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Soil Microbial Diversity of Dibru-Saikhowa Biosphere Reserve Forest of Assam, India

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Abstract - An experiment was carried out in five different sampling sites viz. Kalia, Miripathar, Bordubi, Holokhbari and Surkey area of Saikhowa range of Dibru-Saikhowa Biosphere Reserve (DSBR) Forest of Assam, India in respect of seasonal and depth wise variations in bacterial and fungal populations. The Biosphere Reserve Forest is situated in flood plain area of the mighty Brahmaputra and the Dibang rivers. Bacterial and fungal population was highest during spring season in all sites respectively. The highest microbial counts were recorded in the top soil (0-10 cm) layer except during the summer season when the population was greater in the subsurface (10-20 cm) layer. Altogether, 26 soil micro-fungal forms were recorded from five sites. Aspergillus and Penicillium were the abundant genera in all sites. Over all, soil was sandy loam and slightly alkaline in all sites. Soil organic C, total N and available P decreased with increasing soil depth. Parameters viz. water holding capacity, soil moisture content, pH, organic C, total N and available P had correlated with the microbial colony forming units (cfu). Available P revealed a significant positive correlations with bacterial and fungal cfu as well as inverse correlations with water holding capacity, organic C and organic matter.

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I. Introduction

Soil is a dynamic, biological system that is an essential part of the terrestrial ecosystem. The functions of soil biota are central to decomposition processes and nutrient cycling. Soil microorganisms play an important role in soil processes that determine plant productivity. Soil fungi make a very important part of the ecosystem along with other microbes in turnover of the biomass (James & Hyde1998).

There is an urgent need to conserve biodiversity at global level to preserve the endemic and endangered species, both microscopic and macroscopic which plays vital role for the maintenance of sustainable environment, agriculture and forestry (Jha *et al.* 2002). Microbial diversity plays a dominant role in the maintenance of ecosystem. Soil microorganisms are the major organisms responsible for controlling the amount of nutrient cycling and for controlling the amount of nutrient available to plants (Hernot & Robertson 1994; Singh & Rai 2004; Jain *et al.* 2005). The soil microbes decompose the plant and animal residues entering the soil and convert them into soil organic matter, which influences on soil physical, chemical and biological properties and on creating a complimentary medium for biological reactions and life support in the soil environment (Olson *et al.* 2000). Soil fertility status is dependent upon soil microbial component and their mediated processes (Lynch 1984).

This study deals with the seasonal and depth wise variations in soil bacterial and fungal populations in relation to the micro-environmental and soil nutrient variability in Dibru-Saikhowa Biosphere Reserve (DSBR) Forest of Assam, India. North eastern region of India including Assam is a potential biodiversity area and so it is included in 19 mega diversity regions of the world and 12 diversity areas of the country. A number of studies on the distribution of forest soil micro fungi in terrestrial ecosystem. Some studies dealt with depth effects (Arunachalam *et al.* 1997) and others attempted to examine seasonal trends (Kennedy *et al.* 2005). However, the rate at which organic matter is decomposed by the microbes is interrelated to the chemical composition of the substrate as well as environmental conditions. Substantial works for conservation of diversity and preservation of their genetic resources have done but works on microbial dynamics is very meager in this region. To go about the aforementioned approach, data on the seasonal and depth wise variations in bacterial and fungal populations in DSBR forest had consummated and discussed in this paper to derive some conclusions regarding the influence in relation to micro-environmental and soil nutrient variability on the microbial population dynamics.

II. Materials and Methods

a) Study Area

Dibru-Saikhowa Biosphere Reserve forest is located between 27^0 35' - 27^0 50' North latitude and 95^{0}10' to 95^0 40' East longitude. It is a safe haven for many extremely rare and endangered species of wild life including 300 avifauna and various species of shrubs, herbs and medicinal plants. Wetlands cover sixty percent of the total area while forest and grassland covers 25 and 15 percent respectively. The mean annual

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rainfall ranges between 2300 to 3600 mm in different localities within the sampling areas. The temperature remains moderate and the annual temperature varies from 6^0 to 36^0C while the humidity varies from 64 to 68 percent respectively in all the study sites experiencing a humid tropical climate (Dutta *et al.* 1997). The reserve forest is mainly consisted of semi wet evergreen forests and tropical moist deciduous forest Champion *et al.* 1968). Total area of the reserve forest is 340 sq km comprising two ranges *viz*. Dibru and Saikhowa.

b) Soil Sampling and Analysis

Soil from three depths (*viz*. 0-10, 10-20 and 20-30 cm) were sampled during January, March, May and November that are the representative months of winter, spring, summer and autumn seasons. In each stand, five replicates of soil samples were collected aseptically in sterilized polythene bags using a steel corer (6.5cm inner diameter) and were used for the isolation of bacteria and fungi within 24 h. The water holding capacity, pH and soil moisture content were determined by standard method Trivedi *et al.* 1987. The remaining soil samples were air dried and taken to the laboratory at the Department of Life Sciences, Institute of Advanced Study in Science and Technology, Guwahati, Assam for analysis of soil physical and chemical properties. Particle size composition was determined using the hydrometer method Bouyoucos 1962; organic carbon by the Walkley-Black method (Walkley & Black 1934), after which values obtained were multiplied by 1.72 (Pluske *et al.,* 2009) to convert to organic matter; total nitrogen by the Kjeldahl method (Bremner & Mulvaney 1982); available phosphorus was determined by the method of Bray & Kurtz 1954.

c) Isolation of Bacterial and Fungal Population

Soil bacterial population was estimated by the method of Waksman 1952 using the nutrient agar medium at 10^5 dilutions. The inoculated petri-dishes were incubated at 30± 1^0C for 24 h for 5 days for bacterial colonies. For isolation and characterization of fungi dilution plate method was used at 10^3 dilutions in distilled water. Potato dextrose agar, czapek dox agar and fungal agar media were used as basal medium to isolate and characterization of fungal species (Cappucino & Sherman 1983, Warcup1955). The inoculated petri-dishes were incubated at 25 ± 1^0C for 5 days for growing the fungal colonies. Representative isolates of fungi were identified under the microscope with the help of standard manuals (Gilman 1995, Barnett & Hunter 1972).

d) Data Analysis

To calculate the populations of bacteria and fungi, colonies developed on petri dishes were counted with the help of digital colony counter and expressed as number of colony forming units (cfu) gm^{-1} of dry soil.

III. Results

a) Physico-Chemical Properties

The highest (64.36) and the lowest (30.82) percentage of water holding capacity and moisture content (32.2) and (15.5) were recorded in Bordubi and Miripathar area respectively. The maximum (8.52) and minimum (7.65) pH were recorded in Surkey and Kalia area respectively and both the highest (0.91) and lowest (0.32) percentage of organic carbon was recorded in Kalia area. The highest (1.575) and lowest (0.563) percentage of soil organic matter were also recorded in Kalia area. The range of available phosphorus was recorded as 1.124 to 1.893 ppm and nitrogen content was recorded as 0.164 to 0.398 gm^{-1} of soil. (Table 1).

b) Microbial Population Dynamics

Table 2 revealed that the bacterial and fungal counts were greater in the surface (0-10 cm) layer of the soil as compared to others. The maximum bacterial and fungal population was recorded in spring season in all the sites and minimum during winter. During rainy season, maximum microbial count was, however, recorded in the subsoil (10-20 cm) layer. Both bacterial and fungal cfu's were more in Kalia, Miripathar and Surkey sites than in Holokhbari and Bordubi. Quantitatively, bacterial counts were always high as compared to fungal population in all the sites. In general, similar trend was observed in the distribution of soil microorganisms in all the sites. (Table 2).

c) Microbial Population

During the investigation period, about 26 nos. of different dominant fungal species were observed in all the study sites of Dibru-Saikhowa Biosphere Reserve Forest. The relative proportion of different fungal taxonomic groups was almost identical in all the five sites. *Penicillium* and *Aspergillus* were the most abundant group of species in all the five sites. However, some species were restricted to a particular site. *Aspergillus fumigatus, Trichoderma viride* and *Rhizopus* sp. were exclusively found in Kalia, while *Curvularia* sp. was encountered only in Miripathar area. (Table 3).

d) Correlation Analysis

Over all, soil was sandy loam and slightly alkaline in all sites (Table 1). Among the soil physico-chemical properties, available P show significant positive correlations with bacterial and fungal cfu (<0.05); water holding capacity, organic C and organic matter showed negative correlations. The soil moisture content, pH and total N showed both positive and negative correlations equally for bacterial and that of fungalpopulations. (Table 4).

IV. Discussion

Top layer soil (0-10 cm) contains high organic matter, which in the presence of adequate moisture supply is acted upon by the microorganisms to decompose the complex organic residues into simpler forms; hence, microbial counts are generally higher in the surface soil layer as compared to the lower depths (Shamir & Steinberger 2007). However, the distribution of soil microbial population is determined by a number of environmental factors like pH, moisture content and soil organic matter (Kennedy *et al.* 2005). Higher bacterial population in the topsoil (0-10 cm) layer during spring season in present study is in agreement with the observation who recorded higher populations during spring and post-rainy seasons (Jha *et al.* 1992a. However, peak in bacterial population was recorded during rainy season may be attributed to favorable soil moisture and temperature conditions that coincide with greater microbial activity and decomposition. The litter and other plant residues are decomposed faster during rainy season and sufficient soil organic matter and humus accumulates that may have enhanced the colonization of the soil microbes in subsequent period (Arunachalam 1997). Maximum population in the subsoil (10-20 cm) layer during rainy season in all the five sites studied correlated (Classen *et al.* 2007) who pointed out that during hot summer months, the sub-layer of soil occasionally harbors more fungal populations caused by temperature and moisture regimes than the topsoil layer. Higher rate of infiltration in the loamy sand may also in part have contributed to this phenomenon. Further, overall reduction in microbial population in the lower soil depths was attributed to fewer amounts of minerals, low oxygen content and increased carbon-dioxide concentration (Shukla *et al.* 1989). Consequently fungal flora noted in the deeper depths of soil was significantly low in all the five sites. During winter, low moisture content might have slowed down microbial activity and organic matter decomposition and thus resulted in a low microbial population.

A total of 26 soil microfungi were isolated (Table 3). However, only a few fungal species were found to be dominant and basically no marked variations in the composition of species were noticed in different seasons of the year across the sites. The species like *Aspergillus, Penicillium* and *Rhizopus* were common to all sites. Dominance of the genus *Penicillium* and *Aspergillus* in the present study sites may be due to their greater rate of spore production and dispersal and partly due to their resistance over extreme environmental conditions (Schimel 1995). The fungal species richness recorded in the present study was higher than those (13 species) reported from subtropical humid forest soils in north-east India and 26, 21 and 27 species, respectively from soils of valley land, terrace and slopes in this region (Kennedy *et al.* 2000; Shukla & Mishra 1992).

The topsoil layer (0-10 cm) had 20 species which was greater than the subsoil layer (10-20 and 20-30 cm) (Table 3). Fungi grow slowly in the deeper soil layers due to shortage of mineral nutrients and compaction of soil along depth (Dkhar 1983). The rate of change in fungal population was attributed to the type of vegetation growing on a particular area (Entry & Emmingham 1996), variation in physico-chemical characteristics of the soil and environmental complex of the locality (Bossio *et al.* 2005). Higher counts of bacterial and fungal population in Kalia, Miripathar and Surkey sites may be attributed to the dense growth of plants and greater availability of nutrients on account of greater accumulation of litter and may also be due to spreading of other biodegradable wastes in the system. Contrarily, low microbial population in Bordubi and Holokhbari sites may be because addition of plant remains and other organic wastes in these sites was comparatively poor that also resulted in low organic matter content in the soil. Many workers have also recorded a correlation between fungal species composition and the species composition of the aboveground vegetation (Chung *et al.* 2007). Several microbes promote plant growth and many microbial products that stimulate plant growth. They were described the conditions under which bacteria live in the rhizosphere. To exert their beneficial effects, bacteria usually must colonize the root surface efficiently. They also described several mechanisms by which microbes can act beneficially on plant growth are described *viz.* biofertilization, stimulation of root growth, rhizoremediation and plant stress control (Lutenberg & Kamilova 2009).

V. Conclusion

The present study concludes that the population of bacteria and fungi in the soils of Dibru-Saikhowa Biosphere Reserve Forest are influenced by vegetation, physico-chemical properties and species composition. However, the role of macro and micro-climatic seasonality and soil nutrient status cannot be completely ruled out. It is also understood that the quality of plant residues accumulating in these sampling sites are furthermore important and may play a vital role in soil nutrient management within the system through microbial decomposition.

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Table 1 : Physico-chemical parameters of soil samples collected from Saikhowa Range of Dibru-Saikhowa Biosphere Reserve Forest

Sampling Sites	Soil depth (cm.)	Texture	WHC (%)	SMC (%)	pH	Organic Carbon (%)	Soil Organic Matter (%)	Available P (ppm)	Nitrogen (gm^{-1})
Kalia area	0-10	SL	61.10±0.315	28.8±0.10	7.65±0.015	0.91±0.015	1.575±0.026	1.636±0.031	0.383±0.007
	10-20		54.10±0.374	24.2±0.05	7.89±0.015	0.34±0.006	0.598±0.010	1.478±0.005	0.372±0.008
	20-30		34.77±0.392	19.4±0.10	8.33±0.010	0.32±0.015	0.563±0.026	1.312±0.003	0.266±0.013
Miripathar area	0-10	SS	30.82±0.224	15.5±0.05	8.27±0.012	0.33±0.030	0.575±0.053	1.243±0.003	0.324±0.011
	10-20		53.64±0.395	25.3±0.06	8.04±0.015	0.54±0.040	0.942±0.070	1.154±0.005	0.297±0.019
	20-30		58.71±0.180	25.8±0.05	8.25±0.020	0.63±0.015	1.092±0.027	1.142±0.004	0.174±0.013
Bordubi area	0-10	SS	63.47±0.225	23.1±0.17	8.01±0.013	0.43±0.017	0.669±0.096	1.323±0.009	0.164±0.010
	10-20		63.53±0.402	32.1±0.15	8.01±0.015	0.60±0.015	0.951±0.090	1.452±0.004	0.379±0.015
	20-30		64.36±0.267	32.2±0.15	7.74±0.005	0.43±0.015	0.753±0.026	1.433±0.004	0.383±0.009
Holokhbari area	0-10	SS	54.30±0.157	26.8±0.10	8.42±0.015	0.84±0.005	1.459±0.009	1.268±0.003	0.310±0.018
	10-20		40.73±0.083	25.7±0.05	8.45±0.020	0.74±0.021	1.287±0.035	1.561±0.001	0.398±0.018
	20-30		60.53±0.166	29.4±0.15	8.31±0.015	0.79±0.010	1.362±0.017	1.586±0.002	0.224±0.011
Surkey area	0-10	SL	59.80±0.125	25.8±0.06	8.13±0.015	0.51±0.015	0.885±0.026	1.893±0.002	0.168±0.007
	10-20		44.25±0.119	25.6±0.06	8.52±0.025	0.56±0.015	0.977±0.026	2.124±0.106	0.336±0.009
	20-30		52.13±0.125	21.3±0.15	8.02±0.013	0.61±0.015	1.112±0.053	1.323±0.004	0.266±0.013

CD at 5% level, SL=Sandy Loam; SS= Sandy Silt; WHC= Water Holding Capacity; SMC= Soil Moisture Content
Values presented in the table are mean of three replications and SE

Table 2 : Seasonal variations of bacterial cfu ($\times 10^5$ g^{-1} dry soil) and fungal cfu ($\times 10^3$ g^{-1} dry soil) at three soil depths (cm)

Sampling Sites	Soil depths	Bacterial (cfu) A	W	SP	S	Fungal (cfu) A	W	SP	S
Kalia area	0-10	112.67	105.20	145.67	135.10	46.00	67.00	110.33	104.00
	10-20	100.72	98.67	112.67	106.33	100.33	58.33	114.00	69.00
	20-30	38.60	13.10	61.70	53.00	12.10	41.10	50.00	23.33
Miripathar area	0-10	118.60	145.00	169.67	99.52	109.69	93.00	146.67	129.33
	10-20	104.00	42.00	97.33	88.00	18.00	43.10	100.33	56.00
	20-30	42.00	21.10	41.33	33.33	22.10	36.10	100.00	64.15
Bordubi area	0-10	91.00	97.67	123.00	114.33	88.00	56.30	156.00	100.33
	10-20	83.00	60.00	93.33	95.67	23.33	54.67	118.00	65.33
	20-30	12.00	10.00	34.33	45.67	25.00	42.20	36.33	56.00

Holokhbari area	0-10	109.20	110.00	173.33	133.67	103.10	55.00	116.33	108.33
	10-20	47.00	56.00	87.00	98.30	29.67	33.00	68.00	46.33
	20-30	19.00	10.50	63.00	60.00	19.17	30.00	35.00	38.60
Surkey area	0-10	89.10	99.00	171.33	169.20	141.10	106.67	156.33	148.33
	10-20	68.00	76.00	101.00	111.33	40.00	65.60	78.33	98.33
	20-30	9.67	23.00	34.00	22.00	23.10	15.10	56.00	28.00

A: Autumn; W: Winter; SP: Spring; S: Summer;

Values presented in the table are mean of three replication

Table 3 : Microfungi isolated from three soil depths in five sampling sites of Saikhowa Range of Dibru-Saikhowa Biosphere Reserve Forest

Name of the species	Kalia area			Miripathar area			Bordubi area			Holokhbari area			Surkey area			No. of colonies (gm^{-1} of soil)	Percent Contribution
	0-10	10-20	20-30	0-10	10-20	20-30	0-10	10-20	20-30	0-10	10-20	20-30	0-10	10-20	20-30		
Absidia spinosa Lendner	-	+	-	+	-	-	+	-	-	+	-	-	-	+	-	12.00±0.08	1.10
Aspergillus Mich. ex. Fr.	+	-	+	-	-	-	-	-	-	-	-	-	+	-	-	81.00±3.06	7.42
Aspergillus clavatus Desm.	-	-	-	-	-	-	-	+	-	+	-	-	+	-	-	74.65±0.81	6.84
Aspergillus flavus Linker Gray.	+	+	-	+	-	-	+	-	-	-	+	-	-	-	-	27.34±0.50	2.51
Aspergillus fumigatus Fres.	-	+	-	-	-	-	-	+	-	-	+	-	-	+	+	69.32±0.45	6.35
Aspergillus niger Van Tieghem.	+	-	-	+	-	-	+	-	-	+	-	-	+	+	-	51.30±0.42	4.70
Aspergillus terreus	+	-	-	-	-	-	+	-	-	+	+	-	-	-	-	78.00±1.70	7.15
Biospora Corda.	+	-	-	+	-	+	-	-	-	-	+	-	+	-	-	10.66±0.35	0.98
Curvularia Boedijn.	+	-	-	+	-	-	+	+	-	+	-	-	-	+	-	39.42±0.74	3.61
Curvularia lunata (Wakker) Boedijn.	-	+	-	+	-	-	-	+	-	+	+	-	-	-	-	23.43±1.33	2.15
Fusarium moniliforme Sheld.	+	-	-	-	-	-	+	+	-	+	-	-	-	-	-	34.20±0.32	3.13
Fusarium oxysporum Schl.	-	+	-	+	+	-	+	-	-	-	-	-	+	+	-	63.13±1.22	5.78
Mucor Mich. ex. St. -Am.	+	+	-	+	-	-	-	+	-	+	+	-	+	-	-	42.33±0.67	3.88
Mucor mucedo Mich. ex. St. -Am.	+	+	-	-	-	-	-	+	-	-	+	-	-	+	-	43.53±0.74	3.99
Penicillium Link. ex. Fr.	-	+	-	+	+	-	+	+	-	-	-	-	+	-	-	73.00±1.56	6.69
Penicillium chrysogenum Thom	+	+	+	-	+	+	-	+	+	-	+	+	+	+	+	20.11±0.72	1.84
Penicillium claviforme Bain.	+	-	-	-	-	-	+	-	-	-	+	-	-	+	-	66.00±0.51	6.05
Penicillium sacculum Dale.	+	-	-	+	-	+	-	+	-	+	-	-	+	-	-	7.33±0.54	2.50
Penicillium spinulosa Thom.	-	+	-	-	-	-	+	-	-	-	+	-	-	-	-	13.10±0.45	1.20
Rhizoctonia solani Kuhn.	-	-	-	+	-	-	-	+	-	+	-	-	+	+	-	38.10±1.57	3.49
Rhizopus Ehrenb.	+	+	-	-	-	-	+	-	-	-	-	-	+	-	+	60.38±1.91	5.53
Rhizopus stolonifer Exrenb. ex. Link.	-	+	-	-	+	+	-	+	-	-	+	+	-	-	+	42.65±0.50	3.91
Sclerotium rolfsii Sacc.	+	-	-	-	-	+	+	-	-	-	+	-	-	+	-	6.23±0.16	0.57
Trichoderma Pers. ex. Fr.	+	+	-	+	-	-	-	-	-	+	+	-	+	-	-	59.16±1.39	5.42
Trichoder ma koningi	+	-	-	-	+	-	+	-	-	-	-	-	-	+	-	11.36±0.41	1.04
Trichoderma viride Pers. ex. Fr.	-	+	-	+	-	-	-	-	-	+	-	-	+	-	-	23.65±0.25	2.17

Sign '-' and '+' indicates absence and presence of microfungi respectively Values presented in the table are mean of three replications and SE

Table 4 : Correlation coefficient (r) for the relationships between bacterial and fungal cfu and soil physico-chemical parameters of the collected soil samples

Soil depths	WHC	Soil Moisture	PH	Organic C	Organic Matter	Available P	Total N
Bacteria							
0-10	-0.985**	-0.874	0.375	-0.532	-0.483	0.395	0.346
10-20	0.311	-0.323	-0.530	-0.970**	-0.955*	0.112	-0.161
20-30	-0.472	-0.206	0.836	-0.060	-0.115	0.061	-0.485
Fungi							
0-10	-0.307	-0.435	0.455	-0.690	-0.653	0.368	-0.574
10-20	0.382	-0.107	-0.460	-0.888*	-0.906*	0.260	-0.042
20-30	0.381	0.258	-0.037	0.058	0.031	0.673	-0.358

** $p<0.05$; *$p<0.01$, N=5