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Characterization of Microbial Indicators to Assess the Health of Degraded soil in Cherrapunjee, India - Highest Rainfall Area of the World

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Abstract

Soil management practices can influence both the level and quality of soil microorganisms. The effect of deforestation and land degradation on soil microbes, soil organic carbon, soil pH, soil temp, bacterial and fungal CFU were investigated from two contrasting sites : one representing the conserved protected Sacred forest and the other highly degraded but world`s highest rainfall receiving soil system of Cherrapunjee, India. Microbial diversity indices can function as bio-indicators of community stability and impact of anthropogenic stress on soil system. The two different soil types showed great variability in physical and chemical parameters including soil composition, temperature, pH, soil carbon content and water availability. The bacterial colony forming units (CFU) per gram of dry soil from the soil samples of sacred forest are found to be higher as compared to the bacterial CFU/ gm dry soil from the soil samples of degraded land of Cherrapunjee. The population count of fungi is also higher in sacred forest soil than degraded land of Cherrapunjee. *Chromobacterium* sp. and *Trichoderma harzianum* are found to be indicator species of the degraded soils of Cherrapunjee which are not characterized from the protected forest.

Keywords: Microorganisms, diversity, Sacred grove, degraded land, bioindicator, Cherrapunjee.

Introduction

Ecosystem processes are strongly affected by biodiversity. Changes in ecosystem processes may themselves lead to a decline in biodiversity and thus to further

reductions in ecosystem function. Microbial diversity indices can function as bio-indicators of community stability and impact of anthropogenic stress on soil and soil biota. Soil sustains an immense diversity of prokaryotic microbes, exceeding that of eukaryotic organisms. Microorganisms exist in every conceivable place on earth and one gram of soil may harbor up to 10 billion microorganisms of possibly thousands of different species (Roello-Mora and Amann, 2001). The capacity of a soil to function in a productive and sustained manner is dependent on activity and diversity of microorganisms. Microbial diversity describes complexity and variability at different levels of biological organization. Microorganisms play an important role on nutritional chains that are an important part of the biological balance in the life in our planet. Without bacteria, soil would not be fertile and organic matter would accumulate within a short time (Kummerer, 2004). Soil organisms are assumed to be directly responsible for soil ecosystem processes, especially the decomposition of soil organic matter and the cycling of nutrients (Wardle and Giller 1996). These processes are regarded as major components in the global cycling of materials, energy and nutrients. Genetic diversity is essential to life, since it permits adaptation through the creation of new organisms by genetic transfer and mutations. Diversity statistics can also indicate the ability of a community to recover from disturbance and utilize resources efficiently (Chapin et al, 1997).

Many anthropogenic activities, such as city development, agriculture, dispersal of pesticides and other chemical pollutants can potentially affect soil microbial diversity (Kirk et al 2004; Forney et al 2004). Stress, both chemical and physical, can reduce microbial biomass and diversity (Atlas 1984). Ecosystem functioning before and after disturbance can be governed by soil microbial population dynamics (Kennedy and Smith 1995). Biomass, community structure, and specific functions of soil microorganisms appear to be of major importance for general soil functions and if detectable could serve as sensitive soil quality indicators. Soil quality is strongly influenced by microbe-mediated processes, and its function can be related to microbial diversity. It is likely that microbial community structure will have the potential to serve as an early indication of soil degradation or soil improvement. Since soil microbial communities strongly depend on the conditions of the habitat they colonize, microbiological characteristics of a soil may provide indicators, which integrate short-, middle- and long term changes in soil quality. The richness (number) and evenness (relative abundance or structure) of biological communities reflect selective pressures that shape biodiversity within communities. Measuring these parameters is most useful when assessing treatment effects (e.g., physical disturbance, pollution, nutrient addition, predation, climate change etc.) on community diversity. Therefore, there is growing evidence that soil microbiological and biological parameters may possess potential as early and sensitive indicators for soil ecological stress or reparation (Dick 1992, 1994; Dilly and Blume 1998). However, microbial diversity indicators have been minimally used to assess the effect of disturbance and technogenic pollution on the soil microbial communities (Nordgren *et. al*. 1985, Zvyagintsev 1989). An important factor limiting greater use of these indices is the absence of detailed information on the microbial species composition of soil environments and dynamics of populations of ecologically important microorganisms.

Cherrapunjee is one of the wettest spots in the world, with an average annual rainfall of 11,000 mm (the global being 800 mm). It was once part of a subtropical forest, but large scale deforestation has led to severe erosion. Weakened by loss of trees, soil got washed away by the torrential rains. As a result, Cherrapunjee is now a virtual desert. The present work aims at a comparative analysis of microbial diversity and composition of undisturbed protected Sacred forest and deforested degraded soil which experiences highest rainfall in the world so as to designate microbial indicator species for this unique rainfall receiving but degraded soil.

Materials and method

Site and sampling

The study was conducted from May 2007 through Apr 2009 in two contrasting sites- Sacred forests of Mawsynram ($25^0$17` North Latitudes and $91^0$34` East Longitudes) and degraded areas of Cherrapunjee ($25^0$15` North Latitudes and $91^0$43` East Longitudes) in Meghalaya, India. Soil samples were collected in four different seasons during the study (May-Jul, Aug-Oct, Nov-Jan and Feb-Apr). Soil samples were collected aseptically in sterile sample container and kept refrigerated until processing. All the rocks and debris present in the samples was aseptically removed. Soil temperature, atmospheric temperature and relative humidity were recorded at the time of sample collection. Global Positioning System (GPS) was used to obtain the exact coordinates of sampling sites.

Estimation of moisture content

Soils samples were weighed and dried in the oven (24 hours at 105^0C) and the dried soils were weighed. The difference in their weight was recorded and calculated as moisture content (%)

Measurement of soil pH

It was determined by adding dry soil and distilled water in the ratio 1:5 and pH measured in pH meter (Systronics).

Soil temperature

It was measured by the soil thermometer at the time of soil sampling.

Soil carbon

It was estimated following the method of Walkley and Black (1934) and calculated as soil carbon (%).

Enumeration of microbial population

Enumeration and population count of bacteria was done on nutrient agar media by serial dilution agar plating method using 0.85% normal physiological saline as diluents. Dilutions were made up to $10^{-7.}$ Bacterial population was counted as colony forming unit per gram of dry soil (CFU/gm).

Enumeration and population count of fungi was done on potato dextrose agar media by serial dilution agar plating method using the same diluent. Fungal population was counted as colony forming unit per gram of dry soil (CFU/gm).

Preservation of the isolates

The bacterial isolates were preserved in nutrient agar containing 15% glycerol and kept at -80^0C. The fungal population were preserved in sterile distilled water and refrigerated.

Morphological and Physiological characterization of the bacterial and fungal isolates

Morphological characterization of the bacterial isolates was done by examining the colony morphology. Physiological characterization was done by biochemical tests such as staining reaction, oxidase test, catalase test, Imvic tests, amylase production test, gelatin hydrolysis test, urease test, H_2S production test, oxidation fermentation test of glucose for carbohydrate utilization, carbohydrate fermentation test etc. Fungal isolates were readily identified by their characteristic morphology such as shape, size, colony morphology, nature of conidiophores, sporangiospores, spores. and hyphae under microscopic observation(Leica DM 1000, Germany)

Results

Soil parameter

The soil temperature of the protected sacred grove was found to be lower than the degraded land. The data showed that carbon and moisture content of the forest soil were higher in comparison to the degraded land soils. The pH of the forest soil was found to be acidic than that of the degraded land soil (Table 1).

Total bacterial count in soil

The total bacterial count (CFU/gm soil) in sacred grove fluctuated from $5.96x10^7$ (May-Jul) to $6.23x10^7$ (Nov-Jan). However, the total bacterial count in degraded land ranged from $1.87x10^6$ (May-Jul) to $1.97x10^6$ (Nov-Jan). These results indicate that the total bacterial count was higher in Sacred protected forest. Higher population was observed at both the sites in warmer seasons than in colder seasons.

Identification of bacterial isolates and their frequency

A total of 39 different bacterial strains were isolated from both the sites (26 isolates were from sacred forest and 13 isolates from degraded land). The bacterial isolates were purified and identified at the genus level by standard procedures following Bergey's Manual of Systematic Bacteriology. Among Gram-positive bacteria, *Bacillus* was the most frequently genus isolated from sacred forest. However, *Staphylococcus* and *Micrococcus* were recovered from sacred forest and degraded land with different frequencies during hot and cold seasons. The genus *Serratia* and *Pseudomonas* were frequently isolated from degraded land soil. *Chromobacterium* sp. was the most prevalent isolate characterized from the degraded land soil. Gram

positive bacteria are prevalent in the undisturbed forest soils, whereas more Gram negative bacteria dominated the degraded land.

Total fungal count in soil

The total fungal count (CFU/gm soil) in Sacred grove fluctuated from $6.0x10^6$ (May-Jul) to $6.23x10^6$ (Nov-Jan). However, the total fungal count in degraded land ranged from $1.96x10^4$ (Aug-Oct) to $2.4x10^4$ (Feb-Apr). These results indicate that the total fungal count was higher in Sacred protected forest. Warmer seasons harboured lower population of fungi as compared to the colder seasons.

Identification of fungal isolates and their frequency

Total fungal isolates were found to be 16 out of which 10 were from sacred grove and 6 were from degraded land. The genus *Penicillium, Aspergillus* and *Fusarium* were most frequently isolated from sacred grove. *Trichoderma harzianum* was found to be the most prevalent indicator species of the degraded soils of Cherrapunjee. A rare species—*Syncephalastrum racemosum* was isolated from protected sacred forest soil only.

Table 1: Comparative dataset of seasonal variation in soil parameters for Mawsynram Sacred Grove and Degraded Land of Cherrapunjee.

Sampling Period	Sampling Sites	Soil Temperature (^oC)	Soil pH	Soil Moisture Content (%)	Soil Carbon Content **(%)**	Bacterial CFU/g dry soil	Fungal CFU/g dry soil
May-Jun	**Sacred Forest-Mawsynram**	**22.1**	**4.1**	**26.2**	**3.84**	**$5.96x10^7$**	**$6.0x10^6$**
	Degraded Land-Cherrapunjee	**28.1**	**5.6**	**18.2**	**1.92**	**$1.87x10^6$**	**$2.1x10^4$**
Aug-Oct	**Sacred Forest-Mawsynram**	**23.2**	**4.1**	**25.2**	**3.61**	**$6.1x10^7$**	**$6.1x10^6$**
	Degraded Land-Cherrapunjee	**27.6**	**5.95**	**18.0**	**1.72**	**$1.91x10^6$**	**$1.96x10^4$**
Nov-Jan	**Sacred Forest-Mawsynram**	**20.4**	**4.5**	**23**	**3.7**	**$6.23x10^7$**	**$6.23x10^6$**
	Degraded Land-Cherrapunjee	**24.1**	**5.85**	**11.3**	**1.15**	**$1.97x10^6$**	**$2.23x10^4$**
Feb-Apr	**Sacred Forest-Mawsynram**	**19.4**	**4.2**	**24**	**3.8**	**$6.0x10^7$**	**$6.1x10^6$**
	Degraded Land-Cherrapunjee	**27.2**	**5.8**	**12.6**	**1.2**	**$2.1x10^6$**	**$2.4x10^4$**

Table 2: Comparative dataset of seasonal microbial diversity for Mawsynram Sacred Groove and Degraded Land of Cherrapunjee.

Sampling Period	Sampling Sites	Fungal Species	Bacterial species
May-Jun	**Sacred Forest - Mawsynram**	*Syncephalastrum racemosum* *Fusarium oxysporum* *Penicillium janczewskii* *Penicillium pinophilum* *Penicillium oxalicum* *Aspergillus niger,Mucor sp.* *Curvularia sp.,* *Microsporum sp.*	*Bacillus aerophilus, Bacillus megaterium ,Bacillus pumilus, Bacillus subtilis, Bacillus aerophilus, Brevibacterium sp., Clavibacter sp., Curtobacterium sp., Arthrobacter sp, Staphylococcus aureus, Staphylococcus sp., Micrococcus sp., Cellobiococcus sp,. Planococcus sp, Pseudomonas auroginosa, Pseudomonas sp., Erwinia sp., Aeromonus hydrophila, Flavobacterium sp., Pantoea sp. , Acinetobacter sp.,Alkaligenes sp*
	Degraded Land- Cherrapunjee	*Trichoderma harzianum* *Trichophyton sp.* *Penicillium sp.*	*Bacillus aerophilus , Bacillus subtilis, Arthrobacter sp.,Micrococcus sp, Chromobacterium sp.,Aeromonus veronii, Pseudomonas sp., Acinetobacter sp*
Aug-Oct	**Sacred Forest - Mawsynram**	*Syncephalastrum racemosum* *Fusarium oxysporum* *Penicillium janczewskii* *Penicillium nalgiovense* *Penicillium pinophilum* *Penicillium oxalicum* *Aspergillus niger, Mucor sp., Curvularia sp.,Microsporum sp.*	*Bacillus megaterium, Bacillus pumilus, Bacillus subtilis, Bacillus aerophilus,Brevibacterium sp.,* *Staphylococcus aureus, Staphylococcus sp., Micrococcus luteus, Micrococcus sp., Cellobiococcus sp., Planococcus sp., Pseudomonas auroginosa,*

			Pseudomonas sp., Flavobacterium sp., Kluyvera sp, Pantoea sp.,Acinetobacter sp., Alkaligenes sp.
	Degraded Land- Cherrapunjee	*Trichoderma harzianum Trichophyton sp. Penicillium sp.*	*Micrococcus sp, Chromobacterium sp., Pseudomonas sp., Acinetobacter sp*
Nov-Jan	**Sacred Forest - Mawsynram**	*Syncephalastrum racemosum, Fusarium oxysporum , Penicillium janczewskii, Penicillium nalgiovense*	*Bacillus cereus, Bacillus megaterium, Bacillus subtilis,Bacillus aerophilus, Micrococcus luteus., Cellobiococcus sp. Planococcus sp., Pseudomonas, auroginosa., Yersinia sp., Acromobacter sp., Flavobacterium sp, Kluyvera sp.*
	Degraded Land- Cherrapunjee	*Trichoderma harzianum, Trichophyton sp.*	*Bacillus subtilis, Chromobacterium sp, Aeromonus veronii*
Feb-Apr	**Sacred Forest - Mawsynram**	*Syncephalastrum racemosum Fusarium oxysporum*	*Bacillus megaterium, Bacillus aerophilus, Brevibacterium sp., Clavibacter sp., Curtobacterium sp, Arthrobacter sp, Micrococcus luteus, Micrococcus sp.,Pseudomonas sp.,Erwinia sp., Aeromonus hydrophila, Yersinia sp., Acromobacter sp.*
	Degraded Land- Cherrapunjee	*Trichoderma harzianum, Penicillium sp,*	*Arthrobacter sp., Micrococcus sp, Chromobacterium sp,Pseudomonas sp., Acinetobacter sp.*

Table 3: Comparative dataset of bacterial community of two contrasting sites

Sampling site	Bacterial community				
	Gram +ve Rod	**Gram +ve Cocci**	**Gram -ve Rod**	**Gram -ve Coccobacilli**	**Gram -ve Cocci**
SACRED FOREST-MAWSYNRAM	*Bacillus cereus* *Bacillus megaterium* *Bacillus pumilus* *Bacillus subtilis* *Bacillus aerophilus* *Brevibacterium sp.* *Clavibacter sp.* *Curtobacterium sp.* *Arthrobacter sp.*	*Staphylococcus aureus* *Staphylococcus sp.* *Micrococcus luteus* *Micrococcus sp.* *Cellobiococcus sp.* *Planococcus sp.*	*Pseudomonas aurogінosa* *Pseudomonas sp.* *Erwinia sp.* *Aeromonus hydrophila* *Yersinia sp.* *Acromobacter sp.* *Flavobacterium sp.* *Kluyvera sp.* *Pantoea sp.*	*Acinetobacter sp.* *Alkaligenes sp.*	Not Observed
DEGRADED LAND OF CHERRAPUNJEE	*Bacillus aerophilus* *Bacillus subtilis* *Arthrobacter sp.*	*Micrococcus sp.*	*Chromobacterium sp.* *Aeromonus veronii* *Pseudomonas sp.*	*Acinetobacter sp.*	

Table 4: Comparative dataset of Fungal community of two contrasting sites (Sacred forest and degraded land).

Sampling sites	Fungal species
SACRED FOREST – MAWSYNRAM	*Syncephalastrum racemosum* *Fusarium oxysporum* *Penicillium janczewskii* *Penicillium nalgiovense* *Penicillium pinophilum* *Penicillium oxalicum* *Aspergillus niger* *Mucor spp.* *Curvularia spp.* *Microsporum spp.*
DEGRADED LAND OF CHERRAPUNJEE	*Trichoderma harzianum* *Trichophyton spp.* *Penicillium spp.*

Table 5: Most frequent microbes at the two contrasting sites.

Microbial species	**SACRED FORESTOF MAWSYNRAM**	**DEGRADED LAND OF CHERRAPUNJEE**
Bacterial isolates	*Bacillus megaterium* *Bacillus aerophilus*	*Chromobacterium spp.*
Fungal isolates	*Aspergillus niger* *Penicillium oxalicum* *Syncephalastrum racemosum* *Fusarium oxysporum* *Microsporum spp.*	*Trichoderma harzianum*

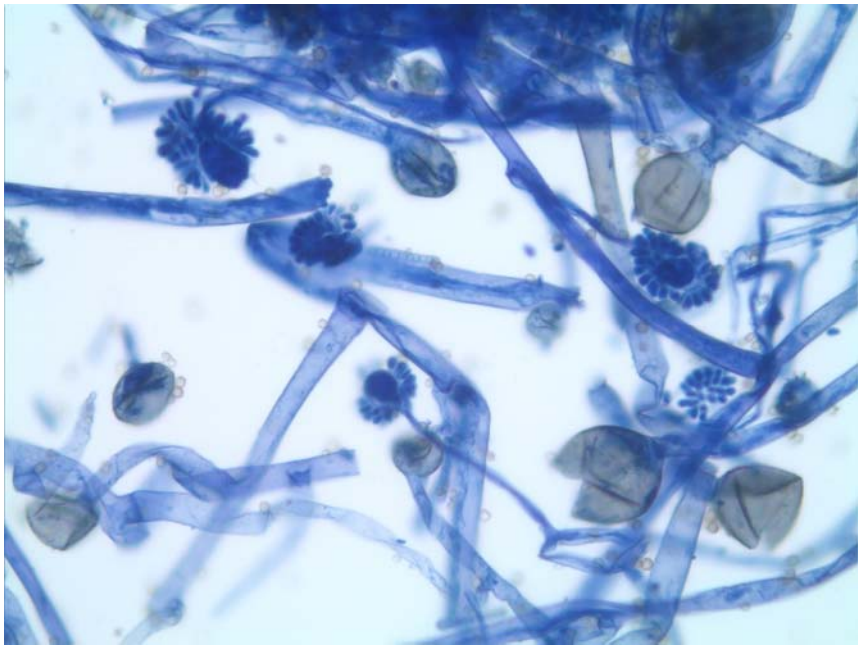

Figure 1

Syncephalastrum racemosum

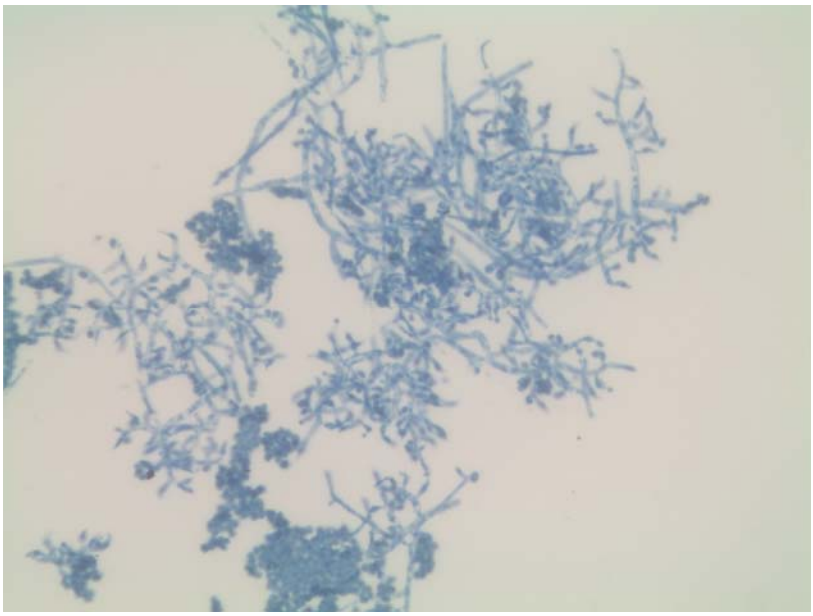

Figure 2

Trichoderma harzianum

Discussion

Soil moisture, soil temperature, and/or substrate availability have been given as the most important factors that influence soil microbial growth and population density. Soil microbial diversity is reported to decrease with disturbance (Liu *et al.* 2000).

The two different soil types showed great variability especially in physical and chemical parameters which include diverse soil composition, temperature, pH, carbon content and water availability. The disturbed Cherrapunjee soils showed higher temperature and less moisture content than the pristine forest soils. This may due to presence of minimum vegetation in these areas. Most of the areas were barren lands with no vegetation at all. Some areas cover only grasslands. But all degraded areas were covered by rocks. The pH of this kind of soil also showed slightly more acidic than undisturbed soil.

A number of studies have addressed soil microbial growth, population density, diversity, and/or activities in disturbed ecosystems (Atlas 1984; Atlas *et al.* 1991; Joshi *et al.* 1991).

The bacterial colony forming units (CFU) per gram of dry soil from the soil samples of sacred forest are found to be higher as compared to the bacterial CFU/gm dry soil from the soil samples of degraded land of Cherrapunjee. Environmental stresses brought by drought could be the factor for reduction in microbial population and diversity in degraded land soils. Plant species composition is a factor influencing microbial community composition (Carney *et al.* 2006). This is because there are strong interactions between aboveground and belowground biotic communities, as revealed by correlations between metabolic and plant taxonomic diversities (He et. al, 2008). Plant species and soil type are two important characteristics affecting the structure of the total bacterial community (Garveba *et al.* 2008). Human alteration of soil microbial communities via the alteration of plant community composition is mediated in part by changes in soil C quality (Joshi *et al.* 1991). Moreover ,16S rRNA gene and phospholipid fatty acid analyses have revealed shifts in the total microbial community in response to the different management regimes, indicating that deliberate management of soils have a considerable impact on microbial community structure and function in tropical soils (Bossio 2005). The extremely dry condition of soil with minimum water holding capacity at Cherrapunjee probably inhibited the growth of soil microorganisms. The diversity and richness of soil bacterial communities differed by ecosystem type, and these differences could largely be explained by soil pH. The acidic soil pH of forest soil support good growth of fungi than disturbed soil.

There is a considerable variation in the soil characteristics and microbial community between the two sites. *Chromobacterium spp.* and *Trichoderma harzianum* prevalent in all seasons are found to be indicator species of the degraded soils of Cherrapunjee which could be because of their better adaptability compared to other species. These species are found to be absent in the undisturbed soils of Sacred Grove which might be due to their poor competitive ability among the prevalent microbes at undisturbed soils. Number of Gram positive bacteria declined sharply in degraded soils. Rainy season was found to be the most favorable season for the growth of microbial species. The distinct seasonal changes of microbial diversity and

activity indicated that moisture, temperature and soil exposure were important factors for soil microorganisms.

The present study demonstrated that increased disturbances had an adverse affect on the microbial population and its diversity. The results emphasize the importance of microorganisms being more responsive to soil conditions demonstrating their usefulness as indicators of soil quality in highly degraded areas like Cherrapunjee.

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