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Ecological Evaluation of Ascomycotina Species Associated with Anacardium occidentale in Coastal Sand Dunes of Orissa, India

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Abstract: Abundance of Ascomycotina was studied from soil in coastal sand dunes of Orissa for a period of two years covering three distinct seasons. A total of 16 species of fungi belonging to 9 genera were enumerated of which site without vegetation surface soil had a share of 8 genera and 10 sp. while sub surface soil produced 7 genera and 10 species. Site with *Anacardium* plantation; the surface soil contributed 5 genera and 7 species while sub surface soil contributed 5 genera and 9 species. The diversity index varies from 3.6 to 3.74 (Shannon) and 0.32 to 0.35 (Simpson). Similarity index shows that barren sand dune is less akin to sand dune with monoculture plantation of *Anacardium*. Despite poor fungal population and nutrient composition in site without vegetation in comparison to soil with Anacardium plantation, more species were recorded from barren coastal sand dunes, which may be due to less competition with other fungi.

Key words: Ascomycotina, coastal sand dune, diversity indices, sac fungi

INTRODUCTION

Defining the number of fungi on earth has always been a point of discussion and several studies have focused on enumerating the worlds fungal diversity (Crous, 2006). From the late 1940s there has been a growing interest in soil mycology and soil borne fungal diseases of plants and this too has motivated the studies on soil fungi and their ecology (Subramanian, 1986). There are over 1.5 million fungal species distributed widely throughout the globe (Hawksworth, 2004) and many of these fungi, particularly sac fungi, the members of Ascomycotina are the chief agents of cellulose decomposition; have great potential in the daily life of human beings besides their utilization in industry, agriculture, medicine, biotechnology, in recycling nutrients and decomposing the dead organic matter in soil and litter (Cowan, 2001; Gates, 2005; Manoharchary et al., 2005). Recent evidence suggests that out of 1.5 million fungi, about one third exist in India and of this only 50% are identified until now (Manoharchary et al., 2005; Swapna et al., 2008). Patent literature in this field indicates that most of the work was carried out in forest soils (Behera et al., 1991; Behera and Mukherji 1985; Mohanty and Panda 1994a; Nilima et al., 2007). Relatively few reports have been published in view of the role of beach plantation on abundance, composition and diversity of fungal flora particularly in coastal sand dunes (Panda et al., 2007, 2008, 2009; Manoharchary, 2008). It is especially true in case of Orissa coast with around

480 km long barren coastline filled with sand dunes only. Presently, monoculture plantations of *Casuarina equisetifolia* L. and *Anacardium occidentale* are created along coastline to check windblast and erosion of sand dunes. Although, it has solved the purpose to some extent, the effect of *Anacardium occidentale* on occurrence and distribution of sac fungi are yet to be studied. Hence a study was made with reference to sac fungi in coastal sand dunes of Orissa.

MATERIALS AND METHODS

The study site was in Kanishi block under Ganjam district of Southern Orissa (19°15'N and 84°50'E) having 60km of coastline along the Bay of Bengal at a height of 6-8m above MSL. The climate of the region is monsoonal with coastal characteristics. The atmosphere temperature ranges from 37°C in summer to 13°C in winter. The annual rainfall is about 130 cm. Some of the unproductive uplands and coastal sand dunes are extensively covered by Casuarina and Cashew (Anacardium occidentale) plants. Cashew plantation at the inner belt of study site covers an area of about 1500 ha extending 4-5 km with a width of 250-450 m, varying at places and a shelter belt cum wind break vegetation of Casuarina about 30-40 rows covering 15-20 m in the outer belt along the coast of the sea. Cashew plant has been preferred over many others because of its physiological adaptation to tolerate extreme drought conditions, good growth in nutritionally poor soils, extensive near surface lateral roots and dense

Table 1: Eda	phic factors an	d fungal p	opulation of stud	ly site						
Sites		Temp	Moisture	pН	Total organic	Total	C/N ratio	Phosphorous	Potassium	Fungal number
		(°C)	c on te nt (%)		carbon (%)	nitrogen (%)		(mg/100g)	(mg/100g)	x10*
Site without vegetation	Surface soil	34.1	.699	6.2	0.21	0.016	13.8	0.45	1.53	54
0	Sub-surface soil	32.3	1.56	6.1	0.131	0.01	12.7	0.74	0.99	40
Site with Anacardium	Surface soil	30.28	1.26	6.9	0.403	0.0246	17.2	0.2	1.7	66
plantation	Sub-surface soil	28.74	2.03	6.3	0.275	0.0195	14.97	0.31	1.1	45
Table 2: AN	OVA									
Sources	DF		SS		MSS	F-value		р	-value	
Varieties	3 n		1164		388	7.8		4.8 * 9	.8 **	23.7***
Season	2n		614		307	6.2		5.1* 1	0.9**	27.0***
Error	6n		298		49.7					
Total	11									

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*: p<0.05, **: p<0.01, ***: p<0.001

canopy due to broad leaf and horizontal growth. Two sites of about one hectare each were selected for the investigation. First one on the sea shore without any vegetation and the second along a coastal sandy bed with 6-8 yr old plantation of Anacardium occidentale without any undergrowth. The study was conducted for a period of two years from June 2002 to May 2004. Soil samples from surface and sub-surface (15 cm depth) were collected from two sites in sterilized test tubes by randomly sampling at monthly intervals. The samples were temporarily stored in an ice chest prior to isolation of microbes. The micro fungi were isolated by dilution (Waksman, 1927) and pour plate (Warcup, 1950) techniques using PDA medium. Fungi were studied after 3-7 days of incubation. Fungi were identified by adopting standard procedures (Barnett and Hunter, 1972; Ellis 1971, 1976; Gilman, 1966; Sarabhoy, 1983; Subramanian 1962, 1971). Physico-chemical properties of soils were estimated as per Jackson (1967).

Statistical analysis: The following indices of diversity were calculated based on species level identification (Krebs, 1989; Ludwig and Reynolds, 1988).

Shannon-Wiener index H = -
$$\sum_{i=0}^{s} Pi \ln Pi$$

where Pi is the proportion of the individual found in the *i*th species, *ln* denotes natural logarithm and H is the Shannon -Wiener index.

Simpson's index
$$D = \sum_{i=0}^{s} (Pi)^2$$

where, Pi is the proportion of the individual found in the i^{th} species and D Simpson's index

Evenness index (E) =
$$H/lnS$$

where, H is the Shannon -Wiener index of diversity, S total number of species and ln is the natural logarithm.

Jacquard's index
$$S_{ab} = S_{AB} / (S_A + S_B - S_{AB})$$

where, S_{AB} is the number of species shared by two locations (A and B), S_A the total number of species in location A and S_B the total number of species in location B. S_{ab} is the extent of similarity between the species in location A and B.

Richness index (Margalef, 1963) R = S-1/lnN

where, S is the total number of species and N is the sampling number.

RESULTS AND DISCUSSION

A comparative study on composition of soil status at two sites revealed that soils from site with Anacardium plantation had low temp, high moisture, better nutrient status and therefore harboured more fungi (Table 1). Micro-fungi of both soils showed a positive correlation with soil moisture and total organic carbon but were negatively correlated with soil temp. The qualitative and quantitative differences of genera and species at the two sites indicated that surface vegetation as well as nutrient composition influenced microfungal inhabitants of the soil (Buresh and Tian, 1997; Christensen, 1969; Corre, 1991; Lavelle and Spain, 2001; Manlay et al., 2000; Mohanty et al., 1991; Panda, 2009; Panda et al., 2009). Similar results have been obtained from the soils at lower depth in all sampling sites. The higher population associated with plantation site may be ascribed to the greater surface area available for microbial colonization. Fungal number of two sites differed significantly (t test 5.34< p 0.01). Anova clearly indicated significant seasonal difference between the samples of soil (Table 2).

A distinct pattern of fungal community structure was observed in all the samples during the study period. The percentage composition and rank abundances of different fungal species fluctuated (Table 3). The majority was from the genus *Aspergillus*; the next two in order of dominance were *Penicillium* and *Trichoderma*. Earlier reports have indicated that these genera appeared

	Soil fro	m site with	hout vegetati	on			Soil from site with Anacardium plantation						
	Surface			Subsurfa	ice		Surface	Surface			Subsurface		
Fungi	No. of colony	%	Rank	No. of colony	%	Rank	No. of colony	%	Rank	No. of colony	%	Rank	
Absidia butleri	14	1.99	21	15	2.27	19	45	5.32	4	38	5.12	4	
A. glauca	-	-		-	-	-	23	2.72	10	19	2.56	15	
A. spinosa	-	-		-	-	-	17	2.01	20	22	2.96	10	
Alternaria alternata	13	1.85	22	-	-	-	-	-		-	-		
Aspergillus awamori	56	7.98	1	47	7.11	1	57	6.74	1	48	6.47	1	
A. flavus	24	3.2	8	21	3.18	13	24	2.84	9	18	2.43	16	
A. flavus fonsecoeus	-	-	-	-	-	-	21	2.48	11	-	-	-	
A. fumigatus	28	3.99	6	27	4.08	7	25	2.96	8	21	2.83	12	
A. luchuens is	18	2.56	14	21	3.17	14	18	2.13	16	13	1.75	24	
A. niger	43	6.12	2	42	6.35	2	49	5.79	3	46	6.2	2	
A. terreus	19	2.71	13	25	3.78	8	16	1.89	21	16	2.16	18	
Chaetomium homopilatum	22	3.13	10	24	3.63	9	14	1.66	25	14	1.89	22	
C. murorum	-	-	-	14	2.12	20	13	1.54	27	-	-	-	
Cladosporium cladosporoides	18	2.56	15	28	4.24	5	20	2.36	12	26	3.5	9	
C. oxysporum	15	2.14	19	18	2.72	16	16	1.89	22	30	4.0	8	
Curvularia eragrostidis	27	3.85	7	23	3.48	10	-	-	-	16	2.16	19	
C. lun ata	17	2.42	16	12	1.82	21	15	1.77	23	15	2.02	20	
C. pallescens	12	1.71	23	18	2.72	17	_	-	_	14	1.89	23	
Drechslera australiensis	16	2.28	17	13	1.97	23	-	-	-	12	1.62	26	
Fusarium sp.	16	2.28	18	17	2.57	18	20	2.36	13	18	2.43	17	
Mucor sp.	-	-	-	-	-	-	13	1.66	26	-	-	-	
Penicillium citrinum	30	4.27	4	28	4.24	6	44	5.2	5	33	4.45	5	
P. cvaneum	-	-	-	-	-	-	-	-	-	15	2.02	21	
P. javanicum	39	5.56	3	39	5.9	3	32	3.78	7	32	4.3	6	
P. minio-leuteum	22	3.13	11	23	3.48	11	19	2.25	15	21	2.8	13	
P. nigricans	11	1.57	24	12	1.82	22	18	2.13	17	13	1.75	25	
P. oxalicum	20	2.85	12	19	2.87	15	15	1.77	24	22	2.96	11	
P. rubrum	15	2.14	20	11	1.66	24	18	2.13	18	20	2.7	14	
P. rugulosum	-	-	-	-	-	-	19	2.25	14	-	-	-	
P. verruculosum	30	4.27	5	29	4.39	4	52	6.15	2	44	5.93	3	
Rhizopus nigricans	-	-	-	-	-	-	18	2.13	19	12	1.62	27	
Trichoderma viride	23	3.28	9	22	3.33	12	44	5.2	6	32	4.3	7	
Table 4: Total count of fungi	isolated d	uring the s	tudy period										
Sites	To	tal genera		Ascomyco	tinagenera	a	Total s	pecies		Ascom	ycotina sp	ecies	
Site without vegetation													
Surface soil	51			8			112	112			10		
Sub Surface soil	37			7			87				10		
Site with Anacardium plantation	on												
Surface soil	45			5			114				7		
Sub surface soil	41			5			93				9		

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Table 3: Percentage contribution and ranks of some dominant fungi isolated from samples at study sites

abundantly in soils (Mohanty and Panda, 1994b; Panda *et al.*, 2007; Rai and Kumar, 1988). This may be due to the faster growth rate of these fungi in addition to their better intrinsic prolific sporulating capacity to utilize the substrate. Considering the dominant species it is clear that fungal succession in plantation site greatly differed from without plantation.

The surface mycoflora was richer in comparison to sub-surface mycoflora; some species, which were constantly recorded from surface layers were never detected in sub-surface layers (Table 3). Similar observations have been made by Mathur and Mukherji (1985). Moreover, the similarity in species composition between the surface layers was found to be more akin than the subsurface soil. The species composition in soil showed marked differences with a change in habitat and surface vegetation (Table 4). A total of 177 species of fungi belonging to 71 genera were enumerated (Table 5). Species of Deutoromycotina were maximum followed by Zygomycotina and Ascomycotina. Their occurrence might be due to ability of the concerned group of fungi for survival in adverse condition and adjustment with the environment. Fifty-two fungal species were detected common to site without vegetation or with *Anacardium* plantation. Soil without vegetation revealed 26 restricted species in surface and 13 species in subsurface. *Anacardium* plantation soil revealed 19 in the surface and 12 in the subsurface.

Total number of genera and species of Ascomycotina isolated from soils (Table 6) during the present study indicated that they never occur in higher numbers. It is noted that except a few genera, most of the Ascomycotina are not restricted to one or neither the other samples nor they are common to all. This corroborates to the findings of Behera and Mukherji (1985). Their isolation seems to be dependant more on final growth and formation of mature colony in the culture plate than the technique employed for the purpose. During present study it was observed that more species of Ascomycotina were recorded in virgin sand dunes at the first site without any vegetation than in the other site with vegetation. It may be due to low competition with other categories of fungi,

Table 5	: List of fungi isolated from th	e study :	sites								
		Soil fro	om	Soil from	site			Soil from		Soil from	site
		site wit	h	with out				site with		without	
		vegeta	tion	vegetation	1			vegetatio	n	vegetation	1
SL No	Fungi	A 1	A 2	A 1	A 2	SL No	Fungi	A 1	А2	A 1	Α2
1	Absidia butleri	+	0	+	+	89	Melanospora zamiae	0	+	+	+
2	A. glauca	+	0	+	-	90	Meria coniospora	0	+	-	-
3	A. spinosa	+	0	+	+	91	Monodictys levis	-	-	-	+
4	Alisidium resinae	-	0	-	-	92	Mucor hiemalis	0	+	+	+
5	Alternaria alternata	+	0	+	+	93	<i>M</i> . sp.	0	-	+	-
6	A. longipes	+	-	+	-	94	Myrothecium roridum	-	-	+	-
7	A. padwickii	+	-	-	-	95	Neopeckia fulcita	0	-	+	+
8	A. tenuisima Argabrio tus tarrastris	-	-	+	-	96	Nigrospora oryzae	0	-	+	-
9	Arachnioius ierresiris Aspergillus awamori	-	-	+	+	97	N.spnaerica Oidiodendron maeius	0	+	- -	-
11	A caepitosus	-	-	+	_	99	O rhodogenum	0	+	_	-
12	A. candidus	0	+	+	+	100	Paecilomyces fusisporus	0	+	+	+
13	A. carbonarius	0	+	+	+	101	P. varioti	0	+	-	+
14	A. flavipes	0	-	+	-	102	Penicillium atromerotosom	-	-	-	+
15	A. flavus	0	+	+	+	103	P. adametezi	0	-	-	-
16	A. foncecaceous	0	+	+	-	104	P. brefaldianum	0	+	+	+
17	A. fumigatus	0	+	+	+	105	P. canadens	0	-	-	-
18	A. funiculosus	0	+	+	-	106	P. charlesi	-	-	+	-
19	A. humicola	-	+	-	-	107	P. chermesinum	0	-	+	+
20	A. luchuens is	0	+	+	+	108	P. chrysogenum	0	-	+	-
21	A. niger		0	+	+	+	109 P. citrinum	0	0	+	++
22	A. niveus	-	+	+	-	110	P. citroviriae	0	-	-	+
23	A restrictum	-		-	+	112	P cyaneum	0	+	+	+
25	A. repens	-	+	-	-	112	P. decumdens	0	+	+	+
26	A. rugulosum	-	-	+		114	P. diversum	0	+	-	-
27	A. sulphureus	-	+	-	+	115	P. ehrlichii	0	-	-	-
28	A. sydowi	0	-	-	-	116	P. fellutatum	0	-	+	-
29	A. terrestre	-	-	+	-	117	P. funiculosum	+	-	-	-
30	A. terreus	+	+	+	+	118	P. glabrum	+	-	-	-
31	A. terricola	+	-	+	-	119	P. harvei	-	-	-	+
32	Asteromella sp.	-	+	-	-	120	P. implicatum	+	-	-	-
33	Aurobasidium sp.	-	-	+	-	121	P. islandicum	-	+	+	-
34	Beltrania rhombica	-	-	+	-	122	P. janthinelum	+	-	+	+
35	Bispora butulina	-	+	-	-	123	P. javanicum	0	+	+	+
30	B. punciala Potrutitis sinora	-	-	- _	+	124	P. lanosum P. lautaum	+	+	÷	+
38	Botryosphaeria sp	-		+		125	P lepidosum	_	-	-	+
39	Candida albicans	-	-	+	-	127	P. levitum	-	+	-	-
40	Catinula sp.	0	+	+	+	128	P. minio-luteum	0	+	+	+
41	Cephalosporium acremonium	0	+	+	-	129	P. minutissima	+	-	-	-
42	Cheatomium fimeti	-	+	-	+	130	P. nigricans	0	+	+	+
43	C. homopilatum	0	+	+	+	131	P. oxalicum	+	+	0	+
44	C. magnum	-	+	-	+	132	P. purpurogenum	+	+	0	+
45	C. murorum	0	-	-	+	133	P. resticulosum	-	-	+	-
46	C. olivacium	-	+	-	-	134	P. restrictii	-	+	-	+
47	C. trilatera te	1	-	+	-	135	P. roseo-purpureum	-	-	+	+
48	C. sp.	0	-	+	-	136	P. rubrum	+	+	+	-
49	Choanephora cucurbitarum	0	+	+	+	13/	P. rugulosum	0	+	+	+
50	Chrysosporium tropicum	U		-	-	130	1. spinuosum P. turbatum	-+	-	-	T
52	Cladosporium chloro	-	-	-	+	140	P variable	+	+	-	+
53	C cladosporoides	0	+	+	+	140	P verruculosum	+	+	+	+
54	C. herbarum	-	-	_	+	142	P. waksmani	+	+	+	_
55	C. oxvsporum	0	+	+	+	143	Periconia cambrensis	+	_	_	-
56	C. spero	0	-	-	-	144	P. cooki	+	-	-	-
57	Coniothyrium	-	-	+	+	145	P. hypsidula	-	-	+	-
58	Cunninghamella echinulata	0	+	-	-	146	P. glycericola	+	-	-	
59	Curvularia brachyspora	0	+	+	+	147	P. minutissima	+	-	-	-
60	C. clavata	-	-	+	-	148	P. saraswatipurensis	+	+	+	+
61	C. eragrostidis	0	+	+	+	149	<i>P</i> . sp.	-	+	-	-
62	C. lunata	0	+	+	+	150	Pestalotia sp.	+	+	+	+
63	C. lunata aeria	-	-	+	-	151	Phaeotrichonis crotolarie	-	-	+	-
64 65	C. pallescens	0	+	+	+	152	rnoma sp.	+	+	+	+
65 66	Cytosporma sp.	U	+	+	+	153	ritnomyces sacchari Pyanidial form	-	-	+	-
67	Denaropnoma sp.	-	-	+	-	154	r yeniaiai ioim Pyrenochaeta cajani	+	-	-	- -
68	Diploding spicium	0	_	+	+	155	Rhinocladiella sp	_	-	_	+
69	Drechslera avenaceae	0	-	-	-	157	Rhizonus conhii	+	-	-	-
70	D. australiensis	0	+	+	+	158	R. nigricans	+	+	+	+
71	D. cohliobolus heterosporum	-	-	+	-	159	R. oryzae	+	+	+	-
72	D. hawa iensis	0	+	+	+	160	Scolicobasidium constrictum	+	+	+	-

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Table	5: Continued										
73	Ellisiopsis	0				161	Sepedonium	+	+	-	-
74	Em ericilops is hum icola	0	+	+	+	162	Spegazzinia tessarthii	-	-	+	-
75	E. terricola	-	+	-	-	163	Syncephalastrum recemosum	+	+	+	+
76	Epicoccum andropogonis	0	-	+	-	164	Tetraploa aristata	-	-	-	+
77	Eurotium repens	-	+	-	+	165	Thamnidium elegans	+	-	-	+
78	Fusarium liseola	0	-	-	-	166	Theilavia terricola	+	+	+	+
79	F. moniliforme	-	-	+	+	167	Torula calligans	+	+	+	+
80	F. hypocastani	-	+	+	-	168	Trichocladium canadense	+	-	+	-
81	F. oxysporum	0	+	+	+	169	Trichoderma album	+	+	+	+
82	F. solani	0	-	+	+	170	T. glabrum	+	+	-	-
83	<i>F</i> . sp.	0	-	+	+	171	T. koningi	+	+	+	+
84	Gelanispora clereatis	-	-	+	-	172	T. viride	+	+	+	+
85	Gleosporium norvisium	-	-	+	-	173	Trichurus spiralis	+	-	-	-
86	Gilmaniella humicola	0	-	-	-	174	Verticillium glaucum	+	-	-	-
87	Haplosporangium parvum	0	+	+	-	175	Black sterile	-	-	+	-
88	Isaria pulcherima	-	+	-	-	176	Grey sterile	+	+	+	-
						177	White sterile	0	+	+	+

(+) =Presence, (-) =Absence

Table 6: Ascogenous fungi isolated from different sites

	Soil from site with Anac	cardium plantation	Soil from site without vegetation			
Ascogenous fungi	Surface	Subsurface	Surface	Subsurface		
Arachniotus terrestris	-	-	+	+		
Botryosphaeria species	-	-	+	-		
Che atom ium fimeti	-	+	-	+		
C. homopilatum	0	+	+	+		
C. magnum	-	+	-	+		
C. murorum	0	-	-	+		
C. olivacium	-	+	-	-		
C. trilatera te	-	-	+	-		
C. sp.	0	-	+	-		
Em ericilops is hum icola	0	+	+	+		
E.terricola	-	+	-	-		
Eurotium repens	-	+	-	+		
Melanospora zamiae	0	+	+	+		
Neopeckia fulcita	0	-	+	+		
Theilavia terricola	+	+	+	+		
Gelaniospora sp.	-	-	+	-		
(1) B (1) 11						

(+) =Presence, (-) =Absence

Table 8. Dominance, diversity, evenness and richness indices of fungi in different samples at study sites.

Sites	Samples	D	1-D	Н	E	R
Site without vegetation	Surface soil	0.0323	0.968	3.718	0.881	21.08
	Sub surface soil	0.035	0.965	3.528	0.921	14.16
Site with Anacardium plantation	Surface soil	0.032	0.968	3.744	0.869	23.28
	Sub surface soil	0.0333	0.967	3.608	0.904	16.68

Т

D = Simpson dominance index, H = Shannon diversity index, E = Evenness, R = Richness

which are less abundant in this soil compared to monoculture plantation of *A. occidentale*. More over, less number of Ascomycotina were recorded in the present study despite reports on large number of species in tropical forest soils (Mohanty and Panda, 1994b; Behera and Mukherji, 1985).

The β diversity (Jacquard's index) value indicates changes in species composition from one location to another (Table 7). It is similar to the findings of Wilson and Shimda (1984) and Aparajita (2007). Shannon's diversity index is reasonably high varying from 3.608 to 3.744 correspondingly the dominance values are low varying from 0.032 to 0.035 (Table 8). The D value and H value in both the soil indicates many species with maximum diversity. The evenness index varies from 0.869 to 0.921 an indication that species were fairly evenly distributed. The surface layers have the highest species richness where as subsurface shows lowest richness (Table 8). It has also been observed that a change in the habitat variable can effect species composition and

able 7: 0	Comparison	of different :	samples by	coefficients	of comparison	

Samples	A ₁	A ₂	B ₁	В,
A	1.0	0.54	0.55	0.46
A ₂		1.0	0.47	0.5
B ₁			1.0	0.44
D				1.0

 $A_1 = Inside$ plantation surface, $A_2 = Inside$ plantation sub-surface, $B_1 = Outside$ plantation surface, $B_2 = Outside$ plantation sub-surface,

diversity. Thus vegetation is a major factor controlling the distribution and diversity of fungal species and subsequently deforestation can lead to loss of biological diversity as a whole.

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