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INFLUENCE OF ORGANIC MANURE AND WOOD ASH ON THE ABUNDANCE OF SOIL MICROORGANISMS, ORGANIC CARBON AND GRAIN YIELD OF SOYBEAN (*GLYCINE MAX L.* MERRILL) IN ACID SOIL

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ABSTRACT

Organic production methods which include the exclusion of chemical approach of soil amendment have been observed to improve plant growth and preserve the soil health and biodiversity of production environment. A study was conducted at the Federal University of Agriculture, Abeokuta, to examine the influence of organic manure and wood ash on the abundance of soil microorganisms, organic carbon and yield of soybean in acid soil. The study consisted of three rates of wood ash (0, 2.5 and 5.0 t ha^{-1}) and three rates of cow dung (0, 5.0 and 10.0 t ha^{-1}) arranged in completely randomized design, replicated three times. Soybean variety TGX 1485 – ID was sown into 5 kg. The results showed that wood ash application at 5.0 t ha^{-1} significantly increased the soil pH (6.6), organic carbon (7.48) and soil microbial population (110×10^4 cfu g^{-1}) compared to lower rate of 2.5 t ha^{-1} and control, which resulted in highest number of pods (7.7) and grain yield (14.2 g per plant) at harvest. Cow dung treatment of 10 t ha^{-1} significantly had highest soil organic carbon (7.39%) at 8 WAP and soil microbial population (21.1 and 91.4×10^4 cfu g^{-1}) at 4 and 8 WAP respectively. These findings indicate that application of wood ash may be an acceptable alternative liming agent to the inorganic soil amendment and higher soybean productivity.

Keywords: Cow dung, organic carbon, soil microorganisms, soil pH, soybean yield, wood ash

INTRODUCTION

Intensive farming over the years with application of nitrogen fertilizers or manures is one of the causes of acidification in soil. Soils formed under conditions of high annual rainfall are more acidic than any soils formed under more arid conditions. The soils become acidic when basic elements such as calcium, magnesium, sodium and potassium held by soil colloids are replaced by hydrogen ions. Rajapaksha *et al.* (2004) reported that low pH constrains the growth of soil bacteria in favour of more resilient fungi. In contrast, liming which increases the soil pH usually improves the bacteria growth and activities of other microbes in the soil.

Lime is the basis of a good soil fertility program, which does more than just correct soil acidity as it supplies essential plant nutrients like Ca and Mg. Application of wood ash as a lime has high acid neutralizing capacity in soils, due to the formation of hydroxides and carbonates during the combustion and conditioning processes (Steenari*et al*., 1999; Holmberg and Claesson, 2001). Wood ash increased the activities and modified the community composition of microorganisms, with implication for microbial processes and nutrient cycling. Its application in some soils may increase the solubility of organic carbon and CO_2 release (Fritze*et al*., 1994; Zimmermann and Frey, 2002).

Manure is a readily available organic source of essential plant nutrients and a source of energy for soil biota, which influences many of the biological processes of soil (Mullinis *et al.* 2002; Yagoub *et al*., 2015). Incorporation of organic manure such as, green manure, animal and residues manure to the soils have a direct effect on soil organic matter content, improves soil fertility and augment microbial activities (Escobar and Hue, 2008; Wong and Swift, 2003). Animal manure is considered a valuable nutrient source when applied to the soil at rates commensurate with good agronomic practices (Duffera*et al*., 1999).

Soybean [*Glycine max* (L.) Merrill], a leguminous vegetable of the pea family grows in tropical, subtropical, and temperate climates. As a major cash crop widely used in food and feeds, its cultivation is increasing in the savannah and rainforest agro ecological zone of Nigeria (Okpara, *et al*., 2007). The crop is an important grain legume, oil seed and source of vegetable protein, which provide cheap and balanced diet and has now been recognized as a potential supplementary source of nutritious food (Wilcox and Shibles, 2001). Leguminous and actinorhizal plants can obtain their nitrogen by forming a symbiotic association with nitrogen fixing bacteria which promote plant growth and development (Amakiri, 2000; Franche, *et al*., 2009), and possess the ability to fix up to 180 kg N/ha symbiotically, 80% of which is harvested as beans (Williams *et al*., 1980). It represents one of the best characterized legumes species, both physiologically and biochemically and also an excellent model species for legumes in general (Ferguson and Gresshoff, 2009), with outcomes frequently extrapolated to the other important food and feed legume crops, such as bean, pea, chickpea, peanut, clover and lucerne (Rispail *et al*., 2010). It grows quickly, is high yielding, and has a size and stature that are well suited for most field and laboratory studies.

Without the use of fertilizers, world food production could not have increased at the rate it did and more natural ecosystems would have been converted to agriculture (Tilman *et al*., 2002). Although, inorganic fertilizers used in conventional production system readily provide nutrients for plants, the use is associated with increased soil acidity and nutrient imbalance (Adediran *et al*., 2004; Kang and Juo, 1980). Nigerian farmers also have limited access to these fertilizers because of its low production, availability, procurement and poor distribution (NISER, 2003). In view of these challenges, there is need therefore to determine the influence of organic methods of soil amendment on soybean production. The objective was to determine the influence of organic manure using cow dung and wood ash on soybean growth, yield and soil attributes of pH, Organic carbon and microbial population.

MATERIALS AND METHODS

Study Area

The experiment was carried out in the Screenhouse of College of Plant Science and Crop Production, Federal University of Agriculture, Abeokuta (Latitude 7º 16′ N, Longitude 3º 35′E). The soil used was an acid sandy loam (0 – 15 cm) collected from Epe, Lagos State, Nigeria.

Experimental Design and Treatments Combinations

The experiment consisted of three rates of wood ash (0, 2.5 and 5.0 t ha^{-1}) and three rates of cow dung (0, 5.0 and 10.0 t ha^{-1}) arranged in completely randomized design, replicated three times. The test crop used was soybean variety TGX 1485 – ID sourced from International Institute of Tropical Agriculture (IITA) Ibadan. The soil was spread on jute bags, air dried for 3 days and passed through 2 mm sieve. The cow dung used was collected from University animal farms and was processed by spreading on jute bag and sundried until it was fully dried, the lumps were crushed and sieved. The quantities of cow dung were weighed using a weighing balance at different rates of application 5 and 10 t ha^{-1} at 11.16 g and 22.32 g/pot respectively. Wood ash was collected from local bakery, passed through 2 mm sieve and weighed in quantities of .5 t ha^{-1} (5.58g/5kg soil), and 5.0 t/ha (11.16g/5kg soil). Already dried cow dung and wood ash were applied into plastic pots of 6 litre size containing 5 kg soil. The 5 kg soil was measured and thoroughly mixed with respective treatments, moistened to 70% field moisture capacity (FMC) and thereafter left for two weeks before planting to allow for equilibration. Four seeds were planted per pot and thinned to two seedlings per pot at 2 weeks after planting.

Data Collection

Plant parameters and soil samples were taken at 4 and 8 WAP (weeks after planting). Soil samples were analysed for pH, organic carbon and microbial population while the grains yield of soybean was determined at harvest.

Soil and plant analysis

The pH was determined by the glass electrode pH meter in a 1:1 soil: water and in 1:1 KCl suspensions, particle size by the hydrometer method (Bouyoucos, 1951), organic carbon by the chromic acid oxidation procedure (Walkey and Black, 1934), and microbial population by plate count. Grain yield and yield parameter of total seed weight per pot was taken at harvest.

Preparation and Sterilization of Materials

Petri dishes were arranged into canisters and sterilized in the oven at 160°C for one hour. The canister with petri dishes were allowed to cool to about 45°C before being brought out, micro pipette was used, sterilized when hot in a spirit lamp. The media used was nutrient agar, which was prepared according to the manufacturer's specification written on the bottle. Sterilized water was preferred by distributing 9 ml distilled water in scents' copper bottles before sterilization at 120°C for 15 minute (thereby having sterile distilled water).

Microbiological Analysis

Ten-fold serial dilutions of samples were prepared as described by Olutiola *et al.,* (1991). One gram per sample of soil were weighed and poured into separate test tubes + content among 9 millilitre of sterile distilled water to make 10^{-1} dilution. The process was repeated for six other test tubes containing nine millilitres of sterile distilled water to make up 10^{-7} dilution. Dilution of

10^{-3}, 10^{-4} and 10^{-5} were used for pour plating, one millilitre of each dilution was transferred into each sterile petri dishes. Each of the media (Nutrient agar) after being allowed to cool to about 40ºC - 45ºC were poured into each petri – dishes and the content were immediately gently swirled and allowed to solidify on the work bench aerobically. These were finally incubated at 37°C for 24 hours. Total counts were taken after 24 hours.

Statistical Analysis

The data collected were subjected to analysis of variance (SAS, 1990), treatment means for each parameter measured were compared at $p<0.05$ using the least significant Difference (SAS, 1990).

RESULTS

Physical and Chemical Properties of the Soil before planting

The pre-planting physical and chemical properties of the experimental soil are presented in Table 1. The particle size analysis showed that the textural class of the soil was loamy sand and a slightly acidic pH (5.25). It had low total nitrogen of 0.05% and medium organic carbon (C) content of 1.54%. The exchangeable cations (Ca^{2+}, Mg^{2+}, Na^{+} and K^{+}) were low with values of 0.0005 cmolkg^{-1}, 0.001 cmolkg^{-1},8 x 1^{-5}cmolkg^{-1}and 0.001 cmolkg^{-1} respectively. The soil had initial microbial population of 1 x 10^{4} (cfu g^{-1}).

Table 1: Physical and Chemical Properties of the Soil before planting

Soil Property	Unit	Value
Sand	g kg^{-1}	899
Silt	g kg^{-1}	1
Clay	g kg^{-1}	100
Textural Class		Loamy sand
pH		5.25
Total N	%	0.05
Organic C	%	1.54
Organic Matter	%	2.65
K	c mol	0.001
Ca	c mol	0.0005
Mg	c mol	0.001
Na	c mol	8 x 10^{-5}
Microbial population (10^{4}cfu g^{-1})		1

Number of pods and grain yield

The number of pods and grain yield of the soybean were significantly ($p<0.05$) influenced by the rates of wood ash applied to the soil (Table 2). Wood ash rate of 5 t ha^{-1} gave highest number of pods (7.7 pods per plant) and grain yield of 14.2 g per plant at harvest. The number of pods and grain yield were highest (6.4 pods and 11.6 g per plant) in cow dung applied at 10 t ha^{-1}, but no significant effect observed compared to other rates (Table 2).

Table 2: Effect of wood ash and cow-dung on number of pods and grain yield in soybean grown in acidic soil

Treatments	No of Pod per plant	Grain yield (g per plant)
Wood ash (t ha^{-1})		
0	5.1	6.12
2.5	5.8	8.02
5.0	7.7	14.2
LSD	2.01	4.24
Cow-dung (t ha^{-1})		
0	5.9	7.72
5.0	6.2	9.08
10.0	6.4	11.6
LSD	Ns	ns
W×C	Ns	ns

ns: Not significant,

Soil pH

Significant ($p<0.05$) was observed among the wood ash rates on the soil pH at 8 WAP. Wood ash applied at rates of 5 t ha^{-1} significantly increased the soil pH to 6.6 compared to the control (Table 3). Cow dung application had no significant effect on the soil pH at 4 and 8 WAP (Table 3).

Soil Organic carbon

The organic carbon content of the acidic soil significantly ($p<0.05$) increased with increase in the rates of wood ash from 0 to 5 t ha^{-1} (Table 3). Soils treated with wood ash at rates of 5 t ha^{-1} had highest organic carbon of 7.75 and 7.48% at 4 and 8 WAP. Cow dung applied at 10 t ha^{-1} had significantly highest organic carbon of 7.39% at 8 WAP compared to other rates. Interaction effect was observed on the soil organic carbon at 8 WAP. The application of wood ash at 5 t ha^{-1} and cow dung at 10 t ha^{-1} significantly ($p<0.05$) resulted in highest organic carbon (8.21%) in the soil compared to other interaction treatments at 8 WAP (Table 4).

Table 3: Effect of wood ash and cow-dung on soil pH, organic carbon and microbial population

Treatments	pH		Organic carbon (%)		Microbial population × 10^4 (cfu)	
	4 WAP	8 WAP	4 WAP	8 WAP	4 WAP	8 WAP
Wood ash (t ha^{-1})						
0	5.9	6.3	5.55	6.05	12.4	52.3
2.5	6.1	6.4	6.60	6.93	17.6	71.9
5.0	6.0	6.6	7.75	7.48	25.5	110
LSD	Ns	0.21	1.32	0.52	3.63	10.0
Cow-dung (t ha^{-1})						
0	6.1	6.4	6.11	6.26	15.7	67.1
5.0	5.8	6.5	6.79	6.81	18.9	75.7
10.0	6.0	6.5	7.0	7.39	21.1	91.4
LSD	Ns	Ns	ns	0.52	3.63	10.0
W×C	Ns	Ns	ns	0.89	ns	17.4

ns: Not significant, WAP: Weeks after planting

Table 4: Interaction effects of cow dung rates and wood ash rates on organic carbon (%) in soybean cultivated plot at 8 WAP

	Organic carbon (%) at 8 WAP		
	Wood ash		
Cow dung	0 t ha^{-1}	2.5 t ha^{-1}	5 t ha^{-1}
0 t ha^{-1}	5.6	6.37	6.82
5 t ha^{-1}	6.1	6.97	7.41
10 t ha^{-1}	6.5	7.45	8.21
LSD (0.05)		0.89	

Soil microbial population

The soil microbial population was significantly ($p<0.05$) influenced with wood ash and cow dung application at 4 and 8 WAP (Table 3). Highest microbial population 25.5 and 110 × 10^4 cfu g^{-1}were observed in soil treated with wood ash at rate of 5 t ha^{-1}at 4 and 8 WAP respectively. Application of cow dung at rate of 10 t ha^{-1} significantly increased the microbial population (21.1 and 91.4 10^4 cfu g^{-1}) compared to the control (Table 3). The interaction of wood ash and cow dung had significant effect on the microbial population of acidic soil at 8 WAP (Table 5). The interaction of cow dung x wood ash had significant effect on the microbial population of acidic soil at 8 WAP. The highest Microbial population of 132.7 x 10^4 (cfu) was recorded in soybean grown in plot treated with 10 t ha^{-1} of cow dung and 5 t ha^{-1} of wood ash at 8 WAP (Table 5).

Table 5: Interaction effects of cow dung rates and wood ash rates on Microbial population x 10^4 (cfu) in soybean cultivated plot at 8 WAP

	Microbial population x 10^4 (cfu)		
	Wood ash		
Cow dung	0 t ha^{-1}	2.5 t ha^{-1}	5 t ha^{-1}
0 t ha^{-1}	46.7	62.7	92.0
5 t ha^{-1}	51.3	70.3	105.3
10 t ha^{-1}	59.0	82.7	132.7
LSD (0.05)		17.3	

DISCUSSION

Wood ash applications can greatly improve the physical and chemical properties of acidic soils (Goodwin and Burrow, 2006). The effects of applying wood ash as a soil ameliorant is predominantly governed by application rate and soil type (Pitman, 2006). Wood ash form at application is crucial as each form has a distinct chemical composition. The study revealed that the soil pH and organic carbon increased with the wood ash dosage applied to the soil. These observations are consistent with previous reports that application of wood ash can increase soil pH and decrease soil exchangeable acidity (Kahl *et al*., 1996; Demeyer *et al*., 2001; Perucci *et al*. 2008; Kuokannen *et al*. 2009; Saarsalmi *et al*. 2012). Literature reviewed by Goodwin and Burrow (2006) stated that the alkalinity of wood ash could increase soil pH from wood ash addition.

Soil microorganisms are also known to respond to wood ash application to the soil (Houtari *et al.,* 2015). Direct responses of the soil microbes to wood ash addition include increase in their population measured by colony forming units (CFU). The stimulation of the microbial populations in the soil can to a large extent be explained by the increase in soil pH. Microbial communities are highly responsive to soil pH changes (Saarsalmi *et al*. 2012). The soil pH has been known to be the main drive in shaping microbial communities in various soils and changes, determining their survival and population under unfavorable conditions (Lowe *et al*., 1989; Nicholson *et al*., 2000). Wood ash applications combined with an additional N source can potentially increase soil C and N microbial biomass and C mineralization rate, and subsequently lead to increased CO_2 production when applied to acidic soils (Saarsalmi *et al*. 2012).

The soil microbial populations are essential for carrying out processes such as decomposition of organic matter and nutrient recycling (Paul, 2014). Increase in pH, organic carbon and microbial population brought by the application of liming (wood ash) materials may lead to nutrient increase which results in better yield of the soybean grown in the acidic soil. Furthermore, soil organic carbon is a pivotal component of soil fertility, and the level of organic matter is influenced by the application of fertilizer. Furthermore, the organic carbon level is an indicator of the net input in a healthy soil. In our study, cow dung manure enhanced the accumulation of organic carbon, which is consistent with the findings of several other previous studies from many countries (Drenovsky *et al*., 2004; Gregorich *et al*., 1996; Bhandari *et al*. 2002; Hao *et al* 2002). The improved soil organic carbon may be due to a higher humification rate and a constantly lower decay rate, since organic carbon is a nutrition pool that maintains microbial activity. The result demonstrated that application of cow dung manure has more influences on soil microbial population than soil pH and organic carbon.

CONCLUSION

Based on the conducted trial, it can be stated that wood ash can effectively be used for amelioration of acidic soils instead of liming. Wood ash can successfully be used for purposes of nutrient supply to enhance. Utilization of wood ash, which is available in huge amounts, may partially replace expensive soil conditioners and fertilizers. Application of cow dung manure at 10 t ha^{-1} also enhanced the microbial population and yield of soybean in acid soil.

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