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Diversity of Soil Microbes under Different Ecosystem Landuse Patterns

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Abstract The impact of aboveground vegetation and soil characteristics on belowground microbial population was tested by analyzing the soil under three different landuse patterns viz., rice field, coconut plantation and *Ipomea* sp. dominated site at Madurai, Tamilnadu, South India. In the present study there are about 22 different species of bacteria and 41 fungal strains were isolated from all the three land use patterns. Among the 22 different species of bacteria, 9 were unidentified and the remaining 13 species were represented from 9 genera, 5 families, 4 orders, 2 class and 2 phyla. In the case of fungi, 33 strains belonging to 2 phyla, 5 subphyla, 7 classes, 8 orders, 8 families and 16 genera were isolated and identified and the remaining 8 strains were unidentified. The diversity analysis of bacterial population revealed high Shannon and Simpson Index values in coconut plantation and rice field respectively for bacteria. Whereas, the Shannon value was high for fungi in rice field and high Simpson value was recorded in coconut plantation. The relationship between soil moisture, pH and organic matter content indicated the existence of a positive correlation between moisture and organic matter content against Colony Forming Unit (CFUs) and a trend of negative correlation between pH and CFUs. The data obtained for CFUs of bacteria and fungi in three different land use patterns were subjected to ANOVA (one-way) and resulted in non significance at 5% level for bacteria and < 0.05 significance for fungi. A maximum number of CFUs of bacteria, fungi and plant biomass were found in rice field. Among the three different landuse patterns, the rice field harbours rich bacterial and fungal populations due to the availability of optimum pH, high moisture, organic content and aboveground biomass with moderate aboveground plant diversity.

Keywords: *land use patterns, Diversity of Microbial Population, Colony Forming Unit (CFUs), plant biomass, Diversity indices*

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1. Introduction

Soil microbial diversity is significant because it is often regarded as an important index of the health of the soil ecosystem [1]. The role of microorganisms in maintaining the dynamic equilibrium and integrity of the biosphere is so critical that the continued existence of life is dependent on the sustained, microbial mediated transformation of matter in both terrestrial and aquatic environments. Almost all biological processes in the environment, either directly or indirectly, involve microorganisms. The potential benefits of regulating, optimizing and exploiting microbial activity are largely unexplored [2].

Microbially driven soil processes play key roles in mediating global climatic change, by acting as sources sinks and generation of green house gases such as nitrogen oxides and methane [3]. The species composition and activity of microorganisms are largely regulated by soil physico-chemical properties, climatic factors and composition of vegetation [4]. Estimates of the microbial diversity in natural environments must accommodate the spatial and temporal variability in microbial populations. Spatial effects include an assessment of the relationship between community composition and scale. Temporal shifts in microbial diversity are brought about by changes in the environment of the microorganisms and may be induced by the organisms or imposed on the community from outside. A prerequisite to the quantification of diversity in natural species is an understanding of the magnitude and level at which such changes operate [5]. The patterns of microbial populations in soils vary spatially and temporally according to factors such as the nature of the soil, parent material, availability of carbon resources, seasonal and diurnal variations in temperature, porosity, water holding capacity, changes in electrolyte concentration, pH, redox and oxygen availability [6]. Soil type and spatial distribution of resources have been found to be key drivers in the organization of soil communities [7,8,9]. In the present study, the vegetation pattern and a few soil characteristics were used to assess the soil microbial diversity.

2. Materials and Methods

2.1. Study Site

The soil samples were collected from three different landuse patterns (Rice field, Coconut plantation and *Ipomea* sp. dominated site) in sterilized plastic bags at Achampatti, Madurai District, Tamilnadu, South India. Three soil samples from each study sites were collected along a transect at regular intervals. Soil sampling was done up to a depth of 30 cm (0-10 cm, 10-20 cm and 20-30 cm). A composite sample for each depth was made by thoroughly mixing three samples in a given site and triplicate of subsamples were considered for further analysis.

2.2. Soil Analysis

Soil physico-chemical characteristics (moisture, pH and organic content) were analysed by standard methods as suggested by Allen *et al.,* [10] and Waksman [11].

2.3. Microbial Analysis

Microbial analysis was carried out in the soil samples as per the standard protocol suggested by Ingham and Klein [12]. The isolates of bacteria were identified using morphological and biochemical characteristic studies as suggested by Bergey and Holt [13]. The fungal isolates were stained with lactophenol cotton blue and observed under the microscope for identification of mycelial and spore structures. The isolated bacterial species were classified into many groups based on their morphology and biochemical characteristics studies [13,14] and the fungal species were classified based on cultural and microscopic spore charactertics [15,16].

2.4. Plant Biomass

The biomass of herbaceous vegetation was estimated by harvest method followed by Chandrasekaran and Swamy [17]. In this method five 1 m^2 quadrates were placed in each study site and plants were clipped at ground level and sorted out into different components. They were dried at 60°C in hot air oven for a period of 48 hours and the weight of dried plant materials was recorded.

2.5. Statistical Analysis

The results were subjected to statistical analysis such as Pearson's co-efficient correlation and ANOVA (one way) with the help of "Minitab computer software". The diversity indices were worked out using the package provided by Ludwig and Reynolds [18].

3. Results

The moisture, pH and organic matter content of the soil samples analysed were indicated in Table 1. In all the three landuse patterns, top soil (0-10 cm depth) showed a maximum moisture content than the other two layers (10-20 cm and 20-30 cm depths). Moisture was significantly greater ($p<0.05$) in rice field in all the three depths ranges and least was recorded in soil under *Ipomea* sp. dominated site. A significantly greater pH value 8.16±0.03 was recorded in coconut plantation and low pH in rice field. In all the three soil types, low pH was observed in top soil and an increasing trend was noticed in pH of soil with increasing depth. The soil organic matter content was significantly higher ($p<0.05$) in rice field at all the three depths ranges. Whereas, low organic matter content of 0.26±0.02% was recorded in soil under *Ipomea* sp. dominated site.

Table 1. Physico-chemical characteristics of soil under three different landuse patterns at Madurai, South India

Landuse		Rice field	Coconut plantation	*Ipomea* sp. dominated site	F-value ($p<0.05$ level of significant)
Soil physico-chemical characteristics	Depth (cm)				
Moisture (%)	0-10	26.50 ± 0.9	11.43 ± 0.25	8.6 ± 0.1	943.20
	10-20	11.56 ± 0.15	10.46 ± 0.15	8.4 ± 0.15	339.85
	20-30	09.73 ± 0.21	08.16 ± 0.25	7.5 ± 0.2	75.98
pH	0-10	6.17 ± 0.04	7.92 ± 0.03	7.23 ± 0.02	2150.57
	10-20	6.39 ± 0.10	8.07 ± 0.07	7.27 ± 0.02	884.96
	20-30	7.39 ± 0.35	8.16 ± 0.03	7.33 ± 0.03	17.96
Organic matter content (%)	0-10	1.66 ± 0.01	1.26 ± 0.01	1.21 ± 0.01	782.14
	10-20	1.42 ± 0.01	0.81 ± 0.01	0.96 ± 0.02	1052.80
	20-30	1.33 ± 0.02	0.57 ± 0.01	0.26 ± 0.02	2717.06

Table 2. Analysis of Pearson's correlation between soil physico-chemical parameters and CFUs of bacteria and fungi in three different landuse patterns at Madurai, South India

Correlation factors		Rice field		Coconut plantation		*Ipomea* sp. dominated site	
		Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi
Moisture	p-value	0.000	0.002	0.003	0.000	0.000	0.010
	Correlation value	0.943	0.880	0.867	0.950	0.978	0.796
pH	p-value	0.003	0.001	0.000	0.003	0.002	0.010
	Correlation value	-0.865	-0.893	-0.927	-0.855	-0.882	-0.797
Organic matter content	p-value	0.000	0.000	0.000	0.004	0.000	0.006
	Correlation value	0.995	0.962	0.991	0.845	0.996	0.826

A positive correlation was observed between the moisture, organic matter content and CFUs of bacteria and fungi in all the three different landuse patterns. Whereas, a negative correlation was recorded between the pH and CFUs of bacteria, and fungi in all the three different landuse patterns (Table 2).

Qualitative analysis of microbes isolated from the soil samples revealed a total of 22 different species of bacteria, of which 9 are unidentified. The remaining 13 species are classified under 9 genera, 5 families, 4 orders, 2 classes and 2 phyla (Table 3). Among this, 12, 16 and 15 bacterial species were recorded in rice field, coconut plantation and *Ipomea* sp. dominated site respectively (Figure 1).

In the present study fungi out number of bacteria with a total of 41 different strains were isolated, among which 25, 14 and 13 fungal strains were recorded in rice field,

coconut plantation and *Ipomea* sp. dominated site respectively (Figure 1). The isolated fungal population was represented by 2 phyla, 5 subphyla, 7 classes, 8 orders, 8 families and 16 genera. Out of 41 different fungal strains, 33 species were identified and the remaining 8 strains were unidentified (Table 4).

Table 3. Taxonomic classification of isolated bacterial species in three different landuse patterns at Madurai, South India

Phylum	Class	Order	Family	Genus and Species
Proteobacteria	γ-Proteobacteria	Enterobacteriales	Enterobacteriaceae	*E.coli* *Enterobacter* sp. *Proteus* sp. *Salmonella* sp. *Shigella* sp.
		Pseudomonadales	Pseudomonadaceae	*Pseudomonas fluoresences* *Pseudomonas aeruginosa* *Pseudomonas* sp.
		Vibrionales	Vibrionales	*Vibrio cholera* *Vibrio vulnificus*
Firmicutes	Bacilli	Bacillales	Staphylococcaceae	*Staphylococcus aureus* *Staphylococcus* sp.
			Bacilaceae	*Bacillus* sp.

Table 4. Taxonomic classification of isolated fungi in three different landuse patterns at Madurai, South India

Phylum	Subphylum	Class	Order	Family	Genus and species
Ascomycota	Pezizomycotina	Dothideomycetes	Pleosporales	Pleosporaceae	*Alternaria* sp. 1 *Alternaria* sp. 2 *Alternaria* sp. 3 *Bipolaris* sp. *Curvularia* sp.
		Arthoniomycetes	Chaetothyriales	Herpotrichiellaceae	*Cladophialophora* sp. *Rhinocladiella* sp.
		Eurotiomycetes	Eurotiales	Trichocomaceae	*Aspergillus niger* *Aspergillus flavus* *Aspergillus nidulans* *Aspergillus terres* *Aspergillus* sp. 1 *Aspergillus* sp. 2 *Aspergillus* sp. 3 *Aspergillus* sp. 4 *Aspergillus* sp. 5 *Aspergillus* sp. 6 *Paecilomyces* sp. *Penicillium* sp. 1 *Penicillium* sp. 2 *Penicillium* sp. 3 *Penicillium* sp. 4 *Penicillium* sp. 5
	Ascomycotina	Sordariomycetes	Hypocreales	Hypocreaceae	*Fusarium* sp. *Trichoderma* sp. *Trichothecium* sp.
			Microascales	Microascaceae	*Scedosporium* sp.
	Taphrinomycotina	Pneumocystidomycetes	Pneumocystidales	Pneumocystidaceae	*Cephalosporium* sp.1 *Cephalosporium* sp.2
	Saccharomycotina	Saccharomycetes	Saccharomycetailes	Saccharomycetaceae	*Candida* sp.1 *Candida* sp.2 *Yeast* sp.
Zycomycota	Zycomycotina	Zycomycetes	Mucorales	Mucoraceae	*Mucor* sp.

Site1- Rice field; Site2- Coconut plantation; Site3-*Ipomea* sp. dominated site

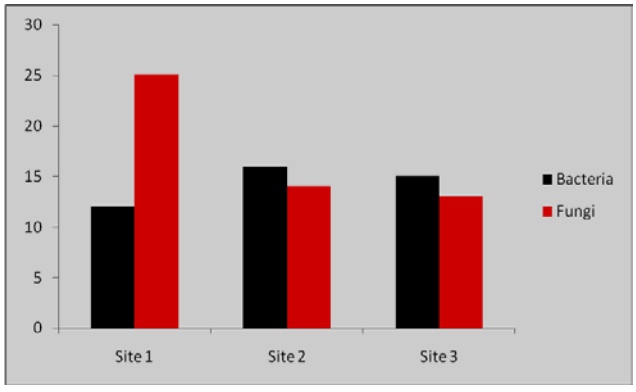

Figure 1. Number of species of bacteria and fungi in three different landuse patterns at Madurai, South India

The quantitative analysis of the isolated microorganisms was also carried out by considering individual colonies as separate units (CFUs). More number of CFUs of bacteria was recorded in rice field (422 CFUs in total) when compared to other landuse patterns such as *Ipomea* sp. dominated site (184 CFUs) and coconut plantation (172 CFUs). A similar trend was also observed for fungi the numbers of CFUs recorded were 190, 31 and 26 in rice field, coconut plantation and *Ipomea* sp. dominated site respectively (Table 5). Among the three study sites there was a non-significant ($p>0.05$) difference for bacteria and a significant ($p<0.05$) difference for fungi (Table 6) were observed.

Table 5. Colony forming units of bacteria and fungi under different landuse patterns at Madurai, South India

Landuse	Depth (cm)	Bacteria	Fungi
Rice field	0-10 10-20 20-30	268 ± 2 127 ± 1 27 ± 1	73 ± 1 68 ± 2 49 ± 1
Total		422	190
Coconut plantation	0-10 10-20 20-30	91 ± 1 47 ± 1 32 ± 1	11 ± 1 9 ± 1 6 ± 1
Total		172	31
Ipomea sp. dominated site	0-10 10-20 20-30	91 ± 2 70 ± 1 23 ± 1	20 ± 1 7 ± 1 4 ± 1
Total		184	26

Table 6. One way ANOVA between CFUs of bacteria and fungi in three different landuse patterns at Madurai, South India

Source of variation		Sum of square	Degree of freedom	Mean square	F-value	Level of significance
Variation between groups	Bacteria	13312.67	2	6656.33	1.180	NS
	Fungi	5899.20	2	2900.10		
Variation within groups	Bacteria	33817.33	6	5636.22	36.40	S
	Fungi	478.00	6	79.70		

NS-Non significant at 5% level; S- p<0.05 significant

Maximum herbaceous biomass was found in rice field with an average of 119.042±13.88 g/m^2 followed by *Ipomea* sp. dominated site (60.46±25.92 g/m^2) and coconut plantation (45.016±11.14 g/m^2). The relationship between plant biomass and CFUs of bacteria and fungi were represented in Figure 2. The highest number of CFUs of bacteria, fungi and plant biomass were recorded in rice field followed by *Ipomea* sp. dominated site and coconut population.

The quantitative data on microbial population recorded in the present study was analysed using various diversity indices and the results were given in Table 7. High Shannon index value for bacteria was obtained in coconut plantation (2.606) followed by rice field (2.430) and *Ipomea* sp. dominated site (2.416) at the soil depth of 0 – 10 cm. High Simpson index value for bacteria was obtained in rice field followed by *Ipomea* sp. dominated site and coconut plantation. The value of similarity index and evenness does not show any obvious change. Shannon Index value for fungi was high in rice field when compared to other three sites. Whereas, Simpson index calculated was high in the coconut plantation and low in rice field. The similarity index and evenness were more or less similar in all the three different landuse patterns.

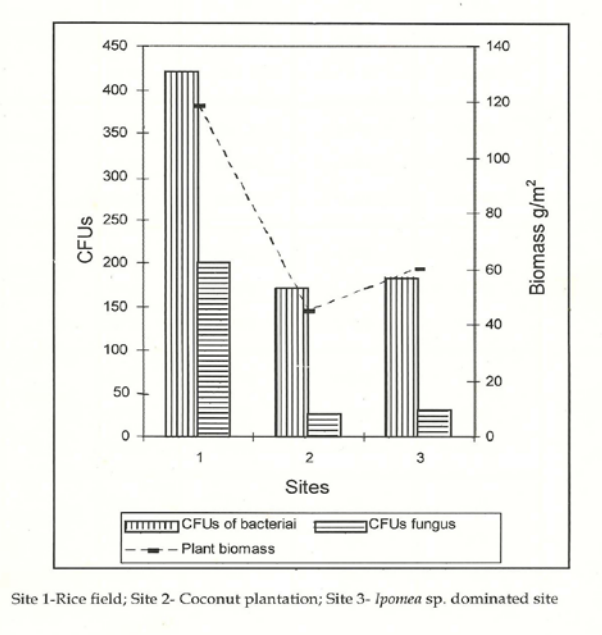

Figure 2. Relationship between plant biomass and CFUs of bacteria and fungi in three different landuse patterns at Madurai, South India

Table 7. Diversity indices of bacteria and fungi in three different landuse patterns at Madurai, South India

Indices	Depth (cm)	Rice field		Coconut plantation		*Ipomea* sp. dominated site	
		Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi
Shannon index	0-10 10-20 20-30	2.43 2.36 2.33	2.824 2.094 2.951	2.626 2.434 2.506	0.00 1.427 1.33	2.416 2.463 2.404	1.277 1.609 0.562
Simpson index	0-10 10-20 20-30	0.117 0.106 0.103	0.072 0.051 0.063	0.092 0.083 0.091	1.000 0.284 0.278	0.099 0.094 0.096	0.306 0.200 0.625
Similarity index	0-10 10-20 20-30	0.883 0.894 0.897	0.928 0.949 0.937	0.908 0.917 0.909	0.000 0.716 0.722	0.901 0.906 0.904	0.694 0.800 0.375
Evenness	0-10 10-20 20-30	0.978 0.950 0.973	0.914 0.961 0.941	0.878 0.947 0.950	0.000 0.887 0.959	0.942 0.960 0.968	0.921 1.000 0.811
Species richness	0-10 10-20 20-30	12 12 11	22 25 23	16 16 16	1 5 4	13 13 12	4 5 3

4. Discussion

Soil ecological research over the past two decades has demonstrated extremely high levels of biological diversity belowground, especially in microbial groups. Microbes exhibit an impressive diversity in their metabolic activities and its diversity is important because it is often regarded as an important index of soil ecosystem health [1]. Though of unquestionable importance in regards to the function of terrestrial ecosystems [19,20,21], our understanding about the structure of microbial communities, their response to the changing environment and the consequences of alterations in microbial community structure on ecosystem functioning is very little. Microbial diversity describes complexity and variability at different levels of biological organization. It encompasses genetic variability within taxons (species), the number (richness), relative abundance (evenness) of taxons and functional groups in communities [22,23].

Bacterial and fungal diversity increases the soil quality by altering the soil agglomeration and increases the soil fertility these two are important in nutrient cycling as well as enhancing the plant health through direct or indirect means. In addition, a healthy rhizosphere population can help plants to deal with biotic and abiotic stresses such as pathogens, drought and soil contamination [24].

The results of the present investigation were corroborates with the many previous research reports. The effect of soil structure and environmental conditions on microbial diversity has been reported by Torsvik and Ovreas [25]. It has been reported that, the population composition and the activity of microorganisms are largely regulated by soil physico-chemical properties [26]. Various parameters like temperature, pH, carbon resources and changes in electrolyte concentration were influence the microbial diversity [6]. Similarly, it has also been reported that changes in soil environment like soil moisture, pH and temperature attributed indirectly by plant characteristics will affect the soil microbial diversity and composition [27,28].

Fierer and Jackson [29] have reported the occurrence of high bacterial diversity in neutral soil and lower in acidic soils. Fierer and Jackson [30] have observed and reported that, the soil pH as a best predictor of bacterial richness. They have also observed some correlation between soil properties including soil moisture, organic carbon content, apart from this; they have also stated the existence of the strong correlation between soil pH and microbial community. In the present study, the least pH value was recorded (pH 6.2) and which lies between the optimal values (6.0 – 7.5 pH) for microbial growth [29]. Atlas and Bartha [31] have stated that many bacteria and fungi have pH optima near neutral.

Soil microbial biomass can be limited by soil moisture under both dry and wet conditions [32]. Several reports have showed that the soil microbial biomass declined upon drying and increase upon rewetting [33,34,35,36]. Pottonen *et al.,* [37] and Redding *et. al.,* [38] have reported the existence of positive correlation between soil moisture and microbial population. Abundance of bacterial and fungal population recorded in rice field with high moisture content in the present investigation correlates with the above findings.

The type and amount of available organic substrates strongly influence the abundance of microbial groups and their functional diversity in soil ecosystems [39,40]. Smit *et al.* [41] have revealed that soil with high content of readily available nutrients harbour bacteria with potentially high growth rate. Brodie *et al.* [42] have studied the microbial dynamics in a temperate upland grassland ecosystem and reported that, the microbial diversity was positively correlated to concentration of the soil organic matter. High diversity and abundance of microbial population in rice field with rich organic content substantiates the significance of available organic carbon which promotes the abundance of microbial diversity. The data collected in the rice field in the present study have been supported by the reports of many authors [39,40,41,43,54]. The relatively low diversity and biomass of microbial community in coconut plantation and *Ipomea* sp., dominated site were supported by the observations of Tilman [45,46]. They have also stated that the resource availability for soil microbial communities is constrained by organic compounds.

The changes in plant diversity and community composition could influence the composition of microbial communities [47]. Wardle *et al.,* [48] and Stephen *et al.* [49] have experimentally investigated the influence of plant diversity on soil microorganisms. The microbial community biomass which significantly increased with the increase in plant diversity in Ceder Creek National History Area was estimated by Zak *et al.,* [50]. Among the three land use patterns, the rice field was observed as rich in above ground biomass and plant diversity. The high above ground biomass and diversity also supports the below ground microbial biomass as evidenced by more number of colony farming units of both bacteria and fungi. Qualitative changes in below ground microbial community were (diversity) due to above ground plant diversity was also evidenced from the studies of Gruter *et al.,* [51], they have stated that several bacterial strains were found only in specific plots with varying plant diversity levels. Verma *et al.,* [52] have reported that, a greater microbial population in the plantation where herb diversity observed was more. This was due to the creation of biological uniformity by monoculture, which leads to the denudation of biological diversity [53]. The low microbial biomass in *Ipomea* sp. dominated site was recorded in the present study indicated the impact of exotic species on the native diversity. Moore [54] has detected a decline in native biota which was coupled with increase in exotic species. Studies of Egunjobi [55] also supported our findings of decreased microbial population in *Ipomea* sp. dominated site.

Quantitatively there was no clear relationship between soil bacterial diversity and plant diversity. Ecosystems with highest levels of bacterial diversity have low levels of plant diversity [56]. Peruvian Amazon had relatively low levels of bacterial diversity but these sites have some of the highest recorded levels of plant diversity on earth (Ter Steege *et al.,* [57]. From the results of the present study it has been concluded that rice field has a rich microbial biomass when compared to coconut plantation and *Ipomea* sp. dominated site, this was attributed mainly by the presence of optimum pH, relatively high moisture content and rich above ground diverse biomass.

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