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Original Research Article

Soil Residual Microbial Population and Chemical Fertility Following Maize Cropping as Influenced by an Organo-Mineral Fertilizer Formulation

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Abstract: Addition of mineral fertilizer to an organic source of nutrients may reduce the lag between application to the soil and nutrient release. A field experiment laid out as randomized complete block design was conducted at the Teaching and Research Farm of the Federal University of Technology, Akure Nigeria to compare the effects of organic, mineral and an organo-mineral fertilizer formulations on soil microbial population, chemical properties and maize (*Zea mays*) performance. Treatments involved three soil amendments, which were: NPK at 250kg/ha; Neem seed-based fertilizer (NSBF) at 300kg/ha, and Compost at 2.5 t/ha. Treatments also included a Control where the soil was not amended at all. At maize harvest, plant height appeared in the order of NSBF > NPK > Compost > control, and they caused 15.9, 15.8 and 6.1 % increases, respectively. Other growth and yield parameters were also affected by the various treatments in similar manner as the plant height. Result also showed that while the Compost and NSBF increased microbial count (both bacteria and fungi), growth of microorganisms was repressed by the application of NPK fertilizer. Soil pH, organic carbon (OC) as well as residual nitrogen, phosphorus and potassium concentrations were significantly increased by both Compost and the NSBF whereas application of mineral fertilizer reduced their concentrations in the soil at the termination of the experiment. It was therefore concluded that organomineral formulations can serve dual purposes of both quick nutrient release as well as leave residual nutrients in the soil for the following cropping.

Keywords: Chemical Fertility, Compost, Microbial Population, Organomineral Fertilizer.

INTRODUCTION

The quality of the soil is an important factor in its productive capacity (Pierzynski *et al*., 1994), which implies the ability of the soil to keep unaltered key ecological functions, such as decomposition and formation of soil organic matter (Doran *et al*., 1996). Soil quality as a very essential fraction of sustainable agriculture (Subbian *et al*., 2000) is therefore adjudged based on the status of its three principal components, which are the physical, chemical and biological properties. Any major alteration in any of these soil components has bearing on the others because of their interconnectivity.

Intensive agriculture was born out of the pressure on food producers to meet the food demands of the world teeming population, which however has caused over exhaustion of soil nutrients hence the need for continuous use of mineral fertilizers. Continuous use of chemical fertilizers on the other hand has been observed to deteriorate soil physical properties (Sharma and Verma 2015). Degradation in soil physical properties resulting from improper crop production practices has in turn produced ripple effects on certain key chemical components of the soil. Some of these include soil pH (Han *et al*., 2010; Li *et al*., 2007), cation exchange capacity, nutrient content and phosphorus availability (fixation) (Dubey *et al*., 2012). The joint effects of physical and chemical deterioration of the soil is that of creating unfavourable environments for microorganism and subsequent threat to beneficial organism such as the nitrogen fixing rhizobium (Stepien *et al*., 2014).

Stepien *et al*., (2014) also observed that excess phosphorus fertilization decreases fungal count in the soil. As an alternative to the use of mineral fertilizers,

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attention is shifting to the use of organic materials as nutrient sources. Most of these organic materials have however, been found to be limited and have not produced the target effects because they appear unstable due to rapid mineralization because of excessive solar radiation and precipitation, which are peculiar to tropical environments (Glaser *et al*., 2002). There is therefore a need to search further for materials, which can both produce nutrients to the crop as well as moderate soil processes that are capable of undermining the quality of the soil.

Improved organic techniques of nutrient supply will undoubtedly contribute to future soil health and productivity (Kumwenda *et al*., 1996). The Neem seed-based fertilizer being an organo-mineral formulation possesses the potential to make nutrients available to the present crop, as well as make nutrient available to soil microorganisms whose principal functions is mineralization of organic materials in the soil.

Organomineral formulations are designed to sustainably improve the nutrient status of tropical soils. They combine the attributes of both organic and inorganic fertilizers (Ayeni, 2008). Extensive research has gone into the effects of organomineral fertilizers on the performance of crops, and they have been found to improve not only the growth and yields of crops over sole application of either mineral or organic manure, but have also been found to improve soil nutrient status (Makinde *et al*., 2010; Okunlola *et al*., 2011; Ojeniyi *et al*., 2012). Literature is however still scanty on their effects on soil microbial activities. The soil microbial biomass, activity, and community structure are useful indicators of soil quality because these parameters are sensitive to changes in cropland management practices (Bending *et al*., 2002). Soil microorganisms through the decomposition of organic matter (Stark *et al*., 2008) are directly connected with ecological functions such as nutrient cycling and formation of soil aggregates. The soil microbial diversity and population have key roles to play in determining the rates of soil processes. Key soil processes such as de-nitrification, nitrification, and nitrogen fixation can all be influenced by little alteration in the soil microbial community structure (Hsu and Buckley, 2008; Philippot *et al*., 2011).

Treatment of soil with organic and inorganic nutrient sources can significantly affect soil microbial population, diversity and activity (Hu *et al*., 2011). Changes in microbial activity and composition can in turn influence plant growth by enhancing nutrient turnover and suppressing or mitigating disease incidence (Zhang *et al*., 2012). Thus, measurement of soil microbial population is considered essential to predict sustainable agricultural production (Wardle *et al*., 1999).

Soil microbial populations can respond much more rapidly to disturbances than other indicators such as soil C or N, and therefore may act as early indicators of changes in soil quality (Kennedy and Papendick 1995). The population size of the soil microorganisms is important to overall soil and usability. Soil organisms contribute to the maintenance of soil quality through their control of many key processes, such as decomposition, nutrient cycling and availability, and soil aggregation. These processes affect erodibility, water infiltration, water storage, and carbon sequestration (Kennedy and Papendick 1995).

Nevertheless, amending soil with a nutrient source, which combines quick nutrient release to crops with ability of prolonged nutrient release to soil microbes and plants is assumed can preserve the microbial competence of the soil following a cropping cycle. The objective of this study was therefore to compare residual microbial population and chemical fertility of the soil following maize cropping when either organic, inorganic, or their blend was added to the soil.

MATERIALS AND METHODS

Study Site

A field experiment was conducted at the Teaching and Research Farm of the Federal University of Technology, Akure to compare the effects of organic, mineral and an organo-mineral fertilizer on soil microbial population, chemical properties and maize (*Zea mays*) performance. Akure ($7^{0}17\ ^{1}$N $5^{0}10^{1}$ E) is located in south-western Nigeria. The soil at the site of experiment is an alfisol (FAO, 1998) derived from the basement complex rock and it is sandy loam. The average annual rainfall range was about 1613mm per annum and the annual mean temperature was 27°C. The vegetation is tropical rain forest with an average relative humidity of between 56 and 59% during the dry season and 51 - 82% during the wet season (IITA, 2002). The chemical properties of the soil show 1.3% organic carbon, 0.30% of soil total nitrogen, 8.5 mg/kg available phosphorus, 0.32, 0.65 and 1.7 Cmol/kg of available potassium, magnesium and calcium respectively. The pH of the soil was found to be 6.02 (1:2 H_2O).

Experimental Design and Treatment

The experiment was a Randomized Complete Block Design (RCBD) comprising four treatments with three replications per treatment. Treatments involved three soil amendments, which were: NPK at 350kg/ha; Neem seed-based fertilizer (NSBF) at 300kg/ha, and Compost at 2.5 t/ha. Treatments also included a Control where the soil was not amended at all. The compost is a compost fertilizer formulation by the Ondo State Waste Management Board, Akure Nigeria (Sunshine Organic Fertilizer ®). This organic formulation contains 7% N and micronutrients. The Neem Seed-Based Fertilizer (NSBF) was a commercial formulation of neem-seed cake blended with mineral fertilizer (Royal Fertilizer Plus ®) containing NPK 7:7:7. The NPK fertilizer used was obtained from local agro dealers in Akure, Nigeria. All the solvents and other chemicals used were analytical grades and obtained from Pascal Chemical Company Ltd. (Akure, Nigeria). Plot size was 4 m x 3 m (12 m^2). Plots were pegged and separated from each other by 1.00 m.

Soil Sampling and Analysis

Prior to treatment application, soil samples were collected randomly from 15 points in the experimental area. The samples were bulked and homogenized to form a composite unit. At maize harvest, soil sampling was done on individual treatment plot at 0-15 cm depth to determine the soil pH and nutrient status using standard methods. The average pH (1:2.5 soil–H_2O (McLean, 1982); available P using the Bray-I method (Bray and Kurtz, 1945); SOC using the Walkley and Black (1934) method; total N content by the Kjeldahl digestion (Nelson and Sommers, 1982); exchangeable cations and CEC using ammonium acetate method (Black, 1965).

Maize Growth and Yield Data

Growth data (plant height and leaf number) were collected on maize at 4, 6, 8 and 12 WAP. Plant height was determined using a measuring tape, while number of leaves per plant was determined by visual count. Data were collected from ten randomly selected plant stands. These sample plants were tagged and consistently sampled all through the period of the experiment. Maize grain yield was assessed from the ten randomly selected plants per plot. The harvested cobs were shelled, weighed and grain weight adjusted to 13% moisture.

Enumeration of Soil Microbial Population

Numbers of microflora were estimated by soil dilution technique on Nutrient and Potato Dextrose Agars as isolation media for bacteria and fungi respectively. To achieve serial dilution, 5 grams of soil was suspended in 150 ml Erlenmeyer flask containing 95 ml of sterilized distilled water to obtain a 10^{-1} dilution and was kept under shaking conditions at 120 rpm for 15 minutes. From the flask 1 ml of suspension was transferred to 9 ml water blank to make 10^{-2} dilution. The water blank was vortexed and then again 1 ml of the suspension was transferred to a new water blank (9 ml) tube to obtain 10^{-3} dilution. In the similar manner dilutions were made up to 10^{-8}. The nutrient agar medium was composed of peptone 5 g, meat extract 3 g, agar agar 15 g and 1000 mL distilled water. For bacterial count 0.1 ml aliquot of the dilution to 10^{-8} was spread plated on Nutrient Agar medium petri plates in triplicates. Then the plates were incubated in an inverted position at 28˚C for 2 days. The constituents of the Potato Dextrose Agar (gL^{-1}) were Peptone 5.0, potato extract 5.0, dextrose 10.0, Agar 20.0, and Distilled water 1000.0 ml at pH 6.5. A mixture of 1g soil and 10mL of saline solution was shaken on a mechanical shaker for 10 minutes to dislodge fungal propagules into the solution. This was followed by serial dilutions to the concentrations of 10^{-5}. 0.5 mL of the aliquot was spread on Potato dextrose extract agars to isolate fungal spores and this was incubated at 28^{0C} for 4 days. Dilution factors of 8 and 5 were used to determine the bacterial colony and fungal spore forming units respectively.

Data Analysis

Data collected were submitted to analysis of variance (ANOVA) and the Tukey test was used to verify the significant differences among treatment means at the 5% probability level**.**

RESULTS

Effects of Fertilizers on Growth and Yield of Maize

The amendments exert significant influences on maize growth over the sampling period. There were no significant differences ($P > 0.05$) in plant height among the soil amendment tested at 4WAP with regards to their effects on maize plant height (Table 1). All the amendments however increased plant height compared to the unamended plot at this time. NPK fertilizer produced the tallest maize stands from the 4th week up to the 8 WAP. This was closely followed by the other two soil amendments as significant differences were not observed among the amendments up till this time of sampling. Compared to the control, NPK and NSBF started to cause significant effects on maize height as from 6WAP whereas significant increase in maize height by the compost application was delayed until 8WAP. All amendment provided significant effects on maize height, but the tallest stands were found in plots treated with NPK and NSBF, which were significantly taller than those on plots treated with Compost. At maize harvest, plant height appeared in the order of NSBF > NPK > Compost > control, and they caused 15.9, 15.8 and 6.1 % increase, respectively.

The pattern average number of leaves in maize was influence by the various fertilizer treatments is very similar to their influences on the plant height. NPK and NSBF produced higher number of leaves compared to the compost treatment and all fertilizer treatments engendered significantly higher leave production than the unamended soil. Average number of leaves we observed to increase with time after planting.

Table 1: Effects of fertilizers on Maize plant height

Treatments	Weeks after planting			
	4	6	8	12
Control	26.74a	63.45b	136.08b	167.42c
Compost	32.20a	80.76ab	161.42a	177.58b
NSBF	33.35a	90.46a	165.92a	194.00a
NPK	33.46a	92.98a	175.42a	193.92a

Means that do not share a letter are significantly different

Table 2: Effects of Fertilizers on average number of maize leaves

Treatments	**Weeks after planting**			
	4	**6**	**8**	**12**
Control	**6.00a**	**7.08b**	**9.17b**	**11.17c**
Compost	6.25a	8.25ab	11.25a	13.17b
NSBF	7.00a	8.83a	11.83a	14.00a
NPK	7.33a	9.50a	11.75a	14.08a

Means that do not share a letter are significantly different

Average leaf area of maize was not significantly influenced by the fertilizer treatments (table 3). All fertilizer treatments however increased leaf area relative to the control treatment. The broadest leaves were obtained from the NSBF plots. On the other hand, maize grain yield responded significantly to the application of fertilizers ($P > 0.05$) (table 3). The highest yield was recorded in the NPK treated plots, but this was not significantly higher than the organo-mineral treatment. The compost treatment recorded the lowest yield among the treated plots but yield on these plots were still significantly higher than the untreated plot.

Table 3: Effects of treatments on leaf area and grain yield

Treatments	**Leaf area (cm^2)**	**Grain yield (kg/ha)**
Control	652.32a	617.91c
Compost	738.47a	693.13b
NSBF	768.23a	807.70a
NPK	751.72a	835.89a

Means that do not share a letter are significantly different

Results of the microbial count taken at the end of the experiment (12WAP) indicated that both bacterial and fungal populations were influenced by the application of the various nutrient sources (table 4). However, while the Compost and NSBF increased microbial count (both bacteria and fungi), growth of microorganisms was repressed by the application of NPK fertilizer. This growth reduction by NPK was significant among bacterial colonies. Highest growths were observed when the soil was treated with compost.

Table 4: Effects of treatments on microbial population ($X10^7$)

Treatments	**Bacteria (cfu g^{-1})**	**Fungi (sfu g^{-1})**
Control	7.15b	5.46bc
Compost	7.96a	6.41a
NSBF	7.77b	5.58b
NPK	6.78c	5.22c

Means that do not share a letter are significantly ($P<0.05$) different.

Soil chemical analysis after harvest (Table 6) showed significant differences amongst soil pH, OC and basic nutrient status under the influence of the various fertilizer treatments. Both the organic and organo-mineral fertilizers significantly raised soil pH compared to the control treatment while the soil hydrogen ion concentration was lowered by the application of the inorganic fertilizer (NPK). The highest pH value was recorded in plots that received the application of the NSBF. Organic carbon (OC), nitrogen, phosphorus and potassium were also all influenced the same way as soil pH by the application of the fertilizer treatments. These parameters were significantly increased by both Compost and the NSBF whereas application of mineral fertilizer reduced their concentrations in the soil at the termination of the experiment. Despite the reduction in soil OC caused by NPK relative to the control treatment, N and P concentrations were still higher in the NPK treatment than in the control. The sampled soils were also richer in exchangeable cations (K, Mg and Ca) when Compost and NSBF were applied compared to the control and NPK application. The two treatments however appeared not to significantly differ in the concentration of these exchangeable cations.

Table 5: Effects of fertilizer and soil zone on soil chemical properties

Treatments	**pH (1:2 H_2O)**	**OC (%)**	**N (%)**	**P (mg/kg)**	**K (cmol/kg)**	**Mg (cmol/kg)**	**Ca (cmol/kg)**
Control	5.28b	1.15c	0.16d	7.55c	0.36c	1.14b	2.53c
Compost	5.85a	1.39a	0.22b	9.61a	0.42b	1.38a	2.82a
NSBF	5.93a	1.35b	0.24a	9.67a	0.47a	1.34a	2.83a
NPK	5.21b	0.92d	0.18c	9.44b	0.33d	1.08c	2.64b

DISCUSSION

The study clearly demonstrated that the neem seed-based fertilizer (NSBF) compared favourably with conventional NPK mineral fertilizer for optimum performance of maize in terms of growth and yield attributing parameters. The NSBF at the same time measured up with the Compost treatment in sustainably supporting soil microbial activity.

The observation that fertilizer treatments did not significantly affect maize plant height and leaf numbers in the early stage (4WAP) of crop establishment suggests a lag period for nutrient sources to release their nutrients, regardless of type. The highest increase in the heights of maize plants caused by the NPK treatment at this early stage however is an indication that the mineral fertilizer formulation was the first to release nutrients to the crops. Inorganic, unlike organic fertilizers are usually quick-release formulations making nutrients rapidly available to plants (Makinde *et al*., 2011). Unlike organic manures, chemical fertilizer formulations have a short period to dissolve to release plant nutrients. One of their major advantages over organic fertilizers is that nutrients are immediately available to plants and exact amount of elements to be given can be measured before feeding plants (Stolton, 1997). The NSBF also started to significantly influence maize growth in this study as from the sixth week after planting maize, an equivalence of about 4 weeks after application, which was about half of the time recorded by Akhtar (1994). This author observed that soil supplemented with neem seed powder did show some ammonium nitrogen production, but the production of nitrogen was delayed up to 60 days (8 weeks). Agbenin *et al*., (1999) has also considered N mineralization from NSC as fast because between 31 to 35% of N was mineralized within eight weeks of incorporation into the soil. However, the formulation used in this study (Royal Fertilizer Plus®) is a blend of neem seed cake and NPK fertilizer. This is presumed to have shortened the time for nutrient release to maize plants due to lowered C:N ratio of the formulation for enhanced degradation and mineralization by soil microbes. The C: N ratio of the NSBF used in this study is approximately 2:1, which is ideal for ease of decomposition (Tisdale *et al*., 2003). The C: N ratio of an organic material can be useful in predicting decomposition rate. Usually, the higher the C: N ratio, the slower the decomposition rate. C: N ratio of less than 20 is associated with a release of mineral N early in the decomposition process (Tisdale *et al*., 2003). The results obtained from the analysis of the neem seed cake are within the range of specified values for organic fertilizer (FAO, 1994). Fortification of organic manures with mineral fertilizers may therefore be considered to shorten the period of lag in their nutrient release to crops.

Although variations in maize plant height due to fertilizer treatments were noticed in subsequent weeks, NSBF (Organo-mineral) amendment gave the tallest plant heights and was in favourable competition with the NPK (inorganic fertilizer) treatment**.** Our finding is in agreement with Makinde (2007), Rajeshwari *et al*., (2007), and Ayoola and Makinde (2009) who reported better plant height in maize plants treated with organomineral fertilizers when compared to inorganic and organic fertilizers. Ahmed *et al*., (2007) also observed a similar trend in sorghum. Records of larger leaf area observed in this study with soil fertilizer amendment (regardless of type) compared to the control treatment is also in agreement with the findings of Makinde (2007) and Rajeshwari *et al*., (2007). Grain yield is the result of many complex morphological and physiological processes occurring during the growth and development of crop (Khan *et al*., 2008). Better yields obtained with fertilizer treatments in this study therefore indicated that the increase in grain yield was mainly due to better growth and yield attributing factors, better nutrient use efficiency and better grain development caused by the fertilizer treatments. This is in consonant with several earlier reports (Ayeni *et al*., 2012; Ayoola and Makinde, 2009).

The highest microbial population recorded in this study was engendered by amending soil with compost. This may not be unconnected with the fact that the addition of compost can increase the levels of organic matter and improve soil porosity, structural stability, moisture, and nutrient availability, which are a set of conditions required for improved biological activity in the soil (Francis *et al*., 2010; Wang *et al*., 2011). Bacterial diversity has been shown to be higher in compost-amended farmyard soils (Ge *et al*., 2008), and there are reports of higher microbial functional diversity with manure application than inorganic fertilizer (Mader *et al*., 2002). Other studies have also demonstrated that compost increased bacterial and fungi diversity by increasing the carbon pool of the soil, thus improving the living conditions for indigenous microbial populations (Wu *et al*., 2008; Helgason *et al*., 2010). The Neem seed-based fertilizer was observed to cause an increase in soil bacteria and fungi population, but these increases were not significant. The neem seed fertilizer has been reported to have diverse influences on the soil microbial community. Results obtained by Elnasikh *et al*., (2011) and supported by Last *et al*., (1985) when soil samples were incubated for eight weeks in the presence of neem seed cake showed that the Neem seed cake stimulated the growth of the users of organic nitrogen, but the mineral nitrogen users decreased in population due to addition of Neem seed cake. Gopal *et al*., (2007) also reported that the chemoautotrophic nitrifying bacteria (Nitrosomonas and Nitrobacter) were strongly suppressed by Azadirachtin (an extract from the neem tree). These varying responses of the soil microbial community to the application of this source of nutrient could be responsible for the lack of statistically detectable differences in cfu and sfu of bacteria and fungi, respectively as influenced by this fertilizer treatment.

The depression of both bacterial and fungal populations as a result of NPK application is in line with the findings of other authors. Mineral fertilizers have been found to decrease the number of mineral-assimilating bacteria and ammonifying bacteria. This decrease in microbial indices with fertilizer application was attributed to a change in the quality of the organic matter to a less available substrate for ammonifying bacteria. Oligonitrophilic bacteria population was reduced seven folds following mineral fertilizer application compared to no application (Egamberdiyeva *et al*., 2001). Soil acidification caused by mineral fertilizer application in this study could also be responsible for the low bacteria population because the mineral fertilizer decreased soil pH in this study. This process reduces most of the activities of bacteria and actinomycetes in the soil (Kaur *et al*., 2005) and seems to be the cause of soil cations leaching over the long term (Likens *et al*. 1996; Bailey *et al*., 2005). This acidifying effect of nitrogenous fertilizers is in agreement with the findings of Hati *et al*., (2008) and Darusman *et al*. (1991), who also reported a decline in soil pH with application of N. This is mainly due to the fact that most fertilizers supply N as NH_4^+ first, which upon oxidation releases H^+ ions (Magdof *et al*., 1997).

Soil organic C and N contents provide a measurement of soil organic matter status. The soil organic C content in the compost and NSBF increased significantly compared to the Control soil treatment in this study. Residual soil organic C content was high compared with the unfertilized plot in this experiment presumably because of C addition through the roots and crop residues, higher humification rate constant, and lower decay rate (Kundu *et al*., 2002). In this study, enhanced accumulation of SOC by the combination of organic and inorganic (NSBF) is consistent with many other studies (Hao *et al*., 2008; Banger *et al*., 2009). Studies have also suggested that combining inorganic fertilizers with organic sources can increase P availability (Nziguheba *et al*., 2000). Increased residual N above the control treatment engendered by the NSBF may be partially due to a slow release of N from the formulation, resulting in smaller losses of N.

CONCLUSION

It can be concluded from the results obtained in the present study that organomineral formulations can serve dual purposes of both quick nutrient release for the present crop through positive priming of the organic component, and as well leave the soil with both biological and chemical fertility for the crops following in rotation.

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