

CHARACTERISATION OF MICROBIOTA COMMUNITY IN SOILS OF DIFFERENT RICE ECOSYSTEMS IN MEGHALAYA

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BY

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ABBREVIATIONS

%	Per cent
μ	Micron
μg g ⁻¹	Microgram per gram
μg	Microgram
μl	Micro liter
μM	Micro-molar
16S rRNA	Small sub-unit of ribosomal ribonucleic acid
23S rRNA	Large sub-unit of ribosomal ribonucleic acid
ANOSIM	Two-way analysis of similarity
ANOVA	One-way analysis of variance
<i>a_w</i>	Water activity
BAC	Bacterial artificial chromosome
bp	Base pair
BSA	Bovine serum albumin
C	Carbon
Ca	Calcium
CEC	Cation exchange capacity
CFU	Colony forming units
CLLP	Community level physiology profile
cm	Centimetre
CO ₂	Carbon dioxide
D/W	Drying and wetting
DEA	Denitrifying enzyme activity
DGGE	Denaturing gradient gel electrophoresis
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleosidetriphosphate
EBT	Erichrome black T
EDTA	Ethylene diamine tetra-acetic acid
F-ARISA	Fungal-automated ribosomal intergenic spacer analysis
Fe	Iron
g	Gram
h	Hour
ha	Hectare
HSD	Honestly significance difference
ITS	Intergenic spacer analysis
K	Potassium
kb	Kilo-base
kg	Kilo-gram
KPa	Kilo-Pascal
L	Litre
LFOM	Light fraction organic matter

LSD	Least significance difference
m	Metre
M	Molar
MBC	Microbial biomass carbon
MBN	Microbial biomass nitrogen
MC	Moisture content
Mg	Magnesium
mg	Milligram
MgCl ₂	Magnesium Chloride
Min	Minute
mm	Millimetre
MOB	Methane oxidizing bacteria
MPN	Most Probable Number
msl	Mean sea level
N	Normality
N ₂	Nitrogen
Na	Sodium
NaCl	Sodium chloride
ng	Nanogram
nM	Nanomolar
nm	Nanometer
NMDS	Non-metric multidimensional scaling
°C	Degree celsius
PAGE	Polyacrylamide gel electrophoresis
PCA	Principal component analysis
PCR	Polymerase chain reaction
Pg	Pico-gram
PLFA	Phospholipid fatty acid analysis
PRIMER	Plymouth Routines In Multivariate Ecological Research
R	Rao's statistic
<i>r</i>	Correlation coefficient
RAPD	Randomly amplified polymorphic DNA
rDNA	Ribosomal deoxyribonucleic acid
RISA	Ribosomal intergenic spacer analysis
RNA	Ribonucleic acid
rpm	Rotation per minute
rRNA	Ribosomal ribonucleic acid
SMB	Soil microbial biomass
SOM	Soil organic matter
SPSS	Statistical Package for Social Sciences
sq km	Square Kilo-meter
SSCP	Single strand conformation polymorphism
TAE	Tris-acetate

TN	Total nitrogen
TOC	Total organic carbon
tRFLP	Terminal-restriction fragment length
Tris	Tris (hydroxymethyl) aminomethane
V	Volt
w v ⁻¹	Weight per unit volume
WEOM	Water extracted organic matter
wt	Weight

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CHAPTER 1

Introduction

Soils form on surface of the earth and they act as nature's integrators by means of cycles of matters and transfer of energy. Soils interact reciprocally with the biosphere, hydrosphere, lithosphere and atmosphere (McGill, 2007). Such interactions involve biological, chemical, biochemical, and physical transformations and biological and physical translocations. Microorganisms are key component of soil biota (Pace, 1997) and they are intimately involved in biological translocations and biochemical transformations. Hence, physiology and biochemistry of soil microorganisms is fundamentally important in understanding ecosystem functioning. Ecosystem functioning is governed largely by soil microbiota (Schimel, 2007). The functional processes such as nutrient cycling, residue decomposition, and soil structure formation are regulated by soil microbiota communities and thereby, they regulate the productivity and health of agricultural systems (Kennedy and Papendick, 1995; Pankhurst *et al.*, 1996). Microorganisms not only influence but also regulate the rate of organic matter decomposition. Besides, they are inherently involved in N-transformation reactions including N₂-fixation and N-loss, oxidation-reduction processes, precipitation and mobilization of Fe, clay mineral transformation and alteration in surface charge characteristic as well as aggregation in rice soils (Dobermann and Witt, 2000). These key soil processes affect and redeem nutrient supply to crop roots, normalize the rhizosphere chemistry and root and plant growth. Microorganism and their activities are the main mediators of C and N turnover in the soil. Dynamics of microbial biomass is regulated by substrate and water availability, temperature, climatic regime and soil structure (Insam, 1990) is considered as transformation agent for soil organic matter and a labile pool of organic and inorganic nutrients (Jenkinson and Ladd, 1981). Recent advanced findings on soil biochemistry, microbial eco-physiology and biogeochemical

cycles have strongly indicated that soil organisms especially microbiota community perform the biogeochemical transformations that determine ecosystem C and N cycling rates (Paul, 2007). For example, dynamics of C and N are known to interact closely during decomposition due to simultaneous assimilation of C and N by the heterotrophic soil microbiota (Sall *et al.*, 2007). Hence, its role in providing mineral nutrients and transforming organic nutrients into plant-available forms could be critical, especially in low fertility ecosystem, like tropical rice culture.

Dependence of nutrient remobilization on C mineralization processes has instigated appropriate conceptual models in C and N cycling (Reichardt *et al.*, 2000). These being intimately interlinked by biological functions, explain the dynamic interplay of microbial populations at community levels. This further underlines the regulatory roles played by microbial communities and signifies the interactive credentials of independent microbial groups in providing the nutritional benefits to the plant. Therefore, it is essential to generate information on microbial diversity and quantification of variability in the microbial community composition to better understand their functional role on regional and landscape level differences in biogeochemical cycling. Over last decade, innovative molecular approaches to the study of natural microbial communities and the functional genes responsible for biogeochemical processes have given us new insight into this relationship (Paul, 2007). One such approach is the use of 16S rRNA gene to characterise microbial community diversity. Therefore, 16S rRNA based molecular characterisation of microbial communities would be of enormous value if the presence of a particular sequence or organism could be related unambiguously to its function in the soil.

Rice is staple food for more than half the world's population (Nguyen and Ferrero, 2006). Rice is commonly cultivated under three different eco-systems namely slope land direct-seeded, transplanted upland terrace and valley land/ or low land in the north-eastern hilly states of India (Tripathy *et al.*, 2007). These rice based ecosystems are likely to be different from each other in terms of ecosystem functioning because of differences in soil types, soil water content and

availability, slopes, above- and below-ground biomass, and anthropogenic activities. Previous work has shown that variability in microbial community composition can be caused by alteration in plant species composition (Grayston *et al.*, 1998; Haichar *et al.*, 2008), management practices (Calderon *et al.*, 2001; Reeve *et al.*, 2010), soil type (Schutter *et al.*, 2001), and seasonal variability in water, temperature, and substrate availability (Bardgett *et al.*, 1999; Steinberger *et al.*, 1999). Thus, it was hypothesized that microbial community composition might vary according to rice ecosystems reflecting impacts of management practices and soil attributes on microbial diversity. To date, there is lack of information available on microbial community composition based on 16S rRNA analysis approach in soils of rice ecosystems in the NE region of India.

Keeping the above facts in mind an attempt had been made to characterize the microbial community composition in soils of different rice ecosystems with the following objectives:

- 1. To determine diversity of bacterial and fungal communities in soils of direct-seeded hill slopes and transplanted upland terrace and low land rice fields.**
- 2. To determined interrelationship among soil attributes with bacterial and fungal community structure.**

CHAPTER 2

Review of Literature

“Everything is everywhere, the environment selects.”

Martinus Beijerinck (1913)

Microorganisms are perhaps the most diverse (Torsvik *et al.*, 2002) and abundant (Whitman *et al.*, 1998) type of organism on earth surface. However, distribution of microbial diversity at ecosystem level is poorly understood. It is well known that each unique ecosystem maintains a set of environmental factors that exert influences on biological components of the ecosystem. In the recent years, several studies have attempted to verify the above-stated quote of Beijerinck or specify which environmental factors exert the influences on microbial communities in nature (Wieland *et al.*, 2001; Steenwerth *et al.*, 2002; Papke and Ward, 2004; Horner-Devine *et al.*, 2004; Haichar *et al.*, 2008; Hartman *et al.*, 2008; Wakelin *et al.*, 2008; Jesus *et al.*, 2009). Knowledge on microbial biogeography is relatively weak for soil ecosystems compared to that in aquatic ecosystems, even though the diversity and composition of soil microbial communities is thought to have a direct influence on a wide range of ecosystem processes (Paul, 2007). With the advent of ribosomal DNA analysis methods that permit the characterization of microbial communities without culturing (Pace, 1997; Jackson *et al.*, 2002), it is now possible to examine the full extent of microbial diversity and describe the bio-geographical patterns exhibited by microorganisms at ecosystem level. Hence, recent trends in soil microbial ecology will be reviewed with special emphasis on cataloguing the diversity of soil microbes and documenting how their communities are affected by specific environmental changes or disturbances.

2.1 The concept of a community

The concept of community ecology originally used in plant and animal ecology studies. Communities are defined as multi-species assemblages, in which organism live together in a contiguous environment and interact with each other. This discipline seeks to analyze how biological assemblages are structured, how community structure changes in space and time, and what are their functional interactions. A community can be looked as a “supra organism” which has a well defined level of organization with tight interactions among organisms that comprised a causal system and give rise to emergent properties (Clements, 1916). The alternative individualistic concept of community is that many species co-occur in a habitat because they tolerate similar physical and chemical conditions and do not necessarily react with each other (Gleason, 1926). The practical delineation of ‘community’ may then reflect the interest of the ecologist rather than any inherent characteristics.

2.2 The microbial community ecology

The concerted activity of interacting microbes is crucial in catalysing and controlling of biogeochemical cycles operating within an ecosystem (Konopka, 2009). Revision of recent literatures revealed that current ecosystem simulation models (for example nutrient cycling in agroecosystems) do not include microbial composition, and often neither explicitly considers the effects of environmental conditions on microbial activities nor the interactions between diverse microbial processes (Bardgett *et al.*, 2008). As microbial communities are of primary importance in biogeochemical transformations, an in-depth understanding of microbial composition and their dynamics is necessary in predicting the functioning of an ecosystem. In this context, application of modern technologies in genome-enabled biology and analytical chemistry to concurrently study both the identity and the function of complex microbial communities is need of hour (DeLong *et al.*, 2006; Musat *et al.*, 2008). However, effective application of such

approaches requires a clear delineation of what is meant by a ‘microbial community’ and identification of important characteristics specific to community ecology.

Konopka (2009) while described the microbial community ecology stated that the delineation of a ‘contiguous environment’ and the meaning of ‘interact’ may be problematic in microbial community. Microorganisms can influence conditions in their microenvironments, which usually have length scales of microns rather than millimetres (Young *et al.*, 2008), except in cases of multicellular structures such as fungal hyphae. However, the substrate consumption and metabolic products pattern can generate chemical gradients over meter scales depending on water-saturated sediments, density-stabilized aquatic water columns and water availability in soils (Wakeham *et al.*, 2007). As a result, these functional groups of microbes metabolically ‘interact’ over many meters that lead to spatial variability in microbial community.

Recently, it has been realised that in community ecological studies the biological assemblage cannot be defined without reference to its abiotic environment. A comprehensive understanding of interrelationship between microbes and their microscale physical and chemical environments is particularly important for delineation of microbial communities (O’Donnell *et al.*, 2007). So, it may be useful to define microbial communities not from a macroscale perspective (for example aquatic vs. terrestrial habitats), but rather based on a bottom-up analysis of the physico-chemical characteristics of the microenvironment, with up-scaling to a spatial domain (the ‘contiguous environment’). This approach should ensure adequate analysis of the local physico-chemical environment (Young *et al.*, 2008). Konopka (2006) defined four ecosystems derived by considering the environment from the microbe’s local perspective (Table 2.1). Each has particular characteristics that define important selective forces in that habitat, but which also impact the spatial scale over which microbial interactions occur.

Table 2.1 Microbial-scale ecosystems

Ecosystem type	Examples	Characteristics
Planktonic	Open ocean, lakes	Oligotrophic lifestyle
surface-associated saturated water	Freshwater and ocean sediments, subsurface sediments, microbial mats, biofilms	Gradient lifestyle
Surface-associated, unsaturated water	Surface and vadose zone soils	Water availability as limiting factor for activity, pH and dispersal of patchy nutrient distribution
Macro-organism associated	Gastrointestinal tract, rhizosphere, epiphytes	Co-evolution, specific molecular interactions with surface-associated molecules

Source: Konopka (2006)

2.3 Methods in studying microbial community composition

One gram of soil may contain 10 billion microorganisms and thousands of species (Torsvik *et al.*, 1990), making soil the most biologically diverse habitat on earth. There are more than 10^{16} prokaryotes in a tonne of soil compared to a mere 10^{11} stars in our galaxy (Curtis and Solan, 2005). Not only are soil microbial communities large, but they are also extremely diverse. Torsvik *et al.* (2002) estimated that 100 cm^3 of soil contains 10,000 different microbial species. Recent studies have revealed that, on a species level, soil is an extremely diverse habitat, with species richness estimates ranging between 2,000 and 52,000 (Tringe *et al.*, 2005; Roesch *et al.*, 2007). Microorganisms exhibit an impressive diversity in their metabolic activities and in their interactions with other microbes, plants, and animals. Because of such high intrinsic structural and functional diversity, that in the past, it was nearly impossible to discern underlying patterns of microbial communities within natural and agricultural ecosystems. Historically, soil microbiologists lacked the tools needed to examine these communities.

2.3.1 Cultural method

Microbial ecologists commonly used counting techniques such as the plate count technique or the most probable number (MPN) technique for estimation of microbial diversity before the advent of ribosomal DNA based methods, (Bakken, 1997; Johnsen *et al.*, 2001). Thus investigators have determined the changes in the number of specific taxonomic or functional groups by plating on agar media, and have assessed precisely the culturable diversity by isolating colonies from these media followed by identification by a variety of typing methods (Vandamme *et al.*, 1996). They have assessed the gross diversity of culturable microorganisms by plotting the different colonies identified on the medium against incubation time (Ishikuri and Hattori, 1985). However, the approach of determining microbial diversity from the number of isolates has become less popular, not only because a limited number of microorganisms can be cultured

but also because the procedures are laborious. As a consequence, an increase number of molecular techniques are now popular because they do not rely on cultivation of microorganisms (Johnsen *et al.*, 2001). It is well established that plate counts estimate only 1-10% of the overall soil microflora (Amann *et al.*, 1995). The discrepancy is essentially due to the interdependency of different organisms upon each other, to the inability to create in pure culture the environmental conditions faced by microorganisms in the soil environment, and to the fact that some microbial species are cultivable only under certain physiological conditions (Bakken, 1997; Muyzer and Smalla, 1998; Heuer *et al.*, 2001). Generally the choice of the growing medium markedly affects the colony formed (Sorheim *et al.*, 1989; Johnsen and Neilsen, 1999). Small bacteria cells (dwarf cells or ultramicrobacteria) are not culturable, that is, they cannot form colonies in agar. By considering that larger cells are supposed to account for about 80% of the bacterial volume. Bakken (1997) hypothesized that the abundant culturable bacteria have more ecological significance in soil than that which appeared from their small number.

Direct counting by fluorescent microscopy can give 100-1000 times more than the numbers obtained by plate counting (Johnsen *et al.*, 2001). Bloem *et al.* (1995) improved the direct counting method with a video camera on an epifluorescence microscope. However, this procedure does not allow counting specific microbial species, and some of the stains used do not discriminate between living and dead microbial cells.

2.3.2 Culture independent method

A range of molecular methods based on analysis of ribosomal rRNA genes (the ultimate molecular chronometers, Woese, 1987) as phylogenetic marker are contributing to our understanding of the diversity of both culturable and unculturable microbes in various habitats over the last three decades. Carl Woese demonstrated that the sequence of small subunit of ribosomal RNA molecule could be used as a molecular chronometer to analyze the phylogenetic

relationships among all forms of life. Norman Pace took this seminal idea and applied it to the analysis of ‘who’s there’ in natural environments. That is, by isolation of nucleic acids (*in-situ* lysis or cell extraction methods) from a natural environment, amplification of small subunit rRNA gene sequences by polymerase chain reaction (PCR), DNA sequencing of the amplicons, and comparison of the retrieved sequences to the ever-growing database of sequences from pure cultures and environmental nucleic acids. Moreover, rRNA genes have highly conserved sequence domains interspersed with hypervariable regions and it is these variable domains that can distinguish one microbe from another and therefore, can be used as molecular markers to discriminate among taxa. Some of the commonly used DNA fingerprinting techniques in analysing microbial community composition are denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), terminal restriction fragment length polymorphism (tRFLP), automated ribosomal intergenic spacer analysis (ARISA) (Muyzer *et al.*, 1993; Muyzer and Smalla, 1998; Heuer *et al.*, 1997; de Liphay *et al.*, 2004; Martin-Laurent *et al.*, 2001; Ranjard *et al.*, 2001; Luna *et al.*, 2006; Thakuria *et al.*, 2009, 2010).

Depending on sensitivity of methods one can resolve microbial diversity at species, genera, families or even higher taxonomic level. Low-resolution methods include the analysis of base distribution of DNA and the determination of rates at which denatured single stranded DNA reassociates when temperature is lowered to approximately 25⁰C below the DNA melting point (Torskvik *et al.*, 1994; 1996). These techniques give an overall indication of microbial diversity and can be used to monitor overall changes in the composition of microbial communities after stress or changes in environment.

Detection of microbial community at the species and sub-species level can be achieved by employing high resolution method that generate ‘fingerprints’ of non-coding DNA regions or involve the sequencing of both coding and non-coding regions. These techniques include polymerase chain reaction (PCR) amplification of sequences between repetitive elements, ribosomal intergenic spacer analysis (RISA, which is based on the length polymorphism of the spacer

region between 16S and 23S rRNA genes, Borneman and Triplett, 1997), and randomly amplified polymorphic DNA (RAPD) which does not require a preliminary knowledge of the genome (Nei and Li, 1985; Harry *et al.*, 2001). In RISA, PCR products are separated by gel electrophoresis, and the separated bands can be sequenced. A limit of this technique is the number of spacer sequences in the database. Borneman (1999) monitored microbial community composition by RISA fingerprinting using bromodeoxyuridine labeled soil DNA to observe the impact of nutrient addition to soil. Recently, Thakuria *et al.* (2010) used automated RISA technique to analyse bacterial community compositions associated with gut-walls of various feeding groups of earthworms.

Among the intermediate resolution methods, DGGE and TGGE are widely used for characterizing microbial communities in environments (Heuer *et al.*, 2001). They are rapid and simple (Muyzer and Smalla, 1998; Heuer *et al.*, 2001). The electrophoretic mobility of DNA fragments in polyacrylamide gels within a linear denaturant gradient depends on the base composition of the DNA (Muyzer and Smalla, 1998; Heuer *et al.*, 2001). These techniques allow us to differentiate two molecules differing at the level of single base (Muyzer and Smalla, 1998). In addition to the simplicity and rapidity, another advantage of DGGE or TGGE is that the identity of bands can be investigated by hybridization with specific probes or by gel elution and re-amplification followed by cloning and sequencing (Muyzer and Smalla, 1998).

Recent development in molecular microbial ecology studies includes the creation of bacterial artificial chromosomes (BAC) for metagenomic approach and the DNA microarrays (Rondon *et al.*, 2000; Tiedje *et al.*, 2001). The former technique clones high molecular weight soil DNA which can be analysed at a phylogenetic and functional levels. Microarrays of DNA are powerful for rapidly characterizing the composition and function of microbial communities because a single array can contain 500-1000 different DNA spots with the possibility of very broad hybridization with a broad identification capacity (Tiedje *et al.*, 2001). Analyses of 16S rRNA gene sequences from environmental DNA enable us to

discover the extraordinary richness of phylotypes in many microbial habitats (Ley *et al.*, 2006; Walker and Pace, 2007).

2.3.3 Denaturing gradient gel electrophoresis (DGGE) fingerprinting

In principle, DGGE fingerprinting method is the separation of a mixture of about equal size 16S rDNA fragments based on sequence variation by electrophoresis in a polyacrylamide gel containing linearly increasing gradient of denaturants (Muyzer *et al.*, 1993; see Fig. 2.1 that depicts flow diagram of steps involved in DGGE fingerprinting). Unlike most commonly used electrophoresis methods that separate nucleic acid fragments by size, DGGE separates DNA strands by their sequence composition (Hovig *et al.*, 1991). Differential migration of equal size fragments occurs because more denaturant is needed to separate sequences with higher guanine (G) + cytosine (C) content due to differences in the number of H bonds between complementary nucleotides holding DNA strands together. There are three H bonds between guanine and cytosine, and only two between adenine and thymine. As DNA strands separate, their migration becomes retarded in the gel. To produce sharp bands, fragment migration is stabilized by adding a high GC sequence (GC clamp) to the end of one PCR primer, thereby preventing complete strand separation (Sheffield *et al.*, 1989). If a mixture of PCR products with different sequences is amplified from a sample, then this type of gel will separate them during electrophoresis. The resulting genetic profiles or fingerprints represent the community structure, an approximation of the numbers of populations (represented by each band) and their relative abundance (represented by band intensity) within the amplified community. Because PCR is the first step in DGGE, the fingerprint profiles are representative of the proportion of PCR products, which may not directly correlate to the proportion of that population within the community (Chandler *et al.*, 1997).

Although there are many advantages to using PCR-DGGE for microbial community analysis, method limitations must be recognized for correct result interpretation. The greatest challenge in performing DGGE and attaining

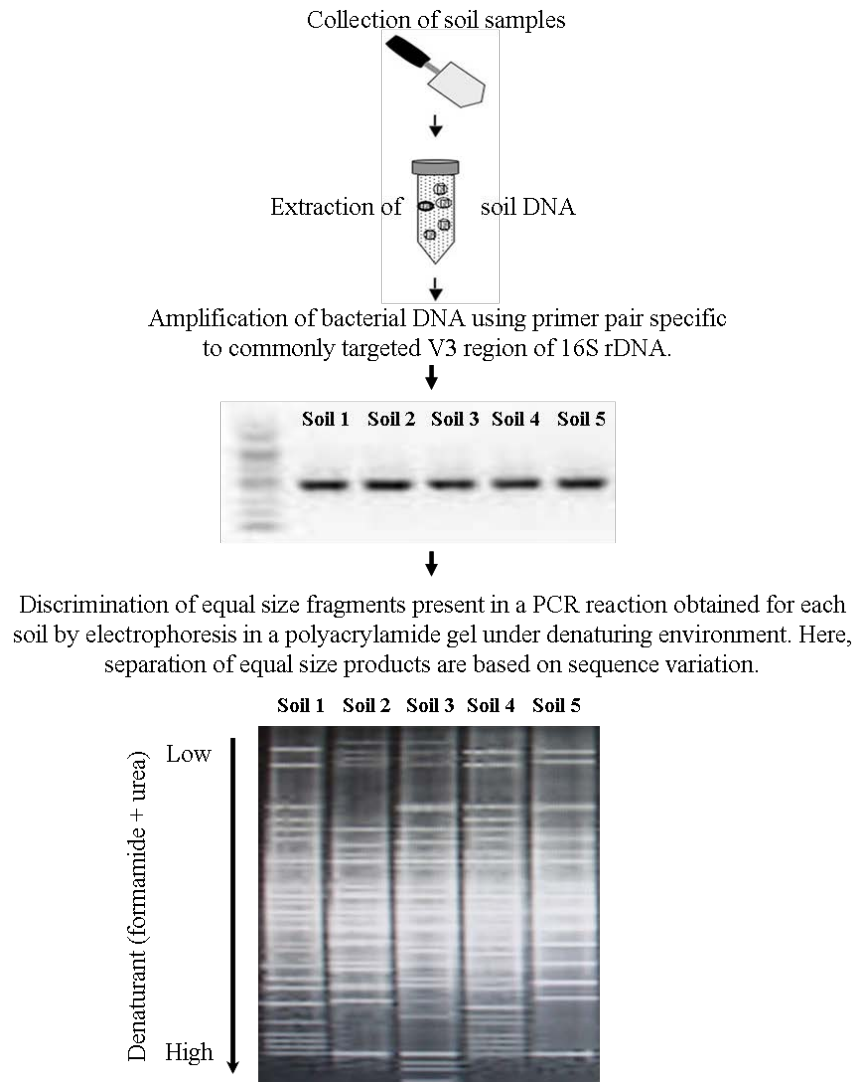


Fig. 2.1 Flow diagram showing the steps involved in developing denaturing gradient gel electrophoresis fingerprinting for analysis of microbial community composition (Adapted from Thakuria *et al.*, unpublished data)

reproducible results is minimizing variation between gel gradients (Fromin *et al.*, 2002). It is very difficult to exactly reproduce gel gradients; this must be kept in mind when performing between-gel comparisons. Another limitation of DGGE is that the number of bands observed in a profile cannot be interpreted to be the exact numbers of populations in a community. In some cases investigators have found that a single laboratory isolate can produce multiple bands by DGGE (Nubel *et al.*, 1996; Satokari *et al.*, 2001), and conversely a single band may represent multiple populations (Young and Crowley, 2000). Combining DGGE profiling with other techniques, such as sequencing of bands or hybridization with probes can reduce the ambiguity of band identification (Stephen *et al.*, 1998). Nevertheless, the method can estimate of richness that enables researches to determine subsequent analyses that can be conducted and a means of choosing samples representing unique or representative communities (Joynt *et al.*, 2006).

2.4 Factors of change in soil microbial community composition

2.4.1 Land use type

Agricultural practices and changes in environment have different effects on microbial community composition. Recently, Zornoza *et al.* (2009) studied the changes in soil microbial community structure following the abandonment of agricultural terraces in mountainous areas of Eastern Spain. Authors observed a substantial level of differentiation in microbial community structure according to land use. The microbial communities of forest soils were highly different from that of abandoned agricultural soils. However, they have not found any physical or chemical soil property capable of explaining the differences in soil microbial communities between agricultural and abandoned agricultural soils. The cessation of the perturbation caused by agriculture and shifts in vegetation may have led to changes in the microbial community structure. PLFAs indicative of fungi and ratio of fungal to bacterial PLFAs were higher in abandoned agricultural soils, whereas the relative abundance of bacteria was higher in agricultural soils.

Jesus *et al.* (2009) demonstrated the impacts of agricultural management practices on structure and composition of amazonian soil bacterial communities. Main differences in bacterial community structure were related to changes in the soil attributes that, in turn, were correlated to land use. McCulley and Burke (2004) reported that there were no differences in microbial biomass at the landscape-scale, and the only alteration in microbial community composition between upland and lowland landscape positions was a shift toward more nonspecific bacteria in lowlands. Conventional and no-till farming systems may support different soil microbial communities (Petersen *et al.*, 2002).

2.4. 2 Soil moisture content

Perhaps the most important physiochemical factor that affects microbes in soil is an adequate supply of available water. As stated earlier, microbes are essentially aquatic organism and even in soil required a sufficiently high water activity (a_w) for growth. Except for short periods after rain, snowmelt, flood or irrigation, most of the soil contains insufficient available water to support microbial activity, as the water is rapidly drained by gravity. Primarily the clay fraction, because of its high surface charge retains water and bestows a degree of permanence to microbes present in this water. Consequently, the apparent correlation between microbial activity and the amount and types in soil may be the result primarily of the fact that this is where the water is, and the role of clays in concentrating nutrients, removing inhibitors, direct surface interactions, may only be secondary in importance (Stotsky, 1986). However, some of the water retained by clays is tightly bound that is a_w is too low for used by microbes (e.g; temperature in excess of 160°C are necessary to removed this water). The amount of such tightly bound water depends on the structure of clay minerals. Consequently, the total amount of water present in soil (as determined by drying) or the amount that a soil can hold (the water holding capacity) does not indicate accurately the amount of water available to microbes. Microbes function best at a water potential of -33-KPa (which is very closed to the field capacity, which is

defined as the amount of water retained by a soil 48 hours after saturation), where no more water will be removed by gravity, and the balanced between the amount of available water and O₂ is optimal for growth. But the water holding capacity of an organic soil (85% organic matter) was 262% (w/w), and this water is tenaciously held and not all is available to microbes (Stotzsky, 1974)

Different groups of microbes have different tolerance to a_w (e.g; fungi can metabolized at asignificantly lower a_w than bacteria). The water content of soil is easily controlled by irrigation, but fine tuning of the manipulation of this physiochemical factor for the differential control of microbes will required considerably more study. In many cases, the effects on microbes of changes in the water content of soil are the result of changes in the O₂ content.

2.4.3 Plant species composition

Plants may release up to 20% of their photosynthesis products into soil, providing the basis for the establishment of plant–microorganism interactions that benefit plant growth by, for example, increasing the availability of mineral nutrients, production of phytohormones, degradation of phytotoxic compounds and suppression of soil-borne pathogens (Bais *et al.*, 2006). These considerations demonstrate the importance of studying the functional properties of soil microbial communities and the ways in which plant species influence bacterial diversity and microbial activities. Several studies indicate that plants have a strong effect on rhizosphere bacterial community composition (Smalla *et al.*, 2001; Kowalchuk *et al.*, 2002; Costa *et al.*, 2006). Haichar *et al.* (2008) determined the bacterial communities assimilating carbon source in the rhizosphere of four plant species viz. wheat, maize, rape and clover using stable isotope probing. They reported that *Enterobacter* and Rhizobiales are generalist colonizers, whereas Sphingomonadales are specific to monocotyledons. *Acidovorax* and *Variovorax* bacteria are actively associated with root exudates of rape, and *Burkholderia* and *Sphingomonadaceae* are associated with root exudates of maize plants.

Soil microbial respiration, biomass and activity were higher under zero tillage, but significant improvement in P-mineralization and increase P-pool are related to increase microbiological activities in reduced tillage soil (Zibelske and Bradford, 2003; Meriles *et al.*, 2009); Recently, Vishwakarma *et al.* (2009) reported that the rhizospheric microbial community undergoes a perceptible influence due to soil type, plant age and cultivation pattern.

2.5 Soil physicochemical properties

2.5.1 Soil reaction (pH)

Soil pH is one of the most influential variables in soil, and is powerful factor in influencing the size, activity and community structure of the soil microbial community (Rousk *et al.*, 2010). Bacterial community structure changed significantly along gradients of pH, base saturation and Al^{3+} concentration and these attributes accounted for about 31% of the observed variation within bacterial community composition in soil (Jesus *et al.*, 2009). Frostegård *et al.* (1993) studied the effects of lime and wood ash application on microbial community structure and demonstrated that bacterial community structure changes were correlated with soil pH changes. Increased pH caused a shift in the bacterial community to more gram-negative and fewer gram-positive bacteria PLFAs. Gram-negative bacteria PLFAs also increase with increasing soil pH (Wu *et al.*, 2009). Similarly, prolonged applications of simulated acid rain, which changed soil pH from 3.83 to 3.65, the structure of soil microbial community changed to a community that better adapted to soil acidity (Pennanen *et al.*, 1998). In general, fungi predominate in acidic soils (below pH 5.5), whereas eubacteria, including actinomycetes, predominate in near neutral or moderately alkaline soils. The apparent lower numbers of fungi in the latter soils might be due to the fact that bacteria are efficient competitors at these pH values and prevent the establishment and proliferation of fungi. In contrast, the lower numbers of bacteria in acidic soils is the result of their intolerance to the elevated

concentration of H^+ that fungi can tolerate, and therefore the fungi can proliferate in the absence of competition (van Elsas *et al.*, 1997). Soil pH can affect the solubility, availability, and toxicity of mineral nutrients, the speciation of heavy metals, the net surface charge of amphoteric materials and the negative charge of ionisable materials. Such effects can influence the adsorption and subsequent binding of microbial cells and organics on surfaces that may lead to uniqueness in the community composition (Babich and Stotzky, 1985; Collins *et al.*, 1989). Rousk *et al.* (2010) took an attempt to elucidate the mechanisms related to pH on microbial community composition. In the experiment, plant materials that especially promote fungal growth (i.e. straw) or bacterial growth (i.e. alfalfa) was added to soil samples maintained at a pH gradient in 5 days laboratory incubations. Also bacterial growth was specially inhibited using bronopol along the entire pH gradient to investigate whether competitive interaction caused the shift in the decomposer community along the pH gradient or not. The author observed a shift in fungal:bacterial growth along pH gradient; however such shifts unaffected by substrate addition. In response to bacterial growth inhibition by bronopol, there was stimulation of fungal growth up to pH 7, but not beyond pH 7, both for alfalfa and straw addition. Authors concluded that the low fungal growth at high pH in an arable soil was caused to a large extent by bacterial competition, and not substrate limitation. From practical view point, manipulation of soil pH is routinely done, although primarily to enhance the growth of plants, by the addition of lime to raise the pH or gypsum or some other relatively cheap sources of sulphur to reduce the pH. A prime example of the manipulation of pH of soil for biological control is the reduction in pH to control a disease (scab) of potato caused by *Streptomyces Scabies*.

2.5.2 Soil organic matter (SOM)

Changes in land use/cover can have marked effect on organic carbon present in soil as a result of the interactions between changes in plant residues and the immobilization of organic carbon mediated by microorganisms (Tate, 1987).

Plant roots are an important source of labile-C (root exudates and other rhizodeposits (Hutsch *et al.*, 2002), and several studies have shown that when plants are present, SOM decomposition can increase up to 380% compared to soil incubations lacking plants (Cheng, 2009). There are only a few studies available which show the influence of changed carbon quality and quantity on microbial community structure. For example, Griffiths *et al.* (1999) demonstrated that the microbial community composition changed with increasing C-substrate supply. Recalcitrant substances within water extractable organic matter (WEOM) may favour those organisms capable of degrading them, while other compounds may act as inhibitors for specific bacterial groups (McNamara and Leff, 2004). Likewise, the composition of WEOM may modulate the microbial community (Bending *et al.*, 2002). Authors reported that the addition of labile-C led to a strong decrease in litter decomposition rates, associated with consistent shifts in the composition of saprotrophic microfungal communities, and to an increase in the overall density of culturable fungal species (r-strategists), but not of specialized cellulolytic microfungi (k-strategists), isolated on Hutchinson agar. Sucrose addition did not affect the number of species isolated but the dominance structure was shifted toward the domination of the 2–4 most abundant species.

2.5.3 Soil texture

Soil texture has profound influence on growth and activity of microbial population (van Veen *et al.*, 1984; Ladd *et al.*, 1992; Muller and Hoper, 2004). The clay fraction is important in imparting specific physical properties to soils, to microbial life, and to plant activity via nutrient availability (Coleman, 2004). No matter what size the clay particle is, microorganisms in unsaturated soil exist in a world dominated by the presence of extensive surfaces. It is possible that labile constituents of organic matter are preferentially adsorbed on to fine clay particles and may be significant source of energy for the soil microbes (Anderson and Coleman, 1985). The thickness of water films in unsaturated conditions allows the microbes little option except to adhere to the surfaces (Mills, 2003). Hence, there

seem to be a general advantage to microbes living at these interfaces in terms of enhanced nutrient concentrations and the potential to use many of the physical substrates themselves as energy or nutrient sources. It is well known that fine textured soils generally have higher organic carbon and nitrogen contents than their coarse-textured counterparts developed under similar vegetation and climate conditions (Jenny, 1941). Such phenomena are significantly differed according to soil textural composition and hence, soil texture can act as a strong determinant of microbial community composition.

Microbial population in different soils varies in composition, age, and physiological and metabolic vigour (Swift, 1976; Kennedy and Gevin, 1997). This is partly shown by the marked variations in the specific respiratory activity of microbial biomass carbon for different soils. Franzluebbers *et al.* (1999) found that coarse textured soils had a more active population than fine-textured soil. On the other hand, many studies confirmed faster turnover rate of soil microbes in sandy soil as compared to clayey soil (Saggar *et al.*, 1999; Muller and Hoper, 2004). Gelsomino *et al.* (1999) found a relationship between the grouping of DNA fingerprints of 16 soils from a broad geographic distribution (from different continents) and soil texture. Soil texture had a larger effect on microbial community composition and activity than did plant species differences in a study of four grasslands across four sites in the northeast United States (Groffman *et al.*, 1996). In another experiment, Johnson *et al.* (2003) found that bacterial DNA fingerprints were significantly correlated with soil texture.

2.5.4 Substrate diversity and quality

Organic substrate quality in soil is an important determinant in regulating the structure and functioning of soil microbial communities (Wardle and Giller, 1996). Quality reflects the biochemical composition of the substrate and the physical availability of those components to degradative microbial community (Swift *et al.*, 1979). Biochemical quality is a key determinant of the rate of decomposition processes, and reflects a number of interacting components. In the

case of crop residue, nitrogen content and structural polymers, notably lignin, interact to control microbial N mineralization-immobilization processes during decomposition (Heal *et al.*, 1997). Defence compounds including phenolics and terpenoids can also influence rates of litter decomposition, by means of direct inhibitory effects on saprophytic organisms (Palm and Sanchez, 1991). Rates of litter decomposition can also be influenced by basic cation content, which may control litter pH during decomposition, affecting the relative activities of bacterial and fungal communities (Cornelissen and Thompson, 1997).

A variety of litter physical quality attributes have been shown to affect rates of mineralization processes. Leaf toughness can correlate highly with decomposition rate, reflecting the mechanical defences against fungal and invertebrate colonization (Cornelissen and Thompson, 1997). Particle sizes can also affect rates of decomposition, although the nature of longevity of such effects appears to depend on the biochemical quality of the substrate (Bending and Turner, 1999). Despite the importance of quality for determining substrate availability to soil microbes, and the well documented effects of quality on rates of mineralization processes, little is known of the impacts of quality of organic matter addition on microbial community composition (Gunapala *et al.*, 1998; Thompson *et al.*, 2001). To date, it is unclear whether such properties affect the structure of communities contributing to decomposition. A number of studies have used metabolic printing techniques to investigate the impacts of additions of simple organic compounds on microbial communities. Such methods characterize substrate utilization by *in situ* microbial communities (Degens, 1998a) or by part of the community that is culturable in BIOLOG[®] GN microplates (Garlands, 1996a) to produce community level physiology profiles (CLLP). These can be used to derive information on ‘functional’ metabolic diversity and to indicate shifts in microbial community structure and functioning.

In the case of rhizosphere, the quantity and composition of plant derived exudates has been shown to influence soil CLLP (Garland, 1996b). Additionally Degens (1998b) showed that the addition of simple organic substrates to soil changed the metabolic profiles and metabolic diversity of soil microbial

communities, with the characteristic of the response varying according to the nature of substrates added. The structure and metabolic diversity of soil microbial communities has been shown to be affected by agricultural management regimes (Lupwayi *et al.*, 1998; Bending *et al.*, 2000) and vegetation cover (Zak *et al.*, 1994).

2.6 Rice ecosystems

Rice is the staple food for the half of world's population. It is grown in agro-ecosystems that range from uplands to the flood prone and coastal wetlands. Rice is the major cereal crop for the people of the N E India and rice cultivation is practiced in three distinct ecosystems. They are upland terrace, lowland and slope lands (Tripathi *et al.*, 2007). These rice based ecosystems are likely to be different from each other in terms of ecosystem functioning.

Waterlogged (lowland) rice ecosystems harbour a unique microbial ecology. They remain flooded during a major part of the crop growing period and are predominantly anaerobic. These ecosystems are different from upland soils in several physicochemical and biological properties (Leisack *et al.*, 2001; Adhya and Rao, 2005). Considerable amounts of aquatic biomass are likely to be produced phototrophically in the floodwater (Roger, 1996) and even chemo-heterotrophically in the presence of inorganic electron donors and CO₂ along the redox gradients in waterlogged rice soils (Revsbech *et al.*, 1999). Thus, flooded soils represent a changed dynamics of microbial biomass and activity as compared to upland soils. About 75% of the global rice volume is produced in the irrigated lowlands (Maclean *et al.*, 2002). In eastern India, where approximately 80% of the 20 million hectares of rice is grown in rainfed lowlands (CRRI, 1990; Widawsky and O'Toole, 1990), there have been low but continuous increases in rice yields (David and Otsuka, 1994). The factors known to affect the indigenous soil nutrient supply in irrigated rice systems are soil drying, length of fallow, crop rotation, and residue management (Witt *et al.*, 1998). In the principal rainfed lowland rice regions, farmers are very poor, tend to be risk-averse and grow

traditional varieties using traditional methods. Many of these cultivars are sensitive to submergence and drought, and are susceptible to diseases (Mackill, 1986; Mackill *et al.*, 1996). Rainfed lowland rice farmers apply very low rates of fertilizer, probably because the potential yield is at great risk in the event of a flood or drought (CRRI, 1990). In general, moisture stress is most damaging near the reproductive stages of the rice crop (Widawsky and O'Toole, 1990). Weeds are increasingly among the most severe biotic constraints, while losses to pests and diseases are seen to be less severe in rainfed systems than in irrigated systems (David and Otsuka, 1994; Widawsky and O'Toole, 1990).

Upland rice is grown as a subsistence crop under rainfed conditions with no fertilizer inputs. It has traditionally been grown under slash-and-burn systems with long fallows, which restore soil fertility and reduce insect and weed pressure. However, increasing population density and government policies aimed at reducing the area under slash-and-burn have reduced fallow cycles to as little as two or three years between rice crops. Sloping fields, combined with heavy tillage, strong winds, and lack of ground cover, lead to extensive erosion and rain-runoff events. Changes in tillage, residue, and rotation practices induce major shifts in the number and composition of soil fauna and flora, including both pests and beneficial organisms (Bockus and Shroyer, 1998; Andersen, 1999). Productivity and qualities of the agricultural soils depend on the microbial functionality which generally decreases with environmental stress (Atlas *et al.*, 1991, Pankhurst *et al.*, 1996).

In hill agriculture where the places are sloppy, flat terraces are essential for cultivation of rice. But this mechanical terracing is an aggressive strategy that results physical alterations in the soil, such as compaction. The surface horizons can disappear or suffer serious disruption, resulting in the loss of a large part of the microbial population and thus, its activity. Soil management is sustainable only when the quality is maintained or improved (Garcia *et al.*, 1997). Bastida *et al.* (2007) studied the influence in the long-term (13 years) of two types of physical management (terracing and strip planting) in the reforestation of a mountainside, together with the addition of an organic amendment. The results

indicate that terracing in semi-arid, if not accompanied by the addition of organic matter, can have a negative effect on soil microbiological and biochemical properties.

Agricultural use of soil can affect its chemical, physical and biological properties. Comparison of soils between the natural vegetation and the cultivated top soils has revealed that prolonged agricultural land use can alter the magnitude, diversity, and spatial variability of some soil properties, mostly those related to fertility (Paz-Gonzalez *et al.*, 2000). Continuous cultivation with frequent tillage results in a rapid loss of organic matter (Balesdent *et al.*, 1999), and reduces microbial populations, activities and different enzymatic activities (Shepherd *et al.*, 2001; Ibekwe *et al.*, 2002; Carpenter-Boggs *et al.*, 2003).

2.7 Microbial community composition in rice ecosystems

Microbial community is highly diverse in rice fields. Reichardt *et al.* (2001) determined microbial population dynamics in rice fields and found that soil microbial community exhibit profound population shifts during a rice cropping cycle on irrigated fields. Greenhouse experiments were designed to examine the effects of the less stable environmental conditions in submergence- and drought-prone rainfed rice cropping systems on soil microbiota. Authors reported that total bacterial cell counts declined toward the end of the cropping period, irrespective of water regimes. Population of copiotrophic bacteria peaked at the flowering stage. This trend was most pronounced under drought conditions, but also significant under submergence. Fungi as primary microbial digesters of crop residues showed a negative correlation with the soil water content and were predominant only under drought or post-harvest fallow conditions. Population of N-remobilizing, proteolytic bacteria declined strongly in the flooded field, whereas drought reversed this trend in the greenhouse. Observations of successive increases of anaerobic guilds in the field were confirmed under permanent flooding. Post-season puddling practices promoted denitrifiers population. Ammonium oxidizers and nitrifiers showed extremely low population under

submergence, but also in planted trials under drought. This suggested a negative rhizosphere effect under water limitation.

Sequencing analysis of the most common DGGE bands retrieved from Japanese paddy fields revealed the presence of Type I, Type II methanotrophs, and β -proteobacterial ammonia oxidizers in rice bulk soils (Jia *et al.*, 2008). The high diversity of methane oxidizing bacterial (MOB) composition suggested that rice bulk soils might serve as an ideal reservoir for the dynamic changes of MOB in a rice field ecosystem in response to environment changes. Nakayama *et al.* (2006) isolated two hundred and seventy five bacterial strains from the floodwater of a Japanese paddy field during rice cultivation. Out of these strains, the 205 strains were negative for Gram stain. Sequencing of partial 16S rDNA of these strains were compare with DNA clones obtained from floodwater using the polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) method. The authors reported that these isolates belonged to α -, β -, and γ -Proteobacteria and the *Cytophaga-Flavobacterium-Bacteroides* (CFB) group as well as high-GC and low-GC Gram-positive bacteria, with proportions of 47.9, 7.6, 11.8, 13.4, 4.2 and 15.1%, respectively. Bacterial communities in the floodwater were estimated to include floodwater-specific members as well as members associated with other habitats in the paddy fields. Asakawa and Kimura (2007) assessed bacterial communities at different habitats in a Japanese paddy field ecosystem to understand the bacterial world in the ecosystem as a whole by analyzing data of the DGGE band patterns and the sequenced DGGE bands. Different habitats were floodwater, percolating water, microcrustacean inhabiting in floodwater, plow layer soil, rice roots, rice straw and rice straw compost incorporated in soil, rice straw placed on the soil surface, plant residues in paddy fields, and rice straw under composting process (Tanahashi *et al.*, 2004; Sugano *et al.*, 2005; Shibagaki-Shimizu, 2006; Kikuchi *et al.*, 2007). Authors observed higher phylotype richness near plant residues, rice straw buried in soil and rice straw placed on soil surface compared to the phylotype richness at microcrustacean and rice straw compost buried in soil. The samples from plow layer soil and rice straw compost buried in soil showed considerably higher index

values for diversity, evenness, and stability, while those from rice straw placed on soil surface and microcrustacean had lower values of the indices than other habitats. Distribution of bacterial members to phylogenetic taxa was different among the respective habitats. Inhabitants in plow layer soil were most widely distributed among the groups (nine phyla: Proteobacteria, Chloroflexi, Chlorobi, Verrucomicrobia, Acidobacteria, Nitrospira, candidate division OP10, Cyanobacteria, and Actinobacteria), while those in floodwater and microcrustacean were restricted to only three phyla (Proteobacteria, Bacteroidetes, and Actinobacteria). Proteobacteria and Bacteroidetes were found at all the habitats and the habitats except for plow layer soil, respectively, whereas abundant members belonged to Chloroflexi and Actinobacteria in plow layer soil. With the aid of multivariate analyses, it was revealed that bacterial communities at the respective habitats were distinct and different in the diversity and stability to each other. Hatamoto *et al.* (2008) estimated the succession and phylogenetic composition of the eukaryotic communities responsible for the decomposition of rice straw incorporated under flooded conditions during the cultivation period of rice in Japanese paddy fields. They observed that eukaryotic community attached to decomposition rice straws mainly composed of fungi including *Ascomycota*, *Zygomycota* and *Chytridiomycota* and protozoa including *Ciliophora*, *Euglyphida* and *Dactylopodida*. In the northeastern alluvial plains of India, the bacterial community compositions as revealed by DGGE banding patterns in rice soils under bacterial bioinoculants included integrated nutrient management were found to be related with the states of C and N dynamics in acidic rice soils (Thakuria *et al.*, 2009).

2.8 Response of microbial community composition to seasonal variation

Microbial activity is greatly influenced by seasonal variation in temperature. Mergeret *et al.*, (2001) found peaks of denitrifying bacteria in the autumn and winter/early spring and cited freezing/thawing as a contributing factor. Specifically, Wolsing and Prieme (2004) used T-RLFP to reveal that nirS

and nirK denitrifiers showed a significant seasonal shift from spring to fall in an agricultural soil and attributed this to the combined effect of abiotic seasonal changes. Multiple factors such as soil moisture and temperature are known to affect community diversity. Avrahami and Bohannan (2007) found that the abundance of amoA ammonia oxidizers increased with increasing soil moisture (30-60%) and decreased with increasing temperature. Within a temperature range (low 4⁰C to high 30⁰C), it was observed that lowest diversity of ammonium oxidizers occurred at both low and high temperature range as analysed by DGGE profiling of amoA genes (Avrahami *et al.*, 2003; Avrahami and Conrad, 2005). Nutrient availability in spring and fall was related with an increased in total bacterial community diversity at these times and a decreased in diversity in July due to warm, dry and nutrient limited conditions (Smith *et al.*, 2001). Also, Pelletier *et al.* (1999) conducted a field experiment to study how seasonal changes in temperature affect denitrifying enzyme activity (DEA) in a cropped humic gleysol located in a cold temperate climate (Quebec, Canada). The authors observed that DEA show significant seasonal variation with the peak activity in the coldest month of the year. In general, seasonal pattern of DEA in the field show decreasing rates during the summer months due to low soil moisture (Limmer and Steele, 1982; Tiedje *et al.*, 1989) and increasing rates after spring thaw (Christensen and Tiedja, 1990) and in fall when soil moisture increases (Liang and MacKenzie, 1997). These divergent results could be due to the capacity of denitrifiers to adapt to their environmental temperature or to temperature-induced shifts in the structure of the soil denitrifiers community (King and Nedwell, 1984). On the other hand, Gogoi *et al.* (2003) reported that seasonal variation was significant in case of bacteria, fungi, ammonifiers and nitrosomonas. Except for nitrifiers, other microbes attained population peak in monsoon season. Similar observation was made by several authors (Das *et al.*, 1991; Singh *et al.*, 1999; Khokar *et al.*, 2001). After decomposition of simple substances by bacteria and actinomycetes, the left over hard to decompose substances are utilized by fungi and as a result fungal population builds up at later stages of decomposition (Nishio and Kusano, 1983). A comparative study of

seasonal variation in specific groups of microbes showed that phosphate solubilizers maintained a higher population in pre-monsoon, post-monsoon and winter seasons than ammonifiers (Gogoi *et al.*, 2003). It is most probably because many bacteria and fungi that can solubilise phosphate from soil can grow well in presence of each other not because of an interaction but due to lack of competition.

The seasonal changes in atmospheric condition also influence number (Diaz-Ravina *et al.*, 1993) and mass (Granatstein *et al.*, 1987) of soil microbes. Franzluebbers *et al.* (1994) found that soil MBC changed significantly during cropping seasons and tillage regimes. Seasonal fluctuation of MBN was also observed in various forest ecosystems (Maithani *et al.*, 1996; Bohlen *et al.*, 2001; Ruan *et al.*, 2004). Feng *et al.* (2009) reported that soil MBC generally decreases from growing seasons (July-October) through the dormant seasons (November-April). The seasonality of soil MBC varies with environmental factors and tree physiology (Myers *et al.*, 2001). In a mixed oaked forest with warm moist summer and cool dry winter, MBC had significant positive correlation with both soil moisture and mean air temperature (Devi and Yadava, 2006). It was reported that, turnover of nutrients and microbial biomass occurs more rapidly in tropical soils (Franchini *et al.*, 2007) than in a temperate regions (Wardle and Ghani, 1995; Wardle *et al.*, 1999).

2.9. Conclusion

Based on the above literature, it can be concluded that the vast diversity of microorganisms represent the riches stock of molecular and chemical diversity in nature. Soil microbial diversity and community composition are found to be influenced by various soil attributes including land uses/covers, above- and below-ground biomass depending on ecosystem types. However, little efforts have been made towards quantifying and conserving microbes that define/regulate the ecosystem functioning. Microorganisms play an important role in various soil processes and soil functions but our understanding of the links between microbial

diversity and soil functions is very limited, although molecular techniques allows us to detect unculturable microorganisms. Perhaps, the link between microbial diversity and soil function requires an in depth study of the relationship between genetic diversity and community structure and between community structure and functions. To date, microbial ecology and eco-physiology research attempting to establish relationship between microbial community composition and soil functions is very limited in Indian context. In fact research in this direction using molecular based approach (16S rRNA gene analysis) is yet to initiate in the northeastern region of India. Considering the influence of biotic and abiotic factors on soil microbial population and the extent of variability in microbial community composition that one encounters in short distances, the question arise is, how one can catalogue and conserve this “unseen diversity”. Therefore, we need to adopt a systematic approach in studying microbial community diversity so that information generated will be of use in defining soil processes and soil health at defined agro-ecosystem levels.

CHAPTER 3

Materials and Methods

3.1 Description of the sampling site

Meghalaya is predominantly hilly and geographically known as “Meghalaya Plateau”. The two villages viz. Saiden and Kyrdemkulai located in the Ri-Bhoi district of Meghalaya were selected for sampling of soils. The elevation of Saiden village is 533 m and Kyrdemkulai village is 808 above mean sea level. Both elevations represent the lower to middle hill elevation range of the state.

3.2 Climate and Soil

Ri-Bhoi district experiences different types of climate ranging from tropical climate in the areas bordering Assam to the temperate climate adjoining the East Khasi Hills District. The areas bordering Assam experience hot-humid weather during summer seasons with average temperature of 30°C especially during the month of May to July and in other areas like Lum Raitong and Lum Sohpetbneng Plateaus, the climate is severely cold during the winter months and is pleasant during the summer period. The usual range of temperature is 25.3⁰C maximum and 23.6° minimum. The normal annual rainfall recorded during the year 2000-2010 is 760.0 mm.

Soil of Ri-Bhoi district is classified into hill and plain soils. Patches of red loamy soil and lime silt constitute the major portion. The soils adjoining Assam also consist of heavy loam while in other areas some stones and chips also present. The geological structure of Ri-Bhoi district is composed of Archaean

Gneissic complex and Granite is one of the major mineral of this district which has the economic value and will be marketable if properly processed.

3.3 Soil sampling and processing

Rice is the main crop of Ri-bhoi district. It occupies an area of 11,364 ha out of 26,921 ha net sown area. The rice fields of Saiden and Kyrdemkulai villages are selected as study area. The rationale behind selection of these two villages is that each village represents one mega-environment - the Kyrdemkulai site and the valley areas of Manipur state considered one mega-environment, and the Saiden site and Kolashib district of Mizoram constitute another mega-environment. Based on the GGE biplot analysis of multi-locational data of rice varietal trials, these mega-environments were reported (Tripathy *et al.*, 2007). Another reason for selection of these two villages was that rice is usually cultivated in three distinct ecosystems namely hill slopes, transplanted upland terrace and narrow lowland strips between two hills. Out of three rice ecosystems, six soil sampling sites were selected. These sites were: Saiden slope land, Saiden upland terrace-1, Saiden upland terrace-2, Saiden lowland, Kyrdemkulai lowland and Kyrdemkulai upland terrace. Characteristics of these sampling sites are presented in Table 3.1. The soil samples were collected in post-summer time (September 05, 2009) and post-winter time (February 05, 2010) from the above sites. All six sampling sites were occupied by rice crop at the time of collection of soil samples in post-summer. Whereas, rice residues were present in soils at the time of sampling in post-winter except the Saiden low land, where transplanted chilli crop was present in raised beds.

Within each sampling site, 5 random rice plots were selected and from each rice plot 4 random soil samples were collected at 0-15 cm depth. Approximately 1.0 kg of soil sample is collected from each sampling spot and immediately placed on ice box and carried to the laboratory within 8 h of collection. Each soil sample was divided into 2 splits: one part was used for microbiota diversity analysis and other part was processed for determination of

Table 3.1 Agricultural management practices at soil sampling sites

Sites	Management of above ground biomass	Use history of inputs	Crops grown
Saiden slope land	Burning of above ground weed biomass practiced every year.	Fertilizers and pesticides never applied.	Traditional rice and occasionally turmeric
Saiden upland terrace-1	Newly created terrace (1 year old). No biomass observed.	DAP was applied @ 30 kg ha ⁻¹	Rice monocrop
Saiden upland terrace-2	Weed biomass incorporated into soil at the time of ploughing. 20 years old terrace.	Fertilizers and pesticides never applied	Rice-vegetables
Saiden low land	-do- (Valley between two hills)	Compost and fertilizers applied occasionally (amounts not defined)	Rice – vegetables
Kyrdemkulai low land	-do- (Valley between two hills)	Fertilizers and pesticides never applied	Only rice
Kyrdemkulai upland terrace	-do- (7 years old terrace)	Compost and fertilizers applied to vegetables only (as per recommended practice)	Rice and vegetables

soil physico-chemical properties namely soil texture, soil moisture content, pH, cation exchange capacity, and total carbon and nitrogen. Soil samples used for analysis of physico-chemical parameters were air dried, grinded and passed through a sieve of 1-mm opening. The soil moisture content was determined in fresh sample.

3.4 Physicochemical analysis

3.4.1 Soil reaction (pH)

The pH of the soils were measured in soil water suspension (1:2.5) using a standard pH meter (Mettler Toledo, Switzerland) as given by Jackson (1973).

3.4.2 Soil Moisture Content

Soil moisture content was determined gravimetrically by oven drying the soil samples at 105° C to a constant weight (wt). The moisture was expressed in percentage (%).

$$\text{Soil moisture content (\%)} = [(\text{Fresh soil wt} - \text{Dry soil wt}) / \text{dry soil wt}] \times 100.$$

3.4.3 Total Organic Carbon (TOC)

Finely grinded air dry soils (0.5 g) was used to determine TOC by following wet oxidation method described by Walkley and Black (1947). Soil sample (0.5 g) was mixed with 10 ml of 1N K₂Cr₂O₇ and 20 ml of conc. H₂SO₄ in a 500 ml capacity conical flask and left for 30 min. After oxidation, 200 ml distilled water was added to the conical flask. 5 ml H₃PO₄ and AgSO₄ (5g AgSO₄ added per litre basis) was added to the flask. The residual K₂Cr₂O₇ was titrated with freshly prepared 0.5 M ammonium ferrous sulfate in the presence of diphenylamine indicator using a digital burette (Eppendorf Make, Germany).

TOC content in soil was expressed in $\text{g } 100\text{g}^{-1}$ soil (%). Inorganic carbon fractions in acidic rice soils were negligible and hence, TOC content was used to determine C: N ratio in the soils.

3.4.4 Total Nitrogen (TN)

Air dried and finely grinded soil sample (1g) was used for determination of TN by regular Kjeldahl method as described by Bremner and Mulvaney (1970). In brief, 1g soil sample was taken in digestion tube and to this 10ml distilled water was added to moisten the sample. Approximately 2g of digestion mixture (Composition: 100 part of K_2SO_4 (100g) 10 part of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (10g) and 1 part of Selenium powder (1g) mixed together and grinded it to get fine powder) and 10 ml of conc. H_2SO_4 were added and then suspension was digested in an aluminium heating block (KEL Plus, Pelican Equipment, India) at 360°C for 2 hour . After completion of digestion (the end point was thick clear copper coloured soup at the bottom of the digestion tube) 40 ml each of 0.32% KMnO_4 and 40% NaOH and 5 ml of paraffin wax (heavy liquid) were added to the tube and distillation was carried out for 6 min using an automatic distillation chamber (Classic DX, Pelican Equipment, India). Ammonia generated during distillation was collected in 20 ml of 2% boric acid solution containing few drops of mixed indicator (Composition of mixed indicator: 0.1g bromocresol with 0.07g of methyl red and dissolved this mixture in 100 ml ethanol). Finally, amount of boric acid used for absorption of ammonia was determined by titrating with 0.1 N H_2SO_4 using a digital burette (Eppendorf Make, Germany). TN was expressed in kg ha^{-1} .

3.4.5 Cation Exchange Capacity (CEC)

A finely grinded air dry soil sample (10g) was incubated with 100 ml of 1N NH_4OAC (pH 7.0) solution for 30 min at 200 rpm shaking speed. This helps to saturate the cation exchange site with NH_4^+ . After incubation soil suspension

was quantitatively transferred to a Buchner funnel fitted with a Whatman No. 42 filter paper. The residual electrolytes including free NH_4^+ present in the suspension were removed successive washing with 99% isopropyl alcohol. Then, the washed soil along with filter paper placed inside a N-distillation tube and distilled NH_4^+ by Kjeldahl distillation method by absorbing released ammonia in 2% H_3BO_3 . The boric acid containing ammonium borate was titrated by standard acid in the presence of bromo-cresol green-methyl red mixed indicator to find out the meq of exchangeable NH_4^+ adsorbed by soil sample. CEC of soil was expressed in meq per 100 g soil.

3.4.6 Soil particle distribution

The relative proportion of sand, silt and clay in a soil sample was determined by hydrometer method as given by Buoyoucos (1962).

3.5 Soil biological analysis

3.5.1 Enumeration of cultural diversity of fungi

Rose Bengal agar medium (pH 4.5) supplemented with streptomycin sulphate was use to grow the fungal colonies (Subba Rao, 1999). Fresh soil sample (10 g) was added to a conical flask containing 100 ml of sterile distilled water. The suspension was vortex at maximum speed for 5 minute to get a uniform soil suspension. An aliquot (100 μl) was spread over the rose Bengal agar medium using a sterile L spreader and the plate was incubated at 30 $^{\circ}\text{C}$ constant temperature for 5-7 days. For each soil sample, 3 replicates plates were used. After incubation, distinct colonies (colonies were distinguished in terms of colony size, shape, colour, vigour and days to appear) were recorded for each soil sample. Then, a binary matrix based on presence or absence of fungal colonies was generated for all the soil samples for analysis of culturable fungal diversity.

3.5.2 Extraction of soil DNA

Soil DNA was extracted based on the principle of *in-situ* lysis DNA extraction method. An amount (0.25 g) of fresh soil was used for extraction of soil DNA using the PowerSoil™ DNA isolation kit (MoBio Laboratories Inc., Carlsbad, USA) according to the manufacturer's protocol, with the following modification to improve DNA yield. The PowerBead tube with soil sample was incubated at 60°C for 15 min in a Thermomixer compact (Eppendorf Make, Germany) at 60°C for 15 min and at 300 rpm. After incubation, rest steps were followed as per manufacturer's instruction. DNA was eluted in 50 µl elution buffer (10mM Tris + 1mM EDTA buffer, pH 8.0) and stored at -20°C until.

3.5.3 Agarose gel electrophoresis

Quality in terms of molecular size of extracted soil DNA was determined by electrophoresis of 5 µl of DNA aliquot in an agarose gel (0.8% w v⁻¹ containing Gel-Red fluorochrom stain with a working strength of 0.06X, Biotium, USA) using a mini gel electrophoresis assembly (HU10, Sci-plas, Hongkong). Gels were visualized and images captured using gel documentation system (BioRad, CA, USA). EcoRI/*Hind*III-cut bacteriophage lambda DNA molecular size marker (250 ng; Invitrogen, UK) was included on gels as standard and the amount of DNA in samples was determined by comparing the fluorescence of genomic DNA bands to that of ladder and was expressed as µg g⁻¹ dry wt of soil (Zhou *et al.*, 1996).

3.5.4 Spectrophotometry

The absorption spectrum of DNA extracts (230-280nm) was determined using a Nano-drop® 2000 spectrophotometer (Thermo Scientific, USA) according to the manufacturer's instructions. DNA was quantified based on the absorption at 260 nm and expressed as µg g⁻¹ (dw) soil. A decrease in absorption

ratio at 260/230 and 260/280 nm was used as indicator of humic acid, polysaccharide and protein impurities (Krsek and Wellington, 1999; Stach *et al.*, 2001; Thakuria *et al.*, 2008).

3.5.5 Polymerase Chain Reaction (PCR)

3.5.6 PCR amplification of bacterial 16S rRNA genes from soil DNA

Extracted soil DNA was amplified in a Gradient Master Cyclor 5331 (Eppendorf Make, Germany) with primer pair (27f and 1492r) specific to bacterial domain (Lane *et al.*, 1991). This primer pair is specific to 16S rRNA genes of the bacterial domain and yields amplified product size of approximate 1465 bp. The amplification reactions were carried out in a 25µl volume containing 25 ng soil DNA as template, 100 nM of each oligonucleotide primers [27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3')], 12.5 µl of Go-Taq DNA polymerase (Promega,USA) along with bovine serum albumin (BSA concentration 0.1 µg µl⁻¹; New England Biolabs, UK) and the required quantity of PCR grade water (HiMedia, India). The PCR reaction condition included an initial 3 min denaturation at 94°C, and was followed by 30 thermal cycles of 1 min at 94°C, 1min at 62°C and 2 min at 72°C. Amplification was completed with the final extension step at 72°C for 7 min. All PCR products were examined by electrophoresed in an agarose gel (1% w v⁻¹) using a 100 bp DNA ladder (New England Biolabs, UK).

3.5.7 PCR amplification of bacterial 16S rDNA products

Amplified bacterial DNA obtained using primer pair 27f and 1492r was used as template DNA for amplification of 16S rDNA fragments at *E. coli* positions 968 to 1401 (V6-V8 regions of 16S rRNA gene; Li *et al.*, 2009) using another set of primer pair 984f and 1378r (984f: CGCCCGGGGCGCGCC

CGGGCGGGGCGGGGGCAGGGGGGAACGCGAAGAACCTTAC and 1378r: CGGTGTGTACAAGGCCCGGGAACG; underlined sequence is a GC-clamp, Heuer *et al.*, 1997). Amplification was carried in a 50µl reaction volume using a Gradient Master cycler 5331 (Eppendorf Make, Germany). The reaction composition consisted of 50 ng amplified PCR product (amplified with 27f and 1492r) as template DNA, 1X PCR buffer, 1.5 mM MgCl₂, 200 µM dNTPs (all reagents from New England Biolabs, UK), 100 nM of each of 984f and 1378r primers, 5.0 µl BSA (concentration 0.1 µg µl⁻¹), 1U of Taq-DNA polymerase. PCR reaction conditions consisted of an initial denaturation step (94°C, 3min) followed by 30 cycles of 1 min of denaturation at 94°C, 1min at 60°C for primer annealing, and 2 min at 72°C for primer extension, followed by final step at 72°C for 7 min and hold at 4°C. Products were first analyzed by electrophoresis in an agarose gel (1% w v⁻¹). Due to the presence of BSA, PCR products were purified by PCR purification spin columns as per manufacturer's instruction (QIAquick; Qiagen GmbH, Germany).

3.5.8 PCR amplification of fungal internal transcribed region from soil DNA

Fungal DNA was amplified from soil DNA extracts using primer pair specific to the internal transcribed spacer ITS1- 5.8S-ITS2 region (2234C/3126T; Sequerra *et al.*, 1997; Anderson and Cairney, 2004). Fungal DNA amplification reactions (25µl) contained 20 ng of soil DNA or pure culture DNA, 1U Taq-DNA polymerase, 1X PCR buffer, 1.5mM MgCl₂, 200 µM dNTPs (all reagents from New England Biolabs, UK), and 100 nM each of the relevant forward and reverse primers. To this mixture, BSA was added at a concentration of 0.1 µg µl⁻¹. All amplifications were performed at least twice for each DNA extract using Gradient Master cycler 5331 (Eppendorf Make, Germany). PCR reactions conditions consisted of an initial denaturation step (94°C, 3min) followed by 30 cycles of 95°C for 1min, 60°C (F-ARISA) for 45s and 72°C for 1min, followed by a terminal extension step at 72°C for 7min. Products were first analyzed by electrophoresis in an agarose gel (1% w v⁻¹).

3.5.9 Denaturing gradient gel electrophoresis (DGGE) fingerprinting of bacterial community composition

Denaturing gradient gel electrophoresis (DGGE) was performed using Ingeny PhorU2 system (Ingeny International BV, The Netherlands). The PCR products (amplified with DGGE primer: 984f and 1378r) were loaded on to 8% ($w v^{-1}$) polyacrylamide gel with a denaturing gradient of 40% and 60% (100% denaturant tends for 7M urea plus 40% formamide in 1 X TAE buffer). Approximate 500 ng of PCR product was loaded into each well and electrophoresis was carried out for 18h at constant 90V in presence of 1X TAE buffer (242 g Trizma, 57.1 ml glacial acetic acid and 100 ml 0.5M EDTA and volume made up to 1 l; pH adjusted to 8.0) at a constant temperature of 60°C. After electrophoresis the gel was stained with Gel-Red fluorescent nucleic acid dye (working concentration 0.6X; Biotium, USA) for 30 min. The stained gel was visualized and image was captured using gel documentation system (BioRad, CA, USA). Triplicate DGGE profiles were generated for each soil sample.

Within a site, three composite soil samples were analysed independently for bacterial community composition resulting three independent DGGE profiles for each site. Thus, three DGGE fingerprints were obtained and each independent DGGE fingerprint contained six numbers of DGGE profiles for six sites. From the image of each DGGE fingerprint a reference DGGE profile was created that contained all maximum possible bands out of six DGGE profiles. Then, DGGE profiles were scored visually for presence or absence of bands comparing to the reference profile. Thus, a binary presence-absence matrix was developed for each DGGE fingerprint (Wilbur *et al.*, 2002; Kropf *et al.*, 2004). Altogether, three binary presence-absence matrices were obtained. These binary matrices were exported to Excel worksheet 4.0 (Microsoft Office Excel, 2003) and arranged in a single sheet according to band numbers. This combined binary matrix (3 replicates DGGE score profiles x 6 sites x 2 seasons = 36 DGGE score profiles) was finally exported to PRIMER v6.1.9 software (Primer-E Ltd, Plymouth, UK) and dataset was standardised by total method (Magurran, 2004). This standardised

dataset finally used for diversity indices and all other multivariate analyses as described below in the section 3.7.

3.5.10 Ribosomal internal-transcribed spacer analysis (RISA) fingerprinting of fungal community composition

The fungal PCR products derived from the partial 18S and 26S rRNA genes including the internal transcribed spacer (ITS) region were used to develop SSCP fingerprinting. Amplified PCR product (7.5 µl) of each soil sample was mixed with 1.5µl of 6X loading dye (10% Ficoll, 0.25% each of xylene cyanol and bromophenol blue) and this mix was loaded into the well of 6% (w v⁻¹) non-denaturing polyacrylamide gel (PAGE). The gels were electrophoresed at constant 60 V for 12h at a constant temperature of 40°C in 1X tris-borate-EDTA buffer using Ingeny PhorU2 system (Ingeny International BV, The Netherlands). After electrophoresis the gel were stained with Gel-Red fluorescent nucleic acid dye (0.6X; Biotium, USA). Gel was visualized and image captured using gel documentation system (BioRad, CA, USA). Triplicate RISA profiles were generated for each soil sample.

For analysis of fungal community composition, scoring of RISA fingerprints were done similar to that for DGGE fingerprints as described in the section 3.5.9.

3.6 Analysis of diversity indices

Richness and diversity parameters were estimated for both bacteria and fungal communities using the Margalef's richness and Shannon's diversity indices. Shannon's diversity is biased towards abundant community in a population (Magurran, 2004). DGGE fingerprint approach for microbial community structure analysis mostly targets abundant community (Nakatsu, 2007). Hence, shannon's diversity index is preferred over other diversity indices in case of DGGE fingerprint profiles.

Shannon's diversity, $H' = -\sum P_i \ln P_i$, where P_i is the relative intensity of band i ($= N_i / N$; intensity of band i , N , sum of the intensities of all bands) (Magurran, 2004).

Margalef's richness index is a measure of the number of species present for a given number of individuals (N).

Margalef's richness, $d = (S-1) / \log N$, where N = total number of individuals (total number of bands in the reference DGGE profile which represented total maximum possible bands out of all soils), S = total number of species (total number of bands in each DGGE profile of a soil).

3.7 Statistical analysis

3.7.1 Univariate statistics

All univariate statistical analyses were performed using SPSS v12.0 (Statistical Packages for Social Science Inc., Chicago, IL, USA). Each physico-chemical parameter i.e. sand content, silt content, clay content, moisture content, pH, total organic carbon, total nitrogen, cation exchange capacity analysed for different seasons or within a parameter i.e. DNA yield, DNA quality, Margalef's richness and Shannon's diversity irrespective of sites and seasons were normally distributed as determined using the Kolmogorov-Smirnov test and wherever, normality was not observed the data were subjected to mild transformation (square root transformation). Paired t-test was performed to determine for any significant differences between seasons within a site. For determination of any significant differences within a parameter irrespective of sites and seasons was performed by one way analysis of variance (ANOVA) [incorporating the Levene Statistic to test for the equality of group variances, and the Tukey's Honestly Significance Difference (HSD) test at $P < 0.05$ for pairwise comparisons].

3.7.2 Multivariate statistics

All multivariate analyses results presented in this study were computed using PRIMER v6.1.9 software (Primer-E Ltd, Plymouth, UK).

Non-metric multidimensional scaling ordination (NMDS) of community data:

A Bray-Curtis resemblance matrix was generated using the square-root transformed standardised presence-absence binary matrix (Bray and Curtis, 1957; Clarke, 1993). The resemblance matrix was plotted in 2-dimensions by NMDS ordination (50 restarts, 0.001 minimum stress, Kruskal fit scheme 1 and Shephard step-wise function) (Kruskal and Wish, 1978). Dendrograms were constructed by hierarchical cluster analysis (group-average linking) using the Bray-Curtis resemblance matrix (Clarke, 1999). Clusters were superimposed on the NMDS plot to form ellipses at arbitrary resemblance levels of slices drawn through the dendrograms. One-way analysis of similarity (ANOSIM) was performed on Bray-Curtis resemblance matrix (incorporating 999 permutations for Global R statistics) to determine the significance of differences between microbial community structure with respect to rice field sites.

Relationship study of community data and environmental parameters using principal component analysis:

The environmental parameter data matrix (soils attributes as rows and the different sampling sites as columns) was normalized to eliminate the effects of different units and then square-root transformed while performing principal component analysis (PCA). The square root transformed normalized data matrix was ordinated in 2 dimensions based on the scores of the variables (environmental parameters) in the first two principal components and the euclidean distance was used as a measure of dissimilarity between sites.

To know how DGGE /or RISA bands relate to the status of a physicochemical parameter in different rice fields, the binary data matrix of DGGE /or RISA bands were superimposed on the PCA plot. Then individual environmental parameter was selected to display their plot values as bubbles in the PCA plot and the DGGE / or RISA bands were highlighted as vector lines based on their correlation (Pearson correlation) with principal axes. By following bubble plot technique, relationships between DGGE/ or RISA bands and individual soil physico-chemical parameter were determined.

CHAPTER 4

Experimental findings

Six rice fields that represented three rice ecosystems (direct-seeded hill slope, transplanted upland terrace, and lowland) were selected from two villages viz. Saiden and Kyrdemkulai of the Ri-Bhoi district of Meghalaya in this investigation for determination of bacterial and fungal community compositions. These rice ecosystems were compared in terms of bacterial and fungal communities and relationships of the members of such communities with soil attributes {sand, silt and clay contents, moisture content (MC), pH, total organic carbon (TOC), total nitrogen (TN) and cation exchange capacity (CEC)} were studied.

4.1 Variability in rice fields in terms of soil attributes

Sand, silt and clay contents, textural class, MC, pH, TOC, TN and CEC in soils of six rice fields are presented in Table 4.1. Soils of upland terraces, lowlands and slope lands were loamy, silt to clay loam and sandy clay loam, respectively. Soil moisture content in rice fields varied greatly depending on land use types. Moisture content in soils of the direct seeded slope land rice fields was the lowest (14-28%) in post-summer and post-winter. Soils of lowland rice fields were waterlogged during both seasons except the soil moisture content during post-winter in Saiden lowland field i.e. 62.7%. Moisture content in soils of Saiden upland terrace-2 rice fields was optimum (53.2-60%) in both seasons, whereas the moisture content in soils of Kyrdemkulai upland terrace rice fields was lower i.e. 22% in post-winter as against 61.4% during post summer.

Soil pH in the rice fields varied in the range from 4.80 to 5.60 (Table 4.1). Soil pH in post-winter was found to be significantly higher (paired t- test, $P < 0.01$)

Table 4.1 Some soil properties of sampling sites under different rice ecosystems^γ

Site	Soil particle distribution (%)			Textural class	MC (%)	pH (1:2.5 soil water ratio)	TOC (%)	TN (%)	CEC (cmol P kg ⁻¹)
	Sand	Silt	Clay						
Saiden slope land	50.0	25.0	25.0	Sandy clay loam	28.1±0.4 ^a	4.85±0.05 ^a	1.82±0.03 ^b	3706±155 ^a	8.47±0.50 ^a
	ND	ND	ND		13.9±0.6^b	4.80±0.05^a	1.61±0.02^a	3813±146^a	9.14±0.40^b
Saiden upland terrace-1	38.0	40.0	22.0	Loam	32.1±0.8 ^a	5.41±0.03 ^a	1.69±0.04 ^a	2967±142 ^b	7.27±0.80 ^a
	ND	ND	ND		32.0±1.2^a	5.60±0.08^b	1.65±0.05^a	3528±144^a	7.34±0.55^a
Saiden upland terrace-2	36.5	42.5	21.0	Loam	60.0±4.3 ^a	5.27±0.04 ^a	1.98±0.06 ^a	5587±65 ^b	9.83±0.83 ^a
	ND	ND	ND		53.2±3.7^b	5.30±0.06^a	2.05±0.08^a	5800±219^a	10.06±0.28^a
Saiden low land	34.5	38.5	27.0	Clay loam	waterlogged	5.04±0.02 ^a	2.48±0.03 ^a	5829±141 ^b	12.32±1.08 ^a
	ND	ND	ND		62.7±0.9	5.10±0.04^b	2.87±0.02^b	6196±194^a	12.64±0.58^a
Kyrdemkulai low land	26.0	52.0	22.0	Silt loam	waterlogged	4.92±0.06 ^a	2.94±0.07 ^a	5905±90 ^a	9.93±0.69 ^a
	ND	ND	ND		waterlogged	5.09±0.02^b	3.20±0.04^b	6071±146^b	10.24±0.46^a
Kyrdemkulai upland terrace	40.0	34.5	25.5	Loam	61.4±1.2 ^a	5.20±0.04 ^a	2.13±0.04 ^a	5263±76 ^b	9.21±0.88 ^a
	ND	ND	ND		22.0±0.4^b	5.40±0.02^b	2.12±0.06^a	5720±116^a	9.95±0.50^a

MC - moisture content; TOC – total organic carbon, TN – total nitrogen, CEC – cation exchange capacity, ND – not determined because within a season soil textural class is hardly changed; ^γ values in bold face for post-winter and values in plain face for post-summer. Within a site for each parameter, values that differ significantly ($P<0.01$) are followed by different letters, as determined by paired t-test.]

as compared to that in post-summer in all rice fields, except the Saiden slope land and Saiden terrace-2, where seasonal variation was not significant. Soil pH in rice fields was observed in the textural class order of loam (pH range 5.2-5.6) > clay loam to silt loam (pH range 4.92-5.1) > sandy loam (pH range 4.82-4.85).

Within Kyrdekulai sampling location, lowland rice soil contained 44% more TOC over that in upland terrace soil. Similarly, within Saiden sampling location, lowland rice soil contained 33%, 55% and 60% more TOC over that in upland terrace-2, slope land and upland terrace-1, respectively (Table 4.1). TOC content in soils of different rice fields were varied significantly between seasons depending on rice ecosystems. TOC content in soils of lowland rice ecosystems was significantly higher in post-winter as compared to that in post-summer ($P<0.01$). Whereas, TOC content in soil of Saiden slope land rice field was significantly higher in post-summer as compared to that in post-winter season ($P<0.01$). No significant seasonal variations in TOC in soils of Saiden upland terrace-1 and terrace-2 and Kyrdekulai upland terrace rice fields were observed ($P>0.05$).

Irrespective of seasons, maximum soil TN content was recorded (average 6000 kg N ha⁻¹) in soils of lowland rice fields followed by 5592 kg N ha⁻¹ in soils of upland terrace rice fields, 3760 kg N ha⁻¹ in slope land rice field and the lowest (3248 kg N ha⁻¹) in soil of Saiden upland terrace-1. Except the direct seeded slope rice fields where seasonal variation in TN was non-significant ($P>0.05$) TN content in soils of all rice fields was significantly higher in post-winter as compared to that in post-summer.

The CEC in soils of lowland rice fields and matured upland terrace (Saiden upland terrace-2 and Kyrdekulai upland terrace) did not show significant seasonal variations ($P>0.05$; Table 4.1). However, saiden slope land rice field showed significant seasonal variations in terms of soil CEC indicating higher exchange capacity in post-winter than in post-summer. CEC in soils of rice fields showed similar trend with that of TOC irrespective of seasons, indicating the contents in the order of lowland > old upland terrace > slope land > young upland terrace.

4.2. Yield and quality of soil DNA

Result of DNA yields (quantified in agarose gel using *Hind*-III digest lamda DNA mass ladder) showed that yield of soil DNA was found to be significantly higher in lowland rice fields (10-12 $\mu\text{g g}^{-1}$ dry wt of soil) compared to that in other rice fields ($P < 0.01$, Table 4.2). The soil DNA yields in terrace and slope land rice fields were comparable ($P > 0.05$). Irrespective of fields; soil DNA yield was higher in post-summer than in post-winter.

DNA extracts obtained from soils of different rice fields were comparable in terms of DNA quality ($A_{260/230}$ and $A_{260/280}$ ratios as determined by Spectrophotometry; $P > 0.05$; Table 4.2). Electrophoretic analysis of soil DNA in agarose gel indicated that soils of all rice fields yielded high molecular weight DNA (> 10.0 to 23.1 Kb; Fig. 4.1).

4.3 Bacterial and fungal diversity indices

Bacterial and fungal diversity indices are presented in Table 4.3. Soils of different rice fields were varied significantly in terms of Margalef's richness and Shannon's diversity indices ($P < 0.01$). Bacterial richness and diversity indices were found to be higher in post-summer compared to that in post-winter irrespective of rice fields. Bacterial richness and diversity indices were found in the order of lowlands $>$ matured terrace $>$ young terrace $>$ slope land. Fungal richness and diversity indices were observed higher in soils of Saiden slope land and upland terrace-1 in post-summer than that in post-winter, whereas reverse trend was observed in lowlands, Saiden upland terrace-2 and Kyrdemkulai upland terrace.

4.4 Bacterial community composition

Bacterial genomic DNA within soil DNA amplified successfully using the bacterial domain specific primers (27f and 1492r) is presented in Fig. 4.2. In order

Table 4.2 Yield and quality of soil DNA extracted from soils of different rice ecosystems^γ

Site	Soil DNA yield ($\mu\text{g g}^{-1}$ oven dry soil)	DNA quality	
		$A_{260/280}$	$A_{260/230}$
Saiden slope land	7.30 $\pm$ 0.30 ^{ab}	2.03 $\pm$ 0.180 ^a	1.25 $\pm$ 0.06 ^a
	6.90$\pm$0.45^a	1.96$\pm$0.09^a	1.23$\pm$0.07^a
Saiden upland terrace-1	8.23 $\pm$ 0.50 ^{bc}	2.05 $\pm$ 0.18 ^a	1.24 $\pm$ 0.07 ^a
	7.63$\pm$0.50^{ab}	2.06$\pm$0.13^a	1.25$\pm$0.08^a
Saiden upland terrace-2	9.60 $\pm$ 0.60 ^{cd}	2.02 $\pm$ 0.13 ^a	1.19 $\pm$ 0.03 ^a
	9.43$\pm$0.50^{cd}	2.09$\pm$0.11^a	1.23$\pm$0.07^a
Saiden low land	11.87 $\pm$ 0.55 ^{fg}	2.07 $\pm$ 0.15 ^a	1.51 $\pm$ 0.60 ^a
	10.93$\pm$0.45^{ef}	2.02$\pm$0.07^a	1.21$\pm$0.06^a
Kyrdemkulai low land	12.50 $\pm$ 0.25 ^g	2.03 $\pm$ 0.16 ^a	1.25 $\pm$ 0.06 ^a
	10.47$\pm$0.57^{de}	2.04$\pm$0.06^a	1.21$\pm$0.11^a
Kyrdemkulai upland terrace	9.87 $\pm$ 0.50 ^{de}	2.05 $\pm$ 0.11 ^a	1.22 $\pm$ 0.08 ^a
	8.50$\pm$0.30^{bc}	2.06$\pm$0.14^a	1.18$\pm$0.07^a

^a Values presented are the means $\pm$ SD (n = 5).

Within a column, values followed by different letters are statistically significant at $P < 0.05$ as determined by one-way ANOVA.

^γ values in bold face for post-winter and values in plain face for post-summer.

Table 4.3 Bacterial and fungal diversity indices in soils of different rice ecosystems^γ

Site	Margalef's richness		Shannon's diversity index	
	Bacteria	Fungi	Bacteria	Fungi
Saiden slope land	4.89±0.02 ^{ab}	2.16±0.16 ^g	2.84±0.06 ^{ab}	2.39±0.09 ^e
	4.73±0.06^a	1.89±0.55^{de}	2.76±0.08^a	2.21±0.08^{cd}
Saiden upland terrace-1	5.12±0.11 ^{ab}	1.67±0.09 ^{cd}	2.93±0.10 ^{ab}	2.15±0.05 ^c
	5.08±0.06^b	1.54±0.05^{bc}	2.83±0.08^{ab}	2.05±0.07^{ab}
Saiden upland terrace-2	5.53±0.13 ^{bc}	1.42±0.11 ^{ab}	3.11±0.03 ^b	1.97±0.03 ^{ab}
	5.45±0.09^{bc}	1.53±0.03^{bc}	3.03±0.11^b	2.03±0.05^{ab}
Saiden low land	7.09±0.46 ^f	1.30±0.04 ^a	3.37±0.07 ^c	1.91±0.04 ^a
	6.08±0.15^{de}	1.97±0.11^{ef}	3.17±0.20^{bc}	2.30±0.03^{de}
Kyrdekulalai low land	6.37±0.07 ^e	1.77±0.04 ^{de}	3.34±0.10 ^c	2.11±0.09 ^{bc}
	6.15±0.16^{de}	2.11±0.14^{fg}	3.14±0.12^{bc}	2.39±0.09^e
Kyrdekulalai upland terrace	5.77±0.08 ^{cd}	1.48±0.07 ^{ab}	3.16±0.04 ^{bc}	2.02±0.06 ^{ab}
	5.46±0.04^{bc}	1.81±0.05^{de}	3.10±0.01^b	2.25±0.05^{cd}

^γ values in bold face for post-winter and values in normal face for post-summer. Within a column, values that differ significantly ($P<0.01$) are followed by different letters, as determined by one-way analysis of variance (ANOVA).



Fig. 4.1 DNA yields and their molecular weight ranges in 0.8% agarose gel, those extracted by using PowerTM Soil DNA Kit (MoBio, USA) from soils of various rice ecosystems; Kyrdekulai lowland samples (Lanes 1 and 12), Saidu lowland samples (Lanes 4 and 10), Kyrdekulai upland terrace (Lanes 2 and 11), Saidu upland terrace-1 (Lanes 3 and 8), Saidu upland terrace-2 (Lanes 5 and 9) and Saidu slope land (Lanes 6 and 7) in post-winter (Lanes 1 to 6) and post-summer (Lanes 7 to 8). Lane M, 245.6 ng of EcoRI- *Hind*III- cut bacteriophage lambda DNA molecular size marker. 5 µl of soil DNA extract per well.

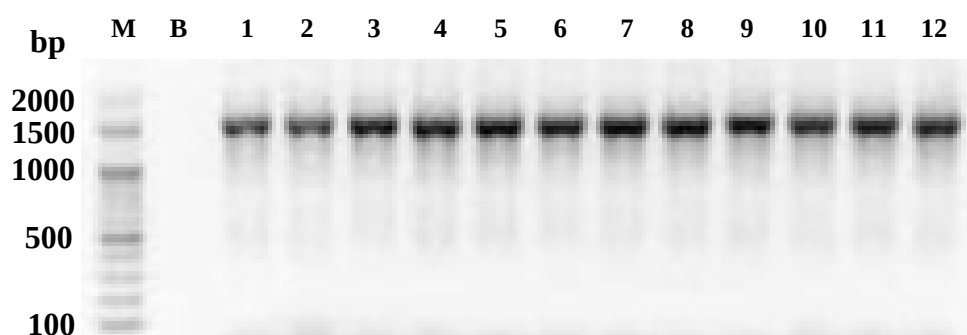


Fig. 4.2 Eubacterial amplified polymerase chain reaction products obtained from soil DNA extracts (10 ng template DNA) using the primer pair 27f and 1492r specific to 16S ribosomal gene (Lane, 1991) in 1.0% agarose gel. Kyrdekulai lowland samples (Lanes 1 and 12), Saiden lowland samples (Lanes 4 and 10), Kyrdekulai upland terrace (Lanes 2 and 11), Saiden upland terrace-1 (Lanes 3 and 8), Saiden upland terrace-2 (Lanes 5 and 9) and Saiden slope land (Lanes 6 and 7) in post-winter (Lanes 1 to 6) and post-summer (Lanes 7 to 8). Lane M, 100 bp DNA ladder. 5 μ l of soil DNA extract per well. B indicates negative control reaction without soil DNA extract as template.

generate DGGE-PCR products, the 27f-1492r derived PCR products were used as template and were amplified using DGGE primer pair (984f and 1378r) that yielded 16S rDNA products of approximate 433 bp (Fig. 4.3). DGGE fingerprints depicted in Figure 4.4 clearly revealed that the abundant bacterial community compositions varied between rice fields. Altogether, maximum possible 46 distinct abundant bacterial phylotypes (DGGE bands) were detected among the rice fields studied. Number of bacterial DGGE bands detected in post-summer and post-winter were in the order of Saiden lowland (29 to 24) > Kydemkulai lowland (25 to 26) > Kydemkulai upland terrace (22 to 24) > Saiden upland terrace-2 (22 in both seasons) > Saiden upland terrace-1 (21 in both seasons) > Saiden slope land (20 in both seasons) (Fig. 4.4).

The NMDS plot generated using the presence-absence matrix of DGGE fingerprints clearly showed the variability among rice fields in terms of abundant bacterial community composition (Fig. 4.5). Hierarchical clusters of DGGE profiles superimposed on the NMDS plot generated 4 grouping at 70% similarity level, irrespective of seasons (Fig. 4.5). Post-summer bacterial communities of Saiden upland terrace-1 and Saiden slope land soil and post-winter bacterial communities Saiden and Kyrdemkulai lowlands constituted a single cluster. Bacterial communities of Kyrdemkulai lowland in both seasons and post-winter Saiden lowland constituted the second cluster. The third cluster was consisted of bacterial communities of post-summer bacterial communities of Kyrdemkulai upland terrace and Saiden upland and lowlands, while the post-winter bacterial communities of Saiden upland terrace-1 alone formed a cluster. However, ANOSIM test for pairwise comparisons among bacterial communities of different rice fields indicated that the groupings of abundant bacterial communities within the NMDS plot were significantly different from each other (Global R= 0.91; 999 permutations at $P<0.001$; Table 4.4). See Appendix xx for ANOSIM of the result obtained.

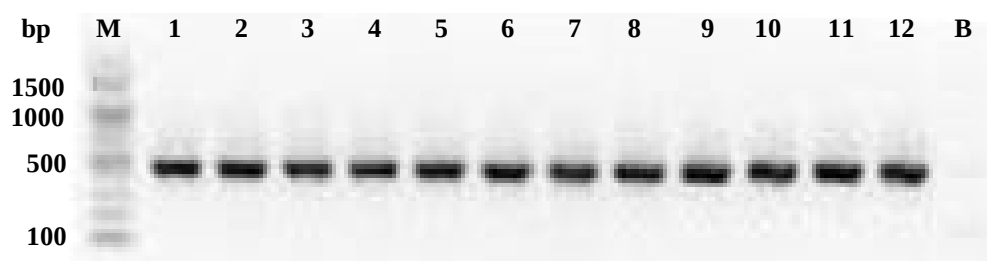


Fig. 4.3 Eubacterial amplified polymerase chain reaction products obtained from 27f-1492r products as template DNA (50 ng) using the primer pair (40 bp GC-clamp) 984f and 1378r specific to V6-V8 region of 16S ribosomal gene (Heuer *et al.*, 1997) in 1.0% agarose gel. Kyrdemkulai lowland samples (Lanes 1 and 12), Saiden lowland samples (Lanes 4 and 10), Kyrdemkulai upland terrace (Lanes 2 and 11), Saiden upland terrace-1 (Lanes 3 and 8), Saiden upland terrace-2 (Lanes 5 and 9) and Saiden slope land (Lanes 6 and 7) in post-winter (Lanes 1 to 6) and post-summer (Lanes 7 to 8). Lane M, 100 bp DNA ladder. 5 μ l of soil DNA extract per well. B indicates negative control reaction without template DNA.

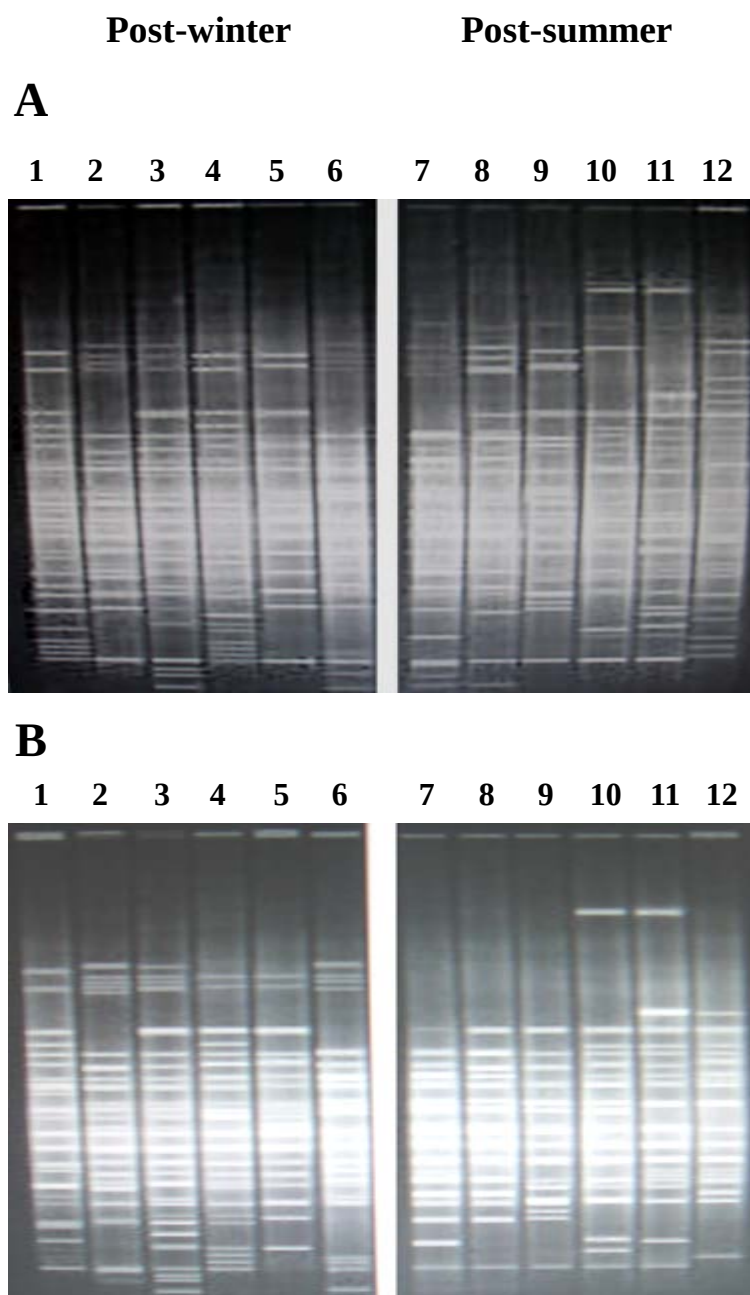


Fig. 4.4 Denaturing gradient gel electrophoresis {DGGE, 8% gel of acrylamide:biacrylamide (37:1) at 40 – 60 % denaturing gradient} fingerprint of bacterial communities in soils of various rice ecosystems; Kyrdekulai lowland samples (Lanes 1 and 12), Saiden lowland samples (Lanes 4 and 10), Kyrdekulai upland terrace (Lanes 2 and 11), Saiden upland terrace-1 (Lanes 3 and 8), Saiden upland terrace-2 (Lanes 5 and 9) and Saiden slope land (Lanes 6 and 7) in post-winter (Lanes 1 to 6) and post-summer (Lanes 7 to 12). Bacterial community pattern was obtained by amplification of 27f-1492r derived eubacterial products as templates using the primer pair (GC)984f and 1397r (Lane, 1991; Heuer *et al.*, 1997). DGGE gel was run at constant 90V and temperature of 60°C for 18h using a Ingeny Mutation PhorU Detection System. A and B indicate replicate DGGE profiles obtained from two independent DNA extracts.

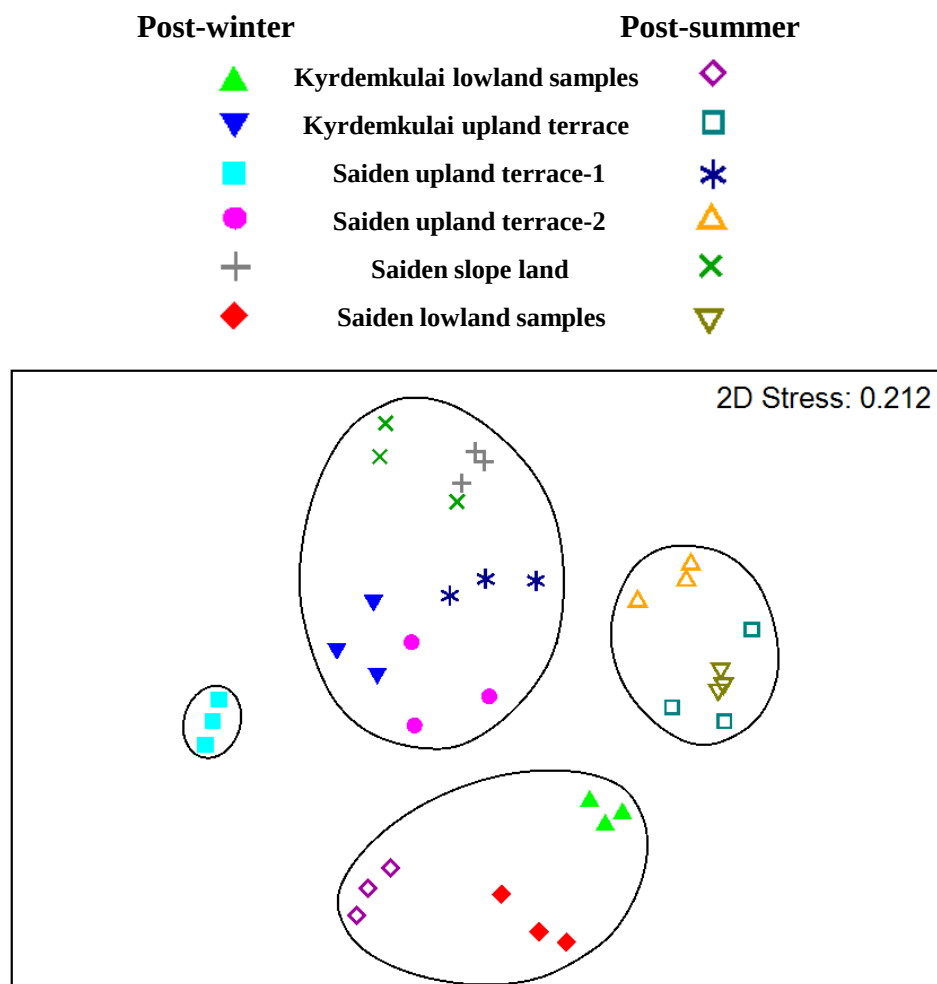


Fig. 4.5 Variability of the abundant bacterial community composition in soils of rice ecosystems. The presence-absence data matrix was generated using denaturing gradient gel electrophoresis analysis of DNA extracted from soils of various rice ecosystems. Non-metric multidimensional scaling ordination plot was derived using a Bray-Curtis resemblance matrix generated on standardised presence-absence data by total method (Clarke, 1999). Results are based on three replicated DGGE profiles obtained from three independent DNA extracts in each rice soil. Ellipses represent superimposed hierarchical clusters (similarity level 70%), deduced using group-average linking based on the Bray-Curtis resemblance matrix.

Table 4.4 Analysis of similarity (ANOSIM^a) test among bacterial communities (plain font) and fungal communities (bold font) in soils of various rice ecosystems

	KLL-W	KUT-W	SUT2-W	SLL-W	SUT1-W	SSL-W	SLL-S	SUT2-S	SUT1-S	SSL-S	KUT-S	KLL-S
KLL-W	0	1.00*	0.92*	0.93*	0.92*	1.00*	0.94*	0.95*	0.93*	0.94*	0.95*	0.92*
KUT-W	0.93*	0	1.00*	0.92*	1.00*	0.94*	0.95*	1.00*	1.00*	0.92*	0.94*	1.00*
SUT2-W	0.85^{ns}	0.72^{ns}	0	0.99*	0.98*	1.00*	1.00*	1.00*	0.96*	0.92*	0.92*	0.94*
SLL-W	0.87^{ns}	0.74^{ns}	0.63^{ns}	0	1.00*	1.00*	0.96*	0.96*	0.98*	1.00*	1.00*	0.97*
SUT1-W	1.00*	1.00*	1.00*	1.00*	0	0.99*	0.92*	0.94*	0.93*	0.96*	1.00*	1.00*
SSL-W	1.00*	1.00*	1.00*	0.94*	1.00*	0	1.00*	1.00*	1.00*	0.94*	0.97*	0.96*
SLL-S	1.00*	0.72^{ns}	0.78^{ns}	0.96*	1.00*	1.00*	0	0.99*	0.95*	1.00*	1.00*	1.00*
SUT2-S	1.00*	0.37^{ns}	0.46^{ns}	0.78^{ns}	1.00*	1.00*	0.22^{ns}	0	1.00*	1.00*	1.00*	1.00*
SUT1-S	0.96*	0.91*	0.96*	0.96*	0.74^{ns}	0.93*	0.9*8	0.96*	0	0.95*	0.96*	0.94*
SSL-S	1.00*	1.00*	0.92*	1.00*	1.00*	1.00*	1.00*	1.00*	0.95*	0	0.99*	1.00*
KUT-S	1.00*	1.00*	1.00*	0.96*	1.00*	1.00*	1.00*	1.00*	0.89*	1.00*	0	1.00*
KLL-S	1.00*	1.00*	0.89*	0.74^{ns}	1.00*	1.00*	1.00*	1.00*	1.00*	1.00*	1.00*	0

^aANOSIM was performed using the Bray-Curtis resemblance matrix (incorporating 999 permutations for R statistics) based on presence-absence data matrix obtained performing denaturing gradient gel electrophoresis fingerprinting for bacterial communities and ribosomal internal-transcribed spacer analysis fingerprinting for fungal communities. R values smaller than the Global R (= 0.91 for bacteria and = 0.89 for fungi) indicated non-significant ^{ns} $P > 0.05$ or otherwise significant at $*P < 0.001$. KKL- kyrdemkulai low land, KUT – kyrdemkulai upland terrace, SLL – saiden low land, SUT1 – saiden upland terrace 1, SUT2- saiden upland terrace 2, SSL – saiden slope land, W – post-winter and S – post-summer.

4.5 Fungal community composition

Altogether 24 distinct fungal colonies were obtained from the six rice fields studied (Fig. 4.6). Number of fungal isolates obtained in rice field ranged from a maximum of 6 isolates in Saiden lowland followed by 3 isolates in Kyrdemkulai lowland, 2 to 3 isolates in upland terraces and only 1 isolates in post-winter slope land. All the 24 fungal isolates were distinct in terms of colony morphologies and the ribosomal internal-transcribed spacer lengths as determined by amplification of genomic DNA of pure culture isolates using fungal specific primer pair 2234C and 3126T (Fig. 4.7).

Fungal genomic DNA within soil DNA amplified successfully using the fungal domain primers (2234C and 3126T specific to ribosomal internal-transcribed region between 18S and 26S) and the amplified products were discriminated in a non-denaturing polyacrylamide gel resulting RISA fingerprint of fungal communities (Fig. 4.8). RISA fingerprint clearly revealed that the fungal community compositions varied between rice fields. Altogether, maximum possible 27 distinct fungal phylotypes (RISA bands) were detected in the six rice fields studied. Number of fungal phylotypes detected were in the order of lowlands (9) > upland terrace (7 to 8). In case of slope land, post-summer fungal phylotypes detected were 11 in comparison to 7 phylotypes in post-winter (Fig. 4.8).

The NMDS plot generated using the presence-absence matrix of RISA fingerprints clearly showed the variability among rice fields in terms of fungal community composition (Fig. 4.9). Hierarchical clusters of RISA profiles superimposed on the NMDS plot generated 5 grouping at 60% similarity level, irrespective of seasons (Fig. 4.9). Fungal communities of all lowlands and Saiden upland terrace-2 in both seasons, Kyrdemkulai upland terrace in post-summer constituted a single cluster. Fungal communities of Saiden upland terrace-1 in both seasons formed a single cluster. Post-winter fungal community of Kyrdemkulai upland terrace formed an individual cluster distinctly separated from

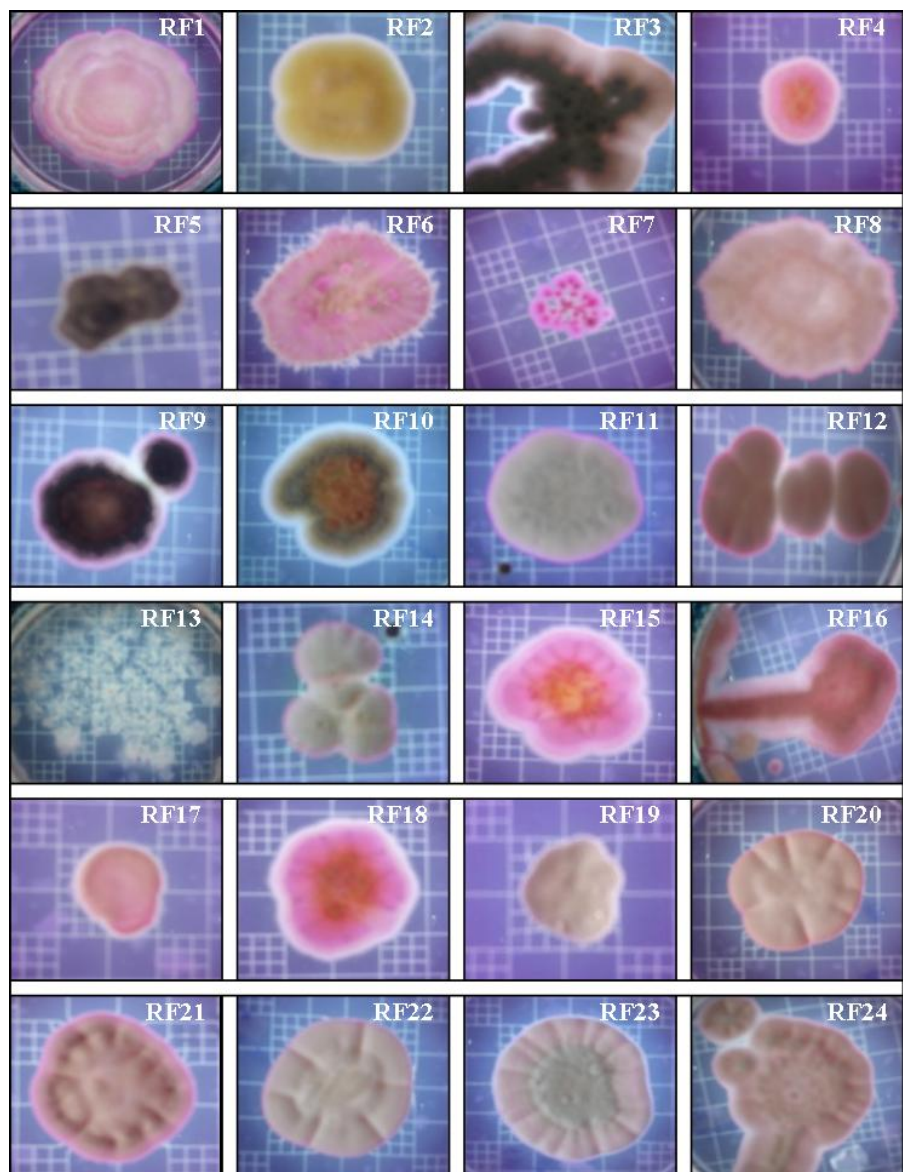


Fig 4.6 Diversity of fungal colony morphology in Streptomycin Rose Bengal Agar medium isolated from soils of various rice ecosystems.

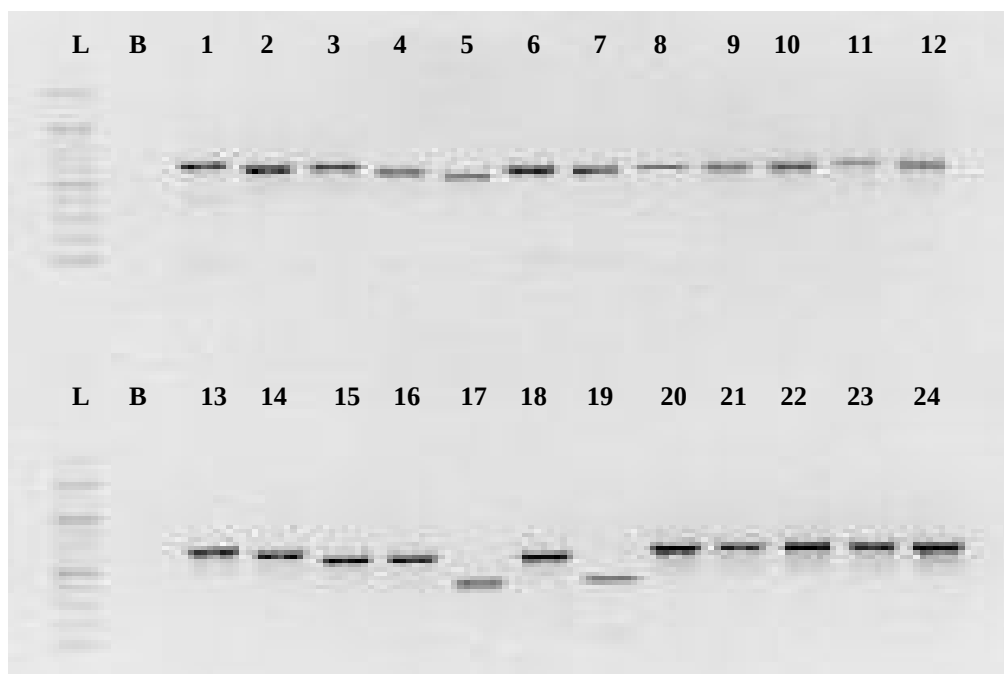


Fig. 4.7 Ribosomal internal transcribed spacer (ITS1-5.8S-ITS2) length analysis (in 1% w v⁻¹ agarose gel) of fungal pure culture isolates obtained from soils of different rice ecosystems. Fungal genomic DNA was amplified using the primer pair 2234C and 3126T specific to internal-transcribed region between 18S and 26S ribosomal genes ([Brown *et al.*, 2005](#)). Altogether 24 fungal isolates were detected. L indicates 100 bp DNA ladder and B indicates negative control PCR reaction where no template DNA was added in PCR mix.

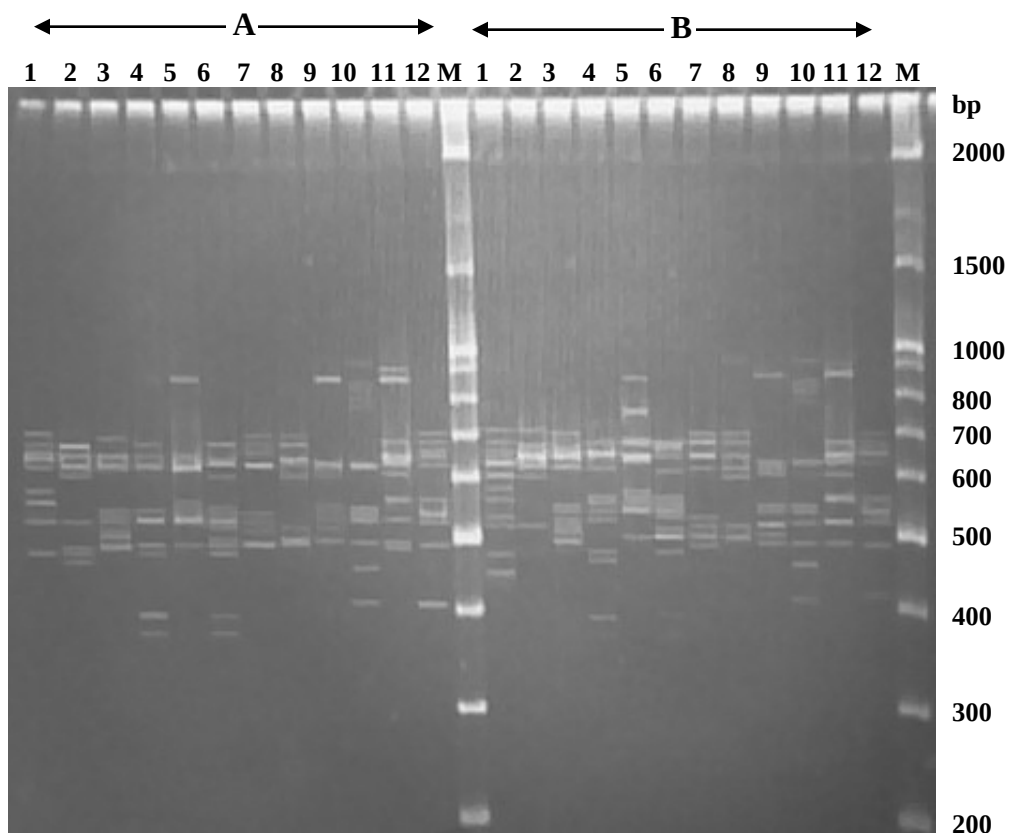


Fig 4.8 Ribosomal internal-transcribed spacer analysis {RISA, 6% acrylamide:biacrylamide (37:1) non-denaturing gel} fingerprint of fungal communities in soils of various rice ecosystems; Kyrdekulai lowland samples (Lanes 1 and 12), Saiden lowland samples (Lanes 4 and 10), Kyrdekulai upland terrace (Lanes 2 and 11), Saiden upland terrace -1 (Lanes 3 and 8), Saiden upland terrace-2 (Lanes 5 and 9) and Saiden slope land (Lanes 6 and 7) in post-summer (Lanes 1 to 6) and post-winter (Lanes 7 to 12). Fungal community pattern was obtained by amplification of soil DNA aliquot using the primer pair 2234C and 3126T specific to internal-transcribed region between 18S and 26S ribosomal genes (Brown *et al.*, 2005). RISA gel was run at constant 70V and temperature of 40°C for 20h using a Ingeny Mutation PhorU Detection System. A and B indicate replicate RISA profiles obtained from two independent DNA extracts. M indicates 100 bp DNA ladder.

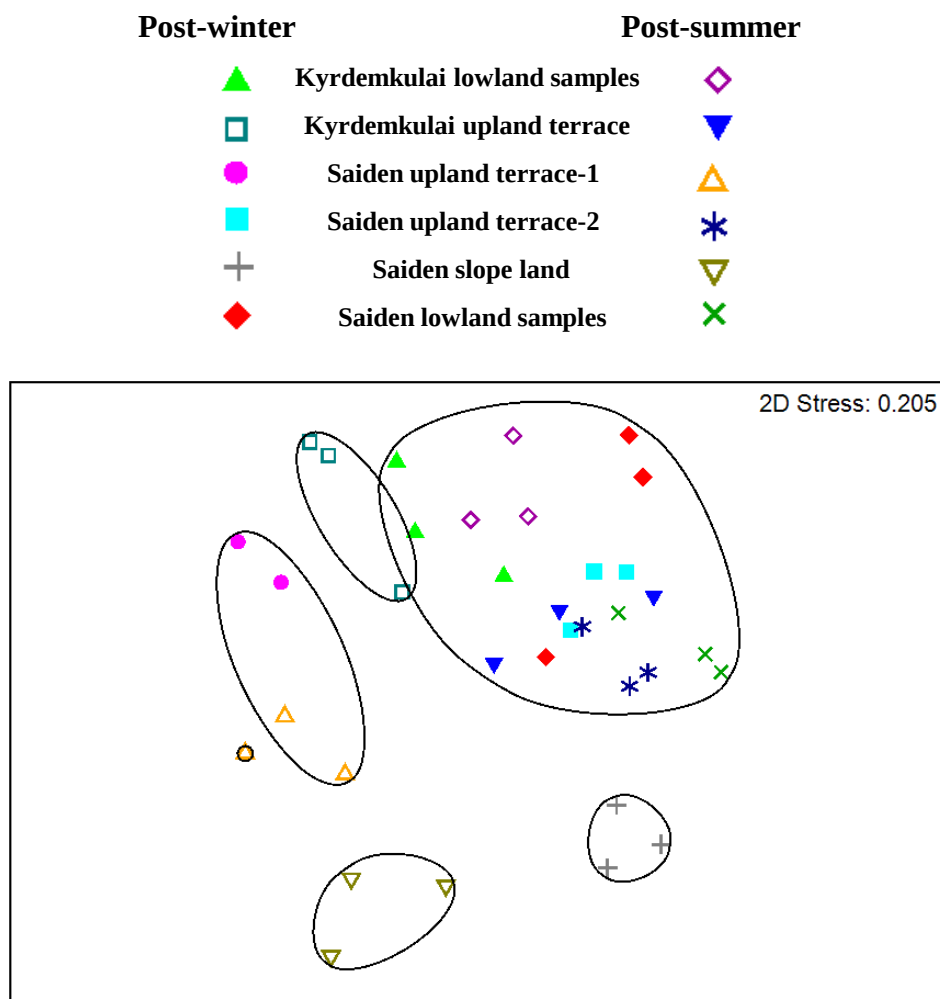


Fig. 4.9 Variability of the fungal community composition in soils of rice ecosystems. The presence-absence data matrix was generated using ribosomal internal transcribed spacer analysis in non-denaturing polyacrylamide gel (6% w v⁻¹) electrophoresis of DNA extracted from soils of various rice ecosystems. Non-metric multidimensional scaling ordination plot was derived using a Bray-Curtis resemblance matrix generated on standardised presence-absence data by total method (Clarke, 1999). Results are based on three replicated RISA profiles obtained from three independent DNA extracts in each rice soil. Ellipses represent superimposed hierarchical clusters (similarity level 60%), deduced using group-average linking based on the Bray-Curtis resemblance matrix.

its post-summer fungal community. Likewise, fungal communities of Saiden slope land formed two distinct clusters according to seasons and were distinctly separated from fungal communities of other rice fields (Fig. 4.9). The ANOSIM test for pairwise comparisons among fungal communities of different rice fields indicated that no significant variation was observed among the post-winter fungal communities of Saiden lowland, Saiden upland terrace-2, Kyrdekulai lowland and Kyrdekulai upland terrace (Global R= 0.89; 999 permutations at $P < 0.001$; Table 4.4). Whereas, fungal communities of other rice fields were significantly differed from each other. See Appendix xx for ANOSIM of the result obtained.

4.6 Relationship of the members of bacterial community with soil attributes

Two-dimensional PCA plot generated using the data matrix (various soil attributes as rows and rice fields as columns) clearly showed that the PC axis-1 (explained 50.3% variability) strongly separated Kyrdekulai lowland and upland terrace rice fields from Saiden slope land rice field and the PC axis-2 (explained 27.1% variability) separated Saiden lowland rice field from Saiden upland terrace-1 rice field (Fig. 4.10; Table 4.5). Saiden upland terrace-2 and Kyrdekulai upland terrace rice fields were situated midway of the spaces between Kyrdekulai lowland and Saiden slope land or Saiden lowland and Saiden upland terrace-1. The superimposed DGGE community profile on PCA plot indicated that out of 46 DGGE bands detected, the DGGE bands 3, 7, 12, 14, 15, 16, 17, 18, 19, 29, 30, 31, 33, 37, 40, 43 and 45 were mainly responsible in discriminating rice fields (Fig. 4.10) and these DGGE bands showed significant correlation with both or one of the two PC axes (Table 4.6). The DGGE bands 1, 9, 20, 21, 22, 23, 24, 25, 26, 27, 32, 34 and 44) were common to all rice fields (Fig. 4.4).

PCA aided with bubble plot was used to study the relationship of each individual soil attribute with DGGE bands (those significantly correlated with PC axes). PCA bubble plot indicated that DGGE bands 7, 16, 19 and 45 were prevalent under rice soil conditions characterized by low MC, low pH, low TOC,

low TN, high sand content, low to medium silt content, high clay content and low to medium CEC (Fig. 4.11 and 4.12; values of soil attributes were within the ranges reported in Table 4.1). On the other hand, DGGE bands 3, 12, 14, 15, 17, 18, 29, 30, 31, 33, 37, 40 and 43 were prevalent under rice soil conditions characterized by medium to high MC, high pH, high TOC, high TN, low to medium sand content, medium to high silt content, low to medium clay content and medium to higher CEC (Fig. 4.11 and 4.12; values of soil attributes were within the ranges reported in Table 4.1).

4.7 Relationship of the members of fungal community with soil attributes

The superimposed RISA community profile on PCA plot (generated using the data matrix of various soil attributes as rows and rice fields as columns) indicated that out of 27 RISA bands detected, the RISA bands 1, 2, 3, 4, 8, 9, 10, 11, 13, 18, 19, 20, 22, 24 and 26 were mainly responsible in discriminating rice fields (Fig. 4.13) and these RISA bands showed significant correlation with both or one of the two PC axes (Table 4.6). The RISA bands 6, 7, 15, 18, 21 and 23 were common to all rice fields (Fig. 4.8).

PCA aided with bubble plot was used to study the relationship of each individual soil attribute with RISA bands (those significantly correlated with PC axes). PCA bubble plot indicated that RISA bands 8, 10, 18, 19 and 20 were prevalent under rice soil conditions characterized by low MC, low pH, low TOC, low TN, high sand content, low to medium silt content, high clay content and low to medium CEC, with an exception was that RISA band 8 was prevalent towards high pH (Fig. 4.14 and 4.15; values of soil attributes were within the ranges reported in Table 4.1). On the other hand, RISA bands 1, 2, 3, 4, 9, 11, 13, 22, 24 and 26 were prevalent under rice soil conditions characterized by medium to high MC, low to medium pH, high TOC, high TN, low to medium sand content, medium to high silt content, low to medium clay content and medium to higher CEC (Fig. 4.14 and 4.15; values of soil attributes were within the ranges reported in Table 4.1).

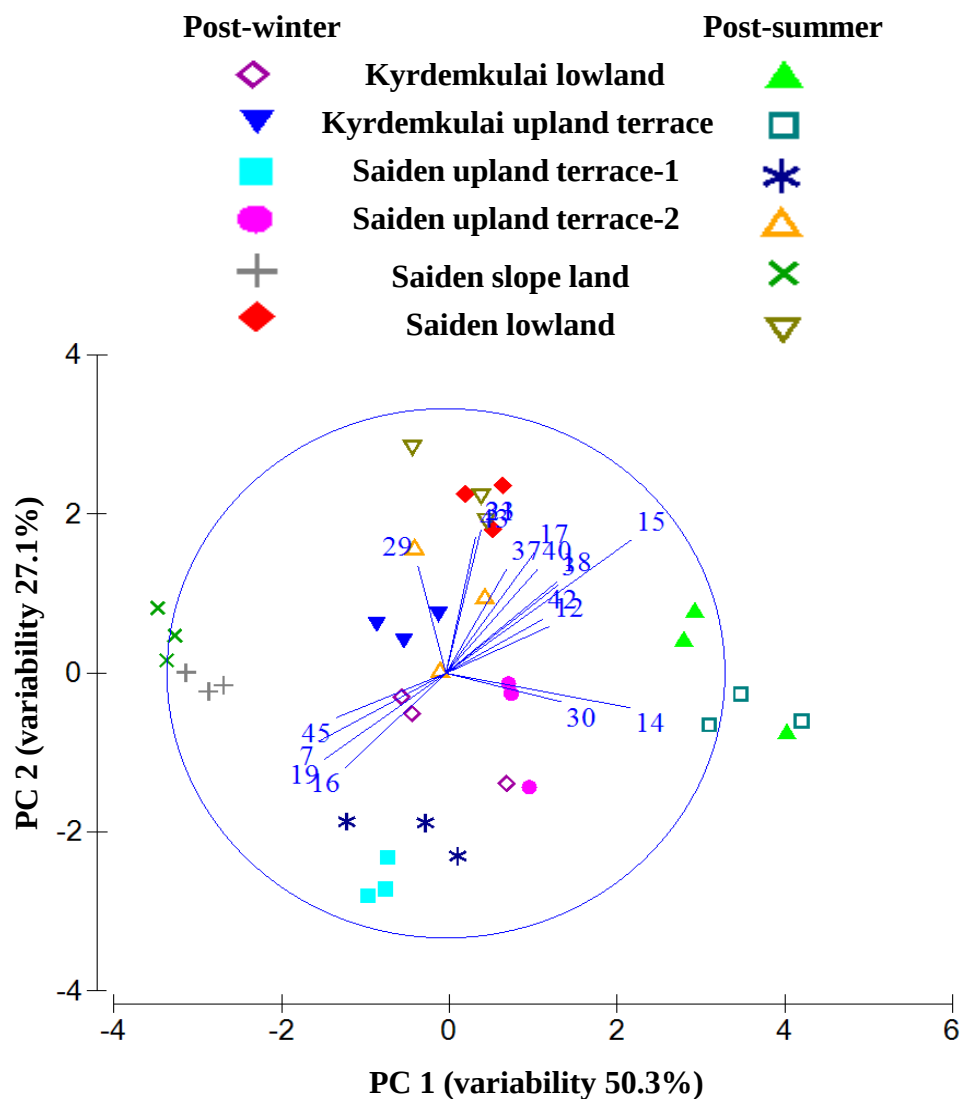


Fig. 4.10 Principal component analysis plot depicted variability among rice fields in terms of soil attributes (moisture content, pH, sand, silt and clay contents, total organic carbon, total nitrogen and cation exchange capacity). Principal axes 1 and 2 expressed variability of 50.3% and 27.1%, respectively. The presence-absence data matrix of bacterial phylotypes (DGGE bands) was superimposed and the significant correlation between DGGE bands and principal axes 1 and 2 were shown as vectors ($r \geq 0.32$ and 0.41 at $P < 0.05$ and $P < 0.001$, respectively).

Table 4.5 Pearson correlation coefficient (r) of principal component axes with soil attributes as variables those responsible for discrimination of rice fields in principal component plot^γ

Soil attributes	Principal component	
	Axis-1	Axis-2
Moisture content	-0.62**	-0.26^{ns}
pH	-0.16	0.34*
Total organic carbon (%)	-0.40*	-0.30^{ns}
Total nitrogen (Kg ha ⁻¹)	-0.32*	-0.42**
Sand content (%)	0.58**	-0.09^{ns}
Silt content (%)	-0.46**	0.24^{ns}
Clay content (%)	0.30^{ns}	-0.49**
Cation exchange capacity (meq 100g ⁻¹ soils)	0.02^{ns}	-0.50**

^γ Values presented are (r ≥ 0.32* and 0.41** at $P < 0.05$ and $P < 0.01$, respectively where n =36). Principal component analysis was performed on data matrix considering various soil attributes as rows and rice fields as column.).

Table 4.6 Pearson correlation coefficient (r) of principal component axes with bacterial (DGGE bands) and fungal phylotypes (RISA bands) those responsible for discrimination of bacterial and fungal community compositions between rice fields^γ

Bacterial DGGE bands	Principal component		Fungal RISA bands	Principal component	
	Axis-1	Axis-2		Axis-1	Axis-2
DGGE-3	-0.40		RISA-1	-0.88	
DGGE-7	0.45		RISA-2		0.54
DGGE-12	-0.40		RISA-3		0.77
DGGE-14	-0.66		RISA-4	-0.85	
DGGE-15	-0.68	-0.70	RISA-8	0.51	
DGGE-16	0.40	0.41	RISA-9	0.40	
DGGE-17		-0.46	RISA-10	-0.45	
DGGE-18	-0.40		RISA-11		0.42
DGGE-19	0.44		RISA-13		0.75
DGGE-29		-0.41	RISA-18		-0.44
DGGE-30	-0.41		RISA-19		-0.46
DGGE-31		-0.54	RISA-20		-0.40
DGGE-33		-0.51	RISA-22	-0.68	
DGGE-37		-0.40	RISA-24	-0.53	
DGGE-40	-0.42	-0.40	RISA-25	-0.63	
DGGE-42	-0.44		RISA-26		-0.40
DGGE-43		-0.51			
DGGE-45	0.40				

^γ Values presented are ($r \geq 0.4$, $P < 0.001$). Principal component analysis was performed on data matrix considering various soil attributes as rows and rice fields as column and then scored data matrix of bacterial DGGE fingerprint was superimposed on the PCA plot to observe the correlation between PC axes with DGGE bands).

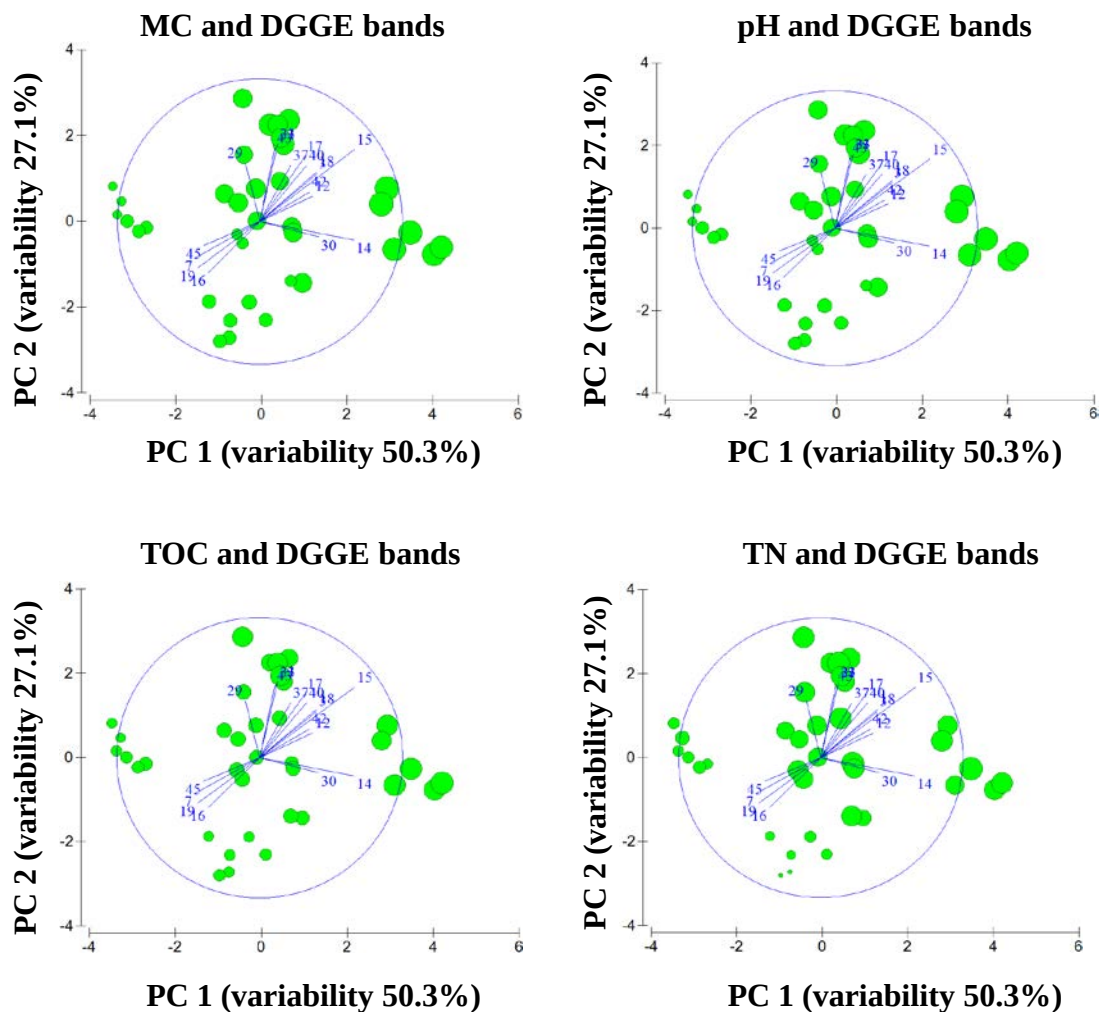


Fig. 4.11 Principal component analysis plot (generated using soil attributes as variables, [Fig.4.10](#)) aided by bubble plot technique depicted the variability of individual soil attribute (moisture content, pH, total organic carbon, total nitrogen) based on their differing values (i.e. high values with large bubble area) according to rice field. The bacterial phylotypes (DGGE bands) data-matrix superimposed on PCA plot depicted the relationship of significant DGGE bands ($r \geq 0.32$ and 0.41 at $P < 0.05$ and $P < 0.001$, respectively) with the soil attribute among rice fields.

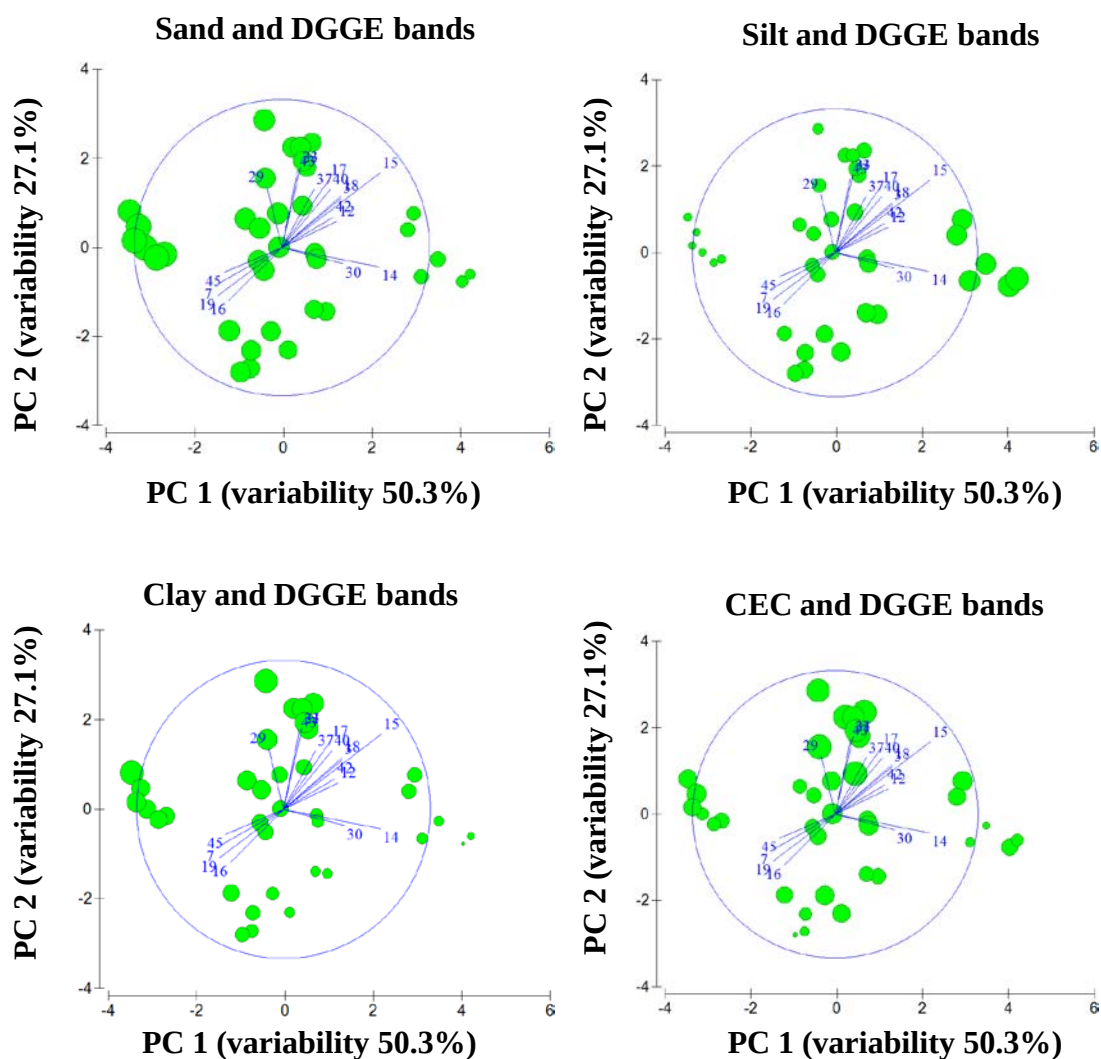


Fig. 4.12 Principal component analysis plot (generated using soil attributes as variables, Fig. 4.10) aided by bubble plot technique depicted the variability of individual soil attribute (sand, silt and clay contents and cation exchange capacity) based on their differing values (i.e. high values with large bubble area) according to rice field. The bacterial phylotypes (DGGE bands) data-matrix superimposed on PCA plot depicted the relationship of significant DGGE bands ($r \geq 0.32$ and 0.41 at $P < 0.05$ and $P < 0.001$, respectively) with the soil attribute among rice fields.

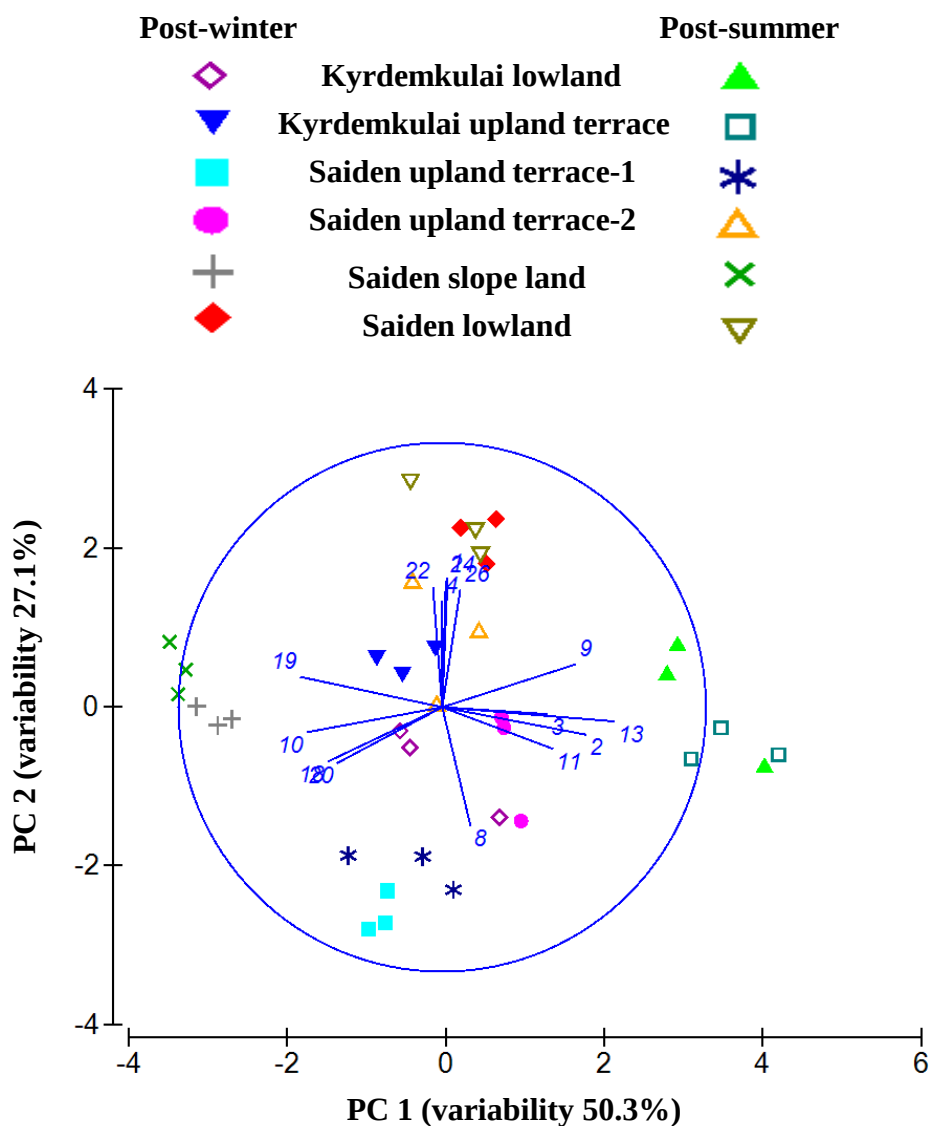


Fig. 4.13 Principal component analysis plot depicted variability among rice fields in terms of soil attributes (moisture content, pH, sand, silt and clay contents, total organic carbon, total nitrogen and cation exchange capacity). Principal axes 1 and 2 expressed variability of 50.3% and 27.1%, respectively. The presence-absence data matrix of fungal phylotypes (RISA bands) was superimposed and the significant correlation between RISA bands and principal axes 1 and 2 were shown as vectors ($r \geq 0.32$ and 0.41 at $P < 0.05$ and $P < 0.001$, respectively).

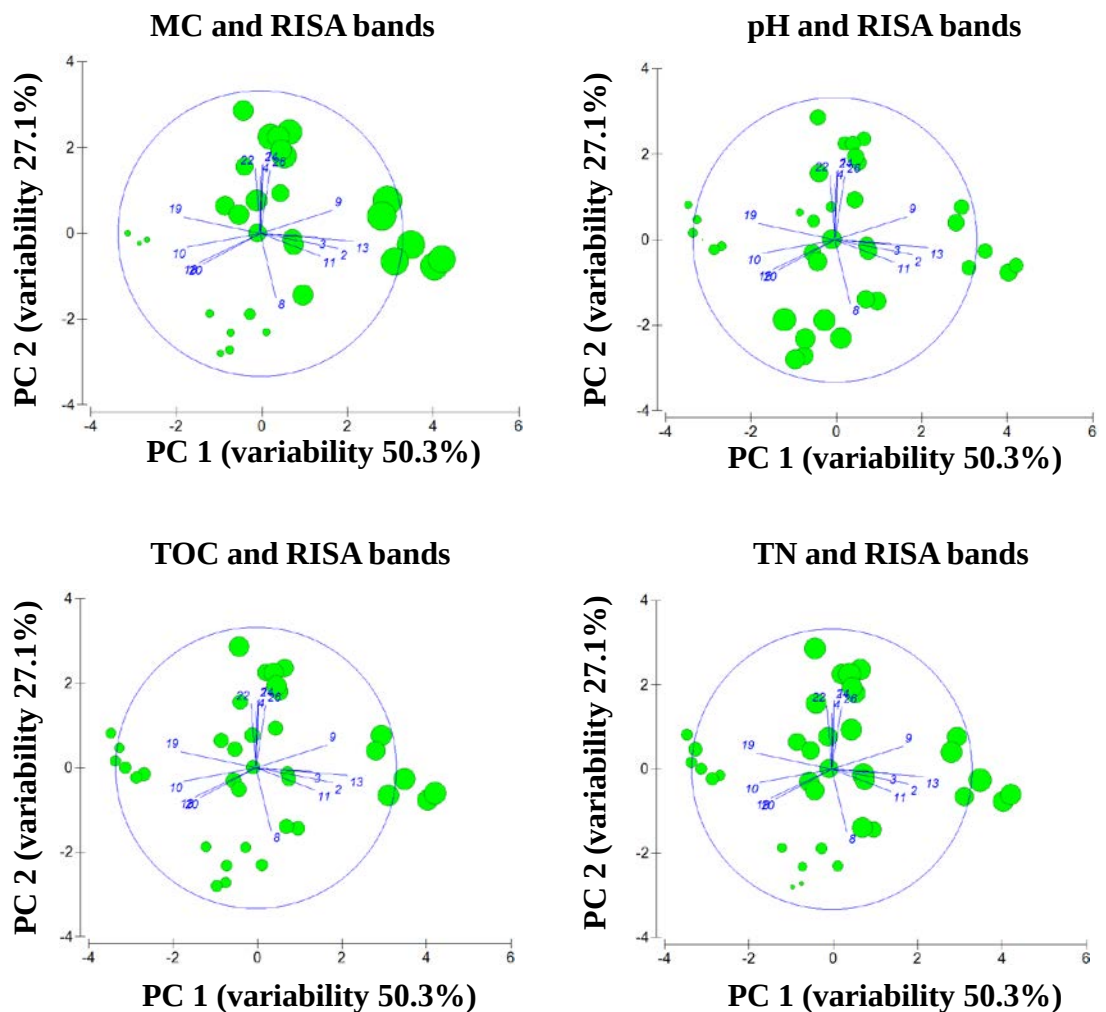


Fig. 4.14 Principal component analysis plot (generated using soil attributes as variables, Fig. 4.13) aided by bubble plot technique depicted the variability of individual soil attribute (moisture content, pH, total organic carbon, total nitrogen) based on their differing values (i.e. high values with large bubble area) according to rice field. The fungal phylotypes (RISA bands) data-matrix superimposed on PCA plot depicted the relationship of significant RISA bands ($r \geq 0.32$ and 0.41 at $P < 0.05$ and $P < 0.001$, respectively) with the soil attribute among rice fields.

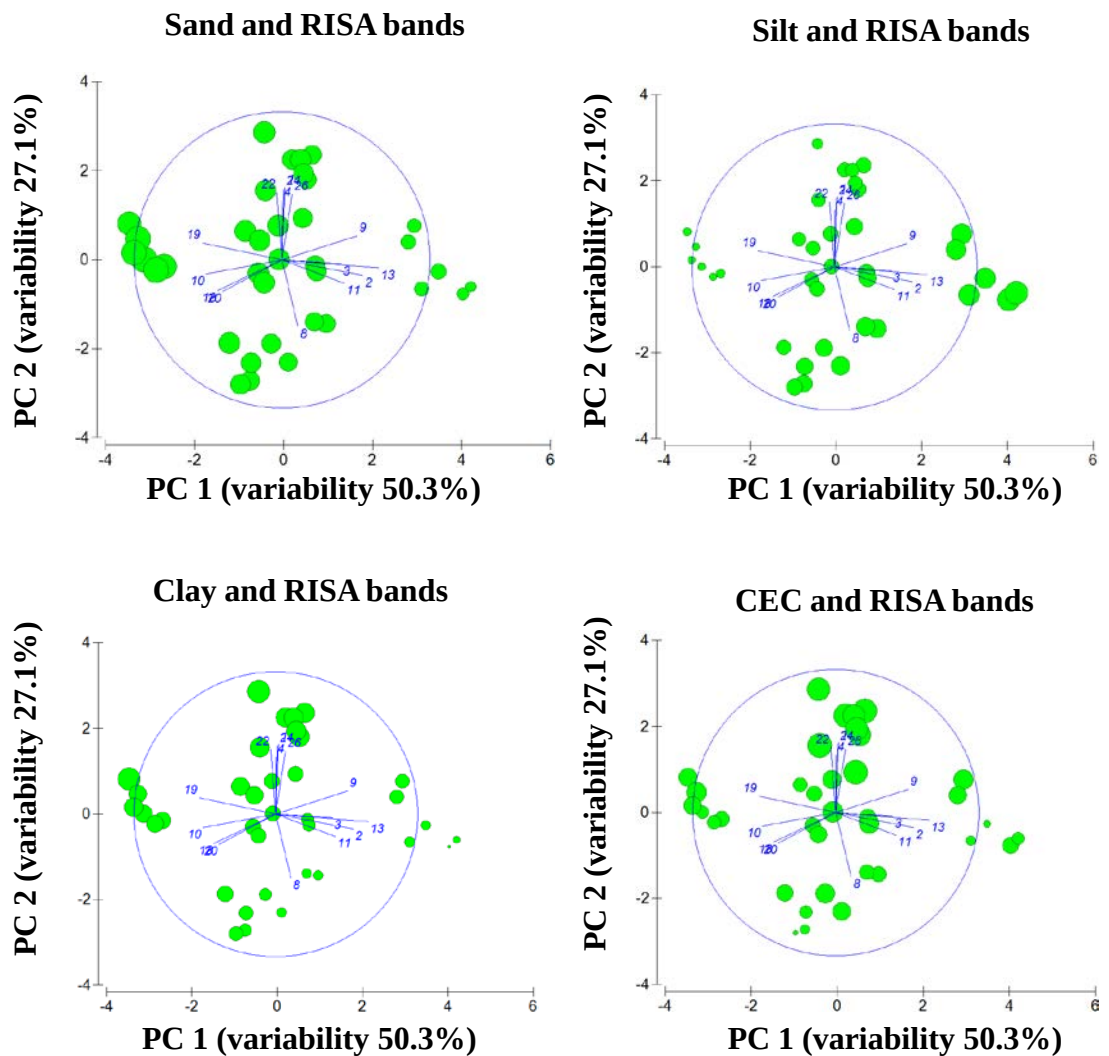


Fig. 4.15 Principal component analysis plot (generated using soil attributes as variables, [Fig.4.15](#)) aided by bubble plot technique depicted the variability of individual soil attribute (sand, silt and clay contents and cation exchange capacity) based on their differing values (i.e. high values with large bubble area) according to rice field. The fungal phylotypes (RISA bands) data-matrix superimposed on PCA plot depicted the relationship of significant RISA bands ($r \geq 0.32$ and 0.41 at $P < 0.05$ and $P < 0.001$, respectively) with the soil attribute among rice fields.

CHAPTER 5

Discussion

Soil microorganisms exhibit great species diversity. Diverse microbial species explore their habitats by adjusting their population abundance and activities to environmental factors. Microorganisms are key components of the soil biota community and are of crucial importance in biogeochemical cycles (Paul, 2007). So, a deeper understanding of microbial community compositions and dynamics at ecosystem levels is essential to refine predictions regarding how the biosphere modulates and responds to future environmental conditions (O'Malley and Dupre, 2007). To date, total microbial community compositions and dynamics of different hill rice ecosystems prevalent in the northeastern region of India are not well known. So, the present investigation assessed bacterial and fungal community compositions in soils of three distinct rice ecosystems viz. direct-seeded hill slope, transplanted upland terrace and transplanted lowland prevalent in Meghalaya by both cultural and the culture-independent method, DGGE profile.

5.1. Soils of different rice ecosystems varied in terms of soil attributes

Three rice ecosystems were represented by six rice fields and these rice fields were grouped as lowland, mature upland terrace, young upland terrace and slope land according to agricultural management practices (Fig. 3.1). Soils of lowland rice fields were silt to clay loam. Relative higher proportion of silt and clay particles in lowland might be due to deposition of transported silt and clay particles from upper elevation through the process of soil erosion. Slope land soil was sandy clay loam and contained higher proportion of sand. Regular slash and burn practice in slope land induces soil erosion, which is a root cause of poor soil aggregate stability. Under such situation silt particles are more vulnerable to

erosion loss leaving higher proportion of sand in slope land. Soil texture of upland terrace rice fields was loam, which is an intermediate range between lowland soil texture (i.e. clay loam) and slope land soil texture (i.e. sandy clay loam).

Soil moisture status in these rice ecosystems varied to a great extent. Soil moisture content in these rice ecosystems was in the order of lowland > mature upland terrace > young upland terrace > slope land. The lowland rice fields were waterlogged and maintained an anaerobic situation throughout the year, and supported a diverse aquatic weed community and produced large quantity of above- and below-ground biomass. Soils of lowland rice fields contained a dense root mat up to few centimeters below from the soil water interface. The slope land rice field contained low soil moisture (13.9-28.1%) in both post-summer and post-winter and represented an aerobic condition. The young upland terrace rice field also contained low soil moisture (32%) in both seasons. This terrace land was newly constructed (1 year old) and not yet recovered from its physical, chemical and biological alterations. The mature upland terrace rice fields contained optimum soil moisture levels i.e. 60% in post-summer and 53.2% in post-winter. This terrace land was constructed 20 years back and the soil supported large quantity of above- and below-ground weed biomass. Soil moisture content in Kyrdemkulai upland terrace differed significantly between seasons (61.4% in post-summer and 22.0% in post-winter). This terrace was constructed 7 years ago and supported a relatively less diverse above- and below-ground weed biomass compared to that in lowland rice ecosystem. Results revealed that soils of these rice fields markedly varied in terms of soil moisture and oxygen status, and above- and below-ground biomass communities.

Findings revealed that soils of rice fields markedly varied in terms of soil pH, TOC, TN and CEC. Seasonal fluctuation in soil pH was observed in lowland and upland terrace land, whereas soil pH in slope land was stable in both seasons. The cause of low soil pH in post-summer in comparison to that in post-winter in lowlands and upland terraces might be the presence of organic acids in soil solution during anaerobic decomposition of weed biomass under high temperature and excessive moisture conditions in post-summer. Such activities considerably

reduced with the decrease in soil temperature in post-winter and hence, increase in soil pH was noticed. This was evident because of low basal respiration in soils of these rice ecosystems in post-winter as compared to that in post-winter (Sangma, 2011). Brady and Weil (2007) reported that the fluctuation in soil pH between summer and winter is because of the movement of salts into and out of the soil zones as the soil moisture moves up and down through the soil profile and the other reason is the intense organic matter decay with onset of warm temperature and rain. But change in soil pH was not significant in Saiden mature upland terrace-2, which might be due to stable soil moisture content in both seasons. Soil erosion and removal of basic cations by excessive runoff rain water might be responsible for low soil pH in slope land compared to the other rice fields. On the other hand, high soil pH in Saiden young upland terrace-1 might be the result of lower level of reduced Al forms due to oxidation of Al during physical alteration of soil layers.

The large quantities of above- and below-ground biomass (including both aquatic weeds and rice roots) produced during summer contributed towards TOC pool in soils of rice ecosystems; however amount of such contribution is relatively higher in lowland rice soils leading to higher TOC as compared to that in upland terrace and slope land rice soils. The lower content of TOC in slope land soils in post-winter in comparison to that in post-summer might be the impact of burning of above-ground biomass in slope land coupled with the loss of surface soil layer due to erosion. The comparative lower amount of TOC in post-summer than that in post-winter in rice soils (exception was the Saiden slope land) might be due to gradual accumulation of large amount of partially decomposed organic matter during anaerobic decomposition in summer season (Brady and Weil, 2007).

Approximate 2 times less TN content in soils of Saiden slope land and Saiden upland terrace-1 compared to that in lowland rice fields indicated that disturbances to ecosystem had significant negative impact on TN content. Saiden slope land was under the influence of periodic burning and exposed to soil erosion and on the other hand Saiden upland terrace-1 was newly constructed (1-year old). TN content in soils is highly dependent on the degree of soil disturbance or

tillage systems (Ren and Hu, 2010). Higher TN content in soils irrespective of rice field types in post-winter might be the result of gradual accumulation of summer grown above- and below-ground biomass in soils.

Cation exchange capacity in soils of rice ecosystems maintained a significant positive correlation with TOC ($r = 0.68$, $P < 0.01$), while a weak negative correlation with soil pH ($r = -0.38$, $P < 0.05$). This suggested that soil organic matter play a strong role in determining CEC of rice soils. Higher TOC content in lowland and mature upland terrace rice fields might retain more basic cations as compared to that in soils of slope land and young upland terraces. Irrespective of rice fields, the probable reasons for low CEC in post-summer as compared to that in post-winter (though seasonal effect was non-significant) are: 1) higher soil moisture content in summer might cause dilution effect, and 2) increase concentration of basic cations in post-winter due to gradual release of basic cations bounded by biomass through decomposition process during summer months.

5.2 Diversity and compositions of soil microbial community varied between rice ecosystems

Diversity indices of bacterial and fungal communities were estimated using fingerprints of DGGE and RISA, respectively in soils of various rice fields. Fingerprint methods like DGGE analysis may underestimate the diversity of microbial community because of low resolution for sequences of rare species, when targeting whole members belonging to domain bacteria in soil (van Verseveld and Röling, 2004). To minimize such effect, whole bacterial community DNA was first amplified using a domain specific primer pair (27f-1492r) followed by amplification of products obtained from first primer pair as template DNA using the DGGE-PCR primer pair (984f-1378r). The present study, however, uncovered the relationship of bacterial community compositions in soils of different rice ecosystems by comparing DGGE fingerprint, and as a result could reveal the characteristics of abundant (dominant) bacterial communities, even though missed rare species. RISA fingerprint has much higher resolution

(high resolution for sequences of rare species also) compared to that of DGGE fingerprint and hence, chances of underestimating fungal community diversity was much less (Brown *et al.*, 2005; Nilsson, 2009).

Soil bacterial and fungal diversity, as estimated by phylotype (DGGE band for bacteria and RISA bands for fungi) richness (Margalef's index) and diversity (Shannon index) varied across rice ecosystem types. MC, TOC, TN, silt content and CEC were good predictor of bacterial richness ($r = 0.0.85, 0.79, 0.74, 0.57$ and 0.76 , respectively; $P < 0.01$) and diversity ($r = 0.0.84, 0.76, 0.81, 0.60$ and 0.71 , respectively; $P < 0.01$) indicating higher index values corresponded to high values of these soil attributes in rice fields. With the increase of sand content in rice soils bacterial phylotype richness and diversity decrease ($r = -0.73$ and -0.74 , respectively $P < 0.01$). Above findings clearly demonstrated that bacterial phylotype richness and diversity was higher in lowland followed by upland terrace and the lowest was in slope land. In this study, a clear relationship was not observed between bacterial diversity and clay content. The reason might be due to the fact that variation in clay content among the soils of different rice fields examined was little. Soil moisture content exerts strong influence on microbial biomass and activity (Wardle, 1992). Stotzky (1986) also reported that microbes are essentially aquatic organisms and even in soil required a sufficiently higher water activity for growth. Recent study has demonstrated that diversity of soil bacterial communities is often strongly correlated with soil pH (Fierer and Jackson, 2006; Hartman *et al.*, 2008; Jenkins *et al.*, 2009; Lauber *et al.*, 2009) and this relationship was observed both for overall bacterial community composition (Fierer and Jackson, 2006; Baker *et al.*, 2009; Lauber *et al.*, 2009) and for the composition of individual bacterial groups (Nicol *et al.*, 2008; Davis *et al.*, 2008; Jenkins *et al.*, 2009; Jones *et al.*, 2009). Such relationship were exclusively studied in arable lands and seems to hold across a variety of spatial scales, including continental scales (Fierer and Jackson, 2006; Lauber *et al.*, 2009) and across land-use types at a given location (Lauber *et al.*, 2008; Jenkins *et al.*, 2009). However, in this study, bacterial phylotype richness and diversity did not show significant correlation with soil pH and reasons may be due to the fact that

soil attributes like water availability, substrate diversity, Al activity, alternate oxic-anoxic situation, above- and below-ground plant community etc. greatly differed between arable and waterlogged rice ecosystems. All these soil attributes might mask the effects of pH on bacterial phylotype richness and diversity in the rice fields examined. Bacterial phylotype richness and diversity were known to influence strongly by soil moisture regime with drastic changes in DGGE pattern during rice cultivation (Asari *et al.*, 2007). Therefore, it can be concluded that relationship between soil pH and bacterial phylotype diversity holds for arable land can't be expected in soils of rice ecosystems. Again, seasonal variation in phylotype richness and diversity of bacteria within each rice field examined might be the cumulative effects of soil moisture content and temperature. Conrad *et al.* (2009) recently confirmed that temperature conditions defined structure and function of the methanogenic microbial community in rice soils.

Interestingly, of the soil attributes (sand, silt and clay contents, pH, MC, TOC, TN, CEC) examined, only soil pH by far was correlated negatively with both fungal richness ($r = -0.48$, $P < 0.01$) and diversity ($r = -0.37$, $P < 0.01$) indicating the higher index values corresponded to high acidity across rice fields. Other soil attributes did not show any significant relationship with fungal diversity indices. The reason for higher fungal richness and diversity in slope land and young upland terrace rice fields might be the accumulative effects of low pH rice and low soil moisture.

The two dimensional NMDS plot (Fig. 4.5) clearly revealed that grouping of communities of abundant bacteria corresponded to the observed patterns with MC, TOC, TN and CEC across rice fields. For examples, the bacterial communities of Saiden lowland in post-summer and Kyrdekulai lowland in both seasons together formed a group at 70% similarity level, which generally have waterlogged condition in soils. Likewise, the bacterial communities of Saiden lowland in post-winter and Saiden upland terrace-2 and Kyrdekulai upland terrace in post-summer together formed another group at 70% similarity level, which have soil moisture content in the range between 60-63%. Each of the land use types viz. lowland, mature upland terrace, young upland terrace and slope

land rice fields maintained a unique soil physicochemical environment (as evident from the soil attributes examined), which might shape the bacterial community composition according to land use type. Our results are consistent with studies where land use and soil attributes (pH, Al activity, plant types, substrate quality, soil texture etc.) were found as important factors determining the diversity and structure of bacterial communities (Hartman *et al.*, 2008; Lauber *et al.*, 2008; Wakelin *et al.*, 2008; Jesus *et al.*, 2009). Toxic effects of Al to microbes and plants are well known in sub-tropical and tropical soils, where soils are rich in Al due to acid alfisoils (minerals with Al interlayers) (Wood, 1995; Jones *et al.*, 2005). Soils of the rice fields examined were predominantly alfisols (Patiram and Ramesh, 2008). Again flooding rice soils exhibit asymptotic behaviour with respect to pH dynamics (Poonomperuma, 1985). Hence, it might be possible that Al activity and soil moisture content would covary and correlate with microbial community changes in the land use types examined in this investigation. This might be the most appropriate explanation for the fact that soil pH did not exhibit strong correlation with bacterial diversity and community composition in rice field ecosystems; otherwise, soil pH is a strong determinant of change in bacterial community composition in most arable soils as reported by several workers (Fierer and Jackson, 2006; Hartman *et al.*, 2008; Lauber *et al.*, 2008; Jenkins *et al.*, 2009; Lauber *et al.*, 2009).

Similarly, the two dimensional NMDS plot (Fig. 4.9) clearly revealed that grouping of fungal community compositions closely related to the observed pattern with soil moisture content and covariant soil pH across rice fields. For examples, the fungal community compositions of Saiden and Kyrdekulai lowlands, Saiden upland terrace-2 in both seasons and Kyrdekulai upland terrace in post-summer together formed a group at 60% similarity level, which generally have 4.92-5.30 pH ranges in soils and soil moisture was near saturation to waterlogg. The fungal community compositions of Saiden slope land and Saiden upland terrace-1 formed separate clusters at 60% similarity level which have 4.8-4.85 and 5.42-5.60 soil pH, respectively and soil moisture content ranged between 14.0-32.0%. Of all the rice fields and soil attributes examined,

NMDS plot indicated that soil pH and the covariant soil moisture content was the best predictor of soil fungal community compositions across rice ecosystems. Hamman *et al.* (2007) demonstrated that variation in fungal community composition could be explained by three environmental variables like soil pH, temperature and carbon substrate availability. Sangma (2011) characterised the biological pools of carbon, nitrogen and phosphorus in soils of four land use types considered in this study and observed that each land use type maintained unique set of biological pools of C, N and P. These findings also provided an indirect indication on existence of microbial community composition specific to land use type. Ross *et al.* (1999) reported that land-use change modulates soil C, N and P pools and fluxes in three adjacent ecosystems viz. broadleaf-podocarp forest, grazed pasture and *Pinus radiata* forest. Overall, the above findings indicate that the soil management practice is a key factor in defining the structure of bacterial communities, whether under natural (Fierer and Jackson, 2006) or under anthropic conditions, including *jhum* cultivation (sites considered in this investigation) and conventional agriculture (Hartman *et al.*, 2008; Wakelin *et al.*, 2008). Recently, Reeve *et al.* (2010) demonstrated that soil physicochemical characteristics were primarily determined by soil textural type (coarse vs fine-textured), but biological and functional gene microarrays (FGA) measures were more influenced by management (organic vs conventional). Organically managed soils consistently showed greater functional activity as well as FGA signal intensity (SI) and diversity. The authors further suggested that management was the dominant determinant of soil biology as measured by microbial gene frequency and diversity, which paralleled measured microbial processes.

5.3 Relationship of soil attributes with bacterial and fungal phylotypes

PCA plot generated using soil attributes as variables (Fig. 4.10) indicated that MC, sand and silt contents, TOC and TN were better predictors as compared to pH, clay content and CEC in terms of discriminating rice fields in PCA plot. This was evident because MC, sand and silt contents, TOC and TN maintained

strong correlation with PC axis-1 that explained 50.3% variability existed among rice fields; whereas pH, clay content and CEC maintained correlation with PC axis-2 that explained only 27.1% variability (Table 4.5). In this study, ranking of soil attributes as better predictor in discriminating rice fields were in the order of MC > sand content > silt content > TOC > TN > CEC > clay content > pH. Results of superimposition of either DGGE or RISA data matrix on PCA plot derived using soil attributes as variables clearly demonstrated that prevalence of phylotypes of bacterial and fungal communities markedly varied depending on soil attributes. Bacterial phylotypes (DGGE bands) 3, 7, 12, 14, 15, 16, 18, 19, 30, 40, 42 and 45 were heavily correlate with PC axis-1 indicating strong influence of MC, sand and silt contents, TOC and TN on prevalence of these phylotypes (Table 4.5 and 4.6, Fig. 4.11 and 4.12). On other hand, prevalence of bacterial phylotypes 15, 16, 17, 29, 31, 33, 37, 40 and 43 were strongly influenced by pH, clay content and CEC (Table 4.5 and 4.6, Fig. 4.11 and 4.12). Likewise, the occurrence of fungal phylotypes (RISA bands) 1, 4, 8, 9, 10, 22, 24 and 25 were strongly influenced by MC, sand and silt contents, TOC and TN; whereas, occurrence of fungal phylotypes 2, 3, 11, 13, 18, 19, 20 and 26 were strongly influenced by pH, clay content and CEC (Table 4.5 and 4.6, Fig. 4.14 and 4.15). The above findings confirmed that rice ecosystems were distinct in terms of both bacterial and fungal community compositions. Previous work also demonstrated that variability in microbial community composition can be caused by seasonal variability in water, temperature and substrate availability (Bardgett *et al.*, 1999; Steinberger *et al.*, 1999), alteration in soil texture (Schutter *et al.*, 2001) and management practices (Calderon *et al.*, 2001) and types of plant host habitat. The distinct microbial community composition observed under lowland and matured upland terrace rice fields compared to that on slope land and young upland terrace might be associated with plant species diversity in these ecosystems. In natural ecosystems, different levels of plant species diversity can influence composition and biomass of microbial communities (Wardle and Nicholson, 1996; Kowalchuk *et al.*, 2002; Ladygina and Hedlund, 2010). Recently, Haichar *et al.* (2008) established that plant host habitat and root exudates can shape the soil microbial

community structure. Again, Gelsimo *et al.* (1999) observed a relationship between the grouping of DNA fingerprints of 16 soils from a broad geographic distribution and soil texture. Similarly, Johnson *et al.* (2003) compared the composition of microbial communities in 47 agricultural soils and adjacent land-use and reported that bacterial DNA fingerprints were significantly correlated with soil texture. Jesus *et al.* (2009) reported that distribution of sequences among and within bacterial phyla differed among land use systems likely through the effects of soil attributes. For example, Acidobacteria and Proteobacteria were the most abundant phyla in most soils, including soils of the eastern and western Amazon. *Clostridium* was present only in the primary forest and old secondary forest. The authors further reported that Acidobacteria group III sequences were observed in the acidic forest soil (pH 4.5 and 4.7) as well as pasture soils (pH 5.2 and 5.1). In the present study, bacterial DGGE bands 1, 9, 20, 21, 22, 23, 24, 25, 26, 27, 32, 34 and 44 and fungal RISA bands 6, 7, 15, 18, 21 and 23 were found common to all rice ecosystems. Having determined that soils of rice ecosystems harbour common and unique bacterial DGGE and fungal RISA bands according to rice ecosystems, the next step is to determine the taxonomic identities of these bacterial and fungal phylotypes. Future challenge will be to define functional significance of these bacterial and fungal phylotypes, particularly those whose occurrence was ecosystem dependent. Current understanding of microbial community compositions in soils of different rice ecosystems through this investigation and determining probable functions of the members of these communities through future research will help designing apt management practices for modern day agriculture. Moreover, understanding the effects of determinants (soil attributes) on occurrence and abundance of bacterial and fungal phylotypes in soils rice ecosystems will certainly maximise our ability to better manipulate microbial community composition through adjustment in soil attributes.

CHAPTER 6

Summary

Perhaps microbes are most diverse life on earth. Microbes are known to orchestrate biogeochemical transformations in natural, managed and engineered ecosystems. Recently, Microbial Ecologists has paid attention to explore vast microbial diversity of various ecosystems. Findings of microbial ecology and eco-physiology studies generated evidences that changes in land-use/ land-cover, agricultural management practice, plant species composition etc. have significant impacts on soil conditions and microbial communities are likely to respond to these changes. Moreover, soil physicochemical properties directly or indirectly affect microbial community composition. Therefore, assessment of microbiota community composition in soil and their relationship with various soil attributes are pre-requisite in understanding the functioning and stability of an ecosystem.

Three rice ecosystems viz. slope land, upland terrace and low land prevalent under hill agriculture in the northeastern region of India varies markedly in terms of agricultural productivity. Such variability in productivity between rice ecosystems are thought to be linked with the factors like soil water content, soil type, slope aspect, above- and below-ground biomass and anthropogenic activities (Tripathy *et al.*, 2007). These factors are also expected to influence soil microbial community composition. It is thus necessary to formulate ecosystem specific research strategy for rice (Barah and Pandey, 2005, Bhowmick *et al.* 2005) so that determinants of microbial community composition in rice soils can be determined. This will help in formulating apt management strategies in maintaining soil health and thereby achieving higher agricultural productivity from rice ecosystems. Keeping the above view in mind, the present investigation characterized the bacterial and fungal community compositions in soils of three distinct rice ecosystems and studied the relationship of sequence based members of the communities with soils attributes at spatial and temporal scales.

Findings of this investigation are summarized below:

- Soil moisture content was found to be a critical variable of rice ecosystems examined and higher soil moisture content observed was in the order of lowland > old upland terrace > young upland terrace > slope land. Distribution of sand, silt and clay particles markedly varied between rice ecosystems indicating higher proportion of silt and clay was in soils of lowland and higher sand proportion was in slope land. Soils of upland terrace contained soil particles at an intermediate level between lowland and slope land.
- Finding revealed that TOC, TN and CEC showed similar trend with soil moisture content, indicating the higher values in the order of lowland > old upland terrace > slope land > young upland terrace. Seasonal fluctuation of TOC was pronounced indicating significant higher values in post-winter season in all rice fields with only exception in slope land.
- Soil pH of rice fields ranged within 4.8 to 5.6. An increase in soil pH was observed in post-winter than that in post-summer. Soil pH was relatively higher in lowlands compared to that in slope land.
- Total organic carbon content in soils of rice ecosystems played a strong role in determining CEC in soil. This was evident because CEC maintained a significant positive correlation with TOC ($r = 0.68$, $P < 0.01$), whereas a weak negative correlation maintained with soil pH ($r = -0.38$, $P < 0.05$).
- Soils of lowland rice fields showed significantly higher yield of soil DNA yield compared to other rice fields.
- Bacterial richness and diversity were found to be higher in soils of lowland followed by soils of upland terrace and the lowest richness and diversity in soils of slope land rice fields.

- MC, TOC, TN, silt content and CEC were good predictors of bacterial phylotypes richness ($r = 0.085, 0.79, 0.74, 0.57$ and 0.76 , respectively; $P < 0.01$) and diversity ($r = 0.084, 0.76, 0.81, 0.60$ and 0.71 , respectively; $P < 0.01$) indicating corresponded of higher index values to high values of these soil attributes in rice fields. With the higher sand proportion in soils, bacterial richness and diversity decreased ($r = -0.73$ and -0.74 , respectively $P < 0.01$). However, these soil attributes did not show correlation with fungal phylotypes richness and diversity.
- Soil pH was not a good predictor of bacterial phylotypes richness and diversity in rice soils. Reasons might be masking effect of factors such as soil water content, substrate diversity, alternate oxic-anoxic situations in rice soils on effects of soil pH. Interestingly, soil pH showed a strong negative correlation with both fungal phylotypes richness ($r = -0.48$, $P < 0.01$) and diversity ($r = -0.37$, $P < 0.01$) indicating corresponded of the higher index values to high acidity across rice fields. Again, this relationship might be the cumulative effects of soil pH and its covariant MC; because soils of low pH (slope land) had low soil MC or soils of relative higher pH (lowland) had high MC.
- Rice ecosystems were varied in terms of abundant bacterial community composition as evident from NMDS plot. At 70% similarity level, abundant bacterial communities formed four clusters in NMDS plot according to the observed patterns of MC, TOC, TN and CEC across rice fields i.e. the cluster-1 represented by waterlogged situation, cluster-2 represented by 60-63% soil MC and cluster-3 and 4 represented by soil MC < 32%. Soil MC is the strongest determinant of TOC, TN and CEC in soils. Hence, it can be stated that soil MC is strongest determinant of abundant bacterial community composition, which can override the effect of location on bacterial community composition.
- Of all the rice fields and soil attributes examined, the NMDS plot of RISA fingerprint indicated that soil MC and its covariant pH were the best predictor of soil fungal community composition.

- Ranking of soil attributes as better predictor in discriminating rice fields were in the order of MC > sand content > silt content > TOC > TN > CEC > clay content > pH. This was evident because MC, sand and silt contents, TOC and TN maintained strong correlation with PC axis-1 that explained 50.3% variability existed among rice fields; whereas pH, clay content and CEC maintained correlation with PC axis-2 that explained only 27.1% variability.
- Revelation of the results of superimposed bacterial or fungal presence-absence data matrix on PCA plot of soil attributes showed that occurrence of phylotypes of bacterial and fungal communities markedly varied according to the observed patterns of soil attributes in PCA plot.
- Out of the 46 DGGE bands detected across rice fields, the bands 3, 7, 12, 14, 15, 16, 18, 19, 30, 40, 42 and 45 were strongly correlated with the PC axis-1 indicating stronger influence of MC, sand and silt contents, TOC and TN on occurrence of these phylotypes. On other hand, occurrence of bacterial phylotypes 15, 16, 17, 29, 31, 33, 37, 40 and 43 were influenced by pH, clay content and CEC.
- Out of the 27 RISA bands detected across rice fields, the bands 1, 4, 8, 9, 10, 22, 24 and 25 were strongly influenced by MC, sand and silt contents, TOC and TN; whereas, occurrence of bands 2, 3, 11, 13, 18, 19, 20 and 26 were strongly influenced by pH, clay content and CEC.

CHAPTER 7

Conclusion

Soils of lowland rice ecosystem harboured higher bacterial phylotype richness and diversity compared to that in upland young terrace and slope land rice ecosystems indicating negative impact of disturbed ecosystem on bacterial diversity. In contrast, higher fungal phylotype richness and diversity observed in soils of slope land corroborated the previously established fact that fungal diversity is higher in disturbed ecosystem. The three rice ecosystems viz. slope land, upland terrace and low land varied markedly in terms of bacterial and fungal community compositions and such variation in community composition followed the observed patterns of soil attributes to their corresponding rice ecosystem. Among the soil attributes examined, MC was the strongest determinant of bacterial and fungal community in rice ecosystems. The NMDS and PCA plots indicated that soil MC override the effects of locational difference in bacterial and fungal community compositions. Soil MC and management factors associated with rice ecosystem strongly influenced TOC, TN, sand and silt content, and CEC and hence, these soil attributes also found to be co-determinants of bacterial and fungal community composition in rice soils. One interesting observation was that soil pH is not a strong predictor of bacterial diversity in soils of rice ecosystems. Results suggested that the structures of soil bacterial and fungal communities are not random at ecosystem scale and that diversity and composition of soil bacteria and fungi in soils of rice ecosystems can largely be predicted based on our knowledge on management practices, soil MC and the covariants of soil MC like TOC, TN, soil texture and CEC. Such prediction data may be useful in determining unique abundant bacterial and fungal phylotypes that may be of high value as bioinoculants for specific rice ecosystem. The community fingerprints of bacteria and fungi generated from soils of different rice ecosystems will be useful as a baseline dataset in monitoring biological soil quality and to study impacts of climate change and agricultural management practices on microbial diversity in future study.

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