Seasonal and Depth-wise variation in Microfungal Population Numbers in Nameri forest soil, Assam, Northeast India

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Soil microflora was isolated by serial dilution plate method using different culture media. The highest fungal population was recorded in spring at the topsoil (1–9 cm), and decreased in other seasons with increasing depths. Twenty-one fungal species belonging to 14 genera were recovered from all depths throughout the seasons with the highest population and relative abundance of *Aspergillus flavus* (8.4%), followed by *Penicillium chrysogenum* (8.0%) and lowest by *Rhizopus oryzae*, *R. nodosus* and *Trichophyton* sp. (2.8% each). Phycomycetes (80.1%) were dominant in the study site followed by Zygomycetes (14.1%), Ascomycetes (3.7%) and sterile mycelia (2.1%). Soil pH, moisture content, organic C (C_{org}), total N concentration (N_{tot}) and available K had positive correlations (p<0.05 and <0.01) with the fungal colony forming unit. Difference in soil depth and seasonal variation have an influence on the microfungal population numbers as well as their species composition in Nameri forest soil.

Key words – colony forming unit – population and relative abundance – serial dilution plate method

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Introduction

Natural forests are sites of high biodiversity, where complex relationships among fauna, flora and microflora are maintained due to structural richness of the habitat (Tsai et al. 2007). Soil microorganisms are essential components of the biotic community in natural forests, responsible for the breakdown of organic materials, mobilization of nutrients, maintenance of soil-plant quality and ecosystem biogeochemistry (Hackel et al. 2004). Soil microorganisms are also known to influence the soil physical, chemical and biological properties (Tangjang & Arunachalam 2009). Fungi are an important component of soil microbiota, contributing more soil biomass than bacteria (Ainsworth & Bisby 1995). They are present in soil both as actively growing organisms and dormant propagules (Warcup 1957). The role of fungi in soil is extremely complex and is fundamental to the entite soil ecosystem (Bridge & Spooner 2001). Soil microfungi play a crucial role in the decomposition of plant structural polymers such as cellulose, hemicellulose and lignin, thereby contributing to the maintenance of the global nutrient cycle. Some twenty functions of soil fungi were described by Christensen (1989) with one of the main functions being their significant role as primary degraders. The rate at which organic matter is decomposed by this group of soil microorganism is interrelated to the chemical composition of the substrate as well as environmental conditions (Arunachalam et al. 1997). Micro fungi are also fundamental for soil ecosystem functioning, especially in forest soils, which

differ from other soils in physical, chemical and biological characteristics as these soils gets supplied (agricultural soils, for instance are contaminated) with large amounts of pesticides, fungicides etc. for a long time.

Microfungal diversity, using cultivation-dependent approaches, has been investigated (Viaud et al. 2000, Taylor et al. 2002). Cultivation techniques might be biased by the media selection and disruption of the fungal mycelia. With the application of cultivation-dependent methods multiple samples can also be analyzed concurrently, making it possible to follow changes in the microfungal population numbers even with increasing soil depths. There have been several studies on the distribution of soil microfungi in the surface soil of forest ecosystems (Berg et al. 1998, Panda et al. 2010). The microfungal composition of the surface horizon has been studied more because it is supposed to be the most active one, whereas little attention has been paid to the deeper horizons of the soil profile (Agnelli et al. 2004). However, microbes in deeper horizons play an important role in ecosystem biogeochemistry, soil formation and maintenance of groundwater quality (Konopka & Turco 1991) because of the unique conditions they harbour. Therefore, the study on diversity and community dynamics of soil microfungi in the unique subsurface environment of soil is required since little is known about the composition and activities of most of these novel soil microbial communities. The study on microfungal diversity in pristine locations such as subsurface soil has the potential to answer questions on how microbial communities function and how they shape and are shaped by the environment (Kostka et al. 2002). However, it is not clear whether the subsurface microfungal community is closely tied to the surface soil microfungal community or is an independent ecosystem with a distinct assemblage of microorganisms. In the present study, we investigated the diversity and community dynamics in microfungal population numbers from Nameri forest soil, using a cultivation-based approach from different depths throughout four seasons (spring, summer, autumn, winter). Since studies of microfungal diversity from the surface and subsurface soils in different seasons is lacking, an integrated approach to the dynamics of microfungal population numbers from different depths of soil collected from North-east India, throughout different seasons was needed to afford an insight into the population dynamics of this important group of soil decomposers as influenced by the soil organic matter and nutrient build up. The present investigation is the first report on the diversity and community dynamics in microfungal population numbers from different depths of forest soil in North-east India, part of the Indo-Burma mega biodiversity hotspot.

Methods

Selection, climate and description of the study area

The study was conducted from December 2009 to October 2010 in Nameri forest (26° 40′N, 92°58′E), Assam, India. The study site is characterized by a climate with most rainfall occurring during the summer months (May-July) with relatively little or scanty rainfall during the winter months. The average annual rainfall varies from 670–1100 mm and the mean minimum and maximum soil temperatures range from 17–36°C in this humid tropical climate. The study area was mostly inhabited by the plants *Alstonia scholaris*, *Arundo* sp., *Dalbergia sissoo*, *Dellinia indica*, *Dipterocarpus macrocarpus*, *Gmelina arborea*, *Mangifera indica*, *Messua ferrea*, *Phoebea atenuata*, *Shorea robusta*, and *Tectona grandis***.**

Collection of soil samples

Soil was sampled from three different spots at six depths: OA (1–9 cm), B (10–15 cm), C (16–30 cm), D (31–50 cm), E (51–100 cm), and F (101–200 cm) using a sterilized hand auger. Collections were made in December, March, July and October, the representative months for winter, spring, summer and autumn. Randomly collected soil samples were mixed thoroughly and the composite sample was taken to the laboratory for isolation of fungi. The soil samples were kept in a refrigerator at 4±1°C till isolation procedure was completed.

Analysis of soil physico-chemical properties

The pH of the soil samples were measured with an electrical digital pH meter in 1:5

(w/v) soil-water suspensions. Soil temperature at different depths was recorded with a soil thermometer at the time of sampling. Soil moisture content was determined by drying 10 g of fresh soil in a hot air oven at 150°C for 24 h. For chemical analysis, samples were air dried, ground, and sieved through a 0.2 mm sieve. A rapid titration method (Walkley & Black 1934) was followed for the determination of C_{org}. N_{tot} was estimated by indophenol blue method while molybdenum blue method was followed to determine the available soil phosphorous. Soil potassium was extracted from the soil in an ammonium acetate solution (pH 7) and was measured with a digital flame photometer.

Growth media, isolation and culture conditions

Five media were used for isolation of fungi: Czepak-Dox agar (CDA), potato dextrose agar (PDA), V8 juice agar, fungi kimming agar base and malt extract agar using the serial dilution plate technique (Johnson & Curl 1972) and 10^5 dilutions. Fungi were grown at 25±1°C for 5 days. Three replicates were maintained in each case. Pure colonies were then transferred to PDA slants, overlaid with mineral oil and stored at 4°C in the culture collection laboratory for further identification. All the media for isolation of fungi were procured from HiMedia Laboratories, Mumbai, India.

Identification of soil microfungi

Fungal isolates were characterized based on cultural and morphological characteristics of spore and hyphae mounted in lactophenol and identified by consulting taxonomic monographs (Gilman 1957, Barnett & Hunter 1972, Domesch et al. 1980, Subramanian 1971). Fungi that did not produce spores were categorized as sterile mycelia and if they did not show distinct morphological characters for identification were included as 'unidentified strain'.

Data analysis

Correlation coefficients (r) between fungal population and various physico-chemical characteristics were analysed by using Pearson's correlation coefficient. *P* values <0.01 and <0.05 were considered as significant.

Results

Analysis of cultured soil microfungi

Twenty one fungal species belonging to 14 different genera were isolated from the study site. Fungal counts were greater in surface soil at OA (1–9 cm) in spring than at other depths or seasons (Fig. 1). The study site also showed a marked variation in fungal species composition. The fungal population comprised 80.1% Phycomycetes, 14.1% Zygomycetes, 3.7% Ascomycetes and 2.1% sterile mycelia. Distribution of fungal species in the study area with respect to depth is shown in Table 1. *Aspergillus*, *Mucor* and *Penicillium* were the most abundant fungal genera at most depths while species of *Absidia*, *Apophysomyces*, *Aureobasidium*, *Chaetomium*, *Curvularia*, *Efloccosum*, *Fusarium*, *Rhizopus*, and *Trichophyton* showed distribution restricted to particular depths. The frequency of isolation using different media is shown in Table 2. V8 juice agar media was the most effective for maximum recovery of fungal species.

The population and relative abundance (%) of fungal species (g^{-1} dry soil × 10^5) in different seasons is shown in Table 3. Highest population and relative abundance throughout all seasons was exhibited by *Aspergillus flavus* (8.4%) and the lowest by *Rhizopus oryzae*, *R. nodosus* and *Trichophyton* sp. (2.8% each).

Physico-chemical properties of soil

Soil properties of different depths and seasons are presented (Fig. 2a–h). Correlation coefficients between fungal population numbers and physico-chemical characteristics at different depths in Nameri forest soil is shown in Table 4. The soil was acidic (pH 5.46–6.42). Topsoil in winter was more acidic (pH 5.46) than subsurface horizons (Fig. 2a). The pH of deeper horizons was higher (pH 5.86–6.42) than that of the organic layer. Soil moisture content was maximum during autumn in surface soil (32.48%) and decreased with increasing depths irrespective of season (Fig. 2b). Soil temperatures ranged from 20.1°C to 35.4°C. Maximum soil temperature was recorded in summer (35.4°C) in surface soil. The soil temperatures with increasing depths

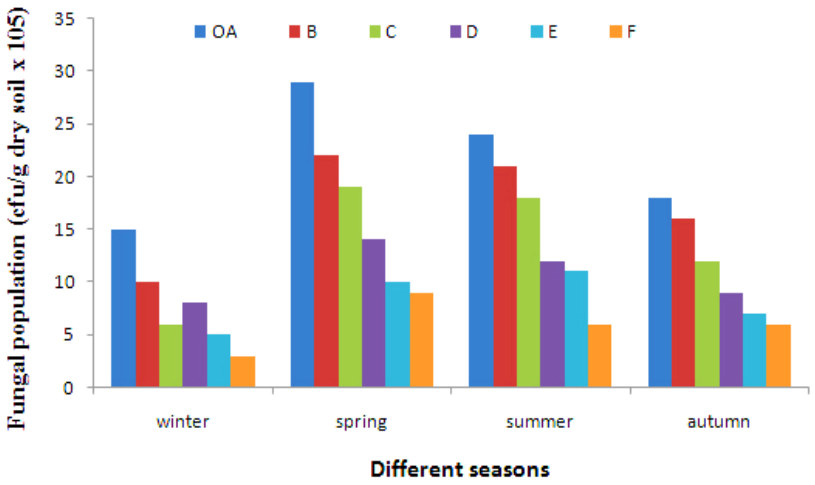

Fig. 1 – Seasonal and depth (OA = 1–9 cm, B = 10–15 cm, C = 16–30 cm, D = 31–50 cm, E = 51–100 cm and F = 101–200 cm) variation in fungal population number (cfu/g dry soil × 105) in Nameri forest soil.

Table 1 Distribution of microfungal species at different depths in Nameri forest soil.

Fungi	**Depth level**					
	OA	**B**	**C**	**D**	**E**	**F**
Absidia sp.	+	-	–	–	+	–
Apophysomyces sp.	–	–	–	+	–	+
Aspergillus flavus	+	+	+	+	+	+
Aspergillus niger	+	+	+	+	+	+
Aureobasidium sp.	–	+	–	–	–	+
Chaetomium globosum	+	+	–	–	–	–
Curvularia geniculata	+	–	+	–	–	+
Curvularia lunata	+	–	–	–	+	–
Curvularia tetramera	+	–	+	–	+	–
Efloccosum sp.	–	–	+	–	+	–
Fusarium moniliformae	+	+	–	+	–	+
Geotrichum sp.	–	–	+	–	–	–
Mucor racemosus	+	+	+	+	+	+
Penicillium chrysogenum	+	+	+	+	+	+
Penicillium digitatum	+	+	+	+	+	+
Penicillium luteum	+	+	+	–	+	+
Rhizopus nigricans	+	–	–	–	+	+
Rhizopus nodosus	–	+	–	–	–	+
Rhizopus oryzae	–	–	+	+	–	–
Trichoderma album	+	–	–	–	+	–
Trichophyton sp.	+	–	–	–	–	–

were lower than that of the organic layer (Fig. 2c). The C_{org} and N_{tot} contents were highest in summer followed by spring, autumn and winter respectively. Further, C_{org}, N_{tot} and the C/N ratio were high in the organic layer and low in deeper layers (Fig. 2d–f). Available K was maximum in spring in surface soil (243 ppm/g soil) and decreased with increasing depth (Fig. 2g). Available P was maximum in autumn topsoil (22 ppm/gm soil) and decreased with increasing soil depths (up to 6 ppm/gm soil) (Fig. 2h). Significant positive correlations were observed between soil pH, soil moisture content, organic C, total N and available K with fungal population (p<0.01 and <0.05), while soil temperature, available P showed negative correlations with that of fungi (Table 4).

Table 2 Frequency of isolated microfungi from soil using different media.

Fungi	Media					
	PDA	**CDA**	**V8**	**MEA**	**RBA**	**FKA**
Absidia sp.	+	++	++	+	+	–
Aspergillus niger	++	+	+++	–	++	-
Aureobasidium sp.	-	+	+	++	-	+
Penicillium chrysogenum	++	+++	+++	++	+	-
Penicillium digitatum	+	+	++	+	–	++
Penicillium luteum	+	++	++	-	+	–
Rhizopus nigricans	+	–	++	+	++	++
Rhizopus nodosus	+++	+	++	-	+	+
Rhizopus oryzae	+	+	+	+	-	–

*****PDA = Potato dextrose agar, CDA = Czepak dox agar, V8 = V8 juice agar, MEA = Malt extract agar, RBA = Rose Bengal agar, FKA = Fungi kimming agar, + = >1–3 colonies, ++ = >4–5 colonies, +++ = >6–7 colonies.

Table 3 Population and relative abundance (%) of fungal species (g^{-1} dry soil × 10^{5}) in Nameri forest soil in different seasons.

Fungi	Season				Total	Relative abundance (%)
	Spring	**Summer**	**Autumn**	**Winter**		
Absidia sp.	5	2	–	2	10	3.6
Apophysomyces sp.	4	2	6	2	14	5.6
Aspergillus flavus	12	5	4	–	21	8.4
Aspergillus niger	7	4	–	3	14	5.6
Aureobasidium sp.	3	8	–	3	14	5.6
Chaetomium globosum	5	3	8	–	16	6.4
Curvularia lunata	9	1	2	–	12	4.8
Efloccosum sp.	–	4	5	1	10	4.0
Fusarium moniliformae	–	5	3	–	8	3.2
Geotrichum sp.	10	5	2	1	18	7.2
Mucor racemosus	7	–	3	2	12	4.8
Penicillium chrysogenum	5	7	5	3	20	8.0
Penicillium digitatum	6	8	–	1	15	6.0
Penicillium luteum	6	–	4	3	13	5.2
Rhizopus nigricans	8	4	5	–	17	6.8
Rhizopus nodosus	–	4	2	1	7	2.8
Rhizopus oryzae	–	6	–	1	7	2.8
Trichoderma album	4	3	7	2	16	6.4
Trichophyton sp.	–	4	2	1	7	2.8
Total	91	75	58	26	250	–

Table 4 Correlation coefficients between fungal population numbers and physico-chemical characteristics at different depths in Nameri forest soil.

Sources of variation	Depth (cm)					
	1–9	**10–15**	**16–30**	**31–50**	**51–100**	**101–200**
pH	0.778^{**}	0.719^{**}	0.777^{**}	0.611^{*}	0.875^{**}	0.627^{**}
Moisture (%)	0.614^{*}	0.770^{**}	0.623^{*}	0.593^{*}	0.634^{*}	0.582^{*}
Temperature (ºC)	0.554	0.491	0.488	0.316	0.413	0.140
Organic C (%)	0.829^{**}	0.805^{**}	0.841^{**}	0.861^{**}	0.840^{**}	0.689^{*}
Total N (%)	0.721^{**}	0.671^{*}	0.646^{*}	0.581^{*}	0.728^{**}	0.662^{*}
Avail.P(ppm/g soil)	0.131	0.300	0.055	0.109	0.054	0.037
Avail.K(ppm/g soil)	0.917^{**}	0.917^{**}	0.854^{**}	0.860^{**}	0.879^{**}	0.811^{**}

*, ** = P<0.05 and 0.01, respectively. Without asterisk statistically insignificant at these levels.

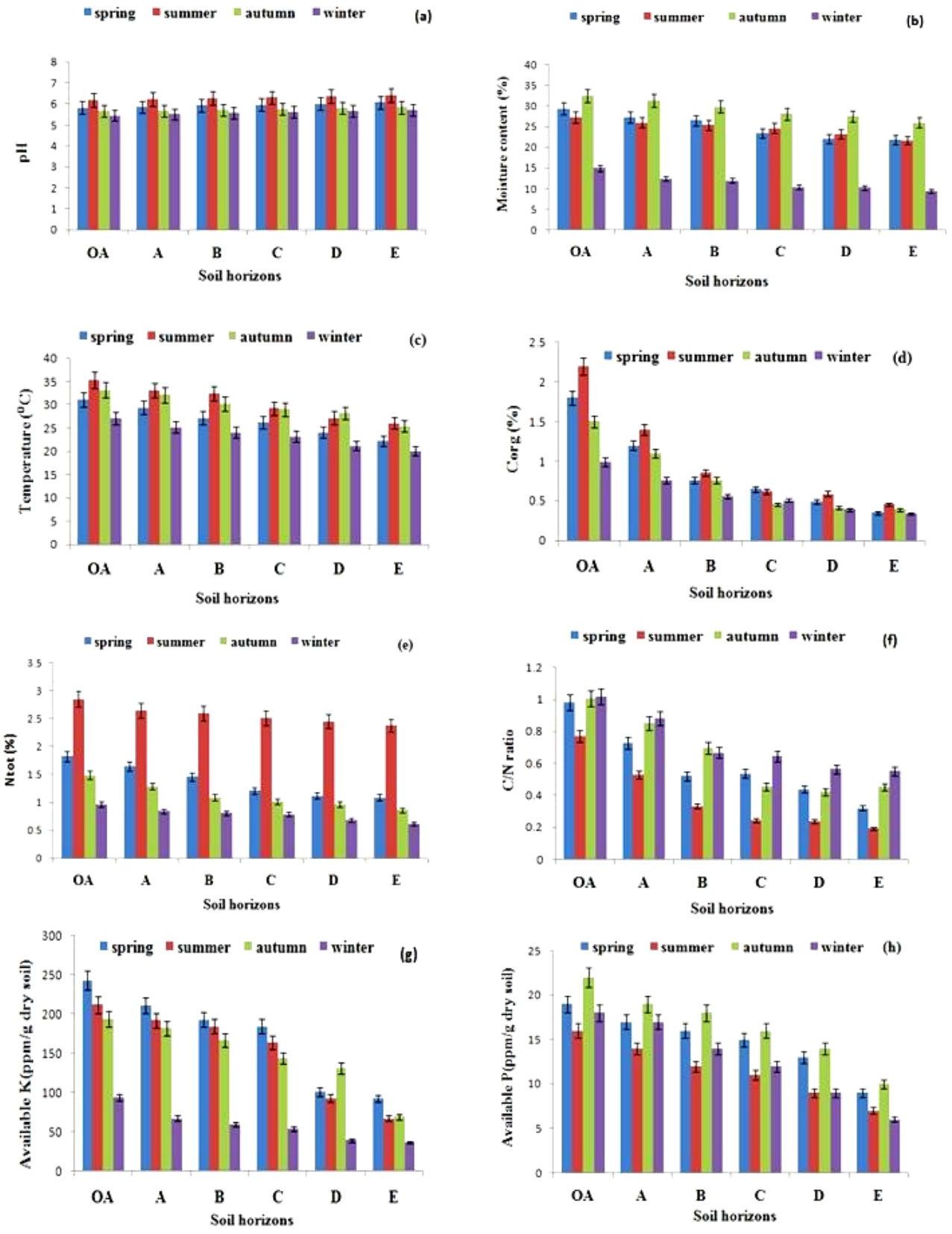

Fig. 2 (a–h) – Physicochemical characteristics of the soil samples in different depths, OA (1–9 cm), B (10–15 cm), C (16–30 cm), D (31–50 cm), E (51–100 cm) and F (101–200 cm) and seasons. **a** pH, **b** moisture content, **c** temperature, **d** total organic carbon (C_{org}), **e** total nitrogen (N_{tot}), **f** C/N ratio, **g** available K and **h** available P. Error bars have been constructed at 95% level.

Discussion

Fungal population was always higher in surface soil, which might be due to high amounts of C_{org}, higher aeration and favourable moisture. Similar observations were also reported by Yamamoto & Glenn (1985). The decline in fungal population numbers with increasing depths observed in this study agreed with Tangjang & Arunachalam (2009) and variation in physico-chemical properties of soil might play an important role in this feature (Bossio et al. 2005, Kennedy et al. 2005). According to Dkhar (1983) fungi grow slowly with increasing depths due to shortage of mineral nutrients and compaction of soil. Significant decrease in C_{org} and N_{tot} with increasing depth might be due to low organic matter availability at greater depths. The soil pH increased slightly with increasing depth in our present investigation from the surface soil to 101–200 cm, which might be due to abundant leaf litter in the surface region, incomplete decomposition of the litter and accumulation of organic acids (Yang et al. 2003). However, Sharmir & Steinberger (2007) stated that it might be high amount of organic matter, which in presence of adequate moisture supply was acted upon by the microorganisms present in topsoil, resulting in higher fungal colony forming units. While overall reduction in microfungal population numbers with increasing soil depths might be attributed to fewer nutrients and low oxygen availability (Shukla et al. 1989).

Higher fungal density during spring and summer in the present investigation might be due to increase in soil moisture content while the drop during winter might be due to comparatively low pH and total N availability of the soil.

The correlationship between soil pH, C_{org}, moisture content, N_{tot} and available K with fungal population numbers reflects the major role played by these physico-chemical factors in changing the population number and relative abundance of fungal species. Influence of soil moisture enhancing the microfungal activity by making availability of the C_{org} has been well demonstrated (Schreven 1967). According to Tiwari et al. (1982) the quantity and quality of organic carbon present in soil could govern the microbial population numbers by affecting the microbial activity. Further, since the correlations between fungal population numbers and abiotic factors like pH and soil moisture were positively significant in the present study, we suggest that this site would be appropriate to monitor different environmental effects such as acid rain.

The presence of one or a few dominant fungal species in the present study was in agreement with that of Jha et al. (1992) who pointed out that for a given community only a few species are numerically predominant and may strongly affect the environmental conditions for the others. Dominance of *Aspergillus*, *Mucor* and *Penicillium* in the present study might be due to their greater rate of spore production and dispersal as well as their resistance in existing environmental condition. Similar observation was made by Schimel (1995). According to Entry & Emmingham (1996) the significant changes in soil microfungal population might be attributed to the type of vegetation growing on a particular area. In addition, topography might influence the quantity and diversity of fungal population numbers in soil (Tsai et al. 2007).

We chose a cultivation-based approach to isolate and characterize the soil fungi since this approach can permit the laboratory cultivation of fungal isolates from natural environments and identify them by classical techniques, including analysis of morphology and physiological characteristics. A wide variety of culture media were used to maximize the recovery of diverse fungal groups, of which, V8 juice agar media appeared the best regarding the maximum recovery of fungal isolates. Alterations of some of the nutrients in the culture media used might have some promotional effects on the recovery of fungi from soil. The present study concludes that the population of microfungi in surface and subsurface soil of Nameri forest is highly influenced by physico-chemical factors such as soil pH, moisture, C_{org}, N_{tot}, and available K, which changes seasonally and with increasing depth of the soil. The quality of plant residues accumulating in this forest is also of fundamental importance and may play a vital role in soil nutrient management through microbial decomposition.

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