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Original Article

Spatiotemporal Variation of Microbial Load in High Altitude Shola Soils of Tropical Montane Forest, Kerala, South India: A Function of Important Soil Physicochemical Characteristics

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ABSTRACT
Microbial population control many of the crucial processes on which the very maintenance and survival of tropical forests depend. Microorganisms in the soil are strongly influenced by various chemical and physical factors, including nutrient availability, organic matter, soil moisture, temperature etc. Therefore in the present study we analyzed the spatiotemporal variation of microbial load and their relationship with important physicochemical characteristics of shola forest soils of tropical montane forest, Kerala, South India. The study revealed that there was a spatial and temporal variation of microbial load in the soils of shola forest. Shola soils were weakly acidic and the microbial load was characterized by high load of bacteria followed by fungi and actinomycetes. Bacterial, fungal and actinomycetes load was high during pre monsoon followed by post monsoon and monsoon period. The microbial load varied with important soil properties and there was significant (0.01 level) positive correlation between bacterial and fungal load with total organic carbon and NPK. Actinomycetes showed significant (0.01 level) negative correlation with moisture content.

KEYWORDS: Shola forest, Soil Bacteria, Fungi, Actinomycetes

INTRODUCTION

Healthy soil is a living body made up of inorganic material, decaying organic matter, water, air and billions of living organisms and is alive with organisms of all shapes and sizes. Soil microbes are essential components of the biotic community in natural forests and are largely responsible for ecosystem functioning. They are the driving force for nutrient supply to plants in both agricultural and forest lands not only because of their ability to carry out biochemical transformations but also due to its fast turnover. The physicochemical soil properties (amount and quality of soil organic matter, mineral nutrients, pH, soil texture, temperature, moisture, etc.) are of particular importance in determining the size and activity of microbial population. When corroborated with other ecological indicators (e.g., biomass and species diversity measurements), changes in the numbers of these organisms can also provide an indication of changes in soil health and productivity.

Organism abundance and doings are not arbitrarily distributed in soil, but differ both horizontally and vertically through the soil profile. Diverse groups of organisms show different spatial patterns, since they each respond to soil environment in dissimilar ways (Klironomos et al. 1999). There is small in-depth information existing on the spatial distribution of soil biota. Information on spatial patterns can be used to plan new statistically powerful experiments, to progress our understanding of how soil communities build up, and to decide what factors are vital for regulating and maintaining soil function.

It has been hypothesized that high levels of soil biodiversity are attributable to spatial heterogeneity in resource accessibility as influenced by land-use patterns and plant community dynamics (Ettema and Wardle 2002). Spatial heterogeneity can be high even in soils that appear relatively homogeneous at the plot or field scale (Franklin and Mills 2003). In the present work an attempt was made to study the spatiotemporal variation of microbial load in the shola soils of tropical montane forest in relation with important soil physicochemical characteristics and nutrients.

MATERIALS AND METHODS

Study area

Study area is located at the top areas of Eravikulam National park lies between 10°05'N - 10º20'N latitude and 77º0'E - 77º10'E longitude in Idukki district, Kerala, South India at an altitude of 1900 m to 2400 m above MSL. Majority of the land in this area is covered by grass lands and shoal forest. For the present study, six sites in shola forest at different altitudes were selected for sample collection.

Soil Sample Collection

Soil samples were collected from prefixed sites (SH1-SH6) of shola forest at different altitude or different microclimate. Samples were collected at a depth of 15 to 20 cm from the surface after removing the litter. For each of the sampling sites, sub-samples of soil were collected from different locations, pooled together and homogenized so as to obtain representative sample. Sampling was carried out during post-monsoon, pre-monsoon and monsoon seasons. Samples were collected using a spade that is thoroughly cleaned and disinfected between sampling so as to prevent cross-contamination.

Physicochemical Characteristics of Soil

Soil temperature was determined *in situ* with the help of mercury bulb thermometer. Soil moisture content was determined by gravimetric method. pH of the soil samples were determined potentiometrically using pH meter. Available nitrogen was determined by Microkjeldahl method and the available phosphorus by Bray and Kurtz method (Jackson, 1973). Available potassium in the soil samples were extracted using neutral normal ammonium acetate as extractant and determined by using flame photometer (Morgan, 1941). Soil organic carbon was determined by Walkey and Black Method (Jackson, 1973).

Enumeration of Microorganisms

Enumeration of Microorganisms (bacteria, fungus and actinomycetes) was carried out by standard serial dilution plate technique. Briefly, 10 grams of fresh soil was transferred to 90 ml sterile distilled water and agitated vigorously. Different aqueous dilutions, up to 10^{-7} of the suspensions were prepared and spread plated on Soil extract Agar, Potato malt Agar and Kusters Agar for bacteria, fungus and actinomycetes respectively. Nystatin (50µg/ml) or Amphotericin (75µg/ml) and Streptomycin (25µg/ml) were added to the Kusters Agar plates in order to prevent fungal and bacterial contamination respectively. Inoculated plates were incubated at 37^{o}C for 24 – 44 hrs. After incubation microbial colonies were counted and the load was expressed as number of colony forming units (CFU) per gram of soil.

Statistical Analysis

Correlation between microbial load and soil physicochemical characteristics were carried out using SPSS software.

RESULTS AND DISCUSSION

Soil Physicochemical Characteristics: Results of important physico-chemical properties of the soil such as temperature, pH, moisture content, organic carbon, available nitrogen, available potassium and available phosphorous during the study period is presented in Table 1. Shola forest soils of the selected study area were weakly acidic in nature (5.1-6.4) and the study showed that there was a spatial and temporal variation of soil physico-chemical characteristics. Soil temperature ranged from 10-15ºC and the values of soil moisture content ranged between 29.91 and 63.11%. Maximum organic carbon of 5.7% was recorded during premonsoon season from SH5 and the minimum of 2.2% was observed from SH6. Soil nutrients also showed high level of spatiotemporal variations.

Physicochemical properties of the soil varied according to the terrain of the study area and in the present study samples were collected both from slopes of different degrees of angles and valleys. Similar observations were reported by different researchers; Miller et al. (1988) reported variation in soil organic carbon with slope position. Variation of soil properties within a distinct climatic area may also result from topographic heterogeneity (Brubaker et al. 1993, Feldman et al. 1991). Another reason of the spatio-temporal variations of soil physicochemical characteristics is found to be the vegetation structure (Johnston 1986, Kumar et al. 2004) as the regions with high density of plants lead to the accumulation of large amount of organic matter, cooling effect *etc.*, which leads to changes in the physicochemical characteristics of the soil such as organic carbon and soil temperature.

Weather patterns also cause the drift in soil physicochemical characteristics (Paudel and Sah 2003). For example precipitation cause changes in soil moisture content, soil temperature, pH etc. In the present study, the soil moisture content in monsoon season was higher than other two seasons, mainly attributed to the rain during June-September. The pH of the shola soil was found to be weakly acidic due to the high leaf litter on the surface, as the decomposition of leaf litter leads to the accumulation of organic acids (Chen and Yang 2000) which is reflected in acid pH of the soil. Soil pH was slightly lower in pre monsoon season than in post monsoon and monsoon season. This could be due to relatively higher soil temperature in pre monsoon season which can result in an increased microbial activity and resultant increase in organic acid accumulation (Berg et al. 1998).

The amount of NPK and organic carbon is a function of mineralization and immobilization of these characters in the soil (Bengtsson *et al.* 2003). It is used as an index of the quality of substrate (Enruez et al. 1993, Bossuyt et al. 2001) and decomposition rate (Hobbie and Vitousek 2000). Organic carbon and nitrogen content have strong relationships with distribution and density of plants in the sampling locations. In the present study samples were collected from different sites having diverse plant composition and biomass. The percentage of NPK and organic carbon were found to be lower in the soil samples collected during monsoon season. Higher uptake of nutrients by plants during monsoon periods as well as the dilution of the soil nutrients by rain water may be probable reasons. Similar trend of soil nutrients was recorded by Sumathi *et al.* (2008).

Table 1. Variation of soil Physico-chemical properties of Shola forest during Pre monsoon, Monsoon and Post monsoon

Sampling sites	Season	Variation in soil physico-chemical parameters						
		Soil Temperature (°C)	Moisture Content (%)	pH	Organic Carbon (%)	Available Potassium (Kg/ha)	Available Phosphorous (Kg/ha)	Available Nitrogen (Kg/ha)
SH1	Pre monsoon	14	43.6	5.1	5.1	897.44	60.3	1254.4
	Monsoon	13	55.4	5	4.6	577.92	25.2	1003.52
	Post monsoon	11	47.1	6.1	4.9	752.64	42	1066.24
SH2	Pre monsoon	15	31.18	5.73	4.7	876.56	39.6	1379.84
	Monsoon	14	36.89	5.6	4.1	779.52	8.4	501.76
	Post monsoon	12	33.41	6.4	4.3	719.52	24.1	752.64
SH3	Pre monsoon	15	29.91	5.6	4.4	913.92	36.4	1003.52
	Monsoon	13	34.91	5.6	4.1	174.92	22.4	627.2
	Post monsoon	11	32.01	5.9	4.1	934.08	36.4	878.08
SH4	Pre monsoon	15	53.11	5.3	5.5	998.88	70.2	2132.48
	Monsoon	12	61.84	5.3	4.9	893.76	46.8	564.48
	Post monsoon	10	56.02	5.4	5.3	873.76	64.4	627.2
SH5	Pre monsoon	13	54.01	5.2	5.7	1004.4	67.3	1881.08
	Monsoon	12	63.11	5.1	5.3	920.64	42	815.36
	Post monsoon	10	59.89	5.3	5.5	860.16	60.0	878.02
SH6	Pre monsoon	14	31.52	5.6	2.9	713.92	41.6	878.08
	Monsoon	12	46.44	5.7	2.2	373.4	36.4	627.26
	Post monsoon	11	37.50	5.9	2.4	438.03	51.21	734.5

Spatiotemporal Variation of Microbial Load

In the present study microbial load showed variation both spatially and temporally. Bacterial load ranged from 88×10^5 to 224×10^5 CFU/1gf.w and the highest count recorded from SH5 and the lowest count recorded from SH6 in all the seasons. Fungal population ranged from 30×10^2 (in SH6 during monsoon) to 66×103CFU/1gf.w (SH5 during pre monsoon). Actinomycetes load ranged from 21×10^2 to 40×103 CFU/1gf.w (Table 2). In the entire seasons studied generally bacterial load was found to be high followed by fungus and actinomycetes (Figure 1, 2 and 3). Although the load of culturable microbes was large during the study periods, the microbial load was comparatively higher in pre monsoon season. This indicates that decomposers are most active during the pre monsoon season because of better soil temperature compared to other seasons, high organic content which might favour the microbial activity. Increase of microbial load is also associated with optimal relation between water and air in the soil. Increase of soil moisture content leads to high oxygen content in the soil cause an increase in the number of aerobic bacteria. The organic carbon content and nutrient availability are reported to be important factors in the determination of bacterial and fungal load spatially and temporally (Eiland et al. 2001, Marschner et al. 2003).

Microbial load was lower in monsoon season perhaps due to competition for nutrients by plants and protozoan predation of microorganisms (Bhatt and Pandya, 2006). Some constituents of soil characteristics have negative relationships with microbial communities. Microbial load have strong relationships with plant composition and density because organic matter contribution by plants and root exudates play an important role in the soil biota. Soil microbial communities rely on materials produced by plants as energy sources for growth and reproduction. Low plant cover and biomass would affect the activity of microbial communities (Bardgett 1997). Declined and degraded plant cover means a lower soil organic matter added to the soil, which will inhibit the activity of soil microorganisms (Garcia 2002). In the present study we have collected soils from different altitude with different plant composition and biomass.

These differences must lead to changes in soil organic carbon, available nutrient and other soil physicochemical characteristics; which in turn could cause variation in the microbial load in different sampling locations. Temporal variation is mainly due to the environmental factors like precipitation, air temperature etc. These environmental factors in turn affect the soil temperature, soil moisture content and other soil properties.

Table 2. Bacterial, Fungal and actinomycetes load during pre monsoon, monsoon and post monsoon (CFU/1g f.w)

Sampling sites	Season	Bacteria (CFU/1gf.w)	Fungus (CFU/1g f.w)	Actinomycetes (CFU/1g f.w)
SH1	Pre monsoon	202×10^5	56×10^3	25×10^3
	Monsoon	121×10^5	14×10^3	9×10^3
	Post monsoon	172×10^5	21×10^3	4×10^3
	Pre monsoon	193×10^5	48×10^3	19×10^3
SH2	Monsoon	114×10^5	9×10^3	24×10^2
	Post monsoon	163×10^5	17×10^3	8×10^3
	Pre monsoon	174×10^5	41×10^3	36×10^3
SH3	Monsoon	101×10^5	6×10^3	6×10^3
	Post monsoon	156×10^5	11×10^3	11×10^3
	Pre monsoon	211×10^5	61×10^3	11×10^3
SH 4	Monsoon	136×10^5	17×10^3	21×10^2
	Post monsoon	180×10^5	26×10^3	37×10^2
	Pre monsoon	224×10^5	66×10^3	16×10^3
SH5	Monsoon	144×10^5	22×10^3	21×10^2
	Post monsoon	188×10^5	33×10^3	40×10^2
	Pre monsoon	161×10^5	36×10^3	40×10^3
SH6	Monsoon	88×10^5	30×10^2	11×10^3
	Post monsoon	140×10^5	7×10^3	18×10^3

CFU – Colony Forming Units, f.w – Fresh Weight

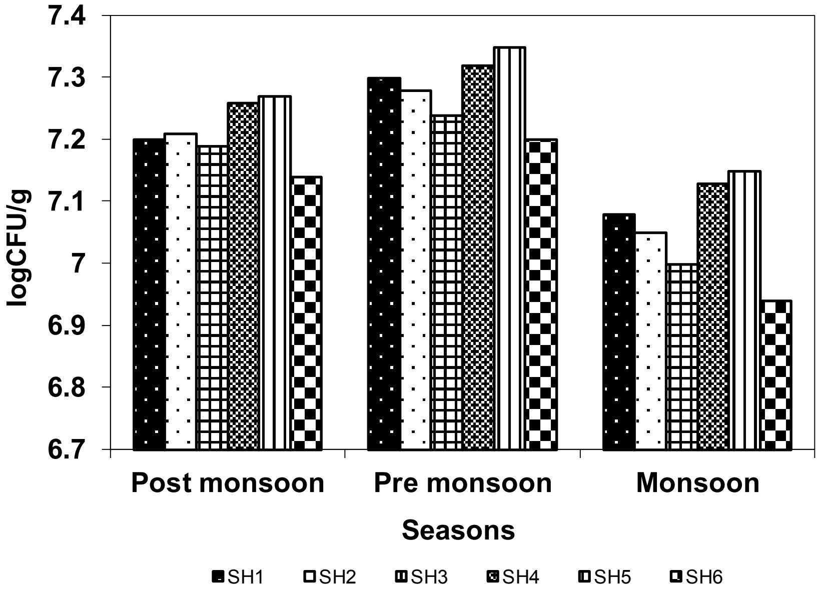

Figure No. 1 Spatiotemporal variation of bacterial population in shola soils of Tropical Montane forest (logCFU/1g f.w)

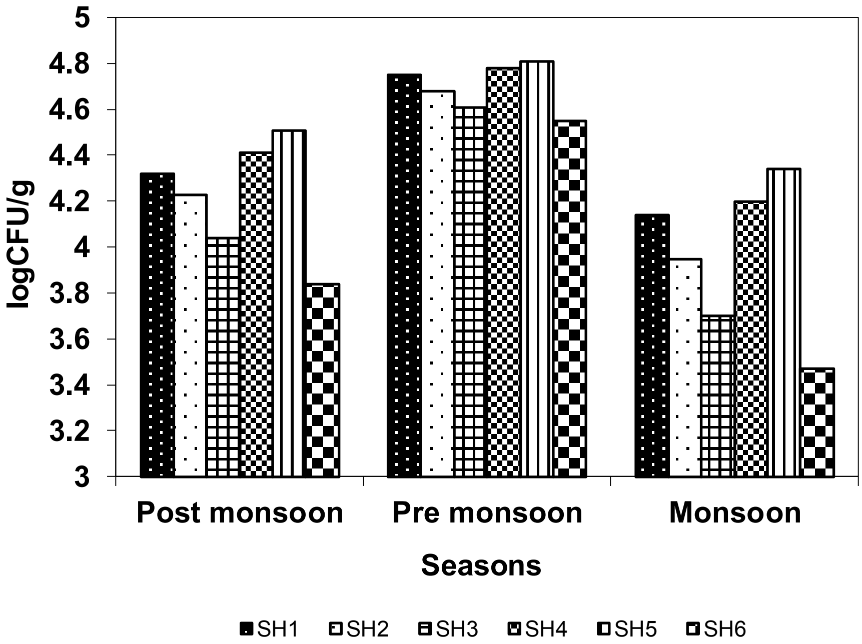

Figure No. 2 Spatiotemporal variation of fungal population in shola soils of Tropical Montane forest (logCFU/1g f.w)

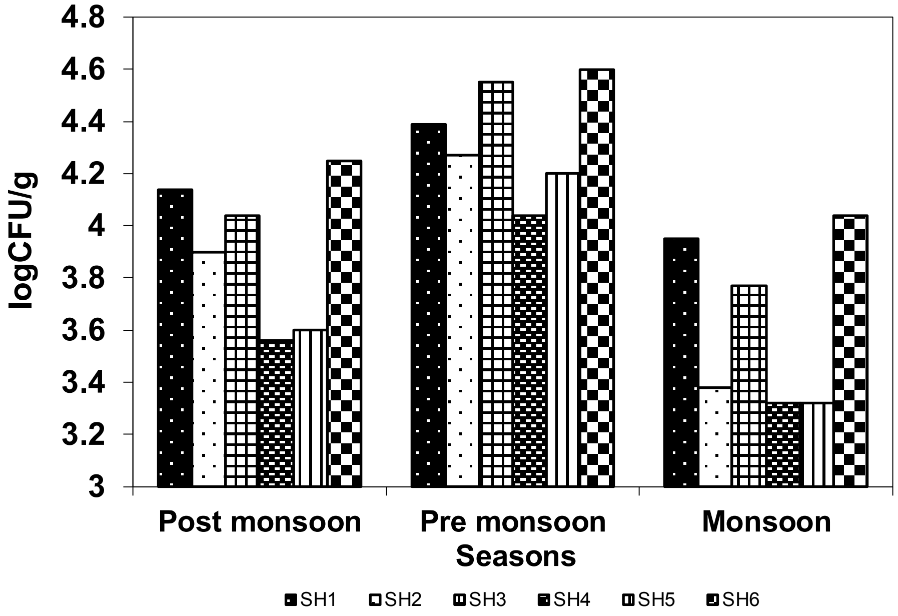

Figure No. 3 Spatiotemporal variation of Actinomycetes population in shola soils of Tropical Montane forest (logCFU/1g f.w)

Correlation of Physicochemical Properties of Soil with Microbial Load

Correlation of microbial load with soil physicochemical properties such as soil temperature, moisture, pH, Organic carbon, available nitrogen, phosphorous and available potassium *etc.* were studied (Table No. 3). The distribution of soil microbial population is determined by a number of factors like pH, temperature, moisture content, nutrients, soil texture and soil organic matter (Kennedy et al. 2005). The soil temperature in the present study ranged between 10^{o}C and 15^{o}C and it positively correlated with microbial load, as observed previously by Cho (2008). From the present study it was observed that the load of bacteria, fungus and actinomycetes were high during pre monsoon season because of comparatively high soil temperature. Chhonkar and Tarafdar (1984) reported that soil temperature had great impact on soil microorganisms.

The soil moisture varies from 29.91% in site SH3 during pre monsoon season to 63.11% in SH5 during monsoon season. The correlation studies revealed that fungal load showed positive correlation and the actinomycetes load showed significant negative correlation with moisture content. Actinomycetes prefer dry soil than wet and the load were reported to be high in desert soil. Most of them are resistant to heat and the water logging of the soil is unfavorable for the growth of actinomycetes (Zenova et al. 2007).

Soil pH was found to be moderately to weakly acidic. In the present study load of fungus was higher when compared to that of actinomycetes. Actinomycetes are intolerant to low pH and their numbers reported to decline at pH below 5 (Basilio 2003), fungus prefer acidic soil (Cho 2008) than neutral and alkaline soil. In one of his studies Basilio (2003) observed that the highest numbers of actinomycetes strains were obtained from pH 7 to 11. Present study showed a positive correlation between the load of actinomycetes and soil pH and a negative correlation between fungus and soil pH (not significant).

Carbon availability also may influence microbial community. Of the three seasons studied, site SH5 showed highest percentage of organic carbon content (5.7) and site SH6 showed the least (2.2). There was a significant positive correlation between bacterial and fungal population with organic carbon content. Nohrstedt (1985) have also concluded that the soil organic carbon from several forests was the factor positively influencing microbial load. There was significant positive correlation between the available nitrogen, available potassium and phosphorous with bacterial and fungal load. Actinomycetes also showed positive correlation with available nitrogen and potassium but not significant. Allen and Schlesinger (2004) stated that the number and species of microbes in the soil varies instantly with nutrient availability.

Table No. 3 Correlation between soil physico-chemical parameters and microbial load

	Bacteria	**SOC**	**pH**	**ST**	**SMC**	**N**	**P**	**K**
Bacteria	1							
SOC	0.638**	1						
pH	-0.147	-0.470*	1					
ST	0.139	0.018	-0.192	1				
SMC	0.095	0.544*	-0.650**	-0.398	1			
N	0.695**	0.480*	-0.277	0.474*	0.117	1		
P	0.784**	0.686**	-0.276	0.145	0.270	0.493*	1	
K	0.707**	0.429	-0.390	-0.163	0.487*	0.583*	0.483*	1
	Fungi	**SOC**	**pH**	**ST**	**SMC**	**N**	**P**	**K**
Fungi	1							
SOC	0.749**	1						
pH	-0.351	-0.460*	1					
ST	0.372	0.055	-0.199	1				
SMC	0.120	0.470*	-0.643**	-0.399	1			
N	0.707**	0.504*	-0.288	0.480*	0.109	1		
P	0.828**	0.736**	-0.291	0.167	0.240	0.518*	1	
K	0.576**	0.422	-0.395	-0.154	0.482*	0.588**	0.481*	1
	Actinomycetes	**SOC**	**pH**	**ST**	**SMC**	**N**	**P**	**K**
Actinomycetes	1							
SOC	-0.324	1						
pH	0.226	-0.470*	1					
ST	0.437	0.018	-0.192	1				
SMC	-0.592**	0.544*	-0.650**	-0.398	1			
N	0.437	0.480*	-0.277	0.474*	0.117	1		
P	-0.028	0.686**	-0.276	0.145	0.270	0.493*	1	
K	0.170	0.429	-0.390	-0.163	0.487*	0.583*	0.483*	1

**Correlation is significant at the 0.01 level (2-tailed)
*Correlation is significant at the 0.05 level (2-tailed)

SOC: *Soil organic carbon,* ***ST:*** *Soil temperature,* ***SMC:*** *Soil moisture content,* ***N:*** *Available nitrogen,* ***P:*** *Available phosphorus,* ***K:*** *Available potassium*

CONCLUSION

In conclusion, shola forest soils were weakly acidic and the microbial load were characterized by high load of bacteria followed by fungi and actinomycetes and showed both temporal and spatial variation. Bacterial, fungal and actinomycetes load were higher during pre monsoon followed by post monsoon and monsoon. Microbial load varied with the soil properties and there was a significant positive correlation between bacterial and fungal load with total organic carbon and NPK. Actinomycetes showed significant negative correlation with moisture content.

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