Current Research in Environmental & Applied Mycology 5 (2): 101–119(2015) ISSN 2229-2225

www.creamjournal.org **Article** CREAM

Online Edition

Doi 10.5943/cream/5/2/4

Fungal diversity of twelve major vegetational zones of Arunachal Himalaya, India

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Sharma D, Gosai K, Dutta J, Arunachalam A, Shukla AK 2015 – Fungal diversity of twelve major vegetational zones of Arunachal Himalaya, India. Current Research in Environmental & Applied Mycology 5(2), 101–119, Doi 10.5943/cream/5/2/4

Abstract

Soil microfungal diversity was studied with the objective to investigate variations in fungal communities along 12 diverse vegetation zones located at different altitudinal gradients in Arunachal Pradesh and to check whether the environmental conditions have an effect on the soil fungal community. Ten soil samples were collected from 0-30cm depth in each forest type and their physico-chemical properties such as pH, temperature, bulk density and organic carbon content analyzed using standard techniques. Serial dilution methodology was used for the isolation of soil fungi in Rose Bengal agar media. A total of 112 fungal types under 59 genera and 88 species were recorded from the selected soils. Altitudinal gradient and bulk density was found to have a negative effect, while soil temperature and soil pH had positive effects on the soil fungal communities. Sub-tropical evergreen forests showed maximum fungal diversity followed by tropical evergreen forests. Overall, *Oidiodendron* followed by *Acremonium*, *Cladosporium*, *Humicola*, *Aspergillus* and *Penicillium* were found dominant fungal genera in majority of soil samples. Distribution of *Beauveria*, *Blastomyces*, *Cercospora*, *Metarrhizium* and *Rhizomucor* were limited to particular soil type. Altitudinal gradient together with associated vegetation and soil physico-chemical parameters determine soil fungal distribution.

Key Words – altitudinal gradient – soil fungi – forests – biodiversity – vegetation

Introduction

Fungi are one of the most important functional groups of soil microbes and perform essential role for functioning of the ecosystem (Doran & Parkin 1994, 1996, Hawksworth et al. 1996). Due to their capability to decompose complex macromolecules they are vital for making the nutrients like C, N, P and S accessible in the soil. Moreover the fungal mycelium plays an important role for stabilization of soil and helps to increase the water-holding capacity (Kennedy & Gewin 1997). Despite their well documented role in ecosystem functioning, it is estimated that only 5% of fungal species have been described (Hawksworth et al. 1996) and actual species richness is

Submitted 19 August 2014, Accepted 18 April 2015, Published online 31 May 2015
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likely to be much higher (Schmit & Mueller 2007). Moreover, little is known about their dynamics, community structure and diversity.

Although there are many examples from the literature on studies of the distribution of soil microfungi, until recently there had been few attempts to directly relate the occurrence of these microfungi to environmental conditions (Van Maanen et al. 2000, Gourbiere et al. 2001, Cabello & Arambarri 2002, Schmit & Mueller 2007, Shivakumar et al. 2012, Zhang et al. 2012). Limited references are available demonstrating the changes in fungal assemblages along altitudinal gradients (Raviraja et al. 1998, Buckova et al. 2000, Slavikova & Vadkertiova 2000) in different parts of the world. In India, studies on soil fungal diversity in relation to habitat, climate and altitudinal gradient is rare (Pandey et al. 2006, Satish et al. 2007). Furthermore in North Eastern part of India, especially from Arunachal Pradesh, no significant studies on the role of environmental gradient on soil microfungal diversity was carried out till date.

Arunachal Pradesh is situated in the far North Eastern part of India adhering to China in North, Bhutan in South West, Myanmar in South East and Assam in South. Arunachal Pradesh is in the Indo-Burma Biological hotspot region and high diversity of plants and animals has already been reported from this region (Fig. 1). It harbors major tropical, subtropical, temperate and alpine forests within a small geographical area which favors about 40% of the floral and faunal species of India, many of which are endemic to the region. However, there is no record of microbial diversity of the region and present study may therefore provide valuable information on the soil fungal diversity of the state. The state has great variability in environmental aspects and cover major forest types viz., Tropical, Sub-tropical, Temperate and Alpine/Sub-alpine within a 100km range. Most of the tribes of the state practice shifting cultivation which is also known as "slash and burn system of cultivation". Fungal study of jhum land has not been carried out in this part of the country. This study will provide fungal distribution along altitude and vegetation.

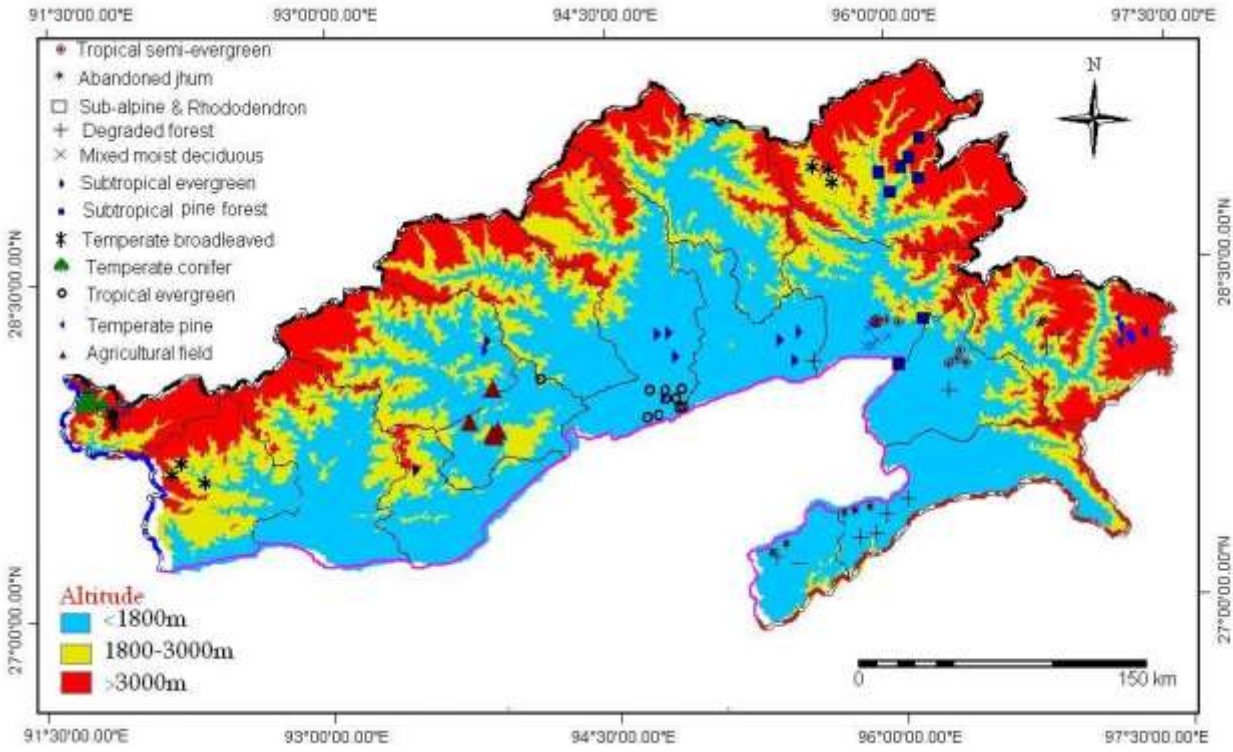

Fig. 1 – Map showing soil sampling sites in different districts of Arunachal Himalaya

Understanding the altitudinal distribution of microfungal assemblages has the potential to provide useful information on the future responses of biodiversity and functioning of ecosystems to environmental changes. Furthermore, much remains unknown about the patterns of changes with

altitude in the diversity and species composition of microfungal assemblages associated with micro-climatic conditions and soil physico-chemical properties. Studies on the analysis of vegetation changes along known environmental gradients may yield valuable insights into the interactions between community structure and environment (Bellis et al. 2007).

Purpose of the present study was to investigate the influence of vegetation, altitude and soil physico-chemical properties on fungal distribution in 12 major vegetational zones situated at altitude ranges from 135 to 4228msl. We expected to find that fungal diversity will vary between different vegetational zones due to the effect of altitude, temperature and soil physico-chemical factors.

Materials and Methods

Study site and sample collection

Microfungal diversity was studied in the soil samples collected from 12 major vegetational zones. The sampling sites in different vegetational zones were Likabali (Tropical evergreen forests); Roing and Tezu (Tropical semi-evergreen forests); Sagalee, Along, Basar and Pasighat (Sub-tropical evergreen forests); Anani (Sub-tropical pine forests); Roing (Mixed moist deciduous forests); Aniani, Tawang and Mandala Top (Temperate broadleaved forests); Walong (Temperate pine forests); Zimithang (Temperate conifer forests); Ptsho Lake (Sub-alpine and Rhododendron forests); Ziro (Sub-tropical agricultural field); Tirap, Changlang, Lohit and Anjaw (Degraded forest land); Tirap, Changlang and Anjaw (Abandoned jhum) located at the altitude ranging from 135 to 4228 meters above mean sea level.

Forest types categorization was done on the basis of altitude, climate, vegetation, followed for survey and sample collection, as described by Kaul & Haridasan (1987). The dominant plant species recorded from tropical forests were *Altingia excels* (Hamamelidaceae), *Dipterocarpus retusus* (Dipterocarpaceae), *Duabanga grandiflora* (Sommeratiaceae), *Mesua ferrea* (Calophyllaceae), *Terminalia indica* (Combretaceae), *Bombax ceiba* (Malvaceae), *Elaeocarpu*s sp. (Elaeocarpaceae), *Quercus* sp. (Fagaceae), *Gmelina arborea* (Lamiaceae). In subtropical forests, *Actinodophne obovata* (Lauraceae), *Illicium griffithii* (Schisandraceae), *Quercus* sp. (Fagaceae), *Michelia oblonga* (Magnoliaceae), *Pinus roxburghii* (Pinaceae) and *Pinus wallichiana* (Pinaceae) were dominant. Plant species like *Abies spectabilis* (Pinaceae), *Acer pectinatum* (Sapindaceae), *Alnus nepalensis* (Betulaceae), *Castanopsis indica* (Fagaceae), *Pinus roxburghii* (Pinaceae), *Rhododendron arboretum* (Ericaceae) were dominant in temperate forests. In sub-alpine and rhododendron forests, *Alnus nephalansis* (Betulaceae), *Rhododendron nivale* (Ericaceae), *Rhododendron anthopogon* (Ericaceae), *Sedum* sp. (Crassulaceae), *Rhodiola* sp. (Crassulaceae) were common. Likewise, in degraded forest and abandoned jhum, plants like *Citrus* sp. (Rutaceae), *Callicarpa arborea* (Lamiaceae), *Macranga denticulate* (Anacardiaceae), *Clerodendrum* sp. (Lamiaceae), *Chromolaena odorata* (Asteraceae) were common.

Soil samples upto 0-30cm of dept were collected randomly from the above selected vegetational soils during 2008-2009 using soil corer (inner diameter 5.5cm). Samplings were done during dry seasons (i.e., from September to March), as most of these study sites are inaccessible during rainy summer. In order to record maximum possible population and diversity, 10 replicates were selected in each vegetation type and two from each location at a distance of 5 meters. The geographical location of each sample collection site was recorded using digital GPS (Germin). Most of the soil samples were collected from vegetation cover area including the soil from degraded land and abundant jhum (slash and burn farming system). Nonetheless, the adjacent vegetation of collection sites was recorded during sampling. In forest soil with litter deposition, samples were collected after removing the top litter layer. Fresh soil samples were used for analysis of fungi and soil pH, and the remaining samples were air dried and stored for further analysis.

2. *Physico-chemical analysis of soil*

Soil temperature was determined using soil thermometer and soil pH was determined in a 1:2 soil water suspensions. Bulk density was determined following Blake & Hartge method (1986)

using soil corer, while soil organic carbon was determined using Walkley & Black's rapid titration method as described in Tropical soil biology and fertility (Anderson & Ingram 1993).

3. *Fungal isolation and identification*

Soils were collected from 10 different locations in each vegetational zone under sterile conditions with the help of 5.5 cm iron core. Two replicates were collected from each spot up to 30cm depth and were mixed properly before analyses. Fungi were isolated by the serial dilution method (Smith & Dawson 1944). Standard level of dilution (1:10,000) was selected to give optimum number of colonies on a single 10 cm Rose Bengal Agar (RBA) plate maintained at 25^{o}C. Each colony was sub-cultured on RBA prior to being maintained on potato dextrose agar (PDA) at 25^{o}C. About 50 µl of streptomycin (30 mg/ml stock) was added to 50 ml of the medium to avoid bacterial contamination (Grigorova & Norris, 1991). Heat and cold treatment were given as and when required for the better development of vegetative and reproductive structures. Standard procedures based on colony, spore and structural morphology were followed for identification at the generic and species level (Domsch et al. 1980, Ellis 1976, Gillman 1975).

4. *Statistical analysis*

Statistical analysis like ANOVA and Regression tests were carried out using statistical software like SPSS 16.0 and MINITAB 11.12. ANOVA was carried out to study the variation in soil physico-chemical parameters among study sites. Regression analysis was done to visualize the effects of soil physico-chemical parameters on soil fungal distribution.

Fungal diversity was analyzed using Shannon and Hills H1diversity indices. The equation for the log series (Log_{10}) distribution is adopted as it seems to provide extrapolating figure with least error. Shannon diversity index (1949) was calculated with the following equations:

$$H' = -\sum_{i=1}^{n} P_i \log P_i$$

Where, p_i = proportion of individuals in sample that belong to species i.

Hills N1 diversity (1973) was calculated as:

$$N_1 = \exp\left[-\sum_{i=1}^{n} P_i \log (P_i)\right]$$

Where, s= total number of species in each site, p_i= proportion of individuals in sample that belong to species i.

Similarities in fungal distribution were analyzed by Bray-Curtis cluster analysis (single link) using Biodiversity Professional statistical software (version 2).

Results

Variations were recorded in physico-chemical properties and fungal count of the soil samples studied from different vegetation zones. Soil pH was maximum in sub-tropical evergreen forests (5.5±1.2) followed by mixed moist deciduous forests (5.2±0.8). Soil temperature was maximum in tropical evergreen forests (30.6±1.5) followed by abandoned jhum (29.5±2.2) and lowest in sub-alpine & rhododendron forests (12.3±5.5). Similarly, soil organic carbon was maximum in samples collected from sub-alpine and rhododendron forests (5.5±0.1) followed by temperate pine forests (5.4±0.03) and tropical evergreen forests (4.4 ±0.1) and lowest level was recorded in degraded forest land (1.0±0.1). Likewise, bulk density was maximum in soil samples from abandoned jhum (1.6±0.18 gm^{-3}) followed by degraded forests (1.5±0.15 gm^{-3}) and lowest in mixed moist deciduous forests (0.50±0.01 gm^{-3}). Statistical analysis of soil physico-chemical parameters, altitude and fungal diversity of the samples collected from different vegetational zones showed significant variation in soil pH (F=7.89, P=0.000), soil temp (F=54.83, P=0.000), soil organic carbon (F=167.04, P= 0.0000), bulk density (F=88.12, P=0.000), altitude (F=120, P=0.000) and fungal diversity (F=8.32, P= 0.000) (Table 1).

Table 1 ANOVA test of soil physico-chemical parameters and fungal count among different sites.

Parameters	F value	P value	Degree of freedom (df)	Pooled Standard deviation
Soil pH	7.89	0.000	11	0.68
Soil temperature	54.83	0.000	11	2.75
Soil Organic carbon	167.04	0.000	11	1.61
Altitude	120.00	0.000	11	327.1
Soil bulk density	88.12	0.000	11	0.12
Soil fungal count (CFU)	8.32	0.000	11	42.15

In tropical evergreen forests, altitude was found to have significant negative correlation on soil fungal distribution (r=-0.632, p=0.05) while temperature was negatively correlated with altitude (r=-0.994, p=0.000). Soil pH, organic carbon content and bulk density showed negative but insignificant correlation with fungal distribution (Table 2). Similarly, in tropical semi-evergreen forests, soil fungal distribution was negatively correlated with altitude (r=-0.930, p=0.000) and positively correlated with soil temperature (r=0.904, p=0.000). Altitude and soil temperature was negatively correlated (r= -0.963, p=0.000). In sub-tropical evergreen and temperate pine forests, soil fungal distribution was negatively correlated with altitude (r= -0.752, p=0.012 & -0.970, p=0.000) and positively correlated with soil temperature (r= 0.716, p=0.020 & 0.979, p=0.000). In both these forests, soil temperature was found negatively correlated with altitude (r= -0.938 & -0.781, p=0.000). However, in temperate pine forests, soil organic content was positively correlation with soil fungal distribution (r=0.764, p=0.010). Soil fungal distribution showed insignificant correlation with altitude and soil temperature while soil temperature was significantly correlated with altitude (r=-0.997, p=0.000) in sub-tropical pine forests. In temperate conifer and sub-alpine and rhododendron forests, soil fungal distribution was negatively correlated with altitude (r=-0.719, p=0.019 & r=-0.879, p=0.001) and positively correlated with soil temperature (r=0.764, p=0.010 & r=0.881, p=0.001). Bulk density was negatively correlated with soil organic content (r=-0.872, p=0.001) in temperate pine forest. In degraded forests and abandoned jhum, soil fungal distribution was negatively correlated with altitude (r= -0.866, p=0.001 & r=-0.794, p=0.006) and positively correlated with soil temperature (r=0.836, p=0.003 & r=0.773, p=0.009). However, in abandoned jhum, soil pH was fund to have significant positive effect on fungal distribution (r=573, p=0.006) and in degraded forests, soil organic content showed positive correlation with soil fungal distribution (r=0.636, p=0.048). In mixed moist deciduous forests, significant correlation was recorded between soil fungal distribution and altitude (r= -0.790, P=0.007). In agriculture field and temperate broadleaved forests, on significant correlation was recorded between fungal distribution and soil physico-chemical parameters. Nevertheless, statistical analysis using data from all the sites showed that soil fungal distribution has negative correlation with altitude (r= -0.378, $p<0.05$) and bulk density (r= -0.116, $p>0.05$) while positive correlation with temperature (r= 0.460, $p<0.05$), pH (r= 0.204, $p>0.05$) respectively. Altitude was negatively correlation with soil temperature (r= -0.478, $P<0.05$), while organic carbon was negatively correlation with bulk density (r= -0.545, $P<0.05$).

A total of 112 fungal types under 59 genus and 88 species were recorded in above studied forests and agricultural soils collected from different altitude (Annexure 1). Ascomycota was the largest phylum with 16 order/group and 94 fungal types followed by Zygomycota with two orders/groups and three fungal types (Table 3). Sordariomycetes has the highest relative abundance in low altitude forest soil however, in higher altitude forests like sub-tropical, temperate conifer and sub-alpine & rhododendron forests, Leotiomycetes was abundant (Fig. 2). Hypocreales was the most diverse fungal groups/order followed by Eurotiales, Leotiomycetales and Pleosporales. Among Ascomycota, diversity indices was highest for *Aspergillus* (0.818) followed by *Penicillium* (0.789).

Table 2 Spearman's correlation coefficient test of soil physico-chemical parameters and fungal count in different vegetation zones.

Vegetation zones	Spearman's Correlation coefficient						
	Altitude Vs Fungal counts	**Temp. Vs Fungal counts**	**Sol pH Vs Fungal counts**	**Soil organic carbon Vs Fungal counts**	**Bulk density Vs Fungal counts**	**Altitude Vs Soil temperature**	**Organic carbon Vs Bulk density**
Tropical evergreen	r= -0.632* p= 0.050	r= 0.599 p= 0.067	r= -0.542 p= 0.106	r= -0.470 p= 0.171	r= -0.191 p= 0.598	r= -0.994** p= 0.000	r= -0.617 p=0.057
Tropical semi-evergreen	r= -0.930** p= 0.000	r= 0.904** p= 0.000	r= 0.591 p= 0.072	r= 0.290 p= 0.417	r= 0.486 p= 0.154	r= -0.963** p= 0.000	r= -0.104 p= 0.775
Sub-tropical evergreen	r= -0.752* p= 0.012	r= 0.716* p= 0.020	r= 0.626 p= 0.053	r= -0.316 p= 0.708	r= 0.636* p= 0.048	r= -0.938** p= 0.000	r= 0.191 p= 0.596
Sub-tropical pine	r= -0.265 p= 0.459	r= 0.282 p= 0.430	r= 0.178 p= 0.623	r= 0.441 p= 0.202	r= 0.019 p= 0.959	r= -0.997** p= 0.000	r= -0.669* p= 0.034
Temperate pine	r= -0.970** p= 0.000	r= 0.979** p= 0.000	r= -0.080 p= 0.826	r= 0.764** p= 0.010	r= -0.590 p= 0.073	r= -0.781* p= 0.008	r= -0.648* p= 0.043
Temperate conifer	r= -0.719* p= 0.019	r= 0.764* p= 0.010	r= 0.208 p= 0.564	r= 0.427 p= 0.219	r=-0.872** p= 0.001	r= -0.383 p= 0.275	r= -0.624 p= 0.054
Sub-alpine and Rhododendron	r= -0.879** p= 0.001	r= 0.881** p= 0.001	r= -0.123 p= 0.735	r= 0.758** p= 0.001	r= -0.626 p= 0.053	r= -0.685* p= 0.029	r= -0.504 p= 0.137
Degraded forest	r= -0.866** p= 0.001	r= 0.836* p= 0.003	r=- 0.505 p= 0.137	r= 0.636 p= 0.048	r= -0.255 p= 0.475	r= -0.872** p= 0.001	r= -0.219 p= 0.544
Abandoned jhum	r= -0.794* p= 0.006	r= 0.773* p= 0.009	r= 0.573* p= 0.006	r= 0.450 p= 0.192	r= -0.644 p= 0.044	r= -0.450 p= 0.192	r= -0.625 p= 0.053
Mixed moist deciduous	r= -0.790* p= 0.007	r= -0.098 p= 0.785	r= -0.198 p= 0.584	r= 0.208 p= 0.564	r= 0.220 p= 0.542	r= -0.500 p= 0.141	r= 0.221 p= 0.540
Agricultural field	r= -0.321 p= 0.366	r= -0.193 p= 0.594	r= -0.207 p= 0.565	r= 0.468 p= 0.172	r= -0.131 p= 0.717	r= -0.307 p= 0.125	r= 0.213 p= 0.554
Temperate broadleaved	r= 0.195 p= 0.590	r= 0.080 p= 0.827	r= 0.420 p= 0.227	r= 0.021 p= 0.953	r= 0.281 p= 0.431	r= 0.091 p= 0.802	r= -0.494 p= 0.147

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

Table 3 Taxonomic grouping of fungi isolated from different vegetation zones.

Phylum	Class	Order/group	Genus with number of species
Ascomycota	Eurotiomycetes	Eurotiales	*Aspergillus* (9), *Paecilomyces* (1), *Penicillium* (14)
		Onygenales	*Arthroderma* (2), *Blastomyces* (1), *Chrysosporium* (1)
		Chaetothyriales	*Exophiala* (1)
	Sordariomycetes	Hypocreales	*Acremonium* (4), *Beauveria* (1), *Cladosporium* (3), *Cylindrocarpon* (1), *Fusarum* (2), *Gliocladium* (1), *Metarrhizum* (1), *Sesquicillium* (1), *Staphylotrichum* (1), *Trichoderma* (3), *Trichothecium* (1)
		Trichosphaeriales	*Humicola* (3), *Nigrospora* (1)
		Sordariales	*Chaetomium* (1), *Trichocladium* (2)
		Phyllachorales	*Verticillium* (5)

		Sordariomycetidae incertae sedis	*Apiospora* (1)
		Microascales	*Doratomyces* (1), *Scopulariopsis* (1)
	Dothideomycetes	Pleosporales	*Cochliobolus* (3)/ *Curvularia* (3), *Phoma* (3)
		Capnodiales	*Carcospora* (1), *Isariopsis* (1)
		Dothideales	*Aerobasidium* (1)
	Saccharomycetes	Saccharomycetales	*Candida* (1), *Geotrichum* (1)
	Leotiomycetes	Helotiales	*Botrytis* (1), *Trichosporiella* (1)
		Leotiomycetes incertae sedis	*Oidiodendron* (4)
	Lecanoromycetes	Teloschistales	*Bispora* (1)
	Not assigned	Incertae sedis	*Ramichloridium* (1)
Zygomycota	Zygomycetes	Mortierellales	*Mortierella* (3)
		Mucorales	*Absidia* (1), *Mucor* (3), *Rhizopus* (2)
Basidiomycota	Urediniomycetes	Sporidiales	*Torula* (1), *Pseudotorula* (1)
	Agaricomycetes	Cantharellales	*Rhizoctonia* (1)
Heterokontophyta	Oomycetes	Pythiales	*Pythium* (1)

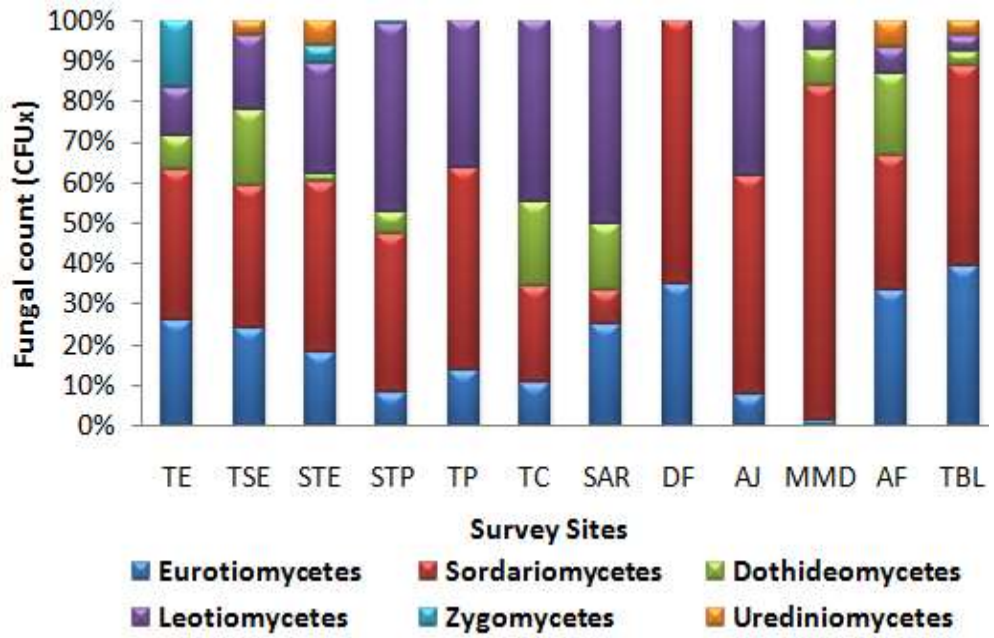

Fig. 2 – Distribution of fungal taxa in different forest and agricultural soils. Abbreviations: TE= Tropical Evergreen, TSE= Tropical semi-evergreen, STE= Subtropical evergreen, STP= Subtropical pine, MMD= Mixed moist deciduous, TB= Temperate Broadleaved, TP= Temperate pine, TC= Temperate Conifer, SAR= Sub-alpine and Rhododendron, DF= Degraded forest, AJ= Abandoned Jhum, AF= Agriculture field

Soil fungal community varied from tropical to sub-alpine forests however, maximum diversity was recorded in the intermediate altitude above 1800 msl (51 types under 31 genus; H′=1.408) followed by tropical evergreen (37 types under 25 genus; H′=0.389) and tropical semi-evergreen forests (25 types under 16 genus; H′=1.251) (Fig. 3). Significant variation was found in fungal distribution among different forest types (F=8.32, P=0.000). Similarities were recorded in the fungal distribution between temperate pine and temperate conifer forests (42.1% by Bray-Curtis cluster analysis). Both Shannon and Hills diversity indices reported highest diversity fungi in subtropical evergreen followed by tropical evergreen forests (Table 4).

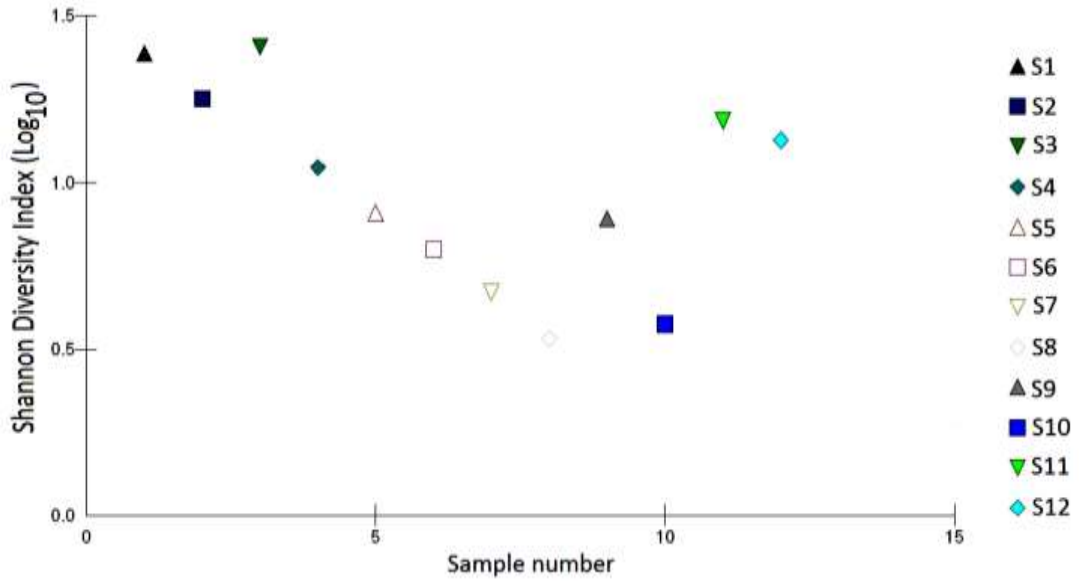

Fig. 3 – Shannon diversity indices of the fungal distribution in different forests and agricultural soils. Abbreviations: S 1= Tropical Evergreen, S2= Tropical semi-evergreen, S3= Subtropical evergreen, S4= Subtropical pine, S5= Mixed moist deciduous, S6= Temperate Broadleaved, S7= Temperate pine, S8= Temperate Conifer, S9= Sub-alpine and Rhododendron, S10= Degraded forest, S11= Abandoned Jhum, S12= Agriculture field

Table 4 Comparative analysis of result using various diversity indices.

Sampling Sites	Shannon base 10	Hills no. H1
1	**1.389**	**145.331**
2	1.251	92.005
3	**1.408**	**154.951**
4	0.532	8.439
5	0.67	13.35
6	0.91	29.602
7	0.893	27.978
8	1.186	74.087
9	0.575	9.738
10	0.799	20.476
11	1.045	46.492
12	1.127	60.989

About 60% fungal species was found aggregated in few selected soils and the rest 40% showed random distribution pattern (Fig. 4). The dominant fungal genera recorded from majority of soil samples were *Oidiodendron* followed by *Acremonium, Cladosporium, Humicola, Penicillium* and *Aspergillus*. However, different forest soils were dominated by diverse group of fungi viz. *Humicola*, *Aspergillus* and *Penicillium* were dominant in tropical forests; *Oidiodendron* and *Acremonium* in subtropical forests; *Verticillium*, *Penicillium*, *Oidiodendron* and *Cladosporium* in temperate forests; *Acremonium* and *Oidiodendron* in sub-alpine & rhododendron forests. Degraded forests and abandoned jhum was dominant by *Cladosporium*, *Acremonium*, *Verticillium* and *Penicillium* whilst agricultural forests were dominated by *Acremonium*, *Penicillium*, *Humicola* and *Aspergillus* (Fig. 5). Common litter decomposers like *Acremonium*, *Aspergillus*, *Oidiodendron*, *Humicola* and *Trichoderma* are dominant in tropical and sub-tropical forest soils. Distribution of *Beauveria*, *Blastomyces*, *Cercospora*, *Metarrhizium* and *Rhizomucor* were limited to sub-tropical evergreen and tropical evergreen forests.

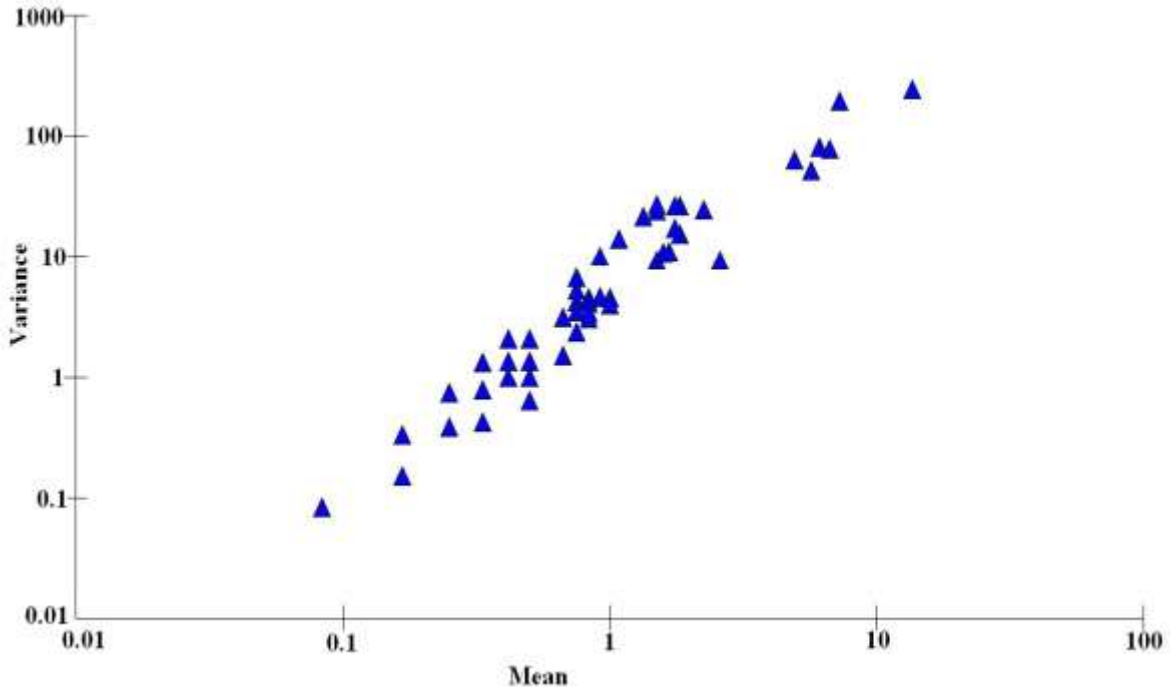

Fig. 4 – Species distribution pattern in forest and agricultural soils.

Species level distribution showed domination of *Oidiodendron truncatum* in sub-tropical forests and *Acremonium butyri* in sub-tropical as well as in degraded forests land. Similarly, *Acremonium kiliense* was common in tropical and sub-tropical soil. Agricultural soil was dominated by *Acremonium murorum*, *Humicola brevi* and *Penicillium expansum*. *Aspergillus* species were commonly distributed in tropical and sub-tropical forests. *Cladosporium herbarum* and *Cladosporium cladosporioides* were the dominant *Cladosporium* species found mainly in tropical, sub-tropical and few temperate forest soils. Among *Humicola* species, *Humicola fuscoatra*, *Humicola brevi* and *Humicola grisea* were common in tropical and sub-tropical forests. *Penicillium chrysogenum* was found in tropical, sub-tropical and temperate soils. In sub-alpine and rhododendron forests, *Acremonium kiliense*, *Humicola fuscoatra* and *Oidiodendron truncatum* were dominant species. Distribution pattern of several fungi were similar in some forests and agricultural soil. Bray-Curtis clustering of fungi in different forests and agricultural soils showed close similarities in distribution pattern of fungal species like *Cladosporium cladosporioides* and *Torula herbarum* (>80% similarities), *Acremonium kiliense* and *Oidiodendron truncatum* (>60% similarities), *Acremonium butyri* and *Cladosporium herbarum* (>60% similarities), *Aspergillus flavus* and *Aspergillus fumigatus* (>60% similarities) (Fig. 6).

Whilst soil fungal diversity was compared by grouping 12 vegetation types studies above into four ecological regions i.e., Tropical (including tropical evergreen and semi-evergreen), Sub-tropical (including sub-tropical evergreen, subtropical pine, subtropical agricultural field and mixed moist deciduous forests), Temperate (including temperate broadleaved, temperate pine, temperate conifer) and Alpine (including sub-alpine and rhododendron forests), maximum fungal concentration (both species and CFU) were recorded in sub-tropical region followed by tropical and lowest in alpine region. However, the soil fungal diversity of sub-tropical region was significantly correlation with temperate region ($r=0.9702$, $p=0.030$).


Fungal species
S1 S2 S3 S4 S5 S6 S7 S8 S9 S10 S11 S12
Absidia glauca
Acremonium butyric
Acremonium kiliense
Acremonium mumorum
Acremonium strictum
Aerobasidium sp.
Apiospora montagnei
Arthroderma quadrifidum
Arthroderma tuberculatum
Aspergillus candidus
Aspergillus fumigatus
Aspergillus flavus
Aspergillus nidulans
Aspergillus niger
Aspergillus restrictus
Aspergillus sydowi
Aspergillus terreus
Aspergillus versicolor
Beauveria bassiana
Bispora sp.
Blastomycessp.
Botrytis cinerea
Botrytis sp.
Candida sp.
Cercospora sp.
Chaetomium indicum
Chaetomium sp.
Chrysosporium merdarium
Cladosporium cladosporiode
Cladosporium herbarum
Cladosporium macrocarpam
Cladosporium sp.
Cochliobolus geniculata
Cochliobolus lunatus
Cochliobolus sativus
Curvularia interseminata
Curvularia lignicolom
Curvularia subulata
Cylindrocarpon magnusianum
Doratomyces sp.
Exophiala jeanselmei
Fusarium flocciferum
Fusarium sporotrichioides
Fusarium sp.
Geotricum candidum
Geotrichum sp.
Gliocladium sp.
Humicola brevi
Humicola fuscoatra
Humicola grisea
Isariopsis sp.
Metarrhizum flavoviride
Metarrhizum sp.
Mortierella alpine
Mortierella bisporales
Mortierella elengata
Mucor circinelloides
Mucor haemilis
Mucor racemosus
Nigrospora sphaerica
Oidiodendron echinulatum

Fig. 5 – Distribution of different fungal species in different vegetation soils. S 1= Tropical Evergreen, S2= Tropical semi-evergreen, S3= Subtropical evergreen, S4= Subtropical pine, S5=Temperate pine, S6= Temperate Conifer, S7= Sub-alpine and Rhododendron, S8=Degraded forest, S9= Abandoned Jhum S10= Mixed moist deciduous, S11= Agriculture field, 12= Temperate Broadleaved

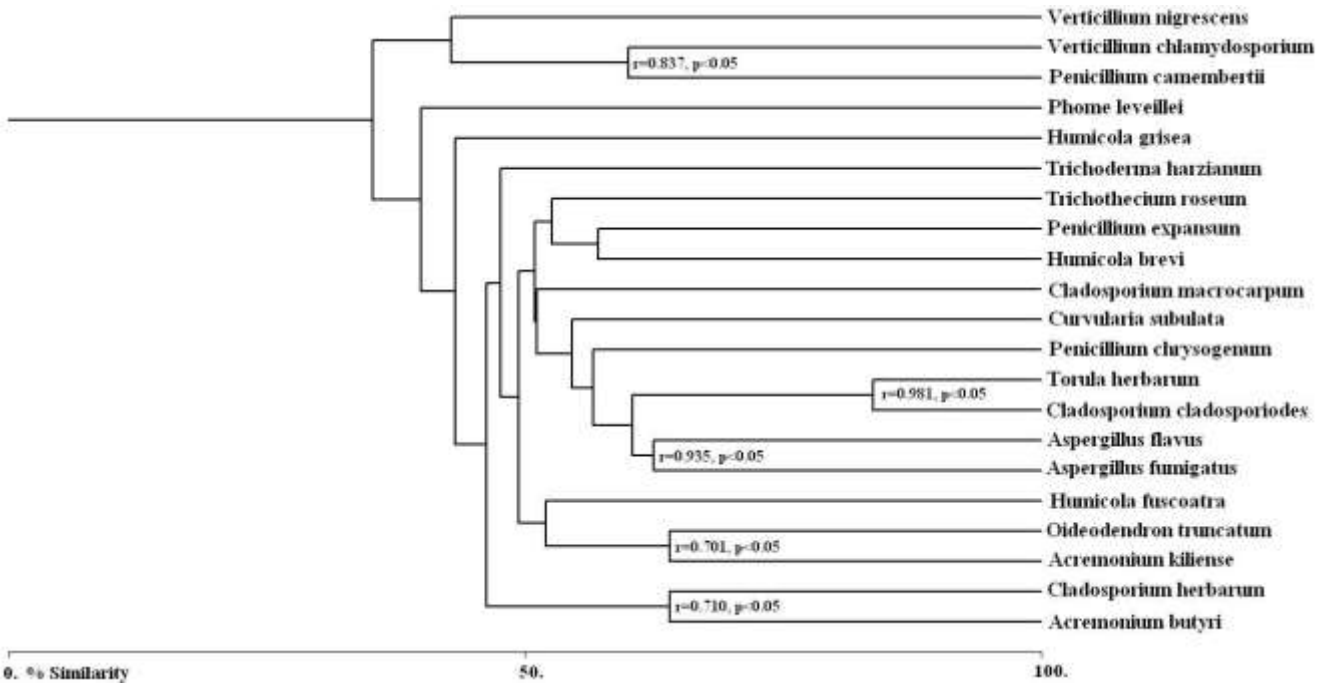

Fig. 6 – Bray-Curtis single link cluster analysis of distribution pattern among different fungal types in 12 selected vegetational soil of Arunachal Himalaya.
Note: r =correlation and p = level of significance

Discussion

Microclimatic condition of study area may also influence the abundance and diversity of soil fungi of any region though the level of association may vary from area to area (Talley et al. 2002). Edaphic as well as climatic conditions affect the number and nature of microbial diversity in general, factors like root exudates and age of the host plants affect the microflora associated with a given rhizosphere, in addition (Pandey et al. 2006). The present study showed negative correlation between the fungal diversity and altitudinal gradient however the level of correlation is not highly significant as maximum population and varieties were recorded in sub-tropical forests, followed by tropical and degraded forests. Higher competition for resources and suitable space at lower altitudes under more favorable environments and more severe environmental stresses at higher altitudes lead to maximum microfungal concentration at intermediate altitudes (Jackson et al. 1991, Raviraja et al. 1998, Osono & Hirose 2009). Supporting the present study, Devi et al. (2012) also reported maximum fungal diversity at intermediate altitude. Maximum fungal diversity at medium altitude is also reported by Widden & Abitol (1980). Higher fungal diversity and abundance in sub-tropical forests as compared to the tropical forests could be due to the favorable physico-chemical properties of sub-tropical soil and stimulatory effects of associated vegetation on soil fungi (Christensen 1969, Widden 1987).

Abundance and activity of soil microorganism of any region is regulated by plant species to a great extent (Wubet 2012, Xu et al. 2013). The trees of sub-tropical and temperate regions are reported to have stimulatory effect on the rhizospheric microorganisms and hence support greater fungi diversity (Christensen 1969). Moreover, plant materials that are used by fungi constitute an important and decisive resource for the life of the different species (Bissett & Parkinson 1979a, Schmitz et al. 1989, Zhang 2010). Complex vegetation provides various kinds of substrata, thereby allowing different fungal species to coexist (Wicklow & Whittingham 1974, Christensen 1984). Even relatively homogeneous plant communities in some cases, support complex mycota (Apinis 1972). This implies that various fungal species could share the space of a single substratum (Ogawa et al. 1996). In the present study although litter and non-litter soils were not categorized, but maximum vegetational soils except degraded and agricultural soils were litter rich. Higher fungal diversity recorded in forest soil as compared to agricultural field and degraded/abundant jhum

could be due to the fact that the complex conditions of the litter-humus layer in forest soil support various kinds of microfungal species, as it consists of a mixture of decaying leaves in various stages and is exposed to air and spore vectors (Novak & Whittingham 1968).

Supporting the present study, Devi el al. (2012) and Hangwitz (2013) also reported abundance of Ascomycota followed by Zygomycota in forest soil. Hangwitz (2013) reported domination of Helotiales and Eurotiales in the forest soil however, in our findings Hypocreales, Eurotiales and Leotiomycetes incertae sedis were found dominant. Domination of Hypocreales and Eurotiales in northeast India's soil is also reported by Devi et al (2012). Fungal species like *Oidiodendron truncatum, Acremonium kiliense, Acremonium butyri, Cladosporium herbarum* and *Humicola fuscoatra*, were found abundant in the above study. Distribution of other fungal species including *Acremonium butyri, Aspergillus nidulans, Aspergillus flavus, Cladosporium cladosporioides, Penicillium chrysogenum, Phoma levellei*, *Trichothecium roseum* and *Torula herbarum*, were found associated with some vegetational soils. *Oidiodendron*, *Acremonium*, *Aspergillus* and *Humicola* were the most abundant genera among those characterizing lower altitudes (tropical and sub-tropical). From higher altitude (above 1800 msl), *Acremonium*, *Cladosporium*, *Penicillium*, *Verticillium* and *Trichothecium* were dominant. Domination of *Aspergillus, Acremonium, Cladosporium, Penicillium, Oidiodendron* in forest soil is reported from different forests and vegetation zones (Bhatt 1970, Baath 1981, Widden 1987, Pandey et. al. 2006, Barbaruah et. al. 2012, Oliveira et. al. 2013). The present study recorded higher number of *Oidiodendron* species, particularly *O. truncatum*, followed by *Acremonium kiliense, Acremonium butyric, Cladosporium cladosporioides, Humicola fuscoatra* and *Penicillium chrysogenum* at higher altitudes. Several of these fungal species are frequently reported from higher altitude soil (Domsch et al. 1980, Maggi et al. 2005, Osono & Hirose 2009). *Penicillium*, *Cladosporium* and *Trichoderma* are commonly known as late-stage colonizers in decomposing litter (Hudson 1968, Osono & Takeda 2007) and domination of these saprobic fungi indicates faster decomposition and recycling of dead organic materials and litter particles, hence maintaining soil nutrient status (Baath 1981). Although *Oidiodendron* species were reported mostly from temperate regions (Domsch et al. 1980, Hambleton et al. 1998, Sigler & Flis 1998) and were found uncommon in tropical and sub-tropical soils (Rice & Currah 2005). In contrary, present study showed domination of *Oidiodendron* in sub-tropical soils besides its distribution in tropical and temperate region. Importantly, these *Oidiodendron* species are reported to produce varieties of enzymes, including pectinases, lipases, gelatinases, and polyphenol oxidases, that potentially allow them to degrade a variety of plant, fungal, and animal-based substrates, including those found in soils (Rice & Currah 2005). Variations in present distribution pattern from earlier report could be due to the disparities in climatic, edaphic and vegetational effects from area to area.

pH and soil organic carbon does not appear to be a conclusive pattern since alterations in pH and soil carbon in several cases has insignificant effects on fungal dominance. Several researchers have reported similar effects of pH and soil carbon on fungal distribution (Hogberg et al. 2007, Strickland & Rousk 2010). Similarities in fungal diversity patterns in different vegetational zones shown by Shannon and Hills diversity indices in the present study could be due to the fact that both are a family of intrinsic diversity indices. Shannon weighted towards species richness and Hill describes the relationship between a numbers of intrinsic diversity. Since both sub-tropical and tropical forest soil recorded higher fungal diversity with maximum intra-specific variations, hence they showed similarities in diversity and distribution of fungi. Hashemi & Kafaki (2009) also reported correlation between Shannon and Hills diversity indices. They also reported that Shannon and Hills diversity indices are suitable for predicting diversity along altitude.

Similarities in fungal diversity in sub-tropical and temperate forests as recorded above could be due to the presence of some similar plant species which are known to provide stimulatory effect on microbial species and support higher fungal populations (viz, *Taxus, Quercus*, *Pinus*). Pandey et al. (2006) and Xu et al. (2013) have also reported that the conifer of sub-tropical and temperate locations, namely *Pinus*, *Quercus* and *Taxus* support relatively higher microbial population in

Annexure 1 Average colony forming unit (CFU × 10^3) of fungi, identified from different vegetational soils of Arunachal Pradesh

Fungal species	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
Absidia glauca Hagem			2									
Acremonium butyric W.Gams	3	1	20	14	10					25		
Acremonium kiliense Grutz	16	5	49	6				1	5		1	4
Acremonium mumorum (Corda) W.Gams												16
Acremonium strictum W.Gams.		3								1		
Aerobasidium species		3										
Apiospora montagnei Sacc.			1									
Arthroderma quadrifidum Dawson & Gentles			1									
Arthroderma tuberculatum Kuehn			1									
Aspergillus candidus Link ex. Link			2									7
Aspergillus fumigatus Fres.	5		4									1
Aspergillus flavus Link ex Gray	8	1	12									1
Aspergillus nidulans (Eidam) Winter	13											
Aspergillus niger van Tieghem			17									1
Aspergillus restrictus G. Sm.				2								
Aspergillus sydowi Thom & Church	5											
Aspergillus terreus Thom	2											
Aspergillus versicolor (Vuill.) Tiraboschi		1										
Beauveria bassiana (Bals.) Vuill.			2									
Bispora species			2									
Blastomyces species			1									
Botrytis cinerea Pers. Ex Nocca & Balb.		3	7									
Botrytis Mich Ex Fr.				6	3							
Candida species									1			
Cercospora species			1									
Chaetomium indicum Corda	1											
Chaetomium sp.		1										
Chrysosporium merdarium Carm.			4									
Cladosporium cladosporiode (Fres) de Vries		1	18	1	2							
Cladosporium herbarum (Pers. Link ex Gray	17		11	3	7	9	1			29	1	2
Cladosporium macrocarpam Preuss			5	6								1
Cladosporium Link ex. Fr..	11											
Cochliobolus geniculata R.Nelson	4											1
Cochliobolus lunatus R.Nelson & Haasis						3					1	
Cochliobolus sativus Drechsler ex Dastur											1	1
Curvularia interseminata (Berkeley and Ravenel)				6		5						
Curvularia lignicolom		1										
Curvularia subulata (Nees) Boedijn	2		4							6		

Cylindrocarpon magnusianum Wollenw.	1												
Doratomyces Corda	1												
Exophiala jeanselmei McGinnis & Padhye			1										
Fusarium flocciferum Corda			2										
Fusarium sporotrichioides Sherb.			1										
Fusarium species	2												
Geotricum candidum Link ex Leman			8		1								
Geotrichum Link										2			
Gliocladium Corda				3	3								
Humicola brevi	8		1									1	8
Humicola fuscoatra Traaen vr. Fuscoatra	24	17	10				1			3		1	3
Humicola grisea Traaen var. grisea	8			2	9								
Isariopsis species	2												
Metarrhizum flavoviride W.Gams & Rozsypal													1
Metarrhizum Sorok.			1										
Mortierella alpine Peyronel	3												
Mortierella bisporales (Thaxt.) Bjorling			1										
Mortierella elengata Linnem.			1										
Mucor circinelloides van Tiegh.	1												
Mucor haemilis f.corticola (Hagem) Schipper	13												
Mucor racemosus Fres. f. racemosus	5			1									
Nigrospora sphaerica (Sacc.) Mason	6		3										
Oidiodendron echinulatum Barron			16										
Oidiodendron griseum Robak			9										
Oideodendron rhodogenum Robak.		1											
Oideodendron truncatum Barron	19	6	45	44	13	17	6			5	5	1	2
Penicillium brevicompactum Dierckx			4										
Penicillium camembertii Thom								1		1		2	
Penicillium canescens Sopp			1										
Penicillium chrysogenum Thom	6	6	7	5				6					1
Penicillium citrinum Thom			3										
Penicillium cyclopium Westling												1	
Penicillium daleae Zaleski.						1							
Penicillium expensum Link ex. Gray											1	2	7
Penicillium frequentans Westling.													3
Penicillium jensenii Zaleski	2												
Penicillium janthinellum Biourge				1									
Penicillium nigricans Bain. Ex Thom	2					3							
Penicillium oxalicum Currie & Thom		3	1										
Penicillium variabile Sopp		1		1									
Penicillium Link ex Fr.		1			6		3						

Phoma eupyrena Sacc.	6	2										
Phoma leveillei Boerema & Bollen		2	1				2				1	
Phoma medicaginis Malbr. & Roum. var. *pinodella* (L.K.Jones) Boerema		2										
Pseudotorula species		2										
Pythium intermedium de Bary	1											
Pythium Pringsheim						1						
Ramichloridium schulzeri (Sacc.) de Hoog			1									
Rhizomucor pusillus (Lindt) Schipper	3											
Rhizopus oryzae Went & Prinsen Geerligs			2									
Rhizopus stolonifer (Ehrenb. Ex Link) Lind	2		6									
Rhizoctonia de Candolle				1								
Scopulariopsis brumptii Salvanet-Duval				1								3
Scopulariopis Bain							1					
Sesquicillium candelabru (Bonord.) W. Gams	1											
Staphylotrichum coccospoium J.Meyer & Nicot			2								1	
Torula herbarum Pers. Ex. Gray			18								1	2
Trichoderma harzianum Rifai		2	5						2			
Trichoderma koningii Oudem.			3									
Trichoderma viride Pers ex Gray.												1
Trichoderma Pers. Ex Fr.			1							1		
Trichosporiella cerebriformis W. Gams	1											
Trichothecium Link ex Gray				9				12				
Trichothecium roseum Link ex Gray	10	7									2	1
Trichocladium asperum Harz	2											
Trichocladium opacum (Corda) Hughes	1											
Verticillium albo-atrum Reinke & Berthold			1									
Verticillium chlamydosporium Goddard				1					2		3	
Verticillium dahlie Kleb.							18					
Verticillium lecanii (Zimm.) viegas			1									
Verticillium nigrescens Pethybr.			3			2					3	
Zygosporium species		1	1									
Sterile hyphae		5		17			1		4			
Unidentified		6	22	10	4	8	1	16			1	

S1= Tropical Evergreen, S2= Tropical semi-evergreen, S3= Subtropical evergreen, S4= Subtropical pine, S5= Mixed moist deciduous, S6= Temperate Broadleaved, S7= Temperate pine, S8= Temperate Conifer, S9= Sub-alpine and Rhododendron, S10= Degraded forest, S11= Abandoned Jhum, S12= Agriculture field

Note: Fungi were identified to species level on the basis of morphological characteristics (colony colour, shape and size; spore size, pattern; hyphal arrangement, shape, size; conidiophores shape, pattern and phialides arrangement, shape, size). For easy reference the species names are given with the authors name and year of report.

comparison to non coniferous species. Slower decomposition activities owing to lower temperature conditions at higher altitudes can limit fungal growth and consequently diminish the pace of changes in chemical composition during decomposition which may promote fungal succession on forest litter (Osono 2005). This may lead to an extension of duration of early stages of decomposition and a shift in microfungal assemblages to early-successional species.

Fungi can serve as indicators of environmental changes or disturbances resulting from natural or anthropogenic causes, including elevated carbon dioxide levels and global warming (Van Maanen et al. 2000, Gourbiere et al. 2001, Cabello and Arambarri 2002). Effects of climatic change and resulted faster snow melt from the indo-china glacier have been recorded recently in Himalayan range (Rasul 2008, Jain et al. 2010). Studies on the affect of these changes on the microbial communities may provide knowledge on fungal community structure. Increases in temperature, for example, may lead to the establishment of species such as *Aspergillus*, *Trichoderma*, *Mucor*, and *Penicillium* at higher altitudes and the replacement of microfungal species currently present at higher altitudes. Studies on these aspects will help in understanding the changes in functional aspects of fungal assemblages at higher altitudes.

Acknowledgements

Authors are indebted to Council of Scientific and Industrial Research (CSIR) for providing financial assistance during the tenure of the present investigation.

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