Temporal and spatial variability of rhizosphere and rhizoplane fungal communities in grasses of the subfamily Chloridoideae in the Lakkavalli region of the Western Ghats in India

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Rhizosphere fungal species in the rhizosphere and rhizoplane of perennial grasses of the subfamily Chloridoideae in Lakkavalli region of the Western Ghats in India were characterized by culturing on potato dextrose agar medium. Anamorphic ascomycetous fungi were frequently isolated. Certain fungal communities are spatially distributed, as they are specific to rhizosphere and rhizoplane of certain grass species. Fungal communities were isolated more during winter than other seasons suggesting that fungal communities are temporally distributed. Although species richness of fungal communities was similar, the diversity and evenness of fungal assemblages differed depending on the grass species. Certain fungal species were commonly encountered, while, certain other fungal species and non-sporulating fungi were grass species-specific. The present study indicated that perennial grasses are reservoirs of diverse groups of fungi that could be exploited for beneficial purposes.

Key words – anamorphic ascomycetes – diversity – fungal assemblages – non-sporulating fungi – perennial grasses

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Introduction

A great array of root-microbe interactions result in the development of a dynamic environment known as the rhizosphere, where microbial communities also interact amongst themselves (Smalla et al. 2001, Kuske et al. 2002, Dunfield & Germida 2003, Garbeva et al. 2004). The rhizosphere supports an abundance of diverse saprophytic microorganisms. This could be due to high input of organic carbon compounds into the soil through the process of rhizodeposition (Rovira 1956, Merckx et al. 1987). Rhizodeposition not only sustains multitrophic rhizosphere food webs (Phillips et al. 2003), but also mediates signal traffic between roots of competing plants and between roots and beneficial or detrimental rhizosphere microorganisms. The existence of such diverse below-ground communications have been discussed (Bais et al. 2004).

The role of fungi in soil is extremely complex and is fundamental to the soil ecosystem (Bridge & Spooner 2001). Soil fungi play an important role in nutrient cycling, and plant health and development (Thorn 1997, Bridge & Spooner 2001, Martin et al. 2001). Some fungi cause a range of plant diseases (Jarosz & Davelos 1995, Thorn 1997), while others antagonize plant pathogens, decompose plant residues, provide nutrients to plants, and stimulate plant growth. Information on the knowledge of the diversity and structure of

fungal communities in bulk and rhizosphere soils help in better understanding of their roles in soil ecosystem and in improving plant health.

Grasses form an important component in plain and montane ecosystems, which will rejuvenate every year. In each growing season, they produce fresh fibrous root system and thus might activate diverse microbial flora in the rhizosphere and rhizoplane regions. These regions in different grass species supported populations of fungal communities of anamorphic ascomycetes, teleomorphic ascomycetes, zygomycetes and certain non-sporulating fungi (Vasanthakumari et al. 2007). The occurrence of these fungal communities in certain grasses was shown to vary depending on the season, root region and soil nutrient situation. (Vasanthakumari & Shivanna 2011). The main objective of the present study was to characterize fungal species and to determine changes in the diversity, evenness and richness of fungal communities in the rhizosphere and rhizoplane regions of certain grass species of subfamily Chloridoideae, resulting from the variability in the season and rhizosphere effects. Grasses and their rhizosphere regions have been a neglected research area with respect to the characterization of fungal communities. There is some evidence on the occurrence of the fungal communities in the rhizosphere and rhizoplane regions of cultivated grass species (Hyakumachi et al. 1992, 1993, Smit et al. 1999). However, there has been far less research work on the fungal communities (Abdel-Hafez 1982, Gomes et al. 2003) in rhizoplane and rhizosphere regions of wild grass species in different seasons (Vasanthaku mari & Shivanna 2011). Hence, these aspects were studied in perennial grasses of subfamily Chloridoideae growing in Karnataka, India.

Materials and methods

Study area

Lakkavalli state forest region (13°34' – 13°39'N latitude, 75°36' – 75°39'E longitude), an area adjoining Bhadra reservoir and situated in the peripheral region of the Bhadra Wildlife sanctuary, in the Western Ghats of Karnataka was selected as the study area. The area is situated at an elevation of 810m a.s.l. and receives an annual rainfall of 1500 to 2500 mm and temperatures ranging from 14.5–31.5°C. The study area is characterized by well-drained red loam soil with coarse quartz grains. It is rich in several species of perennial and annual grasses in addition to herbs, shrubs and trees.

Three study sites at a distance of two kilometers from each other were established. In each site, three quadrats (1×1 m) representing three replicates were established. Experiments were conducted during July, November and March that represented rainy, winter and summer seasons. Experiments were conducted during the above seasons of 2004–2005 and repeated during 2005–2006.

Selection of perennial grass species

Grass species in the study area were identified (Vasanthakumari et al. 2010) based on the methodology suggested by Bhat & Nagen dran (2001). The morphological and floral characteristics of grass species were compared with those described in standard manuals and flora (Bor 1960, Yoganarasimhan et al. 1982, Sreekumar & Nair 1991). Voucher herbarium specimens were prepared and deposited in the Department of Applied Botany, Kuvempu University for reference. Five perennial grass species of subfamily Chloridoideae were selected for the study based on their availability through different seasons: *Chloris barbata* Sw. (swollen fingergrass), *Cynodon dactylon* (L.) Pers. (Bermuda grass), *Dactyloctenium aegyptium* (L.) P. Beauv. (Egyptian grass), *Eleusine indica* Gaertn. (Indian goosegrass), and *Eragrostis unioloides* (Retz.) Nees ex Steud. (Chinese lovegrass).

Collection and analysis of soil samples

The grass species were located in the study sites and labelled for soil sample collection. The zone of soil immediately adjacent to roots of each plant upto 15–20 cm depth from the surface was collected in triplicate (three samples/replicate) in sterilized polypropylene covers. Triplicate soil samples were air-dried and pooled and 200g of the sample was used for the determination of the soil pH, organic carbon, and available N, P and K (Subbaiah & Asija 1956, Jackson 1967, Baker & Amacher 1982).

Preparation of rhizosphere and rhizoplane samples

From each study site, 10 replicate grass plants of each species were uprooted carefully from the soil using a trowel, and roots with adhering soil samples were collected in sterilized polypropylene covers and taken to the laboratory. The root system of the grasses were carefully removed from the soil and gently shaken to remove superfluous soil. The rhizosphere sample was collected by gentle scrapping of the soil particles closely adhering to the root surface by using a sterile spatula and by brushing with a camel hair brush. The root system without soil particles was considered as the rhizoplane sample. Both the samples were used for experimentation within four hours.

Determination of fungal communities from rhizosphere and rhizoplane samples

Rhizosphere samples were subjected to dilution (10^{-4}) plating on potato dextrose agar or Czapeck dox agar (PDA/CZA, Himedia Laboratories, Mumbai) amended with streptomycin (100 mgL^{-1}) (Dhingra & Sinclair 1993). The inoculated plates were incubated in an incubation chamber under 12/12 h regimes of light and darkness at 23 ± 2°C for 5–6 days. Root samples of each grass species (100 roots /plant species) were washed in slow running tap water, blotted, and the root along the axis was segmented into root base, middle and root tip regions, and each region was again fragmented into 1-cm- long segments (Meera et al. 1994). Root segments of each region (300/ species) were incubated as described above on streptomycin amended PDA/CZA to obtain fungal communities. The fungal species were identified based on cultural characteristics, fruiting bodies, and spores using standard identification manuals (Barnett 1960, Booth 1971, Domsch & Gams 1972, 1980, Ellis 1976, Sutton 1980, Arx 1981). The species nomenclature was confirmed from Index Fungorum (www.indexfungorum.org). Fungal isolates that failed to produce reproductive propagules on PDA/CZA were sub-cultured on malt extract agar (Himedia Laboratories, Mumbai) or water agar (2%) or subjected to near-UV (350–400 nm) light/darkness cycle of 12/12 h at 23 ± 2°C for 5–6 days. Colonies that failed to sporulate after these treatments were considered as non-sporulating fungal (NSF) isolates. The NSF isolates were observed for hyphal septation, formation of crozier or clamp connection to identify them to the group level. All fungal isolates were assigned with voucher numbers, documented and stored at 5°C until use. The number of fungal colonies (cfu g^{-1} soil) formed on dilution plates in each sample was determined. The root colonization frequency (%) and predominantly occurring fungi (%) were calculated (Fisher & Petrini 1987, Kumaresan & Suryanarayanan 2002).

Statistical analysis

Inoculated plates were arranged in a randomized complete block design (RCBD). The replicate trials of two years were subjected to homogeneity of trials by ANOVA. The diversity, evenness and species richness of fungal species in the rhizosphere and rhizoplane regions of grass species were analyzed by Simpson and Shannon indices. Jaccard's similarity co-efficient and rarefaction indices were also calculated using the PAST software version, 1.00 (Hammer et al. 2001).

Results

Results of repeated trials indicated that there was no significant ($P_{0.05}$) variation in the incidence of fungi isolated from the two plant regions in different study sites. Hence, the data of two years were averaged season-wise and subjected to further statistical analyses.

The reproductive stage of grass species *Chloris barbata*, *Dactyloctenium aegyptium* and *Eleusine indica* was during January-March, while that of *Cynodon dactylon* and *Eragrostis unioloides* was during September-November. Post-rainy months favored robust fibrous root production in all the grass species.

Soil characteristics

The available organic carbon and N, P and K content in soil samples varied in different seasons but were almost similar during the respective seasons of the two years trial. The NPK availability was higher in winter than in summer and rainy seasons (Table 1).

Table 1 Mineral nutrient composition of soil sample collected from soil adjacent to grasses of the subfamily Chloridoideae[1].

Seasons	pH	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})
Rainy	5.60	0.36	16.59	09.51
Winter	5.32	4.05	23.07	10.12
Summer	5.47	4.05	15.98	07.69

Note: [1]Data based on the values of three study sites and three seasons of two trials. Available organic Carbon (0.99–1.00%)

Media composition and fungal characterization

A large number of fungal species were isolated from the rhizosphere and rhizoplane regions. Most fungal isolates occurred on both PDA and CZA media, however, fungal incidence was higher on PDA than on CZA. Some species such as *Cochliobolus spicifer* RR. Nelson, *Fusarium chlamydosporum* Wollenw. & Reinking, *Mammaria echinobotryoide*s Ces., *Memnoniella echinata* (Rivolta) Galloway, *Myrothecium verrucaria* (Alb. & Schwein.), *Periconia atropurpurea* (Berk. & M.A. Curtis) M.A. Litv., *Stachybotrys chartarum* (Ehrenb.) S. Hughes, *Stenocarpella maydis* (Berk.) B. Sutton, *Rhizopus arrhizus* A. Fisch., and *Chaetomium aureum* Chivers were recorded exclusively on PDA, while others such as *Helicocephalum sarcophilum* Thaxt., *Chrysonilia sitophila* (Mont.) Arx, *Paecilomyces variotii* Bainier, *Cochlonema verrucosum* Dreschler, and *Syncephalastrum racemosum* Cohn ex J. Schröt were recorded only on CZA. The NSF produced hyaline or colored, septate hyphae, cottony aerial or submerged mycelia and lacked in crosiers or clamp connections. The NSF were also media-specific with NSF isolates-CB28, SD30, E9 and E13 occurring only on PDA.

Fungal communities in the rhizosphere region

The rhizosphere samples from all the grass species yielded 1216 fungal isolates per gram of soil on PDA. The number of fungal isolates in the rhizosphere varied depending on the soil sample and grass species. *Cynodon dactylon* supported high fungal incidence (304 cfu g^{-1} soil) in its rhizosphere through all seasons followed by *Chloris barbata* (283 cfu g^{-1} soil), while *Eragrostis unioloides* supported the few species of fungi (187 cfu g^{-1} soil) (Table 2).

In total 62 species (from 42 genera), and 18 NSF isolates were found. The fungi could be grouped into anamorphic ascomycetes (66.8%) (Table 3), teleomorphic ascomycetes (19.7%), zygomycota (4.7%), and NSF (8.7%) (Table 4). Some of the prominent anamorphic ascomycetes occurring in the rhizosphere of grasses included species of *Alternaria*, *Aspergillus*, *Clonostachys*, *Fusarium*, *Myrothecium*, *Penicillium* and *Trichoderma*. All, except species of *Alternaria* and *Acremonium* occurred in more than three grass species (Table 3).

Species of *Chaetomium* and *Cochliobolus* among teleomorphic ascomycetes, *Cunninghamella* species among zygomycota occurred predominantly in more than three grass species (Table 4). The season-wise occurrence of the fungal species of different groups is shown in Fig. 1. More anamorphic ascomycetous fungi were isolated during the rainy season than in winter or summer seasons. But for NSF isolate 67 which occurred in both *Dactyloctenium aegyptium* and *Eleusine indica*, all other NSF were grass species-specific (Table 4).

The number of species ranged from 24–30 on PDA (Table 5). The Shannon diversity (H') and Shannon evenness (J') indices as well as Simpson diversity (D') and Simpson evenness (E') indices of rhizosphere fungal community was highest (H'=3.27; J'=0.96; D'=24.76 and E'=0.82) for *Cynodon dactylon* and lowest (H'=2.96; J'=0.90; D'=17.54 and E'=0.64) for *Eragrostis unioloides* (Table 5). Jaccard's similarity co-efficient showed moderate to high similarity of assemblages of fungal communities from the rhizosphere regions of different grass species in different seasons ranging from 0.58 to 0.93 (Table 6). The rarefaction index indicated that the expected number of fungal species always increased with the increase in the number of isolations from the rhizosphere

Table 2 Occurrence of fungal species in the rhizosphere and rhizoplane regions of certain grasses of the subfamily Chloridoideae on PDA during different seasons[1].

Grasses	Rhizosphere (cfu g^{-1}soil)			Rhizoplane (% colonization frequency)		
	Rainy	Winter	Summer	Rainy	Winter	Summer
Chloris barbata	84.9	119.7	77.7	624.0	823.0	489.0
Cynodon dactylon	92.7	119.7	91.7	681.0	844.0	588.0
Dactyloctenium aegyptium	74.7	93.0	73.0	612.0	773.0	495.0
Eleusine indica	60.3	88.3	57.0	521.0	668.0	406.0
Eragrostis unioloides	56.0	79.0	52.0	510.0	676.0	394.0
LSD[2]	$P_{0.01}$= 0.020, $P_{0.001}$= 0.026			$P_{0.01}$= 1.00, $P_{0.001}$= 1.34		

Note: [1]Data based on the values of three study sites and three seasons of two trials.
[2]For comparing treatment means of occurrence of rhizosphere and rhizoplane fungi among seasons or grass species.

Table 3 Frequency of anamorphic ascomycetes isolated from the rhizosphere of grass species of the subfamily Chloridoideae on PDA.

Sl. No.	Fungi	Frequency of occurrence[1] (%)				
		Chloris barbata	***Cynodon dactylon***	***Dactyloctenium aegyptium***	***Eleusine indica***	***Eragrostis unioloides***
1	*Acremonium strictum*	0.0	0.0	0.0	6.8[2]	0.0
2	*Aspergillus* spp.[3]	13.8(3)[4]	17.2(5)	14.4 (3)	10.5 (2)	16.7(4)
3	*Cladosporium* spp.[5]	9.9(2)	4.6(1)	8.6(2)	7.8(1)	11.0(2)
4	*Clonostachys rosea*	7.4	5.9	3.2	7.1	0.0
5	*Colletotrichum dematium*	0.0	3.5	0.0	0.0	0.0
6	*Fusarium* spp.[6]	6.9(1)	0.0	6.2(1)	5.7(1)	8.4(2)
7	*Gilmaniella humicola*	0.0	2.4	0.0	0.0	0.0
8	*Graphium penicillioides*	2.0	0.0	0.0	0.0	0.0
9	*Myrothecium* spp.[7]	3.7(1)	3.1(1)	5.3(1)	3.2(2)	2.7(1)
10	*Penicillium* spp.[8]	11.6(2)	12.0(3)	12.6(2)	9.5(2)	15.1(3)
11	*Pestalotiopsis* spp.[9]	3.0(1)	0.0	0.0	0.0	1.6(1)
12	*Spegazzinia tessarthra*	0.0	0.0	0.0	4.7	0.0
13	*Trichoderma* spp.[10]	4.5(1)	7.5(2)	5.9(1)	15.6(2)	10.5(2)
14	*Torula herbarum*	2.5	0.0	0.0	0.0	6.2
	Total frequency	67.3(18)	57.5(16)	62.3(15)	70.9(14)	76.4(20)

Note: [1]Frequency of fungal occurrence was calculated based on colony forming units of a particular fungus over sum of colony forming units and represented as percentage.
[2]Data is an average of three replicates, each with 81 samples.
[3]*Aspergillus* species = *A. flavus* (5.5–6.6), *A. nidulans* (1.2–2.5), *A. brasiliensis* (5.0–6.2), *A. ochraceus* (0–2.7), *A. terreus* (0–3.5).
[4]Figures in parenthesis indicate total number of species of genera which may vary in different grasses.
[5]*Cladosporium* species = *C. cladosporioides* (5.3–7.8), *C. herbarum* (2.8–4.6).
[6]*Fusarium* species =*F. chlamydosporum* (0–2.0), *F. oxysporum* (5.7–6.9).
[7]*Myrothecium* species= *M. roridum* (1.8–3.7), *M. verrucaria* (0–1.4).
[8]*Penicillium* species = *P. chrysogenum* (3.2–6.5), *P. citrinum* (5.3–6.3), *P. commune* (4.3–7.6), *P. decumbens* (4.3–7.6), *P. islandicum* (1.6–6.6).
[9]*Pestalotiopsis* species = *P. glandicola* (0–1.6), *P. mangiferae* (0–3.0).
[10]*Trichoderma* species = *T. harzianum* (3.8–8.7), *T koningii* (0–3.7), *T. pseudokoningii* (0-3.6), *T. viride* (0–6.8).
Anamorphic ascomycetes – *Alternaria alternata*, *Cylindrocladiella parva*, *Geotrichum candidum*, *Histoplasma capsulatum*, *Macrophomina phaseolina*, *Mammaria echinobotryoide*s, *Memnoniella echinata*, *Periconia atropurpurea*, *Stachybotrys chartarum*, *Stenocarpella maydis*, *Robillarda sessilis* and *Xepicula leucotricha* with < 2% frequency are also included in the total frequency of respective grass species.

Table 4 Frequency of teleomorphic ascomycete, zygomycota and non-sporulating fungi isolated from rhizosphere of grass species of the subfamily Chloridoideae on PDA.

Sl. No.	Fungi	Frequency of fungal occurrence[1] (%)				
		Chloris barbata	***Cynodon dactylon***	***Dactyloctenium aegytium***	***Eleusine indica***	***Eragrostis unioloides***
	Teleomorphic ascomycetes					
1	*Chaetomium* spp.[2]	5.3(1)[3]	6.7(2)	5.1(2)	5.0(1)	0.0
2	*Cochliobolus* spp.[4]	0.0	3.5	0.0	0.0	0.0
3	*Gibberella fujikuroi*	0.0	5.7	0.0	2.4	0.0
4	*Khuskia oryzae*	5.8	3.6	4.6	4.2	5.1
5	*Plagiostoma* sp.	3.0	2.5	4.2	2.7	0.0
	Total frequency	19.0(5)	26.3(7)	21.7(6)	19.9(6)	11.7(3)
	Zygomycota					
1	*Cunninghamella* spp.[5]	4.8(1)	3.8(1)	3.5(1)	4.3(1)	4.6(1)
2	*Zygorhynchus moelleri*	0.0	2.3	0.0	0.0	0.0
	Total frequency	4.8(1)	6.1(2)	3.5(1)	4.4(1)	5.0(2)
	Non-sporulating fungi					
1	NSF (isolate 54)	2.3	0.0	0.0	0.0	0.0
2	NSF (isolate CB28)	2.6	0.0	0.0	0.0	0.0
3	NSF (isolate 161)	0.0	3.5	0.0	0.0	0.0
4	NSF (isolate 19)	0.0	2.3	0.0	0.0	0.0
5	NSF (isolate DA24)	0.0	0.0	2.4	0.0	0.0
6	NSF (isolate 59)	0.0	0.0	2.7	0.0	0.0
7	NSF (isolate DA29)	0.0	0.0	6.0	0.0	0.0
8	NSF (isolate E9)	0.0	0.0	0.0	4.5	0.0
9	NSF (isolate 89)	0.0	0.0	0.0	0.0	3.7
	Total frequency	8.4(4)	10.0(5)	12.3(5)	6.3(2)	6.8(2)

Note: [1]Frequency of fungal occurrence was calculated based on colony forming units of a particular fungus over sum of colony forming units and represented as percentage.
[2]*Chaetomium* species = *C. aureum* (0–4.4), *C. bostrychodes* (0–1.0), *C.globosum* (4.2–5.3), *C. indicum* (0–2.3).
[3]Figures in parenthesis indicate total number of species of the genera which may vary in different grasses.
[4]*Cochliobolus* species = *C. spicifer* (0.2–3.8), *C. lunatus* (0.2–3.8).
[5]*Cunninghamella* species = *C. echinulata* (3.5–4.8), *C. elegans* (0–4.3)
Teleomorphic ascomycetes – *Neodeightonia subglobosa*, *Sordaria fimicola*, *Thielavia terricola* var. minor, species of zygomycota (*Rhizopus arrhizus*) and NSF (isolates 58, 62, CD 29, 21, CD 30, 71, 67, E13) with < 2% frequency are included in the total frequency of respective grass species.

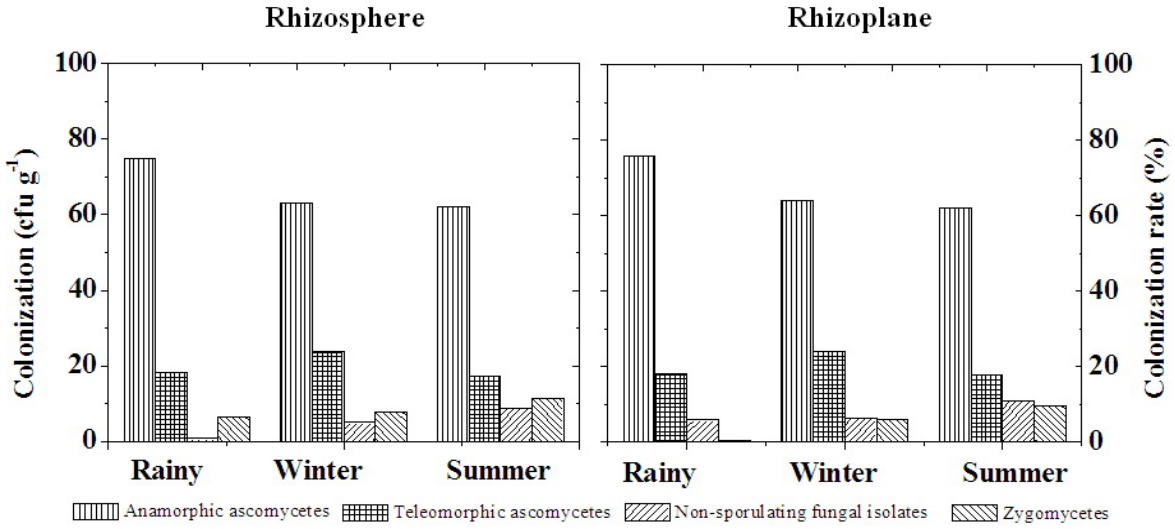

Fig. 1 – Seasonal distribution of fungal groups in rhizosphere and rhizoplane regions of grasses of subfamily Chloridoideae.

Table 5 Species richness, diversity and evenness indices of fungal communities in the rhizosphere and rhizoplane regions of grasses of the subfamily Chloridoideae on PDA.

Root regions/grass species	**Species richness**	**Diversity index**		**Evenness index**	
		Shannon (H')	**Simpson (D')**	**Shannon (J')**	**Simpson (E')**
Rhizosphere[1]					
Chloris barbata	29.0	3.12	20.44	0.93	0.70
Cynodon dactylon	30.0	3.27	24.76	0.96	0.82
Dactyloctenium aegyptium	27.0	3.13	20.82	0.95	0.77
Eleusine indica	24.0	3.02	18.75	0.95	0.78
Eragrostis unioloides	27.0	2.96	17.54	0.90	0.64
Rhizoplane[2]					
Chloris barbata	30.0	3.19	21.73	0.94	0.72
Cynodon dactylon	30.0	3.27	24.36	0.96	0.81
Dactyloctenium aegyptium	29.0	3.20	22.42	1.07	1.12
Eleusine indica	25.0	3.03	18.83	0.94	0.75
Eragrostis unioloides	28.0	3.11	19.83	0.93	0.70

Note: [1] Data is an average of three replicates, each with 81 samples.
[2] 300 root segments/grass species.

Table 6 Jaccard's similarity co-efficient of fungal communities in the rhizosphere and rhizoplane regions of grasses of the subfamily Chloridoideae during different seasons.

Grass species	Rhizosphere[1]			Rhizoplane[2]		
	Rainy	Winter	Summer	Rainy	Winter	Summer
Chloris barbata	0.76	0.66	0.58	0.80	0.69	0.56
Cynodon dactylon	0.89	0.90	0.80	0.80	0.69	0.60
Dactyloctenium aegyptium	0.93	0.84	0.83	0.74	0.84	0.70
Eleusine indica	0.83	0.85	0.70	0.74	0.84	0.70
Eragrostis unioloides	0.71	0.82	0.66	0.62	0.68	0.41

Note: Similarity co-efficient will range from 0 for complete dissimilarity to 1.00 for complete similarity.
[1] Data is an average of three replicates, each with 81 samples.
[2] 300 root segments/grass species.

regions of grass species. The number of isolations was highest in the winter followed by rainy and summer seasons in all the grass species (Fig. 2).

Fungal communities in the rhizoplane region

Incubation of the root segments of five grass species yielded 9014 fungal isolates on PDA. The colonization frequency of these isolates was 51.7%. The rhizoplane regions of all grass species yielded 68 species (in 45 genera) and 17 NSF. As with the rhizosphere, rhizoplane regions of *Cynodon dactylon* and *Chloris barbata* also harboured more fungal species (844 and 823, respectively) during winter than in the other seasons (Table 2).

The rhizoplane fungal isolations from all the grass species were also grouped into anamorphic ascomycetes (71%) (Table 7), teleomorphic ascomycetes (16.3%), zygomycota (5.3%) and NSF (7.4%) (Table 8). The anamorphic ascomycetes also dominated the rhizoplane region during rainy season followed by winter and summer seasons (Fig. 1). Species of *Chaetomium, Plagiostoma* and *Cunninghamella* occurred in more than three grass species, while NSF were specific to a few grass species (Table 8).

The fungal species with a frequency of >5% occurring in more than three grass species were considered as dominant fungal species. For example, *Aspergillus flavus, A. brasiliensis*, *Cladosporium cladosporioides*, *Clonostachys rosea*, *Penicillium chrysogenum, P. citrinum* and *Trichoderma harzianum* (Table 7).

Generally, similar fungal species colonized different root regions of grass but their population varied. However, the preference of these fungi to grow in specific root regions differed depending on the type of grass species and season. Segments in the middle root region were colonized to the greatest extent by most species of fungi (375) followed by root tip **(**327) and root base (283). *Aspergillus flavus, A. brasiliensis*, *C. globosum*, *C. rosea*, *C. cladosporioides*, *Cunninghamella echinulata*, *Fusarium oxysporum*, *Myrothecium roridum*, *Khuskia oryzae*, *P. chrysogenum*, *Plagiostoma* sp. and *T. harzianum* colonized all the root regions of most grass species (data not shown).

The NSF colonized mainly the middle root region (data not shown). Some of the fungal isolates were exclusively found in the root regions of certain grass species. For example, *Chloris barbata*, *Cynodon dactylon*, *Dactyloctenium aegyptium*, *Eleusine indica* and *Eragrostis unioloides* harboured 15, 13, 13, and 8 and 12 fungal species, respectively. Notable among them were *Graphium penicilloides* and four NSF from *Chloris barbata, Chaetomium indicum, Gilmaniella humicola*, *Trichoderma pseudokoningii* and four NSF from *C. dactylon, Chaetomium bostrychodes* and five NSF from *D. aegyptium*, *P. decumbense* and *T. viride* and two NSF from *Eleusine indica* and, *P. islandicum* and *T. koningii* and two NSF from *E. unioloides*.

The number of fungal species from the rhizoplane regions of grasses ranged from 25 to 30 (Table 5), with maximum number of fungal isolates from the rhizoplane of *C. barbata* and *C. dactylon* and the least from *E. indica*. The Shannon diversity (H') and Simpson diversity (D') indices of rhizoplane fungal communities were highest (H'=3.27 and D'=24.76) for *C. dactylon* and lowest (H'=3.03 and D'=18.83) for *E. indica*. Shannon evenness (J'), and Simpson evenness (E') indices were highest (J'=1.07 and E'=1.12) for *D. aegyptium* and lowest (J'=0.93

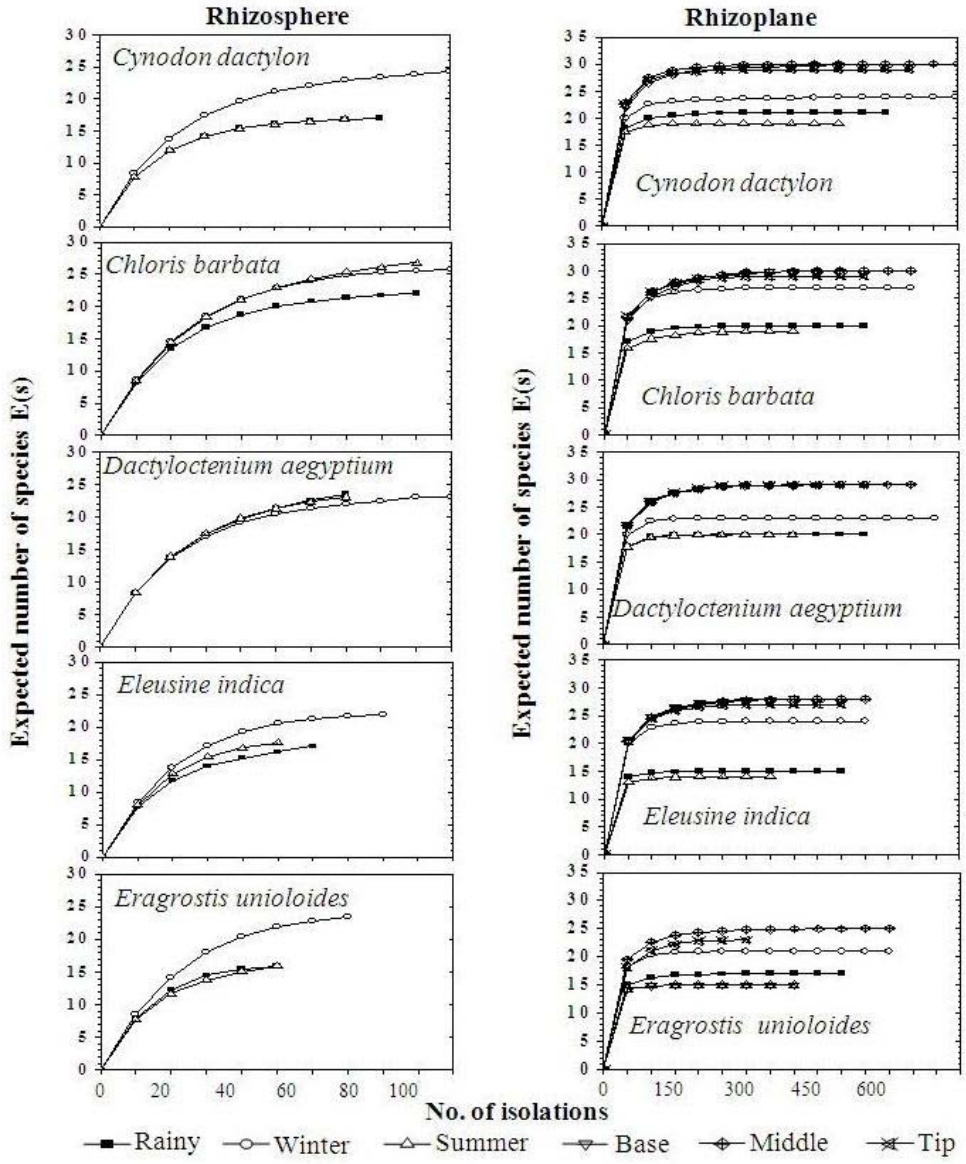

Fig. 2 – Rarefaction curves of rhizosphere and rhizoplane fungi of grasses of subfamily Chloridoideae during rainy, winter and summer seasons.

and E'=0.70) for *E. unioloides* (Table 5). As in the case of the rhizosphere, there was a moderate to high (0.41–0.84) similarity among fungal assemblages in different seasons (Table 6). The expected number of species (E_S) of rhizoplane fungal community always increased with increase in the number of isolations from different root regions of grass-species, but up to 100 isolations. Thereafter, it remained almost static irrespective of the season and root region (Fig. 2).

Discussion

The fungal population in soil and root samples of two-year trials did not vary much, which could be due to the prevalence of an almost uniform rainfall pattern (data not presented) in the study area in these two years. There was also little variation among sites, as they are located only two kilometers apart.

Soil nutrient availability in different seasons

The study area typically falls under the dry deciduous forest category. The soil type of the study area experienced run-off during rainy season with low water holding capacity. An abundant vegetative growth of grass species during post-rainy season could be due to the solublization of minerals present in soil layers during rainy season. Soil microorganisms also play a great role in enhancing the nutrient bioavailability (Bridge& Spooner 2001) and

Table 7 Frequency of anamorphic ascomycetes isolated from the rhizoplane of grass species of the subfamily Chloridoideae on PDA.

Sl. No.	Fungi	Frequency of fungal occurrence[1] (%)				
		Chloris barbata	***Cynodon dactylon***	***Dactyloctenium aegyptium***	***Eleusine indica***	***Eragrostis unioloides***
1	*Acremonium* spp.[2]	0.0	0.0	0.0	3.7	0.0
2	*Alternaria alternata*	0.0	0.0	2.4	0.0	2.0
3	*Aspergillus* spp.[3]	12.5(3)[4]	17.3(5)	11.0(3)	12.1(2)	17.1(4)
4	*Cladosporium* spp.[5]	7.7(2)	4.3(1)	9.0(2)	6.2(1)	10.5(2)
5	*Clonostachys rosea*	7.0	6.0	4.1	6.8	0.0
6	*Colletotrichum dematium*	0.0	4.0	0.0	0.0	0.0
7	*Fusarium* spp.[6]	5.5(1)	6.2(1)	6.4(1)	9.3(1)	8.9(2)
8	*Gilmaniella humicola*	0.0	2.0	0.0	0.0	0.0
9	*Histoplasma capsulatum*	0.0	0.0	2.0	0.9	0.0
10	*Myrothecium* spp.[7]	4.3(1)	3.7(1)	6.2(1)	3.4(2)	2.0(1)
11	*Penicillium* spp.[8]	10.4(2)	10.2(3)	11.1(2)	8.6(2)	15.8(3)
12	*Pestalotiopsis* spp.[9]	3.8(1)	0.0	0.0	0.0	2.1(1)
13	*Spegazzinia tessarthra*	0.0	0.0	0.0	4.8	0.0
14	*Trichoderma* species[10]	7.5(1)	7.4(2)	6.6(1)	14.4(2)	11.5(2)
15	*Torula herbarum*	3.2	0.0	0.0	0.0	6.8
16	*Xepicula leucotricha*	–	–	2.3	–	–
	Total frequency	68.9(20)	56.5(16)	62.7(17)	70.2(16)	79.2(20)

Note: [1]The colonization frequency of each fungus was calculated based on the number of root segments colonized by a fungus over the total number of segments assessed and represented as percentage.
[2]*Acremonium* species=*Acremonium strictum* (0–3.7), *Acremonium implicatum* (0–0.6)
[3]*Aspergillus* species = *A. brasiliensis* (4.5–6.8), *A. candidus* (0–1.4), *A. flavus* (4.3–6.8), *A. nidulans* (1.3–2.2), *A. ochraceus* (0–2.1), *A. terreus* (0–1.8), *A. versicolor* (0–4.7).
[4]Figures in parenthesis indicates total number species of the genera which may vary in different grasses.
[5]*Cladosporium* species = *C. cladosporioides* (5.1–6.2), *C. herbarum* (2.5–4.4).
[6]*Fusarium* species =*F. chlamydosporum* (0–1.6), *F. oxysporum* (5.5–7.3).
[7]*Myrothecium* species = *M. roridum* (2.1–6.2), *M. verrucaria* (0–0.8).
[8]*Penicillium* species = *P. chrysogenum* (3.3–7.1), *P. citrinum* (5.1–5.4), *P. commune* (3.4-5.5), *P. decumbens* (0–1.4), *P. islandicum* (1.7–7.1).
[9]*Pestalotiopsis* species = *P. glandicola* (0–3.8), *P. mangiferae* (0–2.1).
[10]*Trichoderma* species = *T. harzianum* (5.8–7.6), *T koningii* (0–4.7), *T. pseudokoningii* (0–1.7), *T. viride* (0–6.8).

Table 8 Frequency of teleomorphic ascomycetes, zygomycota and non-sporulating fungi isolated from rhizoplane of grass species of the subfamily Chloridoideae on PDA.

Sl. No.	Fungi	Frequency of fungal occurrence[1] (%)				
		Chloris barbata	***Cynodon dactylon***	***Dactyloctenium aegyptium***	***Eleusine indica***	***Eragrostis unioloides***
	Teleomorphic ascomycetes					
1	*Chaetomium* spp.[2]	5.0(1)[3]	7.3(2)	4.7(2)	4.7(1)	0.0
2	*Cochliobolus spicifer*	0.0	4.3	0.0	0.0	0.0
3	*Gibberella fujikuroi*	–	6.2	–	2.0	–
4	*Khuskia oryzae*	6.1	4.0	4.0	5.0	4.7
5	*Plagiostoma* sp.	2.5	3.4	3.7	3.1	–
6	*Sordaria fimicola*	0.0	0.0	0.0	0.0	2.5
	Total frequency	14.3(4)	25.3 (6)	14.2(5)	6.4(2)	3.8(2)
	Zygomycota					
1	*Cunninghamella* spp.[4]	5.0(1)	3.9(1)	3.8(1)	4.9(1)	3.2(1)
2	*Zygorhynchus moelleri*	0.0	2.1	0.0	0.0	0.0
	Total frequency	5.0(1)	6.4(3)	3.8(1)	4.9(1)	5.0(3)
	Non-sporulating fungi					
1	NSF (isolate 62)	2.8	0.0	0.0	0.0	0.0
2	NSF (isolate CB28)	2.0	0.0	0.0	0.0	0.0
3	NSF (isolate 161)	0.0	3.4	0.0	0.0	0.0
4	NSF (isolate 59)	0.0	0.0	2.1	0.0	0.0
5	NSF (isolate DA29)	0.0	0.0	5.5	0.0	0.0
6	NSF (isolate E9)	0.0	0.0	0.0	2.4	0.0
7	NSF (isolate EU28)	0.0	0.0	0.0	0.0	2.1
	Total frequency	8.0(4)	7.1(4)	12.4(5)	4.0(2)	3.6(2)

Note: [1]The colonization frequency of each fungus was calculated based on the number of root segments colonized by a fungus over the total number of segments assessed and represented as percentage.

[2]*Chaetomium* species = *C. aureum* (0–4.4), *C. bostrychodes* (0–1.0), *C.globosum* (4.2–5.3), *C. indicum* (0–2.3).

[3]Figures in parenthesis indicate total number species of the genera which may vary in different grasses.

[4]*Cunninghamella* species= *C. echinulata* (3.1–4.4), *C. elegans* (0–4.9).

Teleomorphic ascomycetes – *Ajellomyces crescens*, *Neodeightonia subglobosa*, *Thielavia terricola* var. minor., species of zygomycota – *Helicocephalum sarcophilum*, *Rhizopus arrhizus*, and NSF (isolates 58, 54, CD 29, 19, 21, DA24, 71, 67, E13, 89) with < 2% frequency are included in the total frequency of respective grass species.

promoting plant health and development (Martin et al. 2001).

Growth stages of grass species, culture media and fungal communities

Irrespective of their growth stages, most grass species harboured enhanced fungal communities during winter months followed by rainy season. Wilson & Carroll (1994) correlated greater colonization of plants with endophytes to winter and monsoon seasons. Waldrop & Firestone (2006) attributed seasonal shifts in microbial community structure to a shift in the carbon substrate availability following scanty above ground vegetation cover in oak woodland system. The increase in fungal communities in certain grasses during their flowering stages might reflect the changes in the rhizosphere deposits as suggested by Houlden et al. (2008).

PDA, as compared to the other media tested, was suitable for the isolation of a large number of fungal communities from the rhizosphere and rhizoplane of grasses. This medium has also been the most favored for the isolation and characterization of fungal species from plant tissues and soil system (Tejesvi et al. 2005, Oyeyiola 2009). This could be due to the availability of required carbon source and other biochemicals for excellent growth and development of most fungal species. On the other hand, CZA supported fewer fungal species. This might suggest the use of more than one culture media for fungal isolation. In the present study, morphological criteria were mostly depended on for fungal characterization, since more than 9000 fungal isolates were collected from five grass species. Certain NSF could not be identified solely on morphological criteria. Such fungi failing to sporulate on different culture media, need characterization by molecular techniques. There have been attempts in this direction (Read & Gregory 1997, Promputtha et al. 2005, Porras-Alfaro et al. 2008). Certain NSF isolated from zoysiagrass rhizosphere (Hyakumachi et al. 1993) was later characterized as a species of *Phoma* (Shivanna et al. 1996b).

Most species documented in the present study are anamorphic ascomycetes fungi. They were the most frequently isolated fungal communities followed by teleomorphic ascomycetes and other classes from the rhizosphere (Mandeel 2002, Jamiolkowska & Wagner 2005, Al-NurEl-Amin & Saadabi 2007). Among the most frequently occurring fungal species in the rhizosphere and rhizoplane of grasses, *Aspergillus flavus*, *Clonostachys rosea*, *Penicillium citrinum* and *Trichoderma harzianum* have also been frequently isolated from other species of grasses (Abdel-Hafez 1982, Al-NurEl-Amin & Saadabi 2007).

Fungal community structure in the rhizosphere and rhizoplane regions

The increase in rhizosphere and rhizoplane fungal incidence during some seasons might suggest the prevalence of host and season specificity of fungal communities. *Cynodon dactylon* and *Chloris barbata* always supported high fungal population. This is reflected by the species richness of the fungal community, diversity and evenness indices of Shannon and Simpson. *Dactyloctenium aegyptium* and *E. indica* appear to be the poor supporters of fungal communities in the rhizosphere and rhizoplane as indicated by low species richness and species diversity and evenness. This supported the findings of Houlden et al. (2008). On the other hand, certain fungal communities including NSF might show host-specific preferences. Krishnamurthy et al. (2008) made a similar observation of enhanced diversity of fungi in some medicinal plant species.

The species richness, diversity and evenness of fungal community could be influenced by the selective determination ability of the host root exudates. The root exudates are known to play a great role in the determination of fungal communities in rhizosphere and rhizoplane regions (Broeckling et al. 2008). Neumann & Romhild (2001) suggested that the shift in fungal community pattern in soil could be due to root morphology and exudation pattern during plant development. Host plants also contribute to the seasonal variation in fungal isolates and their frequency of colonization (Collado et al. 1999, Gao et al. 2005). Responses of fungal communities, in the present study, might largely depend on the season, as winter was most suitable for their expression in most of the grass species.

Jaccard similarity co-efficient indicated the association of fungal assemblages, which are almost similar in most grass species, suggesting that the same fungal community could occur in different seasons but in varying number of populations in the rhizosphere and rhizoplane. However, in the case of *E. unioloides* and *E. indica*, the variation in the occurrence of fungal communities is only marginal but the population of such fungal communities was very high. This suggests that certain grass species might support specific groups of fungal communities. The number of fungal communities in rhizosphere was high during winter, however, with respect to rainy season, the similarity of the fungal species was high. This might be due to the occurrence of similar groups of fungal communities in all the grass species during favorable conditions. Such a similarity could be limited during summer when the water potential is low affecting the nutrient availability in the soil. This could trigger the occurrence of communities tolerant to water stress during summer. Wang & Guo (2007) reported the high degree of similarity in the composition of fungal assemblages between two sites. On the other hand, Tejasvi et al. (2005) noted a very low similarity between two locations. These observations suggest that similarity of fungal assemblages might vary depending on the location or even season.

There were high incidences of the fungal species during the winter in most grass species. This kind of increase in the expected number of species with increase in the number of isolations also has been recorded in mangrove plants (Ananda & Sridhar 2002) and *Terminalia* species (Tejesvi et al. 2005). The rarefaction curves also indicated that some grasses like *C. dactylon* and *Chloris barbata* could provide shelter for a wide range of fungal species in the rhizosphere and rhizoplane regions. The increase in the expected number of species in the middle root region of most grasses that increased with increase in the number of isolations suggested that the middle region is the zone of active fungal colonization in all seasons. The localization of fungal communities in the root region might vary depending on the amount of exudate production, the microbial association and the soil type. Meera et al. (1994) reported maximum colonization of root base of cucumber seedlings, while Yang & Crowley (2000) noted that the zone of elongation behind the root tip supported the growth of primary root colonizers. The difference in carbon sources in nutritionally distinct sites along the root might result by the selective support of fungal communities in the root. The composition of the root exudates has been shown to differ depending on the plant species and the stage of plant development (Jaeger et al. 1999, Neumann & Romhild 2001).

The present study indicated that species of *Aspergillus*, *Clonostachys*, *Chaetomium*, *Penicillium* and *Trichoderma* dominated the rhizosphere and rhizoplane regions in high frequencies. The above species are cosmopolitan and have been tested for their biological control abilities (Whipps 2001, Berg et al. 2005). There are also reports of other fungal species that are not frequently isolated but were shown to possess biocontrol abilities (Meera et al. 1994, Shivanna et al. 1996a, Berg et al. 2005). Since most biocontrol agents have not yet yielded the required results, documentation of the knowledge of diversity and structure of fungal communities in the rhizosphere is necessary. The present study is an attempt to identify fungal communities in the larger interest of identifying novel biological control agents for use in future.

Results of the study indicated that wild perennial grasses harbour diverse groups of fungi in different seasons. The fungal community structure varied to some extent depending on the season. Among the fungal groups, anamorphic ascomycetous fungi topped the list with large number of fungal species. Diversity and evenness indices suggested that certain grasses harboured diverse and abundant fungal assemblages. Some of these fungal species are known as potential biological control agents. However, the occurrence of certain fungal species including NSF, only in certain grass species, is indicative of the existence of host species-specific fungal assemblages. These observations suggested that the distribution of fungal communities in the rhizosphere and rhizoplane of grasses vary temporally and spatially and hence there is scope for isolation of large number of distinct groups of fungi that could be exploited for their biological control potential. Experiment on the antagonistic, root

colonizing and saprophytic abilities of some of these isolates has yielded promising results (Vasanthakumari & Shivanna, Unpublished), which suggest the need to tap the potential of these fungal isolates for future use.

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