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Spatial and Temporal Variation of Microbial Population in the Grassland Soils of Tropical Montane Forest: Influence of Soil Physico-Chemical Characteristics and Nutrients

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Abstract

Present study is focused on the spatiotemporal variation of the microbial population (bacteria, fungus and actinomycetes) in the grassland soils of tropical montane forest and its relation with important soil physico-chemical characteristics and nutrients. Different physico-chemical properties of the soil such as temperature, moisture content, organic carbon, available nitrogen, available phosphorous and available potassium have been studied. Results of the present study revealed that both microbial load and soil characteristics showed spatiotemporal variation. Microbial population of the grassland soils were characterized by high load of bacteria followed by fungus and actinomycetes. Microbial load was high during pre monsoon season, followed by post monsoon and monsoon. The microbial load varied with important soil physico-chemical properties and nutrients. Organic carbon content, available nitrogen and available phosphorous were positively correlated with bacterial load and the correlation is significant at 0.05 and 0.01 levels respectively. Available nitrogen and available phosphorous were positively correlated with fungus at 0.05 level significance. Moisture content was negatively correlated with actinomycetes at 0.01 level of significance. Organic carbon negatively correlated with actinomycetes load at 0.05 level of significance.

Key words: Season, monsoon, actinomycetes, fungi, bacterial load

Introduction

Productive forests need healthy and living soil, clean air, and water. Healthy soils are active with organisms of all shapes and sizes with bacteria, insects, worms, fungi, and animals. Some organisms form partnerships with tree roots helping them to extract nutrients from the soil. Others are significant in breaking down organic matter and cycling nutrients, making them accessible to the coming generation of plants and animals. Forest soil microorganisms and fauna powerfully influence the nutrient cycling of ecosystem through regulating the dynamics of organic matter decomposition and plant nutrient availability. Environmental factors that affect and potentially alter soil microbial assemblage structure and function are complex. It is well known that environmental variables, such as temperature, moisture, nutrient availability and soil pH, can influence the composition and activity of microbial assemblages[1-4]. As biological activity of the soil is directly linked to many ecosystem-level processes such as decomposition, nutrient mineralization etc., the study of soil microorganisms is very important. Soil accommodates one of the most complex and dynamic microbial assemblies in the entire biosphere. Different sizes of soil particles and aggregates in infinite combination result in a highly diverse physical environment with heterogeneity readily displayed at very fine scale[5,6]. The range of physical characteristics of soil can harbor a large diversity of microorganisms in close proximity due to the minute size microorganisms. Furthermore, the relationships between microorganisms and soil particles are fully interactive: while soil particles control the survival and biological activity of microorganisms[7]. The chemical composition of soil is also highly heterogeneous in both vertical and horizontal dimensions[8,9].

Spatial variations in soil-related variables have different patterns[10]. These differences are rooted in the complex relationships between each soil-related variable and the aspects of the soil that they describe[11]. In this work, we explore the spatial and temporal variation of bacteria, fungus and actinomycetes population in the grasslands soils of tropical montane forest of Kerala, India. We place our focus on soil microbial variation, but study some physico-chemical characteristics and nutrients of the soil which, we feel, should affect this variation.

Experimental

Study area and sample collection

The study area is located at the top areas of Eravikulam National park in Idukki district (Anamudy Region) of Kerala: highest area of south India at an altitude of 1900 m to 2400 m above Mean Sea Level (MSL). The geographical location lies between 10º05' - 10º20' North latitude and 77º0' - 77º10' East longitude. Most of the land in this area is covered by grass lands and shoal forest. Soil samples were collected from six sites located at increasing altitude (1900m, 2000m, 2100m, 2200m, 2300m and 2400m above MSL) during premonsoon, monsoon and post monsoon seasons. At each site, 6 individual random samples were collected and pooled together and homogenized so as to obtain representative sample. Samples were collected at a depth of 1–2 cm (to exclude plant litter) to 20 cm using a spade that is thoroughly cleaned and disinfected between sampling so as to prevent cross-contamination.

Physico-chemical characteristics of soil

Temperature was determined *in situ* with the help of mercury bulb thermometer. Soil moisture content was determined by gravimetric method. pH of soil samples were determined potentiometrically using soil pH meter. Available nitrogen in the soil samples was determined by Microkjeldahl method[12]. Available phosphorus was estimated by Bray and Kurtz method[12]. Available potassium in the soil samples were extracted using neutral normal ammonium acetate as extractant and determined by using flame photometer[13]. The organic carbon was determined by Walkey and Black Method[12].

Enumeration of microorganisms

Enumeration of Microorganisms (Bacteria, Fungus and Actinomycetes) was carried by standard serial dilution plate technique. Ten grams of soil was transferred to 90 ml sterile distilled water and agitated vigorously. Different aqueous dilutions, 10^{-1} 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 (50 g/ml) or Amphotericin (75 g/ml) and Streptomycin (25 g/ml) were added to the Kusters Agar plates in order to prevent fungal and bacterial contamination respectively. 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 physico-chemical characteristics were carried out using SPSS software.

Results

Physico-chemical characteristics of soil

Various physico-chemical properties of the soil such as temperature, moisture content, pH, organic carbon, available nitrogen, available phosphorous and available potassium has been studied and the results are presented in Table 1. Soil temperature ranged between 11ºC and 17ºC during the study period and the maximum temperature of 17ºC was recorded during pre monsoon period from GR5 and GR6 sites and the minimum of 11ºC was recorded from Gr3 and Gr4 during post monsoon period. Soil samples collected from GR4 and Gr3 recorded highest moisture content of 56.01% and 51.19% respectively during monsoon and the lowest moisture content recorded was 8.41% during pre monsoon period from Gr6.

Table 1. Physico-chemical properties of Grassland soils during Pre monsoon, Monsoon and Post monsoon

Sampling sites	Seasons	Soil physico-chemical parameters						
		Soil temperature (°C)	Moisture (%)	pH	OC (%)	AN (Kg/ha)	AP (Kg/ha)	AK (Kg/ha)
Gr 1	Pre monsoon	16	15.57	6.1	4.7	537.6	64.4	871.70
	Monsoon	15	28.40	6.1	4.7	188.16	30.8	752.64
	Post monsoon	13	23.11	6.2	4.8	383.04	19.6	752.64
Gr 2	Pre monsoon	16	13.88	6.3	4.9	668.80	40.4	997.50
	Monsoon	15	26.14	6.1	4.8	490.56	22.4	627.20
	Post monsoon	12	20.44	6.1	5.0	544.32	30.8	564.48
Gr 3	Pre monsoon	15	42.44	5.1	6.1	927.36	86.8	1073.41
	Monsoon	16	51.19	5.3	5.4	418.24	47.6	501.76
	Post monsoon	11	46.11	5.9	5.9	577.92	86.8	689.92
Gr 4	Pre monsoon	14	51.32	5.6	5.9	719.04	80.1	1412.54
	Monsoon	15	56.01	5.5	5.6	604.80	47.6	752.64
	Post monsoon	11	54.03	5.7	6.0	833.28	55.1	501.76
Gr 5	Pre monsoon	17	11.99	6.4	2.3	779.52	39.2	778.08
	Monsoon	16	21.01	6.3	1.7	537.60	25.2	752.64
	Post monsoon	14	18.40	6.8	2.0	443.52	30.8	544.48
Gr 6	Pre monsoon	17	08.41	6.4	2.1	557.36	70.1	652.64
	Monsoon	16	15.10	6.3	1.2	551.04	22.4	627.20
	Post monsoon	15	13.03	6.5	1.9	524.16	48.4	376.32

Gr = Grassland; OC = Organic carbon; AN = Available Nitrogen; AP = Available Phosphorus; AK = Available Potassium

The pH values ranged from 5.1 to 6.8 and the highest pH recorded from Gr5 during post monsoon and lowest pH recorded from GR3 during pre monsoon period.

In the present study highest organic carbon of 6.1% recorded from Gr3 site during pre monsoon period and lowest organic carbon of 1.16% from Gr6 during monsoon period. High values of available nitrogen recorded during pre monsoon (Gr4) and lowest during post monsoon period (Gr6), in post monsoon period it was ranged from 376.32 - 752.64 Kg/ha, in pre monsoon 652.64-1412.54 Kg/ha and during monsoon season 501.76 – 752.64 Kg/ha. Soil sample from Gr3 showed the highest available potassium of 927.36 kg/ha in pre monsoon and the least available potassium of 188.16 kg/ha recorded from Gr1 during the monsoon period. The highest value of available phosphorous of 86.8 kg/ha recorded from Gr3 and the least value 19.6 kg/ha from Gr1 during post monsoon period.

Dynamics of Microbial Load

Bacterial load was higher during pre monsoon season followed by post monsoon and monsoon and it ranged from 134 $\times 10^5$ to 168×10^5 CFU/g during post monsoon, from 160×10^5 to 228×10^5 CFU/g during pre monsoons and from 89×10^5 to137×10^5 CFU/g during monsoons. The fungal load of the grassland soils ranged from 17×10^3 to 41×10^3 CFU/g in post monsoon, from 29×10^3 to 61×10^3 CFU/g in pre monsoon, from 8×10^3 to 40×10^3 CFU/g in monsoon. Actinomycetes load varied between 4.1×10^2 and 26×10^3 CFU/g in post monsoons, from 17×10^3 to 44×10^3 CFU/g in pre monsoon and from 3.0×10^2 to17×10^3 CFU/g in monsoon. Bacterial, fungal and actinomycetes load were higher during pre monsoon followed by post monsoon and monsoon. In all the sites bacterial load was higher followed by fungal and actinomycetes load with exception at two sites where actinomycetes came second to bacteria (Table 2).

Correlation between Microbial load and Soil Physicochemical Characteristics

Organic carbon content, available nitrogen and available phosphorous were positively correlated with bacterial load and the correlation is significant at 0.05 and 0.01 levels respectively. Moisture content was positively correlated with bacterial load

Table 2. Spatiotemporal variation of microbial population in Grassland soils of Tropical Montane forest

Sampling sites	Seasons	Microbial load (CFU/g)		
		Bacterial load(x 10^5)	Fungal load(x 10^3)	Actinomycetes load (x 10^3)
Gr 1	Pre monsoon	191	54	30
	Monsoon	121	17	06
	Post monsoon	154	31	14
Gr 2	Pre monsoon	200	40	24
	Monsoon	111	08	10
	Post monsoon	166	27	20
Gr 3	Pre monsoon	209	61	17
	Monsoon	130	27	3.6
	Post monsoon	168	41	4.1
Gr 4	Pre monsoon	228	51	20
	Monsoon	137	21	3.0
	Post monsoon	168	41	4.1
Gr 5	Pre monsoon	177	34	44
	Monsoon	89	40	17
	Post monsoon	146	23	20
Gr 6	Pre monsoon	160	29	35
	Monsoon	101	34	11
	Post monsoon	134	17	26

but did not show any significance. Temperature and pH were negatively correlated with bacterial load but not significant (Table 3). Available nitrogen and available phosphorous were positively correlated with fungus at 0.05 level significance. Temperature, pH, and available potassium were negatively correlated with fungus but no significant. Organic carbon and moisture content were positively correlated with fungal load but not show any significance (Table 4). Moisture content was negatively correlated with actinomycetes at 0.01 level of significance. Organic carbon negatively correlated with actinomycetes load at 0.05 level of significance. Soil temperature and available nitrogen were positively correlated with actinomycetes load and did not show any significance. Available phosphorous and available potassium were negatively correlated with actinomycetes load but not significant (Table 5).

Discussion

Physico-chemical characteristics of soil

Grassland soils of tropical montane forest were feebly acidic and the pH ranged from 5.1 to 6.8. Physico-chemical characteristics of soil showed variation in both spatially and temporally. Spatial variations of soil characteristics are mainly due to the topography of the study area; samples were

Table 3. Correlation between bacterial load and physicochemical characteristics o soil

	B L	ST	SMC	pH	SOC	N	P	K
B L	1							
ST	-0.251	1						
SMC	0.246	-0.447	1					
pH	-0.302	0.189	-0.870**	1				
SOC	0.541*	-0.518*	0.743**	-0.775**	1			
N	0.629**	0.091	0.297	-0.458	0.404	1		
P	0.675**	-0.155	0.518*	-0.521*	0.445	0.561*	1	
K	0.122	-0.055	0.117	0.037	0.046	0.006	-0.089	1

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

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

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

Table 4. Correlation between Fungal load and physicochemical characteristics of soil

	F L	ST	SMC	pH	SOC	N	P	K
F L	1							
ST	-0.030	1						
SMC	0.183	-0.447	1					
pH	-0.281	0.189	-0.870**	1				
SOC	0.159	-0.518*	0.743**	-0.775**	1			
N	0.566*	0.091	0.297	-0.458	0.404	1		
P	0.548*	-0.155	0.518*	-0.521*	0.445	0.561*	1	
K	-0.039	-0.055	0.117	0.037	0.046	0.006	-0.089	1

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

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

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

collected from both valleys and slopes of different angles. A number of studies have exposed that even minute scale topographical landforms can change environmental conditions[14,15]. The same results were observed by different researchers where the organic carbon showed to vary with slope position and variation of soil properties within a distinct climatic area may also result from topographic heterogeneity[16, 17,18].

Another important reason for the spatial variation may be the vegetation cover. The difference in vegetation should affect the soil environment in many ways, including changing light intensity, wind, temperature, and soil moisture and nutrients; these should lead to remarkable changes in soil microbial diversity and structure[19,20]. In the present work vegetation differs considerably between the sampling locations because of samples were collected from different altitude of the grassland. Influences of plant composition and diversity on soil physico-chemical characteristics are reported by many researchers[21,22].

Temporal variations of the soil physico-chemical characteristics are mainly attributed to climatic factors. Seasonally variable environmental factors like temperature, precipitation etc. influence soil characteristics[23], for example precipitation cause changes in soil moisture content, soil temperature, pH etc. In the present study soil moisture content in monsoon seasons was higher than in other two seasons, mainly attributable to the raining season during June – September. The pH of the grassland soils was weakly acidic due to the high plant organic matter on the surface, the decomposition of plant debris leads to the accumulation of organic acids[24]. The pH values were slightly lower in pre monsoon seasons than in post monsoon and monsoon seasons due to the better temperature in pre monsoon season for active microbial activity and it leads to organic acid accumulation[25]. The low pH during

Table 5. Correlation between Actinomycetes load and physicochemical characteristics of soil

	A L	ST	SMC	pH	SOC	N	P	K
A L	1							
ST	0.398	1						
SMC	-0.735**	-0.447	1					
pH	0.551*	0.189	-0.870**	1				
SOC	-0.497*	-0.518*	0.743**	-0.775**	1			
N	0.267	0.091	0.297	-0.458	0.404	1		
P	-0.005	-0.155	0.518*	-0.521*	0.445	0.561*	1	
K	-0.084	-0.055	0.117	0.037	0.046	0.006	-0.089	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, AL: Actinomycetes load, N: Available nitrogen, P: Available phosphorus, K: Available potassium monsoon season may be attributed to high rainfall, which is sufficient to leach basic cations from the surface horizons of the soil.

Dynamics of micro flora

Microbial population of the grassland soils of tropical montane forest are characterized by comparatively high load of bacteria followed by fungus and actinomycetes. Microbial load exhibited both temporal and spatial variations and the microbial load was high during pre monsoon season, followed by post monsoon and monsoon seasons. Spatial variation of the microbial load attribute to variation of the soil characteristics and vegetation structure due to the difference in terrine of the study area. Number of studies has shown that even small scale topographical landforms can alter environmental conditions, which in turn retard or accelerate the activity of organisms[15]. The effects of topographical landforms on species composition, productivity, environmental conditions, and soil characteristics have been well investigated[14]. Seasonal variation in microbial population is due to change in climatic factors. Seasonally-variable environmental factors, like temperature and soil moisture, may influence the soil microbial community and activity and control the organic matter decomposition process [26].

Correlation of microbial load with physiochemical characteristics

The pH of the grassland soils was weakly acidic due to the high amount of plant debris on the surface; the decomposition of which leads to the accumulation of organic acid resulting in acidity of the soil[24]. Actinomycetes showed significant positive correlation with pH. Basilio et al.[27] reported that actinomycetes prefer neutral and alkaline pH and the fungus prefer acid soils. In the present study, acidic pH of the soil samples may be one of the reasons for fewer loads of actinomycetes compared to fungus population. While the relationship between microbial load and temperature between different sites were not significant, significant seasonal variation of the microbial load with temperature was observed during the study period. From the present study it was observed that the load of bacteria, fungus and actinomycetes were high in pre monsoon season because of comparatively high soil temperature. Increase in the bacterial community during the pre monsoon has been attributed to increase in the temperature[28]. The correlation studies revealed that fungal and bacterial load showed positive correlation with moisture content and actinomycetes load showed significant negative correlation with moisture content. It is reported that the actinomycetes load was high in desert soil and most of them are resistant to heat and the water logging of the soil is unfavorable for the growth of actinomycetes[29].

Results of the present study showed positive correlation between bacterial and fungal loads with organic carbon content. Nohrstedt[30] have also concluded that the soil organic carbon from several forests was the factor positively influencing microbial population. Negative correlation between the actinomycete load and organic carbon content may be due to the influence of other soil factors and soil topography. There was also a significant positive correlation between the available nitrogen and phosphorous with bacterial and fungal load. Report of Allen and Schlesinger[31] is comparable with the present study about the nutrient correlation with microbial load. In conclusion Grassland soils of tropical montane forest were weakly acidic and the microbial load was characterized by high number of bacteria, followed by fungi and actinomycetes. Bacterial, fungal and actinomycetes load was higher during pre monsoon followed by post monsoon and monsoon. There was a spatial and temporal variation of microbial load during the study period and it showed significant correlations between important soil physicochemical characteristics.

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