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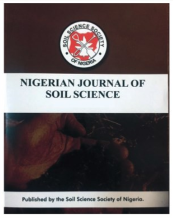

Soil Microbial Activity under Complementary Applications of Organic and Inorganic Amendments

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ABSTRACT

Enhancing soil microbial properties through balanced inputs of organic and inorganic amendments are key to sustainable soil and crop productivity. A laboratory incubation study was conducted to determine microbial respiration, population and nutrient (N and P) mineralization patterns in an Ultisol amended with sole Urea at 56 kg N ha−1 or Urea (at 0 or 28 kg N ha−1) combined with 5 t ha−1 organic amendment (poultry manure Enhancing soil microbial properties through balanced inputs of organic and inorganic amendments are key to sustainable soil and crop productivity. A laboratory incubation study was conducted to determine microbial respiration, population and nutrient (N and P) mineralization patterns in an Ultisol amended with sole Urea at 56 kg N ha^{-1} or Urea (at 0 or 28 kg N ha^{-1}) combined with 5 t ha^{-1} organic amendment (poultry manure compost, biochar or their mixture). The treatments were arranged in a completely randomized design, replicated three times and incubated for a period of 12 weeks at 30 ^{0}C. Microbial respiration, and mineralized N and P were measured fortnightly, while the heterotrophic bacterial and fungal populations were determined at the 2nd, 6th and 12th weeks of the incubation. The results showed that microbial respiration, and mineralized N and P increased in response to the organic amendments. Being richer in most measured nutrient elements, poultry manure compost, followed by its combination with biochar fortified with and without inorganic N, had the highest values of the evolved CO_2, and mineralized N and P. Further, addition of compost, biochar or their mixture with and without fertilizer N when compared with sole inorganic N or the control without any nutrient addition, significantly increased bacterial and fungal populations in the soil. It was concluded that, sole and complementary applications of biochar and compost improve microbial activity, and can be adopted for soil health enhancement.

1.0 Introduction

Soil microbial communities play a crucial role in nutrient cycling through their processes of organic matter decomposition, nutrient mineralization and immobilization (Bailey et al. 2002; Waring et al. 2013). Soil microbes influence the soil biogeochemical cycles by altering nutrient availability through solubilization, chelation and oxidation/reduction processes (Lin et al. 2019; Meng et al. 2022). However, the microbiota, their distributions, as well as their maintenance of soil processes and ecosystem services are affected by the quality of organic materials added to the soil (Bonanomi et al. 2018b). Various organic materials of plant and animal origins can be used to improve the physical, biological, and chemical properties of soil and consequently, its productivity (Mangalassery et al. 2019). These wastes can particularly be beneficial to tropical soils with low organic matter contents and dominance of low-activity clays (Agbede and Ojeniyi, 2009). Even though the use of inorganic fertilizers to increase yield can be effective as a short-term solution, their prices, without government subsidies are often unaffordable to the lowincome and small-scale farmers. Continuous use of inorganic fertilizers can also have hazardous environmental implications (Rahman and Zhang, 2018; Lin et al. 2019). Further, the soil microbial diversities and their soil processes can be adversely affected (Makinde et al. 2001; Agbede, 2010). Thus, organic amendments are important as alternatives or complements to inorganic fertilizers.

Many organic wastes are rich in essential plant nutrients such as nitrogen (N), phosphorus (P), potassium (K), and micronutrients (Rogeri et al. 2016). They release nutrients steadily over a longer period and improve the soil fertility status by activating the soil microbial biomass (Makinde et al. 2001). However, organic amendments can be bulky, costly to transport and slow to mineralize (Oshunsanya, 2011). They are also required in rather large quantities to meet up with crops' nutrient requirements and rarely available to small-scale farmers in the required quantities. Hence, combined use of both organic and mineral fertilizers can help to overcome the challenges posed by their sole applications (Atere and Olayinka, 2012a, b) and constitute a sound soil fertility management strategy.

Among the organic wastes that can be used to complement inorganic fertilizers are poultry droppings. They are generated in large amounts due to the poultry industry's growing expansion; but poor management of the wastes can lead to adverse effects on water bodies and the environment (Rahman and Zhang, 2018; Ilahi et al. 2020). Although it contains considerable amounts of essential plant nutrients, the application of fresh poultry manure to soils poses a risk of pathogenic contamination, foul smell, high ammonia and moisture contents, and leaching of N into water bodies (Alegbeleye *et al*. 2018). Composting is widely practiced to mitigate the negative effects of fresh animal manure. Composting animal manure eliminates toxic chemicals and pathogenic microorganisms as well as introduces beneficial microorganisms (Bernal et al. 2009). Its effectiveness in the stabilization of essential plant nutrients, including micronutrients has also been established. Compost application also improves soil water-holding capacity and results in a better soil structure with well-developed aggregates and many voids that improve rainwater infiltration, thus reducing run-off and soil loss (Mangalassery et al. 2019).

Biochar is another organic amendment for soil fertility improvement, potential toxic element adsorption, and climate change mitigation (Paustian et al. 2016). It is a carbon-rich material obtained from thermochemical conversion (slow, intermediate, and fast pyrolysis or gasification) of biomass in an oxygen-limited environment. It can be produced from a range of feedstock, including forest and agriculture residues, such as straw, nutshells, rice hulls, wood shavings, tree bark, and switch grass (Ennis et al. 2012).

Several studies exist on improving crop yields through combined applications of inorganic fertilizers and organic manure. However, studies have not been extensively carried out on the influence of combined application of N fertilizer, compost and biochar on microbial mineralization of nutrients in the rain forest zone of Nigeria. Hence, the objective of this study was to evaluate the effect of composted poultry manure and biochar enriched with inorganic N on CO_2 evolution, nutrient mineralization and microbial population in an Ultisol

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2.0 Materials and Methods

2.1 Experimental Site and Samples

Top soil (0–15 cm) samples of an Ultisol (Iwo soil series) with sandy loam texture were obtained from an unfertilized plot at the Obafemi Awolowo University Teaching and Research Farm (OAUTR&F) (Latitude 7° 30'–7° 35' N and Longitude 4° 30'–4° 35' E). The soil samples were bulked, air-dried, and passed through 2 mm sieve to remove coarse particles. The portion that passed through the 2 mm sieve was used for laboratory analyses and incubation study.

2.2 Composting of Organic Wastes and Pyrolysis of Wood Shavings

The freshly collected poultry droppings were composted for a period of 8 weeks using a composting bucket at the OAUTR&F, Ile-Ife with regular turning while the wood shavings were pyrolized using top lit updraft gasification technique at a temperature of 400 ^oC (Maican et al. 2016). Thereafter, the composted material was compounded with and without biochar and N fertilizer (28 kg N ha^{-1} as urea).

2.3 Analytical Techniques

The physicochemical properties of the soil were determined, following standard methods. Soil particle size distribution analyses was determined using the hydrometer method (Bouyoucos 1962). Soil pH was determined potentiometrically in a soil–solution ratio of 1:2 in 0.01 M calcium chloride ($CaCl_2$) using a glass electrode pH meter. Organic carbon (OC) was analyzed using the Walkley–Black dichromate method (Nelson and Sommers, 1996); the total N contents were determined using micro-Kjeldahl digestion, distillation, and titration processes (Bremner, 1996), and available P was extracted using the Bray-1 method and colorimetrically determined at 660 nm wavelength after the development of the molybdenum blue color (Bray and Kurtz, 1945). Exchangeable potassium (K), calcium (Ca), magnesium (Mg), and sodium (Na) were extracted with 1 N ammonium acetate (Helmke and Sparks, 1996). Sodium and K concentrations were determined using a flame photometer, while Ca and Mg were determined using Atomic Absorption Spectrophotometer. The following chemical properties: pH, OC, total Nitrogen, available P and basic cations were determined in the composted poultry manure and biochar.

The physical and chemical properties of the soil are as follows: particle size analysis of 767, 90 and 143 g kg^{-1} sand, silt and clay, respectively; Field Moisture Capacity (FMC) of 11.50; pH of 4.9; organic C (OC) of 8.20 g kg^{-1}, total nitrogen (N) of 0.77 g kg^{-1}, available P of 16.10 mg kg^{-1}, exchangeable Ca, Mg, K and Na of 9.11, 0.99, 0.04 and 0.01 cmol kg^{-1}, respectively. Fresh poultry droppings were obtained from the poultry unit at OAUTR&F. Wood shavings and Urea fertilizer were obtained from a saw milling industry and an agrochemical store at the Ile-Ife, Osun State, Nigeria. The chemical properties of the organic amendments are shown in Table 1.

2.4 Amendment of Soil Samples

Five hundred gramme (500 g) portions of soil were amended with composted poultry droppings, biochar and Urea fertilizer at the rate of 5 t ha^{-1} (on oven-dry basis). There were seven treatments replicated three times and arranged in a completely randomized design. The treatments included the following: Soil only (Control) – (C); Soil + 5 t ha^{-1} Biochar - (B); Soil + 56 kg N ha^{-1} Urea - (F1); Soil + 5 t ha^{-1} B + 28 kg N ha^{-1} Urea - (BF2); Soil + 5 t ha^{-1} composted poultry manure - (PM); PM + 28 kg N ha^{-1} Urea (PMF2); Soil + 5 t ha^{-1} 2:1 PM + biochar - (PMB); and Soil + PMB + 28 kg N ha^{-1} Urea - (PMBF2)

2.5 Microbial Respiration, N and P Mineralization

The treatments were poured into 500 ml incubation glass jars in triplicates, moistened with distilled water to 70% of its field moisture capacity, and arranged in a completely randomized design. With the aid of lengths of thread, vials containing 10 mls of 1 M NaOH solutions were suspended into the jars and capped tightly. The evolved carbon dioxide (CO_2) was determined fortnightly for 12 weeks using the double acid titration method (Anderson, 1982).

The CO_2 concentration was calculated using the following equation:

$$\text{Mg C/100 g} = \text{Titre} \times \text{N } H_2SO_4 \times 30/1 \times 12/60$$

Where, 30 is equivalent of CO_3^{2-} in mg; 12/60 is ratio of C to the mass of CO_3^{2-}

The incubated soil, in the absence of CO_2 traps, was repeated for the assay of N and P mineralized, which were determined using the steam distillation technique of Bremner and Mulvaney (1982) and Bray-1 extraction technique (Kuo, 1996), respectively. The experiment lasted for 12 weeks.

2.6 Microbial Population Counts

The microbial populations were estimated using the pour plate method (Dubey and Maheshwari, 2012). The total heterotrophic bacteria (THB) and Total Heterotrophic Fungi (THF) were cultured with sterilized Nutrient Agar and Saboraud Dextrose Agar. The microbial counts were taken and expressed as the log number of colony-forming units (log cfu) per gram.

2.7 Statistical Analysis

Data generated were analyzed using one-way analysis of variance technique. Means were separated using Duncan's New Multiple Range Test (DNMRT) at 5% level of probability with SAS 9.0 Software Package

3.0 Results

3.1 Effects of biochar and poultry manure compost with or without biochar on carbon dioxide evolution during twelve weeks of laboratory incubation

The carbon dioxide evolution which is an index of microbial activity was much higher in the 2nd week and 6th weeks (among B, PM and PMF2) than in the other weeks of incubation across all treatments (Figure 1a). At the end of the 2nd week of incubation, the CO_2 evolved in all the organically-amended soils, were significantly ($p<0.05$) higher than those in the control and fertilizer, with PMB, PMBF2, BF2 and PMB2 recording the highest values F1. At the 4th week of incubation, the organically amended soils recorded higher ($p<0.05$) CO_2 evolution over the control and F1, with the increase over the control ranging from 53–162%. The highest increase was, however, obtained with PMBF2. At the 6th week of incubation, except for F1 and BF2 which were similar to the control, the increases recorded by the organic amendments over the control ranged from 11–50%. At the 8th - 12th weeks of incubation PM- and PMB-treated soils produced higher ($p<0.05$) CO_2 evolution than the control and others. There was a progressive reduction in CO_2 evolution with incubation length, especially from week six of incubation. The CO_2 evolution in PM and PMB were generally higher ($p<0.05$) than in control and other treatments throughout the period of incubation. The mean CO_2 values were higher ($p<0.05$) in PM and PMB than in the control and other treatments (Figure 1b)

3.2 Nitrogen Mineralization

The amount of N mineralized was increased ($p< 0.05$) by composted poultry manure with or without biochar and inorganic fertilizer over the control throughout the period of incubation (Figure 2a). The treatments F1, PM, PMB and PMBF2 and PMF2 were significantly higher ($p< 0.05$) than the control, B and BF2 in the concentration of N mineralized at each sampling point throughout the incubation period. Generally, there was a progressive increase in N mineralized from the soils until week 8 of incubation, with a slight decrease from week 10. The mean N mineralized at the end of the twelve (12) weeks of incubation were significantly ($p< 0.05$) higher in all the organically amended treatments by18–74% than the control (Figure 2b). The highest percentage increases were obtained with PM, PMBF2 and PMF2.

3.3 Phosphorus Mineralization

Phosphorus mineralization was significantly ($p< 0.05$) higher in PM, PMB, PMBF2 and PMF2 than other treatments throughout the incubation period (Figure 3a). There was a progressive increase in the concentrations of P mineralized across the weeks of incubation until the 10th week, A slight decline, was however observed at the 12th week. The mean P mineralized at the end of twelve weeks increased between 11 and 200% in the amended soils compared with the control (Figure 3b). The highest mean P mineralized was obtained with PM.

3.4 Microbial Population

Data on microbial population in response to the application of the amendments at the 2nd, 6th and 12th weeks of laboratory incubation are shown in Table 2. The total heterotrophic bacterial and fungal populations were significantly increased by the application of the amendments. The mean bacterial counts at the end of twelve weeks of incubation were increased by PMBF2 (8 and 14%), PMB (8 and 14%), PMF2 (7 and 12%), BF2 (6 and 11%) and PM (7 and 13%) over the control and F1, respectively. Total fungal counts were significantly increased by PMF2, PMBF2, PMB and PM over the control and F1 at the ends of 2nd, 6th and 12th weeks of incubation. The mean total heterotrophic fungal counts were increased by PMF2 (2 and 3%), BF2 (3 and 4%), PMBF2 (3 and 4%), PMB (3 and 4%) and PM (3 and 4%) over the control and F1.

4.0 Discussion

The flush of microbial activity observed in the treated soils at 2nd week of incubation could be attributed to the activation of microbial activity following re-wetting of air-dried soil and the susceptibility of the added organic materials to microbial attack, resulting in rapid mineralization of C (Oladipo et al. 2010; Erhunmwunse and Olayinka, 2018, 2019; Atere et al. 2020). The gradual reduction in CO_2 evolution with week of incubation could be attributed to exhaustion of readily decomposable C and increasing re-

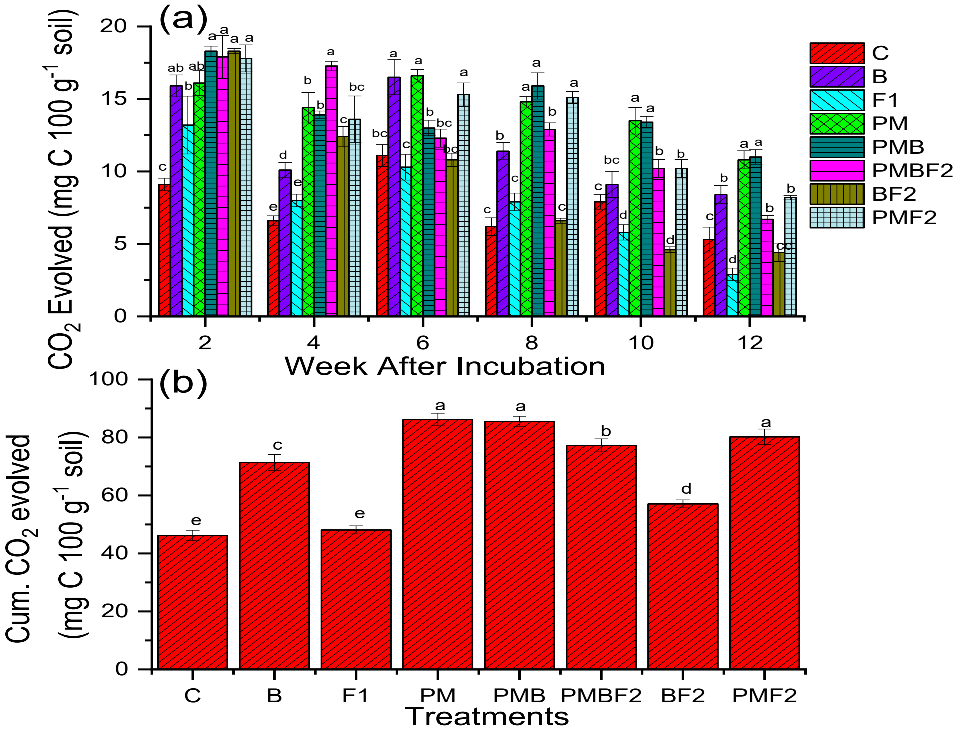

Figure 1. CO_2 evolution from soils treated with organic and inorgganic amendments over a period of 12 weeks of incubation in the laboratory

Where, C = control, B = Biochar, F1 = 56 kg N ha^{-1}, PM = Poultry manure compost, PMB = 5 t ha^{-1} 2:1 PM + biochar, PMBF2 = 5 t ha^{-1} 2:1 PM + biochar + 28 kg N ha^{-1}, BF2 = biochar + 28 kg N ha^{-1}, and PMF2 = PM + 28 kg N ha^{-1} Urea. Line on a bar represents standard error of the means.

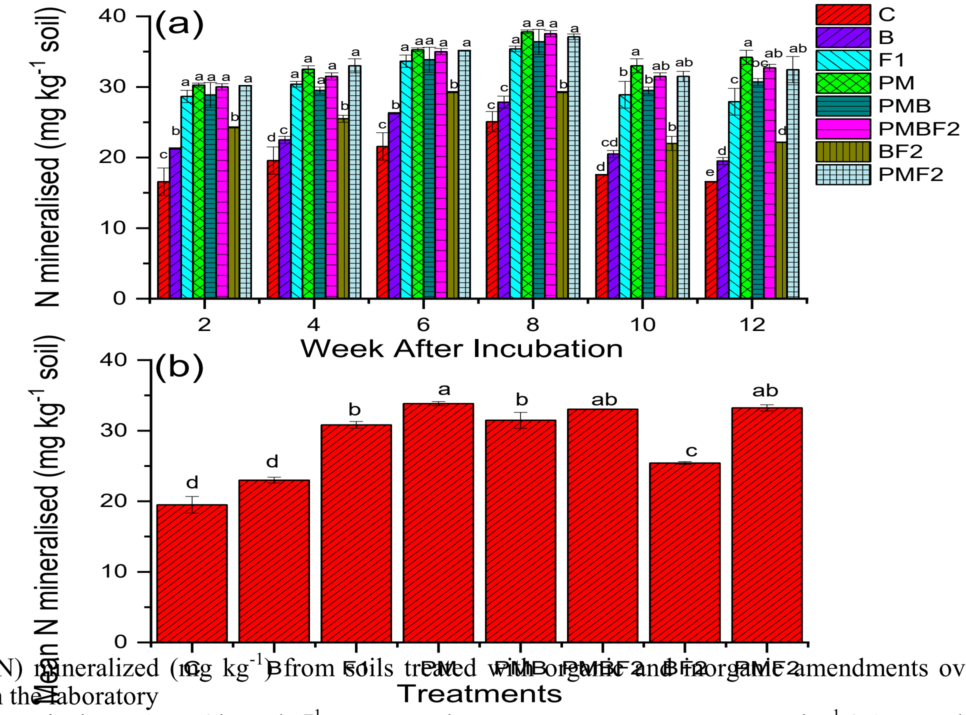

Figure 2. Nitrogen (N) mineralized (mg kg^{-1}) from soils treated with organic and inorganic amendments over a period of 12 weeks of incubation in the laboratory

Where, C = control, B = Biochar, F1 = 56 kg N ha^{-1}, PM = Poultry manure compost, PMB = 5 t ha^{-1} 2:1 PM + biochar, PMBF2 = 5 t ha^{-1} 2:1 PM + biochar + 28 kg N ha^{-1}, BF2 = biochar + 28 kg N ha^{-1}, and PMF2 = PM + 28 kg N ha^{-1} Urea. Line on a bar represents standard error of the means.

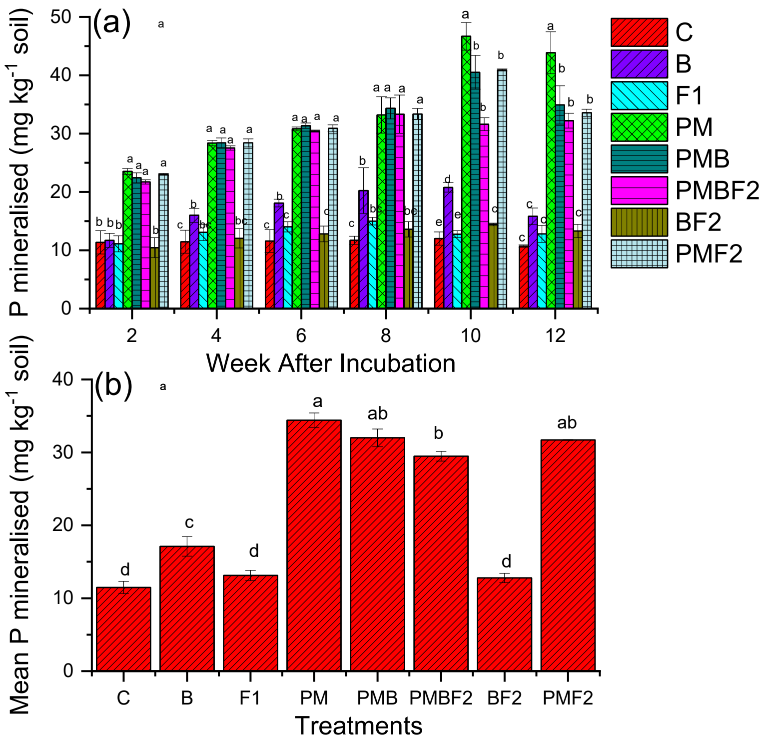

Figure 3. Phosphorus (P) mineralized (mg kg^{-1}) from soils treated with organic and inorganic amendments over a period of 12 weeks of incubation in the laboratory
Where, C = control, B = Biochar, F1 = 56 kg N ha^{-1}, PM = Poultry manure compost, PMB = 5 t ha^{-1} 2:1 PM + biochar, PMBF2 = 5 t ha^{-1} 2:1 PM + biochar + 28 kg N ha^{-1}, BF2 = biochar + 28 kg N ha^{-1}, and PMF2 = PM + 28 kg N ha^{-1} Urea. Line on a bar represents standard error of the means.

sistance of the residual organic C pool to decomposition (Olayinka, 1996). The increase in CO_2 evolution resulting from the organic amendments, especially, poultry manure compost with or without biochar was due to availability of nutrient and carbon sources for increased microbial activity. On the contrary, the treatments which contained inorganic fertilizer (Urea) reduced microbial respiration, even more than the control, which could be due to the acid-yielding nature of urea fertilizer, which could depress soil microbial activity (Justin and Jason 2011; Atere and Olayinka, 2013). Further, the control and inorganic fertilizer treatments released lower CO_2 amounts as a result of lower organic matter content and resistance of the remaining native soil organic matter to microbial decomposition (Olayinka, 1990).

The progressive increase in the amounts of N mineralized in the soils amended with composted poultry manure with or without biochar and inorganic fertilizer up till 10th week of incubation can be attributed to the mineralization of the added compost and the released N from the added inorganic fertilizer. The PM and PMBF2 being rich in C and other nutrients gave the largest amounts of mineralized N among all the treatments. Preusch et al. (2002) had earlier reported that composts affected soil N mineralization due to the presence of stable N compounds.

Phosphorus is called the “key to life” by being directly involved in most living processes. It is a constituent of Adenosine triphosphate (ATP) and plays important roles in various physiological processes and energy transformation in plants. Hence, it is important that the organic materials added to the soil are able to release substantial amounts of P for plant uptake. In the current study, the added organic amendments, namely, PM, PMB and PMBF2 released larger amounts of mineralized P than the control across the incubation week indicating the ability of organic materials to release the substrate as well as the native P (fixed in the soil). The low C: N ratio of the composted materials enhanced nutrient release through decomposition and mineralization activities of soil microorganisms (Alori et al. 2017). The progressive increase in P mineralized in the organically amended soil across week of incubation could be attributed to the ability of the organic materials to decrease P fixation in soil by coating soil mineral surfaces, thereby blocking sites that fix P and increasing P availability (Azeez and Van Averbeke, 2010).

Microorganisms most especially bacteria and fungi play a significant role in the breaking down of organic materials in the soil, releasing the locked-up nutrients and recycling them. The total heterotrophic bacteria and total heterotrophic fungi were significantly increased by the application of biochar and poultry manure compost with or without fertilizer during the twelve weeks of laboratory incubation. This could be attributed to the presence of organic matter which acted as a source of energy, thereby stimulating the growth of microorganisms in the soil. The results showed the highest microbial populations in treatments containing composted poultry manure showing effect of nutrient on microbial growth. Application of composts, especially those with low C/N ratio have been considered to improve the growth of bacteria and fungi (Six et al. 2006; Chang et al. 2007; Ndubuisi-Nnaji et al. 2011).

5.0 Conclusion

Combined application of composted poultry manure with or without biochar and inorganic N significantly increased microbial respiration, population, and N and P mineralization. The application rate of 5 t/ha of poultry manure compost with or without biochar and urea best promoted the measured soil microbial parameters which are key indicators of soil health and potential to release nutrients for plant growth.

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