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An ecological study of soil mycoflora in coastal sand dunes of Orissa covered with cashewnut plantation

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Seasonal dynamics of soil microfungal populations were studied in a monoculture plantation of *Anacardium occidentale* L. in coastal sandy belt of Orissa for a period of two years. Maximum population density of mycoflora was observed in the rainy season followed by winter and summer. Higher microbial populations were encountered in plantation soil than the barren sand. They corresponded to the fluctuation of prevailing temperature, moisture and total organic carbon content of the said habit. The C/N ratio was inversely proportional to the fungal numbers. A total of 177 species of fungi belonging to 71 genera were ennumerated. The majority were from the genus *Aspergillus* ; the next two in order of dominance were *Penicillium* and *Trichoderma*. The diversity index varied from 3.6 to 3.74 (Shannon) and 0.32 to 0.35 (Simpson). Similarity index showed that barren sand dune was less akin to sand dune with monoculture plantation of *Anacardium*. Introduction of predominant decomposing microorganisms isolated from the present study can help to increase the nutrient status of the sandy sterile mass under focus.

Key words: Coastal sand dune, diversity indices, fungi, monoculture, succession

INTRODUCTION

Coastal ecosystem has long been a natural resource of mankind by virtue of its utility, in terms of economic, aesthetic and ecological aspects. This ecosystem is considered as one of the most unproductive and sterile ecosystem of the world, where the rate of primary productivity is quite low; but it supports numerous diversified soil organisms. All these organisms, especially microbial flora play an important role in the degradation of foliage of beach plantation which is continuously shed and decomposed in the coastal substratum and forms the major source of energy and nutrients on which horizon of consumers depend. Besides decomposition, the variety and galaxy of fungi also perform unique and indispensable activity in industry, agriculture, medicine, biogeochemical cycles (Cowan, 2001; Gates *et al.*, 2005; Manoharchary *et al.*, 2005) and many other ways on which human depends. India has a rich diversity of fungi and forms an important geographical region for fungal distribution (Subramanian, 1962). It is especially true in Orissa coast of about 480 km of stretched coast line filled with sand dunes only. Some years ago, phase wise monoculture plantations of *Casuarina equisetifolia* and *Anacardium occidentale* are created along the coastline to minimize the effect of cyclonic sea stroms, a regular phenomenon due to its geographical location.

The efficiency and potentially of microbes in decomposition depend upon their abundance and composition. It is well known that the prevailing climate and above ground vegetation influence the quality and quantity of these microbes in a particular soil (Behera *et al.*, 1991; Mohanty and Panda, 1994a). General ideas about species diversity suggest that habitat heterogeneity and prevailing climate are major factors controlling the quality and quantity of microbes in a particular soil (Gentry 1988; Behera *et al.*, 1991; Mohanty and Panda, 1994a). Though numerous species of fungi have been reported from forest soils (Mohanty *et al.*, 1991; Mohanty and Panda 1994 a ; Nilima *et al.*, 2007;) and some foliar and soil fungi are reported from sand dunes of Orissa (Panda *et al.*, 1996;

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Mohanty and Panda, 1998; Panda, 2009) and Andhra Pradesh (Monoharachary *et al.,* 2008) associated with *Casuarina equisetifolia* but there appears to be no study in sand dune stabilized with monoculture plantation of *Anacardium occidentale.* Though, the purpose of cylonic sea stroms has solved to some extent, the effect of this plantation on occurence and distribution are yet to be studied. Hence a study has been made to find out the occurence, distribution, dominance and variation of soil fungi in the noted belt and the factors influencing their ecophysiology. The purpose of this study is to identify and culture the dominant decomposers of the region, which can be introduced successfully in coastal sandy belt to enhance the nutrient status of the soil.

MATERIALS AND METHODS

The study site was in Ganjam district of Orissa (19°15'N and 84°50'E) having 60 km of coastline along the Bay of Bengal at a height of 6-8 m above MSL. The climate of the region is monsoonal with coastal characteristics. The air temperature ranges from 37°C in summer to 13°C in winter. The annual rainfall is about 130 cm. The region is subject to cyclones during the wet season and coastal areas are affected by the resulting strong winds and intense rainfall. 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 study site covers an area of about 1500 hectares extending 4-5 km in length 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 canopy due to broad leaf and horizonal growth. Two sites of about one hectare each were selected for the investigation. First one (soil B) is a big patch of sand dune situated adjacent to *Anacardium* plantation comprising few grasses only and the second (soil A) 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. First series of soil samples were collected from two sites in sterilized test tubes by randomly sampling at monthly intervals by inserting sterile glass tubes at depths of 0.3 cm (surface), and 8-15 cm (sub-surface) for microbial analysis. Each tube was allowed to contain 10-15 g of soil and stoppered tightly. Second series of samples was collected for estimation of physico-chemical properties. Samples were brought to the laboratory in sterilized polythene packets along with identification tags in sealed condition. The samples were temporarily stored in an ice chest prior to isolation of microbes. The microfungi 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 (Gilman,1966;Ellis,1971;Subramanium, 1971; Barnett and Hunter, 1972; Ellis, 1976; Sarbhoy, 1983). 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 (Ludwig and Reynolds, 1988; Krebs, 1989). Shannon – Wiener index – $H = \sum_{i=0}^{s} PilnPi$; where P is the proportion of the invidual found in the ith 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/*ln*S; 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 similarly between the species in locations A and B; Richness index (Margalef, 1963) R = S-1/*ln* N,where S is the total number of species and N is the sampling number.

RESULTS

Major differences were reported between the two soils under this study. While having a comparable fungal growth profile to that of the nutrient it revealed that sites with low temperature, high moisture and better nutrient status harbored more fungi and bacteria (Table 1).

Table 1: Characteristics and fungal number (cfu/g) in soil with *Anacardium* plantation (average of two years)

Months	Temp (°C)	Moisture content (%)	Total organic Carbon (%)	Total Nitrogen (%)	C/N Ratio	pH	Phosphorous (mg/100 g)	Potassium (mg/100 g)	Fungal number x 10^{-4}
Jun.	32.6	1.16	0.423	0.034	15.48	6.9	0.24	1.41	50
	31.8	1.33	0.354	0.028	13.61	6.6	0.34	1.11	36
Jul.	30.1	1.55	0.44	0.034	12.94	6.9	0.26	1.39	48
	29.5	2.26	0.337	0.029	11.62	6.3	0.34	0.96	47
Aug.	30	2.18	0.553	0.033	16.75	6.7	0.21	1.68	81
	28.8	2.97	0.337	0.028	12.0	6.2	0.38	1.23	53
Sept.	29.3	3.35	0.523	0.034	15.38	7.1	0.16	1.78	98
	26.8	4.12	0.317	0.028	11.32	6.6	0.29	1.26	66
Oct.	28.5	2.2	0.473	0.03	15.7	7.3	0.14	1.89	77
	26.4	3.7	0.333	0.021	15.85	6.8	0.22	1.29	59
Nov.	27	0.85	0.333	0.022	13.5	7.4	0.13	1.88	72
	26.3	3.1	0.242	0.02	12.1	6.8	0.22	0.78	44
Dec.	25.9	0.54	0.285	0.019	14.8	7.0	0.13	1.44	64
	24.8	1.16	0.218	0.016	13.62	6.4	0.17	0.75	38
Jan.	26.2	0.46	0.426	0.021	20.28	6.5	0.09	1.67	71
	25.2	1.15	0.234	0.014	16.71	6.0	0.19	0.98	42
Feb.	28.3	0.54	0.382	0.02	19.25	6.7	0.15	1.98	63
	27	0.99	0.242	0.015	16.13	5.9	0.28	1.45	48
Mar.	31.5	1.18	0.36	0.018	20	6.4	0.28	1.79	52
	30.3	1.67	0.239	0.015	15.9	6.0	0.37	1.17	42
Apr.	35.2	0.63	0.34	0.016	21.6	6.8	0.25	1.73	48
	33.6	1.29	0.239	0.012	19.9	6.2	0.42	0.98	35
May	38.7	0.44	0.292	0.014	20.8	7.0	0.33	1.72	42
	34.4	0.71	0.205	0.0098	20.9	6.3	0.54	1.12	29
Correlation	r=0.643*	r=+0.767	r=+0.725	r=+0.62*	r=0.52*	r=+0.299	r=0.625*	r=+0.197	
	r=0.556*	r=+0.863	r=+0.56*	r=+0.63*	r=0.59*	r=0.289	r=0.389	r=+0.484	

Upper line = Surface soil, lower line = Subsurface soil *P<0.05, **P<0.01,***P<0.001

On the other hand fungal population in soil A was poor and there was substantial drop in nutrient composition (Table 2). The poor colonization of fungi on soil A may be due to low moisture and high temperature which served as an important defense against fungal attacks and / or colonization.Fungal population in both the sites and both the layers had positive correlation with soil moisture, total nitrogen and total organic carbon, while the temperature was negatively correlated (Tables 1 and 2). The seasonal variations seem to influence the density of an individual fungus and population as a whole. The rainy period carried higher population followed by winter and summer. Higher moisture content and temperature of sand corresponding to the rains and summer might be the reason for such fluctuation.

The species composition at two sites showed marked differences with change in habit and surface vegetation. A total of 177 species of fungi belonging to 71 genera were enumerated of which surface soil A had a share of 846 colonies, 45 genera and 114 spp. while sub surface soil produced 742 colonies, 41 genera and 93 species. Soil B; the surface soil contributed 702 colonies, 51 genera and 112 species while sub surface soil produced 661 colonies, 37 genera and 87 species. The medium in our study favoured Dueteromycotina the most, followed by Ascomycotina and Zygomycotina (Table 3).The order of occurence might be due to ability of the fungi for survival of adversity and adjustment with the environment. A distinct pattern of fungal community structure was observed in all

Table 2: Characteristics and fungal number (cfu/g) in soil without plantation average of two years

Months	Temp (°C)	Moisture content	Total organic Carbon (%)	Total Nitrogen (%)	C/N Ratio (%)	pH	Phosphorous (mg/100 g)	Potassium (mg/100 g)	Fungal number x 10^{-4}
Jun.	34.9	0.71	0.152	0.018	11.8	6.4	0.375	1.19	43
	33.6	1.5	0.115	0.0104	11.1	6.2	0.58	0.74	31
Jul.	33.8	1.04	0.174	0.017	10.2	6.0	0.365	1.27	62
	32.7	2.12	0.135	0.0105	12.9	5.7	0.58	1.02	41
Aug.	33.3	1.39	0.205	0.015	13.7	6.1	0.38	1.63	70
	32.0	2.8	0.169	0.013	12.9	6.0	0.71	1.12	44
Sept.	32.6	1.8	0.362	0.024	15.1	6.3	0.49	1.63	72
	31.3	3.39	0.183	0.016	11.9	6.2	0.77	1.23	57
Oct.	32.3	1.06	0.284	0.02	14.2	6.7	0.44	1.73	74
	31.0	3.39	0.15	0.013	12.0	6.6	0.77	0.83	62
Nov.	32.3	0.58	0.221	0.016	13.8	6.7	0.495	1.48	58
	30.2	1.52	0.12	0.011	11.3	6.6	0.63	0.77	37
Dec.	31.7	0.38	0.208	0.014	14.9	6.0	0.355	1.48	50
	29.5	0.895	0.113	0.0085	13.5	6.2	0.78	0.76	33
Jan.	32.0	0.45	0.164	0.013	12.6	5.9	0.22	1.53	52
	29.2	0.615	0.121	0.01	12.1	6.0	0.58	1.13	40
Feb.	32.7	0.21	0.223	0.015	14.9	6.1	0.37	1.49	55
	30.8	0.95	0.139	0.01	13.9	6.0	0.96	1.13	54
Mar.	35.4	0.42	0.216	0.016	13.5	6.2	0.46	1.65	43
	34.6	0.69	0.129	0.01	12.9	6.0	0.91	0.99	36
Apr.	38.1	0.18	0.165	0.0098	16.8	6.2	0.635	1.7	38
	35.4	0.43	0.094	0.0065	14.4	6.0	0.98	0.89	25
May	40.3	0.17	0.142	0.01	14.2	6.1	0.805	1.58	32
	37.8	0.36	0.112	0.0079	14.2	6.1	0.67	1.21	20
Correlation	r=0.739*	r=+0.822***	r=+0.727**	r=+0.734**	r=-0.127	r=+0.31	r=0.458	r=0.157	
	r=0.606*	r=+0.758**	r=+0.794**	r=+0.79**	r=0.411	r=+0.28	r=0.127	r=+0.185	

Upper line = Surface soil, lower line = Subsurface soil *p<0.05,** p<0.01,*** p<0.001

Table 3: Special group distribution of fungus sp. in the study sites (by both methods)

	Site without vegetation				Site with *Anacardium* plantation			
	Surface		Subsurface		Surface		Subsurface	
Name of the groups	Number of Genera	Number of species	Number of Genera	Number of species	Number of Genera	Number of species	Number of Genera	Number (mg/100 g) species
Zygomycotina	5	9	5	6	7	12	6	9
Ascomycotina	8	10	7	10	5	7	5	9
Deuteromycotina	38	93	25	71	33	95	30	75
Moniliales	25	80	17	63	23	85	22	67
Sphaeropsidales	7	7	6	6	6	6	5	5
Melanconiales	3	3	1	1	2	2	1	1
Mycelia sterilia	3	3	1	1	2	2	2	2
Total	51	112	37	87	45	114	41	93

the samples during the study period. The percentage composition and rank abundances of different fungal species fluctuated (Table 4). The majority was from the genus *Aspergillus*; the next two in order of dominance were *Penicillium* and *Trichoderma*. Considering the dominant species it was clear that fungal succession in plantation site greatly differed from without plantation. Moreover, the similarity in species composition between the two sites was found to be very low (Table 5). The

occurrence of good number of sugar fungi in plantation site may be accounted for their greater affinity toward the simple carbohydrates. Fungal number of two sites differed significantly (t test 5.43 < P 0.01). Anova clearly indicated significant seasonal difference between the samples of soil (Table 6). Shannon's diversity index was reasonably high varying from 3.608 to 3.744 correspondingly the dominance values were low varying from 0.032 to 0.035 (Table 7). The D value and H value in both the soil indicated many species with maximum diversity. The evenness index varied from 0.869 to 0.921 an indicates that species were fairly evenly distributed. The surface layers had the highest species richness where as subsurface showed lowest richness (Table7).

Table 4: Percentage contribution and ranks of some dominant fungi isolated from samples at study sites

	Soil from site without vegetation						Soil from site with *anacardium* plantation					
	Surface			Subsurface			Surface			Subsurface		
Fungi	No. of colony	%	Rank	No. of colony	%	Rank	No. of colony	%	Rank	No. of colony	%	Rank
Absidia butleri Lendner	14	1.99	21	15	2.27	19	45	5.32	4	38	5.12	4
A. glauca Hagem	-	-	-	-	-	-	23	2.72	10	19	2.56	15
A. spinosa Lender	-	-	-	-	-	-	17	2.01	20	22	2.96	10
Alternaria alternata Kessler	13	1.85	22	-	-	-	-	-		-	-	
Aspergillus awamori Nakazawa	56	7.98	1	47	7.11	1	57	6.74	1	48	6.47	1
A. flavus Link	24	3.2	8	21	3.18	13	24	2.84	9	18	2.43	16
A. fonsecaceus Tnom and Raper	-	-	-	-	-	-	21	2.48	11	-	-	-
A. fumigatus Fres	28	3.99	6	27	4.08	7	25	2.96	8	21	2.83	12
A. luchuensis Inuy	18	2.56	14	21	3.17	14	18	2.13	16	13	1.75	24
A. niger Van Teigh	43	6.12	2	42	6.35	2	49	5.79	3	46	6.2	2
A. terreus Thom	19	2.71	13	25	3.78	8	16	1.89	21	16	2.16	18
Chaetomium homopilatum Ames	22	3.13	10	24	3.63	9	14	1.66	25	14	1.89	22
C.murorum Corda	-	-	-	14	2.12	20	13	1.54	27	-	-	-
Cladosporium cladosporoides De Vries	18	2.56	15	28	4.24	5	20	2.36	12	26	3.5	9
C.oxysporum Berkland and Curt	15	2.14	19	18	2.72	16	16	1.89	22	30	4.0	8
Curvularia eragrostidis Meyer	27	3.85	7	23	3.48	10	-	-	-	16	2.16	19
C. lunata Boedijn	17	2.42	16	12	1.82	21	15	1.77	23	15	2.02	20
C.pallescens Boedijn	12	1.71	23	18	2.72	17	-	-	-	14	1.89	23
Drechslera australiensis Subram and Jain	16	2.28	17	13	1.97	23	-	-	-	12	1.62	26
Fusarium oxysporum Sehlecht	16	2.28	18	17	2.57	18	20	2.36	13	18	2.43	17
Mucor hiemalis Wehmer	-	-	-	-	-	-	13	1.66	26	-	-	-
Penicillium citrinum Thom	30	4.27	4	28	4.24	6	44	5.2	5	33	4.45	5
P.cyaneum Biourge	-	-	-	-	-	-	-	-	-	15	2.02	21
P. javanicum Van Beyma	39	5.56	3	39	5.9	3	32	3.78	7	32	4.3	6
P.minio-leuteum Dierckx	22	3.13	11	23	3.48	11	19	2.25	15	21	2.8	13
P.nigricans Thom	11	1.57	24	12	1.82	22	18	2.13	17	13	1.75	25
P. verruculosum Peyrowl	30	4.27	5	29	4.39	4	52	6.15	2	44	5.93	3
Rhizopus nigricans Jensen	-	-	-	-	-	-	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 5. Comparison of different samples by coefficients of comparison

Samples	A_1	A_2	B_1	B_2
A_1	1.0	0.54	0.55	0.46
A_2		1.0	0.47	0.5
B_1			1.0	0.44
B_2				1.0

A_1=Inside plantation surface A_2=Inside plantation sub-surface
B_1=Outside planatation surface B_2=Outside plantation sub-surface

Table 6. ANOVA

Sources	DF	SS	MSS	F value		p Value	
Varieties	3n	1164	388	7.8	4.8*	9.8**	23.7***
Season	2n	614	307	6.2	5.1*	10.9**	27.0***
Error	6n	298	49.7				
Total	11						

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

Table 7. Dominance, diversity, evenness and richness indices of fungi in different samples at study sites

Sites	Samples	D	1-D	H	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

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

DISCUSSION

Sandy soils have diverse physical, chemical and biological constraints : poor structural stability, poor nutrient holding capacity and low microbial community (Peiri, 1992; Parotta, 1999; Szott *et al.*,1999; Sall *et al.*, 2003). In these soils organic matter is the main determinant of fertility, nutrient storage and microbial activities. It is the main source of ecosystem energy and plays a major role on soil plant relationship; especially in sand dunes (Buresh and Tian, 1997;Feller and Beare, 1997; Lavelle, 1997; Lavelle and spain, 2001; Diallo *et al., 2005). The colonization of fungi in diversified* habitats is of much significance as their secondary metabolites are of much relevance to the human welfare. The qualitative and quantitative differences of microbial populations at two sites indicate that surface vegetation as well as nutrient composition influences microfungal populations of the soil. Plantations are often linked to a strong increase in the total soil organic matter content and soil microorganisms of the sand dunes (Corre, 1991; Panda *et al.*, 1996; Buresh and Tian, 1997; Manley *et al.*, 2000; Monoharachary *et al.*, 2008).Thus in this ecological context, stability of edaphic factors is one of the important factors governing the activity and diversity of fungi. Mycoflora differ in its composition from an ecological niche to the others as have been reported by Mohanty and Panda (1994a)., Panda *et al.* (1996) and Monoharachary *et al.* (2008). The correlation between the fungal population and soil moisture and temperature was reported by Behera *et al.* (1991), and Panda *et al.* (2007). The pH and other factors proved insignificant. The C/N ratio was inversely proportional to the fungal numbers. The results are in general agreement with some workers (Staff and Bert,1982; Uma devi and Monoharachary, 1991; Panda 2009). Marginal variations in pH at sites fail to influence the fungal population due to its trifle role (Panda *et al.*, 1996; Mohanty and Panda, 1998). When we compare our results with those reported in earlier studies on *Casuarina* population density, we find fair agreement as far as fungal numbers concerned, but the extent of numbers we see is significantly higher than that reported previously in *Casuarina* plantation (Monoharachary *et al.*, 2008; Panda, 2009).

Earlier reports have indicated that *Aspergillus, Penicillium* and *Trichoderma* appeared abundantly in soils (Rai and Kumar, 1988; Mohanty and Panda, 1994b; Panda *et al.*, 2007). 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.

It can be concluded from the present study that the above ground vegetation in coastal sandy belt is essential to maintain a productive environment to enhance microbial growth. The nutrient status of the sands can be enhanced either through introduction of the predominant microbes isolated from the present study biotechnologically or through proper mixing of litter with sands by intermittent ploughing or by the application of both.

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