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An appraisal of physico-chemical and microbiological characteristics of Nanmangalam Reserve Forest soil

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Abstract

A detailed evaluation was performed on the soils of Nanmangalam Reserve Forest (NRF) in order to understand its physico-chemical, microbiological and enzymatic characteristics. The results of analysis showed that soil pH was directly proportional to the soil depth and the soil moisture content was irreversibly related to varying soil depth. Soil organic carbon was positively correlated with ($p < 0.01$) with total nitrogen, total bacterial count, cellulytic microbes, nitrogen–fixing bacteria, microbial biomass carbon, dehydrogenase activity and soil respiration. During summer, microbial population in the organic layer was more diverse than in the deepest layer. Analysis showed that NRF had low organic carbon content (less than 1%), microbial biomass, nutrient and functional microbes. The overall results of the analysis reinstate that Nanmangalam forest soil is undergoing degradation.

Key words

Degraded forest, Microbial biomass, Nutrients, Soil organic carbon, Total and functional microbes

Introduction

Soil comprises the major terrestrial pool of organic carbon on the earth's surface.The soil matrix characterizes the intricate balance of organic matter additions, losses, transformation, and translocations (Jobbagy and Jackson, 2000; Moritz *et al*., 2009). Particularly, the vibrant and active component of soil organic matter (SOM) is restricted by intricate interrelationships between quality and quantity of carbon accumulation and C mineralization, residence time etc, in addition to climatic and edaphic factors (Feller and Beare, 1997; Zech *et al*. ,1997). The crucial carbon inputs in soil systems are plant material, litters, root exudates, and soil microbial residues (Kogel-knabner, 2002; Lindsey *et al*., 2009). In general, the microbial communities in soil persuade soil fertility and soil formation, through which it manages the dynamics of organic matter decay and plant nutrient accessibility (Nannipieri *et al*., 2003; Hackl *et al*., 2004). The diversity of microbial species is directly linked with nutrient availability, environmental conditions, soil texture, and different kind of vegetation (Allen and Schlesinger, 2004). Generally, forest degradation changes the soils physico-chemical and micro biological characteristics harmfully (Behera and Sahani, 2003), as it leads to the formation of unproductive, degraded soils. Such degraded forest soils need appropriate ecological therapy through which soil can be ameliorated to enhance biological productivity. Plantation forestry is one of method by which degraded soils can be reinstated back to sustain soil fertility (Lamb and Gilmour, 2003). Analyzing the soil physico- chemical and biological characters are very essential before forest rehabilitation. Till date, in India, there are not much detailed analysis on physico- chemical and microbiological characteristics of forest soil. So this study attempts to bridge the research gap by the analyzing the physico-chemical and microbiological characteristics of the forest soil.

Materials and Methods

The study area Nanmangalam Reserve Forest lies in the southern part of Chennai and spreads in an area of 321 ha, with latitudinal extension from 12°55'5" N to 12°56'13" N and longitudinal extension from 80°9'46" E to 80°10'57" E. This Nanmangalam soil has got an undulated topography with hillocks, two fresh water ponds, floodplains and plains consisted of scrub forest, and plantations. The major species found in this scrub jungle ecosystem are *Gymnosporia Montana, Pamburus missionis, Vitex negund, Ficus racemosa*, *Terminalia arjuna*, *Azadirachta indica* (Fig.1).

Soil analysis : A systematic sampling procedure was followed, dividing the entire study area into 40 grid points with a grid space of 0.25km^2. Soil samples were collected from different soil strata based on different depths like organic soil from the top layer, topsoil from 0-15 cm, subsoil from 16-30 cm depth for all the twelve months in the year of 2010. Then the data were split into four four seasons for statistical analysis: January and Febraury (Winter season); March, April and May (Summer season); June, July, Augest and September (South West monsoon); October, November and December (Post monsoon).

Soil texture was analysed following the methodology of Gee and Bauder (1986), and moisture was analyzed by Robinson pipette, and gravimetric method, while soil pH was determined by soil thermometer. Total carbon (C), and total nitrogen (N) were quantified by CHNS-O elemental analyzer (Vario EL cube) as described previously (Wang and Anderson, 1998). Total phosphorus and available phosphorus was analyzed by the molybdenum-blue colorimetric method using an auto analyzer method suggested by Allen *et al*. (1974).

The total and functional microbes were isolated using pour plate method. The microbes were incubated at room temperature on nutrient agar, rose bengal agar, glycerol yeast extract agar Mendals reese medium, pikovskayas medium, and nitrogen- free mannitol medium as used by Owen *et al*., (2003). Fumigation extraction method was used to analyze microbial biomass carbon (MBC) , nitrogen (N) and phosphourous (P) proposed by Anderson and Ingram, (1993) and were calculated, by using the formula proposed by Vance *et al*. (1987)

Soil respiration was measured by estimating released CO_2 during 24-hr incubation at room temperature by absorption and titration method, using a phenolphthalein indicator (Macfadyen, 1970).

Urease activity was analysed by the Indophenol blue method, and the optical density of the blue colored solution was read spectrophotometrically at 630nm. The phosphatase activity (Tabatabai and Bremner, 1969) of the soil was also assessed. The p-nitrophenol produced due to phosphatese activity using substrate disodium p-nitropheneyl phosphate gives yellow color under alkaline conditions for which the intensity was measured spectrophotometrically at 420nm. β- glucosidase activities in the soil samples were determined colorimetrically using a spectrophotometer. The method was based on the cleavage of the substrate p-nitrophenyl- β- Glucoside (buffered at pH 6) by beta glucosidase to release p-nitrophenol. In this method, the released p-nitrophenol under alkaline conditions was quantified by measuring the absorbance at 410nm (Dick *et al*.,1996).

Statistical analysis : The soil physico-chemical and microbiological data were analyzed using correlation analysis of bivariate tests using SPSS ver. 11.5. Mean and standard deviation were also calculated for different depths and different seasons of soil samples.

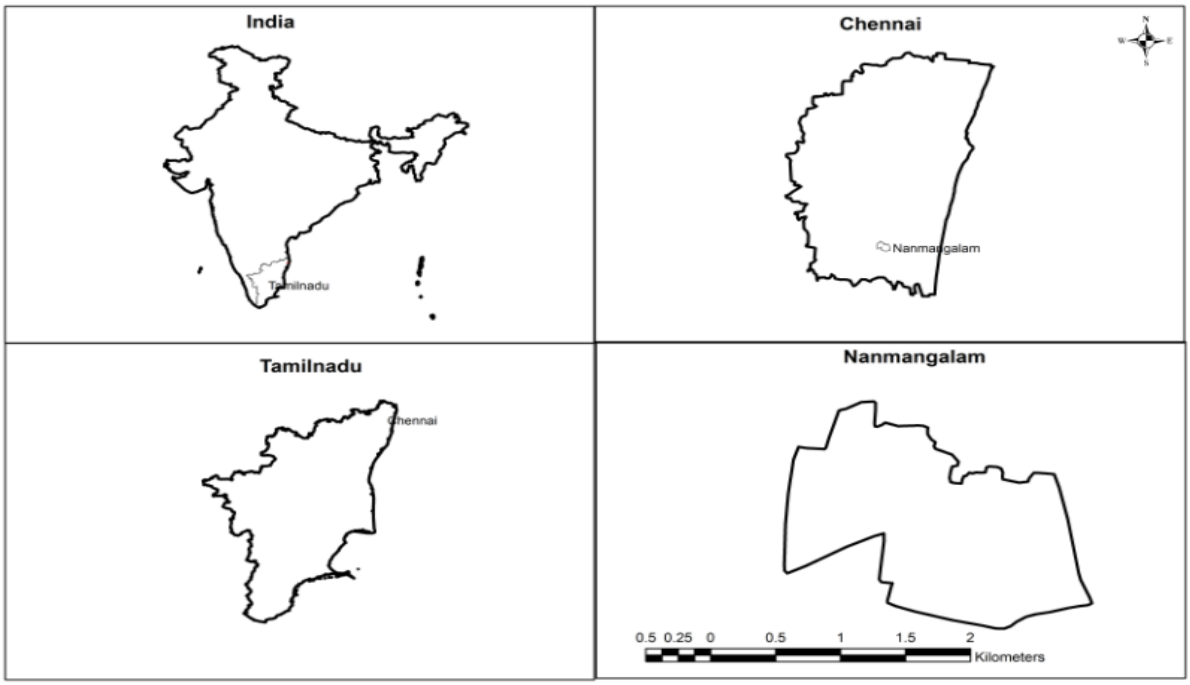

Fig.1 : Study Area - Nanmangalam Reserve forest

Results and Discussion

The monthly mean air and soil temperature varied from 24.5 to 32.3º C and 31.7 to 33.5º C respectively. The average annual air temperature was 29.4º C. January and February had low temperature (24.5-25º C), while April, May and June had high temperature (30.4 – 32.3 0 C). The average of soil temperature at different depths were 31.5, 28.6 ºC, 26.1 ºC. The highest air

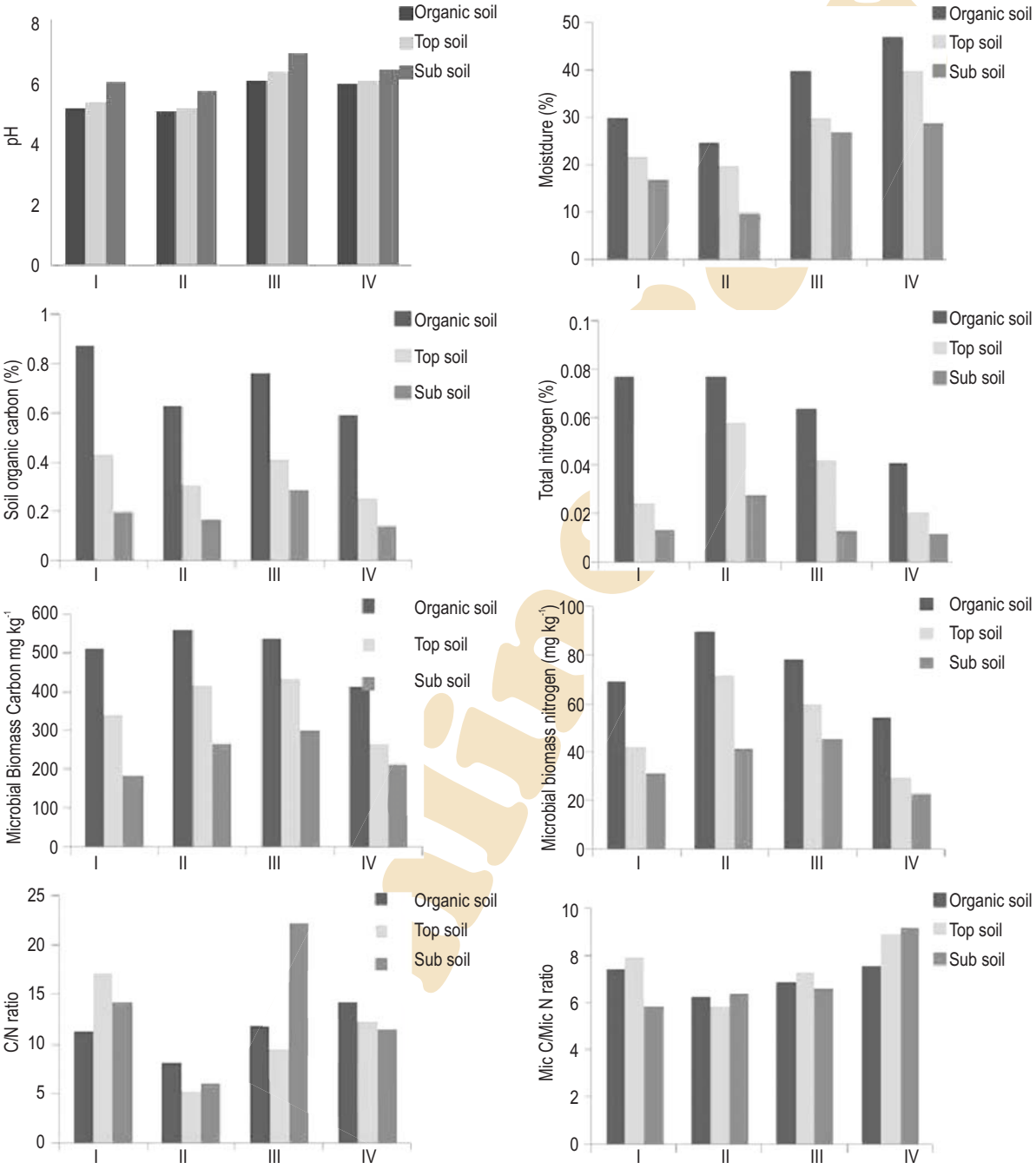

Fig. 2 : The pH, moisture, soil organic carbon, total nitrogen, microbial biomass carbon, microbial biomass nitrogen, carbon nitrogen ratio, Mic C and Mic N ratio of Nanmangalam reserve forest soils. I-January, Febraury, II-March, April, May, III-June, July, August, September, IV-October, November

temperature was observed in summer and lowest in winter, however the soil temperature was higher in summer but, a divergence in soil temperature was observed during winter's. It may be due to the high heat retention capacity of the soil. The annual average precipitation of the forest was 153.1mm. The cation exchange capacity was shown 363µmolc g^{-1} While. The clay content of the study area was 28%, respectively.

The soil pH was in the acidic range (pH 5.01-6.89), and was lower in organic layer as comprised to subsoil. The pH values ranged from 5.01 to 6.89 and average soil pH in soil was 5.51 to 6.22, respectively. Variation in pH was observed during different seasons. In contrast to pH, the moisture content decreased in the subsoil and ranged from 20.75 to 35.25. The moisture content decreased in deeper horizons(Fig. 2).

Soil organic carbon (SOC) and total nitrogen (TN) were less than 1% and 0.1%. SOC and TN decreased with increased depth. Conversely, the decreasing trend of SOC was higher as compared to TN. The SOC content ranged from 0.14% to 0.87% and 0.12% to 0.41%. The C/N ratio of the forest soil was 11.41, 11.12 and 8.13 in organic, topsoil and subsoil, respectively. The conflicting decrease in SOC and TN with increasing soil depth influenced the C/N ratio and caused difference in the tested soils (Fig 2).

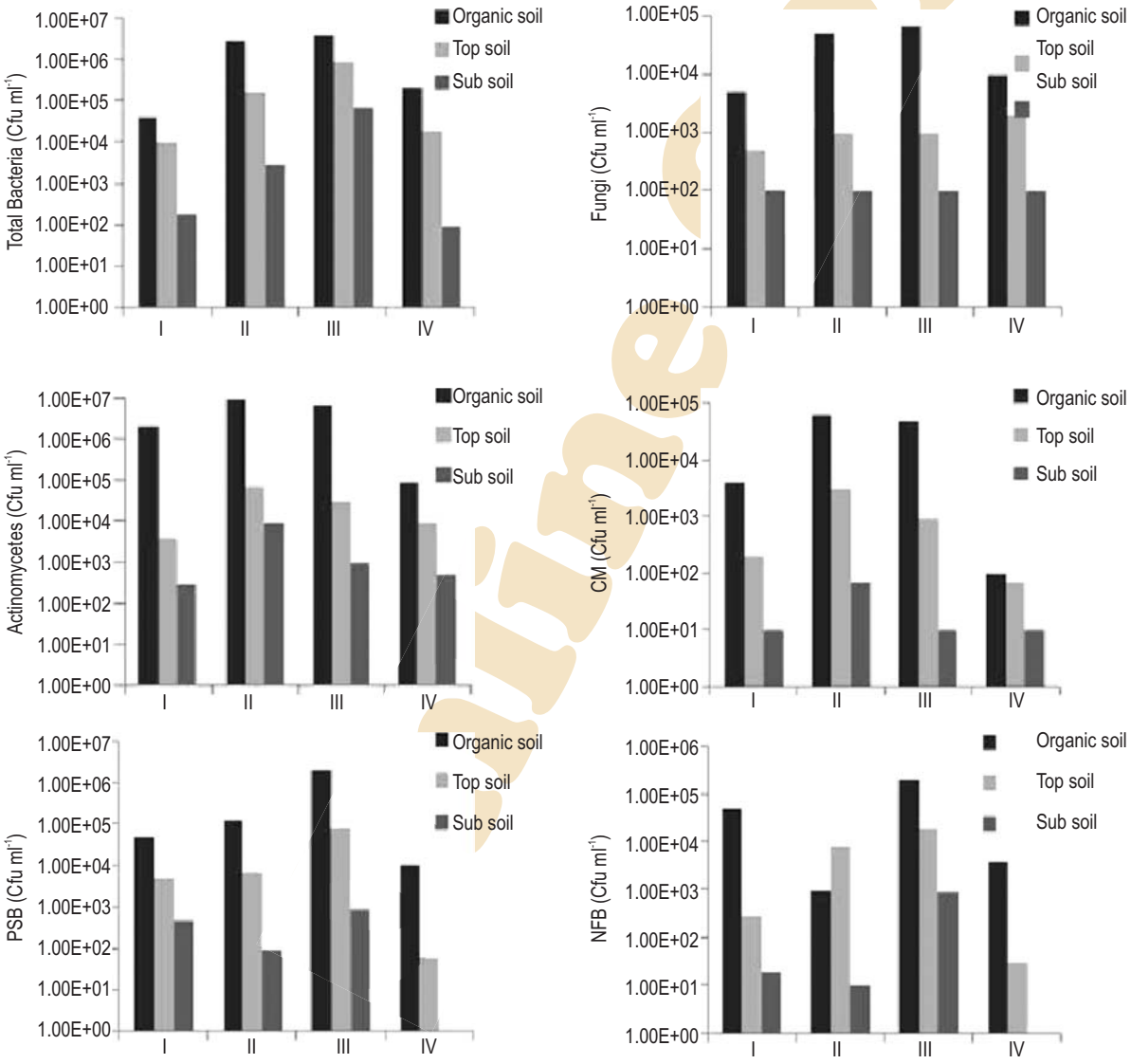

Fig. 3 : The total bacteria, fungi, actinomycetes, Cellulytic bacteria (CB), Phosphate solubilizing bacteria (PSB), Nitrogen fixing bacteria (NFB) I-January, Febraury, II- March, April, May, III- June, July, August, September, IV- October, November, December

Table 1 : Enzymatic activities in Nanmangalam Reserve Forest Soil

Enzymatic activities	Organic soil	Top soil	Subsoil
Dehydrogenase (µg INF $g^{-1}2h^{-1}$)	146.16±58.21	54.16±8.12	12.23±5.89
â –Glucosidase(mg PNP g^{-1} h^{-1})	99.34±16.23	26.34±6.45	9.6±0.23
Phosphatase(mg PNP g^{-1} h^{-1})	210.23±34.89	134±12.50	98.23±6.21
Urease(µg NH_4–N g^{-1} $2h^{-1}$)	43.45 ±3.45	20.36±1.13	8.67±2.5

The average bacterial population of NRF soil ranged from $2.1x10^3$ to $4.1x10^5$ cfu g^{-1} of dry organic soil, from $1.9x10^2$ to $9x10^4$ cfu g^{-1} of dry top soil, $1.1x10^2$ to $7x10^4$ cfu g^{-1} of dry sub soil, respectively. The highest population was observed in organic soil as organic soil contains a huge amount of organic matter, the required oxygen concentration and sunlight in the organic soil. Highly significant differences were observed from March to September and less significant differences were observed from December to February. This may be due to low air mean temperature.

The fungal population ranged from $1x10^1$ to $2x10^5$ cfu/ g of dry soil. In each gram of dry organic, top, and subsoil fungi at populations were $1x10^3$ to $2x10^5$, $2x10^2$ to $2x10^4$, $1x10^2$ to $2x10^2$ cfu g^{-1} of dry soil respectively. On the whole, the NRF soil showed a less fungal population. However, the fungal population was equal to bacterial population. Usually acidic pH enhances the fungal growth, The forest soil has an acidic pH which is responsible for the fungal growth. During summer (March to May), the highest fungal population was observed. Warm temperature also increased the fungal growth. Statistical analysis of the organic soil sample showed $p<0.01$significance in sub soil and seasonal variations showed $p < 0.05$ significance level (Fig. 3).

The actinomycetes populations were between $9X10^4$ to $1x10^7$ cfu g^{-1} of dry organic soil $4x10^3$ to $7x10^4$ cfu g^{-1} of dry topsoil and $3x10^2$ to $9x10^3$ cfu g^{-1} of dry sub soil, respectively. The organic soil sample had higher population than that of top and subsoil. Statistical analysis showed that the differences in the count of actinomycetes population between organic soil and top soil at $p < 0.05$ significance level (Fig. 3).

Functional microbes are very important in the elemental cycle and in plant nutrition of the forest soils. The population of functional microbes differed during different seasons and soil depth. Cellulytic microbes in the dry soils were $4x10^1$ to $2.8x10^3$ cfu g^{-1} in the Nanmangalam Reserve Forest soils. The highest population was observed from March to May and the lowest population was observed during October to December. The cellulytic microbes also decreased with increase in soil depth. The seasonal difference of microbial population and cellulytic microbe counts were insignificant. Phosphate solubilizing microbes were $5x10^2$ to $5.9x10^5$ cfu g^{-1} dry soils, respectively. Nitrogen-fixing microbes were $5x10^2$ to $5.9x10^5$, $2.4x10^2$± 6.810^4 ± .96 cfu g^{-1} dry soils, respectively. Variation in the soil depths has not exhibited any significant seasonal variation in the microbial population and nitrogen fixing microbes. The highest phosphate solubilizing bacteria and nitrogen fixing bacterial population were observed higher during south west monsoon season (June to September) (Fig. 3).

In both horizontal and vertical distribution of the soil, microbial biomass was very less and decreased with increasing depth. The C_{Mic} ranged from 241.75mg kg^{-1} to 505.44 mg kg^{-1} dry soil. The highest C_{Mic} was observed during summer (March-May). The microbial biomass nitrogen ranged from 35.68 to 72.94 mg kg^{-1} dry soil respectively. The C_{Mic}/N_{Mic} ratios ranged from 6.99 to 7.49, of forest soils, respectively. The C_{Mic}/N_{Mic} ratios of organic soil, top soil and sub soil was in descending order, during winter (January and February) and south west monsoon (June-September) .The function of microbial biomass at different depths were observed. This result is in contradictory to many other previously reported research findings. However, the differences were significant in the subsoil. The soil results explained that the microbial biomass carbon and nitrogen contents were not significant during different seasons (Fig. 3).

The results indicate a lack of relationship between phosphatase and soil pH. Dehydrogenase, β glucosidase, urease activity was also very less. Phosphatase activity was high as compared to other enzymatic reactions. The enzymatic reaction decreased with increase in soil depth. In forest soil, the dehydrogenase activity ranged from 12.23±5.89 to 146.16±58.21µg INF $g^{-1}2h^{-1}$ and β – Glucosidase activity was 9.6 ±0. 23 to 99.34±16.23 respectively. Phosphatase and urease activities ranged from 98.23±6.21 to 210.23±34.89 and 8.67±2.5 to 43.45±3.45 respectively. In sub soil, all the enzymatic activities were very less when compared to organic soil (Table 1).

Most of the soil properties were negatively correlated with soil pH and were statistically significant as well. In contrast, soil parameters were positively correlated with soil moisture, except with soil pH. In contrast, moisture content showed positive correlation with the other soil properties, except for soil pH, moisture content was highly significant ($P <0.01$) with soil organic carbon, actinomycetes, dehydrogenase, soil respiration, and also showed a positive significant correlation level of ($P<0.05$) with total nitrogen, bacteria, fungi, cellulytic microbes, nitrogen - fixing microbes and urease. Correlation between C_{Org} and N_{Tot} was statistically significant ($P<0.01$). Actinomycetes were significantly correlated with ($P<0.01$) MBC, phophatase and soil respiration. Results of most of the soil analysis explained high significance

with (P<0.01) soil respiration. Soil pH was positively and significantly correlated with soil respiration and dehydrogenase activity and showed significant negative correlation with fungi population. Soil organic carbon was positively correlated with high significance level with microbial biomass, carbon, total bacteria and functional microbes except for phosphate solubilizing microbes (Table 2).

The forest soil was acidic and reason for the acidity of soil can be attributed to organic matter decay, nature of the parent material, leachate and rainfall (Chiu *et al.,* 2005). The pH increased with depth of the soil from organic layer. This occurrence was also observed in Shang–Hsing Mountain (Wu and Chen, 2005; Tsai *et al.,* 2007). The deficiency in the annual rainfall might have played an important role in affecting the soil pH. In this study, physico-chemical properties and microbial activities are highly influenced by the soil moisture and negatively influenced by the pH. The similar results have been reported by Mishra (2010) for mixed pine and pine forests of Meghalaya North east India.

SOC is essential for controlling different soil biological activity and nutrient cycle. The increased rate of organic matter decomposition is responsible for high organic carbon in the organic soil of forest. As a result, total nitrogen and C/N ratio were markedly higher. During dry periods the microbial biomass was found to be lower and increased during the wet period. Different studies have reported that soil moisture content controls the microbial biomass in the soil (Chen *et al.,* 2005, Devi and Yadava, 2006). Yang *et al.* (2003) pointed out that the soil moisture depends on the environmental conditions and soil texture. On the whole, soil organic carbon and total nitrogen were less in forest soil. The decreasing pool of organic carbon can not support sustainable microbial activity and soil respiration. The negative impact of low carbon content in the forest soils, on reducing microbial soil respiration, could be treated as one of the major indicators for suggesting appropriate management practices for restoring the biological quality of soils (Quemada and Menacho, 2001; Schloter *et al*., 2003; Nourbakhsh, 2007).

Available nitrogen is a very common limiting factor in forest ecosystem (Fisher and Binkley, 2000; Ekienci, 2006). High organic matter is responsible for the formation of high quality of organic carbon and nitrogen in the mineral soil. In forest soil, the nitrogen content is very less, due to inadequate tree cover, and less litter fall. Additionally, high evaporation rate leads to moisture deficiency in the soil, which slows down the decomposition and subsequent mineralization of limited litter in the soil (Selvam *et al*., 2010). Even though, the difference of total microbes was larger during the tested periods, microbial populations were low in subsoil samples due to low total organic carbon (less than 1%, 0.16 to 0.7%) and low total nitrogen (0.01 to 0. 06%). The functional microbes were less in the tested samples. But other studies (Yang *et al.,* 2003, Selvam *et al*., 2010) have explained that the huge number of functional microbes like cellulytic microbes and nitrogen-fixing bacteria were observed in tested soils. In the present study, cellulytic microbes, nitrogen - fixing bacteria were less, where as phosphate solubilizing bacteria were high in population; this could be due to the naturally abundant levels of phosphorous in the study area.

Soil microbial biomass is a very important property in terms of soil health, because it has a close relationship with

Table 2 : Correlations between physical, chemical, biological and enzymatic activities of Nanmangalam reserve forest

Variables	pH	MC	org C	TN	Bacteria	ACT	Fungi	CM	PSB	NFB	MBC	MBN	Urease	Phosphatase	Dehydrogenase	SR
Ph	1															
MC	-0.21	1														
org C	-0.32	0.89**	1													
TN	-0.32	0.67*	0.76**	1												
Bacteria	-0.42	0.64*	0.61*	0.37	1											
Actinomycetes	-0.15	0.80**	0.43	0.49	0.78**	1										
Fungi	-0.67*	0.60*	0.54	0.45	0.71*	0.01	1									
CM	-0.23	0.72*	0.63*	0.29	0.73**	0.24	0.25	1								
PSB	-0.58*	0.26	0.32	0.37	0.30	0.23	0.42	0.67*	1							
NFB	-0.31	0.70*	0.63*	0.87**	0.49	0.27	0.83**	0.35	0.68*	1						
MBC	-0.18	0.23	0.83*	0.7	0.68*	0.56*	0.73*	0.34	0.68*	0.74*	1					
MBN	-0.28	0.46	0.73*	0.81**	0.77**	0.21	0.58*	0.26	0.34	0.69*	0.79**	1				
Urease	-0.63*	0.62*	0.49	0.78**	0.30	0.42	0.22	0.37	0.68*	0.78**	0.45	0.11	1			
Phosphatase	-0.57*	0.37	0.35	0.37	0.47	0.74*	0.34	0.34	0.22	0.69*	0.78**	0.24	0.76**	1		
Dehydrogenase	-0.11	0.86**	0.79**	0.34	0.23	0.51	0.87**	0.71*	0.33	0.34	0.48	0.39	0.69*	0.39	1	
SR	0.12	0.87**	0.79**	0.57*	0.68*	0.57*	0.61*	0.86**	0.67*	0.88**	0.91**	0.78**	0.43	0.67*	0.38	1

MC-moisture content; Org C- Organic Carbon; TN-total nitrogen; ACT–Actinomycetess; CM-Cellulolytic microbes; PSB-Phosphate solubilizing Bacteria; NFB-Nitrogen fixing bacteria; MBC-Microbial biomass carbon; MBN-Microbial biomass nitrogen; SR-Soil respiration; Symbols *and ** indicate the significance of the Pearson correlations at p<0.05 and p<0.01, respectively

microbial diversity, soil and plant quality (Ekinci *et al.,* 2006). The decreasing trend of biomass carbon was observed from organic soil to sub soil. The C_{Mic} / N_{Mic} ratio clearly depicts the present condition of the microbial community in the soil. Campbell *et al*. (1991) reported that a high proportion of fungi population was noted when the high C_{Mic} / N_{Mic} ratio and at this low ratio was noted there will be a high proportion of the bacterial population. C_{Mic} and N_{Mic} ratio were found to be low in the forest soils. The soil properties such as moisture content, texture and pH have a bearing on C_{Mic} / N_{Mic} ratio (Moore *et al*., 2000). Taylor *et al.* (2002), stated that dehydrogenase activity was assayed as an estimation of overall microbial activity due to its presence in all the microorganisms.

SOC was significantly associated ($p<0.01$) with dehydrogenase activity but, was not correlated with bacterial and fungal population. Albeit, the possibility of regulation of dehydrogenase activity by both SOC and microbial population was divulged by Shukla *et al*. (1989) and it was also mentioned that dehydrogenase activity is affected more by fungi than bacteria. Statistical analysis revealed that the correlation coefficients for dehydrogenase activity of fungal populations were highly significant ($P<0.01$) in the forest soil. So the enzyme activity was seen to be highly depended on fungal population than bacteria and actinomycetes population. The soil respiration was positively correlated ($P<0.01$) with urease activity in Nanmangalam soil; similar result has been reported from North East China by Brohon *et al.* (2001); Kaur *et al*. (2002). Schoenholtz *et al.* (2000) confirm that if the SOC value was equal to 1 % or < 1% then soil could be considered as degraded and created a negative impact on the productivity, with no further regeneration prospects.

In conclusion, this study helped to evaluate the present status of physico-chemical and microbiological charecteristics of Nanmangalam Researve Forest soil. The results from the analysis affirms that soil nutrient, SOC content, microbial biomass and population in the NRF soil is meager which confirms that the Nanmangalam forest soil is in the state of degradation.

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