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Diversity of Community Soil DNA and Bacteria in Degraded and Undegraded Tropical Forest Soils of North-Eastern India as Measured by ERIC–PCR Fingerprints and 16S rDNA-DGGE Profiles

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ABSTRACT

The analysis of the denaturated gradient gel electrophoresis (DGGE) of the polymerase chain reaction (PCR) amplified 16S rDNA fingerprints demonstrated a high diversity of bacterial communities in the soils from the three study sites at the surface and subsurface soil layers. The undegraded site possessed maximum number of 16S rDNA fingerprints than the degraded and undegraded sites at both soil depths. The surface soil of the degraded site displayed minimum 16S rDNA fingerprints thereby suggesting the reduced bacterial diversity in this site as a consequence of the long term utilization of the soil through shifting cultivation in the past. The cluster analysis of the DGGE bands of 16S rDNA genes revealed a clear separation of the degraded and moderately degraded sites from that of the undegraded site in terms of bacterial genomic communities.

Keywords: Community Soil DNA, DNA fingerprinting, microbial diversity, soil degradation, Tropics

INTRODUCTION

Microorganisms possess a large size of diversity in terms of number and genetic makeup while constituting an essential part of the biological diversity on the earth. As in higher organisms, microorganisms have some values economic, agricultural, medicinal, food, fibre, industrial, ecological, etc. (Dilly and Munch 1998; Handelsman and Wackett 2002; Hunter-Cevera 1998; Robe et al 2003; Robert and Szakacs 1998; Rondon et al 1999: Roose-Amslag et al 2001, Lauber et al 2009). So, their understanding and maintenance is of equal importance to that of higher organisms. Despite vast size in number of expected species our knowledge on the number of described species of these organisms is less than five percent of the total expected species (DIVERSITAS; Groombridge1992). On the other hand these organisms are apparently depleting from the biosphere due to changes made by natural and manmade activities (Ahn et al 2009, Caracciolo et al 2011). Lodge et al (1996) described various threats to microbial diversity in tropical forests which include forest fragmentation, loss of hosts caused by logging and other human activities, air pollutants, fungicides, disturbances that alter microclimates and exposure to sunlight, global climatic changes, etc. Deforestation and intensification of agricultural practices have been the most important causes among various manmade activities for fragmentation of natural habitats leading to rapid decline in natural forests, subsequent soil erosion, accelerated loss of fertile top soil, nutrient depletion, lowered crop productivity, loss of rare flora and fauna including the microorganisms (Girvan et al 2003; Saxena and Ramakrishnan 1986; Singh 2002; Tiwari et al 2002). Understanding the changes in microbial diversity and their activities in functioning of the tropical forest ecosystems following perturbations is of crucial importance. This is because of the reason that microorganisms in soil play a major role in ecosystem functioning though the microbial estimates do not necessarily reflect the characteristics of microbial populations or processes under field conditions (Dilly and Munch 1998). Moreover, microbial diversity of soil is important to sustainable agriculture because microbes mediate many processes that support agricultural productions and even may indicate disturbances or beneficial effects of amendments or management strategies (Lupiwayi et al 1998; Sharma et al 1998).

Studying the microbial components of the soil systems to understand their role in ecosystem functioning require using both the traditional cultivation, biochemical and recently developed molecular techniques. As an outcome of efforts being made during the last few decades, to explore the complex microbial resources, a number of techniques have been developed based on nucleic acid (DNA and RNA) probes and improvements have been made over the existing methods for screening, isolation and characterization of

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microorganisms (Hill et al 2000; Ogram 2000; Robe et al 2003; Schloter et al 2000; Trosvik 1980; Volossiouk et al 1995; Yeates et al 1998). The use of DNA based techniques have been proved to be better by many folds than the traditional plate culture methods but a polyphasic approach comprising of both the techniques while studying microbial diversity and their role in soil ecosystems is a prerequisite (Crecchio et al 2004; Hugenholtz and Pace 1996; Roose-Amsleg et al 2001; Trosvik 1980).

The small-subunit ribosomal DNA gene (16S rDNA) which codes for 16S rRNA in prokaryotic ribosomes has been used as a useful bio-molecular marker and presently employed as routine technique for culture-independent technique in microbial ecology. The sequence analysis of the gene, isolated and purified from many environmental samples, has shown this molecular probe as one of the most powerful tools in microbial diversity research, molecular evolution and phylogenetic classification of the living organisms (Zhou et al 1997, Sorensen et al 2005, Liebner et al 2008). The analysis of the 16S rDNA nucleotide sequence after isolation and purification from the community soil DNA with the help of polymerase chain reaction (PCR) can easily determine the identity and diversity of the bacterial communities in soil as compared to the traditional plate culture methods which is considered to underestimate culture irresponsive species (Dunbar et al 2000). Some of the reasons for use of 16S rDNA as a molecular marker in microbial diversity studies are due to the universal distribution of this gene in all the communities of the domain bacteria, structural and functional conservation and the size which allows for sufficient sequence divergence (Ludwig and Schliefer 1994; Goebel 1995). The 16S rDNA gene has regions which are highly conserved while other regions display considerable sequence variation even within closely related taxa (Gobel 1995; Santros and Ochman 2004). These conserved sequences have been used as a phylogenetic marker for classification of bacteria into different taxa. The inference of phylogenies based on the comparative sequence analysis of the 16S rDNA provides, from the deepest separation of the different branches of life to the genus or even species or strain level, and facilitates identification and classification of microorganisms with little effort (Ludwig and Schliefer 1994; Olsen et al 1986, 1994). In fact, categorizing of the 16S rDNA gene from the community DNA of environmental samples has become a popular alternative to characterise microbial communities because it avoids the limitations of cultivability and directly provide information on phylogenetic diversity (Zhou et al 1997). However, the cloning and sequencing strategies are rather cost, time and labour consuming and thus not suitable for monitoring a large number of samples, e.g., in studies on the succession of microbial communities during the growing season, or following shifts of microbial communities after perturbations (Heuer and Smalla 1997). Recently use of the denaturing gradient gel electrophoresis (DGGE) of the PCR amplified DNA fragments has become a new approach to study the structural diversity of microbial communities which overcomes the disadvantages in cloning and sequencing of the DNA fragments. DGGE was initially developed for use in the medical research for detection of point mutations (Fisher and Lerman 1983; Myers et al 1985, 1987) but it was introduced in the microbial ecology by Muyzer et al (1993).

Enterobacterial repetitive intergenic consensus (ERIC) sequences are short interspersed repetitive elements found in the genome of eubacteria (Gillings and Holley 1997). ERIC elements are 126 bp in size and are distributed throughout extragenic regions of the genomes of many gram negative enteric bacteria and closely related phyla including vibrios (Giovanni et al 1999a; Hulton et al 1991; Versalovic et al 1991; Wilson and Sharp 2006). The unique locations of ERIC elements in bacterial genomes allows discrimination at genus, species, and even strain level based on the electrophoretic pattern of amplification products (de Bruijn 1992). PCR amplified ERIC elements generates amplicons of varying sizes ranging from approximately 50 to 3000 bp which collectively constitute a DNA fingerprint (Giovanni et al 1999b). Selective amplification of ERIC elements using oligonucleotide primers and comparative study of electrophoretic patterns of the fingerprints are used for identification, discrimination and classification of bacterial strains or communities (Bhattacharya et al 2003; de Bruijn 1992; Giovanni et al 199b; Niemann et al 1999).

Application of these techniques had been successfully employed in diverse microbial habitats on the biosphere ranging from agricultural and Siberian tundra soils in the terrestrial ecosystems towards marine sediments and hot springs in aquatic ecosystems (Cifuentes et al 2000; Dunbar et al 2000; Ennahar et al 2003; Hobel et al 2005; Zhou et al 1997). There has been numerous reports on successful use of PCR based DGGE profiles of 16S rDNA as a technique in studying described and undescribed microbial diversity in agricultural soil (Ovreas et al 1998; Girvan et al 2003), grassland soil (McCaig et al 2001), rhizosphere soil (Duineveld et al 2001; Heuer and Samalla 1997), marine sediments (Ogram et al 1987), polluted soil (Kirk et al 2004; Maila et al 2005), water biofilms (Lyauley et al 2005), pine forest soil (Laverman et al 2005), etc. Similarly,

ERIC-PCR has been used generally to identify and discriminate pathogenic bacterial strains or mixed bacterial communities and including the rhizosphere bacterial communities of genetically modified organisms (Finger et al 2006; Hulton et al 1991; Giovanni et al 1999a, 199b). However, irrespective of the type of methods employed, remarkably very little has been published on the numerous and often crucial ways in which microorganisms (fungi and bacteria) influence tropical forest ecosystems (Lodge et al 1996). Moreover, reports are scarce on application of such molecular based techniques (16S rDNA DGGE and ERIC- PCR) in studying the microbial communities in sub-tropical humid forest soils where the practices of shifting cultivation is a prominent system which leads to large scale environmental degradation problems of north-eastern region. Therefore, the present study was made to investigate the impact of soil degradation due to shifting cultivation and selective logging on distribution of community soil DNA and bacterial diversity by using ERIC-PCR fingerprints and 16S rDNA-DGGE profiles.

MATERIALS AND METHODS

Study site description

Banderdewa forest reserve in the Papum-pare District of Arunachal Pradesh (north-eastern India) was the area of the reported study. The area is located between 27°6' N latitude and 93°49' E longitude at an elevation of 350m amsl. The average annual (maximum) ambient temperature and average total annual rainfall were 26°C and 2609 mm. The vegetation consists of evergreen to semi-evergreen mixed natural forests of varying tree species ranging from the primitive Magnolia to newly introduced teak plantations.

Two study sites, namely degraded forest (DF) and moderately degraded forest (MDF) sites were selected based on the level of disturbance due to agricultural practice (shifting cultivation or "jhum") and selective logging. A protected tract in the reserve forest was selected as an undegraded natural forest (UDF) as control site in this study. The various criteria for classification of the two sites includes land use history, living tree stump density, dominant tree species composition, exposure of soil surface, thickness of soil profile (Ah) and important physico-chemical properties of soil (tables 1 and 2).

Table 1. Textural class and percentage degradation levels of three study sites of DF, MDF and UDF sites at surface and sub-surface soil layers

Parameters	DF	MDF	UDF
Soil texture (%) Sand	72.8	73.0	74.9
Silt	20.1	18.0	18.1
Clay	7.1	9.0	7.0
Textural Class	Loamy sand	Loamy sand	Loamy Sand
Soil profile thickness (A_h, cm) Tree density (trees/100 m^2)	3-5 12	7-10 84	13-17 121
Porosity at surface layer (%)	49	52	55
Land use history (years)*	20	0	0
Erosion class[#]	3	1	0
Extent of degradation[$]	Dominant (.5)	Common (.3)	0

*Agriculture practices
[#]Soil Survey Manual: Handbook No. 18 (USDA, Revised Edition, 1995)
[$]Sehgal and Abrol (1994)

Table 2. Physico-chemical characteristics of soil in DF, MDF and UDF sites at surface (S) and sub-surface (SS) soil layers

Parameters	DF		MDF		UDF	
	S	SS	S	SS	S	SS
Bulk density (g/cm^3)	1.3±0.06	1.34±0.07	1.1±0.09	1.2±0.05	1.0±0.07	1.12±0.05
Moisture content (%)	19.7±1.2	16.6±0.38	20.9±0.98	17.8±1.3	22.7±0.34	20.5±1.6
pH	5.5±0.38	5.2±0.21	5.7±0.23	5.3±0.21	6±0.45	5.7±0.44
Organic C (%)	1.7±0.51	1.2±0.42	2.4±0.41	1.5±0.46	3.1±0.45	2±0.44
Total N (%)	0.22±0.14	0.17±0.13	0.33±0.18	0.21±0.14	0.43±0.20	0.29±0.15

(Values are means of triplicate analysis with standard deviation)

DF was a nine years old regenerating jhum fallow when the research was conducted in 2002. This site had been used for cultivation of rice, maize, finger millet and tuber crops etc. for about fifteen years without proper input of nutrients based on either organic or inorganic fertilizers. Thus, the nutrients available to the crops were obtained only from the debris of plant residues and ash after burning of dried slash prior to cropping in each jhum cycle. This site has not been used for further cultivation of crops since last jhum practiced in 1993 due to lower crop productivity and was lying as an abandoned regenerating fallow land. Luxuriant growth of fern, weeds and grasses covers the soil surface during summer rainy season while dried plant residues cover the soil surface in winter season. MDF was a forest tract where removal of mature and large sized timber trees and introduction of new plantations i.e. selective logging were practiced by forest personnel as routine silvicultural operations. Annual cutting and removal of trees, cleaning, burning of dried grasses and weeds on forest floor are the main causes of soil disturbance in this site. However, no sign of agricultural practice, either in the form of jhum or other practice, was observed form this site. The soil in the study site falls under Karsingsa series, which is a member of mixed loamy sand of hyperthermic family, typic Haplustalfs i.e. class of Alfisol (Singh 1999). The geology of the soil consists of sedimentary (sandstone) parent rock which is drained by small tributaries of Dikrong River towards Brahmaputra River in Assam.

Soil sampling and laboratory analysis

Soil sampling was done from triplicate plots of 50 m2 each from all the three sites in the middle of April 2002. Soil samples were collected separately using metal soil corer having a diameter and height of 6cm and 20cm respectively from two soil depths i.e. surface (0-20 cm) and subsurface (20-40 cm.) layers after discarding approximately 0.5 cm of soil in between the two depths. A total of 15 soil core samples were collected from each triplicate plots of DF, MDF and UDF sites and were mixed to obtain a composite sample for each site. The field moist soil collected from each study site was processed and passed through a 2mm mesh screen for further analyses. The physico-chemical properties of soil were determined on field fresh soil or after air drying of the soil samples (Okalebo et al 1993).

Isolation of community DNA from Soil

Isolation of community soil DNA was done using the Fast DNA® SPIN Kit for Soil (BIO 101). Approx. 0.5 g of fresh soil (stored at -20°C) was taken in a 2 ml E-tube containing lysing mixture. 978 µl SPB (sodium phosphate buffer) and 122 µl MT buffer were added in the tube and homogenized at maximum speed for 1 minute. The suspension was centrifuged for 1 minute at 14000x g and the supernatant was transferred into a clear 2 ml tube and 250µl PPS (protein precipitation solution) was added followed by mixing the tube for 2 minutes. Then the tube was centrifuged again for 5 minutes at 14000x g and the supernatant was transferred into a 15 ml tube. 1 ml of binding matrix suspension was added and the tube was turned upside down for at least 5 times to allow binding of DNA to the matrix. About 500 µl of the supernatant at the surface layer of the tube was discarded and resuspended the remaining supernatant in the binding matrix. The slurry was transferred into a Spin Tube in two aliquots of 500 µl each and centrifuged to discard the waste liquids. Finally, the DNA in the Spin filter was washed with 500 µl of SEWS-M (salt/ethanol wash solution, DNase-free) at 14000 x g for 1 minute and the filtrate was discarded. The Spin was removed from the tube and dried for 5 minutes at room temperature. The Spin was replaced into a fresh catch tube and 50 µl of DNA eluting solution (DES, Dnase /Pyrogen free water)

was added while gently stirring the filter membrane with the pipette tip. Then, the tube was centrifuged at 14000 x g for 1 minute to elute the DNA into the catch tube. The DNA content of the extract was checked at 1% agarose gel.

Enterobacterial repetitive intergenic consensus (ERIC)-PCR

The DNA sample was amplified using ERIC-I universal primer (5'ATGTAAGCTCCTGGGGATTCAC-3'; Versalovic et al 1991). The PCR reaction mixture of 50µl contained 5µl 1x PCR buffer, 2.5µl of 2.5µM $MgCl_2$, 5µl of 3% bovaine serum albumin (BSA), 5µl 0.2 mM dNTP, 2µl of ERIC-I universal primer, 29 µl RNase free water, 1µl DNA and 0.5 µl Taq DNA polymerase (Invitrogen). The PCR was done in two reactions, first with 10 min hot start at 95°C, pause at 80°C and add Taq DNA polymerase. Second reaction starts with denaturation at 94°C for 1 min, annealing at 53°C for 1 min, elongation at 65°C for 2 min for a total of 30 cycles followed by final stabilisation of the products at 65°C for 10 minutes. The PCR product was electrophoresed in 1% agarose gel to check the presence of DNA amplicons followed by further electrophoresis of the ERIC-PCR products on a polyacrylamide gel electrophoresis (PAGE) for analysis of microbial community soil DNA

16S rDNA amplification for DGGE

16S rDNA gene was selectively amplified using the eubacterial primers with GC-clamp, F968-GC (5'-CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG G-AA CGC GAA GAA CCT TAC-3': Engelen et al 1998) and R1401-G (5'-CGG TGT GTA CAA GGC CC-3') for the analysis of bacterial community in the soil samples by DGGE. 1µl of the DNA sample was used for amplification of 16S rDNA in a PCR reaction volume of 50 µl (5µl 1x PCR buffer (Invitrogen), 3 µl 1.5 mM $MgCl_2$, 5µl 3% BSA (Sigma), 5µl 0.2 mM dNTP (Fermentas), 2.5 µl 5 % DMSO, 1µl 10 pM F968-GC primer, 1 µl 10 pM R1401-G primer, 26 µl RNAse free water (Sigma), and 0.5 µl Taq DNA polymerase (Invitrogen). The PCR was completed in two reactions steps, first with 10 min hot start at 95°C, pause at 80C and add Taq DNA polymerase. Second reaction starts with denaturation at 93°C for 1 min, annealing at 62C for 1 min, elongation at 72°C for 1 min for a total of 30 cycles followed by final stabilisation of the products at 72°C for 10 minutes. The amplified PCR products of 16S rDNA was checked for presence of 473 bp16S rDNA band and purified using the QIAquick PCR purification kit protocol (QIAGEN).

Denaturing Gradient Gel Electrophoresis (DGGE)

DGGE was performed by using 6% acrylamide gel (ratio of acrylamide to bisacrylamide, 37:1, Bio-Rad) with a 45 to 65% denaturing gradients {the 100 % denaturing solution comprised of 15 ml of 40 % PAA dissolved in 40 ml of formamide with 42 g of urea (Sigma) and 2 ml of 50x TAE buffer and the final volume maintained at 100 ml with milli- Q water}. 15 µl of the purified 16Sr DNA was loaded in the gel using one-third volume of the DGGE buffer dye in each lane for the six different samples. The gel was electrophoresed at 60°C for 17 h at a constant voltage (70V) by using the universal mutation detector system (Bio-Rad). The gel was fixed in 25% glacial acetic acid solution for 30 min. followed by three times washing for 2 min. each with milli-Q water. Then, the gel was stained in silver nitrate solution ($AgNO_3$, SIGMA) for 25 min, washed twice with milli-Q water for 30 sec. and developed the images in sodium thiosulphate+sodium carbonate solution. The gels were then put in Na-EDTA solution for 10 min to stop darkening of the gel. The gel was dried at 45 to 50°C for 48 hrs in dark and the images were captured using HP Scanjet II scanner.

Gel compare analysis

The DGGE images were processed for removal of background colors and for sharper bands before analysis using Gel Compare-II software (Applied Maths, Belgium).

RESULTS

ERIC profiles of community soil DNA

The community soil DNA profiles of the soil samples from surface and subsurface soil layers of the three study sites as revealed by the ERIC-PCR are depicted in figure 1. The fingerprint of the ERIC elements shows the presence of a variety of community soil DNA derived from various organisms predominantly microorganisms in these soils. The surface soil layer (DF) of the degraded site showed minimum number of ERIC bands as compared to other samples. The surface (MDF and UDF) and subsurface (mdf and udf) soil layers of moderately degraded and undegraded forest sites displayed more dense and higher number of ERIC bands than other soil samples. Gel compare analysis (Dice similarity coefficient-UPGMA based dendrogram) of the community soil DNA from ERIC-PCR fingerprints resulted in two groups of genomic diversity (figure 3A). Group I consists of the repetitive intergenic consensus sequences from surface (DF) and subsurface (df) soil layers of the degraded site. Group II consists of the community soil DNA from surface (MDF and UDF) and subsurface (mdf and udf) soil layers of moderately degraded and undegraded sites respectively. The surface layers formed the subgroup IIa while the the ERIC bands from subsurface soil layers formed subgroup IIb. There was a subdivision of the two soil layers into subgroups, IIa and IIb respectively. Therefore, there was a clear discrimination between the soil layers layers of moderately degraded and undegraded sites and between degraded site and other two sites in terms of community soil DNA composition represented by ERIC-PCR fingerprints.

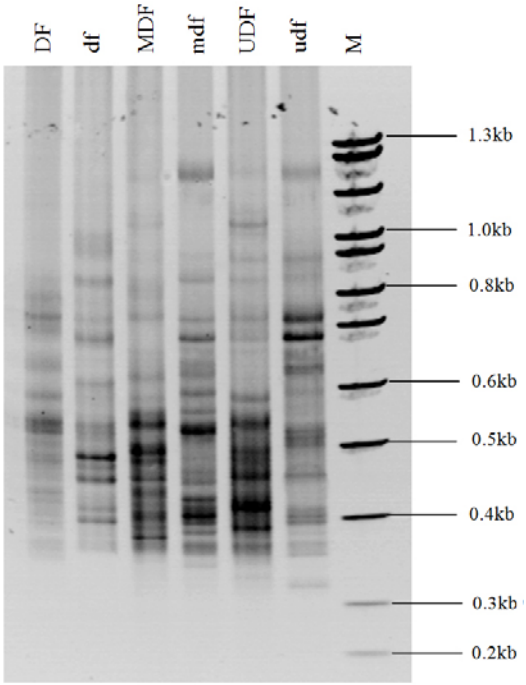

Figure 1. Community soil DNA profiles of six soil samples on PAGE after ERIC-PCR. Surface soil layer (DF) and subsurface soil layer (df) of degraded site; surface soil layer (MDF) and subsurface soil layer (mdf) of moderately degraded site; surface soil layer (UDF) and subsurface soil layer (udf) of undegraded site. M=1.3 kb DNA ladder

DGGE profiles of 16S rDNA

The PCR products of 16S rDNA from the surface and subsurface soil layers of the three study sites produced a clear band of 473 bp against a 1kb ladder as shown in figure 2(A). The purified product of this PCR amplicon was used for separation of the different 16S rDNA nucleotide sequences by DGGE.

The DGGE profiles of the 16S rDNA sequences of the six soil samples are shown in figure 2(B). Gel compare analysis of the 16S rDNA sequences in all the soil samples showed clear variation among the soil samples of the three study sites (figure 3B). The dendrogram of the 16S rDNA-DGGE bands resulted in three different clusters. The group I consist of 16S rDNA bands from surface layer (DF) of degraded site. The cluster II is formed by surface (UDF) and subsurface (udf) layers of undegraded forest site. Similarly, the cluster III is formed by three soil samples and subdivided into two subgroups, IIIa and IIIb respectively. The subgroup IIIa consists of surface (MDF) and subsurface (mdf) soil layers of the moderately degraded site. The subgroup IIIb consists of only 16S rDNA bands derived from the subsurface (df) soil layer of degraded site. There was a 91% similarity index of the banding patterns (16S rDNA bands) between the cluster II and III and 86% among the three cluster groups in this analysis.

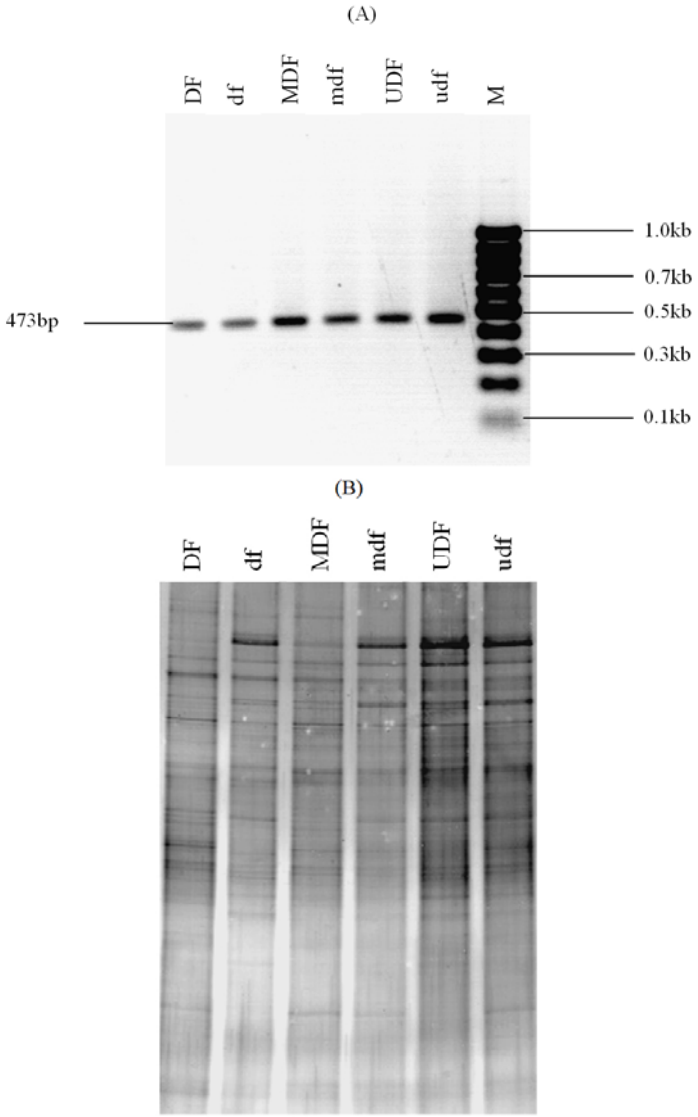

Figure 2. (A) 16S rDNA band (473bp) on 1% agarose gel. (B) DGGE profiles of 16S rRNA bands. Surface soil layer (DF) and subsurface soil layer (df) of degraded site; surface soil layer (MDF) and subsurface soil layer (mdf) of moderately degraded site; surface soil layer (UDF) and subsurface soil layer (udf) of undegraded site.

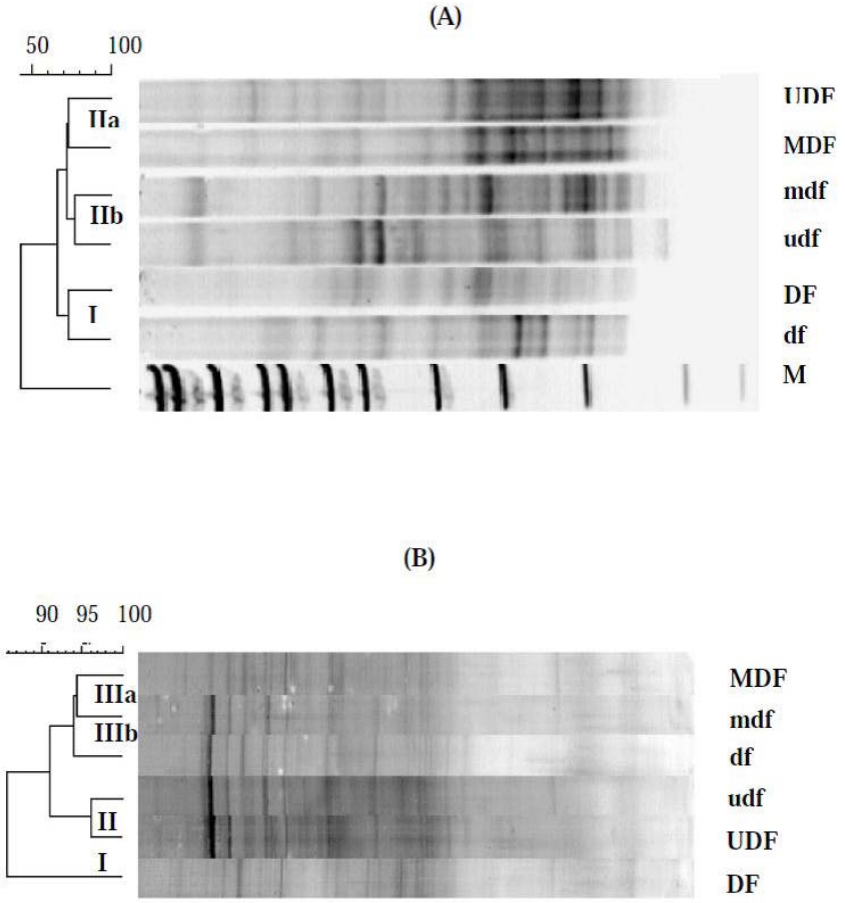

Figure 3. (A) Dendrogram of ERIC-PCR amplified DNA bands (Dice similarity coefficient- UPGMA). (B) Dendrogram of 16S rDNA bands. Surface soil layer (DF) and subsurface soil layer (df) of degraded site; surface soil layer (MDF) and subsurface soil layer (mdf) of moderately degraded site; surface soil layer (UDF) and subsurface soil layer (udf) of undegraded site. M = DNA ladder (1.3 Kbp)

DISCUSSION

Diversity of community soil DNA

Generally, ERIC bands are expected to be generated from microbial genomes, particularly gram negative enteric bacteria and closely related phyla (Giovanni et al 1999a; Hulton et al 1991; Versalovic et al 1991). However, presence of the ERIC elements only in the bacterial genome had been disagreed since ERIC primers sometimes acts as RAPD primers to amplify any DNA under low stringency PCR conditions (Gillings and Holley 1997; personal communication with G.D. Di Giovanni). Generally, soil is a heterogenous matrix of various genomes comprising of DNA derived from plant, animal and predominantly microorganisms, the ERIC elements obtained in the present study were considered to be total community soil DNA with more emphasis on bacterial sources.

Use of Dice similarity coefficient index takes into account of the band position together with more weight on matching bands when the data was analysed for discrimination of variables to form groups with highly similar fingerprints in such a way that the fingerprints in different groups are as much as dissimilar as possible (Rademaker and de Bruijn, 1997). The results from the gel compare analysis of ERIC-PCR products clearly indicated that the soil in the degraded forest site is quite less in composition in terms of total community soil

DNA bands as compared to moderately degraded and undegraded forest sites. The degraded site with comparatively less number of ERIC bands are separated from moderately degraded and undegraded forest sites with more number of ERIC bands. The surface and subsurface soil layers of degraded site in cluster I revealed presence of approximately less number of microbial genomes and less diverse groups in terms of community soil DNA contents represented by ERIC elements. However, the separation of the moderately degraded and undegraded forest sites in a separate cluster (group IIa & IIb) suggests that more number of ERIC bands and similar diversity of community soil DNA existing in these two sites despite clear difference between the surface and subsurface soil layers. It can be mentioned here that the degraded site in the present study was a regenerating jhum fallow land (shifting cultivated fallow). This site had been used for about 15 years for jhum during which the soil had been disturbed with repeated burning, tillage and cropping. Moreover, this site being a hill slope, the top soil has been lost considerably during the course of cultivation in addition to runoff losses through heavy rainfall during summer seasons in the region. The surface layer of the soil shows very thin Ah horizon and characterised by increased bulk density. Vazquez et al (1993) have reported suppresses growth of aerobic bacterial population due to compaction of soil following forest burning of an Atlantic soil as compared to unburnt soil. Changes in physiological groups and even complete sterilization of microorganisms have been reported after forest wild fire in an Atlantic soil and soil heating in a pine forest stand. The degraded site supports no more favourable soil environment conditions for growth and multiplication microorganisms in absence of required moisture and nutrients in the form of humus or organic matter as revealed by reduced organic carbon contents (table 2). These cnditions might have caused decline in community soil DNA population and diversity in this site as compared to undegraded forest site where no soil disturbance has occurred.

The moderately degraded forest site with no cultivation history in the past was disturbed by selective logging of forest timber and routine silviculture practices. There was removal of ground vegetal cover through cleaning and subsequent burning of the dried biomass on the forest floor for every year or alternate year by forest personnel. These practices must have a profound impact on the distribution of community DNA in the soil resulting in an altered diversity status as compared to the subsurface layer where impact of fire and other disturbance are less. The undegraded forest site on the other hand had a similar composition of total genomic composition to the moderately degraded site at both the surface and subsurface soil layers. This suggests that selective logging has lower impact on the distribution of soil community DNA as compared to shifting cultivation or jhum. Decline in population and diversity of bacteria and fungi as measured by cultivation and culture independent methods have been reported from degraded forest soils (Singh 2002).

Bacterial diversity as revealed by 16S rDNA–DGGE profiles

The 16S rDNA sequence pattern of the different soil samples showed different profiles of bacterial genomic diversity. Since, the DNA sequence of the 16S rDNA is derived from the bacterial communities using specific primer, the DNA bands are expected to represent the available bacterial communities in the soil samples. The gel compare analysis uses the number of DNA bands available in a particular lane which represents a soil sample in the gel image. The cluster analysis is based on the number and similarity of bands in each lane with respect to other lanes. Therefore, the resulting classifications of the clusters are the indices of similarity or dissimilarity of the bacterial genomic compositions among the samples. The similarity index of the cluster analysis reveals that 91% of the total genomic diversity is same or 9% dissimilarity among degraded, moderately degraded and undegraded forest sites at both the surface and subsurface layers. However, the surface and the subsurface soil layers of the undegraded site had the maximum similarity index of 96% or the least dissimilarity value of only 4%. This reveals that the bacterial communities between the two soil samples were more similar than the rest of the samples in terms of genetic makeup. This suggests that the microbial communities in the undegraded site possess intact natural population and higher diversity while degraded and moderately degraded sites supports altered microbial communities in terms of 16S rDNA profiles following shifting cultivation and selective logging practices. The surface soil layer of the degraded site displayed minimum 16S rDNA bands in comparison to the other soils thereby revealing reduced microbial composition as a result of consistent jhum cultivation in the past. The lower percentage of similarity index (86%) or higher value of dissimilarity index (14%) in the number of bands and banding patterns of soil samples in this site than other soil are direct influence of lowered microbial population as well as less diversity of bacterial community inhabiting in this disturbed site. These results are in correspondence with the lower bacterial population of the degraded site

determined by cultivation method in this site (Singh 2002). Clear variation in bacterial count, physiological function and molecular diversity (16S rDNA-DGGE) have also been reported from intensively managed horticultural site and another recently established organically farmed soils in southern Italy (Crecchio et al 2004).

The surface and subsurface soil layers of the moderately degraded site and subsurface layer of degraded site were clustered in one group revealing that these soils contained similar bacterial communities different from the other soil samples. It may be mentioned that the surface soil layer of moderately degraded site has also been disturbed for every year or for alternate year by clear-cutting of ground vegetation and burning of the forest floor biomass for proper growth of the new plantations. This practice must have some detrimental impact on the bacterial communities at the surface soil layers though the impact was comparatively lower at the subsurface soil layers, hence less disturbance in the bacterial community structure. Lundgren (1992) also reported a strong and long term detrimental effect of clear cutting of forest on the bacterial population of Ao horizon as compared to an undisturbed natural forest stand.

Therefore, a conclusion may be drawn that shifting cultivation or jhum practice, as a dominant agricultural practice in the north-eastern hill forest soils, has a long term detrimental impact on the distribution of community soil DNA and bacterial genomic diversity as revealed by ERIC-PCR and 16S rDNA-DGGE profiles. The cluster analysis of the DGGE bands of 16S rDNA fragments displayed a clear separation of the degraded and moderately degraded forest sites from that of the undegraded site in terms of community soil DNA content and bacterial diversity. It is clear from the present study that shifting cultivation causes a severe impact than selective logging practices on the distribution of total soil DNA content as well as bacterial genomic diversity in the long term. The use of ERIC-PCR and 16S rDNA-DGGE as culture independent techniques can be successfully applied to the assessment of impact of environmental degradation on microbial communities in soil.

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