Soil Microbial Population and Activity Affected by Fertilizer and Manure Addition in a Coconut Growing Sandy Regosol

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ABSTRACT

Increasing nutrient inputs into terrestrial cropping system has been a major contributor in enhancing the productivity of agricultural systems. While fertilizers play a key role in yield enhancement they have an impact on below ground microbial community. A pot experiment was conducted to evaluate the effect of applying inorganic fertilizer alone and organic manure supplemented with inorganic fertilizers on soil microbial population and activity in a coconut growing Sandy Regosol soil. Treatments were, T_1-No fertilizer (Control), T_2-Inorganic Fertilizer (Urea, Eppawala Rock Phosphate, Muriate of Potash (MOP), and Dolomite) T_3- Poultry manure supplemented with MOP-, T_4- Poultry manure supplemented with Sulfate of Potash (SOP) and T_5- ½ dose of poultry manure plus ½ fertilizer as in T_2. Fertilizers and manures were applied based on the current coconut fertilizer recommendation rates of adult palms. Pots were maintained at 60% water holding capacity. Soil samples were collected on the 7th, 14th, 28th, and 57th days after treatment application and analyzed for soil pH, organic carbon, soil bacterial and fungal population and basal respiration. The initial soil pH of 4.84 increased to a range of 5.99 - 6.66 after the fertilization. The addition of organic manure as full dosage nearly doubled the initial soil organic carbon and thus has positively impacted on soil microbial population and basal respiration. However, addition of SOP has a negative impact on microbial population in comparison to manure added with MOP treatment. The inorganic fertilizer application did not affect the soil microbial population, however have affected the microbial activity two weeks after the application. The findings of this study provide an insight on the effects of fertilization on soil microbial populations in the studied soil and directions for future research.

Keywords: *Basal Respiration; Coconut; Organic Manure; Inorganic Fertilizers; Soil Bacterial and Fungal Population*

INTRODUCTION

External supply of nutrients is an essential practice in the present intensive agricultural systems. Inorganic fertilizer application is preferred by the growers for its convenient use and quick response. With the prolonged application of inorganic fertilizers, soils have lost half or more than its original levels of soil organic matter (Matson *et al.*, 1998). Consequently, the soil microbial community which depends on C for their nutrient and energy source (Fontaine *et al.*, 2003) is adversely affected, wiping the living organisms from the productive soil ecosystem. Soil microbial processes are crucial for the plant nutrient supply, mainly through their role on soil organic matter decomposition and nutrient dynamics (Paul, 2007).

Sustainable agricultural systems would ideally produce good crop yield without a cost to the environment (Tilman, 1999). Management of soil fertility through organic fertilizers is a promising practice in sustainable agricultural systems. According to the 2009 statistics reported by International Federation of Organic Agriculture Movements (IFOAM) – Organics International on world organic agriculture, coconut has been listed among the key crops which are been organically cultivated with an extent of at least 28,000 hectares worldwide (Willer and Kilcher, 2009).

Sustainable soil fertility management either via organic agriculture system alone or organic plus inorganic fertilization systems requires large quantity of organic fertilizers to supply the plant nutrient requirement. Yet the unavailability of organic fertilizers in adequate quantity to supply the entire plant nutrient requirement is a challenge, especially, for a crop like coconut which has a higher demand for potassium. Because of this reason, Coconut Research Institute (CRI) recommends application of organic nutrient sources such as cattle manure, poultry manure and goat manure together with Muriate of Potash (MOP) for conventional farming systems or Sulphate of Potash (SOP) for certified organic coconut farming systems to supply the deficit amount of potassium. With the same understanding, Philippine Coconut Authority- Davao Research Center also recommends combined applications of organic and inorganic fertilizers in orde to obtain consistent positive response on growth nd yield of coconut (Mantiquilla *et al.*, 1994).

Organic fertilizers such as cattle, goat and pou try manures and compost are applied to the soil for sustaining fertility through improvement in ove all physical, chemical and biological properties of soil (Tennakoon *et al.*, 1995). It has shown that oil microbial population and activity are significar ly favored by organic fertilizer applications (Ma er *et al.*, 2002; Leita *et al.*, 1999). Also, Lazca no *et al.* (2013) observed improvement in microbial properties with integrated fertilizer (inorganic plus organic) management than only inorganic fertilizer treatment in short term.

However, the effects of applying inorganic fertilizers and organic fertilizers in combination with MOP or SOP on soil microbial population and activity under coconut cultivation in Sri Lanka are less explored. Therefore, aim of this study was to evaluate the effect of applying inorganic fertilizer alone and organic manure supplemented with inorganic fertilizers on soil microbial population and activity in Sandy Regosol, a common coconut growing soil in Sri Lanka.

METERIALS AND METHODS

Soil and experimental setup

The study was conducted as a pot experiment under greenhouse environment. The soil used in the pots was collected from the top soil (0-30 cm) at three random locations of the center squares of mono cropped coconut plantation in the Low country- Intermediate zone of Sri Lanka. The soil was a sandy textured, Madampe series soil which belongs to the Great Soil Group of Sandy Regosol (Aquic Quartzipsamments) soils

Before filling out the pots, soil was mixed thoroughly and passed through 6 mm sieve. The initial soil pH, organic carbon content, total populations of fungi and bacteria and basal soil respiration were determined. Prior to treatment application, experimental pots (34 cm diameter and 26 cm height) were filled with 18 kg of soil and wetted to achieve, approximately sixty percent of the water holding capacity of the soil, which was previously determined by moistening a soil column and allowing to drain freely. The experimental setup was laid with five treatments: T_1- no fertilizer (Control), T_2-inorganic fertilizer (6.26 g Urea, 7.04 g ERP, 12.52 g MOP, 7.83 g Dolomite), T_3- manure supplemented with MOP (142.43 g Poultry manure - 5.78 g MOP), T_4- manure supplemented with SOP (143.43 g Poultry manure - 6.9 g SOP) and T_5- ½ dose of manure plus ½ dose of inorganic fertilizer (71.21 g Poultry manure, 3.13 g Urea, 3.52 g ERP, 9.15 g MOP, 3.91 g Dolomite) replicated thrice in a Complete Randomized Design (CRD) under natural illumination in green house condition.

The treatment combinations were based on the CRI fertilizer recommendation for adult palms in wet and intermediate zone of Sri Lanka (CRISL, 2016). The reason for using poultry manure is, its availability and higher nutrient content (Tennakoon and Bandara, 2003) and it is also widely used as manure in coconut plantations. Fertilizer and manure application rates were determined based on the nutrient composition of fertilizers and manures used (Table 1) to match the current fertilizer recommendation considering the mass of the soil used for pots proportionate to the soil mass of manure circle. The treatments were applied on the surface of the soil of pots and were well mixed up to a depth 15cm. The pots were maintained at 60 % of the WHC by adding water periodically.

Soil sampling and processing

Soil samples were collected on the 7, 14, 28, and 57 days after treatment application from a 0-15 cm depth in each plot. Soil samples were collected from 4-5 borings across the pot and were composited to represent each pot. Each soil sample was separated into two parts. One part was air dried, passed through a 2-mm sieve and stored at room temperature for determining soil chemical properties. The other part was immediately stored at 4°C for the measurement of soil microbial properties.

Soil chemical analysis

The pH of the soils was measured in a 1:5 soil/water suspension and Soil Organic Carbon (SOC) content was measured in finely ground soil samples by dichromate oxidation method (Walkley and Black, 1934).

Table 1. Mean pH and nutrient contents of poultry manure and different fertilizers on dry weight basis (Mean ± Standard Deviation, n=3)

Parameters	Poultry Manure	Urea	Eppawala Rock Phosphate	MOP	SOP	Dolomite
pH (1:5 v/v)	8.48 ± 0.01	-	-	-	-	-
N %	2.16 ± 0.05	47 ± 1	-	-	-	-
P_2O_5 %	2.34 ± 0.01	-	30 ± 1.5	-	-	-
K_2O %	3.03 ± 0.5	-	-	61 ± 2.7	51 ± 1.3	-
MgO %	2.83 ± 0.04	-	-	-	-	19.7 ± 1.5

Microbiological analysis

Dilution plate method was used to measure the population of culturable bacteria and fungi. Total culturable bacteria and fungi were enumerated by plating of soil suspensions (100µl) on 0.3 % Tryptic Soy Broth (TSB) agar and Rose Bengal –Streptomycin Agar plates respectively. The spread plates were incubated at 30°C for 2 days for bacteria and 5 days for fungi. The colony forming units (CFU) of total bacteria and fungi are expressed per gram dry soil (CFU g^{-1} dry soil).

Basal soil respiration was determined using sealed jar incubation method, which employed a trap of 0.5 M NaOH alkali to trap the respired CO_2 (Anderson, 1982). After one week of incubation period, the lid was opened, the alkali trap was removed, and the solution was back-titrated with 0.1 M HCl in order to assess CO_2 released from the soil.

Statistical analysis

Statistical analyses were carried out using the R statistical software. One-way ANOVA at the 0.05 level was used for analysis of significant difference among treatments at each sampling time. Mean separation was done using Fishers Least Significant Difference (LSD) test to separate the means.

RESULTS AND DISCUSSION

Chemical properties of soil

The initial properties of soil used for the pot experiment are given in Table 2. The soil is very low in soil organic carbon content and slightly acidic (Table 2). Loganathan *et al.* (1984) have also reported similar levels of soil soil organic carbon (0.15-0.22 %) and pH (4.9-5.3) for Sandy Regosol soils of Madampe Series which developed from old marine sands in the coast.

Table 2. Initial Properties of the soil used for pot experiment (Mean ± Standard Deviation, n=3)

Parameters	Value
pH	4.84 ± 0.01
Organic Carbon	0.18 ± 0.01 %
Total Fungi	0.18 ± 0.04 $\times 10^4$ CFU g^{-1} dry soil
Total Bacteria	0.33 ± 0.17 $\times 10^6$ CFU g^{-1} dry soil
Basal Soil Respiration	13.69 ± 2.51 µg CO_2 g^{-1} dry soil day^{-1}

The manure treatments were added to the pot soil taking in account of the nutrient content of the manure used, by balancing the nitrogen requirement of coconut. As coconut is a high potassium demanding crop, deficit potassium was supplied by either MOP or SOP. As a result, organic manure added treatments received surplus of organic carbon, relative to the inorganic fertilizer treated and control pots. The organic carbon content of the treatments with full dosage of manure with MOP and SOP almost doubled from the initial carbon content of 0.24 %. This is in agreement with the finding of Kumar *et al.* (2000) where the initial OC % of 0.36 increased as high as 0.61 % in organic manure treated soil with crop sequence of mustered (*Brassica juncea*)-rice (*Oryza stativa*).

Table 3. Soil organic carbon content and pH of soils one week after the treatment application. (Mean ± Standard Deviation, n=3)

Treatment	Soil Organic Carbon (%)	pH
Control	0.24 ± 0.07^{c}	4.98 ±0.04^{b}
Inorganic Fertilizer	0.30 ± 0.02^{bc}	6.34 ±0.11^{a}
Manure/MOP	0.57 ± 0.10^{a}	6.66 ±0.14^{a}
Manure/SOP	0.46 ± 0.17^{ab}	5.99 ±0.09^{a}
½ Manure + ½ Fertilizer	0.35 ± 0.13^{bc}	6.41 ±0.31^{a}

Mean±SD in columns followed by the same letter do not significantly differ according to LSD at $p \leq 0.05$

The initial soil pH of 4.84 (Table 2) increased to a range of 5.99 - 6.66 after one week of treatment application (Table 3) in manure/fertilizer treated soil. Soil pH of the control pot showed significantly low values compared with other treatments. The one unit increase of initial soil pH is accounted to the application of dolomite in inorganic fertilizer treated soils. Similar effect has been observed in soil pH of manure treated soils as the pH of the applied poultry manure was 8.48. Change of soil pH towards a neutral pH level favors biological process and availability of most of the nutrients in soil.

Microbial population

Soil bacterial and fungal population changes after the application of treatments during the study period is shown in Figure 1a and 1b. Soil bacterial and fungal populations of the Manure-MOP treated soils have shown a rapid increase following the application of treatments while the population of control remains significantly low throughout. It is observed that the Manure-MOP treatment has boosted the bacterial population by 18 fold and the fungal population by 30 fold, 7 days after the treatment application (DAT). On the 14th DAT the bacterial population further increases (Figure 1a) and later declined rapidly to the level of control soil. Lundquist *et al.* (1999) has reported that the microbial biomass carbon rapidly increase by the first week of incorporation of organic substance and later continuously drop to a level of pre-incorporation within 4 weeks of time. This is in agreement with the bacterial population changes observed in the Manure-MOP treatment over the period of time in this study. Once the heterotrophic bacterial population receives organic carbon as high as double the amount of initial soil OC (Table 3) the population rapidly boosts up by utilizing the available source of carbon, and then later subsides when the labile reserves deplete.

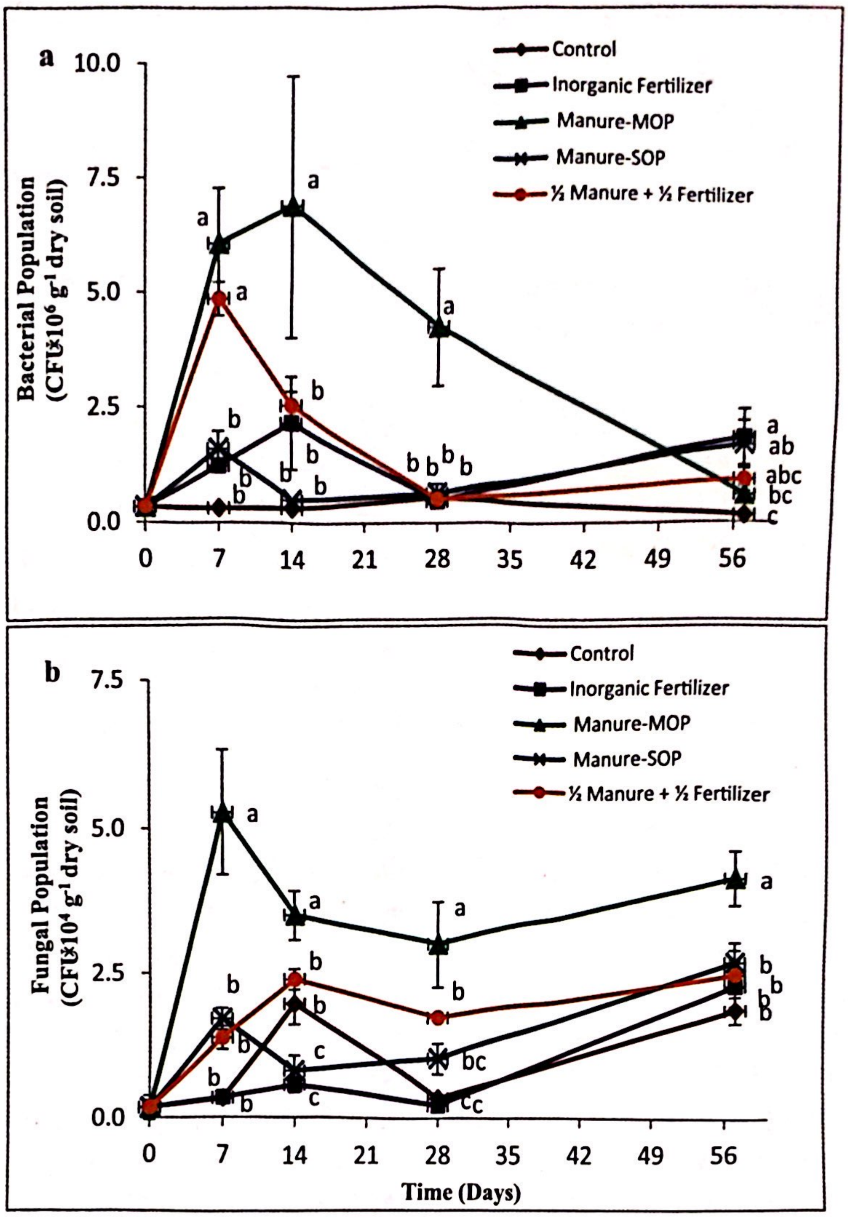

Figure 1. Changes in soil Bacterial (a) and Fungal (b) population after the application of treatments to the soil, during the 57 days of pot experiment. Error bars ± one standard error, n=3. Different letters at each sampling time (days) indicate significantly different means at $p \leq 0.05$.

Contrasting to the rapid changes observed in bacterial population in Manure-MOP treatment within the study duration, the fungal population declined slightly on the 14th DAT but continued to remain significantly high throughout the 57 days of the study period (Figure 1b). Fungal populations of agricultural soils have been positively impacted by organic amendments (Swer *et al.*, 2011; Nakhro and Dkhar, 2010).

The Manure-MOP treated soil and the ½ manure plus ½ fertilizer treated soil showed the significantly highest bacterial population on the 7th DAT (Figure 1a), but those were not significantly different at $p \leq 0.05$, irrespective of the manure amount received. However, at 14 DAT there was a significant difference between bacterial abundance in Manure-MOP treated soil and the ½ manure plus ½ fertilizer treated soil and this prevailed until 28 DAT.

When consider about fungal population, throughout the study period, the fungal population of the ½ dose manure treatment remained significantly lower compared to manure-MOP treatment (Figure 1b). After 14 DAT, the fungal population follows the same trend as in the Manure-MOP treatment while having half the population size. Similar behavior of bacterial and fungal population in the presence of organic manure has been reported previously (Lazcano *et al.*, 2013). They have found that bacterial biomass increase with the addition of manure but not proportional to the dosage, while the fungal biomass responded to the amount of manure added. Accordingly, in this study bacterial population has responded to the added organic manure and the population size of fungi has responded according the dosage of C added with the treatment.

Surprisingly, the bacterial and fungal populations of Manure-SOP treated soil which received the same amount of manure as in Manure-MOP showed significantly lower population. In Manure-SOP treatment, the bacterial population 7 DAT was significantly lower than the Manure–MOP and ½ dose treatment and later became insignificant compared to ½ dose treatment (Figure 1a). While, fungal population of Manure-SOP treatment on the 2nd week (14th DAT), was significantly lower than ½ dose treatment, but later recovered to a same population size as of ½ dose treatment on 57th DAT (Figure 1b). Soil organic carbon content and pH between Manure-MOP and Manure-SOP treatments were not significantly different (Table 3). The prominent difference between these treatments is the applied potassium source. Potassium in MOP is in the form of chloride while in SOP, it is sulfate. Sulfate of Potash contains about 18% of sulfur as sulfate. Therefore, it indicates that the sulfate added with SOP might have an inhibitory effect on the bacterial and fungal populations. Even though there are no straight forward evidences on the effect of sulfate on soil microorganisms, while sulfur as elemental sulfur is an active ingredient of fungicides to control soil borne pathogens. Furthermore, slight reduction in soil pH due to added sulfate may also have affected on both fungal and bacterial population even though difference in pH values was not statistically significant among fertilizer added treatments. Soil pH has been previously demonstrated in several studies to be the strongest influence on the soil microbial communities (Shen *et al.*, 2012; Rousk *et al.*, 2010; Lauber *et al.*, 2009).

The bacterial population of the Inorganic fertilizer treated soil had no significant difference ($p \leq 0.05$) with control soils except an increase in its population at the 57th DAT (Figure 1a). Likewise, fungal population in inorganic fertilizer treated soil was also observed to be same as control except for a significant reduction on the 14 DAT which later leveled up to the population size of control soil (Figure 1b). According to the findings of Khonje *et al.* (1989) application of fertilizers directly stimulate the growth of total soil microbial populations by supplying nutrients but they affect the individual microbial communities differently. However, the result of this study does not show any stimulating effect of inorganic fertilizers on both bacterial and fungal population over the study period. Also, the behavior of fungal population to the added inorganic fertilizer was different from the observations of Nakhro and Dkhar (2010) who have reported a significant increase in fungal population to the added inorganic fertilizer than control soil in paddy cultivation. A meta-analysis too reveled an increase in soil microbial biomass carbon over the long periods of fertilization in agricultural soil (Geisseler and Scow, 2014). Despite to these positive findings, contrasting negative impacts of fertilization on soil microbial communities have also been reported (Lu *et al.*, 2011; Treseder, 2008). However, in this study, the present recommendation of inorganic fertilizer application for coconut does not show any significant effects on soil bacterial and fungal populations over period of this study.

Soil microbial activity

Same as in microbial populations the Manure-MOP added treatment has significantly increased the basal respiration of the soil at 14 DAT (Figure 2). This indicates that organic manure addition along with MOP not only increase soil microbial population it also increases their activity. Contrast to the observation on the microbial population of Manure-SOP treatment, the basal respiration remains similar or higher than ½ dose treatment at all the time points. The Basal respiration of Manure-SOP remains lower than Manure-MOP but follows the same trend throughout the sampling points (Figure 2). However, the inorganic fertilizer treated soil has shown a decrease in soil basal respiration after the 14th day, which has significantly dropped below the control soil at 28th and 57th DAT (Figure 2). The ½ manure plus ½ fertilizer treatments on the 28th day increased slightly than the control, and later on the 57th day it decreased parallel to control (Figure 2). This observation on microbial activity is in par with the findings of Kang *et al.* (2005) saying that organic applications increased nutrient status and microbial activity while the use of only chemical fertilizer resulted in a poor microbial activity in wheat based cropping system.

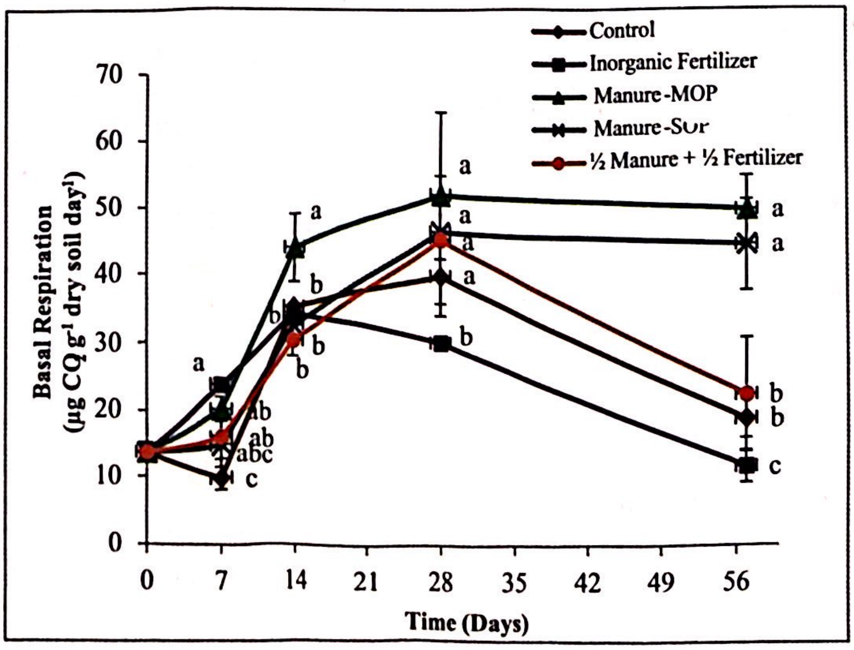

Figure 2. Changes in the microbial activity measured as basal respiration after the application of treatments to the soil, during the 57 days of pot experiment. Error bars ± one standard error, n=3. Different letters at each sampling time (days) indicate significantly different means at $p \leq 0.05$..

CONCLUSIONS

Addition of organic manure increases soil organic carbon and thus, have a positive impact and on soil microbial population and activity. This is evident from the treatment of manure supplemented with MOP. However, SOP has an inhibitory effect on microbial population and activity as seen in the treatment of manure supplemented with SOP. The possible reason for this should be the sulfate added with SOP, but further research is needed to ascertain this. Results of this study also indicate that the inorganic fertilizer application did not affect the soil microbial population, but have affected the microbial activity two weeks after the application. Thus, this study provides an insight to the effect of fertilization on microbiological properties of soil with present coconut fertilizer recommendations and thereby provides implications for sustainable fertilizer programs in future.

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REFERENCES

Anderson, J. P. (1982). Soil Respiration: Methods of soil analysis Part 2: Chemical and microbiological properties. Madison, Wisconsin, USA. pp 83-871.

CRISL, 2016. Coconut Research Instotute of Sri Lanka. [online] available at: http://www.cri.gov.lk/web/index.php?option=com_content&view=article&id=72&itemid=140&;long=en.[Accessed 05 May 2015]

Fontaine, S., Mariotti, A. and Abbadie, L. (2003). The priming effect of organic matter: a question of microbial competition?. Soil Biology and Biochemistry 35: 837-843.

Geisseler, D., and Scow, K. M. (2014). Long-term effects of minereal fertilizers on soil microorganisms- A review. Soil Biology and Biochemistry. 75: 54-63.

Kang, G. S., Beri, V., Rupela, O. P., and Sidhu, B.S. (2005). A new index to assess soil quality and sustainability of wheat based cropping systems. Biology and Fertility of soils 41: 389-398.

Khonje, D. J., Varsa, E. C. and Klubek, B. (1989). The acidulation effects of nitrogenous fertilizers on selected chemical and microbiological properties of soil. Communications in soil science and plant analysis 20: 1377-1395

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 mustered (*Brassica juncea*)-rice (*Oryza stativa*) cropping sequence. Indian Journal of Agriculture Science 70: 69-72.

Lauber, C. L., Hamady, M., Knight, R., and Fierer, N. (2009). Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Applied Environtal Microbiology 75: 5111–5120.

Lazcano, C., Gómez-Brandón, M., Revilla, P. and Domínguez, J. (2013). Short-term effects of organic and inorganic fertilizers on soil microbial community structure and function: A field study with sweet corn. Biological Fertility of Soils 49:723–733.

Leita, L., De Nobili, M., Mondini, C., Muhlbachova, G., Marchiol, L., Bragato, G. and Contin M. (1999). Influence of inorganic and organic fertilization on soil microbial biomass, metabolic quotient and heavy metal bioavailability. Biological fertility of Soils 28:371–376

Loganathan, P., Dayaratne, P. M. N., and Shanmuganathan, R. T. (1984). Evaluation of phosphorus status of some coconut growing soil of Sri Lanka. Cocos 29-34.

Lu, M., Yang, Y., Luo, Y., Fang, C., Zhou, X., Chen, J., Yang, X., and Li, B. (2011). Response of ecosystem nitrogen cycle to nitrogen addition: a meta-analysis. New Phytologyst, 189: 1040-1050.

Lundquist, E. J., Jackson, L. E., Scow, K. M. and Hsu, C. (1999). Changes in microbial biomass and community composition, and soil carbon and nitrogen pools after incorporation of rye into three California agricultural soils. Soil Biology and Biochemistry 221-236.

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.

Mantiquilla, J. A., Canja, L. H., Margate, R. Z. and Magat, S. S. (1994). The Use of Organic Fertilizer in Coconut (A Research Note). Philippine Journal of Coconut Studies

Martin, J. P. (1950). Use of acid, rose Bengal and streptomycine in the plate method for estimating soil fungi. Soil Science 69: 215-233.

Matson, P. A., Naylor, R. and Ortiz-Monasterio, I. (1998). Integration of environmental, agronomic and economic aspects of fertilizer management. Science 280:112-115.

Nakhro, N., and Dkhar, M.S. (2010). Impact of organic and inorganic fertilizers on microbial populations and biomass carbon in paddy field soil. Journal of Agronomy 9:102-110.

Nelson, D. W. and Sommers, L.E. (1982). Total carbon, organic carbon and organic matter. (*In*) Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties-Agronomy Monograph no. 9, 2nd Edn. A.L. Page *et al.* Eds. American Society of Agronomy, Madison, WI, USA. pp 539-579.

Paul E. (2007). Soil microbiology, ecology, and biochemistry, 3rd ed. Elsevier Academic Press.

Rousk, J., Bååth, E., Brooks, P. C., Lauber, C. L., Lozupone, C., Caporaso, J. G. (2010). Soil bacterial and fungal communities across a pH gradient in an arable soil. The ISME journal 4:1340-1351.

Shen, C. C., Xiong, .J. B., Zhang, H. Y., Feng, Y. Z., Lin, X. G., Li, X. Y. (2012). Soil pH drives the spatial distribution of bacterial communities along elevation on Changbai Mountain. Soil Biology and Biochemistry 57:204–211.

Swer, H., Dkhar, M.S. and Kayang, H. (2011). Fungal population and diversity in organically amended agricultural soils of Meghalaya, India. Journal of Organic Systems 6: 1-12.

Tennakoon, N. A., Mahindapala, R. and Widanapathirana, S. (1995). Effects of organic manure on the quality of coconut soils. Journal of National Science Council Sri Lanka 23: 171-182.

Tilman, D. (1999): Global environmental impacts of agricultural expansion: The need for sustainable and efficient practices. Proceedings of National Academy of Science 96: 5995-6000.

Treseder, K. K. (2008). Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. Ecology Letters 11: 1111-1120.

Walkley, A., Black, A., 1934. Determination o organic matter. Soil Science 37: 29-38.

Willer, H. and Kilcher, L. (2009). The World o Organic Agriculture. Statistics and Emergin Trends 2009, IFOAM, Bonn, and FiBL, Frick.