

Cow Dung Forms and their Effect on Microbial Community of Degraded Ultisols of some Smallholder Women Farmers' Fields in the Rainforest Zone of Nigeria

Onwuka, M. I.^{1*}, Okereke, J. C.¹ and Fashola, M. S.²

¹Department of Soil Science and Meteorology, College of Crop and Soil Sciences, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria.

²Department of Microbiology, College of Natural Sciences, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria.

*Corresponding Author E-mail: ifyonwuka2000@yahoo.com

Received 30 March 2019; Accepted 13 May, 2019

Soil microorganism is one of the indices of a healthy soil and one of the ways to boost its increase in the soil is by adding organic manure. It is important to know the form of the animal manure that will increase the soil microbes. It is against this backdrop that a scientific investigation was conducted at the research field of the College of Crop and Soil Sciences, Michael Okpara University of Agriculture Umudike. The pot study was aimed to assess the effect of cow dung forms on the microbial community of the Ultisols of smallholder women farmers in the Rainforest zone of Nigeria. The treatments were composted cow dung (CCD), normal cow dung (CD), cow dung ash (CDA), cow dung biochar (CDB) and a control (CTRL), which did not receive any amendment. They were applied at the rate of 5 t ha⁻¹ (equivalent to 1.4 kg) to 10 kg of soil and replicated three times. The microbial analysis was done before treatment application, at 1, 2, 3 and 4 weeks after treatment

application (WAA) using the standard laboratory procedures. The results obtained showed that the main microbes, present in the soil were bacteria and fungi. At 1 weeks after treatment application, CCD significantly ($P \leq 0.05$) increased the value of bacterial from 137060 (cfu g⁻¹) before treatment application to 774000 (cfu g⁻¹). The highest bacterial population was obtained at 3WAA with a value of 1200000 (cfu g⁻¹) given by CCD. Composted cow dung gave 863000 (cfu g⁻¹), which was the highest value obtained for fungi at 4WAA. Soil pH correlated positively with the soil bacteria. It will be concluded that if the smallholder women farmers will apply CCD, the microbes will be increase and this will improve the soil health of the degraded soil.

Keywords: Cow dung forms, fungi, bacteria, degraded soil, women smallholder farmer, rainforest

INTRODUCTION

Most of the farm activities carried in Africa, such as slash and burn, complete removal of crop residues, continuous cropping of same piece of land without a fallow period or crop rotation affect the soil physical, chemical and biological properties. For example crop residue removal reduces input of organic binding agents essential for the formation and stability of aggregates (Canqui and Lal, 2009). One of the ways stable aggregates are formed is through microbes. These organisms produce substances that bind the soil aggregate and organic colloids through physical binding and chemical secretion (Chotte, 2005; McMahan and Simard, 2018). Soil microbes as an example of soil biological properties enhance the soil

productivity. According to Brookes, (2001) and Bending *et al.* (2002), soil microbial biomass carbon and activity are useful indicators of soil quality and health. Microorganisms such as bacteria and fungi help in nutrient cycling, formation of soil aggregates, among others. These improve the soil structure; formation of organic matter through mineralization and degradation of pollutant (Stark *et al.*, 2008; Fortuna, 2012).

Use of organic fertilizers on agricultural land has great positive effect on the soil's bacterial communities (Tayyab *et al.*, 2018). Organic fertilizers like animal manure and compost are known to increase the microbes especially the bacterial diversity (Ge *et al.*, 2008).

Soil microorganisms' population are increased in substrates that have carbon source of energy (Hoorman and Islam, 2010). Hartmann *et al.*, (2015) stated that livestock wastes can be used to manage, the soil microbial community for the presence of beneficial and absence of detrimental microorganism. The types and form of the animal manure has great influence on the soil microbial community. Das *et al.*, (2017) reported that composted cattle manure stimulated the growth of soil microbes more than composted swine manure. Composted cattle manure increased the microbial biomass carbon and the microbial species; this was attributed to the incorporation of easily degradable organic matter and other nutrients, which stimulates the growth of the microorganisms of the soil (Das *et al.*, 2017). Soil inputs like compost, biochar and ash has been found to increase the soil pH, soil nutrients such as nitrogen, phosphorus, organic carbon and the cations (Onwuka and Nsofor 2011; Onwuka and Nwangwu, 2016). Increased soil pH, nitrogen, carbon and phosphorus stimulate the positive bacterial genera (Tayyab *et al.*, 2018). Composted cattle manure has been reported to increase C, N, P and improved pH and this in turn increase the microbial biomass (Das *et al.*, 2017).

The soils of most smallholder women farmers' farms in the Sub Saharan Africa are degraded. This is generally caused by continuous cropping, frequent and intensive rainfall, acidity, depleted organic matter among others. One of the cheapest and affordable ways of restoring the health of the degraded soil is by using animal manure. Determining the form of animal manure, especially cow dung (that is becoming more abundant in Nigeria) in increasing the soil microbes, is one way of assisting the poor resource farmers in Nigeria improve their soil microbial population.

We hypothesized that converting cow dung into other forms such as compost, biochar and ash rather than using it in the ordinary form will increase the soil microbes. We used soil microbial population and identification as indices of the performance of the cow dung forms. In this study, we investigated the response of soil microbes to the forms of cow dung and some soil chemical properties.

MATERIALS AND METHODS

Site description

The study was a pot experiment carried out at the research field of College of Crop and Soil Sciences, Michael Okpara University of Agriculture Umudike, Abia State Nigeria (05° 29'N, 07° 33'E). The research area is in a tropical rain forest zone; with a mean annual rainfall of 2200 mm, and a monthly minimum air temperature range of 20 to 24°C and monthly maximum air temperature

ranges of 28 to 35°C.

Experimental procedure and design

Five treatments using different cattle dung forms were designed. Cow dung treatments were established as follows: no cattle dung which served as the control (CTRL), composted cow dung (CCD), cow dung biochar (CDB), cow dung ash (CDA) and normal cow dung (CD). The treatments were prepared as follows: CCD was composted in a composter for 90 days. When the maturity test showed that it has matured for use, it was then air dried, crushed and passed through a 2 mm sieve mesh. CDB was made using a self-made 210 litre capacity pyrolysis drum with temperature of 450°C. About 10 kg of the dung was pyrolyzed each time and the heating lasted for 4 hours; afterwards the biochar was cooled for 24 h. The biochar were thoroughly mixed and ground to pass through a 1mm sieve mesh. CDA was prepared by subjecting the dried cow dung feedstock, to combustion in an open incinerator. The ash was also allowed to cool for 24 h before being used. The pH and total nitrogen for CCD, CDB, CDA and CD were 7.3, 8.6, 9.8, 6.9; 1.62%, 0.20%, 0.19% and 2.09% respectively.

The experimental pots of 12 litre (L) sizes, measuring 30.3 cm (diameter) and 29.2 cm (height) were used for the experiment. The soils were randomly collected from ten smallholder women farmers' farms at Nkwoegwu, Umuahia North L.G.A in Abia State (05° 33'N, 07° 28' E). The soil samples were collected at a depth of 0-20cm from the farms that were previously cultivated with cassava, maize and vegetables. The soils were air dried at room temperature and pass through a 2 mm sieve mesh, before using for the experiment. Each pot received 10 kg of the composite soil sample thoroughly mixed with the amendments and packed to a bulk density of 1.40 g cm⁻³. The amendments were applied on a dry weight basis at the rate of 1.4 kg (equivalent to 5 t ha⁻¹) to the soil, and replicated three times in a Completely Randomized Design. The soil for the experiment had a loamy sand texture (832 g kg⁻¹ sand, 88 g kg⁻¹ silt and 80 g kg⁻¹ clay), pH 5.50, total nitrogen 0.12%, organic carbon 1.52 % and available phosphorus 23.20 mg kg⁻¹.

Sampling procedure

Soil sample was collected from the experimental pots before the treatments were applied and at one, two, three and four weeks after treatments application. The samples were passed through a 2 mm sieve, moistened to 60% water holding capacity, and stored immediately at temperature of 4°C for the measurement of soil microbial properties (Zhen *et al.*, 2014).

Plate count of cultivable microorganisms

The total number of cultivable bacteria and fungi were

counted as colony forming units (CFUs) on agar plates using the dilution plate method. About 20 ml of nutrient agar medium was added in 90 mm diameter sterile Petri dishes and then enumerated (Pace, 1999). After the serial dilution plates of the samples, they were incubated to 48 hours to grow the microbial colonies properly.

Soil properties analysis

The pH of the soil was determined in 1:2.5 soil water ratios, using the glass electrode method of (Mclean, 1982). Exchangeable acidity and exchangeable aluminium were determined by the method of Mclean (1986) as outlined by Udo *et al.*, (2009), using 1 N KCl as the extractant and titrating with 0.05 N NaOH. Organic carbon was determined by dichromate-oxidation method of Walkley and Black wet oxidation method as outlined by Nelson and Somner, (1982). Total nitrogen in the soil sample was determined by Macro-Kjeldahl method (Bremner, 1965).

Statistical analysis

All statistical analyses were performed by using GENSTAT software package 18th Edition (GENSTAT Rothamsted Research Centre, United Kingdom). Significant differences were obtained by the one-way analysis of variance (ANOVA) for experiment in Completely Randomized Design (CRD) with means separated using Fisher's Least Significant Difference at probability level of 5%. The correlation was analysed with the Pearson test (two-tailed) at $P \leq 0.05$. Any differences between the mean values at $P \leq 0.05$ were considered statistically significant.

RESULTS

Soil microbes' population before treatment application

The result of the identified soil microbes and their population are shown on (Figure 1). The two main indigenous soil microbes identified in the farms of the smallholder women farmers' were fungi and bacteria. The population of the fungi was 9900 (cfu g⁻¹) while that of the bacteria was 137060 (cfu g⁻¹) before treatment application. These were identified before the cow dung forms were applied to the soil.

Effect of treatment on bacteria population at weeks after treatment application

There were large variations in the number of bacteria

among the various treatments (Figure 2). The colony-forming units (CFUs) of bacteria in all cow dung amended pots were significantly ($P \leq 0.05$) higher, than those observed in the CTRL treatment at the weeks after treatment application (WAA). At two weeks after treatment application, CCD gave the highest bacteria value of 1087000 cfu g⁻¹, there was a 40.44% increase of the bacteria population from 1 WAA to 2 WAA. Composted cow dung had the highest significant ($P \leq 0.05$) colony-forming units (CFUs) of bacteria at 3 WAA, though it was statistically at par with the values obtained for CD. The highest amount of the cultivable bacteria was obtained at 3 WAA by CCD with a value of 1200000 cfu g⁻¹. At the 4 WAA, the value of bacteria population obtained at CCD reduced to 1040000 cfu g⁻¹. This gave a reduction percentage of 13.33% from the values obtained at 3 WAA compared to the values obtained at 4 WAA.

Effect of treatment on fungi population at weeks after treatment application

Cow dung forms greatly influenced the amount of soil fungi (Figure 3) obtained after treatments application. For instance, the amounts of fungi in all amended soils were remarkably higher than that in the CTRL at WAA. At 1 and 2 WAA, the amount of fungi in the CD was same with that of CCD statistically. At 3 WAA, CD treatment had the highest fungi population than all the other treatments, while CCD and CDB had same number of fungi statistically. The numbers of fungi in CCD were considerably, highest at 4 WAA when compared to other treatments. Meanwhile the amount of cultivable fungi in the CD treatments initially increased, but eventually decreased at the fourth week. The value of the fungi population decreased from 703,000 cfu g⁻¹ at the third week to 407,000 cfu g⁻¹ at the 4th week after treatment application. Similarly, CCD values increased from 373,000 cfu g⁻¹ at the first week after application to 863,000 cfu g⁻¹ at 4 WAA, which was the highest value for CCD within the period of the experiment.

Correlation of soil nutrients and microbes

The result of the correlation between the soil microbes and some selected soil properties are shown in (Table 1). Bacteria correlated positively but non-significantly with pH, while fungi had a negative and non-significant correlation with pH. Bacteria negatively correlated with exchangeable acidity however, fungi correlated positively and significantly ($P \leq 0.5$) with exchangeable acidity. Total nitrogen had non-significant differences with bacteria and fungi, however, while it positively correlated with bacteria, it negatively correlated with fungi. Bacteria and fungi had non-significant differences with organic carbon;

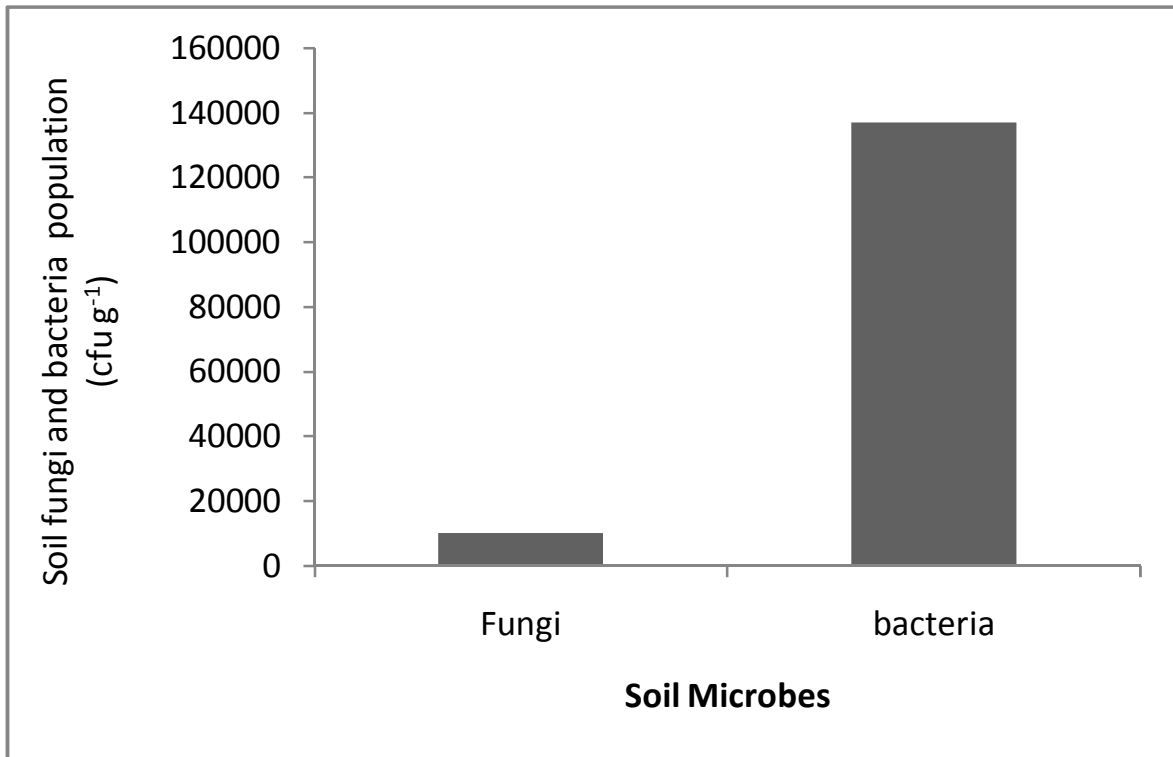


Figure 1. Identified soil microbes and population before treatment application in the soil.

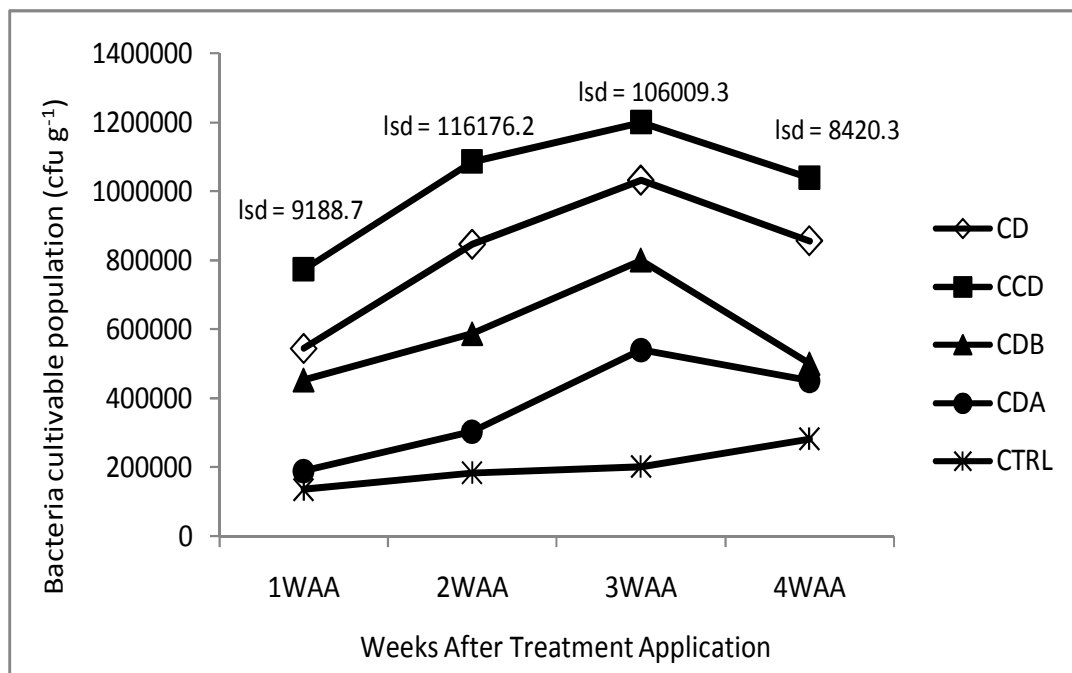


Figure 2. Treatments effect on bacteria population at weeks after treatment application. Vertical bars represent Lsd ($P \leq 0.05$).

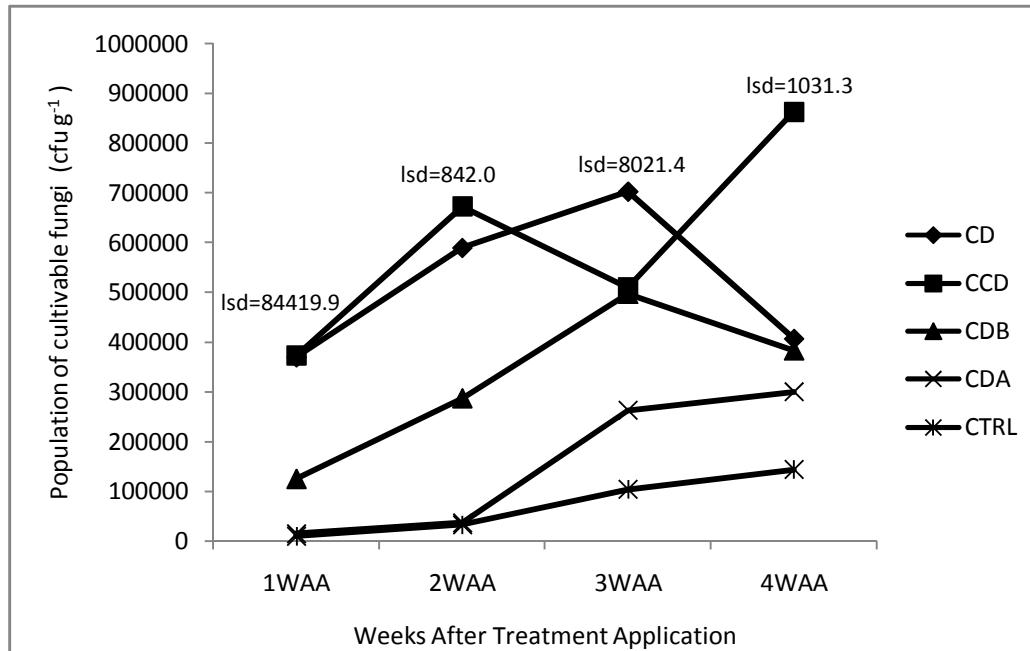


Figure 3. Treatments effect on fungi population at weeks after treatment application.

Table 1. Correlation between the identified soil microbes and some selected soil properties.

Soil Properties	Soil Microbes	
	Bacteria	Fungi
pH	0.121 ^{ns}	-0.149 ^{ns}
Exchangeable Acidity	-0.784 ^{ns}	0.790*
Total Nitrogen	0.691 ^{ns}	-0.674 ^{ns}
Organic Carbon	-0.719 ^{ns}	0.684 ^{ns}
Available Phosphorus	0.614 ^{ns}	-0.736**

bacteria negatively correlated with organic carbon while fungi positively correlated with it. Bacteria correlated positively with available phosphorus with a value of 0.614, while fungi had a negative correlation with available phosphorus.

DISCUSSION

This study investigated response of soil microbial communities to cow dung forms that were applied to the degraded soils of smallholder women farmers. It was observed that all the forms of cow dung; CD, CCD, CDB and CDA significantly increased the population soil microbes over the control. The reason could be that the nutrients required by the microbes to rapidly grow and multiply were supplied by CD, CCD, CDB and CDA. Among the forms of cow dung used in the investigation, composted cow dung had increased population of

bacteria and fungi; this finding is in consonant with the result obtained by Zhen *et al.*, (2014). Animal manure compost was able to increase the bacteria and fungi diversity due to increasing carbon pool of the soil (Ge *et al.*, 2008; Hoorman and Islam 2010; Zhen *et al.*, 2014). As the carbon pool is increased, the living condition of the indigenous microbial are increased. Similarly, plants depends on microbes to access the nutrients in the substrates, this is because as these microbes increase in size and number, they are able to depolymerize and mineralize the organic forms of nutrients especially N, P and S through the metabolic machinery they possessed (Jacoby *et al.*, 2017).

The values of CD and CCD being at par at 3 WAA could be that the bacteria in CD were at their log phase. At the log phase, the population of the bacteria double at a constant rate and this leads to the number of cells and population rates doubling (Rolfe *et al.*, 2012). It was observed that CCD had the highest value for the bacteria

population at 3 WAA, which thereafter decreased at 4 WAA. It could be that the bacteria entered their stationary phase at 4 WAA. The stationary phase occurs due to the depletion of the essential nutrients, exhaustion of the external environment and other various stress factors (Pletnev *et al.*, 2015), which results to the growth rate of the bacteria being equal to their death rate.

The positive correlation that existed between pH and bacteria showed that pH has influence on bacteria. Soil pH has been known to affect the growth and increase of bacteria as well as fungi. According to Rousk *et al.*, (2009) increased bacterial growth and decreased fungal growth were affected by higher pH. The negative correlation between nitrogen and fungi could be because fungi are assumed to have lower N demands than bacteria (Keiblinger *et al.*, 2010). That means the lower the nitrogen, the better the growth of fungi. According to Paterson *et al.*, (2011) the decomposition of the nitrogen poor substrates are mostly done by fungi, while bacteria depend on the labile C sources. This may also be the reason why there was a positive correlation between the organic carbon and bacteria.

Conclusion

This work was aimed at ascertaining the effect forms of cow dung on soil microbes. Composted cow dung (CCD), normal cow dung (CD), cow dung ash (CDA), cow dung biochar (CDB) and a control (CTRL) were the treatments tested. Bacteria and fungi were the identified microbes with the population of bacterial being highest. All the cow dung forms increased the population of the identified soil microbes. Normal cow dung and composted cow dung, consistently resulted in higher population of soil cultivable bacteria and fungi, while cow dung biochar and cow dung ash showed lower influence on the soil microbes when compared with CD and CCD. The soil microbes increased to a peak and declined during the weeks after treatment application. Soil total nitrogen, available phosphorus and pH correlated positively with bacteria but negatively with fungi. From the collective results gotten from this investigation, converting cow dung into composted cow dung can be used as an environmentally friendly and rapid measure for restoring the health of the degraded soils of the smallholder women farmers' farm.

Authors' Declaration

We declare that this study is an original research by our research team and we agree to publish it in the journal.

REFERENCES

Bending GD, Turner MK, Jones JE (2002). Interactions between crop residue and soil organic matter quality and the functional diversity of soil microbial communities. *Soil Biol Biochem* 34: 1073–1082.

- Bremner JM (1965). Total Nitrogen In: C. A. Black, Ed., *Methods of Soil Analysis, Part 2, American Society of Agronomy*, Madison, pp. 1149–1178.
- Brookes P (2001). The soil microbial biomass: Concept, measurement and applications in soil ecosystem research. *Microb. Environ.* 16: 131–140.
- Canqui HB, Lal R (2009). Crop residue removal impacts on soil productivity and environmental quality. *Critical Reviews in Plant Sciences* 28(3):139-163.
- Chotte J (2005). Importance of microorganisms for soil aggregation. In: F. Buscot and A. Varma (Eds.), *Microorganisms in Soils: Roles in Genesis and Functions*. New York, New York: *Springer*. Pp. 107–122.
- Das S, Jeong ST, Kim PJ (2017). Composted cattle manure increases microbial activity and soil fertility more than composted swine manure in a submerged rice paddy. *Front. Microbiol.* 8: 1–10.
- Fortuna A (2012). The Soil Biota. *Nature Education Knowledge* 3(10):1
- Ge Y, Zhang JB, Zhang LM, Yang M, He JZ (2008). Long-term fertilization regimes affect bacterial community structure and diversity of an agricultural soil in northern China. *J. Soil Sediment* 8: 43–50.
- Hartmann M, Frey B, Mayer J, Mader P, Widmer F (2015). Distinct soil microbial diversity under long-term organic and conventional farming. *ISME J.* 9:1177–1194. doi: 10.1038/ismej.2014.210
- Hoorman JJ, Islam R (2010). Understanding Soil Microbes and Nutrient Recycling. *Ohioline* <https://ohioline.osu.edu> [Accessed on 27 March 2019].
- Jacoby R, Peukert M, Succurro A, Koprivova A, Kopriva S (2017). The Role of Soil Microorganisms in Plant Mineral Nutrition—Current Knowledge and Future Directions. *Front Plant Sci.* 8: 1617 doi: 10.3389/fpls.2017.01617
- Keiblinger KM, Hall EK, Wanek W, Szukics U Hämmerle I, Ellersdorfer G, Böck S, Strauss J, Sterflinger K, Richter A, Zechmeister-Boltenstern S (2010). The effect of resource quantity and resource stoichiometry on microbial carbon-use-efficiency. *FEMS Microbiol Ecol* 73: 430–440.
- Mclean EO (1982). Soil pH and lime requirement: In Payer, A.L., (editor), *Methods of soil Analysis*, 2nd ed. Agron Monogr Vol. 9. Madison, WI: ASA and SSSA. Pp. 149 – 157.
- McMahan K, Simard S (2018). *Soil Transplants as a Mine Reclamation Technique for Rehabilitating Soil Microbial Communities*. British Columbia Mine Reclamation Symposium. <https://www.researchgate.net/publication/331274576> [Accessed Mar 27 2019].
- Nelson DW, Sommers LE (1982). Total Carbon, Organic Carbon and Organic Matter. In: Page AL, editor. *Methods of soil analysis part 2*. 2nd ed. Agron Monogr, Vol. 9. Madison, WI: ASA and SSSA. Pp. 539-579.
- Onwuka MI, Nsofor HO (2011). The effect of kitchen residue ash, poultry manure and goat dung on soil properties and yield of cowpea (*Vigna unguiculata*) in a Typic Kandiodult of Southeast Nigeria. *Journal of Agriculture and Food Science. Vol 9 (1)* 12-21.
- Onwuka MI, Nwangwu BC (2016). Characterization of biochar produced from diverse feedstocks used as amendment on acidic Ultisols at Umudike, Abia State. *Nigeria Journal of Soil Science* ISSN 2201-4357 Volume 9 (2): 158-174.
- Pace JL (1999). Analytical approaches to the characterization of samples of microbial communities using patterns of potential C source utilization. *Soil Biol. Biochem.* 28: 213- 221.
- Paterson E, Sim A, Osborne SM, Murray PJ (2011). Long-term exclusion of plant-inputs to soil reduces the functional capacity of microbial communities to mineralise recalcitrant root-derived carbon sources. *Soil Biol. Biochem.* 43: 1873–1880.
- Pletnev P, Osterman I, Sergiev P, Bogdanov A, Dontsova O (2015). Survival guide: *Escherichia coli* in the stationary phase. *Acta Nat.* 7:22–33.
- Rolfe MD, Rice CJ, Lucchini S, Pin C, Thompson A, Cameron AD, Alston M, Stringer MF, Betts RP, Baranyi J, Peck MW, Hinton JCD (2012). Lag phase is a distinct growth phase that prepares bacteria for exponential growth and involves transient metal accumulation

- J. Bacteriol., 194: 686-701
- Rousk J, Brookes PC, Bååth E (2009). Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. *Appl. Environ. Microbiol.* 75: 1589–1596.
- Stark CH, Condon LM, O'Callaghan M, Stewart A, Di HJ (2008). Differences in soil enzyme activities, microbial community structure and short-term nitrogen mineralisation resulting from farm management history and organic matter amendments. *Soil Biol Biochem* 40: 1352–1363.
- Tayyab M, Islam W, Khalil F, Ziqin P, Caifang Z, Arafat Y, Hui L, Rizwan M, Ahmad K, Waheed S, Tarin MWK, Hua Z (2018). Biochar: An efficient way to manage low water availability in plants. *Appl. Ecol. Environ. Res.* 2018, 16, 2565–2583.
- Udo EJ, Ibia TO, Ogunwale JA, Ano AO, Esu IE (2009). Manual of Soil, Plant and Water analyses. Published by *Sibon books Ltd*, Lagos, Nigeria. Pp. 31-33.
- Zhen Z, Liu H, Wang N, Guo L, Meng L., Ding N, Wu G, Jiang G (2014). Effects of Manure Compost Application on Soil Microbial Community Diversity and Soil Microenvironments in a Temperate Cropland in China. *PLoS One.* 9(10): doi: 10.1371/journal.pone.0108555.