Assessment of the Short-term Effect of Different Fertilizers on Soil Microbial Population, Biomass Carbon and Microbial Diversity in Ginger (*Zingiber officinale*) Based Soil

Iwoh, E. F.[1,2], Onyegbula, O. D.[2], Ibok, I. U.[3] and Peter U. U.[4]

[1]*Department of Microbiology, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria*
[2]*National Root Crops Research Institute Umudike, P.M.B. 7006 Umuahia, Abia state, Nigeria*
[3]*Akwa Ibom State Polytechnic Ikot Osurua, Akwa Ibom State, Nigeria*
[4]*Department of Microbiology, Federal University of Lafia, Nigeria*

***Abstract:* - A field experiment was conducted to examine the short-term impacts of replacing inorganic by organic fertilizers on the soil microbial community and biomass carbon relevant for soil fertility and crop yield, using randomized complete block design; with Ginger (*Zingiber officinale*) as the test plant. Three types of fertilization regimes were compared as treatments: Nitrogen Phosphorous Potassium (NPK) fertilizer, Poultry manure (PM), Lime (LM) and non-fertilized (Control). Soil samples were collected from the surface (0-20 cm) soil for physicochemical properties, and microbial analysis before and after treatments at monthly intervals for 3 months. The data were subjected to Duncan new multiple range's test at $p \leq 0.05$. Cultural morphology and biochemical identification were carried out using standard microbiological techniques. Results obtained showed that the organically treated plot recorded the maximum microbial population counts (bacterial and fungal) and microbial biomass carbon, followed by the inorganically treated plot and control. A significant variation in bacterial population was found between control and treated plots (organic and inorganic) at $p \leq 0.05$. Organic plot also exhibited a significant variation in fungal population with the inorganically treated plot and control ($p \leq 0.05$). The application of organic fertilizers increased the organic carbon content of the soil and thereby increasing the microbial counts and microbial biomass carbon as well as microbial diversity trait. The use of inorganic fertilizers resulted in low organic carbon content, microbial counts and microbial biomass carbon of the soil, although it increased the soil's NPK level which could be explained by the rates of fertilizers being applied. Microbial isolates and percentage occurrence in the soil sample were; Staphylococcus aureus (100%), Bacillus cereus (100%), Escherichia coli (100%), Pseudomonas aeruginosa (86.7%), Streptococcus pyogenes (86.7%), Proteus mirabilis(53.3%), Klebsiella pneumoniae (100%), Enterobacter cloacae (53.3%), Rhizobium (60.0%), Clostridium sp (53.3%), Fusarium (53.3%), Clasdosporium (53.3%), Penicillium nalgiovense (60.0%) and Aspergillus niger (80.0%). A link between different fertilizers application and soil microbial components was tentatively established, but needs to be verified in further studies.**

***Keywords:* Fertilizer, ginger, microbial activity, biomass carbon, soil physicochemical, microorganisms, soil, yield.**

I. INTRODUCTION

Application of fertilizers is one of the most significant and essential practice in modern agricultural production and determination of soil quality and sustainable use (Nayak *et al.*, 2018). Application of organic and inorganic fertilizers enhances the soil nutrient availability of plants, besides improving the physical, chemical and biological properties (Lui *et al*., 2007; Yu *et al*., 2015). Fertilizer can directly stimulate the growth of microbial populations as a whole by supplying nutrients and may affect the composition of individual microbial communities in the soil. Extensive use of chemical fertilizers in relation to organic fertilizers has led to decrease in soil organic carbon and soil quality (Kumar *et al*., 2018). Soil microorganisms are important element to soil ecosystems and play a key role in the restoration and sustainability of ecosystem (Potthoff *et al*., 2006).Long term application of inorganic fertilizers affects soil quality and productivity, composition of microbial community and functional diversity. These changes are believed to have significant influences; it can be used to predict the effect of ecosystem perturbations by organic and conventional management practices, which might affect soil processes such as nitrogen fixation, nitrification and denitrification (Levy-booth *et al*., 2014). These soil microorganisms are critical to the maintenance of soil function and structure formation, decomposition of organic matter, toxin removal, and in various biogeochemical cycling of carbon, nitrogen, phosphorus, and sulphur for soil fertility and plant yields (Nannipieri *et al*., 2003). The extent of soil microbial diversity in agricultural soils makes it critical for the maintenance of soil health and quality, growth and yield of crops (Mele and Crowley, 2008). Soil microbial community structure and function are commonly used as indicators for soil quality and fertility (Islam *et al*., 2011). Inclusion of organic fertilizers reduces the harmful effects of chemical fertilizers and enhances the soil metabolic activities (Li *et al*., 2015). Organic applications increased nutrient status, microbial activity and productive potential of soil while the use of only chemical fertilizers in the cropping system resulted in a poor microbial activity and productive potential of soil (Kang *et al*., 2005). Therefore, the judicious and efficient use of mineral fertilizers (e.g. Nitrogen, Phosphorus, Potassium (NPK), Lime (LM) etc.) and organic fertilizers (Poultry Manure (PM), cow dung, etc.) can be practicable and adopted by farmers when the approach has considered some microbiological studies. This study was conducted with an aim to assess the short-term impact of organic and inorganic farming practices on soil microbial activity and yield of ginger on an ultisol of South Eastern Nigeria.

II. MATERIALS AND METHODS

Study site

The study was carried out on a loamy soil at the agricultural experimental field of National Root Crops Research Institute Umudike, Nigeria (longitude 07^0 33' E, latitude 05^0 29' N and altitude 122 M). Umudike is in the low-land humid tropics of south eastern Nigeria. The research plots were established in May 2017.

Experimental design

The field experiment was conducted in plots set-up as randomized complete block design (RCBD), consisting of organic and inorganic fertilizer treatments. According to the type of fertilizer treatments, each of the experimental plots was designated as PM, NPK, LM and control (without fertilizer). Replicates were maintained for each treatment. Each plot size measured $15m^2$ (3 m x 5 m) and the spacing between the plot was 1 m. The test plant used in the experiment was ginger *(Zingiber officinale)*. Treatments comprised: NPK fertilizer at 400 kg/ha, Poultry manure (PM) at 5 t/ha, Lime (LM) at 2t/ha, and control was also set up without the addition of any fertilizers. Ginger seed were planted at a spacing of 0.7 m between rows and 0.25 m within row, respectively.

Soil Sampling

Soil sampling was done in accordance with the method of Saeki and Toyota, (2004). Soil samples were collected aseptically from the plots from the top soil depth of 0-20 cm before treatment and after treatments at monthly intervals: 1^{st} month (before treatments application); 2^{nd} month (after plant emergence in May); 3^{rd} month (after flowering in June); and 4^{th} month (at the end of the growing season in August). Soil samples were collected at four randomly selected locations in each of the experimental plots and were mixed thoroughly to get a homogenous mixture. Composite soil samples of about 10 kg were collected from each plot and stored in a sterile polypropylene bag and kept cool using coolers during field sampling for microbiological analysis; and the remaining were air dried and sieved twice using meshes (2.0 and 0.2 mm) for determinations of physicochemical properties of the soil. Samples were subsequently processed within 24 - 48 hour.

Physicochemical properties

Soil temperature was recorded using soil thermometer at the time of sample collection. Soil pH was taken using an electronic digital pH meter in 1:5 soil-water suspensions. The moisture content of the soil samples were determined gravimetrically by weighing, drying in hot air oven at 105°C for 24 h and then reweighing. Organic carbon (C) was determined by the method of Anderson and Ingram (1993) Total nitrogen (N), available phosphorus (P) and exchangeable potassium (K) were determined by Kjeldahl distillation (Jackson, 1973)

Enumeration of microbial population

Isolation and estimation of microbial populations, (bacteria and fungi) were carried out using nutrient agar media and Sabouraud dextrose agar (SDA) media for bacteria and fungi, respectively. Media were prepared according to the composition and sterilized in autoclave. One gram (1g) of each of the soil samples was weighed and agitated in 9 ml of distilled water, to dislodge the organisms from the soil particles. An aliquot of 1 ml was serially transferred from each sample into series of test tubes containing sterilized distilled water to obtain dilution of 10^{-1} and 10^{-10}. The microbial population was enumerated, which involved determination and count of total viable microorganisms in the experiment using the method of dilution on specific solid media spread plate technique series of 10^{-}fold serial dilution. Aliquot (0.1ml) of 10^{-2}, 10^{-4}, 10^{-6} and 10^{-8} was inoculated into nutrient agar plated in triplicates (Chikere *et al*., 2009). Total soil fungi were enumerated by inoculating aliquots (0.1ml) and determined using Sabouraud dextrose agar (SDA) supplemented with streptomycin (1 mg/100 ml) to suppress bacterial growth with the diluted soil samples of 10^{-3}. The inoculated plates were all incubated at temperature 30^0 C for 24 hours (bacteria) and 96 hours (fungi), respectively. The resulting colonies were counted and recorded as colony forming units per gram (CFU/g) using colony counter (Onifade *et al*., 2007). The counts were characterized based on cultural characteristics, staining reaction and biochemical tests.

Microbial biomass carbon

Soil microbial biomass carbon (MBC) was determined using the chloroform fumigation extraction method according to Anderson and Ingram (1993).

Statistical analysis

Analysis of Variance (ANOVA) for soil microbial population and physico-chemical properties were analysed using Statistica version 6, Duncan new multiple range test (DNMRT) at 5% probability level performed for comparison of treatment means.

III. RESULTS AND DISCUSSION

Physicochemical properties of soil

Results of the soil physicochemical properties of the experimented site showed that the highest organic carbon content was recorded in organically treated plot and the least in control throughout the sampling period, with a significant variation, ($p \leq 0.05$); (Table1). The changes in these properties were associated with the organic and inorganic fertilizer management practices at each plot. Poultry manure plot showed an increase in organic carbon content compared to other plots, this might be due to the addition of organic amendments as they are the sources of nitrogen and carbon to soils. This agrees with the findings of Kumar *et al*., (2000)that organic materials applied alone or in combination with inorganic fertilizer gave greater residual soil fertility in terms of increase in organic carbon content from 0.36 to as high as 0.61% and the available N, P and K in the 2-years cropping cycle. Also, Kang *et al*., (2005) showed that application of organic manures significantly increased the soil organic carbon content whereas, chemical fertilizers had no effect, and the increase in soil organic carbon content depended on both organic inputs as well as high crop yield.

Inorganically treated plot amended with Nitrogen Phosphorus Potassium (NPK) fertilizer at 400 kg/ha showed highest content of total nitrogen, available phosphorus and exchangeable potassium, followed by the organically treated plot amended with Poultry manure (PM) at 5 t/ha, and Lime (LM) at 2t/ha; and least in control during the sampling, with significant variation between organically and the inorganically treated plot ($p \leq 0.0$), as shown in Table 1. Nitrogen content was higher in plot amended with inorganic fertilizer compared to organic and control plots. This may be due to the addition of NPK fertilizer (400 kg/ha) which is destined for use as nitrogen fertilizer, whereas the lower value of total nitrogen in organic plots could be as a result of crop uptake, immobilization by microorganisms and nitrogen loss through volatilization. Available P was higher in the inorganically treated plot compared to organic. The increase in available P in the inorganic plot could be due to the addition of NPK fertilizer which comprises of nitrogen phosphorus and potassium. Exchangeable K was also found to be higher in inorganic (NPK) fertilizer treated soil.

Table 1: Soil physicochemical properties of the soil samples on treatments application

Parameters	NPK	Soil Samples PM	LM	Control
Soil pH	5.35 ± 0.12^{e}	5.12 ± 0.16^{a}	5.38 ± 0.18^{b}	5.55 ± 0.26^{bc}
Moisture content (%)	43.34 ± 0.13^{d}	44.36 ± 33^{c}	46.83 ± 0.93^{b}	47.87 ± 0.73^{a}
Organic Carbon (%)	2.16 ± 0.03^{b}	2.59 ± 0.09^{a}	1.88 ± 0.08^{c}	1.84 ± 0.09^{c}
Organic Matter (%)	2.81 ± 0.05^{b}	2.83 ± 0.09^{a}	2.49 ± 0.03^{d}	2.68 ± 0.09^{c}
Total Nitrogen N (%)	0.68 ± 0.04^{a}	0.58 ± 0.09^{b}	0.45 ± 0.08^{c}	0.34 ± 0.07^{d}
Available Phosphorus P(Mg/kg)	18.45 ± 0.09^{a}	16.90 ± 0.13^{b}	13.59 ± 0.09^{d}	13.13 ± 0.95^{c}
Exchangeable PotassiumK (Cmol/kg)	0.29 ± 0.29^{a}	0.18 ± 0.63^{b}	0.02 ± 0.03^{c}	0.01 ± 0.81^{d}

Values show mean of triplicate analysis ± standard deviation figure with superscripts in the row which shows significantly different according to Duncan new multiple range's test at $P \leq 0.05$

Table 2: Effect of different fertilizer application on soil bacterial mean count (CFU/g) and root yield of ginger in 2017 cropping season

	Sampling period			
Treatments	**1st month**	**2nd month**	**3rd month**	**Root yield (t/ha)**
PM	117.43 x10^{6}	112.43 x10^{6}	98.00 x10^{6}	12.8
NPK	88.63 x10^{6}	77.43 x10^{6}	72.00 x10^{6}	9.1
LM	52.33 x10^{6}	56.66 x10^{6}	63.66 x10^{6}	5.6
NPK+PM+LM	102.66 x10^{6}	106.00 x10^{6}	95.33 x10^{6}	10.8
Control	68.70x10^{6}	68.70x10^{6}	68.70x10^{6}	5.6

Values show mean of triplicates analysis ± standard deviation. Figure with different superscripts down the column were significantly different according to Duncan new multiple range test at $P \leq 0.05$.

Table 3: Effect of different fertilizers application on soil fungal mean count (CFU/g) and root yield of ginger in 2017 cropping season

	Sampling period			
Treatments	**1st month**	**2nd month**	**3rd month**	**Root yield (t/ha)**
PM	16.33 x10^2	9.66 x10^2	8.66 x10^2	12.8
NPK	10.00 x10^2	12.66 x10^2	10.33x10^2	9.1
LM	5.66x10^2	4.66x10^2	4.66x10^2	5.6
NPK+PM+LM	8.33x10^2	11.00x10^2	10.66x10^2	10.8
Control	5.33x10^2	5.33 x10^2	5.33 x10^2	5.6

Values show mean of triplicates analysis ± standard deviation. Figure with different superscripts down the column were significantly different according to Duncan new multiple range test at P≤0.05.

Table 4: Microbial isolates from the Soil sample and their percentage occurrence (CFU/g)

Isolates	Number of Isolates	% occurrence
Bacillus cereus	15	100%
Staphylococcus aureus	15	100%
Escherichia coli	15	100%
Klebsiella sp	15	100%
Pseudomonas aeruginosa	13	86.7%
Streptococcus pyogenes	13	86.7%
Aspergillus niger	12	80.0%
Enterobacter cloacae	11	73.3%
Penicillium nalgiovense	9	60.0%
Rhozobium sp	9	60.0%
Clostridium sp	8	53.3%
Proteus mirabilis	8	53.3%
Fusarium	8	53.3%
Clasdosporium sp	6	40.0%

Microbial biomass carbon

Microbial biomass carbon in the organically (PM) treated plot was significantly higher than inorganically treated as well as control plots. The highest microbial biomass carbon was observed in a plot amended with poultry manure (5 t/ha) followed by the inorganically treated plot amended with nitrogen phosphorus potassium (400 kg/ha), lime (2 t/ha) and the least in control plot. A significant variation in microbial biomass carbon was observed between organically and inorganically treated plots at p≤0.05. Increased microbial biomass carbon content recorded in the organically treated plot might be due to suitable soil pH and environmental conditions for microbial growth, which acted as a good substratum for microbial activity and cycling of organic matter and nutrients in the soil. This finding might be attributable to the competition of carbon and nitrogen source between soil microorganisms and crops.

Microbial Analysis of the soil samples

Soil microbial population increased in organically amended plots compared to inorganic plots as well as control (untreated) plot. This increase may be attributed to addition of organic amendments that have large impact on the size and activity of microbial population. Organic amendment with reduced chemical fertilizer will promote soil microbial development and nutrient availability, and improved morphological growth of plant. The bacterial population was at maximum in a plot amended with poultry manure (PM) and at minimum in control plot. A significant variation in bacterial population was found between control plot and treated plots (organic and inorganic) p≤0.05; (Table 2). This increase in poultry manure (PM) amended plot might be as a result of suitable conditions which acted as a good substratum for microbial activity. Poultry manure (PM) also provided organic substrates that proliferated bacterial population and activities in the soil, as they breakdown soil organic matter in the soil, with the highest bacterial count recorded in the 2nd month of sampling compared to 1st month and 3rd month. This study is in line with findings by(Shubha, *et al*., 2014; Devi *et al*., 2012), who reported poultry manure and vermicompost are good sources of nutrients and might provide more substrates for utilization by microorganisms due to its maximum metabolic activity. Other researchers have also shown that incorporation of organic amendments increased soil microbial activity, microbial diversity and densities of bacteria (Girvan, *et al*., 2004). Poultry manure promoted biological and microbial activities, which accelerated the breakdown of organic substances in the soil with evidence in relatively high carbon content and enzyme activities. Further increase was recorded with plot amended with NPK fertilizer, which might be attributed to the significant roles that nitrogen and phosphorus play in symbiotic and mycorrhizal relationships with soil microorganisms. The effects of chemical fertilizer on soil biological activity have been reported (Emtsev *et al*., 2010). Lowest in bacterial population was recorded from plots amended with lime; this may be attributed to the modification of the soil rhizosphere to an alkaline condition by the lime applied, which might be antagonistic to bacterial growth.

Fungal population recorded under different treatments also exhibited similar trend with the maximum value recorded in the organic (poultry manure) amended plot. PM promoted fungal growth in the soil with a significant variation recorded in organically and inorganically treated plot as well as control plot, respectively at $p \leq 0.05$ (Table 3). The decrease in fungal population value noticed in the inorganic (NPK) plot might be as a result of supply in organic carbon content for microbial growth and utilization, since inorganic fertilizers do not directly supply carbon, thereby causing a drop in microbial population, microbial biomass carbon and ginger yield. Lowest values of fungal count were recorded on plots that received LM treatment at all sampling periods; LM inhibits fungal growth in the soil. The mean values of fungal numbers per gram of soil were observed across the 3 sampling periods.

Results from Tables 2&3, showed that soil microbial population increased in organically amended plots compared to inorganic and control plots, which may be due to the addition of organic amendments that might have large impact on the size and activity of microbial population. This finding is in line with that of Mader *et al*., (2002), who reported that soils under organic farming had enhanced microbial functional diversity in comparison with the soils of conventional farming. In general, the bacterial population was higher than the fungal population throughout the sampling period. Parham *et al*., (2003) reported that cattle manure application promoted the growth of bacteria, but not fungi when compared with the control soil. Studies showed that increased soil pH in the acidic range caused a shift towards dominance of the bacterial community, while fungal communities were unaffected (Pennanen, 2001). Li *et al*., (2007) reported that addition of animal or green manures on organic farms provided a significantly greater input of organic carbon, which increased bacterial populations, while the use of excessive chemical fertilizers reduced microbial functional diversity and enzymatic activity in the soil.

Microbial diversity recorded (Table 4) indicates that total heterotrophic bacteria, total heterotrophic fungi and nitrogen fixing bacteria were isolated from the experimental soil. The relative abundance of Gram-negative and positive bacteria recorded was highest in PM amended plot, compared to NPK and LM plots; suggesting that soil microbial growth might be greatly stimulated by the application of organic manure but reduced by the application of mineral NPK fertilizer. Kourtev *et al*., (2003) found that the ratio of Gram-negative and Gram-positive bacteria was related to quality of organic matter and its content in the soil and this might reflect the loss of easily decomposable materials. Changes in nutrient composition have the potential to directly or indirectly affect the microbial community in the soil, stimulate the development of certain group of microorganisms, and increase microbial count and microbial diversity. The present investigation showed that organic fertilizer applied had great impact on soil microbial community, activity and physicochemical properties, and these would help provide a better understanding of the importance of organic and inorganic fertilizers in promoting the soil microbial biomass, activity and diversity and thus enhancing crop growth and production.

IV. CONCLUSION

This study has shown that partial replacement of inorganic fertilizers by organic manure had a significant short-term impact on soil physicochemical properties and soil microbial community structure, as well as ginger yield. Beneficial effects occurred even when the organic amendments were only a small portion of the total amounts supplied to the soil throughout the 3 months, suggesting that a shift to more sustainable production systems could significantly improve soil fertility while maintaining crop yield at all levels comparable to inorganically fertilized soil. The combination of modern microbiological techniques with the knowledge of soil ecological processes would provide a unique opportunity to study and understand the impact of these chemical fertilizers on microbial community, agronomic practices and other edaphic factors. Poultry manure can be successfully used in the integrated ginger production system to promote biological and microbial activities, optimize fertilizer management and to maximize crop yields while reducing environmental impacts. Future research should be directed towards increasing and understanding the Long-term negative impacts of inorganic fertilizers on soil microbial processes, enzymatic activity and nutrient cycling among others, in order to increase crop yields under sustainable production systems.

REFERENCES

[1] Anderson, J. M. and Ingram, J. S. (1993). Tropical Soil Biology and Fertility: A Handbook of Methods. 2nd Edn., CAB. International, Oxfordshire, Wallingford, UK., ISBN: 0-85198-821-0.

[2] Chikere, C.B., Okpokwasili G.C and Ichiakor O. (2009). Characterization of hydrocarbon utilizing bacteria in tropical marine sediments. *AfricanJournal of Biotechnology* 8(11): 2541-2544.

[3] Devi, S., Sharma, C. R and Singh, K.. (2012). Microbiological biodiversity in poultry and paddy straw wastes in composting systems. *Braz . J. Microbiol*. 43: pp. 288-296

[4] Emtsev, VT, Sokolova A. Y., Selitskayam, O.V. (2010). Protective effect of *Klebsiella* bacteria on lawn grasses under conditions of soil salinization. Eurasian Soil Sci. 43(7): 771-776.

[5] Girvan, M.S., Bullimore, J., Ball, A.S., Pretty, J.N and Osborn, A.M. (2004). Response of active bacterial and fungal communities in soil under winter wheat to different fertilizer and pesticide regiments. Applied Environ. Microbiol., 70: 2692-2701.

[6] Islam, M. R., Chauhan, P.S., Kim, Y., Kim, M and Sa, T. (2011). Community level functional diversityand enzyme activitiesin paddy soil under different long-term fertilizer managementpractices. Biol. Fert. Soils. 47:599-604.

[7] Jackson, M. L. (1973). Soil Chemical Analysis. 1st Edn., Prentice Hall Ltd., New Delhi, India, Pages: 498.

[8] Kang, G.S., V. Beri, O.P. Rupela and Sidhu, B.S. (2005). A new index to assess soil quality and sustainability of wheat based cropping systems. Biol. Fertil. Soils, 41: 389-398.

[9] Kourtev, P., Ehrenfeld, J and Häggblom, M. (2003). Experimental analysis of the effect of exotic and native plant species on the structure and function of soil microbial communities. *Journal of Soil Biology and Biochemistry*, 35:895–905.

[10] Kumar, U., Nayak, A.K., Shahid, M., Gupta, V.V.S.R., Panneerselvan, P., Mohanty, S., Kaviraj, M.,Kumar, A., Chatterjee, D., Lal, B., Gautam, P., Tripathi, R and Panda, B.B. (2018). Continuous application of inorganic and organic fertilizers over years in paddy soil alters the the bacterial community structure and its influence on rice production. Agric. Ecosyst. Environ. 22: pp 5-75.

[11] Kumar, V., Ghosh, B.C. and Bhat, R. (2000). Complementary effect of crop wastes and inorganic fertilizers on yield, nutrient uptake and residual fertility in mustard (*Brassica juncea*)-rice (*Oryza sativa*) cropping sequence. *Indian J. Agric. Sci*., 70: 69-72.

[12] Levy-booth, D. J., Prescott, C. E and Grayston, S. J. (2014). Microbial functional genes involved in nitrogen fixation, nitrification and denitrification in forest ecosystems. Soil Biol. Biochem. 75: pp 11-25.

[13] Li, J., Yang-ting, L., Xiang-dong, Y., Jian-jun, Z., Zhian, L and Zhao, B. Q. (2015). Microbial community structure and functional diversity are associated with organic carbon availiability in an agricultural soil. *J. Int. Agric.* Pp 61229-61231

[14] Li, Z. P., Wu, X. C and Chen, B. Y. (2007). Changes in transformation of soil organic C and functional diversity of microbial community under different land uses. Agr. Sci. china. 6:pp 1235-1245.

[15] Liu, B., Gumpertz, M. L., Hu,S and Ristaino, J.B. (2007). Long term effects of organic and synthetic soil fertility amendments on soil microbial communities and development of southern blight. Soil Boil. Biochem. 39: pp. 2302-2316.

[16] Mader, P., Fliebbach, A., Dubois, D., Gunst, L., Fried, P. and Niggli, U. (2002). Soil fertility and biodiversity in organic farming. Science, 296: 1694-1697.

[17] Mele, P.M. and Crowley, D. E. (2008). Application of self-organizing maps for assessing soil biological quality. *Agric Ecosyst Environ* 126:139–152.

[18] Nannipieri, P., Ascher, J., Ceccherin,I M.T., Landi, L., Pietramellara, G and Renella, G. (2003). Microbial diversity and soil functions. *Eur J Soil Sci* 54:655–670.

[19] Nayak, P. K., Nayak, A. K.., Panda, B.B., Lal, B., Gautam, P., Poonam, A., Shahid, M., Tripathi, R., Kumar, U., Mohapatra, S.D and Jambhulkar, N.N. (2018). Ecological mechanisms and diversity in rice based integrated farming system. Ecol. Indic., 91: pp. 359-375.

[20] Onifade, A. K., Abubakar, F. A. and Ekundayo, F. O. (2007). Bioremediation of crude oil polluted soil in Niger Delta arear of Nigeria using enhanced natural attenuation. *Research Journal of Applied Sciences*. 2:498-504

[21] Parham, J. A., Deng, S. P., Da, H. N., Sun, H.Y and Raun, W.R. (2003). Long-term cattle manure application in soil. II. Effect on soil microbial populations and community structure. Biol. Fertil. Soils, 38: 209-215.

[22] Pennanen, T. (2001). Microbial communities in boreal coniferous forest humus exposed to heavy metals and changes in soil pH- a summary of the use of phospholipid fatty acids biolog and ^{3}H-thymidine incorporation methods in field studies. Geoderma, 100: 91-126

[23] Potthof, M., Steenwerth, K. L., Jackson, L. E., Drenovsky, R. E., Scow, K. M and Joergensen, R. G. (2006). Soil microbial community compositionas affected by restoration practices in california grassland. Soil Boil. Biochem. 38: pp 1851-1860.

[24] Saeki, M and Toyota, K. (2004). Effect of bensulfuron-methyl on the soil bacterial community of a paddy soil microcosm, Biol. Fertile.soils. (40) 110-118.

[25] Shubha, S., Devakumar, N., Rao, G.G.E., Gowda, S.B., Rahmann, G., A and Aksoy, U. (2014). Effect of seed treatment, panchagavya application and organic farming systems on soil microbial population, growth and yield of maize. Proceedings of the 4th ISOFAR Scientific conference, ISOFAR, Instanbul. pp 631.

[26] Yu, C., Hu, W., Deng, W.,Li, Y., Xiaong, C., Ye, C., Han, G. M and Li, X. (2015). Changes in soil microbial community structure and functional diversity in the rhizosphere surrounding mulberry subjected to long- term fertilization. Appl. Soil Ecol. 8:pp 30-40.