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Abundance and Diversity of AM Fungi across a Gradient of Land Use Intensity and Their Seasonal Variations in Niligiri Biosphere of the Western Ghats, India

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ABSTRACT

The impact of land use intensity on the abundance and diversity of arbuscular mycorrhizal fungi (AMF) was investigated at six land use types *viz*., natural forest, grassland, acacia plantations, cardamom plantations, coffee plantations and paddy fields in the Niligiri Biosphere of the Western Ghats in South India. There was no significant difference in AMF root colonization in different land use types during pre-monsoon but there was a significant difference in AMF root infection ratings between different land use types during post-monsoon season, where it was higher in natural forests and grasslands. The AMF spore density and infective propagules were significantly higher in grasslands and acacia plantations compared to all other land use types during both seasons. Except for paddy fields, the spore density and number of infective propagules were higher in post-monsoon season compared to pre-monsoon season in other land use types. The numbers of AMF species identified were 56 during pre-monsoon and 67 species during post-monsoon season suggesting seasonal variations in diversity. During both seasons *Glomus fasciculatum* was recorded in maximum number of sampling points across the landscape followed by *G. geosporum* during pre-monsoon and *G. mosseae* during post-monsoon season. The species diversity was highest in natural forests and grasslands as compared to other land use types in both seasons. The species richness index for AMF was highest in natural forests and least in paddy fields during both study periods. The sand content, bulk density, total N, organic C, alkaline and acid phosphatases positively correlated with AMF activity while clay, silt, K, total P and available P were negatively correlated.

Keywords: AMF, Infective propagules, Land use types, Root colonization, Spore density.

INTRODUCTION

A major component of the soil mycobiota in most agro-ecosystems is the arbuscular mycorrhizal fungi (AMF). AMF exist inside the root and in the soil. The intraradical mycelium consists of hyphae and other fungal structures, such as arbuscules and vesicles; the extraradical mycelium forms spores, explores soil and new areas for colonization and absorbs nutrients (Tommerup and Sivasithamparam, 1990).

In the recent system of classification, all the AM fungal species are placed in four orders *i.e.* Archaeosporales, Diversisporales, Glomerales and Paraglomerales which comprise 13 families and 19 genera that belong to class Glomeromycetes of the phylum Glomeromycota (Sieverding and Oehl, 2006; Oehl *et al*., 2008; and Palenzuela *et al*., 2008).

Since these fungi are obligate symbionts, their population and diversity may be determined by the plant species present in the given ecosystem. Apart from plant species, human activities also affect these fungi which play an important role in

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carbon allocation, nutrient cycling and maintenance of diversified ecosystems (Doss and Bagyaraj, 2001). The presence of these fungi and their genetic and functional diversities are important for both plant community and ecosystem productivity. Improved plant growth due to inoculation of plants with AM fungi has been demonstrated especially under P deficient condition. The growth improvement is mainly because of enhanced P uptake. AMF can also enhance tolerance or resistance to root pathogens and abiotic stresses such as drought and metal toxicity (Clark and Zeto, 2000; Auge, 2004).

Western Ghats of India is identified as one of the biodiversity hotspots of the world (Myers *et al*., 2000). Information on the distribution and frequency of occurrence of specific AM fungi in the Nilgiri biosphere of the Western Ghats area is very scarce. A few studies performed so far have recorded the occurrence of *Glomus* species in the root zone soils of different tree species (Muthukumar and Manian, 1993; Vasanthakrishna *et al.,* 1994). Similarly, Lakshmipathy *et al.* (2004) recorded *Glomus etunicatum* in the root zone soil of cashew. The mycorrhizal activity in terms of root colonization was examined in 59 different forest tree species and the intensity of colonization was found to be high in four species, moderate in 23 species and low in 32 species (Byra Reddy *et al.,* 1994). Patricia *et al*. (2009) recovered a total number of 24 AM fungal species in pristine forest ecosystem of Amazon. Santhaguru *et al*. (1995) observed mycorrhizal root colonization in twenty species of tree legumes in the Eastern Ghats and found altogether 21 species of AMF belonging to six genera *viz. Acaulospora, Entrophospora, Gigaspora, Glomus, Sclerocystis* and *Scutellospora.* Therefore, it appears that occurrence of AM fungi in different forest tree species defines ecological niches.

In tropical soils, application of organic matter stimulated the proliferation of AMF. This was attributed to the low organic matter content in tropical soils (Harinikumar and Bagyaraj, 1989). The addition of organic amendments such as paddy straw, maize straw and pongamia leaf increased the mycorrhizal activity. Of the three amendments used, addition of pongamia leaf improved AM fungal infection to the maximum, followed by maize straw (Harinikumar and Bagyaraj, 1988). High P availability is reported to be negatively correlated with AM fungal activity (Krishna and Bagyaraj, 1982). Balakrishna *et al*. (2001) reported that application of inorganic fertilizers negatively affected native AM fungal spore density, infective propagule numbers and per cent root colonization in a finger millet- maize-fallow crop rotation system. Furthermore, studies conducted on the effect of mono and mixed cropping systems on AMF population in soil have revealed that mixed cropping of soybean and maize stimulated the proliferation of AM fungi compared to mono-cropping of maize or soybean (Harinikumar *et al.*, 1990).

Seasonal fluctuations also seem to have a considerable impact on the AM fungal development in the soil. Harinikumar and Bagyaraj (1988) and Mallesh and Bagyaraj (1991) showed that AM fungi sporulate during winter in the tropics. The optimum temperature for sporulation by mycorrhizal fungi appears to be around 25°C. To our knowledge, the AMF abundance and diversity occurring over broad land use types and in different seasons under tropical conditions have not yet been investigated in India. Thus, the present study was undertaken in the Niligiri Biosphere of the Western Ghats of Karnataka in South India.

MATERIALS AND METHODS

Study Area

The benchmark area, Koothy village of Somwarpet taluk is located in the Kodagu district of Karnataka (Figure 1). A wide range of land use types with a diversified plant species composition is found in the region. This benchmark area is situated in the Nilgiri Biosphere Reserve of the northern region and lies between 12°40' 03" N–12° 42' 19" N and 75° 47" 10" E–75° 79' 14" E. The annual rainfall of the area ranges from 2,000 mm to 3,500 mm. Most of the rainfall is drawn from southwest monsoon during June-August period. Four seasons can be clearly distinguished; summer from March to May, monsoon from June to September, post-monsoon during October and November, and winter during December to February (Temp. 6.2-12.5°C). The temperature begins to increase from March to April, with a

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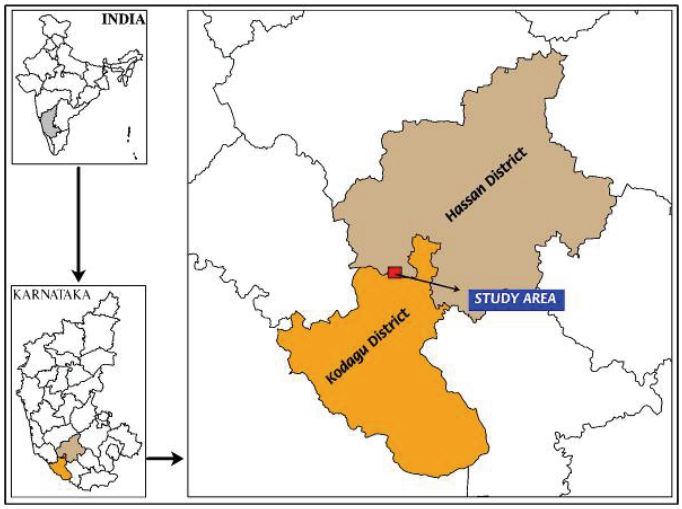

Figure 1. Location of the study area-Koothy, Somvarpet taluk, Kodagu, Karnataka.

mean daily maximum of 28.6°C and mean daily minimum of 17.8°C and reaching as high as 32 to 35°C during April or May.

Coffee and cardamom plantations cover a major part of the study area. The natural forests at the periphery of the plantations are evergreen with varying levels of degradation. A few patches of *Acacia auriculiformis* plantations (monoculture) and grassy patches are found adjacent to the forests. Rain-fed agriculture is practiced in the valleys with one paddy crop every year during the rainy season. Additionally, crops like chilly and short duration grain legumes are also grown in the summer season utilizing the residual moisture and sparse rainfall of northeast monsoon.

Land Use Pattern

Six land use–land cover types could be distinguished in the study area using a satellite database of the year. A brief description of these land use types is presented in Tables 1 and 2.

The soil samples were collected in pre-monsoon season (February) and post-monsoon season (October) for the analysis of soil physical, chemical, biochemical and microbiological properties.

Soil Sampling

A triangle of 50×50×50 m was laid at each sampling point. From the center of the triangle at

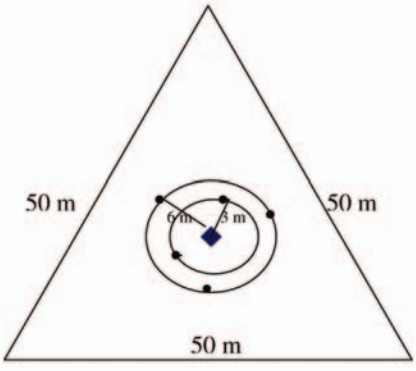

Figure 2. Soil sampling procedure for studying soil properties and AM fungi.

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Table 1. Dominant vegetation types and management practices of the different land use types in the study area.

Land use type	Major vegetation	Fertilization (Kg ha^{-1})	Plant protection	Cultivation intensity
Natural forest	These forests are little disturbed and are adjacent to coffee and cardamom plantations. There are as many as 153 species of plants. Dominated by *Caryota urens*, *Olea dioica*, *Canthium dicoccum, Artocarpus heterophyllus* and *Dimoacarpus longan* trees, shrubs like *Leea indica, Dichapetalum gelonioides, Flacourtia indica, Nilgirianthus heyneanus* etc. and herbs like *Brachiaria milliformis, Justisia trinervia, Blumea barabata, Alysicarpus vagnailis, Archidendron monodelphum, Derris indica, Derris scandens, Pongamia pinnata* were the dominant on the forest floor.	None	None	None
Grasslands	These are the open patches found in slightly elevated regions of the Ghats and adjoining agricultural fields. Dominated by grasses like *Panicum repens, Sporobolus diander* and herbs like *Stachytarpheta indica, , Borreria articularis, Oldenalnadia corymbosa, Derris seandens, Desmodium triquetrum, Mimosa pudica, Zornia gibbosa* etc.	None	None	None
Acacia plantations	These are the monoculture plantations of *Acacia auriculiformis* of 15-25 years old raised in the open and disturbed areas. The ground flora is mainly dominated by species like *Stachytarpheta indica, Brachiaria milliformis, Centella asiatica* and shrubs like *Lantana camara, Maesa indica, Randia dumatorum.*.	None	None	None
Coffee plantations	About 106 species of plants belonging to trees, shrubs and herbs were recorded in addition to coffee plantations. The most dominant tree species are *Grevilia robusta, Caryota urens, Artocarpus heterophyllus, Acrocarpus fraxinifolius and Derris scanden, Mucuna prurienss*. Most of the plantations have naturally occurring species maintained as shade trees and in a few plantations *Grevilia robusta* is planted as shade trees. The *Brachiaria molliformis* grass is the most dominant ground flora.	Mineral and farmyard manure as per the University recommendation	Chemical: Fungicides and Insecticides twice a year	Very low, since it is a perennial crop
Cardamom plantations	One hundred and thirty four species of plants belonging to trees, shrubs and herbs were recorded. The plant species like *Olea dioica, Litsea floribunda*, *Caryota urens,* C*innamomum zeylanicum, Acacia concinna, Acrocarpus fraxinifolius, Albizia lebbeck, Albizia odoratissima, Dalbergia latifolia, Derris scandens and Mucuna prurienss* were the most dominant flora with *Brachiaria molliformis* grass as the dominant ground flora.	Mineral and farmyard manure as per the University recommendation	Chemical: Fungicides and Insecticides twice a year	Very low, since it is a perennial crop
Paddy fields	The agricultural crop grown in that region is mainly rain fed rice. The ground flora varies with the season and as many as 47 species of herbs are recorded. The dominant herbaceous weeds present in the area are *Panicum repens*, *Grangea maderaspatana, Centella asiatica, Blumea barbata.*	Mineral and farmyard manure	Chemical: Fungicides and Insecticides 2 times for each crop.	High:2-3 crops/year i.e. paddy followed by pulses

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Table 2. Chemical soil parameters of the different land use types differing in land use intensity.

Land use types	pH		Organic C ($g\ kg^{-1}$)	Total N ($mg\ kg^{-1}$)	Total K ($mg\ kg^{-1}$)	Total P ($mg\ kg^{-1}$)	Avail. P ($mg\ kg^{-1}$)
	H_2O	KCl					
Natural forests	6.20	5.24	15.5	46.90	232.0	1.9	0.63
Grasslands	5.57	4.37	11.8	40.88	175.5	2.47	0.80
Acacia plantations	5.51	4.43	13.8	42.35	240.0	1.74	1.04
Cardamom plantation	6.16	5.20	10.4	95.80	261.0	3.58	1.38
Coffee plantations	6.16	5.03	11.5	47.90	283.5	3.46	1.23
Paddy fields	5.30	4.21	7.7	37.24	189.0	3.86	1.48

a distance of three and six meters two concentric circles were drawn as shown in the figure. At equidistance from each other three soil cores (6.25 cm dia.) of 0-20 cm depth were collected on the circumference of each of the circle as shown in the (Figure 2) avoiding the litter above the ground. The six soil samples thus collected were mixed together and a composite soil sample was drawn using quaternary technique. The soil sample was collected along with the root bits, the root bits were separated and soil was divided into two parts. The root bits collected were immediately transferred to a fixative. One part was air dried and used for chemical analysis and the other part was stored at $5^{o}C$ in a refrigerator. The soil samples were collected at nine sampling points from natural forests, eight from grasslands, six from acacia plantation, thirteen from cardamom plantation, sixteen from coffee plantations and eight from paddy fields.

Soil Physical, Chemical and Biological Properties

The clay, silt and sand were quantified by mechanical analysis as outlined in the 'TSBF Hand Book of Methods' by Anderson and Ingram (1989). The organic carbon was determined by the modified Walkley-Black method (Anderson and Ingram, 1989). The total nitrogen in the soil samples was analyzed by semi micro-Kjeldhal method (Jackson, 1973) using a Gerhardt auto analyzer. The total phosphorus content of the soil samples was determined by vanado-molybdo phosphoric yellow colour method in nitric acid system (Jackson, 1973). The soil samples used in this study were acidic and hence Bray's extracting solution containing 0.03 N NH_4F in 0.025 N HCl was used (Jackson, 1973). The available potassium was determined by following the ammonium acetate method as described by Merwin and Peach (1951). The bulk density was determined following standard procedures (Anderson and Ingram, 1989). The acid and alkaline phosphatase activities were estimated as per the procedure (Eivazi and Tabatabai, 1977). The results of the soil analysis of different sites are given in the Table 2.

Assessment of the Mycorrhizal Parameters

The following mycorrhizal structures were determined: root colonization was determined by gridline intersection method (Giovannetti and Mosse, 1980) by staining the root bits with 0.05% trypon blue. Spore density was determined by extracting spores from soil by wet sieving and decantation method (Gerdemann and Nicolson, 1963) and infective propagule numbers by 10 fold MPN method (Sieverding,1991).

Identification of the Diversity of AM Fungi

Spores extracted directly from soil can be used for identification, but better identification of AMF is possible by "host baiting technique" or "trap pot culturing". Because spores from baits are in a better state, they can be identified to species level by using spore morphological charecteristics (Bagyaraj and Sturmer, 2008).

The composite samples obtained from the sampling points were transferred to the

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laboratory. The soil test sample was mixed with sterile sand soil (1:1) mix (50% soil test sample+50% sterile sand soil mix) and planted with suitable trap crops such as a mixture of sorghum and cowpea under glass house conditions. Watering was done every other day. After 3-4 months, the potting mix was wet sieved and the spores were observed under a compound microscope. Morphologically similar spores were picked and the population of each spore type was enumerated from a respective field sample. The spores of each type were brought into pot culture by funnel technique after surface sterilization of the spores (Nicolson, 1967) with an aqueous solution containing 200-ppm streptomycin sulphate and 2% chloramine T. The spores were mounted on a glass slide in lacto-glycerol. They were later identified with the help of "Manual for identification of VA mycorrhizal fungi" by Schenck and Perez (1990) and the INVAM website by Joe Morton: http://invam.caf.wvu.edu.

Statistical Analysis

The data collected from the field experiments were subjected to statistical analysis following the statistical package Excel and SYSTAT version 10.2. Arc sin transformations were done (Snedecor and Cochran, 1968) wherever necessary. The treatment means were separated by the Duncan's Multiple Range Test and by Probability Matrix. The Shannon-Weiner diversity index, Jakknife's species richness index, Sorenson similarity index for AMF between different land use types were calculated (Krebs, 1989).

RESULTS

Mycorrhizal Root Colonization

AMF root colonization was studied during two different seasons *viz.* pre and post-monsoon. Significant differences in root colonization between different land use types were observed only during post-monsoon season (Table 3). AMF root colonization was significantly higher in roots collected from natural forests and a grassland compared to other land use types and was lowest in paddy fields. The root colonization was significantly higher in post-monsoon season compared to pre-monsoon in natural forests, grasslands and acacia plantations while it was more during pre-monsoon season in coffee plantations, cardamom plantations and paddy fields.

Spore Density and Infective Propagules

In both seasons, the spore density in soils collected from acacia plantations and grasslands was significantly higher as compared to all other land use types but it was the lowest in case of paddy fields (Table 3). The average spore density of all land use types in two different seasons was higher during post-monsoon season compared to pre-monsoon season.

The infective propagule numbers in soils collected from acacia plantations and grasslands were on par with each other and significantly higher as compared to the other land use types

Table 3. AMF root colonization, Spore load and Infective propagules in different land use types in pre and post-monsoon seasons.

	AMF root colonization (%)		Spore density (No 50 g^{-1} soil)		Infective propagules (No g^{-1} soil)	
Land use types	Pre-monsoon	Post-monsoon	Pre-monsoon	Post-monsoon	Pre-monsoon	Post-monsoon
Natural forests	59	72^{a}	201^{cd}	374^{c}	305^{c}	559^{c}
Grasslands	64	68^{ab}	641^{ab}	654^{a}	690^{ab}	839^{a}
Acacia plantations	57	64^{b}	767^{a}	616^{ab}	775^{a}	737^{ab}
Cardamom plantations	64	49^{d}	176^{cdef}	275^{d}	177^{cd}	407^{d}
Coffee plantations	66	57^{cd}	258^{d}	316^{d}	260^{cd}	443^{d}
Paddy fields	48	33^{e}	184^{cde}	201^{e}	215^{cd}	184^{e}
F test at 0.05%	NS	*	*	*	*	*
Seasons mean	60	57	371	405	404	528

* Significant.

Mean values followed by the same superscript in each column do not differ significantly at P= 0.05 level by DMRT.

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during pre-monsoon season (Table 3). The paddy fields recorded the least. A similar trend was observed in the post-monsoon season too. The average numbers of infective propagules in soils collected from all land use types were higher during post-monsoon season compared to pre-monsoon season.

Effect of Soil Properties on AM Fungi

The influence of soil physical, chemical and bio-chemical properties on different mycorrhizal parameters was calculated. Among the soil physical properties, sand and bulk density had a positive influence on root colonization, spore density and infective propogule numbers, while the clay content was negatively correlated to root colonization, spore density and infective propagules (Table 4). The chemical properties of the soils, such as organic C, had a significant positive influence on the formation of AMF in soil while the total P and available P had a negative influence on AMF formation. The bio-chemical properties of soil such as the acid and alkaline phosphatase activities were positively correlated with mycorrhizal root infection.

Species Abundance and Diversity of AM Fungi in Different Land Use Types

Altogether, a total of 67 species of AM fungi were isolated from all six different land use types across the sampling site, out of which 56 were collected during pre-monsoon and 67 species during post-monsoon season (Table 5). There were 12 species belonging to the genus *Acaulospora*, one to *Archeospora*, 48 to *Glomus,* one to *Intraspora,* one to *Pacispora* and 3 to *Gigaspora. G. mosseae, A. lacunosum, G. fasciculatum, A. bireticulata* and *G. geosporum* were found virtually in all land use types either in the pre- or post monsoon seasons. Some species such as *G. geosporum, G. citricolum, G. heterosporum* and *G. halonatum* were common to natural forests, grasslands, acacia plantations and coffee plantations in both seasons. *G. fulvum* was associated with only cardamom plantations in both seasons and was present in natural forests only in the pre-monsoon season. The species *Arccheospora trappei* was recorded only in the grasslands in both seasons. *G. australe, G. intraradices, A. mellea, G. hoi, A. spinosa, G. magnicaulis* and *A. morrowe,* were recorded both in the pre- and post monsoons. The species, *G. phansihalos, Gi. albida, G. pachycaulis* and *P. scintillans* were recorded only during pre-monsoon while others such as *G. reticulatum, G. caledonium, A. delicata, I. schenckii, G. diaphanum* and *G. pustulatum* were recorded only during post-monsoon season and almost confined to grasslands.

AMF Species Abundance across the Landscape Over Two Different Seasons

The abundance of different AMF species over different land use types indicated a higher incidence during post-monsoon season than in pre-monsoon season (Table 6). During post-monsoon season, the majority of the species produced spores abundantly in field soil compared to pre-monsoon season. A few species

Table 4. Correlation matrix between soil properties and components of AM fungi in different land uses types.

Soil properties	Root colonization (%)	Spore density 50 g^{-1} soil	Infective propagules (IP g^{-1} soil)
Clay (%)	-0.039	-0.067	-0.086
Silt (%)	-0.203	-0.237	-0.142
Sand (%)	0.122	0.151	0.091
Bulk density (g cm^{-3})	0.068	0.302*	0.446*
Alkaline phosphatase (µg g^{-1} soil hr^{-1})	0.647**	0.456*	0.400*
Acid phosphatase (µg g^{-1} soil hr^{-1})	0.836**	0.486*	0.465*
Org. carbon (g kg^{-1})	0.796**	0.379*	0.465*
Nitrogen (mg kg^{-1})	0.115	0.058	0.103
Potassium (mg kg^{-1})	0.065	-0.073	-0.079
Total phosphorus (mg kg^{-1})	-0.279*	-0.308*	-0.232
Available phosphorus (mg kg^{-1})	-0.441*	-0.366*	-0.224

* Significant at P= 0.05 level. ** Highly significant at P= 0.01.

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Table 5. Occurrence and spore numbers (No 50 g^{-1} soil) of AMF species in different land use types in pre- (PRM) and post-monsoon (POM) seasons.

Sl. No	AMF species	Natural forest		Grasslands		Acacia plantations		Cardamom plantations		Coffee plantations		Paddy fields	
		PRM	POM	PRM	POM	PRM	POM	PRM	POM	PRM	POM	PRM	POM
1	*Glomus mosseae*	15	526	69	366	44	620	84	319	527	854	38	215
2	*Acaulospora lacunosa*	36	132	306	491	138	203		55	178	198	91	27
3	*G. fasciculatum*	219	776	401	1013	696	897	278	881	573	795	50	
4	A. bireticulata	65	180	502	749	383	293		72	166	384	24	
5	**G. geosporum**	82	297	230	407	67	102	251	612	379	612	114	
6	*G. citricolum*	78	403	9	119	90	209		52	2	128		
7	*G. heterosporum*	29	117	419	273					42	77		
8	*G. halonatum*	67	101	202	456					121	181		
9	*A. scrobiculata*	18	88					53	241	210	523		24
10	*G. aggregatum*	3	150				114			151	845	48	544
11	*G. globiferum*	8	44			34	73						
12	*G. maculosum*	92	155	149	157	154	402	197	152			78	182
13	*G. multicaulis*	26	187			96	293	128	329	21	410		
14	*A. nicolsoni*	41	133			276	67						
15	*G. manihotis*	59	141			85	81					23	75
16	*G. albidum*	101	161	342	368	272	288						
17	*G. etunicatum*	39	286	51	93		151	171	199				
18	*G. monosporum*	124	192					30	30	80	68		
19	*G. canadense*	67	71						46				
20	*G. delhiense*	25	366										
21	*G. boreale*	100	180										
22	*G. multisbstensum*	27	58										
23	*G. dimorphicum*	17	42										
24	*G. versiforme*	27	146										
25	*S. calospora*	12	105										
26	*G. verrucosa*	23	129										
27	*G. fulvum*	19						228	259				
28	*G. australe*		96	454	178								
29	*G. intraradices*		120	88	459						137		
30	*A .appendicula*		55	115								28	34
31	*A. laevis*		87		78				33		58		
32	*G. claroideum*		97									47	41
33	*G. leptotichum*		24										
34	*G. ambisporum*		80				377						
35	***A. mellea***			225	217		118	319	590				
36	*G. hoi*			121	225	77	145	334	123	14	98	7	
37	*A. spinosa*			60	124	459	311	3	43				
38	*G. magnicaule*			116	102					213	160		
39	*A. morrowae*			188	231								
40	*Ar. trappei*			475	160								
41	*G. phansihalos*			99		85	137	36	81				
42	*G gaspora albida*			58		272							
43	*G. pachycaulis*			218				28	103				
44	*pacispora scintillans*			58									
45	*G. pustulatum*			17	109								
46	*G. reticulatum*				554								
47	*G. caledonium*				78				504				
48	*A. delicate*				44			244	39				
49	*Intraspora shenkii*				40								
50	**G. diaphanum**				56	51	96			96	50	7	66
51	*G. segmentatus*					90	98						
52	*S. persica*					138	258					21	
53	*G. gerdemannii*					45	61						
54	*G. macrocarpum*					99	380						
55	**G. radiatum**					138	138		41	113	386		
56	*G. lacteum*					230	128	75	234	28	42	69	194
57	*A. dilatata*							16	131	234	263		
58	*G. invermaium*							34	159	252	153		
59	**G. constrictum**								21	375	325		
60	*G. clavispora*									13	58		
61	*G. tenebrosum*									209			
62	*G. deserticola*									73			
63	*Scutellospora heterogama*										52		
64	*Gi .margarita*										48	144	69
65	*G. tortuosum*											41	51
66	*Gi .rosea*												39
67	*G. clarum*				97								

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Table 6. Abundance of spores of different species of AM fungi across sampling site during pre- and post-monsoon seasons in different land use types.

	Spore abundance (No 50 g^{-1} soil)					
	Pre-monsoon			Post monsoon		
A.bireticulata	1140	±	216	1678	±	269
A.lacunosa	749	±	110	1106	±	167
A.scrobiculata	28	±	83	852	±	206
A.nicolsoni	317	±	111	200	±	56
A.appendicula	143	±	27	89	±	24
A.laevis	ND[a]	±	ND	256	±	38
A. meellea	544	±	143	854	±	231
A.marrowae	188	±	77	231	±	94
A.dilatata	250	±	94	394	±	110
A.delicata	244	±	100	83	±	28
A.spinosa	522	±	109	478	±	115
Ar.trappei	475	±	64	160	±	64
G.albidum	515	±	137	817	±	151
G. ambisporum	457	±	63	ND	±	ND
G.australe	454	±	63	274	±	82
G.aggregatum	202	±	61	1653	±	343
G.borrealis	100	±	41	180	±	73
G. caledonium	ND	±	ND	502	±	202
G.canedense	113	±	27	71	±	31
G.citricolum	179	±	42	911	±	142
G.claroideum	47	±	19	138	±	40
G. clarum	ND	±	ND	97	±	40
G.clavispora	13	±	5	58	±	24
G.constrictum	375	±	153	346	±	131
G.delhiens	25	±	10	366	±	149
G.deserticola	73	±	30	ND	±	ND
G.diaphanum	154	±	40	268	±	38
G.dimorphicum	17	±	7	42	±	17
G.etinicatum	261	±	66	729	±	113
G.fulvum	247	±	92	259	±	106
G.geosporum	1123	±	121	2030	±	256
G.gerdimanii	45	±	18	61	±	25
G.globiferum	42	±	14	117	±	32
G.halonatum	390	±	83	738	±	179
G.heterosporum	490	±	166	467	±	108
G.hoi	553	±	127	591	±	87
G.intraradices	88	±	32	716	±	178
G.invermaians	402	±	101	598	±	82
G.lacteum	179	±	86	588	±	99
G.leptotichum	ND	±	ND	24	±	10
G.macrocarpum	99	±	203	380	±	155
G.magnicaule	329	±	90	262	±	70
G.manihotene	167	±	36	297	±	59
G.monosporum	234	±	52	290	±	75
G.mosseae	777	±	251	2900	±	233
G.multisubstensum	27	±	11	58	±	24
G.multicaulis	27	±	46	1219	±	141
G.pachycaulis	317	±	11	103	±	42
G.phansihalos	220	±	59	218	±	57
G.pustulatum	17	±	7	109	±	44
G.radiatum	251	±	65	565	±	153
G.reticulatum	554	±	143	ND	±	ND
G.segmentatus	90	±	37	98	±	40
G.tenebrosum	209	±	85	ND	±	ND
G.tortuosum	117	±	17	51	±	88
G.versiforme	27	±	11	146	±	60
G.verrucosa	23	±	9	129	±	53
Gi.albida	715	±	109	817	±	115
Gi.margarita	144	±	59	117	±	31
Gi.rosea	ND	±	ND	39	±	16
I.schenckeii	ND	±	ND	40	±	16
P.scintillans	58	±	24	ND	±	20
S.calospora	15	±	5	105	±	43
S.heterogama	ND	±	ND	52	±	21
S.persica	158	±	55	258	±	105

[a] Not Detected.

such as *A. delicata, A. morrowae, G. albidum, G. fulvum* and *G. magnicaule* produced more spores during pre-monsoon than in post-monsoon season. Spores of *A. laevis*, *Intraspora. schenckii, G. caledonium*, *G. clarum, G. leptotichum, G. reticulatum, G. ambisporum, Gi. Rose* and*, S. heterogama* were noticed only during post-monsoon season while spores of *G. deserticola* and *G. tenebrosum* were observed only during pre-monsoon season.

Diversity of AM Fungi in Different Land Use Types

Shannon-Wiener diversity index during pre-monsoon season was higher in natural forests compared to grasslands, acacia plantations, coffee plantations, cardamom plantations and paddy fields. A similar trend also existed in the post-monsoon season and the diversity was significantly higher in natural forests compared to acacia plantations, cardamom plantations, coffee plantations and paddy fields. The diversity index was significantly higher in post monsoon as compared to pre-monsoon season. Even within land use types, the diversity index was significantly higher in post-monsoon as compared to pre-monsoon season except in paddy fields (Table 7).

Jacknife's species richness index for AMF in different land use types during pre-monsoon season was higher in natural forests and the least in cardamom plantations. During post-monsoon season the AMF species richness index followed a similar trend. Seasonal variations in species richness index were higher during post-monsoon as compared to pre-monsoon season in all land use types except in paddy fields (Table 8).

DISCUSSION

The present investigation in the Nilgiri Biosphere of the Western Ghats in South India has shown that more intensive land use can have a negative impact on AMF, and thus in principle, some earlier studies on similar subjects e.g. Oehl *et al*. (2003) confirmed a decrease in AMF species richness with increasing land use intensity in Central Europe. Our studies also considered the effects of monsoon periods in different land use systems.

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Table 7. AM fungal species diversity in different land use types during pre and post-monsoon seasons.

	No. of AMF species		Shannon-Weiner diversity index	
Land use types	Pre-monsoon	Post-monsoon	Pre-monsoon	Post-monsoon
Natural Forests	27	33	4.41[a]	4.59[a]
Grasslands	24	27	4.20[ab]	4.45[ab]
Acacia Plantations	22	26	3.99[b]	4.26[b]
Cardamom Plantations	19	26	3.57[cd]	4.08[bc]
Coffee Plantations	23	25	3.80[c]	4.04[bc]
Paddy Fields	16	13	3.26[d]	3.01[d]
F test at 0.05%	-	-	*	*
Season mean			3.87	4.07

Note: Values in parenthesis represent Error Mean Sum.
* Significant.
Mean values followed by the same superscript in each column do not differ significantly at P=0.05 level by DMRT.

Table 8. Jakknife's species richness index for AM fungi in different land use types during pre and post-monsoon seasons.

	Species richness index	
Land use types	Pre-monsoon	Post-monsoon
Natural Forest	43.00	51.67
Grasslands	37.13	48.50
Acacia plantations	38.67	41.83
Cardamom plantations	24.38	37.08
Coffee plantations	30.44	34.37
Paddy fields	25.63	20.87

Although there were no significant differences in mycorrhizal root colonization between land use types during pre-monsoon season, significant differences were observed during post-monsoon season. The relative high root colonization in natural forests and grasslands may be related to the low disturbance of these ecosystems as compared to the other land use types. Also, the P contents may play an important role in mycorrhizal colonization and AMF spore production. Several studies have indicated that high available P-content (> 9 ppm) reduced mycorrhizal colonization and spore production (Koide, 1991; Lakshmipathy *et al.,* 2002, 2003). The available-P in soils of the Indian land use types (Table 1) is lower compared to other land uses. This could be one of the reasons for variations in mycorrhizal root colonization in different land use types in this study. Pande and Tarafdar (2004) suggested that increased rainfall and relative air humidity increased AMF colonization. With the onset of rains in the post-monsoon season significant moisture prevailed in the soil that favoured better root growth of plant species and enhanced AMF root colonization.

AMF Spore Density and Infective Propagules

The spore density and infective propagules were significantly more in grasslands and acacia plantations compared to natural forests, cardamom and coffee plantations with the lowest in paddy fields in both seasons. The floor of acacia plantations in the study area is also covered with patches of graminaceous species and acacia itself being a leguminous plant, could have caused higher spore density and infective propagules. Mycorrhizal population can also be affected by several agricultural practices such as ploughing, application of fertilizers, pesticides and herbicides which reduced the mycorrhizal spores and infective propagules (Boddington and Dodd, 2000; Balakrishna *et al.*, 2001; Kabir,

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2005). These factors may be responsible for the lower number of spores and infective propagules in paddy fields, cardamom and coffee plantations.

Seasonal variations may also influence spore density and species diversity. Several workers (Mallesh and Bagyaraj, 1991; Picone, 2000) have reported earlier that the spore density and infective propagules were higher in winter than in summer suggesting that the mycorrhizal activity is favored during winter season. In this study, also such variations were observed between land use types.

Influence of Soil Properties

Soil physical, chemical and biochemical properties influenced AMF root colonization, spore density and infective propagule numbers. Sand and bulk density had a positive influence, while clay and silt contents had a negative influence on AM fungi. In sandy soils, plant roots proliferated better because of good aeration; thereby possibly enhancing spore production. Similar observations have also been reported earlier (Pande and Tarafdar, 2004). Organic C content in soils is known to favor mycorrhizal infections (Johnson and Wedin, 1997; Pande and Tarafdar, 2004). The organic-C and N content of the soils in the study area showed a positive influence on AMF. Therefore, it suggests that the status of organic matter content in soils is important for mycorrhizal activity. The available and total P contents in soil play an important role in mycorrhizal colonization and spore production. Several studies have indicated that high available P-content (> 9 ppm) reduced mycorrhizal colonization and spore production (Koide, 1991; Lakshmipathy *et al.,* 2002, 2003). The total P and available P contents in soils in the study area seem to have a significant negative influence on AMF activity.

AMF Species Distribution

Variations in AMF species numbers during different seasons can potentially be attributed to changes in moisture regimes in soil and other climatic conditions. More AMF species were recorded during rainy season as compared to summer season (Lovelock *et al.,* 2003; Husband *et al.,* 2002). There was a strong shift in mycorrhizal communities and their numbers over time. They suggested that, during wet season a large number of newly germinated plant seedlings prevail, hence there were more species present. Few plant species, which may regenerate only during wet season, may vanish during dry season. Variations in species composition in different seasons are quite obvious in this study owing to the reasons already explained.

During both seasons, higher numbers of AMF species were recorded in natural forests, grasslands and acacia plantations than in the cardamom and coffee plantations and paddy fields. Shi *et al.* (2007) reported higher species diversity in natural forests than in forest plantation. Oehl *et al.* (2003) while studying the impact of land use types have also reported that the AMF species composition was the highest in grasslands, lower in the low and moderate input arable lands and the lowest in the lands with intensive continuous maize mono-cropping. Certain other studies have also indicated that the number of species was higher in undisturbed areas than the disturbed areas. Picone (2000) while studying the AMF species composition in Nicaragua and Costa-Rican forests and pastures, found similar species composition in both forests and pastures, while Mendez *et al.* (2001) noticed higher AMF species composition in pasture soils than in cultivated soils. The results of the present study follow a similar pattern as the natural forests and grasslands and to some extent acacia plantations are more or less undisturbed, because these plantations are almost 10 years old. Coffee plantations and paddy fields on the other hand are more intensively cultivated which may have led to lower AMF species numbers.

AMF Species Spore Abundance over Landscape

AMF species spore abundance was more during post-monsoon than during pre-monsoon season. The majority of the species produced more spores during post-monsoon than during pre-monsoon season. This kind of variation in AMF species composition over different seasons could be attributed to the adaptation of specific AMF species to a particular climatic condition and soil moisture regimes. Sampling of soils over

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seasons has revealed that some AMF species sporulated better during wet season while some species sporulated during dry season. Lovelock (2003) also observed that the relative abundance of spores of *Acaulospora* was lower than that of *Glomus*, during wet season and found that *Glomus* produced relatively more spores at the highest seasonal rainfall. Further, he also suggested that during wet season a number of plant species with profuse rooting favored the sporulation of AMF species. Sanders and Fitter (1992) opined that the composition of plant community might also affect mycorrhizal fungi causing differential reproduction and survival, which will definitely act as a selective force on the composition of AM fungi. Schenck and Kinloch (1980) also noticed incidence of AMF species over different periods of time over the years; the spores of some species increased, while the spores of few species decreased. Spores of few species observed during one season disappeared in the next season.

Diversity of AM Fungi in Different Land Use Types

Mycorrhizal fungi are likely to be affected by plant community composition (Janos, 1980; Kormanik *et al.,* 1980). The AMF populations may readily respond to the proportion of mycotrophic plants in a community because the fungi cannot live without hosts. In the present study, variations in the Shannon-Wiener diversity index of AMF species in different land use types were noticed. This kind of variation in the diversity of AMF in different land use types and during different seasons was also noticed in earlier studies. Picone (2000) and Helgason *et al.* (2007) have reported that the AMF diversity indices for forest and pastures were similar. Also, in this study, the diversity index of AMF in natural forests and grasslands was on par with each other. This suggests that AMF diversity in undisturbed systems as that of forests and grasslands is less affected. In contrast, the diversity index was quite different in less undisturbed grasslands and more disturbed paddy fields. Oehl *et al.* (2003) in their study also recorded the highest mycorrhizal diversity index in grasslands compared to moderate and low input aerable lands and intensive continuous maize mono-cropping. Carpenter *et al.* (2001) in their study on spore density and diversity of AMF in different land uses found that diversity of AMF changed due to change in land use types.

The AMF species richness between different land use types and seasons followed a similar trend as that of diversity. The lower species richness index for paddy fields could be due to intensive agricultural practices. Earlier studies have shown that application of fertilizers, pesticides, tillage and other soil disturbances decreased AMF diversity and species richness (Kruckelmann, 1975; Hayman, 1980, Gemma, 1988, Attitchabi *et al.,* 2008). Further, low fertility status of the soils in natural forests, acacia plantations and grasslands probably required an increased dependence of plants on mycorrhizae for growth and survival, which facilitated the growth of a diverse species of AMF. Blaszkowski (1994) also made similar observations with species richness for different plants grown in different places of Hel peninsula of Poland, where the species richness was more in low fertile soils sampled under Cupressaceae followed by Rosaceae and less in Gramineae. The fertility status of soil was more in soils under Rosaceae and Gramineae.

CONCLUSIONS

This study shows that disturbance of a particular land use type leads to the destruction of flora, which in turn alter/reduce the population and diversity of mycofauna.

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فراوانی و تنوع قارچ‌های AMF در دامنه‌ای از شدت کاربری اراضی و تغییرات فصلی آن در بیوسفر نیلیگیری در منطقه گات غربی، هندوستان

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چکیده

اثر شدت کاربری اراضی بر روی فراوانی و تنوع قارچهای AMF در شش نوع کاربری اراضی شامل جنگل طبیعی، علفزار، مزرعه آکاسیا، مزرعه هل، مزرعه قهوه و مزرعه برنج در بیوسفر نیلیگیری در منطقه گات غربی، در جنوب هندوستان بررسی گردید. در فصل پیش از مونسون تفاوت معنی داری بین مقدار AMF ریشه در کاربری-های مختلف مشاهده نشد در حالی که در فصل پس از مونسون این تفاوت معنی دار بود و مقادیر در جنگل طبیعی و علفزار بیشتر بودند. تراکم هاگ AMF و اندامهای زایشی آلوده درعلفزار و مزرعه آکاسیا در هر دو فصل نسبت به دیگر کاربری‌ها بیشتر بود. به استثنای مزرعه برنج، در بقیه کاربری‌ها تراکم هاگ AMF و اندام‌های زایشی آلوده در فصل پس از مونسون از فصل پیش از آن بیشتر بود. تعداد گونه های AMF شناسایی شده در فصل پیش از مونسون ۵۶ و در فصل پس از مونسون ۶۷ عدد بود که نشان دهنده تغییرات فصلی در تنوع می‌باشد. در هر دو فصل *Glomus fasciculatum* بیشترین تعداد را در منطقه داشت و پس از این گونه *G. geosporum* در فصل پیش از مونسون و *G. mosseae* در فصل پس از مونسون بیشترین مقدار را داشتند. در هر دو فصل، تنوع گونه ای در جنگل طبیعی و علفزار نسبت به کاربری‌های دیگر بیشتر بود. در هر دو دوره زمانی مورد مطالعه، شاخص غنای گونه‌ای برای AMF در جنگل طبیعی حداکثر و در مزرعه برنج حداقل بود. میزان ماسه، دانسیته ظاهری، نیتروژن کل، کربن آلی و فسفاتازهای قلیایی و اسیدی با فعالیت AMF رابطه مثبت و میزان رس، سیلت، پتاسیم، فسفر کل و فسفر در دسترس با آن رابطه منفی داشتند.

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