

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

By  
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THE NORTH-EASTERN HILL UNIVERSITY  
October, 2002

DECLARATION

I, Sorokhatiam 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 original and has not been published elsewhere, and that the contents of this thesis are the work done by me, that the contents of this thesis are not the work of any other person, and that the thesis is not being submitted to any other University for the award of the degree.

*Dedicated to  
the Loving Memory of my  
Grandfather  
& my beloved Parents*

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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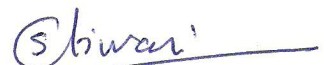
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## Abbreviations

<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>MODF</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

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

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

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## **GENERAL INTRODUCTION**

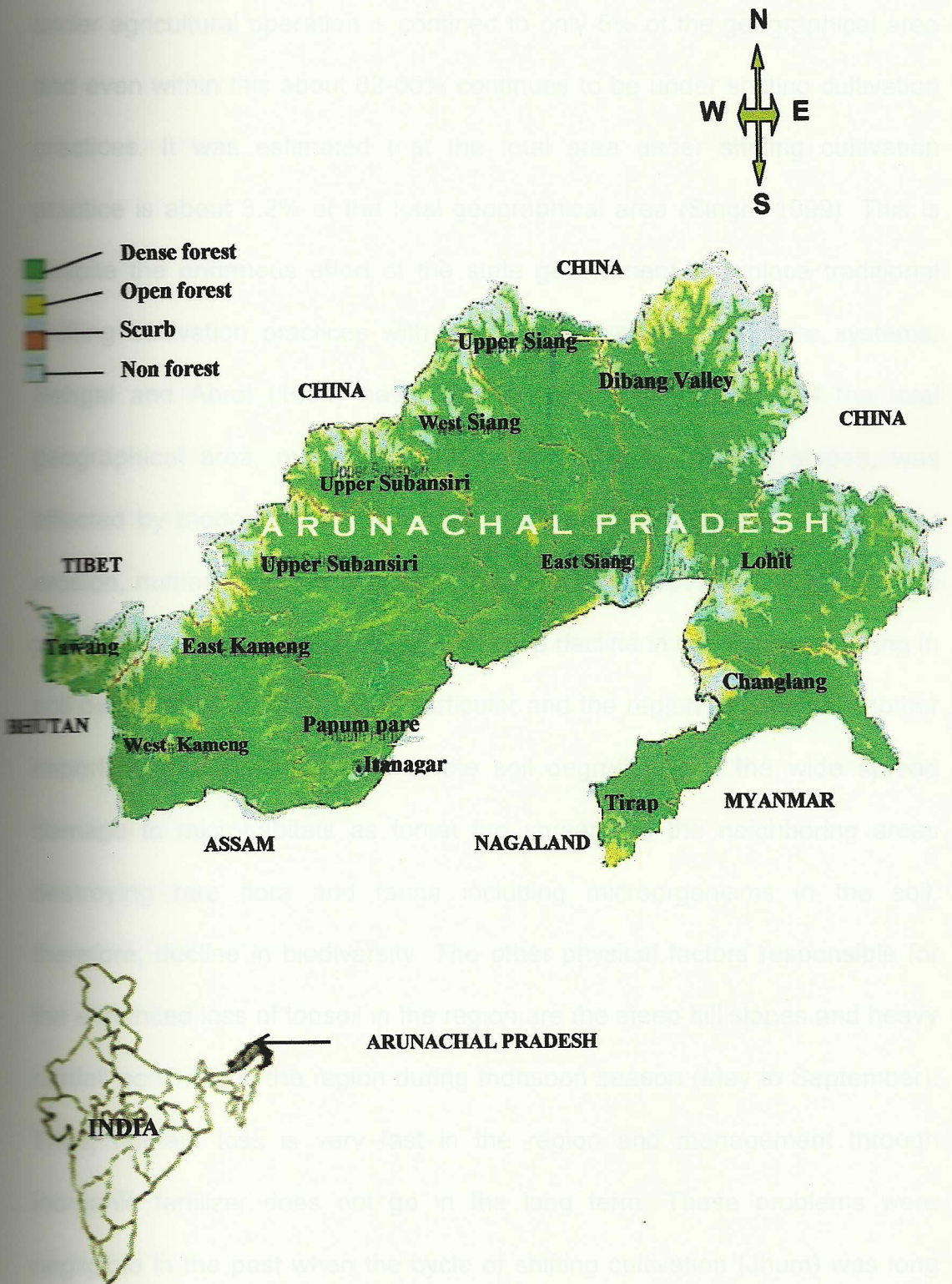
Soil is the natural resource on which the entire terrestrial life depends for food, fiber and shelter. Most of the arable lands on the earth is under tremendous pressure of intensive agricultural practices and other developmental activities such as urbanization and industrialization. This has caused 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



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

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

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  $\text{CO}_2$  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  $\text{CO}_2$  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  $\text{CO}_2$  flux (Wagai *et al.*, 1998) as a measure of microbial activity. Therefore, soil disturbance activities like clear-cutting of

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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.