieties were statistically different in the amount of N_2 fixed and Percentage of N_2 Derived from the Atmosphere (% Ndfa) with TGx 1951-3F fixing the highest amount of nitrogen. This could be due to the fact that the variety TGx 1951-3F is a medium variety with a maturity date of 105 to 110. This also supports the fact that soybean maturity date affects the amount of N_2 fixed in soybean. N_2 fixation has been reported to increase with increasing crop duration (Ogoke *et al*., 2006). This is because longer growth duration allows for a longer period of N_2-fixation in the nodules. Increased crop duration in the field means a longer period of nodule activity. Soybean has been estimated to fix 15-162 kg N / ha (Giller and Wilson, 1991). Based on the experimental site used, amount of N_2 fixed was in the range of 34.3 to 37.2 kg N / ha in the soybean varieties studied. This is lower than the 41-51 kg N / ha reported by Yusuf *et al*. (2008) in a field trial in Samaru, northern guinea savanna of Nigeria. The N_2 derived from the atmosphere in this study ranged between 37-38% against the 70-90 % reported by Ogoke *et al*. (2006). This might be due to fact that the varieties used by Yusuf *et al*. (2008) and Ogoke *et al*. (2006) were different and also included late maturing varieties. There was no significant interaction between phosphorus and variety in N_2 fixed and % Ndfa.

Table 3: Effects of Phosphorus rate and Variety on Amount of N_2 Fixed and Percentage of N_2 Derived from the Atmosphere (% Ndfa).

Treatment	N_2 fixed (Kg/ha)	%Ndfa
Phosphorus rate (P; kg P_2O_5/ha)		
0	25.58d	27.32d
30	54.76a	56.10a
60	34.82b	36.35b
90	29.18c	30.60c
LOS	**	***
SE±	0.877	0.868
Variety (V)		
TGx 1951-3F	37.16a	38.85a
TGx 1448-2E	35.77ab	37.29ab
TGx 1987-10F	34.31b	37.77b
TGx 1987-62F	37.10a	38.46a
LOS	NS	NS
SE±	0.877	0.868
Interaction		
V x P	NS	NS

This means within the same column and treatment group followed by the same letter(s) are not significantly different at a 5% level of probability using DMRT. ** Significant at P < 0.01. *** Significant at P < 0.001. NS= Not significant

Effects of Phosphorus rate and Variety on Grain Yield of Soybean

The result presented in Table 4 shows that there was a significant (P< 0.01) difference between the P treatments and between the varieties. The plots that received 30 kg P_2O_5/ha gave significantly (p < 0.05) the highest grain yield (2402.78 kg/ha) while the control plot had the least (1122.22 kg/ha). In addition, grain yield decreased significantly as the P level increased from 30-90 kg P_2O_5. The yield increase suggests that the application of 30 kg P_2O_5/ha may serve as a prophy-

lactic measure that helps the crop physiologically by increasing its vigour and enhancing its ability to withstand environmental stress (Chinke, 1999). Previous field experiments have established that the application of phosphorus increases legume grain yield (Yusuf *et al*., 2008, Omeke, 2016). The higher grain yield obtained from 30 kg P_2O_5/ha compared to 60 and 90 kg P_2O_5/ha could be the quick response of soybean to P applied as the soil P is low (Table 1). This agrees with the findings of Shahid *et. al.* (2009) who reported relatively high yield and seed quality in soils testing low in P when P was applied.

The soybean varieties were statistically ($P< 0.05$) different from one another with TGx 1951-3F giving the highest grain yield (1680.78 kg/ha). The grain yields obtained in this study is similar to the average soybean yields (550-2200 kg/ha) reported by Javaheri and Baudouin (2001) in Benin. There was no significant interaction.

Table 4: Effects of Phosphorus rate and Variety on Grain Yield of Soybean

Treatment	Grain yield (kg/ha)
Phosphorus rate (P; kg P_2O_5/ha)	
0	1122.22d
30	2402.78a
60	1527.78b
90	1280.56c
LOS	**
SE±	38.521
Variety (V)	
TGx 1951-3F	1680.78a
TGx 1448-2E	1569.44ab
TGx 1987-10F	1505.56b
TGx 1987-62F	1627.56a
LOS	*
SE±	38.521
Interaction	
V x P	NS

Means within same column and treatment group followed by the same letter(s) are not significantly different at a 5% level of probability using DMRT. NS= Not significant. * Significant at $P < 0.05$. ** Significant at $P < 0.01$.

4.0 Conclusion

There was a general decrease in microbial biomass (C, N and P), nitrogen fixation and grain yield with an increase in phosphorus rate. Results showed that microbial biomass carbon, nitrogen and phosphorus were consistently higher at 30Kg P_2O_5/ha level of phosphorus than 60Kg P_2O_5/ha and 90Kg P_2O_5/ha. TGx 1951-3F had the highest microbial biomass carbon and phosphorus while TGx 1987-62F had the highest microbial biomass nitrogen. From the results obtained from the study, it can be inferred that adding phosphorus fertilizer beyond 30 Kg P_2O_5/ha would be detrimental to soybean production.

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