

**STUDIES ON  
MICROBIAL COMMUNITIES AND THEIR  
ACTIVITIES IN DEGRADED AND UNDEGRADED  
FOREST SOILS OF ARUNACHAL PRADESH**

By  
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IN FULFILMENT OF THE DEGREE OF  
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*Dedicated to  
the Loving Memory of my  
Grandfather  
& my beloved Parents*

**THE NORTH-EASTERN HILL UNIVERSITY**  
**October, 2002**

**DECLARATION**

I, **Sorokhaibam Sureshkumar Singh**, hereby declare that the subject matter of this thesis entitled "**Studies on microbial communities and their activities in degraded and undegraded forest soils of Arunachal Pradesh**" is the record of work done by me, that the contents of this thesis did not form basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other University/ Institute.

This is being submitted to the North-Eastern Hill University, Shillong for the award of the degree of Doctor of Philosophy in Botany.



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## **CONTENTS**

	<b>Page</b>
<b>Acknowledgements</b>	i
<b>List of figures</b>	vii
<b>List of tables</b>	viii
<b>Abbreviations</b>	ix
<b>List of plates</b>	x
<b>Chapter-1. GENERAL INTRODUCTION</b>	1
<b>Chapter-2. STUDY SITE DESCRIPTION</b>	13
<b>Chapter-3. PHYSICO-CHEMICAL CHARACTERISTICS OF SOILS</b>	
<b>Distribution of soil temperature, moisture, bulk density, water holding capacity, pH, total organic C, total N &amp; P, ammonium and nitrate-N in degraded and undegraded soils</b>	
<b>3.1. Introduction</b>	19
<b>3.2. Review of literature</b>	20
<b>3.3. Methodology</b>	28
<b>3.4. Results</b>	35
<b>3.5. Discussion</b>	
<b>3.5.1. Influence of shifting cultivation and selective logging on physical characteristics of soil</b>	60
<b>3.5.2. Influence of shifting cultivation and selective logging on chemical characteristics of soil</b>	64
<b>Chapter-4. BIOLOGICAL CHARACTERISTICS OF SOILS</b>	
<b>4.1. Soil respiration, bacterial and fungal populations in degraded and undegraded forest sites</b>	
<b>4.1.1. Introduction</b>	71
<b>4.1.2. Review of literature</b>	73
<b>4.1.3. Methodology</b>	81
<b>4.1.4. Results</b>	83
<b>4.1.5. Discussion</b>	
<b>4.1.5.1. Influence of soil degradation on soil respiration</b>	91
<b>4.1.5.2. Influence of soil degradation on bacterial population</b>	95
<b>4.1.5.3. Influence of soil degradation on fungal population</b>	98

<b>4.2. Diversity of vesicular-arbuscular mycorrhizal fungi (VAMF) as affected by soil degradation</b>	
<b>4.2.1. Introduction</b>	101
<b>4.2.2. Review of literature</b>	103
<b>4.2.3. Methodology</b>	107
<b>4.2.4. Results</b>	113
<b>4.2.5. Discussion</b>	114
<b>4.3. Distribution of microbial biomass C and N in degraded and undegraded forest soils</b>	
<b>4.3.1. Introduction</b>	117
<b>4.3.2. Review of literature</b>	118
<b>4.3.3. Methodology</b>	127
<b>4.3.4. Results</b>	128
<b>4.3.5. Discussion</b>	133
<b>Chapter-5. MICROBIAL COMMUNITY STRUCTURE OF SOILS AS DETERMINED BY MOLECULAR TECHNIQUES</b>	
<b>5.1. Genomic diversity of soil community DNA and structural diversity of bacterial communities as measured by ERIC-PCR and 16S rDNA profiles</b>	
<b>5.1.1. Introduction</b>	138
<b>5.1.2. Review of literature</b>	140
<b>5.1.3. Methodology</b>	141
<b>5.1.4. Results</b>	145
<b>5.1.5. Discussion</b>	
<b>5.1.5.1. Gel compare analysis of microbial community by ERIC-PCR amplified soil DNA</b>	150
<b>5.1.5.2. Bacterial diversity of soil as revealed by DGGE analysis of the PCR amplified 16S rDNA fragments</b>	151

<b>5.2. Microbial community structure of soils as measured by phospholipids fatty acid (PLFA) profiles</b>	
5.2.1. Introduction	154
5.2.2. Review of literature	155
5.2.3. Methodology	158
5.2.4. Results	161
5.2.5. Discussion	
5.2.5.1. Impact of soil degradation on the PLFA profiles as a measure of microbial community structure	171
 <b>Chapter-6. BIOCHEMICAL CHARACTERISTICS OF SOILS</b>	
<b>Soil dehydrogenase, acid phosphatase and urease enzyme activities in degraded and undegraded forest soils</b>	
6.1. Introduction	177
6.2. Review of literature	180
6.3. Metodology	193
6.4. Results	195
6.5. Discussion	
6.5.1. Spatial variation of dehydrogenase, acid phosphatase and urease activities	203
6.5.2. Seasonal variation of dehydrogenase, acid phosphatase and urease activities	205
6.5.3. Depth-wise variation of dehydrogenase, acid phosphatase and urease activities	207
 <b>Chapter-7. STEPWISE DISCRIMINATE FUNCTION ANALYSIS</b>	
<b>Evaluation of forest soil degradation by stepwise discriminant function analysis</b>	
7.1. Introduction	210
7.2. Methodology	211
7.3. Results	214
7.4. Discussion	218

<b>Chapter-8. GENERAL DISCUSSION</b>	
<b>8.1. Physico-chemical characteristics</b>	222
<b>8.2. Biological characteristics of soils</b>	229
<b>8.3. Molecular microbial diversity soils</b>	238
<b>8.4. Biochemical characteristics of soils</b>	241
<b>8.5. Evaluation of forest soil degradation types by using         stepwise discriminant function analysis (DFA)</b>	246
<b>8.6. Impact of soil degradation on soil characteristics in         degraded and moderately degraded forest sites as         compared to the undegraded forest site</b>	248
<b>Chapter-9. CONCLUSION</b>	
<b>9.1. Soil degradation class</b>	251
<b>9.2. Microbial communities and their activities</b>	253
<b>9.3. Fertility status of soils</b>	255
<b>9.4. Discriminant function analysis</b>	255
<b>Chapter-10. SUMMARY</b>	257
<b>References</b>	264
<b>Appendix-I (PLATES)</b>	
<b>Bio-data</b>	

## List of Figures

<b>Nos.</b>	<b>Figure description</b>	<b>Page</b>
<b>Fig.1.1.</b>	Map of Arunachal Pradesh showing distribution of forest cover	4
<b>Fig.2.1</b>	Map the Banderdewa Forest Reserve of Papumpare district in Arunachal Pradesh	15
<b>Fig.2.2.</b>	Climatogram of mean monthly variation of rainfall and maximum air temperature of degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites	16
<b>Fig.3.1.</b>	Soil temperatures of three study sites at surface and subsurface layers	38
<b>Fig.3.2.</b>	Soil moisture content in DF, MDF and UDF sites at surface and subsurface layers	39
<b>Fig.3.3.</b>	Soil bulk density in DF, MDF and UDF sites at surface and subsurface layers	40
<b>Fig.3.4.</b>	Water holding capacity of soil in DF, MDF and UDF sites at surface and subsurface soil layers	41
<b>Fig.3.5.</b>	Distribution of soil pH at surface and subsurface layers of DF, MDF and UDF sites	42
<b>Fig.3.6.</b>	Organic C content of soil at surface and subsurface layers of DF, MDF &UDF sites	43
<b>Fig.3.7.</b>	Total N content of soil at surface and subsurface layers of DF, MDF and UDF sites at surface and subsurface soil layers	44
<b>Fig.3.8.</b>	Distribution of ammonium-N content of soil in DF, MDF and UDF sites at surface and subsurface soil layers	45
<b>Fig.3.9.</b>	Distribution of nitrate-N content of soil in DF, MDF and UDF sites at surface and subsurface soil layers	46
<b>Fig.3.10.</b>	Total P content of soil at surface and subsurface soil layers in DF, MDF and UDF sites	47
<b>Fig.4.1.1.</b>	Soil respiration (CO <sub>2</sub> evolution) in DF, MDF and UDF sites at surface and subsurface soil layers	85
<b>Fig.4.1.2</b>	Bacterial population in soils of DF, MDF and UDF sites at surface and subsurface soil layers	86
<b>Fig.4.1.3</b>	Fungal population in soils of DF, MDF and UDF sites at surface and subsurface soil layers	87
<b>Fig.4.2.1 (ab)</b>	Distribution of vesicular-arbuscular mycorrhizal fungi (VAMF) in soils of DF, MDF and UDF sites at surface and subsurface soil layers	112
<b>Fig.4.3.1.</b>	Microbial biomass C in soils of DF, MDF and UDF sites at surface and subsurface soil layers	130
<b>Fig.4.3.2.</b>	Microbial biomass N in soils of DF, MDF and UDF sites at surface and subsurface soil layers	131
<b>Fig.5.1.1.</b>	Total genomic DNA profiles of DF, MDF and UDF sites for surface and subsurface layers	146
<b>Fig.5.1.2.A</b>	473 bp 16S rDNA band on Agarose gel of DF, MDF and UDF sites	147
<b>Fig.5.1.2.B</b>	DGGE profile of PCR-amplified 16S rDNA fingerprints of DF, MDF and UDF sites	147
<b>Fig.5.1.3.A</b>	Gel compare dendrogram of ERIC-PCR amplified DNA bands of DF, MDF and UDF sites	148
<b>Fig.5.1.3.B</b>	Gel compare dendrogram of PCR amplified 16S rDNA bands of DF, MDF and UDF sites	148
<b>Fig.5.2.1. A-C</b>	Distribution of total phospholipids fatty acids (PLFA) and {subfractions, saturated (SATFA) and monounsaturated ( MUFA)} in DF, MDF and UDF sites	162
<b>Fig.5.2.1. D-F</b>	Distribution of PLFA fractions, polyunsaturated (PUFA), hydroxyl substituted phospholipids (PLOH) and hydroxy-unsubstituted (UNSOH)fatty acids	163
<b>Fig.6.1.</b>	Dehydrogenase activity surface and subsurface soil layers of DF, MDF and UDF sites	196
<b>Fig.6.2.</b>	Acid phosphatase activity in soils of DF, MDF and UDF sites at surface and subsurface soil layers	197
<b>Fig.6.3.</b>	Urease activity of DF, MDF and UDF sites at surface and subsurface soil layers	198
<b>Fig. 8.1.</b>	Percentage reduction of soil physico-chemical, biological and biochemical characteristics in DF and MDF forest sites as compared to UDF forest site.	250

## List of Tables

<b>Nos.</b>	<b>Table description</b>	<b>Page</b>
<b>Table 2.1.</b>	Site characteristics of degraded (DF) moderately degraded (MDF) and undegraded (UDF) forest sites	17
<b>Table 2.2.</b>	Distribution of dominant tree species in DF, MDF and UDF sites	17
<b>Table 3.1.</b>	One-way ANOVA of soil physico-chemical characteristics among the three study sites at surface and subsurface soil layers	48
<b>Table 3.2.</b>	One-way ANOVA of physico-chemical characteristics between surface and subsurface soil layers of three study sites	49
<b>Table 3.3.</b>	Pearson's correlation coefficient ( <i>r</i> ) values among various soil characteristics of DF, MDF and UDF sites at surface soil layers	50
<b>Table 3.4.</b>	Pearson's correlation coefficient ( <i>r</i> ) values among various soil characteristics of DF, MDF and UDF sites at subsurface soil layers	53
<b>Table 4.1.1.</b>	One-way ANOVA of soil biological characteristics (SR, BP and FP) among the three study sites at surface and subsurface soil layers	88
<b>Table 4.1.2.</b>	One-way ANOVA of soil biological characteristics (SR, BP and FP) between surface and subsurface soil layers of three study sites	88
<b>Table 4.2.1.</b>	Indices of general diversity ( <i>H'</i> ) and dominance ( <i>C</i> ) of vesicular-arbuscular mycorrhizal fungi (VAMF) in DF, MDF and UDF forest sites	108
<b>Table 4.2.2.</b>	Correlation coefficient ( <i>r</i> ) values between VAMF spore population and selected soil characteristics of DF, MDF and UDF forest sites	108
<b>Table 4.2.3.</b>	Distribution of VAMF species in DF, MDF and UDF forest sites	109
<b>Table 4.2.4.</b>	Restricted distribution of VAMF in DF, MDF and UDF forest sites	111
<b>Table 4.3.1.</b>	One-way ANOVA of soil microbial biomass ( $C_{mic}$ and $N_{mic}$ ) among the three study sites at surface and subsurface soil layers	132
<b>Table 4.3.2.</b>	One-way ANOVA of soil biochemical characteristics ( $C_{mic}$ and $N_{mic}$ ) between surface and subsurface soil layers of three study sites	132
<b>Table 5.2.1a</b>	Distribution of saturated phospholipid fatty acids (SATFAs) in DF, MDF and UDF sites	164
<b>Table 5.2.1b</b>	Distribution of monounsaturated phospholipids fatty acids (MUFAs) in DF, MDF and UDF sites	165
<b>Table 5.2.1c</b>	Distribution of polyunsaturated phospholipids fatty acids (PUFAs) in DF, MDF and UDF sites	166
<b>Table 5.2.1d</b>	Distribution of hydroxyl (-OH) substituted phospholipids fatty acids (PLOHs) in DF, MDF and UDF sites	166
<b>Table 5.2.1e</b>	Distribution of hydroxyl (-OH) unsubstituted phospholipids fatty acids (UNSOHs) in DF, MDF and UDF sites	168
<b>Table 6.1.</b>	One-way ANOVA of soil biochemical characteristics (DHA, PA and UA) among the three study sites at surface and subsurface soil layers	199
<b>Table 6.2.</b>	One-way ANOVA of soil biochemical characteristics (DHA, PA and UA) between surface and subsurface soil layers of three study sites	199
<b>Table 7.1.</b>	Variables and their characteristics in step 0 of discriminant function analysis (DFA)	215
<b>Table 7.2.</b>	Summary of stepwise DFA	215
<b>Table 7.3.</b>	Canonical discriminant functions (CDFs) after DFA	216
<b>Table 7.4.</b>	Structure matrix of variables in CDF1 and CDF2	217
<b>Table 9.1.</b>	Erosion class, degree, extent, severity and degradation class DF and MDF forest sites as compared to UDF forest site	252

## Abbreviations

<b>ANOVA</b>	Analysis of variance
<b>CDF</b>	Canonical discriminant function
<b>C<sub>mic</sub></b>	Microbial biomass C
<b>DF</b>	Degraded forest site
<b>DFA</b>	Discriminant function analysis
<b>DGGE</b>	Denaturing gradient gel electrophoresis
<b>DHA</b>	Dehydrogenase enzyme activity
<b>DMDS</b>	Dimethyldisulfide
<b>DNA</b>	Deoxyribonucleic acid
<b>ERIC</b>	Enterobacterial repetitive intergenic consensus
<b>FAME</b>	Fatty acid methyl ester
<b>H'</b>	General diversity index
<b>LSD</b>	Least significance difference
<b>MDF</b>	Moderately degraded forest site
<b>MUFA</b>	Monounsaturated PLFA
<b>N<sub>mic</sub></b>	Microbial biomass N
<b>PA</b>	Phosphatase enzyme activity
<b>PAGE</b>	Polyacrylamide gel electrophoresis
<b>PCR</b>	Polymerase chain reaction
<b>PLFA</b>	Phospholipid fatty acid
<b>PLOH</b>	Hydroxy substituted PLFA
<b>PUFA</b>	Polyunsaturated PLFA
<b>rDNA</b>	Ribosomal DNA
<b>SATFA</b>	Saturated PLFA
<b>SL</b>	Surface soil layer
<b>SSL</b>	Subsurface soil layer
<b>TMSI</b>	Trimethylsilyl
<b>UA</b>	Urease enzyme activity
<b>UDF</b>	Undegraded forest site
<b>UNSOH</b>	Hydroxy unsubstituted PLFA
<b>VAMF</b>	Vesicular arbuscular mycorrhizal fungi

## **LIST OF PLATES**

**Plate 1: Different views of the degraded forest (DF) site**

**Plate 2: Different views of the moderately degraded forest (MDF) site**

**Plate 3: Different views of the undegraded (UDF) forest site**

**Plate 4: Comparative views of the forest soil surface in degraded (DF),  
moderately degraded (MDF) and undegraded forest (UDF) sites**

**Plate 5: Comparative views of the forest soil profile in degraded (DF),  
moderately degraded (MDF) and undegraded forest (UDF) sites**

# Chapter 1

## **GENERAL INTRODUCTION**

Soil is the natural resource on which the entire terrestrial life depends for food, fiber and shelter. Most of the arable lands on the earth is under tremendous pressure of intensive agricultural practices and other developmental activities such as urbanization and industrialization. This has caused undesirable changes in various physico-chemical, biological and biochemical characteristics leading to the problem of soil degradation globally. This includes loss of structure and an increase in soil compaction (Vazquez *et al.*, 1993) commonly associated with soil organic matter reduction and ash formation (Prieto-Fernandez *et al.*, 1993). It has been reported that many of the world's ecosystems are in various state of decline as evident by erosion, low productivity and poor water quality caused by forest clearing, intensive agricultural production and continuous use of soil resources that are not sustainable (Kennedy and Smith, 1995). Though the problems of soil degradation were serious in the past it was confined to small areas of some countries only. But the condition has been changing because of ever-increasing human population resulting in food deficit, environmental pollution, decline in soil quality, lower fertility and global warming. At present, the problem of soil degradation becomes the subject of environmental concern attracting attention of scientific communities of the world (Sehgal and Abrol, 1994) for successful restoration and rehabilitation of the degraded lands.

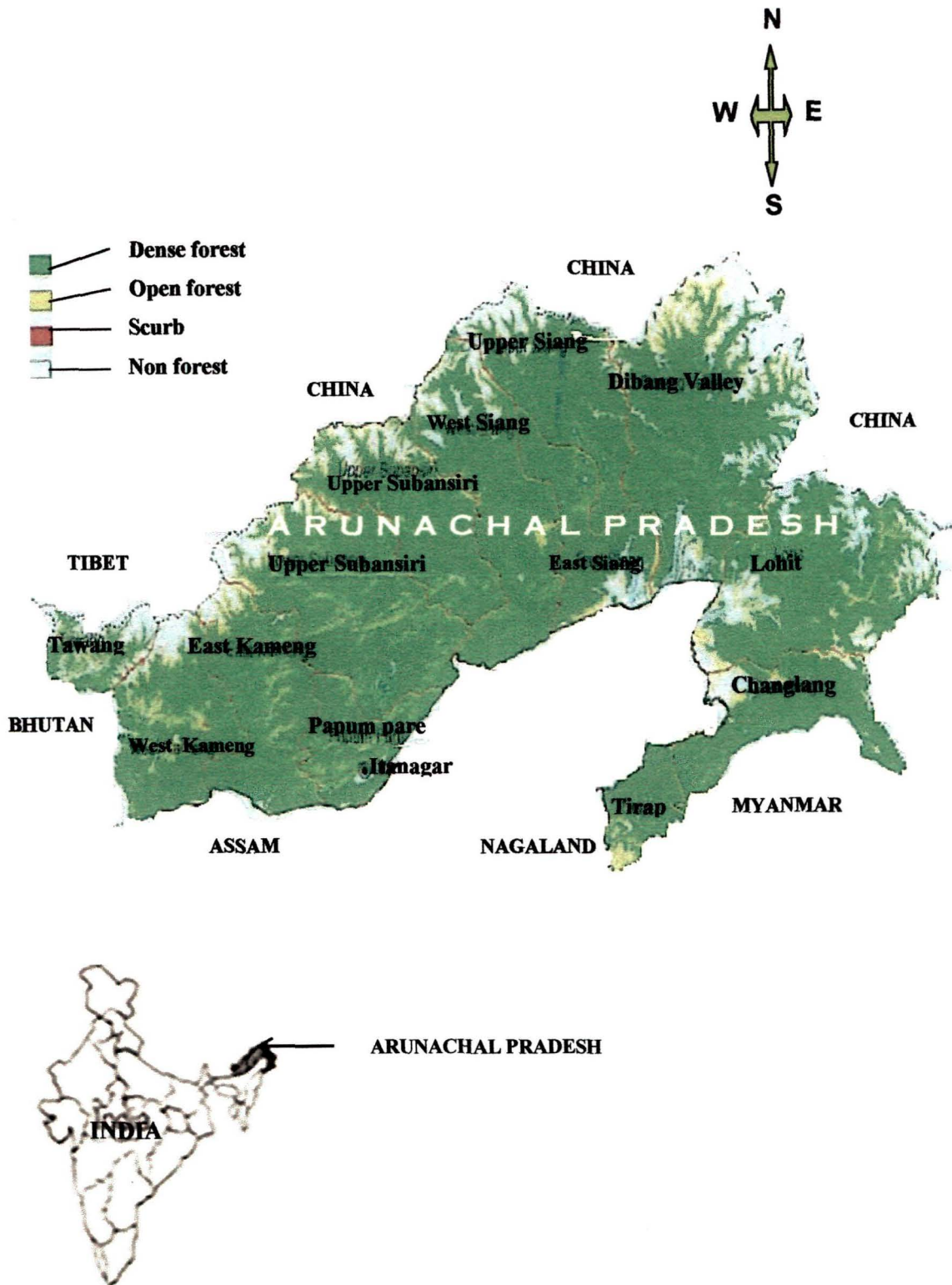
The various causes of soil degradation include the over exploitation of soil resources for intensive agricultural practices, deforestation, wildfire (natural and man-made), salinization, discharge of industrial effluents, heavy metal contamination etc. Agricultural intensification and deforestation are the two most important factors which are responsible for rapid decline in natural forest areas and subsequent erosion of soil, accelerated loss of fertile topsoil, nutrient depletion, lower crop productivity, loss of rare flora and fauna including microorganisms and decrease in their activities. Such a degraded land, at a consequent stage, turns into an unproductive land and even may undergo desertification in extreme conditions. These lands are unable to support good vegetation re-growth until restoration measures are taken up.

In India, the problem of soil degradation due to various causes has reached to an alarming rate affecting more than 50% of the total geographical area (Abrol and Sehgal, 1992). This has caused a significant decline in socio-economic status of the people and leading to ecological imbalance in various ecosystems including natural forests. The total dense forest cover area in the country was expected to be 11.48% and total forest cover including dense, open and mangrove forests is 19.39% of the total geographical area of the country (Forest survey of India, 1999). Major portions of the forest cover are mostly confined in the states of Madhya Pradesh (20.68%) followed by Arunachal Pradesh (10.80%), Orissa (7.35%), Maharashtra (7.32%) and Andhra Pradesh (6.94%). The extension of the rapid agricultural practices including shifting cultivation (Jhum) towards new

forest areas, deforestation and logging for timber extraction are the main causes of forest depletion. A report has revealed that the total area under shifting cultivation practice in the country is about 4.36 mha of which 2.7 mha is confined to the north-eastern region only (Venkataswarlu, 1995). It is because of the reason that shifting cultivation is a dominant agricultural system and logging for timber extraction from forests is the common practice in this hilly region. These two factors are responsible not only for rapid depletion of forest resources but also the root cause of soil degradation and rapid decline in biodiversity resource of the region.

Arunachal Pradesh is the largest state in terms of geographical area and forest cover distribution in the north-eastern region. The state has a large area of dense forests (Fig.1.1) inside the interior parts of hilly terrain which are inaccessible to the local people. According to Forest Survey of India (1999), the total forest area was 82.21% (68,847 sq. km.) of the total geographical area (83,743 sq. km.). But the vegetal cover distribution of the state has been thinned out in a number of places due to shifting cultivation through deforestation and logging to meet the increasing demand of timber and natural disaster etc. There was a net increase of total forest cover (3,601 sq. km) in 1999 mainly due to conversion of open, scrub and non-forests to dense forests. This is despite the fact that there was a net decrease of 19 sq. km forest cover in 1997 against the total forest cover recorded in 1995. Economic review of the state (1995) reported that about 70% of the total geographical area constitutes broad and narrow valleys, 20% snow clad peaks and 10% constitutes foothills and flat areas. The areas

under agricultural operation is confined to only 5% of the geographical area and even within this about 62-65% continues to be under shifting cultivation practices. It was estimated that the total area under shifting cultivation practice is about 3.2% of the total geographical area (Singh, 1999). This is despite the enormous effort of the state government to replace traditional shifting cultivation practices with permanently settled agriculture systems. Sehgal and Abrol (1994) have reported that about 26-50% of the total geographical area, mostly in the arable peripheral foot hill slopes, was affected by moderate to severe loss of fertile top soil as a result of water erosion, nutrient deterioration and terrain deformation. This has caused the problems of low crop productivity and sharp decline in soil fertility resulting in soil degradation in this state in particular and the region in general. Another important problem arisen out of the soil degradation is the wide spread damage to microhabitats as forest fire spreads to the neighboring areas destroying rare flora and fauna including microorganisms in the soil, therefore, decline in biodiversity. The other physical factors responsible for the enhanced loss of topsoil in the region are the steep hill slopes and heavy rainfall occurring in the region during monsoon season (May to September). Thus, nutrient loss is very fast in the region and management through inorganic fertilizer does not go in the long term. These problems were negligible in the past when the cycle of shifting cultivation (Jhum) was long (20 to 30 years) and enough arable land was available. However, it has become serious at present due to shortening of the jhum cycle (1 to 5 years only) and increased human population imposing expansion of agricultural



**Fig.1.1. Map of Arunachal Pradesh (India) showing distribution of forest cover (Forest Survey of India, 1999)**

lands. Moreover, short jhum cycle does not allow restoration of nutrients depleted during the period of cultivation since the regenerating vegetation utilizes the available nutrients coupled with the fast runoff from the loose soils. In the meantime, the local farmers in the region are continuously practicing this agricultural system without proper input of nutrients based on either organic or inorganic fertilizers. Therefore, the fertility problem has become a serious concern in the state in particular and the region in general requiring restoration and rehabilitation of the degraded lands through proper evaluation of soil resources utilization.

The successful restoration of the degraded lands requires an in-depth understanding of the various processes occurring in the soil environment. Therefore, understanding of these processes and their responses to soil management practices and disturbances caused by anthropogenic activities becomes prerequisite. The major soil processes are the physical, chemical, biological and biochemical, which are interlinked to each other, and take place simultaneously in the soil environment. Whenever there occurs any disturbance in any of these processes, it will cause a series of changes in other processes leading to undesirable changes in soil characteristics.

Soil physical conditions play an important role in determining the environment in which biological processes takes place (De Vos *et al.*, 1994) while chemical characteristic determines maximum quality of a particular soil (Hassink, 1997). Nowadays, much attention is paid to the study of biological processes in soil (De Vos *et al.*, 1994) because of the reason that nutrient transformation processes make soil a dynamic part of the biosphere with the

vital role of soil microorganisms and invertebrates. For this particular reason we seek biological and biochemical indicators which can sensitively respond to anthropogenic and environmental stresses on soil as dynamic system (Filip, 1998). Therefore, research studies in the last two decades have revealed that soil quality may be assessed using selected indicators related to soil microorganisms (Staddon *et al.*, 1998). The important microbiological parameters consist of population dynamics, diversity, soil respiration, microbial biomass C and N and enzyme activities. These parameters are considered as bioindicators of soil quality and use as group of indices as they are quickly responsive and sensitive to changes occurring in the soil environment and could illustrate the effects of anthropogenic activities and other disturbances in soil (Dick, 1994, Turco *et al.*, 1994; Kennedy and Papendick, 1995; Filip, 1998; Trasar-Cepeda *et al.*, 1998; Bending *et al.*, 2000 and Palma *et al.*, 2000).

In the natural ecosystems, mature forest soils harbour higher number of microbial populations (bacteria and fungi) and their activities. These microbial populations being one of the most important components of soil and thus exert considerable influence on soil fertility and plant growth (Tiwari *et al.*, 1991). These communities are inherently stable but at the same time are dynamic structures (Campbell, 1983). The changes in the microbial population structures as a result of agricultural practices, ecosystem management and global climatic change can have pronounced impact on ecosystem dynamics (Bossio and Scow, 1995). Generally, microbial populations and their activities are reported to be higher in no tillage soils

than in conventional tillage soils and disturbance activities like clear-cutting of forest affect microbial populations and their activities.

Microorganisms exist in complex communities and are responsible for the important mineralization reactions that recycle important nutrients which may be disturbed by alterations in the soil environment leading to ecosystem instability. In fact, when a forest is clear-cut, the roots die and root decomposition accelerates and the debris from leaves and branches on the soil surface of the disturbed soil serves as food for microbial growth (Coyne, 1999). Thus, there occurs an increase in microbial growth and their activity for a short period. However, clear cutting of forests and subsequent tillage practice following burning of dried slash, as in case of shifting cultivation, has a detrimental effect on these microorganisms and their activities in the long term. Sharp decline in microbial populations and their activities occurs at the surface soil layers immediately following burning of dry slash in the shifting cultivated soil systems. One advantage for burning of the slash prior to cultivation is the release of nutrients in the form of ashes, which may act as a source of fertilizer in the first year of cropping and because of this reason people continue to burn every time cropping is done. But it may be noted that the microbial populations could be reduced drastically following burning of the slash. Nutrient transformation processes, decomposition of litter and mineralization, may be disturbed and may cause lowered soil fertility subsequently. The shifting cultivation and logging of timber along with other unsustainable agricultural practices also have considerable impacts on biological health of soil systems. Any type of agricultural system, which

eliminates beneficial soil microflora, are unlikely to be sustained in the long term. At the same time, crop production in a subsistence cropping system such as shifting cultivation depends on the natural fertility of soils and depletion of nutrients during cultivation is the main reason for land abandonment. This rapid decline in the nutrient flux of soils is the limiting factor for agricultural sustainability in shifting cultivation systems (Brand and Pfund, 1998). The main reason for rapid nutrient depletion is due to alterations in nutrient transformation processes of the disturbed soil as a result of disturbances in microbial activities. Since microbes complete their life cycle in the soil, nutrients taken up by plants return again and the same nutrient can be used over and over (Thompson and Troeh, 1979).

Soil respiration is the evolution of CO<sub>2</sub> from the soil surface as a result of microbial and root respiration (Schlenter and Cleve, 1985) and its measurement can be utilized to assess relative productivity and fertility of soils (Upadhyaya *et al.*, 1997). The soil surface CO<sub>2</sub> flux is a major transfer of carbon from terrestrial ecosystems to the atmosphere and land use practices significantly affect this process. The microbial portion of the soil respiration has been reported to be significantly highest and most quantifiable component of the overall soil respiration (Kelting *et al.*, 1998 and Coyne, 1999). This is because of the reason that vegetation type influences the microenvironment, microbial biomass and fine root biomass which together control soil surface CO<sub>2</sub> flux (Wagai *et al.*, 1998) as a measure of microbial activity. Therefore, soil disturbance activities like clear-cutting of

forest, selective logging and shifting cultivation practices must have marked influences on soil respiration.

Microbial biomass is an important parameter of soil microorganisms. It has a dual role in soil, first as an agent of transformation through which passes all natural organic matters that enters soil and also as a small, but labile, reservoir of C, N, P and S (Jenkinson and Ladd, 1981 and Singh *et al.*, 1989). Microbial biomass C is an important ecological parameter because it acts as a source-sink in nutrient cycling processes and regulates organic matter transformations (Jordan and Beare, 1991). Though, microbial biomass is relatively small fraction of the total biomass in terrestrial ecosystem, the microbial activity is of paramount importance in the nutrient cycling and energy flow (Diaz-Ravina *et al.*, 1993ab). Usually, the microbial biomass of the tropical environments is sensitive to land use changes and even appears to be a sensitive indicator of both soil carbon content and background nitrification. The conversion of the humid forests into other land uses results in remarkable decline in the amounts of soil nutrients and microbial biomass C, N and P (Srivastava, 1992 and Taylor *et al.*, 1999). Following the removal of vegetation and clear-cutting of forests, the changes in microbial biomass can provide an early indication for a slower and less easily detectable soil organic matter and soil fertility as well (Henrot and Robertson, 1994 and Maithani *et al.*, 1996). It has been revealed that a close relationship exists between the physico-chemical properties and other parameters related to soil microorganisms which is altered when the soil ecosystem is subjected to perturbations.

The physiology and metabolism of soil microorganisms are driven by enzymes and the microbial habitat in soil is affected by these soil enzymes. Despite living organisms being the only source of enzymes, once these organisms die, some enzymes can persist and retain their activity in soil for long periods (Coyne, 1999). These soil enzymes are responsible for the processes that occur in the environment such as mineralization and immobilization of nutrients, nitrogen fixation, etc. It is well known that processes of organic matter transformation in soil are catalyzed by soil enzymes (Khan, 1970). The activity of a particular enzyme in the soil is a composite of activities associated with various biotic and abiotic components (Burns, 1982). Measurement of enzymatic activities have been used as a measure of total microbiological activity and soil fertility levels (Stevenson, 1959; Tiwari *et al.*, 1988ab and Chander and Brookes, 1991). These soil enzyme assays have been considered as potential component of group of indices to assess soil quality (Kennedy and Papendick, 1995). Thus, any disruption in the soil microbial activity as shown in the level of soil enzymes can serve as an estimate of the ecosystem disruption (Tate, 1995). Another important aspect of soil enzyme activity is their ability to indicate potential of a soil to support biochemical processes, which are essential for maintaining soil fertility (Dkhar and Mishra, 1983). Existence of close relations between enzyme activity and other microbial attributes like soil respiration, microbial biomass and microbial population numbers have been reported (Frankenberger and Bingham, 1982). Among the various types of soil enzymes dehydrogenase (Oxido-reductase), phosphatase and urease

(Hydrolases) are most widely studied due to their importance in soil management and agricultural practices.

Extensive research data are available on the above parameters of soil microorganisms from different ecosystems of the world and some parts of the country including north-eastern region. However, no significant work has been done on these parameters with respect to various degree of disturbances due to shifting cultivation and selective logging in this hilly remote state of the north-eastern India despite its recognition as one of the important “Hot Spots” of World’s biodiversity reserve. Therefore, the present study aims to investigate the population, community structure and activities of soil microorganisms coupled with few soil physico-chemical properties from different study sites with varying degree of degradation status in humid tropical regions of Arunachal Pradesh, north-eastern India. This will help in providing the required information for constructing an important strategy for a successful restoration and rehabilitation measures of degraded soils in the region in particular and other soils in general.

The present study aims i) to analyze the activities and community status of microorganisms by measuring of the biological and biochemical characteristics along with few physico-chemical characteristics of soil under a regenerating jhum fallow and a selectively logged forest in comparison to an undisturbed forest, and ii) to identify a few soil characteristics which are sensitive and quickly responsive to soil degradation to use them as indices for evaluation of fertility and degradation status of forest soils.

## **Chapter 2**

### **STUDY SITE DESCRIPTION**

#### **Location of the study site**

The Banderdewa forest range (Fig.2.1) in Papum pare District of Arunachal Pradesh was selected as study site for the present study. The study site is located between 27°6' N latitude and 93°49' E longitude at an elevation of 350 masl. The selection of study sites namely, degraded forest (DF), moderately degraded forest (MDF) and an undegraded natural forest (UDF) was done on the basis of land use history, living tree stump density, soil profile thickness, dominant tree species composition, etc. (Tables 2.1. and 2.2).

#### **Soil**

The soil in the study site falls under Karsingsa series, which is a member of mixed loamy sand of hyperthermic family, typic Haplustalfs i.e. class of Alfisol (Singh, 1999). The geology of the soil consists of sedimentary (sandstone) parent rock, which is drained by small tributaries of Dikrong River towards Brahmaputra River in Assam.

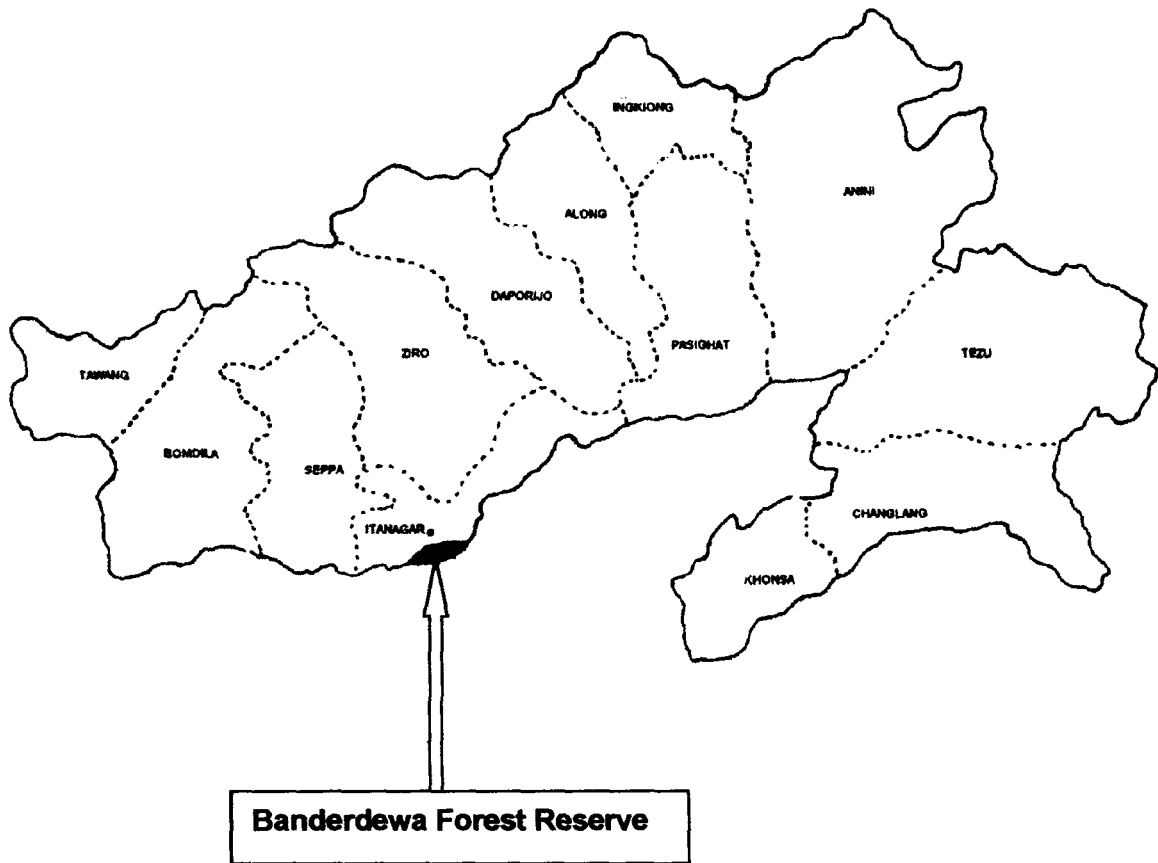
The textural class and profile (Ah horizon layer) thickness of the soils in these sites were measured at the beginning of the study which revealed predominantly sandy loam texture class of the soils in all sites. There was a decline in thickness of the soil profile with the increase in severity of forest site disturbance. Maximum thickness of Ah horizon was recorded from the undegraded site which was followed by moderately degraded and minimum

thickness of Ah layer was recorded from the degraded site in which shifting cultivation was practiced for a long period of time (<20 years) without proper input of nutrients either based on organic or inorganic fertilizers.

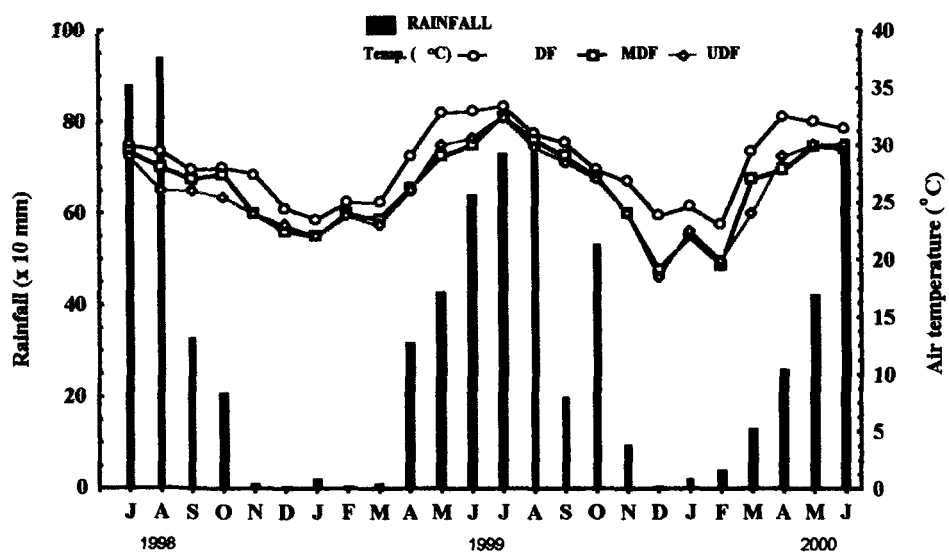
### **Vegetation**

The natural vegetation consists of evergreen to semi-evergreen mixed natural forests consisting of varying tree species from a primitive Magnolia to newly introduced Teak plantations. The presence of a protected forest area in the range allowed to select an undegraded natural forest (UDF) as control site and two others, degraded forest (DF) and moderately degraded forest (MDF) sites for the present study.

Degraded forest site was a 5 years old regenerating jhum fallow when the research was started in 1998. This site had been used for cultivation of rice, maize, finger millet and tuber crops etc., from 1980 to 1993 without proper input of nutrients based on organic and inorganic fertilizers. Thus the available nutrients were obtained only from the debris of plant parts and ashes after burning of dried slash prior to cropping in each jhum cycle. This site has not been used for further cultivation of crops since last jhum cultivation practiced in 1993 due to lowered crop productivity and is currently lying as an abandoned fallow land (Plate 1). Luxuriant growth of fern (*Adiantum sp.*), weeds (*Ageratum conizoides*, *Boreria boralis*, *Mikania mikranthes*, *Oplismenus sp.*, *Paspalum sp.*, *Sida cordifolia* and grasses (*Cyperus, sp.*) etc. covers the soil surface during summer rainy season while dried plant residues cover the soil surface in dry winter season. The tree density is only 21 trees 100 sq.m.



**Fig. 2.1. Map showing the Banderdewa Forest Reserve in Papum pare District of Arunachal Pradesh**



**Fig. 2.2. Climatogram showing mean monthly variation of rainfall and maximum air temperature in degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites**

**Table 2.1. Site characteristics of degraded (DF) moderately degraded (MDF) and undegraded (UDF) forests**

Soil Properties	DF	MDF	UDF
Land use history (yr)	<20	0	0
Degree of soil degradation* (%)	<80	<30	00
Slope (%)	4	4-7	4-7
Aspect (direction of slope)	NE	NE	NE
Texture- Sand (%)	72.8	73.0	74.9
Silt (%)	20.1	18.0	18.1
Clay (%)	7.1	9.0	7.0
Textural class	Loamy sand	Loamy sand	Loamy sand
Particle density	2.56	2.37	2.32
Porosity (%)	49.00	52.00	55.00
Soil profile (Ah) thickness (cm)	3-5	07-10	13-17

\*Percentage degree of soil disturbance was calculated on the basis of numbers of living tree stumps present in each site

**Table 2.2. Distribution of dominant tree species in degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites.**

Sp. No.	Name of tree species	DF	MDF	UDF
1	<i>Ailanthus grandis</i> Prain.	-	+	+
2	<i>Altinga excelsa</i> Noron.	-	+	+
3	<i>Arthocarpus chaplasha</i> Roxb.	-	-	+
4	<i>Bombax ceiba</i> Linn.	-	+	+
5	<i>Canarium strictum</i> Roxb	-	+	+
6	<i>Chukrasia tabularis</i> Andr. Juss.	-	-	+
7	<i>Dillenia indica</i> Linn	+	+	+
8	<i>Duabanga grandiflora</i> (Roxb.ex.D.C.) Walp	+	+	+
9	<i>Gmelina arborea</i> Roxb.	+	+	+
10	<i>Kydia calycina</i> Rob.	-	+	+
11	<i>Manglieta caveana</i> HK. f. & Th	+	+	+
12	<i>Phoebe goalparensis</i> Hutch	-	+	+
13	<i>Pterospermum acerifolium</i> Willd.	-	-	+
14	<i>Tactona grandis</i> Linn.f.	+	+	-
15	<i>Toona ciliata</i> Roem.	-	+	+

Tree species present (+) or absent (-)

## **Climate**

The climate of the study site is humid tropical characterized by high rainfall and humidity. The mean monthly rainfall and maximum monthly air temperature in the three study sites are depicted in figure 2.2. The 80% of total rainfall is generally received during monsoon season from May to August and 20% during September to March.

## **Soil sampling and analysis and laboratory**

The present study was conducted for two years starting from July 1998 to June 2000 at monthly intervals in the selected sites. Soil sampling was done from three replicate plots of 50 m<sup>2</sup> each for all the three sites in the middle of every month, at least 24 h later, if rain had occurred. Soil samples were collected separately using metal soil core sampler having diameter and height of 5.5 and 20 cm respectively from two soil depths i.e. surface (0-20 cm) and subsurface (20-40 cm.) layers after discarding approximately 0.5 cm of soil in between the two depths. A total of 15 soil core samples were collected from each replicate plot of degraded, moderately degraded and undegraded forest sites and were mixed to obtain a composite sample for each replicate plot for every month before analysis. The field moist soil collected from each study site was passed through a 2 mm mesh screen before processing for further analyses. The parameters requiring fresh soil samples (available-N, biological and biochemical properties) were determined immediately otherwise stored below 4°C until further use. However, total organic C, N and P were determined after air drying of the soil samples.

## **Chapter 3**

### **Distribution of soil temperature, moisture, bulk density, water holding capacity, pH, total organic C, total N & P, ammonium-N and nitrate-N in degraded and undegraded forest soils**

#### **3.1. INTRODUCTION**

The climate, vegetation, topography and type of parent rock are the important factors which govern the physico-chemical characteristics of the soils (Tiwari, 1988). The physico-chemical properties can determine the suitability of a soil for production of different crop plants (Brady, 1995). The physical conditions and chemical characteristics of a soil play important roles in determining the environment in which biological processes take place and can be defined at different spatial and temporal scales (Tiwari, 1988 and De Vos *et al.* 1994). These physical properties of soil are considered as important factors which affect the growth and activity of soil microorganisms (Frankenberger and Dick, 1983).

The existence of favourable physical conditions and availability of chemical nutrients can determine the occurrence of microbial communities and their activities in a soil environment. It is well known that the physico-chemical characteristics such as soil temperature, moisture, bulk density, water holding capacity, pH, total organic C, N and P, ammonium-N and nitrate-N regulate the population structure and activities of microorganisms (Tiwari, 1988 and Arunachalam *et al.*, 1996 and 1997).

Status of physico-chemical characteristics of shifting cultivated land, monoculture plantations, agricultural fields, grasslands, degraded and

regenerating forests, and natural forests have been reported in detail during the last three decades (Singh, 1980; Deka, 1981; Ewel *et al.* 1981; Ramakrishnan and Toky, 1981; Dkhar, 1983; Dkhar and Mishra, 1987; Tiwari, 1988; Tiwari *et al.*, 1988ab; Nayak and Srivastava, 1995; Singh *et al.*, 1995; Hajabbasi *et al.*, 1997; Noordwick *et al.*, 1997; Zech *et al.*, 1997; Brand and Pfund, 1998; Saikh *et al.*, 1998 and Garcia-Oliva *et al.*, 1999).

The dynamic nature of the physico-chemical characteristics and their possible relationship with microbial communities and their activities has been reported from jhum fallows (Deka, 1981), maize fields (Dkhar, 1983) and pineapple orchard (Tiwari, 1988) soils of north-eastern India. However, research data on these relationships from shifting cultivated jhum fallow, selectively logged and undegraded soils of humid tropical hill forests are scarce.

Therefore, aim of the present research work is to investigate the temporal, spatial and depth-wise variation of soil physico-chemical characteristics in a shifting cultivated jhum fallow and a selectively logged forest soils as compared to an undegraded forest soil of humid tropics in Arunachal Pradesh, north-eastern India.

### **3.2. REVIEW OF LITERATURE**

The soil physical conditions play an important role in determining the environment in which biological processes take place (De Vos *et al.*, 1994). The chemical characteristics on the other hand, make a significant contribution in determining the quality and may even determine the maximum quality of a particular soil (Hassink, 1997). Due to these reasons

physico-chemical properties of soil systems have been investigated in general apart from their physiological importance in applied agricultural practices.

Yadav and Badolka (1973) reported that soils of deodar forests of Uttar Pradesh (India) have a high water holding capacity and cation exchange capacity.

Vegetation type of a particular ecosystem influences the soil pH in complex ways because it produces organic matter and influences leaching. The formation of organic acids in decomposition of organic matter and litter causes lowering of percent base saturation and pH. Usually acidic conditions are common in soils of humid climates which favour higher leaching. The organic P content of the soil is related to the organic matter content of soil, pH, climate and cultivation practices (Thompson and Troeh, 1979).

The measurement of soil bulk density have been done to evaluate the impact of agricultural practice, tillage, soil management practices and other disturbances. Bulk density of the A-horizons of the mineral soils is usually between 1.0 to 1.6 g cm<sup>-3</sup> which varies according to its degree of compaction and total pore space present in the soil. The bulk density usually increases when cultivation causes a loss in soil organic matter from a soil associated with decrease in soil aggregate stability and size.

Ewel *et al.* (1981) have reported a 30% reduction in soil C and 23% reduction in N at upper soils after burning in Costa Rican wet forests

Ramakrishnan and Toky (1981) studied the soil nutrient status of hill agroecosystems in north eastern India and reported a significant ( $P < 0.05$ )

reduction in the carbon content in the surface soil and this was more pronounced in sites with older jhum cycles in comparison to younger ones immediately after burning. Their study concluded that the maintenance of soil fertility in hot, humid and highly rainfall areas is a serious problem and is more severe in situation when the cycle becomes short, due to poor recovery of soil fertility and increased intensity of weed competition.

Baruah (1983) investigated the physico-chemical characteristics of rice field soils and recorded the higher concentration of C, N and P from the soils which decreased with increase in depth.

Tiwari (1988) reported presence of higher amounts of total C, total N and P at upper soils of older pineapple plantations in comparison to younger plantations at similar soil layers. He also observed higher soil temperature of surface soils in pineapple orchards of different ages in north-eastern India. He reported that higher soil temperature at upper layer could be due to solar radiation heating. Further, he observed positive correlation of soil temperature with air temperature. Further, his study reported higher percentage of moisture content at upper layers than lower layers of old aged pineapple plantations in comparison to younger plantations. He observed positive correlation between soil moisture and other physico-chemical and microbiological properties of the orchard soils. The fluctuation in distribution of soil moisture was related to rainfall in the study.

Tiessen *et al.* (1992) observed a significant reduction of soil C, N and P by 30% when shifting cultivation was done for 6 consecutive years in north-eastern Brazil.

Tiwari *et al.* (1992) reported seasonal variation in P contents of soils which showed that the concentration reached to maximum peak during spring which might be related to rapid release of the nutrients from the litter. Further they reported temporal and depth wise variation in organic C, total N and P from pineapple orchard stands of north-eastern India.

Prieto-Fernandez *et al.* (1993) studied the short-term effect of forest wildfire on nitrogen status and its mineralization pattern in an Atlantic forest soil. They found an increased amount of total N content in the surface layer following wildfire, the N content of the burnt soil in the form of  $\text{NH}_4^+\text{-N}$  increased at surface and subsurface soil layers whereas  $\text{NO}_3^-\text{-N}$  content, which was very low, only increased in the subsurface layers.

Natural forests or savannas contain a wide spectrum of dead plant materials (leaf litter, twigs, roots and woods) with different rates of decomposition. Hence, in strongly seasonal environments with rapid decomposition rates, the high mass of varied composition of litter fall protects the soil surface throughout the year and promotes high soil organic matter (Brown *et al.*, 1994).

The conversion of natural forests of humid tropics into pastures and agricultural lands is expected to produce, in the long term a decline in soil organic matter content and soil fertility. All these lead to a reduction in ecosystem carbon storage due to the immediate removal of above ground biomass and a gradual subsequent reduction in soil carbon (Henrot and Robertson 1994).

A linear decrease in soil pH up to 4 was reported after forest clearing which resulted inhibition of plant growth in Nigeria even after 13 years of regeneration period (Juo *et al.*, 1995).

A study on temperature dependence of soil organic matter decomposition rate have revealed that a 1°C increase in temperature could ultimately lead to a loss of over 10 % of soil organic C in the regions of world with an annual mean temperature of 5°C, whereas the same temperature increase could lead to a loss of only 3% of soil organic C for a soil at 30°C (Kirchbaum, 1995).

Nayak and Srivastava (1995) reported predominantly acidic conditions of humid sub-tropical soils under shifting cultivation of Arunachal Pradesh, north-eastern India, which ranged from pH 4 to 6.5 at surface layers and decreased with increase in soil depth. They concluded that the acidic reactions of the soil are due to the intensive leaching of bases and presence of exchangeable  $Al^{+3}$ . They found higher status of organic carbon content in the surface horizon which could be ascribed to slow microbial activity under low temperature and acid environments of the shifting cultivated lands of this region.

Singh *et al.* (1995) found presence of higher N content at upper soil layers which decreased with increase in soil depth in the natural forest soils followed by bamboo forest and jhum fallow of north-eastern India.

Arunachalam *et al.* (1996) reported a decreasing trend of soil moisture content, bulk density and water holding capacity with increase in degree of forest disturbance in pine forests of Meghalya, north-eastern India.

The water holding capacity of a soil is an important soil physical parameter which depends on texture, organic matter content, soil moisture and soil depths.

Breland and Hansen (1996) studied the influence of soil compaction and increased bulk density on organic matter decomposition and net N-mineralization in a sandy loam soil. They observed that soil compaction by  $1.4 \text{ g cm}^{-3}$  bulk density had reduced the net N mineralization of clover  $^{15}\text{N}$  by 18% as compared to uncompacted soil, a reduction corresponding to 4% of added  $^{15}\text{N}$ . They also observed increase  $^{15}\text{N}$  retention in organic matter by 8% and microbial biomass by 1% of added  $^{15}\text{N}$  following soil compaction.

Maithani *et al.* (1996) and Arunachalam *et al.* (1997) have reported higher water holding capacity in older jhum fallows than in younger jhum fallows of north-eastern India. They noted higher water holding capacity being closely related to organic matter contents in the older jhum fallows.

Study on nutrient status under continuous cultivation have revealed a reduction of 38% of organic carbon, 41% and 39% of total and organic N, respectively, following continuous cultivation without fertilization in comparison to the natural site which had significantly higher C, N and P contents (Agbenin and Goladi, 1997).

Gracia-Oliva *et al.* (1999) have reported that slash and burn conversion of forests can result in significant disruption of soil C cycling. They noticed 32% decrease in soil organic C associated with macroaggregates due to combustion during burning as a result of slash and burn conversion of tropical deciduous forests to pastures.

Deforestation of natural forests and their subsequent tillage practices in Central Zagorous mountain of Iran resulted in almost 50% decrease in organic matter content and total nitrogen, a 10-15% decrease in soluble ions when compared to the undisturbed forest soils (Hajabbasi *et al.*, 1997).

A study on the effects of deforestation on physico-chemical properties of soil revealed 20% increase in bulk density following deforestation of natural forests and subsequent tillage practices in Iran (Hajabbasi *et al.*, 1997). They found that the decreased organic matter content was due to typically high temperature, humidity and rainfall of the region which increases soil bulk density.

Holscher *et al.* (1997) reported a significant increase in soil pH of 4 to 8 years old slash and burn plots of Eastern Amazon as a result of shifting cultivation practices in the past.

Sierra (1997) examined the combined effect of temperature and soil moisture on net N mineralization using undisturbed soil samples. He found that net N mineralization was more responsive to temperature than it was to moisture.

A balance exists between carbon input (above ground and belowground plant residues) and output due to mineralization and leaching of dissolved organic matter (Zech *et al.*, 1997). Almost by definition, decomposition rates of C in matured forests are approximately equal to C fixation rates (Noordwick *et al.*, 1997). In fact, some evergreen forest soils contain exceptionally large amount of organic matter contents (>7%) probably due to a number of factors such as leaf fall, high soil moisture

content and the presence of the rich soil fauna which helps in litter decomposition (Saikh *et al.*, 1998). However, there is a net C removal from the atmosphere in younger ecosystems such as a forest plantation or forests regenerating from impacts of logging, fire or other disturbances (Noordwick *et al.*, 1997).

During the last few decade of the 20<sup>th</sup> Century, tropical environments have become increasingly disturbed by forest destruction due to grazing, cropping and industrial purposes (Zech *et al.*, 1997).

Arunachalam *et al.* (1998) reported maximum P input during winter and spring seasons in the 7, 13 and 16 years old forest re-growths of north eastern India. The level of P was more or less same in all the four seasons under the study.

A recent study on soil degradation and landscape suitability of high altitude soils of Arunachal Pradesh, north-eastern India, revealed that base leaching due to higher rainfall and presence of organic acids influence towards acidity reaction of soils at low and high hill slopes (Bhattacharya *et al.*, 1998).

Brand and Pfund (1998) studied the nutrient dynamics of soils under shifting cultivation in eastern Madagascar. They observed that nutrient depletion is an important limiting factor for agricultural sustainability in shifting cultivation systems. They have suggested an urgent need for improvement of current sustainable agriculture with regard to nutrient status and biodiversity.

Maithani *et al.* (1998) have found lowest values of clay content, water holding capacity, soil organic matter and total kjeldhal-N in younger (1 year old forest stands) and highest in the older (16 year old) forest stands of subtropical humid forests of north-eastern India.

Deforestation and cultivation activities have resulted in drastically and statistically significant reduction in soil organic C in the tropical regions (Saikh *et al.*, 1998).

Saikh *et al.* (1998) reported significant reduction in organic C, total N, and C: N ratios but no significant changes in total P levels though reduction in C:P ratio was observed as a result of deforestation and cultivation in Simlipal National Park in India.

Each microbial group functions over a range of temperature in which they grow best. As a rule, the largest microbial populations are found at moderate soil temperatures (Coyne, 1999).

Hakensson and Lipiec (2000) emphasized dry bulk density and total porosity as the most frequently used parameters to characterize the degree of compactness while taking into account of the soil layer. However, they concluded that the measurement of degree of compactness while taking account into soil moisture is a more useful parameter than bulk density or porosity studies of biological affects of soil compaction.

### **3.3. METHODOLOGY**

#### **Physical properties**

Soil texture was determined by Boyuocos soil hydrometer method on air dried samples. 100 g air dried (2 mm sieved) soil sample was taken into a

500 ml glass beaker (borosil) and saturated with distilled water. 10 ml of 5% calgon solution (sodium hexa-metaphosphate) was added into the beaker and stirred for a minute with a clean glass rod. The soil suspension was then transferred into a soil cup (400 ml) and mixed thoroughly for 2 minutes by using a tissue homogenizer. The suspension was then transferred carefully into a measuring cylinder using distilled water and made up the volume up to 1350 ml after inserting the soil hydrometer. The measuring cylinder was then turned upside down for at least ten times after closing the mouth with a rubber bung. A few drops of amyl alcohol were added to diffuse the bubbles, then the soil hydrometer was inserted at 20 seconds and reading was taken at 40 seconds. A clinical thermometer was also inserted along with the hydrometer to record the temperature of the suspension. The cylinder was again turned upside down for ten times and kept undisturbed. After 2 hours, a few drops of amyl alcohol were added and the soil hydrometer reading was recorded along with temperature of the suspension. After temperature correction of the hydrometer readings, the percent sand, silt and clay contents were calculated as follows:

$$\text{Sand (\%)} = \frac{\text{Hydrometer reading at 40 seconds}}{\text{Weight of sample (100 g for sandy soil)}} \times 100$$

$$\text{Clay (\%)} = \frac{\text{Hydrometer reading at 2 hours}}{\text{Weight of sample (100 g for sandy soil)}} \times 100$$

$$\text{Silt (\%)} = 100 - (\% \text{ sand} + \% \text{ clay})$$

Soil temperature was determined by inserting a soil thermometer inside the soil at a depth of 10 and 30 cm for surface and subsurface soil layers respectively, for each study site.

Moisture content was determined by gravimetric method. 10 g fresh soil was dried at 105°C for 24h. Dry weight of the soil was recorded and percent weight loss was calculated as follows:

$$\text{Moisture content (\%)} = \frac{(W_2 - W_3) - W_1}{W_2 - W_3} \times 100$$

{Where,  $W_1$  = Weight of empty moisture can (g),  $W_2$  = Weight of fresh soil + moisture can (g);  $W_3$  = Oven dry weight of soil + moisture can (g)}.

The bulk density was determined by slight modification of the method given by Okalebo *et al.* (1993). A metal cylinder of 5 cm diameter and 5.5 cm height having known weight and volume was inserted into the soil surface after removing the top 0-1 cm of litter layer. The soil around the cylinder was excavated with a knife and cut at the bottom of the tube. Excess of soil was removed from both the ends using a knife and dried the cylinder for 48 h at 105°C. The dry weight of the cylinder and soil was recorded to calculate the bulk density of the soil as follows.

$$\text{Bulk density (g cm}^{-3}\text{)} = \frac{(W_2 - W_3) - W_1}{V}$$

{Where,  $W_1$  = Weight of empty cylinder (g),  $W_2$  = Weight of fresh soil + cylinder (g);  $W_3$  = Dry weight of soil and cylinder (g),  $V$  = Volume of the cylinder}

Water holding capacity was determined by using soil cup. Air dried and 1 mm passed soil sample was put into the soil cup containing a filter paper of known weight and the rim of the cup was trimmed horizontally with a glass rod. The cup was transferred to its stand and poured water on the

tray below the stand up to 1 cm of the cup from the bottom so that water can be soaked by soil. Then the cups were left for overnight till saturation of the soil. The cup was removed and drained excess of water with blotting papers from the bottom. The saturated weight of soil along with the cup and filter paper was recorded. The cup was dried in a hot air oven at 105°C for 48h and dry weight was recorded to calculate water holding capacity.

$$\text{WHC (\%)} = \frac{(W_2 - W_3) - W_4}{W_2 - W_1} \times 100$$

{Where,  $W_1$  = Weight of cup and filter paper (g);  $W_2$  = Saturated weight of soil with cup and filter paper (g);  $W_3$  = Oven dry weight of soil with cup and filter paper (g);  $W_4$  = Weight of saturated filter paper (g)}

### **Chemical properties**

Soil pH was measured in soil: water suspension (1:2.5 w/v) using a digital pH meter. 20 g fresh soil (2 mm sieved) sample was put in a 100 ml glass beaker and 50 ml distilled water was added. The beaker was shaken for 10 minutes at 100 rotations per minute on a horizontal shaker and kept standing for 30 minutes. The beaker was shaken again for two minutes and pH reading was read out with an electronic digital pH meter (Systronics-335).

Organic C, Total N and P were determined with little modification of the methods of Okalebo *et al.* (1993). 300 mg air dried (passed through 300  $\mu$ ) soil sample was taken into a 100 ml conical flask. 5 ml of 1M  $K_2Cr_2O_7$  solution and 7 ml concentrated  $H_2SO_4$  were added carefully into the conical flask containing soil sample. The sample in the beaker was digested for 30 minutes at 150°C and cooled thereafter. The digested soil mixture was titrated against the 0.2M ferrous ammonium sulfate until the colour changes

from bluish green to brown which is the end point using phenanthroline monohydrate indicator. The used  $K_2Cr_2O_7$ , the difference between the added and residual  $K_2Cr_2O_7$ , gives a measure of organic C content of soil calculated as follows:

$$\text{Organic C (\%)} = \frac{T \times 0.2 \times 0.3}{W}$$

{Where, T= Difference of titration volume of ferrous ammonium sulfate between blank (5 ml  $K_2Cr_2O_7$  and 7 ml  $H_2SO_4$  without sample) and sample; 0.02 = Strength of ferrous ammonium sulfate solution and 0.03 = Milliequivalent weight of C in grams; W= Sample dry weight (g)}

Total N and P were analyzed after acid digestion of the soil sample. 300mg of oven dried ( $70^\circ C$ ) soil sample was taken in to a 50 ml volumetric flask and digested after adding 4.4 ml digestion mixture at  $350^\circ C$  for 1-3 hours till the colour of soil changes to whitish or colourless. After cooling the mixture 25 ml distilled water was added and again cooled down for some time. Finally the volume is increased upto 50 ml with distilled water and mixed well. After settling down the sediments, the clear solution at the top was separated carefully and analyzed for total N and P separately.

Total N was determined using micro-kjeldahl distillation unit and titration of the distillate. 5 ml of the aliquot was taken into the digestion chamber of distillation unit and 10ml of 40% NaOH was added immediately before the distillation starts. The distillate was collected in a 100 ml conical flask containing 5 ml of 1% boric acid solution. After distillation for 2 minutes or when the colour changed from pink to bright green the flask was removed and titrated immediately against the N/140 HCl solution using mixed indicator (bromocresol green + methyl red + thymol blue) till the colour

changed from green to pink through a gray phase. The total N was calculated as follows:

$$\text{Total N (\%)} = \frac{T \times 0.1}{W}$$

{Where, T= Difference in volume of N/140 HCl between sample and blank.; 0.1 = Strength of HCl ; W= Sample dry weight (g)}

Available ammonium and nitrate-N ( $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$ ) were determined by modifying the methods of Allen *et al.* (1974). 20 g fresh soil (2 mm sieved) was taken into a 250 ml conical flask and 100 ml 2M KCl was added. The flask was then shaken for 30 minutes on a horizontal shaker. The soil suspension was filtered through Whatman No.1 filter paper and the filtrate was used for estimation of ammonium and nitrate-N ( $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$ ) contents respectively.

Ammonium-N was determined by the indophenol blue method. 5 ml of the KCl extract was put into a 50 ml volumetric flask, 8 ml of Rochellis reagent, 1ml sodium nitroprusside, 2 ml sodium phenate and 1ml sodium hypochlorite were added simultaneously. The volume was increased upto 50 ml with distilled water and content was mixed thoroughly. The flask was warmed at 40°C for 20 minutes and cooled thereafter. The intensity of blue colour was read spectrophotometrically at 625 nm. The  $\text{NH}_4^+\text{-N}$  was calculated as follows:

$$\text{NH}_4^+\text{-N } (\mu\text{g } 100 \text{ mg}^{-1}) = \frac{C \text{ (mg)} \times V}{10 \times \text{aliquot} \times W}$$

{Where, C = mg  $\text{NH}_4^+\text{-N}$  obtained from standard curve; V= Solution volume (ml); W= Sample dry weight (g)}

Nitrate-N was determined by phenol-disulphonic acid method. 10 ml of the KCl extract was taken in a 100 ml glass beaker (borosil) and evaporated upto nearly drying. After cooling the beaker, 2 ml of sodium disulphonic acid was added followed by addition of 20 ml distilled water. Then freshly prepared ammonium hydroxide (1:1) solution was added into the beaker and filtered the solution into a 50 ml volumetric flask using Whatman No.1 filter paper. The volume was increased upto 50 ml and the yellow colour was read in the spectrophotometer at 410 nm.

$$\text{NO}_3^- \text{-N } (\mu\text{g } 100 \text{ mg}^{-1}) = \frac{\text{C (mg)} \times \text{V} \times 100}{\text{Aliquot (ml)} \times \text{W}}$$

{Where, C = mg NO<sub>3</sub><sup>-</sup>-N obtained from standard curve; V= Extractant volume (ml); W= Sample dry weight (g)}

Total P was determined from the digested aliquot (same extract of total N estimation) with the help of spectrophotometer. 10ml of the aliquot extract was taken into a 50 ml volumetric flask and 0.2ml of 5% *p*-nitrophenol indicator solution was added. Then the solution was made alkaline by drop wise adding of 6N NH<sub>3</sub> (aqueous ammonia) solution till the colour changed to yellow while shaking. Dilute 1N HNO<sub>3</sub> was added till colourless while shaking carefully. Finally, 5 ml ammonium molybdate/ ammonium vanadate mixed reagent was added and made up the volume upto 50ml with distilled water. The flask was shaken for a while and kept undisturbed for 30 minutes. The intensity of the yellow colour was read spectrophotometrically at 400 nm. The total P content was calculated from the standard curve as follows:

$$\text{Total P (\%)} = \frac{C \times 0.025}{W}$$

{Where, C =Corrected reading of P from the standard curve (ppm); W=Sample weight (g)}

### **Statistical analysis**

The relationships among studied parameters for all the sites were analyzed by calculating simple correlation coefficient (*r*) values. Monthly variation of the various physico-chemical properties in the three study sites were analyzed by calculating the least significance differences (LSD) at  $P < 0.05$ . The variation of studied parameters among the three sites and between the soil layers was analyzed statistically using one way analysis of variance (ANOVA).

## **3.4. RESULTS**

### **Soil temperature**

Soil temperature varied from 17.8°C to 31.8°C in all the three study sites (Fig. 3.1). Maximum soil temperature was recorded from the degraded site in the month of June 1999 while the minimum was recorded from the moderately degraded site in the month of January 2000. Soil temperature was higher during summer rainy season (April to October) and lower during the winter dry periods (November to March) in most cases. In general, surface soil layer showed higher soil temperature than the subsurface soil layers in all study sites. The degraded site showed slightly higher soil temperature at surface and subsurface layers in comparison to the moderately degraded and undegraded sites.

The calculated values of least significance differences (LSD) of soil temperature among the sampling periods of the three study sites at both soil depths are shown in Figure 3.1. The variation in soil temperature between degraded, moderately degraded and undegraded sites at surface layers were insignificant ( $P < 0.05$ ). However, the variation was significant at subsurface layers of the three study sites except between degraded and moderately degraded sites at  $P < 0.05$  (Table 3.1.).

### **Soil moisture**

Soil moisture content varied from a minimum of 3% in the degraded site to a maximum of 35% in the undegraded site at surface soil layer (Fig. 3.2). The moderately degraded site had intermediate value of soil moisture between the degraded and undegraded sites at surface soil layer. Similar pattern of soil moisture distribution was recorded from the subsurface layers of all study sites. The maximum soil moisture content was recorded from the undegraded site while the minimum was recorded from the moderately degraded site. Soil moisture content was markedly influenced by seasonal changes in all study sites during the entire period of study. Generally, lowest moisture content was recorded from all study sites during dry winter months (November to February) which increased to highest during summer rainy seasons (March to October). Maximum moisture content were recorded from the undegraded site at surface and subsurface soil layers in the month of June 2000. The minimum moisture content was recorded from the degraded site during February 1999. In general, soil moisture content was higher at the

surface layer than the subsurface soil layers in all study sites. LSD of soil moisture in all the study sites at both soil depths are depicted in Figure 3.2. No significant variation of moisture content was recorded from all the study sites at surface soil layers though the variation were significant ( $P<0.05$ ) at subsurface soil layers (Table 3.1.).

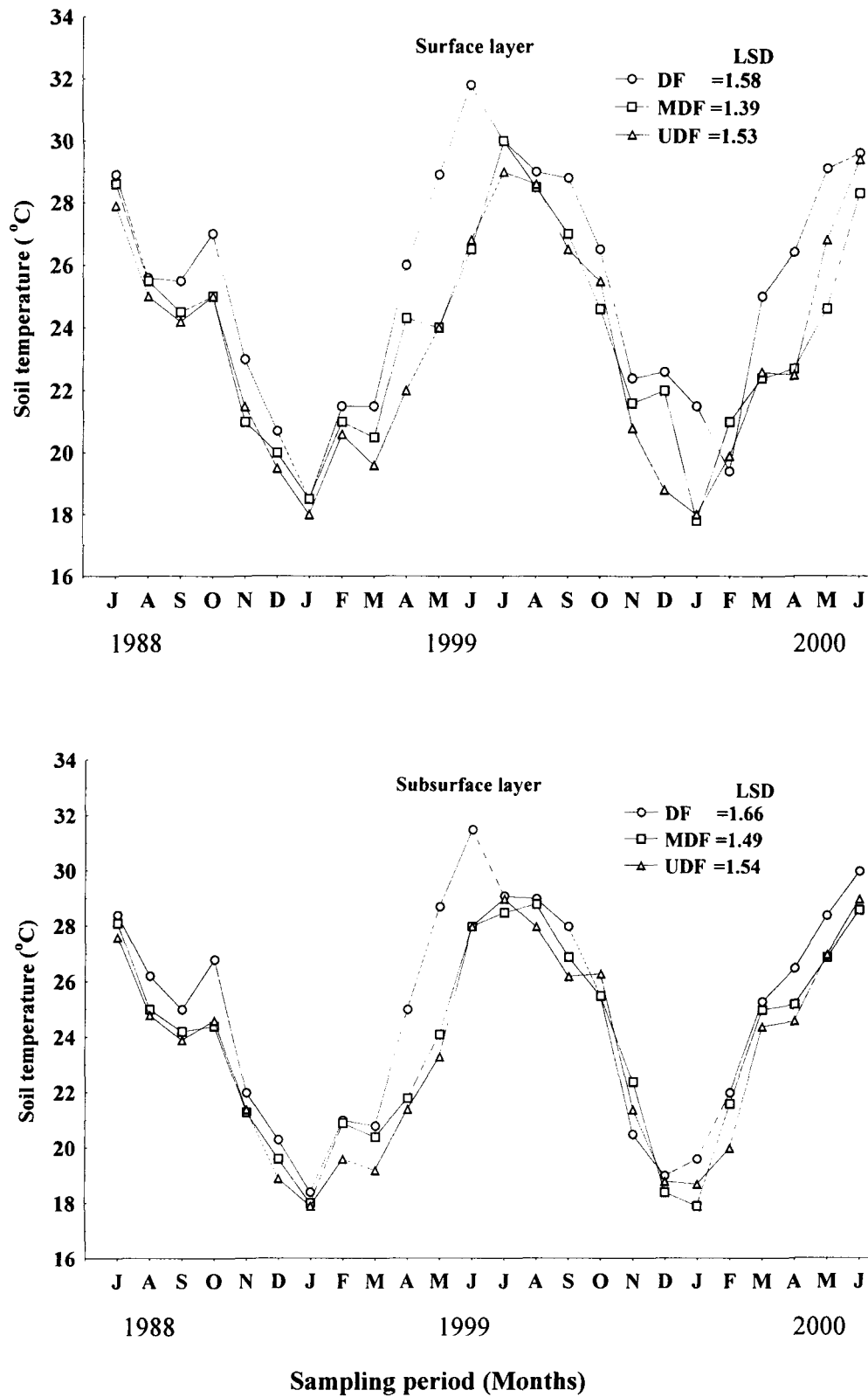
### **Bulk density**

The bulk density of the study sites ranged from 0.84 to 1.43 g cm<sup>-3</sup> at surface soil layer and from 1.03 to 1.46 g cm<sup>-3</sup> in the subsurface soil layers of the degraded, moderately degraded and undegraded forest sites (Fig. 3.3). The maximum value of bulk density was recorded from the degraded site while the lowest was recorded from the undegraded site at both the soil depths.

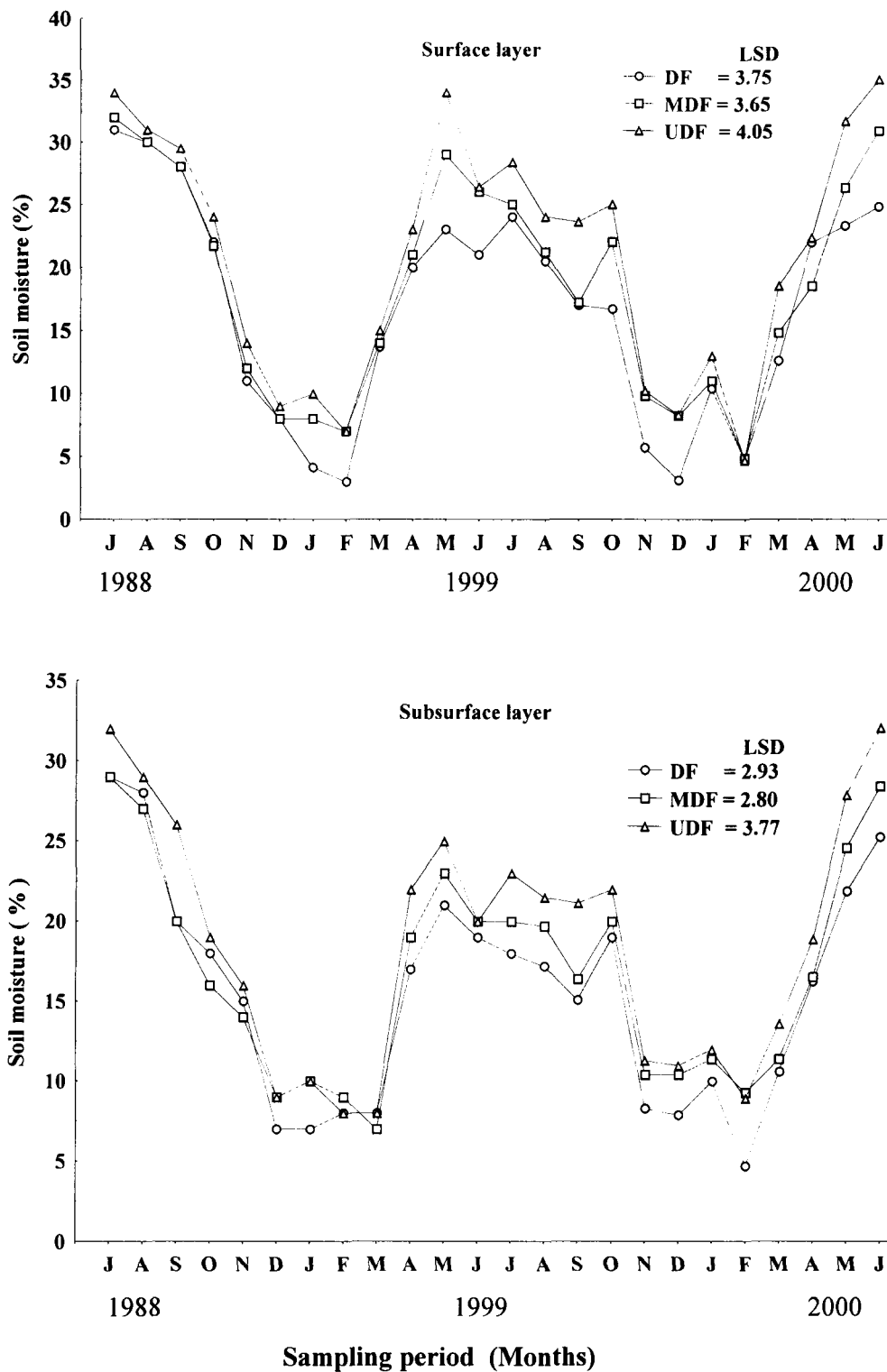
There was a significant ( $P<0.05$ ) variation in the bulk density of soil between all sites and at both soil depths (Table 3.1.) but no definite trend was observed with seasonal climatic changes (Fig. 3.3). The surface soil layer had lower soil bulk density than the subsurface soil layers of moderately degraded and undegraded sites though the variation was insignificant in case of degraded site (Table 3.2.).

### **Water Holding Capacity**

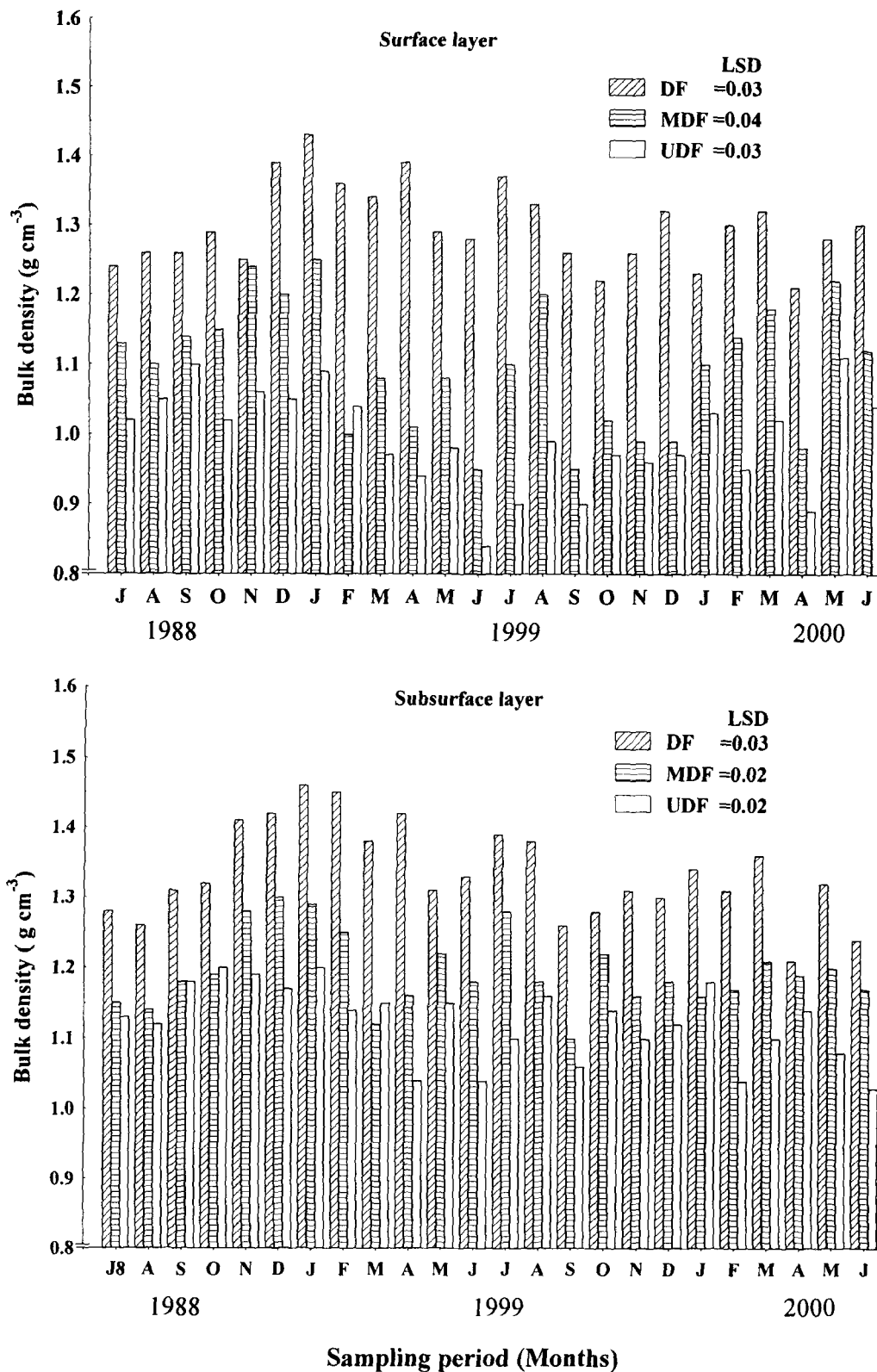
The water holding capacity of soils in all the study sites varied from a minimum of 43% to a maximum of 61% during the entire sampling period of two years (Fig. 3.4). The maximum water holding capacity was recorded from the undegraded site and minimum from the degraded site at both the



**Fig.3.1. Soil temperatures of degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites at surface and subsurface layers.(LSD;  $P < 0.05$ )**



**Fig.3.2. Soil moisture of degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites at surface and subsurface layers. (LSD;  $P < 0.05$ )**



**Fig. 3.3. Soil bulk density in degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites at surface and subsurface soil layers. (LSD,  $P < 0.05$ )**

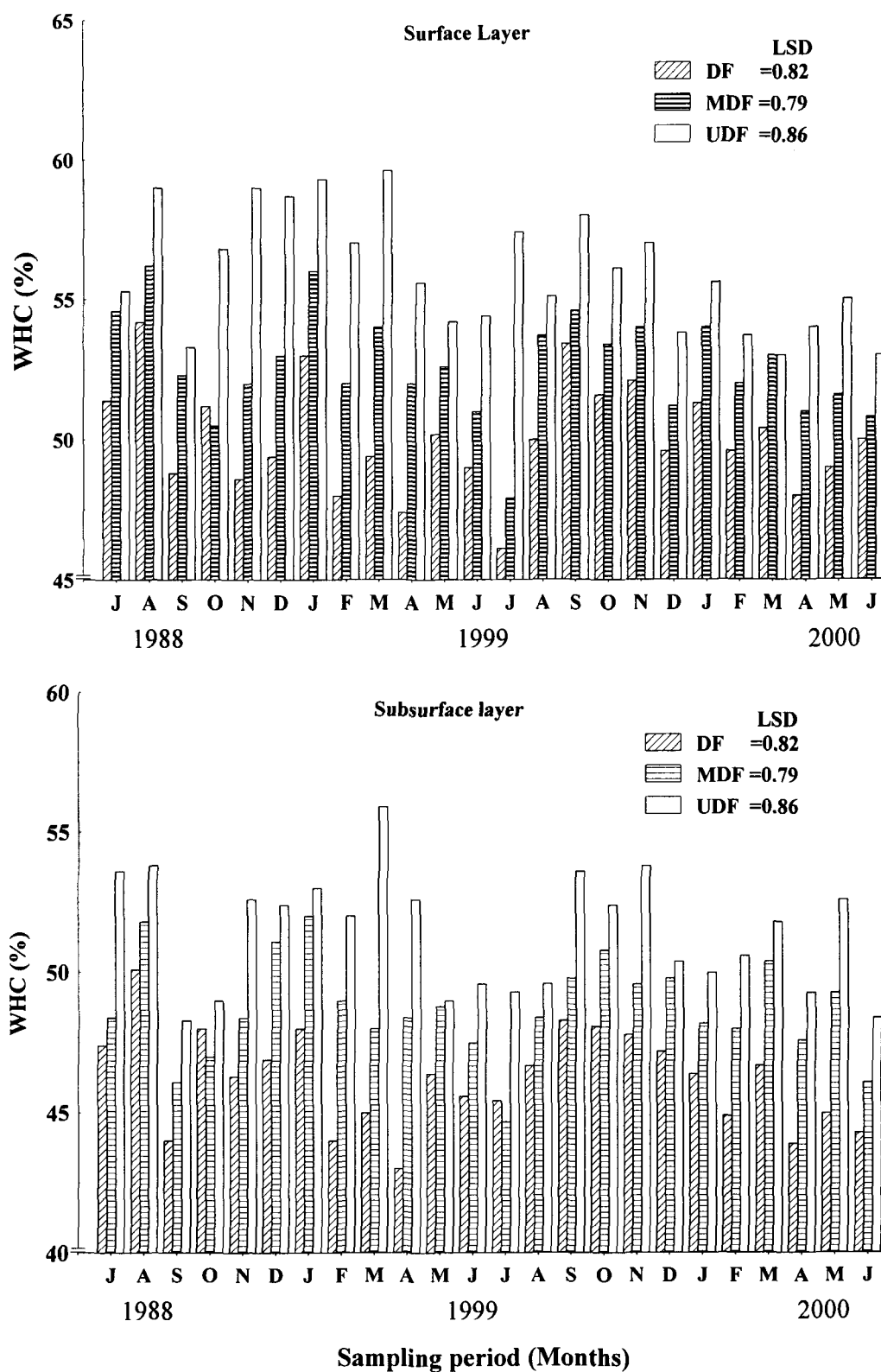
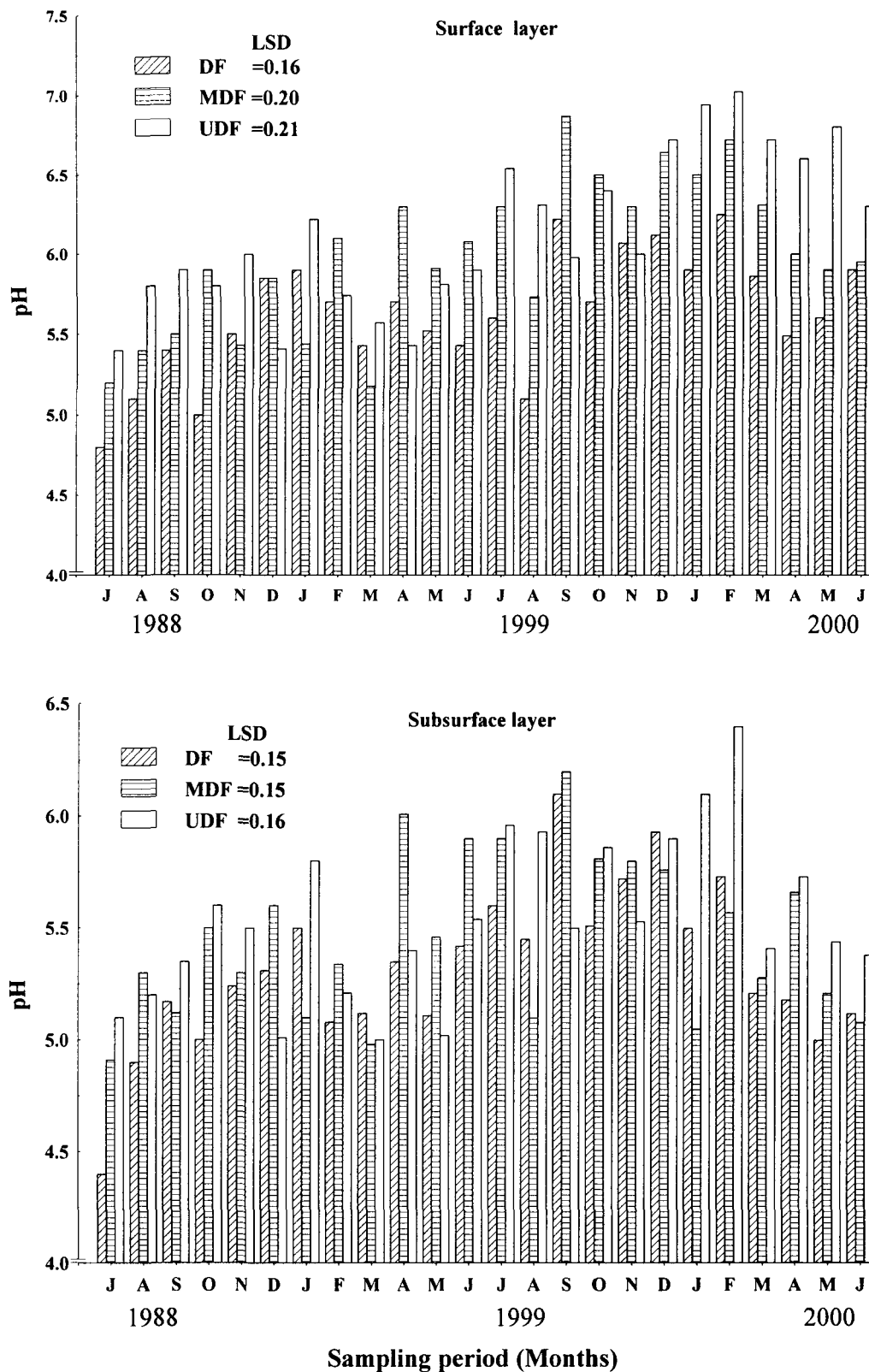
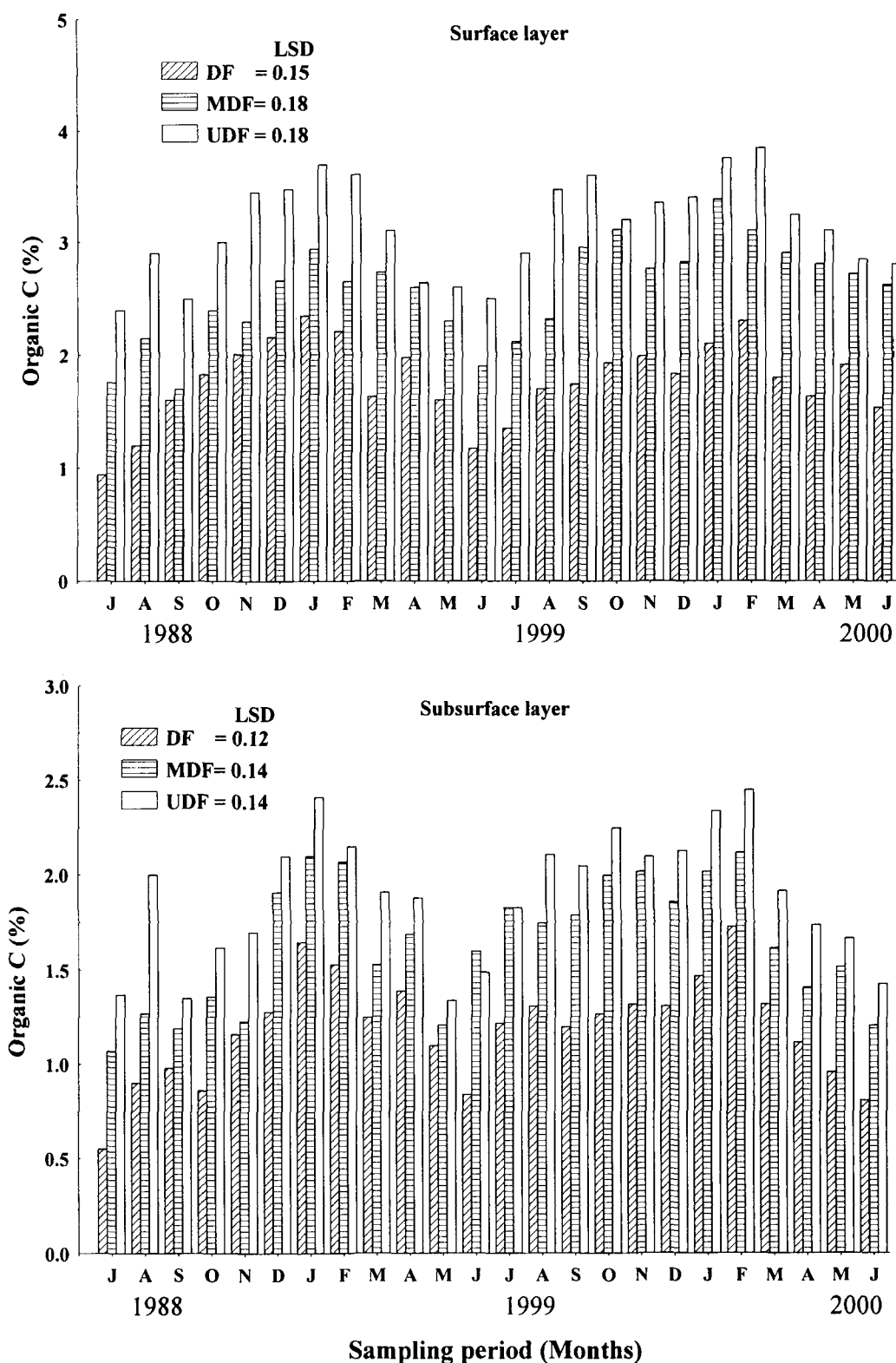


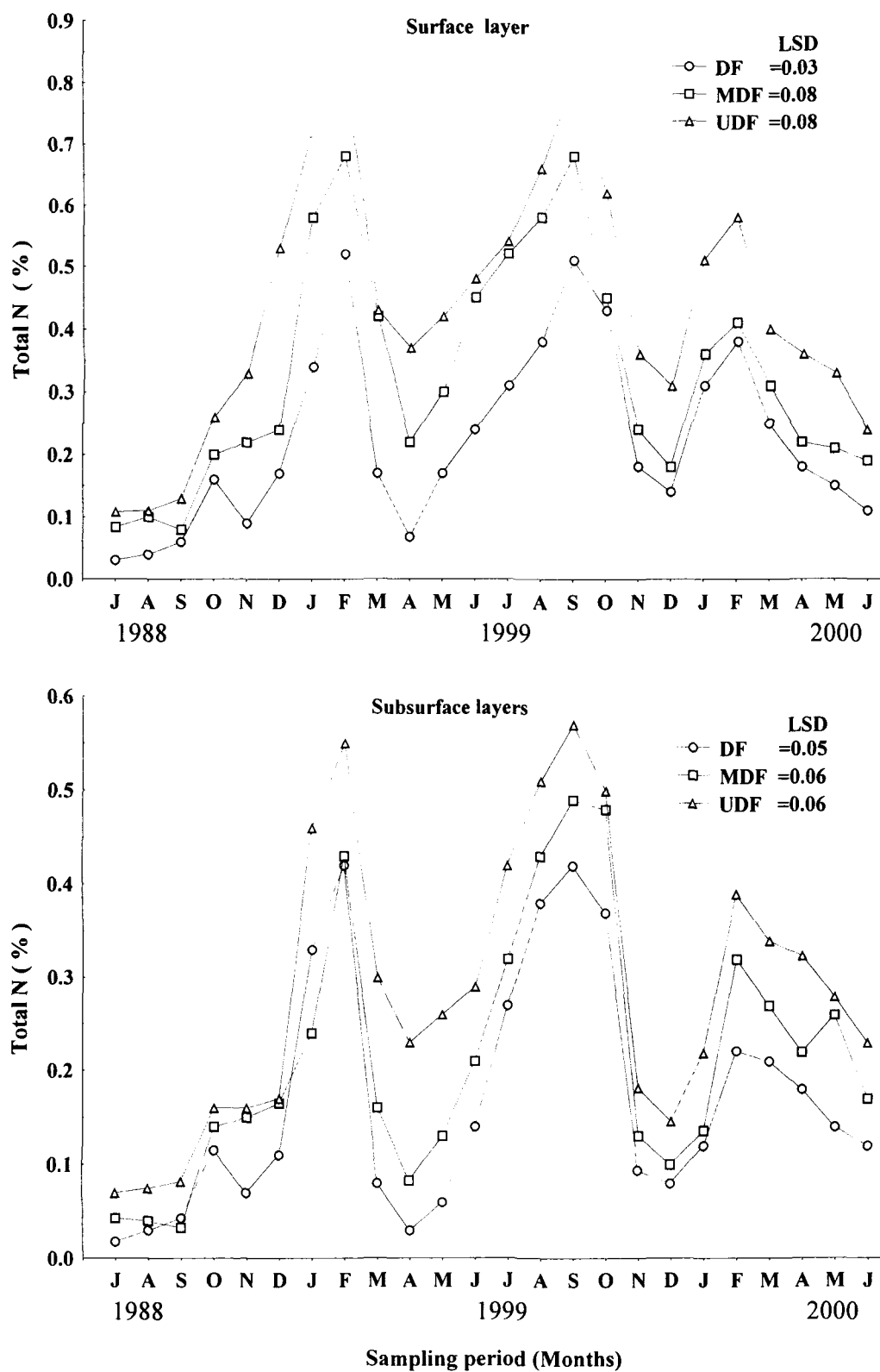
Fig.3.4. Water holding capacity of soil in degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites at surface and subsurface layers. (LSD,  $P < 0.05$ )



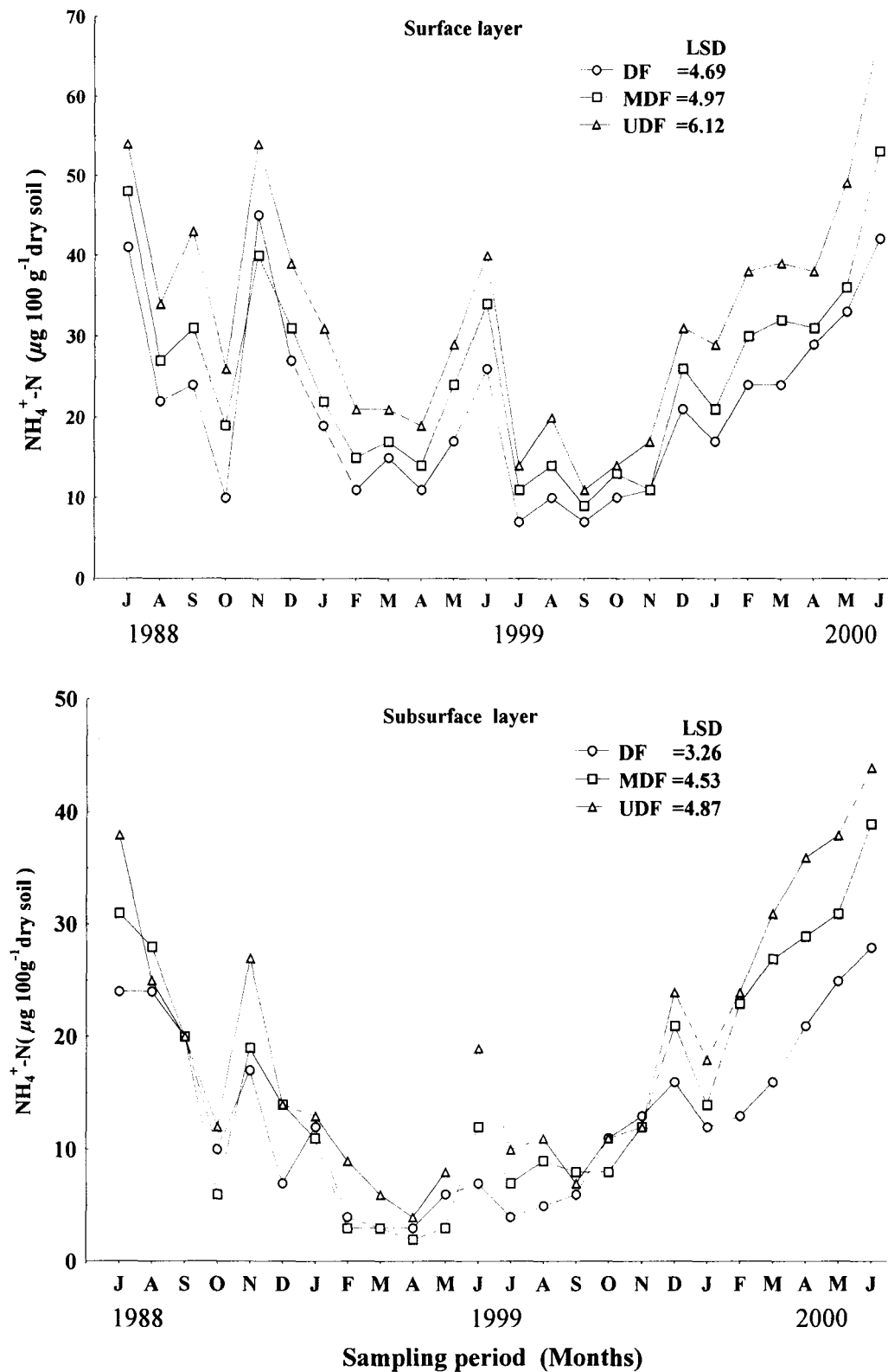
**Fig. 3.5. Distribution of soil pH at surface and subsurface soil layers of degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites. (LSD,  $P < 0.05$ )**



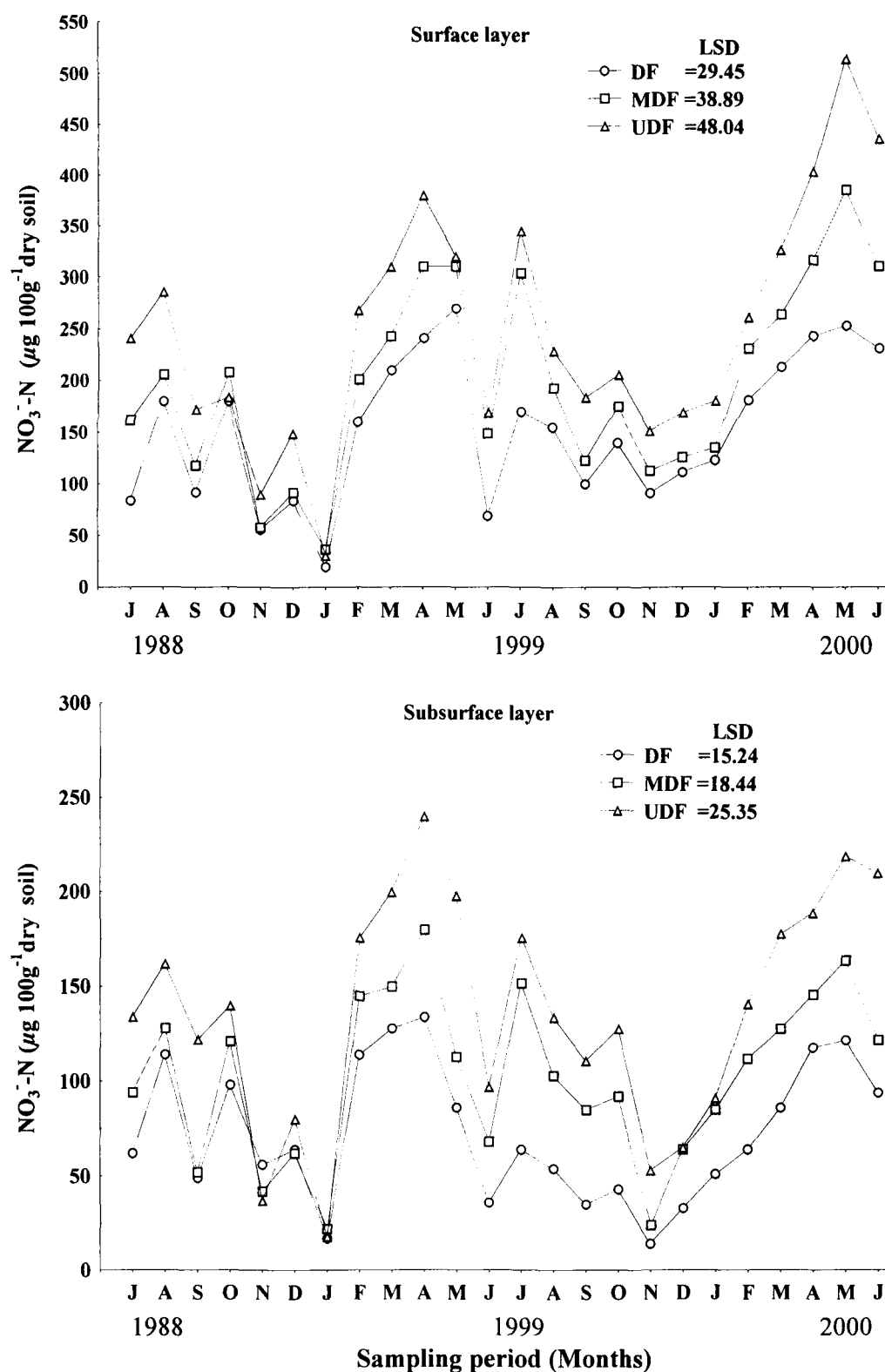
**Fig.3.6. Organic C content of soil at surface and subsurface soil layers of degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites. (LSD,  $P < 0.05$ )**



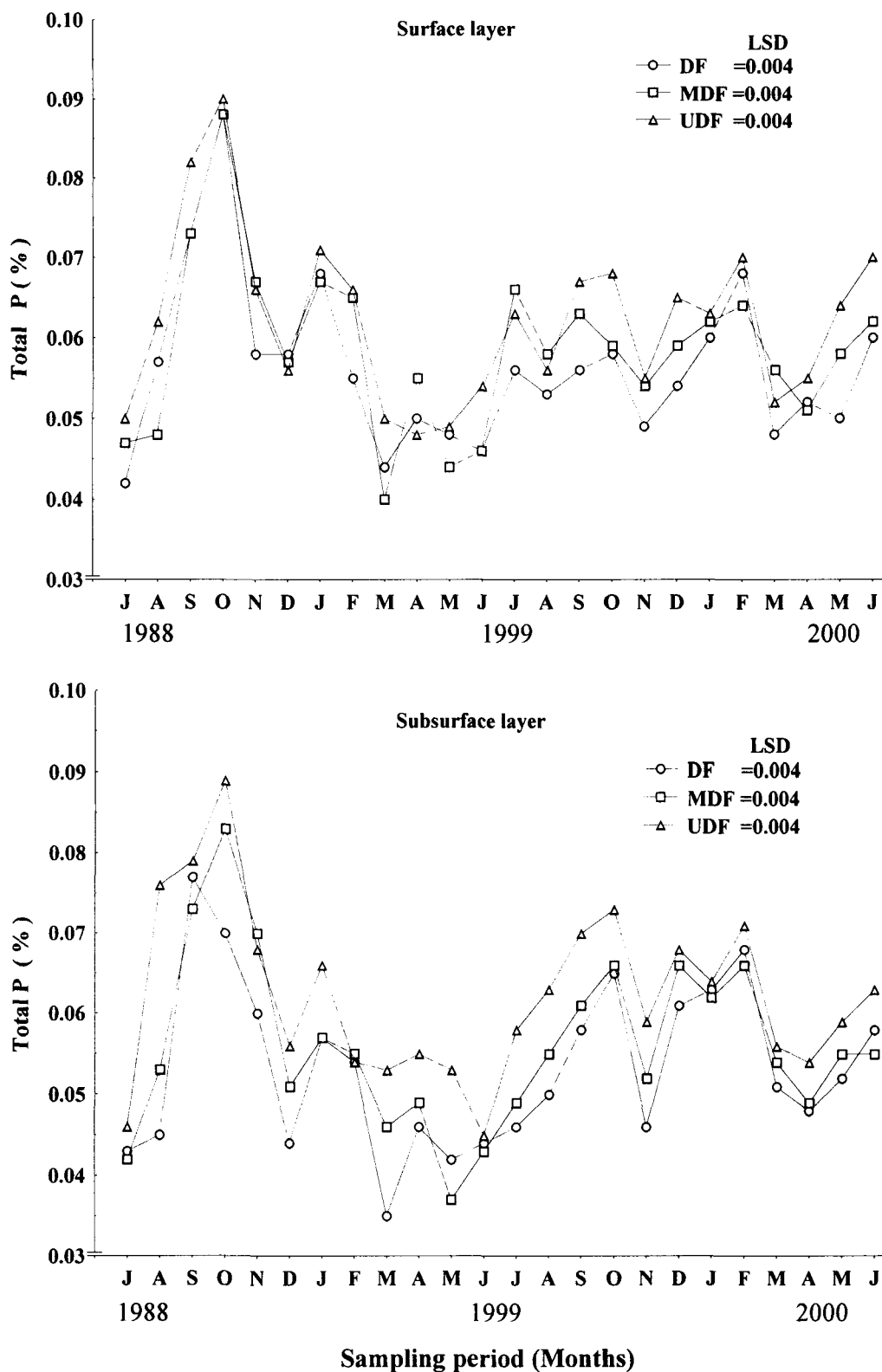
**Fig.3.7. Total N content of soil at surface and subsurface soil layers of degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites. (LSD,  $P < 0.05$ )**



**Fig.3.8. Distribution of ammonium-N content of soil in degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites at surface and subsurface soil layers. (LSD,  $P < 0.05$ )**



**Fig.3.9.** Distribution of nitrate-N content of soil in degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites at surface and subsurface soil layers. (LSD,  $P < 0.05$ )



**Fig.3.10. Total P content of soil at surface and subsurface soil layers of degraded (DF), moderately degraded (MDF) and undegraded forest (UDF) sites. (LSD,  $P < 0.05$ )**

**Table 3.1. One-way analysis of variance (ANOVA) of the physico-chemical characteristics of soil in degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites at surface and subsurface soil layers ( $P < 0.05$ ).**

Soil properties	Source of Variation	Surface layer		Subsurface layer	
		F-ratio	P-level	F-ratio	P-level
Soil Temperature	DFxMDFxUDF	-	-	18.725	$3.14 \times 10^{-6}$
	DFxMDF	-	-	-	-
	MDF x UDF	-	-	21.561	$2.789 \times 10^{-5}$
	DF x UDF	-	-	23.971	$1.45 \times 10^{-5}$
Soil moisture	DFxMDFxUDF	-	-	57.997	$1.665 \times 10^{-15}$
	DFxMDF	-	-	-	-
	MDF x UDF	-	-	119.589	$2.198 \times 10^{-14}$
	DF x UDF	-	-	108.656	$1.069 \times 10^{-13}$
Bulk density	DFxMDFxUDF	101.968	0.050	82.748	0.05
	DFxMDF	81.918	$8.790 \times 10^{-12}$	64.990	$2.399 \times 10^{-10}$
	MDF x UDF	18.212	$9.764 \times 10^{-5}$	21.816	$2.629 \times 10^{-5}$
	DF x UDF	268.081	0.05	147.500	$5.551 \times 10^{-16}$
Water holding capacity	DFxMDFxUDF	52.754	$1.254 \times 10^{-14}$	42.750	$8.361 \times 10^{-13}$
	DFxMDF	21.941	$2.516 \times 10^{-5}$	23.725	$1.354 \times 10^{-5}$
	MDF x UDF	32.736	$7.556 \times 10^{-7}$	22.118	$2.364 \times 10^{-5}$
	DF x UDF	94.429	$6.148 \times 10^{-13}$	76.648	$2.339 \times 10^{-11}$
pH	DFxMDFxUDF	8.579	$4.706 \times 10^{-4}$	3.476	0.0364
	DFxMDF	8.781	0.00481	-	-
	MDF x UDF	-	-	-	-
	DF x UDF	17.168	$1.45 \times 10^{-4}$	6.559	0.0138
Organic C	DFxMDFxUDF	59.126	$1.110 \times 10^{-15}$	30.683	$2.931 \times 10^{-10}$
	DFxMDF	46.015	$9.390 \times 10^{-8}$	25.895	$6.522 \times 10^{-6}$
	MDF x UDF	19.166	$6.843 \times 10^{-5}$	7.063	1.0108
	DF x UDF	124.248	$1.154 \times 10^{-14}$	61.894	$4.644 \times 10^{-10}$
Total N	DFxMDFxUDF	10.445	$1.089 \times 10^{-4}$	-	-
	DFxMDF	11.003	0.00178	-	-
	MDF x UDF	-	-	-	-
	DF x UDF	20.888	$3.697 \times 10^{-5}$	4.326	0.0431
NH <sub>4</sub> <sup>+</sup> -N	DFxMDFxUDF	7.016	0.0017	8.666	$4.388 \times 10^{-4}$
	DFxMDF	4.563	0.0380	5.694	0.0211
	MDF x UDF	-	-	-	-
	DF x UDF	14.406	$4.293 \times 10^{-4}$	20.952	$3.575 \times 10^{-5}$
NO <sub>3</sub> <sup>-</sup> -N	DFxMDFxUDF	73.873	0.05	-	-
	DFxMDF	-	-	-	-
	MDF x UDF	74.018	$3.876 \times 10^{-11}$	-	-
	DF x UDF	83.408	$6.715 \times 10^{-12}$	-	-
Total P	DFxMDFxUDF	-	-	-	-
	DFxMDF	-	-	-	-
	MDF x UDF	-	-	-	-
	DF x UDF	4.729	0.0348	8.659	0.0051

Note: Insignificant values are denoted by “-” sign

**Table 3.2. One-way analysis of variance (ANOVA) of the physico-chemical characteristics of soil between surface and subsurface soil layers of degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites ( $P < 0.05$ ).**

Soil properties	Study Sites	F-ratio	P-level
Soil temperature	DF	-	-
	MDF	-	-
	UDF	-	-
Soil moisture	DF	-	-
	MDF	-	-
	UDF	-	-
Bulk Density	DF	-	-
	MDF	20.304	$4.515 \times 10^{-5}$
	UDF	50.82	$5.842 \times 10^{-9}$
Water holding capacity	DF	52.082	$4.314 \times 10^{-9}$
	MDF	55.359	$1.20 \times 10^{-9}$
	UDF	41.032	$7.12 \times 10^{-8}$
pH	DF	8.510	0.005
	MDF	19.787	$5.447 \times 10^{-5}$
	UDF	16.706	$1.732 \times 10^{-4}$
Organic C	DF	35.310	$3.534 \times 10^{-7}$
	MDF	63.928	$3.003 \times 10^{-10}$
	UDF	11.140	$2.687 \times 10^{-14}$
Total N	DF	-	-
	MDF	8.057	0.006
	UDF	9.1088	0.004
NH <sub>4</sub> <sup>+</sup> -N	DF	5.678	0.021
	MDF	-	-
	UDF	6.933	0.011
NO <sub>3</sub> <sup>-</sup> -N	DF	21.673	$2.766 \times 10^{-5}$
	MDF	12.830	$8.195 \times 10^{-4}$
	UDF	16.829	$1.652 \times 10^{-4}$
Total P	DF	-	-
	MDF	-	-
	UDF	-	-

Note: Insignificant values are denoted by “-” sign

**Table 3.3a.** Correlation coefficient (*r*) values among various physico-chemical, biological and biochemical characteristics of soils in degraded forest (DF) site at surface soil layer ( $P \leq 0.05$ ).

Soil properties	ST	SM	BD	WHC	pH	OC	TN	TP	AN	NN	SR	BP	FP	C <sub>mic</sub>	N <sub>mic</sub>	DHA	PA	UA	RF
AT	0.927 <sup>c</sup>	0.773 <sup>c</sup>	-	-	-0.414 <sup>a</sup>	-0.686 <sup>c</sup>	-	-	-	0.415 <sup>a</sup>	0.587 <sup>b</sup>	0.387 <sup>a</sup>	-	0.361 <sup>a</sup>	0.433 <sup>a</sup>	-0.505 <sup>b</sup>	-	-	0.720 <sup>c</sup>
ST		0.795 <sup>c</sup>	-	-	-0.439 <sup>a</sup>	-0.738 <sup>c</sup>	-	-	-	-	0.484 <sup>b</sup>	0.348 <sup>a</sup>	-	-	-	-0.478 <sup>b</sup>	-	-	0.776 <sup>c</sup>
SM			-	-	-0.695 <sup>c</sup>	-0.803 <sup>c</sup>	-0.467 <sup>b</sup>	-	-	0.338 <sup>a</sup>	0.697 <sup>c</sup>	0.420 <sup>a</sup>	-	-	-	-0.478 <sup>b</sup>	-0.382 <sup>a</sup>	-	0.827 <sup>c</sup>
BD				-	-	0.347 <sup>a</sup>	-	-	-	-	-0.354 <sup>a</sup>	-	-	-	-	-	-	-	-
WHC					-	-	-	-	-	-	-	-	-	-	-0.362 <sup>a</sup>	-	-	-	-
pH						0.622 <sup>c</sup>	0.429 <sup>a</sup>	-	-	-	-0.457 <sup>a</sup>	-0.369 <sup>a</sup>	-	-	-	0.369 <sup>a</sup>	0.536 <sup>b</sup>	-0.478 <sup>a</sup>	-0.580 <sup>b</sup>
OC							0.427 <sup>a</sup>	-	-	-	-0.498 <sup>b</sup>	-0.476 <sup>b</sup>	-	-	-	0.450 <sup>a</sup>	0.411 <sup>a</sup>	-	-0.793 <sup>c</sup>
TN								-	-0.563 <sup>b</sup>	-	-0.364 <sup>b</sup>	-0.351 <sup>a</sup>	-	-	-	-	0.409 <sup>a</sup>	-0.376 <sup>a</sup>	-
TP									-	-	-	-	-	-	-	-	-	-	-
AN										-	-	0.485 <sup>b</sup>	-	-	0.361 <sup>a</sup>	-	-	-	-
NN											-	-	-	-	0.506 <sup>b</sup>	-0.502 <sup>b</sup>	-	-0.370 <sup>a</sup>	-
SR												0.419 <sup>a</sup>	-	-	-	-	-	-	0.505 <sup>b</sup>
BP													0.372 <sup>a</sup>	-	-	-	-	-	0.548 <sup>b</sup>
FP														-	-	-	-	-	-
C <sub>mic</sub>															0.586 <sup>b</sup>	-	-	-	-
N <sub>mic</sub>																-	0.379 <sup>a</sup>	-	-
DHA																	-	-	-
PA																		-	-
UA																			-

(Note: AT=ambient temperature, ST=soil temperature, SM=soil moisture, BD=bulk density, WHC=water holding capacity, C=organic C, N=total N, P=total P, AN=ammonium-N, NN=nitrate-N, SR=soil respiration, BP=bacterial population, FP=fungal population, C<sub>mic</sub>=microbial biomass C, N<sub>mic</sub>=microbial biomass N, DHA=dehydrogenase activity, PA= acid phosphatase activity, UA=urease activity)

Values marked with a, b and c are significant at  $P \leq 0.05$ ,  $P \leq 0.01$  and  $P \leq 0.001$  respectively for 23 degrees of freedom; insignificant values are marked with ‘-’

**Table 3.3b.** Correlation coefficient ( $r$ ) values among various physico-chemical, biological and biochemical characteristics of soils in moderately degraded forest site (MDF) at surface soil layer ( $P \leq 0.05$ ).

Soil properties	ST	SM	BD	WHC	pH	OC	TN	TP	AN	NN	SR	BP	FP	C <sub>mic</sub>	N <sub>mic</sub>	DHA	PA	UA	RF
AT	0.858 <sup>c</sup>	0.842 <sup>c</sup>	-	-	-	-	-	-	-	0.485 <sup>b</sup>	0.755 <sup>c</sup>	0.359 <sup>a</sup>	-	0.379 <sup>a</sup>	0.463 <sup>b</sup>	-0.436 <sup>a</sup>	-	-	0.796 <sup>c</sup>
ST		0.789 <sup>c</sup>	-	-	-	-	-	-	-	0.377 <sup>a</sup>	0.586 <sup>b</sup>	-	-	0.337 <sup>a</sup>	-	-0.432 <sup>a</sup>	-	-	0.854 <sup>c</sup>
SM			-	-	-0.338 <sup>a</sup>	-0.338 <sup>a</sup>	-	-	-	0.450 <sup>a</sup>	0.794 <sup>c</sup>	0.412 <sup>a</sup>	-	-	-	-0.500 <sup>b</sup>	-0.419 <sup>a</sup>	-	0.869 <sup>c</sup>
BD				-	-0.508 <sup>b</sup>	-0.508 <sup>a</sup>	-	0.431 <sup>a</sup>	0.372 <sup>a</sup>	-	-	-	-	-	-	-	-	0.520 <sup>b</sup>	-
WHC					-	-	-	-	-	-0.462 <sup>b</sup>	-	-	-	-0.366 <sup>a</sup>	-0.498 <sup>b</sup>	-	-	0.416 <sup>a</sup>	-
pH						0.999 <sup>c</sup>	-	-	-0.388 <sup>a</sup>	-	-0.342 <sup>a</sup>	-	-	-	-	0.355 <sup>a</sup>	-	-0.558 <sup>b</sup>	-
OC							-	-	-0.388 <sup>a</sup>	-	-0.342 <sup>a</sup>	-	-	-	-	0.355 <sup>a</sup>	-	-0.558 <sup>b</sup>	-
TP								-	-0.589 <sup>c</sup>	-	-	-	-	-	-	-	0.508 <sup>b</sup>	-	-
AN									-	-	-	-	-0.382 <sup>a</sup>	-	-	-	-	-	-
NN										-	-	-	-	-	-	-	-	-	-
TP															0.692 <sup>c</sup>	-0.423 <sup>a</sup>	-	-0.390 <sup>a</sup>	0.354 <sup>a</sup>
SR												0.384 <sup>a</sup>	-	-	0.379 <sup>a</sup>	-0.417 <sup>a</sup>	-	-	0.669 <sup>c</sup>
BP													0.535 <sup>b</sup>	0.446 <sup>a</sup>	-	-	-	-	0.490 <sup>b</sup>
FP														-	-	-	-	-0.393 <sup>a</sup>	-
C <sub>mic</sub>															0.640 <sup>c</sup>	-	-	-	0.346 <sup>a</sup>
N <sub>mic</sub>																-	0.429 <sup>a</sup>	-0.396 <sup>a</sup>	-
DHA																	-	-	-0.369 <sup>a</sup>
PA																		-	-0.338 <sup>a</sup>
UA																			-

(Note: AT=ambient temperature, ST=soil temperature, SM=soil moisture, BD=bulk density, WHC=water holding capacity, C=organic C, N=total N, P=total P, AN=ammonium-N, NN=nitrate-N, SR=soil respiration, BP=bacterial population, FP=fungal population, C<sub>mic</sub>=microbial biomass C, N<sub>mic</sub>=microbial biomass N, DHA=dehydrogenase activity, PA= acid phosphatase activity, UA=urease activity)

Values marked with a, b and c are significant at  $P \leq 0.05$ ,  $P \leq 0.01$  and  $P \leq 0.001$  respectively for 23 degrees of freedom; insignificant values are marked with '-'

**Table 3.3c.** Correlation coefficient ( $r$ ) values among various physico-chemical, biological and biochemical characteristics of soils in undegraded forest (UDF) site at surface soil layer ( $P \leq 0.05$ ).

Soil properties	ST	SM	BD	WHC	pH	OC	TN	TP	AN	NN	SR	BP	FP	C <sub>mic</sub>	N <sub>mic</sub>	DHA	PA	UA	RF
AT	0.881 <sup>c</sup>	0.843 <sup>c</sup>	-	-	-	-0.626 <sup>b</sup>	-	-	-	0.483 <sup>b</sup>	0.684 <sup>c</sup>	0.543 <sup>b</sup>	-	0.373 <sup>a</sup>	0.436 <sup>a</sup>	-	-	-	0.764 <sup>c</sup>
ST		0.844 <sup>c</sup>	-	-	-	-0.584 <sup>b</sup>	-	-	-	0.416 <sup>a</sup>	0.612 <sup>b</sup>	0.454 <sup>a</sup>	-	0.365 <sup>a</sup>	0.366 <sup>a</sup>	-	-	-	0.857 <sup>c</sup>
SM			-	-	-	-0.814 <sup>c</sup>	-0.473 <sup>b</sup>	-	-	0.496 <sup>b</sup>	0.759 <sup>c</sup>	0.428 <sup>a</sup>	-	-	-	-	-	-	0.834 <sup>c</sup>
BD				-	-		-	0.404 <sup>a</sup>	0.438 <sup>a</sup>	-	-	-	-	-	-	-	-	-	-
WHC					-0.447 <sup>a</sup>		-	-	-0.359 <sup>a</sup>	-0.405 <sup>a</sup>	-	-	-0.309	-0.516 <sup>b</sup>	-0.500 <sup>b</sup>	-	-	-	-
pH						0.411 <sup>a</sup>	-	-	-	-	-	-	-	0.583 <sup>b</sup>	0.595 <sup>c</sup>	0.549 <sup>b</sup>	0.495 <sup>b</sup>	0.508 <sup>b</sup>	-
OC							0.659 <sup>c</sup>	-	-	-0.419 <sup>a</sup>	-0.582 <sup>b</sup>	-	-	-	-	0.426 <sup>a</sup>	0.477 <sup>b</sup>	-	-0.646 <sup>c</sup>
TN								-	-0.571 <sup>b</sup>	-	-	-	-	-	-	-	0.466 <sup>b</sup>	-	-0.353 <sup>a</sup>
TP										-	-	-	-0.528 <sup>a</sup>	-	-	-	-	-	-
AN										-	-	-	-	-	-	-	-	-	-
NN										-	-	-	-	0.519 <sup>b</sup>	0.607 <sup>c</sup>	-	-	-	0.373 <sup>a</sup>
SR												0.538 <sup>b</sup>	-	-	-	-	-	-	0.597 <sup>c</sup>
BP													0.530 <sup>a</sup>	0.391 <sup>a</sup>	-	-	-	-	0.589 <sup>c</sup>
FP														-	-	-	-	-0.578 <sup>b</sup>	-
C <sub>mic</sub>															0.855 <sup>c</sup>	-	0.392 <sup>a</sup>	-	-
N <sub>mic</sub>																-	0.483 <sup>b</sup>	-	-
DHA																		-	-
PA																		-	-
UA																		-	-

(Note: AT=ambient temperature, ST=soil temperature, SM=soil moisture, BD=bulk density, WHC=water holding capacity, C=organic C, N=total N, P=total P, AN=ammonium-N, NN=nitrate-N, SR=soil respiration, BP=bacterial population, FP=fungal population, C<sub>mic</sub>=microbial biomass C, N<sub>mic</sub>=microbial biomass N, DHA=dehydrogenase activity, PA= acid phosphatase activity, UA=urease activity)

Values marked with a, b and c are significant at  $P \leq 0.05$ ,  $P \leq 0.01$  and  $P \leq 0.001$  respectively for 23 degrees of freedom; insignificant values are marked with ‘-’

**Table 3.4a.** Correlation coefficient ( $r$ ) values among various physico-chemical, biological and biochemical characteristics of soils in degraded forest (DF) site at subsurface soil layer ( $P \leq 0.05$ ).

Soil properties	ST	SM	BD	WHC	pH	OC	TN	TP	AN	NN	SR	BP	FP	C <sub>mic</sub>	N <sub>mic</sub>	DHA	PA	UA	RF
AT	0.920 <sup>c</sup>	0.738 <sup>c</sup>	-0.395 <sup>a</sup>	-	-	-0.627 <sup>c</sup>	-	-0.343 <sup>a</sup>	-	-	0.595 <sup>b</sup>	0.394 <sup>a</sup>	-	0.549 <sup>b</sup>	0.509 <sup>b</sup>	-0.538 <sup>b</sup>	-	-	0.720 <sup>c</sup>
ST		0.790 <sup>c</sup>	-0.448 <sup>a</sup>	-	-	-0.681 <sup>c</sup>	-	-	-	-	0.573 <sup>b</sup>	0.424 <sup>a</sup>	-0.441 <sup>a</sup>	0.468 <sup>b</sup>	0.435 <sup>a</sup>	-0.659 <sup>c</sup>	-	-	0.803 <sup>c</sup>
SM			-0.505 <sup>b</sup>	-	-0.578 <sup>a</sup>	-0.844 <sup>c</sup>	-	-	0.482 <sup>b</sup>	-	0.763 <sup>c</sup>	0.441 <sup>a</sup>	-0.379 <sup>a</sup>	0.347 <sup>a</sup>	0.407 <sup>a</sup>	-0.518 <sup>b</sup>	-0.391 <sup>a</sup>	-	0.875 <sup>c</sup>
BD				-	-	-0.527 <sup>b</sup>	-	-	-0.580 <sup>b</sup>	-	-0.481 <sup>a</sup>	-	-	-	-	-	-	-	-0.407 <sup>a</sup>
WHC					-	-	-	-	-	-0.451 <sup>a</sup>	-	-	-	-	-0.463 <sup>b</sup>	0.384 <sup>a</sup>	-	0.371 <sup>a</sup>	-
pH						0.612 <sup>c</sup>	0.443 <sup>a</sup>	-	-0.416 <sup>a</sup>	-0.566 <sup>b</sup>	-0.345 <sup>a</sup>	-	-	-	-	0.482 <sup>b</sup>	-	-	-0.400 <sup>a</sup>
OC							0.476 <sup>b</sup>	-	-0.499 <sup>b</sup>	-	-0.583 <sup>b</sup>	-	0.415 <sup>a</sup>	-0.402 <sup>a</sup>	-0.394 <sup>a</sup>	0.526 <sup>b</sup>	0.480 <sup>b</sup>	-	-0.661 <sup>c</sup>
TN								-	-0.392 <sup>a</sup>	-	-0.352 <sup>a</sup>	-	-	-	-	-	0.499 <sup>b</sup>	-	-
TP									-	-	-	-	-	-	-	0.359 <sup>a</sup>	-	-	-
AN										-	0.483 <sup>b</sup>	-	-	0.513 <sup>b</sup>	-	-	-	0.455 <sup>a</sup>	-
NN											-	-	-	-	0.468 <sup>b</sup>	-0.528 <sup>b</sup>	-	-	-
SR												0.512 <sup>b</sup>	-0.476 <sup>b</sup>	-	-	-	-	-	0.574 <sup>b</sup>
BP													-	0.358 <sup>a</sup>	-	-	-	-	0.600 <sup>c</sup>
FP														-	-	-	-	-	-
C <sub>mic</sub>															0.552 <sup>b</sup>	-	-	-	0.354 <sup>a</sup>
N <sub>mic</sub>																-	-	-	-
DHA																	-	-	-0.523 <sup>b</sup>
PA																		-	-
UA																			-

(Note: AT=ambient temperature, ST=soil temperature, SM=soil moisture, BD=bulk density, WHC=water holding capacity, C=organic C, N=total N, P=total P, AN=ammonium-N, NN=nitrate- N, SR=soil respiration, BP=bacterial population, FP=fungal population, C<sub>mic</sub>=microbial biomass C, N<sub>mic</sub>=microbial biomass N, DHA=dehydrogenase activity, PA= acid phosphatase activity, UA=urease activity)

Values marked with a, b and c are significant at  $P \leq 0.05$ ,  $P \leq 0.01$  and  $P \leq 0.001$  respectively for 23 degrees of freedom; insignificant values are marked with ‘-’

**Table 3.4b.** Correlation coefficient ( $r$ ) values among various physico-chemical, biological and biochemical characteristics of soils in moderately degraded forest (MDF) site at subsurface soil layer ( $P \leq 0.05$ ).

Soil properties	ST	SM	BD	WHC	pH	OC	TN	TP	AN	NN	SR	BP	FP	C <sub>mic</sub>	N <sub>mic</sub>	DHA	PA	UA	RF
AT	0.918 <sup>c</sup>	0.776 <sup>c</sup>	-	-0.380 <sup>a</sup>	-	-0.512 <sup>b</sup>	-	-	-	0.384 <sup>a</sup>	0.643 <sup>c</sup>	0.504 <sup>b</sup>	-	0.440 <sup>a</sup>	0.627 <sup>c</sup>	-0.433 <sup>a</sup>	-	-	0.796 <sup>c</sup>
ST		0.763 <sup>c</sup>	-	-0.393 <sup>a</sup>	-	-0.461 <sup>a</sup>	-	-	-	-	0.610 <sup>c</sup>	0.491 <sup>b</sup>	-	0.537 <sup>b</sup>	0.548 <sup>b</sup>	-0.454 <sup>a</sup>	-	-	0.818 <sup>c</sup>
SM			-	-	-	-0.668 <sup>c</sup>	-	-	0.414 <sup>a</sup>	-	0.741 <sup>c</sup>	0.654 <sup>c</sup>	-	0.383 <sup>a</sup>	0.344 <sup>a</sup>	-0.518 <sup>b</sup>	-	-	0.901 <sup>c</sup>
BD				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
WHC					-	-	-	-	-	-	-	-	-	-0.418 <sup>a</sup>	-0.486 <sup>b</sup>	-	-	0.372 <sup>a</sup>	-
pH						0.352 <sup>a</sup>	-	-	-0.368 <sup>a</sup>	-	-	-	-	-	-	-	-	-	-
OC							0.544 <sup>b</sup>	-	-0.416 <sup>a</sup>	-	-0.745 <sup>c</sup>	-0.352 <sup>a</sup>	0.353	-	-	0.573 <sup>b</sup>	0.410 <sup>a</sup>	-	-0.486 <sup>b</sup>
TN								-	-	-	-	-	-	-	-	-	0.577 <sup>b</sup>	-	-
TP								-	-	-	-	-	-0.502 <sup>a</sup>	-0.356 <sup>a</sup>	-	-	-	-	-0.346 <sup>a</sup>
AN									-	-	0.474 <sup>b</sup>	-	-	0.584 <sup>b</sup>	-	-	-	0.365 <sup>a</sup>	-
NN										-	-	-	-	0.398 <sup>a</sup>	0.407 <sup>a</sup>	-	-	-0.346 <sup>a</sup>	-
SR												0.539 <sup>b</sup>	-	0.383 <sup>a</sup>	0.348 <sup>a</sup>	-0.446 <sup>a</sup>	-	-	0.592 <sup>c</sup>
BP													-	0.364 <sup>a</sup>	-	-	-	-	0.722 <sup>c</sup>
FP														-	-	-	-	-0.417 <sup>a</sup>	-
C <sub>mic</sub>															0.544 <sup>b</sup>	-	0.375 <sup>a</sup>	-	0.357 <sup>a</sup>
N <sub>mic</sub>																-	-	-0.401 <sup>a</sup>	-
DHA																	-	-	-0.488 <sup>b</sup>
PA																		-	-
UA																			-

(Note: AT=ambient temperature, ST=soil temperature, SM=soil moisture, BD=bulk density, WHC=water holding capacity, C=organic C, N=total N, P=total P, AN=ammonium-N, NN=nitrate-N, SR=soil respiration, BP=bacterial population, FP=fungal population, C<sub>mic</sub>=microbial biomass C, N<sub>mic</sub>=microbial biomass N, DHA=dehydrogenase activity, PA= acid phosphatase activity, UA=urease activity)

Values marked with a, b and c are significant at  $P \leq 0.05$ ,  $P \leq 0.01$  and  $P \leq 0.001$  respectively for 23 degrees of freedom; insignificant values are marked with ‘-’

**Table 3.4c.** Correlation coefficient ( $r$ ) values among various physico-chemical, biological and biochemical characteristics of soils in undegraded Forest (UDF) site at subsurface soil layer ( $P \leq 0.05$ ).

Soil properties	ST	SM	BD	WHC	pH	OC	TN	TP	AN	NN	SR	BP	FP	C <sub>mic</sub>	N <sub>mic</sub>	DHA	PA	UA	RF
AT	0.883 <sup>c</sup>	0.769 <sup>c</sup>	-	-	-	-0.620 <sup>c</sup>	-	-	-	0.463 <sup>a</sup>	0.653 <sup>c</sup>	0.484 <sup>b</sup>	-	0.443 <sup>a</sup>	0.484 <sup>b</sup>	-0.442 <sup>a</sup>	-	-	0.764 <sup>c</sup>
ST		0.809 <sup>c</sup>	-0.381 <sup>a</sup>	-	-	-0.554 <sup>b</sup>	-	-	-	0.384 <sup>a</sup>	0.654 <sup>c</sup>	0.408 <sup>a</sup>	-	0.487 <sup>b</sup>	0.561 <sup>b</sup>	-	-	-	0.837 <sup>c</sup>
SM			-	-	-	-0.682 <sup>c</sup>	-	-	0.405 <sup>a</sup>	0.429 <sup>a</sup>	0.769 <sup>c</sup>	0.471 <sup>b</sup>	-	-	0.551 <sup>b</sup>	-0.457 <sup>a</sup>	-0.346 <sup>a</sup>	-	0.858 <sup>c</sup>
BD				-	-	-	-	-	-	-0.423 <sup>a</sup>	-	-	-0.455 <sup>a</sup>	-0.534 <sup>b</sup>	-0.345	-	-0.350 <sup>a</sup>	0.362 <sup>a</sup>	-
WHC					-0.361 <sup>a</sup>	0.345 <sup>a</sup>	-	-	-	-	-	-0.343 <sup>a</sup>	-	-	-	-	-	-	-
pH						0.543 <sup>b</sup>	0.351 <sup>a</sup>	0.339 <sup>a</sup>	-	-	-	-	-	-	-	0.534 <sup>b</sup>	-	-	-
OC							0.478 <sup>b</sup>	-	-0.357 <sup>a</sup>	-0.369 <sup>a</sup>	-0.596 <sup>c</sup>	-0.352 <sup>a</sup>	-	-	-	0.540 <sup>b</sup>	0.400 <sup>a</sup>	-	-0.468 <sup>b</sup>
TN								-	-0.387 <sup>a</sup>	-	-	-	-	-	-	-	0.521 <sup>b</sup>	-	-
TP									-	-	-	-	-0.682 <sup>c</sup>	-0.347 <sup>a</sup>	-	-	-	0.450 <sup>a</sup>	-
AN										-	0.394 <sup>a</sup>	-	-	0.628 <sup>c</sup>	0.514 <sup>b</sup>	-	-	-	-
NN											0.355 <sup>a</sup>	-	-	0.365 <sup>a</sup>	0.487 <sup>b</sup>	-0.443 <sup>a</sup>	-	-0.385 <sup>a</sup>	0.368 <sup>a</sup>
SR												0.427 <sup>a</sup>	-	-	0.419 <sup>a</sup>	-	-	-	0.616 <sup>c</sup>
BP													0.337 <sup>a</sup>	0.341 <sup>a</sup>	-	-	-	-	0.573 <sup>b</sup>
FP														0.453 <sup>a</sup>	-	-	0.492 <sup>b</sup>	-0.535 <sup>b</sup>	-
C <sub>mic</sub>															0.751 <sup>c</sup>	-	0.470 <sup>b</sup>	-	-
N <sub>mic</sub>																-	-	-	0.367 <sup>a</sup>
DHA																	0.380 <sup>a</sup>	-	-0.488 <sup>b</sup>
PA																		-	-
UA																		-	-

(Note: AT=ambient temperature, ST=soil temperature, SM=soil moisture, BD=bulk density, WHC=water holding capacity, C=organic C, N=total N, P=total P, AN=ammonium-N, NN=nitrate-N, SR=soil respiration, BP=bacterial population, FP=fungal population, C<sub>mic</sub>=microbial biomass C, N<sub>mic</sub>=microbial biomass N, DHA=dehydrogenase activity, PA= acid phosphatase activity, UA=urease activity)

Values marked with a, b and c are significant at  $P \leq 0.05$ ,  $P \leq 0.01$  and  $P \leq 0.001$  respectively for 23 degrees of freedom; insignificant values are marked with '-'

soil layers. The moderately degraded site contained water holding capacity value in between the degraded and the undegraded sites. No seasonal trend of water holding capacity was observed from all the sites. However, the water holding capacity varied significantly ( $P < 0.05$ ) among the study sites and between the surface and subsurface soil layers (Tables 3.1 and 3.2). Generally, the surface layer contained higher water holding capacity than the subsurface layers in all the study sites. No significant positive correlation was observed between the water holding capacity and other soil properties in degraded and undegraded sites though it was related to the organic carbon content and urease activity at surface layers of the moderately degraded site.

## **pH**

pH of the soil in the study sites ranged from 4.4 to 7.0 for all study sites at both soil depths (Fig. 3.5). Maximum pH (7.02) was recorded from the surface layer of undegraded site during the month of February 2000 while the minimum (pH 4.4) was recorded from the degraded site in the month of July 1998. Soil pH varied significantly ( $P < 0.05$ ) among the three study sites and between the two soil depths (Tables 3.1 and 3.2). There was a clear pattern in the fluctuations of soil pH corresponding with seasonal changes as supported by significant LSD values (Fig. 3.5). The pH value remained higher at surface soil layer than at subsurface soil layer in all the study sites.

## **Organic C**

Temporal and depth-wise distributions of organic C content in soils of different study sites are depicted in figure 3.6. Generally, surface soil layer contained higher soil organic C in degraded, moderately degraded and undegraded forest sites, which varied from a minimum of 0.55% in the subsurface layer of degraded site during the month of July 1998 to a maximum of 3.84% recorded from the surface layer of undegraded site during the month of February 2000. The moderately degraded site contained organic C content ranging in between the degraded and undegraded sites at both surface and subsurface soil layers. Organic C content declined from highest levels in the dry winter periods to the lowest in the wet rainy periods in all the study sites.

The variation of organic C content among the three study sites were significant ( $P < 0.05$ ) at both soil depths. The calculated values of the LSD also revealed significant ( $P < 0.05$ ) monthly variation of organic C contents of degraded, moderately degraded and undegraded forest sites (Fig. 3.6).

## **Total N**

Distribution of total nitrogen in soil of all the study sites is depicted in figure 3.7. Total N content ranged from 0.018% to 0.82% in all the sites at both surface and subsurface soil layers. Maximum total N was recorded from the surface layer of undegraded site while the minimum was noted from the subsurface layer of degraded site. Two peaks of total N were noted during the entire period of two years, the highest peak appeared in the month of February 1999 followed by another in September 1999 in the three

sites at both the soil layers. In general, the surface soil layer contained higher total N than the subsurface layer in the three sites.

The monthly variation of the total N in degraded, moderately degraded and undegraded sites were significant (LSD,  $P < 0.05$ ) at both soil layers (Fig. 3.7). The variation of total N among the study sites were significant at surface layers (Table 3.1). However, it was significant only in between degraded and undegraded sites at subsurface layer. No significant variation in total N was observed between the soil layers of degraded site though it varied significantly in moderately degraded and undegraded sites.

#### **Ammonium-N**

Ammonium-N contents of soil in the degraded, moderately degraded and undegraded forest sites varied from  $2 \mu\text{g } 100 \text{ g}^{-1}$  dry soil to  $68 \mu\text{g } 100 \text{ g}^{-1}$  dry soil (Fig. 3.8). Maximum ammonium-N content was recorded from the undegraded site at surface layer in the month of June 2000 and the minimum was recorded from the degraded site at subsurface layer in the month of April 1999. Generally, surface soil layer contained higher ammonium-N content than subsurface soil layers of all study sites. There was marked variation in the distribution of ammonium-N among the study sites as supported by significant LSD values (Fig. 3.8). Similarly, distribution of ammonium-N varied significantly among the three study sites and at both soil depths (Table 3.1.). However, the ammonium-N distribution varied significantly between surface and subsurface soil layers of degraded and undegraded study sites only (Table 3.2.).

### **Nitrate-N**

Nitrate-N contents of soil in all study sites ranged from minimum of 14  $\mu\text{g } 100 \text{ g}^{-1}$  dry soil at subsurface layer of degraded site in the month of November 1999 to the maximum of 513  $\mu\text{g } 100 \text{ g}^{-1}$  dry soil at surface layer of undegraded site in the month of May 2000 (Fig. 3.9). The surface soil layer contained higher nitrate-N content than subsurface soil layers of all study sites in all the sampling months. Nitrate-N content varied significantly ( $P < 0.05$ ) among study sites at surface layer. However, no significant variation was observed from the subsurface soil layers of all study sites (Table 3.1.). There was a significant variation of the nitrate-N content between surface and subsurface soil layers of all study sites (Table 3.2.).

### **Total P**

Total P content of the soil ranged from 0.035% to 0.09% in the three study sites and at both soil depths (Fig. 3.10). Highest total P content was recorded during October 1998. Lowest total P content was recorded in the rainy months (March to June 1999) from all study sites. There was no clear trend of seasonal variation in the total P content of soil in the three study sites and at both soil depths (Fig. 3.10) Total P content varied significantly between degraded and undegraded sites at both soil layers (Tables 3.1) though no significant variation was recorded in between the two soil layers of all sites (Table 3.2.).

### **3.5. DISCUSSION**

#### **3.5.1. Influence of shifting cultivation and selective logging on physical characteristics of soil**

Increased soil temperature in the degraded site than moderately degraded and undegraded sites could be due to the heating up of the soil surface by solar radiation heating in absence of tree canopy. On the contrary, the lower soil temperatures at surface and subsurface soil layers of undegraded site might be due to presence of abundant vegetal cover and thick layer of litter on the forest floor which protects direct heating of the soil surface due to insolation leading to lowering of soil temperature at the site. The variation in the soil temperature of the three study sites was positively related ( $P < 0.001$ ) to air temperature revealing a direct relationship between these two parameters (Tables 3.3 and 3.4). Higher soil temperature was recorded from all sites during summer rainy season (April to October) and further declined during the winter season (November to March) in the three study sites at both soil layers. Tiwari (1988) reported higher soil temperature at surface soil layers of pineapple stands during rainy season. This decreased with onset of winter season which is in correspondence to the present findings in which higher soil temperature was recorded from the surface soil layers. He has also reported close positive correlation between the soil temperature and the air temperature in the pineapple orchard soils of north-eastern India. Tiwari *et al.* (1992) have also reported significant ( $P < 0.05$ ) variation of the soil temperature with time and depth in pineapple orchard of different ages in north-eastern India. These reports support the

present findings of significant variation in soil temperatures among the three study sites with varying degree of disturbances and at two soil layers.

Soil moisture content did not vary significantly ( $P < 0.05$ ) among the three study sites at surface layer though it varied significantly at subsurface layer (Table 3.1.). The higher soil moisture content at the surface layer of the study sites was found positively related ( $P < 0.05$ ) with the monthly rainfall, air and soil temperatures of these sites and decrease in the rainfall caused to decline in soil moisture content at both the soil depths. Similar result of fluctuation in soil moisture content had been reported from pineapple orchard soil where the increase or decrease in soil moisture was related to the amount of rainfall (Tiwari, 1988). Maximum moisture content was recorded from the undegraded site at surface soil layer during the peak rainy month of June 2000. This could be due to the occurrence of higher rainfall during this month and more water retention capacity of the soil. Another reason could be due to presence of higher root biomass in the undegraded site. The lower moisture content in the degraded site was the result of quick runoff from the hill slopes and low water retention capacity of the soil in the absence of tree roots as a result of slash and burn cultivation practices in the past in this site.

Das (1980), Baruah (1983) and Dkhar (1983) have reported seasonal and depth-wise variation of soil temperature and moisture content in forest, rice field and maize field soils of north-eastern India. They have found close positive relationship of soil temperature with air temperature and soil moisture with rainfall in their study. Higher soil temperature was recorded

from the surface soil layer, which decreased with increase in soil depth. However, they have noted increase in moisture contents of soils with increase in soil depth during dry winter periods. The higher soil moisture on the surface soil layer of undegraded site in the present study revealed that the presence of a thick litter layer on the forest floor throughout the year might have reduced rapid evaporation of soil water thus increasing the water retention capacity of the soil in this site. However, absence of the thick litter layer could be the reason of rapid evaporation of soil water leading to decline in moisture content of the soil in the degraded site.

The bulk density of the study sites varied significantly ( $P < 0.05$ ) among sites and between soil depths though the variation was insignificant along seasonal gradients (Tables 3.1 and 3.2). The soil in the degraded site had comparatively higher bulk density than the moderately degraded and undegraded sites at both soil layers. This suggests that land abandonment following shifting cultivation practice causes soil compaction and hardening even after 5 years of forest regeneration period in the region. There was an increase in soil bulk density (<10%) in the degraded site in comparison to the undegraded forest as a result of slash and burn cultivation practices in the past. The decrease in soil aggregate stability and gravitational movement of fine soil particles might have caused increase in bulk density. Another reason for increased bulk density in the degraded site could be due to the presence of lower organic matter in comparison to the other two sites. Thompson and Troeh (1979) have also reported that soil bulk density usually increases when cultivation causes a loss in organic matter from soil. Breland

and Hansen (1996) reported the negative effects of soil compaction and subsequent increase in bulk density upto  $1.4 \text{ g cm}^{-3}$  causing a 18%, 8% and 1% reduction in net N mineralization, organic matter content and microbial biomass in comparison to undisturbed soil cores. Hajabbasi *et al.* (1997) reported a 20% increase in the bulk density of soil following deforestation of natural forests and its subsequent tillage practices in Iran. They have also suggested that the increased bulk density was the result of decreased organic matter content due to typically high temperature, humidity and rainfall in the region. In fact, the typically heavy rainfall in the humid tropical regions of north-eastern India, particularly in the Arunachal Pradesh might have contributed to gravitational movement of the finer soil particles from top soil and filling the pore spaces leading to increased bulk density and decreased porosity (Table 2.1).

The water holding capacity varied significantly ( $P < 0.001$ ) among the three study sites (Table 3.1.). The surface soil layer of the undegraded site contained higher water holding capacity than degraded and moderately degraded sites. In general, undegraded natural forest site had higher water holding capacity than the degraded and moderately degraded forest sites. This might be due to the presence of higher organic matter resulting from huge amount of litter fall on the forest floor throughout the year in the undegraded site. Yadav and Badolka (1973) also reported presence of higher water holding capacity of deodar forest soil as compared to other soils in Uttar Pradesh. Similar results of higher water holding capacity have been reported from the old aged forest re-growths of north-eastern India

(Arunachalam *et al.*, 1996; Maithani *et al.*, 1996 and 1998). They further noted that presence of high amount of organic carbon and water holding capacity in the older forest stands in comparison to the younger forest stands.

### **3.5.2. Influence of shifting cultivation and selective logging on chemical characteristics of soil**

Soil pH of the study sites varied from strongly acidic to neutral reactions and was influenced markedly by seasonal climatic changes. The minimum pH of 4.4 was recorded from the subsurface soil layer of degraded site in July 1998 and maximum (pH 7) was recorded from the surface layer of undegraded site in the month of July 1999. There was significant ( $P>0.05$ ) variation of the pH in between the surface and subsurface soil layers. The subsurface soil layers contained more acidic reactions in comparison to the surface soil layers of degraded, moderately degraded and undegraded forest sites revealing increasing trend of the acidity of soil with increase in soil depth. Thompson and Troeh (1979) have reported commonly acid conditions of soil in the humid climate which favour higher leaching and subsequent increase in soil acidity. A linear decrease in the soil up to pH 4 was reported after forest clearing which resulted in inhibition of plant growth in a Nigerian soil even after 13 years of fallow period (Juo *et al.*, 1995). These findings are in correspondence with the results of the present investigation where highly acidic condition was recorded from the degraded site in comparison to the undisturbed natural forest. The degraded site had been used for cultivation of crops more than 13 years and presently lying as fallow land (5 years old). Nayak and Srivastava (1995) have also reported predominantly

acidic condition of the soils of shifting cultivated lands of Arunachal Pradesh, where increase in acidity occurred with increase in soil depth. They concluded that the reason for acidic condition being due to intense leaching of bases and presence of exchangeable  $Al^{+3}$ . A recent study also reported base leaching due to higher rainfall and presence of organic acid's influence towards acidic reaction of soils at low and high hill slopes of Arunachal Pradesh (Bhattacharya *et al.*, 1998). However, this is in contrast to the higher value of soil pH recorded from the jhum fallows in hill forest in comparison to bamboo and natural forests of north-eastern India (Singh *et al.*, 1995).

The surface soil layer of the undegraded forest soil was found to contain higher organic carbon content as compared to degraded and undegraded forests sites. The degraded site contained lowest organic carbon content at both the soil layers. Singh (1980) had reported higher value of organic carbon from the surface soil layer of forests which declined with increase in depth of soil. There was significant ( $P>0.05$ ) variation of the organic carbon distribution among study sites and between the soil layers. Higher organic carbon content was recorded from the study sites during winter (September to March) dry periods and lower in the summer rainy season (April to July) in the three sites at both the soil depths. The higher organic carbon recorded during the winter months could be due to addition of organic matter in to the soil after decomposition of litters present throughout the year on the forest floor of undegraded site. Singh (1980), Deka (1981), Tiwari *et al.* (1987) and Tiwari (1988) have also reported presence of

higher organic carbon contents during spring seasons respectively from *Shorea robusta* forest, jhum fallows and pineapple orchard soils of north-eastern India. They have noted lower organic carbon in these soils during the winter months and an increased in summer rainy seasons which is in contrast to the present findings of higher organic carbon during the winter dry periods from degraded, moderately degraded and undegraded sites. The highest organic carbon content in the undegraded site could be due to the rapid accumulation of organic matter and high mass of varied composition of litters protecting the soil surface throughout the year and promoting increase in soil organic matter (Brown *et al.*, 1994 and Singh, 1995). On the other hand, the lowest value of organic carbon content in the degraded site might be due to the removal of vegetation during shifting cultivation which reduces accumulation of litter fall on the soil surface and ultimate decline in soil organic matter. Brown *et al.* (1994); Henrot and Robertson (1994) and Saikh *et al.* (1998) have also reported significant reduction of soil organic matter and organic carbon following conversion of tropical forests into pastures, agricultural fields, tillage practices, logging, and shifting cultivation practices. In fact, deforestation of tropical forests and subsequent cultivation practices have resulted in statistically significant reduction in organic carbon (Saikh *et al.*, 1998) which even reaches up to a 50% reduction in organic matter content and total N in comparison to undisturbed natural forests sites (Hajabbasi *et al.*, 1997, Saikh *et al.*, 1998). The degraded site had been used for shifting cultivation practices for 13 years and the available organic matter and nutrients would have depleted during the entire period of

cultivation and the declined in soil fertility and subsequently lower crop productivity led to abandonment of the site as fallow land. Brand and Pfund (1998) also reported that the nutrient depletion is an important limiting factor for agricultural sustainability in shifting cultivation systems. They also noted a net loss of 20-22% soil fixed C and N after burning practices indicating an urgent need for improvement of current unsustainable agriculture systems, such as slash-and-burn agriculture, with regard to nutrient restoration status and biodiversity conservation.

The percent total N content of the soils in the degraded, moderately degraded and undegraded forest sites remain higher at surface soil layers in comparison to subsurface layer in all the sampling months. There was a marked variation in the distribution of total N in all sites with the changes in seasons. The highest total N was recorded from the surface soil of undegraded site in the months of February 1999 and 2000 and September 1999. The lowest total N was recorded from the degraded site at both soil layers. There was a reduction of total N of more than 47% in the degraded site in comparison to the undegraded site. Singh (1980) and Deka (1981) have also reported lower values of total N contents during dry winter periods which is in correspondence to the present findings of higher total N in the winter periods in all three study sites.

Agbenin and Goladi (1997) reported 38% C and 41% and 37% total N and nitrate-N reduction in soils as a result of continuous cultivation for long time without proper nutrient input in comparison to the native site which had significantly higher C, N and P. The lower N content of soils in all the sites

during the rainy season could be due to faster decomposition rate of organic matter as a result of higher temperature and rapid surface runoff (Ramakrishnan and Toky, 1981) leading to decline in nutrient concentration of the cultivated soils. Another reason for the rapid loss of the total N from the degraded site may be due to heavy rainfall occurred in the sites causing nutrient runoff from the hill slopes.

The ammonium-N ( $\text{NH}_4^+$ -N) content of the study sites was significantly influenced by seasonal changes in the three sites. The minimum ammonium-N was recorded from the study sites in winter dry periods, which increased with the onset of spring and highest was recorded in rainy season. The positive correlation between the ammonium-N and the microbial biomass N reveals that large amount of the available N is immobilized in the microbial biomass in the spring season. There was significant ( $P < 0.05$ ) variation of the ammonium-N among the study sites at both soil layers.

Nitrate-N ( $\text{NO}_3^-$ -N) content was significantly influenced by the seasonal climates of the study sites at surface and subsurface soil layers. The surface soil layer of the study sites contained higher nitrate-N than the subsurface layers. There was positive correlation ( $P < 0.05$ ) between nitrate-N and soil moisture in all sites whereas microbial biomass N was related to the nitrate-N in all the sites at both soil layers.

There was no definite pattern of total P distribution in all the three sites and at two soil layers with the changes in seasonal climate. The variation of total P was significant ( $P < 0.05$ ) between degraded sites and undegraded sites at both the surface and subsurface soil layers (Tables 3.1

and 3.2). The surface layers contained higher total P than subsurface layer in all sites. However, no significant variation in total P distribution was observed between surface and subsurface layers in all sites. The positive correlation of the total P with the organic C in the degraded site at surface soil layer reveals that total P is present as an integral part of the organic matter.

Tiwari (1988) and Tiwari *et al.* (1992) reported significant reduction in organic C, total N and P contents in younger pineapple plantations in comparison to older plantations. They also noted that higher value of these nutrients from the surface soil layers of older plantations than younger ones. They concluded that the higher nutrient concentration of surface soil layer was due to presence of higher organic matter in the surface soil layer which is continuously enriched by nutrients released from the decomposition of the litters.

Tiessen (1992) reported a significant reduction of soil C, N and P by 30% when shifting cultivation was practiced for 6 consecutive years in north-eastern Brazil. Tiwari *et al.* (1992) found marked seasonal variation in P contents of pineapple orchard soils with higher P content at surface soil layers during spring season due to rapid release of nutrients and due to higher concentration of organic matter and higher microbial activity. However, insignificant seasonal variation of total P distribution in the regenerating jhum fallows of different ages in north-eastern India (Arunachalam *et al.* 1998) and between the cultivated field, grassland and natural forests of Simlipal National Park in India (Saikh *et al.*, 1998). They

have concluded that the nature of vegetation has no significant influence in total P distribution of soils. Moreover, it was found that organic substances in the soil are not the primary P sources rather most of P is probably in the inorganic form even in forest soils. Saikh *et al.* (1998) reported lack of significant correlation between C and P levels in all types of soils i.e. cultivated field, natural grasslands and evergreen forests revealing existence of bulk of P in the organic form. There was neither total P nor available P forms decreased significantly on deforestation and cultivation. They further reported that the nature of vegetation also does not have any significant influence in the P contents in soil. Moreover, it appeared that organic substances are not the primary P sources in the soil i.e. most of the P is probably in the organic form even in forest soils.

## **Chapter 4**

### **4.1. Soil respiration, bacterial and fungal populations in degraded and undegraded forest sites**

#### **4.1.1. INTRODUCTION**

Microbial communities, particularly bacteria and micro-fungi constitute an essential component of biological characteristics in soil ecosystems. The important functional attributes of soil microorganisms include population number, biomass, respiratory efficiency and diversity patterns. These microorganisms are responsible for breakdown of organic matter and nutrient transformations (Tiwari, 1988), influence plant growth and are considered crucial to the functioning of soil ecosystems. Apart from their indispensable role in biogeochemical cycling of nutrients they are reported to be useful indicators of fertility and quality of soil since they are sensitive to changes occurring in the soil environment. In fact, soil microorganisms play very important role in soil fertility not only because of their ability to carry out biochemical transformations but also due to their importance as a source and sink for mineral nutrients (Jenkinson and Ladd, 1981).

Soils are rarely manipulated for microbial growth. However, if a soil is altered to affect plant growth, which is the essence of agriculture, these manipulations affect the distribution and types of microbial populations. Thus, the microorganisms in turn affect plant growth (Coyne, 1999). Generally, distribution of microorganisms in soil is influenced by physico-chemical characteristics of soil namely, nature of the particles of which the soil is composed i.e. nutrients, aeration, temperature, precipitation, organic

matter content, moisture, pH and depth (Deka, 1981; Baruah, 1983; Campbell, 1983; Behera and Mukerji 1985; Tiwari, M.B. *et al.*, 1986; Tiwari, S.C. *et al.*, 1987ab; Tiwari, 1988; Arunachalam *et al.*, 1997; Berg *et al.*, 1998 and Coyne, 1999). Climatic regimes, seasonal variation of the year, type of vegetation, soil management practices and deforestation are other important factors which influence the distribution and diversity of microorganisms in soil (Kauri, 1982; Lundgren, 1982; Behera and Mukherji, 1985; Hassink, 1997; Tiwari, S.C. *et al.*, 1991; Jha *et al.*, 1992; Vazquez *et al.*, 1993; Bossio and Scow, 1995; Acea and Carballas, 1996; Maithani *et al.*, 1996; Maithani and Tripathi, 1996; Gorres *et al.*, 1998; Lupiwayi *et al.*, 1998 and Zeller *et al.*, 2001).

The natural forest soils are reported to harbour higher number of microbial populations and their activity. Being one of the most important components of soil environment, microorganisms exert considerable influence on soil fertility and plant growth (Tiwari *et al.* 1991). These microbial communities are inherently stable but at the same time are also dynamic structures (Campbell, 1983). The changes in microbial communities resulting from agricultural practices, ecosystem management and global climate change can have profound impacts on ecosystem dynamics (Bossio and Scow, 1995).

Generally, microbial populations and their activities in no tillage soils are greater than in conventional tillage soils. But environmental disturbances like clear-cutting of forests affect microbial populations and their activities. When a natural forest is clear-cut, the roots die and root decomposition

takes place and the debris from leaves and branches on the soil surface enters into the disturbed soil serving as food for microbial growth (Coyne, 1999). Thus there is an increase in microbial growth and their activity in the short term. However, clear cutting of forests and subsequent burning of dried slash for agricultural purposes, as in case of shifting cultivation practices, has a detrimental effect on these microorganisms and their activities in the long term. Soil disturbance activities such as shifting cultivation and others related to agriculture and forestry has considerable impact on biological health of soil systems. In fact any type of agricultural system either based on organic or inorganic nutrients (fertilizer) input that eliminates beneficial soil microflora are unlikely to be sustained in the long term.

Microbial activity is essential for release of plant nutrients from the organic and inorganic residues and without such release available nutrient supply would soon be used up and the soil becomes sterile. Since microbes complete life cycle in the soil, nutrients taken up by plants return again and the same nutrient can be used over and over (Thompson and Troeh, 1979)

#### **4.1.2. REVIEW OF LITERATURE**

##### **Soil respiration (CO<sub>2</sub> evolution)**

Soil respiration is the evolution of CO<sub>2</sub> from soil surface as a result of microbial and root respiration (Schlenter and Cleve, 1985; Wagai *et al.*, 1998) and its measurement can be utilized to assess relative productivity and fertility of soils (Upadhyaya *et al.*, 1997). Soil respiration is considered as one of the indices of microbial activity in soil (Anderson and Domsch,

1975; MacFadyen, 1970 and Tiwari, S. C. *et al.*, 1989ab) and biological activity of soils.

Upadhyaya *et al.* (1981) reported seasonal variation in soil respiration of different tropical grassland communities. They have found changes in soil respiration with change in soil temperature and rainfall. They noted higher respiration with high temperature and declined with decrease in temperature. They also noted highest soil respiration, which was obtained from the grassland communities in the month of July when the temperature was moderate. They further reported increase in soil respiration of the grassland communities with increase in root biomass.

Keeney *et al.* (1985) studied the effect of CO<sub>2</sub> concentration in soil nitrification and associated nitrous oxide production. They found increase in CO<sub>2</sub> evolution in arable soils containing high amount of nitrifiable-N, could result increased N<sub>2</sub>O production during nitrification.

Rout and Gupta (1989) observed higher rates of carbon dioxide evolution during rainy seasons and lower during winter and summer months in three sites namely, mixed deciduous forests, pine forests and scrub forest of Siwaliks in northern India. They concluded that high rate of soil respiration during rainy season could be attributed to rapid organic matter mineralization by microorganisms due to favourable soil water, litter moisture and moderate soil temperature.

Orchard and Cook. (1983) found that a higher soil nutrient availability increased the capacity of microorganisms to utilize higher carbon inputs from

litter fall resulting in increased rate of respiration. They have suggested that this was due to a change in microbial population.

Chang and Trofymow (1996) reported that clear cutting of old forest re-growths and consequent site preparation for land use practices may have a long lasting effect on soil respiration and may contribute greatly to the green house effect.

Upadhyaya *et al.* (1997) studied soil respiration in disturbed forest ecosystems of central Himalayan and found a significant positive correlation between organic carbon content of the soil and soil respiration reflecting lower organic matter content in the damaged sites and higher at undamaged sites.

Keith *et al.* (1997) reported that seasonal pattern of CO<sub>2</sub> efflux generally follows that of soil temperatures as reported from many temperate forests. They also noted that a change in moisture content had a greater effect at higher temperature and vice-versa. However, temperature had the greatest effect on CO<sub>2</sub> efflux, but the response at higher temperatures was reduced by moisture limitation and in particular caused large fluctuations in soil respiration. They further reported that the nutrient availability in soil and nutritional quality of litter influence microbial utilization efficiency of organic compounds. The changes in nutrient availability in soil can also cause changes in the composition of microbial population, and thus alter soil CO<sub>2</sub> efflux.

Santruckova and Smith (1997) studied the effect of soil CO<sub>2</sub> evolution on microbial biomass and found a close positive correlation where

decreased in CO<sub>2</sub> in the soil was followed by decrease in microbial biomass. They also noted that increased in CO<sub>2</sub> concentration of soil air caused temporary suppression in soil respiration as well as decrease in microbial biomass.

Lomoander *et al.* (1998) observed significant increase in soil CO<sub>2</sub> evolution with increasing temperature and moisture. They also found reduction of the CO<sub>2</sub> evolution rate to half in subsoil as compare to topsoil.

CO<sub>2</sub> evolution from the soil surface arises from at least three sources; microbial, microfaunal and root respiration of this microbial respiration has been reported to be the highest and most quantifiable component of the overall soil respiration (Schlenter and Cleve, 1985 and Kelting *et al.*, 1998). However, micro-environment variables such as soil temperature, rainfall, season, moisture content, microbial diversity and biomass, and fine root biomass together control soil surface CO<sub>2</sub> flux as a measure of microbial activity.

Soil surface CO<sub>2</sub> flux is a major transfer of carbon from terrestrial ecosystems to the atmosphere and land use practices, vegetation type, organic matter content, pool fractions, fine root biomass and biodiversity in both plant and microbial communities significantly affect CO<sub>2</sub> flux (Wagai *et al.*, 1998).

Piao *et al.* (2000) reported that when the air temperature is low, soil microbial biomass was highest and soluble organic C in the soil declines, resulting in the lowest rate of CO<sub>2</sub> flux from the soil surface of Karst areas in China. They further noted that at higher air temperature, soil microbial

biomass is lower, soluble organic C tends to increase and soil CO<sub>2</sub> efflux is highest.

### **Microbial populations (Bacterial and fungal populations)**

Soil disturbance and management practices such as clear-cutting of forests and tillage systems are reported to have a profound impact on the distribution and activities of soil microorganisms. Lundgren (1982) reported a strong detrimental effect of clear-cutting of forest on the bacterial population of A<sub>0</sub> horizon. He observed a pronounced increase in bacterial biomass at the clear-cut area as compared to the natural pine stand. However, there was rapid decrease in bacterial biomass in comparison to a reference stand and the results from an old clear-cut area (ca. 10 year) indicated long lasting detrimental effects on the size of the bacterial population.

The main determinant of microbial distribution on the small scale is the nature of the particles of which the soil is composed i.e. nutrients. They are also sensitive to changes in the levels of temperature, light, pH, organic and inorganic nutrients, carbon dioxide, oxygen, water etc. and extreme levels may well have more effect on microorganisms than they do on higher plants and animals which are in some way more isolated from the environment (Campbell, 1983).

Tiwari *et al.* (1987ab) have reported wide differences on distribution of microbial populations, their activities and biochemical transformations in soils of various moisture regimes. They found significant variation in fungal population with different moisture contents. They concluded that soil

moisture status not only regulates the population and activity of microbes but also modifies the relationship between various parameters.

Tiwari *et al.* (1991) have reported maximum occurrence of fungal population during spring-summer season while minimum was recorded during winter season at three soil depths of pineapple orchard plantations in north-eastern India. They also observed higher fungal population at 10-20 cm layer than 0-10 cm and 20-30 cm layers of the pineapple plantations.

Hassink, 1997 have reported that events that occur within a growing season, such as plant growth and draught, determine the dynamics of microbial populations, rather than historical or present agricultural precautions. There was considerable change in characteristics of the microbial population with time. They noted decreased diversity of the bacterial and fungal populations in July and August, the decrease occurring in this rhizosphere of the conventional fields than in reduced input systems. However, they concluded that seasonal variations and farming systems on the composition of the microbial populations appeared to be very small as compared to the quantitative effects.

Bloem *et al.* (1992) have reported increased microbial number and activity in integrated (reduced input farming) than conventional management (high input farming) systems in Netherlands.

Jha *et al.* (1992) have studied the microbial population and enzyme activities in relation to altitude and forest degradation in north-east India. They recorded higher fungal and bacterial population numbers in the less degraded forests than the more degraded one at both higher and lower

altitudes. Further they found greater enzyme activity of less degraded forest soils in comparison to more degraded forest stands.

Vazquez *et al.* (1993) have reported increased soil microbial populations by burning in the short-term but adversely affected the population size of different groups. They have observed more bacterial numbers than fungi following burning revealing the ability of the soil bacteria to survive soil heating better than fungi. They also observed that the soil compaction following the burning was another factor for suppression of aerobic bacterial population.

Interest in quantifying impacts on the biotic component of the soil ecosystem has increased with concern for the sustainability of our agricultural systems as agricultural and industrial pollutants have become more prevalent in the environment (Bossio and Scow, 1995).

Acea and Caraballas (1996) reported significant changes in the physiological groups of microorganisms as a result of forest wild fire in a pine forest stand in Spain. They observed decrease in cellulase producers while increase in amylase producers and ammonifying number in the short time. However, increasing population of cellulase producers was found after one year of wild fire and reduction in amylase producers and ammonifying microbes in field conditions.

Arunachalam *et al.* (1997) recorded maximum bacterial population during rainy season and minimum during the winter, while fungal population was maximum during autumn and minimum during winter in four forest re-growths (7, 10, 13 and 16 years old) in north-east India. There was increase

in microbial population with increasing age of the forest re-growths and also at top layer (0-10 cm).

The soil microorganisms live in complex communities and are responsible for the important mineralization reactions apart from mediating many processes that are essential to the agricultural productivity of soil (Bossio and Scow, 1995 and Lupiwayi *et al.*, 1998).

Dilly and Munch (1998) have pointed out that microorganisms in soil play a major role in ecosystem functioning though it has been suggested that many microbiological estimates do not necessarily reflect significant characteristics of microbiological populations or processes under field conditions.

Berg *et al.* (1998) have reported higher bacterial and fungal counts in litter and fragmented litter than the humus layer of Scots pine forests. They found that abundance of bacteria and fungi was influenced by water content and temperature.

Lupiwayi *et al.* (1998) and Sharma *et al.* (1998) have reported that microbial diversity of soil is important to sustainable agriculture because microbes mediate many processes that support agricultural productions and may even indicate disturbances or beneficial effects of amendments or management strategies. They have also noted reduction in microbial diversity in bulk soil during cropping. However, the reduction varied with growing season. They have concluded that it is probably safer to adopt agricultural practices that preserve or restore microbial diversity than to adopt practices that diminish this component of total diversity.

The population number, diversity and distribution of microorganisms reflects overall soil productivity (Coyne, 1999). It was also reported that the effects of clear-cutting of forests on the population and activities of soil organisms revealing a short term increase in soil respiration and enhanced release of nutrients such as nitrogen that are found in organic matter. Unfortunately this can also lead to nitrogen loss in streams because for a brief period, there can be no roots around to take up the nutrients that are being released by microorganisms. It has been reported that microbial population and their activities in no tillage soils are greater than in conventional tillage soils. This may be because no-tillage soils are generally more moist than conventionally tillage soils and have more organic C (Coyne, 1999).

Acea and Caraballas (1999) reported a complete sterilization of soil microorganisms upto the top few centimeters following soil heating upto 200<sup>0</sup>C. The recovery of natural microbial population was absent even after incubation period, however the organic residue application of wheat straw and poultry manure could neither improve the proliferation nor change the order of the size of various sub groups added following burning.

#### **4.1.3. METHODOLOGY**

##### **Soil respiration**

Microbial soil respiration (CO<sub>2</sub> evolution) was determined by alkali absorption method of Macfadyen (1970). The CO<sub>2</sub> released was collected in a 100 ml glass beaker containing 20 ml of 0.1N KOH solution after incubating 1 kg field moist soil (2 mm sieved) in a rectangular glass jar for 24

h at room temperature ( $25 \pm 2^\circ\text{C}$ ) under dark condition. The saturated KOH solution was titrated against 0.1N HCl solution after adding a few drops of 1% phenolphthalein indicator solution.

$$\text{Soil respiration (mg CO}_2\text{-C g}^{-1}\text{ dry soil 24 h}^{-1}\text{)} = \frac{T \times 2.2}{W}$$

{Where, T= Volume of HCl used against saturated KOH solution (ml) - Volume of HCl used against blank KOH solution (ml); 2.2= mg CO<sub>2</sub> equivalent to 1 ml 0.1M HCl (Anderson and Ingram, 1993); W= Weight of oven dry soil}

### **Microbial populations (Bacteria and fungi)**

Bacterial and fungal populations were enumerated following the serial dilution technique (Johnson and Curl, 1972). 10 g fresh soil was suspended in 95 ml sterile water and thoroughly shaken for 15 minutes in a horizontal shaker. 10 ml of the suspension was again diluted in 90 ml sterile water to get a dilution factor of  $10^{-2}$  g soil per ml suspension. Similarly,  $10^{-3}$ ,  $10^{-4}$  and  $10^{-5}$  diluted suspensions were prepared for bacterial and fungal populations.

A  $10^{-5}$  dilution was prepared to enumerate the bacterial colony forming units. Half a milliliter of the suspension was inoculated into sterile Petri dishes containing 15 ml solidified nutrient agar medium. The dishes were then rotated gently in a whirling motion to disperse the inoculum uniformly over the surface of the medium. The inoculated dishes were incubated at  $37 \pm 2^\circ\text{C}$  for 24 h.

$$\text{Bacterial population (CFU x g}^{-1}\text{ dry soil)} = \frac{\text{No. of colony forming units (CFU)}}{W \times 10^{-5}}$$

(Where, W= Dry weight of soil)

Fungal population was determined by inoculating diluted soil suspension on rosebengal agar medium (Martin, 1950). 1 ml of  $10^{-3}$  soil

suspension was inoculated in sterilized Petri dishes and gently rotated to disperse the soil aggregates uniformly on the bottom of Petri dishes. Then approximately 15 ml of molten and cooled (below 45°C) rose-bengal agar supplemented with streptomycin sulfate (30 mg per liter), was poured into each Petri dish. The dishes (three replicate) were gently rotated and solidified. The Petri dishes were incubated at a temperature of 25±2°C for 4-5 days.

$$\text{Fungal population (CFU x g}^{-1}\text{ dry soil)} = \frac{\text{No. of colony forming units (CFU)}}{W \times 10^{-3}}$$

#### **4.1.4. RESULTS**

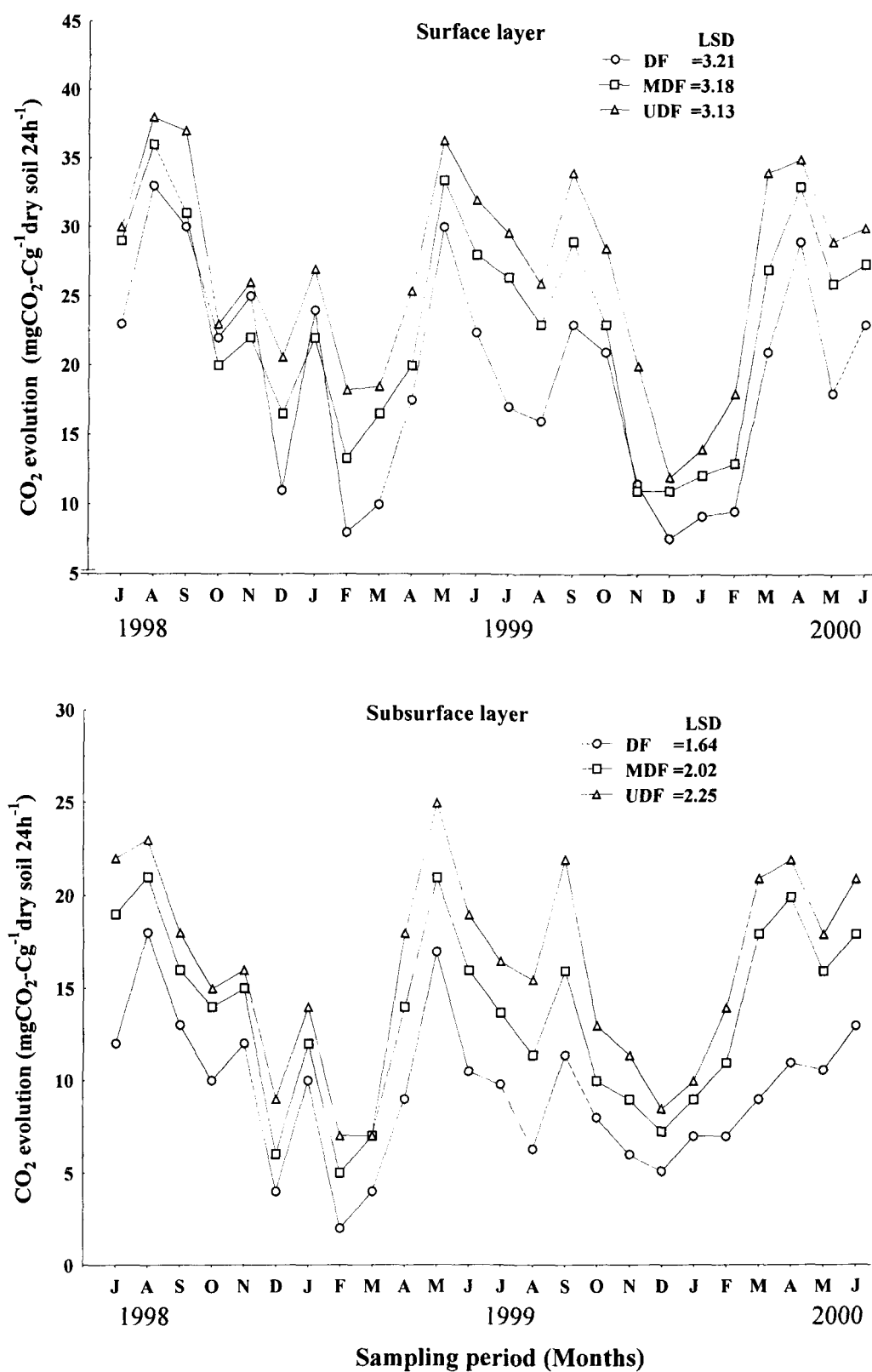
##### **CO<sub>2</sub> evolution**

The CO<sub>2</sub> evolution (soil respiration) of the degraded, moderately degraded and undegraded forest sites showed a marked seasonal variation at both the surface and subsurface soil layers. Four peaks of soil respiration were recorded during the entire period of two years. The highest peak of soil respiration was recorded in the month of August 1998 followed by May 1999, April 2000 and September 1999 respectively (Fig. 4.1.1). The maximum rate of soil respiration (38mg CO<sub>2</sub>-C g<sup>-1</sup> dry soil 24 h<sup>-1</sup>) was recorded from the undegraded site in the month of August 1998 while the minimum was recorded from the degraded site (7.6mg CO<sub>2</sub>-C g<sup>-1</sup> dry soil 24 h<sup>-1</sup>) at surface soil layer in the month of December 1999. Similar seasonal trend of soil respiration was observed at the subsurface layer of the study sites with four peaks of soil respiration. In the subsurface layer also, the maximum soil respiration was recorded from the undegraded site (24 mg CO<sub>2</sub>-C g<sup>-1</sup> dry

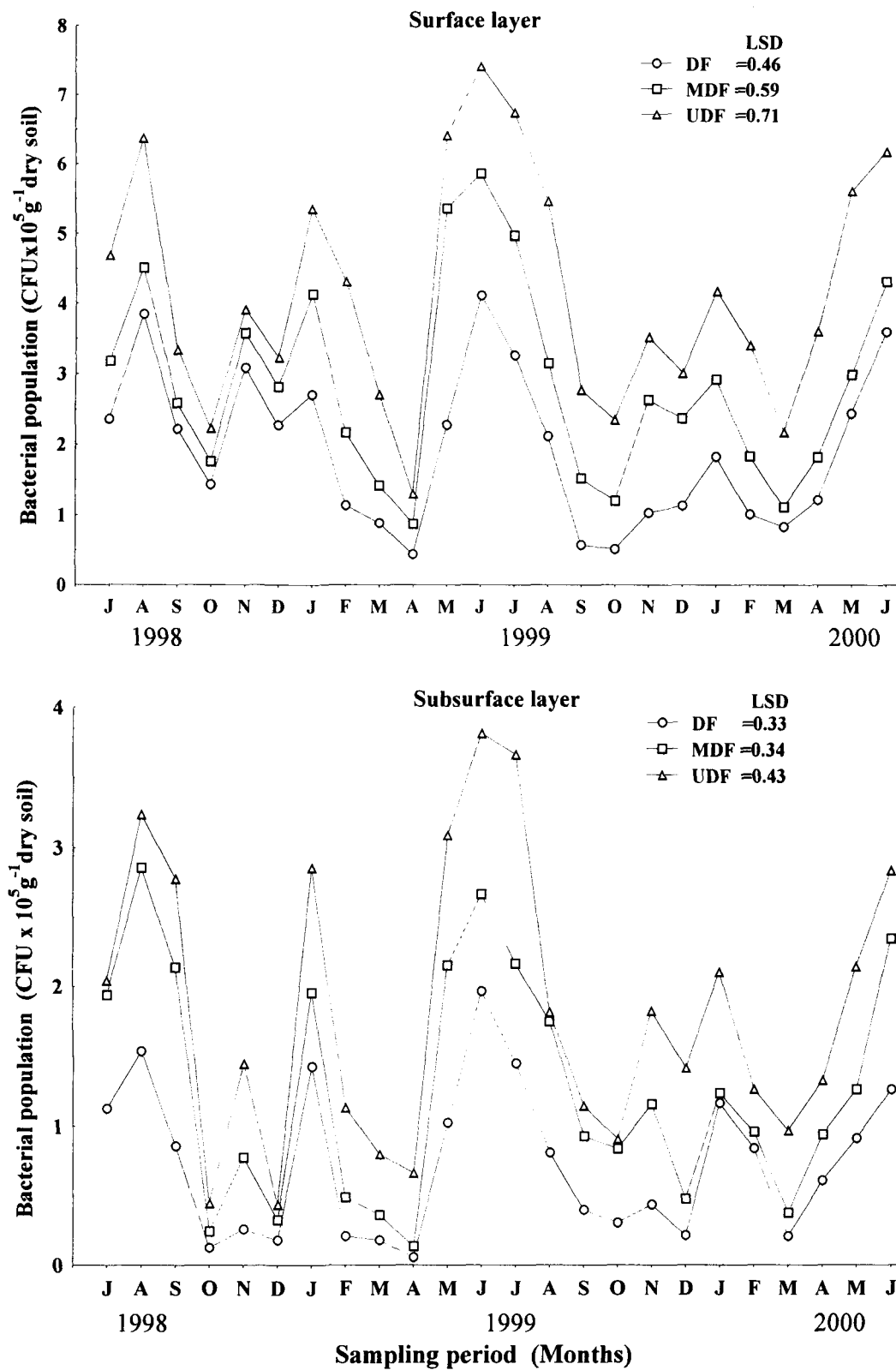
soil  $24 \text{ h}^{-1}$ ) in the month of May 1999 while the minimum was recorded from degraded site ( $2 \text{ mg CO}_2\text{-C g}^{-1} \text{ dry soil } 24 \text{ h}^{-1}$ ) in the month of February 1999.

Least significance difference value of the monthly soil respiration revealed significant ( $P < 0.05$ ) variations at surface and subsurface soil layers of degraded, moderately degraded and undegraded forest sites (Fig. 4.1.1). Soil respiration also varied significantly ( $P < 0.05$ ) among the three study sites at surface and subsurface layers (Table 4.1.1). However, the variation in soil respiration between degraded and moderately degraded sites was significant at subsurface layer only whereas the variation was insignificant between moderately degraded and undegraded forest sites at surface and subsurface subsurface layer only whereas the variation was insignificant between moderately degraded and undegraded forest sites at surface and subsurface layers. The variation in the rate of soil respiration was also significant ( $P < 0.05$ ) between surface and subsurface soil layers of degraded, moderately degraded and undegraded forest sites (Table 4.1.2).

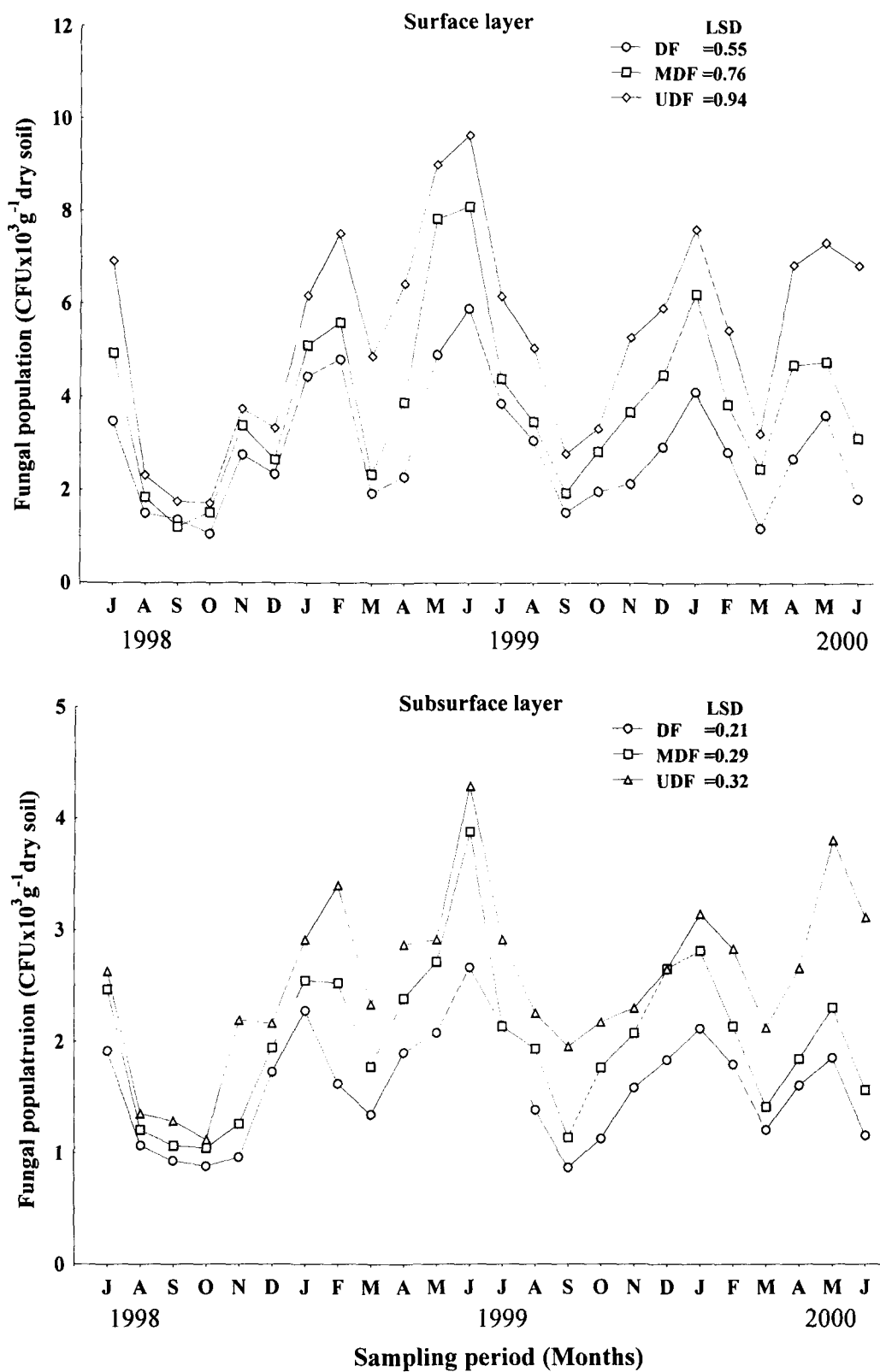
Soil respiration was found positively correlated to air temperature, soil temperature, moisture and rainfall in all the three sites at two soil depths (Tables 3.3 and 3.4). Soil respiration was also correlated positively with bacterial population at surface and subsurface layers of degraded, moderately degraded and undegraded sites. However, it was also correlated positively with the microbial biomass C ( $C_{\text{mic}}$ ) and microbial biomass N ( $N_{\text{mic}}$ ) at subsurface layer of moderately degraded and undegraded sites. Generally, the surface layer contained higher soil respiration than subsurface soil layer at all the three study sites throughout the sampling periods.



**Fig.4.1.1. Soil respiration ( $\text{CO}_2$  evolution) in degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites at surface and subsurface soil layers. (LSD,  $P < 0.05$ )**



**Fig.4.1.2. Bacterial population in soils of degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites at surface and subsurface soil layers. (LSD,  $P < 0.05$ )**



**Fig.4.1.3. Fungal population in soils of degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites at surface and subsurface layers. (LSD,  $P < 0.05$ )**

**Table 4.1.1. One-way analysis of variance (ANOVA) of the biological characteristics of soil in degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites at surface and subsurface soil layers ( $P < 0.05$ )**

Soil properties	Source of variation	Surface layer		Subsurface layer	
		F-ratio	P-level	F-ratio	P-level
Soil respiration	DFxMDFxUDF	6.923	0.002	9.673	$1.91 \times 10^{-4}$
	DFxMDF	-	-	10.199	0.003
	MDF x UDF	-	-	-	-
	DF x UDF	13.391	$6.49 \times 10^{-4}$	18.367	$9.21 \times 10^{-5}$
Bacterial population	DFxMDFxUDF	18.326	$4.14 \times 10^{-7}$	9.251	$2.28 \times 10^{-4}$
	DFxMDF	6.982	0.011	6.568	0.014
	MDF x UDF	12.135	0.001	-	-
	DF x UDF	31.612	$1.20 \times 10^{-6}$	18.129	$1.01 \times 10^{-4}$
Fungal population	DFxMDFxUDF	11.785	$3.95 \times 10^{-5}$	8.448	$5.23 \times 10^{-4}$
	DFxMDF	5.202	0.027	-	-
	MDF x UDF	6.539	0.014	4.299	0.044
	DF x UDF	23.187	$1.62 \times 10^{-5}$	17.399	$1.33 \times 10^{-4}$

Note: Insignificant values are denoted by “-” sign

**Table 4.1.2. One-way analysis of variance (ANOVA) of the biological characteristics of soil between surface and subsurface soil layers of degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites ( $P < 0.05$ ).**

Soil properties	Study Sites	F-ratio	P-level
Soil respiration	DF	23.326	$1.553 \times 10^{-5}$
	MDF	25.117	$8.451 \times 10^{-6}$
	UDF	36.317	$1.989 \times 10^{-7}$
Bacterial population	DF	22.942	$1.773 \times 10^{-5}$
	MDF	24.411	$1.072 \times 10^{-5}$
	UDF	33.263	$6.454 \times 10^{-5}$
Fungal population	DF	18.342	$9.297 \times 10^{-5}$
	MDF	21.412	$3.034 \times 10^{-5}$
	UDF	33.844	$5.430 \times 10^{-7}$

### **Bacterial population**

Monthly variation in bacterial population in all the three study sites followed the pattern of seasonal climatic changes at two soil depths (Fig. 4.1.2). Highest bacterial population peak was recorded in June 1999, followed by another in August 1998 in all sites and at two soil depths. The maximum bacterial population was recorded from undegraded site ( $7.4 \text{ CFU} \times 10^5 \text{ g}^{-1}$  dry soil) followed by moderately degraded site ( $5.9 \times \text{CFU} \times 10^5 \text{ g}^{-1}$  dry soil) while minimum was recorded from degraded site ( $0.64 \text{ CFU} \times 10^5 \text{ g}^{-1}$  dry soil) at surface layers. Similar seasonal fluctuation of bacterial population was recorded at subsurface soil layers of degraded, moderately degraded and undegraded forest sites. The variation of bacterial population number among the study sites and between the surface and subsurface layers were statistically significant ( $P < 0.05$ ; Tables 4.1.1. and 4.1.2).

Bacterial population was positively correlated to soil moisture and rainfall at surface and surface layers of degraded and moderately degraded soils (Tables 3.3). Bacterial population was also related to microbial biomass C in moderately degraded and undegraded sites at both soil layers. However, bacterial population was positively related to microbial biomass at surface soil layer only (Tables 3.3 and 3.4).

### **Fungal population**

The fungal population was influenced by seasonal climatic changes in the degraded, moderately degraded and undegraded sites at surface and subsurface soil layers (Fig. 4.1.3). The maximum fungal population was recorded in the month of June 1999 from the undegraded forest site (9.648

CFU x 10<sup>3</sup> g<sup>-1</sup> dry soil), followed by moderately degraded site (8.10 CFU x 10<sup>3</sup> g<sup>-1</sup> dry soil) while the minimum was recorded from degraded site (1.1 CFU x 10<sup>3</sup> g<sup>-1</sup> dry soil) at the surface layer in the month of October 1998. The subsurface soil layer also showed similar pattern with highest fungal population recorded in the month of June 1999 from degraded, moderately degraded and undegraded sites respectively. The lowest fungal population was (0.9 CFU x 10<sup>3</sup> g<sup>-1</sup> dry soil) recorded from the degraded forest site in the months of October 1998 and September 1999 respectively. The fungal population was highest in the undegraded site followed by moderately degraded site while the lowest was recorded in degraded site at surface and subsurface layers. The surface soil layer harbored higher fungal population than subsurface soil layer in all the three study sites.

The calculated values of least significance difference of fungal population showed significant ( $P < 0.05$ ) variation at both surface and subsurface soil layers (Fig.4.1.3). The variation in distribution of fungal population was also statistically significant ( $P < 0.05$ ) among the three sites whereas the variation was significant for all sites except for between moderately degraded and undegraded sites at subsurface soil layers (Table 4.1.1). Similarly, the variation in mean monthly fungal population of degraded, moderately degraded and undegraded sites were significant between surface and subsurface soil layers (Table 4.1.2).

Fungal population was positively correlated to bacterial population at surface layers of degraded and moderately degraded sites. However, it was related to bacterial population at both soil layers of undegraded site (Table

4.1.3). Fungal population was also positively related to organic C at subsurface layers of degraded and moderately degraded sites while it was related to microbial biomass C at subsurface layer of undegraded site only

#### **4.1.5. DISCUSSION**

##### **4.1.5.1. Influence of soil degradation on soil respiration**

Soil respiration (CO<sub>2</sub> evolution) was highest in the undegraded site followed by moderately degraded and degraded sites. The reason for higher soil respiration in the undegraded site could be due to presence of comparatively high organic matter and microbial population numbers on the forest floor throughout the year. Four peaks of soil respiration were recorded during the rainy and warmer periods of the year. Soil temperature and moisture content was found to be positively correlated ( $P < 0.001$ ) with soil respiration in all the sites at both surface and subsurface soil layers. Tiwari (1996b) has also reported highest soil respiration in the forest soils in comparison to grassland, garden, orchard, fallow and arable soils of north-eastern hill regions of India. However, Tiwari *et al.* (1989b) have observed maximum soil respiration in surface layer (0-10 cm) which decreased with increasing soil depth. The soil respiration was also higher at the surface soil layer of all study sites which declined with the increase in soil depth.

The change in soil respiration was significantly influenced by seasonal changes in the climate. The soil respiration was significantly influenced by seasonal climatic change as the highest peaks were observed during the period when the seasonal rainfall has started in April/ May and ends in August/ September. This reveals that onset of the rainfall during spring

months (April and May) increased the microbial soil respiration followed by decline in the month of June and July when heavy rainfall occurred. After that the soil respiration again increased with the decrease in rainfall in August and September followed by sharp decline in the month of dry period from October to March. These results suggest the inhibiting effect of excessive or absence of rainfall on soil respiratory activity of microorganisms in soils of hill region.

Upadhyaya *et al.* (1987) reported marked seasonal variation of soil respiration in the tropical grassland communities. They have noted higher soil respiratory rate with higher rainfall with moderate temperature than lower soil temperature and short rainfall period of winter month. Similar reports of higher soil respiration in summer rainy seasons and lower in winter dry periods have been reported from central Himalayan disturbed forest ecosystems. Lomoander *et al.* (1998) have also reported significant increase in the soil respiration with increase in soil temperature and moisture. Further, there was decrease in soil respiration upto half of the surface layer with the increase in soil depth.

Lower soil respiration rates in degraded and moderately degraded sites could be ascribed to the type of land use and its subsequent impacts. The degraded site was used for shifting cultivation for a long period would have caused reduction in nutrients and organic matter content leading to lower microbial populations and their activity. The frequent slash-and-burn practice in every cropping season could have destroyed the microbial population structure and their diversity, causing lower soil respiration in the

degraded site. On the other hand, frequent felling of selected trees and subsequent burning of litter and dry grass on the forest floor also disturbed the distribution and activity of microorganisms in the moderately degraded site. These activities must have caused considerable damage to the microbiological components of the soil leading to decline in activity and reduction of soil respiration in these sites.

Chang and Trofymow (1996) have revealed that clear-cutting of old forest re-growths and subsequent preparation for land use practice could have a long lasting effect on soil respiration and contribute greatly to green house gases. The occurrence of maximum soil surface CO<sub>2</sub> flux have been recorded during the summer in conventional till (chisel plow), no tillage corn agro-ecosystem and in native prairie of southern Wisconsin (Wagai *et al.*, 1998). They observed higher rate of soil respiration at surface by 2 fold greater than the deeper layer. More significant impact of soil temperature was observed in comparison to moisture content. Further they noted higher CO<sub>2</sub> flux in prairie soil than in other soil. They have revealed marked influence of vegetation type cover on the microenvironment, microbial biomass and fine root biomass which together controls soil surface CO<sub>2</sub> flux. Similar result was also reported by Mathes and Schriber (1985) with a pronounced positive impact of soil temperature on soil respiration than does by moisture in soils of secondary succession with or without additional management. There was no significant influence of temperature on soil respiration while soil respiration generally followed soil temperature patterns in both ecosystems and showed positive correlation with temperature. Their

study have also concluded that differences in carbon release between both plots must be due to microclimatic differences. Chang and Trofymow (1996) have reported a significantly greater basal soil respiration in old forest re-growths at both decaying litter and humus layers in comparison to the younger plantations. They also noted relationship of the changes in air temperature, precipitation and the soil moisture contents with the basal respiration at the time of respiration.

Keith *et al.* (1997) reported greater effect of soil temperature on the soil respiration in comparison to soil moisture in a *Eucalyptus* forest soil of Australia. They have recorded the greatest effect of temperature on soil CO<sub>2</sub> efflux but the response at higher temperature was reduced by moisture limitations and in particular caused large fluctuations. Piao *et al.* (2000) have found considerable variation of the flux of CO<sub>2</sub> from the soil surface within an annual cycle. A highly positive correlation of the CO<sub>2</sub> flux from soil surface to the average air temperature was also noted. Further they noted highest and lowest CO<sub>2</sub> fluxes in summer and winter respectively in four experimental plots of terraced fields in Guijone Province of China.

The above reports from different ecosystems on the pattern of soil respiration suggest that soil respiration is influenced by the climatic variables like temperature, rainfall, soil moisture and temperature which are further controlled by vegetation type cover and soil management practices. Therefore, it is concluded that the absence of the enough tree canopy and year round vegetal cover distribution caused wide fluctuations in the microclimatic variables and which further influences indirectly to the soil

properties. These factors altogether affect soil respiration in the degraded site and moderately degraded site. On the other hand, the highest soil respiration in the undegraded site could be ascribed to the controlled microclimatic variables and other soil properties which are favourable for the growth of soil microorganisms and their rapid multiplication, decomposition of litter and other activities. These altogether leads to higher soil respiration in the undegraded forest site.

#### **4.1.5.2. Influence of soil degradation on bacterial population of soils**

The bacterial population was markedly influenced by the changes in seasonal climatic regimes. The highest bacterial population was observed during the rainy season (May to August) in all the three sites and at surface and subsurface soil layers. The reason for higher bacterial population during the rainy season might be due to higher soil moisture content in the soils which influenced rapid multiplication of the bacterial cells. Tiwari, M.B. *et al.* (1986) and Tiwari, S.C. *et al.*, 1987ab) also observed higher bacterial population in the wet rainy season (April/ May ) in pineapple orchard soil of different ages in north-eastern India. They also noted decreased bacterial population during the dry winter periods and with increasing soil depth in the 1, 5 and 10 year old orchard stands. Their study concluded that the higher moisture content in the soil was the most important factor for the higher bacterial population in the respective season. Jha *et al.* (1992) have noted highest bacterial population in September and lowest in January. Arunachalam *et al.* (1997) observed maximum bacterial population during the rainy season and minimum during the winter in 7, 10, 13 and 16

year old forest re-growths of north-eastern India. Berg *et al.* (1998) also reported existence of a positive correlation between moisture content and bacterial population however, the abundance of bacteria was influenced by water content.

The decline in the bacterial population in the present study from degraded forest site in comparison to the moderately degraded and undegraded forest sites was significant ( $P < 0.05$ ). This is because of the presence of the evergreen forest cover with thick layer of leaf, twig and wood litter etc. on the forest floor protecting excessive evaporation of soil water, thus creating a favourable environment of rapid microbial growth and multiplication in presence of higher organic matter in the undegraded site. On the other hand, the degraded site had no permanent vegetation cover or grass (only seasonal) on the forest floor causing rapid loss of soil water and nutrients from the top soil layer and subsequently poor growth and multiplication of bacterial cells. Jha *et al.* (1992) reported significant variation in bacterial population due to forest degradation, altitudinal variation and seasonal changes in the north-eastern region. However, minimum bacterial population was observed from the less degraded forests of the lower altitudes in comparison to the more degraded forest at higher altitude. Arunachalam *et al.* (1997) also noted increased bacterial population number with the increase in stand age while a decline was recorded with the increase in soil depth. This reveals that bacterial population number increased with decrease in disturbance of forest soil environments. Further,

they also found that bacterial population being controlled by rainfall and soil moisture contents.

Lundgren (1982) reported significant reduction in bacterial population following forest clear-cutting in the long term. He observed increased bacterial growth upto 2 years after clear cutting but from the 3 years onwards the bacterial population declined significantly and showed detrimental affect even upto 10 years. This is in correspondence to the present findings that the degraded site remained lower in bacterial population than the moderately and undegraded sites even after 5 years of regeneration period. A similar report of higher bacterial population by 25 fold greater than that of the unburnt soil was observed immediately after one month of forest wild fire in an Atlantic soil (Vazquez *et al.*, 1993). The microbial population density diminished significantly after one year onwards in comparison to the unburnt site. However, the burning did not affect the population sizes of the microbial groups. Acea and Caraballas (1996) also reported changes in physiological groups of soil microbes following wildfire in an Atlantic soil. They observed very short time increase in ammonifying bacteria and amylase producers immediately after one month of wildfire. However, cellulase producers dominated in the soils after one year of wildfire. Acea and Caraballas (1999) reported almost complete microbial sterilization of the upper soil layer when heated to 200°C for 1h and the recovery pattern of the inoculated microbes also failed to reach the maximum population in comparison to an unburnt soil.

#### **4.1.5.3. Influence of soil degradation on fungal population of soils**

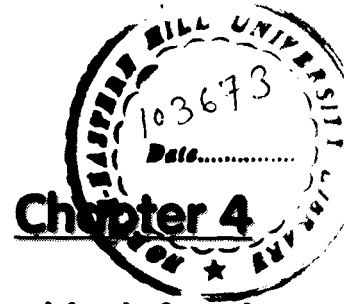
The higher fungal population in the undegraded site would be due to presence of soil properties favourable for growth of fungal communities. Maximum fungal population was also reported from 16 year old forest re-growth followed by 13 and 10 years old stands and lowest was observed in the 7 year old forest re-growth of humid subtropics in north-eastern India (Maithani and Tripathi, 1996). They observed decrease in total number of the fungal species in the soils with increase in soil depth. Their study concluded that species diversity and richness of the soil microfungi are related to forest re-growth age and soil nutrient status. Hassink (1997) observed more change in population characteristics of the fungi in the rhizosphere soil of conventional field than bacterial population under the reduced input systems. Fungal propagules as well as hyphal length growth were clearly depressed by wild fire in the short term (Vazquez *et al.*, 1993). Their study confirmed the long term detrimental effect of burning on fungal population. Tiwari *et al.* (1991) observed decreased in the fungal population with increasing soil depth and related to the organic C content of the soil.

Fungal population was significantly influenced by seasonal variations in the degraded, moderately degraded and undegraded forest sites and at surface and subsurface soil layers. The undegraded site contained higher fungal population throughout the sampling months than other sites at surface layer. The highest peak of fungal population was recorded during the month of June 1999 at surface soil layer of the undegraded site while the lowest population was observed in the month of October 1999. Tiwari, M.B. *et al.*

(1986) and Tiwari, S.C. *et al.* (1987ab) reported lowest fungal population being recorded during dry winter period from a pineapple orchard soil of different ages. Their study concluded the presence of soil moisture content was the major factor controlling the distribution of fungal community. Jha *et al.* (1992) found higher fungal population from the less degraded forests than in the more degraded forests of high and low altitudinal soils. It was revealed that disturbance of soil and vegetation has an adverse affect on the distribution of microbial population number and enzyme activities. The maximum fungal population was recorded from the study sites in the month of July while it decreased to minimum in the winter period. Similarly, declining trend of fungal population in winter months has been reported from a selectively logged humid subtropical forest of north-eastern India (Arunachalam *et al.*, 1997). Tiwari and Mishra (1994) reported highest fungal species occurred during rainy-summer season from pineapple orchard soils of different ages in the north-eastern India. They observed highest fungal species in winter than in autumn in the 10 years old stand. The seasonal variation in the fungal spectrum of the soil might be due to seasonal variations in soil moisture, temperature, pH and organic matter of the soil (Baruah, 1983; Chauhan *et al.*, 1985 and Agarwal and Chauhan, 1988). Tiwari *et al.* (1991) found highest number of fungi in spring-summer season while least number of fungi was recorded during winter season in 1, 5 and 10 year old pineapple plantations. The soil moisture content in the study sites was related to the fungal population and was responsible for the higher population number in the rainy season. The lower number of the fungal

population in their study was due to the lower soil moisture in the dry winter season. Hassink *et al.* (1997) have found decreased fungal population in July and August in conventional till soils than in reduced input system.

It is concluded from the present study that occurrence of bacterial and fungal populations in forest soil are reduced markedly by shifting cultivation and selective logging practices in humid tropical hill forest soils of north-eastern India. The repeated slash-and burn agriculture practiced in the degraded site for every year could have destroyed soil bacterial and fungal propagules in these sites. Similarly, the forest floor of the moderately degraded site was also burnt every 1 or 2 years to remove excessive growth of weeds and grasses to allow proper growth of tree plantations. This could have eliminated microbial propagules resulting in decreased bacterial and fungal population in the long term. These declines in the growth rate of the bacterial and fungal populations might have caused reduced rate of soil respiration from the forest floors of these two sites as compared to the undegraded site. The undegraded site on the other hand contained maximum bacterial and fungal population irrespective of seasonal variations than degraded and moderately degraded forest sites at both the soil depths.



## **4.2. Diversity of vesicular-arbuscular mycorrhizal fungi (VAMF) as affected by soil degradation**

### **4.2.1. INTRODUCTION**

Soil disturbance due to deforestation and associated agricultural practices causes undesirable changes in physico-chemical and biological characteristics of the soil i.e. soil degradation. These problems lead to the ultimate decline in soil fertility and crop productivity (Lal and Cummings, 1979; Ramakrishnan and Toky, 1981; Ramakrishnan, 1994; Stromgaard, 1984 and Tiessen *et al.*, 1992). Usually, degraded lands with poor soil quality are unable to support good vegetation due to slow regeneration potential and may turn into an unproductive wasteland until restoration measures are taken up. Soil microorganisms, being an essential component of soil ecosystem, act as an important index of soil health. This is because of the reason that their abundance and diversity are sensitive to changes occurring in the soil environment (Turco *et al.*, 1994 and Kennedy and Papendick, 1995). Such changes in microbial population following soil disturbance contribute to poor plant growth (Bellgard, 1994). One of the severely disturbed groups of microbes in the soil is mycorrhizal fungi which are symbiotically associated with the roots of the higher plants, internally or externally. Vesicular-arbuscular mycorrhizal fungi (VAMF), found associated within the roots are able to increase the absorptive surface area of plant roots in addition to supply of micro-nutrients particularly phosphorous during plant growth (Sanders and Tinker, 1971 and Jacobson, 1999). One of the

fundamental roles of mycorrhizal fungal hyphae may be to bridge the annular space, producing a physical connection between the root surface and surrounding soil particles (Miller, 1987). Mycorrhizal hyphae are also involved as an important cementing agent in forming soil aggregates and in maintaining its stability (Miller and Jastrow, 1992), thus conserving moisture and plant nutrients. Any undesirable change in the association between these fungi and host plant roots (mycorrhizae) due to soil disturbance leads to reduction in propagule population of mycorrhizal fungi (Ahmad, 1996 and Roldan *et al.*, 1997) as well as in slow forest regeneration as evident by most of the degraded tropical forest soils. It was reported that a decrease in the number of mycorrhizal propagules occurs when soil is disturbed or is partially removed (Miller, 1979). Bellgard (1994) also suggested that density of VAM fungal propagules present in a given soil system will determine to what extent soil disturbance also reduces root colonization. There have been reports on distribution of VAM species under the soil of important tree species in other parts of the north-eastern hilly region of India (Sharma *et al.*, 1986 and Pradhan *et al.*, 1996). VAM species diversity and population status of the fungi in degraded soils of Arunachal Pradesh have not been studied so far where shifting cultivation, locally called "jhum" agriculture, is practiced at a large scale on the hill slopes. Under this type of agricultural practice the area is cultivated for 1 or some times 2 years consecutively and then left abandoned as "jhum fallow" for restoration of nutrients and forest (Tiessen *et al.*, 1992 and Ramakrishnan and Toky, 1981). This shifting cultivation practice is responsible for fast decline in fertility, crop productivity as well as

decrease in biological diversity of the hilly regions associated with enhanced soil erosion. Therefore, it is required to examine to what extent the population status of vesicular-arbuscular mycorrhizal fungi (VAMF) is related to the degree of soil degradation for a successful restoration strategy of these abandoned lands. The present study investigates the distribution pattern of VAM spore population under different degrees of soil degradation in the humid tropical soils of Arunachal Pradesh, north-eastern India.

#### **4.2.2. REVIEW OF LITERATURE**

Most species of plants are capable of associating with fungi of a single zygomycetous family, the *Endogonaceae*, to form vesicular-arbuscular (VA) mycorrhizae (Gerdemann, 1968).

Vascular plants depend to some extent on vesicular-arbuscular (VAM) mycorrhizae for mineral uptake, although a few species do not. Some mycotrophic plants cannot grow without mycorrhizae; others grow better without mycorrhizae in fertile soils, but benefit from mycorrhizae in poor soils. Thus the mycorrhizal fungus content and fertility of soil influence occurrence of plant species (Janos, 1980).

Relatively little specific information is available concerning the responses of vesicular-arbuscular (VA) mycorrhizal fungi to tropical environments and surveys of mycorrhizal hosts and fungi in native tropical vegetation have been sporadic, and recent experimental investigations have dealt primarily with effects on crop growth (Janos, 1987).

Morton (1988) emphasized that the fungal symbionts of mycorrhizae are ubiquitous soil-inhabitants which must colonize plant roots to grow and

reproduce. They have been found in diverse habitats ranging from arctic to the tropics, arid to aquatic environments, and stable plant communities to highly disturbed ecosystems. Arbuscular mycorrhizal (AM) fungi are present and mediate plant root and soil interactions in most terrestrial ecosystems and crop production systems and colonization of plant roots by arbuscular mycorrhizal fungi can greatly increase the plant uptake of phosphorous and nitrogen (George *et al.*, 1995).

Yano *et al.* (1996) proposed that quantitative, structural and functional aspects of root systems forming mycorrhizas also need to be analysed in addition to fungal component in order to fully comprehend the mechanism of the symbiotic benefits. Further, they suggested that plants under nutrient poor conditions give propriety to mycorrhizal roots when partitioning assimilation products within the root system.

An assessment and enumeration of vesicular-arbuscular mycorrhiza propagules in some forest sites of Jenka revealed that spore numbers found in three representative forest areas were low and well below those recorded in other tropical soils (Ahmad, 1996). Evidence in the present study revealed that current forest logging practices influenced significant changes in VAM propagules.

A study on the effect of rotary tillage and no-till on the interaction between indigenous mycorrhizal fungi, *Bradyrhizobium japonicum* and soybean under field conditions revealed that both the nodule function as well as the level of arbuscular mycorrhizal fungi (AMF) colonization was

significantly greater in the plants under no-till than in the plants under rotary till (Antunes and Goss, 1996).

Brundrett *et al.* (1996) reported increased relative abundance and frequency of occurrence of inoculums of VAM and ectomycorrhizal fungi with vegetation cover in older disturbed sites. Further it was found that propagules of VAM fungi were substantially more numerous in some mine site habitats with dense vegetation cover, than adjacent natural habitats.

Smith and Smith (1997) reviewed the distribution of the structural diversity of arbuscular mycorrhizal symbioses in different plant kingdoms. They found that *Paris*-types occur frequently in the plant kingdom than *Arum*-types and predominate in ferns, gymnosperms and many wild angiosperms. The cultivated herbs that are the subject of much experimental work are mostly *Arum*-types.

Roldan *et al.* (1997) suggested that when a soil is put to agricultural use it undergoes a series of physical, chemical and microbiological changes, one most important of which is the changes that affect root-inhabiting microorganisms.

Cuenca *et al.* (1998) studied the roles of arbuscular mycorrhizae in the rehabilitation of fragile degraded tropical lands. They found that the rehabilitation of degraded lands in La Gran Sabana does not seem possible solely with the application of chemical fertilizers rather it was evident that mycorrhizae are required to achieve rehabilitation, given that inoculation of arbuscular mycorrhizae and simultaneous fertilization with triple super

phosphate led to significantly better results than those achieved with treatment of P.

The restoration of degraded sites with tree species is seen as a challenge to identify and reintroduce populations of soil microorganisms which are essential in the functioning of ecosystems (Varma, 1998). Mycorrhizal fungi, which aid the plant in nutrient uptake and assist in protecting roots from soil stresses are seen as a key component of this restoration process.

Yano *et al.* (1998) reported a novel finding that arbuscular mycorrhizal formation in undisturbed soil could promote root elongation despite the fact that soil was seriously compacted.

McGonigle and Miller (1999) reported the capability of extraradical mycelium of arbuscular mycorrhizal fungi to survive in winter freezed conditions in field condition. Further they reported the ability of the extraradical systems of the arbuscular mycorrhizal fungi to colonize test plants effectively.

A recent study on the influence of mycorrhizal fungi on the growth of different tree species and their nutrient uptake in gypsum mine spoils in India revealed that arbuscular mycorrhizal fungi are important symbionts for use in the revegetation of gypsum mine soils (Rao and Tak, 2001).

Duponnois *et al.* (2001) reported markedly variation in the relationship of the various species of mycorrhizal fungi between abundance of mycorrhizal spore population and the physico-chemical characteristics of the soils of different aged fallows in Snegal.

### 4.2.3. METHODOLOGY

#### VAMF population

The VAMF spores were isolated by wet sieving and decanting method of Gerdemann and Nicholson (1963) modified by Singh and Tiwari (2001). The spores were identified up to species level with the help of VAM fungi identification manual of Schenck and Perez (1987) under stereo-zoom microscope.

The species composition of VAM fungi were assessed using the methods for study of trophic structure of ecosystem. VAM fungi species and propagule populations (No. of spores 100 g<sup>-1</sup> dry soil) were measured and described. The variations in the VAM fungal population and number of species between disturbed and undisturbed sites were tested statistically by calculating least significant differences (LSD). Pearson's correlation coefficient (*r*) was calculated between VAMF spore numbers and important physico-chemical parameters in for all sites at both soil layers. Using the data obtained, the following indices of VAMF species structure were assessed:

**(1). Index of general diversity (*H'*); Shannon and Weaver (1949) cited in Odum (1971).**

$$H' = - \sum ( n_i / N \log_e n_i / N )$$

(where *n<sub>i</sub>* is the importance value of each species and *N* is the total importance value)

**(2). Index of dominance ( *C*); (Simpson 1949);**

$$C = \sum ( n_i / N )^2$$

(where *n<sub>i</sub>* is no. of individuals of each species and *N* is total no. of individuals in that location)

**Table 4.2.1. Distribution of Vesicular-arbuscular mycorrhizal (VAM) fungi in degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites**

Parameters	DF	MDF	UDF
No. of VAM fungi species	14	25	35
Species restricted in each site*	2	7	14
General diversity index, H'	0.81	0.85	0.99
Index of dominance, C	0.16	0.15	0.12

\*Out of the 44 VAM species 21 species were common to the three sites

**Table 4.2.2. Correlation coefficient (*r*) between VAMF spore population and selected soil characteristics of DF, MDF and UDF forest sites**

Soil properties	VAM spore population	
	Surface layer (0-20cm)	Subsurface layer (20-40cm)
Bulk density	-0.914174*	-0.977491**
SMC	0.997479**	0.849782*
WHC	0.966693**	0.998243**
pH	0.92814*	0.993169**

SMC=Soil moisture content; WHC=Water holding capacity

\* values are significant at  $P=0.05$

\*\* values are significant at  $P=0.01$

**Table 4.2.3. Distribution of VAM fungi species in degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites**

Species name	DF	MDF	UDF
1. <i>Acaulospora delicata</i> Walker <i>et al.</i> <sup>f</sup>	+	+	+
2. <i>A. denticulata</i> Sieverding & Toro <sup>f</sup>	+	+	+
3. <i>A. elegans</i> Trappe & Gerd <sup>b</sup>	-	+	-
4. <i>A. foveata</i> Trappe & Janos <sup>d</sup>	+	-	+
5. <i>A. lacunosa</i> Morton <sup>a</sup>	-	-	+
6. <i>A. spinosa</i> Walker & Trappe <sup>d</sup>	+	-	+
7. <i>Glomus albidum</i> Walker & Rhodes <sup>d</sup>	+	-	+
8. <i>G. clarum</i> Nicol. & Schenck <sup>f</sup>	+	+	+
9. <i>G. boreale</i> (Thaxter) Trappe & Gerd <sup>f</sup>	+	+	+
10. <i>G. canadense</i> (Thaxter) Trappe & Gerd <sup>a</sup>	-	-	+
11. <i>G. claroideum</i> Schenk & Smith <sup>a</sup>	-	-	+
12. <i>G. constrictum</i> Trappe <sup>f</sup>	-	+	+
13. <i>G. diaphanum</i> Morton & Walker <sup>d</sup>	-	+	-
14. <i>G. fasciculatum</i> (Thaxter)Gerd., & Trappe <sup>e</sup> emend. Walker & Koske <sup>e</sup>	-	+	+
15. <i>G. fulvum</i> (Berk & Broome) Trappe & Gerd <sup>e</sup>	-	+	+
16. <i>G. globiferum</i> Koske & Walker <sup>c</sup>	+	-	-
17. <i>G. glomerulatum</i> Sieverding <sup>b</sup>	-	+	-
18. <i>G. heterosporum</i> Smith & Schenck <sup>f</sup>	-	+	+
19. <i>G. intraradices</i> Schenk & Smith <sup>a</sup>	-	-	+
20. <i>G. lacteum</i> Rose & Trappe <sup>a</sup>	-	-	+
21. <i>G. maculosum</i> Miller & Walker <sup>e</sup>	-	+	+
22. <i>G. microaggregatum</i> Koske <i>et al.</i> <sup>e</sup>	-	+	+
23. <i>G. microcarpum</i> Tul. & Tul <sup>e</sup>	-	+	+
24. <i>G. monosporum</i> Gerd. & Trappe <sup>b</sup>	-	+	-
25. <i>G. mosseae</i> (Nicol. & Gerd.)Gerd. & Trappe <sup>f</sup>	+	+	+
26. <i>G. multicaule</i> Gerd. & Bakshi <sup>e</sup>	-	+	+
27. <i>G. pulvinatum</i> (Henn.) Trappe & Gerd <sup>a</sup>	-	-	+
28. <i>G. reticulatum</i> Bhattacharjee & Mukerjee <sup>c</sup>	+	-	-
29. <i>G. rubiformis</i> Gerd. & Trappe <sup>a</sup>	-	-	+
30. <i>G. sinuosa</i> Gerd. & Bakshi <sup>b</sup>	-	+	-

**Contd./ Table 4.2.3.****Table 4.2.3. Distribution of VAM fungi species in degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites**

<b>Species name</b>	<b>DF</b>	<b>MDF</b>	<b>UDF</b>
31. <i>G. tenebrosum</i> (Thaxter) Berch <sup>b</sup>	-	+	-
32. <i>G. tenerum</i> Tandy emend. Mc Gee <sup>f</sup>	+	+	+
33. <i>G. tortuosum</i> Schenck & Smith <sup>f</sup>	+	+	+
34. <i>Gigaspora albida</i> Schenck & Smith <sup>a</sup>	-	-	+
35. <i>G. candida</i> Bhattacharjee et al. <sup>p</sup>	-	+	-
36. <i>G. decipiens</i> Hall & Abbot <sup>a</sup>	-	-	+
37. <i>Sclerocystis rubiformis</i> Gerd. & Trappe	-	-	+
38. <i>Scutellospora aurigloba</i> (Hall) Walker & Sanders <sup>a</sup>	-	-	+
39. <i>S. coralloidea</i> (Trappe et al.) Walker & Sanders <sup>a</sup>	-	-	+
40. <i>S. gregaria</i> (Schenck & Nicol.) Walker & Sanders <sup>f</sup>	+	+	+
41. <i>S. heterogama</i> (Schenck & Nicol.) Walker & Sanders <sup>f</sup>	-	+	+
42. <i>S. pellucida</i> (Schenck & Nicol.) Walker & Sanders <sup>f</sup>	+	+	+
43. <i>S. persica</i> (Koske & Walker) Walker & Sanders <sup>a</sup>	-	-	+
44. <i>S. reticulata</i> (Koske & Walker) Walker & Sanders <sup>a</sup>	-	-	+
<b>Total Number of species in each site</b>	<b>14</b>	<b>25</b>	<b>35</b>

**Note: Species presence (+) and absence(-)**

**a, VAM species found only in the undegraded site**

**b, VAM species found only in the least degraded site**

**c, VAM species found only in the degraded site**

**d, VAM species found common to undegraded and degraded sites**

**e, VAM species found common to undegraded least degraded sites**

**f, VAM species found common to undegraded, least degraded and degraded sites**

**Table. 4.2.4. Restricted distribution of VAM fungi in DF, MDF and UDF sites****VAM fungi species found only in UDF site (14)**

<i>A. lacunosa</i> Morton	-	-	+
<i>G. canadense</i> (Thaxter) Trappe & Gerd	-	-	+
<i>G. claroideum</i> Schenk & Smith	-	-	+
<i>G. intraradices</i> Schenk & Smith	-	-	+
<i>G. lacteum</i> Rose & Trappe	-	-	+
<i>G. pulvinatum</i> (Henn.) Trappe & Gerd.	-	-	+
<i>G. rubiformis</i> Gerd. & Trappe	-	-	+
<i>Gigaspora albida</i> Gerd. & Trappe	-	-	+
<i>G. decipiens</i> Hall & Abbott	-	-	+
<i>Sclerocystis rubiformis</i> Gerd. & Trappe	-	-	+
<i>Scutellospora aurigloba</i> (Hall) Walker & Sanders	-	-	+
<i>S. coralloidea</i> (Trappe <i>et al.</i> ) Walker & Sanders	-	-	+
<i>S. persica</i> (Koske & Walker) Walker & Sanders	-	-	+
<i>S. reticulata</i> (Koske & Walker) Walker & Sanders	-	-	+

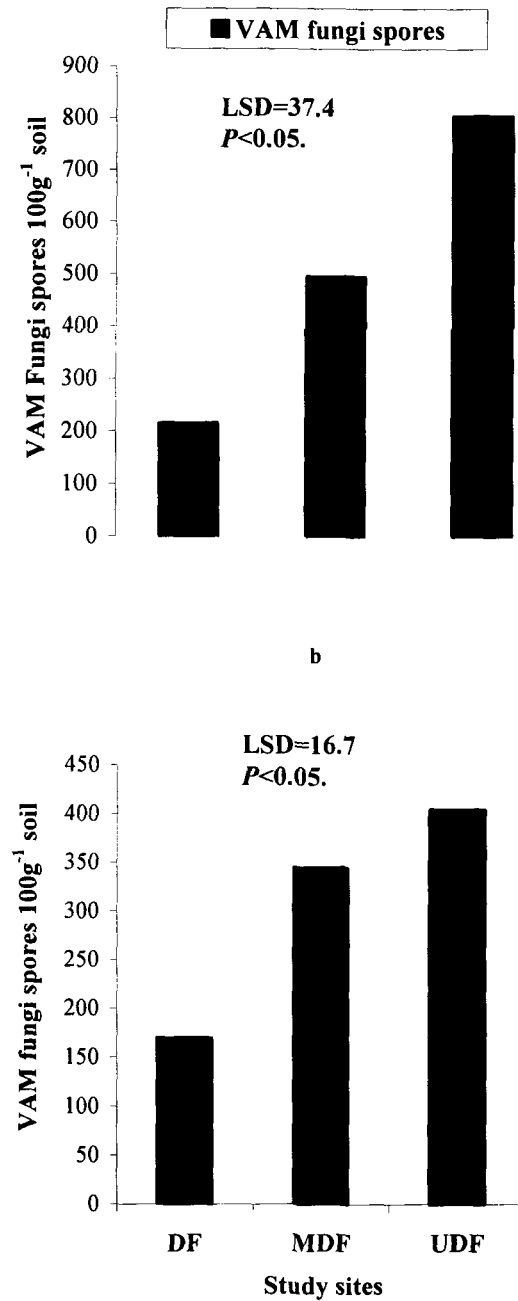
**VAM fungi species found only in MDF (7)**

<i>A. elegans</i> Trappe & Gerd.	-	+	-
<i>G. candida</i> Bhattacharjee <i>et al.</i>	-	+	-
<i>G. diaphanum</i> Morton & Walker	-	+	-
<i>G. diaphanum</i> Morton & Walker	-	+	-
<i>G. glumerulatum</i> Sieverding	-	+	-
<i>G. tenebrosum</i> (Thaxter) Berch	-	+	-
<i>S. sinuosa</i> Gerd. & Bakshi	-	+	-

**VAM fungi species found only in DF site (2)**

<i>G. globiferum</i> Koske & Walker	+	-	-
<i>G. reticulatum</i> Bhattacharjee & Mukerjee	+	-	-

**Note: Species present (+) and absent (-)**



**Fig. 4.2.1(ab).** Distribution of VAM spore population in two different depths of degraded (DF), moderately degraded (MDF) and degraded (UDF) forest soils at surface (a) and subsurface (b) soil layers.

#### 4.2.4. RESULTS

The VAM population was comparatively high in the undegraded site (805 spores  $100\text{ g}^{-1}$  soil) which was followed by the moderately degraded site (495 spores  $100\text{ g}^{-1}$  soil). Lowest population (215 spores  $100\text{ g}^{-1}$  soil) was recorded in the degraded site at the surface soil layer (Fig. 4.2.1a). Similar pattern of VAMF spore population was observed in the subsurface layer of the study sites (Fig.4.2.1b). The subsurface soil layers of undegraded, moderately degraded and degraded sites contained 50%, 30%, and 20% less VAMF spore populations than respective surface soil layers. The index of general diversity and index of dominance of VAM fungi species in degraded, moderately degraded and undegraded forest sites are given in table 4.2.1. The correlation coefficient between the VAMF spores and important soil characteristics are shown in the table 4.2.2.

Qualitative study of soil samples revealed that undegraded soil had the maximum number of VAM fungi species (35) followed by moderately degraded (25) and degraded (14) site respectively. Altogether, 44 species belonging to five genera namely, *Acaulospora* (6), *Gigaspora* (3), *Glomus* (27), *Sclerocystis* (1) and *Scutellospora* (7) were recorded from the three forest sites (Tables 4.2.3 and 4.2.4). Twenty one species were found abundant in all the three sites whereas distribution of fourteen species were restricted to undegraded site, seven species to moderately degraded site and only two species to degraded site (Table 4.2.4).

The undegraded site showed lower (0.12) index of VAM fungi species dominance while the degraded and moderately degraded sites showed

higher indices of dominance (0.16 and 0.15) respectively. The index of general diversity displayed a maximum of 0.99 for undegraded site, 0.85 for moderately degraded site while the lowest (0.81) was noted in degraded site.

#### **4.2.5. DISCUSSION**

The results of the studied physico-chemical parameters and VAM population structure in all the sites revealed that the degree of soil degradation had significant influence on the distribution of VAM spore population. The reason for lower population structure in both depths of the degraded site may be due to compaction and hardening of soil as supported by higher value of bulk density. This might have restricted and delayed penetration of plant roots in the soil system thereby reducing movement and colonization of VAMF spores in the regenerating site. Ahmad (1996) reported 30-50% reduction in VAMF propagules when forest soils were severely disturbed through heavy soil mechanical compaction, exposure and erosion. This is in correspondence to our finding of 30% reduction in moderately degraded site and 70% reduction in degraded site when the soils at these sites were disturbed for selective logging and agricultural use by shifting cultivation in comparison to the undegraded natural forest site. The lower moisture and organic carbon contents and more strongly acidic condition of the degraded site might have also reduced VAM spore germination and subsequent growth of the hyphae in the hardened and compacted soil. The continuity in the life cycle of VAMF in the soil through host plant roots must have been disturbed repeatedly since degraded site was clear cut and burnt for every time prior to cropping. Decline in the rate of

infection and reduced formation and colonization of VAMF propagules were reported by Jasper *et al.* (1987 and 1989); McGonigle *et al.* (1990) and Bellgard (1993) from different disturbed soils. It was found from the present study that there was significant reduction in VAM spore density in the degraded site following slash and burn agriculture in comparison to the undegraded natural forest site. Even after five years of fallow period (1994 to 1998) for restoration of physico-chemical, biological properties and soil fertility, the VAMF were not able to re-establish to its original population level and many species were unable to recover at all. The absence of a number of VAMF species in the degraded site might be due to the impact of uncontrolled burning done for every year or every alternate year, which might have killed VAMF propagules in the soil. Another reason might be that the spores in the soil could not complete their life cycle due to the absence of a suitable host root even if VAMF propagules were present in the site. Roldan *et al.* (1997) also reported decrease in VAMF population following agriculture use of soils and their recovery appearing to be influenced more by the presence of host plants in the semi-arid areas. The study reveals that higher degree of soil degradation due to shifting agriculture practices have a significant adverse influence on the distribution of VAMF in the fragile hilly soils in the humid tropical region in the long term.

The average index of dominance for undegraded site was lower (0.12) indicating shared species dominance of different VAM fungi species while the higher values of 0.16 and 0.15 in degraded and moderately degraded sites indicates dominance by a few species of VAM fungi only. In

case of index of general diversity, the result was converse, a value of 0.99 for undegraded site, 0.85 for moderately degraded site while the lowest of 0.81 from the degraded site suggesting a greater diversity of VAM fungi species in the undegraded site than in degraded and moderately degraded sites respectively. There was a net reduction of 30% and 60% species diversity of VAM fungi in moderately degraded and degraded forest sites in comparison to the undisturbed forest site. This is in conformity with the higher number of VAM fungal species and propagules in undegraded soil than in degraded soils. The diversity of VAMF species was highest in undegraded forest soil followed by moderately degraded site and the degraded forest soil had the minimum species diversity. The distribution of VAM spore population in all the sites demonstrates a clear impact of soil disturbance following shifting cultivation and selective logging in the degraded and moderately degraded sites. The higher values of soil moisture, water holding capacity and soil reaction were positively correlated to the higher population of VAMF spores in undegraded site except for bulk density which showed negative correlation to the distribution of VAMF population in this site.

## **Chapter 4**

### **4.3. Distribution of microbial biomass C and N in degraded and undegraded forest soils**

#### **4.3.1. INTRODUCTION**

Microorganisms are the main mediators of C turnover in soil. By definition they are also part of the organic C and nutrient pool: as they are called microbial biomass which is an important variable in the C cycle (Insam, 1990). Being an important ecological parameter, microbial biomass acts as a source-sink in nutrient cycling processes and a small, but labile reservoir of C, N, P and S which regulates many organic matter transformations (Jenkinson and Ladd, 1981; Singh *et al.*, 1989; Jordan and Beare, 1991 and Arunachalam *et al.*, 1996). Since microbial biomass is critical in regulating soil ecosystem level processes, such as nutrient cycling, and organic matter transformations, there is much interest in understanding the factors which regulate its size, activity and structure (Grayston *et al.*, 2001 and Zeller *et al.*, 2001). The important variables or factors which could affect the microbial biomass content are different physical, chemical and biological characteristics of soil, vegetation cover and agricultural practices, including fertilizer treatment and pesticides, biological additions, heavy metal contaminations, wild fire, deforestation, topographic features, climatic variables such as rainfall, temperature etc. With a growing concern over the importance of microbial biomass and microbial activity in sustainable agriculture and ecosystem stability, research investigations have gained top priority in the field of soil ecology. Microbial biomass dynamics has been

studied in natural forest, grasslands, pastures, savannas, managed ecosystems, monoculture plantations etc. (Diaz-Ravina *et al.*, 1993ab and 1995; Das *et al.*, 1997; Griffith *et al.*, 1997; Holt and Mayer, 1998; Vesterdal, 1998 and Grayston *et al.*, 2001). Effect of seasonal changes, forest cutting and succession, wild fire, climatic regimes such as soil temperature, moisture, fertilization, management, liming, afforestation, cropping systems, long term field experiments, freezing and drying of soil, topsoil removal, depth-wise variation, etc. have been reported in detail (Ross *et al.*, 1982; Insam, 1990; Goyal *et al.*, 1992; Mao *et al.*, 1992; Srivastava, 1992; Lavahun *et al.*, 1996; Maithani *et al.*, 1996; Chotte *et al.*, 1998; Prieto-Fernandez, *et al.*, 1998; Stenberg *et al.*, 1998; Wardle, 1998; Witter and Kanal, 1998; Seiter *et al.*, 1999; Taylor *et al.*, 1999 and Bending *et al.*, 2000). Further, the importance of microbial biomass in soil fertility and their relationship with CO<sub>2</sub> evolution, soil inorganic properties and organic matter contents have been reported (Verstraete and Voets, 1977; Shan-Min *et al.*, 1987; Alvarez *et al.*, 1998 and Puri and Ashman, 1998).

#### **4.3.2. REVIEW OF LITERATURE**

Marumoto *et al.* (1982) investigated the contribution of microbial biomass to the pool of mobile plant nutrients in two soils having different amount of microbial biomass contents. They observed 55-57% of the N mineralized in the soils were derived from the freshly killed biomass. They have suggested that the amount of microbial biomass might be used for estimating the mobile plant nutrient pool in soil.

Shan-Min *et al.* (1987) suggested that measurement of microbial biomass by fumigation–incubation technique requires a short time conditioning period of 5-10 days for freshly sampled soils. Their study also revealed reduced microbial biomass by air-drying at 25°C and subsequent re-moistening of soil in comparison to the original undried soil. The biomass showed significant recovery after 10 days of air-drying and remoistening, however it could not reach to the amount of biomass as before air-drying of soils.

Sparling and Ross (1988) reported a significant increase in microbial biomass and net nitrogen mineralization after air drying and subsequent re-wetting of soils. They also observed that the reason being the release of a large fraction of the increased N was derived from microbial cells killed by desiccation.

Studies on intimate relationship between climatic conditions and the microbial soil C pool and respiratory C flux showed the best possible prediction of microbial biomass C and its ratio to total organic C by using climatic variables such as precipitation / evaporation ratio in soil with equilibrium C content (Insam, 1990). Further he found that longer dry period reduced microbial biomass and increased with increase in moisture content.

A study on the relationship between microbial biomass and background nitrification over a range of soils revealed a proportionate relationship of microbial biomass and background (unamended) soil nitrification. Organic soils contained greatest biomass C followed by the fine and medium textured soils while the sandy soils having lowest biomass C

content (Drury *et al.*, 1991). The microbial biomass appeared to be a sensitive indicator of both the soil C content and background nitrification. Biomass C was highly correlated to organic C contents of the soils.

Srivastava and Singh (1991) while studying the effect of alternate land uses and nutrient flux on microbial biomass C, N and P in dry tropical forests of India observed remarkable reduction in the amounts of microbial biomass C, N and P following conversion of dry tropical forests into other land uses.

Studies on the effects of inorganic fertilizer and organic amendments on organic matter-microbial biomass relationships in field experiments under tropical conditions have shown that soil microbial biomass C and N increased with balanced fertilization (Goyal *et al.*, 1992). They also found that addition of the organic amendments increased microbial biomass even when the organic C content of the soil did not increase.

Mao *et al.* (1992) found rapid improvement of soil microbial properties like microbial biomass C and respiration, soil C and nutrient status by afforestation in tropical China. They noted lowest microbial biomass C in the barren lands and highest in the secondary monsoon forests. Their study confirmed that from the microbiological point of view the mixed forests are more similar to the natural forests than monospecied forests.

Srivastava (1992) recorded minimum microbial biomass nutrients (C, N & P) in a 5 year old mine spoil and maximum in the mixed forest in different seasons. However, he found consistently maximum biomass C, N & P in dry summer periods and decreased significantly to a minimum in the rainy season indicating pulsed turnover of microbial biomass in the soil.

Diaz-Ravina (1993a) reported a relatively high amount of nutrients being mineralized in the biomass of soil microorganisms in forest soils and suggested that the contribution of microbial biomass to soil concentration of available plant nutrients was large for N, important in the cases of P, K and Mg, not significant for Na and it was not possible to obtain any conclusion about Ca.

Diaz-Ravina *et al.* (1993b) evaluated the mineralization pattern of microbial biomass and N in forest soils. They found a significant increase in the average C and N mineralization as a consequence of microbial lysis by fumigation. Their result indicated that the soils have a considerable quantity of microbial biomass but are relatively low metabolic activity and that C and N mineralization may be significantly improved when microbial constituents are liberated into the soil.

Diaz-Ravina *et al.* (1995) reported the significant effects of the season, the type of soil, the interaction between the type of soil and season, but soil type explained most of the variance on microbial biomass and other soil nutrient flush of forest soils. They have also found a substantial contribution of microbial biomass to plant-available nutrients of forest soils. Further, they noted close relationship of microbial biomass with pH, organic C, total N and moisture contents.

A study on the seasonal dynamics of microbial biomass C, N and P during re-growth of a disturbed subtropical humid forest in north-eastern India revealed the occurrence of higher microbial biomass during the winter and lowest during the rainy season at surface and subsurface soil layers

(Maithani *et al.*, 1996). They also obtained significantly high microbial biomass in the surface layer than the subsurface layer of the 7, 10, 13 and 16 years old forest re-growths. There was an increasing trend of microbial biomass from lower in younger stand to the highest in the older stands of the forest re-growths.

Lavahun *et al.* (1996) reported significant variation in the distribution of the microbial biomass C at different depths of a grassland and two arable soils. The biomass C declined from maximum at the upper layer to a minimum at the lower depths in all the study sites. They observed decrease in microbial biomass C associated with decline in organic C content revealing higher organic C mineralized at upper layers leading to higher microbial activity than the lower soil layers.

Griffiths *et al.* (1997) reported existence of significant positive correlation between C, mineralizable-N, and both fungal and bacterial biomass in the forest soils but this was not the case in the pasture soils. They concluded that forested riparian zones have a greater capacity than pasture soils to sequester C and recover nutrients.

Das *et al.* (1997) reported presence of highest microbial biomass N in the winter and lower in the rainy season in a subtropical humid forest of Meghalaya, north-eastern India. It was reported that these forests had higher ammonification and lower nitrification rates.

A clear trend of increase in microbial biomass C was observed with the increase in application of lime and had a similar pattern to soil respiration (Neale *et al.*, 1997). They found decrease in microbial biomass after 4 days

and 8 days, however the amount was higher than the unlimed soils after 101 days. It was concluded that the affect of liming on microbial biomass, CO<sub>2</sub> evolution and net N mineralization was less dependant and varied between two soils.

Alvarez *et al.* (1998) observed active microbial biomass as a fraction of the total soil microbial biomass, increased with depth in the cropland, plow tillage and no tillage systems, and highest in the plow tillage soils. The highest biomass was recorded 3 times higher in pasture than in cropland, but there was no difference between the tillage systems.

Puri and Ashman (1998) studied the relationship between microbial biomass and gross N mineralization in field conditions using <sup>14</sup>N pool dilution technique. They found no significant relationship between gross N mineralization and soil microbial biomass N pool. It was concluded that only a fraction of the microbial biomass was involved in N mineralization.

Prieto-Fernandez *et al.* (1998) investigated the effects of wildfire on microbial biomass of pine forests in Galicia, N.W. Spain. They found a drastic reduction at the upper layer of soil which decreased with increasing depth. Their study demonstrated a detrimental effect of wildfire on microbial biomass recovery up to 4 years and had almost half of the unburned forest by 13 years. They have suggested that improvement of the burned soils in terms of nutrient recovery possible only when re-plantation, organic amendment and microbial inoculation are done.

Holt and Mayer (1998) reported the long term detrimental effect of sugarcane monoculture plantations on soil microbial biomass. They have

found significantly lower amount of microbial biomass in the monoculture plantations than the new plantations which had no history of sugarcane production.

Stenberg *et al.* (1998) noted negligible effects of soil storage on microbial biomass at  $-20^{\circ}\text{C}$  than at  $+2^{\circ}\text{C}$  when the biomass assay was conducted by fumigation incubation method. They also reported that storage at  $-20^{\circ}\text{C}$  for 13 months does not affect the microflora in annually frozen soils in any decisive way.

Witter and Kanal (1998) found close relationship of amounts of microbial biomass C to that of soil C concentration in different soils of a 40 year old field. No constant ratio of  $C_{\text{mic}}$  to  $C_{\text{org}}$  was observed but it increased with soil carbon concentration in the study. They concluded that  $C_{\text{mic}}$  to  $C_{\text{org}}$  ratio between the soils was mainly due to differences in the quality of soil organic matter rather than due to intrinsic differences in microbial efficacy of substrate utilization.

Studies on the effect of temperature on organic matter and microbial biomass dynamics in temperate and tropical soils revealed slower declining trend of microbial biomass in tropical soils at 15 and  $35^{\circ}\text{C}$  than in temperate soils, although at  $15^{\circ}\text{C}$  the differences were minimal (Grisi *et al.*, 1998). They also observed more rapid mobilization of organic matter in the temperate than in the tropical soils at  $35^{\circ}\text{C}$ . It was concluded that the organic matter in the tropical soils are more degraded, or humified than in the temperate soils.

Houston *et al.* (1998) reported no significant changes in C and N mineralization, microbial biomass C,  $C_{\text{mic}}/C_{\text{org}}$ , or metabolic quotients

indicating that little or negligible impact of vegetation management like herbicide or brushshaw treatment were practiced for 2 years in a mixedwood forest soils in Canada.

Wardle (1998) stressed the temporal variability of the soil microbial biomass is an important component of its turnover; and thus contribute to patterns of soil nutrient release and mineralization. The temporal variability of microbial biomass C was most closely related to soil N content in forest, pH and latitude in arable ecosystems, and pH, latitude and soil C contents in grasslands.

Vesterdal (1998) reported highest microbial biomass N and P availability in Oak forest floors than forest floors of other species, namely Norway spruce, Sitka spruce, Douglas fir and beach. Microbial P availability was lowest in the Norway spruce forest floor. Basal respiration was found to be positively related to microbial biomass N and P in his study.

Taylor *et al.* (1999) investigated the microbial biomass in a sequence of northern hardwood forest stands ranging in age since clear cutting from 3 to more than 120 years. Significant variation was observed among sampling periods and was greater in early and late successional stands than mid successional stand. They found that microbial biomass was not very responsive to the environmental factors however, moisture content was found to be the most often contributed to variation in microbial biomass. It was concluded that the lower microbial biomass in the mid successional stand suggesting that microbial dynamics in forest soils are not controlled by factors directly related to forest harvesting.

Bending *et al.* (2000) reported insignificant variation in distribution of microbial biomass N between or within two treatments, grass-clover ley and crop sequence soils following crop harvesting. They suggested that management practice had no effect on soil biomass N, probably because there was no change in the total organic matter content, or the size of most of the labile organic matter pools, which could be considered to representing important energy source for the biomass.

Grayston *et al.* (2001) reported increase in microbial biomass with decrease in soil fertility, moving from the improved to unimproved grasslands. Site characteristics had also a significant input on the broad-scale measure of microbial biomass and respiration in these soils.

Zeller *et al.* (2001) reported that management abandonment has important effect on the structure of soil microbial biomass, namely an increase in the fungal biomass in different sites. It was revealed that the size and structure of the soil microbial biomass varies considerably in grassland with similar management in the study sites. The temporal variation in size of the microbial biomass was generally higher in these soils and pattern of temporal fluctuations, as shown for biomass C, varied significantly between sites. In contrast to these strong effects of site and time of sampling on soil microbial biomass, the impact of land abandonment of management was relatively very less importance.

### 4.3.3. METHODOLOGY

#### Microbial biomass C and N

Microbial biomass C and N were determined by modified fumigation extraction method of Okalebo *et al.* (1993). Fifteen grams of field moist soil in a 50 ml glass beaker was incubated inside a vacuum desiccators containing 25 ml of alcohol free chloroform in a 50 ml beaker. Vacuum was applied till the available chloroform was completely vaporized and kept for 24 h under dark conditions at room temperature. Similarly, unfumigated samples were also incubated for control treatments without chloroform. The desiccators was applied with vacuum to remove excess of chloroform before opening. The soil sample was then extracted with 50 ml of 0.5M  $K_2SO_4$  in a plastic bottle for 30 minutes in a rotary shaker (horizontal). The soil extract was filtered through Whatman No. 42 filter paper and analyzed for microbial C by digesting 5 ml of filtrate with 5 ml of 1M  $K_2Cr_2O_7$  and 7 ml of concentrated  $H_2SO_4$  for 30 minutes at 145-155°C. After cooling for 1 h, the digested mixture was titrated against freshly prepared 0.2 M  $(NH_4)_2FeSO_4 \cdot 6H_2O$  solution.

Microbial biomass C ( $C_{mic}$ ,  $\mu g g^{-1}$  dry soil) =  $C_f - C_o$

{Where,  $C_f$  = biomass C of fumigated sample;  $C_o$  = biomass C of unfumigated sample}

For estimation of N, 10 ml of the filtrate was digested at 350°C with 4.4 ml of digestion mixture (0.42 g selenium powder + 14 g lithium sulfate + 30 ml of 30%  $H_2O_2$  + 420 ml concentrated sulfuric acid) in a micro-kjeldahl digestion tube till the change of colour to whitish (colourless). The mixture was extracted with successive aliquots of distilled water in a 50 ml

volumetric flask. The solution was either centrifuged or filtered through Whatman No.1 filter paper to get a clear solution for estimation of microbial biomass N. 10 ml of clear extract was used to determine ammonium-N released as a result of reaction with 40% NaOH solution in the micro-kjeldahl digestion chamber. The ammonium-N released was collected in 5 ml of 1% boric acid solution till permanent green colour develops. Then, a few drops (2-4 drops) of bromocresol green indicator solution was added into the solution mixture and titrated with N/14 HCl till it turns into pink colour which is the end point.

$$\text{Microbial biomass N}(N_{mic}, \mu\text{g g}^{-1} \text{ dry soil}) = N_f - N_o$$

{Where,  $N_f$  = biomass N of fumigated sample;  $N_o$  = biomass N of unfumigated sample}

#### **4.3.4. RESULTS**

##### **Microbial biomass C ( $C_{mic}$ )**

Microbial biomass C content of soils in degraded, moderately degraded and undegraded forest sites at two depths is shown in figure 4.3.1. Microbial biomass C varied from  $73 \mu\text{g g}^{-1}$  dry soil to  $606 \mu\text{g g}^{-1}$  dry soil in the surface soil layer whereas it ranged between  $30 \mu\text{g g}^{-1}$  dry soil to  $361 \mu\text{g g}^{-1}$  dry soil in the subsurface layer of all study sites. Maximum microbial biomass C was recorded from the undegraded site in the month of May 2000 while the minimum was recorded from the degraded site in the month of October 1998. The microbial biomass C appeared to be higher during rainy months (May to September) and lower during dry winter seasons (October to March) in all the sites and at surface and subsurface layers. Generally,

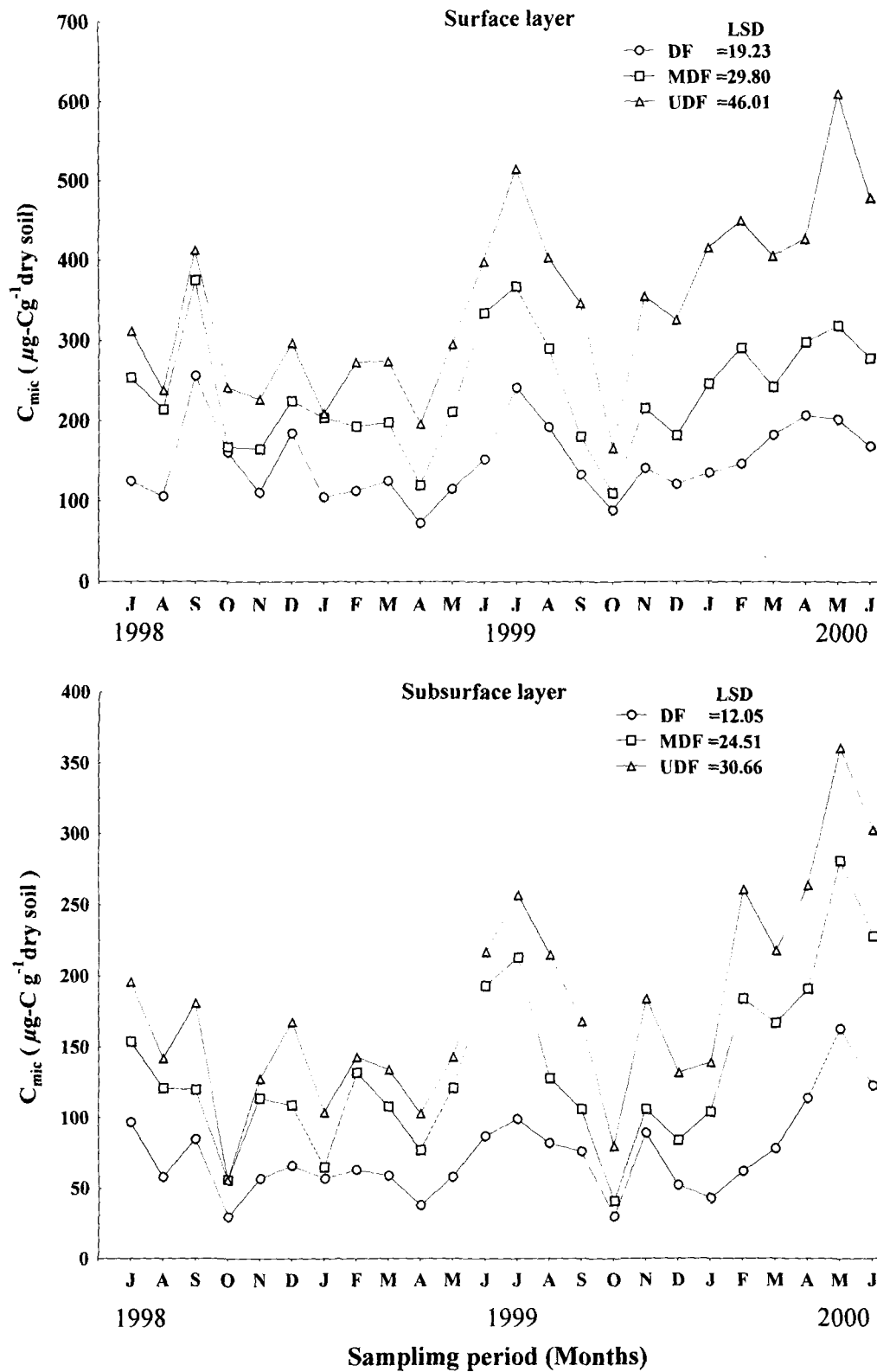
microbial biomass C was higher at surface layer than in subsurface layer of all sites.

One-way-analysis of variance of microbial biomass C content showed significant ( $P<0.05$ ) variation among the study sites at both the surface and subsurface soil layers (Table 4.3.1). Similarly, the variation in microbial biomass C contents between the surface and subsurface soil layers of degraded, moderately degraded and undegraded sites were also significant (Table 4.2.2). Microbial biomass C was positively related to microbial biomass N in all sites at both surface and subsurface layers (Table 3.3 and 3.4). Similarly, microbial biomass C was also related to bacterial population in all sites at both the soil layers except for degraded site where it was related to soil respiration at surface layer only.

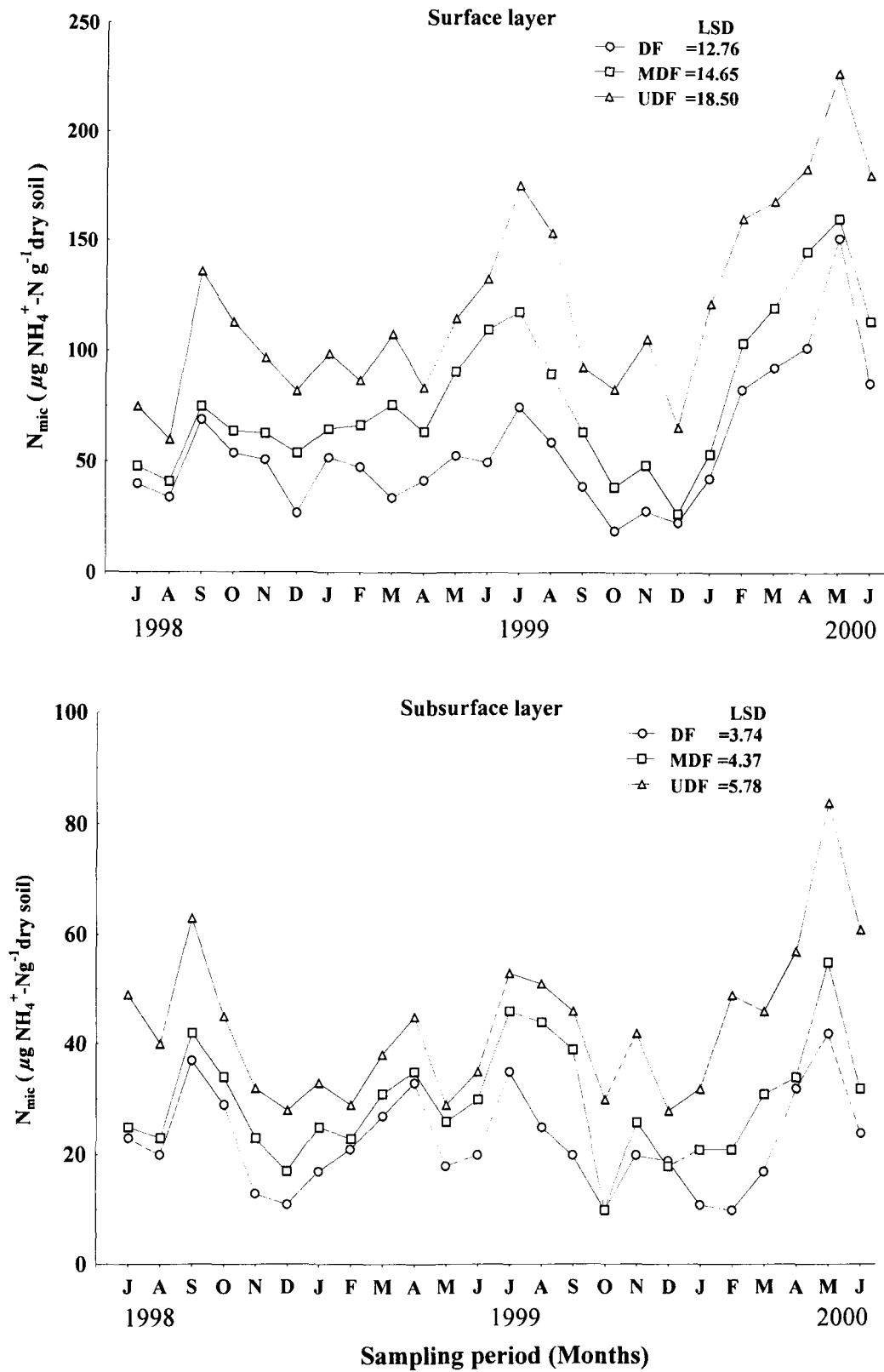
#### **Microbial biomass N ( $N_{mic}$ )**

Maximum microbial biomass N was recorded from the undegraded site followed by moderately degraded site and minimum was recorded from the degraded site at surface and subsurface soil layers. There was a marked variation in distribution of microbial biomass N contents in the three sites with sampling months. Highest peak of microbial biomass N appeared in the month of April 2000 while lowest peak was found in the month of October 1999 in all sites and at both the soil depths. Microbial biomass N was higher in the surface layers than in the subsurface layers of all the three study sites (Fig. 4.3.2).

Microbial biomass N content varied significantly ( $P<0.05$ ) among the three study sites at both the soil depths (Table 4.3.1). The variation was also



**Fig.4.3.1. Microbial biomass C in soils of degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites at surface and subsurface layers. (LSD,  $P < 0.05$ )**



**Fig.4.3.2. Microbial biomass N in soils of degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites at surface and subsurface layers. (LSD,  $P < 0.05$ )**

**Table 4.3.1. One-way analysis of variance (ANOVA) of the biochemical characteristics of soil in degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites at surface and subsurface soil layers ( $P < 0.05$ ).**

Soil properties	Source of variation	Surface layer		Subsurface layer	
		F-ratio	P-level	F-ratio	P-level
<b>Microbial biomass C (<math>C_{mic}</math>)</b>	DFxMDFxUDF	35.676	$2.29 \times 10^{-14}$	2.152	$3.71 \times 10^{-8}$
	DFxMDF	23.431	$1.49 \times 10^{-5}$	20.974	$3.55 \times 10^{-5}$
	MDF x UDF	16.503	$1.87 \times 10^{-4}$	6.189	0.02
	DF x UDF	65.918	$1.98 \times 10^{-10}$	44.149	$2.532 \times 10^{-8}$
<b>Microbial biomass N (<math>N_{mic}</math>)</b>	DFxMDFxUDF	22.663	$2.72 \times 10^{-8}$	16.739	$1.18 \times 10^{-6}$
	DFxMDF	5.890	0.0192	6.789	0.013
	MDF x UDF	16.912	$1.60 \times 10^{-4}$	10.219	0.003
	DF x UDF	42.416	$4.78 \times 10^{-8}$	30.702	$1.40 \times 10^{-6}$

**Table 4.3.2. One-way analysis of variance (ANOVA) of the biological characteristics of soil between surface and subsurface soil layers in degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites ( $P < 0.05$ ).**

Soil properties	Study Sites	F-ratio	P-level
<b>Microbial biomass C (<math>C_{mic}</math>)</b>	DF	47.878	$1.204 \times 10^{-8}$
	MDF	33.458	$6.089 \times 10^{-7}$
	UDF	42.593	$4.691 \times 10^{-8}$
<b>Microbial biomass N (<math>N_{mic}</math>)</b>	DF	24.215	$1.145 \times 10^{-5}$
	MDF	33.715	$5.6642 \times 10^{-7}$
	UDF	59.852	$7.256 \times 10^{-10}$

significant between the surface and subsurface soil layers of degraded, moderately degraded and undegraded forest sites (Table 4.3.2). Microbial biomass N was positively correlated ( $P < 0.05$ ) with available-N contents at both the surface and subsurface layers of the degraded and undegraded sites (Tables 3.3 and 3.4). However, it was related to acid phosphatase activity at surface layers of all study sites.

#### **4.3.5. Discussion**

Microbial biomass C and N contents were reduced in soils of degraded and moderately degraded forest sites as a result of shifting cultivation and selective logging of forest trees in comparison to the undegraded forest site in the present study. Henrot and Robertson (1994) reported marked decline in microbial biomass C as a result of vegetation removal in humid tropical soils. They have observed a rapid loss of microbial biomass C in bare soils in comparison to annual harvest re-growth and 20 years old secondary succession forest. Tiwari *et al.* (2002) also reported decline in microbial biomass C in humid tropical hill forest soils of north-eastern India after forest soil disturbance by shifting cultivation and selective logging practices. Microbial biomass C was reduced by 50% in degraded site and 35% in moderately degraded site in comparison to undegraded forest site at surface layers. Similar reduction in microbial biomass C was also recorded at subsurface soil layer of degraded site while the reduction was less (20%) in case of the moderately degraded site. This reveals the long-term detrimental effect of shifting cultivation and selective logging practices on microbial biomass recovery of humid tropical hill forest soils. The reason

for higher microbial biomass C and N in the undegraded forest soil in the present study could be due to presence of dense vegetal cover and thick layer of litters (leaf and twigs) and decaying woods which harbour greater growth of microbial communities (mostly bacteria and fungi). Arunachalam *et al.* (1996) and Maithani *et al.* (1996) have also reported that relatively dense growth of plants and higher accumulation of the litter on the forest floor and distribution of fine roots in older forest re-growth favour the growth of microorganisms and accumulation of microbial biomass in comparison to younger re-growth forests in humid subtropical forest soils of north-eastern India. Srivastava (1992) reported maximum microbial biomass C and N from mixed forest and minimum from 5 year old mine spoil soil of dry tropical regions of Uttar Pradesh, India. Higher microbial biomass C content was also reported in old-growth forests as compared to 3 and 10 year old forests planted following harvesting in British Columbia (Chang *et al.*, 1995). Similar findings of higher microbial biomass C had been reported from the mixed secondary monsoon forest than in barren land and monospecied forests of tropical China (Mao *et al.*, 1992). They also demonstrated that litter removal altered soil microbial biomass content and further accelerate loss of C and nutrients. Srivastava and Singh (1991) noted remarkable decline in the amounts of soil microbial biomass C and N following conversion of tropical forests into alternate land use systems. Further they found higher biomass nutrients in forests followed by savanna, cropland and lowest in mine spoil soils.

The presence of comparatively higher microbial biomass C at the surface (0-20 cm) layers of all the study sites reveals higher microbial activity (fungal and bacterial populations) and organic matter content at the surface layer. Lavahun *et al.* (1996) also found significant decline in the microbial biomass C with increasing soil depth from 0-10 to 70-90 cm in three sites namely permanent grassland, arable site with neutral pH and arable site with acidic pH. They also noted 77% to 80% of the total biomass stored at the plough layer (0-30 cm) of various sites. Their study confirmed that decrease in metabolic efficiency and increase in metabolic quotients ( $qCO_2$ ) in deeper layers as the probable reason for reduced microbial biomass. Maithani *et al.* (1996) also reported significantly greater ( $P < 0.01$ ) microbial biomass C and N in surface (0-10 cm) layers of disturbed sub-tropical humid forests than in subsurface layers. The reason for this higher microbial biomass in surface soils layer was attributed to the greater accumulation of litter and fine root biomass and presence of relatively dense growth of plants in the forests.

No definite trend of microbial biomass C and N distribution was observed during the entire experimental period of two years (July 1998 to June 2000) in all study sites. However, microbial biomass C and N were recorded higher during summer rainy months and lower during dry winter periods. This suggests the possible influence of soil water in soil microbial biomass accumulation in these study sites since higher biomass C and N were recorded during the periods when soil moisture contents were higher. Similar seasonal variation of microbial biomass with higher amount during rainy season had been reported from pasture and dry tropical forest soils

(Sarathchandra *et al.*, 1984 and Singh *et al.*, 1989). On the contrary, lower microbial biomass C and N were recorded during rainy season and higher during winter dry periods from sub-tropical humid forests of north-eastern India (Maithani *et al.*, 1996). The reason for higher microbial biomass C and N during the rainy season in this study could be due to rapid mineralization of soil nutrients in microbial cells in presence of favourable soil moisture. The presence of higher moisture content in the soils would have caused rapid multiplication of microbial cells and accumulation of microbial nutrients. Mao *et al.* (1992) also reported similar results of higher microbial biomass C content during early rainy season than before onset of rainfall, and they assumed that nutrients derived from litter that accumulated on the soil surface during dry season was immobilized by microorganisms as soon as water availability allowed microbial growth. Despite the seasonal variation in distribution of microbial biomass, variation in amounts of the microbial biomass was more significant between the sites ( $P < 0.05$ ). This reveals the significant impact of site management on soil microbial biomass accumulation in forest soils in the abandoned fallow contained lower microbial biomass than selectively logged forest and undegraded forest sites. However, Zeller *et al.* (2001) have found less importance of management abandonment on soil microbial biomass than effects of site and sampling time suggesting significant influence of site variation and sampling time on microbial biomass distribution. Diaz-Ravina *et al.* (1995) reported significant variation of microbial biomass in forest soils due to soil type and seasonal fluctuations. Their study confirmed that variation in the microbial

biomass of forest soil is mainly due to the type of soil, which explained 71% of the total variation, rather than seasonal variation that accounted only 18% of the variation and 8% by the interaction between the soil type and seasonal changes. Further they concluded that the type of soil as the most important factor for variation in microbial biomass in forest soils. Therefore, microbial biomasses C and N distribution in forest soils depend largely on the type of site and soil rather than temporal variation.

## **Chapter 5**

### **5.1. Genomic diversity of soil community DNA and structural diversity of bacterial community as measured by ERIC-PCR and 16S rDNA profiles**

#### **5.1.1. INTRODUCTION**

The use of small-subunit ribosomal DNA gene (16S rDNA or rRNA) as a bio-molecular marker has become a routine technique for a culture-independent studies of bacterial diversity in molecular microbial ecology. The sequence analysis of the gene isolated and purified from many environmental samples have shown that this molecular probe as one of the most powerful tools in microbial diversity research, molecular evolution and phylogenetic classification of the living organisms (Zhou *et al.*, 1997). The restriction analysis of the 16S rDNA gene after isolation and purification from the total soil DNA with the help of polymerase chain reaction (PCR) can determine the identity and diversity of the bacterial communities very easily in soil as compared to the traditional plate culture methods (Dunbar *et al.*, 1999, 2000). The reasons for the using this 16s rDNA gene as a molecular marker is due to the universal distribution of this gene in all the communities of the domain *bacteria*, structural and functional conservation and size which allows for sufficient sequence divergence (Ludwig and Schliefer, 1994 and Goebel, 1995). The other important characteristics are that the 16S rDNA gene has regions which are highly conserved while other regions display considerable sequence variation even within closely related taxa. This conserved sequence has been used as a phylogenetic marker for

classification of bacteria into different taxa. These characteristics allows the inference of phylogenies based on the comparative sequence analysis of the 16S rDNA gene, from the deepest separation of the different branches of life to the genus or even species or strain level, and facilitates identification and classification of microorganisms with little effort (Ludwig and Schliefer, 1994 and Olsen *et al.*, 1986 and 1994). In fact, categorizing of the 16S rDNA gene from community DNA of environmental samples has become a popular alternative to characterise microbial communities because it avoids the limitations of cultivability and directly provide information on phylogenetic diversity (Zhou *et al.*, 1997). However, the cloning and sequencing strategies are rather time and labour consuming and thus not suitable for monitoring a large number of samples, e.g., in studies on the succession of microbial communities during the growing season, or following shifts of microbial communities after perturbations (Heuer and Smalla, 1997). The use of the denaturing gradient gel electrophoresis (DGGE) of the PCR amplified DNA fragments has become a new approach to the study of the structural diversity of microbial communities which overcomes the disadvantages in cloning and sequencing of the DNA fragments. DGGE was initially developed for use in the medical research for detection of point mutations (Fisher and Lerman, 1983; Myers *et al.*, 1985, 1987 and Sheffield *et al.*, 1989) but it was introduced in the microbial ecology by Muyzer *et al.* (1993). This technique has the potential needed for monitoring technique since it offer the chance to analyse the major constituents of microbial communities by generating fingerprints (Heuer and Smalla, 1997).

### 5.1.2. REVIEW OF LITERATURE

Pace *et al.* (1985) were the first to suggest the use of the 16S rDNA gene as molecular marker for cultivation-independent studies of microbial populations in environmental samples. Since then, considerable amount of works has been done on microbial community studies based on the 16S rDNA gene.

Borneman *et al.* (1996) conducted a culture-independent survey of the soil microbial diversity in a clover-grass pasture in Wisconsin by sequence analysis of the universally clone library of gene coding for the small sub-unit 16S rDNA and found that 98.4% of the 124 clones sequenced were derived from the domain bacteria having a diverse group of genera which have been never identified before.

A similar study on the impact of deforestation on the microbial diversity of southern Amazonia revealed that a shift in microbial diversity occurs when soils are disturbed due to deforestation as shown by reduced bacterial diversity of the pasture soils than mature forest soils in terms of 16S rDNA sequences (Borneman and Triplett, 1997).

Brinkhoff and Muyzer (1997) reported successful monitoring of the sulphur-oxidizing *Thiomicrospora* spp and their diversity in a range of extended habitats by using DGGE of the PCR amplified 16S rDNA fragments followed by hybridization analysis with a primer as a genera specific probe.

Marilley *et al.* (1996) identified specific bacterial strains present in the microhabitats in soil after 16S rDNA gene was amplification and restriction

analysis from the community DNA of different soil fractions under two crops, *Trifolium repens* and *Lolium perenne*. Their study showed that plant root created a selective environment for microbial populations as revealed by diverse 16S rDNA sequences.

A recent study on the bacterial community structure and diversity in rhizosphere soils of two grassland types, improved and unimproved upland grass pastures in UK had shown no significant variation in the 16S rDNA clone sequences though there were underlying differences in the specific components of the populations which may have been related to the community functions in the two soils (McCaig *et al.*, 1999).

Duineveld *et al.* (2001) analysed the bacterial communities of the rhizosphere of *Chrysanthemum* by DGGE of the PCR amplified 16S rDNA. They have identified majority of the bacterial communities mostly, *Pseudomonas*, *Comamonas*, *Vericovorax* and *Acetobacter* after excising the prominent DGGE bands and subsequent sequencing of the gene.

A recent analysis of the 16S rRNA from the two soils by DGGE and canonical variate analysis using weighted and unweighted data demonstrated that there was a clear difference in microbial diversity between different grasslands (McCaig *et al.*, 2001).

### **5.1.3. METHODOLOGY**

Isolation of community DNA was done using the FastDNA<sup>®</sup> SPIN Kit for Soil (BIO 101). Approx. 0.5 g of fresh soil was taken in a 2 ml E-tube containing lysing mixture. 978 µl SPB (sodium phosphate buffer) and 122 µl MT buffer were added in the tube and homogenized at maximum speed for 1

minute. The suspension was centrifuged for 1 minute at 14000x g and the supernatant was transferred into a clear 2 ml tube and 250 $\mu$ l PPS (protein precipitation solution) was added followed by mixing the tube for 2 minutes. Then the tube was centrifuge again for 5 minutes at 14000x g and the supernatant was transferred into a 15 ml tube. 1 ml of binding matrix suspension was added and the tube was turned upside down for at least 5 times to allow binding of DNA to the matrix. About 500  $\mu$ l of the supernatant at the surface layer of the tube was discarded and resuspended the remaining supernatant in the binding matrix. The slurry was transferred in to a Spin Tube in two aliquots of 500  $\mu$ l each and centrifuged to discard the waste liquids. Finally, the DNA in the Spin filter was washed with 500  $\mu$ l of SEWS-M (salt/ethanol wash solution, DNase-free) at 14000 x g for 1 minute and the filtrate was discarded. The Spin was removed from the tube and dried for 5 minutes at room temoerature. The Spin was replaced into a fresh catch tube and 50  $\mu$ l of DNA eluting solution (DES, Dnase/Pyrogen free water) was added while gently stirring the filter membrane with the pipette tip. Then, he tube was centrifuges at 14000 xg for 1 minute to elute the DNA into the catch tube. The DNA content of the extract was checked at 1% agarose gel.

### **Enterobacterial repetitive intergenic consensus (ERIC) PCR**

The DNA sample was amplified by using the universal primers (ERIC-I) to see the presence of DNA amplicons for further restriction analysis of desired genes for microbial community analysis. The reaction mixture of 50 $\mu$ l contained 5 $\mu$ l 1x PCR buffer, 2.5 $\mu$ l of 2.5 $\mu$ M MgCl<sub>2</sub>, 5 $\mu$ l of

3% bovine serum albumin (BSA), 5 $\mu$ l 0.2 mM dNTP, 2 $\mu$ l of ERIC-I universal primer, 29  $\mu$ l RNase free water, 1 $\mu$ l DNA and 0.5  $\mu$ l Taq DNA polymerase (Invitrogen). The PCR was done in two reactions, first with 10 min hot start at 95°C, pause at 80°C and add Taq DNA polymerase. Second reaction starts with denaturation at 94°C for 1 min, annealing at 53°C for 1 min, elongation at 65°C for 2 min for a total of 30 cycles followed by final stabilisation of the products at 65°C for 10 minutes.

#### **16S rDNA amplification for denaturing gradient gel electrophoresis (DGGE)**

16S rDNA gene was selectively amplified using the eubacterial primers, F968-GC (5'-CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG G-AA CGC GAA GAA CCT TAC-3') and R1401-G (5'-CGG TGT GTA CAA GGC CC-3') for the analysis of bacterial community in the soil samples by DGGE. 1 $\mu$ l of the DNA sample was used for amplification of 16S rDNA in a PCR reaction volume of 50  $\mu$ l (5 $\mu$ l 1x PCR buffer (Invitrogen), 3  $\mu$ l of 1.5 mM MgCl<sub>2</sub>, 5 $\mu$ l of 3 % BSA (Sigma), 5 $\mu$ l 0.2 mM dNTP (Fermentas), 2.5  $\mu$ l 5 % DMSO, 1 $\mu$ l of 10 pM F968-GC primer, 1  $\mu$ l 10 pM R1401-G primer, 26  $\mu$ l RNase free water (Sigma), 1 $\mu$ l DNA and 0.5  $\mu$ l Taq DNA polymerase (Invitrogen). The PCR was completed in two reactions steps, first with 10 min hot start at 95°C, pause at 80°C and add Taq DNA polymerase. Second reaction starts with denaturation at 93°C for 1 min, annealing at 62°C for 1 min, elongation at 72°C for 1 min for a total of 30 cycles followed by final stabilisation of the products at 72°C for 10 minutes.

The amplified PCR products of 16S rDNA was checked for the presence of 473 bp 16 rDNA band and purified using the QIAquick PCR purification kit protocol (QIAGEN).

### **DGGE analysis and Gel compare**

DGGE was performed by using 6% acrylamide gel (ratio of acrylamide to bisacrylamide, 37:1, Bio-Rad) with a 45 to 65% denaturing gradients {the 100 % denaturing solution comprised of 15 ml of 40 % PAA dissolved in 40 ml of formamide with 42 g of urea (Sigma) and 2 ml of 50x TAE buffer and the final volume maintained at 100 ml with milli-Q water}. 15  $\mu$ l of the purified 16Sr DNA was loaded in the gel using one-third volume of the DGGE buffer dye in each lane for the six different samples. The gel was electrophoresed at 60°C for 17 h at a constant voltage of 70V by using the universal mutation detector system (Bio-Rad). The gel was fixed in 25 % Glacial acetic acid solution for 30 min. followed by three times washing for 2 min. each with milli-Q water. Then the gel was stained in silver nitrate solution ( $\text{AgNO}_3$ , SIGMA) for 25 min, washed twice with milli-Q water for 30 sec. and developed the images in sodium thiosulphate + sodium carbonate solution. The gels were then put in Na-EDTA solution for 10 min to stop darkening of the gel. The gel was dried at 45 to 50°C for 48 hrs in dark and the images were captured using HP Scanjet II scanner.

### **Gel compare analysis**

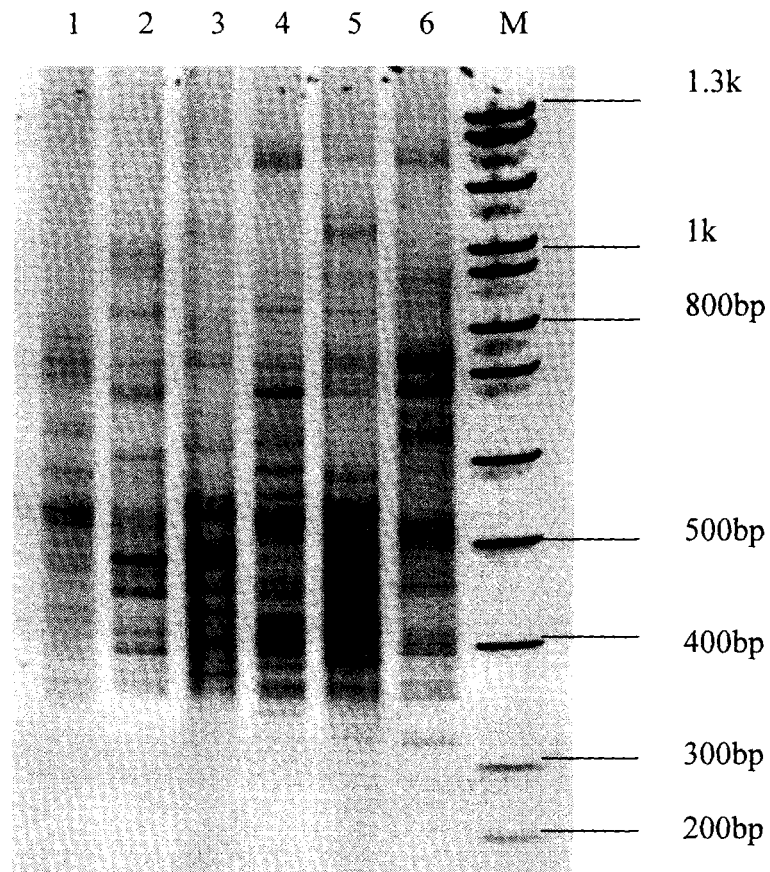
The DGGE images were processed in microsoft photo editor for removal of background colors and for sharper bands before analysis using Gel Compare-II software (Applied Maths, Belgium).

#### **5.1.4. RESULTS**

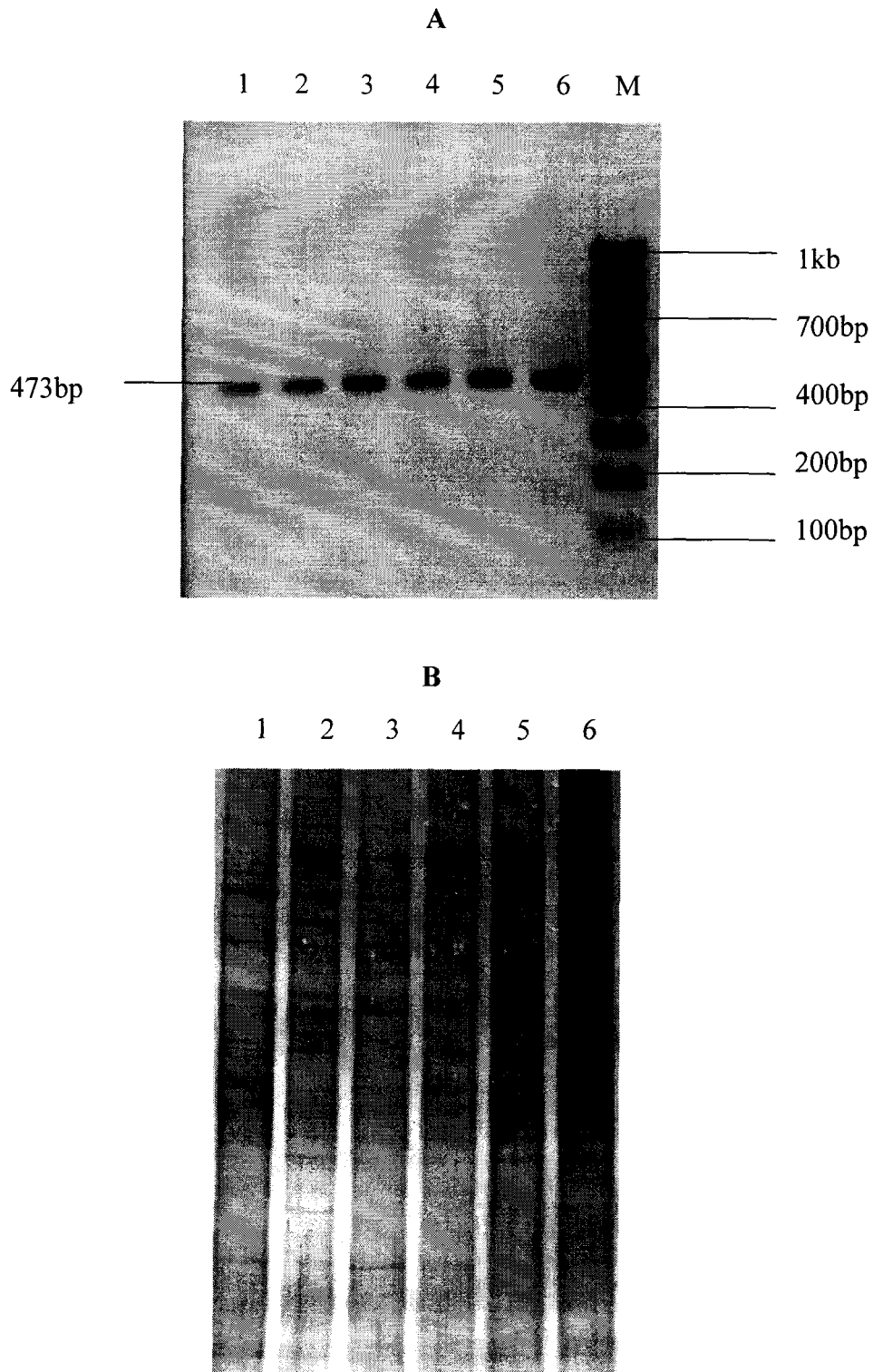
##### **ERIC profiles of total soil genomic DNA**

The total genomic DNA profiles of the soil samples from surface and subsurface soil layers of the three study sites as revealed by the enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) are shown in figure 5.1.1. The fingerprints of the DNA bands demonstrates the existence of a variety of organisms present in these soil ecosystems. The surface soil layer of the degraded forest site showed minimum number of ERIC bands as compared to the other samples. The surface soil layers of the moderately degraded and undegraded forest sites exhibited more number of ERIC DNA bands than the other soil samples.

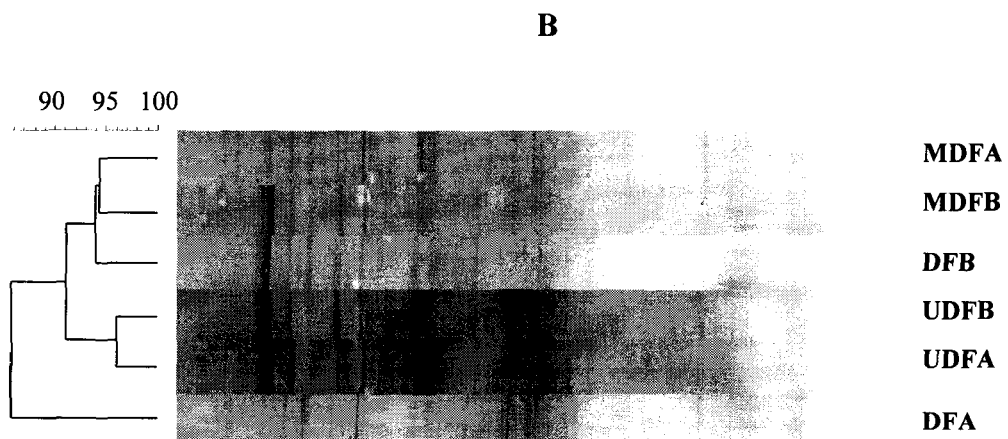
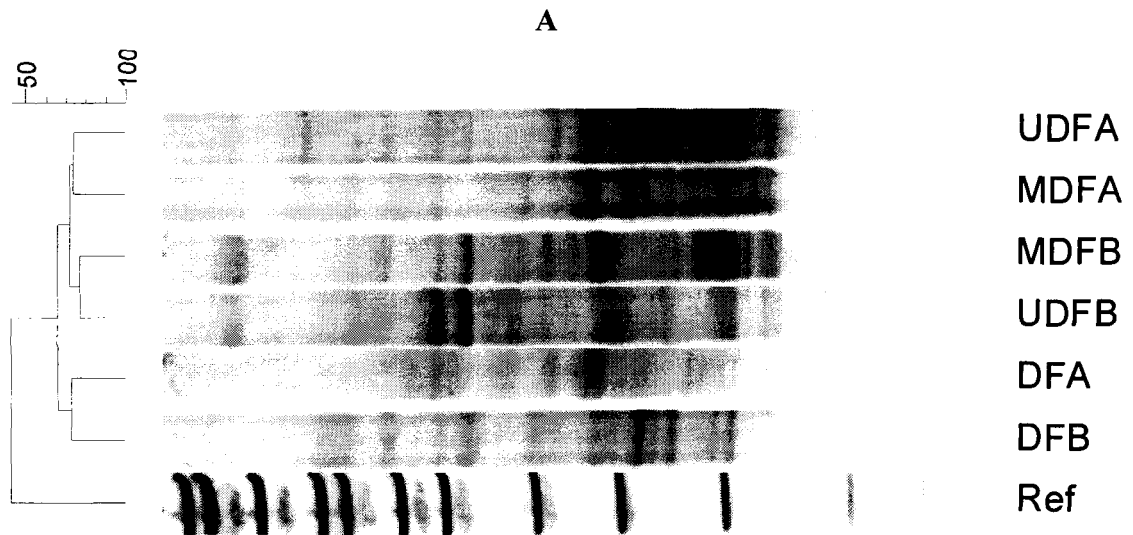
Gel compare analysis (based on Pearson's correlation) of the total soil DNA from ERIC-PCR of soil samples from three study sites resulted two groups of genomic diversity. Group I consists of the repetitive intergenic consensus sequences of all microorganisms from surface (DFA) and subsurface (DFB) soil layers of the degraded site (fig. 5.1.3A). The similarity index of the banding pattern between these two soil samples shows a maximum of 75% revealing that the composition of microbial communities in these two layers were almost same. Group II(ab) consists of the genomic DNA of microorganisms from the moderately degraded and undegraded forest sites at both the surface and subsurface soil layers. There was a clear subdivision of the two soil layers into subgroups, IIa and IIb respectively. The subgroup IIa comprised of the surface soil layers from moderately degraded (MDFA) and undegraded forest sites (UDFA) with 73% similarity index.



**Fig. 5.1.1.** Total genomic DNA profiles of six soil samples on PAGE after ERIC-I PCR. Surface soil layer (1) and subsurface soil layer (2) of degraded site; surface soil layer (3) and subsurface soil layer (4) of moderately degraded site; surface soil layer (5) and subsurface soil layer (6) of undegraded site and 100bp DNA ladder (M).



**Fig. 5.1.2(AB).** 16S rDNA band (473bp) of six soil samples on 1% agarose gel (A) and DGGE profiles of 16S rRNA bands of six soil samples. Surface soil layer (1) and subsurface soil layer (2) of degraded site; surface soil layer (3) and subsurface soil layer (4) of moderately degraded site; surface soil layer (5) and subsurface soil layer (6) of undegraded site.



**Fig. 5.1.3(AB).** Gel compare analysis (Pearson's correlation) of ERIC-PCR amplified DNA bands (A) and 16S rDNA bands (B) of six soil samples. Surface soil layer (DFA) and subsurface soil layer (DFB) of degraded site; surface soil layer (MDFA) and subsurface soil layer (MDFB) of moderately degraded site; surface soil layer (UDFA) and subsurface soil layer (UDFB) of undegraded site. Ref = DNA ladder (100bp)

Similarly, the subsurface layers of moderately degraded (MDFB) and undegraded (UDFB) formed the subgroup IIb with a greater similarity index (80%) than the subgroup IIa. Therefore, there is classification of the soil layers in terms of total genomic composition of the microorganisms in this cluster analysis of the moderately degraded and undegraded forest sites.

The genomic composition of microorganisms in the two groups (all the soil samples from the three sites) in this cluster showed about 65% similarity index.

### **Restriction PCR of 16S rDNA gene and DGGE profiles**

The restricted digestion of the 16S rDNA gene from the surface and subsurface soil layers of the three study sites produced a clear band of 473 bp (fig. 5.1.2A). This product was used for separation of the 16S rDNA genes with different nucleotide sequences by DGGE.

The DGGE profiles of the 16S rDNA genes of the three study sites at two soil layers are depicted in figure 5.1.2B. Comparative analysis of the banding patterns of the 16S rDNA genes in all the soil samples showed clear variation among the soil samples of the three study sites (fig 5.1.3B). The gel compare analysis of the 16S rDNA bands of the soil samples from the three study sites resulted in three cluster groups as shown in figure 5.1.3B. The group I consists of only the bands from surface layer of the degraded site (DFA) with a total banding pattern similarity index of 85% with other soil samples. The group II cluster is formed by surface (UDFA) and subsurface (UDFB) layers of the undegraded forest site. These soils have a total 16S rDNA bands with a similarity index of 96%. Similarly, the group III cluster is formed by three soil samples and subdivided into two subgroups, IIIa and

IIIb respectively. The subgroup IIIa consists of surface soil layer (MDFA) and subsurface soil layer (MDFB) of the moderately degraded site with a 94.5% similarity index of 16S rDNA banding patterns between the two soil samples. The subgroup IIIb consists of only the subsurface soil layer of degraded site (DFB) with a similarity index of 94% banding pattern to that of IIIa. There was a 91% similarity of the banding patterns of the 16S rDNA bands between the groups II and III in this analysis.

### **5.1.5. DISCUSSION**

#### **5.1.5.1. Gel compare analysis of microbial community by ERIC-PCR amplified DNA fragments**

The results from the gel compare analysis of ERIC-PCR products clearly indicated that the soil in the degraded site is quite different in composition of microbial genomic diversity as compared to moderately degraded and undegraded sites. The degraded site with comparatively less number of ERIC bands are separated from the other two sites with more ERIC bands. The distribution of the microbial genomes at the surface and subsurface soil layers of degraded site shows similar microbial diversity at these two layers. However, the presence of the moderately degraded and undegraded forest sites in a separate group revealed that similar genomic diversity of microorganisms existing between the two sites despite clear difference between the two soil layers.

It can be mentioned here that the degraded site in the present study is a regenerating jhum fallow land (shifting cultivated fallow). This site had been used for more than 15 years for jhum during which the soil had been disturbed with repeated burning, tillage and cropping. Moreover, this site

being a hill slope, the top soil has been lost considerably during the course of cultivation and by runoff during heavy rainfall occurred in the region. The surface layer of the soil shows very thin Ah horizon with exposed B or C horizons and characterised by increased bulk density and are not favourable soil environment for microbial growth and multiplication. These might have caused decline in microbial diversity in this site as compared to the other two sites.

The moderately degraded forest site on the other hand has not been used for cultivation in the past though selective logging of forest trees continues till now. However, the lower percentage of similarity index for ERIC-DNA bands in the surface layer than the subsurface layers could be due to the burning of the forest floor for every alternate years by forest personals. This continuous practice of clearing the forest floor and subsequent burning of the dried mass in this site must have a profound impact on the microbial communities in the soil resulting a changed diversity status as compared to the subsurface layer where impact of fire is less.

The undegraded forest site on the other hand had a similar composition of total genomic diversity to the moderately degraded site at both the surface and subsurface soil layers.

#### **5.1.5.2. Bacterial genomic diversity as revealed by DGGE analysis of the PCR amplified 16S rDNA fragments**

The 16S rDNA pattern of the different soil samples showed different profiles of the bacterial genomic diversity. Since, the gene of the 16S rDNA is derived from the bacterial communities, the DNA bands seen in the image are expected to represent the available bacterial communities in the soil. In

fact, the gel compare analysis uses the number of DNA bands available in a particular lane which represents a soil sample in the gel image. The cluster analysis is based on the number and similarity of bands in each lane. Therefore, the resulting classification of the clusters are the indices of similarity or dissimilarity of the genomic compositions among the samples. The similarity index of the cluster analysis reveals that 85% of the total genomic diversity is same for degraded, moderately degraded and undegraded sites at both the surface and subsurface layers. However, the surface and the subsurface soil layers of the undegraded site had the maximum similarity index of 96% which reveals that the bacterial communities between the two soil samples were more similar than the rest of the samples. The surface soil layer of the degraded site on the other hand showed minimum 16S rDNA bands in comparison to the other soils thereby revealing reduced microbial composition as a result of consistent jhum cultivation in the past. The lower percentage of similarity index of the banding patterns in this site than other soil samples suggests that more diverse groups of bacterial community might be inhabiting in this disturbed site. This results are in correspondence with the lower bacterial population of the degraded site determined by cultivation method in this study.

The surface and subsurface soil layers of the moderately degraded site and subsurface layer of degraded site were clustered in one group revealing that these soils contained similar bacterial communities different from the other soil samples. It may be mentioned that the surface soil layer of moderately degraded site has also been disturbed for every year or for alternately by clear-cutting and burning of the forest floor vegetation for

proper growth of the introduced trees. This practice must have some detrimental impact on the bacterial communities at the surface soil layers but the impact was comparatively lower at the subsurface soil layers, hence less disturbance in the bacterial community structure.

Therefore, a conclusion can be drawn from the above results and discussion that the culture-independent analysis of the bacterial genomic diversity as revealed by gel compare analysis of the 16S rDNA gene from the DGGE profiles had a similar results of bacterial community structure to that of the cultivation-dependant studies. The cluster analysis of the DGGE bands of 16S rDNA genes revealed a clear separation of the degraded and moderately degraded sites from that of the undegraded site in terms of bacterial genomic communities.

## **Chapter 5**

### **5.2. Microbial community structure of soils as measured by phospholipids fatty acid (PLFA) profiles**

#### **5.2.1. INTRODUCTION**

Phospholipid fatty acid (PLFA) or fatty acid methyl ester (FAME) profile analysis has become a culture-independent, modern molecular approach, for studying the community structures, diversity and biomass of microorganisms in environmental samples such as soil, sediments, water etc. The PLFA/ FAME profiles of soil samples offer a rapid, inexpensive and reproducible means for characterizing numerically dominant portions of soil microbial communities, including those organisms not culturable and for microbial biomass estimations (Cavigelli *et al.*, 1995; Frostegard and Baath, 1996; Zelles, 1999 and Zelles and Bai, 1993). Isolation and characterization of the marker or signature fatty acid profiles from soil samples and identification of marker or signature fatty acids is the only molecular approach alternative to the ribosomal DNA targeted approaches used as culture-independent methods in understanding the microbial components and their diversity in environmental samples. The quantitative measurement of ester-linked fatty acids in phospholipids and hydroxy fatty acids in lipopolysaccharides has been regarded as one of the most sensitive and reliable chemical measures of microbial biomass and community structures (Zelles and Bai, 1993). The basis for the use of this approach is that viable microbes have an intact membrane which contains fatty acids as components of its phospholipids and these fatty acids can be separated

easily into neutral lipids, glycolipids and phospholipids from the soil samples using organic solvents like chloroform, acetone and methanol on silicic acid columns (Frostegard, 1995 and Zelles, 1999). Mild alkaline methanolysis of this lipid fractions can be further separated into ester-linked (EL-FAME) and non-ester-linked acids (NE-FAME) by gas chromatography after dissolving in isooctane or hexane to give a profile of fatty acids in the soil sample (Frostegard *et al.*, 1993 and Zelles, 1999). This PLFA/ FAME profiles can provide an insight view of the microbial community structure of the soils because of the relative abundance of certain PLFAs which differ considerably among specific group of microorganisms (Zelles, 1999). However, the interpretation of the PLFA/ FAME profiles from environmental samples can be difficult because many fatty acids are common to different organisms and many are extracted from each sample (Cavigelli *et al.*, 1995; and Zelles, 1999). Assigning a fatty acid as biomarker to an organism may lead to the incorrect interpretation of a fatty acid as the indicator of another microorganisms since it is not investigated in the other organism (Zelles, 1995, 1999). Therefore, It is necessary to identify the fatty acid compositions of the individual species from the whole community PLFA profile which makes up the microbial community.

### **5.2.2. REVIEW OF LITERATURE**

Zelles *et al.* (1992) studied eight long-term agricultural monocultures and rotation-crop experimental plots for their fatty acid profiles in either phospholipid or lipopolysaccharide fractions to identify signature fatty acids as indicators of microbial biomass and community structures. More than 100

fatty acids under different fractions were found in the soils of the different plots which had a good correlations to the microbial biomass and activities obtained by different chemical methods indicating a close linkage of these fatty acids to the soil microorganisms.

Zelles and Bai (1993) developed a single phase and extended extraction procedure for the PLFAs in soils and could identify more than 160 PLFAs derived from phospholipids and several dozens from the hydroxy fatty acids derived from the lipopolysaccharides reflecting the complexity of the microorganisms present in the soil and thus diversity.

Cavigelli *et al.* (1995) analysed the FAME profiles of an agronomic plot planted with tilled corn to measure the microbial community structure. Their study showed that 20% of the total 162 soil samples analysed had relatively low and about 10% had relatively high bacterial : fungal ratio. Altogether 56 FAMEs were identified in the study and more than 8% of them were detected in the first 12 samples, indicating that most of the diversity detected in the study was present within a very small portion of the study area.

Frostegard and Baath (1996) studied 15 soils of Sweden and revealed that the amount of the bacterial biomass measured by determining PLFA patterns were much higher than the one measured by acridine orange microscopic counts.

Zelles (1997) investigated the PLFA patterns in selective members of microbial communities to identify "signature" fatty acids using the extended extraction method. The amount of the non-ester linked fatty acids was high as 70% of the phospholipids in some fungi while the *cis-vaccinic* acid

constituted about 50% of the selected bacteria belonging to the alpha subclass *Proteobacteria*. Similarly, presence of the branching at localized position of C other than *iso/ anteiso* were found to be strong indication of Gram-positive bacteria.

Schlöter *et al.* (1998) assessed the microbial diversity in soils of a fallow land and crop field using PLFA analysis revealed a significant variation in structure of the whole microbial community in the soil of the hop field to that of the crop rotation.

Similarly, PLFA profiles of soils as affected by flooding and carbon substrate stress revealed that increased in the monounsaturated fatty acids occurred when C substrates was added or decreased when flooded, therefore, shifts in the aerobic microorganisms in the soil while the branched fatty acids decreased with high substrate condition (Bossio and Scow, 1998).

A sensitively higher bacterial: fungal biomass ratio was recorded in the unfertilised grasslands than in the fertilized grasslands of northern England as measured from the PLFA concentrations of the soils along a gradient of long-term experimental plots having varying management intensities (Bardgett and McAlister, 1999).

Gattinger *et al.* (2002) reported variation in the microbial community structure of different soil zones of a potato field as revealed by total PLFA profiles. The compactin of interrow soil caused increased in bacterial and eukaryotic biomass as measured by total PLFA concentration as well as an increase in total archaeal biomass expressed in total ester-linked phospholipid (PLEL) concentrations.

Ponder and Tadros (2002) reported no variation in bacterial biomass of forest soils between treated and control sites as determined by PLFA analysis. However, their study showed increased in prokaryotic organisms outnumbering the polyenoic organisms by 5 to 1. This difference in the PLFA patterns attributed to the soil disturbance suggested that there occurs an increased PLFA profiles of specific group of organisms over some other groups.

### **5.2.3. METHODOLOGY**

#### **Soil sampling**

Soil samples were collected from the three study sites in the middle of April 2002 for the analysis of PLFA patterns. The procedure of soil collection and soil depths are same with those samples collected for other biological and biochemical characteristics (Chapters 2 and 3).

#### **Extraction of lipids from soil**

PLFAs were extracted from fresh soil samples using the one phase extended method of Zelles and Bai (1993). About 50g soil (dry weight) was put in a flask and 250 ml methanol, 125 ml chloroform, 0.05M phosphate buffer {pH 7.4; 100-moisture content (%) in soil} were added. After shaking for 2 h, 125 ml of H<sub>2</sub>O and 125 ml of chloroform were added into the flask. After a thorough mixing, the soil flask was kept for 24h, the water phase at the top was removed and discarded carefully. The organic phase and slurry were passed through a filtration funnel containing 2 cm Celite 545. The organic phase is transferred to a separatory funnel and separated, dried

over anhydrous sodium sulphate and finally the chloroform-phase is reduced to a small volume (=10ml) after evaporation.

**Separation of lipid fractions by silicic acid (SI) column:**

The  $\text{CHCl}_3$ -phase sample was fractionated in a SI column (2g silicic acid/ 12ml) into neutral lipids with one volume (1V) of chloroform and discarded. Glycolipids and phospholipids were separated with 1V of acetone and 4 V of methanol respectively. The phospholipid fraction was evaporated and reduced to almost dryness.

**Mild alkaline hydrolysis:**

The residue of phospholipid was dissolved in 1 ml of methanol: toluene (1:1, v/v) and 5 ml of 0.2M KOH in  $\text{CH}_3\text{OH}$  (freshly prepared) was added. The pH of the mixture was adjusted at approx. pH 6 with 1M acetic acid after incubated for 15 min at  $37^\circ\text{C}$ . Then 10ml each of  $\text{CHCl}_3$  and  $\text{H}_2\text{O}$  were added and transferred to a centrifugation tube. The tube was shaken for a while at wrist motion and centrifuged at 3000 rpm for 15 min. the  $\text{CHCl}_3$  phase was separated and the  $\text{H}_2\text{O}$  phase was extracted once more with 5ml of  $\text{CHCl}_3$ , centrifuged again and separated the  $\text{CHCl}_3$  phase into the same flask. This  $\text{CHCl}_3$ -phase was dried over  $\text{NaSO}_4$  and reduced to a small volume.

**Separation of FAMES from the OH-FAMES and unsaponifiables by  $\text{NH}_2$  column:**

The  $\text{NH}_2$  column (size 0.5 g/3ml) was conditioned with 1V of hexane :  $\text{CH}_2\text{Cl}_2$  (3: 1, v/v). The sample was dissolved in hexane :  $\text{CH}_2\text{Cl}_2$  (3:1 ; v/v) for separation of unsubstituted FAMES (UNSOH) and eluted with 1 V of

$\text{CH}_2\text{Cl}_2$  : ethyleacetate (9:1, v/v) for OH-substituted FAMES (PLOHs) and with 2V of 2% acetic acid in methanol for unsaponifiables (UNSFAs).

**Separation of FAMES with different double bounds by SCX column impregnated with  $\text{Ag}^+$ :**

SCX column (size 0.5 g/3ml). 0.1g of  $\text{AgNO}_3$  in 1.5 ml of  $\text{CH}_3\text{CN}$  :  $\text{H}_2\text{O}$  (10 : 1, v/v) is put on the column and let passed through by gravity, followed by 2V of  $\text{CH}_3\text{CN}$ , 2V of acetone and 2V of  $\text{CH}_2\text{Cl}_2$  for washing the column. The sample in the  $\text{CH}_2\text{Cl}_2$  : hexane (7:3, v/v) was quantitatively put on the column and then eluted with 2V of  $\text{CH}_2\text{Cl}_2$  : hexane for separation of saturated fatty acid (SATFA), 2V of  $\text{CH}_2\text{Cl}_2$  : acetone (9:1, v/v) for monosaturated fatty acid (MUFA) and 4V of acetone:  $\text{CH}_3\text{CN}$  (9:1, v/v) for polyunsaturated fatty acid (PUFA) fractions.

**Acidic methylation of free fatty acids or unsaponifiables:**

The residue was dissolved in 2ml of  $\text{CH}_3\text{OH}$  :  $\text{CHCl}_3$  : HCL (37%) in the ratio 10:1:1 (v/v/v) ratio in a 12ml vial and was kept for overnight at 60°C. Then the content was transferred to a centrifugation tube and 2 ml of 2% NaCl was added. Then the FAMES were extracted in 4ml x 3 of hexane: toluene (1:1, v/v).

**Trimethylsilyl (TMSI) derivatization:**

0.5ml of a mixture of pyridine : bis (Trimethylsilyl trifluorocis amide, BSTFA): hexamethyldisilazane : trimethylchlorosilane (0.2:1:2:1, by volume) was added to a vial containing the residue to be derived into TMSI. It was kept at 60°C for 15 min and then evaporated by a stream of  $\text{N}_2$ .

**Dimethyldisulfide (DMDS) derivatization:**

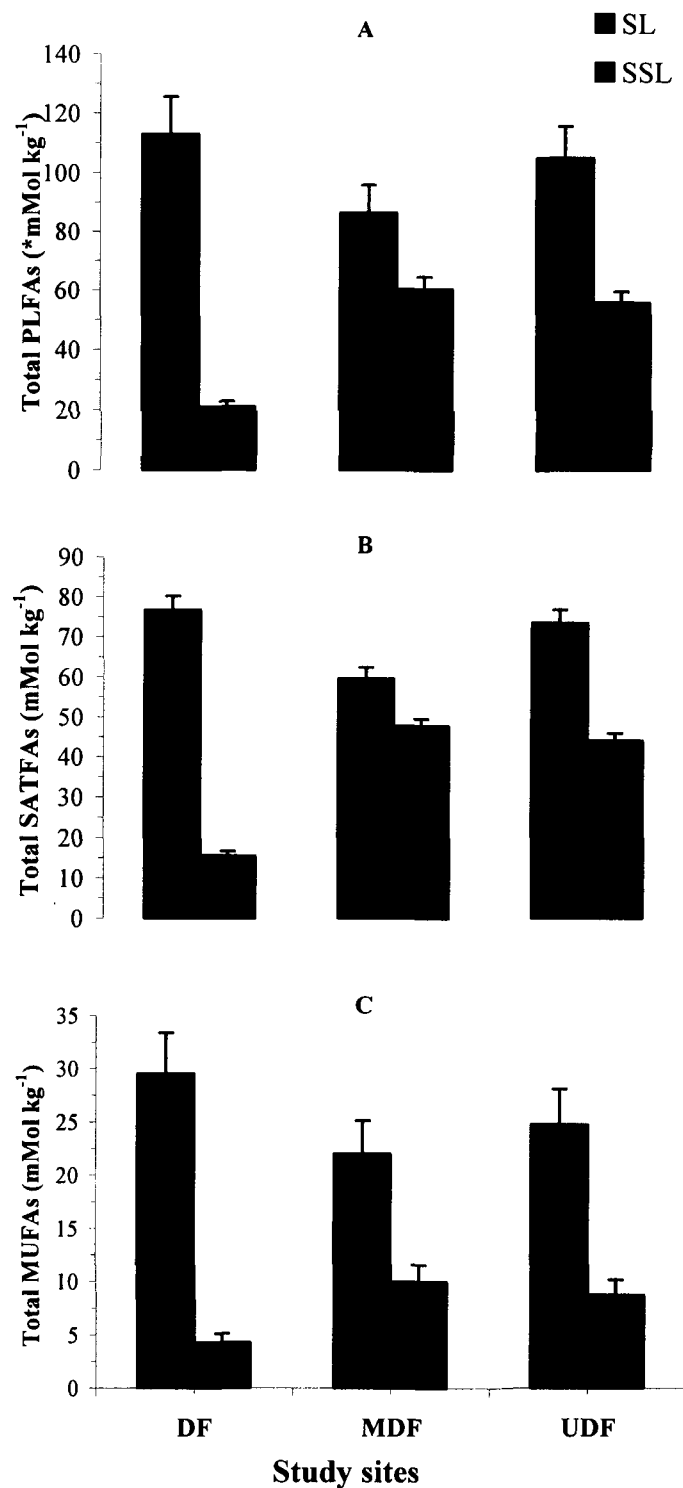
The residue was dissolved in 0.05ml of hexane and 0.1ml of DMDS and 1-2 drops of I<sub>2</sub> (6% in diethylether, v/v) were added. The mixture was kept at 60°C for 72h. The excess of I<sub>2</sub> was removed by addition of 1ml of 5% sodium thiosulfate and the adduct was extracted for 3 times with 1.5 ml of hexane. The hexane phase was evaporated to dryness.

**Measurement of PLFA fractions in GC/MS:**

100µl internal standard (Nonadecanoic acid methyl ester 100ng/µl) was added to each of the 6 PLFA fractions, evaporated to dryness and analysed in a Gas Chromatograph (HP 5890) with mass selective detector (HP 5971).

**5.2.4. RESULTS**

Total PLFA profiles and different fractions extracted from degraded, moderately degraded and undegraded forests soils at surface and subsurface soil layers are shown in figure 5.2.1(a-f). None of the fatty acids under UNSFAs subgroup was detected in the study from all soil samples. A total of 133 fatty acids derived from different phospholipid fractions (ester-linked) and lipopolysaccharides (non ester-linked) were recorded from all samples in this study. The total PLFA content of the soil samples did not vary significantly at surface soil layer though there was significant variation at the subsurface soil layers. The SATFA, MUFA, PUFA, PLOH and UNSOHs of all sites displayed a similar pattern of their distribution to the total PLFAs (Fig. 5.2.1a-f).



**Fig. 5.2.1(A-C).** Distribution of total PLFA (A) and their fractions, SATFA (B) and MUFA (C) in degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites at surface (SL) and subsurface soil (SSL) layers. Bars on histogram represents SD. (\*m= $\mu$ Mol kg<sup>-1</sup>)

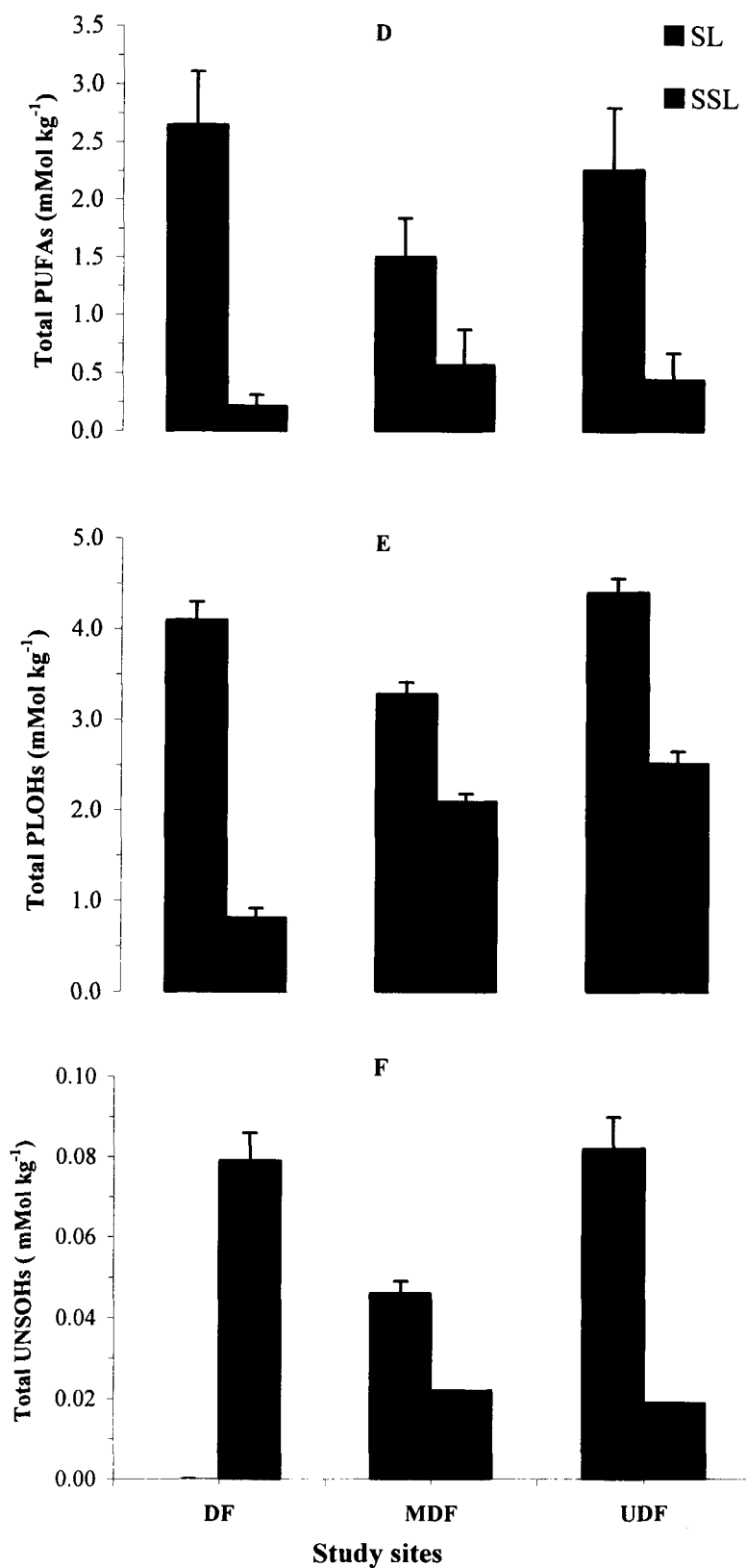


Fig. 5.2.1(D-F). Distribution of PLFA fractions, PUFA (D), PLOH (E) and UNSOH (F) in degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites at surface (SL) and subsurface soil (SSL) layers.

**Table 5.2.1a. Distribution of the SATFAs in degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites at surface (SL) and subsurface soil (SSL) layers. Number in the parentheses denote library reference numbers.**

	SATFAs	DF		MDF		UDF	
		SL	SSL	SL	SSL	SL	SSL
1	8-16:0*(0,672) :	0.00	0.00	0.65	0.98	0.00	0.78
2	10-15:0(0,618) :	1.19	0.00	0.00	0.00	1.43	0.00
3	10-16:0(0,673) :	0.69	0.00	0.00	0.00	0.00	0.00
4	10-16:0(0,708) :	0.00	0.00	0.00	0.00	0.37	0.00
5	10-17:0(0,793) :	0.00	0.00	0.00	0.00	7.75	0.00
6	10-17:0(0,760) :	0.00	0.00	0.00	1.34	0.00	1.02
7	10-18:0(0,842) :	0.00	0.00	0.00	0.00	0.00	0.31
8	10-18:0(0,843) :	2.40	3.99	2.12	3.76	2.08	0.94
9	10-18:0(0,843) :	0.00	0.00	0.86	0.00	0.00	3.53
10	10-18:0(0,846) :	0.75	0.00	0.59	0.71	0.58	0.57
11	10-18:0(0,873) :	0.83	0.00	0.00	0.71	0.75	0.72
12	11-17:0(0,793) :	0.00	7.81	7.59	8.49	0.00	7.16
13	11-17:0&(0,798) :	9.10	1.07	0.83	1.45	0.89	2.07
14	11-17:0&(0,798) :	0.84	0.00	0.00	0.00	0.00	0.00
15	12-17:0(0,756) :	0.27	0.00	0.22	0.01	0.14	0.02
16	12-19:0(0,953) :	4.42	0.00	4.33	3.66	3.99	5.26
17	13-16:0(0,727) :	0.00	0.00	0.00	5.87	0.00	5.37
18	20:0 (1,073) :	0.92	1.05	0.83	0.70	0.86	0.59
19	a15:0(0,645) :	5.12	3.10	9.15	4.77	5.19	9.43
20	a15:0*(0,645)re :	9.15	1.76	5.44	8.76	9.21	5.16
21	a17:0(0,817)ref :	3.15	3.01	3.21	3.56	3.02	3.36
22	b15:0(0,582) :	0.00	0.00	0.00	0.00	0.00	0.88
23	b16:0(0,729) :	5.60	3.39	6.22	0.00	5.48	0.00
24	br10-19:0(0,953) :	0.00	7.71	0.00	0.00	0.00	0.00
25	br16:0(0,713) :	0.44	0.00	0.00	0.00	0.00	0.00
26	br16:0(0,672) :	0.00	0.00	0.00	0.00	0.54	0.68
27	br18:0(0,890) :	0.33	0.00	0.00	0.00	0.33	0.00
28	che-8-12:0(0,93) :	0.68	0.00	0.00	0.00	0.00	0.00
29	cy17:0,9(0,831) :	3.29	1.10	3.63	1.51	3.11	1.40
30	cy18:0(0,880) :	0.00	0.00	0.00	0.00	0.00	0.52
31	cy18:0,11(0,911) :	0.00	0.00	0.00	0.42	0.36	0.56
32	cy18:0(0,928) :	0.00	0.00	0.63	0.71	0.69	0.69
33	cy19:0,11(0,993) :	11.22	26.35	12.31	14.37	13.66	17.15
34	d16:0\$(1,019) :	0.00	0.00	0.29	0.88	0.46	0.58
35	i14:0(0,550)ref :	0.82	0.00	0.87	0.36	1.05	0.43
36	i17:0(0,809)ref :	4.27	4.60	4.05	5.31	4.04	5.17
37	i18:0(0,892)ref :	0.00	0.00	0.00	0.41	0.00	0.48
38	n14:0(0,580) :	0.00	0.00	1.28	0.80	0.00	0.00
39	n15:0(0,667) :	1.00	0.00	1.01	0.80	0.99	0.00
40	n16:0 (0,756) :	19.26	11.62	19.12	14.40	18.84	13.16
41	n17:0(0,839) :	1.24	0.00	1.19	1.00	1.23	0.00
42	n18:0(0,921) :	5.87	6.99	5.85	5.42	5.88	5.27

43	n22:0(1,219) :	0.82	0.80	0.70	0.62	0.73	0.55
44	n24:0(1,351) :	0.56	0.00	0.50	0.36	0.60	0.33
45	2'-22:0(1,073) :	0.07	0.07	0.07	0.05	0.06	0.04
46	p2"-23:0(1,143) :	0.80	0.00	0.77	0.27	0.70	0.00
47	p2"-24:0(1,211) :	0.32	0.00	0.00	0.00	0.00	0.00
48	p2"-25:0(1,278) :	0.29	0.00	0.00	0.00	0.00	0.00
49	p4'-20:0(0,985) :	0.00	0.00	0.31	0.00	0.29	0.00
50	p4'-22:0(1,132) :	0.54	0.00	0.41	0.00	0.36	0.00
51	tubs10-19(0,954)	0.00	0.00	0.00	1.64	0.00	0.00
	<b>Total (%)</b>	<b>62</b>	<b>33</b>	<b>58</b>	<b>62</b>	<b>65</b>	<b>62</b>

**Table 5.2.1b. Distribution of the MUFAs in degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites at surface (SL) and subsurface soil (SSL) layers.**

	MUFAs	DF		MDF		UDF	
		SL	SSL	SL	SSL	SL	SSL
1	15:1,4(1,145) :	0.00	0.00	0.00	0.00	0.54	0.00
2	16:1,7(1,240) :	1.43	0.00	1.49	1.32	1.16	1.24
3	16:1,9(1,209) :	0.71	0.00	0.79	0.00	0.58	0.00
4	16:1,9(1,244) :	0.00	4.43	0.00	6.90	0.00	5.92
5	16:1,9(1,245) :	11.91	0.00	12.81	0.00	12.16	0.00
6	17:1,9(1,283) :	0.00	0.00	0.00	8.08	0.00	8.00
7	17:1,9(1,286) :	8.94	5.20	7.70	0.00	8.46	0.00
8	17:1,9(1,305) :	1.29	0.00	1.03	0.00	0.00	0.00
9	17:1,9(1,308) :	0.00	0.00	0.00	0.00	1.11	0.00
10	18:1t9 (1,382) :	16.30	13.34	16.32	16.27	16.98	15.14
11	16:1,11(1,256) :	11.87	6.87	10.57	9.98	10.99	11.01
12	17:1,11(1,312) :	0.46	0.00	0.00	0.00	0.00	0.00
13	18:1,11t(1,389) :	31.29	23.73	31.99	27.70	30.62	28.03
14	18:1c11(1,382) :	0.25	0.00	0.04	0.00	0.10	0.00
15	18:1,13(1,391) :	3.98	0.00	3.12	2.44	3.17	2.17
	<b>Total (%)</b>	<b>73</b>	<b>33</b>	<b>67</b>	<b>47</b>	<b>73</b>	<b>40</b>

**Table 5.2.1c. Distribution of the MUFAs in degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites at surface (SL) and subsurface soil (SSL) layers.**

	PUFAs	DF		MDF		UDF	
		SL	SSL	SL	SSL	SL	SSL
1	18:2(0,889) :	5.16	0.00	5.60	0.00	5.80	0.00
2	18:2c9,12(0,898) :	35.23	13.48	34.10	30.60	40.85	25.44
3	18:2t9_(0,889) :	6.30	0.00	4.43	0.00	5.43	0.00
4	20:2c11,14(1,05) :	1.61	0.00	0.00	0.00	0.00	0.00
5	20:3-(1,040) :	2.33	0.00	1.50	0.00	1.18	0.00
6	20:3c8,11,14(1. ) :	6.90	0.00	2.41	0.00	3.59	0.00
7	20:4-5,8,11,14( ) :	0.00	3.72	0.00	5.05	0.00	4.71
8	20:5(1,035) :	13.93	0.00	10.31	0.00	8.20	0.00
	<b>Total (%)</b>	<b>87</b>	<b>25</b>	<b>75</b>	<b>25</b>	<b>75</b>	<b>25</b>

**Table 5.2.1d. Distribution of the PLOHs in degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites at surface (SL) and subsurface soil (SSL) layers**

	PLOHs	DF		MDF		UDF	
		SL	SSL	SL	SSL	SL	SSL
1	a14:0re(0,760) :	0	0	0.31	0.19	0.25	0
2	a15:0_(0,813) :	6.07	1.52	0	0	0	0
3	a15:0i(0,813) :	0	0	6.26	3.69	5.86	2.36
4	a15:0_(0,820) :	0	0	0.66	0	0	0
5	a15:0_(0,843) :	0	0	0	0	0.41	0
6	a16:0a_(0,893) :	3.42	0	3.59	1.69	3.24	1.1
7	a16:0re(0,921) :	3.45	0	3.28	3.17	2.99	2.37
8	a17:0_(0,969) :	1.34	0	1.37	0	1.19	0
9	a17:0_(0,970) :	0	2.38	0	2.17	0	3.1
10	a17:0i_(0,953) :	1.15	0	0.74	1.11	0.69	1.08
11	a17:0n_(0,978) :	1.48	0	1.4	0.74	1.32	0.69
12	a17:2(1,074) :	0	2.03	4.34	2.83	3.69	3.26
13	a18:0re(1,071) :	0.33	0	0.3	0.26	0.42	0.38
14	a18:0cy :	1.65	0	0	0	0	0
15	a18:1_(1,058) :	0	0	2.16	0	0	0
16	a19:0(1,100) :	0	0	0	0	1.06	0
17	a19:0cy(1,078) :	1.93	0	0	0	0	0
18	a19:0cy(1,138) :	10.87	16	10.31	14.36	11.39	18.14
19	b14:0re(0,760) :	0	0	0.6	0.71	0.6	0
20	b15:0_(0,813) :	0.25	0.02	0	0	0	0

21	b15:0_(0,820) :	0	0	0.02	0	0.64	0
22	b15:0_(0,843) :	0	0	0	0	0.1	0
23	b16:0a_(0,893) :	0.01	0	0.02	0.02	0.02	0
24	b16:0re(0,921) :	0.65	0	1	0.4	0.77	0.34
25	b17:0_(0,969) :	0.42	0	0.51	0	0.56	0
26	b17:0_(0,970) :	0	0.09	0	0.17	0	0.11
27	b17:0i_(0,953) :	0.01	0	0.01	0.01	0.04	0
28	b17:0n_(0,978) :	0.06	0	0.05	0.02	0.07	0.01
29	b18:0re(1,071) :	1.28	0	1.16	0.63	1.26	0.49
30	b18:1_(1,058) :	0	0	0.03	0.08	0.23	0
31	b19:0cy(1,138) :	0	0	0	0.02	0.01	0.04
32	w11:1*(0,612) :	0	0	0	1.27	0.73	1.13
33	w13:1*(0,781) :	1.16	1.59	1.63	2.1	1.43	1.77
34	w14:0(0,887) :	0.86	0	0	0	0	0
35	w15:1*(0,942) :	1.65	3.04	1.97	2.66	2.32	2.87
36	w16:0(1,043) :	2.32	3.04	3.37	6.86	3.7	5.28
37	w17:1*(1,092) :	1	0	0	1.53	0	2.02
38	w18:0(1,184) :	1	0	0.46	0	0	0
39	w18:1*(1,090) :	0	0	1.06	0	1.53	0
40	w19:1*(1,229) :	1.16	0	1.56	2.98	2.35	3.12
41	w20:0(1,318) :	0	0	0.59	1.06	0.72	1.34
42	w21:1*(1,359) :	0	0	0.98	1.14	1.38	1.54
43	w22:0(1,441) :	2.4	3.13	2.83	4.55	3.41	5.08
44	w-1,11:1(0,477) :	0	0	0	0	0.65	0.55
45	w-3,17:2*(0,961) :	17.46	0	13.14	3.5	12.2	2.6
46	w-10,18:0(1,034) :	0	0	0	0	0.49	0
47	w-12,18:0(1,049) :	2.29	0	2.55	0	3.08	2.18
48	w12,18:0(1,051) :	0	0	0	0.53	0	0
49	w-118:1(1,058) :	0	0	0	0.79	1.82	1.26
50	w-9,18:1(1,051) :	0	0	0	0.83	0	0
51	w-9,18:2(1,066) :	0	0	0	0	0.68	0
52	diOH12:1(0,905) :	2.06	0	0	0.97	1.52	0
53	mid19:0(1,165) :	0	0	0	0.64	0	0
54	119-14:0(0,845) :	0	0	0.54	0	0	0
55	5-16:0(0,919) :	1.35	0	0	0	0	0
56	16:0w12(0,904) :	0	0	1.39	0	0	1.75
57	16:1w-6(0,904) :	0	0	0.6	0	0	0.27
	<b>Total (%)</b>	<b>48</b>	<b>17</b>	<b>60</b>	<b>58</b>	<b>68</b>	<b>50</b>

**Table 5.2.1e. Distribution of the UNSOHs in degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites at surface (SL) and subsurface soil (SSL) layers**

	UNSOHs	DF		MDF		UDF	
		SL	SSL	SL	SSL	SL	SSL
1	w15:1#(0.942) :	0.00	2.56	2.65	2.60	4.18	2.32
2	w-9,14:0(0,785) :	0.00	1.97	2.30	0.00	3.10	0.00
	<b>Total (%)</b>	<b>0</b>	<b>100</b>	<b>66</b>	<b>33</b>	<b>66</b>	<b>33</b>

### **Saturated fatty acids (SATFAs)**

51 SATFAs (*straight chain, cyclic and branched fatty acids*) were detected from all the soil samples. A minimum (33%) of these fatty acids occurred in the subsurface soil layer of degraded site while a maximum of 65% was recorded from the surface soil layer of the undegraded site (Table 5.2.1a). SATFAs with *mid chain branching* at different C positions of methyl group (predominantly at 10<sup>th</sup> C of the fatty acids) and *straight chain* were the largest subgroups in terms of numbers and concentrations ( $\mu\text{Mol kg}^{-1}$ ). SATFAs with methyl branching at *iso* and *anteiso* positions and with *cyclopropyl rings* were the other subgroups with maximum numbers of fatty acid. The n16:0 and the cy19:0 were the only two SATFAs with highest concentrations ( $\% \mu\text{Mol kg}^{-1}$ ) at both the surface and subsurface soil layers of the three study sites.

### **Monounsaturated fatty acids (MUFA)**

Altogether 15 MUFAs were recorded in this study from all the soil samples. These were unsaturated at different C positions ranging from  $\Delta 4$  to  $\Delta 13$  (Table 5.2.1b). Fatty acids unsaturated at  $\Delta 9$  (oleic series) and  $\Delta 11$  were the largest subgroups which represented 53 and 26% of the total MUFAs. The amount of MUFA concentrations ( $\% \mu\text{Mol kg}^{-1}$ ) among the samples ranged from 33% at subsurface soil of degraded site to 73% at surface soil layers of degraded and undegraded sites respectively. The 18:1t9 and 18:1,11t were the two MUFAs which occurred in all soil samples at maximum concentrations followed by the 16:1,9c at the surface soil layers only.

### **Polyunsaturated fatty acids (PUFA)**

This PLFA fraction comprised of 8 fatty acids with 2 to 5 double bonds unsaturated at different C positions of 18 and 20 C fatty acids (Table 5.2.1c). The degraded site contained a maximum (87%) of the total PUFAs followed by 75% in both the moderately degraded and undegraded sites respectively at surface soil layers. The subsurface soil layers contained only 25% of the total PUFAs in all the three sites. The *cis* isomers of the linoleic acid (18:2c9,12) was the only PUFA with maximum concentration ( $\% \mu\text{Mol kg}^{-1}$ ) which occurred in all the soil samples. The arachidonic acid (20:4-5, 8,11,14) was the second subgroup of this PUFA fraction with maximum concentration recorded only from the surface soil layers of the three study sites.

### **OH-substituted fatty acids (PLOH)**

There was a total of 57 PLOHs (predominantly with their hydroxy substitutions at  $\alpha$ ,  $\beta$ ,  $\omega$  and  $\omega$ - positions of the fatty acids) recorded from the soil samples analysed in this study (Table 5.2.1d). A maximum (68%) of the total PLOHs were recorded from the undegraded site followed by moderately degraded and degraded sites with 60% and 48% at surface soil layers. The subsurface layer showed comparatively lower number of PLOHs than surface layer of all sites with the minimum (17%) recorded from the degraded site. The  $\alpha$ 19:0cy and  $\omega$ -3,17:2 were the two PLOHs which occurred in all the study sites with maximum concentrations as compared to others.  $\alpha$  PLOHs formed the maximum of 30% followed by  $\beta$ ,  $\omega$  and  $\omega$ - PLOHs with 22, 20 and 13% of the total fatty acids in this PLFA fraction. The undegraded site contained the

highest numbers of these fatty acids than moderately degraded and undegraded sites at both the soil layers.

#### **OH-unsubstituted fatty acids (UNSOH)**

A very few fatty acids (only 2 fatty acids) of this PLFA fraction were recorded from all the three study sites except in the surface soil layer of degraded forest site which displayed none of the fatty acids (Table 5.2.1e). The two USNOHs,  $\omega$ 15:1 and  $\omega$ -9,14:0 were recorded from the degraded site at subsurface layer and from moderately degraded and undegraded sites at both soil layers.

### **5.2.5. DISCUSSION**

#### **5.2.5.1. Impact of soil degradation on the distribution of PLFA profiles as a measure of microbial community structure:**

There was no significant variation in distribution of the total PLFA contents among the degraded, moderately degraded and undegraded forest sites at surface soil layers. The subsurface soil layers displayed a clear variation of total PLFA contents between the degraded and moderately degraded and between the degraded and undegraded sites revealing a significant decline in PLFAs in this degraded site to the other two sites. This finding suggests that the microbial population status at surface soil layer of the degraded site has restored to its original composition when this site has been left for regeneration for more than 7 years after last shifting cultivation practiced in 1994. However, the minimum amount of PLFAs recorded from the subsurface soil layer in this site revealed the long term detrimental effect of shifting cultivation on microbial community structure as compared to the other two sites.

A close analysis on the distribution pattern of the different fractions of PLFAs among the study sites at the surface and subsurface soil layers revealed a significant variation of microbial community structures. *Straight chain*, branching at *iso / anteiso* and *cyclopropyl ring* containing fatty acids were major subgroups of SATFA recorded from all the soil samples. *Straight chain* SATFAs are universal in distribution including the three microbial domains, *archaea*, *bacteria* and *microeukarya* (Gattinger *et al.*, 2002; Zelles, 1999). Palmitic acid (n16:0) was the only straight chain fatty acid which occurred in all soil samples and displayed the maximum percentage concentration (%  $\mu\text{Mol kg}^{-1}$ ) among all SATFAs. Because of the universal distribution of this fatty acid in most of the eukaryotes and prokaryotes, there is only superficial information on the distribution of microbial communities in the samples analysed (Zelles and Bai, 1994). The distribution of the SATFAs with methyl branching at *iso* position (i14:0; i17:0 and i18:0) as indicators of the Gram-positive and Gram-negative bacteria like *Acetobacter* were detected from all the soil samples though their percentage concentration was lower than other subgroups of SATFAs. Similarly, the fatty acids with methyl branching at *anteiso* positions (a15:0 and a17:0) were found to be distributed in equal percentages (6%) in all the soil samples. These reveals that the microbial community structures of the Gram-positive bacteria, sulphate-reducing Gram-negative bacteria and predominantly those genera *Flavobacterium* and *Cytophaga* (Brennan, 1988; Hack *et al.*, 1994) were not affected in the long term by shifting cultivation in the degraded site or by selective logging in the moderately degraded site. The *cyclopropyl* fatty acids are known to be

indicators of Gram-negative bacteria (*Rhodospirillum*, *Legionella*) and Gram-negative bacteria (*Closteridium* and *Bifidobacterium*) including aerobic and anaerobic bacterial groups (Gattinger, 2002; Ratledge and Wilkinson, 1988 and Zelles, 1999). These microbial communities were found in different diversity percentages ranging from the lowest 4% in the degraded site to the maximum of 8 and 10% in the undegraded site at surface and subsurface layers while the moderately degraded site contained 6 and 8% at both the soil layers. This suggests that there is a long term detrimental effect of shifting cultivation on these microbial groups in the degraded site than in the moderately degraded site. The cy19:0,11 was the only *cyclopropyl* SATFA which occurred in all the samples in highest concentrations next to the *straight chain* SATFA, palmitic acid (n16:0 ) which together represented about 30% of the total SATFA concentrations in all the soil samples except for the subsurface soil layer of degraded site where these two fatty acids represented about 38% of the total SATFAs.

The MUFAs are described the indicators of the Gram-negative aerobic bacteria, strictly anaerobes ( $\omega$ 4; vaccinic type,  $\omega$ 7;  $\omega$ 11), and Gram-positive bacteria and eukaryotes (Oleic series,  $\omega$ 9) (Gattinger, 2002 and Zelles, 1999). The profiles of this PLFA fraction among the different study sites showed similar composition of these microbial communities in the surface soil layer. The subsurface soil layers displayed significantly lower number of these fatty acids thus less microbial diversity. The aerobic bacteria with their fatty acids unsaturated at  $\omega$ 4 was recorded from the surface soil layer of the undegraded site while those with  $\omega$ 7 (vaccinic acid type) which are anaerobic bacteria

(desaturase pathways) were detected from all the soil samples except that this fatty acid was not detected from the subsurface soil of the degraded site. The reason for the absence of this fatty acid in the subsurface soil layer of the degraded site could be due to the increased bulk density as compared to other soils. Majority of the microbial group, Gram-positive bacteria and other eukaryotes including fungi as represented by fatty acids with unsaturation at  $\omega 9$  were dominant microorganisms followed by those aerobic groups ( $\omega 11$ ) in all the study sites and at both the soil layers. Maximum number of these microbial communities with  $\omega 11$  were recorded from the degraded site followed by moderately degraded and undegraded sites at the surface soil and the subsurface soil layers contained comparatively lower to almost half to that of surface soil layers.

The PUFAs are considered as the markers of microeukaryotes (fungi) and cyanobacteria (Bossio and Scow, 1998 and Gattinger, 2002) in the soils. However, these PUFAs are suggested to be predominantly occurring in the eukaryotes and they are considered to be present in exceptional cases in cyanobacteria (Zelles, 1999). The degraded site contained 7 fatty acids with different degrees of unsaturations and represented maximum number of PUFAs than other sites at surface soil layer. Linoleic acid (18:2c,9,12) was the only PUFA which occurred in all the soil samples at maximum per cent concentrations while the subsurface soil layer contained comparatively lower concentrations than the surface layer. Arachidonic acid (20:4-5, 8,11,14) was another PUFA which occurred at the surface soils of the three study sites with high percentage concentrations to the total PUFAs. The absence of this fatty

acid at the surface layers revealed that *archaeotale* communities were mostly aerobic occurring in the surface soil layers only.

The  $\alpha$ -PLOHs as indicators of the Gram-negative bacteria (*Pseudomonas*) and *Actinomycetales* (Galbraith and Wilkinson, 1991; Gattinger, 2002; Yano et al., 1978 and Zelles, 1999) were the largest subgroup in terms of number of fatty acids. The distribution of this subgroup varied significantly among the study sites and between the soil layers. Moderately degraded and undegraded sites contained maximum (22%) and similar composition of fatty acids of this subgroup at the surface soil layer while the degraded site displayed minimum diversity and quantity at both the soil layers. The  $\beta$ -PLOHs as the marker of the bacterial groups, *Pseudomonas*, *E.Coli*, *Rhodococcus*, etc. (Zelles 1997) showed highest diversity and concentration at the surface soil layer of the undegraded site expressed in per cent of the total PLOHs. There was a sharp decline in the diversity and population of these microbes at the subsurface soil layer of the undegraded site as compared to the moderately degrade and undegraded sites which suggests the negative effects of the shifting cultivation on survival and multiplication of these organisms. The number of  $\omega$ -PLOHs and their concentrations did not vary significantly among the three sites at surface soil layer but the subsurface layer of the degraded site contained minimum number and quantity of these PLOHs than other sites. These fatty acids as indicators of fungi (Gattinger, 2002 and Zelles, 1999) did not vary in their composition among the three sites.

UNSOHs are regarded as the general indicators of anerobes and eukaryotes (fungi) including the representatives of the genus, *Closteridium*

(Gattinger, 2002 and Zelles, 1999) which showed a unique distribution pattern among the soil samples studied in this study. The surface soil layer of the degraded site showed no microbial representatives of the above groups due to non detection of any of these UNSOHs while the subsurface layer contained the two UNSOHs. The surface layers of the moderately degraded and undegraded sites contained two fatty acids and the subsurface soil layers with one fatty acid each respectively. These suggests that the subsurface soil layer of the degraded site being disturbed repeatedly with burning and tillage during the course of cultivation lead to complete loss of these microbial communities.

Total PLFA contents of the soils expressed in  $\mu\text{Mol kg}^{-1}$  revealed that there was no significant variation on microbial population numbers among the three study sites at the surface soil layers though the subsurface soil contain lower amounts of the PLFA in general. There was a decline in the microbial diversity status of the degraded sites at both the soil depths in comparison to the moderately degraded and undegraded sites as revealed by the PLFA profiles. The undegraded forest site contained maximum diversity of the PLFA fractions and their individual fatty acids revealing a higher microbial diversity than the moderately degraded and degraded site respectively.

## **Chapter 6**

### **Soil dehydrogenase, acid phosphatase and urease enzyme activities in degraded and undegraded forest soils**

#### **6.1. INTRODUCTION**

Biochemical reactions are the important nutrient transformation processes of organic and inorganic substances in soil environment through the catalytic activity of biomolecules called enzymes. Many of the organic matter transformation processes in soil are catalyzed by enzymes (Khan, 1970) and all biochemical transformations in soil are dependent on, or related to the presence of enzymes. The important sources of enzymes in soil include plant, animal and microorganisms. The quantity of soil enzymatic activity detected in a particular soil sample is the sum of active and potentially active enzymes (Tate III, 1995). However, the activity of a particular enzyme in the soil is a composite of various activities associated with various biotic and abiotic components, e.g. proliferating cells, latent cells, cell debris, clay materials, humic colloids and aqueous phase (Burns, 1982 and Tiwari *et al.*, 1988a).

The measurement of biochemical activity in soil i.e. soil enzyme assays have been done for various reasons particularly, as a measure of soil fertility or productivity (Kiss *et al.*, 1978; Dkhar and Mishra, 1983; Tiwari *et al.*, 1988ab and Verstraete and Voets, 1977), as a measure of microbial biomass (Casida, 1977; Ladd, 1978 and Klose and Tabatabai, 1999), as indicators of vegetation effects of pollutants and capability to conduct bio-

geochemical cycling, total microbial activity (Stevenson, 1959 and Tiwari *et al.*, 1988ab), as a predictor of bioremediation and potential success (Dick *et al.*, 1998), to understand the consequence of rhizosphere effect (Boero and Thien, 1979), as a potential indicator of soil quality (Kennedy and Papendick, 1995; Garcia and Hernandez, 1997; Trasar-Cepeda *et al.*, 1998; Bendick and Dick, 1999; Palma *et al.*, 2000; Pascual *et al.*, 2000 and Trasar-Cepeda *et al.*, 2000). Research work on soil biochemistry during the last two decades appeared to be concentrated towards development of soil quality indices based on these biochemical properties. This is due to the reason that biological and biochemical properties are highly sensitive to environmental stress and thus can be used as indicators of soil quality (Trasar-Cepeda and Gill-Sotres, 1987; Dick and Gupta, 1994; Kennedy and Papendick, 1995 and Ajwa *et al.*, 1999).

Microbial activities in soil, despite their importance in many of the soil processes, are frequently disturbed as shown by altered soil enzyme activities as a result of agricultural exploitations and tillage practices (Tiwari *et al.*, 2002). Disruption in soil microbial activity as shown by changes in levels of metabolic enzymes, can serve as an estimate of ecosystem disruption (Tate, 1995). Among the different types of soil enzymes studied from various objectives of investigations, one oxidoreductase (dehydrogenase) and two hydrolases (phosphatase and urease) are thoroughly studied enzymes due to their specific importance in organic matter transformation processes, phosphorous cycle and agricultural practices. Soil dehydrogenase is an extracellular enzyme which is

considered to be a good tool to measure microbial oxidative activity (Ross, 1971), as an indicator of any disruption caused by pesticide application, trace element discharge and soil management practices (Versraete and Voets, 1977; Burns and Edwards, 1980 and Reddy and Faza, 1989) as a measure of microbial biomass (Ladd, 1978) and measure of soil respiration. Acid and alkaline phosphatase activity assays have been used to our understanding of the phosphorus cycling which is related to the organic matter and its turnover in soil (Speir and Ross, 1978 and Trasar-Cepeda and Gill Sotres, 1987). The abundance and activity of these enzymes in the soil is an indication of the available P as these enzymes are responsible for conversion of organic form of P to inorganic and labile P forms. Urease is another important and thoroughly studied soil enzyme due to the agricultural importance of its substrate, urea. The possible effects of soil properties and land use pattern on urease activity may have significant implications of the efficient use of fertilizers based on urea (O'Toole *et al.*, 1982). However, little research has been done to determine the interaction between urease levels and environmental parameters in the soil (Stott and Hagedon, 1980). Reports have revealed that urease activity is directly related to type of vegetation and quality of incorporated organic materials and with fluctuations in nutrient levels due to associated changes in populations of urolytic microbes in the soil (McGarity and Myers, 1967 and Stott and Hagedon, 1980). Urease activity is an important factor for survival of ammonium fertilizer oxidizers in forest and agricultural soils (Swenson and Bakken, 1998).

The above research findings shows that soil biochemical characteristics in terms of soil enzyme activities are largely affected by change in soil environment induced by disturbances such as deforestation, tillage, soil management and other agricultural practices. Therefore, this research investigation aims to study that if there exists significant variation in distribution of the soil enzyme activities (dehydrogenase, acid phosphatase and urease) in degraded, moderately degraded and undegraded forest soils of humid tropics in Arunachal Pradesh, north-eastern India.

## **6.2. REVIEW OF LITERATURE**

Detailed survey of the available literature on the studies of various aspects of soil enzyme activities reveals an extensive research work done in this field during the last three decades of the 20<sup>th</sup> century.

Research investigation on enzyme activity in gray wooded soil as affected by cropping systems and fertilizer use has shown that growing legumes in rotation resulted in considerably greater total microbiological activity than the wheat-fallow system (Khan, 1970). Further the study demonstrated that the enzymatic activity increased with increase in organic matter content in the soil.

Frankenberger and Bingham (1982) reported the inhibitory effect of increased soil salinity to the enzyme activities that have a specific role in the C, N, P and S cycles of saline soils. They also observed decrease in enzyme activity with increasing electrical conductivity or salinity, however, the degree of inhibition varied among the enzymes assayed and the nature and amounts of salts added. The activity of dehydrogenase was severely

inhibited by salinity, whereas, the hydrolases showed lesser degree of inhibition.

An extensive study on the relationship between enzyme activities and microbial growth and activity indices in different soils have revealed high correlation of the enzyme activities with both microbial respiration and total biomass in soil (Frankengberger and Dick, 1983).

Dormaar *et al.* (1984) studied the impacts of seasons and site management on the enzyme activities of soils in Alberta, Canada. They found highest enzymatic activity of soils in winter months and lower during rainy season. Their results indicated the significant effects of grazing on enzyme activities of soils in two sites.

Bolton *et al.* (1985) reported significantly higher levels of urease, phosphatase and dehydrogenase following growth of winter peas (as green manure crop) in comparison to the soils which received regular applications of anhydrous ammonia, P and S at recommended rates for a period of 30 years in Palouse region of Eastern Washington.

Baruah and Mishra (1986) investigated the effect of three herbicide (2,4-D, butachlor and oxyflurefen) on activities of dehydrogenase, urease and carbon dioxide evolution in submerged paddy fields of north-east India. They noted significant stimulation of the dehydrogenase activity and carbon dioxide output with herbicide treatments. However, they found that the herbicide application on urease activity remained unchanged.

Study on enzyme activity and carbon dioxide evolution from upland and wetland rice soils under three agricultural practices in hilly region of

north-eastern India revealed higher activity of dehydrogenase, urease, and carbon dioxide evolution in wetland (Valley soils), followed by terrace system and hill-slope site respectively (Tiwari, M.B *et al.*, 1989).

Bonmati *et al.* (1991) have reported that urease enzyme has the highest spatial variability followed by phosphatase in 1 year air dried soil samples of a 5 year old legume meadows in Pisa, Italy.

Studies on the depth-wise distribution of enzyme activities revealed a decreasing trend of dehydrogenase, urease and acid phosphatase activities with increase in soil depth in a hilly sandy loam profile of north-eastern India (Tiwari, 1996a). His study demonstrated persistent activities of these enzymes to a depth of 2m in sandy loam soil profile.

Tiwari (1996b) investigated the relationship between enzyme activities, microbial populations and soil respiration in some Indian soils namely, grassland, garden, orchard, fallow and arable soils of north-eastern hill regions. Multiple regression and simple correlation analysis of the studied parameters revealed widest range (40 fold) in urease enzyme activity for various soils whereas the narrowest range (1-15 fold) was recorded for the phosphatase activity. The dehydrogenase activity falls in the range of variation between the two enzymes, urease and phosphatase. The results showed that fungal biomass accounted largely for the variability in dehydrogenase, urease and phosphatase activities.

Marjadori *et al.* (1996) reported the influence of lead (Pb) pollution on two enzyme activities, soil dehydrogenase and phosphatase in four soils of south western Sardinia, Italy. They noted marked influence of lead (Pb) and

soil moisture on the activities of the dehydrogenase and phosphatase but the effect of variation in soil moisture was less for phosphatase activity. They further recorded reduction in activity of these enzymes at very high concentration of lead (i.e.  $6000 \mu\text{g Pb g}^{-1}$  soil) otherwise the doses did not result in clear fluctuations.

Kumari and Charya (1997) found significant positive correlation between soil enzyme activities and microbial population number in four polluted sites of Warangal, Andhra Pradesh, India. They found increased microbial colonies showing increased accumulation of soil enzymes. Positive correlation was observed between enzyme activities and soil nutrients such as nitrates, potassium and organic matter whereas iron and aluminum contents showed negative correlation.

Nagaraja *et al.* (1997) studied the effects of three pesticide applications on the enzymatic activities of dehydrogenase, phosphatase and urease in three different soils of Karnataka, India. They noted inhibition of the enzymes by pesticides in the order of captan > atrajin > aldrin at all concentrations of treatment. The 10 ppm aldrin treatment had no significant impact. Higher inhibitions of the enzyme activities were observed at 100 and 500 ppm concentrations of all pesticides.

Influence of compost addition and inorganic fertilizer treatment on soil biological and yield of crop under a cereal-legume on a typic Haplaustert increased dehydrogenase and alkaline phosphatase activities with addition of organic material whereas no significant influence of inorganic fertilizer treatment was observed (Manna and Ganguli, 1997). They further noted

significant correlation between the crop yield and activities of enzymes in soils.

Rao *et al.* (1997) reported increased activities of dehydrogenase, phosphatase and nitrogenase enzymes in soils with ley farming system in comparison to soils under conventional cultivation farming system in Jodhpur, India. Further, they recorded decreased enzyme activities in the ley and conventional farming (CF) systems with the increase in soil depth. Organic matter content and soil moisture were found to be the prime factors responsible for variation in enzyme activities.

Tiwari and Sharma (1998) recorded increased activities of dehydrogenase and urease soil enzymes with increased altitude upto 1100 masl in two mountain ranges of Arunachal Pradesh, north-eastern India. Correlation coefficient values of the enzyme activities and other soil properties revealed that the soil organic matter content was important factor that regulates the enzyme activities in highland soils.

Naseby *et al.* (1998) investigated the ecological impacts of the biocontrol agent, *Pseudomonas fluorescens* F113 in the rhizosphere of field-grown sugar beet using soil enzymes, acid and alkaline phosphatase, phosphodiesterase and arylsulphatase. They observed significant correlation between these enzyme activities in the rhizosphere highlighting the usefulness of enzyme assays to document variation in soil nutrient cycling.

Staddon *et al.* (1998) described the consequence of acid and alkaline phosphatase and arylsulphatase activities in soils from a jack pine (*Pinus banksiana* Lamb.) ecosystem after clear cutting, prescribed burning and

scarification. They recorded lower enzyme activity after prescribed burning in organic layers as compared to other treatments. They further noted inverse relationship of the acid phosphatase to soil pH and suggested that this enzyme assay may be useful for assessing the impact of fire on soil.

Higher activities of dehydrogenase and alkaline phosphatase enzymes were observed in treatments with tree-crop combination than in the treatment without tree in a 12 year old *Dalbergia sisoo* plantations (Chander *et al.*, 1998). Their study showed that adoption of agroforestry plantation led to improved organic matter status of the soil, which is also reflected in the increased nutrient pool and microbial activities necessary for long-term productivity of the soil.

In an effort to assess soil quality using microbiological and biochemical procedures, Filip (1998) revealed that dehydrogenase activity measurement in soil samples affected by natural and anthropogenic activities may respond as one of the suitable indicators of soil quality. He has demonstrated that the dehydrogenase enzyme activity sensitively indicated the enhanced concentration of lead (Pb) in soddy-podzolic soil.

Gostkowska *et al.* (1998) investigated the suitability of some biochemical and microbiological parameters for the evaluation of the degree of degradation in podzolic soils in the background of its differentiated usage in Lublin, Poland. Their study revealed that microbiological and biochemical changes in soil were more significant than that in chemical status of the soil. Further, they noted much stronger variation of the biochemical activity

(enzyme levels) of the layers of Ap horizon than physical and chemical properties of the studied soils.

Highly sensitive nature of soil biological and biochemical properties to environment stress suggesting their suitability to use in assessment of soil quality have also been reported by Trasar-Cepeda *et al.* (1998). They have demonstrated in their study that a balance existed between the organic matter content of a high-quality native soil and its biochemical and biological properties. Variations in the biochemical quality of a soil may disrupt this balance, in which case the equation may be useful as a biochemical quality index for soils.

Bendick and Dick (1999) studied the effects of field management on soil enzyme activities in vegetable crop rotation plots (VRP) and residue utilization plot (RUP) in Oregon. They observed significant treatment effects on the enzyme activities of the two sites ( $P < 0.05$ ). Enzyme activities (except  $\alpha$ - and  $\beta$ -glucosidase and  $\alpha$ - and  $\beta$ -galactosidase) were generally higher in centrinum grass fields than in cultivated fields. Their study revealed the growing recognition for the need to develop sensitive indicators of soil quality that reflect the effects of land management on soil and assist land managers in promoting long-term sustainability of terrestrial ecosystems.

Tiwari (1999) reported significantly greater ( $P < 0.05$ ) activities of dehydrogenase, urease and acid phosphatase in plots treated with organic manure or with N and P or a combination of both than in the control plots. However, he observed no significant impact of individual treatments of the fertilizer, C, N and P on the activities of these soil enzymes.

Palma *et al.* (2000) stressed the importance of biochemical properties of soil (particularly enzyme activity) as potential indicators of disturbances. Based on the result of their study in two different tillage systems (conventional and non-conventional) and two crop rotations (continuous corn and soybean-corn) it was concluded that enzymatic activities did reflect changes due to management and were suggested as sensitive indicators to different treatments.

### **Dehydrogenase activity**

Dehydrogenase is an extracellular enzyme in the soil and considered to play an important role in the initial stages of the oxidation of soil organic matter by transferring hydrogen or electron from substrates to acceptors (Ross, 1971). Because of its importance in the organic matter transformation processes and its potential to indicate the available microbiological activity in the soil, dehydrogenase has been the subject of chosen biochemical tool in various fields of agricultural and soil science investigations.

Studies on effect of freezing and thawing of some grassland top soils on dehydrogenase activity revealed that storage of soil in frozen condition is useful for minimizing changes in some biochemical activities but it may sometimes result in increased activity when thawed samples are subsequently assayed (Ross, 1970 and 1972). It was concluded that a prolonged thawing period appears to be less essential for estimating dehydrogenase activity, particularly if anaerobic assay conditions are employed.

Reddy and Faza (1989) examined the enzymatic activity of dehydrogenase in sludge amended soil at different incubation periods. Their result indicated significantly inhibition of dehydrogenase activity at 24, 48, 96 h at all concentrations of sludge (40, 80 and 120 ton h<sup>-1</sup>) treatments. The highest dehydrogenase activity in control (no sludge) soils was followed by 40, 80 and 120 ton sludge h<sup>-1</sup> in decreasing order. The lower dehydrogenase activity in the sludge amended soils at all samplings could be due to the heavy metal concentration in sewage sludge (Reddy *et al.*, 1987).

Brezeznska *et al.* (1998) investigated relationship between soil oxygen status and dehydrogenase activity in soils of Lublin, Poland. They noted increased activity of dehydrogenase activity with increase of soil water content and the conditioning temperatures. A combined effect of flooding and temperature to 30°C caused an increased dehydrogenase activity on an average of 129 fold as compared with 15.9 KPa at 10°C treatment. They suggested that soil water content and temperature influence the dehydrogenase activity indirectly by affecting the soil oxidation-reduction status.

Camina *et al.* (1998) measured dehydrogenase activity of acid forest soils rich in organic matter content of Galicia, N.W. Spain and revealed lower activity due to adsorption of formazan. They have suggested use of DMF-Ethanol and reference standards containing soils for determination of dehydrogenase activity at an enhanced recovery of formazan in acid soils rich in organic matter.

### **Phosphatase activity**

Phosphatase activity is essential for conversion of organic substrates containing phosphorus into inorganic form through hydrolysis in the soil. Phosphatase being an important enzyme in soil is an oxidoreductase which plays a key role in P-cycle of the environment.

Since the development of an easy and simple method of assaying phosphatase activity in soil systems by using *p*-nitrophenyl phosphate (Tabatabai and Bremner, 1969) as the substrate of phosphorus hydrolysis in laboratory conditions have brought the research in this field to an emerging field of soil enzymology.

Trasar-Cepeda and Gil-Sotres (1987) studied phosphatase activity of acid soils with high organic matter content in forest soils. They found higher activity of acid phosphatase between pH 5 and 6, which appeared to depend on organic activity of soil suggesting that enzymes originating from litter was progressively inhibited as it penetrated the soil.

Fox and Commerford (1992) examined the acid phosphatase activity in the rhizosphere of slashpine (*Pinus elliotii*) growing in A and Bh horizons of soils from two forested Spodosols. Their results indicated significantly high acid phosphatase activity in the rhizosphere of the Leon A and Bh horizons and Pomona Bh horizon soils. Further they noted decrease in phosphatase activity following application of phosphorus fertilizers.

Deng and Tabatabai (1997) observed significant affects of tillage management and crop residues placement on phosphatase activities. They noted higher activities of phosphatase in soils with no till x double mulch of

corn residues than other treatments. They also recorded significant correlation between phosphatase activities with organic C of the 40 soil samples tested suggesting that organic matter plays an important role in protecting and maintaining soil enzymes in their active forms.

Hysek and Sarapatka (1998) investigated the relationship between phosphatase active bacteria and phosphatase activities in forest soils in Ižera Mountains of Czech Republic. The study reported that the number of acid phosphatase active colonies correlated positively with the number of alkaline phosphatase active colonies in F-AO1 horizon and there was a high, positive correlation between the former and the level of ammonification in the H-AO2 horizon. It was shown that positive correlation between the number of alkaline phosphatase active colonies with organic carbon, the number of ammonification bacteria, and the number of mycomycetes in H-AO2 horizon. Neither acid nor alkaline phosphatase activities correlated with the number of phosphatase active colonies of bacteria.

### **Urease activity**

Urease is a hydrolase enzyme responsible for hydrolytic conversion of the substrate, urea into carbon dioxide and ammonia. The urease enzyme assay is important in understanding mineralization process of N element and its response to the application of inorganic fertilizers, land use systems, tillage and soil management systems particularly its relationship to the agricultural practices has led to the extensive research investigation in the last three decades. As a result, the urease enzyme assay has become an important and routine practice in agricultural systems.

Klein and Kloths (1980) reported higher activities of urease in the no tillage grain-plots in comparison to the other plots. They also noted higher values of urease activity related to moisture and organic matter content of the soils in the order no tillage and tillage practices respectively.

Stott and Hagedon (1980) examined the interrelationship between selected soil characteristics and urease activities under two forest vegetations one native grassland and three clover/grass pastures in Benton County, Oregon. Highly positive correlation was observed between urease activity and soil organic matter ( $r=0.59$ ). Principal component analysis demonstrated that urease activity, which when combined with four other factors, accounted for 65.5% of the observed variables in urease activity. Seasonal fluctuation in the urease activity was recorded where fluctuations in the activity levels were related to moisture and temperature conditions of the soils.

Vlek *et al.* (1980) reported fate of urea application under flooded conditions revealing approximately half of the urea incorporated into flooded water. This urea was hydrolysed largely at the soil-water interface and subsequently returns to the flood water (>80%) or is retained by the soil (<20%). They concluded that the fraction returning to flooded water is either taken by algae or volatilized.

Nor (1982) studied the activity and kinetic properties of urease in several Malaysian soils. He noted a significant correlation between  $K_{max}$  and  $V_{max}$  of urease activities. He also observed inhibition of urease activity very effectively with the use of  $Ag^{3+}$  while  $Cu^{2+}$  was only effective in two soils and

marginally effective in other soils. It was concluded that urease inhibitors have potential applications in reducing volatilization losses of ammonia derived from urea added to soils.

Investigations on the effects of temperature and moisture on urease activity in semi-arid tropical soils revealed that urease activity increased with increase in temperature from 10°C to 60°C (Vertisol) and 70°C (Alfisol). Further increase in the temperature decreased urease activity and completely inhibited at 100°C (Sahrawat, 1984). He also noted increase in urease activity with the increased moisture content up to field capacity beyond which activity declined.

O'Toole *et al.* (1985) studied urease activities in pasture and tillage soils of 10 soil series each. The results indicated higher activities of urease in pasture soils than in tillage soils in 8 of the 10 soil series. They recommended best prediction use of urease activity in grouping of agricultural soils by land use.

Palma and Conti (1990) studied the effects of various treatments of sample and seasonal variation upon urease activities on surface samples of Argentine Agricultural soils. They noted highest urease activity during summer and lowest during winter. The variation in the urease activity in different soil types under different vegetation revealed the activity of urease enzyme is related to the type of vegetation.

Tiwari and Mishra (1995) studied seasonal variation in urease activity in hilly soils under grassland and forests of north-eastern India. Their results

showed higher activity of urease under forest and grassland during rainy summer season and lower during winter season.

Studies on nitrification potential of urease in a mineral sub soil revealed the possible contribution of ammonia oxidizers to a complete hydrolysis of urea (Swenson and Bakken, 1998).

Klose and Tabatabai (1999) studied the relationship between urease and microbial biomass C and N and revealed highly significant relationship between urease activity and microbial biomass C and N. It was noted that urease activity of the microbial biomass, expressed as per cent of total urease activity ranged from 37.1 to 73.1% and the remaining 26.9 to 62.9% was extracellular.

### **6.3. METHODOLOGY**

#### **Enzyme activities**

Dehydrogenase enzyme activity was assayed using modified 2,3,5-triphenyl tetrazolium chloride (TTC) reduction technique (Casida, 1977). Five grams of soil was placed in a test tube (15 x 2cm) and carefully mixed with 0.1g of CaCO<sub>3</sub> and 1.5 ml of distilled water added into the mixture. Then, 1 ml of 1% TTC solution was added and the tubes were incubated at 30°C for 24 h after plugging with cotton. The resulting slurry was transferred on Whatman No.1 filter paper and triphenyl formazan (TPF) was extracted with successive aliquots of concentrated methanol in a 50 ml volumetric flask. The extinction of the pink colour was read out with the help of spectrophotometer (Systronics-106) at 485 nm using methanol as control (without soil).

$$\text{Dehydrogenase activity } (\mu\text{g TPF g}^{-1} \text{ dry soil } 24 \text{ h}^{-1}) = \frac{C \times 50}{W}$$

{Where, C= corrected reading of  $\mu\text{g TPF ml}^{-1}$  from the standard curve; 50= Extractant volume (ml); W= dry weight of soil}

Acid phosphatase activity was measured by *p*-nitrophenyl phosphate (*p*-NPP) reduction method of Tabatabai and Bremner (1969). 0.1g fresh soil sample was taken in a 50ml conical flask and mixed with 4 ml of modified universal buffer (MUB pH-6.5), 0.25ml toluene and 1ml of 0.115 M *p*-NP solution. The flask was swirled for a few seconds and plugged with cotton stopper and incubated for 1 h at 37°C. Then 1ml of 0.5 CaCl<sub>2</sub> and 4ml of 0.5 M NaOH solutions were added simultaneously into the mixture before transferring into Whatman No.12 filter paper. The yellow coloured filtrate of *p*-nitrophenol phosphate (phosphoric acid) was read out with the help of spectrophotometer at 420 nm. For the control, 1ml *p*-NPP was added after CaCl<sub>2</sub> and NaOH were added into the mixture without soil just before filtration.

$$\text{Acid phosphatase activity } (\mu\text{g } p\text{-NPP g}^{-1} \text{ dry soil h}^{-1}) = \frac{C \times 10}{W}$$

{Where, C= corrected reading of  $\mu\text{g } p\text{-NPP ml}^{-1}$  from the standard curve; 10= Solution volume (ml); W= dry weight of soil}

The urease activity was determined by urea reduction method of McGarity and Myers (1967). 10 g of fresh soil was placed in a 100 ml volumetric flask and treated with 1 ml of toluene, 10 ml buffer (pH-7) and 5 ml of 10% urea solution (freshly prepared). After a thorough mixing the flask was incubated for 3 h at 37°C in dark. For the control, 5 ml of 10% urea solution was replaced by 5 ml of sterile distilled water. After incubation the

volume of the flask was made up to 100 ml with distilled water and shaken thoroughly and transferred the filtrate through Whatman No.5 filter paper. The ammonia released as a result of urease activity was measured by indophenol blue method. 0.5 ml of the filtrate was taken into a 25 ml volumetric flask and 5 ml of distilled water was added. Then 2 ml of phenolate solution {mixture of 20 ml of stock A (62.5 g phenol crystals dissolved in a minimum volume of methanol and made up the volume upto 100 ml with ethyl alcohol after adding 18.5 ml acetone) and 20 ml of stock B (27 g NaOH dissolved in 100 ml distilled water and kept in freezer)} was added. Thereafter, 1.5 ml of sodium hypochlorite solution was added. The final volume of the flask was increased upto 25 ml with distilled water and the blue colour was read out with the spectrophotometer at 630 nm.

$$\text{Urease activity (mg NH}_4^+\text{-N g}^{-1}\text{ dry soil 3 h}^{-1}\text{)} = \frac{C \times 25 \times 100}{W}$$

{Where, C= corrected reading of mg NH<sub>4</sub><sup>+</sup>-N ml<sup>-1</sup> from the standard curve; 25= Extractant volume (ml); 100= Total solution volume; W= dry weight of soil}

Data reported anywhere in the text, table and figures for three enzymes (Dehydrogenase, phosphatase and urease) are mean values of triplicate analyses.

## 6.4. RESULTS

### Dehydrogenase activity

Activity of dehydrogenase enzyme ranged from 6-189 $\mu$ g TPF g<sup>-1</sup> dry soil 24 h<sup>-1</sup> in soils of degraded, moderately degraded and undegraded sites at surface and subsurface layers (Fig. 6.1). Maximum dehydrogenase activity was recorded from undegraded site (189 $\mu$ g TPF g<sup>-1</sup> dry soil 24 h<sup>-1</sup>) followed by moderately degraded site (145 $\mu$ g TPF g<sup>-1</sup> dry soil 24 h<sup>-1</sup>) while

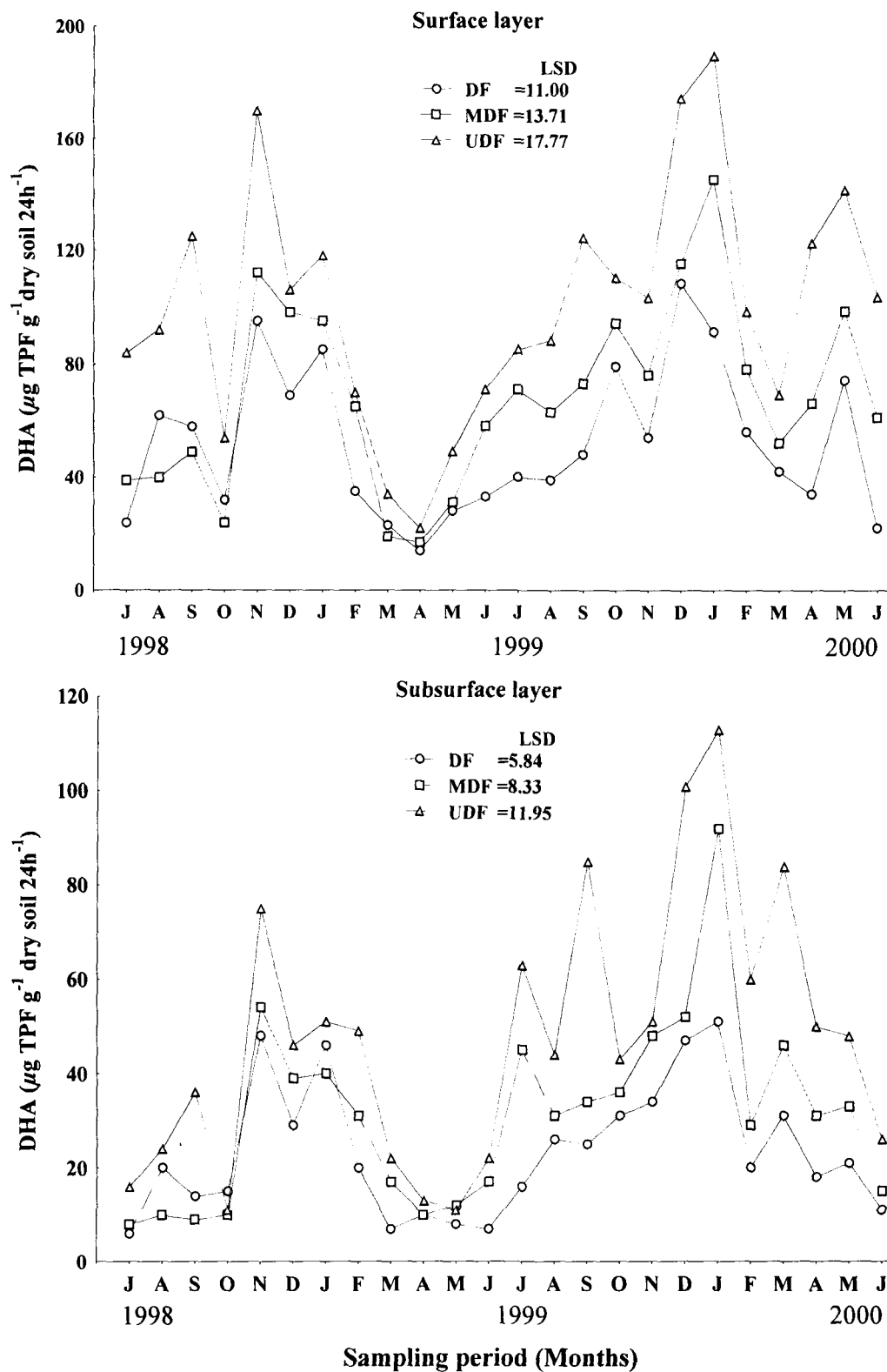
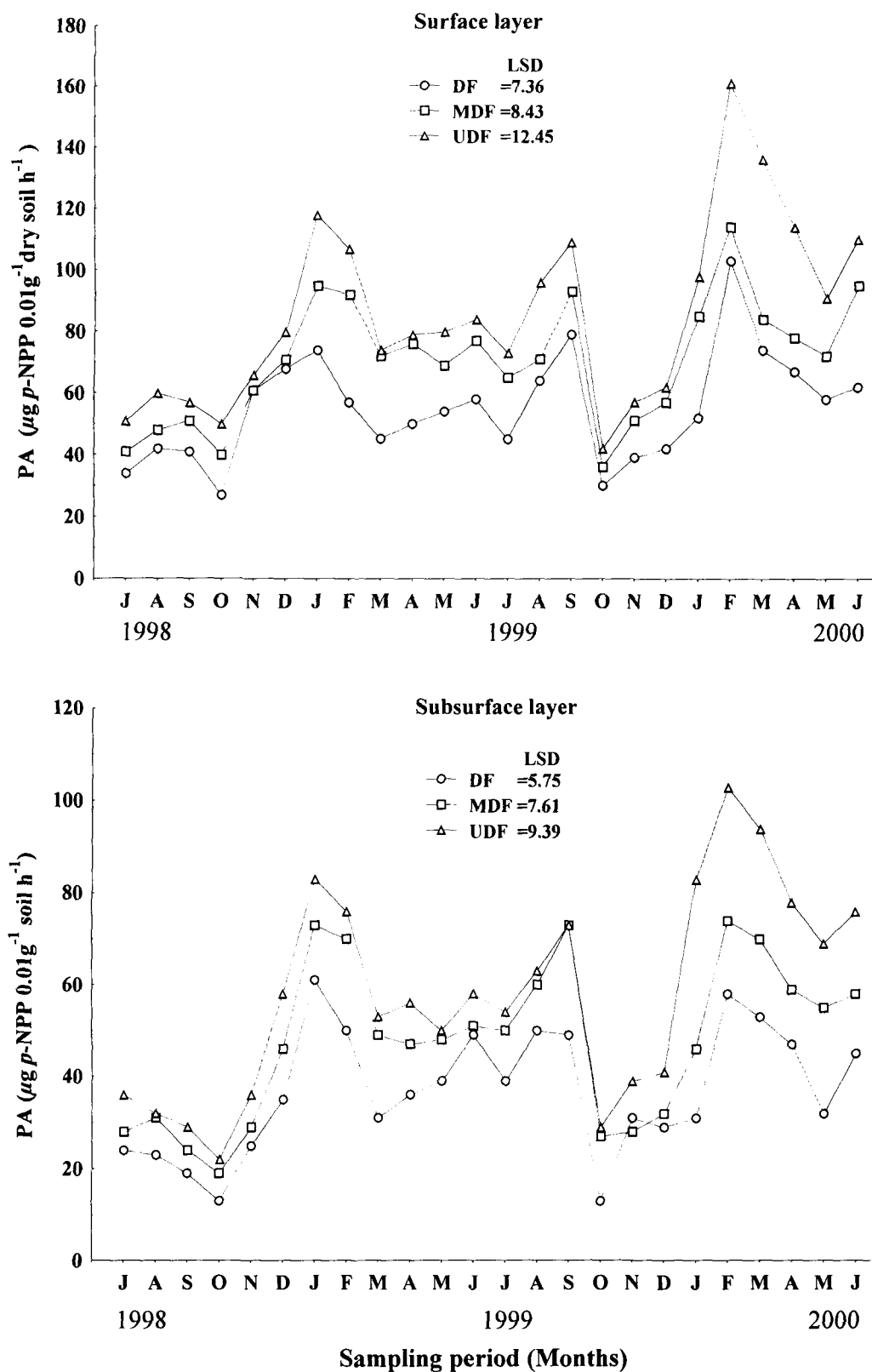
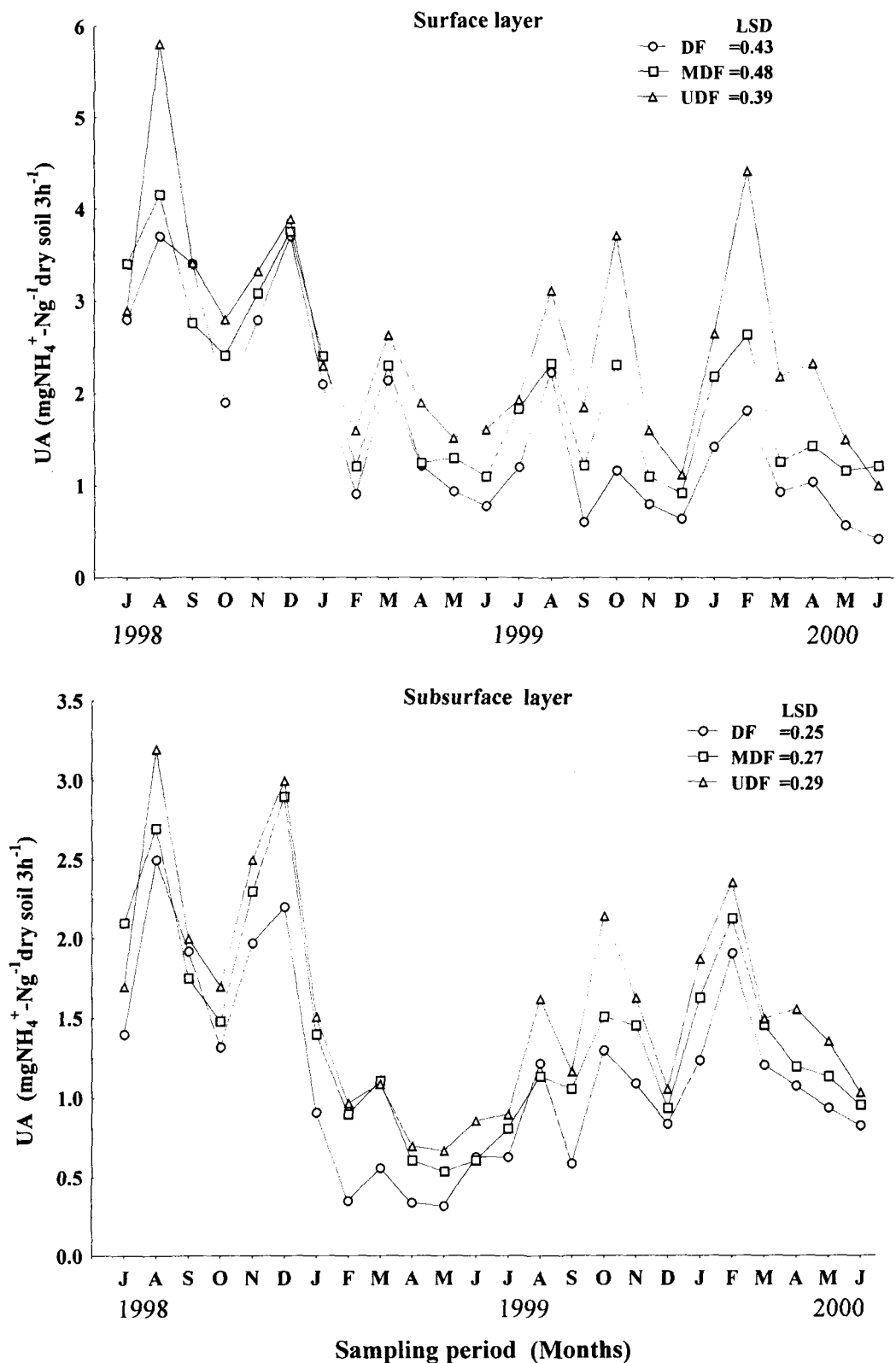


Fig.6.1. Dehydrogenase activity in soils of degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites at surface and subsurface layers. (LSD,  $P < 0.05$ )



**Fig.6.2. Acid phosphatase activity in soils of degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites at surface and subsurface layers. (LSD,  $P < 0.05$ )**



**Fig.6.3. Urease activity in soils of degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites at surface and subsurface layers. (LSD,  $P < 0.05$ )**

**Table 6.1. One-way analysis of variance (ANOVA) of the biochemical characteristics of soil in degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites at surface and subsurface soil layers at  $P < 0.05$ .**

Soil properties	Source of variation	Surface layer		Subsurface layer	
		F-ratio	P-level	F-ratio	P-level
Dehydrogenase activity	DFxMDFxUDF	12.334	$2.63 \times 10^{-5}$	8.735	$4.15 \times 10^{-4}$
	DFxMDF	-	-	-	-
	MDF x UDF	8.573	0.005	5.367	0.025
	DF x UDF	22.749	$1.89 \times 10^{-5}$	15.807	$2.46 \times 10^{-4}$
Phosphatase activity	DFxMDFxUDF	8.579	$4.71 \times 10^{-4}$	7.650	$9.98 \times 10^{-4}$
	DFxMDF	6.816	0.012	5.819	0.020
	MDF x UDF	-	-	-	-
	DF x UDF	14.2766	$4.52 \times 10^{-4}$	14.898	$3.52 \times 10^{-4}$
Urease activity	DFxMDFxUDF	3.281	0.043	-	-
	DFxMDF	-	-	-	-
	MDF x UDF	-	-	-	-
	DF x UDF	5.599	0.022	-	-

Note: Insignificant values are denoted by “-” sign

**Table 6.2. One-way analysis of variance (ANOVA) of the biochemical characteristics of soil between surface and subsurface soil layers in degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites ( $P < 0.05$ ).**

Soil properties	Study sites	F-ratio	P-level
Dehydrogenase activity	DF	26.891	$4.702 \times 10^{-6}$
	MDF	24.616	$1.000 \times 10^{-5}$
	UDF	27.489	$3.872 \times 10^{-6}$
Phosphatase activity	DF	17.4788	$1.288 \times 10^{-4}$
	MDF	15.274	$3.034 \times 10^{-4}$
	UDF	10.89	0.002
Urease activity	DF	-	-
	MDF	8.349	0.005
	UDF	-	-

Note: Insignificant values are denoted by “-” sign

the minimum was recorded from the degraded site ( $14\mu\text{g TPFg}^{-1}$  dry soil  $24\text{ h}^{-1}$ ) at both the soil depths. The maximum activity was recorded in the month of January 2000 from the undegraded site while the minimum was recorded in the month of April 1999 from the degraded site at surface soil layer. However, the subsurface soil layer contained maximum dehydrogenase activity in undegraded site in the month of January 2000 and minimum in degraded and moderately degraded sites in the month of July 1998. In general, the subsurface layer had higher dehydrogenase activity in all the sites and at both the soil layers.

There was a marked seasonal variation of dehydrogenase enzyme activity in all sites and at both the soil depths (Fig, 6.1). The highest activity was recorded during winter dry months (November to January) and lowest in the late spring and early rainy months (February–April). The variation in dehydrogenase activity among the three sites varied significantly ( $P<0.05$ ) at surface and subsurface soil layers except between the degraded and moderately degraded sites in which the variation in dehydrogenase activity was insignificant (Table 6.1). Similarly the variation in dehydrogenase activity of degraded, moderately degraded and undegraded sites between the two soil depths were also significant at  $P<0.05$  (Table 6.2) Dehydrogenase activity was positively correlated ( $P<0.05$ ) with soil pH and organic C in all sites (Table 3.3 and 3.4). Similarly it was also related to phosphatase activity at subsurface layer of undegraded forest site.

### **Acid phosphatase activity**

Acid phosphatase activity of the soil at both the surface and subsurface soil layers of all study sites varied from  $13\mu\text{g } p\text{-NPP g}^{-1} \text{ dry soil h}^{-1}$  to  $180\mu\text{g } p\text{-NPP g}^{-1} \text{ dry soil h}^{-1}$  at both the surface and subsurface soil layers (Fig. 6.2). The undegraded site contained maximum acid phosphatase activity followed by moderately degraded site while the minimum was recorded from degraded site at surface and subsurface soil layers. Generally, surface soil layer contained higher acid phosphatase activity than the subsurface soil layers in all the sites.

The seasonal variation in acid phosphatase activity showed three peaks during the entire period of two years in the three study sites. The highest peak was recorded in the month of February 2000 followed by January and September 1999. The results showed higher acid phosphatase activity during the intermediate period between late winter and early spring seasons and between, late rainy and early winter i.e. at the end of rainfall and winter season the activity was maximum at both soil layers.

The variation in acid phosphatase activity among degraded, moderately degraded and undegraded forest sites were significant ( $P < 0.05$ ) at both soil depths (Table 6.1). The variation in acid phosphatase activity was also significant between the two soil depths of degraded, moderately degraded and undegraded forest sites (Table 6.2). Phosphatase activity was positively correlated ( $P < 0.05$ ) with organic C in all study sites at both soil layers (Table 3.3 and 3.4). Microbial biomass N was also related positively

with phosphatase activity in the surface soil layers of degraded, moderately degraded and undegraded forest sites.

### **Urease activity**

Urease activity varied from the minimum of 0.3 mg NH<sub>4</sub><sup>+</sup>-N 100 g<sup>-1</sup> soil 3 h<sup>-1</sup> in the degraded site to a maximum of 5.81mg NH<sub>4</sub><sup>+</sup>-N 100 g<sup>-1</sup> soil 3 h<sup>-1</sup> in the undegraded site (Fig. 6.3). The maximum urease activity occurred in the month of August 1998 at both the soil layers of undegraded site whereas the minimum was recorded from the degraded site in June 2000 from the surface layer and in May 1999 from subsurface layer respectively.

There was significant variation in urease activity distribution among the three sites and between the degraded site and undegraded site at surface soil layer only (Table 6.1). However, no significant variation was observed between the degraded and moderately degraded sites and between moderately degraded and undegraded sites at both the surface and subsurface soil layers. The variation in urease activity was significant ( $P < 0.05$ ) between the surface and subsurface soil layers of moderately degraded site only but no significant variation of urease activity was recorded from the degraded site and undegraded sites (Table 6.2). Urease activity was positively related to soil pH of undegraded site at surface layer and with available-N in degraded and moderately degraded sites at subsurface layers (Table 3.3 and 3.4).

## 6.5. DISCUSSION

### 6.5.1. Spatial variation of dehydrogenase, acid phosphatase and urease enzyme activities

Dehydrogenase enzyme activity was significantly higher in the soil of undegraded forest site in comparison to the degraded and undegraded sites. The reason for higher dehydrogenase enzyme activity could be due to the presence of higher organic matter on the forest floor and abundant tree cover in this site providing favourable microclimatic conditions for larger microbial growth and accumulation of more enzymes. Tiwari *et al.* (2002) have reported higher dehydrogenase activity in an undisturbed forest site in comparison to a degraded site and a slightly degraded site in humid tropical regions of north-eastern India. They have noted 40% and 25% reduction in dehydrogenase activity of degraded and moderately degraded sites at surface and subsurface soil layers in comparison to the undisturbed site. The decline in dehydrogenase activity in the degraded site in the present study reveals the long-term detrimental effect of shifting cultivation practice and selective logging of forest trees on biochemical characteristics of soil. The removal of the vegetation by clear-cutting prior to the sowing of crops and continuous cutting of selected trees caused significant reduction in dehydrogenase activity and other microbiological properties in soils of these sites. Garcia *et al.* (1997) found that devegetation of soils in semi-arid areas lead to reduction of their biochemical quality in contrast to natural or undisturbed area. As an indication of microbiological metabolic activity, dehydrogenase activity was significantly affected as a result of devegetation in their study.

Acid phosphatase activity was higher in the undegraded forest site than degraded and moderately degraded sites at both the surface and subsurface soil layers. The undegraded site had highest acid phosphatase activity due to the presence of higher organic matter on the forest floor which influenced greater growth of microorganisms and accumulation of more soil enzymes (Dinesh *et al.*, 1998). Another reason could be the favourable soil reaction in the undegraded site which influenced higher enzyme activity and declined with increased soil acidity. Staddon *et al.* (1998) also reported similar correlation of acid phosphatase activity with soil pH in clear-cut, burned and scarified jackpine (*Pinus banksiana* L.) community soils. Increase in acid phosphatase activity with increase in soil pH have been reported in detailed by Dick *et al.* (1998). Similar results of higher acid phosphatase activity was observed in pineapple (*Annanus comosus* L.) orchard soils of different ages (Tiwari, 1988). His study revealed significant relationship between acid phosphatase activity and organic C content in the 1, 5 and 10 year old pineapple orchards.

Urease activity showed significant variation among soils of all the three study sites and at surface soil layers but there was no significant variation among the sites at subsurface soil layers (Table 6.1). However, urease activity, remained highest in the undegraded site during its peak activity periods than degraded and moderately degraded sites at both soil layers. This results suggest no detrimental effect of shifting cultivation and selective logging of trees in the forest soils on urease activity. Earlier reports on urease soil enzyme activity have revealed generally higher urease activity

in older pineapple orchard soils than younger ones (Tiwari, 1988), in forest soils than in grasslands (Tiwari and Mishra, 1995), in pasture than in tillage soils (O'Toole et al., 1985) and in no tillage soils than in plow-grain soils (Klein and Kloths, 1980). Pancholy and Rice (1973) showed that urease activity is related to type of vegetation and the quality of incorporated organic materials in the soil. Similarly, Palma and Conti (1990) also reported significant variation in distribution of urease activity in grassland and forest soils revealing direct relationship between pattern of urease activity to the type of vegetation and impact of organic matter.

#### **6.5.2. Seasonal variation of dehydrogenase, acid phosphatase and urease activities**

Distribution of dehydrogenase activity was significantly affected by seasonal variation in the three study sites and at two soil depths. Dehydrogenase enzyme activity was higher during winter dry period and lower during summer rainy season. The peak of dehydrogenase enzyme activity occurred in the months of November 1998 and another in January 2000 where the soil was dry and temperature was also lower than the average. This suggests that accumulation of dehydrogenase enzyme takes place with the onset of winter season which is against the decreasing moisture gradient in all the three study sites and at two soil depths. Dormaar *et al.* (1984) also reported higher dehydrogenase activity during winter months and lower during summer months under two grazing regimes of two different study site. They did not find close relationship between dehydrogenase activity and soil organic matter in either site though the relationship existed between the two areas. However, they assumed that

hygrothermal conditions (soil moisture and temperature) were the overriding factors of dehydrogenase activity. The results in the present study indicated that higher dehydrogenase activity being controlled by presence of higher organic matter content during the dry winter period and favourable soil pH of the soils.

Acid phosphatase activity was significantly influenced by fluctuations in seasonal climatic variables in soils of degraded, moderately degraded and undegraded forest sites. The peaks of the acid phosphatase activity in the present study appeared in the intermediate period of winter and spring and another in rainy-winter period reveal that this soil enzyme activity is dependent on the moisture availability in all sites and at both the soil depths. However, the sharp decline in the acid phosphatase activity in the month of October in both the years 1998 and 1999 could not be explained despite its positive correlation with soil pH, organic C, total N and microbial biomass N. Higher phosphatase activity was also reported during spring-summer from pineapple orchard soil of north-eastern India (Tiwari, 1988 and Tiwari, S.C. *et al.*, 1989ab) and during peak rainy seasons of acid soil rich in organic matter content in Galicia, NW Spain (Trasar-Cepeda and Gil-Sotres, 1987). However, the results of the present study corresponds to the findings of Dormaar *et al.* (1984) which reported higher acid phosphatase activity during winter months due to higher root biomass and microbial population in the soils.

There was no clear trend in variation of urease activity along seasonal gradients in all study sites though the activity. The monthly variation in

urease activity was insignificant in degraded, moderately degraded and undegraded sites at both soil layers. Therefore, seasonal variation in urease activity remained unchanged throughout the sampling period of the study.

### **6.5.3. Depth-wise variation of dehydrogenase, acid phosphatase and urease enzyme activities**

In general, dehydrogenase activity was higher at the surface soil layers of all sites than the subsurface soil layers. This reveals that dehydrogenase enzyme is produced at the surface soil layer which contains larger amounts of decomposed litter and organic carbon. However, the lower dehydrogenase activity in the subsurface soil layer could be due to lower quantity of dehydrogenase enzyme produced in this layer due to the presence of lower organic matter and microbial populations. Higher dehydrogenase activity was reported from the surface soil layers which declined with increasing depth in pineapple orchard soils of north-eastern India (Tiwari *et al.*, 1987a). The reason for higher dehydrogenase enzyme activity in the surface soil layer was due to presence of higher bacterial population, organic carbon content, favourable moisture content and temperature (Khan, 1970; Das, 1980; Dkhar and Mishra, 1983; Baruah and Mishra, 1984 and Tiwari *et al.*, 1987b). Dehydrogenase enzymes appeared to be linked with microbial activity associated with initial breakdown of organic matter and are dependant on the metabolic state of the soil or on the biological activity of the microbial populations (Ross, 1970). The results in the present study reveals that more accumulation of dehydrogenase enzyme at the surface soil layer having higher microbial population and organic carbon content than at the subsurface layers of all study sites.

The activity of phosphatase was higher at the surface soil and lower at the subsurface soil layer of degraded, moderately degraded and undegraded forest sites. There was decrease in acid phosphatase activity of the soil with increase in the soil depth from highest at the surface to the lowest in the subsurface layer of all sites. This reveals presence of higher soil enzyme at the surface soil layer and lower enzyme accumulation in the subsurface soil layer. The presence of higher organic matter, pH and total N at the surface soil layer could be other important factors for higher acid phosphatase activity in the surface soil layer since these properties of soil were correlated positively to the acid phosphatase activity. Trasar-Cepeda and Gil-Sotres (1987) also reported decrease in soil enzyme activity of acid phosphatase with increase in the soil depth and its positive correlation with organic carbon content. This suggests that acid phosphatase enzyme originated mainly at the surface soil layer, which contained more litter and was progressively inhibited with increase in soil depth, which was associated with a decrease in organic C content. Their results also confirmed significant correlation of phosphatase activity with the soil pH under different tillage and residue managed soils. Higher acid phosphatase was also reported from the pineapple orchard soils of north-eastern India (Tiwari, 1988; 1996ab and Tiwari, S.C. *et al.*, 1989ab). Their results confirmed that enzyme activity of acid phosphatase decreased from surface organic layer to deeper subsurface layers along the decreasing trend of soil organic C, moisture and other inorganic nutrients and lower microbial populations.

Distribution of urease activity was not significantly different between the surface and subsurface soil layers of the degraded and undegraded sites. However, the urease activity varied significantly between two soil depths of moderately degraded site. Singh *et al.* (1995) also reported insignificant variation in the distribution of urease activity along the gradient of soil depth from surface to the deeper soil depths, which is against the earlier reports of the decreasing turnover of urease activity with increase in soil depth (Tiwari *et al.*, 1987a and Tiwari, 1988 and 1996).

## **Chapter 7**

### **Evaluation of forest soil degradation by stepwise discriminant function analysis**

#### **7.1. INTRODUCTION**

Discriminant function analysis (DFA) is an important statistical tool generally used to determine which variables discriminate between two or more naturally occurring groups. DFA includes a set of response variables and a set of one or more grouping or nominally scaled variables (Huberty and Barton, 1989). The use of stepwise methodologies has been sharply criticized by several researches, yet their popularity, especially in educational and psychological research, continues unabated and considered particularly well suited for use in regression and discriminant analysis (Whitaker, 1997). The application of this statistical tool is very common in case of business prediction (Parks and Waldo, 1999), social studies (Hough and Erwin, 1997), medical sciences (Telmissani *et al.*, 1999), forensic science and medico-legal investigations (Kalmey and Rathbun, 1996; King *et al.*, 1998; Holliday and Falsetti, 1999; Tanaka *et al.*, 2000 and Purkait, 2001). Presently, this statistical tool is increasingly used in the field of animal sciences research particularly in predicting the animal behavior (Poulson *et al.*, 1997) and in identifying conservation strategies and habitat location of rare animal species (Pergams *et al.*, 2000 and Hussain *et al.*, 2001).

Very little work has been done on general numerical characterization of microbial communities occurring in soil and in other natural habitats despite considerable popularity of multivariate analysis in synecology of

higher organisms (James and McCulloch, 1990). Generally, multivariate numerical methods are being used in microbiology mainly for numerical taxonomy of Bacteria (Rozycki, 1998). A recent study revealed the successful discrimination of plots under natural sod from that of cropped and plow managed soils with the use of fatty acid methyl esters (FAMES), particularly ester-linked FAMES (EL-FAMES) and phospholipid-linked FAMES (PL-FAMES) as discriminating variables related to soil microorganisms (Drijber *et al.*, 2000). However, literatures are scarce regarding the evaluation or prediction of forest soil degradation types from the databases of various soil characteristics using stepwise discriminant function analysis. Therefore, the present study is an attempt to classify or evaluate soil degradation types in humid tropical forests of Arunachal Pradesh, north-eastern India by using the stepwise discriminant function analysis as statistical tool from the databases of various soil properties collected for a period of two years.

## **7.2. METHODOLOGY**

The data of physico-chemical, biological and biochemical parameters of two soil depths (surface and subsurface layers) collected for a period of 24 months (July 1998 to June 2000) from degraded (DF), moderately degraded (MDF) and undegraded (UDF) forest sites were used for the present DFA. The monthly data of every parameter from the surface and subsurface layers of each site were pooled and their mean value calculated. The mean values were used for stepwise DFA was run in SPSS statistical software Version 6 for Windows using Advanced Statistics Users' Guide (Norusis, 1990) to identify those variables which at the most can predict or can discriminate

forest soil degradation types among the degraded, moderately degraded and undegraded forest sites.

The various soil variables included in the present DFA were physico-chemical variables {soil temperature (ST), soil moisture (SM), bulk density (BD), water holding capacity (WHC), pH, organic C (OC), total N, available-N { $\text{NH}_4^+$ -N (AN) and  $\text{NO}_3^-$ -N (NN)} and total P (TP)}, biological variables {soil respiration or  $\text{CO}_2$  evolution (SR), bacterial population (BP), fungal population (FP), microbial biomass C ( $C_{mic}$ ) and microbial biomass N ( $N_{mic}$ )} and biochemical variables {i.e. activities of dehydrogenase (DHA), acid phosphatase (PA) and urease (UA) soil enzymes} respectively. A variable column named "Type" was included in the analysis where the data in each row of the degraded site was represented by a logical value 0, moderately degraded site by 1 and undegraded site by 2. Then, stepwise discriminant function analysis was conducted to classify the soil degradation types where the variable (soil parameter) having a significant F value at  $P \leq 0.05$  was included in the model and those which does not have a significant F value was removed from the model.

**Preliminary selection of significantly important soil characteristic for inclusion in the analysis:**

A preliminary selection of significantly important parameters from each group of soil characteristics was performed for their inclusion in the final stepwise discriminant function analysis for classification of forest soil degradation type.

The physico-chemical characteristics of soils i.e. ST, SM, BD, WHC, pH, OC, TN, AN, NN and TP were processed for stepwise discriminant

function analysis at  $P \leq 0.05$  to identify those parameters/ variables which are responsible for differentiating the forest soil degradation types. At the end of the analysis, seven (7) physico-chemical characteristics namely, ST, BD, pH, OC, TN, AN and NN were found with significant contribution to the variation in forest soil degradation types between the three sites. Three variables SM, WHC, and TP were removed from the analysis.

The biological characteristics of soil i.e. SR, BP, FP,  $C_{mic}$  and  $N_{mic}$  were tested for their contribution to the variation in forest soil degradation types by stepwise discriminant function analysis. Only  $C_{mic}$  was found selected after step 1 due to its significant contribution in the variation of forest soil degradation types. Four variables were removed from the analysis due to poor significant contribution in group variability of the soil degradation types.

Similarly, three biochemical characteristics of the soil namely, DHA, PA and UA were processed for stepwise discriminant function analysis. DHA was the only variable found entered in the analysis because of its significant contribution in differentiating the forest soil degradation types. PA and UA were removed from the analysis model.

Therefore, altogether ten (10) variables belonging to various groups of soil characteristics i.e. physico-chemical, biological and biochemical characteristics (ST, BD, pH, OC, NN, TN, AN, NN,  $C_{mic}$ , and DHA) were selected for inclusion in the final stepwise discriminant function analysis for classification of forest soil degradation types.

### 7.3. RESULTS

The tolerance limits, F values to enter in the analysis model and corresponding Wilks' Lamda values of each variable at step 0 of the analysis are given in table 7.1 ( $P \leq 0.05$ ). OC had the maximum value of F and corresponding lowest value of Wilks' Lamda followed by BD. The minimum F and maximum Wilks' Lamda value were noted against the variable, ST.

The entire analysis was completed at step 7 where a maximum of seven variables namely, OC, BD, pH, ST, TN,  $C_{\text{cmi}}$  and DHA were entered in the model. The entry of a variable was performed based on the maximum value of F at highest level of significance after the one-way analysis of variance (ANOVA) of the variables in each step. The OC with maximum F value (271.583) in step 0 was entered as first variable in step 1 of the analysis. The F values of the remaining nine variables are calculated by one-way ANOVA before preceding the next step and the variable with maximum F value i.e. BD (153.685) is entered. Simultaneously, pH, ST, TN,  $C_{\text{cmi}}$  and DHA were entered in stem 3 to 7 in accordance of decreasing order of F values. The summary of the stepwise discriminant analysis in table 6.2 shows the steps in which a particular variable was entered or removed in the model along with the values of Wilks' Lamda and significance levels.

Two canonical discriminant functions (CDFs) were obtained from this DFA which together contributed a total variance percentage of 100 (Table 7.3). Canonical discriminant function 1 (CDF1) was the most important function due to its higher Eigenvalue (31.1829) and canonical correlation

**Table 7.1. Variables and their characteristics at step 0 of DFA**

Variable	Tolerance	Minimum tolerance	F to Enter	Wilks' Lambda
BD	1.0000000	1.0000000	119.7685336	0.2236360
AN	1.0000000	1.0000000	4.1679015	0.8922129
CMI	1.0000000	1.0000000	31.7419826	0.5208177
DHA	1.0000000	1.0000000	11.7056832	0.7466614
NN	1.0000000	1.0000000	8.4051105	0.8041000
OC	1.0000000	1.0000000	271.5834452	0.1127144
pH	1.0000000	1.0000000	9.9066778	0.7769102
PH	1.0000000	1.0000000	5.6877132	0.8584713
ST	1.0000000	1.0000000	1.4503897	0.9596558
TN	1.0000000	1.0000000	6.9290541	0.8327489

**Table 7.2. Summary of stepwise discriminant function analysis**

Steps	Variables		Wilks' Lambda	Significance Level ( <i>P</i> )
	Entered	Removed		
1	OC	-	0.11271	0.0000
2	BD	-	0.03282	0.0000
3	pH	-	0.01870	0.0000
4	ST	-	0.01237	0.0000
5	TN	-	0.00799	0.0000
6	C <sub>cmi</sub>	-	0.00581	0.0000
7	DHA	-	0.00507	0.0000

(Note: The sign "-" denotes that no variable was removed in any of the steps)

**Table 7.3. Canonical Discriminant Functions (CDFs) after DFA**

CDFs	Eigenvalue	% of Variance	Cumulative % variance	Canonical Correlation	After function	Wilks' Lambda	Chi-square	Degrees of freedom	P level
CDF 1*	31.1829	85.88	85.88	0.9843	0	0.005070	348.773	14	0.00001
CDF 2*	5.1288	14.12	100.00	0.9148	1	0.163163	119.658	6	0.00001

\* Marks the 2 canonical discriminant functions remaining in the analysis.

**(Note: Eigenvalue:** The ratio of the between group sums of square to within group sums of squares. Large Eigenvalue are associated with good function. **Percent of variance:** When two or more functions are derived in a discriminant analysis, to compare their merits "the percent of total between group variations" attributable to each function, percent of variance is taken into consideration. The remaining function has successively less between variability. **Canonical correlation:** It is the square root of the ratio of between group sums of square to total sums of square. When squared, it is the proportion of the total variability explained by differences between groups. **After function:** It shows the number of functions entered/ remained at the end of analysis. **Wilks' Lamda:** The value of Wilks' Lamda provides a test of the null hypothesis that in the population, the means of all discriminant function in all groups are really equal.)

**Table 7.4.** Structure matrix (standardized canonical function coefficients) of all the variables in the canonical discriminant function 1 (CDF1) and canonical discriminant function 2 (CDF2).

Variables	CDF 1 (85.88%)	CDF 2 (14.12%)
OC	0.50243*	-0.00762
BD	-0.19963	0.65923*
CMI	-0.00547	-0.37283*
DHA	0.02708	-0.24839*
TN	0.03181	-0.18168*
pH	0.04572	-0.13941*
ST	-0.02629	0.06320*

\* denotes largest absolute correlation between each variable and any discriminant function.

(**Note:** The matrix shows the individual contribution of each variables within the function in terms of standardized canonical function coefficients. Variables with large absolute coefficients has the most significant contribution to the particular function (CDF 1 or CDF 2). Negative sign indicates that small functions are associated with the large values of the variables, a case of negative correlation.

(0.9843). This function accounted for a maximum variation in soil degradation types in the present study with a variance percentage of 85.88 as compared to the canonical discriminant function 2 (CDF2) having lower Eigenvalue (5.1288) and canonical correlation (0.0148) accounting for only 14.12 % variation in the forest soil degradation types. The structure matrix of each variable in table 7.4 gives the pooled within group correlations between the discriminating function variables and canonical discriminant functions. The variables in the table are ordered by the size of the correlation with each function.

#### **7.4. DISCUSSION**

Organic C content of the soils in the degraded, moderately degraded and undegraded forest sites was found as the only variable from the CDF1 which had contributed to a maximum of 85.88 % variance of soil degradation types and highest within-groups positive canonical correlation (0.502) in the present study. This suggests that stepwise DFA provide a much better classification of the soil degradation type among the three study sites based on the two discriminant functions, CDF1 and CDF2 as compared to the other visual site characteristics. It is clear from this analysis that the soil in each of the study sites differ significantly ( $P \leq 0.05$ ) in terms of a gradient in organic C content or organic matter in the soils. Maximum organic C content in undegraded forest site varied towards a minimum organic C content in the degraded site through an intermediate value of organic C in the moderately degraded site. The average organic C content of the undegraded site remained higher for the entire sampling period of two years from July 1998

to June 2000 despite seasonal variations. The main reason for this higher organic C content in the undegraded site could be due to absence of forest soil disturbance either for selective logging or shifting cultivation which are the root causes of organic C loss from the hill soils of the region. Another reason might be the rapid accumulation of litter on the forest floor from the evergreen perennial vegetation present in this site resulting in increased organic C content of the soil. On the other hand, the soils in degraded and moderately degraded forest sites were repeatedly disturbed by shifting cultivation and selective logging practices for a period of more than 20 years leading to a decline in organic C contents. The main reason for this decline in soil organic C of the degraded site might be due to the destruction of the soil organic matter at the surface layer since uncontrolled burning of dried slash is done every time prior to crop sowing in the fields during shifting cultivation practices. This uncontrolled burning not only reduces organic C content of soils but also kills microorganisms in the soil ecosystem. In shifting cultivation practices, it is usual that preparation of field for sowing crops is done in the late winter months before the onset of spring or monsoon rain. This causes rapid loss of fertile topsoil and organic matter contents during runoff from the hill slopes, as there occurs heavy rainfall in this region. The recovery of the organic matter in these shifting cultivated soils become slower due to the absence of a permanent vegetal cover and absence of root systems to hold soil particles and organic matter. Similarly, the soil of moderately degraded site contained lower organic matter content as compared to the undegraded site though it contained higher organic

matter content than the degraded site. Despite a permanent vegetal cover in this site, selective logging caused increased tree gap. The removal of forest floor litter through annual clear cutting of grasses and weeds every year or every alternate year followed by subsequent burning of the dried mass by the forest personals for growth improvement of introduced plantations was the main reason for decline in organic C content in this site. In absence of the forest floor vegetation, the rate of runoff increases in this site leading to rapid loss of organic matter content and soil particles from the hill slopes.

The variables in CDF 2 in this DFA comprised of various physico-chemical, biological and biochemical characteristics of soils which together accounted for a total of 14.12% variation in soil degradation types between the three study sites. Bulk density with a maximum canonical correlation value of 0.659 was the most significant variable in CDF 2 which had contributed maximum percentage in discriminating the soil degradation types of the three study sites. The average bulk density varied significantly between the three study sites where highest value was recorded from the degraded site followed by moderately degraded site and lowest bulk density was recorded from the undegraded site. This pattern of variation in bulk density is in contrast to that of organic C contents where maximum organic C content was undegraded site followed by moderately degraded site and minimum was recorded from the degraded site.

The other variables in CDF 2 namely, microbial biomass C, dehydrogenase activity, total N, soil pH and soil temperatures were also important in discriminating the soil degradation types between the three

study sites but their absolute correlation with the discriminant function were negative or comparatively lower to the bulk density.

Therefore, it can be concluded from the present discriminant analysis that the stepwise discriminant function analysis can successfully classify the soil degradation types in degraded, moderately degraded and undegraded forests sites in terms of two canonical discriminant functions comprising of seven variables. These two functions could discriminate the soil degradation types upto a maximum of 100% between the three study sites. The CDF 1 had a single discriminating variable i.e. organic C having the largest absolute positive correlation with the function could contributed to a maximum of about 86% variation in soil degradation types between the sites. The CDF 2 consisting of six variables i.e. bulk density, microbial biomass C, dehydrogenase activity, total N, soil pH and soil temperature could contributed only 14% of the total variation in soil degradation types between the sites. The bulk density with the largest absolute positive correlation with the function was the only important variable in CDF 2, which had contributed to a maximum variation of soil degradation types between the three study sites. Therefore, it may be recommended that determination of the soil organic C content and bulk density with or without few soil characteristics may be used to evaluate or classify the status soil degradation in the humid tropical hill forest soils of north-eastern region in particular and other soils of the country in general.

## **Chapter 8**

### **GENERAL DISCUSSION**

#### **8.1. Physico-chemical characteristics of soils**

***Soil temperature, soil moisture, bulk density, water holding capacity, pH, organic C, total N,  $NH_4^+$ -N,  $NO_3^-$ -N and total P***

Physico-chemical characteristics of the soils in the present study showed a declining trend of their values from highest in the undegraded forest site to the lowest in degraded forest site while moderately degraded site contained an intermediate range of their values at surface and subsurface soil layers. In general, the important soil parameters such as, soil profile (Ah) thickness (Plate 5), moisture content, water holding capacity, pH, organic C, total N, ammonium and nitrate-N and total P showed declining trend with the increase in forest soil disturbance i.e. the maximum values of these parameters were recorded from undegraded forest site followed by moderately degraded site whereas minimum values were recorded from the degraded forest site at both surface and subsurface soil layers. However, the parameters such as, soil temperature and bulk density were found highest in the degraded site followed by moderately degraded site. Lowest values these two soil parameters were noted from the undegraded forest site at surface and subsurface soil layers. This shows that soil disturbance through the intensive agriculture systems like shifting cultivation and selective logging practices caused significant impact on various physico-chemical characteristics of soils in different magnitudes in the humid tropical hill region of north-eastern India.

Higher soil temperature at surface and subsurface soil layer of the degraded site than the moderately degraded and undegraded sites could be due to the heating up of the soil surface by direct exposure of soil surface to solar insolation in absence of tree canopy. The intensity of the increased soil temperature was very prominent during the mid summer season (April to July) in case of degraded site as compared to other sites. The lower soil temperature at surface and subsurface soil layers of undegraded site might be due to presence of abundant vegetal cover and thick layer of litter on the forest floor which protects direct heating of the soil surface due to insolation leading to lowering of soil temperature at the site. Soil moisture content did not vary significantly among the three study sites at surface soil layer though it varied significantly ( $P < 0.05$ ) at subsurface soil layer (Table 3.1). However, variation of soil moisture was significant in each of the study sites. Highest moisture content was recorded from the undegraded site in the month of June 2000 at both soil layers (Fig.3.2). The reason could be due to the ability of soils to retain water and reduction in faster runoff due to the presence of higher organic matter on the surface layer of undegraded site following rainfall. In fact, the forest floor of the undegraded forest site was covered with a thick layer of litter (the fallen leaves, broken twigs and debris of dried seasonal weeds and grasses of the forest vegetation) must have absorbed water and protected excessive evaporation after rain resulting in higher soil moisture content in this site. These properties of soil to retain water and reduce faster runoff after rainfall must have been lost as a result of long term shifting cultivation practices in the degraded site. Similarly, frequent logging

of trees along with cleaning of forest floors for alternate years in the moderately degraded site might have caused lowering of soil moisture content. At the same time, the absence of permanent forest vegetation, litter and exposure of the forest floor to atmosphere must have increased soil surface runoff and excessive evaporation causing decline in soil moisture in these sites.

Bulk density of the degraded site was found significantly ( $P < 0.05$ ) higher than the moderately degraded and undegraded forest sites at both the surface and subsurface soil layers (Table 3.1). There was an increasing trend of bulk density with the increase in degree of forest soil degradation where lowest bulk density was recorded from the undegraded site followed by the moderately degraded site and the highest bulk density recorded from the degraded forest site at both surface and subsurface soil layers. The main reason for the increase in bulk density of the degraded site might be due to enhanced compaction and hardening of the soil while the site was left abandoned as fallow land after continuous utilization for agricultural production through shifting cultivation practice. Usually, soil aggregates become smaller and smaller when it is used for cultivation by tillage practice. Large portions these smaller soil particles become lost through runoff from the hill slopes and some particles enters deeper layers along with gravitational water movement following rainfall during the period of vegetation regeneration for nutrient restoration. These might have caused removal of upper topsoil layer (Ah) and exposure of lower soil horizons (Bo). These causes decreased porosity of the soil leading to increased soil bulk density in

the degraded site. Hajabbasi *et al.* (1997) also reported similar results of 20% increase in bulk density as a result of conversion of tropical forests into agricultural land in Iran. This is in correspondence with present findings of 15% increase of bulk density in the degraded site as compared to the undegraded forest site.

Water holding capacity also varied significantly ( $P < 0.05$ ) among the three study sites at both soil layers though there was no significant variation recorded with change in sampling month. The maximum water holding capacity recorded from the undegraded site could be ascribed to the presence of large tree vegetation having diffused root network and thick layer of litter accumulated (round the year) on the forest floor of this site as compared to degraded and moderately degraded sites (Plate 4). Generally, the surface soil layers had higher water holding capacity than subsurface soil layers of the degraded, moderately degraded and undegraded sites showing a decline in water holding capacity with the increase in soil depth. Arunachalam *et al.* (1996) and Maithani *et al.* (1996; 1998) have also reported higher water holding capacities of old aged forest soils than younger forest re-growths in the north-eastern region. Further, they concluded that increase in forest disturbance through logging practice caused decline in water holding capacity of soils.

Soil reaction of the degraded site revealed strongly acidic condition in comparison to the undegraded forest site at both the surface and subsurface soil layers. There was an increase in acidity of soils in all the there sites with the increase in soil depth from surface towards subsurface soil layers. The

reason for acidic pH in the degraded site might be due to strong leaching of bases from the surface soil through water runoff and presence of the  $Al^{+3}$  in the degraded site following shifting cultivation practices (Nayak and Srivastava, 1995 and Bhattacharya *et al.*, 1998). However, Singh *et al.* (1995) reported more acidic soil pH of jhum fallows as compared to natural bamboo forests of north-eastern India which is in contrast to the present findings of highly acidic soil in the abandoned jhum fallow as compared to an undegraded natural hill forest. The moderately degraded and undegraded sites had slightly acidic pH at surface and subsurface soil layers revealing that these soils have buffering activity to protect higher base leaching due to the presence of large vegetal cover and thick layer of litter on the forest floor.

Organic C content of the undegraded site was highest in comparison to degraded and moderately degraded forest sites at both surface and subsurface soil layers. The main reason for higher organic C content must be due to the presence of permanent vegetation with higher species diversity and accumulation of large amounts of organic residues from the thick layer of litter on the forest floor of undegraded site. Whereas, lower organic C content in the degraded site might be due to the absence of a permanent vegetation cover (Plate 1) to provide forest floor litter other than seasonal weeds, ferns and grasses. Another reason could be the depletion of the soil organic matter, which is the source of nutrients for crops in soil during the period of shifting cultivation. Ultimately, poor nutrient status of the soil led to the abandonment of this site as fallow land. Similarly, the forest floors of the

moderately degraded site was cleaned and burned by forest department of the state for allowing proper growth of newly planted trees could have also led to reduction in organic C content in this site as compared to the undegraded site. Decline in organic C content of soils as a result of conversion of natural forests into agriculture lands, pastures and due to logging practices have been reported from the tropical and sub-tropical forests (Brown *et al.*, 1994; Henrot and Robertson, 1994; Saikh *et al.*, 1998 and Arunachalam *et al.*, 1996, 1997; 1998). The organic C content also declined with the increase in soil depth from surface to subsurface layer of degraded, moderately degraded and undegraded forest sites in the present study.

Total N and available N (ammonium and nitrate-N) contents of the soil in the study sites revealed a declining pattern with the increase in degree of soil degradation. Total and available N contents were comparatively higher in the undegraded site followed by moderately degraded site and lowest was recorded from the degraded forest site. The higher N content in the undegraded site might be due to higher nitrification rate of the organic residues at favourable rainfall, soil temperature, moisture and organic C contents. Significant ( $P < 0.05$ ) positive correlations were observed between these parameters and total N and available N contents at surface and subsurface soil layers of the undegraded site. However, the total N and available N contents were lower in degraded and moderately degraded sites as compared to the undegraded site because these sites had been disturbed for shifting cultivation and selective logging practices followed by burning of

dried slash causing loss of N content along with decline in soil organic matter. Another reason for reduced N content in the degraded and moderately degraded sites could be due to rapid loss of nutrients through surface runoff during rainfall since enough vegetal cover and organic matter content were not there to retain the nutrients.

The distribution of total P did not vary among the three study sites in the present study site though its distribution varied significantly ( $P < 0.05$ ) between degraded and undegraded site at both the surface and subsurface soil layers. However, the undegraded site contained slightly higher P in comparison to the other two sites at both soil layers.

Tiessen *et al.* (1992) and Agbenin and Goladi (1997) reported significant reduction in organic C, total N, available N (ammonium and nitrate N) and total P contents of soils in the long term when cultivation was practiced without proper input of nutrients based on either inorganic or organic fertilizer when compared to an undisturbed natural forest site. Ramakrishnan and Toky (1981) also reported decline in nutrient concentrations of cultivated soils due to faster decomposition rate of organic matter as a result of higher temperature and rapid surface runoff. These reports are in agreement with the present findings of reduced nutrient concentrations in the degraded and moderately degraded forest sites where shifting cultivation and selective logging were practiced for long time as compared to an undegraded natural forest site.

## **8.2. Biological characteristics of soils**

### ***Soil respiration, bacterial and fungal populations***

All the biological characteristics namely, soil respiration, bacterial and fungal populations, vesicular-arbuscular mycorrhizal fungi (VAMF) diversity and microbial biomass C and N was found significantly affected by the degree of soil degradation in the present study.

The respiratory efficiency of the soil showed a declining trend from highest soil respiration in undegraded forest site followed by moderately degraded site to the lowest soil respiration in degraded site at both the surface and subsurface soil layers. The reason for the higher soil respiration in the undegraded site might be due to the presence of favourable soil environments such as, higher soil moisture, organic matter content, soil temperature etc. for rapid multiplication of microorganisms leading to increased soil respiration in this site. However, the degraded and moderately degraded sites were recorded with lower soil respiration since these sites. These micro-environmental conditions required for rapid microbial replication were repeatedly disturbed by shifting cultivation and selective logging practices which led to a decline in efficiency of soil respiration in the long term. Chang and Trofymow (1996) also reported the detrimental effect of forest clear-cutting and subsequent preparation for land use on the respiratory efficiency of soils. This is in correspondence with the present findings of the reduced soil respiration in degraded site which had been used for a long period for shifting cultivation practice.

There was a significant change in rate of soil respiration with the change in sampling months which reveals a clear impact of seasonal climatic change on the respiratory efficiency of soils in all the three study sites at both the soil layers. The maximum rate of soil respiration was recorded from the surface layer of undegraded site in the month of August 1998 where highest rainfall occurred during the entire sampling period of two years. Similarly, degraded and moderately degraded sites also showed maximum rate of soil respiration in this month at surface soil layers. There was significant ( $P < 0.01$ ) positive correlation between the rate of respiration and various soil parameters such as air and soil temperatures, soil moisture, bacterial population and monthly rainfall in all sites and at both surface and subsurface soil layers of the three study sites. This shows that increase in soil moisture, soil temperature increased bacterial multiplication leading to enhanced soil respiration in the present study. On the other hand, the minimum rate of soil respiration was recorded in the month of December 1999 at surface layer and in the month of February 1999 at subsurface soil layer of the degraded site. This could be due to decrease in bacterial population as well as soil moisture, temperature and rainfall during the dry winter season. In general, the respiratory efficiency declined during the dry winter season in all sites and at both the soil layers. The soil respiration was lower in the subsurface layer as compared to the surface soil layers of the three study sites which also revealed decline in bacterial population and other soil physical parameters in the subsurface soil layer leading to decrease in soil respiration. Dkhar (1983), Upadhyaya *et al.* (1987), Tiwari (1988) Lomoander *et al.*

(1998) have also reported seasonal variation in respiratory efficiency of soils in maize fields, tropical grasslands, pineapple orchards and forest ecosystems respectively. Further, they also reported positive correlation between soil respiration and soil moisture, soil temperature and rainfall in their studies.

Shifting cultivation and selective logging practices reduced bacterial and fungal populations in degraded and moderately degraded forest sites in comparison to the undegraded forest site at both the surface and subsurface soil layers. Maximum populations of bacteria and fungi were recorded from the undegraded forest site which was followed by the moderately degraded site while the minimum was recorded from the degraded site. There was significant ( $P < 0.05$ ) decline in the bacterial population in the degraded forest site as comparison to the moderately degraded and undegraded forest sites (Table 2.1). This is because of the presence of the evergreen forest cover with thick layer of leaf, twig and wood litter etc. on the forest floor protecting excessive evaporation of soil water, thus creating a favourable environment of rapid microbial growth and multiplication in presence of higher organic matter in the undegraded site. On the other hand, the degraded site had no permanent cover of vegetation or grass (only seasonal) on the forest floor causing rapid loss of soil water and nutrients from the topsoil layer and subsequently poor growth and multiplication of bacterial and fungal populations. There was decline in bacterial and fungal populations of all the three study sites at subsurface soil layers as compared to the surface soil layers. The main reason for depletion in the population status of bacteria and

fungi in the subsurface layers of degraded, moderately degraded and undegraded forest sites might be due to the decrease in nutrient requirements of these microorganisms and other physico-chemical parameters required for rapid growth and multiplication of the bacteria and fungi in this soil layer. Tiwari (1988) reported significant decline in bacterial and fungal populations in younger aged (1 and 5 year old) pineapple orchard soils as compared to the 10 year old pineapple orchards of north-eastern India. Jha *et al.* (1992), Arunachalam *et al.* (1997) also reported decline in bacterial and fungal populations due to forest degradation, altitudinal variation and seasonal changes and due to forest logging practices in the north-eastern India. Their study reported decreased in bacterial and fungal populations with increase in soil depth. In fact, the degraded site had been continuously used for cultivation of various crops after burning the dried slash prior to cropping for a long period without proper nutrient input. This must have caused depletion of the nutrients required for the growth of microorganisms in the soil environment due to loss of soil organic matter as a result of burning and nutrient utilization by the seasonal crops leading to long term detrimental effect on the restoration of microbial communities in this site. Similarly, the burning of the forest cover for every year or every alternate year along with logging practice continues in the moderately degraded site might have caused loss of soil nutrients preserved in the soil organic matter causing unfavorable conditions for microbial multiplication and growth. Another reason for reduced microbial populations in the degraded site and moderately degraded sites could be due to loss or killing of the microbial

propagules while burning the dried slash (since the fire intensity of field burning reaches upto a height of about 2 to 3 meters above the soil surface and its heat reaches upto a depth of about 10 to 20 cm inside the soil subsurface layers) while preparing for crop cultivation in shifting cultivation systems. Acea and Caraballas (1999) have also reported almost complete microbial sterilization of the upper soil layer when heated upto 200°C for only 1h. This shows that shifting cultivation through forest clear-cutting and selective logging with repeated burning of forest floor caused not only decline in soil organic matter which is the reservoir of various nutrients but also decreased the growth and multiplication of bacterial and fungal communities in degraded and moderately degraded forest sites as compared to an undegraded forest site.

#### ***Vesicular-arbuscular mycorrhizal fungi (VAMF) diversity in forest soils***

The distribution of vesicular-arbuscular mycorrhizal fungi (VAMF) diversity in forest soils of the present study revealed a significant impact of soil disturbance through shifting cultivation and selective logging in the humid tropics. There was a decrease in spore population, species diversity and species abundance of VAMF with the increase in intensity of soil disturbance. The undegraded forest site was recorded with maximum spore population, species diversity and species abundance of VAMF followed by moderately degraded site and minimum was recorded from the degraded site at both the soil layers. There was a reduction in spore population of VAMF in degraded site by 70% and in the moderately degraded site by 30% at surface and subsurface soil layers as compared to the undegraded forest site.

Ahmad (1996) also reported 30-50% reduction in VAMF propagules when forest soils were severely disturbed through heavy soil mechanical compaction, exposure and erosion. Altogether, 44 species belonging to five genera namely, *Acaulospora* (6), *Gigaspora* (3), *Glomus* (27), *Sclerocystis* (1) and *Scutellospora* (7) were recorded from the three forest sites (Tables 4.2.3 and 4.2.4). Out of the 44 VAMF species recorded in the study, 21 were found as abundant in all the sites whereas, 14 species were restricted in undegraded site, 7 in moderately degraded site and 2 species in the degraded site respectively.

The average index of dominance for undegraded site was lower (0.12) indicating shared species dominance of VAMF species while the higher value of 0.16 and 0.15 in degraded and moderately degraded sites indicated dominance by a few species of VAMF only. In case of index of general diversity, the result was converse, a value of 0.99 for undegraded site, 0.85 for moderately degraded site while the lowest of 0.81 from the degraded site suggesting a greater diversity of VAMF species in the undegraded site than in degraded and moderately degraded sites respectively. There was a net reduction of 30% and 60% species diversity of VAM fungi in moderately degraded and degraded forest sites in comparison to the undisturbed forest site. This is in conformity with the higher number of VAM fungal species and propagules in undegraded site than in degraded and moderately degraded sites. The main reason for reduction in spore population, species diversity and species dominance in the degraded and moderately degraded sites could be due to long term practice of shifting

cultivation and selective logging in the degraded and moderately degraded sites. In shifting cultivation system, it is very common that all the vegetation is clear cut and burned the dried slash of the cut vegetation prior to direct sowing of seeds or growing of crops. This causes loss of specific host plants required by VAMF to establish its symbiotic association with the plant roots and at the same time viable propagules and dormant spores of VAMF are also destroyed along with loss of soil organic matter as a result of burning. This burning of the dried slash not only destroyed VAMF propagules and host plant roots but also caused alterations in micro-environmental conditions such as increased soil temperature, lower moisture content, decrease in soil aggregate stability leading to decline in growth of other soil microorganisms. These situations are also found in case of the moderately degraded site since forest floor clearing and burning were practiced every year or every alternate year for removal of excessive grass, weeds and fern growth to improve new forest plantations. Therefore, long term decline in the status of VAMF spore population, species diversity and lower species dominance were occurred in the degraded and moderately degraded forest sites at both the soil layers as compared to the undegraded forest site.

#### ***Microbial biomass C and N***

Microbial biomass C and N contents of the soil in degraded, moderately degraded and undegraded forest sites showed a significant ( $P < 0.05$ ) variation at surface and subsurface soil layers (Table 4.3.1). Maximum microbial biomass C and N contents were recorded from the undegraded site followed by moderately degraded site in all the sampling

months at both soil layers. Degraded forest site contained the minimum microbial biomass C and N at both the soil layers in all the sampling months. There was a decline in microbial biomass C and N contents with increase in soil disturbance from highest microbial biomass C and N in undegraded forest site towards lowest in the degraded forest site. The main reason for decline in microbial biomass C and N in these sites might be due to loss or decline in microbial populations and soil organic matters. The degraded site had been used for shifting cultivation for a period of 13 years continuously without proper nutrient input based on either organic or inorganic fertilizers to compensate the nutrients depleted during cropping period. Therefore, a decline in soil organic matter and decrease in microbial population status occurs as a result of burning of dried slash every time prior to crop sowing in the fields which ultimately led to reduction in soil organic matter, microorganisms and microbial biomass C and N in this site as compared to the undegraded forest site. Similarly, the moderately degraded site had also been disturbed frequently for logging purposes and burned the forest floor to eliminate excessive growth of unwanted grass and other weeds to improve new forest plantations. This must have also caused reduction in litter accumulation, loss in soil organic matter and destroyed active and dormant microbial propagules present on the forest soils that ultimately led to decreased microbial biomass C and N. Another important factor which might have contributed to the loss of soil organic matter and microbial biomass in cultivated fields and selectively logged forest is the faster surface runoff of the soil particles along with larger soil aggregates from the hill slopes during

rainy seasons. This is perhaps the most important problem faced by the farmers since longer period of rainfall occurring in the north-eastern hill regions causing tremendous loss of fertile topsoil which contains the largest amount of organic matter, nutrients and microorganisms in every soil profile. Srivastava and Singh (1991), Henrot and Robertson (1994) and Tiwari *et al.* (2002) have reported decline in soil organic matter and microbial biomass C and N as a result of vegetation removal and subsequent conversion of tropical forests into agricultural lands and due to the practices of shifting cultivation and selective logging in the humid tropics. The reason for higher microbial biomass C and N in the undegraded forest might be due to the presence of significantly higher soil organic matter, favourable micro-environmental parameters such as soil temperature, moisture and nutrients which lead to rapid microbial multiplication and subsequent immobilization of nutrients in the microbial cells. Microbial biomass C and N contents were positively correlated ( $P < 0.05$ ) to the air and soil temperatures, moisture content, pH, ammonium and nitrate-N, fungal population, soil respiration and phosphatase activity in the undegraded site (Tables 3.3 and 3.4).

The microbial biomass C and N of the soil in each site also declined from highest at surface to the lowest at the subsurface layer indicating poor distribution of soil organic matter and microorganisms at deeper soil layers. Decline in the microbial biomass C and N with increasing soil depth in three sites namely, permanent grassland, arable site with neutral pH and arable site with acidic pH had also been reported by Lavahun *et al.* (1996). Similar results of decline in microbial biomass C and N with increase in soil depth

have been reported from the disturbed subtropical forests of north-eastern India (Maithani *et al.*, 1996).

### **8.3. Molecular microbial diversity of soils**

#### ***Bacterial diversity of soils as determined by 16S rDNA fingerprints***

Culture-independent studies of the microbial community structures based on genotypic and phenotypic techniques in the soils of degraded, moderately degraded sites revealed significant variation in distribution of the microbial communities. The analysis of the density gradient gel electrophoresis (DGGE) of the polymerase chain reaction (PCR) amplified 16S rDNA fingerprints demonstrated a high diversity of bacterial communities in the soils from the three study sites at the surface and subsurface soil layers. The undegraded site possessed maximum number of 16S rDNA fingerprints than the degraded and undegraded sites at both soil depths. The surface soil of the degraded site displayed minimum 16S rDNA fingerprints thereby suggesting the reduced bacterial diversity in this site as a consequence of the long term utilization of the soil through shifting cultivation in the past. The gel compare analysis of the DGGE fingerprints of 16S rDNA resulted three clusters (Fig.5.13B) with distinct separation of the study sites. The group I consists of the surface soil layer of degraded site displayed a minimum similarity index of 85% to the other soils while the group II comprised of the surface and subsurface soil layers of undegraded site which showed a maximum of 96% similarity index of the fingerprints. The group IIIa and IIIb were formed by surface and subsurface layers of moderately degraded site and the subsurface soil layer of the degraded site respectively.

This suggests the declining trend of the similarity index from highest in undegraded site to the lowest in the degraded site which means less number of bacterial genera and thus diversity in the degraded site as compared to the undegraded site.

***Microbial community structure of soil as measured by phospholipid fatty acid (PLFA) profile analysis***

Analysis of the phospholipids fatty acids (PLFAs) profiles of soils from the three study sites showed no significant variation in the amount of total PLFA contents. However, there was significant variation in distribution of the individual fatty acids representing indicators of specific microbial groups. A total of 133 fatty acids belonging to various PLFA fractions namely SATFAs, MUFAs, PUFAs, PLOHs (all ester-linked) and UNSOHs (non ester-linked) were recorded from all the soil samples.

The straight chain SATFAs with methyl branching at 10<sup>th</sup> C-atom were largest subgroup. These fatty acids as indicators of actinomycetes in the soil were detected in maximum numbers at the surface layer of the undegraded site while least was detected in the subsurface soil of the degraded site. The fatty acids with methyl branching at *iso* and *anteiso* positions as indicators of Gram-positive and Gram-negative bacteria were detected in all sites and at both the soil layers though their percent concentration was lower than other fractions. These fatty acids are found in the genera *Acetobacter*, *Cytophaga* and *Flavobacterium* (Brennan, 1988 and Hack *et al.*, 1994) and were not affected by the shifting cultivation and selective logging in the long term. The diversity of the genera *Bifidobacterium*, *Closteridium*, *Legionella* and *Rhodospirillum* Gattinger *et al.* 2002; Ratledge and Wilkinson, 1988 and

Zelles, 1999) and as revealed by the *cyclopropyl ring* containing fatty acids showed maximum in degraded site followed by moderately degraded site and least in the degraded site.

No variation in the microbial communities represented by the monounsaturated fatty acids were detected from all the sites. However, the majority of the microbial group, Gram-positive and Gram-negative bacteria and other eukaryotes including fungi represented by the fatty acids with unsaturation at  $\omega 9$  were dominant microorganisms in these soils. The polyunsaturated fatty acids (PUFAs) as the markers of microeukaryotes (fungi) and cyanobacteria (Bossio and Scow, 1998 and Gattinger *et al.*, 2002) were found distributed in all the sites. The degraded site contained 7 fatty acids with different degrees of saturations and represented maximum number of PUFAs than other sites at surface soil layer. Arachidonic acid (20:4-5, 8,11,14) was another PUFA which occurred at the surface soils of the three study sites with high percentage concentrations to the total PUFAs. The absence of this fatty acid at the surface layers revealed that *archaeotale* communities were mostly aerobic occurring in the surface soil layers only.

The  $\alpha$ -PLOHs as indicators of the Gram-negative bacteria (*Pseudomonas*) and *Actinomycetales* (Galbraith and Wilkinson, 1991; Gattinger *et al.*, 2002; Yano *et al.*, 1978 and Zelles, 1999) were the largest subgroup in terms of number of fatty acids. The  $\beta$ -PLOHs as the marker of the bacterial groups, *Pseudomonas*, *E.Coli*, *Rhodococcus*, etc. (Zelles 1997) showed highest diversity and concentration at the surface soil layer of the undegraded site expressed in per cent of the total PLOHs. There was a

sharp decline in the diversity and population of these microbes at the subsurface soil layer of the undegraded site as compared to the moderately degraded and undegraded sites which suggests the negative effects of the shifting cultivation on survival and multiplication of these organisms.

Oh-nsubstituted fatty acids (UNSOHs) are regarded as the general indicators of anaerobes and eukaryotes (fungi) including the representatives of the genus, *Clostridium* (Gattinger, 2002 and Zelles, 1999) which showed a unique distribution pattern among the soil samples studied in this study.

#### **8.4. Biochemical characteristics of soils**

##### ***Dehydrogenase, acid phosphatase and urease soil enzyme activities***

Biochemical characteristics of soil particularly dehydrogenase and acid phosphatase activities in degraded, moderately degraded and undegraded forest sites revealed marked variation due to site, season and soil depths. The urease activity showed marked seasonal variation rather than site and depth-wise variations.

The activity of dehydrogenase enzyme in the soil in degraded and moderately degraded forest sites showed minimum as compared to the undegraded forest sites at both the soil layers. Maximum dehydrogenase activity recorded in the present study from the undegraded site could be due to presence of higher soil organic matter and favourable soil reactions as revealed by significant ( $P < 0.05$ ) positive correlation between the dehydrogenase activity with soil pH and organic C contents at both soil layers. Generally, soil dehydrogenase being an oxido-reductase enzyme is known for its role in initial oxidation of soil organic matter decomposition

(Burns, 1982). Therefore presence of the higher amount of organic matter and favourable soil pH in the surface and subsurface soil layers of undegraded site might have influenced enhanced activity of this enzyme as compared to the degraded and moderately degraded sites. Another reason for higher dehydrogenase enzyme activity could be the presence of abundant and permanent vegetal cover in this site providing favourable microclimatic conditions for larger microbial growth and accumulation of more enzymes on this forest soil. Tiwari *et al.* (2002) have reported higher dehydrogenase activity in an undisturbed forest site in comparison to a degraded site and a slightly degraded site in humid tropical regions of north-eastern India. However, the decline in the dehydrogenase activity of the degraded site might be due to the absence of a permanent vegetation to provide litter on the forest soil which led to poor growth of microbial population and lower enzyme accumulation. The lower amount of organic C and inhibitory effect of acidic pH might be another reason for reduced dehydrogenase activity in degraded site despite its positive correlation with organic C at surface soil layer. However, there was a positive correlation between dehydrogenase activity with organic C and soil pH ( $P < 0.05$ ). This suggests a long term detrimental effect of shifting cultivation practice on dehydrogenase enzyme activity. The moderately degraded site also showed lower dehydrogenase activity in comparison to the undegraded site at both soil depths. This might be due to presence of lower organic matter in terms of litter accumulation on the forest floor since cleaning and burning of litter were continuously practiced along with selective logging in this site leading to destruction of

micro-environmental conditions for microbial growth, thus lower population of soil microorganisms and lesser enzyme accumulation.

Acid phosphatase activity in soils of the three study sites showed an increasing trend with decrease in soil disturbance at both the surface and subsurface soil layers. The undegraded site supported maximum phosphatase activity followed by moderately degraded site and minimum was recorded from the degraded site for both soil layers. The reason for higher phosphatase activity in the undegraded forest site might be due to presence of a permanent vegetation producing higher soil organic matter and creating a favourable micro-environment of soil microorganisms to grow and multiply rapidly for greater enzyme accumulation. Dinesh *et al.* (1998) also reported similar results with higher acid phosphatase activity in soils with higher organic matter content. There was a significant ( $P < 0.05$ ) positive correlation between the available phosphatase activity and other soil characteristics like, soil pH, organic C, total N and microbial N contents which reveals that acid phosphatase activity depends on these soil characteristics. Trasar-Cepeda and Gill-Sotres (1987) also reported higher activity of phosphatase soil enzyme in soils having higher organic matter contents. Acid phosphatase activity also varied significantly ( $P < 0.05$ ) with the seasonal climatic regimes. Generally, higher acid phosphatase activity occurred during the intervening periods of winter and spring seasons (January-March ) and between late rainy season and early winter season (August-September) revealing an increase in phosphatase activity with increase in soil moisture at the beginning of spring seasons when there was an increase in soil moisture and

before decline in moisture with the onset of winter dry season. However, no positive correlation was found between acid phosphatase activity and soil moisture content, rather there was a significant ( $P < 0.05$ ) negative correlation with soil moisture in degraded and moderately degraded sites at surface soil layer. This showed that moisture content of the soils might have inhibitory impact on acid phosphatase activity since its activity declined when the soil moisture was higher during peak rainy season and further its activity declined when the soil moisture is lowest during winter period. Higher acid phosphatase activity was also reported during spring-summer season from pineapple orchard soil of north-eastern India (Tiwari, 1988 and Tiwari, S.C. *et al.*, 1989ab). There was a decline in acid phosphatase activity with the increase in soil depth of the degraded, moderately degraded and undegraded sites. The main reason of this decline in acid phosphatase activity could be due to the decline in soil organic matter and other nutrients required by soil microorganisms and subsequent decline in soil enzyme concentration with the increase in soil depth from surface to subsurface soil layer.

Soil urease activity also declined from its highest activity recorded from the undegraded site to the lowest in the degraded site whereas the moderately degraded site was recorded with an intermediate urease activity. There was a significant variation ( $P < 0.05$ ) in urease activity among the three sites and between the degraded and undegraded sites at surface soil layer only (Table 6.1). However, the variation was insignificant between the degraded and moderately degraded sites and between the moderately degraded and undegraded sites at surface and subsurface soil layers.

Generally, higher urease activity was recorded from the undegraded site and it was followed by moderately degraded, and undegraded sites at both soil layers. The decline in urease activity in degraded and moderately degraded sites could be due to strong acidic reaction of the soil as revealed by its significant negative correlation ( $P < 0.05$ ) with the soil pH at the surface layer. However, undegraded site showed significant ( $P < 0.05$ ) positive correlation with the soil pH at surface soil layers revealing that available urease activity in these sites was controlled by favourable soil reaction in presence of higher soil organic matter. These findings in the present study suggests that shifting cultivation practice and selective logging of forest trees did not have highly significant impact on urease activity of the soils. However, variation in site and vegetal cover distribution might have been responsible for variation in urease activities among the three study sites. Pancholi and Rice (1973) reported higher urease activity related to type of vegetation and the quality of incorporated organic materials in the soil. Similarly, Palma and Conti (1990) also reported significant variation in distribution of urease activity in grassland and forest soils revealing direct relationship between pattern of urease activity to the type of vegetation and impact of organic matter. These reports are in conformity with the present findings that undegraded forest site with a permanent vegetal cover distribution with different tree species and year round accumulation of litter on the forest floor supported higher urease activity than the selectively logged forest and shifting cultivated forest sites. There was significant ( $P < 0.05$ ) variation in distribution of urease activity with the change in seasonal climate (Fig. 5.3). However, no significant variation

was observed between the surface and subsurface soil layers of the degraded and undegraded forest sites though moderately degraded site had shown significant ( $P < 0.05$ ) variation of urease activity between the two soil layers in the present study (Table 6.2).

#### **8.5. Evaluation of forest soil degradation types by using stepwise discriminant function analysis (DFA)**

The data of all variables under physico-chemical, biological and biochemical characteristics were used for determining forest soil degradation types by using discriminant function analysis (DFA) as a statistical tool. The analysis resulted two important canonical discriminant functions (CDFs) which together contributes 100% variation in the sites.

CDF1 with organic C as the only important discriminating variable could differentiate upto 85.88% the forest soil degradation types between degraded, moderately degraded and undegraded sites. The CDF2 comprised of six variables of various soil characteristics namely, bulk density, microbial biomass C, dehydrogenase activity, total N, soil pH and soil temperature. This CDF2 had a maximum contribution of 14.12% in group variation. The structure matrix of the two CDFs in the table 7.4 depicted the largest absolute correlation of the respective variable to its function revealing the best discriminating property of these variables for soil degradation types. Organic C was the only variable in CDF1 with largest absolute positive correlation ( $r = 0.5024$ ). Similarly, bulk density of the CDF2 had the largest absolute positive correlation ( $r = 0.6592$ ) over the other variables.

The selection of CDF1 as the most important function in discriminating the soil degradation types was due to the largest Eigenvalue and other

characteristics (Table 7.3). The significance level of the two functions reveals that CDF1 with maximum Chi-square value (348.773, 14 df) was more significant ( $P < 0.000$ ) than the CDF2 which had lower Chi-square value.

Organic C content of the soils in the degraded, moderately degraded and undegraded forest sites was found as the only variable from the CDF1 which had contributed to a maximum of 85.88 % variance of soil degradation types and highest within-groups positive canonical correlation (0.502) in the present study. This suggests that stepwise DFA provide a much better classification of the soil degradation type among the three study sites based on the two discriminant functions, CDF1 and CDF2 as compared to the other visual site characteristics. It is clear from this analysis that the soil in each of the study sites differ significantly ( $P \leq 0.05$ ) in terms of a gradient in organic C content or organic matter in the soils. Maximum organic C content in undegraded forest site varied towards a minimum organic C content in the degraded site through an intermediate value of organic C in the moderately degraded site.

Bulk density with a maximum canonical correlation value of 0.659 was the most significant variable in CDF 2 which had contributed maximum percentage in discriminating the soil degradation types of the three study sites. The average bulk density varied significantly between the three study sites where highest value was recorded from the degraded site followed by moderately degraded site and lowest bulk density was recorded from the undegraded site. This pattern of variation in bulk density is in contrast to that of organic C contents where maximum organic C content was undegraded

site followed by moderately degraded site and minimum was recorded from the degraded site.

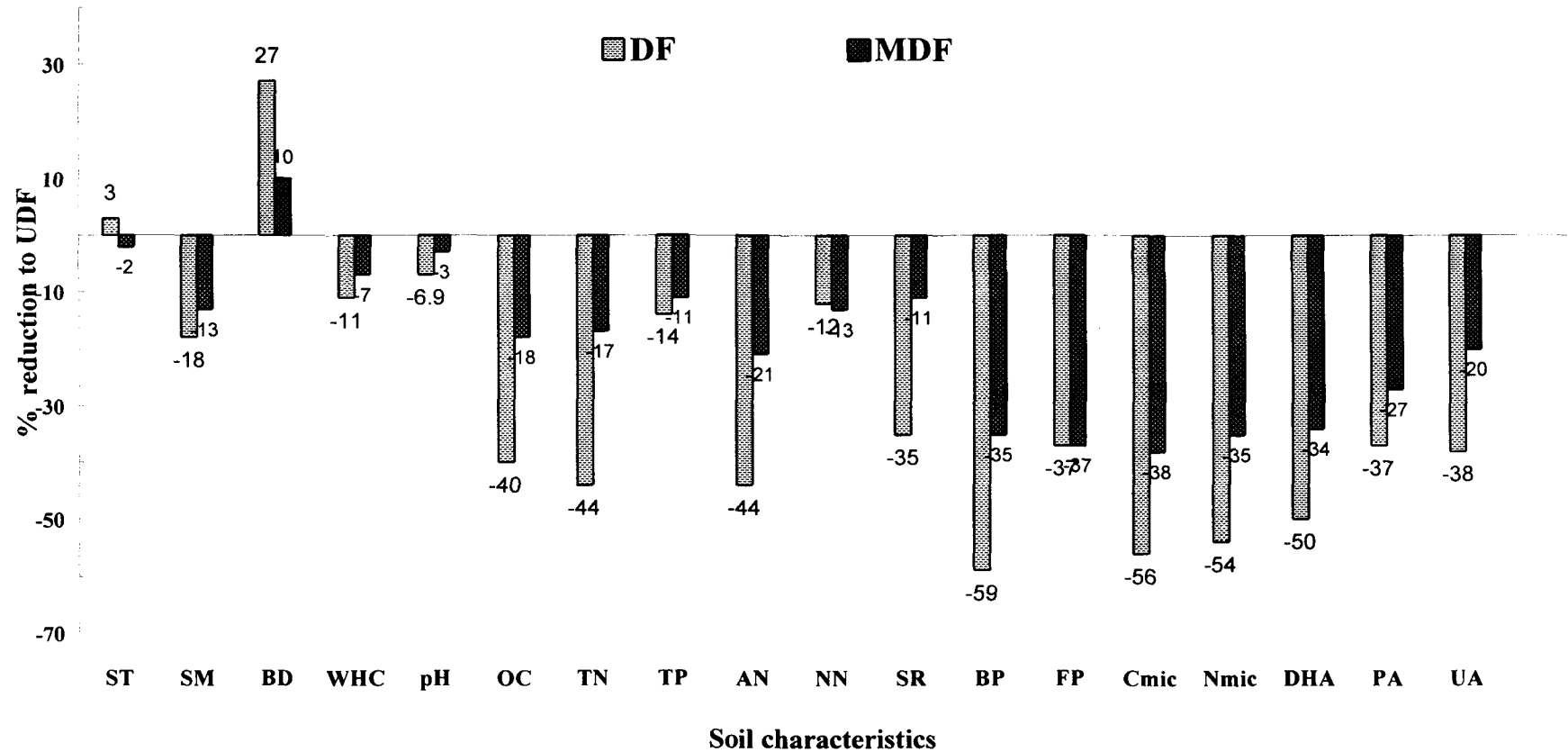
#### **8.6. Impact of soil degradation on soil characteristics in degraded and moderately degraded forest sites as compared to the undegraded forest site**

A comparative analysis of the impact of soil degradation on various soil characteristics, namely physico-chemical, biological and biochemical parameters of degraded and moderately degraded sites revealed marked decline in these soil characteristics in terms of percentage as compared to the undegraded site (Fig.8.1). Most of the soil characteristics showed decline in their values with the increase in soil degradation from undegraded site to degraded site. However, a few soil physical characteristics namely, soil temperature was found to be increased by 3% with the increase in soil degradation in degraded site whereas bulk density was found increased by 27% in degraded site and 10% in moderately degraded site as a consequence of soil degradation through shifting cultivation and selective logging practices in this sites as compared to the undegraded site.

The magnitude of decline in the values of soil characteristics in terms of percentage ranged from a minimum of -2% for soil temperature to a maximum of -37% for fungal population in the moderately degraded site as compared to the undegraded site. In case of the degraded site, the percentage decline was ranged from a minimum of -6.9% for pH to a maximum of -59% for bacterial population as compared to the undegraded site. Among the physico-chemical characteristics of soil, organic C, total N and ammonium-N were found to be declined by -40%, 44% and -44% in the

degraded site revealing most pronounced impact of soil disturbance on these soil chemical characteristics against the undegraded site. Similarly, among the biological and biochemical characteristics particularly bacterial population, microbial biomass C, microbial biomass N and dehydrogenase activity were found declined by -59%, -56%, -54% and 50% respectively in the degraded site as compared to the undegraded site. These results suggests that soil degradation has the most pronounced detrimental impact on biological and biochemical characteristics of soil followed by chemical characteristics while the physical characteristics showed least effect of soil degradation. Thus, it may be suggested that the biological and biochemical characteristics are more sensitive and responsive to the soil disturbance due to shifting cultivation and selective logging practices in the humid tropical forests soils of north-eastern India. Therefore, these biological and biochemical characteristics coupled with few important physicochemical characteristics may be used to identify the status of soil degradation in this region in particular and other soils in general.

**Fig. 8.1. Percentage reduction of physico-chemical, biological and biochemical characteristics of degraded (DF) and moderately degraded (MDF) forest sites as compared to undegraded (UDF) forest site.**



(Note: ST=soil temperature, SM=soil moisture, BD=bulk density, WHC=water holding capacity, C= organic carbon, N=total nitrogen, P=total phosphorus, AN=ammonium, N, NN=nitrate-N, SR=soil respiration, BP=bacterial population, FP=fungal population, C<sub>mic</sub>=microbial biomass C, N<sub>mic</sub>=microbial biomass N, DHA=dehydrogenase activity, PA= acid phosphatase activity, UA=urease activity)

## **Chapter 9**

### **CONCLUSION**

#### **9.1. Soil degradation class**

The soil erosion class, degree, extent and severity of soil degradation of the degraded and moderately degraded sites were evaluated based on the criteria by Sehgal and Abrol (1994) and USDA Soil Survey Manual (1995). Finally, degradation class of the degraded (DF) and moderately degraded (MDF) sites were classified as compared to the undegraded forest (UDF) site in terms of code numbers as given in table 9.1. Soil degradation class of the degraded site and moderately degraded sites are 3(2.5) and 1(1.3) respectively as compared to the undegraded site.

#### **Degraded forest site: 3(2.5)**

##### ***Erosion Class ( 3)***

Soils in the degraded (DF) site have lost >75% of A or E horizons of uppermost 20cm and there is exposure of the materials below A or E horizons to the atmosphere. The soils in this site will be difficult for restoration at farm level

##### ***Degree of degradation (Moderate, 2)***

The soils have greatly reduced agricultural productivity in terms of economic value

##### ***Extent of degradation (Dominant, .5)***

More than 50% of the total area in this site is affected by soil degradation

##### ***High Severity***

The site has a significant loss in 1/3 to 2/3 of agricultural productivity and the affected area not economical to cultivate but can be used as agroforestry system.

**Table 9.1. Erosion class, degree, extent, severity and degradation class of degraded (DF) and moderately degraded (MDF) forest sites**

<b>PARAMETERS</b>	<b>DF</b>	<b>MDF</b>	<b>UDF</b>
<b>Erosion class*</b>	<b>3</b>	<b>1</b>	<b>0</b>
<b>Degree of degradation**</b>	<b>Moderate (2)</b>	<b>Slight (1)</b>	<b>0</b>
<b>Extent of degradation**</b>	<b>Dominant(.5)</b>	<b>Common (.3)</b>	<b>0</b>
<b>Severity of degradation**</b>	<b>High</b>	<b>Low</b>	<b>0</b>
<b>Degradation class</b>	<b>3(2.5)</b>	<b>1(1.3)</b>	<b>0</b>

\*Soil Survey Manual: Hand book No. 18 (USDA, 1962, Revised Edition, 1995)

\*\* Soil Degradation Status & Impacts (Abrol & Sehgal, 1994)

**Moderately degraded site: 1(1.3)*****Erosion Class (1)***

Soils in this site have lost <25% of A or E horizons of uppermost 20cm soil but this soil can be restorable at form level. The surface soil layer thickness of this site is in the normal range.

***Degree of degradation (Slight, 1)***

The soils in this site has somewhat reduced agricultural productivity as compared to the undegraded site

***Extent of degradation (Common, .3)***

About 10-2% of the total area in this site is affected by soil degradation

***Low severity***

The soil has lost up to a maximum of 15% in agricultural productivity which is negligible and easily manageable

**9.2. Microbial communities and their activities**

Microbial populations of bacteria and fungi, including vesicular-arbuscular mycorrhizal fungi (VAMF) population in the sites showed long term detrimental impact of soil utilization by shifting cultivation for agricultural production and selective logging of trees as comparison to the undegraded site. Even after 5 years (1993-1997) of regeneration period, microbial community structure and activities in the degraded site could not restore up to half of the undegraded forest soil. The microbial activities as measured in terms of soil enzyme activities, dehydrogenase, acid phosphatase and urease activities also declined in degraded and moderately degraded forest sites as a result of shifting cultivation and selectively logging practices accompanied by burning of the soil surface. The rate of decline was significantly higher in the degraded site than in moderately degraded site for

all the biological and biochemical characteristics of soil as compared to the undegraded forest site at surface and subsurface soil layers.

Therefore, it may be concluded that the microbiological communities and their activities as measured by population status of bacteria and fungi, vesicular-arbuscular mycorrhizal fungi (VAMF), microbial biomass C and N and soil enzyme activity were drastically decreased in soils which had been used for shifting cultivation for long time without proper input of nutrients either based on organic or inorganic fertilizers. Similarly, there was decline in fertility status of soils in moderately degraded site that has been continuously used for selective logging as comparison to an undegraded natural forest in the north-eastern hill region.

The culture-independent analysis of the bacterial genomic diversity as revealed by gel compare analysis of the 16S rDNA gene from the DGGE profiles had a similar results of bacterial community structure to that of the cultivation-dependant studies. The cluster analysis of the DGGE bands of 16S rDNA genes revealed a clear separation of the degraded and moderately degraded sites from that of the undegraded site in terms of bacterial genomic communities.

Total PLFA contents of the soils expressed in  $\mu\text{Mol kg}^{-1}$  revealed that there was no significant variation on microbial population numbers among the three study sites at the surface soil layers though the subsurface soil contain lower amounts of the PLFA in general. There was a decline in the microbial diversity status of the degraded sites at both the soil depths in comparison to the moderately degraded and undegraded sites as revealed by the PLFA

profiles. The undegraded forest site contained maximum diversity of the PLFA fractions and their individual fatty acids revealing a higher microbial diversity than the moderately degraded and degraded site respectively.

### **9.3. Fertility status of soils**

Based on the results of all measured parameters it can be inferred that the shifting cultivation and selective logging practices in humid tropical hill forest soils caused significant decrease in physico-chemical, biological and biochemical properties and thus affecting soil fertility status. It is clear from the results that there were significant decline in the important physico-chemical, biological and biochemical characteristics of the soils in degraded and moderately degraded forest sites as compared to the undegraded site. The sharp decline in important plant nutrients such as organic C as an indicator of soil organic matter, total N and ammonium N among the chemical characteristics revealing decline in fertility status of the soils in degraded and moderately degraded sites as a consequence of soil degradation due to shifting cultivation and selective logging practices. However, the undegraded site being undisturbed for any type of soil utilization in the past maintained the important soil nutrients along with higher organic C content, thus higher soil fertility in this site.

### **9.4. Discriminant function analysis (DFA).**

Two canonical discriminant functions (CDFs) were obtained from the DFA which together contributed a total of 100 per cent variance among the three study sites (Table 7.3). Canonical discriminant function 1 (CDF1) was the most important function due to its higher Eigenvalue (31.1829) and

canonical correlation (0.9843). This function accounted for a maximum variation in soil degradation types in the present study with a variance percentage of 85.88 as compared to the canonical discriminant function 2 (CDF2). The other variables in CDF 2 namely, microbial biomass C, dehydrogenase activity, total N, soil pH and soil temperatures were also important in discriminating the soil degradation types between the three study sites but their absolute correlation with the discriminant function were negative or comparatively lower to the bulk density. Therefore, the soil characteristics in CDF1 and CDF2 were found to be sensitively responsive to the soil degradation caused by shifting cultivation and selective logging practices in the hill forest soils. These parameters namely, organic C , bulk density, microbial biomass C and dehydrogenase soil enzyme activity may be used to assess the degradation status of soil in the hill region in particular and other soil in general.

## **Chapter 10**

### **SUMMARY**

A study was conducted to determine the microbial community structures and their activities in the soil of three forest sites with varying degree of disturbances namely, degraded forest (a 5 year old regenerating jhum fallow), moderately degraded forest (selectively logged forest) and an undegraded natural forest having no land use history or disturbance in the past. These study sites were classified based on a preliminary field survey with the important site characteristics such as vegetal cover distribution, land use history, number of living trees and diversity, etc.

#### **Physico-chemical characteristics of soil as influenced by forest degradation**

The textural class of the soil in these sites revealed predominantly sandy loam in texture. There was a decline in soil profile ( $A_h$  layer) thickness with the increase in severity of forest site disturbance. Maximum thickness of  $A_h$  layer was recorded from the undegraded site which was followed by moderately degraded site in which selective logging of tree was practiced continuously in the past. Minimum thickness of  $A_h$  layer was recorded from the degraded site in which shifting cultivation was practiced for a long period of time without proper input of nutrients either based on organic or inorganic fertilizers.

The analytical results of the important physico-chemical characteristics showed a declining trend with the increase in soil disturbance where maximum values recorded from the undegraded site followed by moderately degraded and degraded sites respectively. However, there was

an increase in magnitude of few soil physical characteristics, particularly maximum bulk density was recorded from the degraded soil which had the highest degree of soil disturbance due to shifting cultivation.

Moisture content, water holding capacity, pH, organic C, total and available-N (ammonium and nitrate-N) and total P were recorded comparatively higher in the undegraded site than moderately degraded and degraded sites at surface and subsurface soil layers respectively. Soil moisture content was recorded maximum from the undegraded site than moderately degraded site and degraded sites and it varied significantly with change in season in all sites and at two soil layers. Higher soil moisture content was recorded during the rainy months (May to September) and declined to the lowest during winter dry months (November to March) from all sites at both soil layers. Soil of undegraded site showed higher water holding capacity of in comparison to the other two sites at both surface and subsurface soil layer.

The pH of the degraded site showed strongly acidic soil reaction (pH 5) at both the surface and subsurface soil layers as compared to the moderately degraded and undegraded sites. There was a marked difference in organic C content of the soil among the three study sites at both the soil layers. Maximum organic C content was recorded from the undegraded forest site while minimum was recorded from the degraded site. Moderately degraded site contained organic C content ranged in between degraded and undegraded sites at both soil layers. Significantly higher organic C content was recorded during the winter dry months (November to March) from all sites and at both soil layers. In general, surface soil layer contained higher

organic C as compared to subsurface soil layer which showed a decline in organic C content with the increase in soil depth. Total N and available-N (ammonium and nitrate-N) contents were comparatively lower in degraded and moderately degraded sites than undegraded sites at surface soil layer. There was a marked seasonal variation in total N contents of soils than monthly variation. Two peaks of total N content appeared in the months of February and September 1999 respectively. However, ammonium and nitrate-N contents varied significantly ( $P < 0.05$ ) with the change in sampling months at both surface and subsurface soil layers. Total P content did not vary significantly among the three sites but it varied significantly ( $P < 0.05$ ) between degraded and undegraded sites at both soil layers.

#### **Biological characteristics of soil as influenced by forest degradation**

Soil biological characteristics such as soil respiration ( $\text{CO}_2$  evolution), bacterial and fungal population, spore distribution and diversity of vesicular-arbuscular mycorrhizal fungi (VAMF), microbial biomass C and N contents were significantly decreased in the degraded and moderately degraded sites as compared to the undegraded site. These suggest the detrimental effect of shifting cultivation involving burning of dried slash on the forest floor prior to growing crops and selective logging of trees accompanied by frequent cleaning and burning of forest floors on the distribution of microorganisms and their multiplication. There was decline in metabolic efficiency of soils as revealed by decreased soil respiration, poor growth of bacterial and fungal microorganisms and subsequent loss of microbial biomass C and N from the soils of these sites.

Soil respiration was found significantly influenced by variation in soil physical characteristics such as soil temperature and moisture contents in all the three sites as revealed by their significant ( $P < 0.05$ ) positive correlation at surface and subsurface soil layers. The bacterial population was also positively correlated to soil moisture and respiration suggesting significant contribution of bacterial population to overall soil respiration of the soils in degraded, moderately degraded and undegraded study sites at surface and subsurface layers respectively. However, fungal population showed significant correlation with bacterial population, which reveals a possible synergistic relationship in the moderately degraded and undegraded sites which might have also contributed to the increased metabolic activity in terms of soil respiration at surface layer only.

A maximum of 44 VAMF species under five genera namely, *Acaulospora* (6), *Gigaspora* (3), *Glomus* (27), *Sclerocystis* (1) and *Scutellospora* (7) were recorded from the three forest sites (Table 4.2.3). Twenty one species were found abundant in all the three sites whereas distribution of fourteen species were restricted to undegraded site, seven species to moderately degraded site and only two species to degraded site. The undegraded site showed lower index of species dominance than the moderately degraded and undegraded sites indicating dominance by a large number of VAM fungi species while higher values in the moderately degraded and degraded sites revealed species dominance by a few VAM fungi. The results was opposite for indices of VAM fungi diversity. The degraded and moderately degraded sites showed lower indices of species

diversity of VAM fungi as compared to the undegraded site which suggests the higher level of VAM fungi diversity in the undegraded site.

### **Molecular microbial diversity of soil as influenced by forest degradation**

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

Analysis of the phospholipids fatty acids (PLFAs) profiles of soils from the three study sites showed no significant variation in the amount of total PLFA contents. However, there was significant variation in distribution of the individual fatty acids representing indicators of specific microbial groups. A total of 133 fatty acids belonging to various PLFA fractions namely SATFAs, MUFAs, PUFAs, PLOHs (all ester-linked) and UNSOHs (non ester-linked) were recorded from all the soil samples. Total PLFA contents of the soils expressed in  $\mu\text{Mol kg}^{-1}$  revealed that there was no significant variation on microbial population numbers among the three study sites at the surface soil

layers though the subsurface soil contain lower amounts of the PLFA in general. There was a decline in the microbial diversity status of the degraded sites at both the soil depths in comparison to the moderately degraded and undegraded sites as revealed by the PLFA profiles. The undegraded forest site contained maximum diversity of the PLFA fractions and their individual fatty acids revealing a higher microbial diversity than the moderately degraded and degraded site respectively.

### **Biochemical characteristics of soil as influenced by forest degradation**

Biochemical characteristics of the soils showed a significant variation among the three sites and also varied with seasonal changes. Dehydrogenase and acid phosphatase activities declined with the increase in degree of soil degradation in the present study. The maximum activity of these enzymes was recorded from the surface layer of the undegraded site followed by moderately degraded site during all the sampling months. Whereas, degraded site was recorded with minimal activities of these soil enzymes as compared to the undegraded site at both soil layers. Urease activity showed significant variation among soils of all the three study sites and at surface soil layers but there was no significant variation among the sites at subsurface soil layers. However, urease activity, remained highest in the undegraded site than degraded and moderately degraded sites at both soil layers.

### **Comparative variation of soil characteristics in degraded and moderately degraded sites as compared to the undegraded forest site**

A comparative analysis of the impact of soil degradation on various soil characteristics, namely physico-chemical, biological and biochemical parameters of degraded and moderately degraded sites revealed marked

decline in these soil characteristics in terms of percentage as compared to the undegraded site. Most of the soil characteristics showed decline in their values with the increase in soil degradation from undegraded site to degraded site. However, a few soil physical characteristics namely, soil temperature and bulk density were found to be increased in degraded and moderately degraded site as a consequence of soil degradation through shifting cultivation and selective logging practices in this sites as compared to the undegraded site.

#### **Discriminant function analysis for identification of soil characteristics sensitively responsive to soil degradation**

Discriminant function analysis of the data from physico-chemical, biological and biochemical characteristics of soil revealed that organic C in the discriminant function 1 (CDF1) may determine the forest soil degradation type between the degraded, moderately degraded and undegraded sites upto a maximum of 86% total variation. Similarly, bulk density with a largest absolute positive correlation to the function 2 (CDF2) could discriminate only 14% of the total variation of the forest soil degradation types. These findings from the statistical analysis suggest that organic C content and bulk density along with few soil characteristics, such as microbial biomass C and dehydrogenase enzyme activity may be useful in evaluating the degree of forest soil degradation in humid tropical hill soils in particular and other soils in general.

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## Plate 1



Landscape view of the degraded forest (DF) site with few trees only



Exotic growth of weeds and grasses on the forest floor of the site during summer wet rainy season



A part of the forest floor covered with *Mikania mikranthes* L. as a dominant weed species

## Plate 2



**Landscape view of the moderately degraded forest (MDF) site showing sparsely distributed trees and selectively logged stumps**



**Inner view of the site with a selectively logged tree stump**



**A part of the forest floor covered with grasses cleaned up in ridges by forest personals for proper growth of introduced trees**

## Plate 3



**Landscape view of the undegraded (UDF) forest site showing luxuriant growth of different tree species including bamboo**

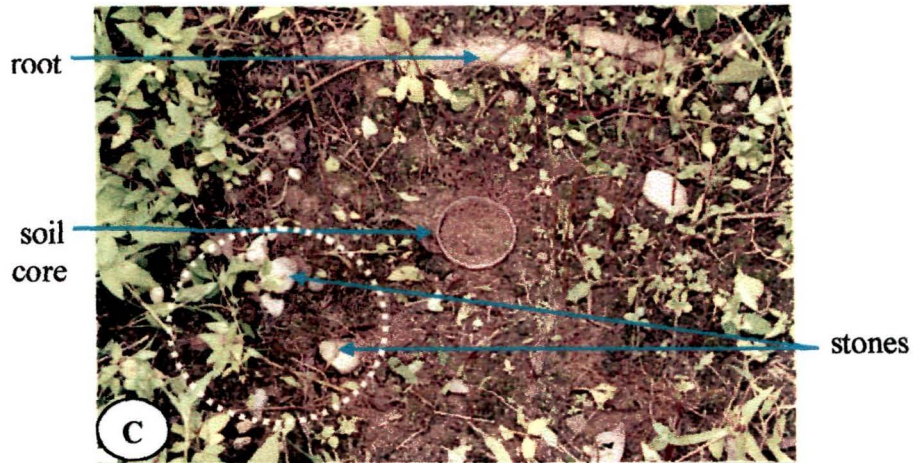


**Inner view of the site with different sized living tree stumps, shrubs and herbs during summer wet season**



**Inner view of the site with different sized living tree stumps, shrubs and herbs during dry winter season**

## Plate 4



**Soil surface of DF site with exposed B/ E horizon, root and interrupted with stones; a metal soil core sampler inserted on the soil surface for collecting sample**



**Soil surface of the MDF site with sparsely distributed litter-fall on the forest floor; a metal soil core sampler inserted on the soil surface for collecting sample**



**Soil surface of the UDF site with thickly distributed litter-fall on the forest floor; a metal soil core sampler inserted on the soil surface for collecting sample**

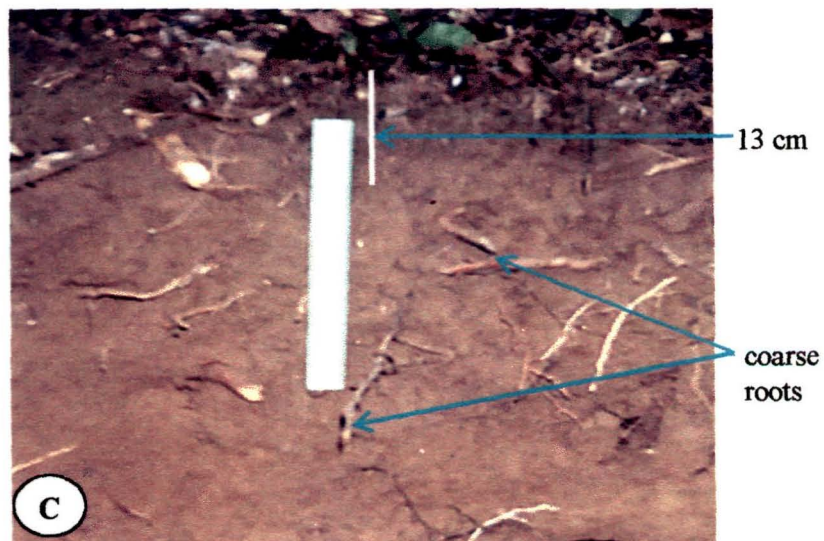
## Plate 5



**Soil profile of the DF site with very thin layer of humus; average thickness of  $A_h$  horizon is 0-3 cm**



**Soil profile of the MDF site with moderately thick layer of humus; average thickness of  $A_h$  horizon is 0-7 cm**



**Soil profile of the UDF site with very thick layer of humus; average Thickness of  $A_h$  horizon is 0-13 cm**

## BIO-DATA

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#### Educational Statistics

YEAR	EXAMS	DIVISION	% MARK	INSTITUTE / UNIVERSITY
1996	*GATE	Qualified	74.32 (percentile)	IISc Bangalore
1996	M.Sc. Botany	1 <sup>st</sup> div.	65.94	<sup>§</sup> NEHU, Shillong
1994	B.Sc. Botany	1 <sup>st</sup> div.	71.92	Manipur University, Imphal

**Scholarship Abroad** Awarded DAAD (German Academic Exchange Service, GAES) Short Term Scholarship-2002 (4 months) in the Institute of Soil Ecology, National Research Center for Environment and Health, Neuherberg, Germany

#### Research

1999-2002 Senior Research Fellow (CSIR), Department of Forestry, <sup>#</sup>NERIST  
1997-1999 Junior Research Fellow (CSIR, Scheme), Department of Forestry, NERIST

**IT skills** MS Office, Origin 4.1, Statistica, SPSS, Gel Compare and Windows 9X, XP  
**Languages** English, Hindi, Manipuri, Assameese

#### Trainings

- DBT sponsored contact training course (21 days) on "MYCORRHIZOSPHERE" at the Unit of Microbiology, School of Life Sciences, JNU, New Delhi in 1999 under the Directorship of Prof. Ajit Varma.
- Training workshop on "Aromatic, Medicinal and Spice plants" at Imphal, Manipur under the sponsorship of Manipur Science and Technology (MASTEC) and Regional Research Laboratory (RRL), Bhubaneshwor from 6-9<sup>th</sup> March 2001.
- Short-term training course on "Application of Computers in Offices" under the Department of Computer Science and Engineering, NERIST in May-June 1999.

\*Graduate Aptitude Test in Engineering conducted by Indian Institute of Science, Bangalore

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## PUBLICATIONS

### A. Papers Published in Research Journals (National and International)

1. S.C. Tiwari, S. S. Sorokhaibam, M.S. Dkhar and R R.Mishra (2002). Soil degradation affects microbial biomass carbon and dehydrogenase activity in humid tropical hilly forest soils. *Asian Journal of Microbiology, Biotechnology and Environmental Sciences* **4** (1): 143-148.
2. S. Sureshkumar Singh, S.C. Tiwari and M.S. Dkhar (2001). Evaluation of soil degradation using physico-chemical, biological and biochemical parameters in humid tropics of Arunachal Pradesh. *Annals of Forestry* **9** (2): 287-292.
3. S. S. Singh and S. C. Tiwari (2001). Modified wet-sieving and decanting technique for enhanced recovery of vesicular-arbuscular mycorrhizal (VAM) fungi from forest soils. *Mycorrhiza News* **12** (4): 12-13.
4. S.C. Tiwari and S. Sureshkumar Singh (2001) Modern molecular techniques in microbial diversity research. *Arunachal University Research Journal*. **4** (1): 27-34.
5. E.N.Singh., N. Angila, S. S. Singh and S.C. Tiwari (2001). Influence of *Tectona grandis* and *Duabanga grandiflora* plantations on soil properties of humid tropics of Arunachal Pradesh, North East India. *Indian Journal of Forestry* **24** (2):135-142.
6. S.C. Tiwari, J.K.Das, B.Loktongbam and S. S. Singh (1999). *Phoebe goalparensis* (Bonsum): A potential species for soil amelioration. *Arunachal Forest News* **17** (1&2): 1-7

### B. Papers in Press/ Revised/ Communicated in Research Journals

7. S.C. Tiwari and S. S. Singh (2002). Impacts of vesicular-arbuscular mycorrhizal (VAM) fungi diversity associated with degradation of soils in humid tropical forests. *Proc. New Horizons in Biotechnology*, Kluwer Academic Press: (In press)
8. S. S. Singh, S.C. Tiwari and M.S. Dkhar (2002). Species abundance and diversity of vesicular-arbuscular mycorrhizal (VAM) fungi in disturbed and undisturbed soils of Arunachal Pradesh, North Eastern India. *Tropical Ecology* (Revised).
9. S. S. Singh, S.C. Tiwari, M.S. Dkhar and M. Schloter (2002). Bacterial diversity in degraded and undegraded tropical soils as determined by PCR-amplified 16S rDNA fragments and denaturing gradient gel electrophoresis. *Plant and Soil* (communicated).
10. S. S. Singh, S.C. Tiwari, M.S. Dkhar, M. Schloter and A. Gattinger (2002). Microbial community structure of degraded and undegraded tropical soils as measured by phospholipid fatty acid (PLFA) profiles. *Applied and Environmental Microbiology* (communicated).

### C. Papers Published in Books

11. S. C. Tiwari and **S. S. Singh** (1998). Influence of *Glomus mossae* and *Frankia* on two plants in nursery. In: *New Trends in Microbial Ecology*. (Eds. B.Rai and M.S.Dkhar), The Computer Composers, Allahabad, pp 326-334
12. J.K Das, L. Bebija, **S.S. Singh** and S.C. Tiwari (1998). Potential forest tree species for amelioration of soil properties in humid tropics. In: *Natural Resources, Conservation and Management for Mountain Development*. (Eds. S.C.Tiwari and P.P. Dabara), International Book Distributors and Publishers, Dehra Dun, pp 371-381.

### D. Papers Published in Proceeding Volumes

13. S.C.Tiwari and **S. S. Singh** (1998). Influence of mycorrhizal and actinorhizal fungi on seedling growth. In: *Proc. of National Workshop on "Perspectives for Planning and Development in North East India*. (Eds. R.C.Sundriyal, Uma Shankar and T.C. Opreti), GBPIHED Occasional Pub. No.11. Bishen Singh Mahendra Pal Singh, Dehra Dun, pp 181-184.
14. **S. S. Singh** and S. C. Tiwari (1998). Microbial diversity-Its Role in Sustainable Forestry and Agriculture. In: *Proc. National Symposium on Plant Diversity Pattern and Bioresource Technology*, Sugarcane Research Institute, Kurnaghat, Gorakhpur, Lead Paper, pp-1,

### E. Popular article

15. **S. S. Singh** and S.C. Tiwari (1998). Microbial diversity and its importance. *Employment News*, dated 10-16<sup>th</sup> January, 1998.

### Seminars / Workshops/ Conferences/ Symposia attended

1. DST sponsored workshop "*Patent Awareness*", NERIST, October 2000.
2. National Workshop on "*Arunachal Pradesh: Environmental Planning and Sustainable Development-Opportunities and Challenges*", Itanagar, December 1999
3. National Conference on "*Mycorrhiza*" held at Barakatulla University, Bhopal, March 1999
4. National Seminar on "*Role of Microbes in Rural Development and Environmental Protection*", NEHU, Shillong, October 1998
5. National Workshop on "*Perspectives of Planning and Development in North East India*", NERIST, April 1998

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