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Full Length Research Paper

Microfungal Diversity in Oak Forests and Plantations of Northeastern India: A Focus on Quercus Serrata

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Fungi play a major role in forest functioning by regulating the process of litter decomposition and nutrient release and are sensitive to management practices. Species richness and abundance of fungi in surface soil and decaying leaf litter of *Quercus serrata* were compared between a subtropical natural mixed oak forest and a managed oak plantation in the eastern Himalayan region. During 12 months of study a total of 106 fungal species were isolated from soil by dilution plate method and decomposing litter by leaf dilution, washed disk and moist chamber methods. Month of samplings and study sites influenced the species composition of soil and litter fungi. Total species richness of soil fungi was significantly ($P < 0.05$) lower during different seasons in managed plantation than in the natural forest. Dominant soil colonizers from both sites were: *Cladosporium cladosporioides, Clonostachys rosea* f. *catenulata*, *Fusarium oxysporum*, *Penicillium indonesiae*, *Penicillium turbatum, Trichoderma* sp. and *Trichoderma koningii*. Different soil and litter fungi showed differential seasonal preferences at two study sites. Type of fungal species recovered from leaf litters was influenced by the isolation method. Total number of litter fungi isolated from both sites was broadly similar, whereas the species compositions were different. Litter fungi between the two sites showed the highest similarity index in winter, while the similarity of soil fungi was high during summer. The management practices at plantation site had no significant effect on species richness of litter fungi although soil fungal diversity was significantly reduced indicating the role of intensive management practices on soil fungal population.

Key words: Fungal diversity, species richness, soil, litter, natural oak forest, managed plantation, Northeastern India.

INTRODUCTION

Fungi are the major decomposers of dead organic matter and contribute significantly in recycling of nutrients in natural and modified ecosystems (Gadd, 2004). Decomposition process brings about change in chemical constituents of organic matter that determines the species composition of successive colonizers and this trend continues until organic substrates are mineralized (Holland and Coleman, 1987). Attempts have been made to review the underlying principle of fungal occurrence by studying the species composition of fungal communities during decomposition of various plant substrates (Hudson, 1968; Frankland, 1998; Osono, 2006). The species richness of a fungal community and relative abundance of individual species have been considered as measures of functional activities of the group in the particular habitat (Kjøller and Struwe, 1982, 1987). However, it appears that each decomposition system has its own characteristic patterns of species assemblage depending on several factors such as substrate quality, fungal species reservoir of the site and environmental characteristics (Swift, 1976).

High species diversity and spatial heterogeneity of decomposer fungi on decaying leaf litters in tropical

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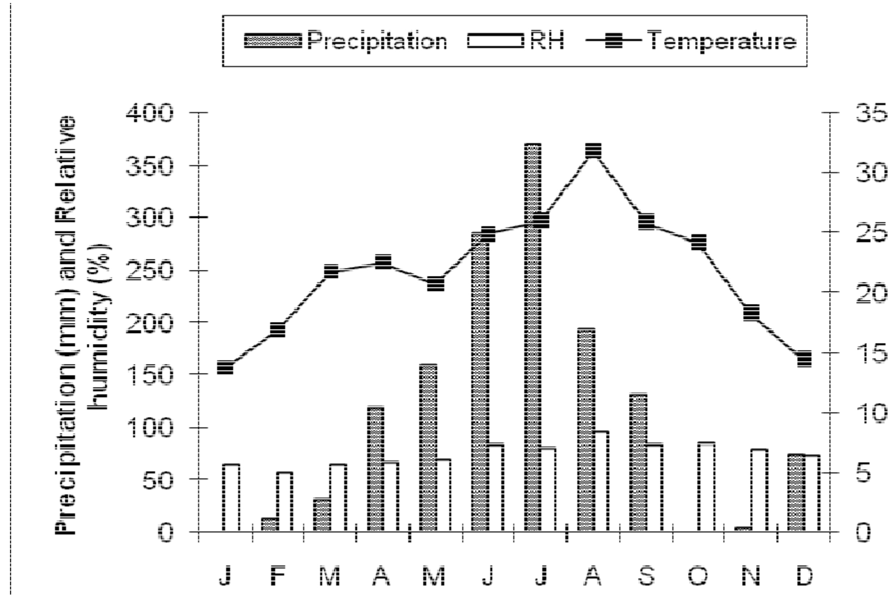

Figure 1. Monthly changes in major climatic variables during the study period.

forests have been reported (Bills and Polishook, 1994a, b; Santana et al., 2005). These decomposer fungi play a vital role in maintaining the fertility and sustainability in the tropical forests (Lodge, 1997). However, as compared to the temperate mycota less attention has been paid to the nature and abundance of saprobic fungi on decaying plant materials in the tropical and subtropical habitats (Bills and Polishook, 1994a, b; Sharma et al., 1995; Polishook et al., 1996; Parungao et al., 2002).

Northeastern India forms an important portion of the Indo-Burma biodiversity hotspots (Pawar et al., 2007). In this region, the practices like shifting cultivation and over-exploitation of wood for timber and fuel are responsible for degradation of natural forests. In degraded areas, large-scale plantations of different species of *Quercus* have been raised since 1970 for the production of economic Tasar silk. Various management practices in oak plantation have been reported to affect the decomposition process, nutrient release patterns and microbial populations of leaf litter as compared to natural oak forest (Pandey et al., 2007). However, the role of different management practices on the fungal diversity of decaying leaf litter and underlying mineralized surface soil is largely unclear. Natural or anthropogenic disturbances can alter the species composition or may have negative effect on species diversity of the decomposer fungi (Lodge, 1997). These changes may directly or indirectly affect the vital functions of the soil such as decomposition and mineralization and may result in the disturbance of the balance between the rate of substrate input and the rate of mineralization.

The present work was designed to study the fungal species diversity and their seasonal preferences on decaying oak (*Quercus serrata* Thunb.) leaves and underlying surface soil in a subtropical natural oak dominated forest and a monospecific, managed oak plantation. Major objective of this study was to assess the impact of management practices on species richness and abundance of microfungi occurring in soil and litter during different seasons in northeastern region of India.

MATERIALS AND METHODS

Site description

The studies were conducted in a natural mixed oak forest and a managed oak plantation site (24°51′ - 24°56′ N Latitude and 93°52′ - 93°58′ E Longitude). Both sites were about 8 km apart from each other towards the north of Imphal city and have similar climatic conditions. The mean minimum and maximum temperature during the study period (January to December, 2003) ranged between 13.7 to 31.8°C in the months of January and August, respectively (Figure 1). Mean relative humidity ranged from 57.7 to 95.6%. Total monthly rainfall varied from 1.15 to 370.4 mm. The year is divisible into three distinct seasons viz. summer (April to June), rainy (July to September) and winter (November to February). March and October are the transitional months between winter and summer and rainy and winter seasons, respectively. The summer and rainy seasons are characterized by high temperature and humidity. Low temperature and sort photoperiod of clear sunny days followed by frosty nights are common in winter.

The natural forest vegetation was dominated by *Q. serrata* along with the other woody associates like *Acacia australiensis, Engelhardtia spicata, Flacourtia cataphracta, Holigarna longifolia,* and *Schima wallichii*. At plantation site, trees of *Q. serrata* were planted for the production of exclusive Tasar silk (Jolly et al., 1974) and are intensively maintained by Regional Tasar Research Station, Imphal since 1970. Management practices of oak plantation consisted of periodical weeding and removal of accumulated litter, and pruning of shoots (during January to February) and incorporation of chemical fertilizer (equivalent to N at

150 kg ha^{-1} , P at 50 kg ha^{-1} and K at 38 kg ha^{-1}), organic manure (equivalent to C at 2450 kg ha^{-1}, N at 126 kg ha^{-1}, P at 17 kg ha^{-1} and K at 25 kg ha^{-1}) and bio-fertilizers (at 20 kg ha^{-1}) into the surface soil once a year (during May to June) for improving plant health and leaf quality for feeding silk moth (Singh et al., 2001). Ages of oak trees were 70 to 80 years at forest and 30 to 32 years at plantation. The ground flora was better developed at forest than plantation, and formed by abundant herbaceous cover. Soil pH at both the sites was slightly acidic (5.6 to 6.5) in nature. Soil organic carbon varied between 1.6 to 2.6% and 2.5 to 3.2% in forest and plantation sites, respectively. Details of soil, litter and vegetation characteristics and management practices at these sites have been reported by Pandey et al. (2007).

Fungal isolation

Nylon net bag technique was adopted for the study of leaf litter decomposition at both sites. Senescent leaves were collected separately from two sites individually on a large nylon net during December 2002 when the maximum litter fall occurred. Ten gram air-dried leaves were placed in each nylon net bag (15 x 15 cm, 1 mm mesh). For each study site a total of 100 litterbags were placed randomly at 5 different locations in the bunch of 20 bags on 15th January 2003. Five litter bags (one bag from each location) and the surface soil (0 to 10 cm depth) underlying nylon bags from each location were collected at monthly interval from both stands and brought to the laboratory. The litter and soil samples collected from different locations of each site were pooled together and processed for further analysis. A total of 12 collections were made till December 2003.

Qualitative estimation of soil fungi was performed by serial dilution plate method (Parkinson et al., 1971). Ten gram freshly collected ground soil was suspended into 100 ml distilled water in a 250 ml Erlenmeyer conical flask and thoroughly shaken for 15 min on a horizontal mechanical shaker (120 throws min^{-1} and 1.5 cm displacement). The suspension was further diluted to 10^{-4} using sterile distilled water. One ml aliquot was inoculated separately into each of five Petri dishes (9 cm diam) and 20 ml molten and cooled (40°C) Martin's agar (Martin, 1950) medium was poured into each Petri dish. Fungal colonies developed after 7 days of incubation in the dark at 25 ± 1°C were identified based on keys and descriptions provided by Domsch et al. (1980), Ellis (1971,1976), Pitt (1979), Subramanian (1971) and Watanabe (2002). The percent relative abundance of each species was calculated. International Mycological Association (2009) was referred for the nomenclature of fungal species (http://www.mycobank.org). Non-sporulating strains were grouped into sterile mycelia.

Three isolation methods viz. leaf dilution plating, washed disk and moist chamber as described by Pandey (1990) were applied for isolation of litter fungi in order to obtain more information regarding the species composition of the litters at two study sites.

For leaf suspensions plating 100 disks (5 mm diam) were punched out from the litters retrieved from 5 bags of each site using a sterile cork borer. The leaf disks were suspended in 100 ml sterile distilled water, shaken thoroughly on a horizontal mechanical shaker and diluted further. One ml aliquot of suspension (10^{-3}) was pipetted separately into five replicated Petri dishes and then 20 ml molten and cooled (40°C) Czapek-Dox + Yeast Extract agar (CYA) (Onions et al., 1981) medium was poured into each Petri plate. After incubation for 7 days in the dark as mentioned above, relative abundance of fungal species was calculated.

In washed disk method 25 leaf disks prepared as above were washed serially in 10 changes of sterilized distilled water (1 mm/ washing) and blotted dry using sterilized blotting papers. Leaf disks were plated in 5 Petri dishes (5 disks/ plate), each dish containing 20 ml solidified CYA medium. The plates were incubated at 25 ± 1°C for 7 days and the fungal colonies developed on the leaf disks were identified. The method removes detachable fungal propagules from leaf surface and isolates only the actively growing mycelia.

Another 25 unwashed leaf disks were used for plating in moist chamber. This method was used for inducing sporulation of those fungal species which cannot compete with fast growing fungi on nutrient media. Five Petri dishes each containing two folds of round filter paper moistened with distilled water were sterilized. In each sterilized Petri dish 5 leaf disks without any prior treatment were placed on filter paper at equal distance (Keyworth, 1951), enclosed separately inside sterile polythene bags to maintain moisture content, incubated at 25 ± 1°C for 30 days and then the leaf disks were examined for fungal identification. The long period of incubation enables the fungal propagules to sporulate properly. In case of washed disk and moist chamber methods any fungal species obtained on a leaf disk was counted as a single isolate of the individual species and the results were expressed in terms of percent frequency of occurrence.

Based on number of months and seasons in which soil and litter fungi of two sites occurred by different methods they were categorized into ubiquitous (isolated in 6 or more months), summer-rainy (recorded in 3 or more months during March to October), winter (occurring in 3 or more months during November to February), nonspecific (occurring in 3 or more months but not included in any categories) and accidental (occurring in less than 3 months) species. Those fungi which occurred in 10 or more months were regarded as most frequent species.

Calculations

Percent relative abundance (RA) of each fungal species was calculated as: (Number of colonies of a fungal species / Total number of fungal colonies) x 100. Percent frequency of occurrence (FO) of each fungus was calculated as: (Number of leaf disks on which a fungal species occurred / Total number of leaf disks observed) x 100. Mean annual percentages of RA and FO of each fungus were calculated by dividing the sum of RA or FO of individual species by the number of observation that is 12. Similarity index (SI, %) of soil and litter fungi isolated by dilution plating was calculated as described by Sørensen (1948) using the formula: SI = 2C/(A+B)/100 where A is the total number of species in one study site, B is the total species number in other site and C is the sum of species common to both sites. Student *t* test was applied to compare the seasonal mean values of total fungal species richness isolated by different methods from soil and litter of two sites.

RESULTS

A total of 106 fungal species belonging to 61 genera was isolated from surface soils and decaying *Q. serrata* leaf litters of forest and plantation sites (Table 1). Soil samples collected from two study sites yielded 1376 and 1231 isolates, respectively. Three cultural methods employed for isolation of litter fungi revealed different proportions of isolates (with overall colonies of 3677 and 4676 at forest and plantation stands, respectively).

Soil-inhabiting fungi

Of the total, 40 and 36 fungal species were isolated from soils of forest and plantation stands, respectively. Out of which, 19 species were common in both sites whereas, 21 species at forest and 17 species at plantation sites

Table 1. Species composition and seasonal occurrence of microfungi in surface soil and decomposing *Q. serrata* leaf litter at natural oak forest and plantation sites in northeastern India.

Fungal species	Soil		Litter						Seasonal occurrence			
	Soil dilution#		Leaf dilution#		Washed disk##		Moist chamber##		Soil		Litter	
	NF	PL	NF	PL	NF	PL	NF	PL	NF	PL	NF	PL
Absidia glauca Hagem	0.0	0.0	2.2	0.0	0.0	0.0	0.0	0.0	-	-	W	-
Acremonium roseogriseum (S.B. Saksena) W. Gams	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	-	-	-	AC
A. strictum W. Gams	0.0	1.9	0.0	0.0	0.0	0.0	0.0	0.0	-	U	-	-
Acrophialophora fusispora (S.B. Saksena) Samson	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	-	-	AC	-
Alternaria alternata (Fr.) Keissl.	1.1	2.8	0.0	1.6	2.7	18.7	2.0	16.3	SR	SR	U	U
Aspergillus candidus Link	0.0	1.2	0.0	0.0	0.0	0.0	0.0	0.0	-	W	-	-
A. clavatus Desm.	0.9	0.7	0.0	0.0	0.0	0.0	0.0	0.0	SR	AC	-	-
A. giganteus Wehmer	0.0	0.0	0.0	0.0	0.0	5.3	0.0	0.0	-	-	-	SR
A. niger Tiegh.	1.5	1.0	1.7	0.0	0.0	0.0	0.0	0.0	U	NS	U	-
A. ochraceus K. Wilh.	0.6	0.0	0.7	0.5	1.7	0.0	0.0	0.0	NS	-	W	S
Aureobasidium pullulans (de Bary) G. Arnaud	0.0	3.4	1.6	3.2	1.3	27.3	0.0	0.0	-	U	U	U
Beltrania rhombica Penz.	0.0	0.0	0.0	0.0	0.0	0.0	4.0	0.0	-	-	NS	-
Bipolaris australiensis (M.B. Ellis) Tsuda and Ueyama	0.0	0.0	1.8	0.0	0.0	0.0	3.0	0.0	-	-	U	-
Bispora catenula (Lév.) Sacc.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	22.3	-	-	-	U
Brachysporium sp.	0.0	0.0	0.0	0.0	1.0	0.0	1.0	0.0	-	-	AC	-
Cacumisporium capitulatum (Corda) *S.* Hughes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.7	-	-	-	S
Cercospora fusimaculans G.F. Atk.	0.0	0.0	0.0	0.0	0.0	0.0	4.7	3.3	-	-	NS	SR
Chaetomium globosum Kunze ex Fr.	0.0	0.0	0.0	0.0	0.0	0.0	13.7	8.7	-	-	U	S
Chaetopsis grisea (Ehrenb.) Sacc.	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	-	-	W	-
Choanephora infundibulifera f. *cucurbitarum* (Berk. and Ravenel) Schipper	0.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	-	SR	-	-
Cirrenalia donnae B. Sutton	0.0	0.0	0.0	0.0	0.0	0.0	0.0	19.7	-	-	-	SR
Cladosporium cladosporioides (Fresen.) G.A. de Vries	10.6	9.9	12.6	15.4	31.7	40.0	27.7	31.0	U	U	U	U
C. herbarum (Pers.) Link	0.0	0.0	5.9	6.2	2.0	20.0	0.0	1.3	-	-	U	U
C. oxysporum Berk. and M.A. Curtis	0.0	0.0	1.7	0.0	0.0	0.0	0.0	0.0	-	-	NS	-
C. variabile (Cooke) G.A. de Vries	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	-	-	AC	-
Clasterosporium sp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.7	-	-	-	S
Clonostachys rosea f. *catenulata* (J.C. Gilman and E.V. Abbott) Schroers	5.0	6.0	6.4	3.2	3.0	26.7	0.0	0.0	U	SR	U	U
Colletotrichum gloeosporioides (Penz.) Sacc.	0.0	0.0	0.0	1.8	0.0	25.7	0.0	0.0	-	-	-	U
Cunninghamella echinulata (Thaxt.) Thaxt. ex Blakeslee	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	W	-	-	-
Curvularia pallescens Boedijn	0.0	0.0	0.0	1.3	0.0	0.0	0.0	12.3	-	-	-	U
Dark sterile mycelia	1.6	0.3	2.0	0.0	6.3	0.0	12.3	0.0	U	NS	U	-
Dictyoarthrinium sp.	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	-	-	AC	-

Table 1. Contd.

Discosia sp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3	-	-	-	W
Epicoccum nigrum Link	0.0	0.0	2.6	0.0	0.7	4.0	27.0	17.7	-	-	U	U
Fusariella obstipa (Pollack) S. Hughes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.0	-	-	-	NS
Fusarium incarnatum (Roberge) Sacc.	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	W	-	-	-
F. oxysporum Schltdl.	3.8	4.8	4.3	0.0	2.3	13.0	3.3	7.0	U	U	U	U
F. solani (Mart.) Sacc.	1.6	2.3	0.0	1.7	18.0	0.0	3.7	0.0	SR	U	W	SR
Gilmaniella humicola G.L. Barron	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.0	-	-	AC	-
Gliocladium sp.	2.7	3.3	1.2	2.9	30.0	38.3	0.0	0.0	SR	U	U	U
Hirudinaria sp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.7	-	-	-	SR
Humicola fuscoatra Traaen	0.0	2.3	2.0	0.0	0.0	0.0	0.0	0.0	-	U	U	-
H. grisea Traaen	2.4	0.0	0.7	1.9	28.3	0.0	20.7	16.7	U	-	U	U
Lasiodiplodia theobromae (Pat.) Griffon and Maubl.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	23.3	-	-	-	SR
Memnoniella echinata (Rivolta) Galloway	0.0	0.0	0.4	0.2	0.0	0.0	5.0	0.0	-	-	NS	NS
Monilia sp.	2.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	S	-	-	-
Monodictys lepraria (Berk.) M.B. Ellis	0.7	0.6	0.0	0.6	1.0	0.0	0.0	20.3	SR	SR	AC	U
M. nigrosperma (Schwein.) W. Gams	0.0	0.0	1.8	0.0	0.0	0.0	0.0	0.0	-	-	U	-
Mortierella sp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.0	-	-	-	W
M. subtilissima Oudem.	0.0	0.0	2.4	0.0	0.0	0.0	0.0	0.0	-	-	W	-
Mucor hiemalis Wehmer	0.0	1.9	0.0	0.0	17.0	17.0	0.0	0.0	-	NS	W	U
M. hiemalis f. *luteus* (Linnem.) Schipper	0.0	0.0	0.0	0.0	0.0	5.7	0.0	0.0	-	-	-	W
M. hiemalis f. *silvaticus* (Hagem) Schipper	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	AC	-	-	-
M. racemosus f. *racemosus* Fresen.	3.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	U	-	-	-
Mycocladus corymbifer (Cohn) Vánová	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NS	-	-	-
Nigrospora sphaerica (Sacc.) E.W. Mason	0.0	2.4	0.8	1.6	11.0	11.3	26.0	14.7	-	SR	U	U
Penicillium sp. 1	0.0	1.4	4.7	1.3	0.0	0.0	0.0	0.0	-	SR	SR	SR
Penicillium sp. 2	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	SR	-	-	-
P. arenicola Chalab.	0.2	0.0	0.0	0.0	1.3	0.0	0.0	0.0	AC	-	W	-
P. capsulatum Raper and Fennell	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	AC	-	-	-
P. chrysogenum Thom	3.7	1.0	0.0	0.0	0.0	0.0	0.0	0.0	U	NS	-	-
P. citreonigrum Dierckx	0.0	0.6	1.1	0.3	0.0	11.7	0.0	0.0	-	SR	W	SR
P. citrinum Thom	0.0	0.0	1.7	0.0	0.0	25.7	0.0	0.0	-	-	U	SR
P. diversum Raper and Fennell	0.0	0.8	1.2	1.5	0.0	0.0	0.0	0.0	-	AC	NS	U
P. duclauxii Delacr.	0.0	0.0	1.3	0.7	0.0	0.0	0.0	0.0	-	-	SR	NS
P. expansum Link	1.9	0.0	0.2	0.0	0.0	0.0	0.0	0.0	AC	-	AC	-
P. funiculosum Thom	0.0	2.3	0.0	2.1	0.0	3.7	0.0	0.0	-	SR	-	SR
P. glabrum (Wehmer) Westling	0.0	2.7	0.0	1.8	18.3	17.3	0.0	0.0	-	SR	U	U
P. glandicola (Oudem.) Seifert and Samson	0.0	3.3	0.0	3.2	0.0	0.0	0.0	0.0	-	SR	-	W

Table 1. Contd.

P. implicatum Biourge	0.6	0.0	0.7	0.0	1.0	12.3	0.0	0.0	NS	-	S	W
P. indonesiae Pitt	3.7	6.7	2.1	2.6	24.7	71.3	0.0	0.0	U	U	U	U
P. klebahnii Pitt	0.0	0.0	0.8	0.0	18.0	0.0	0.0	0.0	-	-	SR	-
P. lineatum Pitt	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	NS	-	-	-
P. nalgiovense Laxa	0.0	1.3	0.0	0.0	4.0	0.0	0.0	0.0	-	W	SR	-
P. rubrum Stoll	2.9	4.4	0.0	2.9	0.0	0.0	0.0	0.0	U	U	-	W
P. rugulosum Thom	2.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	W	-	-	-
P. simplicissimum (Oudem.) Thom	0.0	0.0	1.0	0.0	5.0	4.3	0.0	0.0	-	-	U	W
P. spinulosum Thom	0.0	0.0	0.0	0.0	0.0	32.0	0.0	0.0	-	-	-	SR
P. thomii Maire	0.0	1.1	0.0	0.8	0.0	0.0	0.0	0.0	-	SR	-	SR
P. turbatum Westling	5.1	3.0	0.0	0.4	20.7	21.7	0.0	7.7	U	U	SR	SR
Periconia hispidula (Pers.) E.W. Mason and M.B. Ellis	0.9	0.0	0.0	0.0	4.0	6.3	58.0	86.7	S	-	U	U
Pestalotiopsis disseminata (Thüm.) Steyaert	0.0	11.0	4.7	14.0	29.0	96.3	33.3	39.3	-	U	U	U
Phialophora fastigiata (Lagerb. and Melin) Conant	0.0	0.0	0.0	0.0	0.0	0.0	11.7	0.0	-	-	U	-
Phoma glomerata (Corda) Wollenw. and Hochapfel	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.0	-	-	-	W
Pithomyces chartarum (Berk. and M.A. Curtis) M.B. Ellis	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	-	-	-	AC
Rhizopus oryzae Went and Prins. Geerl.	0.0	0.0	0.0	0.0	3.7	1.7	0.0	0.0	-	-	NS	W
Rosellinia necatrix Berl. ex Prill.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	53.0	-	-	-	U
Rutola graminis (Desm.) Crane and Schokn.	3.5	0.0	2.0	0.0	0.0	0.0	0.0	0.0	U	-	U	-
Septonema sp.	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	-	-	AC	-
Sphaerostilbella aureonitens (Tul. and C. Tul.) Seifert, Samuels and W. Gams	5.4	0.2	1.6	0.6	13.7	0.0	0.0	0.0	U	AC	U	SR
Stachybotrys kampalensis Hansf.	0.0	0.0	0.0	0.0	0.0	0.0	6.3	22.0	-	-	SR	U
Talaromyces luteus (Zukal) C.R. Benj.	0.7	2.7	0.1	0.0	0.0	0.0	0.0	0.0	NS	AC	AC	-
Tetraploa aristata Berk. and Broome	0.0	0.0	0.0	0.0	0.0	0.0	0.7	6.3	-	-	AC	AC
Thielavia terricola (J.C. Gilman and E.V. Abbott) C.W. Emmons	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	SR	-	-	-
Thysanophora penicillioides (Roum.) W.B. Kendr.	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	AC	-	-	-
Torula allii (Harz) Sacc.	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.0	-	-	W	-
T. herbarum (Pers.) Link	0.0	0.0	0.0	0.0	0.0	0.0	2.3	3.0	-	-	W	NS
Trichoderma sp.	5.6	3.0	0.0	2.0	0.0	0.0	0.0	0.0	W	U	-	U
T. koningii Oudem.	10.3	6.6	12.4	16.0	31.7	15.0	0.0	0.0	U	U	U	U
T. viride Pers.	4.5	0.0	7.6	1.6	19.0	0.0	0.0	0.0	U	-	U	U
Trichothecium roseum (Pers.) Link	0.0	0.0	0.0	0.0	0.0	0.0	4.7	0.0	-	-	SR	-
Triposporium elegans Corda	0.0	0.0	0.0	0.0	0.0	0.0	5.7	3.0	-	-	AC	NS
White sterile mycelia	3.1	0.4	3.3	6.4	42.7	14.7	8.0	0.0	U	R	U	U
Xylohypha ferruginosa (Corda) S. Hughes	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	-	SR	-	-
X. nigrescens (Pers.) E.W. Mason ex Dieghton	1.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	SR	-	-	-
Zygosporium gibbum (Sacc., M. Rousseau and E. Bommer) S. Hughes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	75.0	-	-	-	U
Total number of fungal species = 106	40	36	37	31	31	28	29	31	-	-	-	-

[#] Mean relative abundance (%), [##] Mean frequency of occurrence (%); NF = Natural forest, PL = Plantation, U = Ubiquitous, SR = Summer-rainy, S = Summer, R = Rainy, W = Winter, NS = Non-specific, AC = Accidental, - = Absent.

were unique. Thus, total number of fungal species isolated from both soil types was 57. Dominant soil colonizers from both sites with mean RA values of 3 or more were: *Cladosporium cladosporioides*, *Trichoderma koningii*, *Clonostachys rosea* f. *catenulata*, *Fusarium*

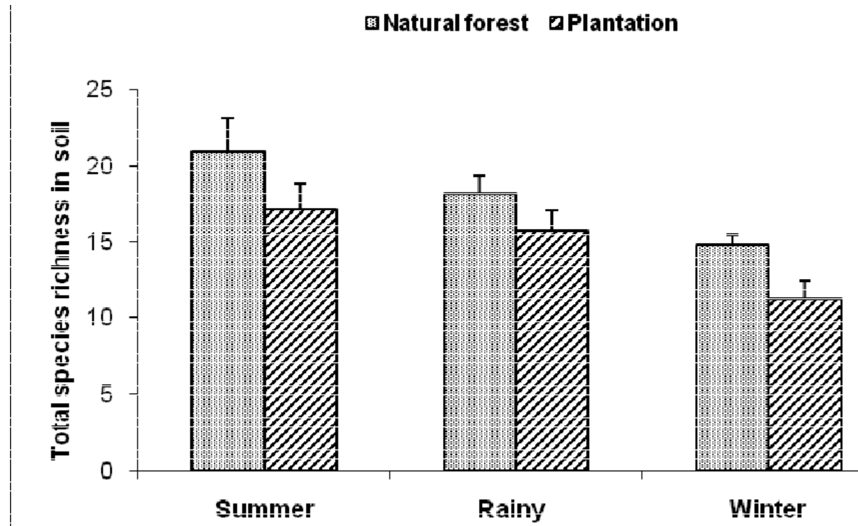

Figure 2. Mean total species richness of soil fungi during different seasons. Bars indicate standard errors.

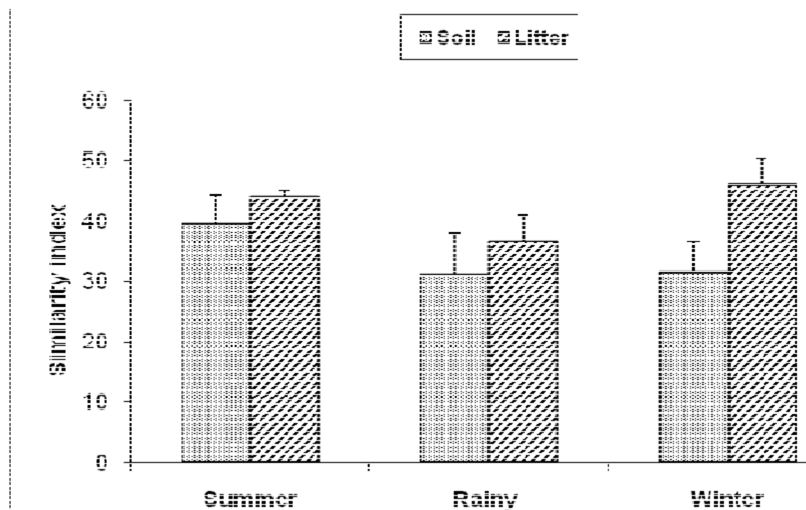

Figure 3. Sørensen's similarity index of soil and litter fungi at two sites during different seasons. Bars indicate SE.

oxysporum, *Penicillium indonesiae*, *Penicillium turbatum* and *Trichoderma* sp. (Table 1). *Trichoderma viride*, *Rutola graminis* and *Mucor racemosus* f. *racemosus* in forest soil and *Pestalotiopsis disseminata*, *Aureobasidium pullulans* and *Penicillium glandicola* in plantation soil were the site specific species with mean abundance values 3. The highest numbers of fungal species in forest (21) and plantation (17) soils were isolated during summer while the lowest was recorded in winter (Figure 2) . Statistically significant differences in species richness of soil fungi were observed between the sites ($P < 0.01$) and seasons ($P < 0.05$). Similarity index between fungal communities of two soil types varied during different sampling months revealing highest and lowest values in summer and rainy seasons, respectively (Figure 3). The numbers of ubiquitous, summer-rainy, winter, non-specific and accidental species in forest soil were 16, 10, 4, 5 and 5, respectively. Corresponding values at plantation soil were 13, 12, 2, 5 and 4, respectively (Table 1). Different fungal species showed differential seasonal preferences at two study sites. For example, *C. rosea* f. *catenulata* was recorded as ubiquitous species at forest while at plantation it was summer-rainy species. Similarly, *Gliocladium* sp. and *Fusarium solani* were summer-rainy species at forest but they were ubiquitous at plantation. Few species like *Alternaria alternata* and

Monodictys lepraria appeared during summer-rainy seasons at both sites. White sterile mycelia were ubiquitous at forest whereas, it was recorded during rainy season at plantation. *Aspergillus niger*, *Penicillium chrysogenum*, *Sphaerostilbella aureonitens* and dark sterile mycelia were ubiquitous fungi in forest soil while they were non-specific and/or accidental species in plantation. *Cunninghamella echinulata*, *Fusarium incarnatum* and *Penicillium rugulosum* at forest site and *Aspergillus candidus* and *Penicillium nalgiovense* at plantation site were isolated during winter season only.

Litter-colonizing fungi

Total number of species colonizing decomposing oak leaf litters of two sites was 87 of which 49 species were exclusively isolated from litters while remaining 38 species were similar to those isolated from soil (Table 1). Among litter colonizers 25 species were specific to forest and 24 species were restricted to plantation. The remaining 38 species were common to both sites. Thus, total number of species colonizing the decaying litters of natural forest and plantation sites were 63 and 62, respectively. The type of species isolated from litter was influenced by the method of isolation. Some species could be isolated by a specific isolation method while others were recovered by at least two or all three methods. Out of total litter fungi, number of species isolated specifically by dilution plating, washed disk and moist chambers were 7, 3 and 28, respectively (Table 1). The remaining 49 species were recorded by combination of methods. Total species richness of litter colonizing fungi isolated by three methods at forest and plantation sites ranged from 11 to 20 species and 9 to 18 species, respectively showing highest number in summer and lowest in winter season (Figures 4a, b, c). Significant differences ($P < 0.01$) in species richness between two sites were observed during summer by moist chamber method and in winter by dilution plating and washed disk methods. Species similarity index on litters of two sites was highest (46.2%) in winter (Figure 3).

Most frequent species isolated by all three methods from litters of both sites were: *C. cladosporioides*, *P. indonesiae*, *Periconia hispidula*, *P. disseminata*, *T. koningii* and white sterile mycelia. Other frequently isolated species of forest litter were: *Bipolaris australiensis*, *C. rosea* f. *catenulata*, *Epicoccum nigrum*, *F. oxysporum*, *Humicola grisea*, *Trichoderma viride* and dark sterile mycelia whereas, species isolated at plantation site were: *A. alternata*, *A. pullulans*, *Colletotrichum gloeosporioides*, *Gliocladium* sp. and *Rosellinia necatrix*.

Numbers of ubiquitous, summer-rainy, winter, non-specific and accidental fungal species on decaying oak litters at forest site were 28, 7, 10, 11 and 6, respectively. Corresponding values at plantation site were 27, 14, 9, 3 and 5, respectively (Table 1). Among litter fungi only *Penicillium implicatum* was exclusively isolated during summer at forest site. At plantation site, the litter fungi isolated exclusively during summer were: *Aspergillus ochraceus*, *Chaetomium globosum*, *Cacumisporium capitulatum*, and *Clasterosporium* sp. *S. aureonitens* and *Penicillium citrinum* were ubiquitous on decomposing litter of natural forest but were summer-rainy species at plantation. However, *P. turbatum* occurred during summer-rainy season on litters of both sites. *F. solani* was isolated during winter at forest but at plantation it was recorded during summer-rainy season. *Monodictys lepraria* and *Penicillium diversum* were classified as accidental and non-specific species, respectively on forest litter but were ubiquitous on plantation litter.

DISCUSSION

Generally, anthropogenic disturbances have been reported to reduce the diversity of fungal species under varied ecosystems (Lodge, 1997; Allison et al., 2007). In the present study, fungal species richness in forest soil was significantly higher than that of plantation soil. Christensen (1981) reported that species diversity of soil fungi is a reflection of multiple factors and appears to be reduced by disturbances and manipulation activities. Wicklow et al. (1974) found positive correlations between species diversity of soil fungi and plant species diversity. Abundance of decaying plant materials for colonization by fungi that mostly includes saprobes and weak plant parasites is an important contributing factor for rich fungal diversity in forest ecosystems (Chaverri and Vilchez, 2006). In general, soils with rich organic matter, less physical disturbance and low nutrient inputs tend to be fungal dominated whereas, frequently tilled soils receiving high inorganic fertilizer inputs are dominated by bacteria (Ritz and Young, 2004). More specifically, N fertilization has been found to reduce significantly the abundance of soil saprobic microfungi (ezá ová et al., 2005) and the total fungal diversity in soil (Allison et al., 2007).

We found a higher abundance of *Gliocladium* sp. and *P. indonesiae* in surface soil and decaying litter at managed plantation site compared to natural forest (Table 1). Among Penicillia, *P. funiculosum, P. glandicola* and *P. thomii* were exclusively isolated from plantation site. Arnebrant et al. (1990) observed an increased isolation frequency of *Penicillium spinulosum* and *T. viride* in NH_4NO_3 treated soil and *P. brevicompactum* and *P. spinulosum* in urea treated plots. Donnison et al. (2000) pointed out that inorganic N, particularly nitrate and ammonium ions may inhibit or stimulate the growth of certain fungal species by inhibiting or promoting the activity of specific extracellular enzymes.

Combinations of climate and soil type are reported to be important factors determining the species composition

Figure 4a. Mean total species richness of litter fungi isolated by dilution plating at two study sites during different seasons. Bars indicate SE.

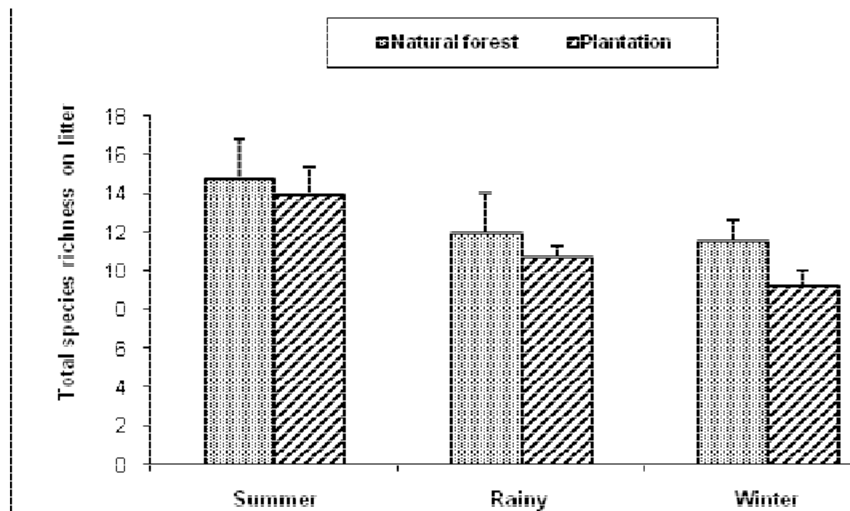

Figure 4b. Mean total species richness of litter fungi isolated by washed disks method at two study sites during different seasons. Bars indicate SE.

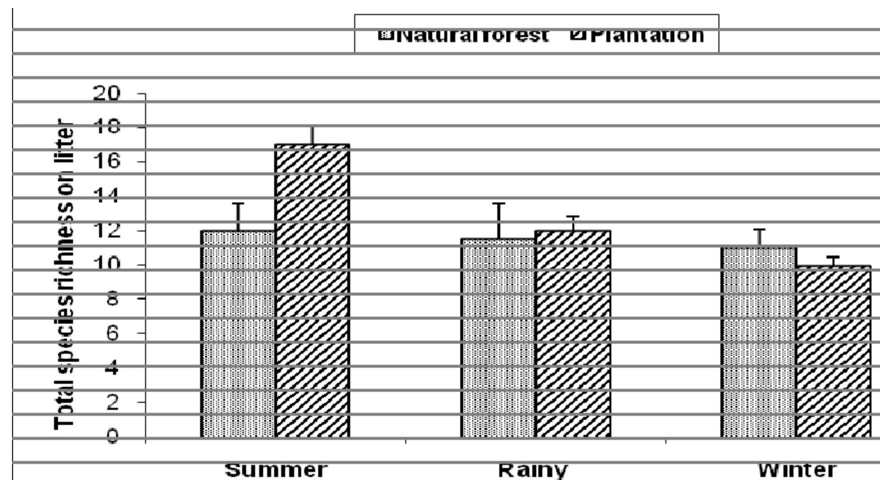

Figure 4c. Mean total species richness of litter fungi isolated by moist chamber method at two study sites during different seasons. Bars indicate SE.

of soil fungi (Mishustin, 1975). When dry soil become moist due to onset of rain during summer season there was an increase in total fungal species richness at both sites. Most of the fungal species revealed their occurrence and abundance to be related to sampling months showing differential seasonal preferences at two study sites. Frequently isolated species of *Aspergillus, Penicillium* and *Trichoderma* grow quickly and produce large number of conidia which are easily dispersed and exhibit wide ecological spectrum (Christensen, 1981; Pandey et al., 1991). Bettucci and Roquebert (1995) also reported the abundance of these species in humid tropics because of their tolerance to the fluctuations in varying environmental conditions.

Though, total species richness of litter fungi isolated from two sites was broadly similar, we observed differences in species compositions of microfungi showing 43.6% species overlapping among culturable fungi. Spatially separate study sites that is natural mixed oak dominated forest and monospecific managed plantation having different microenvironments and other characteristics may contribute to the observed variations in species composition. Bills and Polishook (1994b) have reported differences in microfungal communities associated with decaying litter of same plant species at a distance of about 50 m. Environmental differences can greatly influence the microfungal assemblages in tropical leaf litters (Santana et al., 2005). Climatic variables such as temperature, moisture and humidity have also been found to affect the changes in species composition on decaying litters (Osono and Takeda, 2001, 2007) and abundance of individual fungal species like *Trichoderma* spp. (Shirouzu et al., 2009). The observed seasonal preferences shown by the microfungal assemblages of soils and decaying leaves at both sites may indicate their adaptability to the prevailing climatic conditions in a particular season and their role at different decomposition stages. Pandey et al. (2007) have shown that 68% variability in mass loss rate of *Q. serrata* litter at natural forest site was controlled by relative humidity and mean air temperature while at plantation, fungal and actinomycetes populations in combination with relative humidity and maximum temperature accounted for 93% variability in litter mass loss rate.

Among different categories of litter fungi at two sites ubiquitous colonizers represented the highest number of species. The common ubiquitous species like *A. alternata, A. pullulans, C. cladosporioides, C. herbarum, E. nigrum* and *N. sphaerica* from litters of both sites in the present study were also reported as frequently isolated species from attached leaf surfaces and leaf litters of different plants throughout the world (Frankland, 1998; Kjøller and Struwe, 1982; Osono and Takeda, 2001, 2007; Pandey, 1990; Sadaka and Ponge, 2003). These fungi were reported as primary saprophytes which can withstand on adverse conditions such as desiccation, UV radiation and microbial lysis by producing thick walled pigmented multicellular spores and microsclerotia (Hudson, 1968; Sadaka and Ponge, 2003). In this study, *T. koningii* and *T. viride* were ubiquitous species from *Q. serrata* leaf litters of both sites. Shirouzu et al. (2009) also recorded *T. koningii* and *T. harzianum* as constantly isolated species on well-decomposed leaf litters of *Q. myrsinaefolia*. *Beltrania rhombica*, *C. cladosporioides*, *Clonostachys* spp., *C. gloeosporioides*, *F. solani*, *Gliocladium* sp., *Lasiodiplodia theobromae*, *Mucor heimalis*, *Pestalotiopsis* spp., *Stachybotrys* sp. and *Trichoderma* spp. were reported as cosmopolitan microfungi from tropical leaf litters that support highly varied and extensive mycota (Polishook et al., 1996). White sterile mycelia isolated frequently from both soils and litters must be representing those species which do not produce spores naturally or under cultural conditions including monokaryotic basidiomycetes. These fungi can decompose lignin and lignin like humic substances vigorously in forest soils to produce white-rot humus (Osono, 2006). The highest number of fungal species could be detected by moist chamber compared to other direct isolation methods which emphasize the importance of using a combination of several cultural methods for studying fungal diversity of decaying plant substrates.

Conclusion

Our findings demonstrate the differences in fungal species composition of surface soils and decaying leaf litters at natural oak forest and managed plantation sites. Similar species richness of litter fungi was observed at two sites, while species richness of soil fungi was significantly reduced at plantation suggesting that management practices have greater potential to influence the size and structure of soil fungal community than that of litter mycoflora. Such observations have relevance in explaining the shifts in fungal community structure or reducing the species diversity associated with intensification of management practices like fertilizer amendments which may adversely affect the ecosystem functioning. Further investigations on fertilizer- sensitivity of specific fungal communities will clarify the effect of long term consequences of fertilization on fungal diversity and their ecological significance.

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