Research Paper

Occurrence of culturable soil fungi in a tropical moist deciduous forest Similipal Biosphere Reserve, Odisha, India

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Abstract

Similipal Biosphere Reserve (SBR) is a tropical moist deciduous forest dominated by the species *Shorea robusta*. To the best of our knowledge their rich biodiversity has not been explored in term of its microbial wealth. In the present investigation, soil samples were collected from ten selected sites inside SBR and studied for their physicochemical parameters and culturable soil fungal diversity. The soil samples were found to be acidic in nature with a pH ranging from of 5.1-6.0. Highest percentage of organic carbon and moisture content were observed in the samples collected from the sites, Chahala-1 and Chahala-2. The plate count revealed that fungal population ranged from 3.6×10^4-2.1×10^5 and 5.1×10^4-4.7×10^5 cfu/gm of soil in summer and winter seasons respectively. The soil fungus, *Aspergillus niger* was found to be the most dominant species and Species Important Values Index (SIVI) was 43.4 and 28.6 in summer and winter seasons respectively. Among the sites studied, highest fungal diversity indices were observed during summer in the sites, Natto-2 and Natto-1. The Shannon-Wiener and Simpson indices in these two sites were found to be 3.12 and 3.022 and 0.9425 and 0.9373 respectively. However, the highest Fisher's alpha was observed during winter in the sites Joranda, Natto-2, Chahala-1 and Natto-1 and the values were 3.780, 3.683, 3.575 and 3.418 respectively. Our investigation revealed that, fungal population was dependent on moisture and organic carbon (%) of the soil but its diversity was found to be regulated by sporulating species like *Aspergillus* and *Penicillium*.

Key words: diversity indices, fungal population, SIVI.

Introduction

In the forest ecosystems, the growth and diversity of microorganisms are regulated by different physical and chemical properties like pH, organic carbon, P and K contents of the soil (Wardle *et al.*, 2004; Ogram *et al.*, 2006). Rhizosphere, which is a complex environment of forest ecosystems, is an intense site of microbial activity that harbours a great diversity of microorganisms affecting plant growth and health (Khan *et al.*, 2007). Among various microorganisms, rhizospheric fungi play an important role for decomposition of different macromolecules like celluloses, hemicelluloses and peptides in forest ecosystem (Berg and McClaugherty, 2003; Hanson *et al.*, 2008; McGuire *et al.*, 2010). The population size and distribution of these organisms is often influenced by the abundance and nature of the organic content of the soil, climatic conditions, surface vegetation and soil texture (Marschner *et al.*, 2003).

Recent studies suggested that there may be around 1.5 to 5.1 million extant fungal species and about 1200 new species are being described in each year (Hibbett *et al.*, 2011). Further, O'Brien *et al.* (2005) were of the opinion that less that 2-6% of the fungal species are currently described. This indicated that there are many more fungal species to be explored, named and identified. Therefore, studying fungi in tropical forests, unexplored habitats and lost or hidden species could contribute sustainable to global fungal diversity (Hawksworth and Rossman, 1997). The estimation of fungal species diversity at any given site of any ecosystem may be a difficult task for mycologist because of the fact that fungal communities are highly di-

verse, cryptic and ephemeral in nature. Besides, probability of encountering and recording all species present during any sampling effort is low (Tran *et al.*, 2006; Aime and Brearley, 2012).

Similipal hills located in the midst of Mayurbhanj district of Odisha state, India and located in between 21°28' to 22°08'N latitude and 86°04' to 86°37' E longitude and covering 5,578 km^2 of forest land. The Similipal Biosphere Reserve has been divided into three zones, *i.e.* Core zone (845 km^2), Buffer zone (2174 km^2) and transitional zone (2559 km^2). The climates of Similipal is tropical with warm and humid, summer temperature around 40 °C during peak of the season. The rainy season starts from mid of June to October with a rainfall of about 125 mm in the monsoon. Winter creeps in gradually from mid October and becomes severe in December lowering temperature up to 5 °C in many parts of the hill. This uneven geophysical condition influences the diversity of floral and faunal distribution. Except few sporadic reports (Bahuti *et al.*, 2006; Behera *et al.*, 2009) scientific studies of SBR in terms of microbial wealth is not available in literature. This prompted us to carry out the present investigation to study the fungal diversity of this unique ecosystem in different seasons with respect to their physicochemical factors, in order to explore the importance of fungal population in maintenance of soil quality and to exploit their biotechnological potentials in near future.

Materials and Methods

Collection of soil samples

Hundred soil samples were collected from ten different sites (Chahala-1&2, Bareipani, Natto1-&2, Gurgudia-1&2, Pithabata-1&2 and Joranda) particularly from rhizosphere during a time span of 2 years (2010-2012) representing both summer and winter seasons (Figure 1). All ten sites differ from each other on the basis of physical and chemical nature of soil. The Chahal-1&2 are within core region and covered by dense forest. Natto-1 &2 are characterized by dense vegetation with slope position of 20-30°. Bareipani is located at high elevation of 776 m above sea level. The vegetation of Joranda is characterized by numerous streams and water fall with dense forest cover, Pithabota- 1 &2 are located in between buffer and core areas. The Gurgudia-1&2 are located in the buffer area of the Biosphere reserve. Random sampling was carried out in each plot following the method of Baruah and Barthakur (1998). Each soil sample represents composite of well mixed five sampling units collected from each plot of 20 x 20 m. Soil pH, temperature, humidity, P, K and percentages of organic carbon was determined by following standard soil analysis methods (Alexander, 1972; Baruah and Barthakur, 1998).
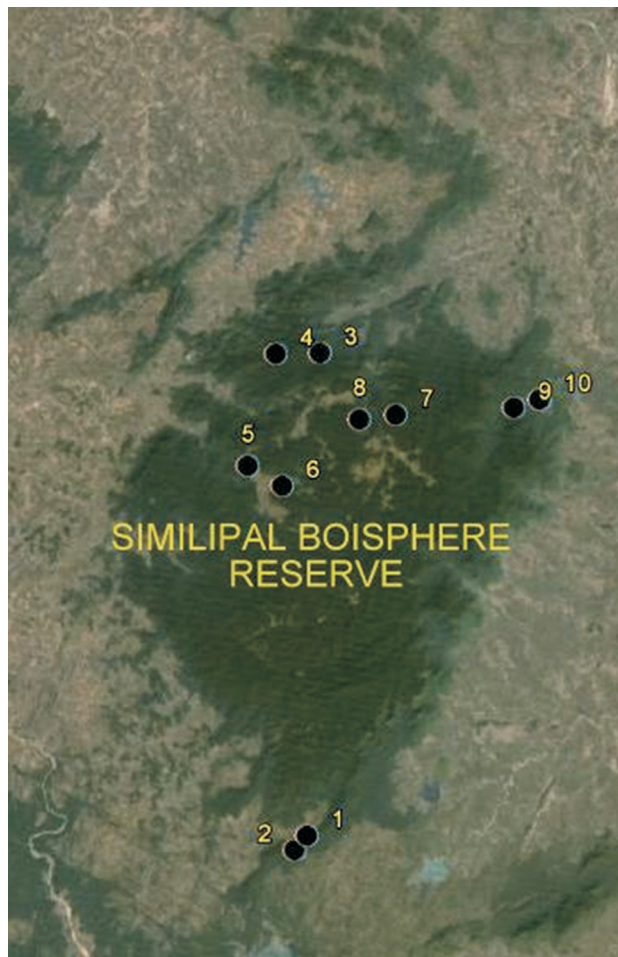

Figure 1 - Soil sample collection sites in Similipal Biophere Reserve: (1) Natto- 1, (2) Natto-2, (3) Chahala-1, (4) Chahala-2, (5) Gurgudia-1, (6) Gurgudia-2, (7) Joranda, (8) Bareipani, (9) Pithabata-1, and (10) Pithabata-2.

Isolation and enumeration of fungi

Fungi were isolated and enumerated following standard microbiological culture technique on Potato Dextrose Agar (PDA) by total plate count method (Cruickshank *et al.*, 1973). The fungal isolates were identified based on their colony morphology, growth pattern on medium and reproductive characters using the standard identification manuals (Gilman, 1971; Barnett and Hunter, 1996).

Quantification of the species (Species Important Value Index, SIVI)

The relative abundance, relative frequency and relative density of species were determined using the following formulae (Dash, 2001).

Relative Abundance (RA) = Abundance of a species per sampling sites divided by total number of abundance value based on number.

Relative Frequency (RF) = Number of sampling sites containing a species divided by sum of frequency of all species.

Relative density (RD) = Number of individuals of a species in all sampling sites divided by number of individuals of all species in all sampling sites.

Species Importance Values Index (SIVI) was calculated by the sum of three parameters (RA+RF+RD).

Diversity analyses

Fisher's α, Shannon's diversity index (H'), Shimpson's diversity index (λ), Margalef's richness index (RI), Menhinick's richness index (R_2), and Evenness indices (E1, E2, E3, E4 and E5) diversity indices were calculated using software of Paleontological Statistics (PAST ver. 2.0; Ryan *et al.*, 1995).

Community ordination techniques

The variations of population size among the sampling area in two seasons were summarized and the relations between sites were determined by Principal Components Analysis (PCA). The logarithmic values of population of each site were arranged in relation to multi condition axes (axis 1, 2 and 3) on PCA. These data were used to calculate the Eigen value based on which co-ordinate axes were represented so as to produce information on the similarity of the habitat types. To test for linearity of population structure among the sites, the data was further evaluated to Detrended correspondence analysis. Thus, genera were calculated to be highest SIVI was considered for determination of inter species relation distribution in two different seasons by measuring Euclidean distance, using PAST.

Results

Physico chemical parameters of the soil

A total of 260 fungal isolates were obtained from 100 soil samples collected from 10 different sites of Similipal Biosphere Reserve in two seasons. The physico-chemical parameters of the soil samples and its associated plant species were studied (Table 1). All the soil samples were found to be acidic in nature, and highest acidity, moisture and organic carbon percentage were observed in the soil sample collected from Chahala-1. Whereas, low moisture content and organic carbon percentage were recorded from Bareripani site. The salinity of the soil samples were found to be even (0.5) except increasing trend was observed in the soil samples collected from Gurgudia. The dominant plant species occurring in the collected sites was *Shorea robusta*.

Fungal population

Occurrence of fungal propagules in the soil samples were enumerated by standard plate count methods (pour and spread plate methods) and expressed in cfu gm^{-1} of soil. Soil fungal population was observed to vary among differ-

Table 1 - Physicochemical analysis of soil samples collected from Similipal Biosphere Reserve.

Collection site	Moisture content % (winter)	Moisture content % (summer)	Temperature (winter)	Temperature (summer)	Organic C % (winter)	Organic C % (summer)	Acidity (winter)	Acidity (summer)	Salinity	P kg/hector	K kg/hector	Dominant plant species/ associated plant
Natto-1	20	16	19	24	0.644	0.654	5.36	5.4	0.5	2.48	138.5	*Shorea robusta*
Natto -2	19.8	15	19.2	25	0.612	0.632	5.64	5.8	0.5	5.88	158	*S. robusta*
Chahala-1	21.4	20.4	14	16	0.676	0.656	5.08	5.1	0.5	2.36	142.6	*S. robusta*
Chahala-2	23.6	21.6	14.8	18	0.6375	0.6415	5.14	5.1	0.5	2.08	407.4	*S. robusta*
Gurguria-1	14.6	12.6	17.5	22.5	0.564	0.555	6.1	5.98	0.7	1.76	394.2	*S. robusta, Ardisea solanacea*
Gurguria-2	14.8	12	16.9	19	0.516	0.51	6.12	6.0	0.5	4.24	407.4	*S. robusta*
Bareipani	12.8	10	18	20	0.428	0.422	5.92	5.8	0.5	2.04	425.4	*S. robusta, Terminalia alata*
Joronda	23.4	19.1	15.6	20.2	0.622	0.63	5.52	5.4	0.5	2.4	397.2	*S. robusta, Mangifera indica*
Pithahata-1	17.6	16.2	15.6	19.6	0.58	0.588	6.04	5.9	0.5	1.8	394	*S. robusta*
Pithahata-2	18.8	15.2	15.9	20.9	0.572	0.55	5.98	5.8	0.5	3.2	391.6	*S. robusta*

ent sites. The fungal population ranged from 3.6×10^4 to 2.1×10^5 in summer and 5.1×10^4 to 4.7×10^5 during winter. In comparison, spread plate method showed higher number of fungal propagules than pour plate technique. Among the sites, highest fungal population was observed in the soil sample collected from Chahala-2 in both the seasons whereas, lowest fungal population was observed in the sample collected from Bareipani (Figure 2).

Species important values index (SIVI)

Species important values index is used to determine the overall importance of identified species in the community structure (Table 2). It gives an idea of the sociological structure of a species in its totality in the community. The result indicated that *Aspergillus niger* and *Aspergillus awamori* showed highest SIVI values. *Aspergillus* species were found to be the most dominant species among the community in all sampling sites. The dominant species of *Aspergillus* were identified as *A. alliceus*, *A. flavus*, *A. fornscecous*, *A. humicola*, *A. luchuensis*, *A. niger*, *A. awamori*, *A. tamarine*, *A. terrus*, *A. wentii*, *A. ustus* and *A. panamensis*.

Species diversity and richness

The species diversity and richness of soil fungi were studied by different diversity indices in different sites and seasons (Tables 3, 4). Shannon diversity index (H) was calculated as it represents the number of individuals as well as number of taxa in each collected site. Maximum value of H was observed at Natto-2 (3.12) and Joranda (2.84) in summer and winter seasons respectively (Figure 3). Simpson diversity index (1-D) was also studied as it measures the dominant species. Highest 1-D was observed at Natto-2 (0.942) during summer while during winter was observed in Chahala-2 (0.903). The Dominance (D) indicates dominancy of a particular species. The value was highest at Pithabata-2 (0.2127) during summer. But in Natto-2, the D value was higher in winter (0.1557) and lower in summer

(0.05746). However, lowest D value was observed at Chahala-2 (0.09694) in winter. Besides, Fisher alpha diversity indicated highest value in the soil sample of Joranda in both seasons.

Sometimes one sample may contain a larger number of taxa, while the other has a larger diversity index. Therefore, a number of diversity indices may be compared to make sure that the diversity ordering is robust. A formal way of doing this is to define a family of diversity indices, dependent upon a single continuous parameter. So the diversity profile was carried out among the samples in different sites. The diversity profile of the sites, Natto-1 and Natto-2, Chahala-1, Chahala-2, Bareipani and Gurgudia did not intercept with each other, indicating the distinctness of their diversity indices (Figure 4). Further, species richness was studied by Menhinick- Margalef diversity indices (Smax). The result indicated that maximum species richness was reported in the sampling site of Joranda but less in Bareipani. Besides, Berger Parker dominance index was calculated which expresses the proportion of the total share that is due to the dominant species. In comparison to the species components in sampling sites with respect to their population size, Berger-Parker dominance index expressed total higher number of species sharing the communities in the sites Pithabata-2, followed by Bareipani, Pithabata-1, Joranda, Chahala-1, Chahala-2, Gurguria-2, Guruguria-1, Natto-1 and Natto-2 during summer season. While, it was found to be highest in Bareipani followed by Pithabata-1, Natto-2 and Chahala-1 during winter.

Species evenness

Species evenness is also an important component of diversity index as it calculates the distribution pattern of species among the sites. Simpson index (1-Shannon index) measures evenness of the community from zero to one. The calculated values indicated approximately towards zero, which means, less evenness species distribution. At Natto-1 and Natto-2 during summer season, the distribution of species was found to be even in nature whereas, in Pithabat-2 species distribution was less even. But during winter, species evenness was more in Pithabata-2 followed by Bareipani and Chahala-2. Little variation of equitability values were observed among the sites.

Component ordination

PCA is used for the reduction of information on a large number of variables into a small set while losing only a small amount of information. It also helps in breaking down or partitioning a resemblance matrix into a set of perpendicular axes or components.

The presences of variables (population of sampling units) in different axis were provided maximum information about the ecological similarities among sites. Each PCA axis or components correspond to an Eigen value is the variance accounted for, by the axes. The Eigen values
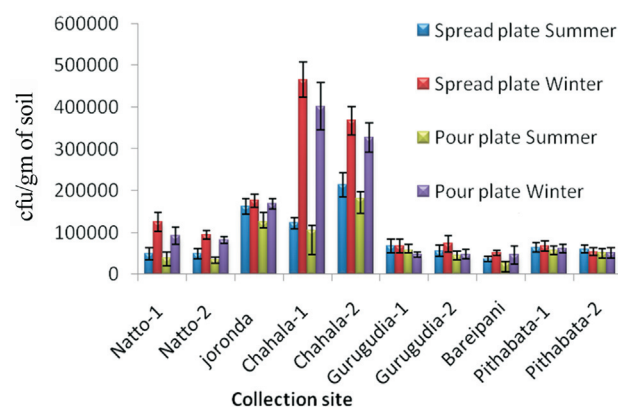

Figure 2 - Enumeration of culturable fungi in different sites of Similipal Biosphere Reserve, through spread and pour plate methods at two different seasons.

Table 2 - SIVI value of different fungal species of two seasons (summer and winter).

Fungal species	Relative density		Relative abundance		Relative frequency		SIVI	
	summer	winter	summer	winter	summer	winter	summer	winter
Absidia sp.	3.4	5.4	2.9	1.9	3.1	3.0	9.4	10.2
Alternaria sp.	1.9	1.4	1.9	0.8	2.7	2.3	6.4	4.6
Aspergillus alliceus	1.9	1.8	1.9	1.5	2.7	2.6	6.4	5.9
Aspergillus flavus	1.7	0.9	1.5	3.0	3.1	3.0	6.2	6.8
Aspergillus fonscecous	2.2	1.6	1.9	1.3	3.0	2.9	7.1	5.8
Aspergillus humicola	2.2	3.2	1.9	17.5	3.0	2.9	7.1	23.6
Aspergillus luchuensis	1.9	1.3	1.8	3.3	3.0	2.7	6.7	7.3
Aspergillus niger	21.9	20.3	18.3	5.2	3.2	3.1	43.4	28.6
Aspergillus panamensis	0.2	0.3	0.3	0.2	1.7	1.5	2.3	2.1
Aspergillus awamori	4.9	3.8	4.5	1.6	3.0	3.1	12.4	8.4
Aspergillus sp.	2.8	5.4	2.6	1.2	2.9	2.8	8.2	9.4
Aspergillus tamarine	0.6	1.4	0.7	0.6	2.3	2.3	3.6	4.3
Aspergillus terrus	0.7	1.3	0.9	0.2	2.1	3.0	3.7	4.5
Aspergillus untii	0.7	0.5	0.9	0.5	1.9	2.3	3.5	3.3
Aspergillus ustus	0.3	0.2	0.3	0.3	2.8	2.6	3.4	3.1
Curvularia sp.	0.1	0.2	0.3	0.1	1.3	2.1	1.7	2.4
Emencellopsis sp.	0.3	0.2	0.3	1.3	2.9	2.8	3.5	4.3
Epicoccum nigrosum	0.1	0.1	0.2	0.4	1.2	1.4	1.6	1.8
Epicoccum sp.	1.9	1.5	1.6	0.5	3.2	3.1	6.7	5.1
Fusarium oxysporium	0.5	0.4	0.4	0.1	2.8	2.7	3.7	3.2
Fusarium sp.	0.7	0.5	1.7	0.2	2.7	2.6	5.0	3.2
Gliocladium catenulatum	0.2	0.1	0.2	0.1	2.6	2.5	2.9	2.7
Gliocladium spp.	0.2	0.2	0.2	0.1	2.7	2.6	3.2	2.9
Homicola brevis	0.1	0.1	0.2	5.6	2.4	2.3	2.7	8.1
Mucor circinelloides	0.2	0.1	0.2	0.3	2.8	2.6	3.1	3.0
Mucor sp.	6.1	6.5	5.1	1.2	3.2	3.1	14.4	10.8
Penicillium coryphilum	0.4	0.2	0.5	0.5	2.1	1.8	2.9	2.6
Penicillium cumminie	1.1	0.8	1.6	1.1	1.7	1.7	4.4	3.5
Penicillium granulatum	0.3	0.2	1.2	4.2	0.8	1.2	2.3	5.7
Penicillium implicatum	1.0	0.7	2.4	3.5	1.2	1.7	4.5	5.9
Penicillium regulosum	1.1	2.7	2.7	1.3	1.1	1.7	4.9	5.6
Penicillium sp.	18.0	18.0	16.1	10.8	3.2	3.1	37.4	31.9
Penicillium stectii	0.6	2.3	1.1	0.6	1.4	1.7	3.0	4.6
Penicillium tardum	1.0	0.7	1.8	15.5	1.5	1.4	4.2	17.6
Penicillium rubrum	0.6	0.4	0.9	1.3	1.7	1.7	3.2	3.3
Rhizoctonia sp.	2.1	1.5	1.7	1.8	3.2	3.1	7.0	6.4
Rhizopus sp.	4.8	5.6	4.0	2.0	3.2	3.1	12.0	10.6
Scopulariopsis brevicaulis	1.7	1.3	2.3	0.5	2.0	1.8	6.0	3.6
Talaromyces sp.	2.6	1.7	2.9	3.5	2.4	2.3	7.9	7.5
Trichoderm aliguorum	0.6	0.4	0.9	1.3	2.1	2.0	3.6	3.7
Trichoderma sp.	6.1	4.1	5.1	2.6	3.2	3.1	14.5	9.8
Zygorhynchus expenes	0.5	0.6	1.3	1.0	1.1	1.1	2.9	2.7

Table 3 - Diversity indices of fungi at different sites of Similipal Biosphere Reserve, during summer season.

Diversity indices	Different Sites inside SBR									
	Natto-1	Natto-2	Joronda	Chahala-1	Chahala-2	Gurugudia-1	Gurugudia-2	Bareipani	Pithabata-1	Pithabata-2
Taxa S	33	35	40	37	33	35	32	28	35	30
Individuals	47050	49390	149150	111650	155230	53300	49100	35750	54550	64950
Dominance D	0.0626	0.05746	0.1242	0.09009	0.1459	0.1091	0.1274	0.1491	0.1643	0.2127
Shannon H	3.022	3.126	2.78	2.971	2.399	2.673	2.531	2.5	2.404	2.08
Simpson 1-D	0.9374	0.9425	0.8758	0.9099	0.8541	0.8909	0.8726	0.8509	0.8357	0.7873
Evenness e^H/S	0.622	0.6509	0.4029	0.5274	0.3338	0.4137	0.3928	0.4353	0.3161	0.2668
Menhinick	0.1521	0.1575	0.1036	0.1107	0.08376	0.1516	0.1444	0.1481	0.1499	0.1177
Margalef	2.974	3.146	3.274	3.097	2.677	3.124	2.87	2.575	3.117	2.617
Equitability J	0.8642	0.8792	0.7536	0.8228	0.6862	0.7517	0.7303	0.7504	0.6761	0.6116
Fisher alpha	3.468	3.683	3.78	3.575	3.044	3.65	3.334	2.981	3.64	3.006
Berger-Parker	0.1275	0.1215	0.3017	0.2508	0.2577	0.2251	0.2444	0.3357	0.33	0.3849

Table 4 - Diversity indices of fungi at different sites of Similipal Biosphere Reserve, during winter season.

| Diversity indices	Different sites inside SBR									
	Natto-1	Natto-2	Joronda	Chahala-1	Chahala-2	Gurugudia-1	Gurugudia-2	Bareipani	Pithabata-1	Pithabata-2
Taxa S	34	35	41	34	35	35	32	28	36	30
Individuals	107650	88890	146150	242850	215050	60900	49100	35750	57650	66900
Dominance D	0.146	0.1557	0.1126	0.1498	0.09694	0.1073	0.1274	0.1491	0.1495	0.1058
Shannon H	2.416	2.481	2.84	2.427	2.696	2.666	2.531	2.6	2.493	2.575
Simpson 1-D	0.854	0.8443	0.8874	0.8502	0.9031	0.8927	0.8726	0.8509	0.8505	0.8942
Evenness e^H/S	0.3293	0.3414	0.4175	0.3332	0.4236	0.4109	0.3928	0.4353	0.3362	0.4377
Menhinick	0.1036	0.1174	0.1072	0.06899	0.07547	0.1418	0.1444	0.1481	0.1499	0.116
Margalef	2.848	2.984	3.363	2.661	2.769	3.086	2.87	2.575	3.193	2.61
Equitability J	0.685	0.6977	0.7648	0.6883	0.7584	0.7499	0.7303	0.7504	0.6958	0.7571
Fisher alpha	3.268	3.446	3.892	3.009	3.144	3.594	3.334	2.981	3.732	2.996
Berger-Parker	0.2787	0.2925	0.2737	0.2882	0.186	0.2299	0.2444	0.3357	0.3122	0.1794
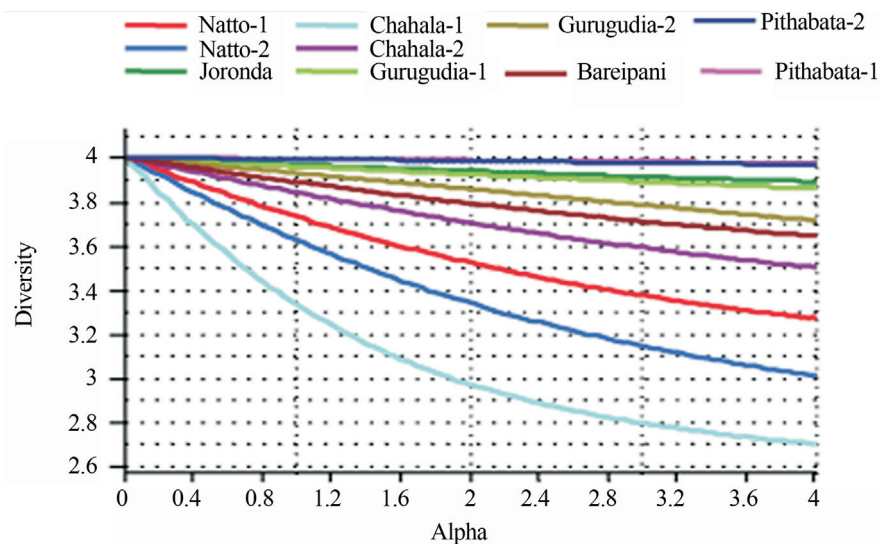
Figure 3 - Depicting the relation between organic carbon and moisture contents with Shannon index.
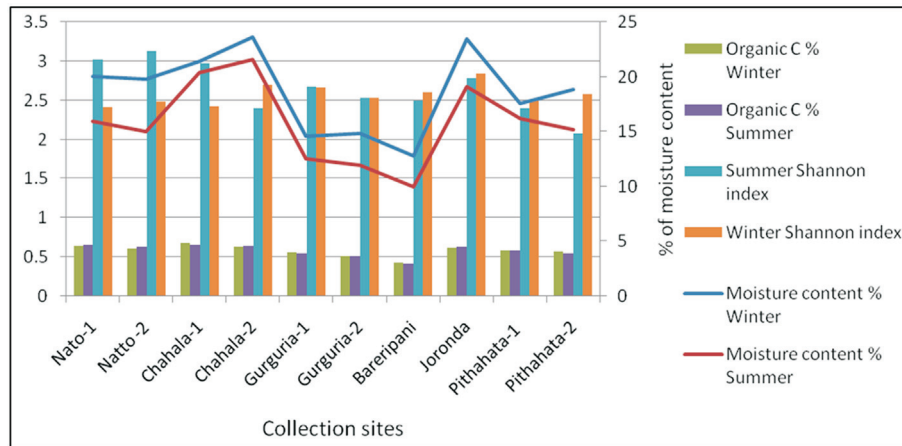
Figure 4 - Depicting the diversity profile of fungi in different sites of Similipal Biosphere Reserve.

are extracted in the descending order of magnitude. Such that the corresponding PCA components represented greater to lesser amount variation in the matrix (Muthukrishna *et al.*, 2012). The values of PCA as co- relation among the sites were represented (Figure 5). The PCA ordination plotting of Component 1 *vs.* Component 2 indicates that the data of the fungal population sites showed highest +ve values for Chahala-1 and Chahala-2 around 0.9 to be static and more significantly modulated the fungal population in this ecosystem. On the other hand Pithabat-2 and Bareipani were positioned towards the -ve sides of the axis and values were less to be -0.40289 and -0.64711 respectively. Whereas, variation in the fungal population was observed in both while studying in spread and pour plates. Plotting Component-1*vs.* Component-3 also showed relatively same effect as that of spread plate and pour plate methods for encountering the fungal population. But, the summer population and winter population fall on alternate sides when Component-1 *vs.* Component-3 and Component-2 *vs.* Component-3 were plotted. Moreover, the populations of fungi in Chahala-2 and Joranda-1 sites were depicted on same sides of plotting represented less variation between the sites.

Detrended correspondence analysis

We have made an attempt to find out the linearity distribution of fungal population among the sampling sites. Detrended correspondence analysis plotting axis 1 and axis 2 and axis 2 and axis 3 indicated non linear arrangement of the samples. The values of the first three axis obtained by computing the detrended correspondence analysis is proved. In pictorial representation (Figure 6) of all the sites were plotted on sheet, to be observed scattered any where suggested that non linearity distribution of taxa among sampling sites.

Community ordination of *Aspergillus* species

The relationships between *Aspergillus* species in the community in different sites and seasons were analysed by community ordination to understand the natural system of grouping of species.

Overall 12 *Aspergillus* spp. reflected the community structure and population size. Dendrogram of the clustering of the species using Euclidean distance during summer is provided (Figure 7). The result indicated that *Aspergillus niger* formed a separate group in the community. Further, *A. awamori* also differed from other group indicating its distinctness. While other species clustered together within the community. Correspondingly, the dendrogram using Euclidean distance during winter also indicated similar results (Figure 8).

Discussion

In terrestrial ecosystem, fungi are important components of microbial communities and play vital role in maintaining soil health and productivity. Studies suggest that soil fungal diversity and composition is governed by wide range of biotic and abiotic factors (Lauber *et al.*, 2008; Barcenas-Moreno *et al.*, 2009; Hawkes *et al.*, 2011). Das and Dkhar (2011) were of the opinion that microbial flora in moist deciduous forest is dependent on moisture content, pH, temperature, Nitrogen, Carbon sources. Several studies surveyed fungal diversity in different forest ecosystems, suggested a strong correlation between fungal and plant diversity, due to fungal host specificity (Peay *et al.*, 2013; Shi *et al.*, 2013). Similipal Biosphere Reserve rich in plant diversity and dominated by *Shorea robusta*. The geography of this Biosphere Reserve is uneven. The soil physico-chemical properties were found to be varied from site to site. Therefore, in this investigation, fungi occurring in soil samples of Similipal Biosphere Reserve were studied in respect to their diversity and physicochemical parameters of the soil. In the present investigation, we studied soil fungi
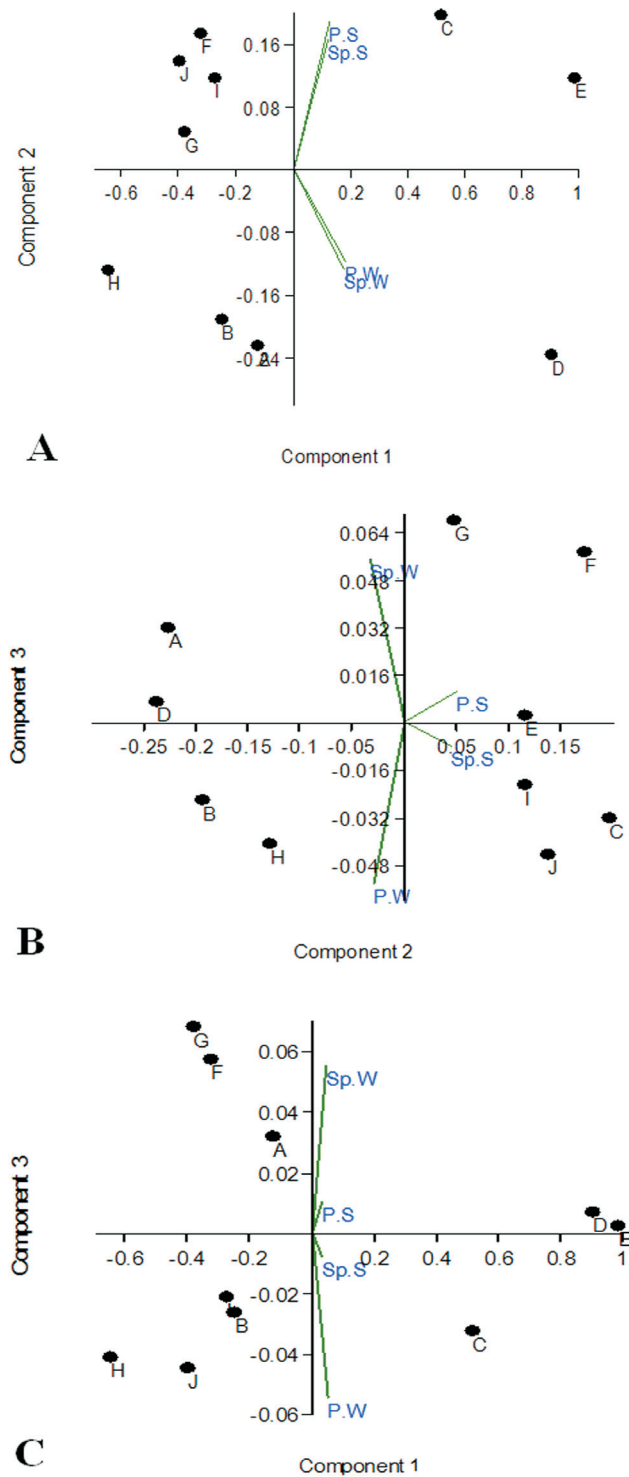
Figure 5 - A,B,C graphs showing the principal component ordination. Position of ten sites based on fungal species abundance. A: Principal Component 1 vs 2. B: Component 1 vs 3. C: Component 2 Vs 3. A (Natto-1), B (Natto-2), C (Joranda) D (Chahala-1), E (Chalala-2), F (Gurgudia-1), G (Gurgudia-2), H (Bareipani), I(Pithabata-1) J (Pithabata-2), Sp.W. (spread plate winter), Sp.S. (spread plate summer) P.S. (pour plate summer) and P.W. (pour plate winter).

of 100 sampling units collected from Similipal Biosphere Reserve, which are categorized into 10 major sites (Table 1). In the other words, the disturbances on fungal communities and population have been poorly studied in tropical regions, because these communities have been considered, likely wrongly, as both resistant and resilient to disturbance (Allison and Martiny, 2008; Da Silva *et al.*, 2012). Fungal population and diversity also varied with respect to the rhizopheric plants species(Hattori *et al.*, 2012). Here, the sampling sites were accomplished to the rhizopheric region of *Shorea robusta as* dominant flora. Gilbert (2005) suggested that the high plant diversity of tropical systems results in a decrease in fungal specificity due to a lack of selective pressure for specialization, often resulting in low species diversity. The Similipal soil was fertilized continuously by deposition of biomass of the plants. Therefore, dominance of fungal species in the soil samples could be recognized by organic substances deposited by dominant plant species. Another factor that, geographic distance monitoring the fungal diversity was reported by Green *et al.* (2004). According to him, fungal community became less similar with increasing geographic distance. Therefore, in our investigation larger variation of population was recorded among the above 10 sites could be attributable to the geographic position of the sites. It was also found that the similar fungal flora were recorded where soil samples were collected from closely located sites *i.e.* Chahala-1 &2, Natto-1 &2, Pithabata 1-&2, Gurgudia-1 &2. The varied population among the sites was also found due to the change in physicochemical parameters of the soil. Highest population was recorded in Chahala-1, followed by Chahala-2, Joranda and Natto-1 in winter season. Among the sites, the fungal population size was dependent on moisture content and organic carbon % of the soil (Table 1, Figure 2). Lowest fungal population was recorded in the site Bareipani *i.e.*, 5.16×10^4 and 3.62×10^4 in winter and summer, seasons respectively.

In PCA analysis study it was summarized and calculated that winter and summer populations to be far change in population size in same place. However, in summer, fungal population fell down to half of the winter population among the sites as recorded could be attributable to the drying of soil. These findings are in corroboration with other workers (Gupta and Bhriguvanshi, 1997; Narsian and Patel, 2009). Yan *et al.* (2000) has drawn a relationship between organic carbon and functional diversity of fungal population, as observed in this investigation. Sharma *et al.* (1997), stated a linear relationship between (Shannon's diversity index) H and microbial biomass. In this context, it was observed that Shannon index to be more in summer season than winter season because of the rise of dominance of single species. It was clearly understood that population size of ecosystem is directly dependent to organic carbon contents and moisture contents of the soil (Figures 2, 4). Both, Dominance and Simpson diversity index were found
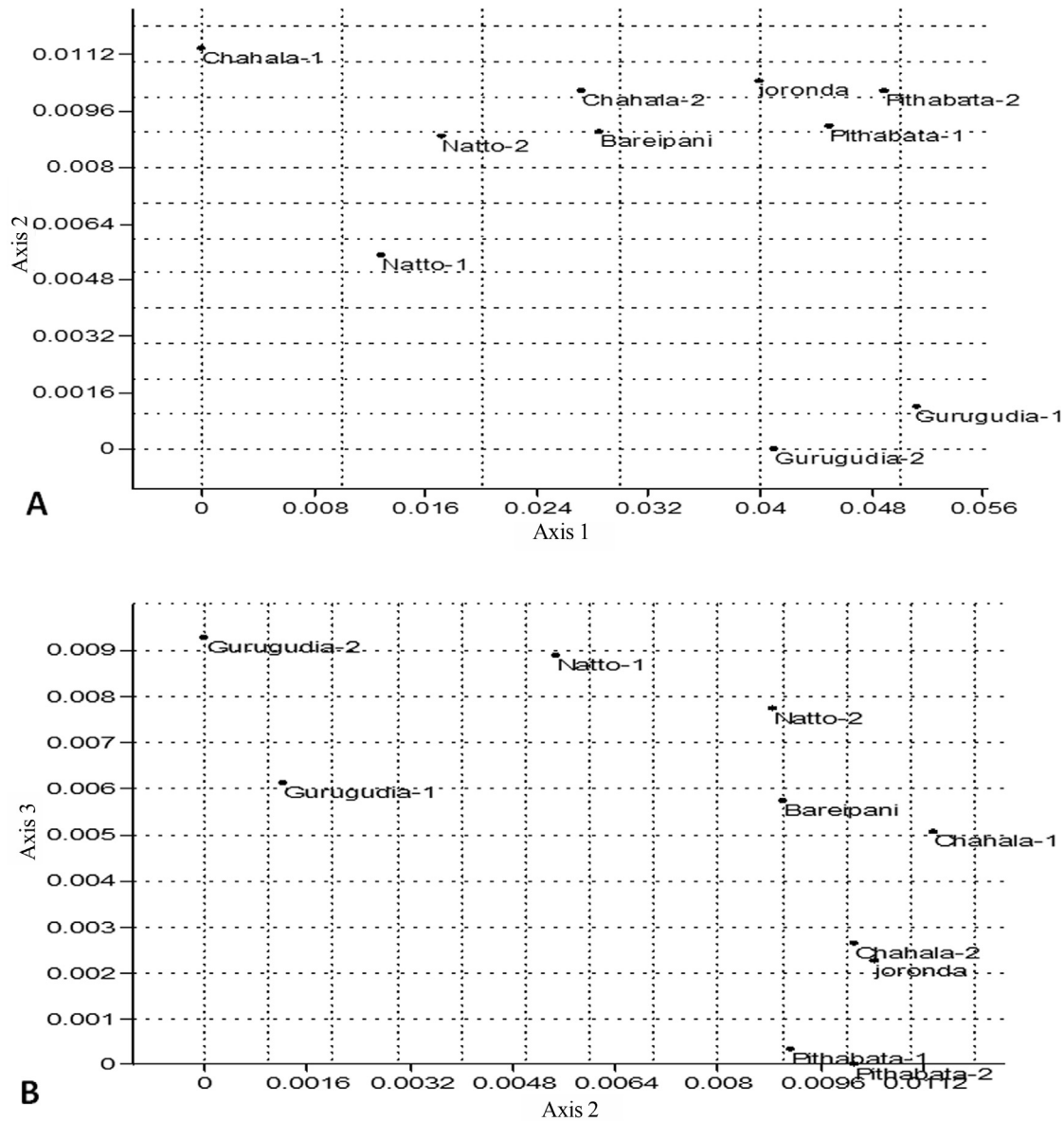
Figure 6 - Deterned correspondence analysis of sampling sites (A: Axis 1vs2 and B: Axis 2vs3).

to vary from site to site, as a result of difference in physicochemical parameters and associated rhizopheric plants. Mycoflora differs in its composition from one ecological niche to the other have also been reported (Monoharachary, 2008). The dominant genera appeared abundantly in Indian soils regulate the relative frequency and relative density of other soil fungal genera (Bhagat and Pan, 2010) and had important role in leaf litter decomposition (Panda *et al.*, 2009). This may be due to the faster growth rate of these fungi in addition to their better intrinsic prolific sporulating capacity to utilize the substrate. Therefore, sporulating fungi were better established and studied by culturable methods. Panda *et al.* (2010) also reported that fungal succession in plantation sites greatly differed from sites without plantation due to the presence of organic materials and

that promote the domination of single species. Therefore, eveness (Evenness e^H/S) of fungal species also varied from site to site as reported by our study due to uneven geography, physico-chemical parameters of soil.

On the other hand, the exact evaluation of fungal population and diversity is very difficult (Smith *et al.*, 2011; Piepenbring *et al.*, 2012). As, fungi are cryptic and hyper diverse organisms that assemble in complex and dynamic communities. Perrone *et al.* (2011) reported that species that produce spore-bearing structures can be easier to discover, although fruiting periods can be short and fructifications are ephemeral. In this investigation, we have measured the fungal population rather than fungal diversity. The species which are more dominant colonized better on the plate (during standard plate count method) than other
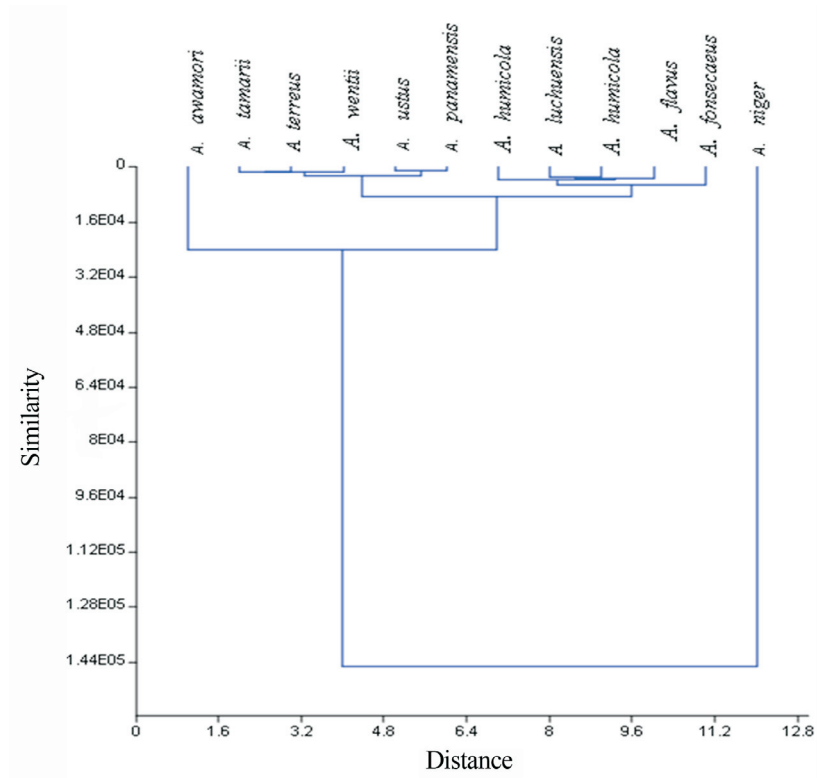
Figure 7 - Dendrogarm clustering the 12 abundant fungal (*Aspergillus*) species of the forest in summer litter using Euclidean distance.
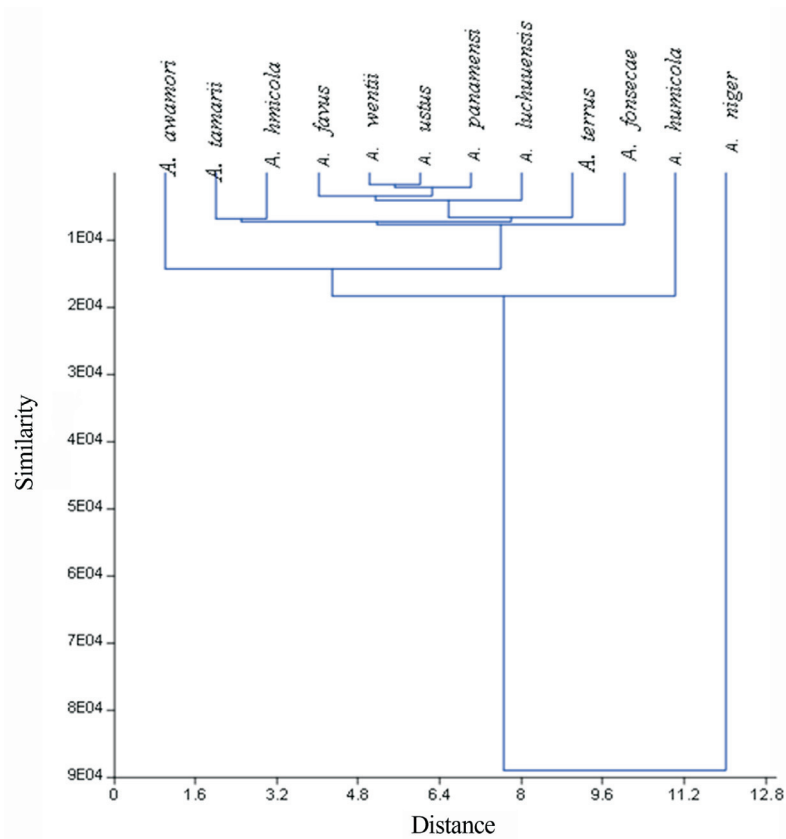

Figure 8 - Dendrogarm clustering the 12 abundant fungal (*Aspergillus*) species of the forest in winter season litter using Euclidean distance.

species. On the other hand these species were better sporu-lating, therefore, soil fungal flora and population were maintained by these group of organisms. Total 17 genera were taken into account during this study, amongst all, *Aspergillus* was observed to be most dominant genera fol-lowed by *Penicillium*. The SIVI value suggested the flo-ristic status of the species (Table 2). The *Aspergillus niger* showed SIVI 43.4 and 28.6 both in summer and winter sea-sons respectively, indicating the population size of the communities among the sampling sites being regulated by these two species, It is also called insurance hypothesis (Yachi and Loreau, 1999). In winter the *Aspergillus niger* was isolated in more numbers than summer season but the SIVI value showed the alternate results due to the well es-tablishment of other species in winter rather than summer season. The dendrogarm of the 12 abundant *Aspergillus* species isolated from the Similipal Biosphere Reserve soil both in winter and summer season litter using Euclidean distance (Figures 7, 8) was drawn based on the SIVI values. The figure showed that *Aspergillus niger* form a separate group in community and minimize the role of other mi-crobes in microbial ecosystem followed by cryptic species (Perrone *et al.*, 2011) like *Aspergillus awamori*. Shimpson's diversity index of the site Chahala-2(0.850) and Bareipani (0.854) was reported to be same but in Chahala-1 the fungal population recorded to be more by two folds, than that of population of Bareipani (Figure 2). It clearly indicated that *Aspergillus niger* have not only better sustainability but has capacity to regulate the microbial structure in moist deciduous forest soil, could be due to the competitively superior under such conditions when there are favourable niches everywhere (Hall *et al.*, 2000), called resource heterogeneity hypothesis. Secondly, the specific association of fungal species in different sites is because of their niche specialization. Senthilkumar *et al.* (1993) re-ported that most of the fungal flora occurring in the later stages of decomposition was efficient degraders of cellu-lose and lignin. In the present study more fungal species were recorded from the sites with more moisture content and leaf litter deposition, indicating that these species could be efficient cellulose and lignin degraders.

A little change of diversity index and dominance in-dex were observed in communities with uniform distribu-tion of taxa among the sampling sites. But large variation of population of taxa among the sites were observed by study-ing the positioning of sites on Detrended correspondence analysis plot of axis-1 vs axis-2 and axis-2 and 3 (Figure 6).

Conclusion

It is concluded from our findings and other investiga-tions, that the determination of complete structure of fungal flora is a difficult chapter. But, dominant and sporulating genera are the determinate of fungal diversity in the soil. The well evolved (sporulating) species are the dominant genera that contribute the maximum population to maintain

the floral structure in the soil ecosystem that is attributable due to well dissemination of their spores in the soil. How-ever, the moisture content and organic C percentage regu-late the viability of their spores as well as the vegetative hyphae, as evidenced in this investigation, maintaining the fungal diversity in the soil ecosystem to a large extent.

Acknowledgments

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